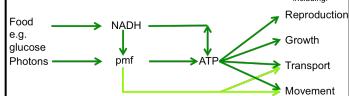
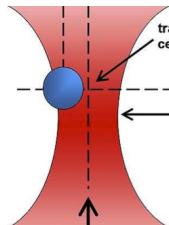


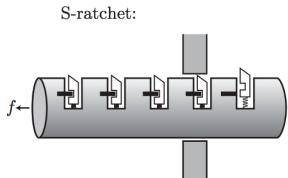
Molecular Motors



Energy Sources:
Protonmotive Force, ATP



Single Molecule Biophysical Techniques :
Optical Tweezers, Atomic Force Microscopy, Single Molecule
Fluorescence Microscopy



Physical Models :
Brownian Ratchets, Smoluchowski Equation

Molecular Motors

Molecular motors are proteins that are able to convert energy stored in ATP or ion gradients into mechanical work

- *Cytoskeletal motor proteins*: myosin, kinesin, dynein
- *Polymerization motors*: actin, microtubules, RecA
- *Ion pumps*: Na-K pump
- *Rotary motors*: ATP-synthase, bacterial flagellar motor
- *DNA motors*: RNA polymerase, helicases

Molecular Motors Functions

Contraction of stress fibres (actin - myosin)

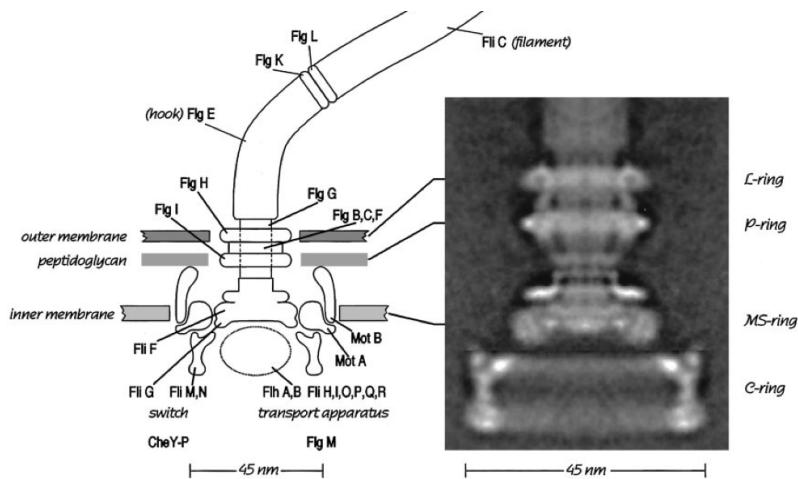
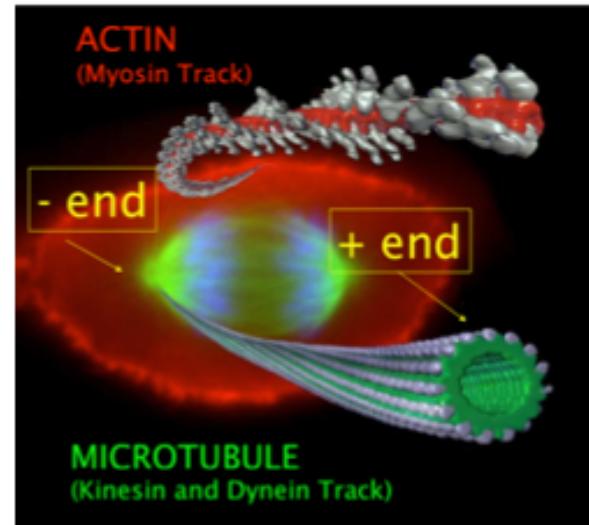
Muscle contraction (actin - myosin)

Cell motility (actin - myosin)

Cell division (actin - myosin)

Separation of chromosomes (MT - dynein)

Transport of cargo (MT - kinesin/dynein)

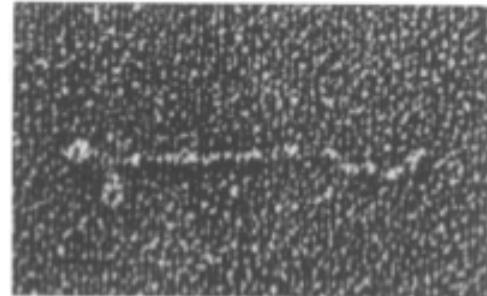


Bacterial chemotaxis (flagellar motor)

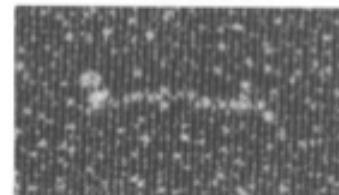
Energy storage in the form of ATP
(ATP synthase motor)

Motor Protein Structures

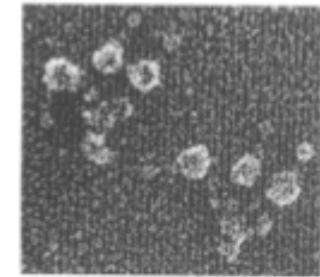
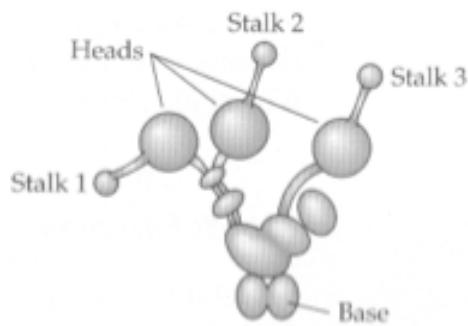
Myosin : moves towards +end of actin



Kinesin : moves towards +end of microtubule

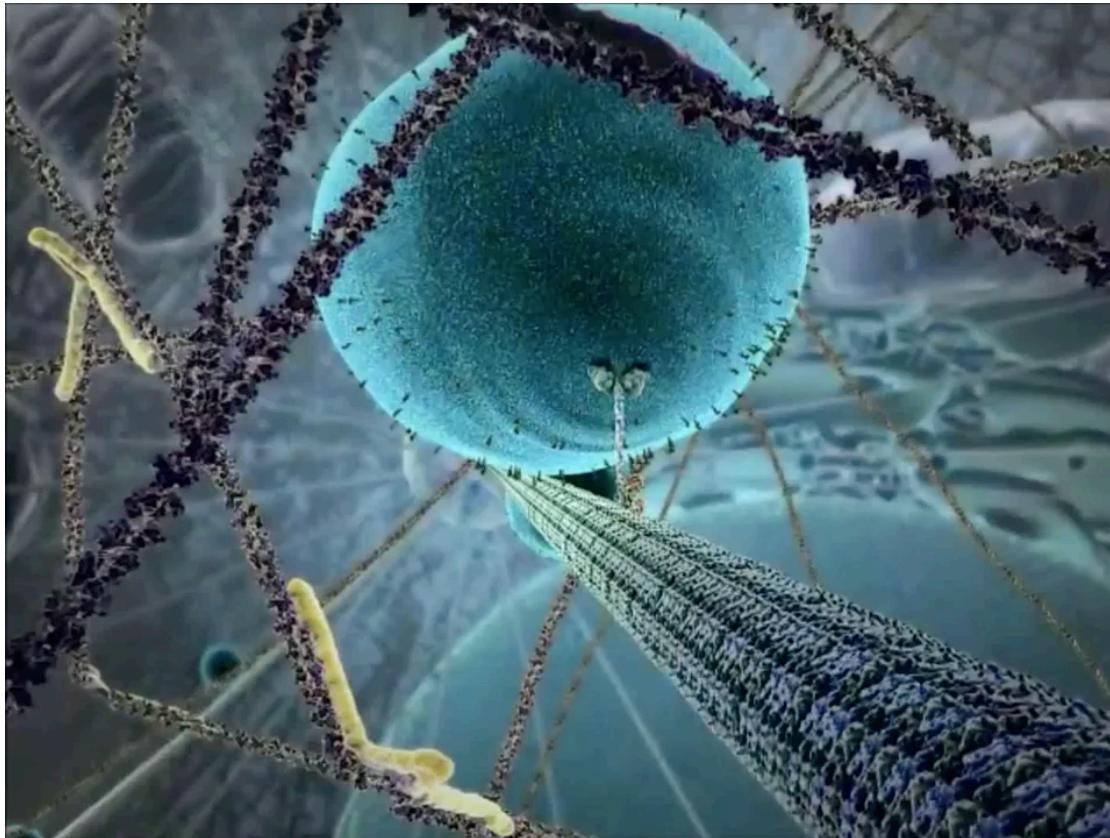


Dynein : moves towards -end of microtubule

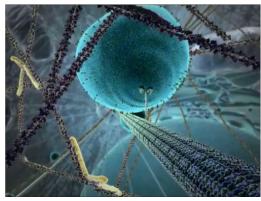


100 nm

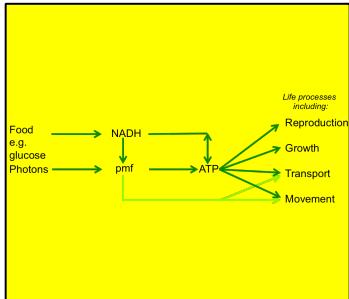
Molecular Motors in action



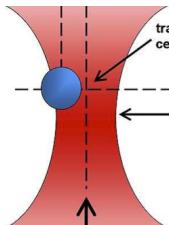
“The Inner Life of a Cell” by Cellular Visions and Harvard



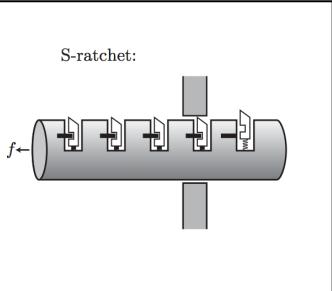
Molecular Motors



Energy Sources:
Protonmotive Force, ATP

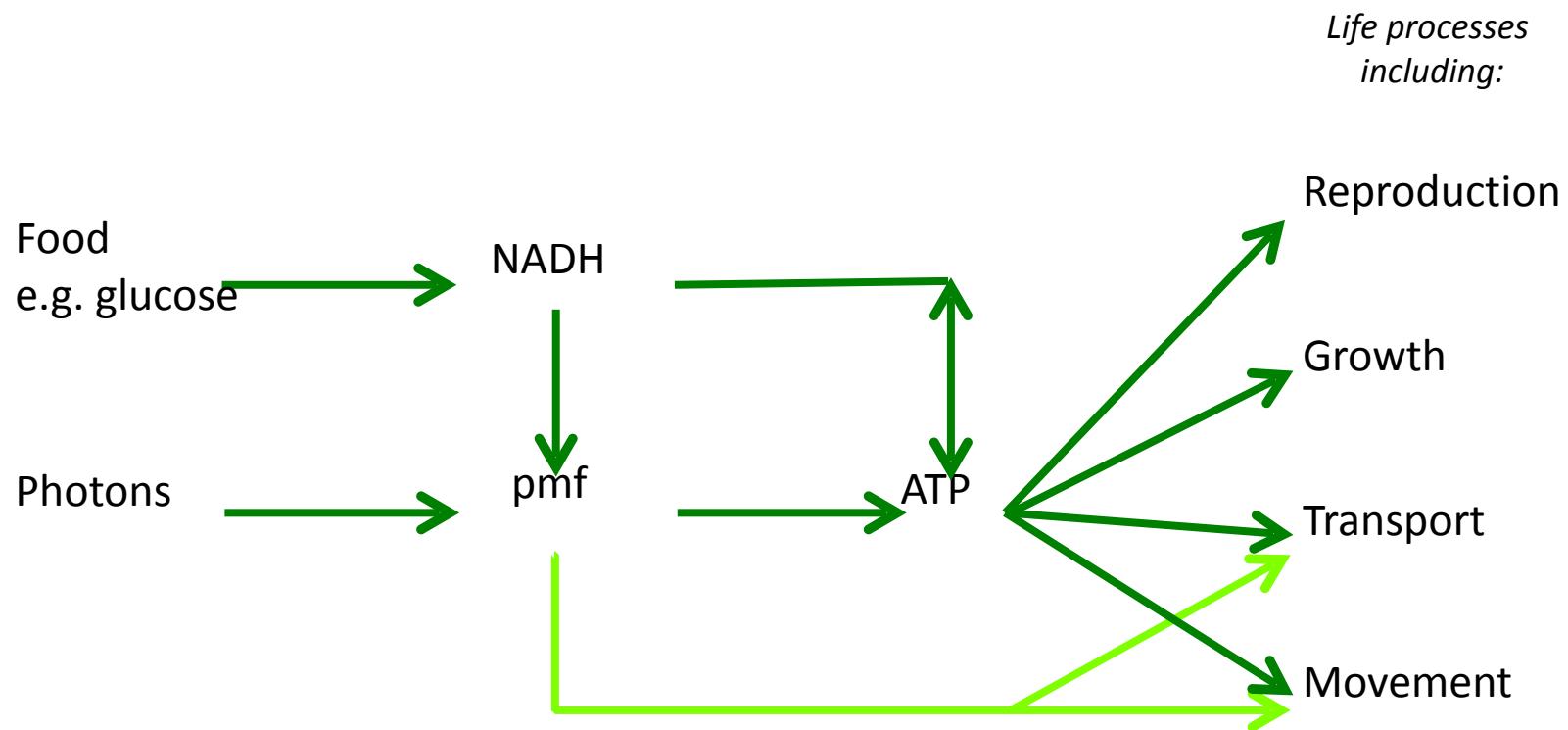


Single Molecule Biophysical Techniques :
Optical Tweezers, Atomic Force Microscopy, Single Molecule
Fluorescence Microscopy

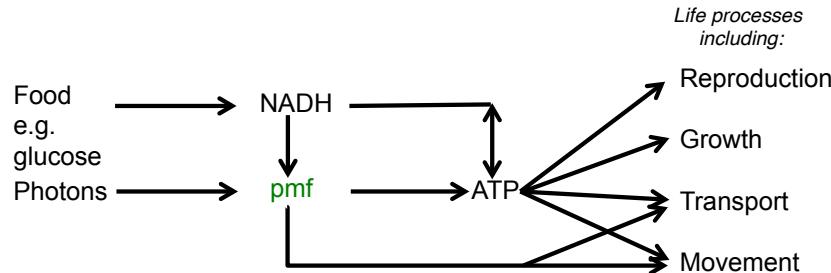


Physical Models :
Brownian Ratchets, Smoluchowski Equation

Energy Flow In Living Systems



Protonmotive Force

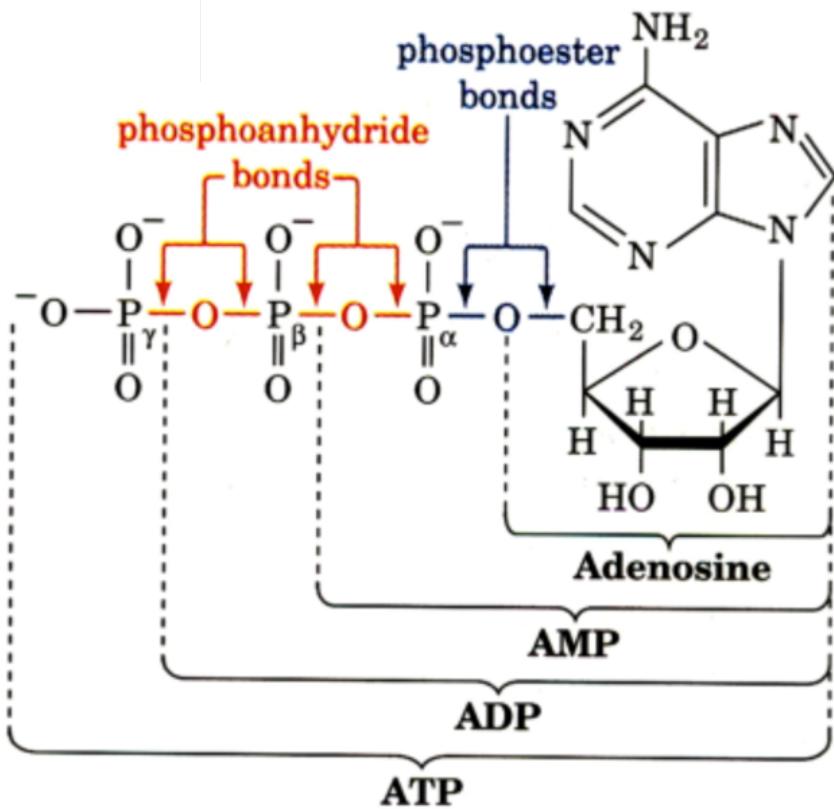


$$pmf = V_m + \frac{\Delta\mu}{e} = V_m + \frac{k_B T}{e} \ln\left(\frac{c_i}{c_o}\right)$$

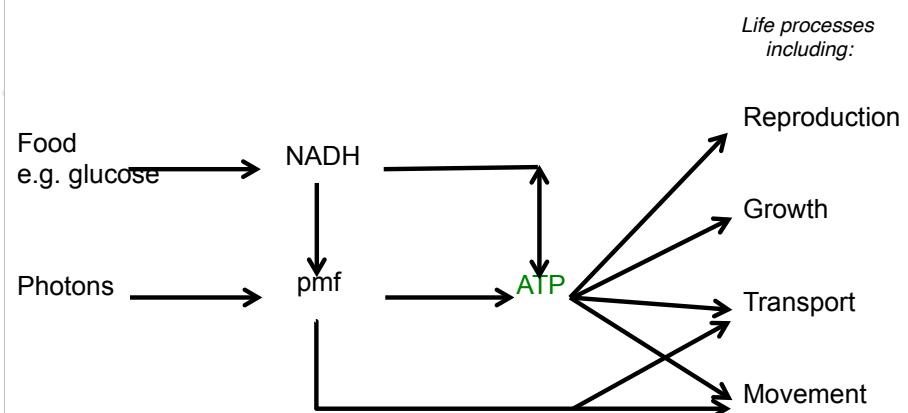
pmf is the extra free energy per unit charge inside the cell versus outside

- Sign convention inside minus outside
- Units of volts
- V_m is the electrical potential, $\Delta\mu$ is the chemical potential
- Typically pmf is in the range -150 mV to -200 mV
- Usually both components negative : inside has lower voltage and lower $[H^+]$
- Powers FLAGELLAR MOTOR and ATP SYNTHASE

ATP



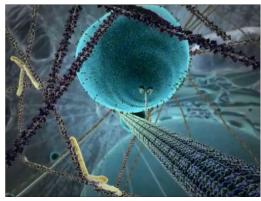
Hydrolysis of ATP : $\Delta G = -20 \text{ k}_\text{B} T$ to $-30 \text{ k}_\text{B} T$



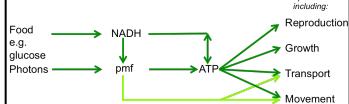
Hydration free energy is larger for the products. In part due to entropy, in part due to better hydrogen bonding and electrostatic screening.

Charge repulsion between phosphate groups

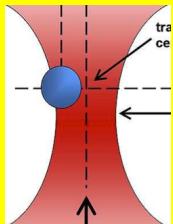
Resonance : partially delocalized electrons free to move between oxygens. The shared oxygen bond reduces the number of arrangements of electrons (entropy) compared to hydrolysis products



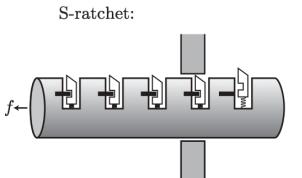
Molecular Motors



Energy Sources:
Protonmotive Force, ATP

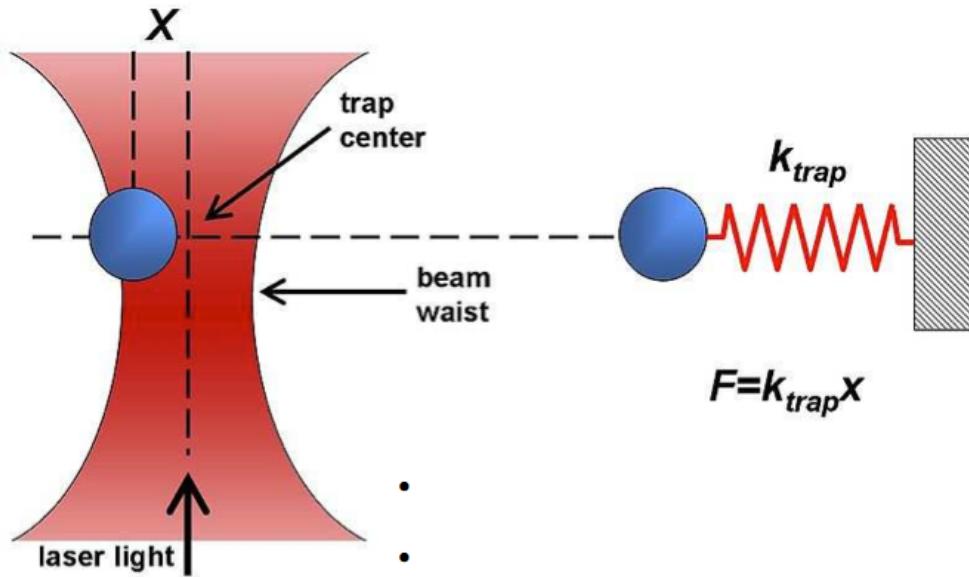


Single Molecule Biophysical Techniques :
Optical Tweezers, Atomic Force Microscopy, Single Molecule
Fluorescence Microscopy



Physical Models :
Brownian Ratchets, Smoluchowski Equation

Optical Tweezers



- 10s – 100s pN
- Can be used to control beads specifically attached to proteins and cells
- Infrared light (low absorbance, cheap lasers)
- Acousto-optic deflectors enable beam
- Multiple traps made possible by time sharing
- Micro-Tetris with 1um glass beads

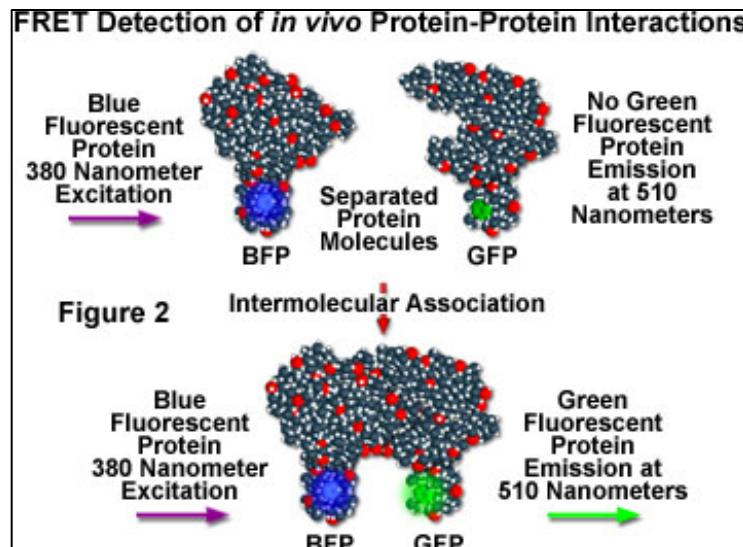
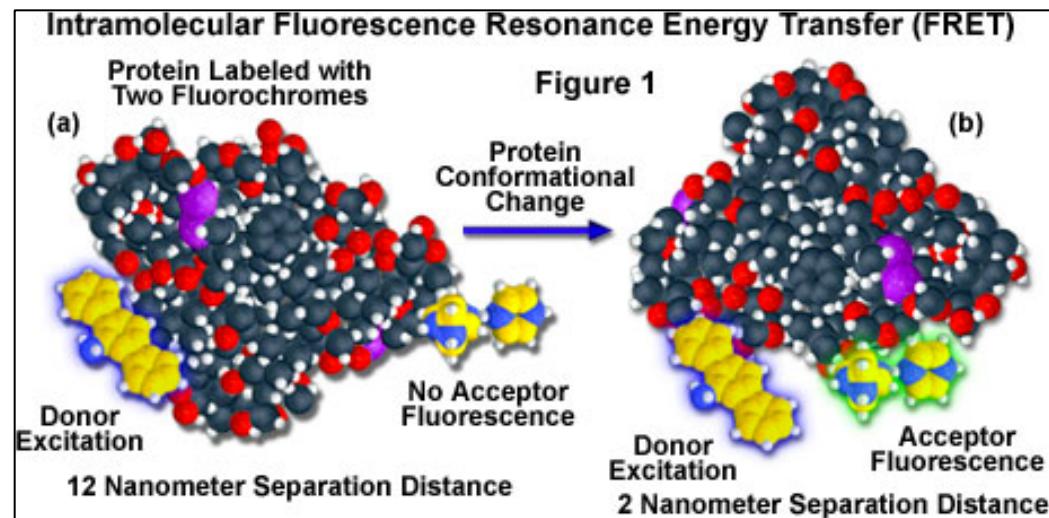
Single Molecule Fluorescence Imaging : FRET

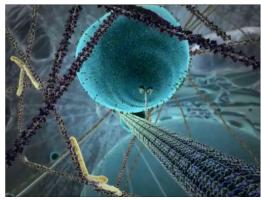
FRET a molecular ruler:
Electronic excitation energy
can be transferred between
two chromophores in close
proximity

Key Use :
Protein-Protein interactions &
Protein conformation changes
at high spatial precision < 10
nm

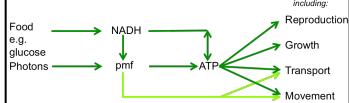
Practical Limitations : Control
of chromophore
concentrations,
Photobleaching, Signal
Separation and Detection

Fernandez *et al* Cell surface distribution of lectin
receptors determined by resonance energy transfer.
Nature **264**, 411–415 (1976)

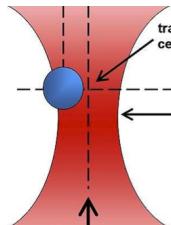




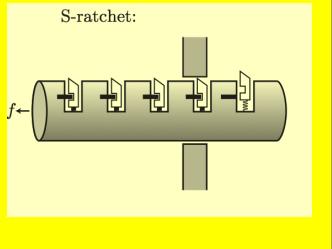
Molecular Motors



Energy Sources:
Protonmotive Force, ATP

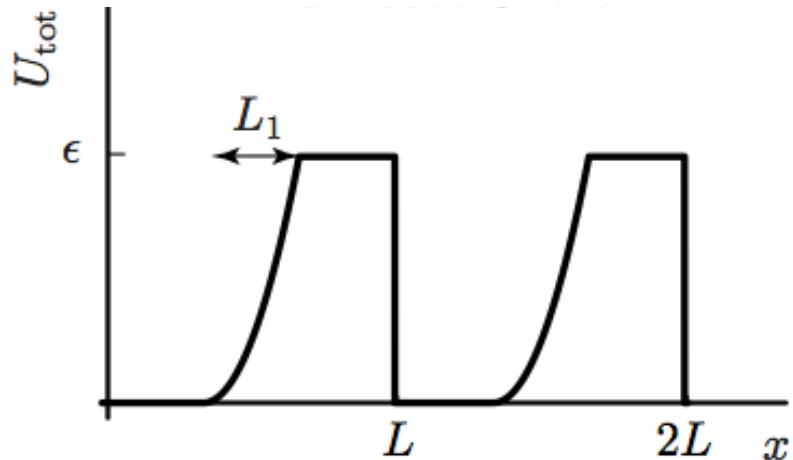
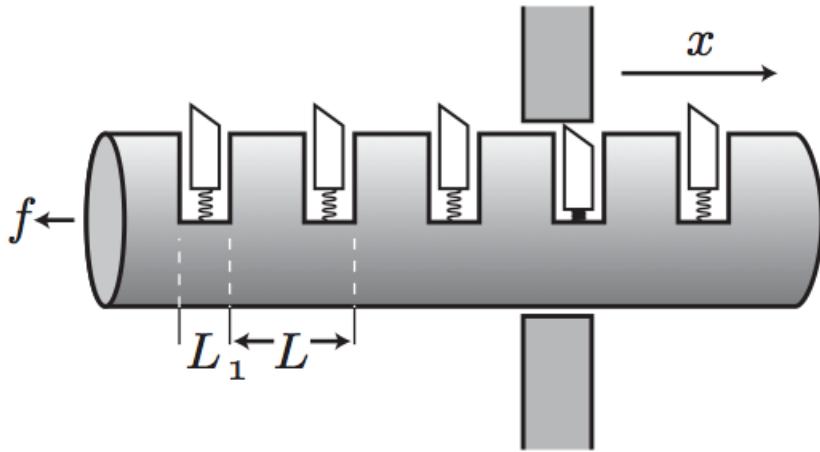


Single Molecule Biophysical Techniques :
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Fluorescence Microscopy

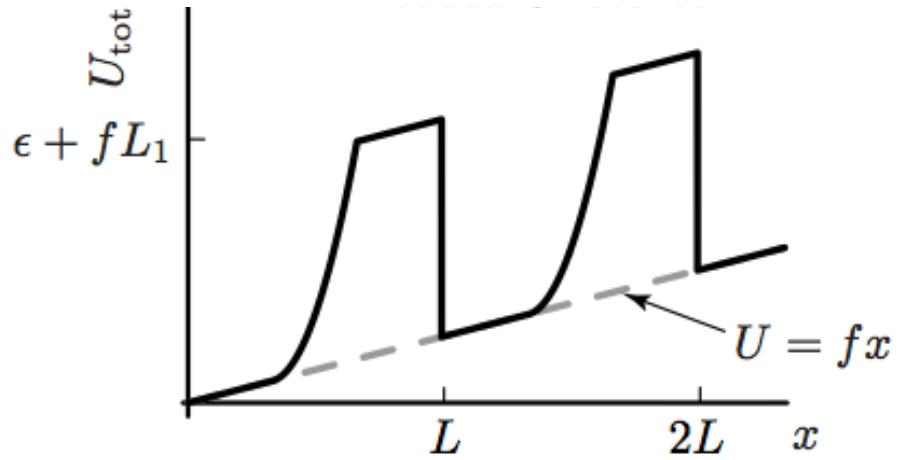


Physical Models :
Brownian Ratchets, Smoluchowski Equation

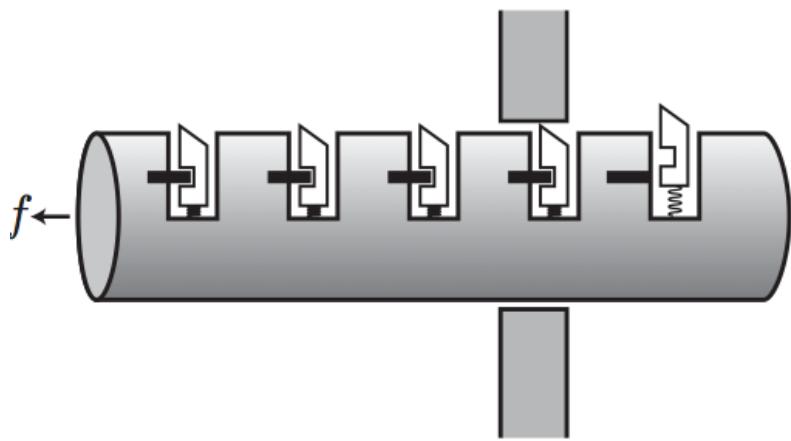
Thermal Ratchet



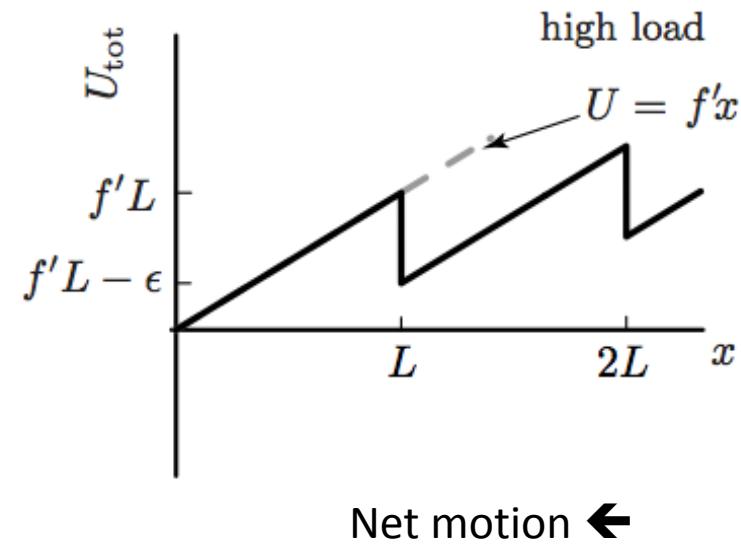
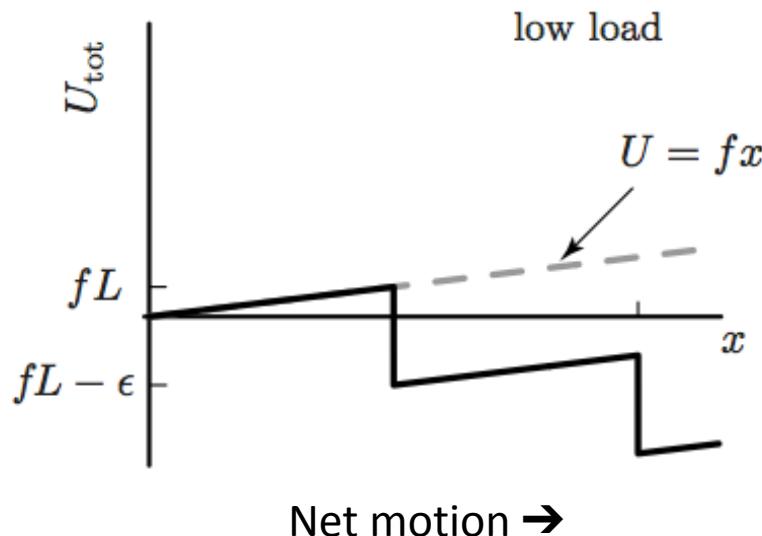
- Consider an idealised nanoscale device operating in a thermal environment subject to random fluctuations : shaft with series of bevelled bolts mounted on springs
- Thermal push $> \epsilon + fL$ will push shaft to right
- Prevented from moving to left by tapered sliding bolts
- Violates 2nd law (loop version)
- If the energy for bolt retraction ϵ comparable to kT the machine can step to the left



Thermal Ratchet with stored potential energy



- Consider a modification in which a latch keeps bolt down when it's to left of wall but releases the bolt on right side
- Movement to right doesn't violate 2nd Law (Why?)
- Rectified Brownian Motion
- Stall force $f = \epsilon/L$



Rectified Brownian Motion

This rectified Brownian ratchet is equivalent to a random walk with absorbing and reflecting boundary conditions.

The ratchet diffuses back and forth until it happens to move a distance L to the right, when the next spring engages. At this time, the ratchet starts at position $x = 0$.

If the free energy drop is very high, the ratchet cannot move to the left i.e. leftward flux is not allowed, so the LH boundary condition (at $x = 0$) is perfectly reflecting. If the free energy drop ϵ is finite, there is a probability of moving leftward, but it's small if $\epsilon/k_B T$ is large

If the ratchet ever reaches position $x = L$, the next spring is triggered and if the free energy drop ϵ is very high the position $x = L$ acts like a perfectly absorbing boundary. If the free energy drop ϵ is finite, the walker simply falls off a very large cliff and is unlikely to be able to return.

This type of problem involves calculating the time it takes for a random walker to reach a specified target and is called a first passage time problem

Diffusion in Force Field

- Consider particles moving under the influence of a constant external force F
- Diffusive motion of the particles modelled by Fick's law:
- External force $F = -\partial\phi/\partial x$ which in the absence of any diffusive motion would impart drift velocity $v = F/\zeta$
- The motion of the particle is the sum of the contributions of diffusion and field-driven drift

$$J_x = \underbrace{-D \frac{\partial c}{\partial x}}_{\text{Diffusion flux}}$$

$$J_x = \underbrace{-D \frac{\partial c}{\partial x}}_{\text{Diffusion flux}} - \underbrace{\left(\frac{D}{k_B T} \cdot \frac{\partial \phi}{\partial x} \right)}_{\text{Drift flux}} c = -D \left(\frac{\partial c}{\partial x} + \frac{\partial(\phi/k_B T)}{\partial x} \cdot c \right) \quad [1]$$

- At equilibrium the flux vanishes : $J_x = 0$. Integrating with respect to x gives the equilibrium concentration of particles in external field $\phi(x)$: $c_{eq} = c_0 e^{-\phi/k_B T}$ (Boltzmann Distribution)

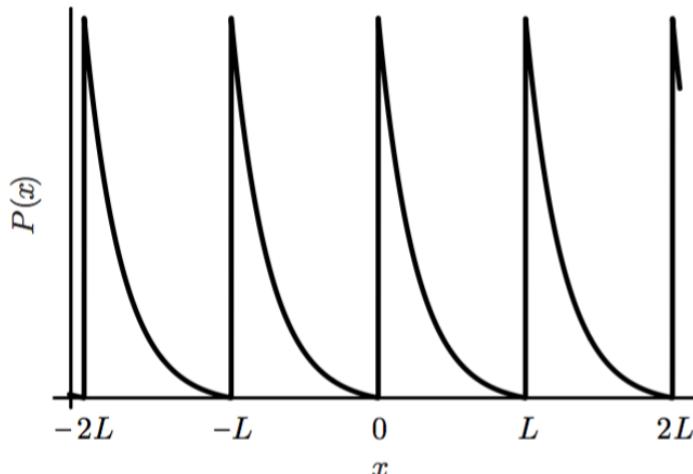
Smoluchowski Equation

- Reframe the problem in terms of the probability of finding a single particle at (x,t).
- Normalize the concentration in the previous slide : $p(x, t) \equiv c(x, t) / (\int_0^L c(x, t) dx)$ [2]
- Conservation of particles implies : $\frac{\partial c}{\partial t} = -\frac{\partial J_x}{\partial x}$ [3]
- Combining [1],[2] & [3] gives the Smoluchowski equation. At steady state LHS is 0 (not necessarily equilibrium)

$$\frac{\partial p}{\partial t} = D \left[\underbrace{\frac{\partial}{\partial x} \left(p \frac{\partial(\phi/k_B T)}{\partial x} \right)}_{\text{Drift}} + \underbrace{\frac{\partial^2 p}{\partial x^2}}_{\text{Diffusion}} \right]$$

Molecular Ratchet : Smoluchowski Equation

- Consider circular ratchet with bolts reset at ($x = x+4L$). Look at motion of ensemble M.
- To find the speed of the ratchet we need to find the flux of particles J_x from which we can calculate the average time it takes for the ratchet to cross a ‘step’ in the potential energy landscape. The speed is the step length divided by this time.
- The steady state probability distribution $p(x)$ for the ‘perfect ratchet’ case where $\varepsilon > K_B T$



Speed of the Ratchet

[Question Sheet Problem]

- Verify $P(x) = C(b e^{-(x-L)F/k_B T} - 1)$ solves Smoluchowski equation for a constant force F . You can assume steady state.
- Find the current flux : Expression in terms of p sub equation [2] in [1]
- Show average speed of the loaded ratchet perfect ratchet is

$$v = \frac{2D}{L} \quad v = \left(\frac{FL}{k_B T} \right)^2 \frac{D}{L} \left(e^{FL/k_B T} - 1 - FL/K_B T \right)^{-1}$$

$$F = 0$$

$$F < 0 \text{ and } FL/kT \gg 1$$

Forward stepping contains exponential activation barrier

Polymerization Ratchet

- Addition of monomers generates a force
- When filament reaches the barrier (e.g. cell wall) fluctuations in position of cell wall or filament will allow another monomer to squeeze through and bind

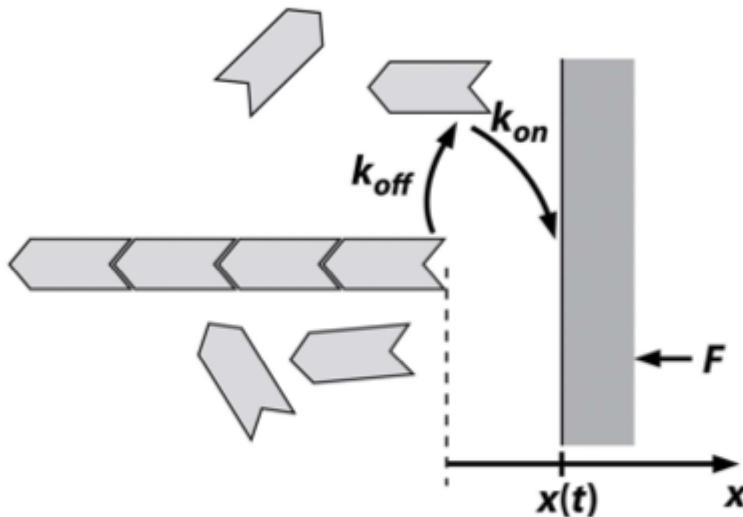


Figure 16.44 Physical Biology of the Cell (© Garland Science 2008)

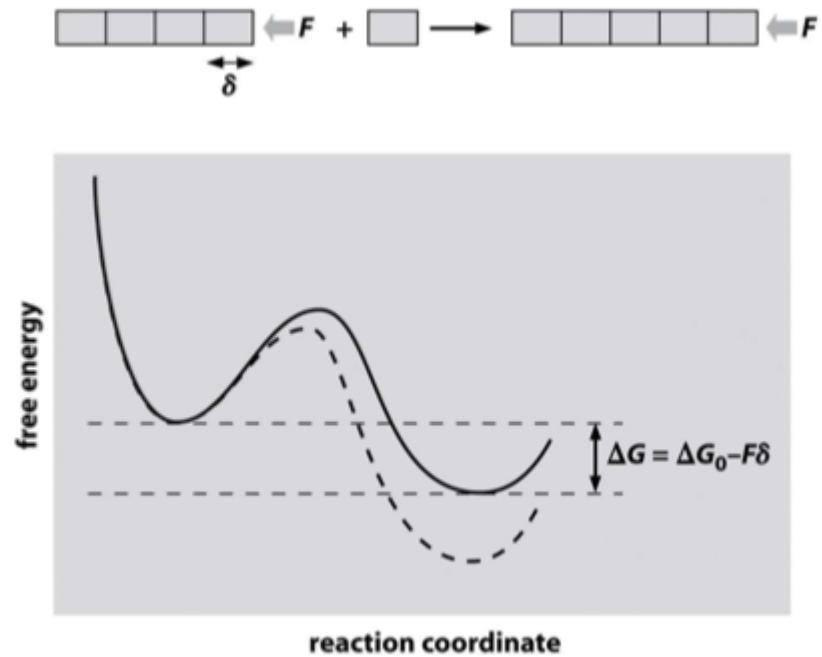
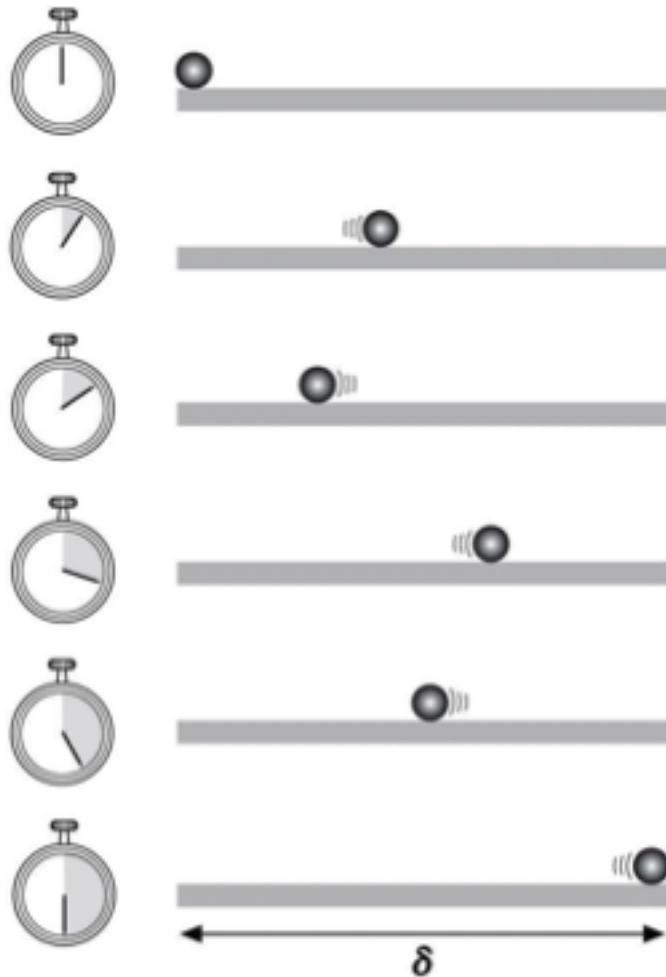


Figure 16.43 Physical Biology of the Cell (© Garland Science 2009)

Polymerization Ratchet contd



- How do we find the velocity (filament polymerization rate)?
Driven diffusion equation
- Want to find $p(x)$ of finding a particle at x in width δ
- Force $p(x)$ to be 1 in $(0, \delta)$ interval
- Mean rate at which particle reaches boundary at $x = \delta$ starting at $x = 0$ is

$$j_0 = -D \frac{\partial p}{\partial x} - \frac{F}{\zeta} p$$

- We want to solve for $p(x)$ in terms of j_0

Figure 16.46 Physical Biology of the Cell (© Garland Science 2009)

Velocity of Polymerization Ratchet

The solution is the sum of the general solution of homogeneous Equation (absence of force) and a particular solution to inhomogeneous equation:

$$p(x) = A \exp(-Fx / k_B T) - j_0 \zeta / F, BC \rightarrow p(\delta) = 0; \int_0^\delta p(x) dx = 1$$

$$\text{Find } j_0, A \Rightarrow j_0 = \frac{1}{k_B T \zeta / F^2 (\exp(F\delta / k_B T) - 1) - \zeta \delta / F}$$

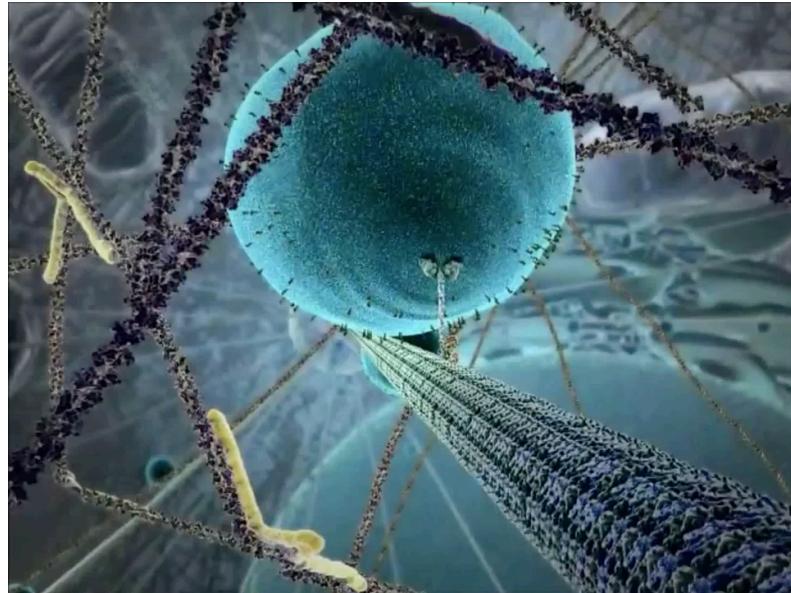
$$\therefore v = \delta j_0 = \frac{D}{\delta} \frac{(F\delta / k_B T)^2}{\exp(F\delta / k_B T) - 1 - F\delta / k_B T}$$

Low Force: $v = \frac{2D}{\delta}$ Same as no force limit – diffusion – first passage problem

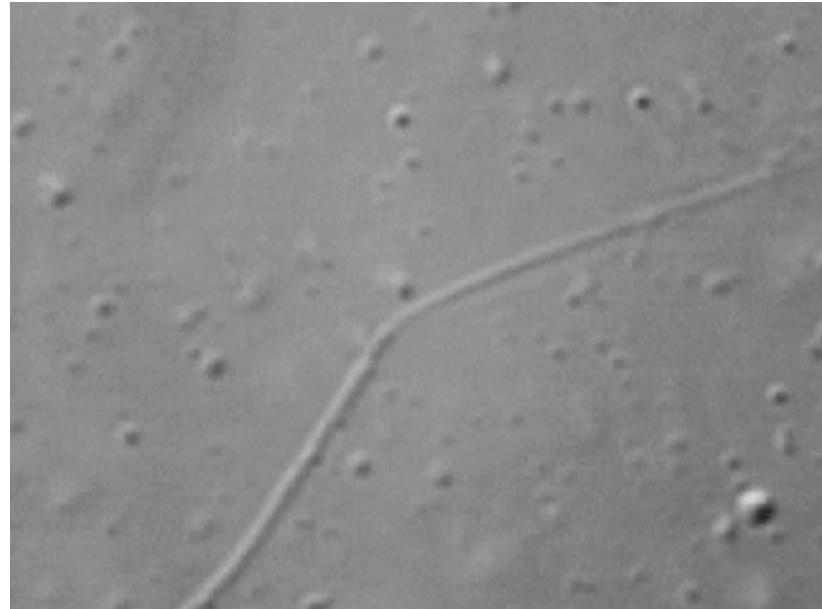
High Force: $v = \frac{F^2 D \delta}{(k_B T)^2} \exp(-F\delta / k_B T);$ Time to diffuse distance $k_B T / F$
 $FPT \rightarrow \tau = \delta / v = [(k_B T / F)^2 / D] \exp(F\delta / k_B T)$ Probability particle will find itself with energy $F\delta$

Motion of Motor Proteins

Kinesin walking along microtubules carrying a lipid vesicle as cargo



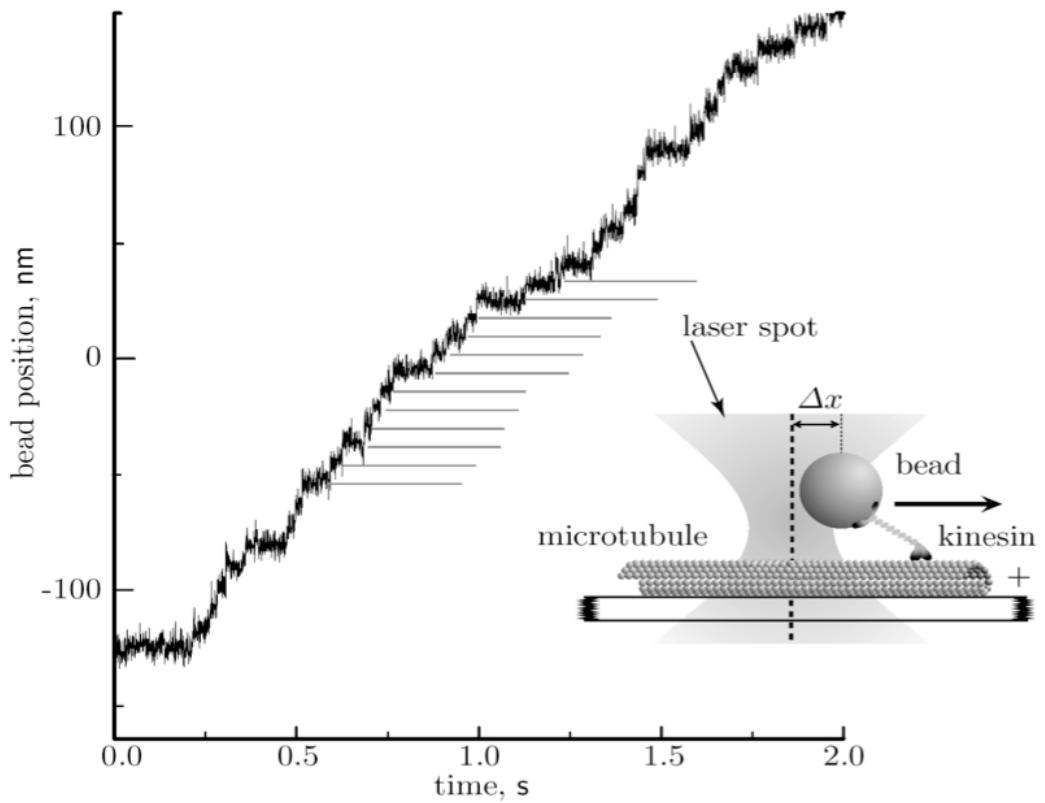
Organelles being carried along by kinesin and dyenin motors



How Proteins Work : Garland Science

Example : Kinesin

- Two headed molecular motor which walks along hollow protein structures called microtubules
- Tightly coupled (one spatial step for each ATP molecule consumed)
- Highly processive (many steps before detachment)
- Nearly 100% duty cycle



A *tightly-coupled* molecular motor with at least one *irreversible step* in kinetics should move at a speed according to MM kinetics (with parameters dependent on the load force)

Models of Specific Motor Proteins

- To build a model of motor movement need to consider :
- (1) Range of possible conformational states of the motor
- (2) Alterations to the energy levels of the conformational states by the biochemical reaction which powers the motor
- (3) Ways in which motor can change from one conformational state to another

One-state & Two state models

- Explore a class of problems where position and state are coupled and state transitions are driven by biochemical reactions
- Calculate average speed v and diffusion D parameters in of the Smoluchowski equation
- Incorporate details of internal states and transitions between them (two-state model).
- Dependence of the transition rates on the applied force explicit - assumed to be in the -ve direction
- Energy consumption factored in by having $k-$ not equal to $k+$ even with zero load

Motor Stepping

Real free energy isn't flat between steps
but more like this:

In principle we could solve the
Smoluchowski eqn for this landscape; in
practice we don't know its details

Rate limiting step is diffusion over a
free energy barrier

Transition rates can be related to barrier
height using Arrhenius relation

$$\frac{k_+}{k_-} = e^{-(\Delta G_{hydrolysis} + fL)/k_B T}$$

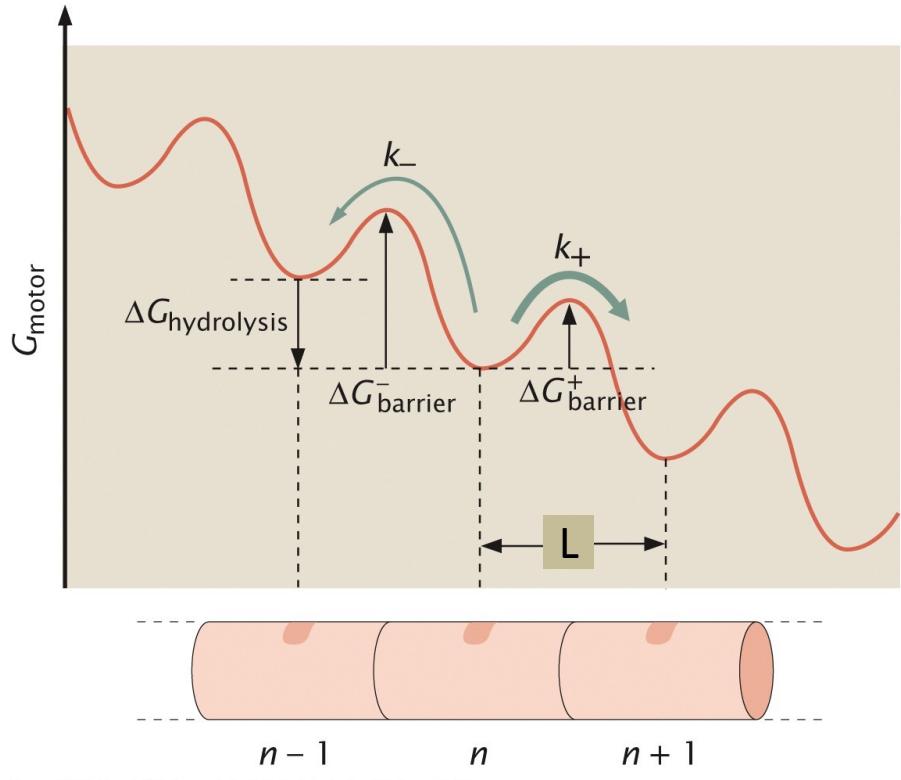


Figure 16.29 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

One-state ratchets

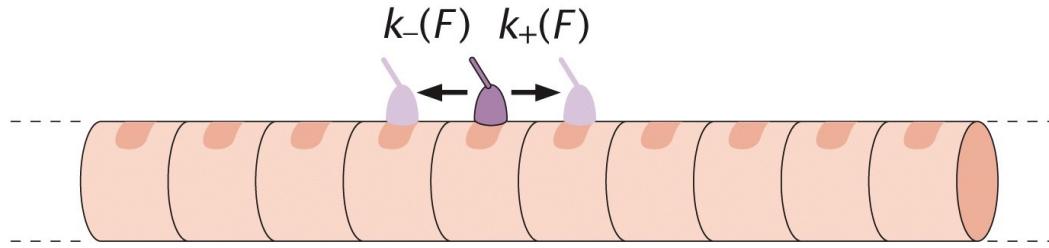


Figure 16.21 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Cartoon picture of e.g
kinesin moving
on a microtubule, 4 nm
periodic protein structure

- Linear motors move along regular, periodic tracks
- One-state model : forward stepping with force dependent rate constant k_+
backward stepping with rate constant k_-
all bound motors equivalent
- Free energy difference between steps:

$$\Delta G_{ATP} = \Delta G_0 + k_B T \ln \frac{[ADP][P_i]}{[ATP]}$$

$V([ATP])$ predictions

Two parameters : forwards and backwards stepping rate constants related by Thermodynamic arguments

$$\frac{k_+}{k_-} = e^{-\Delta G/k_B T} \propto [ATP]$$

Either can change with [ATP] but $v([ATP])$ different depending on which one

All dependence in backward rate

$$k_- \propto \frac{1}{[ATP]} \quad v = a \left(k_+ - \frac{k_-^0}{[ATP]} \right)$$

Speed saturates with [ATP]

All dependence in forward rate

$$k_+ \propto [ATP] \quad v = a \left(k_+^0 [ATP] - k_- \right)$$

Speed is linear with [ATP]

Kinesin : $v([ATP])$

- Kinesin data not consistent with pure k- or k+ modulation
- Kinesin not a simple one state ratchet

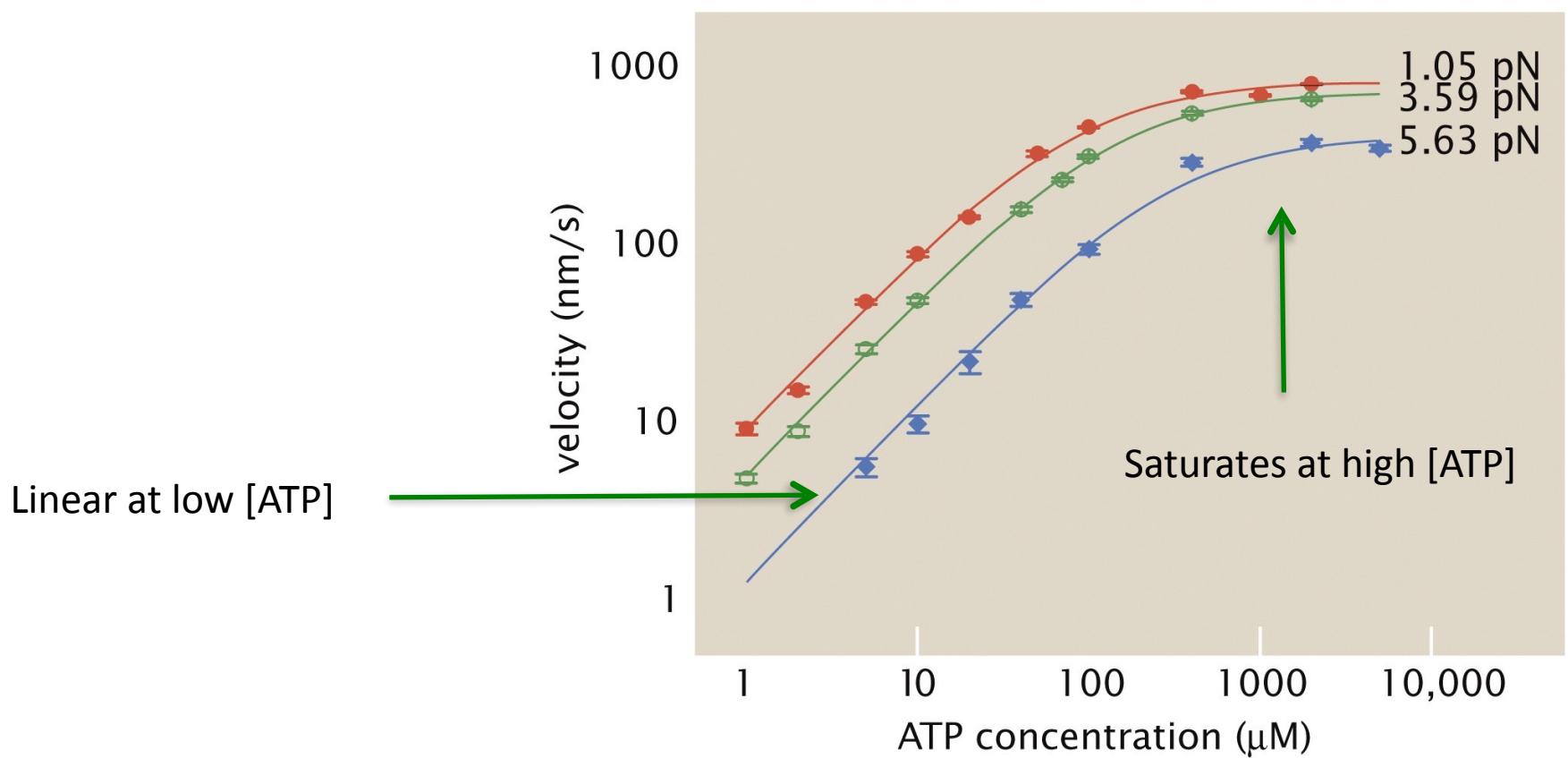


Figure 16.34a Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Dwell time distributions

For transitions controlled by activation
barriers waiting time for transition

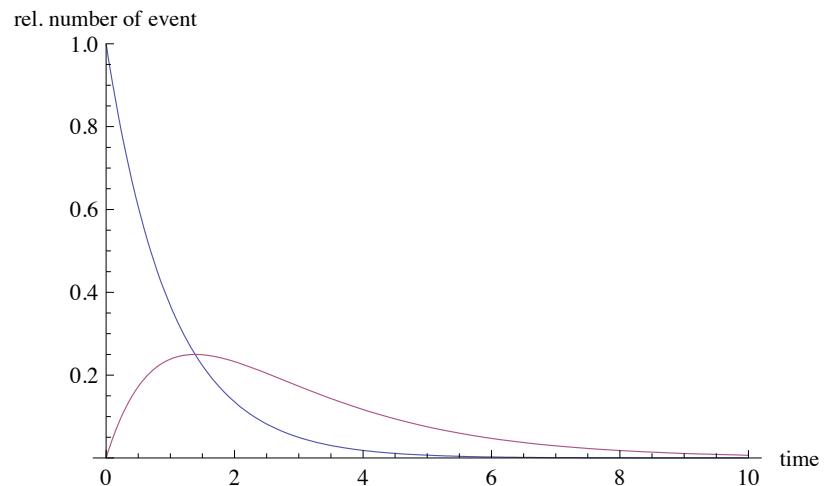
Exponentially distributed with mean time $\tau = 1/k$

Can distinguish between **one-state**

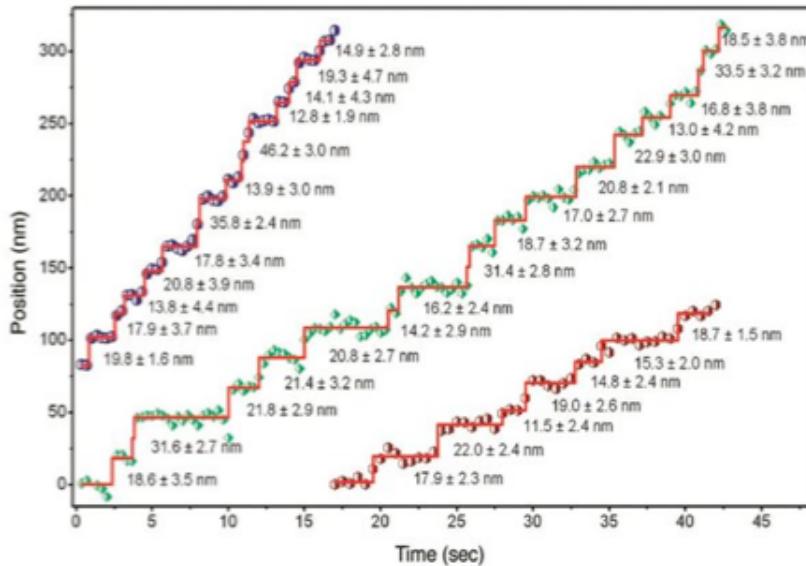
$$p(t) \propto e^{-t/\tau}$$

and **two-state** waiting time distributions

$$p(t) \propto e^{-t/\tau_1} - e^{-t/\tau_2}$$



Experimental Evidence



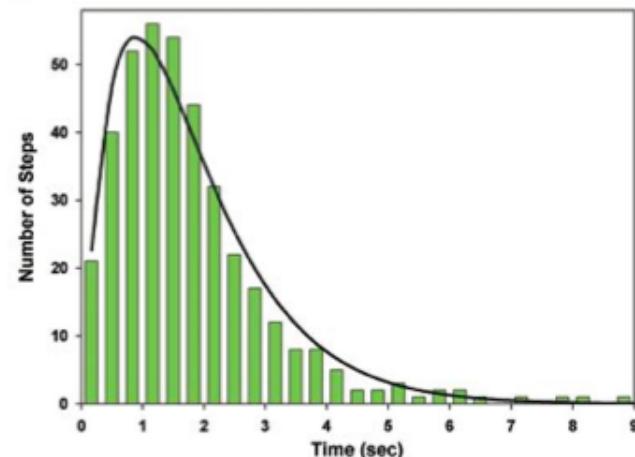
<- Step data from individual kinesin molecules stepping in microtubules

From which a distribution of Waiting times can be obtained |

V

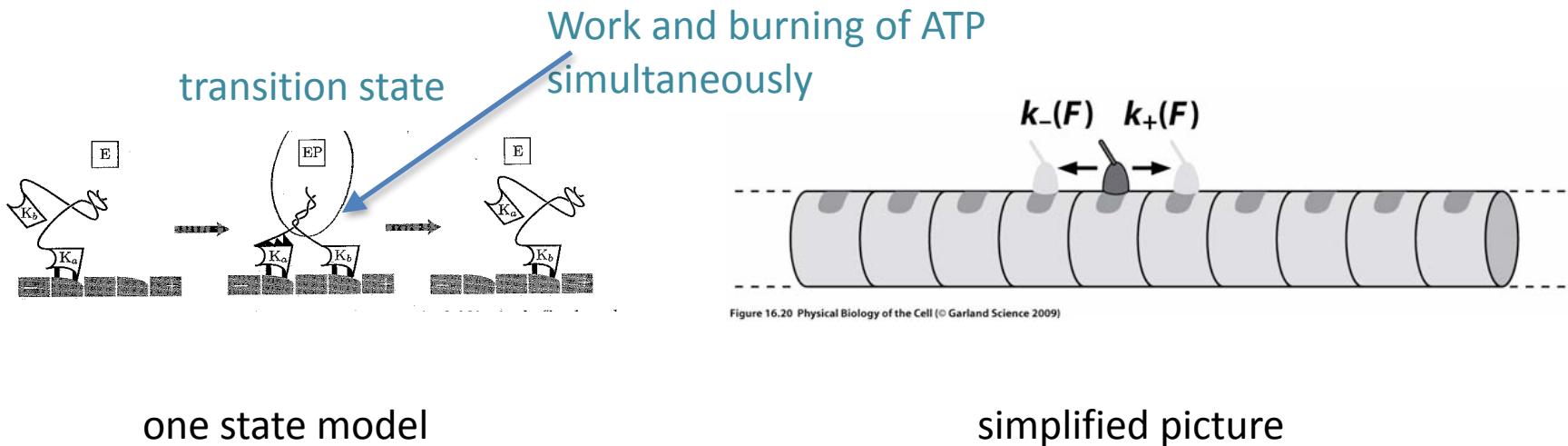
Kinesin has a bi-exponential dwell time distribution: it's at least a two-state ratchet

Myosin V also at least two-state (not shown here)



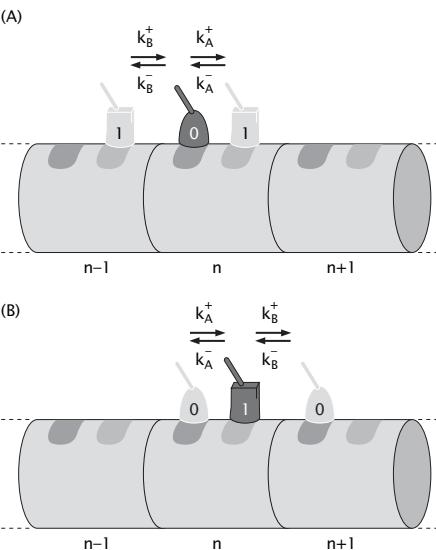
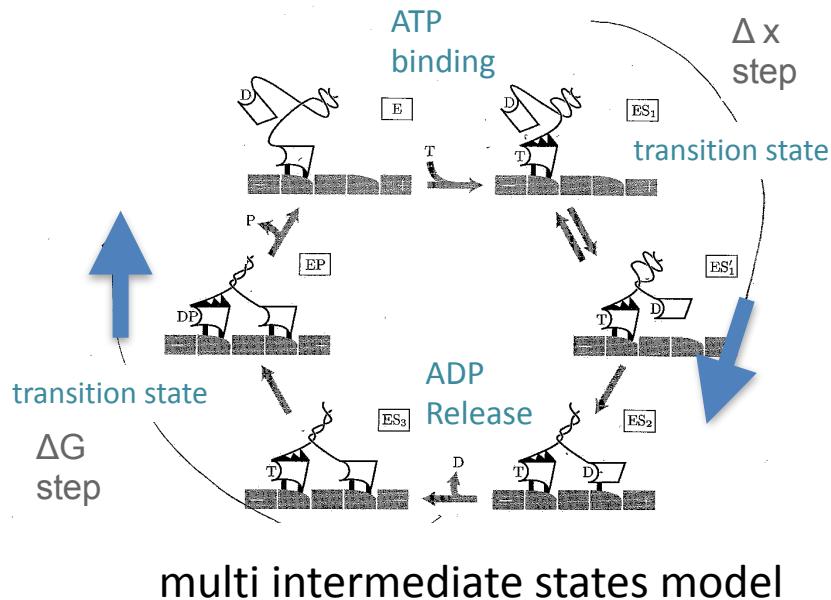
One-State Model Assumptions

- Most motor free energy landscapes cannot be reduced to a single distance reaction coordinate
- Single transition state assumption not accurate



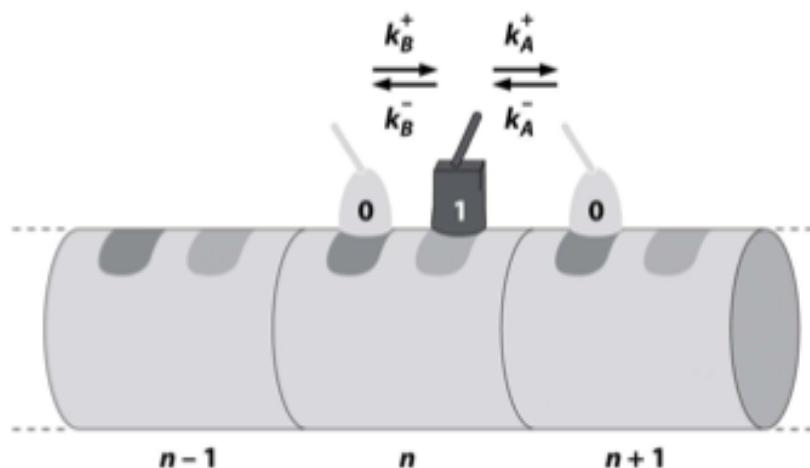
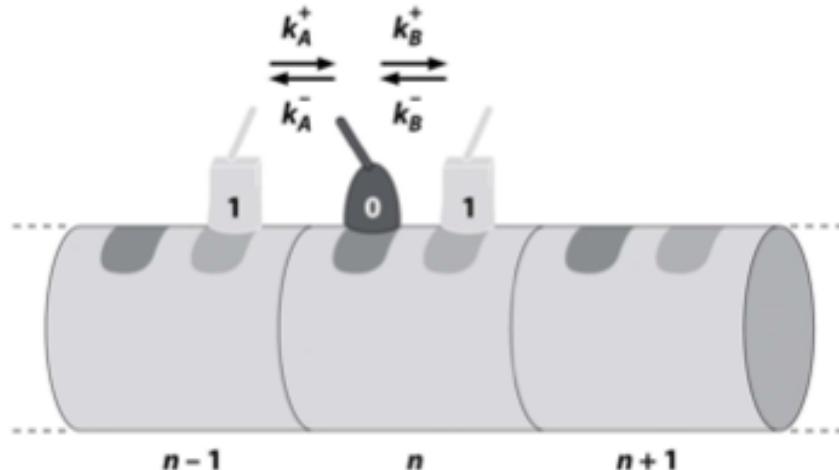
Additional States

- More detailed model with intermediate states required
- One more reaction coordinate
- Decouple ATP hydrolysis from physical movement
- Complete decoupling ATP hydrolysis insensitive to load f , physical movement insensitive to [ATP]



simplified picture

Two State Motor Models



- ⇒ Consider motor as two state system with states 0,1
- ⇒ Find $p_0(n,t)$ and $p_1(n,t)$ protein being in state 0 on subunit n at time t (similarly state 1)
- ⇒ k values give rates of transitions to different internal or position states

Rate equations:

$$\frac{dp_0(n,t)}{dt} = k_A^+ p_1(n-1,t) + k_B^+ p_1(n,t) - k_A^- p_0(n,t) - k_B^- p_0(n,t)$$

$$\frac{dp_1(n,t)}{dt} = k_A^- p_0(n+1,t) + k_B^- p_0(n,t) - k_A^+ p_1(n,t) - k_B^+ p_1(n,t)$$

⇒ Can reduce this to probability of being in state 0 or 1 irrespective of location n

⇒ Parameterized problem for one site is enough to capture dynamics

⇒ Assume : 0 → 1 while remaining on same site gives movement δ
0 → 1 moving to neighbouring site gives movement $a - \delta$
(a subunit size kinesin 8 nm)

$$\frac{dP_0}{dt} = k_A^+ P_1 + k_B^- P_1 - k_A^- P_0 - k_B^+ P_0$$

K_A's change state and n
K_B's change state

$$\frac{dP_1}{dt} = k_A^- P_0 + k_B^+ P_0 - k_A^+ P_1 - k_B^- P_1$$

$$\Rightarrow (k_A^+ + k_B^-)P_1 = (k_A^- + k_B^+)P_0 \text{ at steady state}$$

$$\text{Also, } P_0 + P_1 = 1$$

$$\therefore P_0 = \frac{k_A^+ + k_B^-}{k_A^+ + k_B^- + k_A^- + k_B^+} \text{ and } P_1 = \frac{k_A^- + k_B^+}{k_A^+ + k_B^- + k_A^- + k_B^+}$$

$$v = \delta(P_0 k_b^+ - P_1 k_b^-) + (a - \delta)(P_1 k_A^+ - P_0 k_A^-)$$

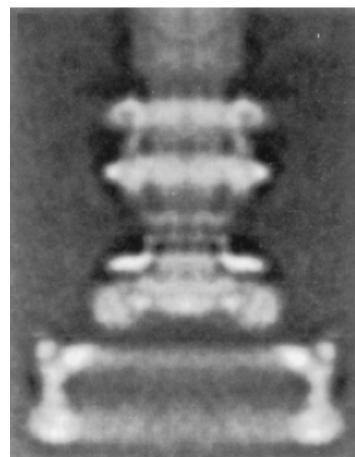
$$\langle v \rangle = a \frac{k_A^+ k_b^+ - k_A^- k_b^-}{k_A^- + k_b^- + k_A^+ + k_b^+}$$

Flagellar Motors - Rotary Molecular Nanomotors

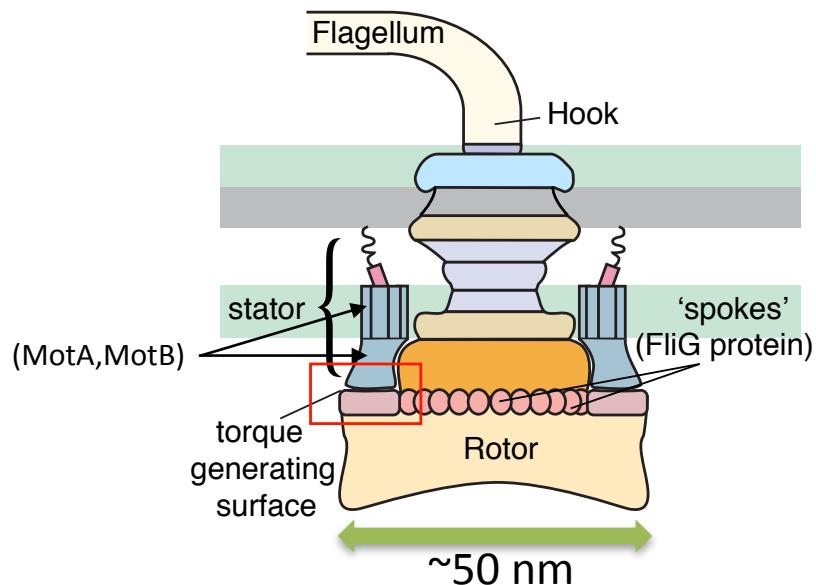


NOVA productions

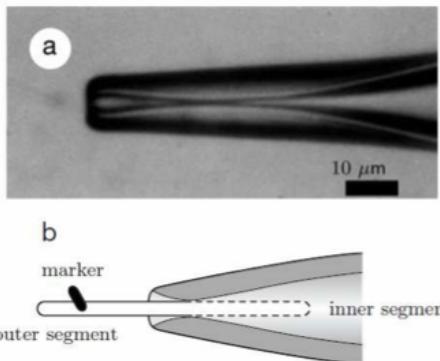
Composite electron micrograph of the flagellum basal body and hook, produced by rotational averaging (Francis *et al.*, 1994)



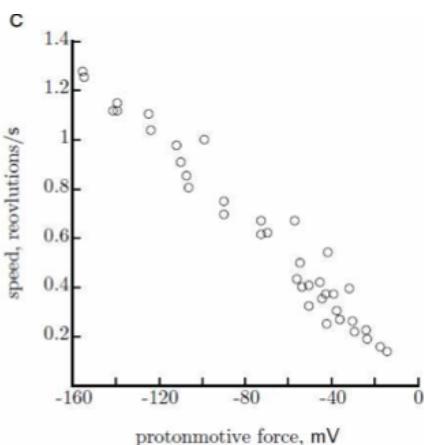
- **Assembly** : Dynamic Self Assembly
- **Production** : famine conditions
- **Driving Force**: H⁺ or Na⁺ electrochemical gradient
- **Gears**: clockwise/anticlockwise rotation
- **Switching Control**: CheY phosphorylation
- **Torque at stall***: $\sim 4 \times 10^{-18}$ Nm
- **Max Speed***: ~ 300 Hz (H⁺) (1700 Hz (Na⁺))
- **Max Power***: ~ 1 fW



Experimental Measurements



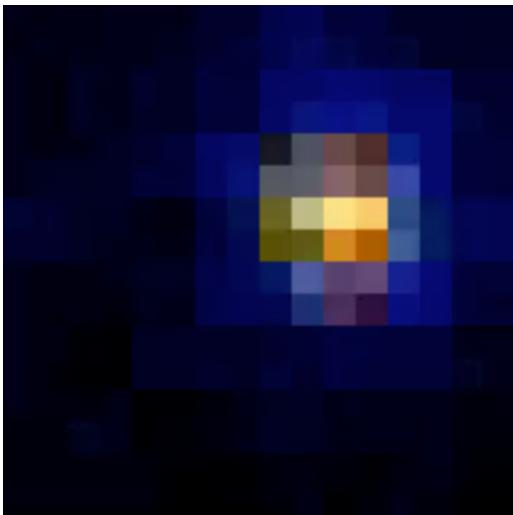
"Biological Physics", Philip Nelson (2007)



Berry Group, Oxford :

High-speed video of a 200 nm fluorescent bead attached to a flagellar motor taking steps

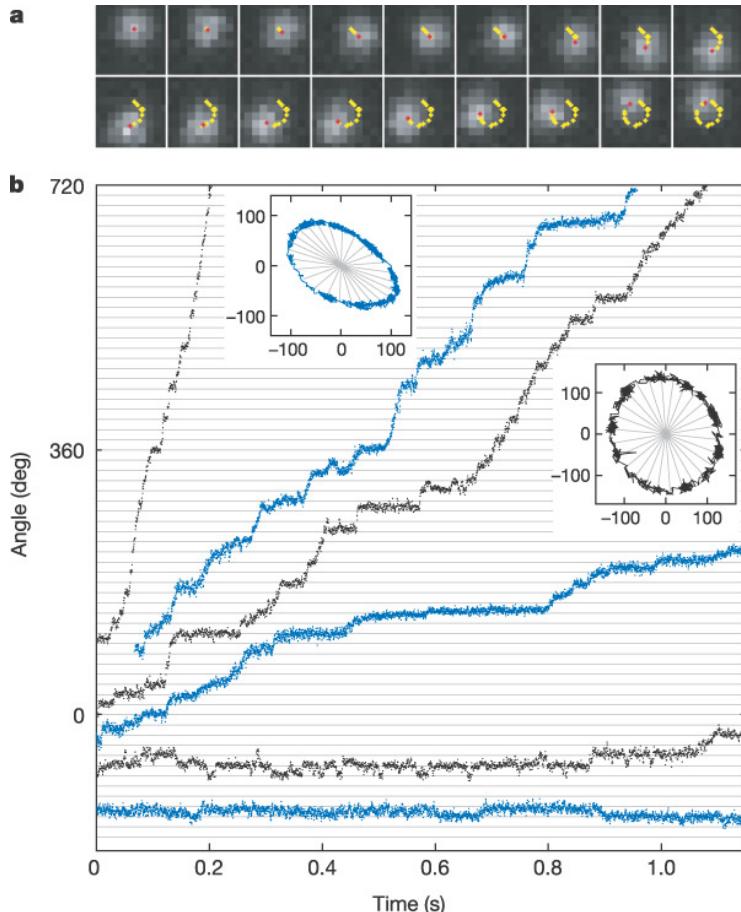
30x slower than real time
2400 fps
position resolution ~5 nm



- ⇒ Marker attached to flagella
- ⇒ Controlled voltage difference across inner and out segment
- ⇒ Speed of motor depends linearly on applied voltage for physiological range (up to -150 mW)
- ⇒ Motor powered by protons/sodium but what's the mechanism?

- ⇒ Fluorescence bead (200 nm) attached to flagellar stub of immobilised cell
- ⇒ Some experiments done with chimera motors (parts of H⁺ and Na⁺ motors fused together)
- ⇒ Individual steps could be resolved for chimera motors where speed is slowed down

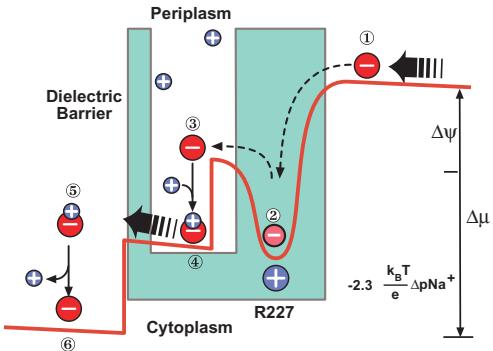
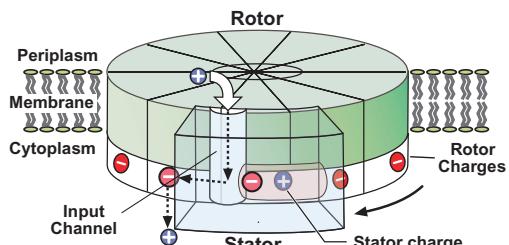
Steps in Flagellar Rotation (organism : E. Coli)



- Na⁺ driven chimaeric motor, low sodium-motive force. ***Small number of force generating units.***
- 26 steps per revolution ($\sim 14^\circ$ per step) - depends on the number of force generating units
- Step size matches periodicity of inner part of flagellar motors (ring of FliG protein)
- Energetics: one ion crossing per step is not enough to explain this

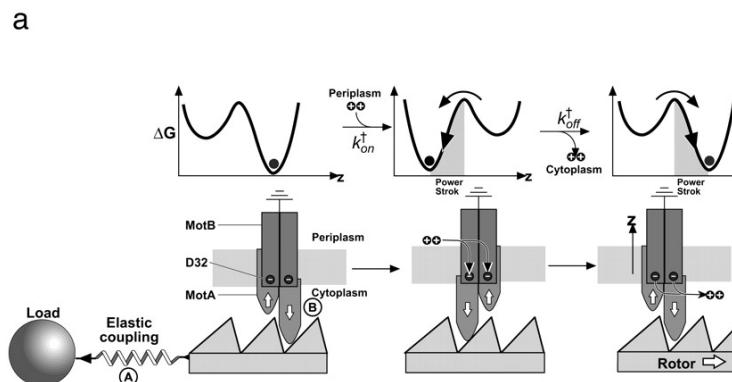
Sowa Y, Rowe AD, Leake MC, Yakushi T, Homma M, Ishijima A, Berry RM. (2005) Direct observation of steps in rotation of the bacterial flagellar motor. Nature. 437:916-919.

Operation of pmf driven Rotors

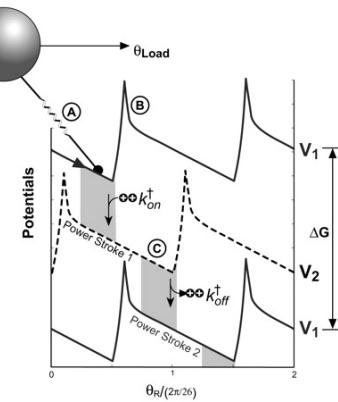


Oster, G. and Wang, H. (2002) How Protein Motors Convert Chemical Energy into Mechanical Work, in Molecular Motors (ed M. Schliwa), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG.

a



b



1. Rotor diffuses to the left, bringing the empty (negatively charged) site into attractive field of the positive stator charge.
2. Site captured, membrane potential biases the thermal escape of the site to the left
3. Site quickly picks up an ion from the input channel neutralizing the rotor.
4. Occupied site being nearly neutral can pass through the dielectric barrier.
5. Upon exiting the stator the site quickly loses its ion. The empty (charged) site binds solvent.

Torque–speed relationship of the bacterial flagellar motor, Xing *et al* PNAS 2006

Bacterial Chemo-taxis Overview

E coli swims up a chemical gradient of aspartic acid (amino acid)

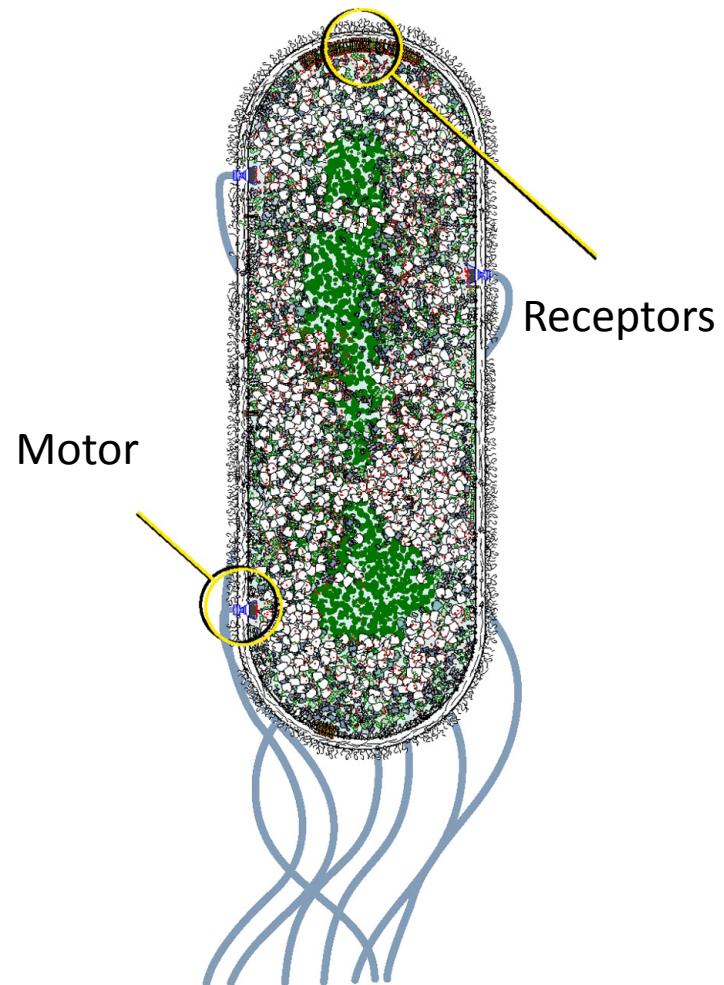
Constraints:

- only 1,000 receptors
- only 1-10 s to evaluate concentration
(due to Brownian rotary motion)

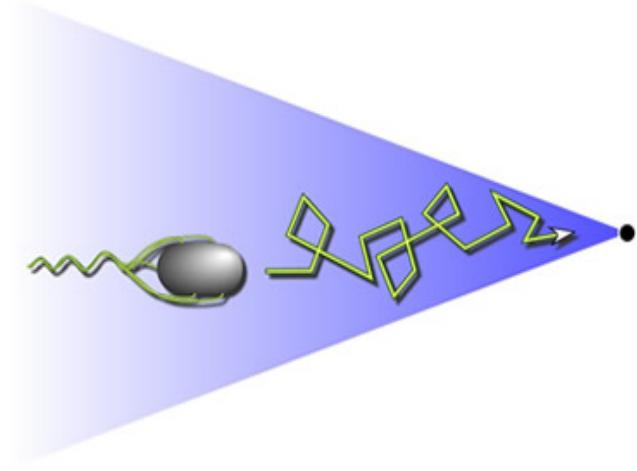
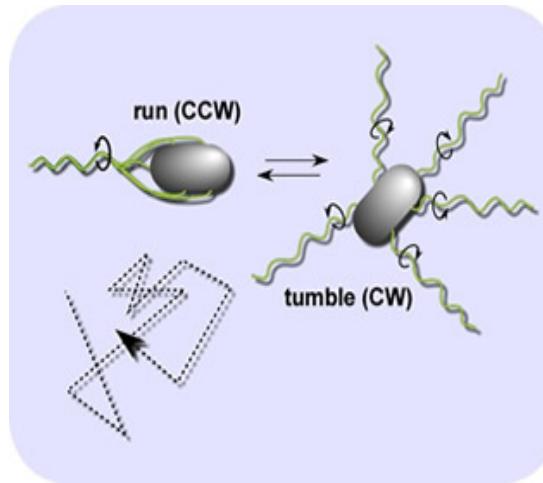
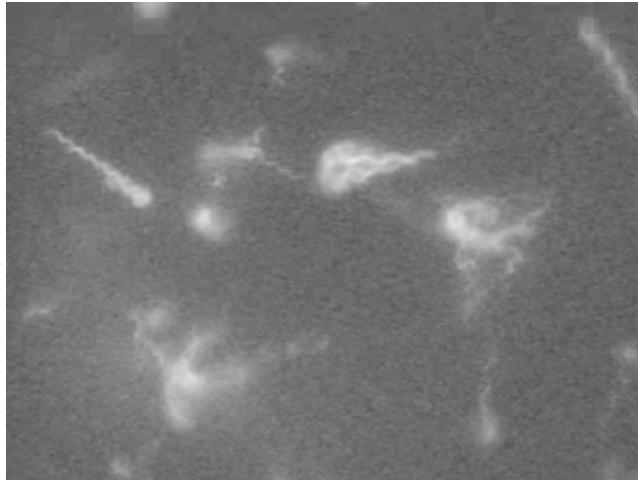
Performance:

- can detect 0.2% change of occupancy
- can operate over 5 orders of magnitude
(2 nM – 1 mM aspartate)

Does behavior require exquisite fine-tuning ...or are some features robust?



Chemo-taxis in Action

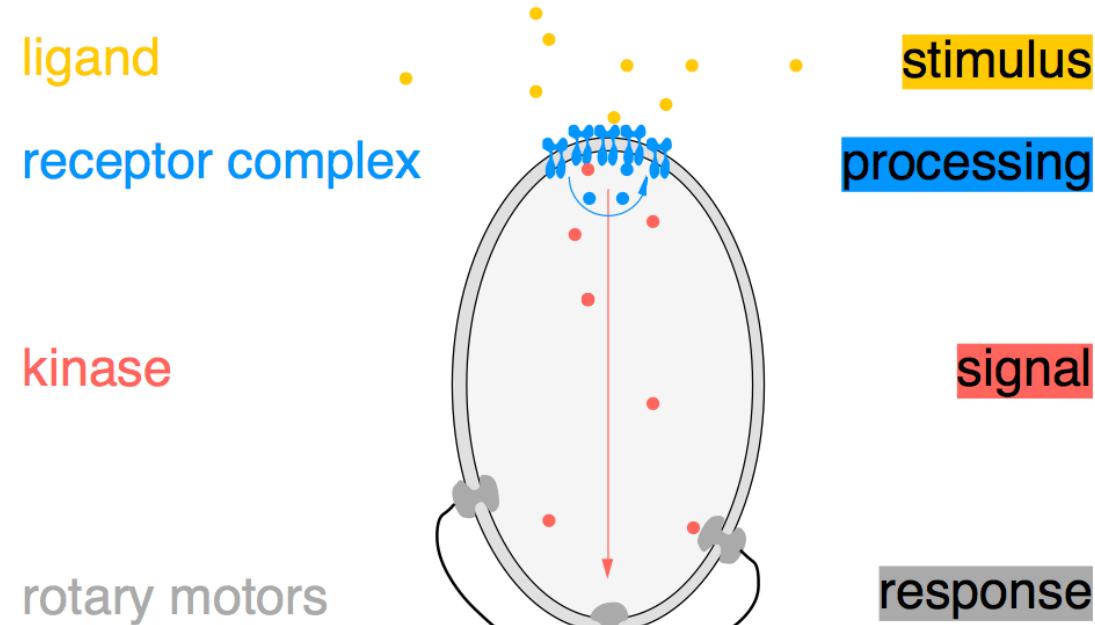


- motor turns **CCW** for **smooth swimming**
- motor turns **CW** for **tumbling**

- rate of **CW** to **CCW** transition modulated by change in ligand concentration

- Comparing last 1 sec with prev. 3 sec
- If concentration does not change
--> adaptation

System Elements



CheA, CheB, ... : **Chemotaxis**

Fla (Fl) : **Flagella**

TarA, ... : **Taxis towards aspartate and away from repellent**

D. Bray (1995) *Protein molecules as computational elements in living cells*. Nature 376:307.

Trans-membrane Signalling

Receptor is an **allosteric protein**

CheA is **kinase** which phosphorylates CheY

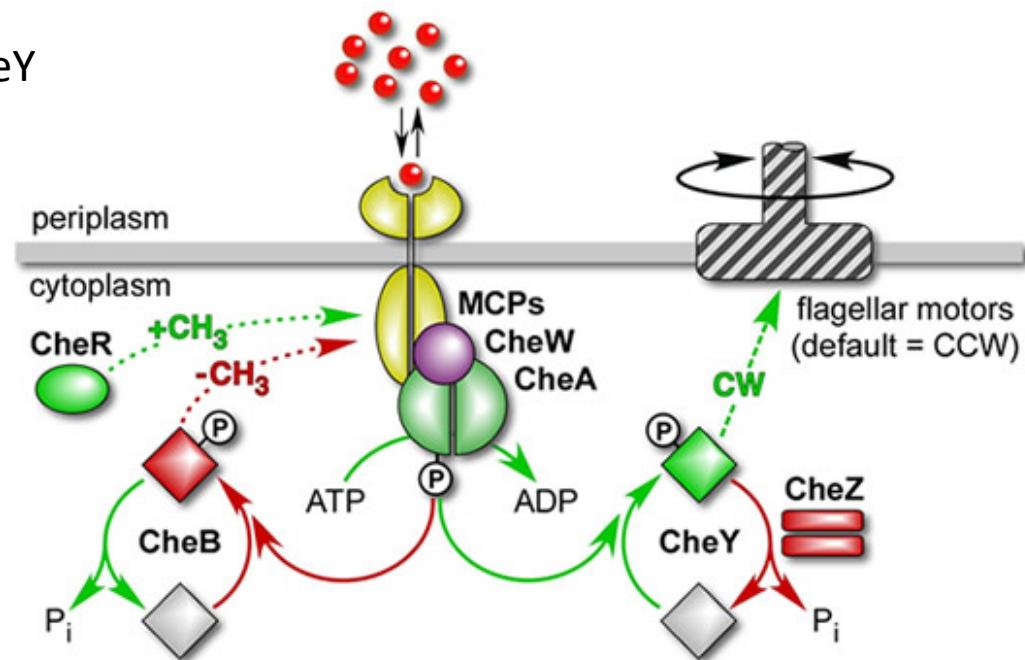
CheA activity depends on two things:

- when ligand bound
- when receptor methylated

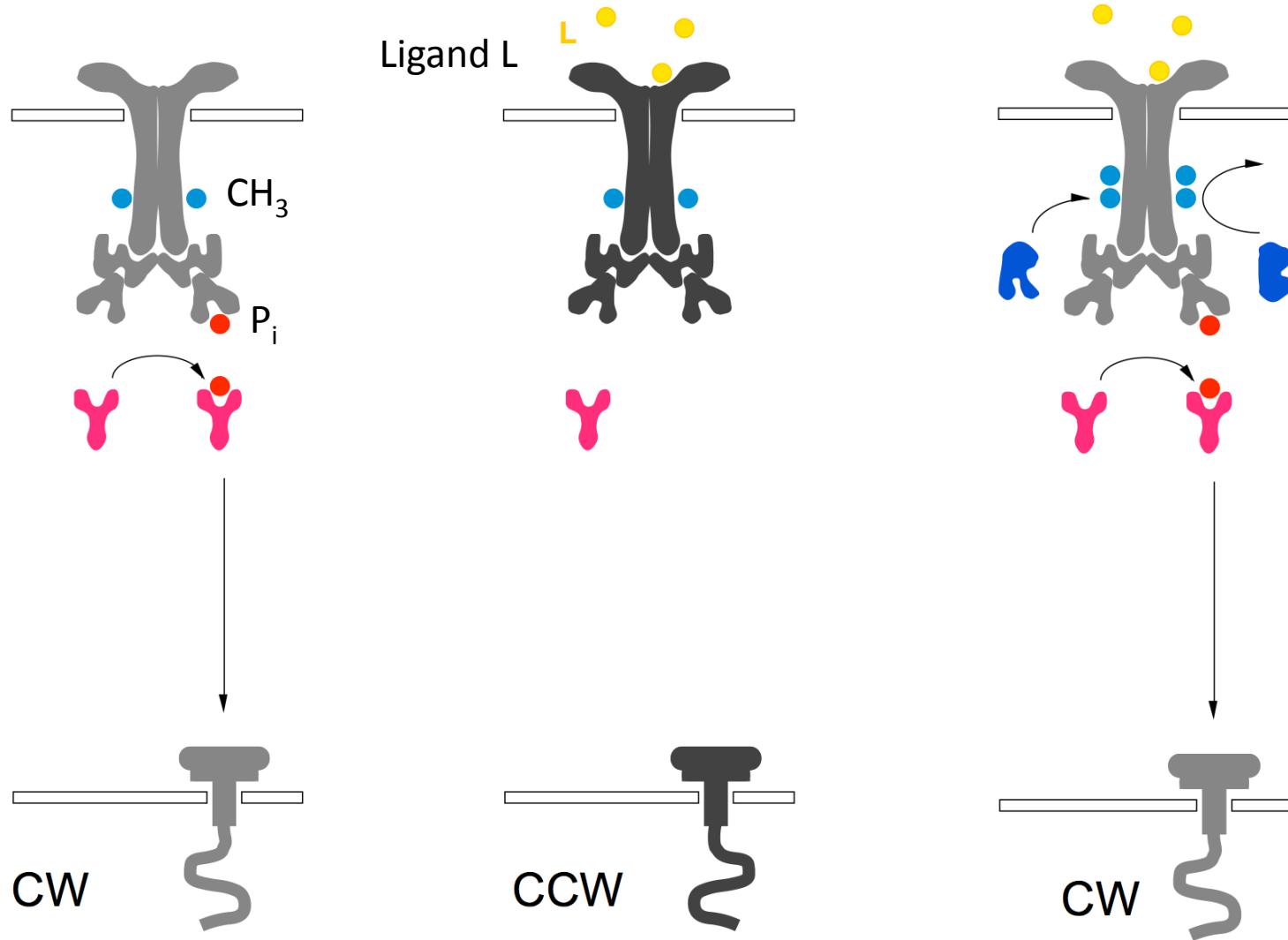
Model: assume 2 conformational states

active : CheA phosphorylation

inactive : no CheA phosphorylation



Protein Networks



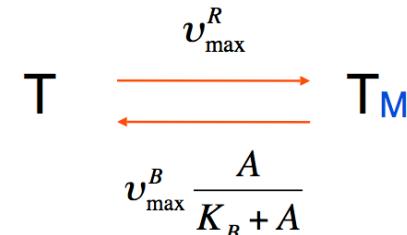
Exact Adaptation

Activity-dependent kinetics permits perfect adaptation

Simplified model:

- Activity A only depends on methylation of receptor T
- CheB activity depends on A
- CheR works at saturation

$$\frac{d[T_M]}{dt} = v_{\max}^R - v_{\max}^B \frac{A}{K_B + A}$$



steady state: $A_{ss} = K_B \frac{v_{\max}^R}{v_{\max}^B - v_{\max}^R}$

→ if activity changes, it will always return to steady state!

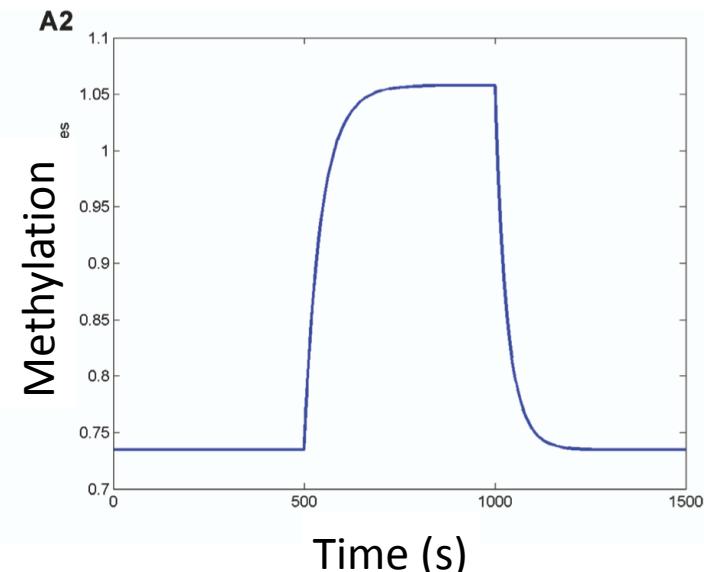
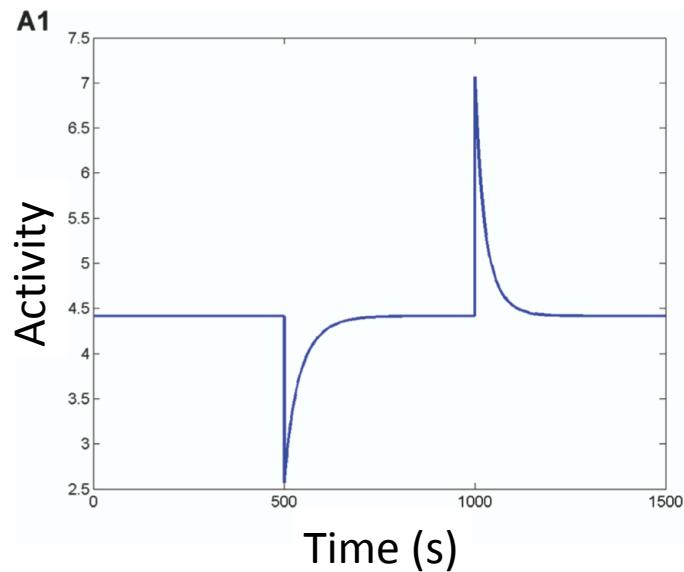
Exact adaptation is a robust property
(insensitive to variation in enzyme concentrations)

but the kinetic aspects depend on the concentrations

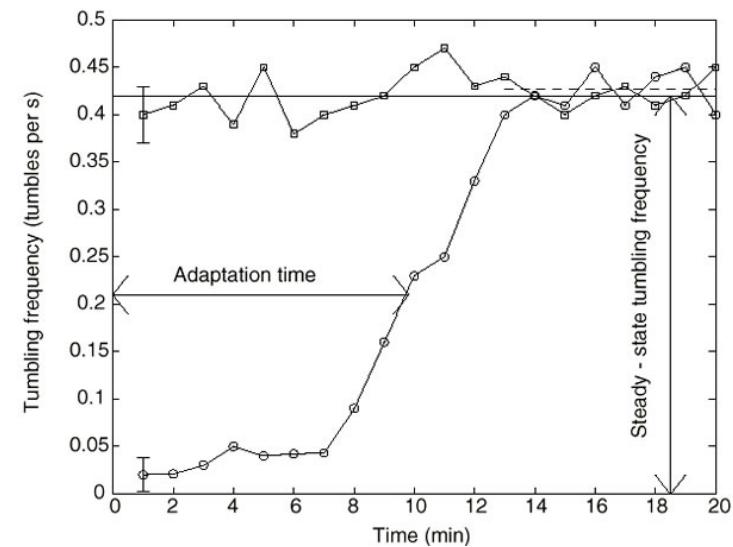
Barkai and Leibler, Nature (1997), 387, 913

Exact Adaption : Experimental Evidence

Protein Level



Cell Level



U. Alon et al. (1999) Nature 397:168.

Exact Adaption : Experiments

Test: Vary expression level of methylating enzyme CheR

Precision of adaptation is independent of [CheR]

Steady-state tumble frequency and adaptation time depend on [CheR]

U. Alon et al. (1999) Nature 397:168.

