Reading for lecture 9

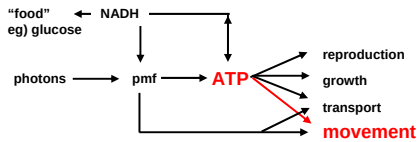
1. Molecular Motors- models

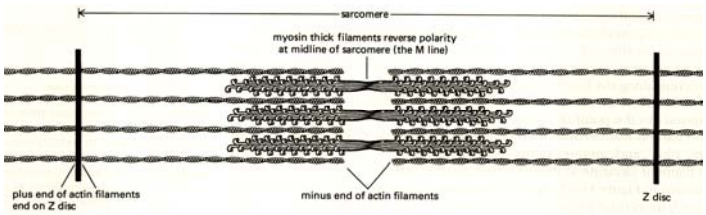
- Howard, Chapters 16, 4 (2, 12-15)

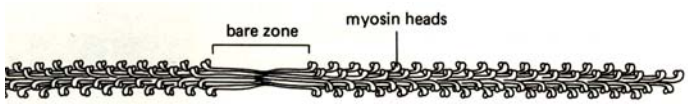
Reviews in Journals

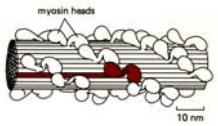
- Berry, R.M. (2000) "Theories of Rotary Motors". *Philos. Trans. R. Soc. Lond. B. Biol. Sci*. 355:503-511.
- C. Bustamante, D. Keller and G. Oster. (2001) "The physics of molecular motors". *Acc. Chem. Res*. 34:412-420 (available online at http://www.cnr.berkeley.edu/~goster/oster/Motors.pdf)

Muscle Contraction









Myosin

[Muscle structure] (2)

Muscles have a repeating unit called the "sarcomere"

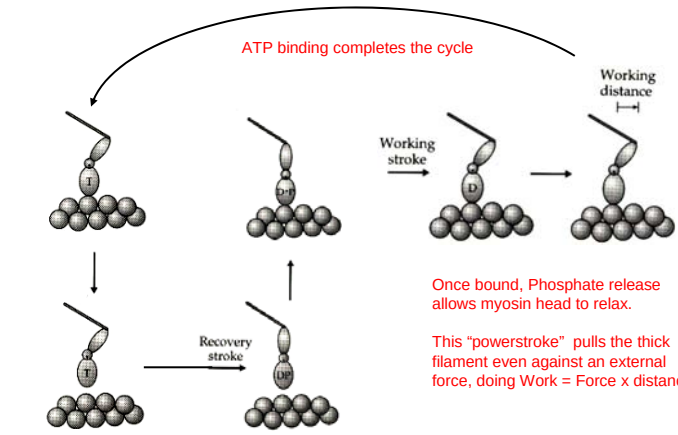
In each sarcomere, thin (actin) filaments slide past think (myosin) filaments, making the sarcomere contract.

High force by multiple sarcomeres in parallel.

Fast contraction by multiple sarcomeres in series.

Sliding filament mechanism, along with nerve action potential and patch clamp, is one of the classic successes of modern biophysics.

Actin-myosin crossbridge cycle



Once bound, Phosphate release allows myosin head to relax.

This "powerstroke" pulls the thick filament even against an external force, doing Work = Force x distance

ATP binding makes myosin release actin

ATP hydrolysis transfers free energy to "strained" straight form of myosin head. This form also binds actin.

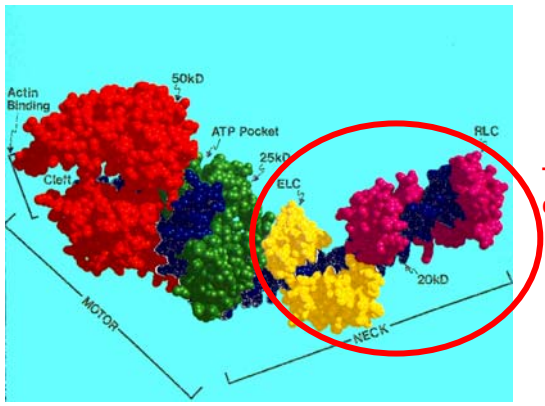
[Crossbridge cycle] (3)

Transitions between states which differ **both** in their chemistry (eg the state of ATP) **and** in the mechanical output variable (here, the relative position of actin and myosin, determined by whether the head is bound and which shape it has) are the essential core of the way molecular motors work.

> This idea is the basis of models of all sorts of molecular motor
>
> Transitions between two states that depend simultaneously on two different types of free energy are the basis of **ANY** free energy coupling mechanism.

Notice that the step in the ATP hydrolysis cycle where the free energy of ATP hydrolysis is converted to work is different from the F1 cycle.

> Myosin, powerstroke is after phosphate release
>
> F1, powerstroke is upon ATP binding
>
> In one or both of these models, free energy is stored somewhere, somehow in the motor, to be released in the appropriate step.



The neck tilts about 20° during the powerstroke

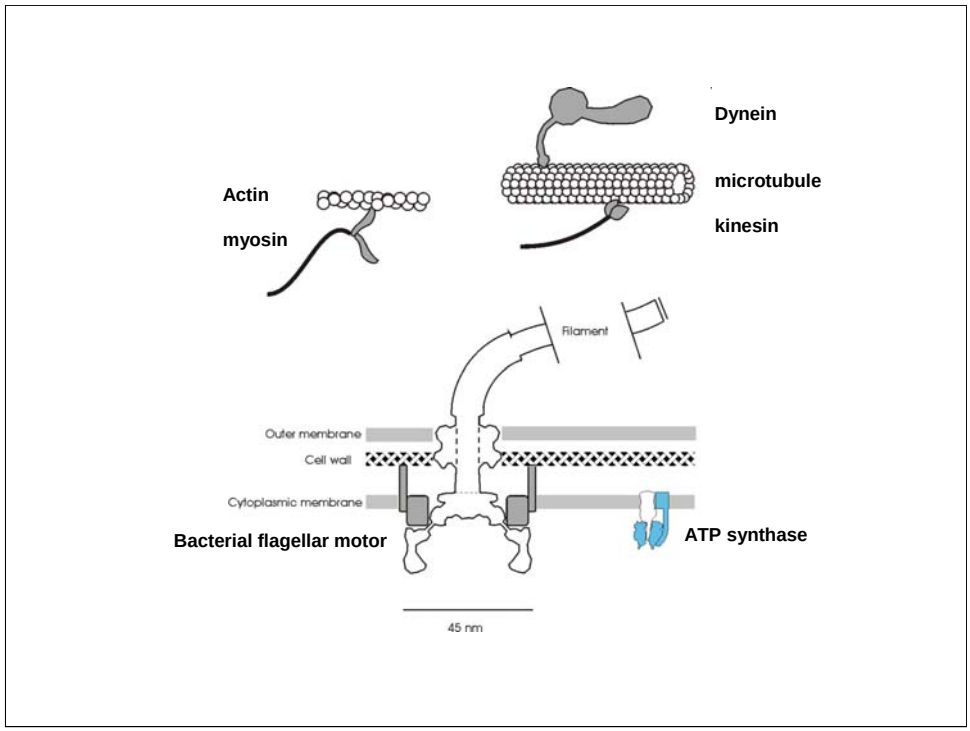
Computer animation

[Myosin head] (4)

There are crystal structures of the myosin head showing the neck titled at different angles, as required by the crossbridge model.

Neck tilting in the powerstroke has been seen directly using polarized fluorescence microscopy.

Single molecule measurements in myosin too. Lecture 10.



Molecular Motors - Models

[Gallery of molecular motors] (5)

These are only some of the better known molecular motors. More and more are being discovered and investigated all the time.

In particular, machines that move on DNA are just beginning to be studied as molecular motors.

This lecture will be about models that attempt to understand how molecular motors work.

Two types of modelling in biological physics…

Large model incorporating all known elements of the system. Example: molecular dynamics. Every atom is modelled, and moves in the potential due to all other atoms. Goal is to understand from the bottom up.

Simplified model with the minimum number of parameters. Goals:

extract and illustrate the key features,

bring quantitative rigour to ideas of how a motor works

generate testable predictions

This lecture will be about the second type of model. Two stages…

Physical model

How are components arranged

What are the mechanical forces and constraints on movements of the motor and its parts

What are the chemical transitions that power the motor, and how are they linked to the mechanical constraints.

Theoretical Representation of the physical model

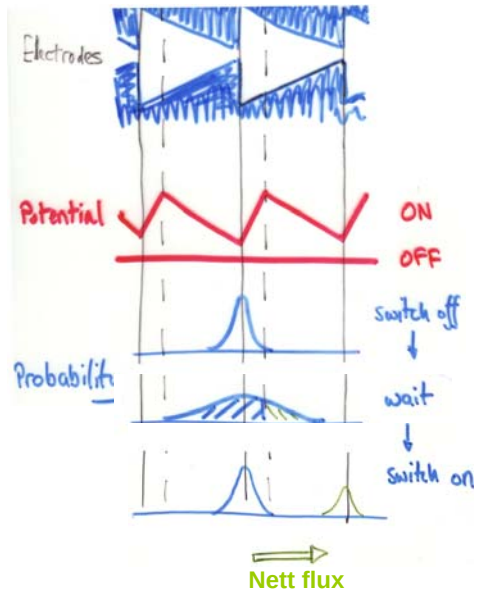
Mechano-chemical cycle.

Kinetics of chemical transitions

Langevin equation to describe the motor mechanically

Usually computer simulation or solution of equations

A Thermal Ratchet



Physical Models

Examples: Binding Change mechanism of F1 (Lecture 7), Myosin crossbridge model (this lecture).

Energy scales of the motor are on the order of kT. Thus it cannot be treated simply as a scaled-down macroscopic motor.

> Negligible inertia (see for example Howard, page 18: swimming bacteria will stop in 0.005 nm ! “Reynold’s Number”, a measure of the relative importance of inertial versus viscous forces, is much less than one).
>
> Diffusive motion, not constant velocities.
>
> Components are in constant motion. Thermal activation of states and transitions is possible, and indeed may be necessary.

An extreme example is the “thermal ratchet” (idea originally attributed to Richard Feynman).

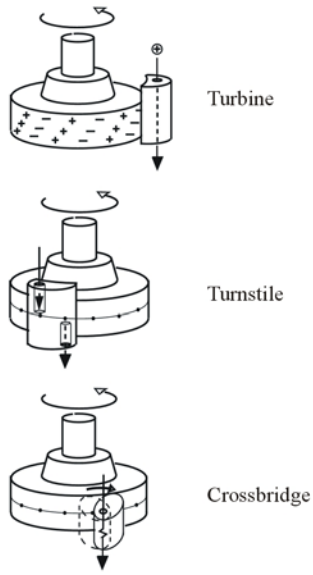
[Thermal Ratchet] (6)

Switching between different states with different energy vs position profiles is a key idea in models of biological molecular motors.

BUT: Real motors are much more sophisticated

> Switches are more precisely controlled as a function of the position.
>
> Switches are coupled to motor chemistry (eg ATP hydrolysis).
>
> Energy profiles of the states are designed to increase motor efficiency, speed and/or power.

Types of model for the bacterial flagellar motor

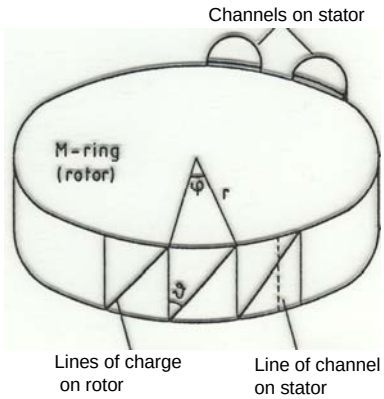


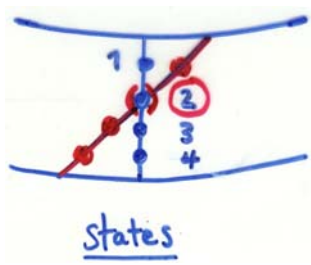
As an illustration, consider models for ion-driven rotary motors (Bacterial flagellar motor, Fo).

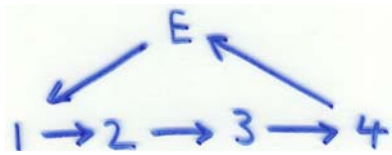
[Types of rotary motor] (7)

Crossbridge model is essentially the same as myosin, but with a circular rotor instead of a linear actin filament, and proton transit instead of ATP hydrolysis coupled to changes of molecule shape.

Proton Turbine Model







[Turbine model] (8)

Ion channel is formed partly by the rotor and partly by the stator.

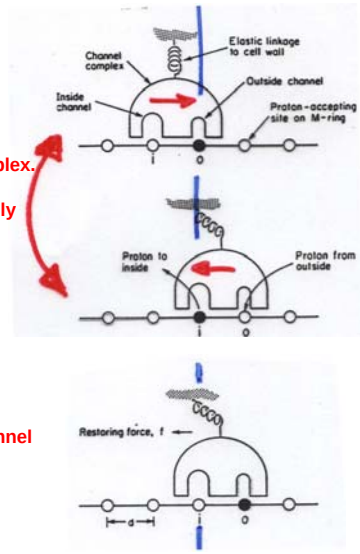
Tilt between rotor and stator channel components couples ion transit to rotation.

"**Powerstroke**": The ion is driven inwards by pmf, leading to a high-energy state which generates torque. For example, due to electrostatic forces between the ion in the stator and charges on the rotor.

Note: The model is still thermally activated, in that ion transit is a series of thermally activated steps (diffusion over energy barriers in the channel).

However, at no stage is the motor dependent upon diffusion of the whole rotor or the whole channel. Unlike the turnstile model…

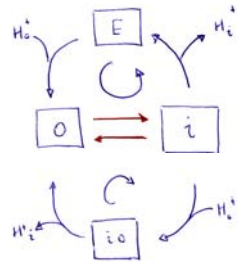
Turnstile Model



Thermally driven motion (diffusion) of channel complex.

Constraint = middle can only cross occupied rotor sites

Proton transit locks channel in high-energy state



[Turnstile model] (9)

Half-channels conduct ions to the rotor from either side of the membrane.

Ion transit requires the rotor to carry them from one half channel to the next.

"Thermal Ratchet": The rotor or the channels must diffuse to carry ions from one half-channel to the other. Ion transit does not generate a high-energy state, but rather "locks-in" thermal motions in the right direction.

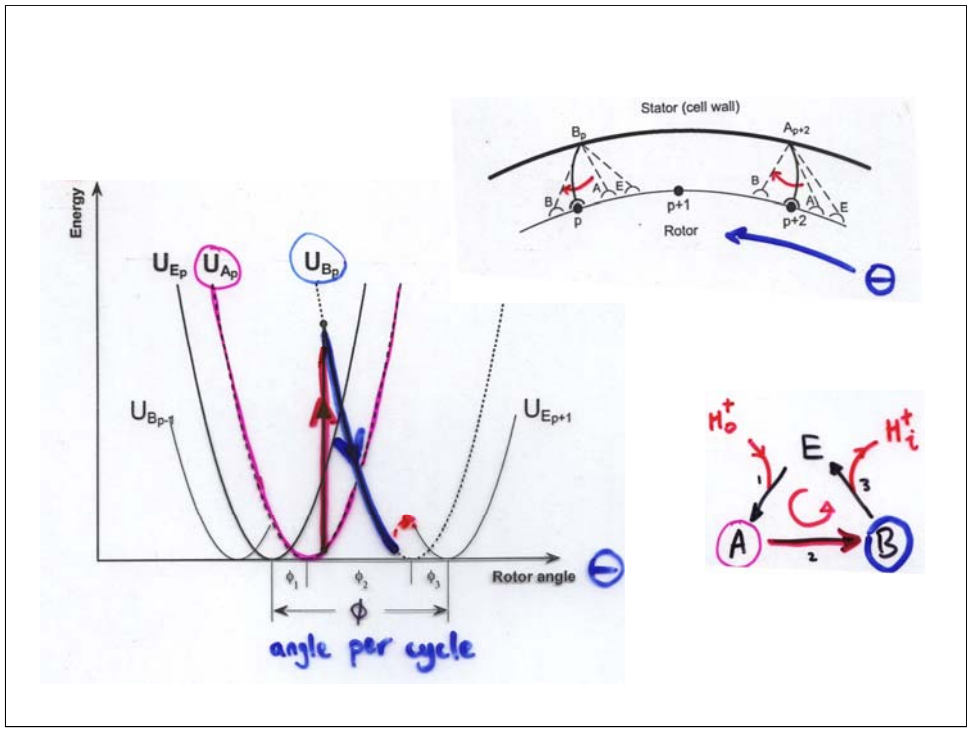
Thermal Ratchet vs Powerstroke models.

In "powerstroke" models the free energy of the ion is stored in the motor as "energy" – eg) electrostatic energy, mechanical strain of the protein.

In "thermal ratchet" models the free energy of the ion is stored in the motor as "entropy" or chemical potential. – eg) arrival of ions form the outside increases the occupancy of the state which allows the rotor to diffuse forwards but not backwards.

Powerstroke models will be faster, because they depend upon diffusion of small things over small distances (like the ions in the channel, or the ATP-binding domain in F1 or myosin).

Thermal ratchet models will be slower, because they depend upon diffusion of large things over large distances(like the whole rotor, or the whole channel).



Mathematical Treatment of Models

So far, we have described motor mechanisms as a series of transitions between states, which differ chemically, physically or both.

> Chemical transitions (eg. ion movements, ATP hydrolysis) are local and fast. Typically nanoseconds.
>
> Mechanical transitions (eg. movements of whole proteins or larger parts of the motor) are global and slow. Typically milliseconds.

To predict motor behaviour on the timescale of motor movements (milliseconds), we can model chemical transitions as instantaneous jumps between discrete states while treating mechanical changes as a continuous variable …

[energy-profiles] (10)

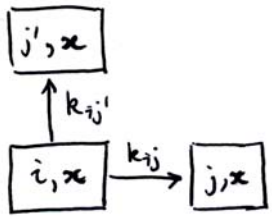
Energy profiles may be due to electrostatic forces or protein elasticity.

Actual profiles have never been measured, but this may soon be possible.

For small displacements from energy minima, profiles are quadratic, like linear spring.

Monte-carlo, chemical transitions

At time ***t***, motor is in state ***i*** at position ***x***.
State ***i*** has chemical transitions to states ***j*** (***j'***)



The probability of being in state ***j*** at time ***t+Δt*** (ie. making the transition to j) is:

$P_j = k_{ij}\,\Delta t$

and with sufficiently small ***Δt***…

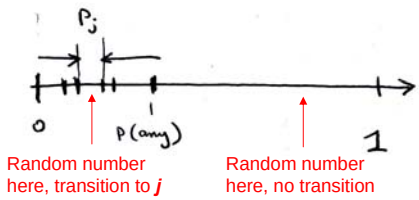
$P(\text{any transition}) = \sum_j k_{ij}\,\Delta t \ll 1$

Rate constants for these transitions obey detailed balance:

$$\frac{k_{ij}(x)}{k_{ji}(x)} = e^{\frac{U_i(x) - U_j(x)}{kT}}$$

(Non-zero rate constants define positions where the motor can switch states.)

A random number between 0 and 1 decides whether any transition should occur in time ***Δt***, and if so which…



Random number here, transition to ***j***

Random number here, no transition

[Monte-Carlo Simulation] (11, 12)

At each time step, random numbers are used to determine the state of the motor at the next step **based on probabilities calculated from the current state of the motor**.

Note, the time step must be small enough that the motor does not move a significant distance, and thus it is ok to calculate rate constants and force (-dU/dx) based on the position before the time step. Similarly, the probability of a transition in a time step must be small, so that it is okay to assume that the motor is in one particular state during the time step.

Such a semi-random process is called “stochastic”.

Using the Einstein relation, it is possible to work out the form of the thermal force ***Ft (t)*** . It is “white noise” with a uniform power density of ***4kTg***. (See Howard chapter 4.)

The output of a Monte-Carlo simulation is a trajectory of the position and chemical state of the motor as functions of time.

Advantage. Individual runs reveal the microscopic behaviour of the motor. Step sizes, distribution times for intervals between steps etc.

Disadvantage. Many or long runs are needed to determine average properties like motor speed, ion flux, dependence upon pmf or external load etc. This can be computer intensive (but still far less so than molecular dynamics simulations).

Sometimes called “Brownian dynamics”.

Monte-carlo, movement

At time ***t***, motor is in state ***i*** at position ***x,*** moving with instantaneous velocity ***v***.

$F = ma$:

"Thermal force" due to collisions with water molecules

$$m\ddot{x} = -\frac{dU_i}{dx} + F_t - \gamma \dot{x}$$

"Langevin Equation"

Inertial forces are negligible:

$$\frac{dx}{dt} = -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right) + \frac{1}{\gamma}F_t$$

The distance moved in time ***Δt*** is…

$$\Delta x = -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right)\int_0^{\Delta t} dt + \frac{1}{\gamma}\int_0^{\Delta t} F_t\, dt$$

The second term is the distance the particle would have travelled by free diffusion in time ***Δt***.

Thus choose a random number drawn from a Gaussian distribution with mean-square **2DΔt**…

$$= -\frac{1}{\gamma}\left(\frac{dU_i}{dx}\right)\Delta t + RND\left(\sqrt{2D\Delta t}\right)$$

"forced" motion at velocity v (deterministic)

Brownian motion (stochastic)

Reaction-Diffusion Equation

Diffusion with an external force: (As in lecture 5, slide 7, but ***P(x),*** the probability of finding a motor at position ***x***, replaces the concentration ***C(x)***)

"Probability flux":

$$J = -D\frac{dP}{dx} + \frac{F}{\gamma}P \qquad : F = \frac{-dU}{dx}$$

Continuity equation:

$$\frac{\partial P}{\partial t} = -\frac{\partial J}{\partial x} = D\frac{\partial^2 P}{\partial x^2} - \frac{\partial}{\partial x}\left[\frac{F}{\gamma}P\right]$$

Fokker-Planck Equation

With allowed transitions between states:

$$\frac{\partial P_i}{\partial t} = D\frac{\partial^2 P_i}{\partial x^2} - \frac{\partial}{\partial x}\left[\frac{F}{\gamma}P_i\right] + \sum_j\left[k_{ji}P_j - k_{ij}P_i\right]$$

Mechanical: Motor movement while in state ***i***

Chemical: Transitions into and out of state ***i*** at position ***x***

Reaction-diffusion Equation

[Reaction-Diffusion Equation] (13)

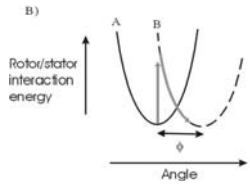
Coupled Partial Differential Equations nearly always have to be solved numerically to obtain probability functions. Average speeds, ion fluxes etc can be derived from probability functions.

Advantage. Average properties obtained faster than Monte-Carlo. True averages, not dependent upon averaging many individual simulations.

Disadvantage. No microscopic information.

What do we mean by "Tight Coupling" ?

- Fixed angle (or distance) for each proton (or ATP)
- Eg), transition from ***A*** to ***B*** is ALWAYS accompanied by rotation of ϕ.



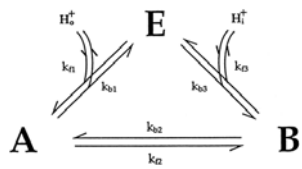
Consider transition ***A*** →***B*** as being from the energy minimum of ***A*** to the energy minimum of ***B***:

$$\Delta G = \Delta G_o + W \qquad : W = \Gamma x \phi \text{ (or } F x d)$$

Chemical ΔG (zero load) — Work = torque x angle (or force x distance)

What do we mean by "Kinetic Model" ?

- Motor can exist in a discrete number of internal states
- There are allowed transitions between states, governed by reaction rates.
- **Reaction rates depend BOTH upon chemical reactions that power the motor AND work the motor has to do against external forces**
- This leads to a cycle that couples the motion to dissipation of chemical energy



Monte-Carlo and reaction-diffusion approaches are mathematically complicated, sometimes a simpler treatment can still give useful predictions of motor behaviour and insight into motor mechanisms.

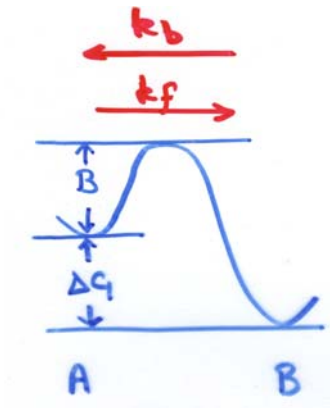
A simple tight-coupled model

[tight coupled kinetic model] (14)

Tight-coupling assumes that chemical transitions are always accompanied by mechanical relaxation to the energy minimum of the new state.

> Chemical flux (eg numbers of ATP hydrolysed or of ions crossing the membrane) is directly proportional to the mechanical flux (distance or angle moved).
>
> Model becomes purely kinetic, with instantaneous transitions describing both chemical changes and the accompanying mechanical motions. In other words, the model is looking at a time-scale slow compared to mechanical changes as well as to chemical changes.

Transition Rates



$k_f = \nu \exp(-B/kT)$

$k_b = \nu \exp(-[B+\Delta G]/kT)$

$\left(\nu = \frac{kT}{h} \approx 6\times10^{12}\,s^{-1}\right)$

(Eyring Theory)

$$\frac{k_f}{k_b} = \exp\left(\Delta G/kT\right)$$

"Detailed Balance"

[Transition rates] (15, 16)

Assumption in the form of the rate constants:

> Chemical and mechanical free energies increase forwards transitions and decrease backwards transitions by the same factor. In other words, transition energy barrier height changes by half of the mechano-chemical DG.
>
> This assumption can be relaxed by introducing another free parameter for the barrier height of each transition.
>
> (However, the fewer free parameters the better. With too many, anything can be modelled.)

This type of model is appropriate in cases such as the bacterial flagellar motor, where relatively little is known about the microscopic details of the mechanism.

The model is designed to predict the dependence of torque and speed in the flagellar motor, for comparison with experimental data (lecture 10).

> Torque appears as a control parameter and speed as the outcome of the calculation.

For each transition, ***i***:

$$\Delta G_i = \Delta G_{oi} + W_i = \Delta G_{oi} + (\Gamma \times \phi_t)$$

Let the rate constants be:

$$k_{fi} = k_i \exp\left(\frac{\Delta G_{oi} - \Gamma \phi_i}{2kT}\right)$$

$$k_{bi} = k_i \exp\left(\frac{-\Delta G_{oi} + \Gamma \phi_i}{2kT}\right)$$

Free parameters for each transition:

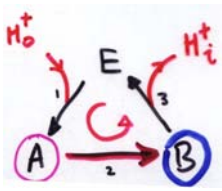
- ΔG_{oi} : Chemical free energy of transition
- ϕ_i : angle moved in transition
- k_i : "Basic" rate constant, without consideration of chemical or mechanical free energy

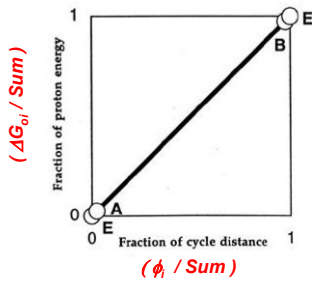
Control Parameter for all transitions:

- Γ : Load torque – resisting the motor.

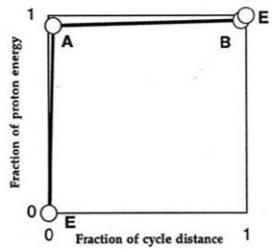
Conservation of energy:

- Sum of ΔG_{oi} = chemical free energy used in one cycle (eg. one ATP, ***n*** protons)
- Sum of ϕ_i = angle moved in once cycle ("step size", eg. 120° in F_1)





Powerstroke model



Thermal Ratchet model

[Powerstroke and Ratchet models] (17)

Powerstroke model – mechanical and chemical energy are released in the same step.

Ratchet model – mechanical and chemical energy are released in separate steps.

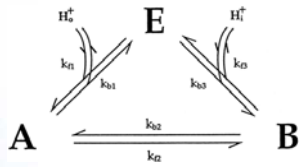
This is a very simple way to distinguish between ratchet and powerstroke models.

steady-state

$$\frac{dP_E}{dt} = k_{b1}.P_A + k_{f3}.P_B - (k_{f1} + k_{b3}).P_E = 0$$

$$\frac{dP_A}{dt} = k_{b2}.P_B + k_{f1}.P_E - (k_{f2} + k_{b1}).P_A = 0$$

$$\frac{dP_B}{dt} = k_{b3}.P_E + k_{f2}.P_A - (k_{f3} + k_{b2}).P_B = 0$$



$$f = k_{f2}.P_A - k_{b2}.P_B$$

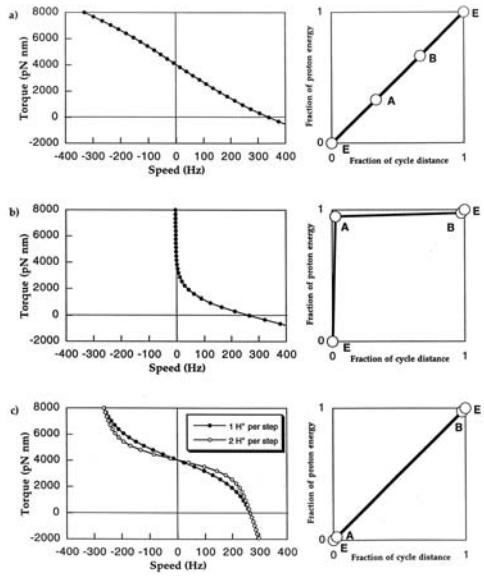
nett flux ∝ speed

[Differential equations] (18)

With the additional assumption of steady-state occupancies, the model reduces to a set of simultaneous linear equations. Solution using standard matrix methods.

Motor speed is proportional to flux around the kinetic cycle, and can be taken from any transition.

> Note: Steady-state is NOT the same as equilibrium, as going once around the cycle consumes chemical free energy, in this case the flux of protons down the electrochemical gradient, with the pmf.



Gradual Powerstrokes model

Thermal Ratchet model

Powerstroke model

[Torque vs speed predictions] (19) /

Ratchet and powerstroke models give qualitatively different torque-speed relationships.

Data from the bacterial flagellar motor match the powerstroke but not the thermal ratchet model (lecture 10).

F1 is more like the gradual powerstroke model, Kinesin is more like the ratchet model (lecture 10).