Introduction into the Theory of Biological Nanomachines

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Lectures 5 & 6
Molecular machines are single-molecule chemical engines. All of them represent enzymes. Now we want to understand how *mechanical work* can be produced by enzymes.
Enzymes are single-molecule protein catalysts

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Here, we have assumed that product $P$ is immediately released and have not included a separate product-enzyme state $EP$. 
Conformational changes within an enzymic cycle

If there are several substrates and (intermediate) products, even more conformational changes may take place.
Thus, an enzyme can repeatedly change its shape in each next catalytic turnover cycle.

These shape changes are induced by binding and dissociation of ligands (substrates and products) and, generally, also by chemical changes in the ligand state in the enzyme.

They represent internal conformational motions within a protein. These internal motions can be used to generate mechanical work.
When a ligand $L$ binds to a protein $P$, additional interactions between the ligand and the protein are established. Thus, a new physical system $LP$ is formed. The equilibrium conformation of ligand-protein complex $LP$ is different from that of a free protein $P$. Therefore, just after ligand binding, this system is not in its correct equilibrium state. The process of conformational relaxation begins. It continues until the equilibrium state of the complex $LP$ is reached.

Similarly, if the ligand is initially present and the complex $LP$ is in the equilibrium state, but then the ligand dissociates, the free protein $P$ is first not in its equilibrium state. It undergoes conformational relaxation (=mechanochemical motion) towards its own equilibrium state.

When the state of the ligand in the complex is changed, $LP_1 \rightarrow LP_2$, this also generally changes the equilibrium conformation of the complex, inducing next conformational relaxation process within it.
Ligand-induced mechanochemical motions in proteins

Adenylate Kinase  Calcium pump ATPase  Cdc42

HCV helicase  Lysine  Sucrose Phosphotase
Ligand-induced mechanochemical motions in proteins

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Whenever cyclic functional mechanochemical motions are involved, the enzyme acts as a biological nanomachine!

For motors, such cyclic motions need be further transformed into study translational or rotational motions.

How to convert a machine into a motor?
Whenever cyclic functional mechanochemical motions are involved, the enzyme acts as a biological nanomachine!

For motors, such cyclic motions need be further transformed into steady translational or rotational motions.
Machine proteins typically have a domain structure

Flexible hinge

Open state
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If the ligand is removed, the protein undergoes reverse relaxation to its initial open state.
substrate → Open state → Closed state → product
A toy dimer model of a protein machine

Dimer: two beads connected by elastic spring

Substrate introduces additional short spring

Dimer contracts

Product is formed

Product is released, dimer returns to its original shape
We assume that product is \textit{instantaneously} released once it has been formed. Then, the dimer has only two states: \( s = 0 \) without the ligand (substrate) and \( s = 1 \) with the ligand (substrate). The mechanical elastic energy of the dimer in these two states is \((x\) is the distance between the beads\):

\[
E(x,s) = \frac{1}{2} k_0 (x - l_0)^2 + \frac{1}{2} s k_c (x - l_c)
\]

\( E_{s=0} = \frac{1}{2} k_0 (x - l_0)^2 \)

\( E_{s=1} = C + \frac{1}{2} k_1 (x - l_1)^2 \)

\[
\begin{align*}
    k_1 &= k_0 + k_c \\
    l_1 &= \frac{k_0 l_0 + k_c l_c}{k_0 + k_c} \\
    C &= \frac{1}{2} \left( k_0 l_0^2 + k_c l_c^2 \right) - \frac{1}{2} \frac{\left( k_0 l_0 + k_c l_c \right)^2}{(k_0 + k_c)^2}
\end{align*}
\]
Macroscopic mechanical model: dynamics

Our toy machine is in a viscous fluid. On length scales below tens of nanometers and time scale above picoseconds, the dynamics is dominated by viscous friction forces and inertial effects are negligible.

\[
\frac{dx}{dt} = -\gamma \frac{\partial E(x,s)}{\partial x}
\]

The dimer performs relaxation motion in a given parabolic potential that depends on the ligand state \(s\).

\[
\frac{dx}{dt} = -\gamma k_0 (x - l_0) \quad \text{if } s = 0
\]

\[
\frac{dx}{dt} = -\gamma k_1 (x - l_1) \quad \text{if } s = 1
\]

Transition \(s = 0 \rightarrow s = 1\) (substrate binding) takes place when \(x = l_0 - \varepsilon\).

Transition \(s = 1 \rightarrow s = 0\) (combined product formation and release) takes place when \(x = l_1 + \varepsilon\).

\[
E_{s=0} = \frac{1}{2} k_0 (x - l_0)^2
\]
\[
E_{s=1} = C + \frac{1}{2} k_1 (x - l_1)^2
\]
Molecular oscillations

The toy machine performs periodic oscillations. Because equations of motion are linear, they can be easily solved. Thus, the profile of oscillations and their period can be determined. We have

\[ T = \gamma \left( \frac{1}{k_0} + \frac{1}{k_1} \right) \ln \left( \frac{l_0 - l_1 - \varepsilon}{\varepsilon} \right) \]

The oscillations are autonomous and stable with respect to perturbations. We have previously discussed autonomous self-oscillations in macroscopic open reactors. Now, we have similar self-oscillations in a single model molecule.

\[ \frac{dx}{dt} = -\gamma k_0 (x - l_0) \text{ if } s = 0 \]

\[ \frac{dx}{dt} = -\gamma k_1 (x - l_1) \text{ if } s = 1 \]
Energetics of the toy machine (macroscopic limit)

Energies of all states of this machine can be easily determined (we assume here that $\varepsilon$ is very small).

$$E_A = E_0(x = l_0) + E_{\text{free}_{\text{substrate}}} = E_{\text{free}_{\text{substrate}}} \quad \text{(before substrate binding)}$$

$$E_B = E_1(x = l_0) + E_{\text{bound}_{\text{substrate}}} = C + \frac{1}{2}k_1(l_0 - l_1)^2 + E_{\text{bound}_{\text{substrate}}} \quad \text{(just after substrate binding)}$$

$$E_C = E_1(x = l_1) + E_{\text{bound}_{\text{substrate}}} = C + E_{\text{bound}_{\text{substrate}}} \quad \text{(after relaxation to equilibrium state with bound substrate)}$$

$$E_D = E_0(x = l_1) + E_{\text{free}_{\text{product}}} = \frac{1}{2}k_0(l_1 - l_0)^2 + E_{\text{free}_{\text{product}}} \quad \text{(just after the reaction and product release)}$$

$$E_{A^*} = E_0(x = l_0) + E_{\text{free}_{\text{product}}} = E_{\text{free}_{\text{product}}} \quad \text{(after relaxation to equilibrium state of free dimer)}$$

Assuming that no dissipation is involved in substrate binding and product release ($E_A = E_B$ and $E_C = E_D$), we find

$$\Delta E = \frac{1}{2}k_1(l_0 - l_1)^2 + \frac{1}{2}k_0(l_1 - l_0)^2 = \frac{1}{2}(k_0 + k_1)(l_0 - l_1)^2$$

This is the (minimal) difference in the chemical potentials of substrate and product, needed to operate such a machine.
When thermal fluctuations are included, the model is stochastic.

- Dimer: two beads connected by elastic spring
- Substrate introduces additional short spring
- Dimer contracts
- Product is formed
- Product is released, dimer returns to its original shape

All transitions are reversible and their rate constants should satisfy the conditions of detailed balance.
Properties of stochastic toy machine

Thermal fluctuating forces need to be included into equations of motion

\[
\frac{dx}{dt} = -\gamma k_0 (x-l_0) + \xi(t), \quad \text{if } s = 0
\]

\[
\frac{dx}{dt} = -\gamma k_1 (x-l_1) + \xi(t), \quad \text{if } s = 1
\]

\[
\langle \xi(t)\xi(t') \rangle = 2\gamma k_B T \delta(t-t')
\]

Transitions (binding of substrate, release of product) will be possible within some windows \((l_0 \pm \epsilon)\) and \((l_1 \pm \epsilon)\).
Conversion from substrate to product will be reversible, taking place inside a window within the cycle.

If energy differences \(\Delta E_0\) and \(\Delta E_1\) are not large as compared to \(k_B T\), this machine can also operate in the opposite direction. It will be then converting "products" into "substrates" if product concentration is high enough.
How to make a motor using a cyclic machine?

Ratchets are mechanical devices used to convert cyclic motion into steady rotation or translation.

Molecular motors are also using ratchet mechanisms.
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Mechanical Ratchets

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Molecular motors are also using ratchet mechanisms.
How to convert a molecular machine into a motor?

This machine cyclically changes its length. Using it, we want to construct a motor that would transport a filament.

This ratchet motor transports the filament in the left direction by distance $\Delta l = l_0 - l_1$ in each its cycle.
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This is the ratchet motion: the worm just cyclically changes its shape.
Inchworm motion mechanism

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We want to make an inchworm from our toy molecular machine

There are molecular motors, such as HCV helicase, that indeed use this translocation mechanism.

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Brownian ratchets

Suppose that the ratchet is very small and the wheel performs Brownian rotational motion. Will they ratchet *rectify* thermal fluctuations and generate mechanical work?

If this were possible, *perpetual mobile* could have been obtained.

R. Feynman showed that this cannot be the case. Not only the cogwheel, but also the handle will undergo thermal fluctuations. As a result, no directed rotational motion will take place.
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Motion in ratchet potentials

\[
\gamma \frac{dX}{dt} = -\frac{\partial V}{\partial X} + F_{\text{ext}}
\]

A small force is enough to move a particle to the right, but, under the same force in the opposite direction, the motion is blocked.
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The answer is NO. Even if the potential has a ratchet form, the equilibrium Boltzmann distribution for the particle coordinate holds. There is no flow in this equilibrium distribution.

\[ p(X) = A \exp \left( -\frac{V(X)}{k_b T} \right) \]

Otherwise, work could have been extracted from thermal fluctuations and *Perpetuum Mobile* could have been made.
Suppose for a while that thermal forces are absent, but a periodic external force is applied:

$$\gamma \frac{dX}{dt} = -\frac{\partial V}{\partial X} + F_{\text{ext}}(t)$$
Within an interval of force amplitudes

\[ F^+ > F_0 > F^- \]

and for sufficiently long periods \( T \), this force will lead to particle translocation into a new trough in each oscillation cycle.

Hence, steady translational motion can be induced by such periodic force!
Fluctuating external force

\[
\gamma \frac{dX}{dt} = -\frac{\partial V}{\partial X} + F_{ext}(t)
\]

Analysing this system, it can be noted that the rectangular shape of oscillations is not important, the same effect can be found for harmonic or other oscillation shapes.

Moreover, it is also not important that the oscillations are strictly periodic and their amplitude is constant.

Actually, statistically persistent translocation of the particle can be produced even by a fluctuating external force, provided that some conditions are satisfied:

Such fluctuating force should not be too strong or too weak. Moreover, it should not too rapidly change.

Note, however, that such fluctuating external force will still have different statistical properties than the thermal noise.
Brownian ratchet

\[ \gamma \frac{dX}{dt} = -\frac{\partial V}{\partial X} + F_{\text{ext}}(t) + \xi(t) \]

Non-thermal fluctuating force \[ \langle F_{\text{ext}}(t) \rangle = 0 \]

Thermal noise

When some statistical conditions are satisfied, non-biased fluctuating external force can induce steady translational motion in an asymmetric (ratchet) potential!
Can molecular machines behave as Brownian ratchets?

\[ \gamma \frac{dX}{dt} = -\frac{\partial V}{\partial X} + F_{\text{ext}}(t) + \xi(t) \]

A machine is weakly bound to the filament and can move along it, experiencing a periodic ratchet potential.

It is subject to equilibrium thermal fluctuations, which cannot themselves induce persistent translational motion.

Additional interactions with the filament are caused by active irregular conformational changes in the machine, powered by the free energy brought under conversion of substrate into product.

As a result, non-thermal forces acting on the machine arise.

If some conditions are satisfied, such actively fluctuating machine is able to persistently translocate itself along the filament.
Two motor operation modes

Brownian ratchets: generation of non-thermal active noise

Deterministic ratchets: ordered conformational oscillations

Characteristic energy of ordered conformational oscillations in deterministic ratchets must be significantly larger than the thermal energy $k_B T$. Therefore, the difference $\Delta \mu = \mu_s - \mu_p$ in chemical potentials of substrate and product must also be large as compared to $k_B T$. In contrast to this, Brownian ratchets may operate even when $\Delta \mu \sim k_B T$. 
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. .... Like the machines invented by humans to deal efficiently with the macroscopic world, these protein assemblies contain highly coordinated moving parts.