Introduction into the Theory of Biological Nanomachines

Alexander S. Mikhailov/Holger Flechsig
Lectures 1 & 2
Amazing Mechanical Machines

YouTube: Amazing molecular machines.mp4
A factory of single-molecule nanoscale machines: motors, ion pumps, enzymes,... Many thousands of such machines are packed inside a volume of about $1 \mu m^3$. The factory is self-regulated and is able to reproduce itself.
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.
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Real-time imaging of walking myosin motors

Myosin motors walking over actin filaments. High-speed atomic force microscopy (AFM). Scan size 130 nm x 65 nm, scan rate 146.7 ms per frame

Kodera, Yamamoto, Ishikawa & Ando „Video imaging of walking myosin V by high-speed atomic force microscopy“ Nature 468, 72 (2010)

Artistic animation
Macroscopic Machines and Motors

- Characteristic sizes: centimeters to meters
- Characteristic times: seconds to minutes
- Material basis: metals
- Energy is continuously supplied
- Ordered, coordinated motions of mechanical parts
- Operate under well-defined environmental conditions
- Robustness against damage: low

Molecular Machines and Motors

- Characteristic sizes: nanometers to fractions of a micrometer
- Characteristic times: milliseconds to seconds
- Material basis: biomolecules
- Energy is discretely supplied in chemical form with ATP molecules
- Ordered, coordinated intramolecular motions of protein domains
- Operate under strong fluctuations in environment
- High robustness against perturbations
Machine operation is autonomous and self-organized.

The motor in this useless machine is constantly spinning, which causes the hammer to rise and fall.
Machine operation is autonomous and self-organized

Industrial revolution: Factory in England, 18th century
**Self-Organization in Living Cells**

Benno Hess and Alexander Mikhailov

A living cell is an open system with a flow of energy passing through it. As shown by Schrödinger (1), the energy flow creates the conditions for strong deviations from thermodynamic equilibrium. This results in the phenomena of self-organization, the parameters of which are set by genetics as well as epigenetic constraints and opens up the possibility of autonomous pattern formation as revealed in the fundamental contributions by Turing (2) and Prigogine (3).

Recently, Lechleiter et al. (4) have observed propagating calcium waves inside single cells from frog eggs with a diameter of about 1 mm. Their properties were very similar to those of the spiral waves in the Belousov-Zhabotinsky reaction (5). Moreover, there are suggestions that stationary Turing patterns could also be found within biological cells (6).

However, it would be misleading to expect that the processes of self-organization in living cells represent simply a reduced copy of the pattern-formation phenomena in macroscopic reaction-diffusion systems. The laws of physics, when applied at a different scale typical for intracellular processes, can influence the mechanisms involved and produce a wealth of new properties, as demonstrated, for instance, by Purcell (7) for the case of the cell’s motion.

The diffusion time for macromolecules in a cell or a cellular compartment with the linear size of 1 μm is about 10 ms. Because the turnover rate for many intracellular enzymatic reactions is a few hundreds per second, it means that within the duration of a single round of the catalysis the molecules can cross the entire reaction volume. This makes spatial pattern formation based on such reactions and diffusion at small length scales practically impossible. The finest spatial details of waves in the experiment (4) were still on the order of 10 μm. The estimates are less restrictive for fast enzymatic reactions with the turnover times below 0.1 ms. The possibility that under high enzyme concentrations these reactions may develop local spatial patterning should be further investigated (6).

The random Brownian motion of macromolecules inside cells is extremely strong. Our estimates of a characteristic traffic time show that within a compartment of a micrometer size, any two molecules meet each other within a time of about 1 s. Thus, simple diffusion fills the cellular compartment space with components always ready to react. At such smaller scales, the chemical system of a living cell is far from resembling a macroscopic unstrained chemical reactor. Rather, it should be viewed as a network formed by a population of active macromolecules that is characterized by a very high degree of “communication” between its members. This implies a different mode of self-organization similar to the collective behavior of insect societies or immune networks (8).

Another special aspect of self-organization in living cells is the abundance of energy contained in thermal fluctuations. For instance, random hydrodynamic flows induced by thermal fluctuations inside a cell have velocities on the order of 10 μm/s in the time range of 10 ms and the characteristic lengths of 1 μm. These strong thermal fluctuations can be employed by far from equilibrium subsystems inside the cells.

The laws of thermodynamics prevent the directed use of thermal fluctuations. However, as shown by Feynman (9) in his analysis of the thermal ratchet (a process allowing motion in one direction only), this does not generally apply to systems with some components that are far from thermal equilibrium (and thus, “Maxwell’s demon”) may operate if it receives and dissipates energy from external sources. The ratchet has already been proposed by Huxley (10) in his explanation of force generation by muscle fibers. Several further examples of rectification of thermal fluctuations by organelles have recently been considered (see, for example (11)). It might be that the ratchet effects are involved to a much larger extent in the function of living cells and the organization of intracellular traffic.

The temporal self-organization of chemical processes, expressed in the generation of different periodicities and interactions between them, plays a fundamental role in living cells. Recent advances in the understanding of the mechanism of calcium oscillations not only revealed its widespread occurrence in cellular systems but showed its inherent response to frequency and coding to be a most important feature (12). This property might well be involved in the phenomenon of synchronization of synaptic boutons (13), in the shuttle streaming of Physarum polycephalum (14), in the propagation of calcium waves in Xenopus oocytes (4), and mitotic cell cycles (15).

In this framework, the dynamic processes in larger cellular structures such as genomic components or the cellular actin and tubulin, the shape producing and controlling entities, should not be overlooked. Whereas the exploration of the first set of structures seems still to have a long way to go (although proper methodologies are coming up (16, 17)), investigations of the second class are already at hand.

The dynamic network of microtubules plays an essential role in the self-organization of cellular structures, turnover, and motility. The knowledge of its mechanisms and of the control of formation and decay (polymerization-depolymerization cycles) are prerequisites for understanding of the cellular interior. The complex organization of the microtubule turnover is revealed in the typical properties of arising dissipative structures. Recently, spatial pattern formation from oscillating microtubules has been observed (18, 19). Depending on the length and the frequency of oscillations, different spatial microtubule patterns might readily be formed, leading to a variety of intracellular structures that fit well to the general size of the cellular reaction space as well as its time domains.

Here, the biological design of suitable control mechanisms is a problem area open for future research. Studies reported by Tabony on p. 245 of this issue (20) clearly demonstrate the influence of reaction-diffusion instabilities and related bifurca-
Plan of this lecture course

1. Self-organisation in non-equilibrium systems
2. Stochastic thermodynamics of nanomachines
3. Mechanochemical motions in proteins, molecular ratchets
4. Methods of computational modelling for proteins (all-atom MD, Go models, elastic networks)
5. Examples of structurally resolved modelling for protein machines
6. Allosteric self-regulation of machine cycles
Entropy is a statistical measure of disorder:
\( W \) is the number of microscopic states that all correspond to a given macroscopic state

\[
\frac{dS}{dt} \geq 0
\]

The Second Law of Thermodynamics:
Entropy increases with time until the state of thermal equilibrium is reached.

All systems tend to reach the state with the maximal possible disorder?
Do biological systems contradict the laws of physics?

Biological organisms seem to retain and even increase their order over time. Does this contradict the Second Law of Thermodynamics?

The Second Law is only applicable to closed systems. It does not hold for a biological organism that permanently exchanges substances and energy with its environment. Even if such an organism is in a steady state, it is not the state of thermal equilibrium. The flows persist in this steady state.

„To maintain a closed system at equilibrium, no work is needed, but work cannot be also performed by a system in such a state. A dammed mountain lake contains much potential energy, but, in absence of outgoing flow, it cannot power a motor or a turbine. To generate work, the system needs to be under a transition to an equilibrium site. To keep the system over a long time under such transition, one has to engineer it like a water power station, supplying new material whose energy is used to produce work.“

Ludwig von Bertalanffy (1901-1972)

In 1926 (at the age of 25!), von Bertalanffy had finished his study of philosophy and art history with a doctoral degree at the University of Vienna and, 2 years later, published his first book on theoretical biology, *Critical Theory of Form Development*, soon followed by two volumes of *Theoretical Biology*.
Thus, biological organisms feed on „negative entropy“, i.e. they import order.

\[ \frac{dS}{dt} = \sigma - h \]

\( \sigma \geq 0 \), but \( h = j_s^{\text{out}} - j_s^{\text{in}} \) may have any sign.

In emigration in Ireland, he had given in 1943 several lectures in the Dublin Institute for Advanced Studies which were published one year later as a book with the title *What is Life? The Physical Aspect of a Living Cell*. The lectures were attended by an audience of about four hundred, both physicists and biologists.
Thermodynamic free energy is defined as

\[ F = E - TS \]

Energy \hspace{1cm} Temperature

Suppose that (internal) energy \( E \) is not consumed or released inside an open system and temperature is fixed. Than, the balance equation for free energy is

\[ \frac{dF}{dt} = -T \frac{dS}{dt} = -T \sigma + j_{in}^F - j_{out}^F \]

In the steady state we have \( \frac{dF}{dt} = 0 \)

Hence \( j_{in}^F = T \sigma + j_{out}^F \geq T \sigma \)

The influx of free energy should counterbalance the effect of persistent entropy production in a non-equilibrium steady state.

A biological organism imports „order“ with the flux of free energy coming with its nutrients, even just to maintain its steady state.
Ilya Prigogine (1917-2003)
Nobel Prize in Chemistry 1977

He was born in Russia and emigrated as a child with his parents first to Germany and then to Belgium. In 1941 he has received a doctoral degree from Free University of Brussels and, in 1947, (at the age of 30!) published his first book *A Thermodynamic Study of Irreversible Phenomena.*

This closed system undergoes relaxation to the equilibrium state with temperature $T_0$. It can perform work only transiently, during the relaxation process.
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This open system cannot reach any thermal equilibrium state. In its steady state, a flow of energy is passing through the system. Work can be persistently performed.
Suppose that the system is in a steady state (but not at thermal equilibrium!). Even though the state is stationary, heat $Q$ would continue to arrive from the bath with the higher temperature and become released into the colder bath.

Because $dQ = TdS$, we have $j_{in}^s = \frac{1}{T_1} \frac{dQ_1}{dt}$ (this is the rate of entropy removal from the hot bath)

and $j_{in}^s = \frac{1}{T_2} \frac{dQ_2}{dt}$ (this is the rate of entropy uptake by the cold bath).

Thus, the Second Law of thermodynamics yields

$$\frac{dS}{dt} = \sigma + j_{in}^s - j_{out}^s = \sigma + \frac{1}{T_1} \frac{dQ_1}{dt} - \frac{1}{T_2} \frac{dQ_2}{dt}$$

The First Law of thermodynamics implies that a balance equation for the internal energy should hold

$$\frac{dE}{dt} = \frac{dQ_1}{dt} - \frac{dQ_2}{dt} + \frac{dR}{dt}$$

where $R$ is the work produced by the system.

If the system changes its volume $V$,

$$\frac{dR}{dt} = -p \frac{dV}{dt}$$

where $p$ is pressure.
Assuming a steady state,
\[
dE/dt = dS/dt = 0
\]
Substituting this into the First Law, we find the rate of work generation (or power)

\[
\frac{dR}{dt} = -T_2 \sigma + \left( \frac{T_2}{T_1} - 1 \right) \frac{dQ_1}{dt}
\]

Thus, the system can operate as a heat engine and generate mechanical work!

Most work is performed if the engine operates near thermal equilibrium, i.e. where the entropy production rate is very small.

The efficiency of the engine is defined as the ratio of work generation (power) to the heat flow from the hot bath

\[
\eta = \frac{dR/dt}{dQ_1/dt}
\]

When \( \sigma \rightarrow 0 \), we find the maximal possible efficiency of the heat engine

\[
\eta = \frac{T_2}{T_1} - 1
\]
Now, we consider an open chemical reactor where molecules X (substrate) are converted into molecules Y (product). This reactor is connected to two external reservoirs (chemostats) where concentrations of molecules X or Y are maintained constant. The reactor is also connected to an external heat bath. The chemical potential in the chemostat for X molecules is higher than in the chemostat for Y molecules.\[\mu_X > \mu_Y\]
We assume that, in the considered chemical reaction, one X molecule is converted into one Y molecule
\[ X \rightarrow Y \]

The First Law of thermodynamics yields
\[
\frac{dE}{dt} = \mu_X j_X - \mu_Y j_Y + \frac{dR}{dt} - \frac{dQ}{dt}
\]
where \( j_X \) is the number of molecules X arriving per unit time into the reactor and \( j_Y \) is the number of molecules Y leaving it per unit time.

The Second Law of thermodynamics yields
\[
\frac{dS}{dt} = \sigma + s_X j_X - s_Y j_Y - \frac{1}{T_0} \frac{dQ}{dt}
\]
Here \( s_X \) and \( s_Y \) are amounts of entropy per molecule X or Y in the respective chemostats. According to thermodynamics, we have
\[
s_X = -\frac{\partial \mu_X}{\partial T}, \quad s_Y = -\frac{\partial \mu_Y}{\partial T}
\]
From the Second Law

\[ \frac{dQ}{dt} = T_0 \sigma + T_0 (s_X - s_Y) j \]

Then, the Second Law yields

\[ \frac{dR}{dt} = -T_0 \sigma + \left[ T_0 (s_X - s_Y) - (\mu_X - \mu_Y) \right] j \]

In the steady state,

\[ \frac{dE}{dt} = \frac{dS}{dt} = 0 \quad \text{and} \quad j_X = j_Y = j \]

Thus, the system can operate as a chemical engine and generate mechanical work!

Most work is performed if the engine operates near thermal equilibrium, i.e. where the entropy production rate is very small.

The efficiency of the chemical engine is defined as the ratio of work generation (power) to the (free) energy flow from the substrate chemostat

\[ \eta = \frac{dR / dt}{(\mu_X - \mu_Y) j} \]

When \( \sigma \to 0 \), we find the maximal possible efficiency of the chemical engine

\[ \eta = \frac{T_0}{\mu_X - \mu_Y} \frac{\partial}{\partial T} (\mu_X - \mu_Y) - 1 \]
Amplitude and shape of such self-oscillations do not depend on initial conditions. The oscillations are not induced by external forces, but autonomously generated by a system itself.
Self-organised dissipative patterns in reaction-diffusion systems

These autonomously formed spatial structures are possible in distributed open chemical reactors. They exist only as long as the energy or material flow passing through a system is maintained. Like in self-oscillations, their amplitude and shape are determined by the system itself, not externally imposed.

A toy model of molecular turbine (motor/pump)

So far, macroscopic engines were considered. Now, we want to see what becomes different when we go to nanoscale...

As an illustration, I use a toy model of molecular turbine proposed by my friend, prof Jose Maria Sancho, from the Barcelona university.


This machine can be used as a motor or as a pump (if it made to rotate in the opposite direction by an external force)
When external force is relatively small, the turbine operates as a motor (mechanical engine). The pressure difference between two compartments pushes the piston in the right direction. When the end of the channel is reached, the piston is replaced by a new one at the opposite side and the cycle is repeated. In each cycle, mechanical work is produced.
A toy model of molecular turbine

When external force is relatively small, the turbine operates as a motor (mechanical engine). The pressure difference between two compartments pushes the piston in the right direction. When the end of the channel is reached, the piston is replaced by a new one at the opposite side and the cycle is repeated. In each cycle, mechanical work is produced.
A toy model of molecular turbine

When external force is strong, the turbine operates as a pump. The piston is dragged to the left until it reaches the end of the channel. Then it is replaced by another piston on the opposite side and the same motion is repeated again. In each cycle, a certain number of particles is transported against their gradient.
A toy model of molecular turbine

When external force is strong, the turbine operates as a pump. The piston is dragged to the left until it reaches the end of the channel. Then it is replaced by another piston on the opposite side and the same motion is repeated again. In each cycle, a certain number of particles is transported against their gradient.
The total force acting on the piston is

$$\bar{F} = (p_1 - p_2)A - F_E$$

where $p_1$ and $p_2$ are pressures in the two compartments.

For the ideal gas $p = k_B T \rho$, where $\rho$ is the density. Therefore,

$$\bar{F} = k_B TA(\rho_1 - \rho_2) - F_E$$

If $\gamma$ is the effective friction coefficient of the piston, the piston velocity therefore is

$$v = \frac{\bar{F}}{\gamma} = \frac{k_B TA(\rho_1 - \rho_2) - F_E}{\gamma}$$

When $F_E < F_{\text{stall}}$, the turbine operates as a motor, when $F_E > F_{\text{stall}}$, the turbine operates as a pump.
Now we calculate the flux of particles $J$. This is the number of particles transported through the channel per unit time. 

If the turbine operates as a motor ($F_E < F_{stall}$), the number of particles transported in each step is $N^+ = V_0 \rho_1$ where $V_0 = AD$ is the channel volume. If $T$ is the step time, we have

$$J^+ = \frac{V_0 \rho_1}{T} = vA \rho_1 \text{ if } F_E < F_{stall}.$$ 

For the pump,

$$J^- = \frac{V_0 \rho_2}{T} = vA \rho_2 \text{ if } F_E > F_{stall}.$$ 

The (Gibbs) free energy transported with each particle in the case of ideal gas is

$$\Delta g = \mu_1 - \mu_2 = k_B T \ln \rho_1 - k_B T \ln \rho_2 = k_B T \ln \frac{\rho_1}{\rho_2}$$

where $\mu_1$ and $\mu_2$ are chemical potentials of particles in two compartments.

The energy fluxes associated with particle flows in two regimes therefore are

$$P^+ = \Delta g J^+, \quad P^- = \Delta g J^-$$
Motor \( F_E < F_{\text{stall}} \)

The mechanical power (work per unit time) is

\[
P_M = F_E v = \frac{1}{\gamma} F_E \left( F_{\text{stall}} - F \right)
\]

The Gibbs free energy used to generate this work per unit time is

\[
P^+ = J^+ (\mu_1 - \mu_2) = A \rho_1 v (\mu_1 - \mu_2)
\]

The motor efficiency is

\[
\eta = \frac{P_M}{P^+} = \frac{F_E}{A \rho_1 (\mu_1 - \mu_2)}
\]
The motor regime is on the right side and the pump regime on the left one. The shaded zone corresponds to the force gap where power and efficiency are exchanged, for low concentration gradients the pump power exhibits the parabola and for larger values of the concentration difference the power behaviour is exchanged, for low concentration difference the pump and motor regimes (fig. 6). For low values of concentration difference, the external force is able to stall the motor, is also interesting to see the fraction of the input energy that is transduced into useful energy. The efficiency for an intermediate external force is large enough to reverse the motion of the piston so no mechanical work can be extracted, and necessary to overcome the external force that is only able to pump particles and a higher concentration difference is needed to extract mechanical work from the turbine. Note that it is worth remarking that in the intermediate leakage zone no useful energy can be transduced. In this regime, the external force is able to overcome the chemical power which is the free energy difference between the reservoirs. This is the free energy lost by the ions crossing the machine: 

\[ P^M = \frac{1}{\gamma} F_E (F_{\text{stall}} - F) \]

\[ \eta = \frac{P^M}{P^+} = \frac{F_E}{A \rho_1 (\mu_1 - \mu_2)} \]

\[ \eta = \frac{P^-}{P^M} = \frac{A \rho_2 (\mu_1 - \mu_2)}{F_E} \]

Dashed curves correspond to the macroscopic theory.

**Motor** \( F_E < F_{\text{stall}} \)

The mechanical power (work per unit time) is

\[
P_M = F_E v = \frac{1}{\gamma} F_E \left( F_{\text{stall}} - F \right)
\]

The Gibbs free energy used to generate this work per unit time is

\[
P^+ = J^+ (\mu_1 - \mu_2) = A \rho_1 v (\mu_1 - \mu_2)
\]

The motor efficiency is

\[
\eta = \frac{P_M}{P^+} = \frac{F_E}{A \rho_1 (\mu_1 - \mu_2)}
\]

**Pump** \( F_E > F_{\text{stall}} \)

The mechanical power (work per unit time) used to operate the pump is

\[
P_M = F_E v = \frac{1}{\gamma} F_E \left( F_{\text{stall}} - F_E \right)
\]

The generated Gibbs free energy used to transport particles against the gradient per unit time is

\[
P^- = J^- (\mu_1 - \mu_2) = A \rho_2 v (\mu_1 - \mu_2)
\]

The pump efficiency is

\[
\eta = \frac{P^-}{P_M} = \frac{A \rho_2 v (\mu_1 - \mu_2)}{v F_E} = \frac{A \rho_2 (\mu_1 - \mu_2)}{F_E}
\]

Both efficiencies reach their maxima at the stall force!
Dashed curves correspond to the macroscopic theory

\[ P_M = \frac{1}{\gamma} F_E \left( F_{\text{stall}} - F \right) \]

\[ P^- = \frac{1}{\gamma} A \rho_2 (\mu_1 - \mu_2) \left( F_E - F_{\text{stall}} \right) \]

\[ \eta_M = \frac{P_M}{P^+} = \frac{F_E}{A \rho_1 (\mu_1 - \mu_2)} \]

\[ \eta^- = \frac{P^-}{P^-} = \frac{A \rho_2 (\mu_1 - \mu_2)}{F_E} \]
What becomes different at nanoscales?

A. The piston will be subject to thermal fluctuations that affect its motion.
B. The number of particles transported per a step may be small and stochastic motions of individual particles have to be resolved.

The piston motion is now described by a stochastic Langevin equation

\[ \gamma \frac{dX}{dt} = \bar{F} + \xi(t) \]

It includes a random thermal force \( \xi(t) \).
According to the Langevin theory of Brownian motion; this noise has Gaussian statistics, its mean value is zero and its correlation function is

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

The frequency spectrum of thermal noise is flat and therefore it is also called "white".
When thermal fluctuations are taken into account, the mean flux of particles through the channel is

\[
J = \frac{F_A}{\gamma} \left[ \rho_2 \exp \left( \frac{FD}{k_B T} \right) - 1 \right] - \rho_1 \left[ 1 - \exp \left( - \frac{FD}{k_B T} \right) \right]
\]

\[
\exp \left( \frac{FD}{k_B T} \right) + \exp \left( - \frac{FD}{k_B T} \right) - 2
\]

where \( \bar{F} = F_{stall} - F_E \)

This leads to two principal effects:

1. Within an interval of forces (gray region in the figure) the nanoturbine does not persistently operate as a motor or a pump, it alternates randomly between the two regimes

2. The efficiencies of the nanoscale motor and pump vanish at the boundaries of this gray zone.

What becomes different at nanoscales?

A. The piston will be subject to thermal fluctuations that affect its motion.
B. The number of particles transported per a step may be small and stochastic motions of individual particles have to be resolved.

The Brownian motion of particles $i = 1, 2, \ldots, N$ that interact with the piston was numerically simulated, together with the stochastic motion of the piston itself

$$\gamma \frac{dX}{dt} = -\sum_i V'_p(x_i - X) - F_E + \xi(t)$$

$$\gamma_p \frac{dx_i}{dt} = V'_p(x_i - X) + \xi_p(t)$$

where the interaction potential was

$$V_p(x_i - X) = H \exp \left[ -\left( \frac{x_i - X}{a} \right)^2 \right]$$

The numerical study was performed for a more efficient, two-piston turbine.

Comparison of analytical predictions and numerical simulation data for the efficiencies of the turbine

Dashed and solid curve are analytical predictions (for one and two pistons) accounting only for thermal fluctuations for the pistons, symbols show numerical simulation results

\[ \eta = 80 \text{ mM}, \rho_2 = 20 \text{ mM}, \]
\[ A = 150 \text{ nm}^2, D = 4 \text{ nm} \]