Lecture "Soft Matter Physics"
(Prof. Dr. R. Seidel)

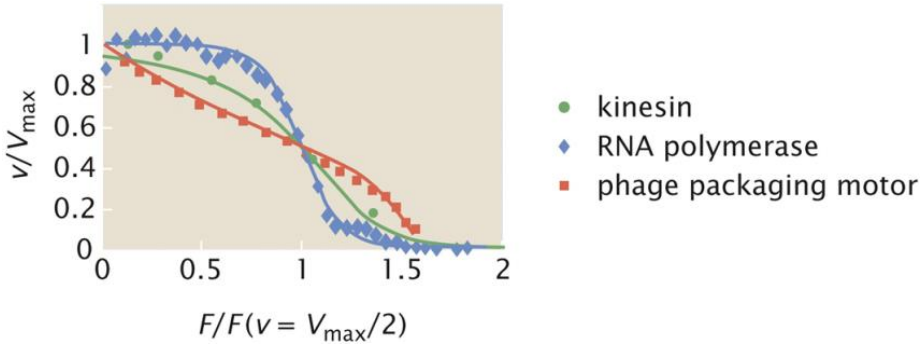
Lecture 8

Dynamics of molecular motors

- Principles and types of molecular motors
- Brownian motors
- Driven walks to describe motor dynamics
- Force-dependence of molecular motors

1) Molecular motors: Introduction

Within a unified framework, molecular motors are tiny machines that convert chemical energy into mechanical work. In principle, the **characteristics of different molecular motors** can be compared as well as the mechanisms of their inner "gearboxes". Like motor from engineering, molecular motors slow down at higher load forces, but there is no universal behavior.
The biological function of a particular motor depends on the way that it converts the chemical energy into a conformational change that causes movement and generates force. The motor speed depends on the applied force and is dependent on the mechanochemical energy conversion mechanism.



What is a machine and what is special about molecular machines/motors? (see slides)
Generally, a machine is a system that converts energy into a desired useful form, such as mechanical work. It is not just a device that only dissipated/consumes energy. Typically, a motor visits during its operation different energy states. Often these states are revisited within a cyclic scheme. In order to make a machine work we require **non-equilibrium conditions**, which can for example be established by:

(i) a spatial energetic bias
(ii) a temporal modulation of the energy landscape, i.e. a temporal modulated energy bias

A fundamental difference between macroscopic and microscopic machines is that the latter can employ so called non-adiabatic mechanisms. Within an **adiabatic mechanism** the entire internal energy change within the motor can in principle be converted into work, as given by an adiabatic expansion. Microscopic machines are often employing non-adiabatic mechanisms, where dissipation, e.g. by viscous drag, and/or diffusion are an integral part of the motor mechanism its-self. Examples are certain low Reynolds number swimmers (see later lectures) and so-called Brownian motors (see slides).

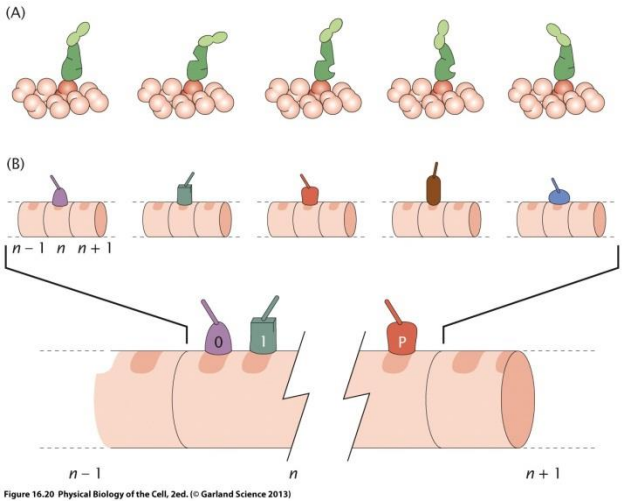
A possible classification of biological motors can distinguish four broad **classes of molecular motors:**

(i) Translational motors: step along linear track (cytoskeletal motors), DNA motors such as helicases, polymerases
(ii) Rotary motors: within cell membrane such as F1F0-ATP synthase or flagellar motor
(iii) Polymerization motors: force generation during (de)polymerization of actin and microtubules
(iv) Translocation motors: threading a structure such as DNA or an unfolded protein through a hole and then pushing it or pulling it through the hole (protein translocation through membranes, DNA conjugation. i.e. DNA exchange between bacteria)
Cytoskeletal motors and rotary motor: see slides

2) Driven random walks to describe motor dynamics

In the following we want to develop simple kinetic models to describe the dynamics of molecular motors. To this end we consider their **periodic track** (actin filament, microtubule, DNA) as a **1D lattice of *n* identical slots**. In each slot the **motor can be in any one of *P* distinct states.** The state space shall comprises thus a set of geometric positions that each include a set of internal structural states at each position.

The internal states are for real molecular motors different conformational states that are often associated with different states of substrate binding (ATP,ADP,P_i)



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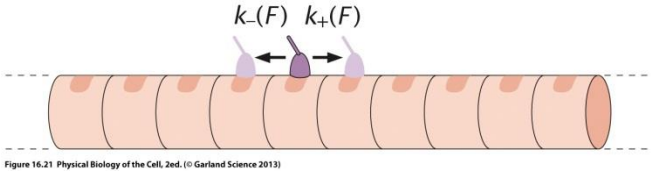
A) One-state model

The simplest model we can consider is the one-state model with **only a single internal state**. Thus, the motor only hops from one site to the next with forward rate $k_+(F)$ and backward rate $k_-(F)$ under the action of the applied force F:



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The probability $p(n, t + \Delta t)$ for the motor to be at position n at $t + \Delta t$ can be obtained by summing over all over all microtrajectories during Δt that could have brought the motor to this position. If Δt is small enough (not more than one step within Δt) we can write:

$$p(n, t+\Delta t) = \underbrace{k_+ \Delta t p(n-1, t)}_{\text{jump from site to left}} + \underbrace{k_- \Delta t p(n+1, t)}_{\text{jump from site to right}} + \underbrace{(1 - k_- \Delta t - k_+ \Delta t) p(n, t)}_{\text{stay put}}$$

Transformation gives:

$$\frac{p(n, t+\Delta t) - p(n, t)}{\Delta t} = k_+[p(n-1, t) - p(n, t)] + k_-[p(n+1, t) - p(n, t)]$$

Replacing the slot number n with a position $x = na$ with a being the length of a slot, i.e. the step size, allows to Taylor-expand the probability:

$$p(x \pm a, t) \approx p(x, t) \pm \frac{\partial p}{\partial x} a + \frac{1}{2} \frac{\partial^2 p}{\partial x^2} a^2$$

In the limit of $\Delta t \to 0$ we get after inserting the Taylor expansion into the probability equation from above:

$$\frac{\partial p}{\partial t} = -(k_+ - k_-)\frac{\partial p}{\partial x}a + \frac{1}{2}(k_+ + k_-)\frac{\partial^2 p}{\partial x^2}a^2$$

since $p(x,t)$ canceles in both brackets on the right side. Defining the terms:

$$V = a[k_+(F) - k_-(F)]$$

$$D = \frac{a^2}{2}[k_+(F) + k_-(F)]$$

we obtain:

$$\frac{\partial p}{\partial t} = -V\frac{\partial p}{\partial x} + D\frac{\partial^2 p}{\partial x^2}$$

This is the so-called Smoluchowski equation, which describes diffusion in the presence of a drift force (as will again encounter later in the lecture series). It can be solved by coordinate transformation into the reference system of the moving motor:

$$\bar{t} = t \quad \text{and} \quad \bar{x} = x - Vt$$

With this we can write for the partial derivative by the time:

$$\frac{\partial p(\bar{x},\bar{t})}{\partial t} = \frac{\partial p}{\partial \bar{t}}\frac{\partial \bar{t}}{\partial t} + \frac{\partial p}{\partial \bar{x}}\frac{\partial \bar{x}}{\partial t} = \frac{\partial p}{\partial \bar{t}} - V\frac{\partial p}{\partial \bar{x}}$$

Similarly can write for the partial derivatives:

$$\frac{\partial p}{\partial x} = \frac{\partial p}{\partial \bar{x}} \quad and \quad \frac{\partial^2 p}{\partial x^2} = \frac{\partial^2 p}{\partial \bar{x}^2}$$

Inserting the derivatives for the transformed coordinates gives:

$$\frac{\partial p}{\partial \bar{t}} = D\frac{\partial^2 p}{\partial \bar{x}^2}$$

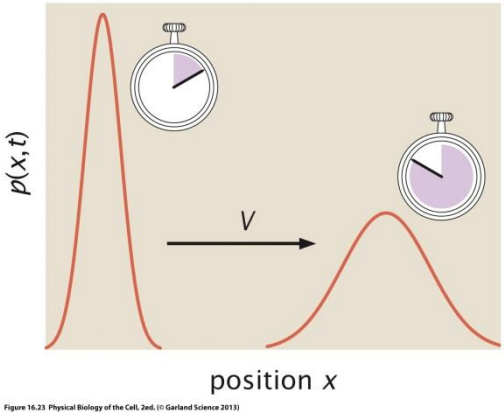
which is an ordinary diffusion equation. Using the initial conditions for the motor $\bar{x} = 0$ at $t = 0$, this equation is solved by a Gaussian function with linearly increasing variance:

$$p(\bar{x},\bar{t}) = \frac{1}{\sqrt{4\pi D\bar{t}}}e^{-\bar{x}^2/4D\bar{t}}$$

as can be confirmed by inserting. After backtransformation of the coordinates we obtain:

$$(x,t) = \frac{1}{\sqrt{4\pi Dt}}e^{-(x-Vt)^2/4Dt}$$

This is a **Gaussian distribution, where the mean position is shifts linearly with time with velocity V while the variance increases linearly with time with $2D$**:



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The broadening of the Gaussian is due to the random stepping of the motor, i.e. its diffusive component. Note: ***D*** **is non-zero even when no backward steps are allowed** ($k_- = 0$). This is **due to the random nature of the individual steps** where the stepping time is not a single fixed time but rather an exponentially distributed random variable. This causes **desynchronization of the pool of individual molecular motors** that would start from a given position at the same time. The diffusive nature of the stepping process relative to the directional movement can be described by the randomness parameter *r*, which is the mean squared diffusive excursion per step taken normalized by the squared step size:

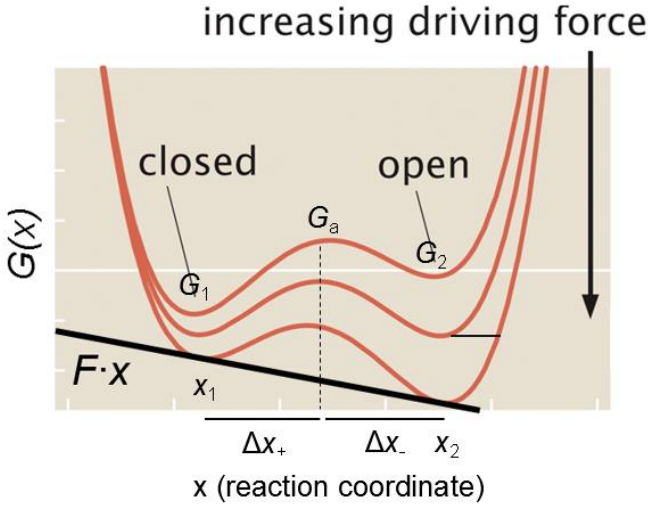
$$\text{randomness} = r \equiv \frac{\Delta x^2}{a^2} = \frac{2D}{Va} = \frac{k_+(F) + k_-(F)}{k_+(F) - k_-(F)}$$

with $\Delta x^2 = 2D\tau = 2Da/V$.

It **approaches 1 for simple forward stepping only** and values **much larger than one for considerable backward stepping**. Values **smaller than one can be obtained for 2- and more-state motor models**. A multistep reaction per motor step provides a smaller relative standard deviation for the stepping time. Thus, by studying the desynchronization of motors during the stepping one can gain inside into their stepping mechanism and their step sizes.

B) Force-dependence of molecular motors

As discussed before, an applied force tilts the energy landscape along the force direction and can thus lower the energies of the final states but also the transition state.



This leads to a change of the forward and backward stepping rates depending on the distances Δx_+ and Δx_- of the transition state from the initial states:

$$k_+(F) = A\, e^{-\beta[\Delta G_{a1} - F\Delta x_+]} = k_+(0)e^{\beta F\Delta x_+}$$
$$k_-(F) = A\, e^{-\beta[\Delta G_{a2} + F\Delta x_-]} = k_-(0)e^{-\beta F\Delta x_-}$$

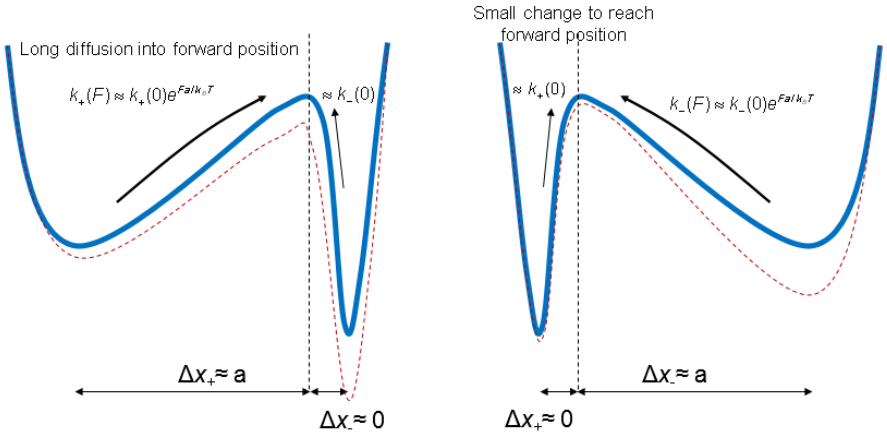
Using these expressions, we can calculate the force dependence of the motor velocity within the 1-step model:

$$V(F) = a[k_+(F) - k_-(F)] = a\left[k_+(0)e^{\beta F\Delta x_+} - k_-(0)e^{-\beta F\Delta x_-}\right]$$

The exact force dependence of the motor velocity depends on the relative position of the transition state with respect to the initial states. There are two extreme cases:

(i) **only the forward step is force-dependent**, i.e. the transition state is far away from the initial state, such that a large diffusion-like conformational change is required to reach it. This **corresponds to a Kramers-like reaction mechanism.**

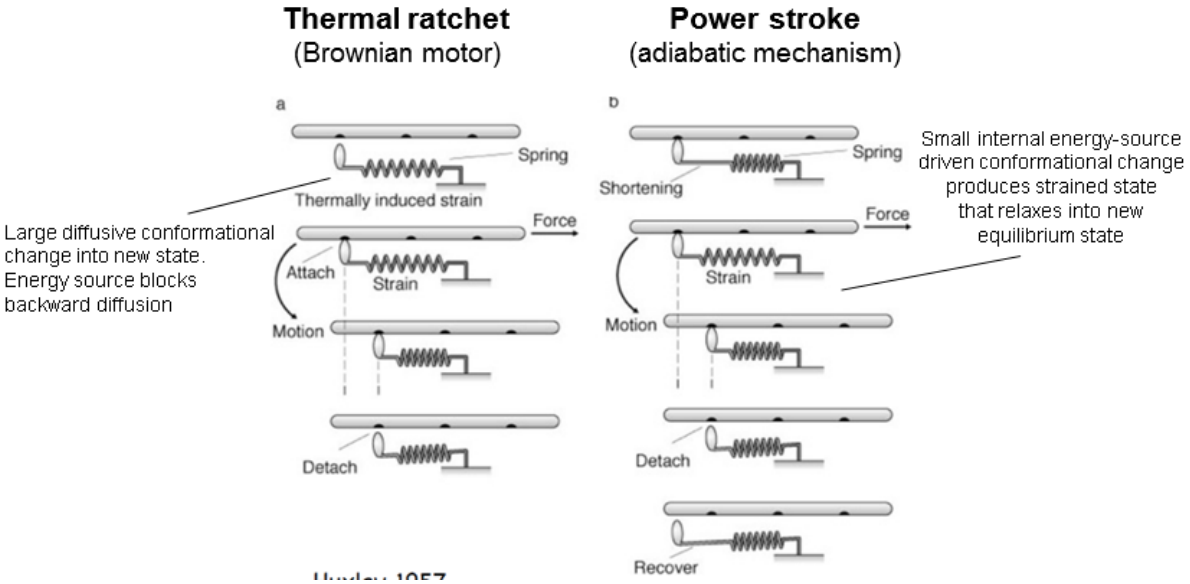
(ii) **only the backward step is force dependent**, i.e. small internal bond breakage allows to reach the transition state, while the final conformational change along the force coordinate is a large-scale (diffuse) relaxation. This corresponds roughly to an **Eyring-like mechanism**. Whether the Eyring theory can really be applied depends on the number of bonds that need to be broken for the reaction.



These cases lead to two different motor mechanisms, the so-called **Brownian ratchet** and the **power stroke** mechanism:

(i) In the **Brownian ratchet mechanism,** the motor head leaves its original position due to thermal fluctuations until it eventually attaches at the new position. Here it becomes locked by an energy consuming step (e.g. due to ATP binding, hydrolysis), that prevents the simple backreaction. The new state becomes thus the initial state for the next step. Energy is thus needed to rectify the motor movement.

(ii) In the **power stroke mechanism,** a small internal conformational change driven by an energy source produces a strained state, which relaxes subsequently into the new state. This represents an adiabatic mechanism, in which an int. energy change is directly coupled to work:



Inserting the two extreme possibilities for the transition state position into the velocity equation provides:

- if the **force-dependence is only on forward stepping**, i.e. $\Delta x_+ = a,\ \Delta x_- = 0$ we have:

$$V(F) = a[k_+(F) - k_-(F)] = a\left[k_+(0)e^{\beta F a_+} - k_-(0)\right] = ak_-(0)\left[k_+(0)/k_-(0)e^{\beta F a_+} - 1\right]$$

  The rate ratio in the brackets can be replaced by the equilibrium constant and thus the free energy of the reaction that is gained, e.g. from ATP hydrolysis

$$V(F) = ak_-(0)\left[e^{-\beta(\Delta_r G_{ATP} - Fa)} - 1\right]$$

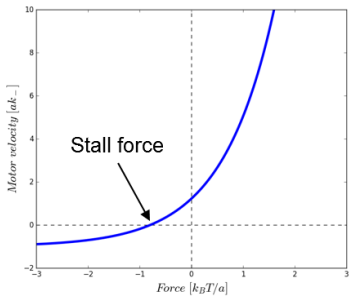
- if the **force-dependence is only on backward stepping**, i.e. $\Delta x_+ = 0,\ \Delta x_- = a$ we have:

$$V(F) = a\left[k_+(0) - k_-(0)e^{-\beta F a}\right] = ak_+(0)\left[1 - k_-(0)/k_+(0)e^{-\beta F a}\right]$$

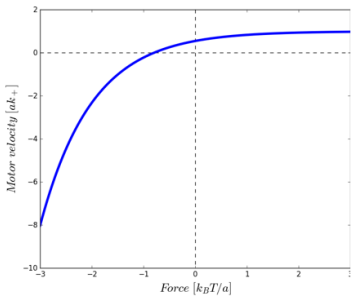
$$V(F) = ak_+(0)\left[1 - e^{\beta(\Delta_r G_{ATP} - Fa)}\right]$$

Plotting provides very different force dependencies:

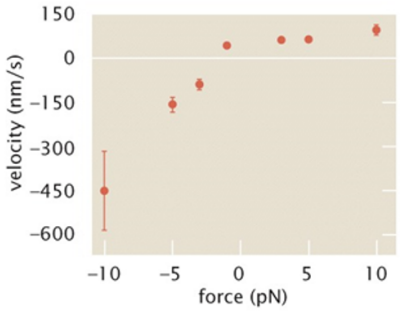
all force-dependence at forward step



all force-dependence at backward step



Myosin V data

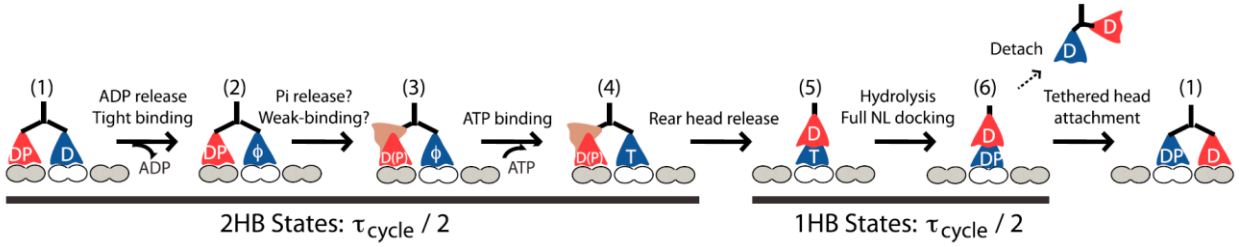


Comparing with data the simplistic model indicates that the force dependence of myosin V is primarily on the backwards step.
Furthermore, the model predicts that there is a backward oriented stall force at which the motor velocity becomes zero. At even large backward forces, the motor will even move backwards. In agreement with even such a simple model, these phenomena have been directly observed for myosin V and kinesin (**see slide**).

3) More complex models for motor dynamics

A) Current understand of the kinesin stepping cycle

The stepping movie of kinesin illustrates the anti-correlated, **i.e. coordinated,** action of both motor heads. ATP binding, hydrolysis, product and force-producing stepping occur in mutual synchronization of the motor heads.
The latest understanding of the stepping cycle of kinesin is illustrated in the following:



- We distinguish the following nucleotide states:

  - strongly-bound empty (Φ) and ATP (T) states
  - weakly bound ADP(D) and ADP-Pi (DP) states
- **Coordination between the heads (step 4)** is achieved by: a **conformational change in front head upon ATP binding that causes a power stroke),** such that the **front head pulls off the weakly-bound rear head in the DP state**, since the stress on the rear head causes fast rear head detachment.

- **Preferential forward movement (step 6)** is obtained by: ATP hydrolysis in the front head promotes preferential „rear" head binding in front of leading head due to a corresponding conformation of the enzyme motor.

B) ATP dependence of motor stepping velocities (not part of lecture)

C) More complex models (not part of lecture)