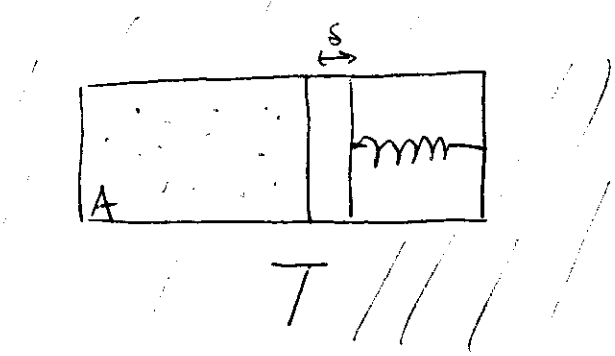
Free Energy –



System A in thermal reservoir

Spring released

$$E_{pot} = F\,\delta$$

$$E_{kin} = \frac{3}{2} k_B T$$

$$E_{tot} = E_{pot} + E_{kin}$$

Total energy (including entropic effects)

$$T\,\Delta S_R = \Delta E_R = -\Delta E_A$$

$$T\,\Delta S_{tot} = T\Delta S_A - \Delta E_A$$

A system A in contact with a thermal reservoir R at temp. T will come to an equilibrium which minimizes the quantity

$$H_A = E_A - TS_A$$

Helmholtz free energy

[Gibbs free energy

$$G_A = E_A + pV_A - TS_A$$ push big system aside costs work

relevant for systems with large ΔV (gases!) but not so much for cells]

Basic facts from Physical Chemistry on the interactions of ions in electrolyte solution with a charged surface.

Interpretation of ion current data through nanopores with excess surface charge requires reviewing information about effects connected with a presence of a charged surface in an electrolyte solution.

Electrostatic interactions of charges in a vacuum or a dielectric medium of a given dielectric constant are long range interactions which decay with distance r as $\sim 1/r$ [42]. The situation in an electrolyte solution is entirely different. Presence of other ions causes a strong screening of these interactions, which results in the short-range character of electrostatic interactions in an electrolyte medium [42].

If we consider a surface with excess surface charge in an electrolyte solution, in order to fulfill the requirement of electroneutrality, the charges on the surface have to be balanced by counterions (ions of opposite sign) from the solution. As a result of that requirement, so called electrical Debye layer is created, consisting of negative charges on the surface and diffused layer of counterions. Concentration of counterions in this Debye layer is related to the surface charge on the pore walls, temperature and bulk concentration, and close to the surface, the counterions concentration can be much higher than in the bulk solution.

The relation between concentration of ions at the surface, concentration of ions at the bulk, and potential at the surface is given by the so-called Graham equation [42]. From the Graham equation one can get very important parameters, namely Debye length and the relation for electric potential at distance x from the surface. Due to this strong screening effect, the potential decays with distance in an exponential way with Debye length characterizing the distance over which the potential dropped by 1/e.

Important implications for the project: (i) surface charge density has to be well-controlled (ii) in order to probe interactions of ions with the pore walls, the diameter of the nanopores has to be comparable to the Debye length.

Modeling of electric potential inside a conical nanopore

Conical and double-conical nanopores in polymer films will be our model nanoporous system into which ionic selectivity will be introduced. Geometrical asymmetry of these pores and excess surface charge is expected to influence the distribution of the electrical potential inside the pores [7,8,31,40].

generalized form of entropy

$S = -k \sum p_i \ln p_i$ Shannon entropy

E.g. message of N letters from an alphabet with M letters

Extreme cases

$p_i = \delta_i$ $S = 0$ total order, minimal entropy

$p_i = \frac{1}{M}$ each letter equally probable

$S = -k N \ln M$

maximal entropy

Probability and free energy

$\langle E \rangle = \sum E_i p_i$ $p_i = \frac{1}{Z} e^{-E_i/k_B T}$

$S = -k \sum p_i \ln p_i$

$$
\begin{aligned}
H &= \langle E \rangle - TS \\
&= \sum p_i \{ E_i + k_B T \ln p_i \} \\
&= \sum \frac{1}{Z} e^{-E_i/k_B T} \left\{ E_i + k_B T \ln \frac{1}{Z} e^{-E_i/k_B T} \right\} \\
&= \frac{1}{Z} \sum e^{-E_i/k_B T} \left\{ E_i + k_B T \left[\ln 1 - \ln Z + \ln e^{-E_i/k_B T} \right] \right\} \\
&= \frac{1}{Z} \sum e^{-E_i/k_B T} \{ E_i - k_B T \ln Z - E_i \} \\
&= -k_B T \ln Z
\end{aligned}
$$

$S = k_B \ln \Omega$ $\qquad\qquad \Omega = e^{S/k_B}$

Consider Small system A in contact with large reservoir R

Goal: Prob. for allowed states of A

$$P_A \propto \Omega_A(E_A) = \Omega_B(E_{tot} - E_A)$$

$$= e^{S_B(E_{tot} - E_A)/k_B}$$

$E_A \ll E_{tot}$

$$S_B(E_{tot} - E_A) = S_B(E_{tot}) - E_A \underbrace{\frac{dS_B}{dE}}_{\frac{1}{T}} + \mathcal{O}(E_A^2)$$

$$P_A \propto \underbrace{e^{S_B(E_{tot})}}_{c} \cdot e^{-E_A/k_B T}$$

Boltzmann distribution

Normalization

$$\sum P_i = 1 \quad \Rightarrow \quad P_i = \frac{e^{-E_i/k_B T}}{\sum e^{-E_i/k_B T}}$$

$$Z = \sum_i e^{-E_i/k_B T}$$

partition function

SELECTIVITY AND GATING IN SYNTHETIC CALCIUM CHANNELS

A. Specific aims

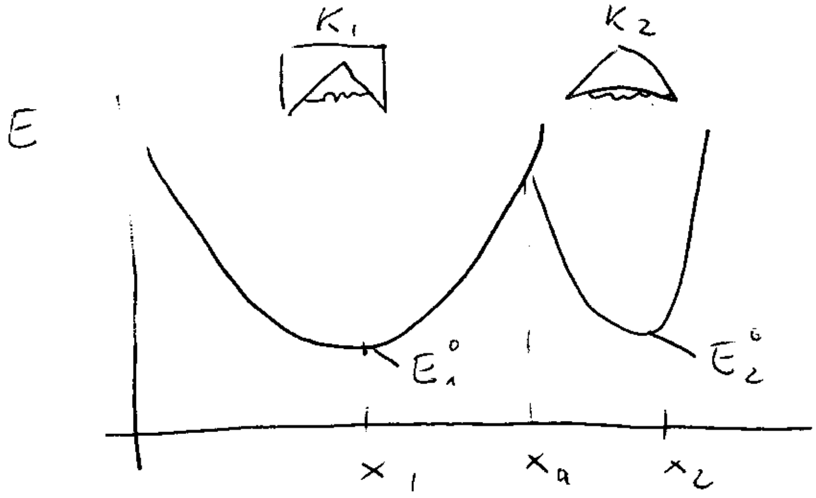
Ion channels in biological membranes are nanodevices that are the basis of most physiological processes in living organisms. Ion selectivity, i.e., the ability to selectively transport one type of ion, enables channels to fulfill their biological function [1]. Calcium channels, for example, which play a crucial role in regulation of heart muscle function, can preferably transport calcium ions although monovalent cations, e.g. sodium ions, are present in extra- and intracellular media in thousand fold excess to calcium. It is thought that the mechanism of selectivity of these channels involves four carboxylate groups, which create a chemical environment similar to that found in many calcium-chelating agents like ethylenediaminetetraacetic acid (EDTA) [2-5]. One of the models for calcium channels points to the importance of the following factors in determination of channel selectivity: (i) negatively charged locus with carboxylate groups, (ii) high binding affinity of calcium ions, and (iii) charge crowding in the channel [6-10]. According to the model, any nanopore could be given calcium selectivity by tuning the properties of the surface charge. Electrostatic interactions have been recently shown to play a very important role in selectivity of voltage-gated channels as well. It has been found that selectivity of a voltage-gated potassium channel is determined not only by pore diameter [11-13] but also the density and dynamics of charged groups on the pore walls [14]. The main goal of this project is to elucidate the basic biophysical phenomena underlying selectivity of two types of channels, (i) L-type calcium channels and (ii) voltage-gated channels. In the studies, we will use single engineered nanopores with diameters down to 1 nm as a model system. Fully controllable chemistry and mechanical stability of the engineered nanopores give unique freedom in performing a wide spectrum of needed experiments at various conditions (e.g. pH, temperature), which would be difficult with fragile biochannels. Specific goals of the project comprise identification of conditions that are crucial for ionic selectivity in biological channels.

<u>Specific goal #1.</u> Examination of the importance of surface charge for ionic selectivity. We will prepare single engineered nanopores with various densities of carboxylate groups and examine selectivity of these synthetic systems for calcium and monovalent cations. The first method of chemical modification will involve electroless deposition of gold on the surface of the membrane and the pore walls, with subsequent chemisorption of thiols. We will also modify the pore walls by direct attachment of various chemical groups to the existing carboxylate groups in polymer films.

<u>Specific goal # 2.</u> Effect of ionic crowding on ion selectivity of calcium and voltage-gated channels. Ionic selectivity of biological channels has been predicted to be directly related to the sub-nano size of the channels. Using engineered nanopores we will be able to tailor the effective volume of the nanopores. Two major pore geometries will be used: tapered-cone and double-conical nanopores.

<u>Specific goal # 3.</u> Influence of dynamics of carboxylate groups on selectivity towards ions. Engineered nanopores with attached polymer oligomers containing carboxylate groups will be used. Different dynamics of these groups will be achieved by performing measurements at elevated temperatures as well as changing length of the polymer oligomers.

<u>Specific goal # 4.</u> Physical modeling of ionic selectivity in ion transport through engineered nanopores. The interplay between experimental data of current-voltage curves and ion current series in time, low-resolution modeling, and atomic-resolution modeling is crucial for the success of this proposal. Mean-field theories and Poisson-Nernst-Planck equation will be applied together with molecular dynamic simulations. Low-resolution models will help to identify conditions for which particularly interesting features of channel behavior can be expected. Atomic resolution modeling will be used as a spotlight to investigate these conditions in more detail. Both low and atomic-resolution modeling will help to guide further experimental studies through predictions, and to



K_1 soft, relaxed

K_2 stiff, extended

$$P_1 = \frac{1}{Z}\int_{-\infty}^{x_a} e^{-E(x)/kT}\, dx \equiv \frac{Z_1}{Z}$$

$$P_2 = \frac{1}{Z}\int_{x_a}^{+\infty} e^{-E(x)/kT}\, dx \equiv \frac{Z_2}{Z}$$

$$Z_1 = \int_{-\infty}^{x_a} e^{-E(x) kT} \qquad Z_2 = \int_{x_a}^{\infty} e^{-E(x) kT}\, dx$$

Free energies

$$H_1 = -kT \ln Z_1 \qquad H_2 = -kT \ln Z_2$$

$$P_i = \frac{1}{Z} e^{-H_i/kT}$$

Different stiffness

$$E_i(x) = E_i^0 + \frac{1}{2} k_i x^2$$

$$E(x) = \begin{cases} E_1(x - x_1) & x \le x_a \\ E_2(x - x_2) & x > x_a \end{cases}$$

analyze complex sets of experimental data, thereby helping to elucidate the mechanism(s) underlying ion selectivity in close interplay with the experimental studies.

B. Background and Significance

Key points of the Background and Significance

We will model selectivity of calcium and voltage-gated channels using synthetic nanopores with diameters down to 1 nm. We will elucidate the importance of electrostatic interactions occurring in a highly restricted volume of nanopores, for ionic selectivity. We will consider interactions between passing ions, and interactions of the ions with excess surface charge on the pore walls. By tailoring surface characteristics, controlling dynamics of structural elements of the nanopores and controlling the pore geometry, we want to render ionic selectivity in these engineered nanopores.

We will also study the importance of restricted volume for the ionic selectivity. For this part of the project we will use polymer nanopores covered with gold by the process of electroless plating; the electroless plating enables reducing the pore diameter to sub-nanometer dimensions.

Summary of facts on ionic selectivity in biological channels

Ion channels are proteins, which enable transport of ions through the otherwise impermeable (for ions) lipid membrane [1,15]. Selectivity is an important property, which is the basis for channels functioning. Biological channels are specific nanodevices selecting one type of ion over other ions by thousands folds. For example calcium channels can preferably transport calcium although monovalent cations e.g. sodium are present in extra- and intracellular media in thousands fold excess to calcium. This feature is even more surprising when one takes into account the fact that sodium and calcium ions have basically the same size: crystal radii are equal to 0.95 Å and 0.99 Å, for Na^+ and Ca^{2+}, respectively [16].

Currently it is believed that ion selectivity in ion channels can be achieved either by presence of a binding site in a pore or by acting as a sieve [17]. In voltage-gated channels, the latter mechanism has been shown to be in operation [11-13]. In calcium channels, on the other hand, it is believed that there are specific binding sites for calcium which make the calcium transport more energetically favorable than sodium or potassium [2-5,8]. It has long been a puzzle a calcium channel can be highly selective for calcium and at the same time be capable of transporting ions at huge rates of over 10^6 ions per second. This classical puzzle seemed even more mysterious when one took into account the apparent values of dissociation constants for calcium known to be in micromolar range. K_d is a ratio of rate constants of unbinding k_{off} and binding k_{on}. Classical models state that k_{on} can have a maximum value of a diffusion limit given by 10^9 $M^{-1}s^{-1}$, one can therefore calculate that k_{off} is ~ 1000 1/s [17]. This k_{off} is too small to account for a high conductivity of the channels. To explain the discrepancy, it has been assumed that there are two binding sites for calcium ions inside the pore. When one site is occupied by calcium, it is harder for the next calcium to get bound, because the sites are so close to each other that the calcium ions repel themselves electrostatically. The apparent dissociation constant for calcium is therefore larger, and much higher fluxes of ions can be observed for higher Ca^{2+} concentrations. A direct observation of this phenomenon is the so-called anomalous mole-fraction effect, recorded as nonmonotonic dependence of ion current on calcium concentration.

$$Z_1 = \int_{-\infty}^{x_a} e^{-E/kT} dx = e^{-E_i^0/kT} \int_{-\infty}^{x_a} e^{-\frac{1}{2} K_i x^2/kT} dx$$

$$\approx e^{-E_1^0/kT} \int_{-\infty}^{\infty} e^{-\frac{1}{2} K_1 x^2/kT} = \sqrt{\frac{2\pi kT}{K_1}}\, e^{-E_1^0/kT}$$

$$\left[\begin{array}{ll} \int_{-\infty}^{\infty} e^{-ax^2} dx = \frac{\sqrt{\pi}}{a} & a = \frac{K_i}{2kT} \\ \int_{-\infty}^{\infty} x^2 e^{-ax^2} dx = \frac{\sqrt{\pi}}{2a^3} & \end{array} \right]$$

$$H_1 = -kT \ln Z_1 = E_1^0 + \frac{1}{2} kT \ln \frac{K_i}{2\pi kT}$$

$$\Delta H = H_2 - H_1 = \Delta E_i^0 + \frac{1}{2} kT \ln \frac{K_2}{K_1}$$

$$K_2 = 4 K_i \quad \Rightarrow \Delta H = kT \ln 2$$

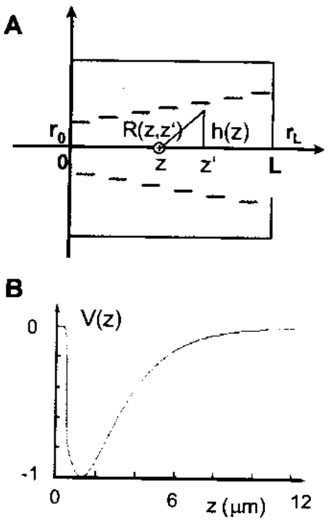


Figure 1. (A) Scheme of an axial cut of a conical pore (not in propotions). (B) Profile of electric potential V(z) inside a tapered-cone pore.

The potential was modeled by assuming Debye type of interactions between ions [31,40]. Figure 1 shows a schematic representation of a conical nanopore together with a profile of electric potential representing interactions of a cation at the pore axis with the negative charges on the pore walls. **We expect that the potential minimum at the tip of the pore will play a role of an 'electrostatic selectivity filter' for cations.**

C. Preliminary Studies

Conical nanopores as ionic rectifiers

In the previous studies we have fabricated synthetic nanopores which exhibited transport properties similar to these of biological voltage-gated channels [33]. The single nanopores were prepared by the track-etching technique. The foils were irradiated with single swift heavy ions and etched chemically (the details of the process are given in the next section). The unique feature of this technique is the single-particle type of recording i.e. it is one heavy ion which creates one track and one track after chemical development results in preparation of one nanopore.

Transport properties of single conical pores were investigated by measuring current-voltage characteristics as well as ion current signal in time. Figure 2 shows current-voltage characteristics of a single tapered-cone shaped nanopore prepared in a polyethylene terephthalate (PET) foil (Hostaphan RN12, Hoechst), recorded for symmetric electrolyte conditions of 0.1 M KCl buffered to pH as indicated in the Figure. [39] The diameter of the pore openings were 3 nm and 600 nm, respectively. To interpret the I-V curves, it is important to note that as a result of the fabrication process, the pore walls have carboxylate groups with the density of ~1 group per nm^2 [33,43]. Presence of these groups allows regulation of the surface charge of the pore walls by immersing the membrane at solutions of various pH values. We have found that the surface charge is the critical parameter, which regulates transport properties of these nanopores [30-32]. This is not unexpected [9,10]: electrical forces are known to have a large effect on permeation and these forces arise, in large measure, from the permanent (i.e., fixed) charge on the wall of channels.