Physics | Lancaster University

Coulomb Blockade Oscillations and AMFE in Calcium/Sodium Biological Ion Channels

Igor Kh. Kaufman[1], Dmitry G. Luchinsky[1,2], William A.T. Gibby[1], Peter. V.E. McClintock[1], Robert. S. Eisenberg[3]

[1]Department of Physics, Lancaster University, UK
[2]SGT Inc., Ames Research Center, Moffett Field, CA, USA
[3]Molecular Biophysics, Rush University, Chicago, USA

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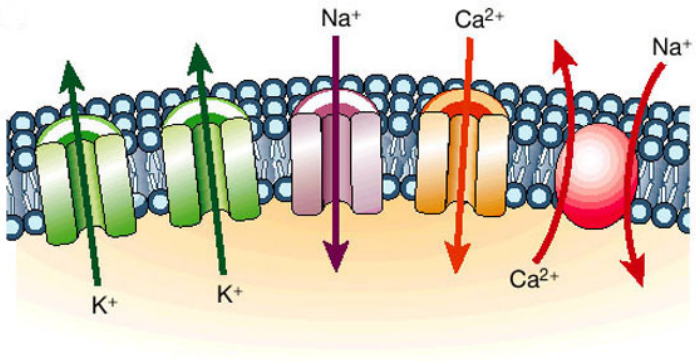
Outline

Ion channels

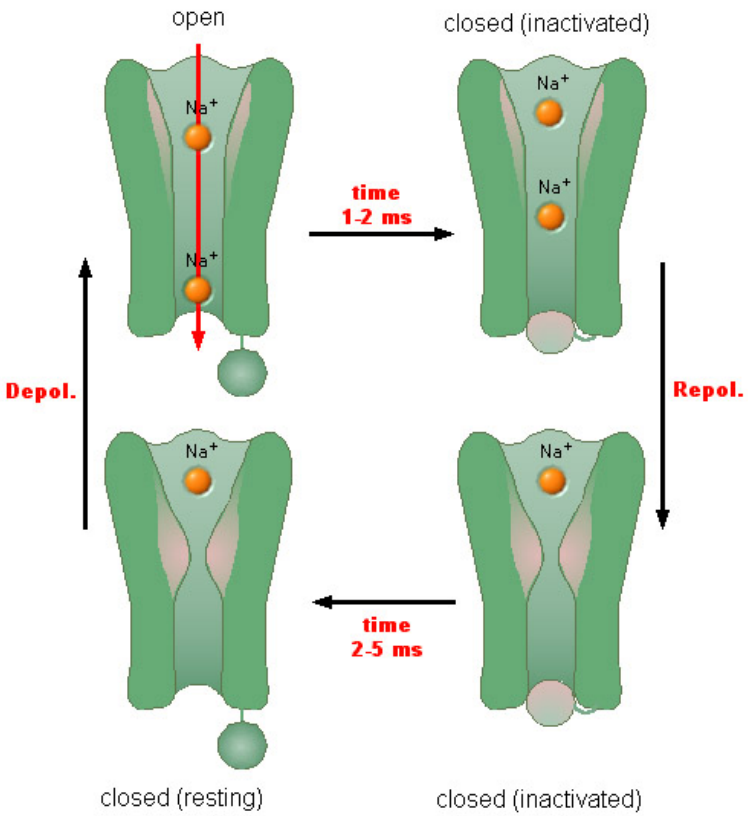


- Biological ion channels are natural nanopores in protein hubs passing through the bilipid membranes of biological cells.
- Fast and selective transport of physiologically important ions (e.g. Na^+, K^+ and Ca^{2+}).

- Essential to physiology – bacteria to humans.
- Target for drugs
- Highly selective for particular ions.
- More than four decades after their discovery a great deal is now known about ion channels.
- Yet there remain many of their basic features are still not properly understood.
- Physical origin of permeation and selectivity - is one of the main long-standing problems.

Calcium and sodium channels

Our study is focused on **voltage-gated calcium and sodium ion channels**.

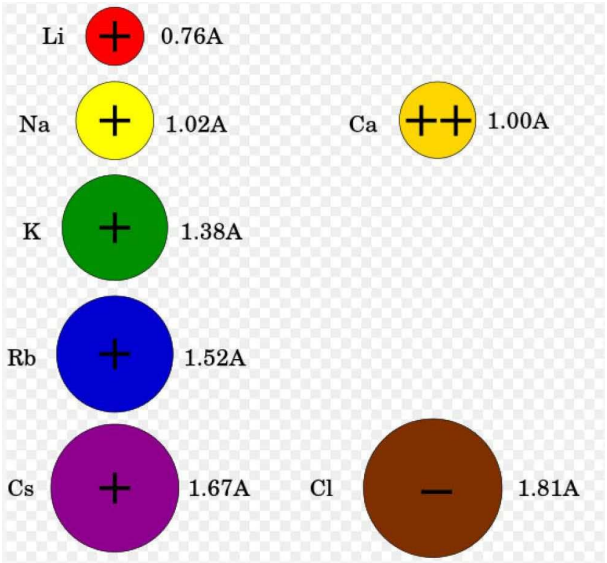


- Voltage-gated Ca^{2+} and Na^{+} channels control muscle contraction, neurotransmitter secretion, and the transmission of action potentials.
- **They are opening on response to cell membrane depolarization.**
- Sodium and calcium channels have very similar structures, but differing SF loci, and hence differing Q_f.
- The mammalian calcium channels supposed to have a EEEE locus (Q_f=4e)
- The mammalian sodium channel has a DEKA locus ($Q_f = 1e$)
- Bacterial sodium channels ChNaBac, NavAB posses a EEEE locus similar to calcium channel.

Ionic selectivity

Selectivity between different ions is the main functional feature of ion channels. Specialized ion channels allow selected species to permeate freely (close to the rate of free diffusion) while largely prohibiting permeation by other ions.

- *Valence selectivity*. Calcium channel favours Ca^{2+} over Na^{+} by up to 1000:1, even though they are the same size.
- *Alike charge selectivity*, Potassium channel strongly disfavours Na^{+}, even though Na^{+} is smaller K^{+}.



Yet the physical origin of selectivity and its relation to channel structure remains a conundrum and it constitutes the central topic of our Project.

Mutation-induced transformation of selectivity

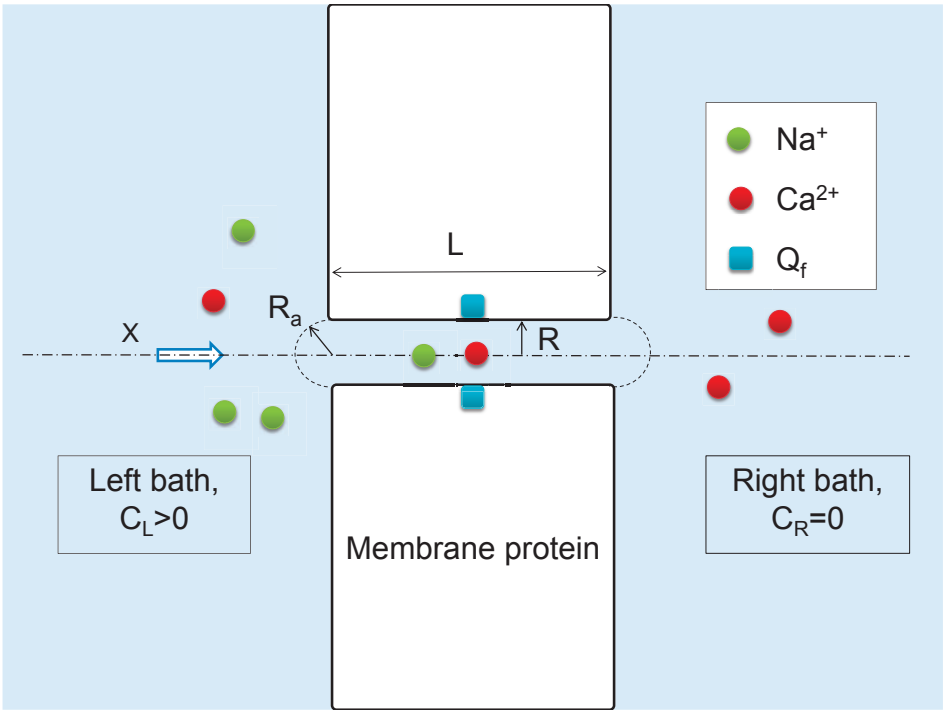
- The charge Q_f at the selectivity filter can be changed by mutating the genes coding for the protein side chains (i.e. DEKA$\rightarrow$DEEA$\rightarrow$DEEE)
- Mutations that influence Q_f usually destroy the channel's selectivity, and hence physiological functionality, leading to "channelopathies".
- **A point mutation of the DEKA sodium channel can convert it into a calcium-selective channel with a DEEA locus, and** *vice versa*.: Heinemann, Nature, 1992.
- **Generally, Calcium selectivity increases with growth of Q_f.**
- Similar transformations occur in bacterial sodium channels.
- Experiments on the mutants show that Q_f is a crucial factor in determining the Ca^{2+} *vs.* Na^{+} valence selectivity.
- **These results are not yet properly explained.**

Research goals

- Despite of structure complexity of ion channels, there is strong experimental evidence that a simple parameter - fixed charge Q_f at the SF controls valence selectivity features. This fact should be confirmed and explained.
- Recently introduced Ionic Coulomb blockade model has been applied to permeation and conductivity of biological ion channels. We are going to investigate ionic selectivity of bacterial sodium channels, wild types and mutant and explain them on the base of Coulomb blockade model.
- We are going to verify CB model and develop multi-level (structural, electrostatic and stochastic) model of bacterial channels.
- **We are going to find direct experimental evidences of Coulomb blockade model of permeation and selectivity of calcium/sodium ion channels.**

Generic electrostatic model. Geometry

- A water-filled, cylindrical hole, radius $R = 3$ Å and length $L = 16$ Å through the protein hub in the cellular membrane.
- The selectivity filter (charged residues) represented by a rigid ring of negative charge $Q_f = 0 - 7e$.
- Water and protein described as continuous media.
- Dielectric constants $\varepsilon_w = 80$ (water) and $\varepsilon_p = 2$ (protein)



Generic electrostatic model. Electrostatics

- The electrostatic field is derived by self-consistent numerical solution of Poisson's equation:

$$-\nabla(\varepsilon\varepsilon_0\nabla u) = \sum ez_i n_i + \rho_f$$

  where ε_0 and ε is the dielectric permittivity of vacuum and the medium (water or protein), u is the electric potential, e is the elementary charge, z_i is the valence, n_i is the number density of ions, and ρ_f is the density of fixed charge.
- This equation accounts for both ion-ion interaction and self-action for all ions in their current positions.
- Dedicated Finite-Volume Poisson Solver accounting for large permittivity mismatch between water and protein.
- One result of the calculations is the axial potential energy profile = the self-consistent Potential of the Mean Force (PMF) used for Brownian Dynamics simulations.

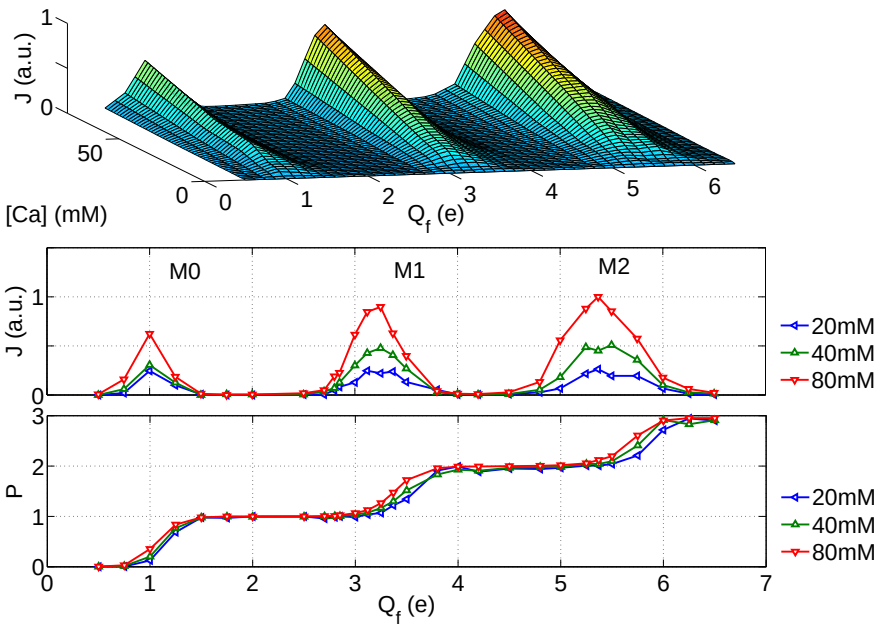
Generic electrostatic model. Brownian dynamics

- The BD simulations use numerical solution of the 1-D overdamped, time-discretized, Langevin equation for the i-th ion:

$$\frac{dx_i}{dt} = -D_i z_i \left(\frac{\partial u}{\partial x_i}\right) + \sqrt{2D_i}\xi(t)$$

  where x_i stands for the ion's position, D_i is its diffusion coefficient, z_i is the valence, u is the self-consistent potential in $k_B T/e$ units, and $\xi(t)$ is normalized white noise.
- Numerical solution is implemented with the Euler forward scheme.
- We use an ion injection scheme that allows us to avoid simulations in the bulk. The arrival rate j_{arr} is connected to the bulk concentration C through the Smoluchowski diffusion rate: $j_{arr} = 2\pi DRC$.

Calcium conduction and occupancy bands



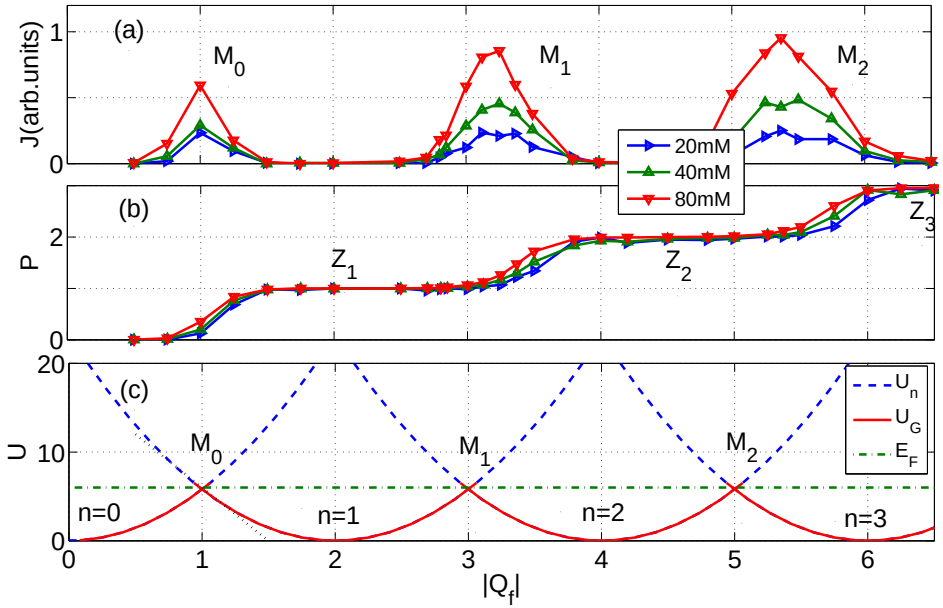
Current J and occupation P as a function of charge Q_f at selectivity filter were simulated for different Ca^{2+} concentrations [Ca].

- Brownian Dynamics simulation revealed an oscillatory pattern of narrow conduction bands and stop bands.
- Conduction bands occur at transitions in channel occupancy *P*.
- Occupancy P represented a Coulomb staircase typical for Coulomb blockade
- Conduction bands correspond to single-ion (M0=1e) and multi-ion (M1=3e, M2=5e) barrier-less conduction.

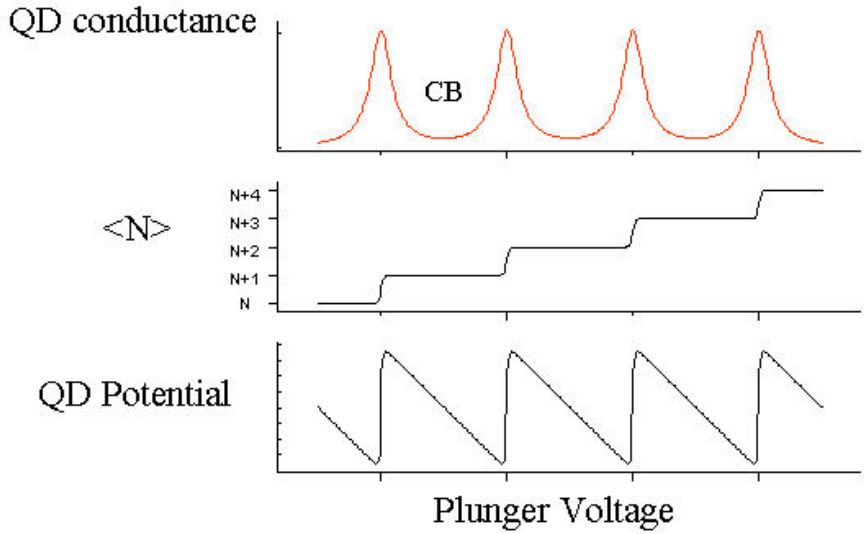
Ionic CB vs electronic CB in quantum dots

The results of simulations are analogous to Electronic Coulomb blockade oscillations in e.g. quantum dots and may be interpreted as Ionic Coulomb blockade oscillations (Krems et al., *J. Phys. Condens. Matter* **25**, 065101 (2013).)

Conduction bands in Ca-channel



CB oscillations in quantum dots



- Ion trapped at SF.
- Periodic conduction bands .
- *Classical* mechanics of ion.
- Stochastic motion.

- Electron trapped in quantum dot.
- Coulomb blockade oscillations.
- *Quantum* mechanics for electron.
- *Quantum tunnelling* for electron.

Ionic Coulomb blockade

- Coulomb blockade appears in low-capacitance, discrete-states systems , provided that the ground state $\{n_G\}$ is separated from neighbouring $\{n_G \pm 1\}$ states by large gap $U_{s,z} \gg k_B T$.
- We approximate U_n as the dielectric self-energy $U_{n,s}$ of the excess charge Q_n:

$$\begin{aligned} U_{n,s} &= \frac{Q_n^2}{2C_s} \qquad (\text{ Electrostatic energy }) \\ Q_n &= zen + Q_f \qquad (\text{ Excess charge }). \end{aligned}$$

- We examine the minimum (i.e. ground state) energy

$$U_G(Q_f) = \min_n(U_n(Q_f, n)) \quad \text{(Ground state energy)}$$

for the ground state occupancy n_G, and the excess charge Q_G, all as functions of Q_f.

Hydration and concentration band shifts

- The total potential energy U_n for a $\{n\}$-channel can be expressed as:

$$\begin{aligned} U_n &= U_{n,s} + U_h \quad (\text{ Potential energy sum }) \\ \mu &= \mu_0 + k_B T \ln P_b \quad (\text{ Chemical potential }) \end{aligned}$$

  where U_n is the electrostatic energy, U_h is dehydration energy, μ_0 is standard chemical potential (we assume $\mu_0 = 0$) and P_b is bulk occupancy related to SF volume.
- Resonant value M_n of fixed charge Q_f for $\{n\} \to \{n+1\}$ transition is defined from shift condition $U_{n+1} - U_n - \mu = 0$:

$$\begin{aligned} M_n &= -ze(n + 1/2) - \Delta M \\ \Delta M &= C_s(U_h - k_B T \ln(P_b) \end{aligned}$$

- Dehydration and concentration terms lead to proper shifts of electrostatic conduction bands. These shifts define different kind of selectivity.

Stair-case transitions and resonant conductance

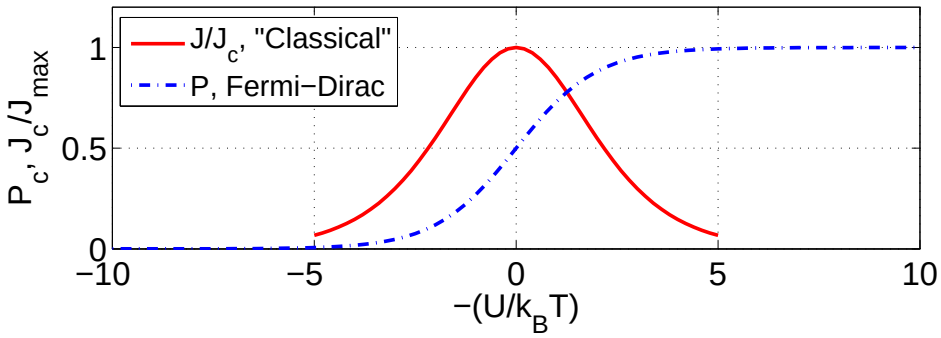
- For Ca^{2+} ions Coulomb gap $\Delta U_n \gg k_B T$ and SF states form exclusive set:

$$m = \{n, n+1\}; \theta_n + \theta_{n+1} = 1;$$

- For this set of states standard derivation via partition function and GCE leads to Fermi-Dirac statistics for an excess occupancy $P_c^* = P_c \bmod 1$:

$$P_c^* = (1 + P_b^{-1} \exp(U_c/k_B T))^{-1},$$

where $U_c = U_{n+1} - U_n$, P_b is a reference bulk occupancy.



- A self-consistent calculation of the conductance via linear response theory leads to the standard CB approximation:

$$J_c \propto (U_c/k_B T) \sinh^{-1}(U_c/k_B T)$$

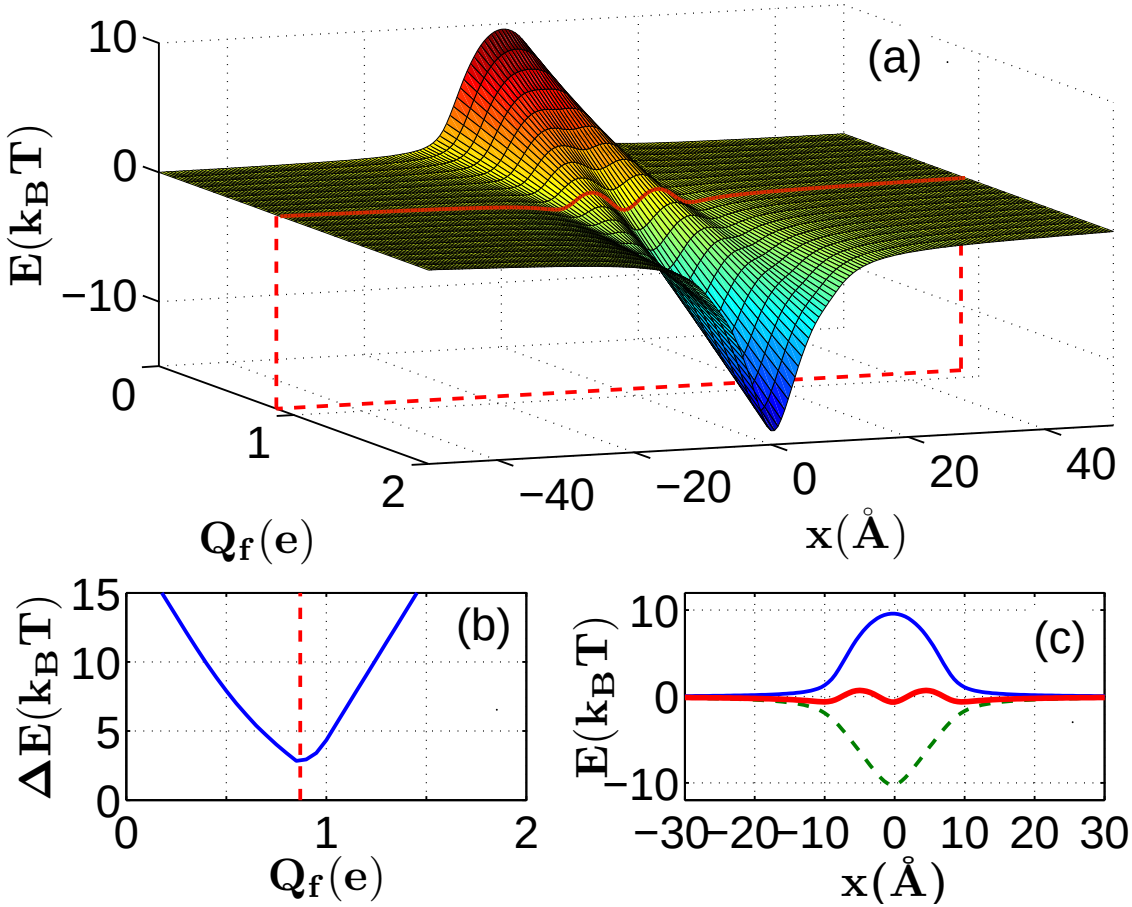
- **The current J_c exhibits a resonant peak similar to that of the tunneling current in a quantum dot.**

Energetics of “barrier-less” conduction for M0 band

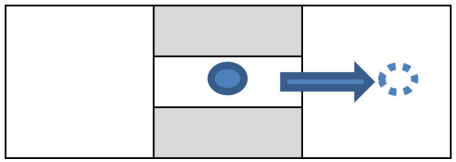
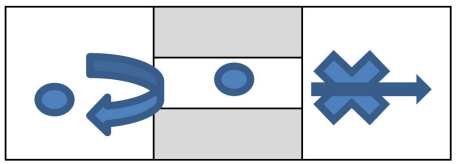
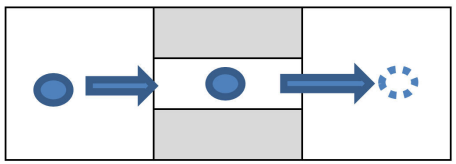
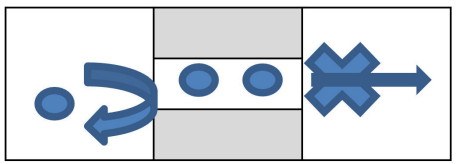
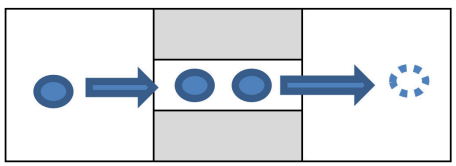
(a) Potential energy along the channel’s $x-$axis plotted *vs.* Q_f.

(b) Energy difference ΔE as a function of Q_f.

(c) “Barrier-less” profile (red) comes from a balance between self-repulsion (blue) and attraction to Q_f (dashed).



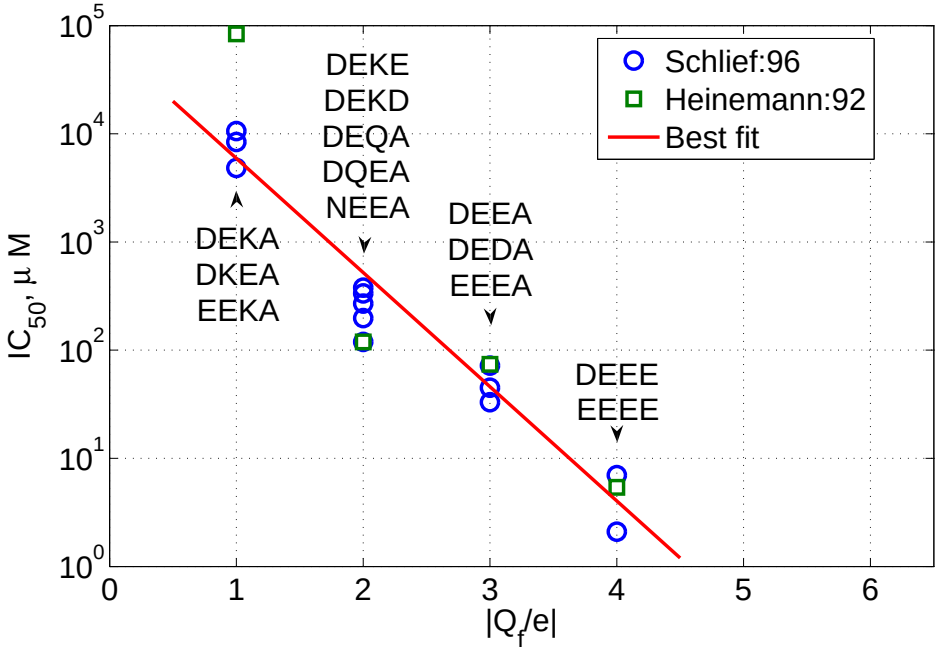
Permeation processes for Ca^{2+}

Singular point	Fixed charge	Conduction mode	Conduction event's scheme	Putative identification
M0	1e	Single-ion barrier-less conduction		OmpF porin, NaK channel
Z1	2e	Single-ion Coulomb blockade		
M1	3e	Double-ion knock-on conduction		L-type calcium channel
Z2	4e	Double-ion Coulomb blockade		
M2	5e	Triple-ion knock-on conduction		RyR calcium channel

Divalent blockade

Coulomb blockade theory (Fermi-Dirac distribution) predicts exponential character for dependence of divalent (calcium) blockade threshold IC_{50} on Q_f.

- Divalent blockade is an example of concentration-related shift.
- Figure show clear exponential dependence of threshold calcium concentration IC_{50} on nominal fixed charge at the selectivity filter.

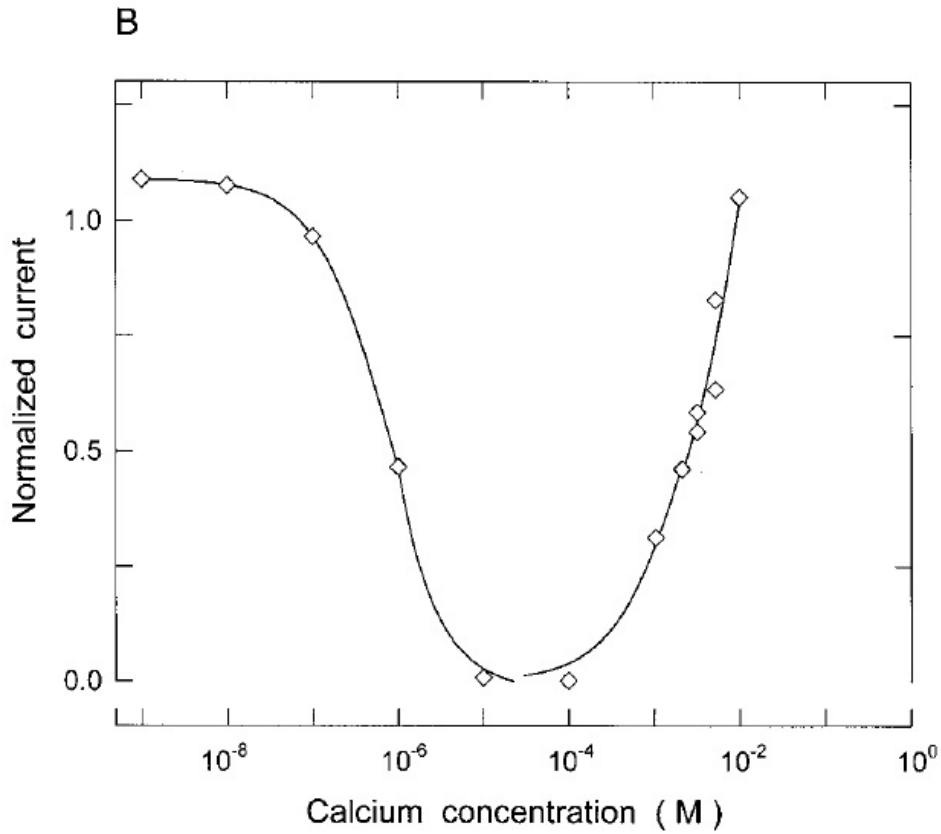


Dependence of IC_{50} on Q_f for calcium and sodium channels and their mutants.

Anomalous mole fraction effect (AMFE)

AMFE is a special phenomenon of valence selectivity manifesting in calcium channels, combining divalent blockade and selective Ca^2 current.

- Calcium channels conduct Na^+ ions at [Ca]=0.
- Divalent Blockade of Na^+ current by μ-molar quantities of Ca^{2+}
- Appearance of Ca^{2+} current at milli-molar Ca^{2+} concentrations.
- Blockade threshold IC_{50} is a significant analytical parameter of channel.



Concentration dependence: Phase diagram of AMFE

- AMFE is exlained as divalent blockade for 1-st Ca^{2+} ion followed by self-sustained 2-ions current starting when 2-nd Ca^{2+} ion arrives.
- The switching lines and AMFE trajectory are shown at the "ion exchange phase diagram" for the calcium channel.
- In "zero ions" area pure sodium conducts ion current.
- The first Ca^{2+} ion provides blockade of channel.
- The second Ca^{2+} ion provides knock-on fast Ca^{2+} conduction.

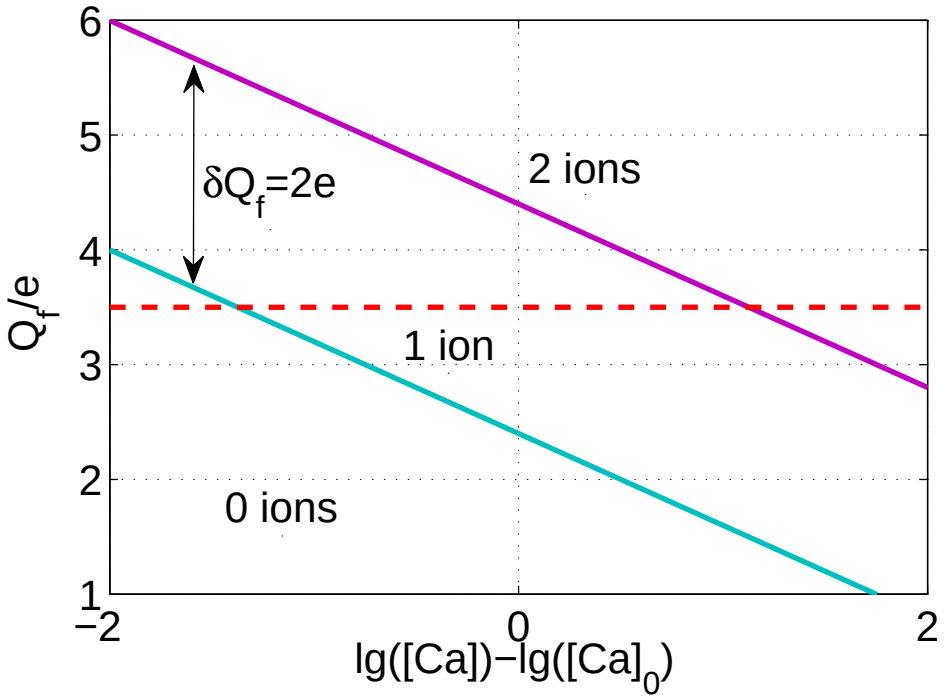
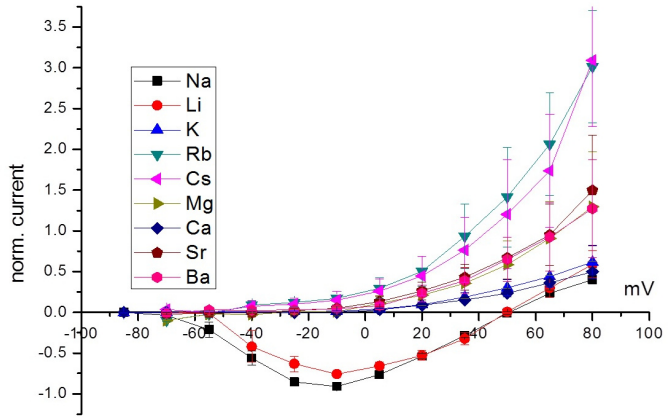


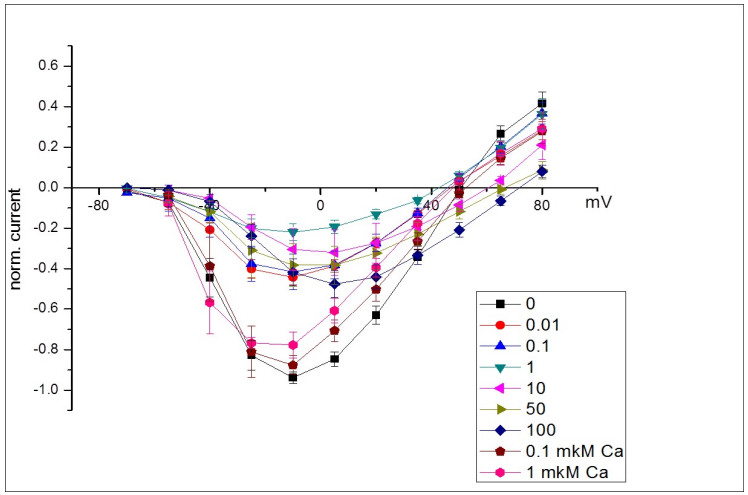
Figure: AMFE in calcium channels (mixed bath). Phase diagram Q_f vs log([Ca]), switching lines are: 0→1 (green) and 1→2 (violet), AMFE trajectory is shown as red dashed.

NaChBac: LESWAS, LEDWAS and LEEWAS

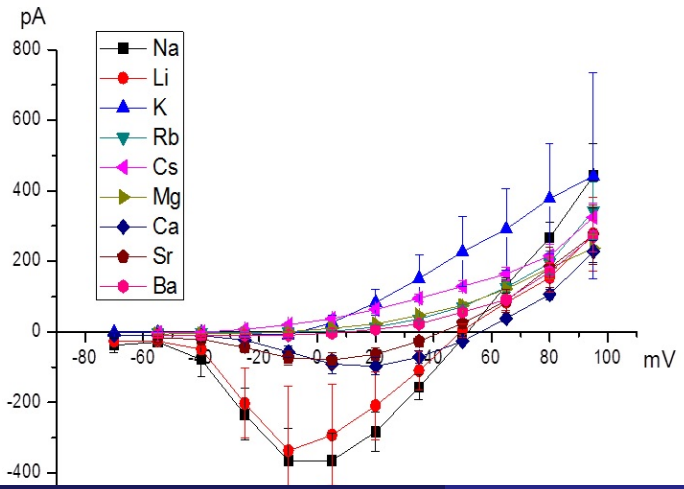
- LESWAS - Selectivity



- LEDWAS - AMFE



- LEEWAS - selectivity



- Selectivity and AMFE patch clamp tracks for NaChBac channels, wild type (LESWAS SF formula) and mutants (LEDWAS and LEEWAS) are reproduced with kind permission of Olena Fedorenko and Stephen Roberts (Division of Biomedical and Life Sciences, Lancaster University, UK)

Summary/conclusions

1. Ionic Coulomb blockade appear in low-capacitance ion channels due to charge/occupancy discreteness.
2. Coulomb staircase is fundamental electrostatic phenomenon related to charge discreteness.
3. Resonant conductivity is derived from Coulomb staircase via fluctuation-dissipation theorem.
4. Hydration energy and concentration-related entropy add appropriate shifts in positions of conduction resonance leading alike and valence selectivity and AMFE.
5. Coulomb blockade model of permeation and selectivity of calcium/sodium ion channels provides general and transparent explanation of divalent blockade and AMFE.
6. Coulomb blockade model is consistent with new mutation studies of NaChBac channels.

Acknowledgement and selected publications

Acknowledgement

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Recent publications

1. I Kh Kaufman, P V E McClintock, R S Eisenberg, ”Coulomb blockade model of permeation and selectivity in biological ion channels”, *New Journal of Physics* **17**, 083021 (2015).
2. I Kh Kaufman, D G Luchinsky, W Gibby, P V E McClintock, & R S Eisenberg, "Coulomb blockade oscillations in biological ion channels", in, *23nd Intern. Conf. on Noise and Fluctuations (ICNF),* Xian, 2-5 June 2015 (IEEE Conf. Proc., 2015), DOI:10.1109/ICNF.2015.7288558.
3. I Kaufman, D G Luchinsky, R Tindjong, P V E McClintock, and R S Eisenberg, “Energetics of discrete selectivity bands and mutation-induced transitions in the calcium-sodium ion channels family”, *Phys. Rev. E* **88**, 052712 (2013).
4. I Kaufman, D G Luchinsky, R Tindjong, P V E McClintock, and R S Eisenberg, “Multi-ion conduction bands in a simple model of calcium ion channels”, *Phys. Biol.* **10**, 026007 (2013).