Electrostatic Basis of Selectivity in Biological Ion Channels

W.A.T. Gibby[1] I.Kh. Kaufman[1] D.G. Luchinsky[1,2]
P.V.E. McClintock[1] R.S. Eisenberg[3]

[1]Department of Physics, Lancaster University, UK

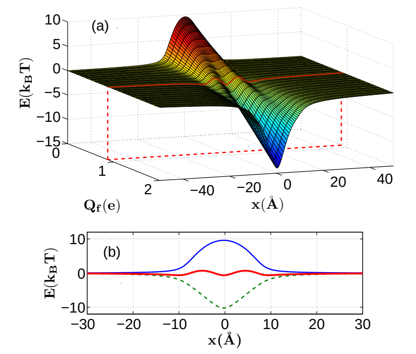
[2]SGT Inc., Ames Research Center, Moffett Field, CA, USA

[3]Molecular Biophysics, Rush University, Chicago, USA

Lancaster – 12 April 2016

Outline

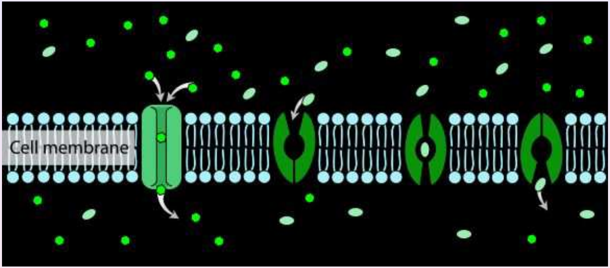
1. Introduction
   - Ion channels
   - Puzzles
2. Model channel
   - Electrostatics
   - Energy
3. Conclusion
   - Summary



Archetypal SR characteristic

How are ions transported *selectively* through Ca^{2+} and Na^{+} channels?

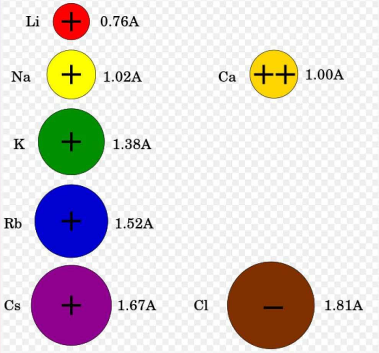
Ion channels



- Cell membrane with ion channels (+ pumps etc.).
- Natural nanotubes through the membrane.
- Allow ion exchange between inside and outside of cell.
- Essential to cell physiology, from bacteria to humans.
- Simple, passive device – but many longstanding puzzles.

Puzzles include...

1. Selectivity for cations?
   - E.g. Calcium channel favours Ca^{2+} over Na^{+} by up to 1000:1, even though they are the same size – example of *valence selectivity*.
   - Also *alike charge selectivity*, e.g. potassium channel strongly disfavours sodium, even though Na^{+} is smaller K^{+}.
2. Fast permeation? Almost at the rate of free diffusion (open hole).
3. AMFE? Na^{+} goes easily through a calcium channel but is blocked by tiny traces of Ca^{2+}.

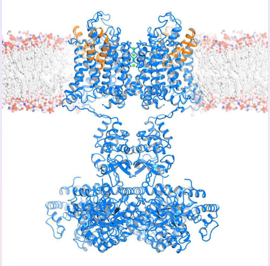


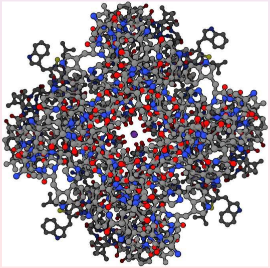
Puzzles

4. Function of the fixed charge at the SF
   - Ion channels have narrow *"selectivity filters"* with fixed negative charge... somehow associated with selectivity.
   - What does the charge do, and how does it determine selectivity?
5. Mutations
   - Mutations that alter the fixed charge (alone) can –
     (a) Destroy the channel (so it no longer conducts), or
     (b) Change the channel selectivity, e.g. Ca^{2+} to Na^{+} or *vice versa*.

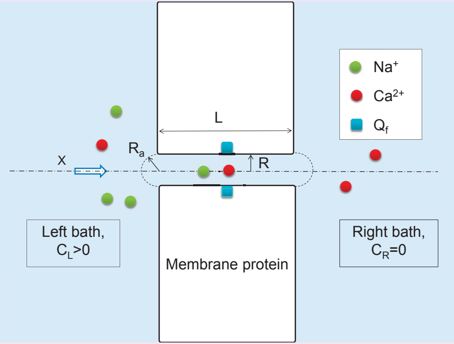
Atomic structure of KcsA Potassium ion channel

- Structure of channel is very complicated.
- Knowledge of the structure did not immediately explain its function – the famous “structure-function problem”.
- To apply physics, need a simplified model.



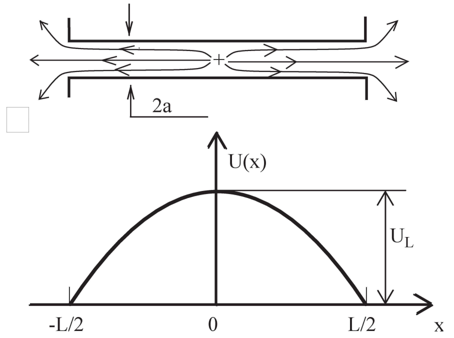


Minimal model of calcium/sodium ion channel



- A water-filled, cylindrical hole, radius $R = 3\,\text{Å}$ and length $L = 16\,\text{Å}$ through the protein hub in the cellular membrane.
- Water and protein described as continuous media with dielectric constants $\varepsilon_w = 80$ (water) and $\varepsilon_p = 2$ (protein)
- The selectivity filter (charged residues) represented by a rigid ring of negative charge $Q_f = 0 - 6.5e$.

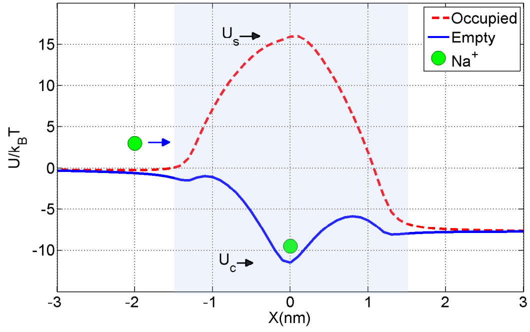
Electrostatic potentials – no fixed charge



- Electric field almost 1-D on account of dielectric mismatch.
- So if fixed charge Q_f is zero, there is a huge potential barrier impeding permeation.
- So finite fixed Q_f is *essential* for conduction.

Electrostatic exclusion principle

- In absence of fixed charge Q_f, self-energy barrier U_s prevents entry of ion to SF.
- But Q_f compensates U_s and allows cation to enter.
- This effectively restores the impermeable U_s for 2nd ion at channel mouth.
- So for this Q_f only one ion can occupy the SF.



Implications

1. The SF's forbidden multi-occupancy is an electrostatic exclusion principle.
2. Like the Pauli exclusion principle in quantum mechanics, it implies a Fermi-Dirac occupancy distribution.
3. For larger Q_f similar arguments apply for occupancies of 2,3...
4. Coulomb blockade!

Energy considerations

Consider "eigenstates" $\{n\}$ for n ions inside SF.

$\{n\} \to \{n+1\}$ is entry of new ion, and
$\{n+1\} \to \{n\}$ is escape of trapped ion.

Total energy U_n for channel in state $\{n\}$ is

$$U_n = U_{n,s} + U_{n,a} + U_{n,int}$$

where $U_{n,s}$ is self-energy, $U_{n,a}$ is energy of attraction, and $U_{n,int}$ is ions' mutual interaction energy.

Approximate U_n as dielectric self-energy $U_{n,s}$ of the excess charge Q_n, apply Gauss's Theorem, and use self-capacitance C_s of channel

$$U_n = \frac{Q_n^2 L}{8\pi\varepsilon_0\varepsilon_w R^2} = \frac{Q_n^2}{2C_s} \quad \text{(Electrostatic energy)}$$

$$Q_n = zen + Q_f \quad \text{(Excess charge).}$$

Identical to electronic Coulomb blockade relations, except for z.

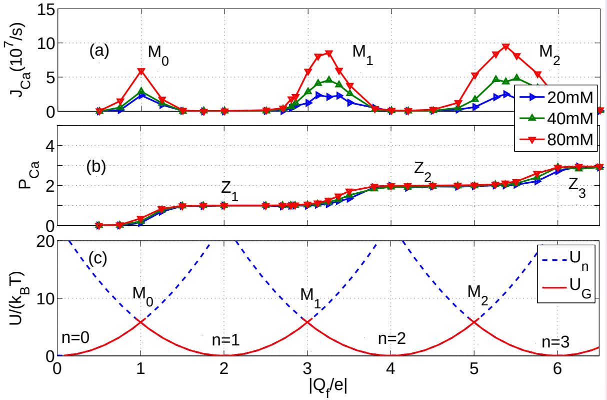
Ground state energy

- Theory can be developed in close analogy to conventional Coulomb blockade, e.g. in a quantum dot.
- Calculate U_n as a function of Q_f for $n = 0, 1, 2, 3$ and seek the minimum, i.e. the ground state energy

$$U_G(Q_f) = \min_n(U_n(Q_f, n))$$

- To test the theory use Brownian dynamics simulations.
- Theory and simulations both reveal conduction bands and stop bands as functions of Q_f.

Brownian dynamics simulations *v.* CB theory



BROWNIAN DYNAMICS

← Current

← Channel occupancy

COULOMB BLOCKADE THEORY

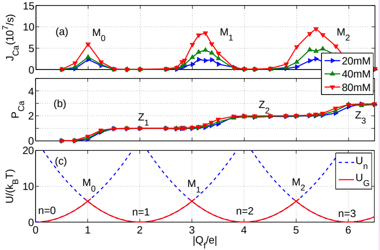
← Ground state energy

$$Z_n = zen \pm \delta Z_n,$$ Coulomb blockade

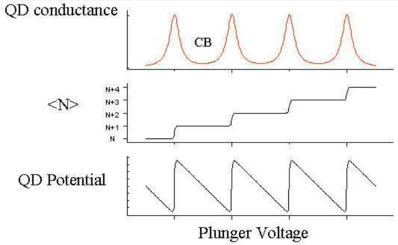
$$M_n = ze(n + 1/2) \pm \delta M_n$$ Resonant conduction

Coulomb blockade: channels *vs.* quantum dots

Ca^{2+} channel



Quantum dot



Ion(s) trapped at SF	⇔	Electron(s) trapped in quantum dot
Periodic conduction bands	⇔	Coulomb blockade oscillations
Steps in occupation number	⇔	Coulomb staircase
Classical mechanics for ion	⇔	Quantum mechanics for electron
Stochastic permeation by ion	⇔	Quantum tunnelling by electron

Comparison with experiment

The conduction bands observed in the Brownian dynamics simulations can be related to real ion channels –

Band	$\approx$Fixed Charge	Channel	Locus	Nominal charge
L0	$0.5e$	Sodium Nav	DEKA	$1e$
M0	$1.0e$	Non-selective OmpF	(K)RRRDE	$1e$
Z1	$2e$	Na-selective mutant	EAEA	$2e$
M1	$3e$	Ca-selective mutant; Ca L-type	EEEA; EEEE	$3e; 4e$
Z2	$4e$	Sodium NaChBac, NavAB	EEEE	$4e$
M2	$5e$	Calcium RyR	DDDD(ED)	$6e$

Results mostly agree with published data, and make sense of observed mutation phenomena.

Amino acids aspartate (D) and glutamate (E) have negatively-charged side chains; lysine (K) and argenine (R) have positively-charged side chains; others are neutral.

What puzzles have we explained?

Well, arguably –

1. Selectivity? (valence selectivity) ✓
2. Fast permeation? ✓
3. AMFE? ✓
4. Role of fixed charge at the selectivity filter? ✓
5. Effect of mutations in the selectivity filter? ✓

But more work is needed to develop a fuller picture...

Where next?

Seeking to determine the fundamental origins of selectivity and conductivity in ion channels –

- Biological experiments on mutants, testing predictions e.g. of effect of changing Q_f (Steven Roberts and Olena Fedorenko, Lancaster).
- Brownian dynamics simulations (Igor Kaufman, Lancaster).
- Molecular dynamics simulations (Igor Khovanov & Carlo Guardiani, Warwick).
- Analytic theory (Dmitri Luchinsky & Will Gibby, Lancaster) especially for situation where hydration energy dominates, e.g. K^+/Na^+ selectivity.

Most results equally applicable to charged artificial nanopores.

Acknowledgement and selected publications

Acknowledgement

We are grateful to the Engineering and Physical Sciences Research Council (UK) for their support of this research.

Recent publications

1. 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).
2. I Kh Kaufman, P V E McClintock and R S Eisenberg, "Coulomb blockade model of permeation and selectivity in biological ion channels", *New J. Phys.* **17**, 083021 (2015).
3. I Kh Kaufman, D G Luchinsky, W A T Gibby, P V E McClintock and R S Eisenberg, "Putative resolution of the EEEE selectivity paradox in L-type Ca^{2+} and bacterial Na^{+} biological ion channels", *J. Stat. Mech.*, in press (2016).