# Coulomb Blockade of Stochastic Permeation in Biological Ion Channels

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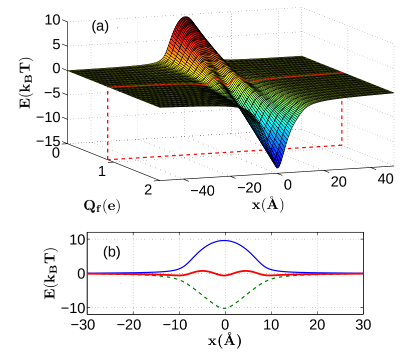
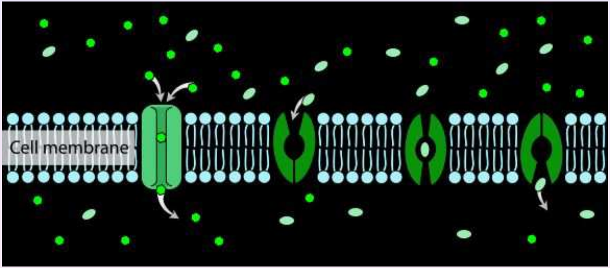
# Outline

1. The unsolved problem
   - Background
   - Electrostatic model
   - Effect of the fixed charge
2. Solving the problem?
   - Modelling – Brownian dynamics
   - Ionic Coulomb blockade
   - Mechanisms of permeation
3. Conclusions
   - Prospects
   - Summary



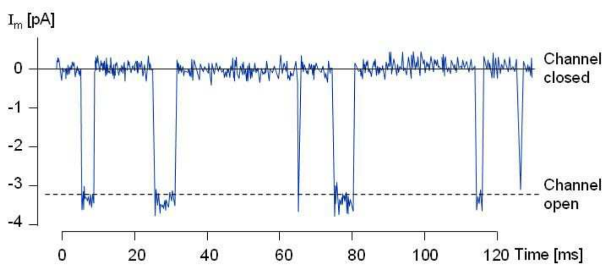

How are ions transported *selectively* through $Ca^{2+}$ and $Na^{+}$ channels?

# Ion channels




- Cellular membrane has numerous ion channels (+ pumps and transporters).
- Ion channel is a natural nanotube through the membrane.
- Allows ion exchange between inside and outside of cell.
- Essential to physiology – bacteria to humans.
- Highly selective for particular ions.

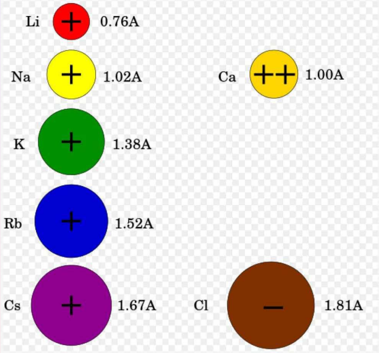
# Gating




- Channels spontaneously "gating" open/shut.
- A stochastic process influenced by e.g. –
  - Voltage
  - Chemicals
- We are interested only in open channels.

# Puzzles

1. Selectivity?
   - 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 $Ca^{2+}$.

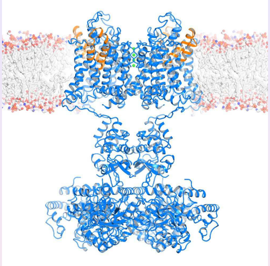


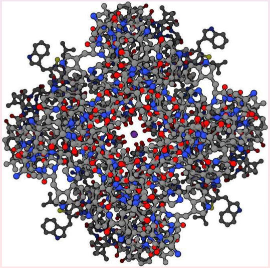
# Puzzles

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*.
6. Gating
   - Why/how does the channel continually open and close?

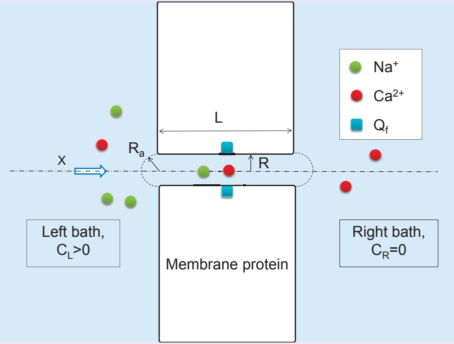
# Atomic structure of KcsA Potassium ion channel

- So-called “crystal structure” of a bacterial ion channel.
- Very complicated.
- Knowledge of the structure does not immediately explain the function – the famous “structure-function problem”.
- Which features are important?
  - Selectivity filter?
  - Generic features of structure?
- Need to pick out aspects important for modelling the permeation process.





# Minimal model of calcium/sodium ion channel




- A water-filled, cylindrical hole, radius $R = 3$ Å and length $L = 16$ 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 $Q_f = 0 - 6.5e$.

# Calcium and sodium channels

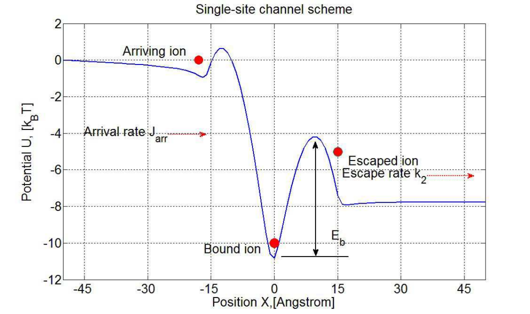
We focus on voltage-gated calcium and sodium ion channels – which control muscle contraction, neurotransmitter secretion, transmission of action potentials) – because:

- They are very similar in structure, but have differing SF loci and hence different $Q_f$.
- The many mutation experiments to change $Q_f$ (destroying the channel or changing its selectivity) are potentially revealing but not yet properly understood.

But results and conclusions are more generally applicable.

# Permeation through an ion channel

- Typically, the fixed charge $Q_f$ provides a strong binding site at the selectivity filter.
- Dimensions & electrostatics enforce single-file motion.




- Arriving ions captured at binding site, then fluctuational escape occurs over the potential barrier $E_b$.

# Electrostatics

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

$$-\nabla(\varepsilon\varepsilon_0\nabla u) = \sum ez_in_i$$

where $\varepsilon_0$ is the dielectric permittivity of vacuum, $\varepsilon$ is the dielectric permittivity of the medium (water or protein), $u$ is the electric potential, $e$ is the elementary charge, $z_i$ is the valence, and $n_i$ is the number density of ions.

- This equation accounts for both ion-ion interaction and self-action for all ions in their current positions.
- One result of the calculations is the axial potential energy profile = the Potential of the Mean Force (PMF).

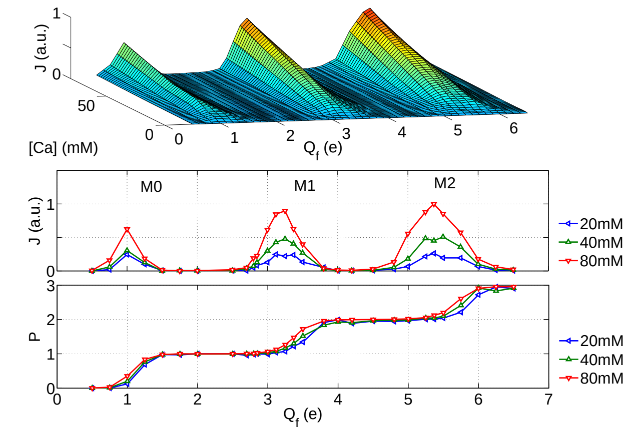
# Brownian dynamics

- 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.

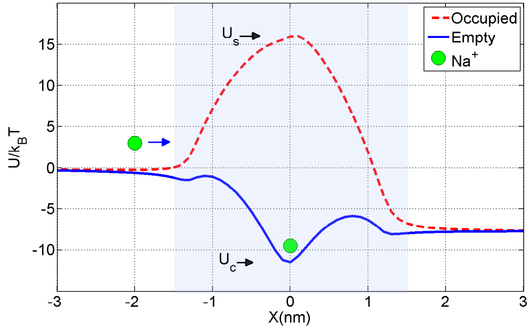
For different $Ca^{2+}$ concentrations $[Ca]$.

We find –

- Pattern of narrow conduction bands and stop bands.
- Conduction bands occur at transitions in channel occupancy $P$.

# 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.

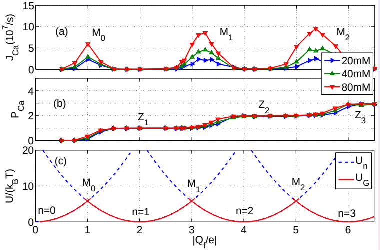



Implications

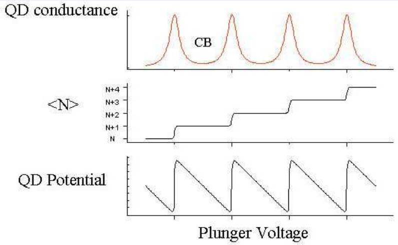
1. The SF's forbidden multi-occupancy is an electrostatic exclusion principle.
2. Like the Pauli exclusion principle in quantum mechanics, it implies a Fermi-Dirac occupancy distribution.
3. For larger $Q_f$ similar arguments apply for occupancies of 2,3...

# Coulomb blockade: channels *vs.* quantum dots

## $Ca^{2+}$ channel

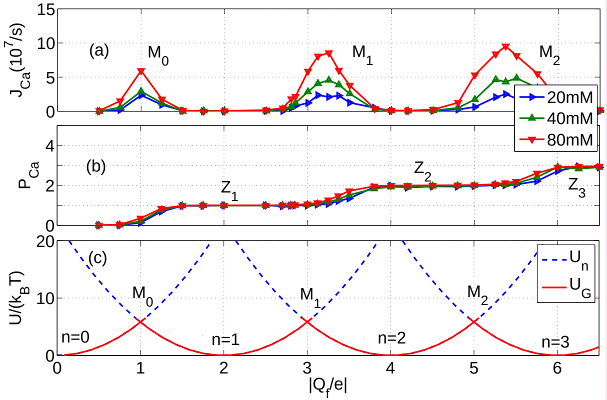



## Quantum dot




| | | |
|---|---|---|
| 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 |

# Singular points in ionic Coulomb blockade



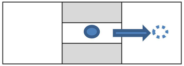
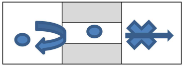
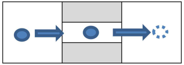
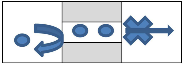
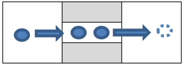

$$Z_n = zen \pm \delta Z_n,$$ Coulomb blockade

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

# Coulomb blockade: ion channels *vs.* quantum dots

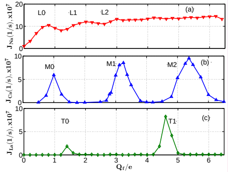
| Feature | Ionic CB (channels) | Electronic CB (q. dots) |
|---|---|---|
| System type | Classical | Quantum |
| Charge carriers | Ions – $Ca^{2+}$, $Na^{+}$... | Electrons |
| Field equation | Langevin | Schrödinger |
| Exclusion principle | Electrostatic | Electrostatic |
| Occupancy statistics | Fermi-Dirac (in channel) | Fermi-Dirac |
| Carrier valence | $z = 1, 2, \ldots$ | $z = 1$ |
| Control parameter | Fixed charge $Q_f$ | Potential $U = Q_f^2/2C$ |
| Occupancy structure | Coulomb staircase | Coulomb staircase |
| Conduction peaks at | $Q_f = ze(n + \frac{1}{2})$ | $Q_f = e(n + \frac{1}{2})$ |
| Peak shape | Landauer approximation | Landauer approximation |

# 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 |

# Where next?

- Develop model to include hydration effects: expect significant corrections for valence selectivity; and the basis of *alike selectivity* e.g. $Na^+/K^+$.
- Mutation experiments (biologist collaborator Stephen Roberts) to "tune" $Q_f$.
  - Will $J$ and $S$ behave as expected?
  - How about trivalent ions?




- Molecular dynamics simulations (collaborator Igor Khovanov in Warwick) – underpinning for our higher-scale Brownian dynamics simulations.

# What puzzles have we explained?

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? ✓
6. Shed light on gating? No, not really, but...

# Acknowledgement and selected publications

Acknowledgement

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Recent publications

1. 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).
2. R S Eisenberg, I Kaufman, D Luchinsky, Tindjong, and P V E McClintock, “Discrete conductance levels in calcium channel models: multiband calcium selective conduction”, *Biophys. J.* **104** 358a (2013).
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, “Coulomb blockade model of permeation and selectivity in biological ion channels”, *New J. Phys.* (in press).