Insights into the molecular workings of ion channels

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NanoBiophotonics course - UIUC
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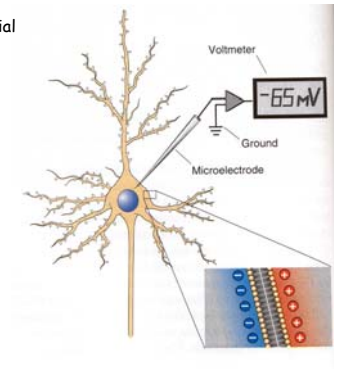
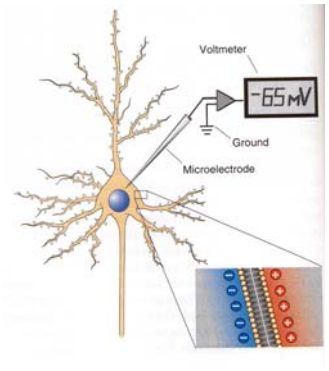
- Nerve and muscle cells communicate via propagation of electrical impulses at velocities of 1-100 m/sec
- The electrical messages are in the form of short impulses: **action potentials**.
- The information is largely encoded by the **frequency** of these impulses.

Nerve cell – resting potential

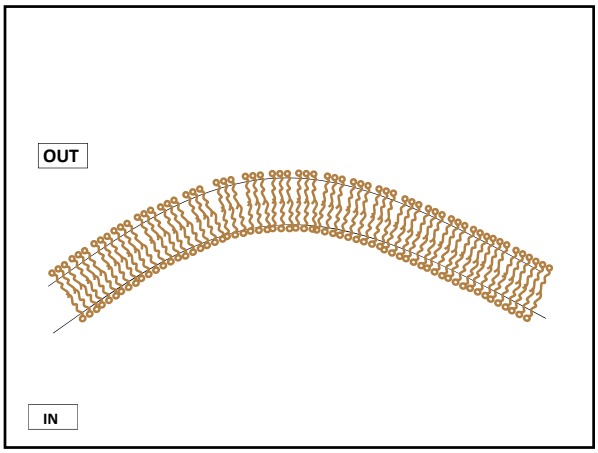


Action potential

= transient change in the membrane potential due to a change in the permeability to ions via changes in the state of ion channel proteins – OPEN or CLOSED to ion fluxes

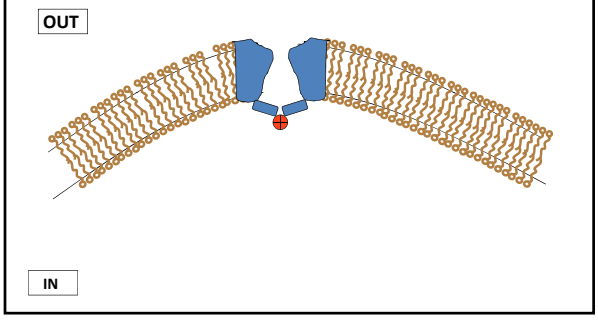


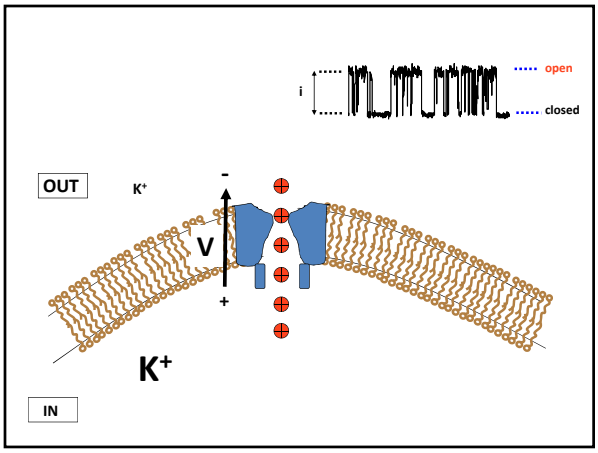
ION CHANNELS



Ion channels are are not just passive holes:

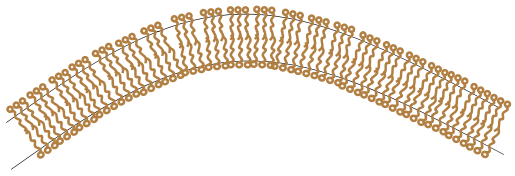
- Their pores select for the desired ion
- They have gates that open and close
- They have domains that modulate these gates





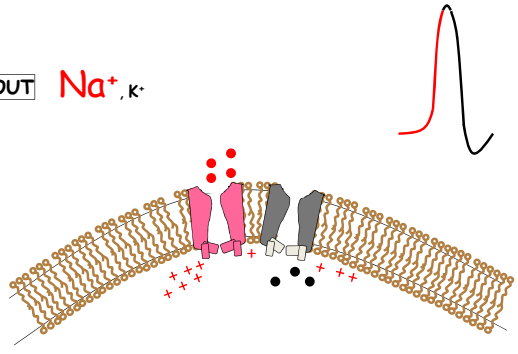
Action Potential Generation

OUT Na^+, K^+



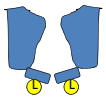
IN K^+, Na^+

OUT Na^+, K^+



IN K^+, Na^+

Research in the lab is focused on how different domains of ion channels function both separately and together to allow these channels to fulfill their physiological roles.



On what protein domain does the ligand bind and what is the chemistry of binding?

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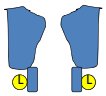

On what protein domain does the ligand bind and what is the chemistry of binding?

How does binding of the ligand open the channel?

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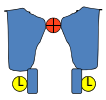
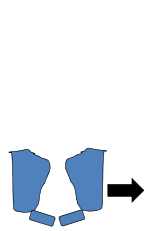


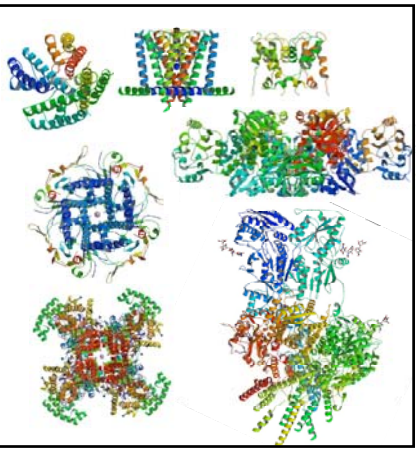
On what protein domain does the ligand bind and what is the chemistry of binding?

How does binding of the ligand open the channel?

How does the pore select for the appropriate ion?



25 unique ion channel structures (till 2/1/2010)



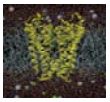
Techniques used to address mechanistic questions about ion channels:

1) Functional studies of channels using either patch-clamp electrophysiology or lipid bilayers.

2) Biochemical and biophysical studies of ion channel properties using radioactive flux assays, ITC, etc.

3) X-ray crystallography of ion channels

4) Electron microscopy of ion channels



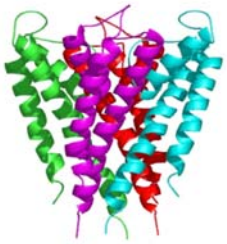
5) Molecular dynamics simulation studies

K^+ Channels

- K^+ channels are **tetramers**
- K^+ channels have similar pore architectures
- K^+ channels are ubiquitous and are a big family (K_v, K_{ir}, K_{ATP}, BK, SK, HCN, KcsA, KvAP etc)
- K^+ channels are remarkably **selective for potassium** ions and the protein region responsible for selectivity is completely conserved among family members

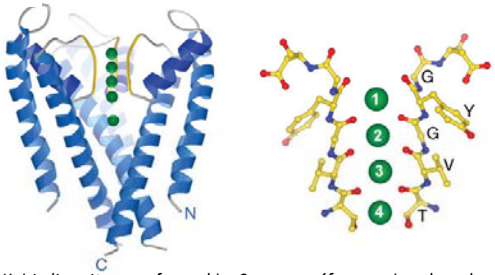
KcsA

- A prokaryotic K^+ channel with a high-resolution crystal structure
- It allows K^+ conduction close to diffusion-limited rates
- Preserves most K+ channel permeation characteristics including **exquisite selectivity against Na^+**



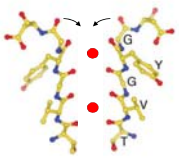
Doyle et.al., Science 1998

Selectivity filter



K^+ binding sites are formed by 8 oxygens (from carbonyls and hydroxyls) that mimic the waters of hydration around a K^+ in solution

Selectivity against Na^+

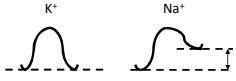


- K dehydration costs are compensated in the filter due to perfect carbonyl positioning
- Na is smaller and carbonyls are too far away to coordinate its binding

Current hypothesis: Na binding in the selectivity filter is an energetically unfavorable process

= K^+ (ionic radius 1.33 A)

= Na^+ (ionic radius 0.95 A)

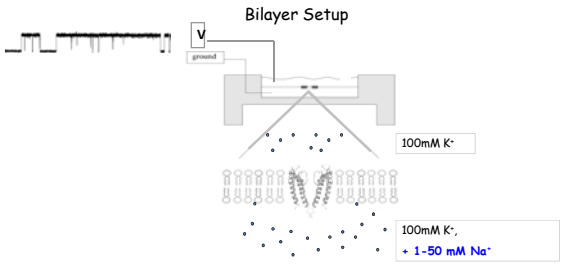


How do KcsA channels select against intracellular Na^+?

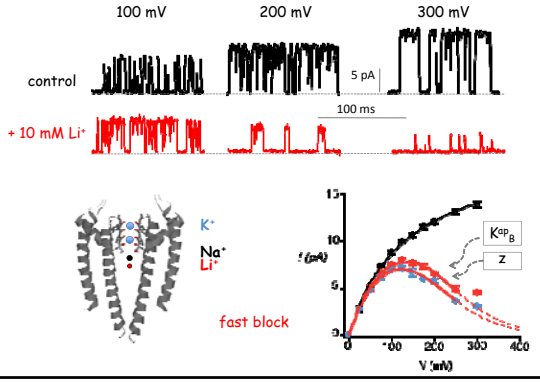
Investigative tools

- Single-channel lipid bilayer recording of purified KcsA channel in lipid vesicles
- Molecular dynamics simulations
- X-ray crystallography of KcsA in Li

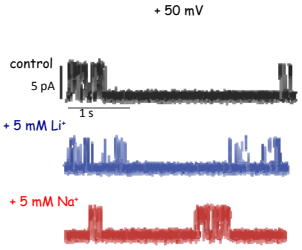
Single-Channel Lipid Bilayer Recording of KcsA



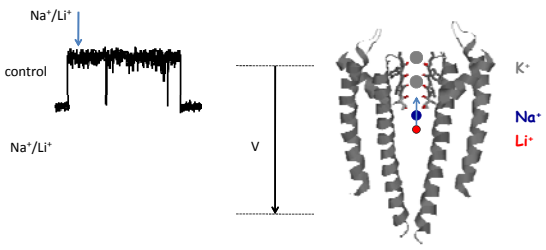
Single-Channel Lipid Bilayer Recording of KcsA



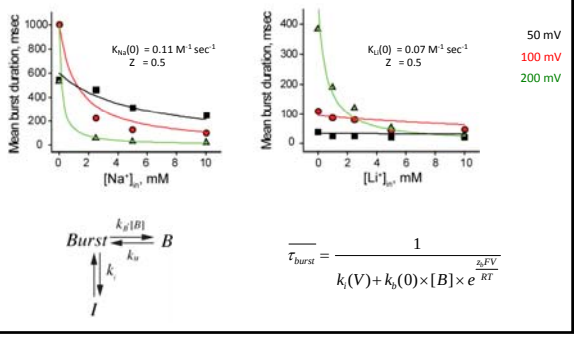
Intracellular Na^+ and Li^+ lead to a dramatic voltage-dependent decrease in open probability



Effects of Na^+ and Li^+ at high voltages are consistent with a slow blocking mechanism occurring on a much longer time scale



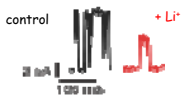
Effects of Na^+ and Li^+ on the burst durations are concentration and voltage dependent



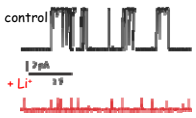
$$\overline{\tau_{burst}} = \frac{1}{k_f(V) + k_b(0) \times [B] \times e^{\frac{z_b FV}{RT}}}$$

Na^+ and Li^+ have two distinct effects on KcsA gating indicating that each ion has at least two binding locations in the KcsA pore

- ***Fast block***, indicative of low affinity fast kinetics binding - cavity
  - Blocking events are so fast that they cannot be captured and the result is an apparent decrease in unitary current



- ***Slow block***, indicative of longer residence binding - filter
  - Blocking events are slow and they lead to discrete, measurable interruptions of the current that lead to a decrease in open probability

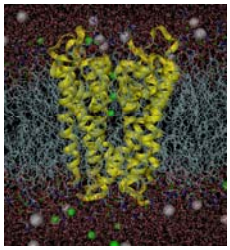


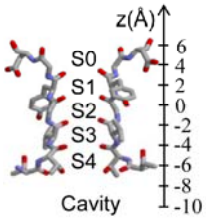
We decided to investigate where in the pore Na^+ and Li^+ bind

- We needed a different technique:
  - X-ray crystallography of KcsA in Li^+
  - Molecular dynamics to find the free energies for Na^+ and Li^+ in the cavity

Molecular dynamics

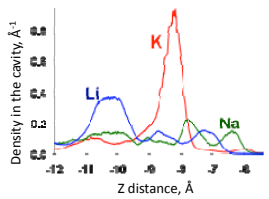
Molecular dynamics simulations of free energy profiles for K^+, Na^+ and Li^+ in the KcsA pore

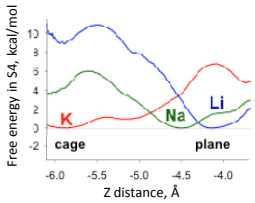


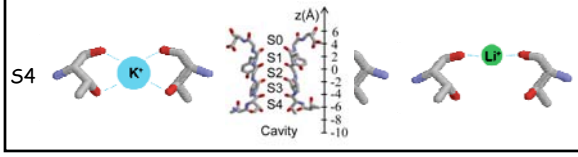


UC Davis
Ilsoo Kim
Toby Allen

Free energy simulations uncover Na^+ and Li^+ ion locations in the cavity and selectivity filter





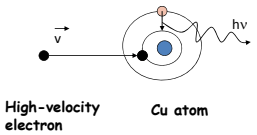


X-ray crystallography

Why do we need X-rays to determine protein structures at atomic resolution?

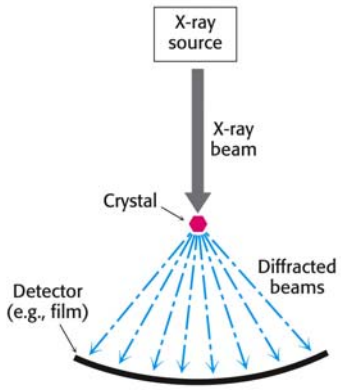
- In order to resolve two objects (capable of scattering light or some other form of radiation), the two objects **must** be separated by a distance **greater** than $\lambda/2$ where λ is the wavelength of the light scattered.
- Peptide bond is 1.3 A, so in order to resolve two consecutive aminoacids we need radiation on the same order of magnitude (X-rays wavelength ~ 1 A depending on the source).
- In contrast, the dimension of a cell is ~ 10 μm which allows it to be seen with light microscopy methods

What are X-rays?



- X-Rays are photons (electromagnetic radiation) with wavelengths in the range of 0.1-100 A. X Rays used in diffraction experiments have typical wavelengths of 0.5 - 1.8 Å.
- X-Rays are generated by accelerating electrons to collide with metal targets. The electrons from the inner shells of the metal are knocked out and as a result, electrons from outer shells will occupy the vacancy emitting a photon in the process. The energy of the emitted photon depends on the metal source used (usually Cu, Mo and W) and is ~ 1 Å.

Crystallographic setup



X-ray crystallography

- X-ray beams directed at crystals allow constructive interference (waves add up in amplitude), destructive interference (waves cancel each other) to produce a diffraction pattern. This diffraction pattern is related to the crystal that produced it by a mathematical operation called Fourier transform.

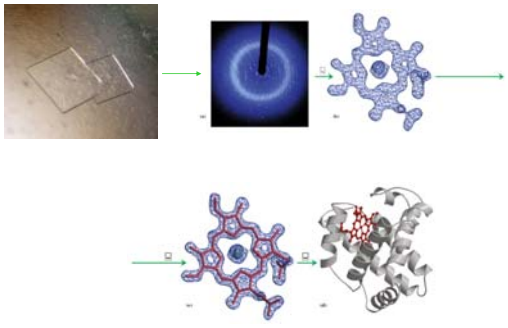
Structure factor $$F(S) = \int dr \rho(r) e^{2\pi i S \cdot r}$$

Electron density $$\rho(r) = (1/V) \int dS e^{-2\pi i S \cdot r} F(S)$$

$$= (1/V) \int dS e^{-2\pi i S \cdot r} |F| e^{i\Phi}$$

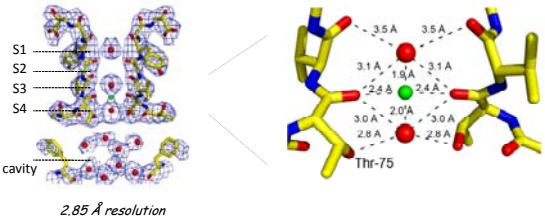
PHASE

Results of a crystallography experiment



X-ray crystallography of purified KcsA in Li^+ only

KcsA-Li^+

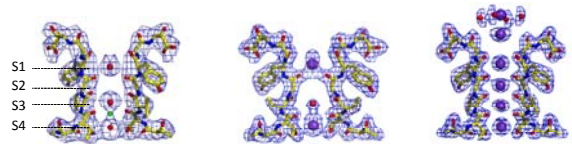


2.85 Å resolution

Vanderbilt University
Tim Panosian
Tina Iverson

X-ray crystallography of purified KcsA in Li^+ only

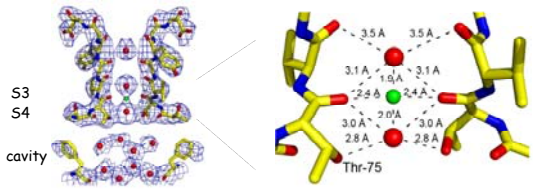
KcsA-Li^+ KcsA-LoK^+ KcsA-HiK^+

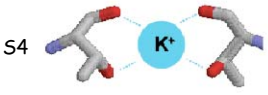


2.85 Å resolution

Zhou et al., 2001,
2.3 Å and 2 Å resolution

Li^+ binds "in-plane" in the selectivity filter





in cage in plane

Binding sites for Na^+ and Li^+ in the S4 region, with occupancy not observed under physiological conditions

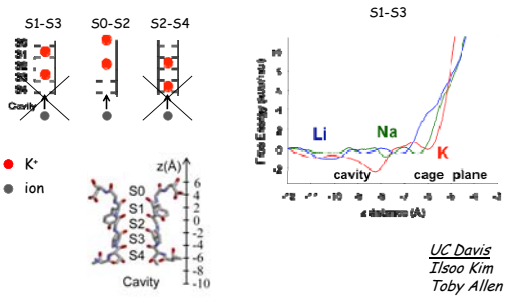
→ an energetically favorable process that occurs infrequently due to large energy barriers

Q1: Are there large barriers?

Q2: What causes these barriers?

Molecular dynamics

Simulations reveal energetic barriers for Na^+ and Li^+ to enter their high affinity sites in the selectivity filter



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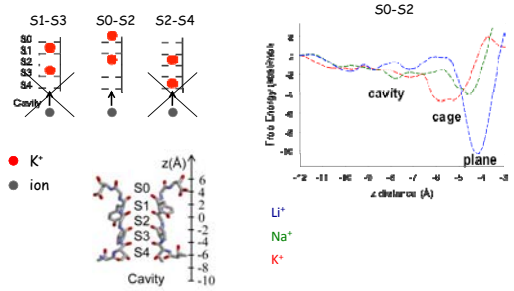
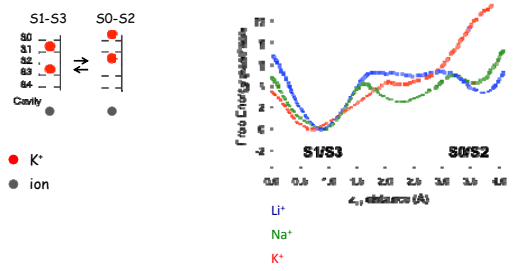


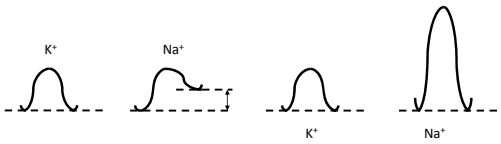
What is the size of the energy barrier required to move K ions from S1-S3 to S0-S2 with an ion in cavity?



Both X-ray crystallography and molecular dynamics indicate:

1. Na^+ and Li^+ low affinity binding sites in the KcsA cavity, consistent with the fast block seen in functional studies;
2. Additional Na^+ and Li^+ binding sites in the S4 site of the selectivity filter, consistent with the slow block phenotype seen at high voltages.

Cartoon conclusion



Current hypothesis for selectivity: Na binding in the selectivity filter is energetically unfavorable

Our hypothesis for selection against Na from inside: Na binding in the selectivity filter is thermodynamically favorable but infrequent due to a large energy barrier

Conclusions

- We propose that intracellular Na^+ and Li^+ are excluded from the KcsA pore by a mechanism of selective exclusion and not selective binding
- Selection is not due to unfavorable binding of Na^+ and Li^+ in the selectivity filter (S4) – we think we found binding sites
- The Na^+ and Li^+ sites are "in-plane", between S3 and S4, while K binds "in-cage" in S4
- Selection occurs because when a Na^+ or Li^+ ion is in the cavity, there is a large energy barrier to move K^+ ions up

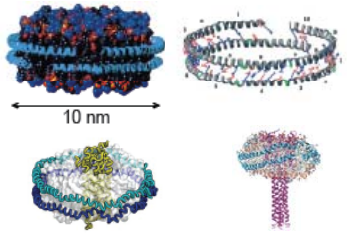
Thompson et al., NSMB, 2009

Can we measure **directly** the binding of ions (K^+, Na^+, Li^+) to the channel?

A few problems with this:

1. The channel has to be completely devoid of ions and protein is believed to fall apart under these conditions;
2. The binding affinities are believed to be low (micromolar) and only detectable using equilibrium measurements for which large protein concentrations are needed;
3. Measurements of binding affinity should be made in a lipid environment and not detergent, and lipid vesicles cannot be concentrated sufficiently.

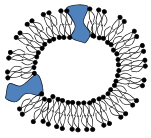
All these problems can be addressed by by taking the ion channel out of detergent and incorporating it in a specialized structure: the nanodisc or NABB (nanoscale apoliporotein bound bilayer)

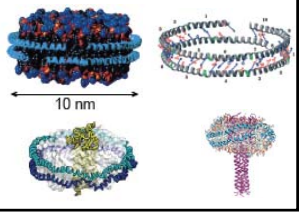


Nath A, Atkins WM *et al.* (2007)
Biochemistry 46:2059-69

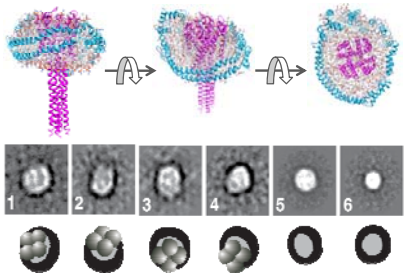
Binding assays in lipid environment

- **Lipid vesicles**
  - Cannot achieve high concentrations
  - Access to one side of membrane only
  - Cannot control orientation

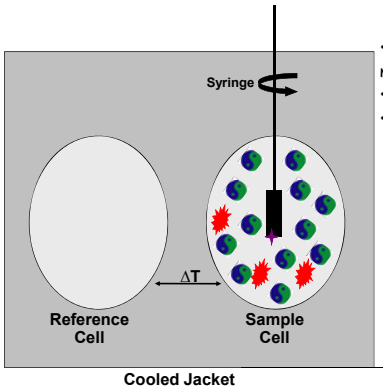




KcsA incorporates successfully into NABBs

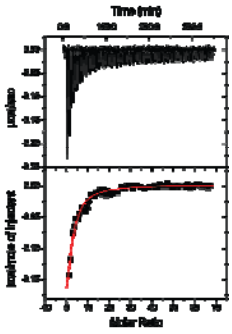


Binding with Isothermal Titration Calorimetry (ITC)



- Heat is related to n, ΔH, K_A
- Calculate $K_D=1/K_A$
- $\Delta G=-RT \ln K_A=\Delta H-T\Delta S$

KcsA binds K^+ ions with a K_D of ~ 160 μM



Thermodynamic parameters for K^+ binding to KcsA in NABBs using ITC

N	1.0
K_D (μM)	158 ± 13
ΔH (cal/mol)	-943.9 ± 28.1
ΔS (cal/mol.K^{-1})	14.2

Next: do the same measurements by titrating Na^+ and Li^+ and determining their binding parameters

Acknowledgements

- **Ameer Thompson**
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- Dina Rubin

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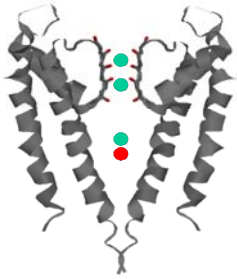
Tina Iverson

UC Davis –*MD*
Ilsoo Kim
Toby Allen

- **NIGMS**
- **AHA**
- **NSF**

Selectivity filter requires ions to dehydrate in order to permeate while the cavity accommodates hydrated ions and is thus fairly non-selective

OUT



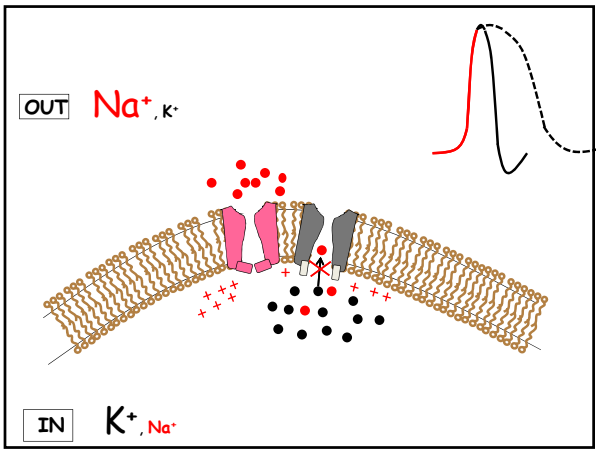
IN

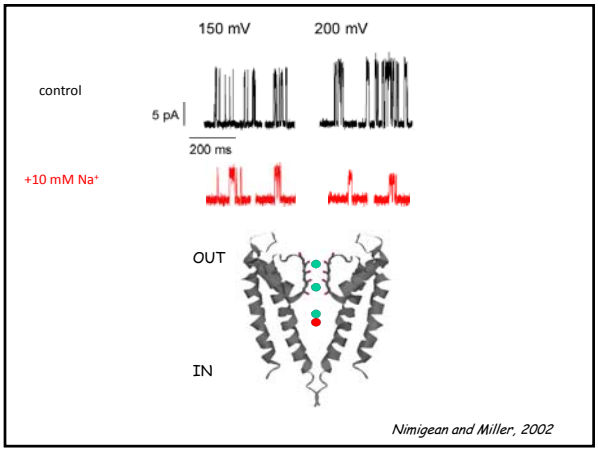
~ 150 mM K^+
~ 10 mM Na^+

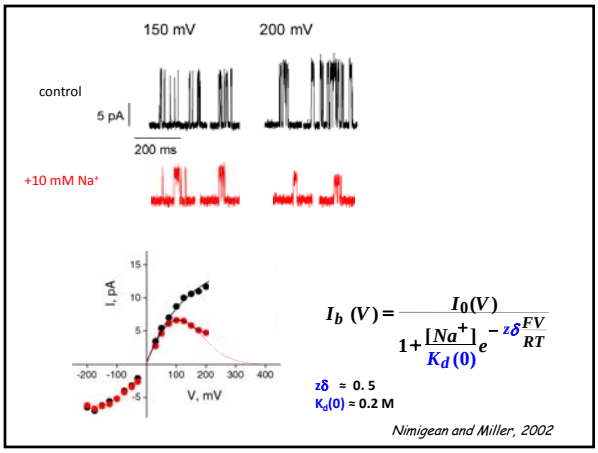
Q:

How do K^+ channels avoid being constitutively blocked in the cavity by intracellular Na^+ as they carry out their biological tasks?

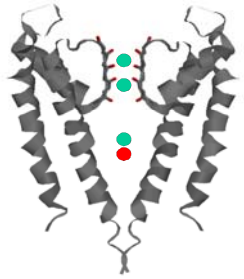
Nimigean and Miller, 2002


OUT
Na+, K+
IN
K+, Na+

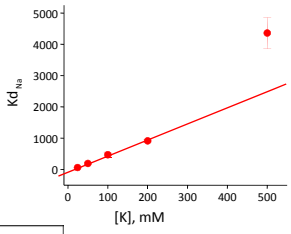

150 mV
200 mV
control
5 pA
200 ms
+10 mM Na+
OUT
IN
Nimigean and Miller, 2002

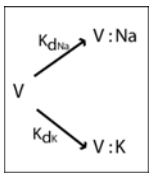

150 mV
200 mV
control
5 pA
200 ms
+10 mM Na+
I, pA
V, mV
$I_b(V) = \frac{I_0(V)}{1 + \frac{[Na^+]}{K_d(0)} e^{-z\delta \frac{FV}{RT}}}$
zδ ≈ 0.5
$K_d(0)$ ≈ 0.2 M
Nimigean and Miller, 2002

OUT



IN





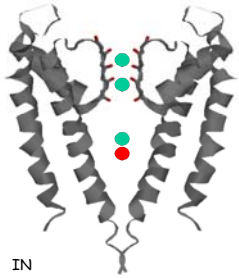
$$Kd_{Na}(K) = Kd_{Na}(0) + \frac{Kd_{Na}(0)}{Kd_K(0)}[K]$$

slope = selectivity for binding in the cavity ≈ 5

Nimigean and Miller, 2002

WHY DOES THE LARGE HYDRATED CAVITY DISPLAY PREFERENCE AMONG IONS?

OUT



IN

~ 150 mM K^+
~ 10 mM Na^+

Does the binding site in the cavity require partial dehydration?

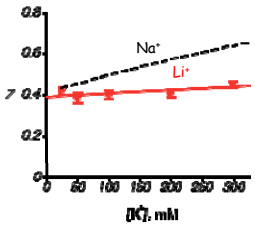
To probe this hypothesis, we used Li^+ as a blocking ion.

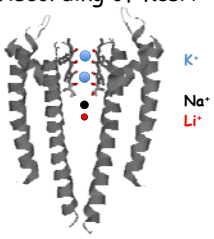
Li^+ is a monovalent cation, much smaller than Na^+ and K^+ (radius of ~0.6Å, compared to 1.33 and 0.95Å for K^+ and Na^+ respectively), and not permeable through K^+ channel pores similar to Na^+.

($\Delta G^0_{hydration}$, in kcal/mol: Li^+ = -123.1, Na^+ = -96.6, K^+ = -79.4)

If our hypothesis is correct and the selectivity comes from partial dehydration, assuming they bind at the same site, then Li^+ should be much more selected against compared with Na^+.

Single-Channel Lipid Bilayer Recording of KcsA

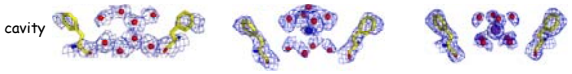




Li^+ movement to its binding site is less well coupled with permeant ions than Na^+, further suggesting they block at different sites.

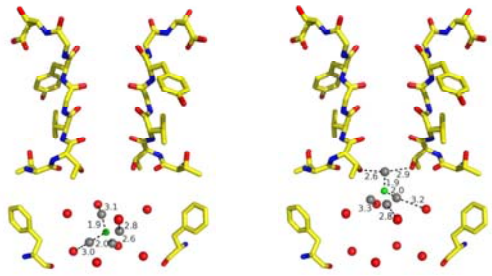
Other binding sites for Li^+

KcsA-Li^+ KcsA-LoK^+ KcsA-HiK^+

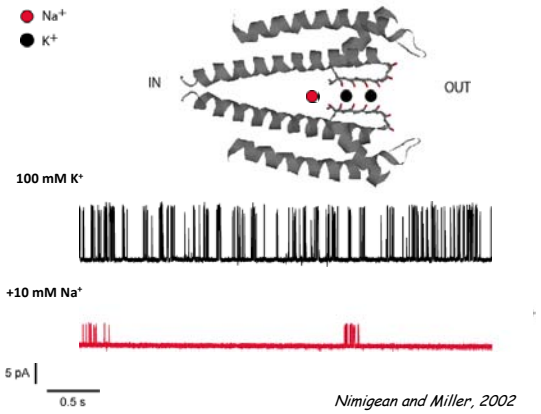


Zhou et al., 2001

Li^+ can also bind in the cavity at two positions

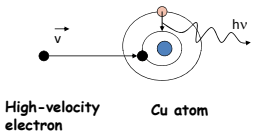


These are the sites where Li^+ can block with low affinity yielding the functional fast block seen with single-channel currents



Nimigean and Miller, 2002

What are X-rays?



High-velocity electron **Cu atom**

- X-Rays are photons (electromagnetic radiation) with wavelengths in the range of 0.1-100 A. X Rays used in diffraction experiments have typical wavelengths of 0.5 - 1.8 Å.
- X-Rays are generated by accelerating electrons to collide with metal targets. The electrons from the inner shells of the metal are knocked out and as a result, electrons from outer shells will occupy the vacancy emitting a photon in the process. The energy of the emitted photon depends on the metal source used (usually Cu, Mo and W) and is ~ 1 Å.

Why do we need crystals to determine protein structure using X-rays? Why is one molecule not enough?

- A biological molecule can absorb X-rays and will be thus destroyed by the high energy X-ray beam.
- Very few X-rays can be scattered by a macromolecule due to their strong penetrating power (high energy).
- Cannot make an X-ray microscope due to lack of materials to focus the beam (too short wavelength).
- X-ray scattering from a single molecule is weak and cannot be detected above the noise level, which include scattering from air and water. A crystal arranges huge numbers of molecules in the same orientation, so that scattered waves can add up in phase and raise the signal to a measurable level. In a sense, a crystal acts as an amplifier.

X-ray crystallography

- X-ray beams directed at crystals allow constructive interference (waves add up in amplitude), destructive interference (waves cancel each other) to produce a diffraction pattern. This diffraction pattern is related to the crystal that produced it by a mathematical operation called Fourier transform.

Structure factor $$F(S) = \int dr \rho(r) e^{2\pi i S \cdot r}$$

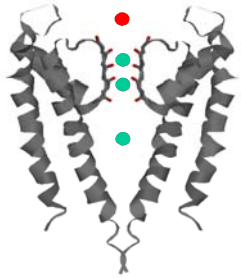
Electron density $$\rho(r) = (1/V) \int dS e^{-2\pi i S \cdot r} F(S)$$

$$= (1/V) \int dS e^{-2\pi i S \cdot r} |F| e^{i\Phi}$$

PHASE

OUT

~ 100 mM Na^+
~ 5 mM K^+

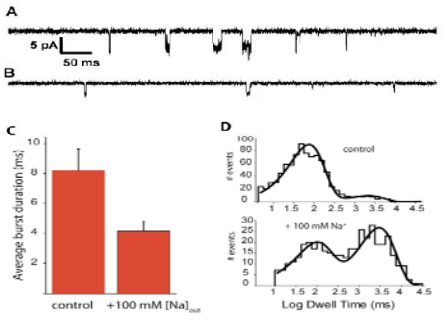


IN

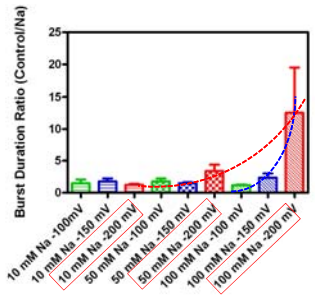
~ 150 mM K^+
~ 10 mM Na^+

Is inward current through KcsA blocked from the outside by Na^+ and Li^+? We do not expect fast block due to the absence of an extracellular cavity but if Na^+ and Li^+ can bind in the outside part of the filter we should see voltage dependent slow block.

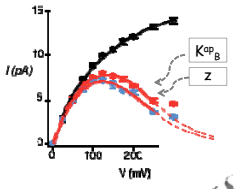
Slow block of inward K^+ currents through KcsA induced by extracellular Na^+

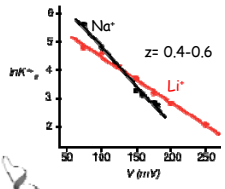


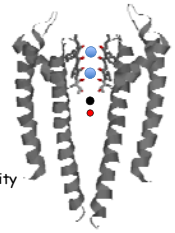
Slow block induced by Na^+ is concentration and voltage-dependent



Single-Channel Lipid Bilayer Recording of KcsA

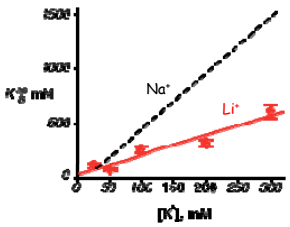






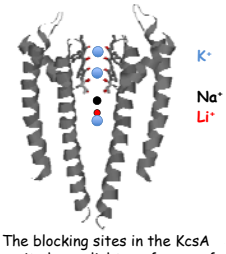
Na^+ and Li^+ block with low affinity and voltage-dependence in the KcsA pore

Single-Channel Lipid Bilayer Recording of KcsA



$$Kd_{Na}(K) = Kd_{Na}(0) + \frac{Kd_{Na}(0)}{Kd_K(0)}[K]$$

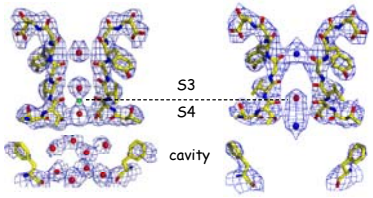
= 5 for Na^+
= 2 for Li^+



The blocking sites in the KcsA cavity have slight preference for K^+ over Li^+ and Na^+ with Li^+ being preferred.

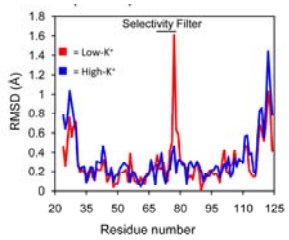
Na^+ and Li^+ may block at different sites in the cavity.

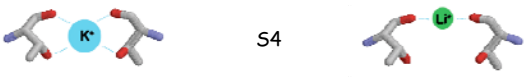
KcsA Na^+ structure has densities "in-plane" at similar positions to the ones inferred for Li^+



Lockless et al., 2007, 3.2 Å resolution

KcsA-Li^+ structure adopts a conductive filter conformation





- *K^+ binds in the S4 cage*
- *Li^+ binds in-plane, straddling S4 and S3 sites*