Potassium channels: conduction, selectivity, blockage, inactivation and gating

NCN at NW: Student Leadership Council Seminar
Northwestern University
October 25, 2006

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Ion channels: Basic concepts

- Selectivity
- Ion conduction

\[ V_{eq} = \left( k_B T / q \right) \ln \left[ C_{ext} / C_{int} \right] \]

\[ I = \Lambda (V_{mp} - V_{eq}) \]
Traditional approaches, such as Eyring Rate Theory or Nernst-Planck continuum electrodiffusion theory, picture the movements of ions across membrane channels as chaotic random displacements in a free energy profile $W(z)$ driven by the transmembrane electric field $F=qE_{mp}$. 
X-ray structure of KcsA (Doyle et al, 1998)
Ion Conduction & selectivity are governed free energy profiles

\[ \Delta \Delta G = \left\{ G(\text{Na}^+ \text{ in channel}) - G(\text{Na}^+ \text{ in bulk}) \right\} - \left\{ G(\text{K}^+ \text{ in channel}) - G(\text{K}^+ \text{ in bulk}) \right\} \]
Molecular Dynamics Simulations

"The molecular dynamics or MD approach consists in, having represented the microscopic forces between the atoms with some potential function, generating a step-by-step trajectory of the atoms by numerically integrating the classical equation of motion of Newton, \( F=MA \)."

From position and velocity at some time, we calculate the position and velocity at a short time step later

\[
R(t + \Delta t) = R(t) + V(t)\Delta t + \frac{1}{2M} F(t)\Delta t^2
\]

\[
V(t + \Delta t) = V(t) + \frac{1}{M} F(t)\Delta t
\]

In fact, the widely used Verlet algorithm is slightly more complicated than this...
Molecular Dynamics simulations of the KcsA K+ Channel

\[ F = MA \]

- 150 mM KCl
- 112 DPPC, 6500 waters
- Over 40,000 atoms
- No cutoff of electrostatics (PME)
- Nanosecond simulations

Bernèche & Roux (Biophys J, 2000)
Ion Conduction
Multi-ion free energy surface $W_{\text{eq}}$

Bernèche & Roux (*Nature*, 2001)
Microscopic mechanism of ion permeation

Bernèche & Roux (PNAS, 2001)
Brownian Dynamics Simulations of K+ in KcsA

Zhou et al, (2001)
Transport Cycle for Ion Conduction

Bernèche & Roux (PNAS, 2001)
Selectivity
The “snug-fit” model of ion selectivity

The size of the rigid pore is such that $K^+$ fits precisely, but $Na^+$ is too small to be well coordinated, so it does not enter the pore (Bezanilla & Armstrong, 1972)
The “paradox” of selectivity

\[ K^+ \ 1.33 \ \text{Å} \]

\[ Na^+ \ 0.95 \ \text{Å} \]

**Thermal fluctuations at 300K \approx 0.75 \ \text{Å}**

- X-ray, NMR, neutron scattering, B-factors of KcsA
- CHARMM (Bernèche & Roux)
- GROMOS (Shrivastava & Sansom; Luzkhov & Åqvist; Allen et al.)
- AMBER (Guidoni & Carloni)
FEP calculations with channel in membrane

Electrostatic repulsion

Including all interactions

Without C=O repulsion

$S_2$

+5.63

-3.34

Becomes selective for Na$^+$ over K$^+$ by 9 kcal/mol !!!
\[ \Delta \Delta G = \Delta G_{\text{hydration}}(K) - \Delta G_{\text{hydration}}(Na) - \Delta G_{\text{interaction}}(K) + \Delta G_{\text{interaction}}(Na) - \Delta G_{\text{strain}}(K) + \Delta G_{\text{strain}}(Na) \]

\[ \Delta \Delta G = 18 \text{ kcal/mol} \]

\[ \Delta \Delta G = 5.6 \text{ kcal/mol} \]

Valinomycin
\[ \Delta \Delta G = 8.2 \text{ kcal/mol} \]

KcsA
\[ \Delta \Delta G = 6.5 \text{ kcal/mol} \]

\[ \Delta G_{\text{hydration}} = +18 \text{ kcal/mol} \]
\[ \Delta G_{\text{interaction}} = -18 \text{ kcal/mol} \]
\[ \Delta G_{\text{repulsion}} = 8 \text{ kcal/mol} \]
Blockage
Internal tetrabuthylammonium (TBA) Blockade

Jose Faraldo-Gomez, Esin Kutluay, Vish Joginni, Yanxiang Zhao, Lise Heginbotham
Structure of KcsA+TBA

- Electron density for ions is same as WT
- Cross wired density corresponds to TBA
- A blob of density is seen below the center of the cavity

Yanxiang Zhao & Vish Joginni
Molecular basis of interaction

- van der Waals interaction with Phe103 and Ile100
- Electrostatic interaction with Thr75
- The corresponding residues in HERG are involved in binding drugs
Conformations of TBA in the cavity
Inactivation
K⁺ current under voltage-clamp conditions

- Fast inactivation (N-type)
- Slow inactivation (C-type)
- Pore gating
- Ball and chain

5 ms
Non-conducting state of the selectivity filter
Gating process in KcsA K+ channel

Close \[\xrightarrow{\text{H}^+} \] Open

Single channel recording of WT-KcsA

\[ P_0 = 0.1-0.15 \]

Close \[\xrightarrow{\text{H}^+} \] Open \[\xrightarrow{\text{H}^+} \] Inactivation
The inactivation gate is located at the selectivity filter

pH jump: Macroscopic current recordings using liposome patch clamp

The inner gate formed by the helix bundle is unaffected by the same mutation affecting the open probability

Cordero, Cuello, Perozo
Crystal structure of E71A mutant – 2 Configurations

Yanxiang Zhao & Vish Joginni
In Shaker W434F inactivates

Gating
Large movement

Small movement

Sliding helix

Paddle

Transporter-like
X-ray structure of Kv1.2 (pdb id 2A79)
Long et al (Science, 2005)

Model of Shaker

http://thallium.bsd.uchicago.edu/RouxLab
“In the three-dimensional model of the open channel conformation, R362 in S4 in one subunit is within atomic proximity of A419 in S5 in the adjacent subunit located in the clockwise direction as seen from the extracellular side of the membrane.”
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<tr>
<td>11.1 Å</td>
<td>12.5 Å</td>
<td>13.7 Å</td>
<td>−29 Å</td>
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<td>Fraction of S4 exposed to lipids</td>
<td>~10%</td>
<td>~20%</td>
<td>~19%</td>
<td>n.a.</td>
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MD simulation of Kv1.2 Channel in membrane

\[ \sim 80,000 \text{ atoms, } 40 \text{ ns (V. Jogini)} \]

S4

\[ \text{Droste-Willis Thermal B-factors (angle^2)} \]

0.5 sigma

SCWRL

X-ray
MD simulation, 40 ns (V. Jogini)
Arg294  Ala351

~9 Å

X-ray

~5 Å rms

Shaker


A419H  R362H  Zn$^{2+}$

~5 Å rms
Microscopic Theory of Gating Charge

Solve numerically the PB-V equation for the open and the closed states to calculated the total gating charge

\[
\Delta Q_{\text{tot}} \quad V_{mp} \quad = \quad \sum_i q_i \phi_{mp}^O(r_i) \quad - \quad \sum_i q_i \phi_{mp}^C(r_i)
\]

Not so “modest” movement...

Models of Shaker (Chanda et al., 2005)

Model of Kv1.2 (Yarov et al., 2006)
“Transporter” model
Chanda et al (2005)

“Modest” movement

“Paddle” model of
MacKinnon

Large movement

Kv1.2 (Long et al, 2005)
Models from Chanda et al (2005)

Ruta et al (Cell, 2005)

- Accessible only to external side
- Accessible only to internal side
- Accessible from both sides
- Inaccessible
Open

Closed

“Transporter” model
Chanda et al (2005)

“Modest” movement

“Paddle” model of
MacKinnon

Large movement

Kv1.2 (Long et al, 2005)
CONCLUSION

Molecular dynamics simulation based on detailed computational atomic models is a powerful tool to incorporate all available information from structural, spectroscopic, and functional data.