Studies of ion channel activation and modulation via computer simulation

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Outline Talk

- Membrane Proteins and Ion Channels
- Computational Techniques
- Examples
Membrane Proteins

- Biological Membrane = Lipid Bilayer + Membrane Proteins
- Selective Permeability due to Channel & Transport Proteins
- Receptors & Recognition
- Membrane Bound Enzymes

http://www.usd.edu/~bgoodman/ReviewFrames.htm
Genomics - Membrane Proteins constitute 25% to 30% of all genes
Membrane Proteins are implicated in many diseases: Diabetes, Parkinson’s, drug resistance (tumours & bacteria) …
Major drug targets
Biosensors
Nobel Prize in Chemistry 1988/2003/2012/2021

Importance of Membrane Proteins

- Predicted Number of Drug Targets
- Membrane Proteins ~ 50%

Drug Discov Today. 2009 14, 1130-5

1,713 unique structures known
(7,770 coordinate files)
Expression of ion channels in tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Effect of Na_v Dysfunction on Physiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Central nervous system</td>
<td>Epilepsy, migraine, autism, ataxia^{36}</td>
</tr>
<tr>
<td>B Retina</td>
<td>Altered visual processing^{62}</td>
</tr>
<tr>
<td>C Olfactory sensory neurons</td>
<td>Anosmia^{40,42}</td>
</tr>
<tr>
<td>D Sensory neurons and vagal sensory neurons innervating airways</td>
<td>Cough^{36,68}</td>
</tr>
<tr>
<td>E Heart muscle</td>
<td>Brugada syndrome, QT syndrome, atrial fibrillation^{59,67}</td>
</tr>
<tr>
<td>F Nerves, musculature involved in ventilation</td>
<td>Respiratory cessation (TTX poisoning)^{69}</td>
</tr>
<tr>
<td>G Pancreatic β-cells</td>
<td>Diabetes^{36}</td>
</tr>
<tr>
<td>H Skeletal muscle</td>
<td>Hyperkalaemic periodic paralysis, paramyotonia congenita, hypokalaemic periodic paralysis^{36}</td>
</tr>
<tr>
<td>I Skin</td>
<td>Pain disorders, paroxysmal itch^{37,39}</td>
</tr>
<tr>
<td>J DRG neurons</td>
<td>Pain disorders, paroxysmal itch^{37,39,51}</td>
</tr>
<tr>
<td>K Metastatic cancer cells</td>
<td>Ovarian, cervical, prostate, breast, colon, small cell lung cancer, melanoma, lymphoma^{35,70,71}</td>
</tr>
</tbody>
</table>
Active vs Passive transport of $K^+$ ions

J. Physiol. (1955) 128, 28–60

ACTIVE TRANSPORT OF CATIONS IN GIANT AXONS FROM SEPIA AND LOLIGO

BY A. L. HODGKIN AND R. D. KEYNES

From the Physiological Laboratory, University of Cambridge, and the Laboratory of the Marine Biological Association, Plymouth

(Received 30 August 1954)

Like many other living cells, nerve and muscle fibres use metabolic energy to move sodium and potassium ions against concentration gradients. In excitable tissues this process is of particular interest because it is essential for building up the concentration differences on which the conduction of impulses depends. When a nerve fibre is stimulated it undergoes rapid changes in permeability which allow first sodium and then potassium to move down concentration gradients. The effect of a train of impulses is therefore to raise the sodium and to lower the potassium concentration inside the cell. In giant nerve fibres

[Diagram of ion movement and concentration changes]
Ion permeation in K⁺-channels

J. Physiol. (1955) 128, 61–88

THE POTASSIUM PERMEABILITY OF A GIANT NERVE FIBRE

BY A. L. HODGKIN AND R. D. KEYNES

From the Physiological Laboratory, University of Cambridge

(Received 30 August 1954)

Experiments with a mechanical model

The very large departures from the independence relation described in this paper can be explained by assuming that K⁺ ions tend to move through the membrane in narrow channels, or along chains of sites such as might be provided by the negatively charged groups of a cation exchange resin. The essential feature of these systems is that the ions should be constrained to move in single file and that there should, on average, be several ions in a channel at any moment. Since it may not be immediately obvious that this

Alan Lloyd Hodgkin

The ionic basis of nervous conduction

Nobel Lecture, December 11, 1963

To begin with we hoped that the analysis might lead to a definite molecular model of the membrane. However, it gradually became clear that different mechanisms could lead to similar equations and that no real progress at the molecular level could be made until much more was known about the chemistry and fine structure of the membrane. On the other hand, the equations
Transport through Cells Membranes

- Channels: ions
- Carriers/Transporters: bigger molecules (sugars, drugs, ions, ...)

First known Structures of $(K^+)$ Channels

KcsA  KirBac1.1/3.1  MscL  MscS
MthK  KvAP  NaK

... vs thousands of structures of globular proteins
Experimental difficulties ...

Water
Membrane Protein
Antibody Fragment

Antibody
Micelle

Cryo-EM
A Kuo, C Domene, LN Johnson, DA Doyle, C Vénien-Bryan
Structure 2005, 13 (10), 1463

Declan Doyle & Catherine Venien
Crystallographers assume in their refinements that if a site is not occupied by an ion, the site is likely to be occupied by a water molecule.

Permeation in $K^+$ channels

Aqvist & Luzhkov, Nature 2000
Berneche & Roux, Nature 2001

- The most favourable pathway for ion translocation is the simple cycling between states $1010(1)$ and $0101(1)$
- Barrier for passage from $1010(1)$ to $0101(1) = 6 \text{ kcal mol}^{-1}$
- Mechanism involving three-ion states are excluded

- Largest free energy barrier $\sim 2-3 \text{ kcal mol}^{-1}$
- Each colour level $=1 \text{ kcal mol}^{-1}$
- Lowest energy pathway= Dotted line
- The position of the ions, $Z_1, Z_2$ and $Z_3$
Classical Mechanism of Permeation in K⁺ channel

- Both mechanisms display similar energy barriers (2-3 kcal/mol)
- In both, movement of one ion assist others to travel in the same direction
- The ‘Atypical’ mechanism agrees with experimental observations, and explains data that did not fit within the Classical model

S. Furini & C. Domene, PNAS 2009, 106 (38) 16074-16077
Ion permeation in K⁺ channels occurs by direct Coulomb knock-on
David A. Köpfer et al.
Science 346, 352 (2014);
DOI: 10.1126/science.1254840

for potassium channels. By analyzing more than 1300 permeation events from molecular
dynamics simulations at physiological voltages, we observed instead that permeation
occurs via ion-ion contacts between neighboring K⁺ ions. Coulomb repulsion between
adjacent ions is found to be the key to high-efficiency K⁺ conduction. Crystallographic
Do different $K^+$-channels adopt different conduction strategies?

MD Simulations were used to compare the mechanism of conduction in a pair prototypical members of 2 distinct families of $K^+$-channels

(a) Canonical Selectivity Filter
(b) Non-Canonical Selectivity Filter
Markov state models are a powerful framework for analysing MD simulations. Discretise trajectories from different replicas can be easily combined to estimate a consensus description of conduction events.

Network of states from the MSM of KcsA-WT

- Total simulation time: 0.1 ms (expensive!)
- +100 mV / +200 mV
- Starting from different loading states:
  - Only ions
  - Ions/Water
- Replicas (4*1 µs)
- Amber ff

**Notation**
Cavity/S4/S3/S2/S1/S0
w = Water
K = potassium ion
- = site is vacant
Conduction strategies in K\textsuperscript{+}-channels is channel dependent

The different occupancy of the SF binding sites for KcsA-WT, KcsA-E71A, and TRAAK supports the hypothesis of non-universal conduction strategies in K\textsuperscript{+}-channels, more likely a family dependent one.

The high functional diversity of K\textsuperscript{+}-channels could be attributed in part, to the differences in conduction properties.

Understanding ion conduction remains a fascinating question!
Critical Assessment of Common Force Fields for Molecular Dynamics Simulations of Potassium Channels

Simone Furini* and Carmen Domene

ABSTRACT: For the last two decades, the KcsA K⁺ channel has served as a case study to understand how potassium channels operate at the atomic scale, and molecular dynamics simulations have contributed significantly to the current knowledge of the atomic mechanisms of conduction, inactivation, and gating in this family of membrane proteins. Currently, microsecond trajectories are becoming the new standard in the field, and consequently, it is critical to assess and compare the performance of the classical force fields ordinarily used in simulations of biological systems as well as to quantitatively assess the agreement with experimental data for trajectories of this order of magnitude. To that extent, we performed classical molecular dynamics simulations with CHARMM36 and AMBER-f14sb force fields using atomic models based on the experimental structure of the KcsA channel in the open/conductive state, at conditions of ionic concentrations and membrane potentials resembling the ones adopted in experiments. In simulations using the CHARMM force field, the experimental open/conductive structure of the channel exhibited high conformational plasticity and fast collapse toward an occluded state. In contrast, in an identical set of simulations using the AMBER force field, no major deviations from the experimental structure were recorded. Force field development is a complex process in which many approximations are typically required and adopted. The results presented here provide additional motivation to further improve the existing models and, crucially, alert practitioners about limitations.
Selectivity in Ion Channels: steric effects & hydration energy

- Experimental Relative hydration free energy between $K^+$ and $Na^+ = 17.2$ kcal/mol

- $Na^+$ has a stronger interaction with its first hydration shell than $K^+$, giving the latter a more flexible structure

Zhou & Mackinnon (2011) J Gen Physiol.137(5), 397
Permeation in Na⁺-channels

- Different from ion conduction in K⁺-channels
- Permeation involves a loosely coupled 'knock-on' movement of 2 'solvated' ions
- Selectivity due to the inability of K⁺ to fit in the middle of the SF with a fully intact hydration shell

Furini S, Barbini P, Domene C. Biophys J. 2014 106(10):2175
Selectivity filter architectures

K^+ 1.2
TRPV1
Na^+ Ab

K^+ -channels
S0
S1
S2
S3
S4

Bacterial Na^+ -channels
S_{HFS}
S_{CEN}
S_{IN}

FxxxTxExW

TRPV1 Non-selective

GMGD

Sensations: cold, hot, spicy, pain

Nature 389, 783 - 784 (1997) DE Clapham

From the web!
https://www.boboandchichi.com/2016/07/spicy-korean-food-make-mouths-water/
Pain prevention and treatment

Affecting 619 million people worldwide in 2020; this number is projected to increase to 843 million by 2050.

https://doi.org/10.1016/S1474-4422(23)00446-5
Structure of the TRPV1 ion channel determined by electron cryo-microscopy

Maochu Liao, Erhu Cao, David Julius & Yifan Cheng

- Non-selective cation channels
- They respond to physical and chemical stimuli
- Modulated by inflammatory agents
- Contributes to acute and persistent pain
- Target for analgesic drugs
Transient Receptor Potential (TRP) Channels

Non-selective cation channels responding to a wide range of chemical and physical stimuli

As of today: 21 unique structures

<table>
<thead>
<tr>
<th>Channel</th>
<th>Species</th>
<th>Resolution</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPV1</td>
<td><em>Rattus norvegicus</em></td>
<td>3.275 Å</td>
<td>2013</td>
</tr>
<tr>
<td>TRPV1</td>
<td>complex with vanilloid agonist RTX</td>
<td>3.8 Å</td>
<td>2013</td>
</tr>
<tr>
<td>TRPV1</td>
<td>in nanodiscs</td>
<td>3.28 Å</td>
<td>2016</td>
</tr>
<tr>
<td>TRPA1</td>
<td><em>Homo sapiens</em></td>
<td>4.24 Å</td>
<td>2015</td>
</tr>
<tr>
<td>TRPV2</td>
<td><em>Oryctolagus cuniculus</em></td>
<td>4 Å</td>
<td>2016</td>
</tr>
<tr>
<td>TRPV2</td>
<td>channel, full-length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPV6</td>
<td><em>Rattus norvegicus</em></td>
<td>3.25 Å</td>
<td>2016</td>
</tr>
<tr>
<td>TRPP1/PKD2</td>
<td>polycystic kidney disease channel in lipid nanodiscs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Homo sapiens</em></td>
<td>3.0 Å</td>
<td>2016</td>
</tr>
</tbody>
</table>
Binding of Capsaicin to the TRPV1 Ion Channel

- TRPV1 is activated by capsaicin
- Identification of this site is not certain in the structural work

**GOAL:** generate a structural model of the capsaicin-channel complex

Understanding capsaicin binding to TRPV1 can potentially impact on the knowledge about regulation by a variety of other agonists, with crucial pharmacological implications.
Computational Studies

- Identify capsaicin modes of binding (not obvious from structural work)
- Study permeation of Na\(^+\), Ca\(^{2+}\) and K\(^+\) (identify binding sites and contacts)
- Selectivity
- Get insight into the flexibility of the selectivity filter
- Identify the effect of Temperature

NAMD/Gromacs
CHARMM ff
Lipid bilayer

- **Permeation:**
  Open channel (PDB 3J5Q)
  Three systems: NaCl /CaCl\(_2\) /KCl
  MD + free energy methods
- **Capsaicin/VGAs Binding:**
  Closed/Open forms (PDBs: 3J5P; 3T5R)
  Docking + MD + free energy methods
- **Temperature Effect:**
  Closed form (PDB 3J5P)
  MD

System Size > 1,000,000 atoms
Capsaicin binding to the TRPV1 ion channel

(1) Mutagenesis studies pinpointed residues that specify sensitivity to capsaicin
(2) Capsaicin (EM) density in close proximity to these residues
(3) The channel has multiple protonation sites

Jordt-Julius

Gavva et al

Docking Solutions

Cluster 1

Cluster 2

Binding modes predicted from docking calculations
Cluster 2: W549-capsaicin NOT observed
Capsaicin binding to the TRPV1 ion channel

Thermodynamic cycle

\[ \Delta G_{\text{bind}} \]

\[ \begin{align*}
R + L & \leftrightarrow R:L \\
\Delta G_{\text{bind}} & \\
\Delta G_{\text{Go,bulk}} & \\
\Delta G_{\text{Gc,bulk}} & \\
\Delta G_{\text{Gp,bulk}} & \\
R + L^* & \leftrightarrow R:L^* \\
\Delta G_{\text{decouple,bulk}} & \\
\Delta G_{\text{couple,site}} & \\
\end{align*} \]

\[ \begin{align*}
\Delta G_{\Theta,\text{site}} & \\
\Delta G_{\Phi,\text{site}} & \\
\Delta G_{\Psi,\text{site}} & \\
\Delta G_{\Theta,\text{site}} & \\
\Delta G_{\Phi,\text{site}} & \\
\Delta G_{\Theta,\text{site}} & \\
\Delta G_{\phi,\text{site}} & \\
\Delta G_{\Theta,\text{site}} & \\
\end{align*} \]

* Ligand restraint; o=orientation; c=conformation ; p=position

Free energy of binding = $-10.6 \pm 1.7$ kcal/mol (up)
= $-12.4 \pm 1.4$ kcal/mol (down)

Free Energy landscape of Capsaicin in the binding pockets of TRPV1 (well-tempered metadynamics; 340 ns)

(i) Direct H-bonds between Y511, T550 & Capsaicin and non-specific hydrophobic contacts of the aliphatic tail with the protein
(ii) ~2.5 (2) and ~1.5 (3) kcal/mol if 1 water bridge, ~3 kcal/mol for (4)
Route taken by Capsaicin to reach the TRPV1 binding pocket: direct or membrane mediated pathway?

\[ \Delta G_{\text{solution/membrane}} = -5 \text{ kcal/mol} \]

VR\text{up} : \ -12.4 \pm 1.4 \text{ kcal/mol}
VR\text{down} : \ -10.6 \pm 1.7 \text{ kcal/mol}

\(~ ??? \text{ kcal/mol}~\)

\ (>1 \text{ms})\]

TRPVs Drug Discovery - yeast-based method

Alex Perálvarez-Marín

*Pichia pastoris* expressing rTRPV2-EGFP

Fura-2 loaded vesicle preparation from yeast membranes
TRPV1 modulation

[Diagram showing various pathways involving TRPV1 modulation, including neurotransmitters, second messengers, and signaling proteins such as PKC, Calcineurin, and NFkB.]

THANKS ...

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Matteo Masetti & Riccardo Ocello, University of Bologna (Italy)