Structural basis of voltage-gated ion channel function

- Introduction to ion channels
- Subunits and their assembly
- Activation gate
- Ion selectivity
- Voltage sensor
- Inactivation gates
Ion channels: general properties

Membrane bound proteins that conduct ions at a rate near the diffusion limit across the plasma membrane, or intracellular membrane of organelles (e.g., mitochondria).

Ions move faster through ion channels than via carriers. Throughput rates for selective ion channels: $10^6 – 10^8$ ions/s. Current equivalent: $10^{-12} – 10^{-10}$ Ampere (1 – 100 pA)

Transfer rate for carriers (Na-K exchanger): 300 Na$^+$, 200 K$^+$/sec. Current equivalent: $1.5 \times 10^{-17}$ A

Frame of reference:
- Electronic circuits: $10^{-2}$ A
- Light bulb: $10^{-1}$ A
Four major breakthroughs in ion channel biology

1. **Ionic conductances**
   - Nobel 1963 (Physiol/Medicine)
   - Andrew F. Huxley
   - Alan L. Hodgkin

2. **Patch clamp methodology**
   - Nobel 1991 (Physiol/Medicine)
   - Erwin Neher
   - Bert Sakmann

3. **ACh receptor channel cloning/sequencing**
   - Shosaku Numa
   - (Kyoto)

4. **K channel structure**
   - Nobel 2003 (Chemistry)
   - Rod MacKinnon
Classification of ion channels

1) **Voltage-gated**: based on ion selectivity
   
   \[(K, Na, Ca, Cl \text{ channels})\]

2) **Ligand-gated**
   
   \[(\text{ligands: glutamate, GABA, ACh, ATP, cAMP})\]

3) **Specialized channels**
   
   \[(\text{connexins - gap junctions, mechanosens. channels})\]
Physiological functions of ion channels

Maintain cell resting potential: *inward rectifier K and Cl channels*

Conduction of electrical signals: *Na and K channels of nerve axon*

Synaptic transmission at nerve terminals: *glutamate, glycine, acetylcholine receptor channels*

Intracellular transfer of ions, metabolites: *gap junctions*

Cell volume regulation: *Cl channels (+ aquaporins)*

Sensory perception: *cyclic nucleotide gated channels of rods, cones*

Oscillators: *pacemaker channels of the heart and central neurons*

Excitation-contraction coupling: *Ca channels of skeletal & heart muscle*

Stimulation-secretion coupling: *release of insulin from pancreas*
Ion channels can be highly localized
Localization of channels

Motor neuron

Neuromuscular Junction

Muscle fiber

Adapted from: Kandel, Schwartz, and Jessell. Principles of Neural Science
Site-specific membrane targeting of ion channels
CatSper Ca channels

(6TM domains/subunit like Kv channels)

ONLY expressed in the principal piece of sperm (end of the tail)

Sperm lacking CatSper are poorly motile (no hyperactivity during capacitation phase) and are unable penetrate zona pellucida and fertilize egg.

Genetic knock out of CatSper makes male mice infertile - target for new contraceptive drugs?
Channel Gating: closed-open-inactivated

CLOSED

outside

K^+

inside

+ + +

27
In response to a change in voltage, single channels can activate (Open), deactivate (Close) or Inactivate:

\[ \text{depolarization} \]

\[ C \leftrightarrow C \leftrightarrow O \]

**TERMINOLOGY:**
- Activation: \( C \rightarrow O \)
- Deactivation: \( O \rightarrow C \)
- Inactivation: \( C \rightarrow I; \ O \rightarrow I \)
- Recovery from inactivation: \( I \rightarrow C; \ I \rightarrow O \)
Single channel currents sum to generate whole cell currents
Magnitude of whole cell current, $I$ can be determined by single channel properties

$$I = N \times P_o \times i$$

where $N$ is the total number of channels in the cell, $P_o$ is the open probability of a single channel, and $i$ is the single channel current amplitude.
Channel structure

Transmembrane, extra- & intra-cellular domains

Gates

Pore and selectivity filter

Voltage sensor
Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence

Masaharu Noda, Shin Shimizu, Tsutomu Tanabe, Toshiyuki Takai, Toshiaki Kayano, Takayuki Ikeda, Hideo Takahashi, Hitoshi Nakayama*, Yuichi Kanaoka*, Naoto Minamino†, Kenji Kangawa‡, Hisayuki Matsuo†, Michael A. Raftery§, Tadaaki Hirose§, Seiichi Inayama§, Hidenori Hayashida∥, Takashi Miyata∥ & Shosaku Numa

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∥ Department of Biology, Kyushu University Faculty of Science, Fukuoka 812, Japan

NATURE VOL. 312 8 NOVEMBER 1984
Molecular Characterization of Shaker, a Drosophila Gene That Encodes a Potassium Channel

Alexander Kamb, Linda E. Iverson, and Mark A. Tanouye
Division of Biology 216-76
California Institute of Technology
Pasadena, California 91125

Channel Structure

Structure of a voltage-gated K channel obtained by 3-dimensional reconstruction of multiple EM images

Side view  cytoplasmic face  extracellular face

100 Angstroms
EM reconstruction of Ryanodine receptor (SR Ca release channel)

RyR viewed from cytoplasm

RyR viewed from side
Subunits and their assembly
Ion channels subunits

α-subunits form ion conduction pore
Accessory subunits (β, γ, δ are usually smaller in size)

α-subunit size:
- 300 amino acids (inward rectifier K channels)
- 5000 amino acids (Ca release channel of SR)
Channel subunits are large proteins

---

HNC2
MDARGGCQPGESPGATP
--- APGP PPPPPPPPFP  PPPPPPPPPPPPPPPPPPPPPPPPPPPPAPLPPPAPAAGCPRG-----
HNC4
MIXKLPPSKRLYSLPQVQVAGAMIMDEEDAAEQRGGRGRRSSRLRDSDEASAGAGTGTESSSALQGAAGECPGARCAGSS

HNC2
ELRDSGQRFPAAFTAAGSTPNECGR
--- GPPC SPAG
HNC4
TGNCDFPFSLSLASHLSPVSCSHTDHGSGSGLHLD&AERLTAAGDRTTPFAAPRPNALAGPFAQPEQPPFFAPA

HNC2
QEEPPQCVGR
--- QEPGPQGaL
HNC4
SCEQPSVDIAK

HNC2
TMLLMVGNLIIIPVITPPKDFTWPIVFNVSDFTPFLDLVNFRTGFRVECNTIIIDPSTKLYSRKLVWDPDSIPVDYIFL
HNC4
TMLLMVGNLIIIPVITPPKDFTWPIVFNVSDFTPFLDLVNFRTGFRVECNTIIIDPSTKLYSRKLVWDPDSIPVDYIFL

HNC2
IVEGKIDSEYKTARALRIVRTKLSSLRLRSRLIRYIQWBEIFHMYTLASAVLMCLTLMSSMLLCHWDGCLQFLVFMLQDFR
HNC4
IVETHLDSEYKTARALRIVRTKLSSLRLRSRLIRYIQWBEIFHMYTLASAVLMCLTLMSSMLLCHWDGCLQFLVFMLQDFR

HNC2
NCWSINQVNSQSERISSLFKSHMLCIGYGRQAPVSMPLWIKTMIVGATCYAMFPIGHATALIQSLSRQQYEBKRYQKEOY
HNC4
ICWSINQVNSQSERISSLFKSHMLCIGYGRQAPVSMPLWIKTMIVGATCYAMFPIGHATALIQSLSRQQYEBKRYQKEOY

HNC2
MSPLKLFIPKQHODYEHRYQKMFEDISILGRELPEENIFNCRKLVAIQLPSANADPNFVTMLTKIVEFQQPGDYYIRBT
HNC4
MSPLKLFIPKQHODYEHRYQKMFEDISILGRELPEENIFNCRKLVAIQLPSANADPNFVTMLTKIVEFQQPGDYYIRBT

HNC2
IGKKMYFQIQGHSVSSLKGKRIEMGDSYFGBCILLTGRATSAVRADTYCRFLSLVDFNVLELYPMRRAFFTVAADLRD
HNC4
IGKKMYFQIQGHSVSSLKGKRIEMGDSYFGBCILLTGRATSAVRADTYCRFLSLVDFNVLELYPMRRAFFTVAADLRD

HNC2
NS1ILLKVQHNDLNSYPFAMQENIQGLEQWYMREVAGCL
HNC4
NS1ILLKVQHNDLNSYPFAMQENIQGLEQWYMREVAGCL

HNC2
PPPQPPQQVTA
--- IATLPCNACP
HNC4
PPPQSSGLGNLPGAGQTFRLHRKRSLSLPSAGLSASPSSPSVTPASSSHIFQASPAGLSSLPLPSSSSSPFPCGSAFPPSAAT

HNC2
QVARLPGPLAC
--- STLVRHPFPAHAAASGPPFASPH
HNC4
QVATTBQFGPHRLGSLSSCGIQPGLGARKGQGAPQAGLPEHFLPPHSSSSSSTSSLGQPGELSLGA

HNC2
APDFALFFP
HNC4
TPKLSTQPPFEPPFLVAGASGAPVGFTRGLSPGHSPPFRTSPAASPRAKLYGSSHGLLLPPASSSSPPQFPQRGTPPLPG

HNC2
RILRASPLASACPVSHPAGPAASSTHRASA
--- SPPTLRPTFAARAN
HNC4
RILQDNLKLASACPVSHPAGPAASSTHRASA
--- AQTRARAQPSSGMAAPFLPRAIDCQGGSGSGLGPRFYQGCTPHTVLPRKTSAGSLPPP

HNC2
--- RSLFEGNL
HNC4
LSLPGARATSSGPPPVTAGPQREPARGEPVWFSLGSLN

---

HNC2
--- 73
HNC4
--- 88

HNC2
--- 145
HNC4
--- 177

HNC2
--- 216
HNC4
--- 267

HNC2
--- 306
HNC4
--- 357

HNC2
--- 396
HNC4
--- 447

HNC2
--- 486
HNC4
--- 537

HNC2
--- 576
HNC4
--- 627

HNC2
--- 666
HNC4
--- 717

HNC2
--- 715
HNC4
--- 807

HNC2
--- 740
HNC4
--- 897

HNC2
--- 799
HNC4
--- 987

HNC2
--- 819
HNC4
--- 1077

HNC2
--- 877
HNC4
--- 1164

HNC2
--- 889
HNC4
--- 1203
General structure determined by hydropathy plots
Transmembrane (TM) domains have \( \alpha \)-helical structure and are more hydrophobic than intracellular or extracellular domains.

Alpha helix: 3.6 residues/turn; 5.41 Angstroms/turn
Plasma membrane thickness ~ 34 Angstroms
~23 amino acids/transmembrane domain
A primitive channel: Kir

Inward rectifier K+ channel

Adapted from Kandel, Schwartz and Jessel *Principles of Neural Science*
Voltage-gated K+ channel

Adapted from Kandel, Schwartz and Jessel Principles of Neural Science
Voltage-gated Na and Ca channel structure

Four motifs (I – IV) in a *single* protein
Auxiliary subunits of the voltage-gated ion channel superfamily
Likely Evolution pattern for the Superfamily of Voltage-Gated Channels
Amino acid relationships of the minimal pore regions of the voltage-gated ion channel superfamily (143 types)
The “Holy Grail – Part II”  
(Clay Armstrong)

The Structure of the Potassium Channel: Molecular Basis of $K^+$ Conduction and Selectivity

Declan A. Doyle, João Morais Cabral, Richard A. Pfuetzner, Anling Kuo, Jacqueline M. Gulbis, Steven L. Cohen, Brian T. Chait, Roderick MacKinnon*

SCIENCE • VOL. 280 • 3 APRIL 1998
KcsA channel co-crystallized with an antibody Fab fragment to stabilize structure & enhance x-ray resolution


2.0 Angstrom resolution!
X-ray crystal structures were first obtained from bacterial channels

- **KcsA**: 2 TM domains/subunit
  - Activated by protons
- **MthK**: 2 TM domains/subunit
  - Activated by intracellular Ca^{2+}
- **KvAP**: 6 TM domains/subunit
  - Activated by voltage

All structures solved in Rod MacKinnon’s lab at Rockefeller Univ
(Nobel Prize in Chemistry, 2003)
KcsA bacterial K channel

View from extracellular side

Side view – within membrane
Inner helices form “inverted teepee” structure

Mutations in *Shaker* that affect function are mapped onto KcsA structure

White: agitoxin2, charybdotoxin binding

Yellow: external TEA binding

Mustard (T74): internal TEA binding

Pink: accessible by intracellular ligand only when channel is open

Green: accessible by intracellular ligand when channel is open or closed

**GYG** – required for K selectivity

Molecular surface of KcsA and contour of the pore (cutaway view)

Blue
Basic (+) residues

Yellow:
Hydrophobic residues

Red:
Acidic (-) residues

$\text{K}^+$
Representation of the inner pore based on nearest van der Waals protein contact

Outer vestibule
Selectivity Filter
Central cavity
Outer vestibule
Ring of aromatic amino acids define the membrane-facing surface

Activation gate
Gates

- Activation
- Inactivation
Bundle crossing of inner helices defines the “activation gate”
Open vs closed state of bacterial K channels

MthK

KcsA

Side view

View from cytoplasm
Channel opening: inner helices bend at “glycine hinge”

MthK (GI: 2622639)  : YWTFTVATTVGVGDYS--PSTPLGMYFTVTLLVELIGTFAVAVERLLEFLIN
KcsA (GI: 2127577)  : WWNVETATTGVDLY--PVTLLLLAVVVMVARATSFGTVTAALATWFVG
Dradio (GI: 6458547) : YWAVVVTTVGVGDYS--PKTGLGKFIATLAMLSGAYAIAVPTGIVTVGLQQ
Ecoli (GI: 400124)  : YFSIETMSSTVGVGDIV--PVSEASRTFTISVIPSTISFMTSIGPLIR
Shaker (GI: 85110)   : WWAVVVTMTTVGVGDYT--PVGFWKIVVSICVATLTPRVPVMSFNY
hDRK1 (GI: 345875)  : WWATITMTTVGVGYI--PKTLLGKIVGGLCCQVTIALPPIVNNFSE
hBK (GI: 2570854)   : YLMVTTMSTTVGVGDYT--AKTGLGRLMVFVFFILGGLAMFASYVPEIELIGN
hSK3 (GI: 15983750) : WLSITFISIGYGDML--PHTYGKGGVCLLTGIAGCTALVVAVVARKELE
hERG2 (GI: 14745363) : YFIFSSTTSVFGNVS--PNTNPSKIFISCVMLILGSLMYASIFGNVSAIQRR
hGIRK2 (GI: 1352487) : LFSITETTTGNYRITDKPERSIGLLIQSFLGSLSTNAMFMCVCOMFKISQ
hIRK1 (GI: 2460307) : LFSIETTTTGYFRCVTDECPATFVMVFQSIIVGCIIDAFIIGAVMAKAK
bCNG1 (GI: 231739)  : YWSTLTTTTG--ETPP-PVRDSEYFFVVADFLILEVFIVTIVGNIGSMISN

Pharmacol Rev 57: 387-395, 2005
Molecular surface of the MthK pore viewed from the intracellular solution

The membrane electric potential across the pore changes on opening. Electrostatic contour plots for KcsA (a) and MthK (b) in a membrane.

- grey region: protein or membrane (dielectric constant 2)
- white regions: aqueous solution (dielectric constant 80)

Ion Selectivity
Selectivity filter
Selective ion permeability

Permeability in K channels

<table>
<thead>
<tr>
<th>Ion</th>
<th>Dehydrated Radius</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>1.31 (Å)</td>
</tr>
<tr>
<td>Rb⁺</td>
<td>1.48 (Å)</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.95 (Å)</td>
</tr>
<tr>
<td>Cs⁺</td>
<td>1.69 (Å)</td>
</tr>
</tbody>
</table>
1. How can a channel be selectively permeable to one cation vs another?

Radius of Na\(^+\) is 0.95 Ang, K\(^+\) is 1.31 Ang
-yet K channels selects for K\(^+\) over Na\(^+\) by a factor of 1000-10,000

2. How can a channel be highly selective, yet paradoxically have an ion throughput rate near the diffusion limit (100 million ions/sec)?

High selectivity suggests high affinity binding to channel - this would be expected to slow the ion throughput rate

*Apparent dilemma solved by x-ray crystallography of a bacterial K-selective channel, KcsA*
Selectivity filter of KcsA channel

MthK (GI: 2622639) : YWTFVTVIATVGYGDS--PSTPLGMYFTVTLIVLGLIGTF
KcsA (GI: 2127577) : WWSVETATTVGYGDLY--PVTLWGRLVAVVVMVAGITSF
Dradio (GI: 6458547) : YWAIVTVTTVGYGDIS--PKTGLGKFIATLAMLSGYAI
Ecoli (GI: 400124) : YFSIETMSTVGYGDIV--PVSESARLFTISVIISGITVF
Shaker (GI: 85110) : WWAVVTMTTVGYGDMT--PVGFWGKIVGSCLCVVAGVLTI
hDRK1 (GI: 345875) : WWATITMTTVGYGDIV--PKTLTGKIVGGLCCICAGVLVI
hBK (GI: 2570854) : YLLMVSTSTVGYGDVY--AKTTLGRLFMVFILLGGLAME
$K^+$ ions inside the filter are dehydrated

- $H_2O$
- $K^+$
K ion dehydration at the extracellular pore entryway and ion hopping

Interpretation: two states

Selectivity filter of KcsA channel crystallized in high and low [K$^+$]

High K structure (conducting)

Low K structure (nonconducting)

Electron density map in low K

Zhou et al. (2001)
Nature 414:43
Two mechanisms by which $K^+$ channel stabilizes a cation in the middle of the central cavity

(1) a large aqueous cavity stabilizes a single $K^+$ in the hydrophobic membrane interior.

(2) oriented pore helices point their partial negative charge (carboxyl end) towards the cavity where a cation is located.
Why is K\(^+\) favored over Na\(^+\)?


The energy for K\(^+\) in water and the selectivity filter is similar (~79 kcal/M)

Coordination of Na\(^+\) in selectivity filter is energetically unfavorable (lower binding affinity than K\(^+\))
Summary: High selectivity and high permeation rate

$10^8$ ions/sec – selectivity filter must allow K ion to dehydrate, enter and cross filter within ~10 ns

- High selectivity:
  - Multiple ion occupancy: optimized geometry of K binding sites (1,3/2,4) in the narrow selectivity filter (customized oxygen cages)

- High permeation: electrostatic repulsion between adjacent K ions (4 M equivalent local concentration)

- A central cavity that is lined by hydrophobic residues
  - with plenty of water and central K+ stabilized by pore helix dipoles
### Ion selectivity in Na and Ca channels

<table>
<thead>
<tr>
<th>K channel (Sh B)</th>
<th>T M T T V G Y G D I Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cardiac</td>
<td>I R L M T Q D C W E R</td>
</tr>
<tr>
<td>II</td>
<td>R I L C G E W I E T</td>
</tr>
<tr>
<td>III</td>
<td>Q V A T F K G W M D</td>
</tr>
<tr>
<td>IV</td>
<td>Q I T T S A G W D G</td>
</tr>
</tbody>
</table>

| Na channel       | I Q C I T M E G W T D  |
| Rabbit skeletal muscle | II Q V L T G E D W N S |
| III              | T V S T F E G W P Q    |
| IV               | R C A T G E A W Q E    |

- **TEA binding sites in K channel**
- **Putative selectivity filter / Ca\(^{2+}\) binding site**
- **Isoform-specific TTX/Cd\(^{2+}\) sensitivity**
- **Na\(^{+}\)/Ca\(^{2+}\) permeability**

Balser (1999)  
Cardiovasc Res 42:327
Voltage sensing

VSD: the voltage sensor domain
Voltage sensor
Voltage-gated ion channel = pore domain + VSD
S4 domain is the primary voltage sensor

Lysine (K)  
\[
\begin{align*}
\text{COO} & \\
\text{H}_2\text{NCH} & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{NH}_3^+ & \\
\end{align*}
\]

Arginine (R)  
\[
\begin{align*}
\text{COO} & \\
\text{H}_2\text{NCH} & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{CH}_2 & \\
\text{NH} & \\
\text{C=NH}_2^+ & \\
\text{NH}_2 & \\
\end{align*}
\]
S4 domains from different channels are similar but not identical

**ShakerB**

**hSkM1**

**Domain I**

**Domain II**

**Domain III**

**Domain IV**

**ShakerB S4**

AILRVIRLVRVFIRIFKLSRHSK

**hSkM1 S4 DI**

ALRYFRVLRALKTITVIPGLKT

**hSkM1 S4 DII**

VLRSFRLLRVRFKLAKSWPTLN

**hSkM1 S4 DIII**

LGPIKSLRTLVARPLRALSR

**hSkM1 S4 DIV**

FRVIRLARIGRVLRLIRGAKGIR
Helical screw motion model of voltage sensor

S4 domain
VSD = S2/S3/S4

- Salt bridge forms between acidic residues in S2/S3 (red spheres) and basic residues of S4 (blue spheres)
- Consistent with helical screw motion

Bezanilla (2008)
Nature Reviews Mol Cell Biol 9:323
A different view of VSD: based on structure of KvAP, a voltage-gated K\(^+\) channel

(from thermophilic archaebacteria, *Aeropyrum pernix*)

Top view

side view

KvAP channel

“Paddle” model of voltage sensor movement

Closed

Open

Nature. 423:42
controversy

Shaker

KvAP
KvAP sequence is similar to Shaker

Comparison of KvAP and KcsA pore domain

KcsA: green  KvAP: blue

S5 (Outer)  S6 (Inner)

Glycine hinge

Electron density and crystal lattice of the Kv1.2–β2 subunit complex, a mammalian K channel

Long et al (2005) Science
The Kv1.2–β₂ subunit channel complex
(back to the traditional VSD model)

Long et al (2005) Science
Model of Kv1.2 in the closed state

Model of Kv1.2 VSD in the closed state: salt bridges form between basic residues in S4 and acidic residues in S2/S3

Side view  view from intracellular side
Neuron 56:124
Model of S4 movements in a Kv channel

(only two subunits shown)

Bezanilla (2008)
Nature Reviews Mol Cell Biol 9:323
Contribution of the S4 domain to gating charge in Shaker K channels

Aggarwal and MacKinnon (1996) Neuron 16, 1169
Figure 7. Summary
Voltage-activated ion channels respond to changes in membrane voltage by coupling the movement of charges to channel opening. A K⁺ channel-specific radioligand was designed and used to determine the origin of these gating charges in the Shaker K⁺ channel. Opening of a Shaker K⁺ channel is associated with a displacement of 13.6 electron charge units. Gating charge contributions were determined for six of the seven positive charges in the S4 segment, an unusual amino acid sequence in voltage-activated cation channels consisting of repeating basic residues at every third position. Charge-neutralizing mutations of the first four positive charges led to large decreases (~4 electron charge units each) in the gating charge; however, the gating charge of Shaker Δ10, a Shaker K⁺ channel with 10 altered nonbasic residues in its S4 segment, was found to be identical to the wild-type channel. These findings show that movement of the NH₂-terminal half but not the CO₂H-terminal end of the S4 segment underlies gating charge, and that this portion of the S4 segment appears to move across the entire transmembrane voltage difference in association with channel activation.
First recording of gating current ($I_g$) for Na channels in a squid giant axon

$I_K$ was eliminated by removing all $K^+$, $I_{Na}$ was reduced by lowering $[Na^+]$. $I_{cap}$ was removed by subtraction, then eliminated with tetrodotoxin (TTX).

Gating currents of cloned \textit{Shaker} K channel

Voltage pulse protocol

Gating currents (ionic currents blocked; or use nonconducting channels)
Integrate gating current to obtain charge ($Q$)
Q = charge
   (gating current)
G = conductance
   (ionic current)
"Accessibility" of residues mutated to Cys used to determine extent of S4 movement during channel activation

In closed state, the Cys residues cannot be modified by Cys reactive agent (MTSET, \( \text{HS}^- \))

In open state, Cys residue *can* be modified
Experiment to probe for extent of S4 domain movement during gating of Shaker K channel
Intramembrane charge displacement

• Measurement of total charge moved ($Q_{\text{tot}}$)/# of channels = 13 $e^o$/channel

Mutate a single Arg group (R362) – outermost charged a.a. in S4 domain of *Shaker K* channel

......reduces Q/channel to 9 $e^o$/channel

(1 $e^o$/subunit of homotetramer)
MTS modification of R362C affects gating currents and $Q_{tot}$ of Shaker K channels

Increasing tether length monotonically decreases charge movement

Ahern and Horn (2005)
Neuron 48:25-29
Inactivation gates
Pronase, a proteolytic enzyme applied internally to squid giant axons eliminates inactivation of Na channels

Looks similar to block of K channels by internal C9:


Removal of “ball and chain” from subunit eliminates inactivation in cloned Shaker K channels