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Sandia National Laboratories

The Role of Ions in Structural Stability of the K^+ Channel Tetramer

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Centro Interdisciplinario de Neurocienca de Valparaiso

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Principal Member of the Technical Staff

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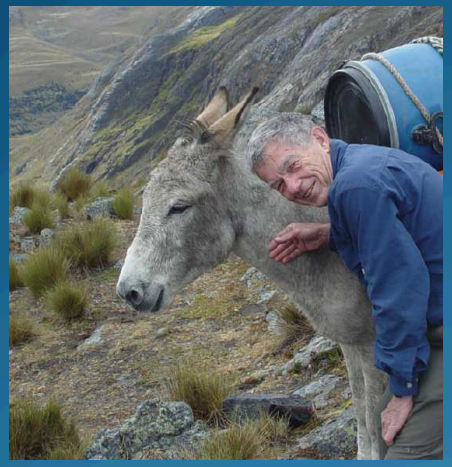
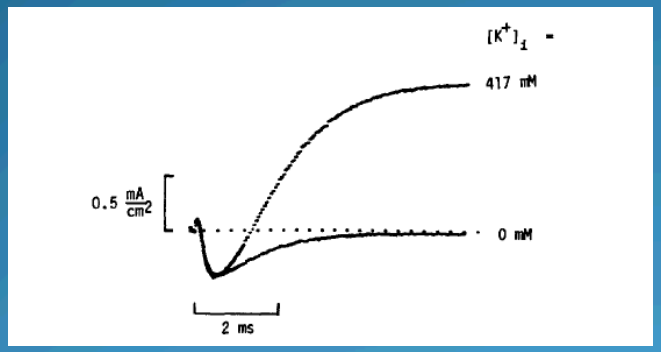
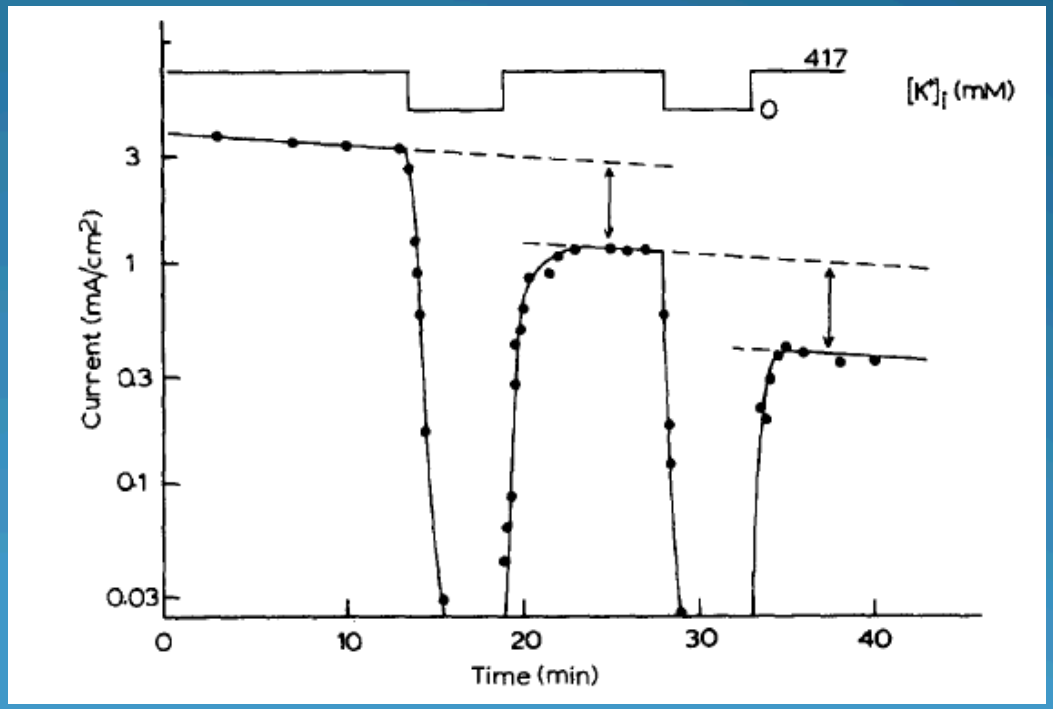


"One difficulty in studying K^+ channels is that they cease to function when internal K^+ is removed for prolonged periods." Almers & Armstrong, 1980





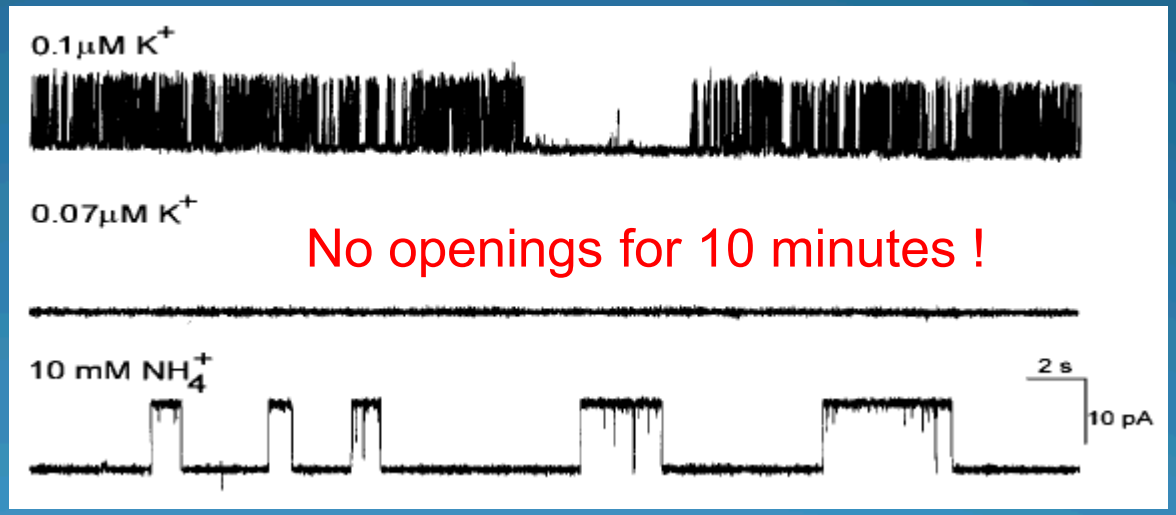
Survival of K^+ Permeability and Gating Currents in Squid Axons Perfused with K^+-Free Media

W. Almers & CM Armstrong. 1980. J Gen Physiol 75: 61-78

"Conductance Collapse" of the BK_{Ca} Channel at low $[K^+]$ out

100 mM KCl ,
125 μM Ca^{2+}
Inside

V = 0 mV



Recovery Ions: Rb^+, Cs^+, NH_4^+

No Recovery: Na^+, Li^+

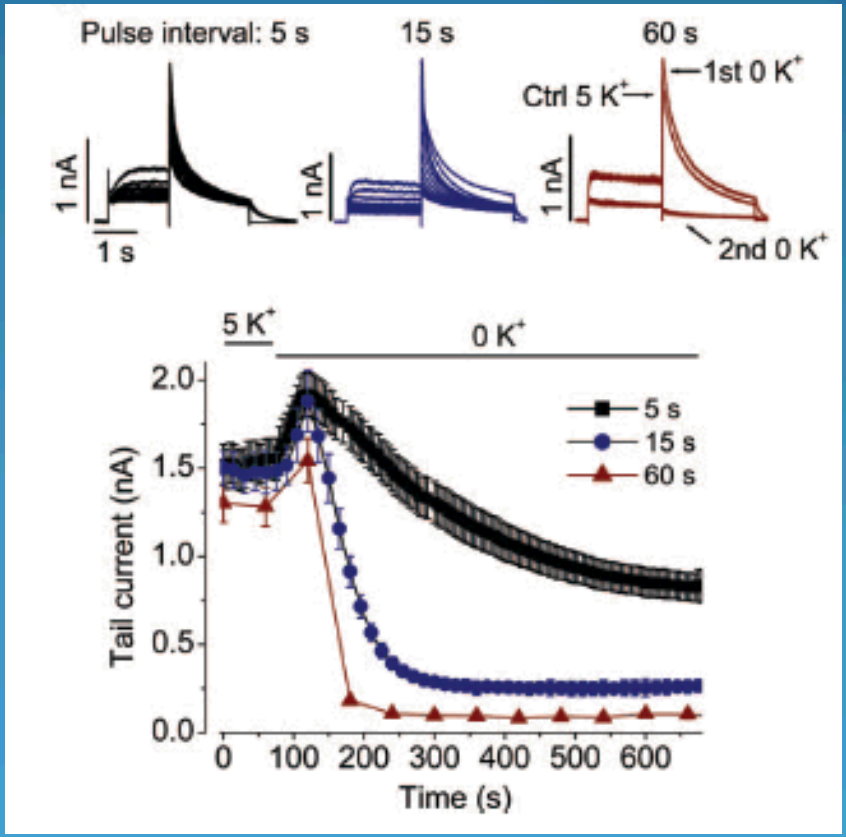
C Vergara, O Alvarez, & R Latorre. 1999. J Gen Physiol 114: 365-376

Extracellular K^+ Is a Prerequisite for the Function and Plasma Membrane Stability of HERG Channels

Hamid Massaeli,* Jun Guo,* Jianmin Xu, Shetuan Zhang

Circ. Res. 2010; 106: 1072-1082.

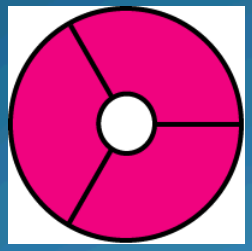
V pulse: -50 → +50 mV



Introduction to the Problem

- Most ion channel proteins have a rosette-like architecture

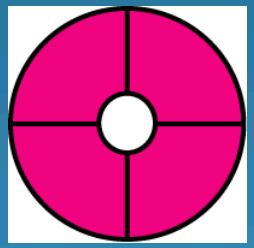
Trimers



Epithelial Na Channels
ENaC
ASIC

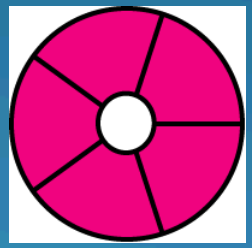
ATP-gated Channels
P2X Receptors

Tetramers



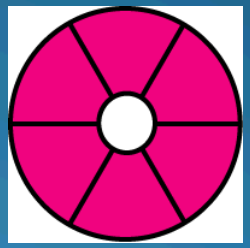
P-Loop Family
K^+ channels
Na_V channels
Ca_Vchannels
CNG channels
TRP channels
GluR channels

Pentamers



LGIC Family
AChR
5-HT_3R
$GABA_A$R
GlyR

Hexamers



Connexins
(Cell-cell Gap Junctions)

- Exceptions: bacterial and mitochondrial porins (β-barrels), ClC Cl^- channels, aquaporins,

K^+ Channel function requires a stable tetrameric protein complex.

What holds the channel together ?

- What is the molecular and energetic basis of tetramer stability of K^+ channel proteins?
- How does tetramer stability correlate with ion discrimination, binding affinity, and unitary conductance?
- How does channel function depend on inter-subunit interactions?

KcsA: What is the basis of tetramer stability?

Lipid Membrane

- N and C termini
- phospholipids
- K^+ / selectivity filter
- protein-protein subunit interface

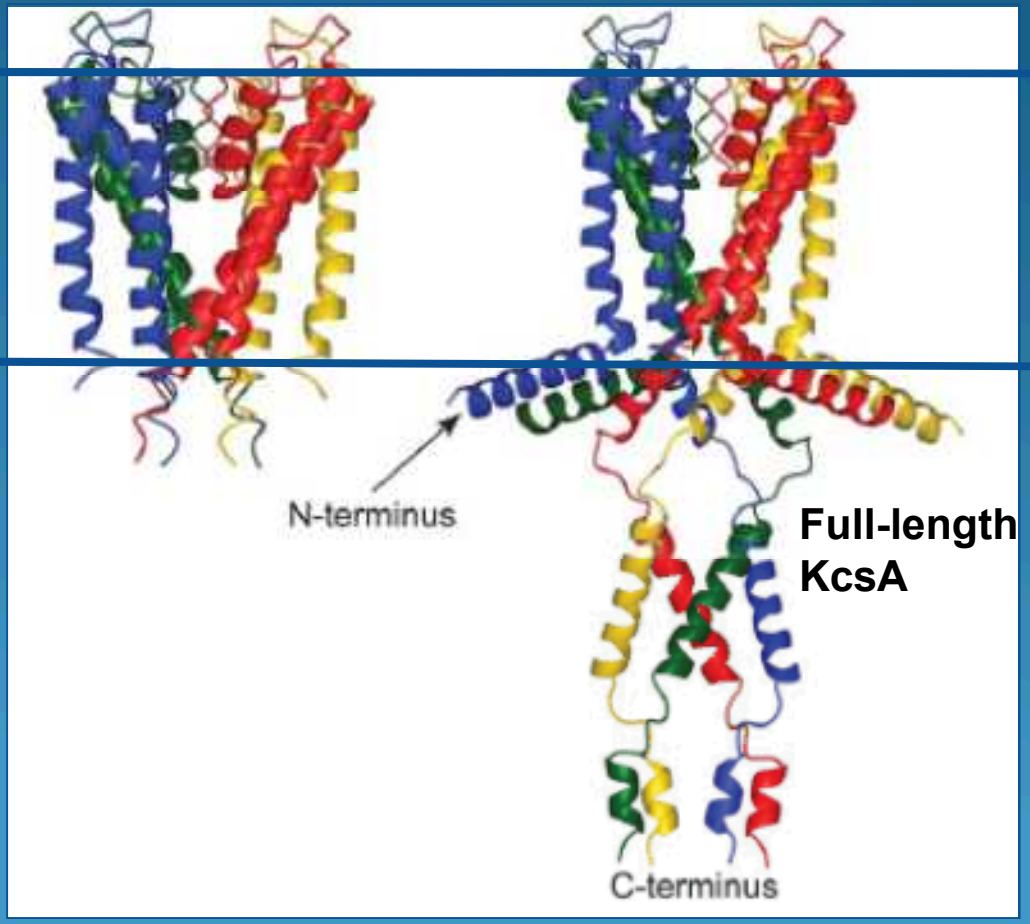
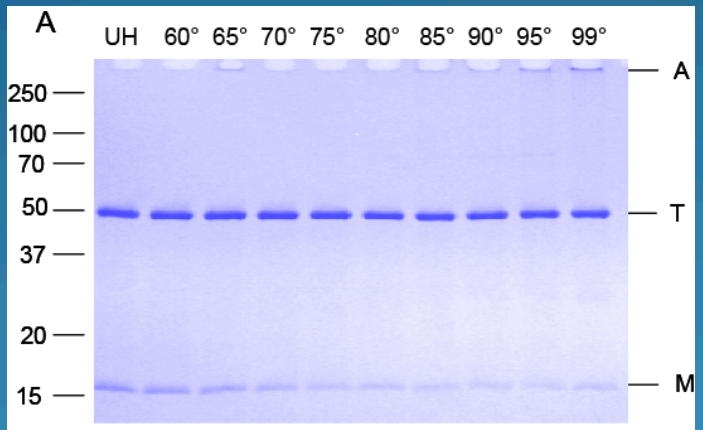


Figure from: JA Ecinar et al. 2005. FEBS Lett. 579: 5199-5204

Thermal stability of KcsA tetramer is ion-dependent

100 mM KCl

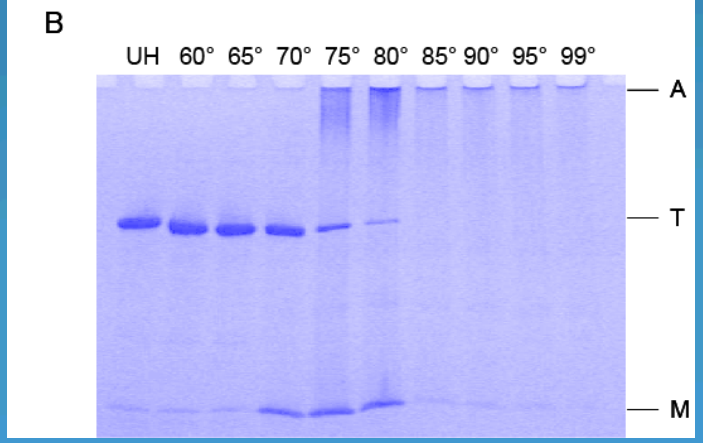


← Aggregate

← Tetramer (74 kD)

← Monomer (18.5 kD)

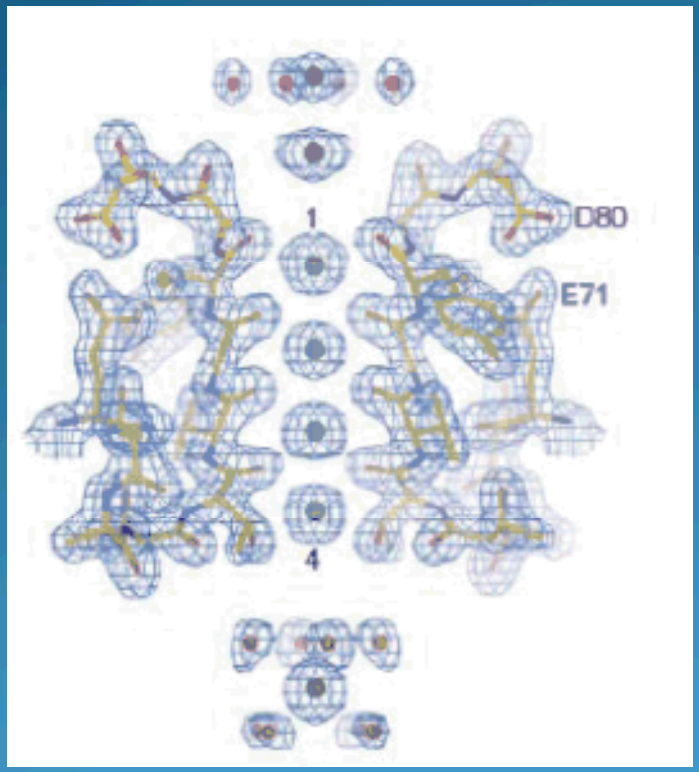
100 mM NaCl

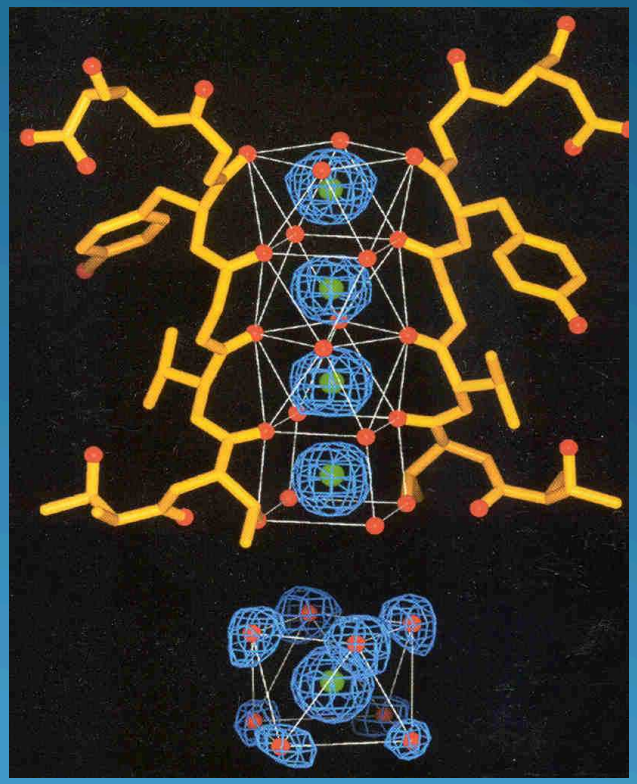


Krishnan et al 2005.
J Gen Physiol 126: 271-285

7 Binding Sites for K^+ in a High Resolution Structure of KcsA

Zhou et al. 2001. Nature 414: 43-48.

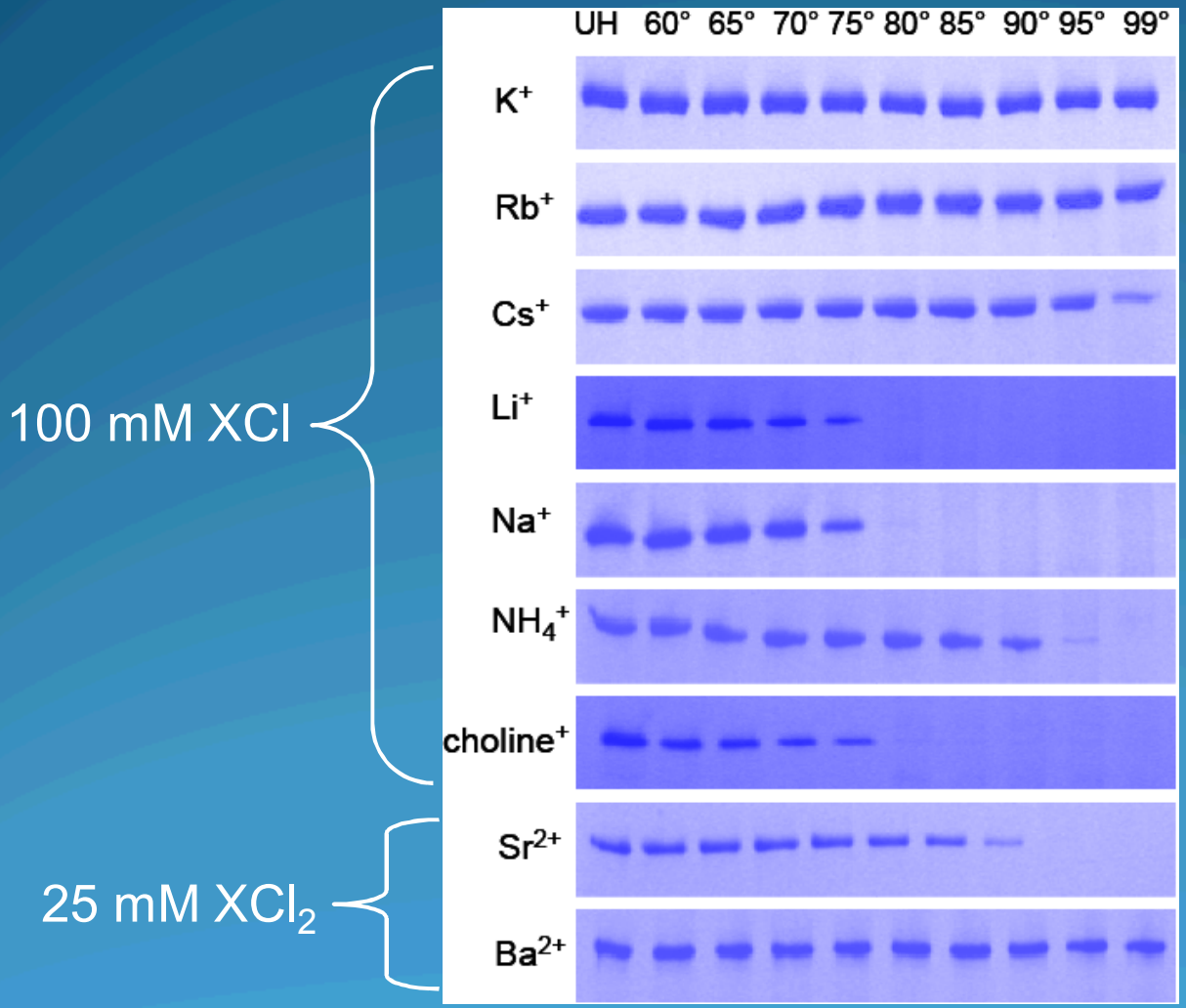




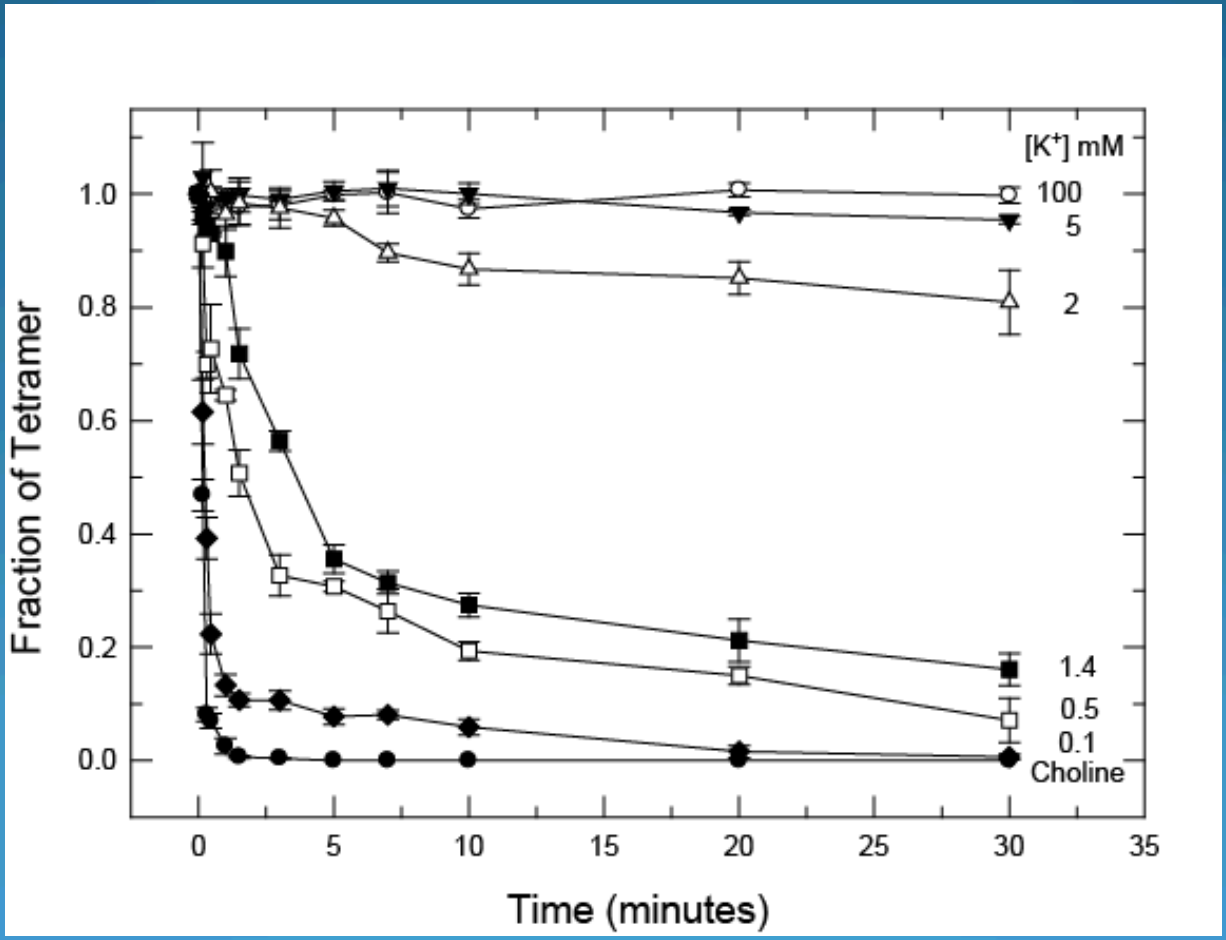
- 2 sites in external vestibule: S_{ext} & S_0
- 4 sites in the filter: S1, S2, S3, S4
- 1 site in the central cavity: S_{cavity}

• 8-fold coordination of K^+ to Oxygen atoms of –C=O, -OH of Thr75, and H_2O

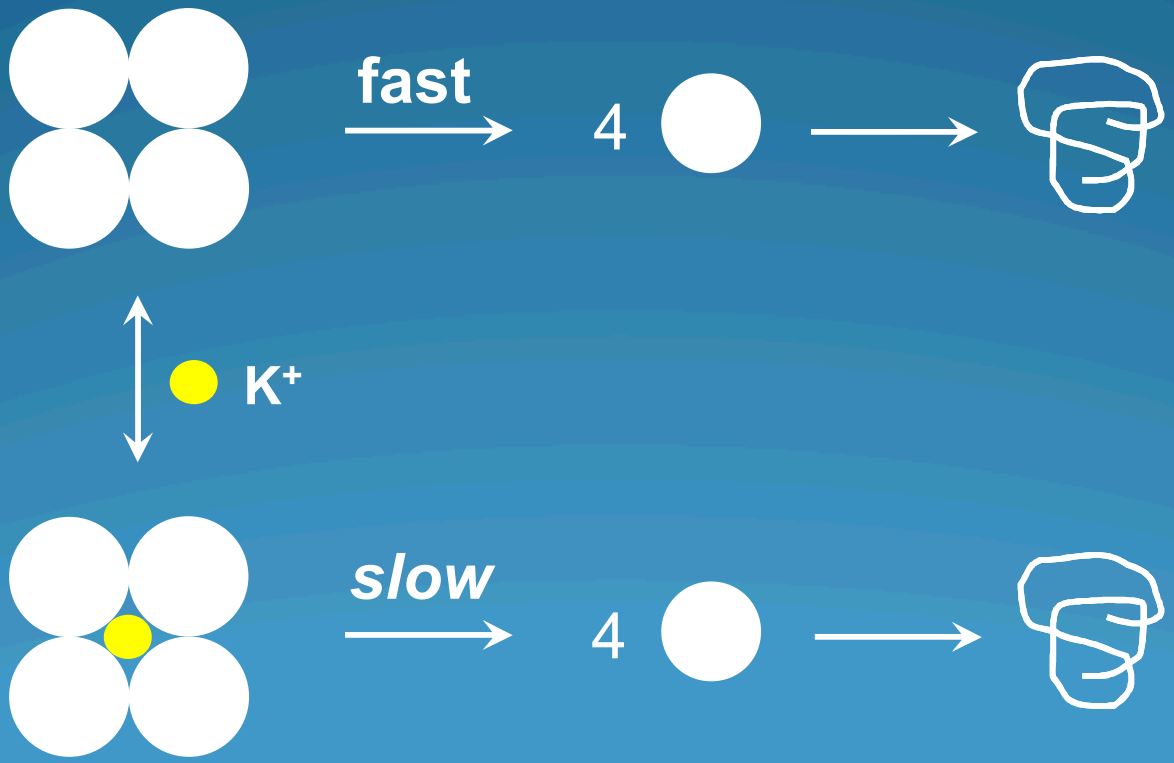
Effect of Cations on Tetramer Stability of KcsA



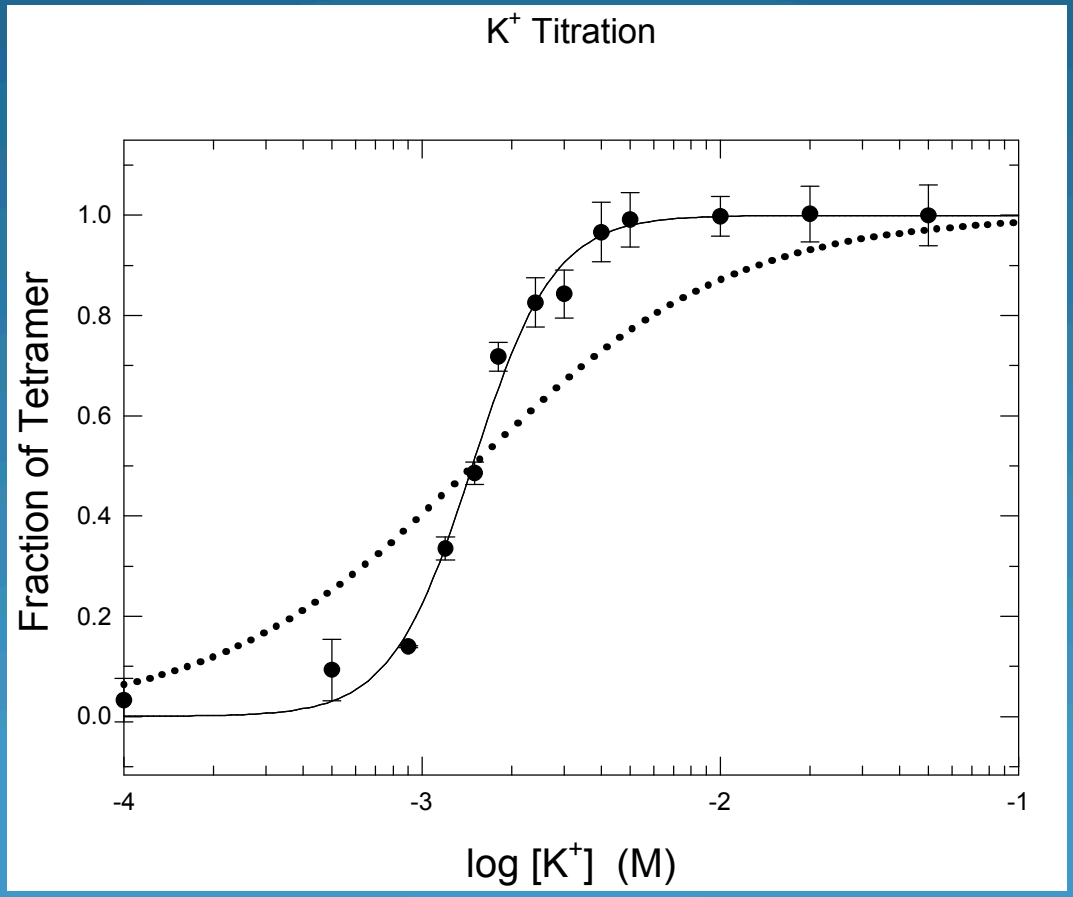
Time Course of Tetramer Dissociation (90°C) is a Function of [K^+]



Model for Protection of KcsA Tetramer Dissociation by K^+

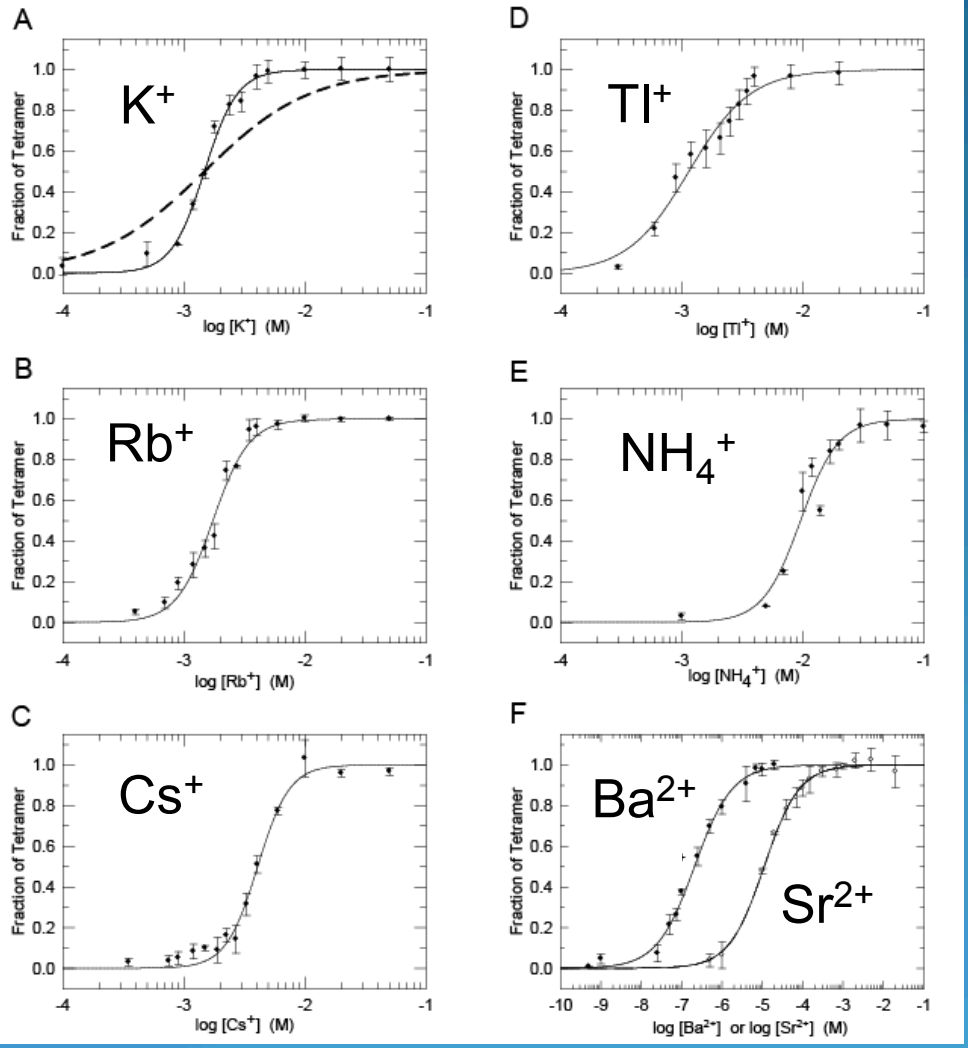


Titration of [K^+] Dependence of Tetramer Stability



- K^+ Protects Against Thermal Denaturation at 90° C in 100 mM CholineCl

Relative "Affinity" of Various Cations for Protection of KcsA Tetramer Against Thermal Denaturation at 90° C

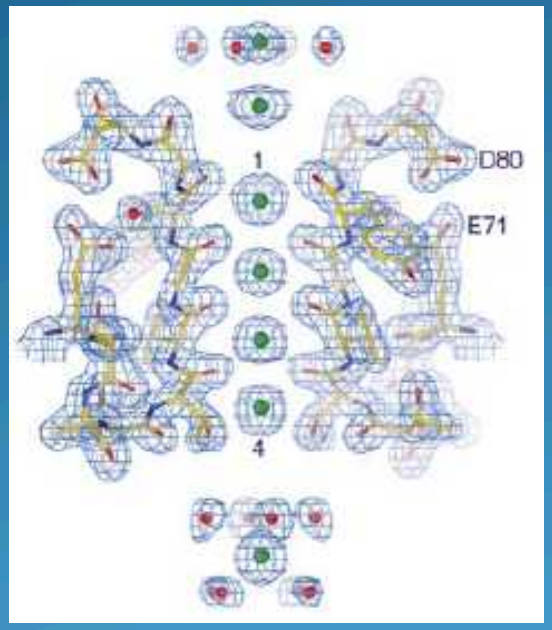


Ion	$K_{0.5}$ Gel	$K_{0.5}$ ITC*
K^+	1.5 mM	0.43 mM
Rb^+	1.7 mM	0.12 mM
Cs^+	4.1 mM	0.44 mM
Tl^+	1.1 mM	
NH_4^+	9.6 mM	
Ba^{2+}	210 nM	0.19 mM
Sr^{2+}	11 μM	

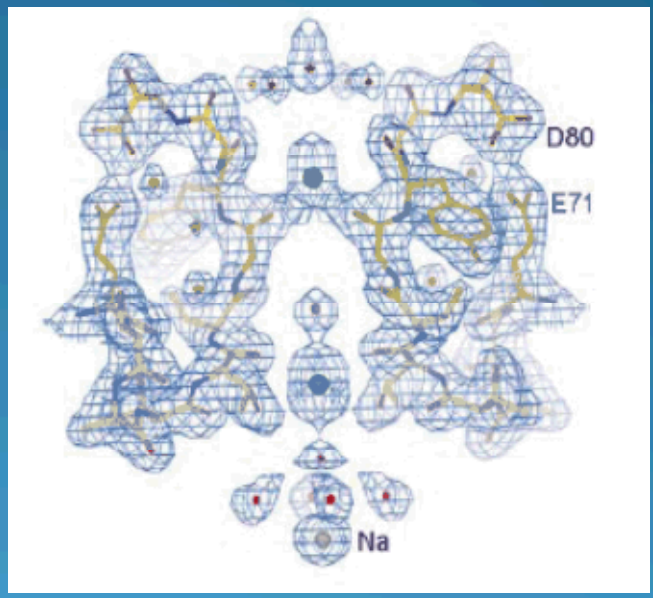
*ITC titration data (22 C, 100 mM NaCl) Lockless et al. 2007. PLOS Biology 5: 1079-88

Effect of Na^+ or Low K^+: Structural Change of the KcsA Filter

200 mM K^+

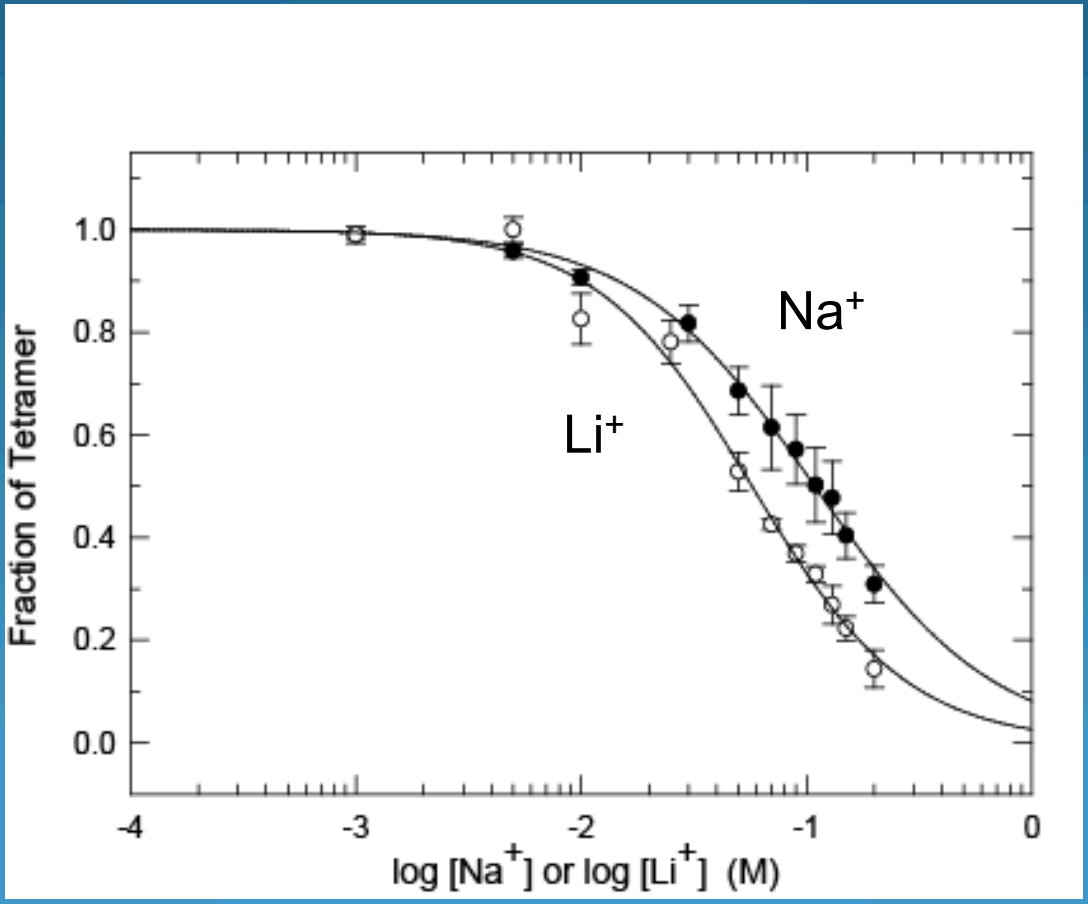


3 mM K^+ + ~200 mM Na^+



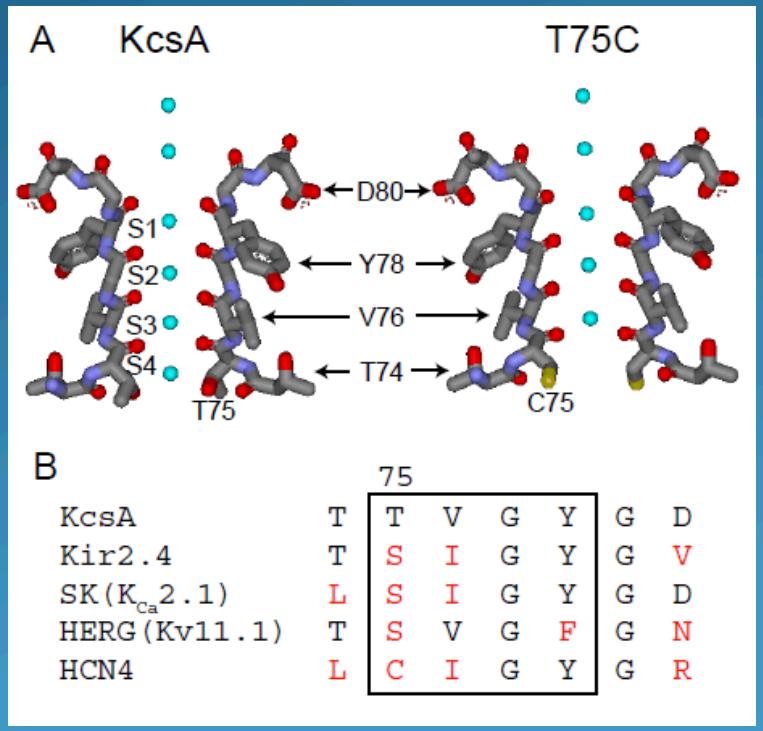
Zhou et al. 2001. Nature 414: 43-48.

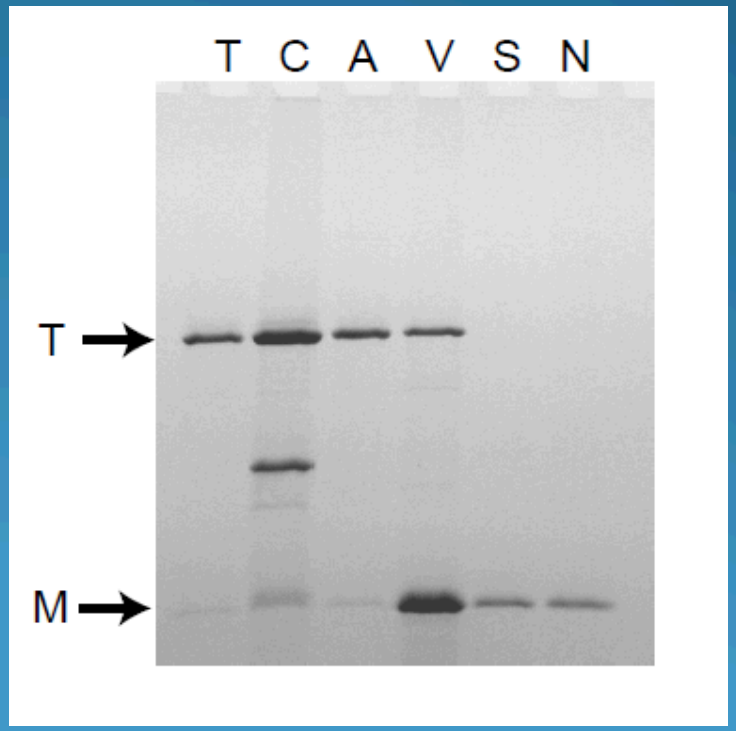
Destabilization of KcsA Tetramer by Na^+ and Li^+ in 300 mM CholineCl and 5 mM KCl



Possible Mechanism: Competition of K^+ and Na^+ Binding

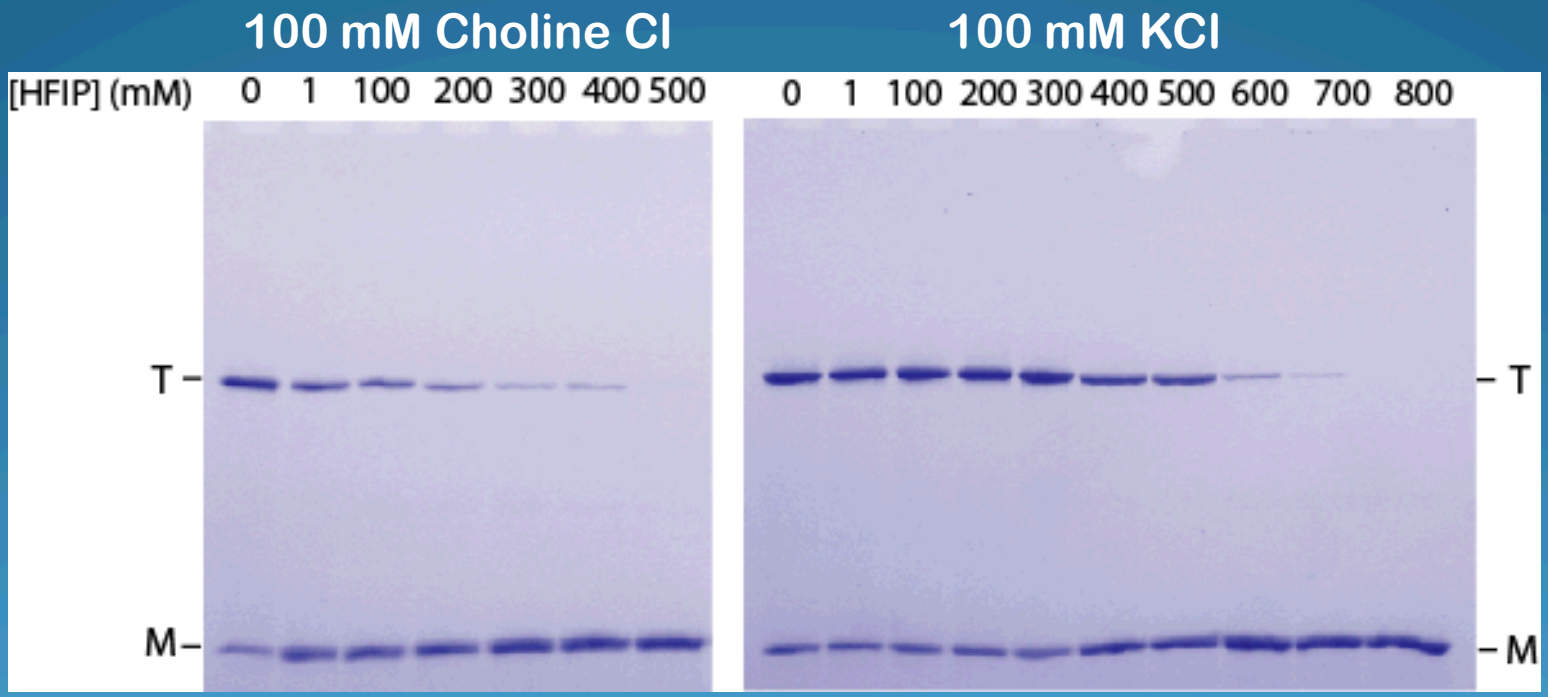
- Mutation of Thr75 destabilizes tetramer formation and alters ion-selectivity





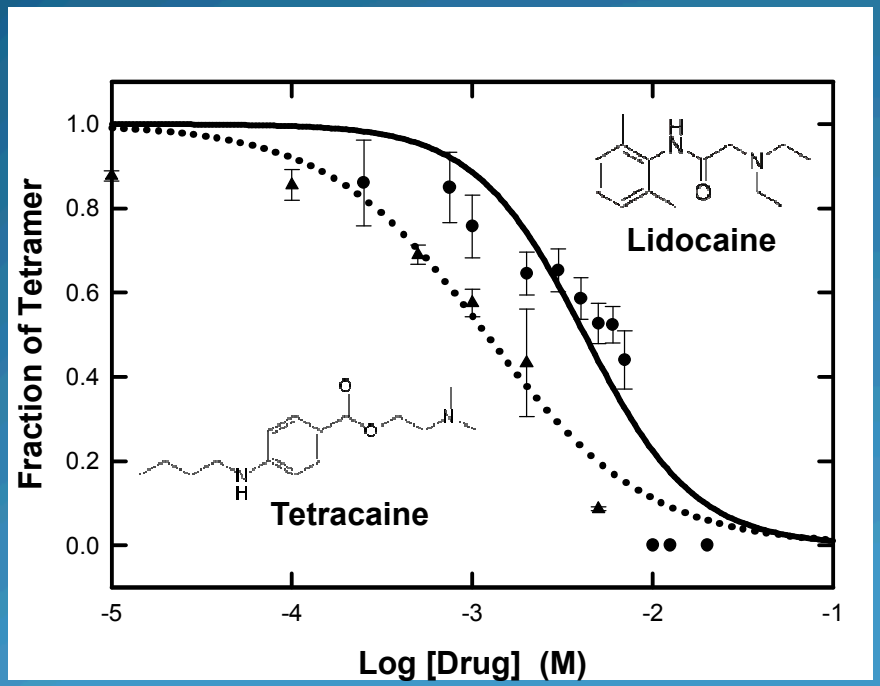
Krishnan et al. 2008. Biochemistry 47: 5354-5367

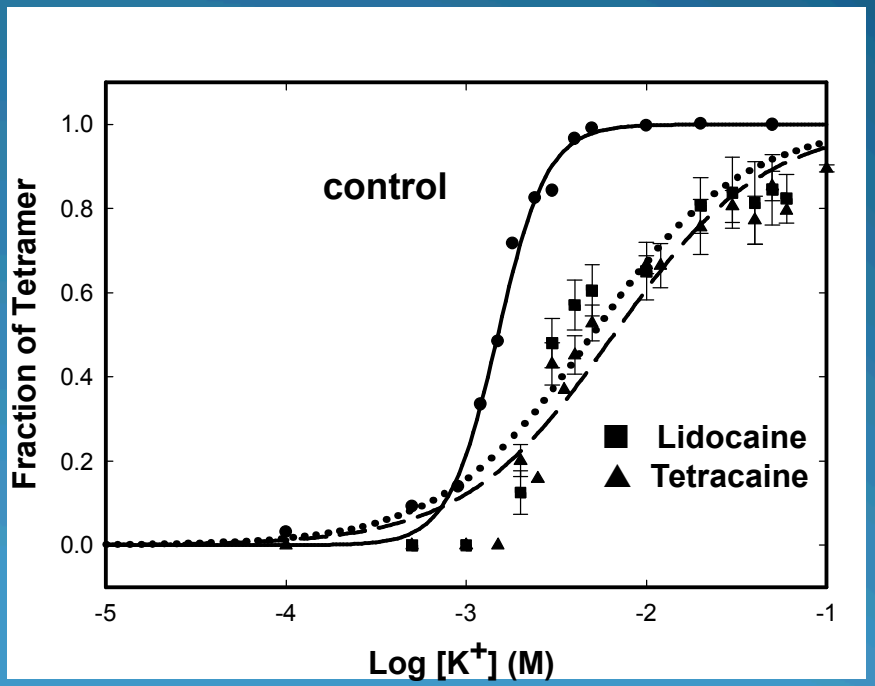
Effect of HFIP (hexafluoroisopropanol) on Tetramer Stability of KcsA: Cation **Dependence**



HFIP = $CF_3 - CH(OH) - CF_3$

- Local anesthetics destabilize tetramer stability of KcsA





The People Behind the Science

Manoj N Krishnan, PhD

Patrick Trombley, MS

Noel Gray, MS

Special Thanks to Lise Thomas (Heginbotham)

HAPPY 70th BIRTHDAY RAMON !!