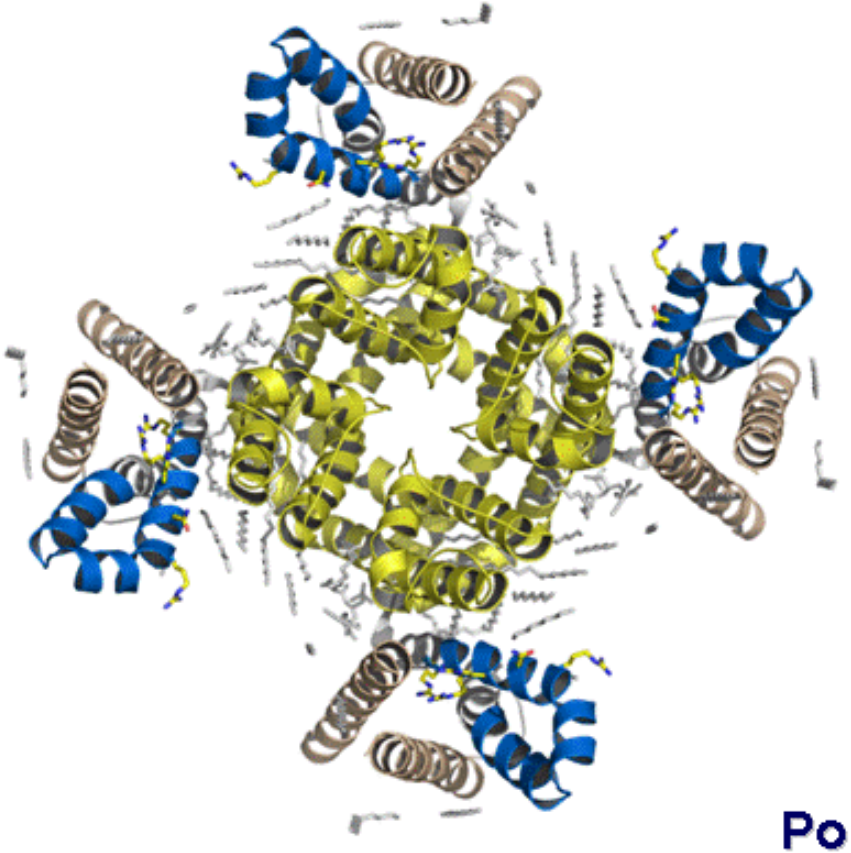
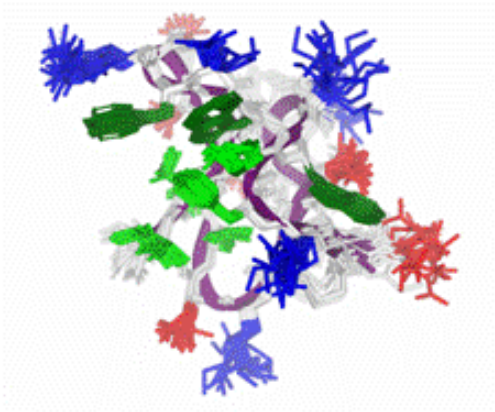
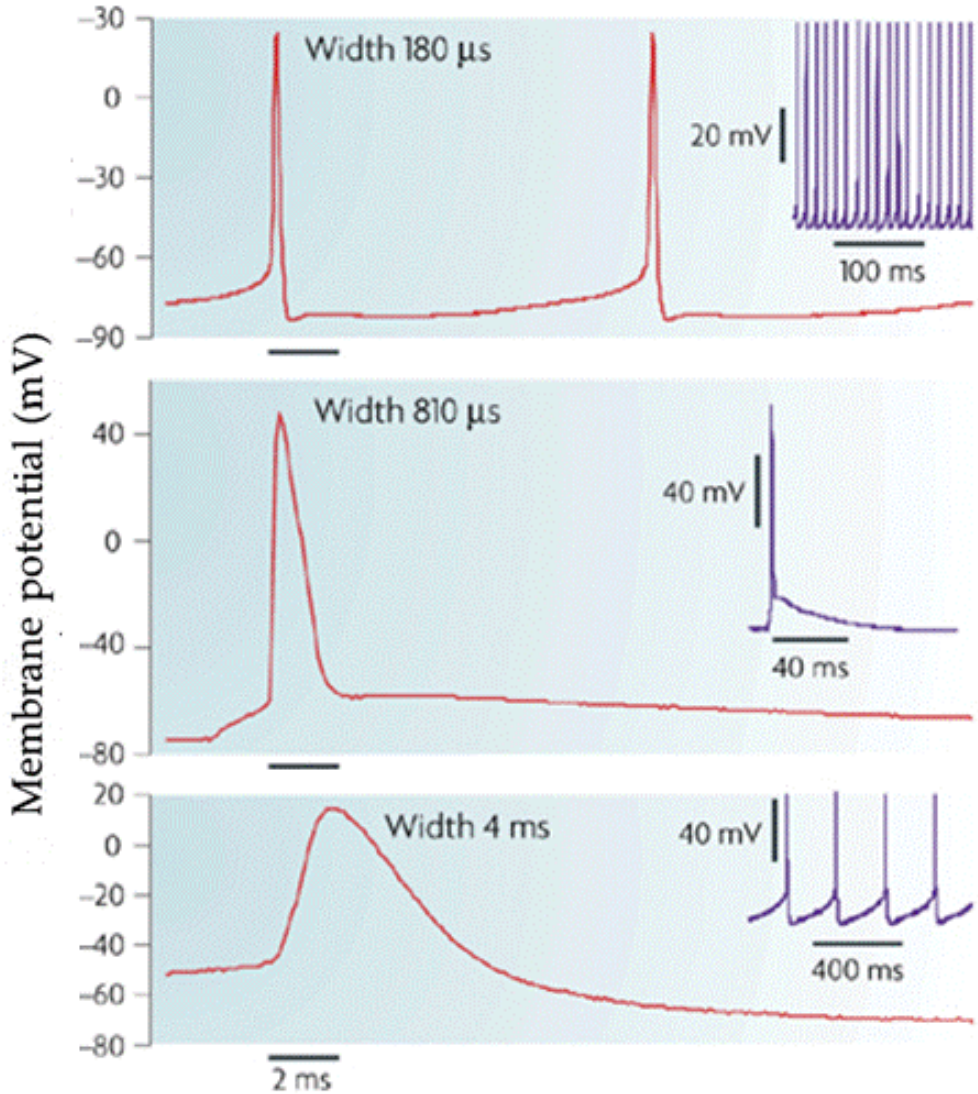
Venom toxins as molecular tools for ion channel research





Mirela Milescu
Porter Neuroscience Research Center
NINDS, NIH

Understanding electrical signaling in the brain



Purkinje neuron

Pyramidal neuron

Dopamine neuron

"Not all spikes are the same" – combination of Na^+, K^+, Ca^{2+} .....currents

Bean (2007), Nat Rev Neurosci, 8, 451

Understanding electrical signaling in the brain

- determine the molecular identity of specific ionic currents in native tissue

- determine the structural and biophysical properties of molecular complexes

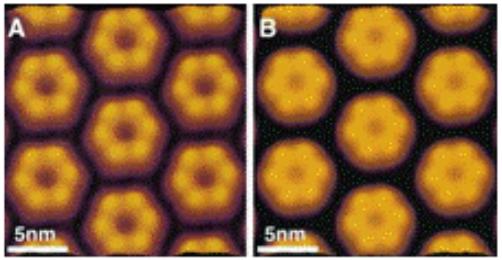
- functionally dissect neuronal networks (silencing specific neurons)

Molecular diversity of ion channels complexes

α subunits > 500

Hexameric

Gap junctions

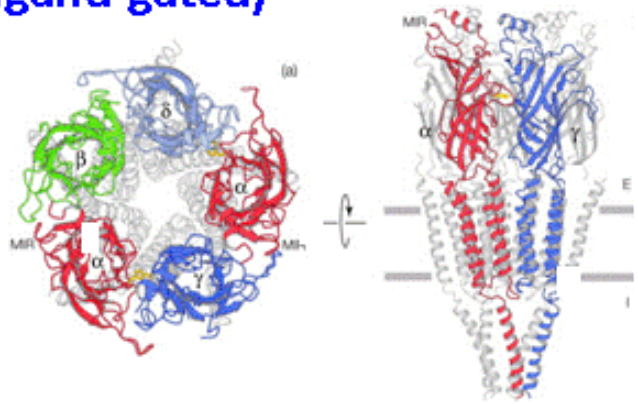


Trimeric (ligand-gated)

P2X
ASIC
ENaC

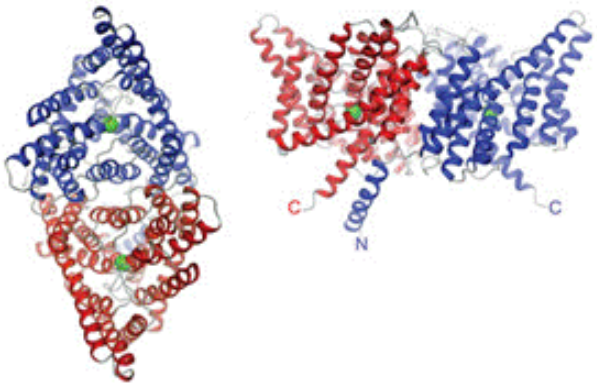
Pentameric (ligand-gated)

nAchR,
5HT,
GABA,
Glycine



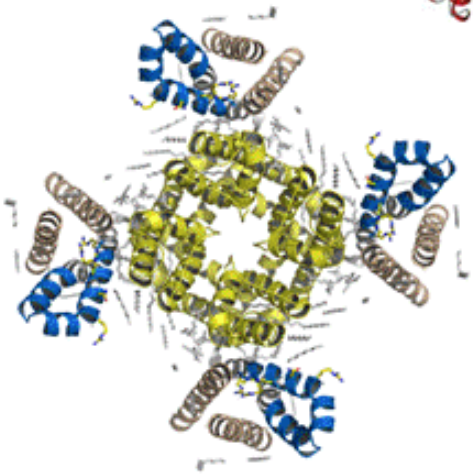
Dimeric Cl^- channels

ClC

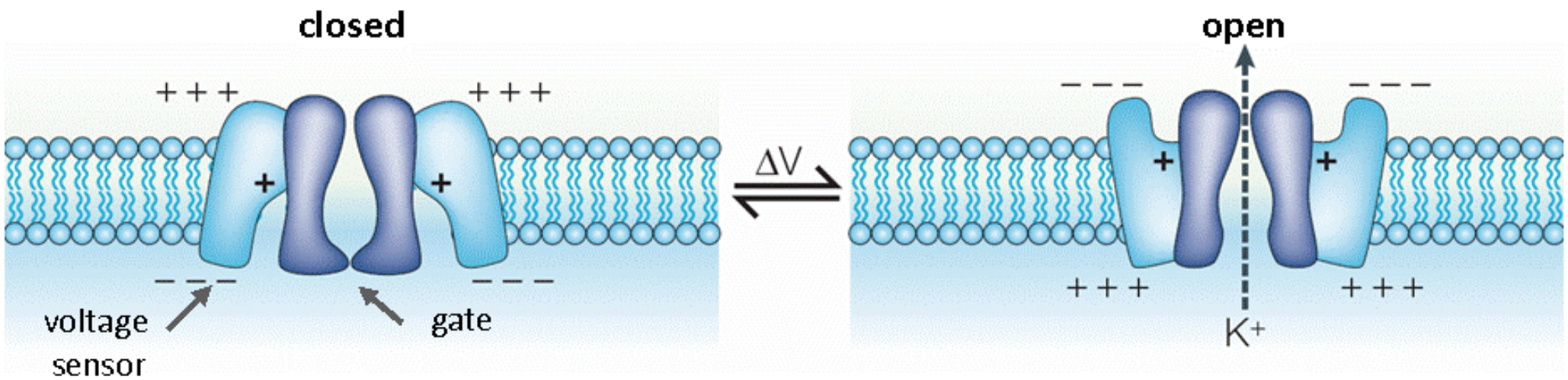


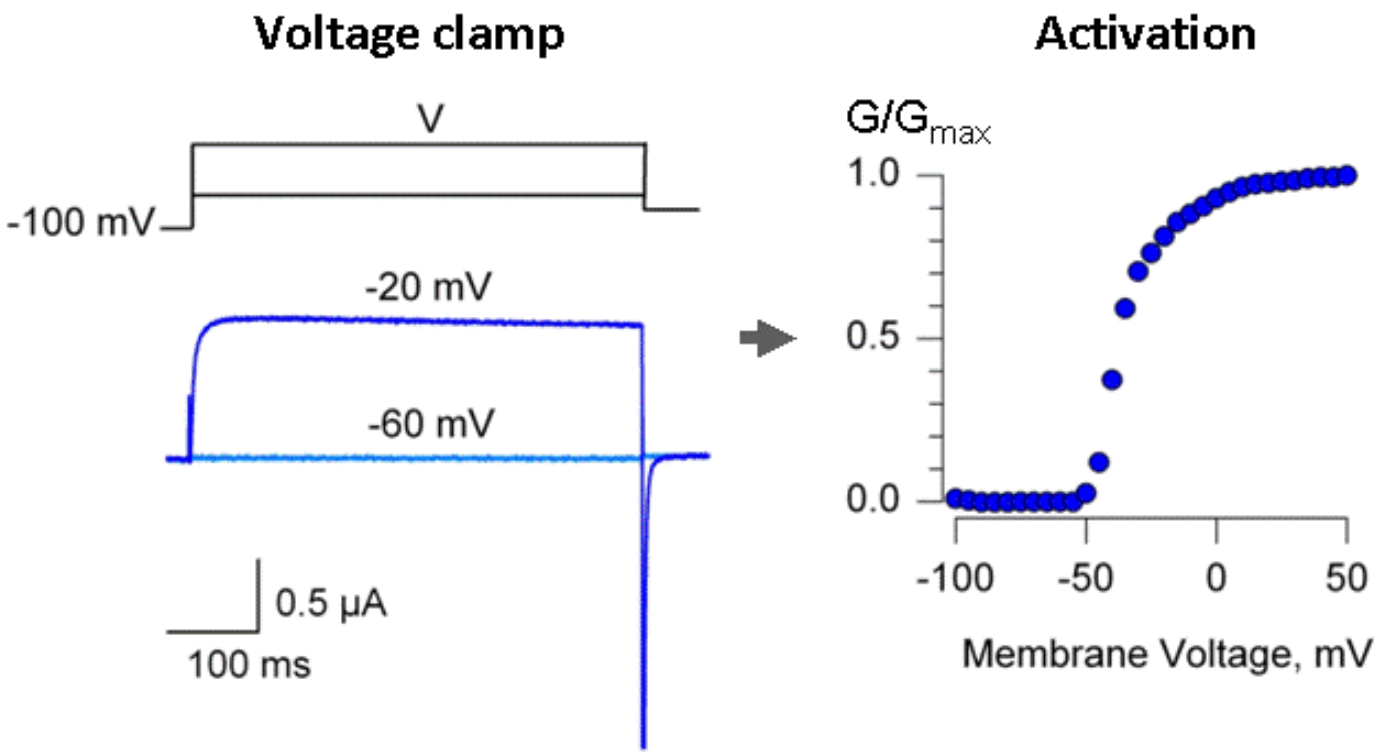
Tetrameric (ligand and voltage-activated)

2 TM	K_{ir}, K_{ATP}	24 TM	Nav
		24 TM	Cav
3 TM	Glutamate		
4 TM	K_{2P}	4 TM	Hv
6 TM	Kv, K_{Ca}, HCN CNG, TRP		



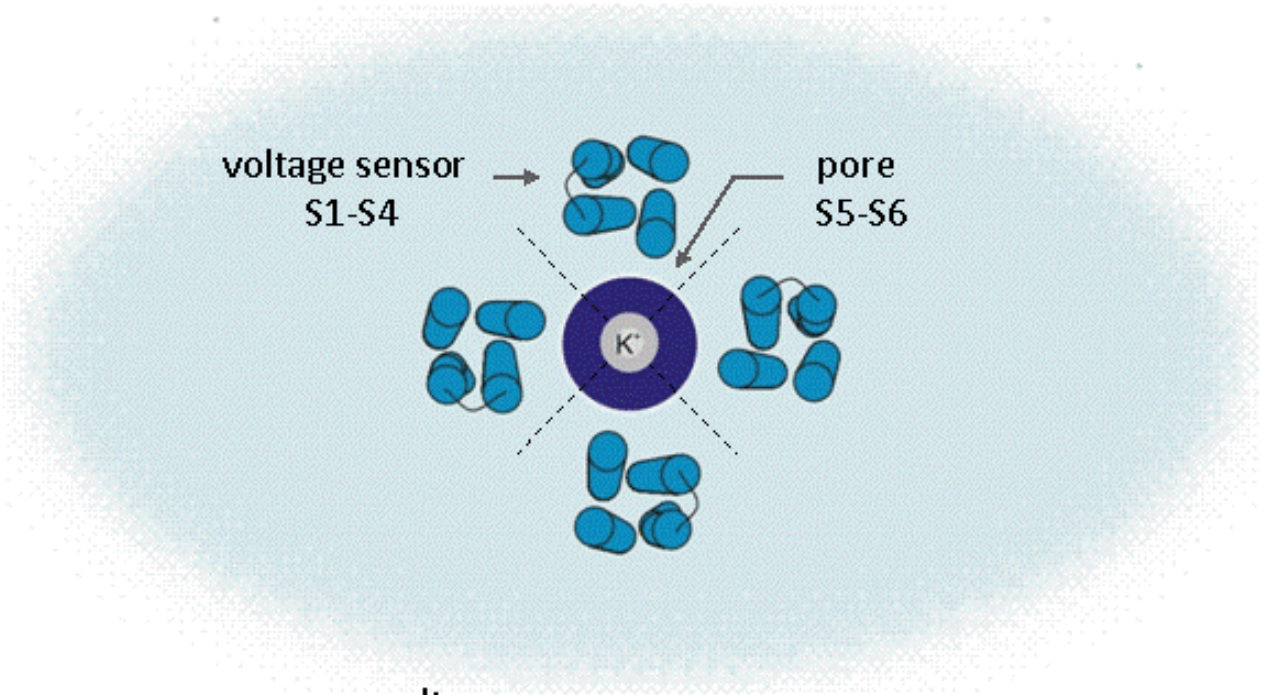
Voltage activated K^+ channels (K_v)



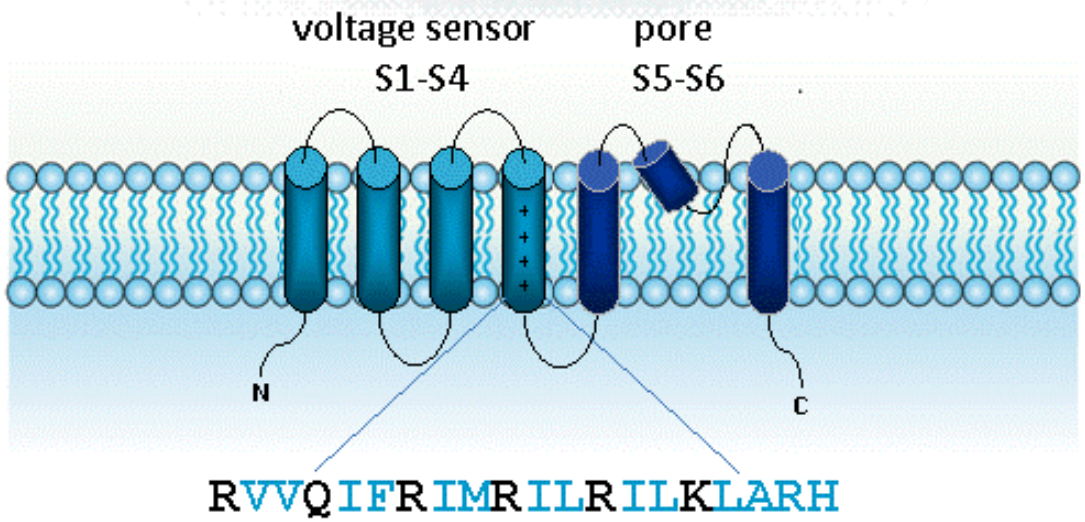


Architecture of voltage-activated K^+ channels (K_v)

top view

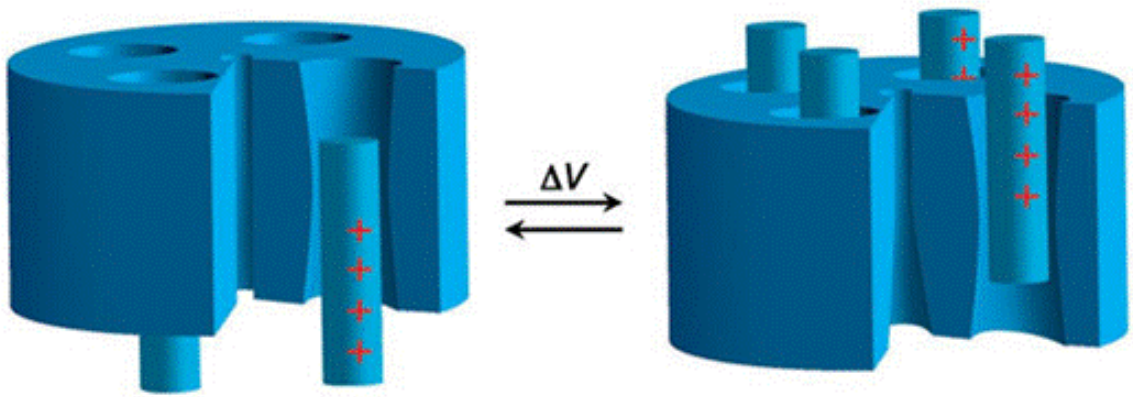


lateral view

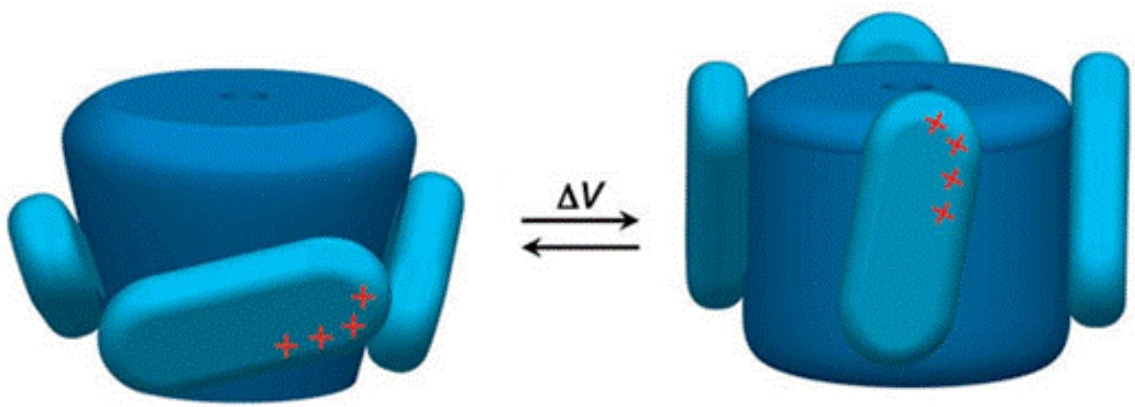


Proposed mechanisms for voltage sensing

Conventional Model

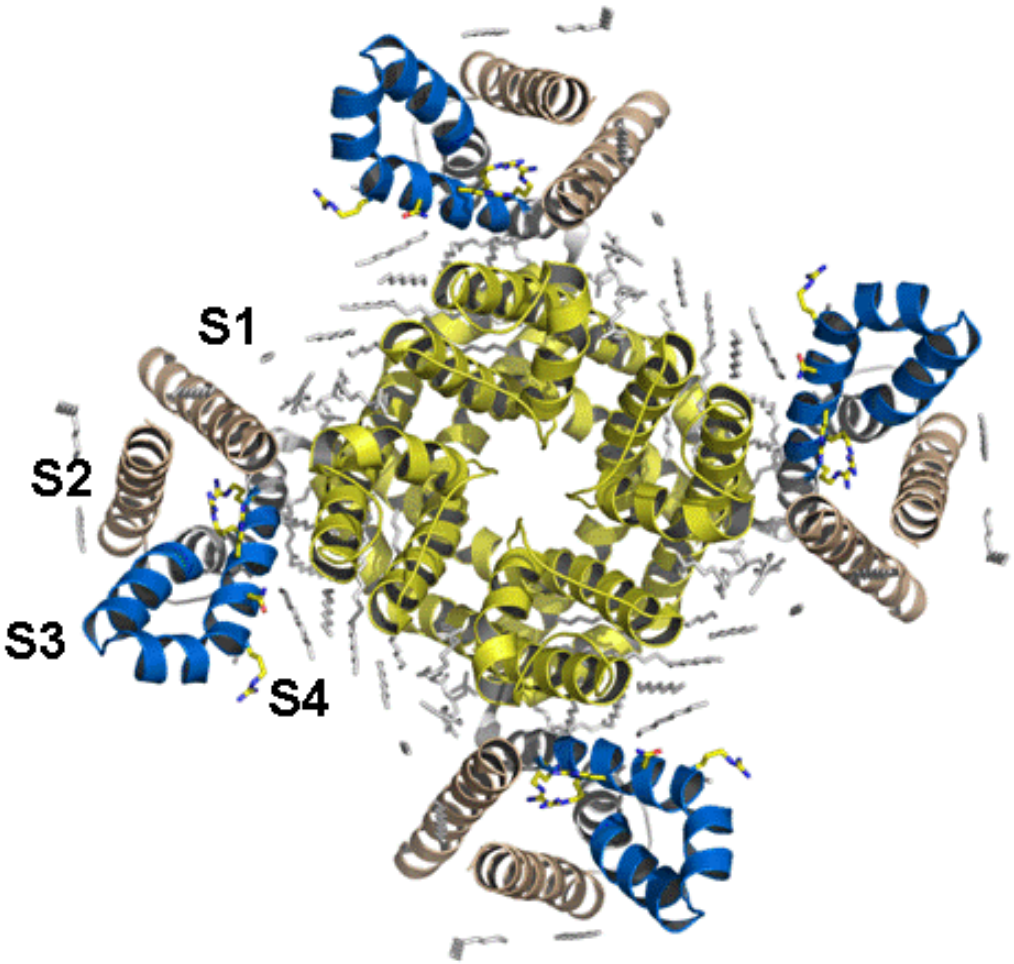


Paddle Model

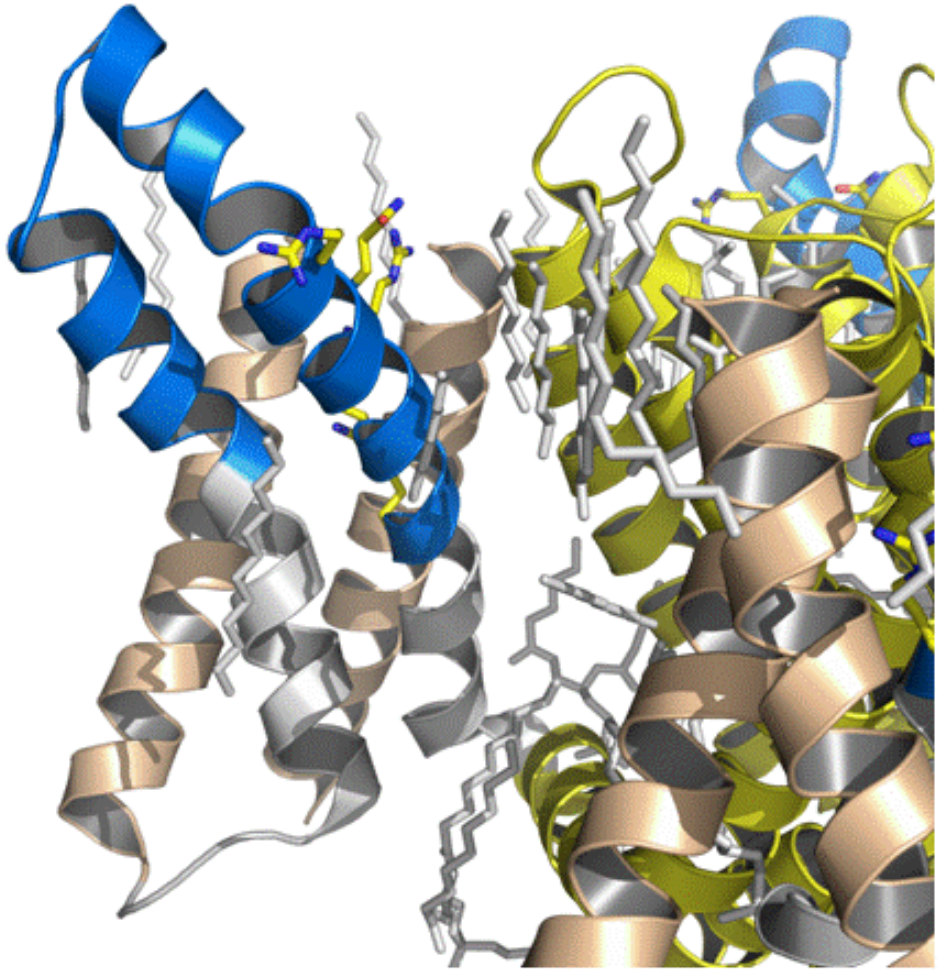


Yang et al. (1996) Neuron 16, 113
Starace and Bezanilla (2004) Nature 427, 548
Jiang et al. (2003) Nature 423, 33

X-ray structure of an eukaryotic Kv channel



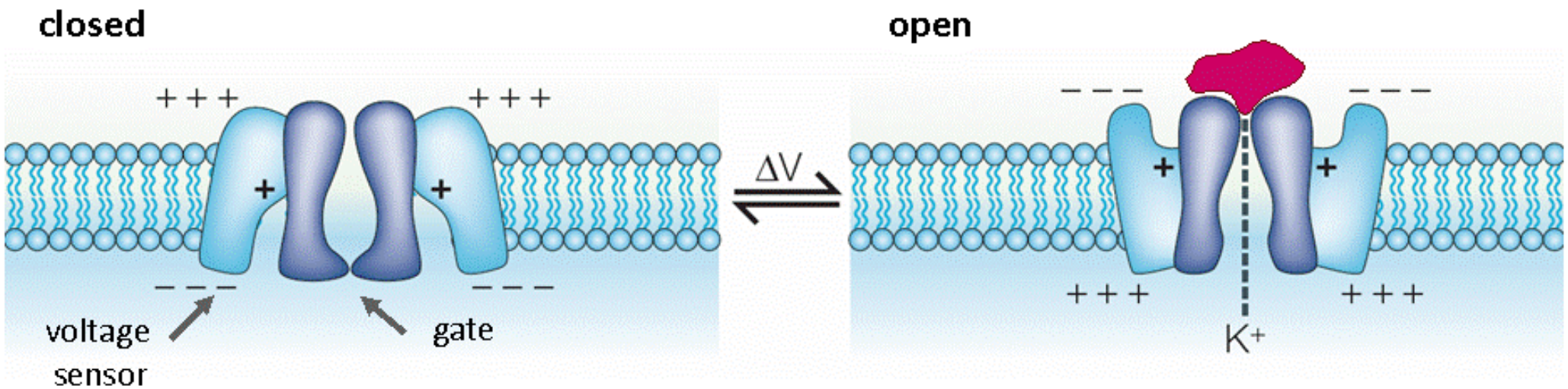
top view

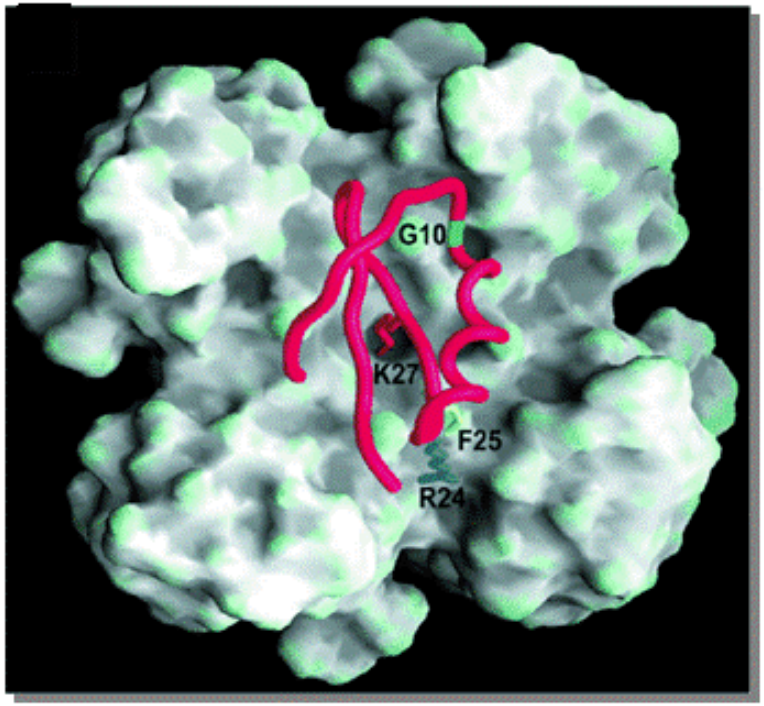


lateral view

Long et al. (2007) Nature 450, 376

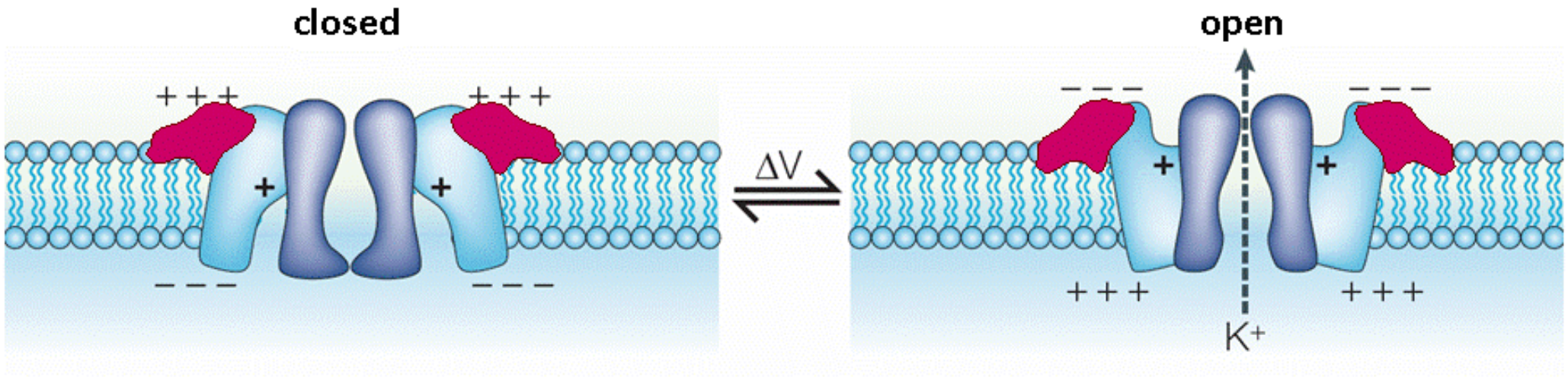
Pore blockers targeting voltage-activated K_v channels

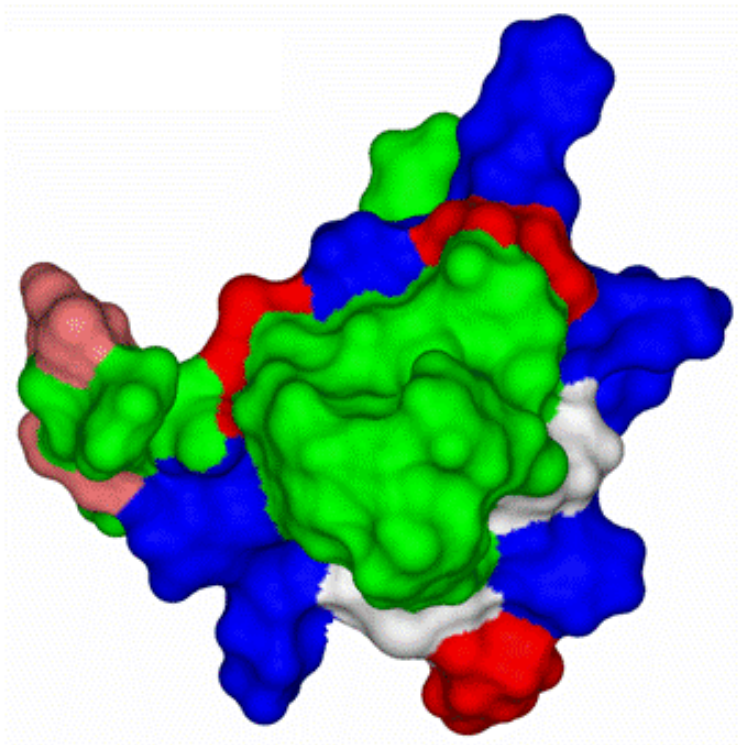




Garcia et al. (1994) Biochemistry 33, 6834

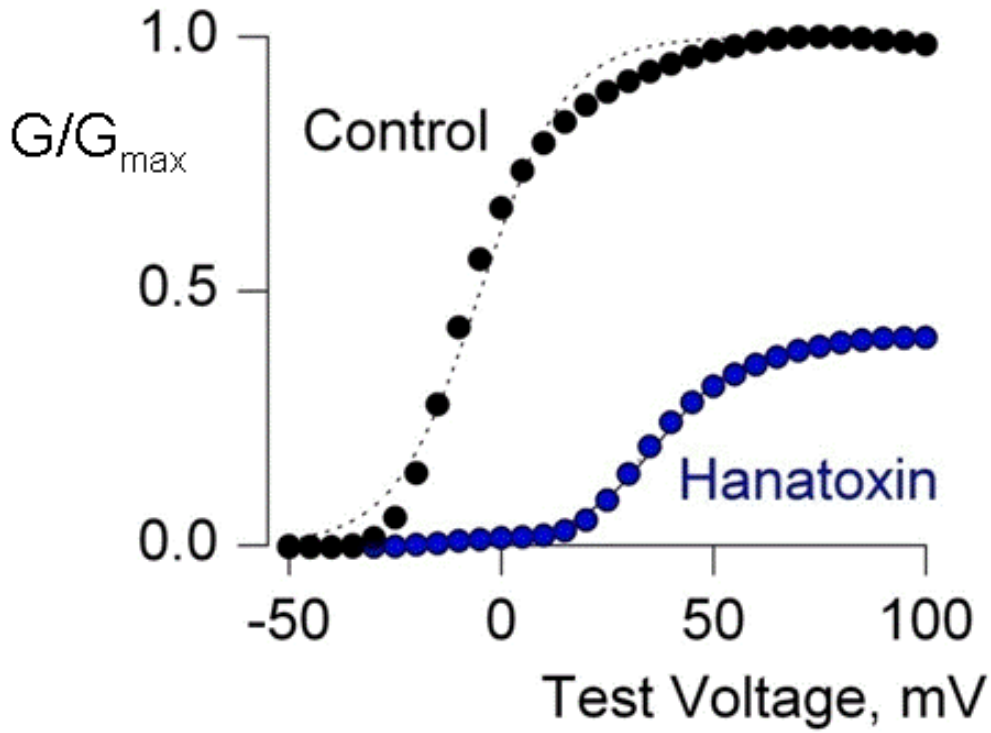
Gating modifiers





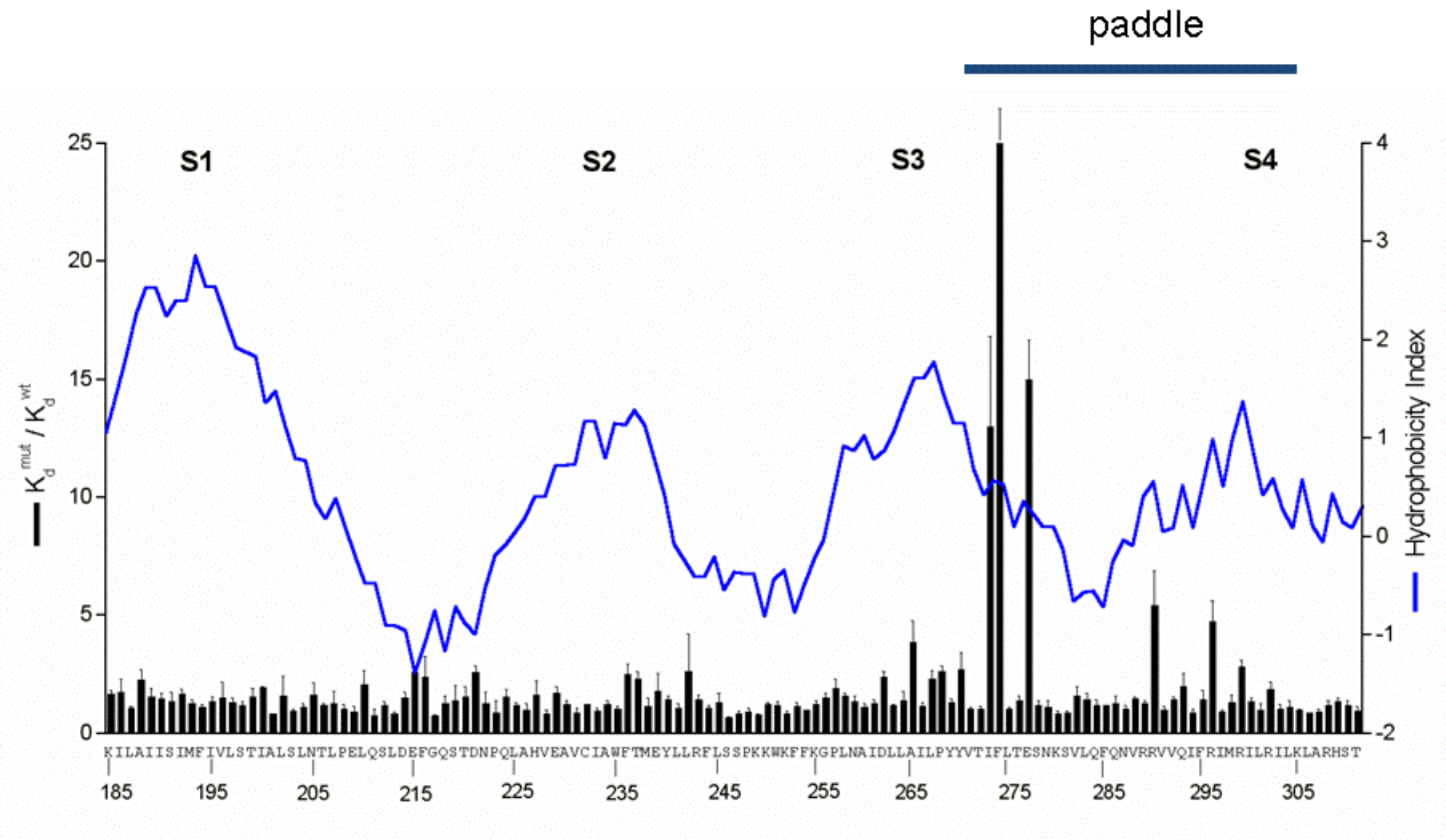
Swartz and MacKinnon (1995) Neuron 15, 941

Gating modifier toxins alter voltage-dependence activation of Kv 2.1 channels



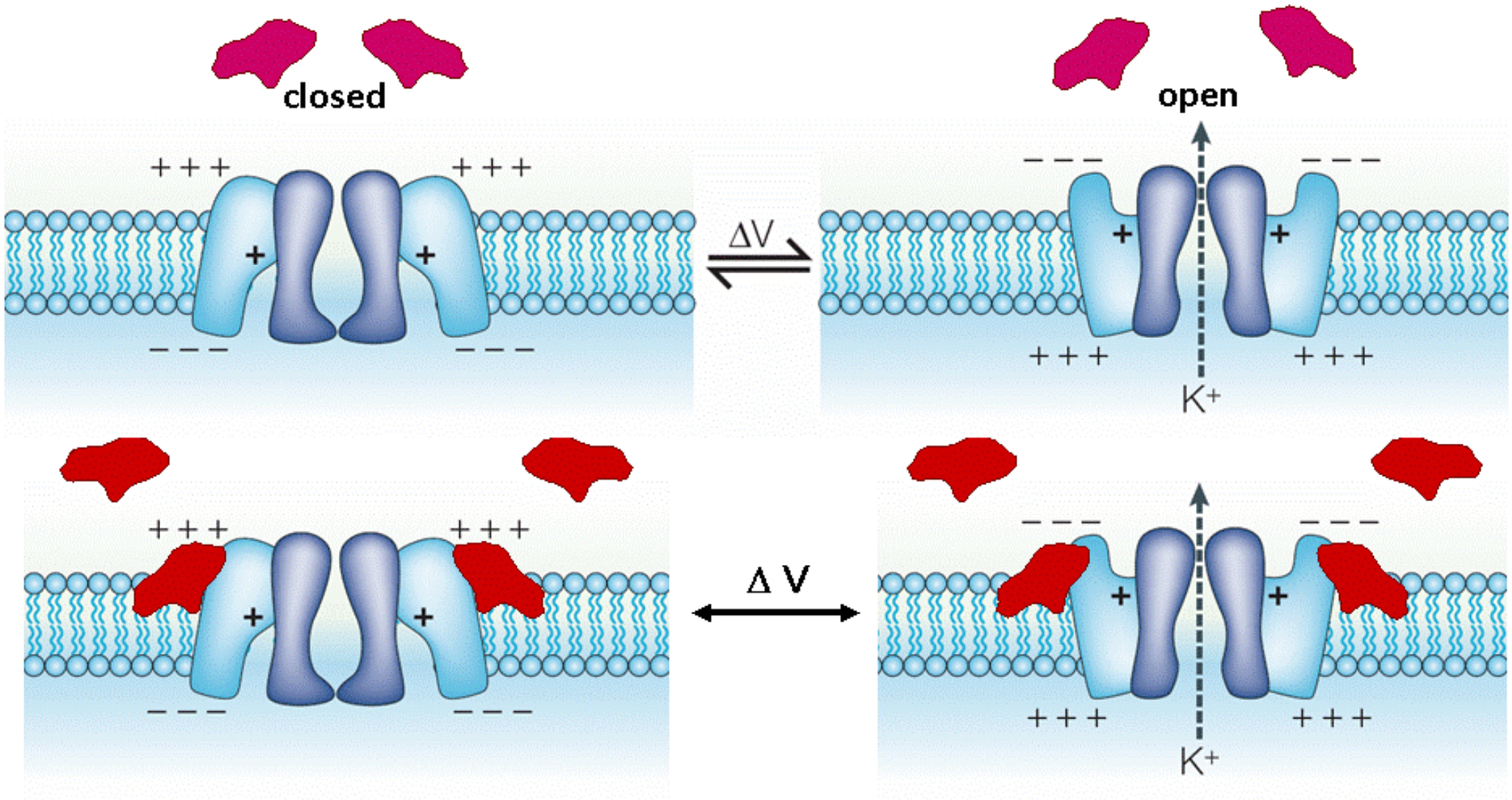
MacKinnon (1997) Neuron 18, 665

Gating modifier toxins bind to the voltage-sensor paddle motif



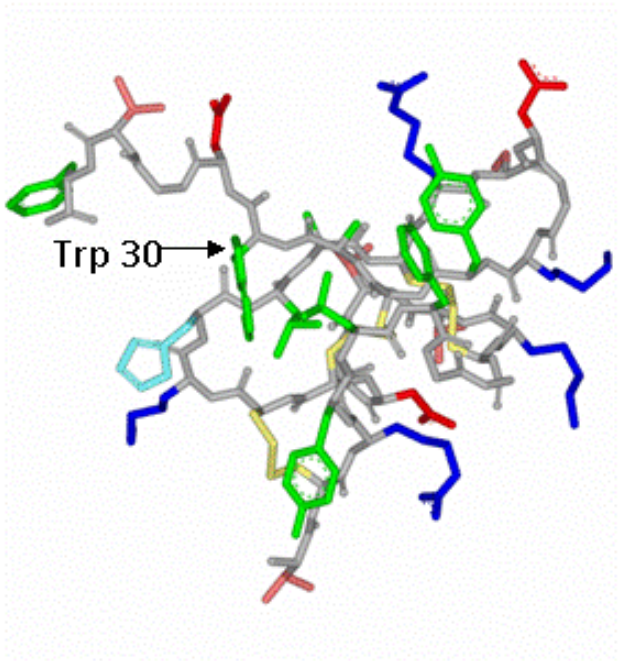
Li-Smerin and Swartz (2000) J Gen Physiol. 115, 673

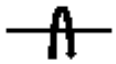
Is the voltage sensor paddle in contact with lipids?



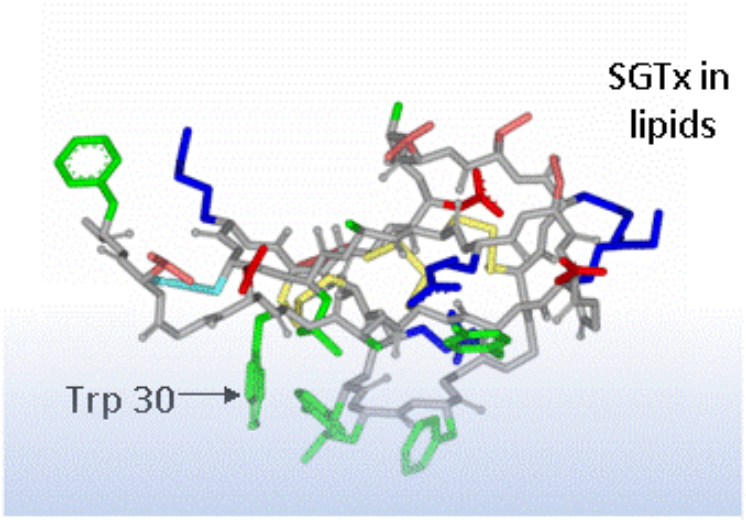
Gating modifier toxins partitioning into artificial lipid membranes

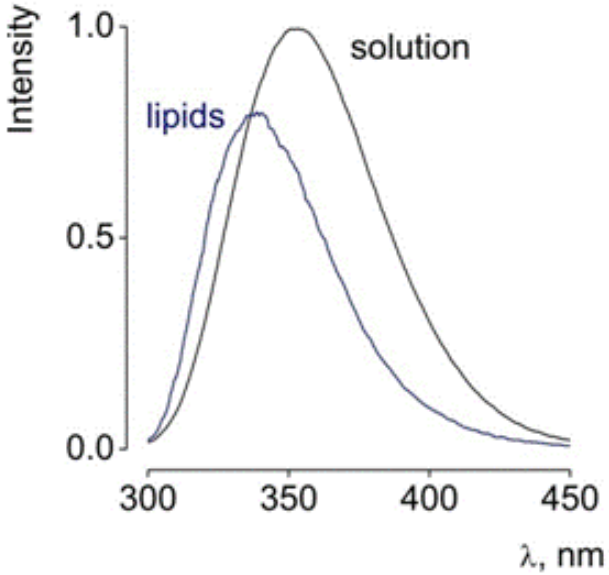
SGTx in solution

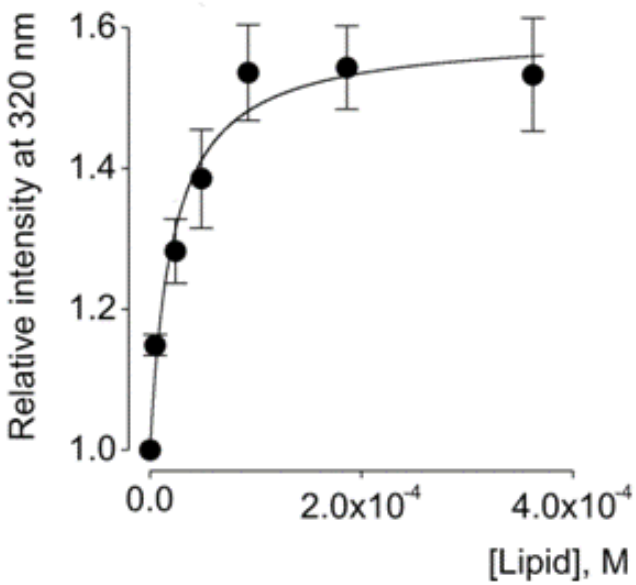




SGTx in lipids

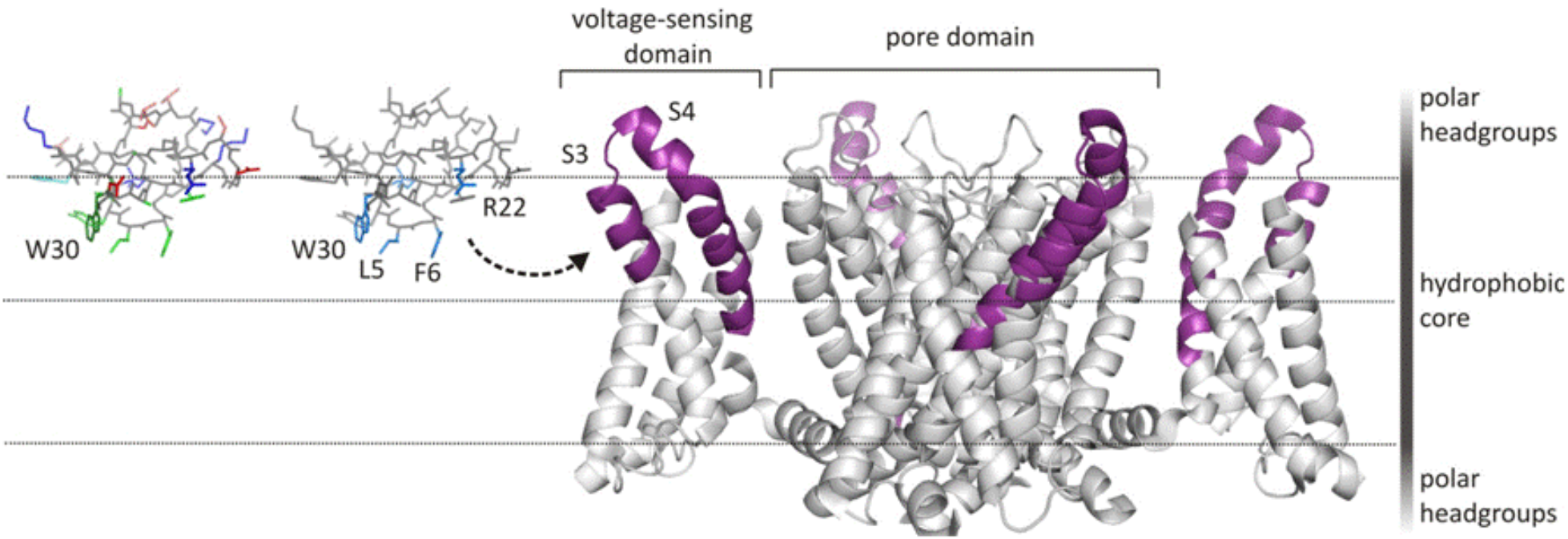




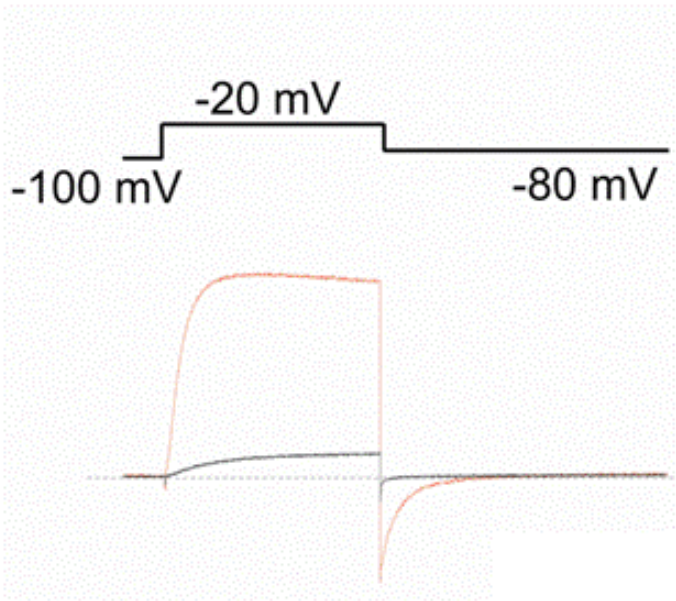


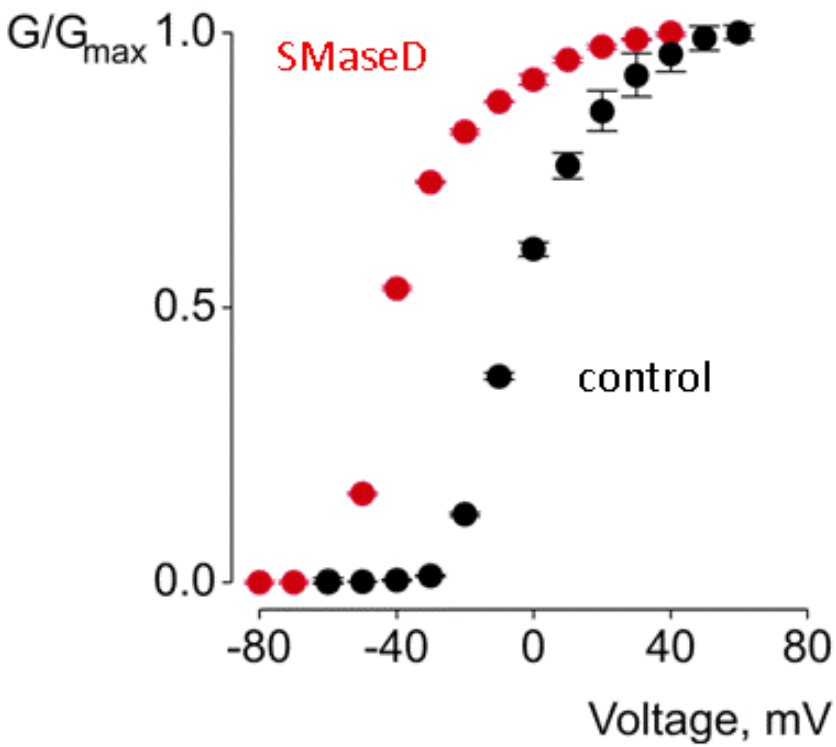
Philips and Milescu (2005) Nature

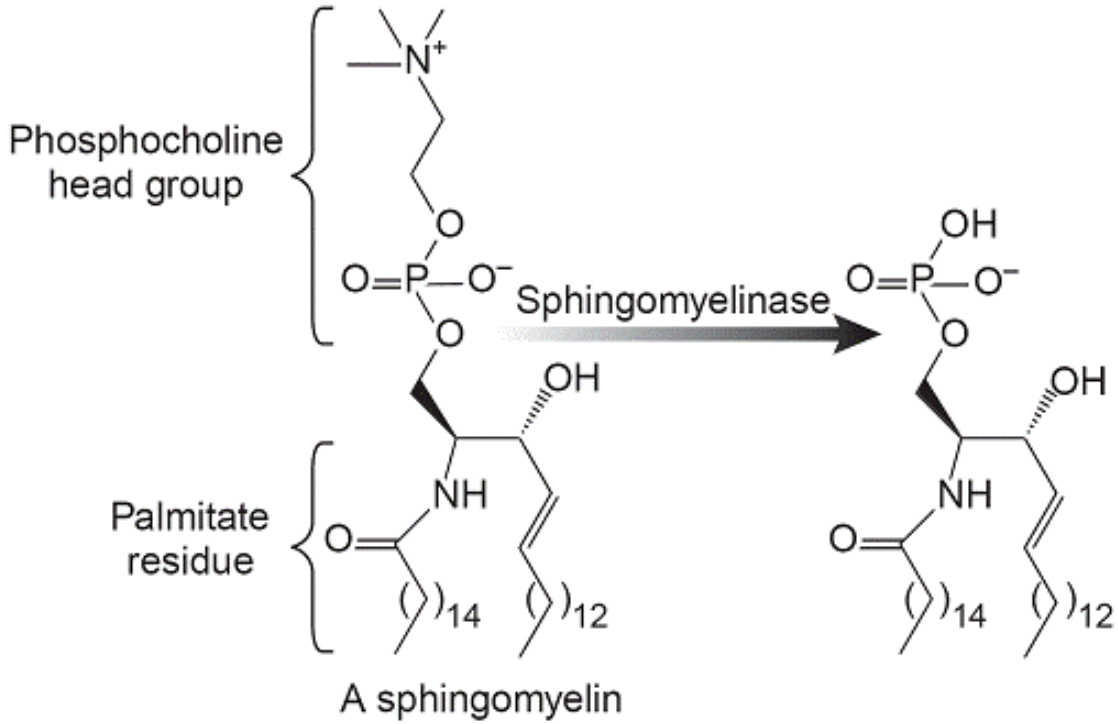
Gating modifier toxins bind to voltage sensor paddle within the membrane



Effect of lipid modifications on channel function

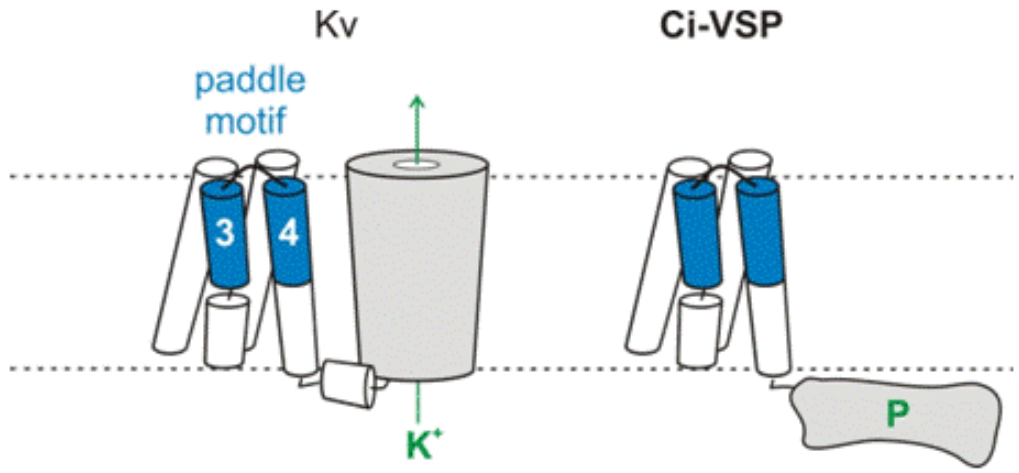


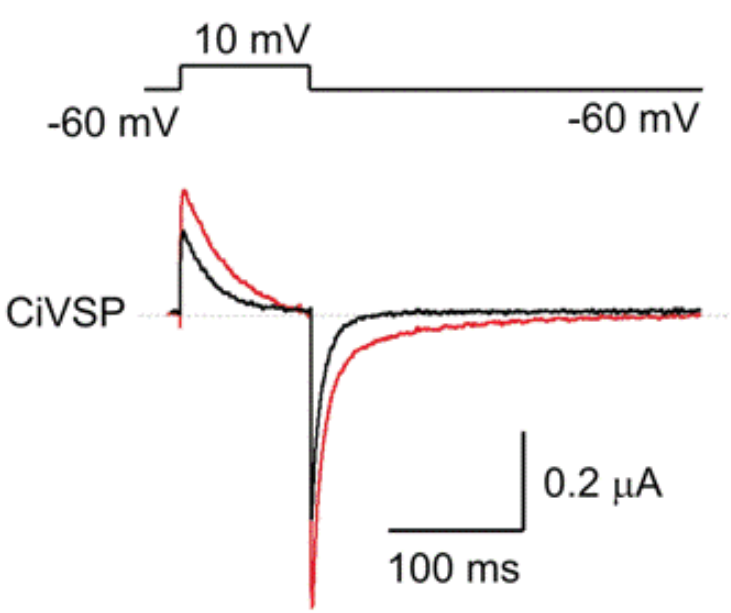


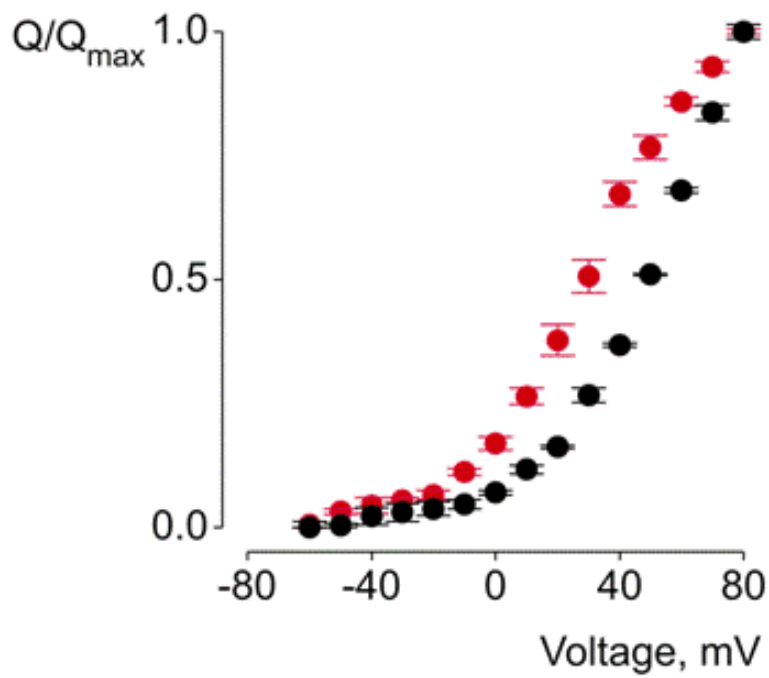


Ramu et al. (2006) Nature 442, 696

Structural determinants responsible for effect of lipid modifications

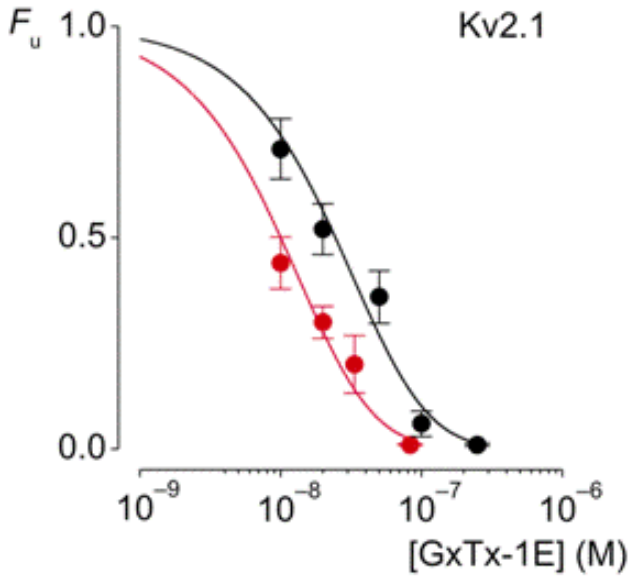


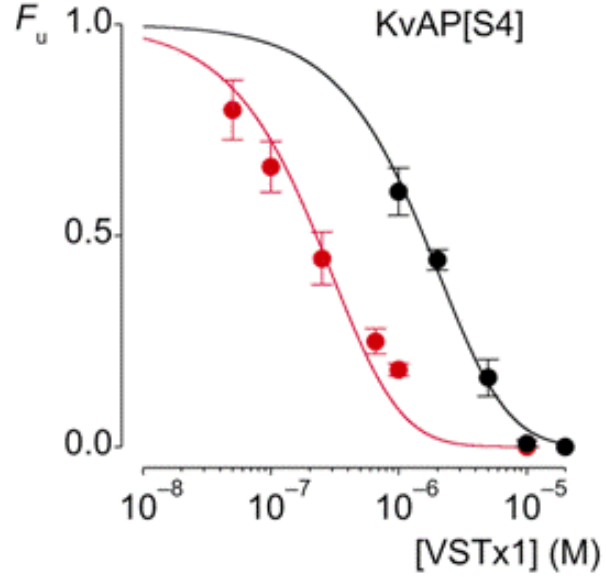


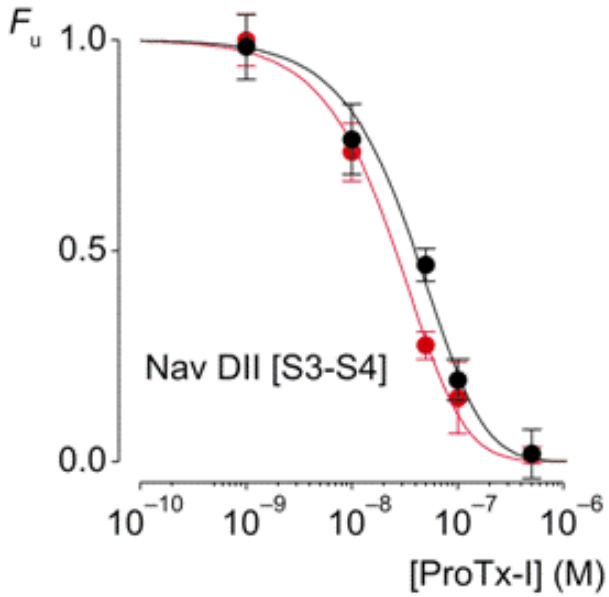


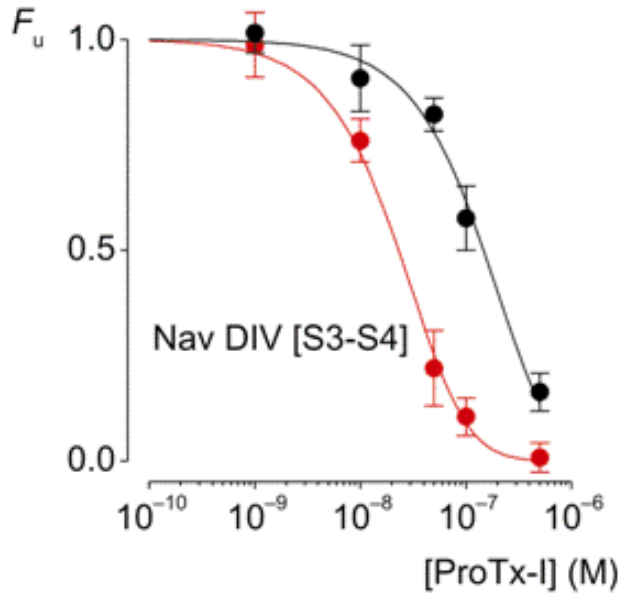
Milescu et al (2009) Nature

Effect of lipid modifications on voltage sensor pharmacology

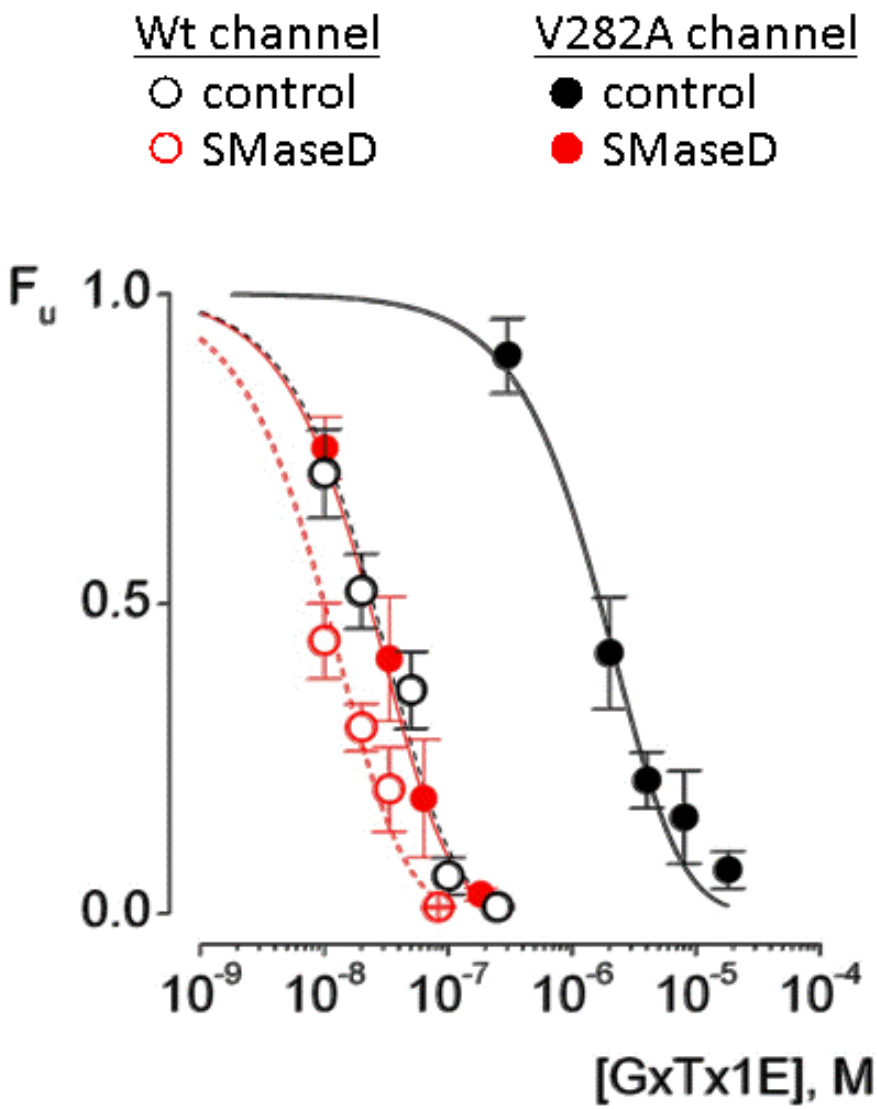




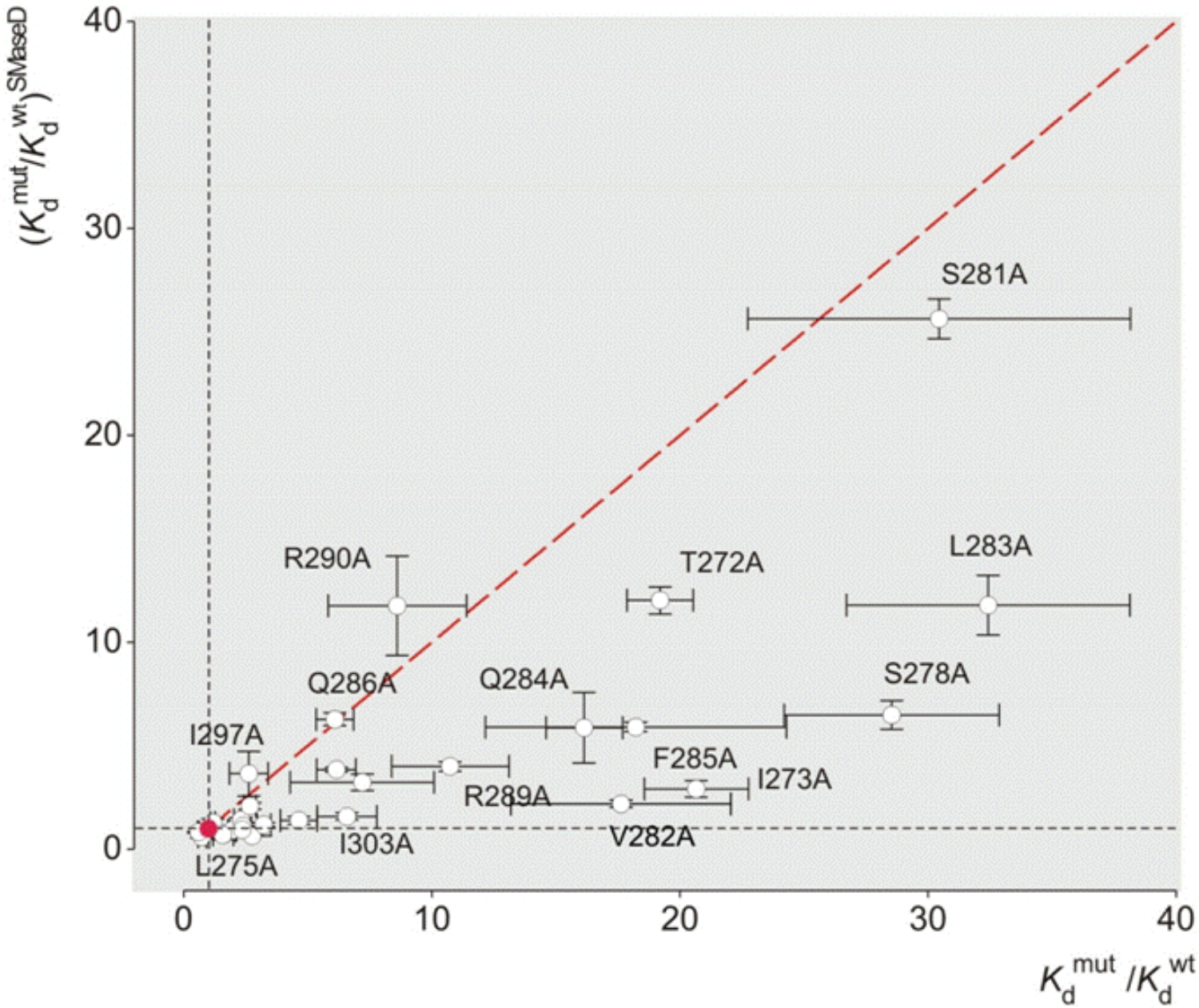


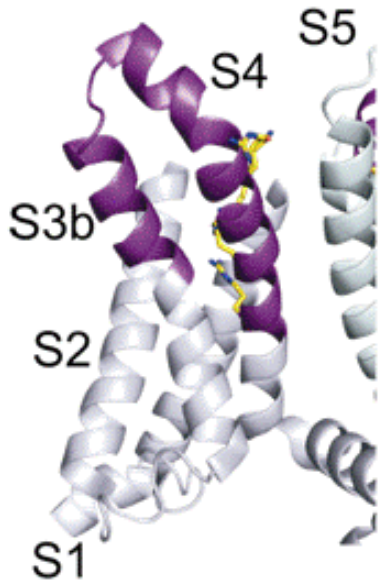


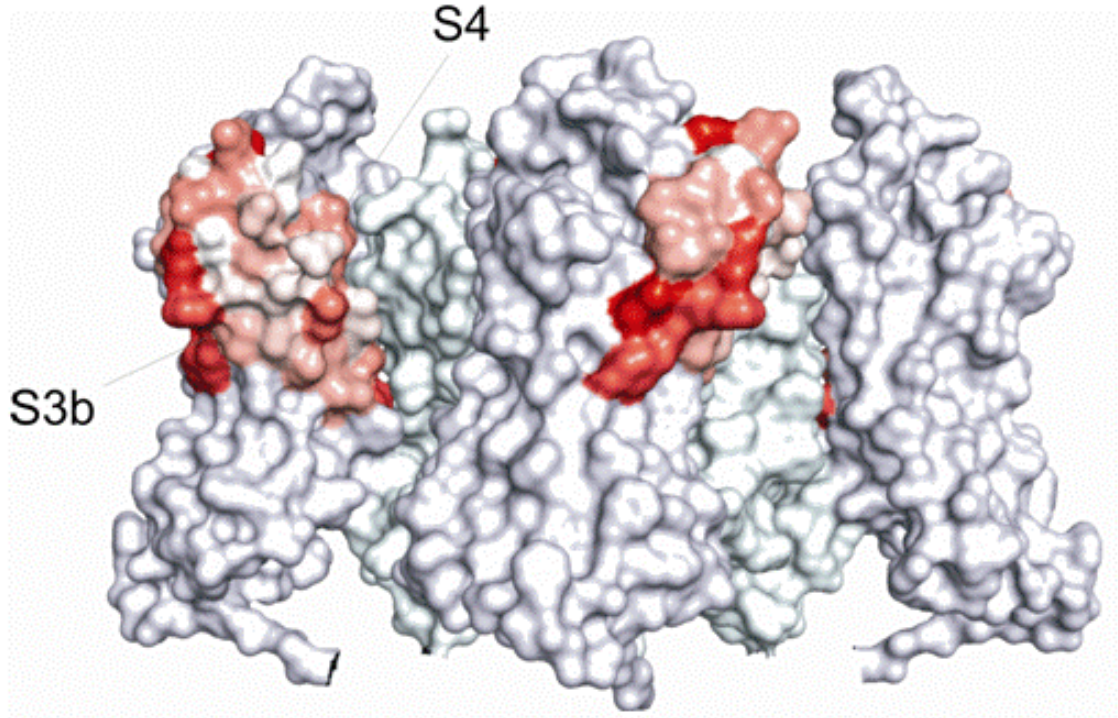
Toxins as detectors of lipid-paddle interactions

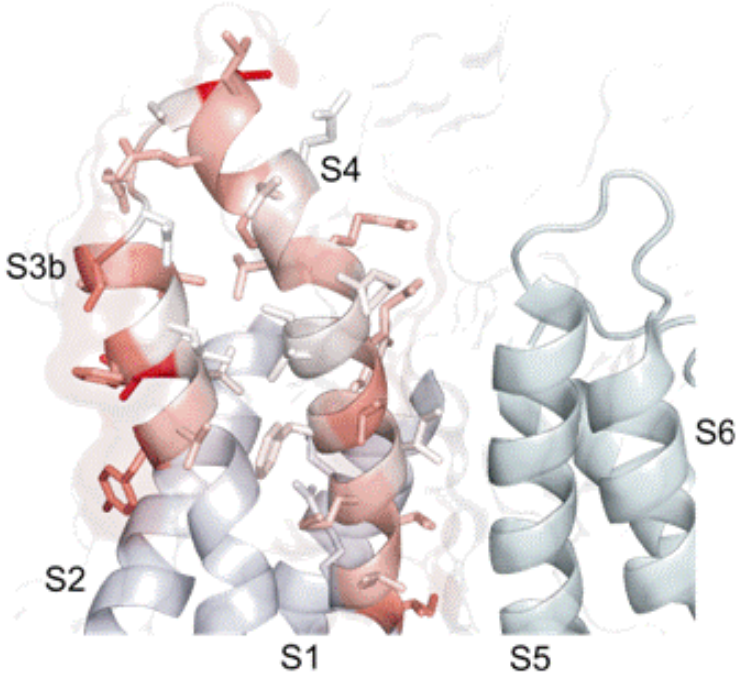


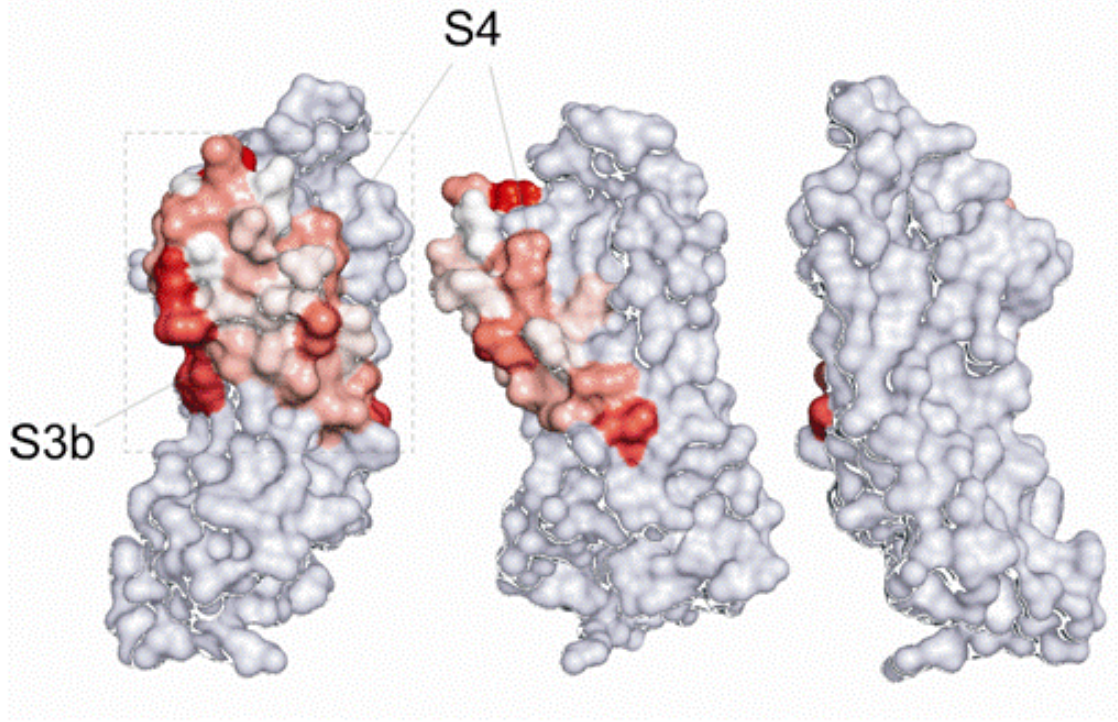
Coupling between lipid modification and paddle mutations



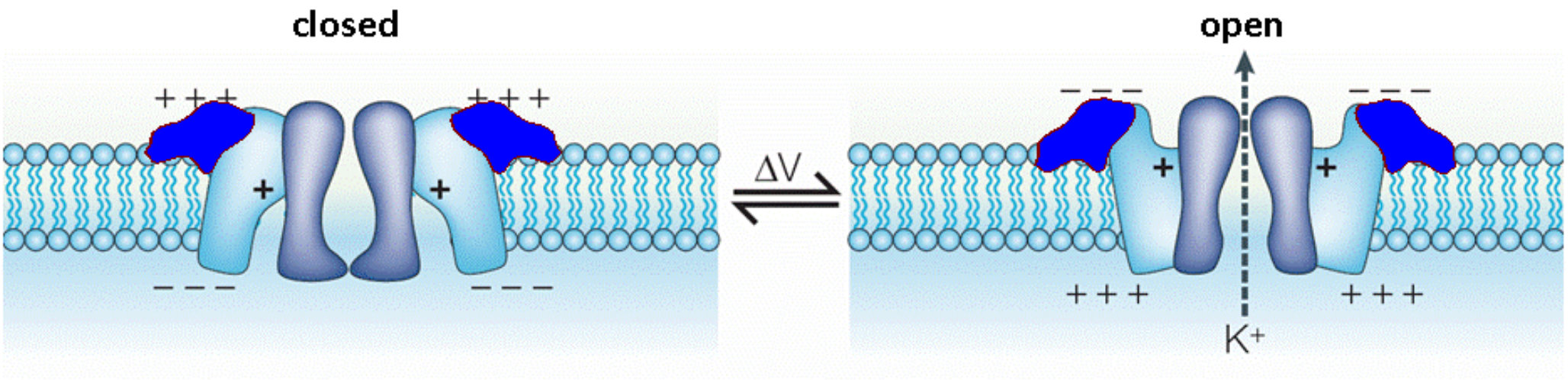

S5
S4
S3b
S2
S1

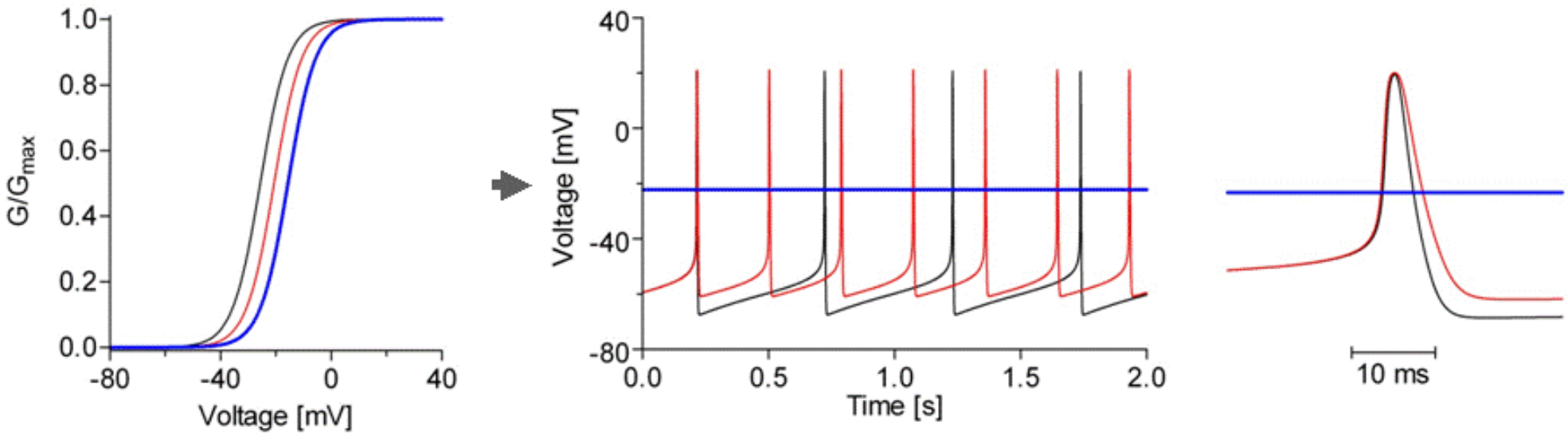

S4
S3b


S4
S3b
S6
S2
S1
S5


S4
S3b

Potential research and therapeutic use





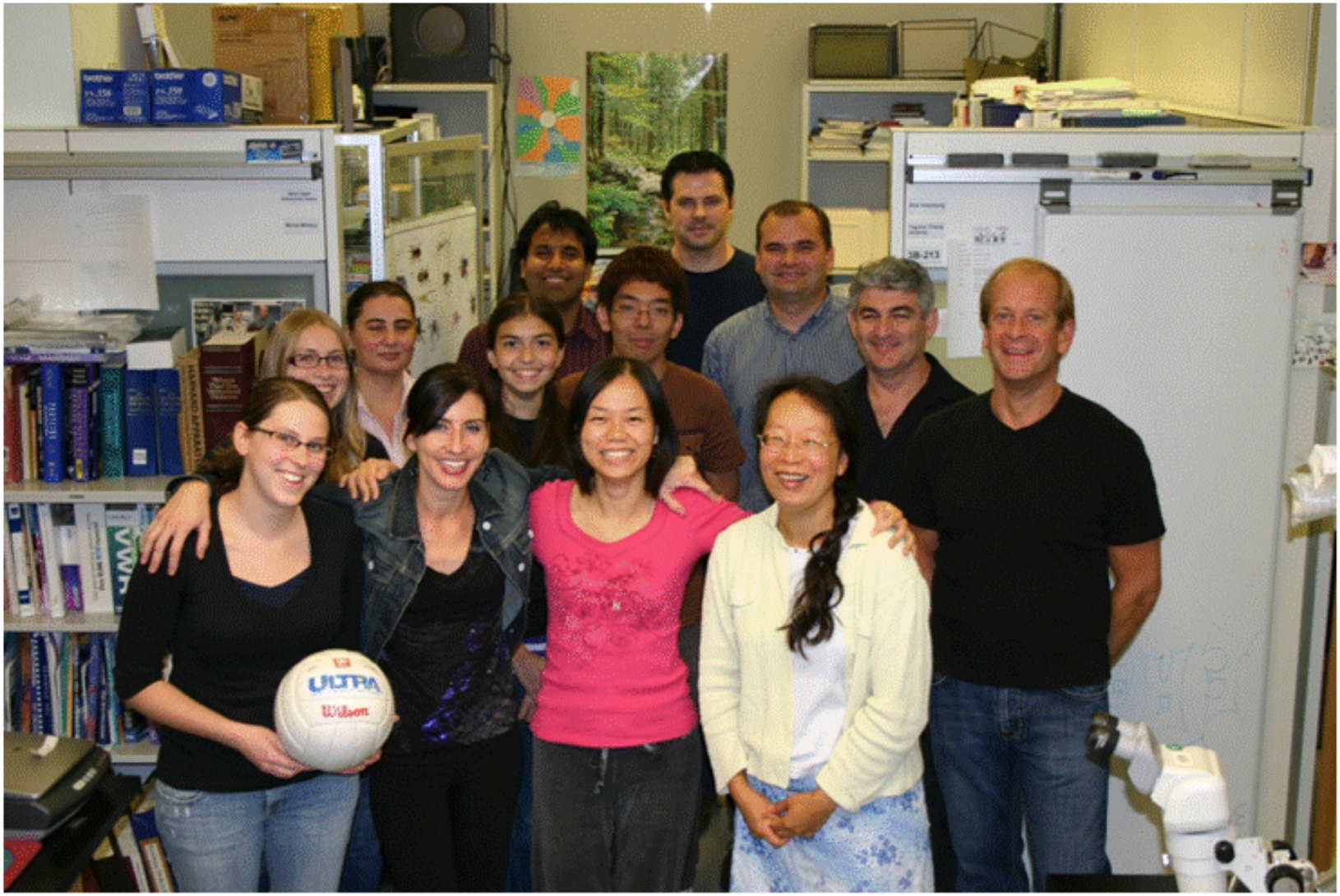
Conclusions

- In physiological membranes the voltage sensor paddle is lipid-exposed and moves at the channel-lipid interface - consistent with the new ideas emerging from the structural data.

- Specific lipid-channel interaction shape the function and pharmacology of the channel (channel-lipids as a functional unit!).

- Gating modifier toxins act through a membrane pathway.
  - Protein-protein interactions as well as protein-lipid interactions are required for their activity.
  - They can be used to probe local lipid environment and even protein-protein interactions between transmembrane proteins (e.g. channel and auxiliary subunits).

Acknowledgements



Kenton Swartz lab, NINDS, NIH