Elucidating protein function and mechanism

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Assistant Member, Structural Biology Program

GSK Core Course

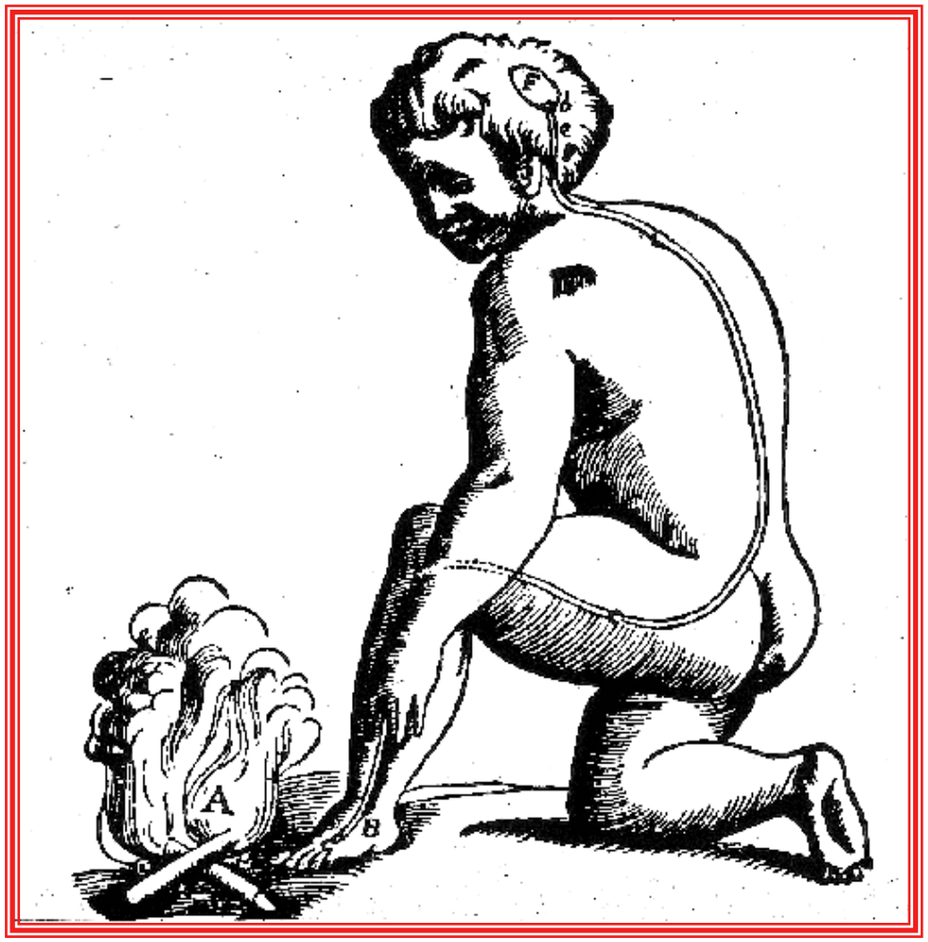
November 12, 2025

Learning Objective: To explore how protein function and mechanism can be elucidated using the discovery and subsequent characterization of somatosensory ion channels as an example.

Pain

Warning and Protective System

- Signals Tissue Injury
- Initiates Protective Reflexes



Plasticity

- Subject to Variation (Often Enhanced)
- Acute and Chronic Phases

Societal

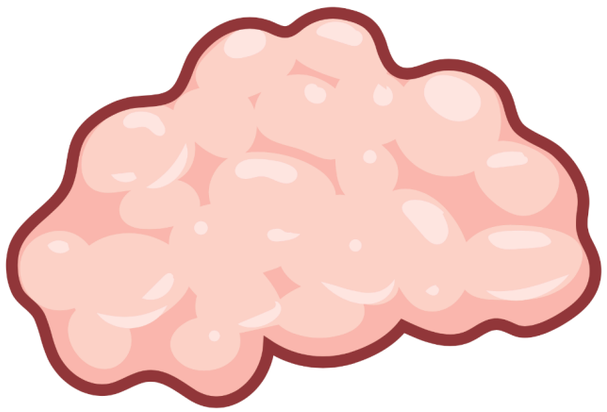
- Most Common Reason for Seeking Medical Help
  (Affects 100 Million People in the United States)

Cancer-related Pain Afflicts the Majority of Cancer Patients

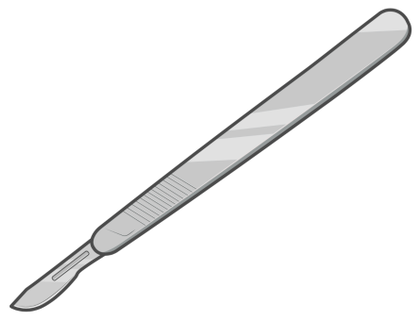
Neuropathic Cancer Pain

Caused by Nerve Damage

Tumor



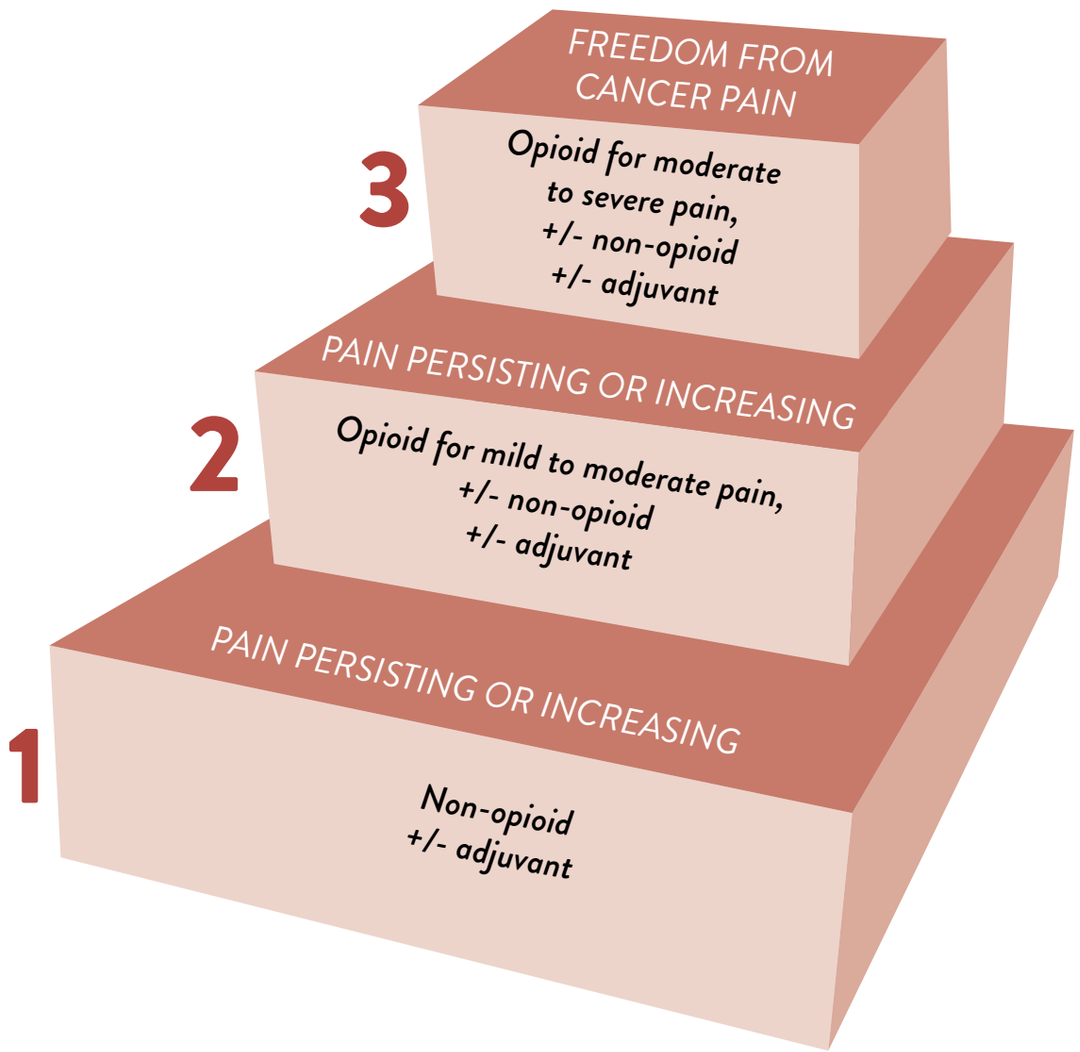
Surgery



Radiation

Chemotherapy

Inadequately controlled in ~50% of suffers.

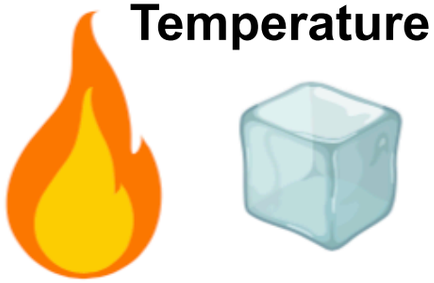


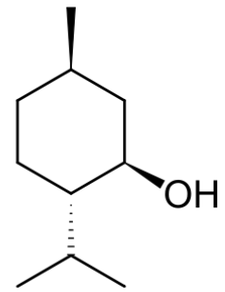
World Health Organization. 2018.

Nociceptors Facilitate the Detection and Response to Environmental Signals

Itch

Temperature

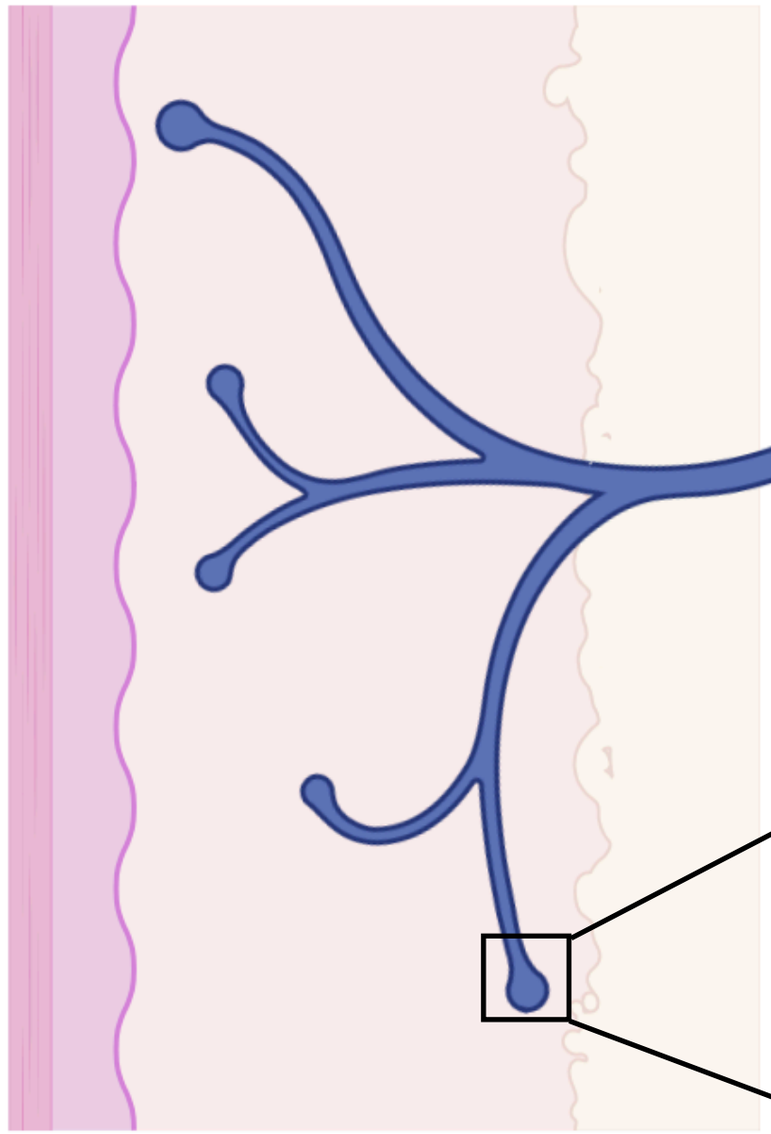




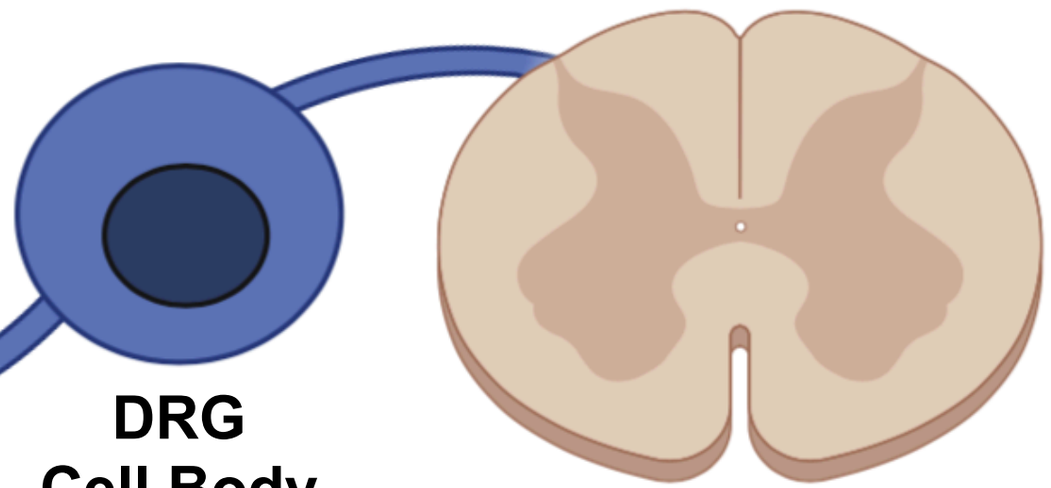
Chemical

Mechanical

Innocuous or Painful Stimuli

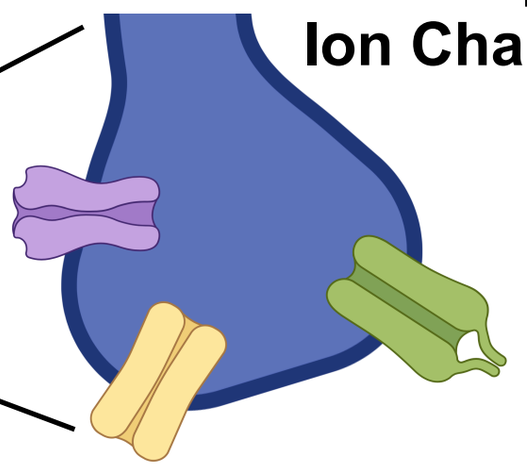


Spinal Cord

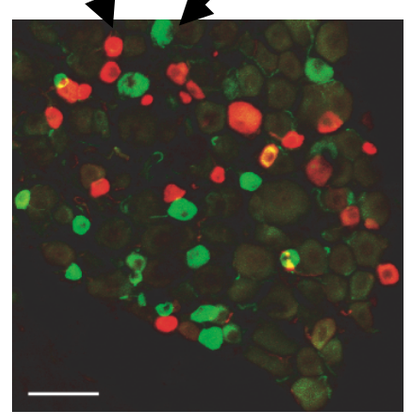


DRG
Cell Body

Nociceptive Ion Channels

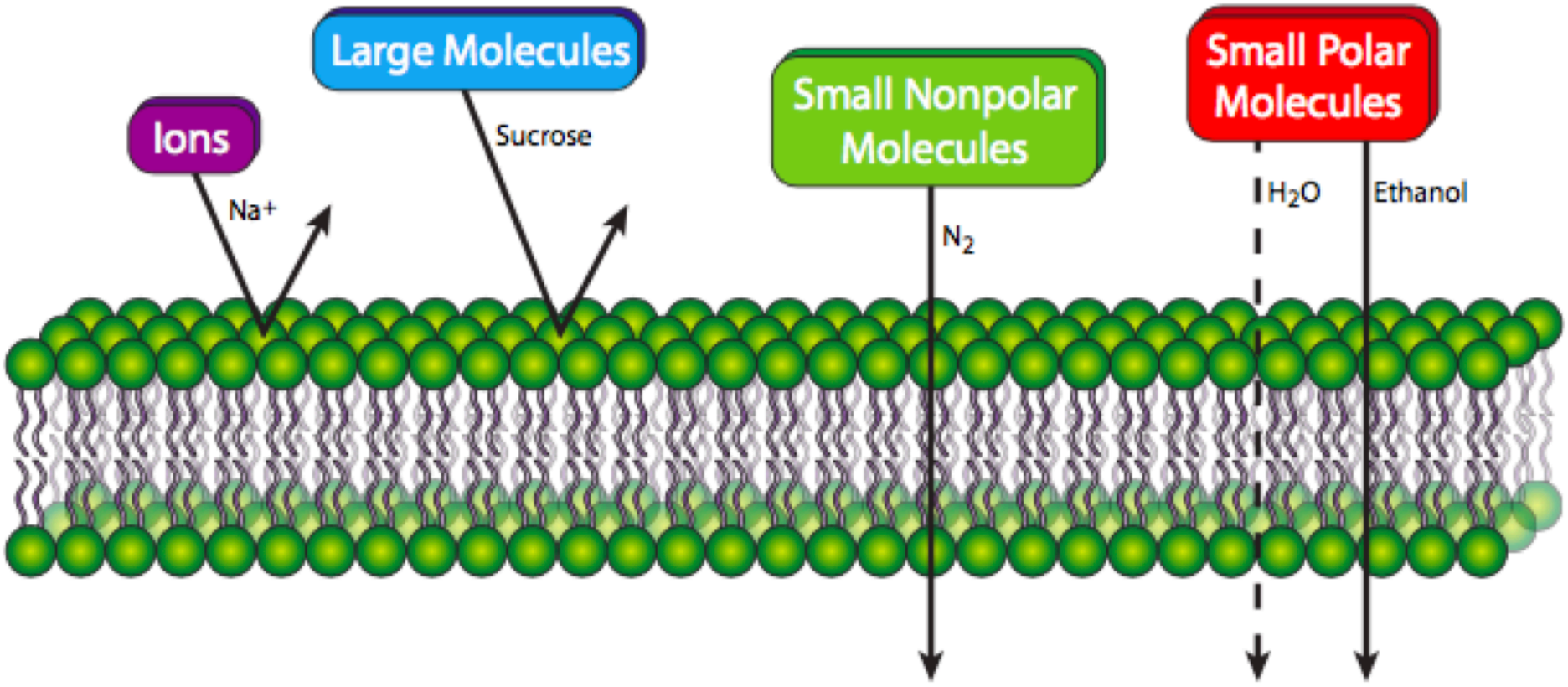


TRPV1 TRPM8



Somatosensory Ion Channels

Cell Membrane is a Barrier that Controls Molecular Transport

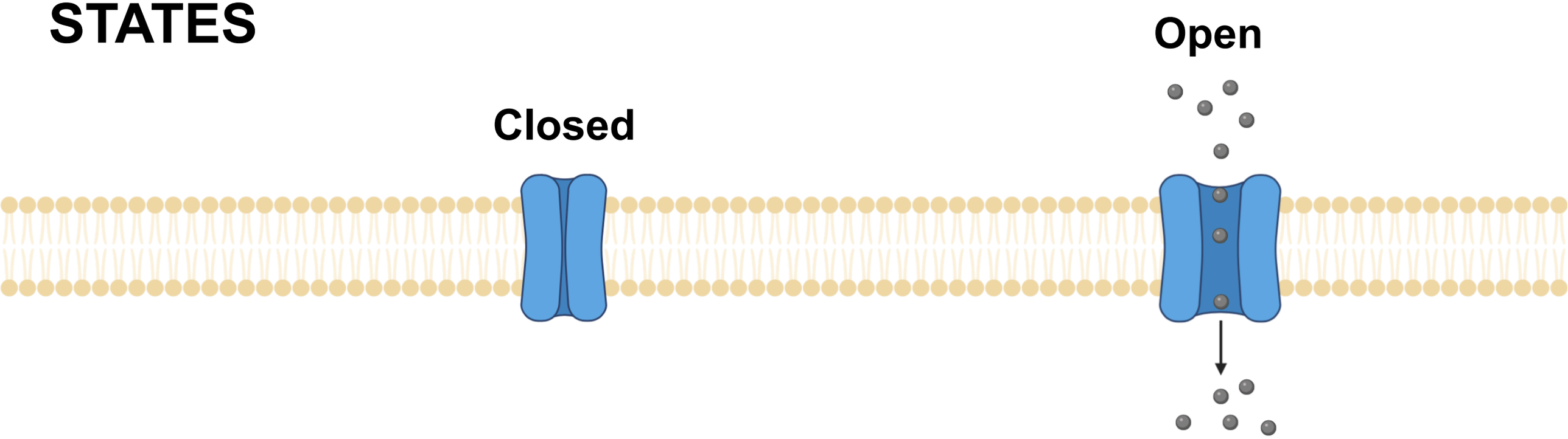


Transport of ions requires proteins - a subset of which are ion channels.

LibreTexts Biology - Figure 4.2.7

Ion Channels Properties - Gating

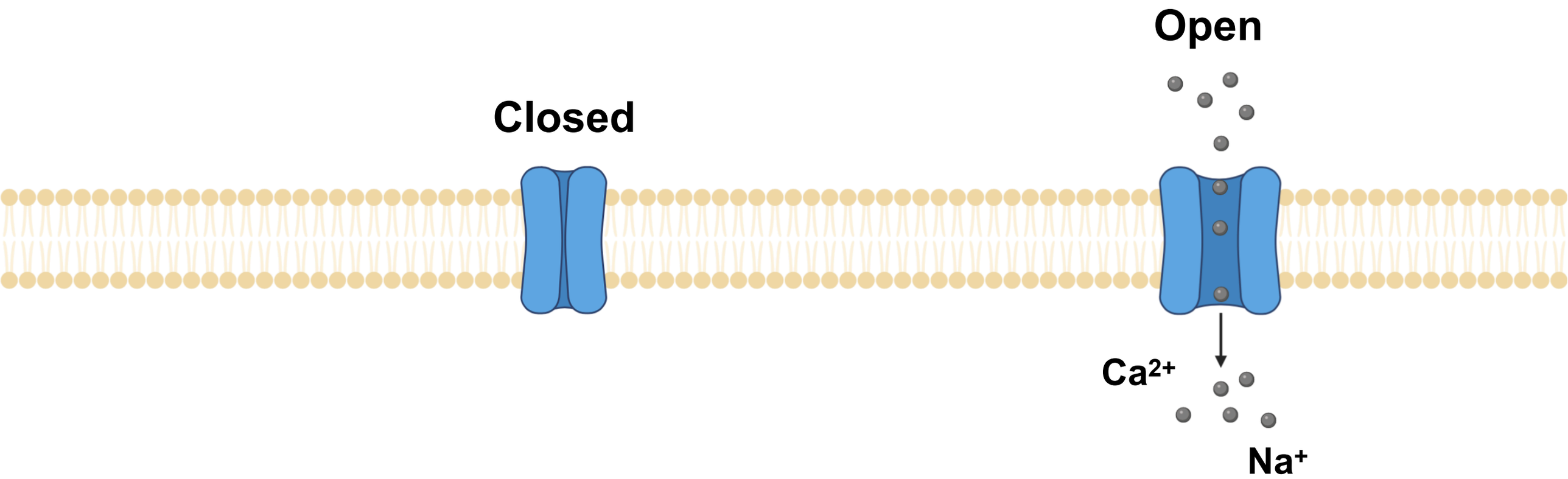
STATES



Ion channels can GATE the flow of ions into or out of the cell.

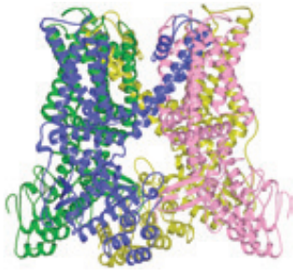
This may be in response to a specific stimuli.

Ion Channels Properties - Selectivity

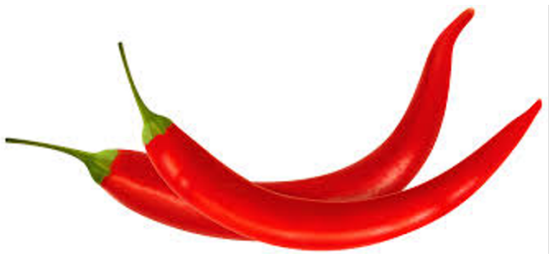


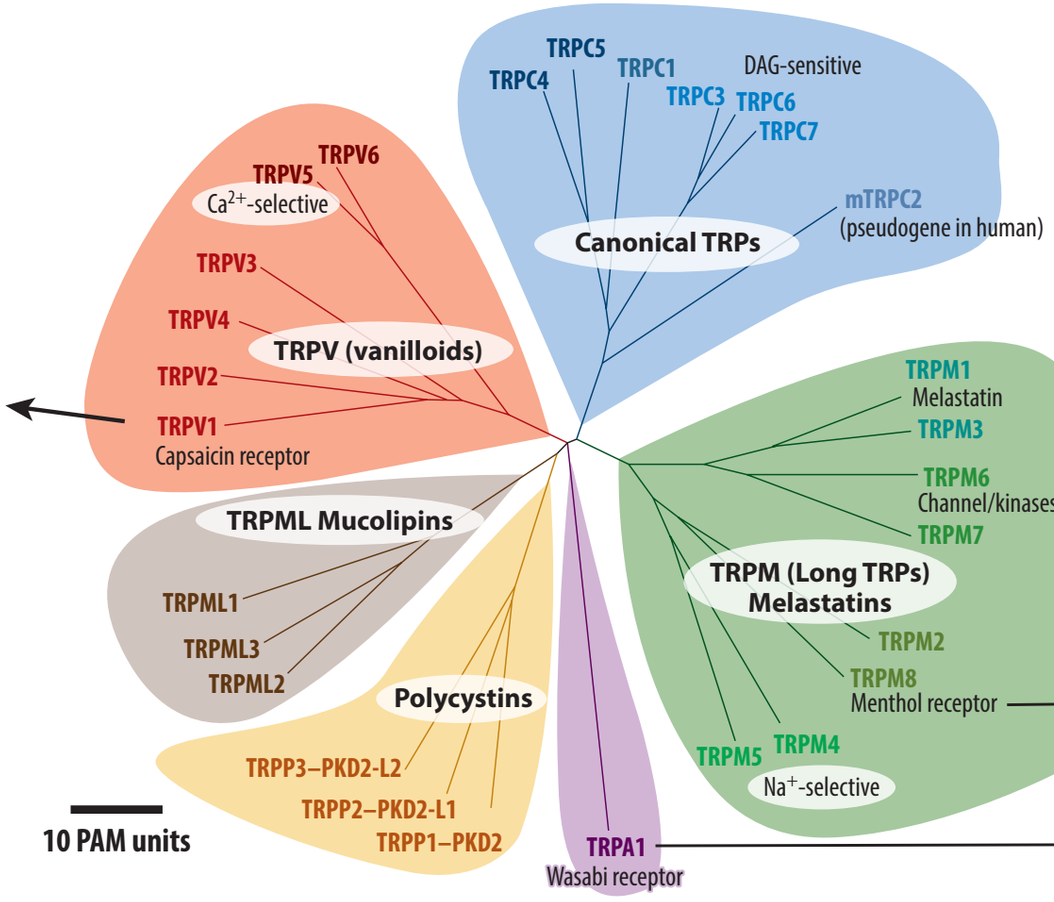
Ion channels show ion SELECTIVITY, permitting some ions to pass, but not others.

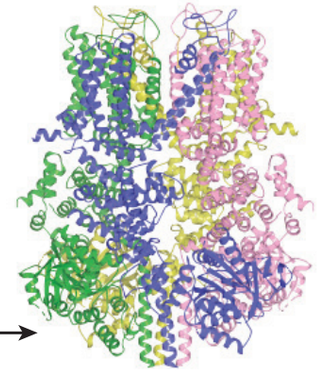
TRP Ion Channel Family



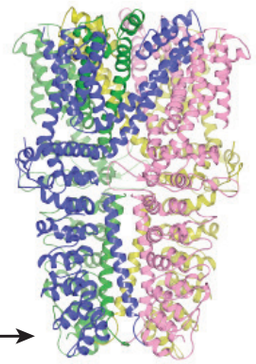
TRPV1: heat activated capsaicin receptor







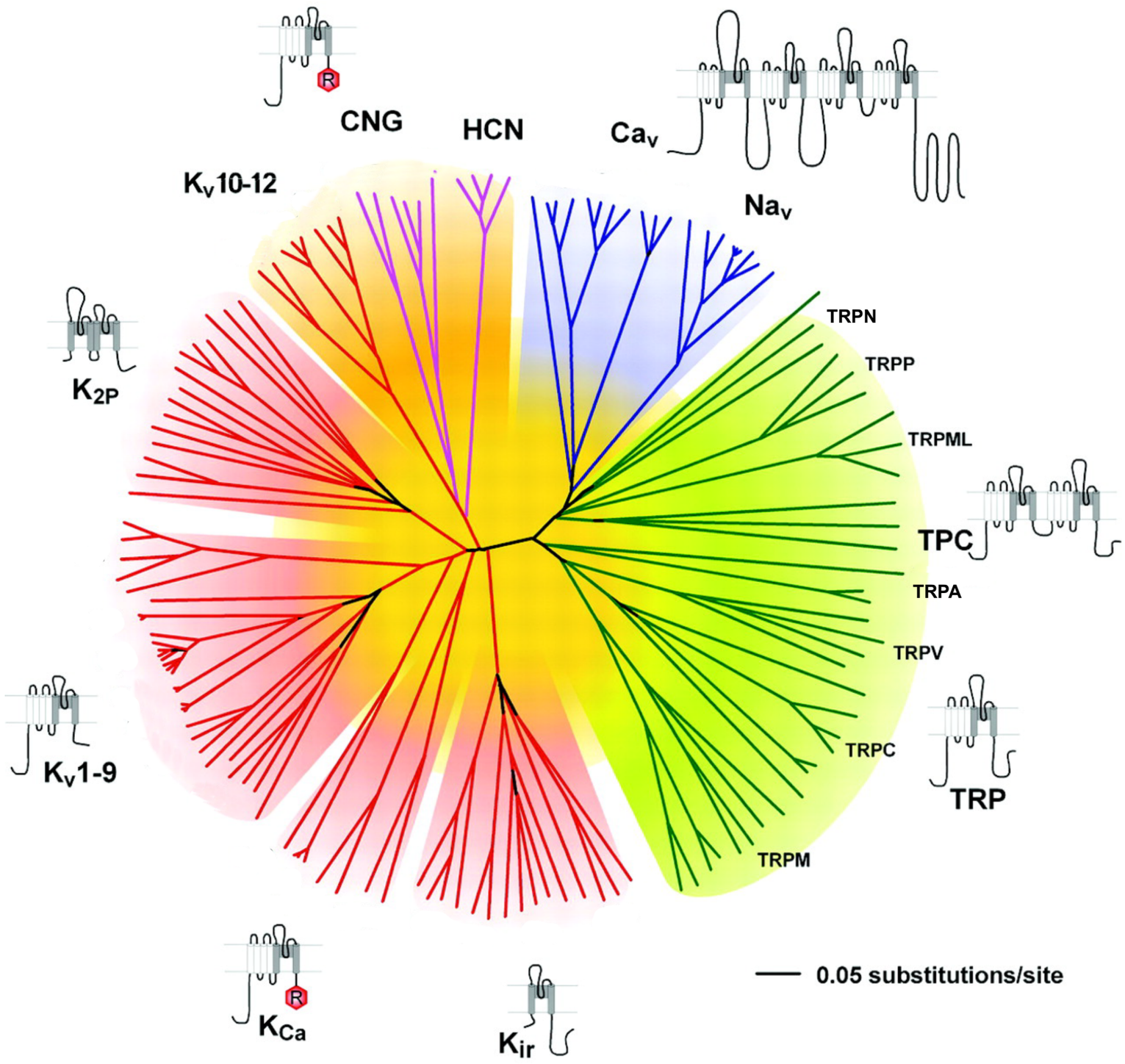
TRPM8: cold activated menthol receptor



TRPA1: electrophile activated Wasabi receptor

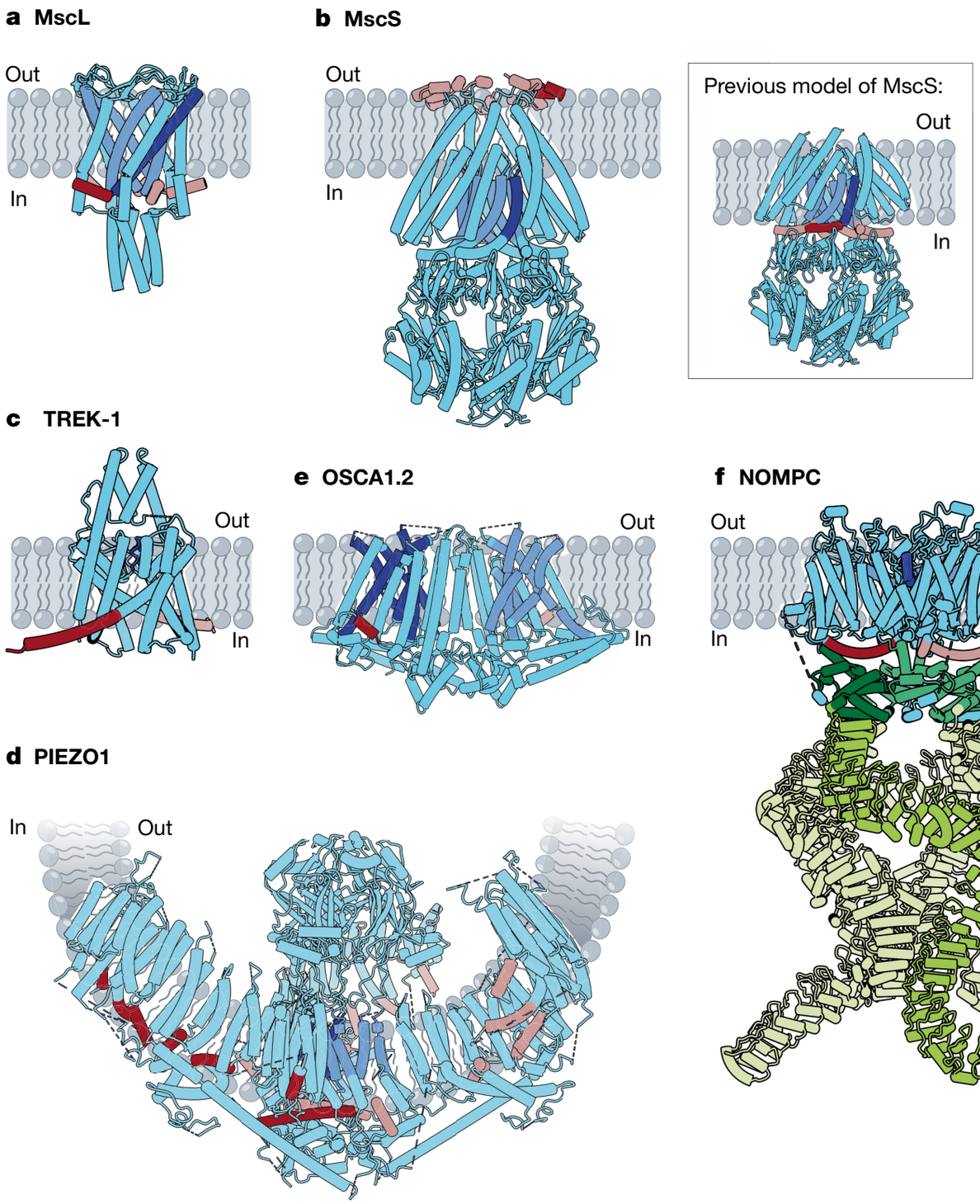
Diver, Lin King, Julius & Cheng. Annu. Rev. Biochem. 2022.

TRP Channels Belong to the Voltage-gated Ion Channel Superfamily



Yu & Catterall. Pharmacol. Rev. 2005.

Mechanosensitive Ion Channel Families



Kefauver, Ward & Patapoutian. Nature. 2020.

The Noble Prize in Physiology or Medicine 2021 was awarded jointly to David Julius and Ardem Patapoutian “for their discoveries of receptors for temperature and touch.”

Ill. Niklas Elmehed © Nobel Prize Outreach

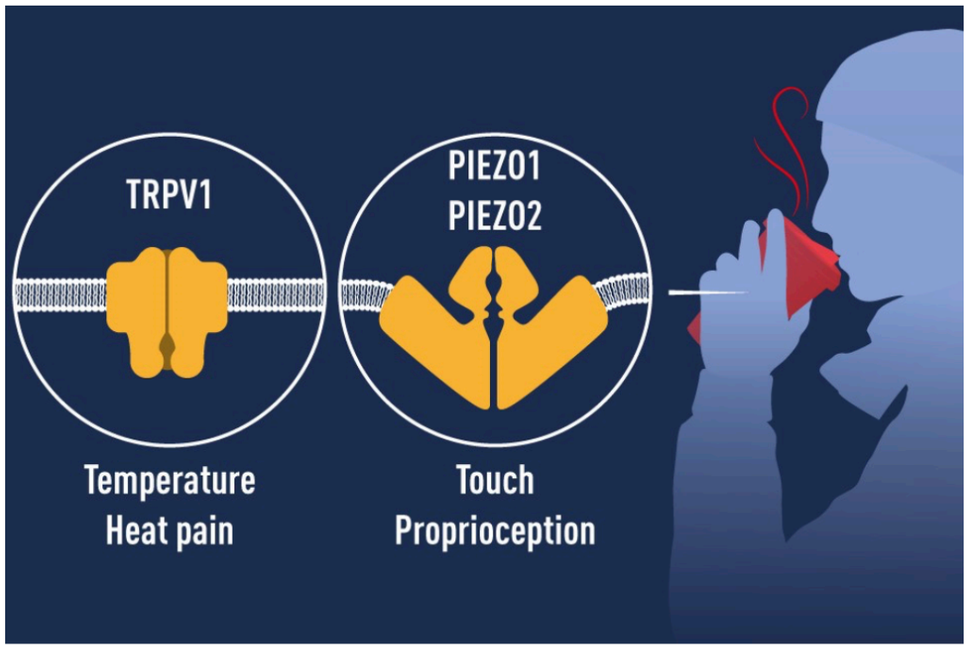
David Julius

Prize share: 1/2

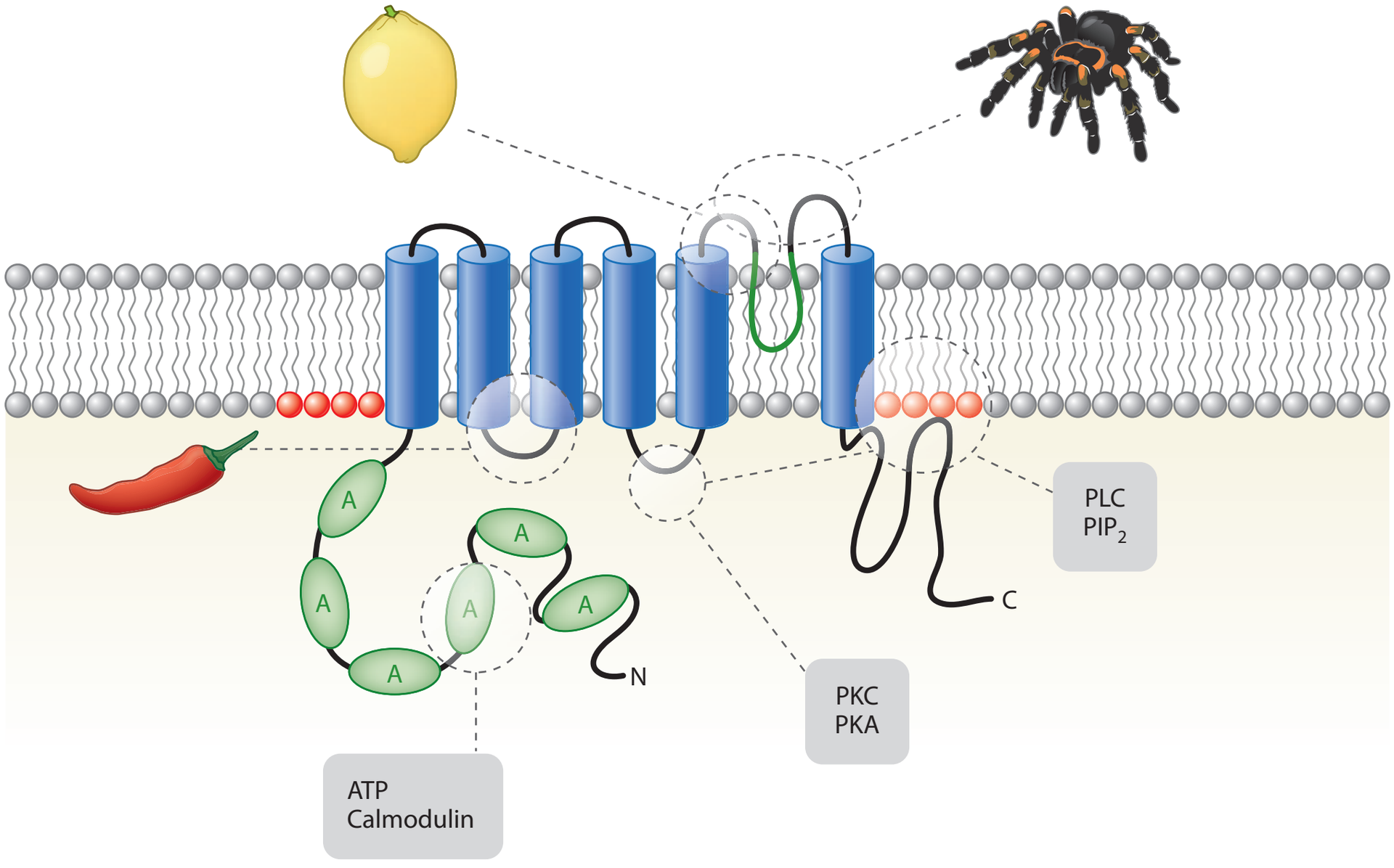
Ill. Niklas Elmehed © Nobel Prize Outreach

Ardem Patapoutian

Prize share: 1/2



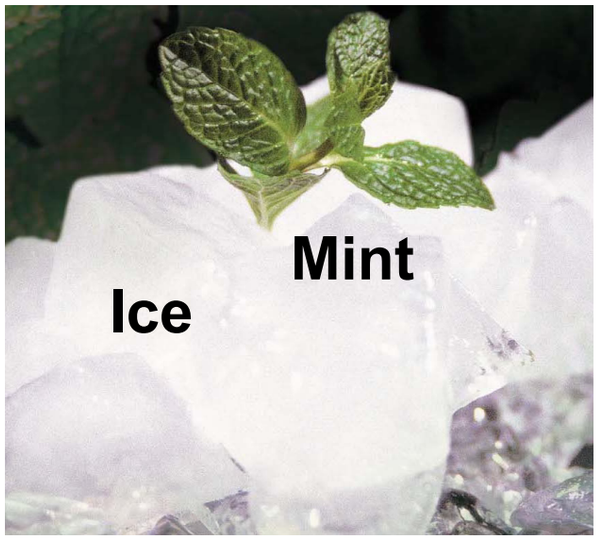
TRPV1 is the Primary Detector of Environmental Heat



Caterina, *et al.* & Julius. Nature. 1997.

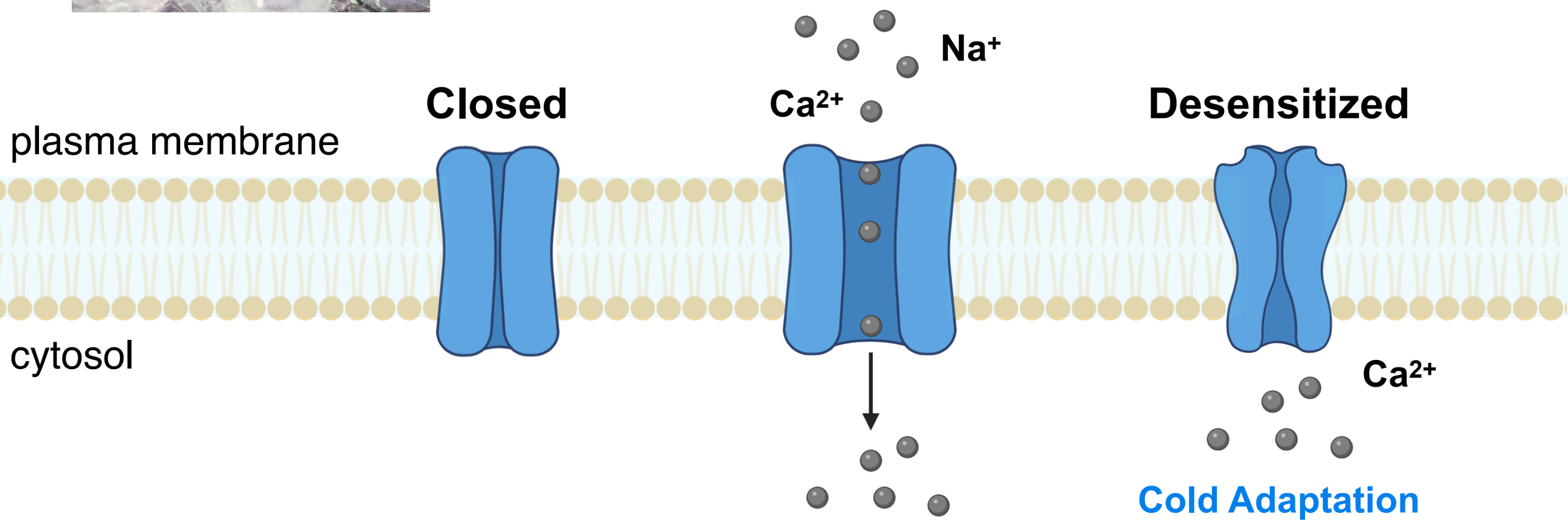
Julius. Annu. Rev. Cell Dev. Biol. 2013.

TRPM8 is the Primary Detector of Environmental Cold



Cold

Natural & Synthetic
Cooling Agents



Nociceptor

McKemy, Neuhausser & Julius. Nature. 2002.

TRPM8 as an Analgesic Target: Agonists Relieve Mechanical Hyperalgesia

TRPM8 Activators

Sports Injuries, Minor Aches and Pains, Colds

Cold

Camphor 4.8%
Eucalyptus Oil 1.2%
Menthol 2.6%

Menthol 1%

Camphor 11%
Menthol 10%

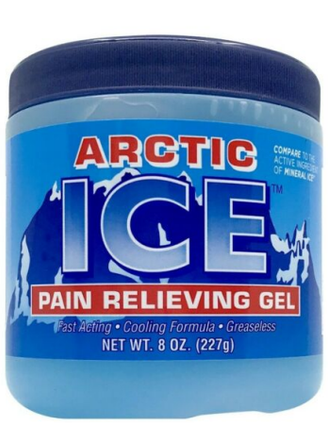
Menthol 5%

TRPM8 as an Analgesic Target: Antagonists Relieve Cold Hyperalgesia

TRPM8 Activators

Sports Injuries, Minor Aches and Pains, Colds

Cold



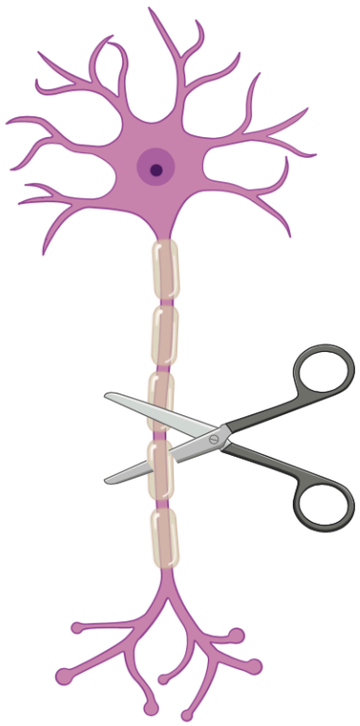
Menthol 1%

Camphor 11%
Menthol 10%

Camphor 4.8%
Eucalyptus Oil 1.2%
Menthol 2.6%

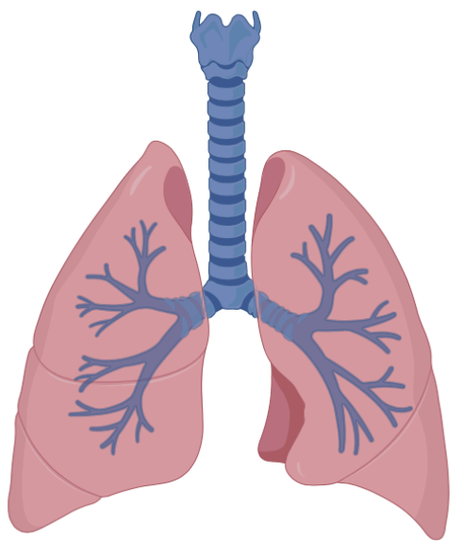
Menthol 5%

TRPM8 Inhibitors

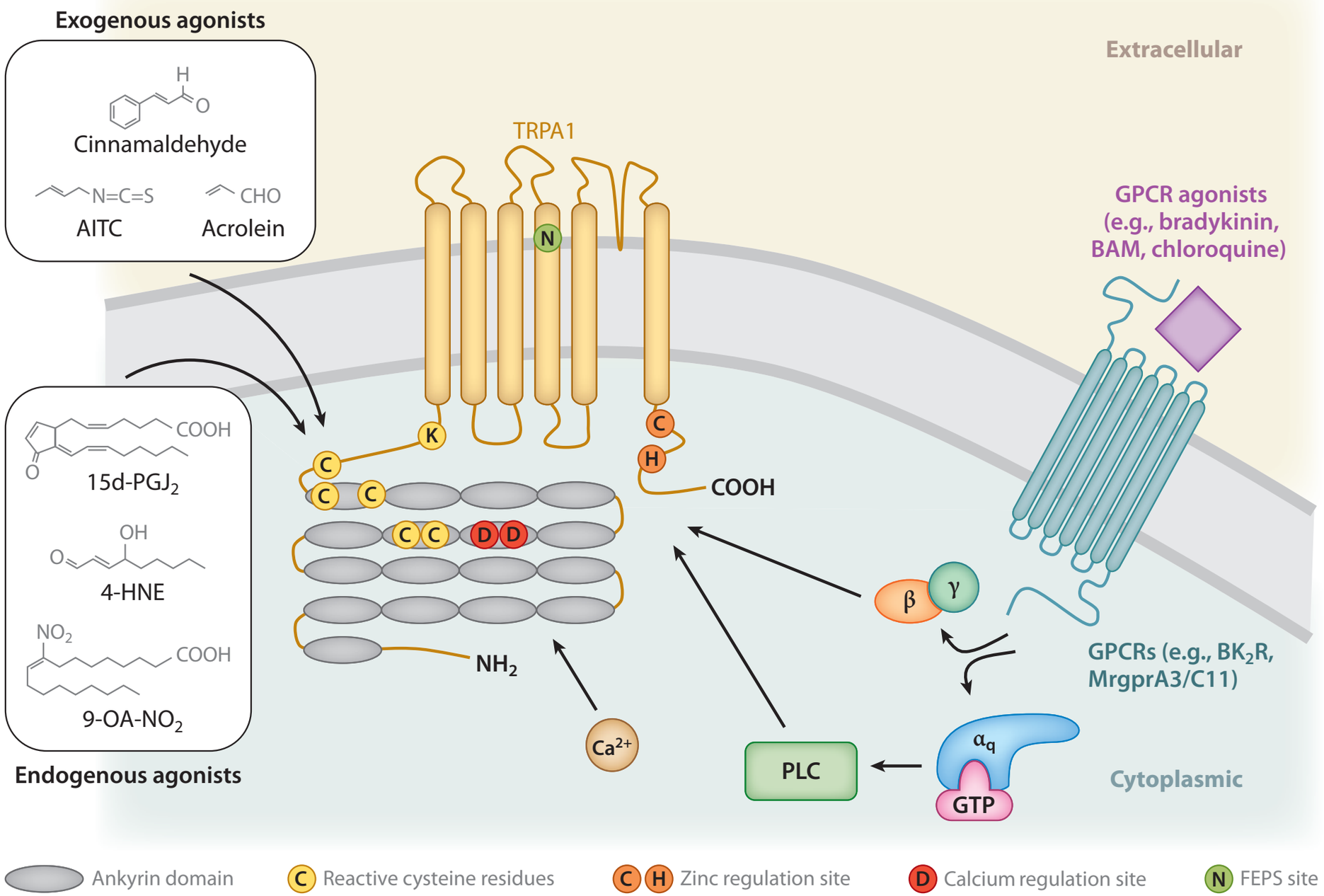


Cold Hypersensitivity due to Nerve Damage

Airway Restricted Diseases (ex. Chronic Cough, Asthma)



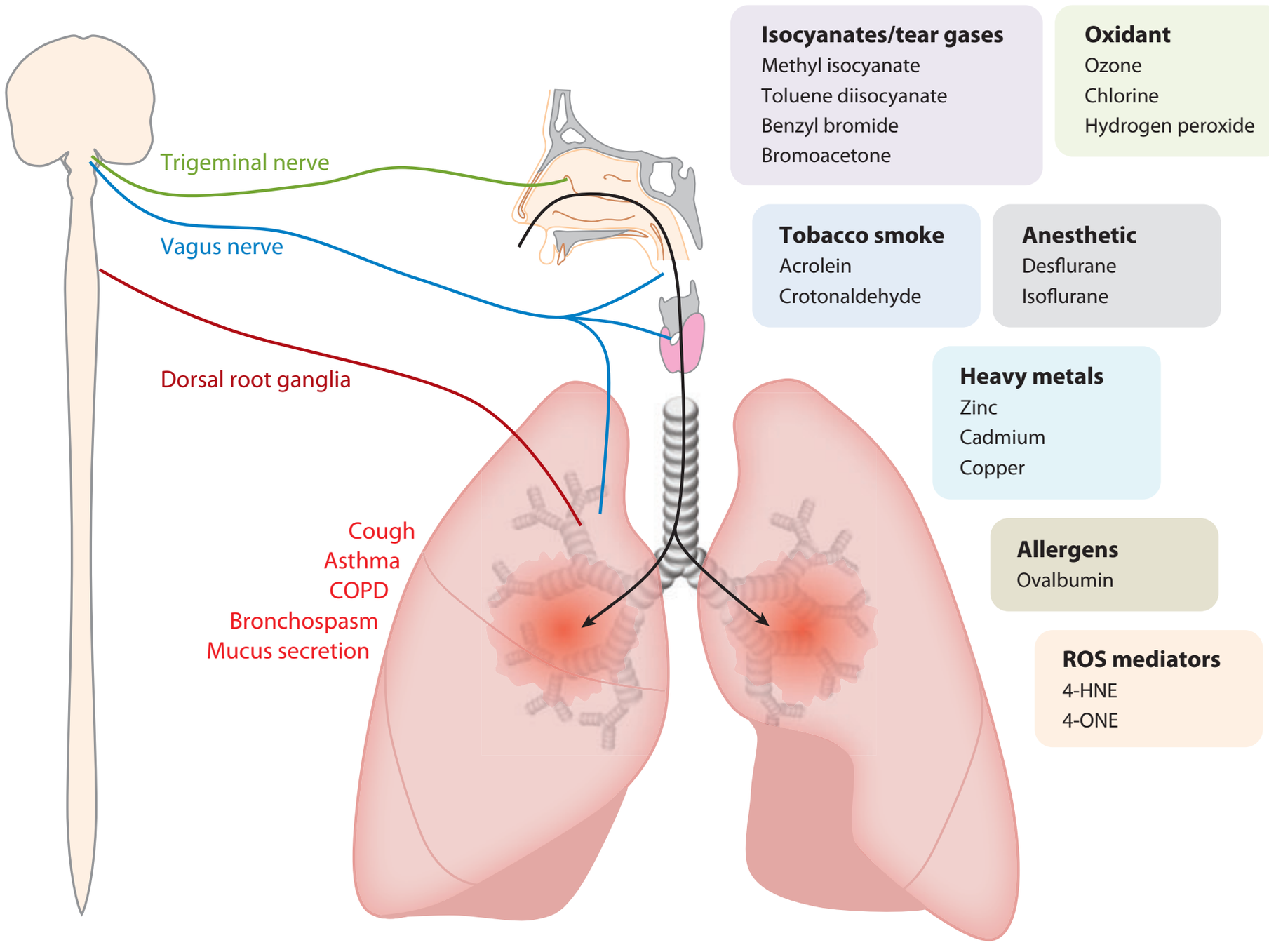
TRPA1 is the Primary Detector of Environmental Irritants



Bautista, *et al.* & Julius. Cell. 2006.

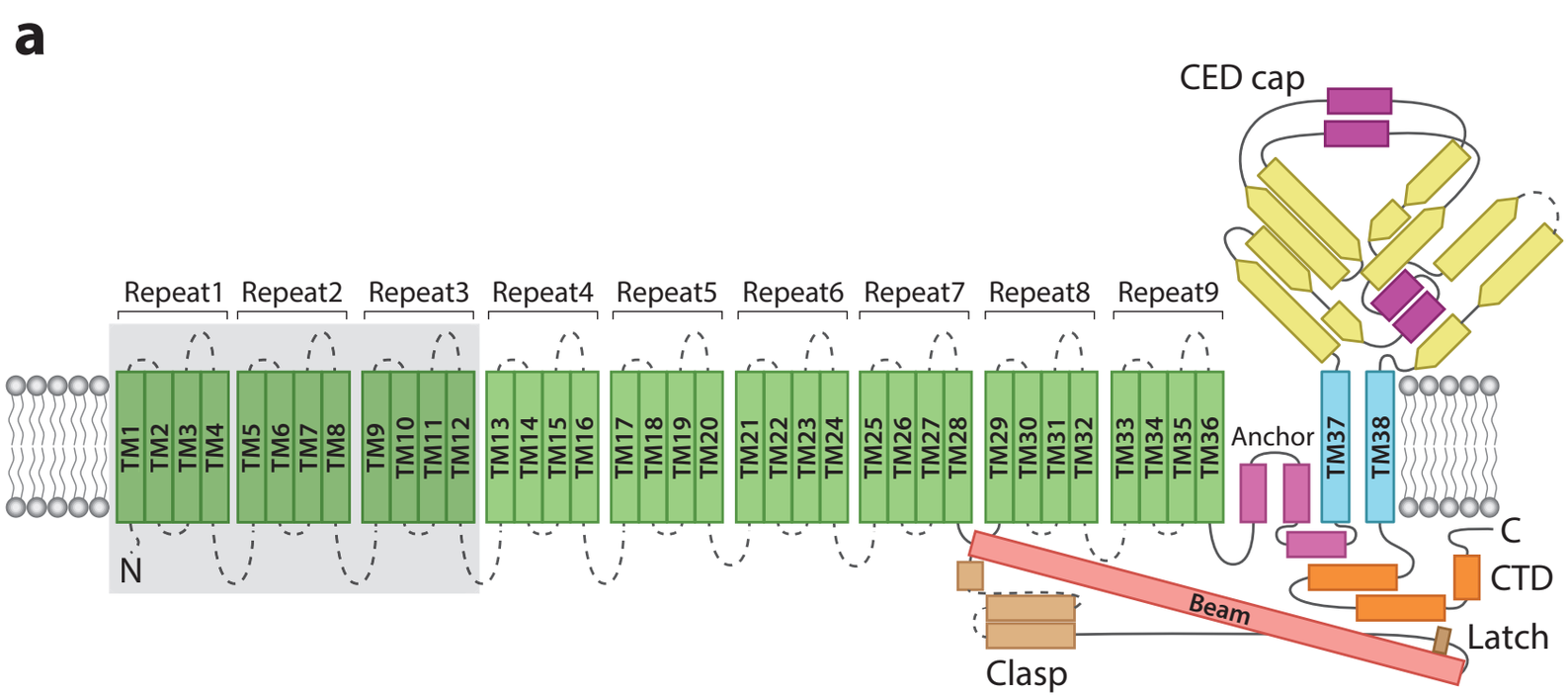
Bautista, Pellegrino & Tsunozaki. Annu. Rev. Physiol. 2013.

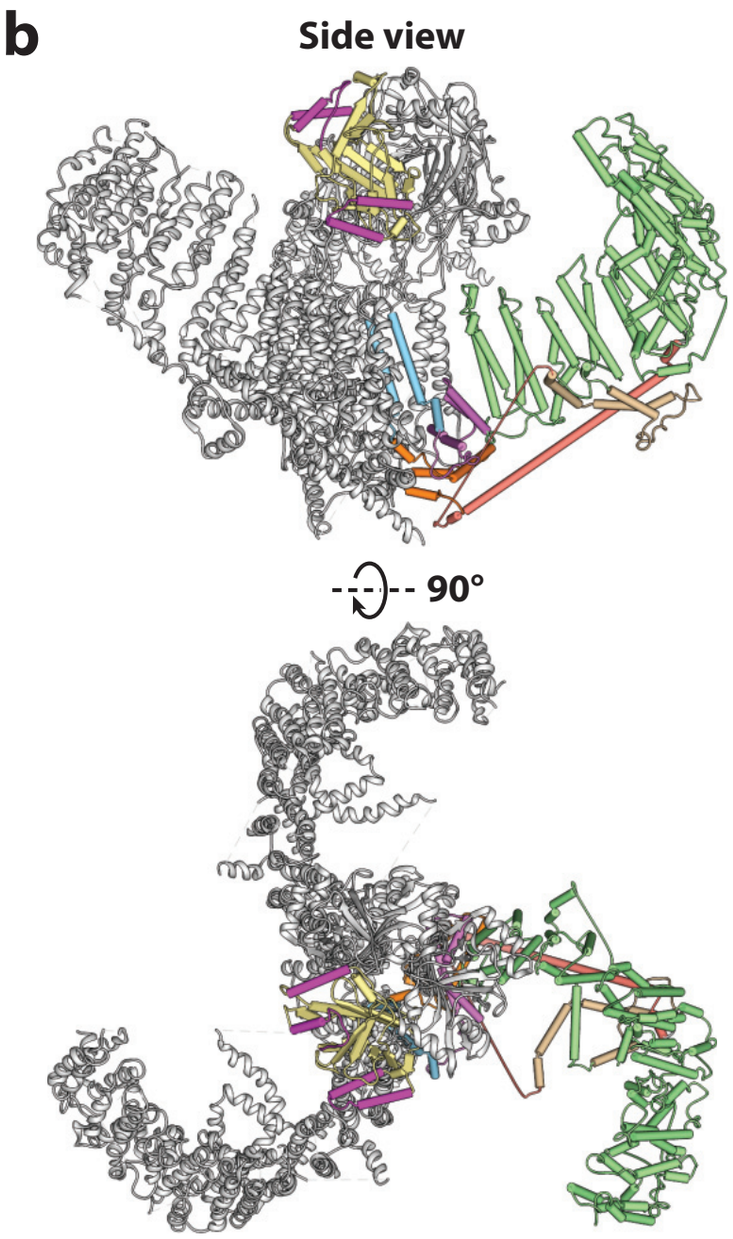
TRPA1 in Airway Inflammation

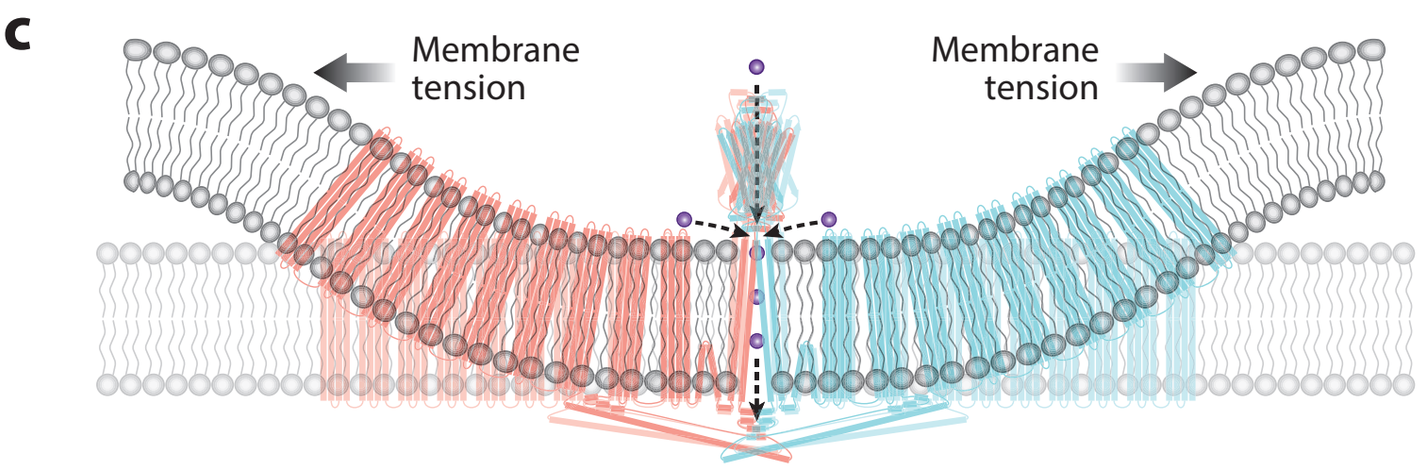


Bautista, Pellegrino & Tsunozaki. Annu. Rev. Physiol. 2013.

Piezo2 is the Primary Detector of Touch and Proprioception

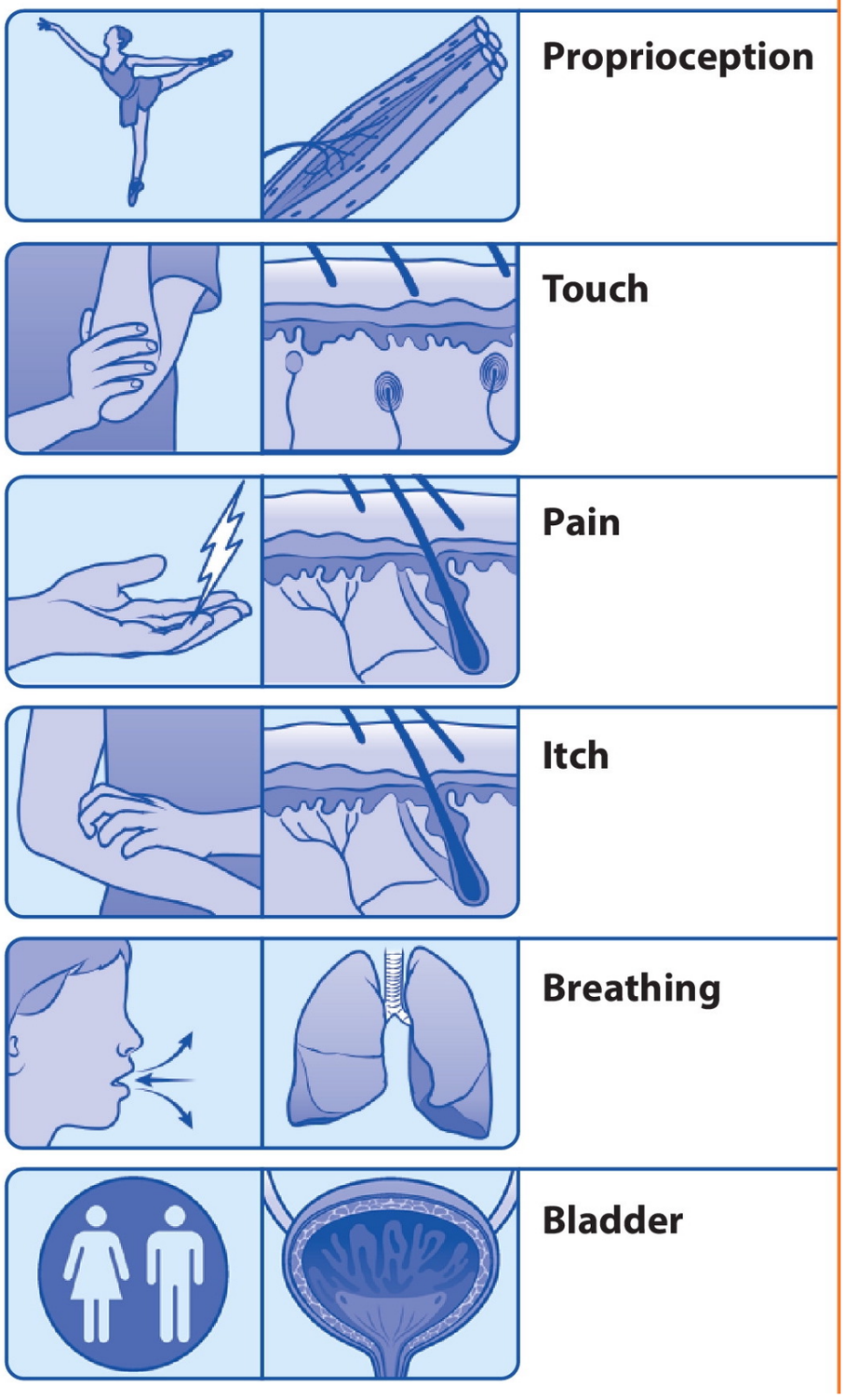


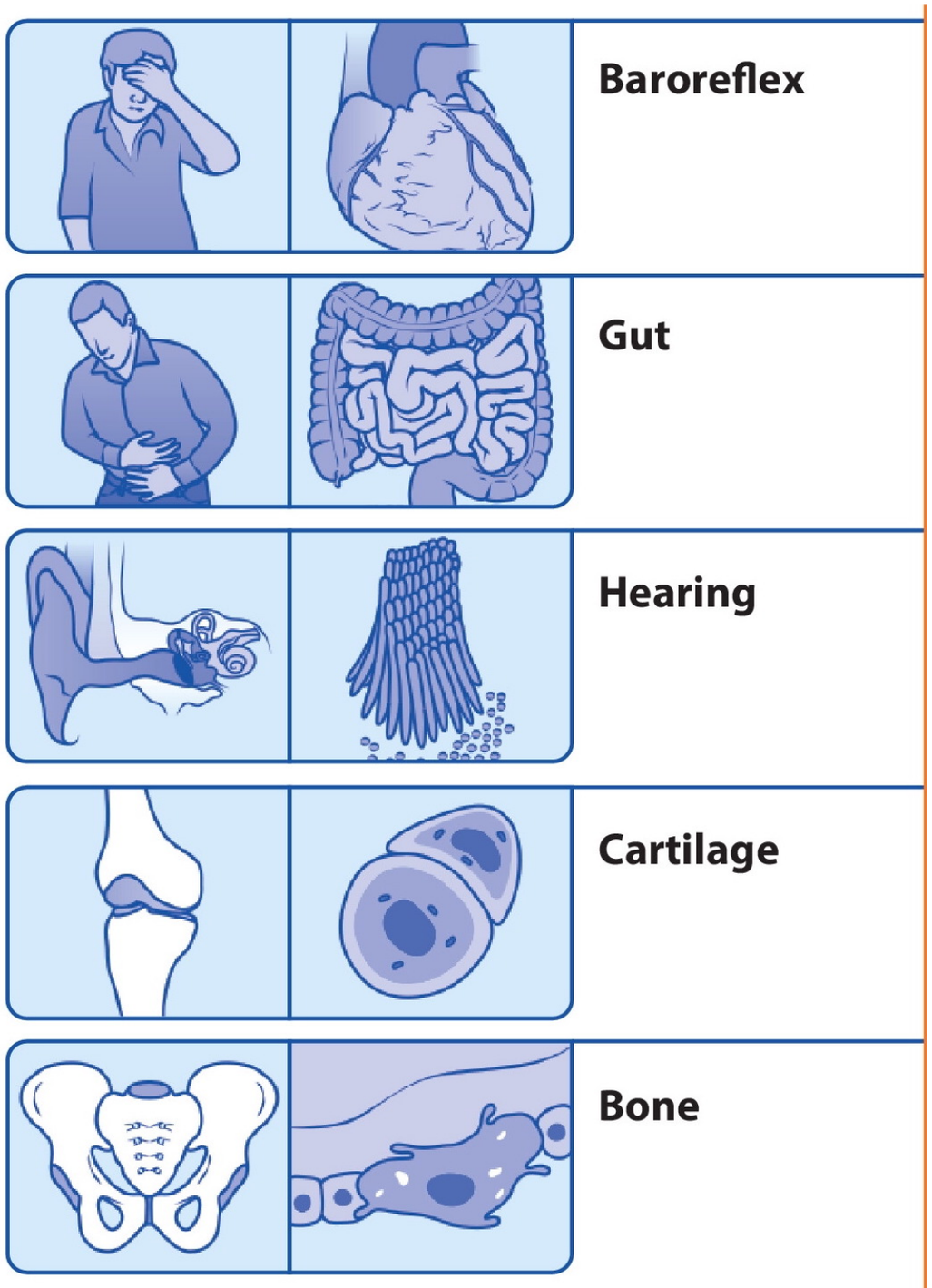




Jin, Jan & Jan. Annu. Rev. Neurosci. 2020.

Physiological Roles of Piezo2



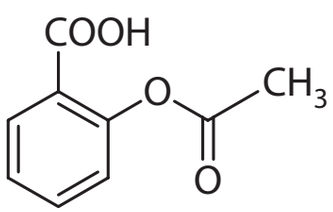


Szczot *et al*. Annu. Rev. Biochem. 2021.

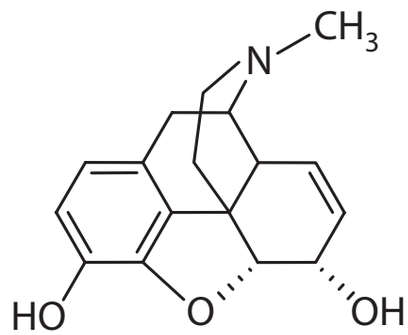
Exploiting Natural Products and Synthetic Chemical Modulators to Probe Protein Function

Natural Products as Probes of the Pain Pathway

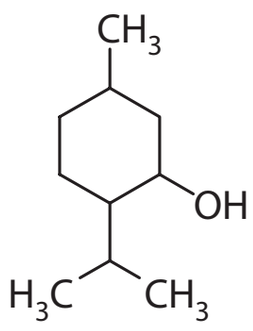
Aspirin

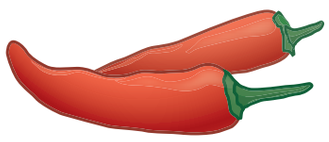


Morphine

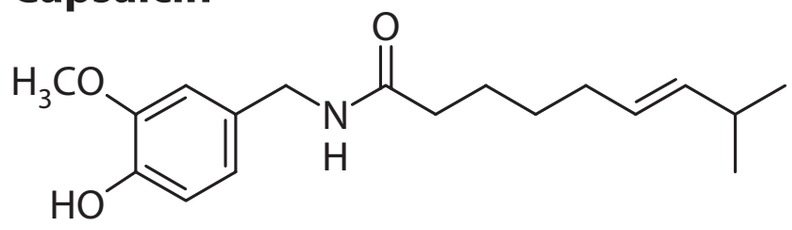


Menthol

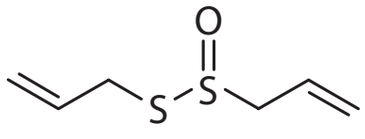




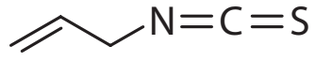
Capsaicin



Thiosulfinates



Isothiocyanates



Julius. Annu. Rev. Cell Dev. Biol. 2013.

Discovery of the Heat and Capsaicin Receptor, TRPV1

articles

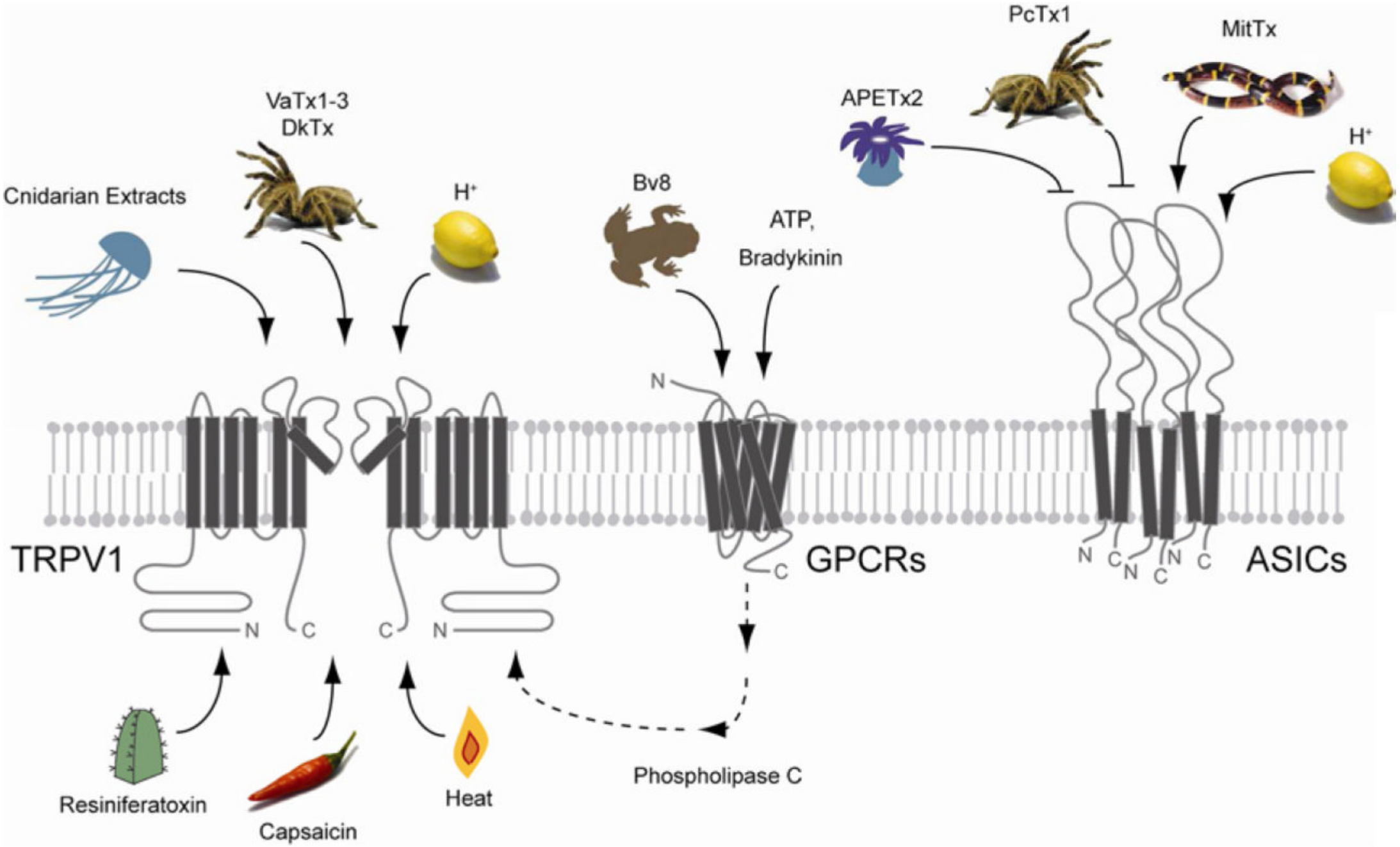
The capsaicin receptor: a heat-activated ion channel in the pain pathway

Michael J. Caterina*, Mark A. Schumacher†‖, Makoto Tominaga*‖, Tobias A. Rosen*, Jon D. Levine‡ & David Julius*

*Departments of * Cellular and Molecular Pharmacology, † Anesthesia, and ‡ Medicine, University of California, San Francisco, California 94143-0450, USA*
‖ These authors contributed equally to this study.

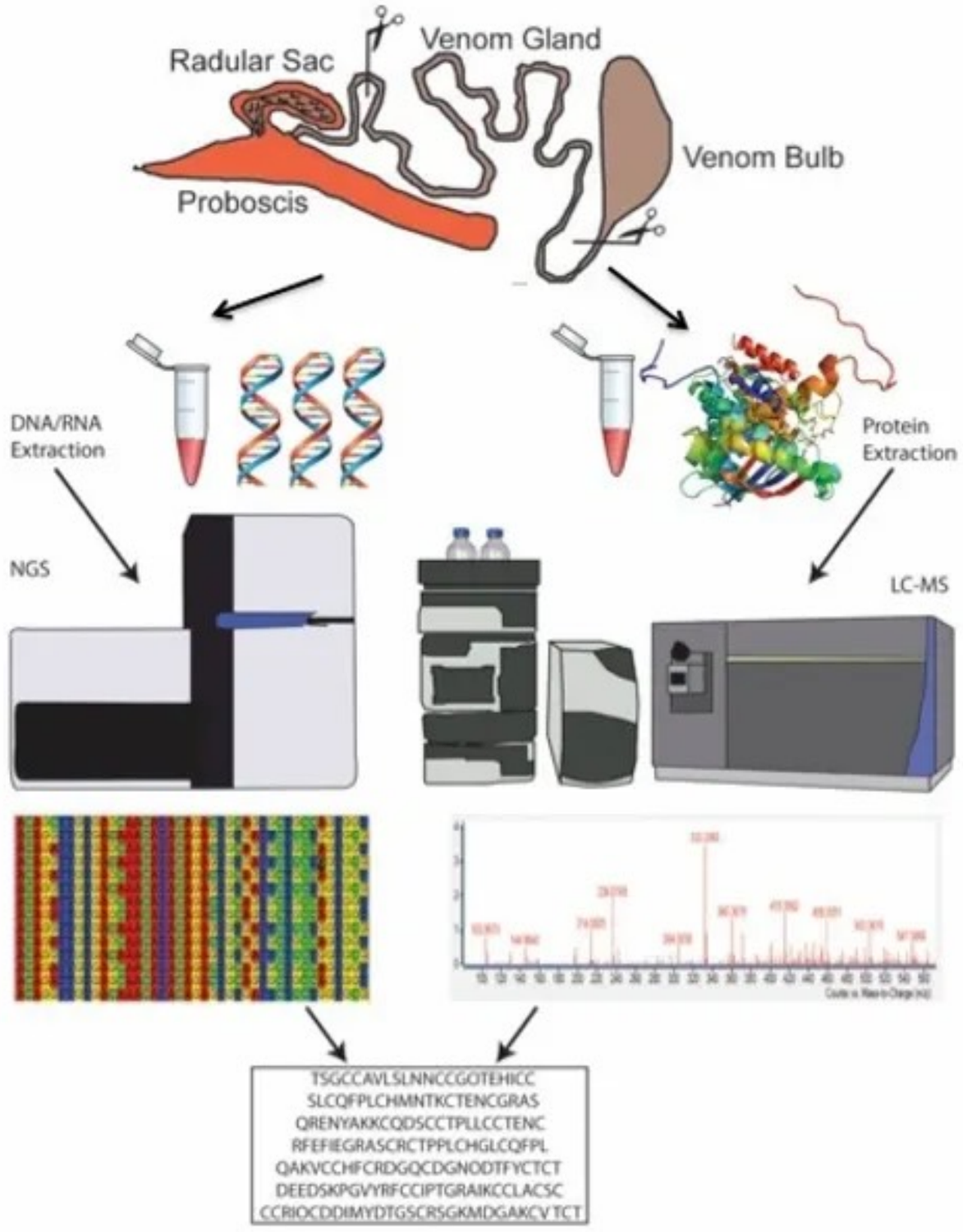
Capsaicin, the main pungent ingredient in 'hot' chilli peppers, elicits a sensation of burning pain by selectively activating sensory neurons that convey information about noxious stimuli to the central nervous system. We have used an expression cloning strategy based on calcium influx to isolate a functional cDNA encoding a capsaicin receptor from sensory neurons. This receptor is a non-selective cation channel that is structurally related to members of the TRP family of ion channels. The cloned capsaicin receptor is also activated by increases in temperature in the noxious range, suggesting that it functions as a transducer of painful thermal stimuli *in vivo*.

Natural Peptide Toxins as Probes of the Pain Pathway



Bohlen & Julius. Toxicon. 2012.

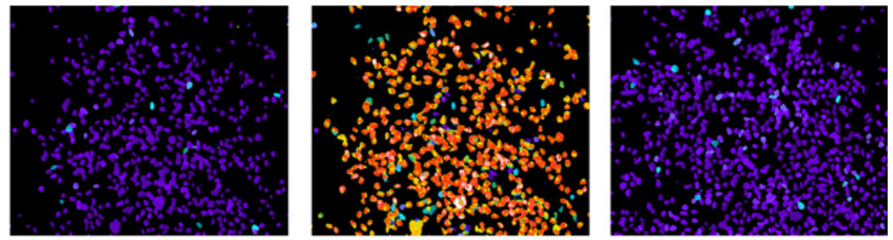
Identification of Natural Peptide Toxins



Mandë Holdford (CUNY Hunter College)

A Bivalent Tarantula Toxin Activates TRPV1

A



B

C

```
DkTx (K1)     DCAKEGEVCSWGKKCCDLDNFYCPMEFIPHCKKYKPYVPVTT-
DkTx (K2)    -NCAKEGEVCGWGSKCCHGLD..CPLAFIPYCEKYR
VaTx1        SECRWFMGGCDSTLDCCKHLS..CKMGLY.YCAWDGTF
VaTx2        GACRWFLGGCKSTSDCCEHLS..CKMGLD.YCAWDGTF
VaTx3         ECRWYLGGCKEDSECCEHLQ..CHSYWE.WCLWDGSF
HaTx          ECRYLFGGCKTTSDCCKHLG..CKFRDK.YCAWDFTFS
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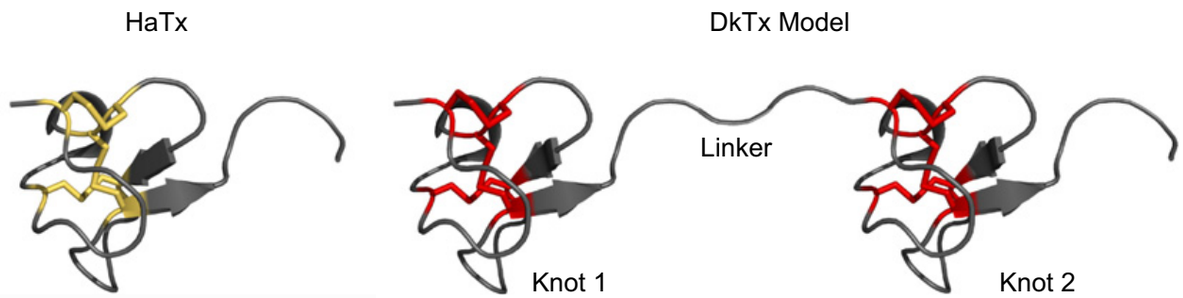
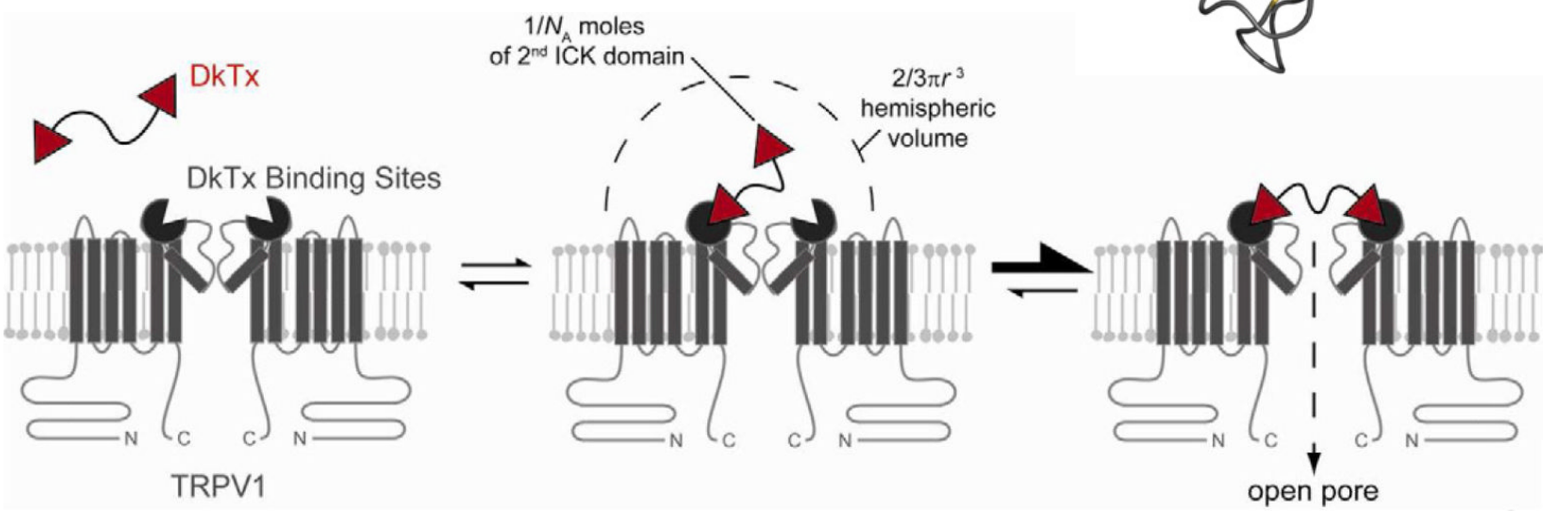
D





Bohlen *et al*. Cell. 2010.

Bohlen & Julius. Toxicon. 2012.

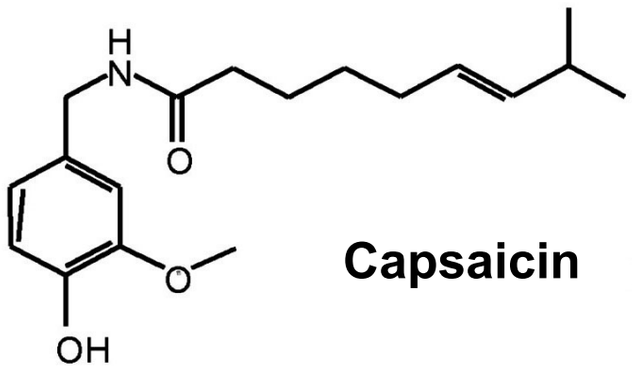
Capturing Unique Structural States of TRPV1 Bound to Natural Peptide Toxins

ARTICLE

doi:10.1038/nature17964

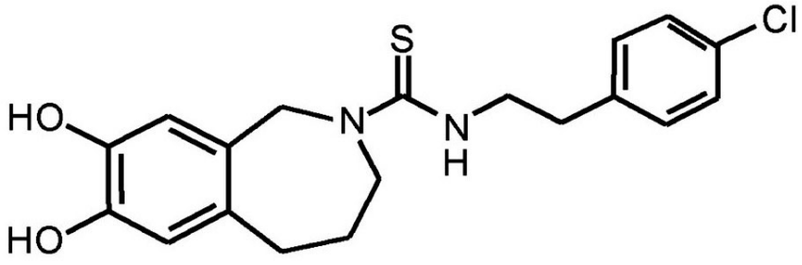
TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action

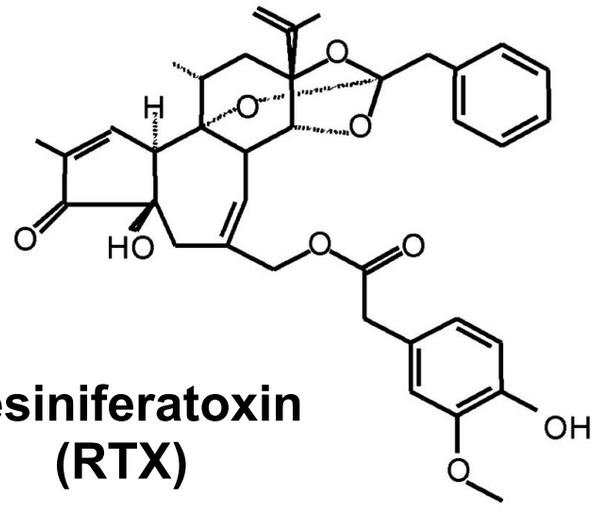
Yuan Gao[1,2], Erhu Cao[1]†, David Julius[1] & Yifan Cheng[2,3]



Capsaicin

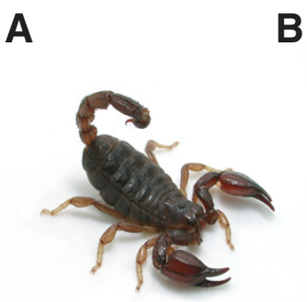
Capsazepine

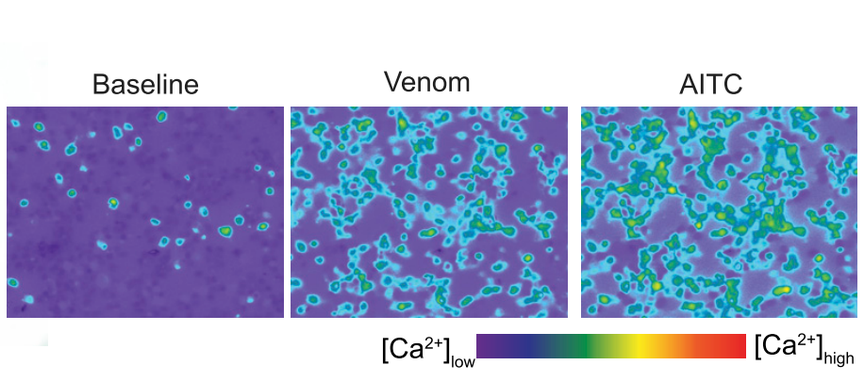


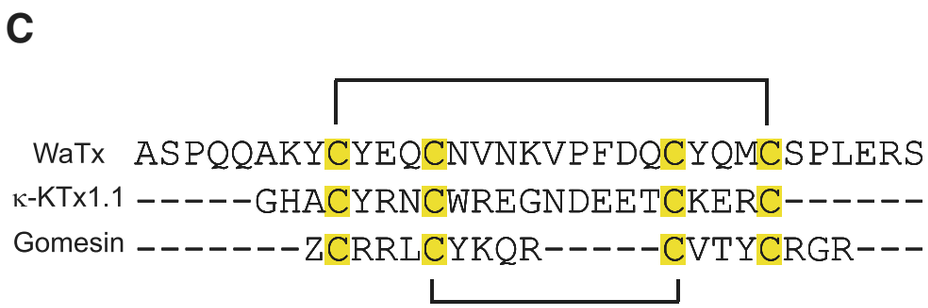


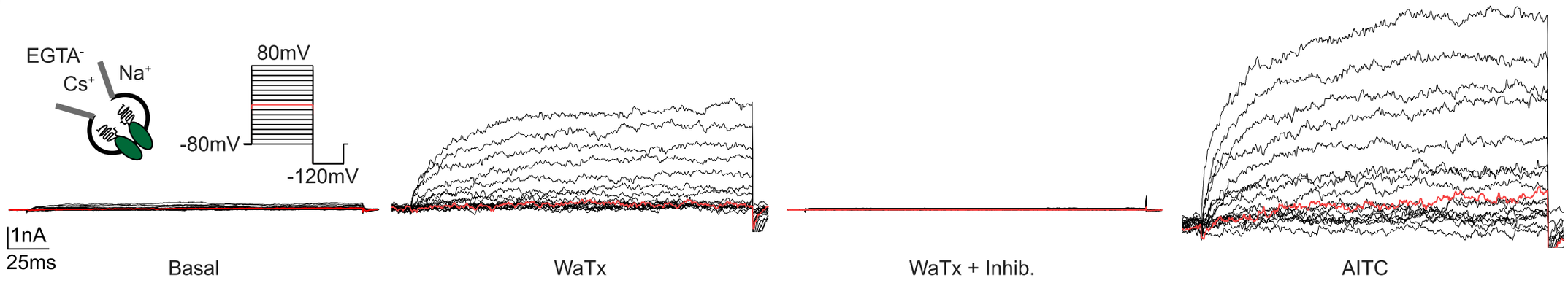
Resiniferatoxin (RTX)

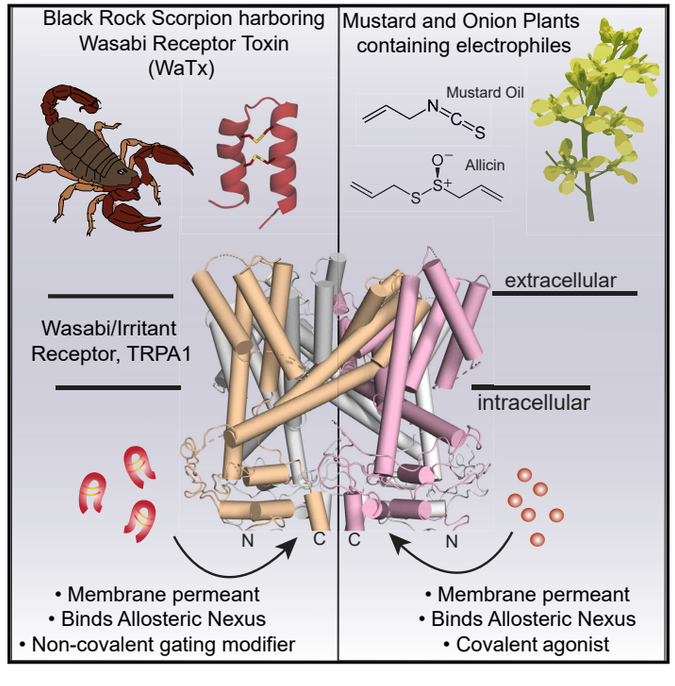
A Scorpion Toxin Activates TRPA1









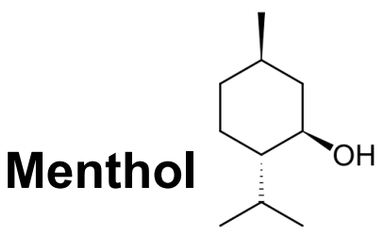


Lin King *et al*. Cell. 2019.

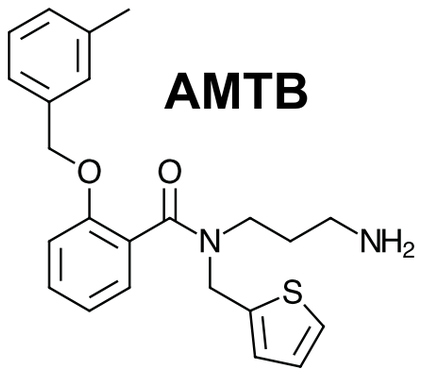
Synthetic Compounds as Probes of the Pain Pathway

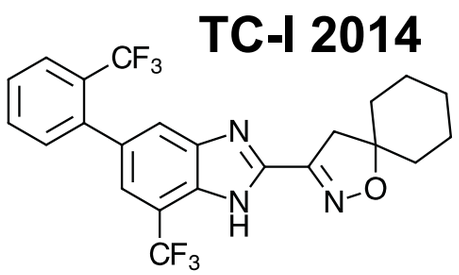
TRPM8

Natural Agonist

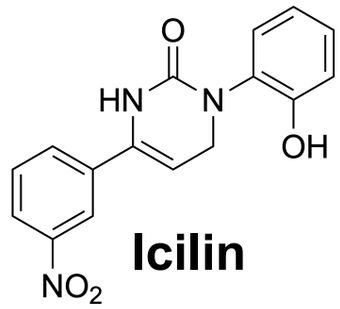


Synthetic Antagonists



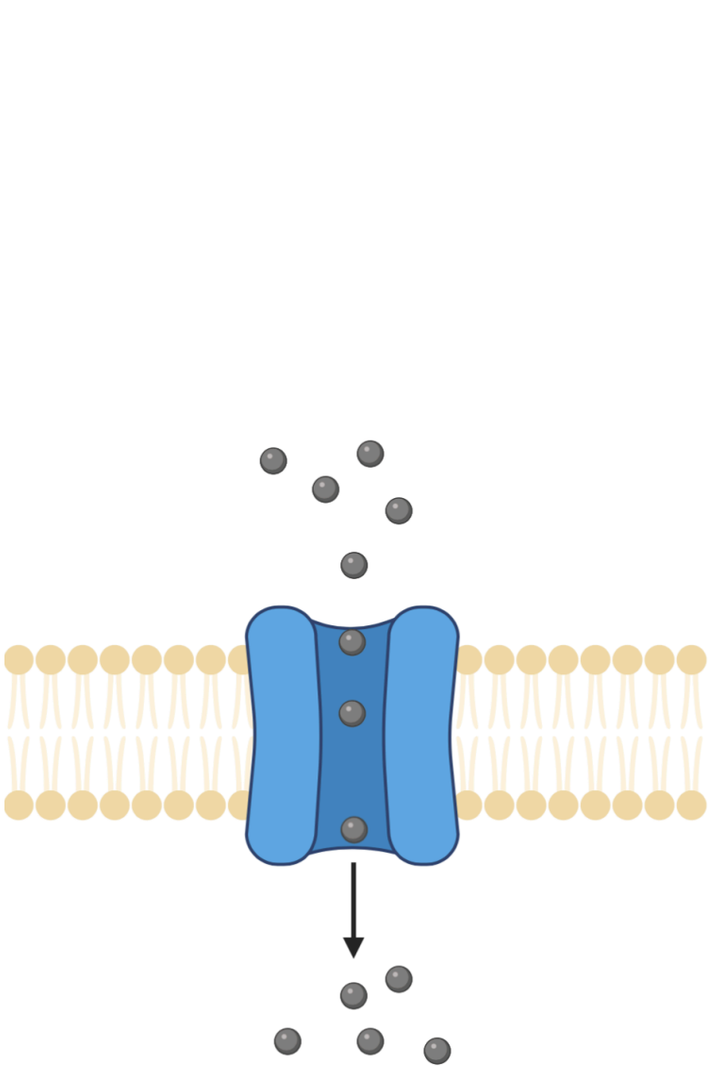


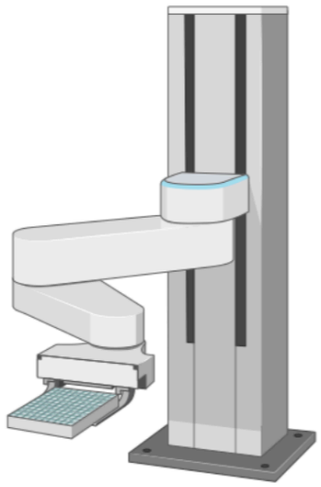
Synthetic Agonist



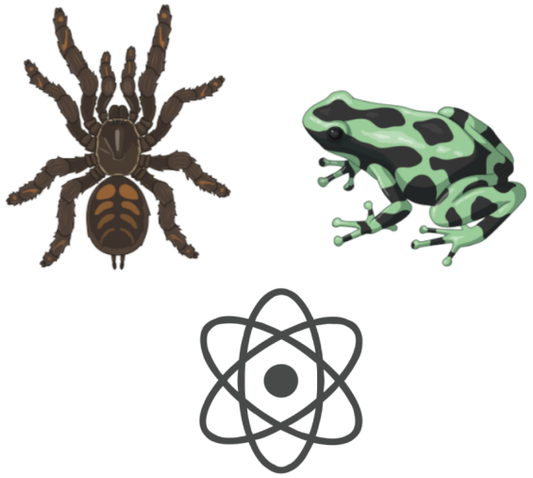
Super-cooling Agent

Access to Specific Chemical Probes that Modulate these Channels is Essential

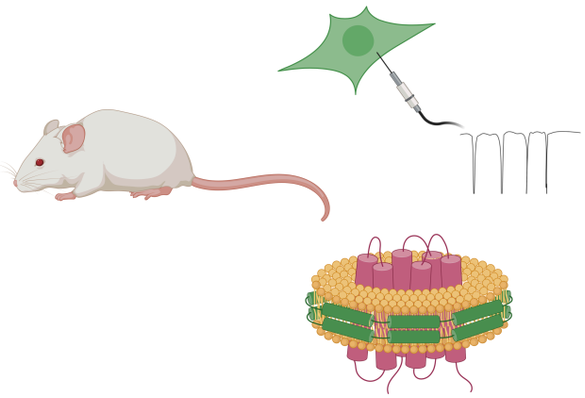




High-throughput Screening Assay



Identify Small Molecular and/or Natural Product Modulators



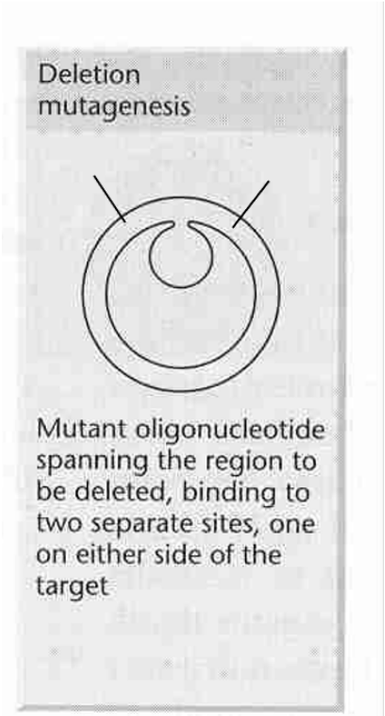
Application of Chemical Tools

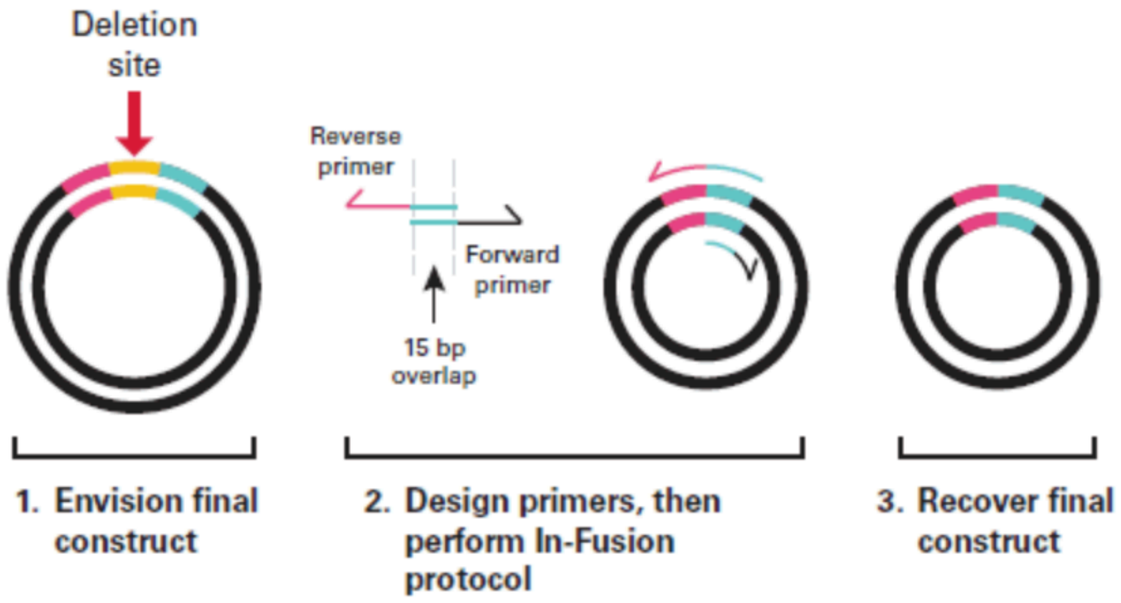
Protein Structure-function Analysis to Elucidate Mechanism

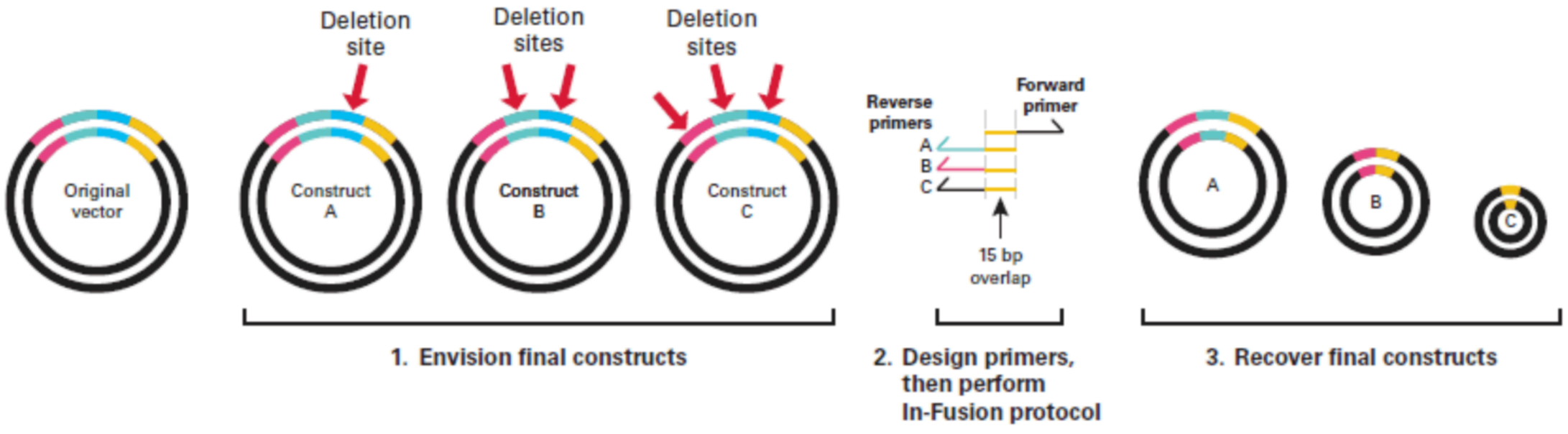
Using Mutagenesis to Probe Function: What's Possible?

- Define the physiological role of a gene
- Determine functionally important regions or amino acid residues
- Improve function
- Alter function

Deletion Mutagenesis



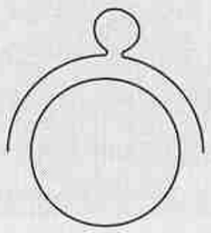




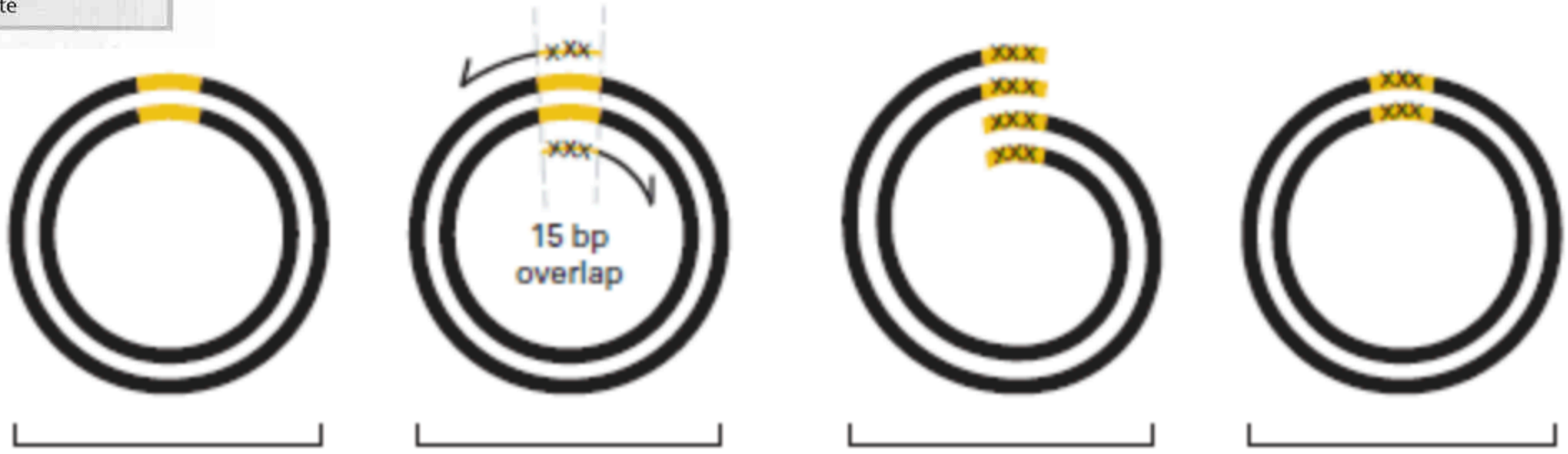
Takara

Insertion Mutagenesis

Insertion mutagenesis



Mutant oligonucleotide carrying a sequence to be inserted sandwiched between two regions with sequences complementary to sites on either sides of the target site in the template

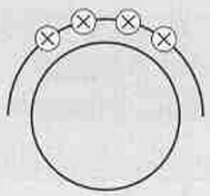


1. Envision final construct
2. Design primers
3. Amplify linear construct
4. Recover final construct

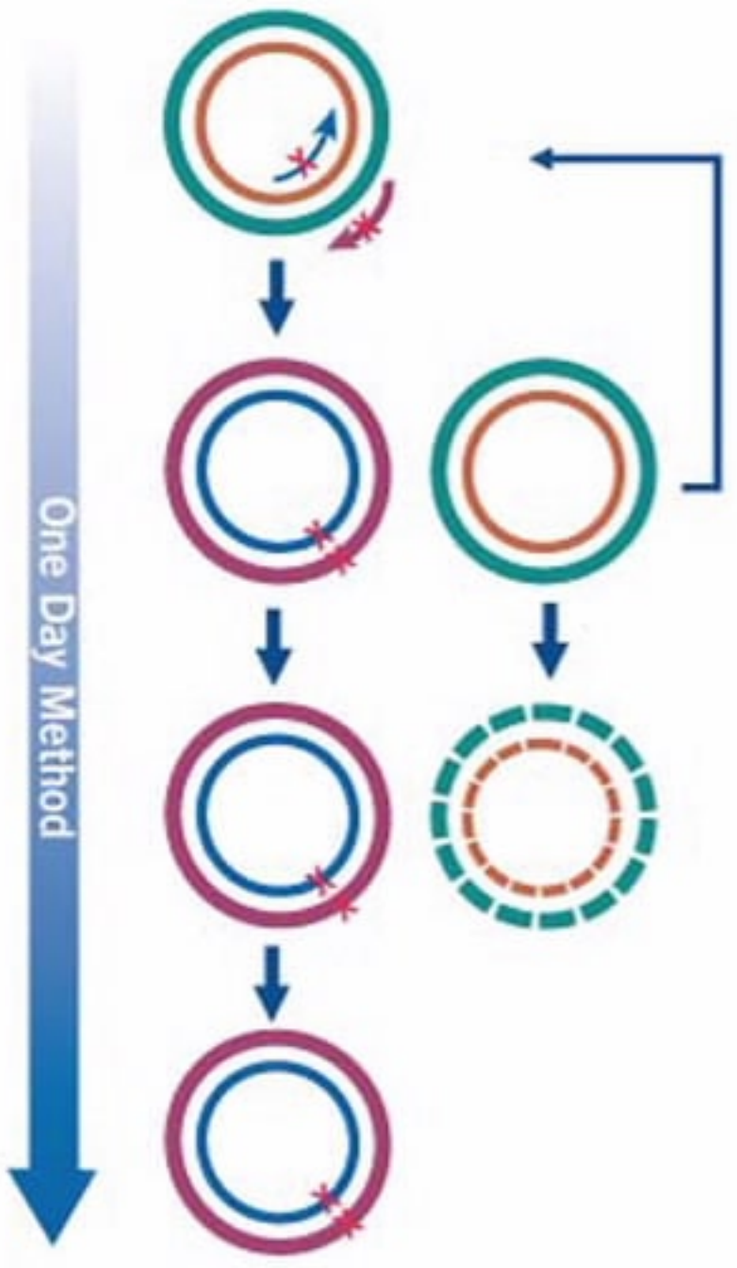
Takara

Site-directed Mutagenesis

Multiple point mutations



Mutant oligonucleotide with multiple (four) single base pair mismatches



1. Mutant Strand Synthesis
Perform thermal cycling to:
- Denature DNA template
- Anneal mutagenic primers containing desired mutation
- Extend and incorporate primers with *high-fidelity* DNA polymerase

2. *Dpn*I Digestion of Template
Digest parental methylated and hemimethylated DNA with *Dpn* I

3. Transformation
Transform mutated molecule into competent cells for nick repair

Agilent

Practical Decisions: Site-directed Mutagenesis

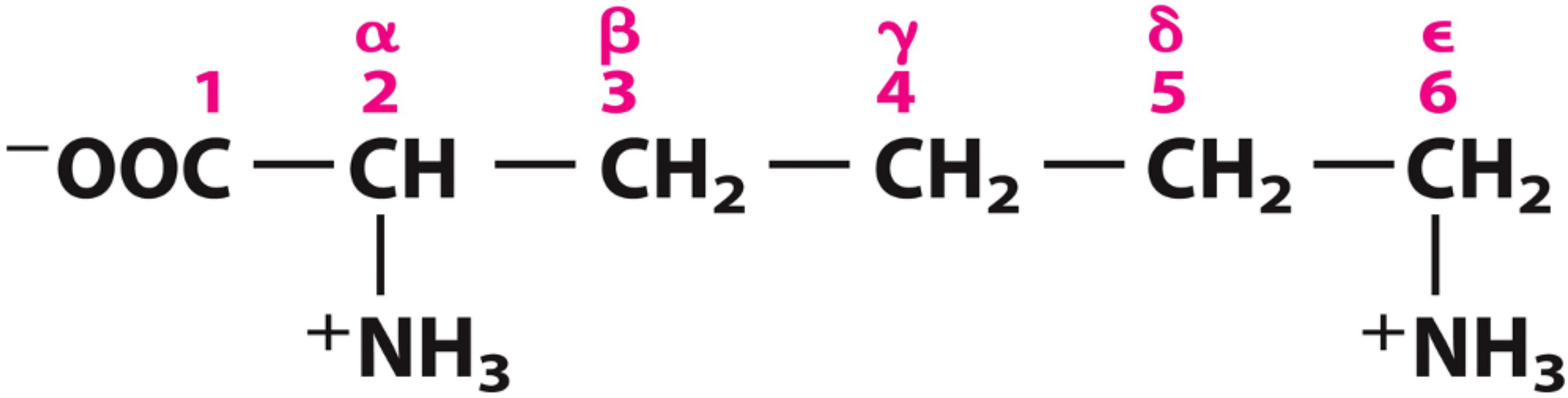
- Rational decisions
- Comparison with family members
- Structural information

Universal Genetic Code

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; **UAA Stop**; **UAG Stop**	UGU, UGC: Cys; **UGA Stop**; UGG Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; **AUG Met**	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

"The genetic code", by OpenStax College, Biology.

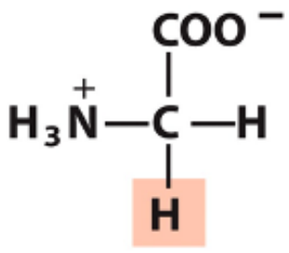
Amino Acids - Numbering of Carbons



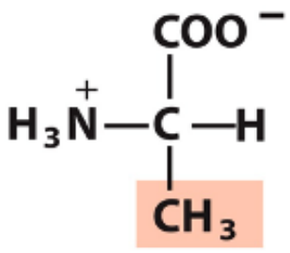
Lysine

Nonpolar, Aliphatic Amino Acids

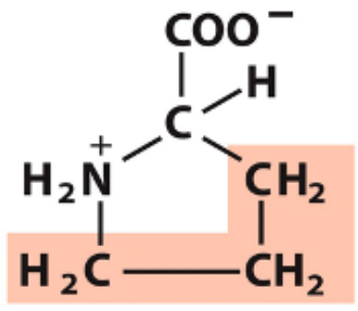
Lack polar functional groups in their side chains. Often cluster together within the interior of proteins, stabilizing protein structure via hydrophobic interactions.



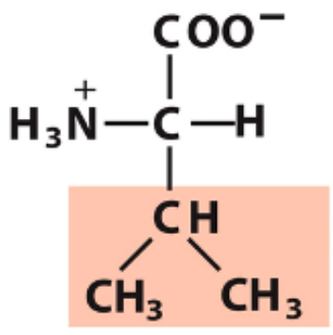
Glycine



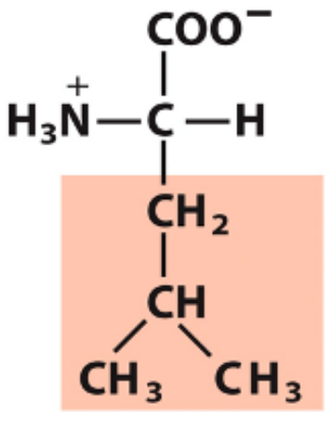
Alanine



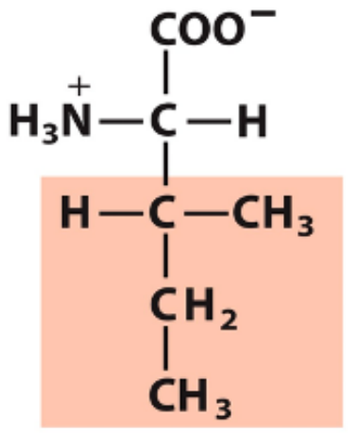
Proline



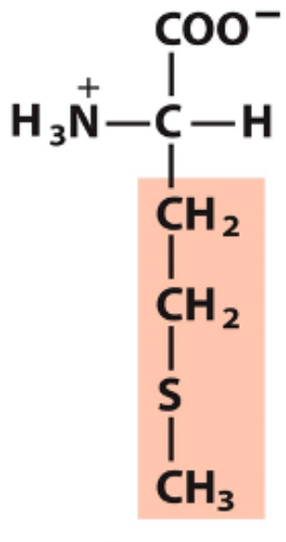
Valine



Leucine



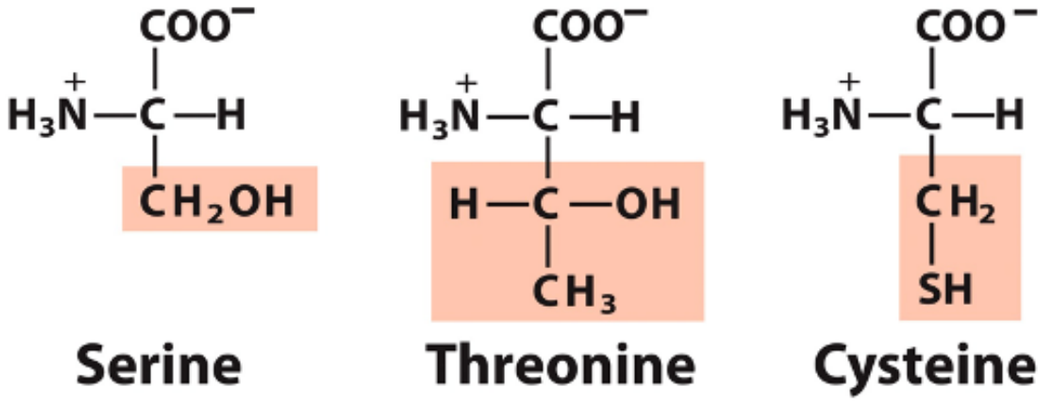
Isoleucine

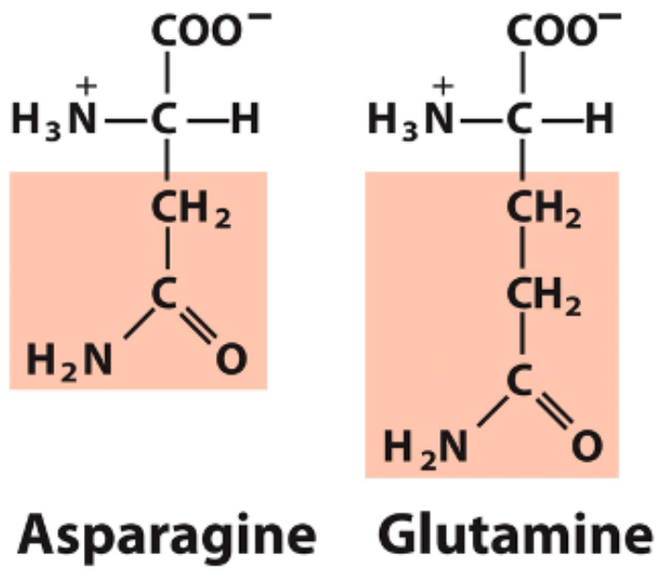


Methionine

Polar, Uncharged Amino Acids

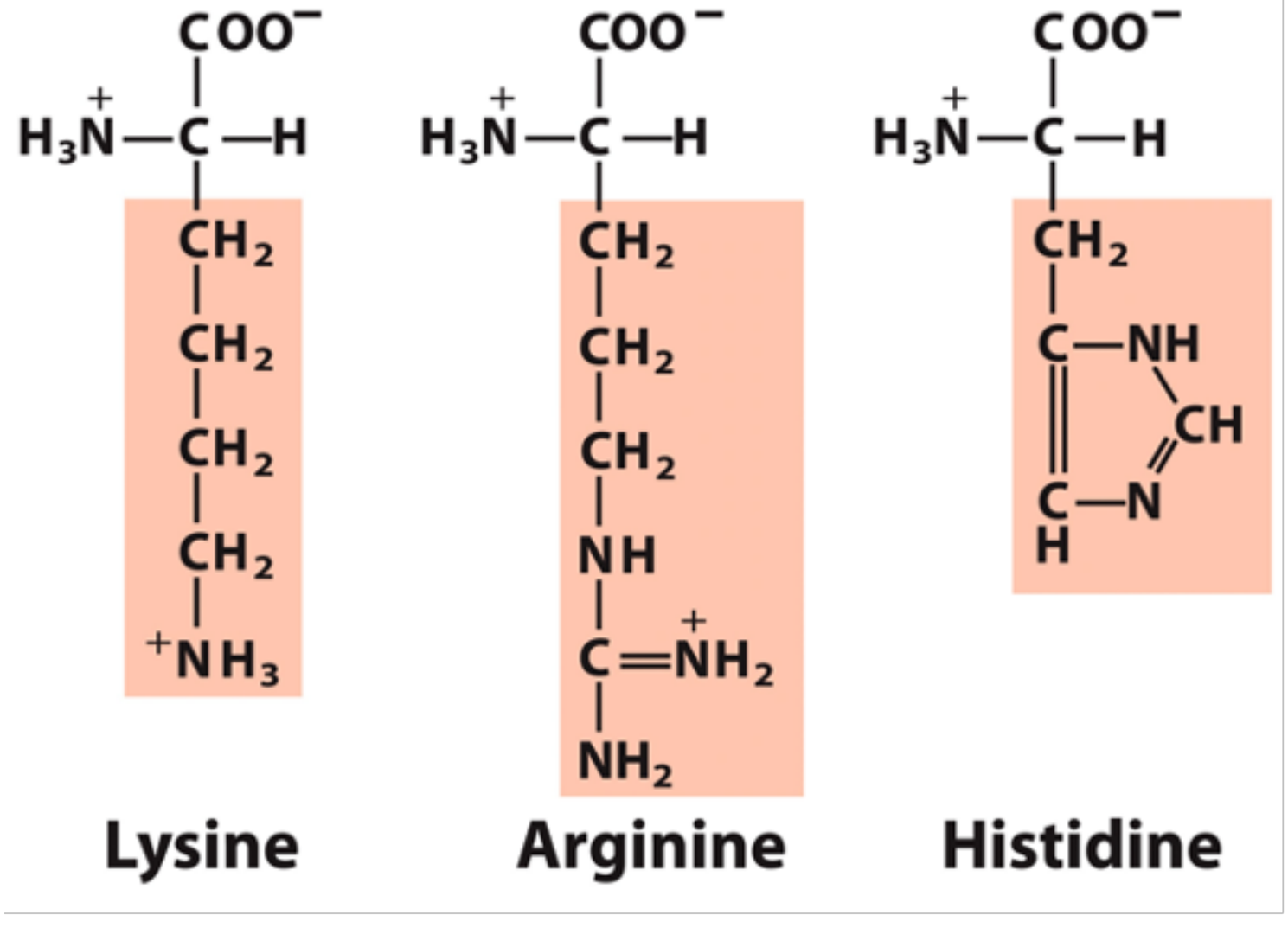
Polar functional groups in their side chains. Able to hydrogen bond with water.





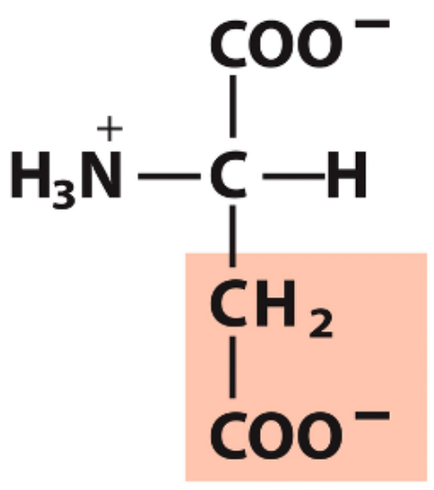
Positively Charged Amino Acids

Hydrophilic R groups with a positive charge. Histidine residues function in many enzymatic reactions as proton donors and/or acceptors.

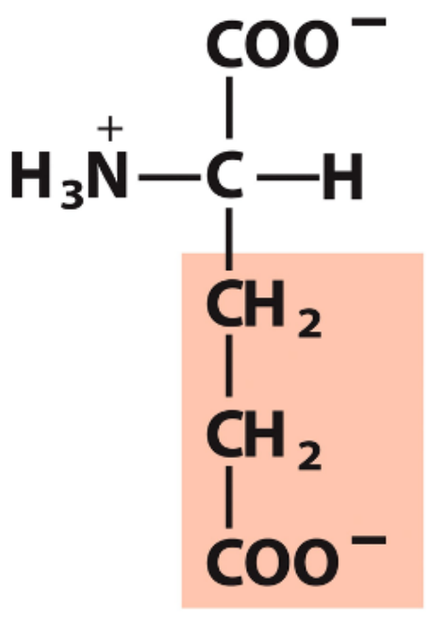


Negatively Charged Amino Acids

Hydrophilic R groups with a negative charge.



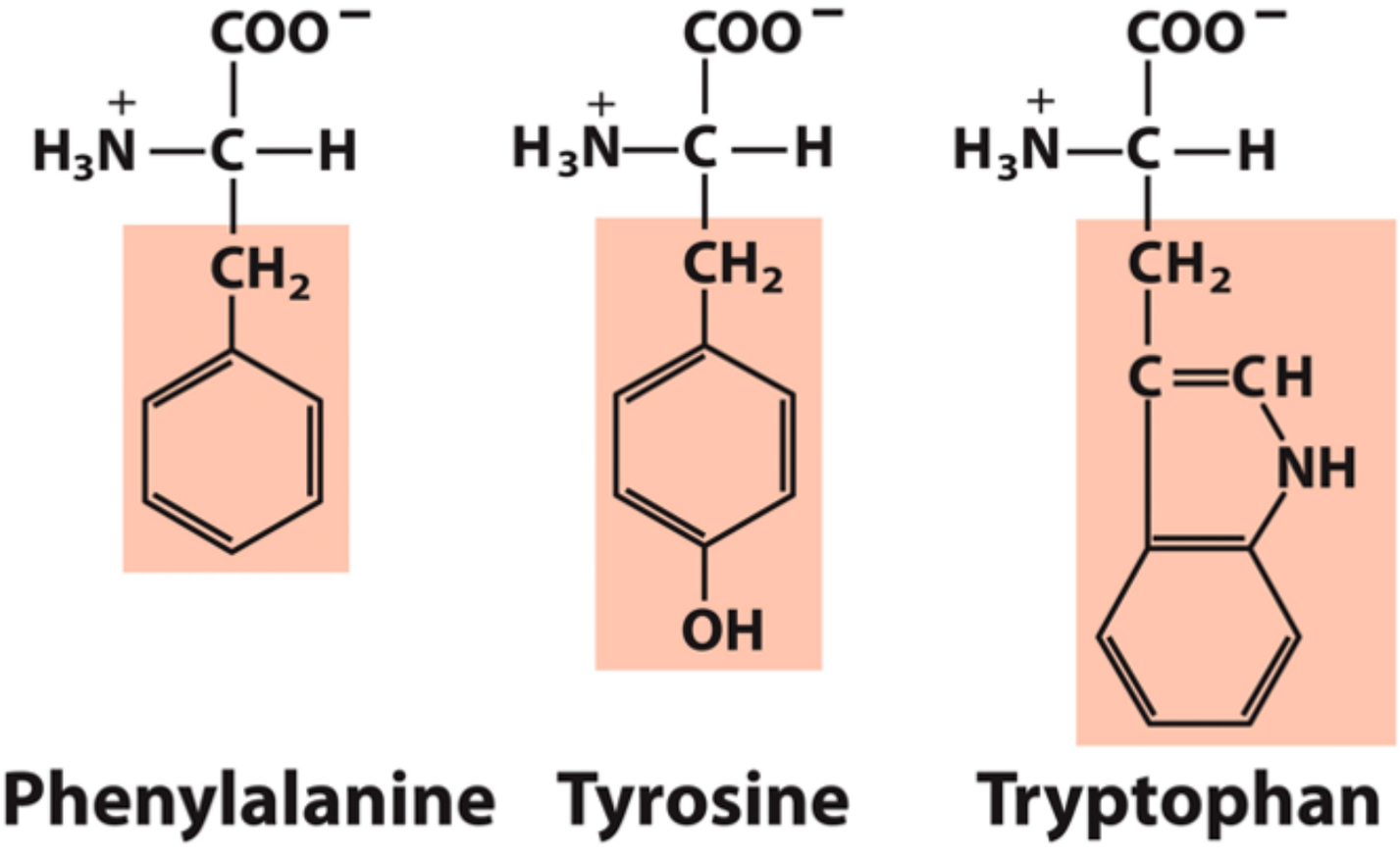
Aspartate



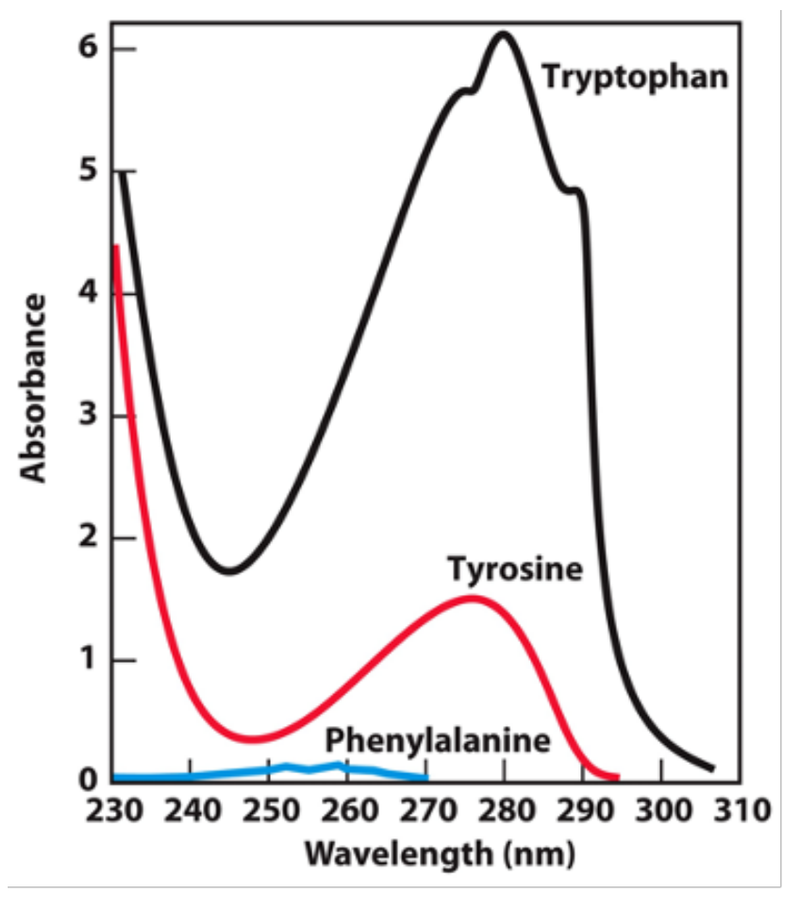
Glutamate

Aromatic Amino Acids

Side chains contain ring structures. The R groups, particularly of tryptophan, absorbs ultraviolet light, which is how proteins are quantified.

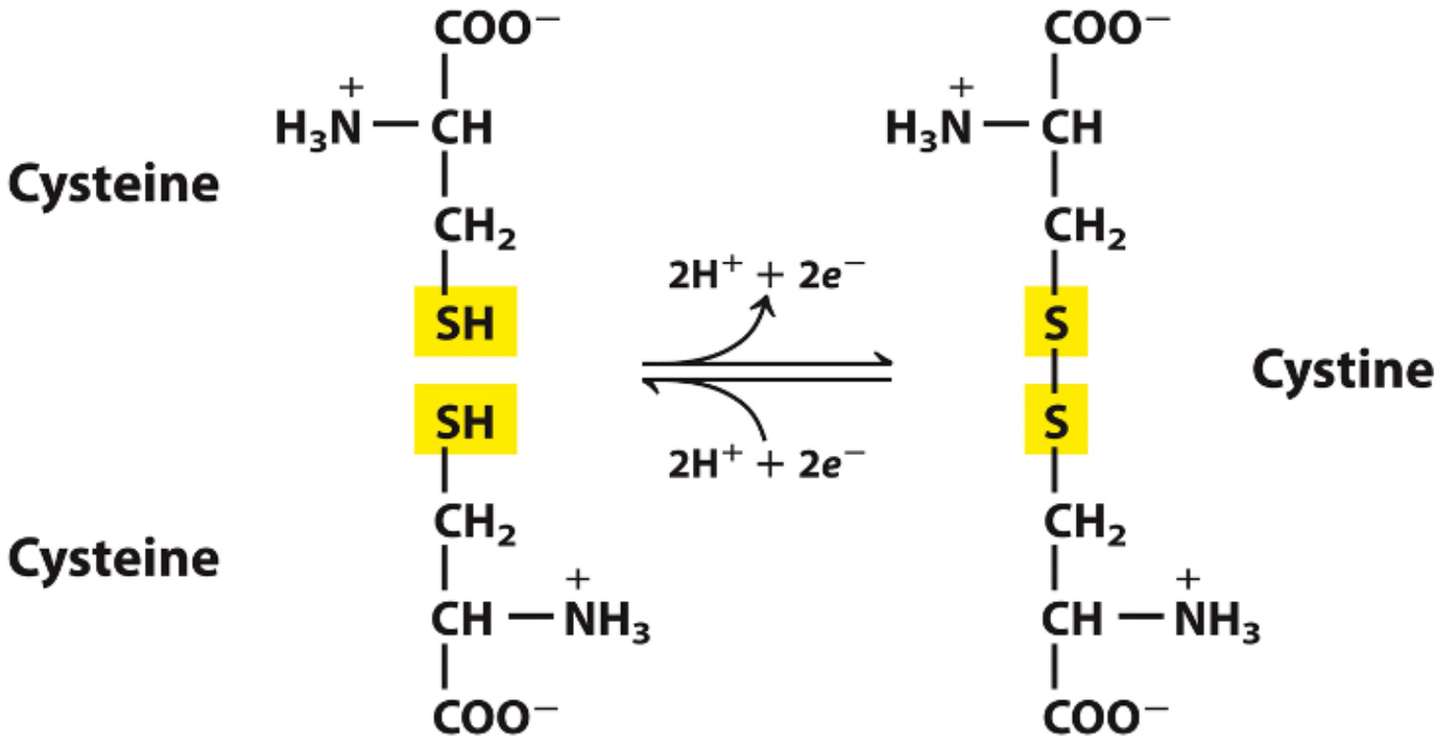


Phenylalanine **Tyrosine** **Tryptophan**



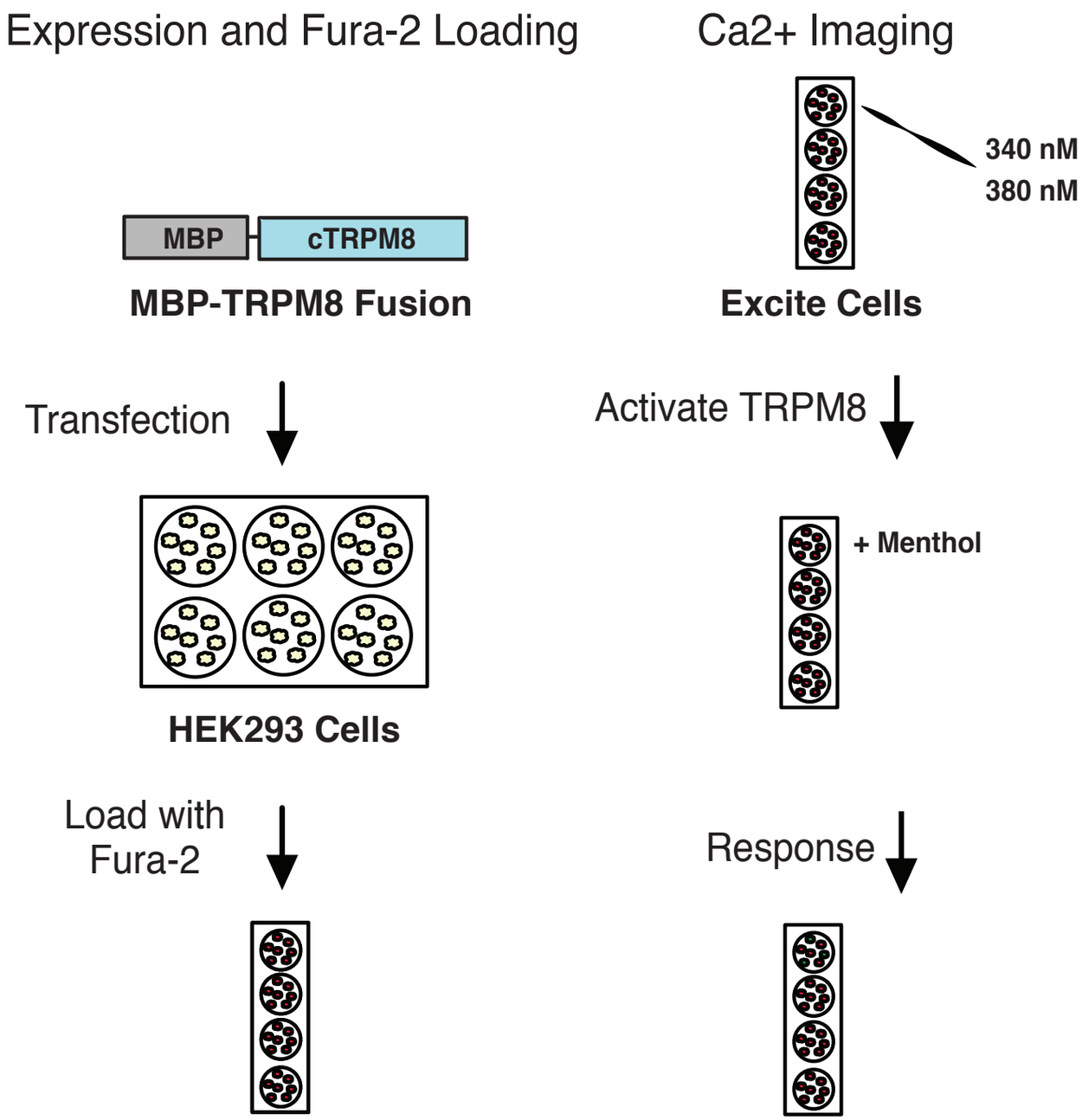
Cysteine and Disulfide Bonds

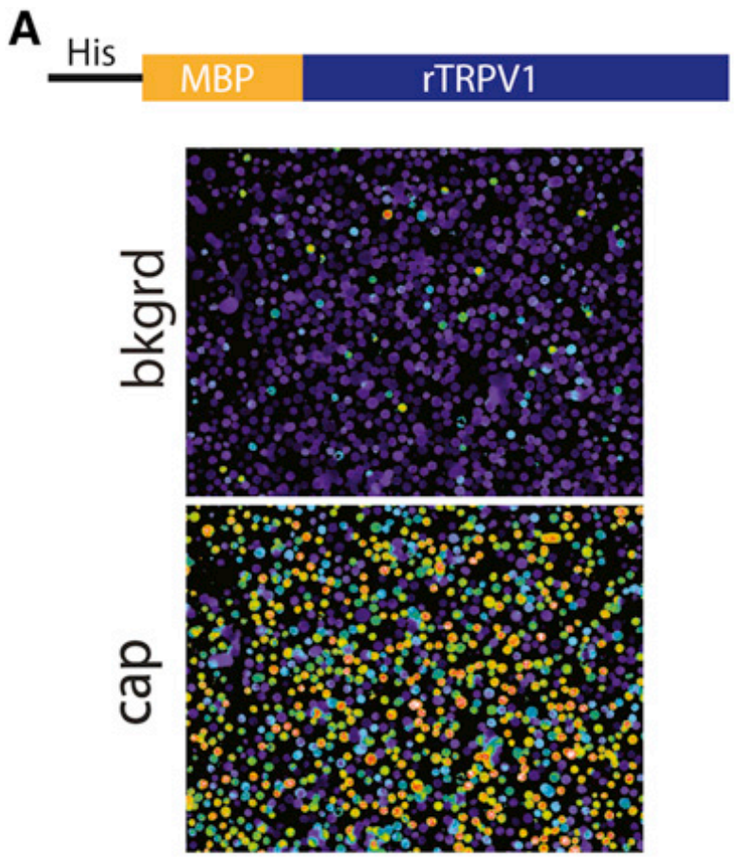
The thiol groups of two cysteine residues are readily oxidized to form a covalently linked disulfide bond.



Assaying
Ion Channel Function

Calcium Imaging

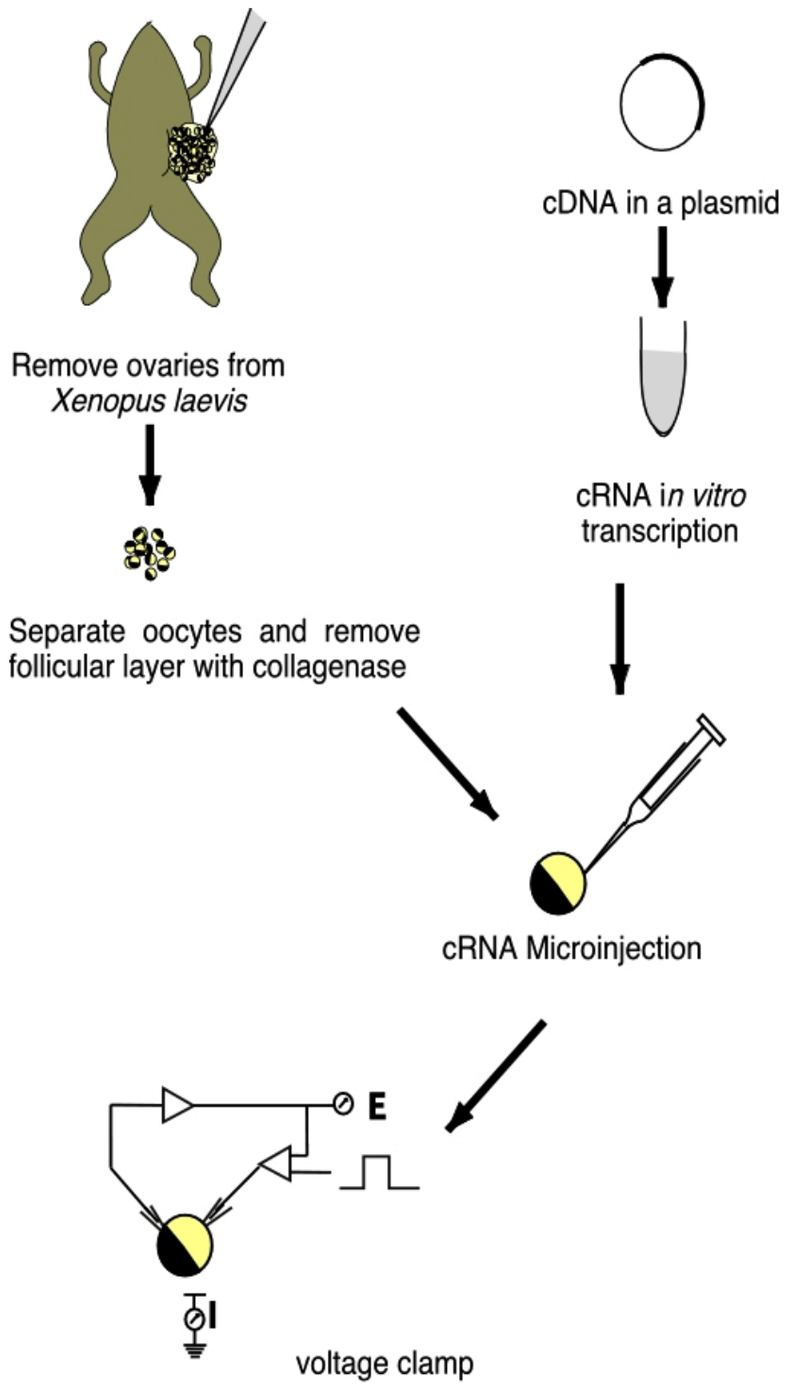




Cao *et al.* & Julius. Neuron. 2013.

Assay Ion Channel Function: Oocyte Recordings

***Xenopus* oocytes**

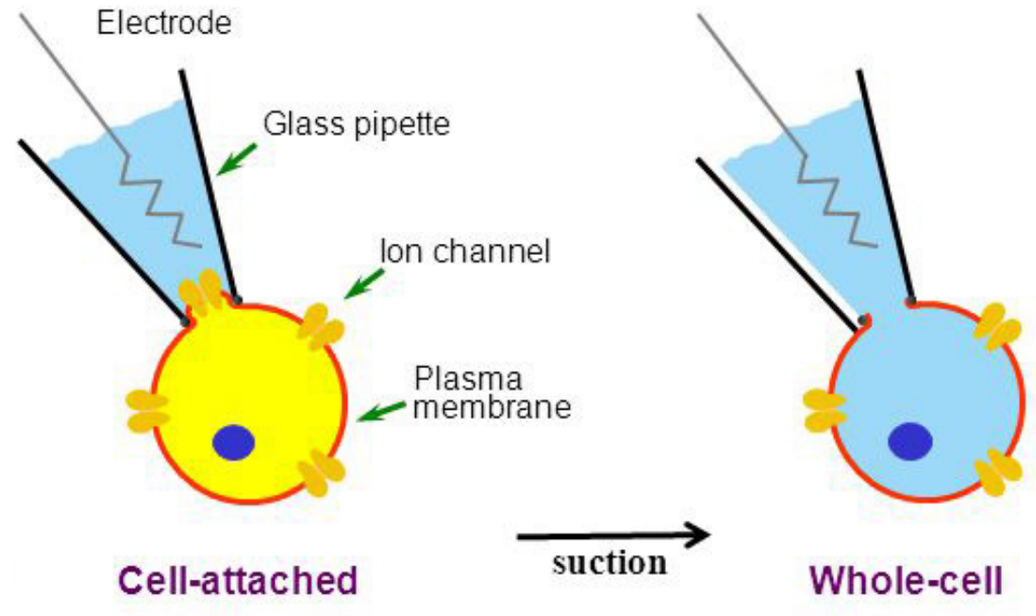


Boccaccio. 2001.

Patch-clamp Electrophysiology: Whole-cell or Single Channel

Patch clamp recording configurations

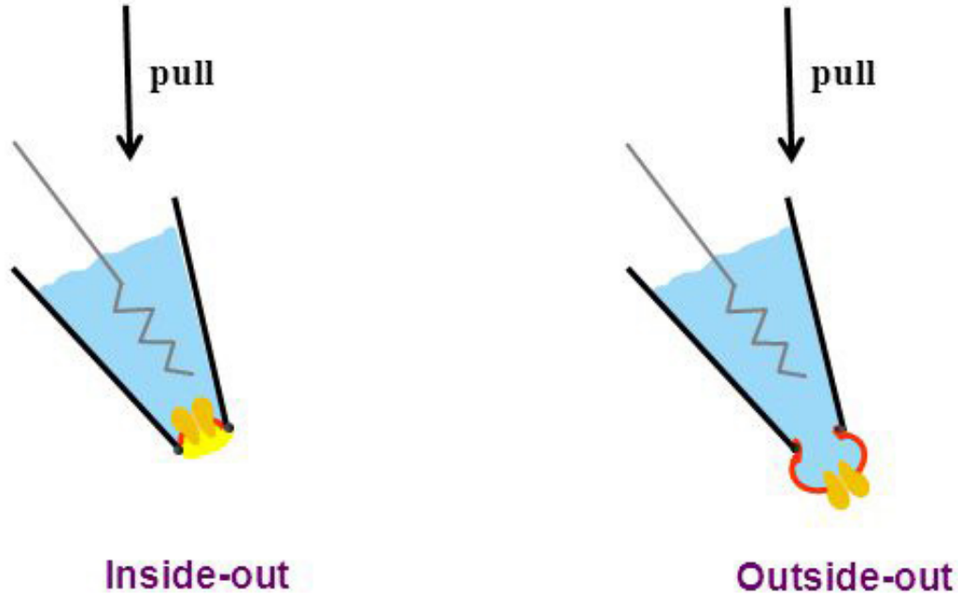
Whole-cell



Most Common

Single Channel

Effect of Cytosolic Factors



Effect of Extracellular Factors

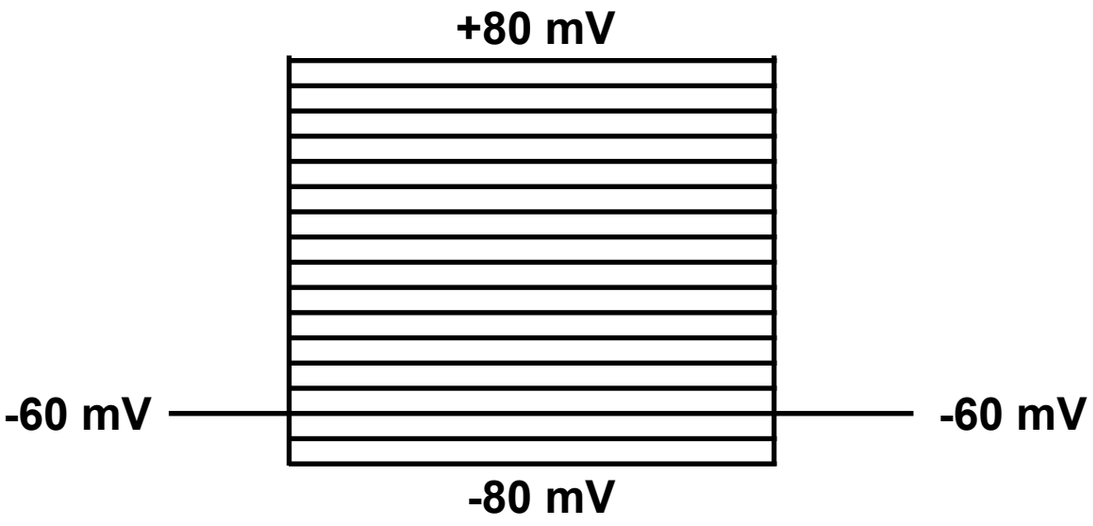
Ion Channel Electrophysiology: What questions can you answer?

Macroscopic Currents

- Ion specificity
- Effect of voltage, pH, (or lipids)
- Effect of drugs
- Functionally important regions or amino acid residues (with mutagenesis)

Voltage Clamp Experiments

By clamping the voltage of an isolated excitable cell membrane, the currents that flow through ion channels may be measured.



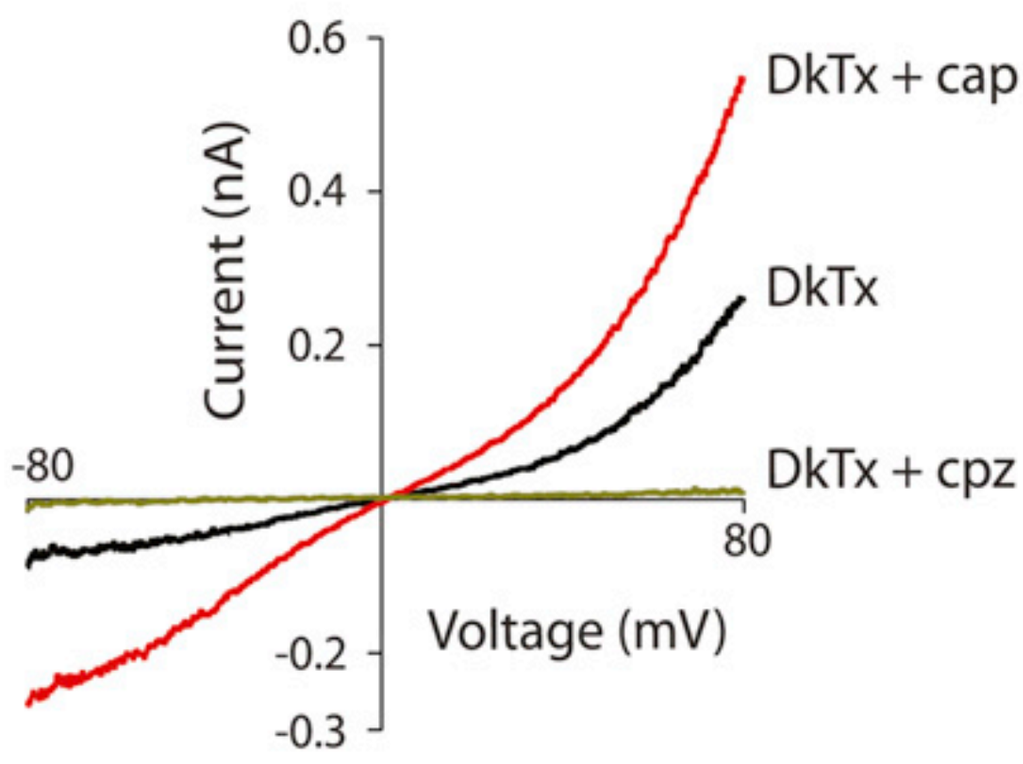
Voltage Ramp

E

Na^+ and Ca^{2+} ions enters the cell.

I-V Curve

Na^+ and Ca^{2+} ions exit the cell.



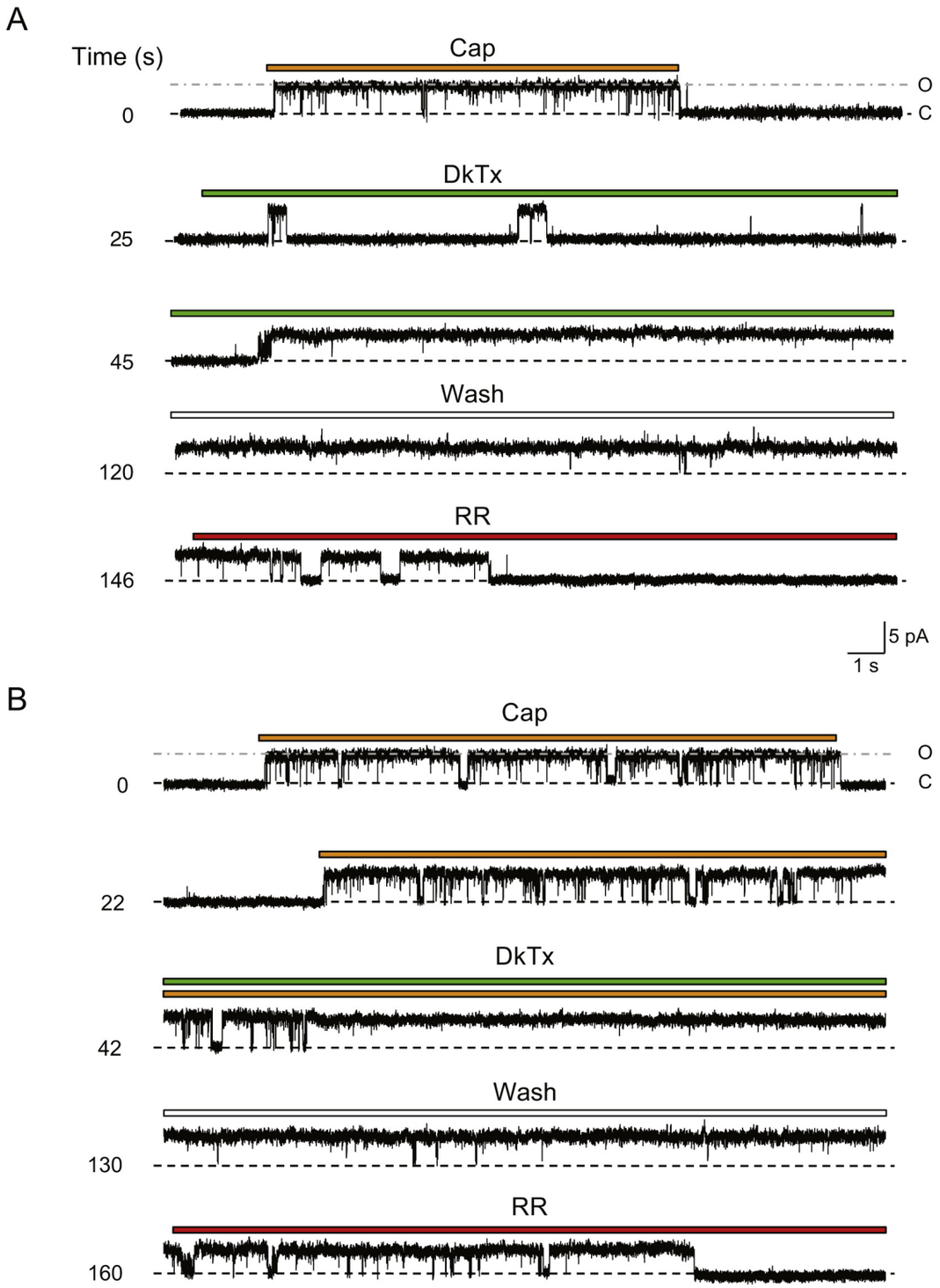
Cao *et al.* & Julius. Neuron. 2013.

Ion Channel Electrophysiology: What questions can you answer?

Single Channel Currents

- Current transmitted by a single channel
- Time spent open/closed

Single Channel Recordings



The the presence of DkTx, TRPV1 often flickers, but once open, the channel stays open for a very long time.

Bohlen *et al*. & Julius. Cell. 2010.

Ion Channel Electrophysiology: How to study ion channels in isolation?

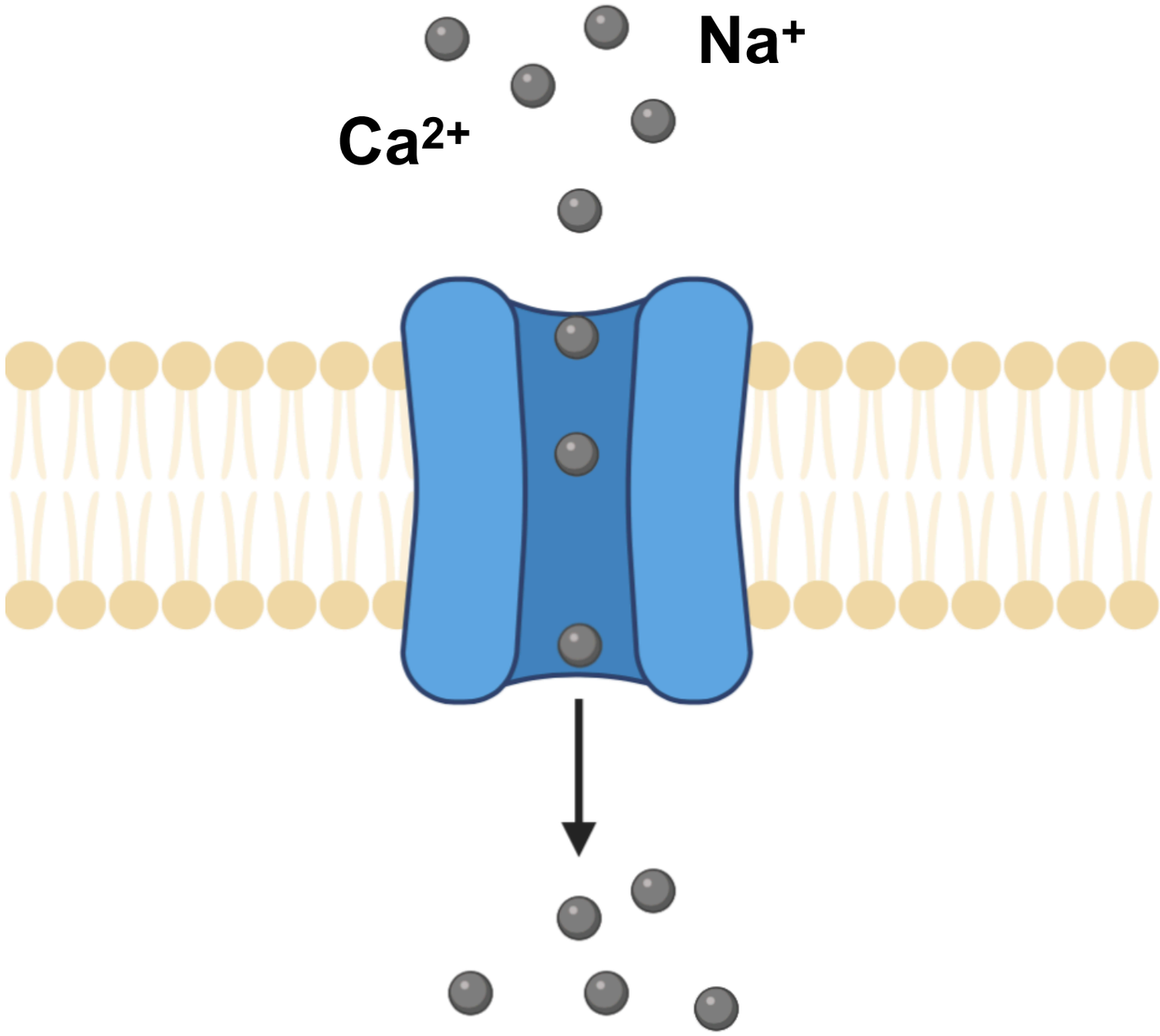
Analyzing Currents in Isolation

- Block the function of confounding ion channels with specific inhibitors

Protein Structure-function Analysis to Elucidate Mechanism of the Cold Receptor TRPM8

Role of Calcium in TRPM8 Physiology

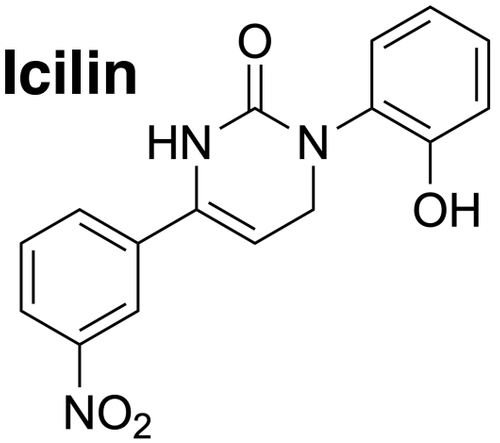
Permeant Ion

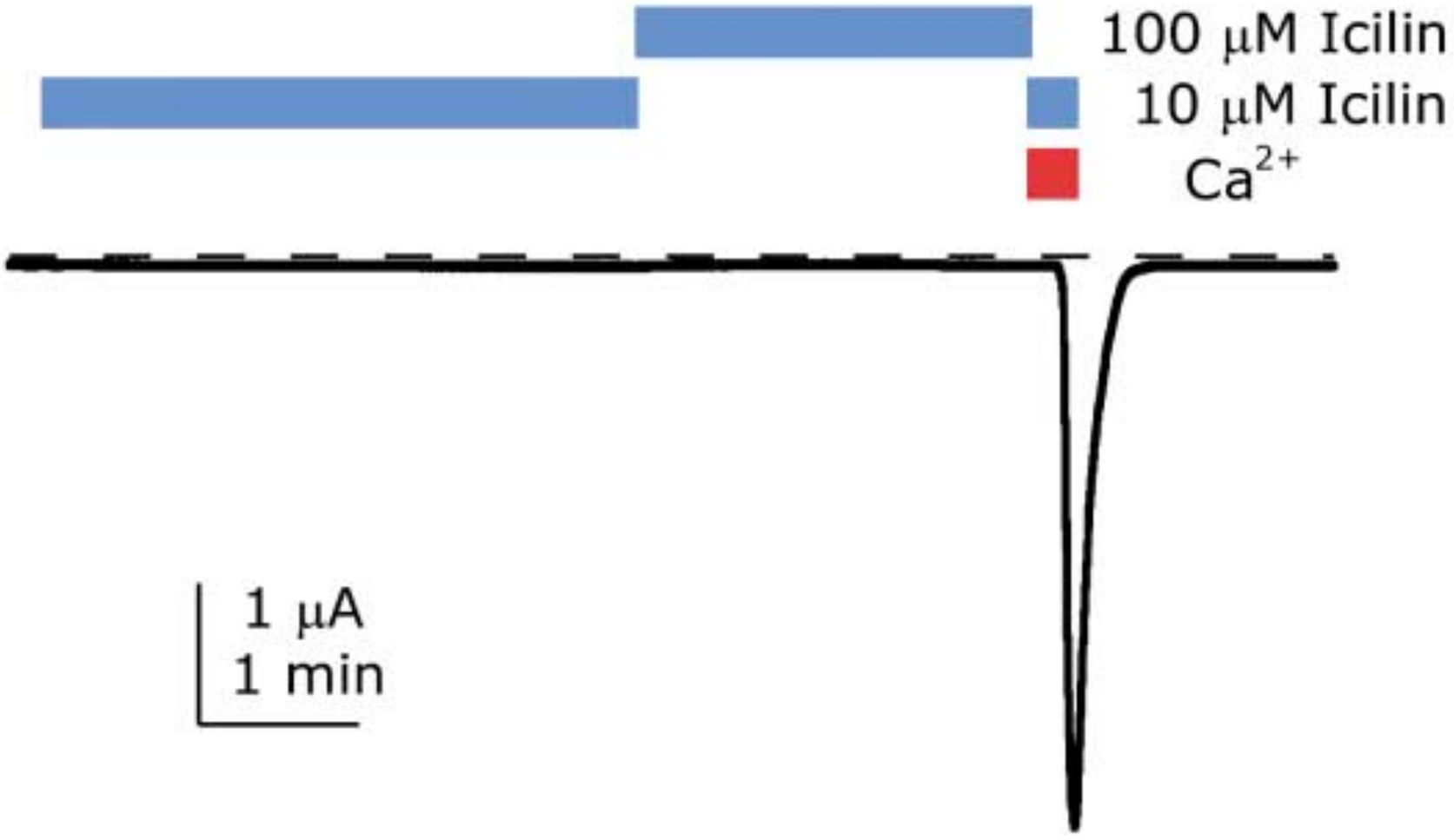


Role of Calcium in TRPM8 Physiology

Co-agonist with Icilin
(Synthetic Super-cooling Agent)

Icilin

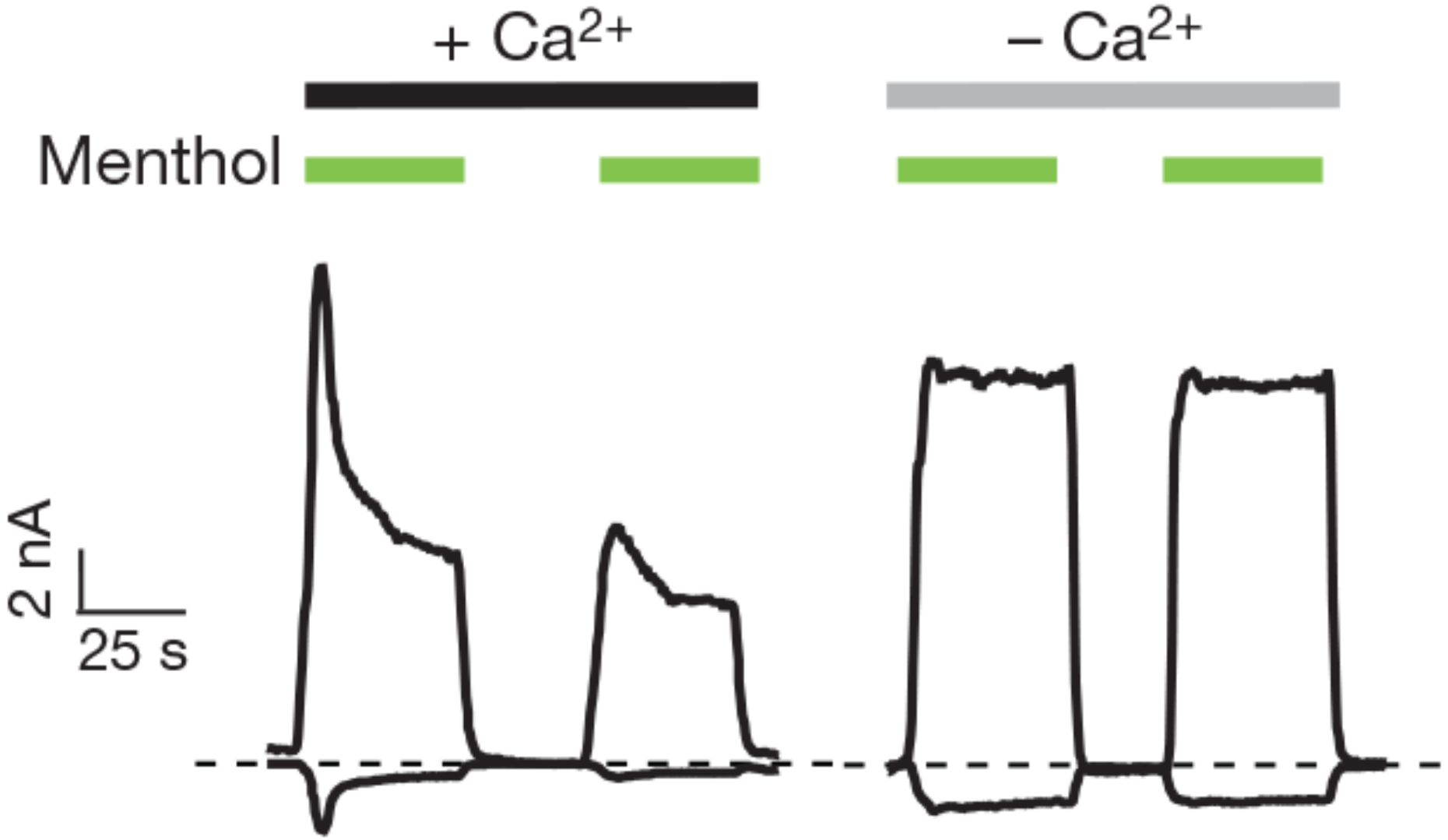




H. H. Chuang *et al.* Neuron. 2004.

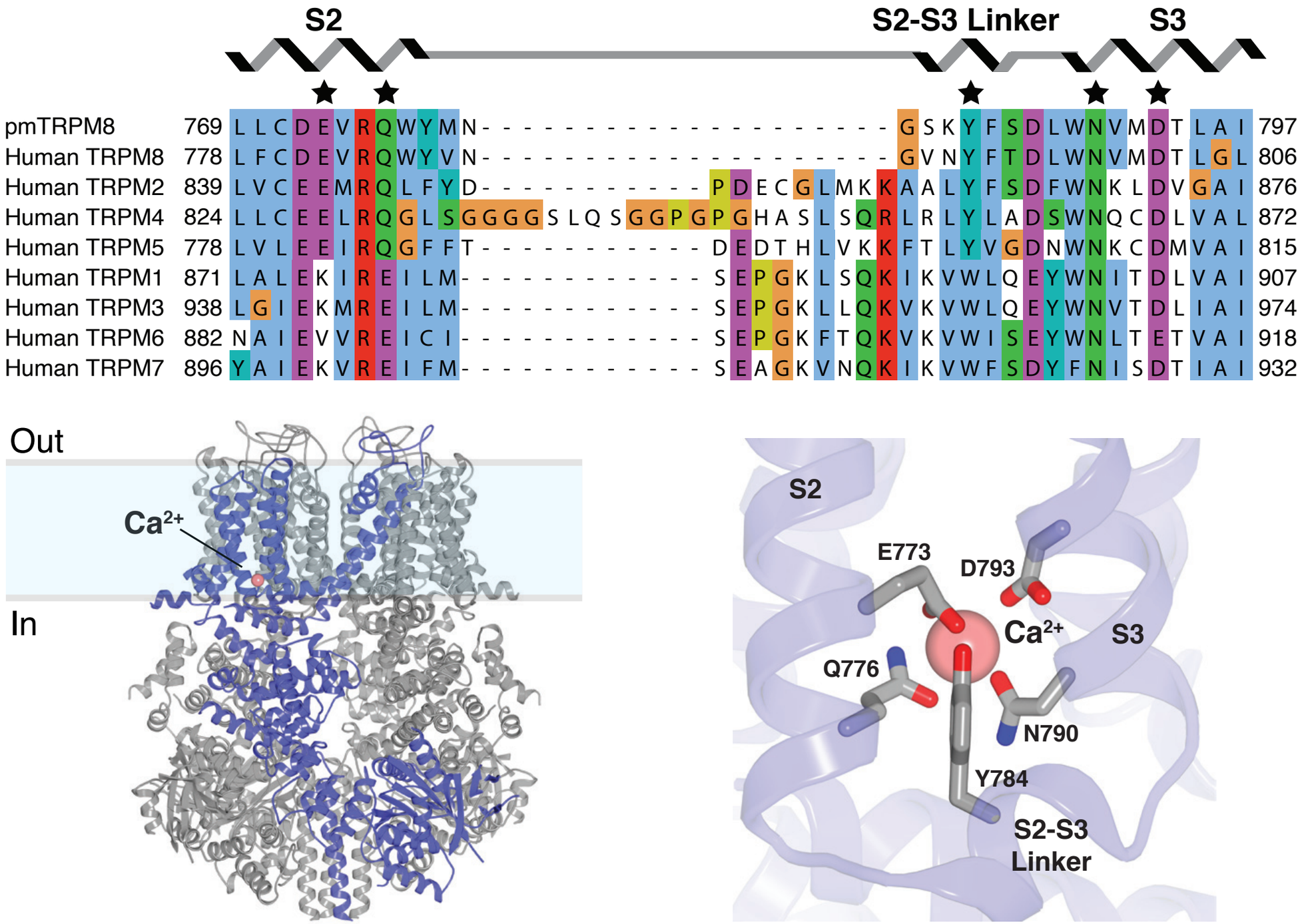
Role of Calcium in TRPM8 Physiology

Causes Desensitization of Current



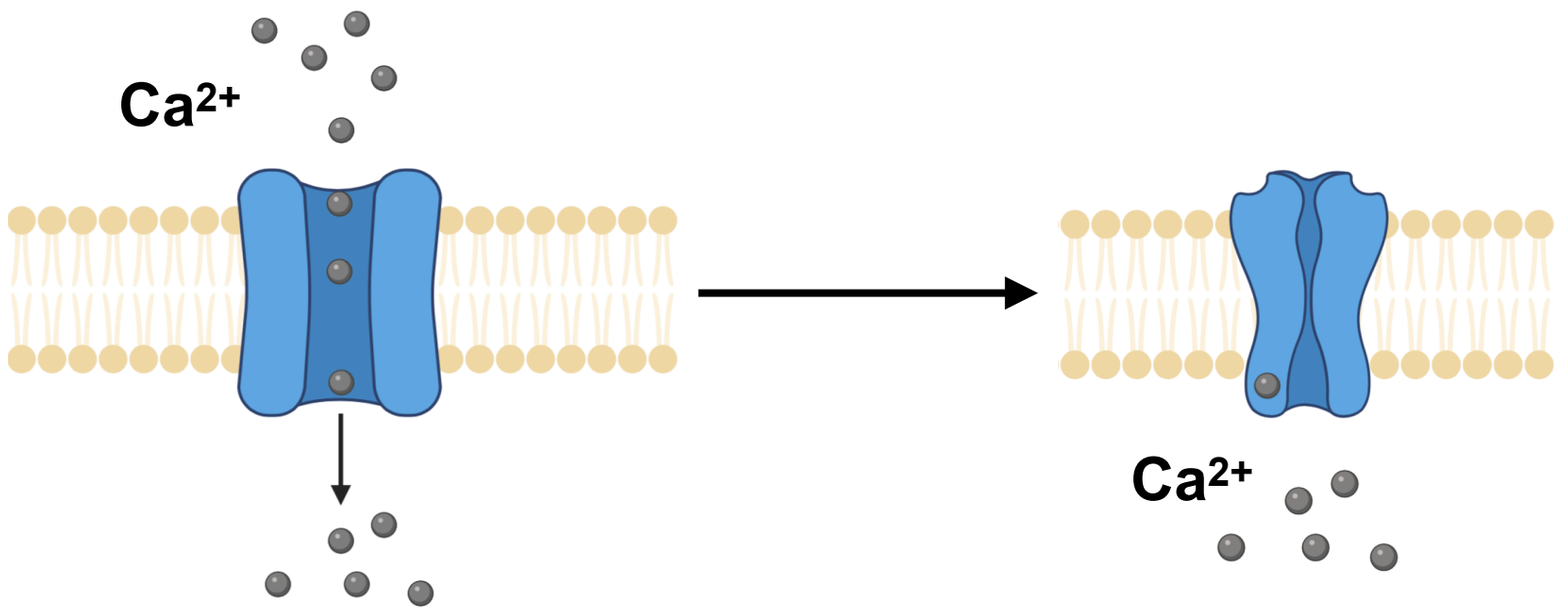
D. McKemy *et al.* Nature. 2002.

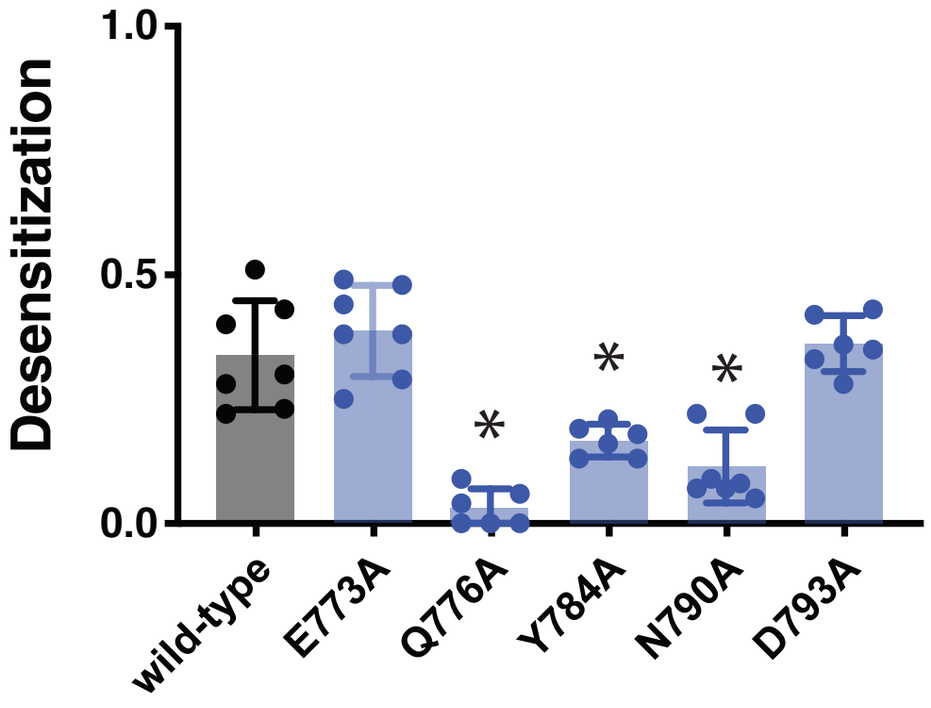
Conserved Calcium Binding Site

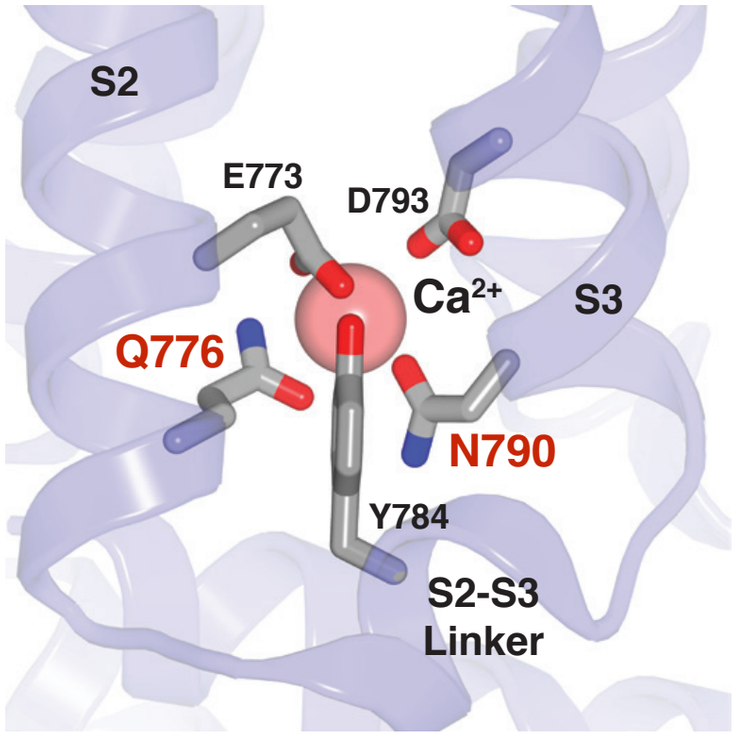


Diver, Cheng & Julius. Science. 2019.

Channel Desensitization Occurs Through Direct Calcium Binding

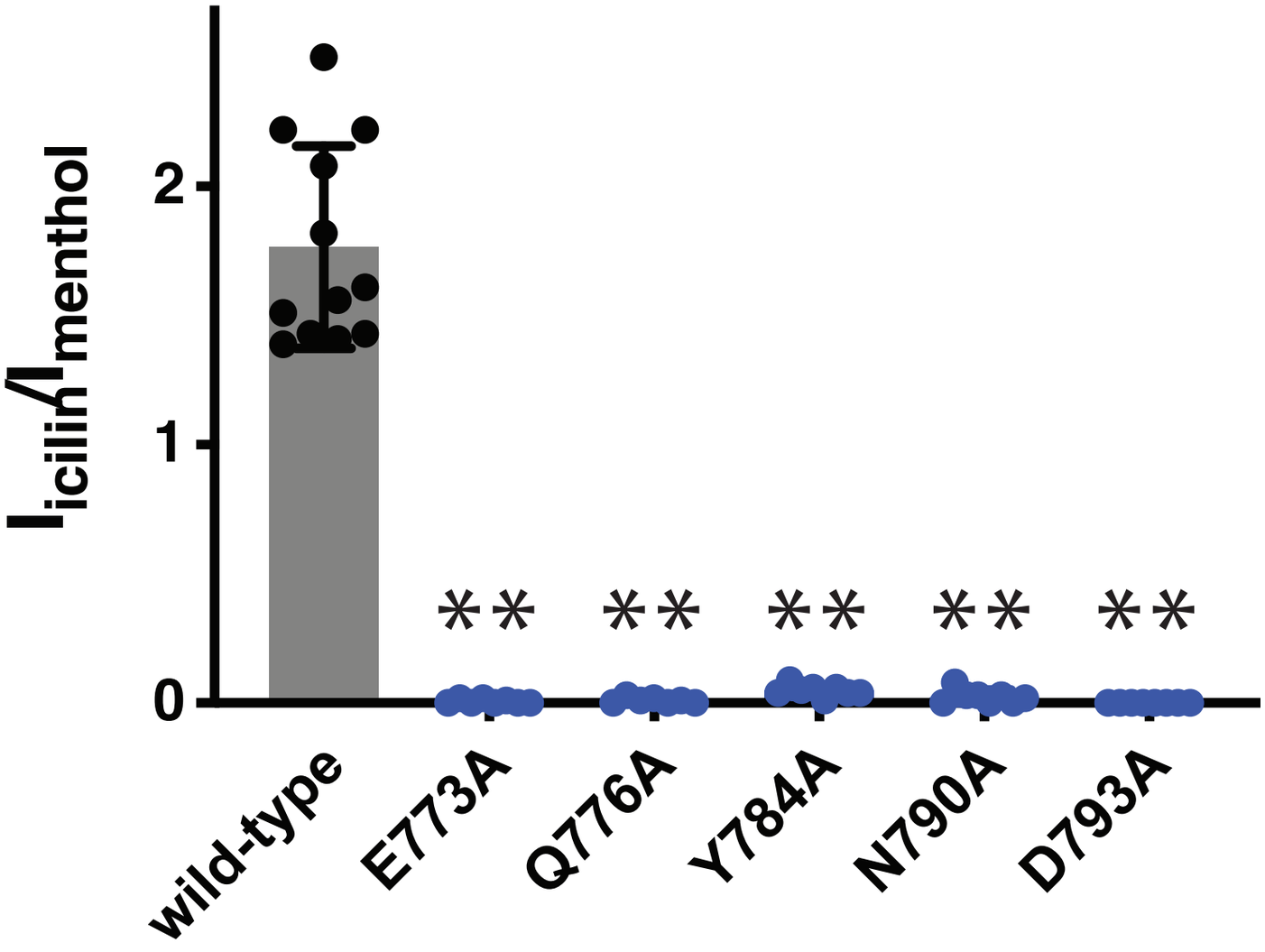


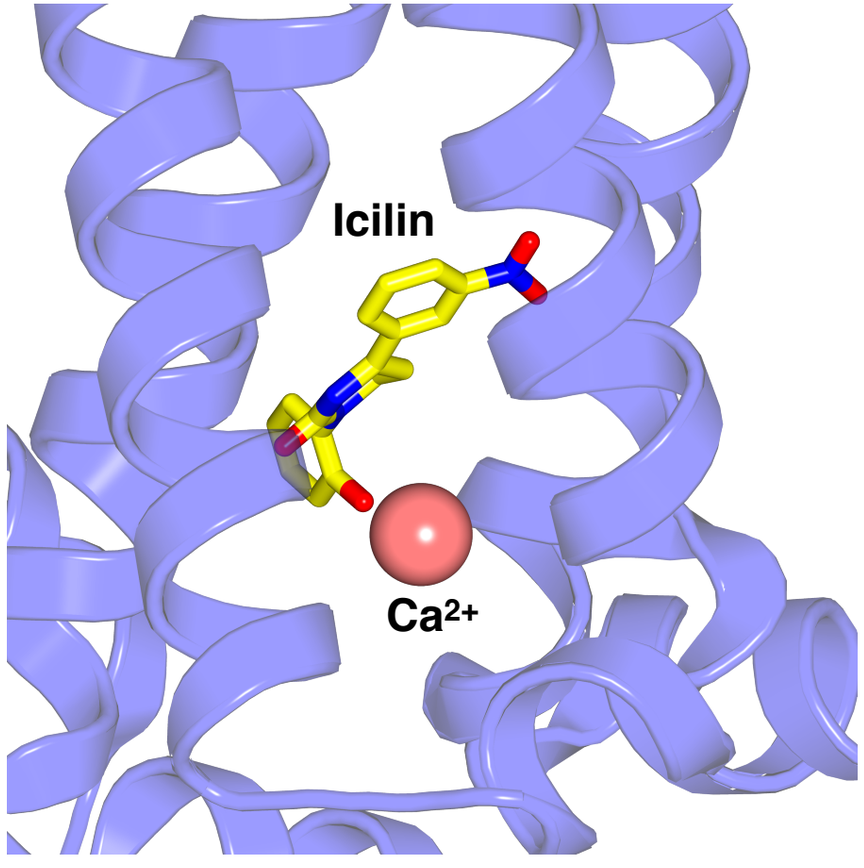




Diver, Cheng & Julius. Science. 2019.

Calcium Binding to the Same Site is Required for Activation by Icilin



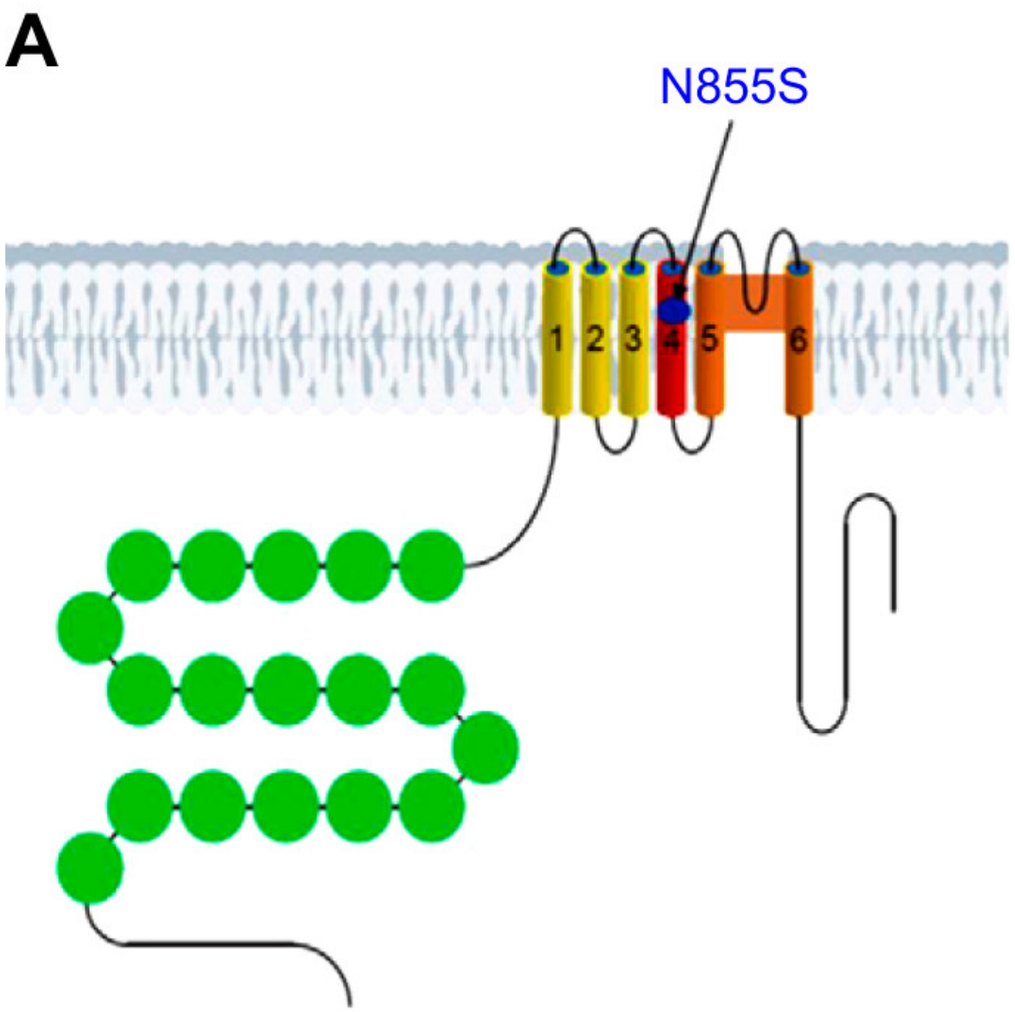


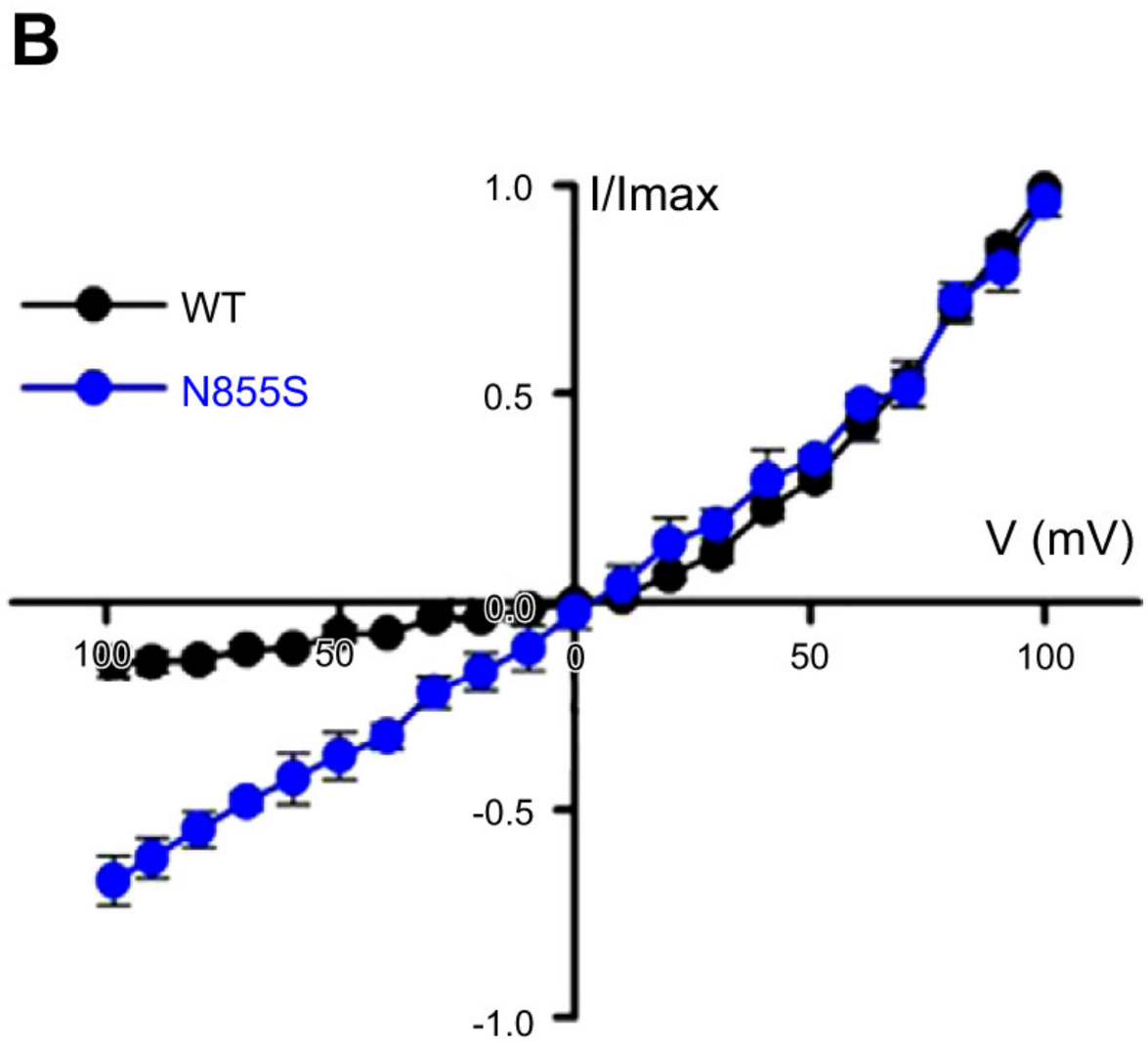
C. Zhao *et al.* Nat. Comm. 2022.

Diver, Cheng & Julius. Science. 2019.

Channelopathies

TRPA1-related Channelopathy: Familial Episodic Pain Syndrome





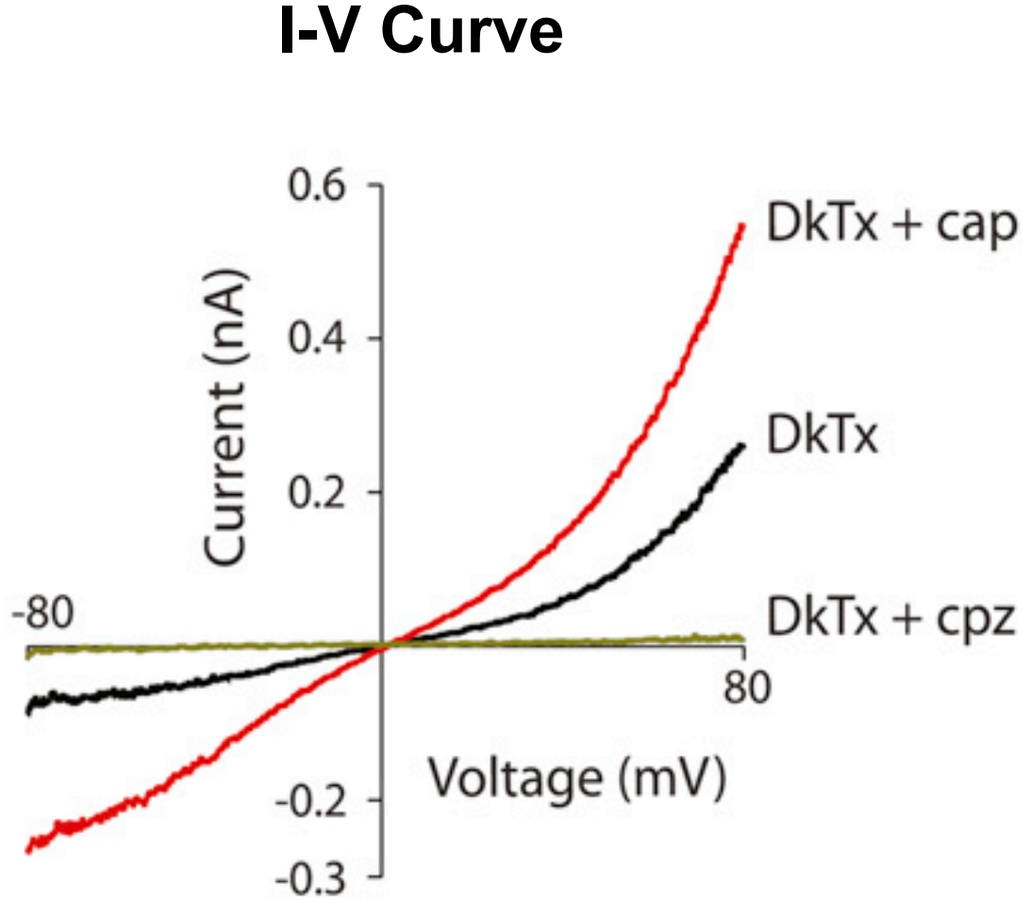
Cause is a mutation in N855S that is activating.

Talavera. Physiol. Rev. 2020.

Voltage Clamp Experiments: Reversal Potential

Reversal potential refers to the membrane potential at which the current changes its flowing direction. Allows the experimenter to determine ion specificity.

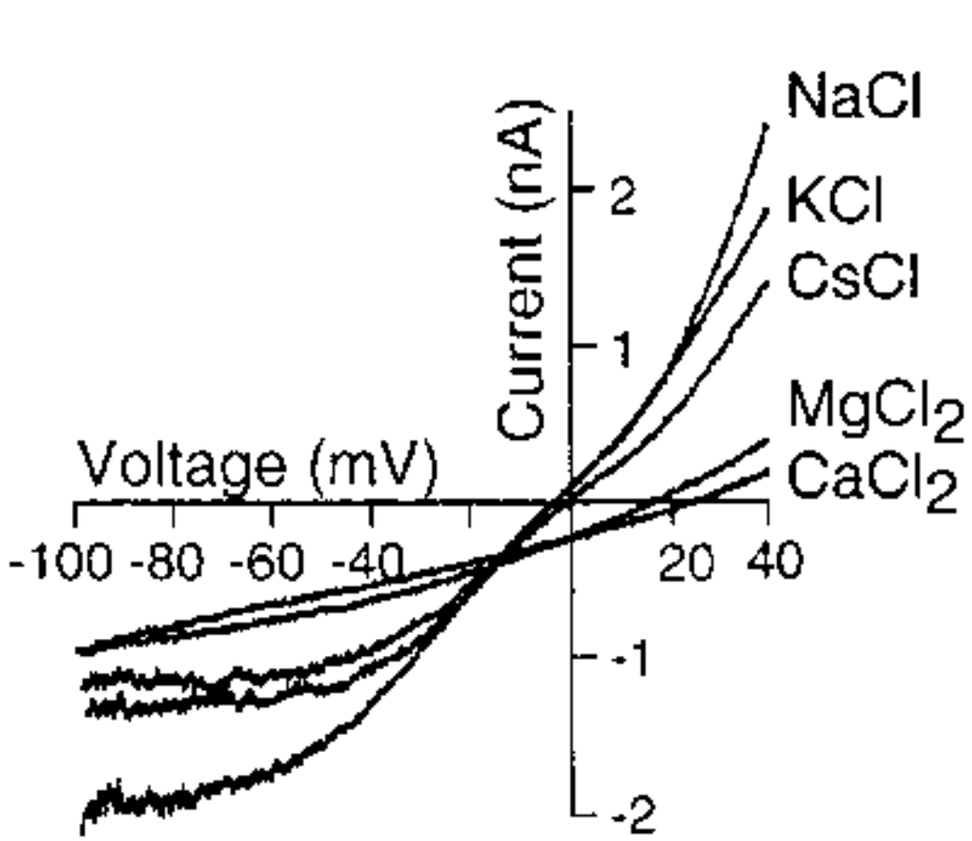
If close to zero, then must be a cation-specific ion channel.



Cao *et al.* & Julius. Neuron. 2013.

Voltage Clamp Experiments: Ion Substitution Experiments

Can obtain a permeability sequence by obtaining current-voltage relationships for cells bathed in solutions of different ionic composition.



Caterina *et al.* & Julius. Nature. 1997.

Inside the Pipette = NaCl

Bath Solution = Different Ionic Composition

TRPV1 is a non-selective cation channel that exhibits notable preference for divalent ions.

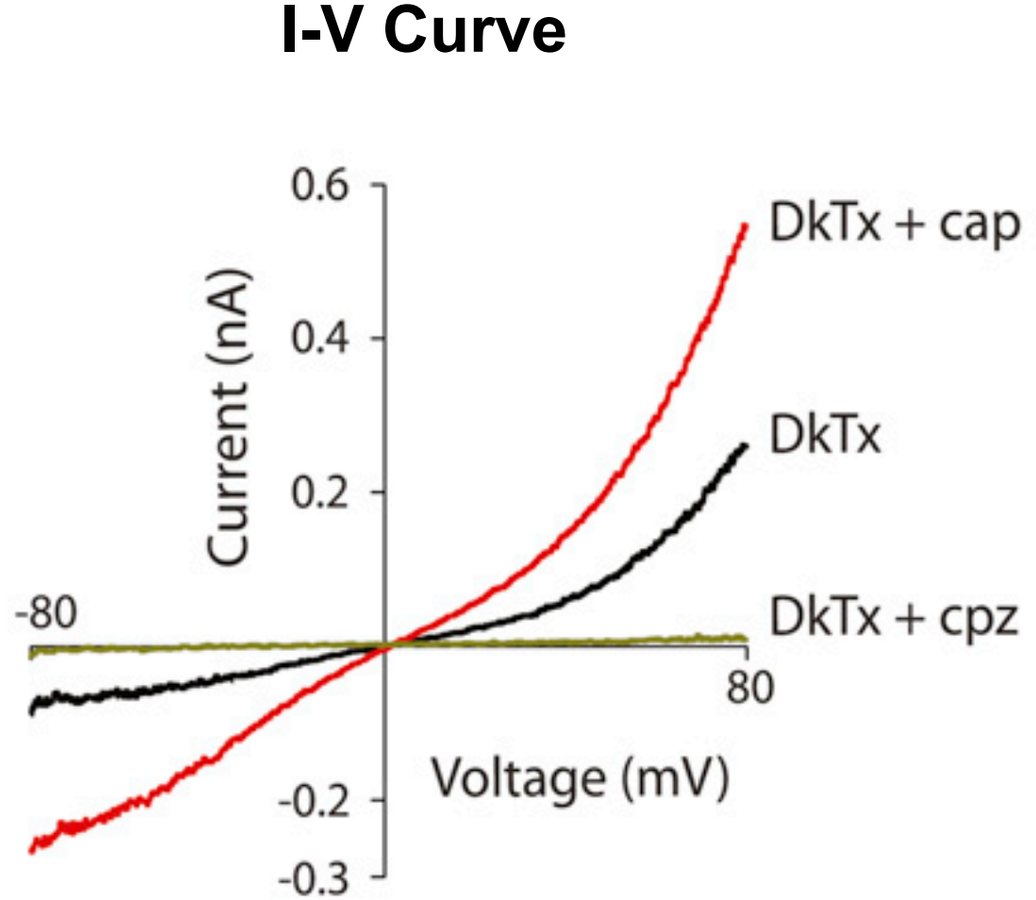
$Ca^{2+} > Mg^{2+} > Na^{+} \approx K^{+} \approx Cs^{+}$

Voltage Clamp Experiments: Rectification

Membrane conductance changes with voltage.

TRPV1 is outward rectifying.

(conducts ions more readily in the outward direction)



Cao *et al.* & Julius. Neuron. 2013.