Transient receptor potential ion channels

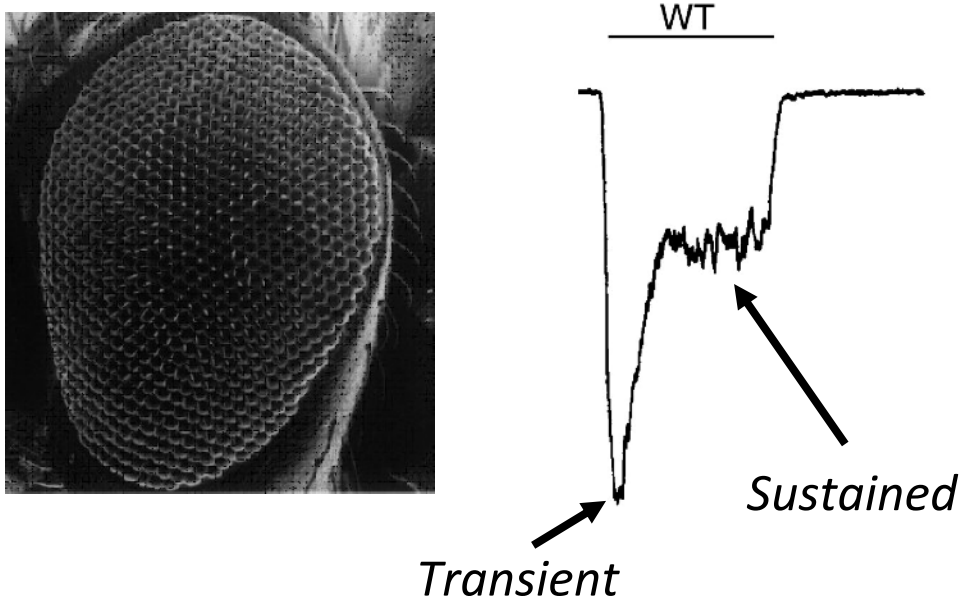
"Multi-valent cation channels"

Some like it "hot" others "cold"

Transient receptor potential proteins

Original observation in 1969 leading to the discovery of TRPs:

Drosophila with mutation in the *trp* gene feature rapid bleaching in strong light (horizontal line) .



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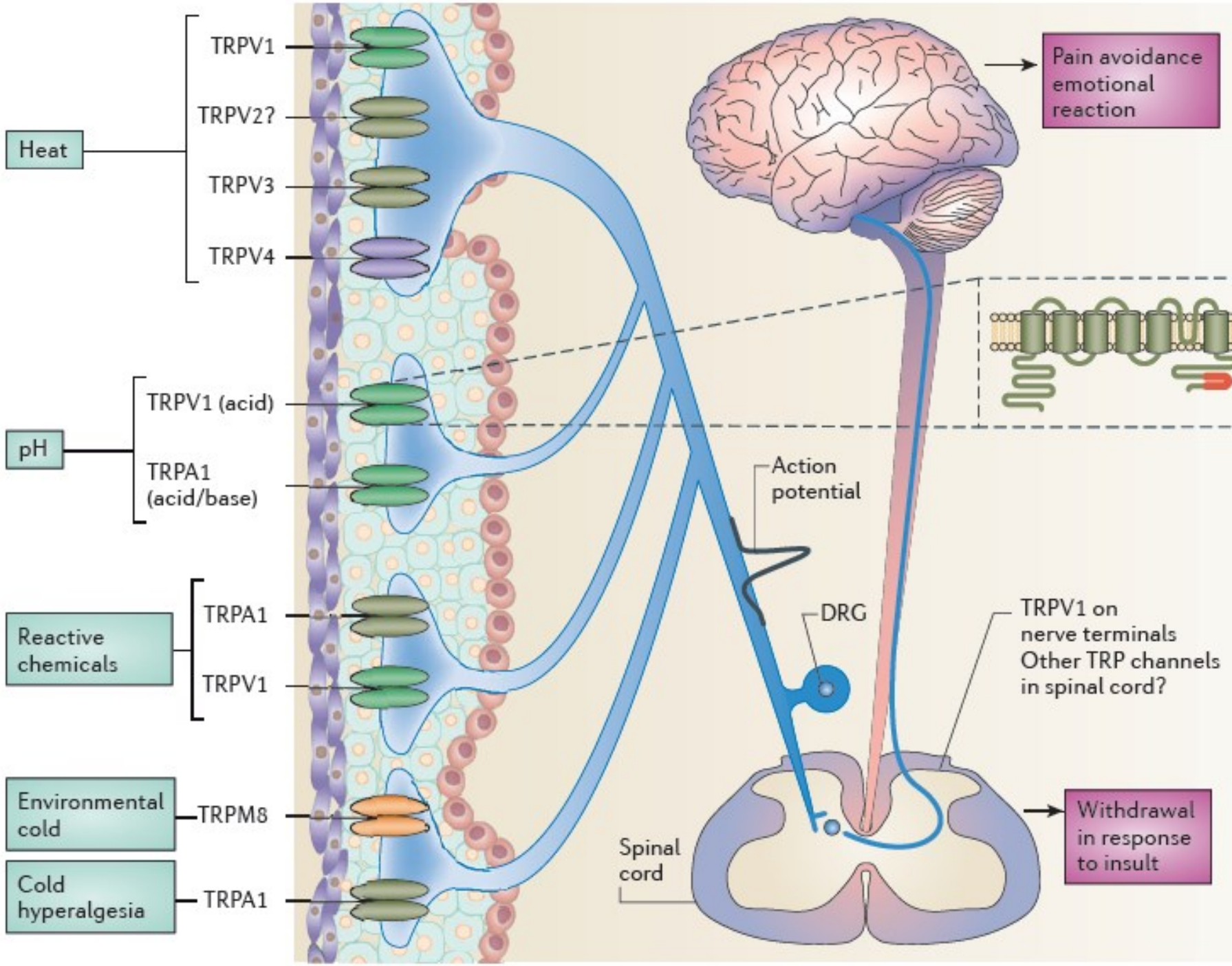
Cosens & Mannin

Pain and temperature perception

Pain:
Alarm
&
Burden



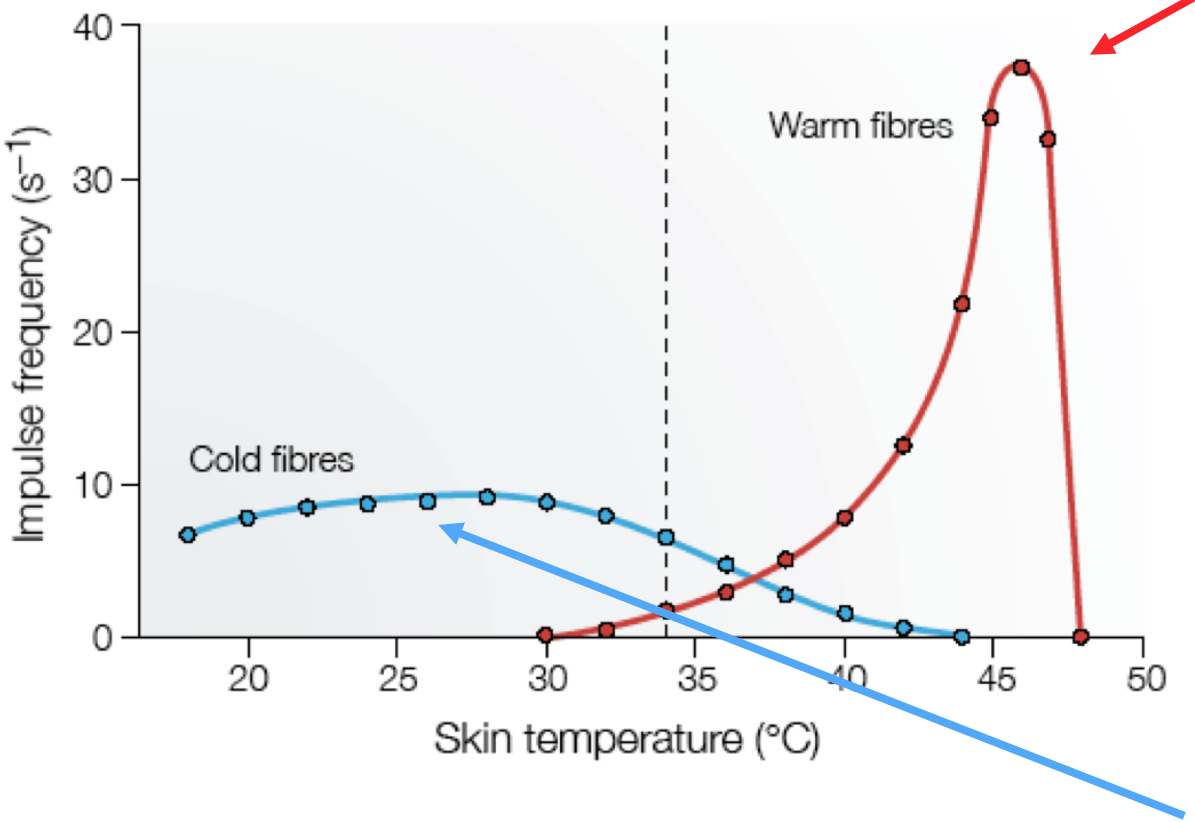
Moran NatRevDrugDisc 2011

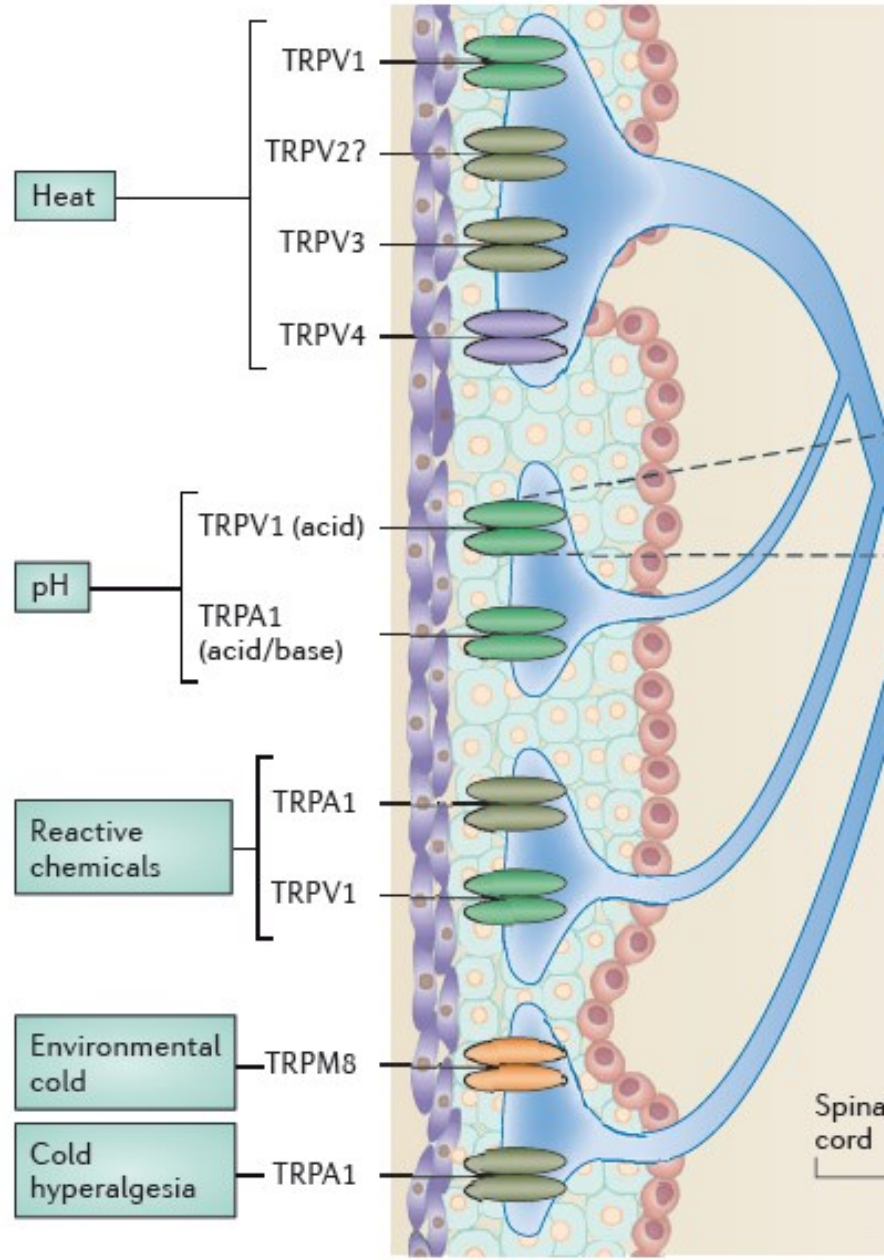
Pain and temperature perception

Pain:

+ alarm

- burden





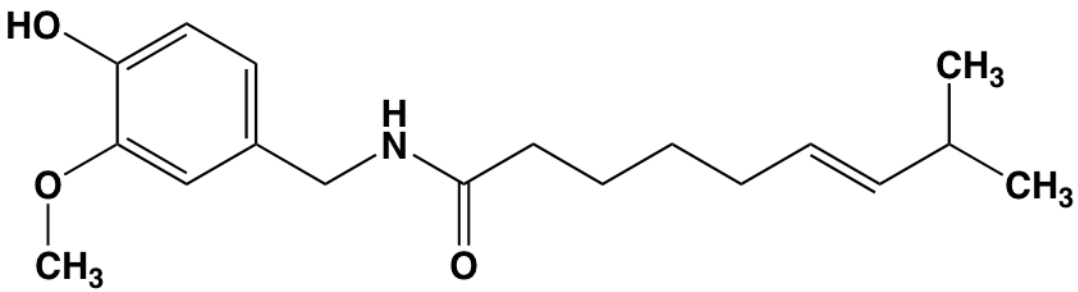
Moran NatRevDrugDisc 2011

Patapoutian, Nature Rev Neurosci 2003

Nociception

=> Quests for molecular mechanism & compounds to reduce pain perception

A case study : Capsaisin, the “burning spiciness” of chili peppers



Observations: - contact with capsaisin evokes a “burning” sensation
- prolonged exposure to capsaisin abolishes “pain”

Expression cloning to link genes and phenotypes

Identification of gene by expression cloning:

i• Construct cDNA library

ii• Transfect fractions of cDNA into cells.

iii• Assay for phenotype :: which?

iv• Pools with positive signals are split

Repeat steps ii to iv until

a single clone “x” remains

=> phenotype is linked to gene “x”

Nociception - Capsaisin receptor TRP_V1

Identification of gene by expression cloning:

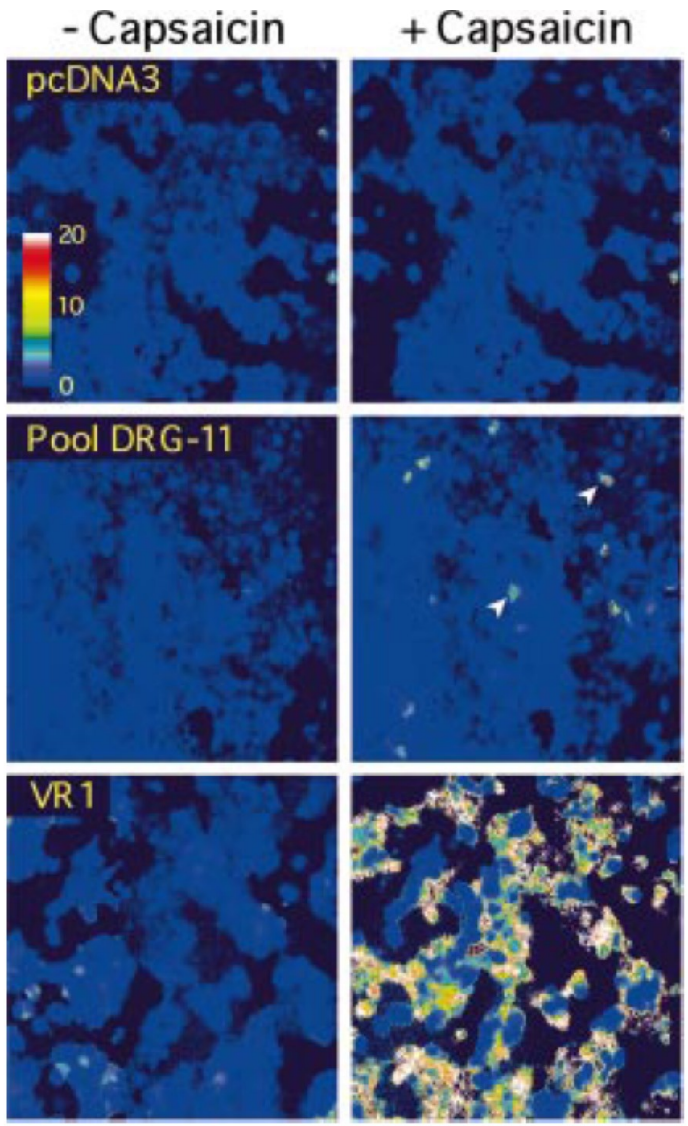
i• Construct cDNA library from nociceptive neurons.

ii• Distinct cDNA fractions transfected into HEK cells.

iii• Measure Ca^{2+}-influx with fluorescent indicator

iv• Pools with positive signals subdivided until single positive cDNA clone found.

=> Positive clone named “vanilloid receptor VR1”.

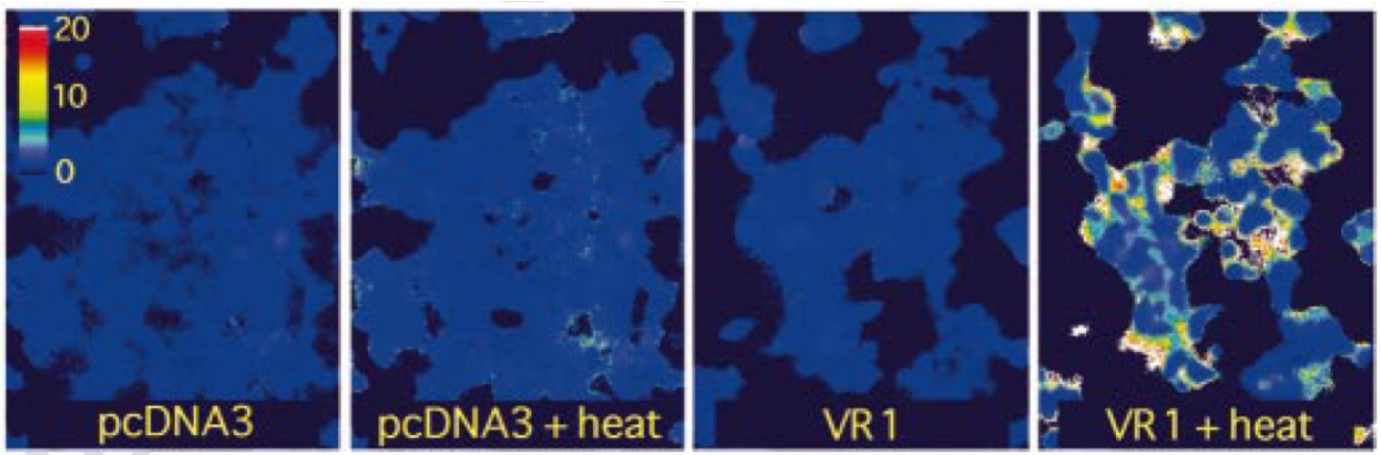


Caterina Nature 1997

Heat perception - Capsaisin receptor TRP_V1

"Hot" detection: both spicy taste and heat are detected by same protein

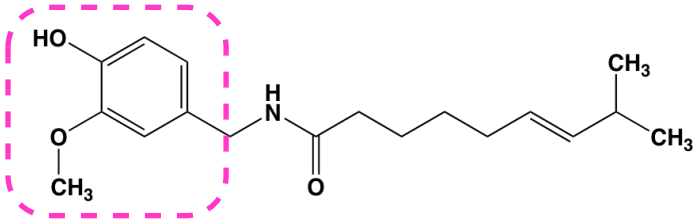
- Calcium signalling assay

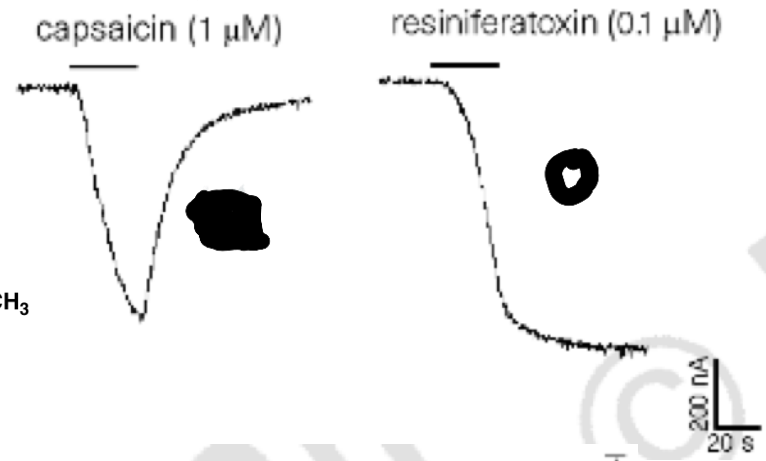


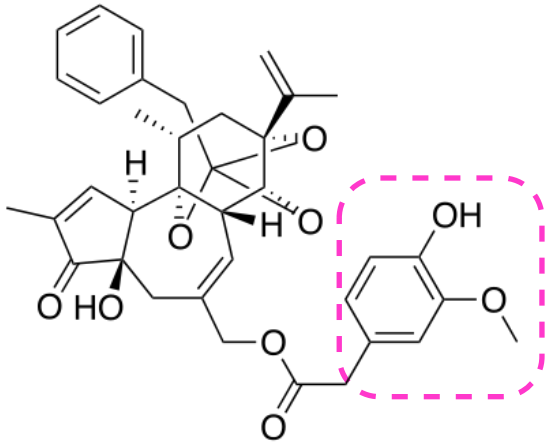
ture 1997

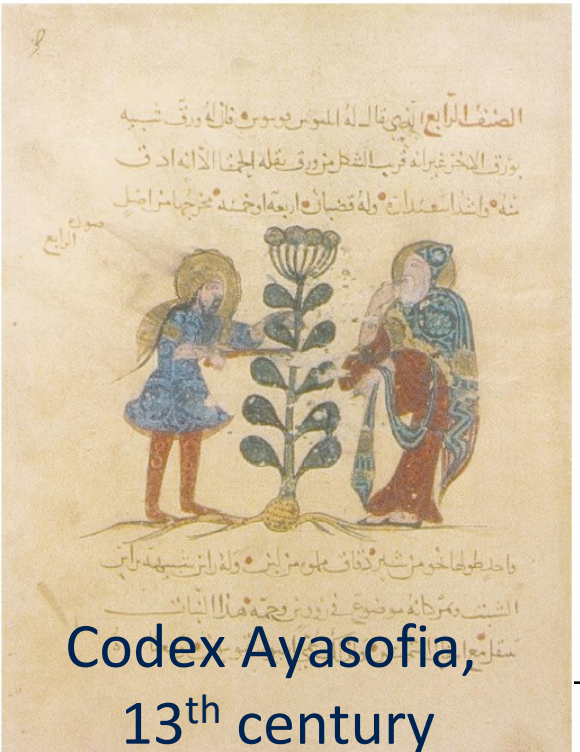
Capsaisin receptor TRP_V1 : A hot pharmacology

Activation by a “hot” component capsaisin (■) and
the ultra-hot resiniferatoxin (o) from the cactus *Euphorbia resinifera*

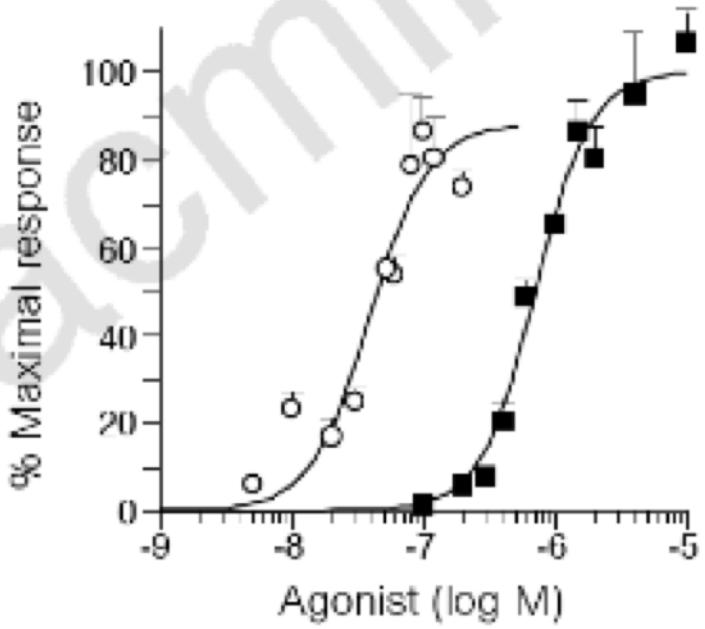






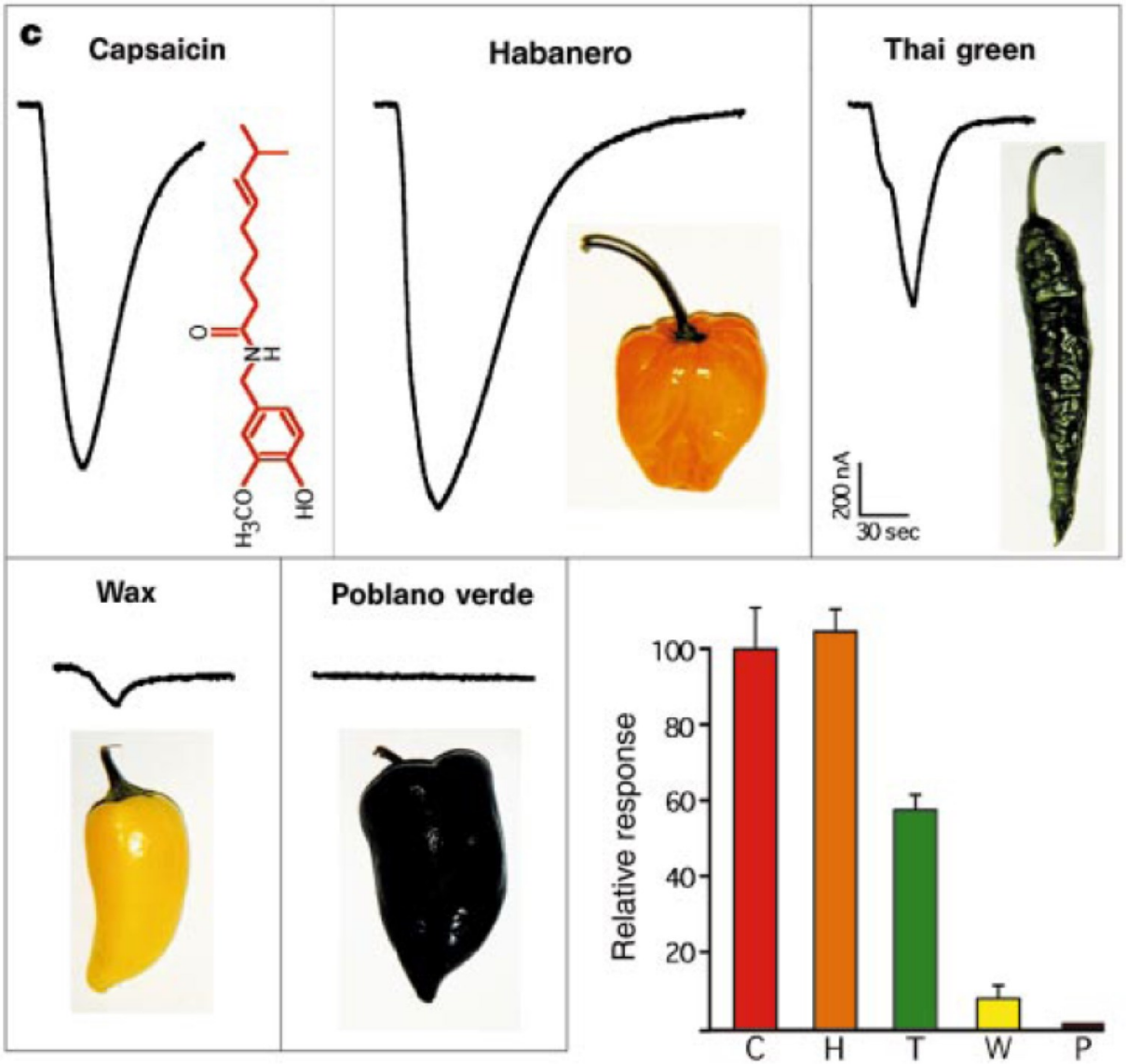


Codex Ayasofia,
13th century



Euphorbus, 50BC - 23AD

Spicy food - Capsaicin receptor TRP_V1



Caterina Nature 1997

Reported pungencies for pepper varieties (in Scoville units) are:

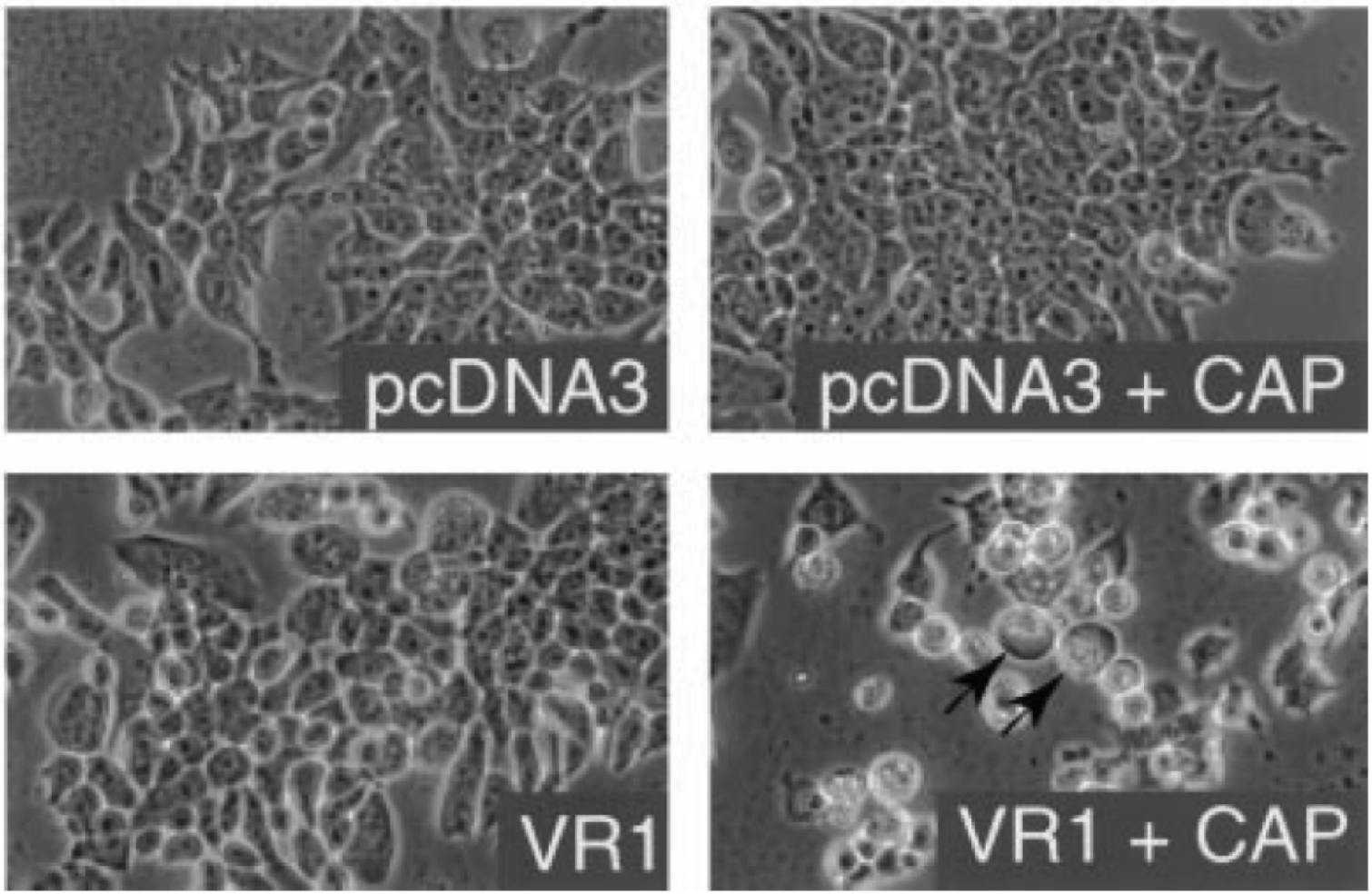
Capsaicin (C)	16’000’000
Habanero (H)	300’000
Thai green (T)	100’000
Wax (W)	10’000
Poblano verde (P)	1’500

Trinidad Moruga scorpion
2’009’231 Scoville units

Wilbur Scoville, 1912

Nociception - Capsaisin receptor TRP_V1

TRP_V1 mediates cell death induced by high concentrations of capsaisin



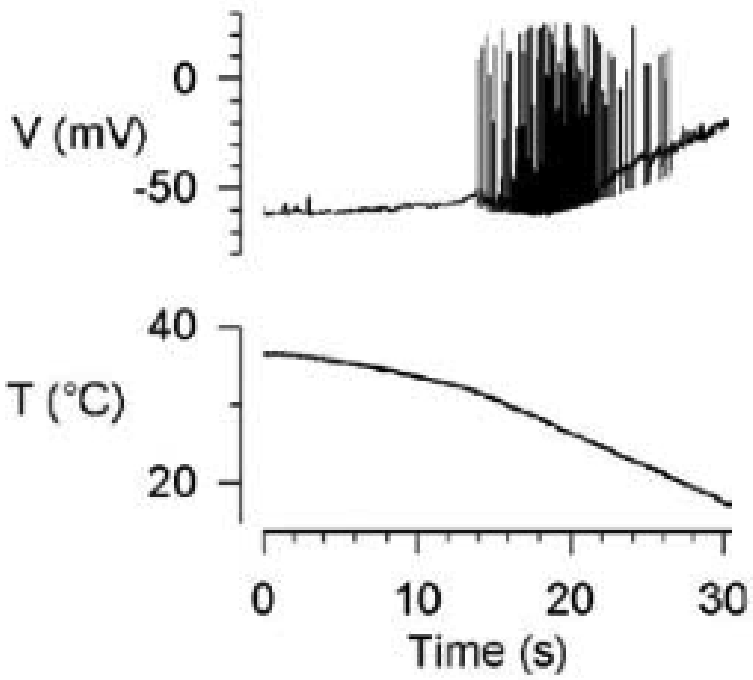
=> pain-sensing neurons are destroyed

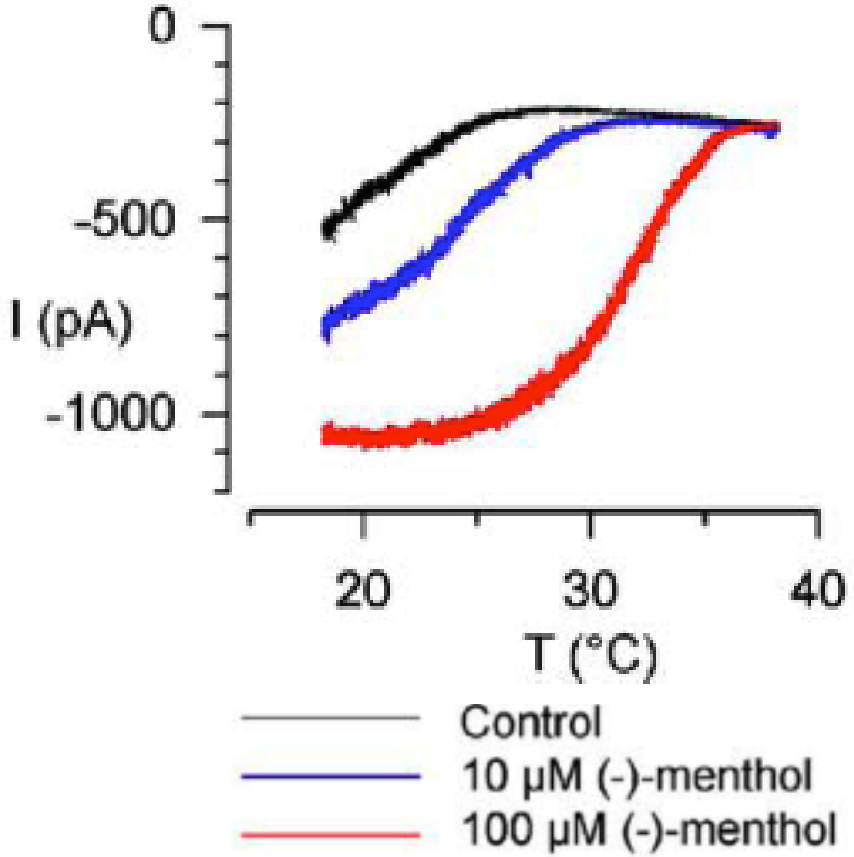
Caterina Nature 1997

What about cold perception ?

Cold is sensed by a specialized small sub-population of neurons.
Menthol affects cold sensation by shifting threshold to higher temperatures;
i.e. cold sensation already at higher temperatures

E.g. electrical activity of cold-sensitive neurons of rat:



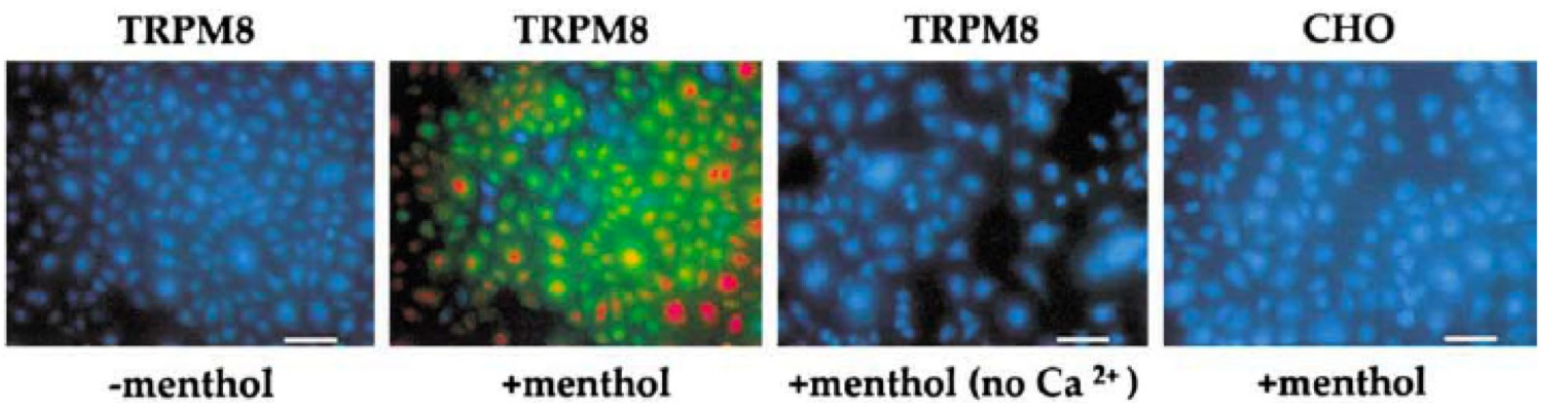


Reid, Eur J Physiol 2005

Cold perception - Also a TRP channel involved ?

i• a cDNA library was transfected into HEK293 cells yielding 10’000 clones.

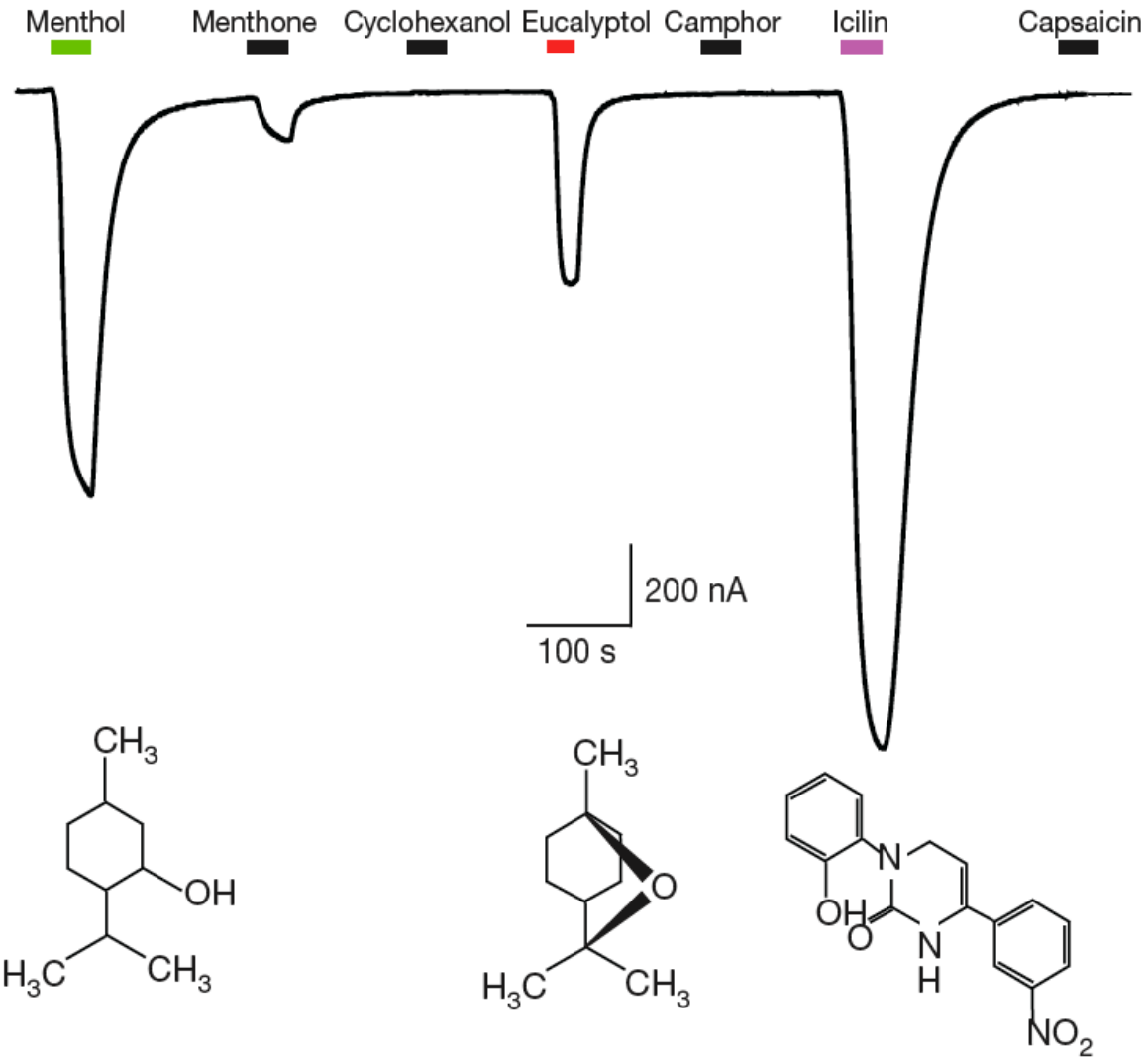
ii• Expression testing identified a single gene TRP_M8/CMR1 that induced in CHO cells a response to “cool”sensations like menthol.

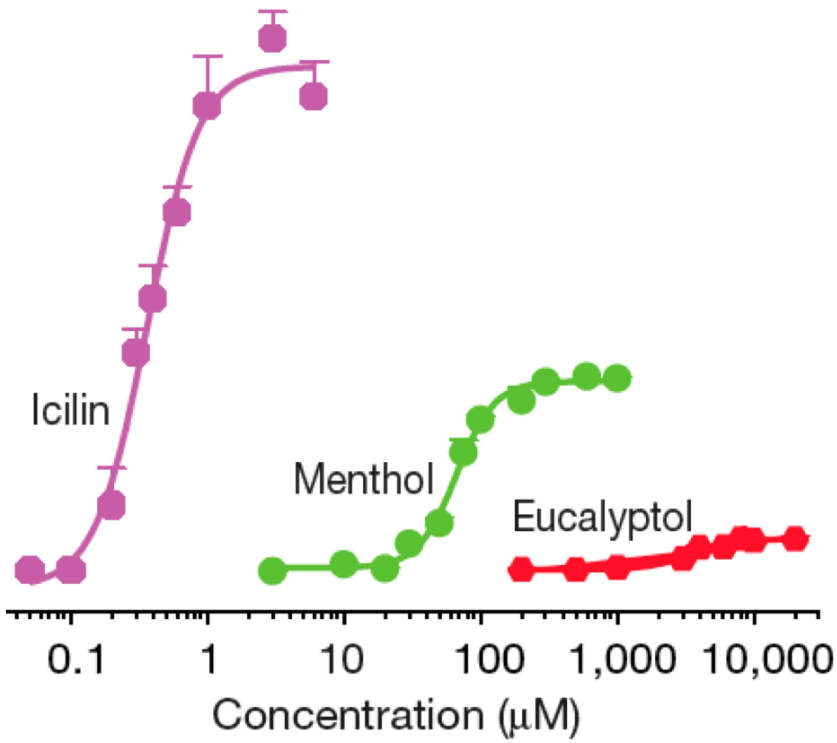


Peier, Cell 2002;
McKemy Nature 2002

“Cold” perception by expressed TRP_M1

Pharmacology

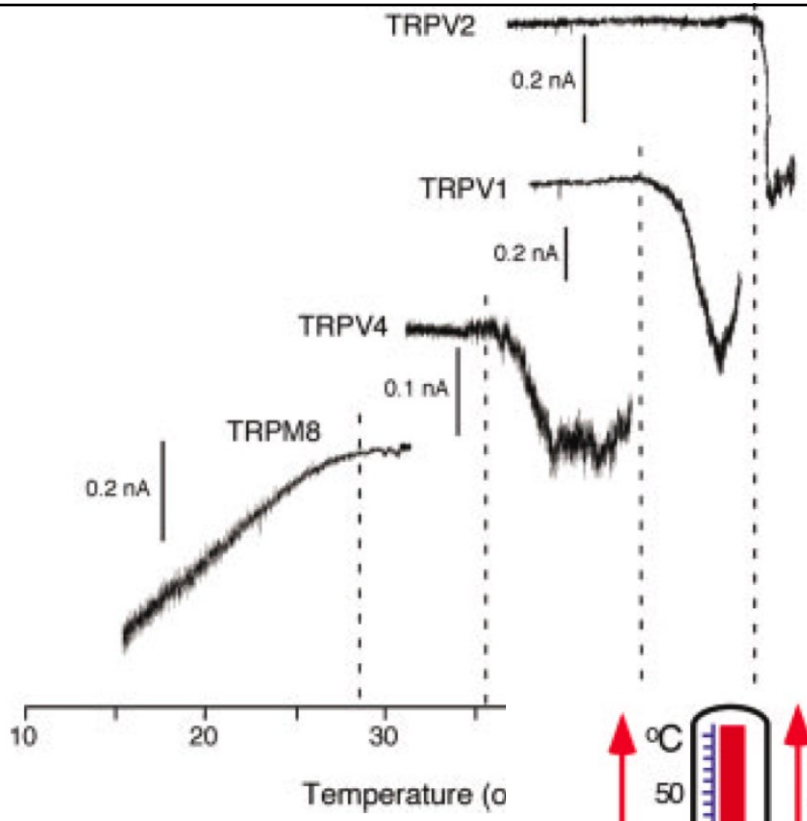


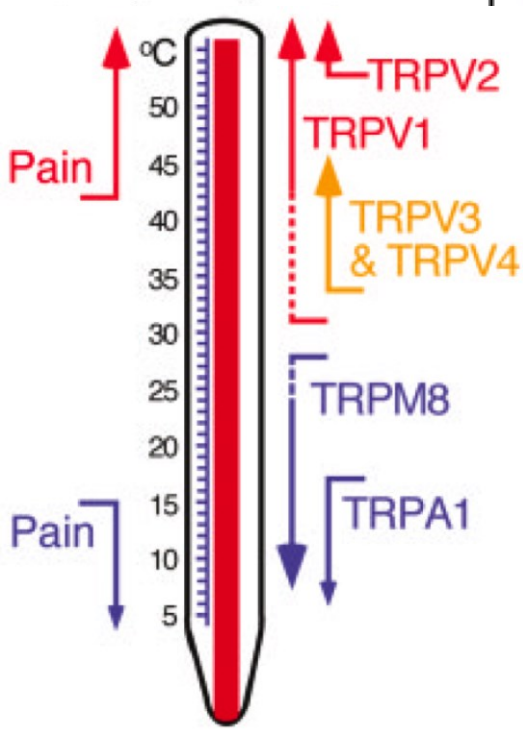


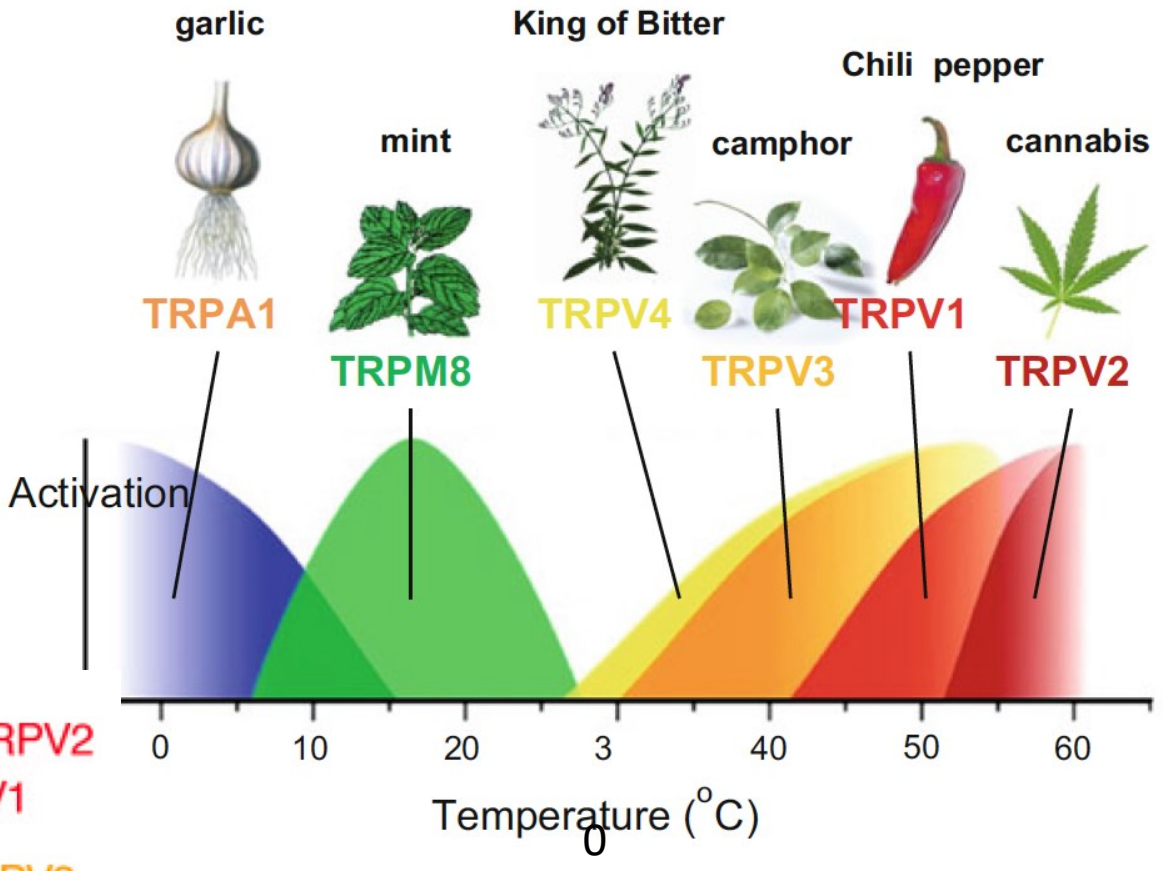
menthol eucalyptol icilin

McKemy Nature 2002

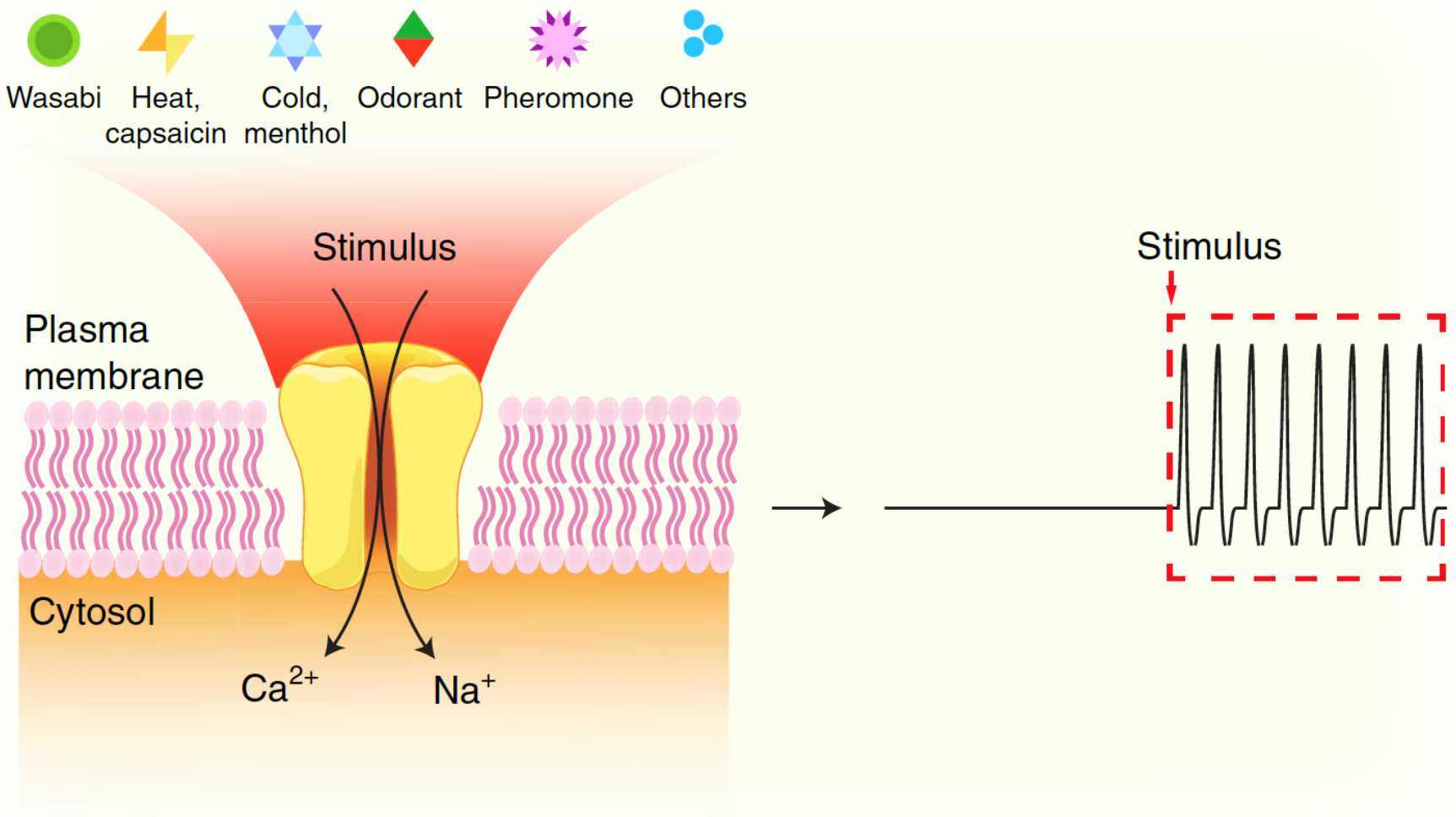
TRP channels and temperature sensation





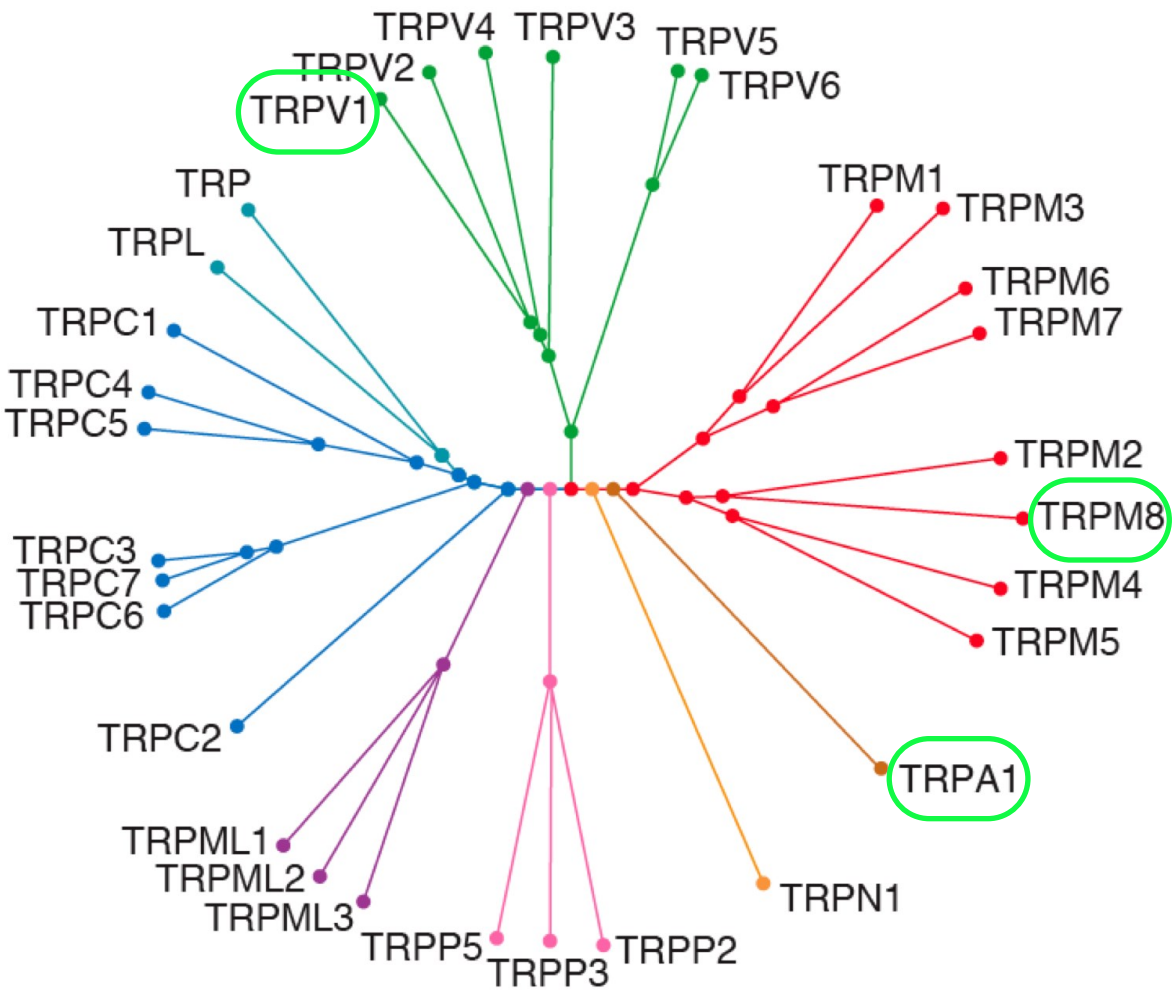


TRP's integrate multiple stimuli into an electrical response



Structural organisation

TRP genes and protein structure



Several sub-families TRP_C classical

TRP_V vanilloid

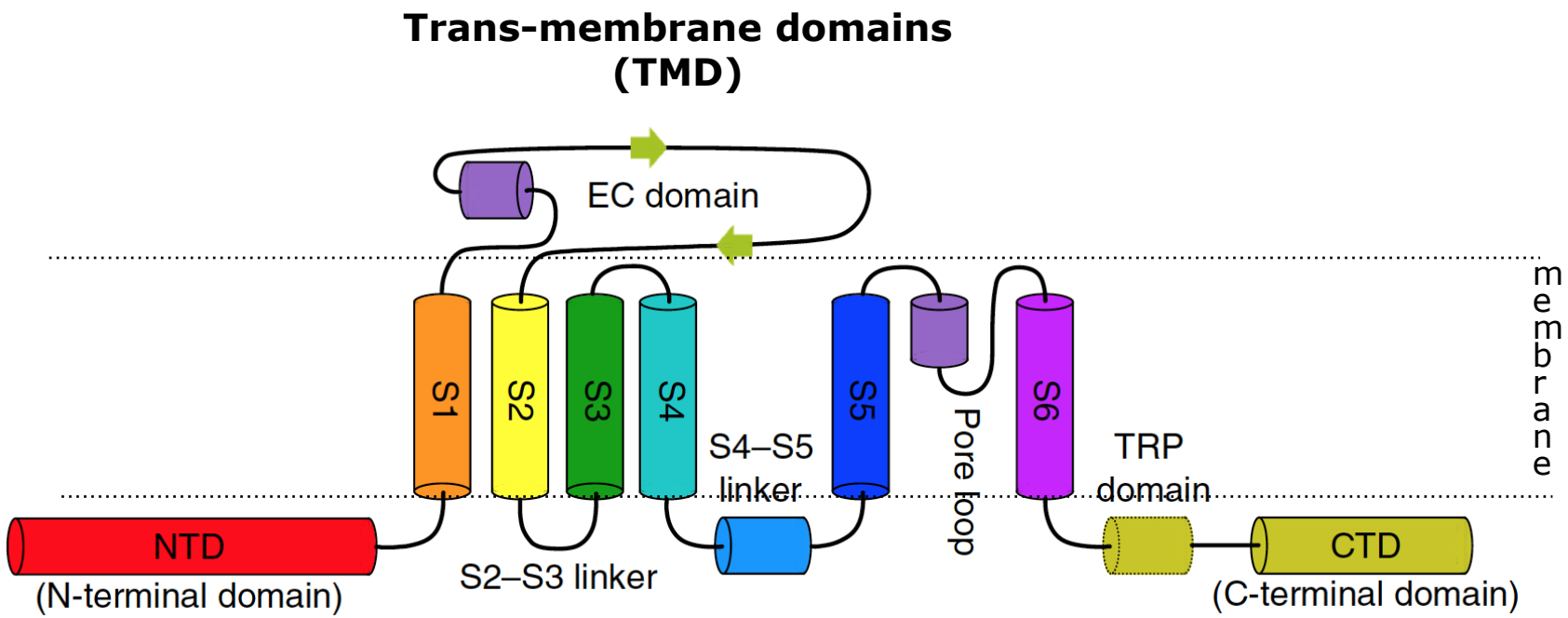
TRP_M menthol

Fleig TiPS 2004

Julius GenBiol 2011

Putney TiCellBiol 2004

TRP proteins: Domains and tertiary structure

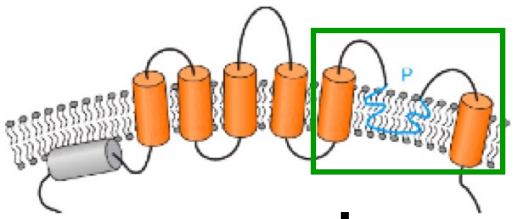


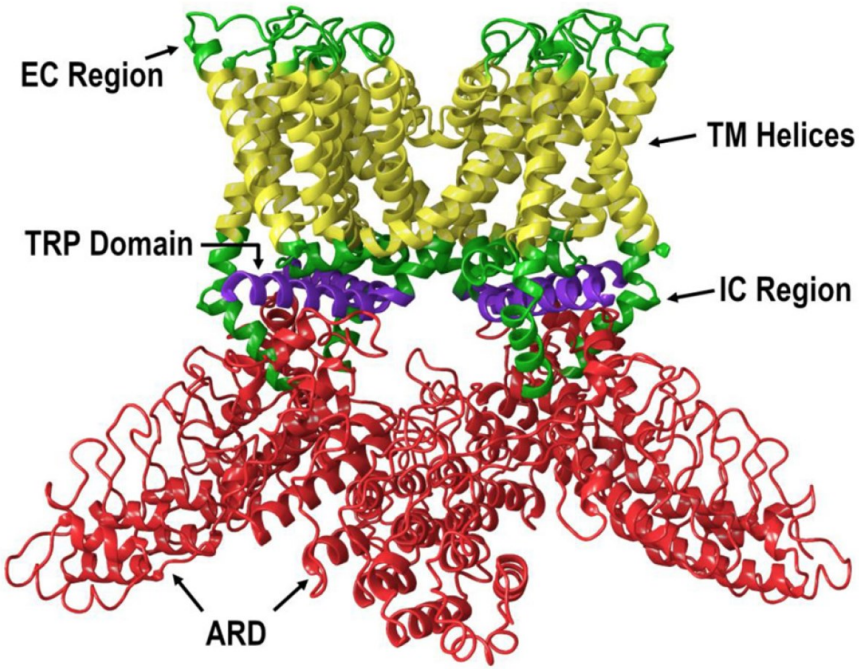
N-terminal domain (NTD):
Ankyrin repeats, mediate interactions
with other proteins

Pore-domain:
TM5-TM6 domain typical for voltage-gated channels

TRP domain:
Highly conserved region
=> lipid interaction

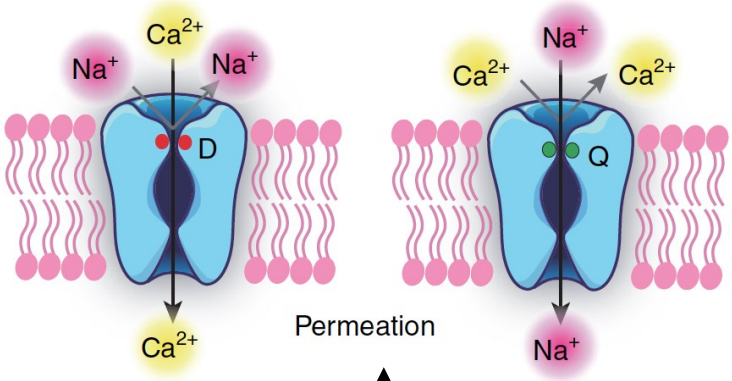
Structure and function of the pore domain

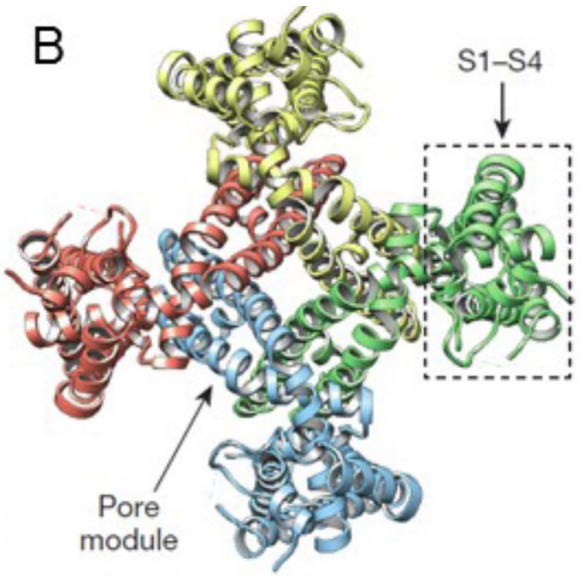




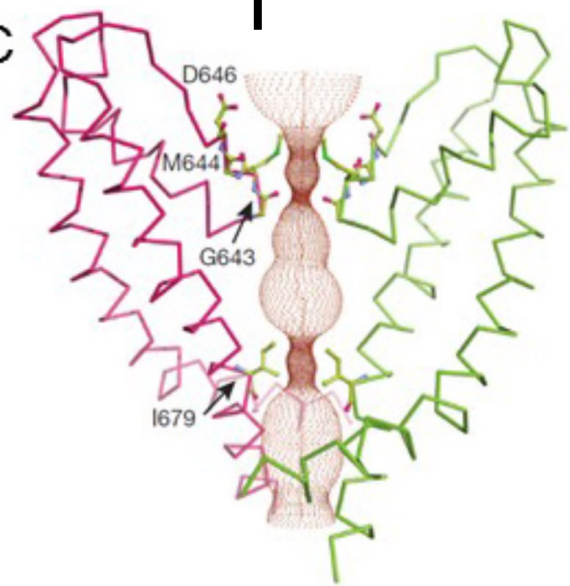
side view

Ion permeability depends pos 646
D => Ca^{2+} Q => Na^{+}



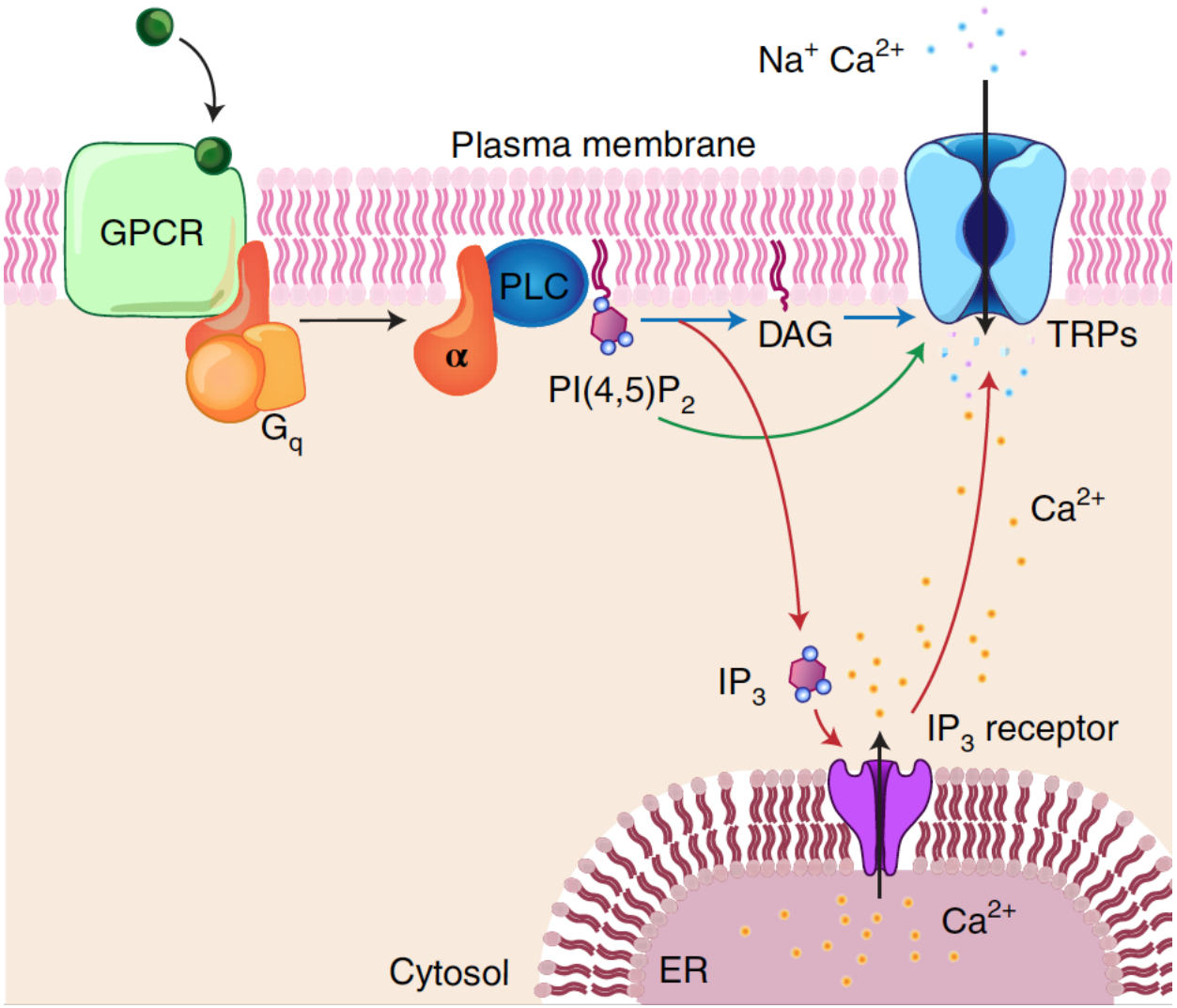


top view



side view of the channel domain

”Metabolic” TRP’s can act as secondary ion channels



In e.g. taste perception of bitter, sweet & umami

=> GPCR as receptors activating PL-C
=> Ca^{2+} response

=> activates TRP’s in the baso-lateral membrane

=> depolarization plasma membrane

=> neurotransmitter release

=>=> signal to taste centre in brain

Structure & Activation by other stimuli

Depolarization activates the TRP ion channel

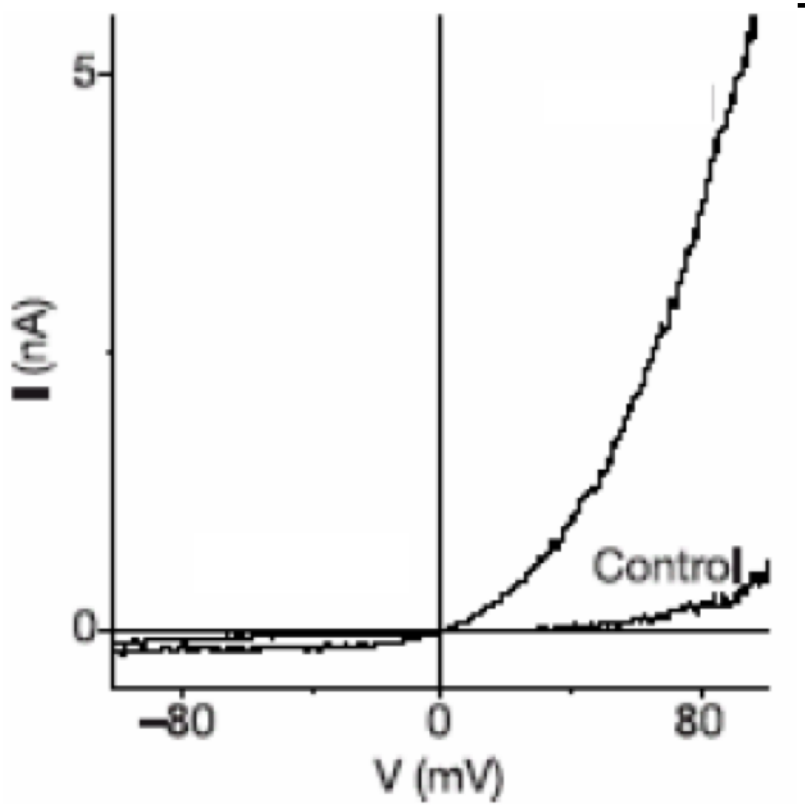
Experiment:

- expression of TRP channel in cells
- measure membrane current

as function of membrane potential

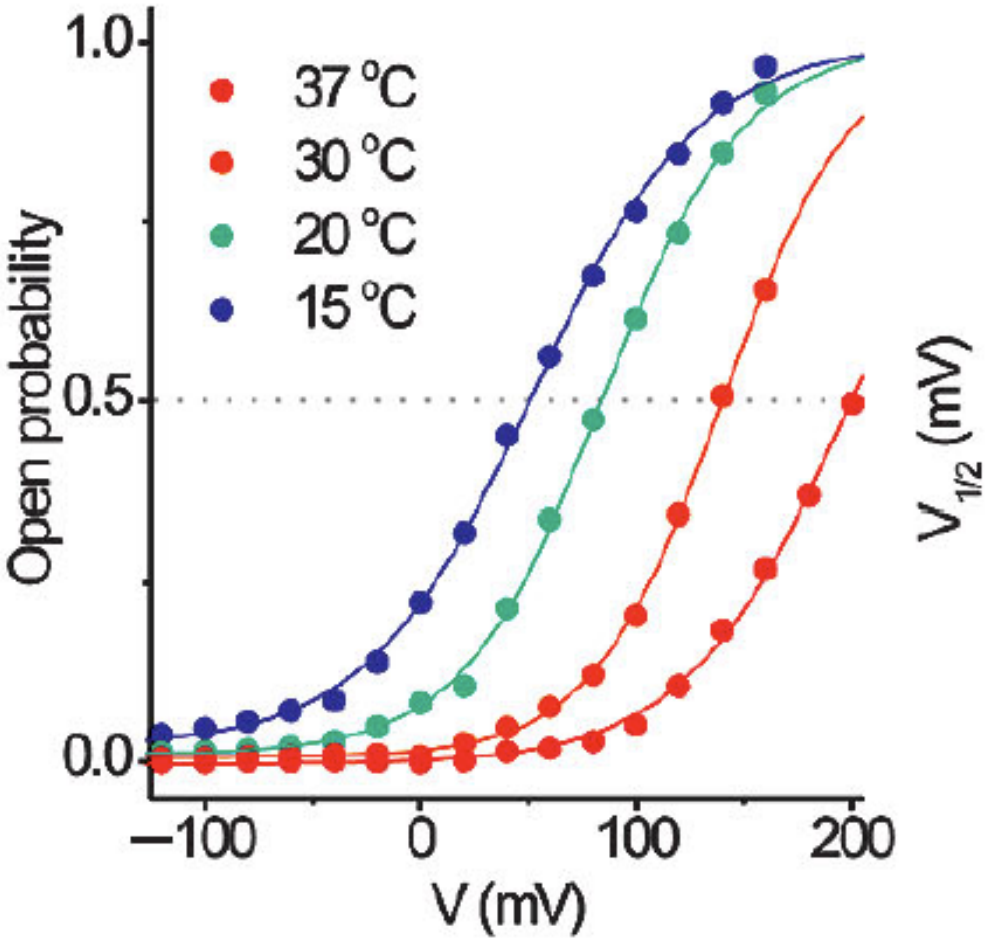
- control: mock-transfected cells

=> TRP's are voltage-gated ion channels activated by depolarisation



TRP channels : Temperature and membrane potential

Channel activity of TRP_M8 (*cold*):
at lower T, a smaller depolarisation
is needed to activate the channel



Voets, Nat (

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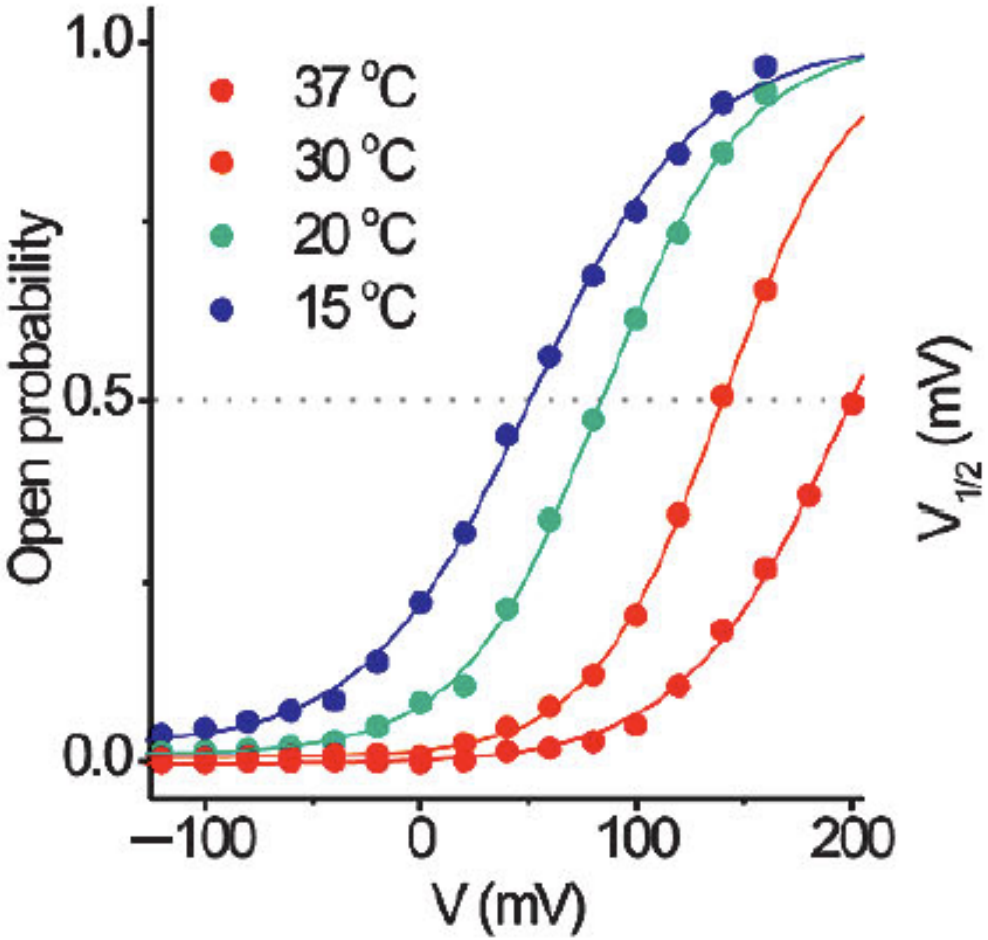
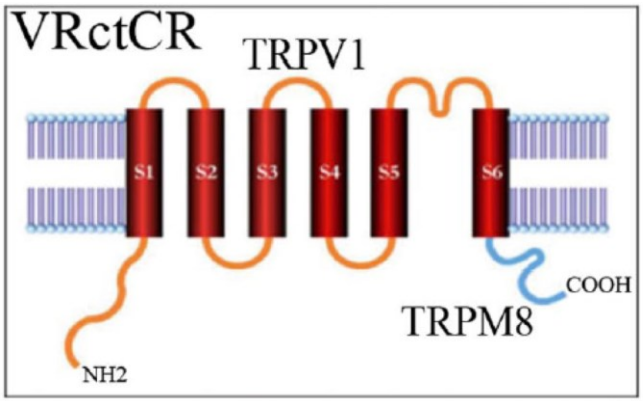
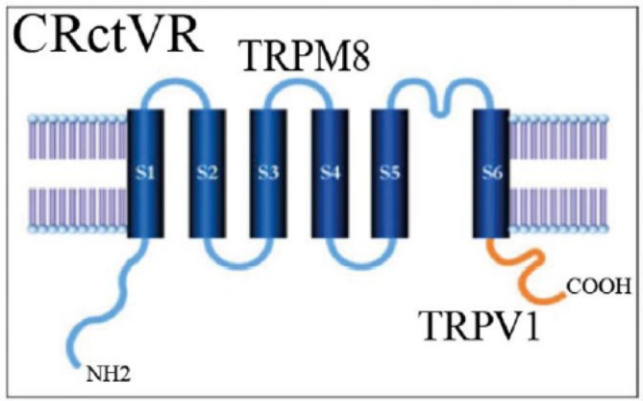


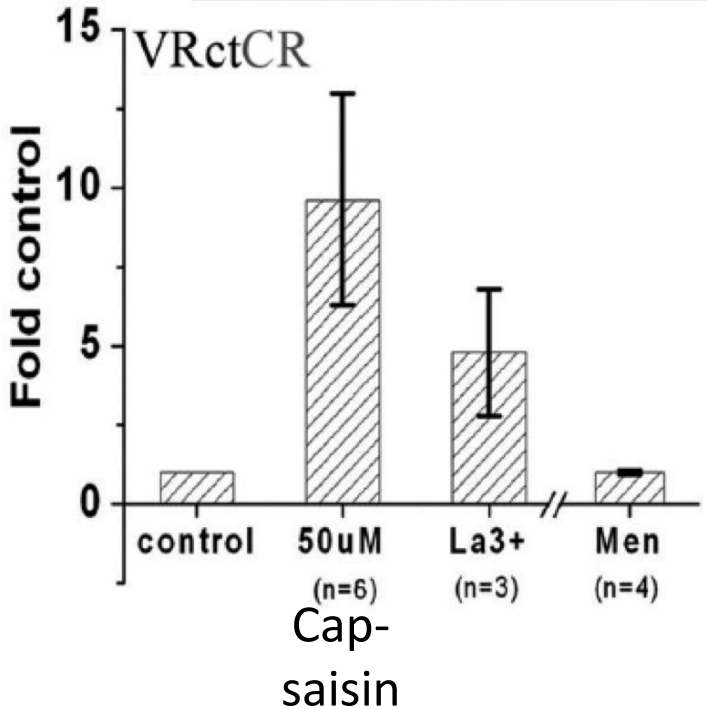
Voets, Nat (

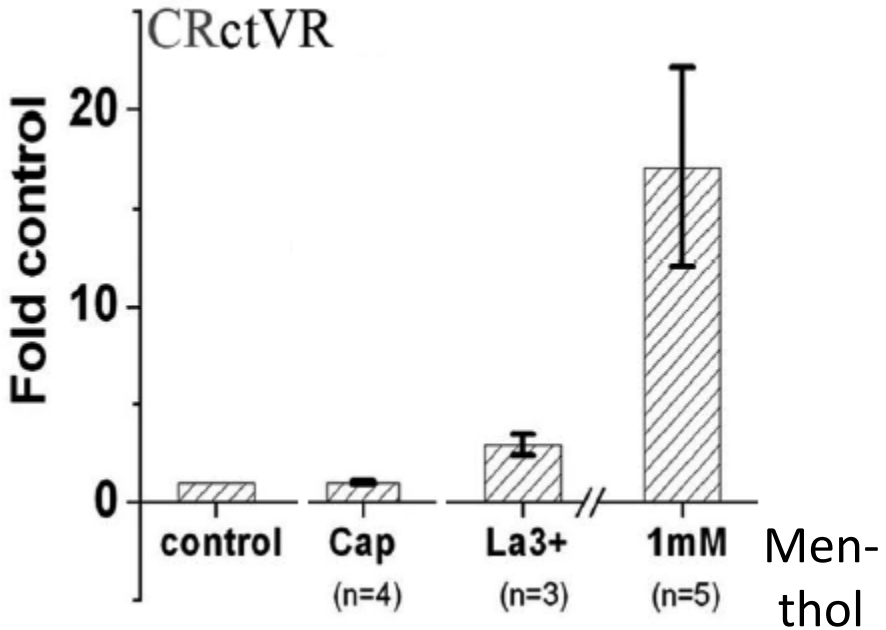
Cold vs heat perception : Which domain decides ?

Chimera's of the TRP_V1 (heat) and TRP_M8 (cold) swapping the C-terminus







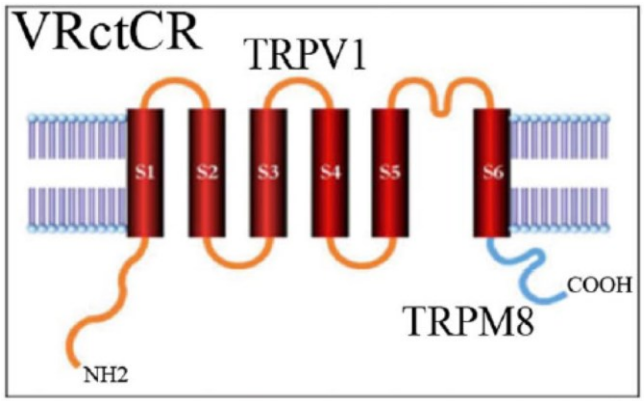


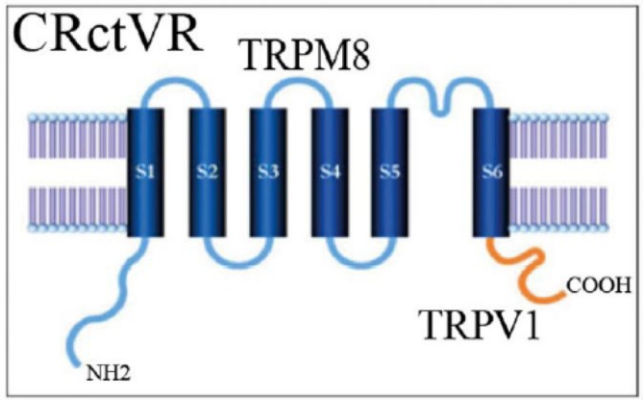
=> C-terminus does not affect ligand activation.

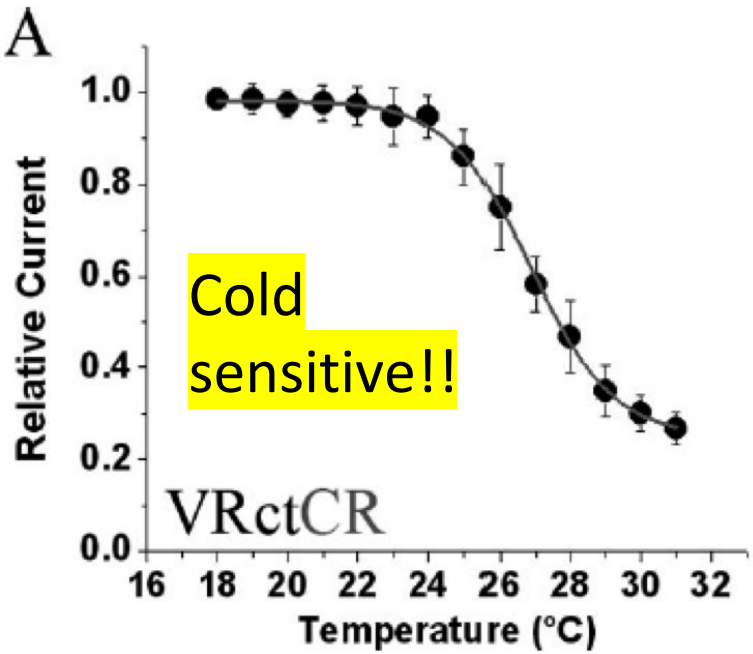
Brauchi, J Neurosci 200

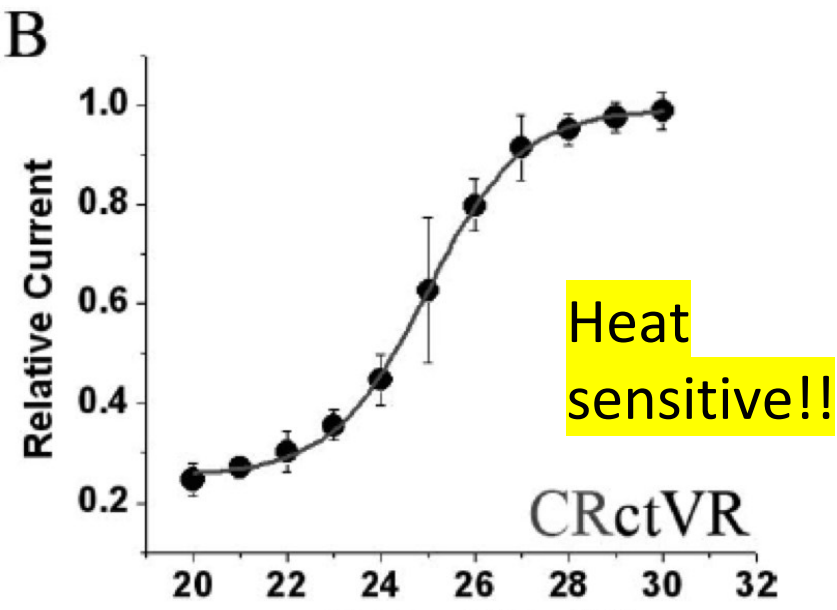
Cold vs heat perception : Which domain decides ?

Chimera's of the TRP_V1 (heat) and TRP_M8 (cold) swapping the C-terminus



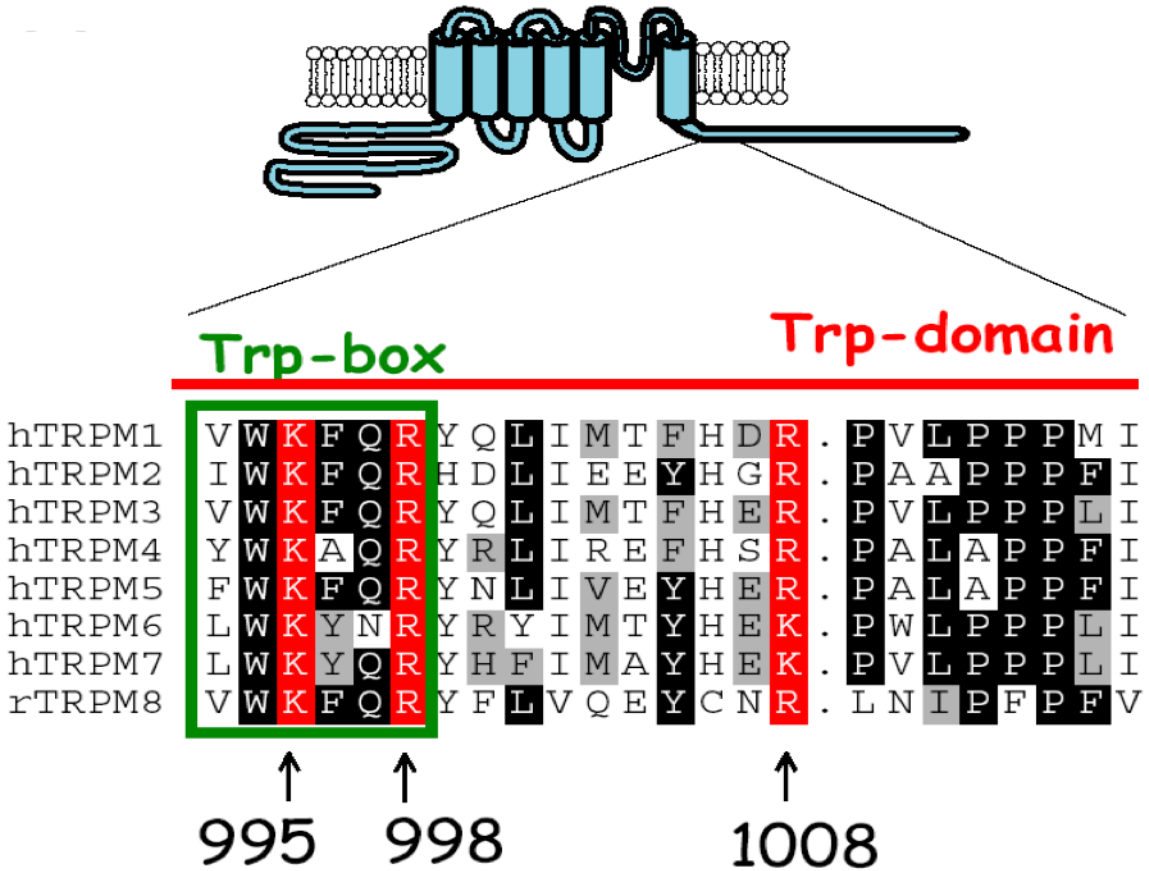






=> C-terminus determines cold or hot sensitiv

TRP-domain and modulation of channel current



Alignment => conservation of 3 basic residues.

Topology => close to the inner surface of the plasma membrane

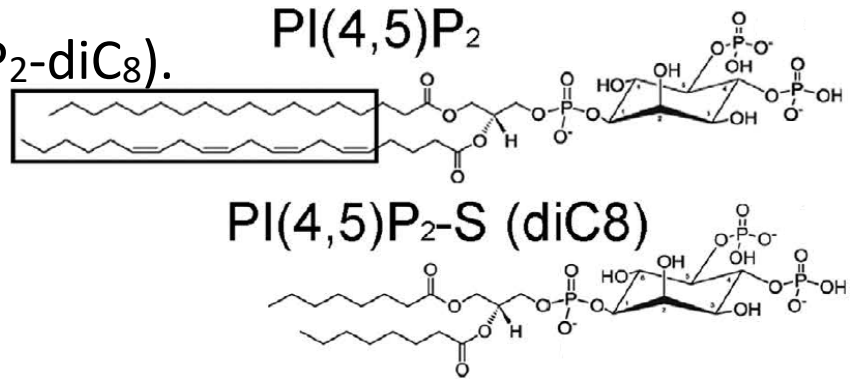
Are negatively charged lipids important, especially inositol phosphates?

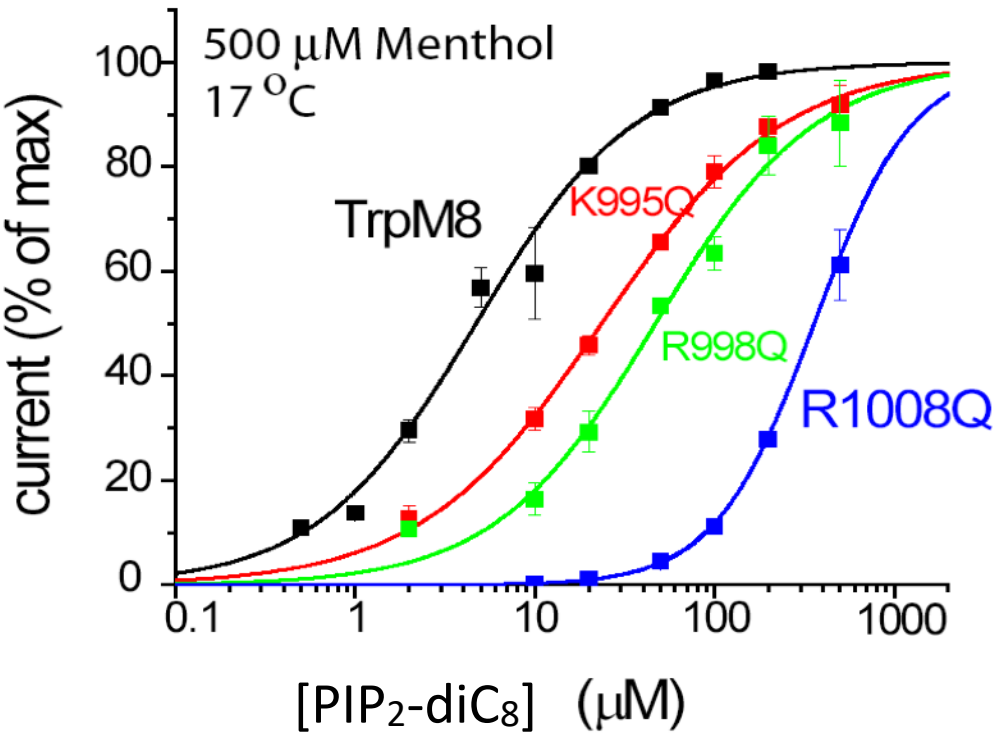
Rohacs, Nature Neurosci 2006

TRP-domain and channel modulation by PIP_2

Experiment:

- express in oocytes TRP_M8 or mutants lacking one basic residue,
- make an inside-out patch and measure current
- stimulate with menthol in presence of
  different concentrations of di-octanoyl-PIP_2 (PIP_2-diC_8).



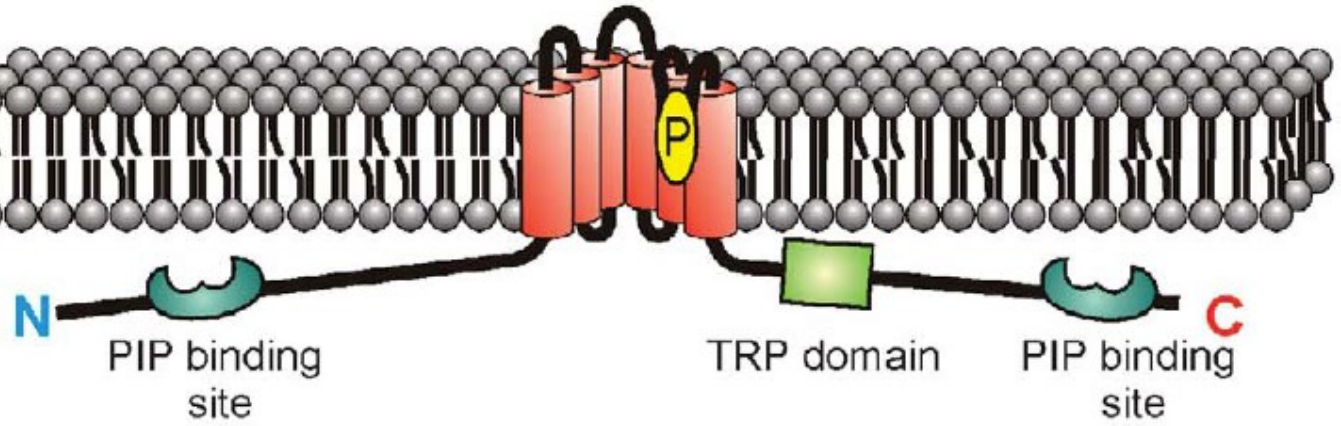


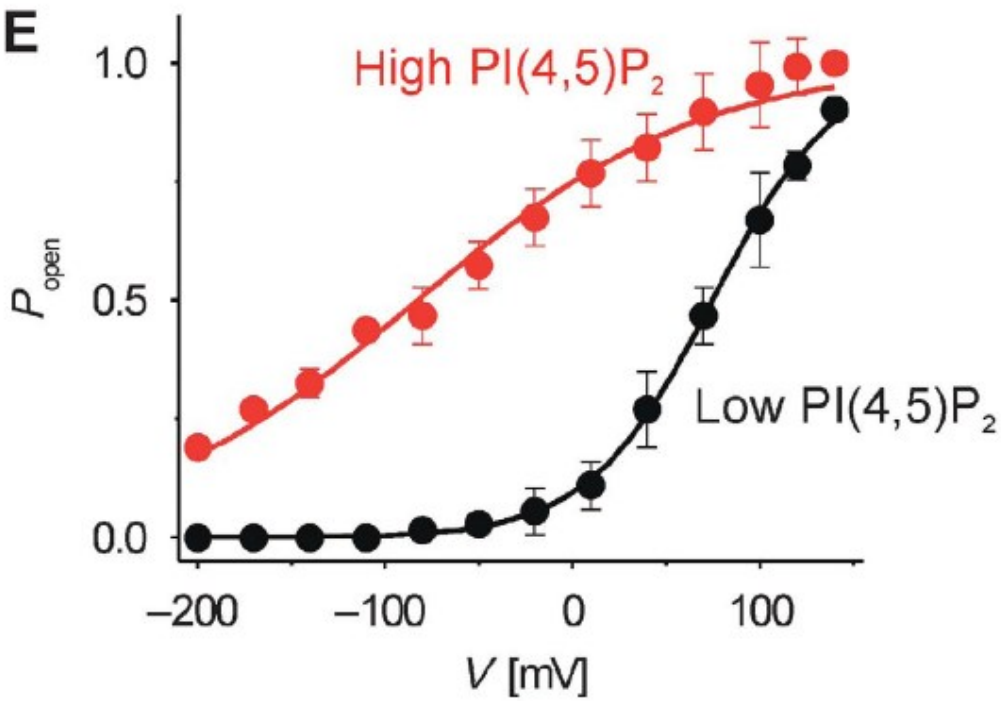
Putative interaction of TRP domain of TRP_M8 protein with PIP_2.

=> activation of currents by PIP_2

Rohacs, Nature Neurosci 2006

TRP-domain and channel modulation by PIP_2





TRP gating - modulation by many stimuli

Changes in

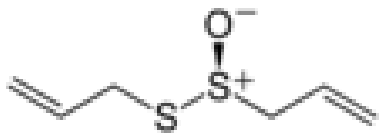
- membrane potential
- intracellular Ca^{2+}
- membrane tension or composition
- temperature
- mechanical stress
- presence of chemicals

or *combination* of these stimuli

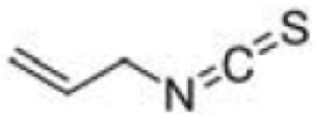
TRP channels: Covalent activation

TRP_A1: activated by pungent compounds that are Cys-reactive
- during activation they make a covalent bond with TRP_1A

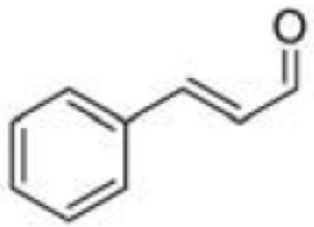
Allicin



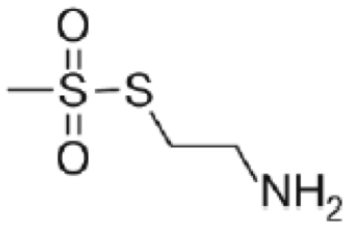
Mustard oil (MO)



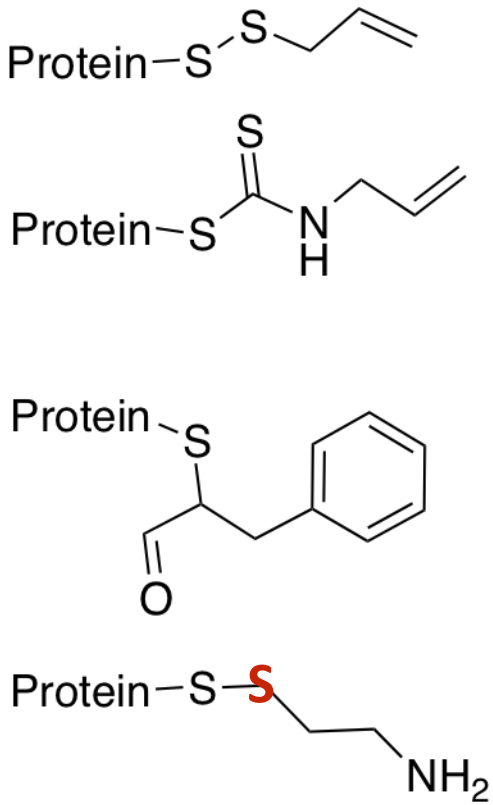
Cinnamaldehyde (CA)



MTSEA



+ Protein-SH



Macpherson, Nature 445 (2007) 541

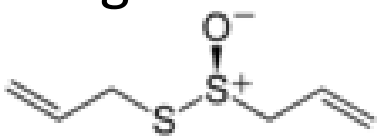
TRP channels: Covalent activation

TRP_A1: activated by pungent compounds that are ***Cys-reactive***

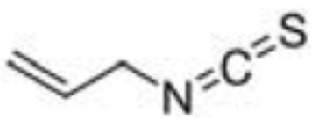
- during activation they make a covalent bond with TRP_1A

=> use mutagenesis to identify Cys!

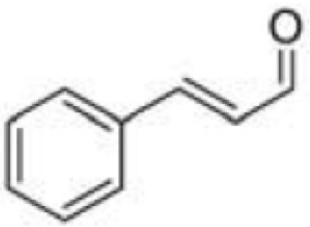
Allicin



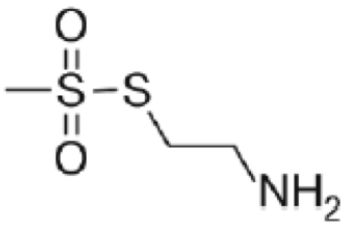
Mustard oil (MO)

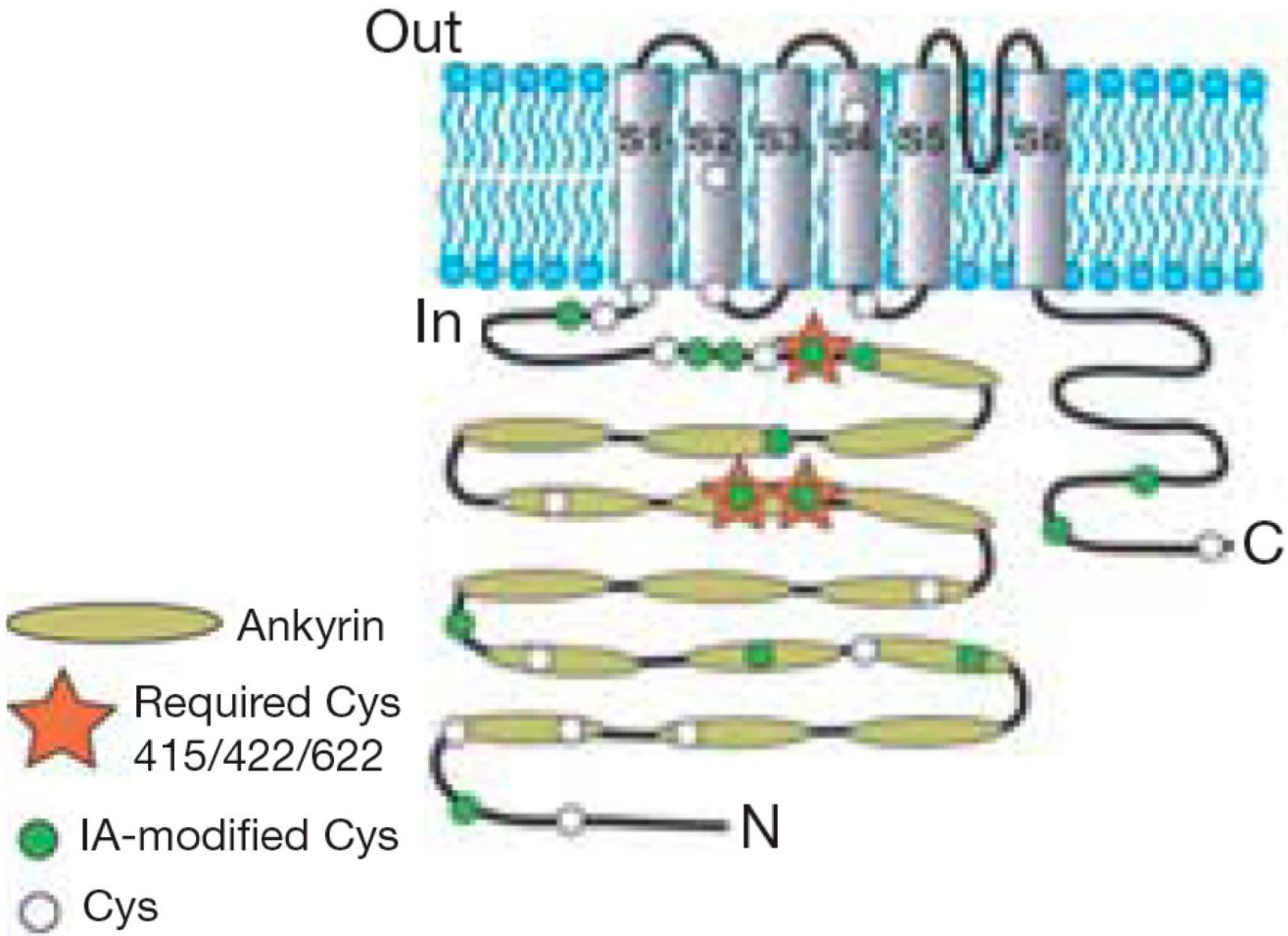


Cinnamaldehyde (CA)



MTSEA

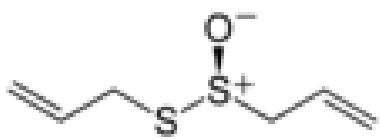


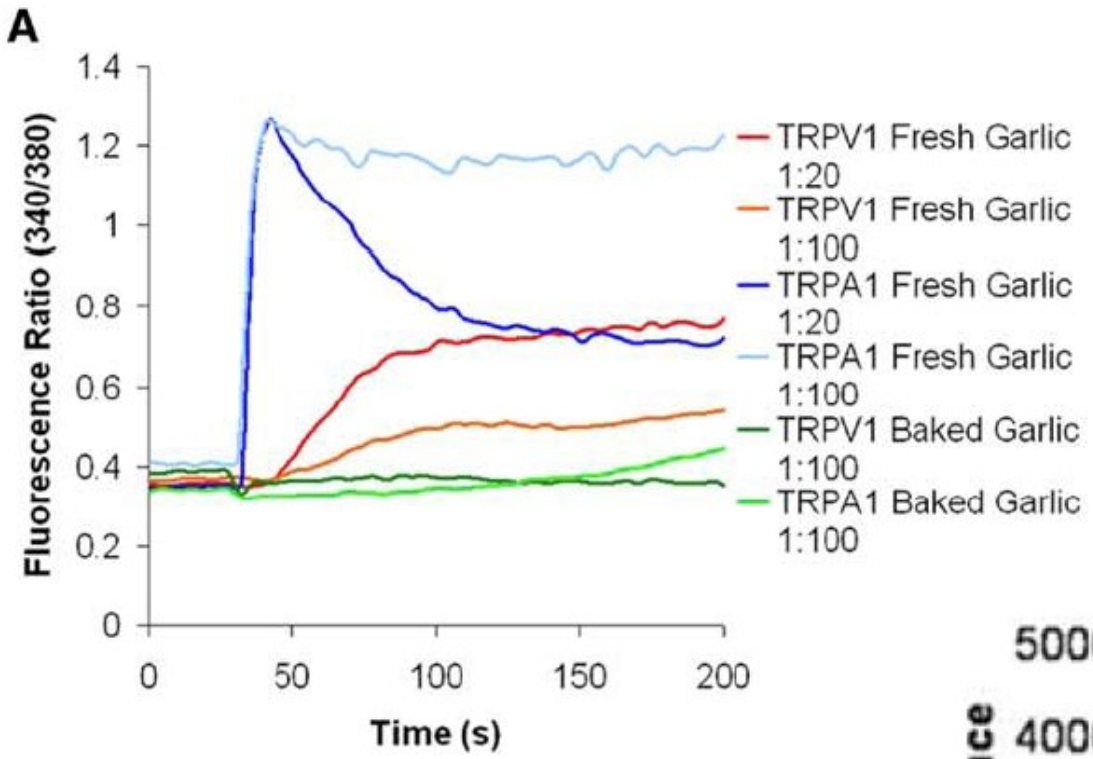


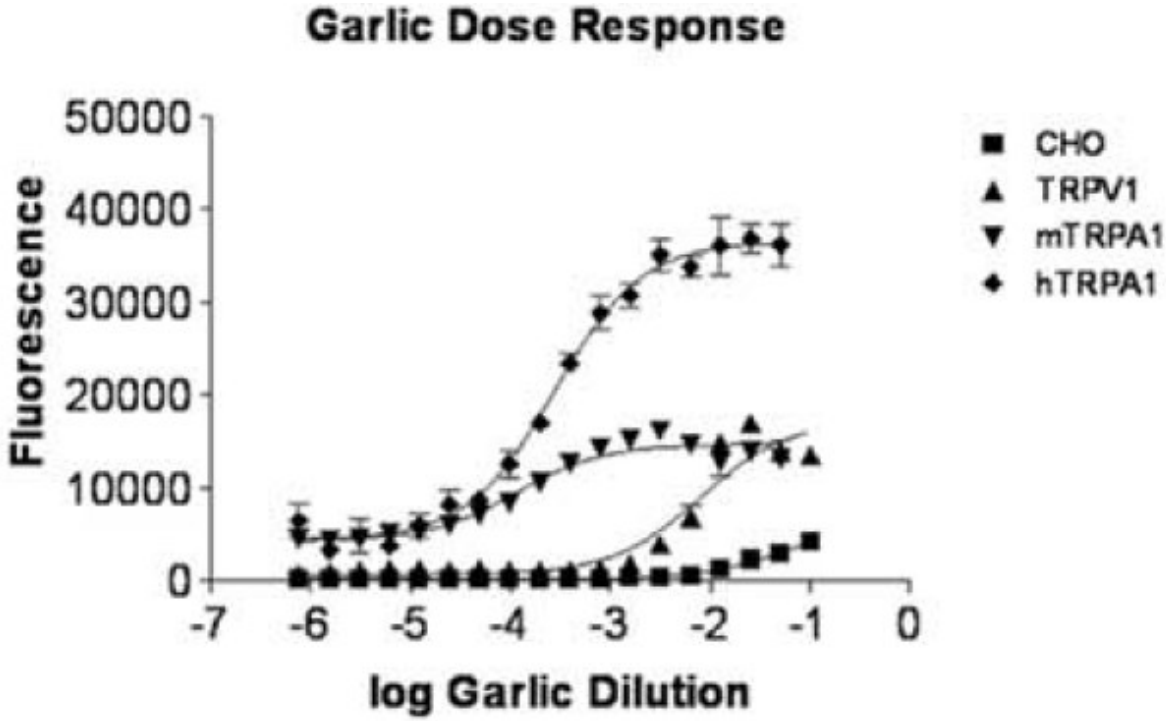
Macpherson, Nature 445 (2007) 541

TRP channels: Covalent activation

TRP_A1: strongly activated by garlic







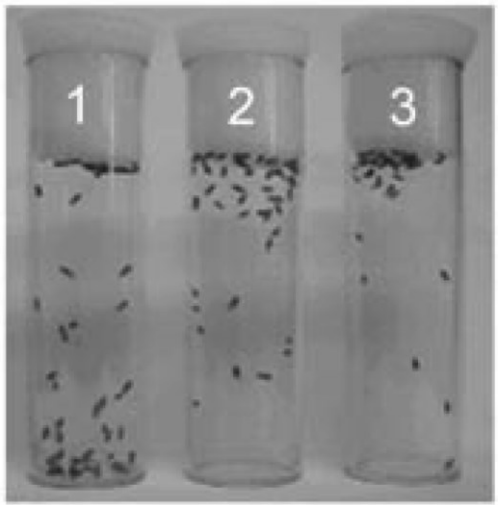
Macpherson, Nature 445 (2007) 541

TRP channel : Mechano-sensation of hearing

In *Drosophila* a TRP_V channel required for hearing.

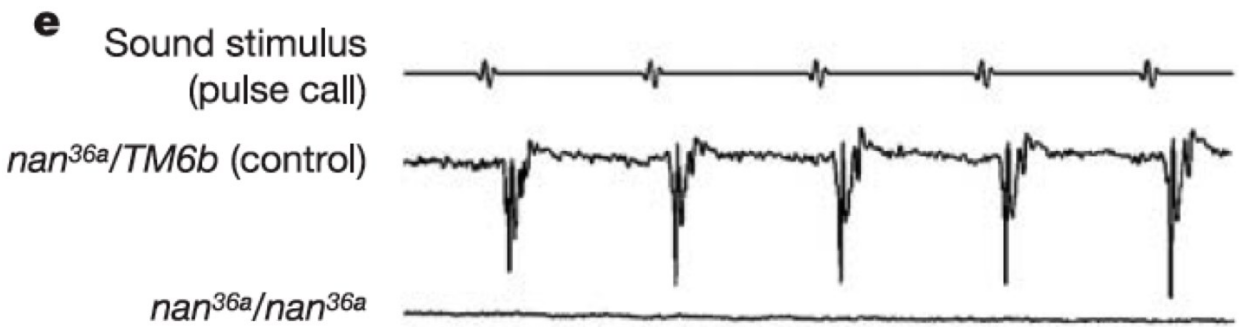
Kim describes a mutant *Drosophila* lacking "Nan" similar to TRP_V
Nan is expressed *in vivo* exclusively in chordotonal neurons and is localized to the sensory cilia.

- Escape from sound



-/- +/- +/+

- Antennal sound-evoked potentials

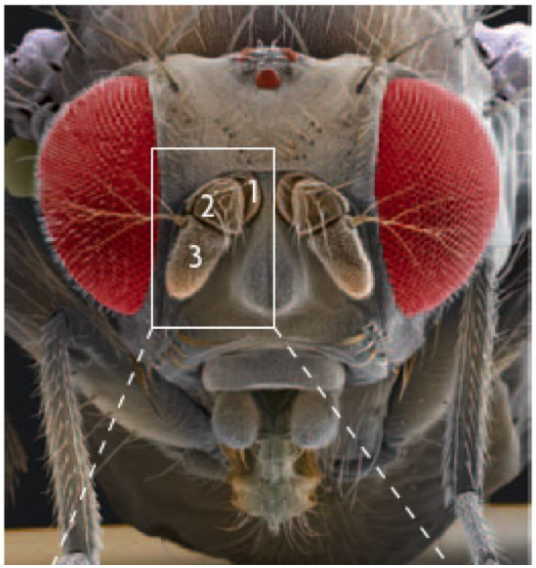


Mutants lacking Nan neither fear sound (-/-) nor show antennal potential (nan^{36a}).

=> Nan is an essential component of the chordotonal mechanotransducer.

Kim, Nature 2003

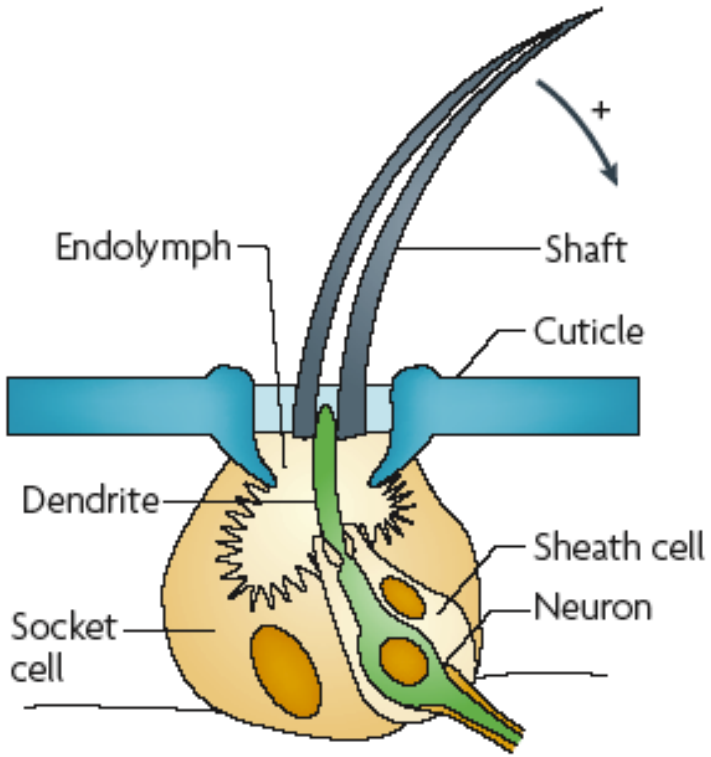
TRP channel : Mechano-sensation

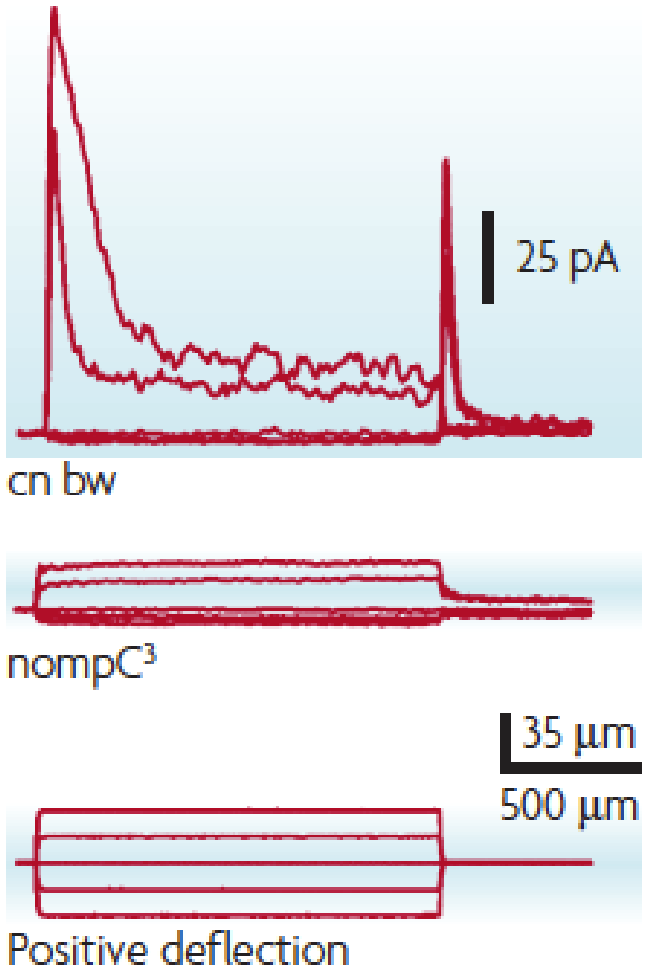


Potential response upon bristle movement

Wild-type (wt) => biphasic response

TrpN1 knock-out (nompC) => no transient spike





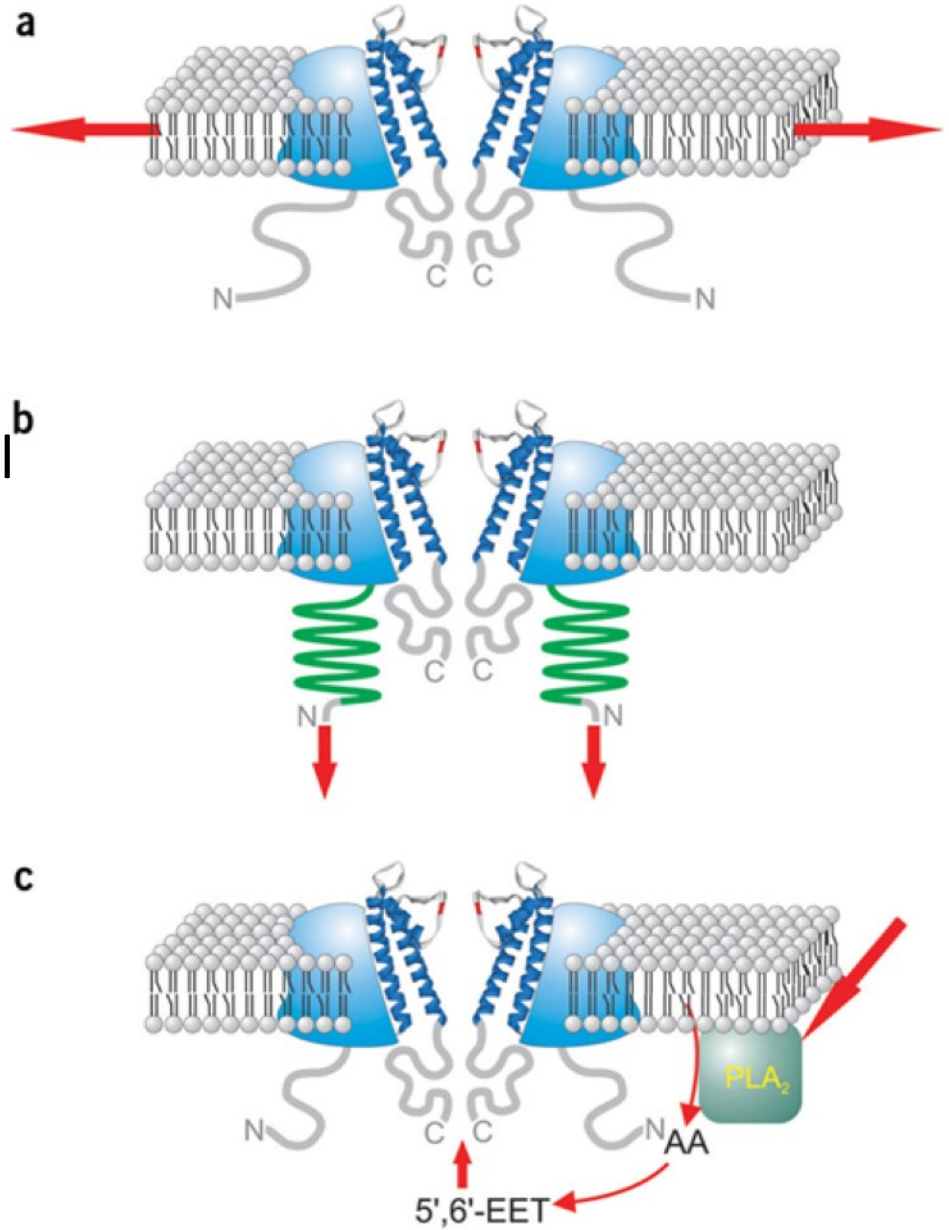
Chistensen, Nat Rev Neuro 2007

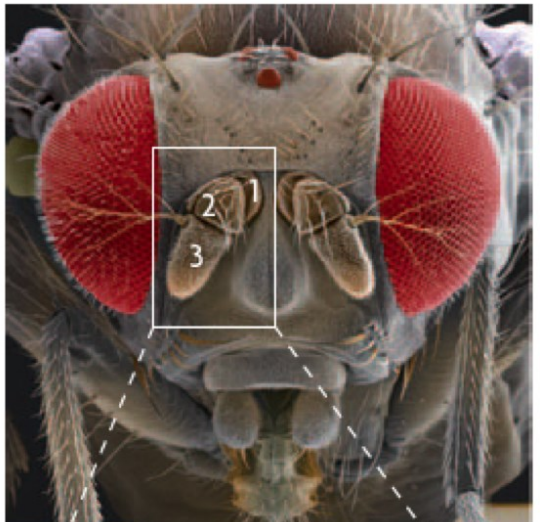
Mechano-sensation

Lateral pressure in membrane

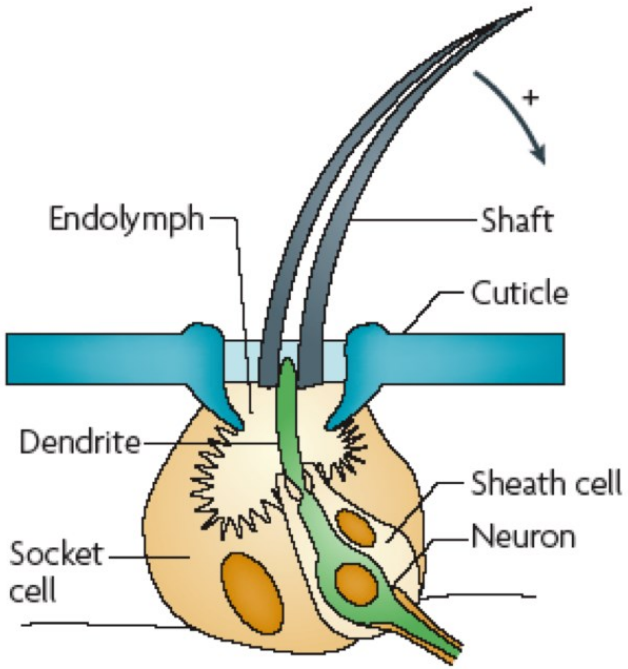
Force on channel

Via second messengers





b



Voets Nat Chem Biol 2005

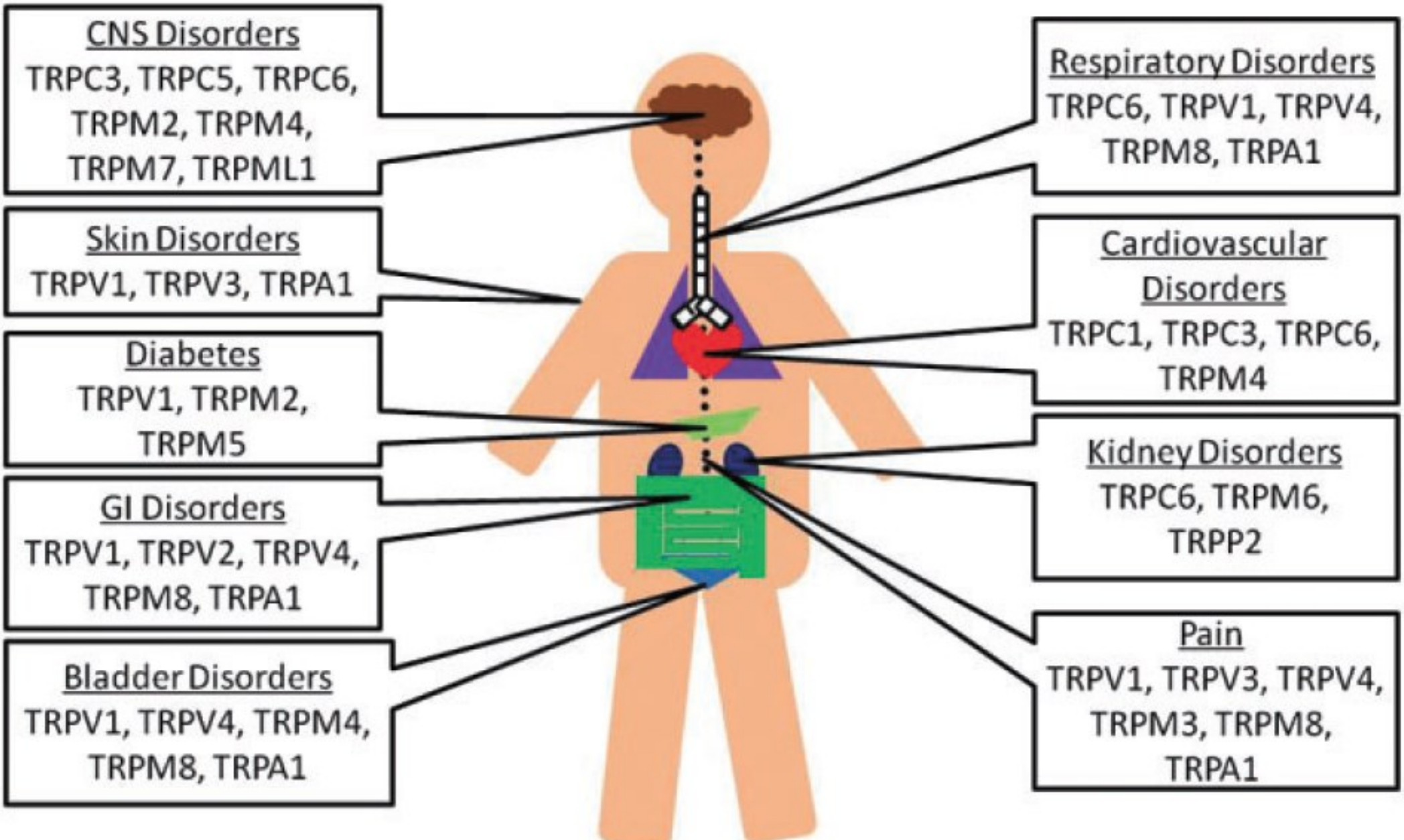
TRP gating - modulation by many stimuli

Changes in

- membrane potential
- intracellular Ca^{2+}
- membrane tension or composition
- temperature
- mechanical stress
- presence of chemicals

or *combination* of these stimuli

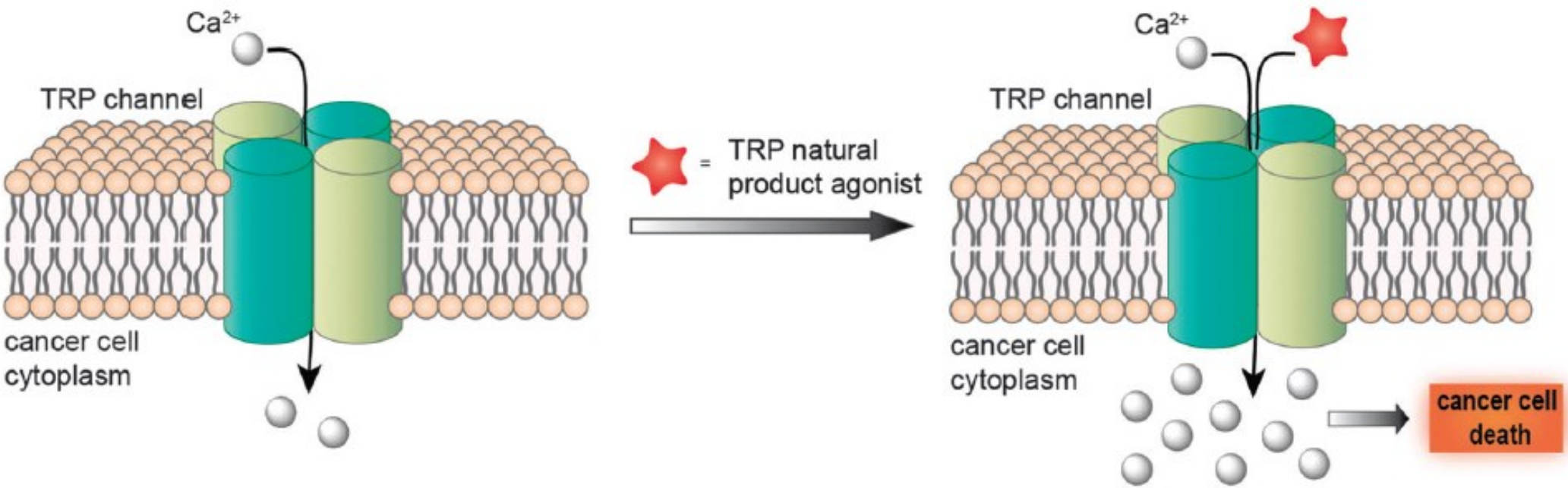
TRP channels : Roles in disease pathogenesis



Kaneko & Szallasi (2014) Br J Pharmacol 171, 2474

TRP channels and cancer treatment

Activate TRP-channels to evoke high-[Ca^{2+}] induced cell death.

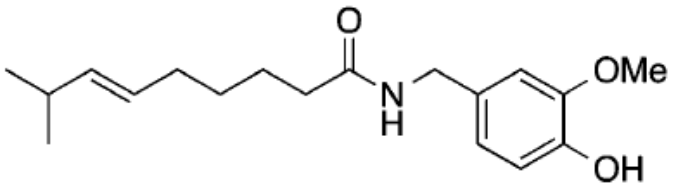


=> a given TRP-channel has to be over- or uniquely expressed in tumours

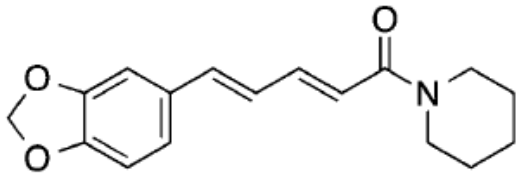
e.g. some TRP_V channels are over-expressed in prostate cancer

Rodrigues et al (2016) Chem Soc Rev

TRP-V1 channels and cancer treatment

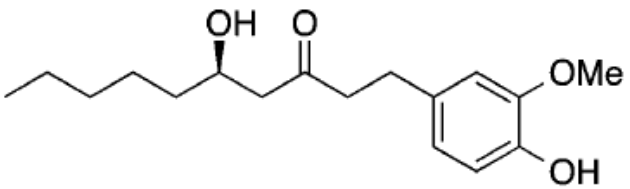


Capsaicin **(40 nM)**

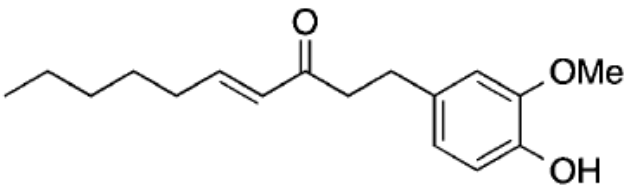


Piperine **(~100 uM)**

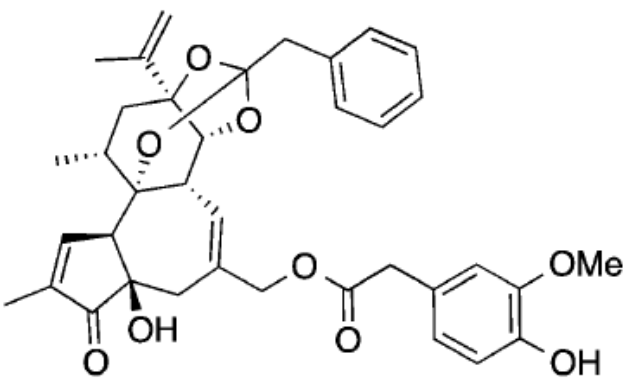
(EC_{50})



6-Gingerol
(~1 uM)



6-Shogaol
(770 nM)



Resiniferatoxin **(~1 nM)** K_d ~25- 500 pM

Fig. 3 Chemical structure of selected TRPV1 natural product agonists with anticancer activity.

TRP channels : Drug development

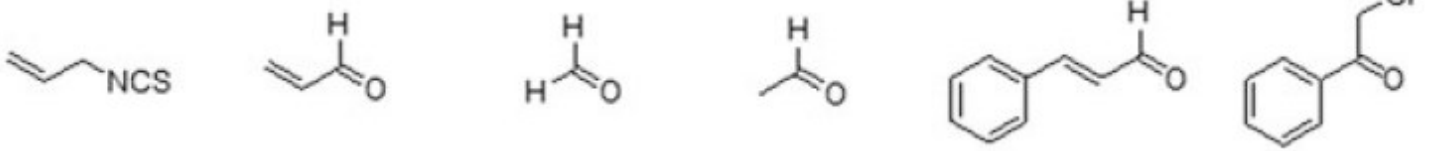
Action	Drug	Company	Therapy Area	Highest development status
TRPV1 agonist	capsaicin	Not Assigned	Pain	Launched
TRPV1 agonist	NGX-4010	Acorda Therapeutics Inc/Astellas Pharma Inc	Postherpetic neuralgia	Launched
TRPV1 agonist	zucapsaicin	Sanofi-Aventis Canada Inc	Osteoarthritis	Registered
TRPV1 agonist	zucapsaicin	Winston Pharmaceuticals Inc	Cluster headache	Phase 3
TRPV1 agonist	MCP-101 (resiniferatoxin)	Mt Cook Pharma	Overactive bladder	Phase 2
TRPV1 antagonist	DWP-05195	Daewoong Pharmaceutical Co Ltd	Neuropathic pain	Phase 2
TRPV1 antagonist	XEN-D0501	Provesica Ltd	Overactive bladder	Phase 2
TRPV1 siRNA	SYL-1001	Sylentis Sau	Ocular pain	Phase 2
TRPV1 antagonist	Mavatrep	Johnson & Johnson Pharmaceutical Research & Development LLC	Osteoarthritis/Pain	Phase 1
TRPV1 antagonist	PHE-377	PharmEste SRL	Neuropathic pain	Phase 1
TRPV1 antagonist	MR-1817	Mochida Pharmaceutical Co Ltd	Pain	Phase 1
TRPV1 antagonist	PAC-14028	Pacific Pharmaceuticals Co Ltd	Atopic dermatitis/IBD	Phase 1
TRPV1 antagonist	SB-705498	GlaxoSmithKline plc	Pruritus	Phase 1
TRPV3 antagonist	GRC-15300	Glenmark Pharmaceuticals Ltd/Sanofi	Neuropathic pain/Osteoarthritis	Phase 2
TRPM8 agonist	Menthol	Not assigned	Carpal tunnel syndrome/Neck pain	N/A
TRPM8 agonist	D-3263	Dendreon Corp	Cancer	Phase 1
TRPA1 antagonist	GRC-17536	Glenmark Pharmaceuticals Ltd	Diabetic peripheral neuropathy/ Respiratory disorders	Phase 2
TRPA1 antagonist	CB-625	Cubist Pharmaceuticals/Hydra Biosciences	Inflammatory disease/ Pain	Phase 1

Kaneko & Szallasi (2014) Br J Pharmacol 171, 2474

TRP-A1 : Development of (re-)active compounds

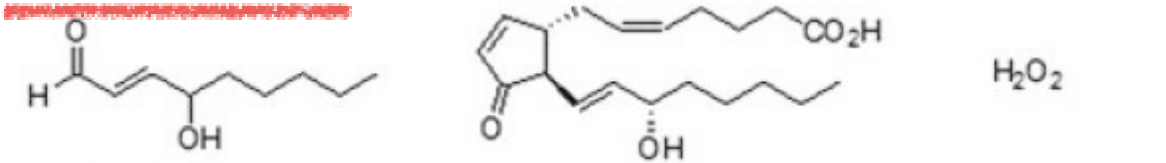
Agonists

Reactive Exogenous Agonists



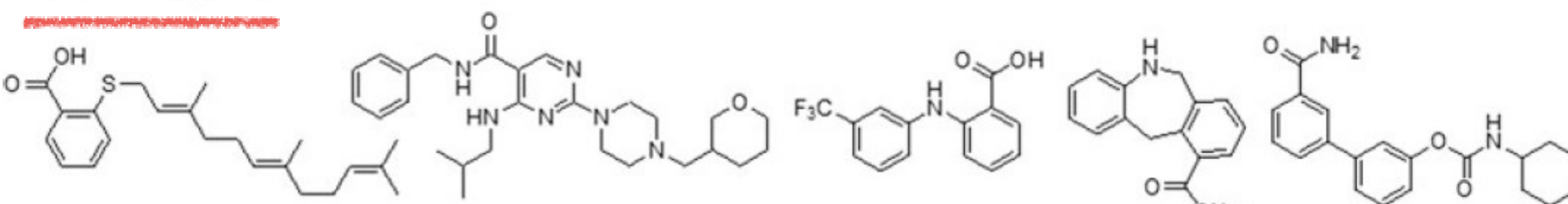
AITC (~10 µM) | Acrolein (1-5 µM) | Formaldehyde (400 µM) | Acetaldehyde (77 µM) | Cinnamaldehyde (~10 µM) | CN Tear Gas (91 nM)

Endogenous Agonists



4-hydroxynonenal (27 µM) | PGJ2 (5 µM) | Hydrogen peroxide (230 µM)

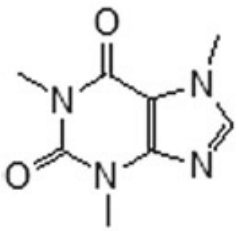
Non-reactive Agonists



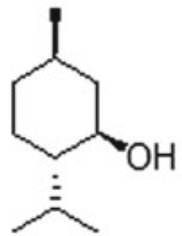
Farnesyl thiosalicyclic acid (5 µM) | Pfizer PF-4840154 (23 nM) | Flufenamic acid (24 µM) | Janssen WO2010/141805 | URB 597 (25 µM)

TRP-A1 : Development of active compounds

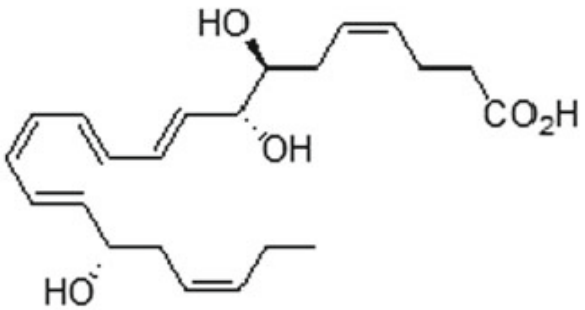
Antagonists - natural



Caffeine



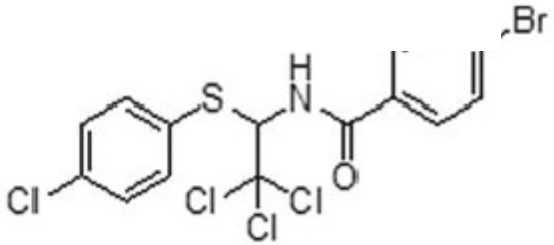
Menthol



Resolvin D1

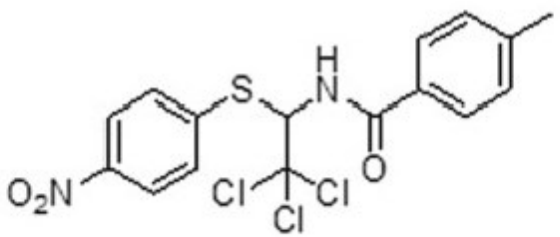
TRPA1 IC_{50} 69 nM

Antagonists - synthetic



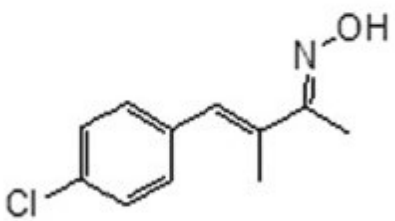
AMG7160

IC_{50} 51 nM



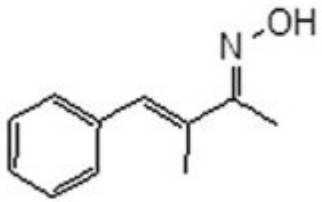
Abbott CMP1

IC_{50} 2.0 µM



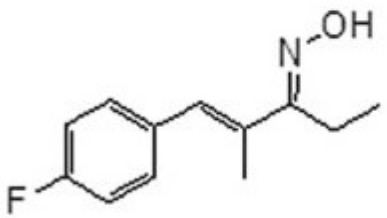
AP18

TRPA1 IC_{50} 3.1 µM



Renovis

TRPA1 IC_{50} 2.7 µM



Abbott

Molecular Pain 2010, 6:14

TRPA1 IC_{50} 67 nM

Transient receptor potential ion channels

- TRPs are cation-selective channels, and
  critical modulators of Ca^{2+} entry into cells

- TRP's are essential for many sensory functions probing the environment:
  - vision
  - hearing
  - taste
  - smell
  - mechano-sensation
  - pheromone sensation
  - thermo-sensation
  - humidity

- TRPs are at the centre of drug development activities

Further reading on TRP's

Hilton Biochemistry 2015

Zhang NSMB 2018