(Mechanosensitive) ion channels

ME-480 Lecture 9

Mechanosensing

Sense of touch: tactile discrimination

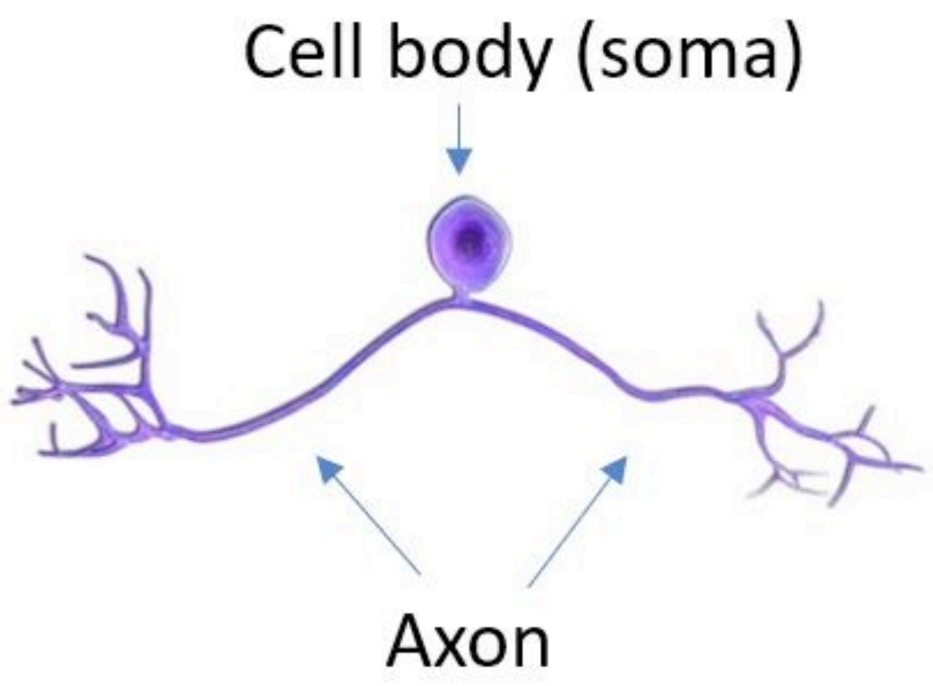
Proprioception

Dorsal root ganglia

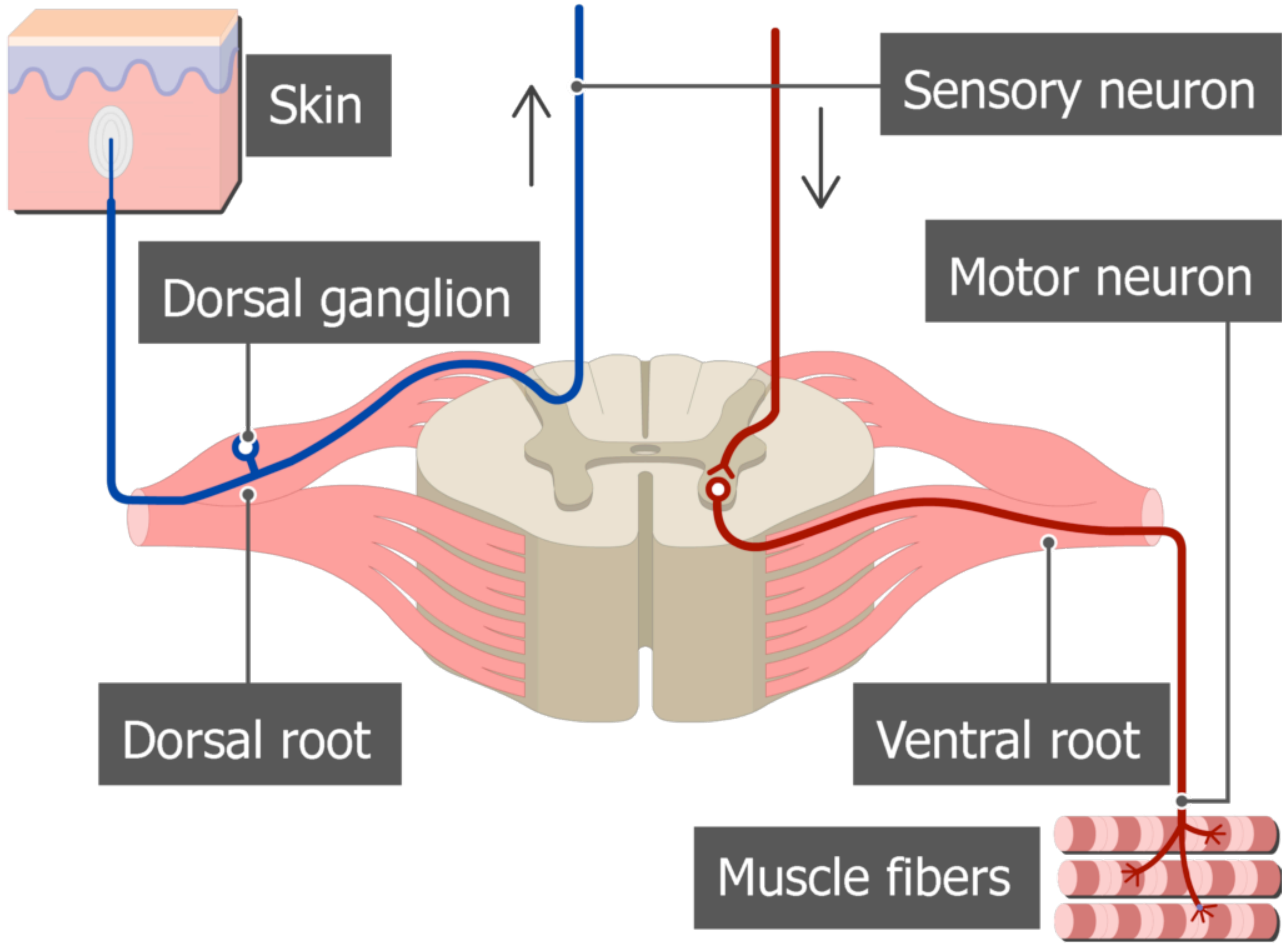
Transmits sensory information (*e.g.* pain) from periphery to CNS

Input from periphery

Receptor?



Synapse in spinal cord



External signals are transmitted along axons to the spinal cord, then relayed to the brain for processing

Signal transmission in neurons

the physical basis of signal propagation in neurons

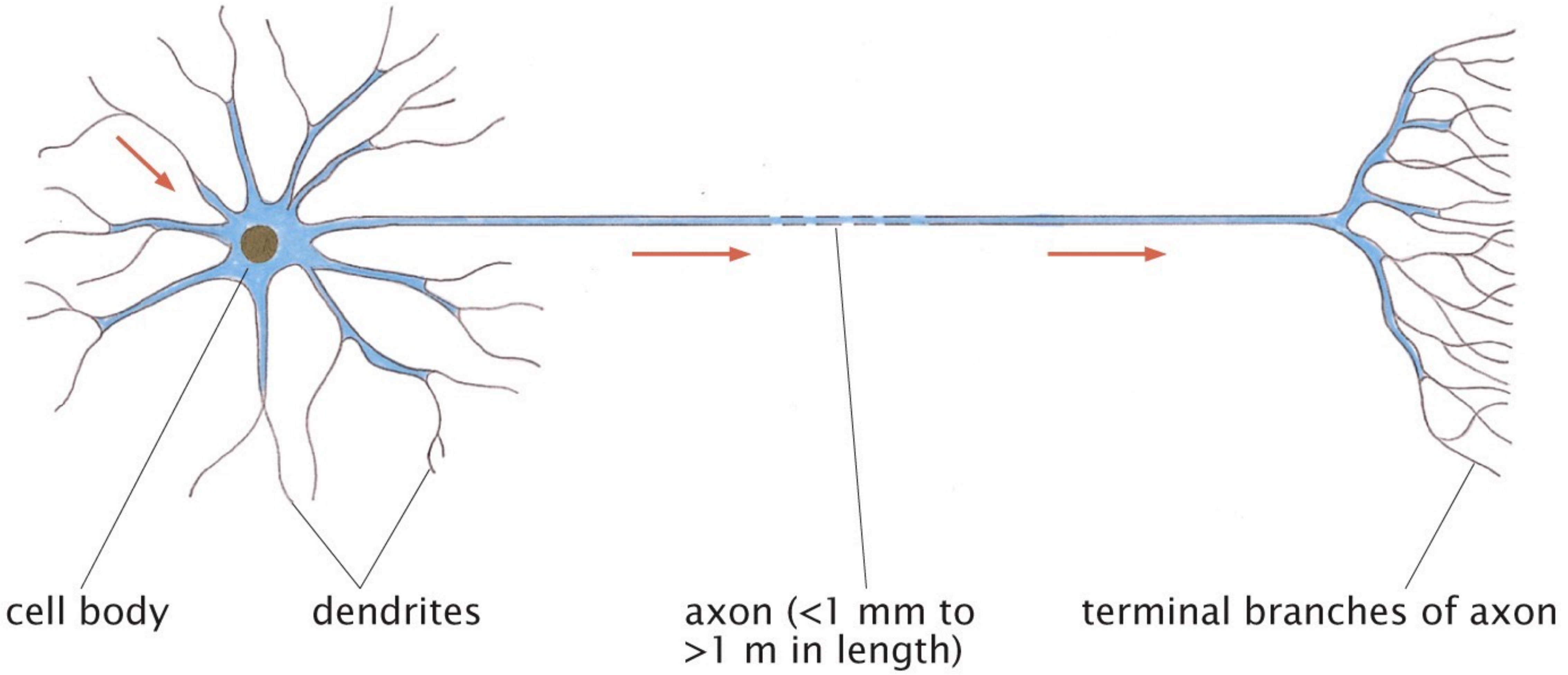
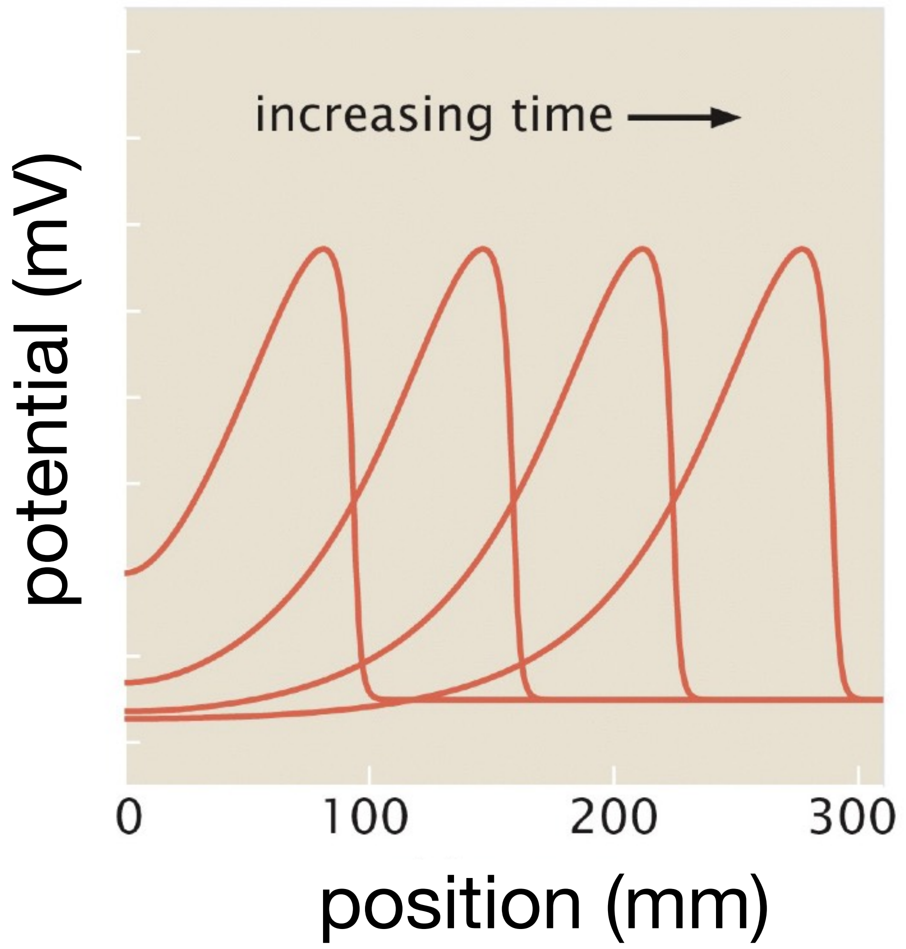
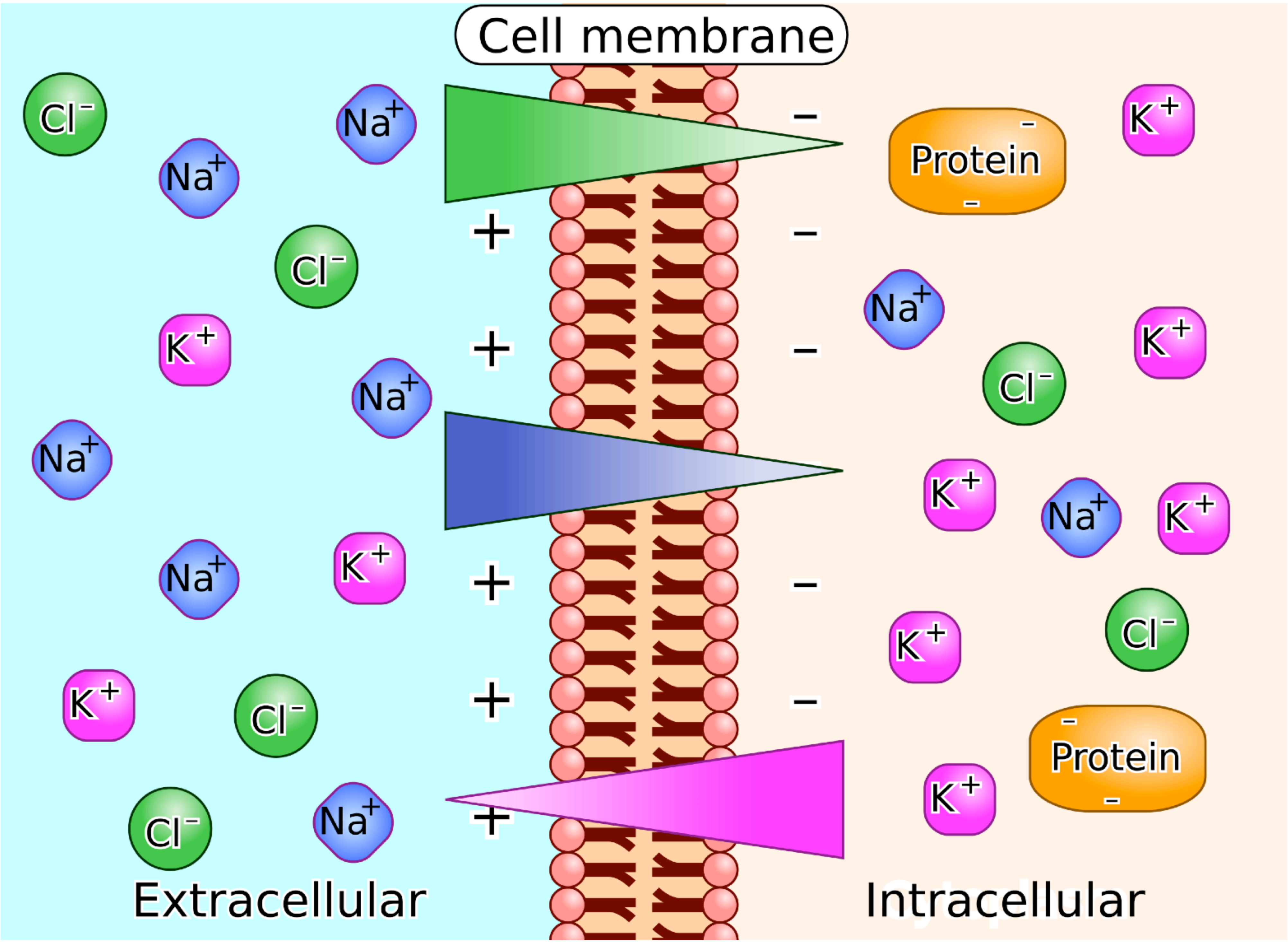


Figure 17.1 Physical Biology of the Cell, 2ed. (© Garland Science 2013)



Information propagates via electrical signals called action potentials

Ionic charge imbalances generate membrane potential



How do cells create gradients at the membrane?

To do that, cells must overcome a huge problem with membranes:

Permeability

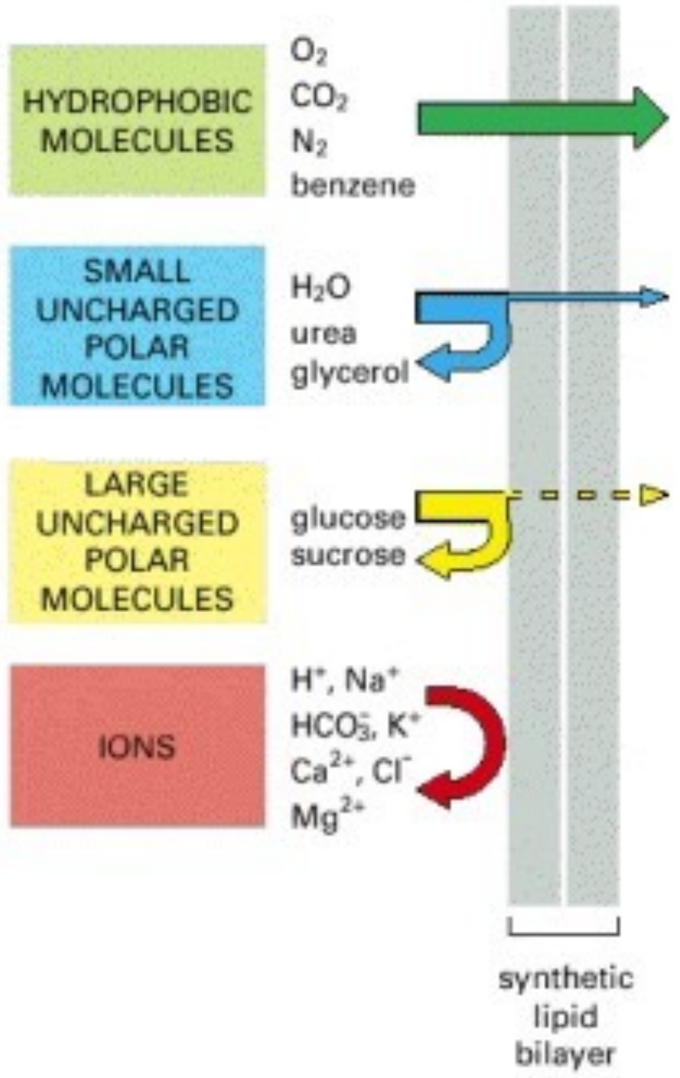
>Molecules

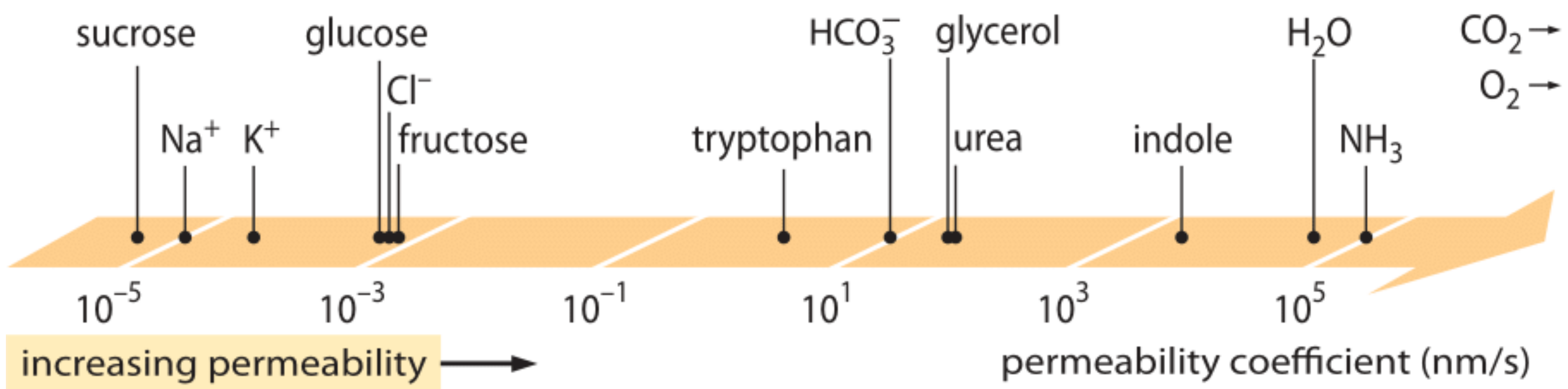
>Information

Ion channels

Solve an important permeability problem of membranes

Cell requires ions for many functions: Na^+, K^+, Cl^-





Flux per unit area through the membrane:

$$j = -p\left(c_{in} - c_{out}\right)$$

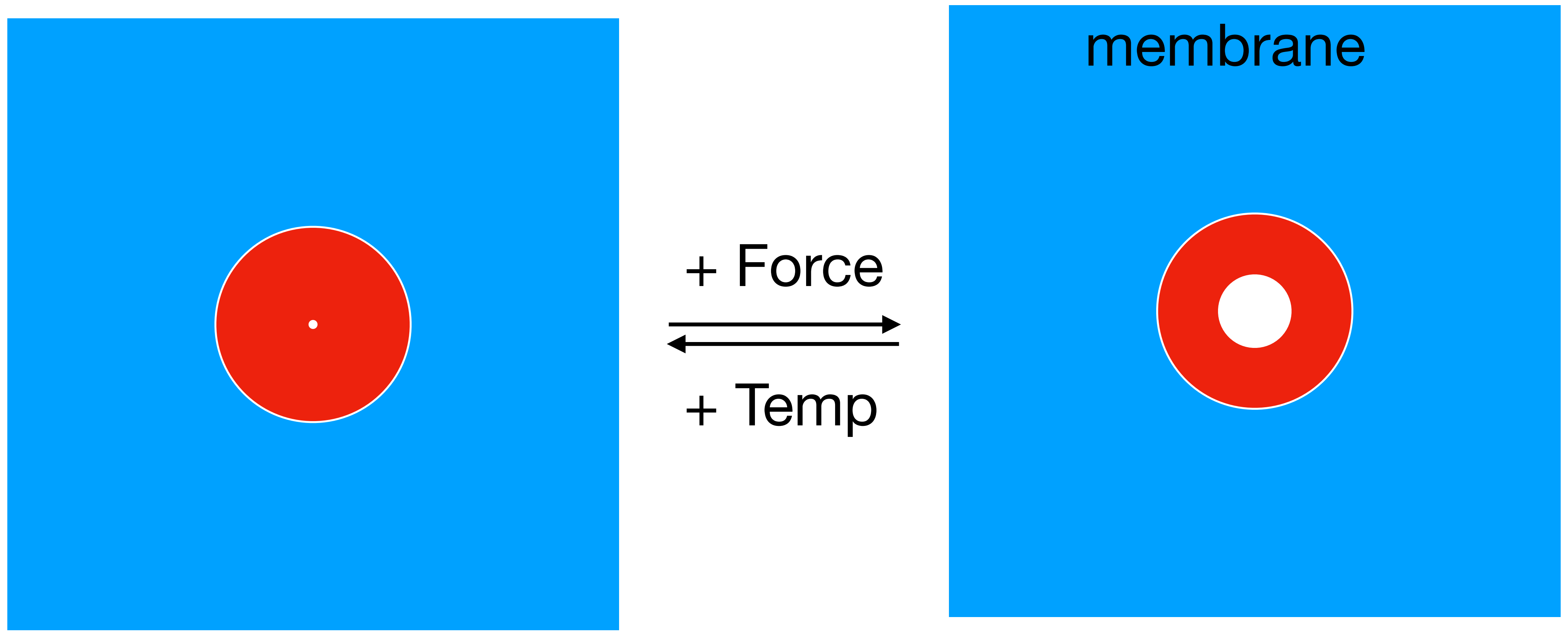
Ion channels increase cell permeability to charged molecules

Lecture outline

- Types of channels
- Biophysical mechanisms
- Molecule structure
- Physiological functions
- Mechanosensitive ion channels in disease

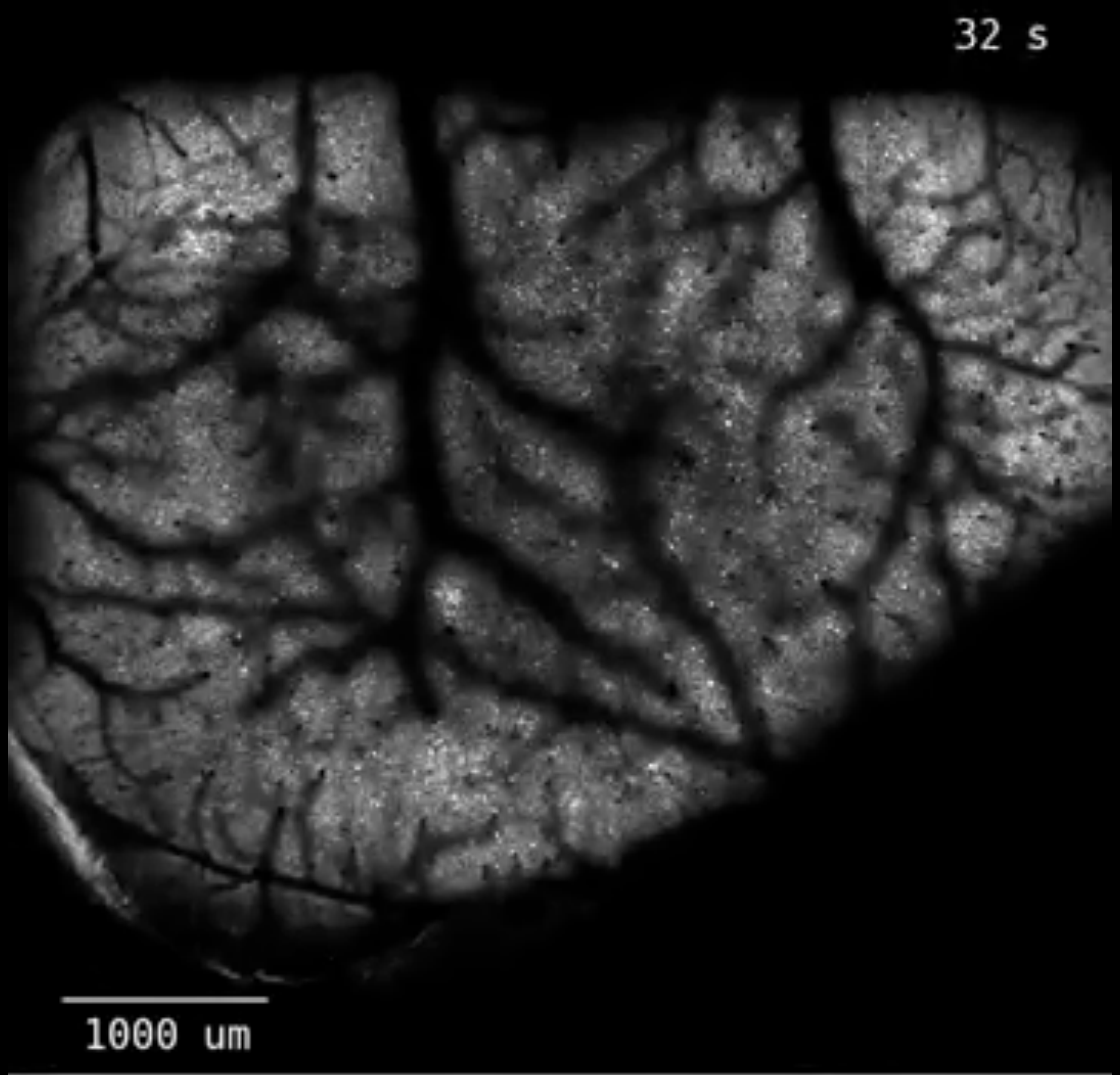
(Mechanosensitive) ion channels

- membrane proteins
- pore-forming
- Can gate in response to mechanical stimuli on membrane

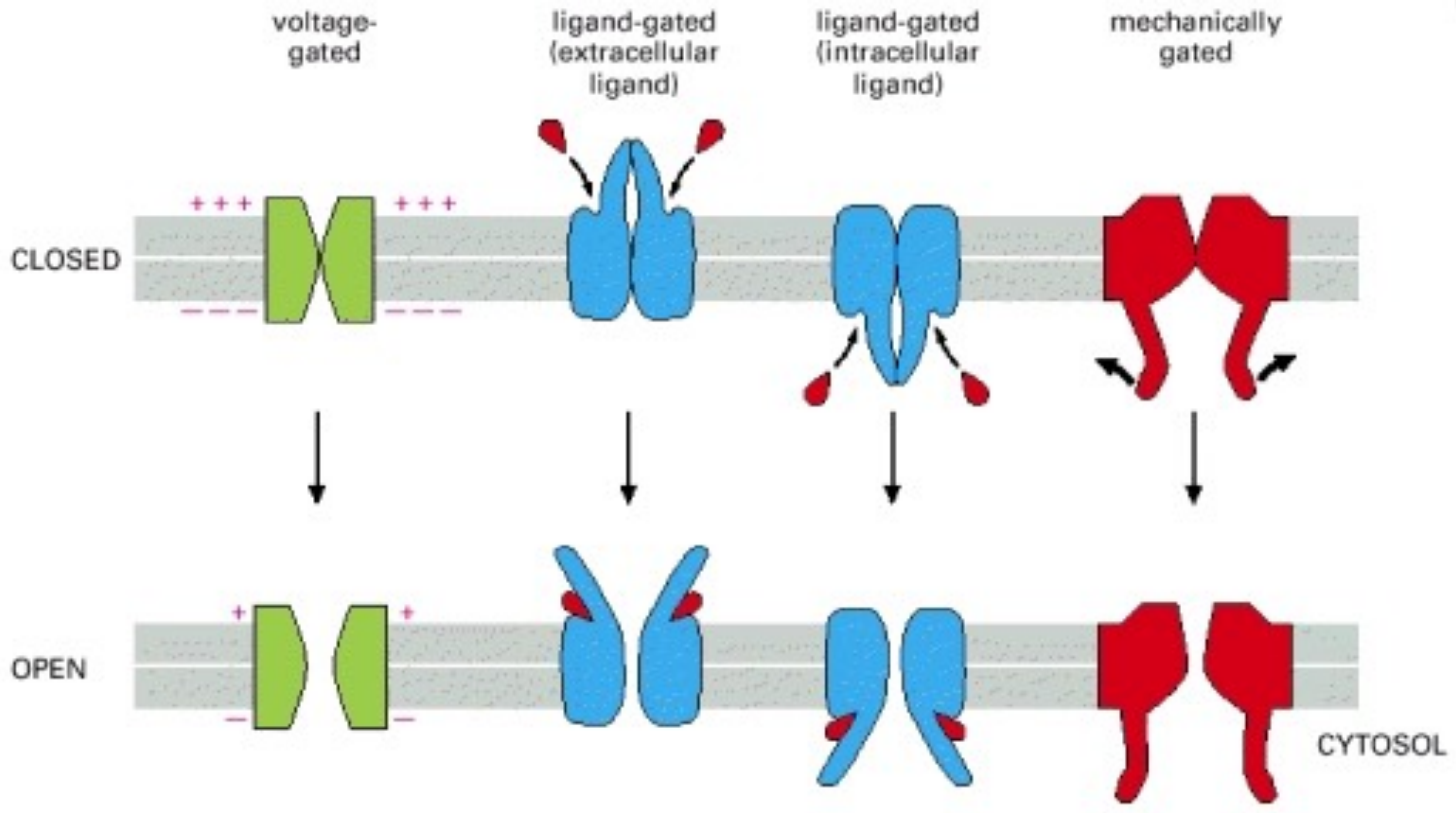


Ion channels enable neuronal signal transmission

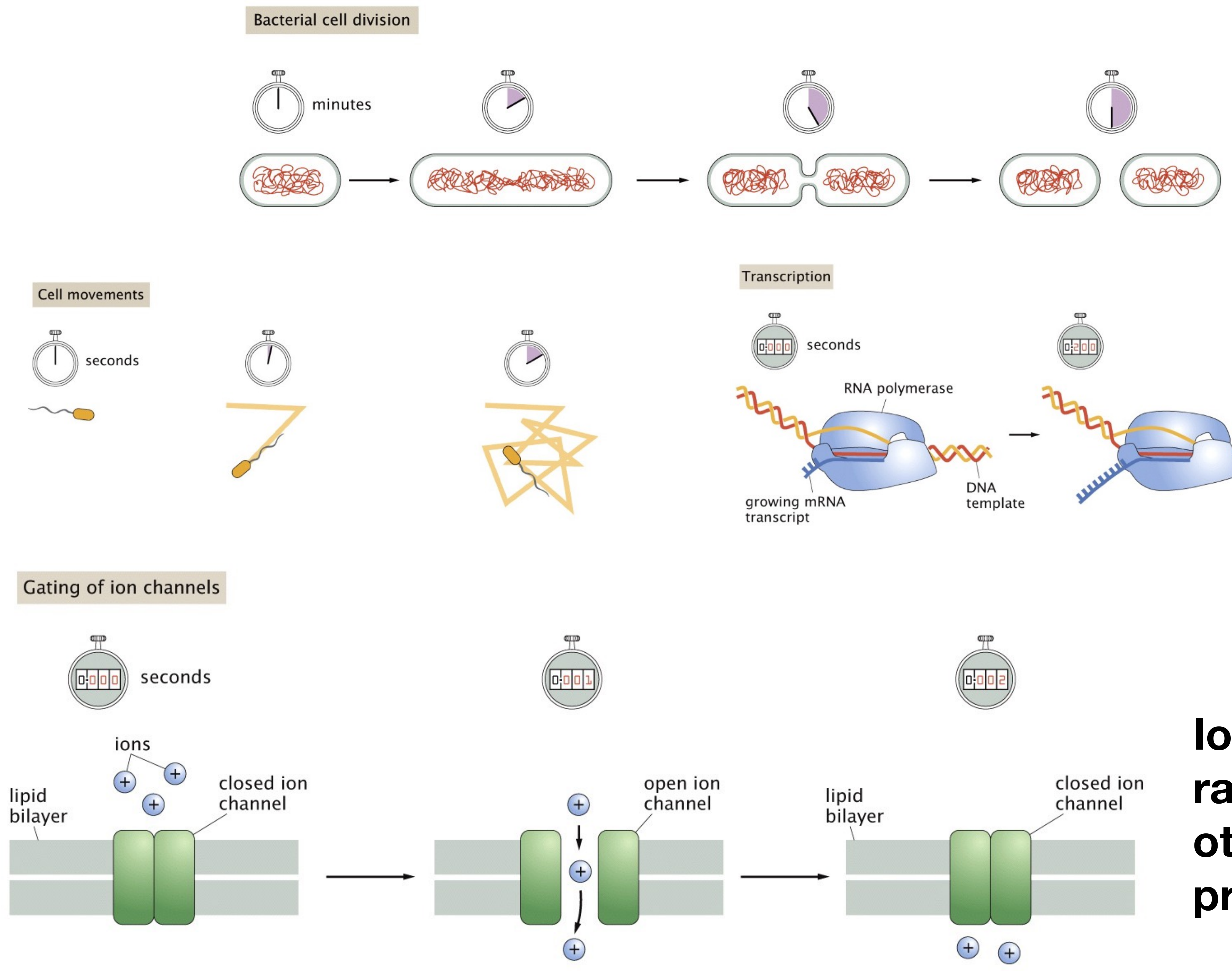
GCaMP calcium indicator



Types of ion channel actuation



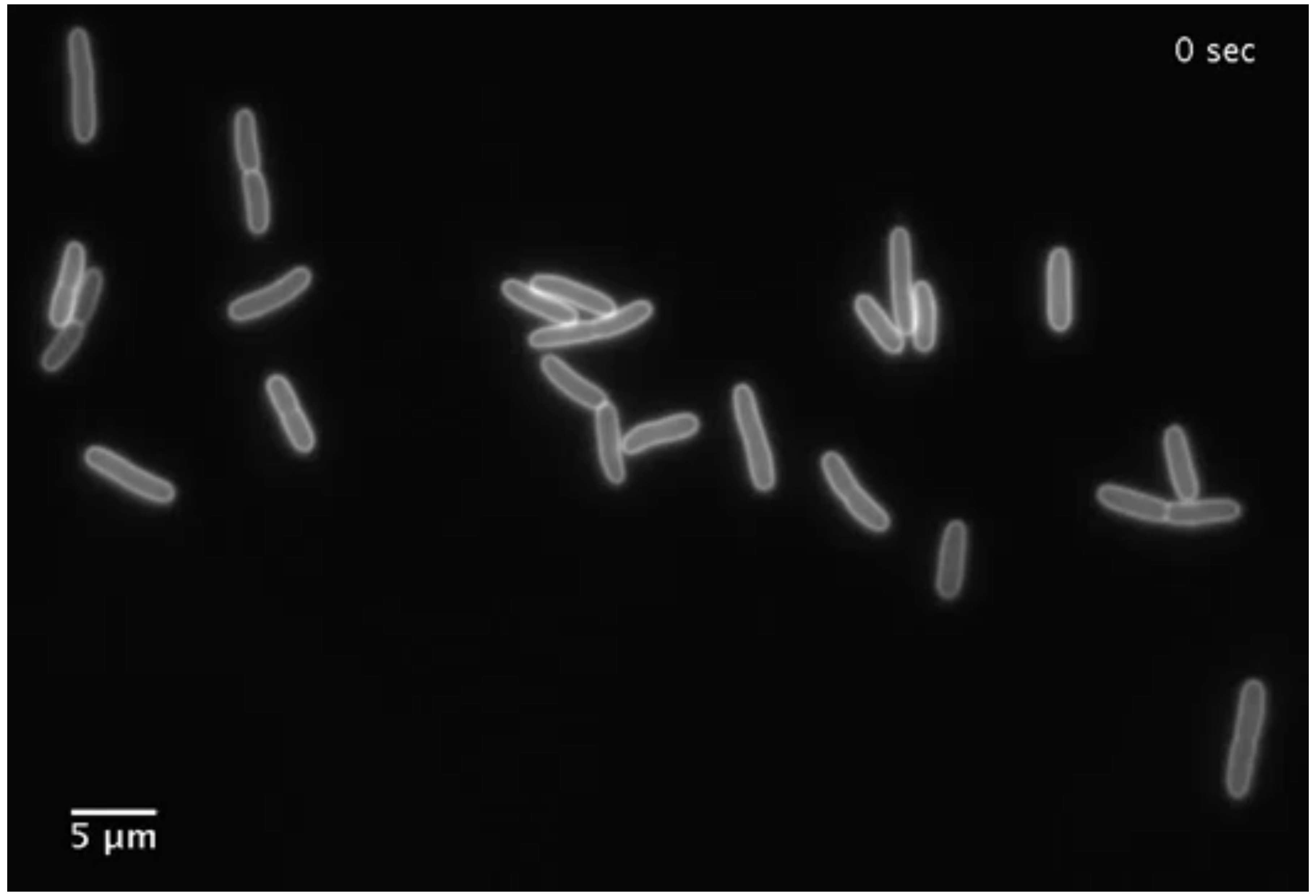
Timescale for ion transport

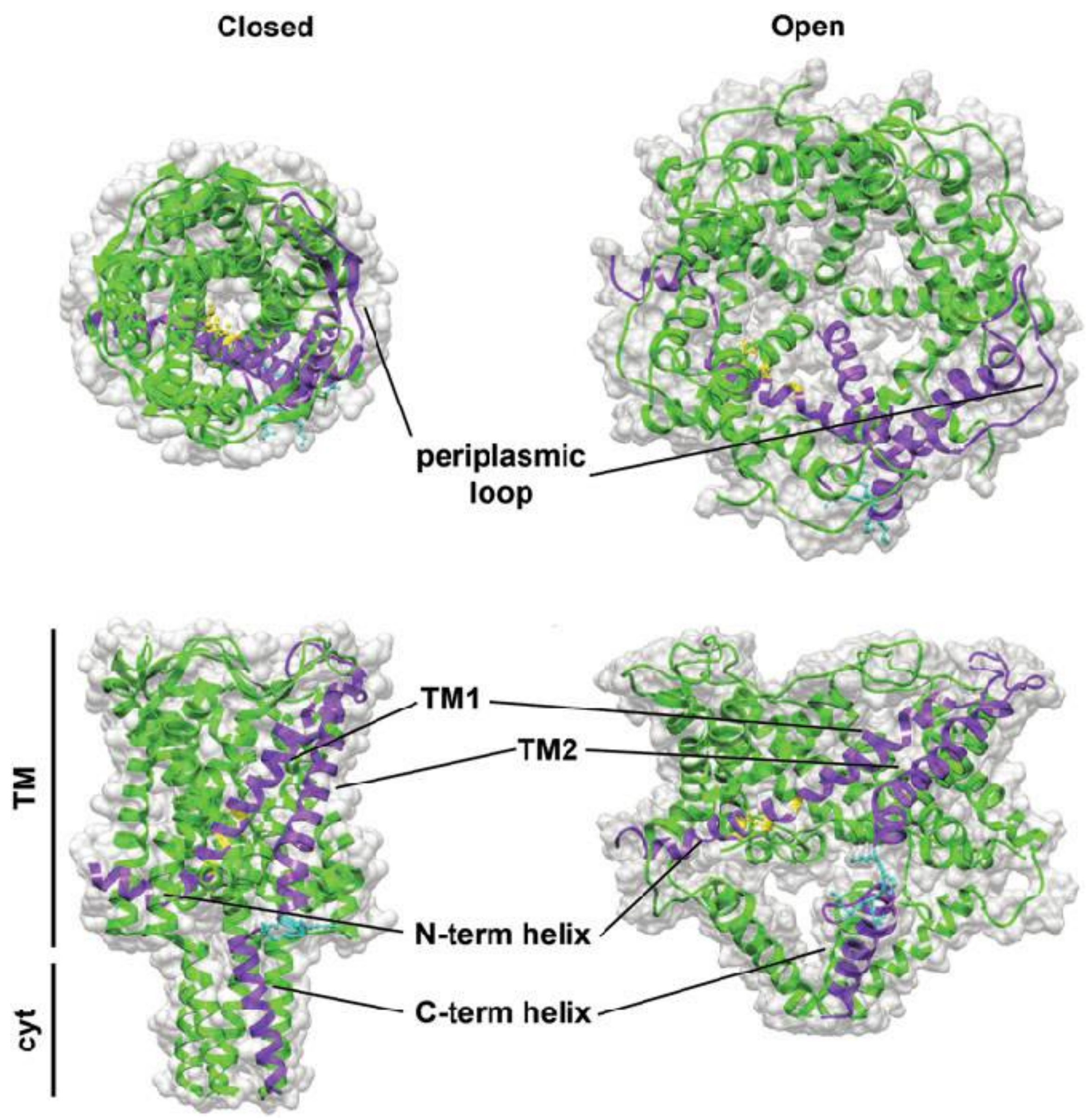


Ion gating is extremely rapid compared to other cellular processes

Mechanosensitive ion channels in bacteria

A convenient model for biophysics

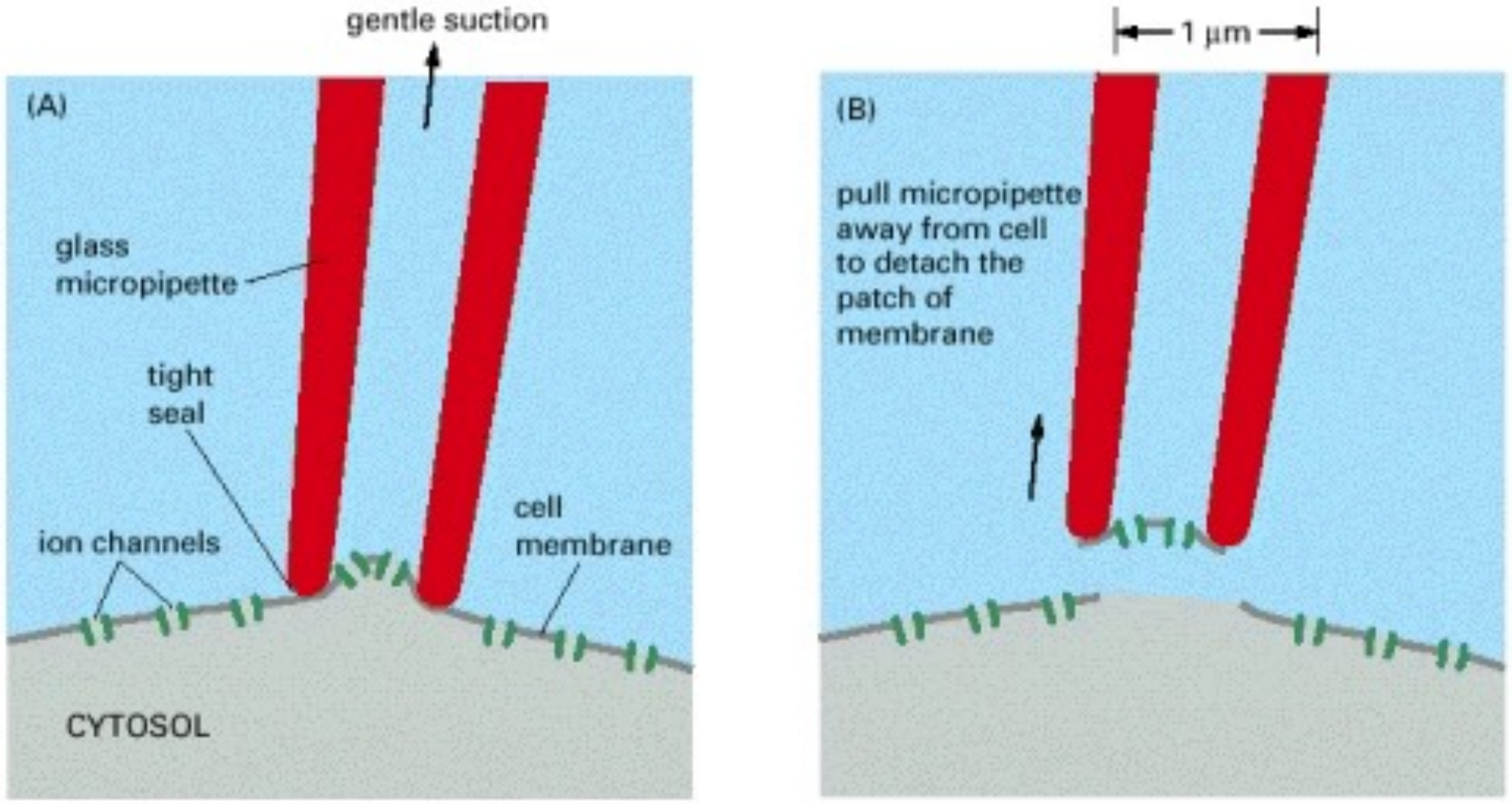


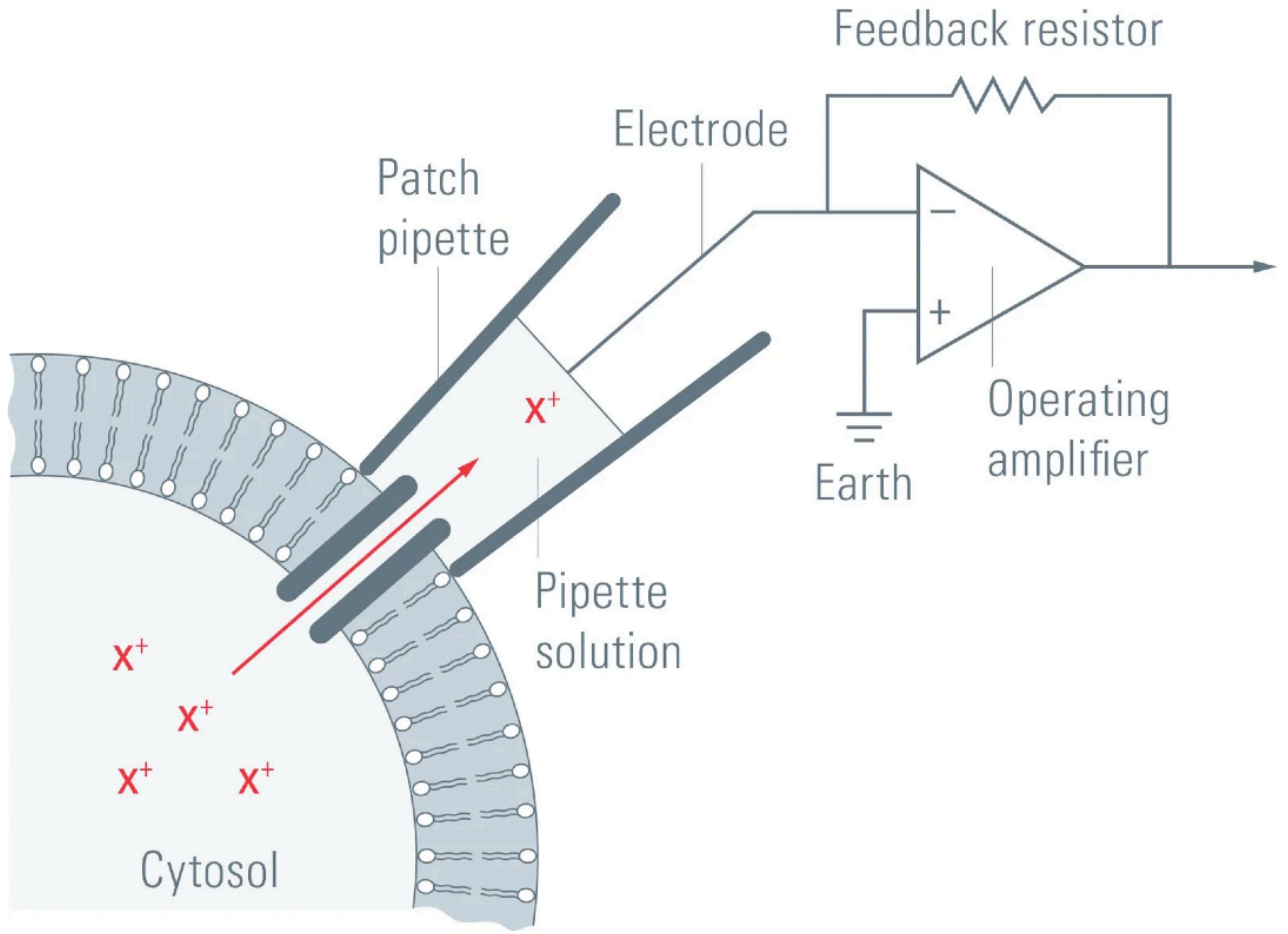


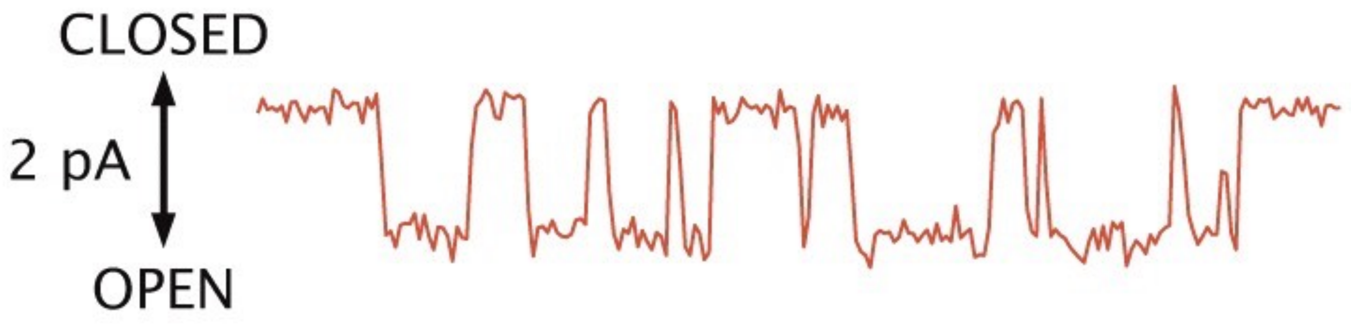
ion channels enable resistance to osmotic shock
function as mechanosensitive “relief valves”

MScL: a model for ion channel
structure and mechanical activation

Patch clamp measurements of ion transport



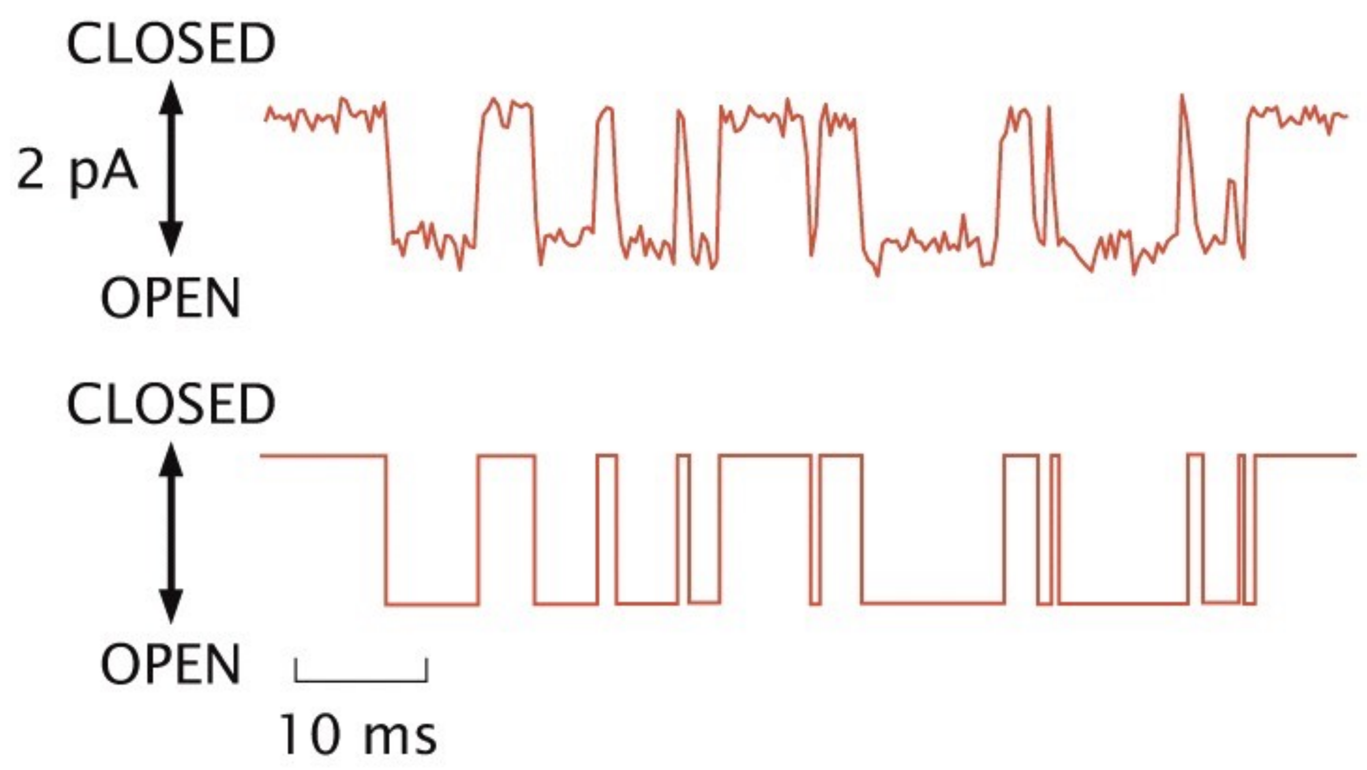




Electrical current measurements (single channel)

Characterizing mechanosensitive channels

- Apply pressure with patch clamp pipette
- Measure current
- Compute open probability



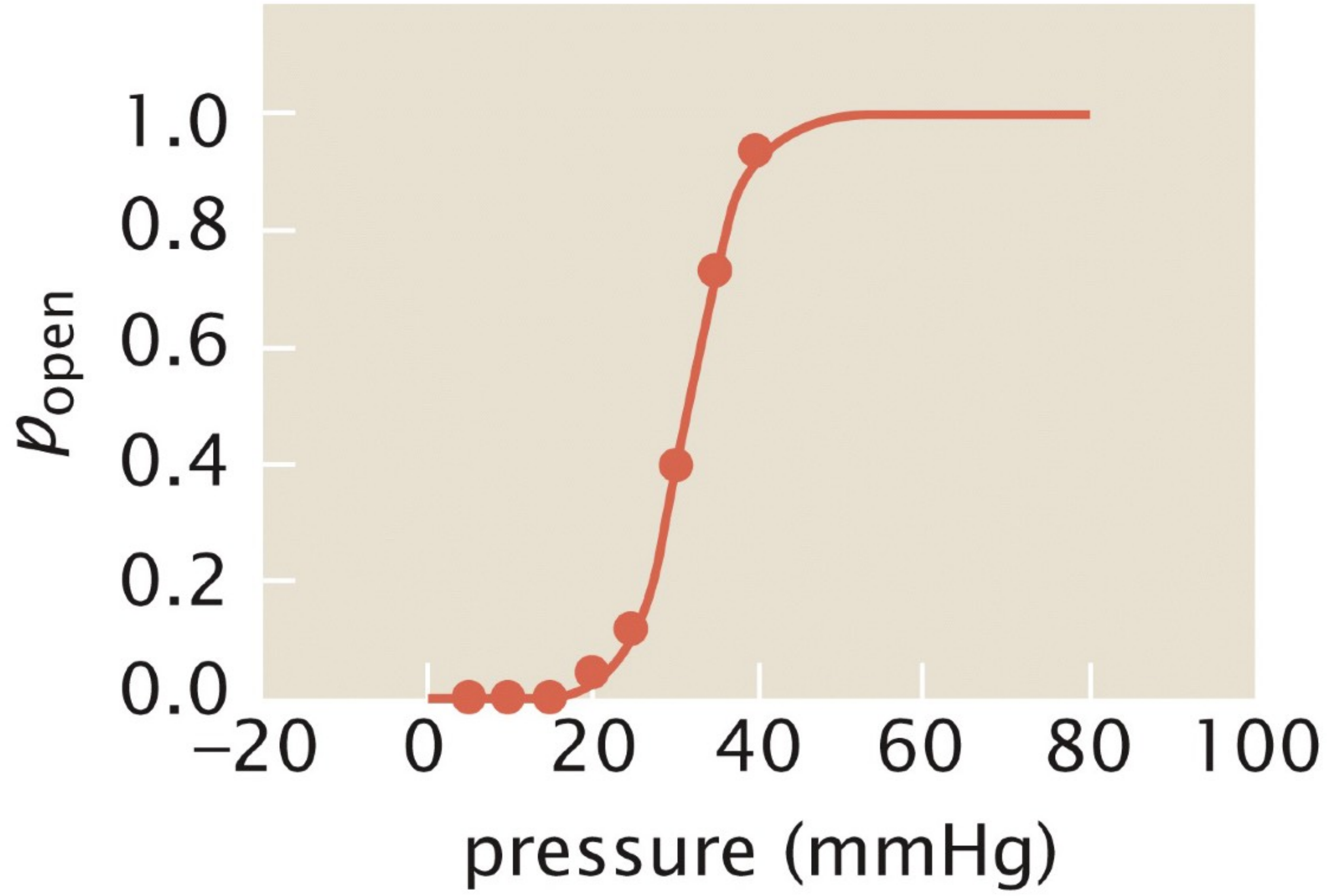


Figure 7.5 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Two-state model for mechanosensitive ion channels

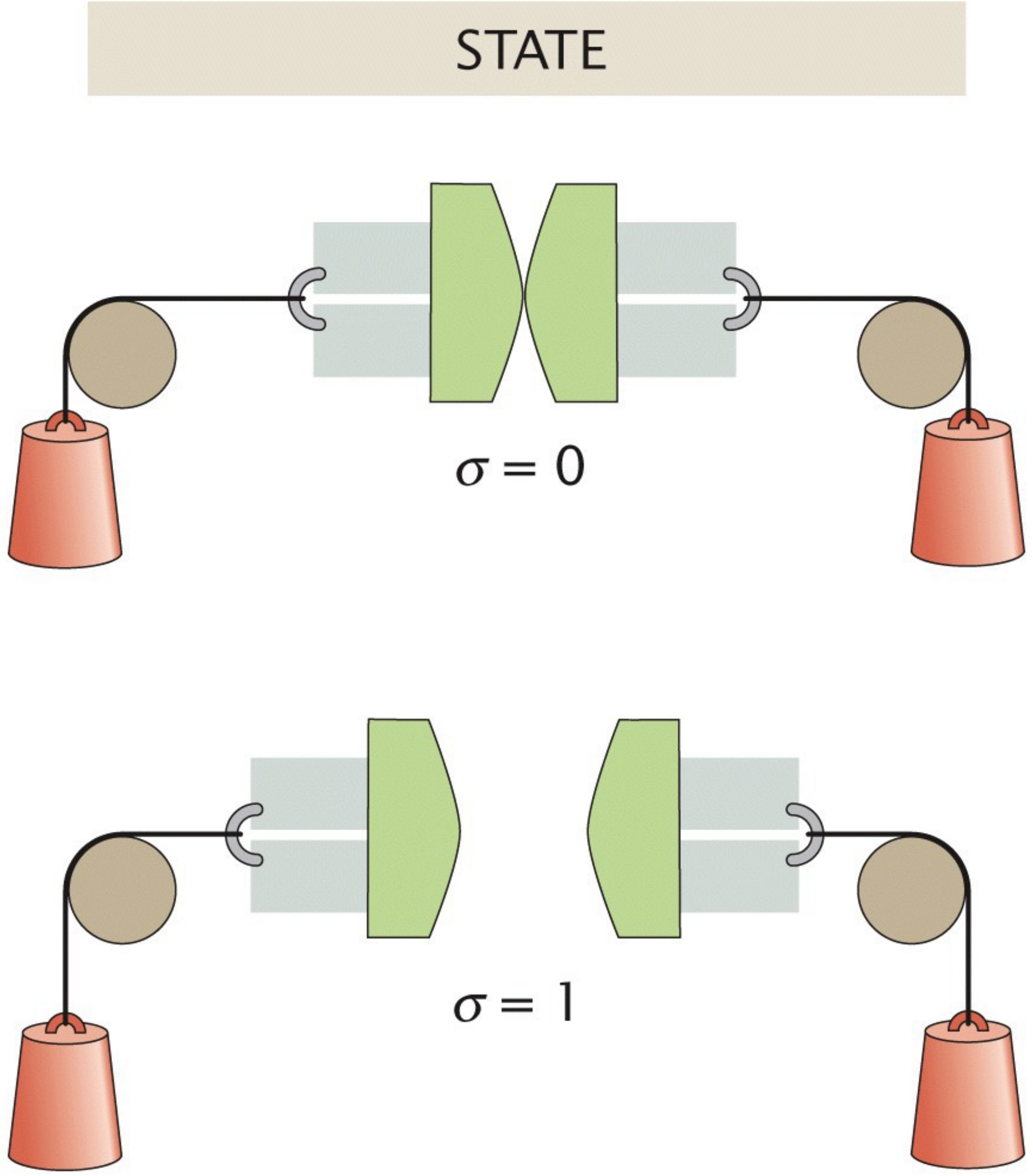


Figure 7.4 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

probability for channel to be open:

$$p_{open} = \frac{e^{-\varepsilon_{open}/k_BT}}{e^{-\varepsilon_{open}/k_BT} + e^{-\varepsilon_{closed}/k_BT}} = \frac{1}{1 + e^{-(\varepsilon_{closed}-\varepsilon_{open})/k_BT}}$$

Modeling free energy changes upon channel opening

effect of membrane tension

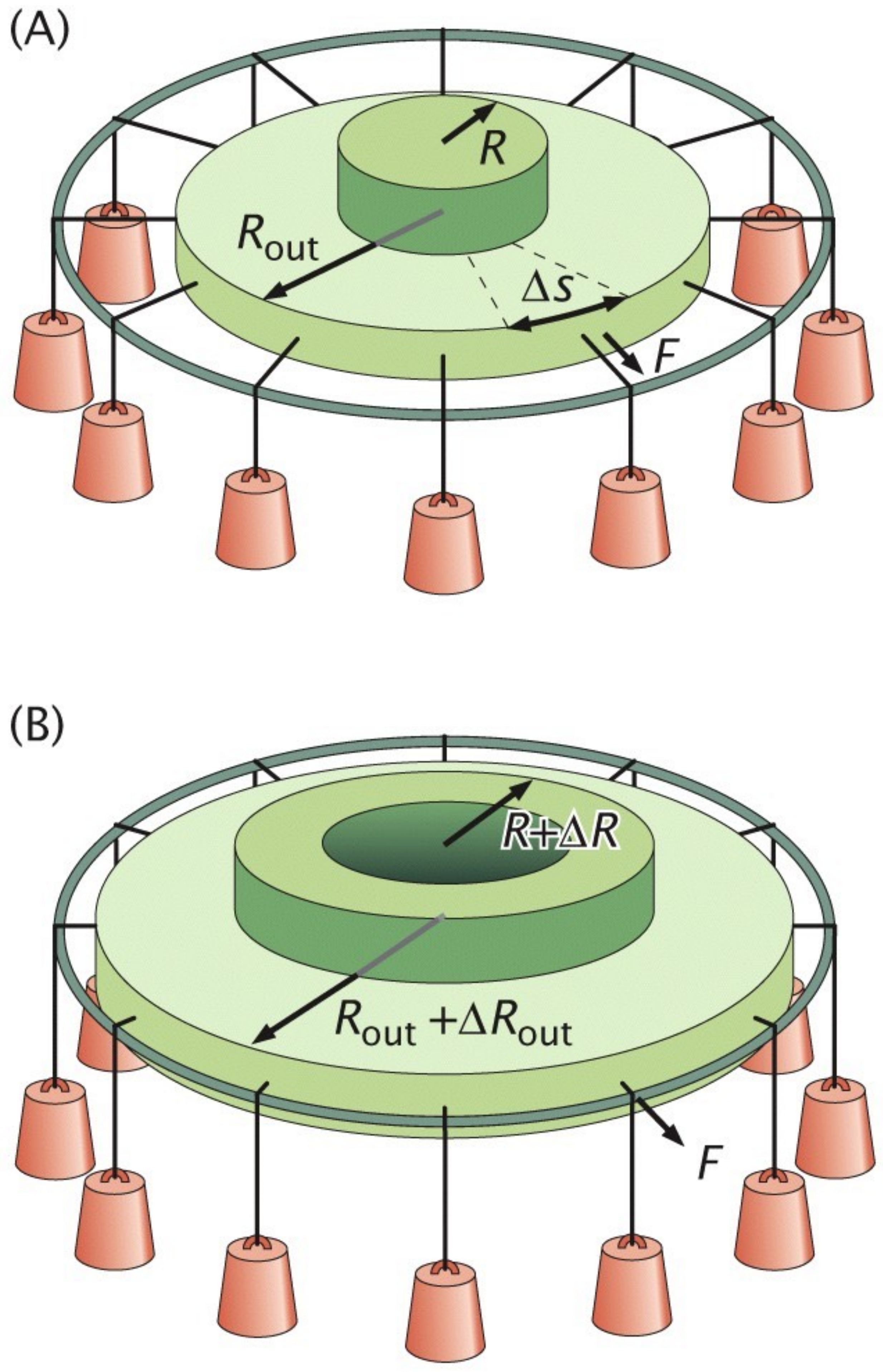


Figure 7.6 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$\varepsilon_{\text{force}} = \tau \times \Delta A$$

With τ = Tension exerted by weights
ΔA = area change generated by opening

$$p_{open} = \frac{e^{-\frac{1}{k_B T}(\varepsilon_{open} - \tau \Delta A)}}{e^{-\frac{1}{k_B T}(\varepsilon_{open} - \tau \Delta A)} + e^{-\frac{1}{k_b T}\varepsilon_{closed}}}$$

Opening under tension

Mechanics modulate channel opening energy landscape

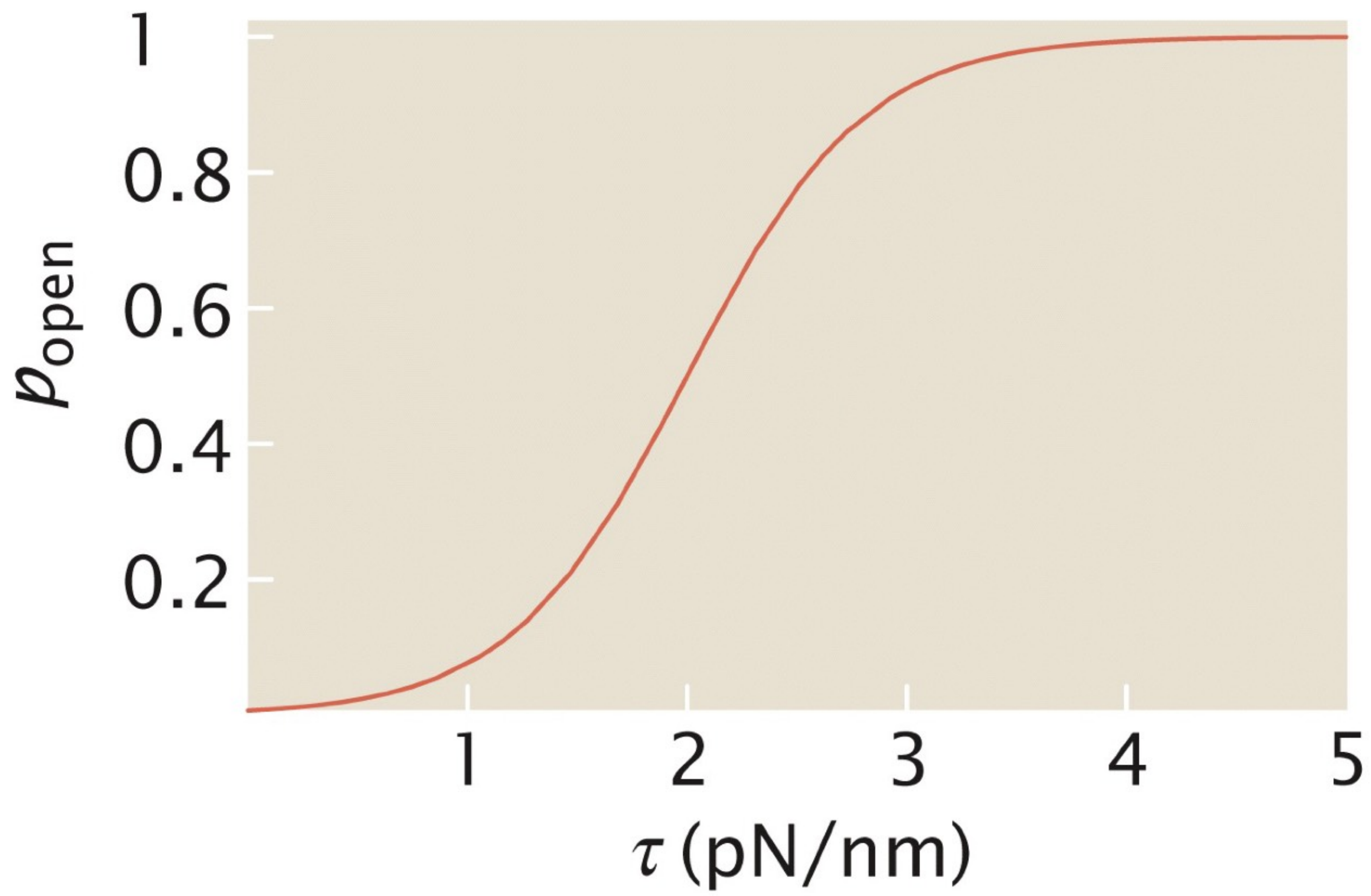


Figure 7.8 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

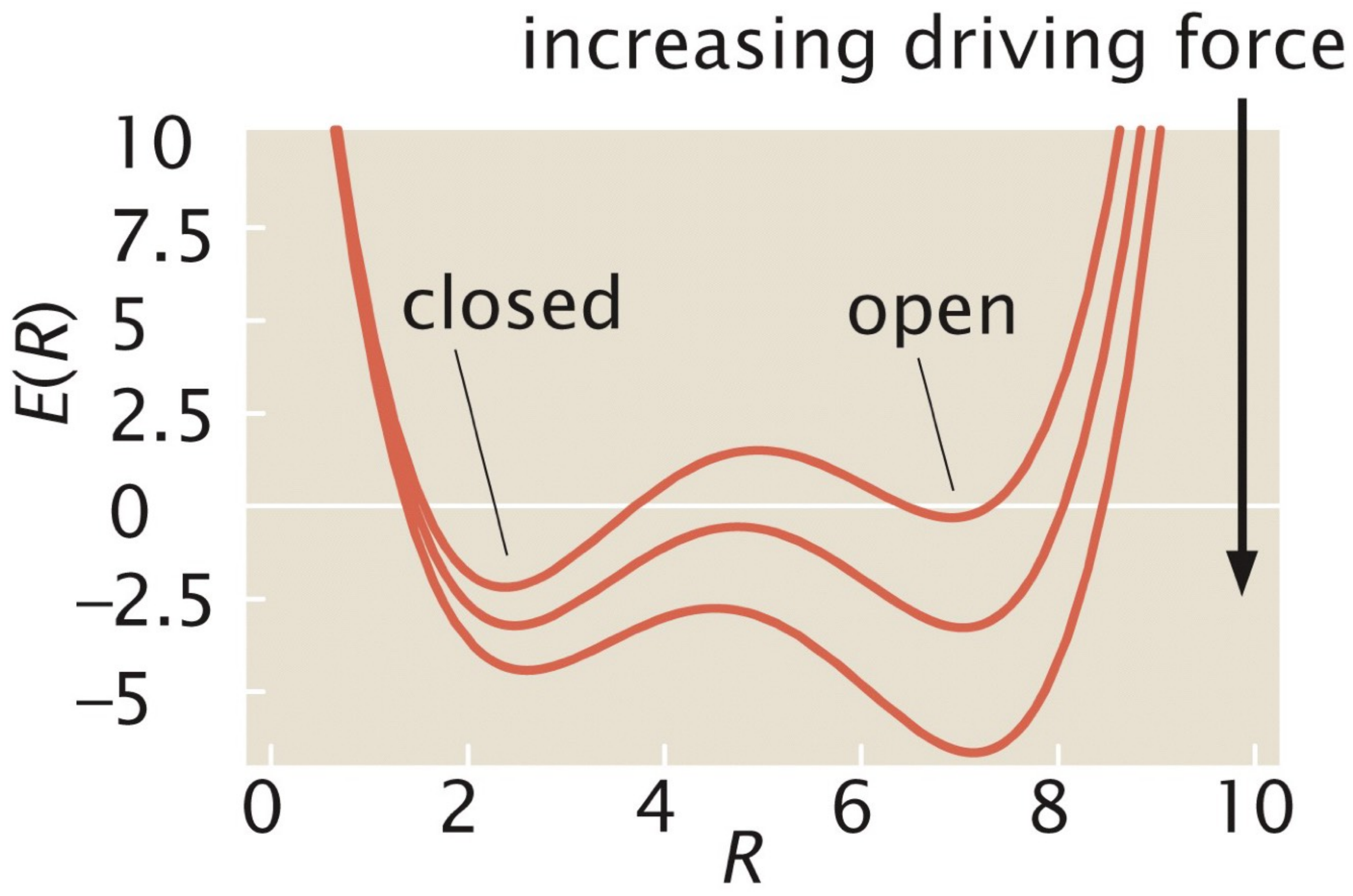


Figure 7.7 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

2021 Nobel Prize in medicine

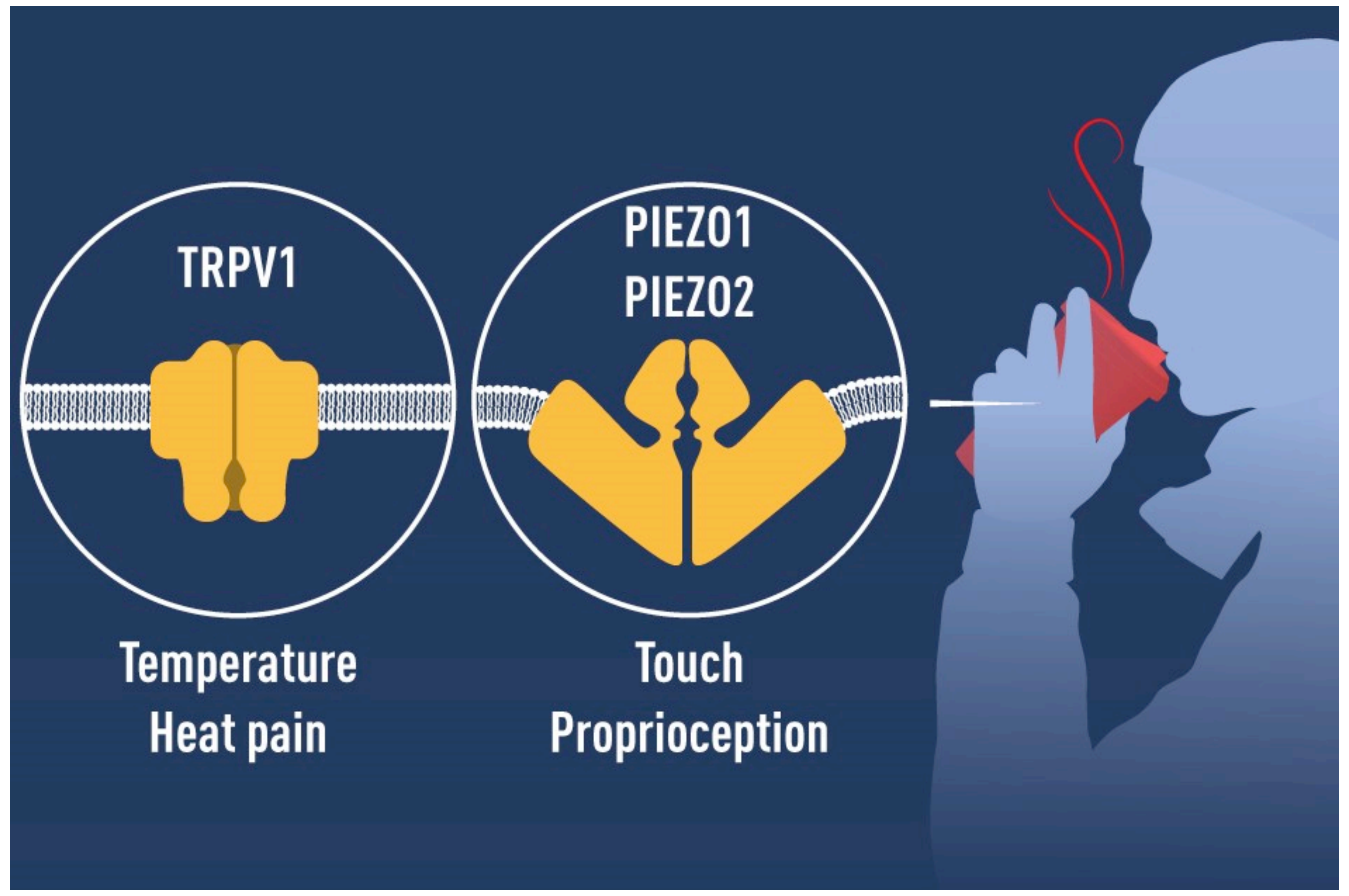
Sensory ion channels

David Julius

Ardem Patapoutian

↓

Talking at EPFL SV
seminar on Jan. 27

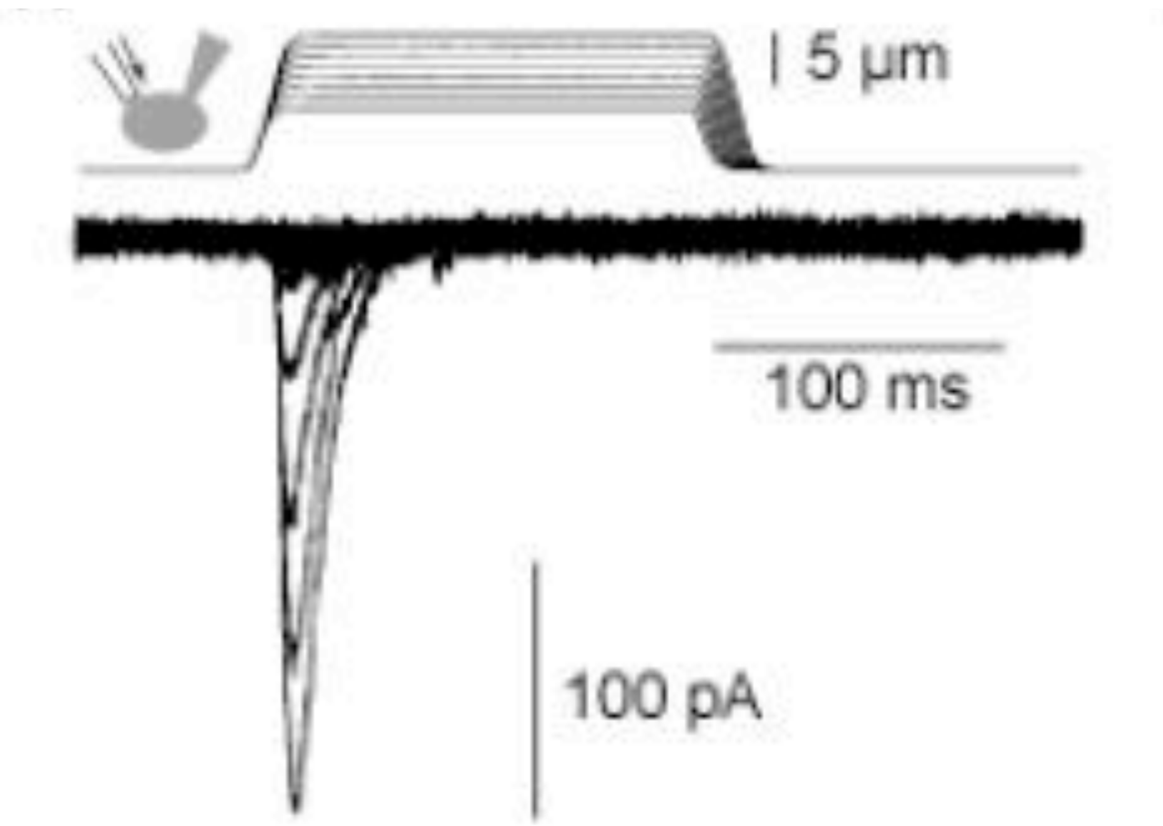


Discovery of Piezo channels

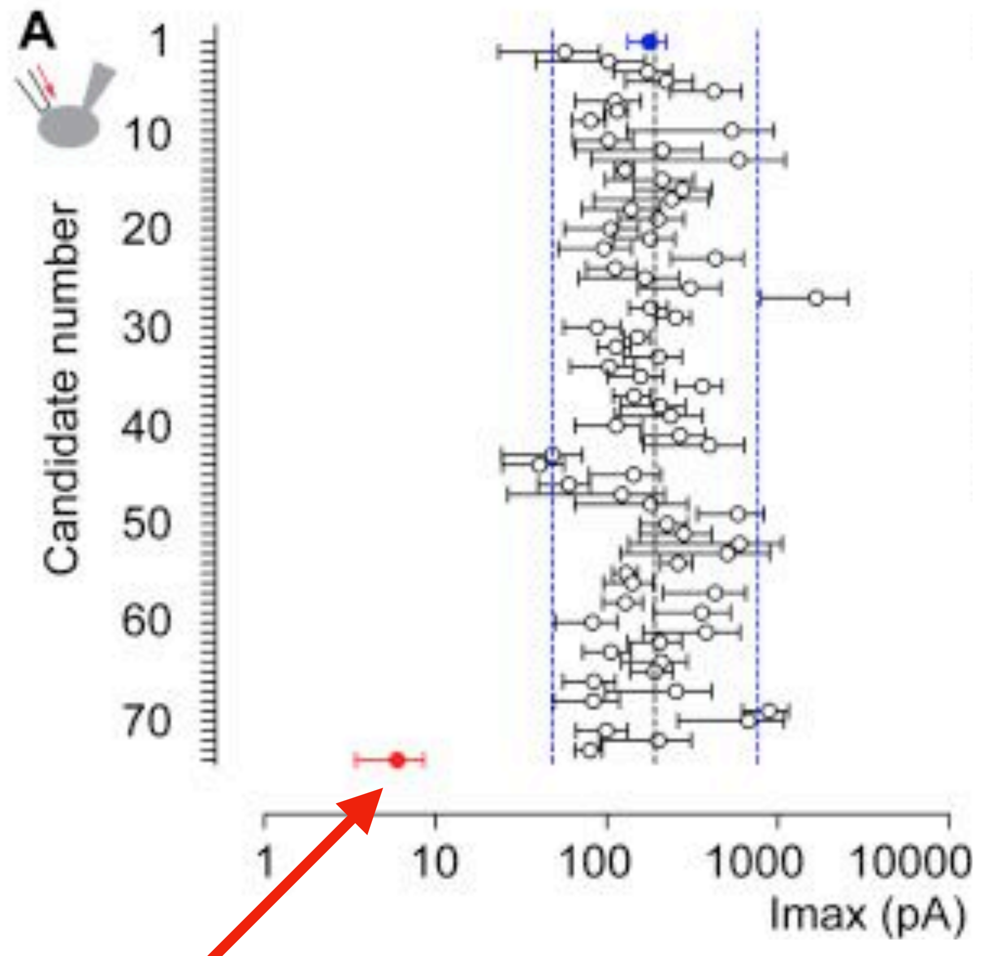
Mechanically stimulate cells

Measure ionic current response
(patch clamp)

Patch clamp



Mutant screen

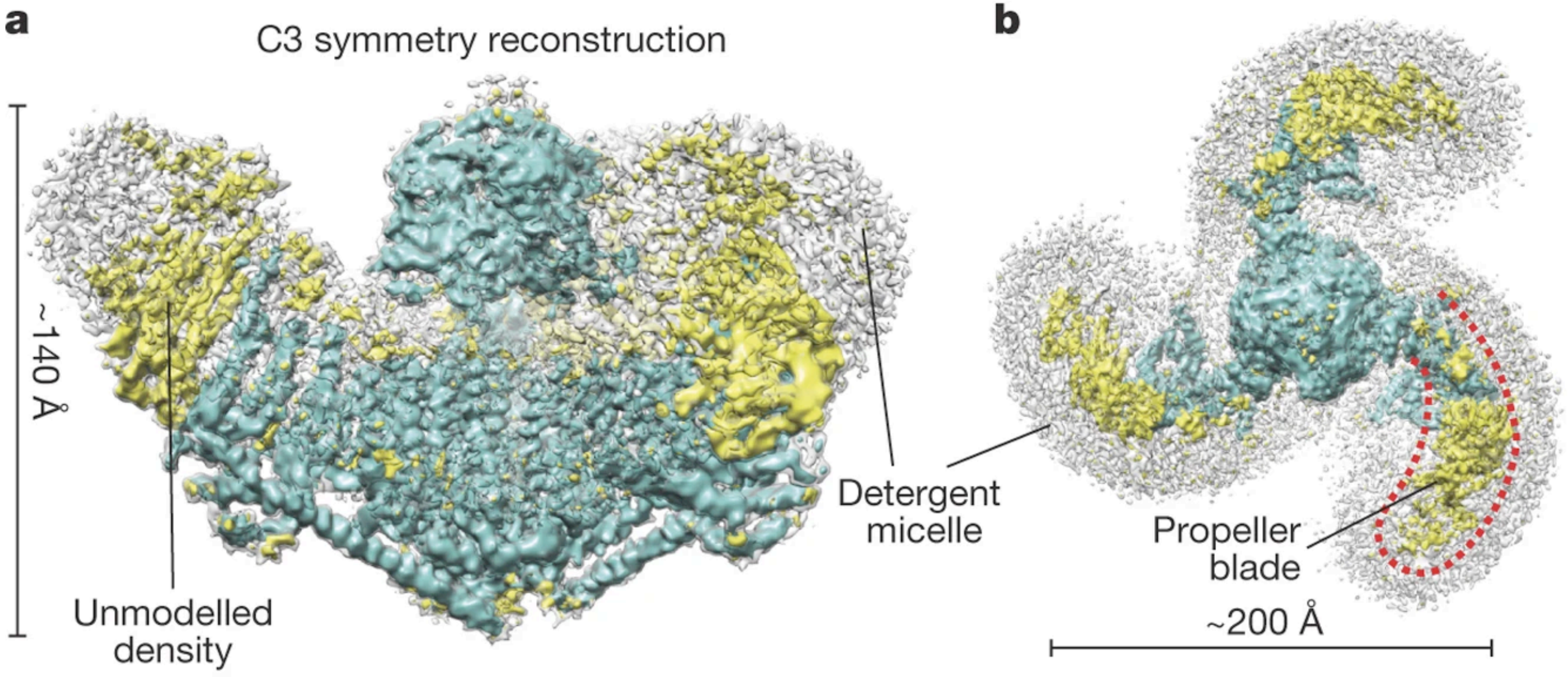


Piezo1

Coste et al., Science 2010

Piezo1 structure

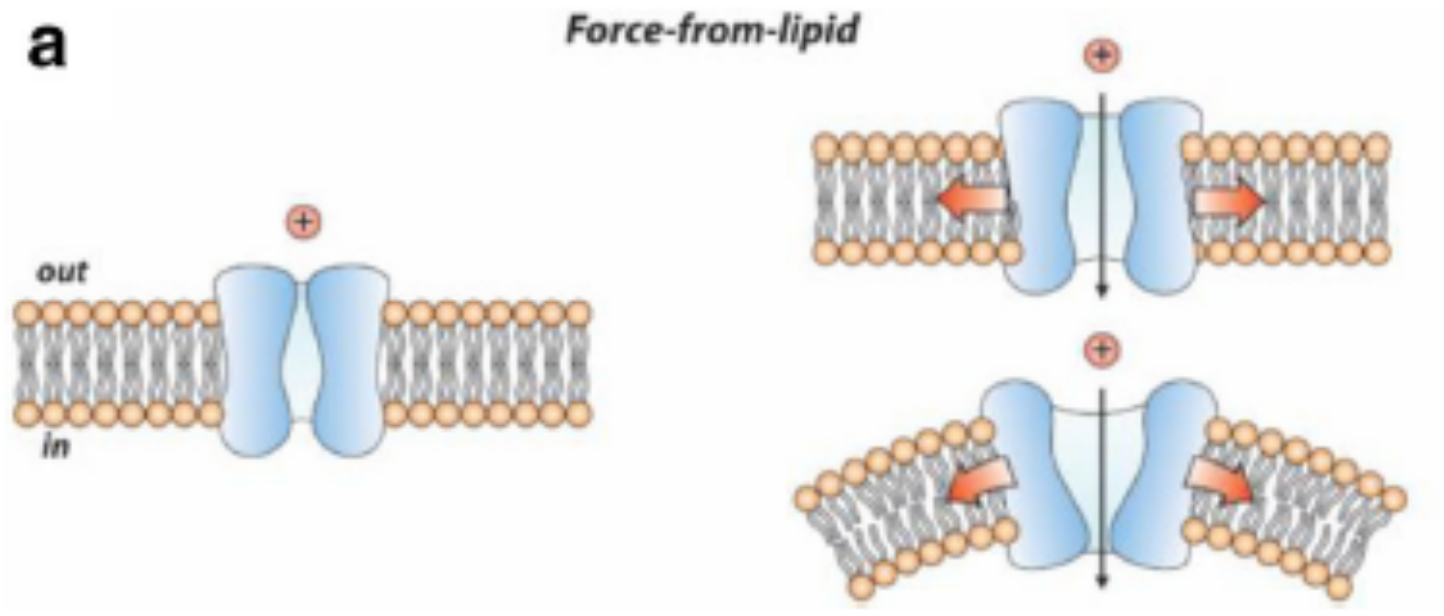
A “propeller blade”-shaped ion channel



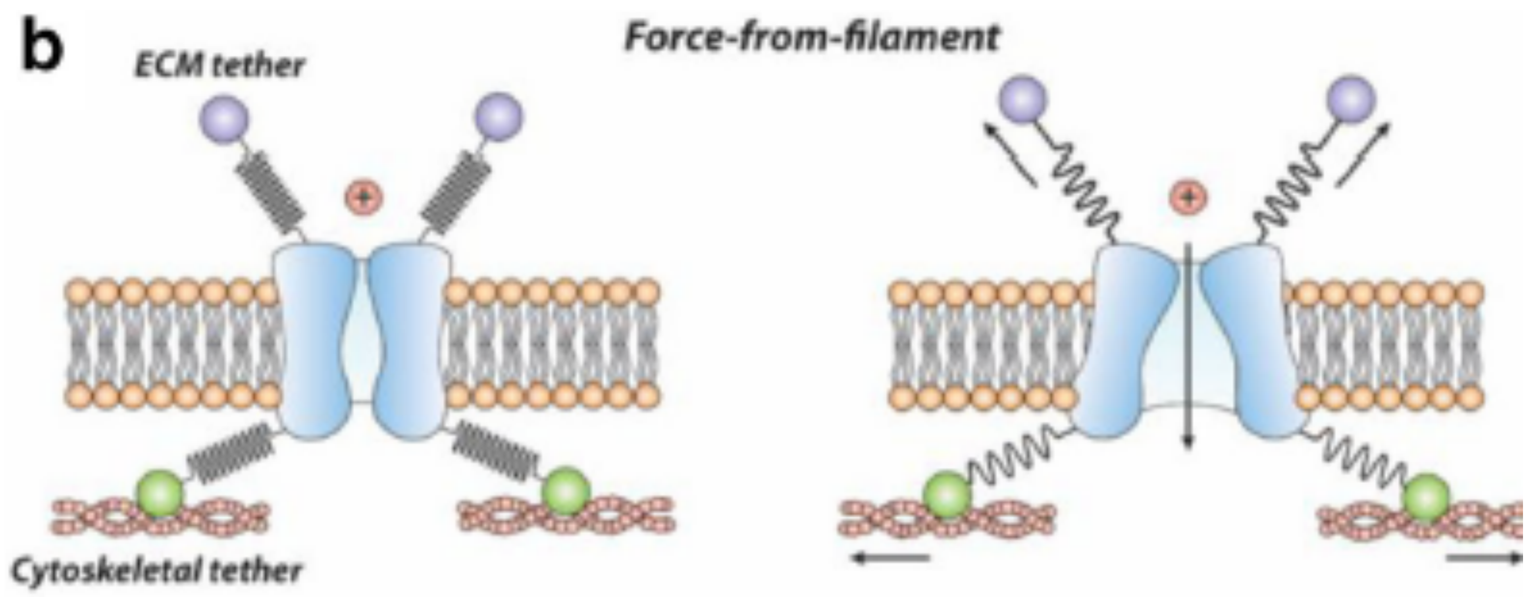
Saotome, Nature 2018

Mechanisms of force transmission to ion channels

two proposed mechanisms



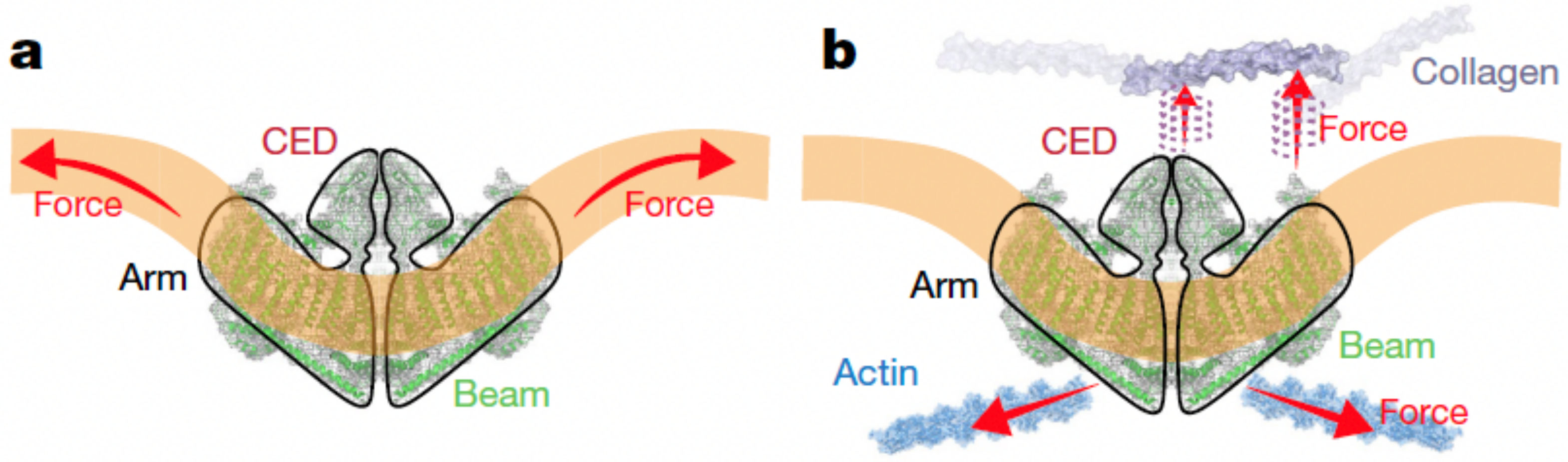
Force from lipid:
mechanical stress transmitted
exclusively from lipid bilayer



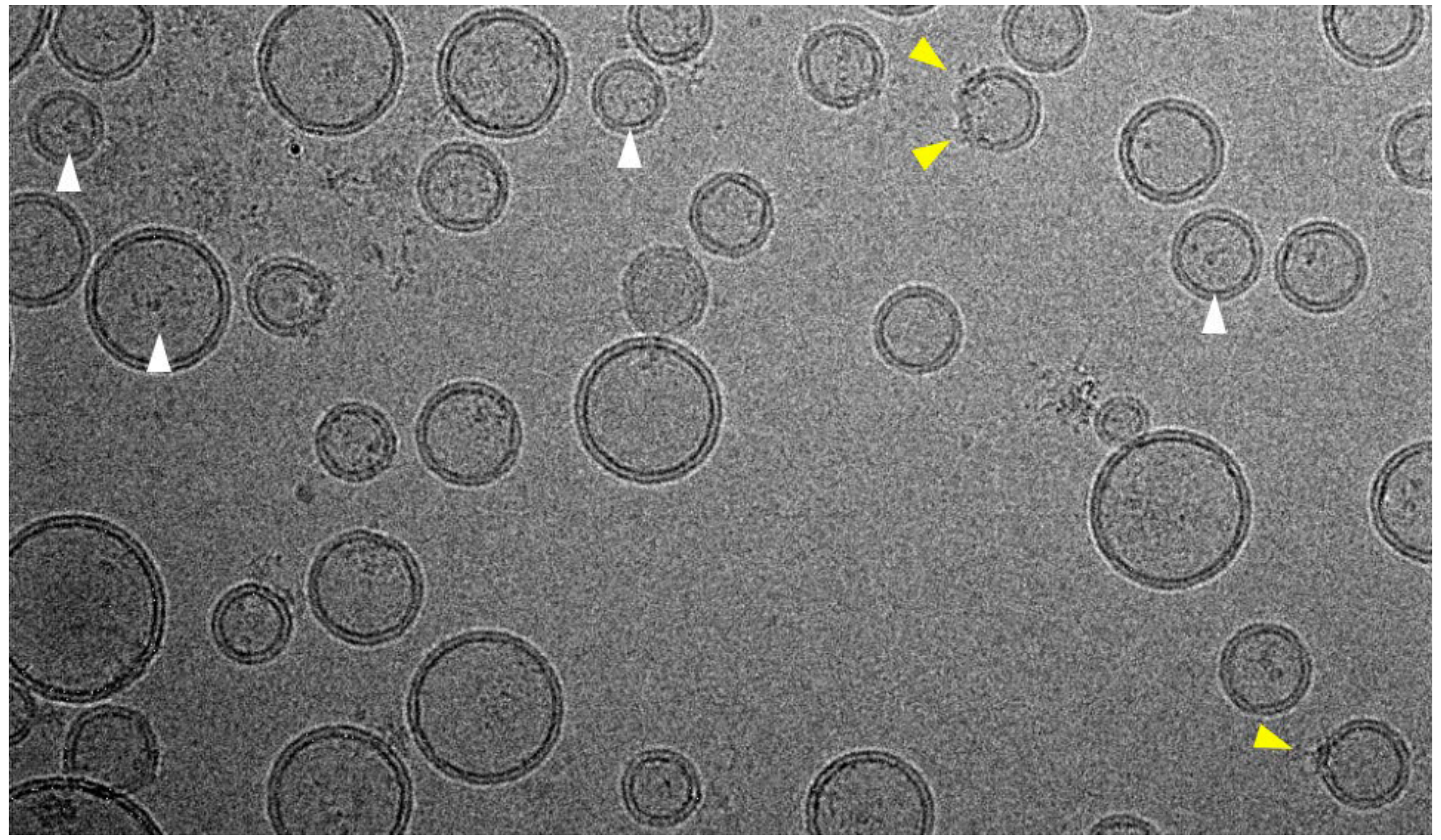
Force from filament:
mechanical stress transmitted from lipid
bilayer and external scaffold support

Force-induced conformational changes in PIEZO1

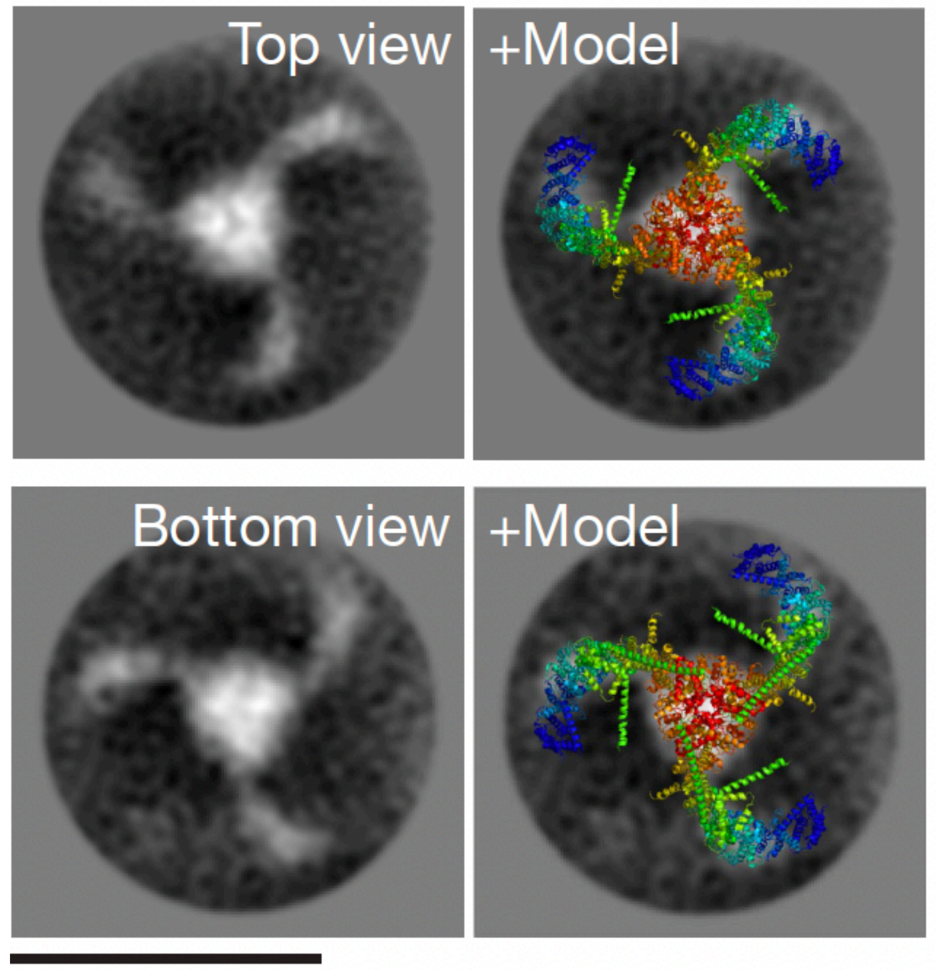
Yi-Chih Lin[1,2,4], Yusong R. Guo[3,4], Atsushi Miyagi[1,2], Jesper Levring[3], Roderick MacKinnon[3]* & Simon Scheuring[1,2]*



Vesicles containing Piezo1 (cryoEM)

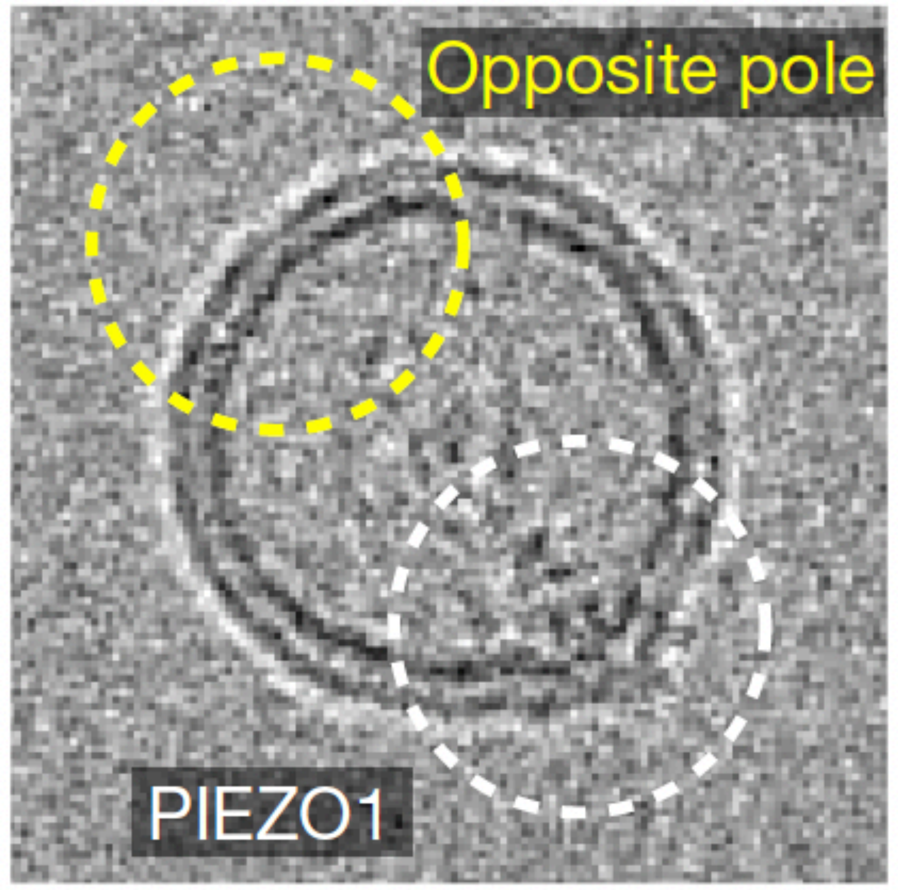


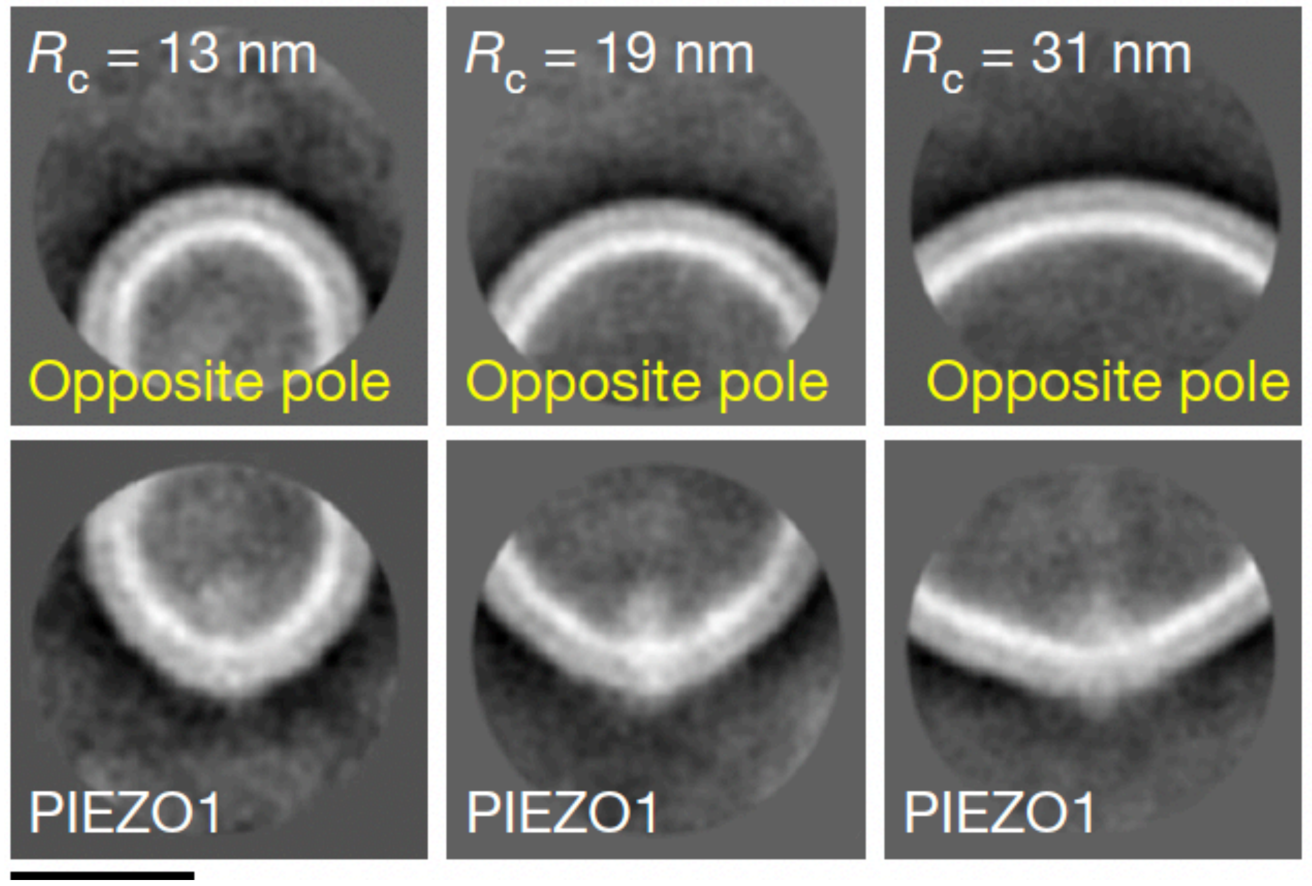
Align and average hundreds of Piezo images

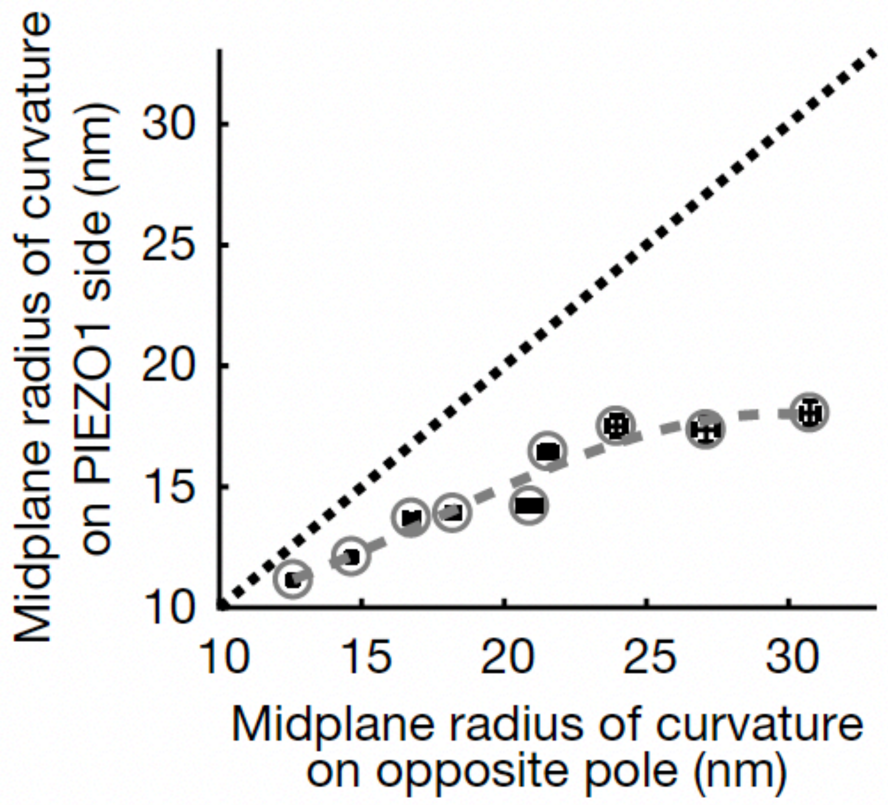


Piezo1 changes conformation under tension

Geometrical changes in vesicle shape



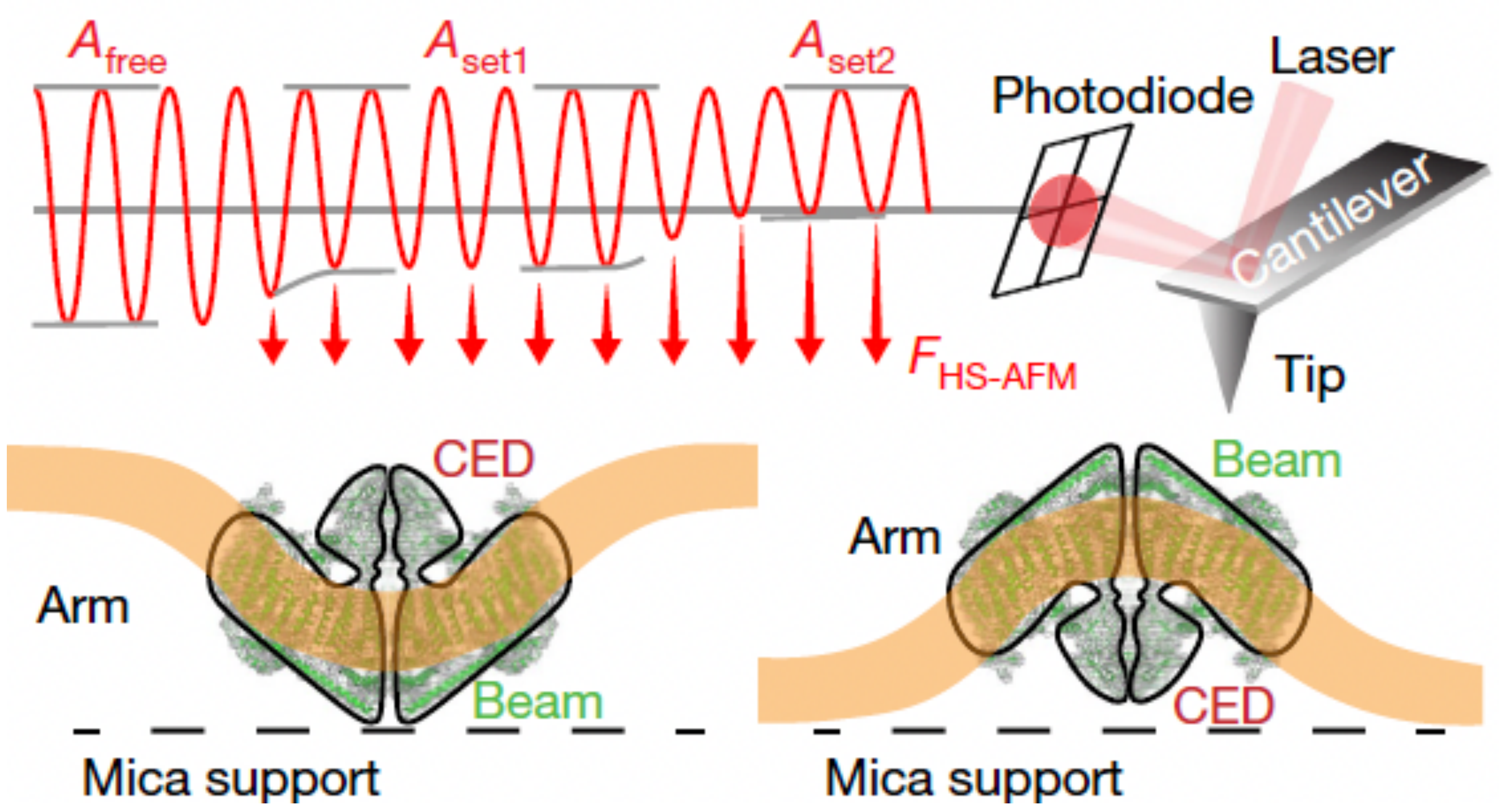


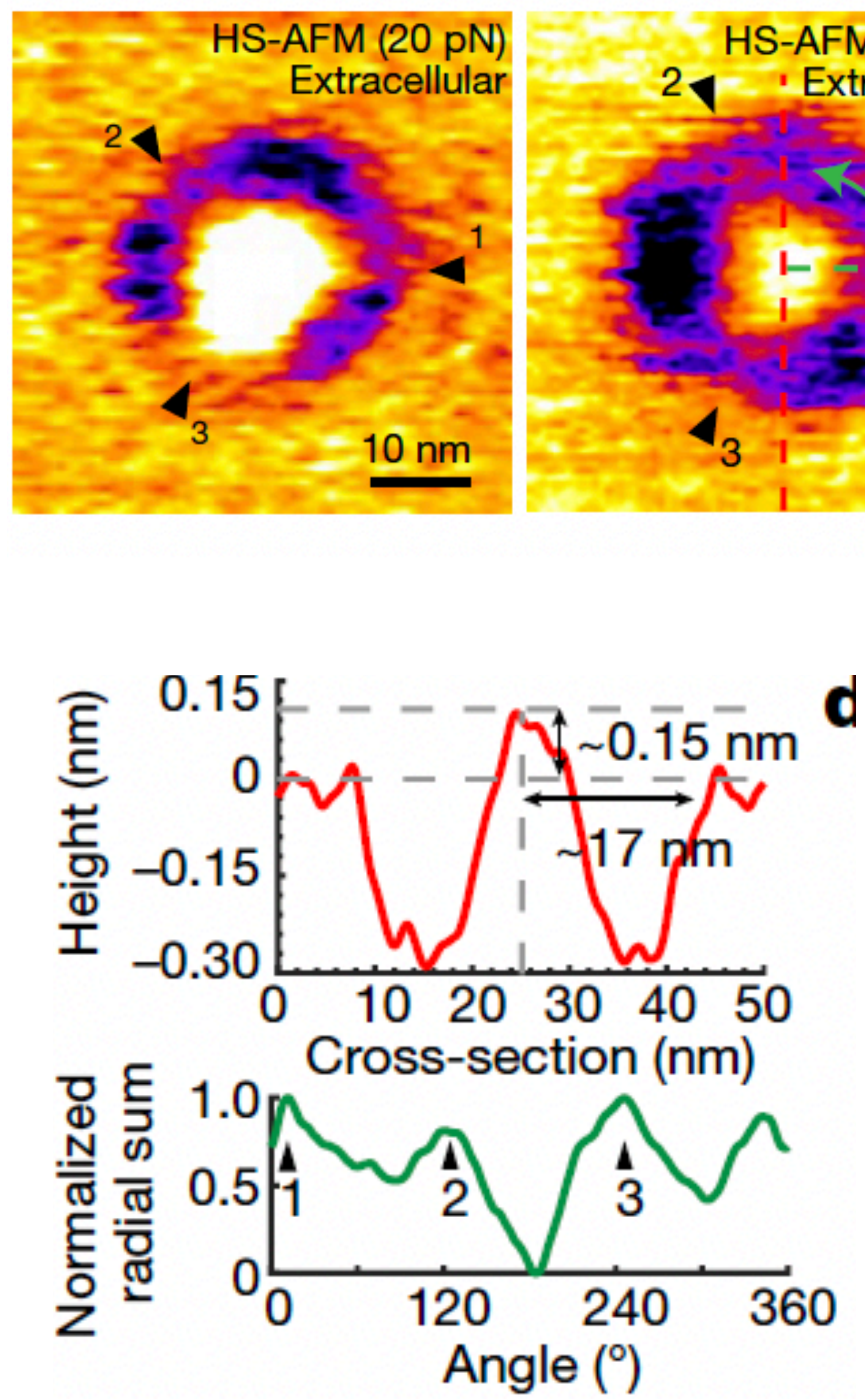


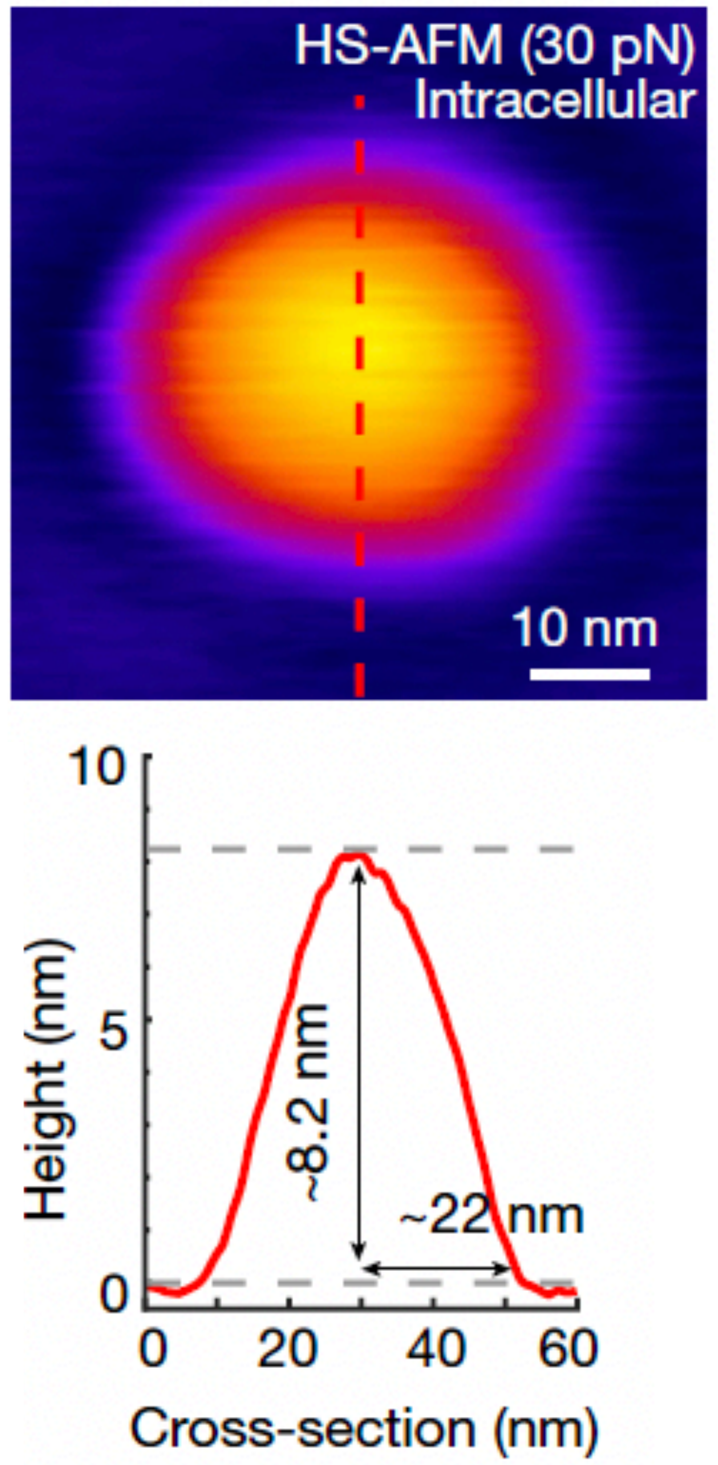
Piezo adopts different curvatures as a function of vesicle size

Piezo force sensitivity

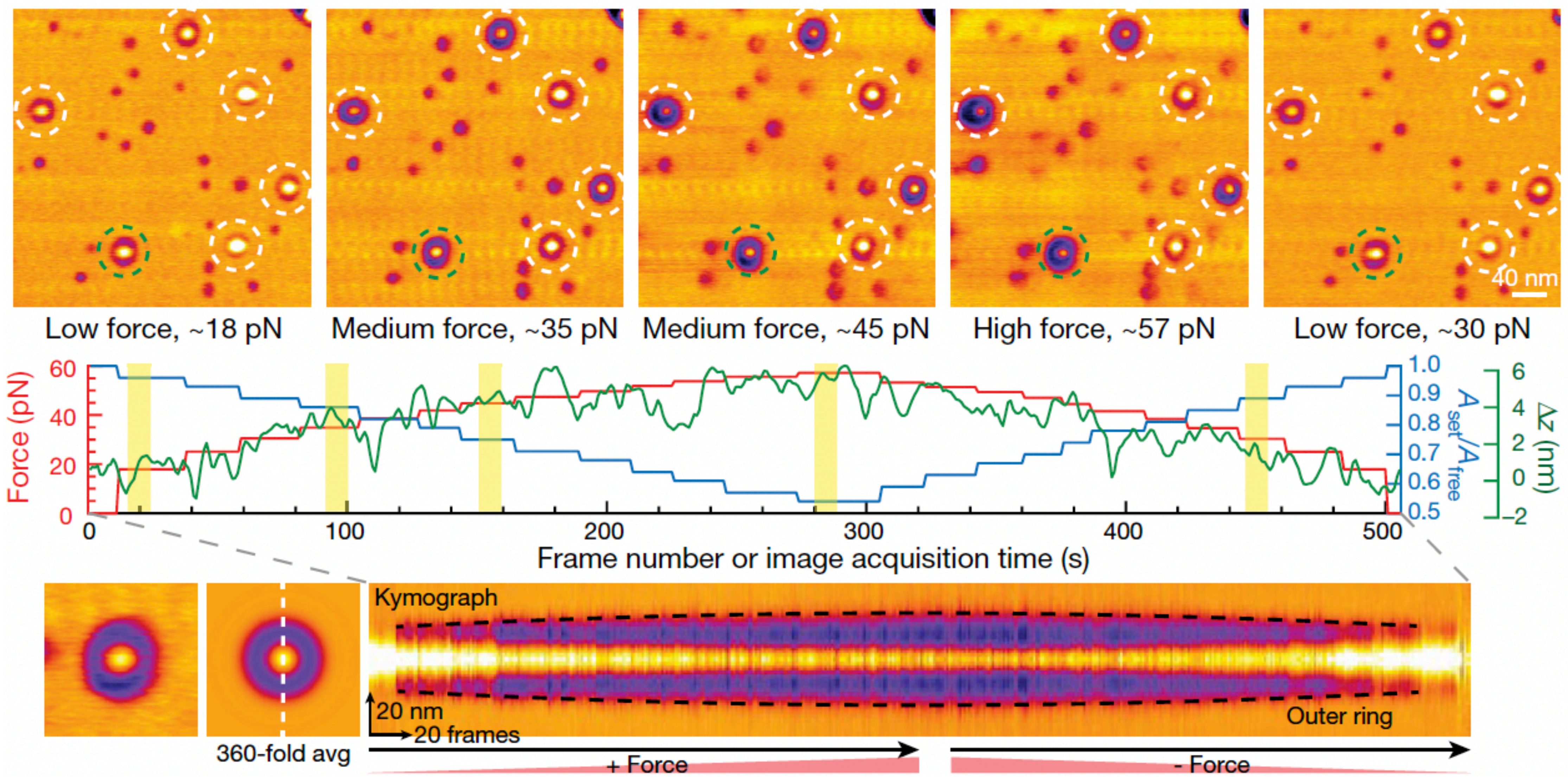
High speed AFM enables sumultaneous imaging and force application

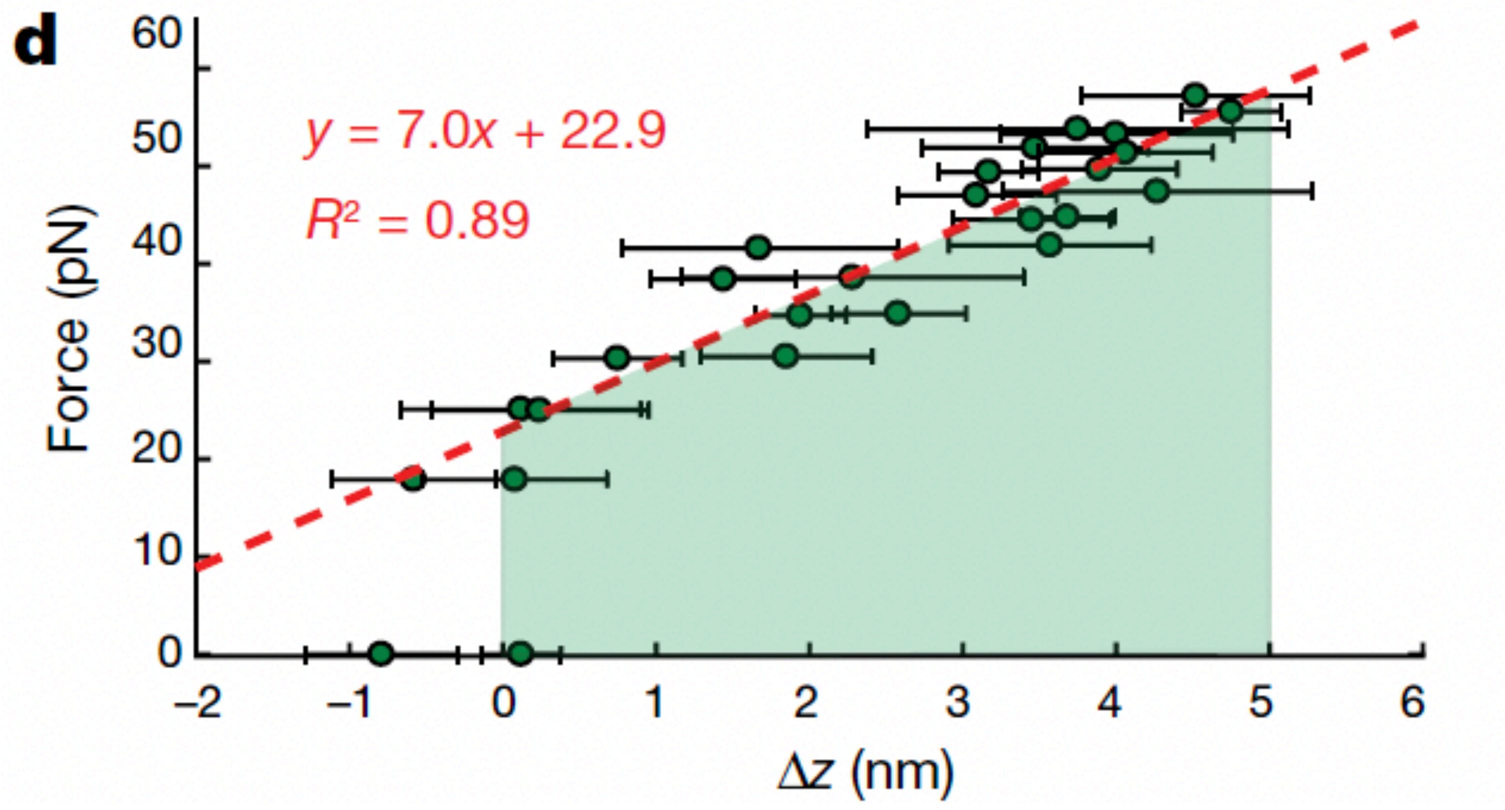


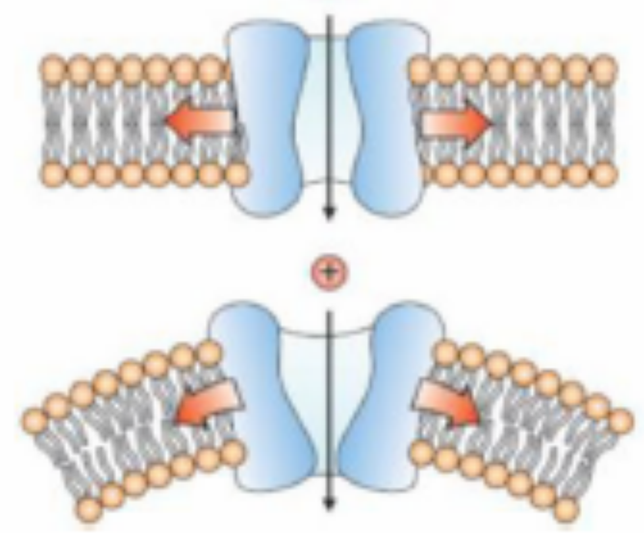




Dynamic imaging of conformational change







Conclusion: force from lipid mechanism is favored model

Functions of Piezo1/2

touch and proprioception

Piezo2 deficient patients
(from mutations)

Functions of Piezo1/2

touch and proprioception

Piezo2 deficient patients
(from mutations)

Piezo in somatosensation (introception)

- respiration
- baroreception
- bladder
- stomach

Mechanosensitive tissue	Disease
sensory neurons	baroreflex failure, pain
inner ear	deafness
blood vessels	hypertension
red blood cells	sickle cell anemia, malaria sensitivity
lung	asthma
eye	glaucoma

How do we feel the urge to urinate?

PIEZO2 in sensory neurons and urothelial cells coordinates urination

Urination patterns of Piezo2 mutations

Patient questionnaire

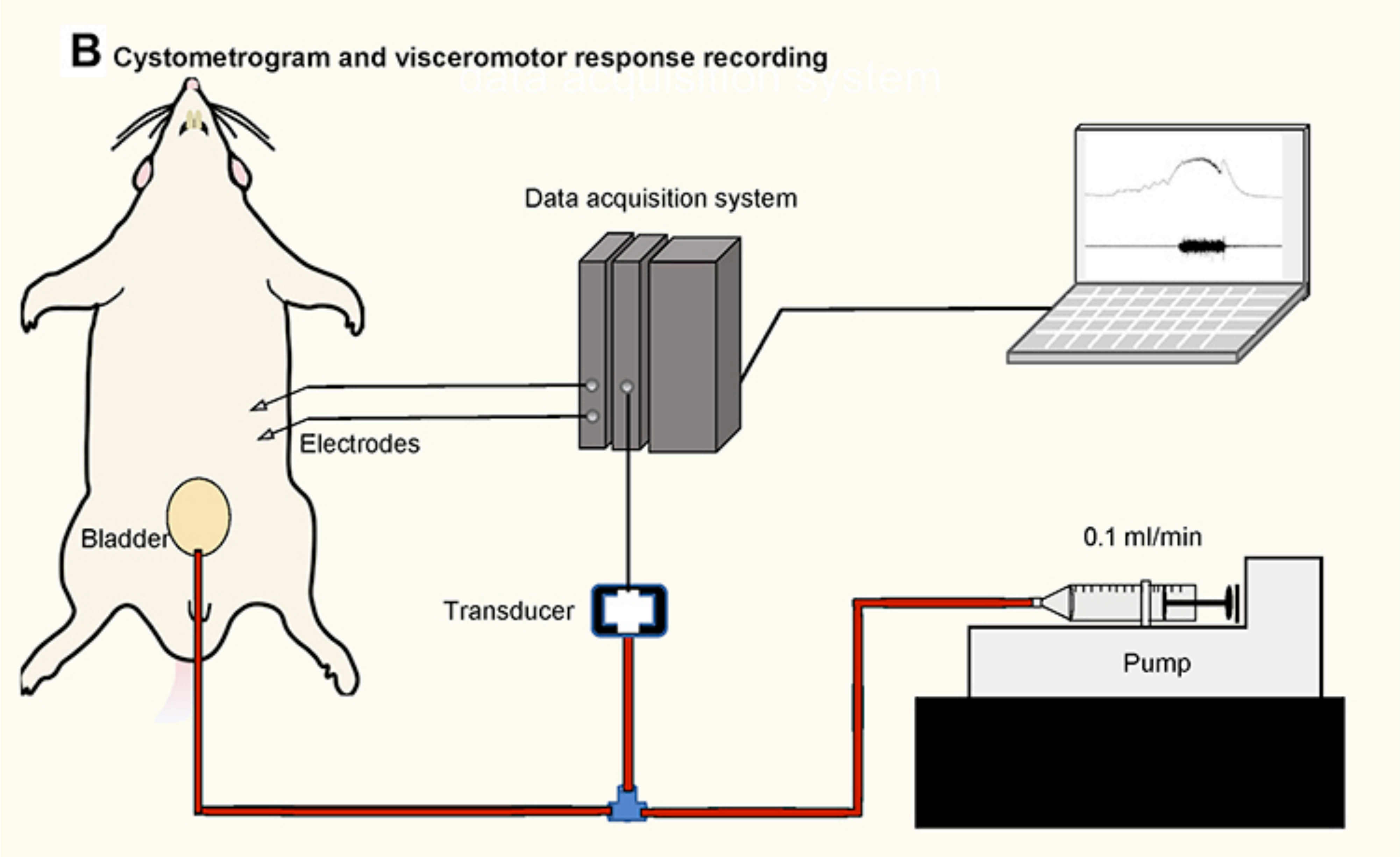
Patient	1	2	3	5	7	9	10	12
Age	23	13	16	18	10	9	5	36
Sex	F	F	M	M	F	F	F	F
In a normal day I go to the washroom to pee (no. of times)	*	3–4	1–2	1–2	1–2	3–4	3–4	5–6†
I pee in my underwear during the day (days per week)				1			1	
When I pee in my underwear, it is				Damp			Damp	
I feel that I have to rush to the washroom to pee								
I hold my pee by crossing my legs or sitting down								
It hurts when I pee								
I wet my bed at night								
I wake up to pee at night (nights per month)	3–4							
When I pee, it stops and starts								
I have to push or wait for my pee to start								

Piezo2-deficient patients show urinary dysfunction

… they don't pee a lot

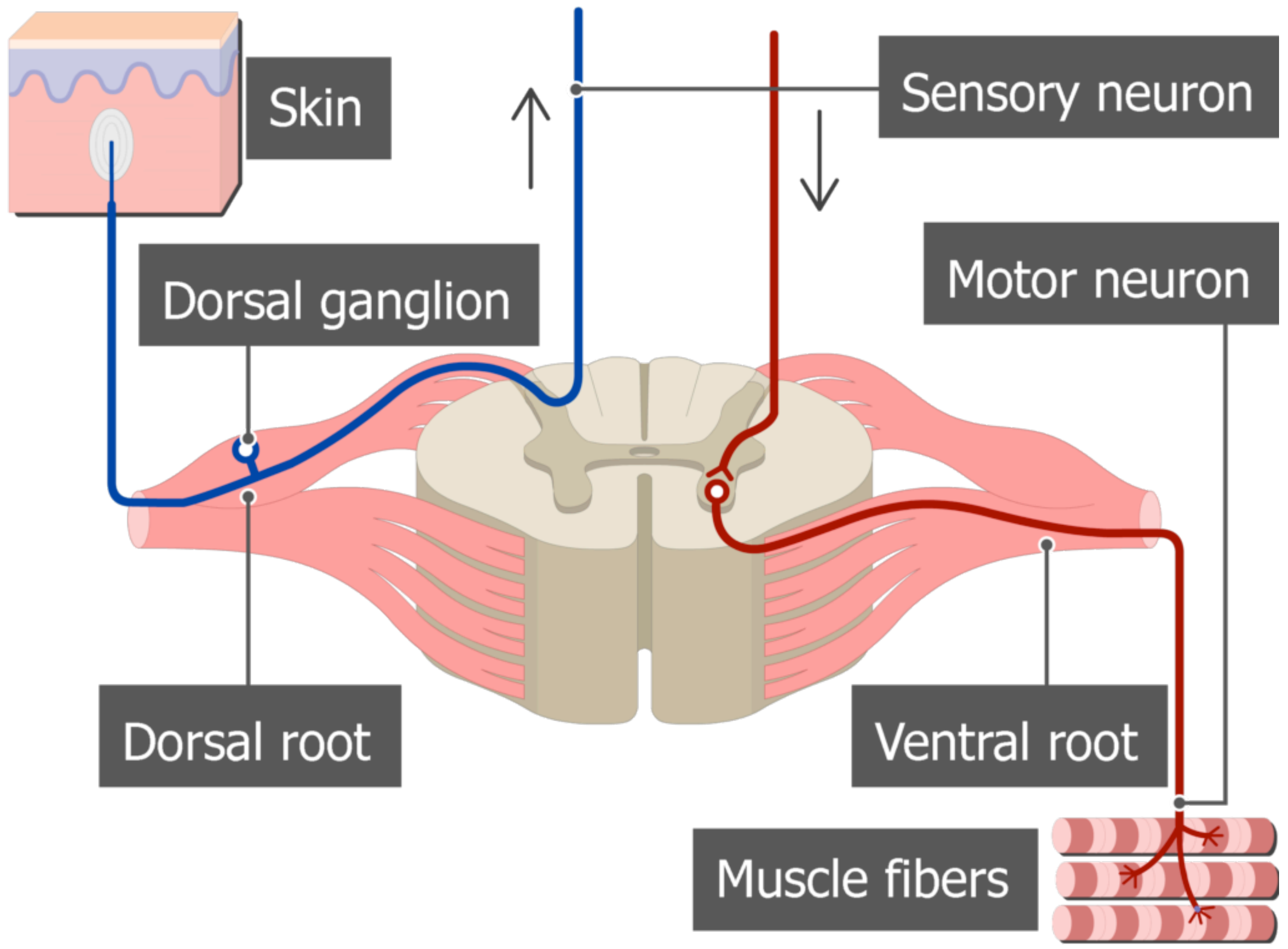
Evaluation of bladder function with cystometry

Inject fluid in the bladder (catheter)
measure flow and pressure as the bladder voids

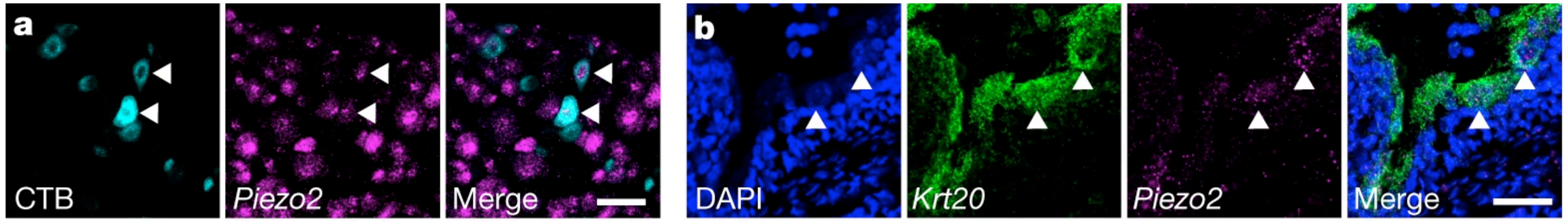


Piezo in the bladder

Focus on sensory neurons



RNA-Fish labeling

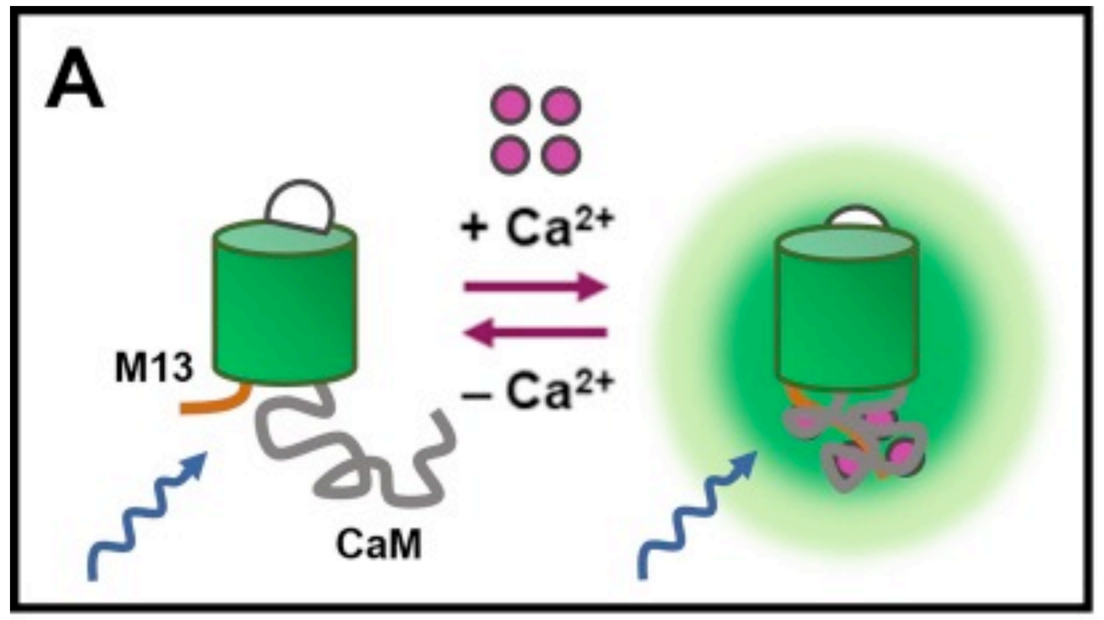


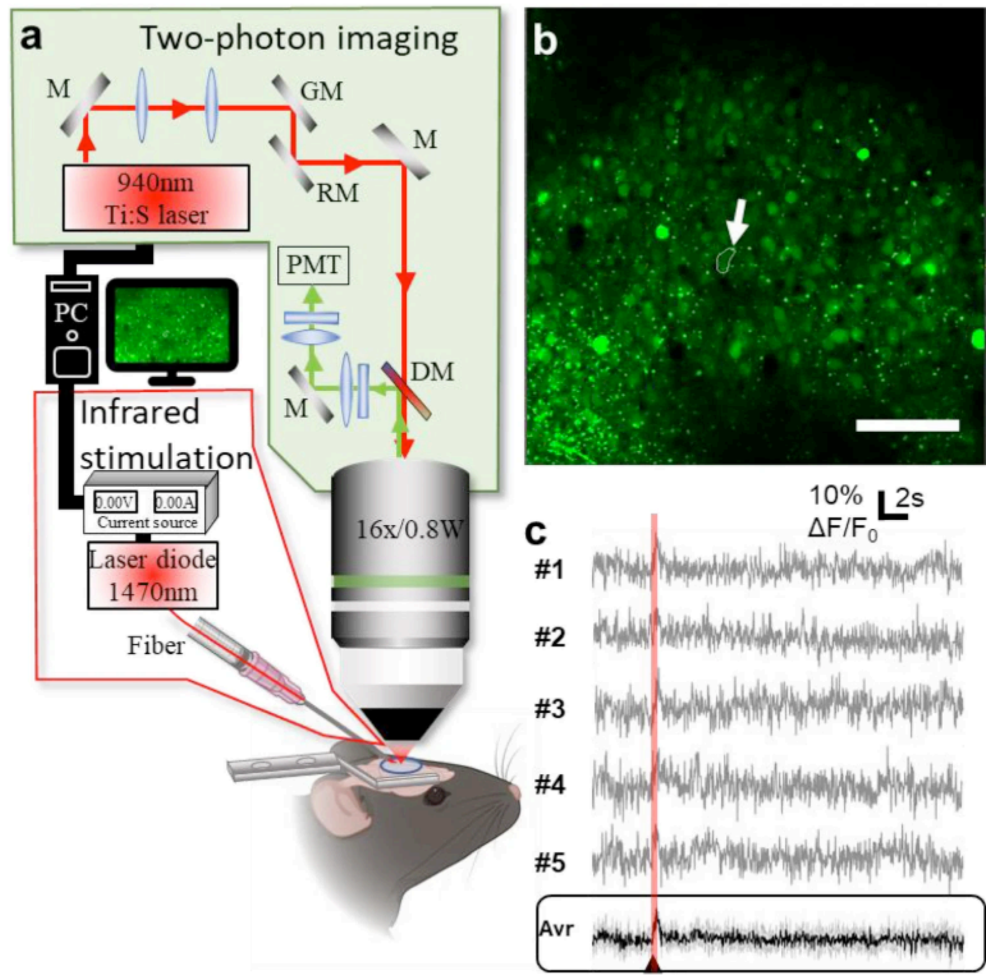
Bladder neurons express piezo2

Piezo2 is expressed in umbrella cells

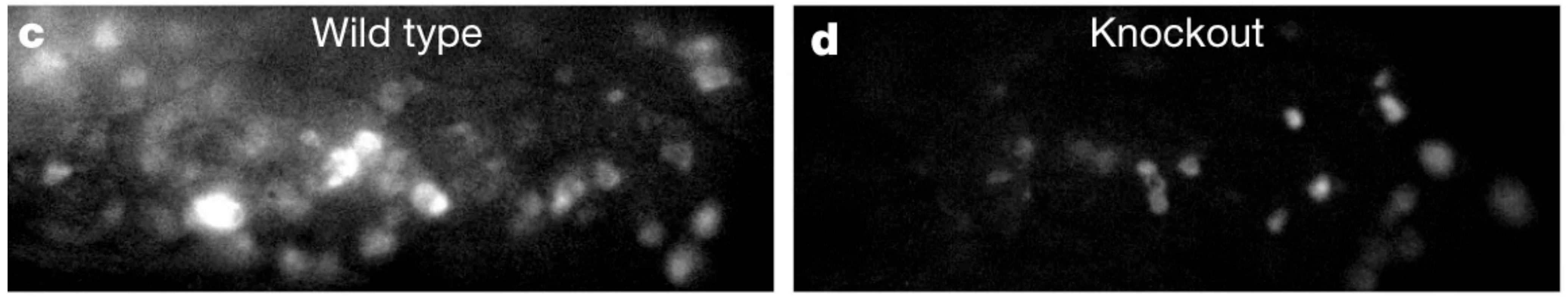
Piezo control of neuronal activity

Output measurement at the single cell level: GcAMP



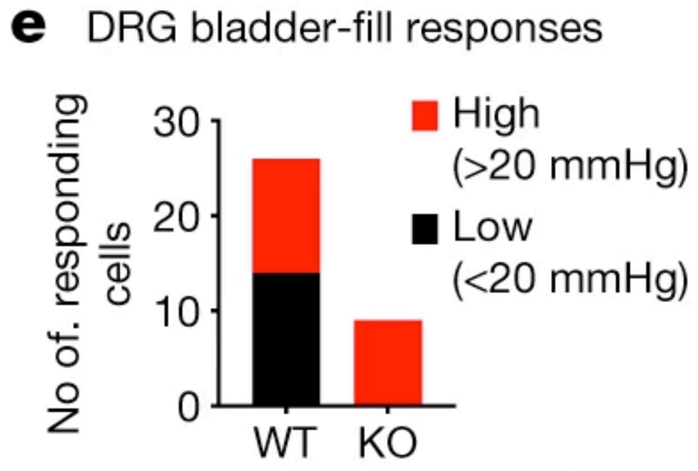


Bladder filing assay:



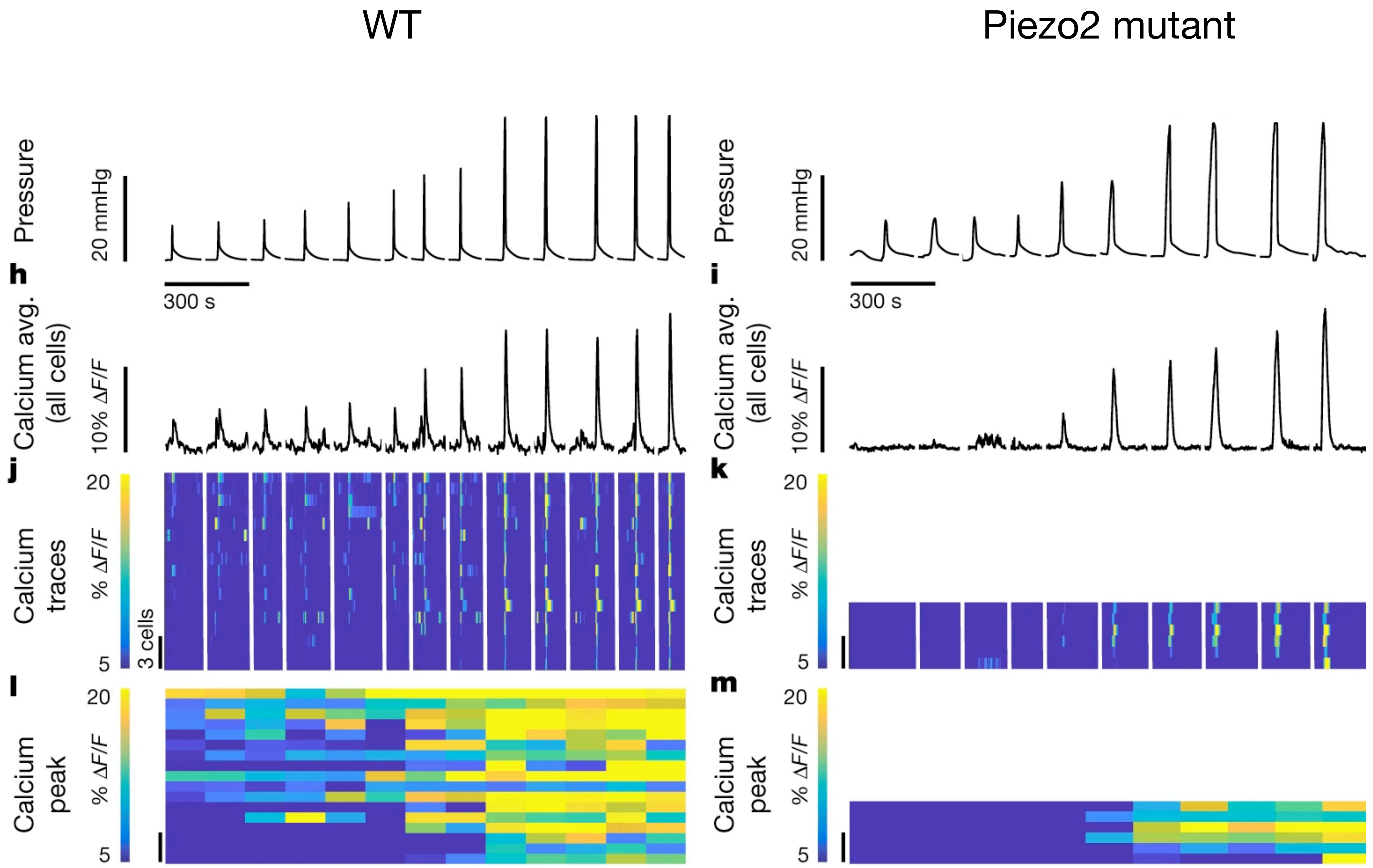
Good response

Reduced response



Count of cells responding to bladder-filling stimuli

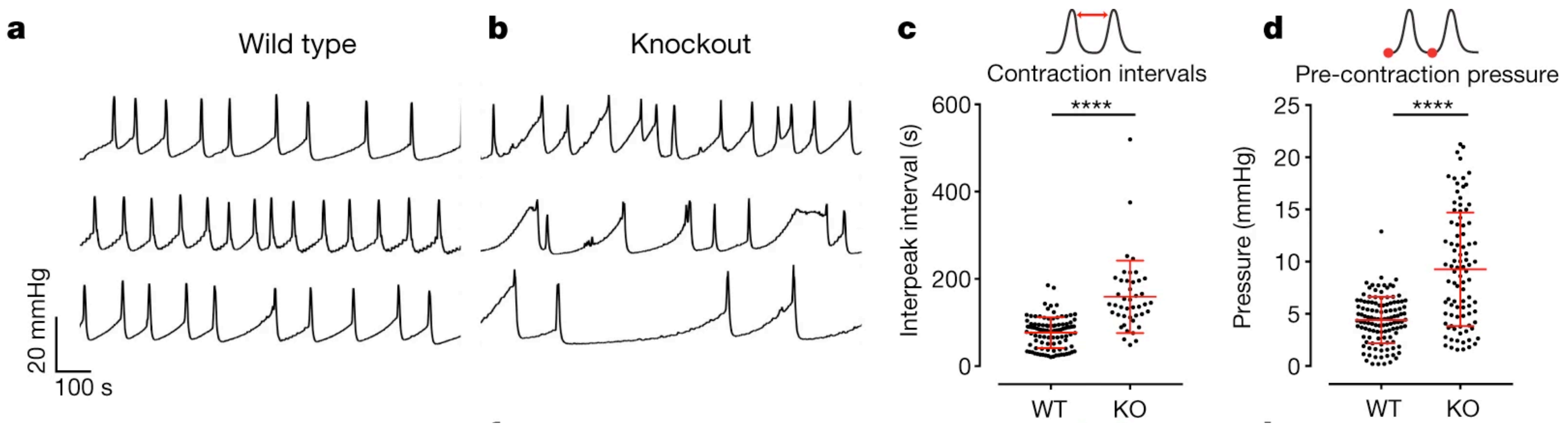
Single cells response to pressure stimulus

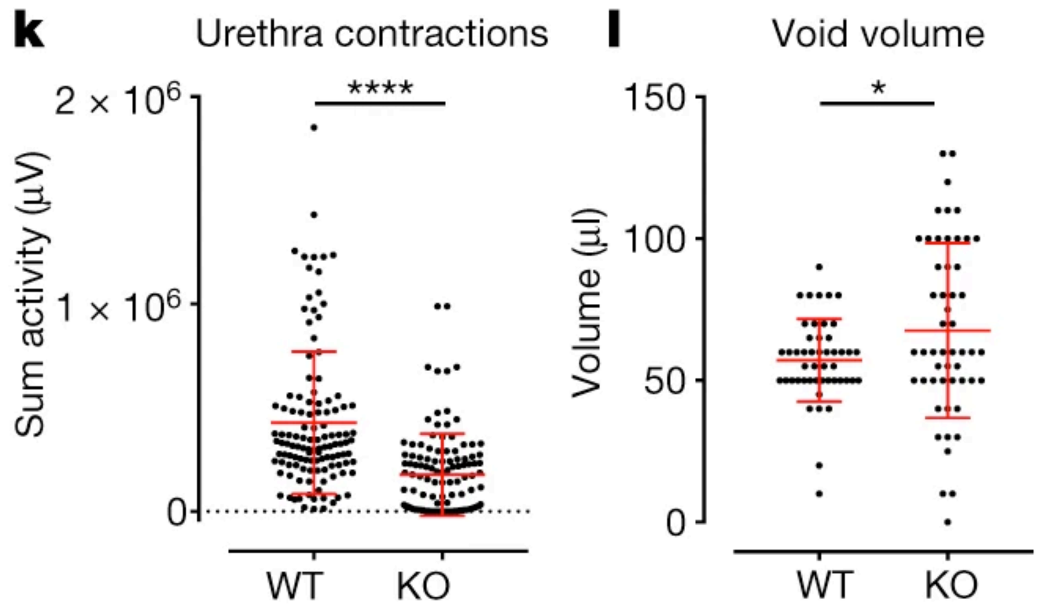


Piezo2 promotes response to bladder filling, most likely is a stretch sensor

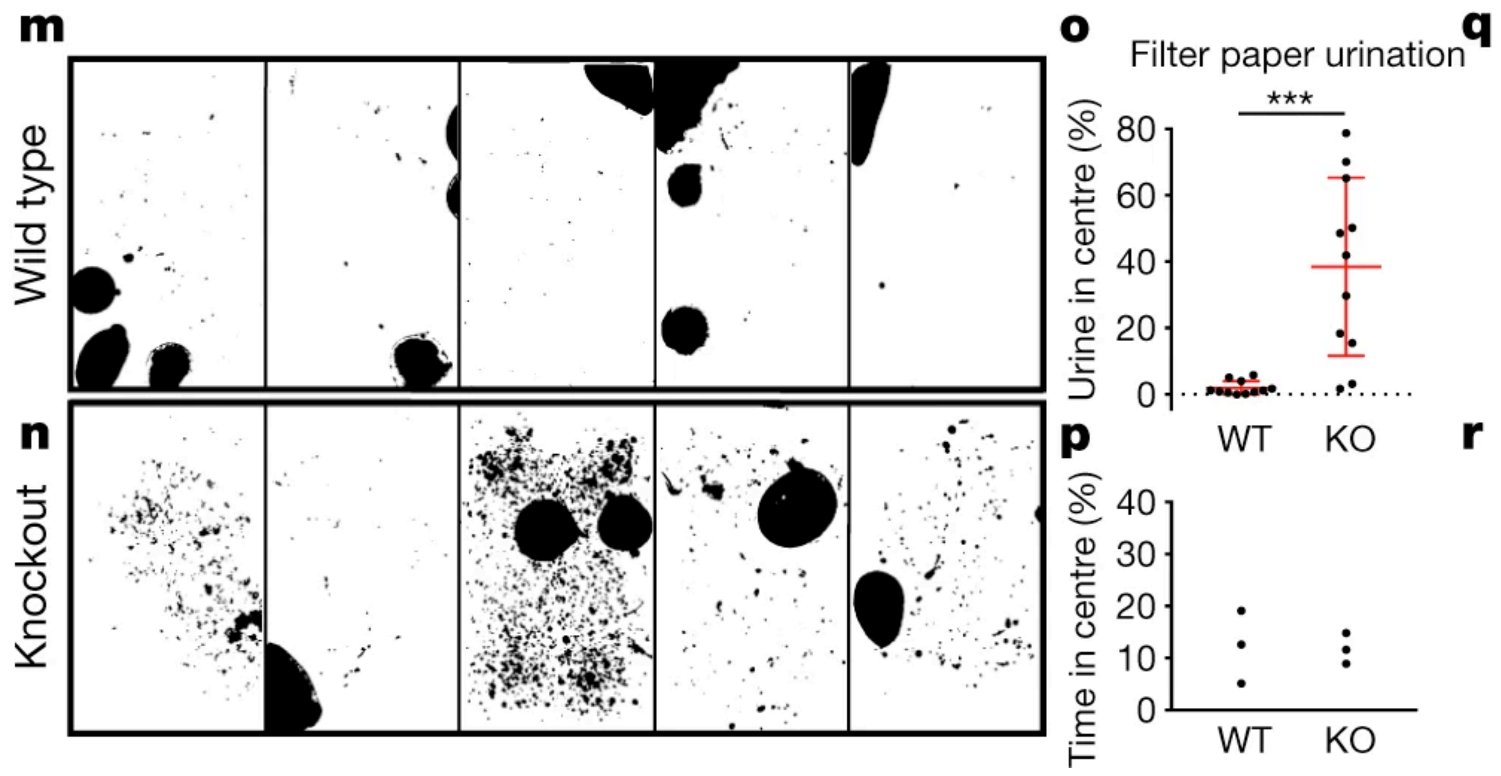
Piezo2 controls urination response

Continuously fill the bladder, measure pressure response





Piezo2: less contractions, larger volumes



Piezo2: spatial pattern of urination is irregular

Pain

acute pain

chronic pain

Temperature sensing

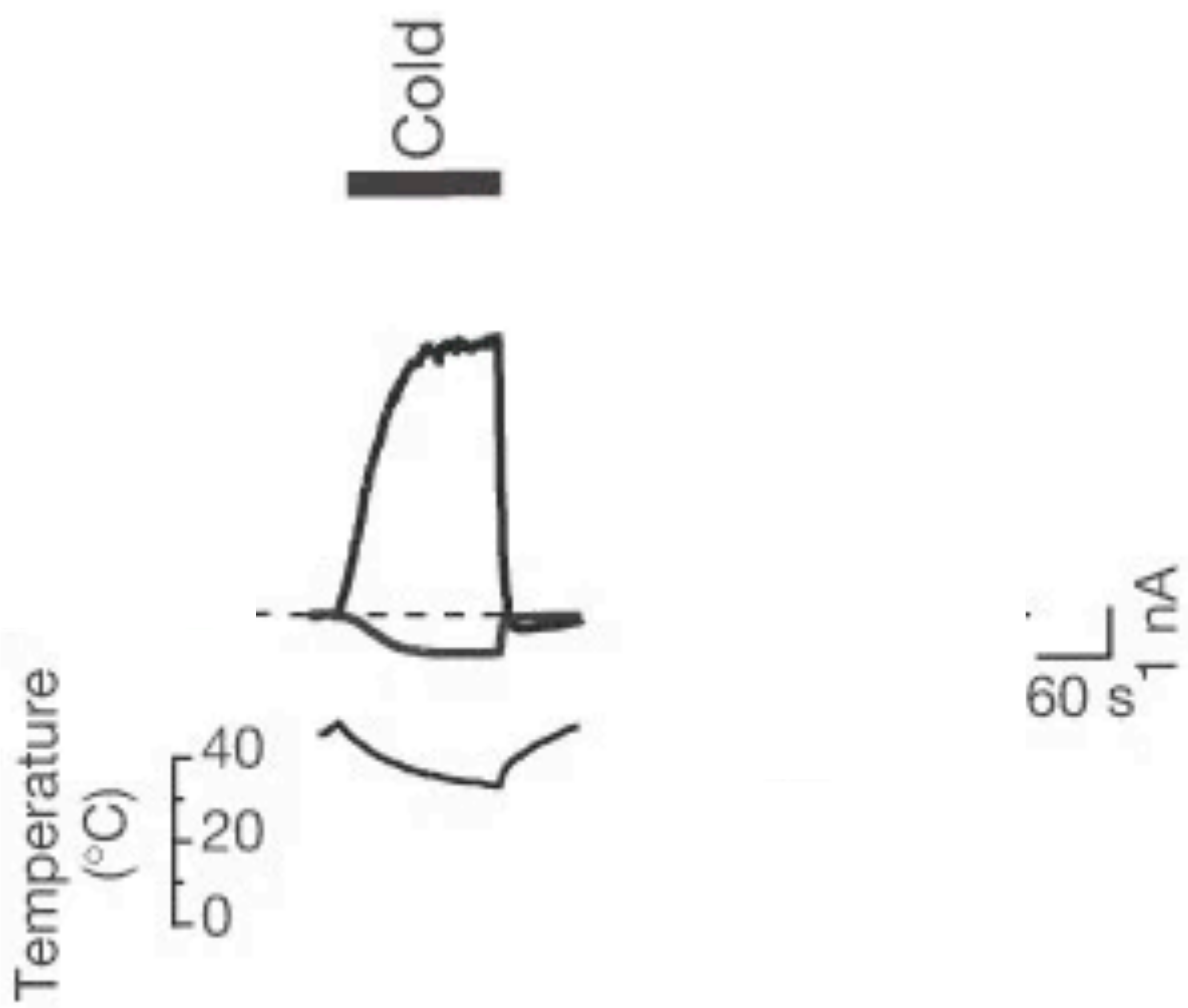
heat sensing

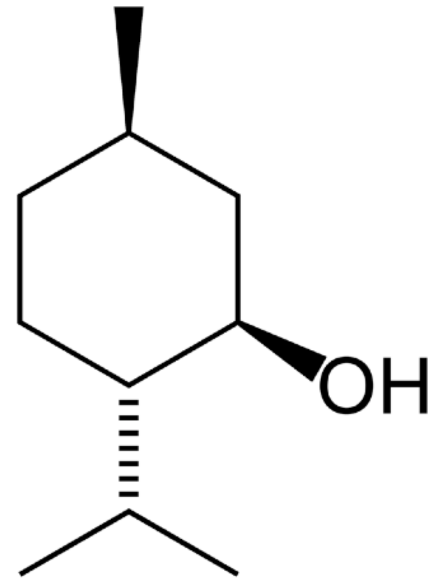
cold sensing

Cold-activated ion channel

TRPM8

Patch clamp recording



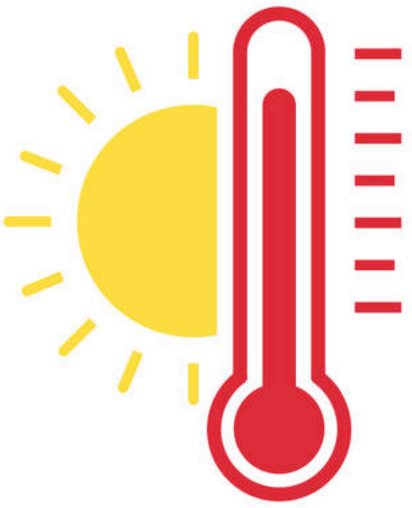


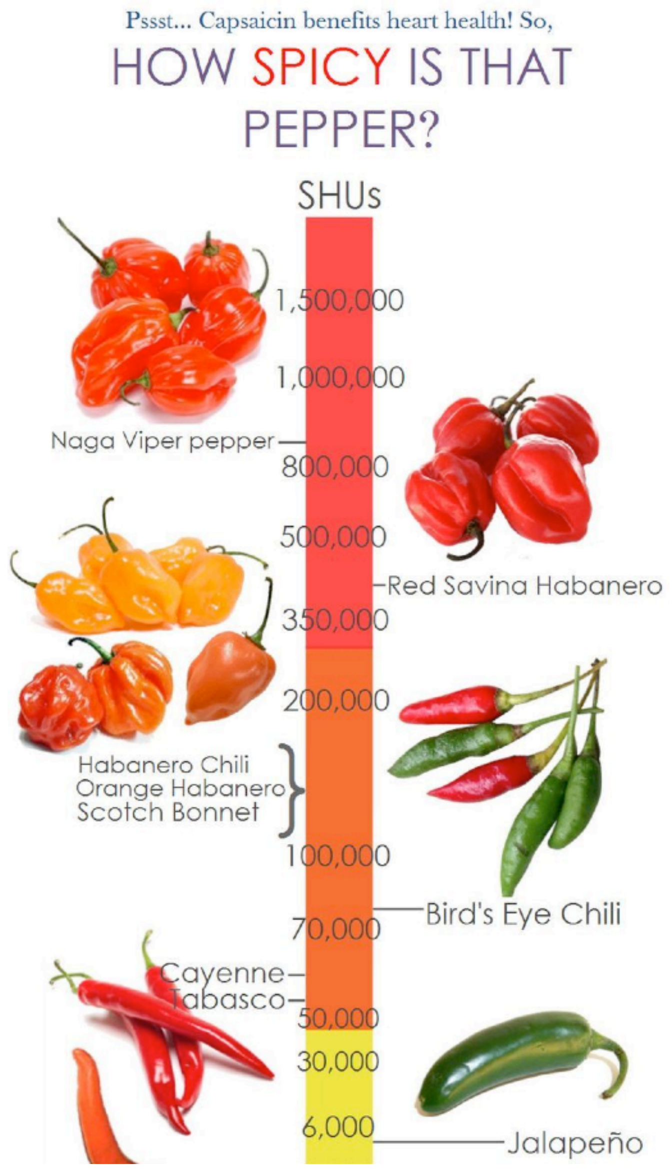
Menthol

heat and pain sensation

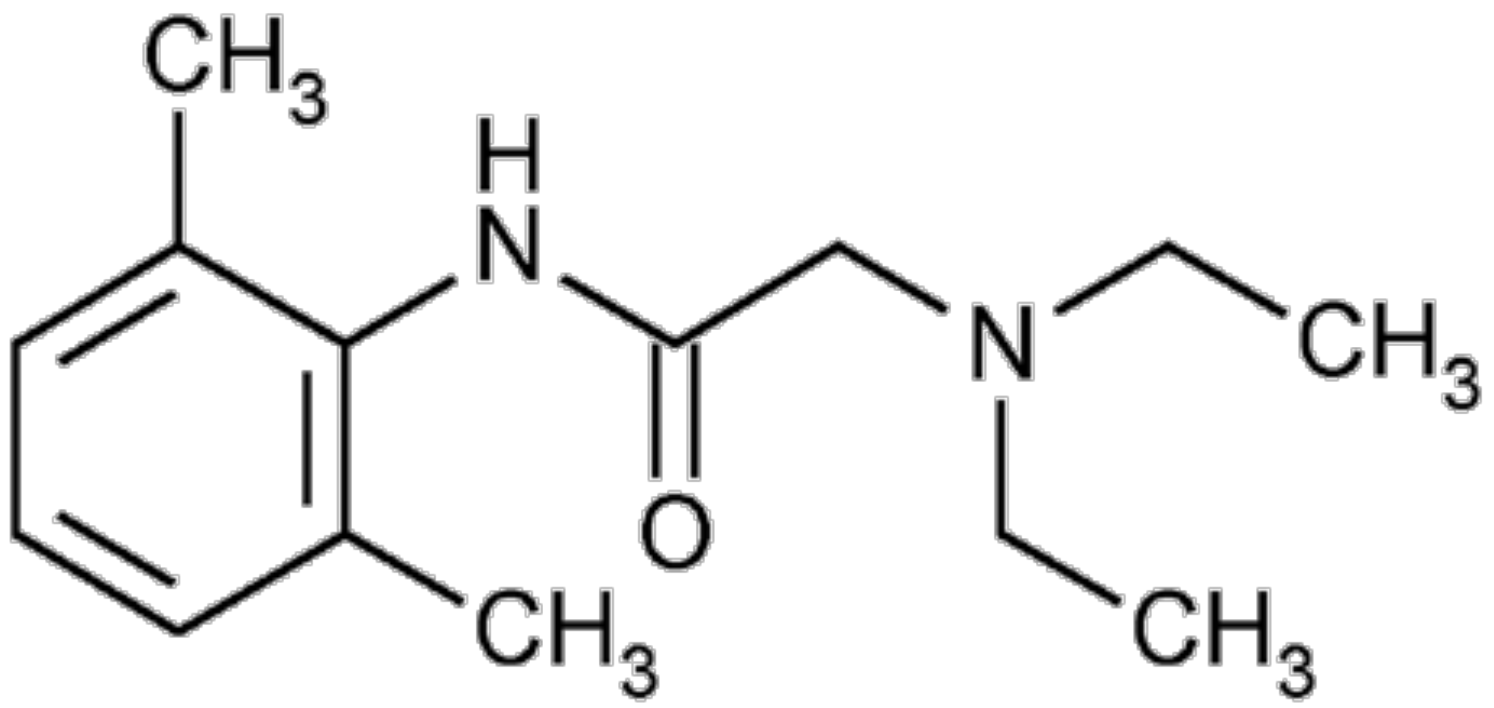
TRPA1

TRPV1

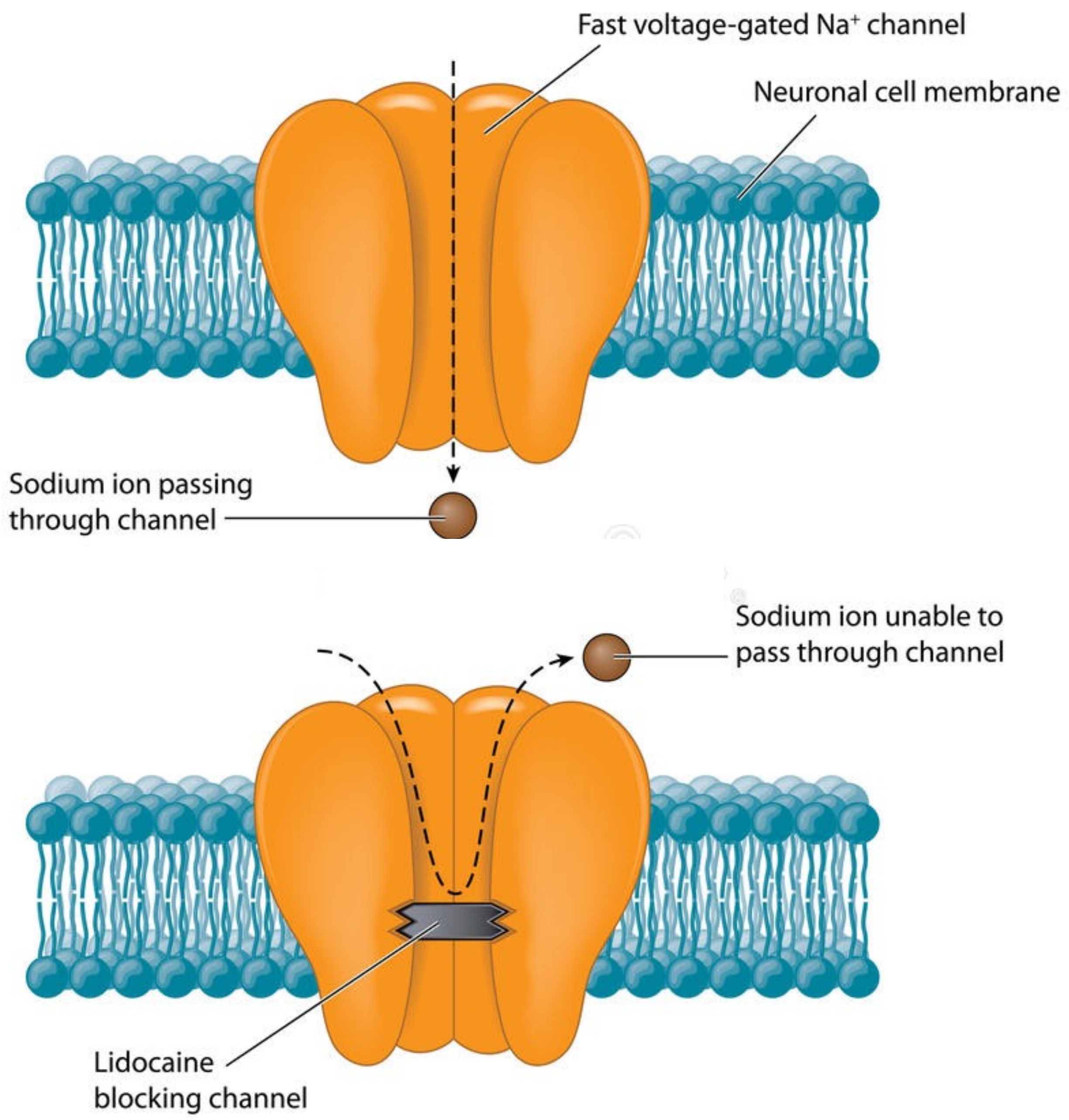




Ion channels and pain



Lidocaine



anesthetics inhibit ion channels,
repressing excitation
and transmission

Predators use ion channel inhibition to capture prey