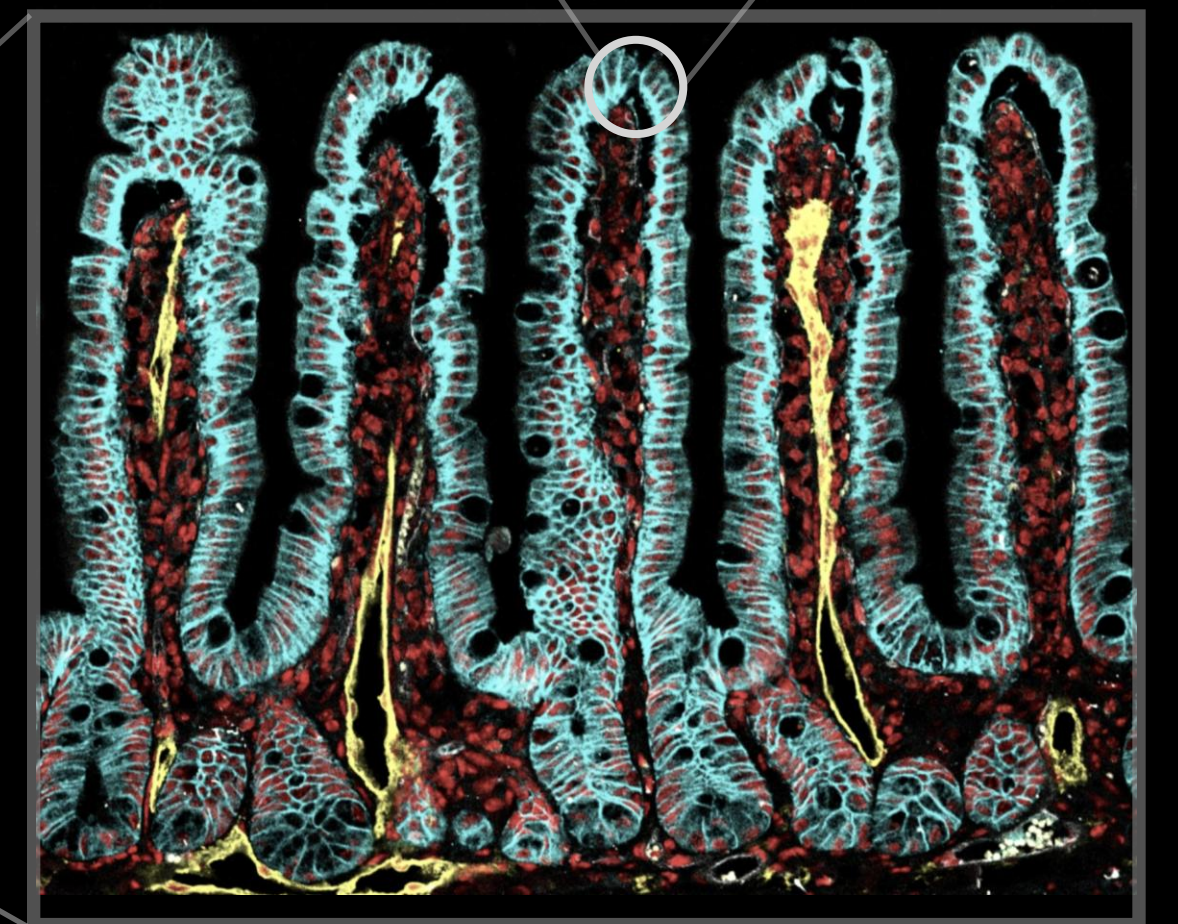
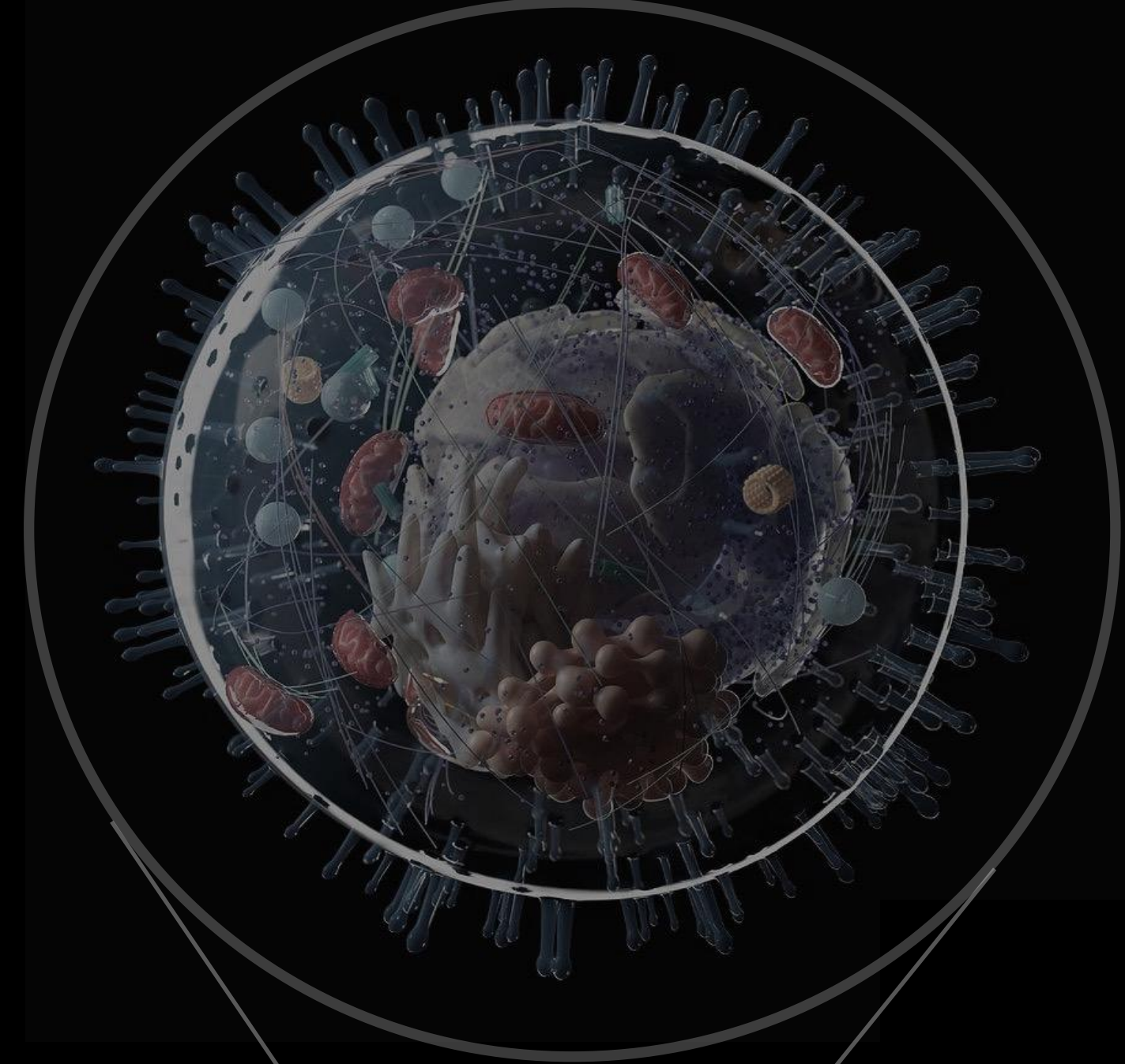
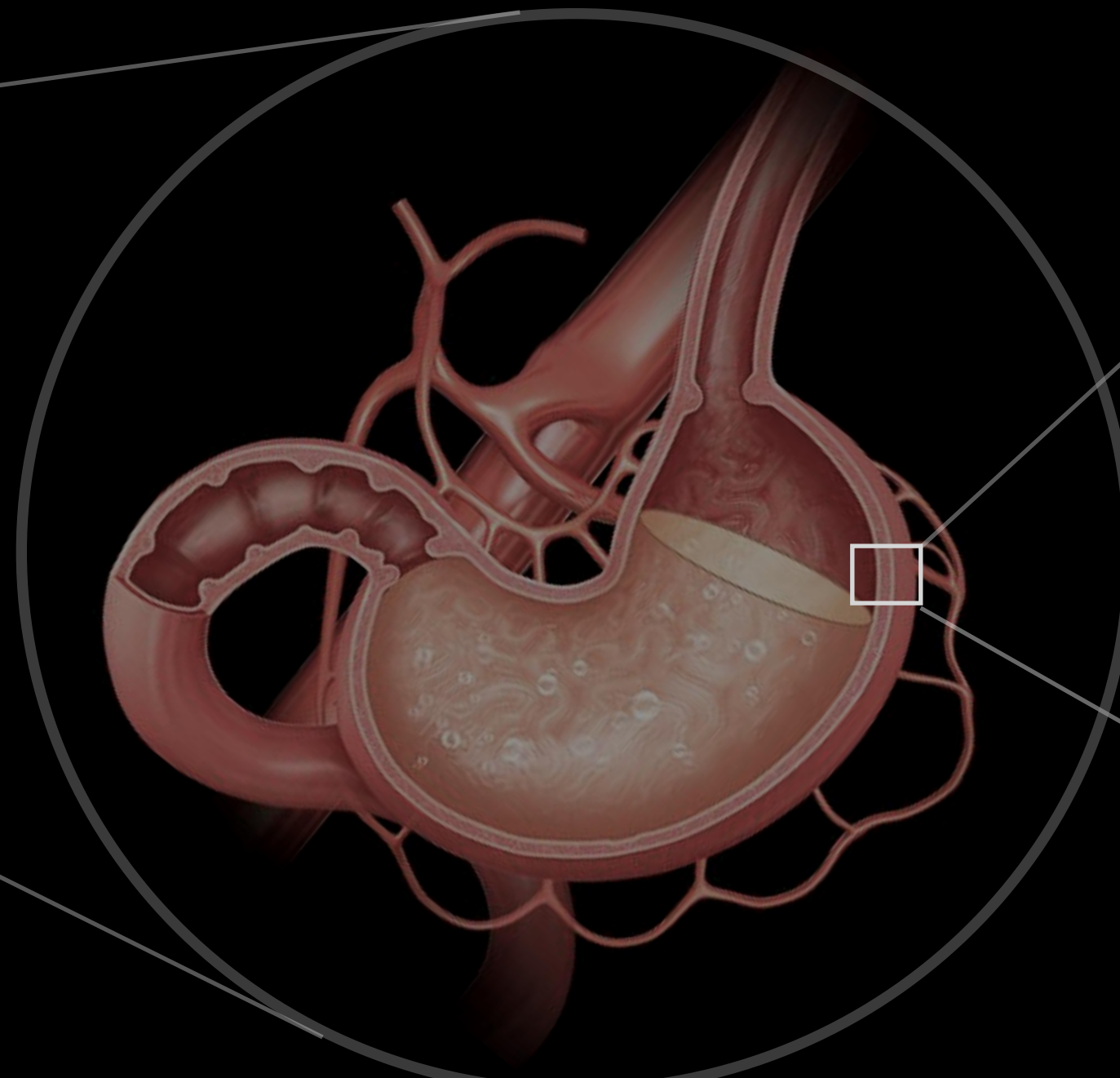
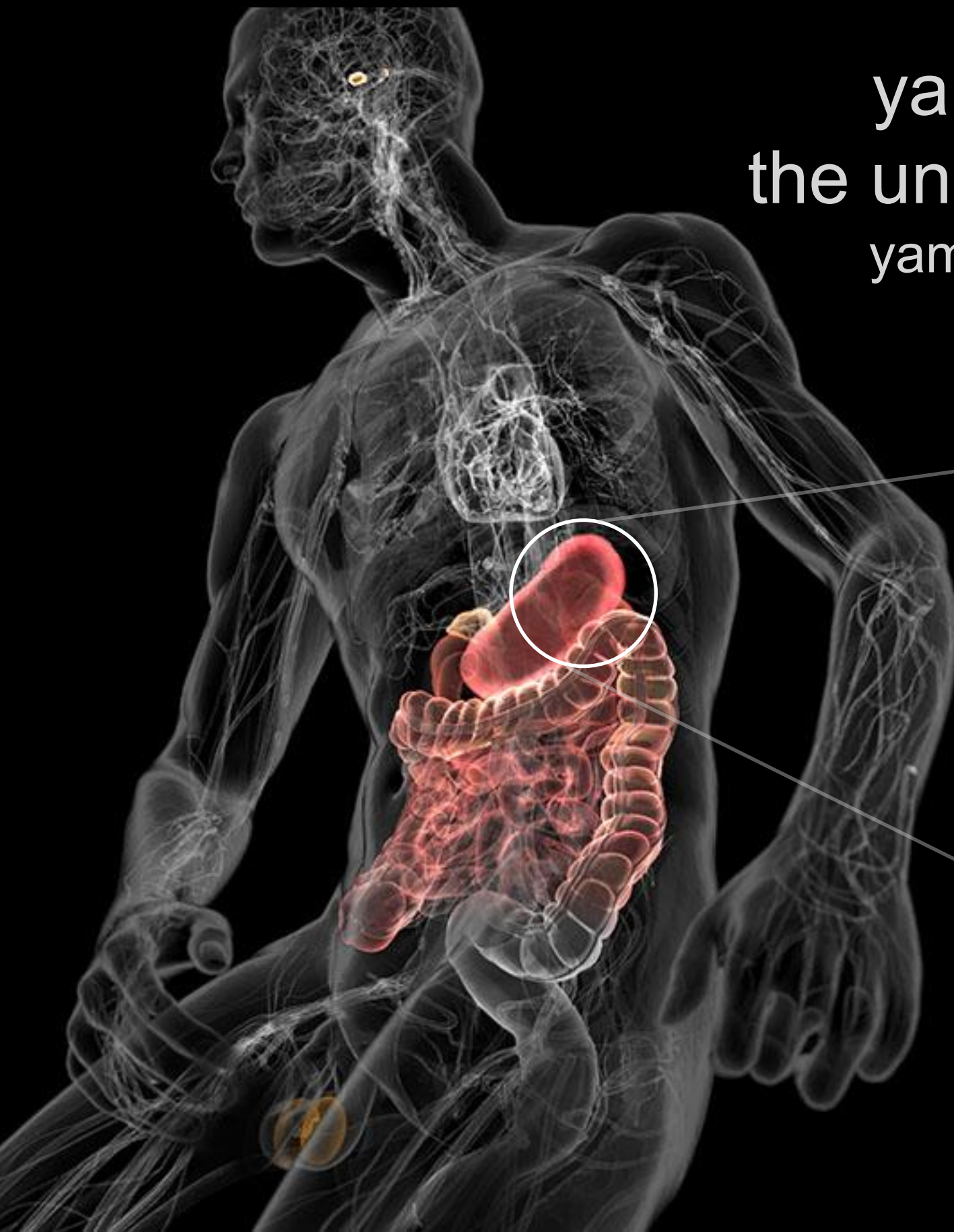
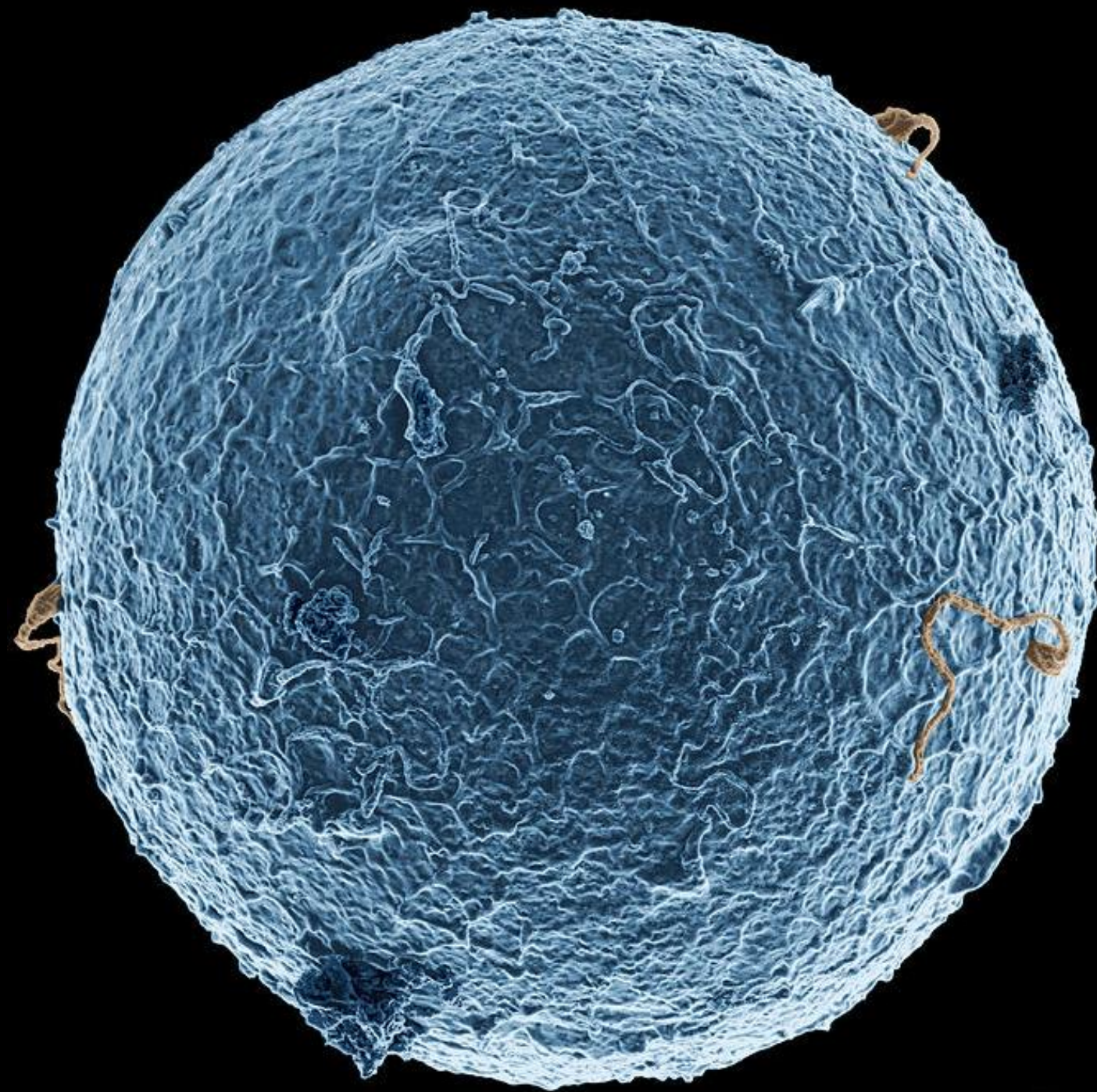


# exploring the inside of the insides of our insides

yamuna krishnan  
the university of chicago  
yamuna@uchicago.edu

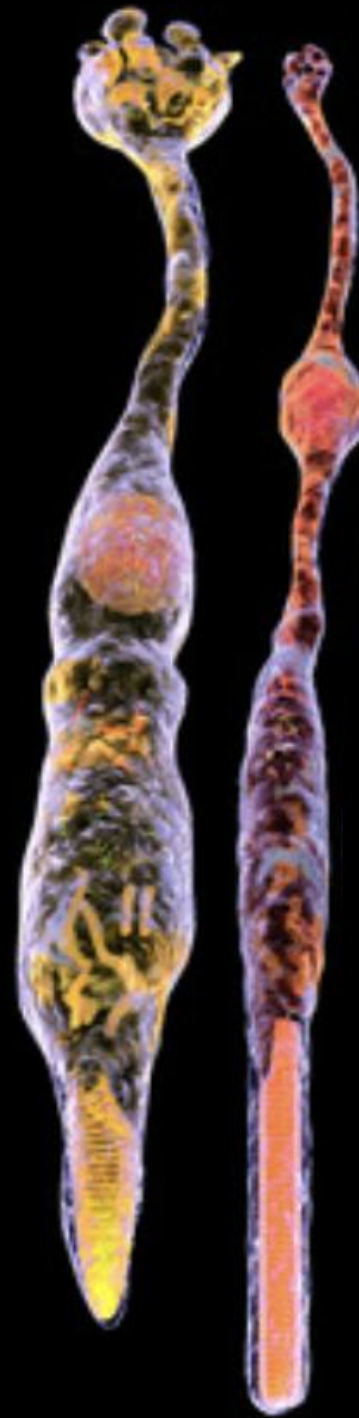


# cells: dramatic differences in size &

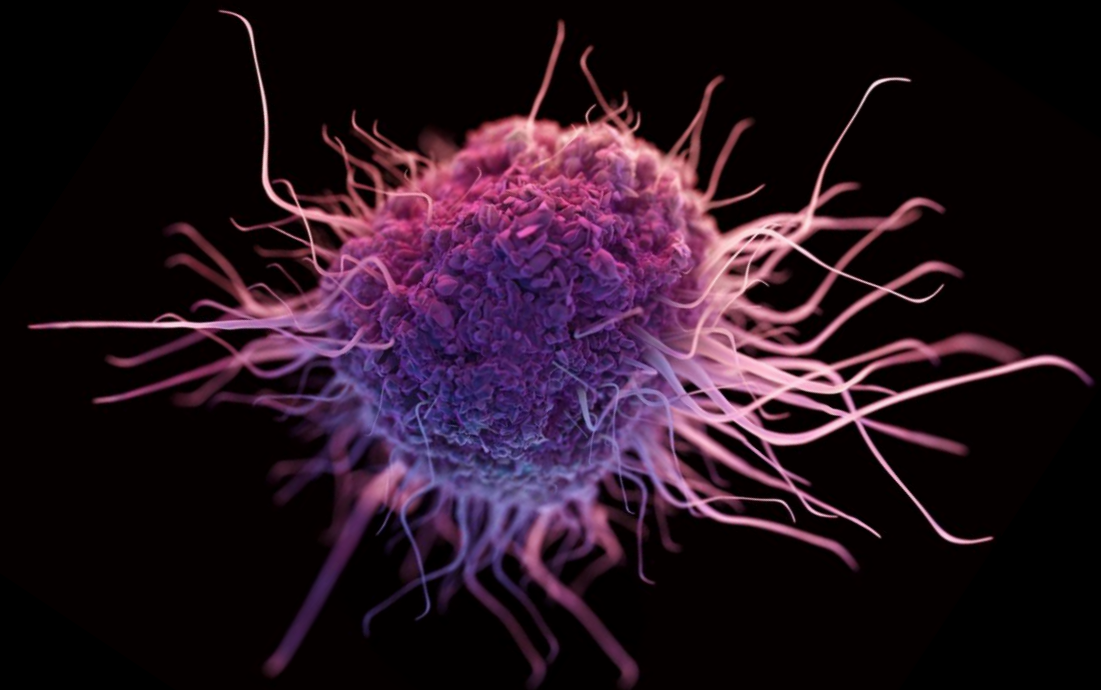


Ovum  
Sperm

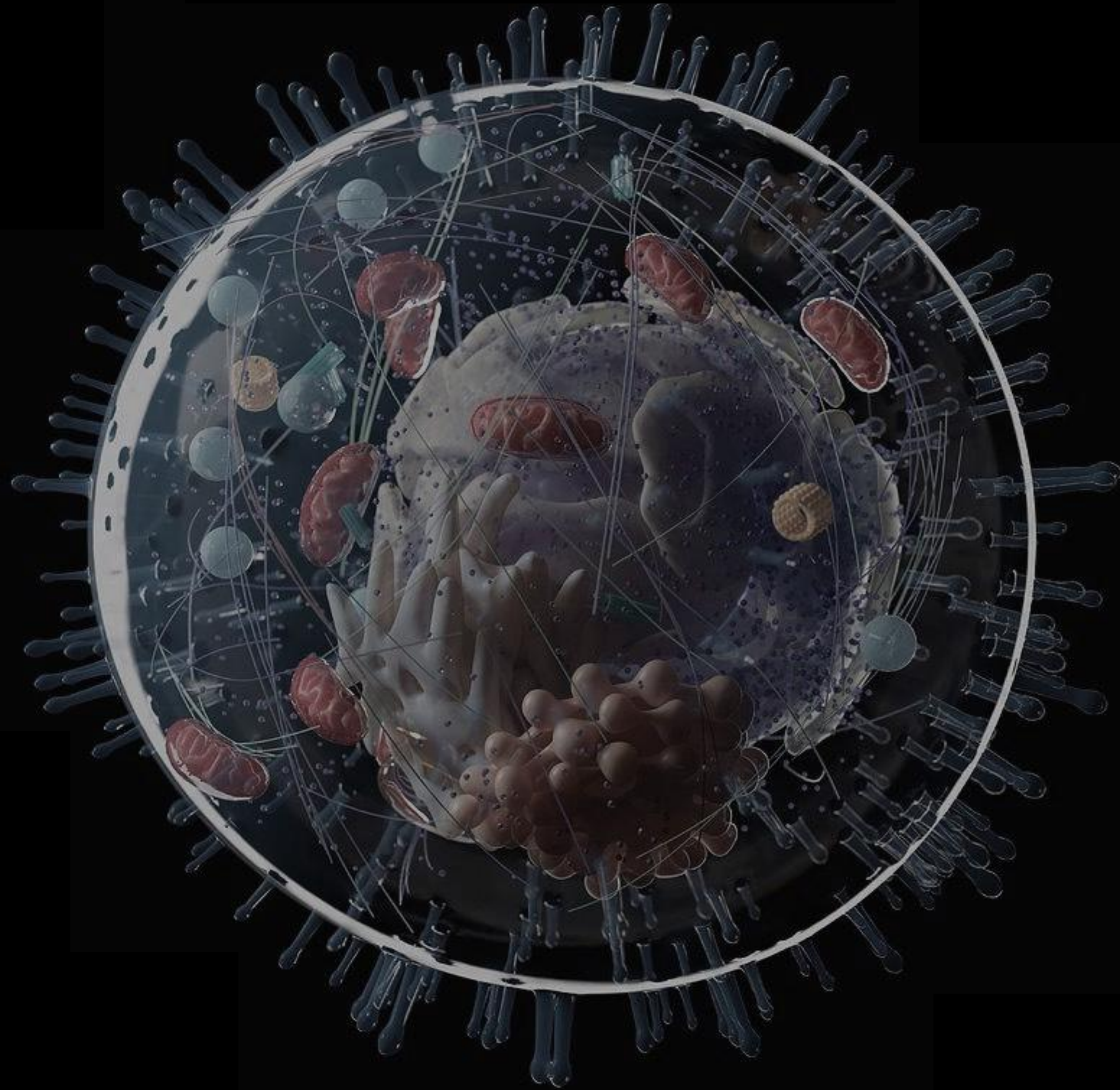
s  
'e



Rod cell  
Cone cell



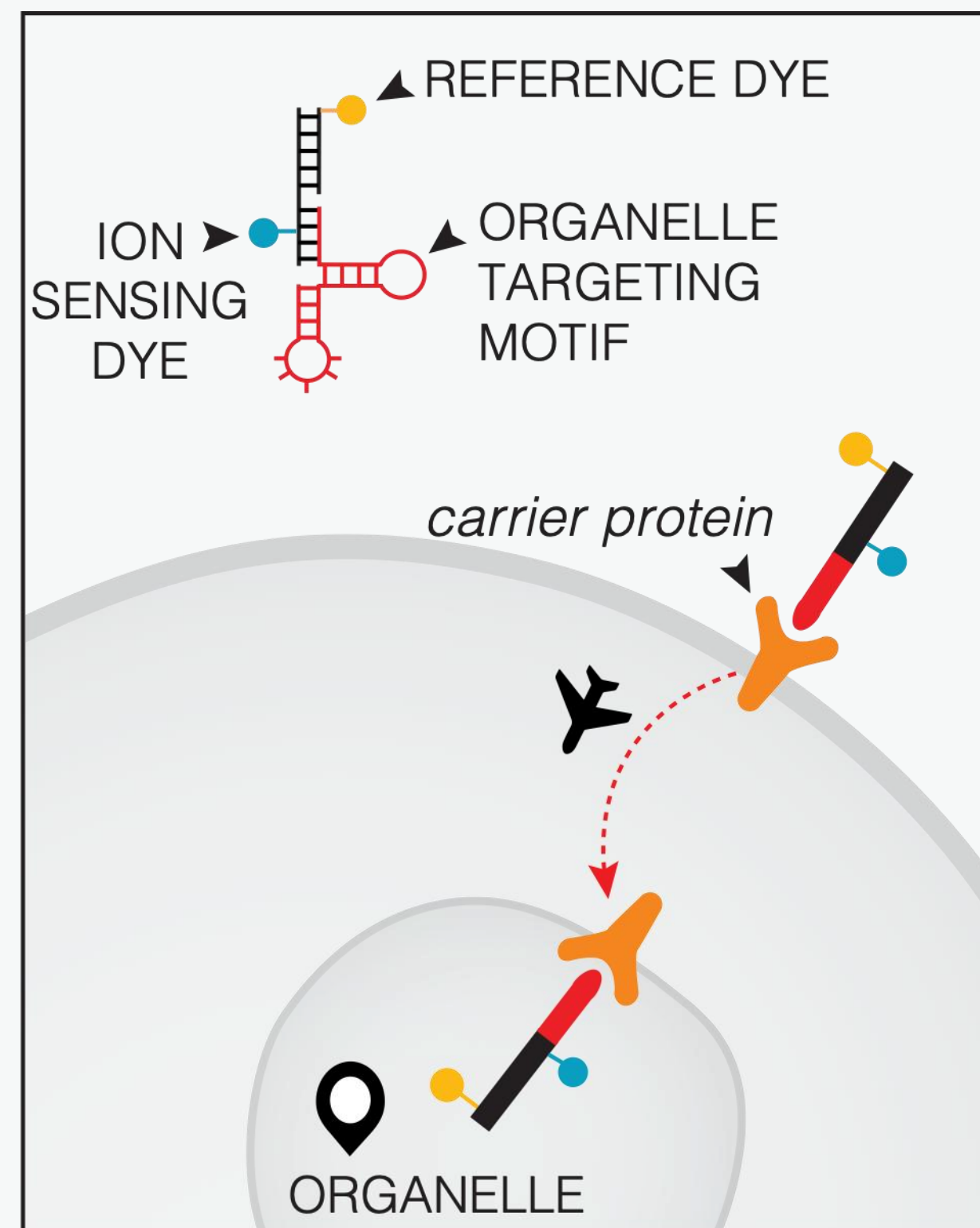
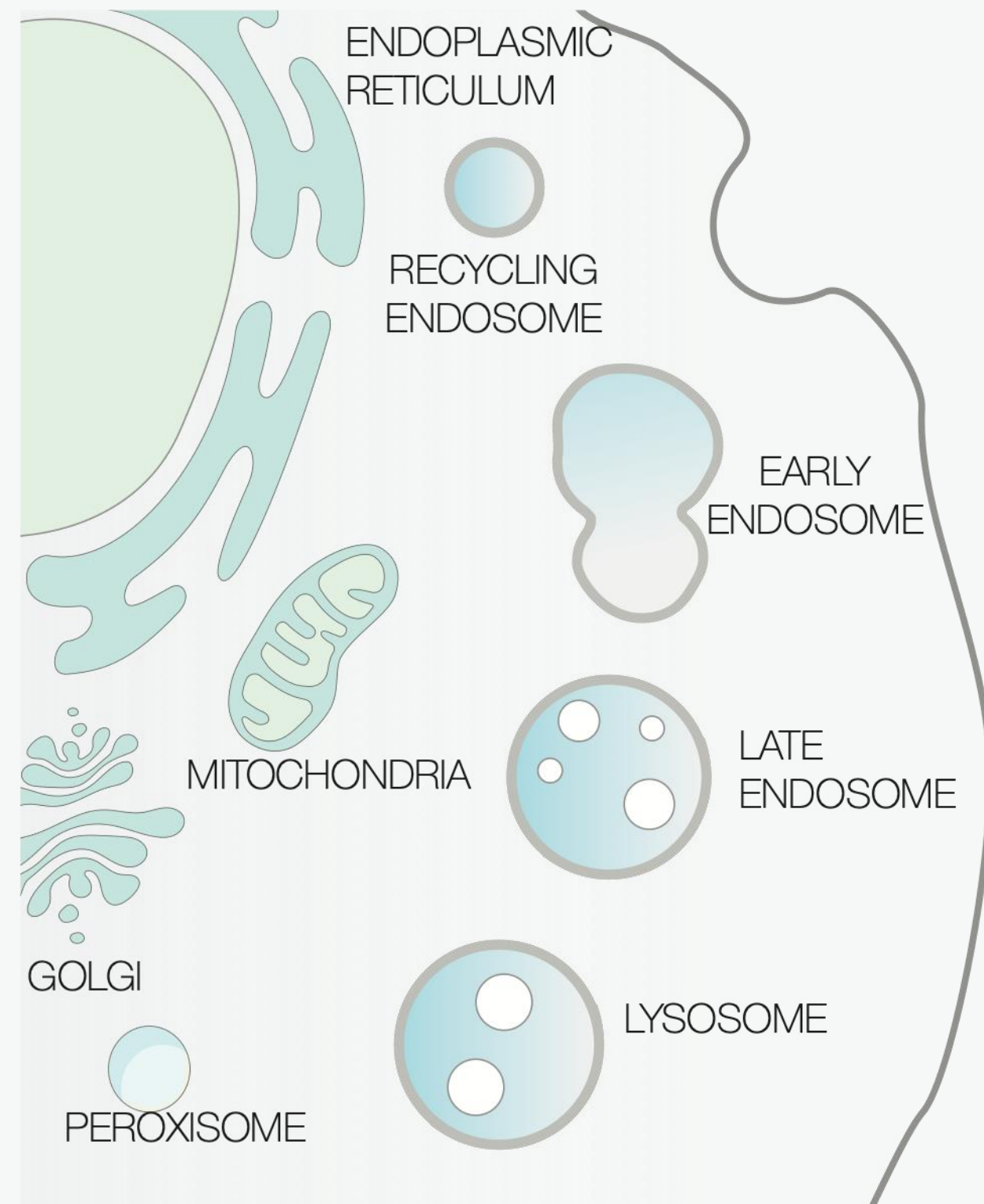
Dendritic cell



# Organelles

- common feature of all cells
- membrane bound structures
- biochemical reactors in series
- many ways to study the outer surface of organelles
- ...but inside?

# unveiling the ionic composition of organelles



$H^+$

Nat. Nanotechnol. 2009  
Nat. Nanotechnol.. 2013

$Cl^-$

Nat. Nanotechnol. 2014  
eLife 2017  
Cell Reports 2021

$Ca^{2+}$

Nat. Methods 2019  
PNAS 2021  
Nat. Nanotechnol. 2021a

$Na^+$

Nat. Biotechnol. 2024a

$K^+$

Nat. Biotechnol. 2024b

mV

Nat. Nanotechnol. 2021b

enzyme activity

Nat. Nanotechnol. 2019a  
Nat. Nanotechnol. 2019b  
PNAS 2021

different organelles

Nat. Chem. Biol. 2020a  
Nat. Chem. Biol. 2020b

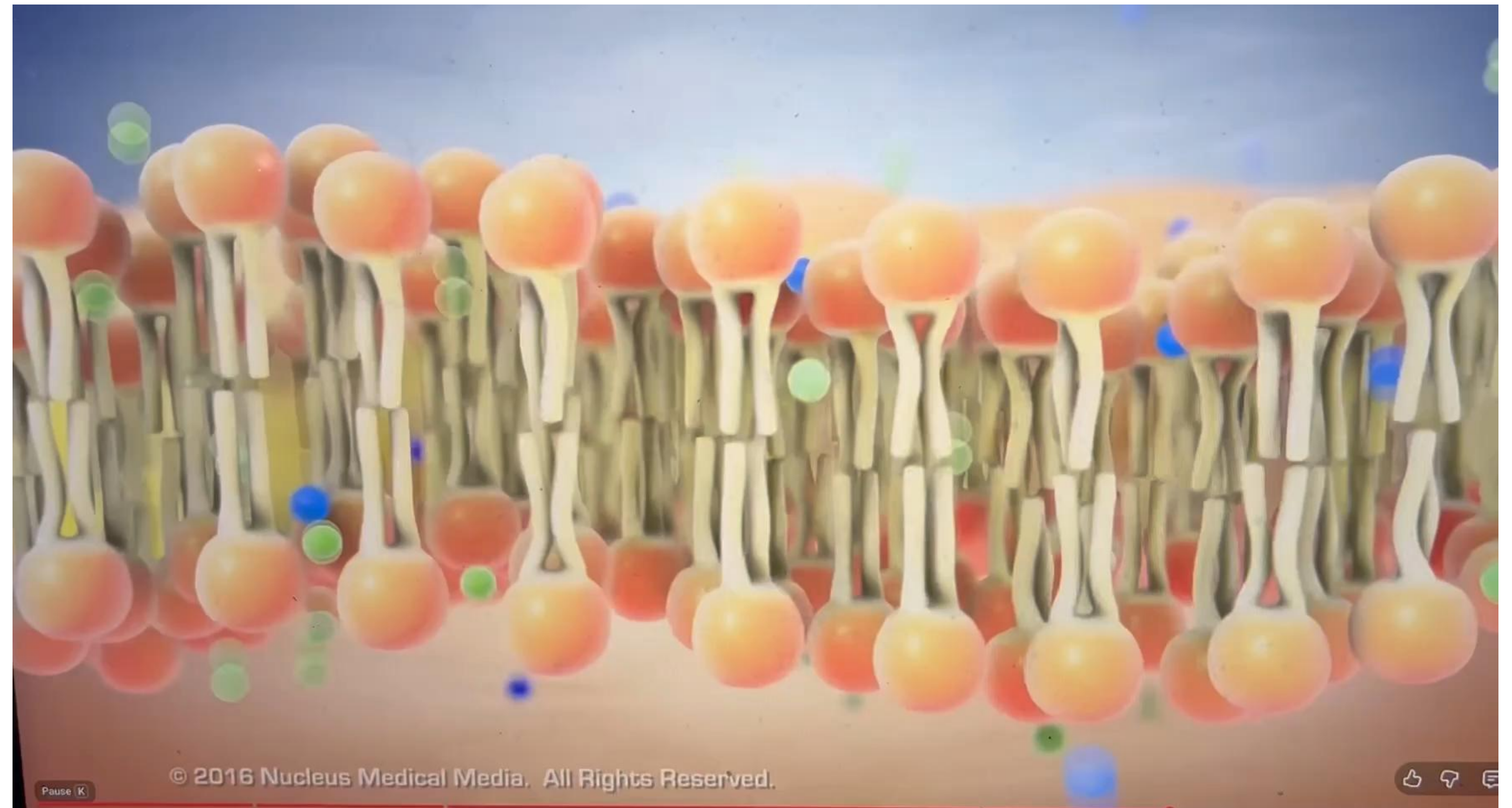
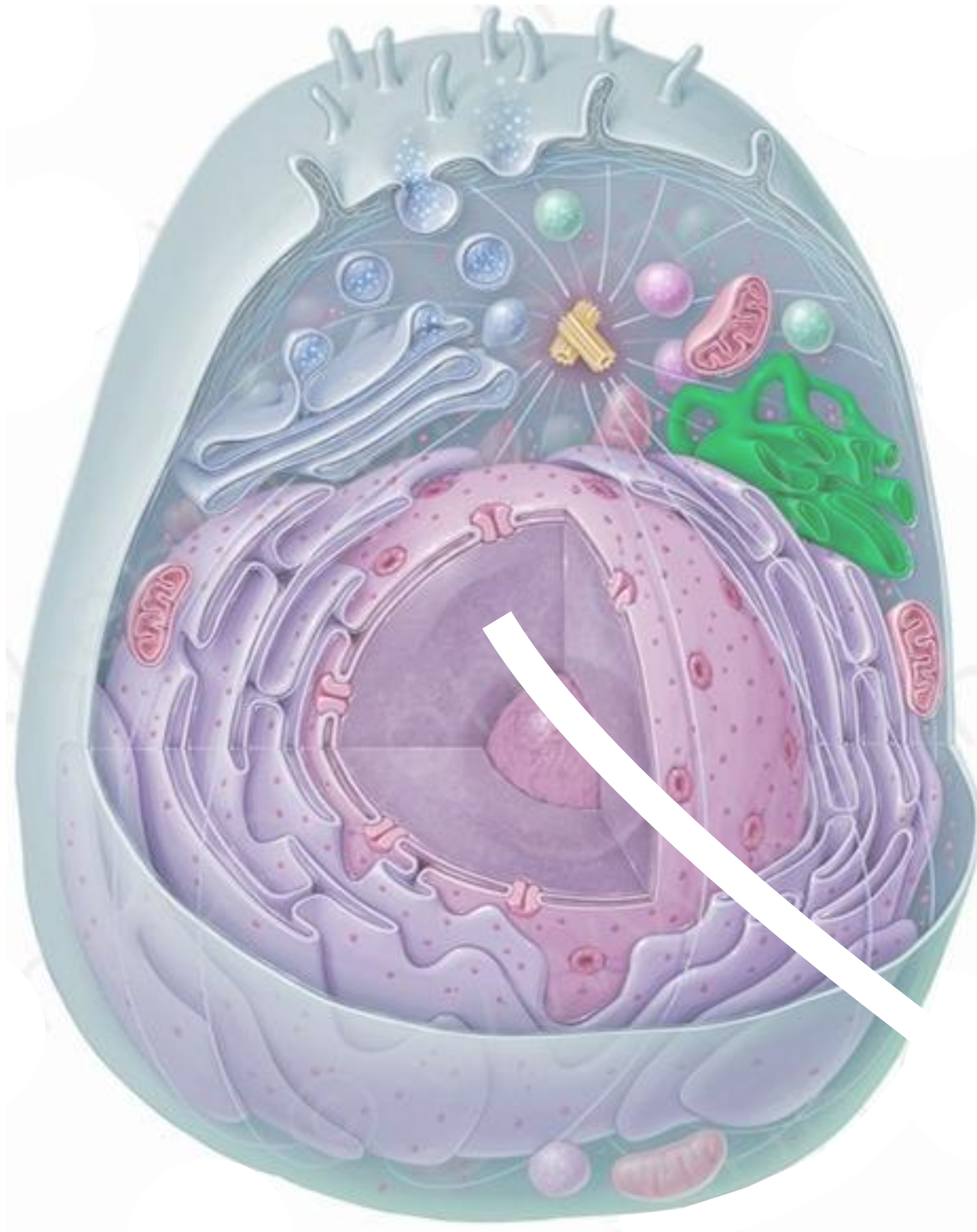
worms, fish, mice

eLife 2021

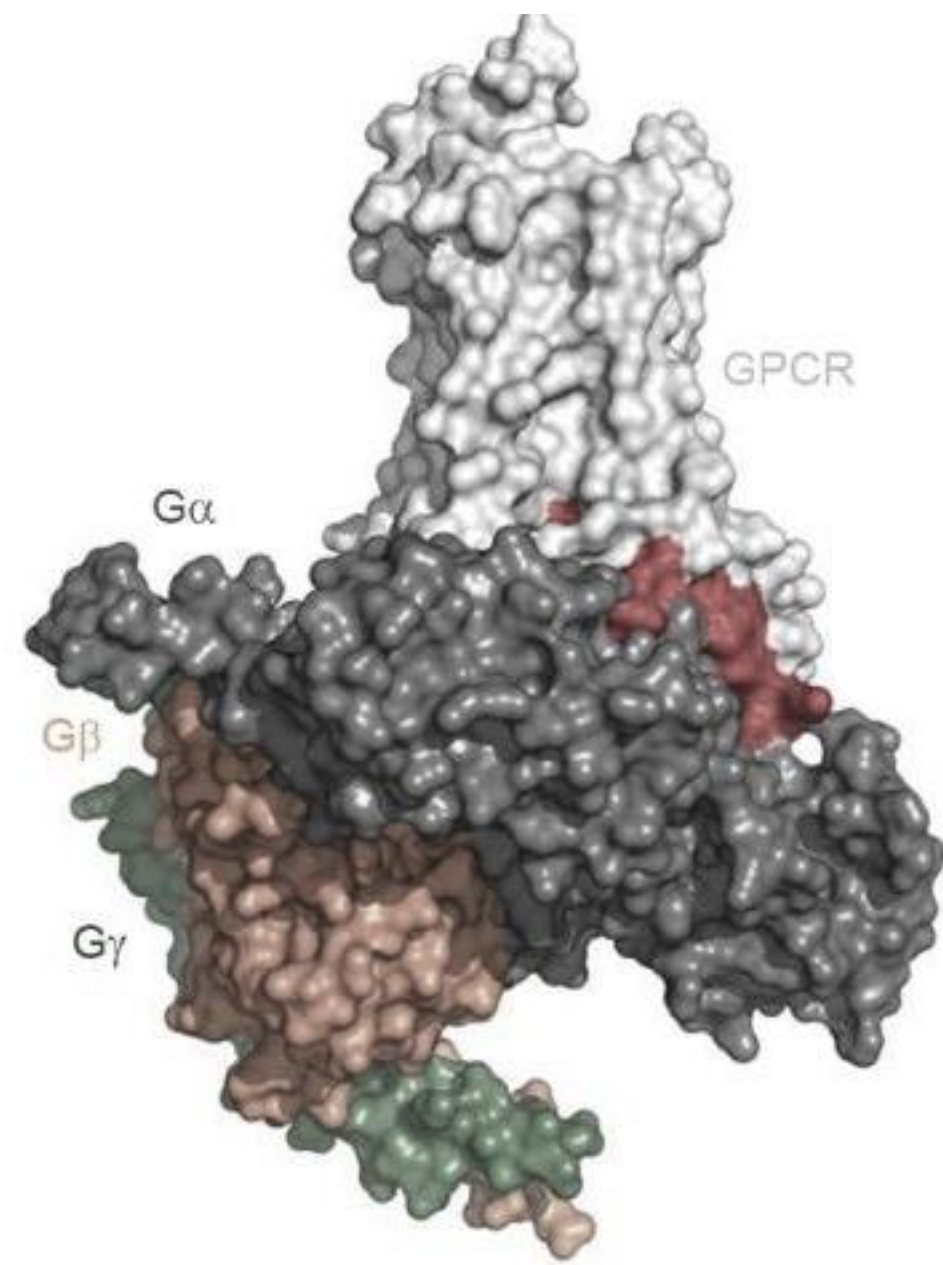
PNAS 2020

Nat. Nanotechnol. 2021b

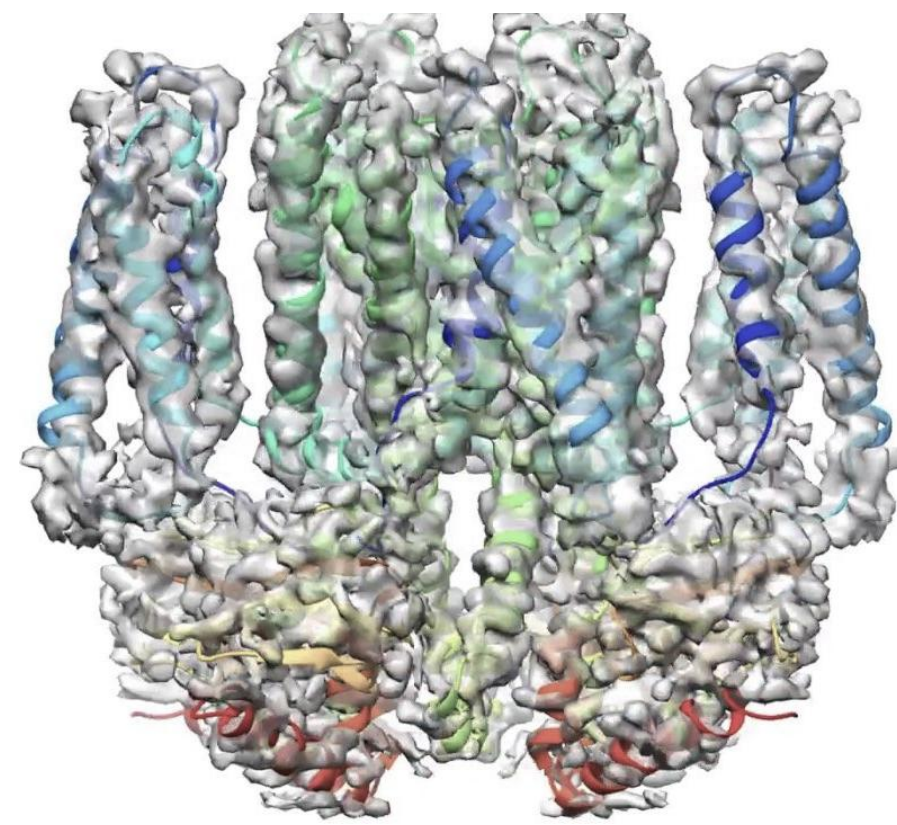
# Ion transporting proteins maintain ion levels inside the cell



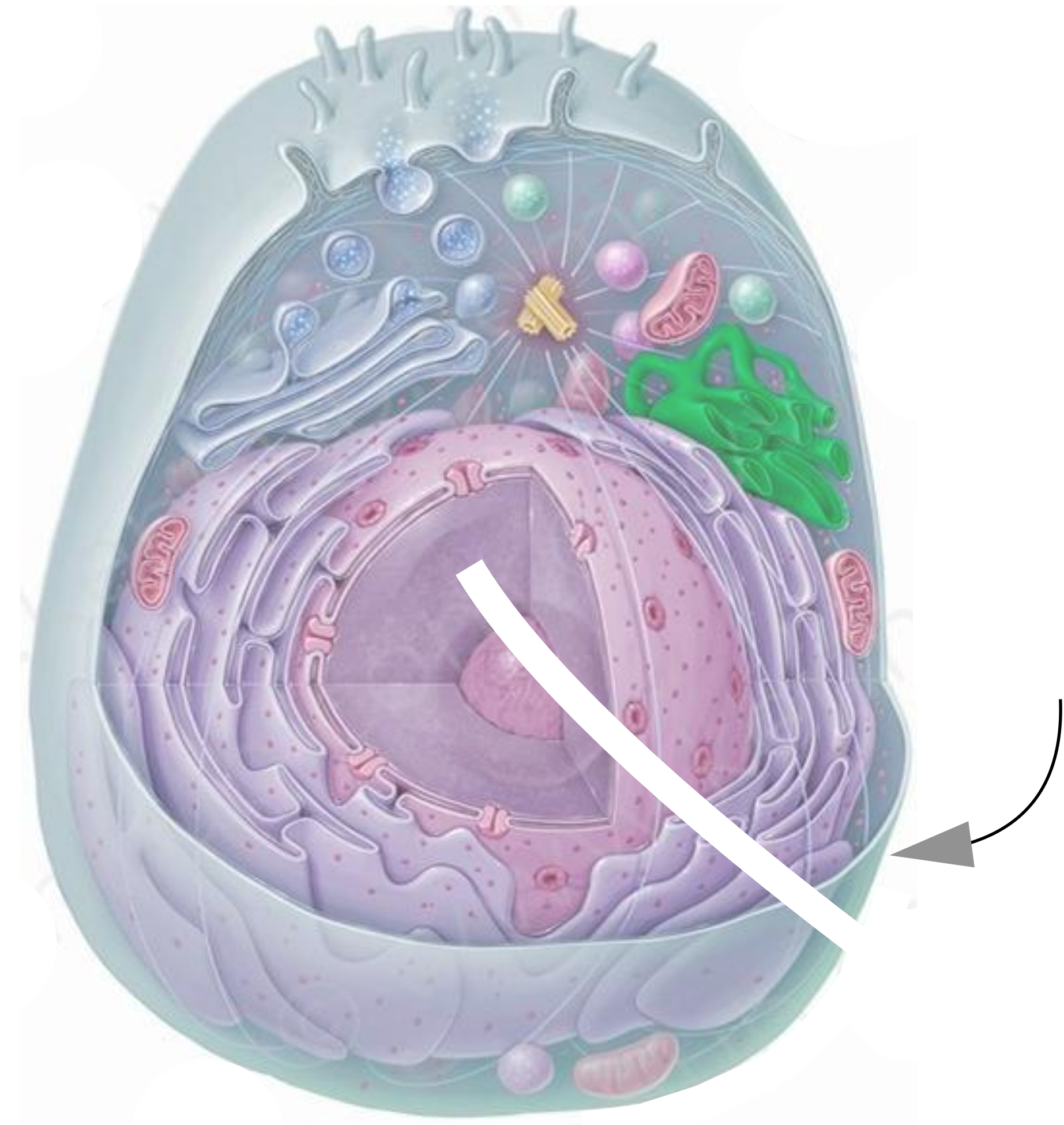
# GPCRs & ion channels: most successful drug targets



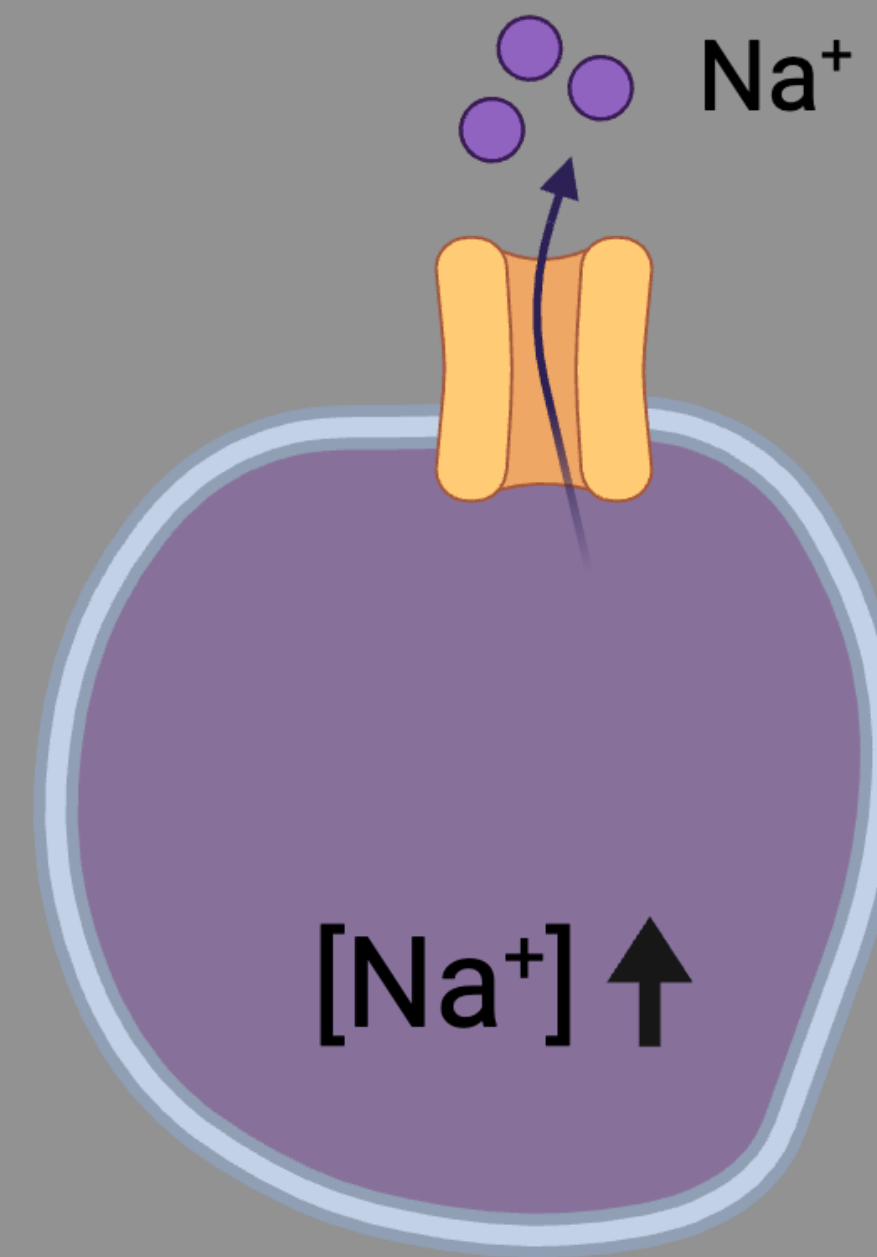
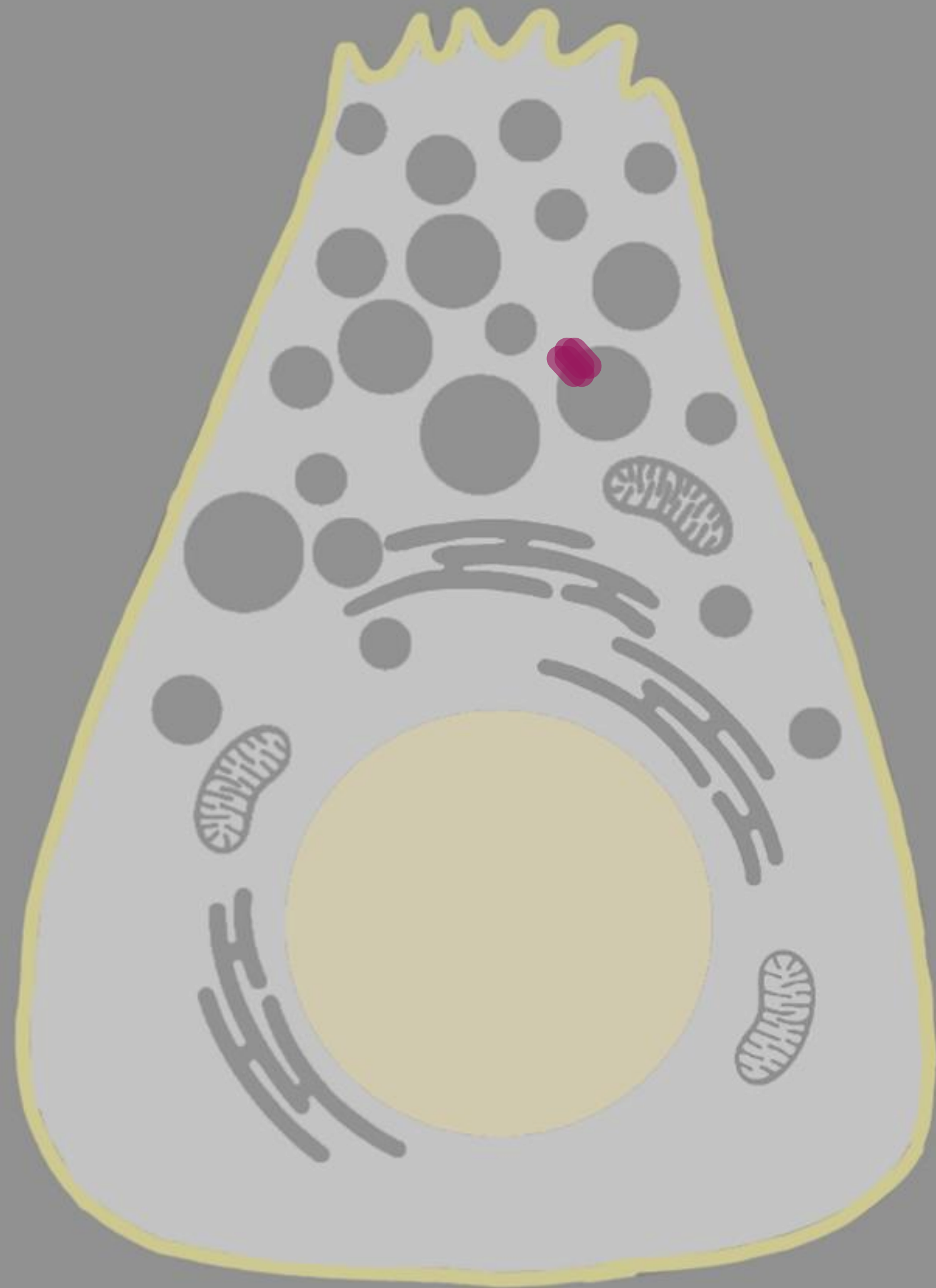
GPCR



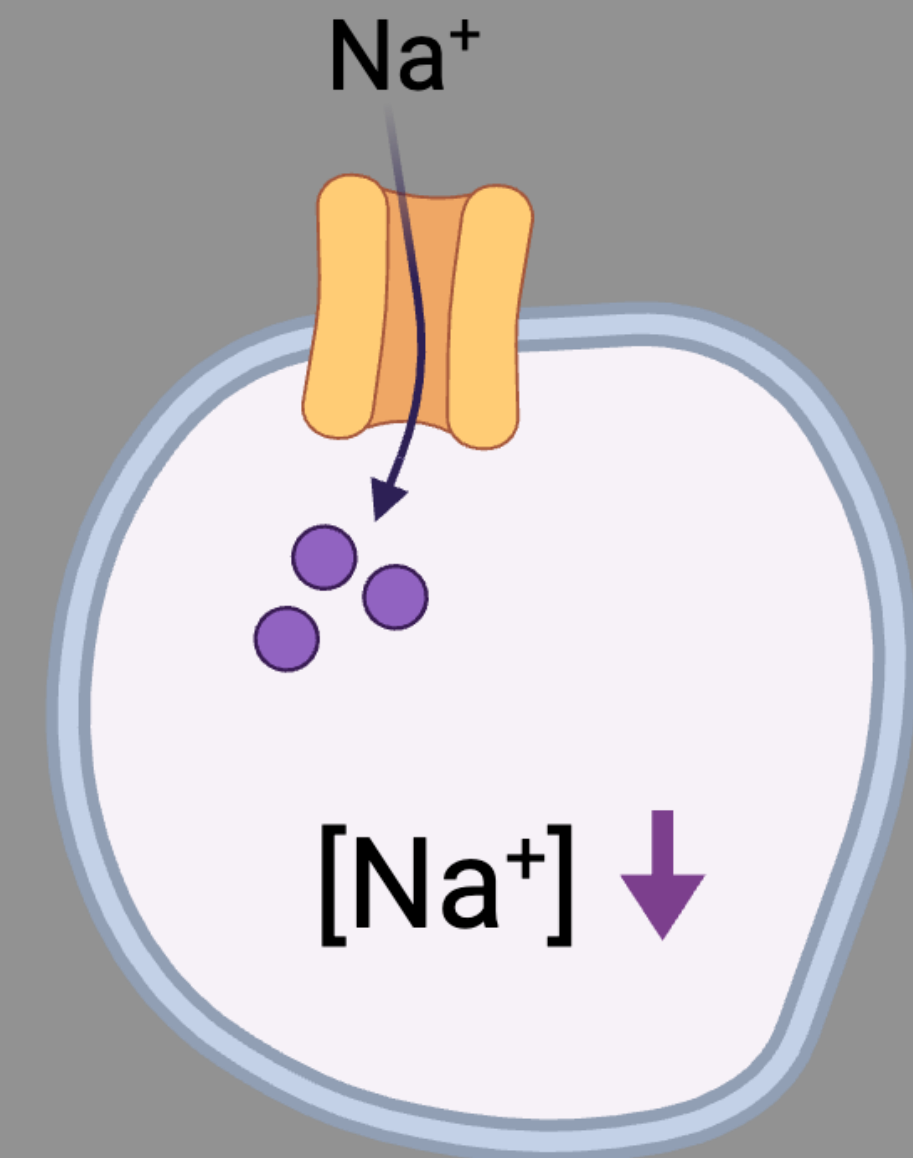
K<sup>+</sup> channel



# The only readout of ion channel activity in native organelles

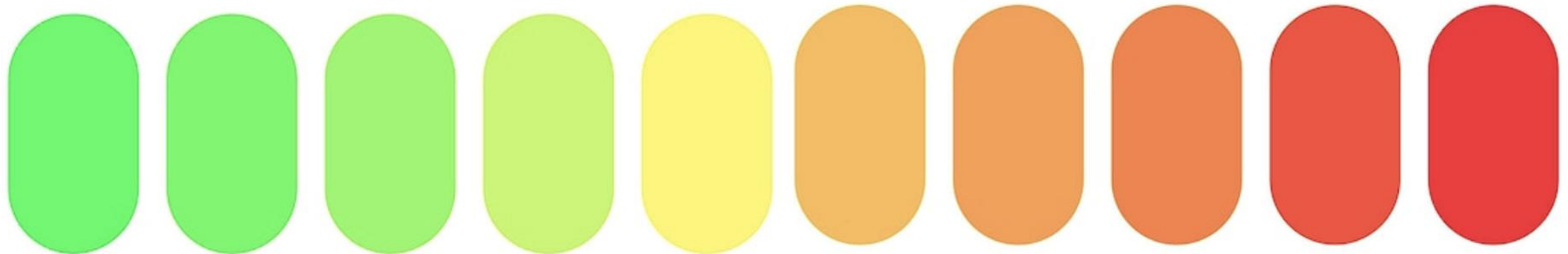


$\text{Na}^+$  efflux

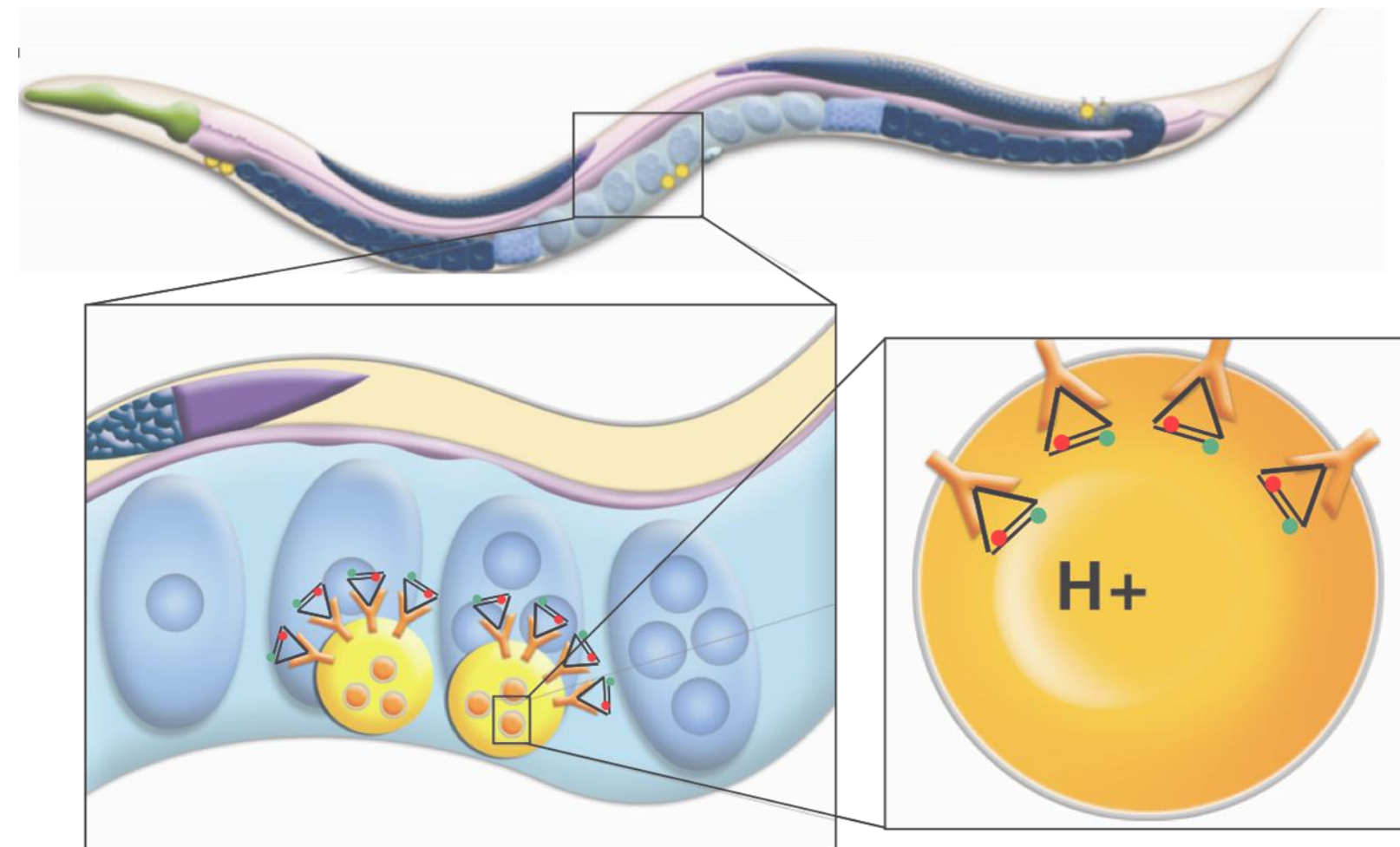


$\text{Na}^+$  influx

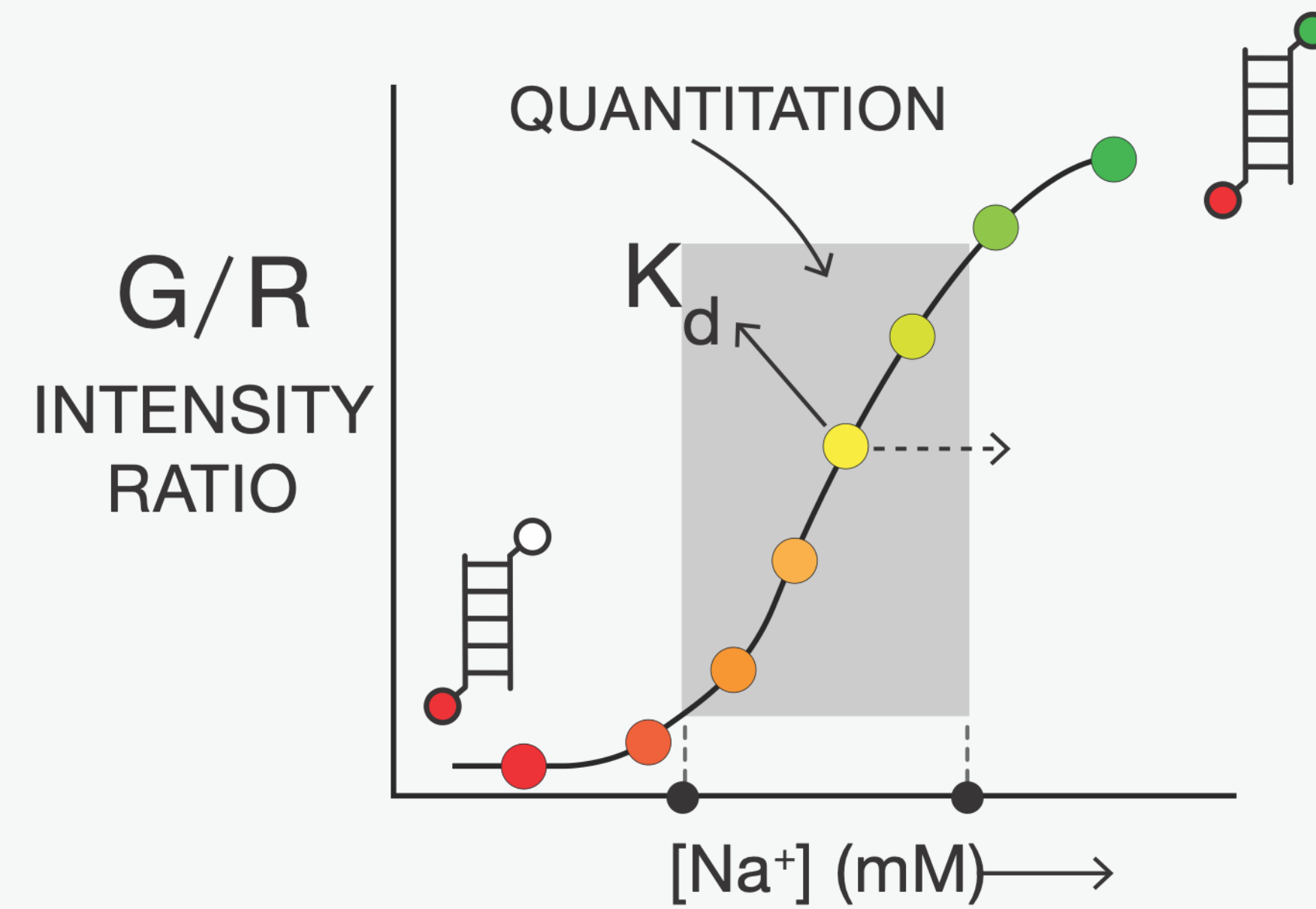
# 1. the *measurement* problem: (measurement vs detection)



## 2. the Russian doll problem: *access*



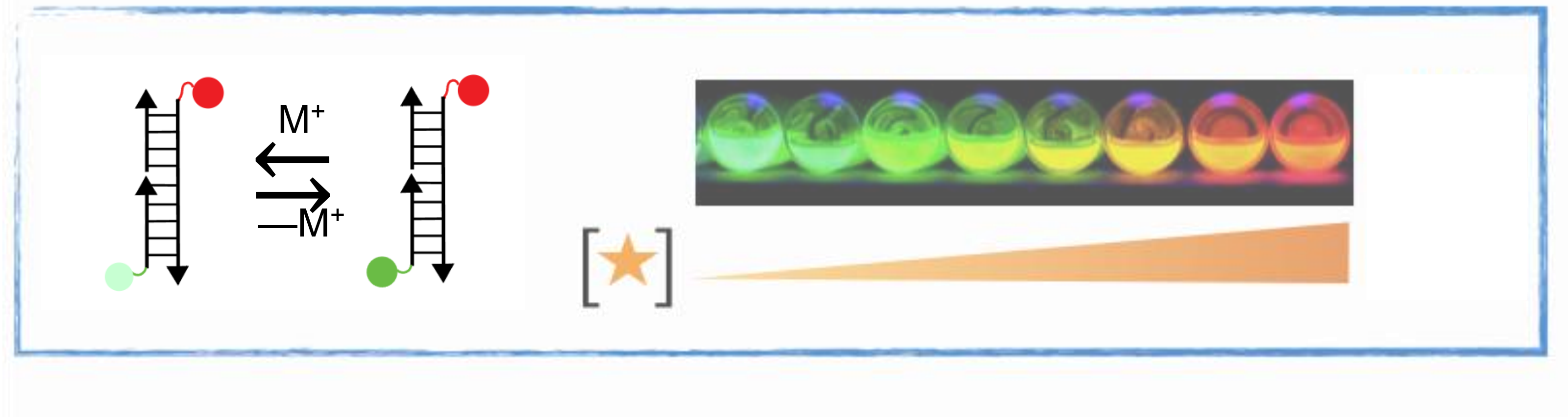
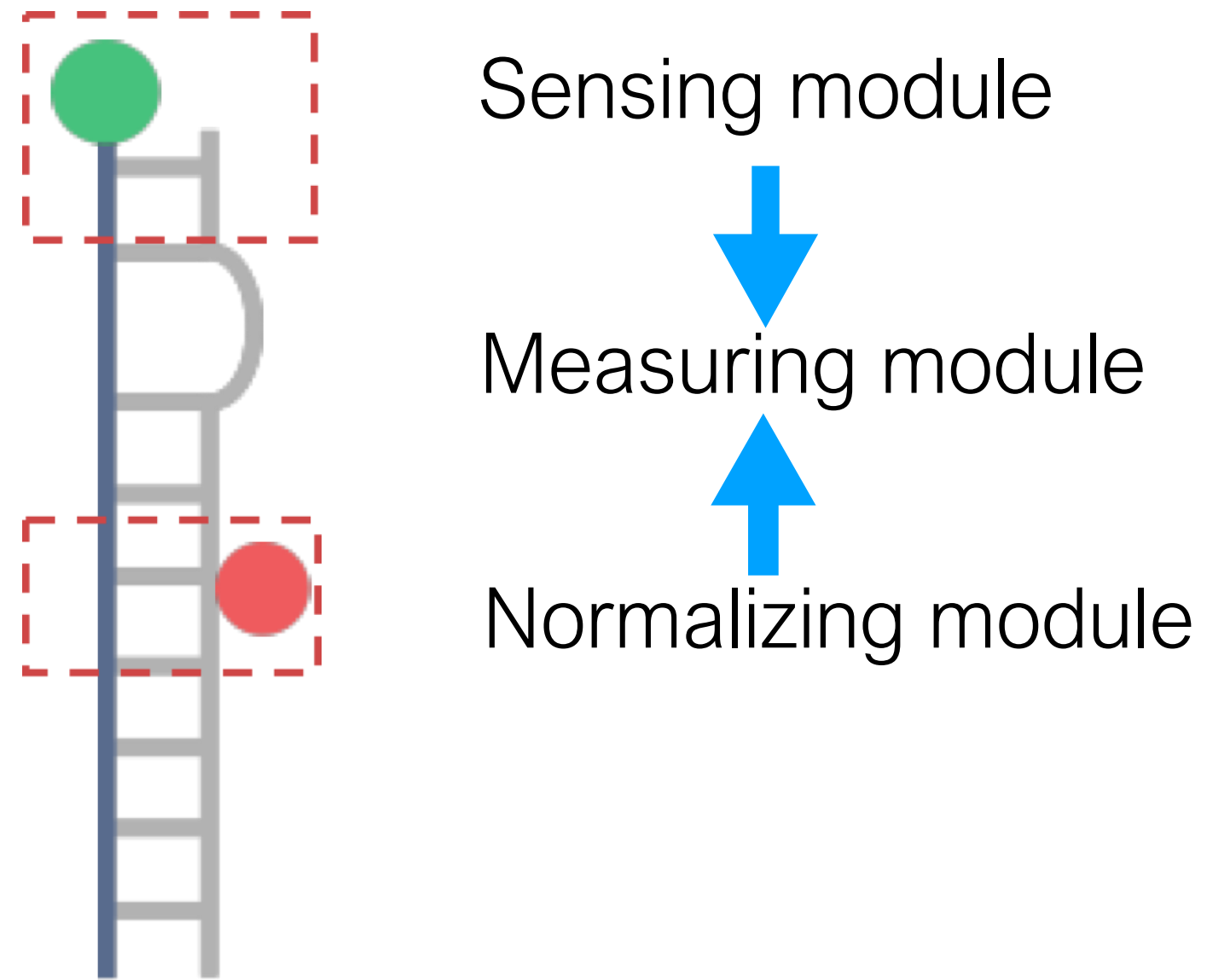
### 3. the black box problem: *designing blind*



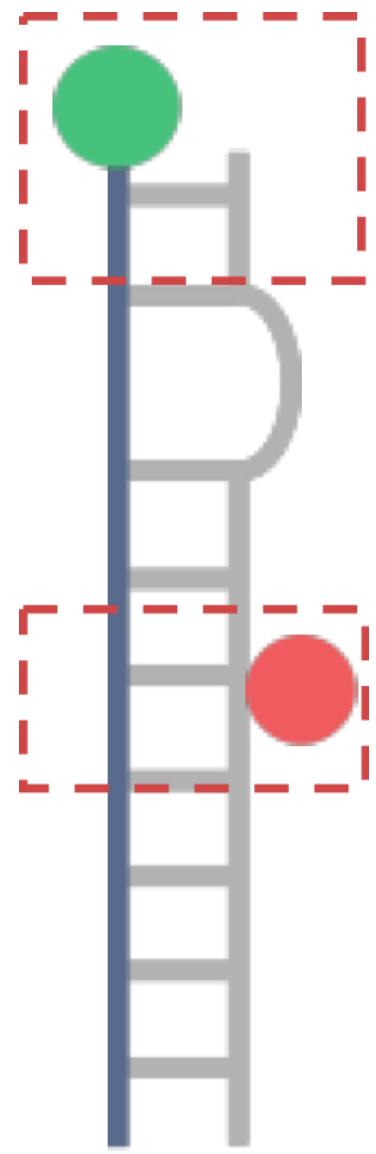
# 4. the acidity problem: *the killer challenge*



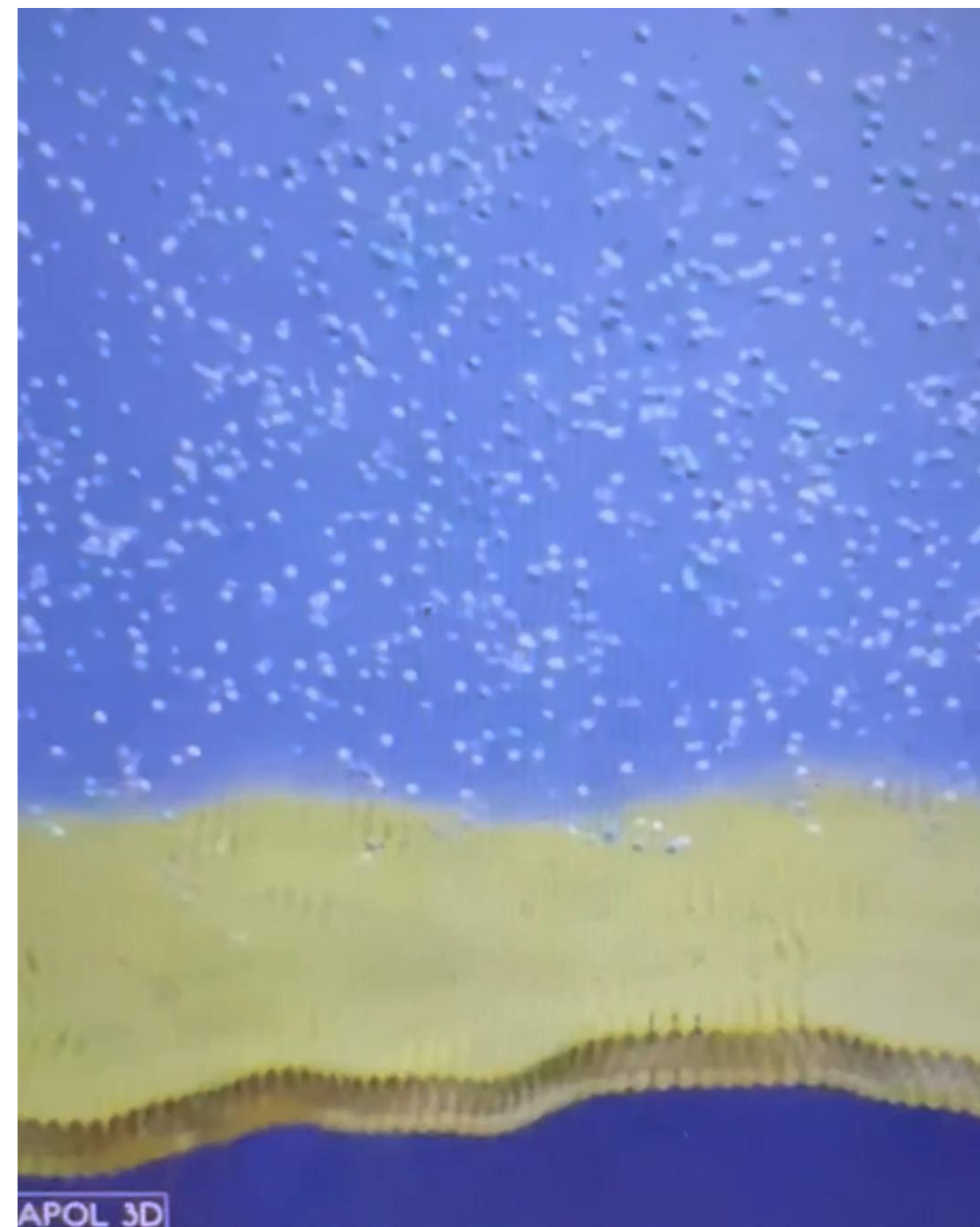
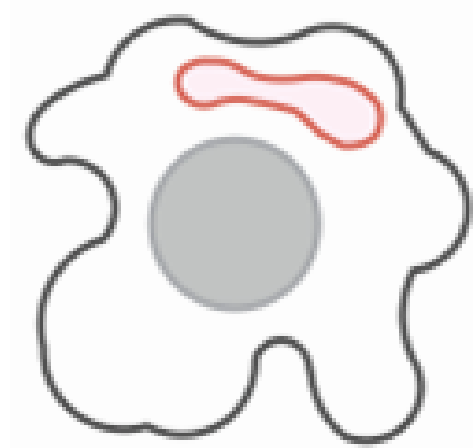
# DNA nanodevices: solving the measurement problem



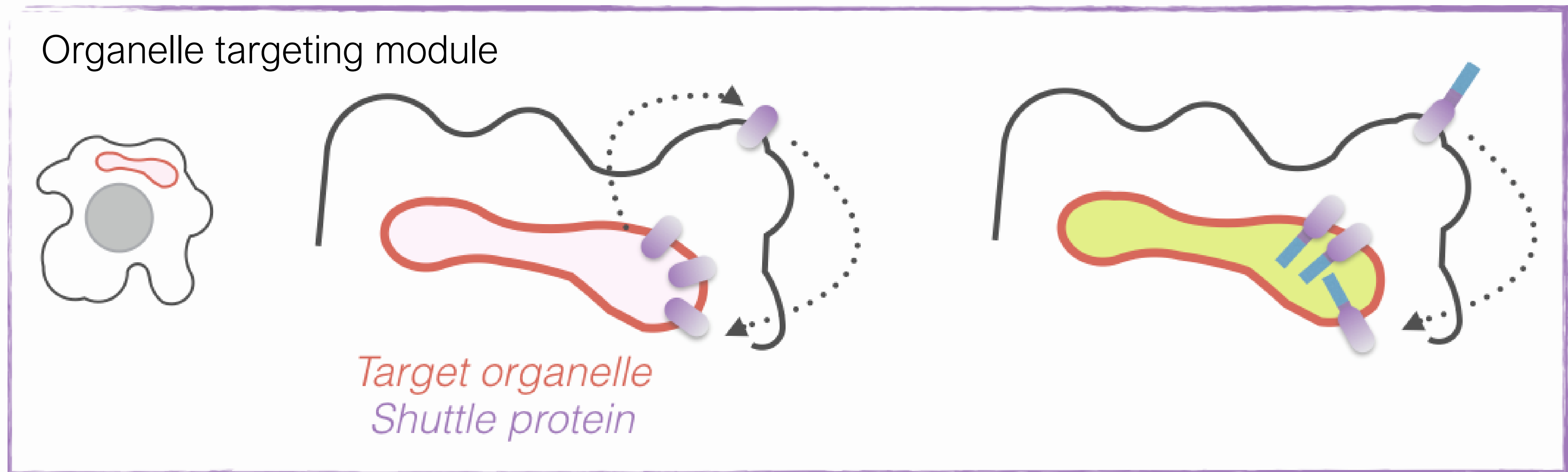
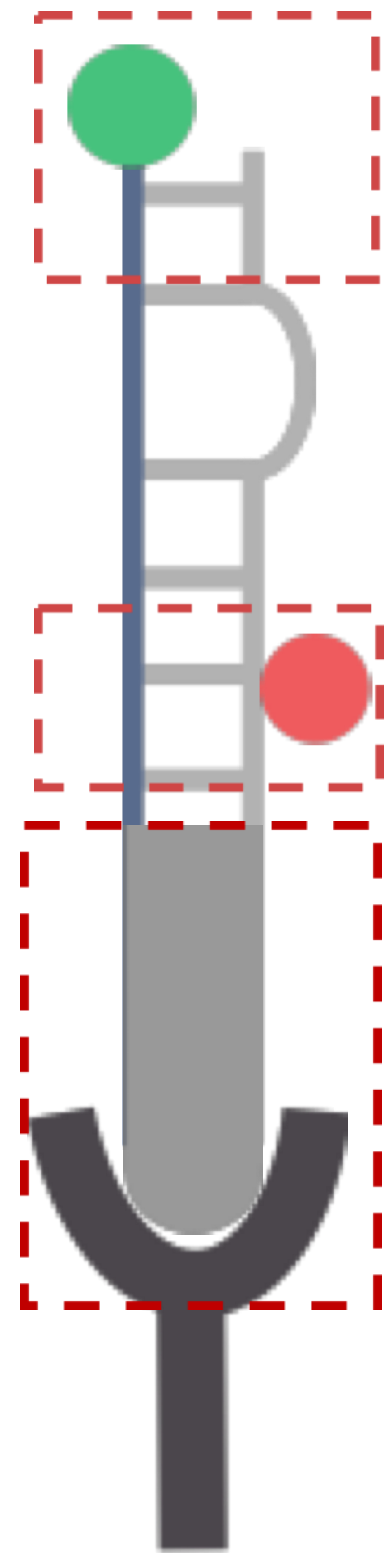
# Targeting nanodevices to organelles: Solving the accessibility problem



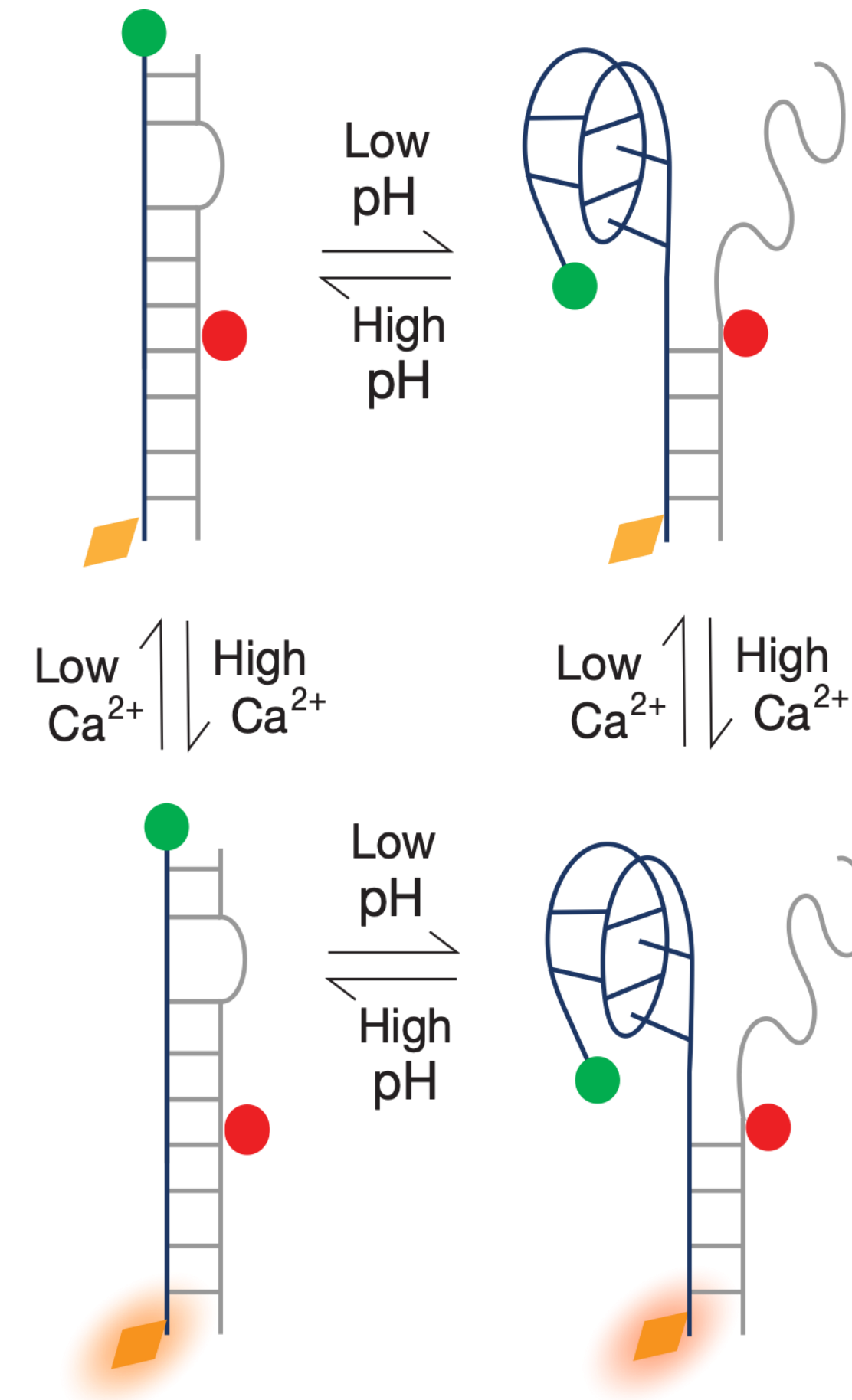
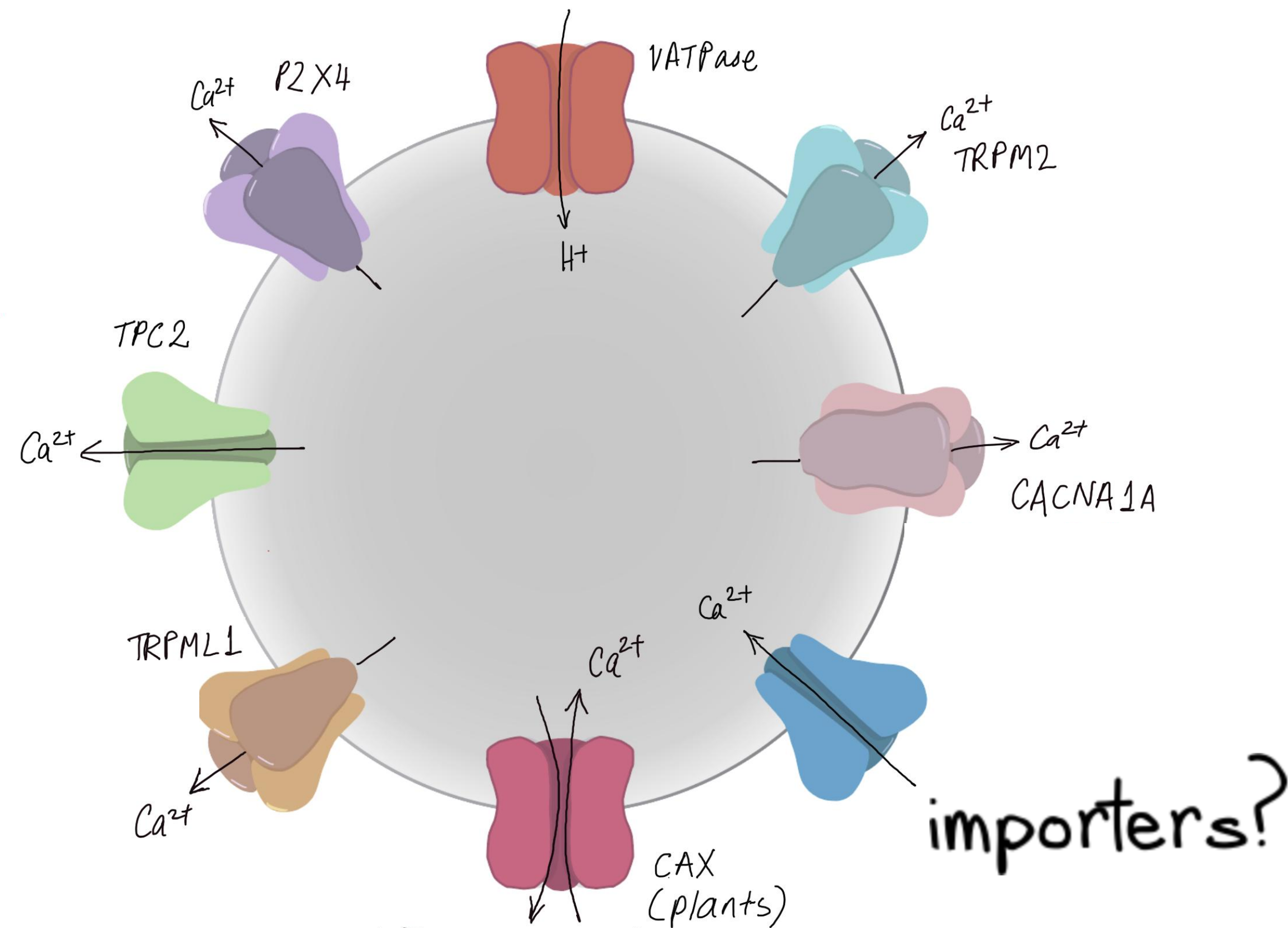
Organelle targeting



# Targeting DNA nanodevices to organelles



# Quantitatively imaging $\text{Ca}^{2+}$ in acidic organelles

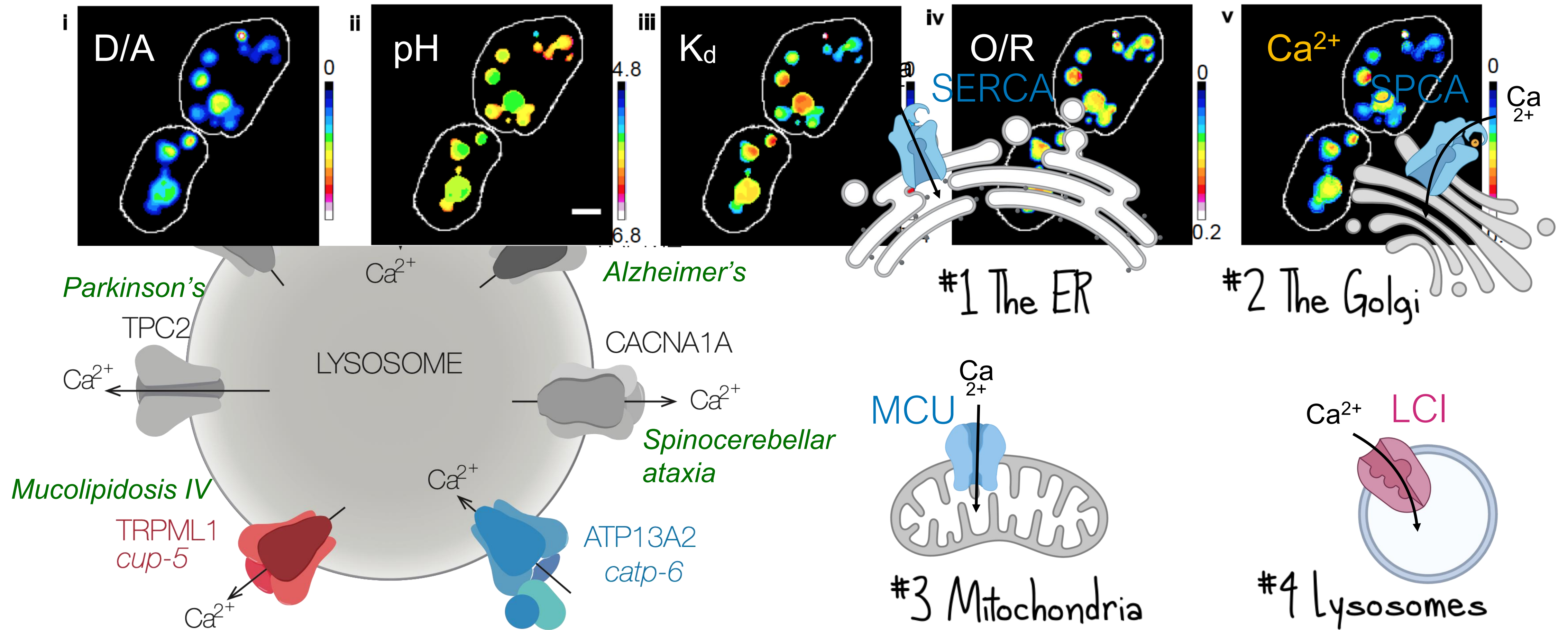


Dr Kasturi Chakraborty @MIT

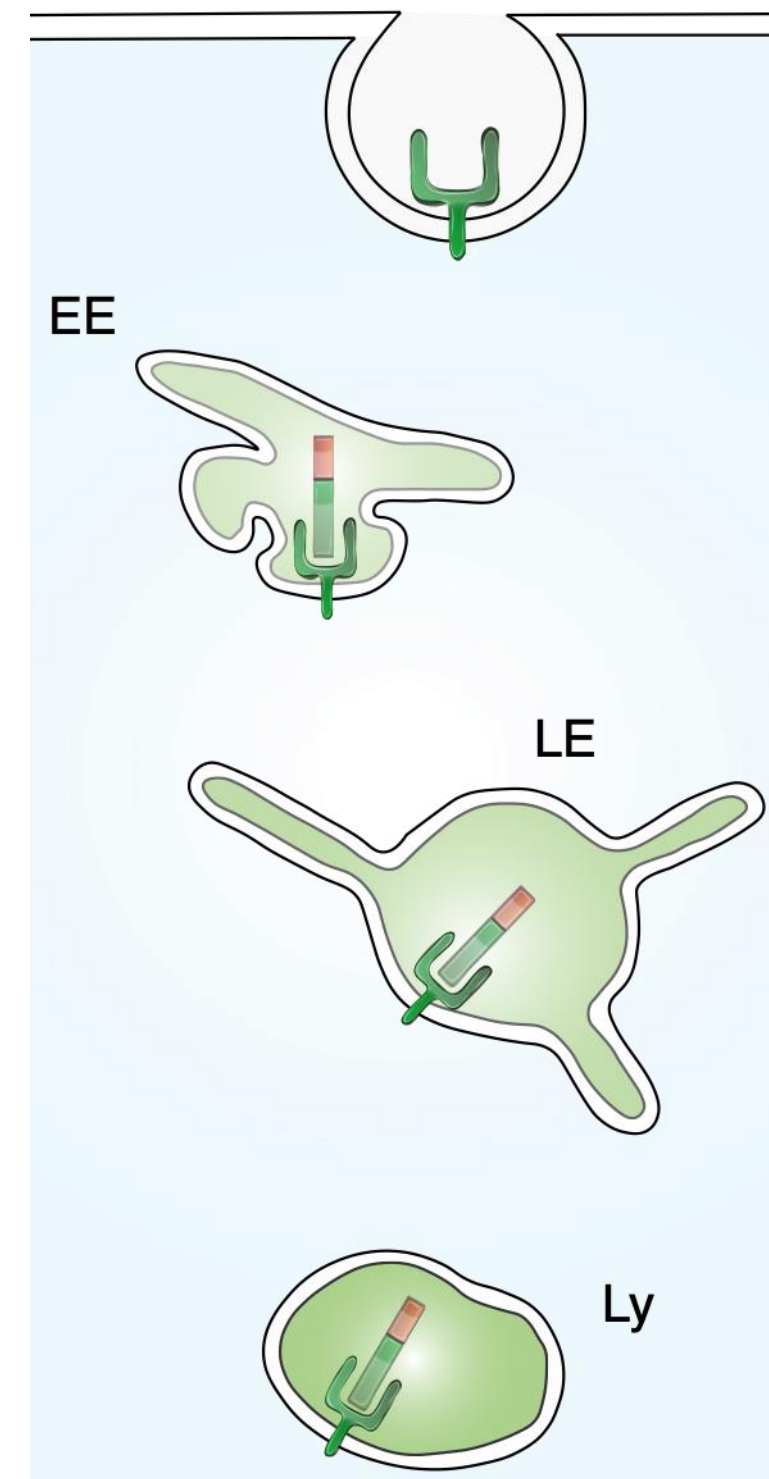
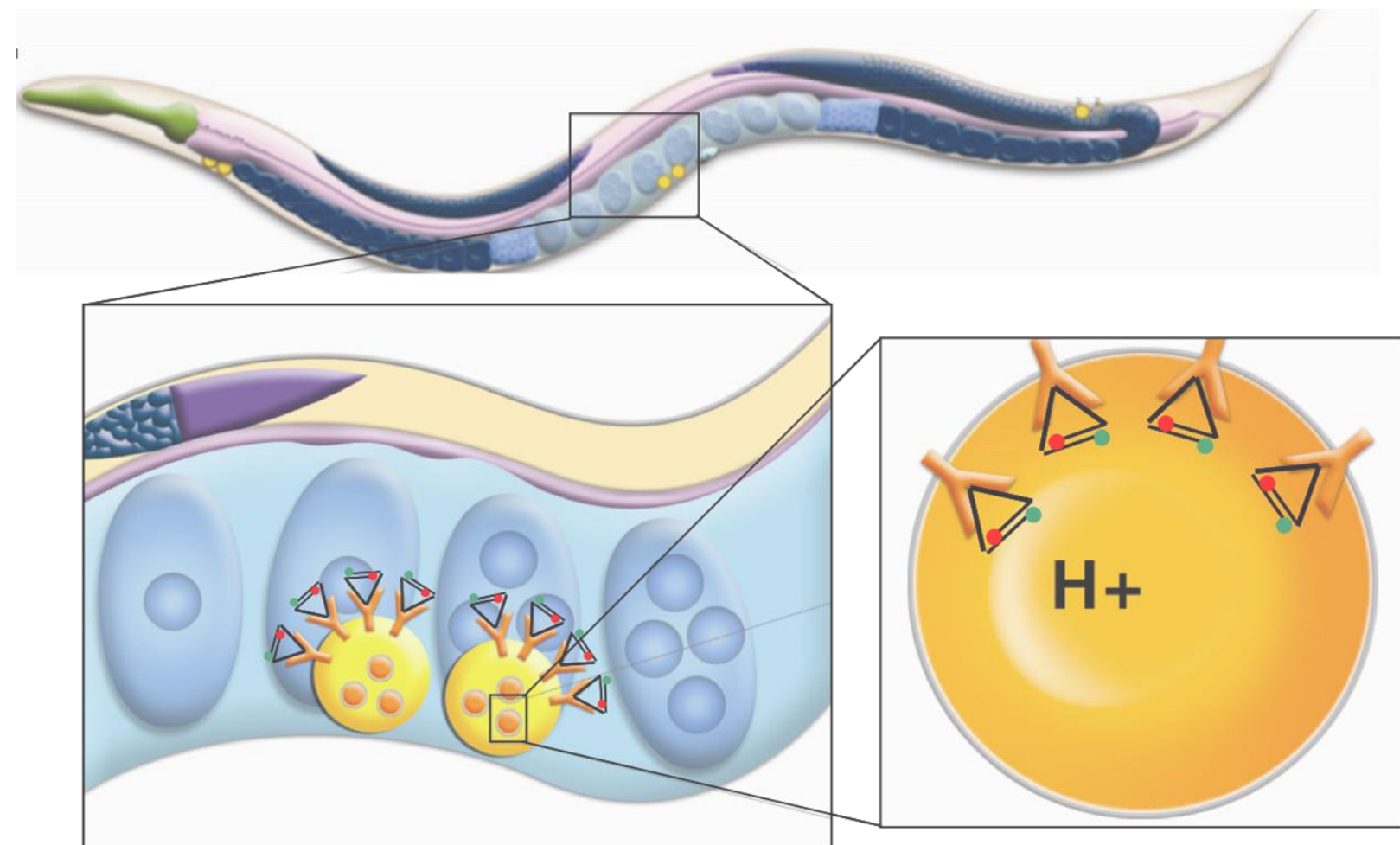


Dr Matt Zajac @AAAS

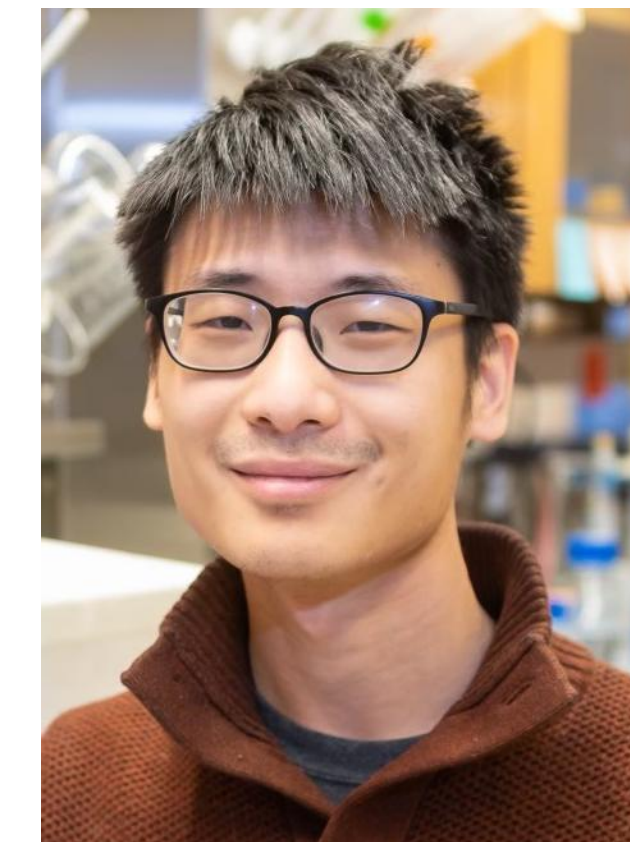
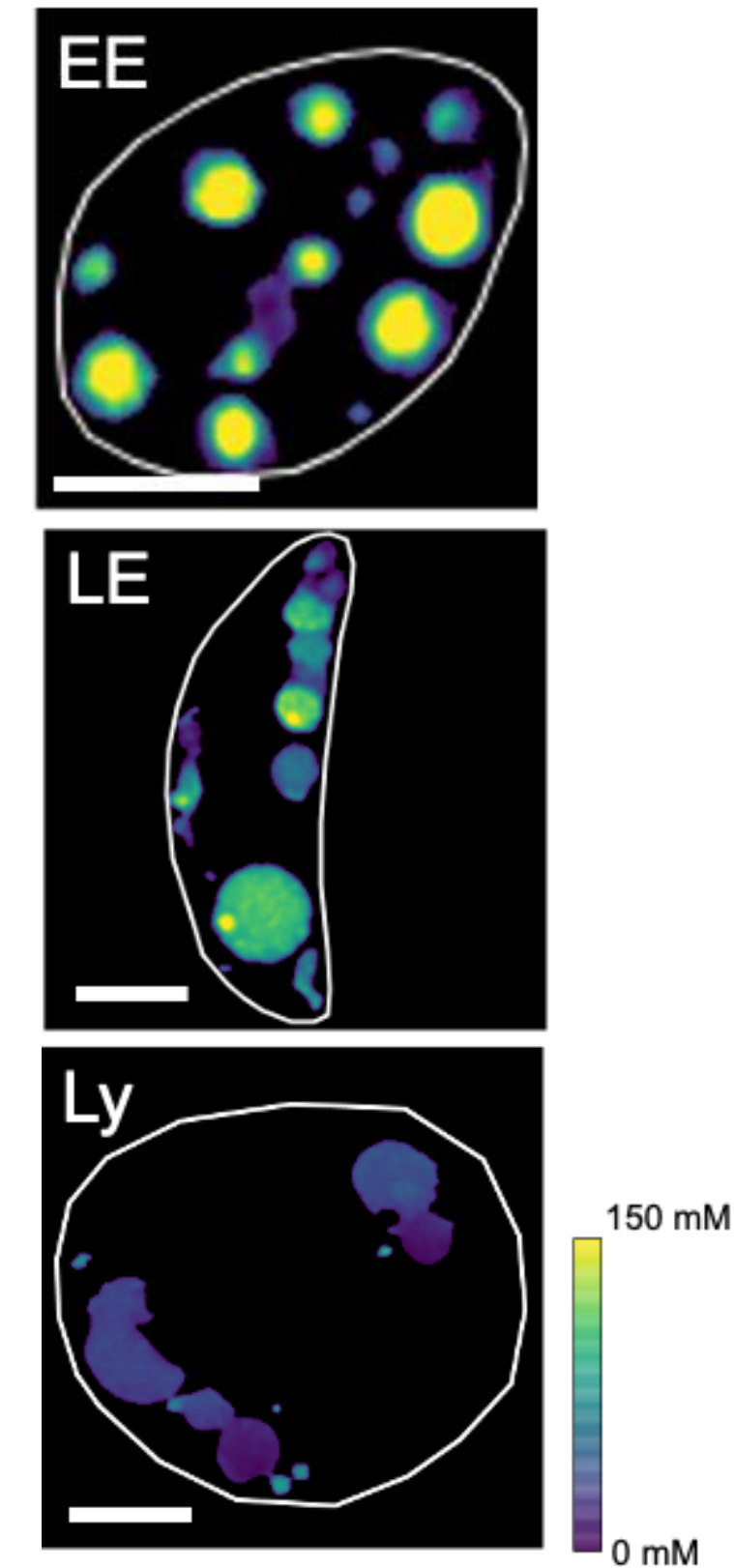
# LCl: the first lysosomal $\text{Ca}^{2+}$ importer described in humans



# Organelle Na<sup>+</sup> : lysosomes help cells counteract salt-stress



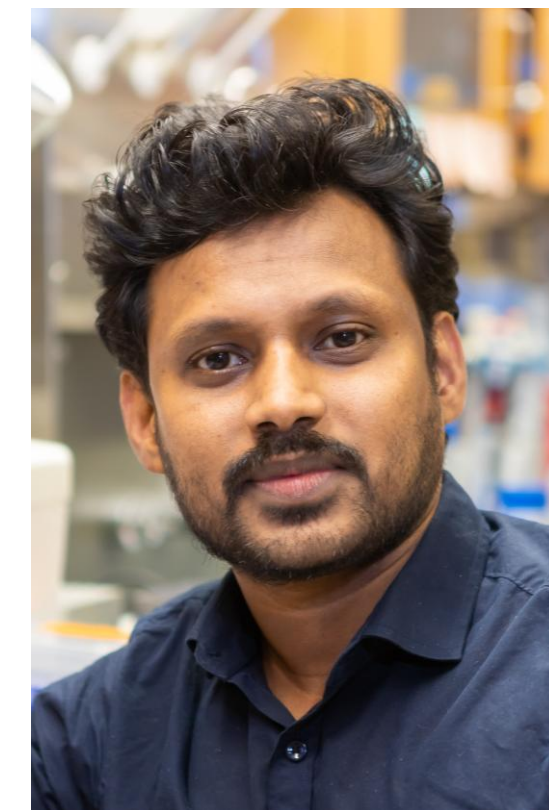
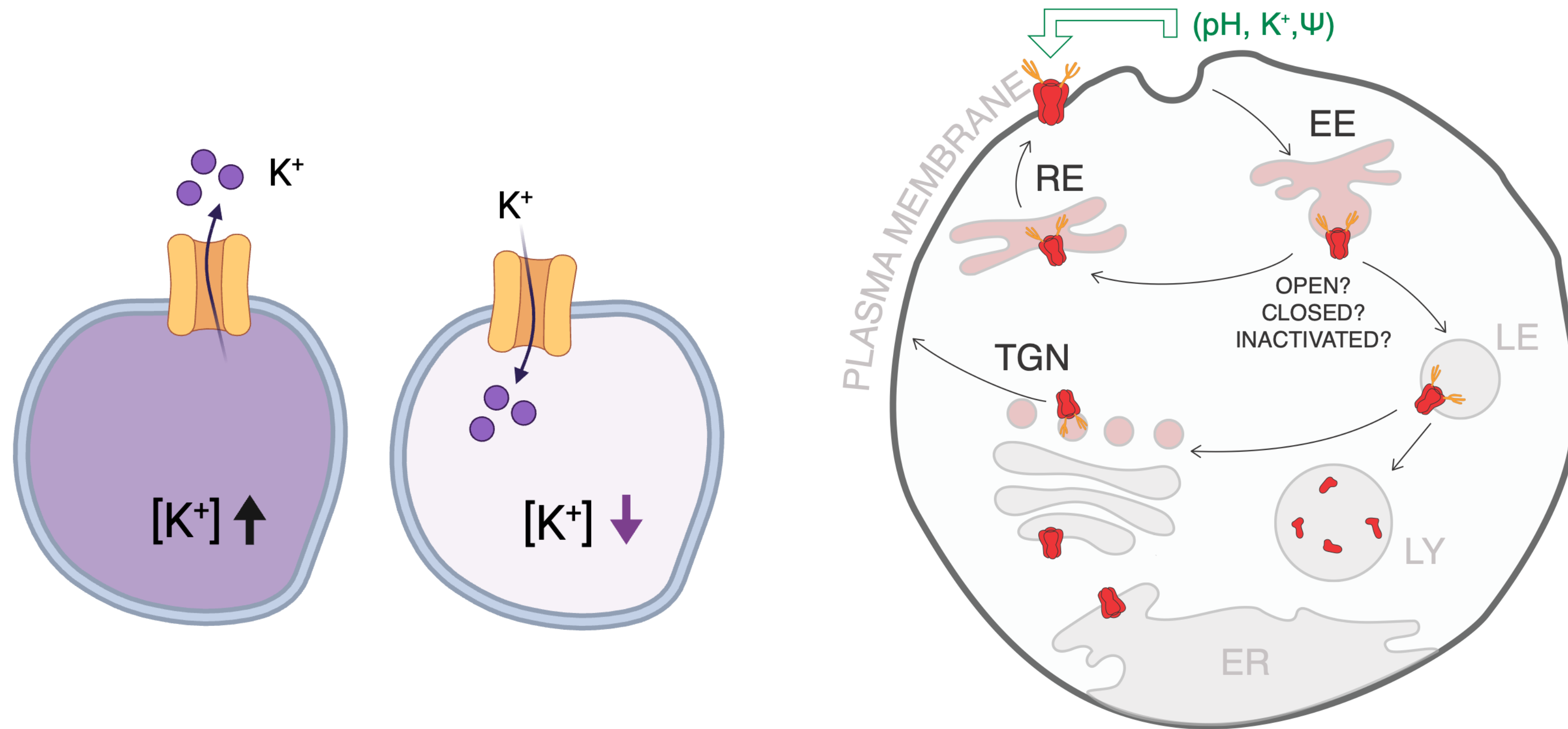
scale: 5 μm



Dr Junyi Zou  
@UChicago

Zou J. ....YK, 2024, Nat. Biotechnol.

# Organelle K<sup>+</sup> : Cell surface K<sup>+</sup> channels work in organelles



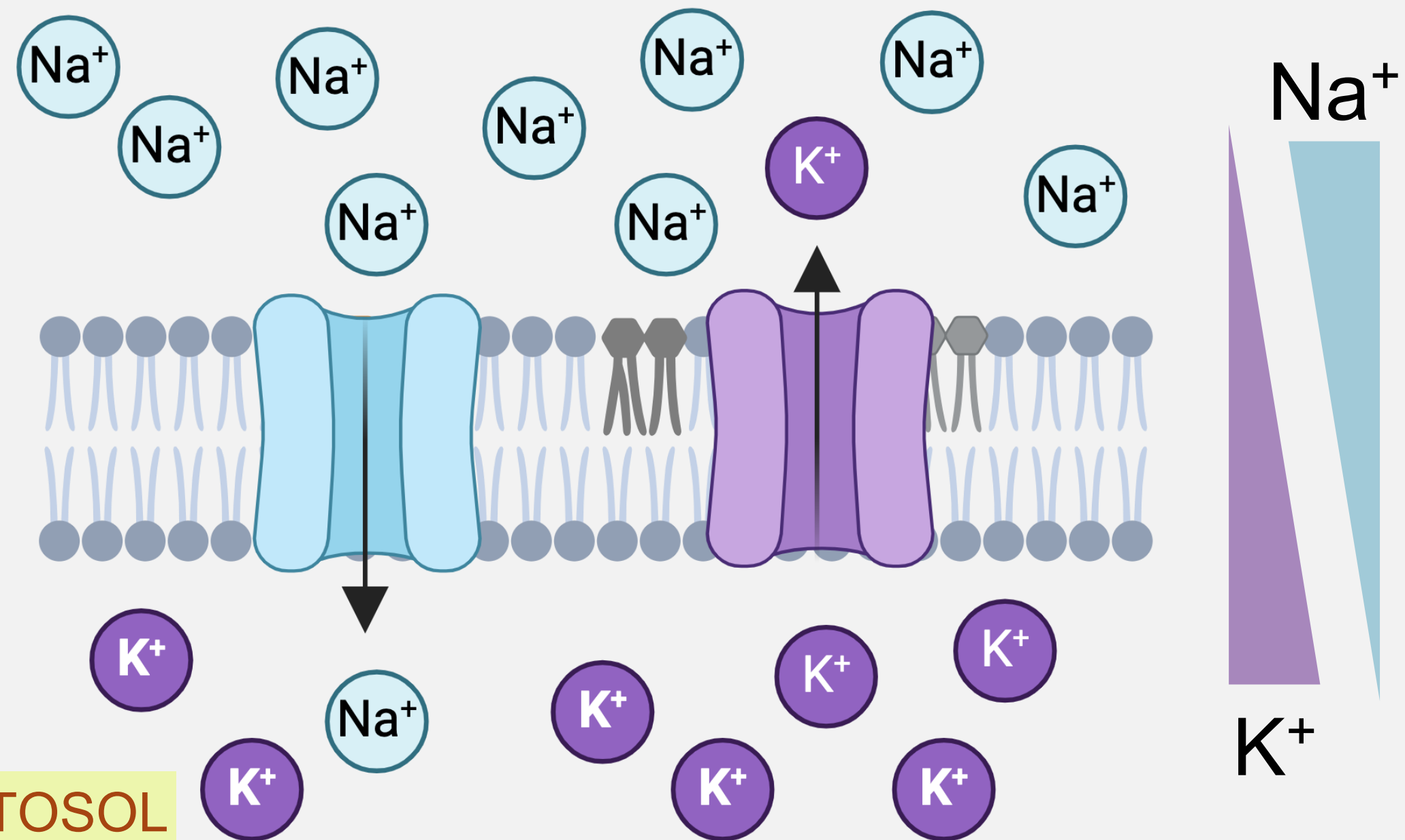
Dr Anees P.  
@IISER -T

Anees, P., ....YK 2024, Nat. Biotechnol.

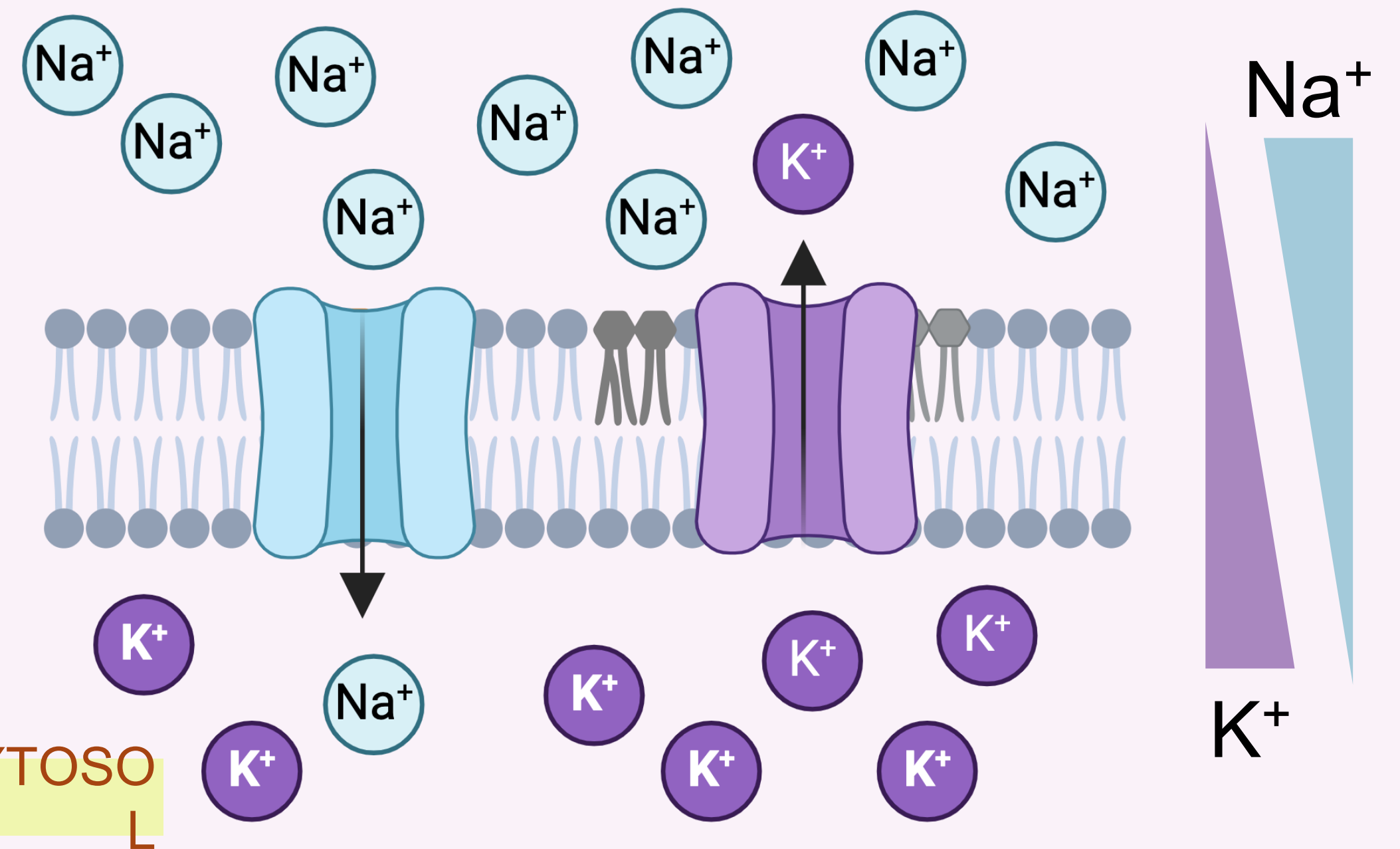
# Plasma membrane membranes

# Organelle

EXTRACELLULAR SPACE



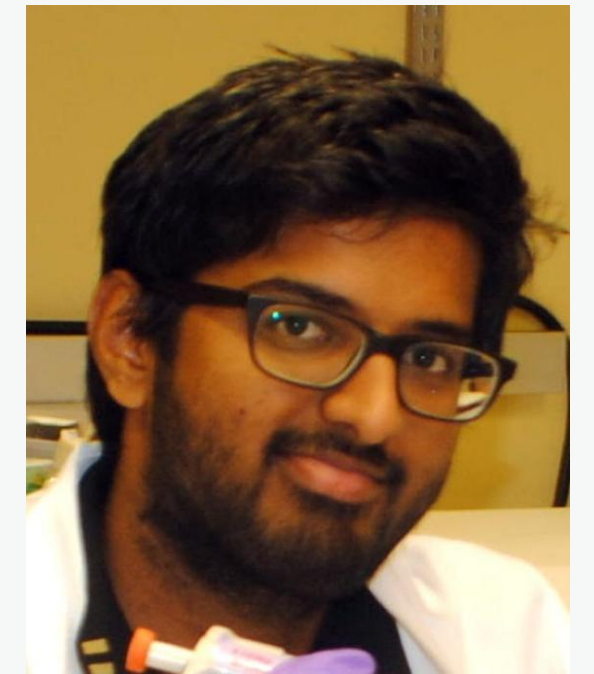
ORGANELLE LUMEN



- o Ion gradient =  $[Ion]_{IN} - [Ion]_{OUT}$ 
  - o Ions must **move**
  - o electricity!

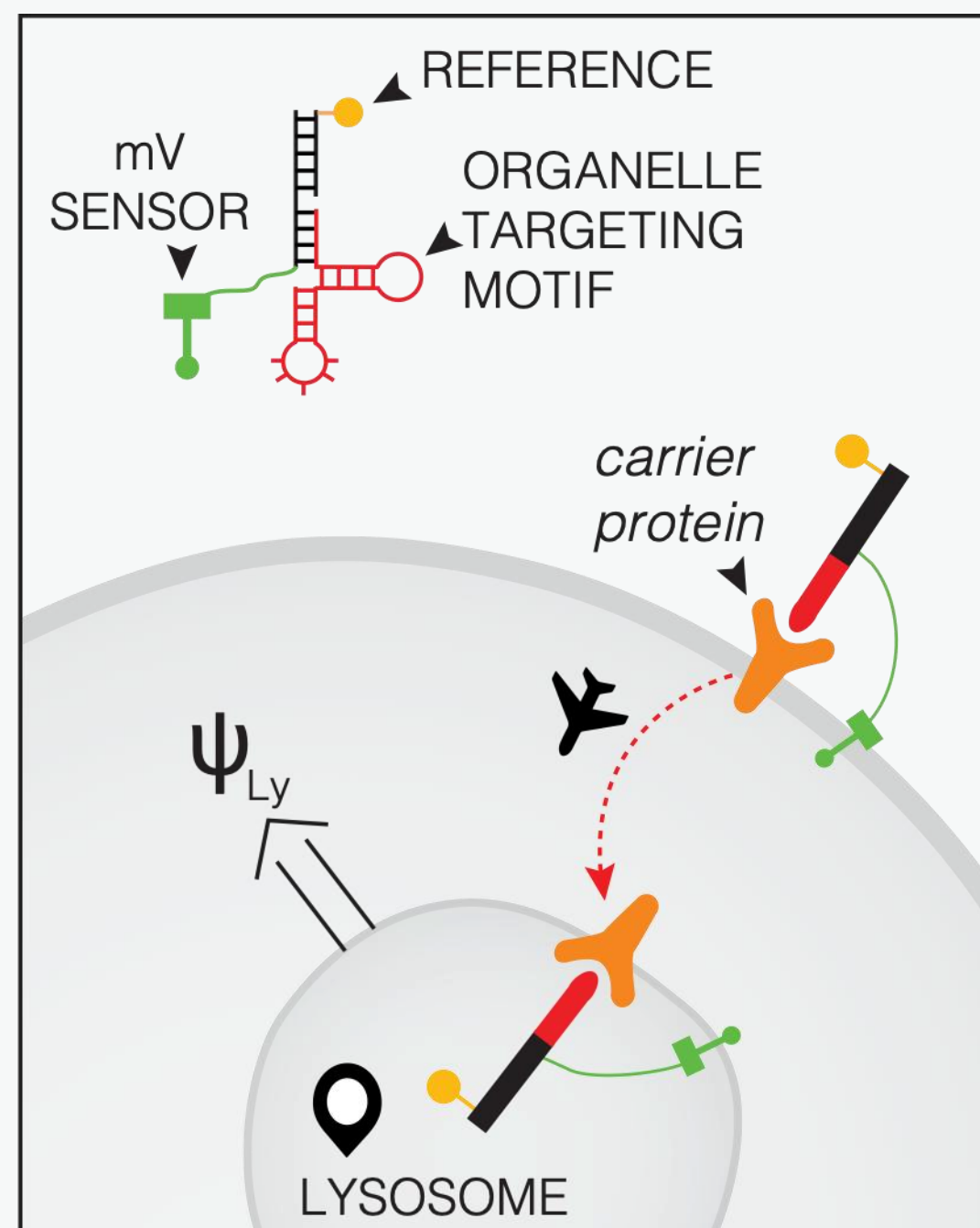
- o Ion gradients ✓
- o Ion transport mechanism ✓
- o electricity ?

# A DNA-based voltmeter for organelles



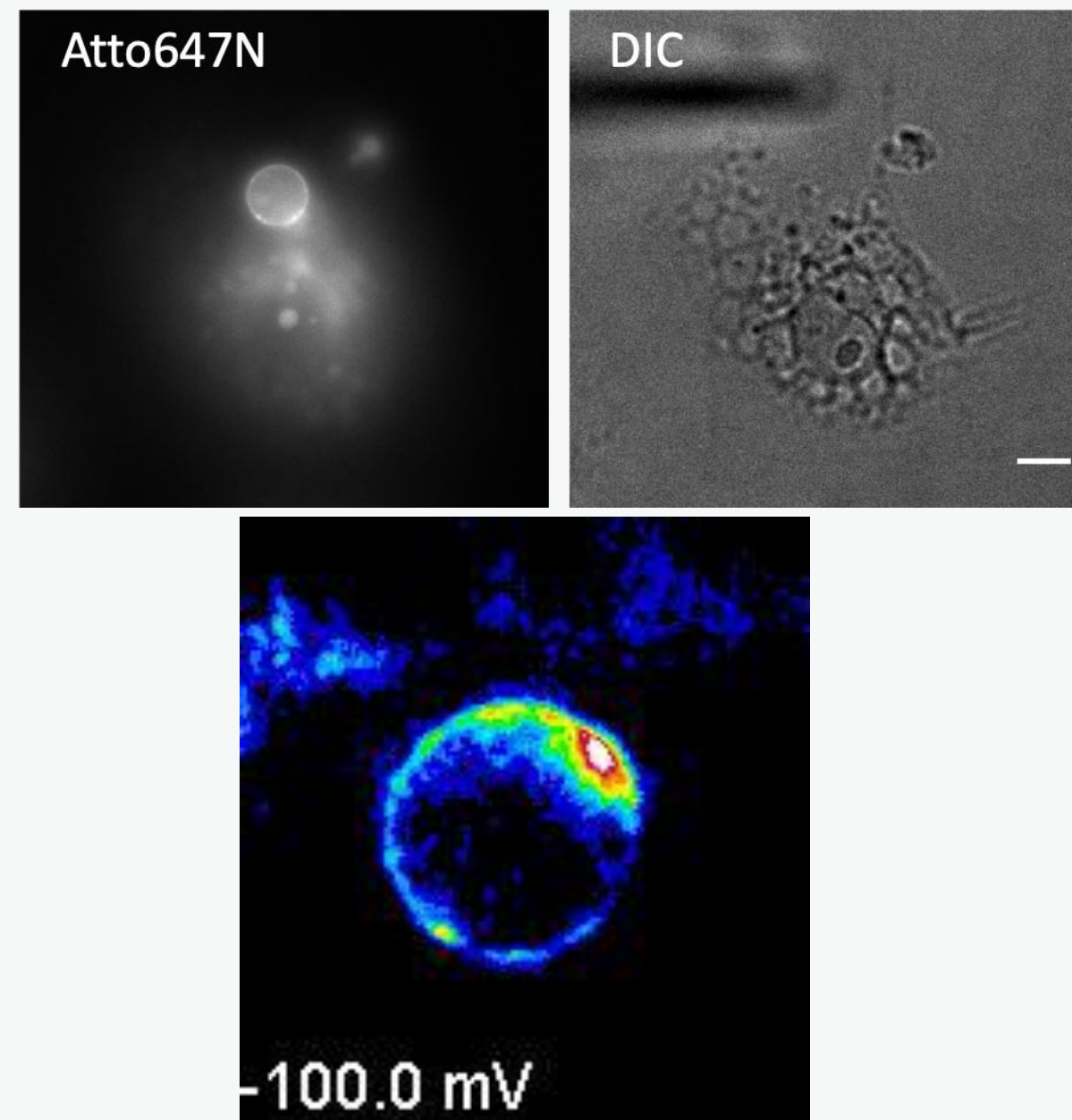
Dr Anand Saminathan @HMS

*organelle-targeted voltmeter*

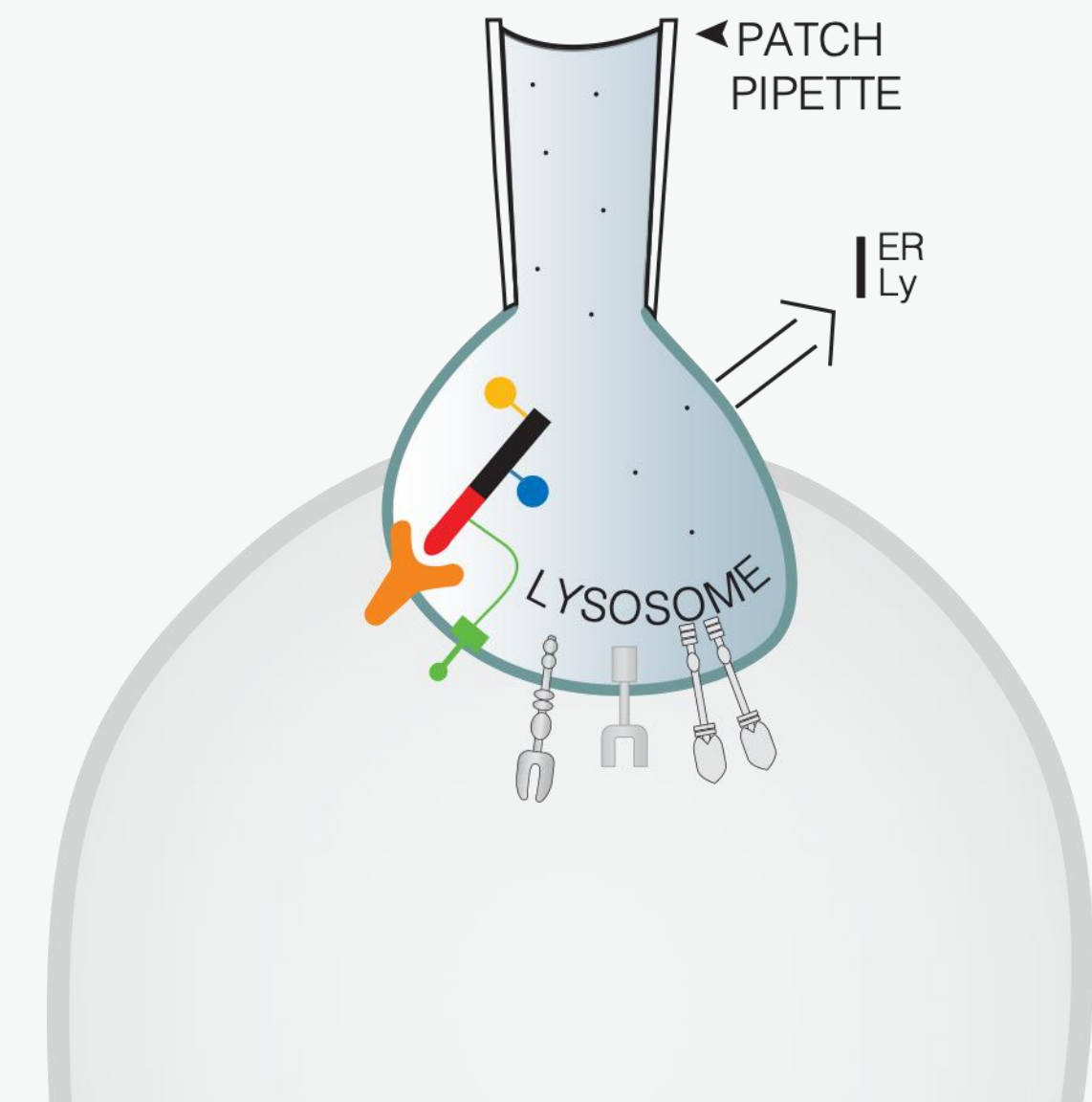


Saminathan, A.,..... YK 2021 *Nat. Nanotechnol.*

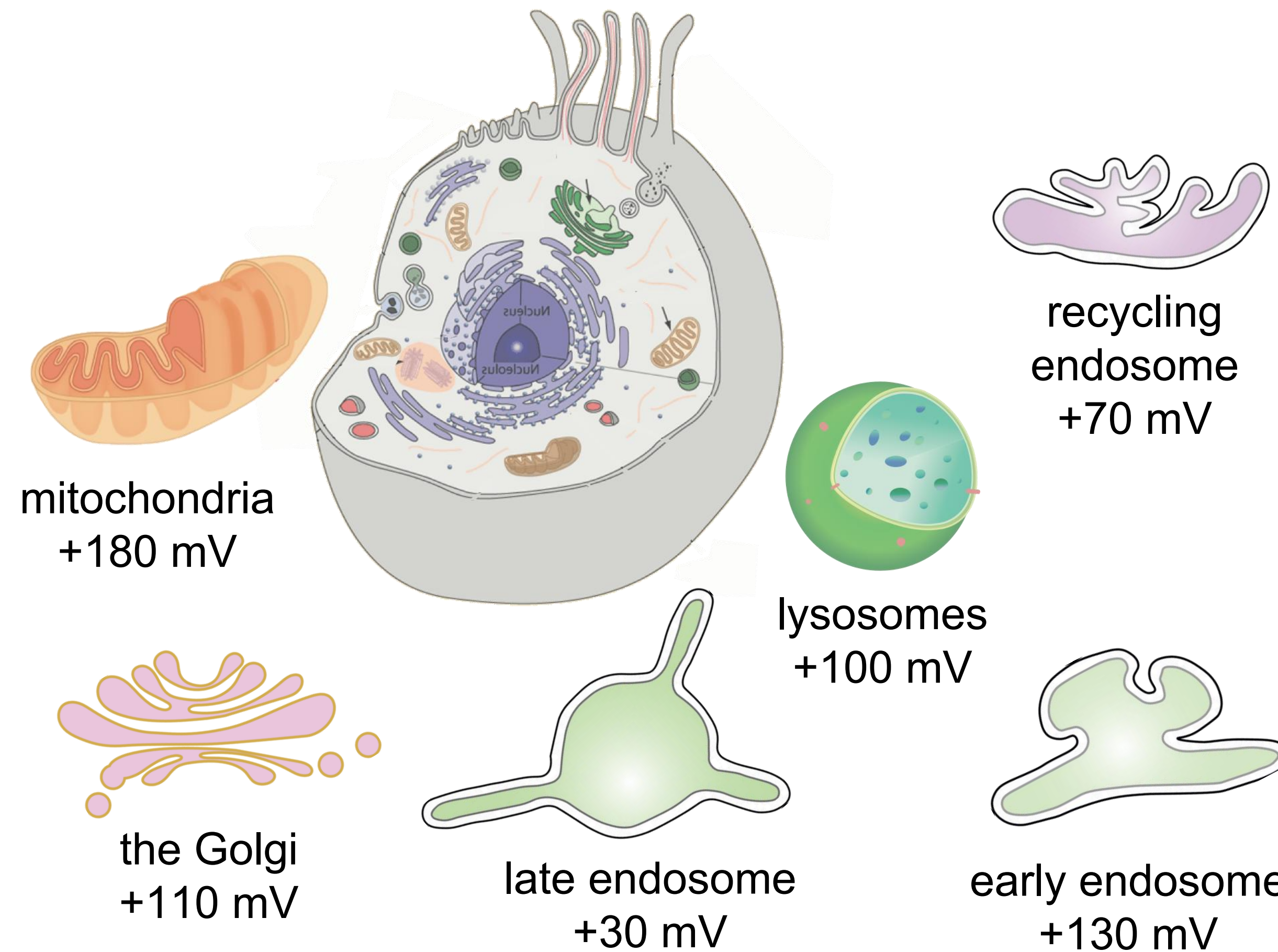
*patch an organelle + image at sub-cellular resolution*



Lysosome patched & voltage-imaged simultaneously

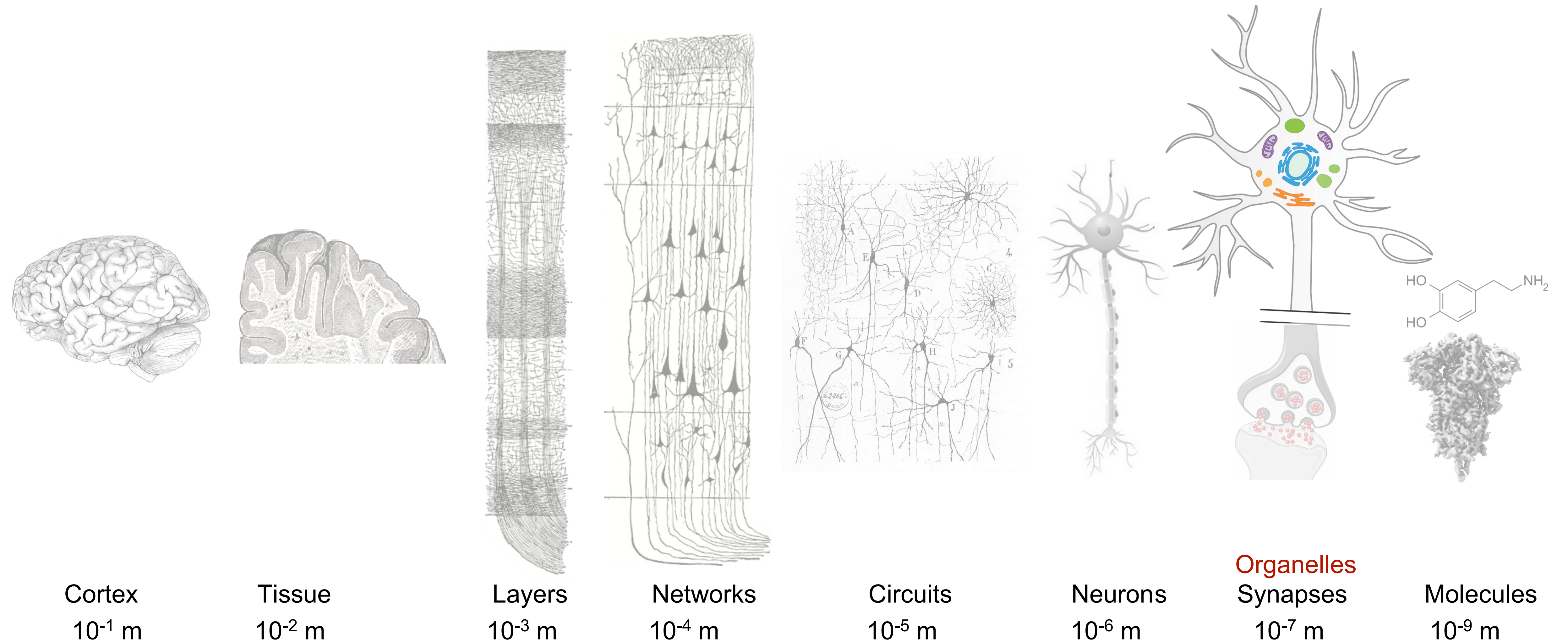


# organelles have electrical behavior

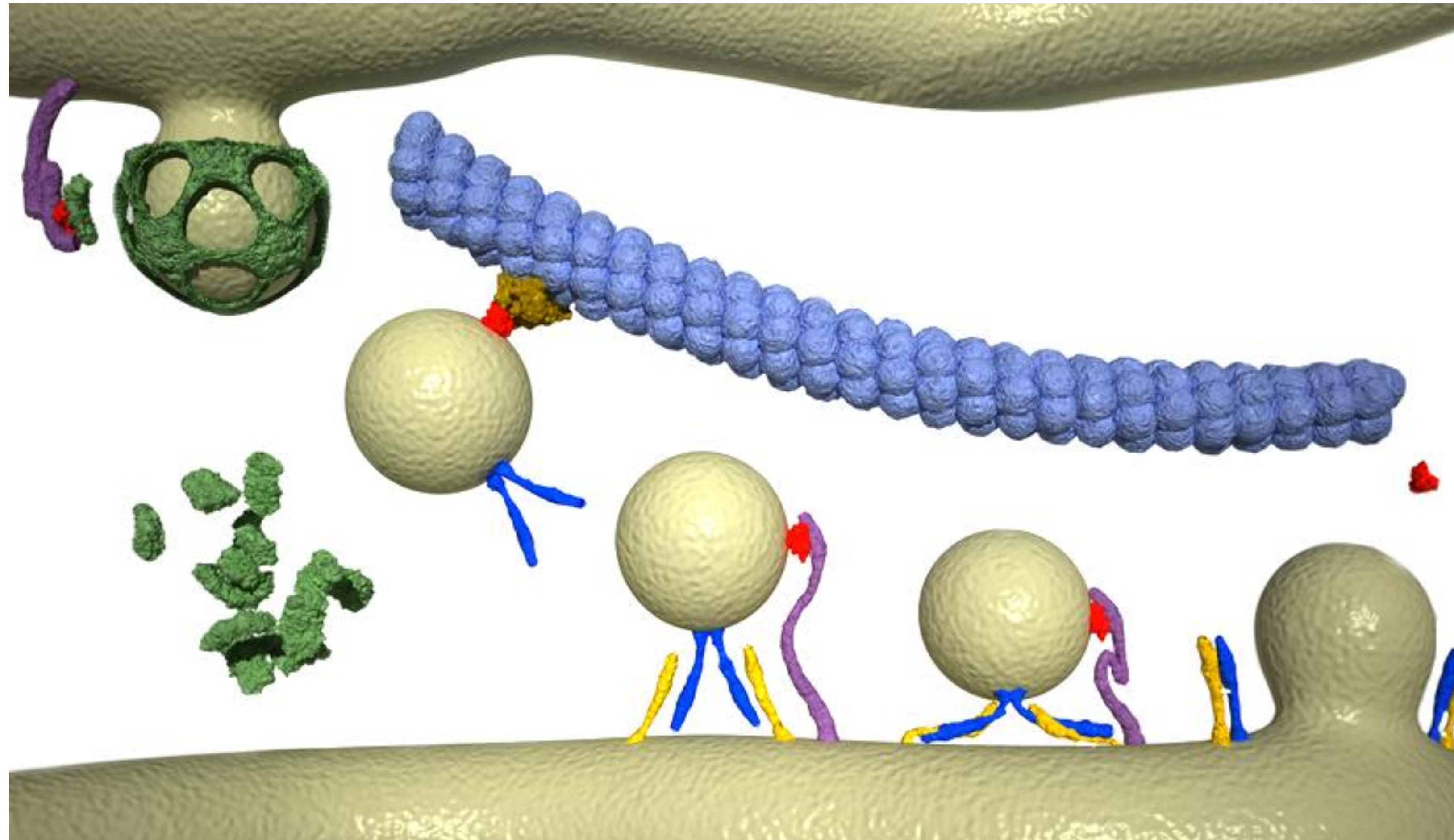


Saminathan, A., et al **2021** Nat. Nanotechnol.

# organelle circuitry: the missing link in our multiscale brain circuitry



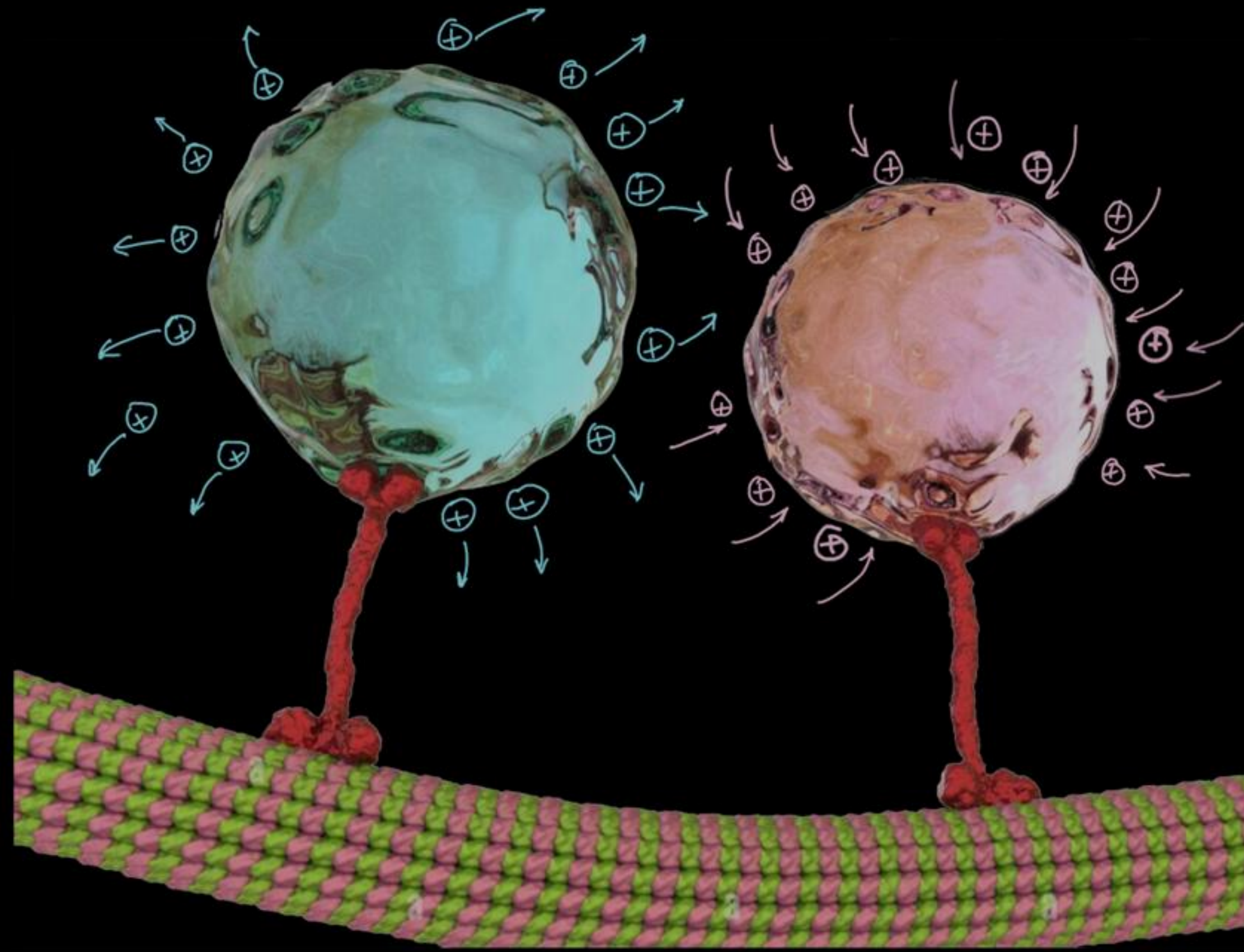
organelle membranes : passive sheaths;  
*proteins* determine organelle fate



# An idea: “fizzing” organelle membranes communicate luminal status to the cytosol

Organelle exists to  
do biochemistry on  
luminal cargo

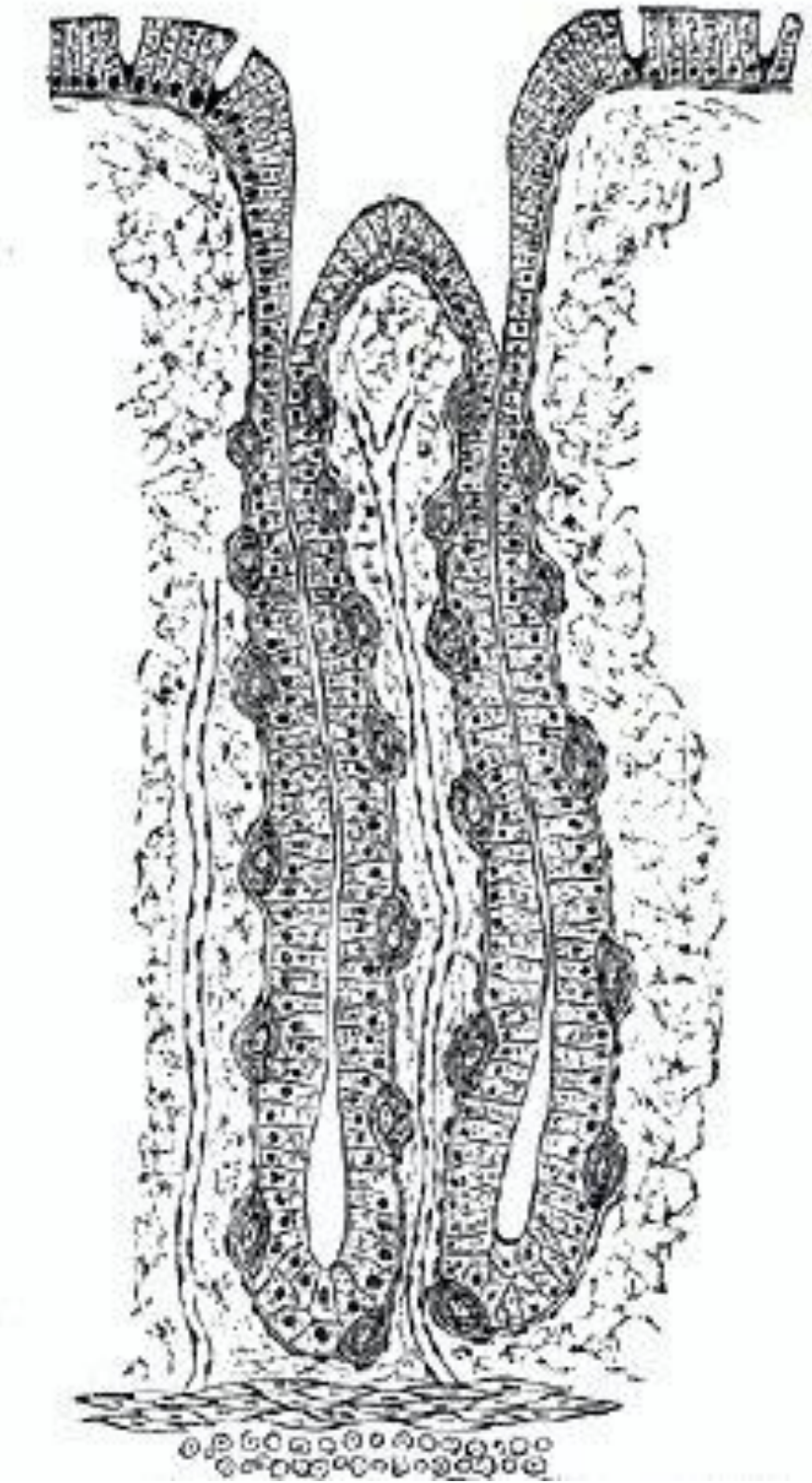
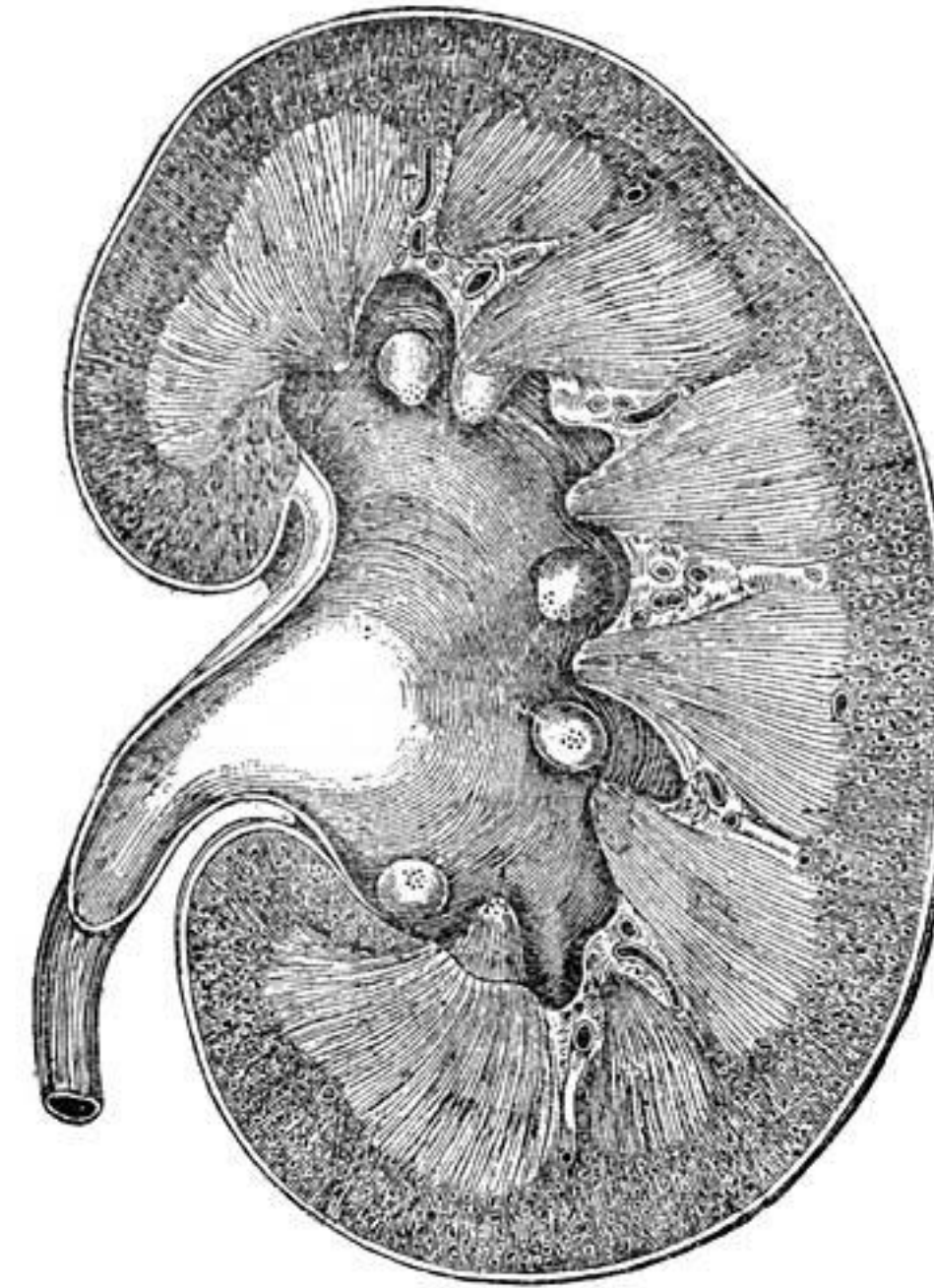
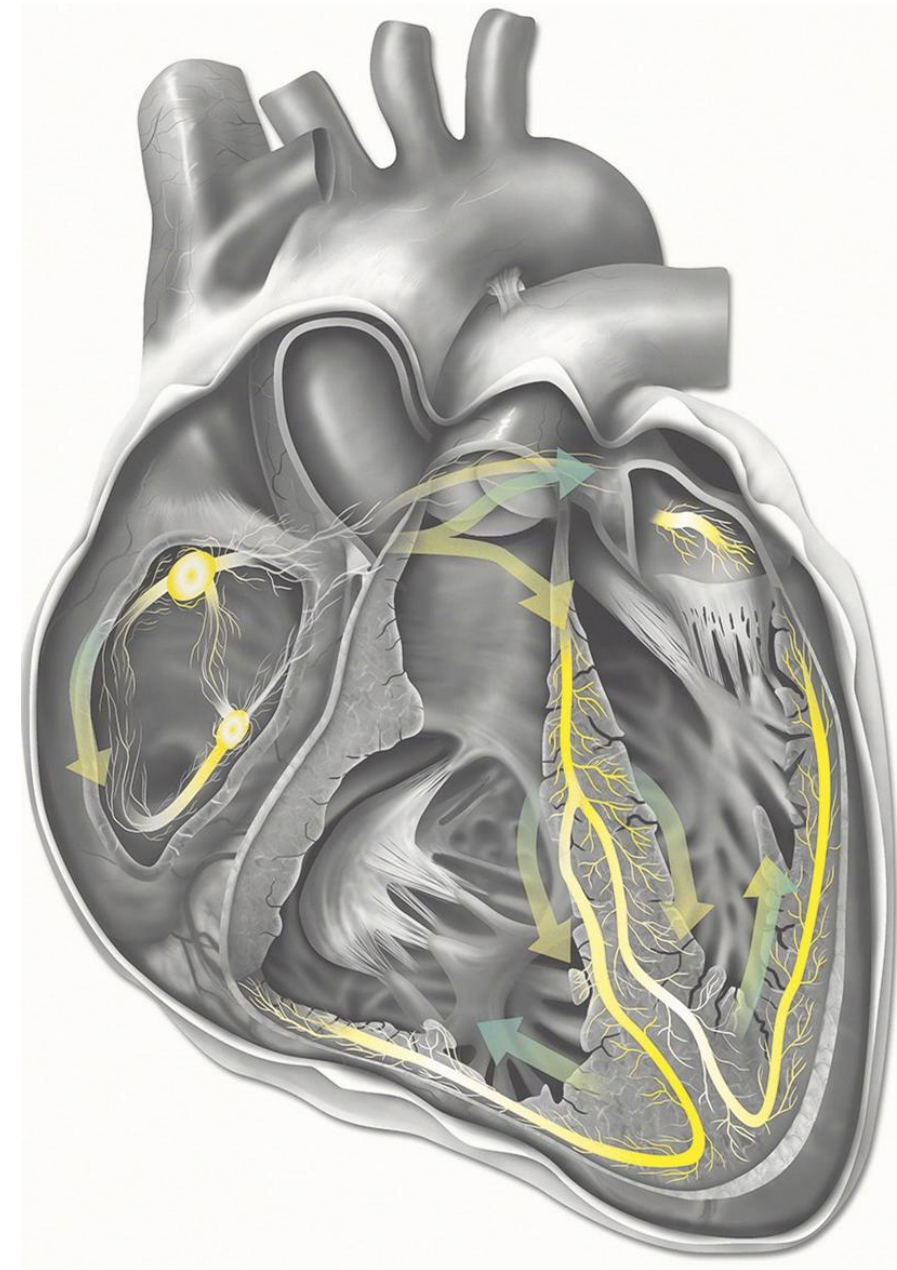
cargo must be  
ready for next step



how is readiness  
conveyed?

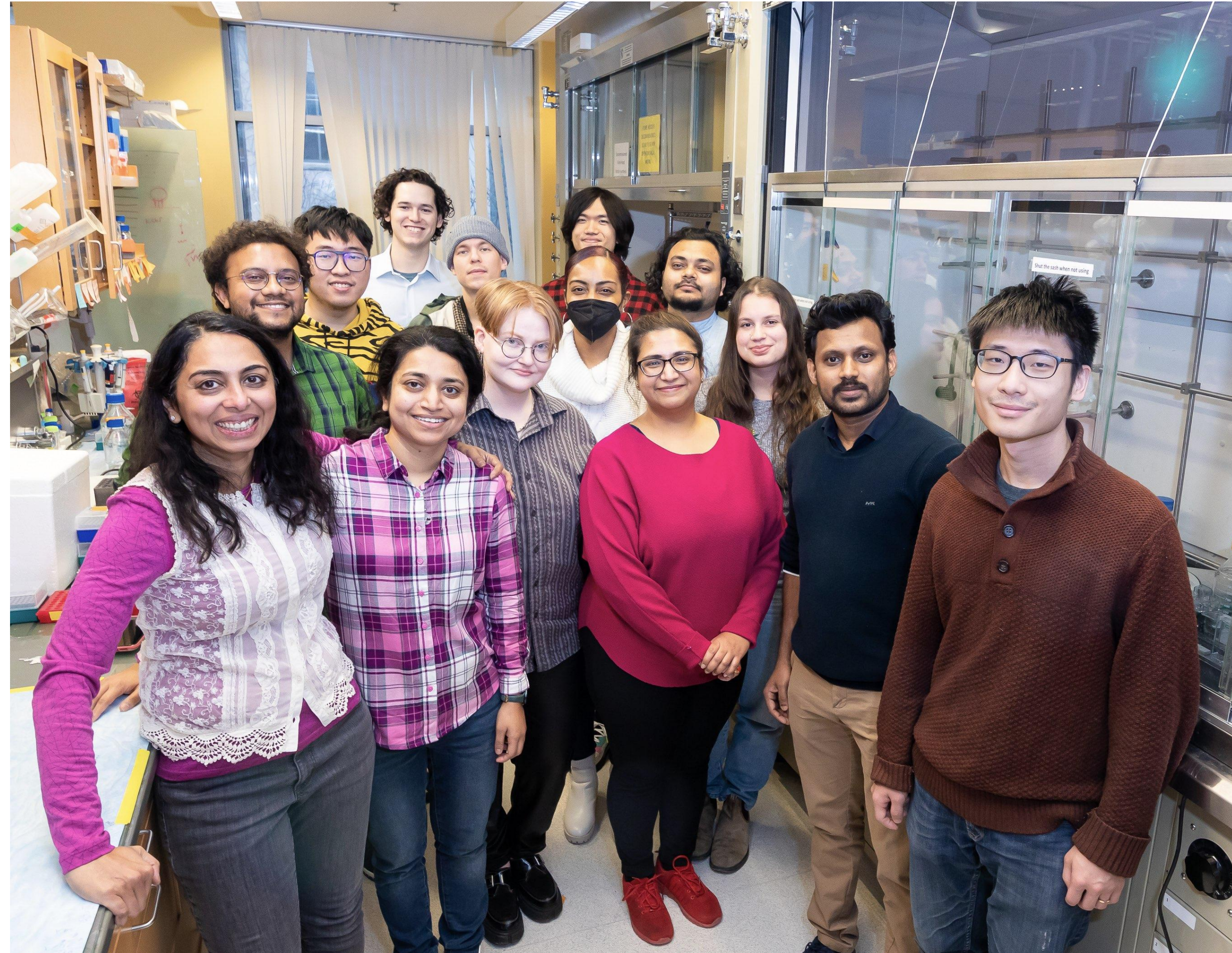
ions could convey  
luminal status

“It’s turtles electricity all the way down.”



# THE GROUP

JoAnn Tinker  
Benton Girdler  
Sangyoon Lee  
Rui Fu  
Avantika Negi  
Alok Apan Swatiputra  
Sandip Chakraborty  
Priyanka Duttagupta  
Yuxin Liu  
Aditya Venkatraman  
Suruchi Sharma  
Rashmi Chandra  
Junyi Zou\*  
Vanity Spruill

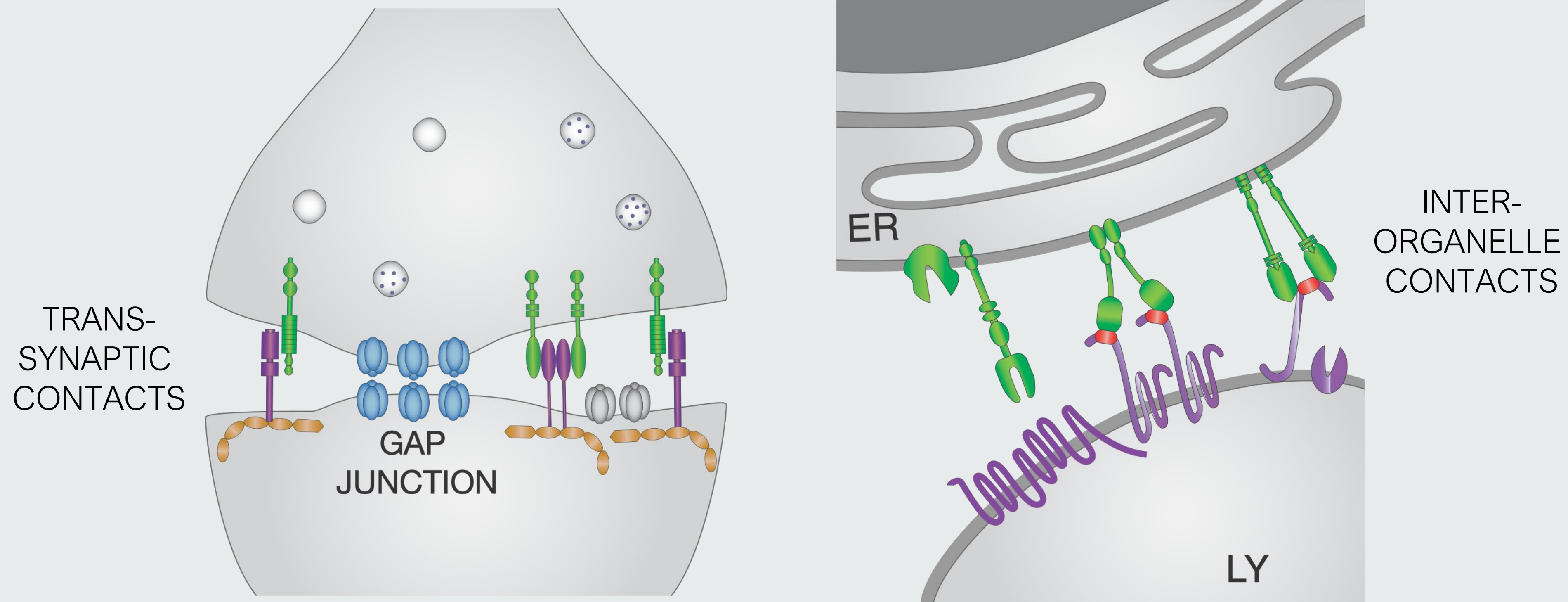


DP1GM149751  
R01GM147197  
R01 NS112139  
R01 HL149993  
R21 HL161825  
R15 GM140392



*YK is co-founder and CSO of Esya Inc & MacroLogic Inc that use DNA nanodevices for diagnostics & therapeutics respectively*

# inter-organelle contacts resemble neuronal synapses



electrochemical state  $\rightarrow$  local  $[M^+]$  increases on cue  $\rightarrow$  ions exchanged

*what will be the insight?*