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FREE ENERGY ANALYSIS OF SELECTIVE CATION TRANSPORT IN CHANNELRHODOPSIN CHIMERA, C1C2

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U.S. DEPARTMENT OF ENERGY | NNSA National Nuclear Security Administration

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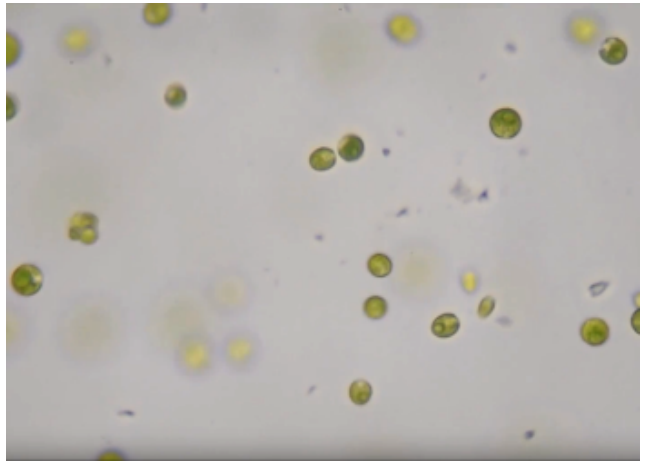
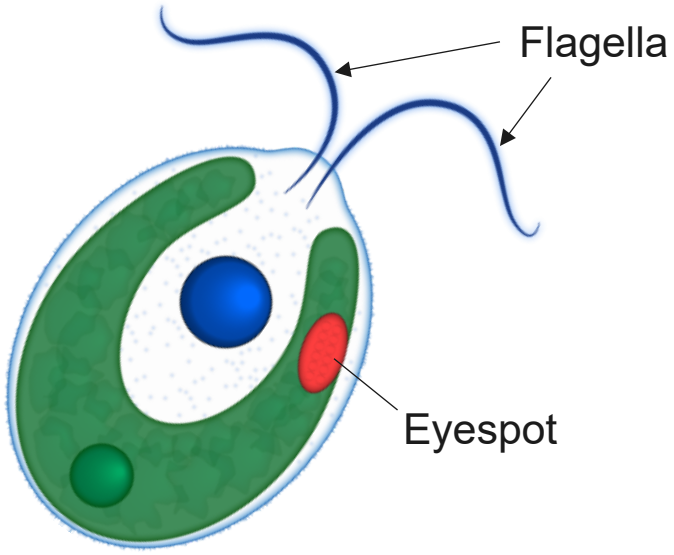
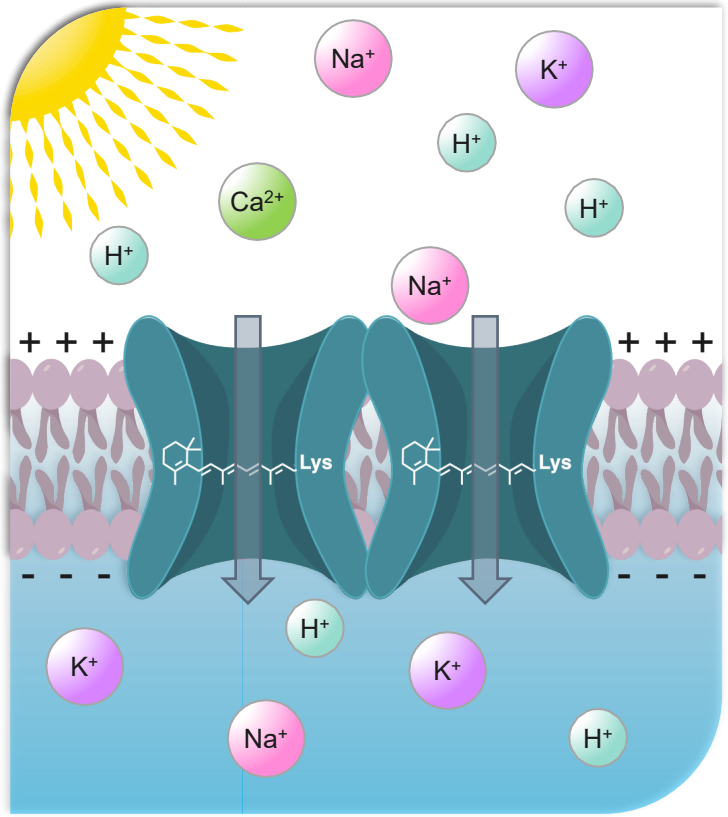
CHANNELRHODOPSINS –LIGHT-GATED ION CHANNELS



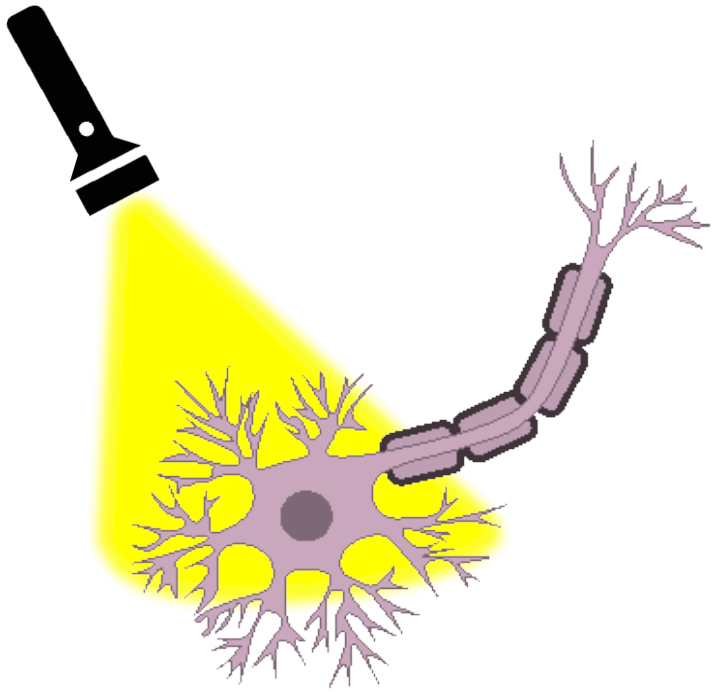
Sineshchekov et al., *PNAS* **99,** 8689–8694 (2002).

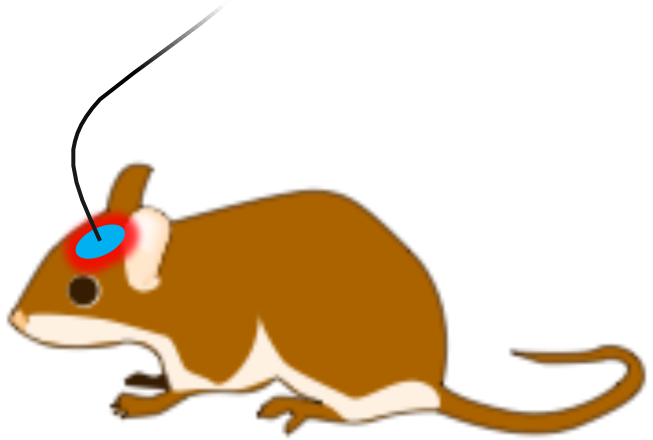


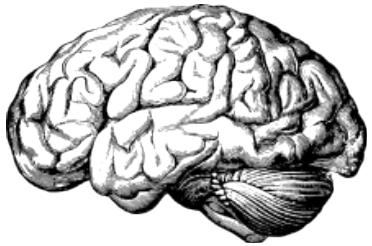


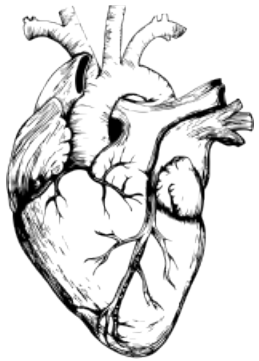
- ***Chlamydomonas reinhardtii***
  - Single-celled alga
  - Well-studied
- **ChR1 & ChR2**
  - Eyespot
  - Initiates phototaxis
  - Nonselective for cations: $H^+ >> Li^+ > Na^+ \approx K^+ > Ca^{2+}$
  - Rapid membrane depolarization & activation of voltage-gated Ca^{2+} channels → flagella move

OPTOGENETICS APPLICATIONS









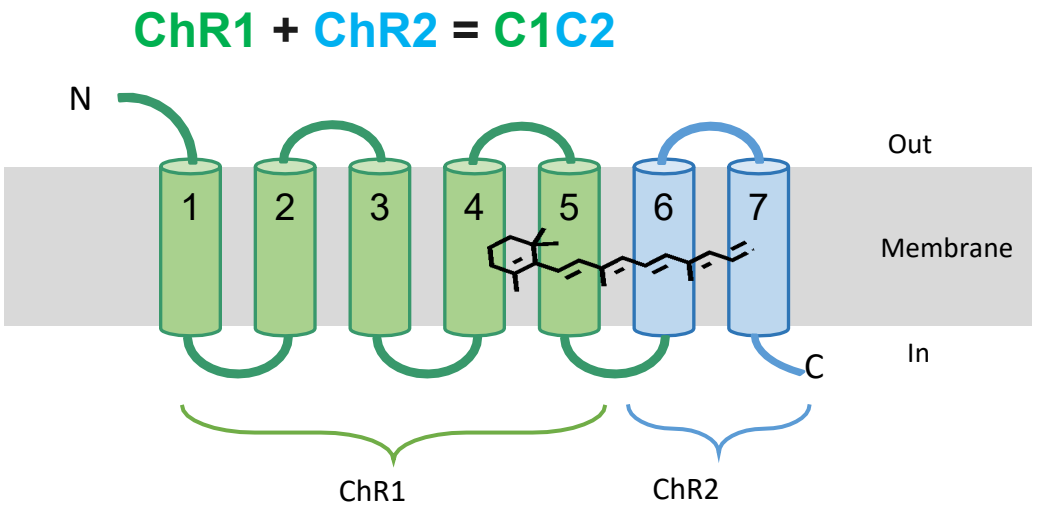
- Control neurons w/ light
- Targeted

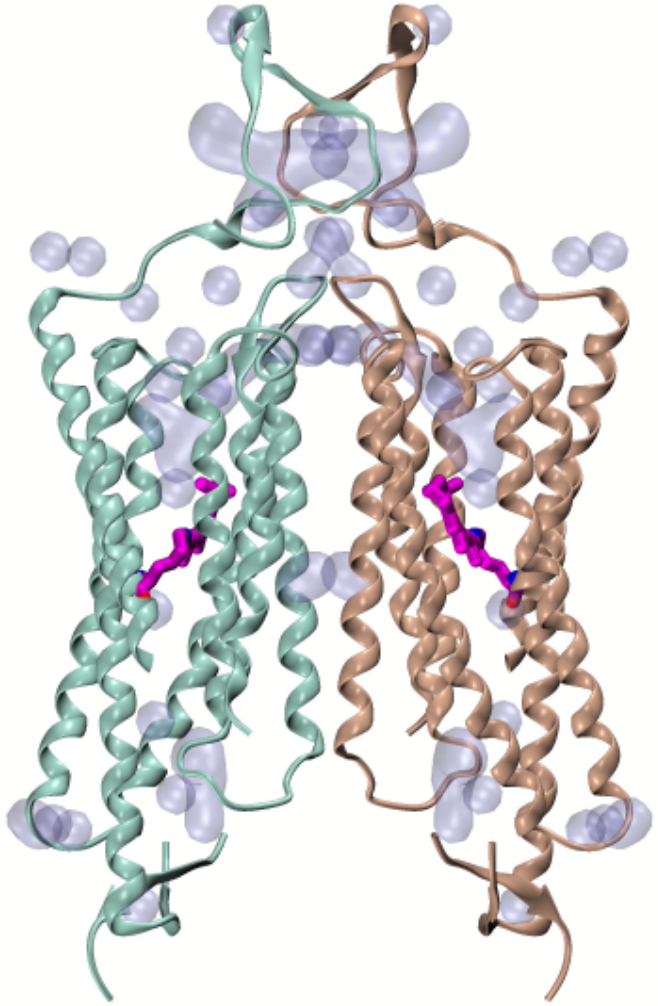
- Map functional neuronal circuits
- Live organisms

- Apply clinically

CHANNELRHODOPSIN CHIMERA (C1C2)

- First ChR crystal structure (2012)
- Few functional studies vs ChR2
- Challenge: No open state structures

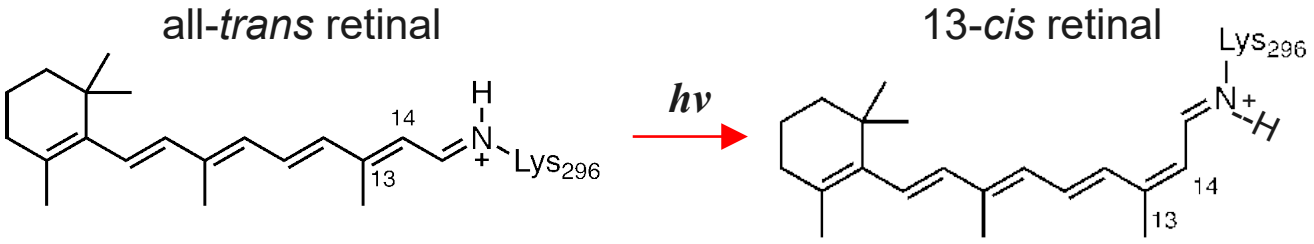


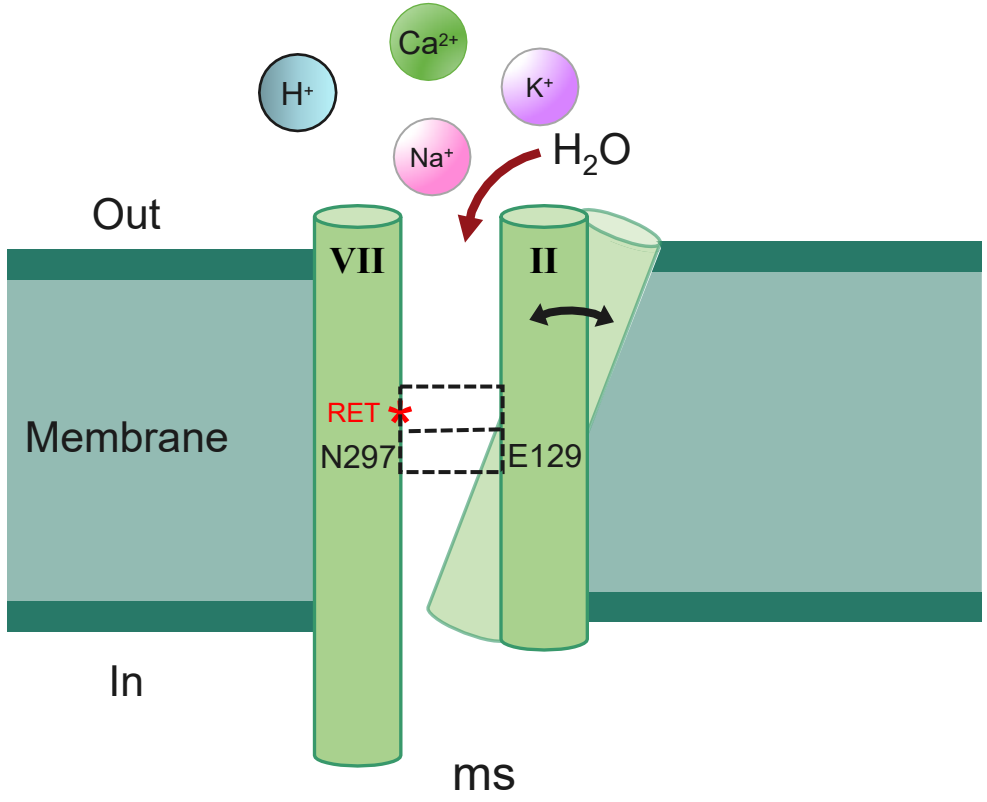


PDB ID: 3UG9

Kato *et al. Nature* **482,** 369–374 (2012).

INFERRED ACTIVATION MECHANISM: HELIX-II-TILT MODEL

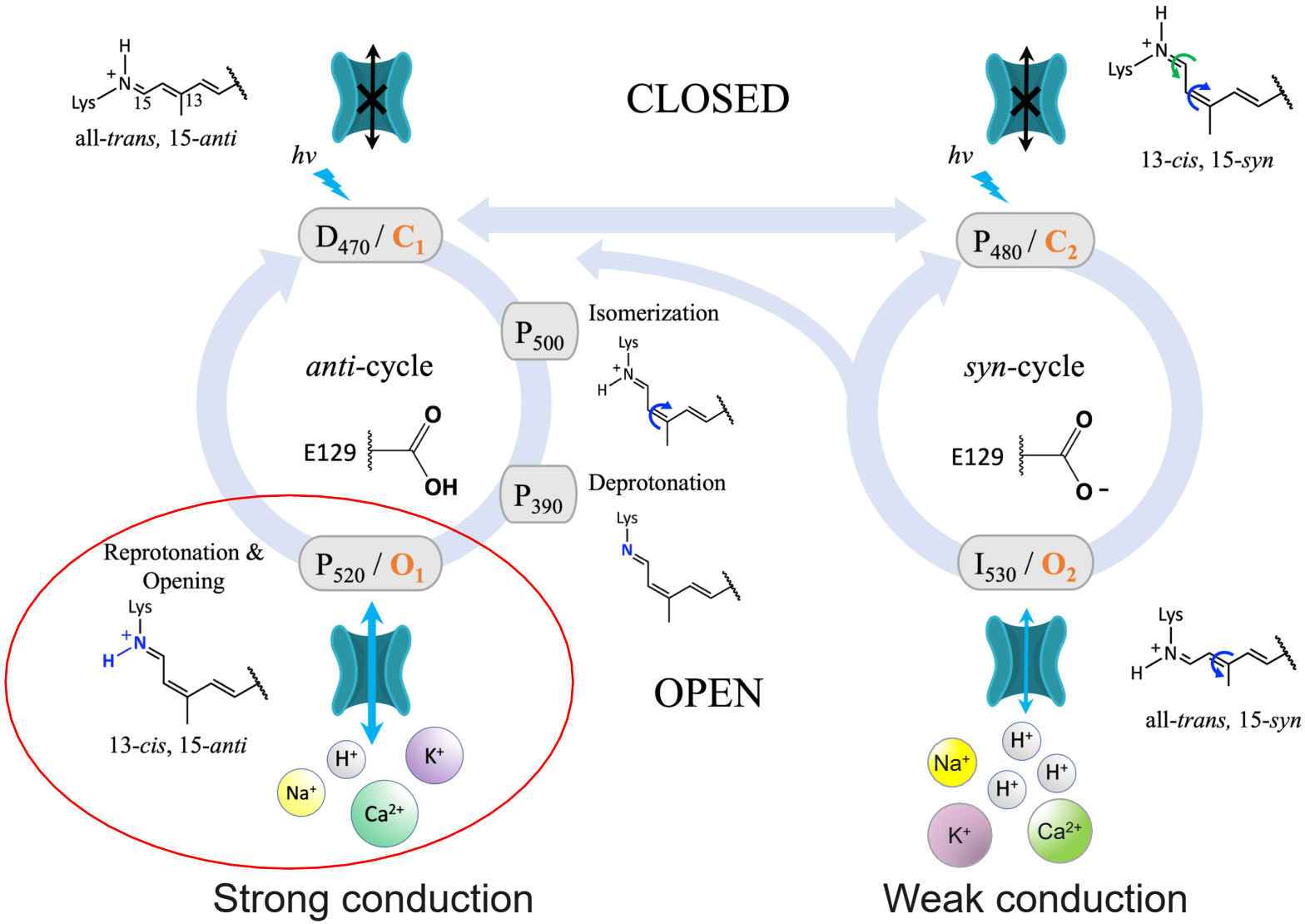




1. Photoisomerization of retinal
2. Proton transfer reactions
3. Helix II tilts & channel opens
4. Water & ion influx

Kuhne, *et al. PNAS* **2019**, *116* (19), 9380.

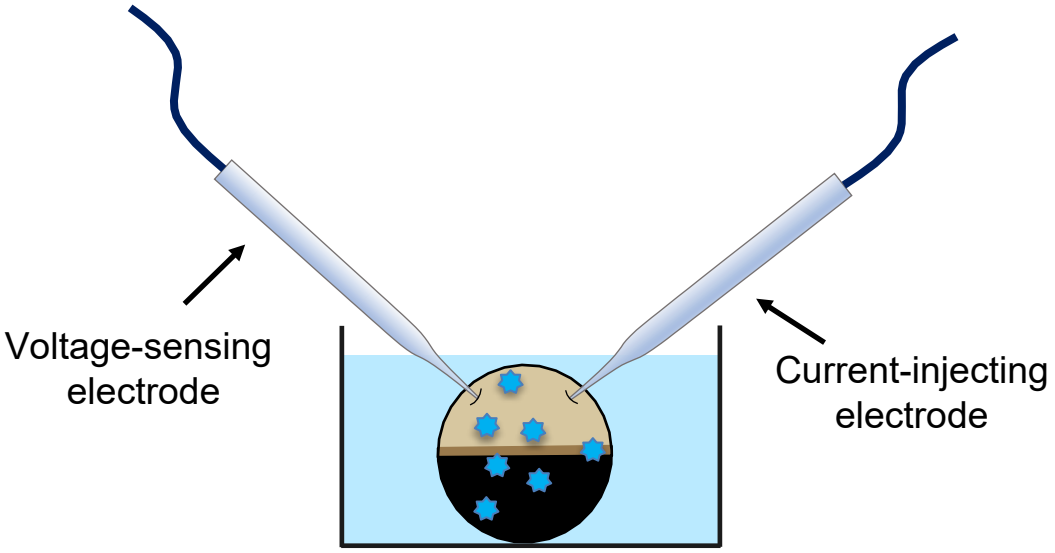
CHR PHOTOCYCLES



Kuhne, *et al. PNAS* **2019**, *116* (19), 9380.

GOALS & APPROACH

- Conduction mechanism: Na^+ vs Ca^{2+}?



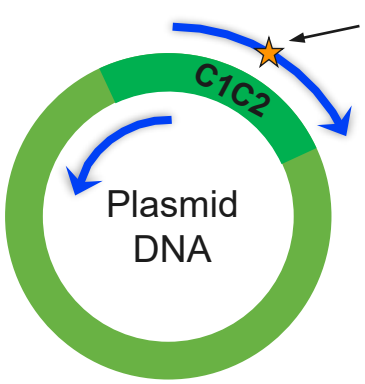
Electrophysiology (IV)

Molecular dynamics (***F***=***ma***)

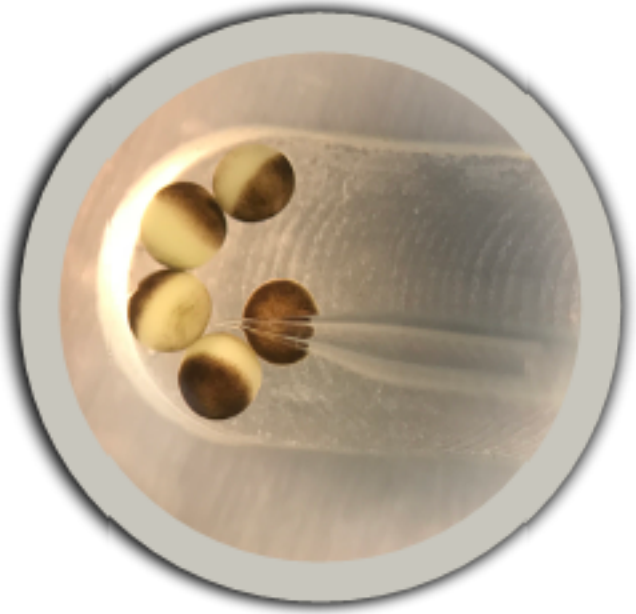
STEP 1: EXPRESS C1C2 IN FROG OOCYTES

African clawed frog

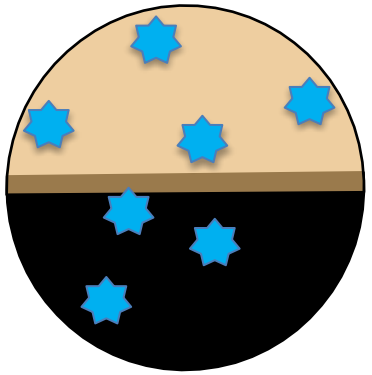
PCR-based
site-directed
mutagenesis



mRNA
synthesis



Expression

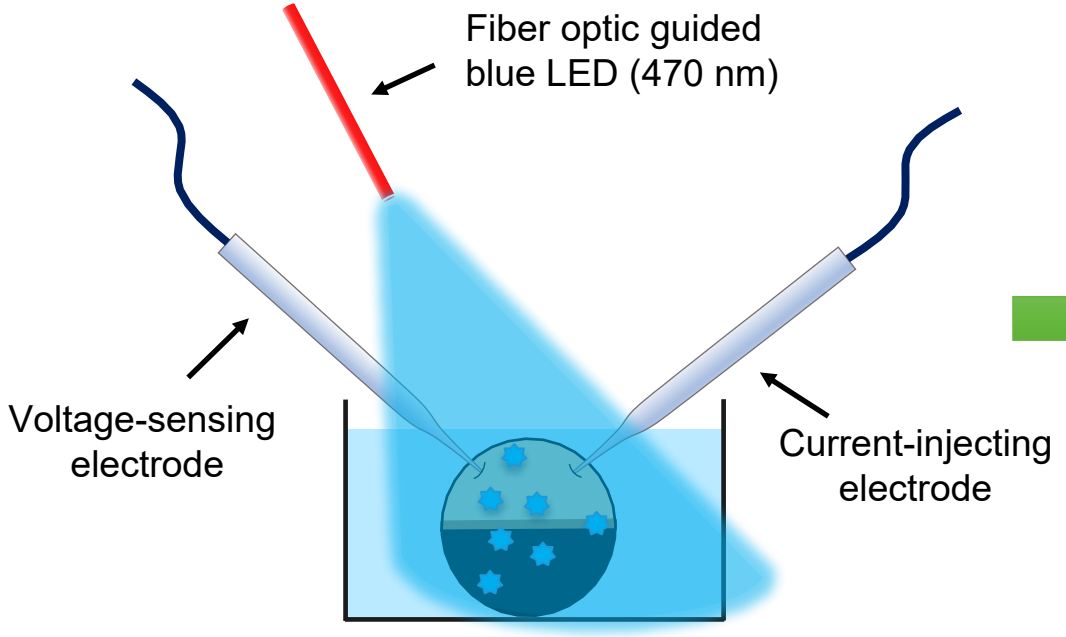


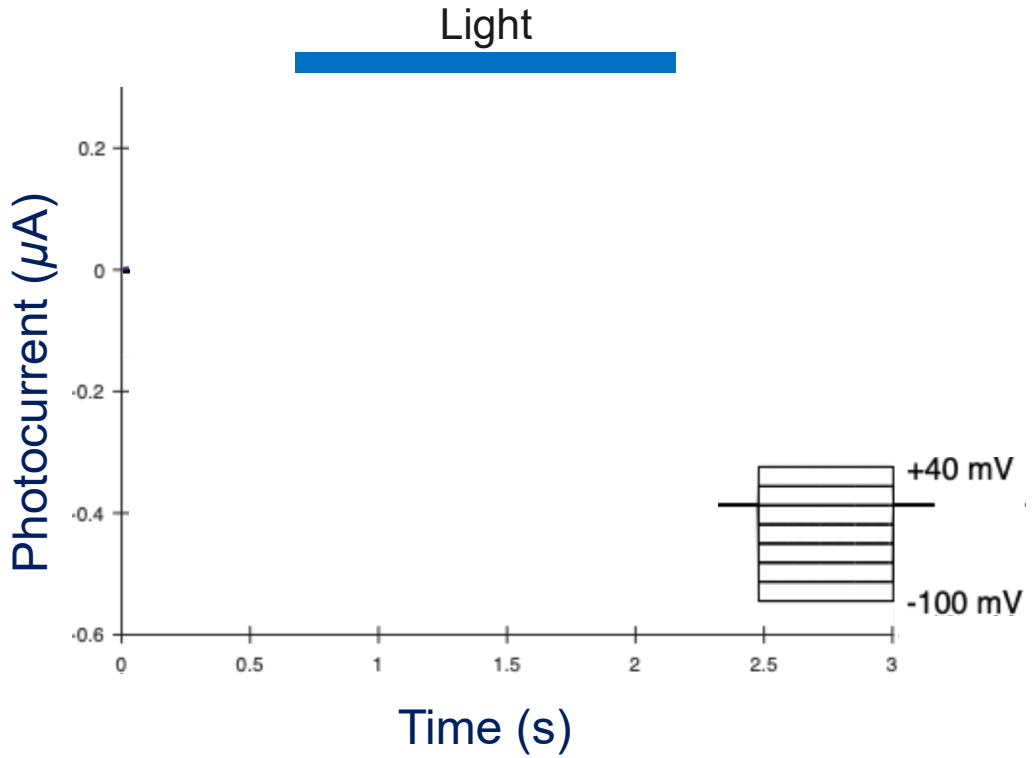
Timing!

TWO-ELECTRODE VOLTAGE CLAMP

- Directly measures whole-cell current across membrane
  - Conductance of ChRs too small for single-channel recordings (~40 fS)
- Oocytes have no endogenous light-activated channels
  - Current recorded through ChR only

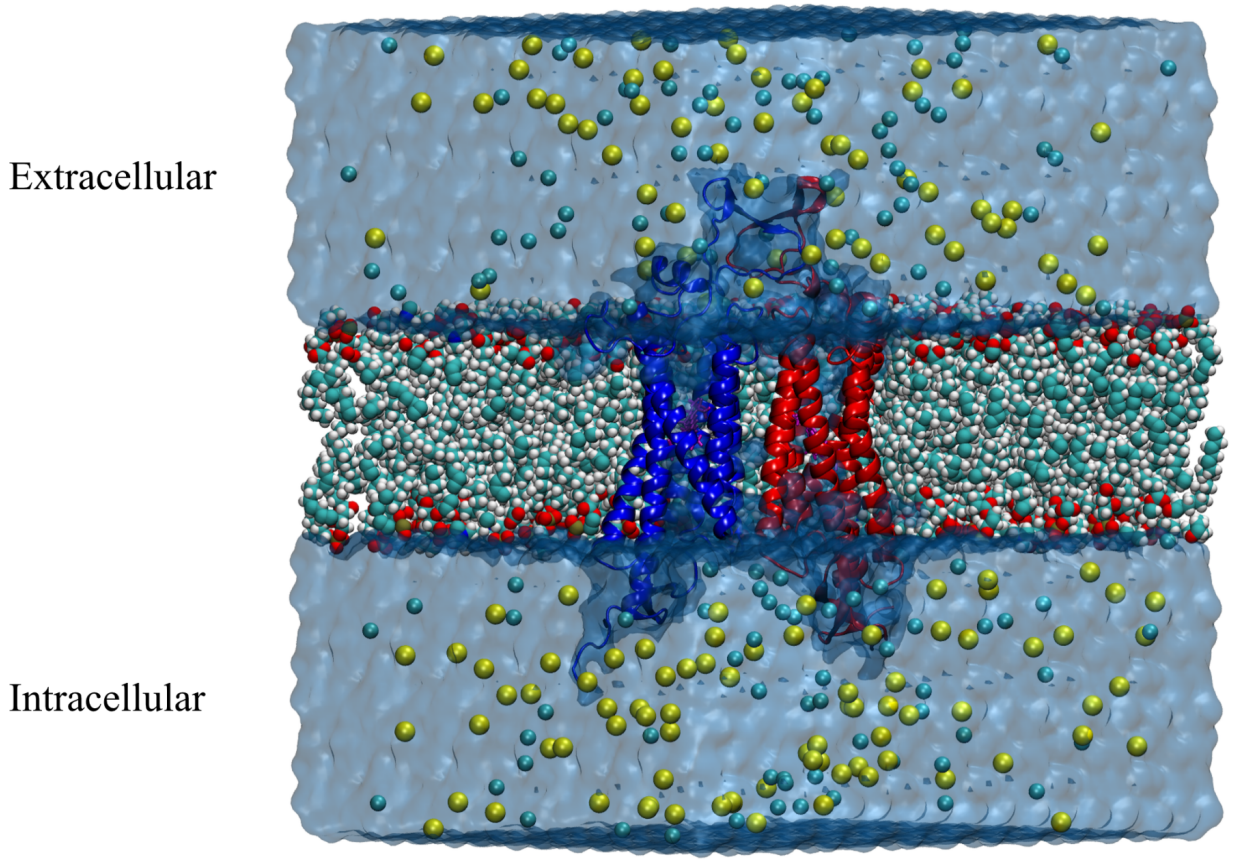
- Selectivity (P_{Na}/P_{Ca}) - peak current





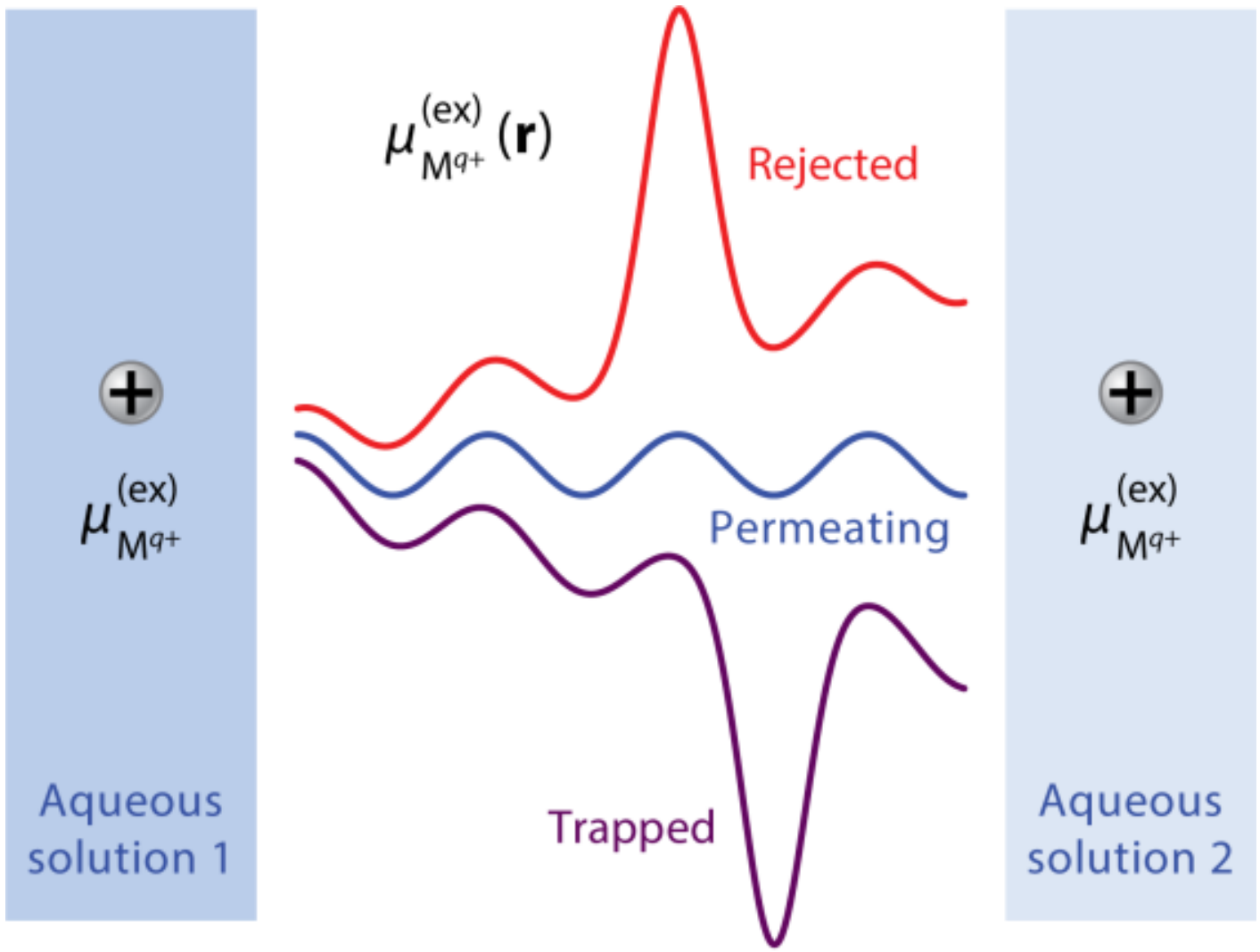
Prignano, L.; Herchenroder, L.; Dempski, R. E. In *Channelrhodopsin*; Dempski, R. E., Ed.; **2021**; Vol. 2191, pp 49.

SIMULATION MODEL



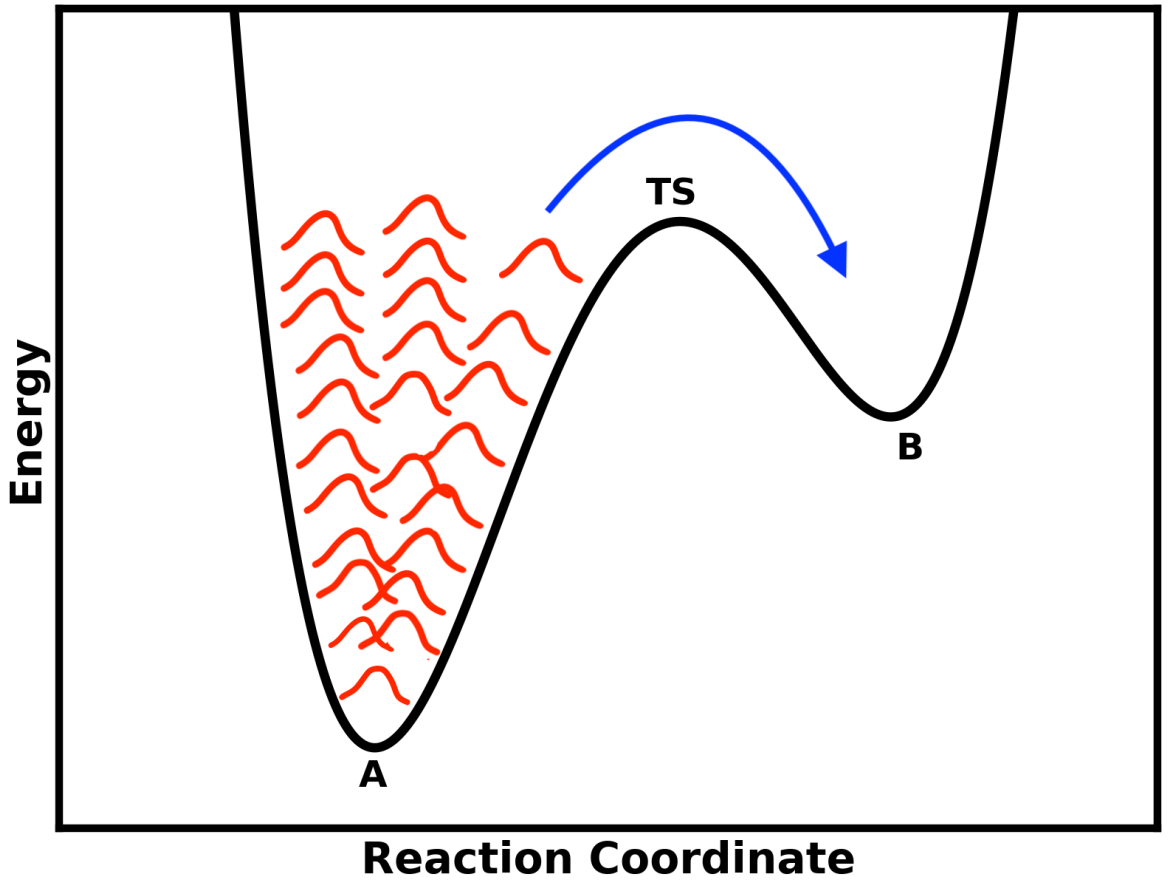
Open State 1 (P_{520}/O1)

FREE ENERGY



Chaudhari MI, et al. 2020.
Annu. Rev. Phys. Chetm. 71:461–84

ENHANCED SAMPLING FOR FREE ENERGY

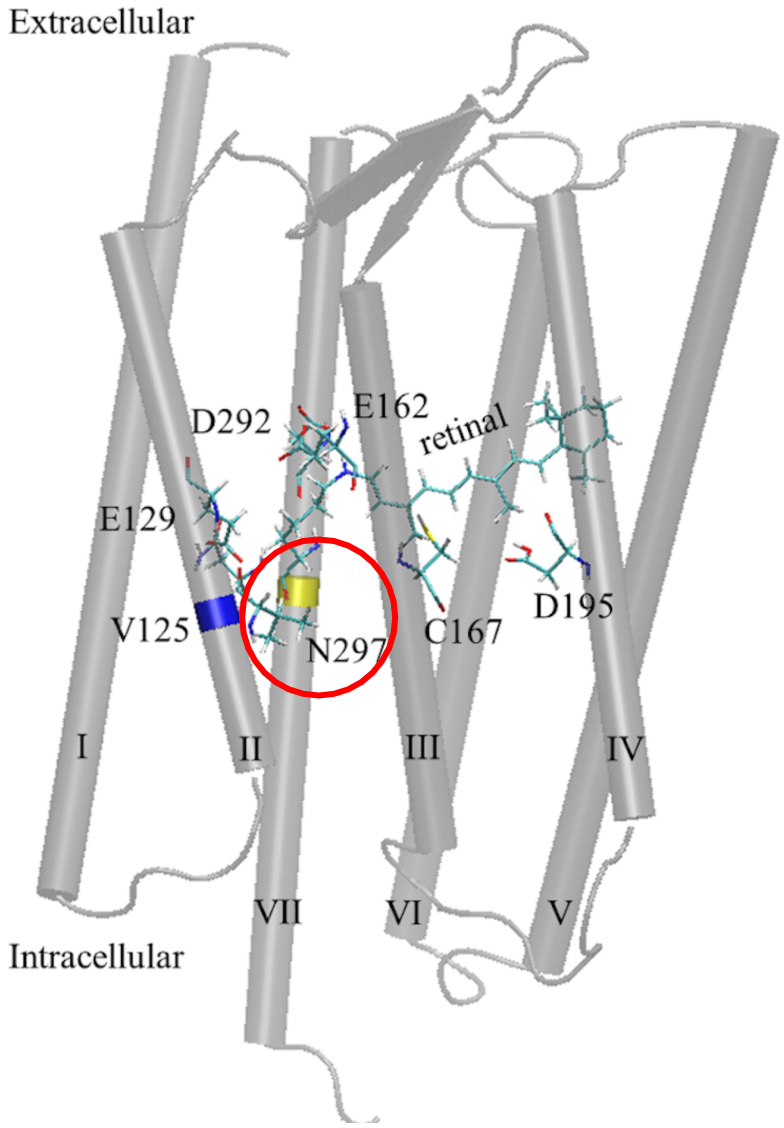


- **Metadynamics** enhances sampling of V(rc)
- Biased V forces system to leave local minima
- Sample low-probability states
- No biases on up/down right/left motions – discover ion path
- **Free energy along discovered rxn coordinate** (PMF)

Poudel

Parrinello & co, *JPCB* (2005)

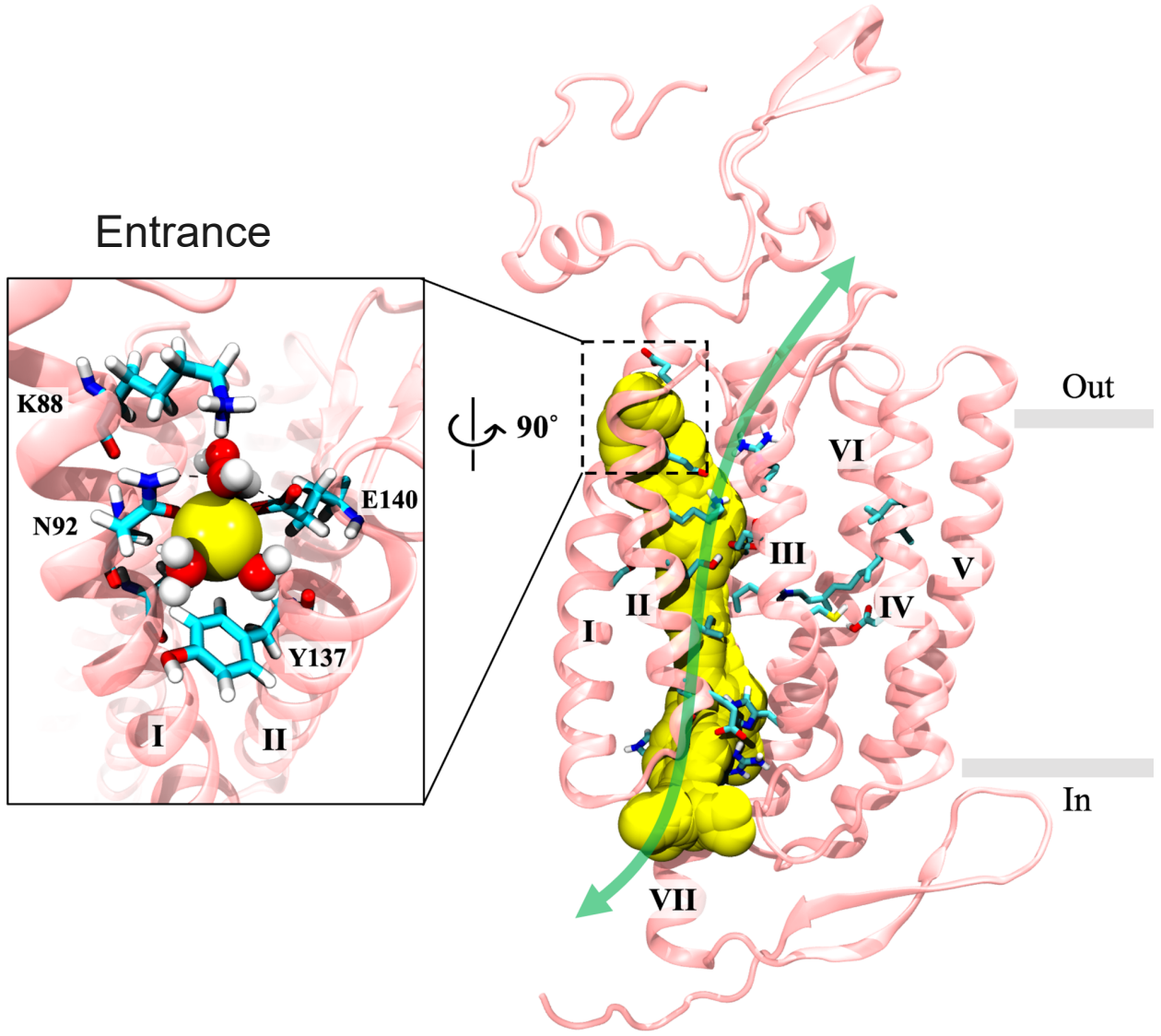
MUTATIONS (N297D)



RESULTS

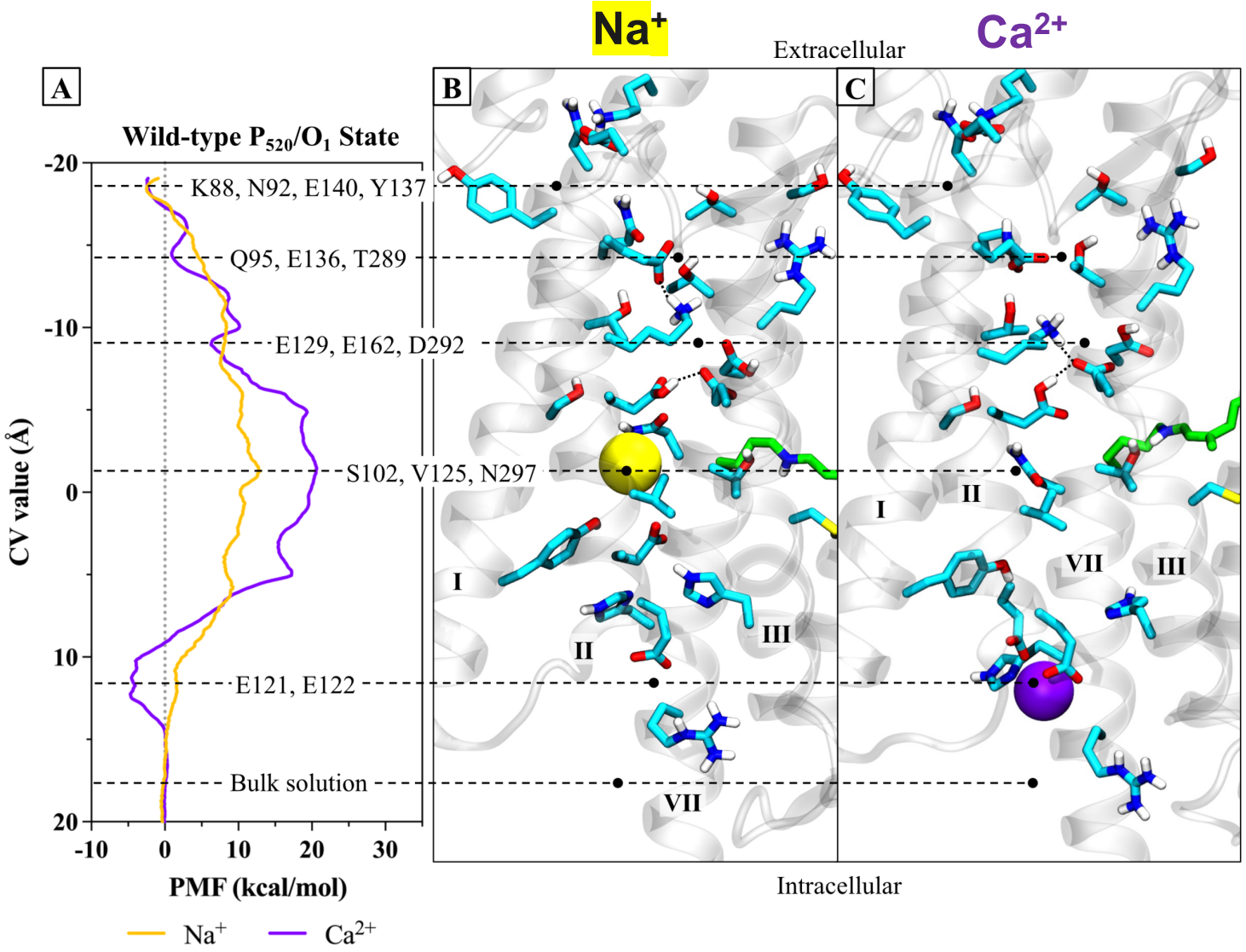
Los Angeles

ION PATH DIFFERS FROM PREDICTIONS



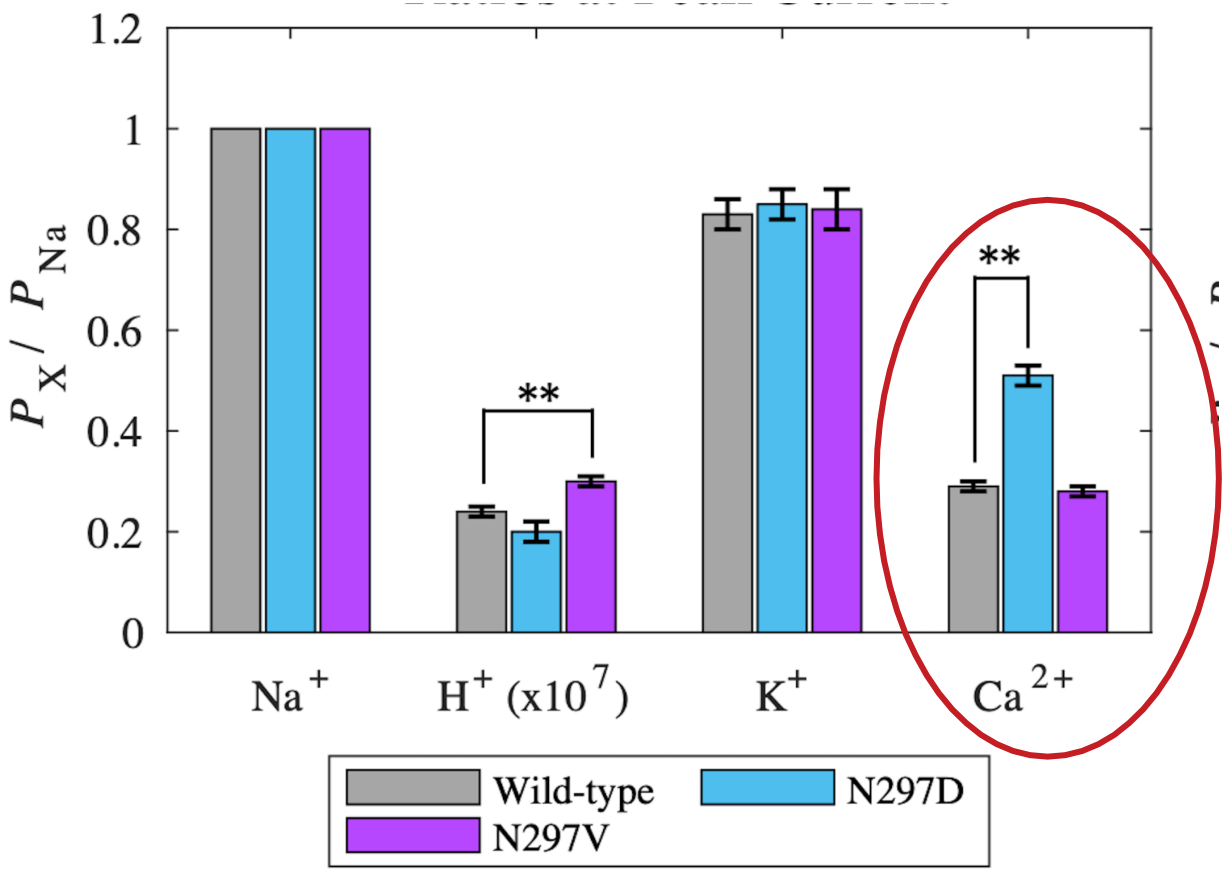
- **Green:** predicted from crystal structure
- **Yellow:** actual Na & Ca path during simulations
- Ions hydrated

STRUCTURE VS FREE ENERGY



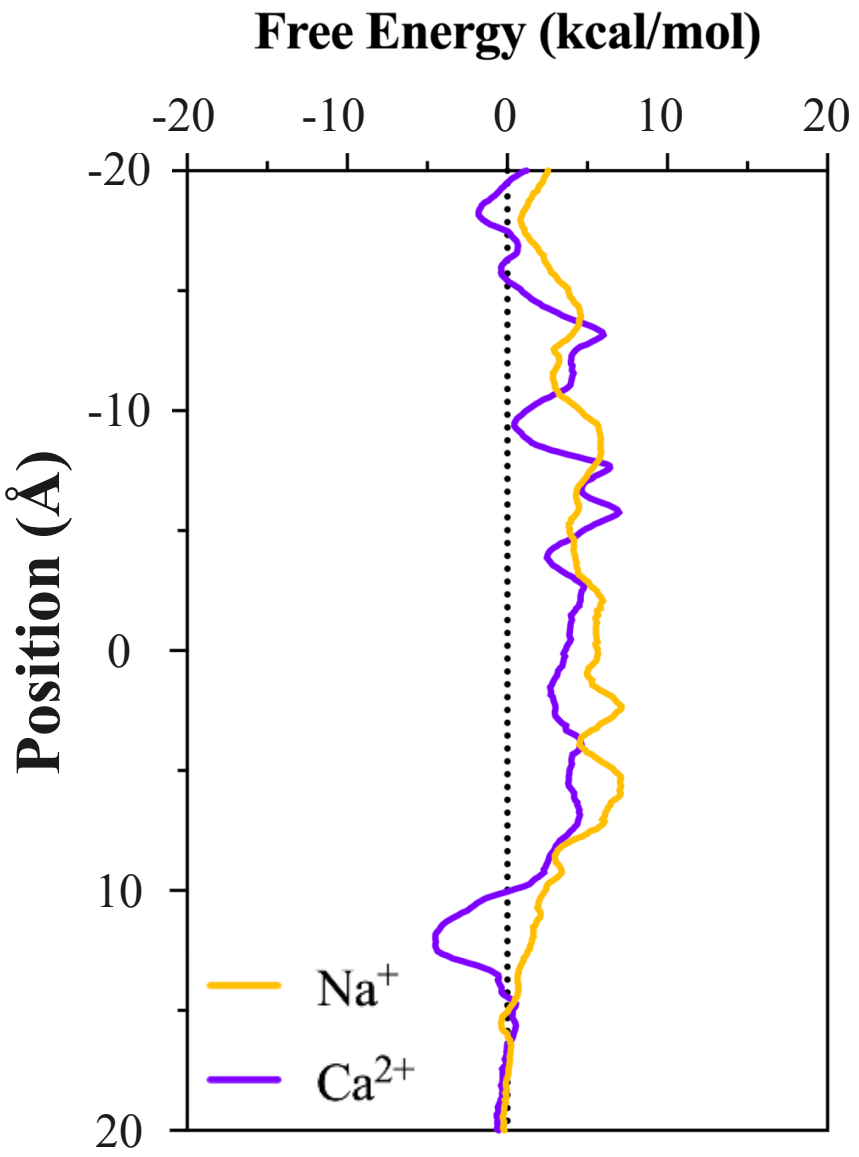
- Lowered barriers at glutamates (**E**)
- Highest barrier at narrowest (**N297**)
- Ca well at exit; binds both **E**

N297D INCREASES Ca^{2+} PERMEATION

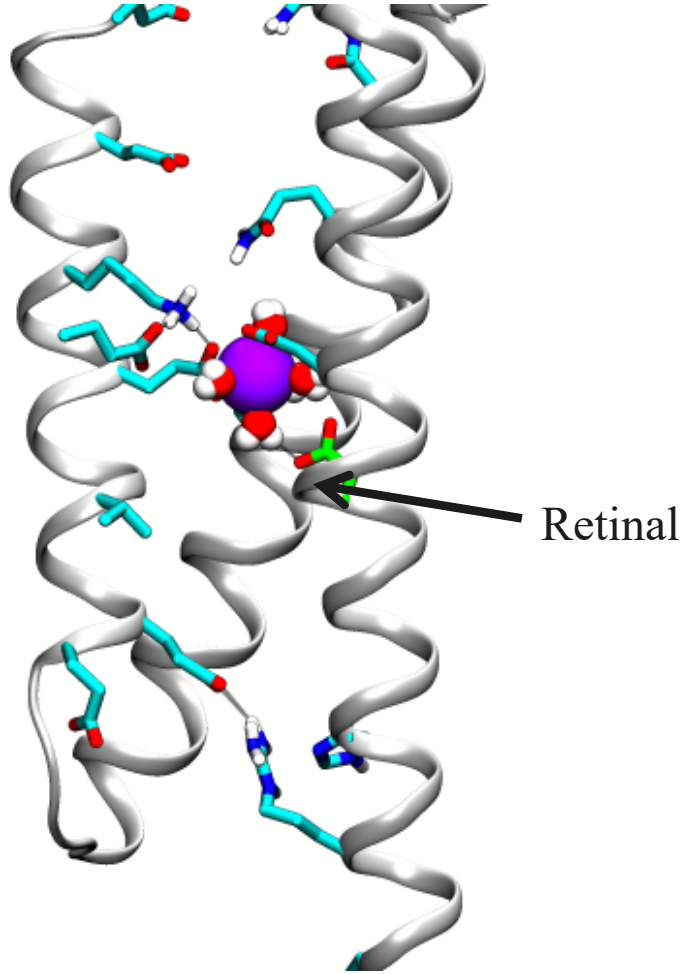


- N297D less selective (Na/Ca)

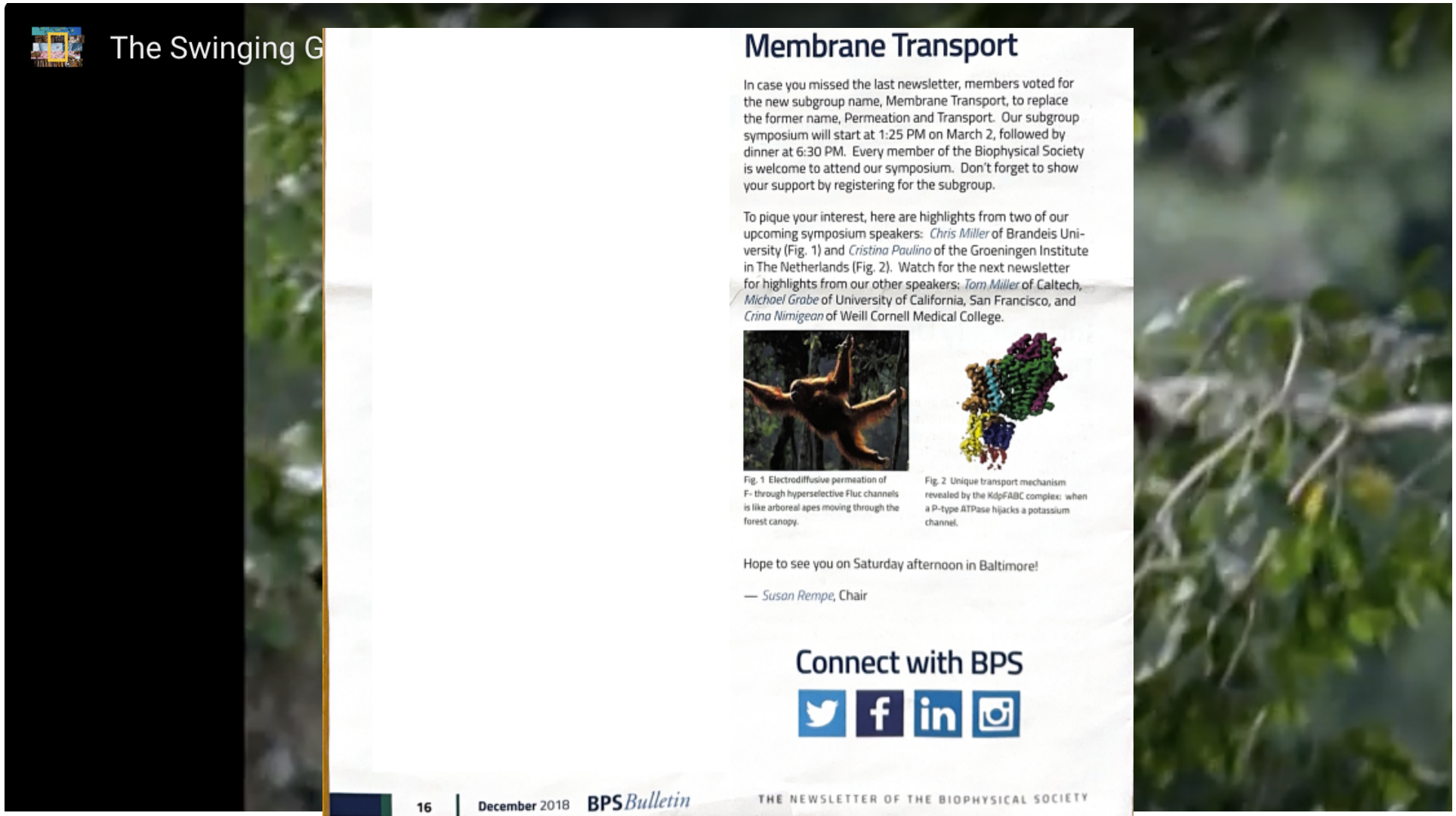
N297D FREE ENERGY



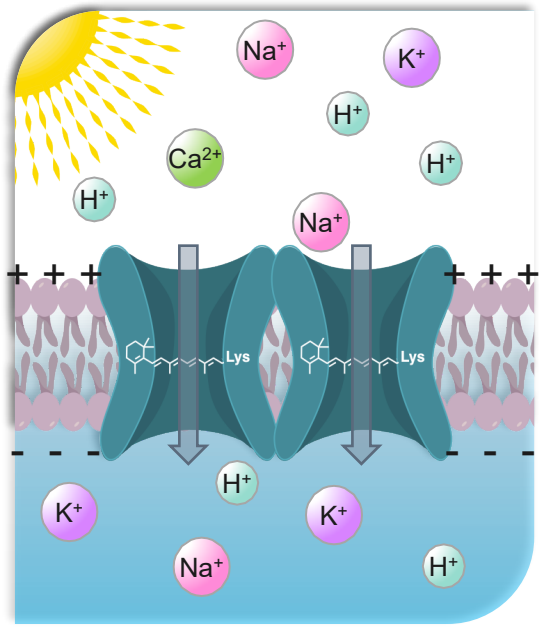
N297D I_{530}/O_2 state



REMINISCENCES



CONCLUSIONS: LIGHT-GATED C1C2



(1) Ion entry – **differs** from crystal predictions

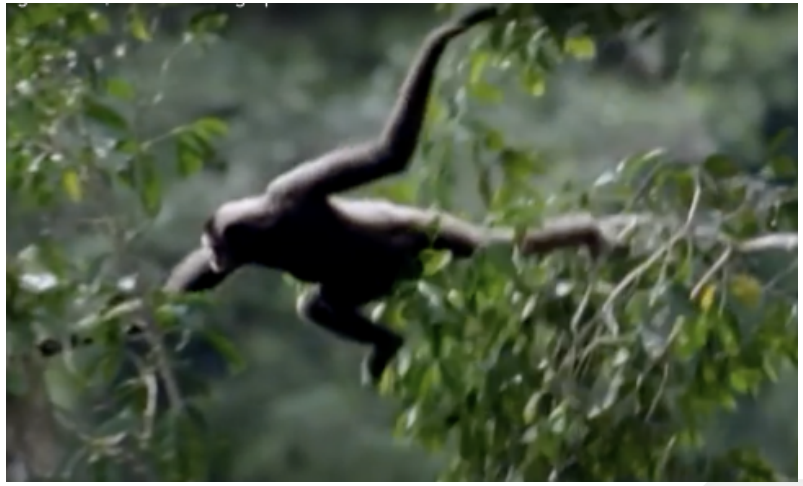
(2) Ion permeation (Na vs Ca): measured selectivity

- free energy barrier **higher** for Ca at narrow central gate (N297)

- free energy well **lower** for Ca at exit w/ glutamates (E121, E122)

(3) N297D: increases Ca permeability by **swinging** of side chains &

reducing free energy profile of Ca relative to Na



ACKNOWLEDGEMENTS

Lindsey Prignano

Center for Integrated Nanotechnologies

Channelrhodopsin; Dempski, Ed.; **2021**; Vol. 219

VanGordon, Prignano, Dempski, Rick, Rempe *Biophys. J*. 120 (**2021**) 1835.

Prignano, Stevens, Vanegas, Rempe,* Dempski* *PLOS ONE* (**2024)**, *19* (9), e0309553.

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N297D FREE ENERGY O1 STATE