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

Susan Rempe¹, Lindsey Prignano², Mark Stevens¹,

Juan Vanegas³, Rob DempSKI²

¹Sandia National Labs

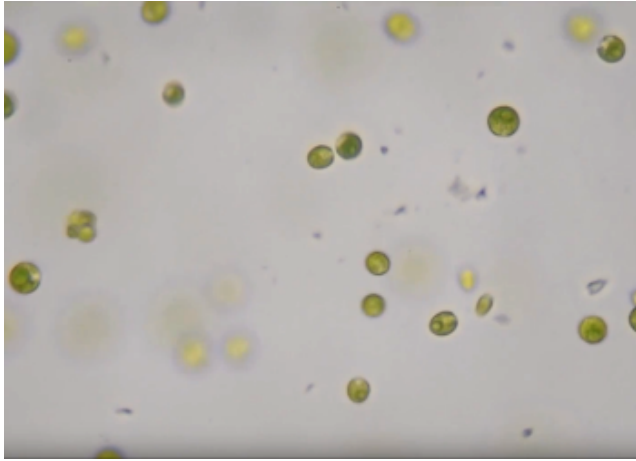
²Worcester Polytechnic Institute

³Oregon State University

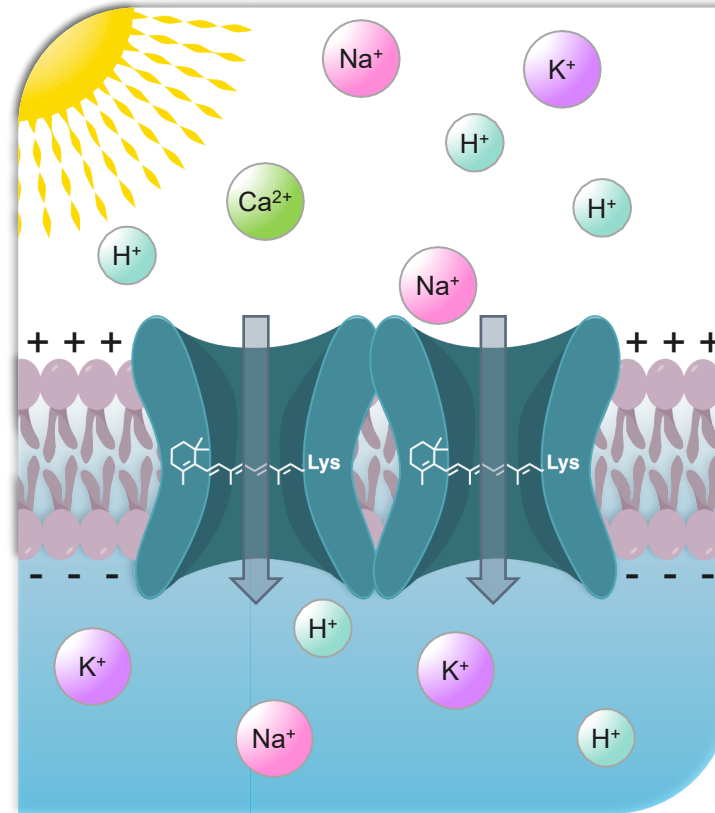


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CHANNELRHODOPSINS –LIGHT-GATED ION CHANNELS



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

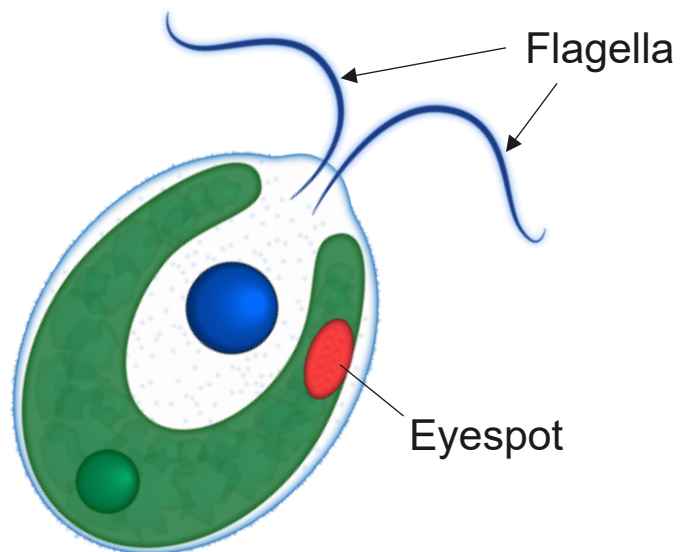


- ***Chlamydomonas reinhardtii***

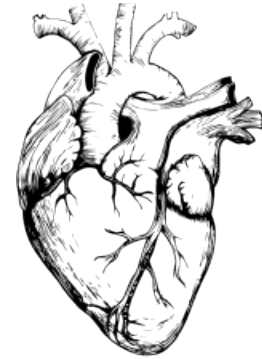
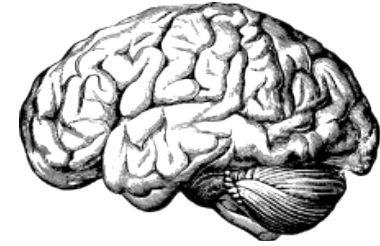
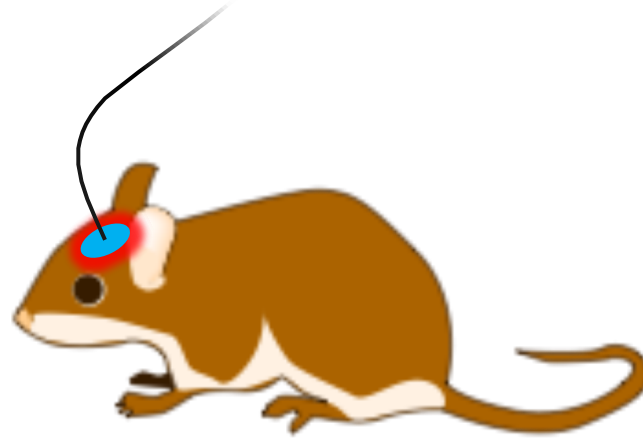
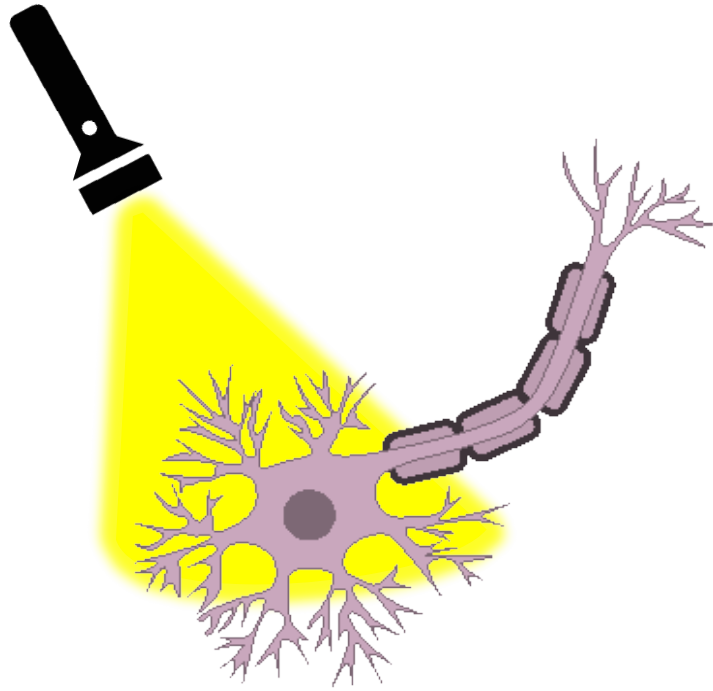
- Single-celled alga
- Well-studied

- **ChR1 & ChR2**

- Eyespot
- Initiates phototaxis
- Nonselective for cations: $H^+ \gg Li^+ > Na^+ \approx K^+ > Ca^{2+}$
- Rapid membrane depolarization & activation of voltage-gated Ca^{2+} channels → flagella move



OPTOGENETICS APPLICATIONS

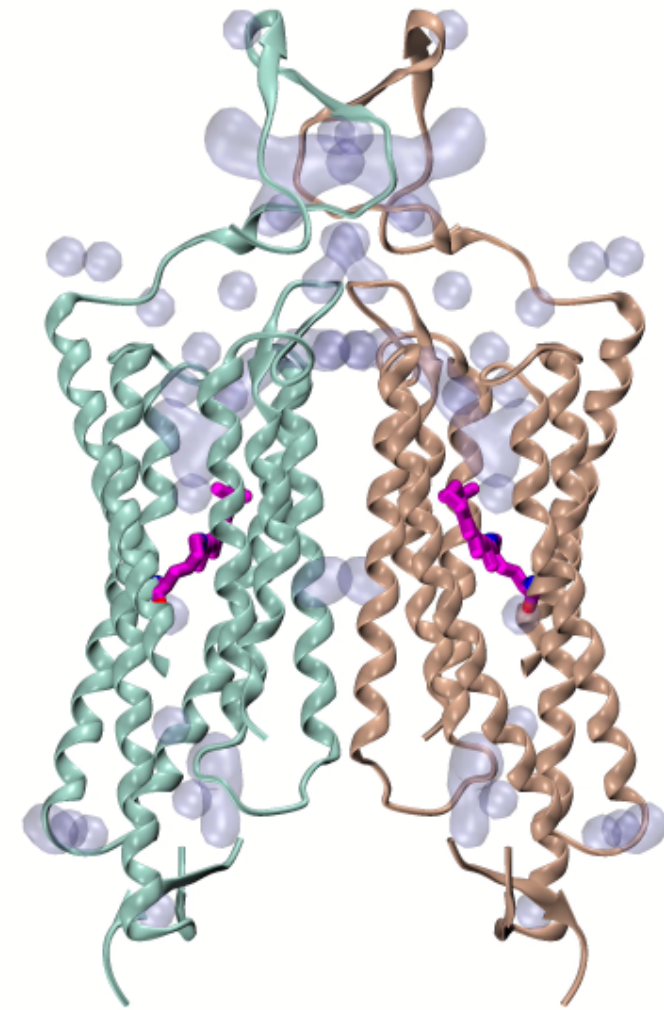
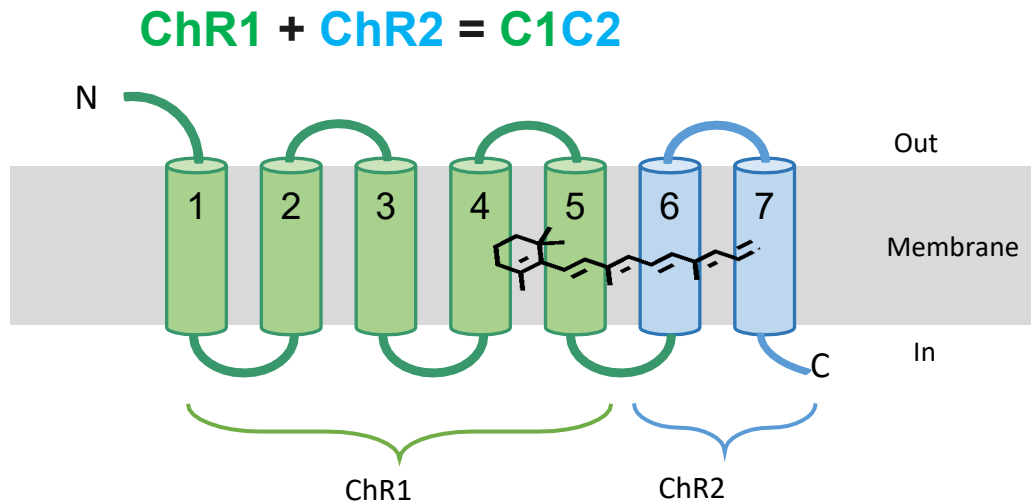


- Control neurons w/ light
- Targeted

- Map functional neuronal circuits
- Live organisms
- Apply clinically

CHANNELRHODOPSIN CHIMERA (C1C2)

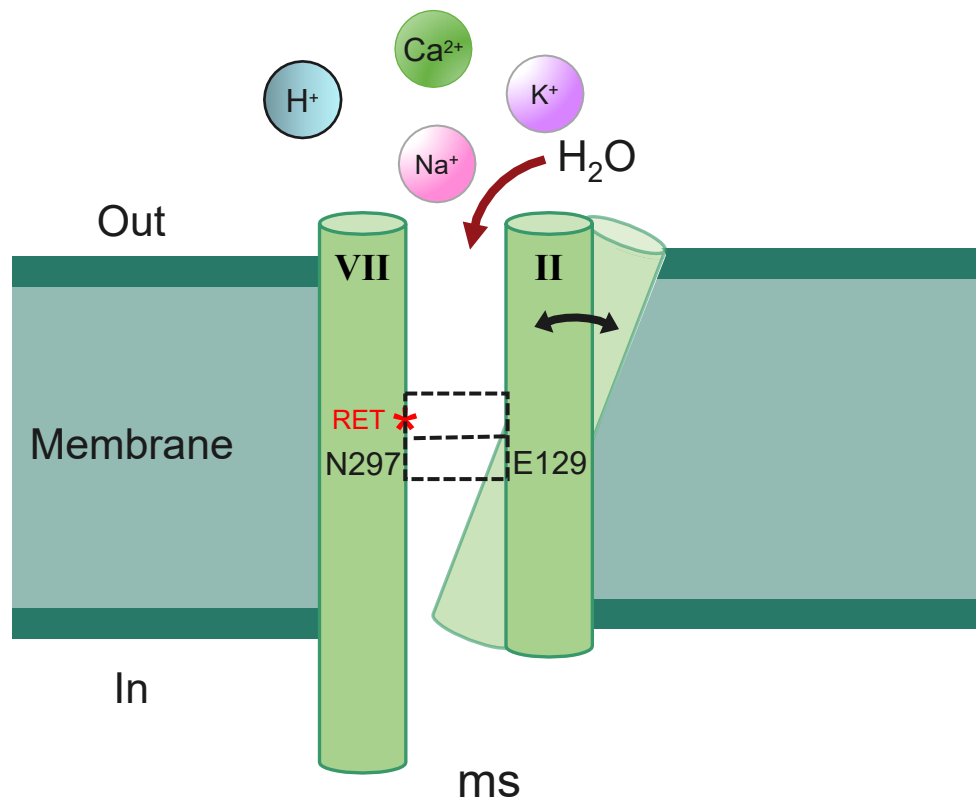
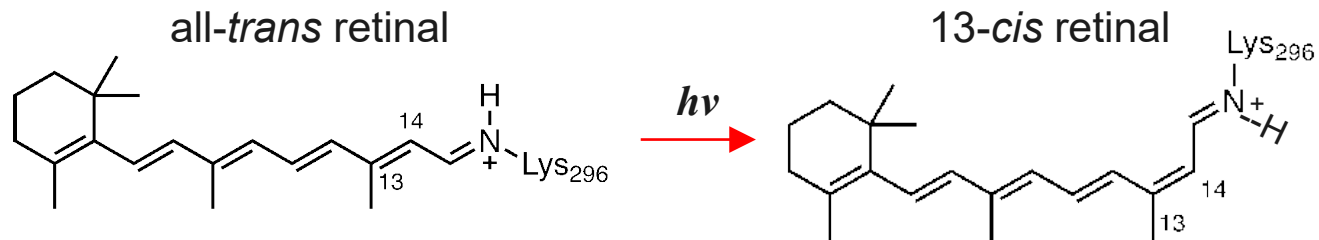
- First ChR crystal structure (2012)
- Few functional studies vs ChR2
- Challenge: No open state structures



PDB ID: 3UG9

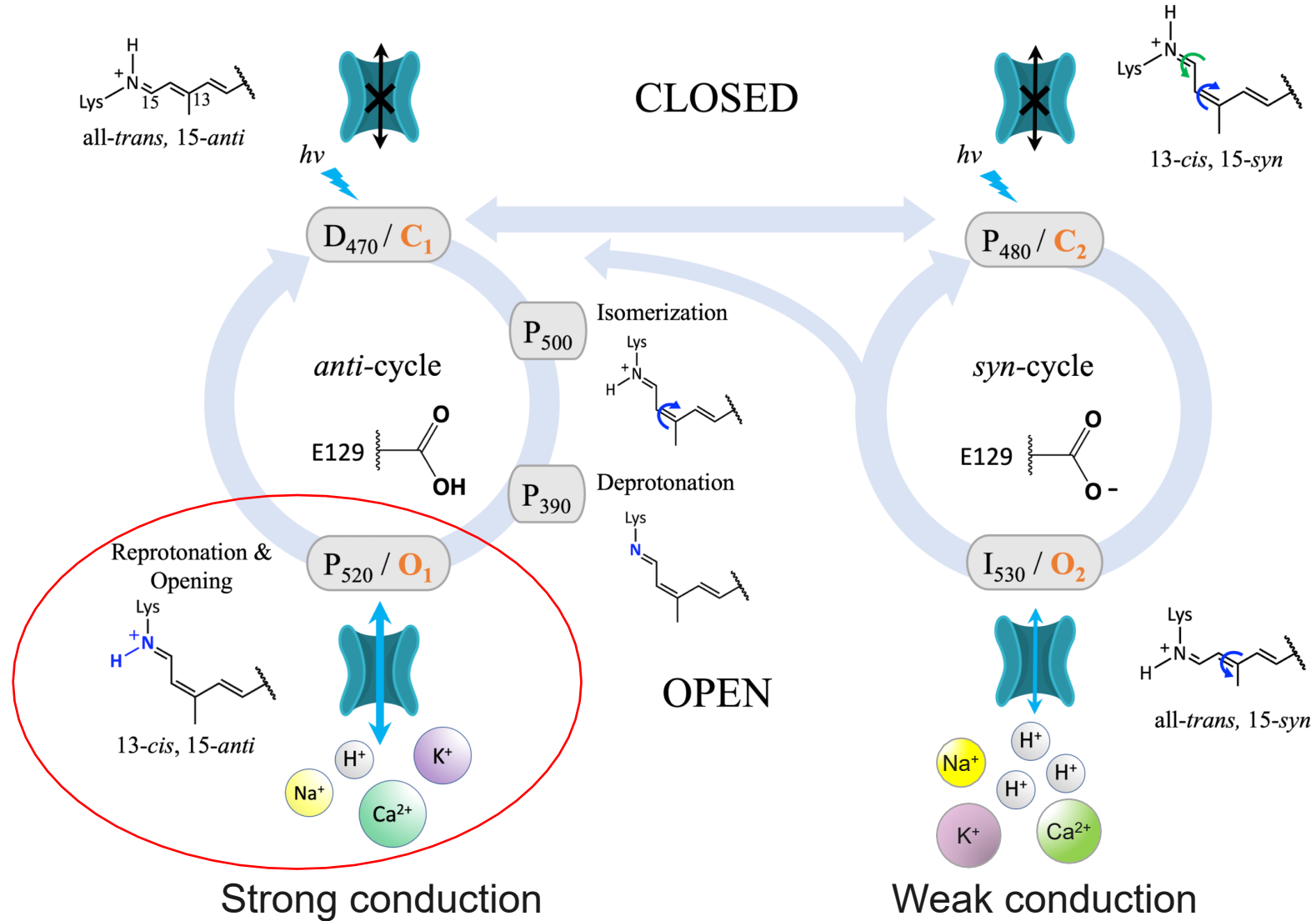
Kato *et al.* *Nature* **482**, 369–374 (2012).

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

CHR PHOTOCYCLES

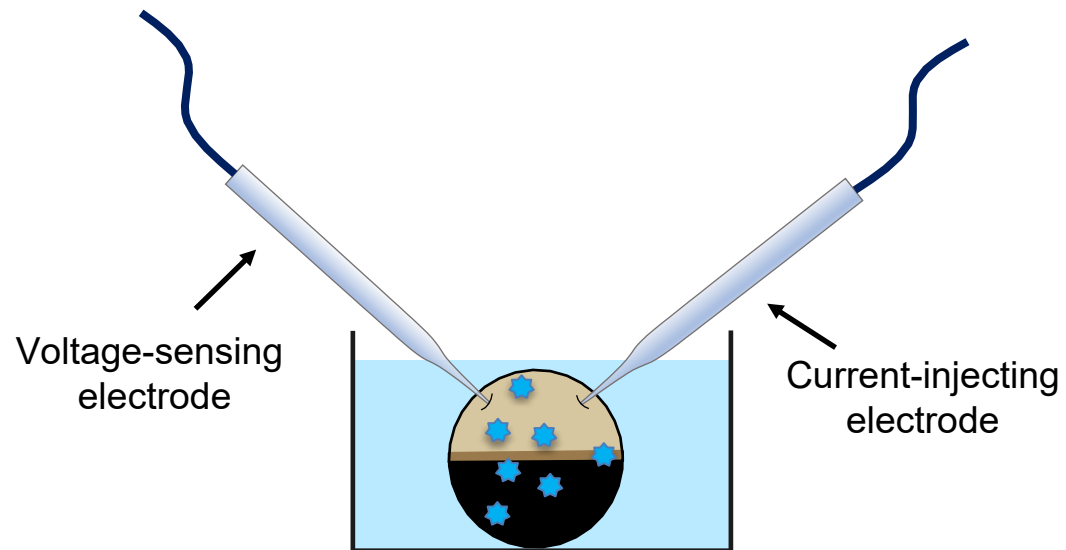


Kuhne, et al. PNAS 2019, 116 (19), 9380.

GOALS & APPROACH



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

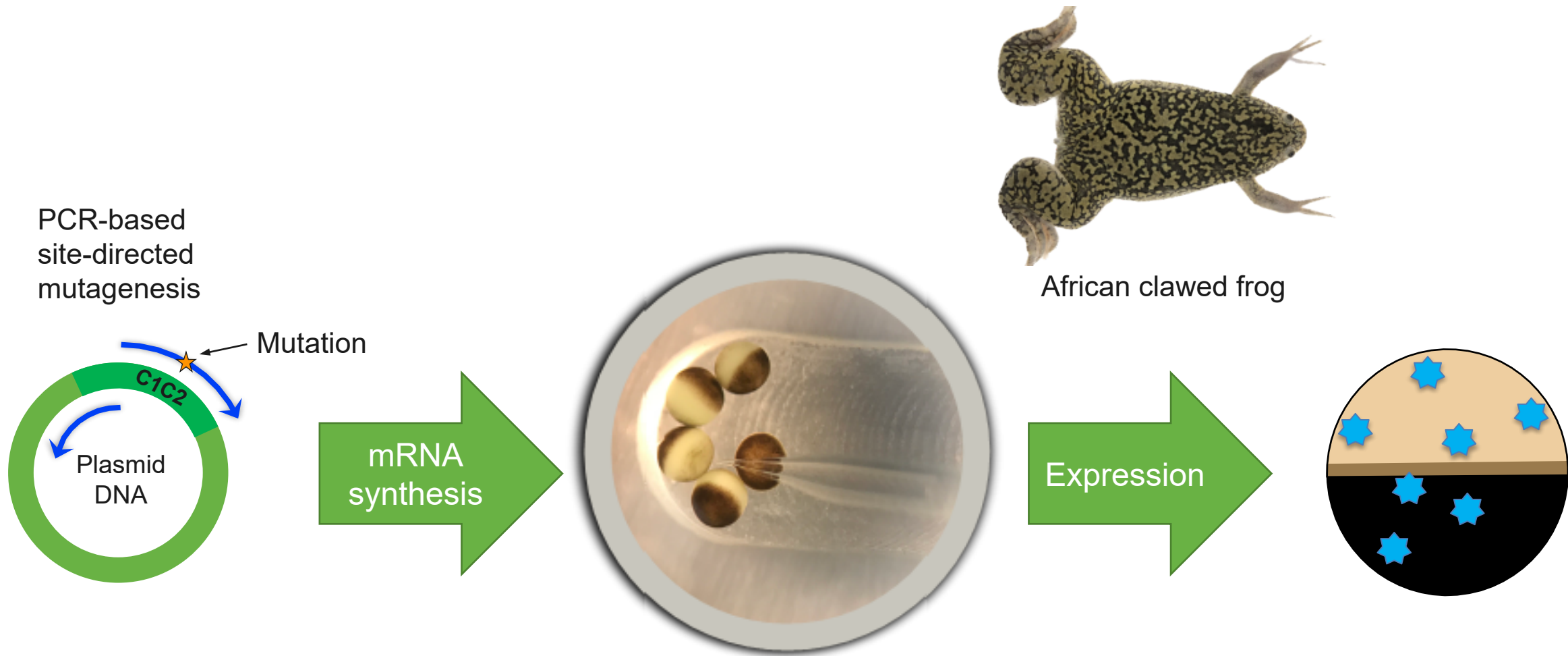


Electrophysiology (IV)



Molecular dynamics ($F=ma$)

STEP 1: EXPRESS C1C2 IN FROG OOCYTES



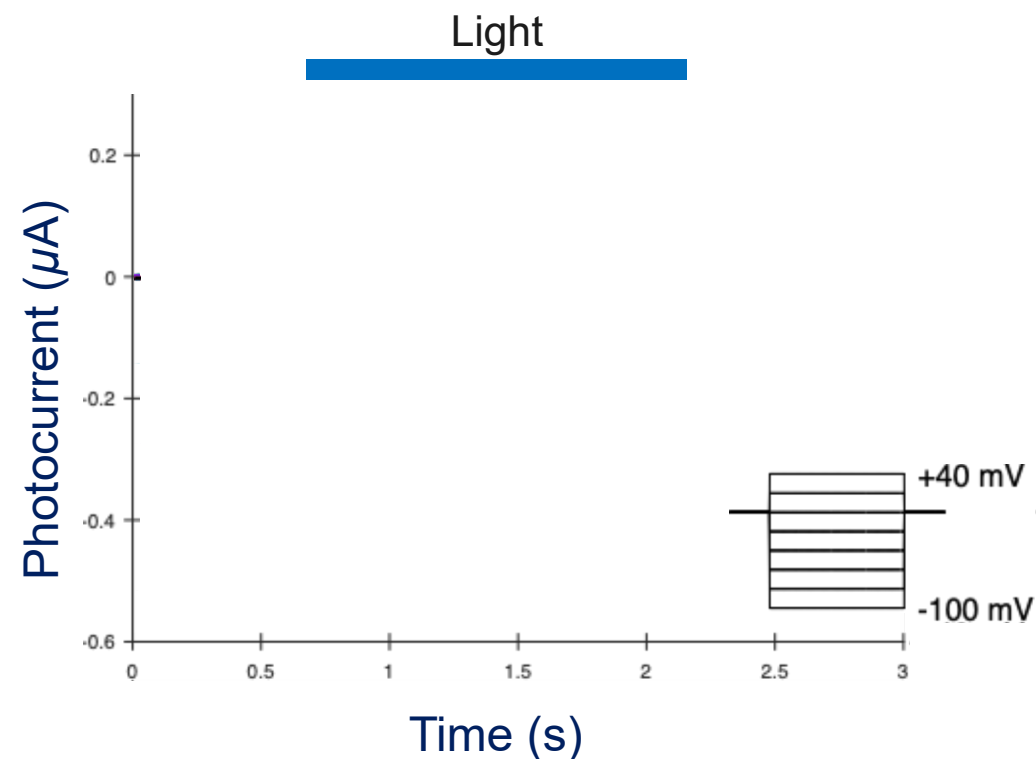
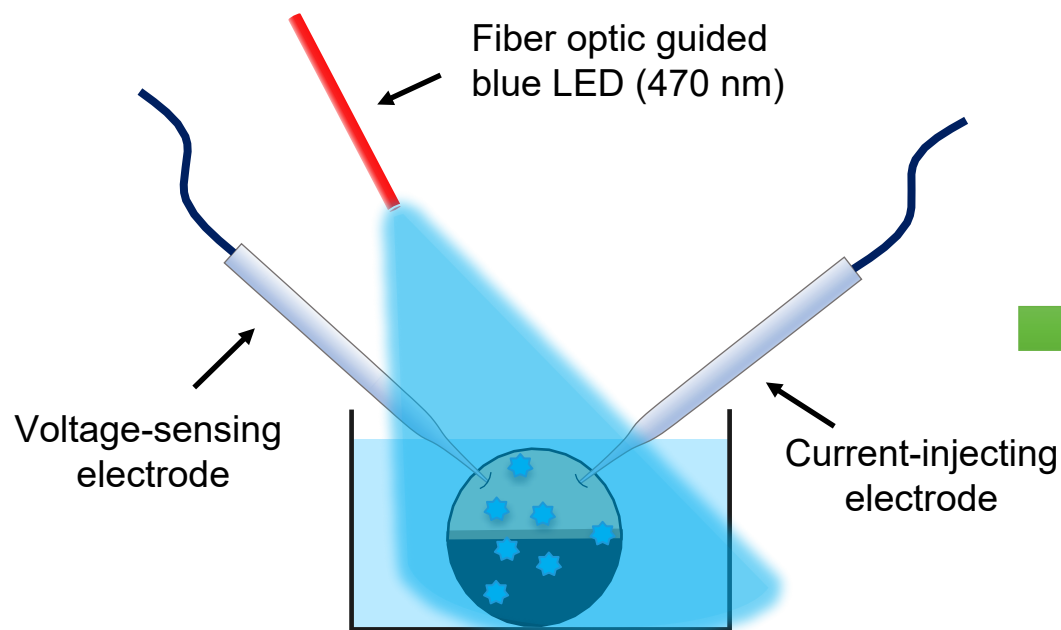
Timing!



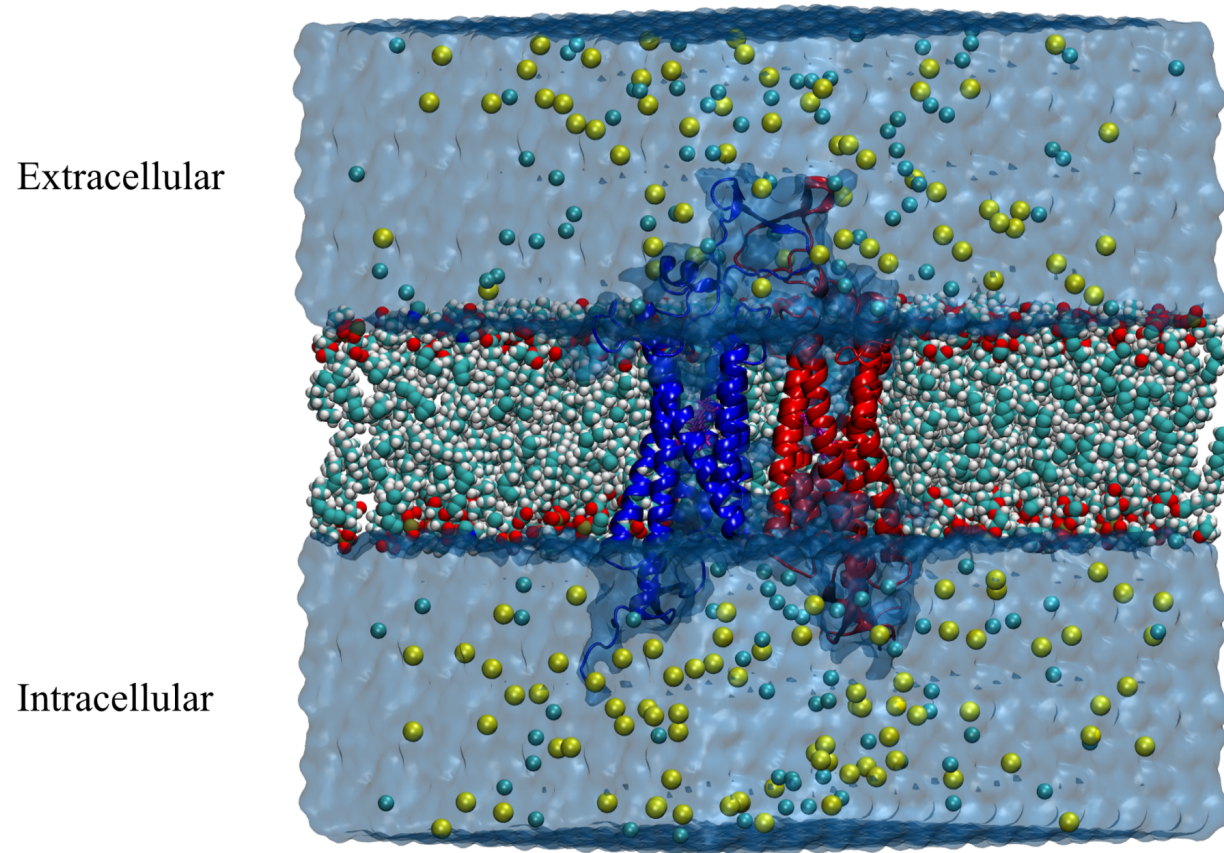
TWO-ELECTRODE VOLTAGE CLAMP

- 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

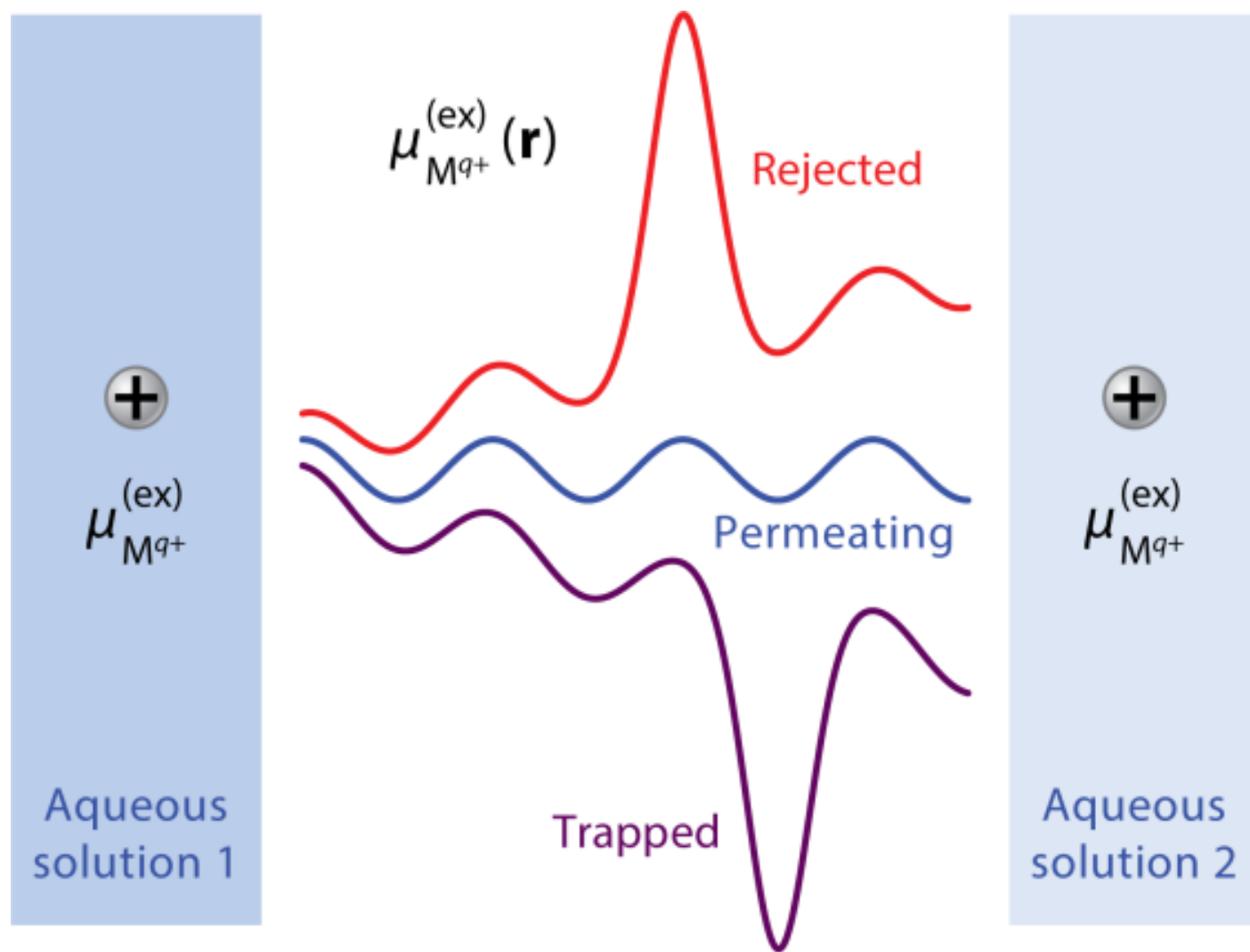


SIMULATION MODEL

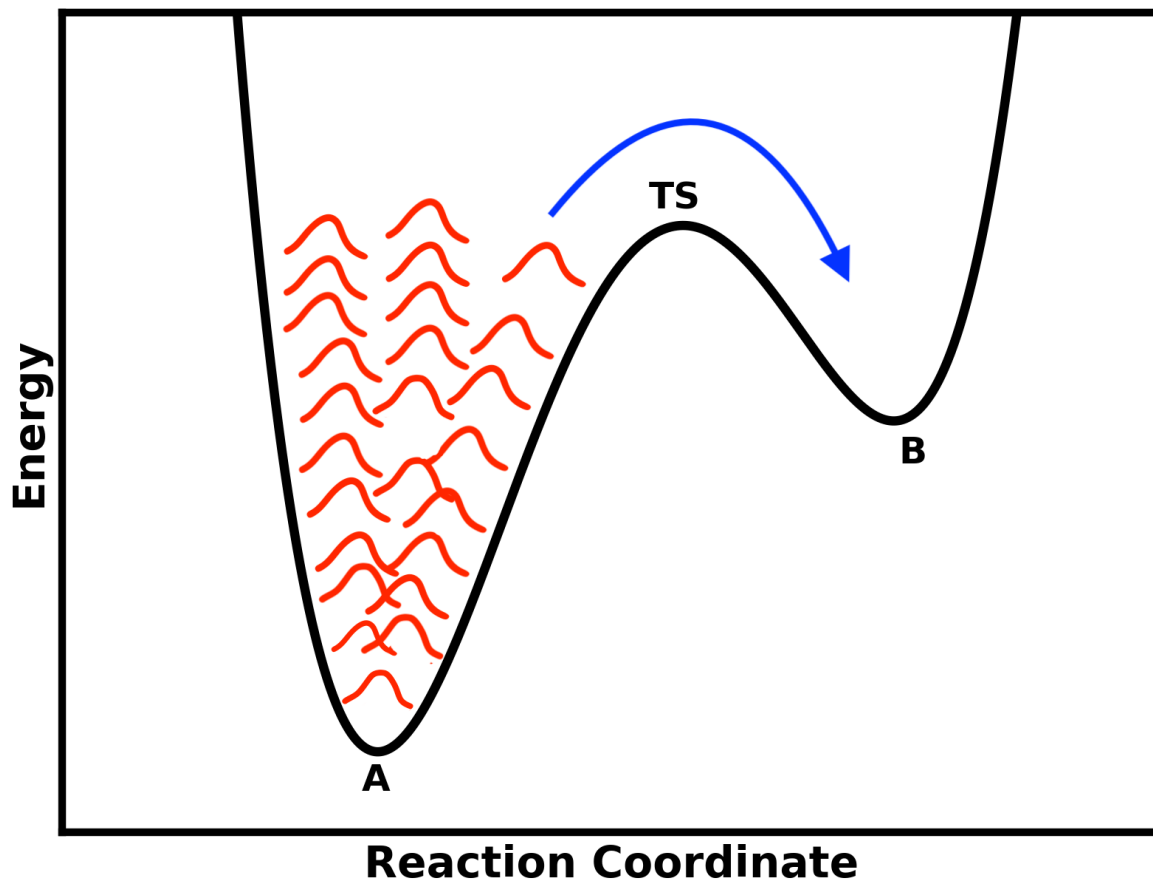


Open State 1 ($P_{520}/O1$)

FREE ENERGY

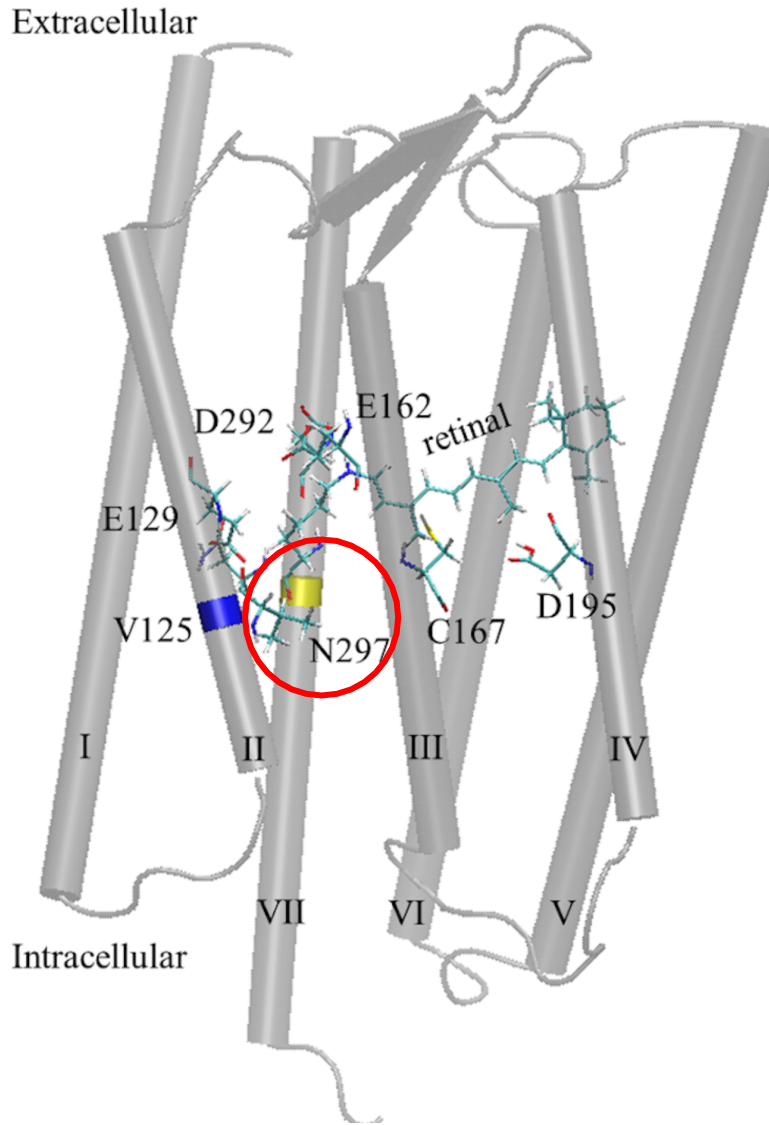


ENHANCED SAMPLING FOR FREE ENERGY



- **Metadynamics** enhances sampling of $V(\text{rc})$
- 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)**

MUTATIONS (N297D)

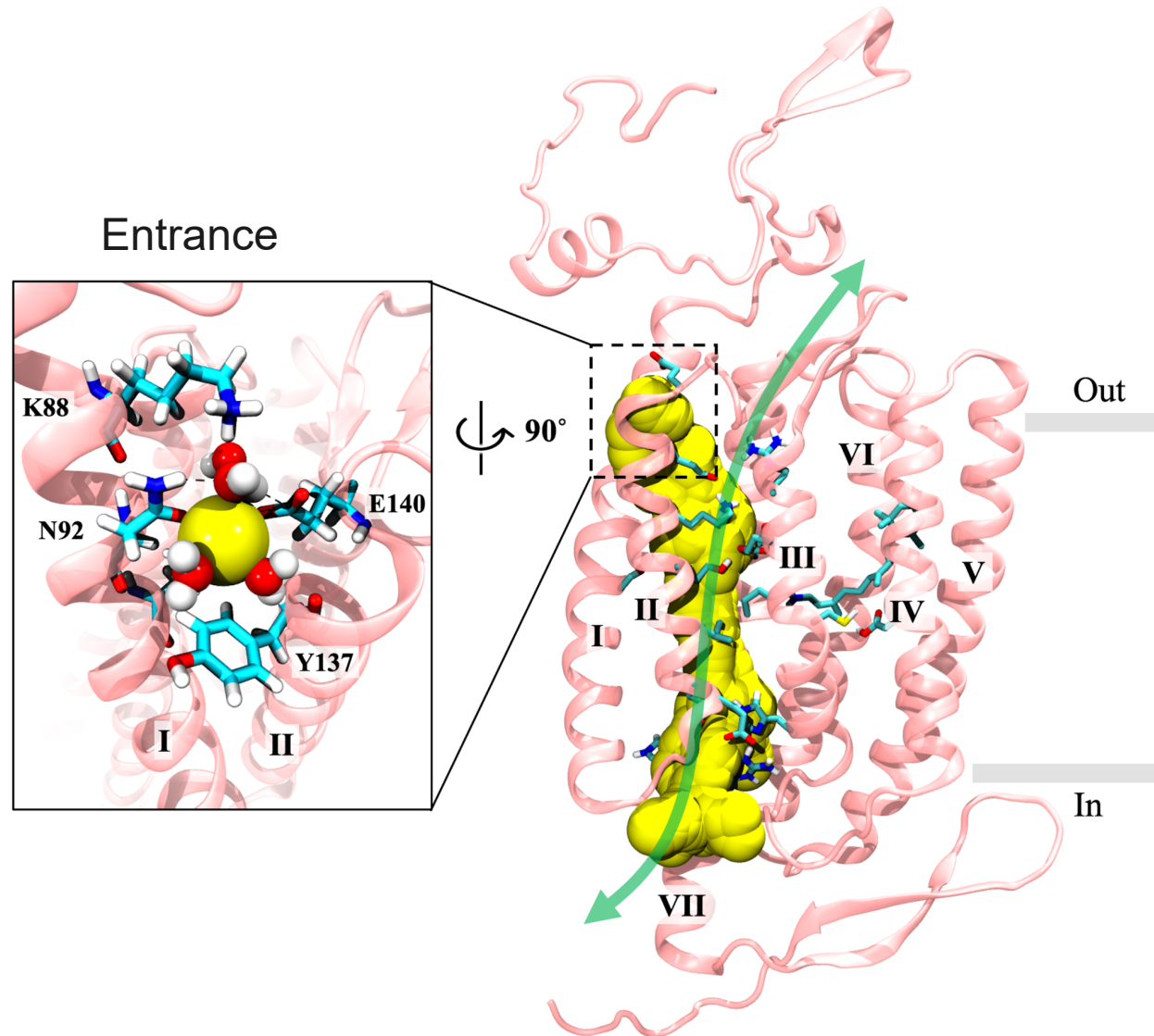


RESULTS



Los Angeles

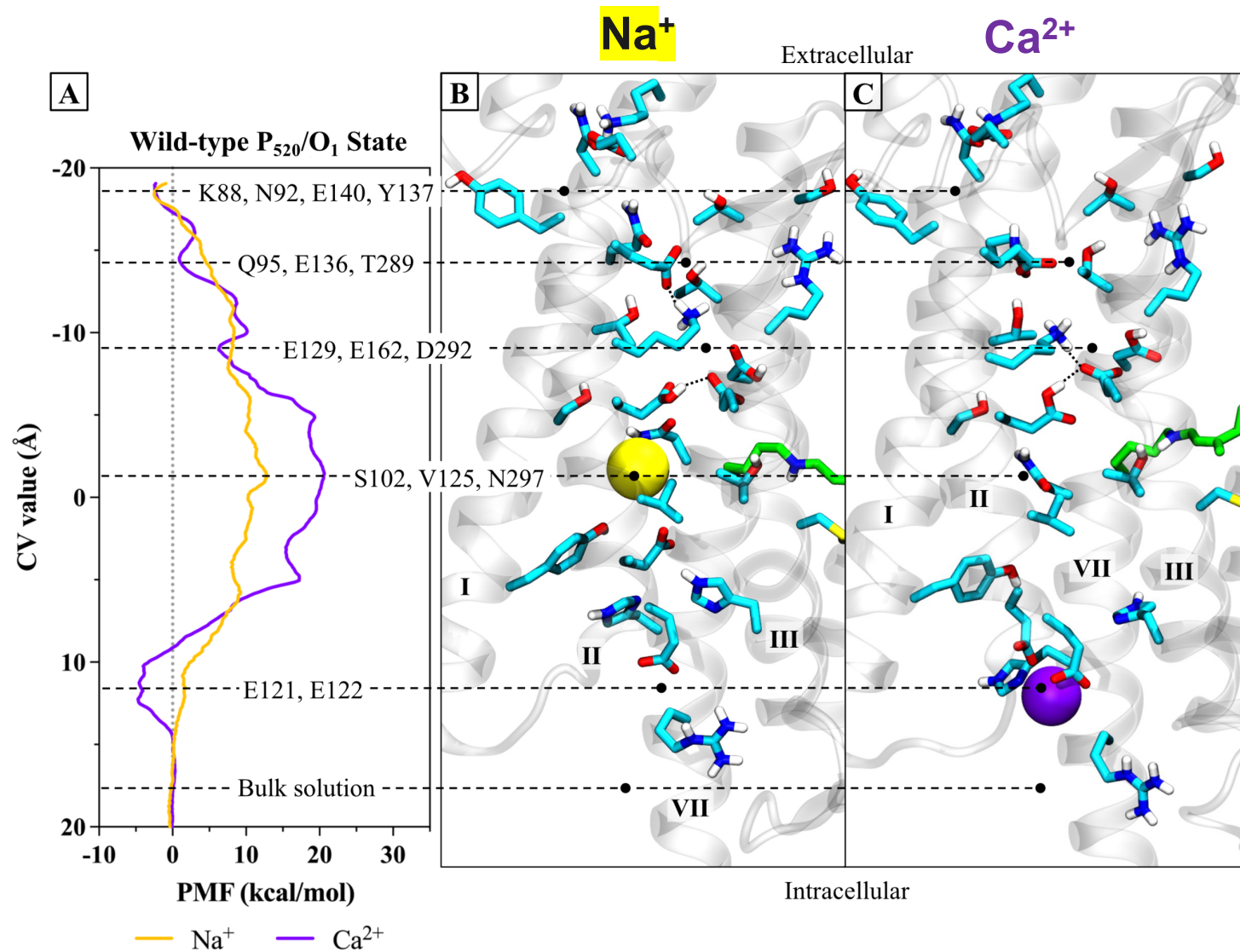
ION PATH DIFFERS FROM PREDICTIONS



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

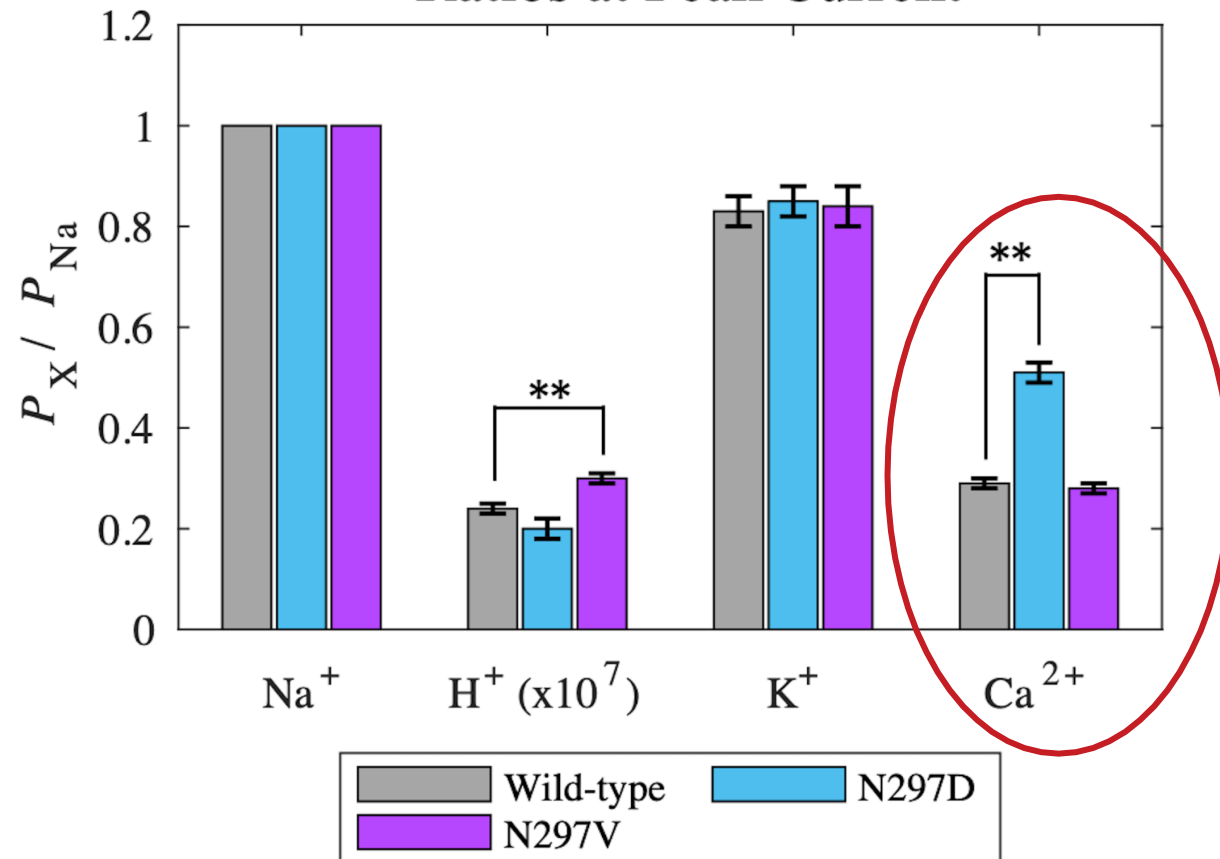


STRUCTURE VS FREE ENERGY



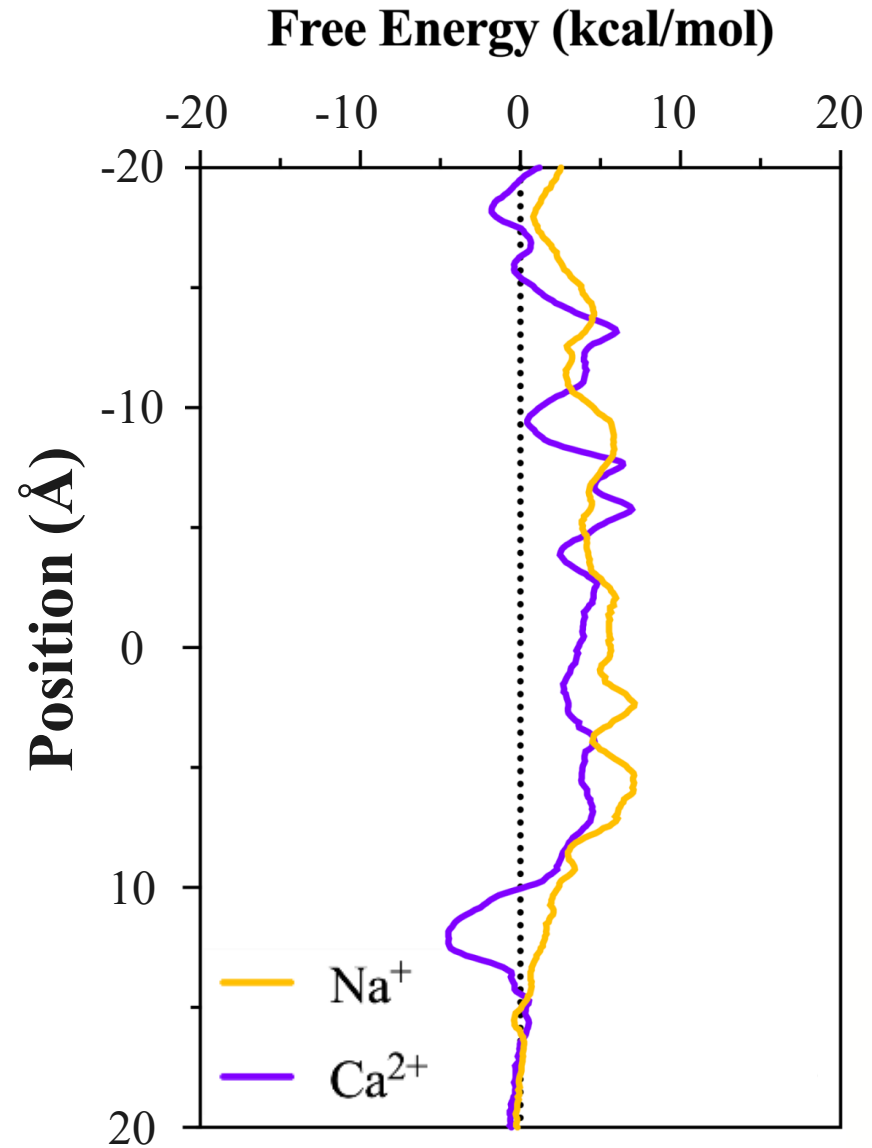
- Lowered barriers at glutamates (**E**)
- Highest barrier at narrowest (**N297**)
- Ca well at exit; binds both **E**

N297D INCREASES Ca^{2+} PERMEATION

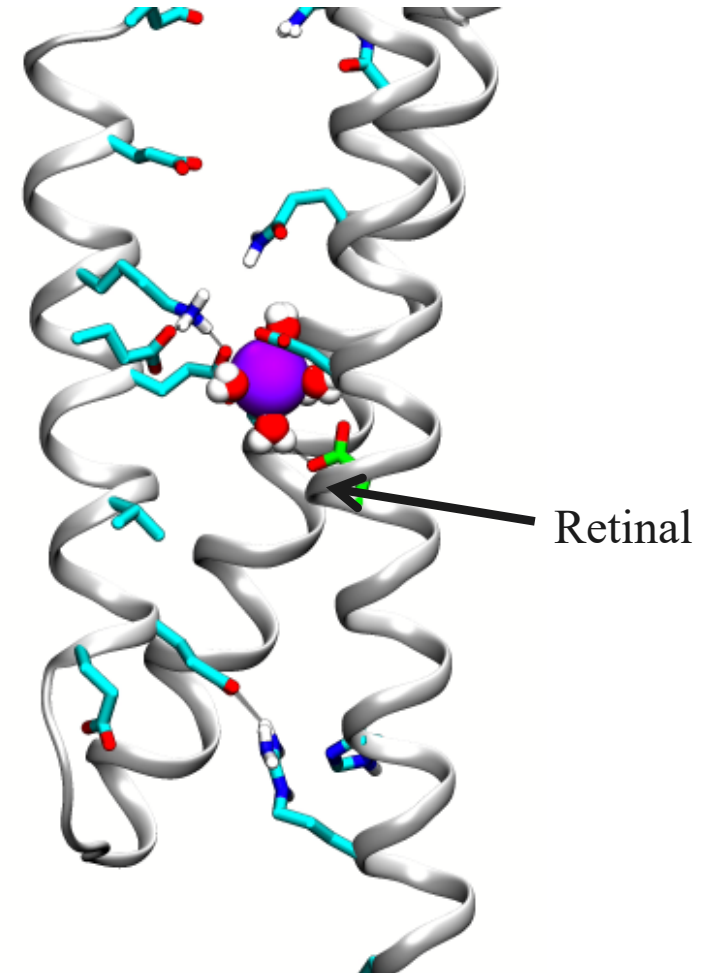


- N297D less selective (Na/Ca)


N297D FREE ENERGY



N297D I₅₃₀/O₂ state





 The Swinging G

Membrane Transport

In case you missed the last newsletter, members voted for the new subgroup name, Membrane Transport, to replace the former name, Permeation and Transport. Our subgroup symposium will start at 1:25 PM on March 2, followed by dinner at 6:30 PM. Every member of the Biophysical Society is welcome to attend our symposium. Don't forget to show your support by registering for the subgroup.

To pique your interest, here are highlights from two of our upcoming symposium speakers: *Chris Miller* of Brandeis University (Fig. 1) and *Cristina Paulino* of the Groeningen Institute in The Netherlands (Fig. 2). Watch for the next newsletter for highlights from our other speakers: *Tom Miller* of Caltech, *Michael Grabe* of University of California, San Francisco, and *Crina Nimigean* of Weill Cornell Medical College.




Fig. 1 Electrodiffusive permeation of F⁻ through hyperselective Fluc channels is like arboreal apes moving through the forest canopy.








Fig. 2 Unique transport mechanism revealed by the KdpFABC complex: when a P-type ATPase hijacks a potassium channel.

Hope to see you on Saturday afternoon in Baltimore!

— *Susan Rempe*, Chair

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16 | December 2018 **BPS Bulletin** THE NEWSLETTER OF THE BIOPHYSICAL SOCIETY

CONCLUSIONS: LIGHT-GATED C1C2

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

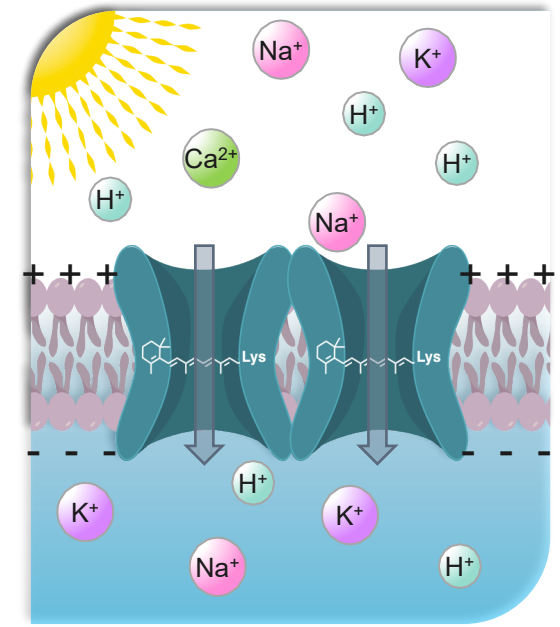
(2) Ion permeation (Na vs Ca): measured selectivity

- 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; Dempksi, Ed.; **2021**; Vol. 219

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

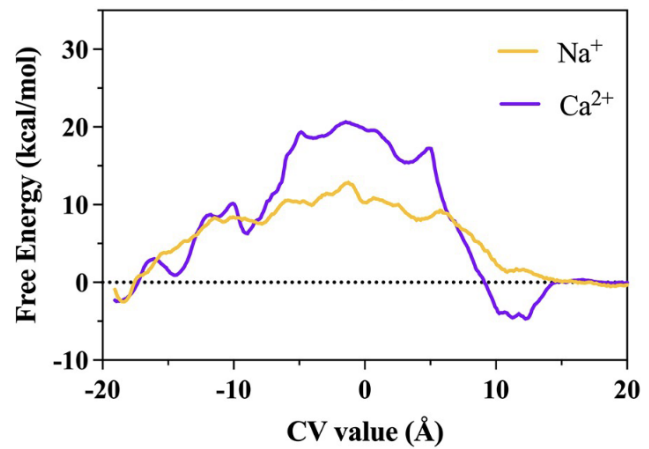
Prignano, Stevens, Vanegas, Rempe,* Dempksi* *PLOS ONE* (**2024**), 19 (9), e0309553.

Susan Rempe slrempe@sandia.gov

N297D FREE ENERGY O1 STATE



Wild-type P₅₂₀/O₁ State



N297D P₅₂₀/O₁ State

