

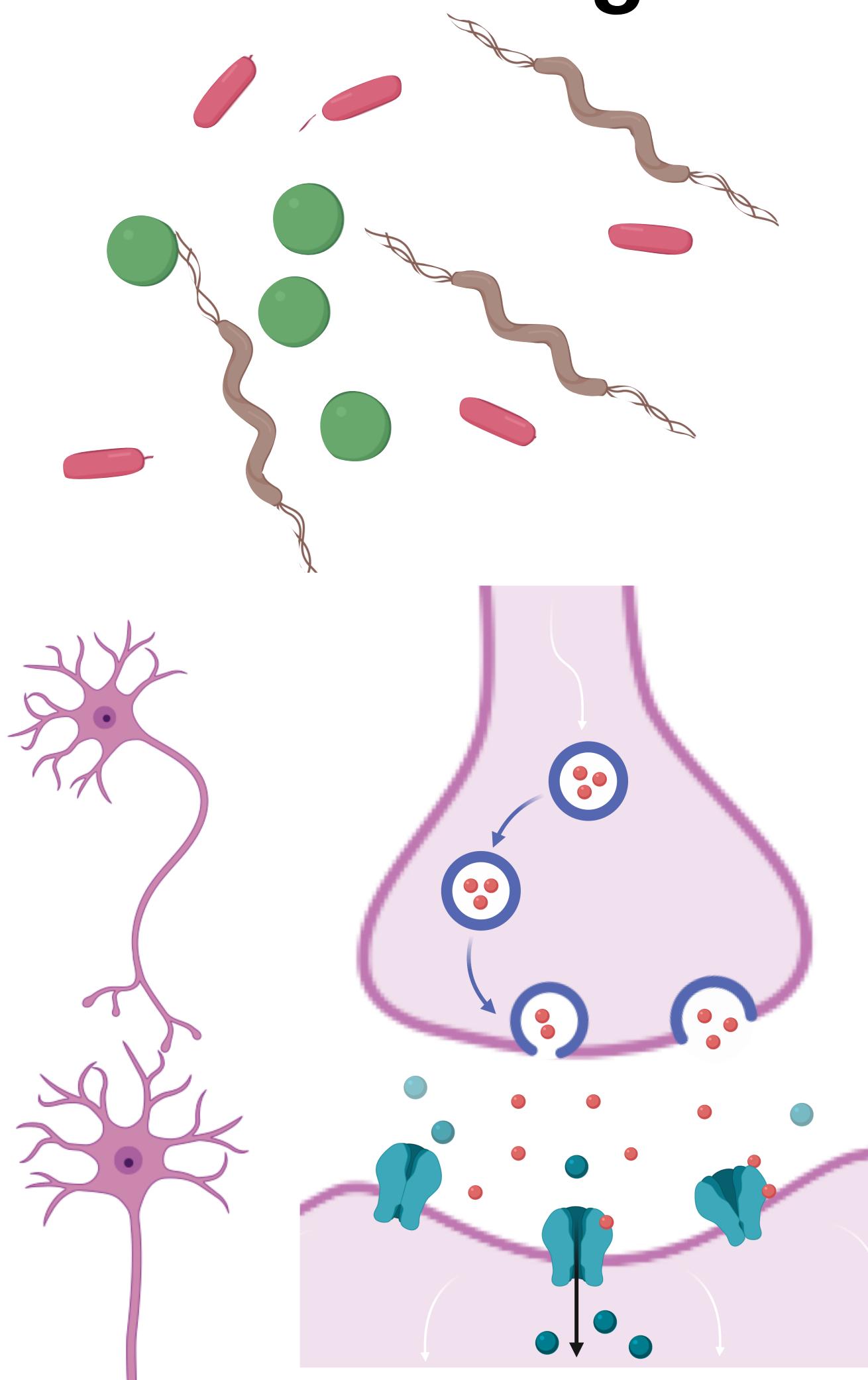
# Specific Lipid-Protein Binding

**Absolute binding affinities of phospholipids  
to Erwinia Ligand-Gated Ion Channel (ELIC)  
by Streamlined Alchemical Free Energy Perturbation  
(SAFEP)**

Ezry Santiago-McRae, Tom Joseph, John T Petroff II, Wayland Cheng, Jérôme Hénin, Grace Brannigan

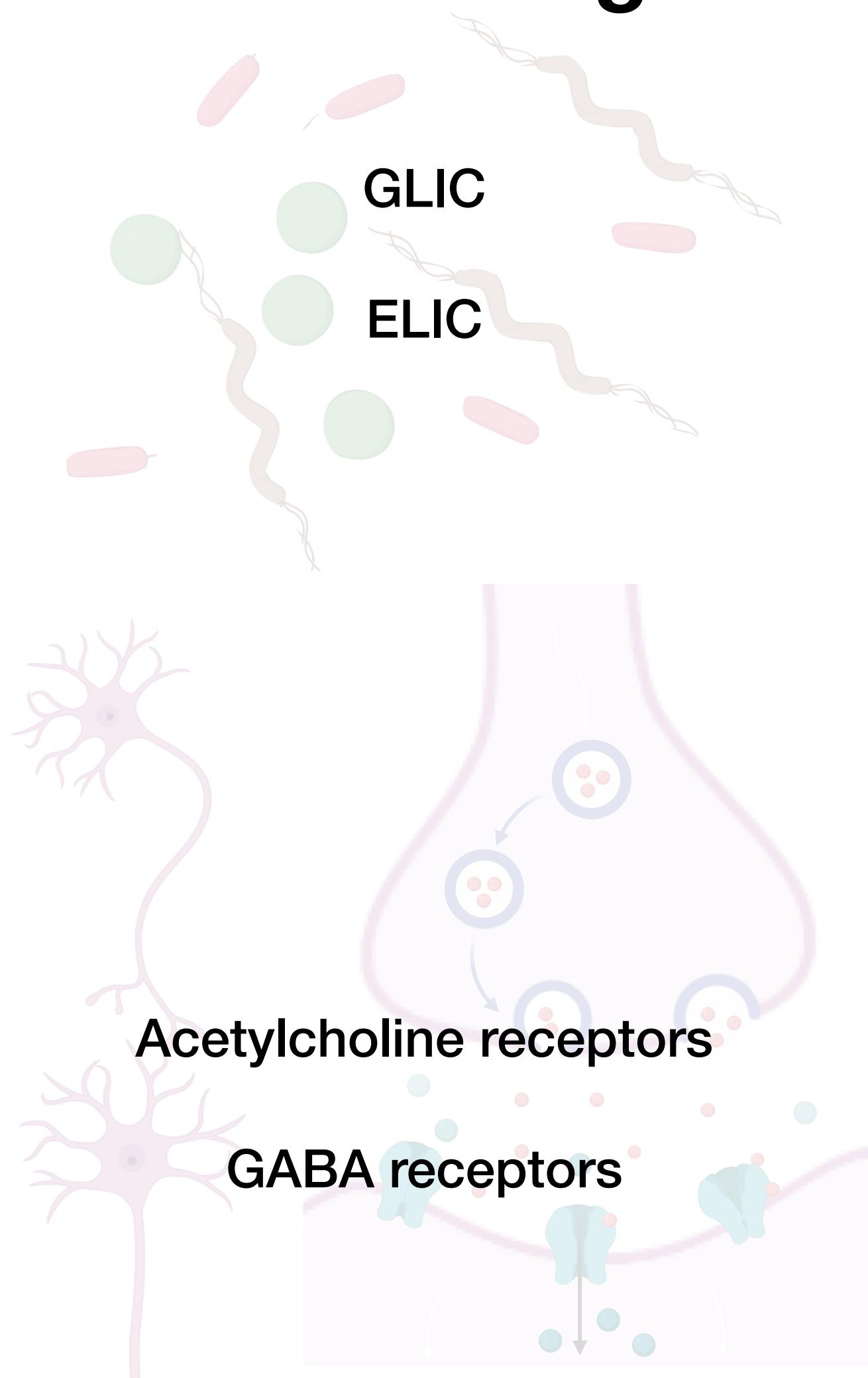
# pLGICs play diverse roles

## Pentameric Ligand-Gated Ion Channels



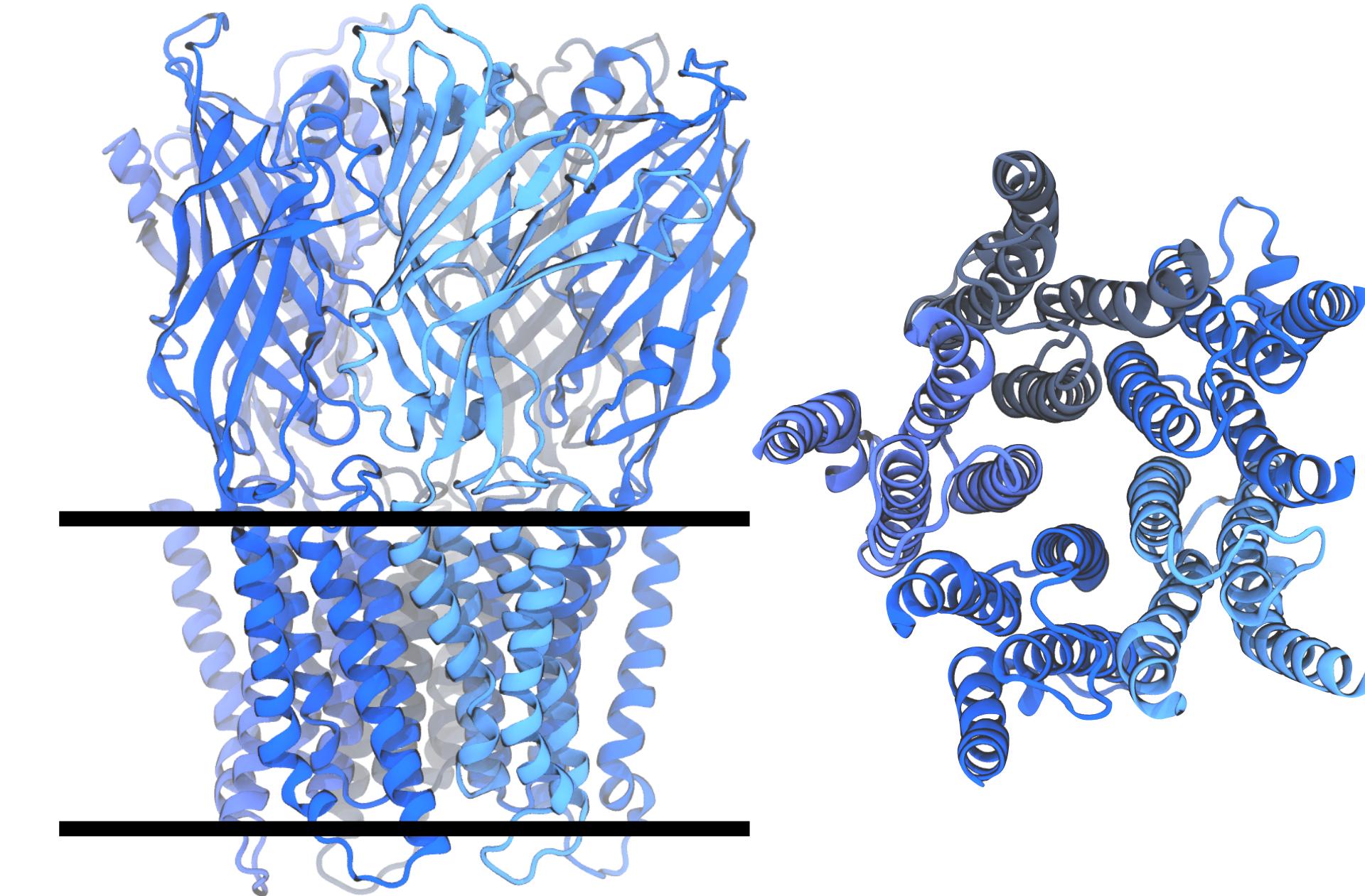
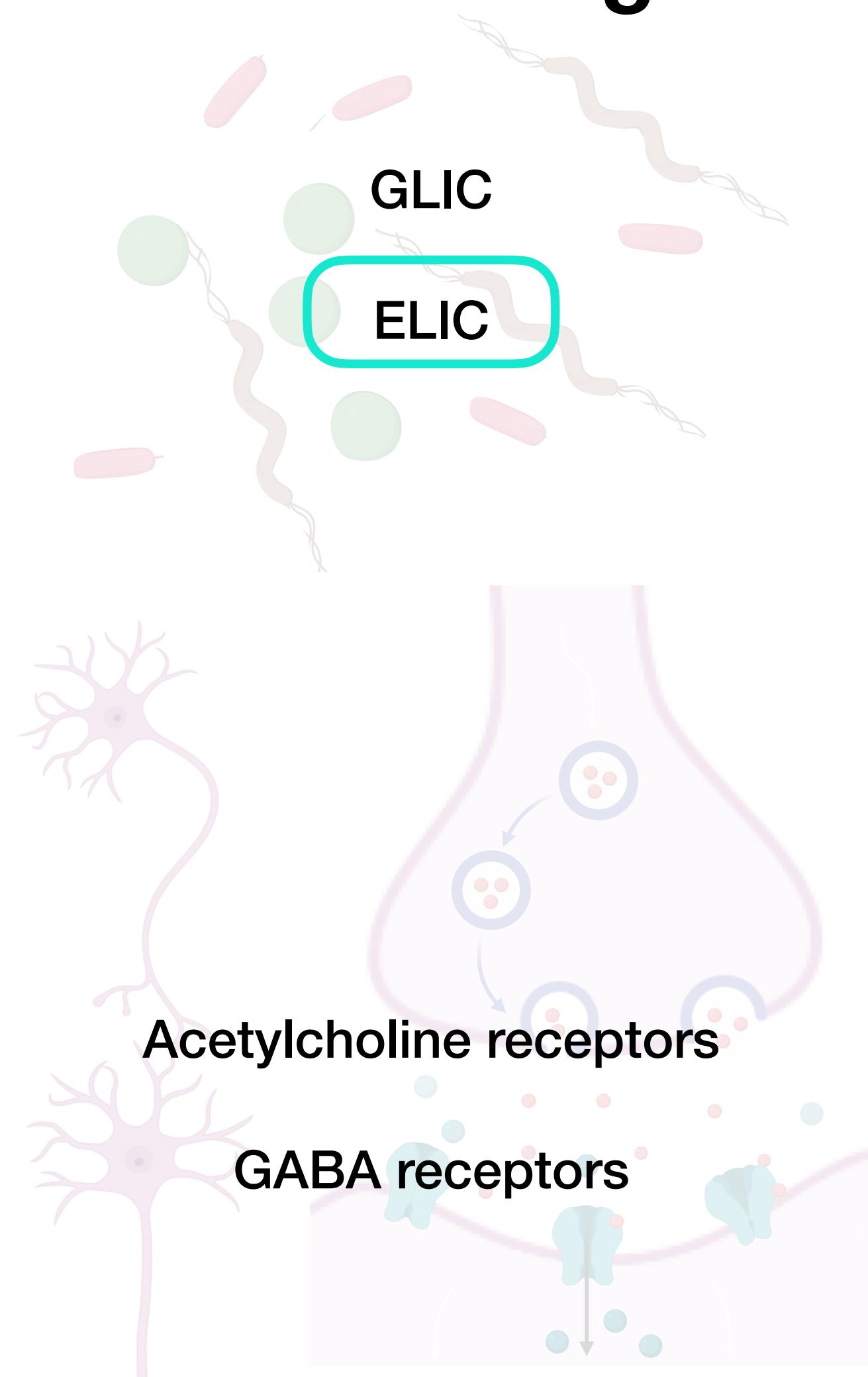
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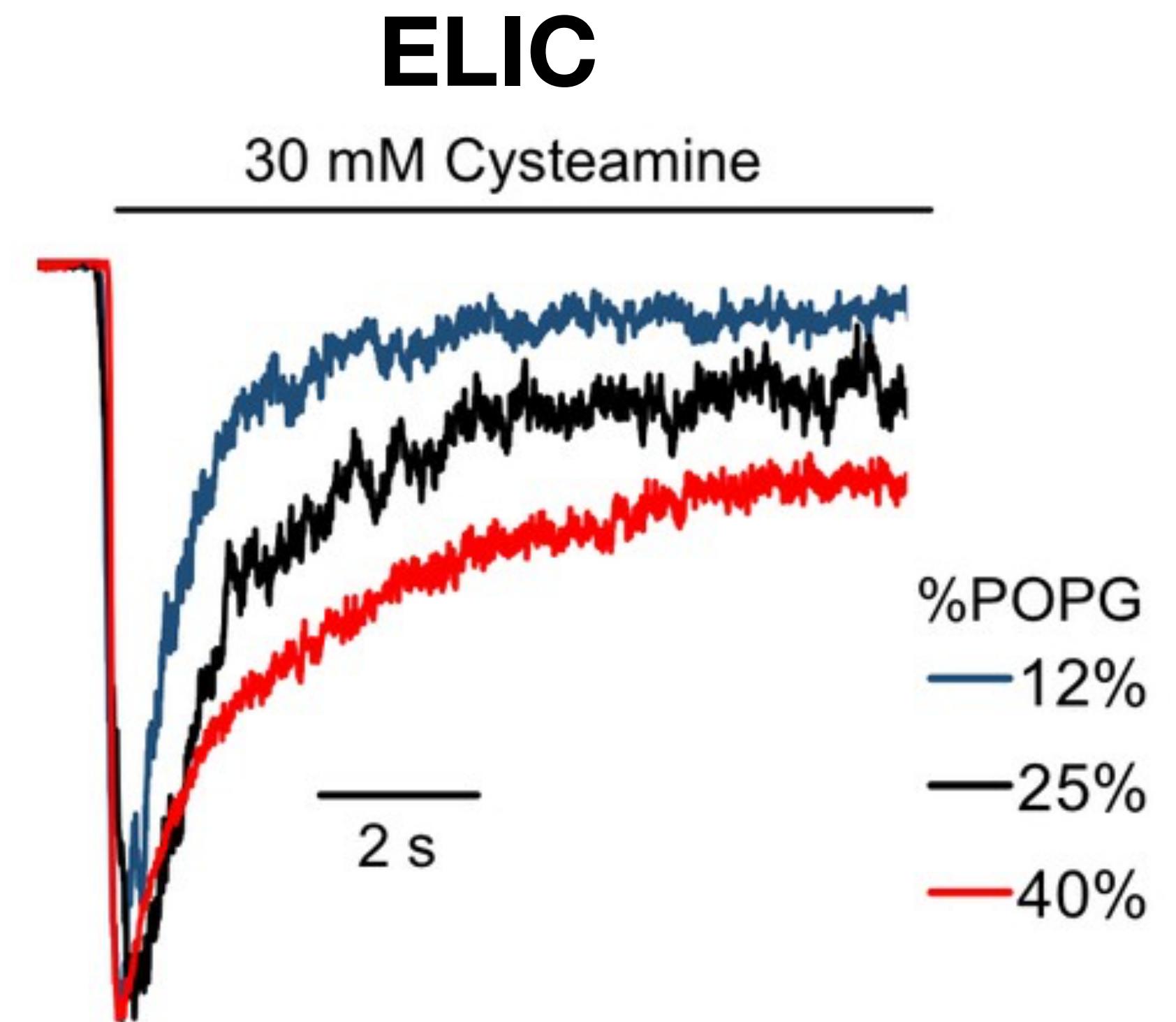
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## Pentameric Ligand-Gated Ion Channels



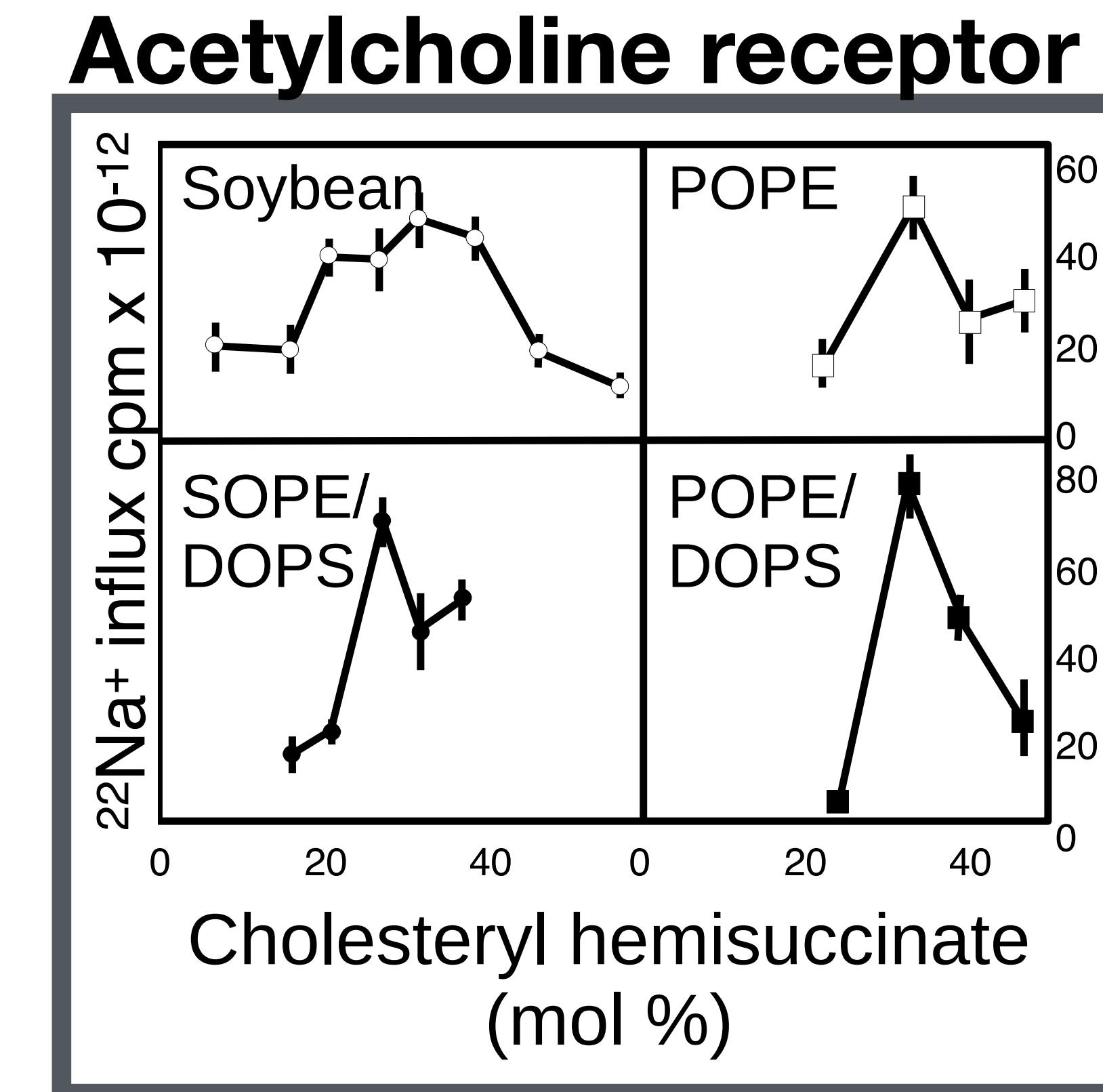
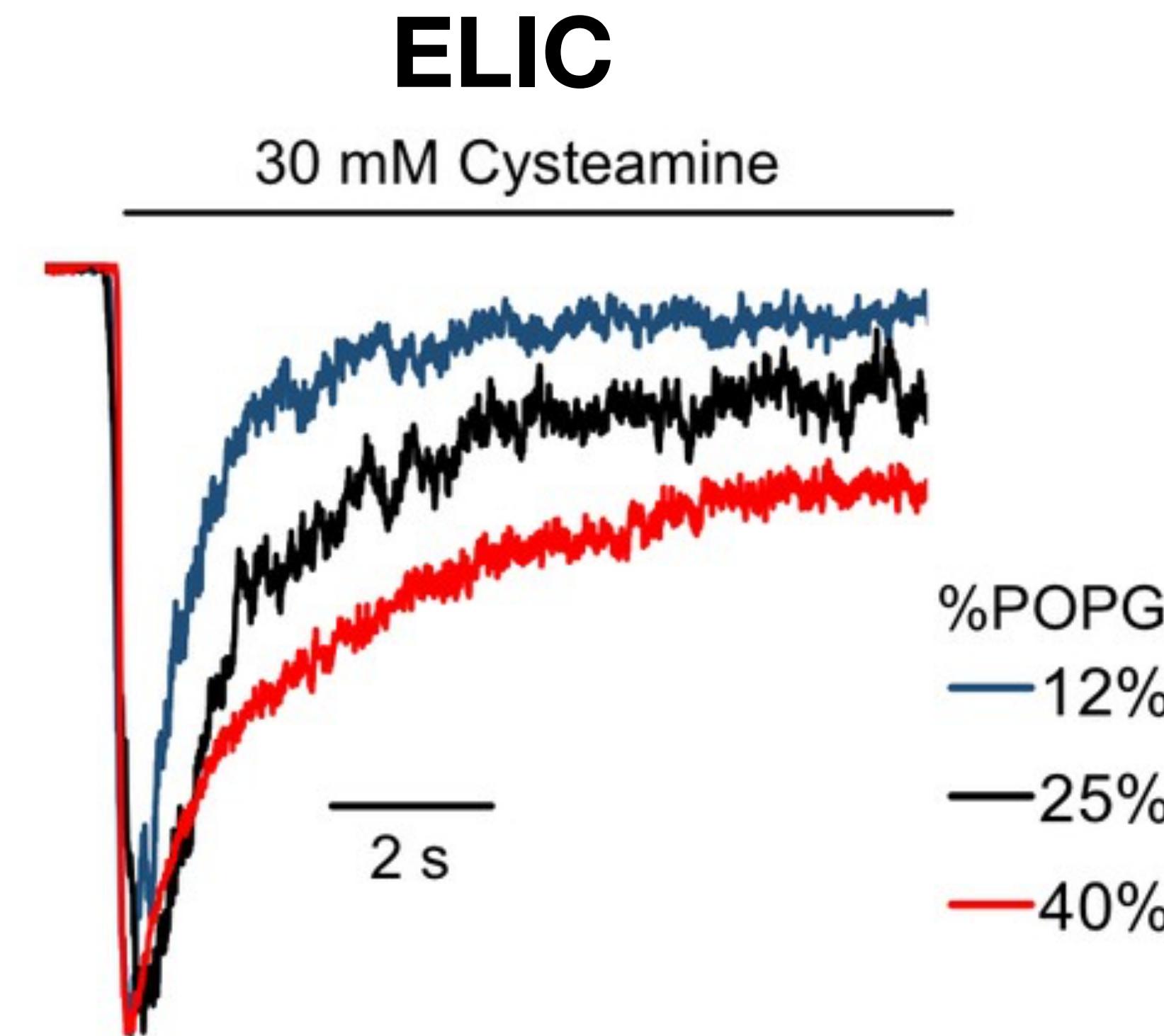
**ELIC: a prokaryotic model**

# pLGICs are lipid-sensitive



Ailing Tong, John T Petroff II, ... ,  
Grace Brannigan, Wayland WL Cheng  
eLife 2019

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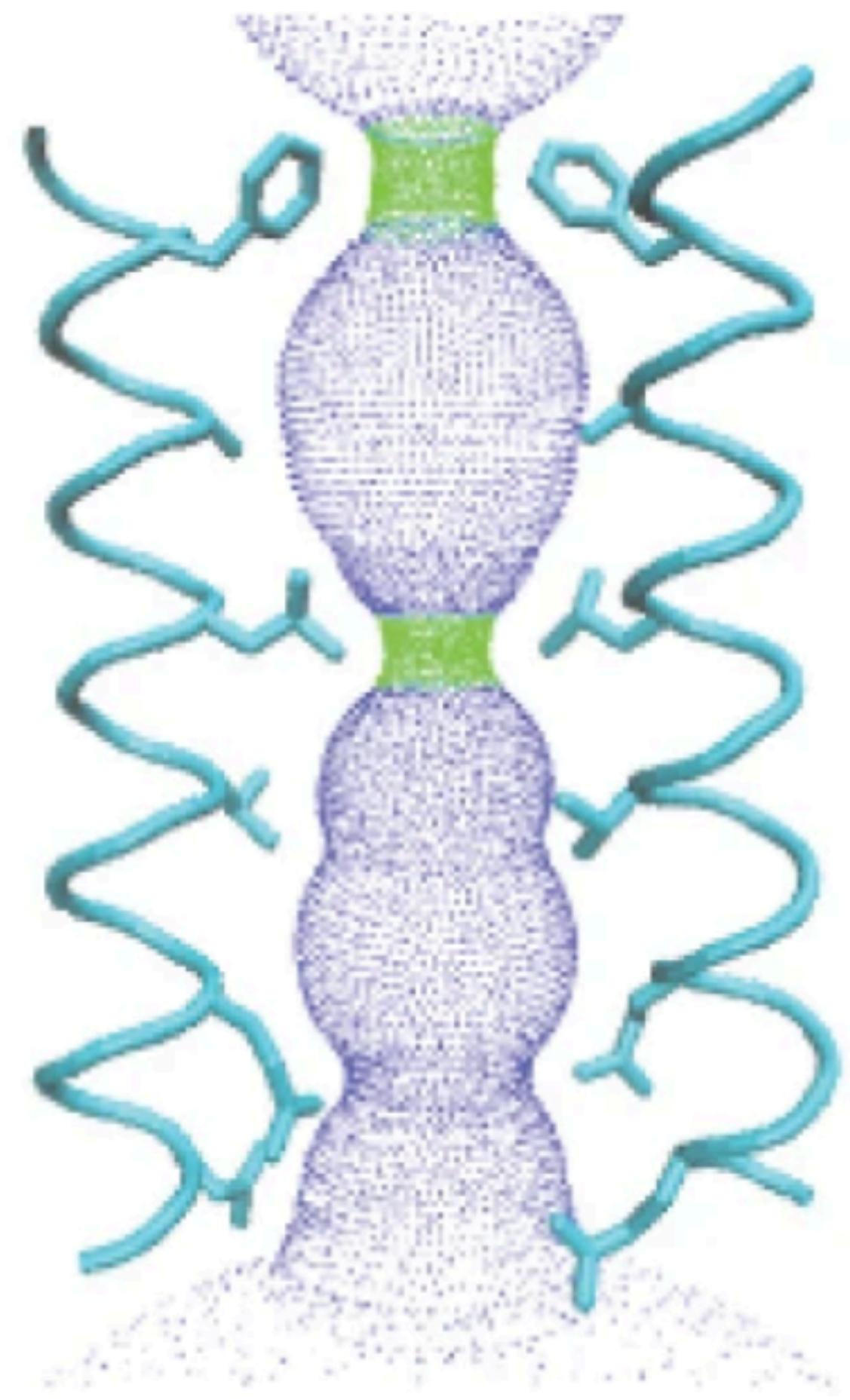


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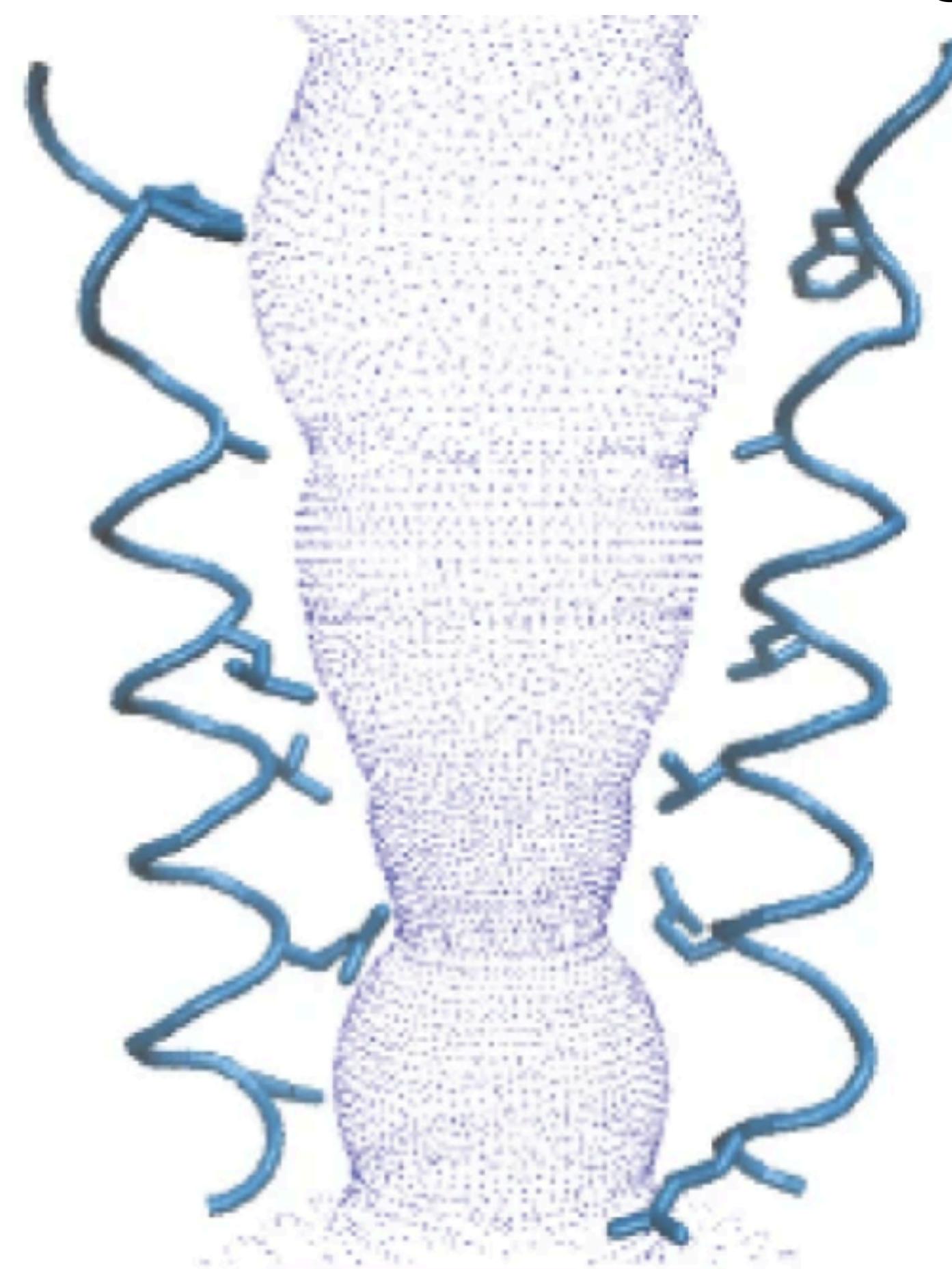
M Criado, H Eibl, FJ Barrantes  
J. Biol. Chem. 1984

# ELIC binds lipids

WT Non-conducting

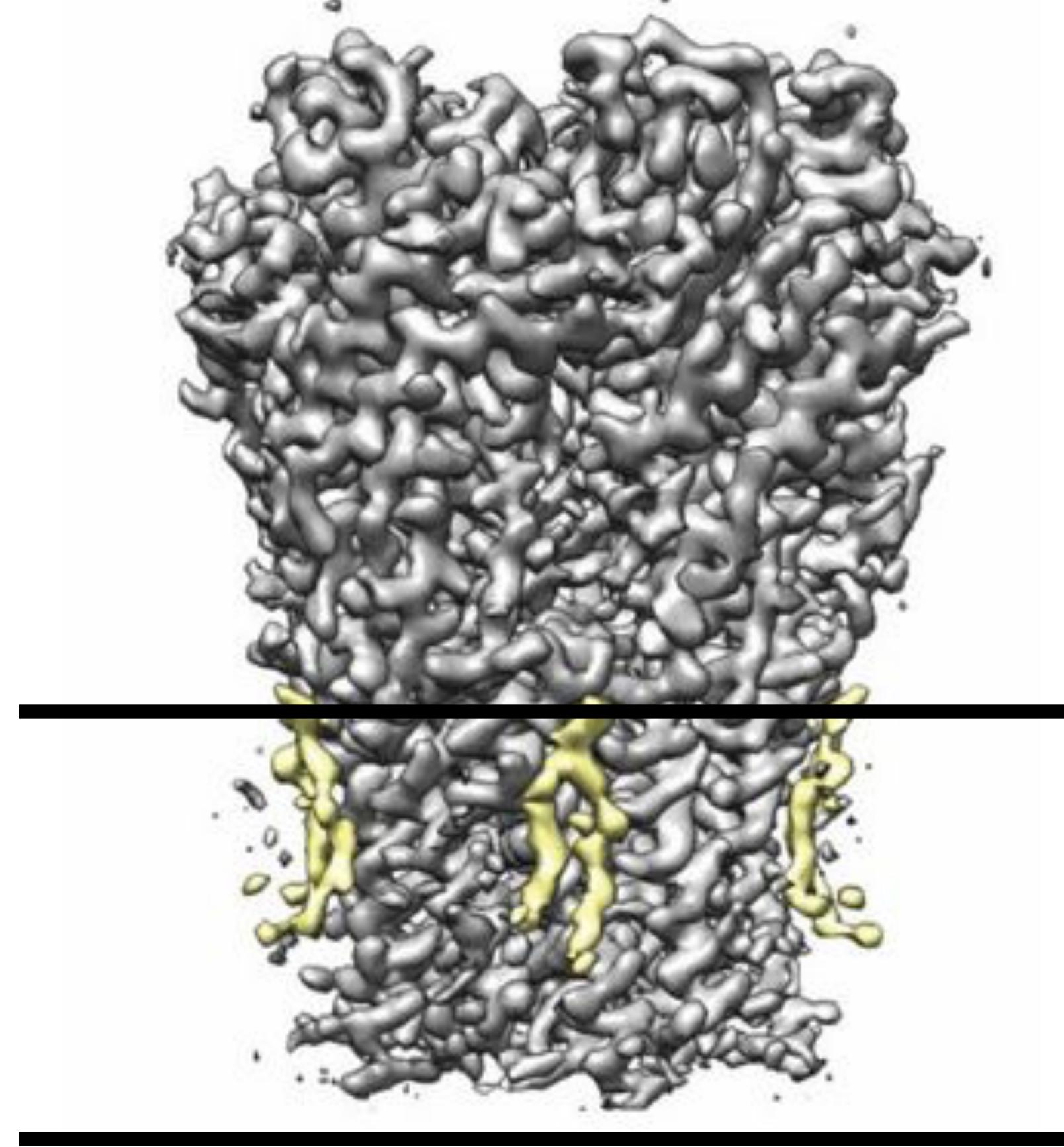


5-mutant Conducting

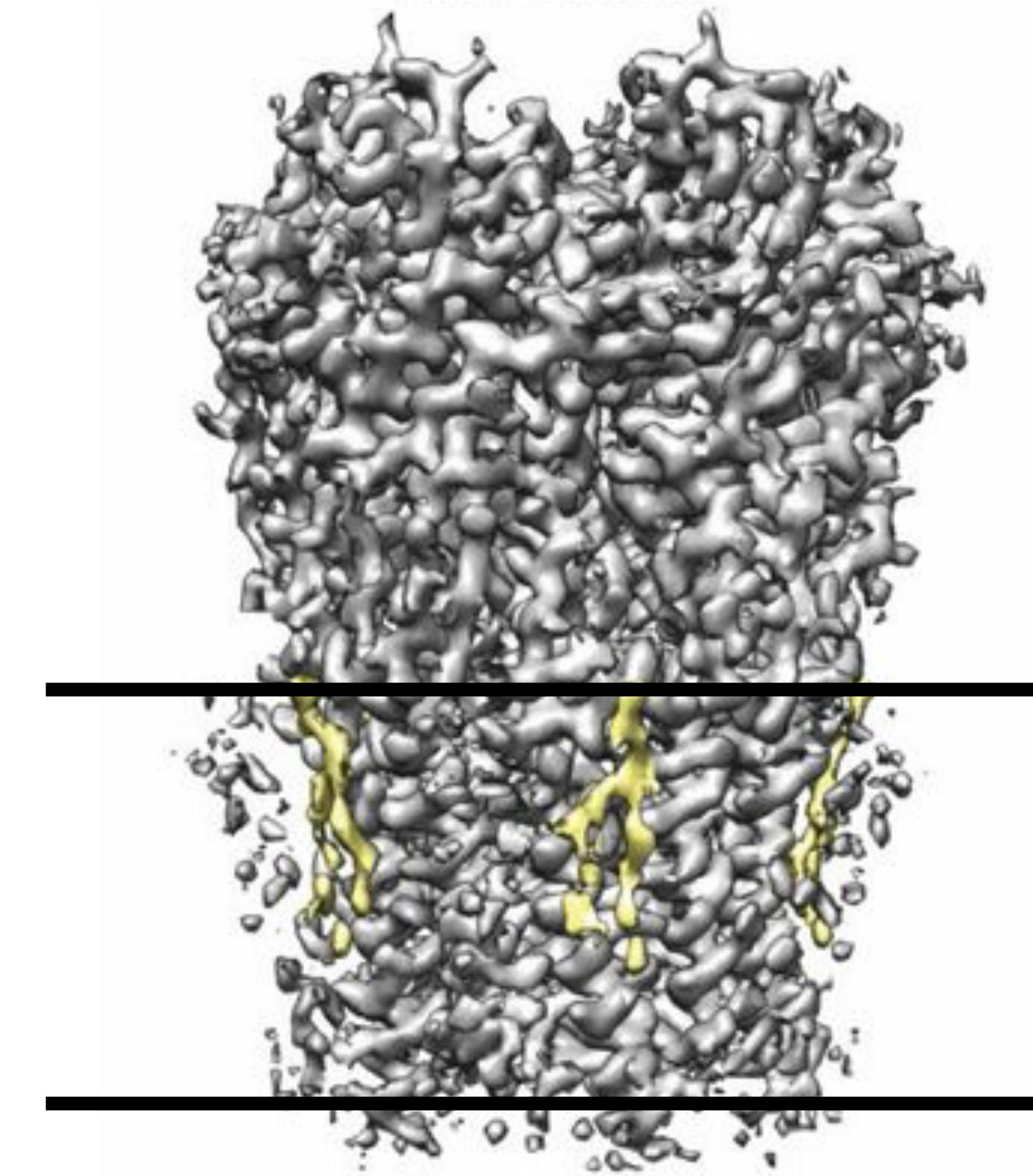


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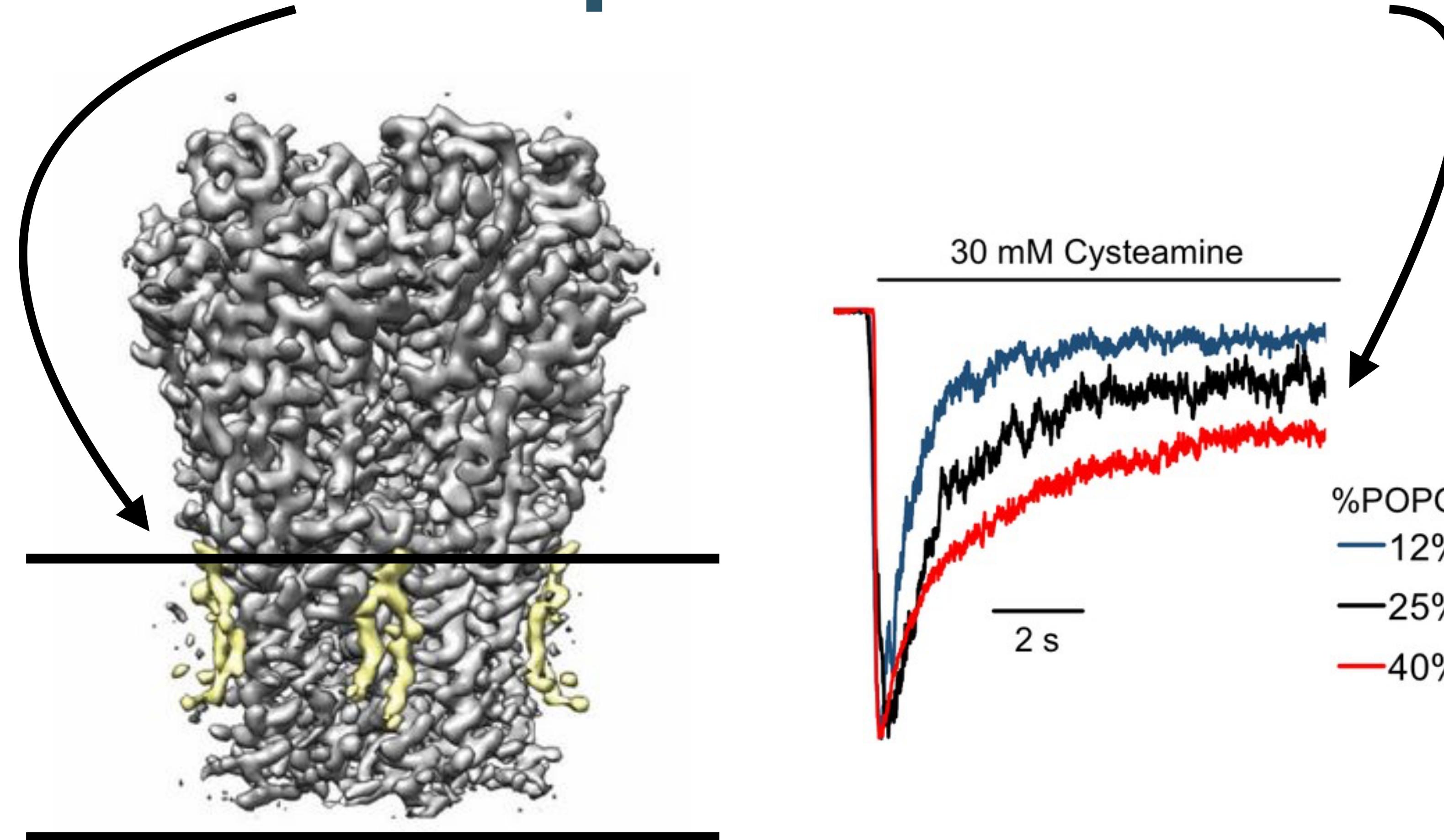
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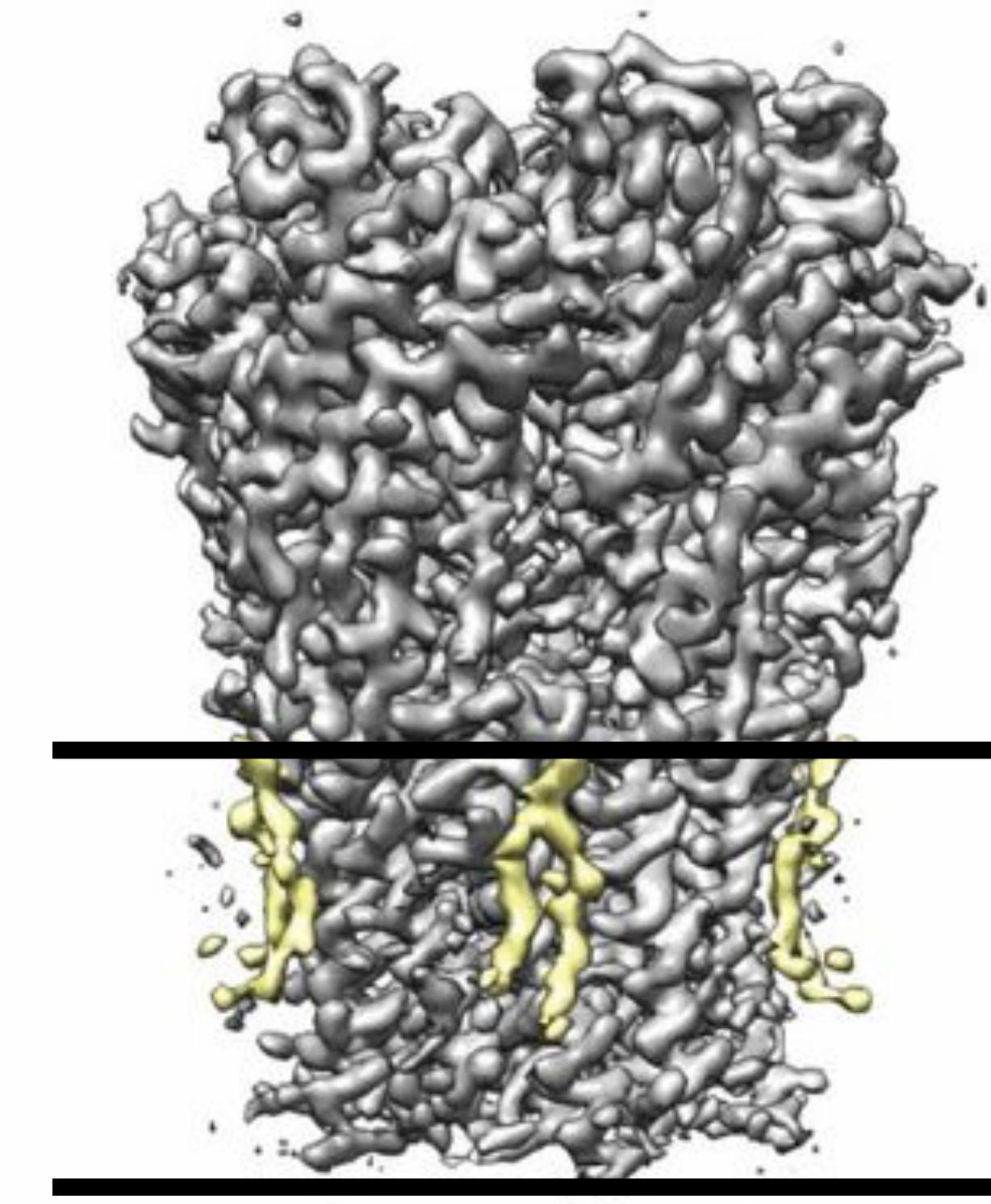


# Are these responsible for these?



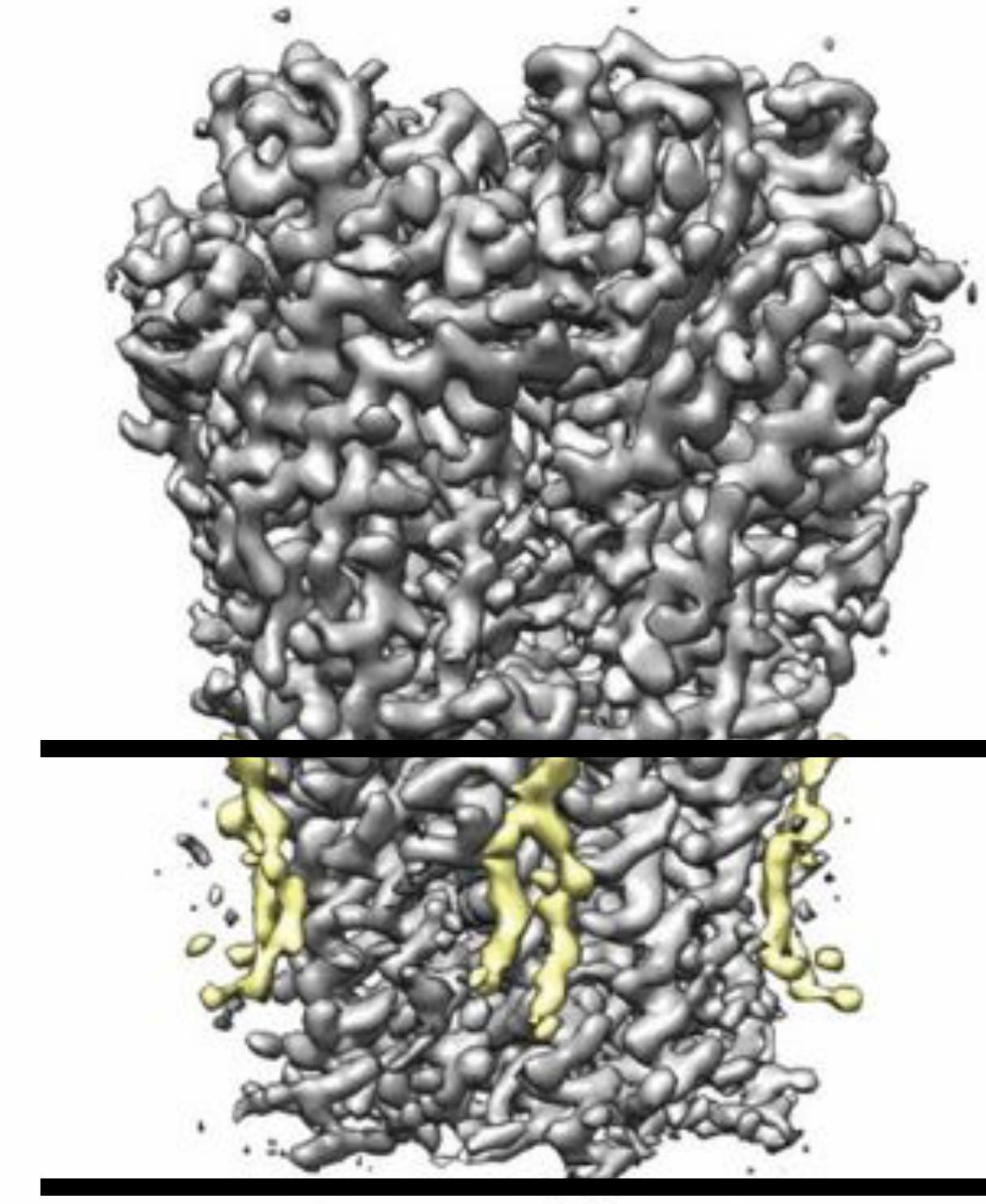
John T. Petroff II, ... Ezry Santiago-McRae, ...  
Tom Joseph, Jérôme Hénin,  
Grace Brannigan & Wayland W. L. Cheng  
Nature Communications 2022

# Experimentally inaccessible questions:



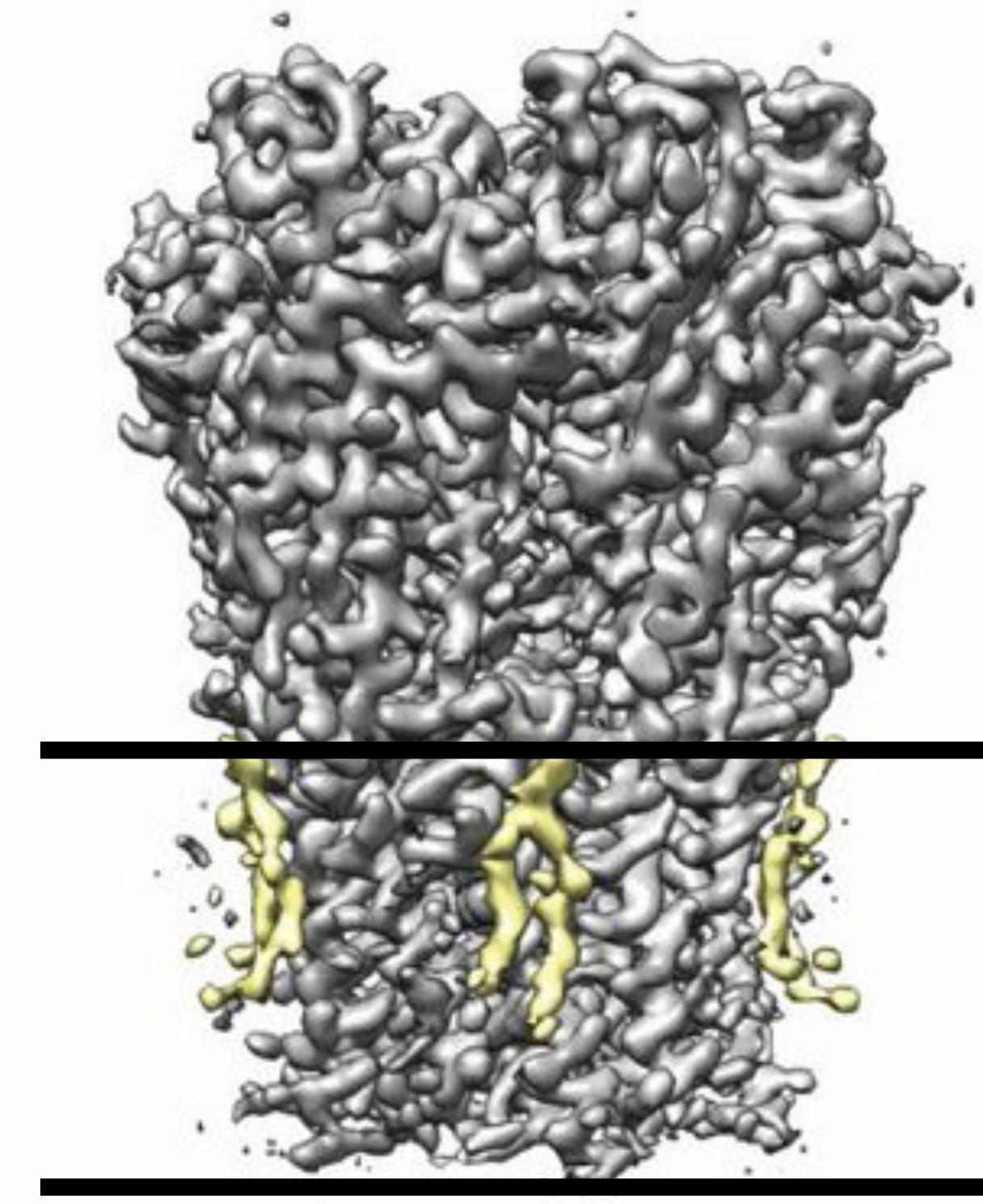
# Experimentally inaccessible questions:

- Are these lipids **specifically** bound?



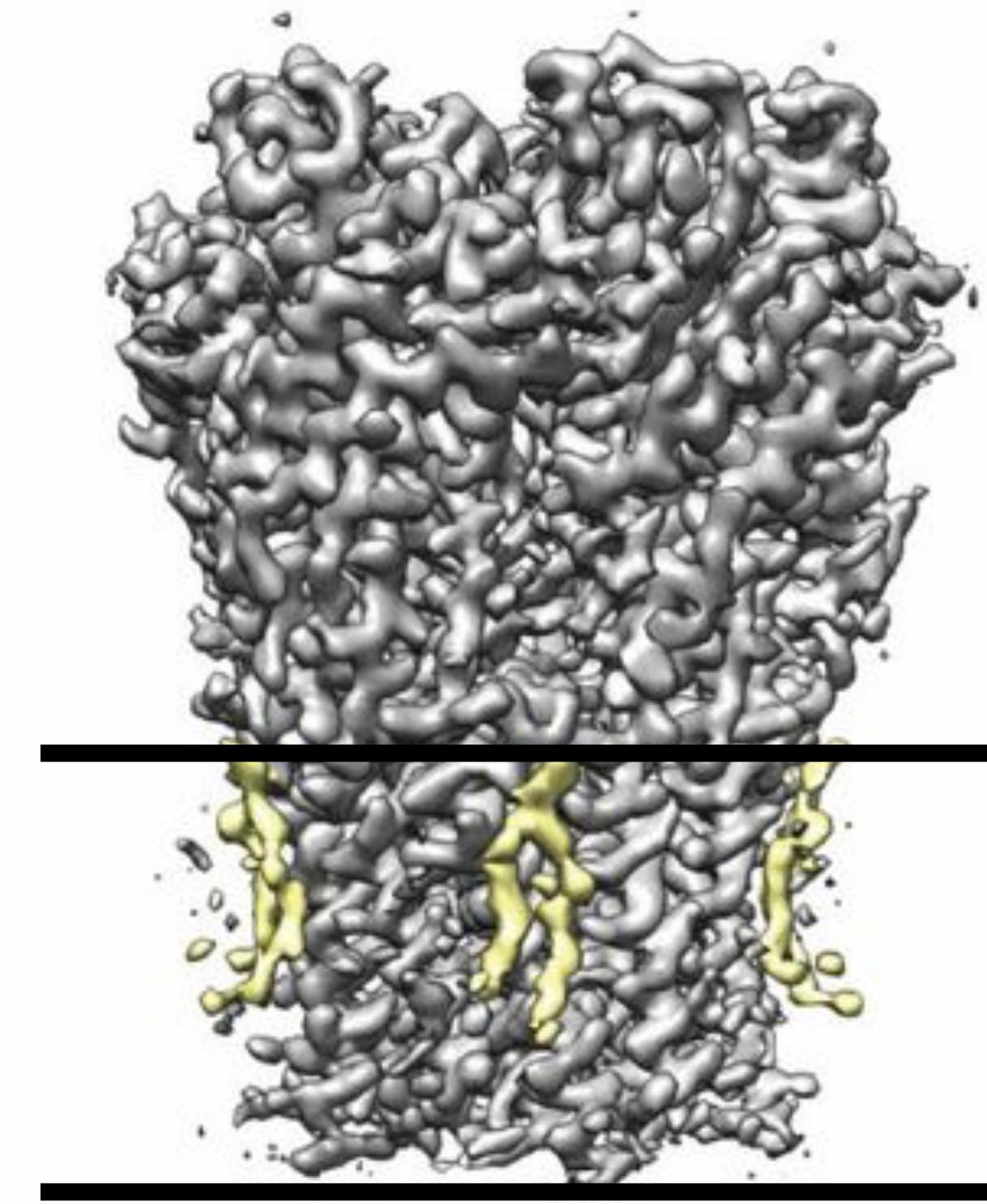
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- Are these lipids **specifically** bound?
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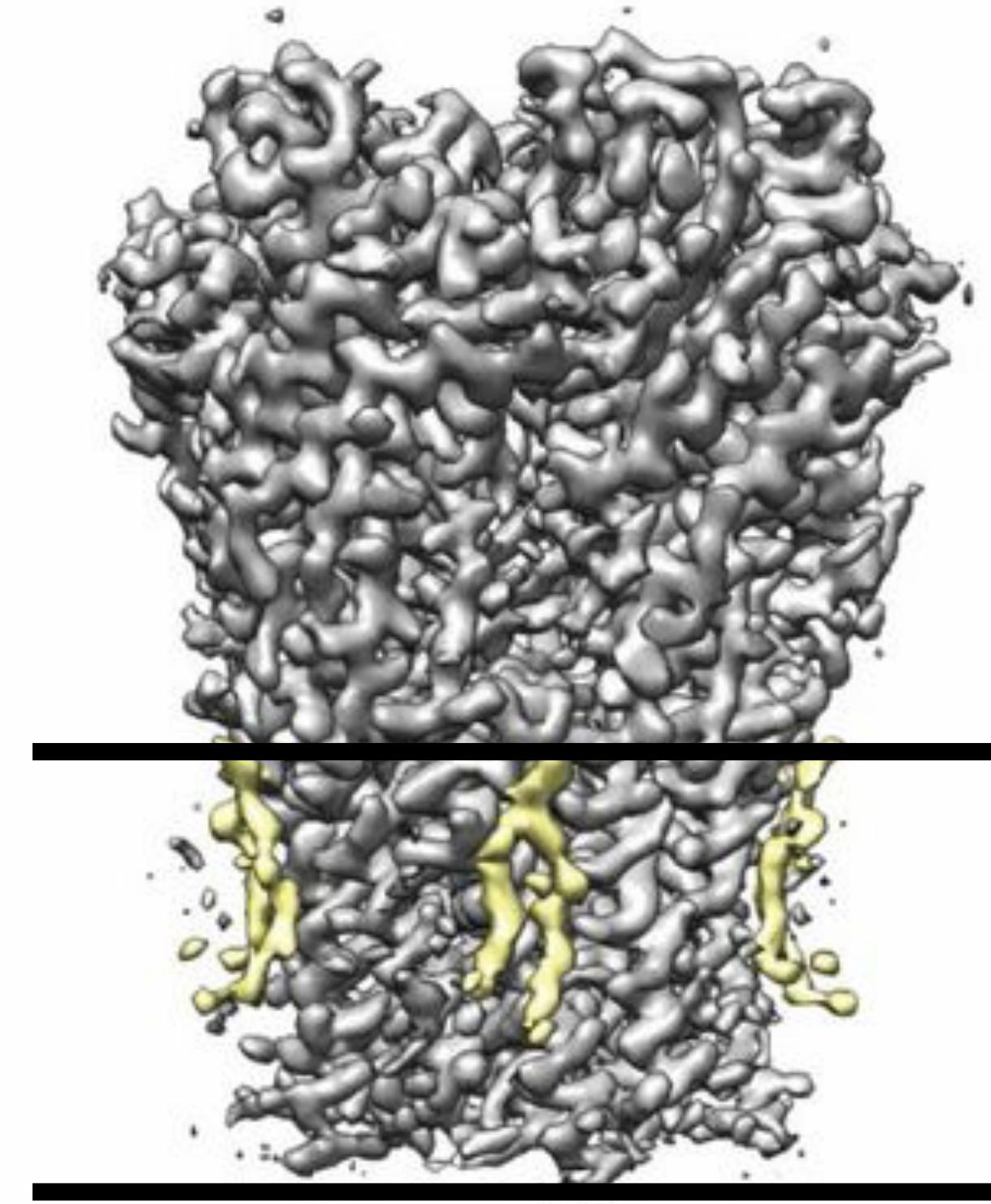
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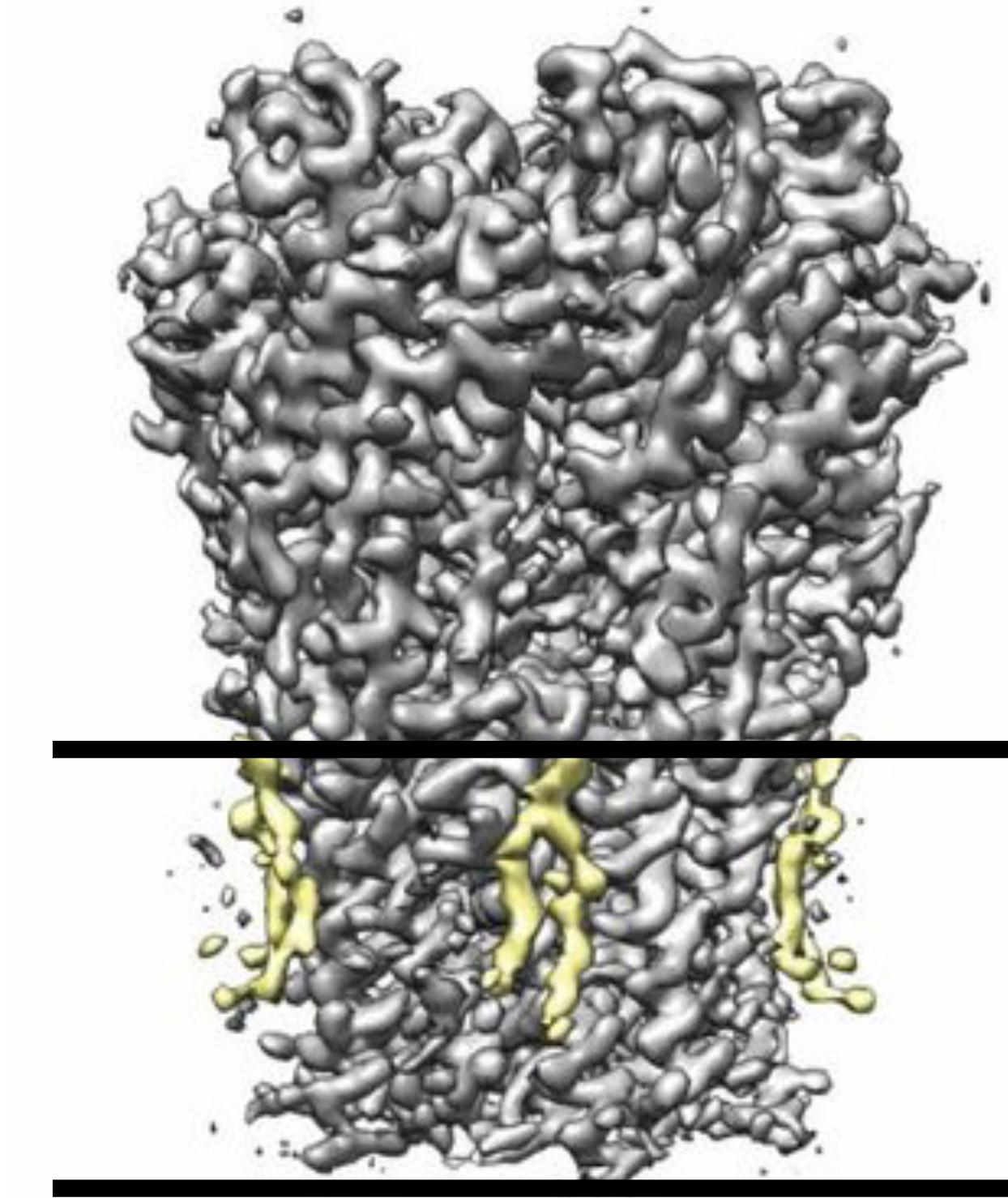
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We have a computational solution:  
**Streamlined Alchemical Free Energy Perturbation  
(SAFEP)**

# **SAFEP is a FEP implementation**

- Two Flavors (both work at atomistic resolution)**

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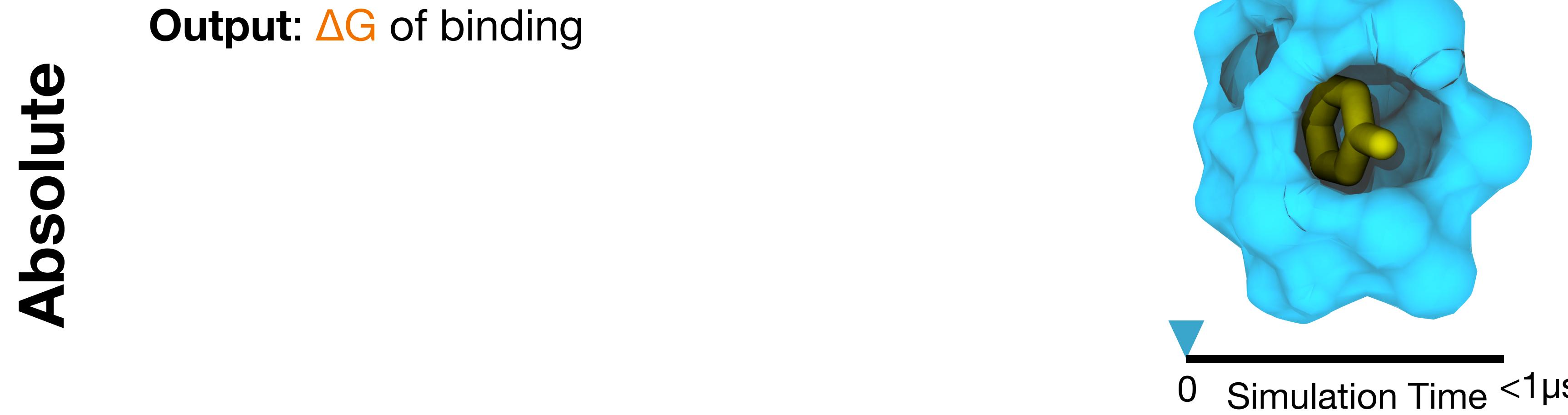
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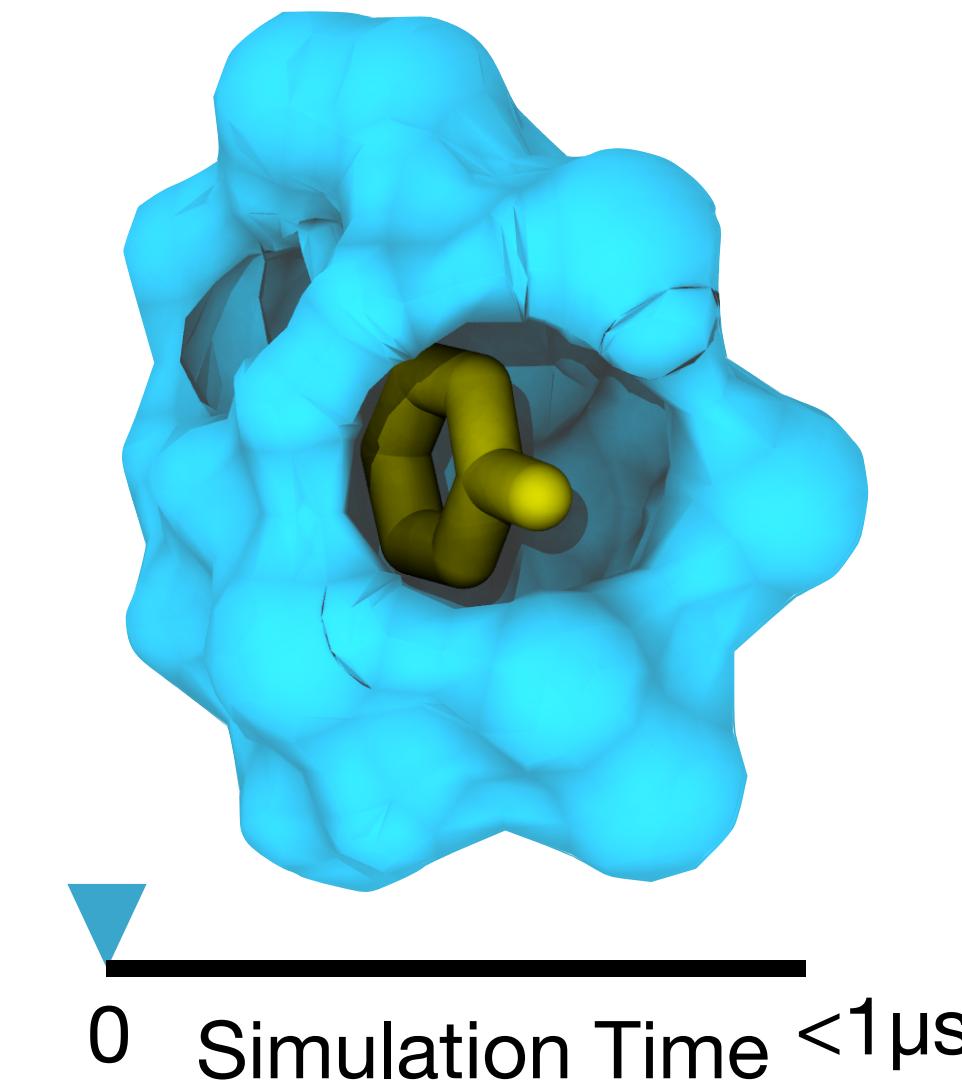
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**Output:**  $\Delta G$  of binding

**How:** Turn off the interactions between the ligand and everything else



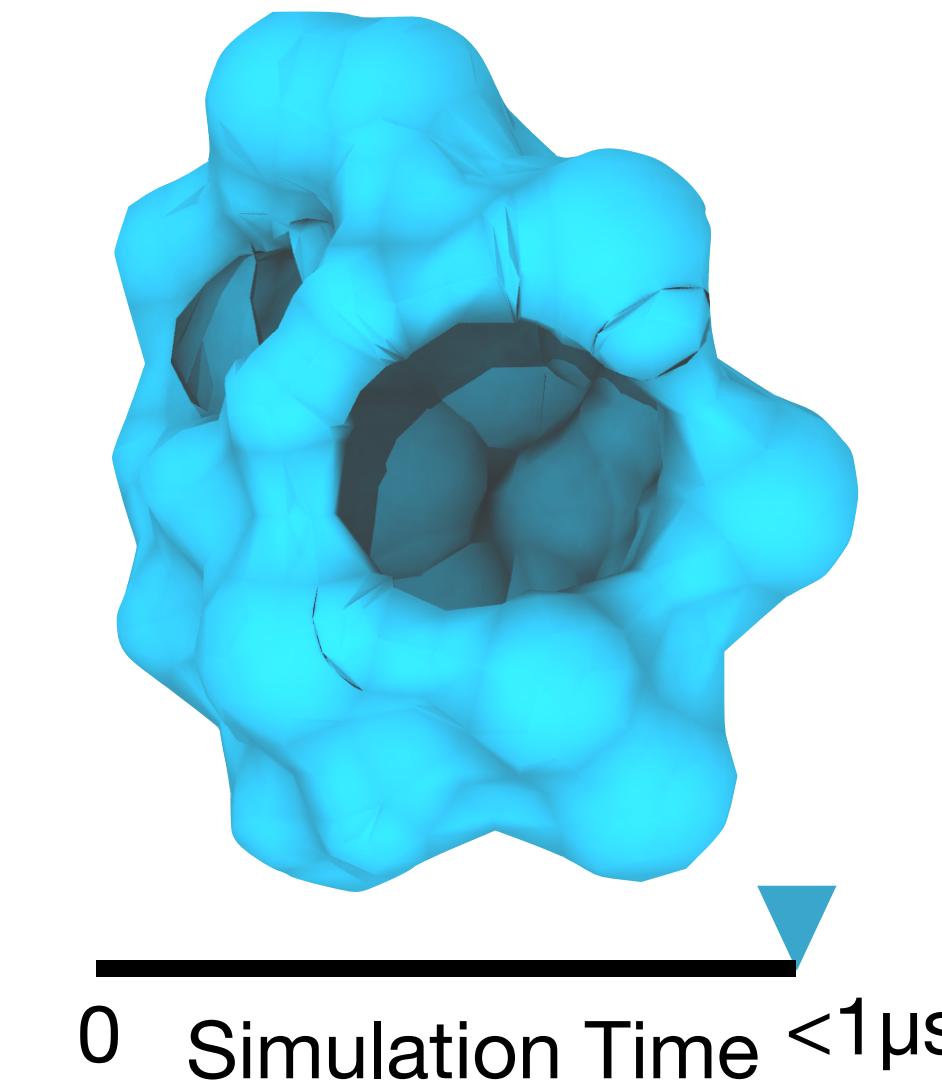
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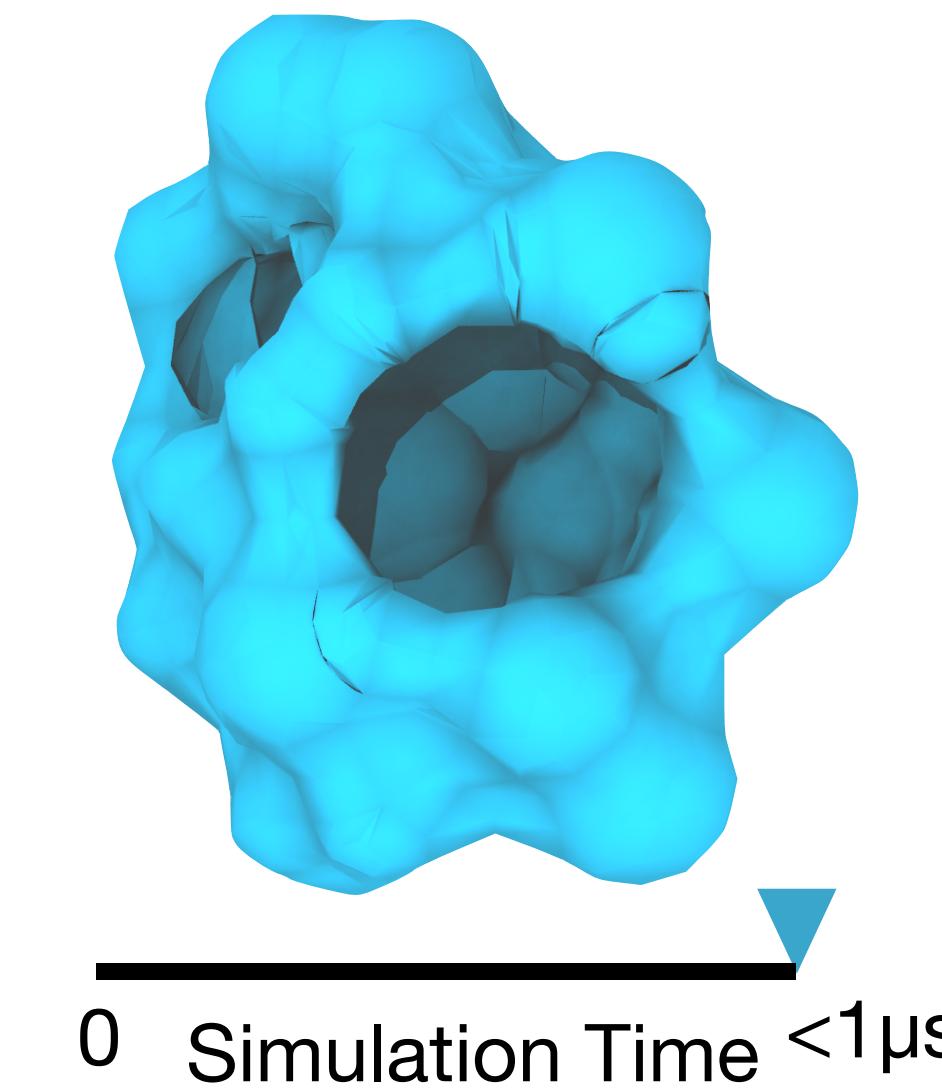
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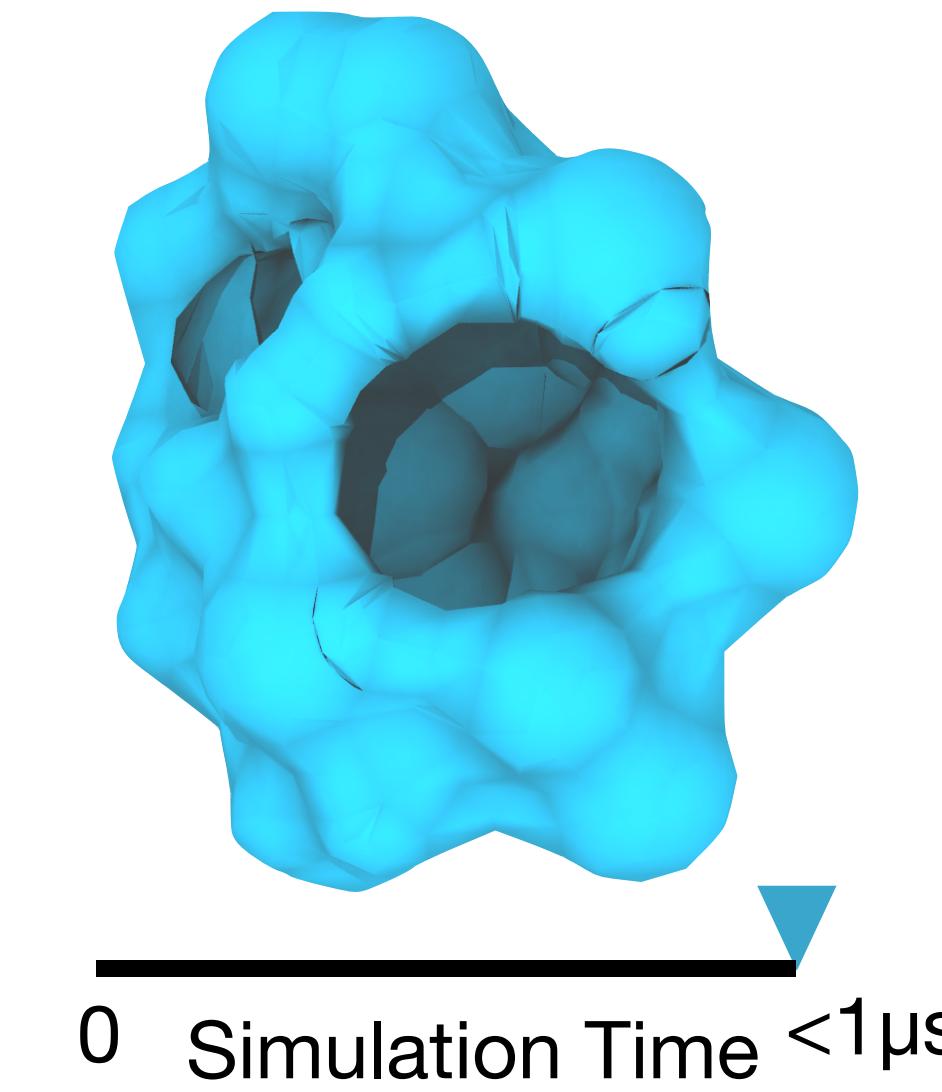
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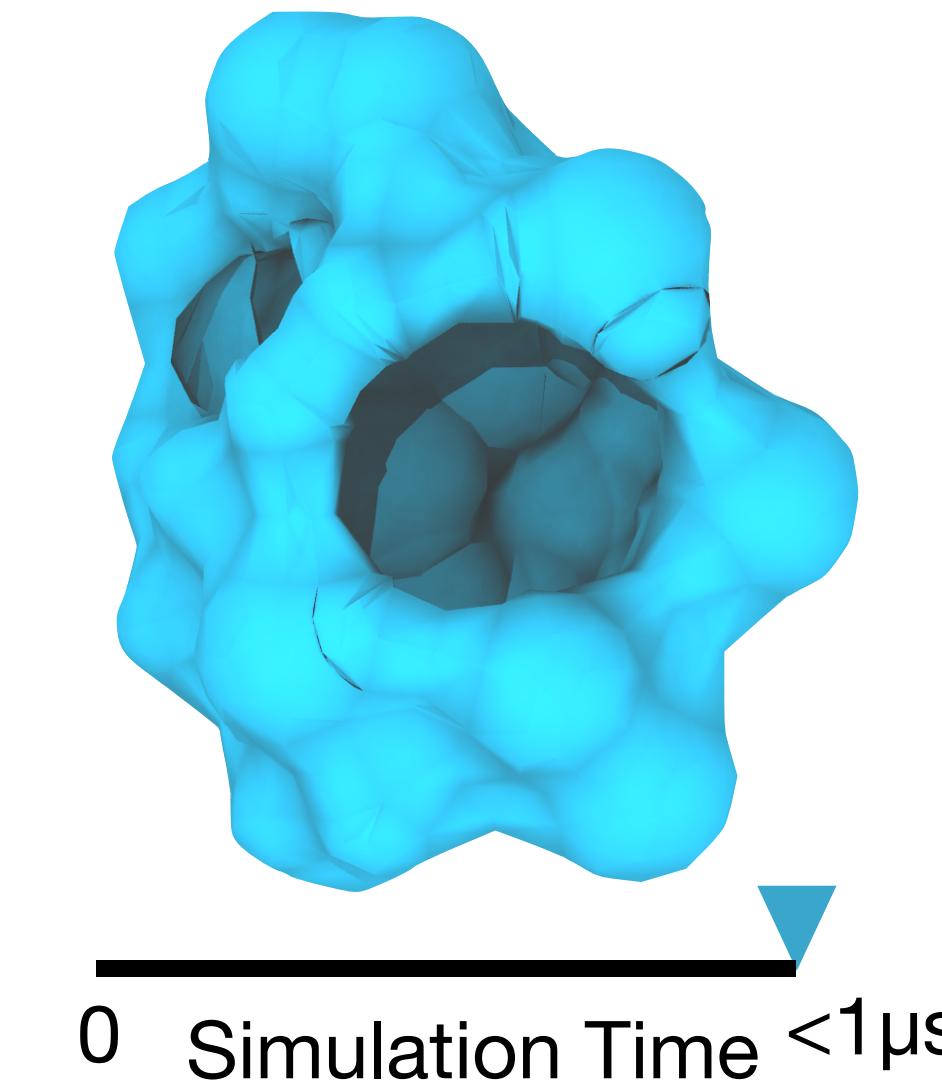
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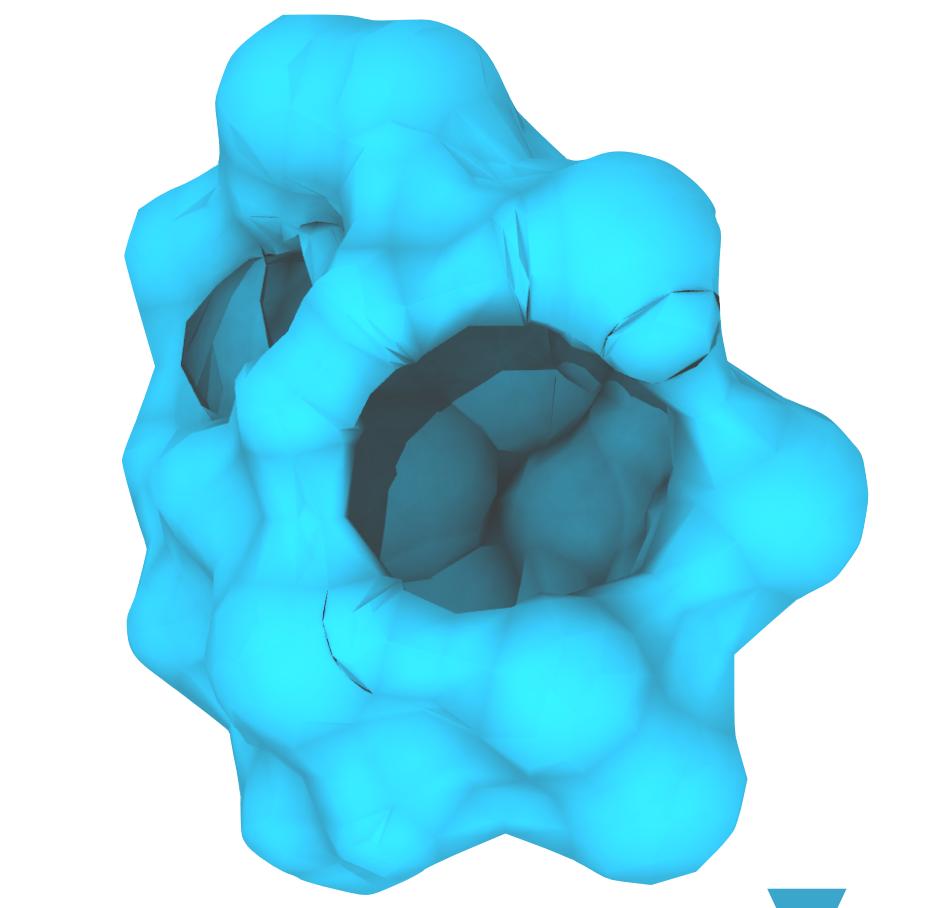
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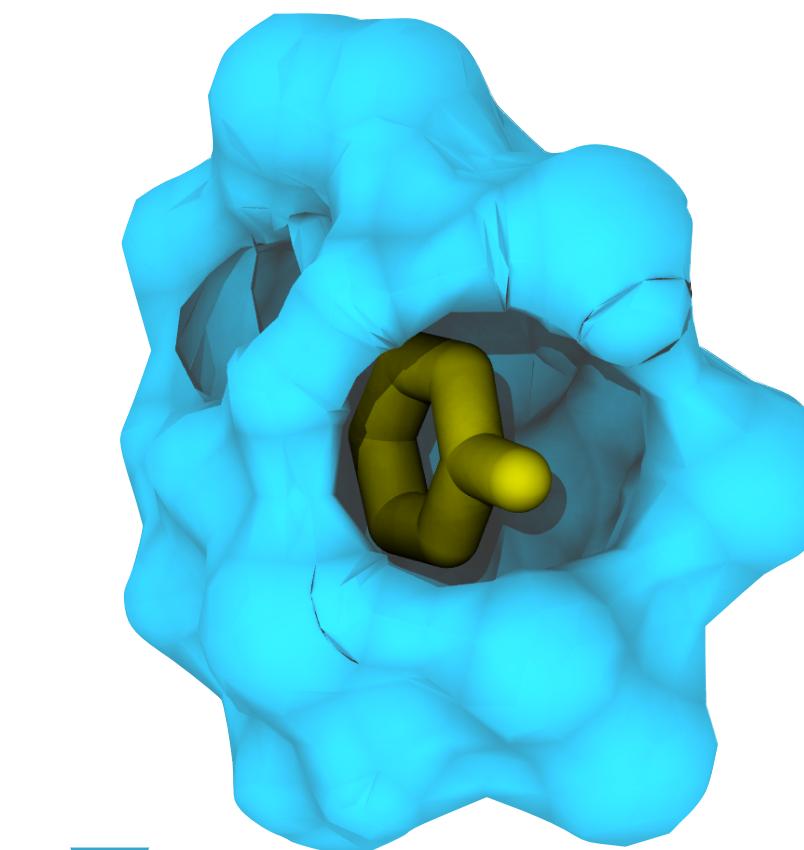
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Relative



0 Simulation Time <1μs



0 ~100ns 8  
Simulation Time

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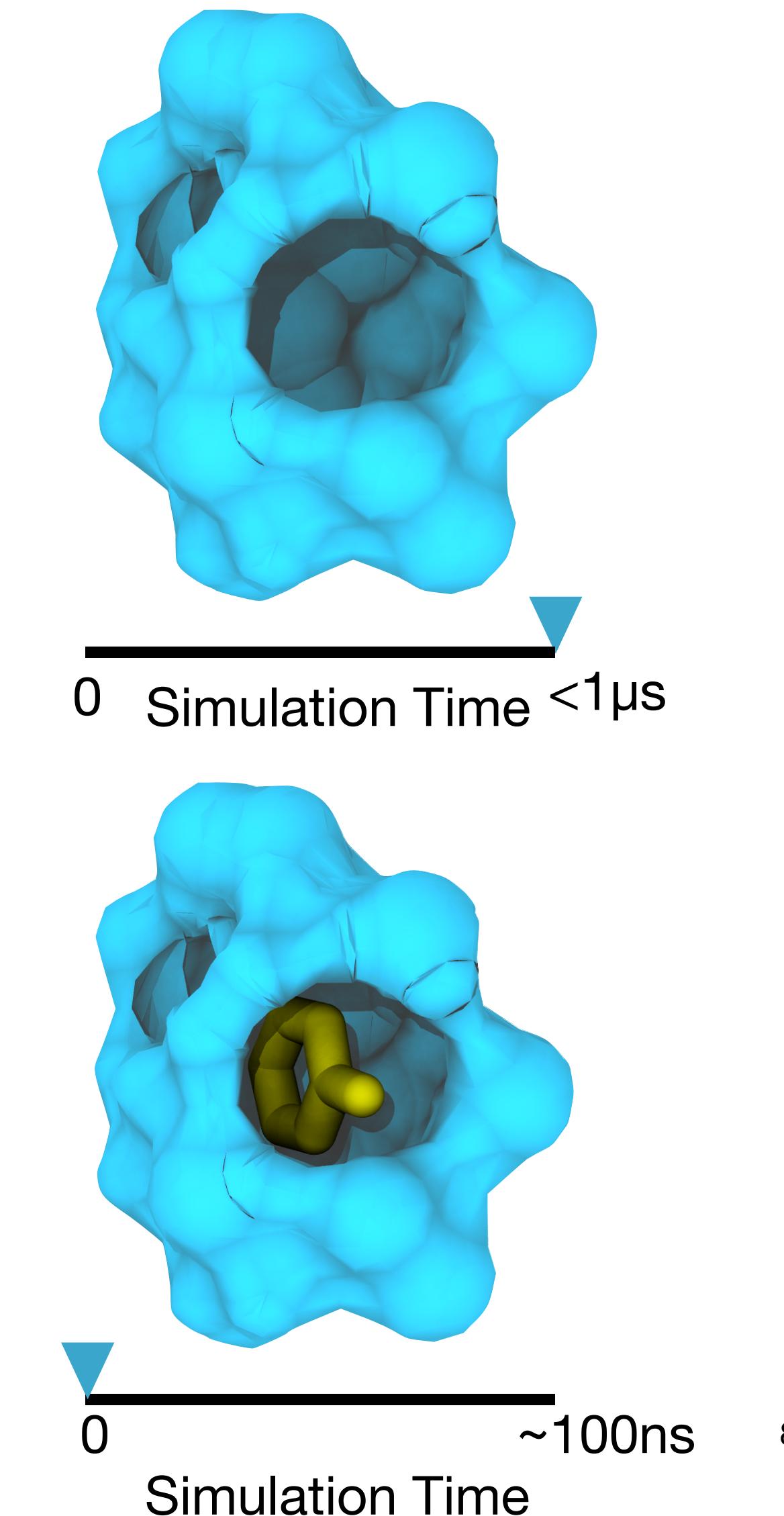
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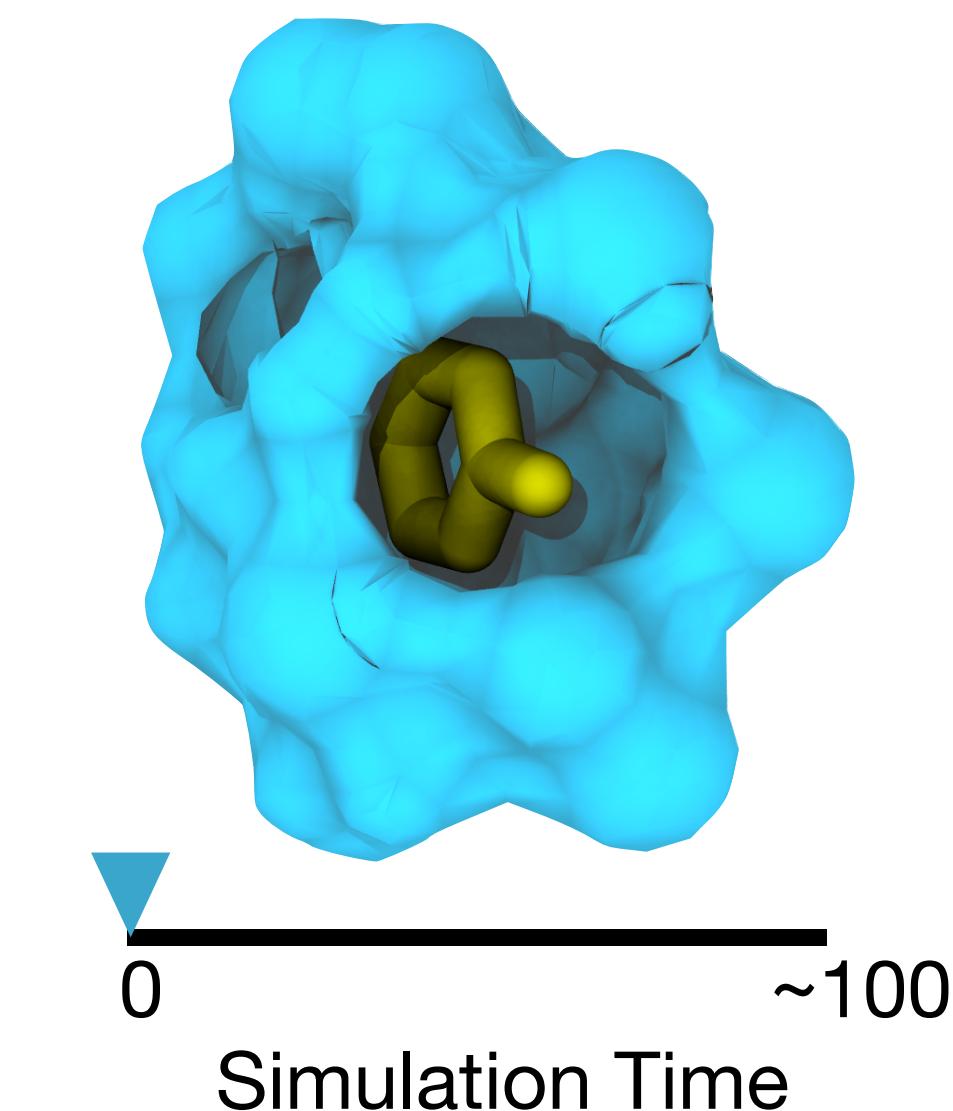
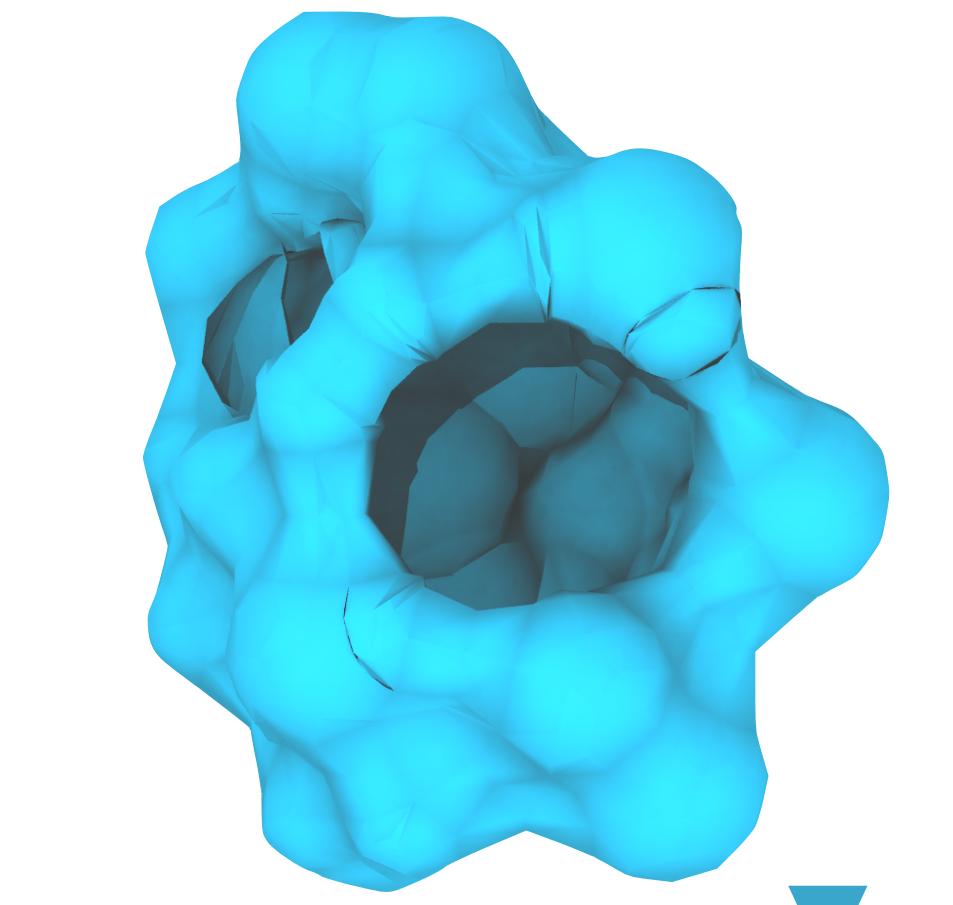
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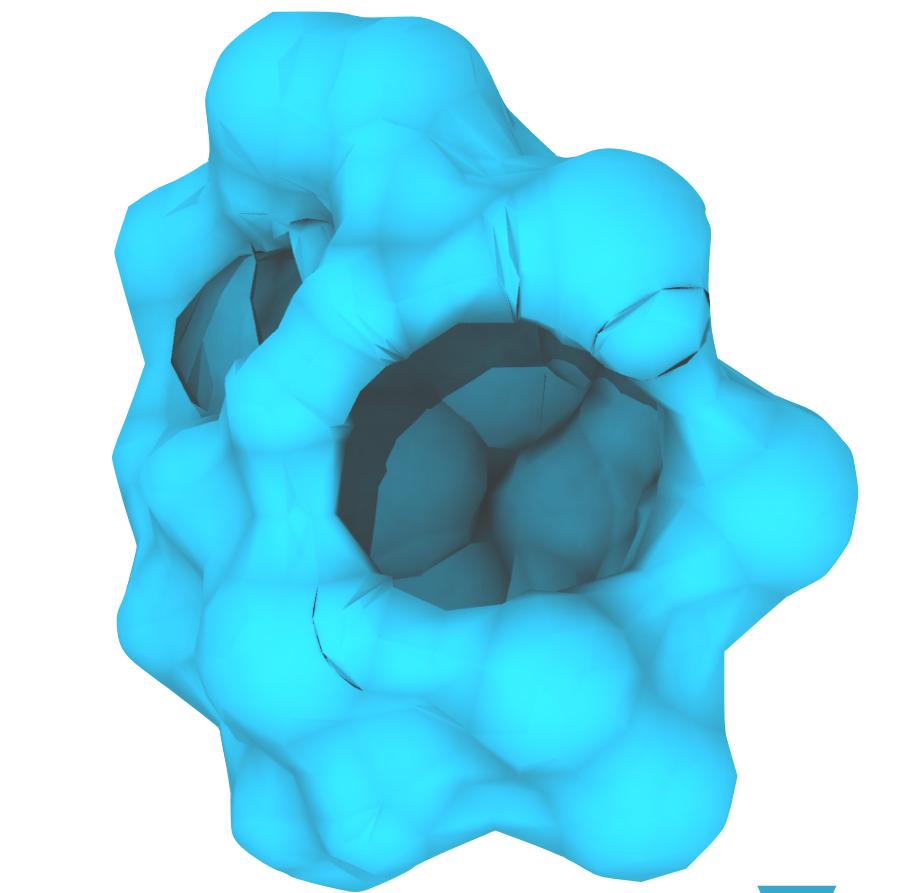
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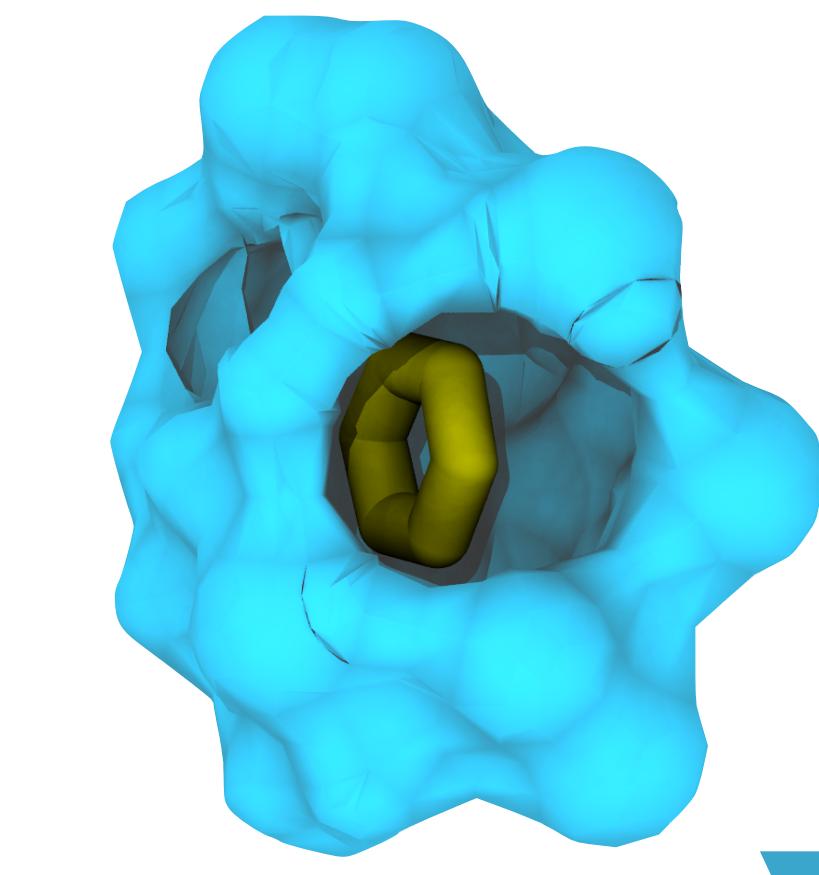


0 Simulation Time <1μs

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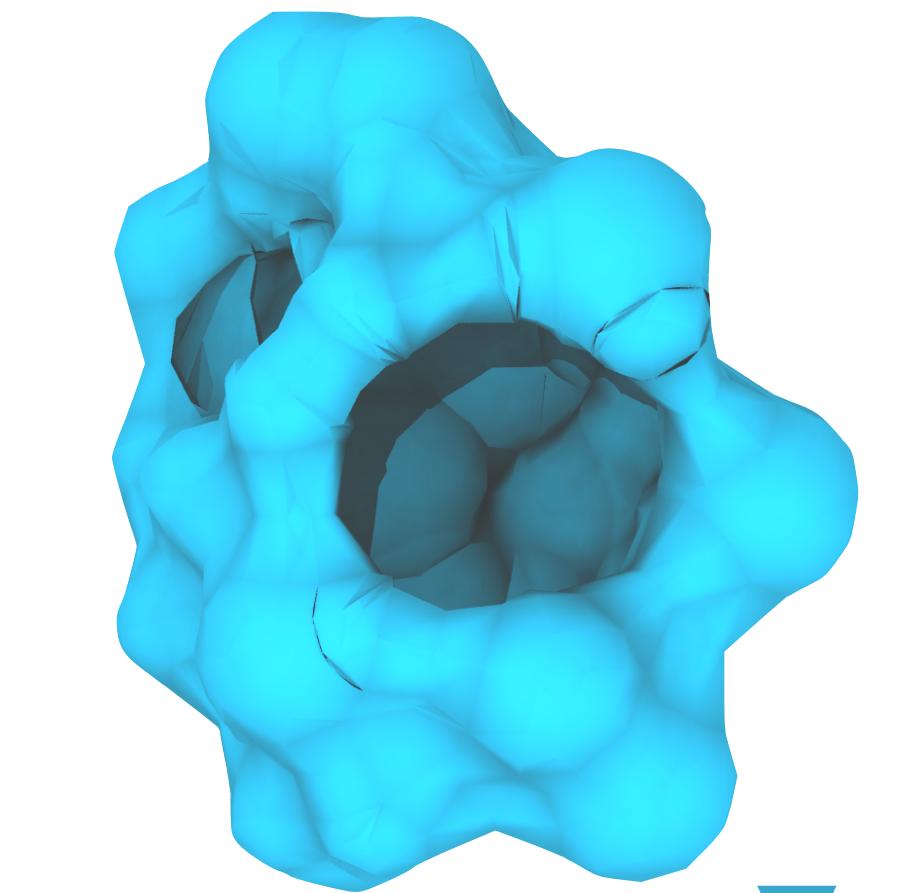
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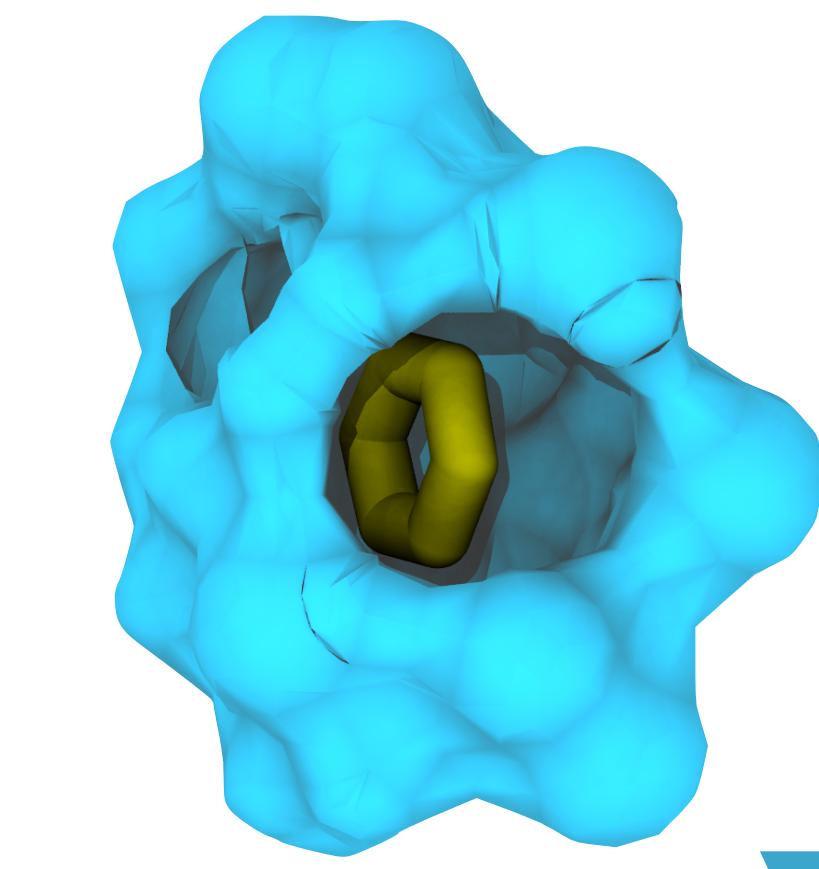
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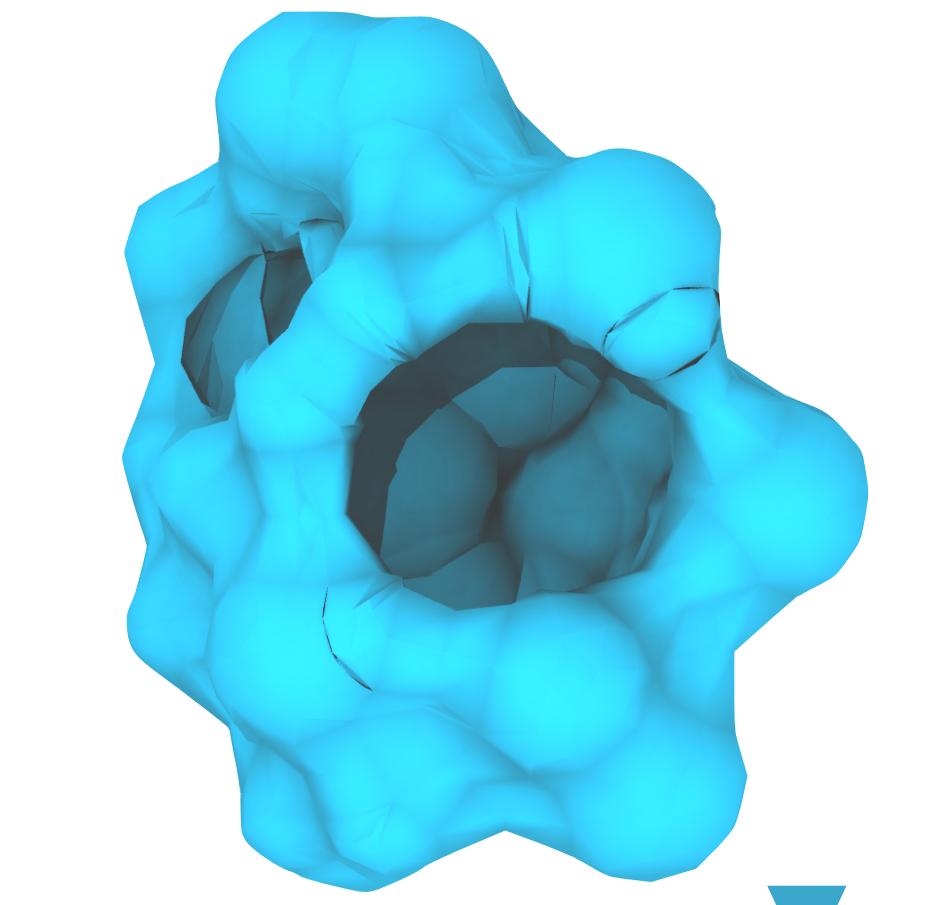
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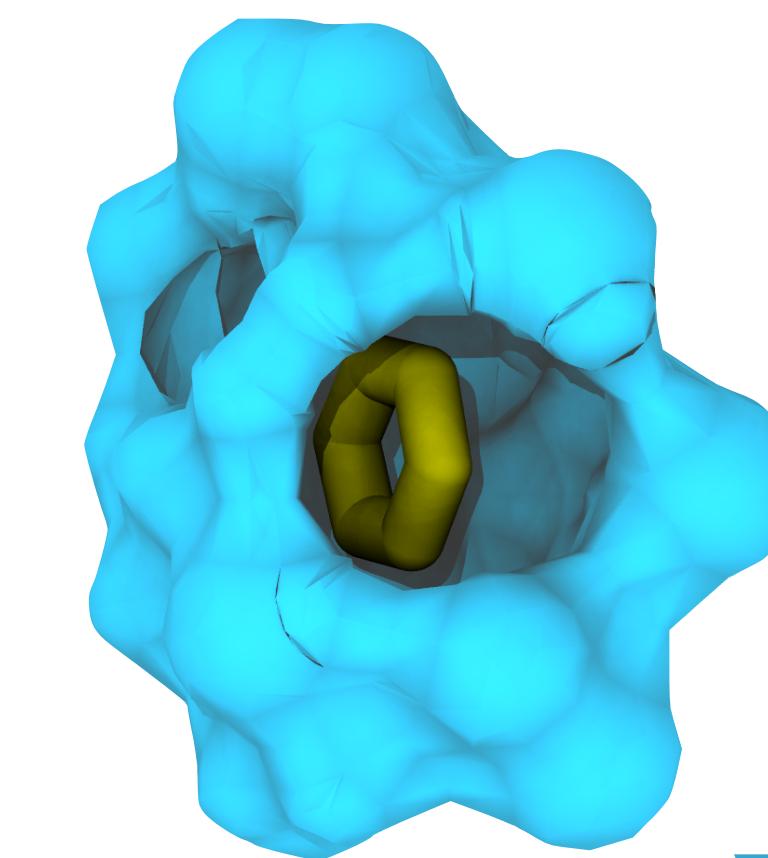
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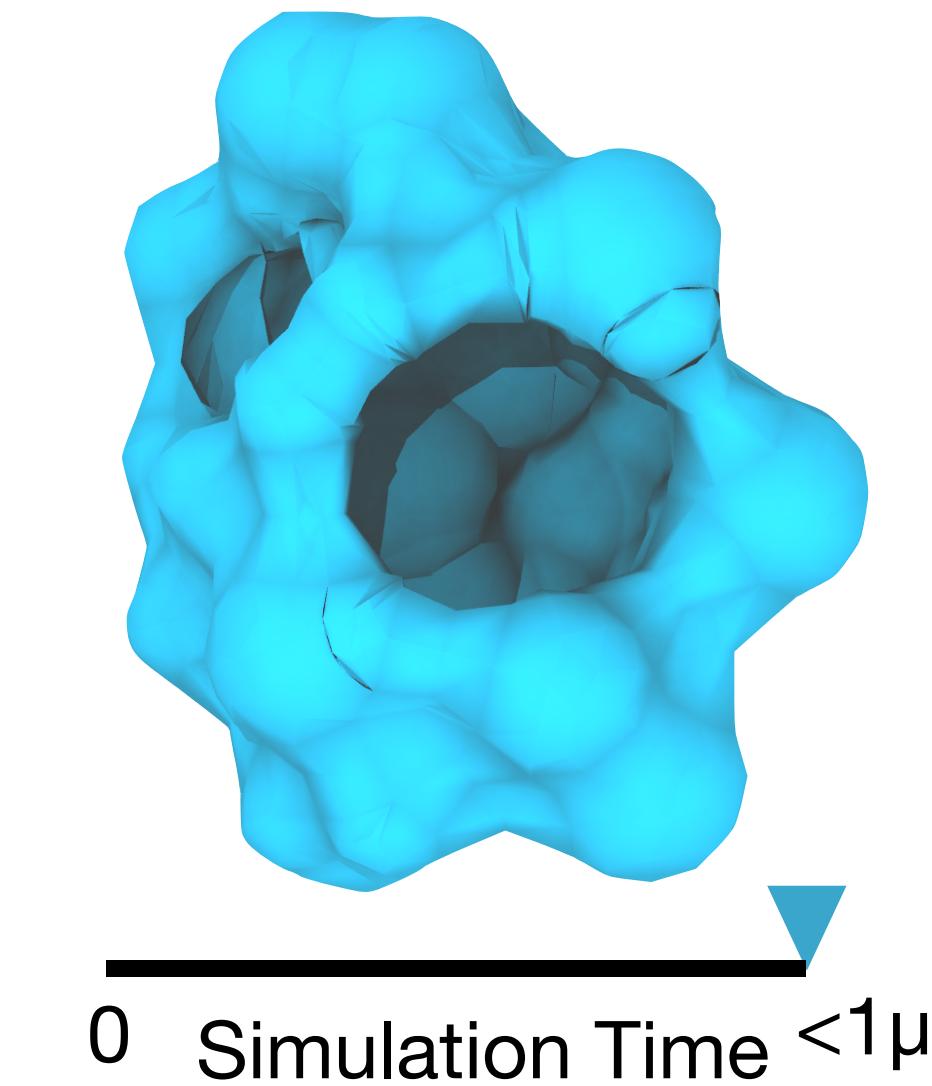
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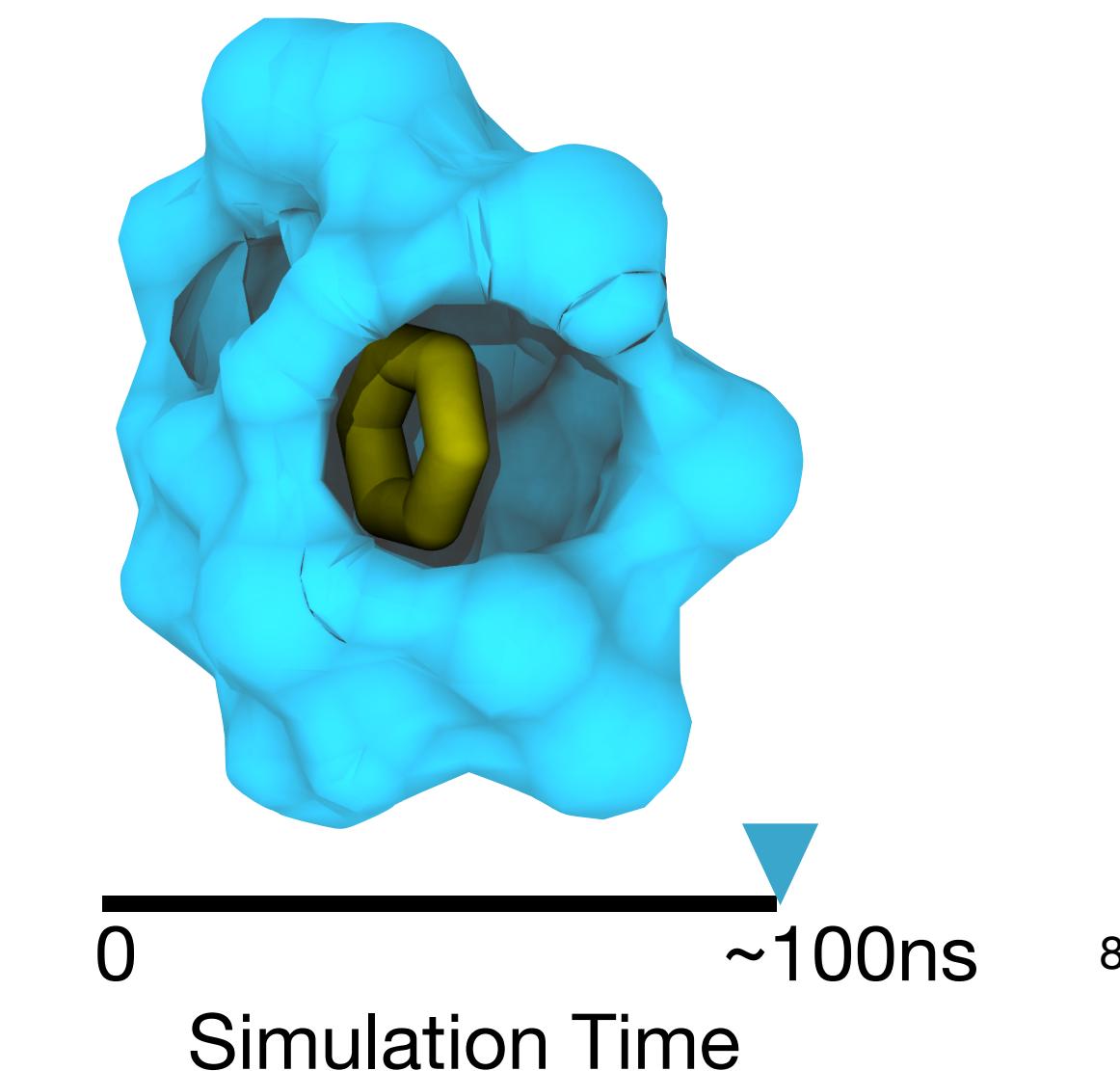
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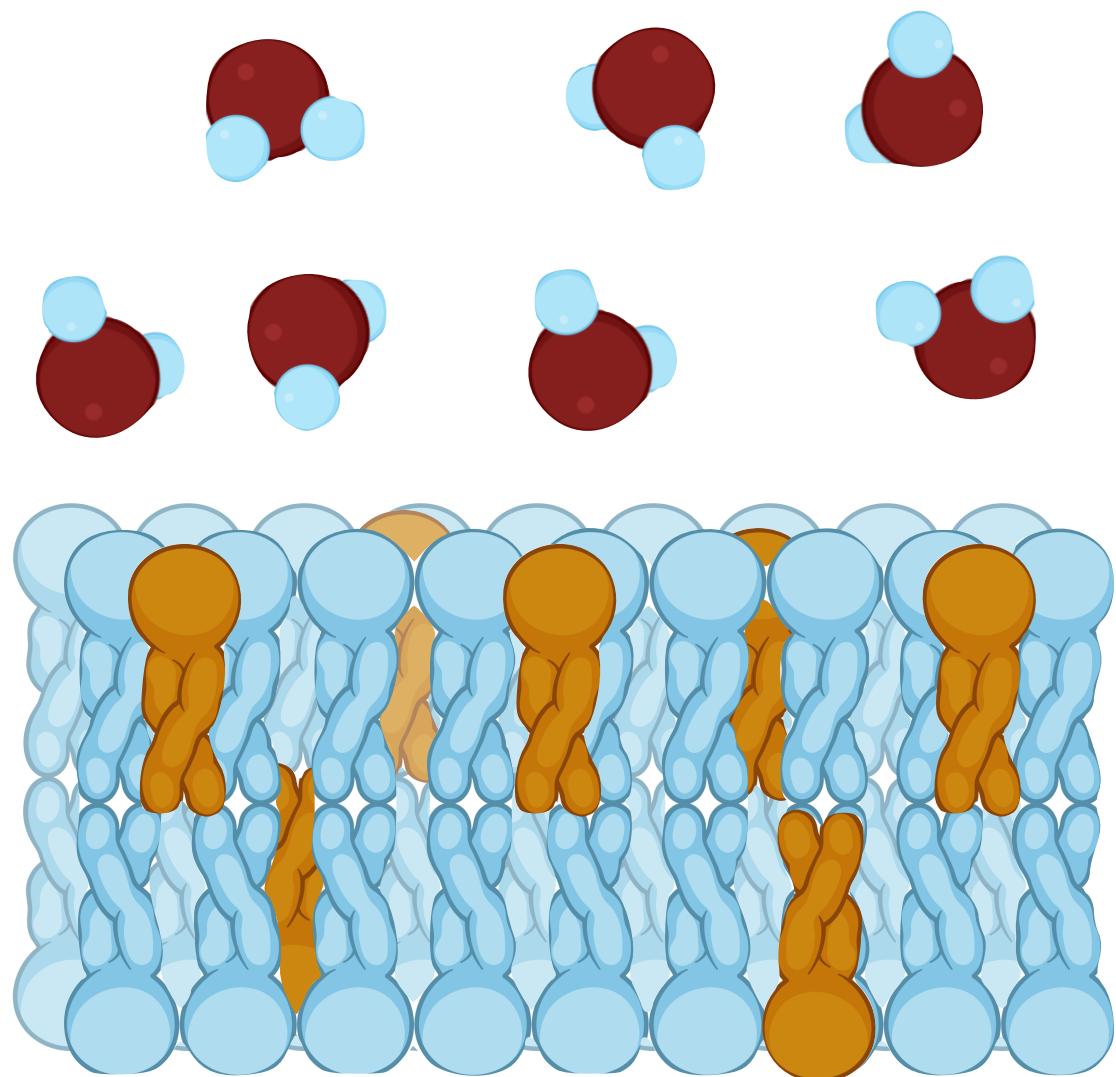
**How:** Transform one ligand into another

**Challenges:**

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2. More parameterization **overhead**

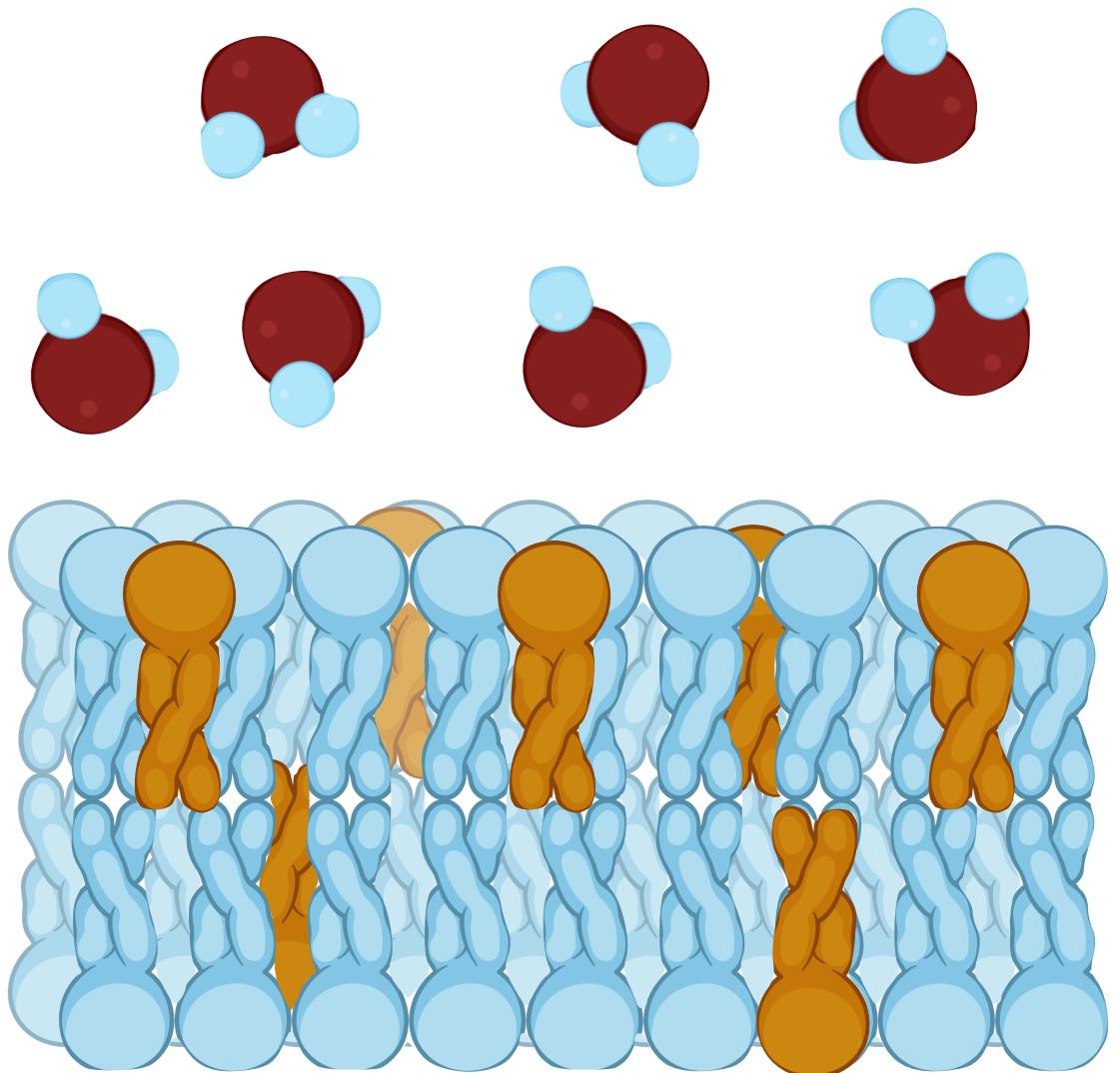


# Challenges of FEP in membranes



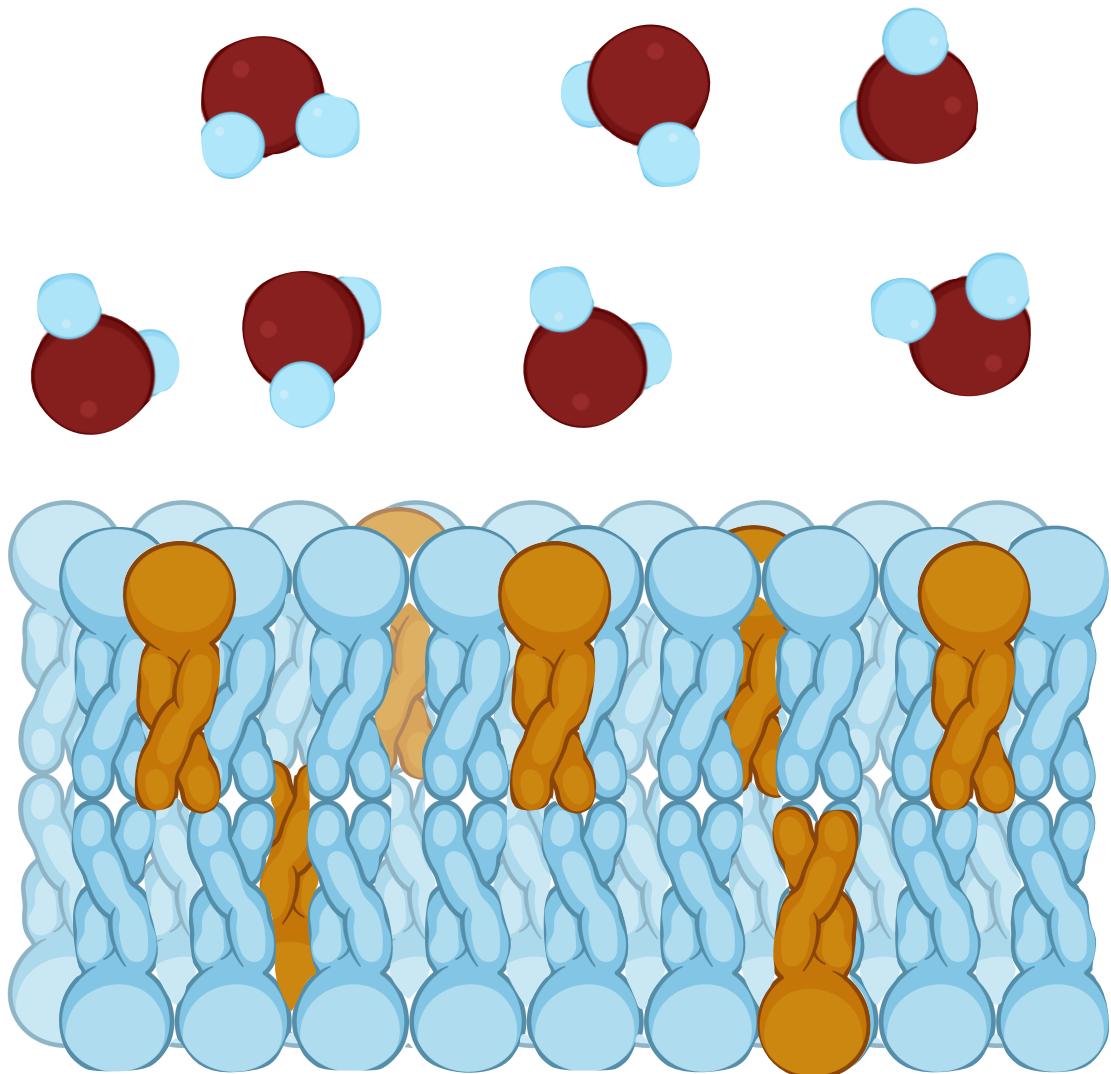
# Challenges of FEP in membranes

**Highly heterogeneous**



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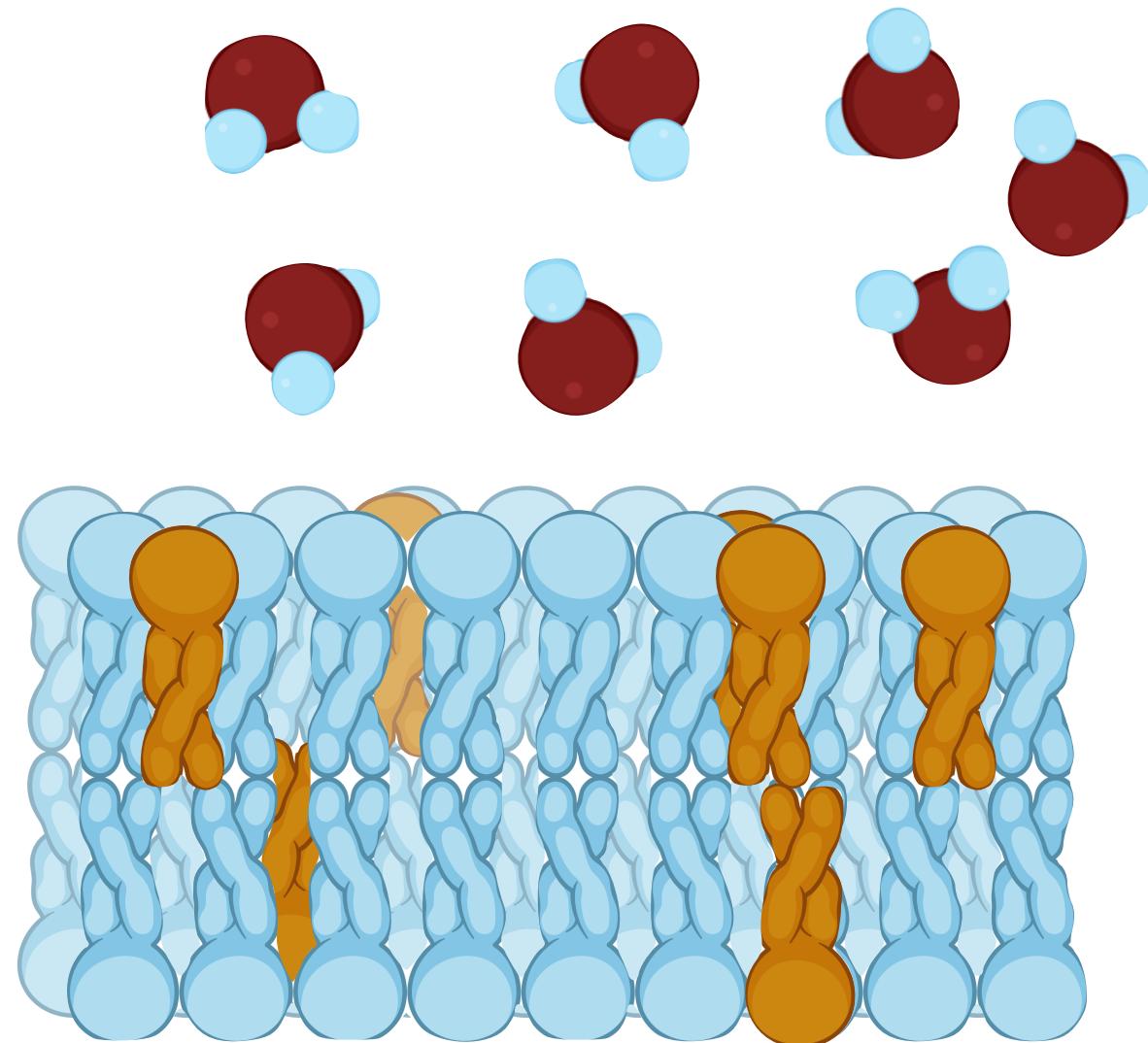
**Highly heterogeneous**



**Lipids are slow**

# Challenges of FEP in membranes

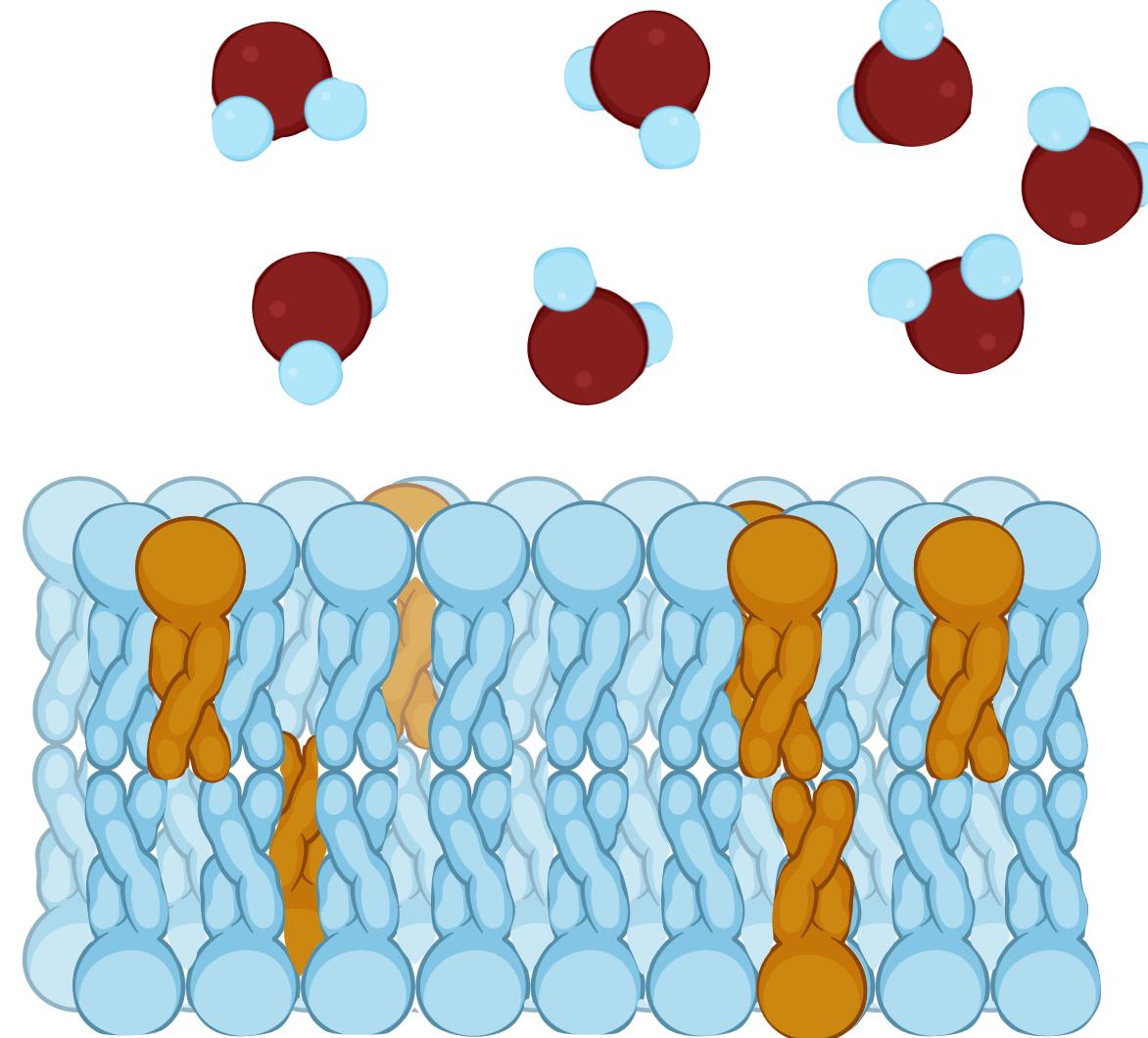
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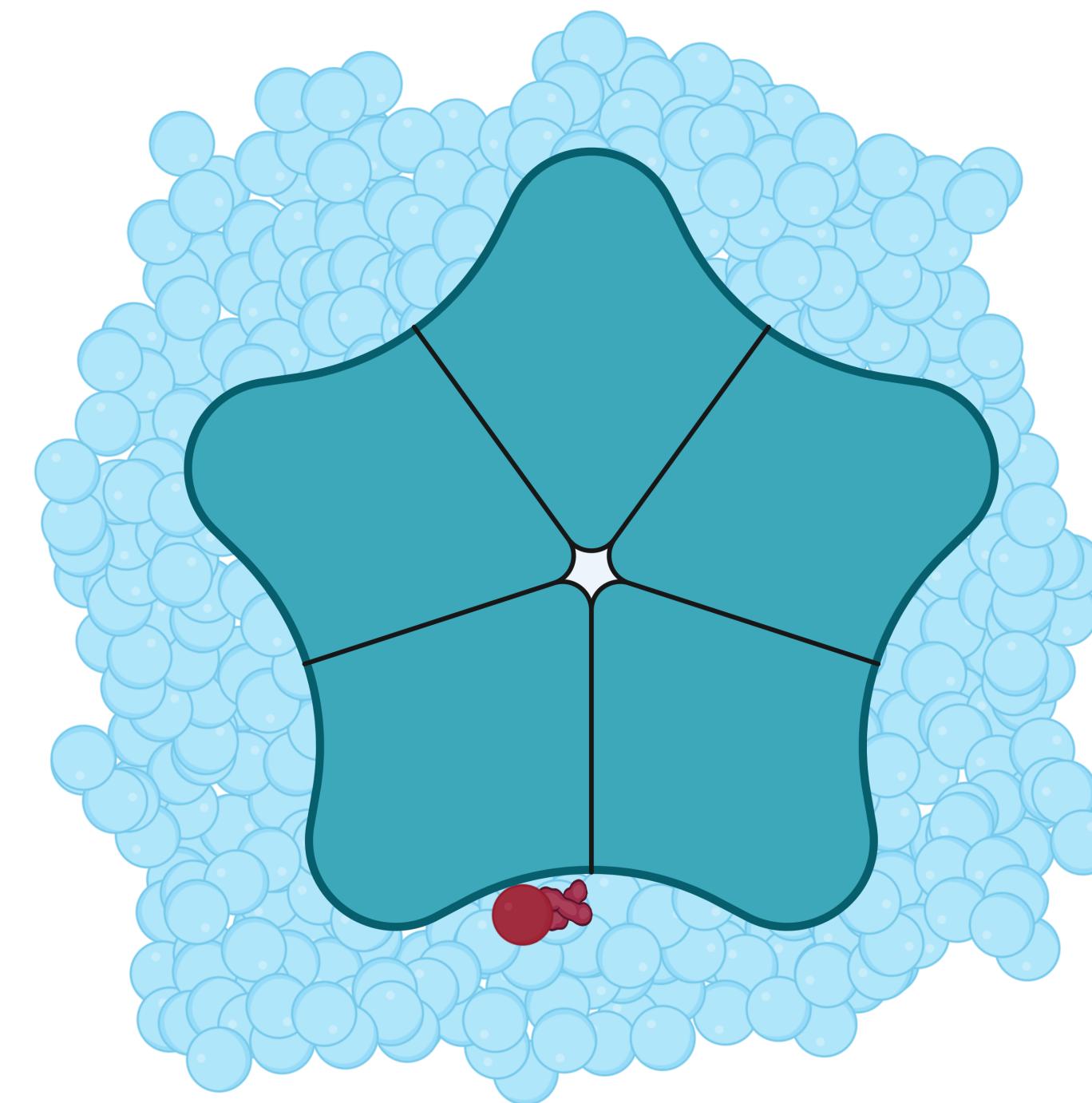
**Lipids are slow**

# Challenges of FEP in membranes

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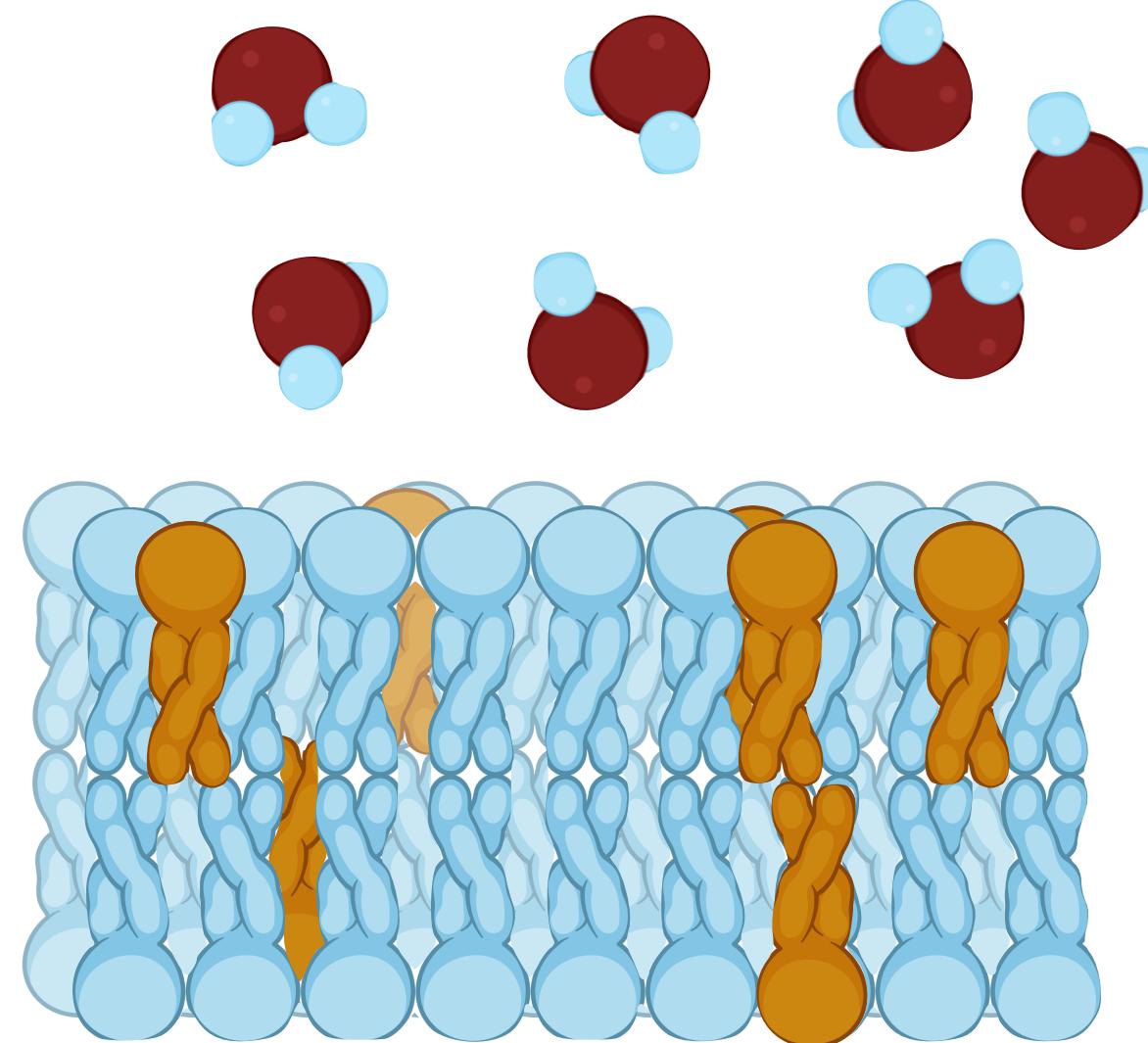
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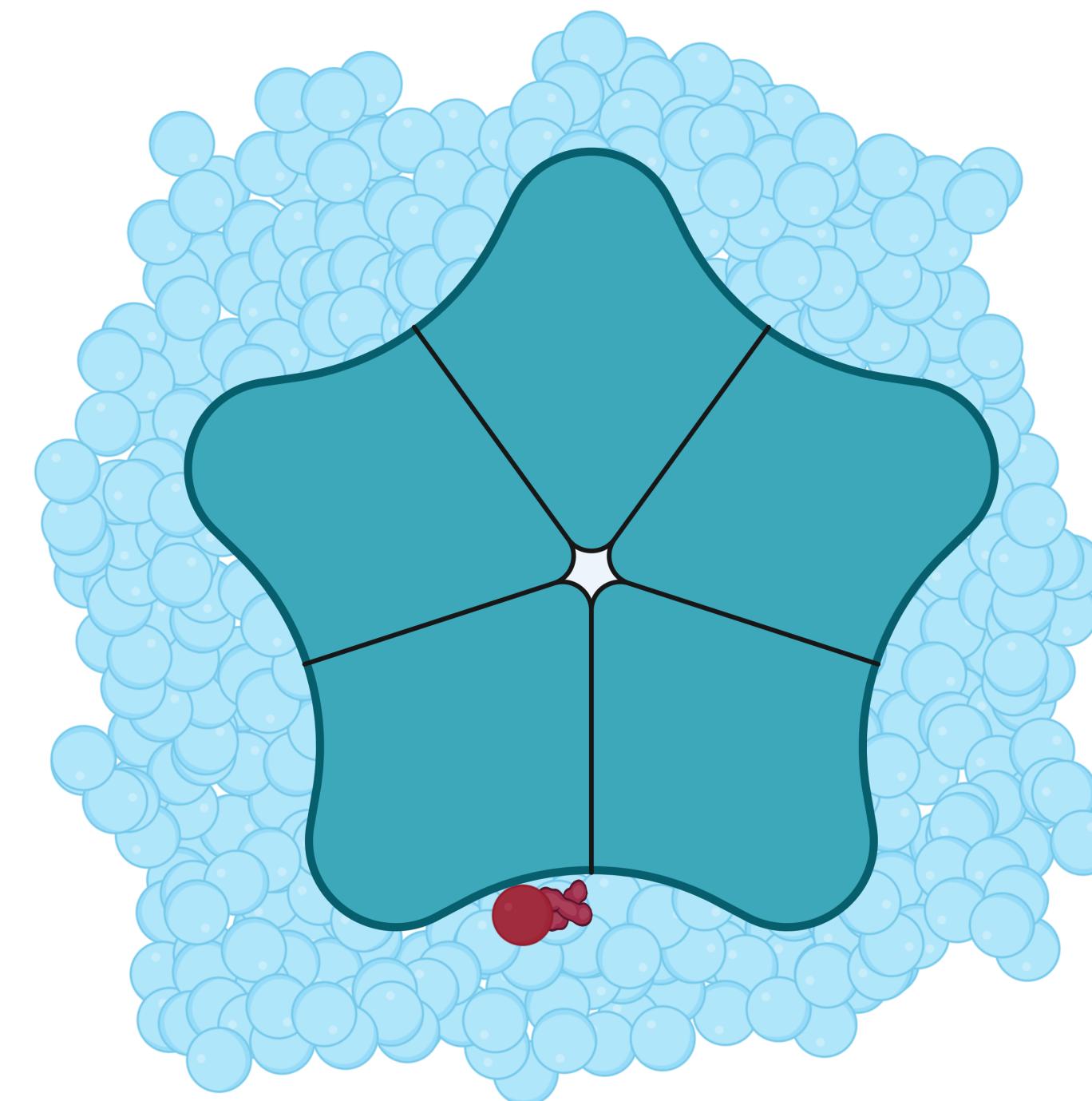
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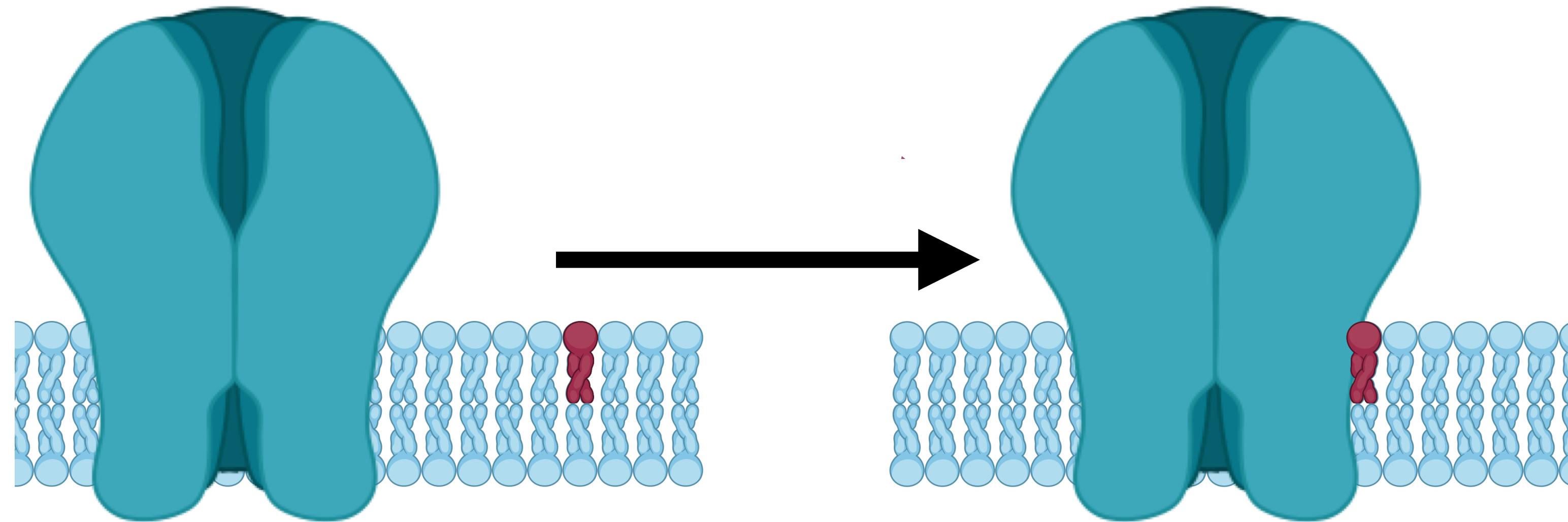
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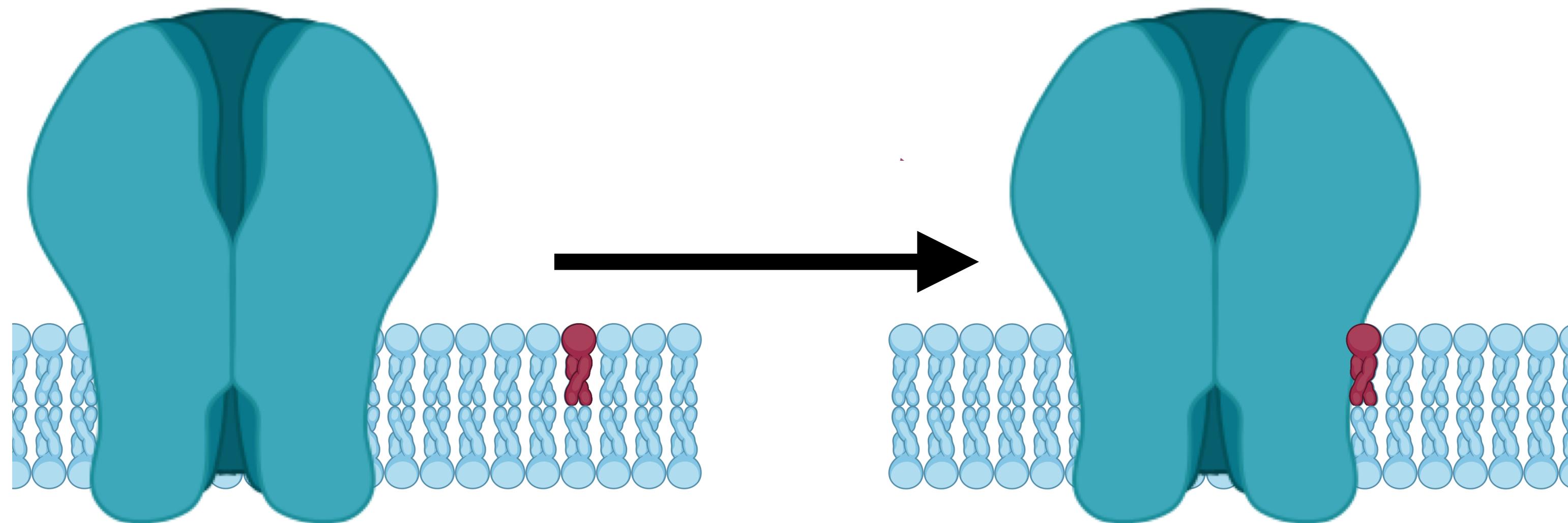


**POPC: solvent or ligand?**

# How can we use $\Delta G$ with these challenges?

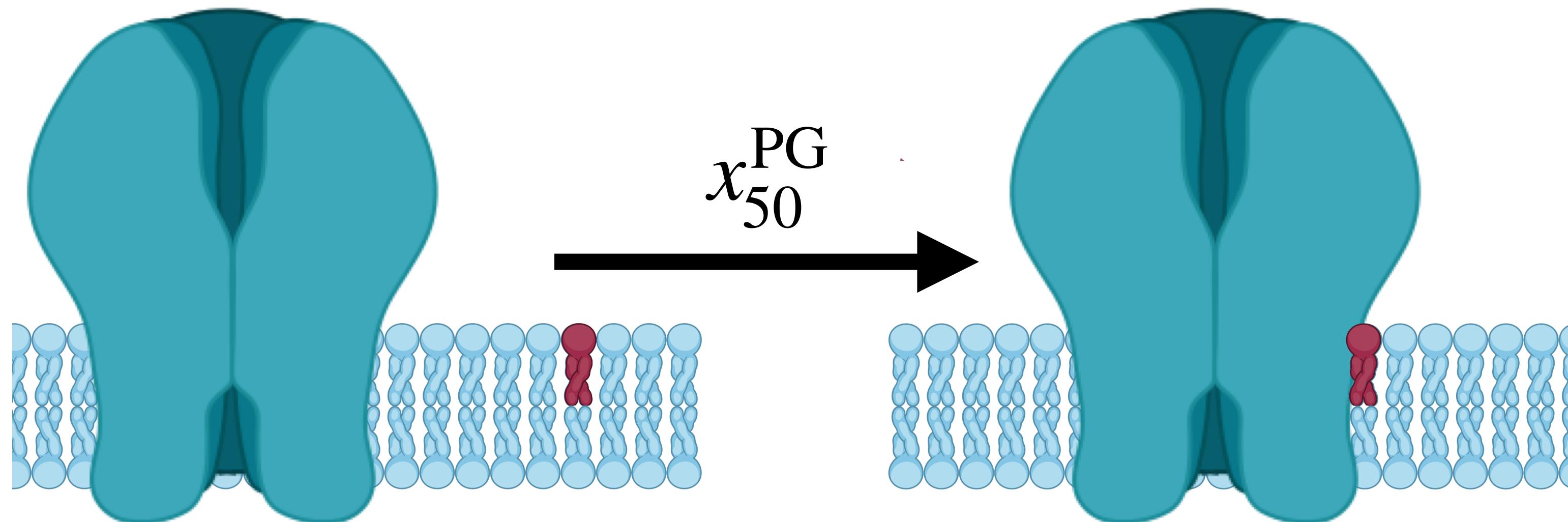


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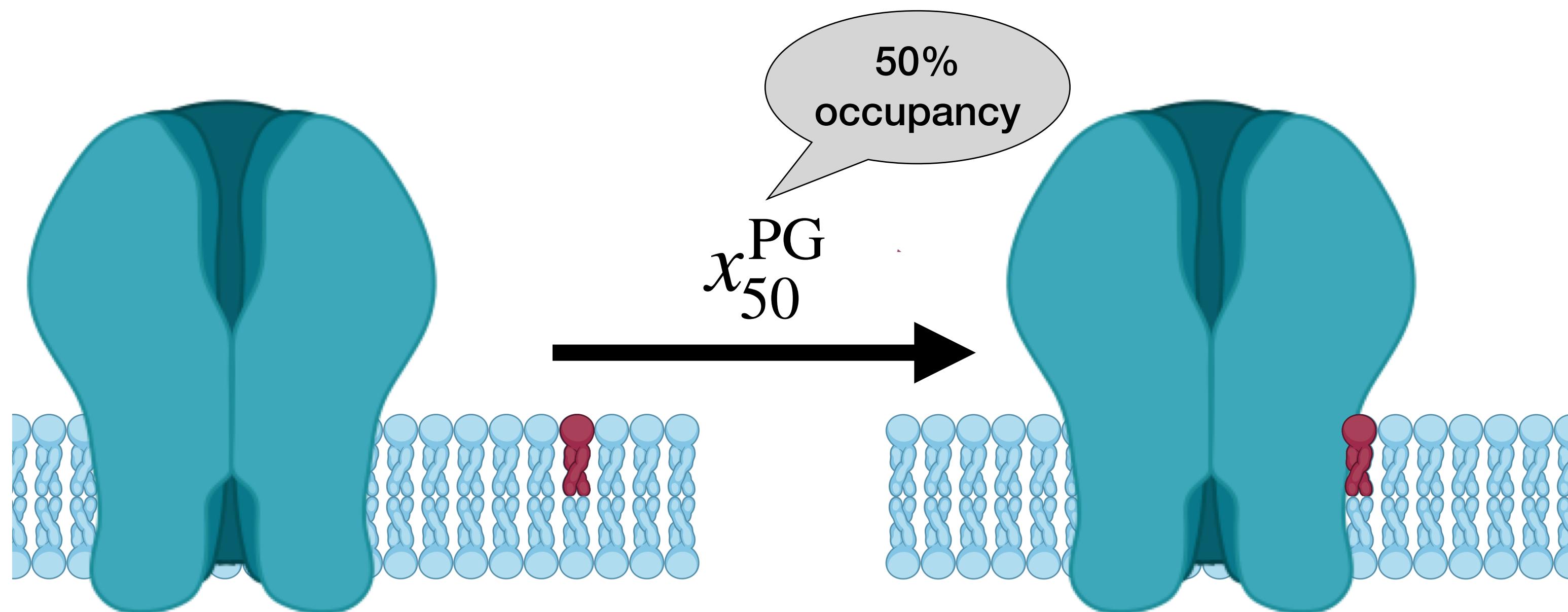
$$\Delta G = -RT \ln \frac{P_{\text{occ}}}{P_{\text{unocc}}}$$

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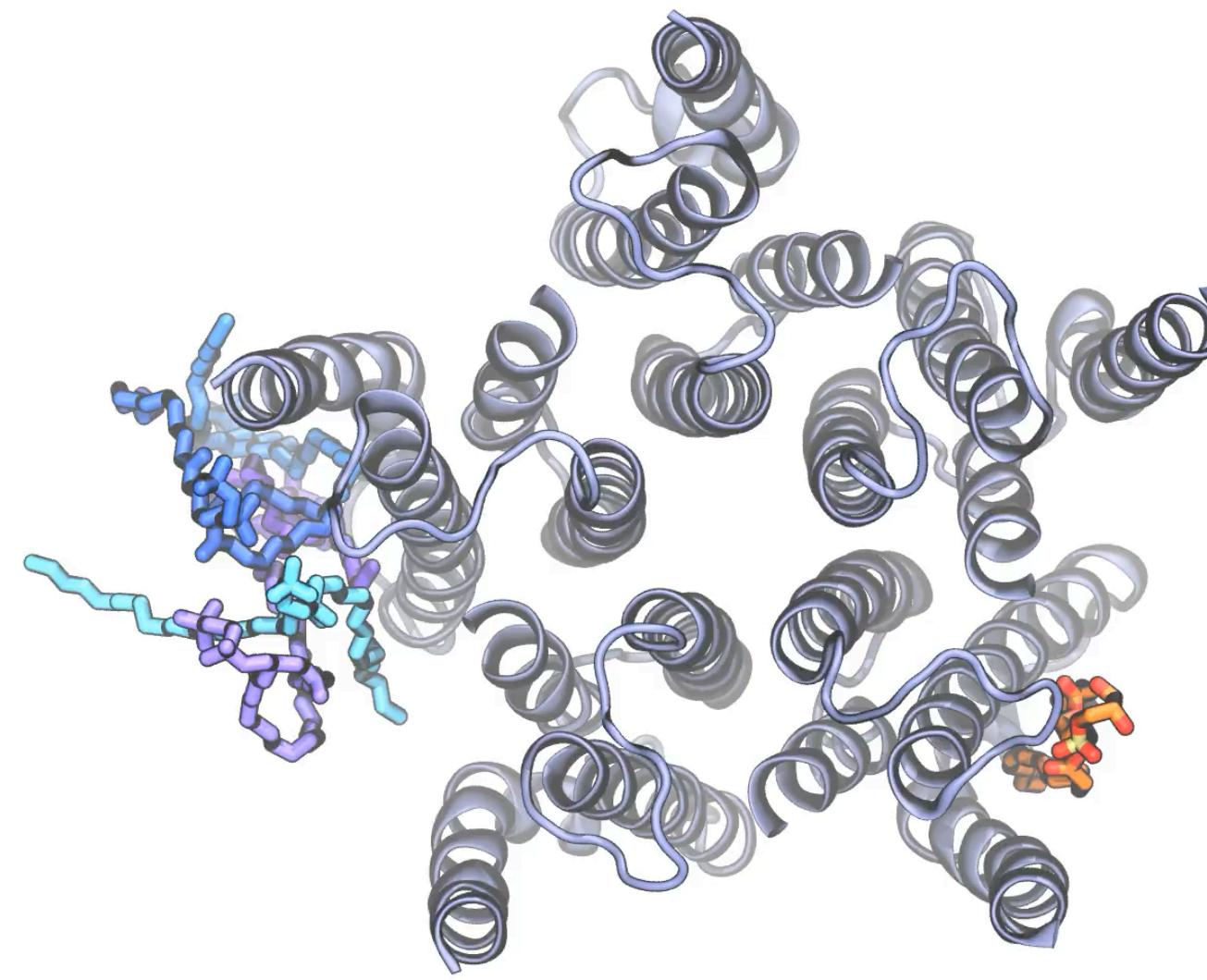
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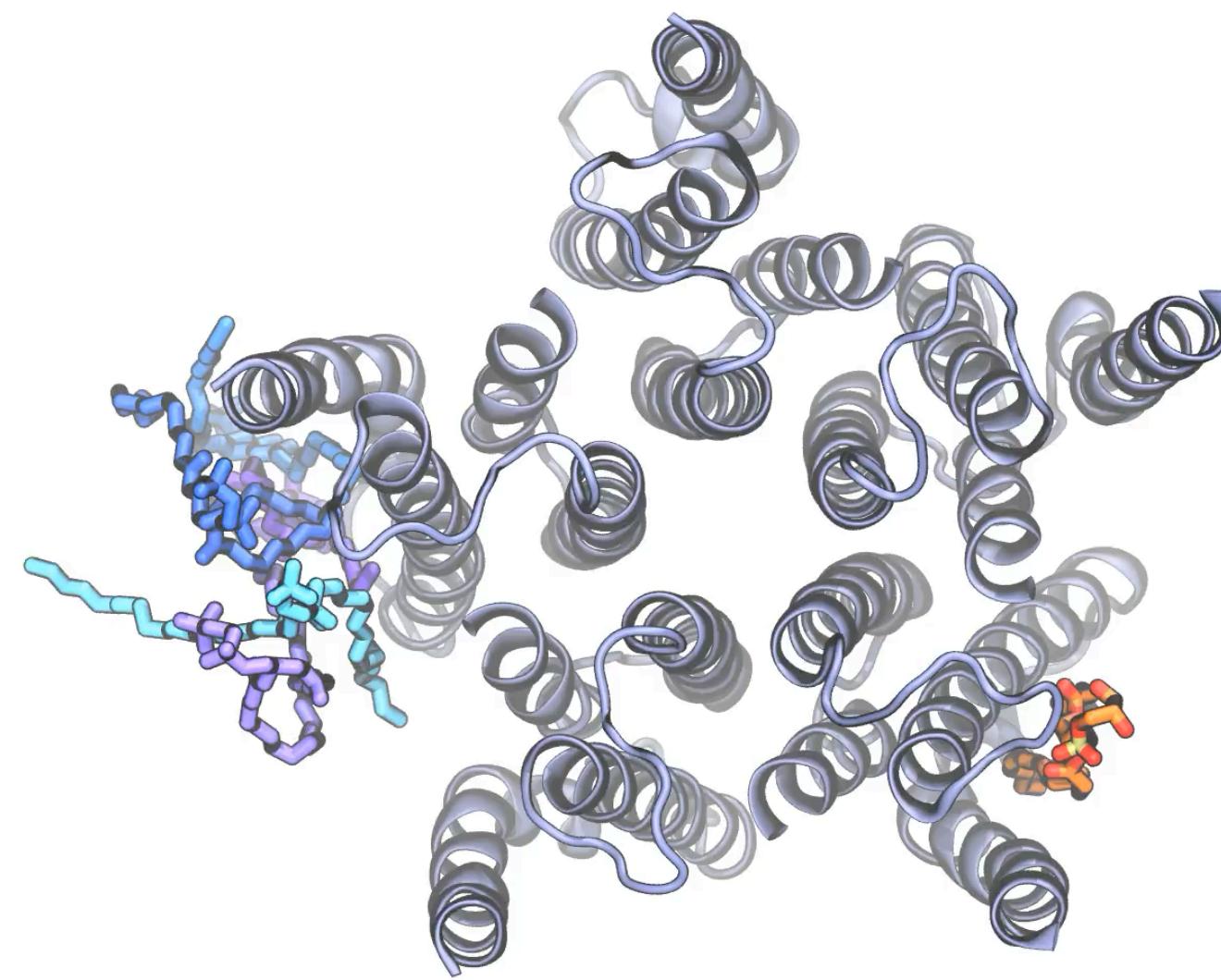
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# But what is “occupancy”?



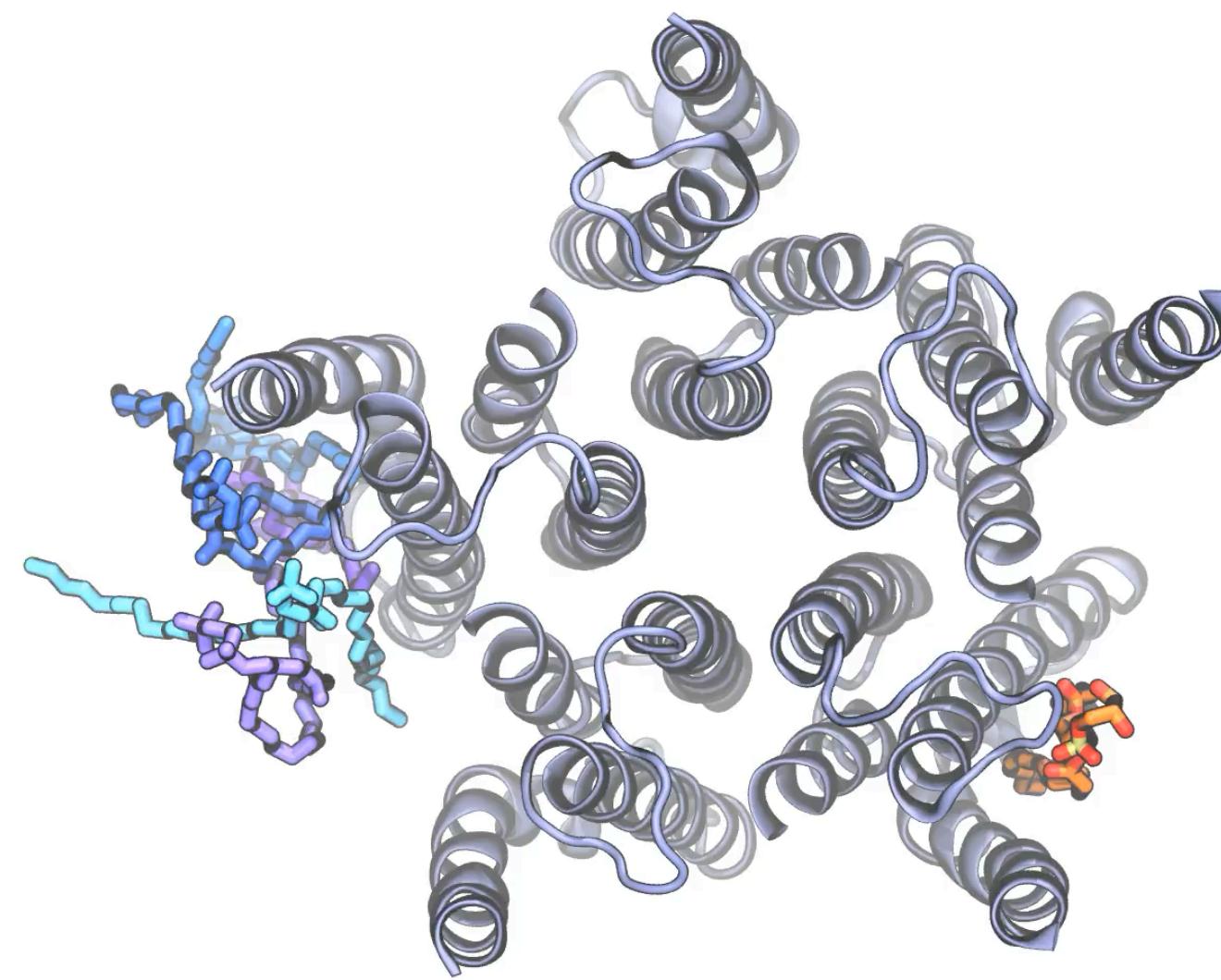
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A **given** lipid species



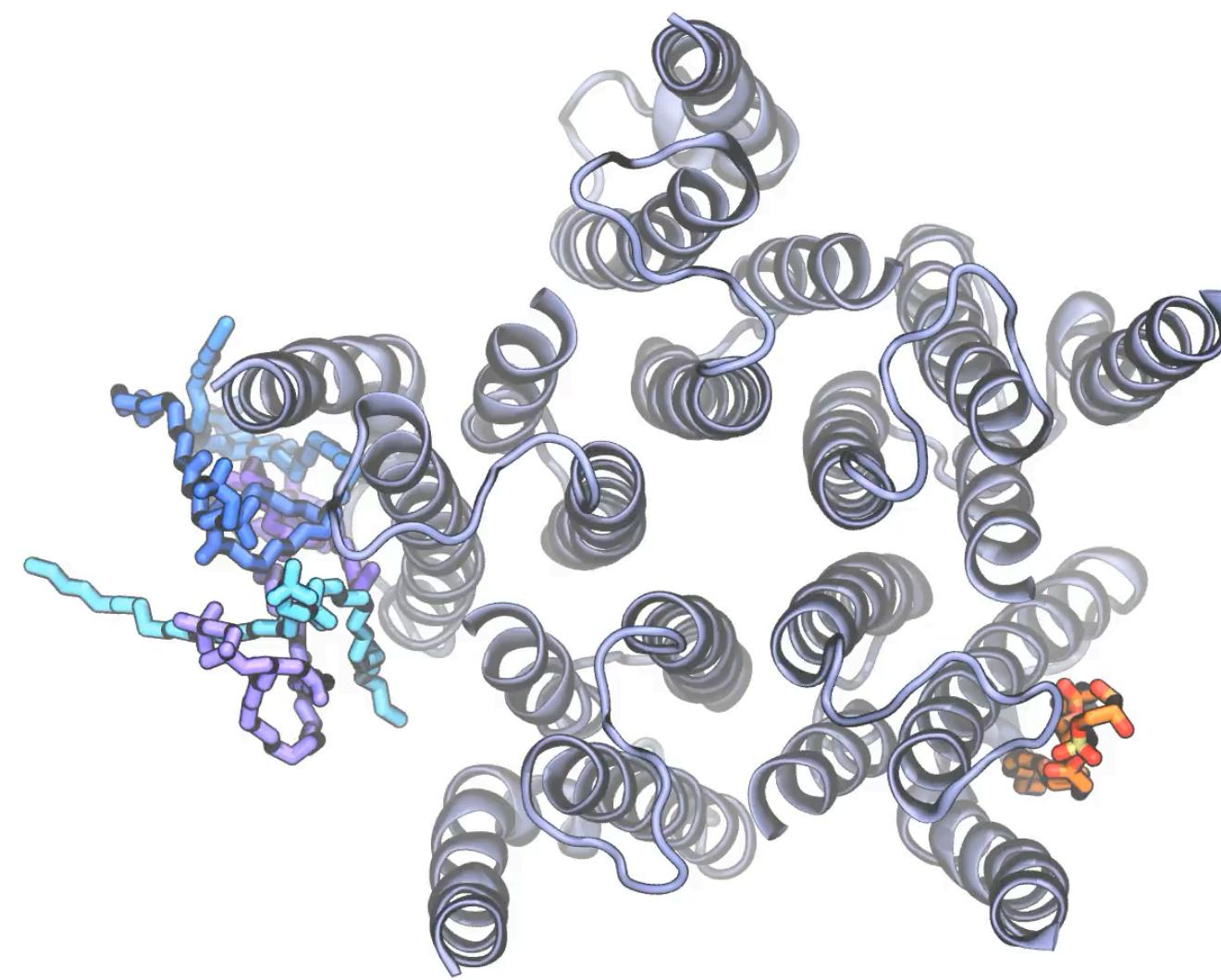
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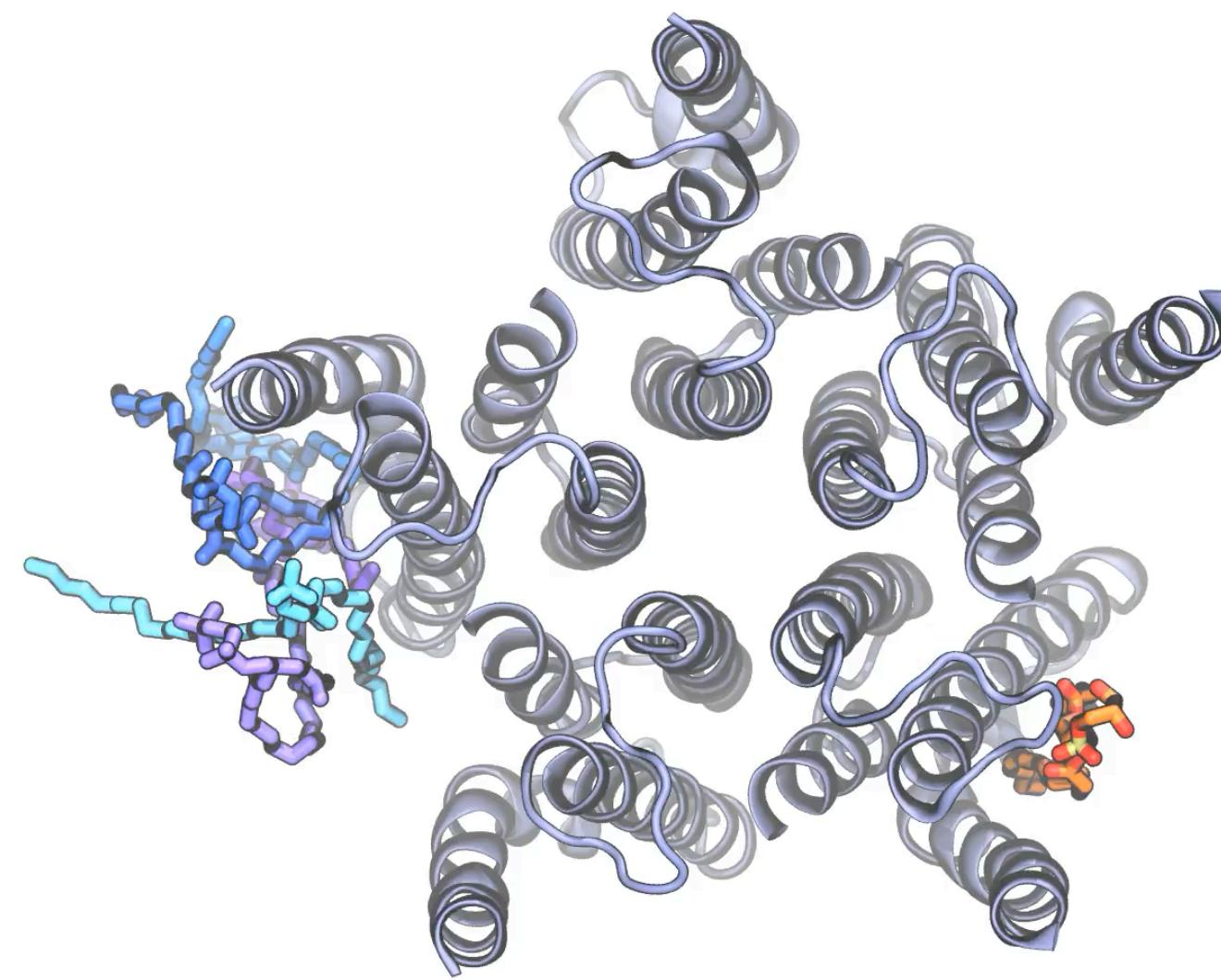
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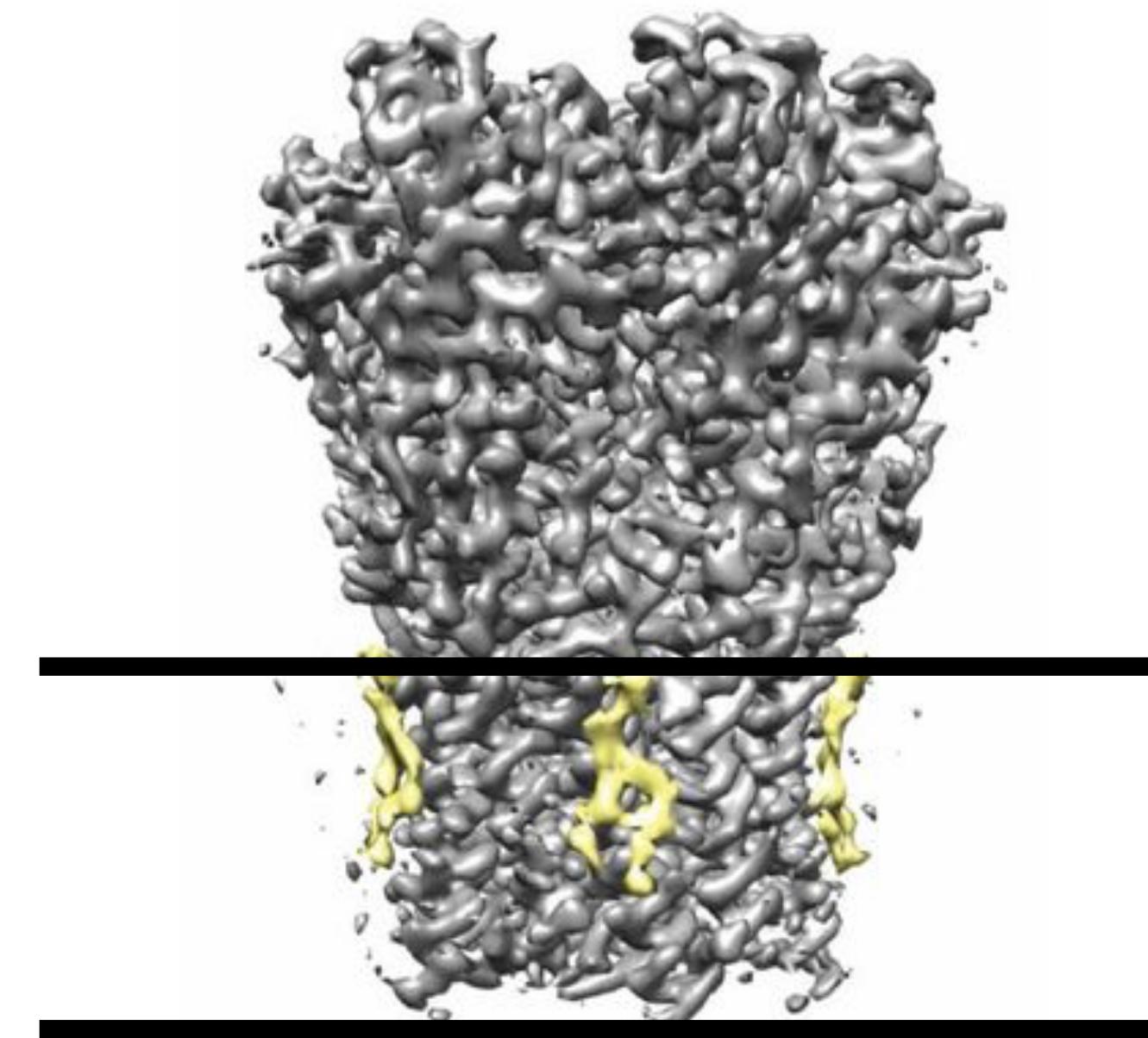
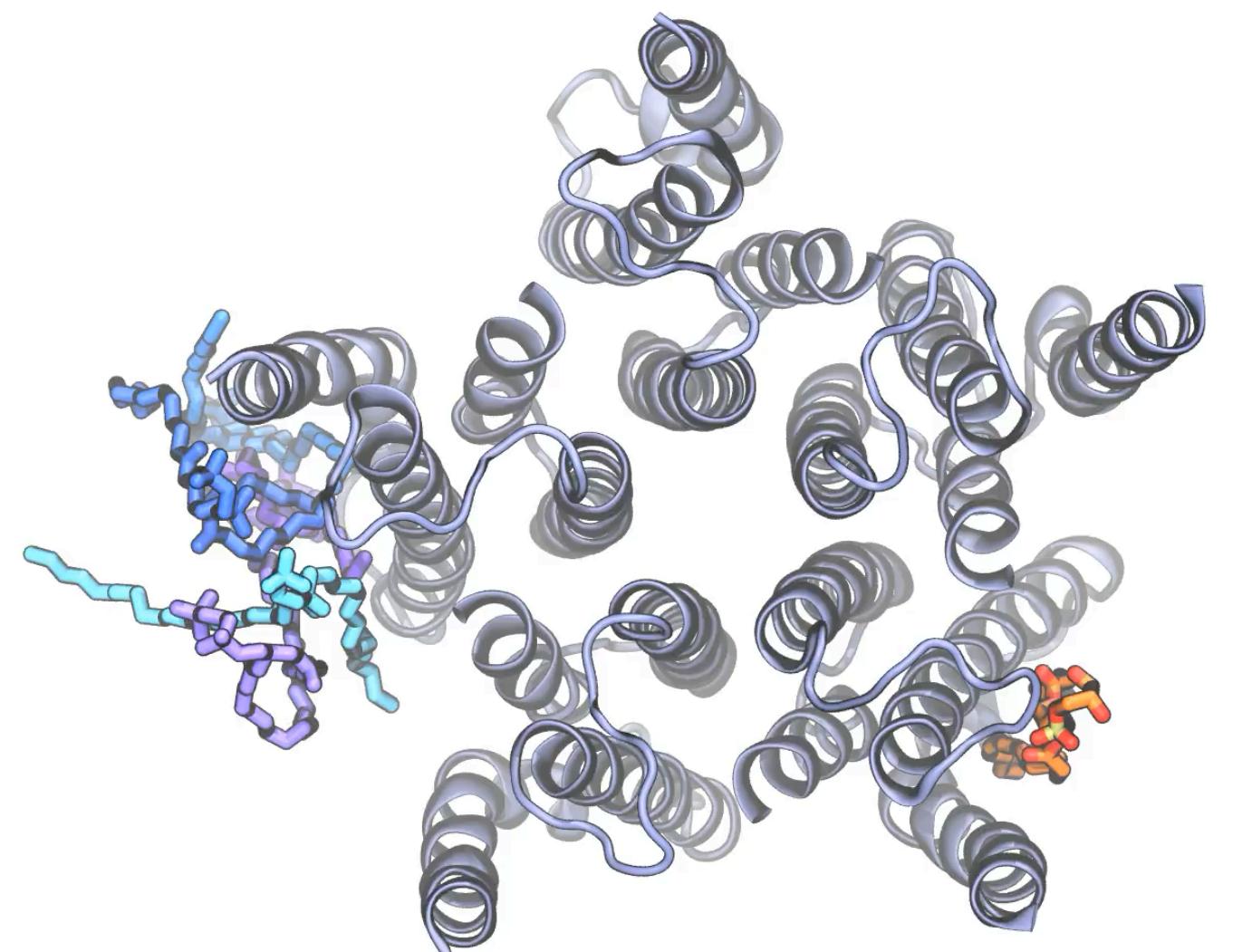
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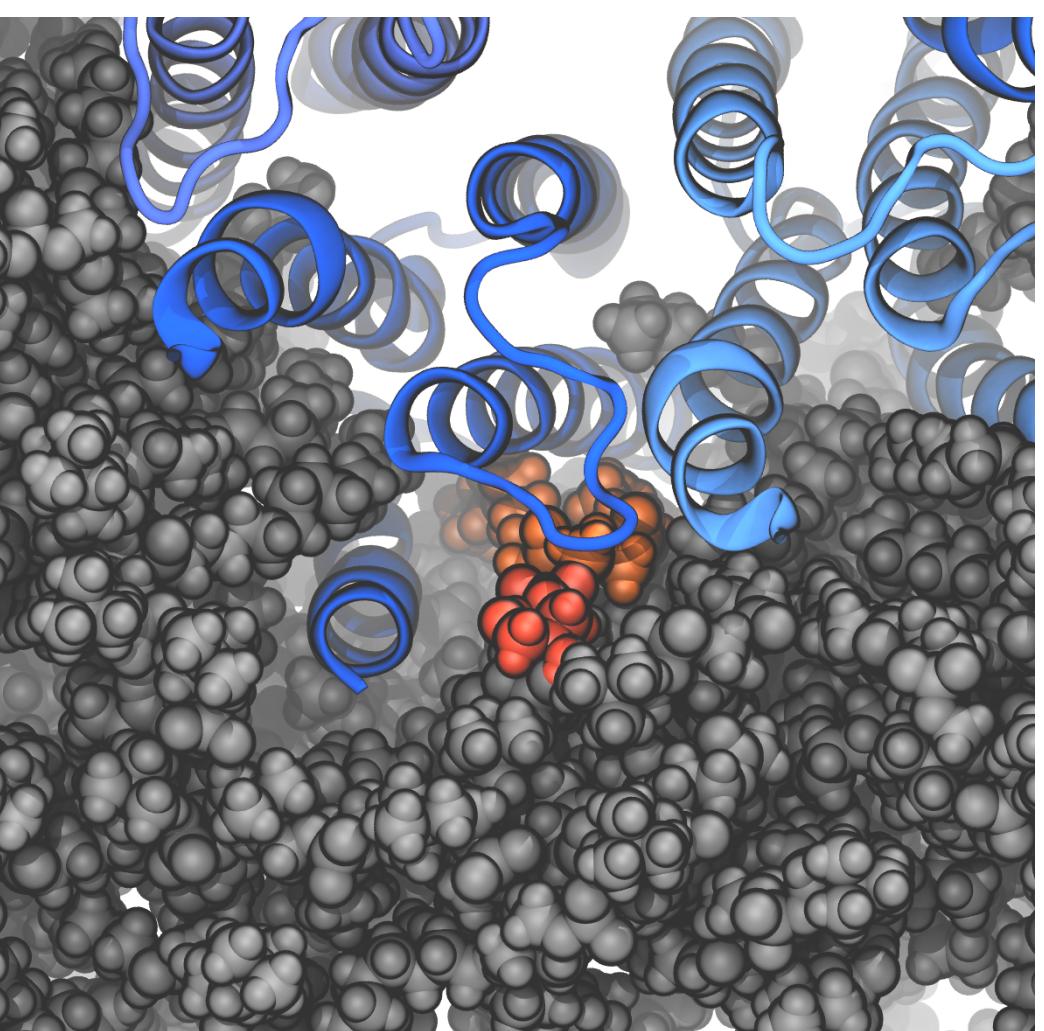
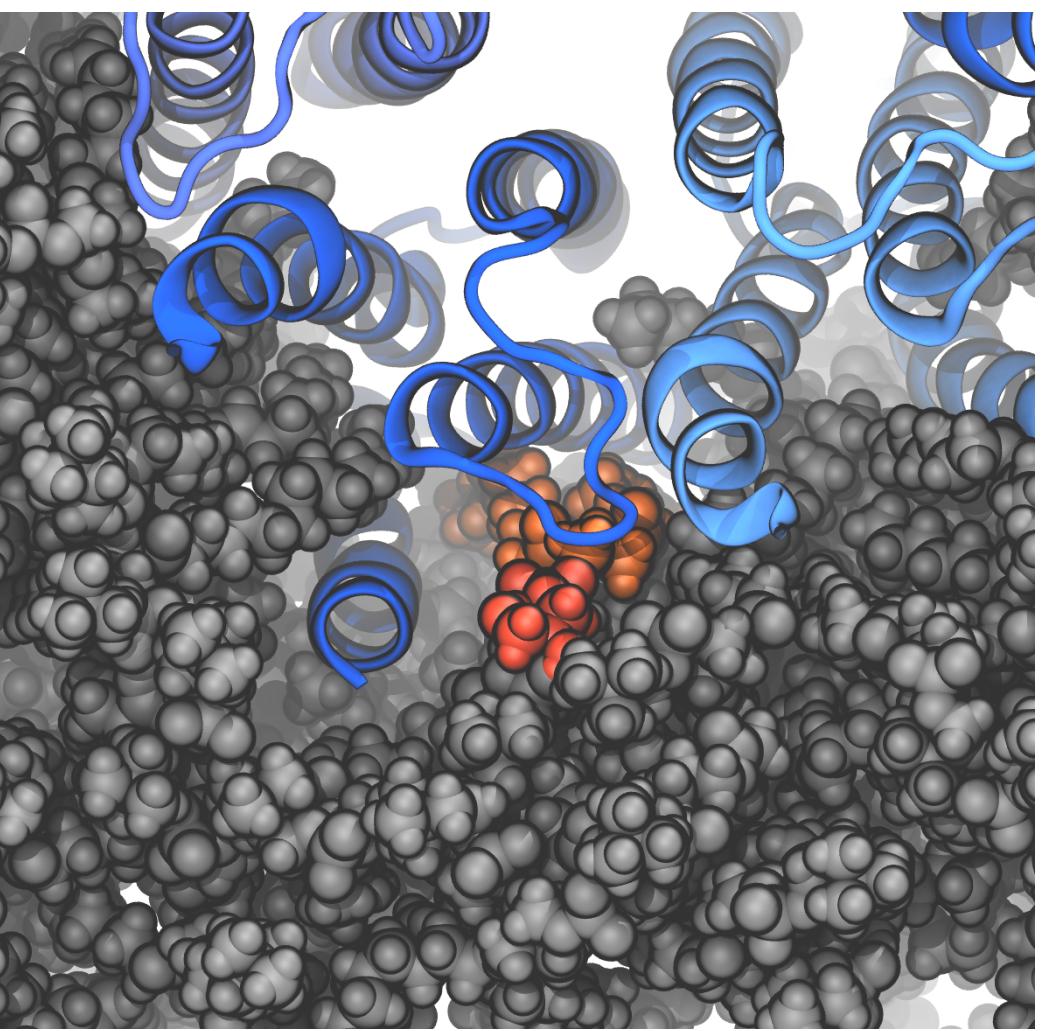


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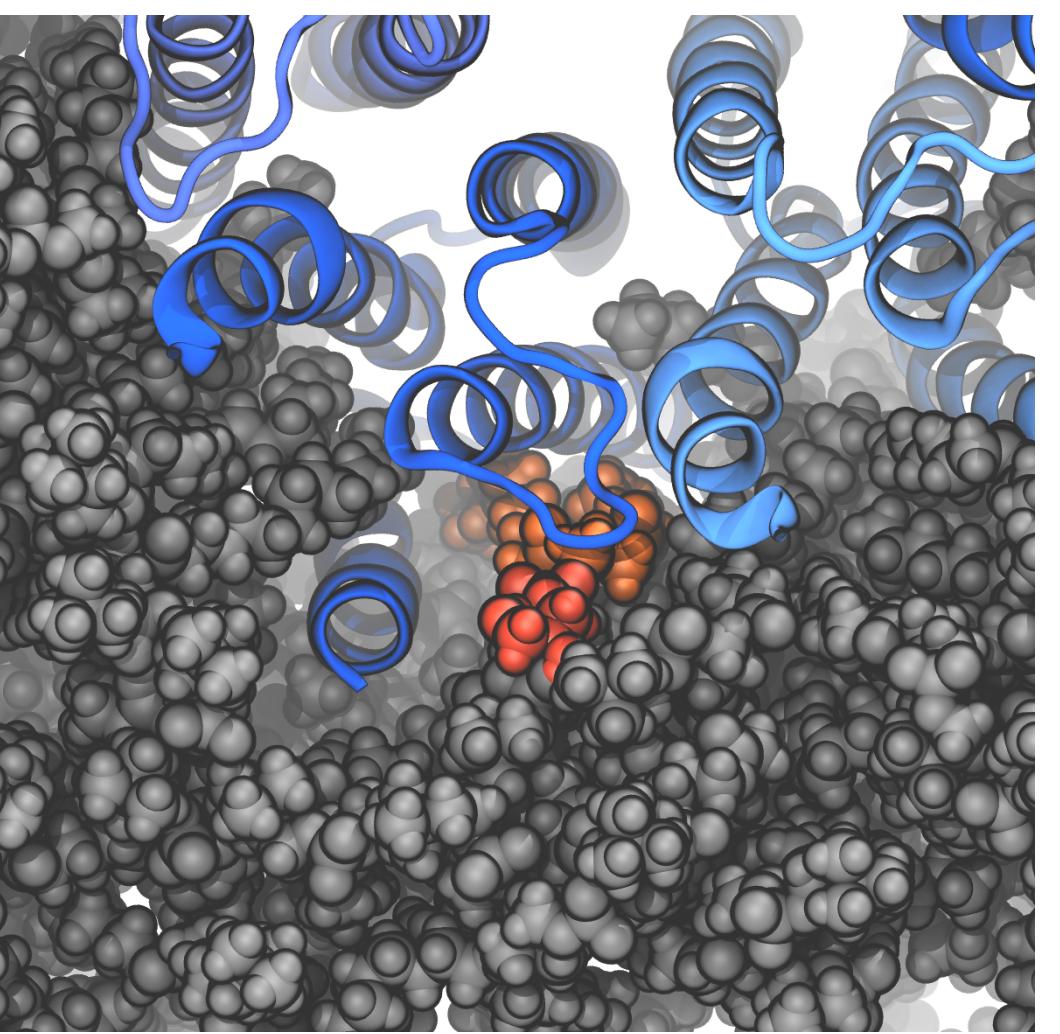
A **given** lipid species  
**has a conformation**  
**and position**  
**consistent with**  
**structural data**



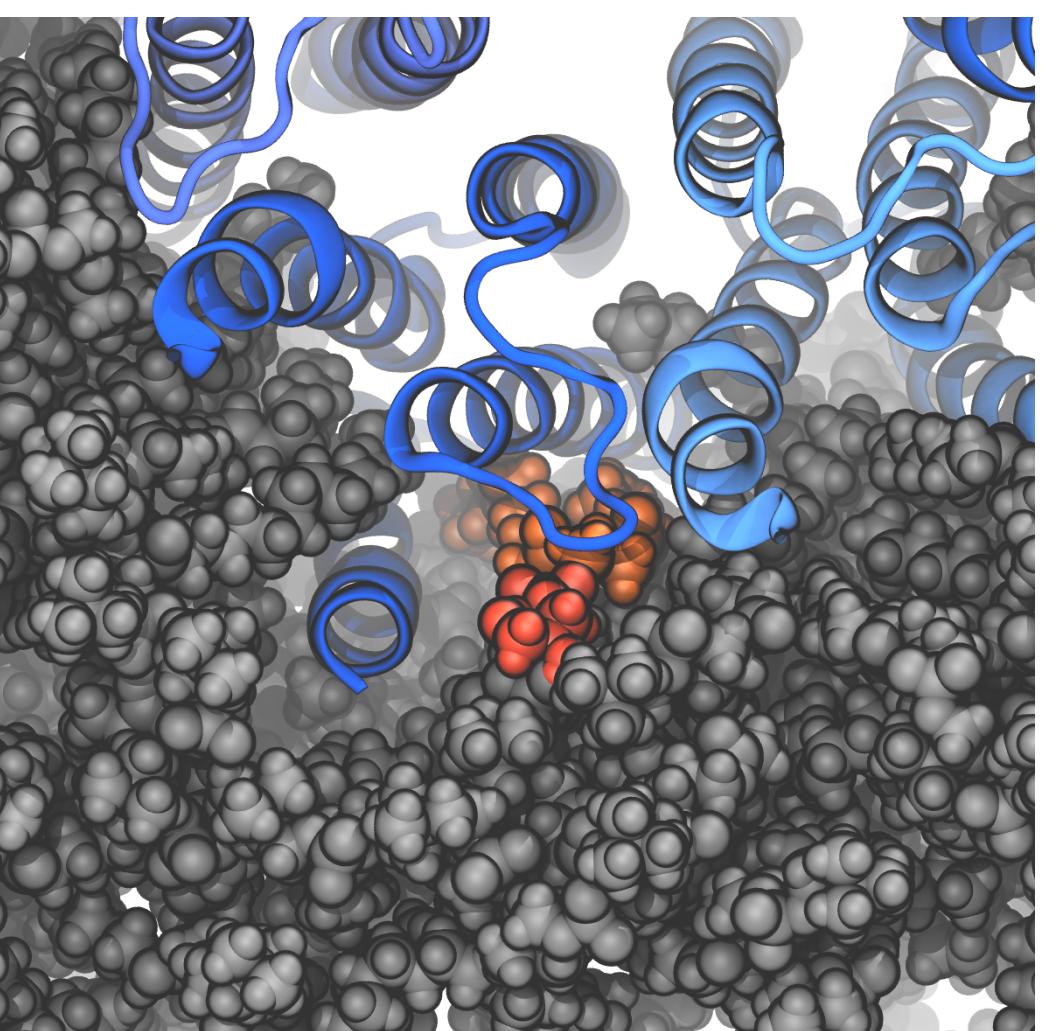
# Which FEP should we use?



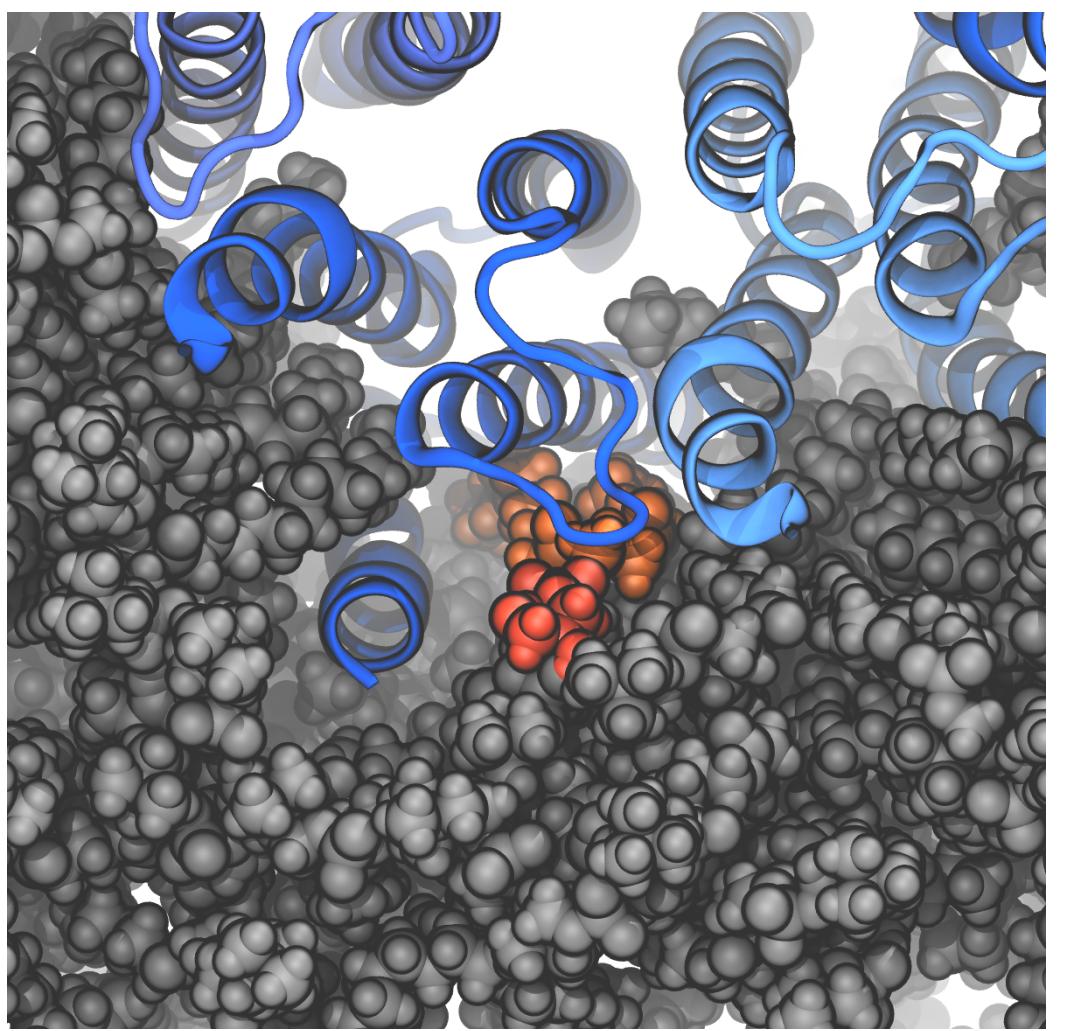
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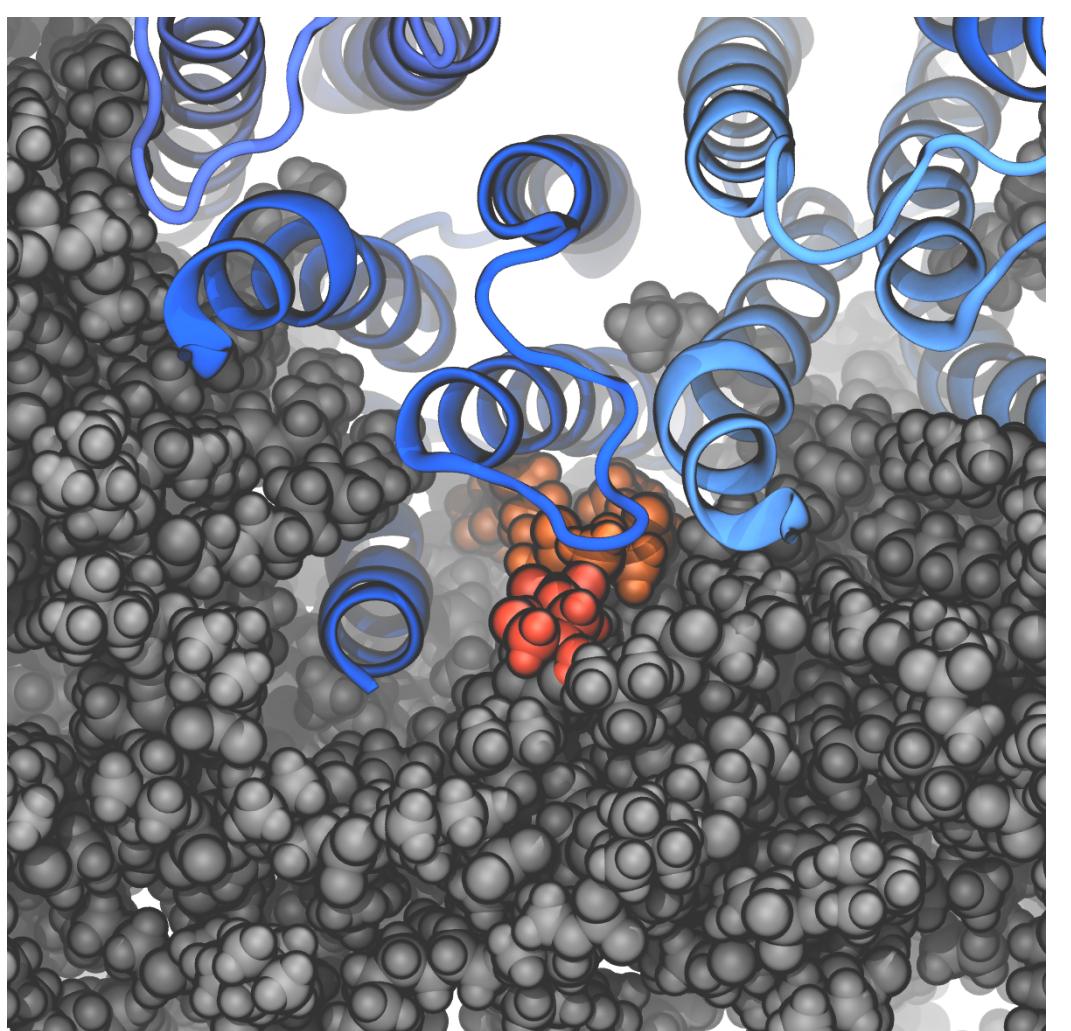


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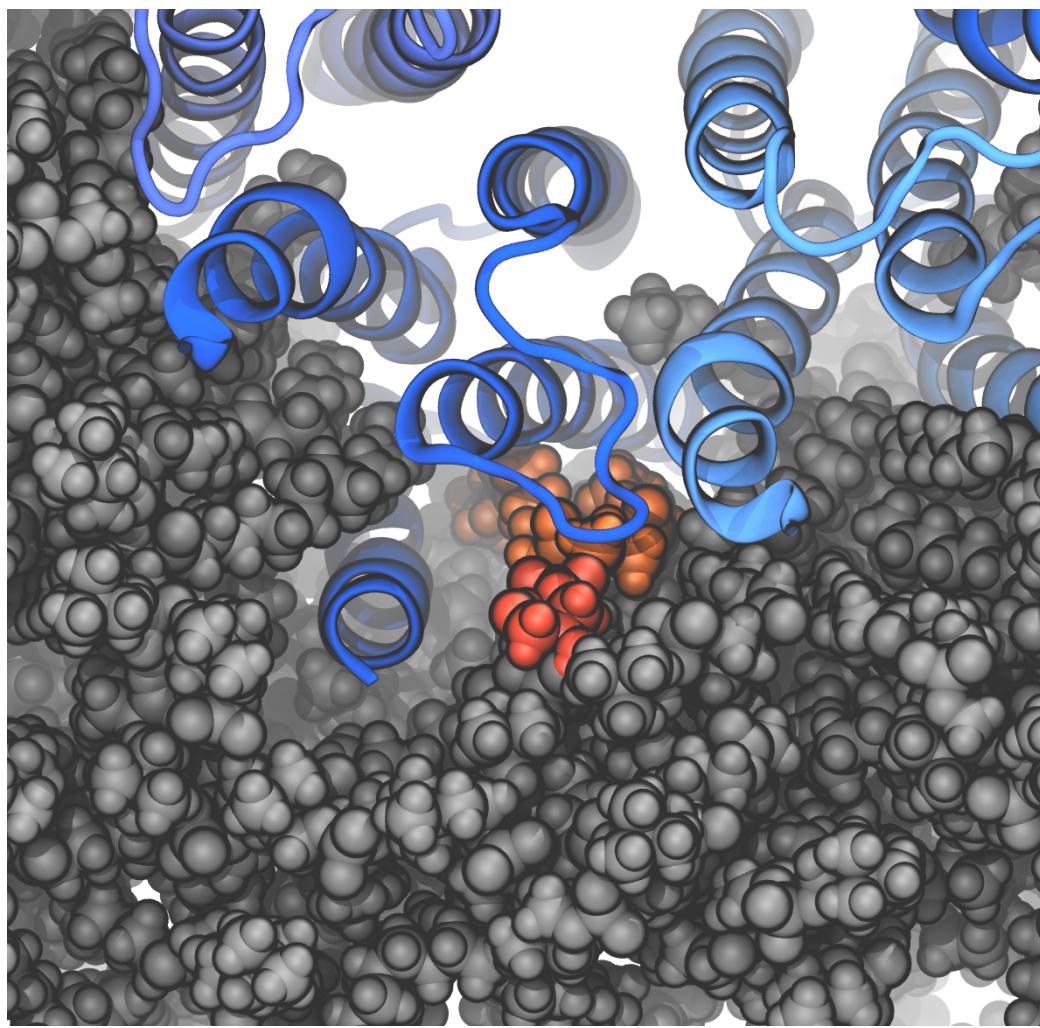


**Relative:**

- **Cheaper**

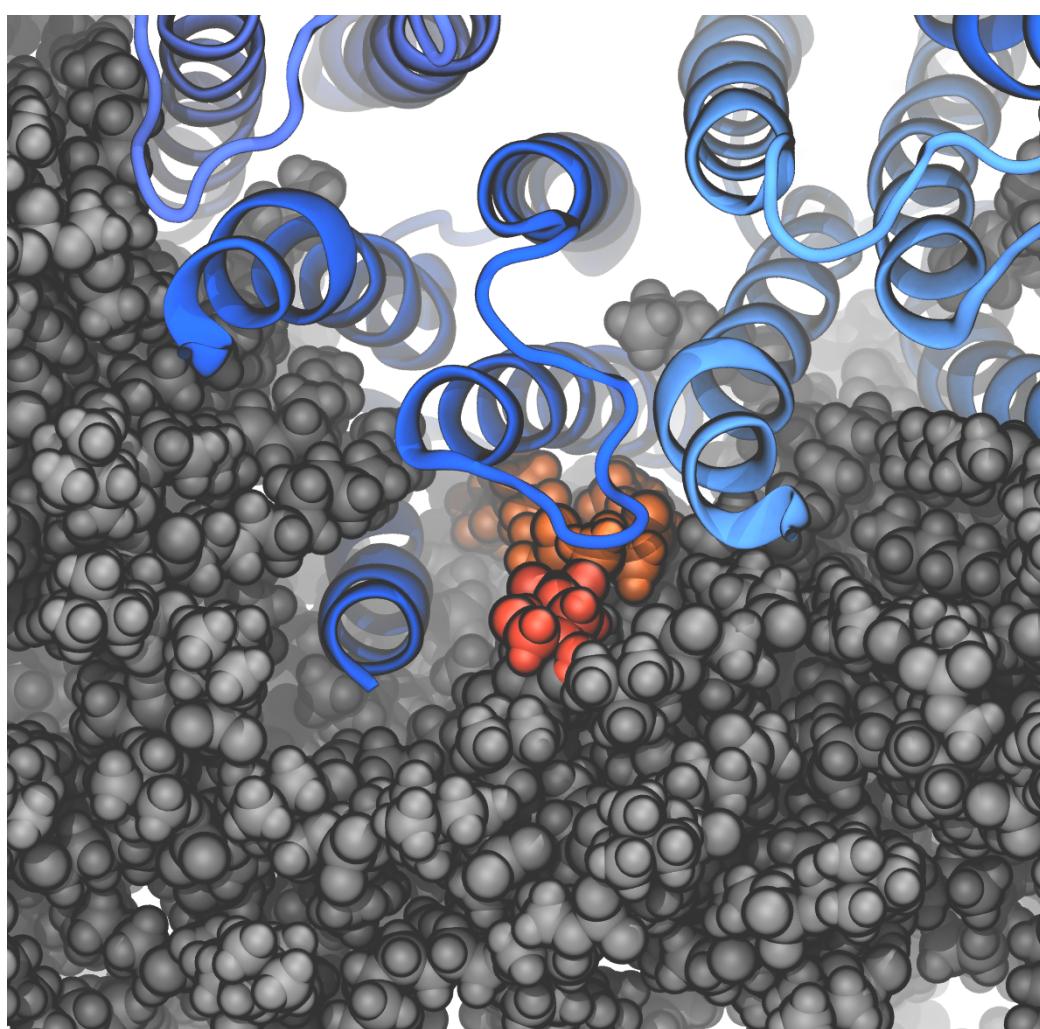


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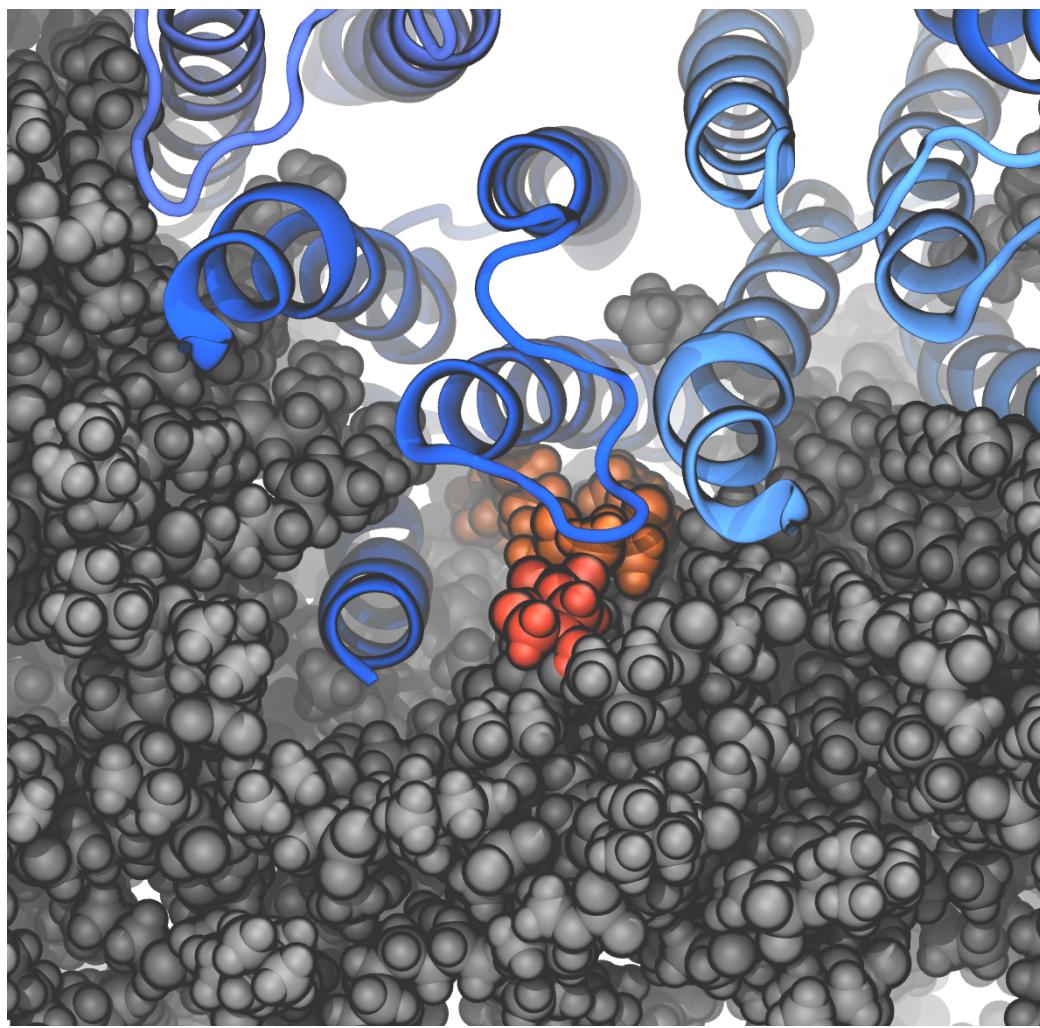


**Relative:**

- **Cheaper**
- **Site is never vacant**

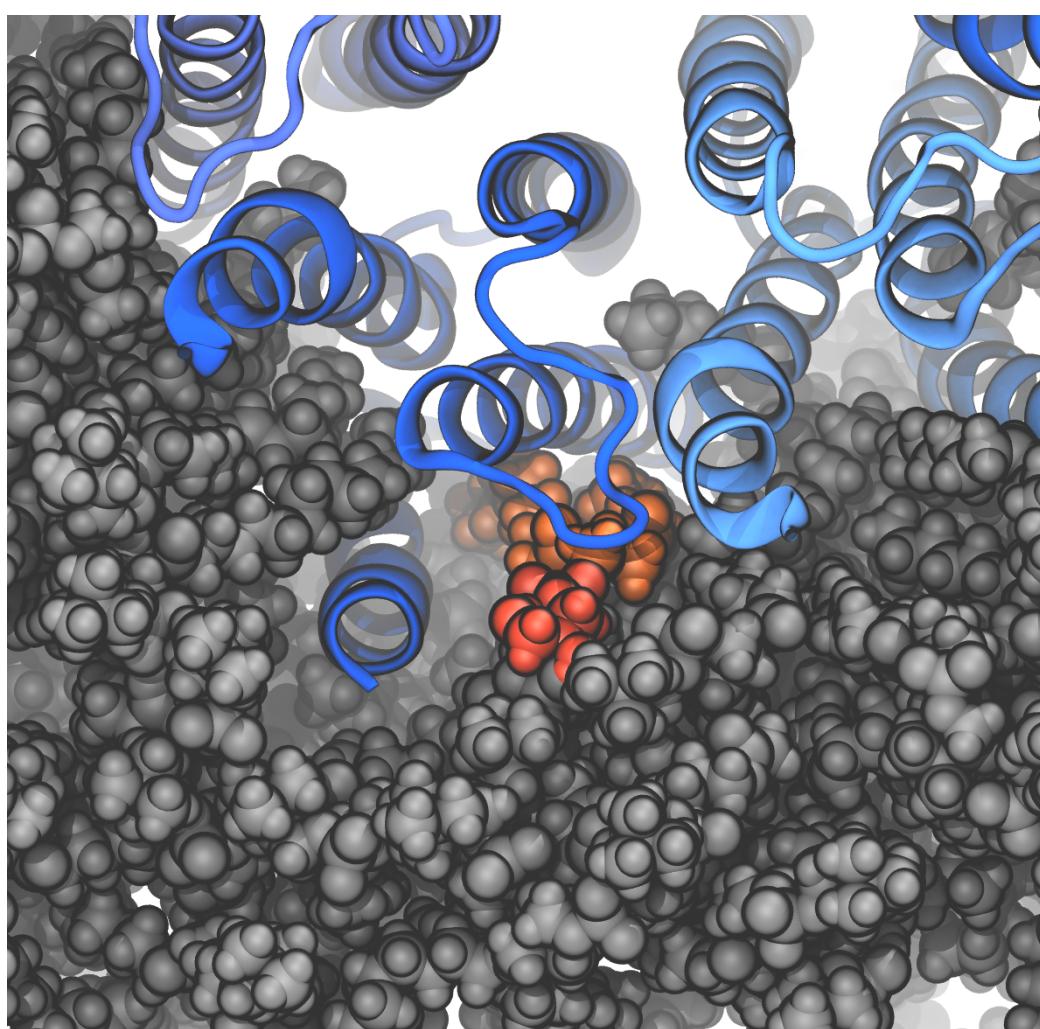


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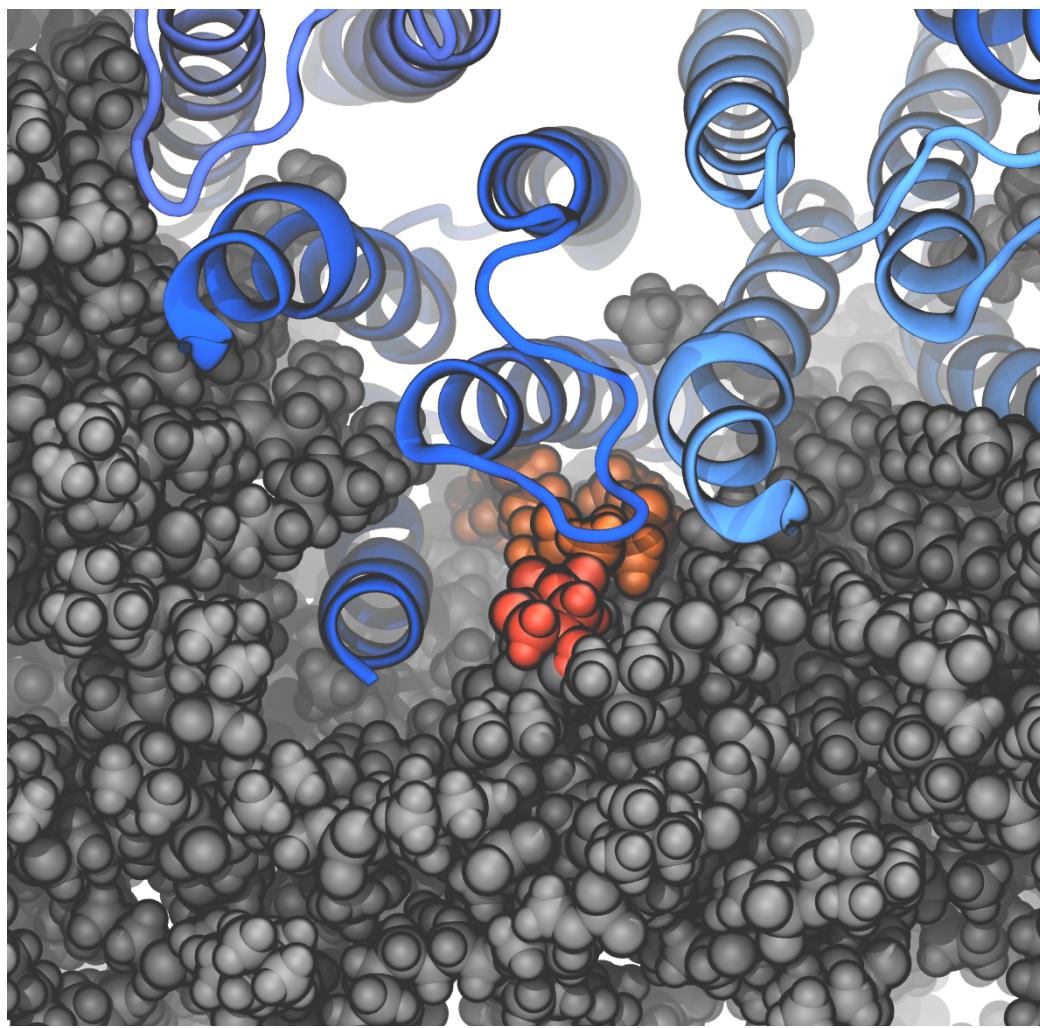


## Relative:

- **Cheaper**
  - **Site is never vacant**
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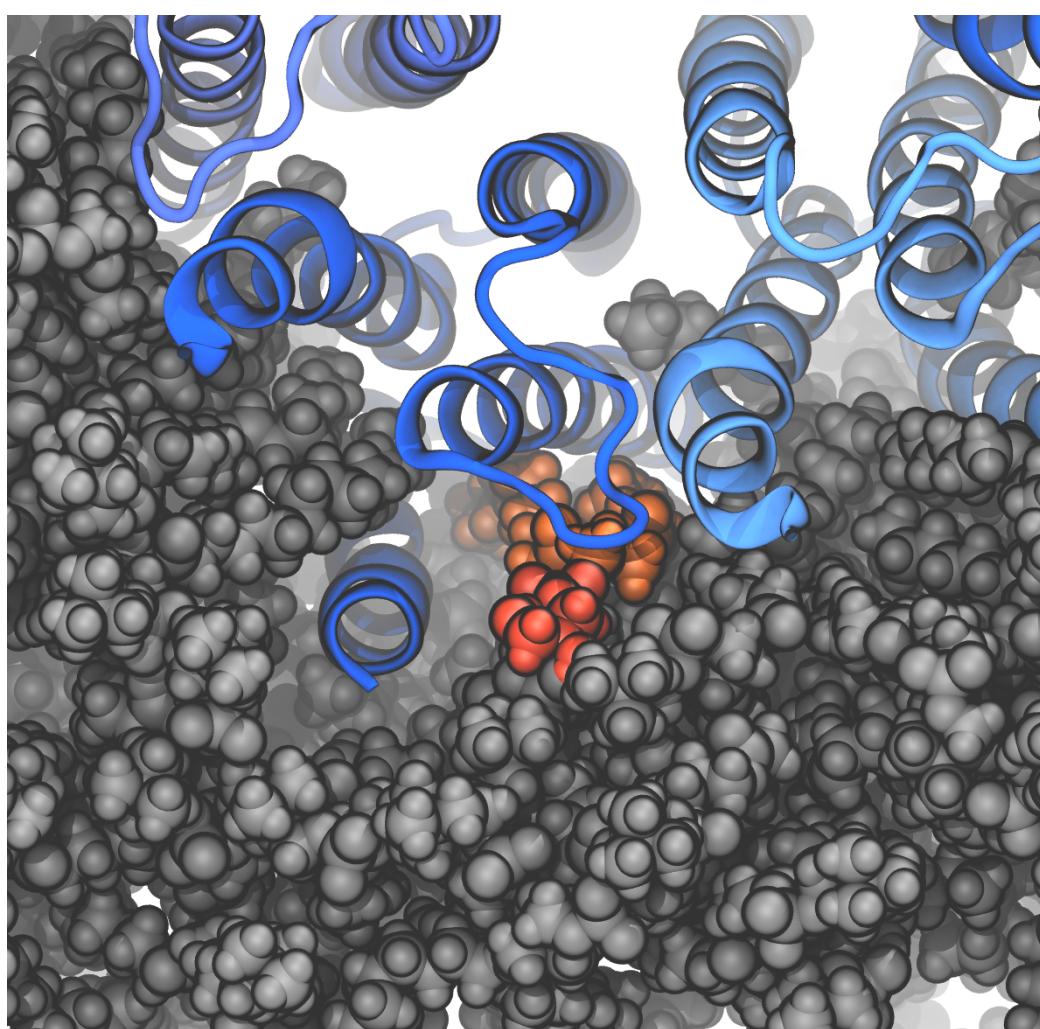


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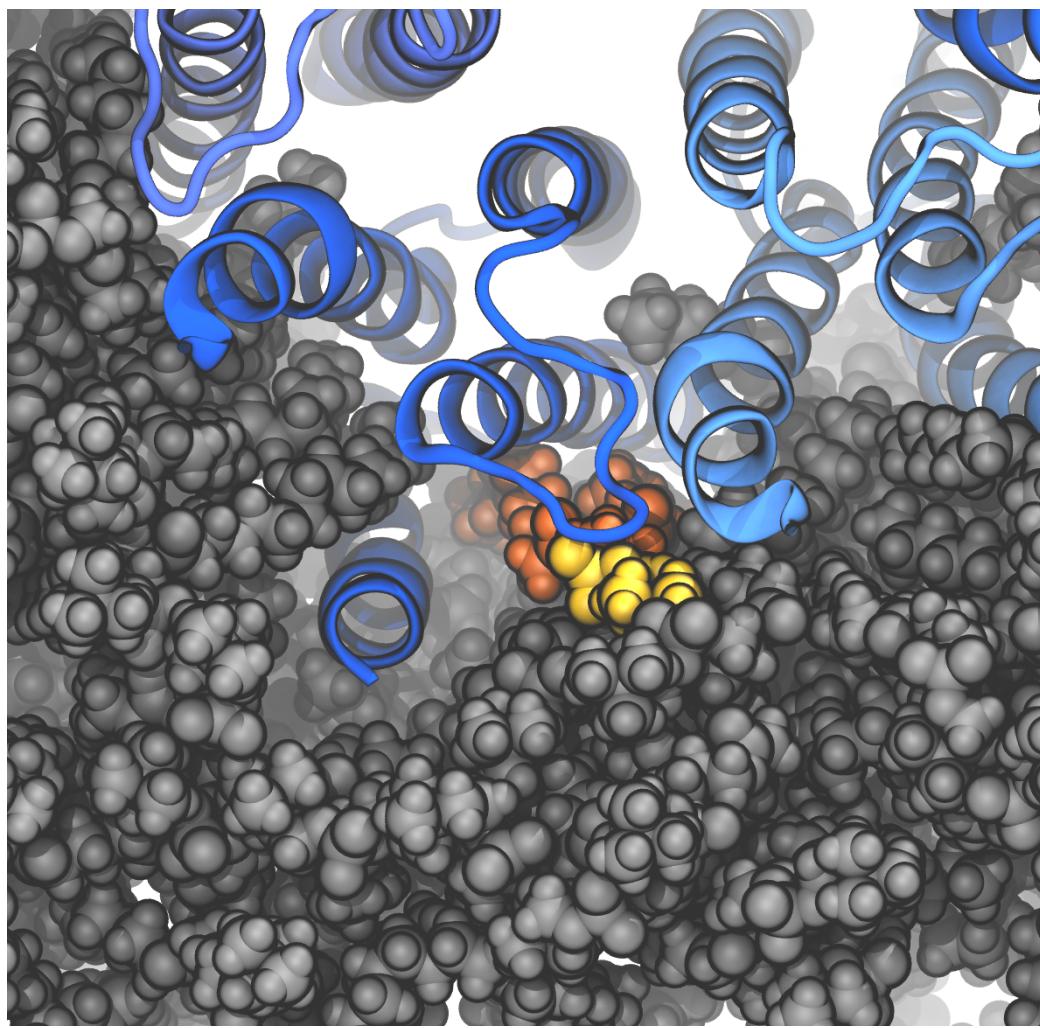


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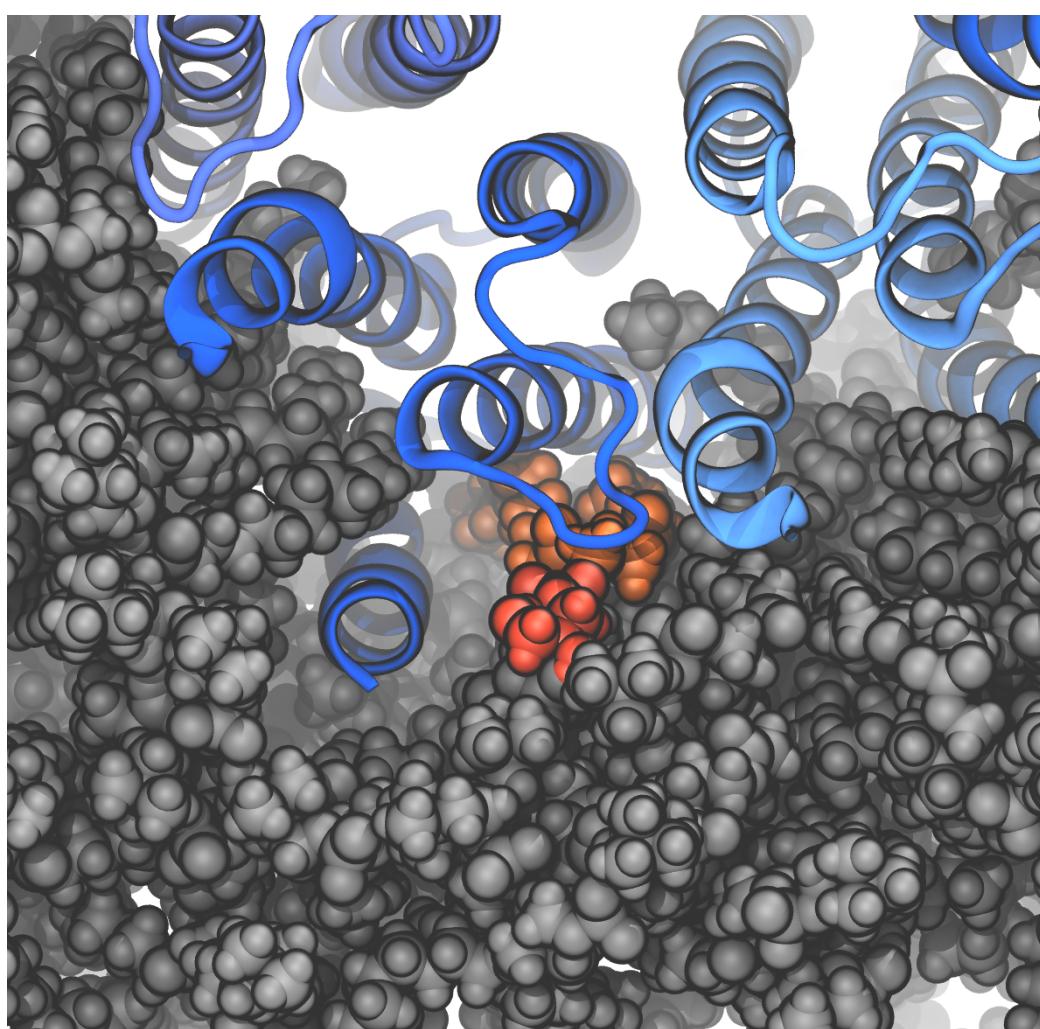


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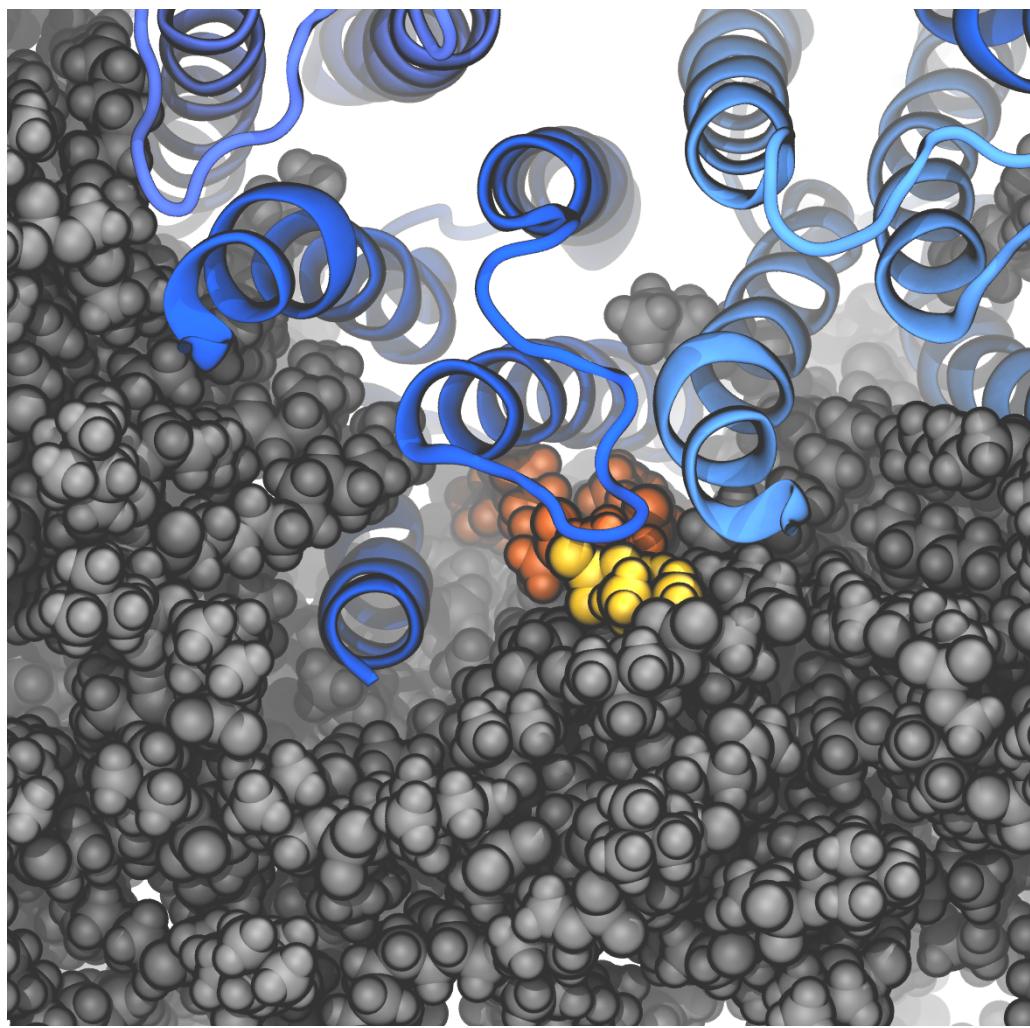


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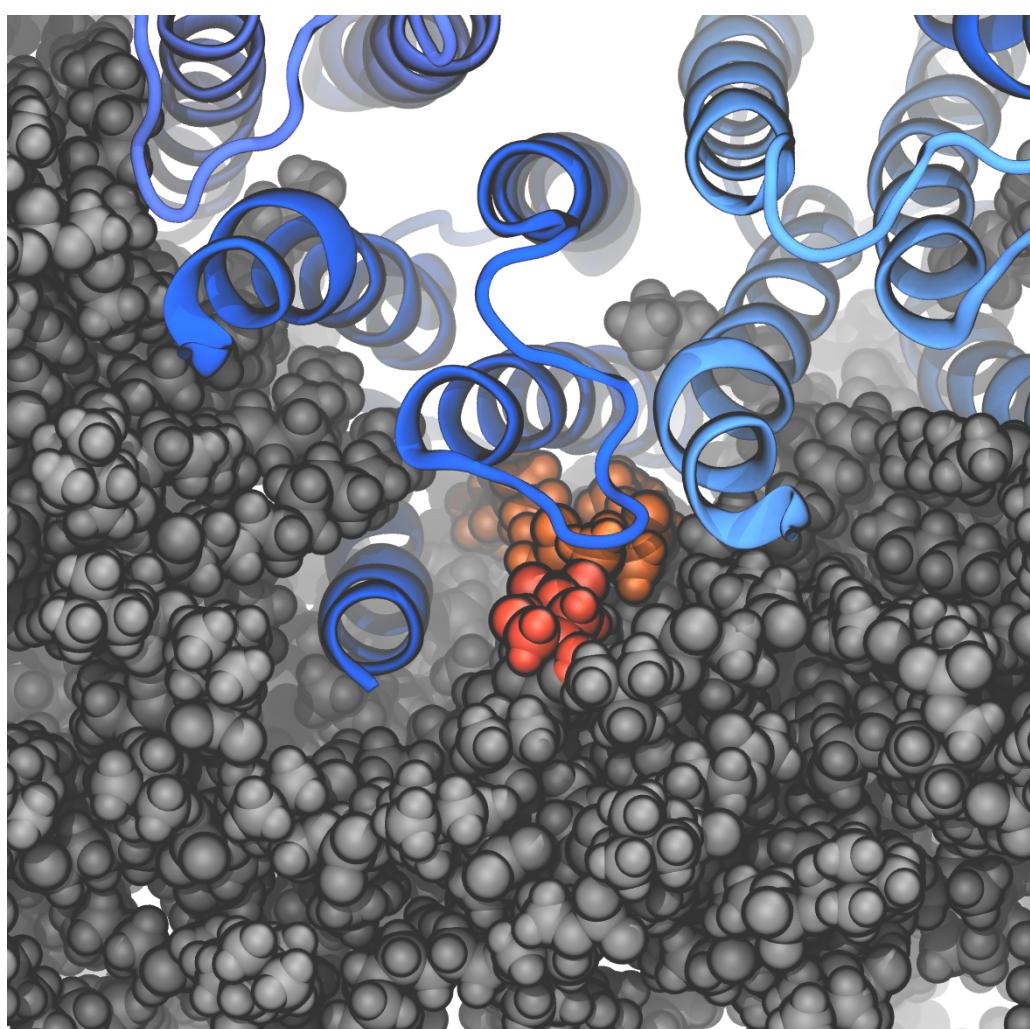


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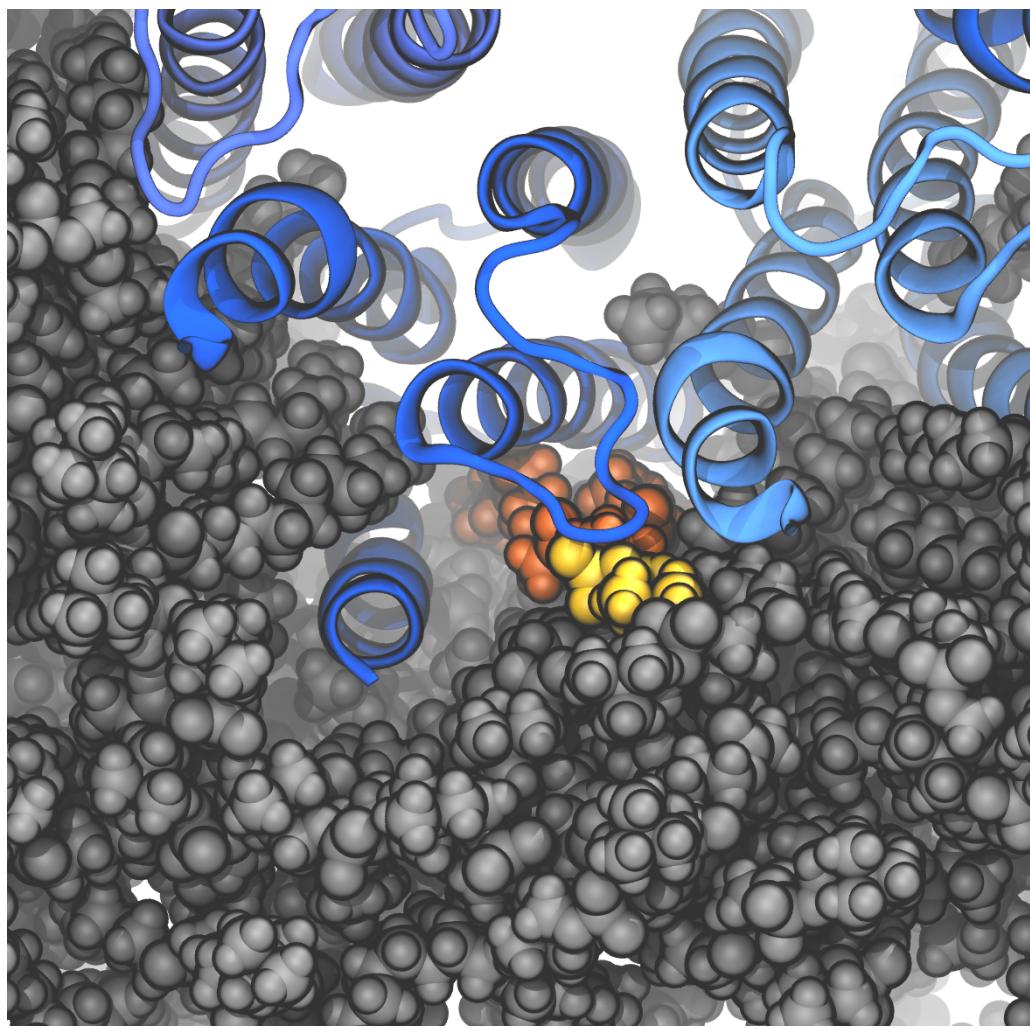


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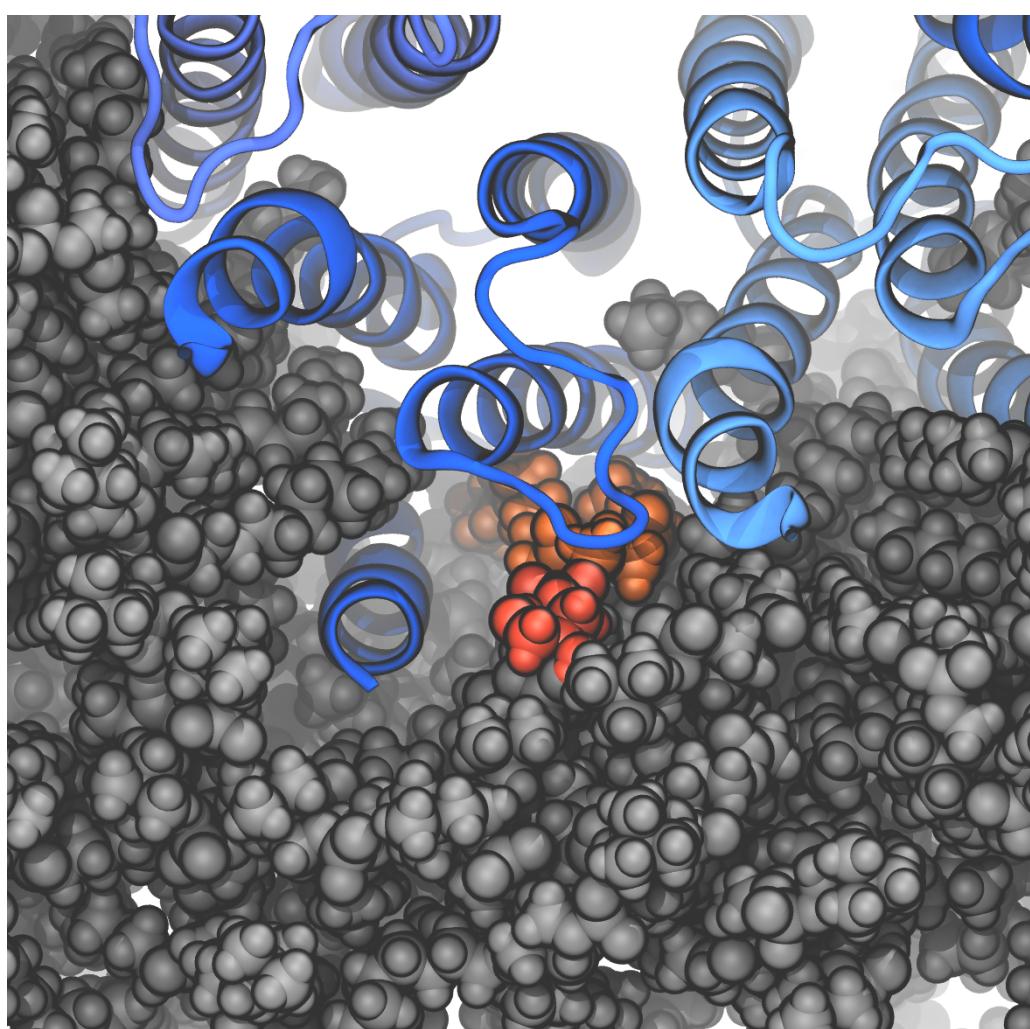


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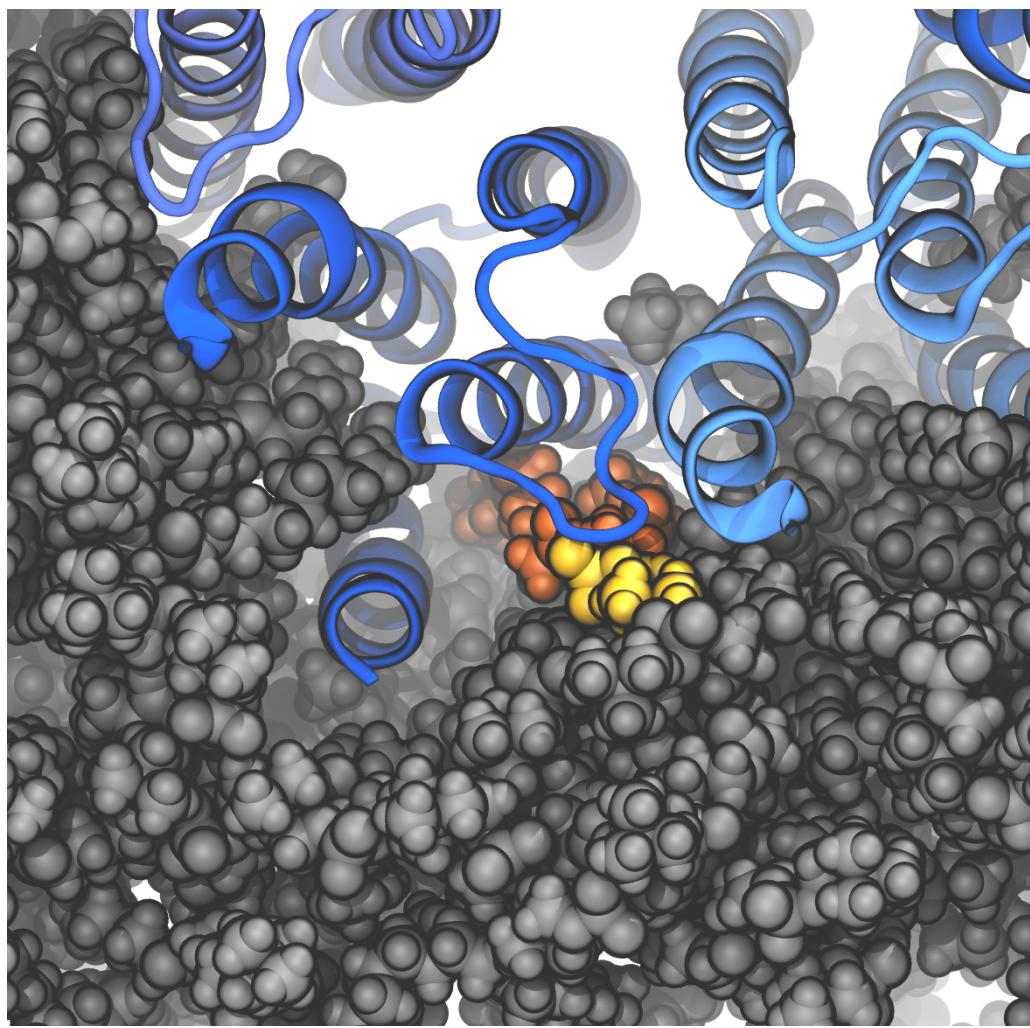
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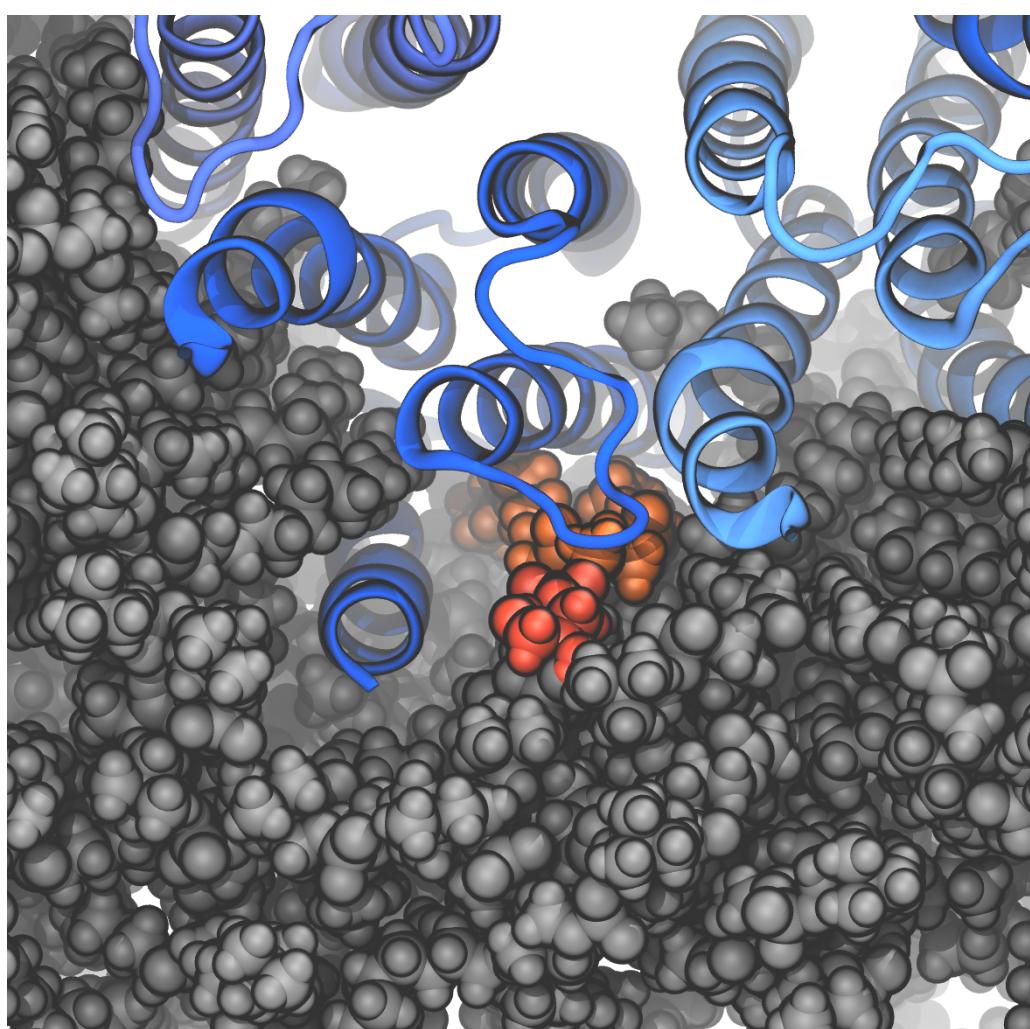
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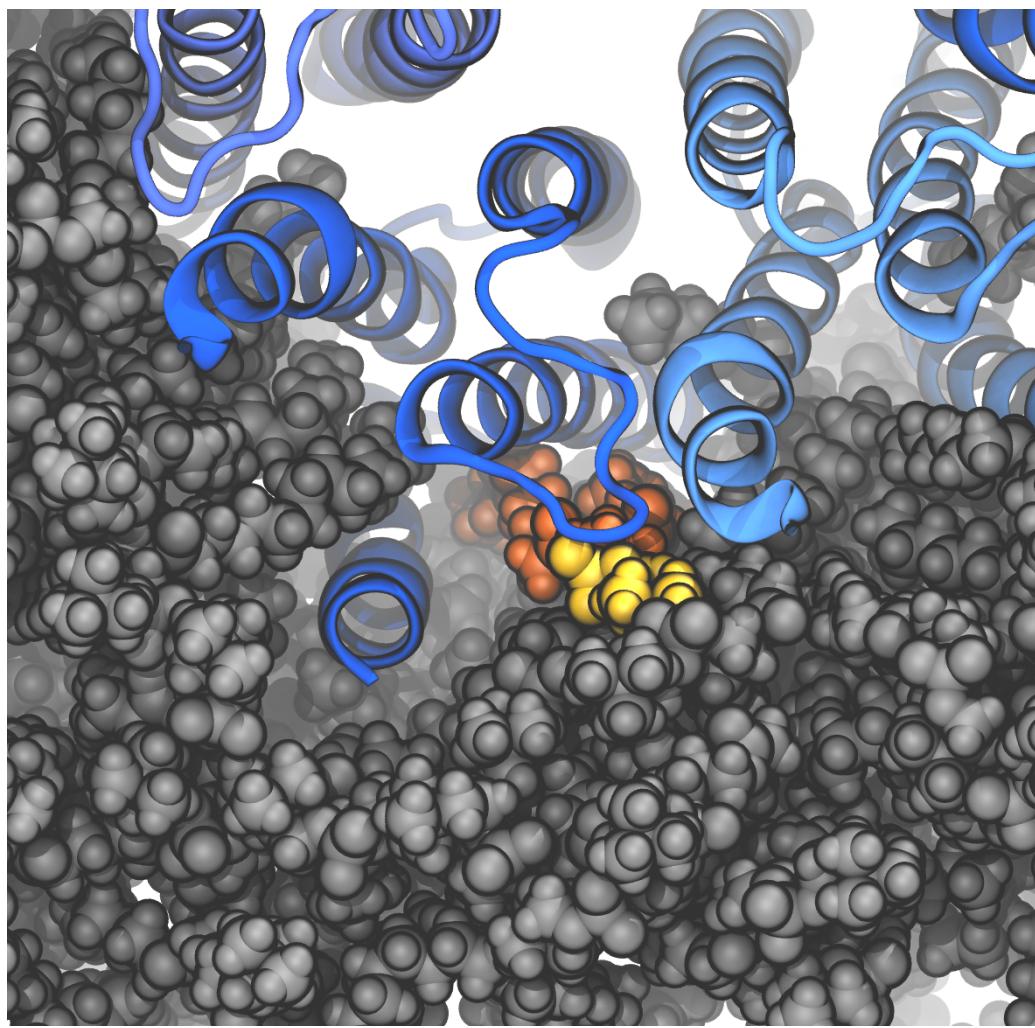
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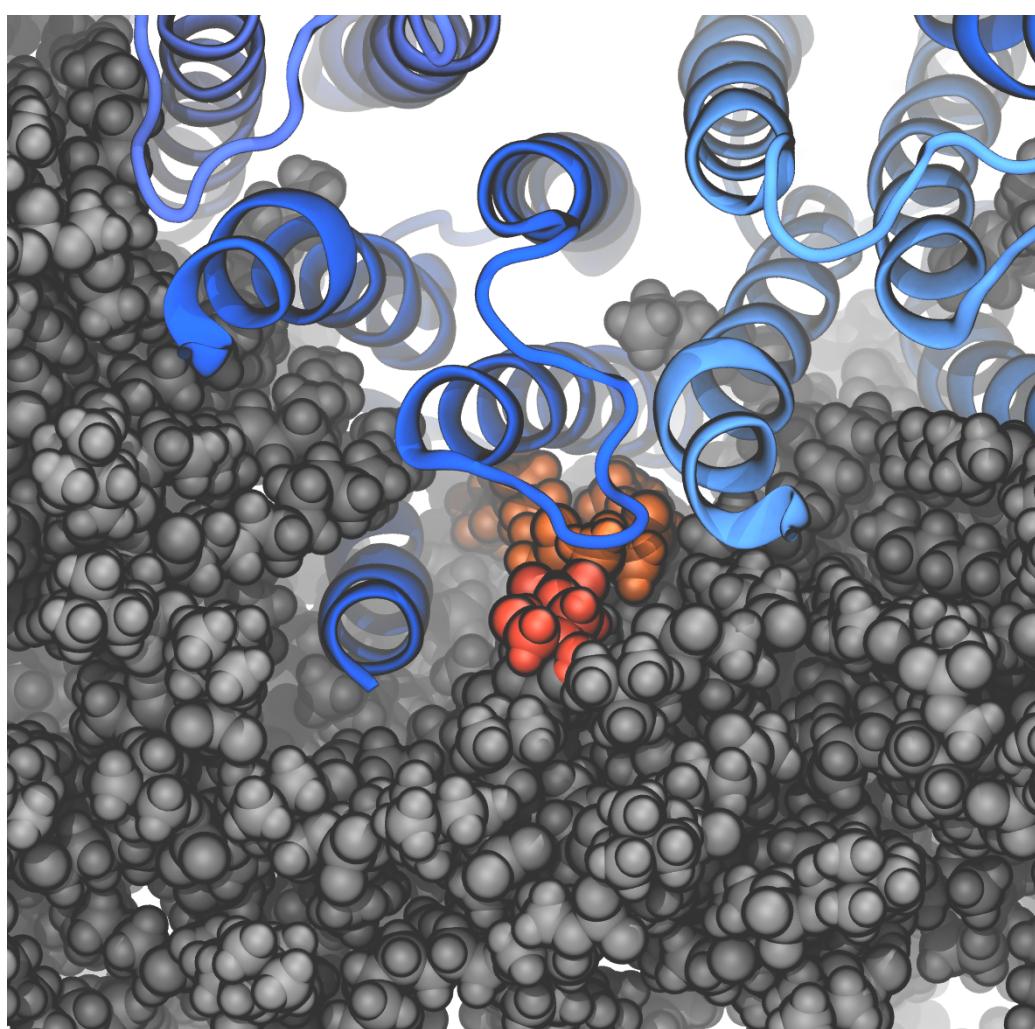
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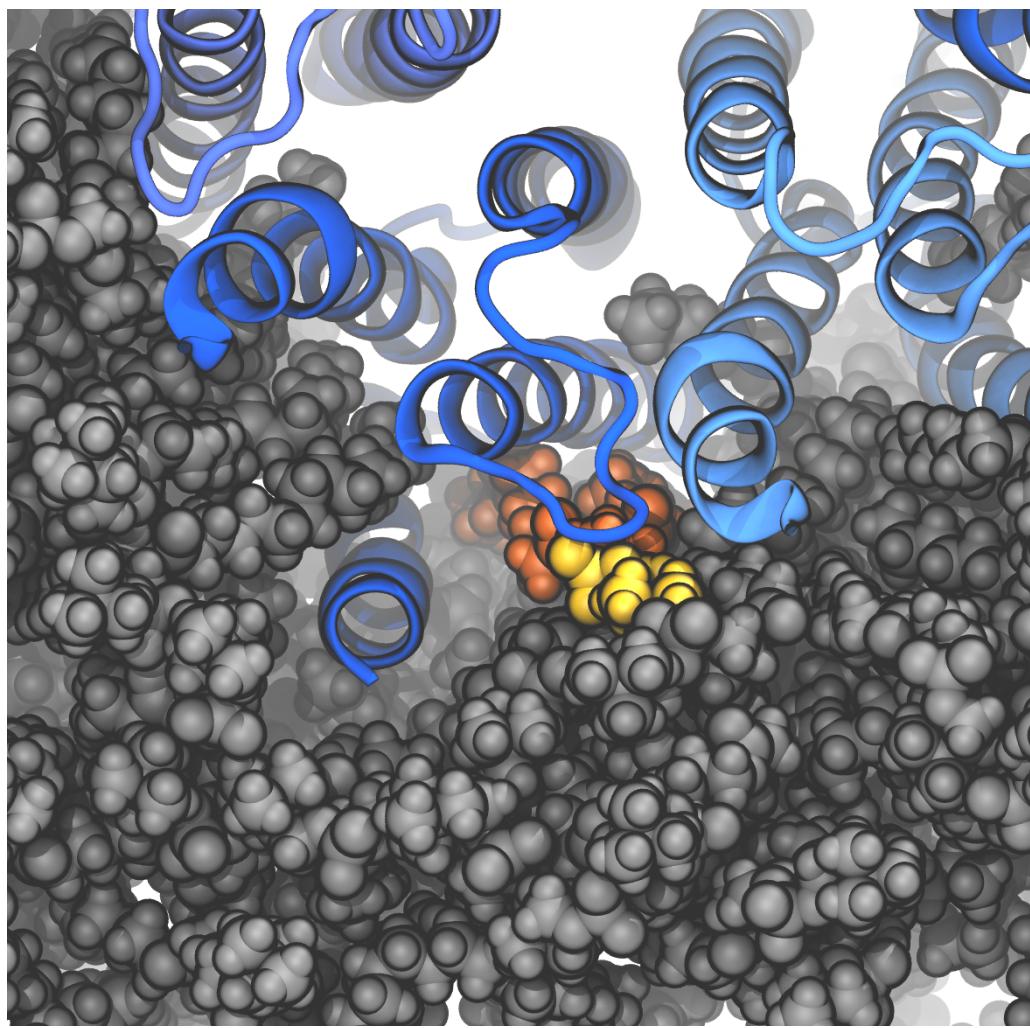
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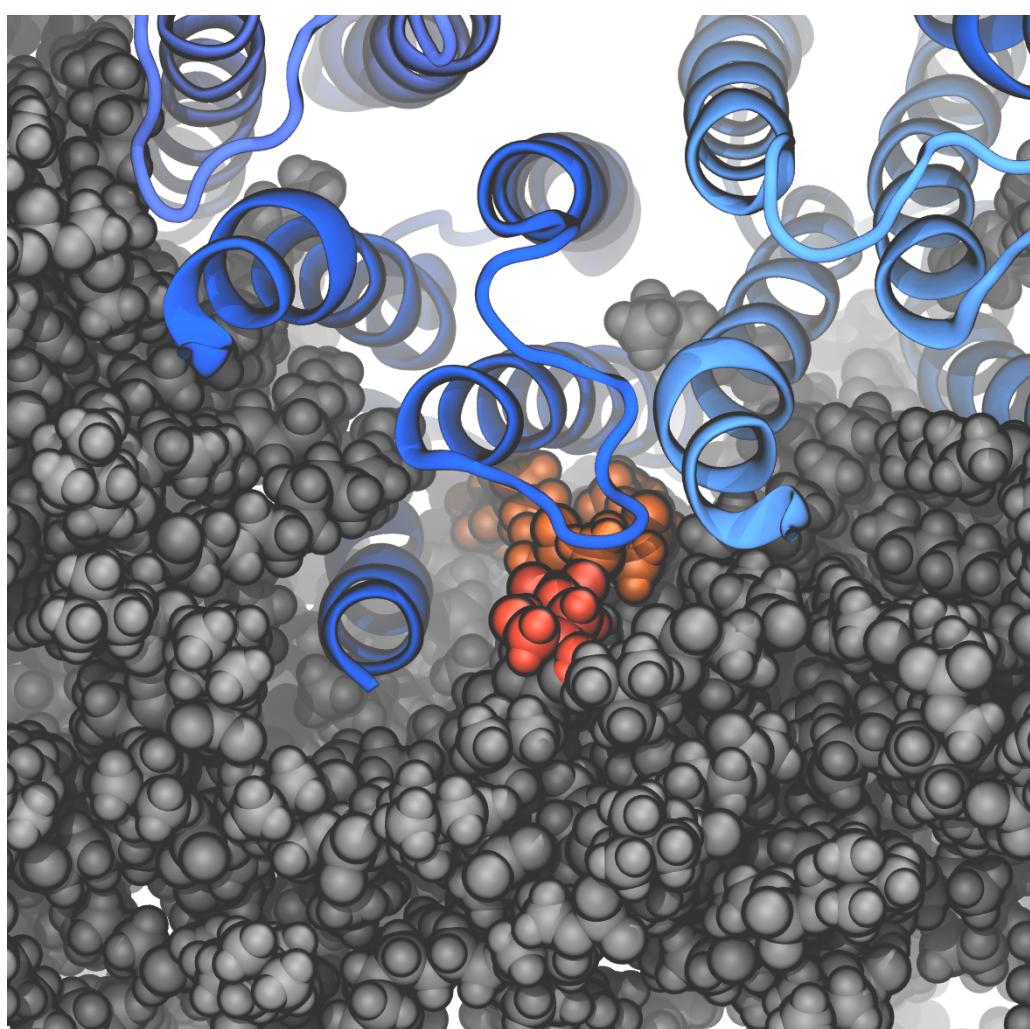
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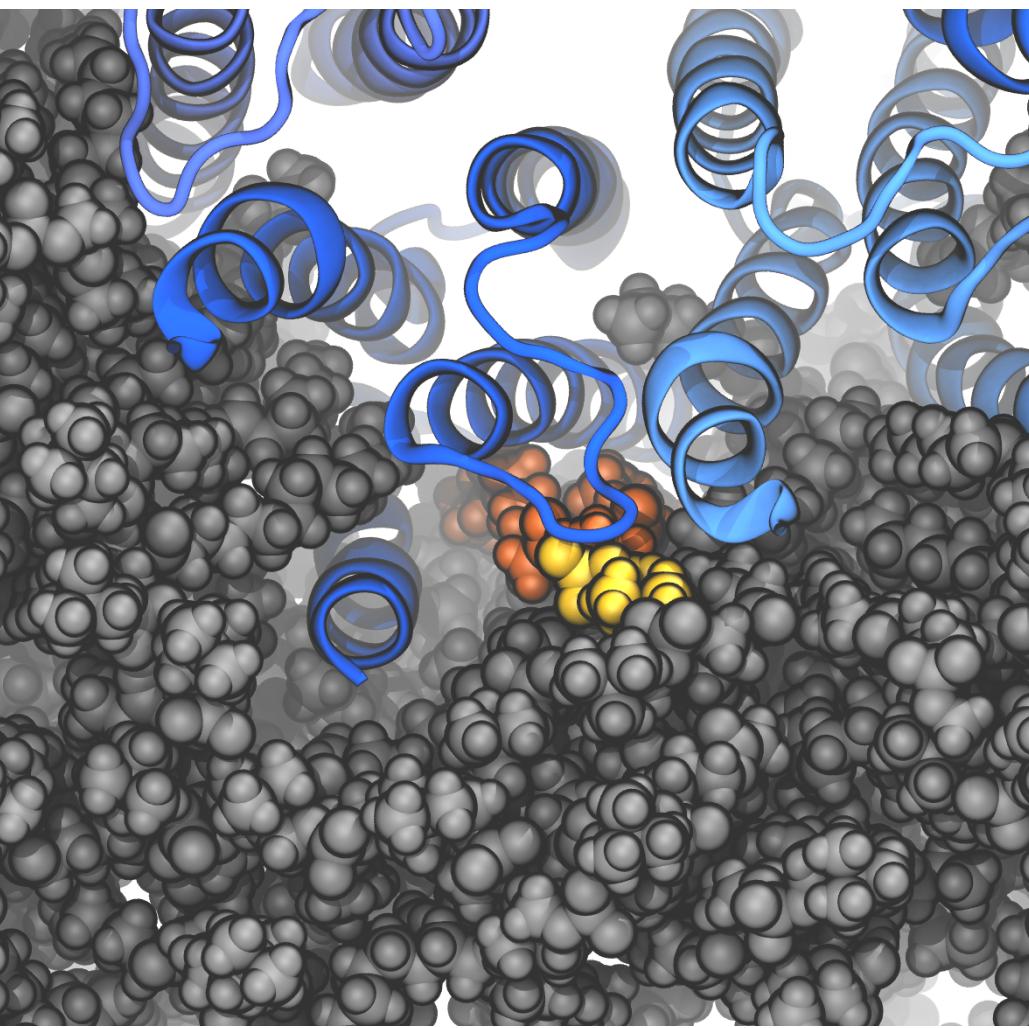
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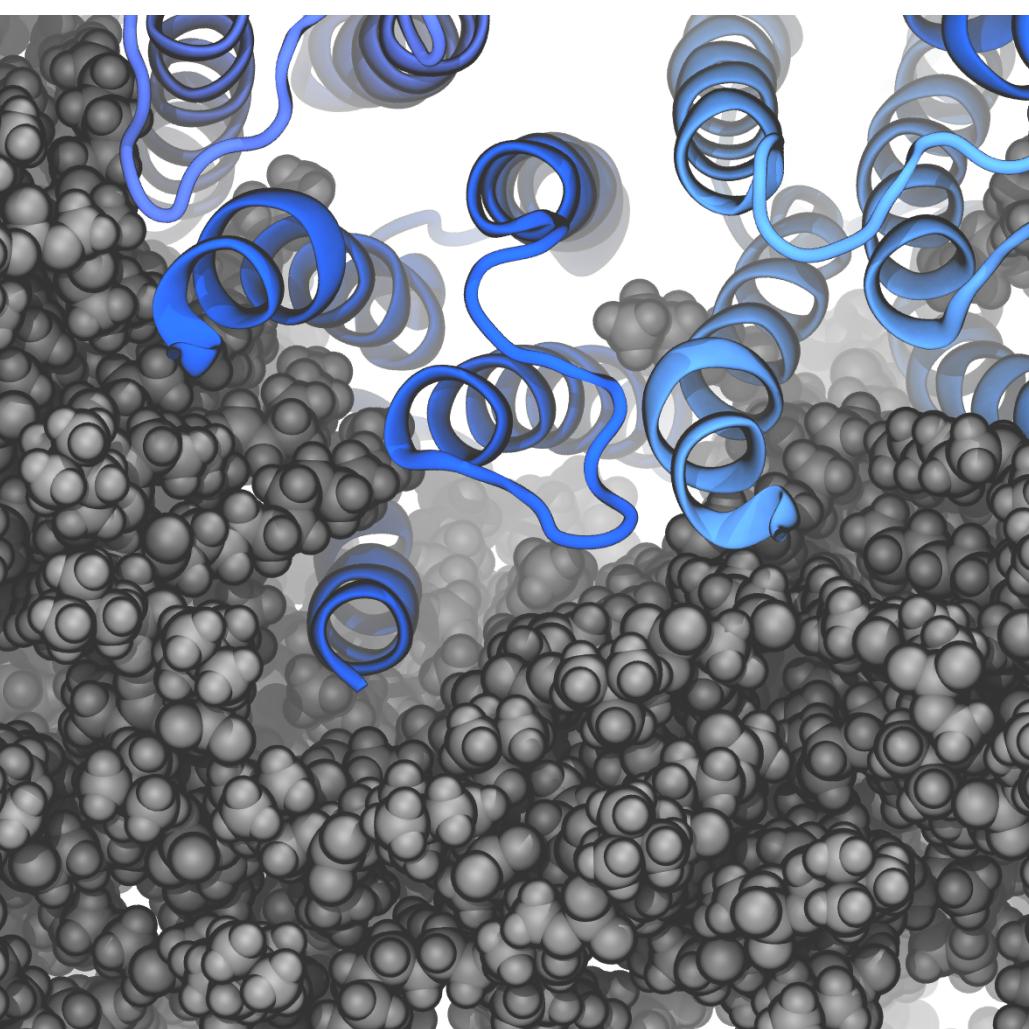
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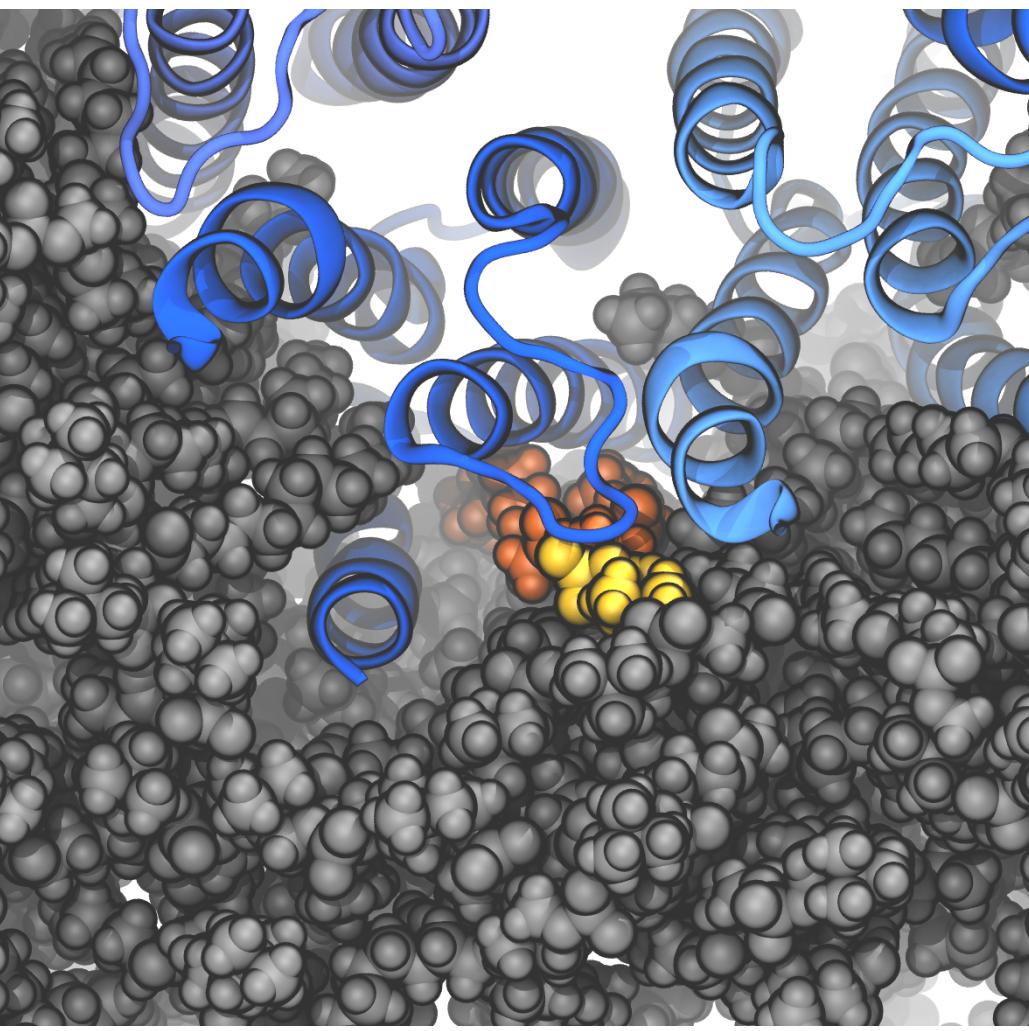
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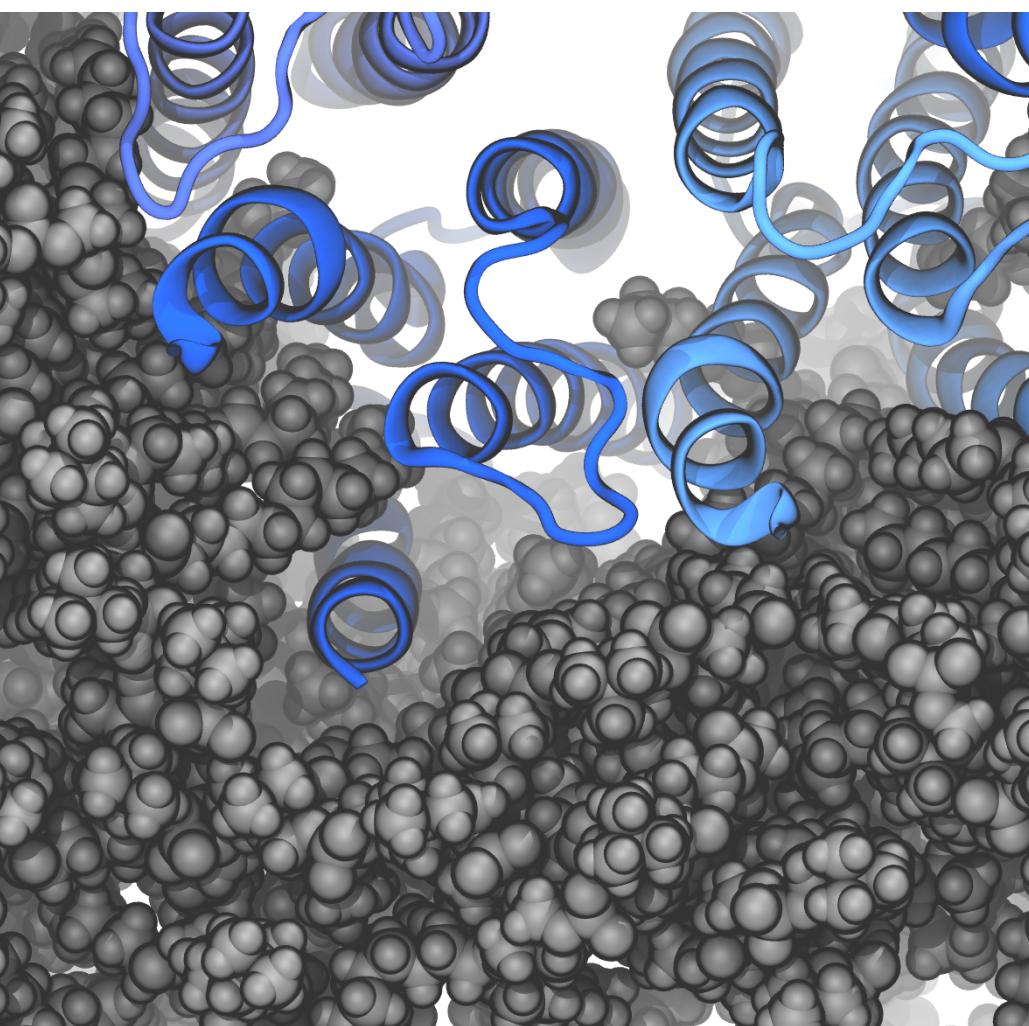
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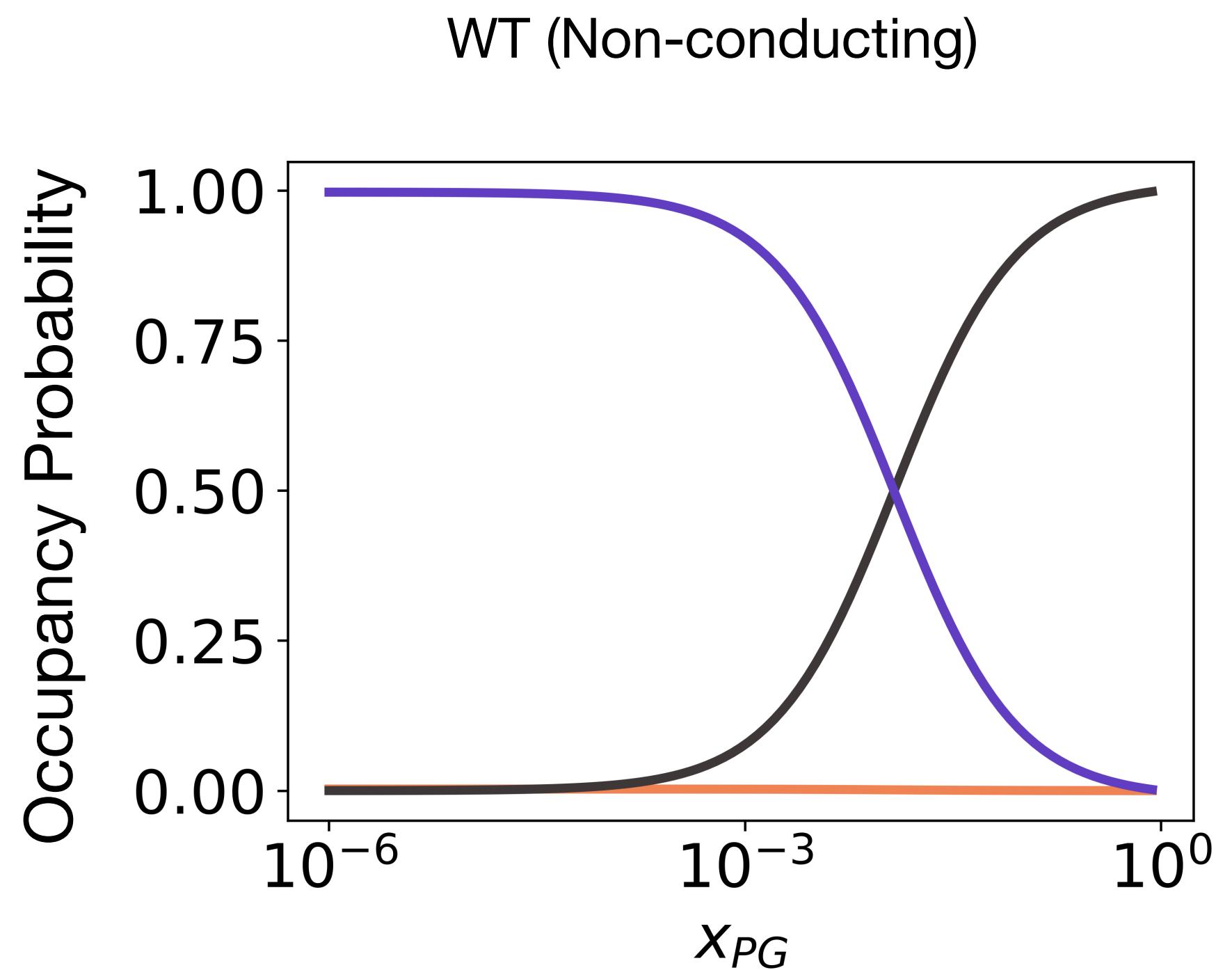


## Absolute:

- **Fewer assumptions**
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- **More expensive (>500ns)**

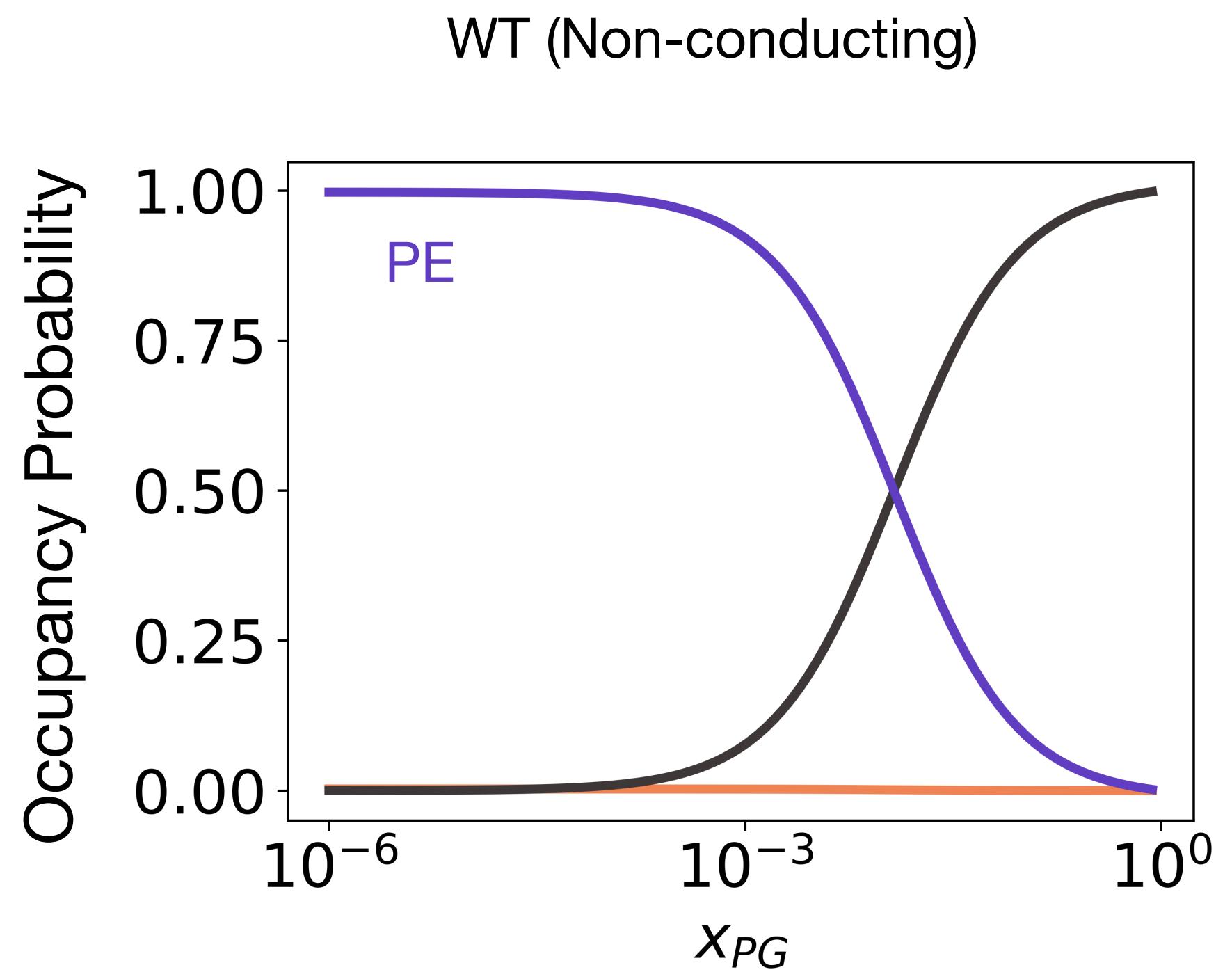
# Occupancy Predicted by Relative FEP

2:1:X PC:PE:PG mixture



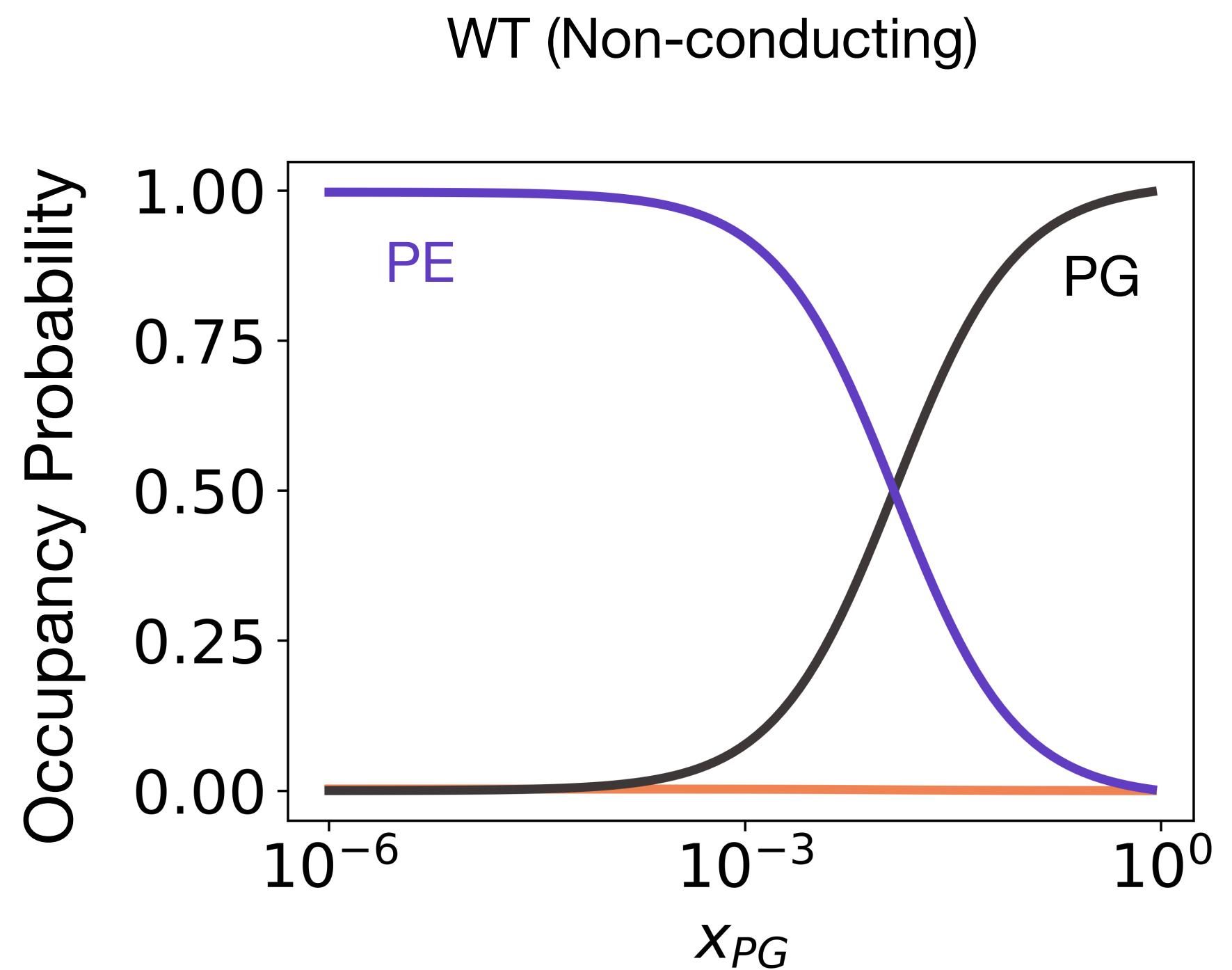
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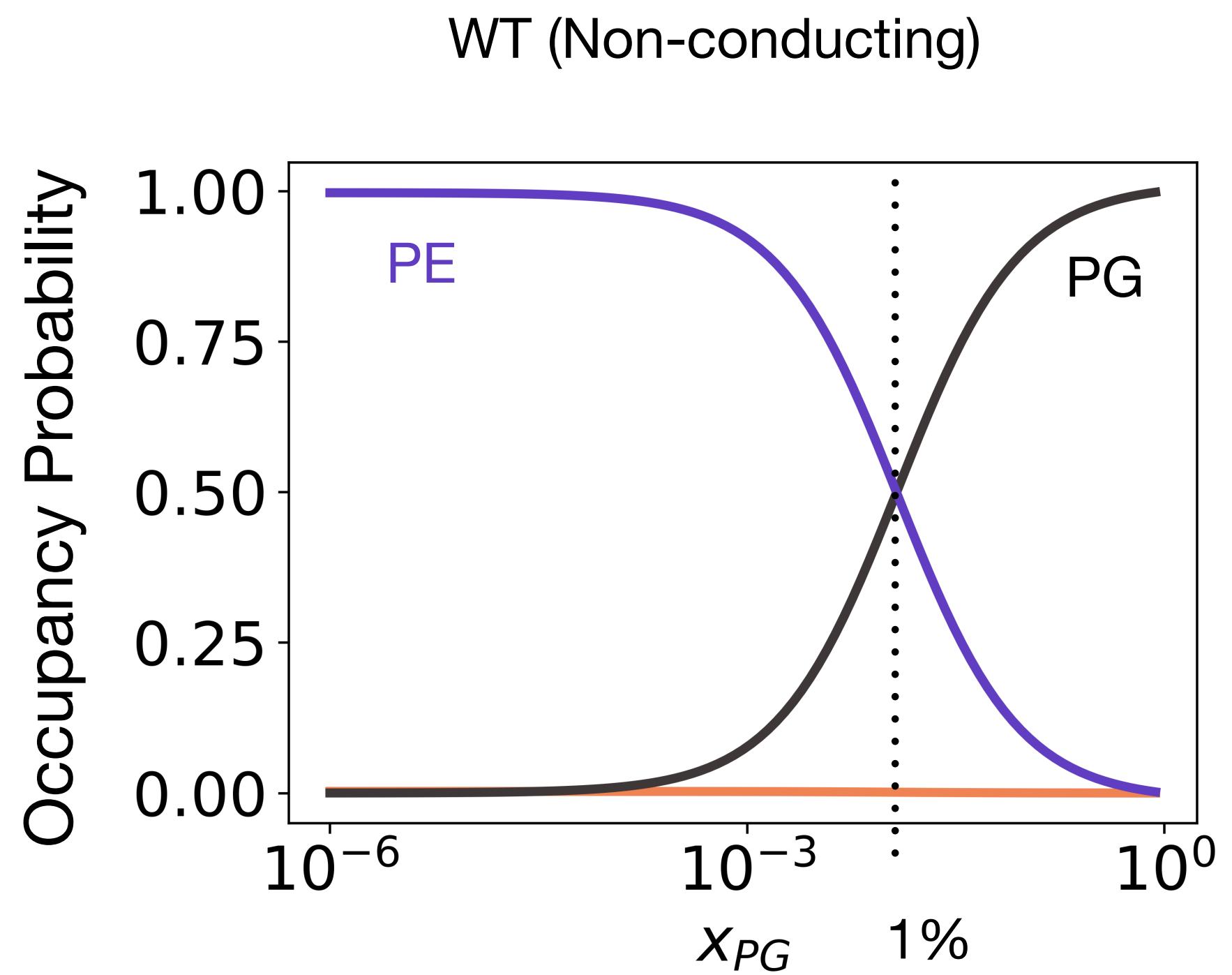
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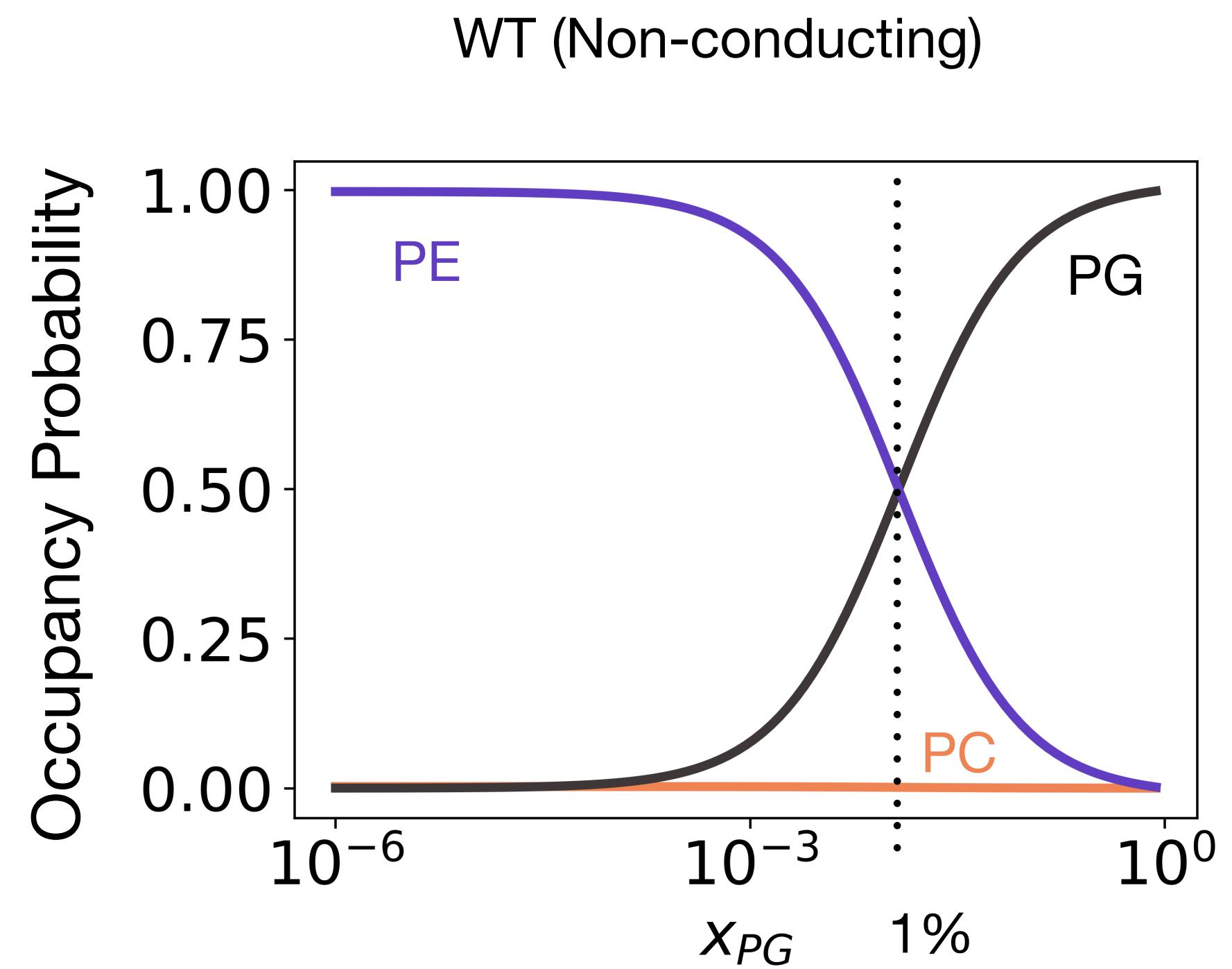
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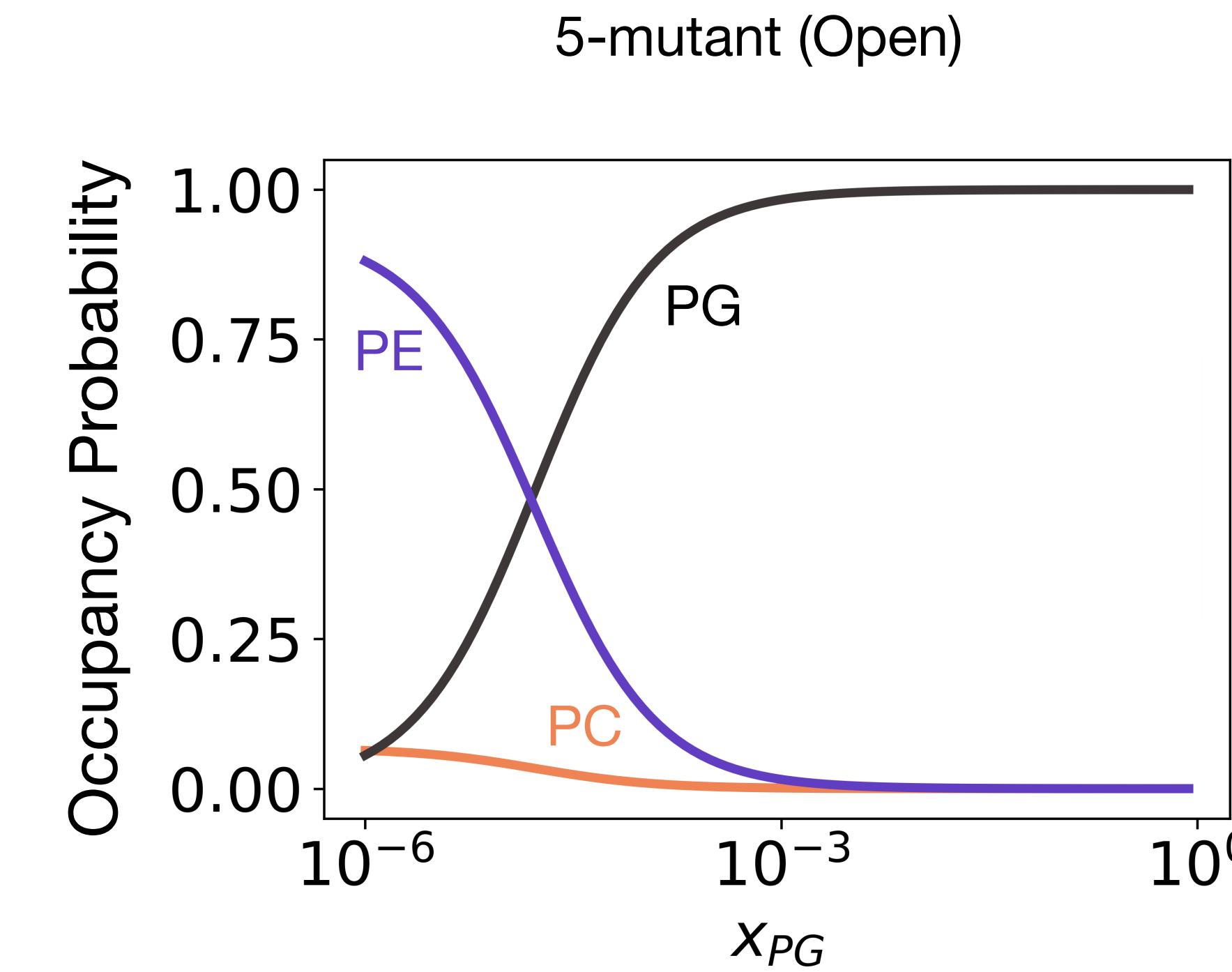
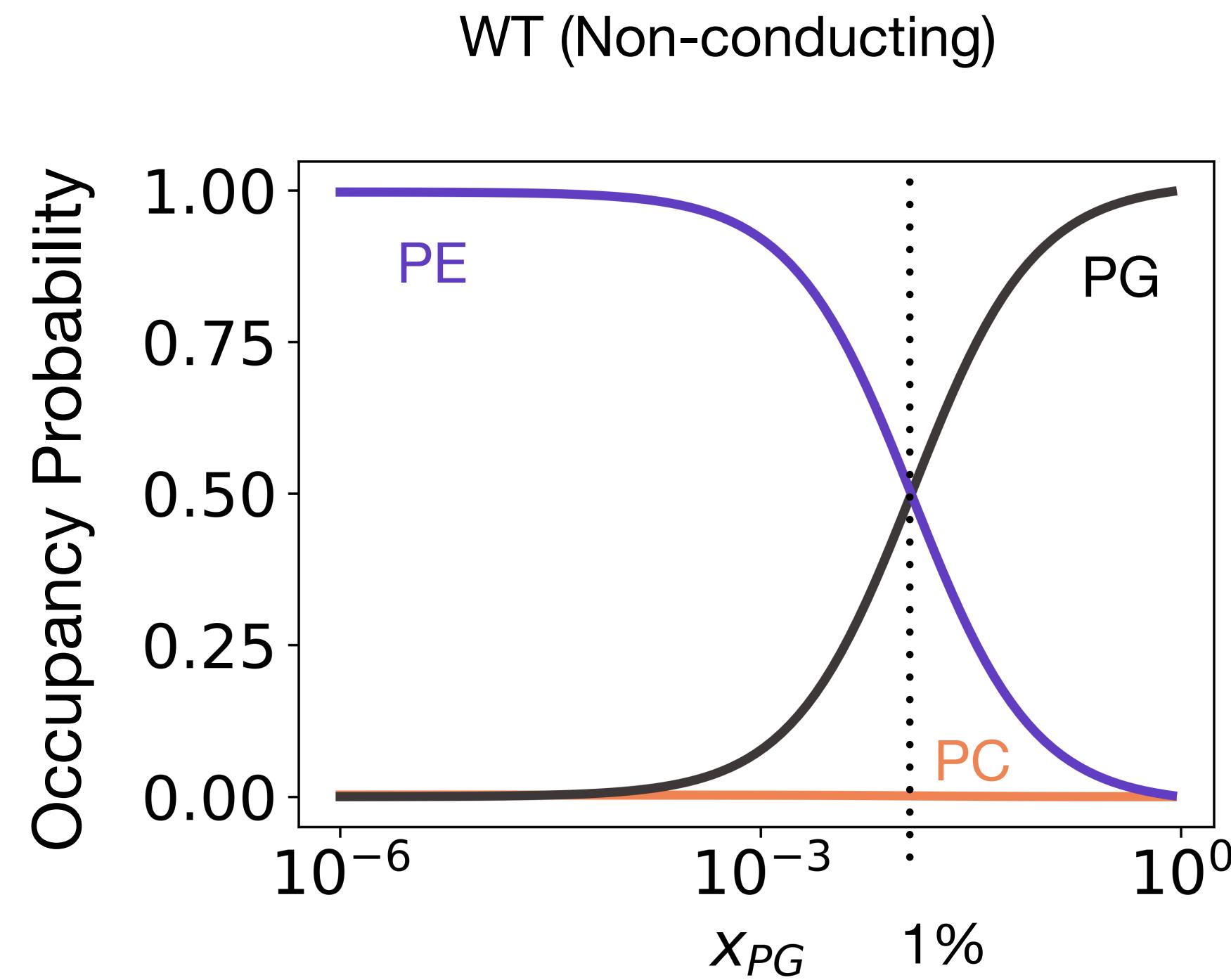
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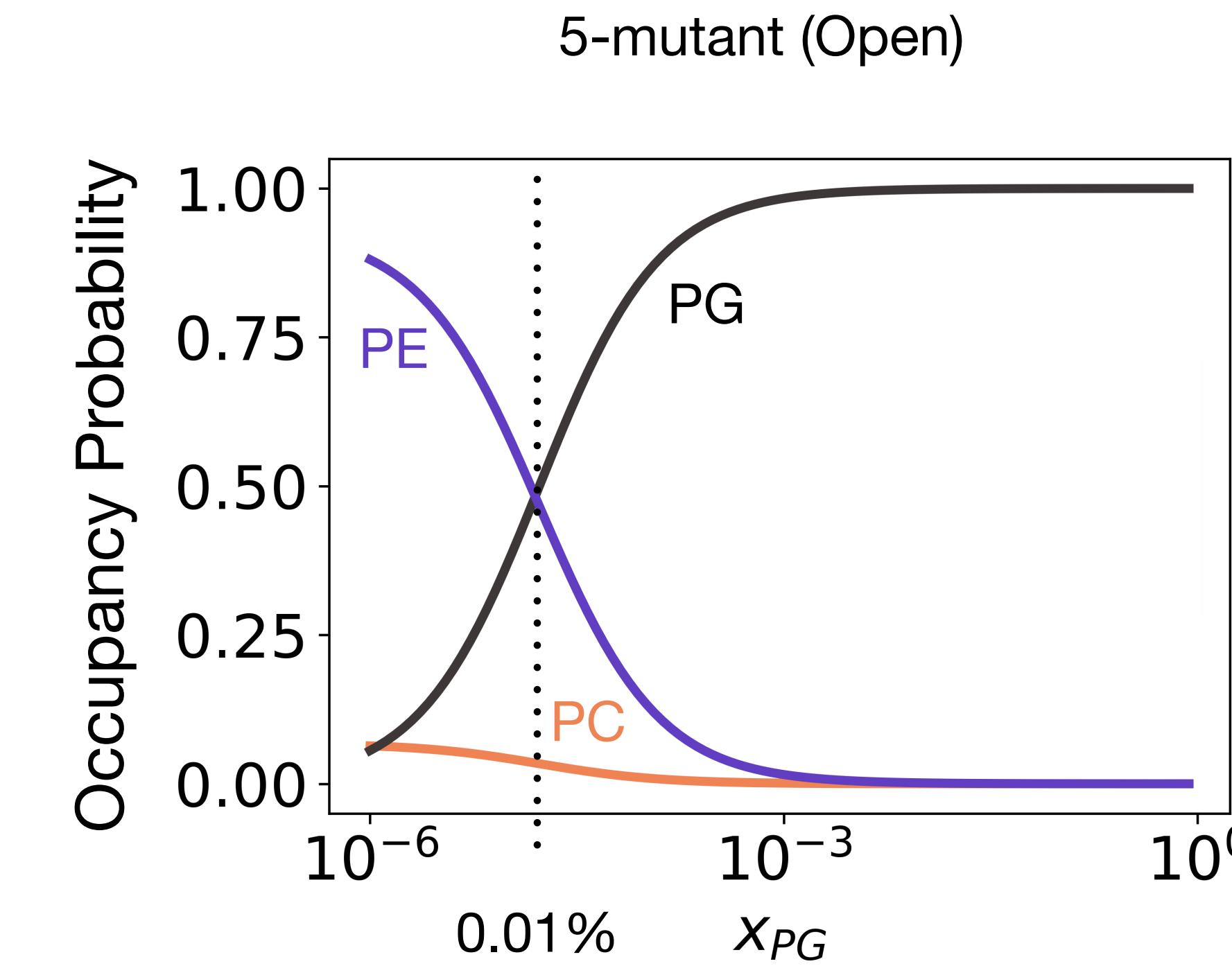
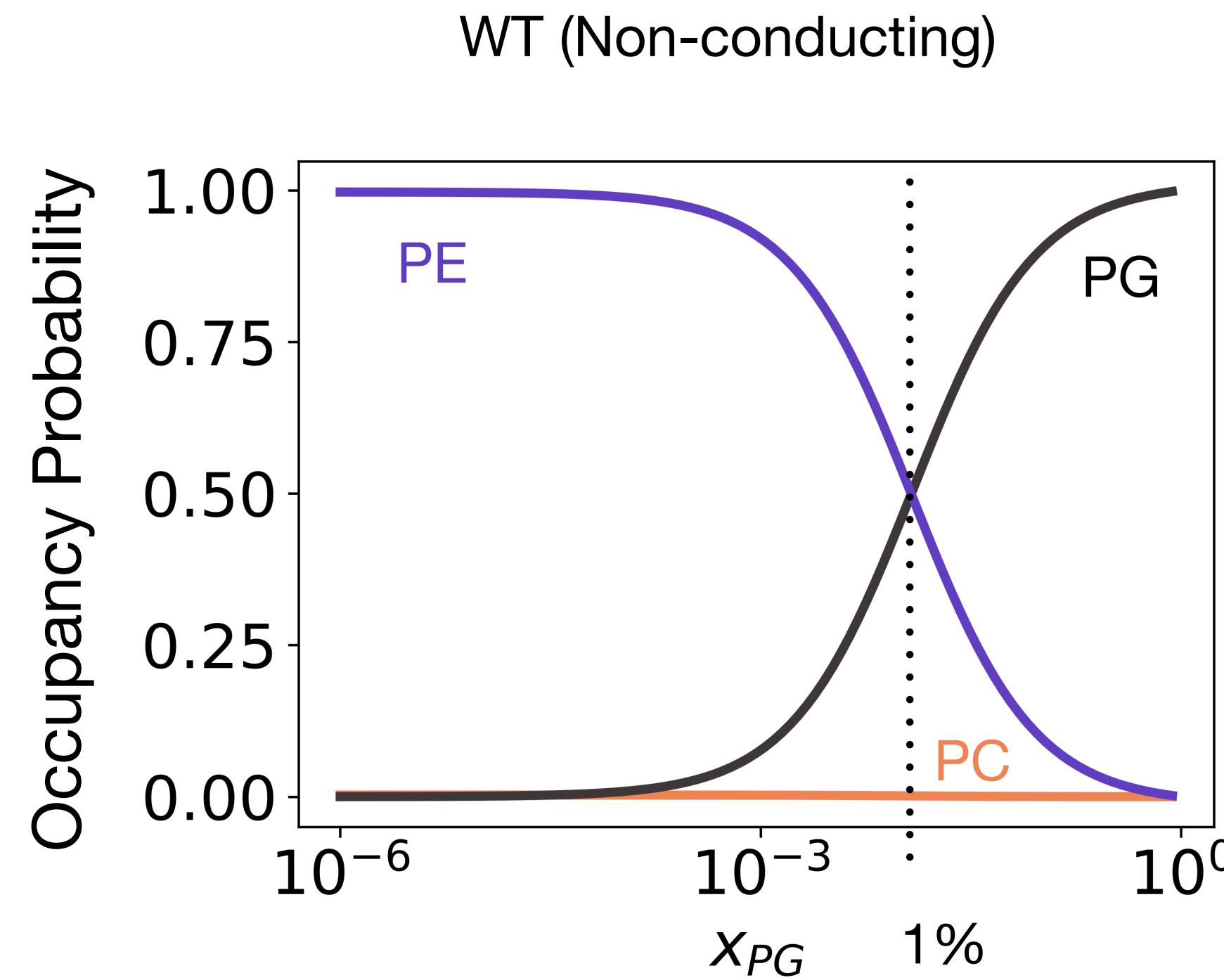
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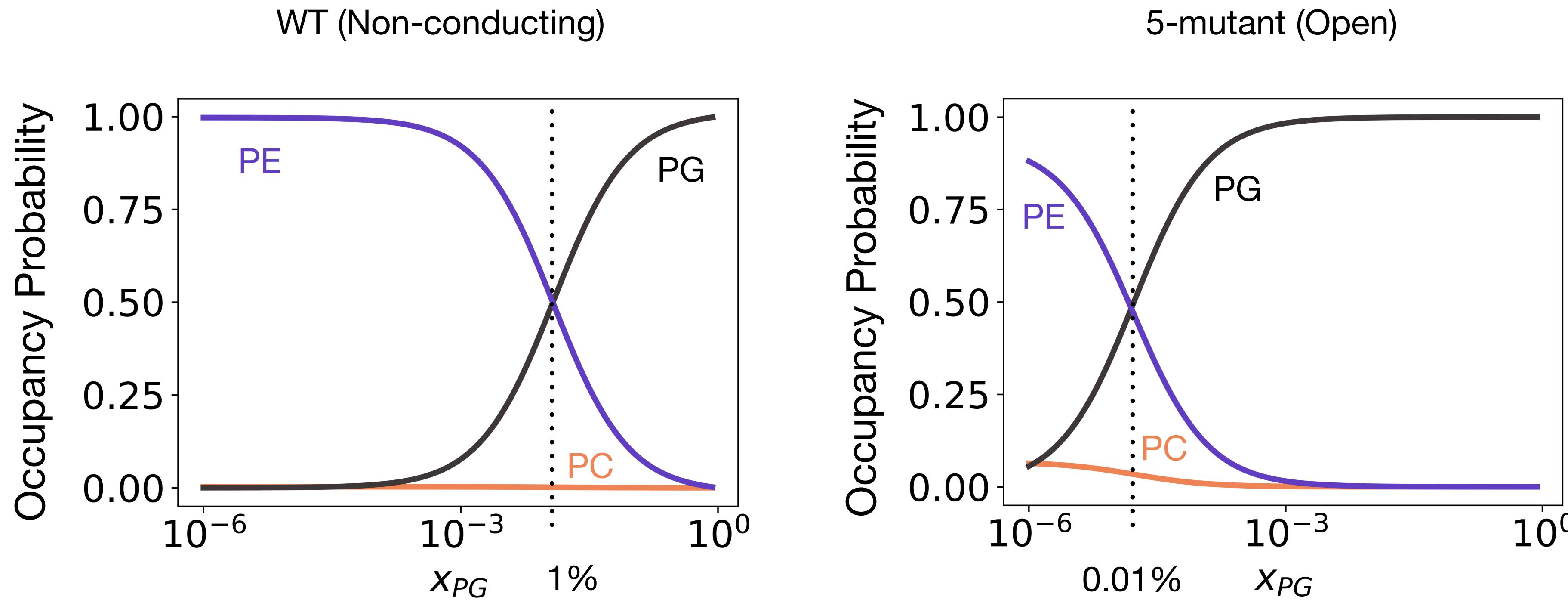
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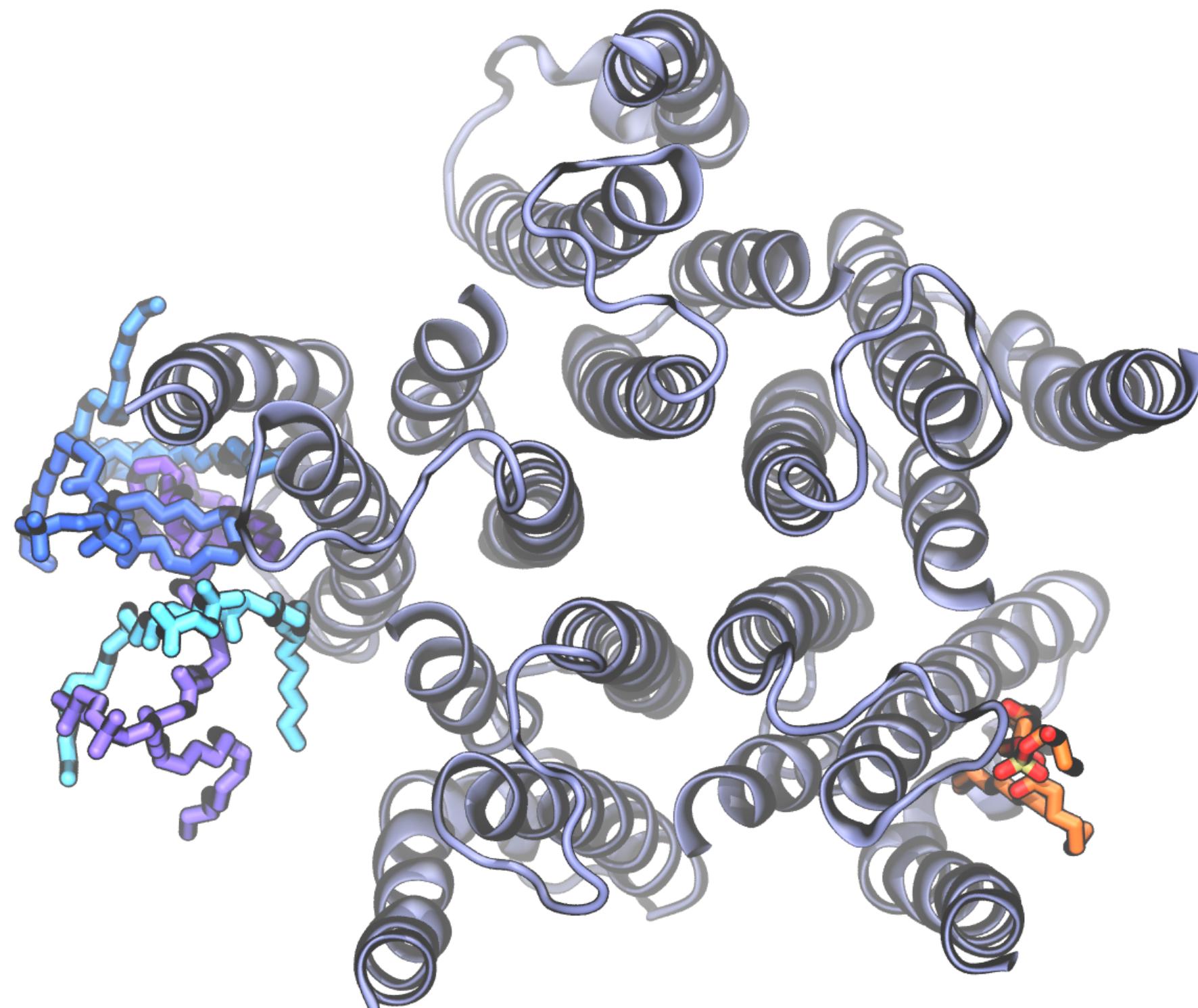
POPC looks like a solvent...

# Binary mixtures: mutating between ligand and solvent

**WT CA (Agonist-Bound)**

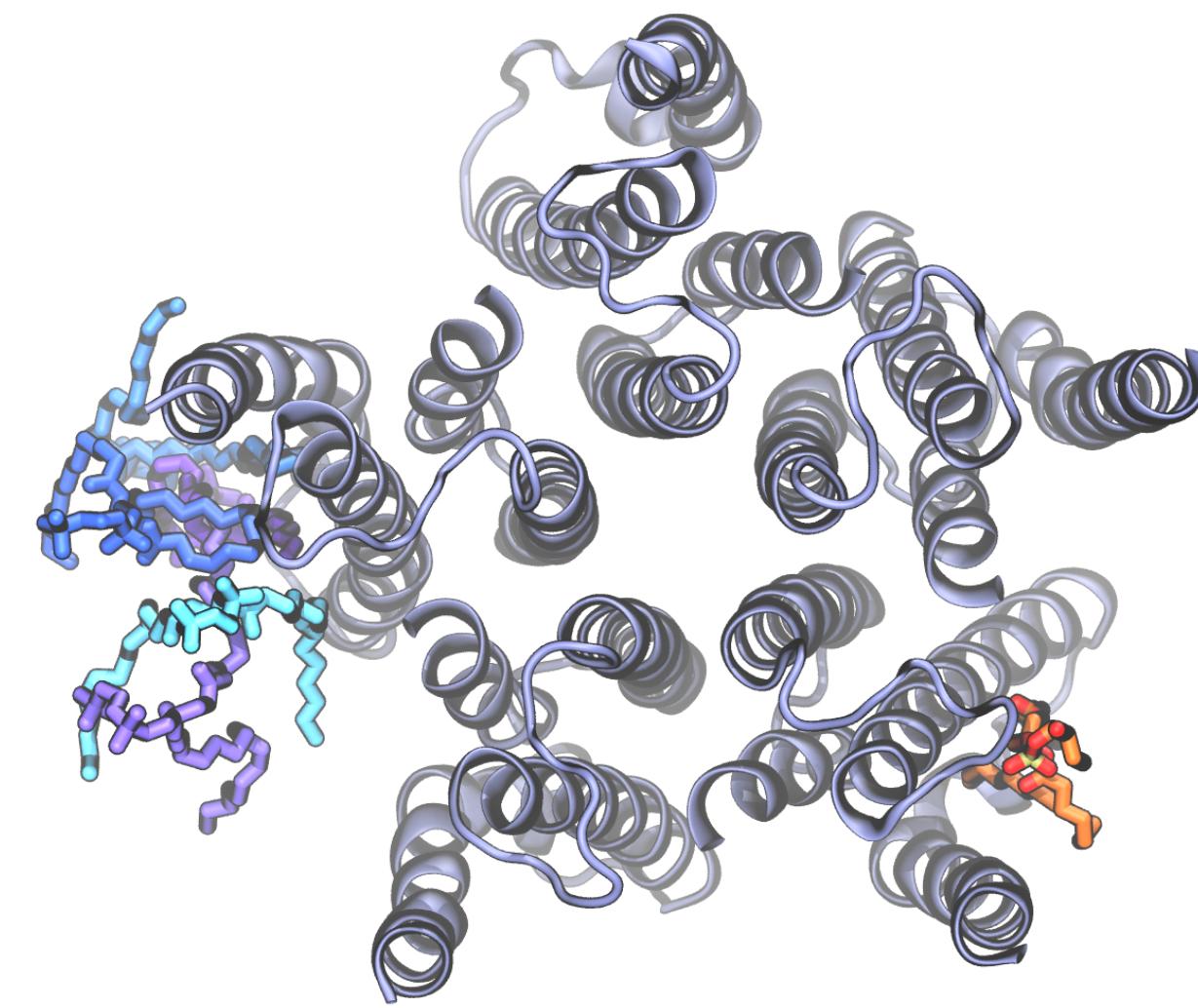
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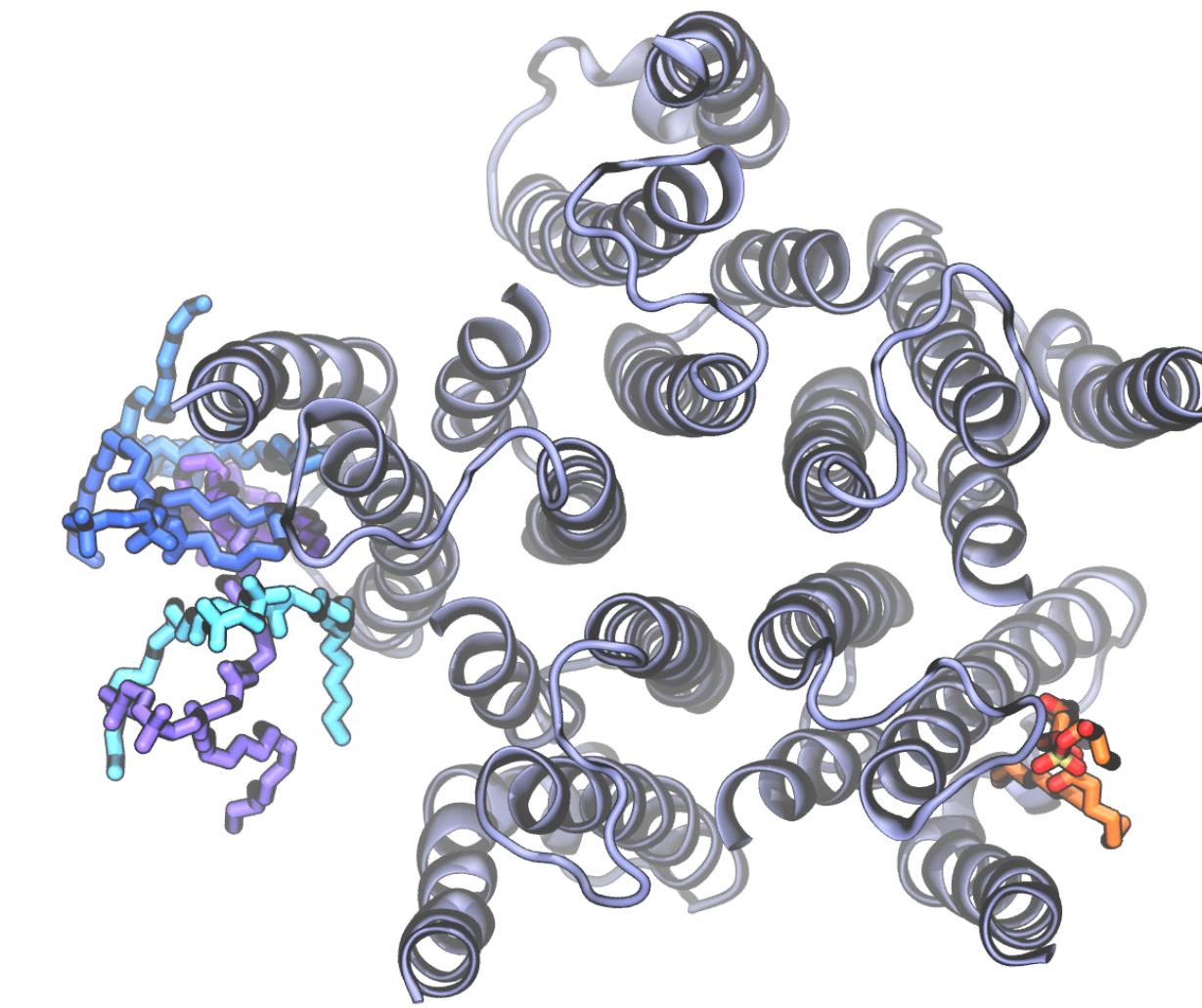
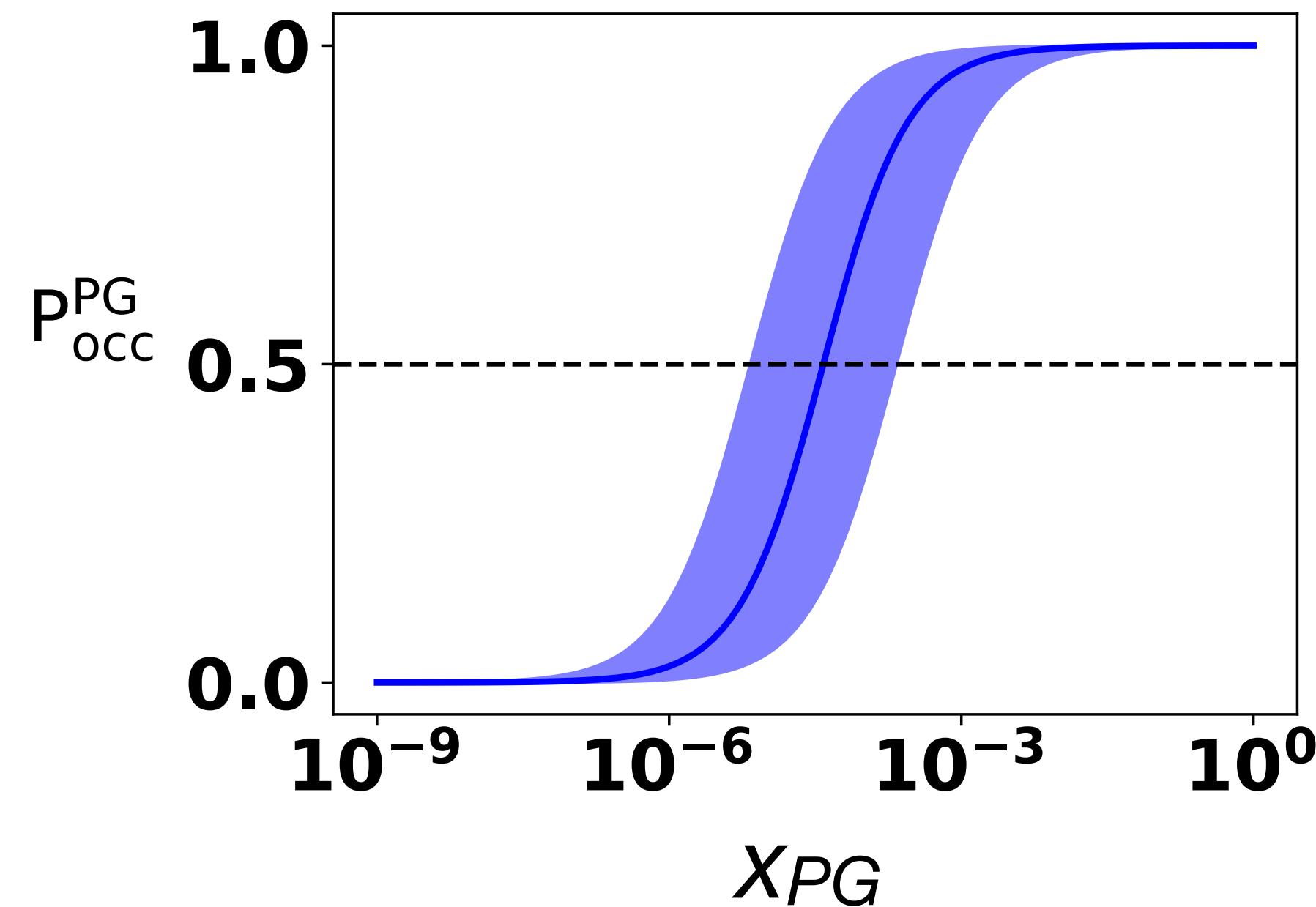
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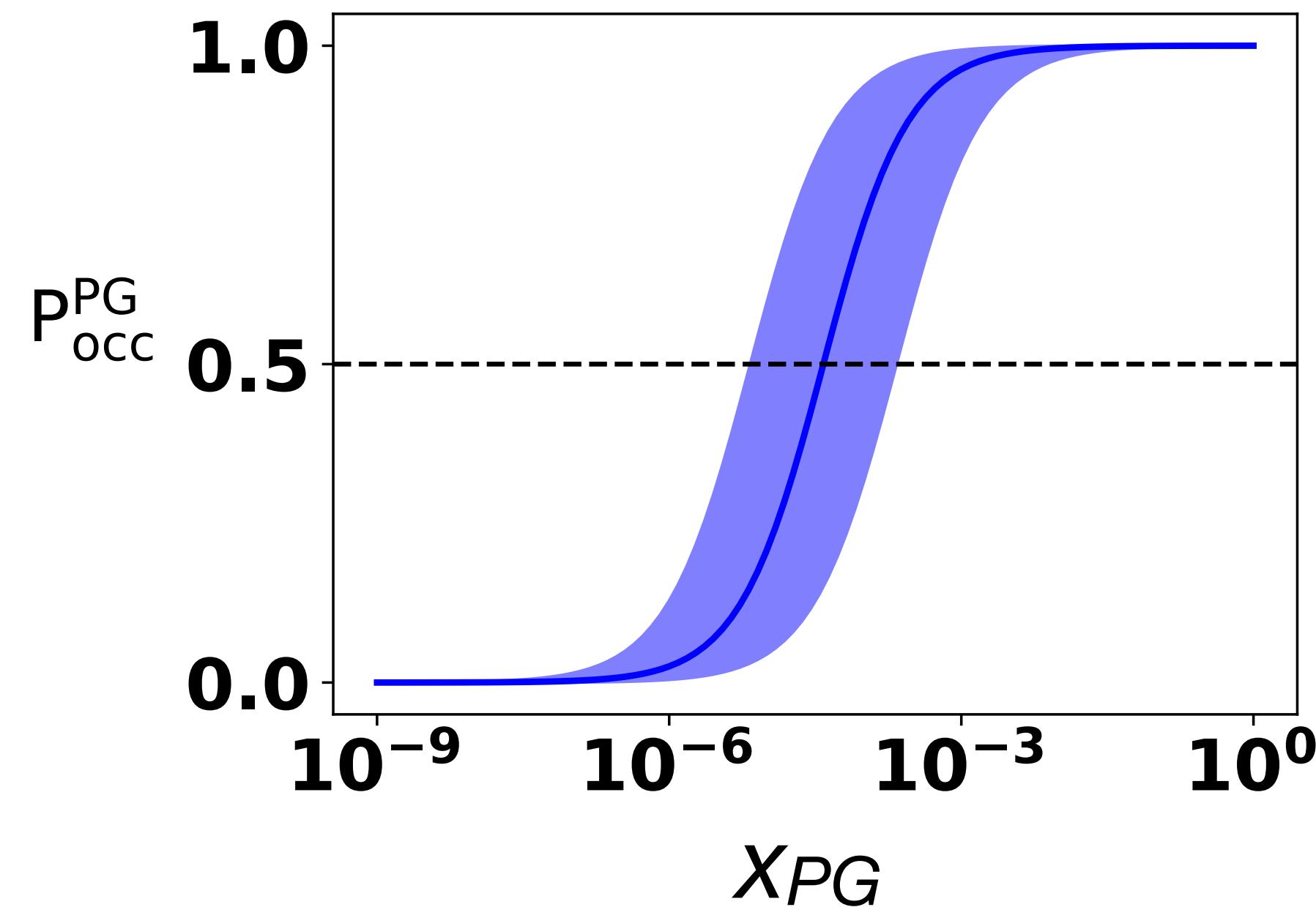
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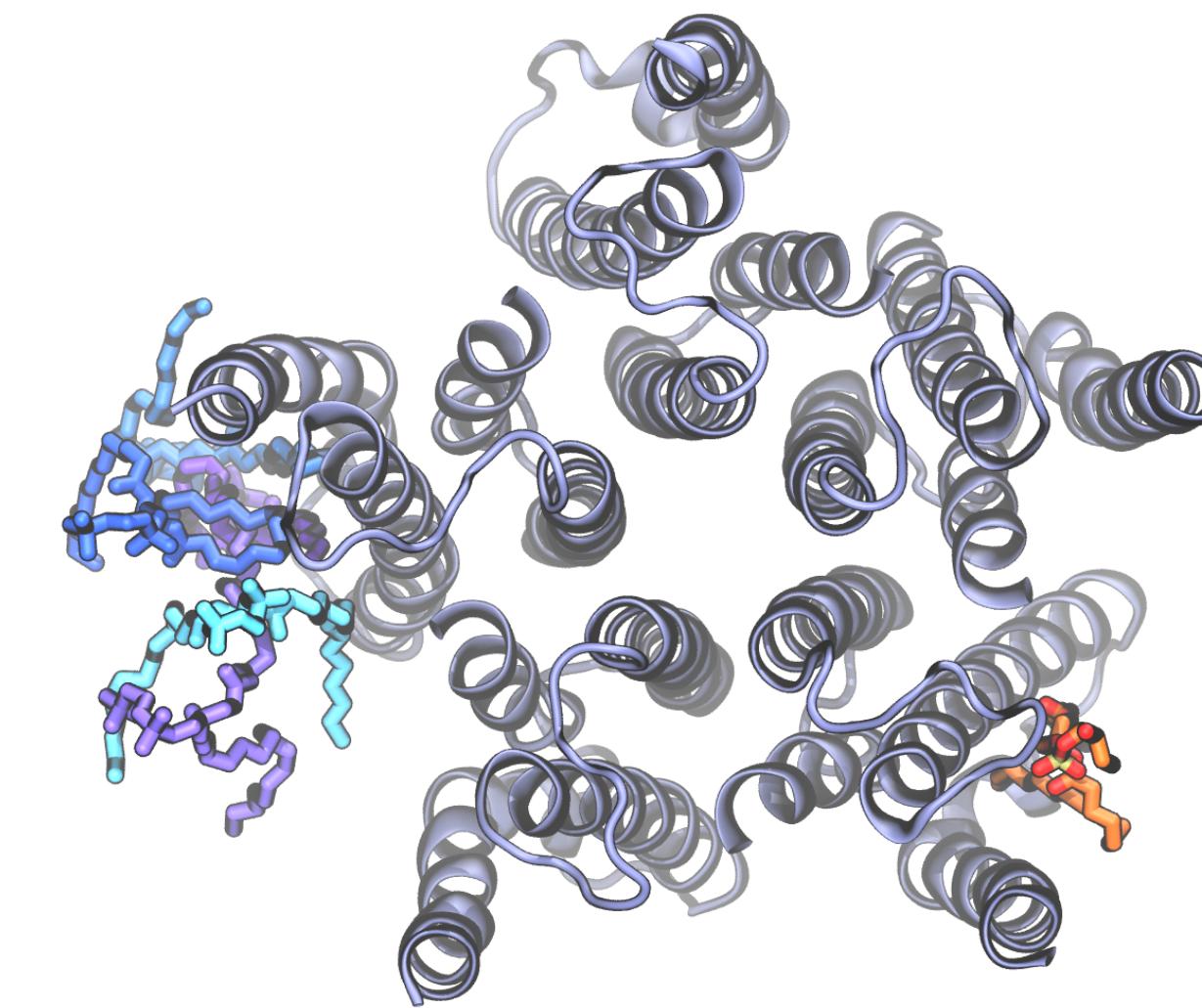


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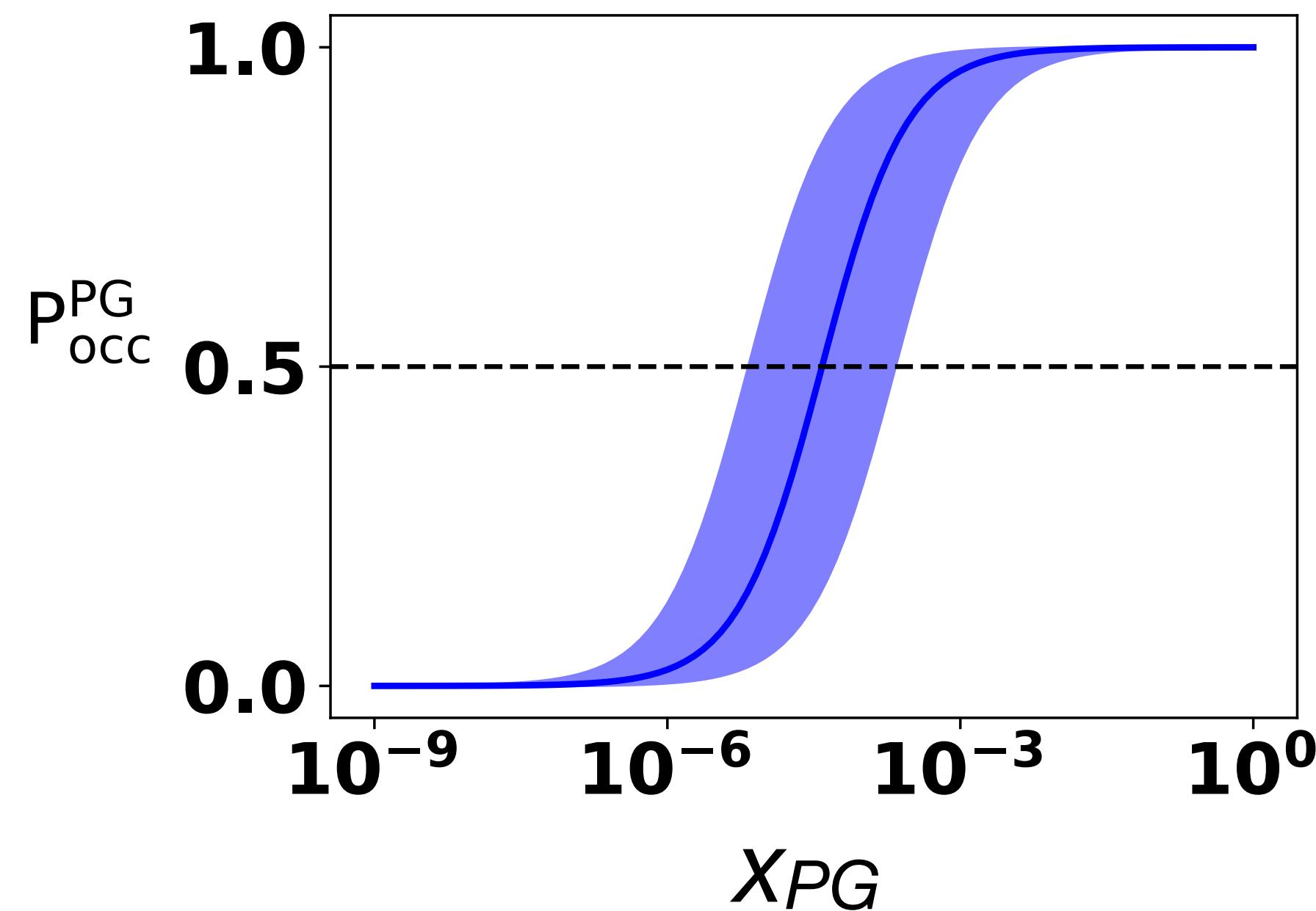


- Error is too high

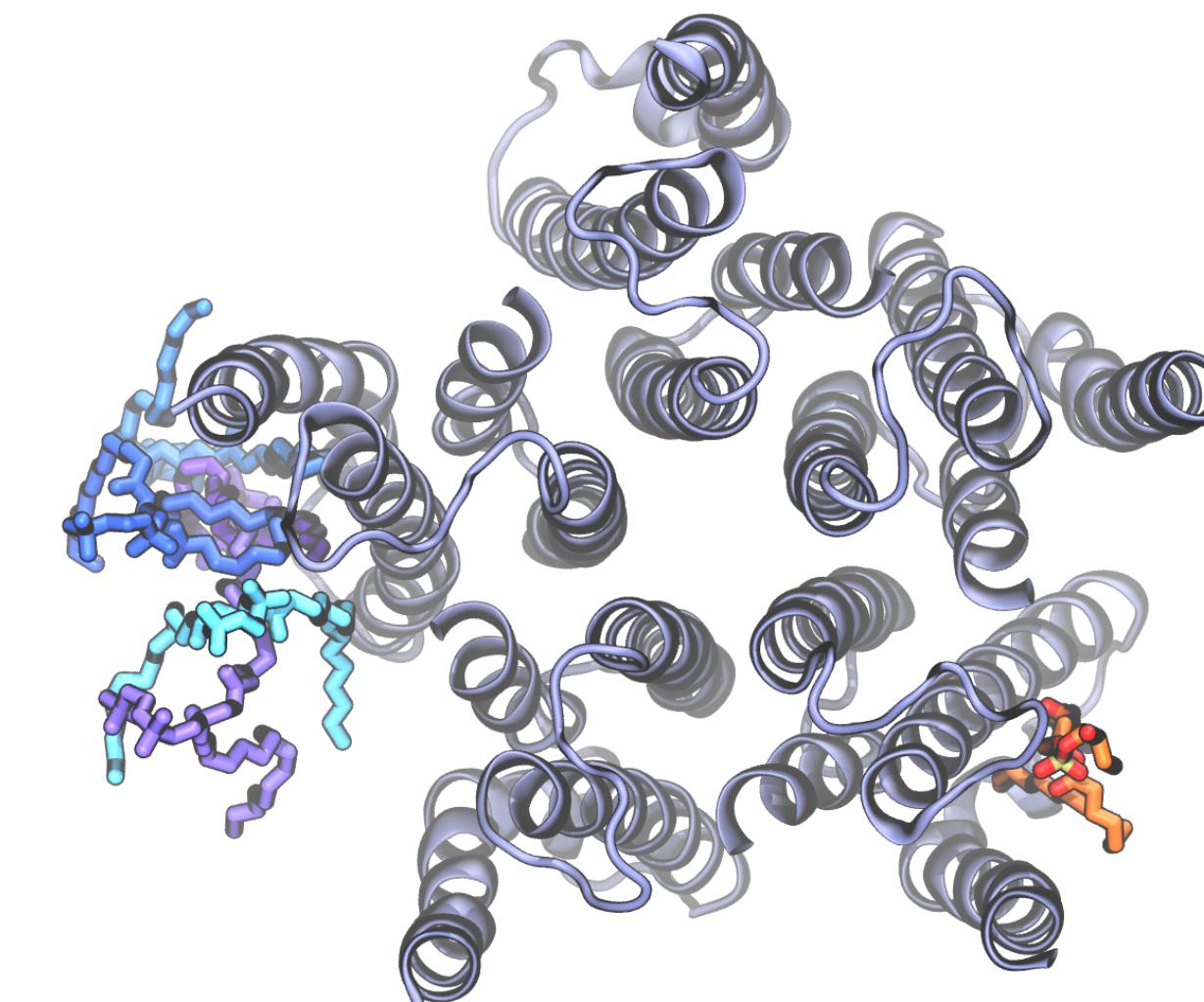


# Binary mixtures: mutating between ligand and solvent

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- Error is too high
- Relative calculations aren't good enough



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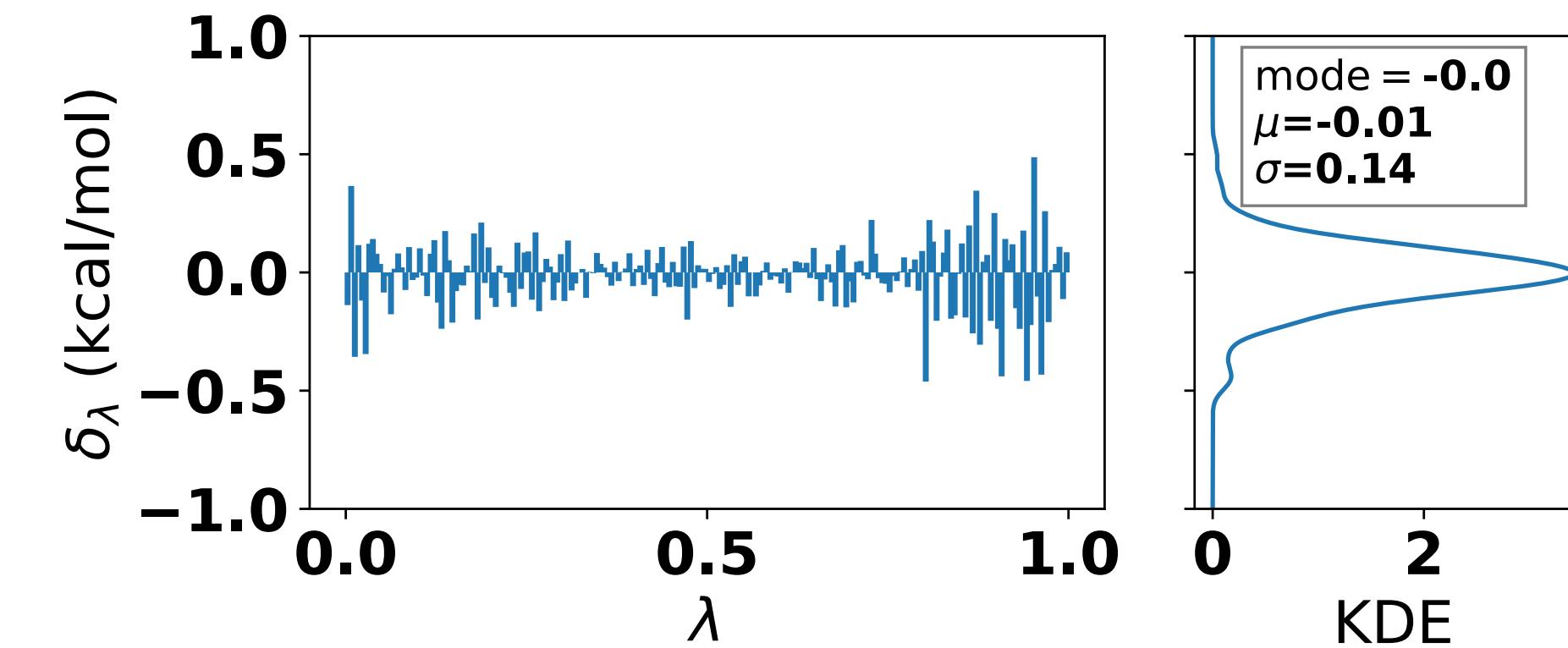
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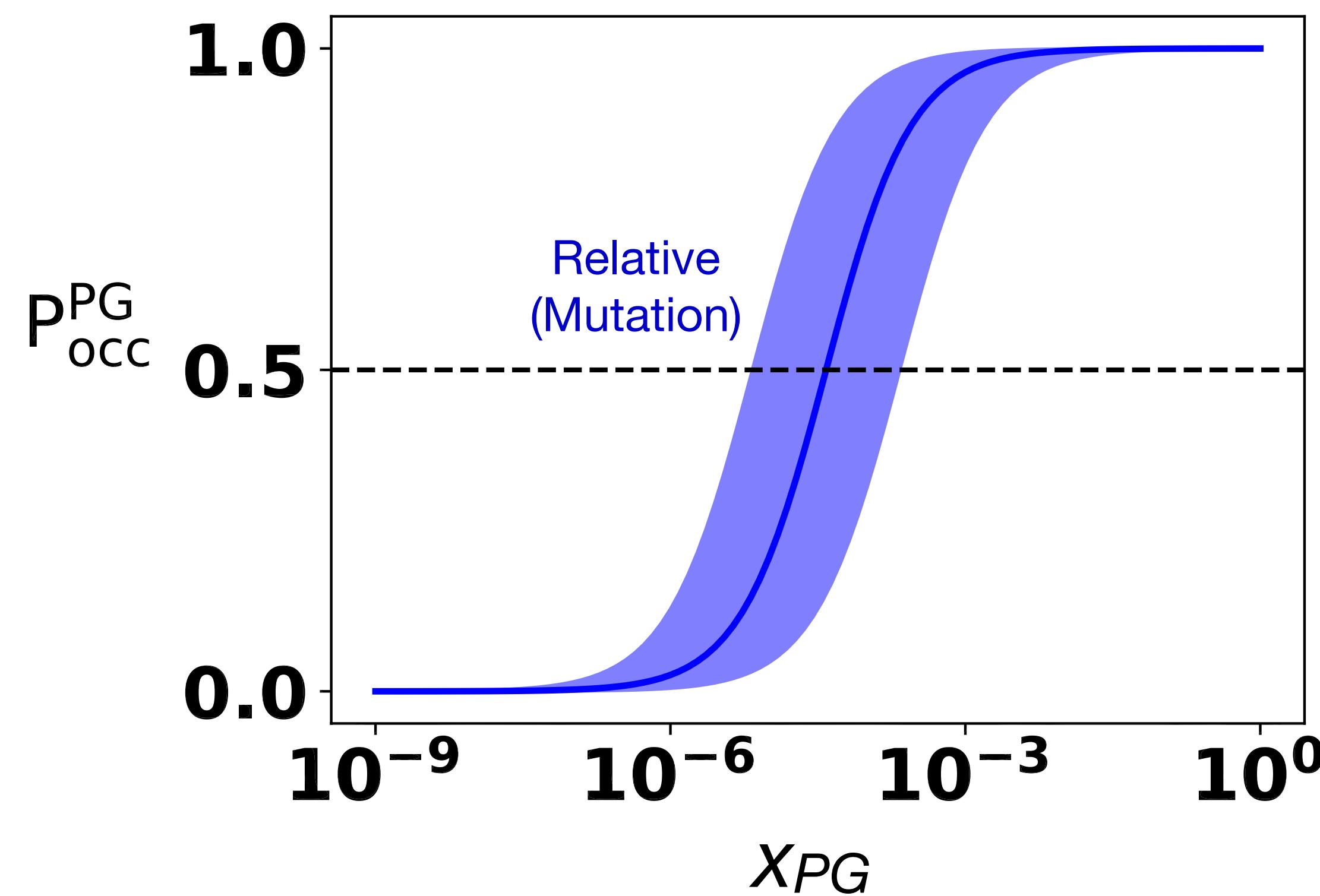
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**Poster B472**  
**Sunday**



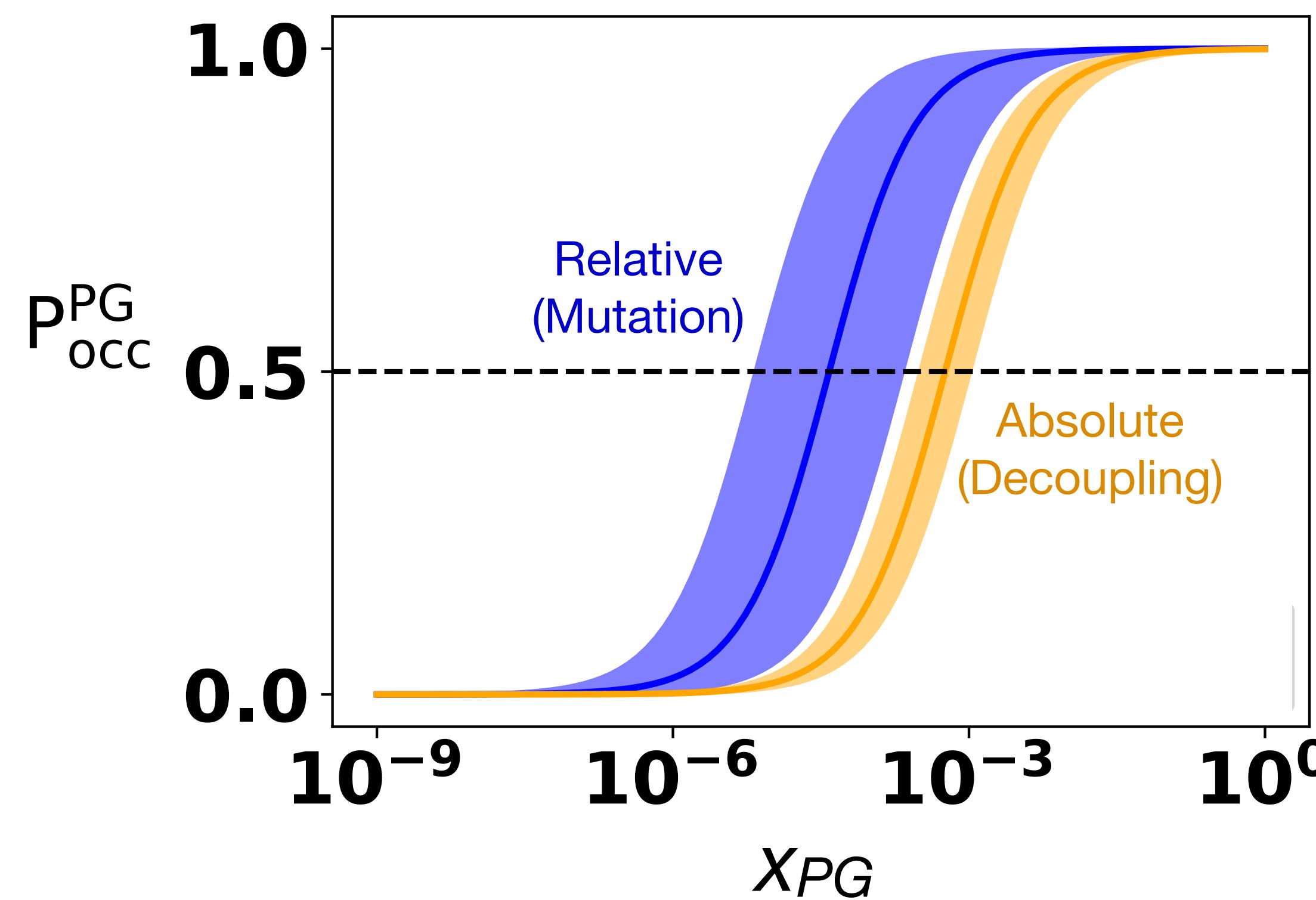
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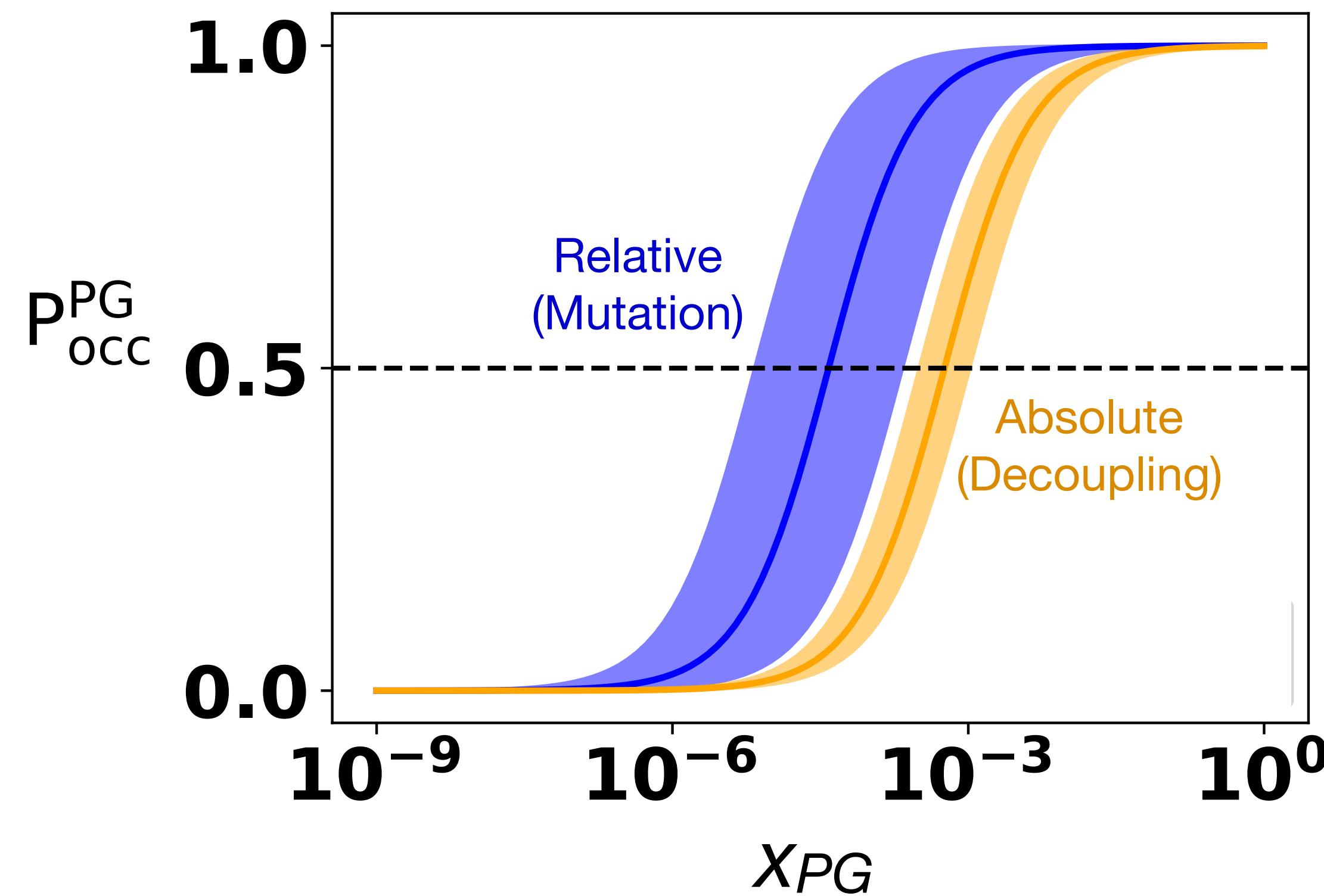
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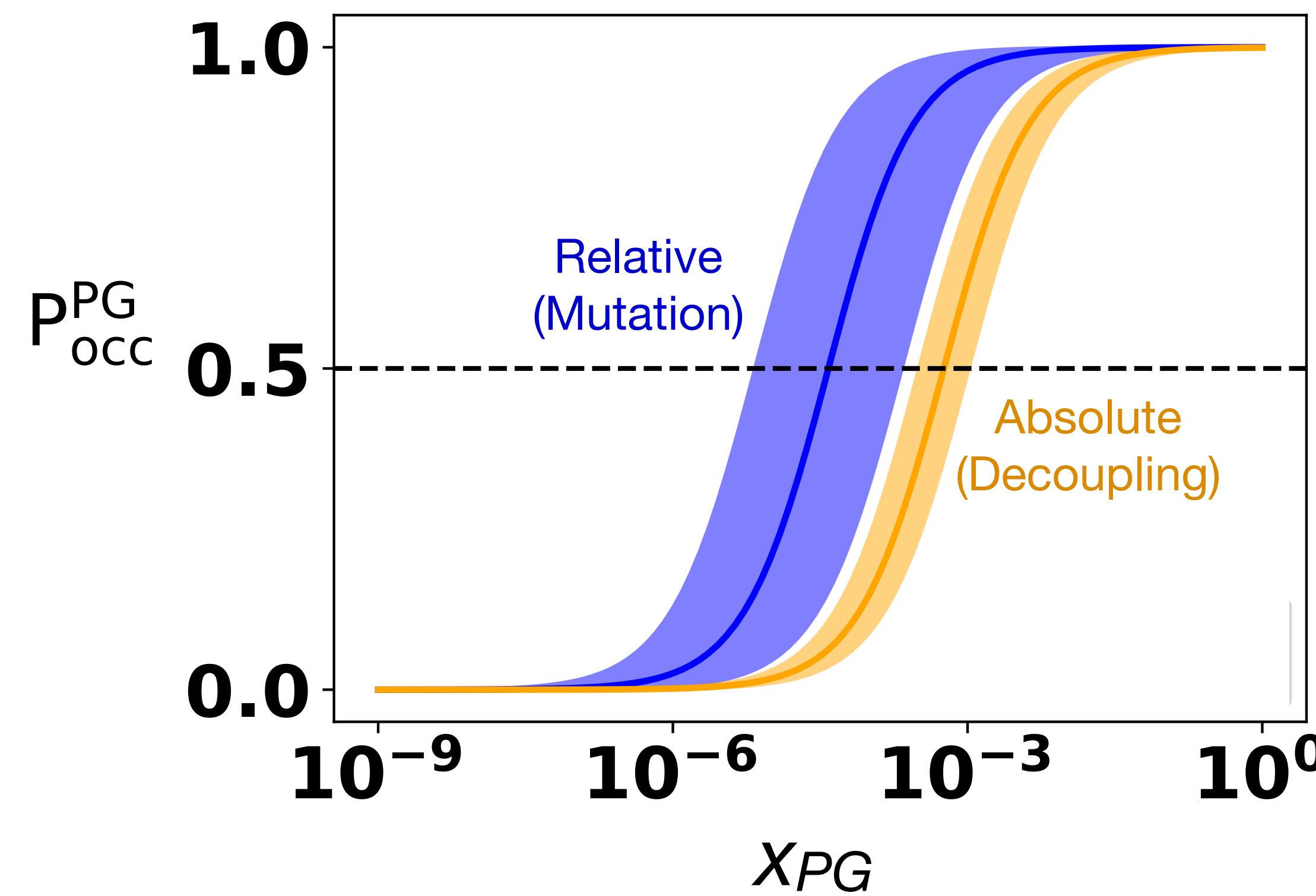


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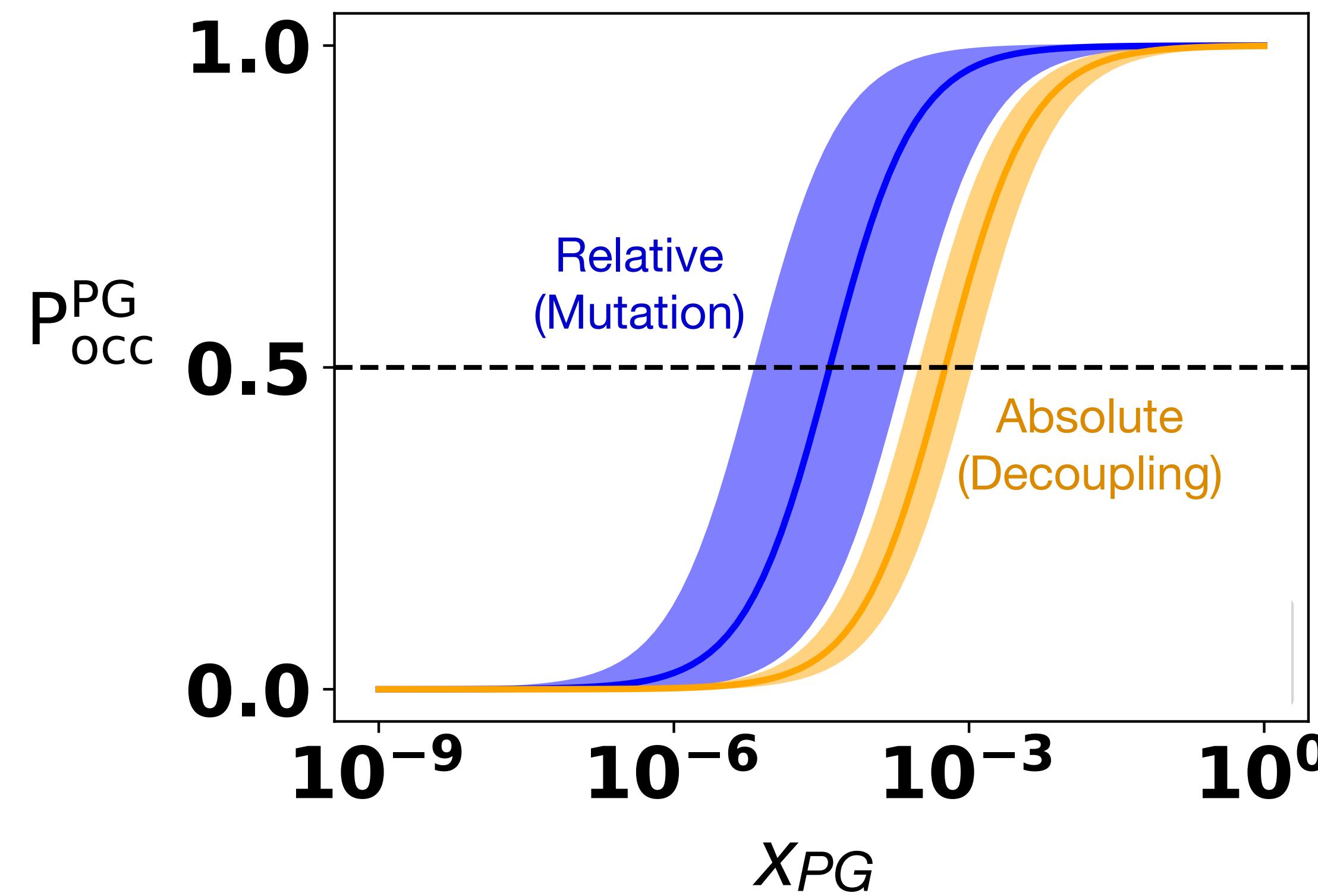
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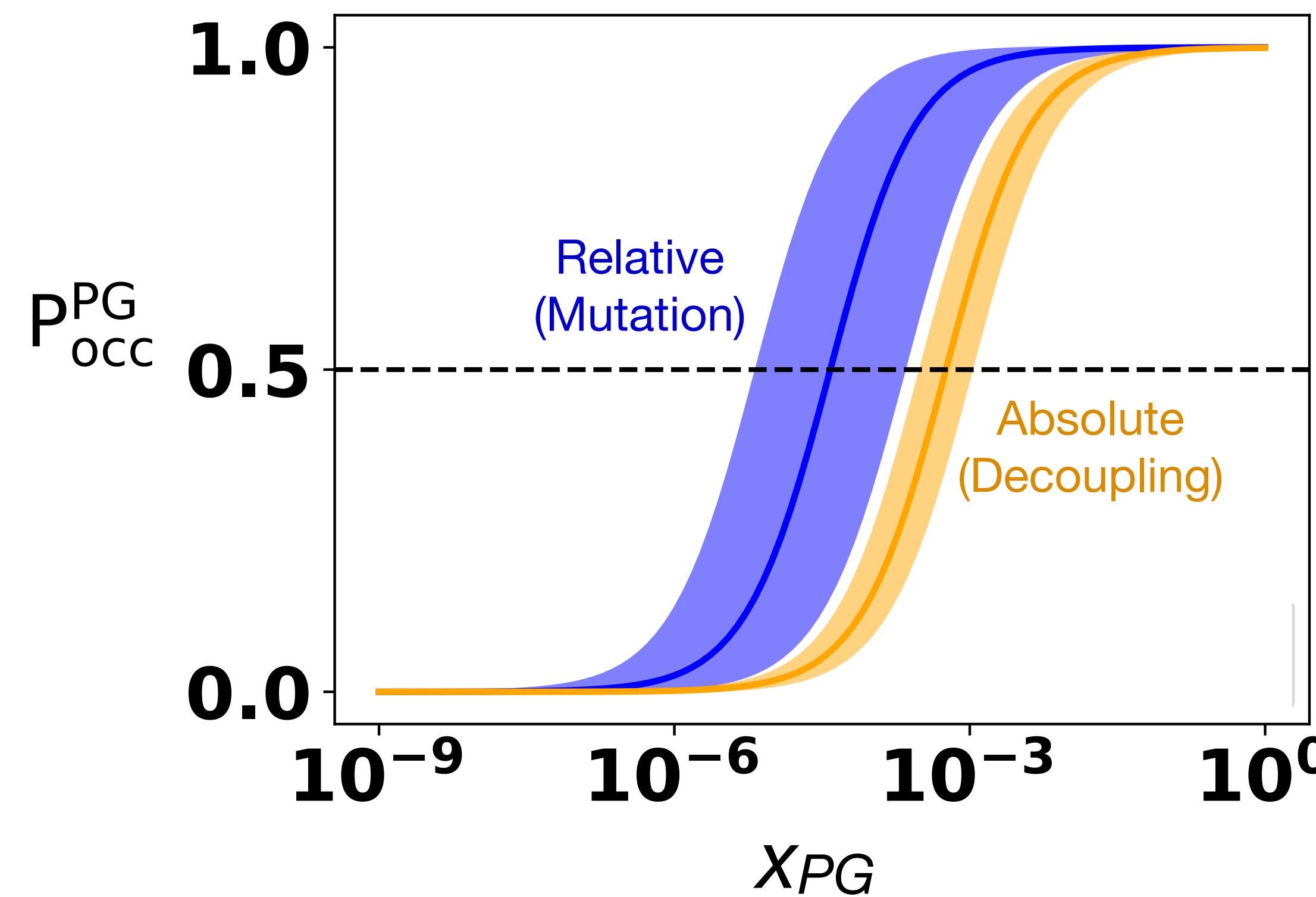
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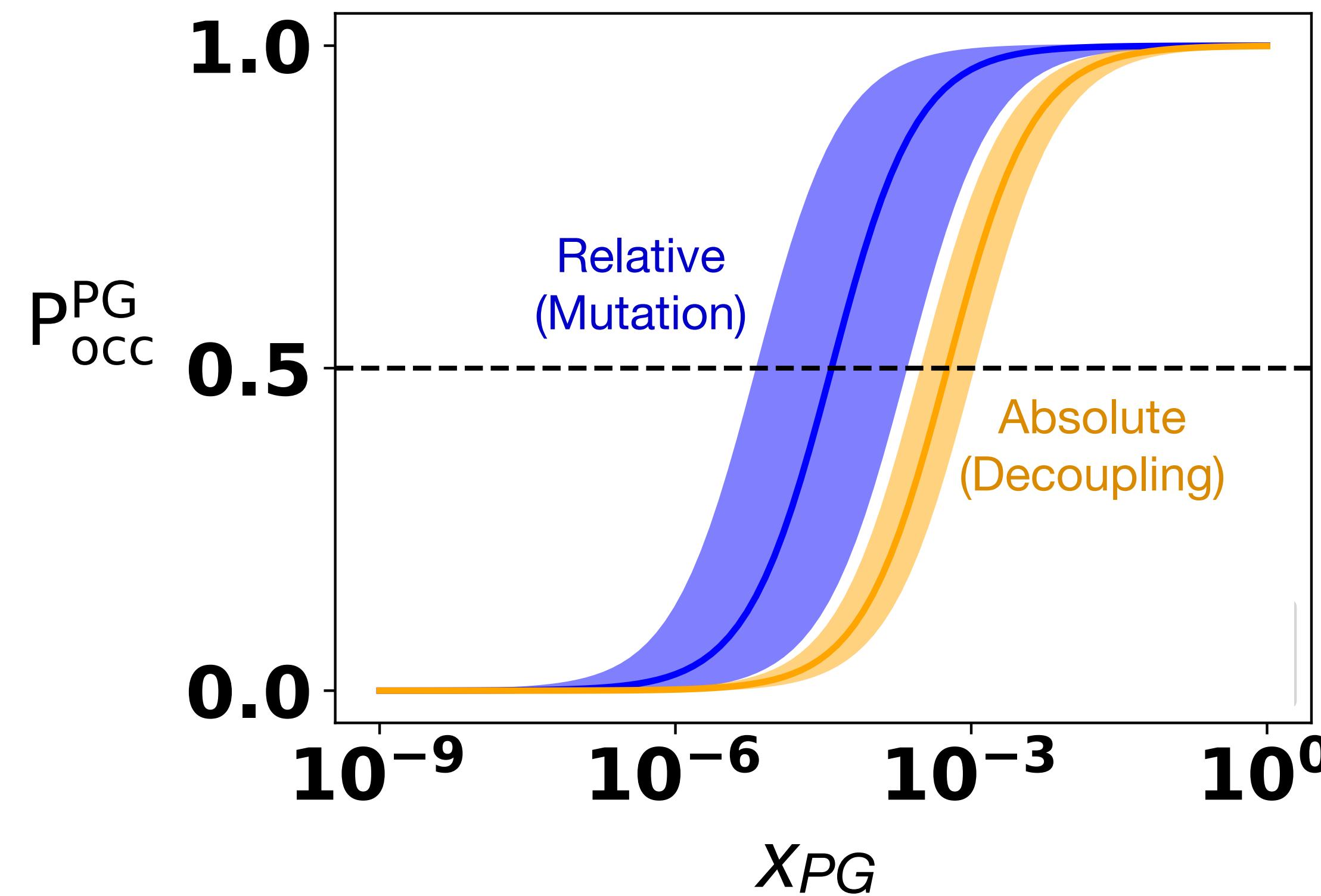
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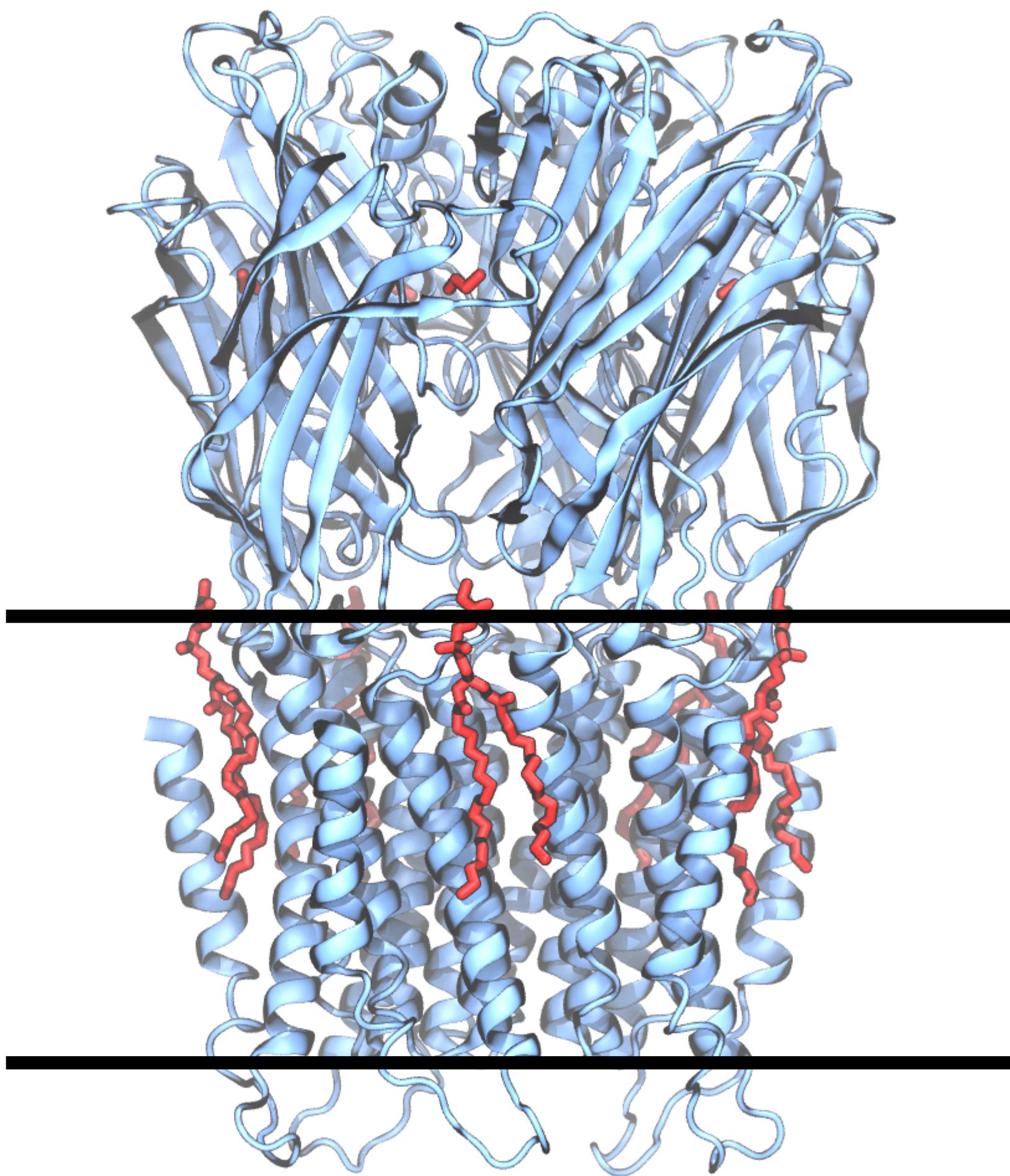
POPC *is* a solvent here:

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Does this hold for other lipids and structure(s)?

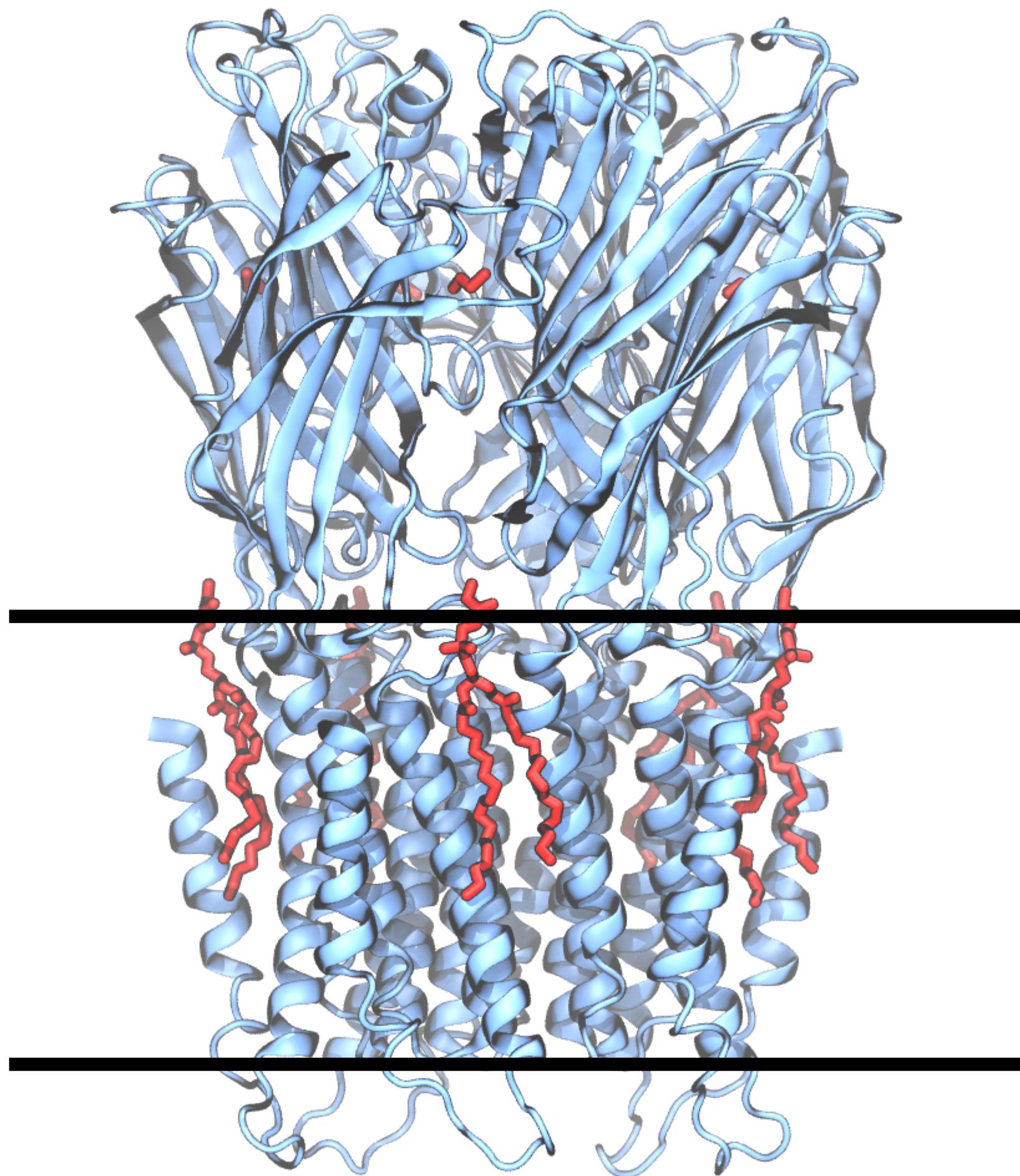


# Summary



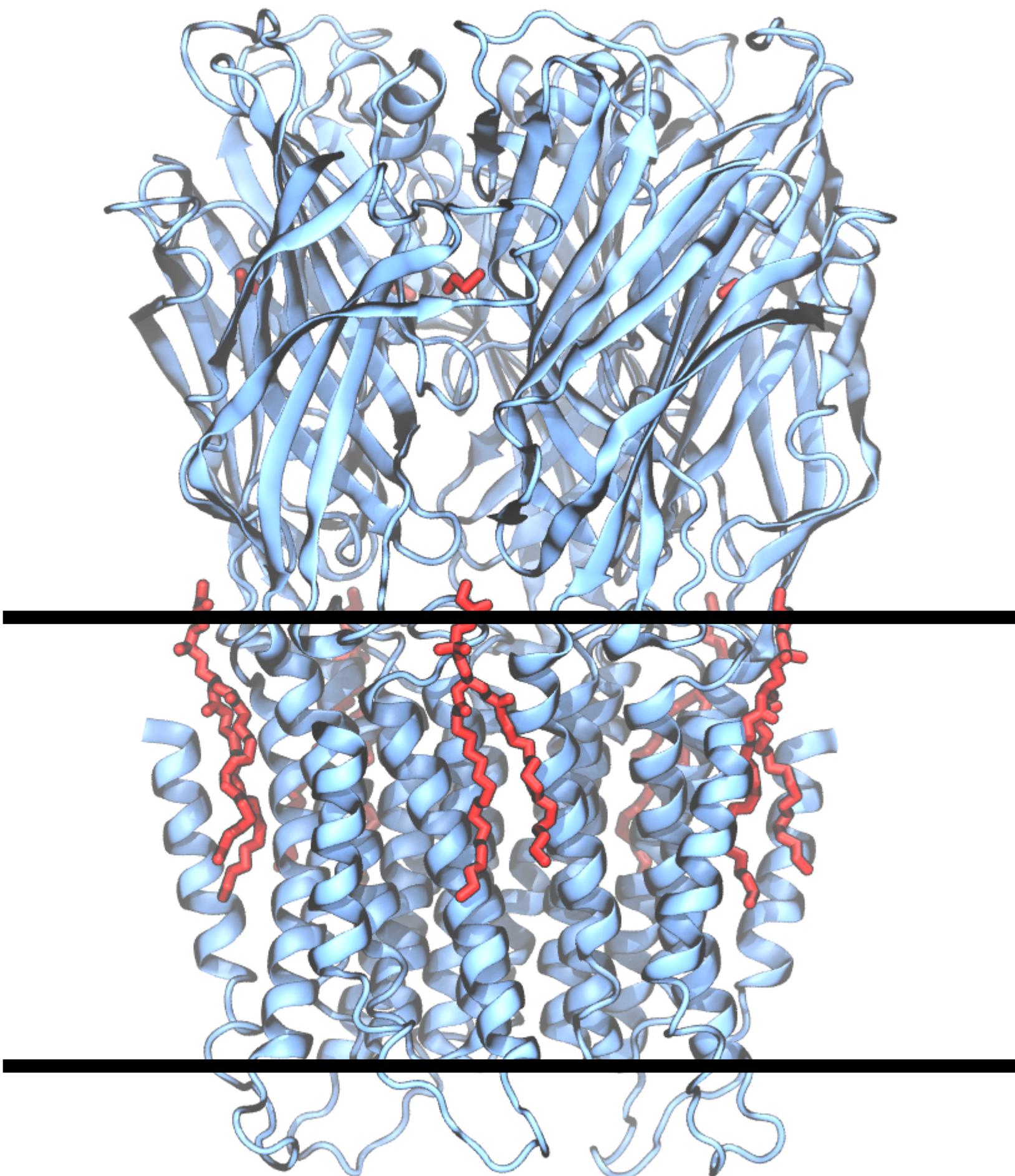
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- Are these lipids **specifically** bound?



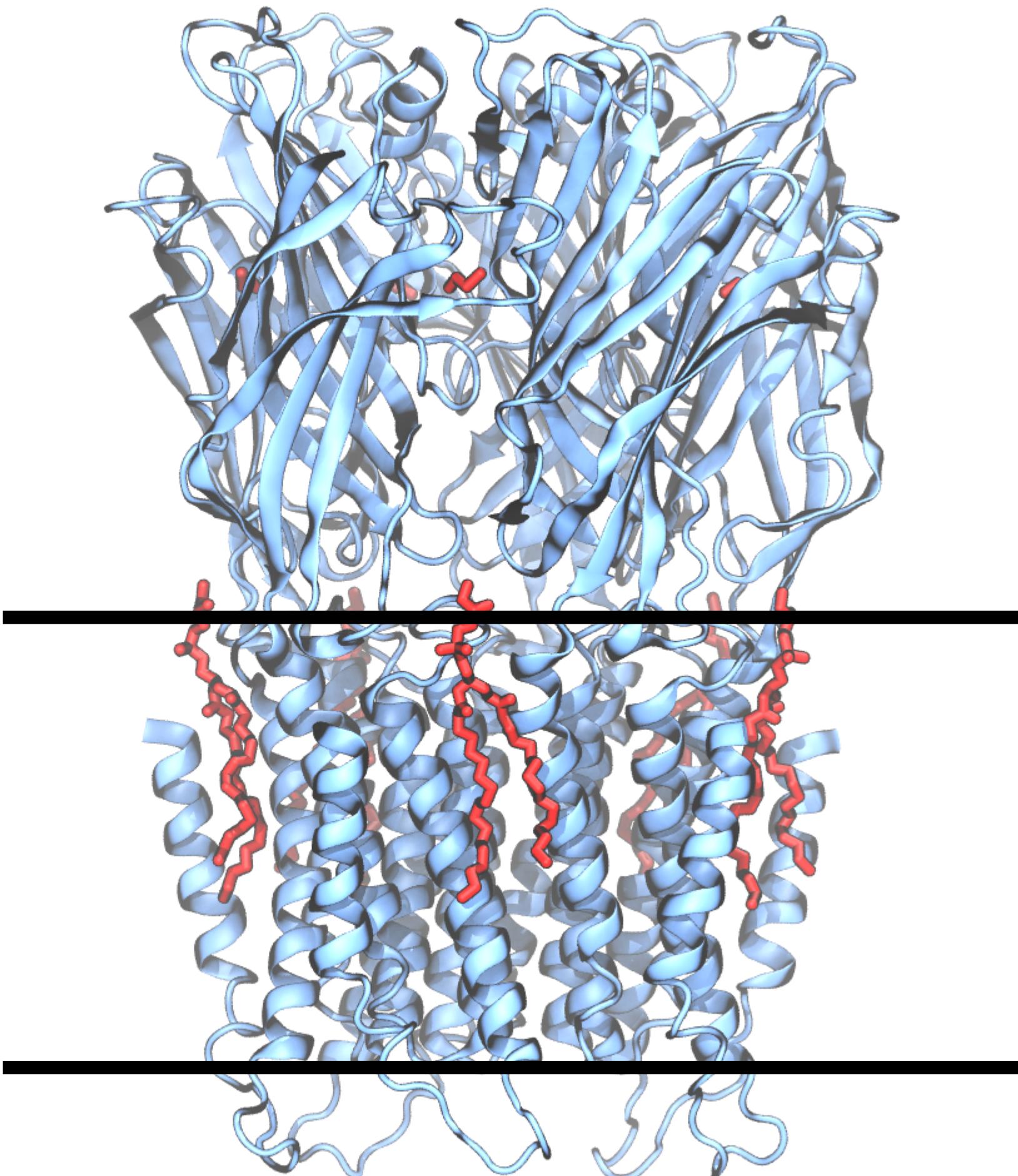
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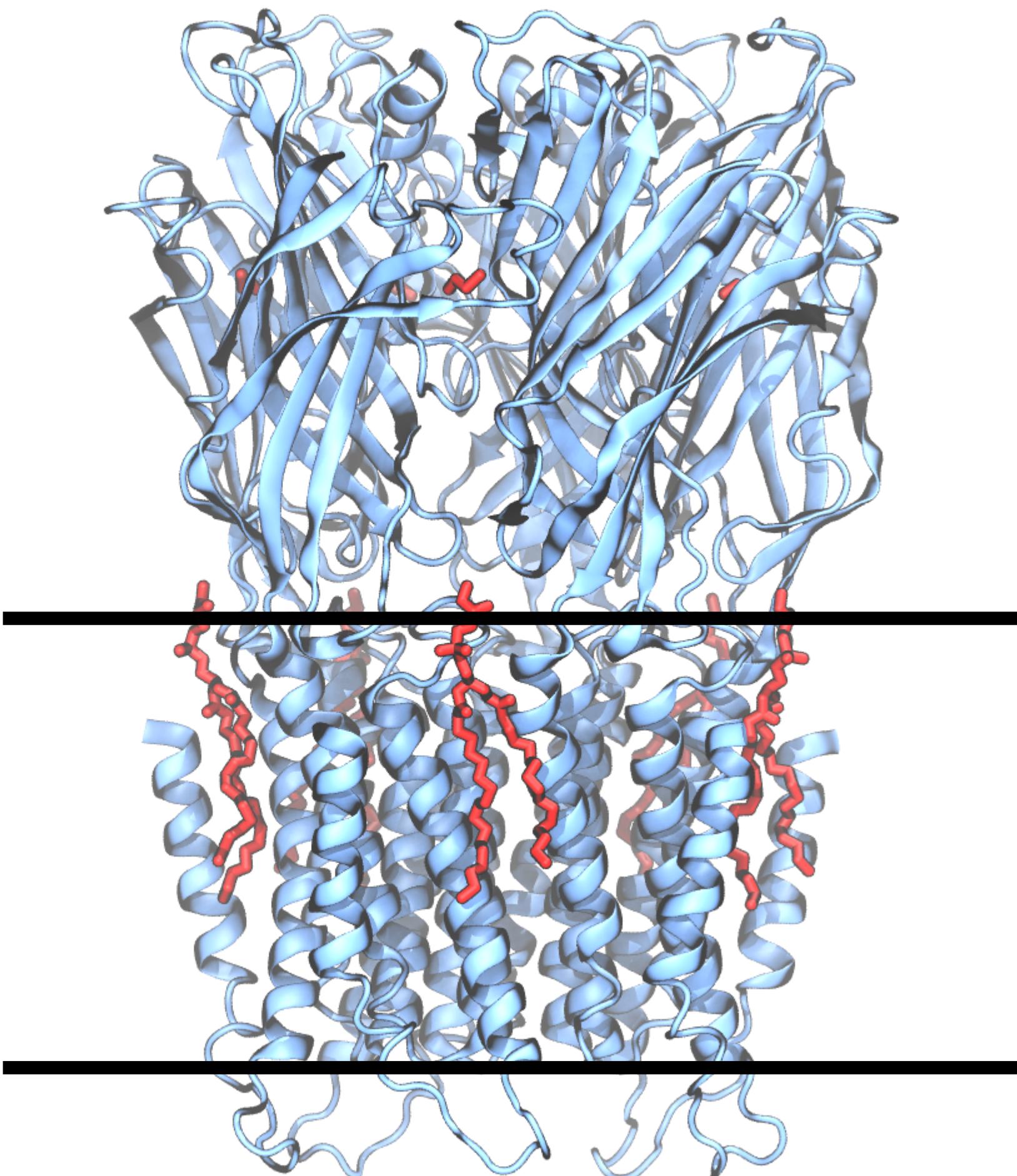
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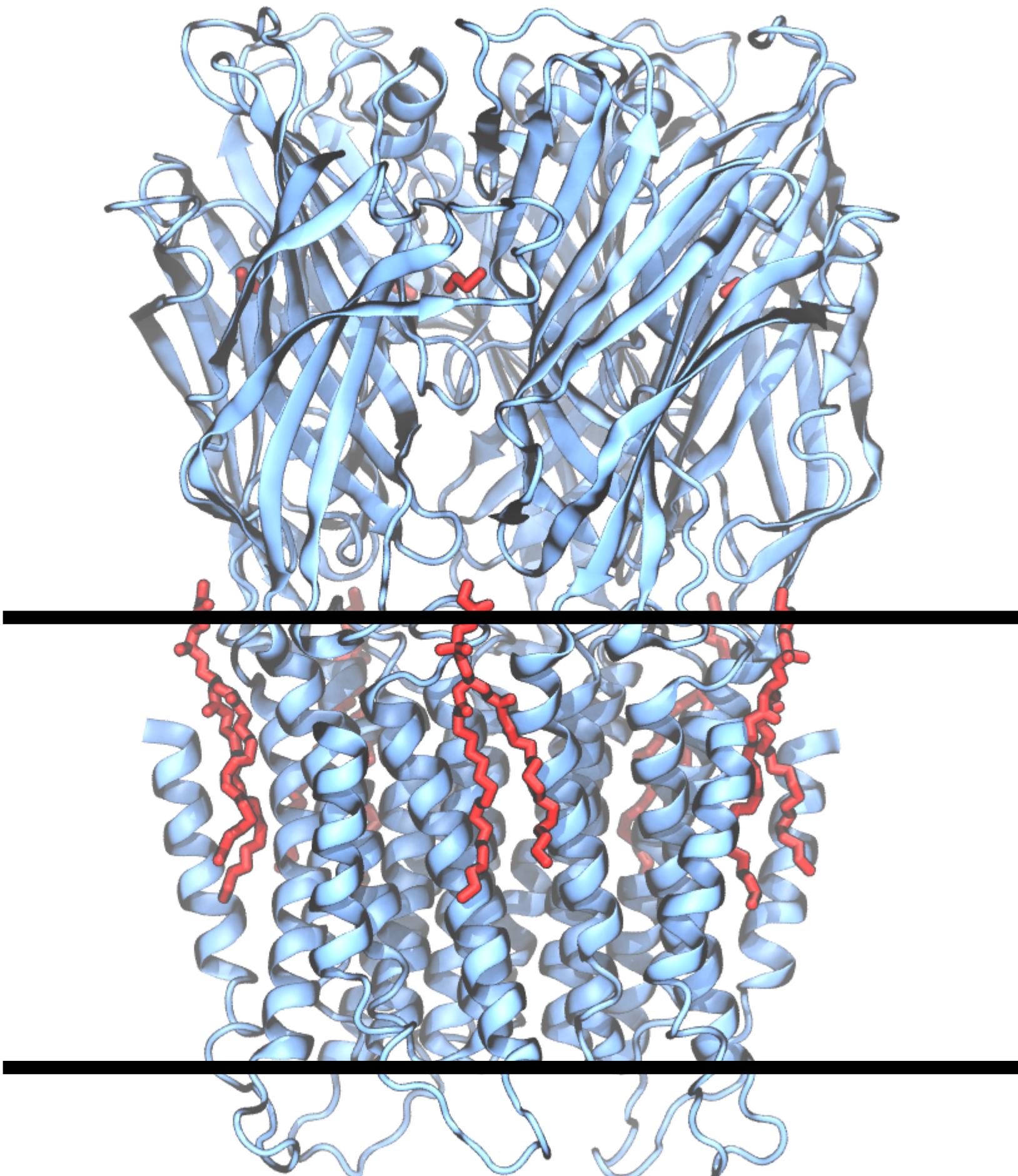
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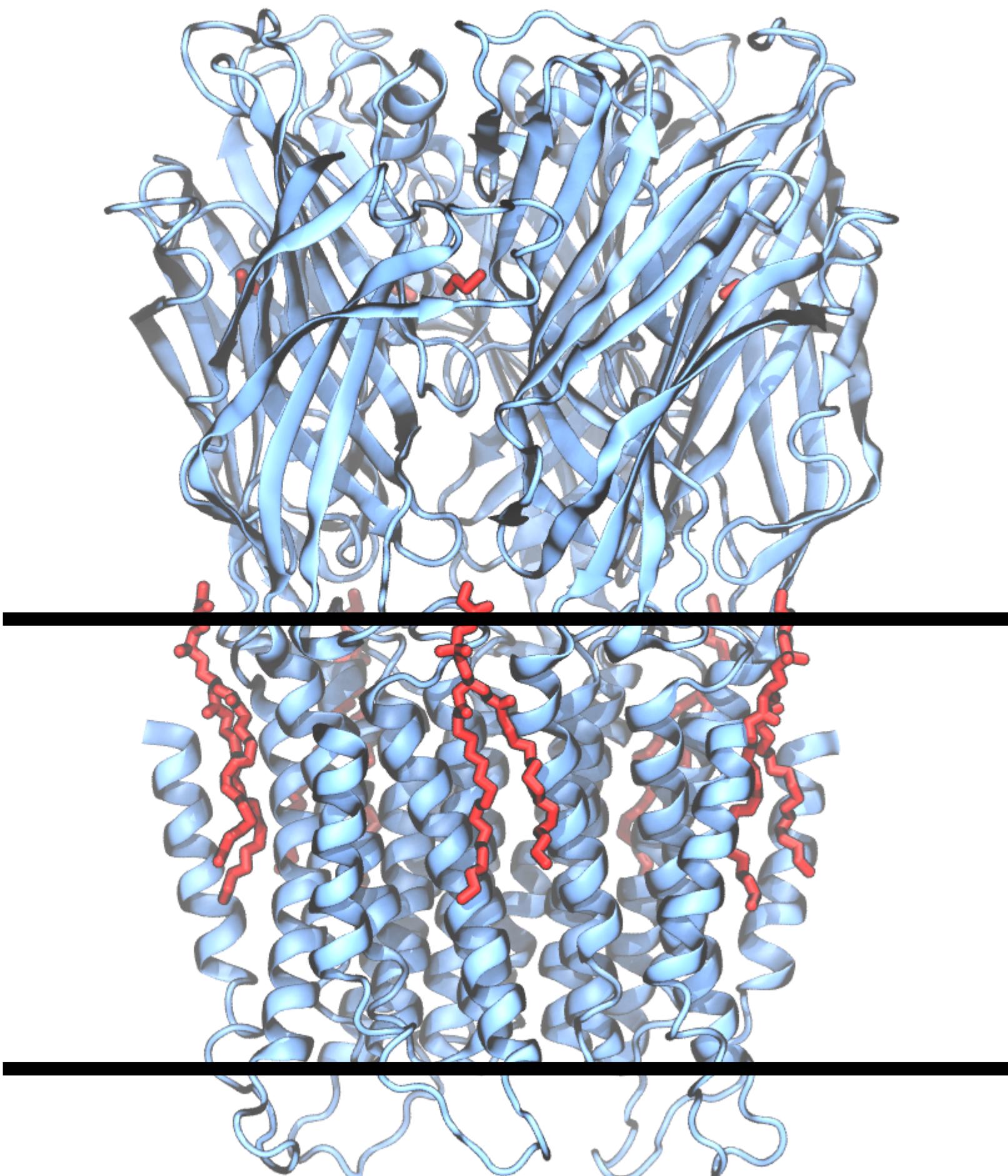
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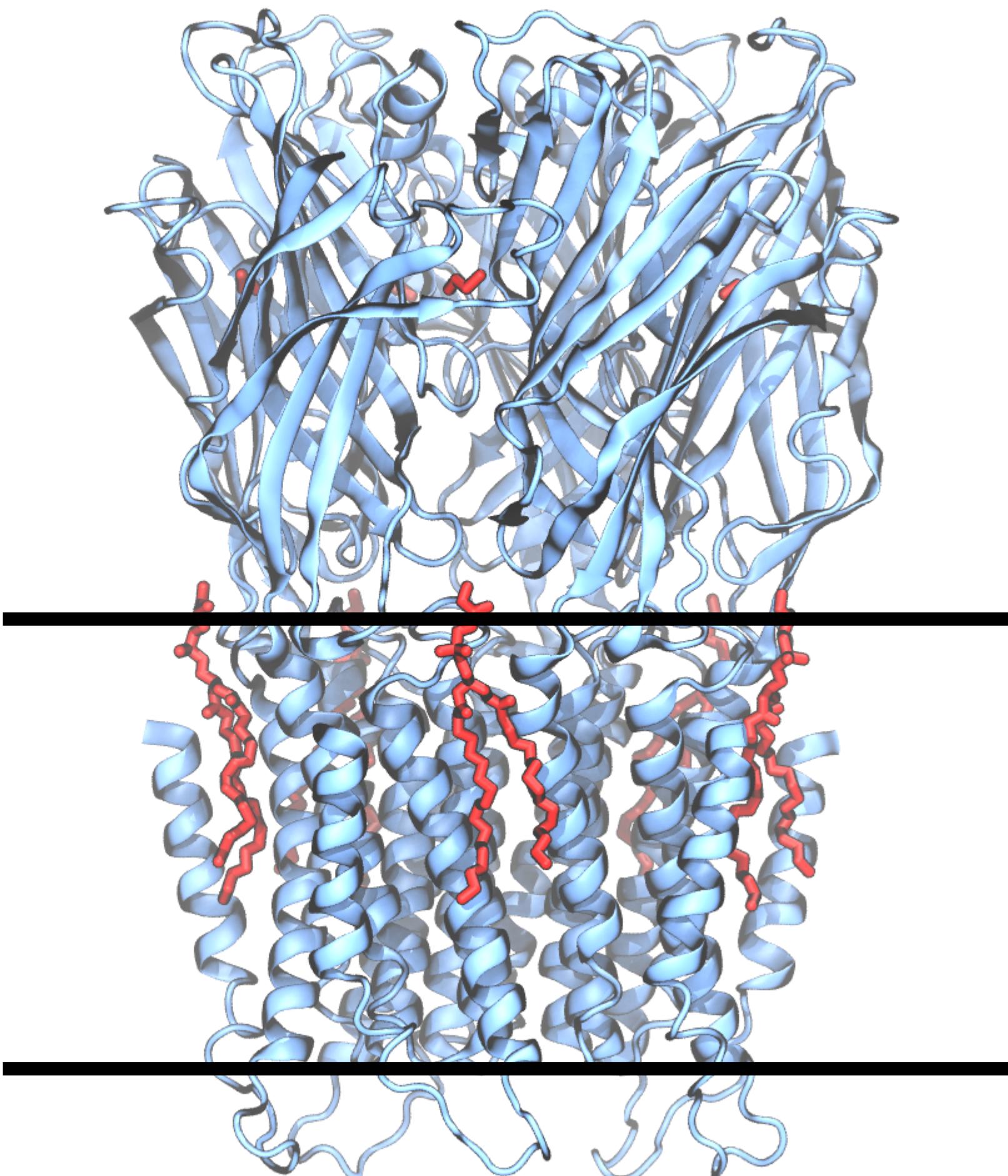
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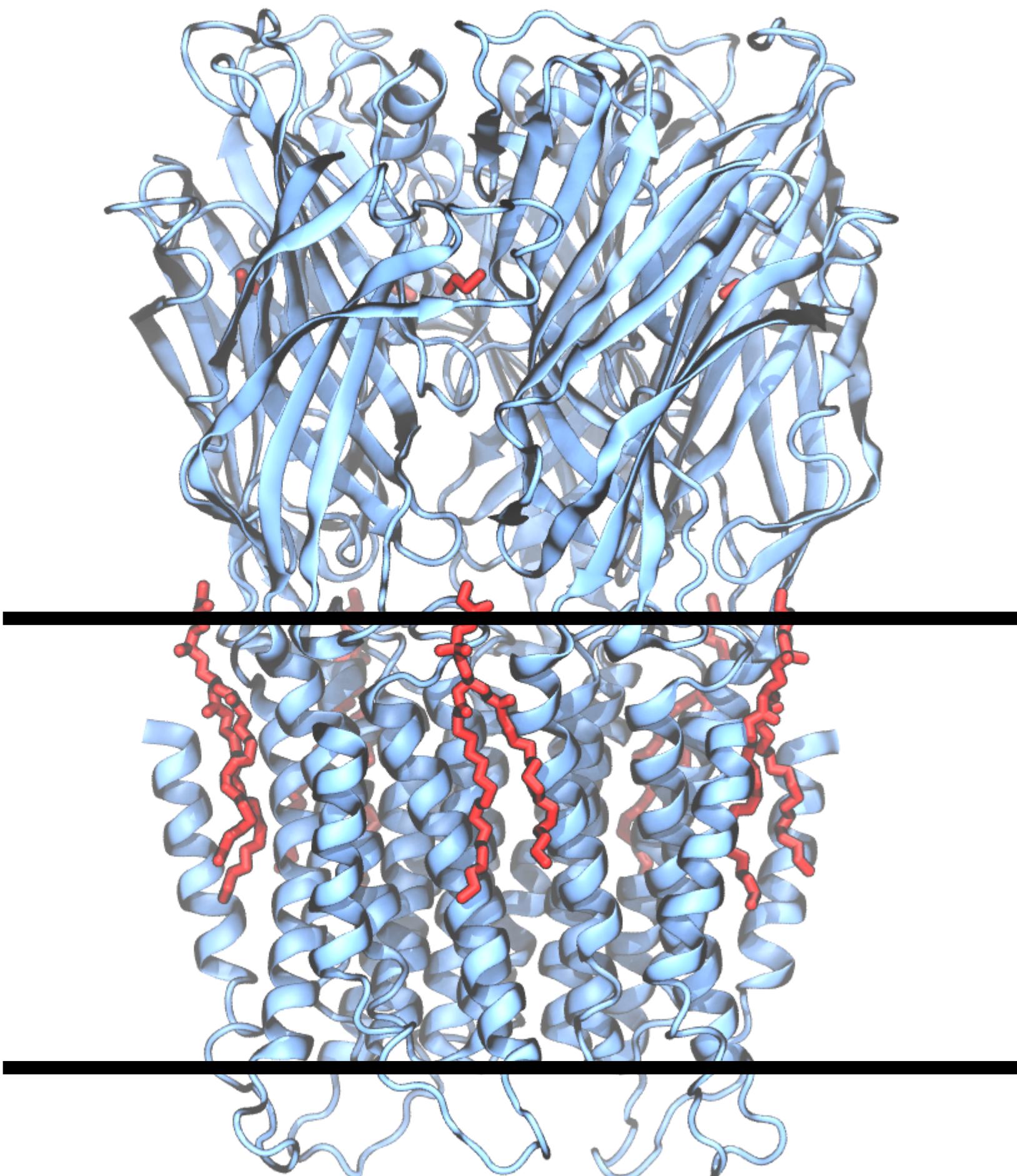
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- Do the answers change with **membrane composition**?

Only when PE and PG compete



# Where can I learn more?

Open Channel  
Structure of ELIC



nature  
communications

SAFEP Tutorial

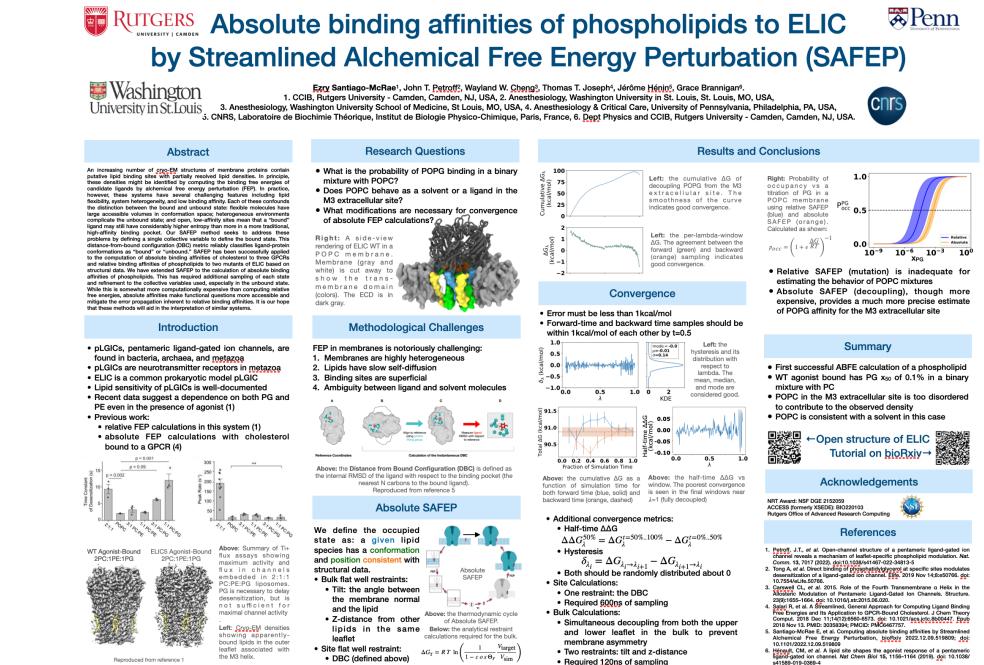


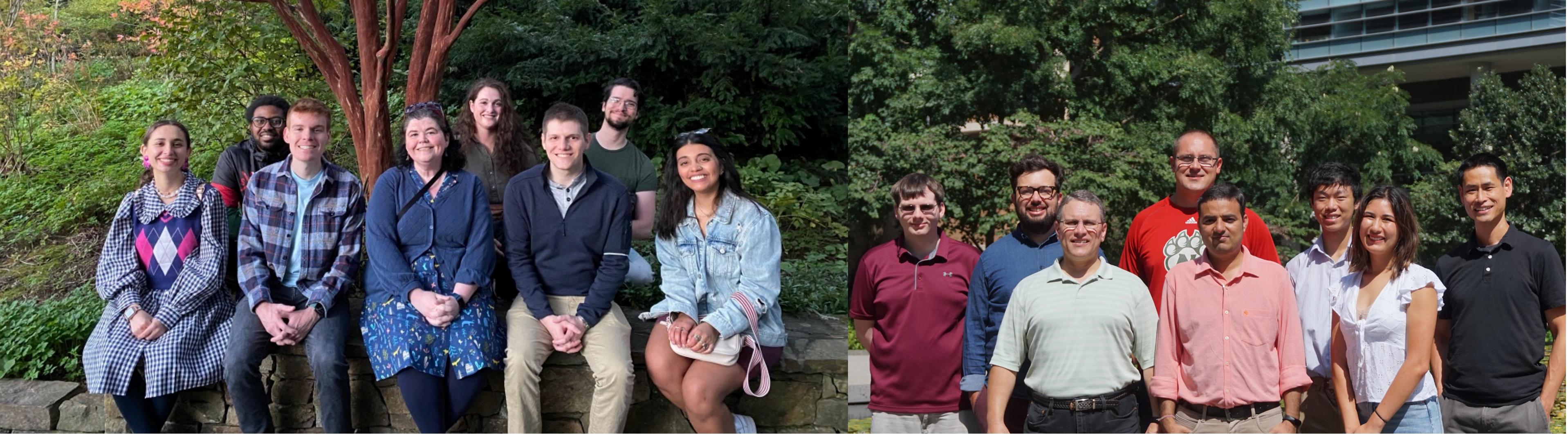
10.1101/2022.12.09.5

bioRxiv

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Poster B472  
Sunday





## Brannigan Lab



## Wayland Cheng's Lab



Jérôme Hénin

## Collaborators



Tom Joseph

