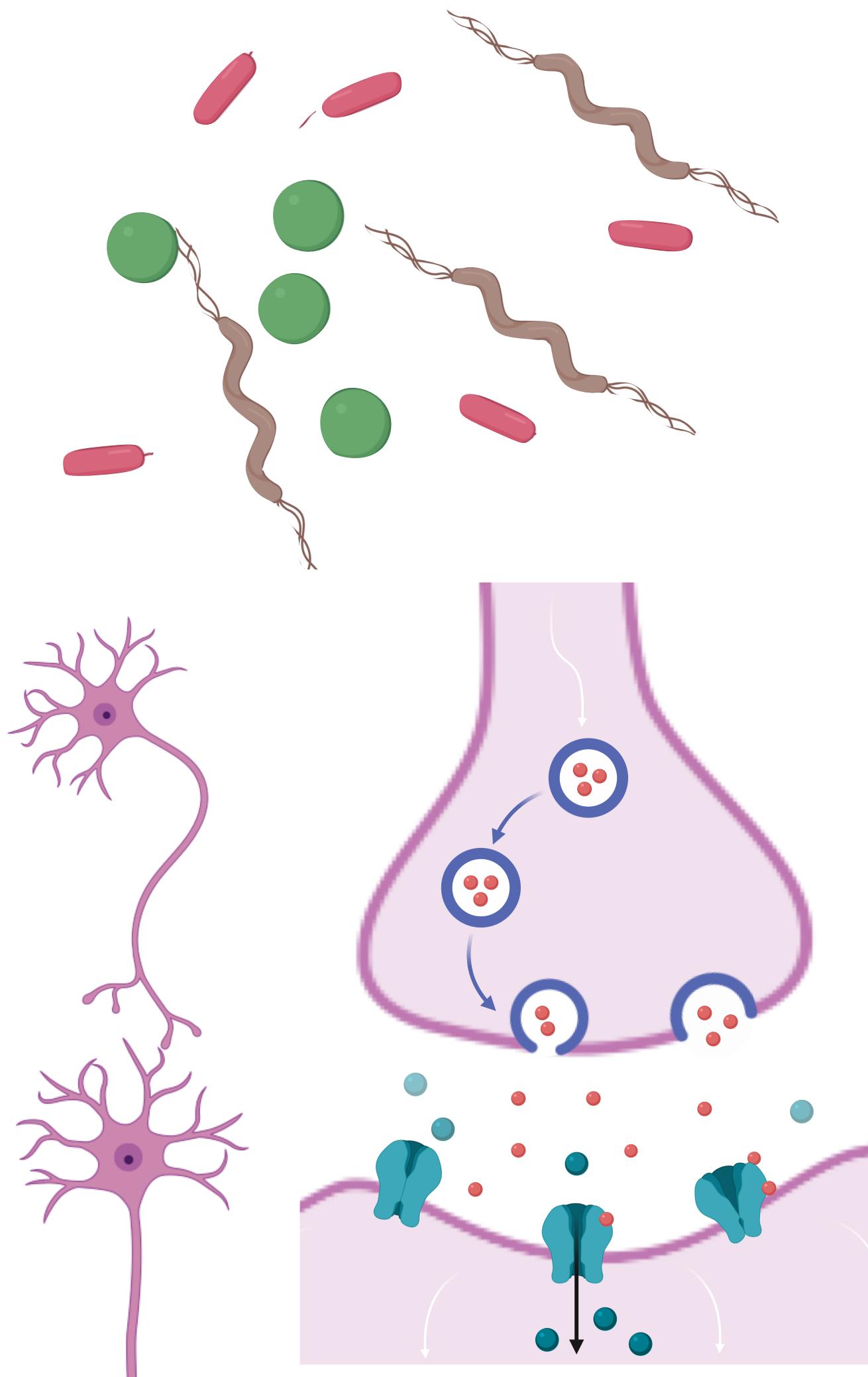


Lipids as Allosteric Modulators of a Pentameric Ligand-Gated Ion Channel, ELIC

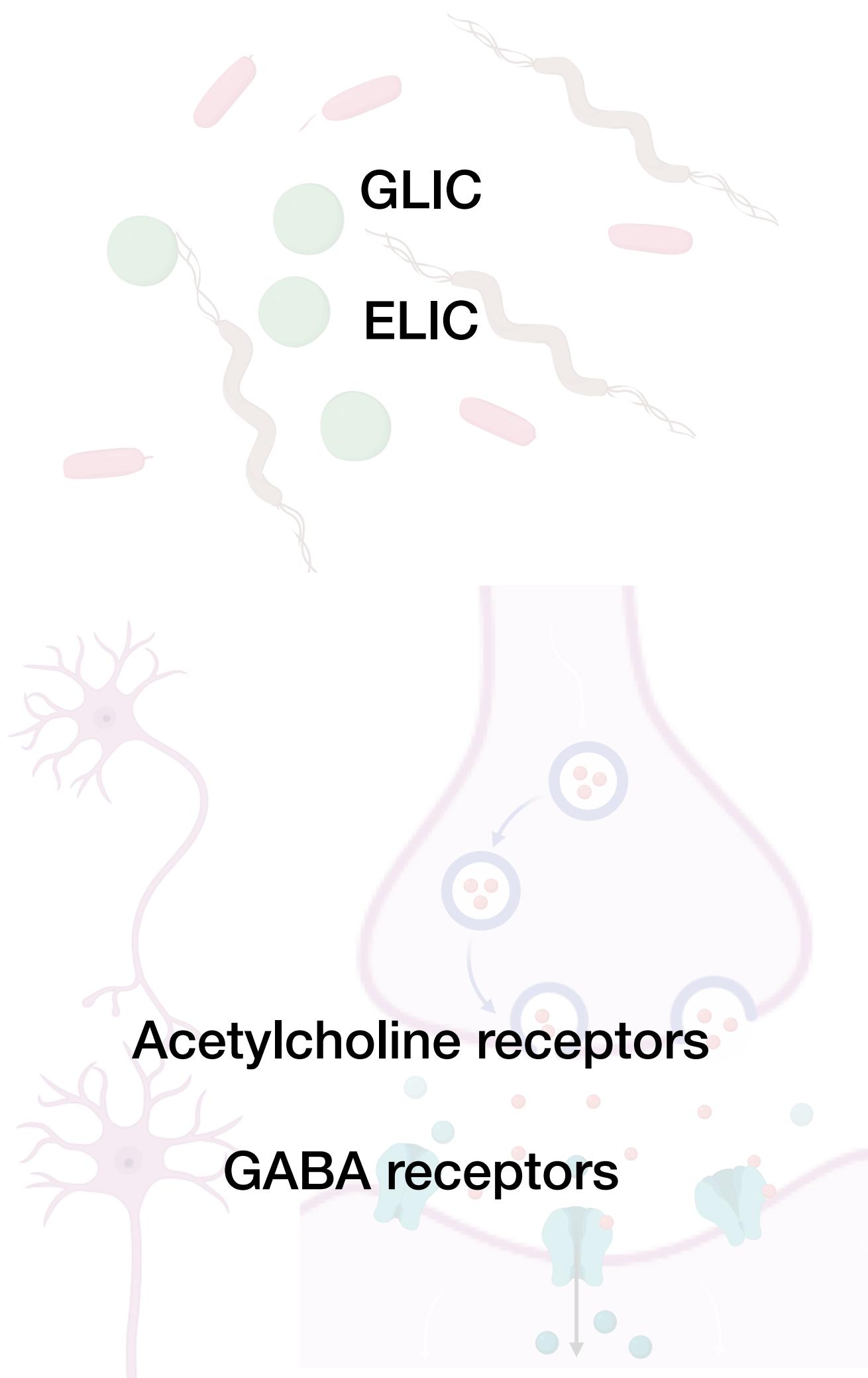
Insights from Free Energy Computation

Ezry Santiago-McRae

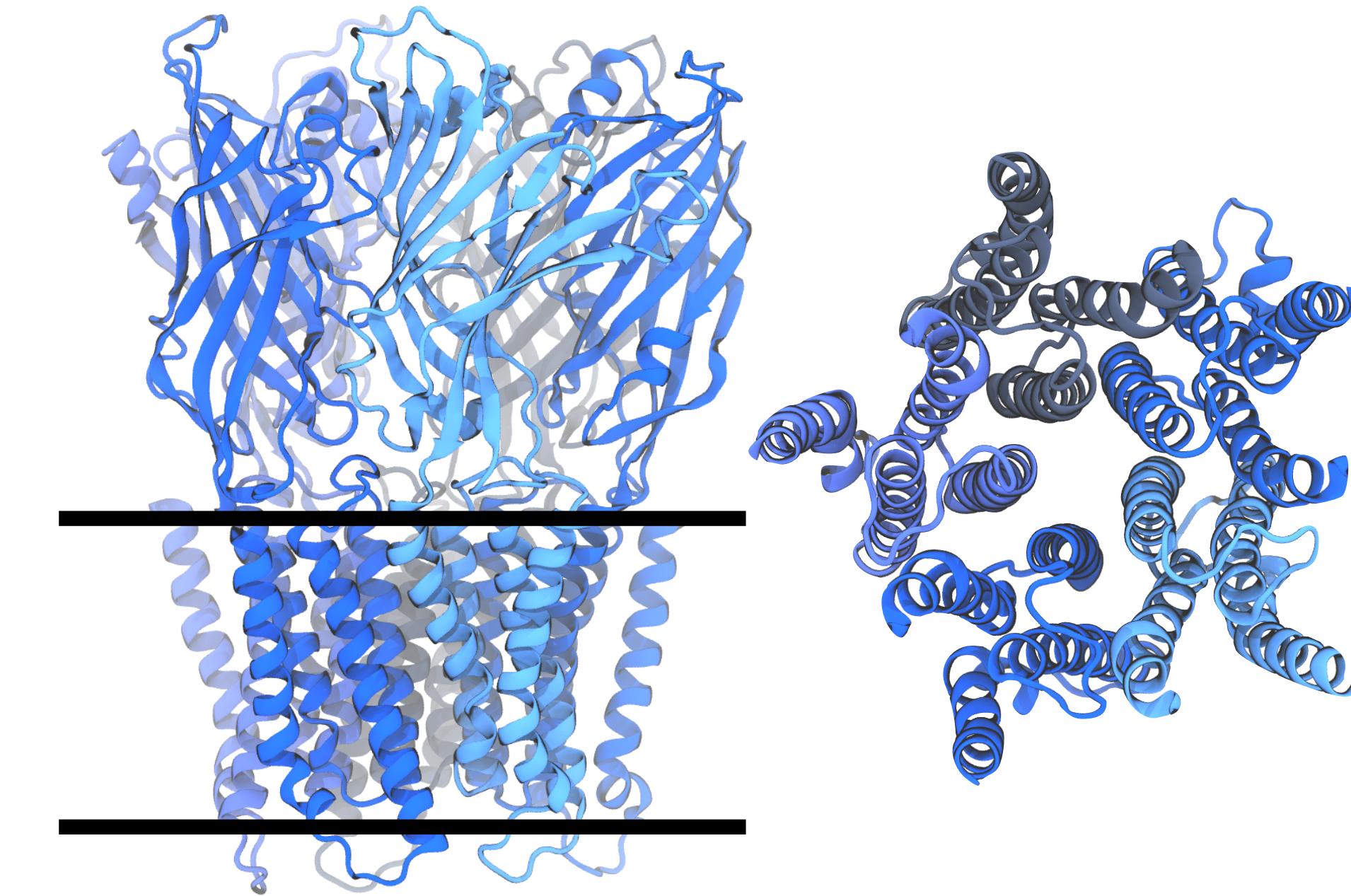
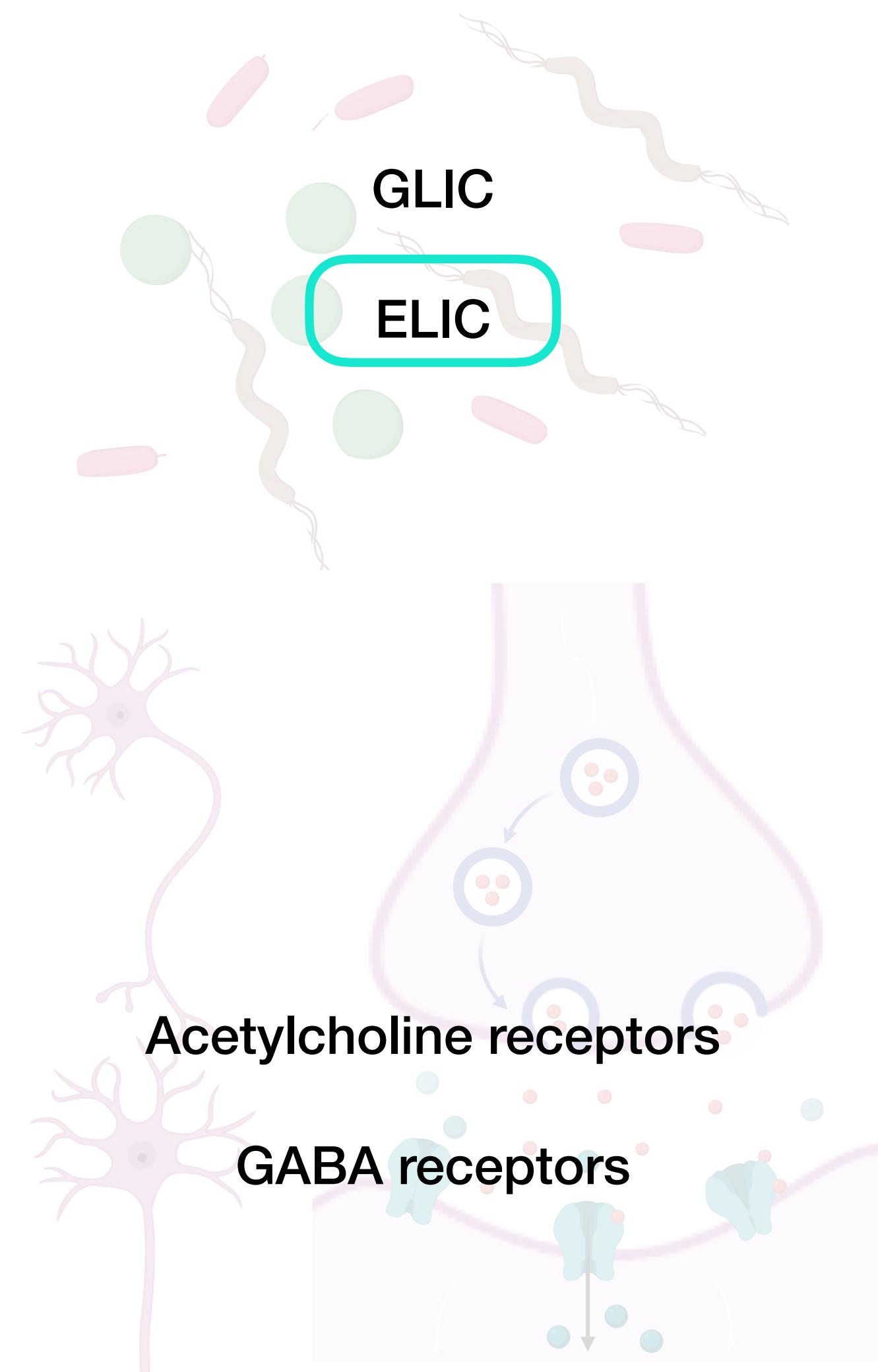
Pentameric Ligand-Gated Ion Channels (pLGICs) are chemosensors



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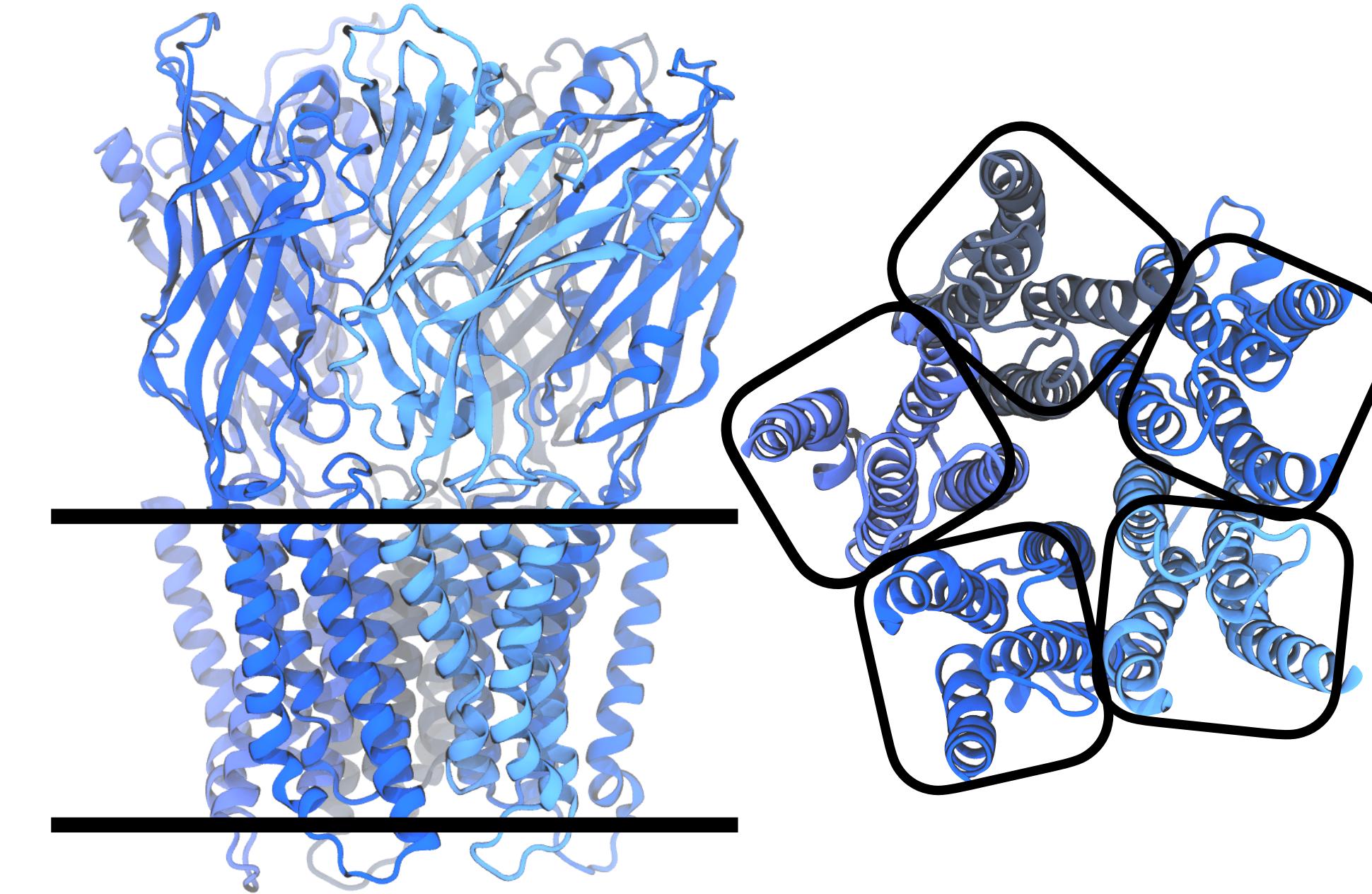
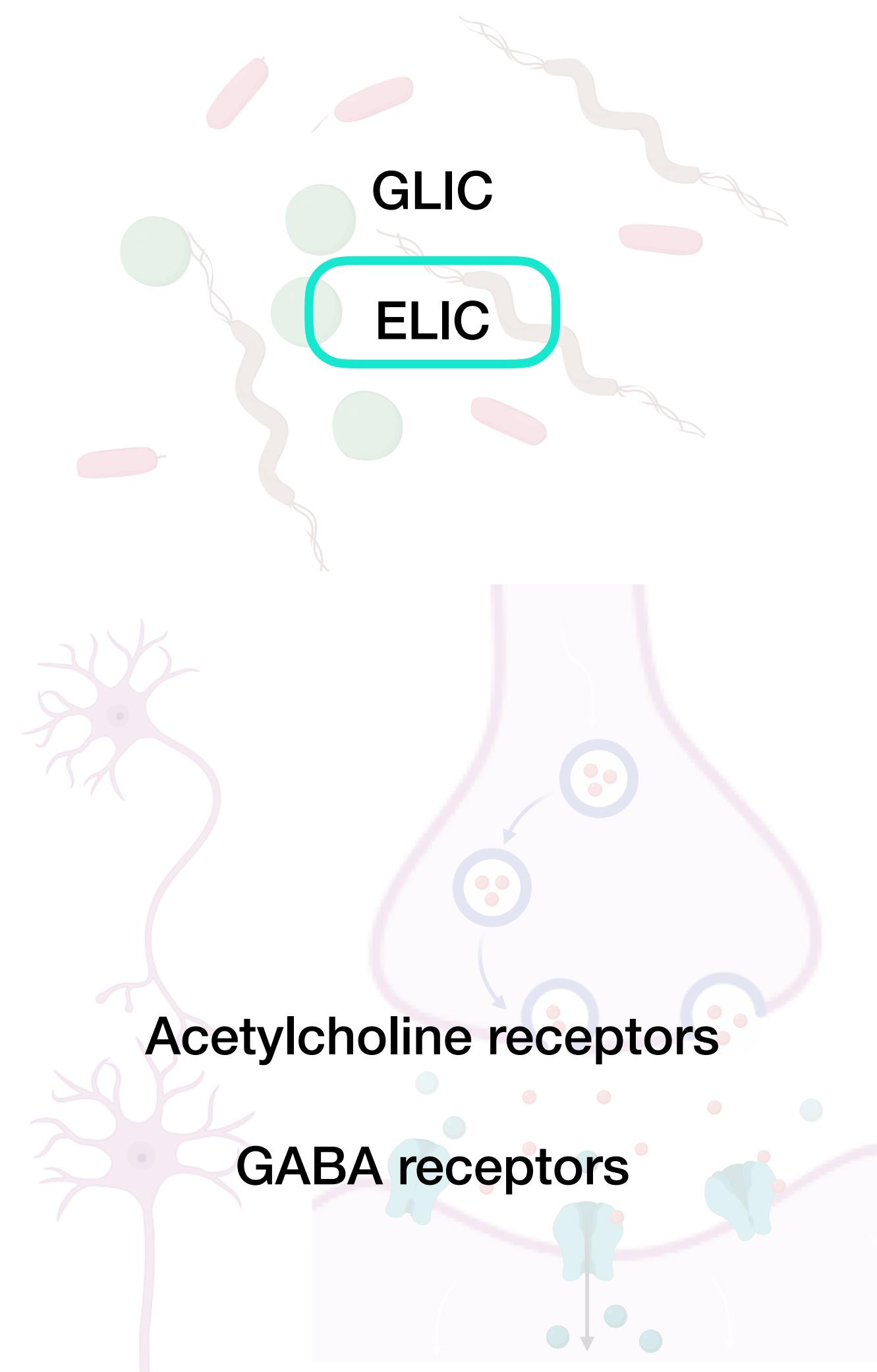


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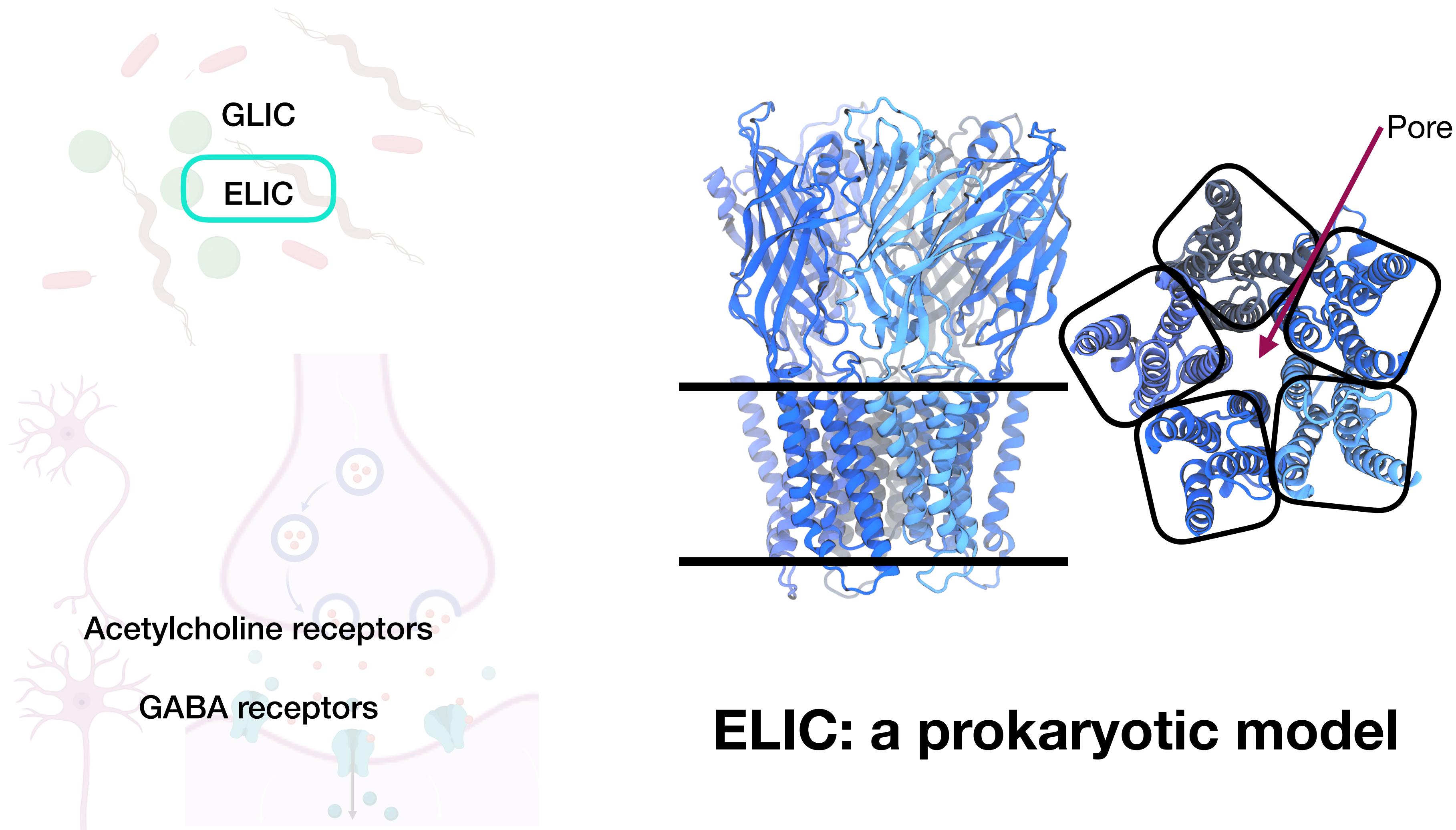
ELIC: a prokaryotic model

Pentameric Ligand-Gated Ion Channels (pLGICs) are chemosensors

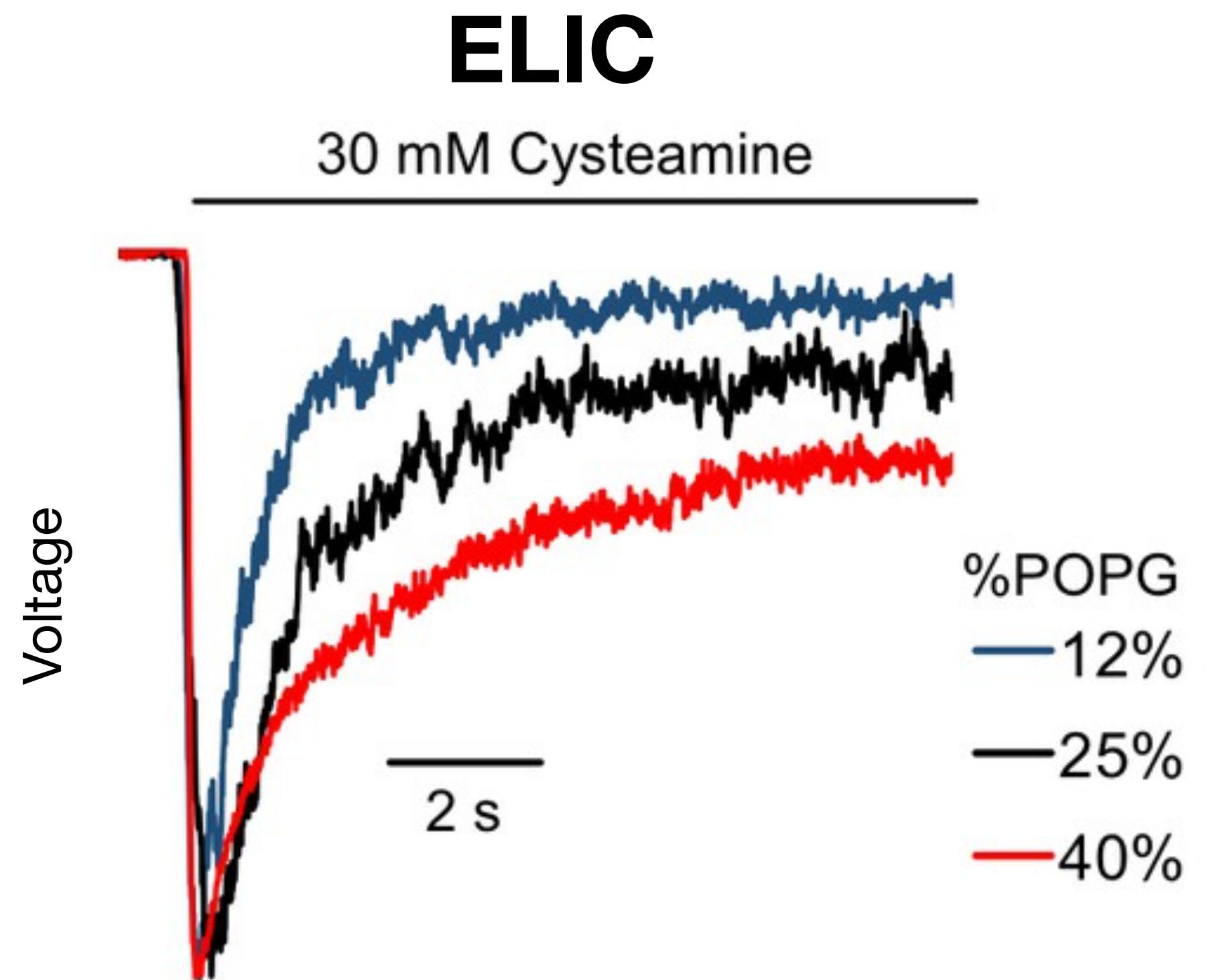


ELIC: a prokaryotic model

Pentameric Ligand-Gated Ion Channels (pLGICs) are chemosensors

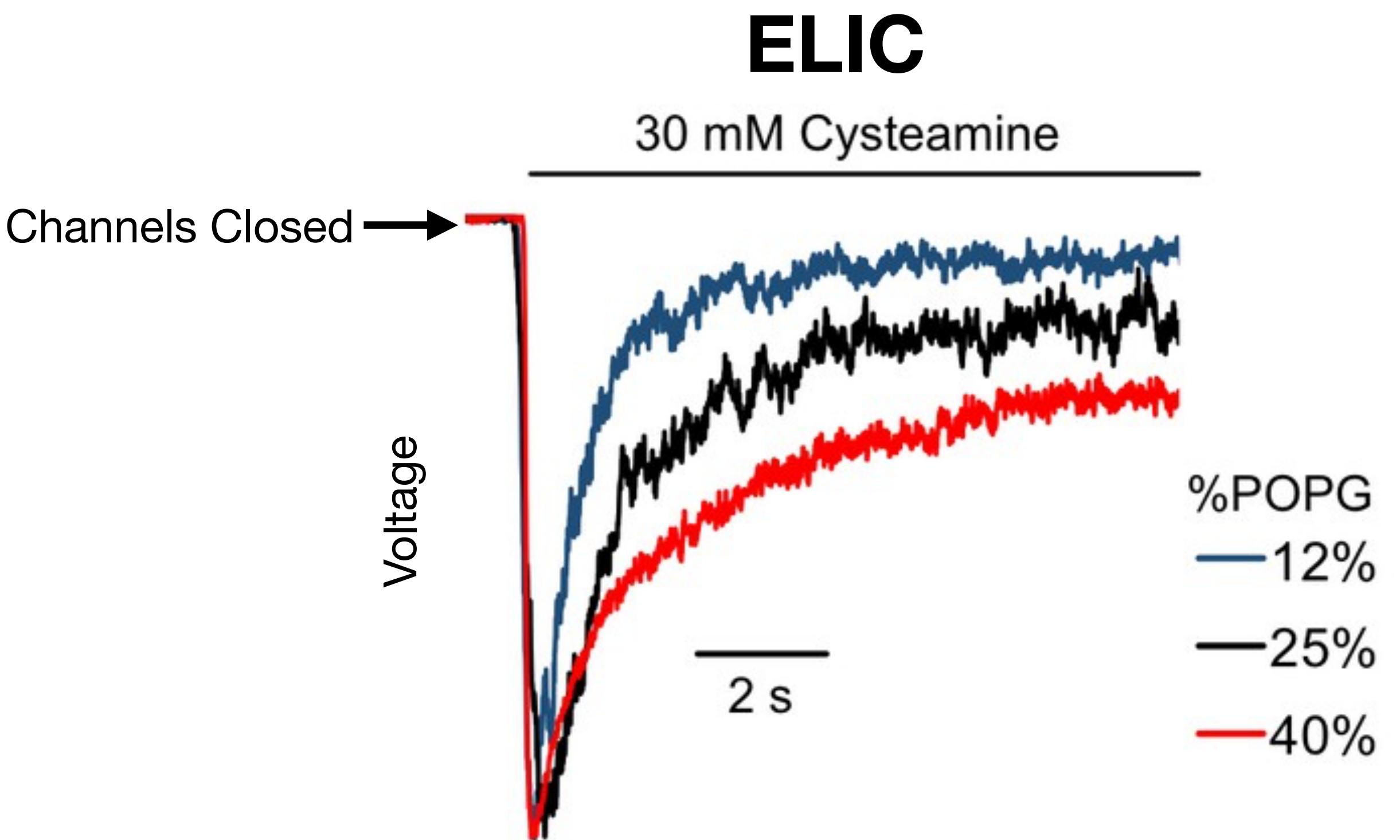


pLGICs are lipid-sensitive



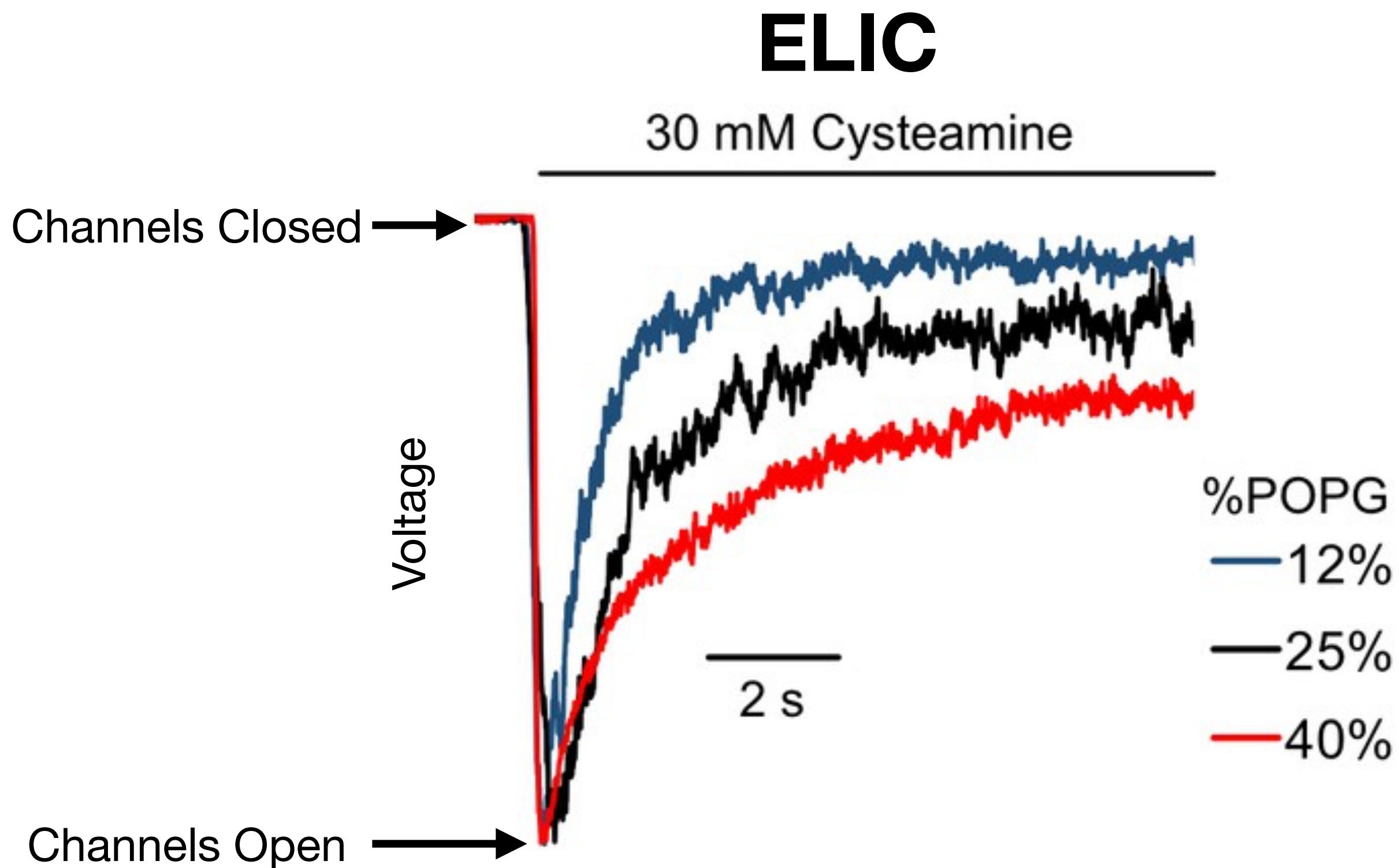
Ailing Tong, John T Petroff II, Liam Sharp, ... ,
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eLife 2019

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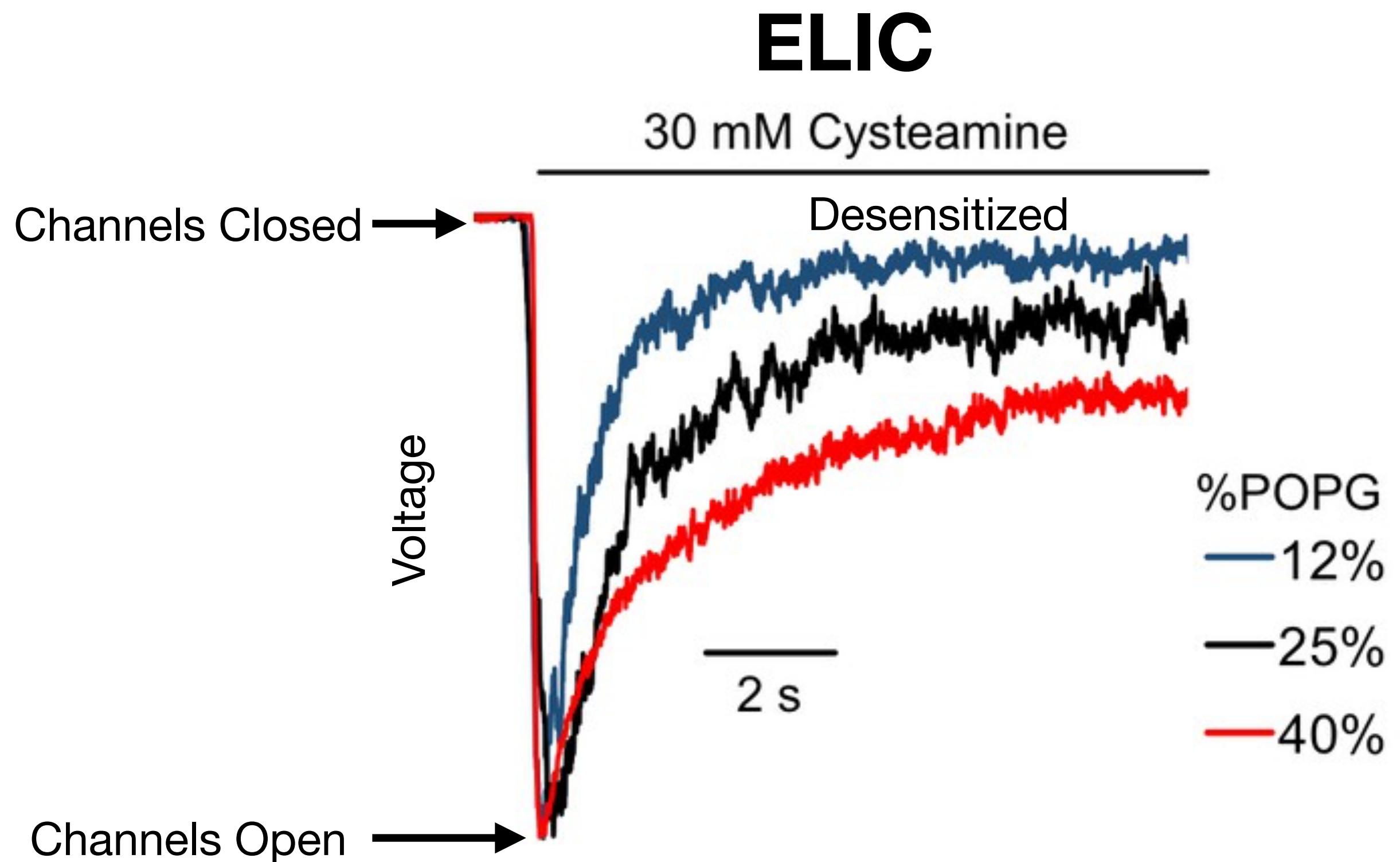
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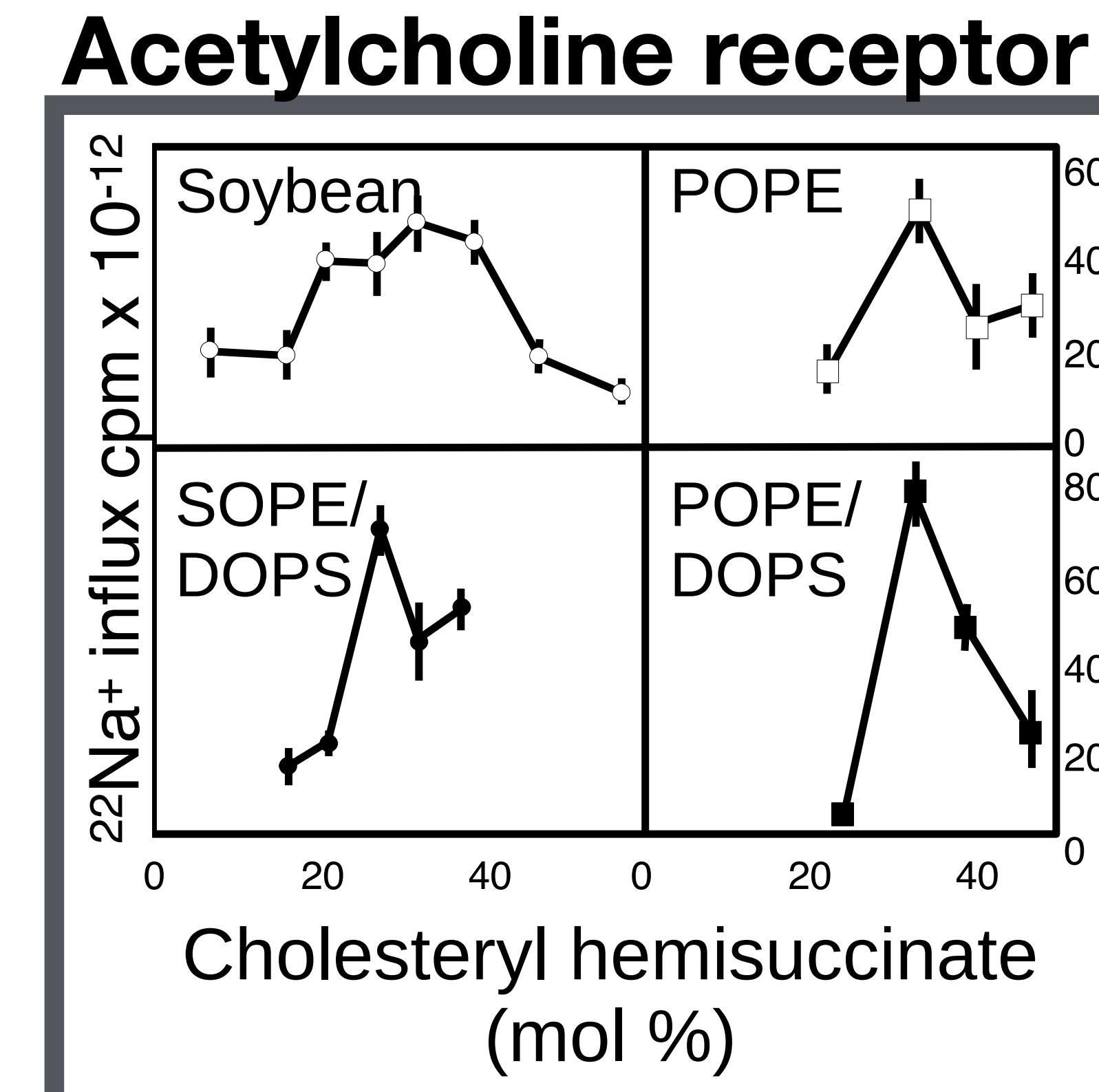
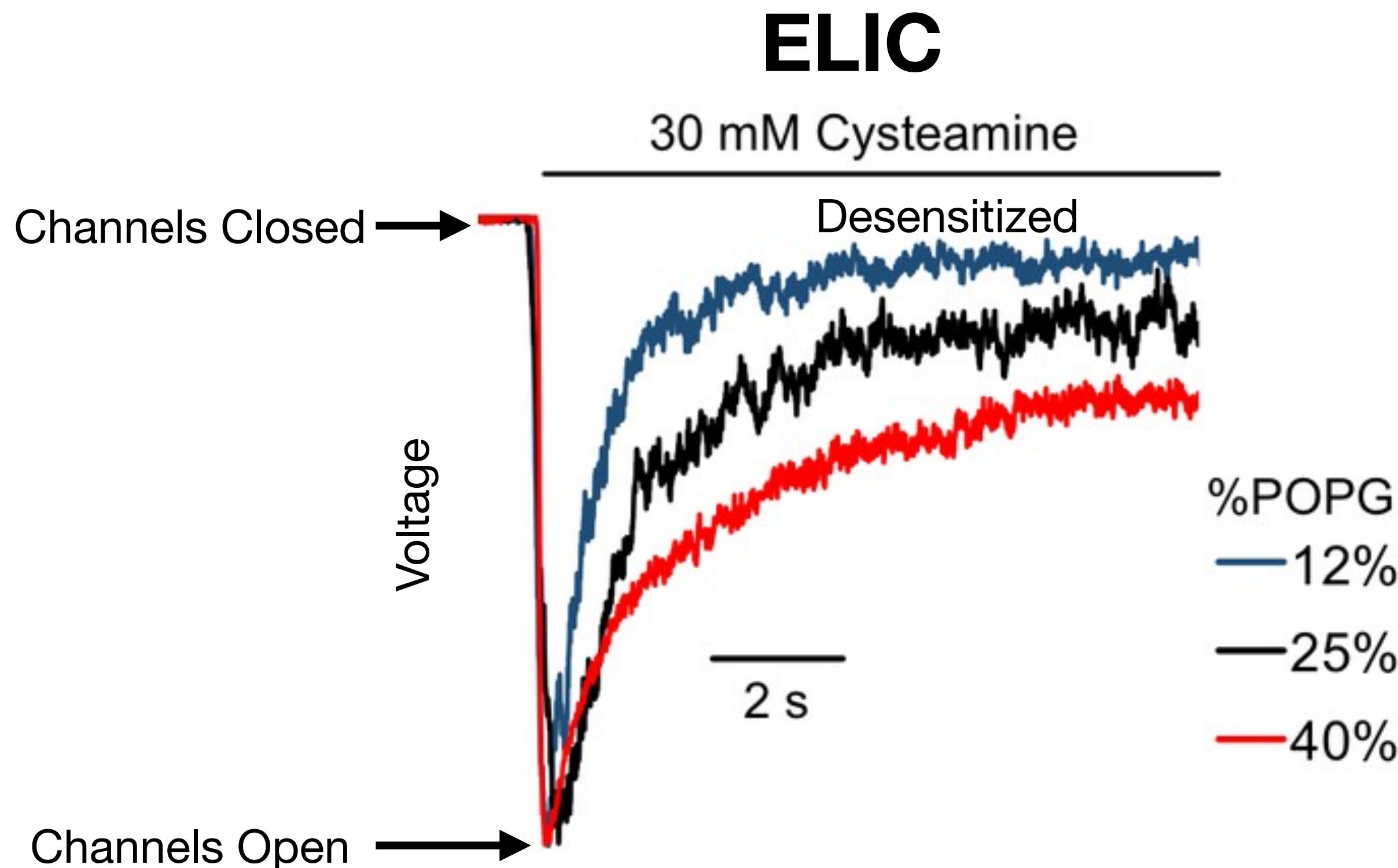
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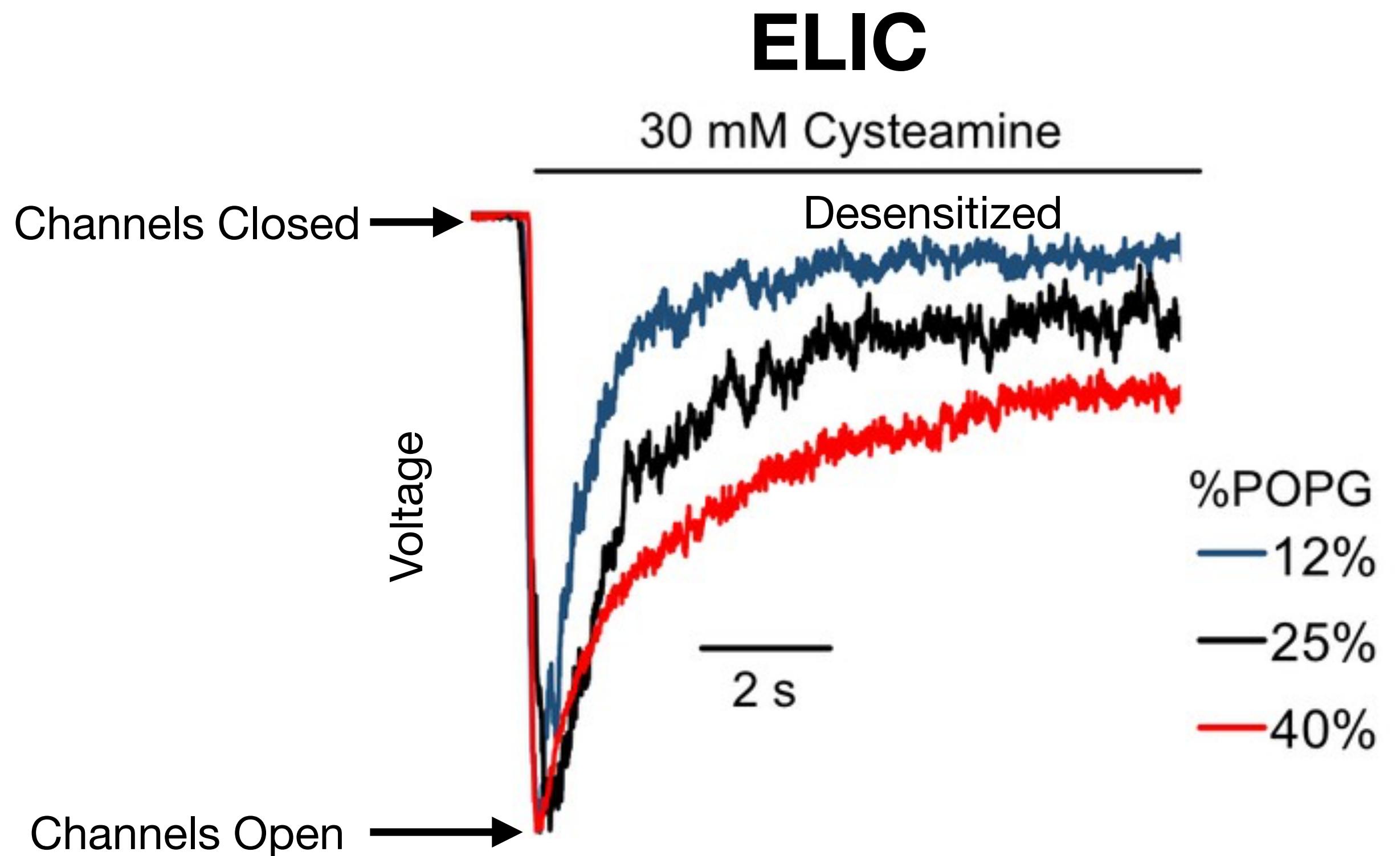
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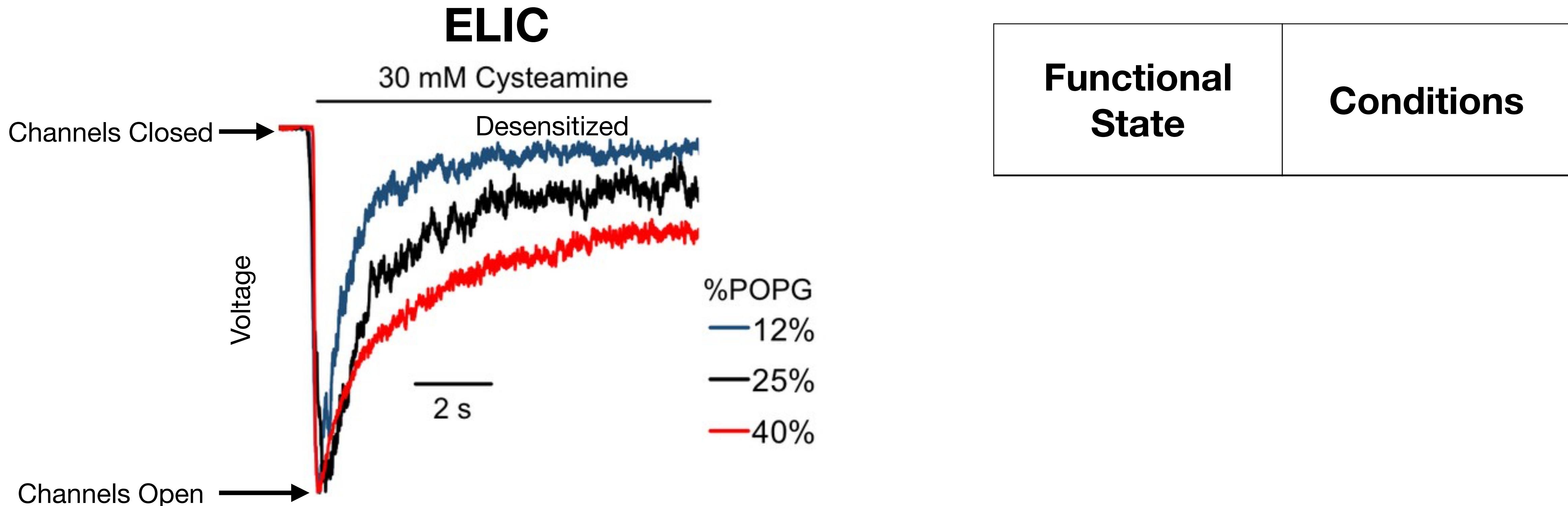
M Criado, H Eibl, FJ Barrantes
J. Biol. Chem. 1984

A Structural Challenge



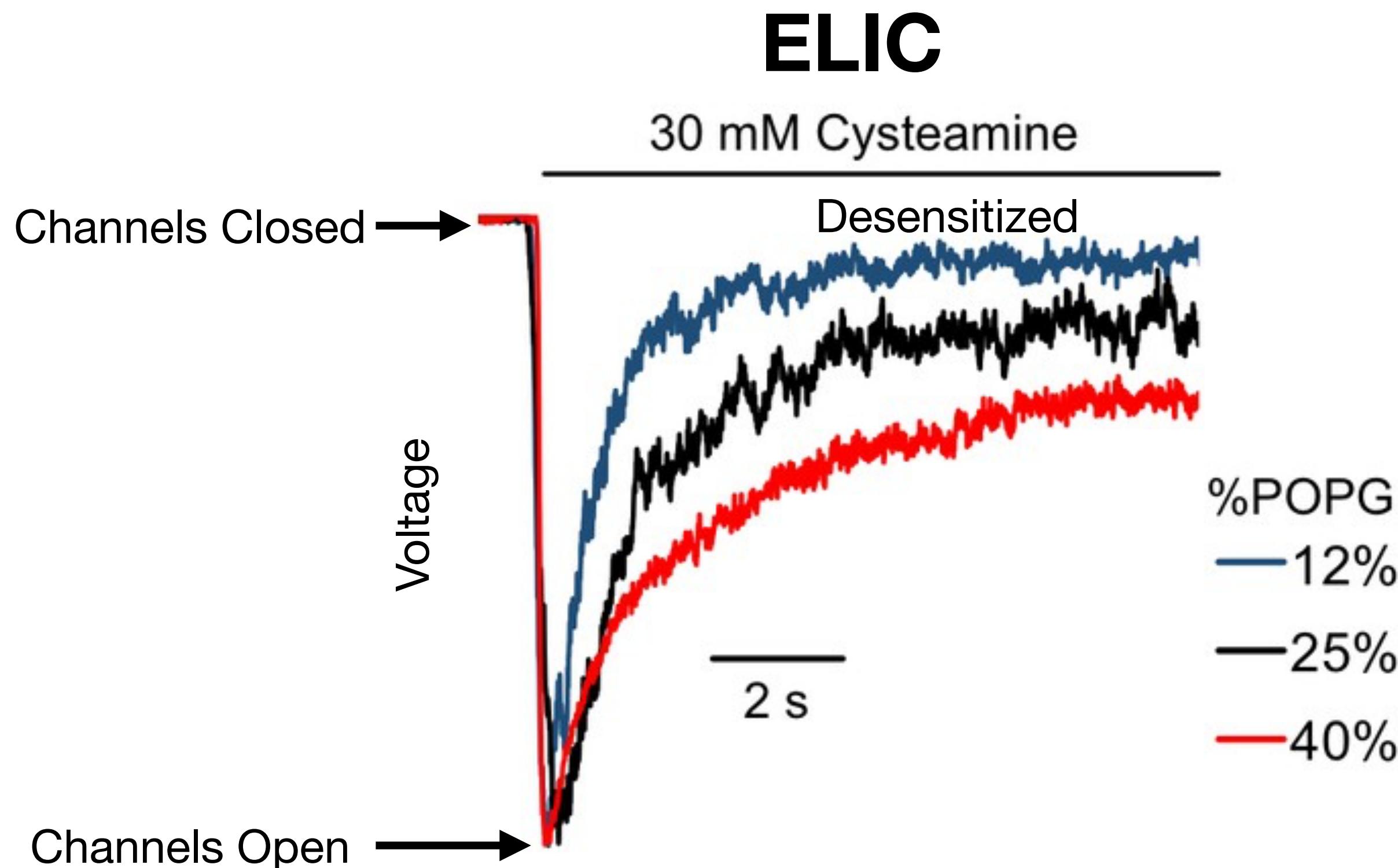
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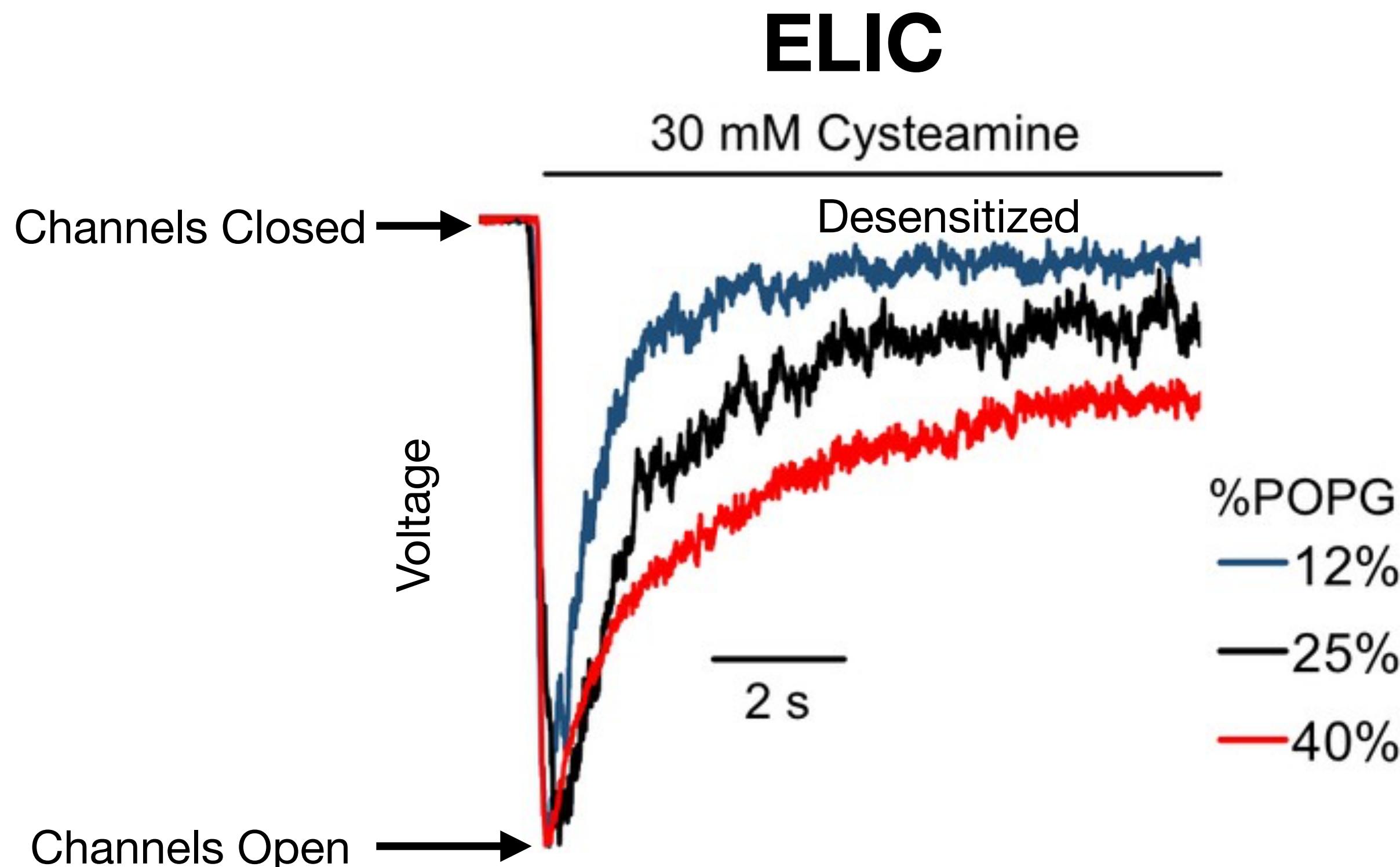
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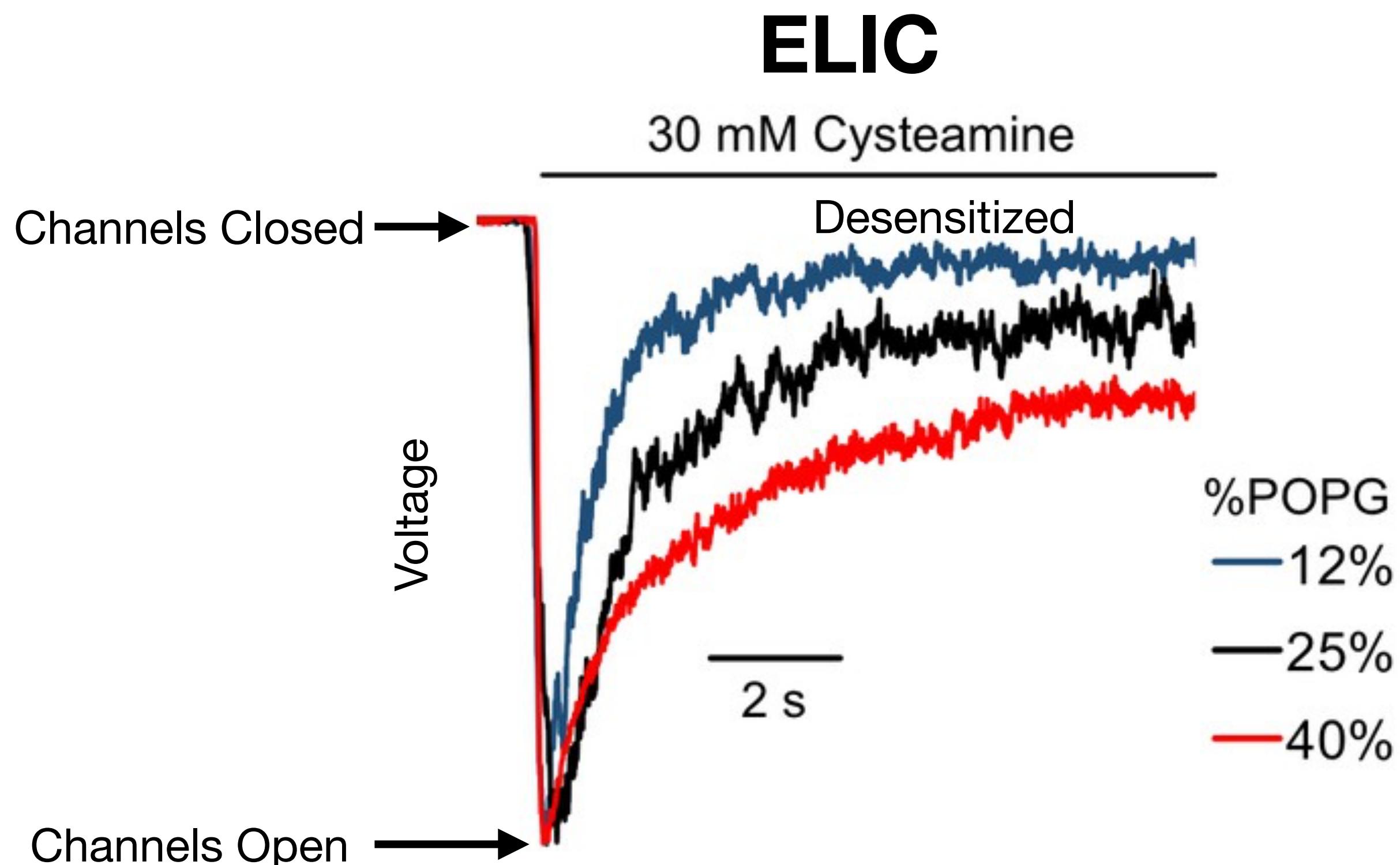
Functional State	Conditions
Ligand-free/ non-conducting	No Ligand

A Structural Challenge



Functional State	Conditions
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A Structural Challenge

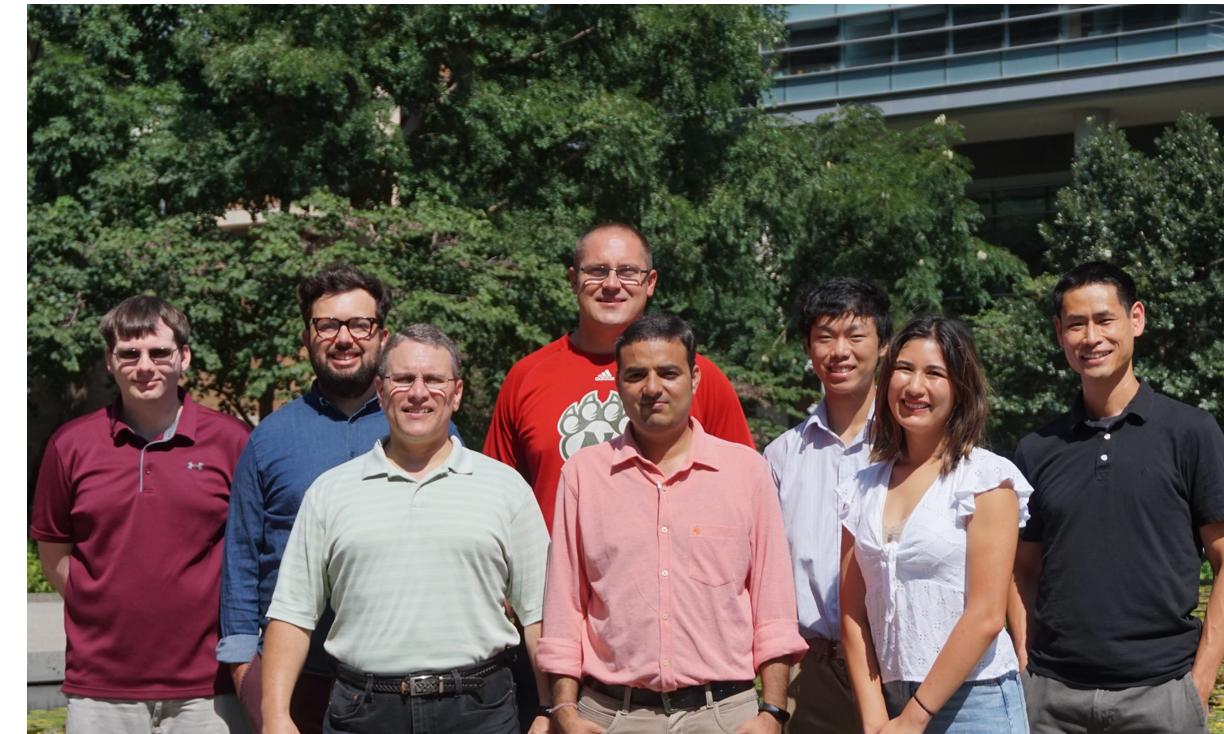


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Conducting	???

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Getting the Open Structure

Wayland Cheng Lab



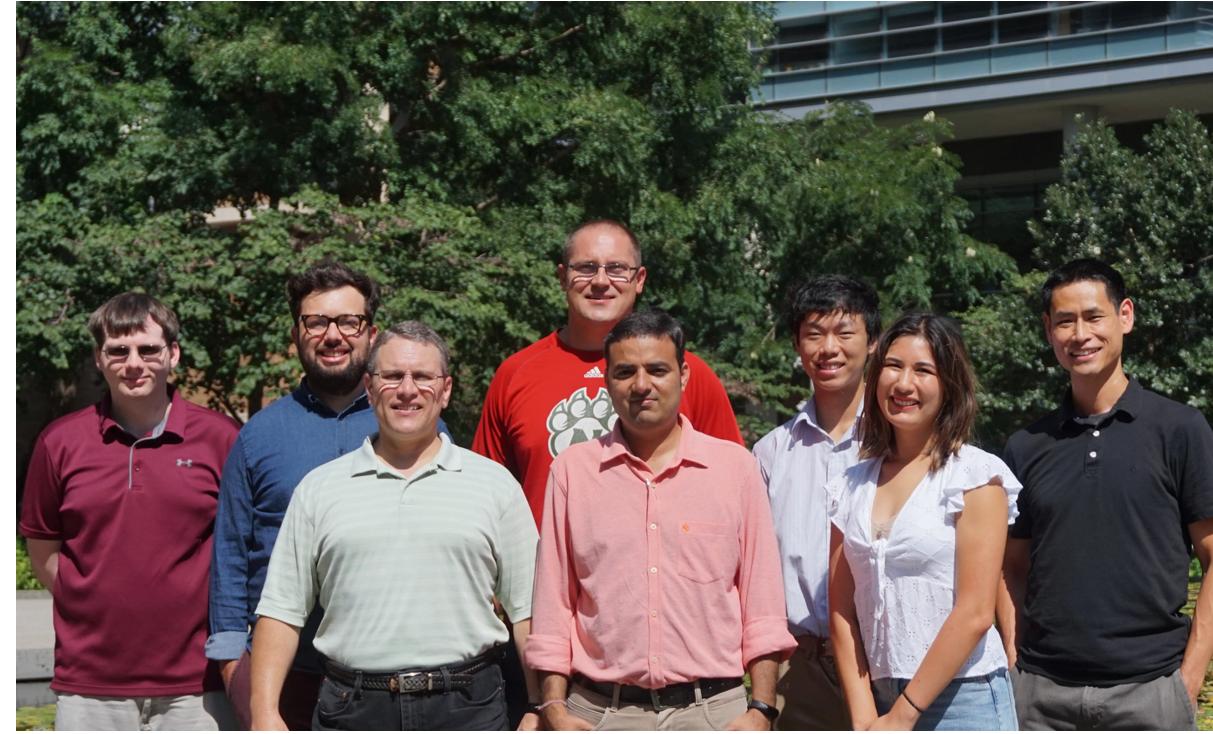
 Washington
University in St. Louis

SCHOOL OF MEDICINE

Getting the Open Structure

Wayland Cheng Lab

- 5 Mutations



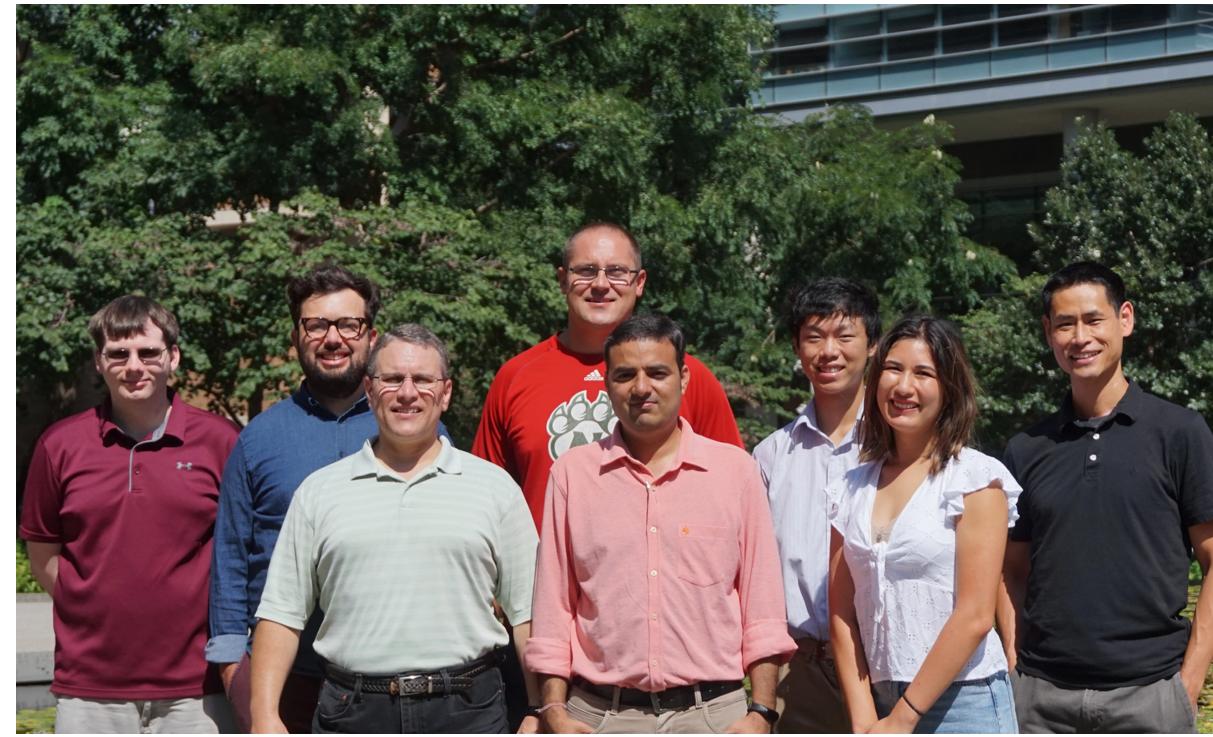
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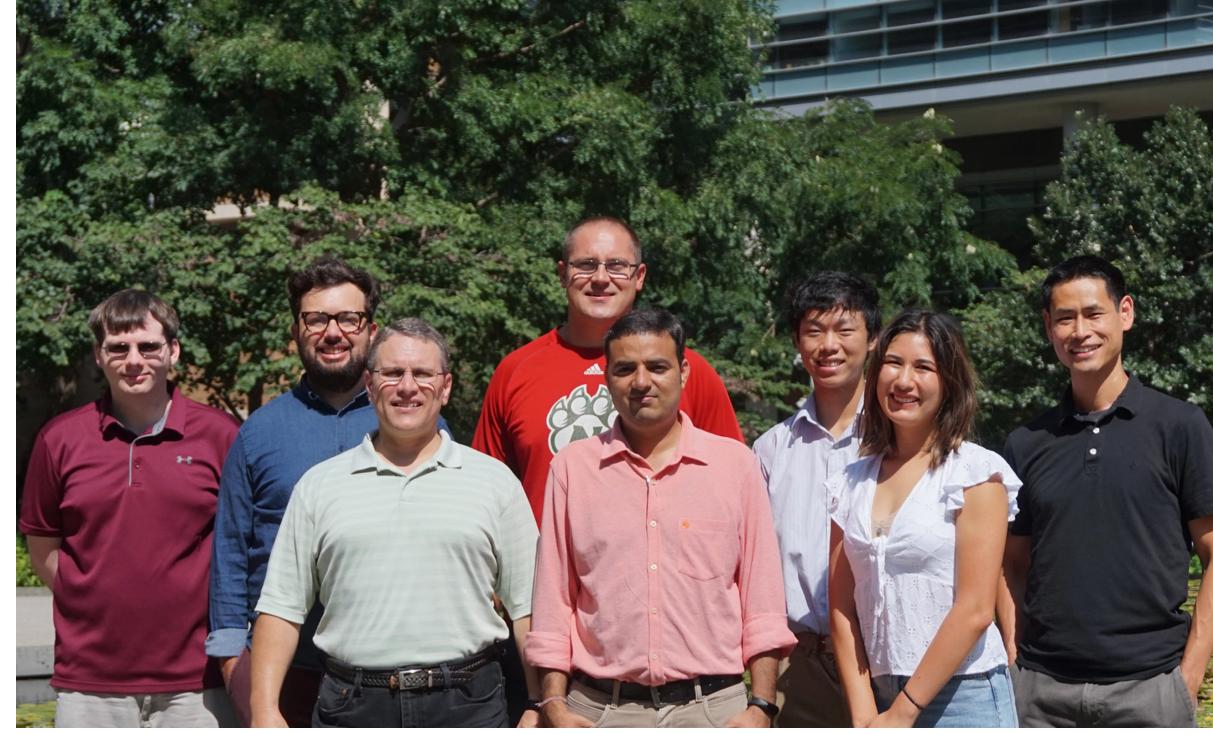
- 5 Mutations
- Ternary Membrane:
 - 2POPC
 - 1POPE
 - 1POPG



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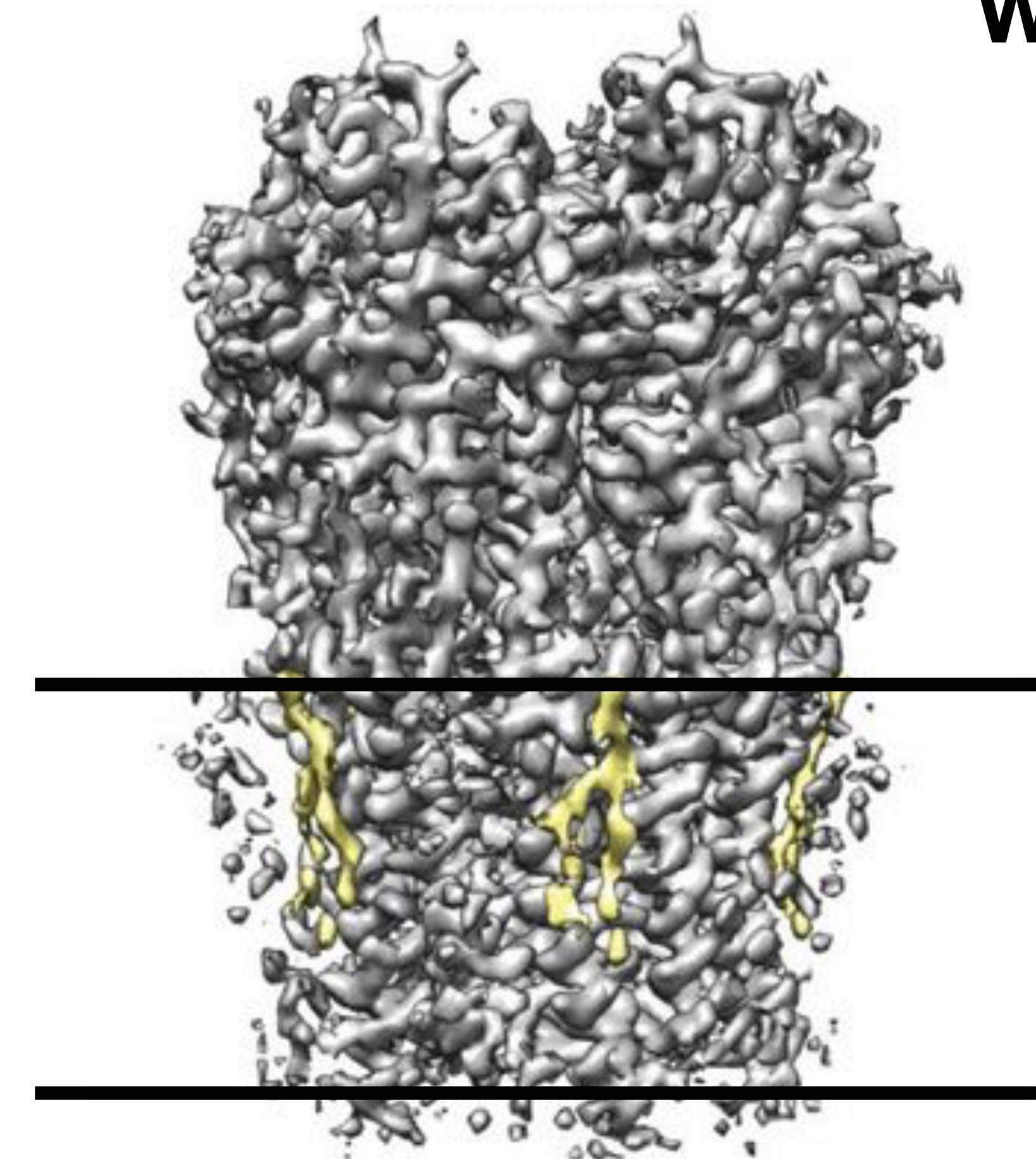


- 5 Mutations
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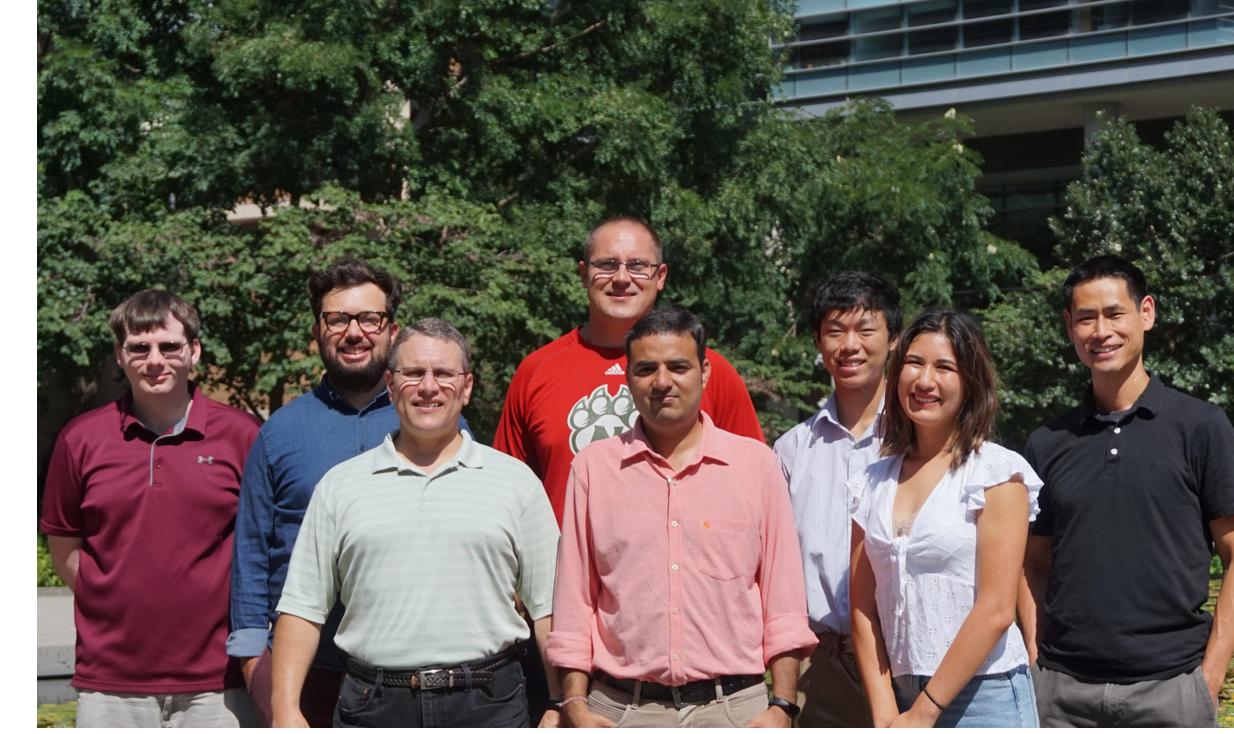
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Nature Communications 2022

Wayland Cheng Lab

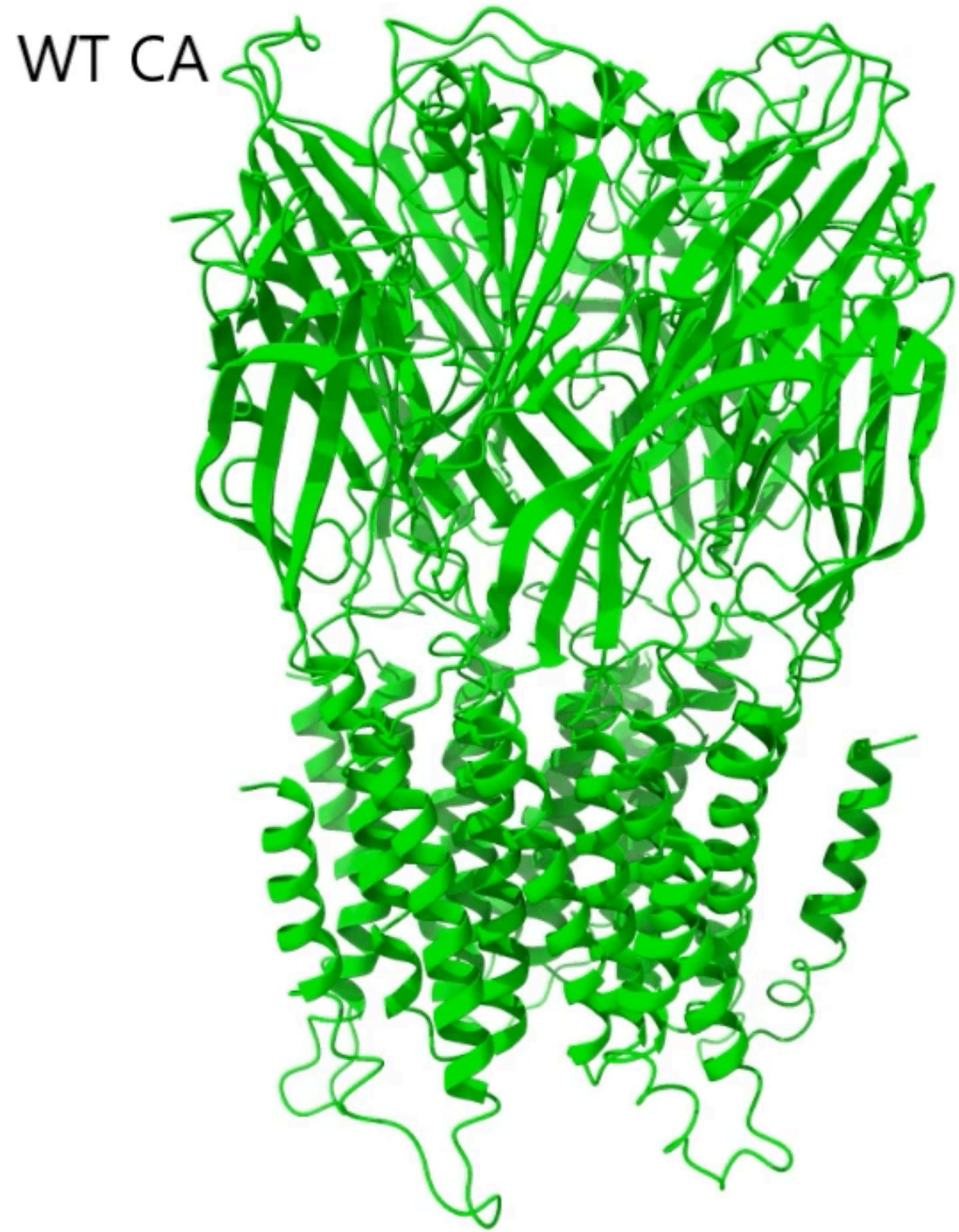


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We can (finally) see an open structure!

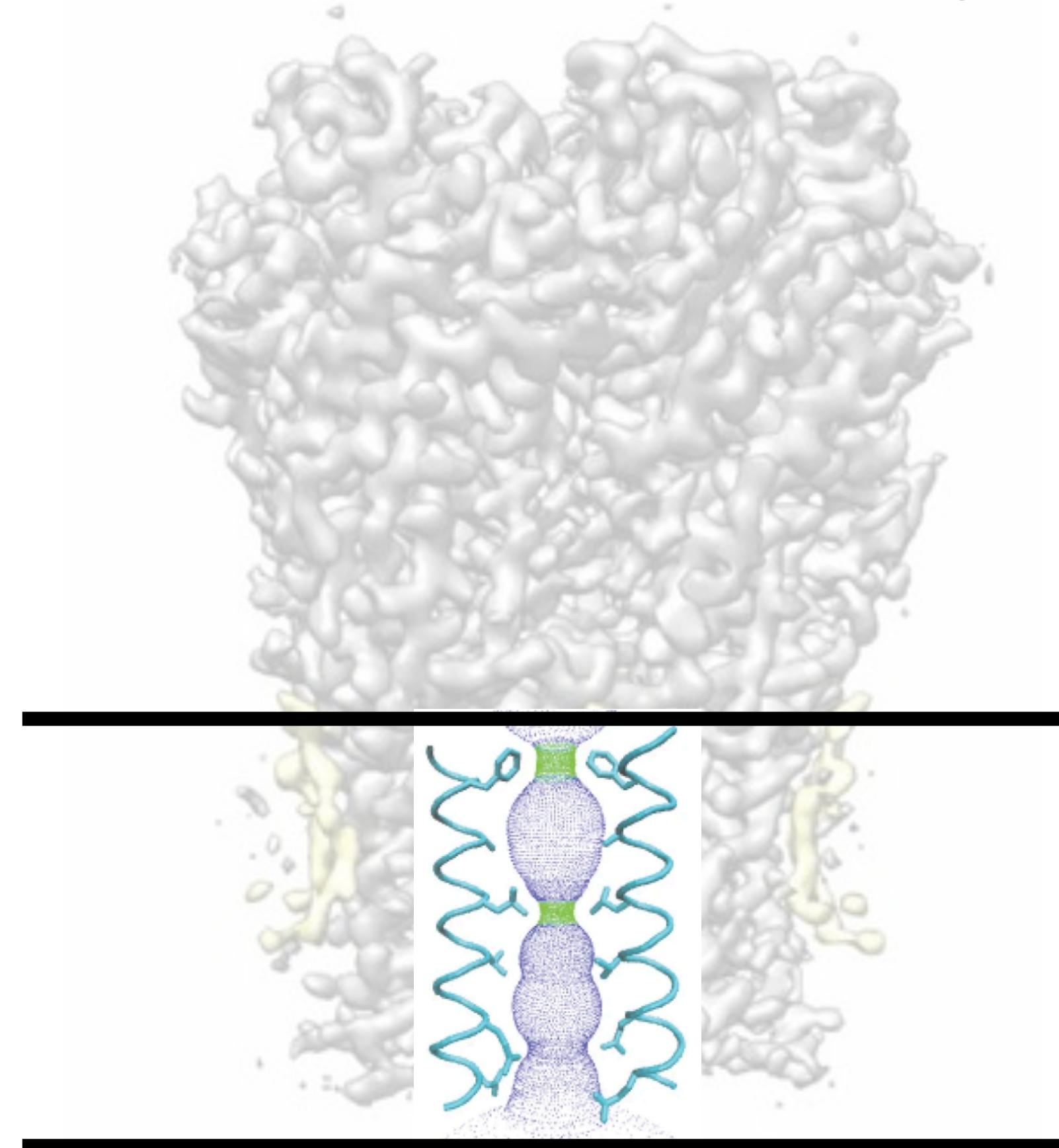


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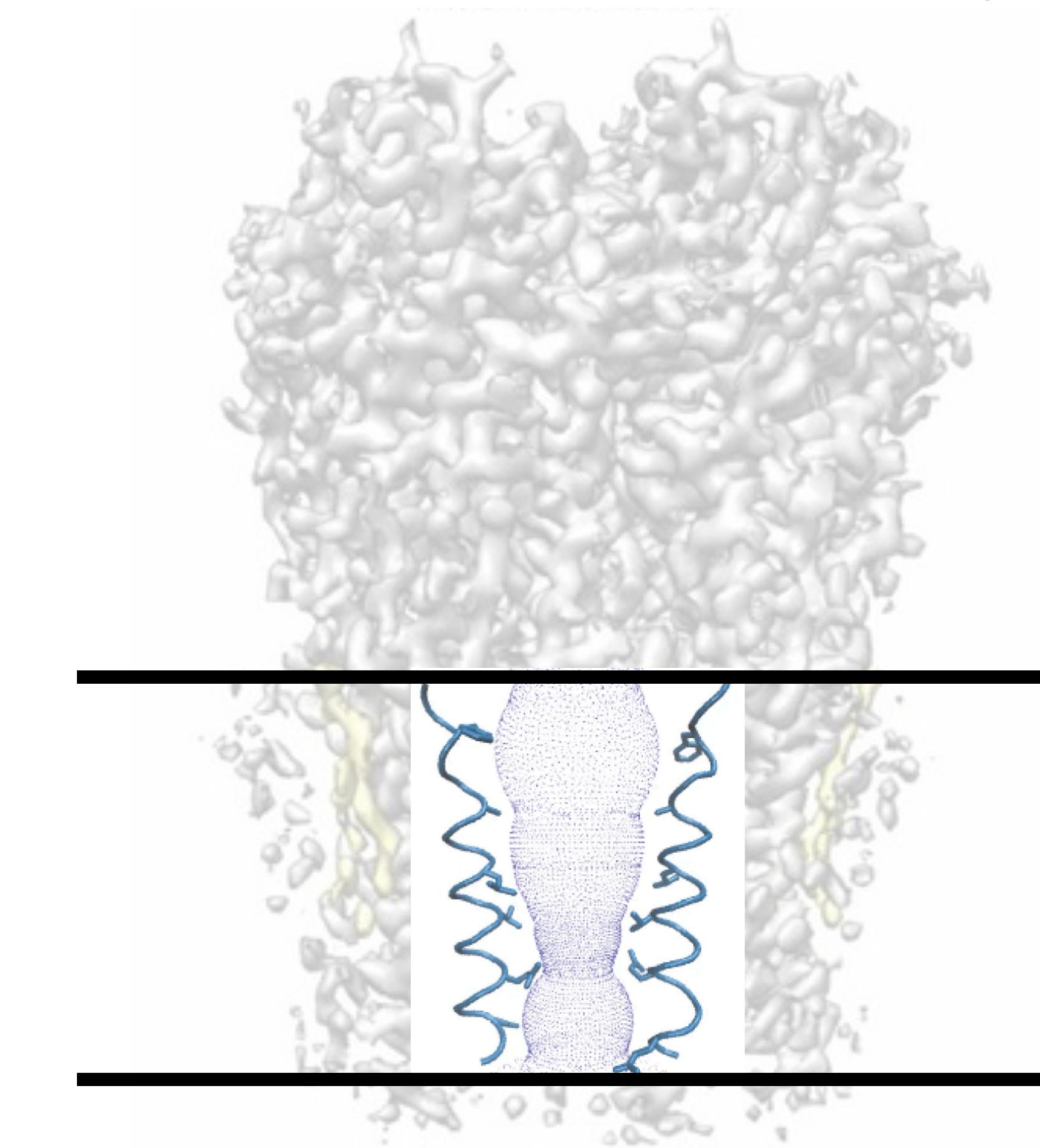


And compare pores

WT Non-conducting

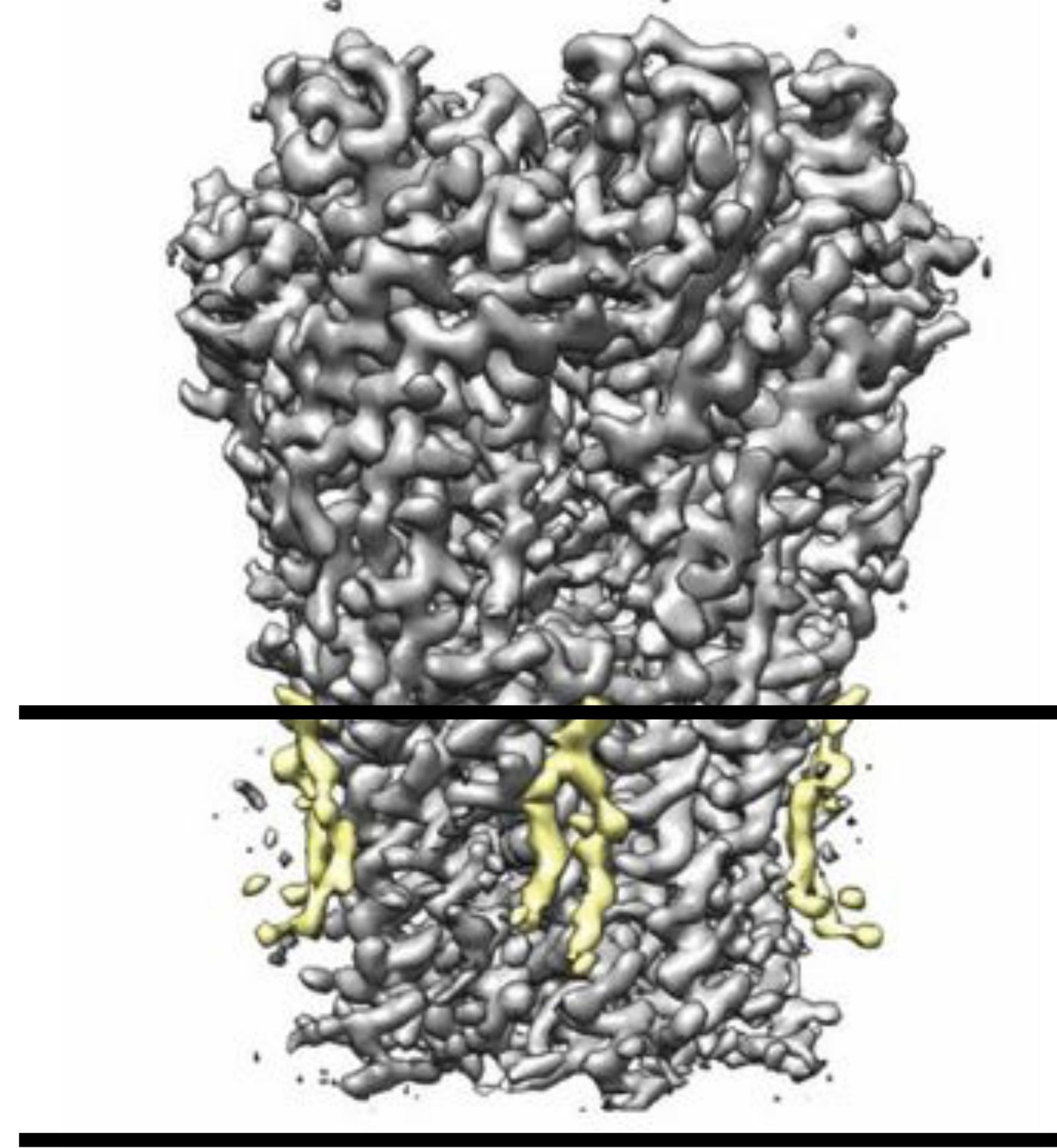


5-mutant Conducting

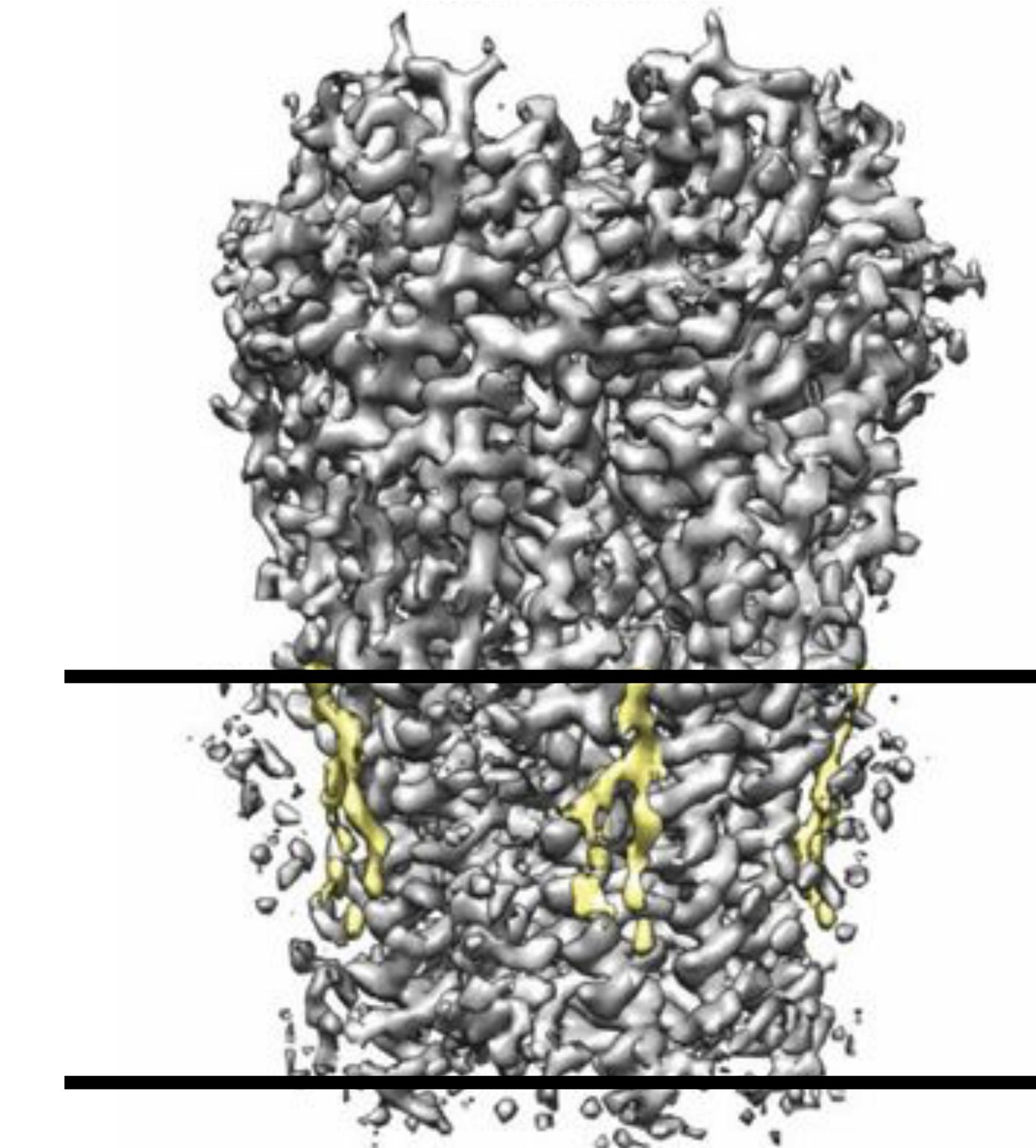


But with surprise densities...

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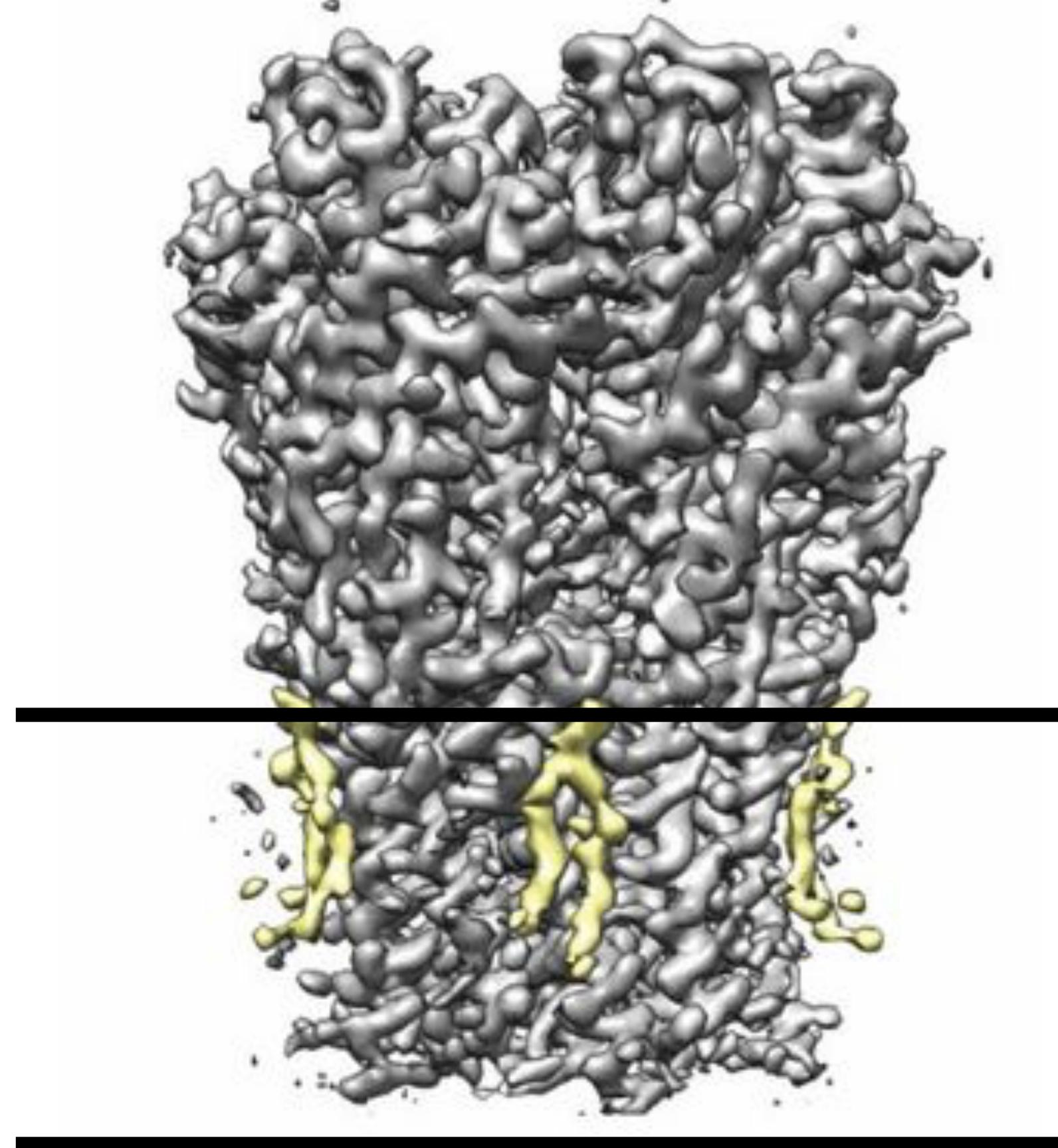


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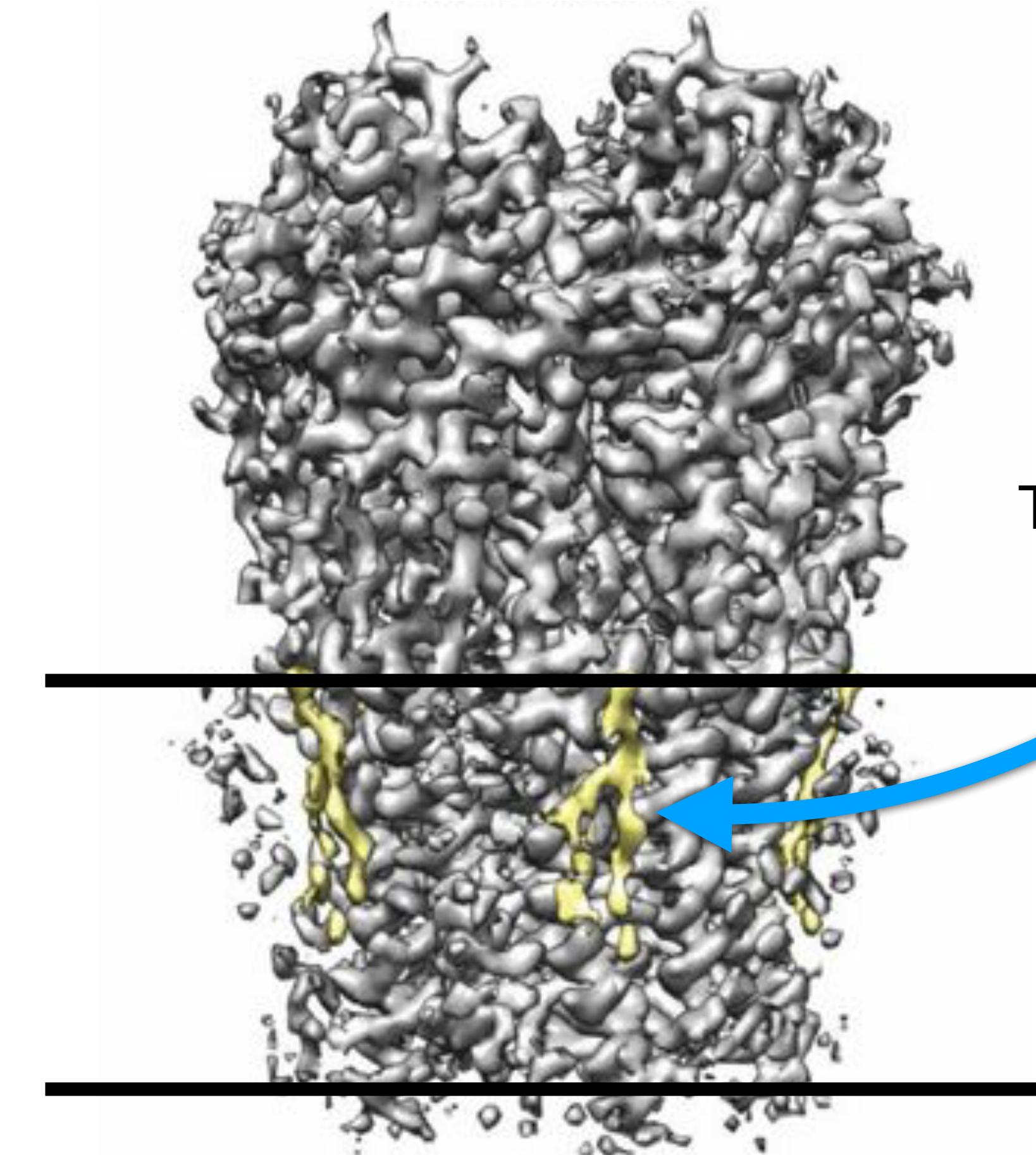


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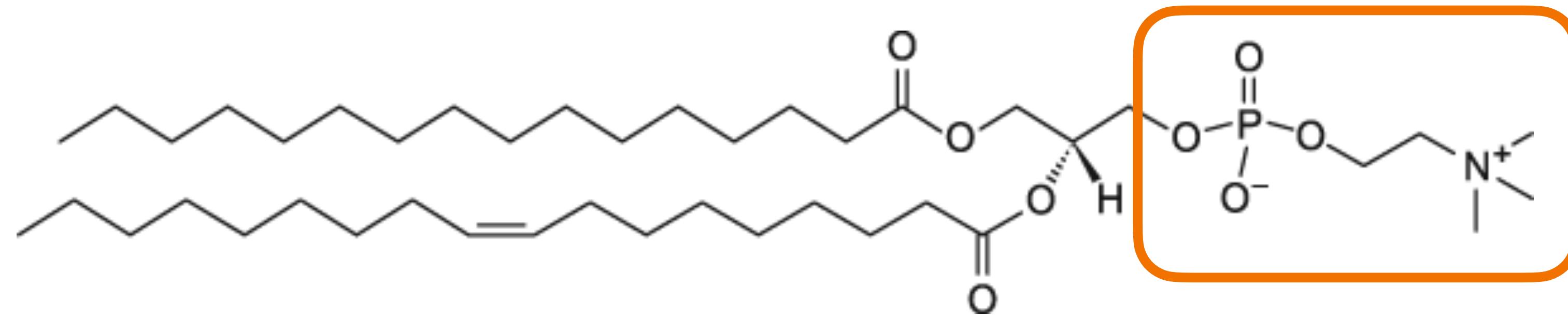


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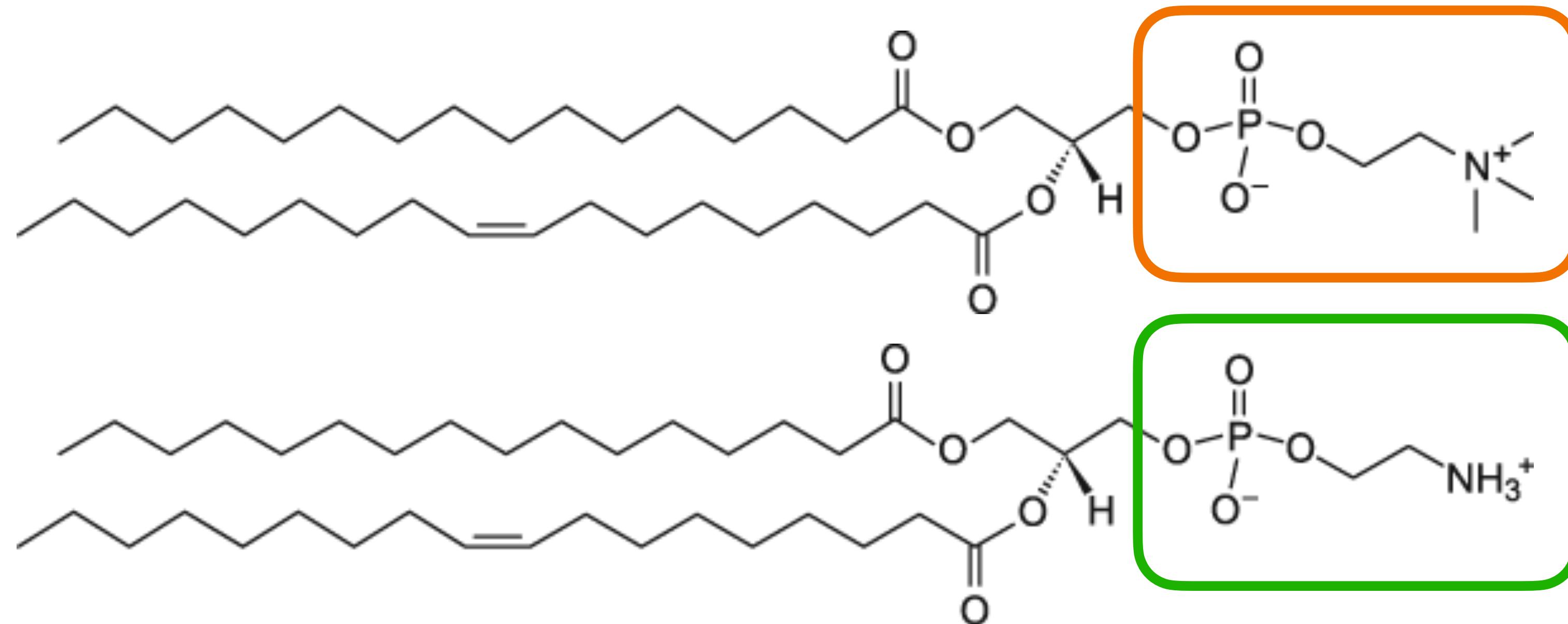
Three Lipids in the Experimental System:

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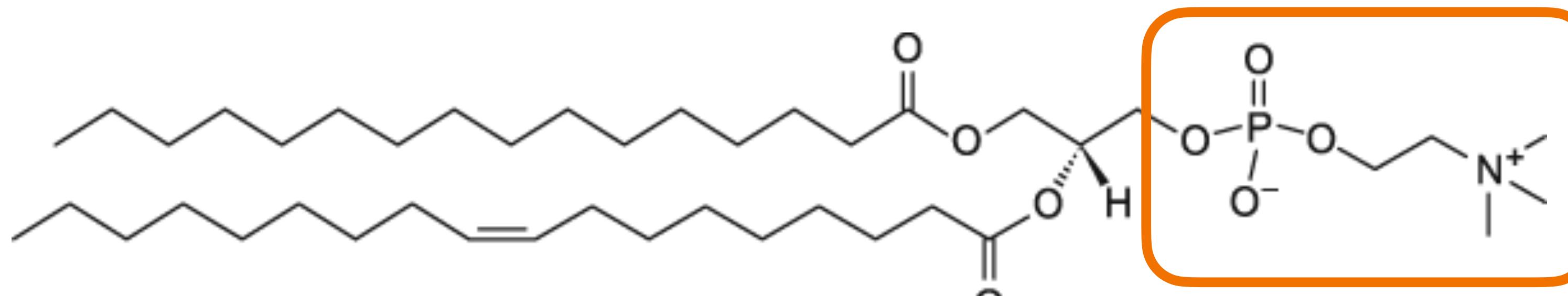


POPC

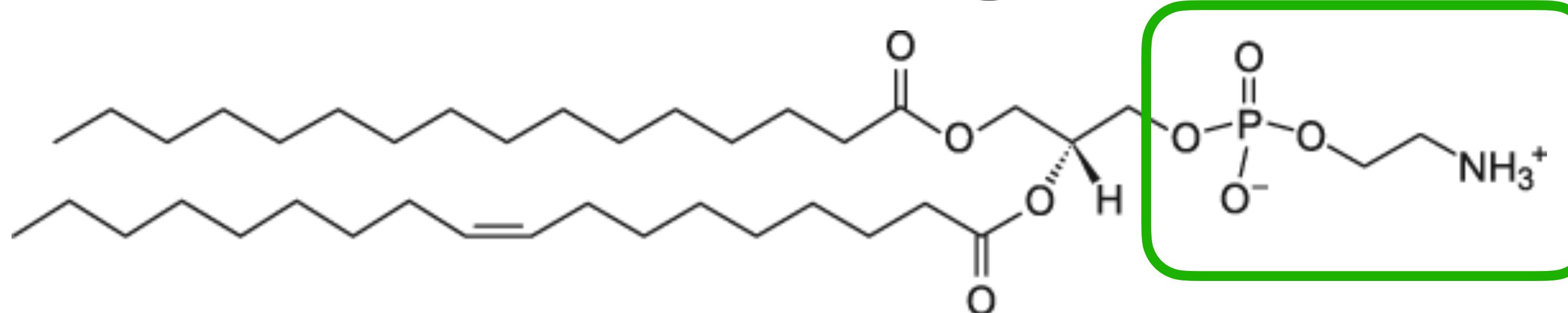
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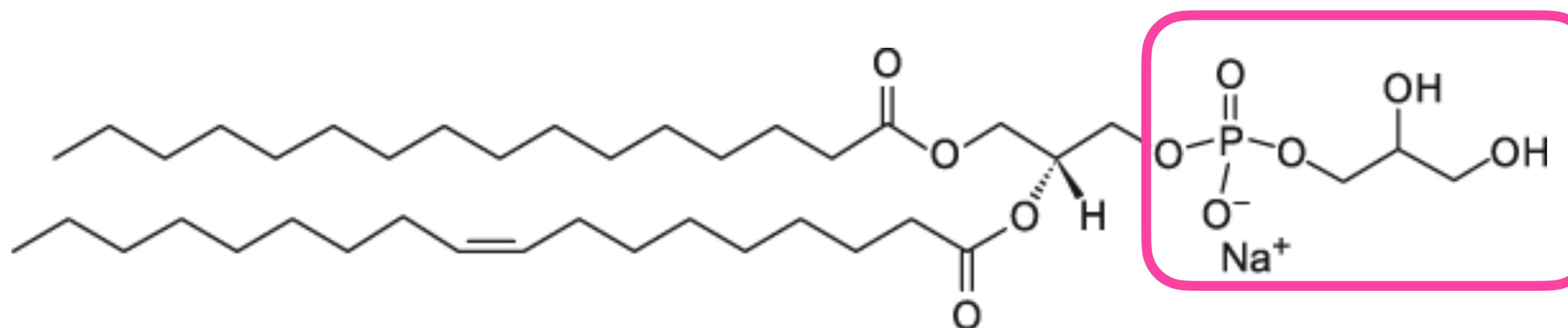
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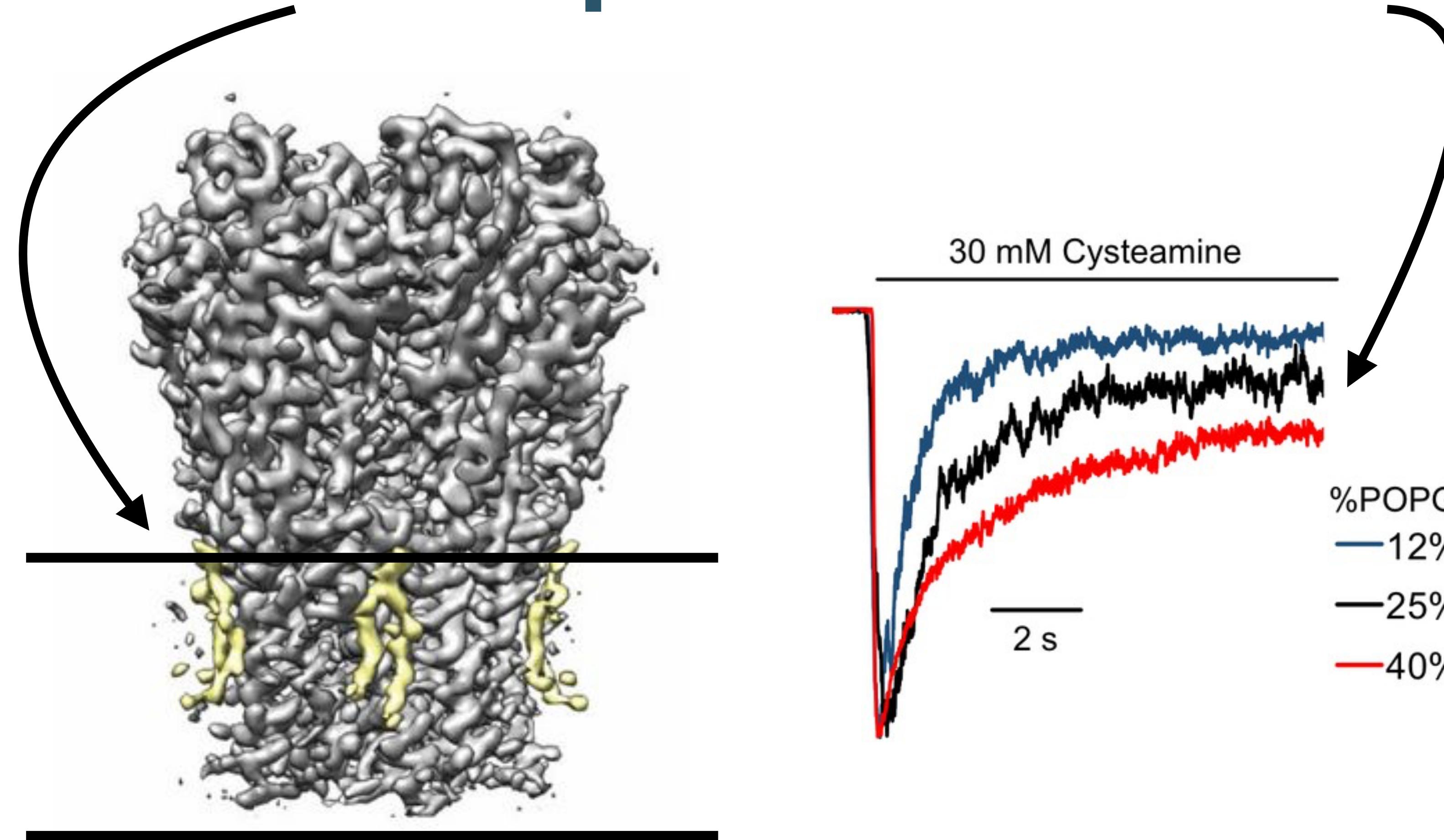


POPE



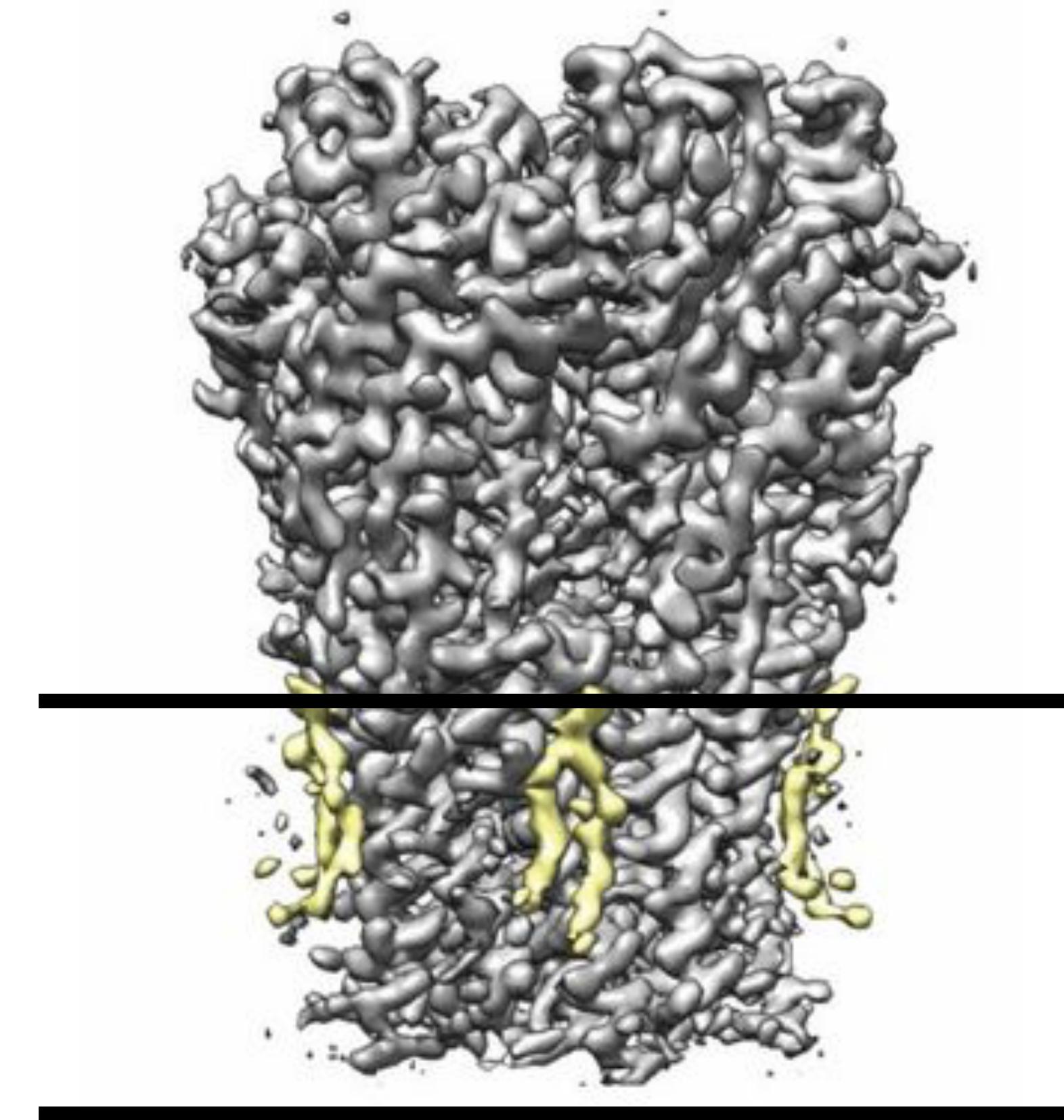
POPG

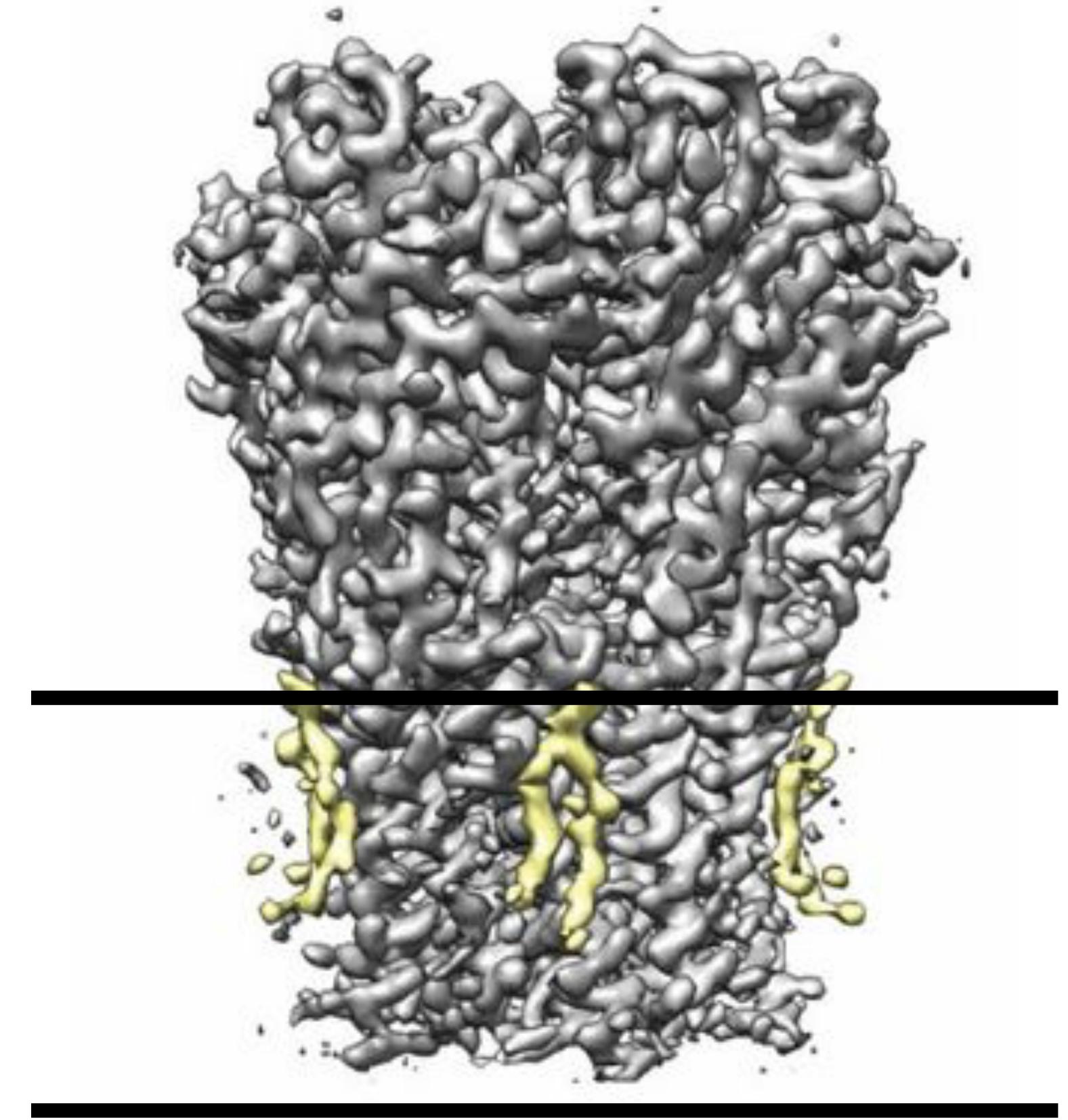
Are these responsible for these?



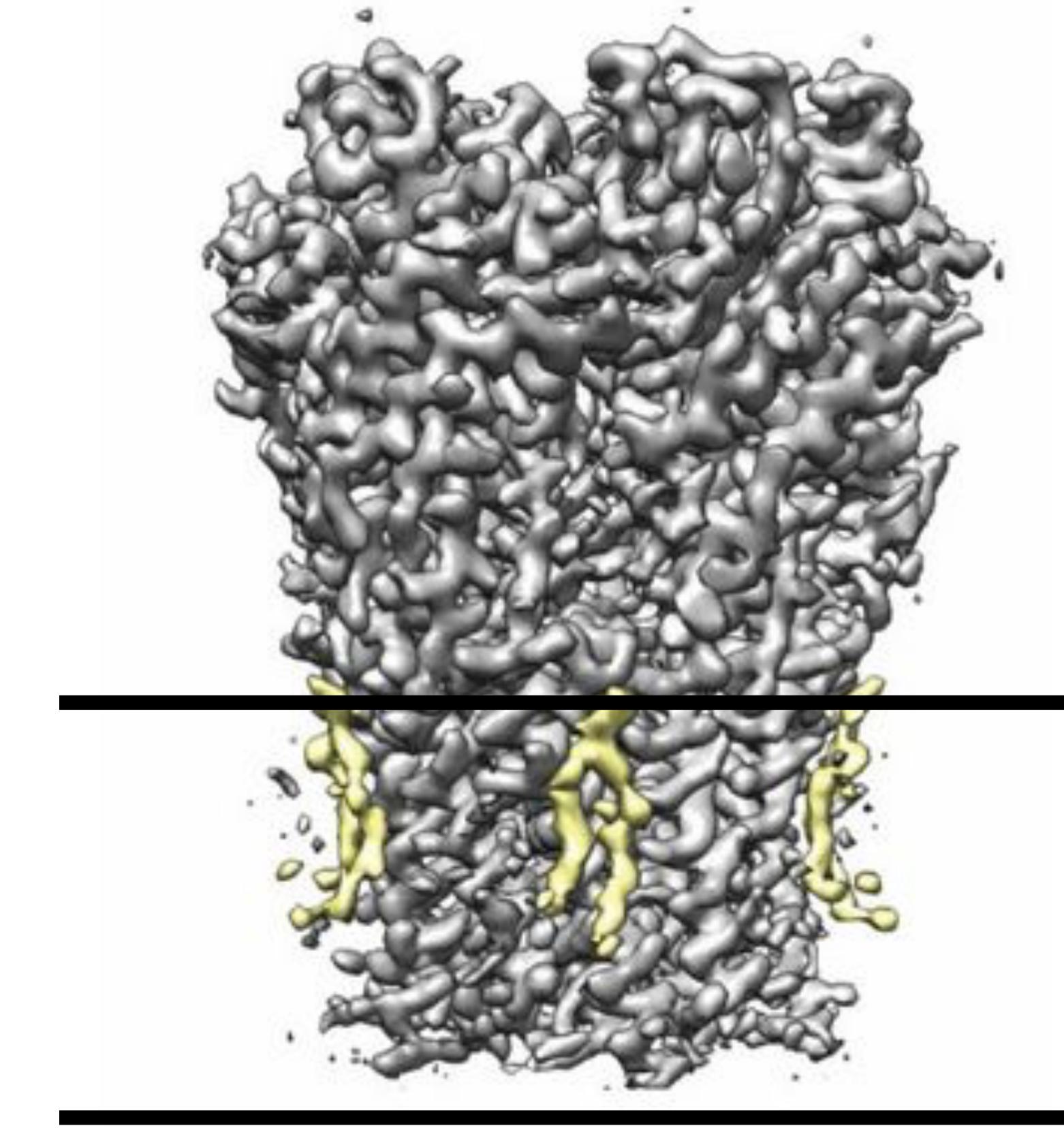
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eLife 2019



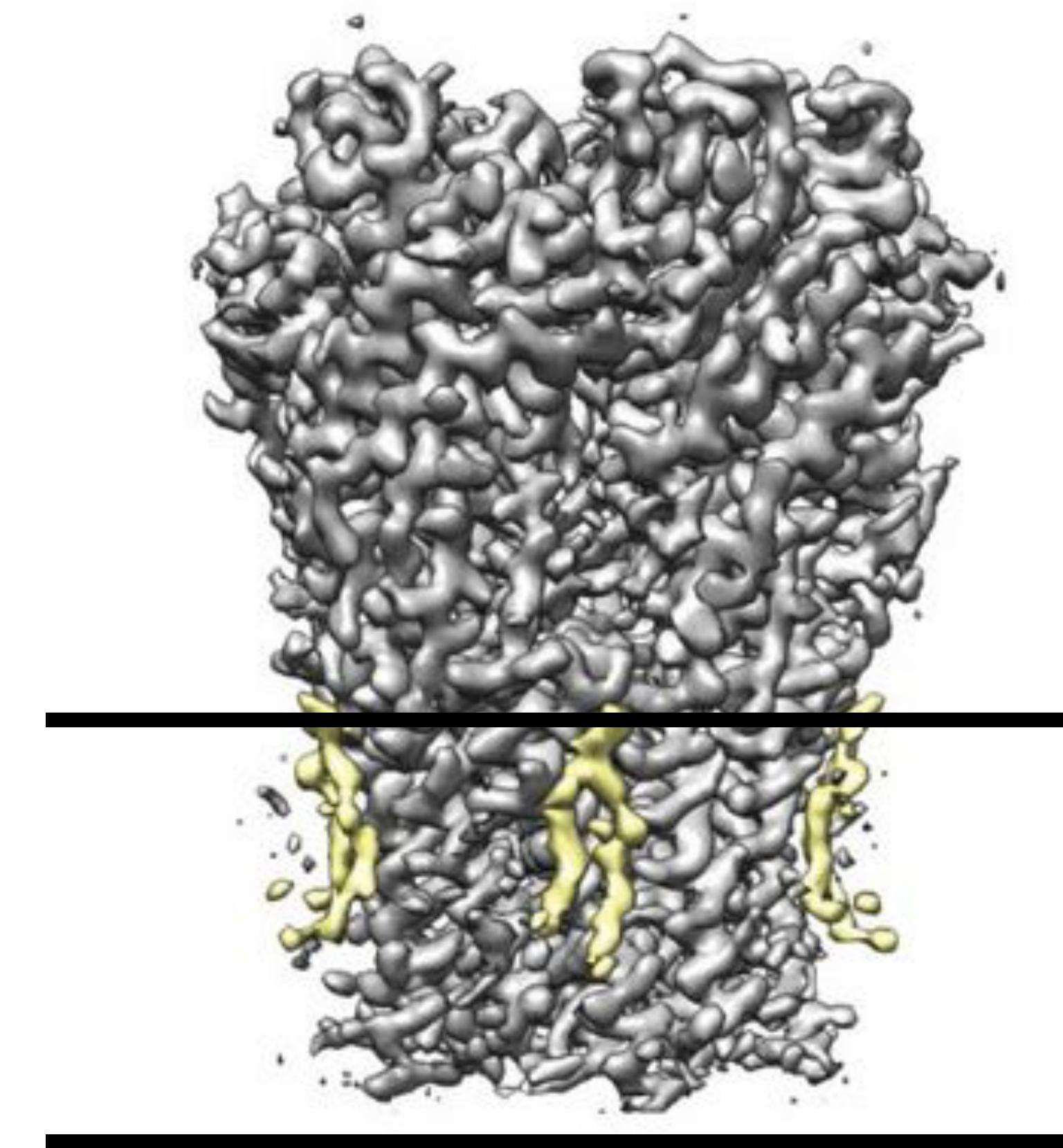


Experimentally inaccessible questions:



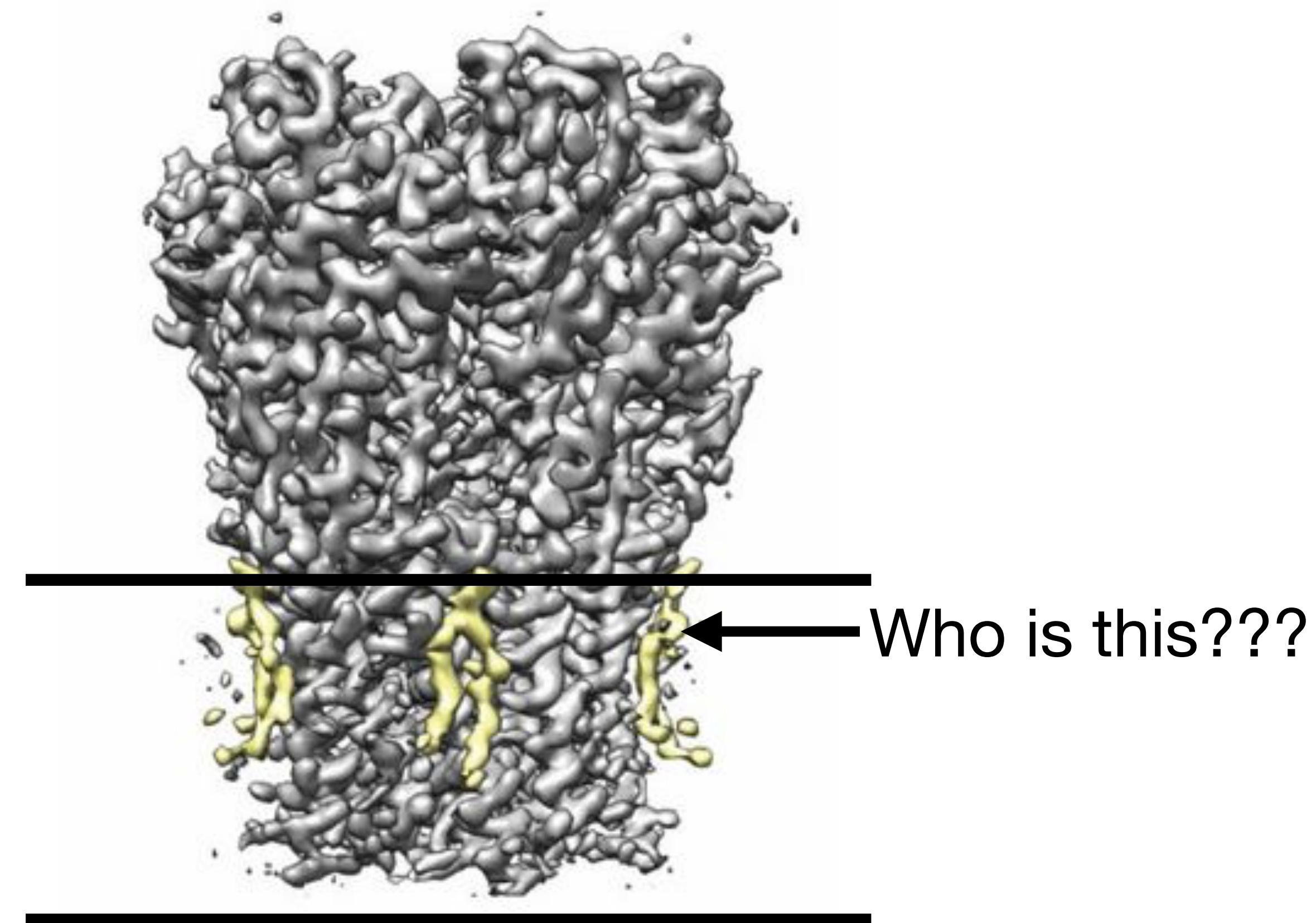
Experimentally inaccessible questions:

- What is the **identity** of these lipids?



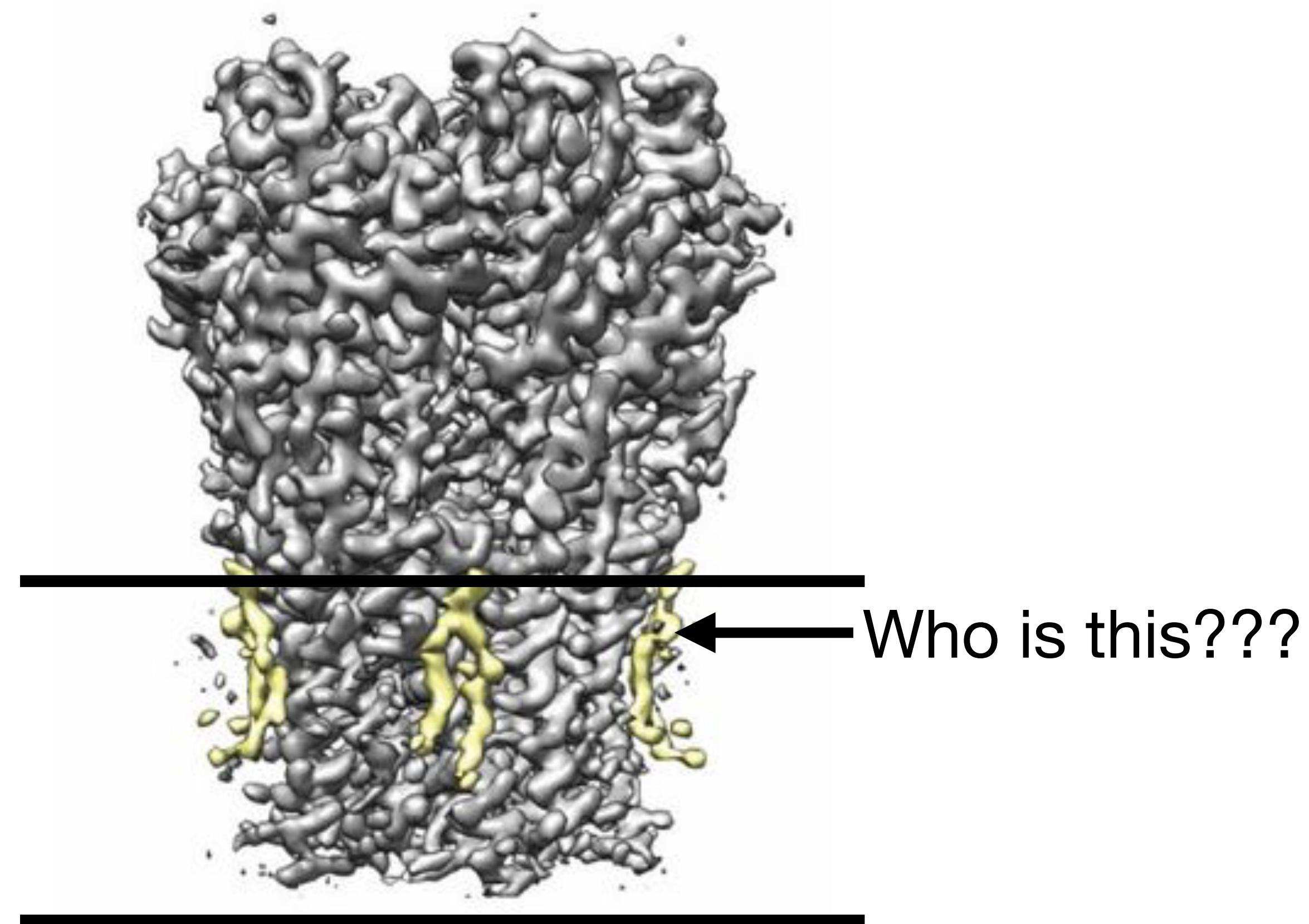
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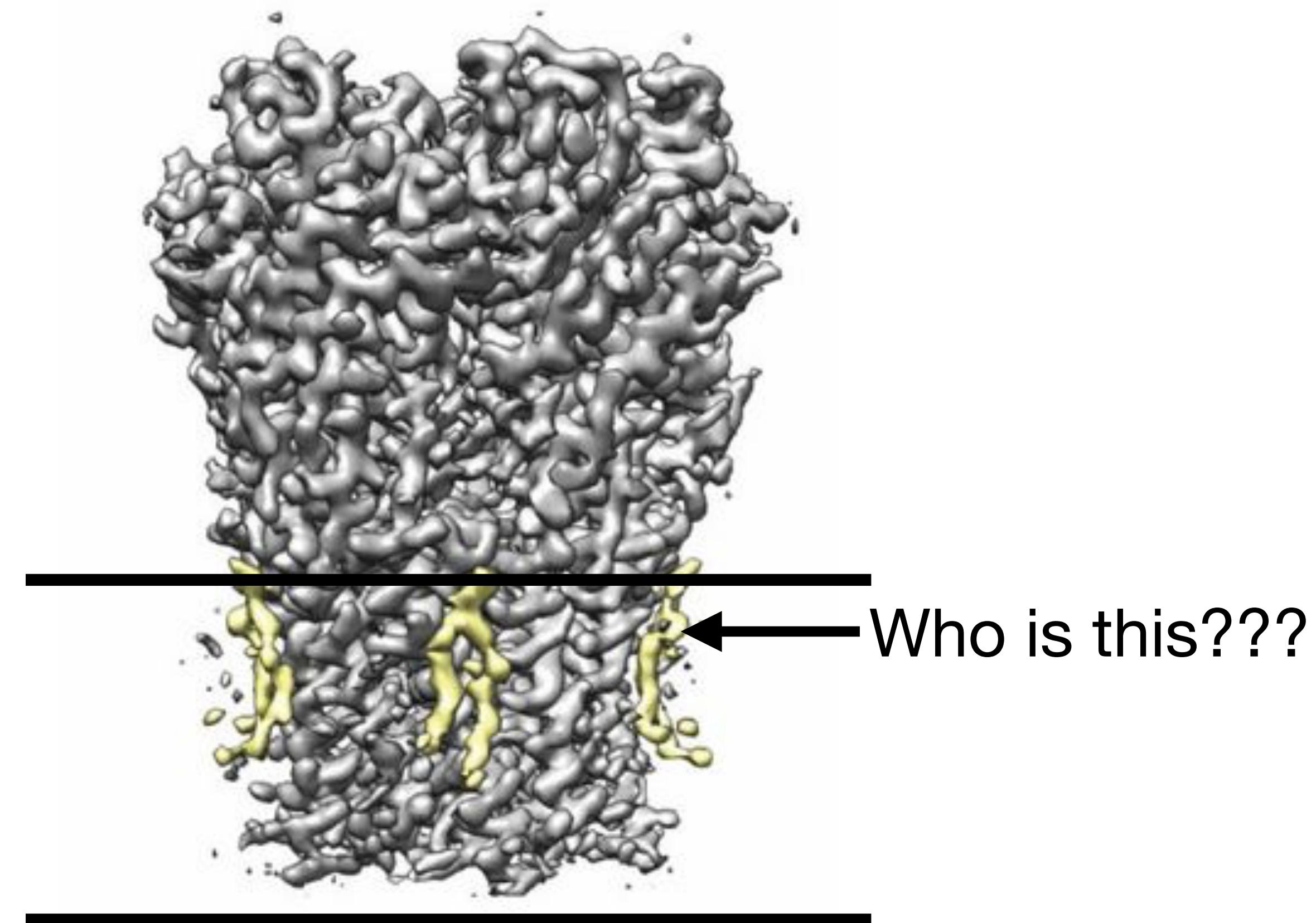
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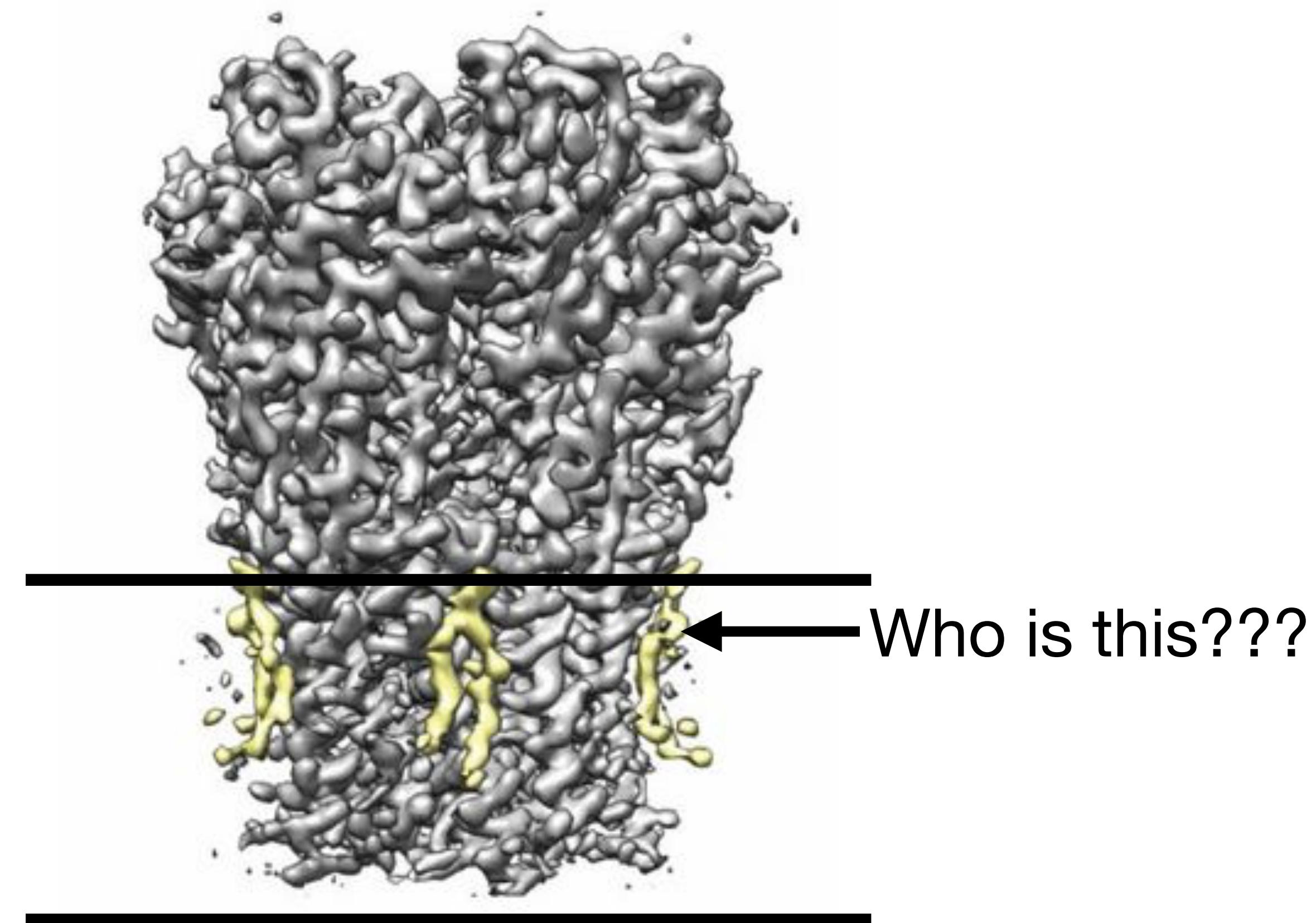
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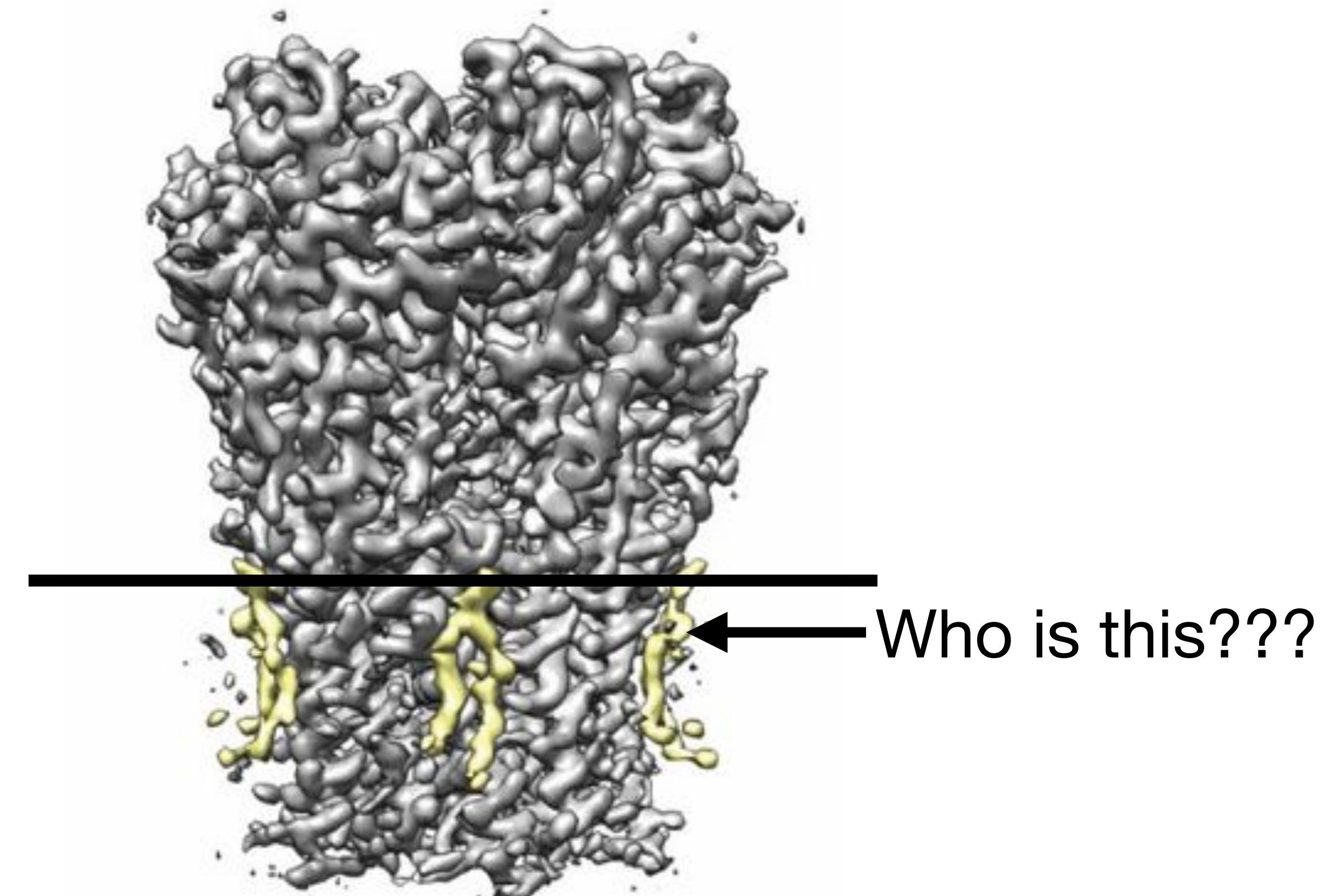
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We have a computational solution:
**Streamlined Alchemical Free Energy Perturbation
(SAFEP)**

SAFEP is a FEP implementation

- Two Flavors

SAFEP is a FEP implementation

- Two Flavors

Absolute

SAFEP is a FEP implementation

- Two Flavors

Absolute

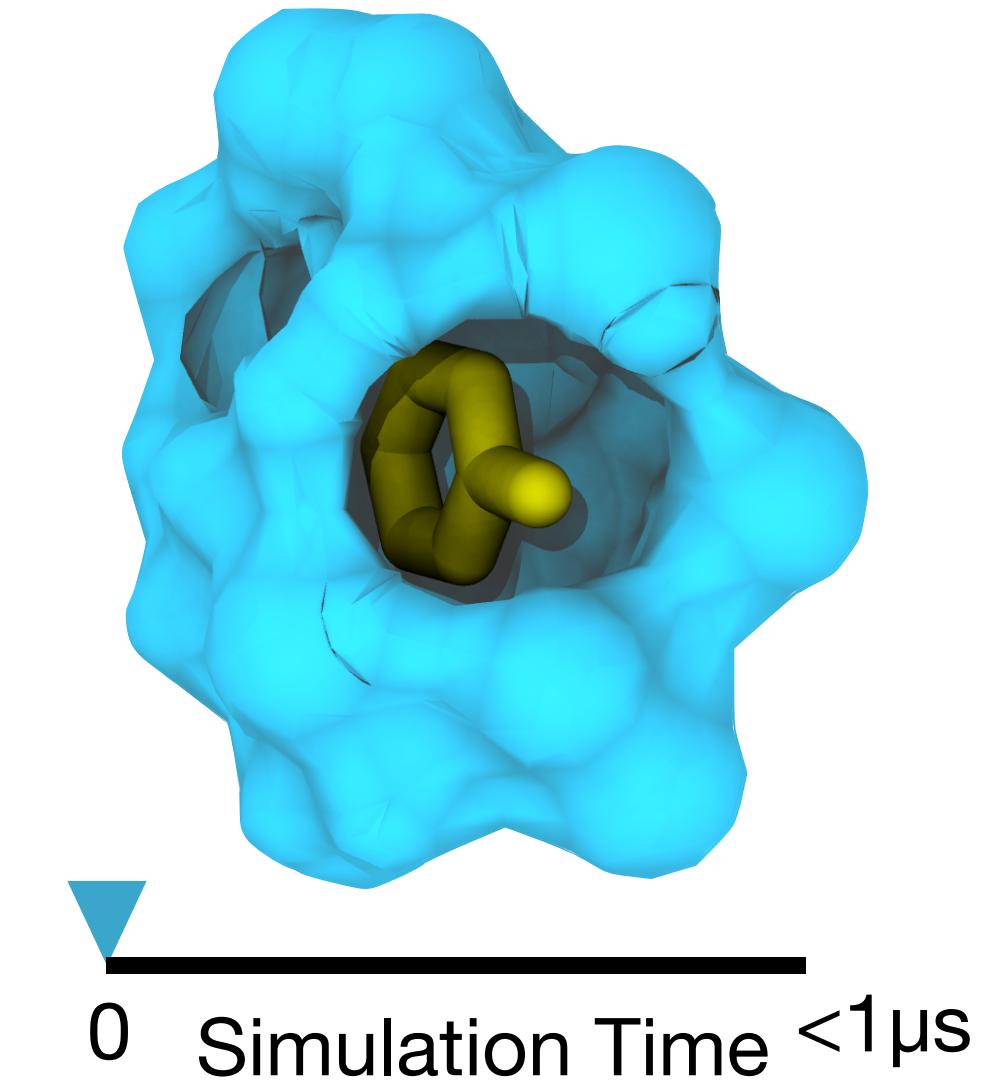
Estimate affinity of one ligand

SAFEP is a FEP implementation

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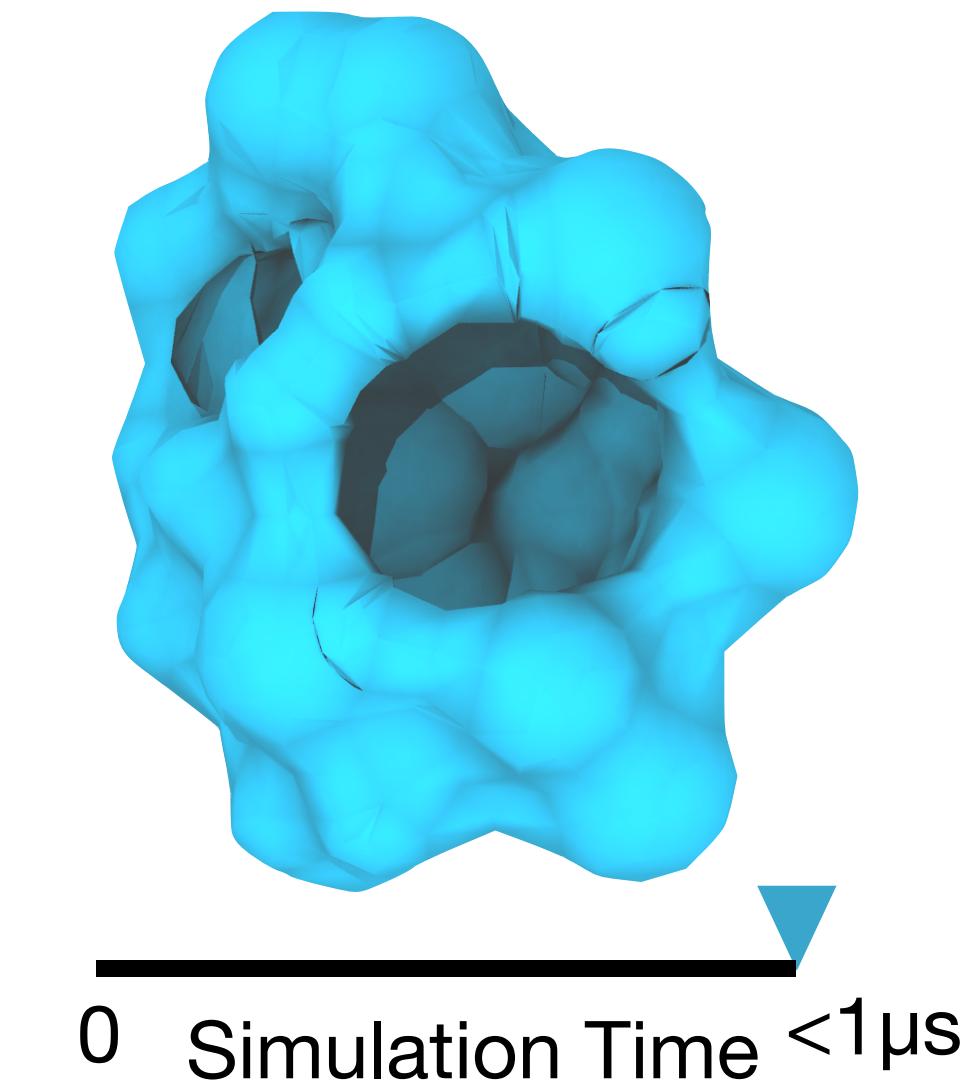
0 Simulation Time <1μs

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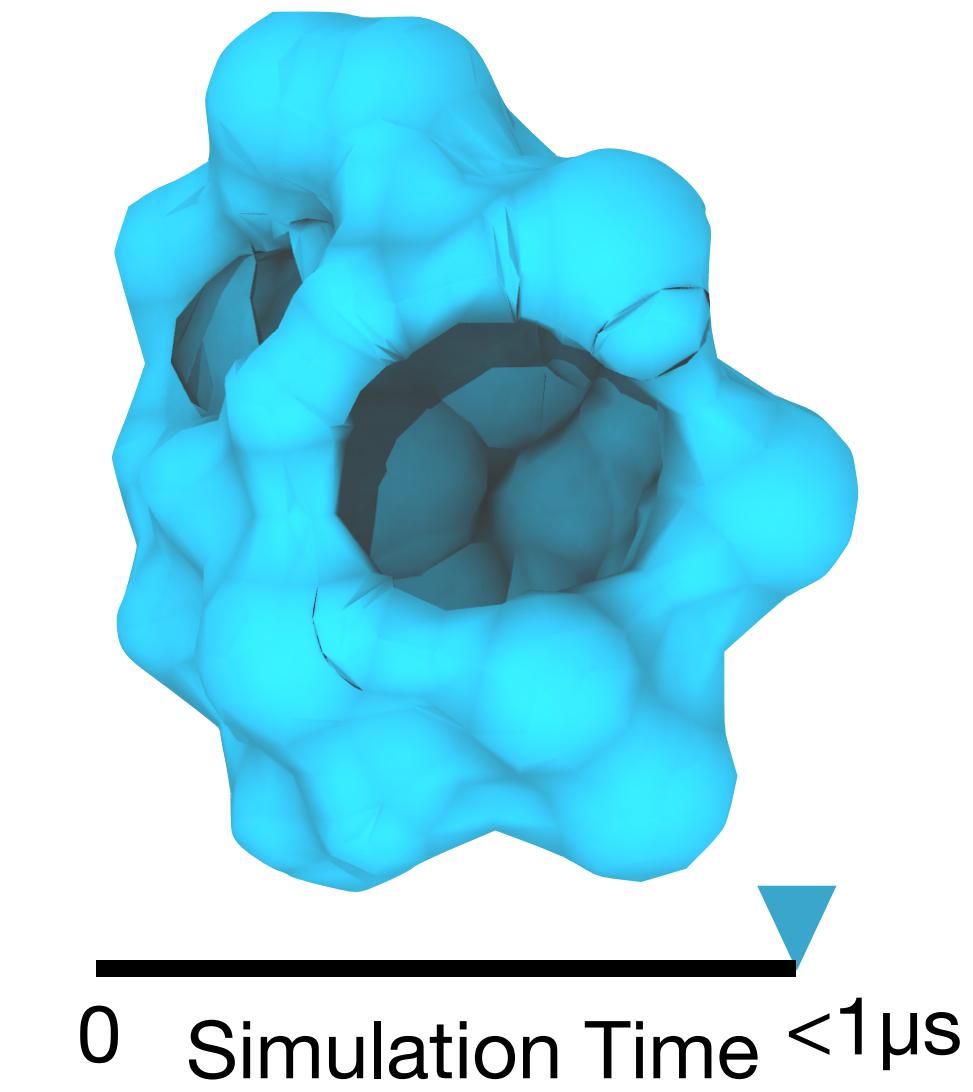
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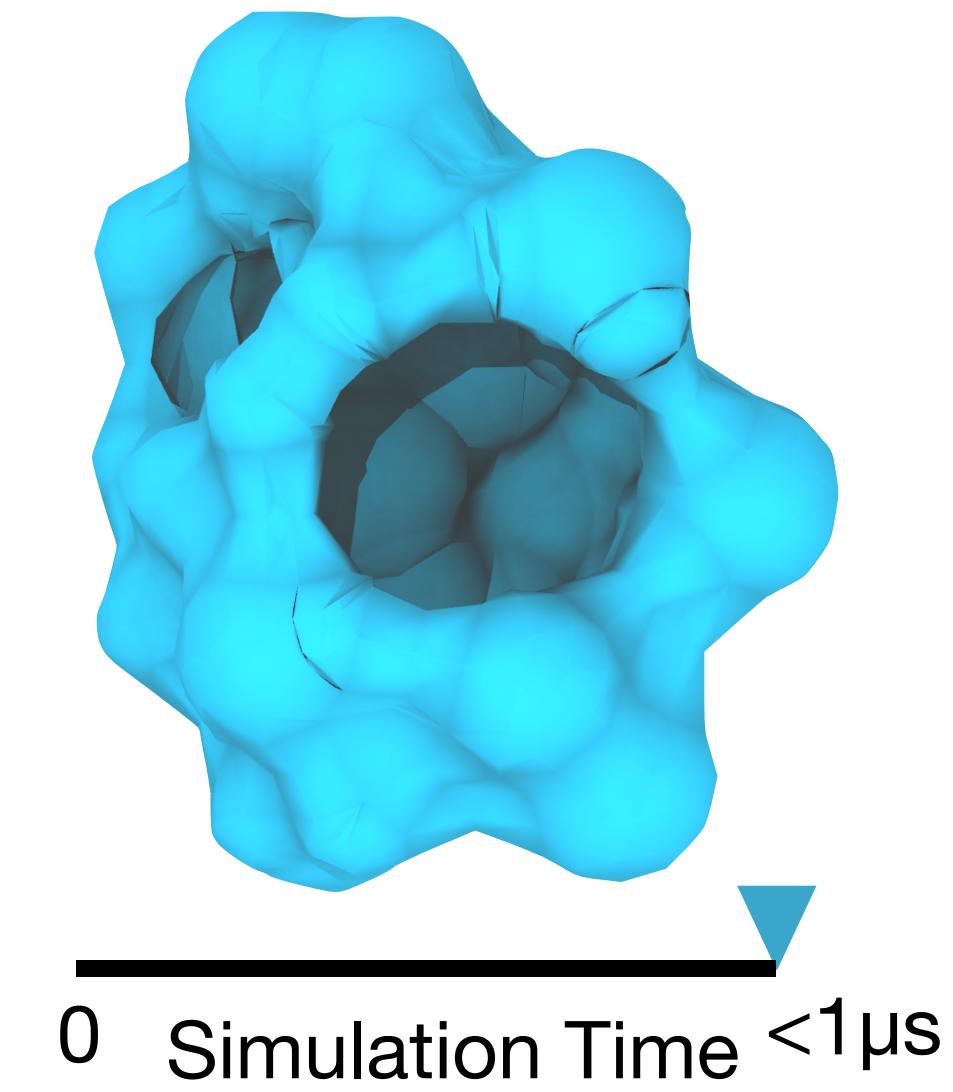
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Con: Long simulations



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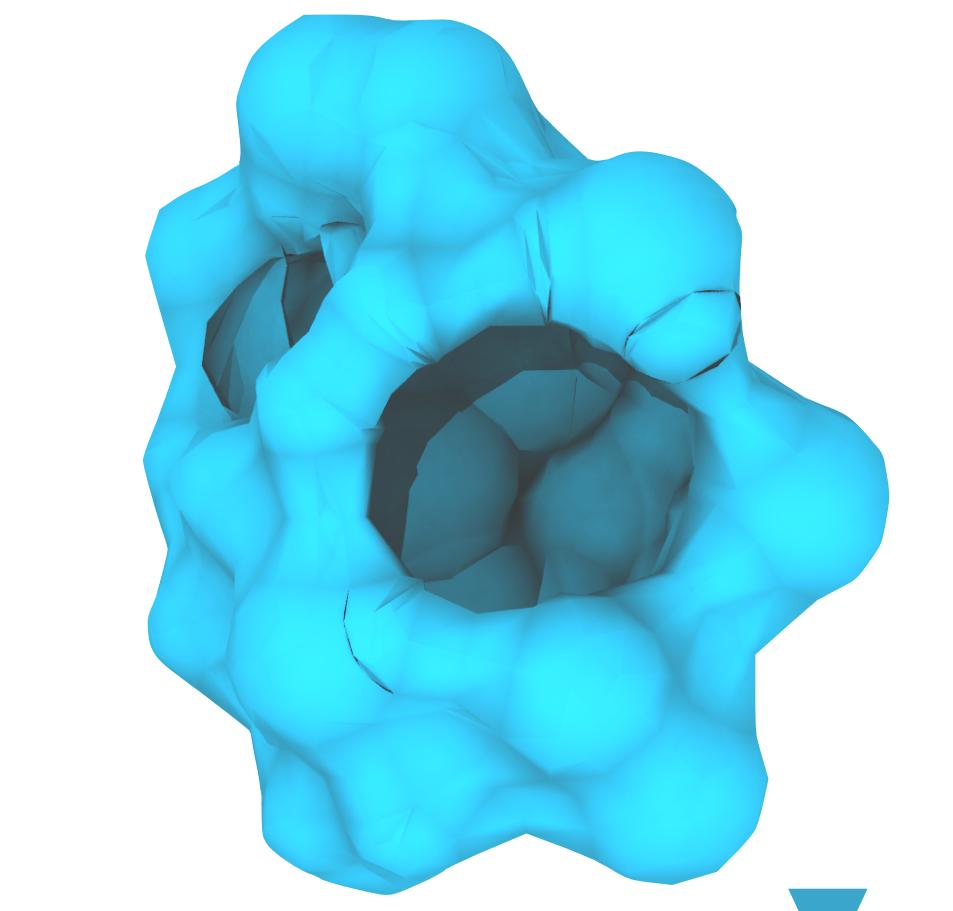
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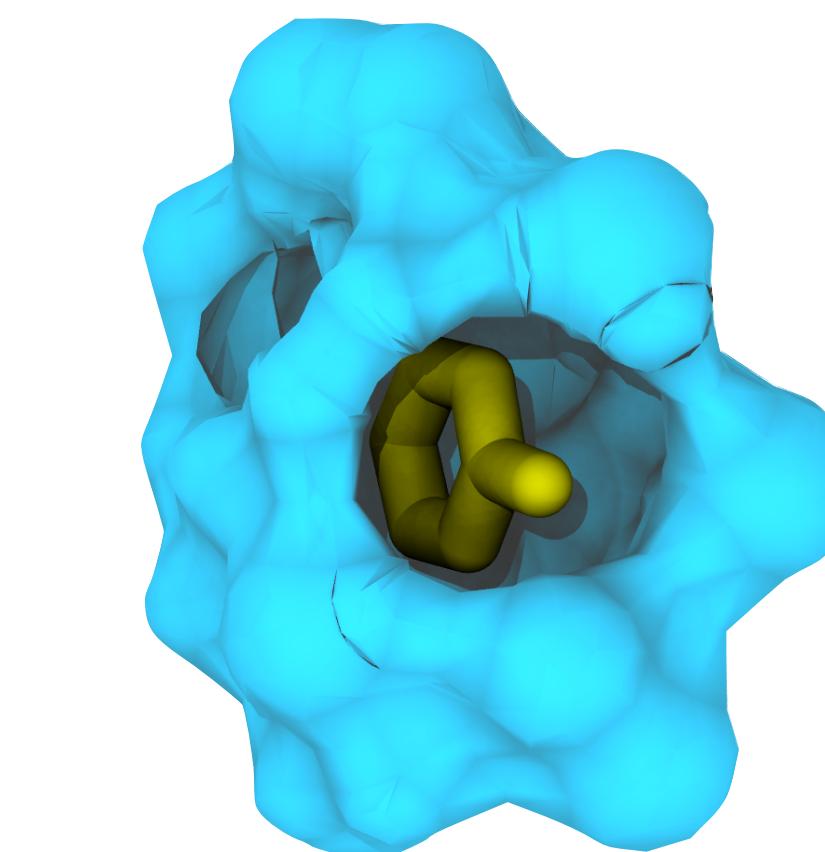
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0 Simulation Time <1μs

Relative



0 ~100ns 13
Simulation Time

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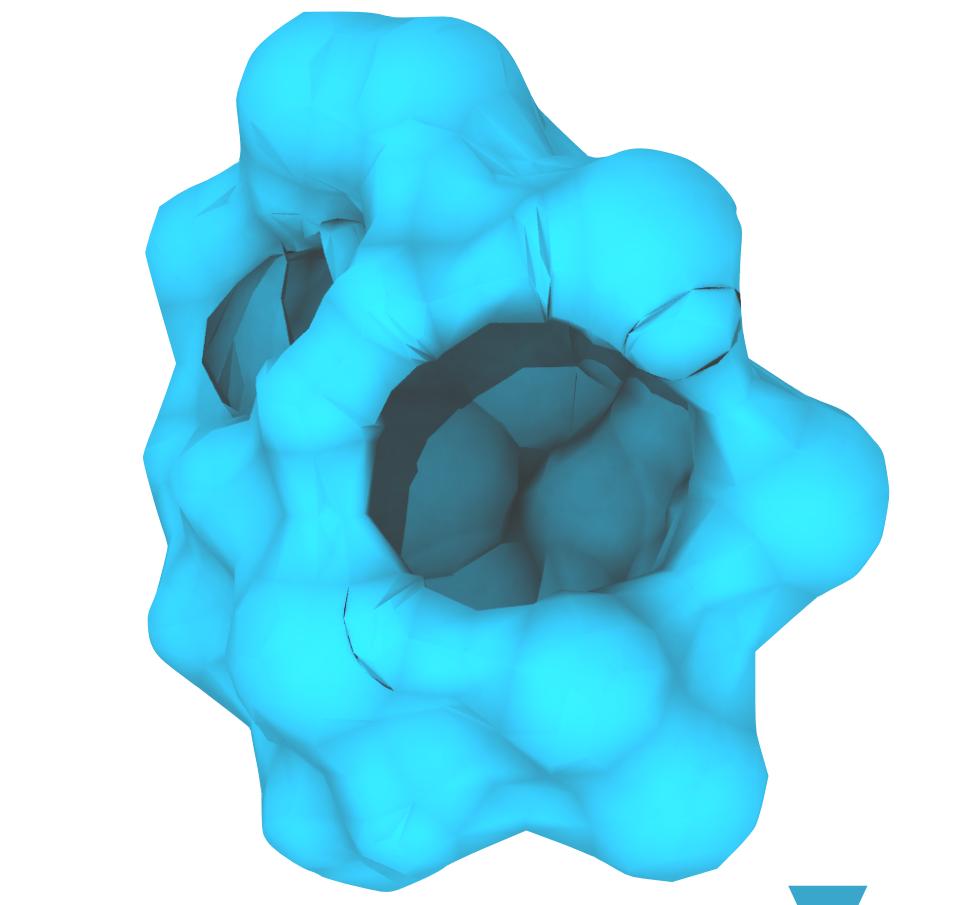
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Estimate affinity of one ligand

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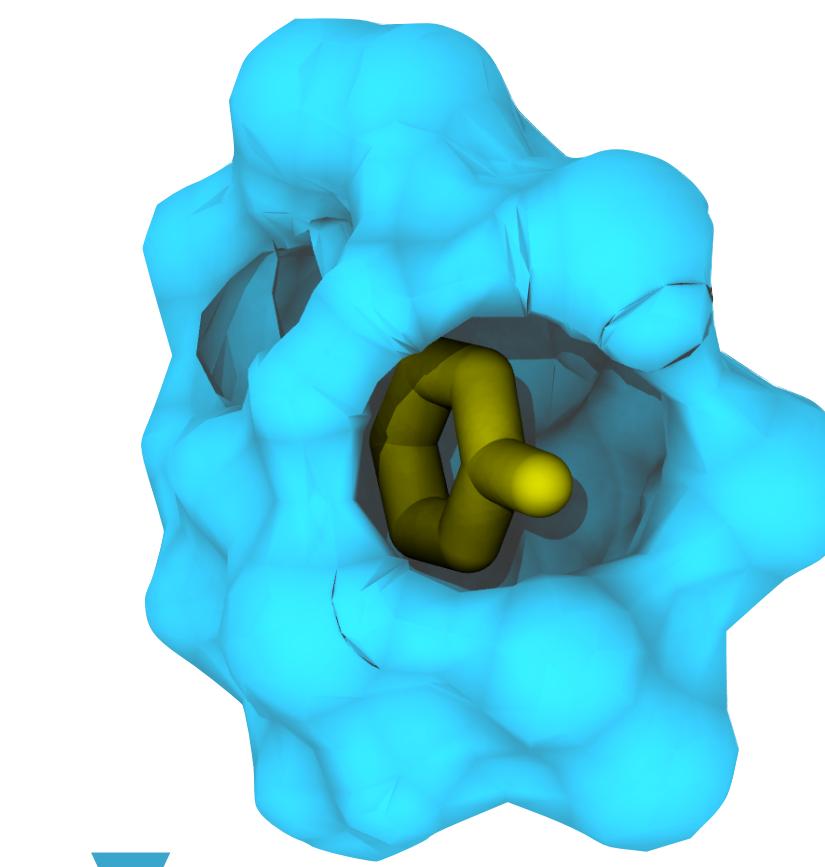
Con: Long simulations



0 Simulation Time < 1 μ s

Relative

Compare affinities of two ligands



0 ~ 100 ns 13
Simulation Time

SAFEP is a FEP implementation

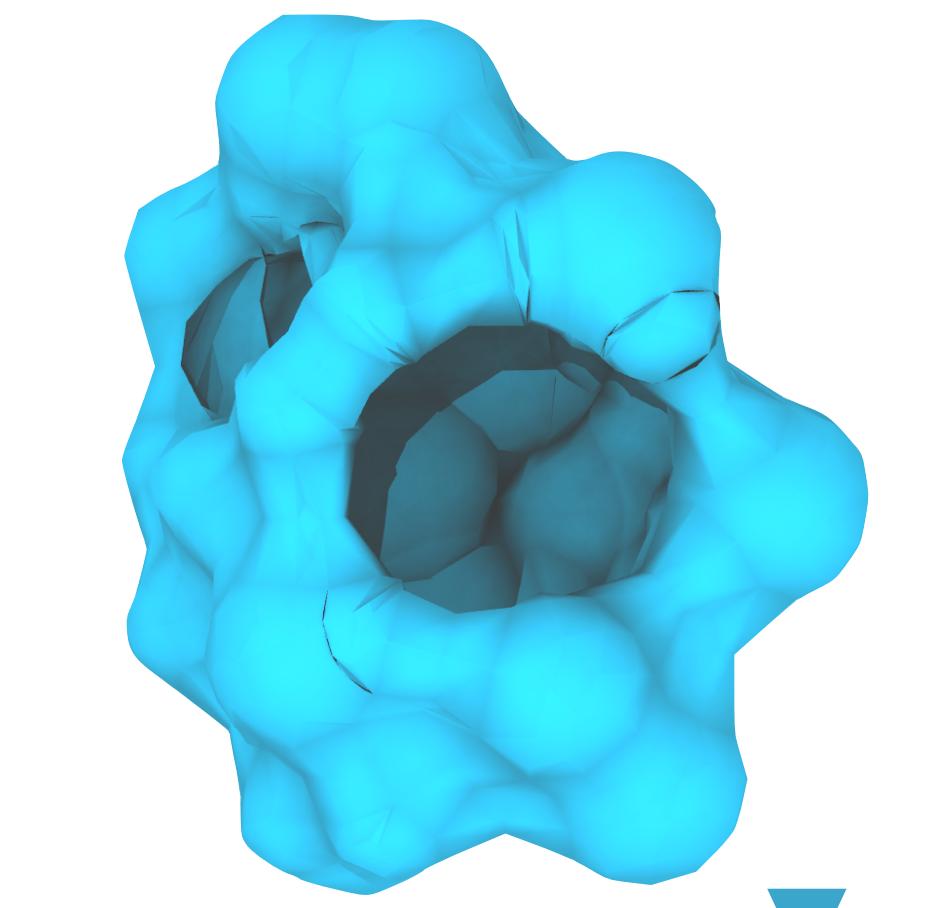
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Absolute

Estimate affinity of one ligand

Pro: Simple system

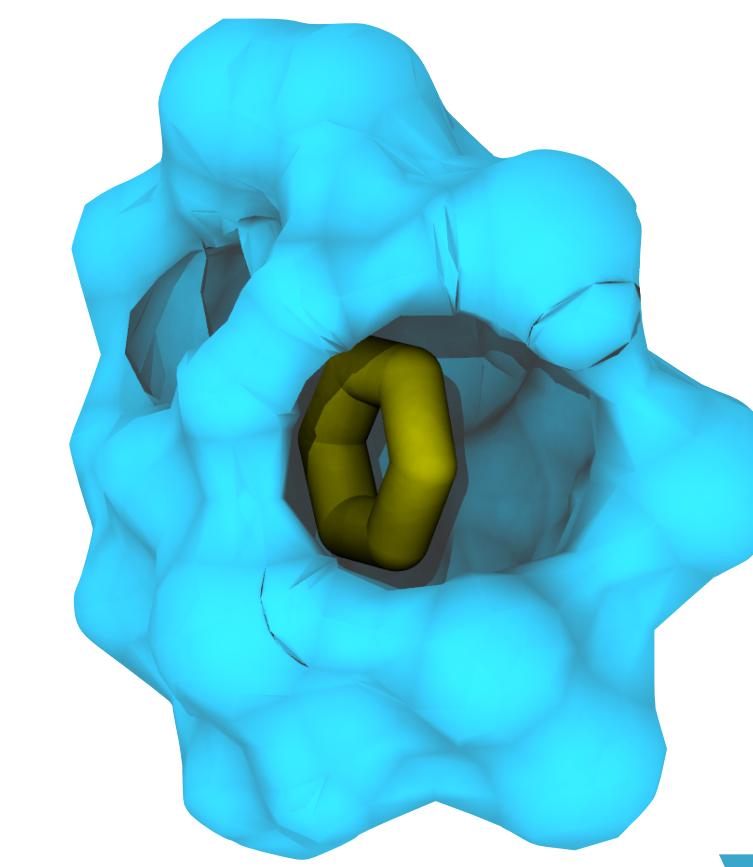
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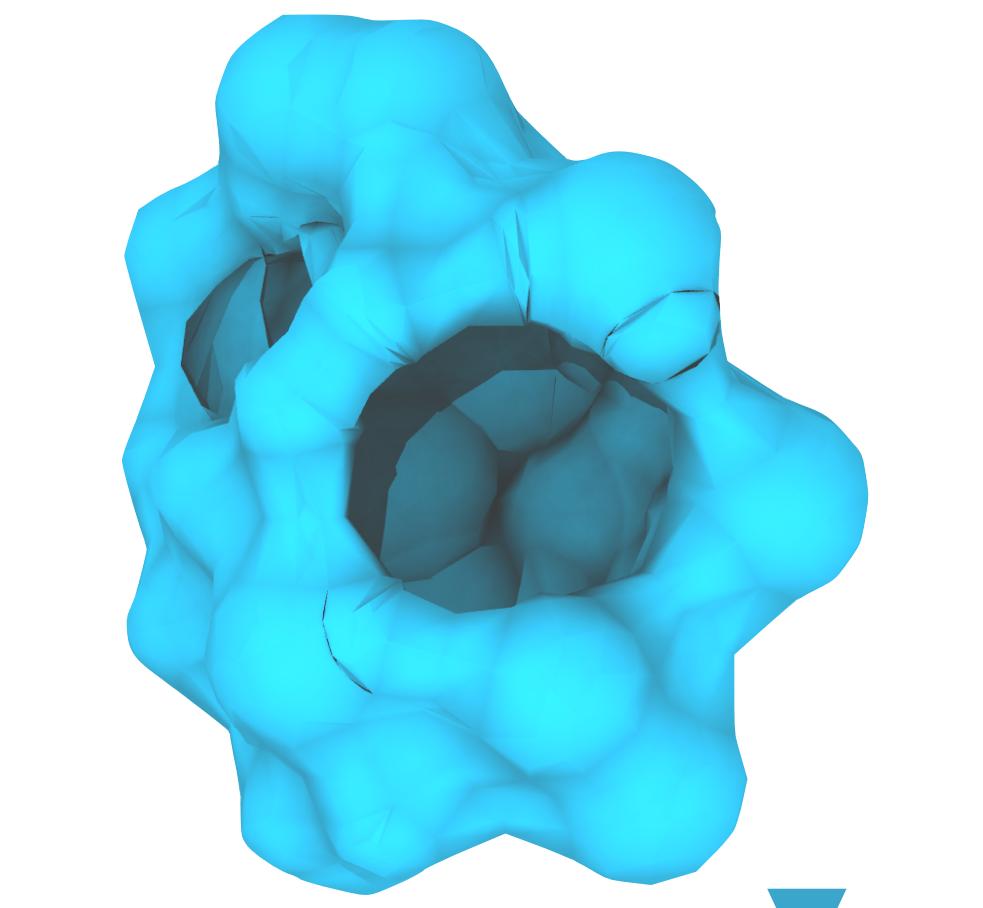
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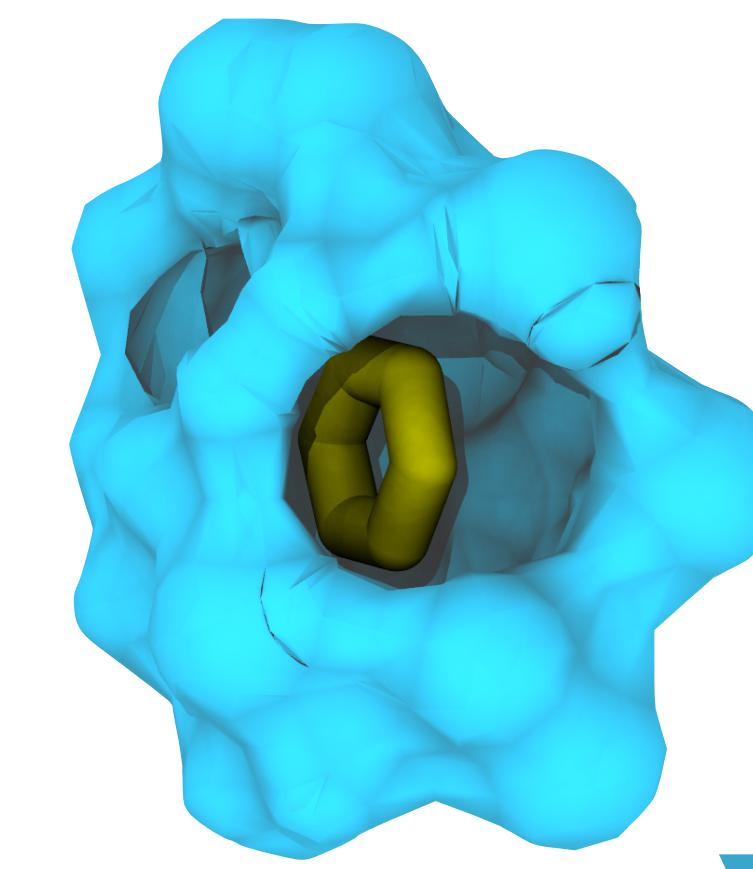


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Relative

Compare affinities of two ligands

Pro: Shorter simulations



0 ~100ns 13
Simulation Time

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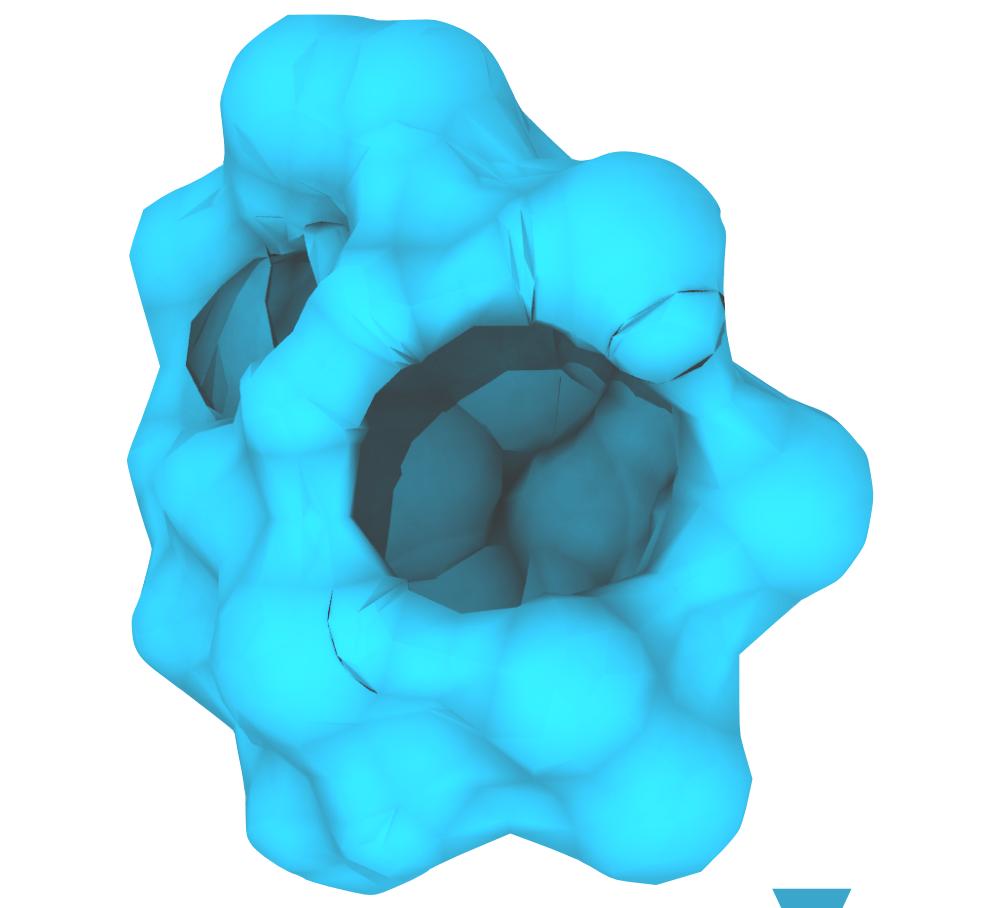
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Estimate affinity of one ligand

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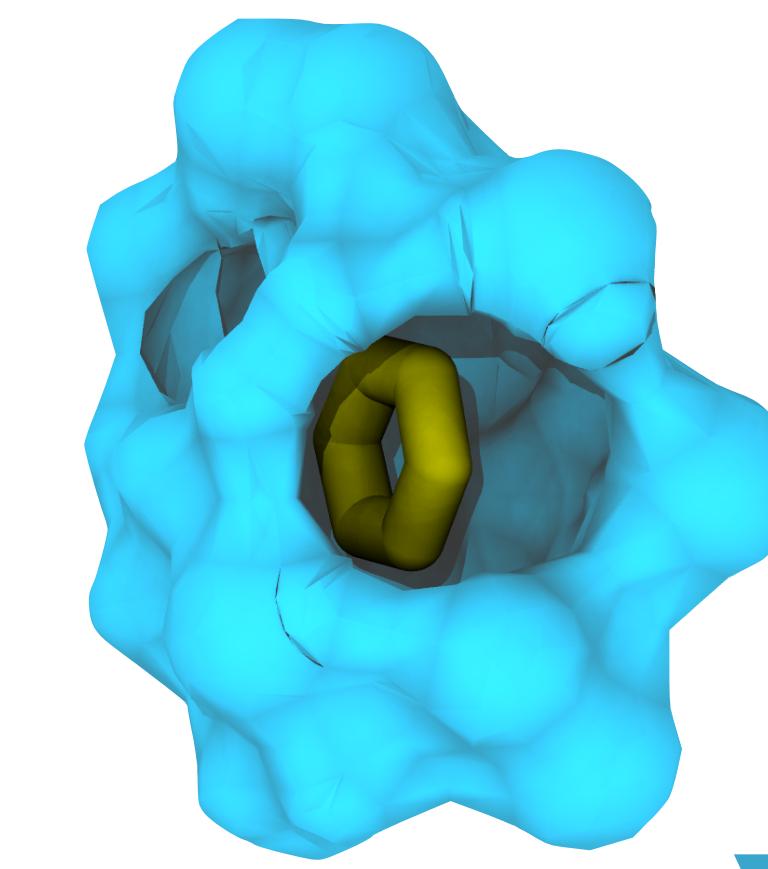
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Relative

Compare affinities of two ligands

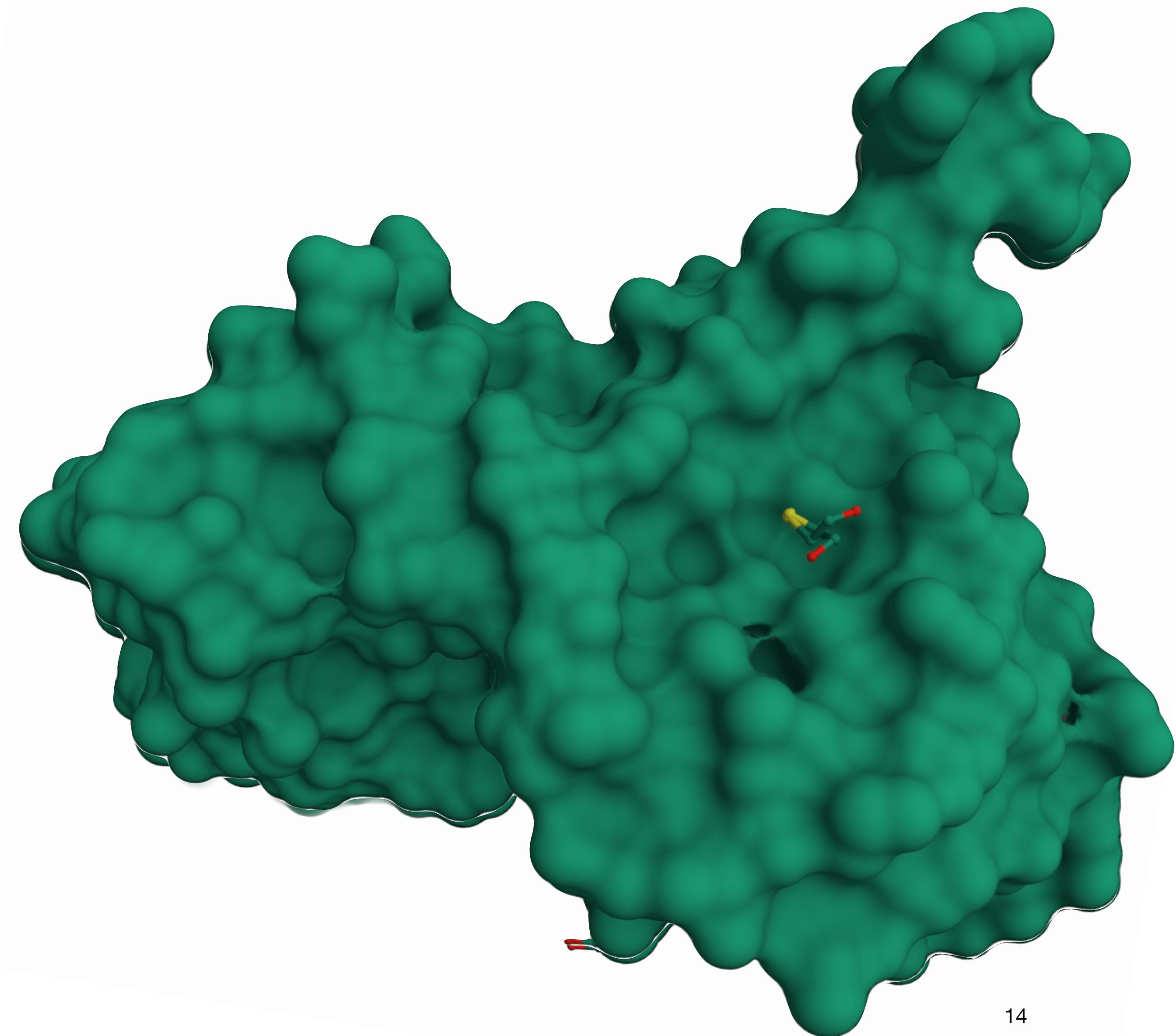
Pro: Shorter simulations

Con: Only good for competition



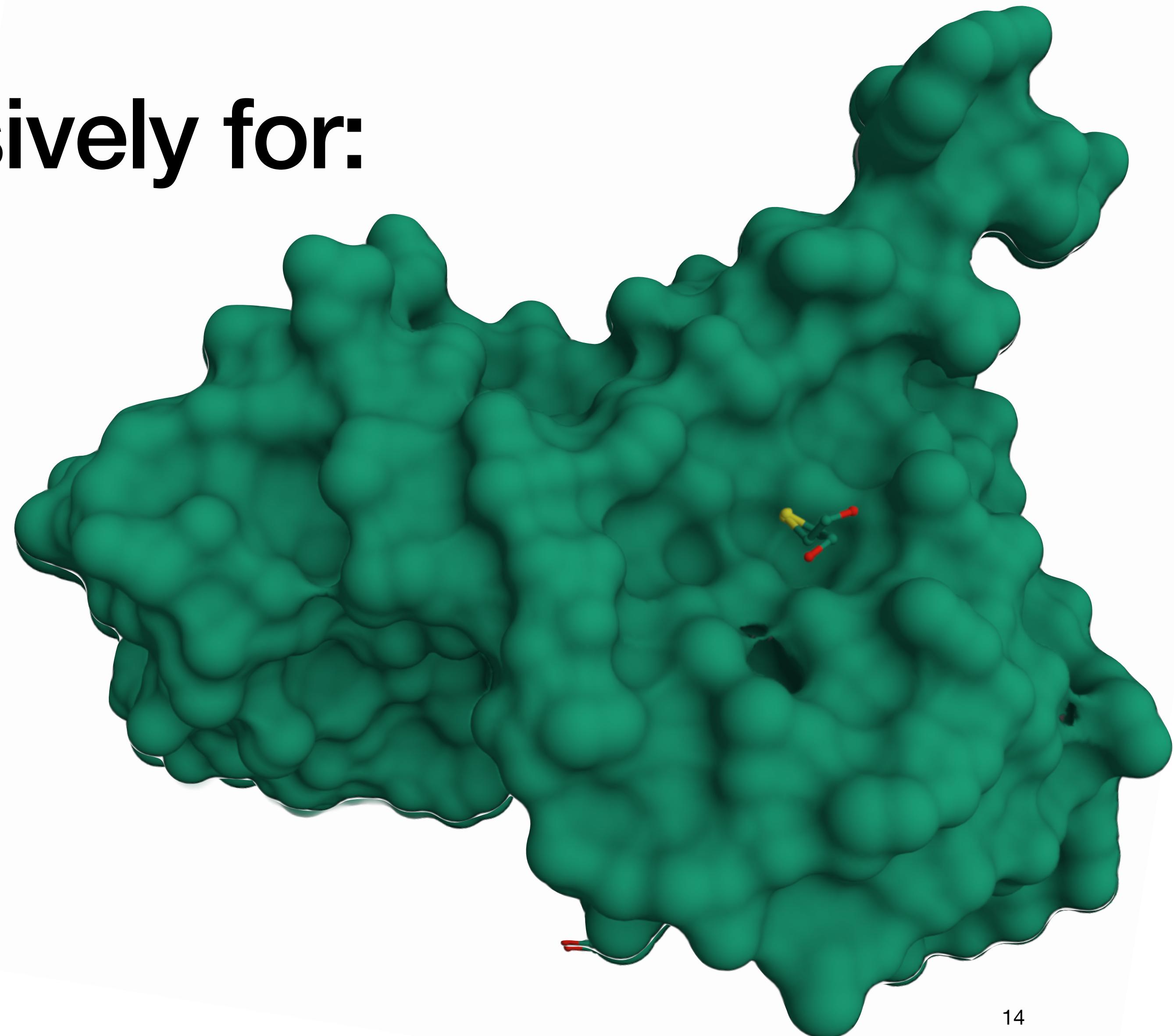
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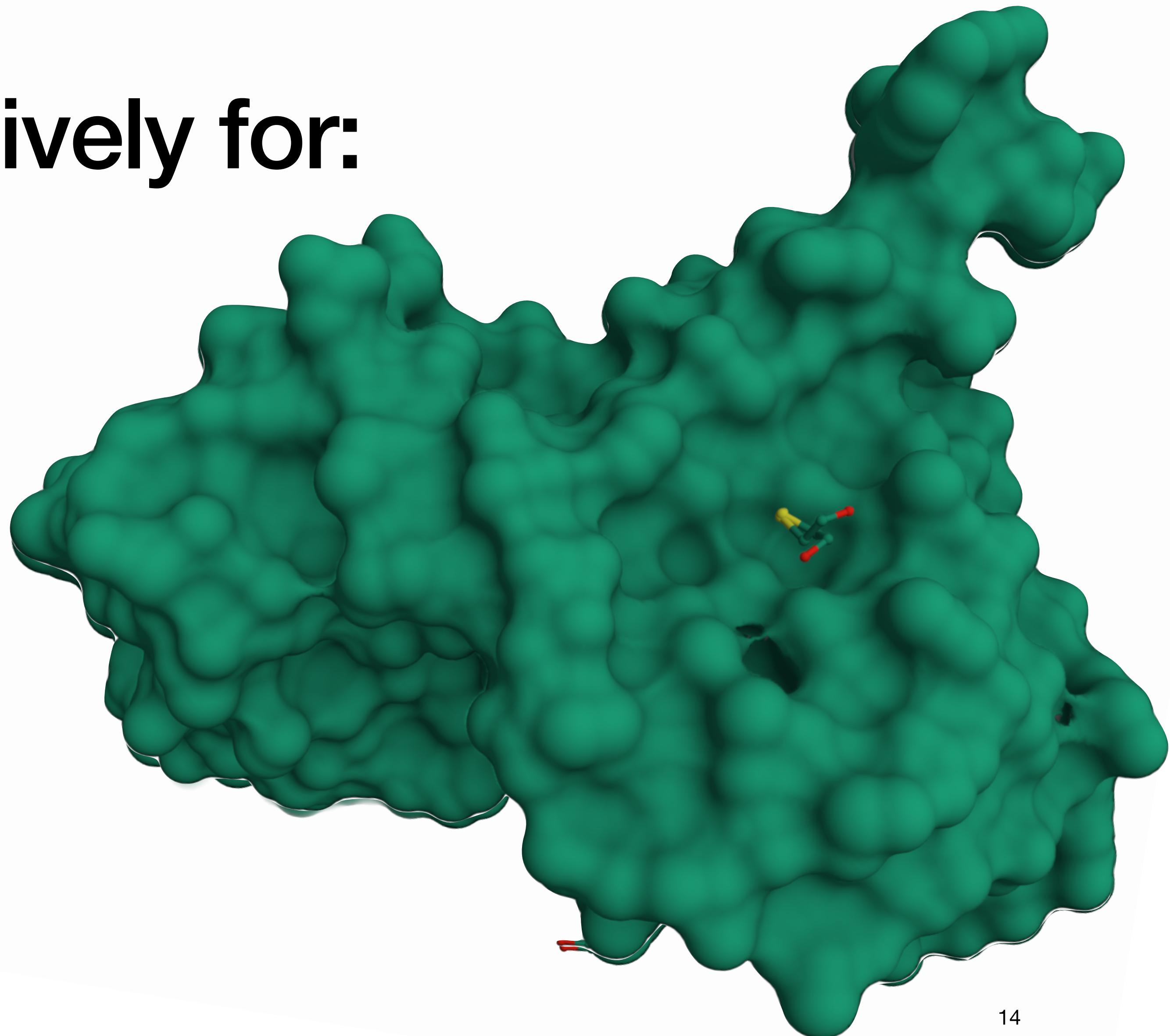
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SAFEP is a FEP implementation

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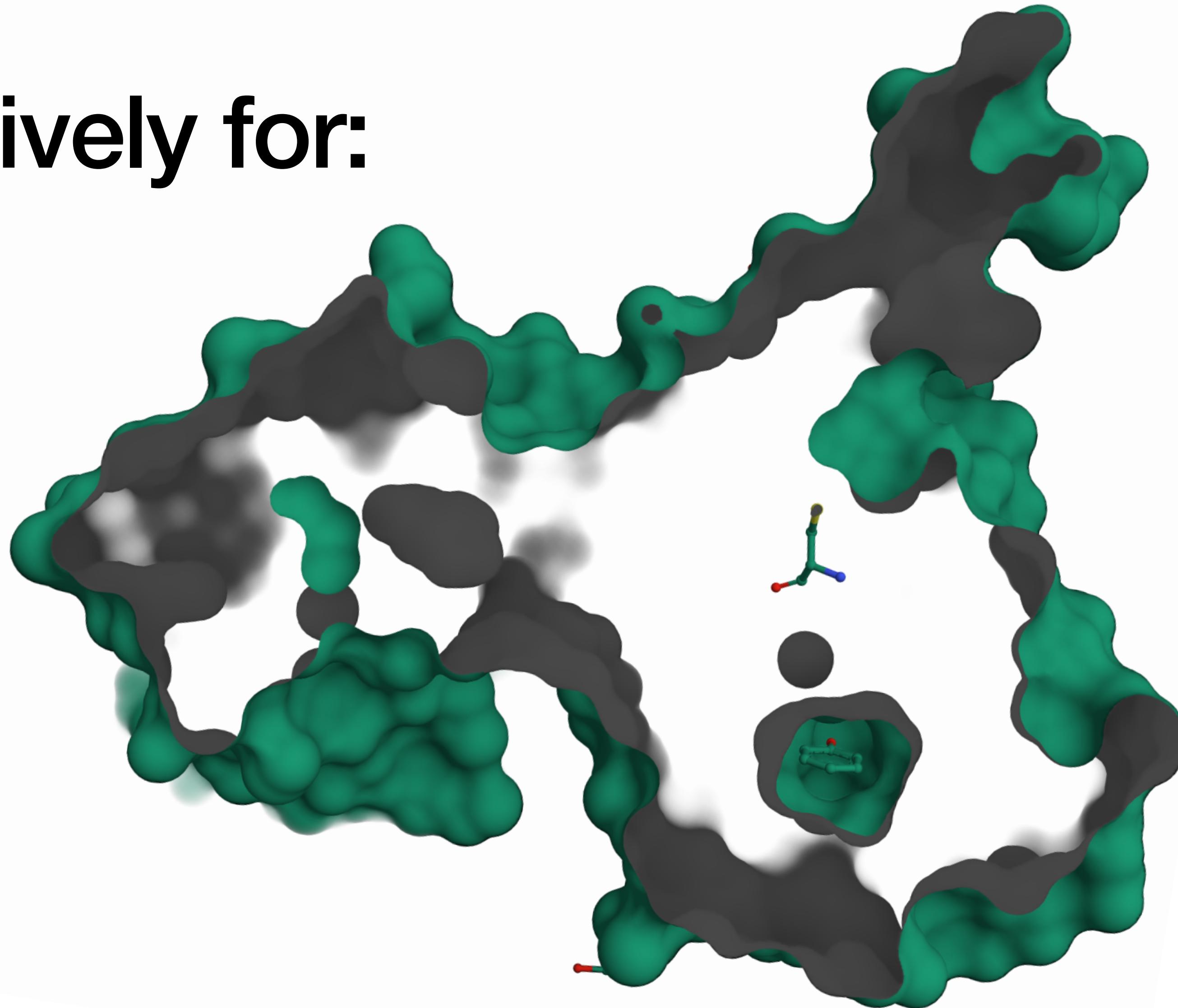
- Soluble proteins



SAFEP is a FEP implementation

FEP is used extensively for:

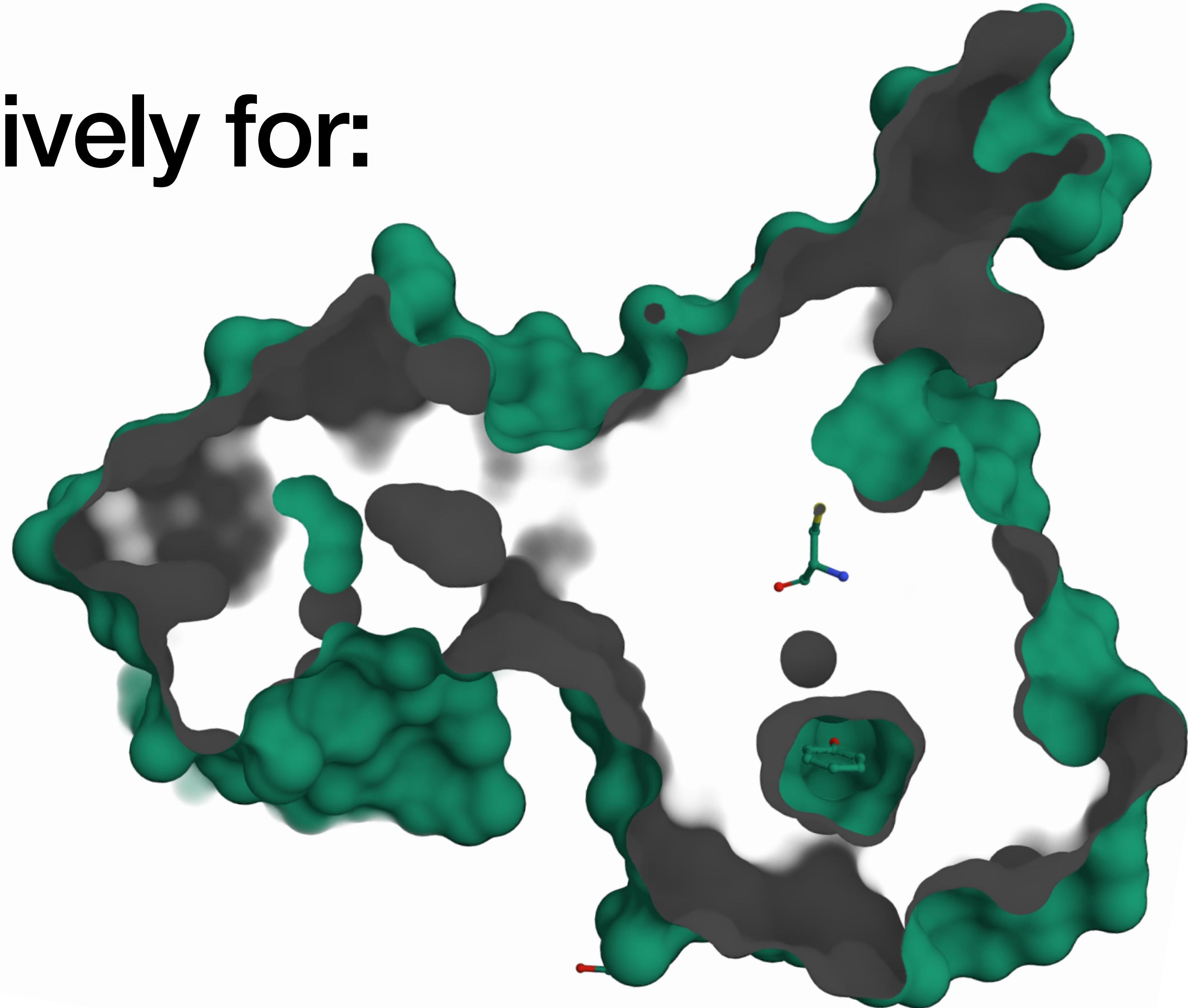
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SAFEP is a FEP implementation

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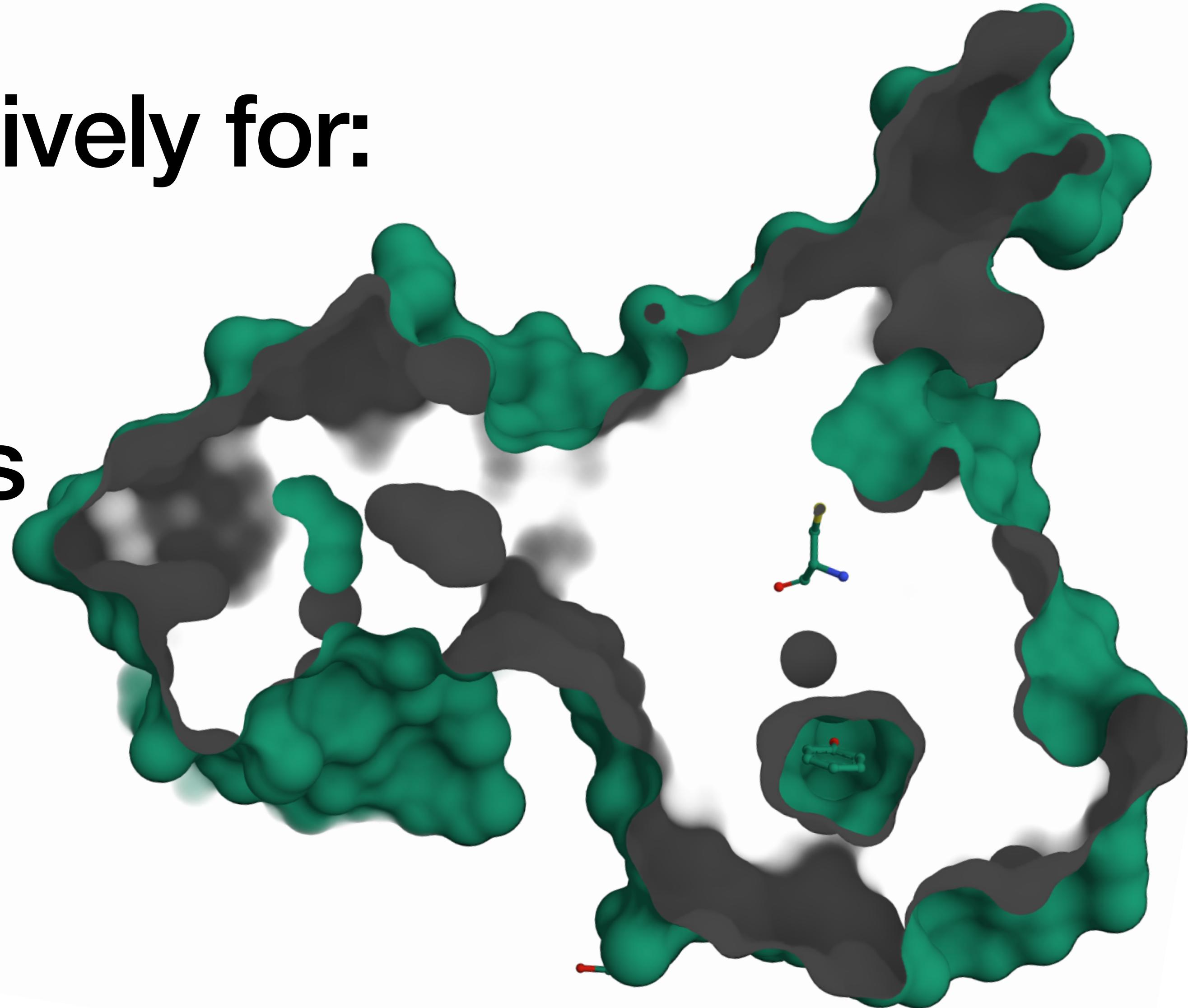
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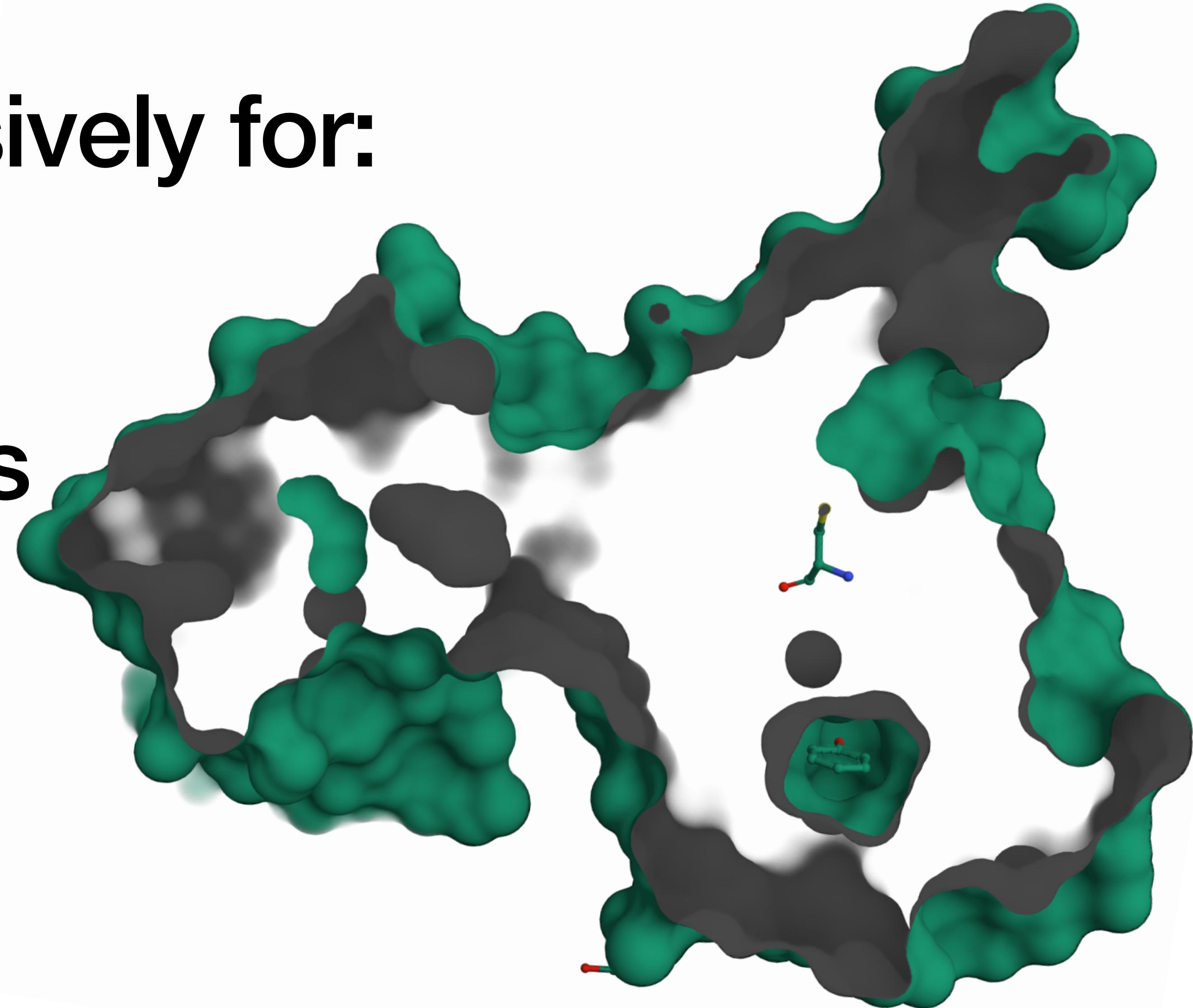
- Soluble proteins
- Rigid ligands
- In buried pockets



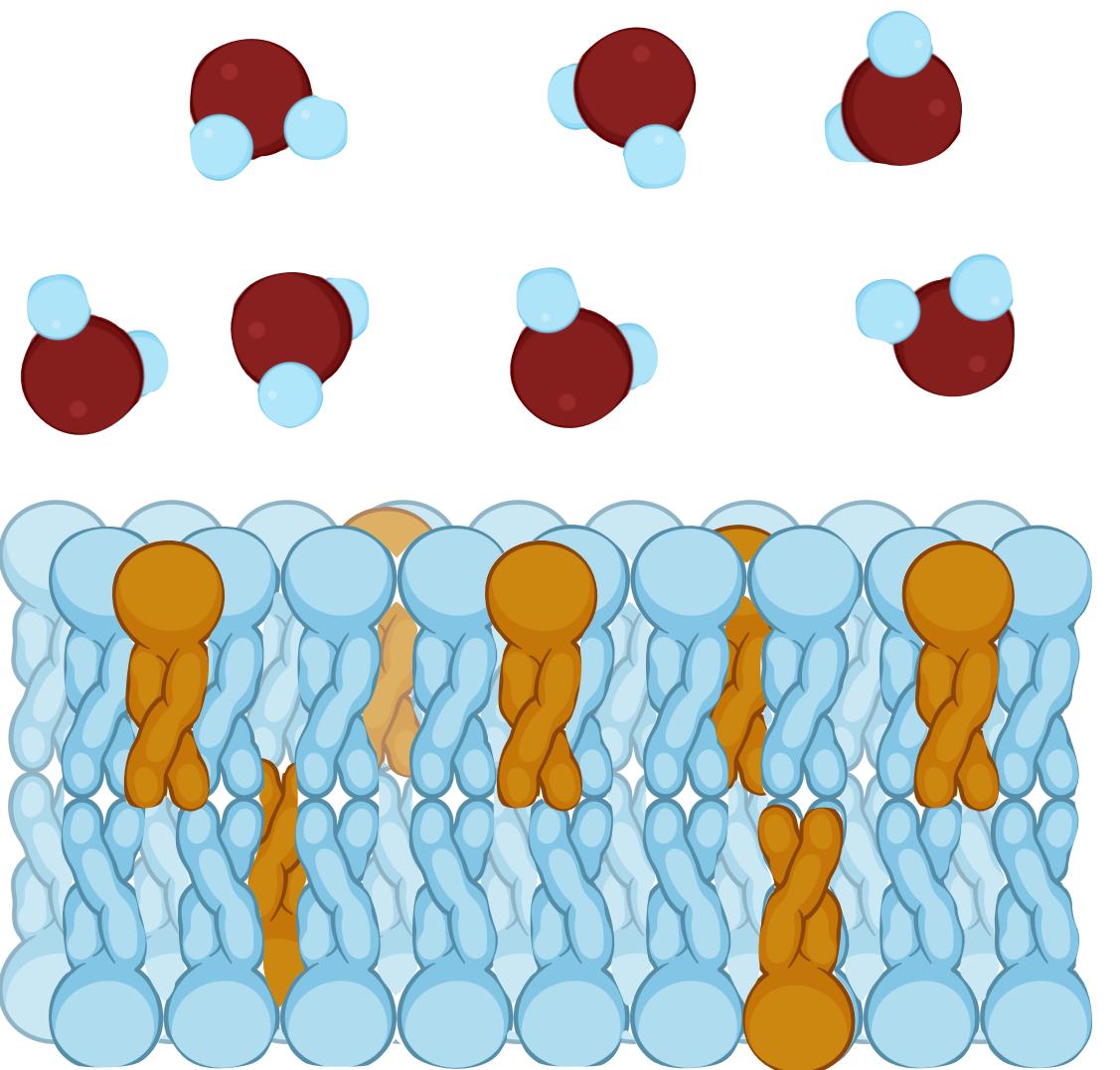
SAFEP is a FEP implementation

FEP is used extensively for:

- Soluble proteins
- Rigid ligands
- In buried pockets
- To get a ΔG_{bind}^0

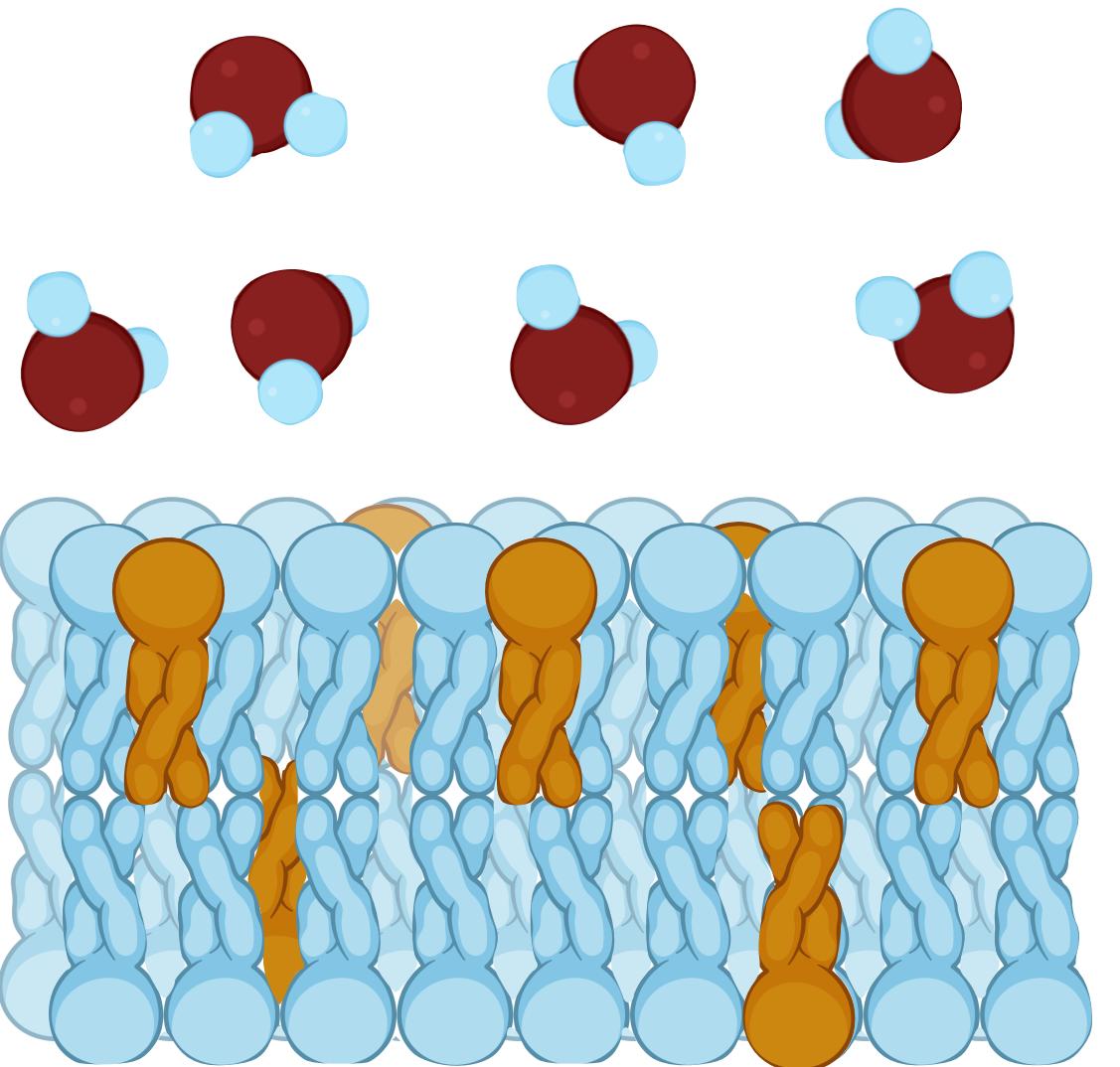


Challenges of binding in membranes



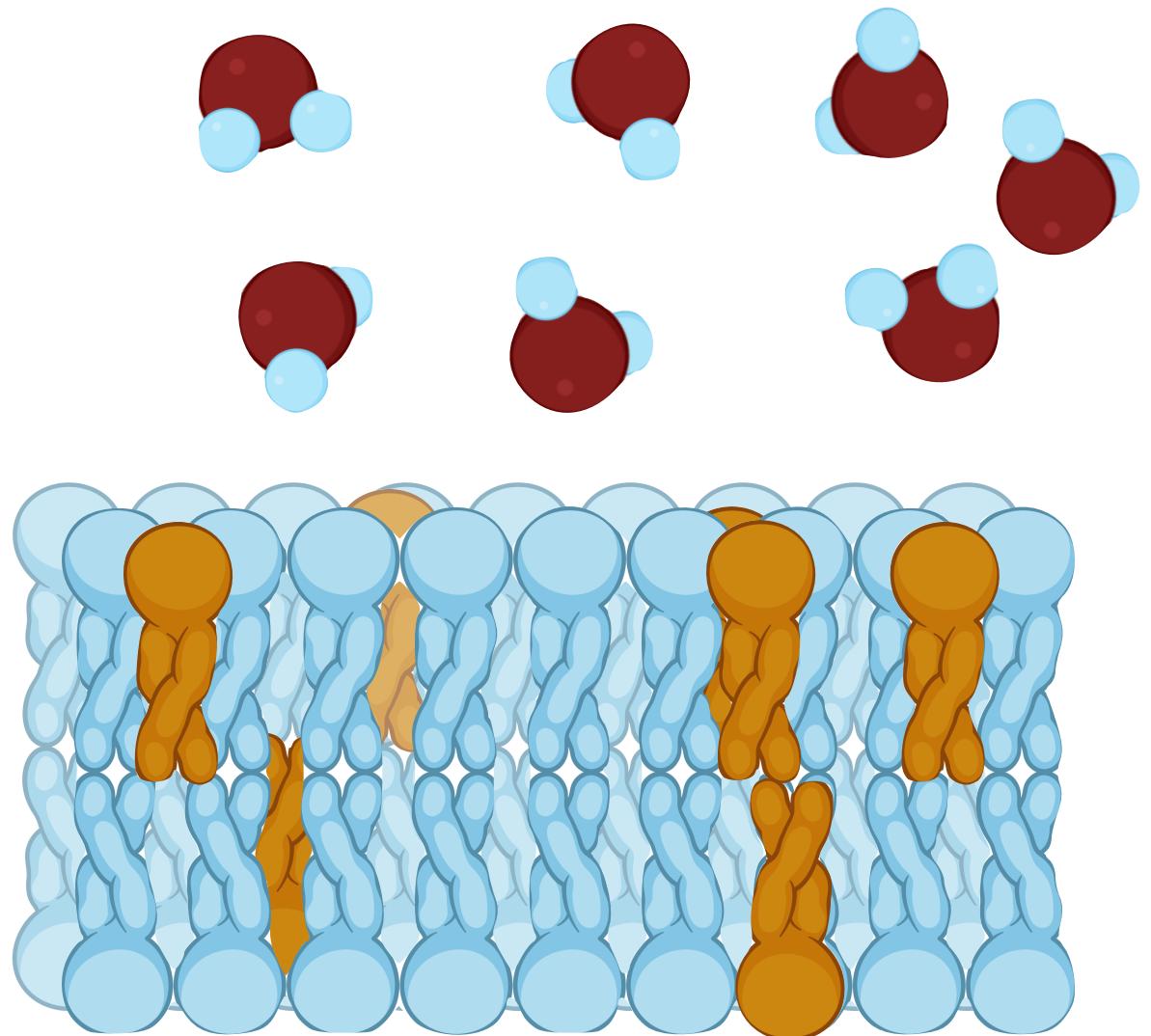
Challenges of binding in membranes

Lipids are slow and flexible



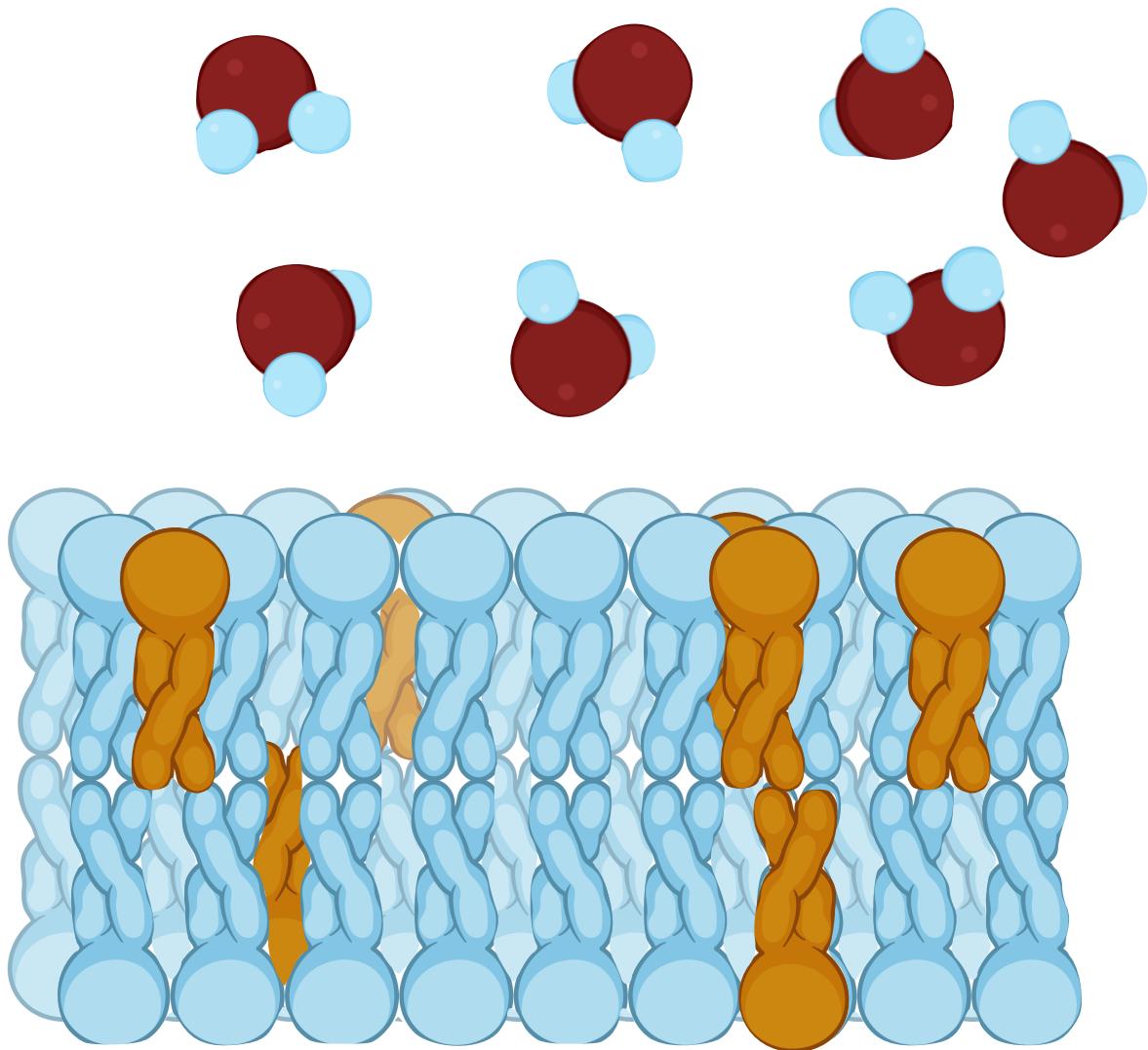
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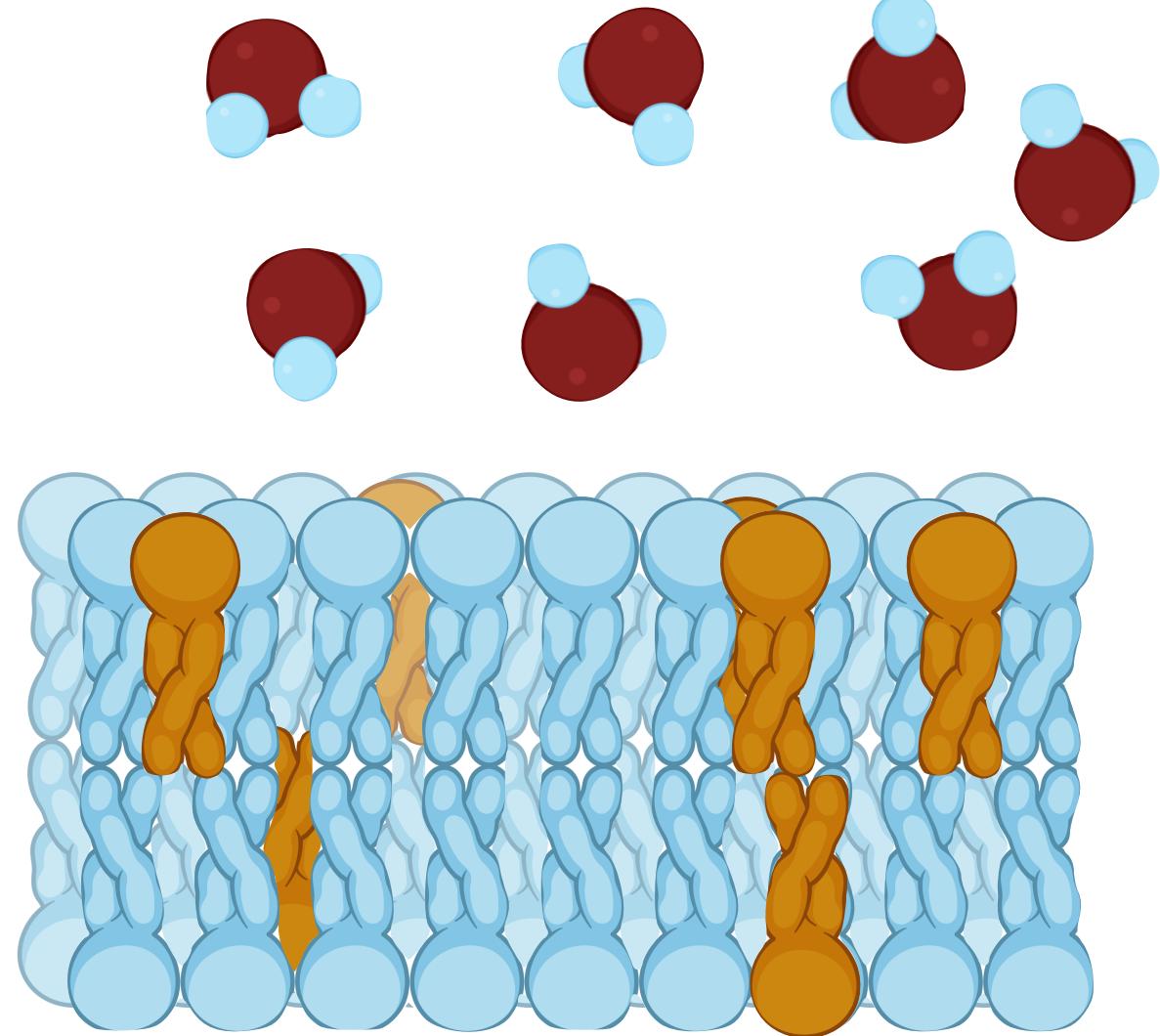
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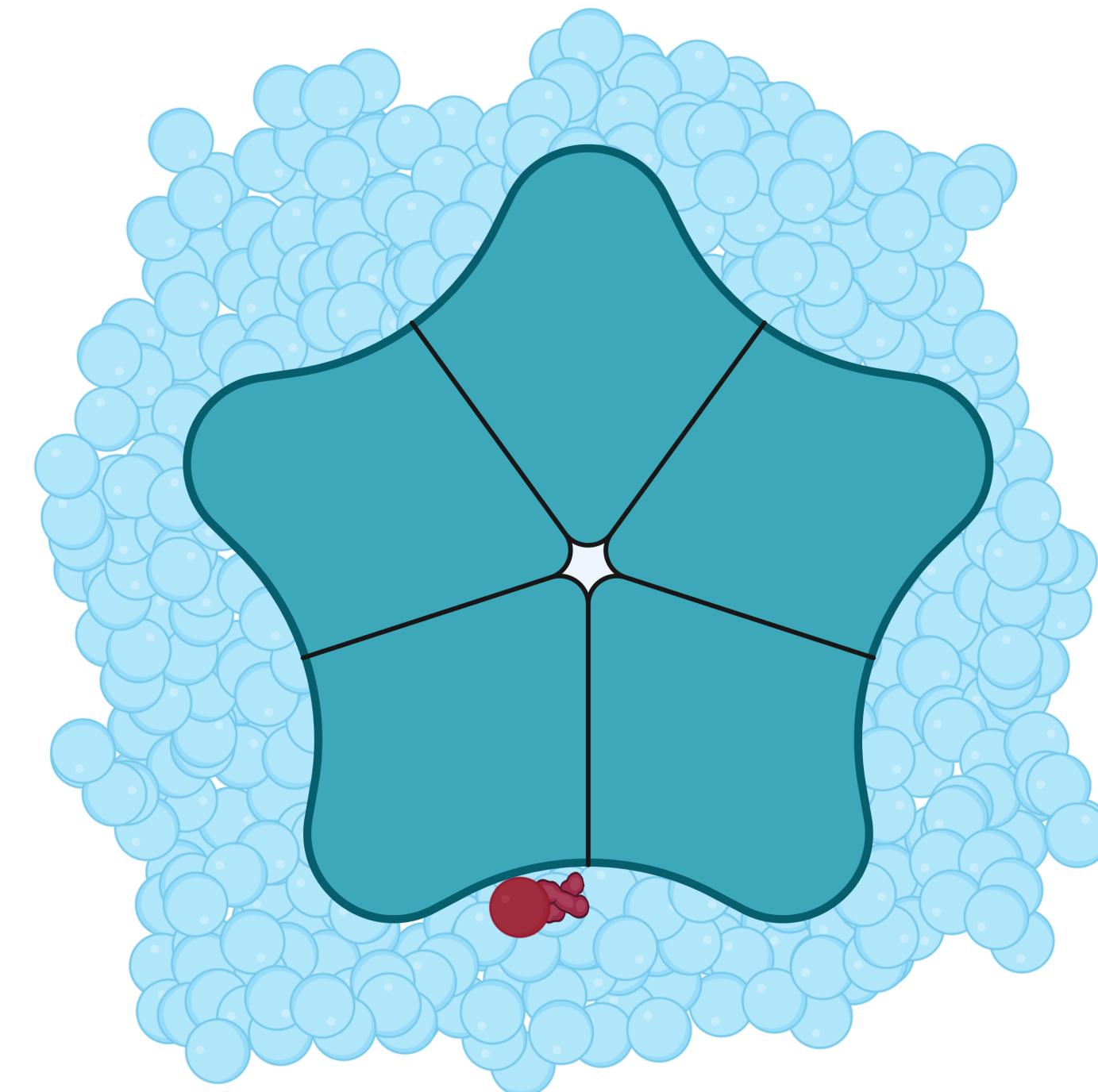
Highly heterogeneous
(Standard state is ill-defined)

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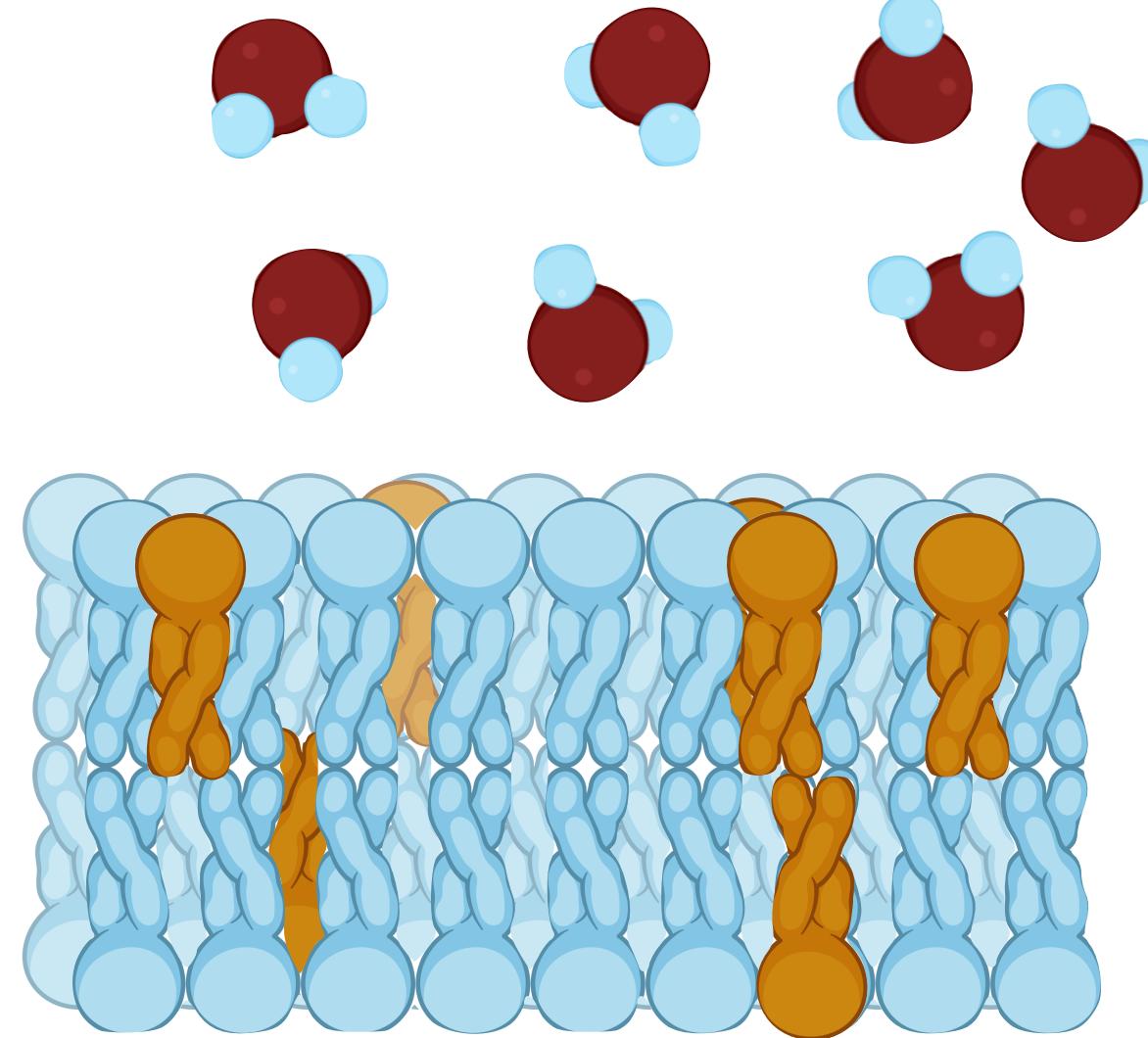
Superficial binding sites



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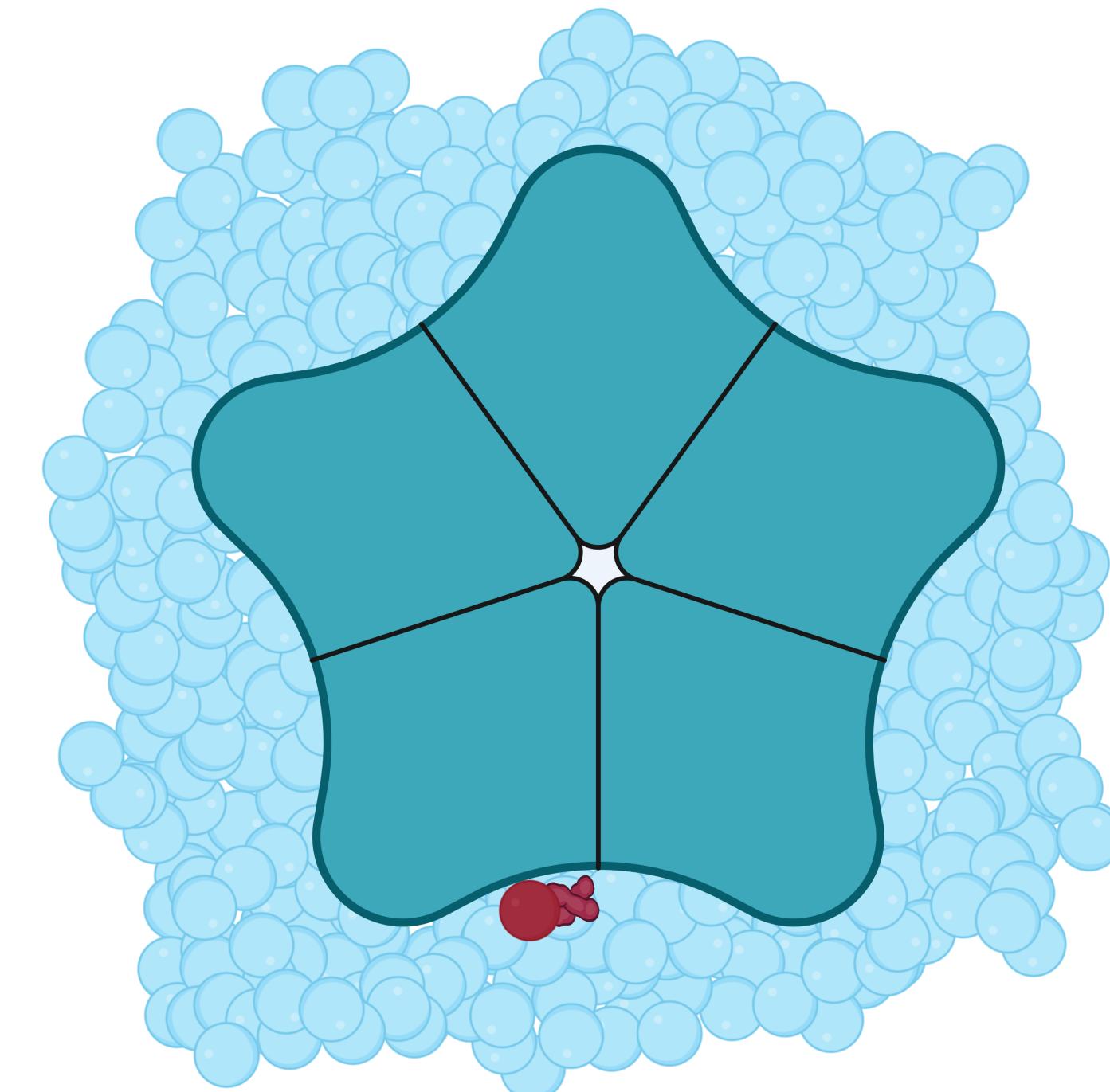
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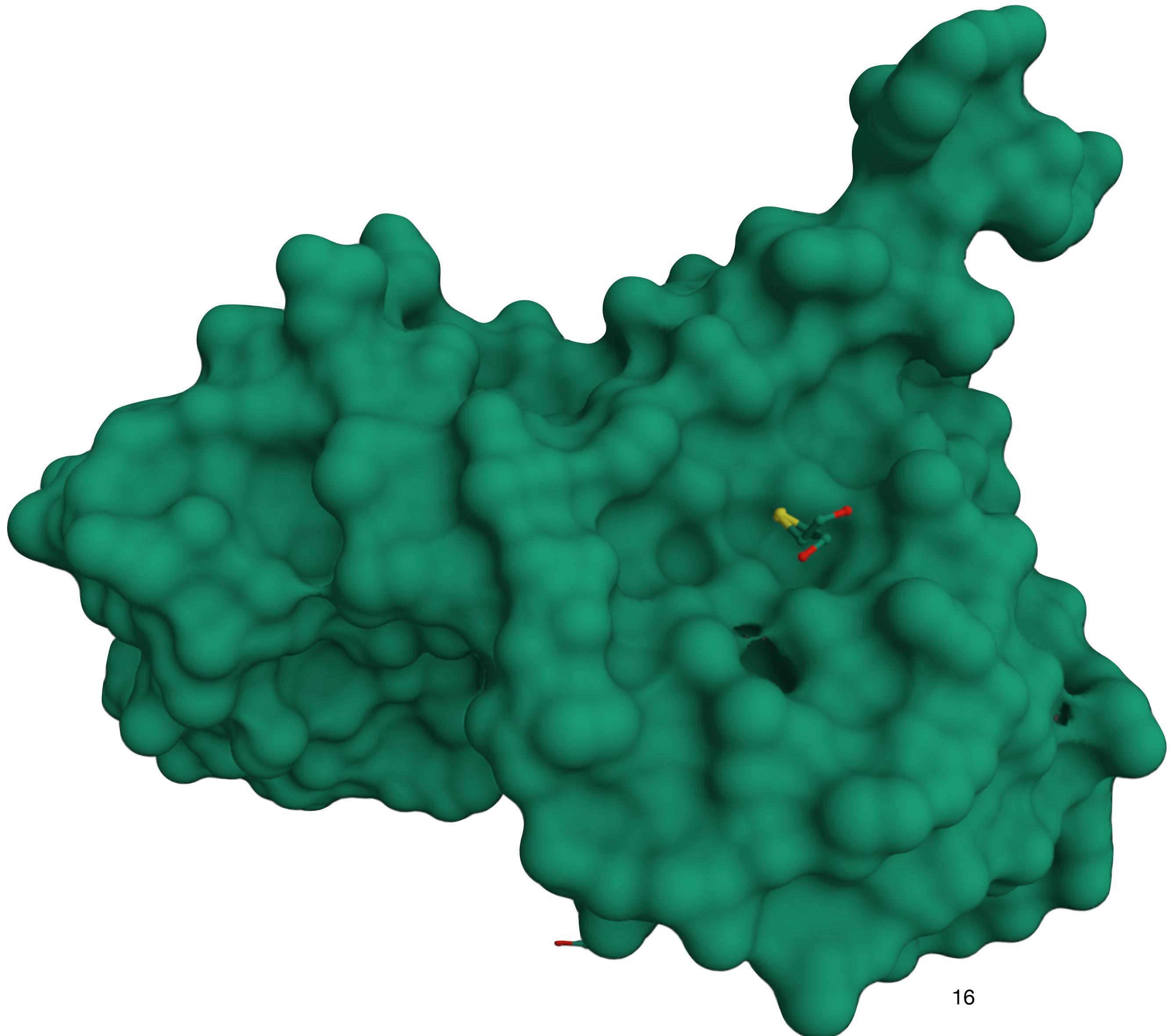
Superficial binding sites



POPC: solvent or ligand?

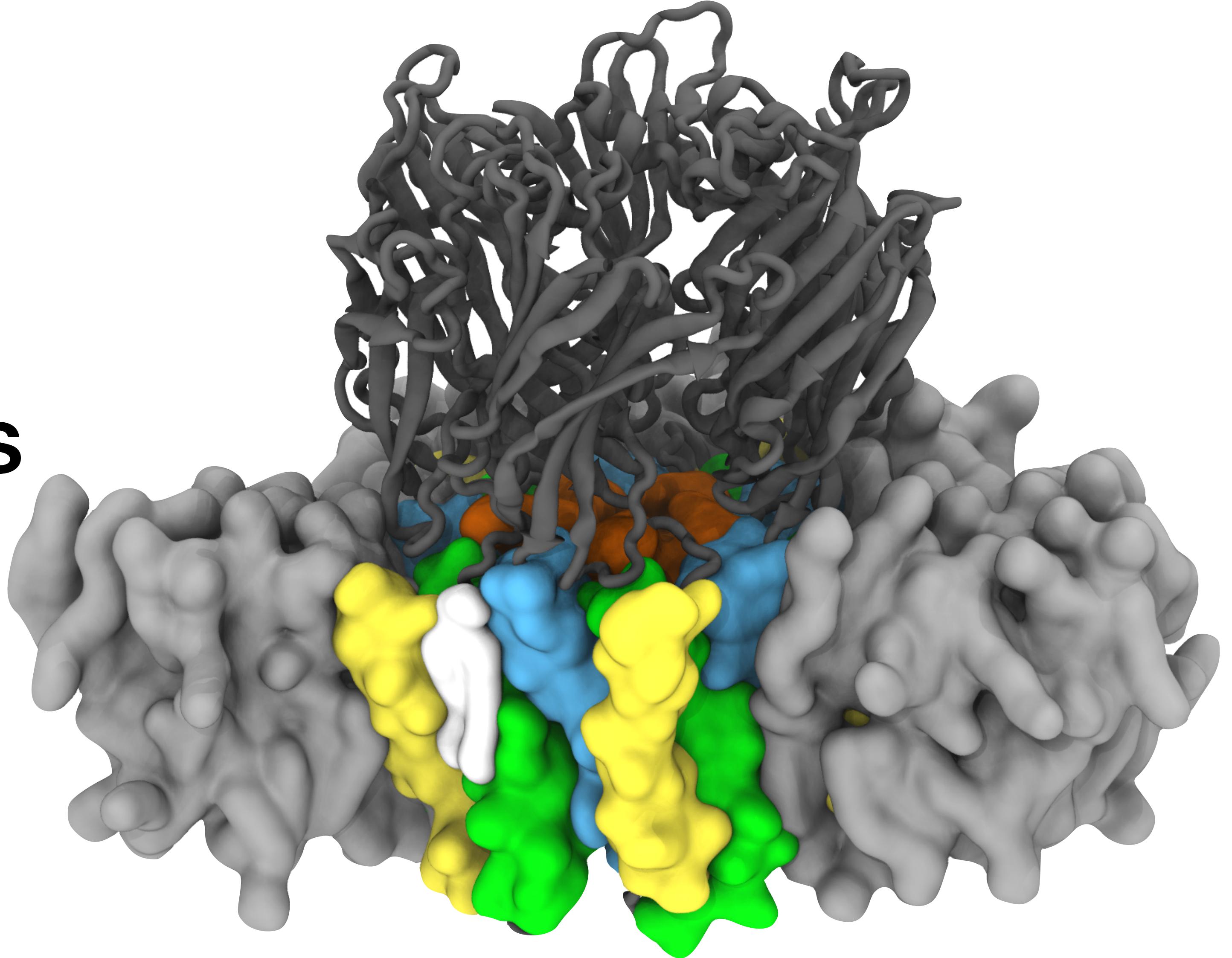
SAFEP is a FEP implementation

- Soluble proteins
- Rigid ligands
- In buried pockets
- To get a ΔG_{bind}^0



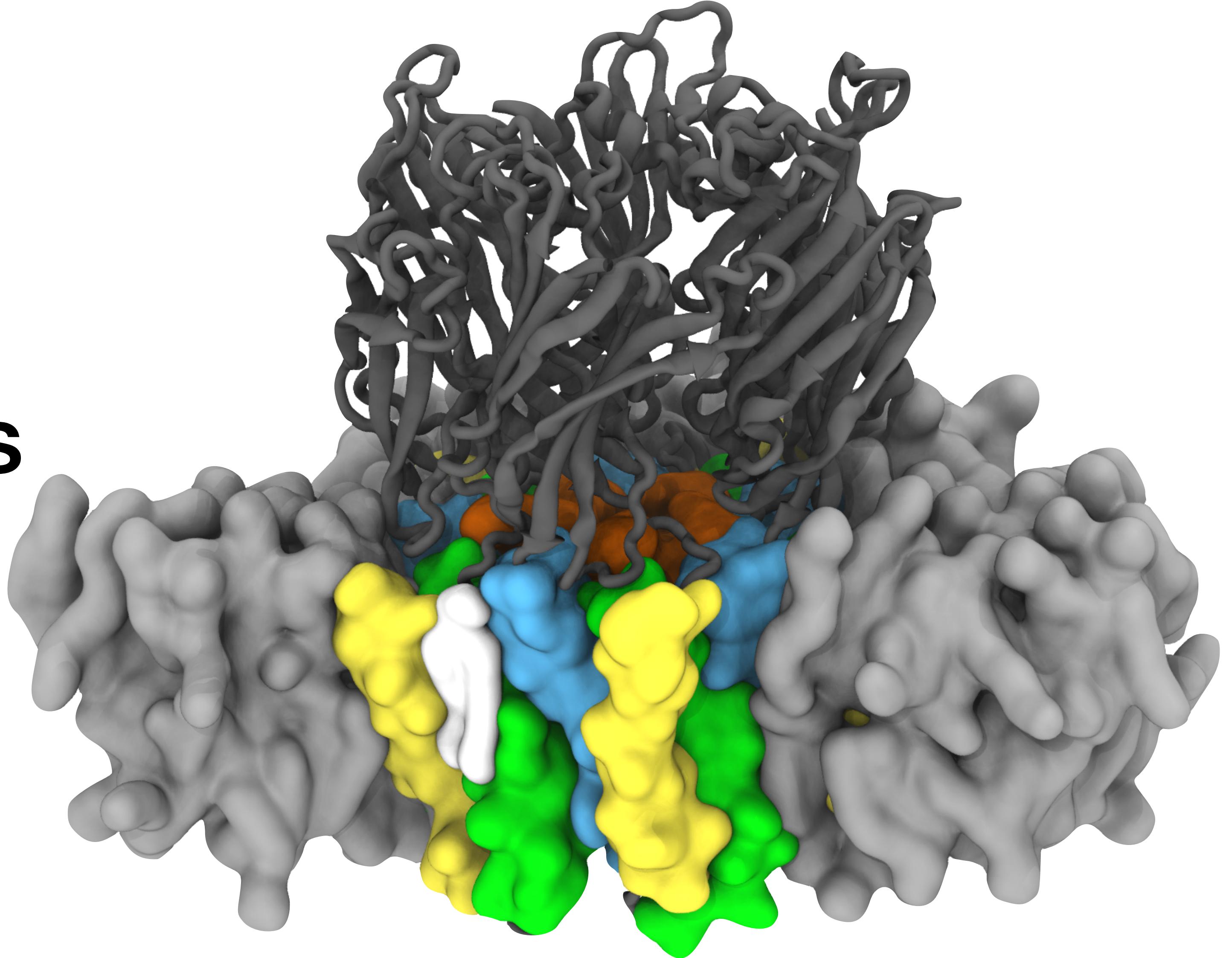
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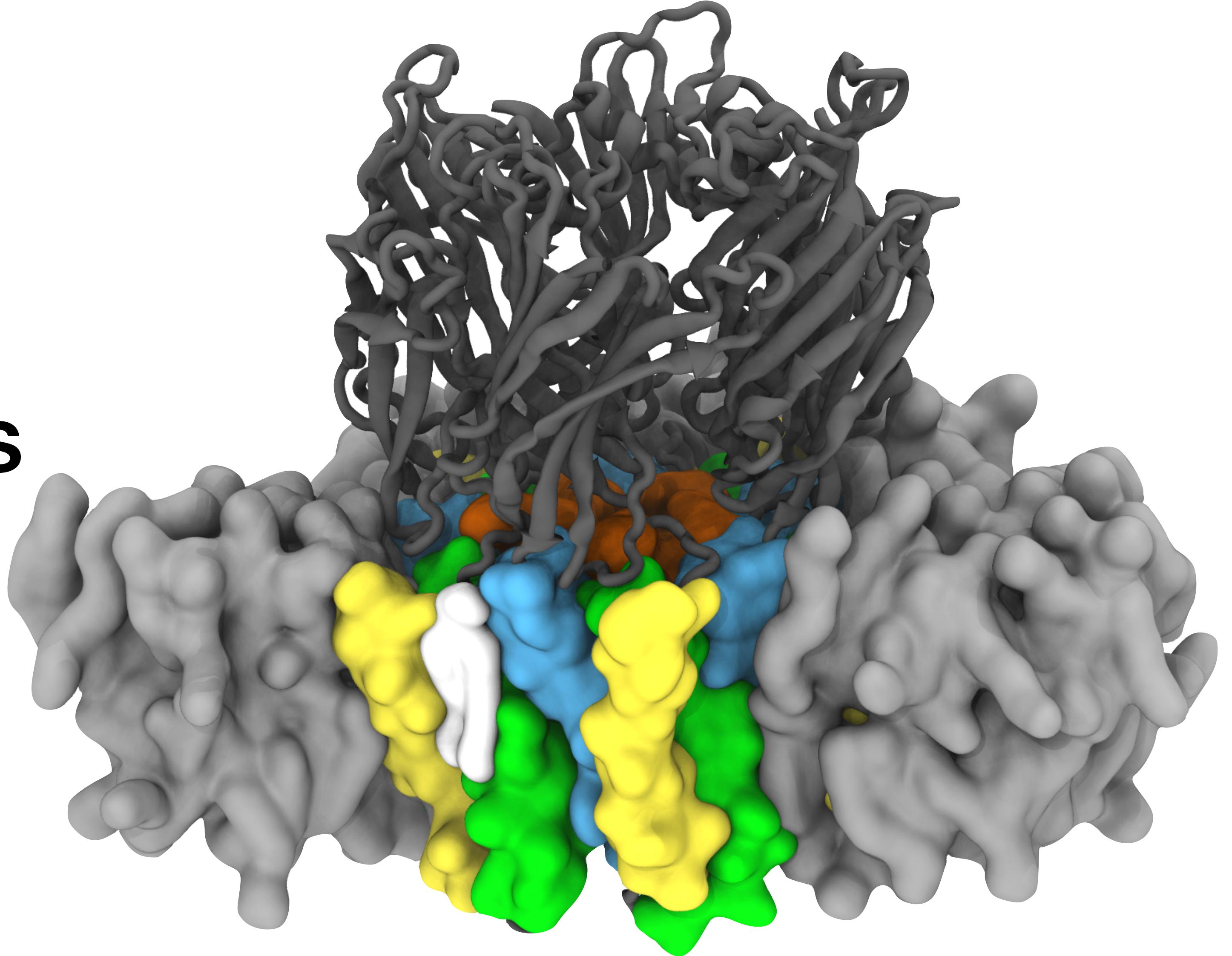
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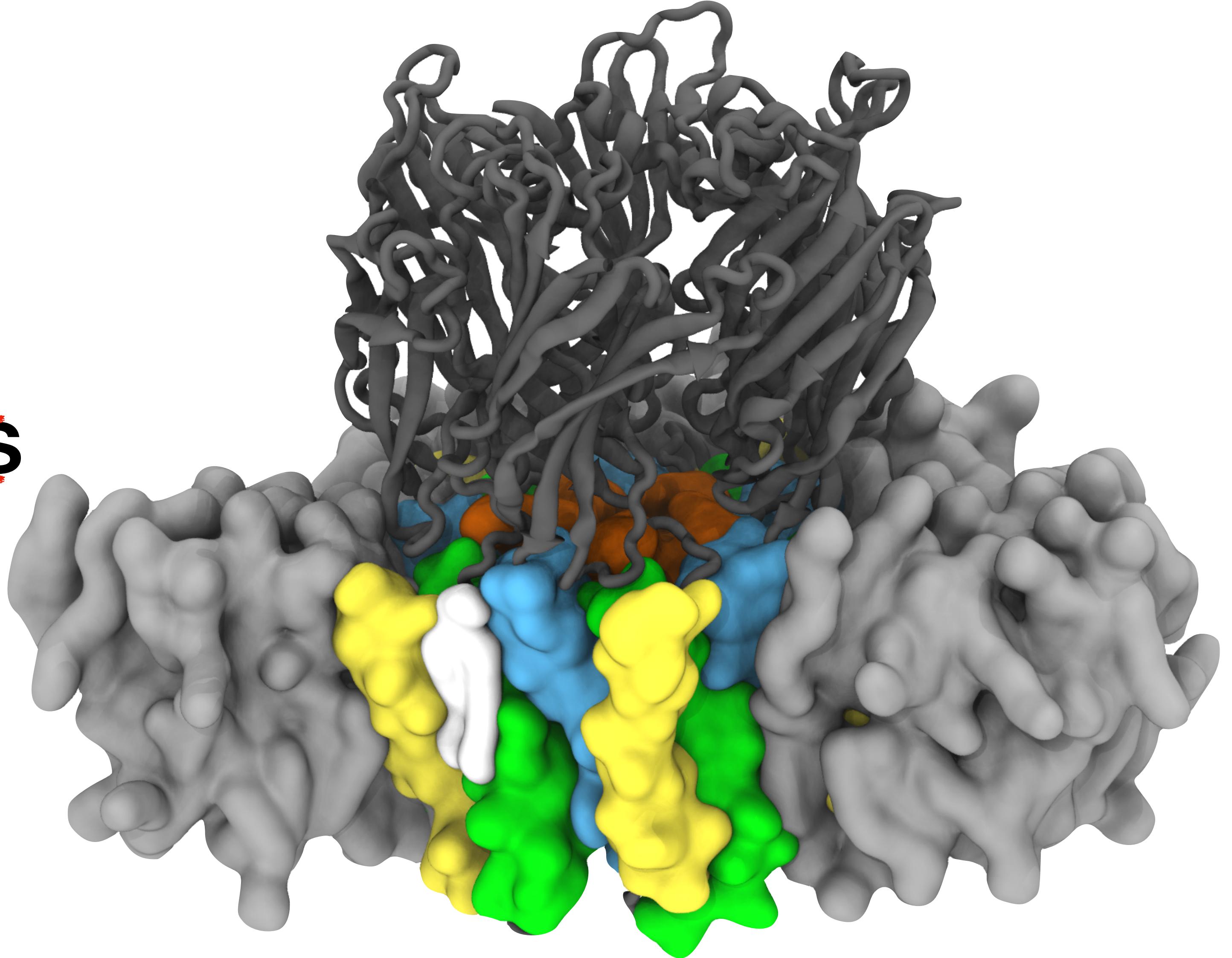
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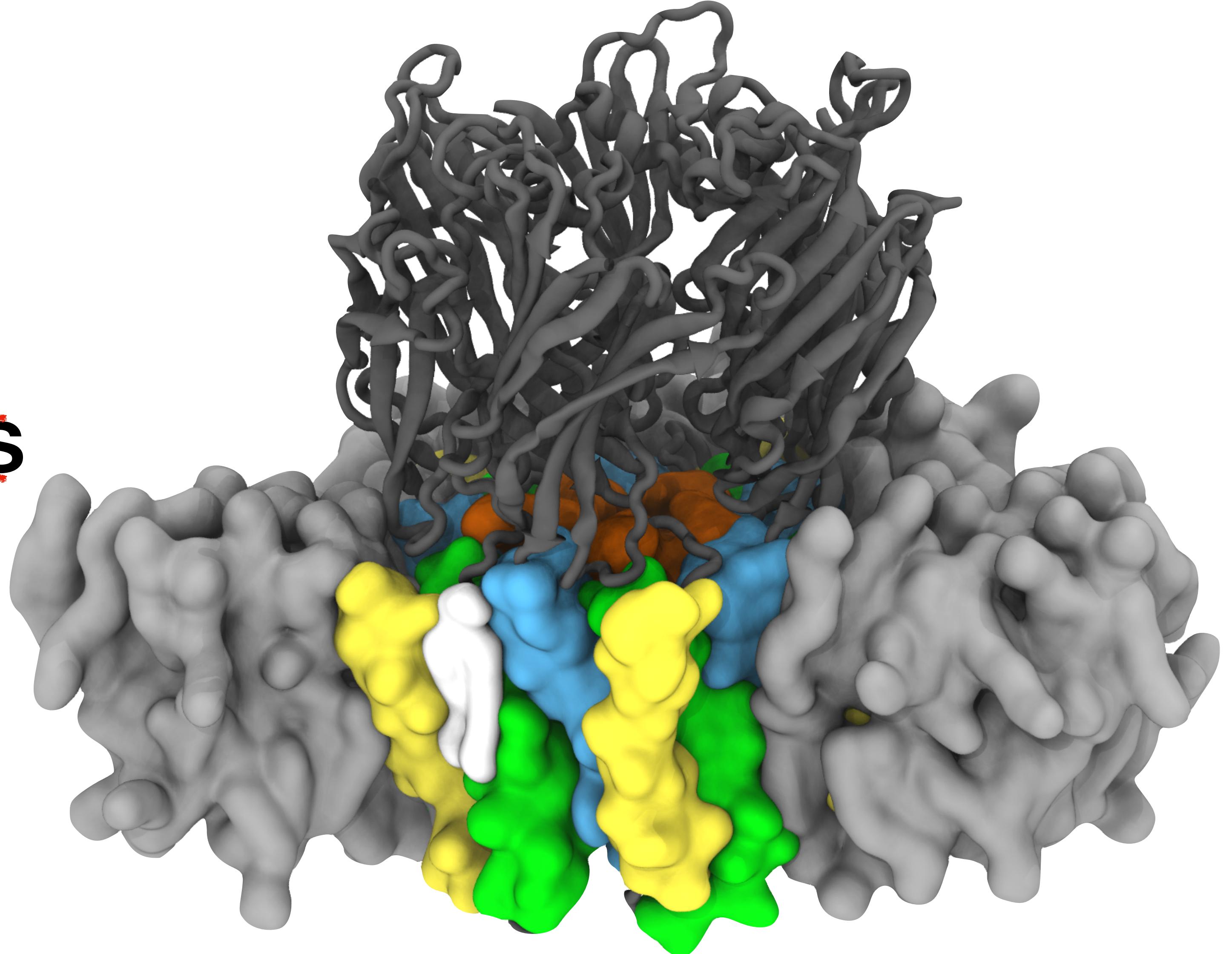
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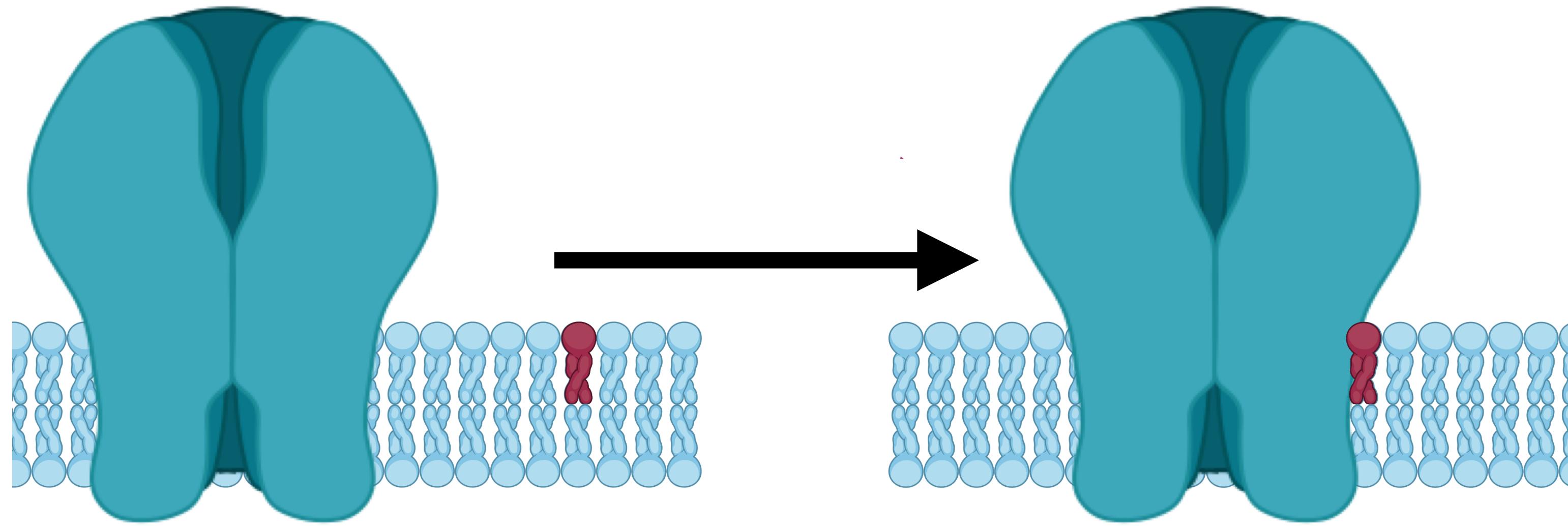


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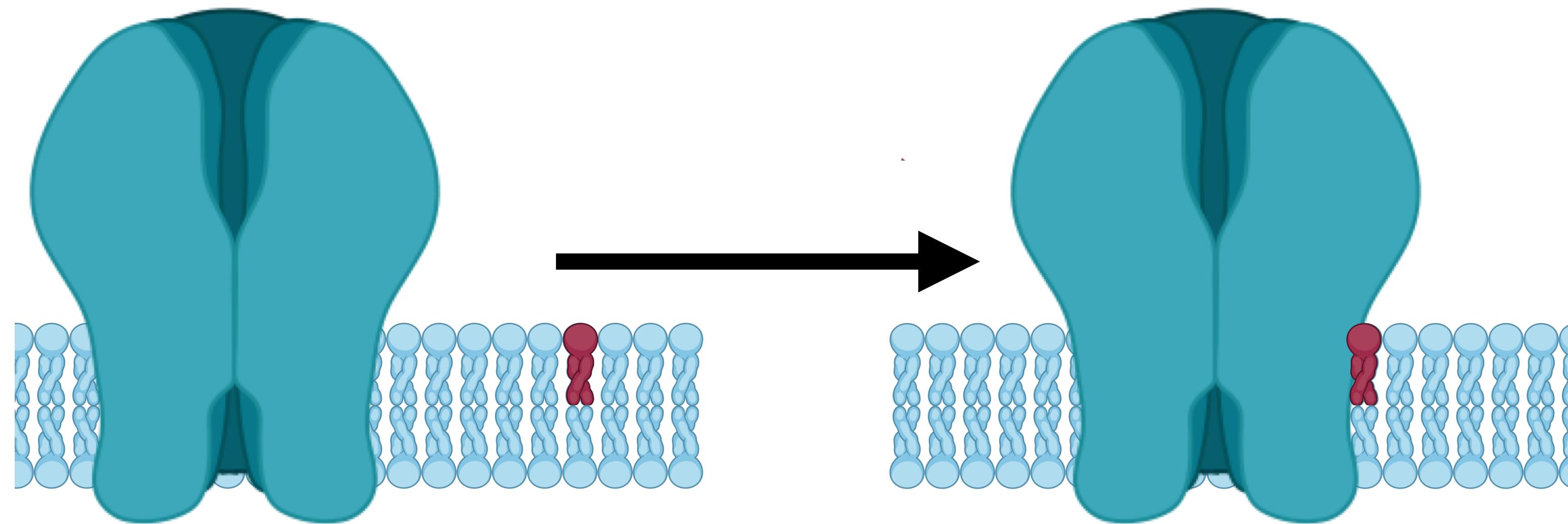
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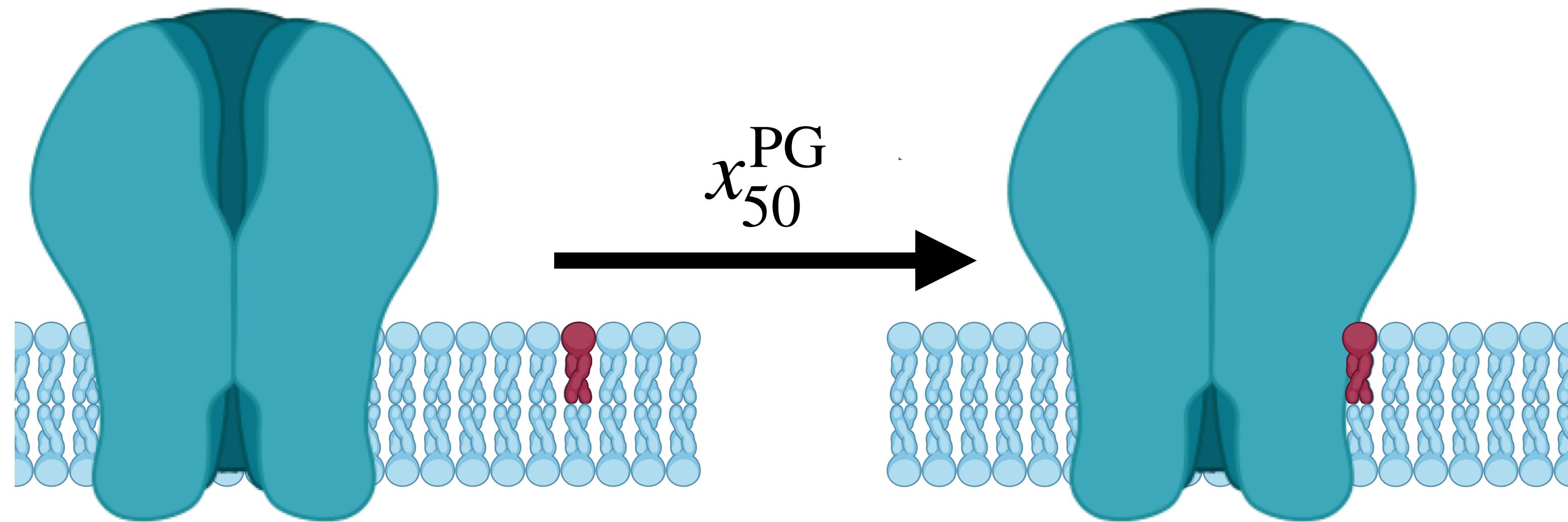


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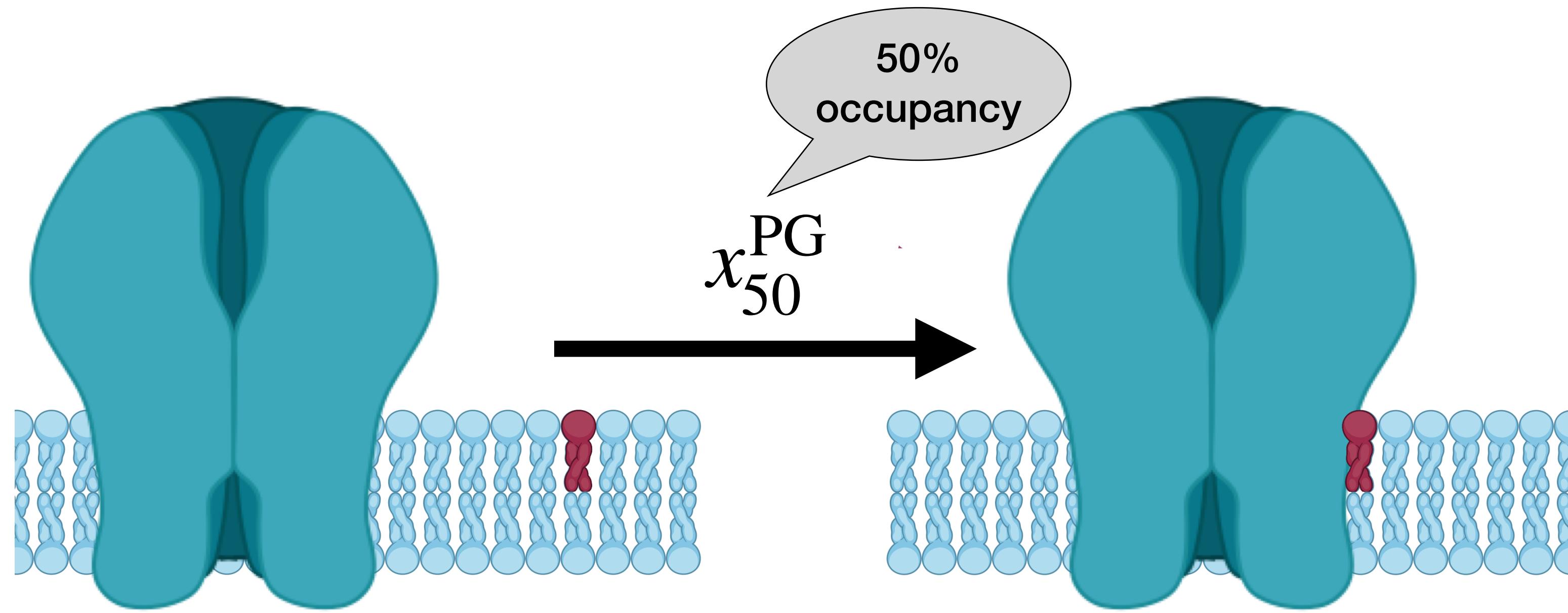
Use an arbitrary
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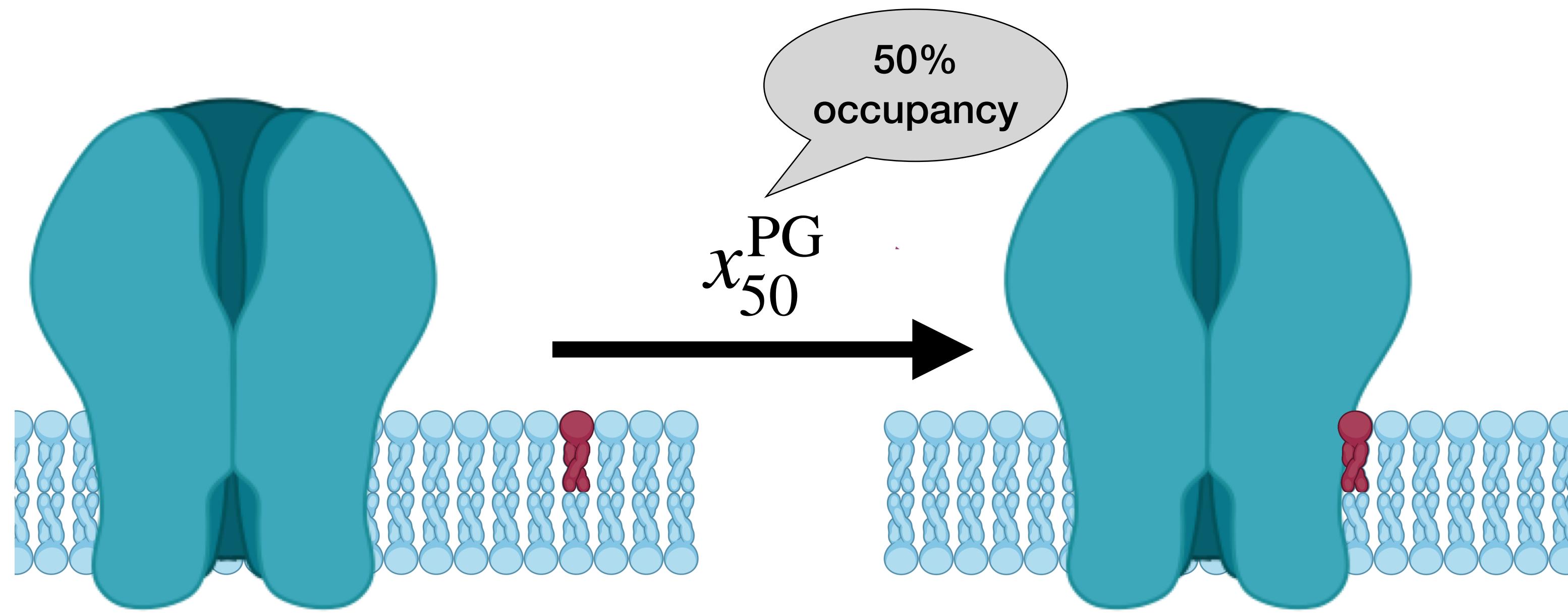
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$$x_{50}^{\text{PG}} = x^* e^{\frac{\Delta G^*}{RT}}$$

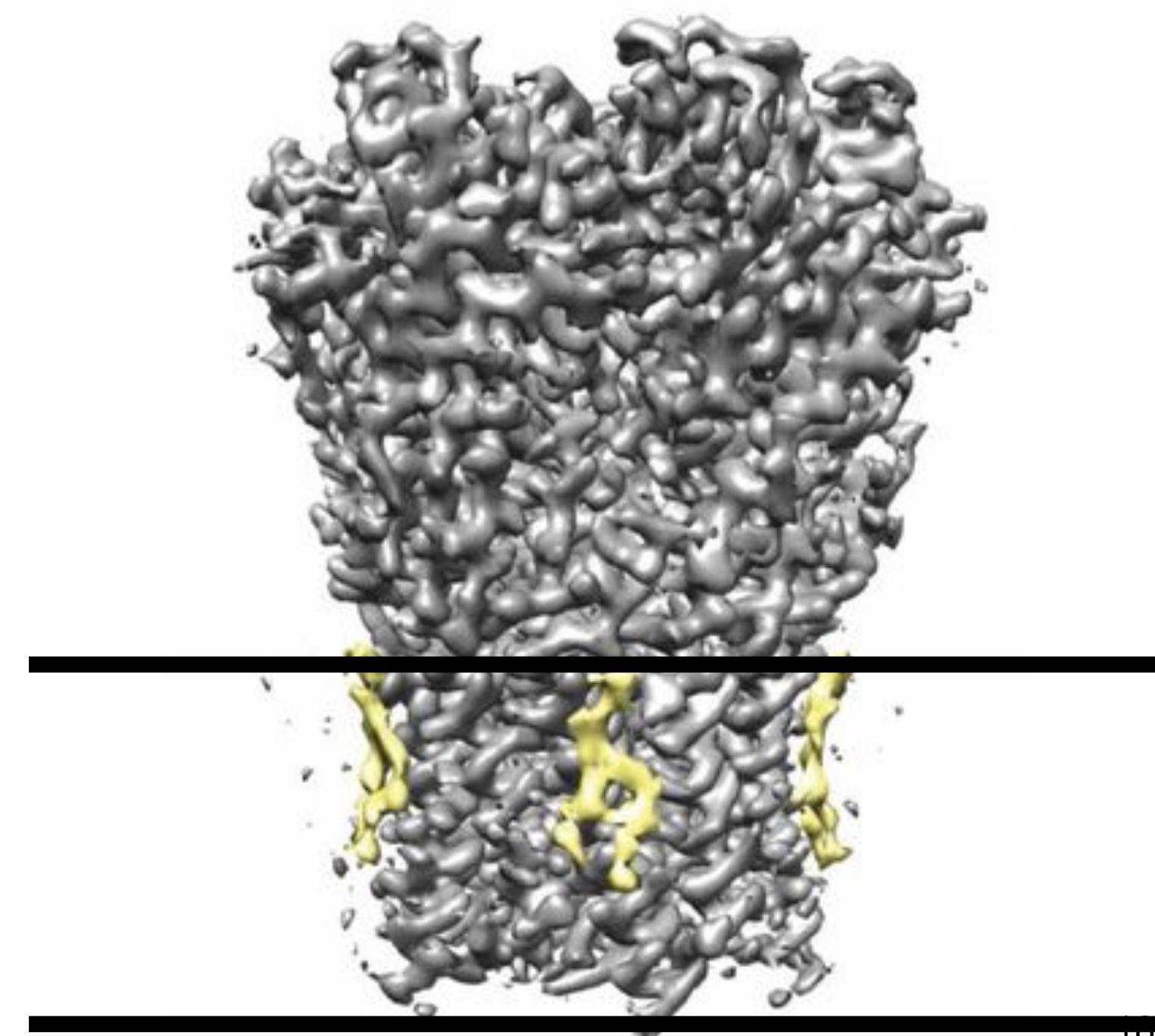
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A given lipid species

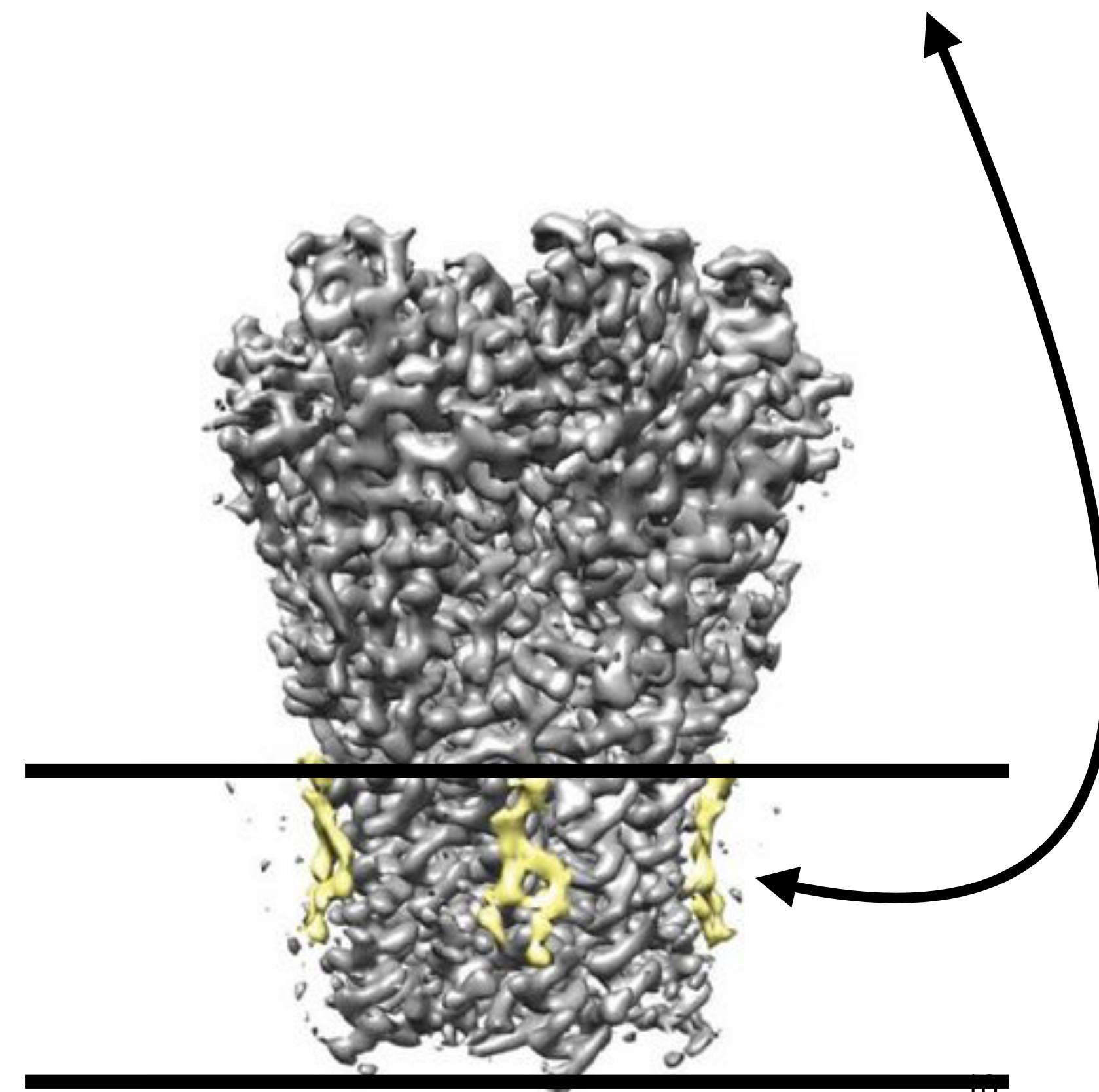
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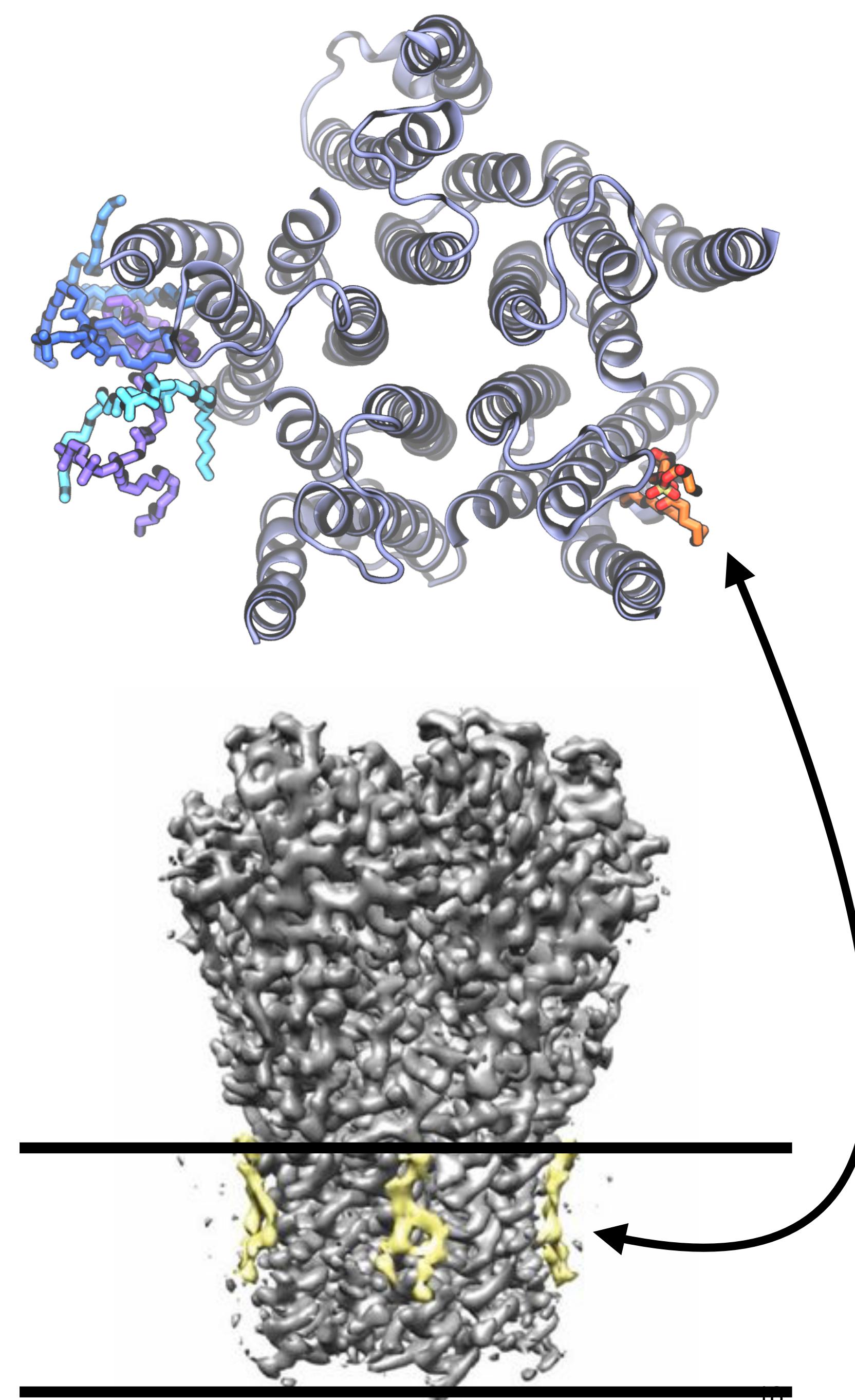
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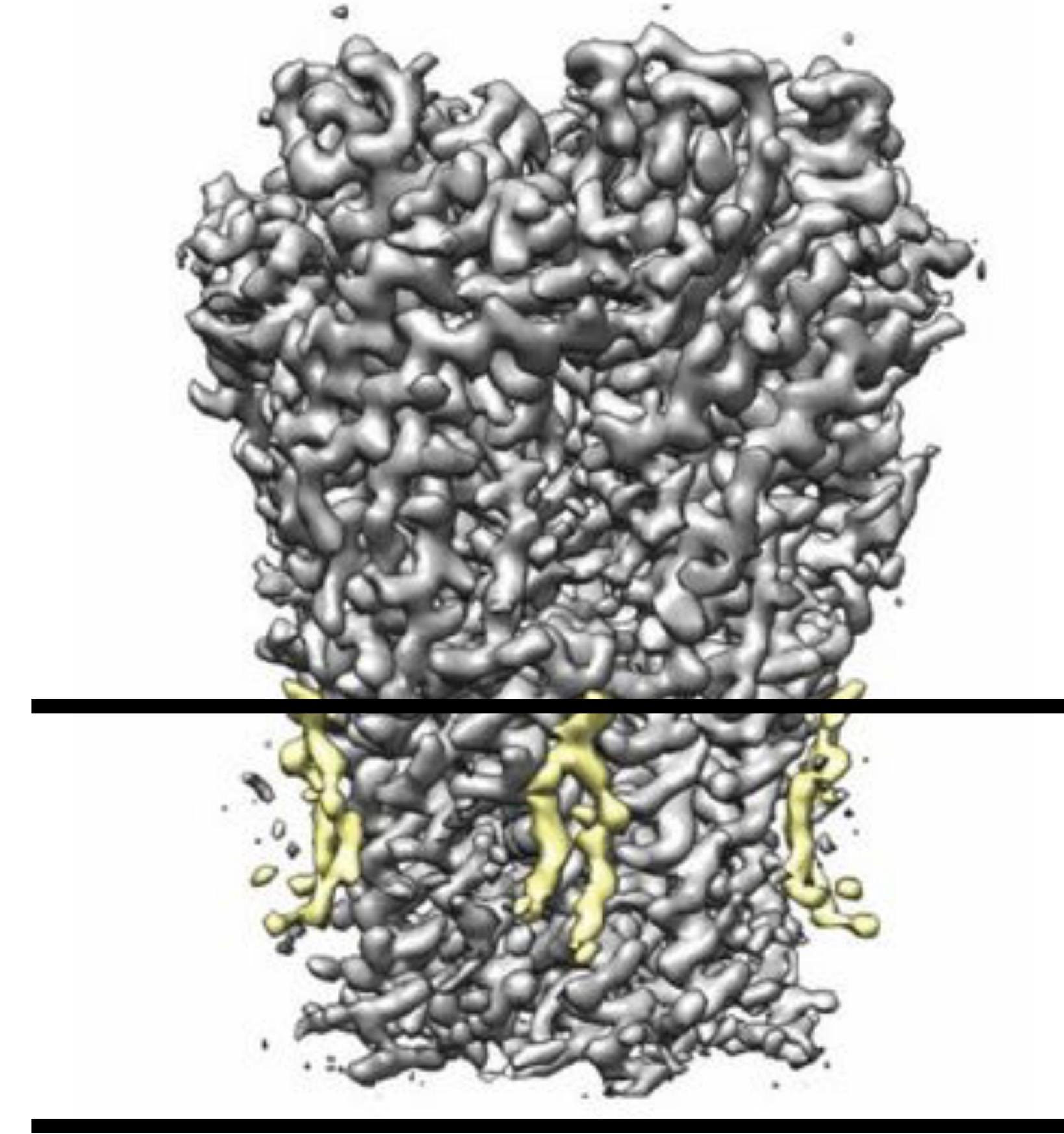


Find the most stable lipid/structure pairs

POPG?

POPC?

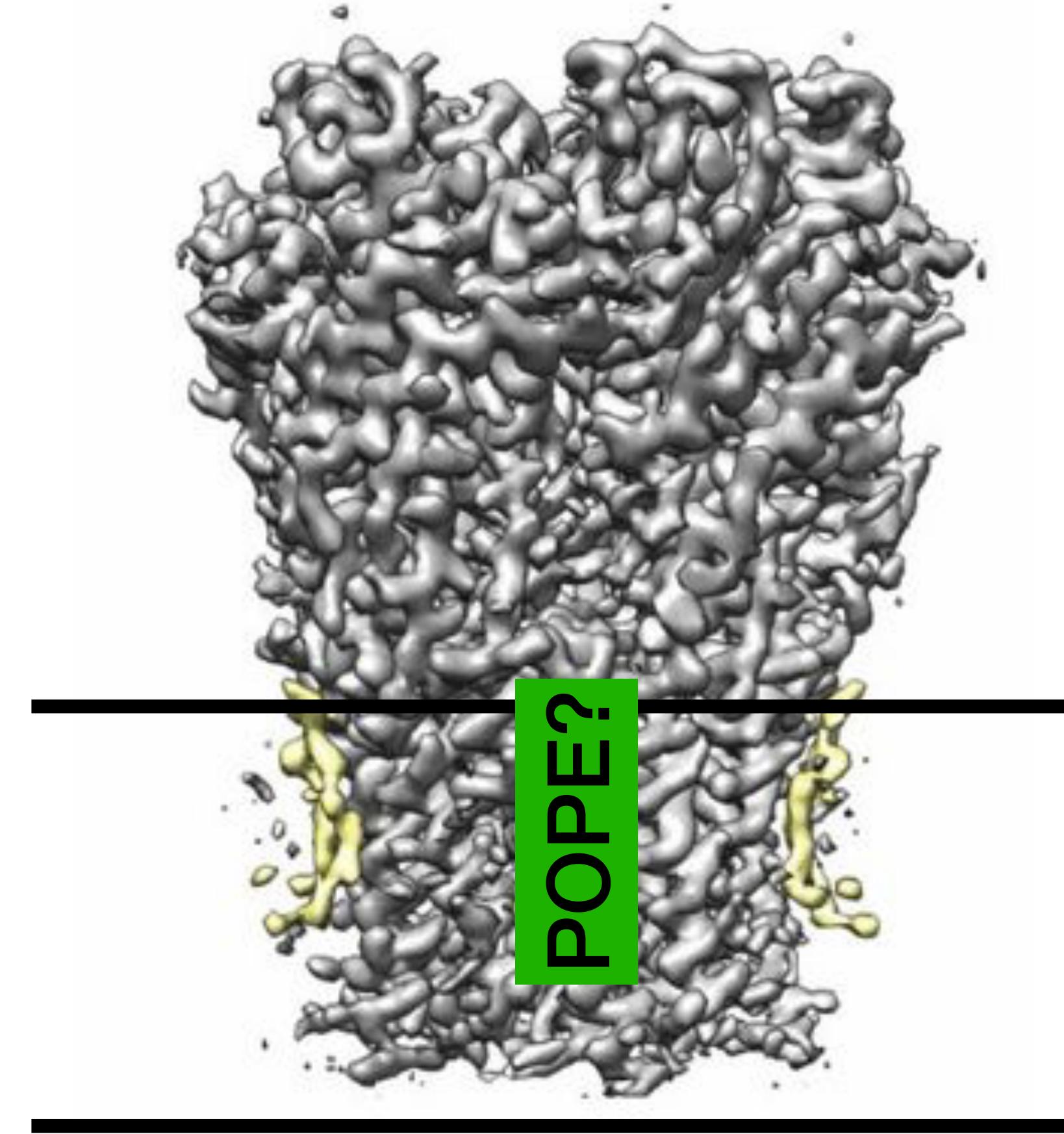
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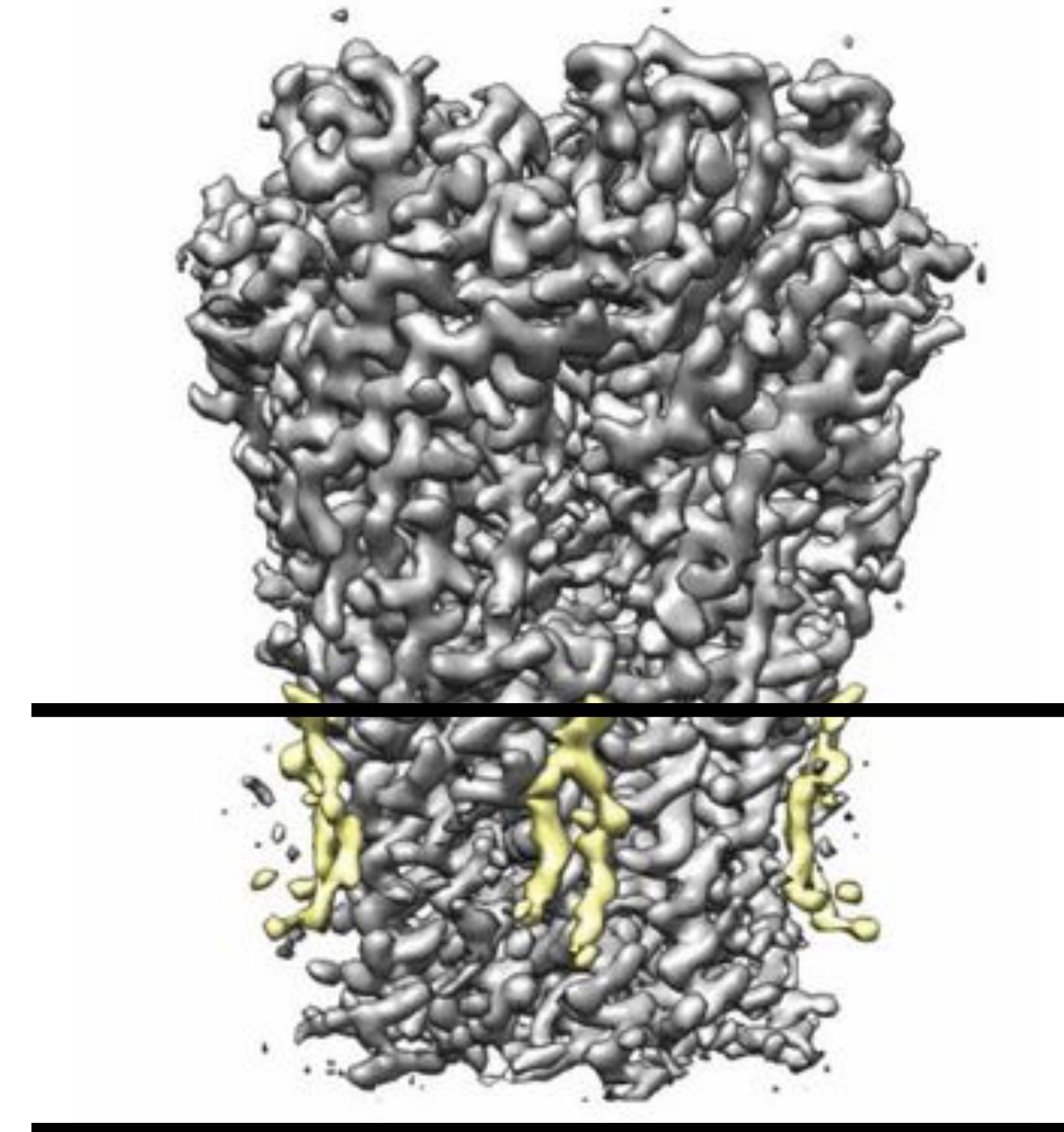


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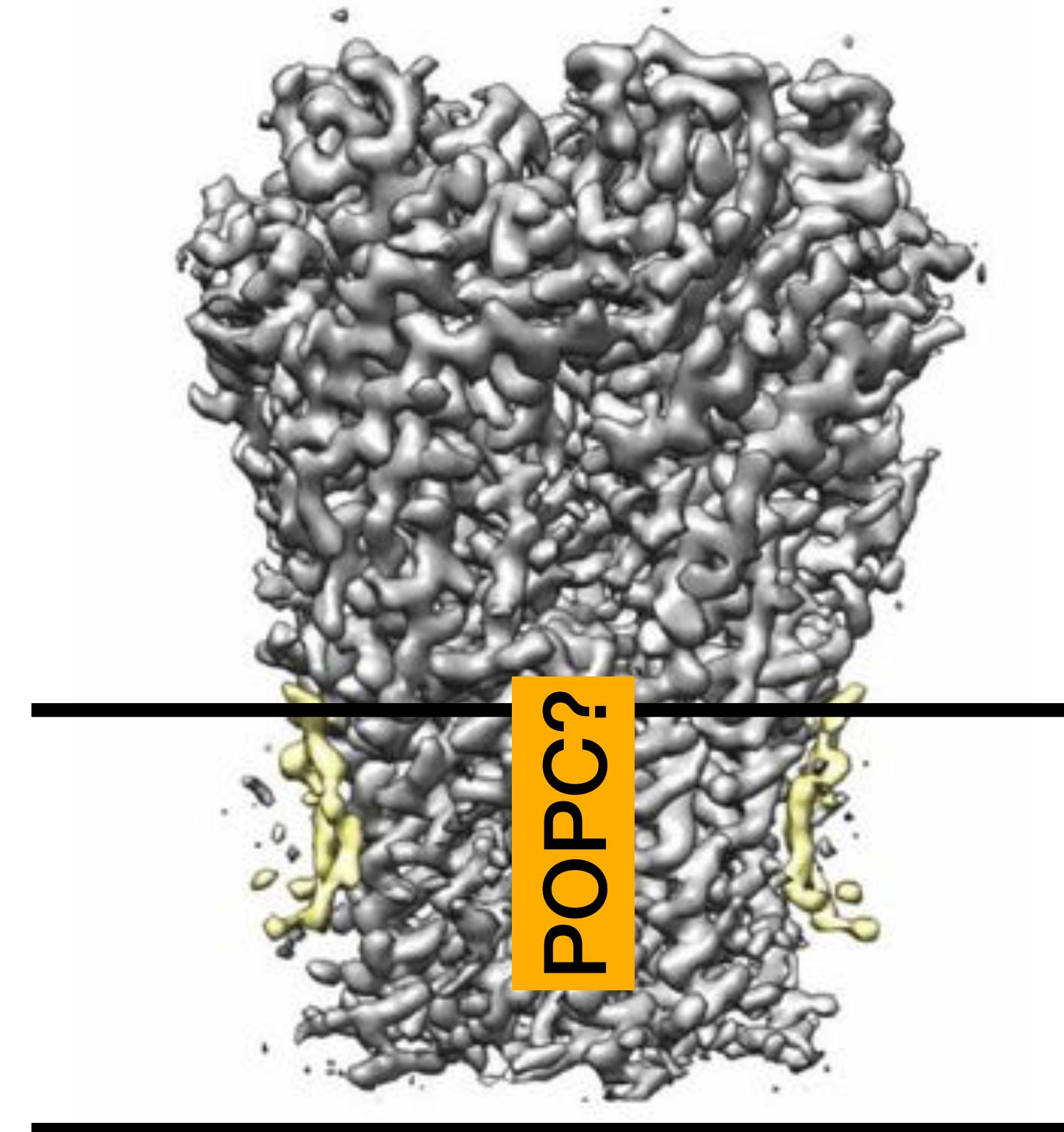


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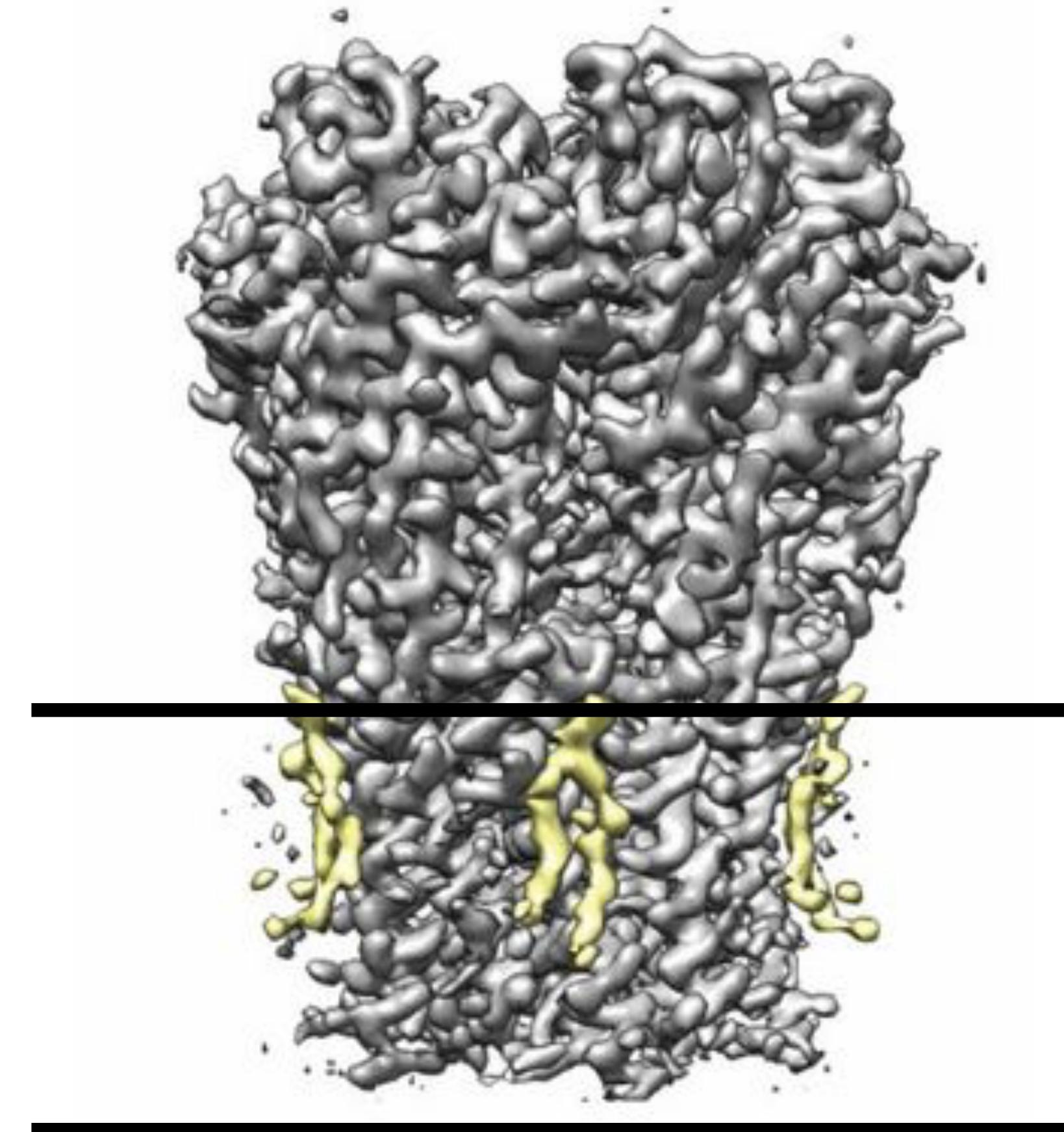
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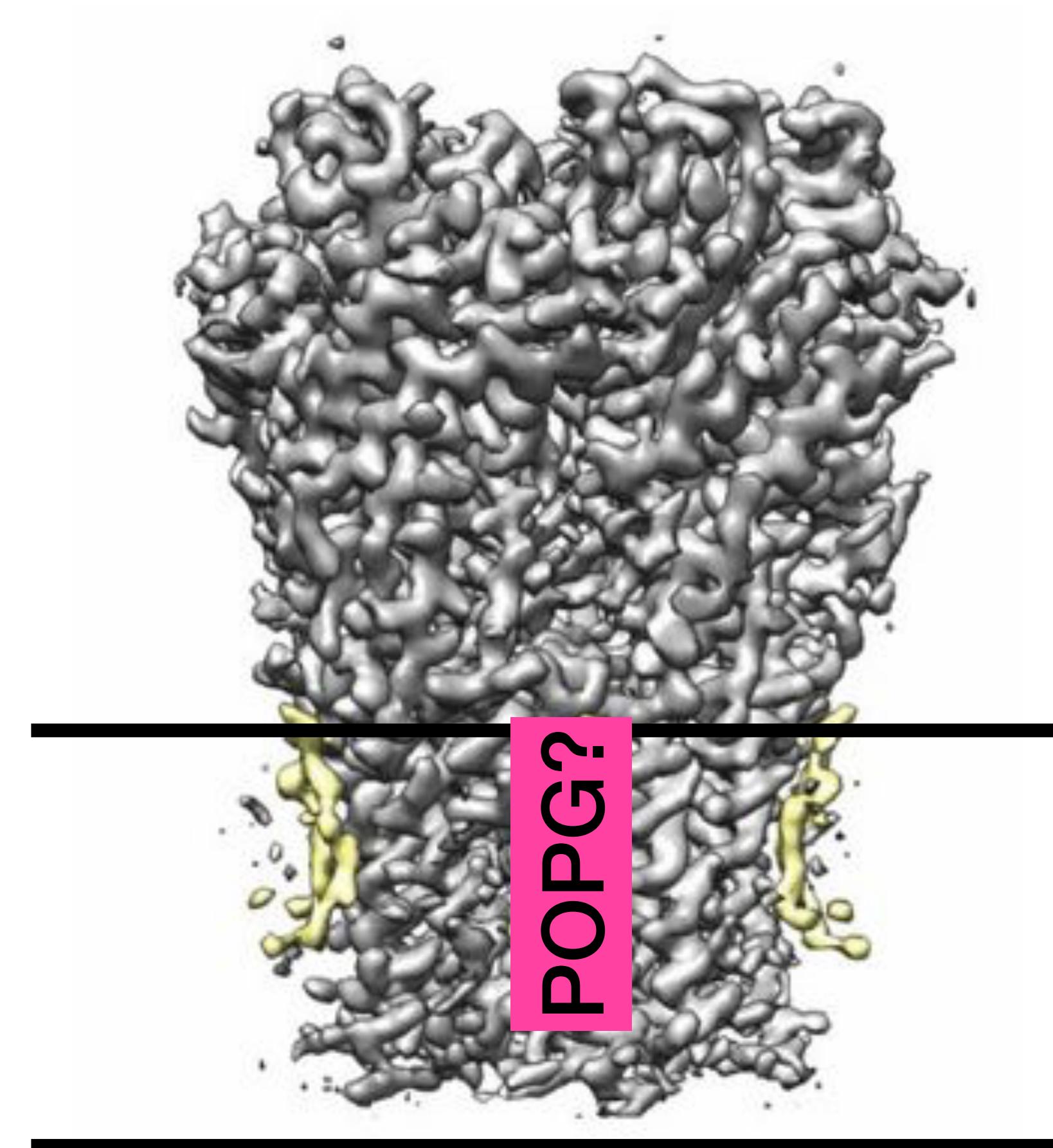
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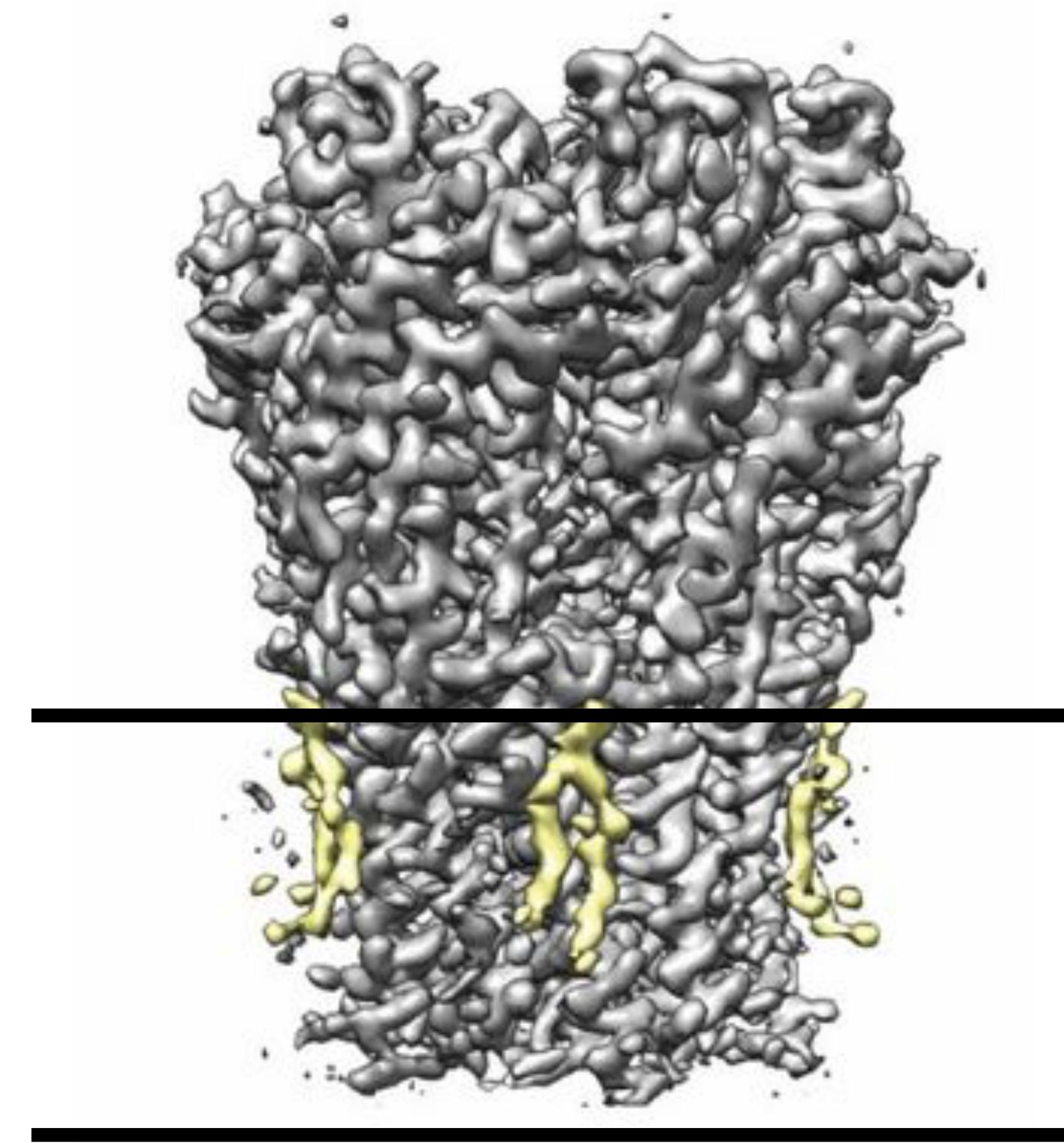
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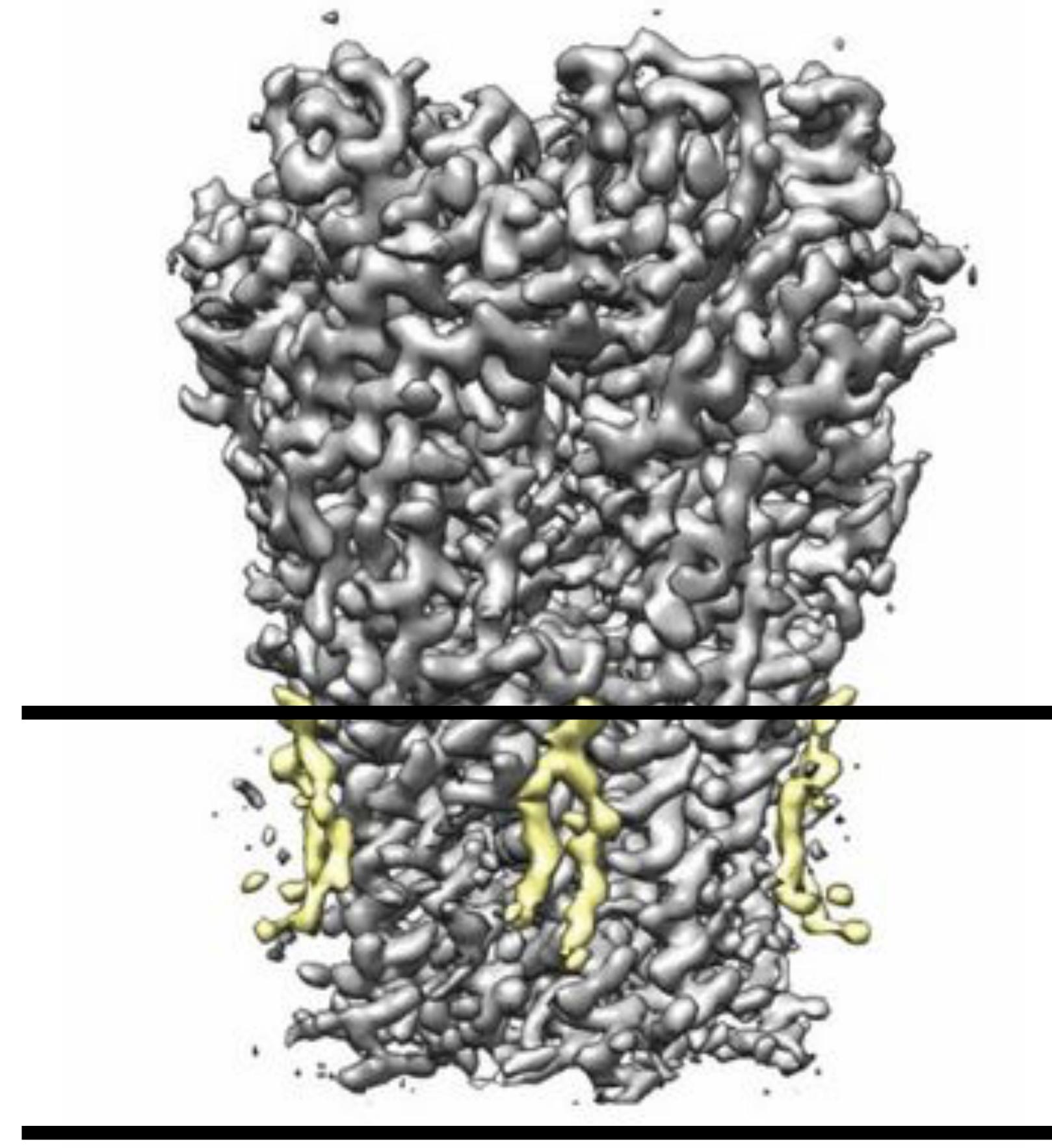
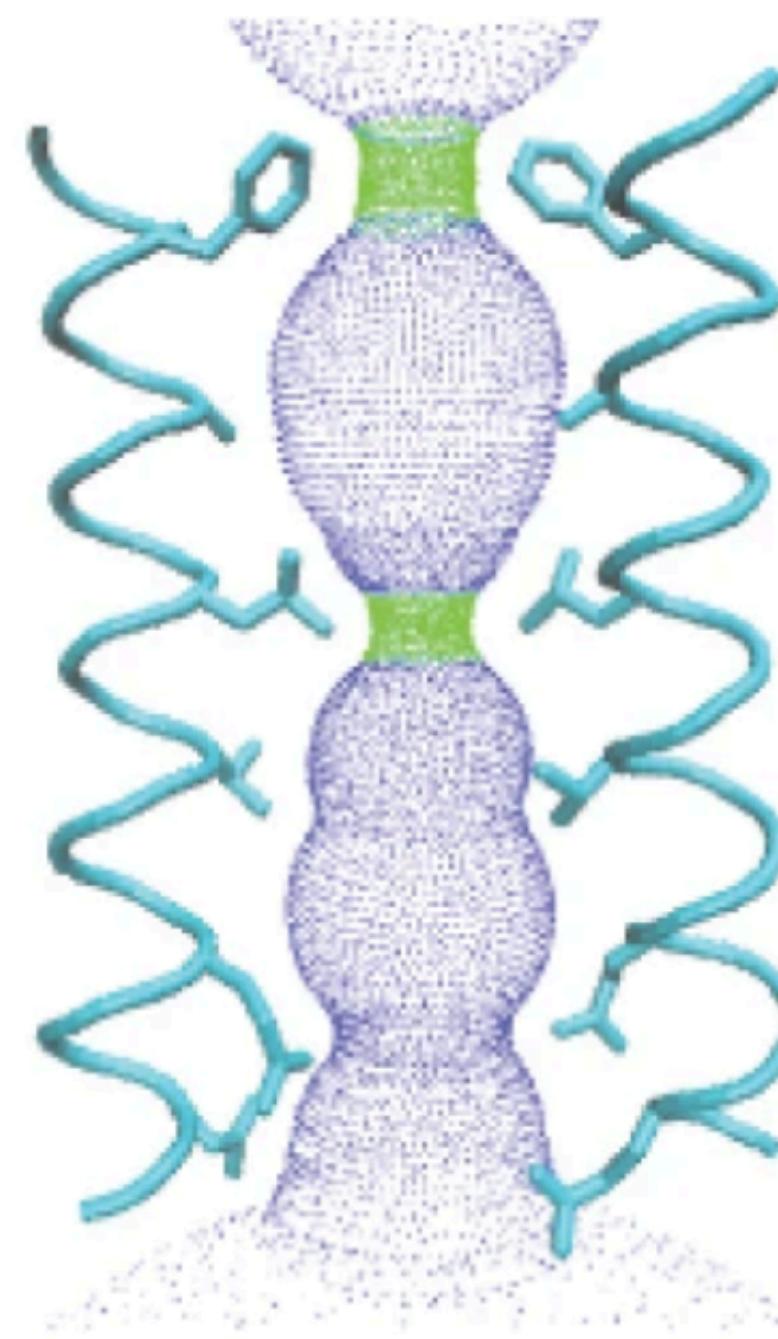


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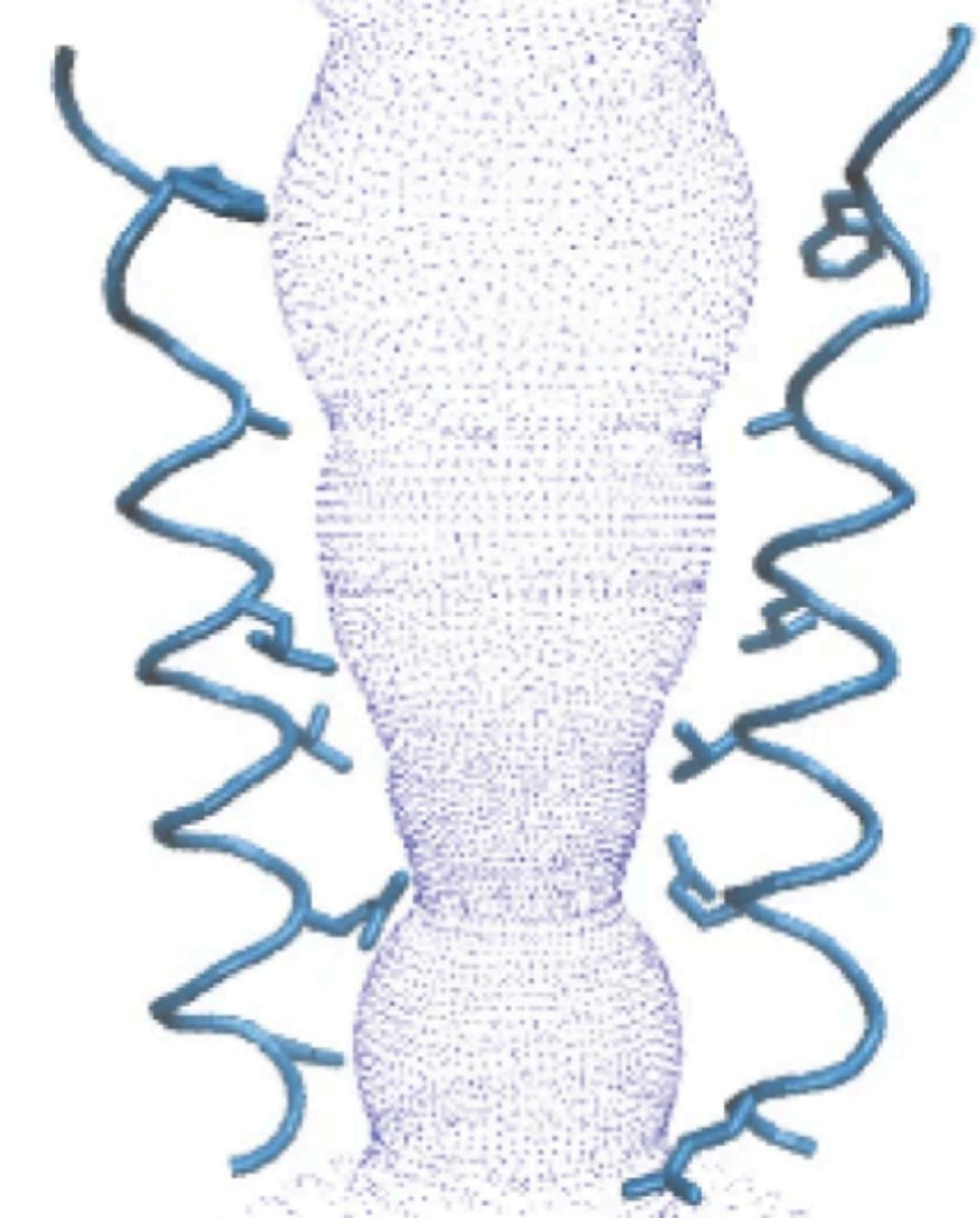
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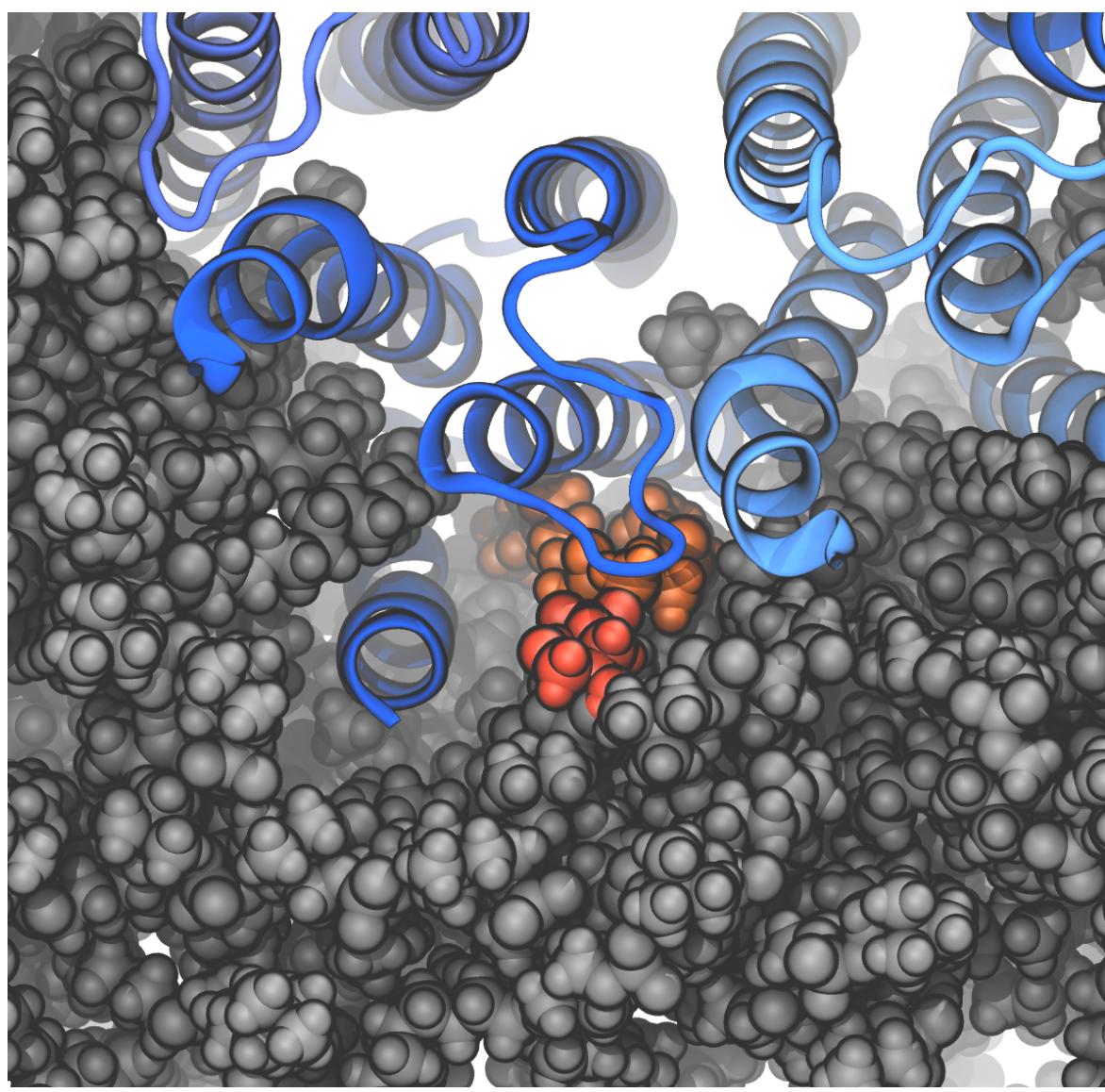
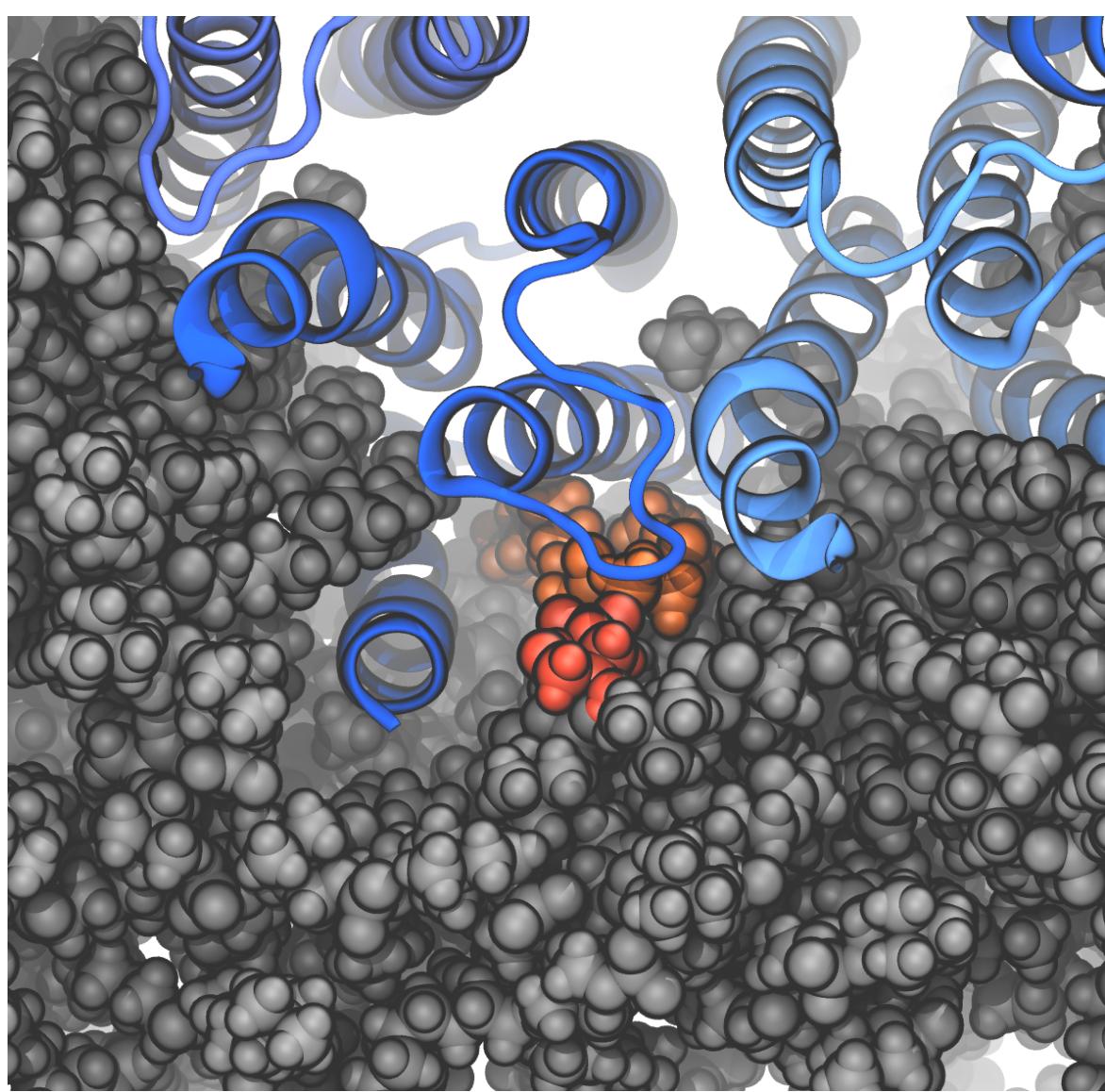
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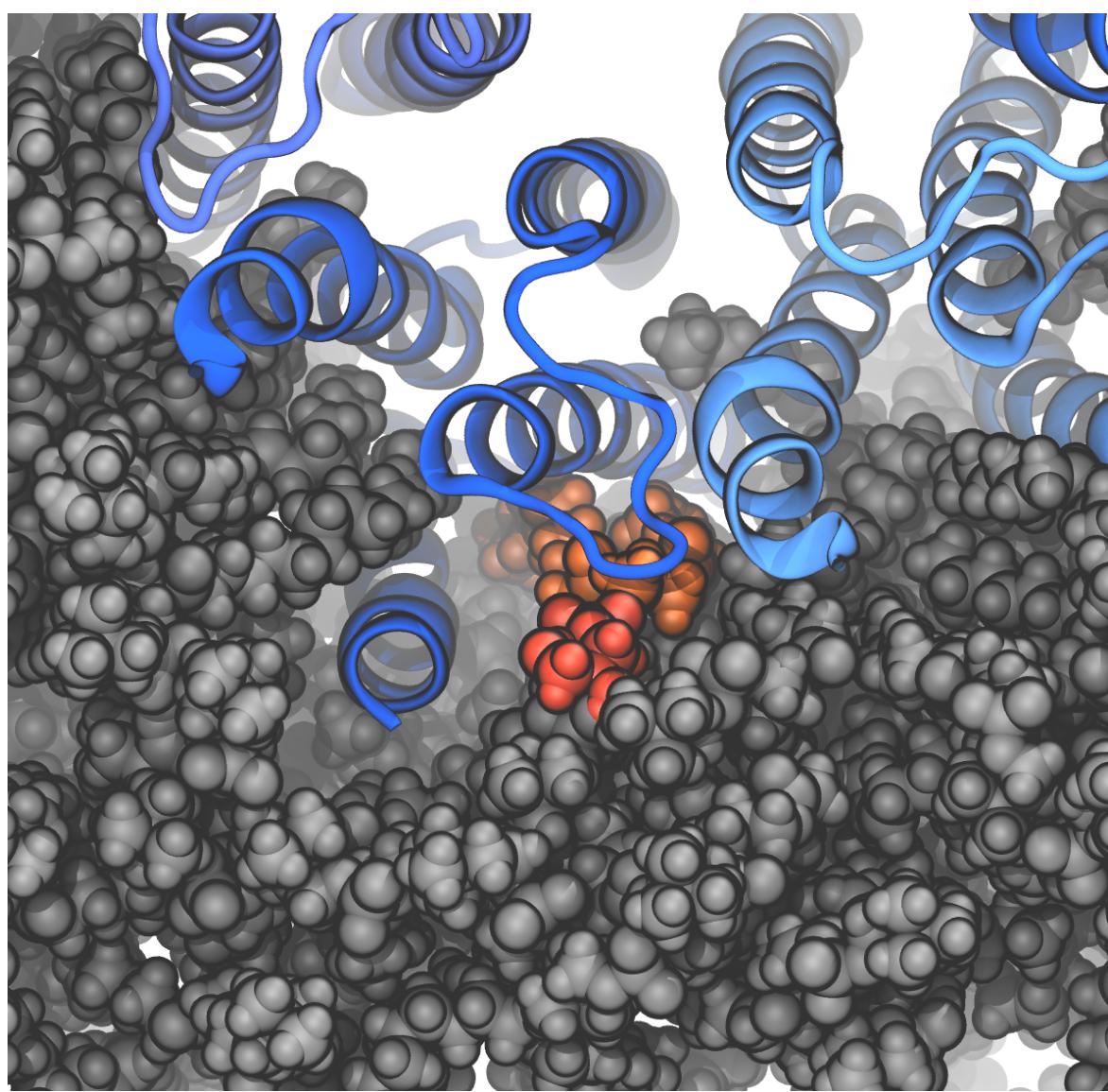
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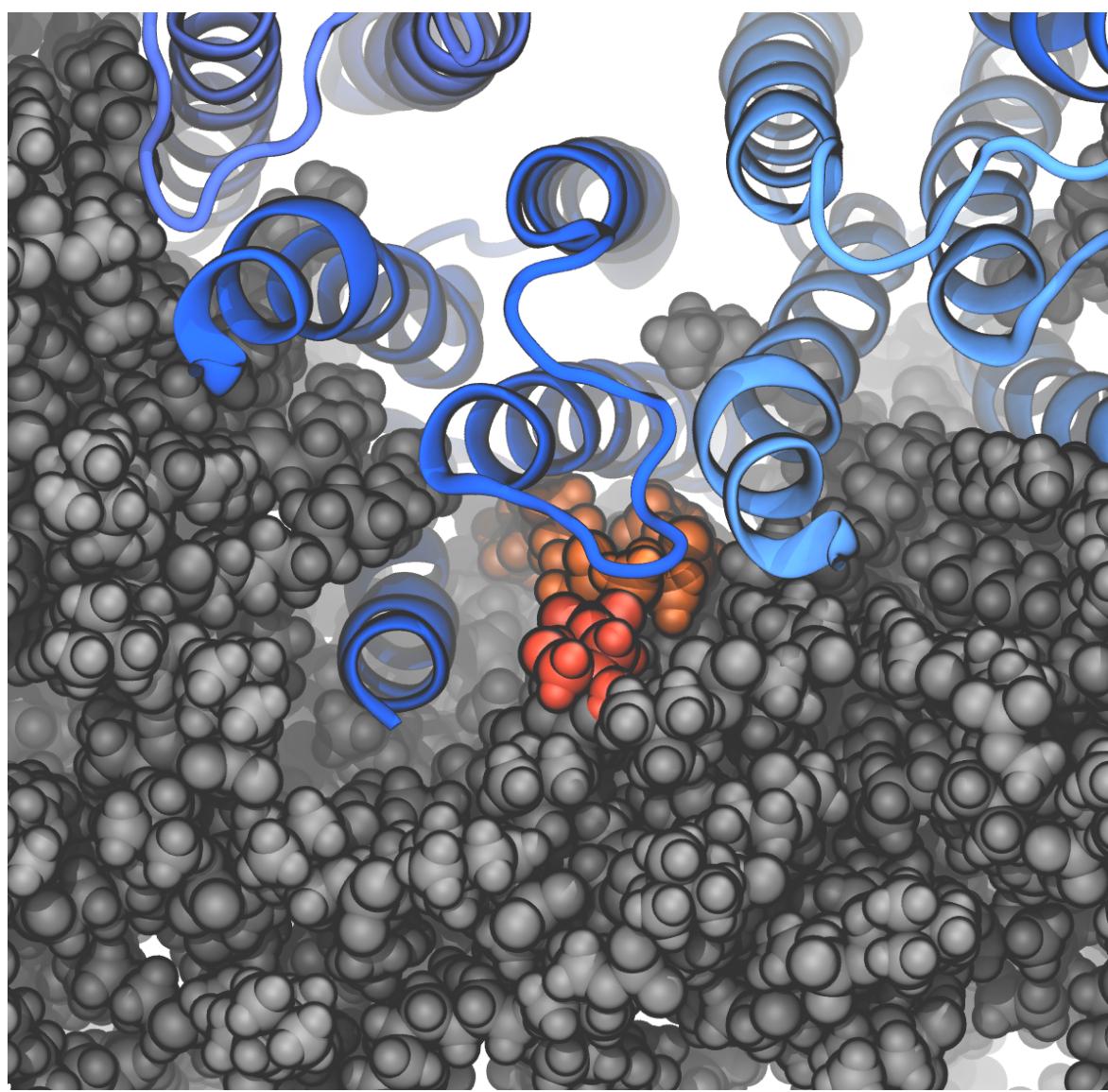
How does FEP look in a membrane?



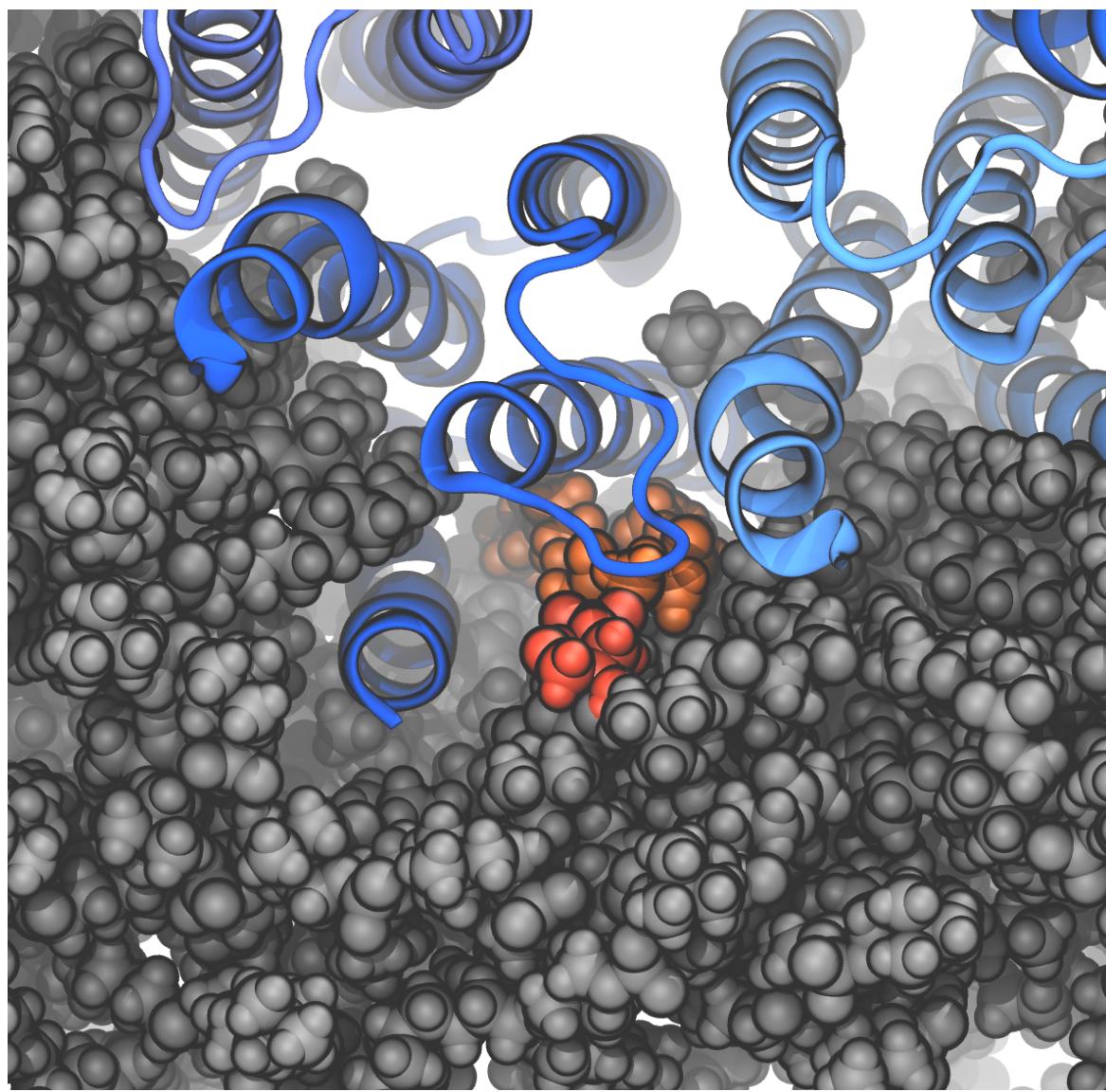
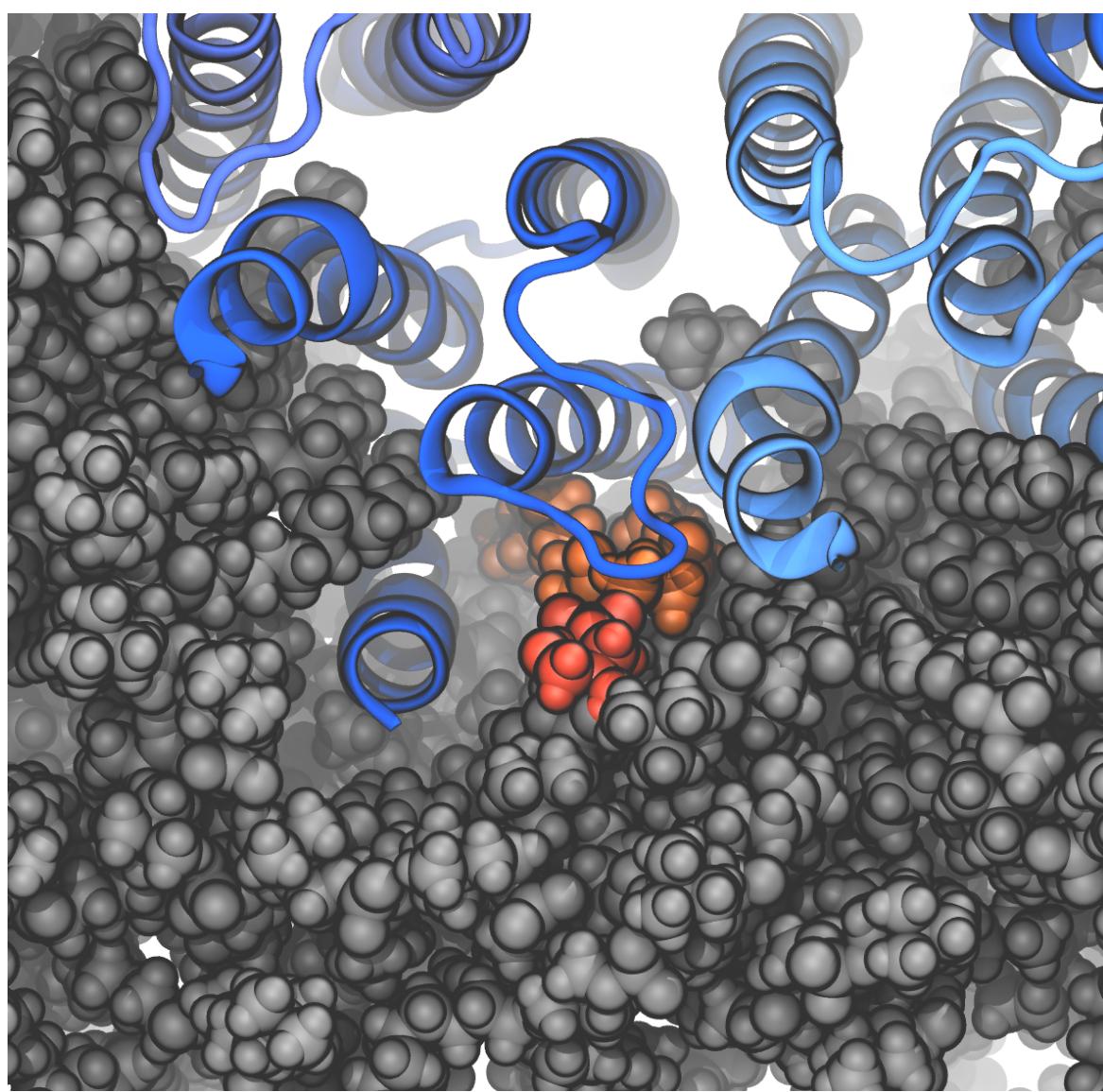
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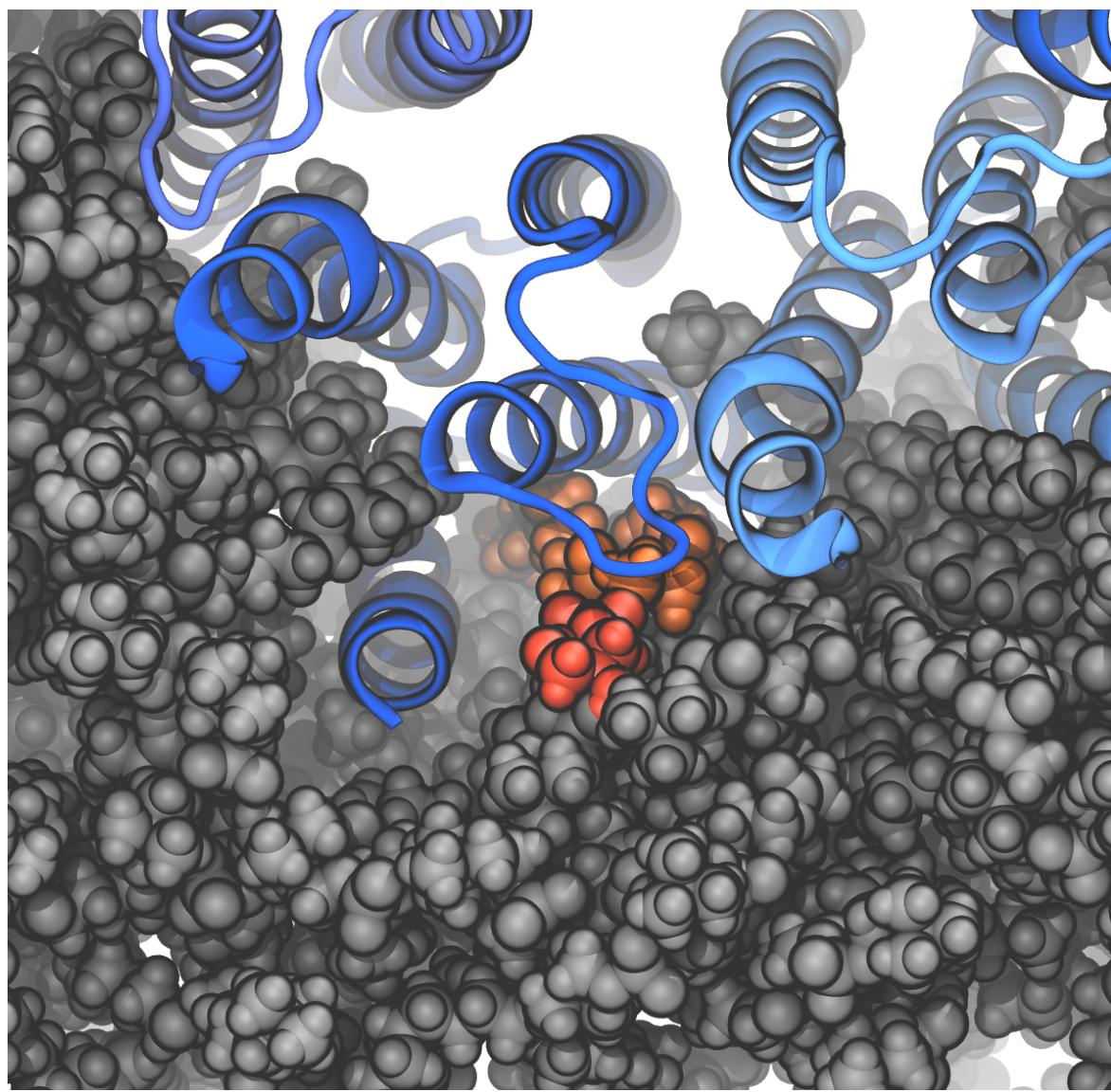
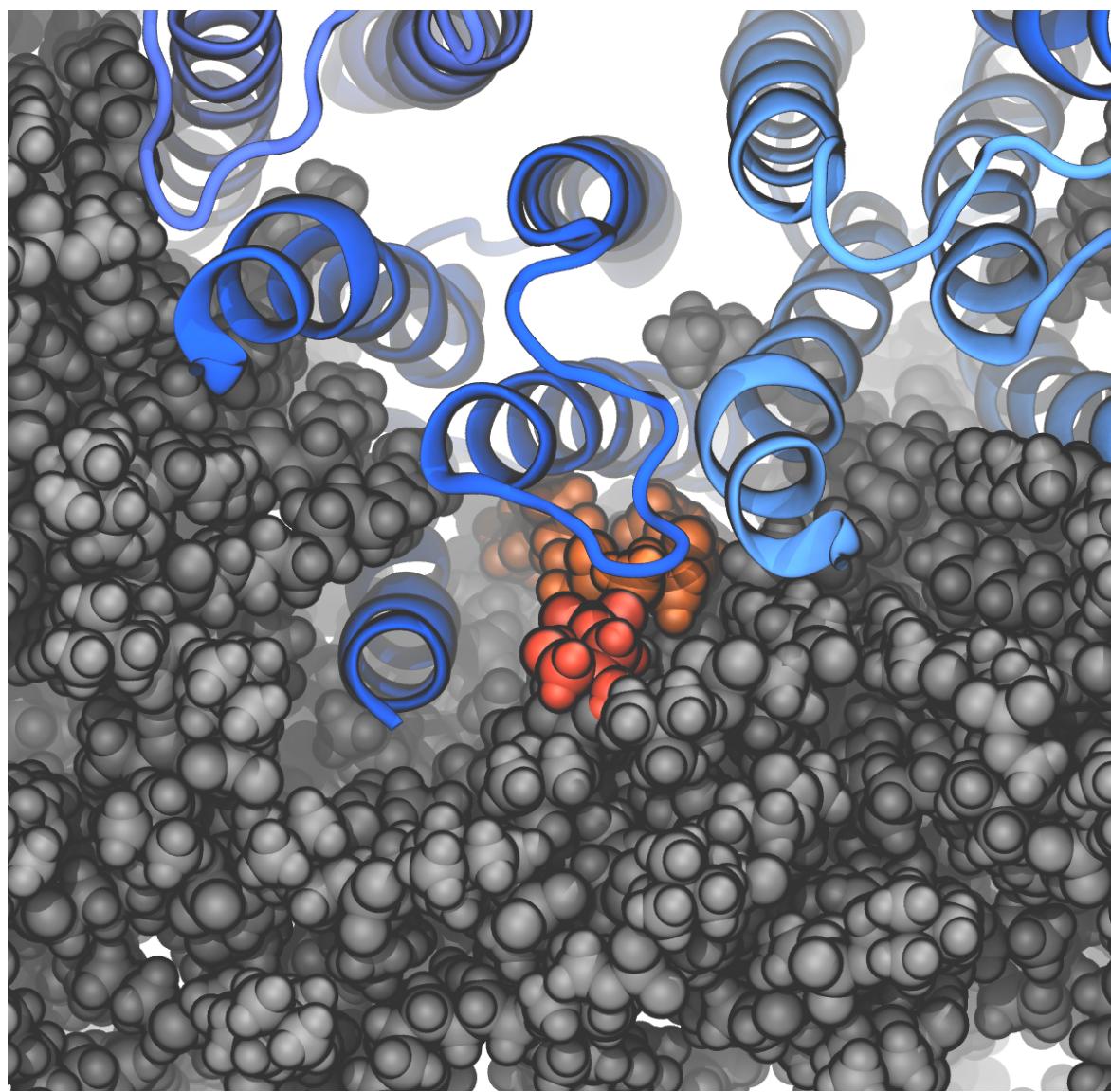


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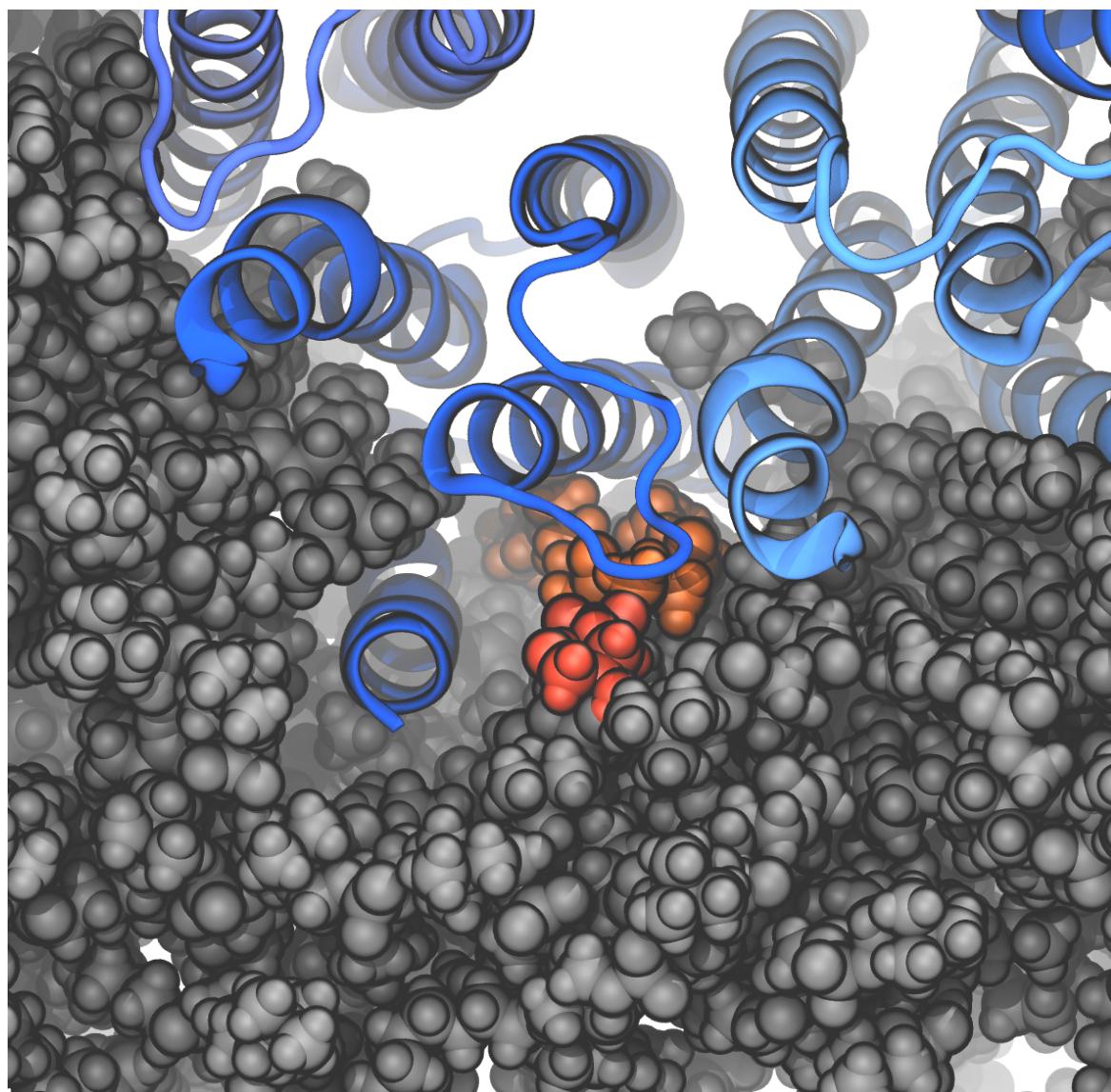
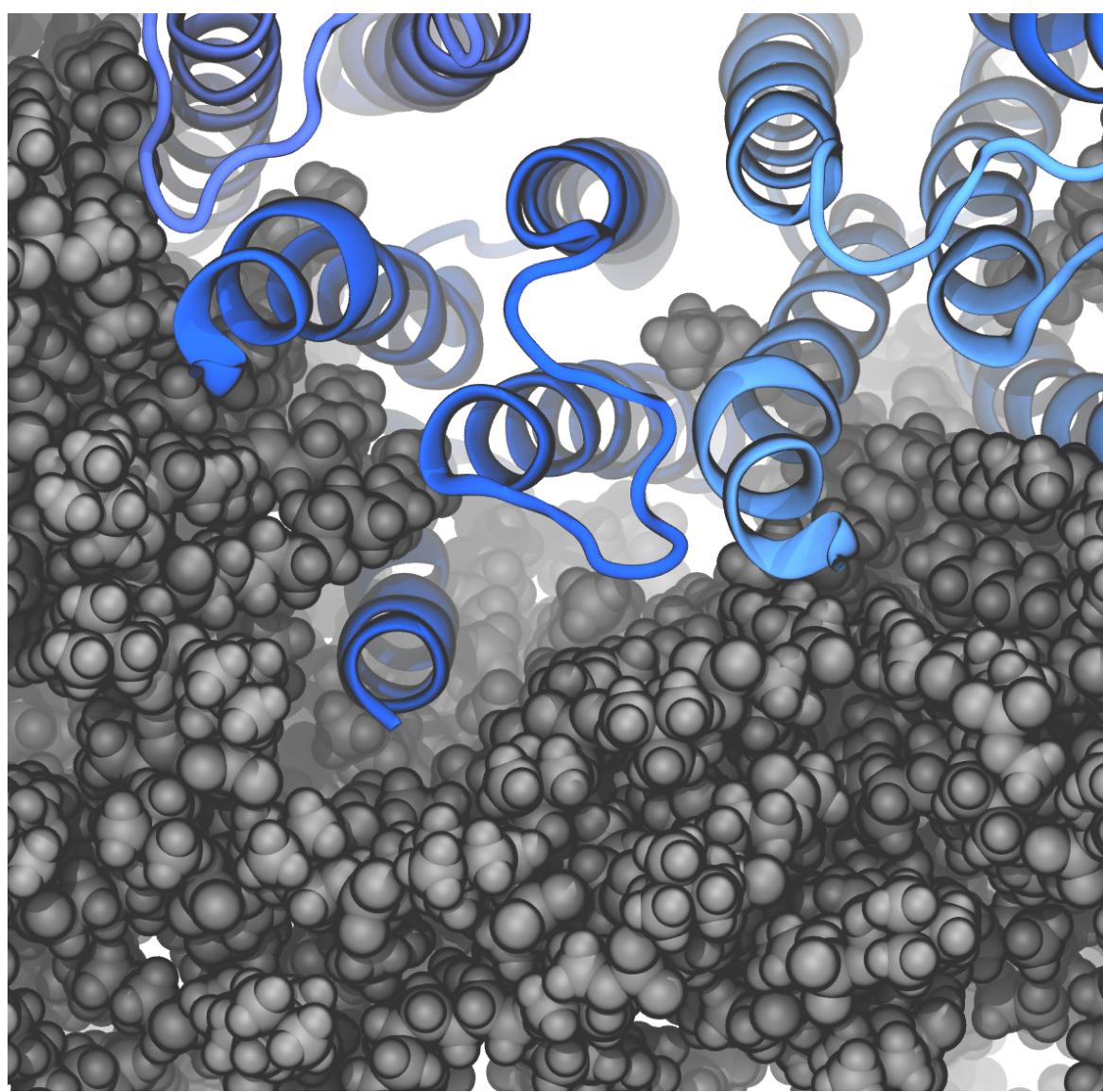
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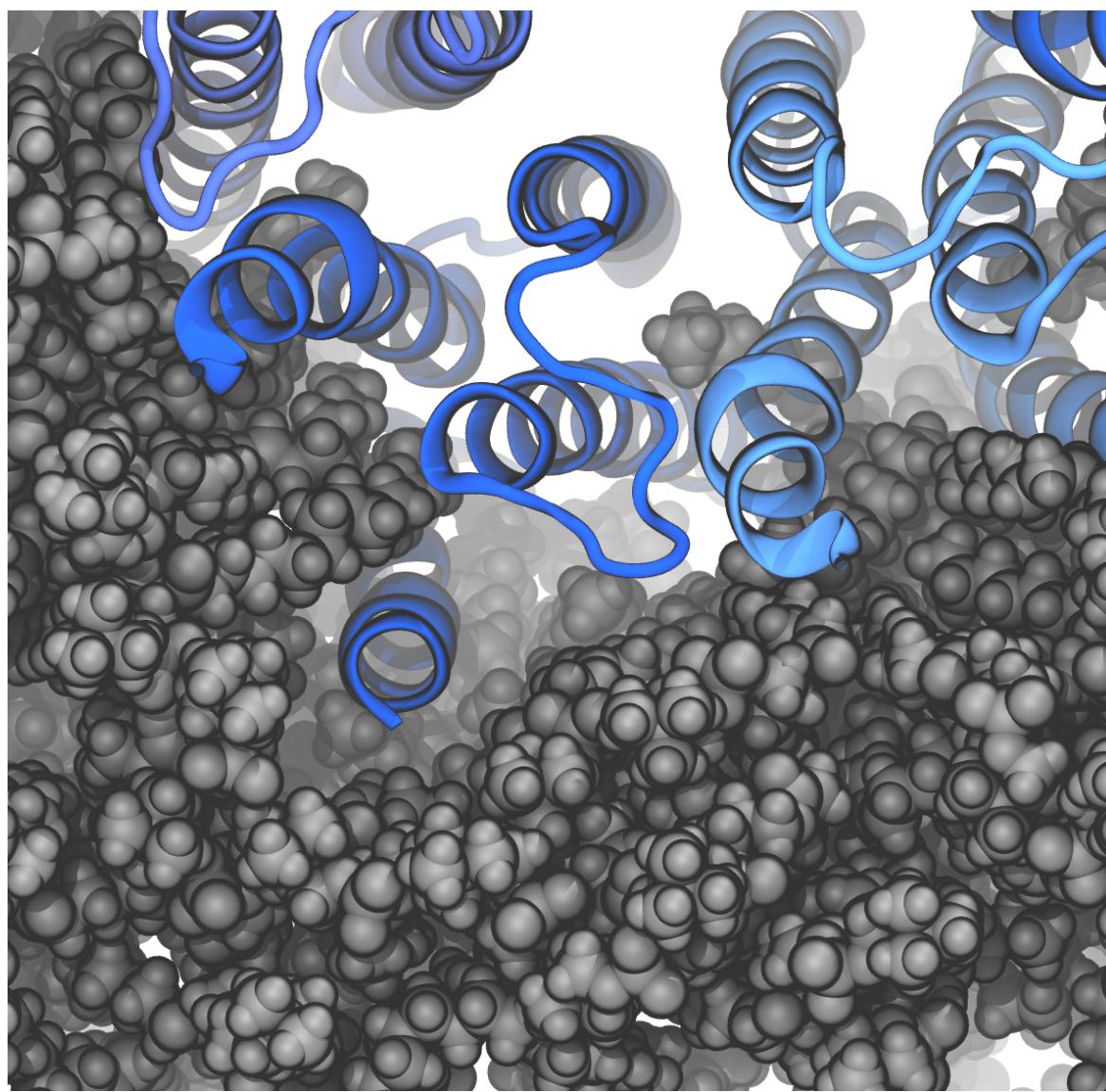
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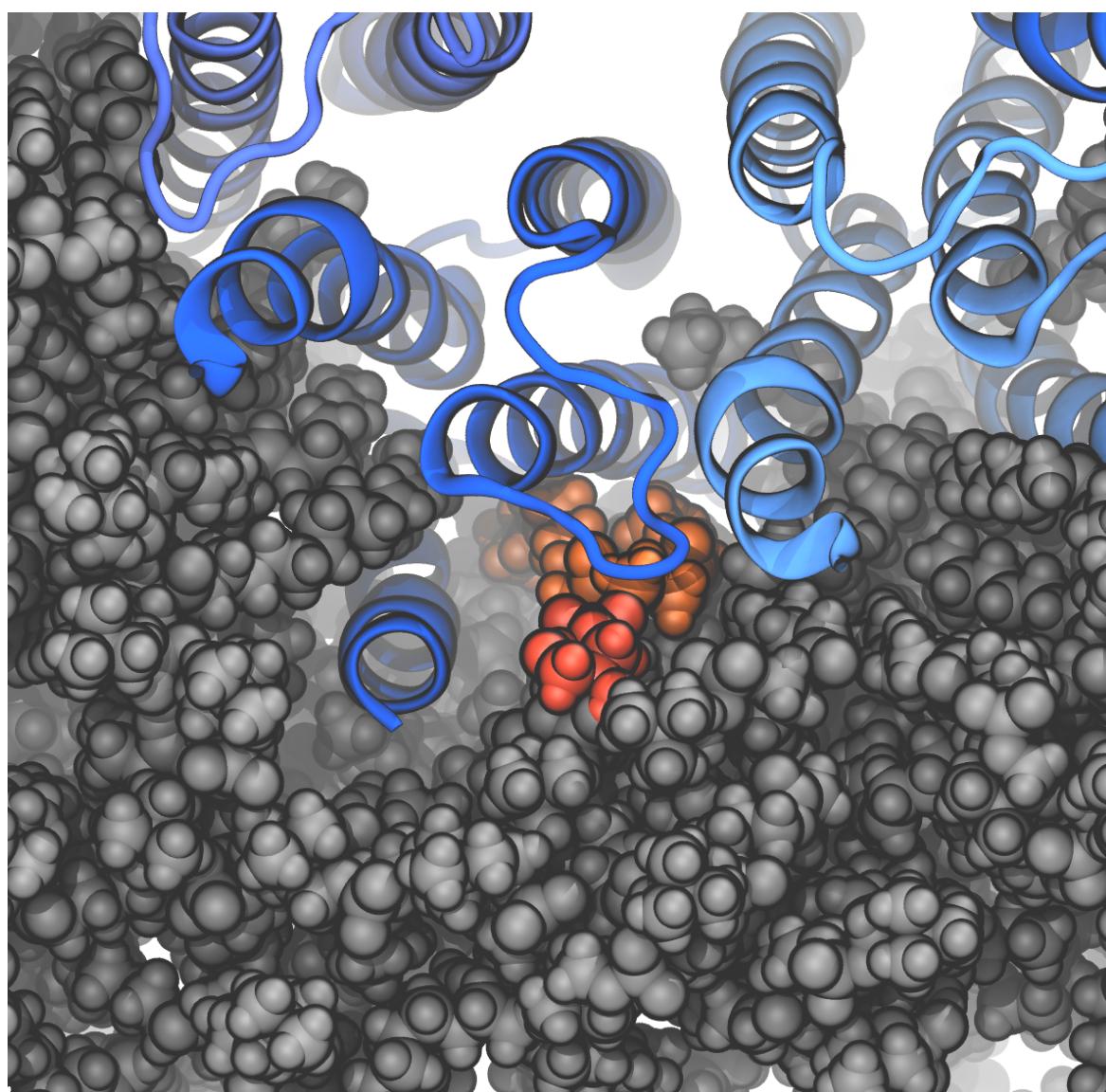
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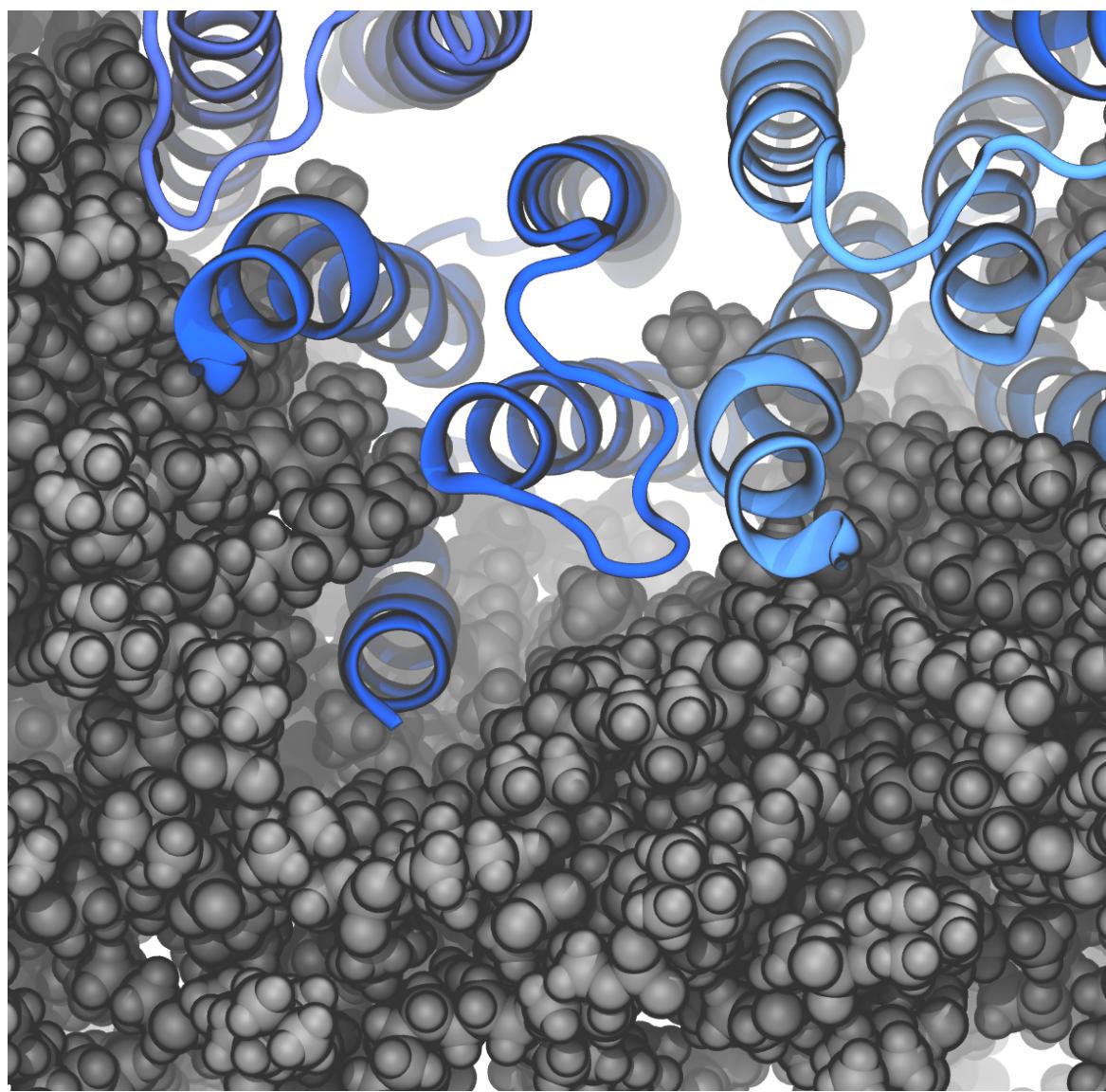
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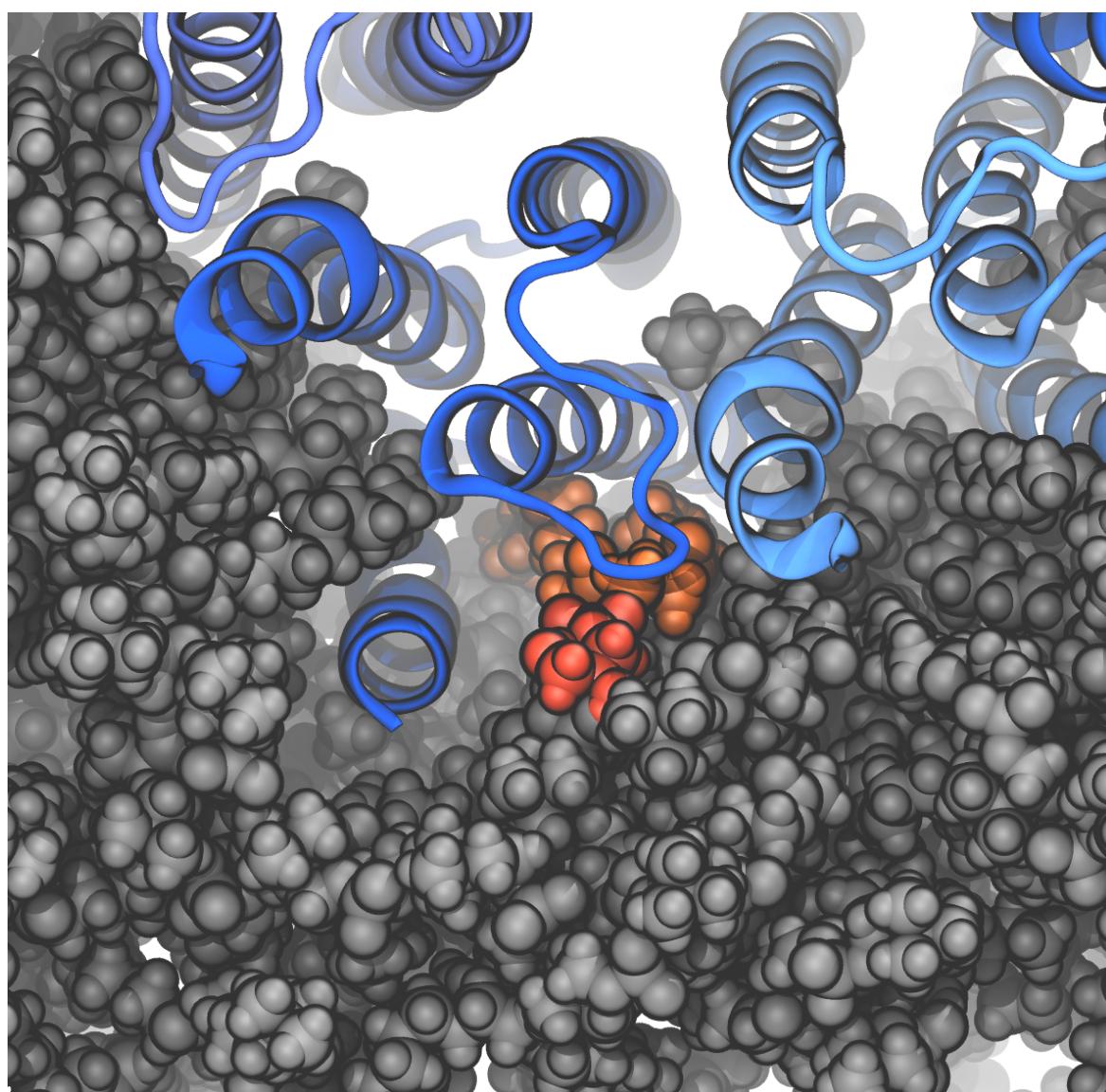
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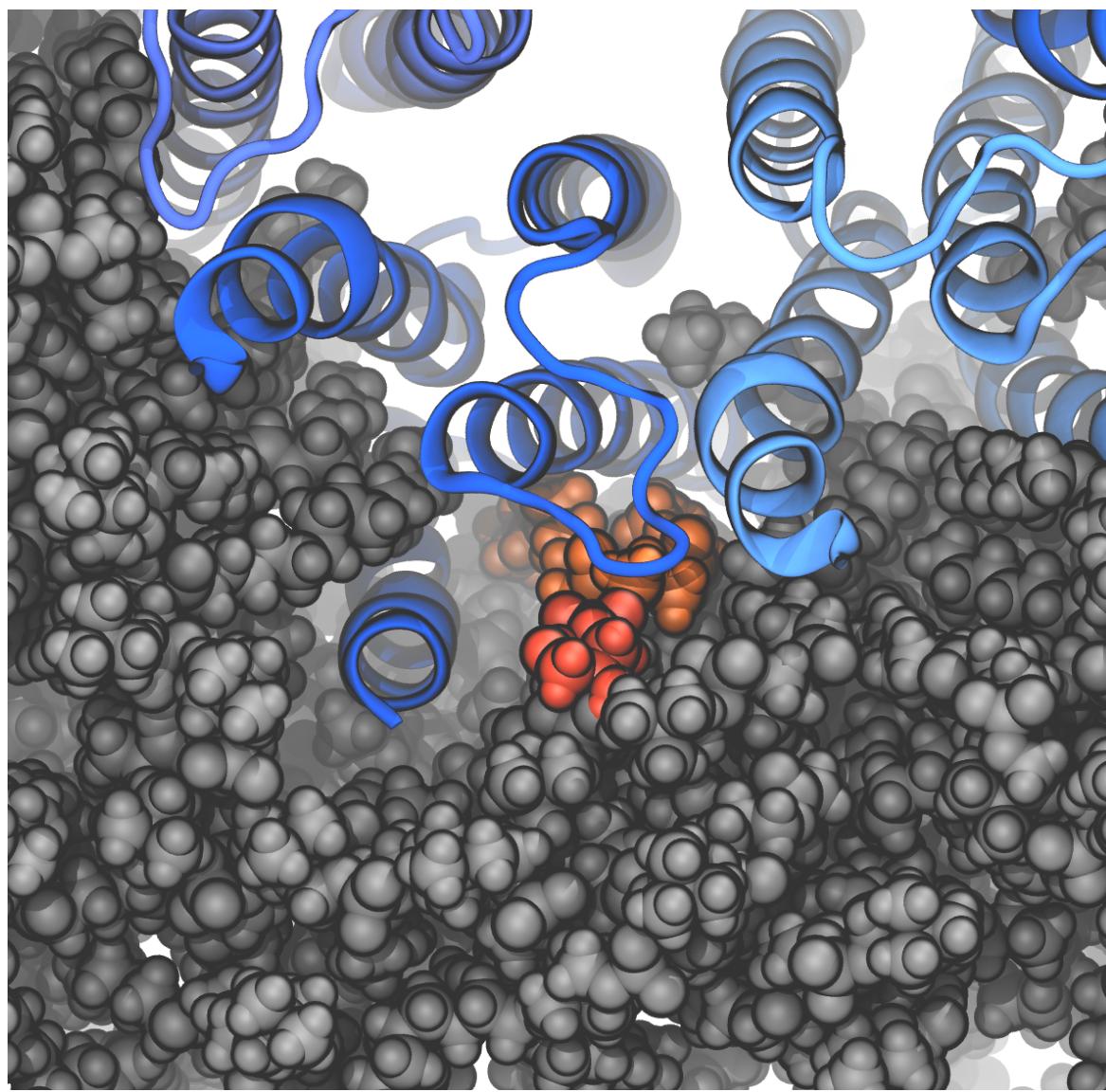
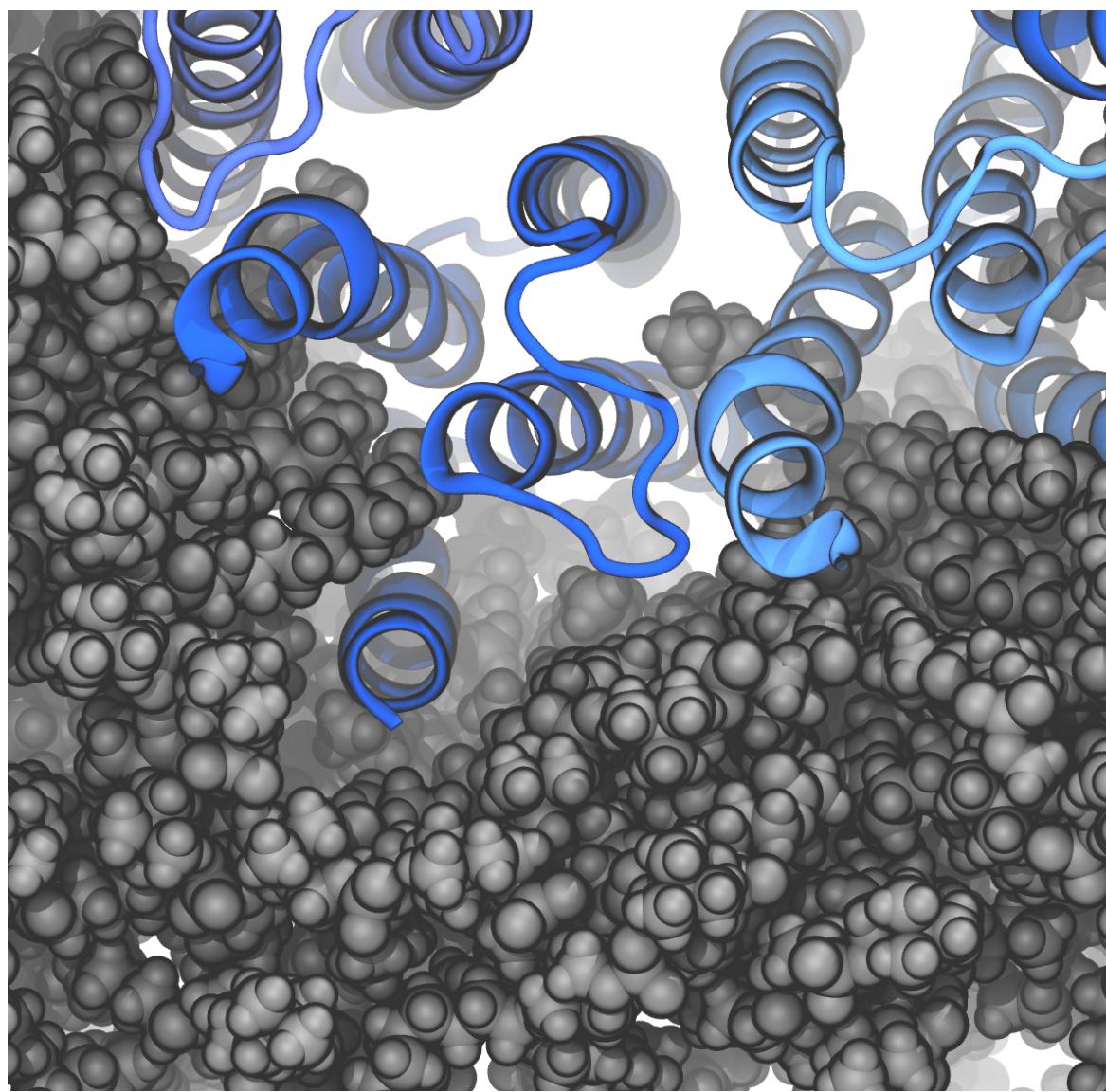
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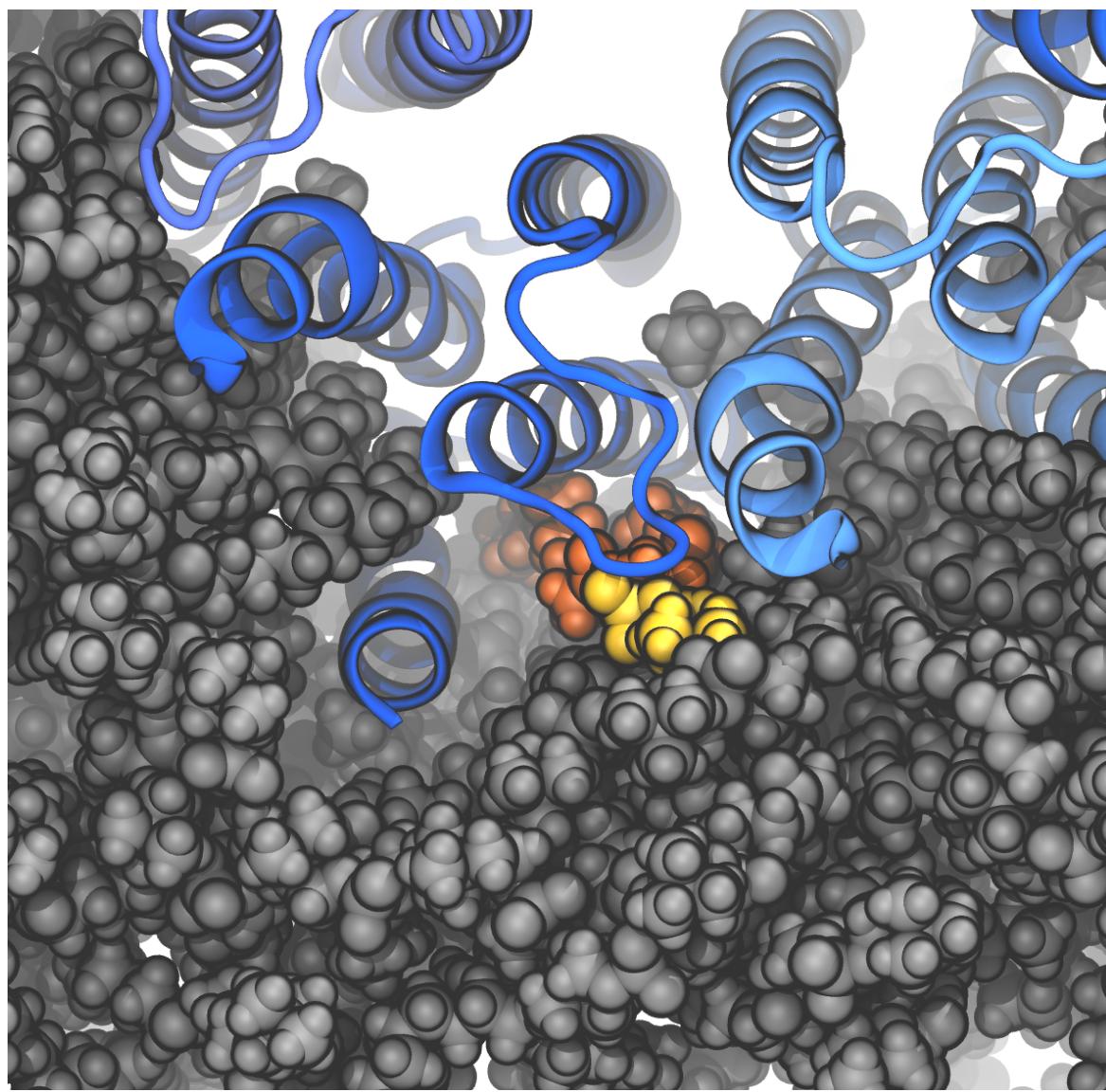
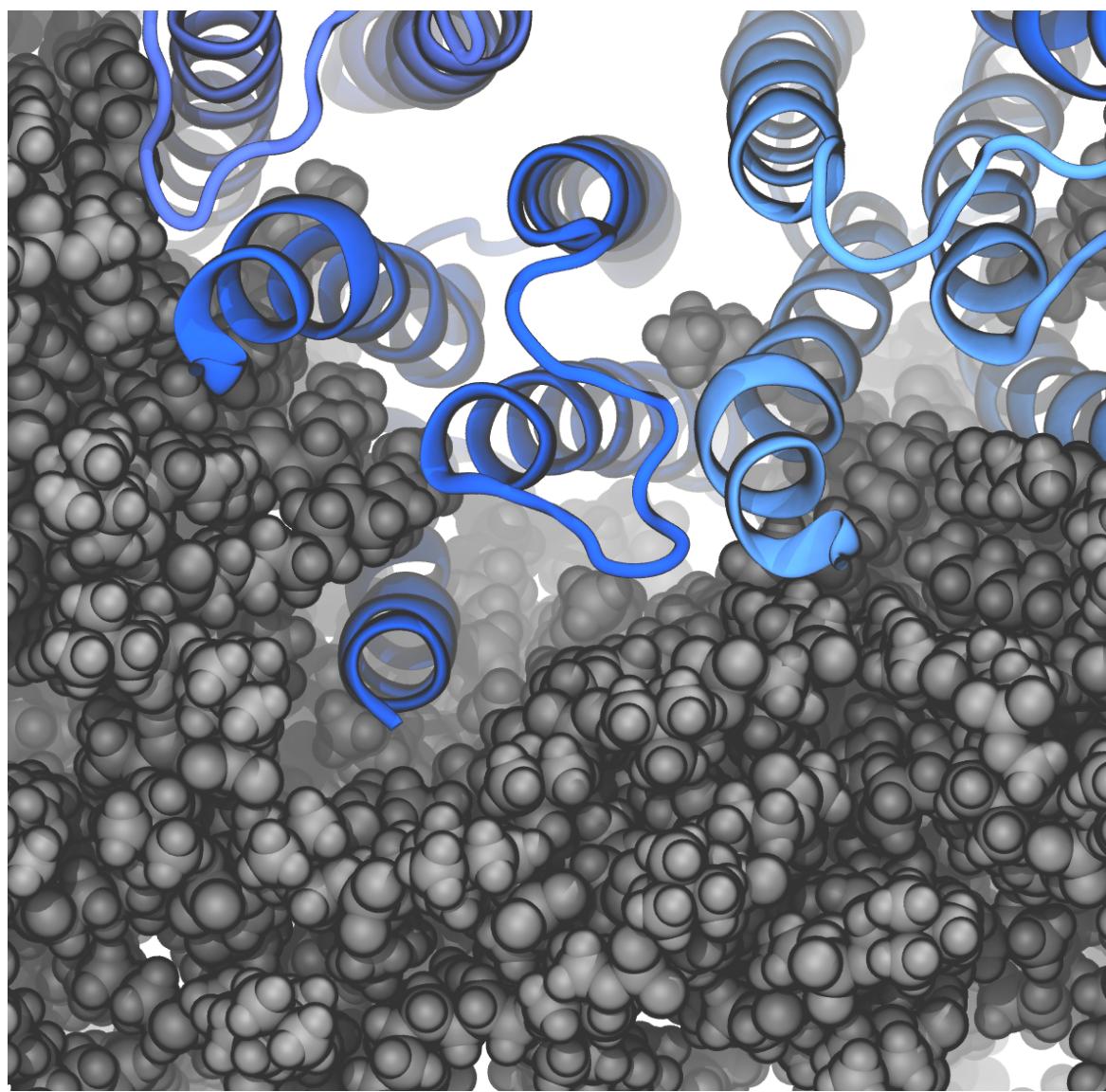
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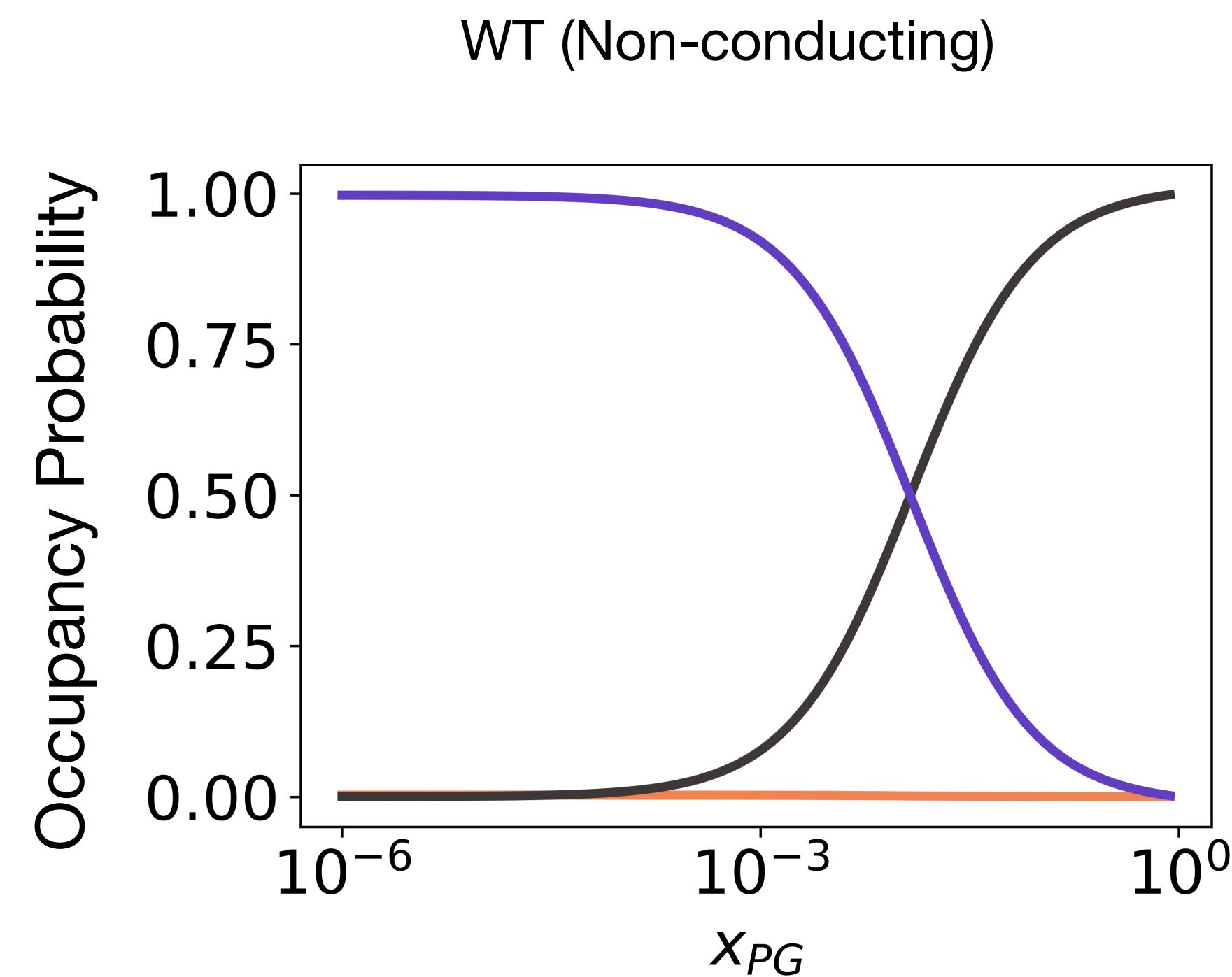
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Virtual Competition Assay Estimates (Relative SAFEP)

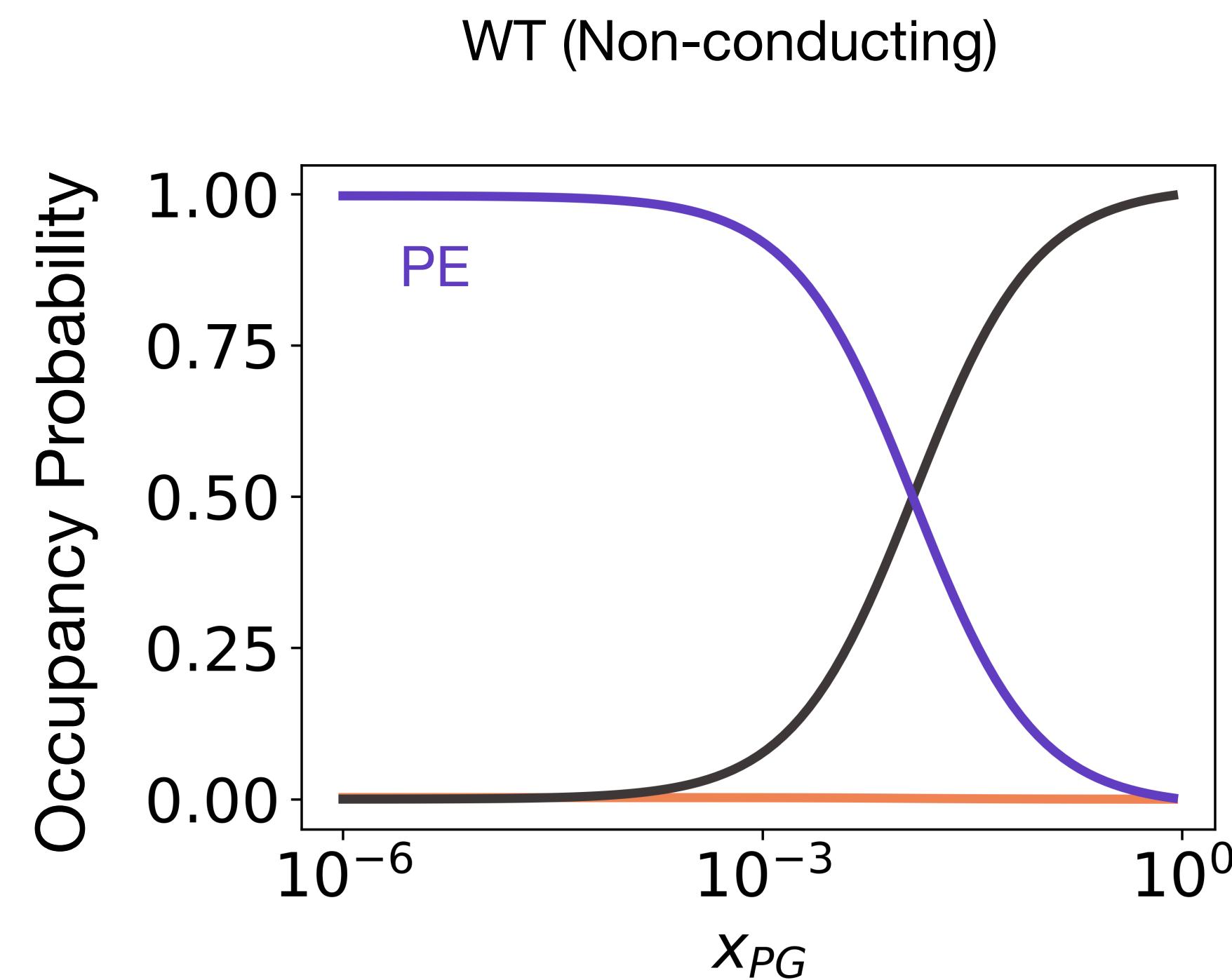
Occupancy Predicted by Relative FEP

2:1:X PC:PE:PG mixture



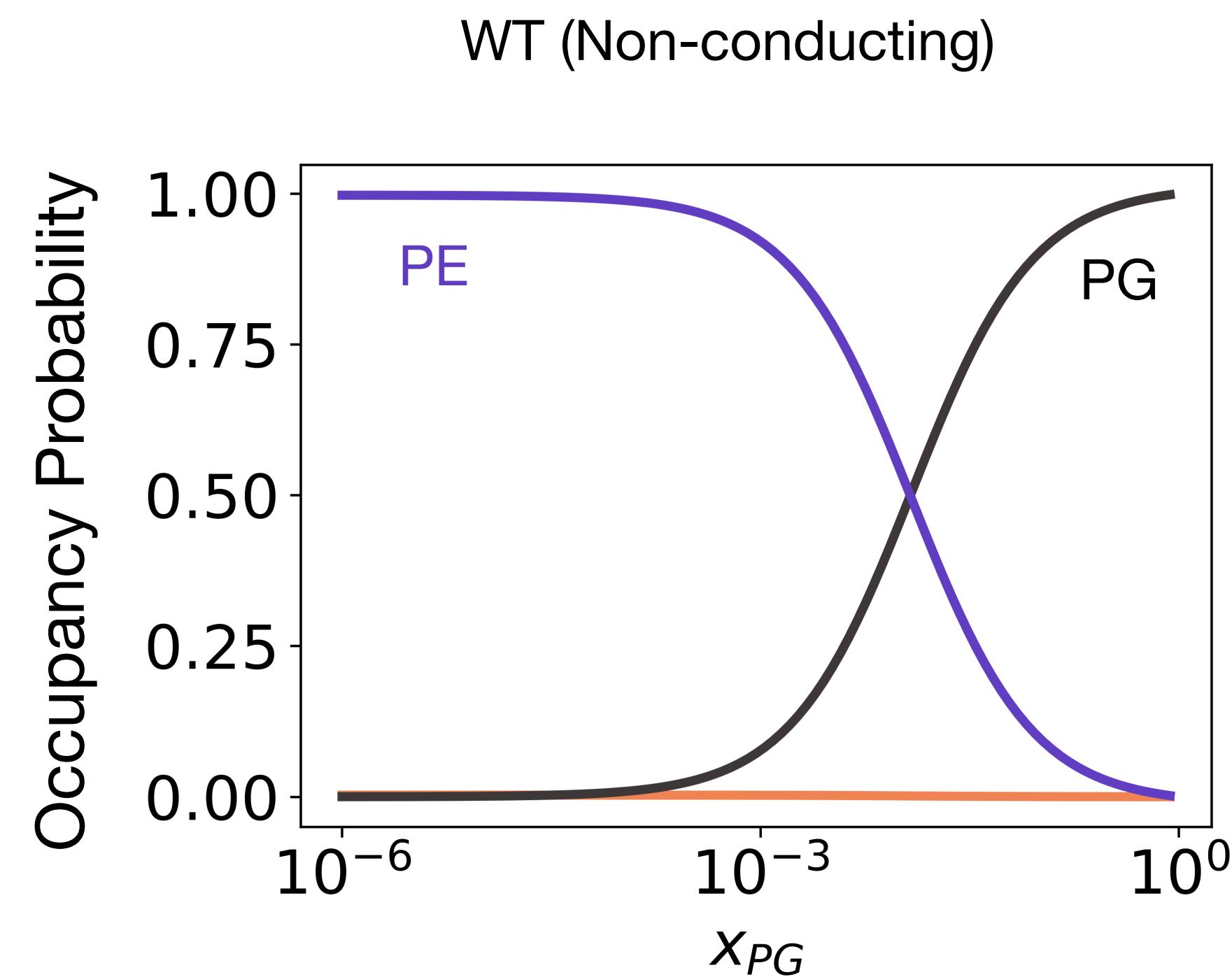
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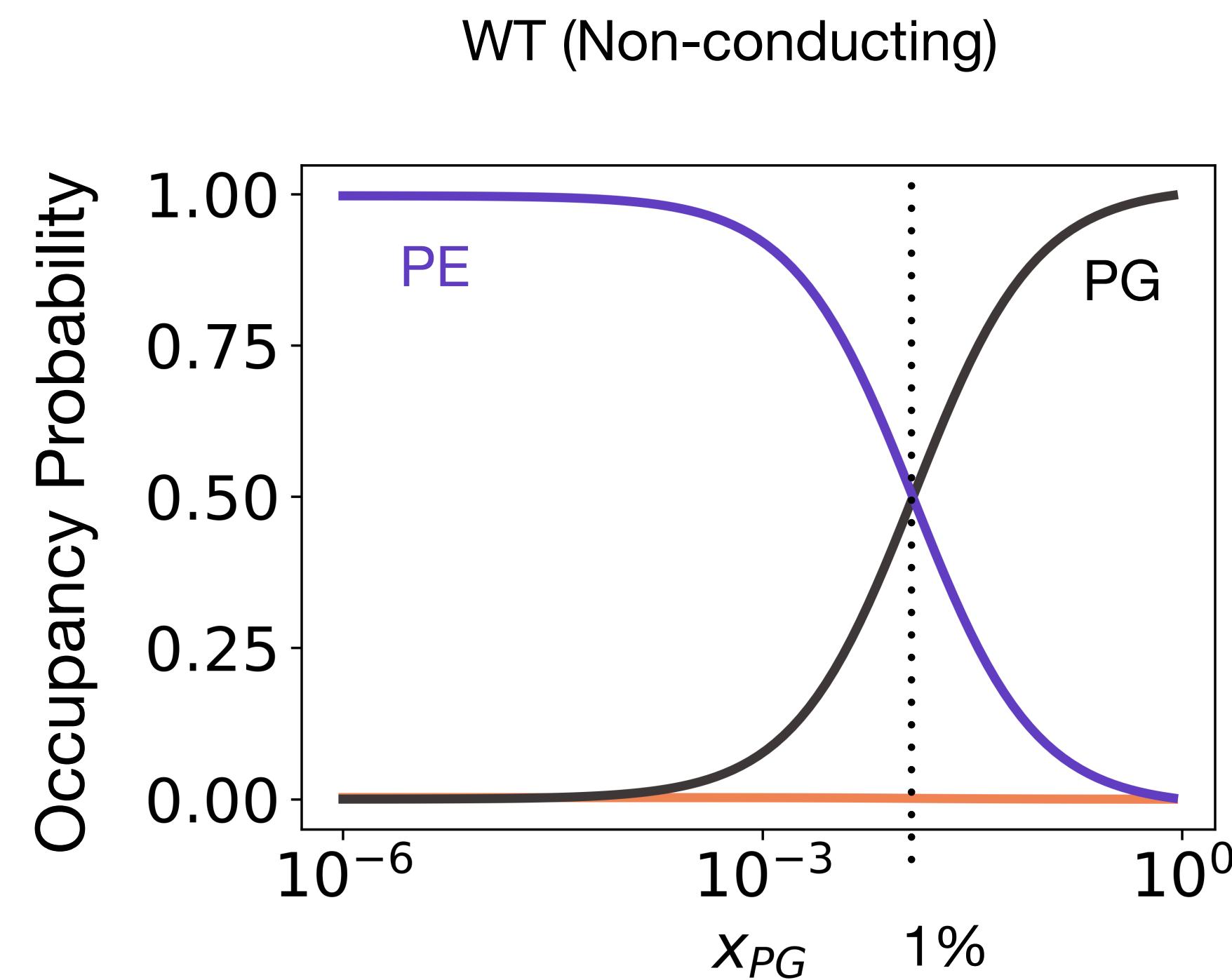
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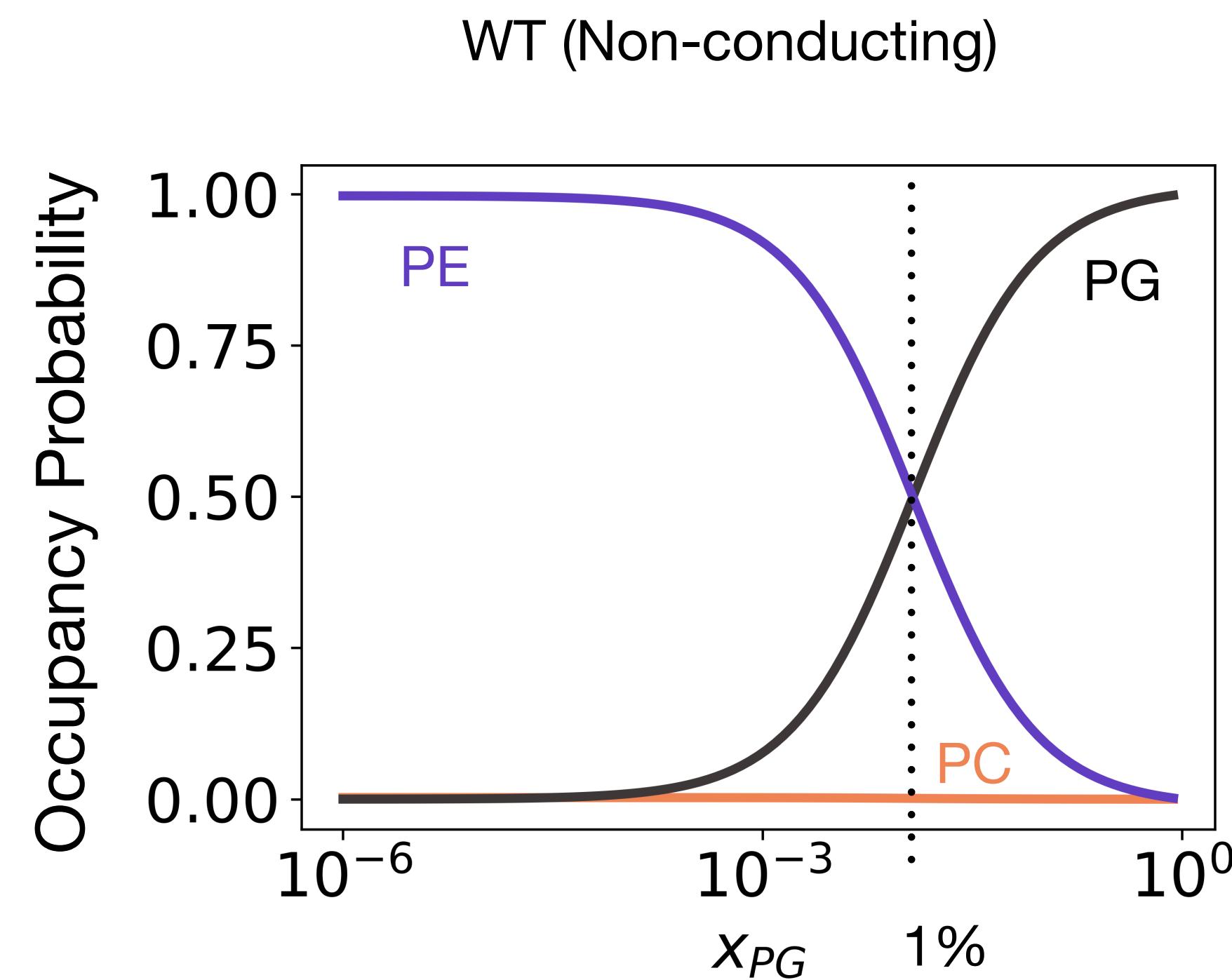
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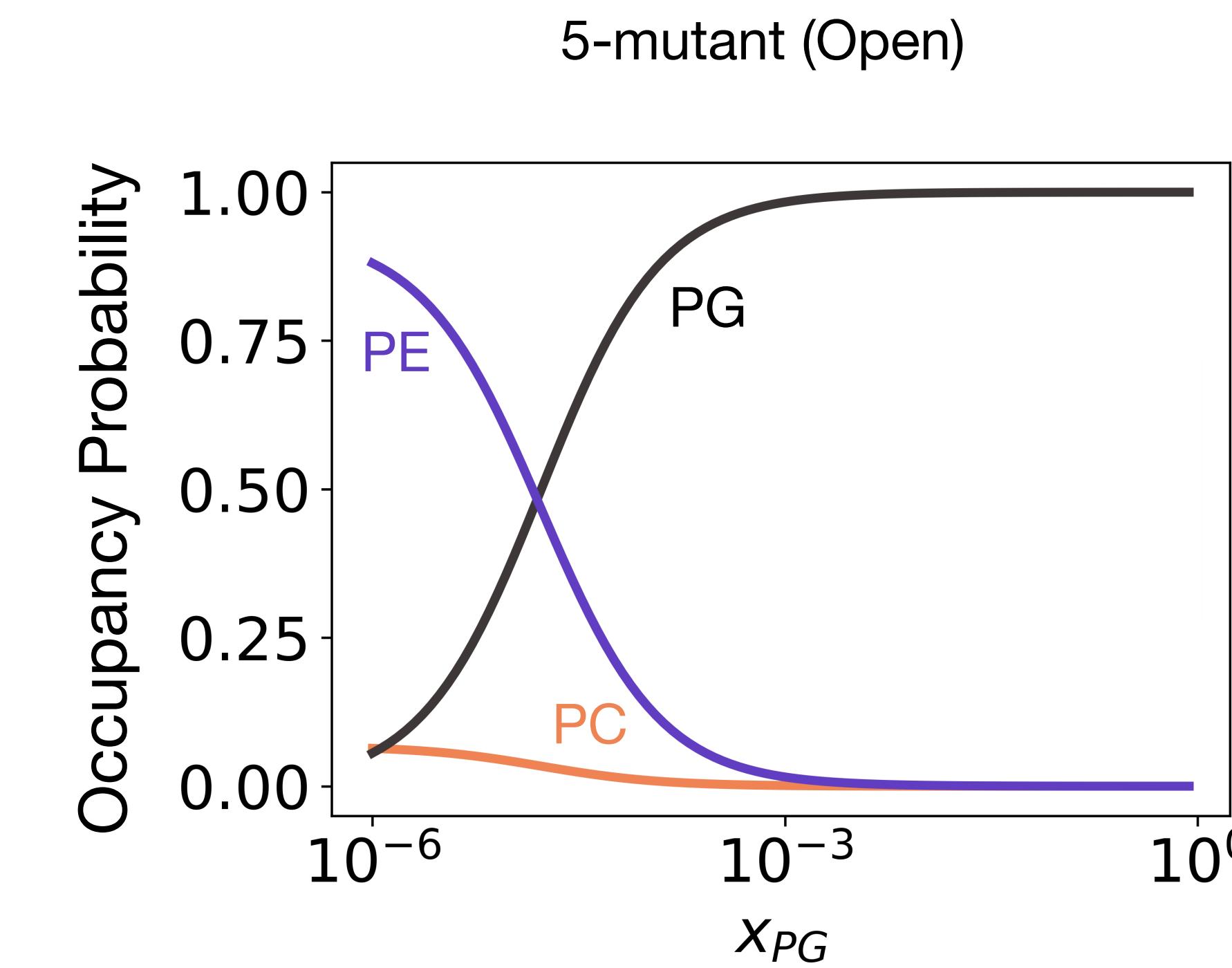
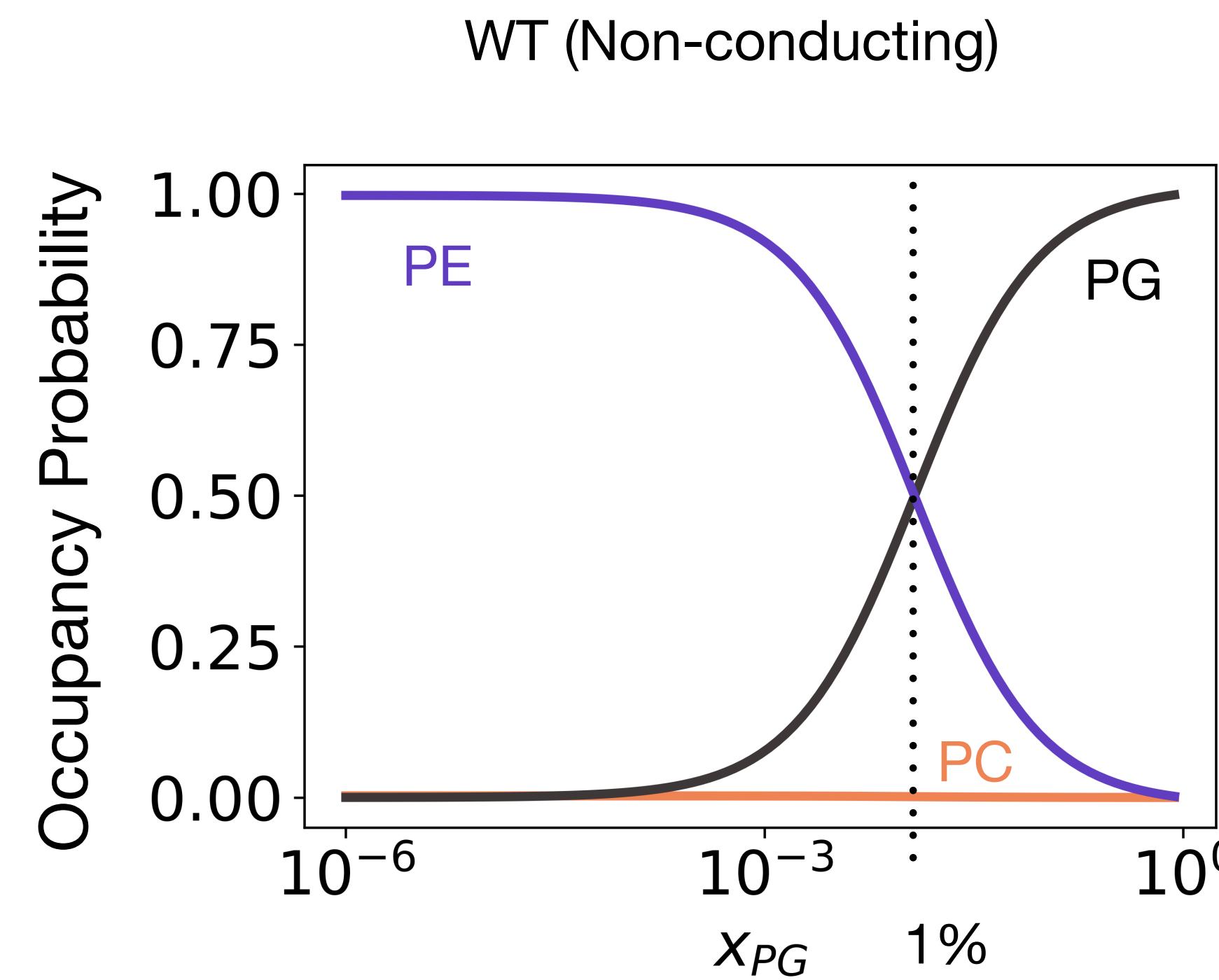
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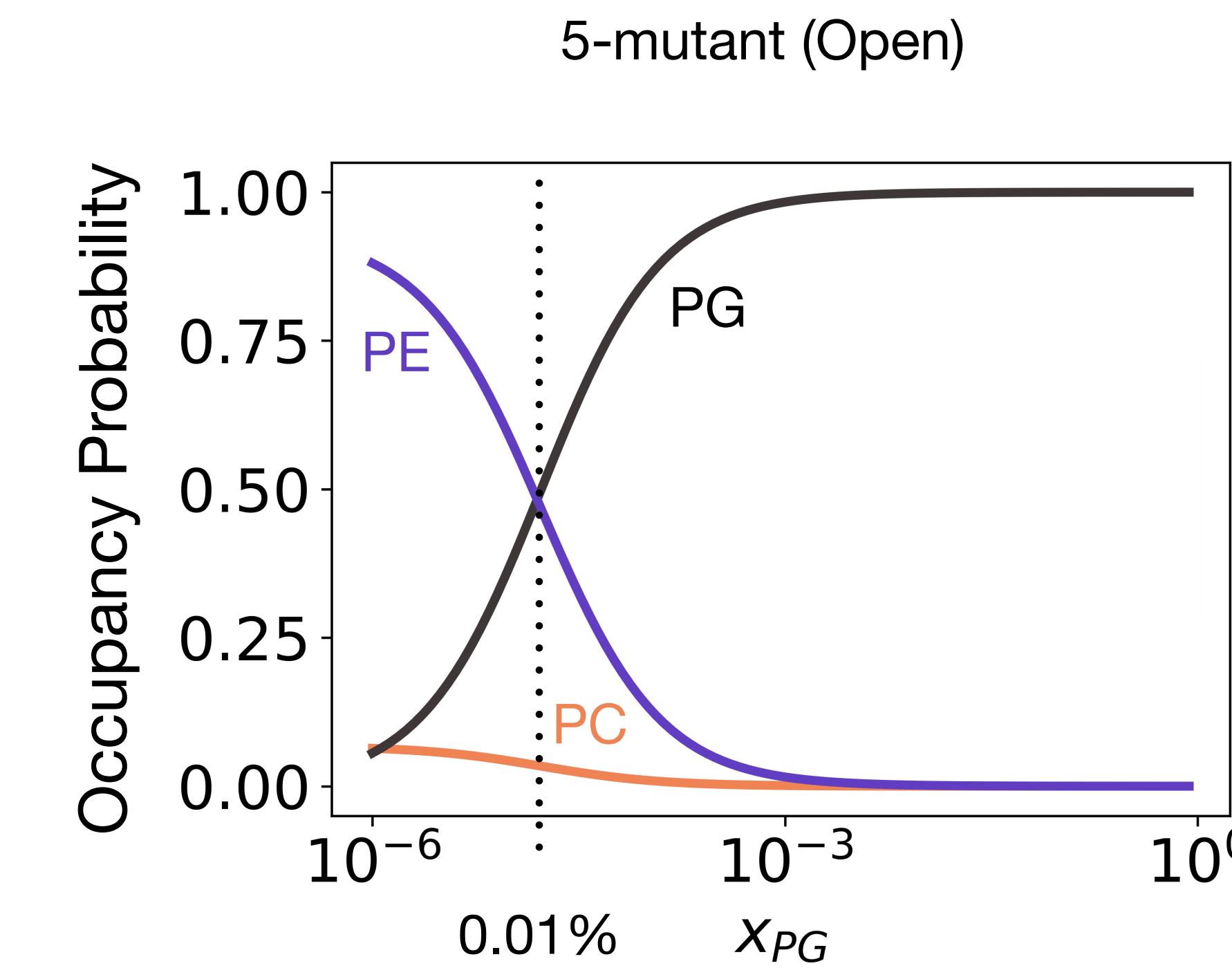
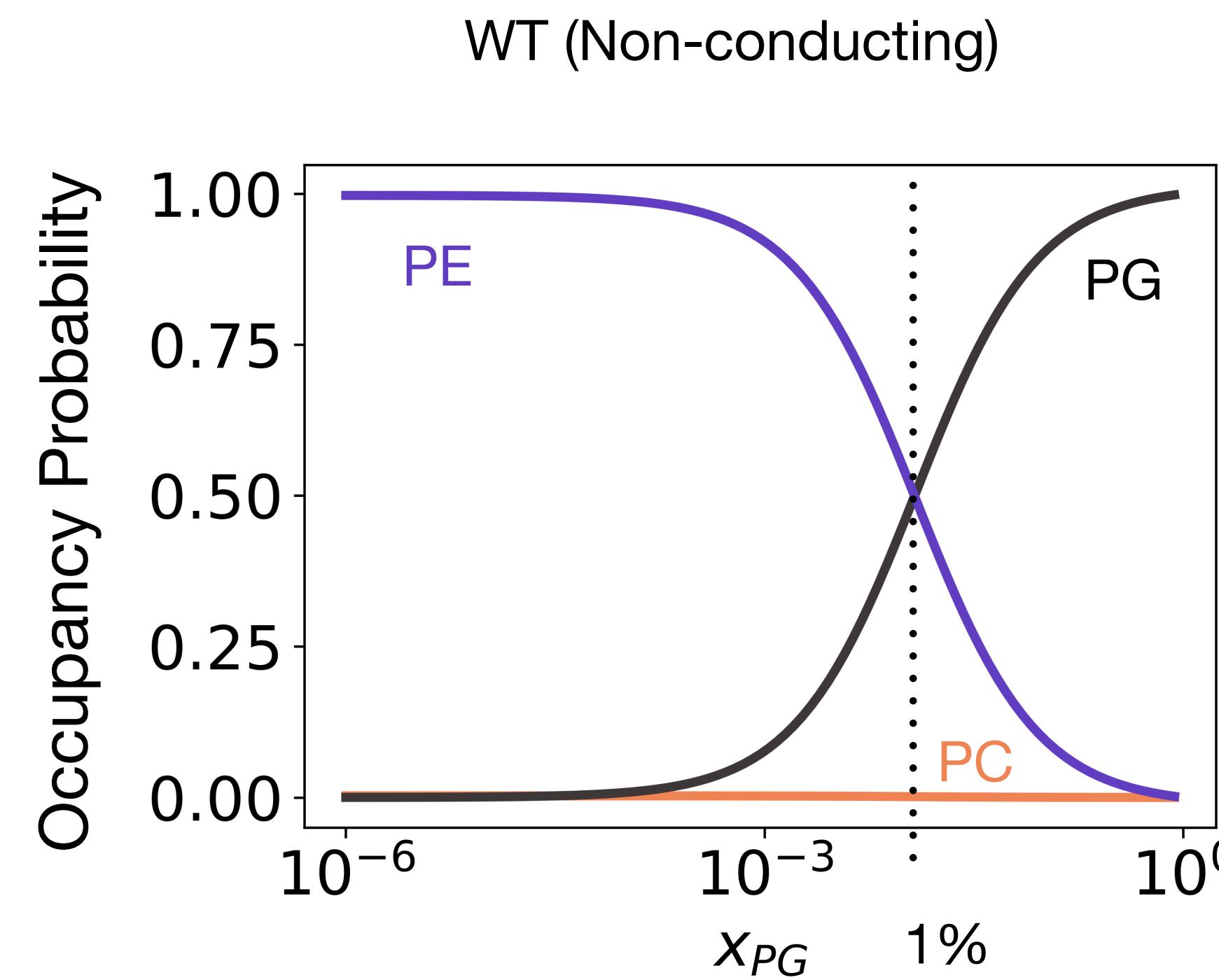
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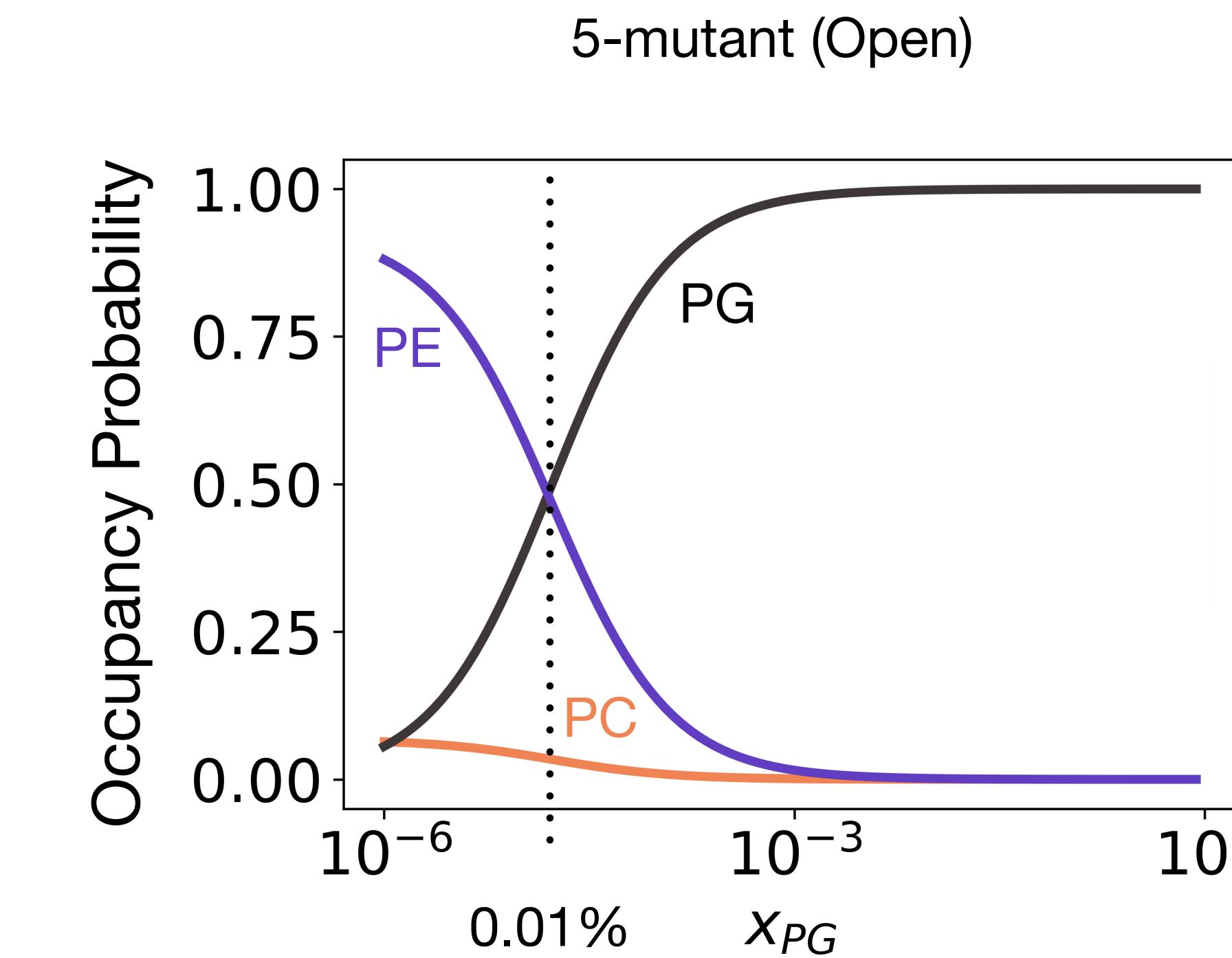
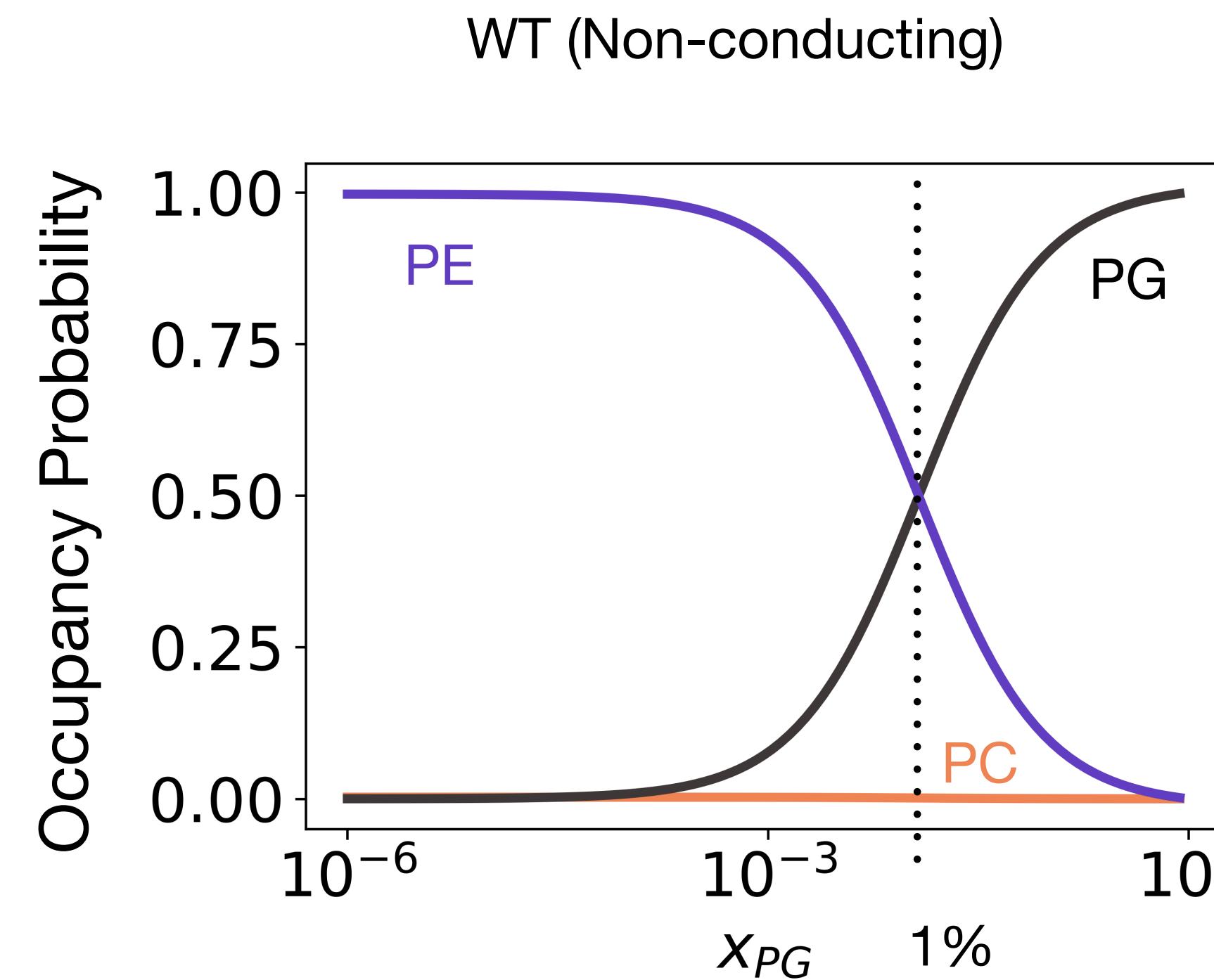
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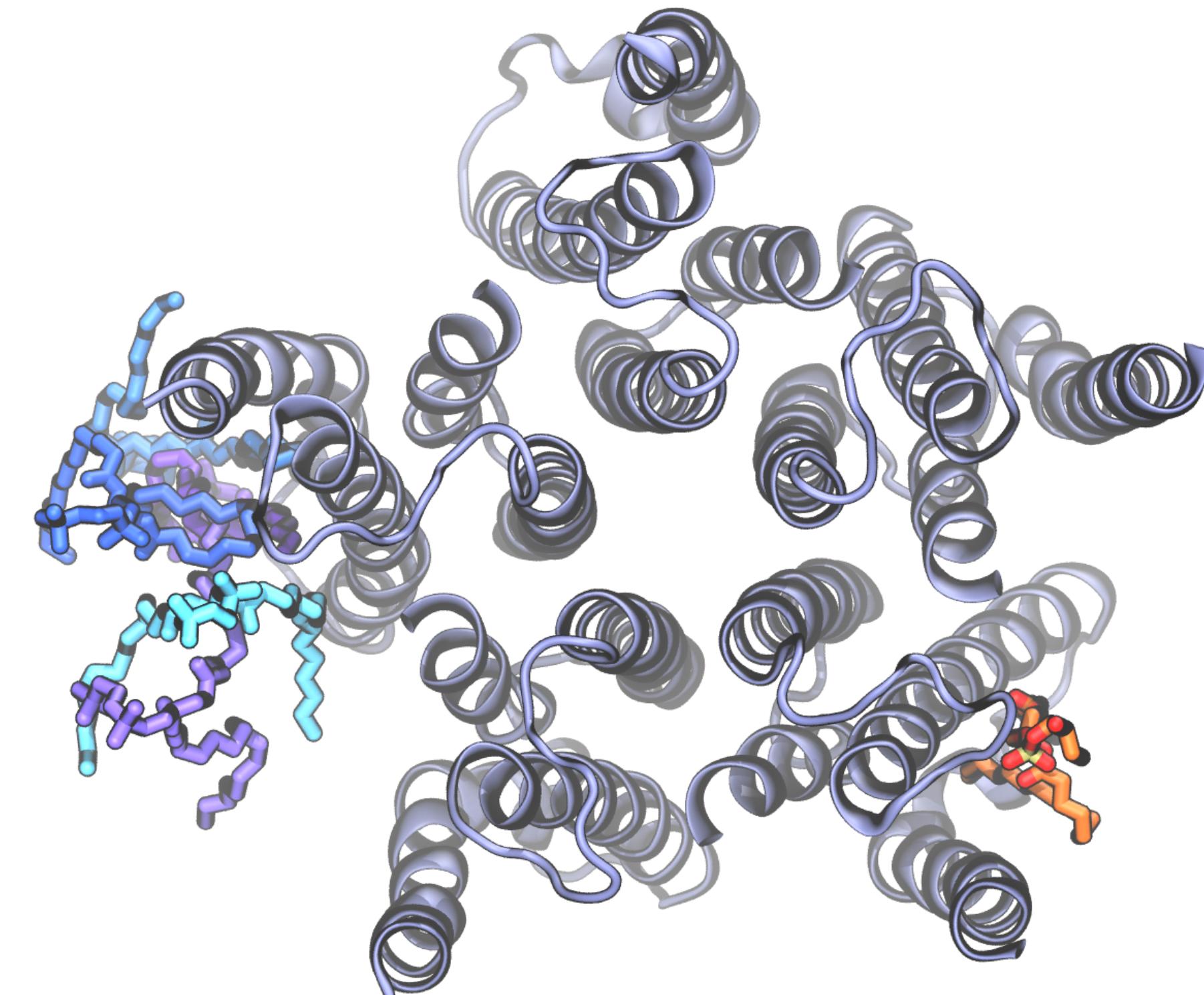
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POPC looks like a solvent...

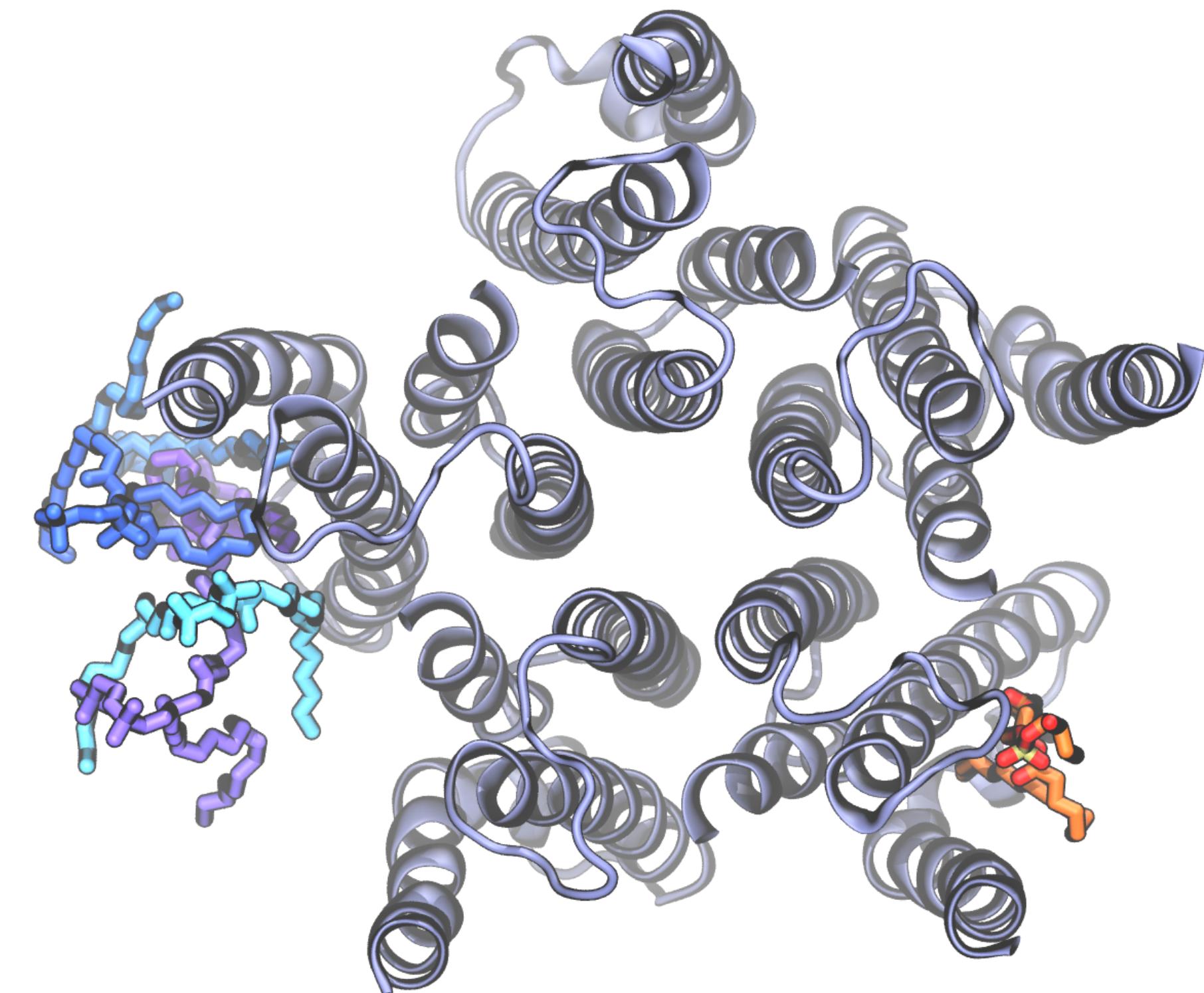
Binary mixtures POPC+POPG: “Competition” between ligand and solvent?

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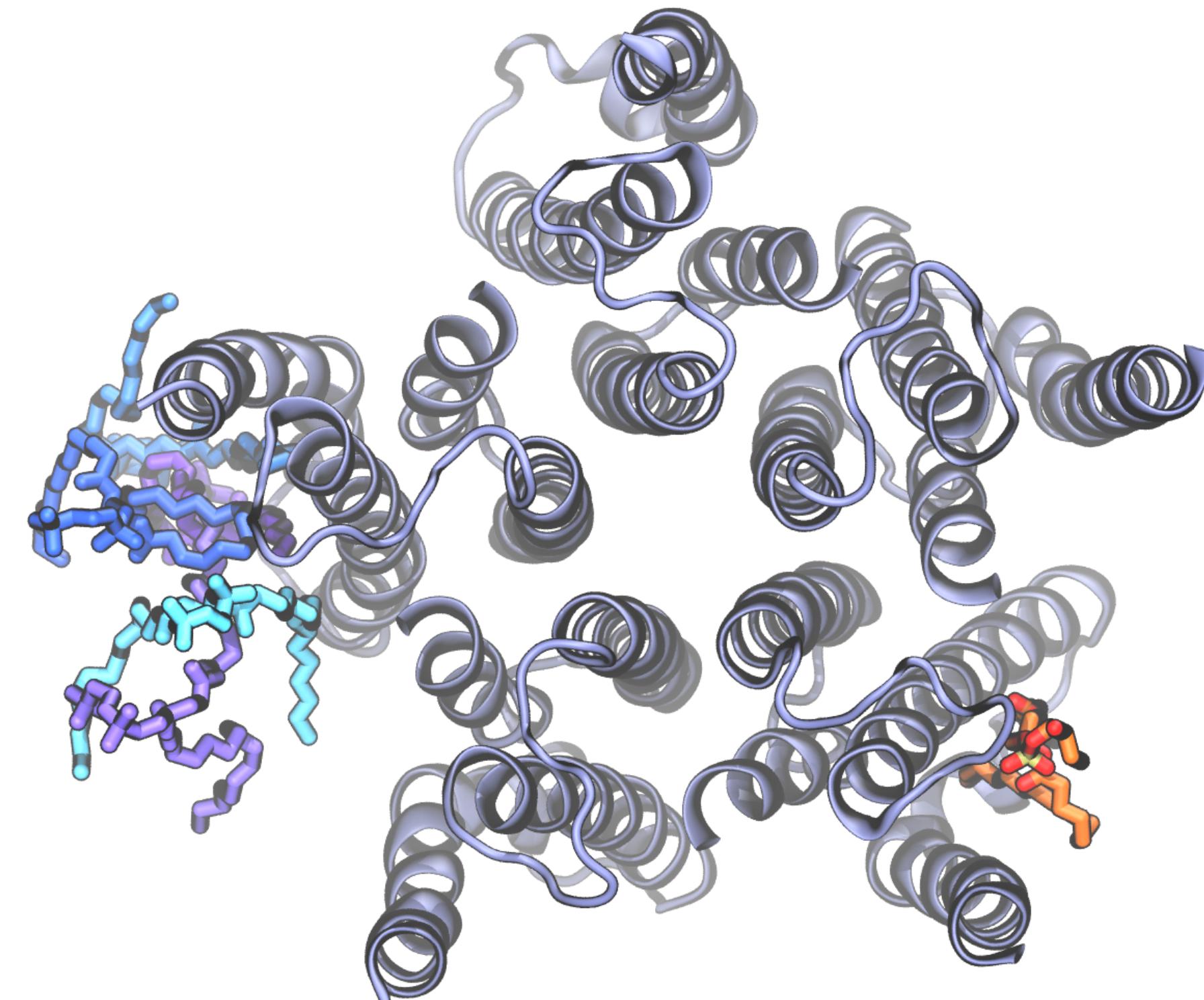
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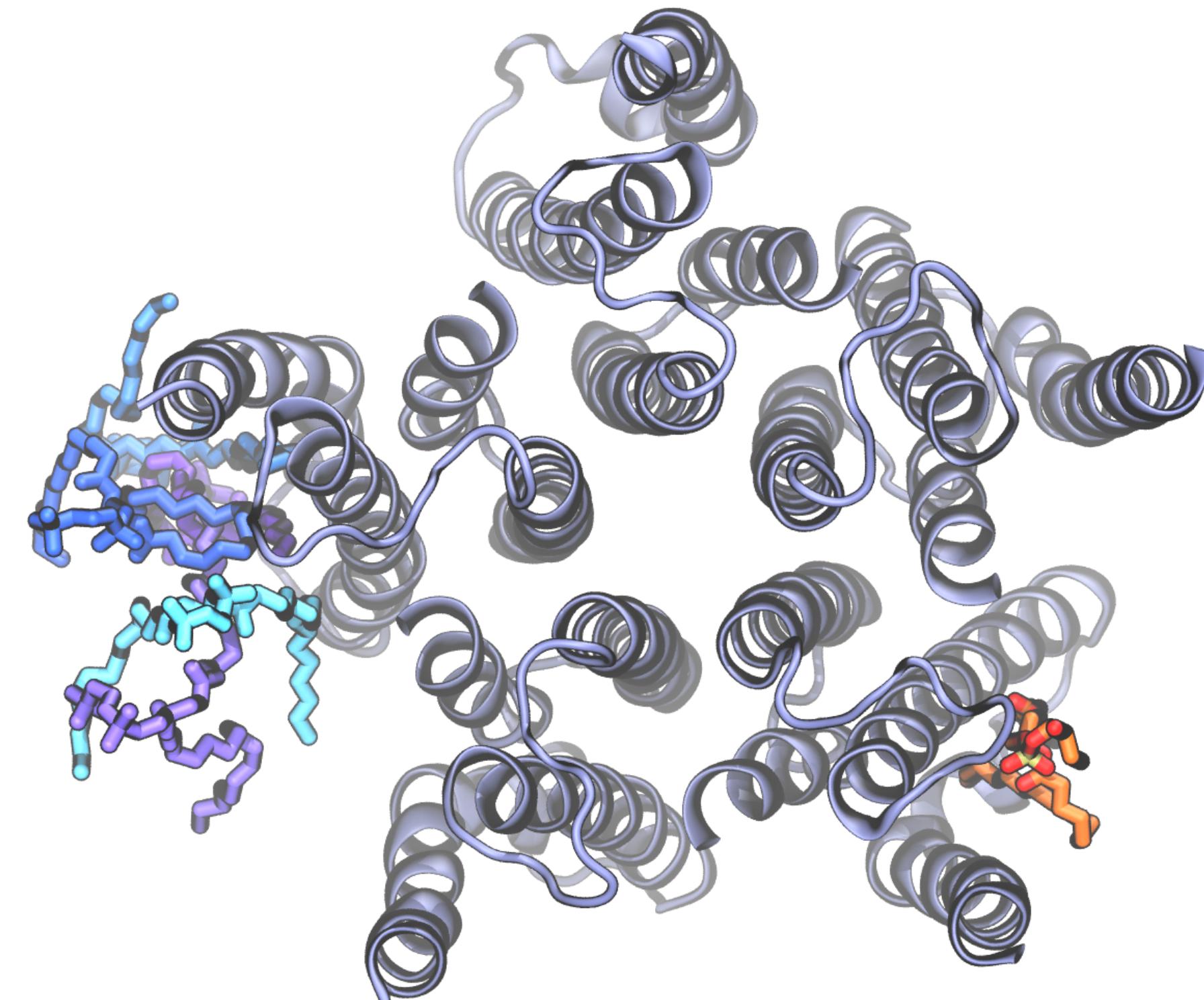
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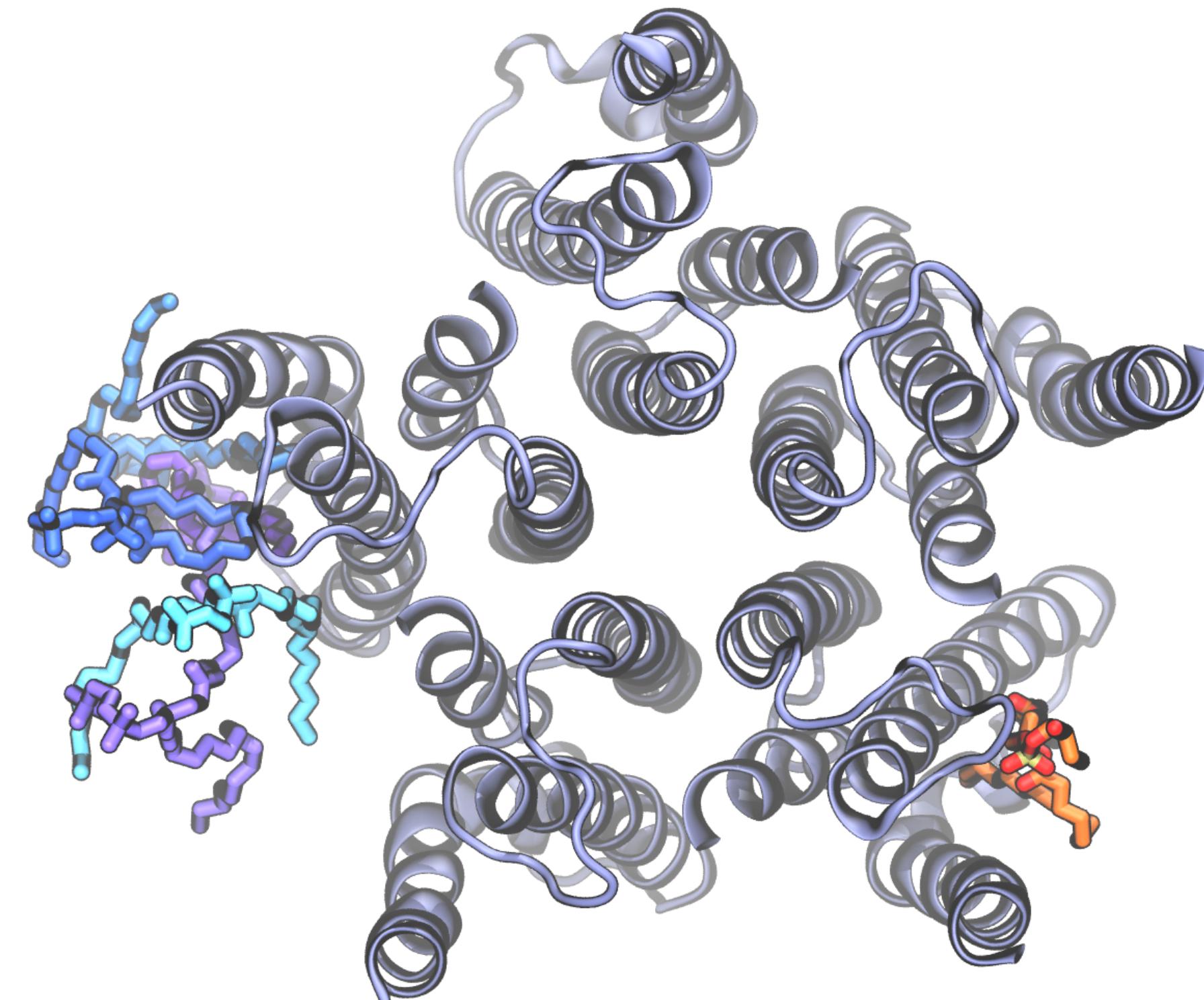
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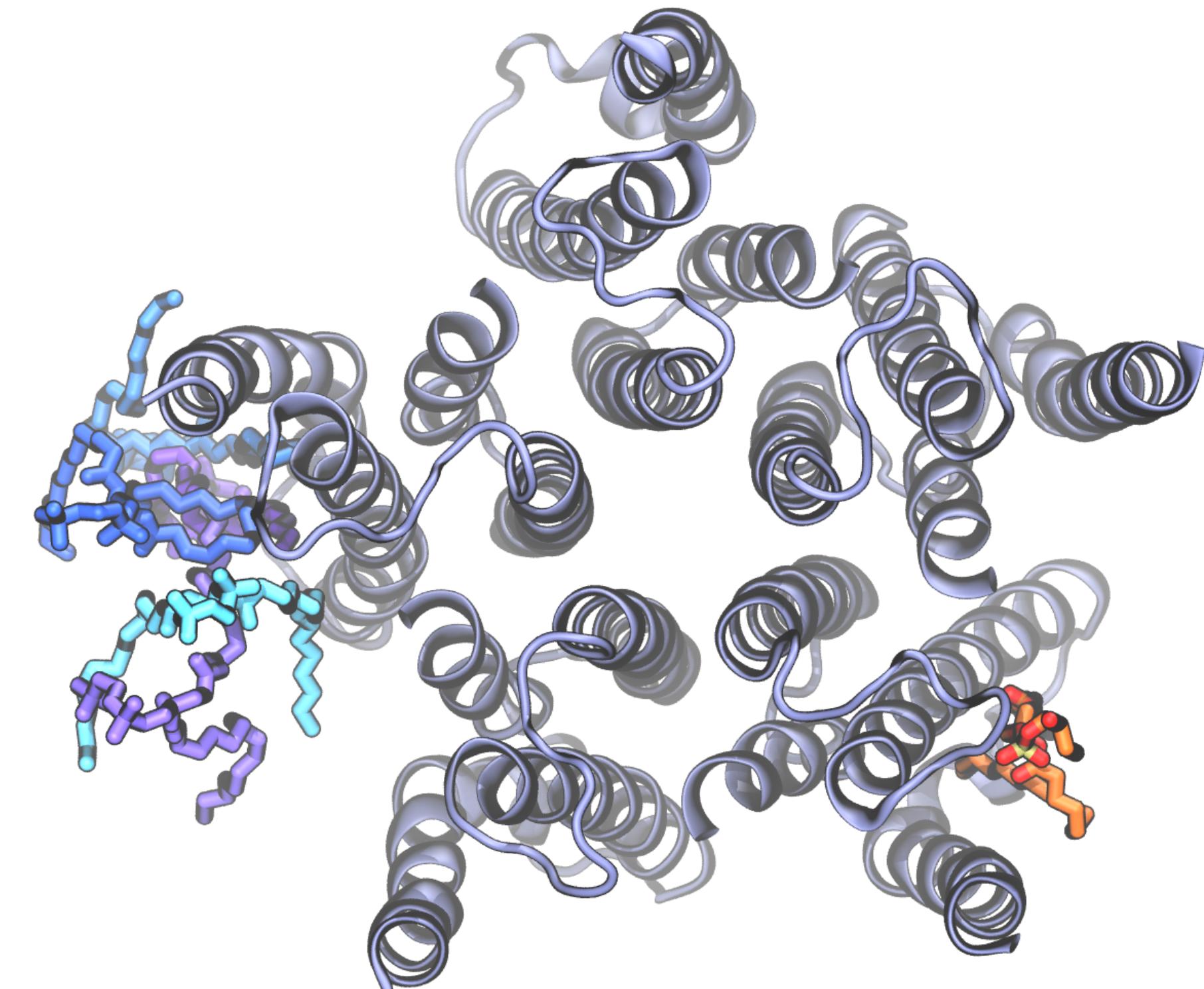
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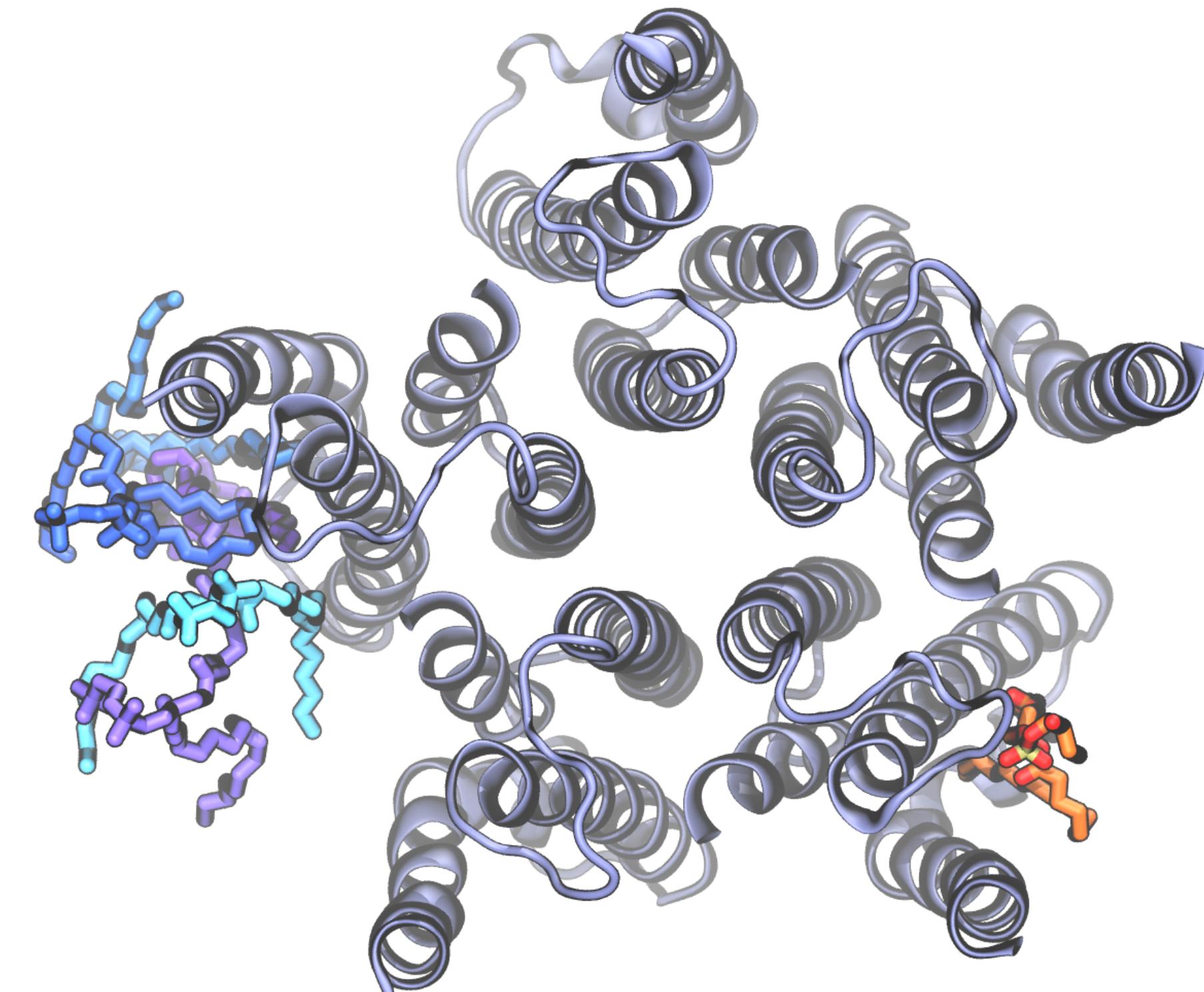
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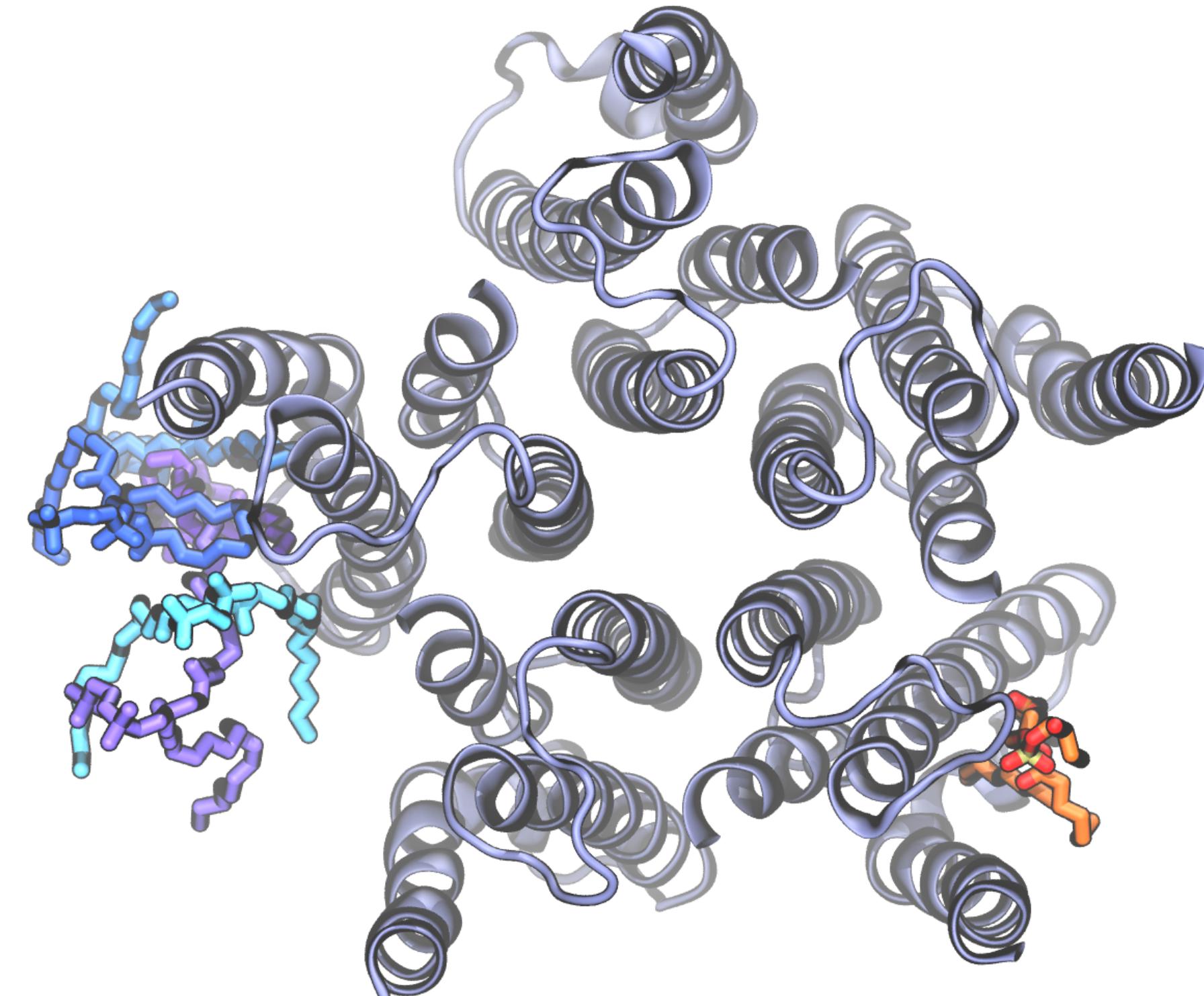


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WT CA (Agonist-Bound)

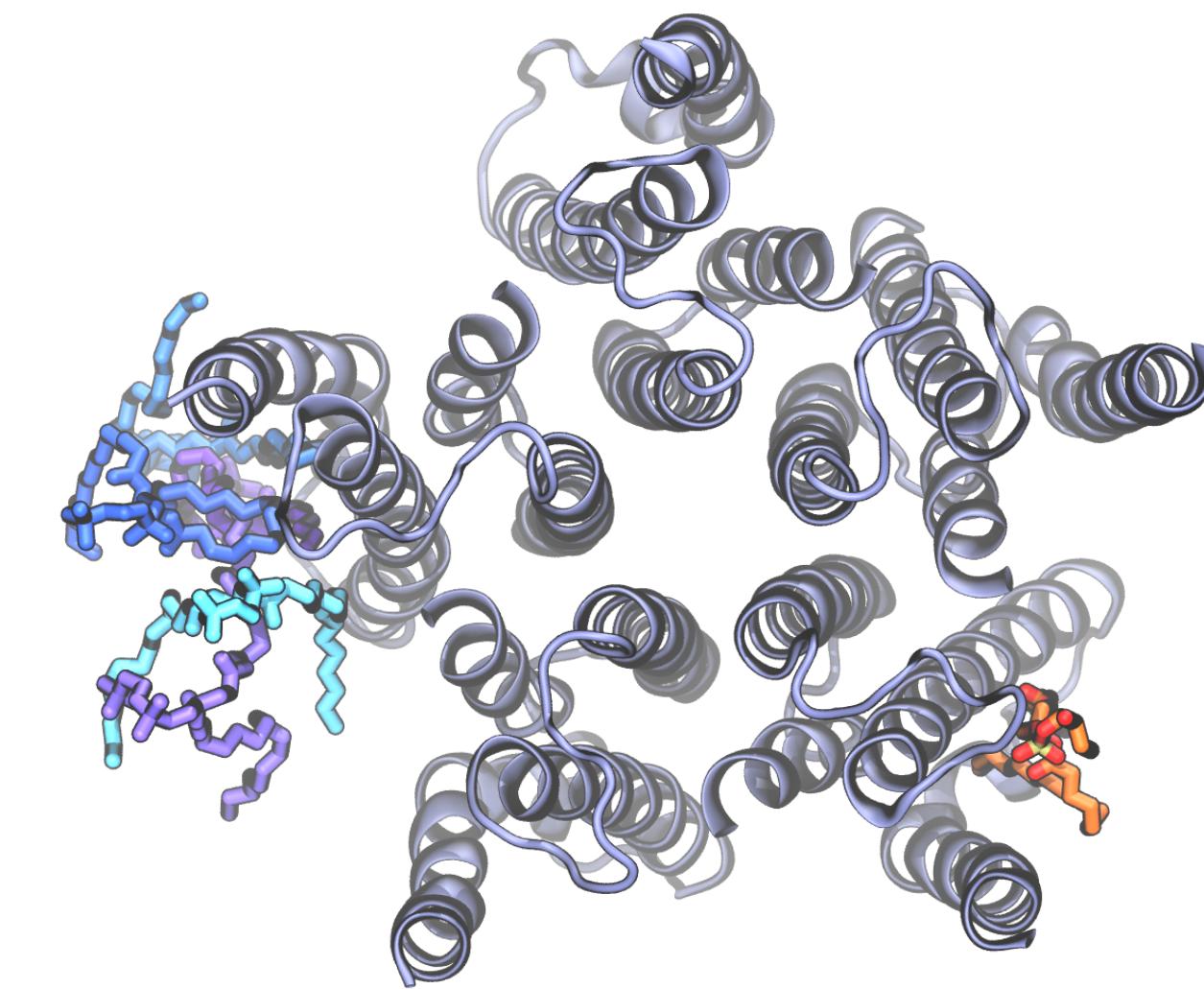
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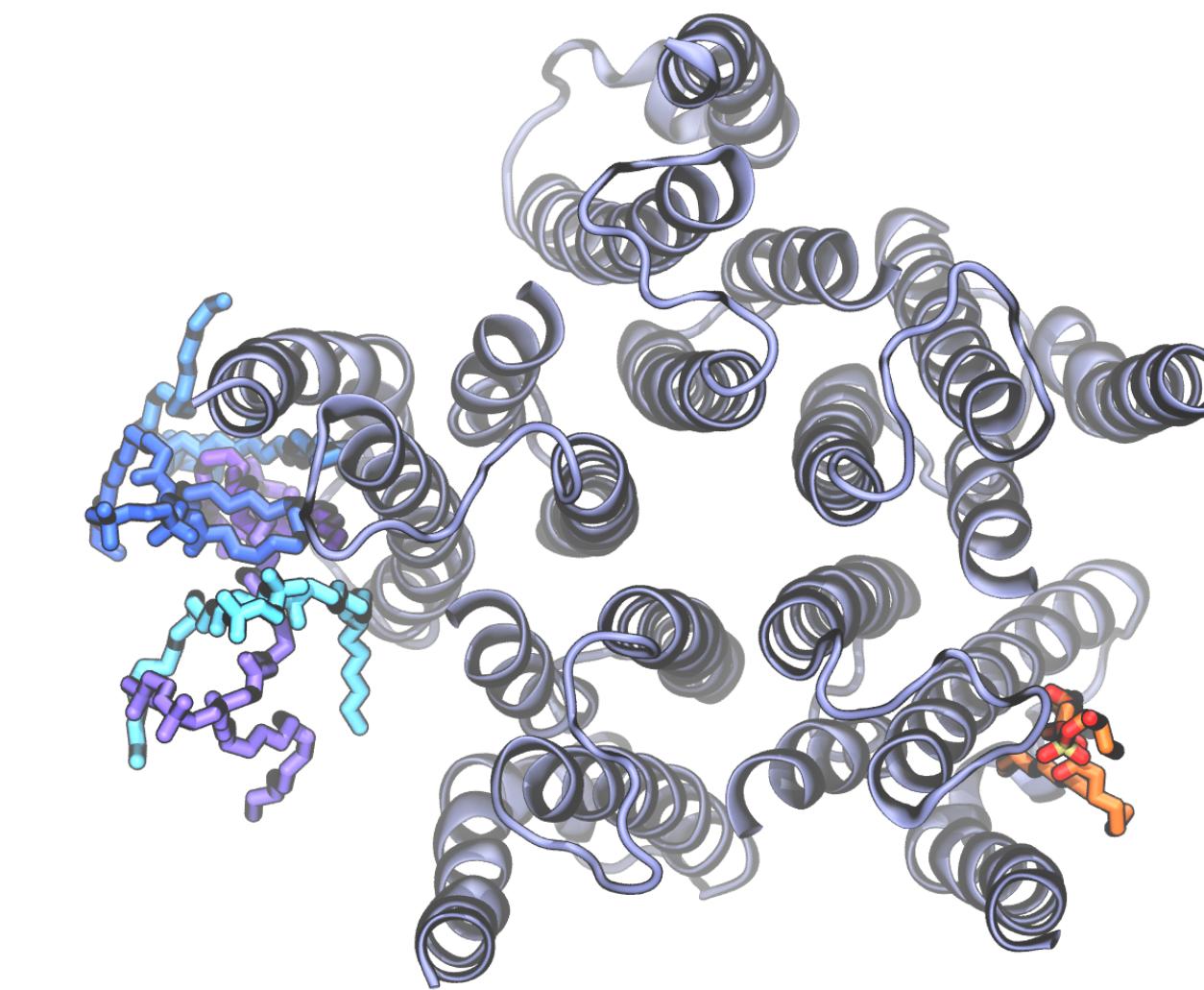
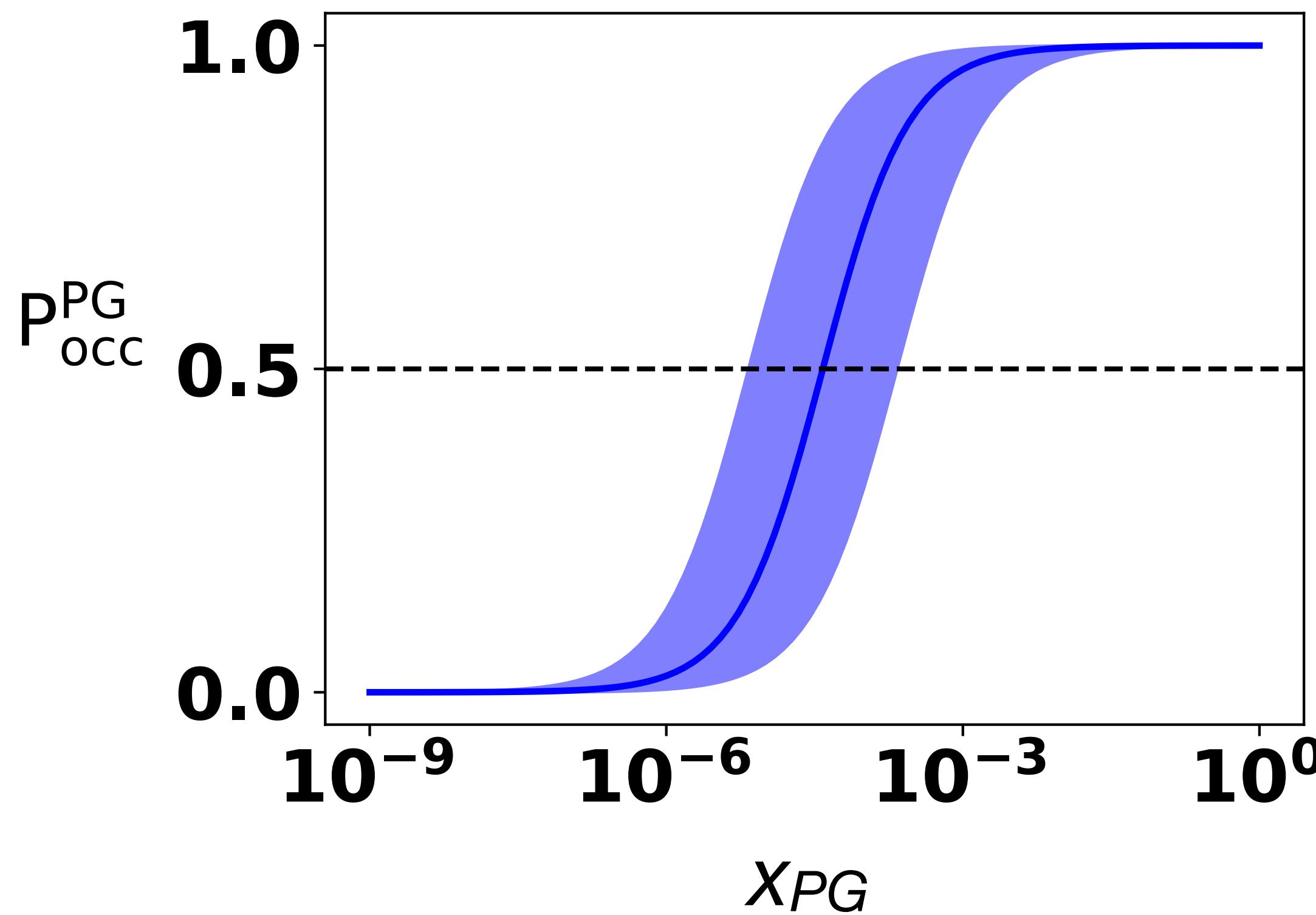
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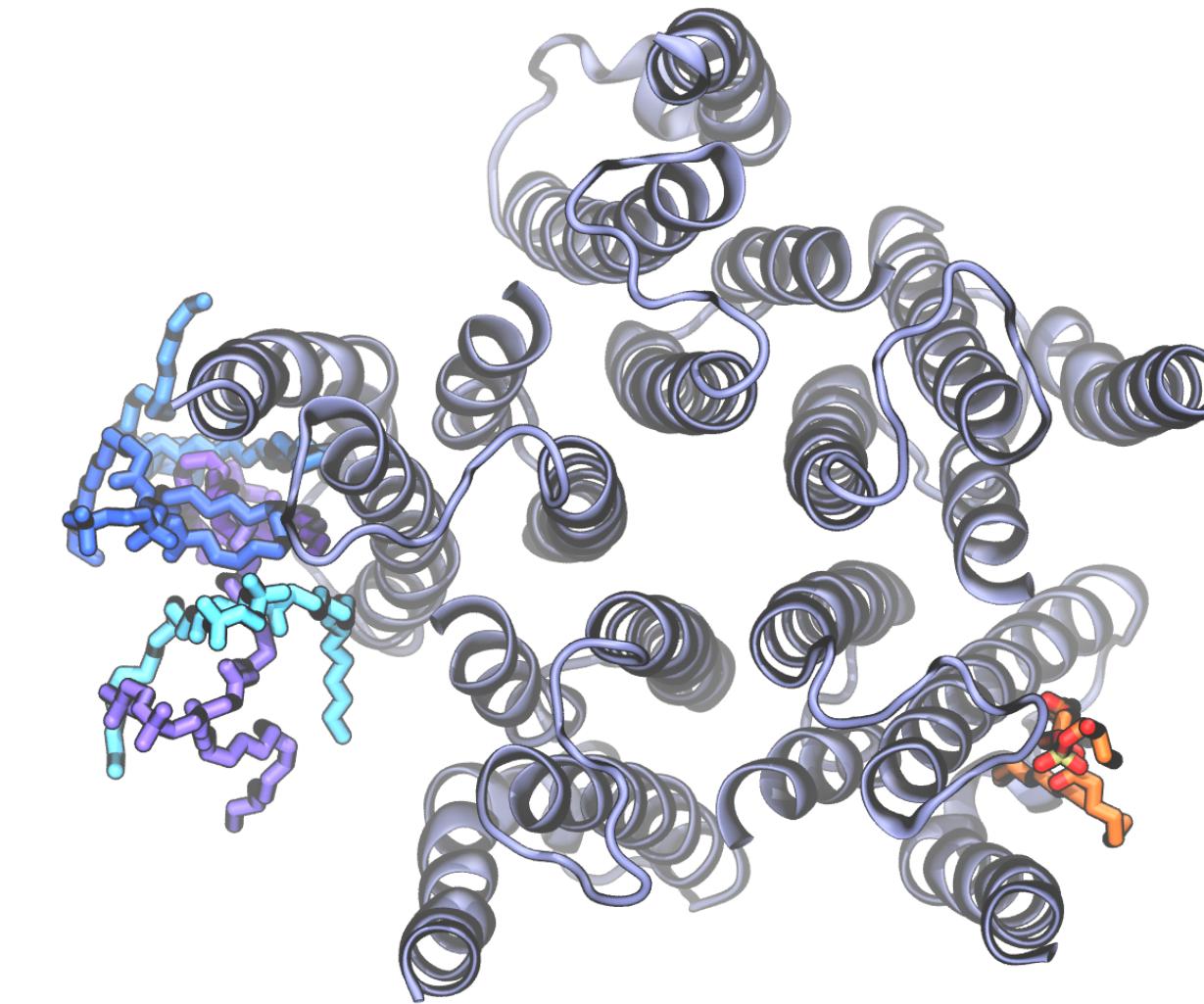
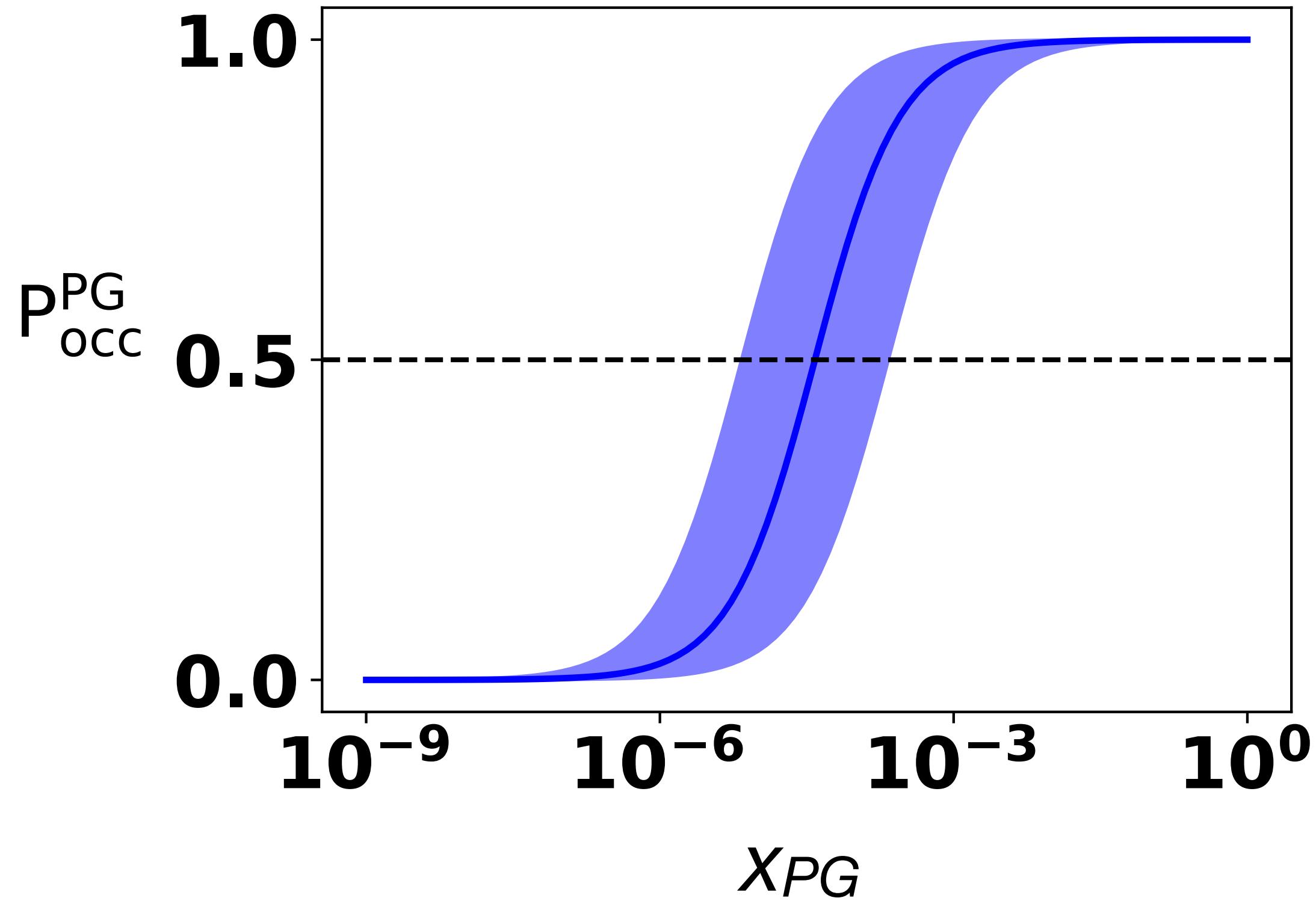
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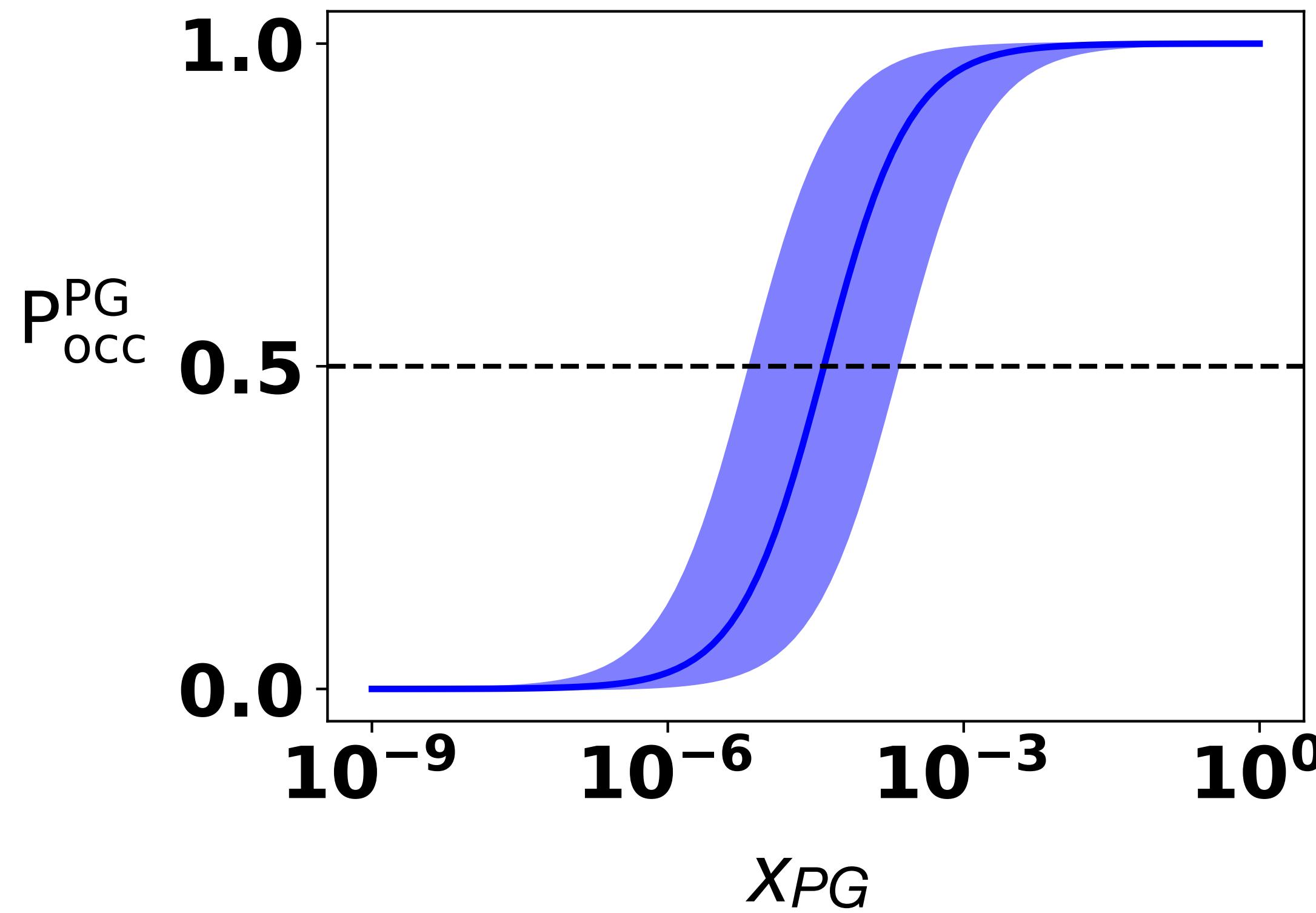
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- Error is too high

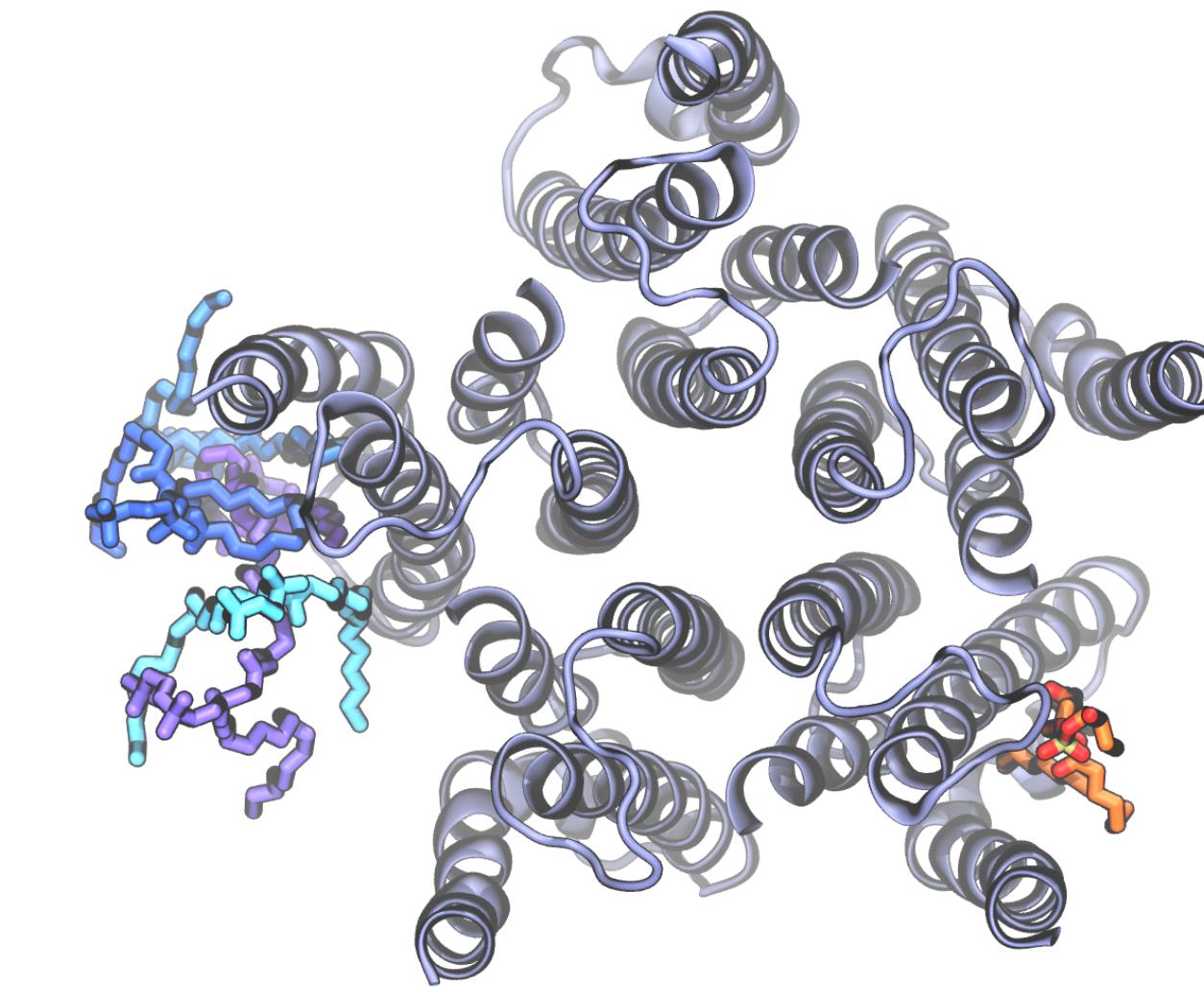


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WT CA (Agonist-Bound)



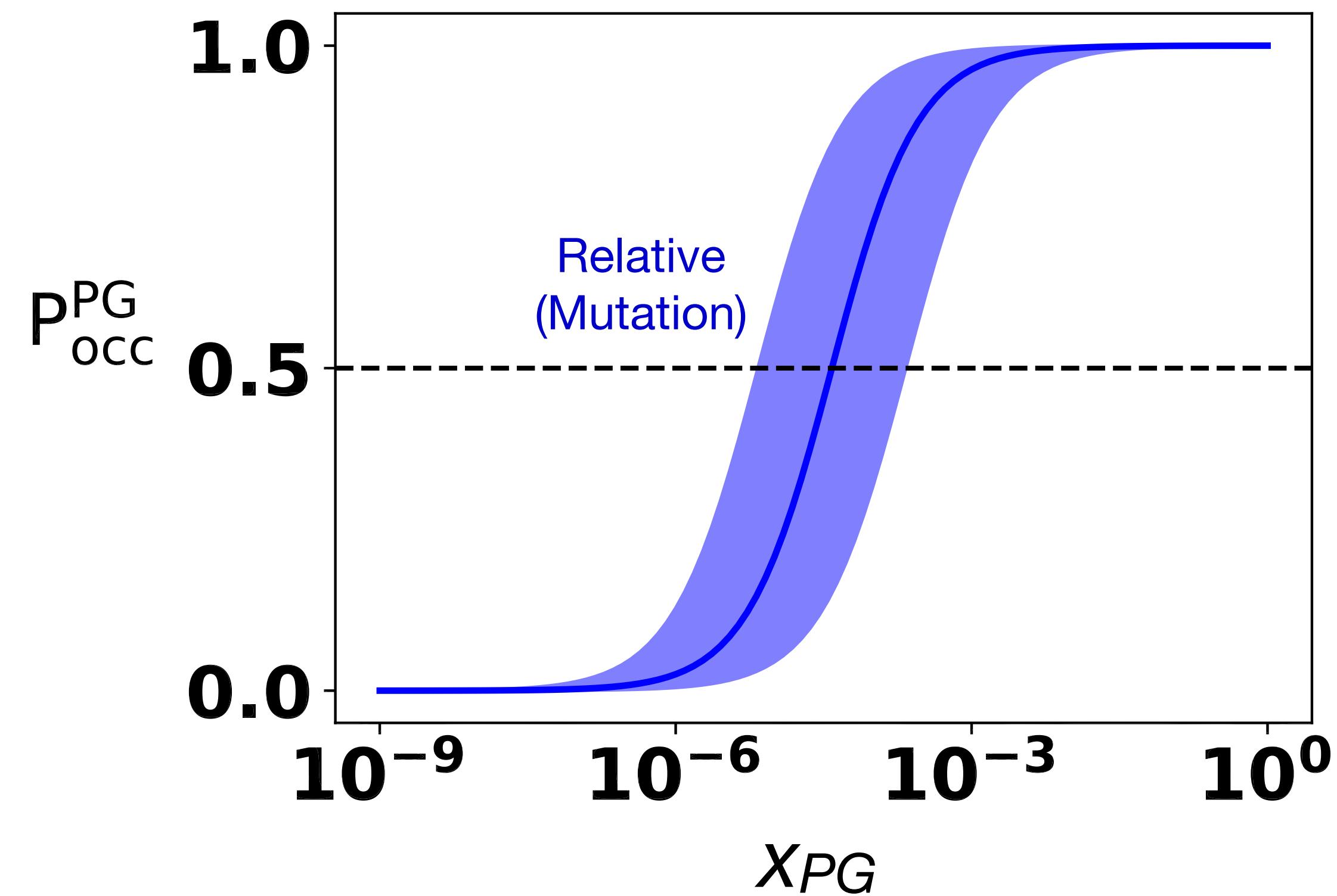
- Error is too high
- Relative
calculations aren't
good enough



Virtual Binding Assay Estimate (Absolute SAFEP)

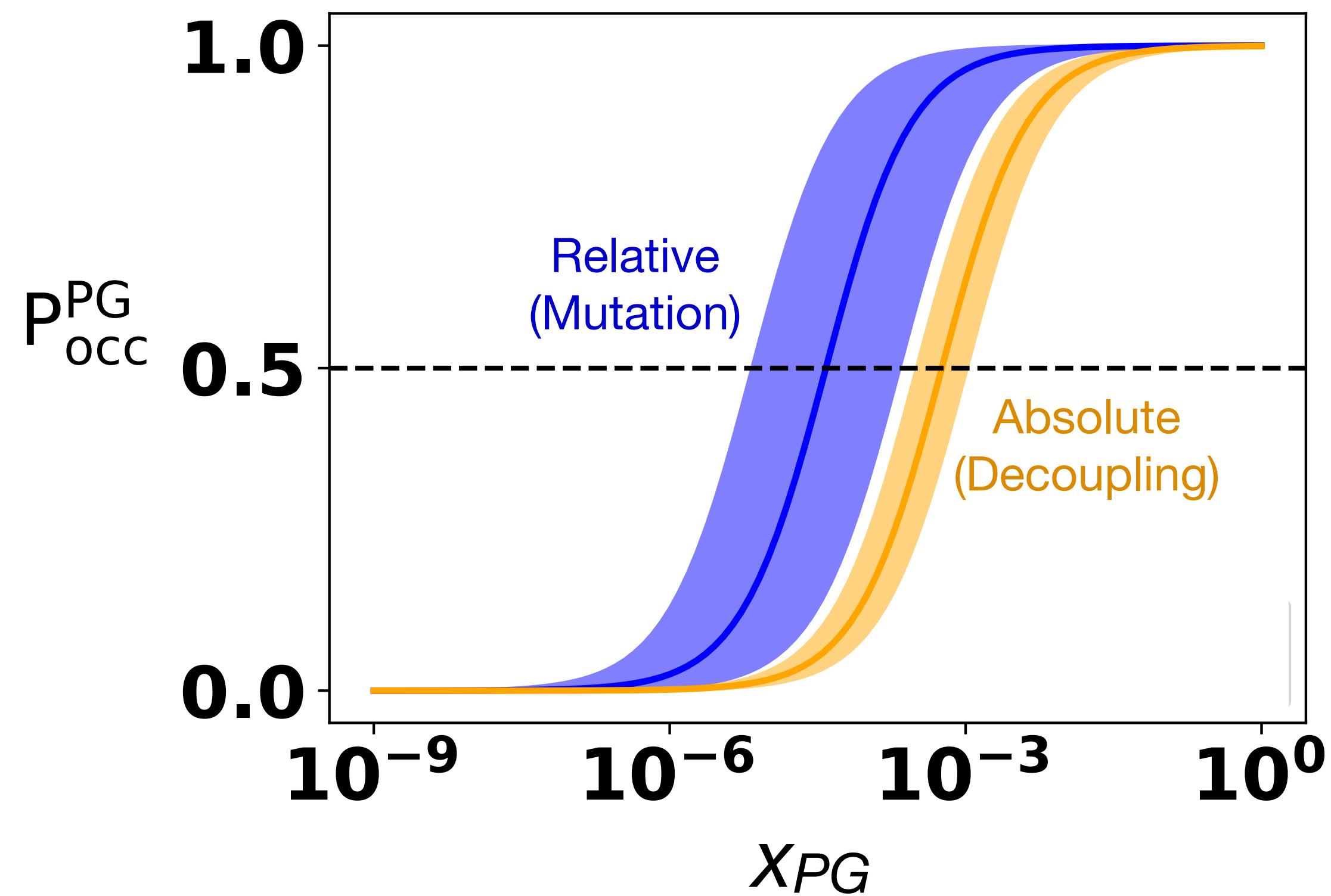
Binary mixture of PC and PG

Calculated by absolute SAFEP



Binary mixture of PC and PG

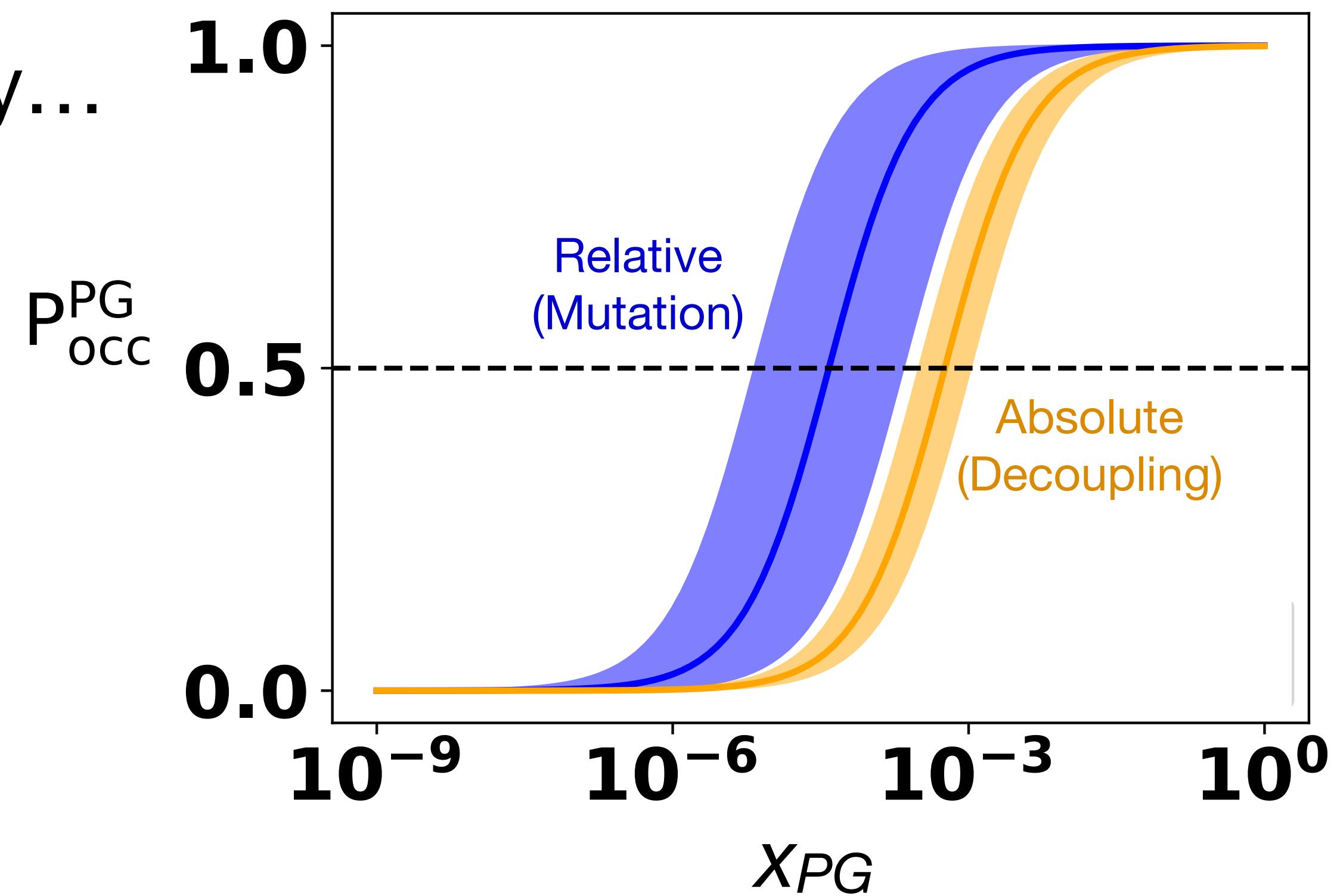
Calculated by absolute SAFEP



Binary mixture of PC and PG

Calculated by absolute SAFEP

WT CA binds PG with high affinity...

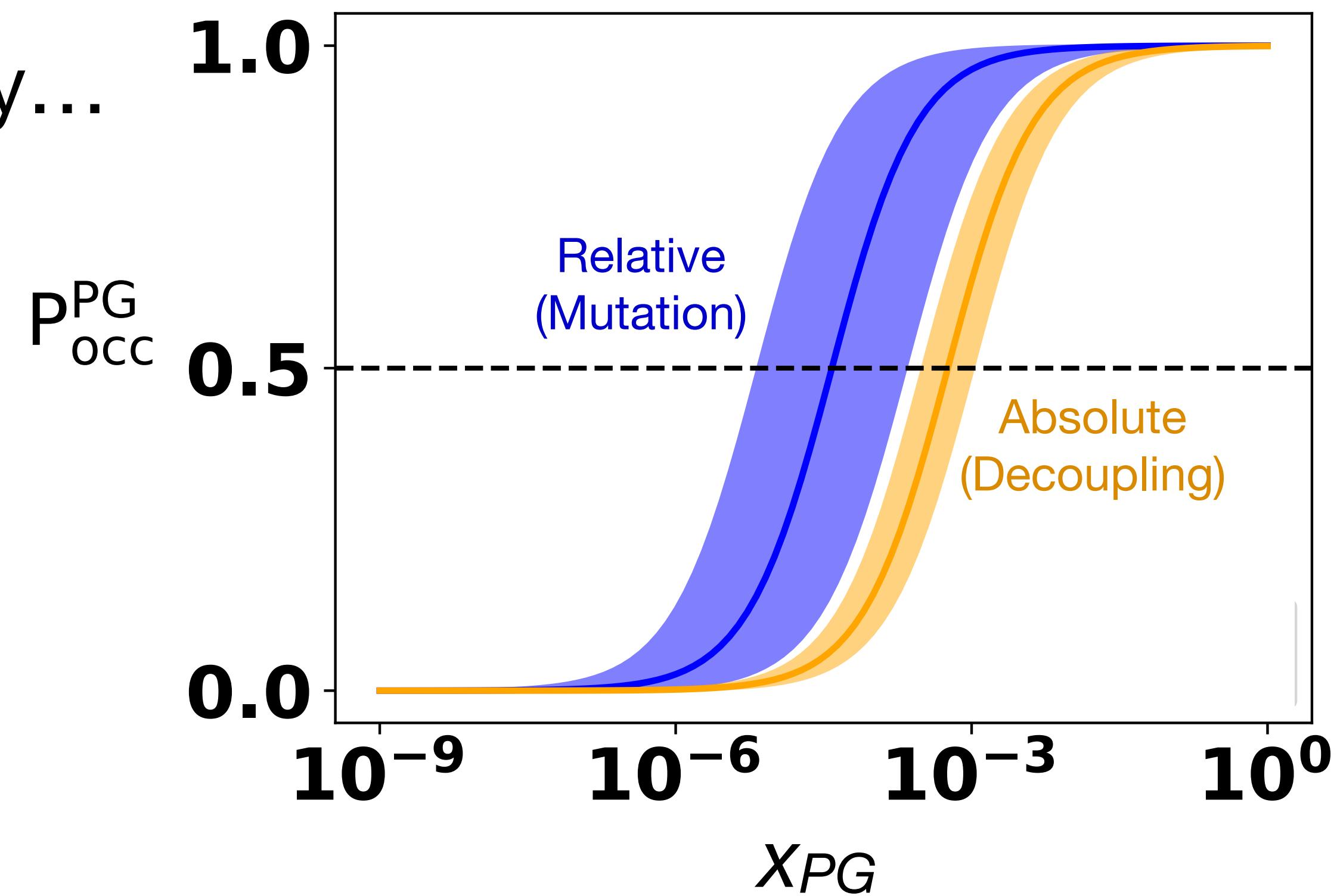


Binary mixture of PC and PG

Calculated by absolute SAFEP

WT CA binds PG with high affinity...

But not as high as the relative calculation would suggest



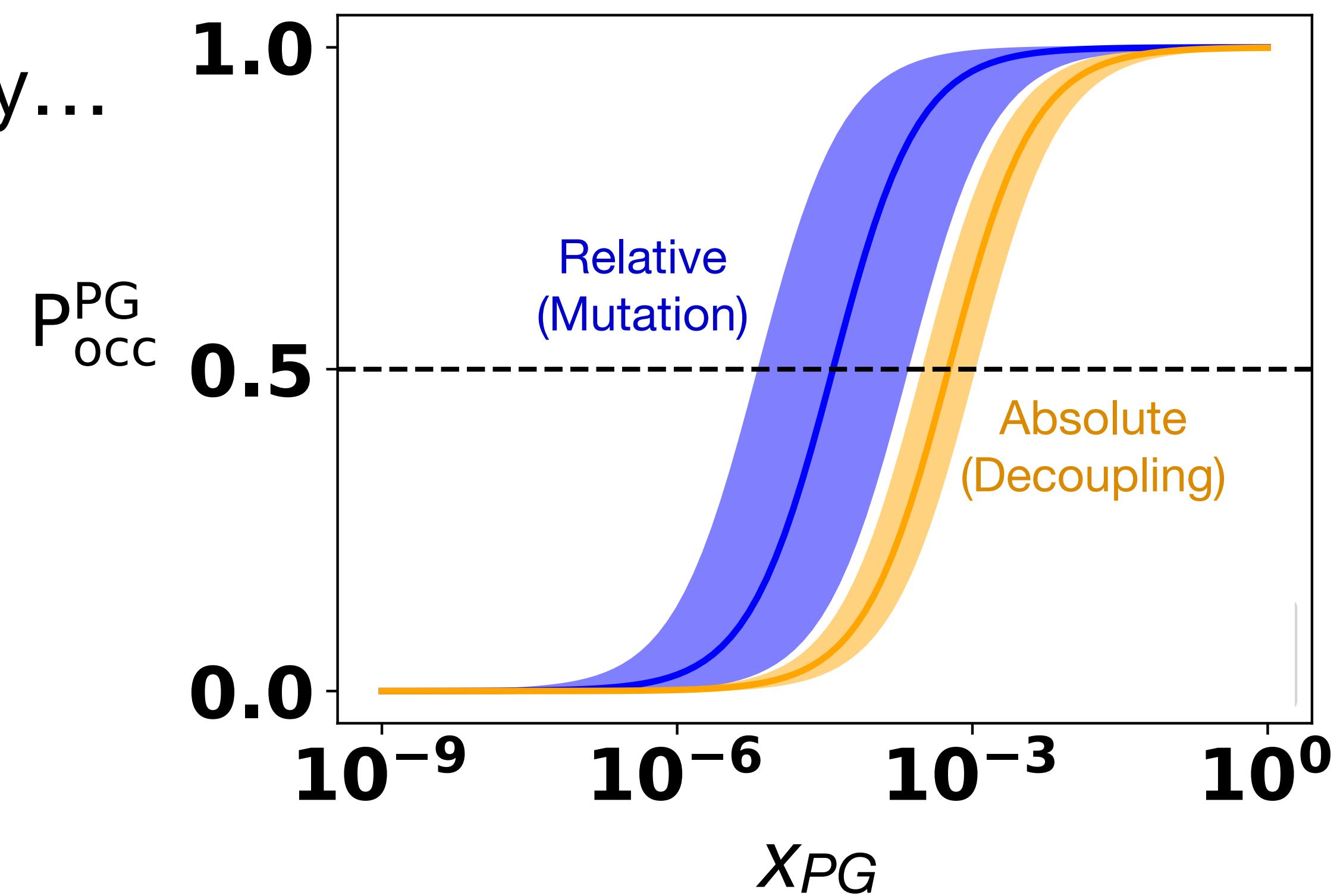
Binary mixture of PC and PG

Calculated by absolute SAFEP

WT CA binds PG with high affinity...

But not as high as the relative calculation would suggest

POPC *is* a solvent in this case:



Binary mixture of PC and PG

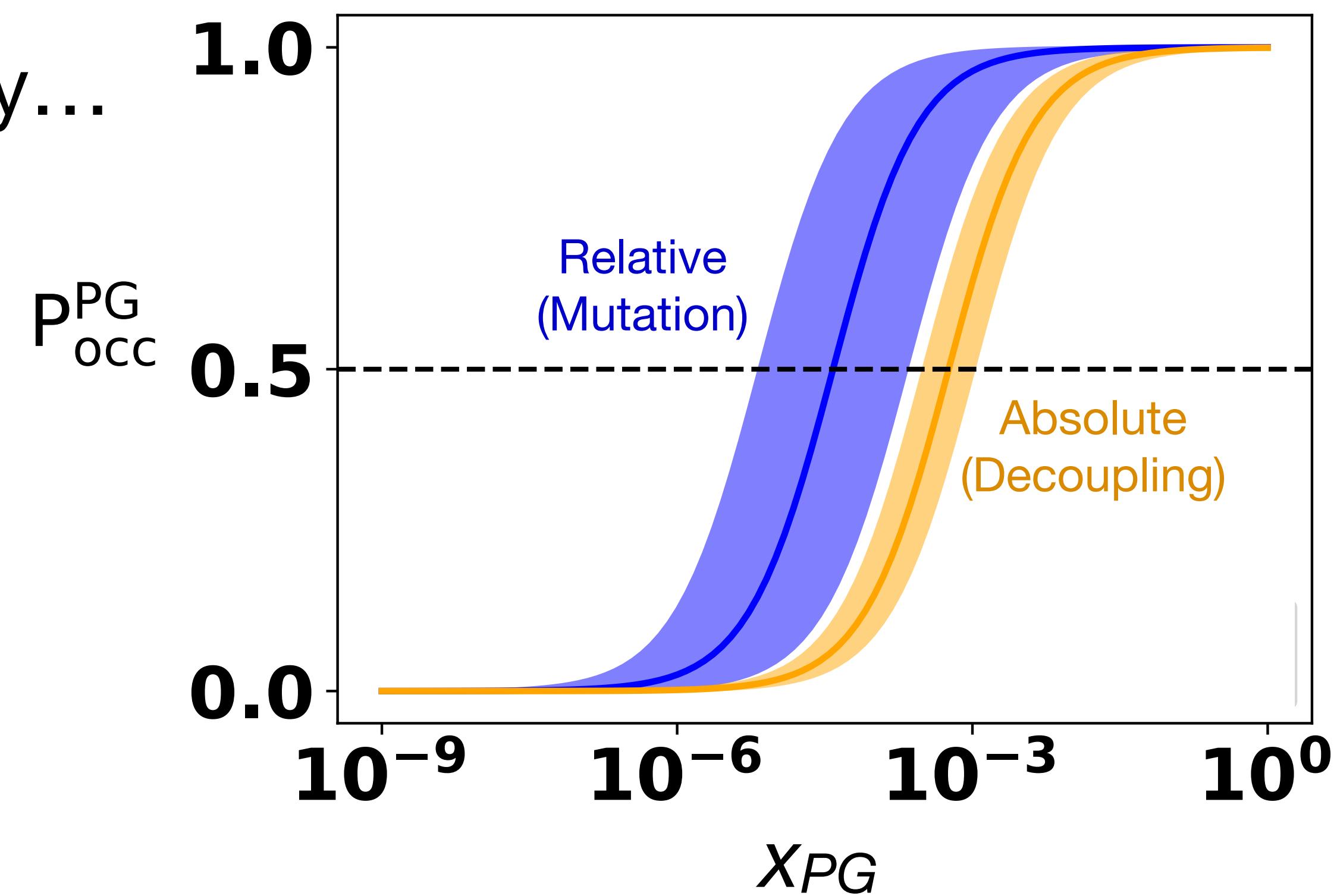
Calculated by absolute SAFEP

WT CA binds PG with high affinity...

But not as high as the relative calculation would suggest

POPC *is* a solvent in this case:

- Wrong place



Binary mixture of PC and PG

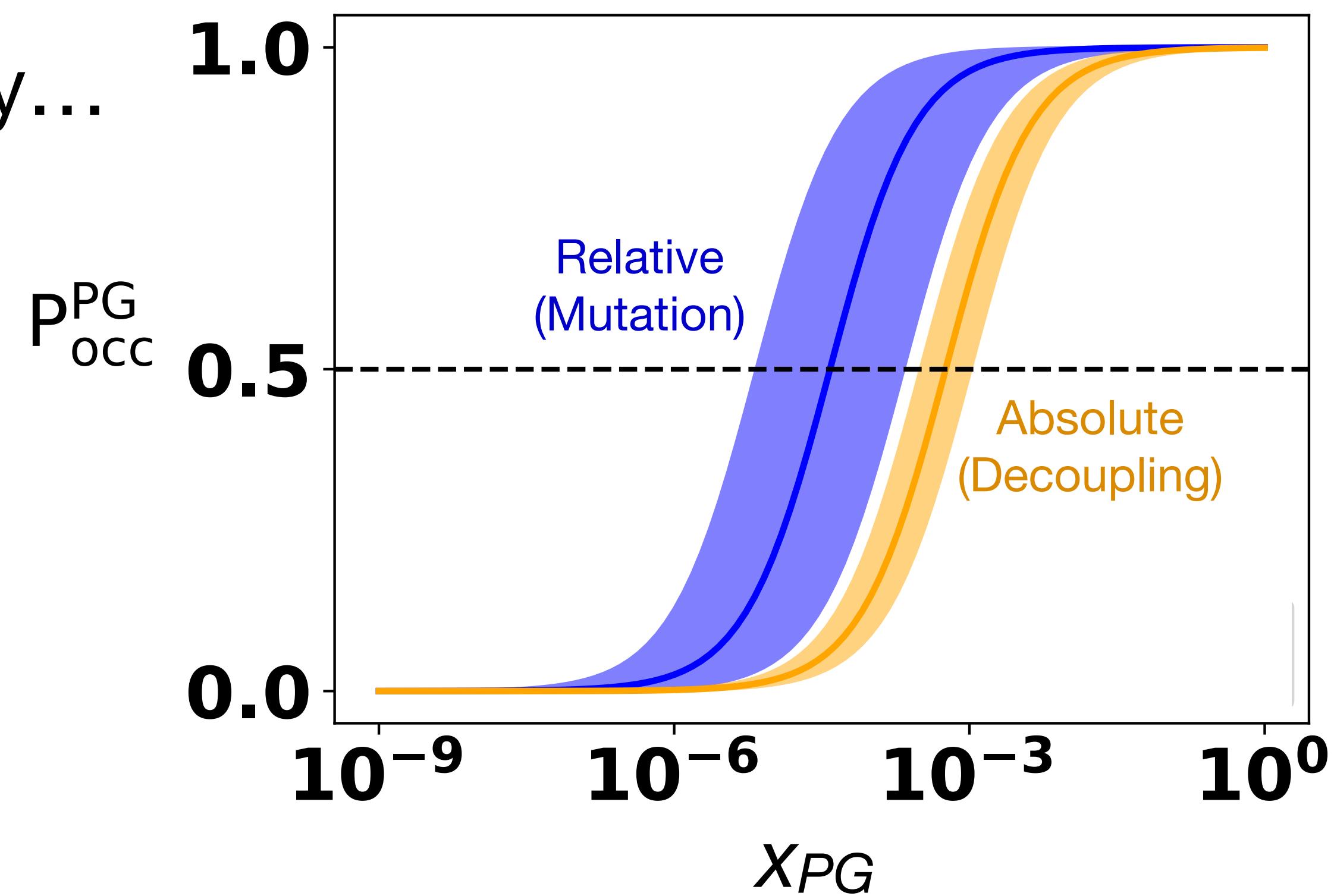
Calculated by absolute SAFEP

WT CA binds PG with high affinity...

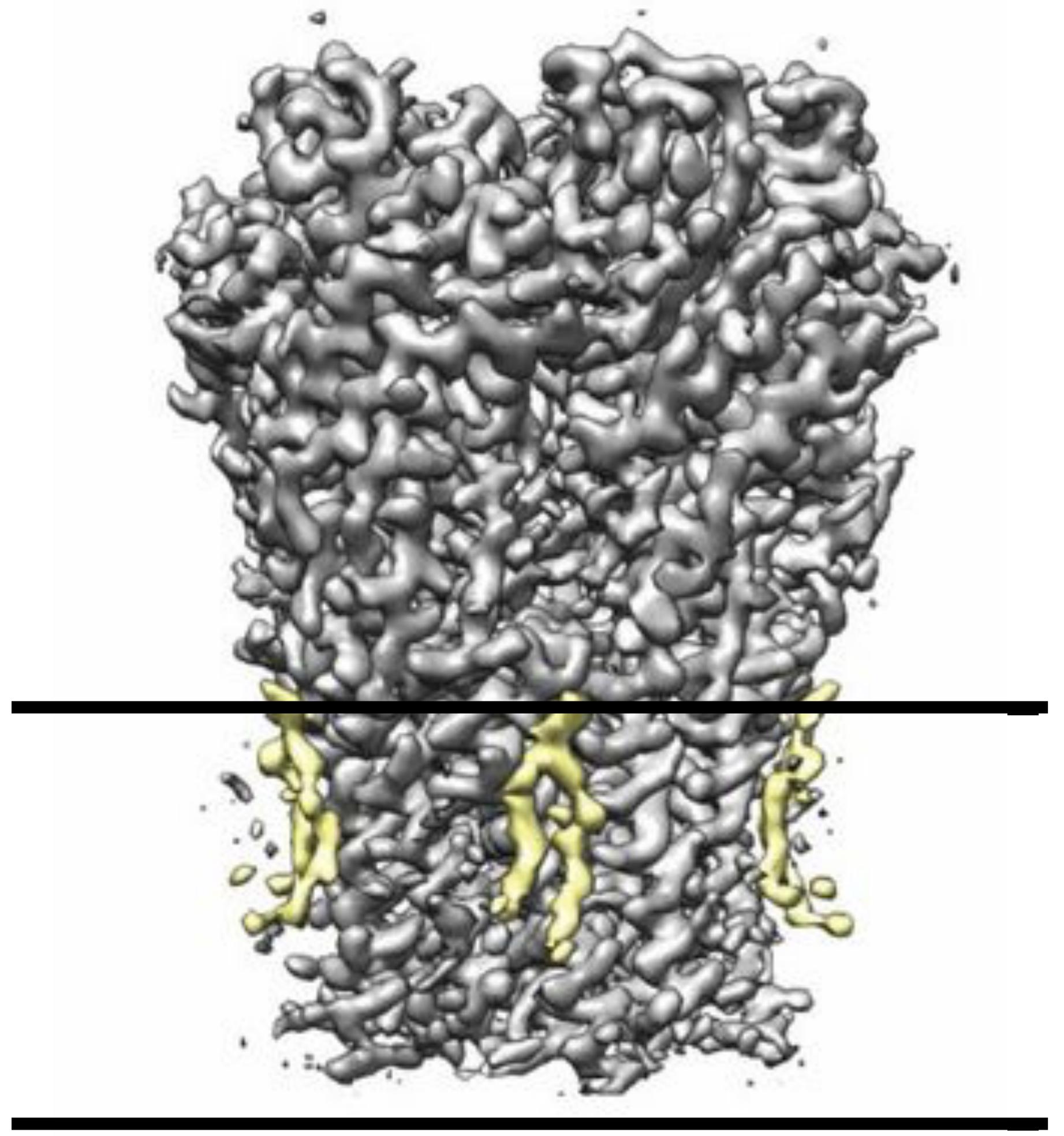
But not as high as the relative calculation would suggest

POPC *is* a solvent in this case:

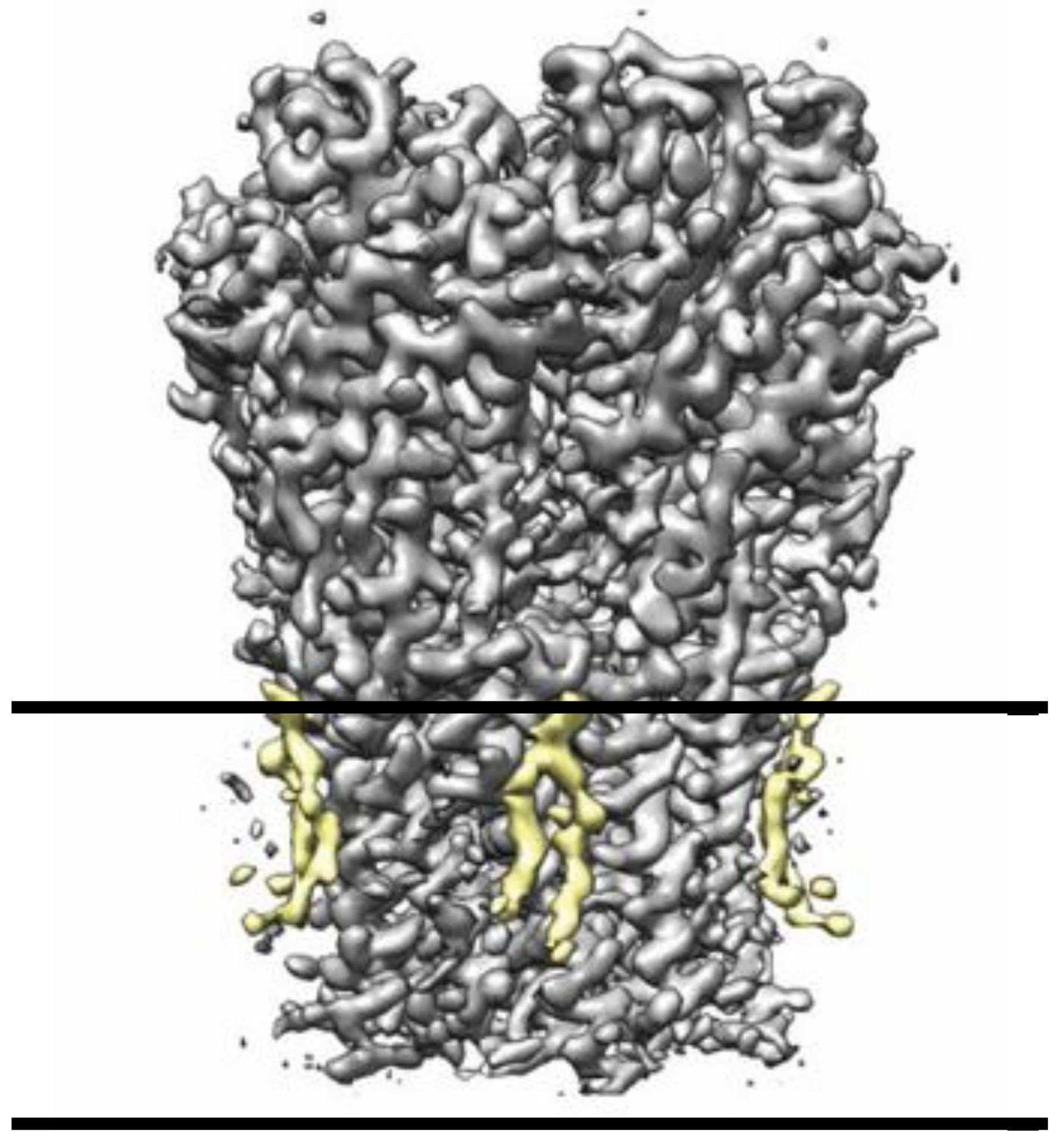
- Wrong place
- Wrong conformation



Summary

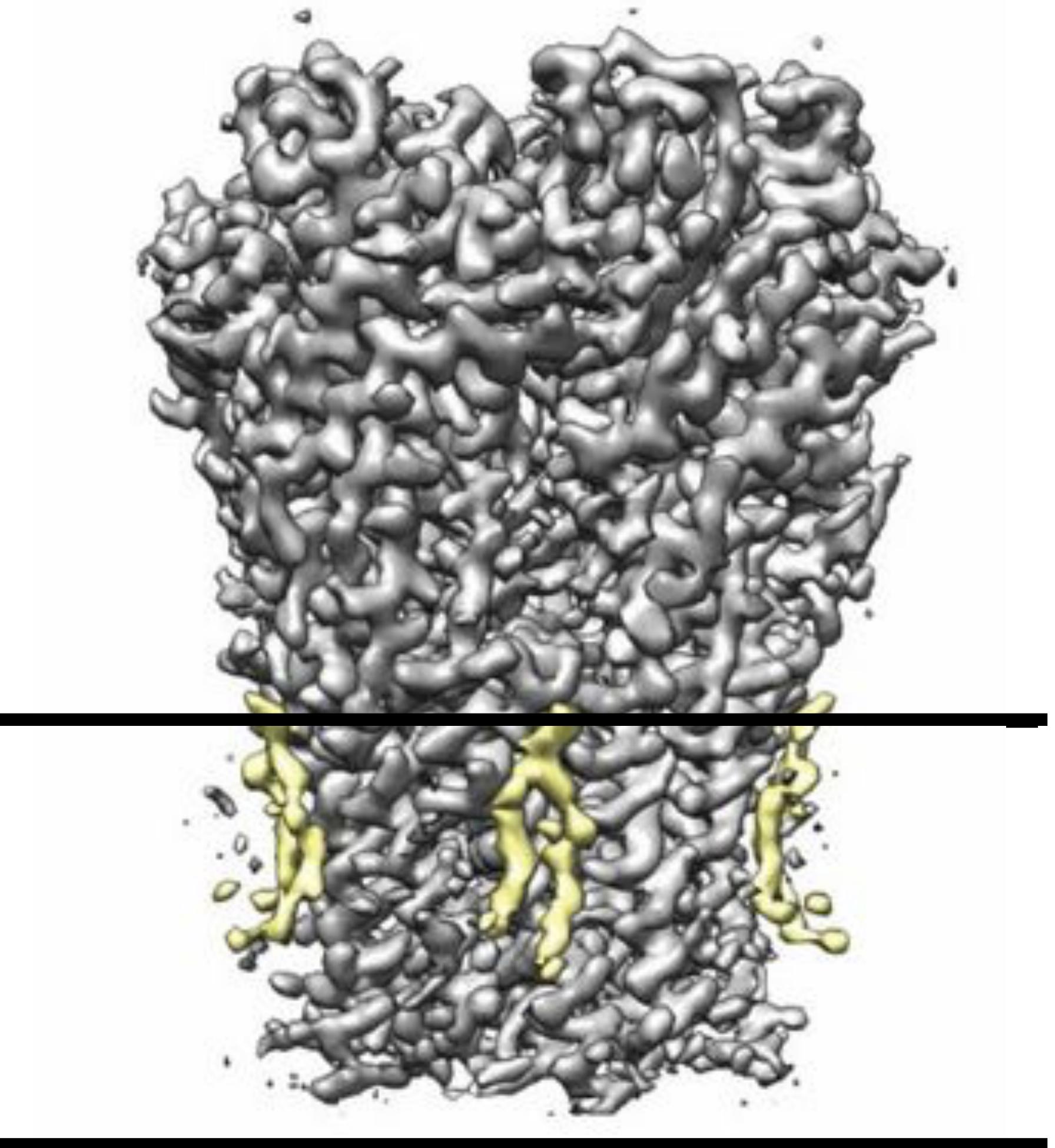


Summary



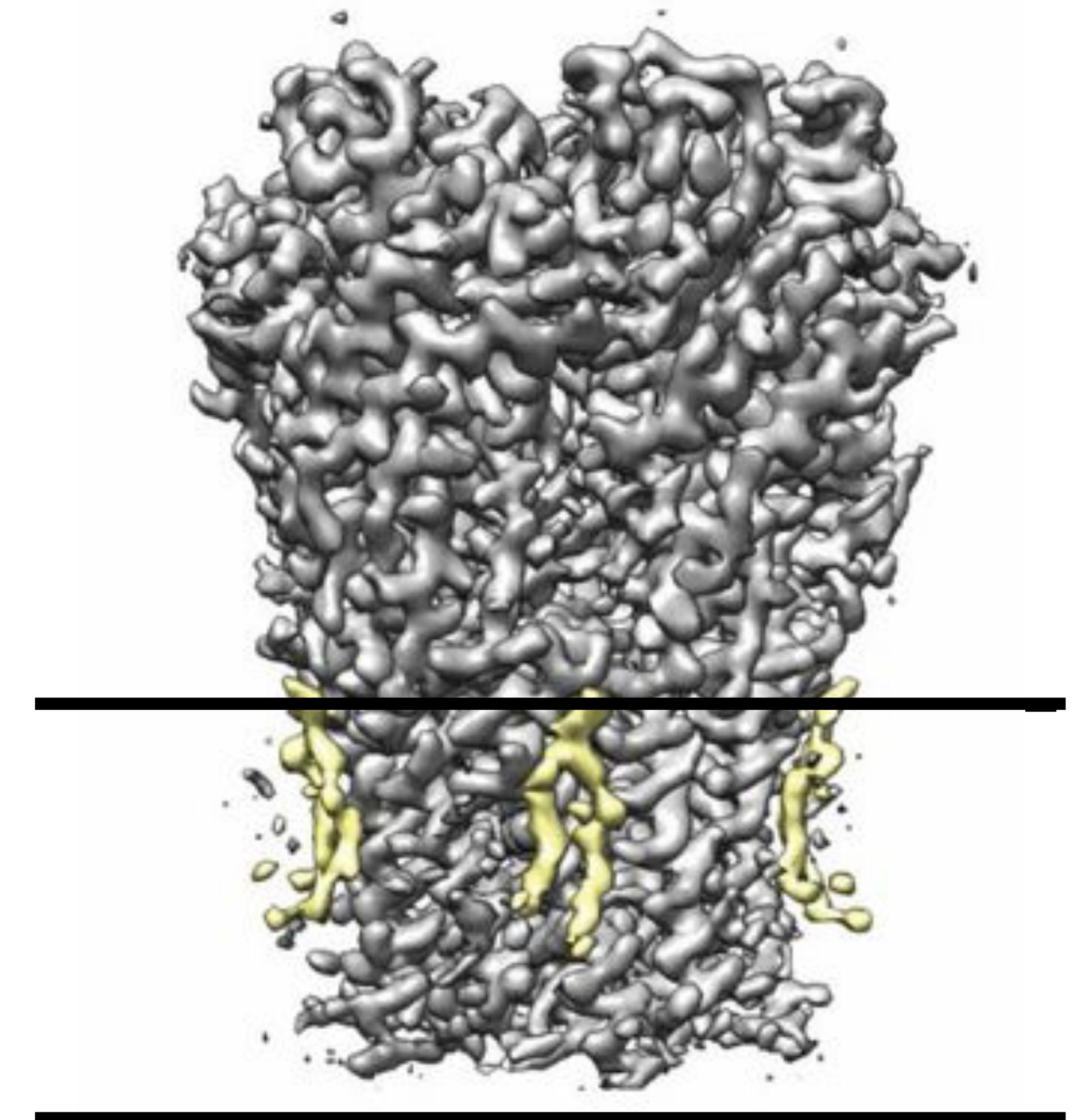
Summary

- POPG and POPE compete for the site



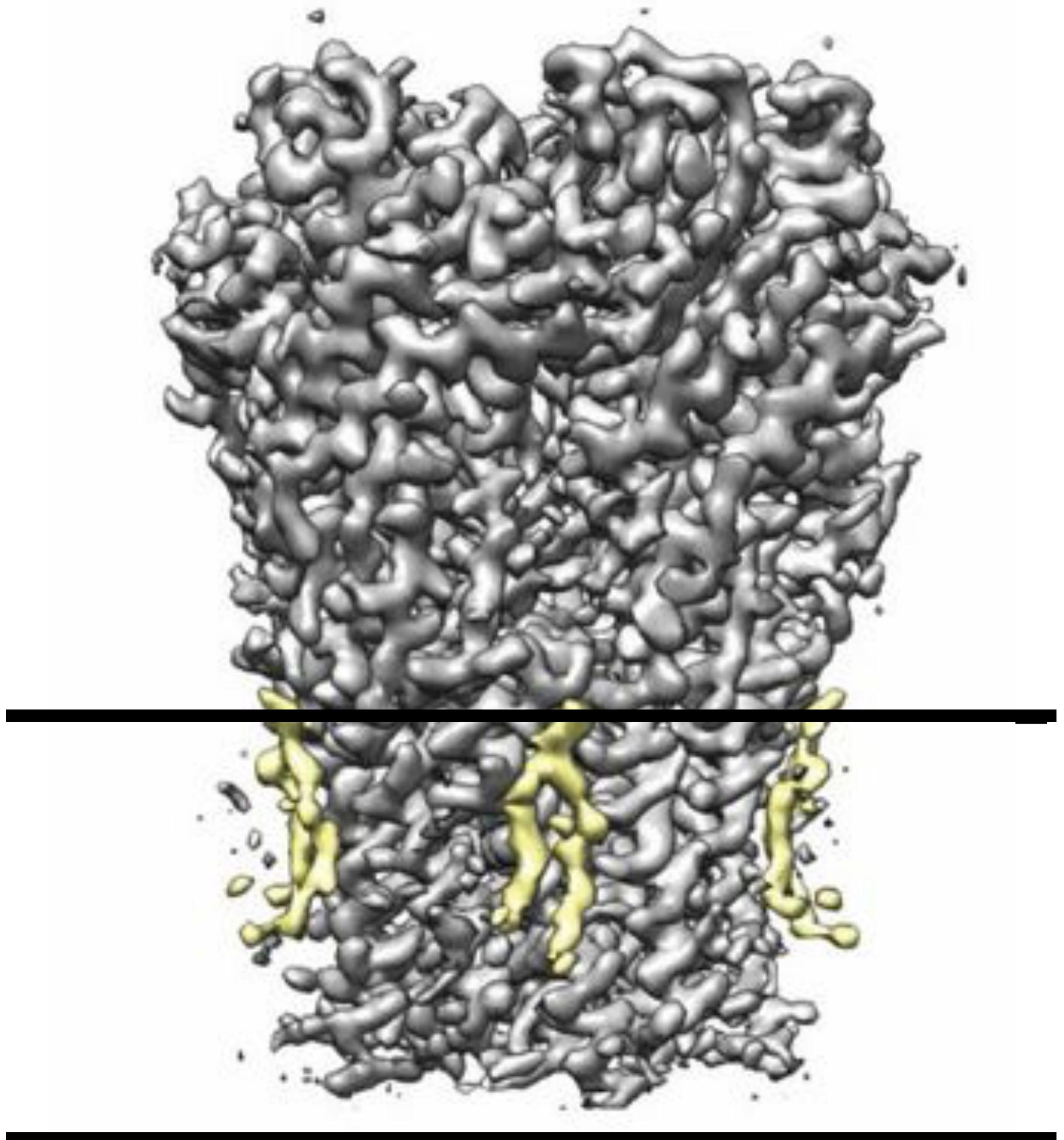
Summary

- POPG and POPE compete for the site
- Binding is **lipid-specific**.



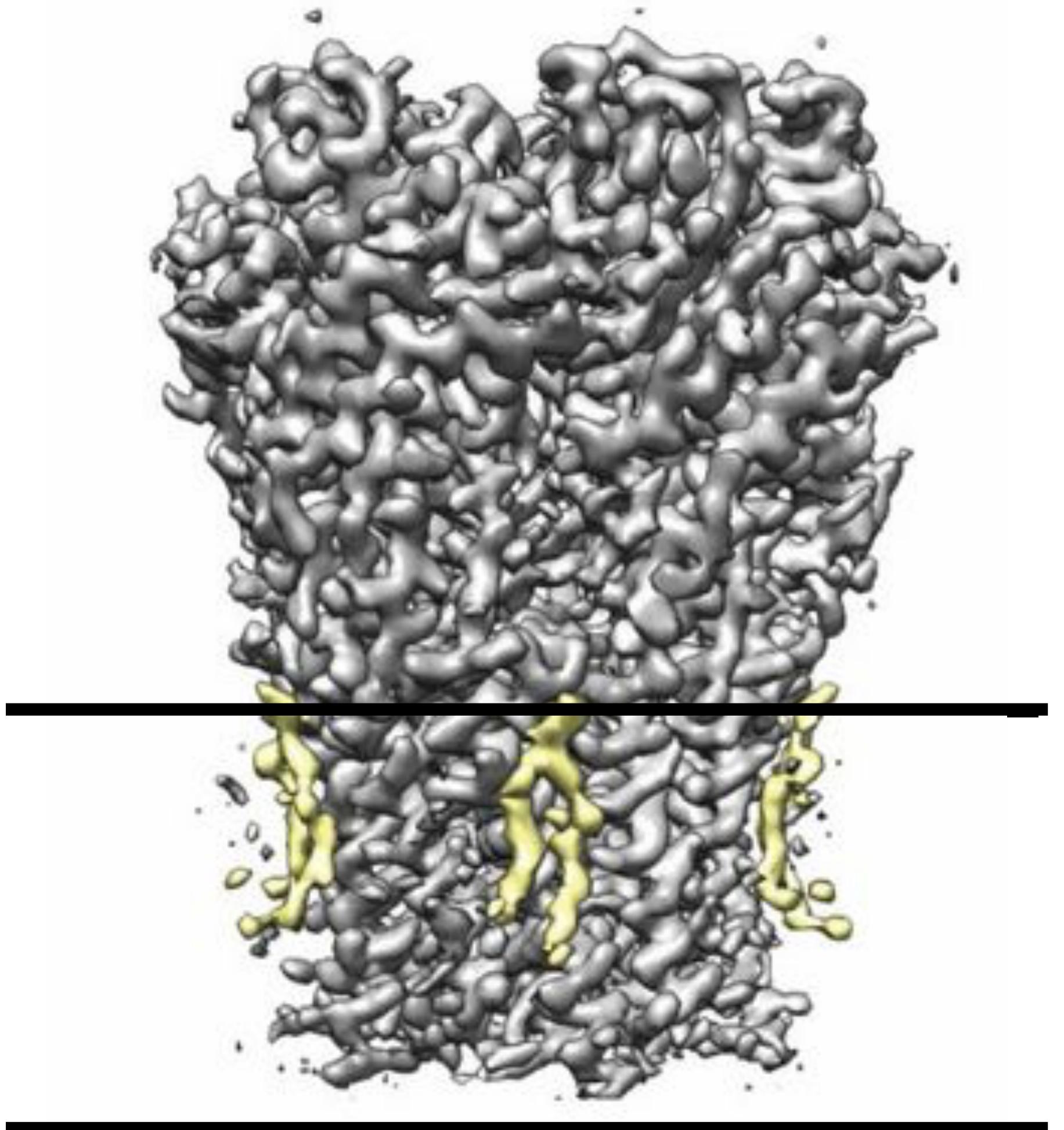
Summary

- POPG and POPE compete for the site
- Binding is **lipid-specific**.
- Affinity is **state dependent**:



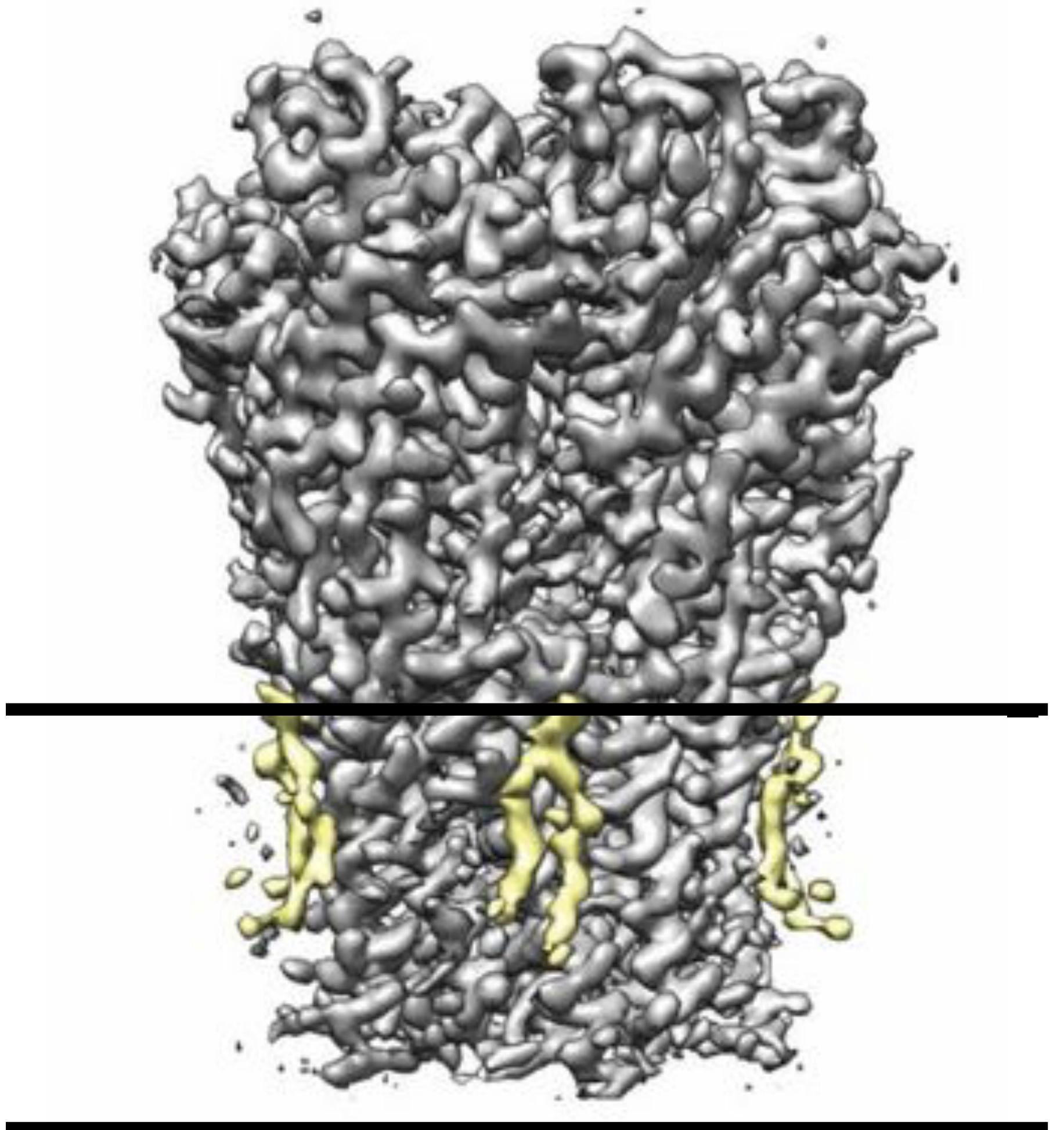
Summary

- POPG and POPE compete for the site
- Binding is **lipid-specific**.
- Affinity is **state dependent**:
POPG favors the open conformation



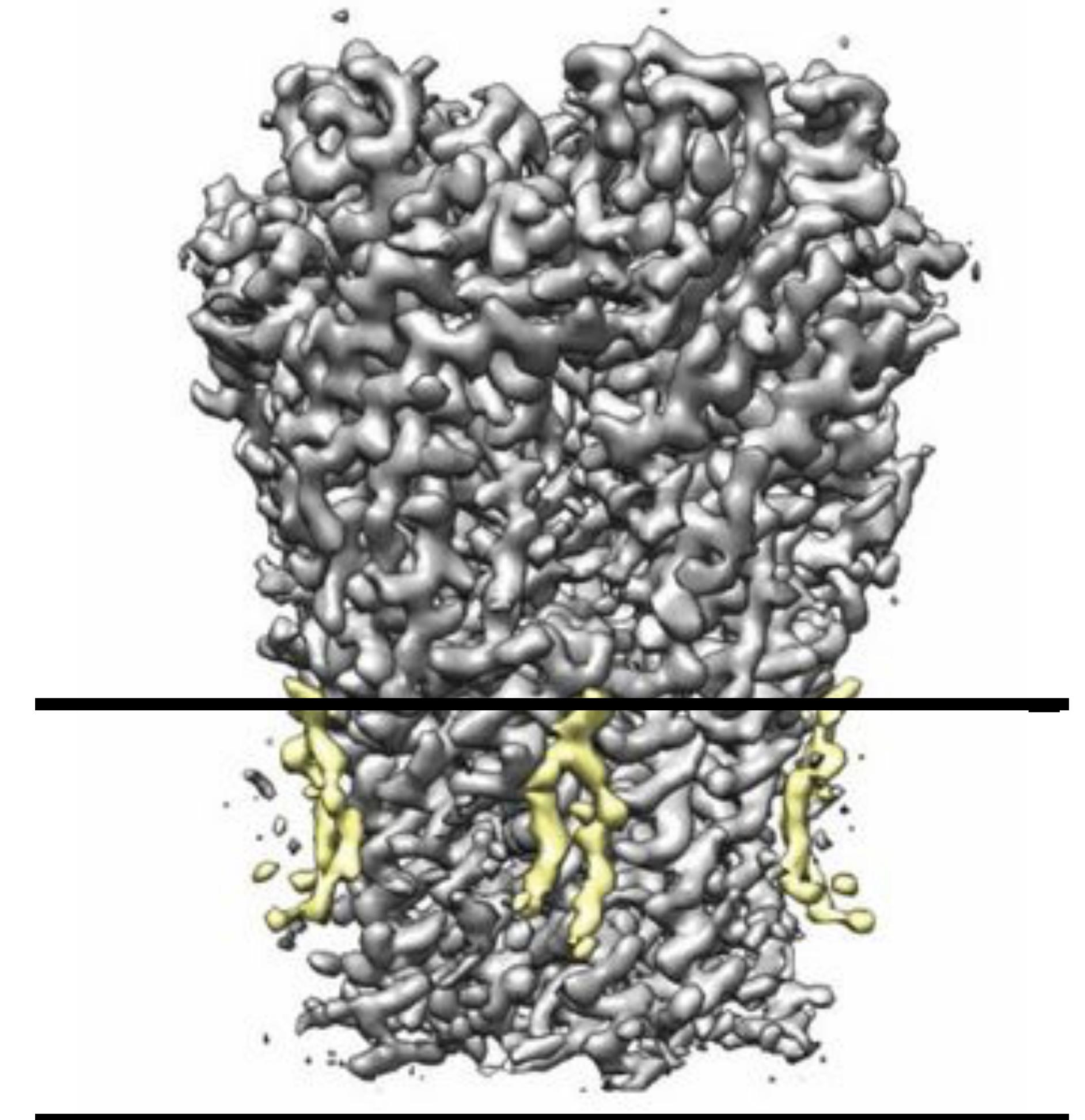
Summary

- POPG and POPE compete for the site
- Binding is **lipid-specific**.
- Affinity is **state dependent**:
POPG favors the open conformation
- **Membrane composition** is important:



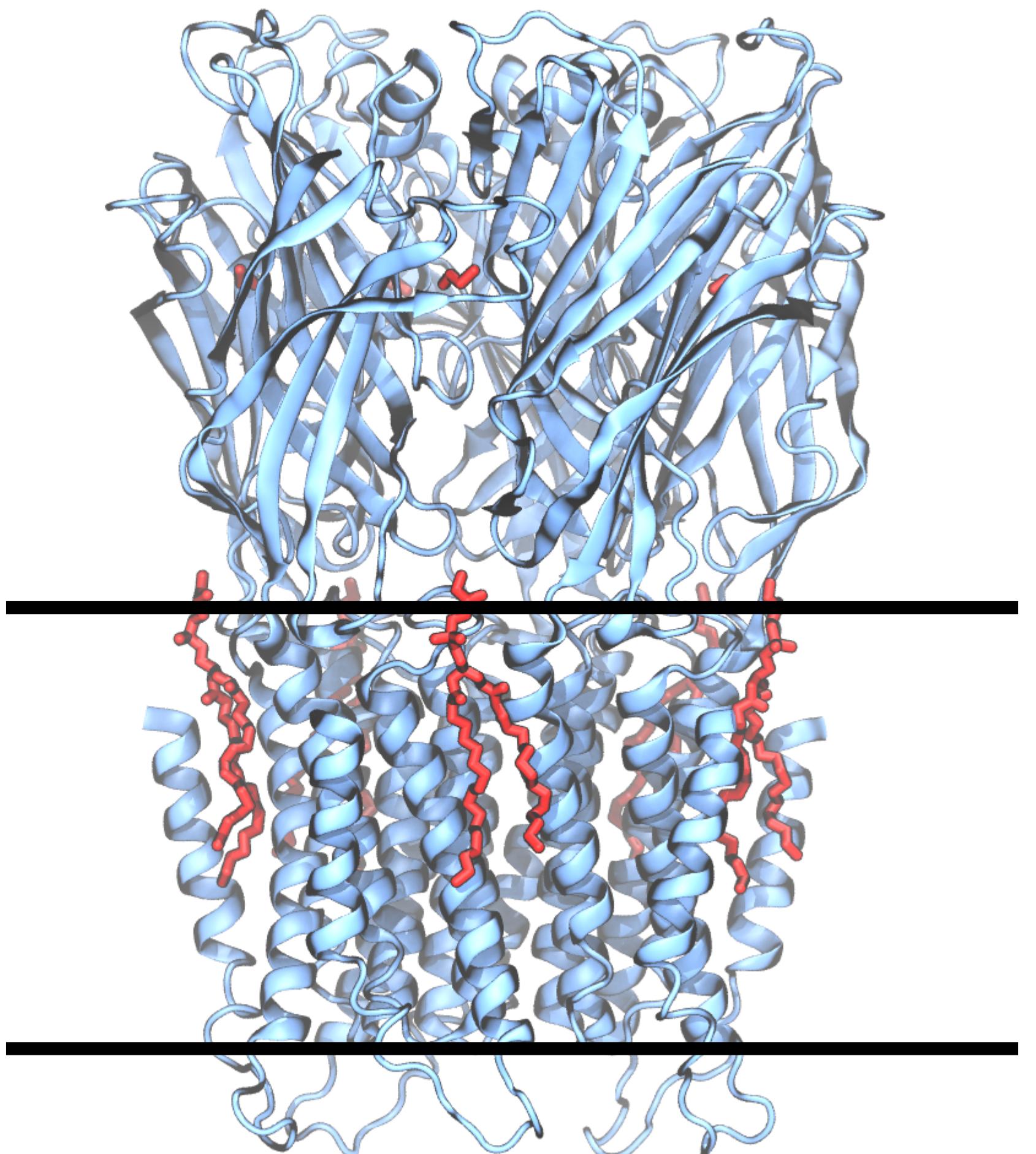
Summary

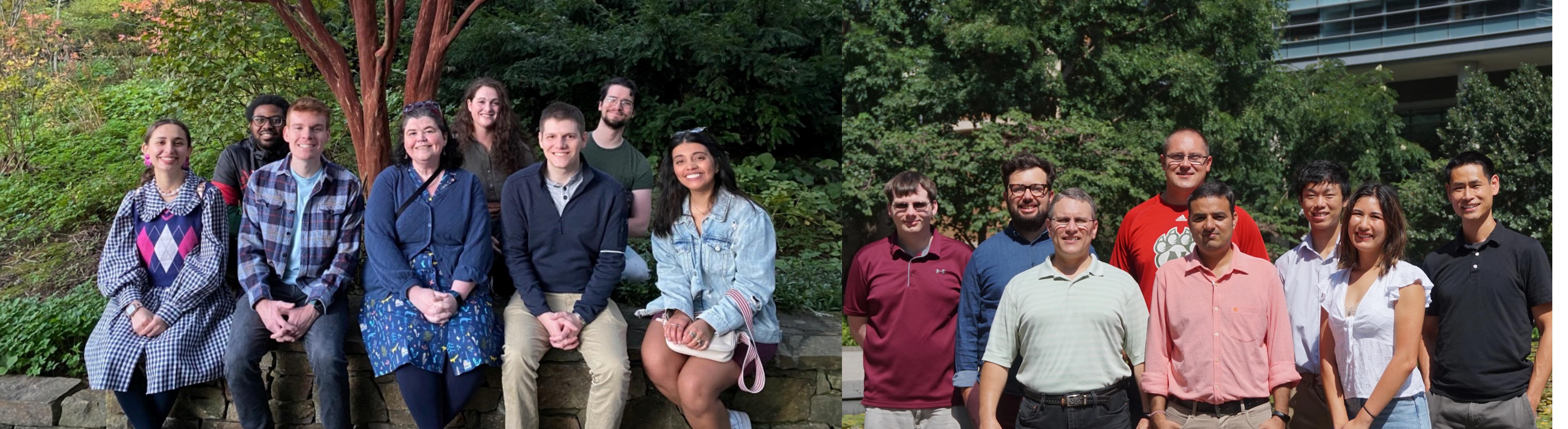
- POPG and POPE compete for the site
- Binding is **lipid-specific**.
- Affinity is **state dependent**:
POPG favors the open conformation
- **Membrane composition** is important:
PE competes with PG



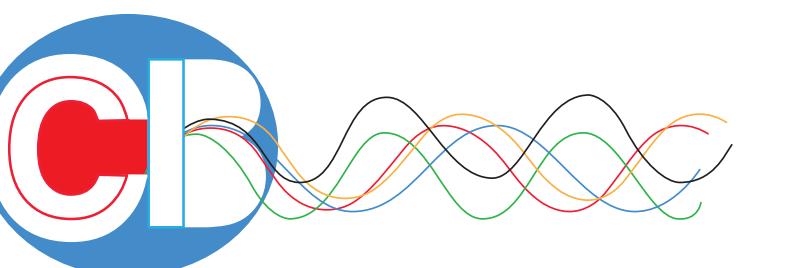
Summary

- POPG and POPE compete for the site
- Binding is **lipid-specific**.
- Affinity is **state dependent**:
POPG favors the open conformation
- **Membrane composition** is important:
PE competes with PG





Brannigan Lab



Wayland Cheng's Lab



Reza Salari



Collaborators



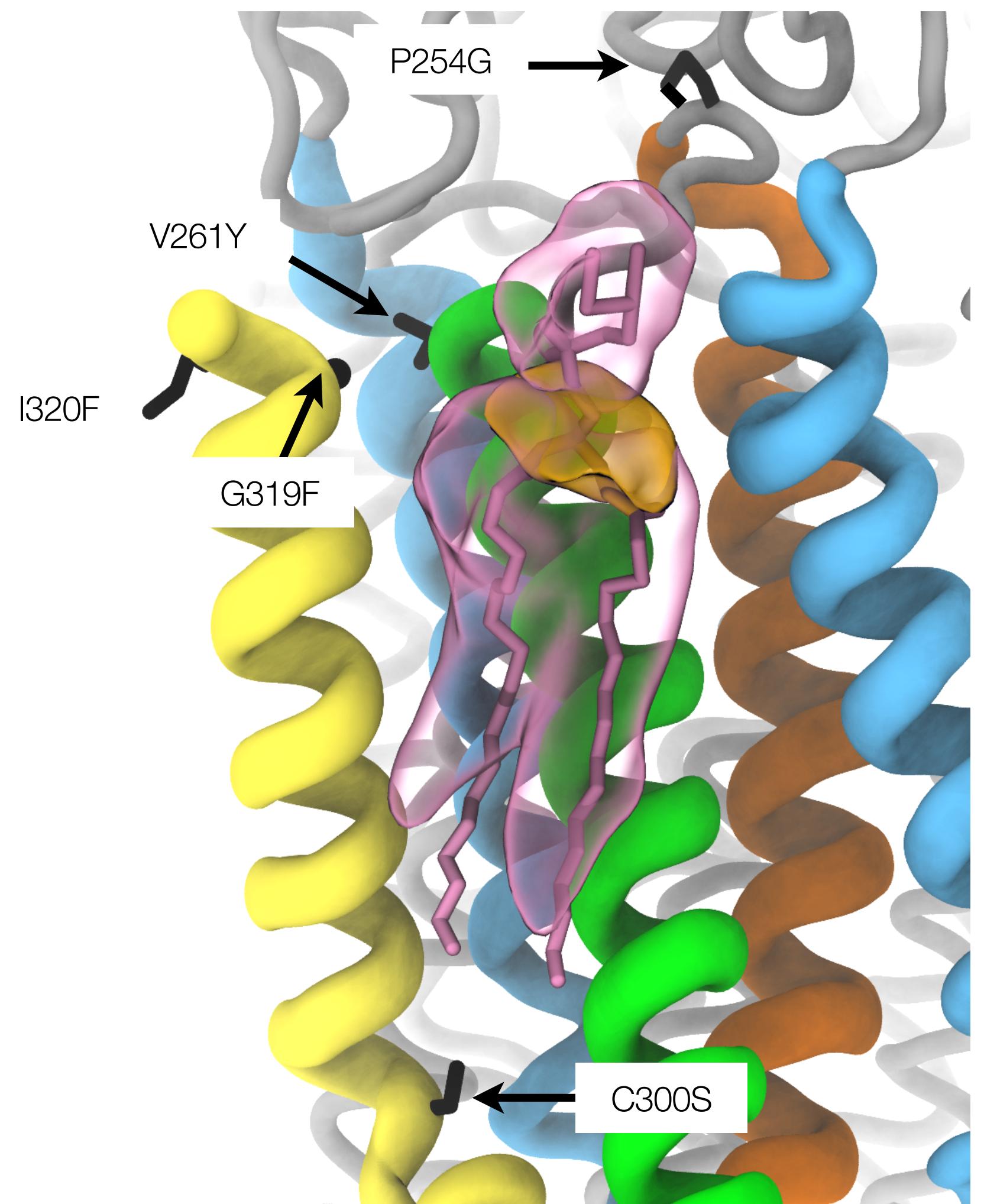
Jérôme Hénin



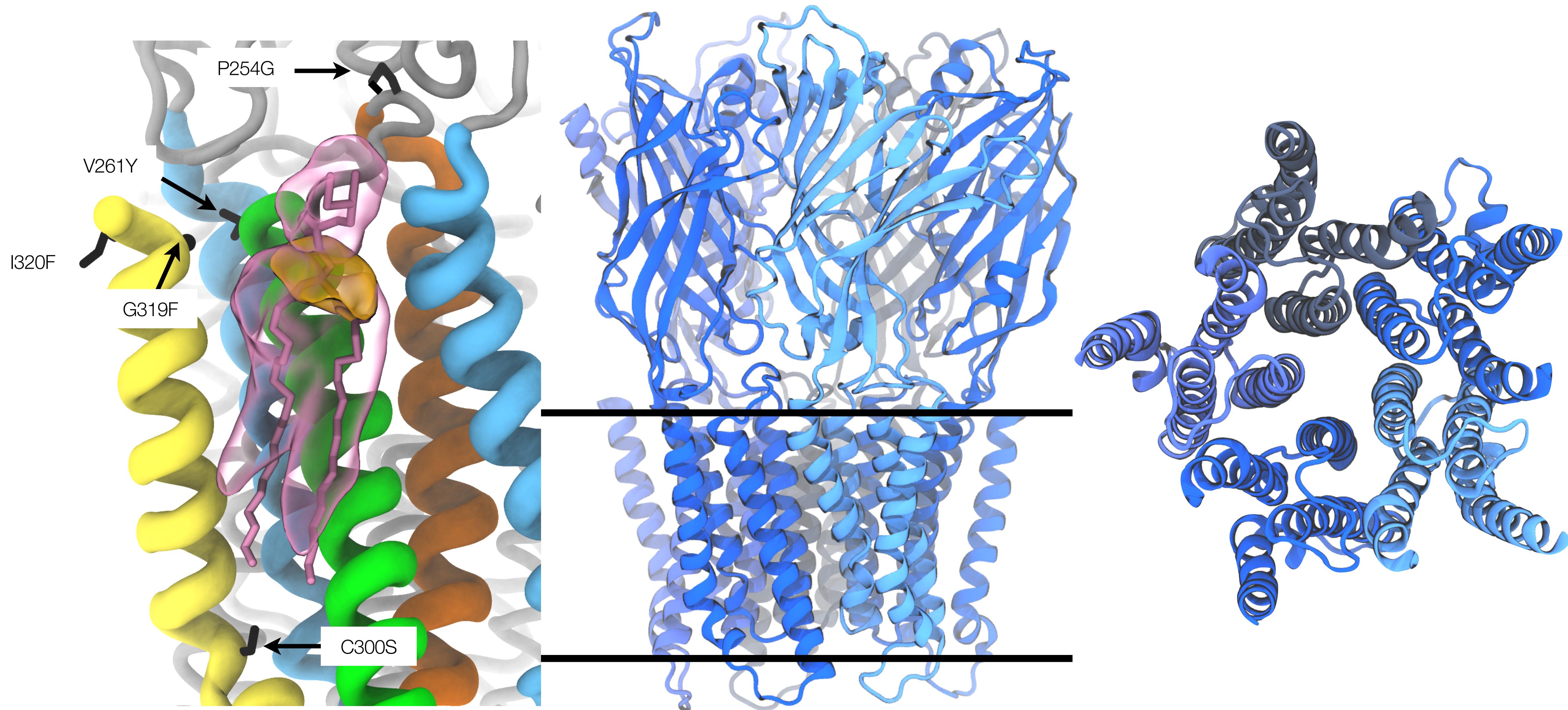
Tom Joseph

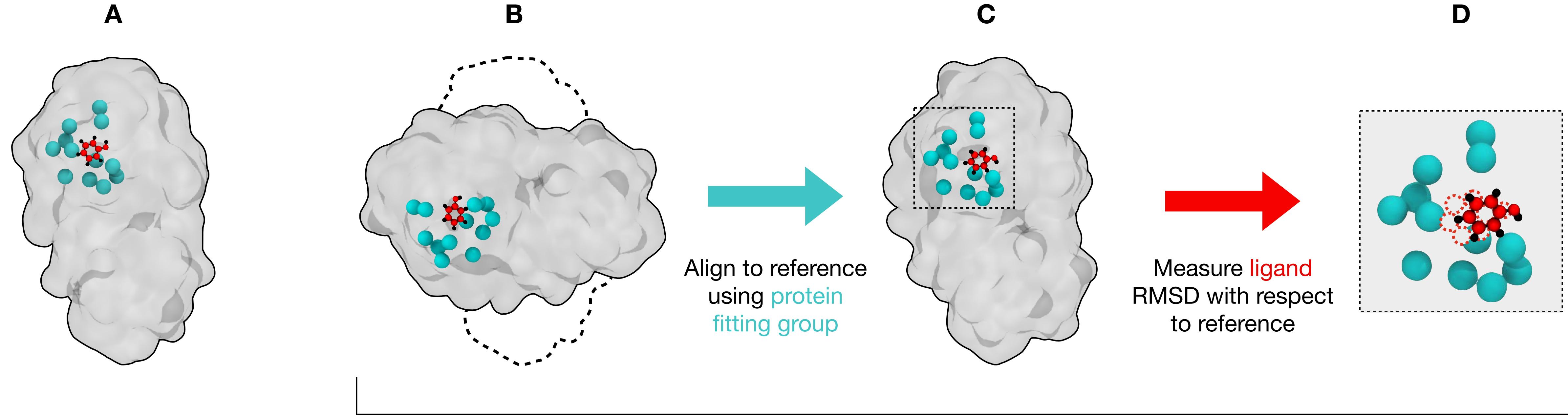


Mutations of ELIC5



Mutations of ELIC5





Reference Coordinates

Calculation of the Instantaneous DBC