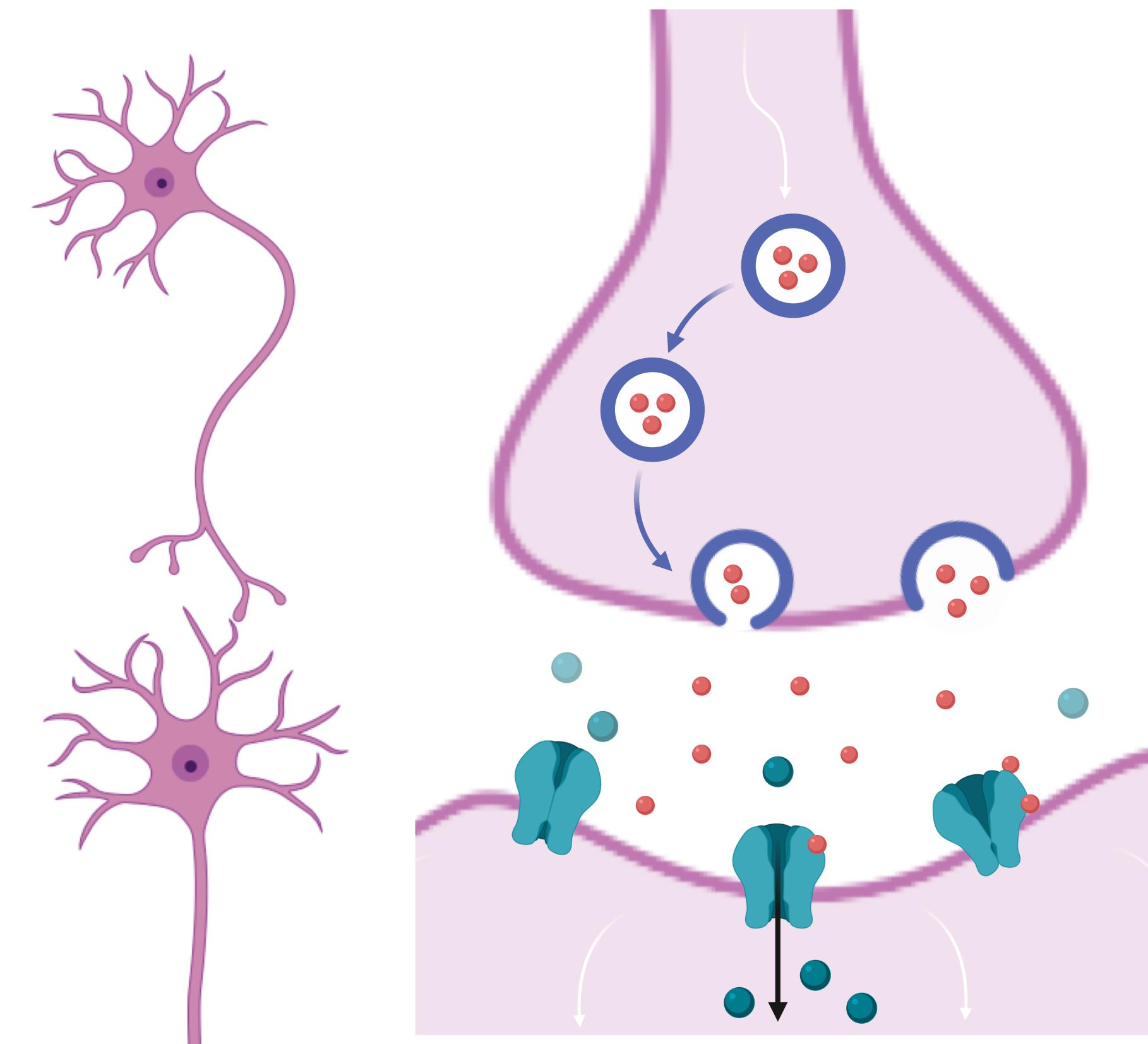


# **Lipid Modulation of a Bacterial “Neurotransmitter” Receptor POPG Binds and Modulates ELIC**

Ezry Santiago-McRae, Tom Joseph, Wayland Cheng, Jérôme Hénin, Grace Brannigan

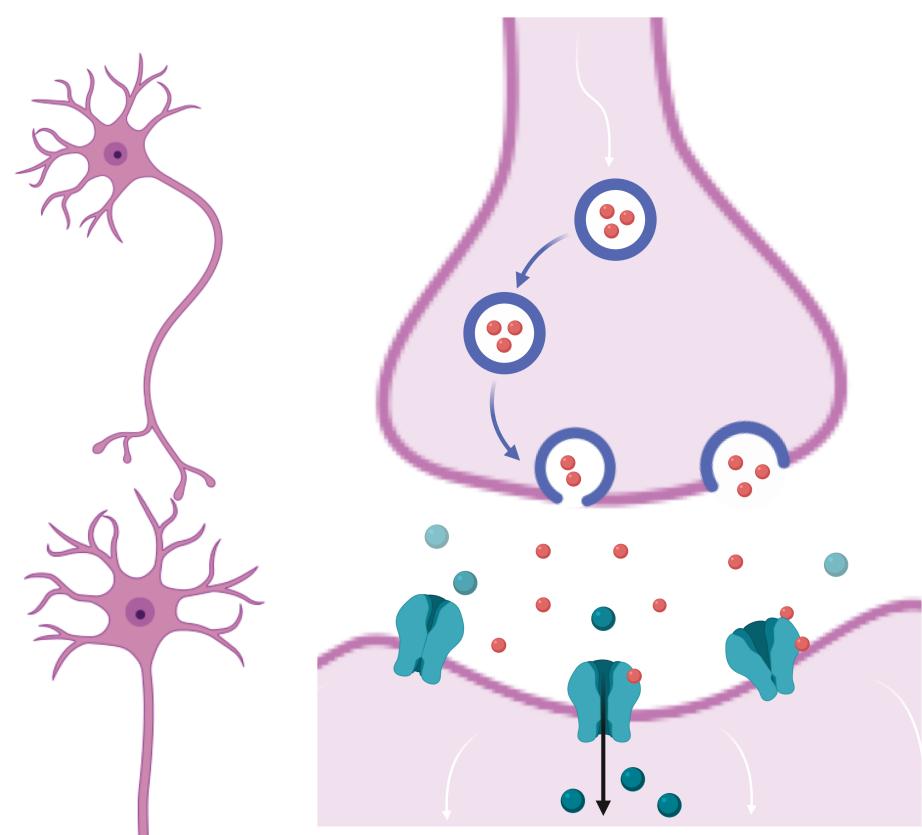
# Pentameric Ligand Gated Ion Channels (pLGICs)

## (Nearly) Ubiquitous Transmembrane Chemosensors



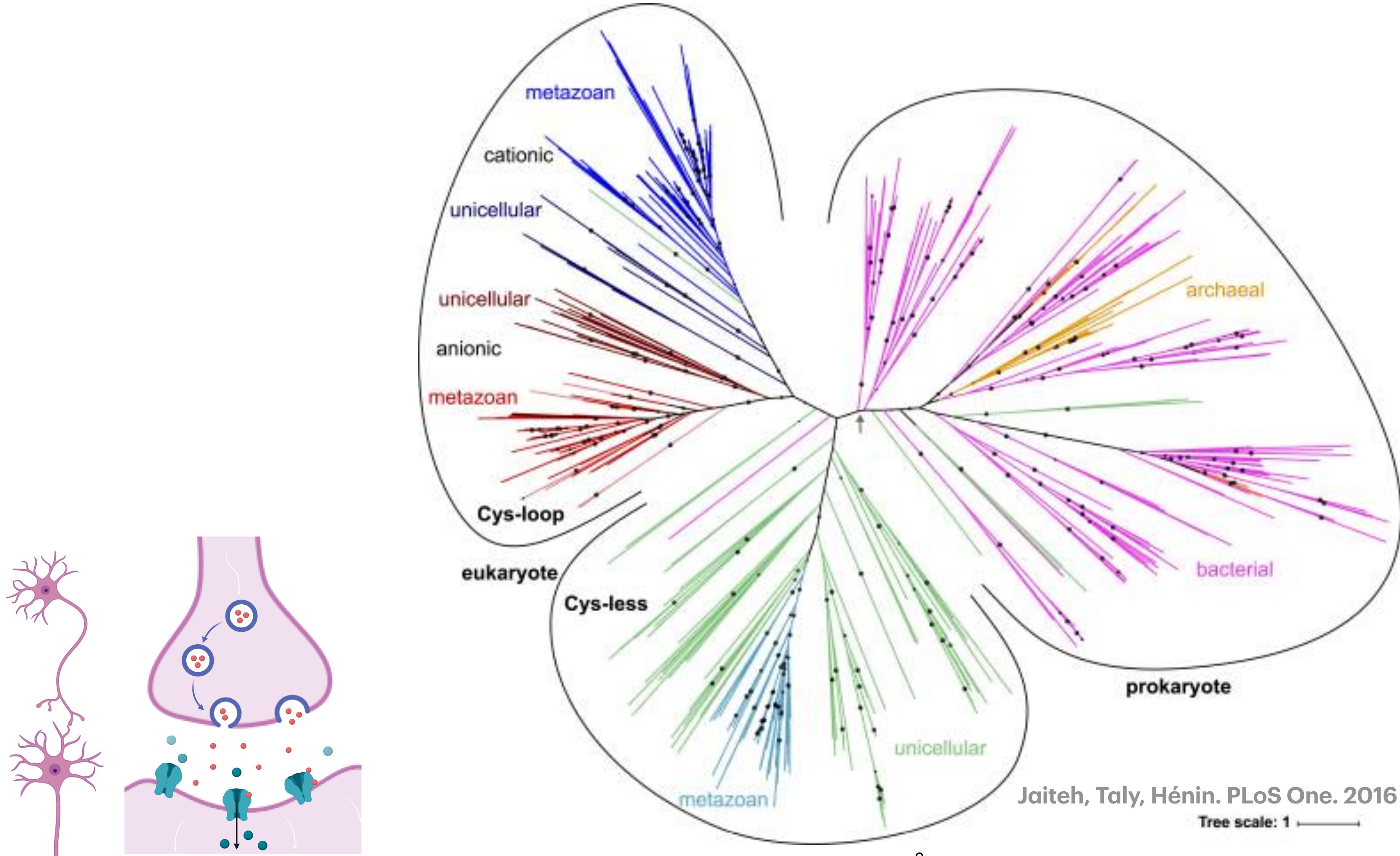
# Pentameric Ligand Gated Ion Channels (pLGICs)

## (Nearly) Ubiquitous Transmembrane Chemosensors



# Pentameric Ligand Gated Ion Channels (pLGICs)

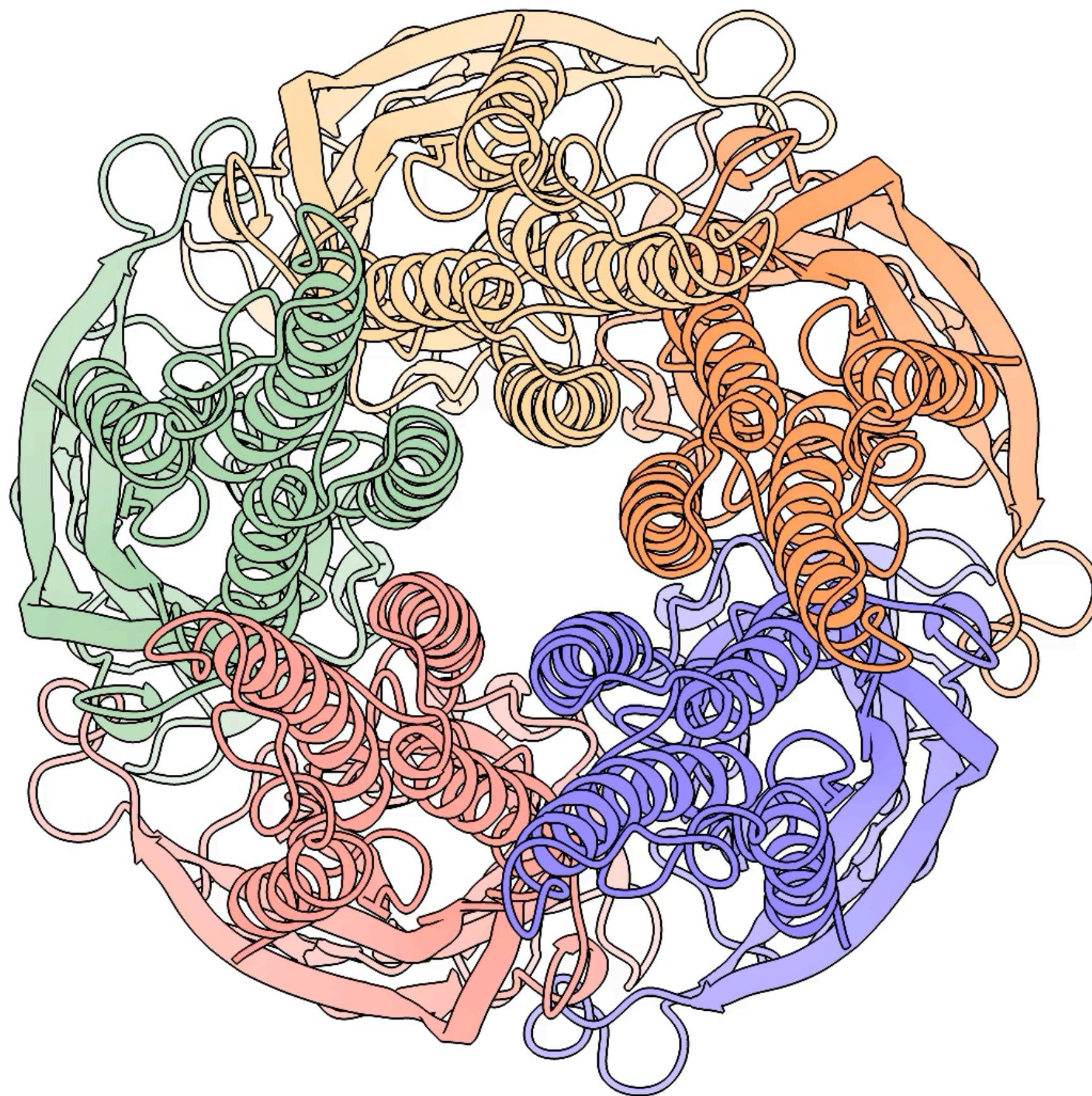
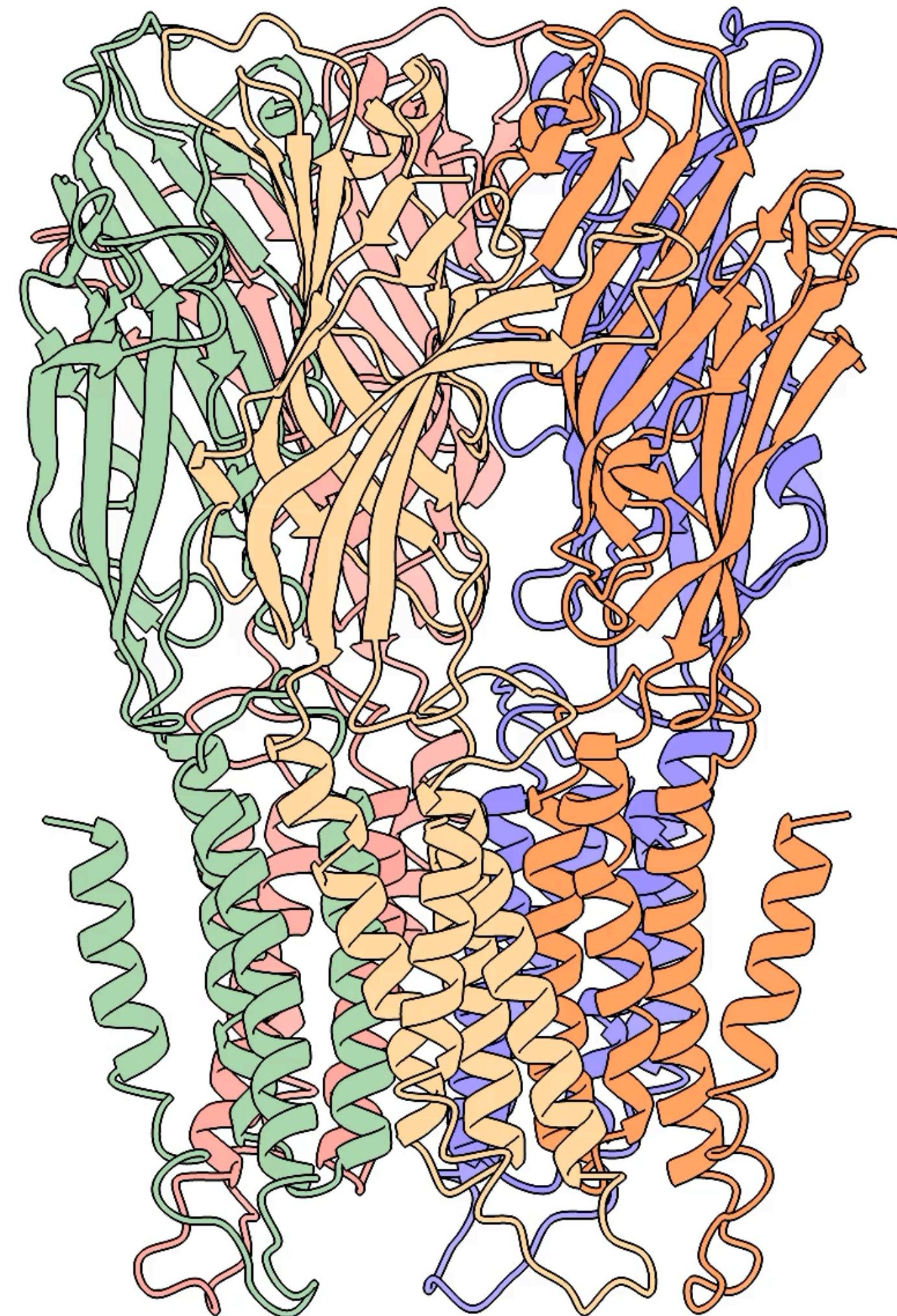
## (Nearly) Ubiquitous Transmembrane Chemosensors



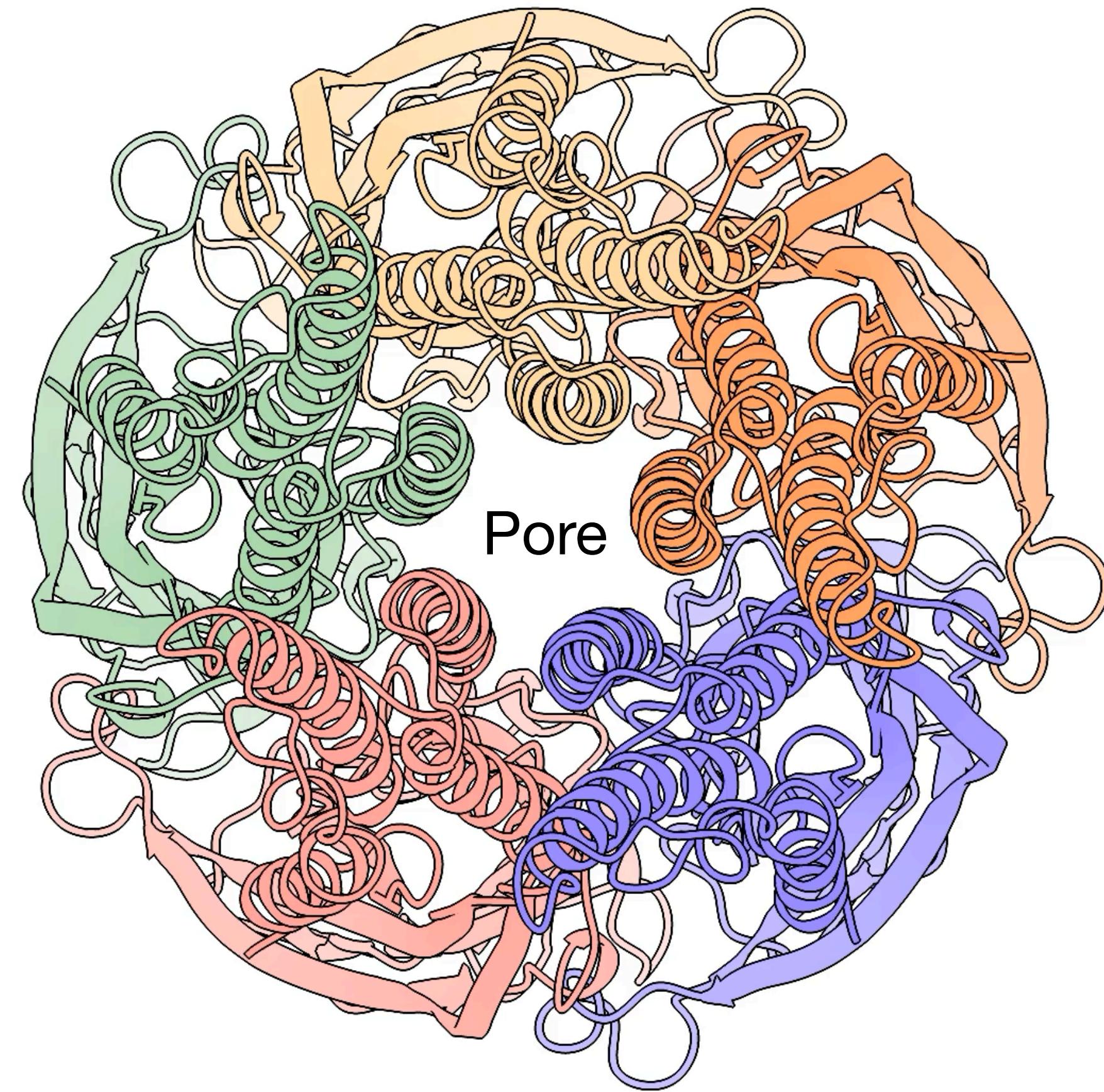
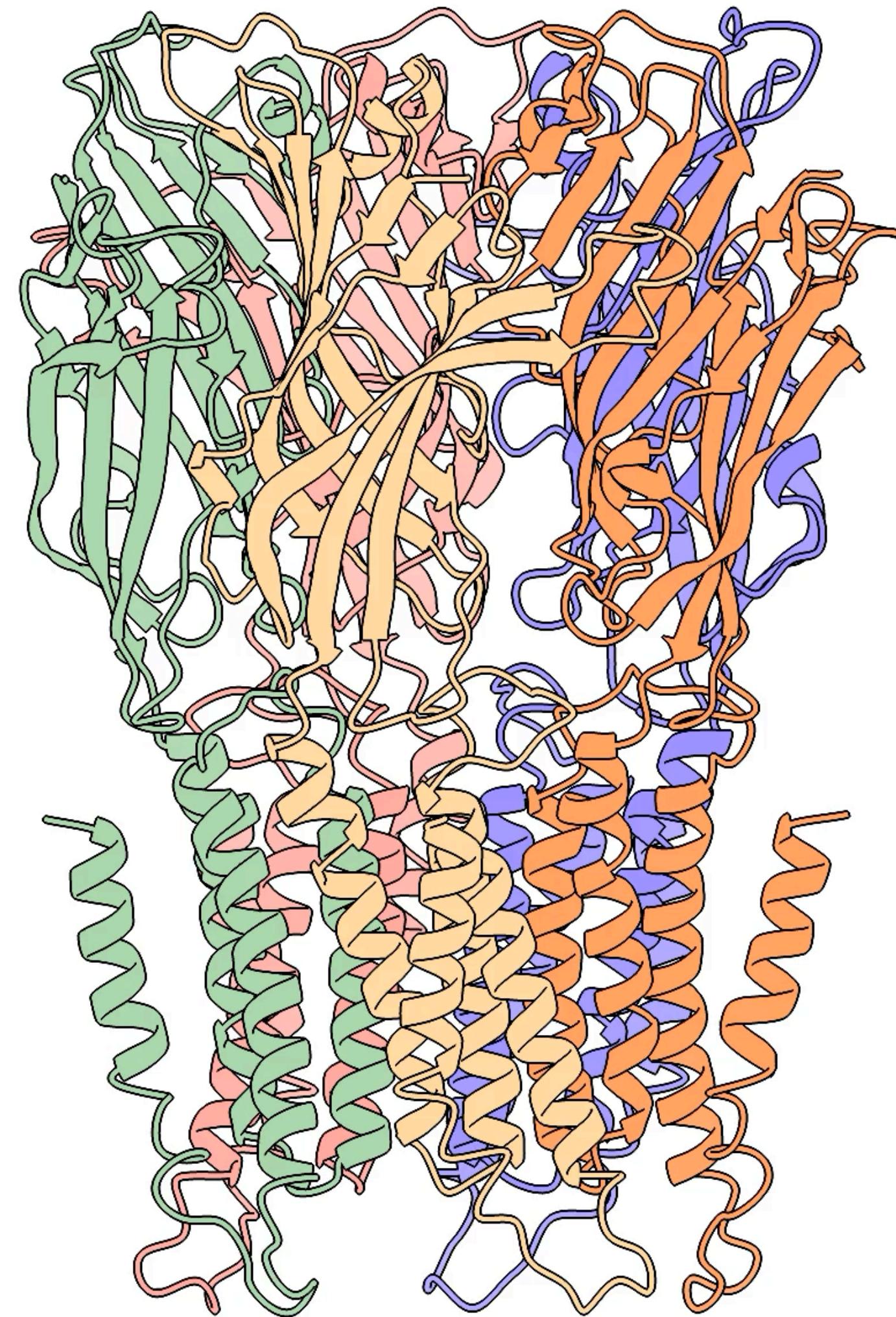
Jaiteh, Taly, Hénin. PLoS One. 2016

Tree scale: 1

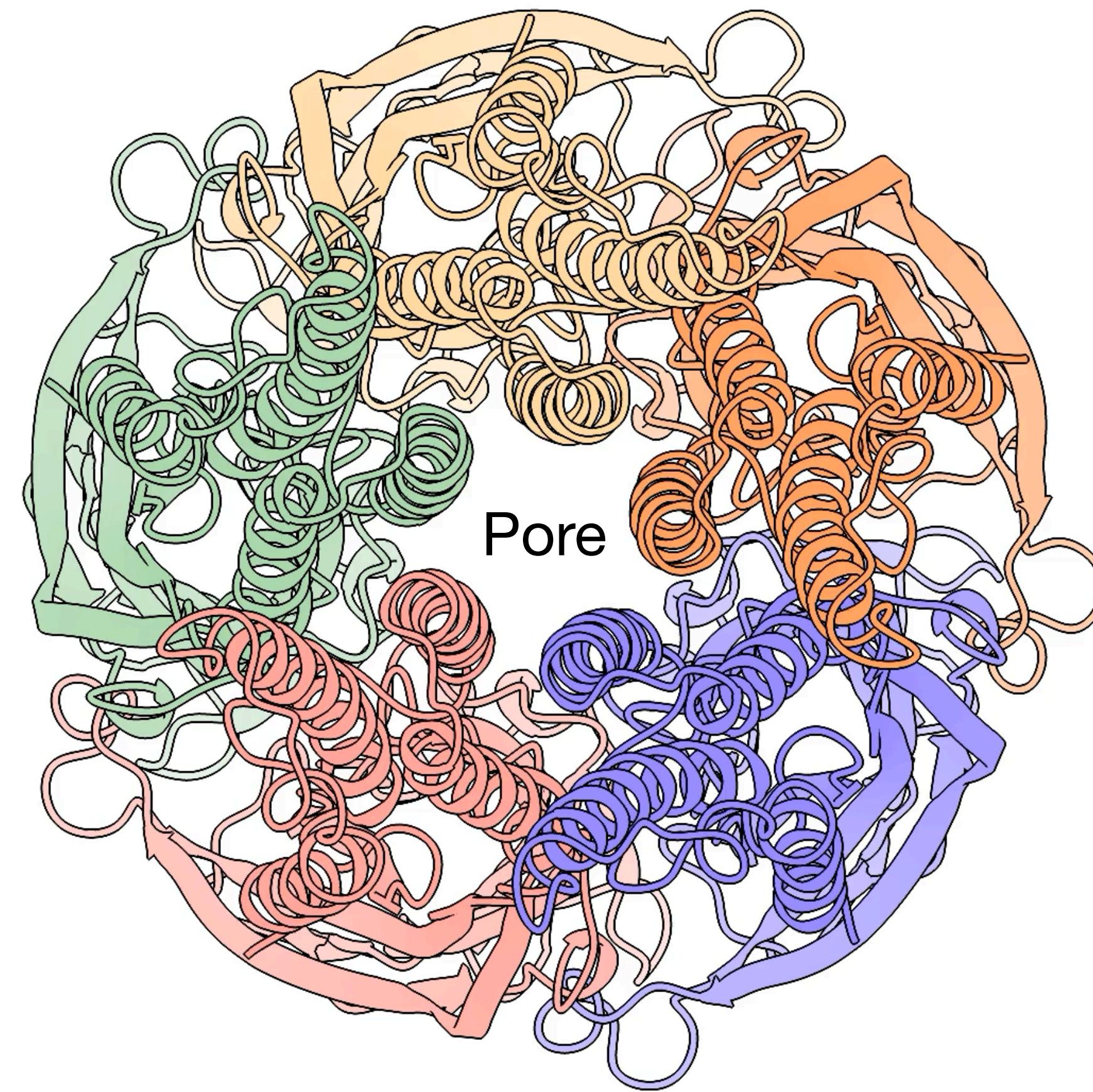
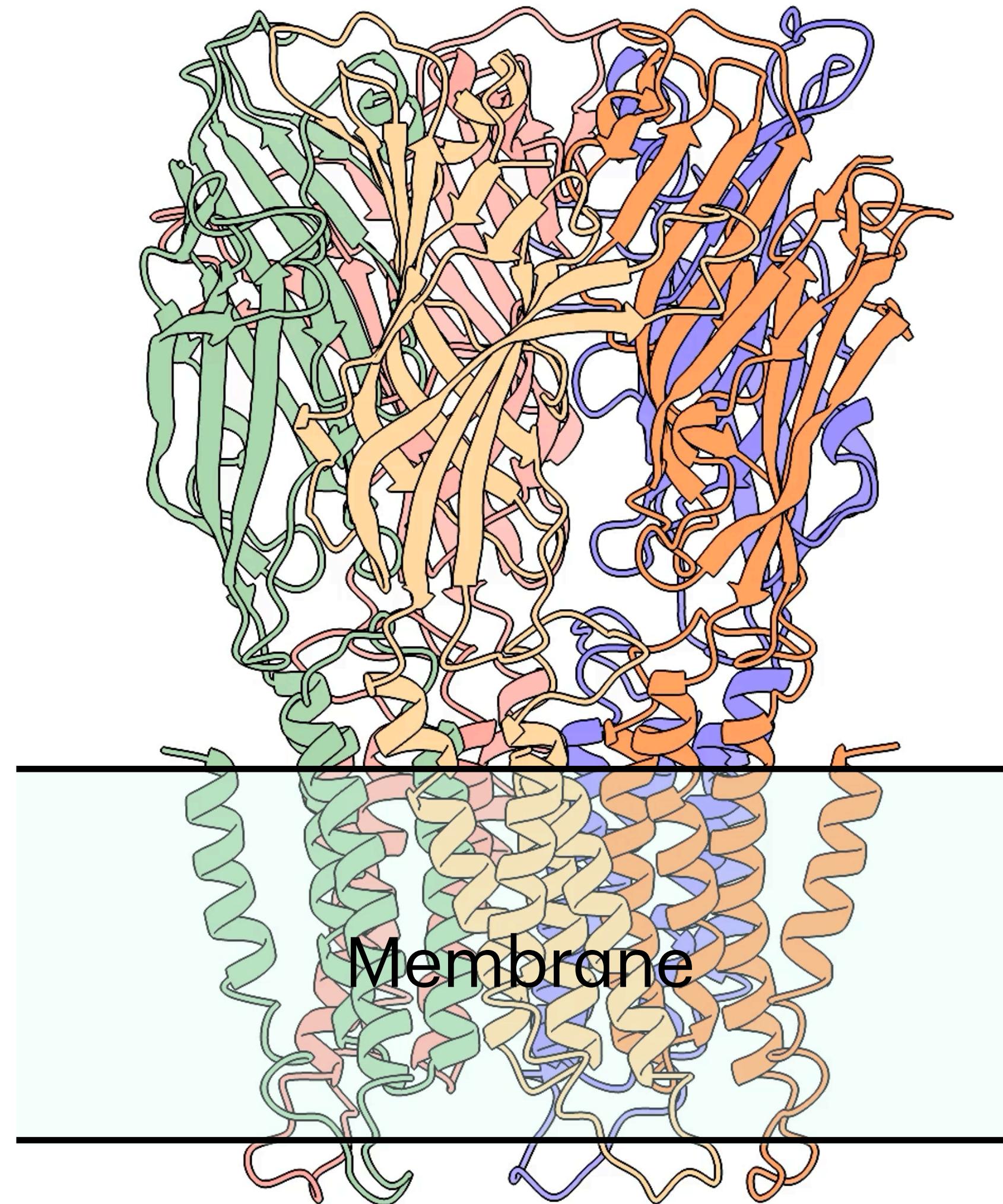
# *Erwinia* Ligand Gated Ion Channel (ELIC)



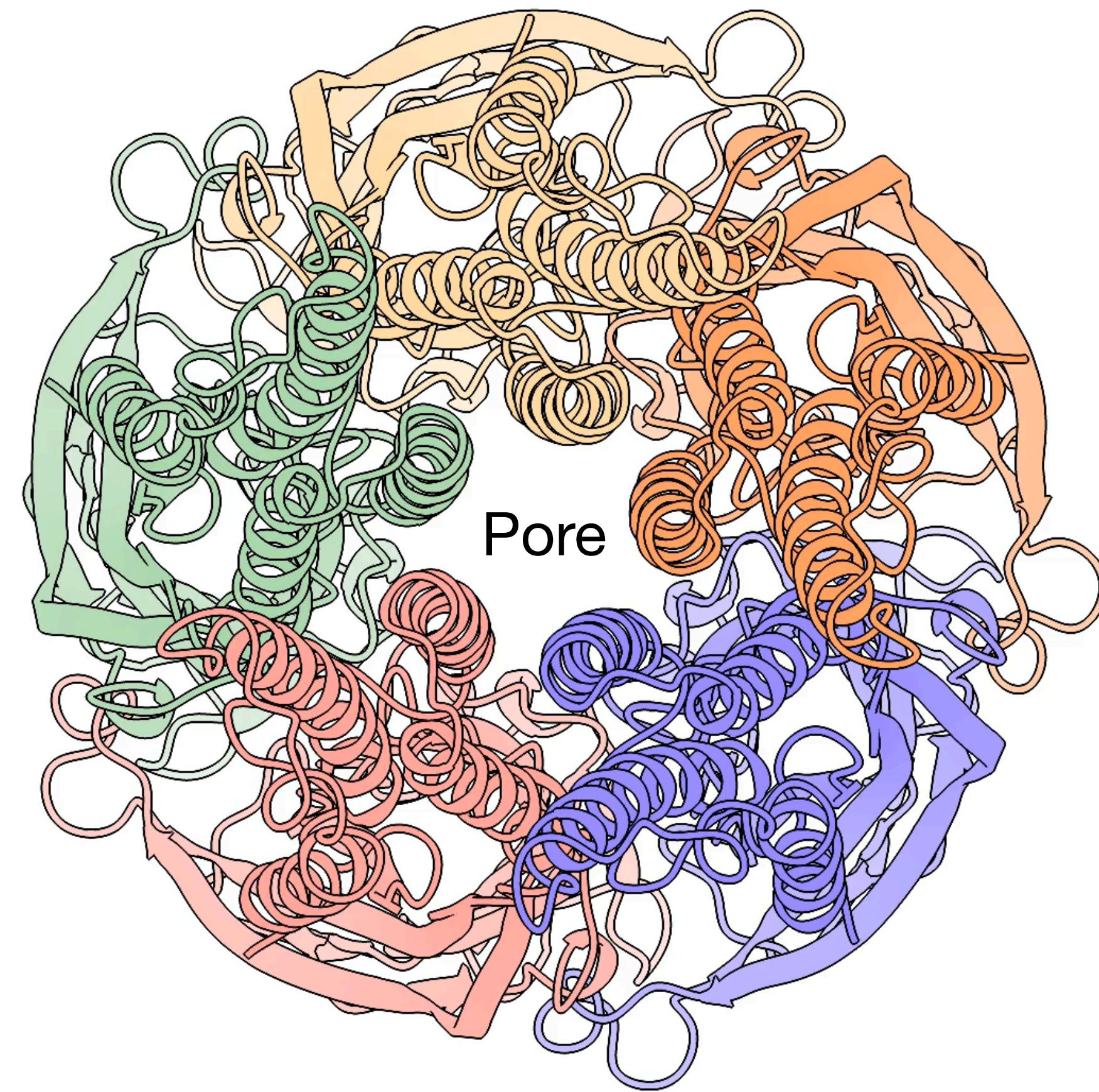
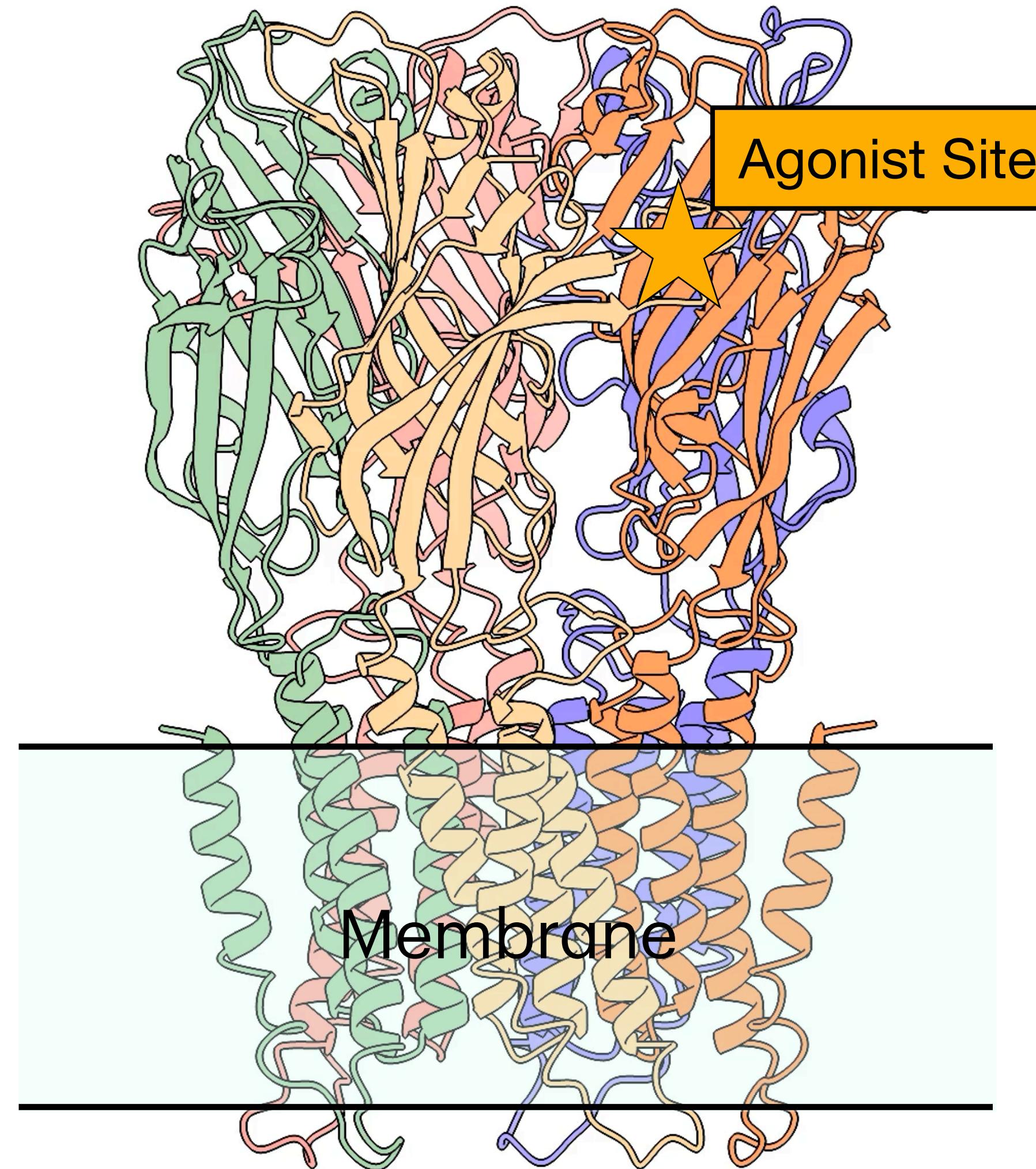
# *Erwinia* Ligand Gated Ion Channel (ELIC)



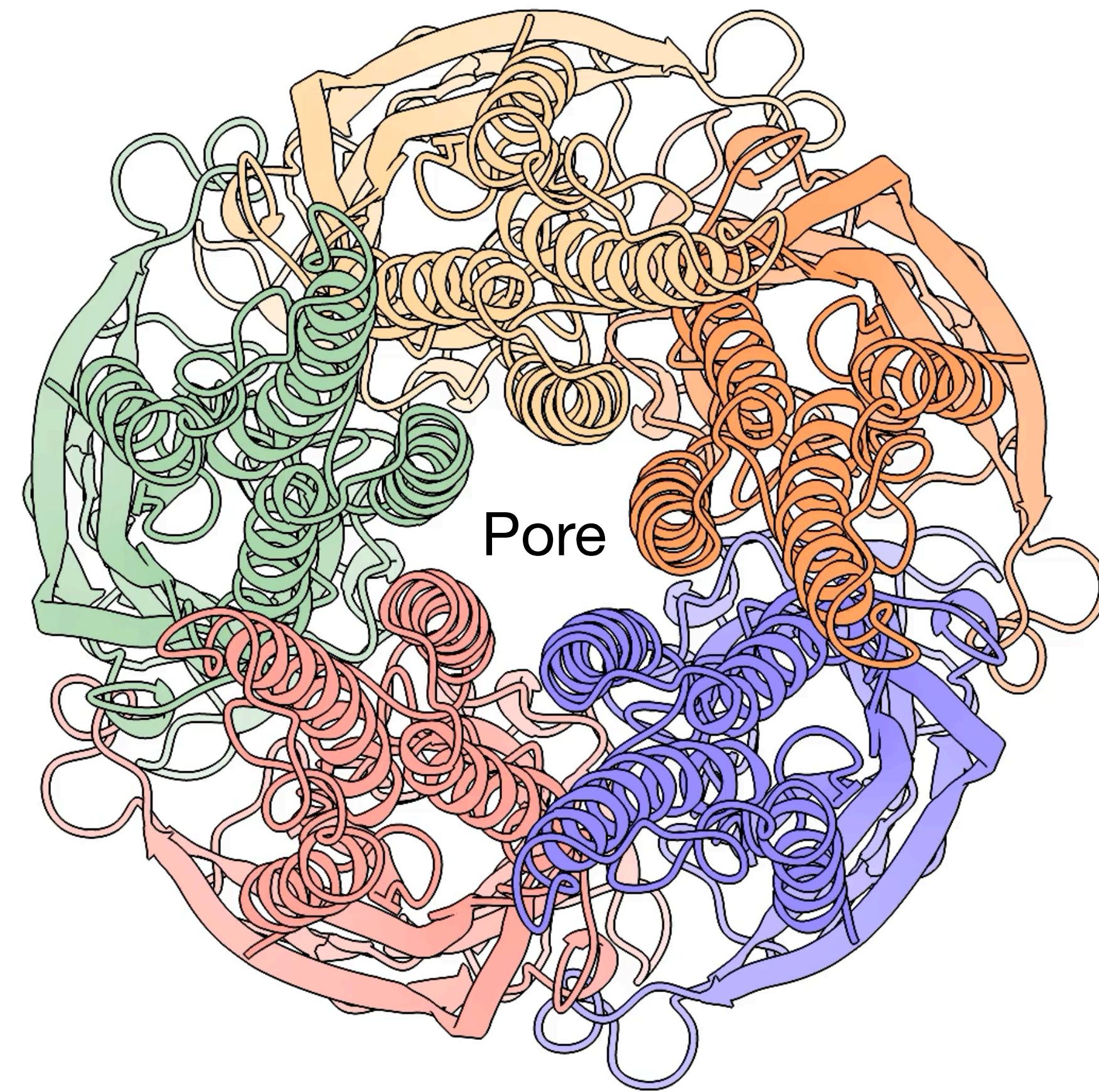
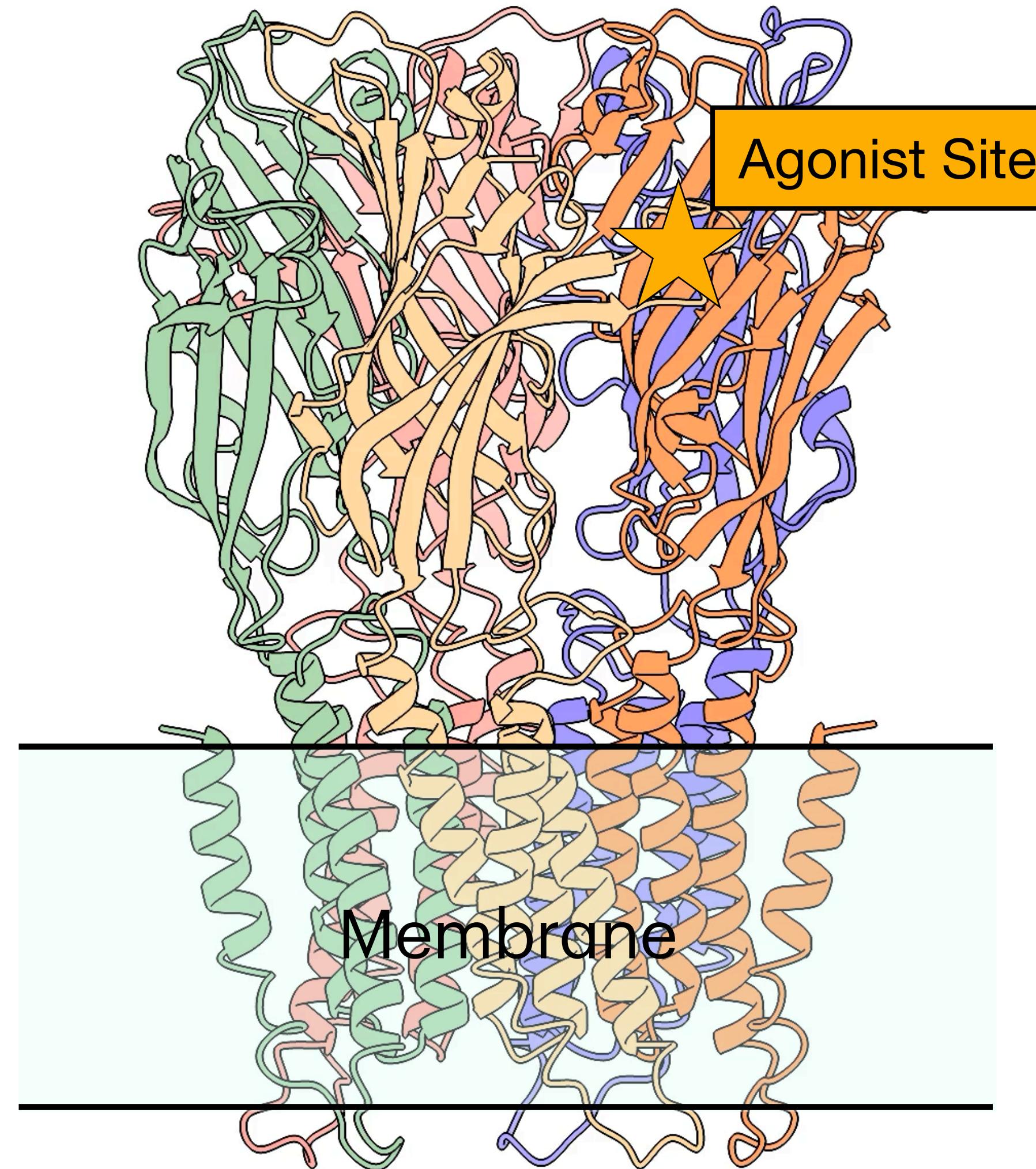
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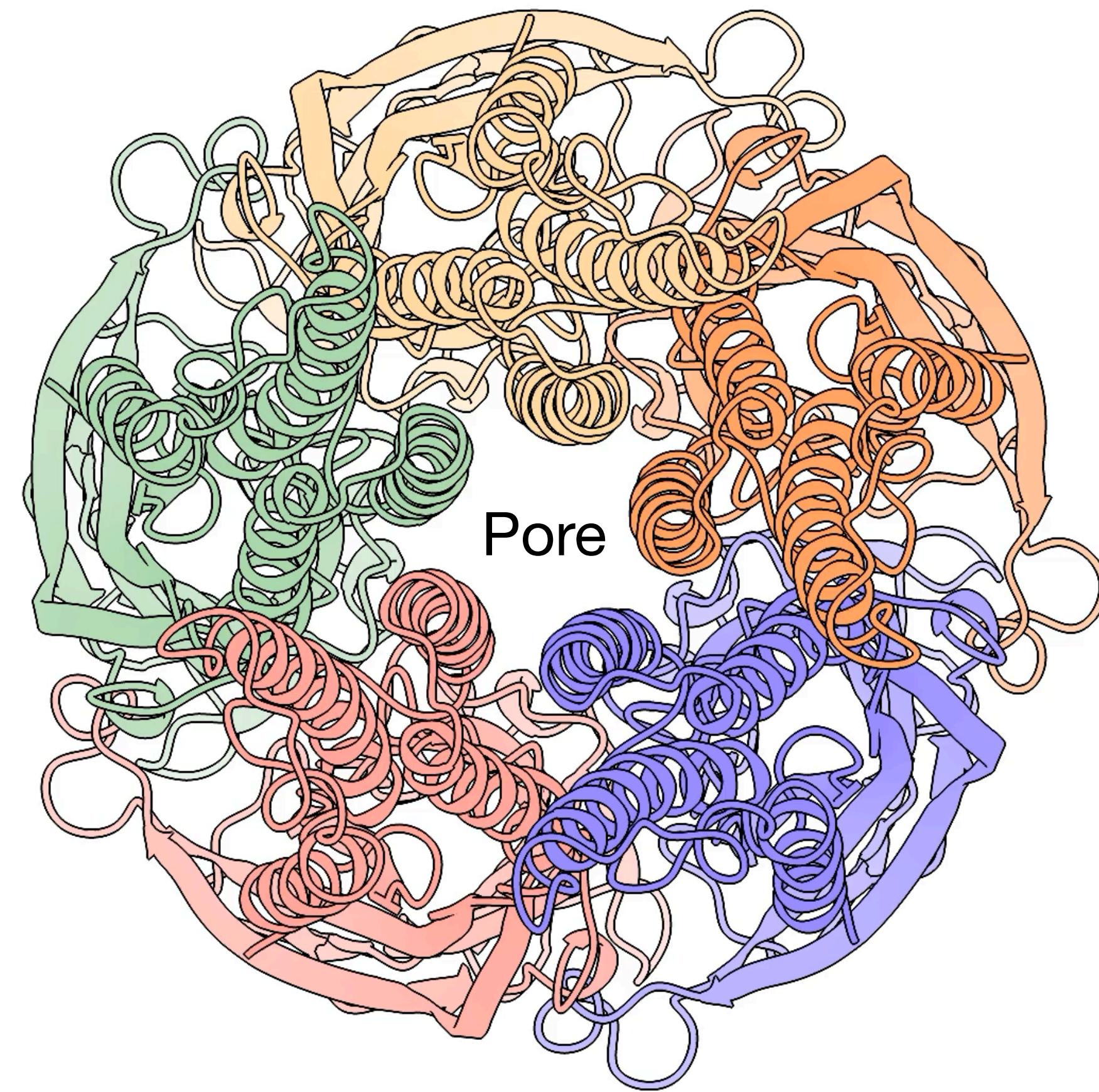
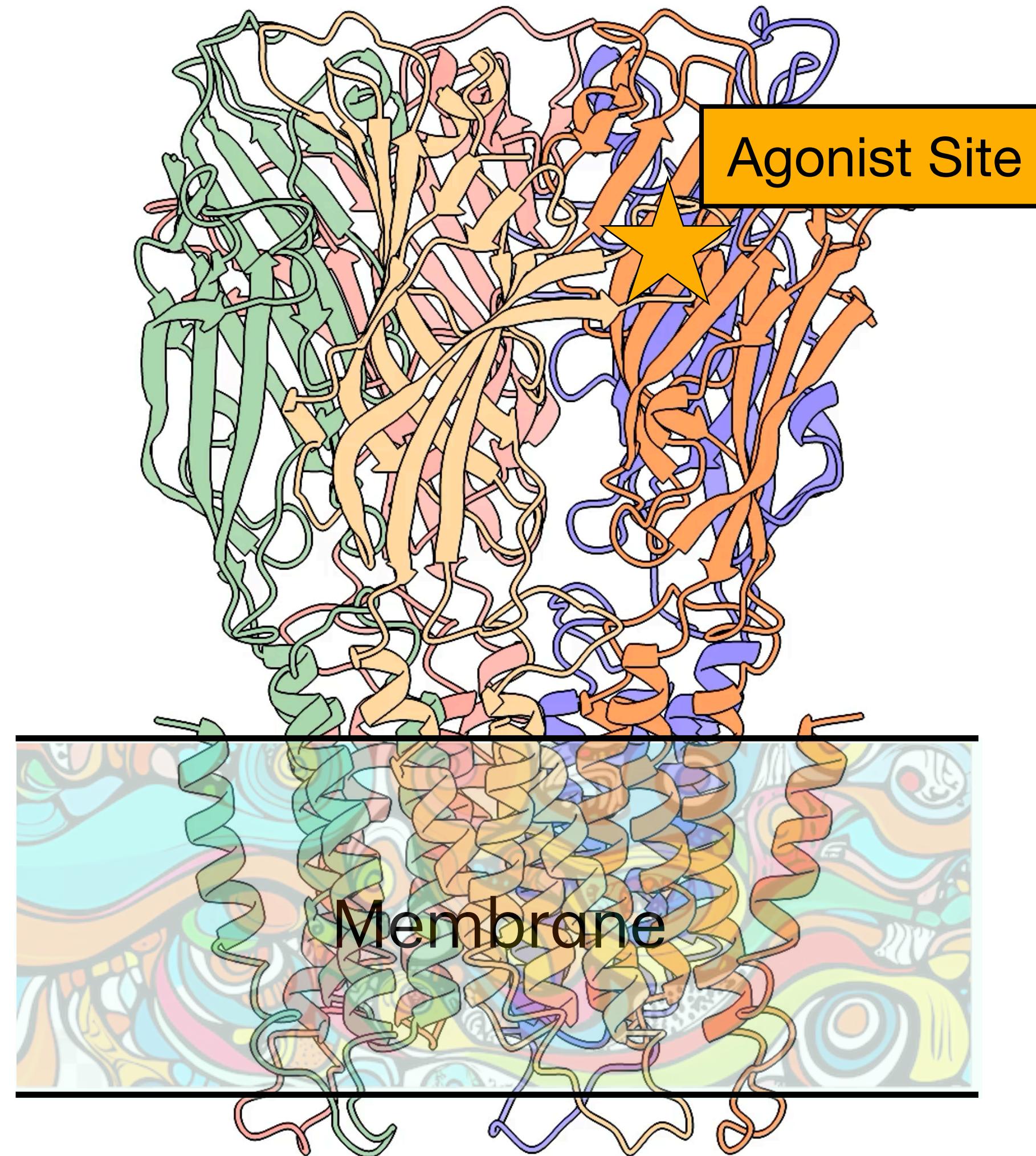
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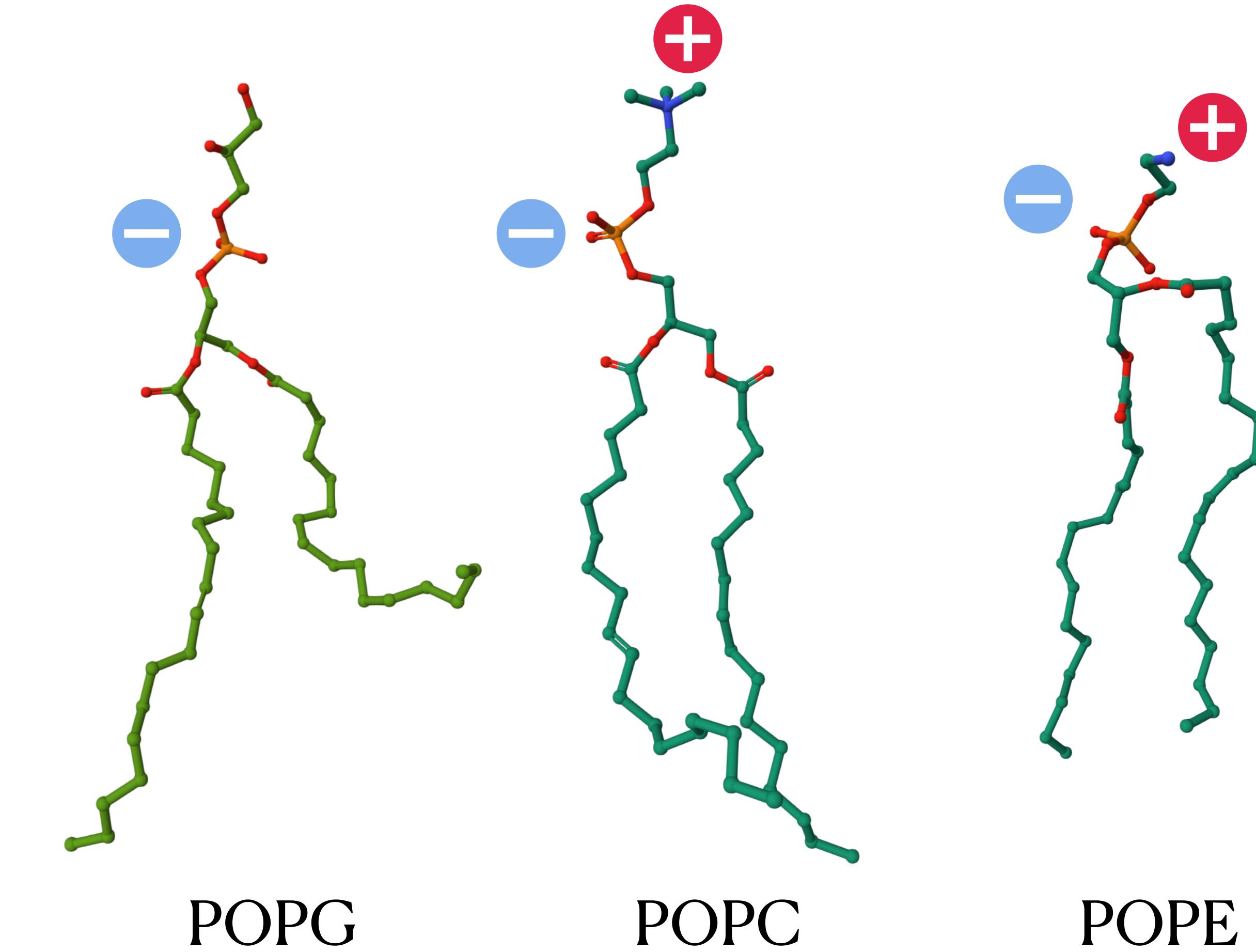


# *Erwinia* Ligand Gated Ion Channel (ELIC)

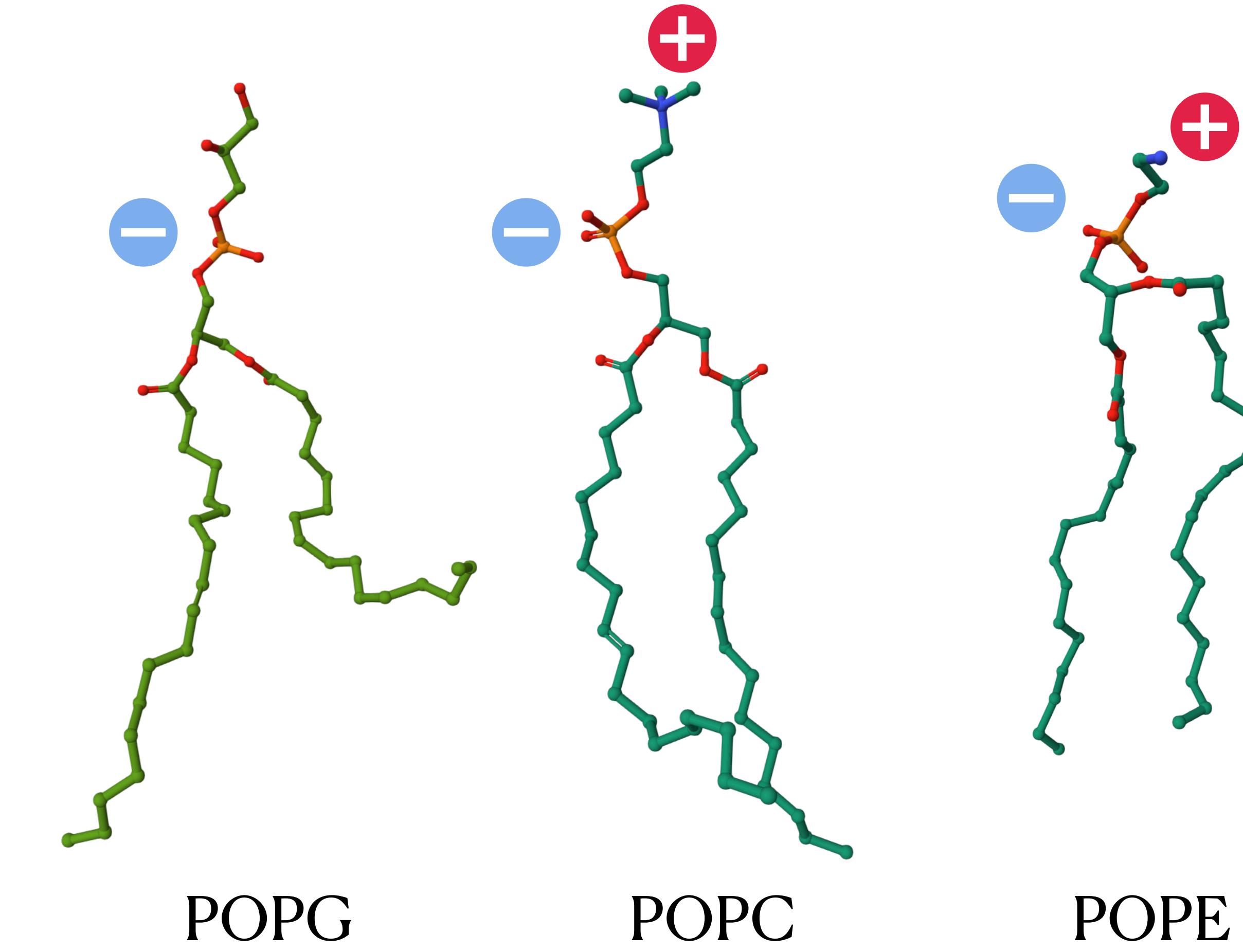
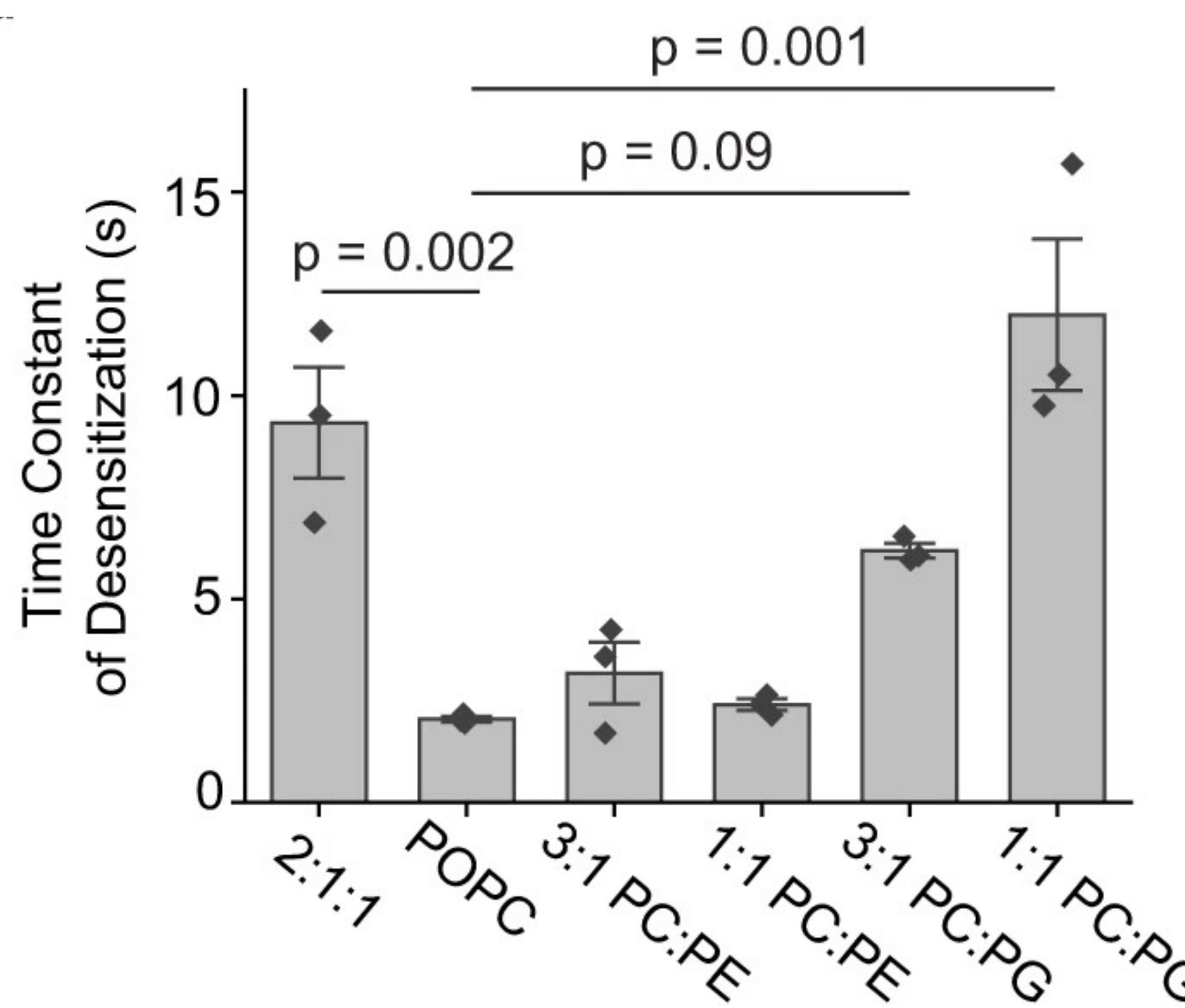


# **ELIC (like many pLGICs) is Lipid Sensitive**

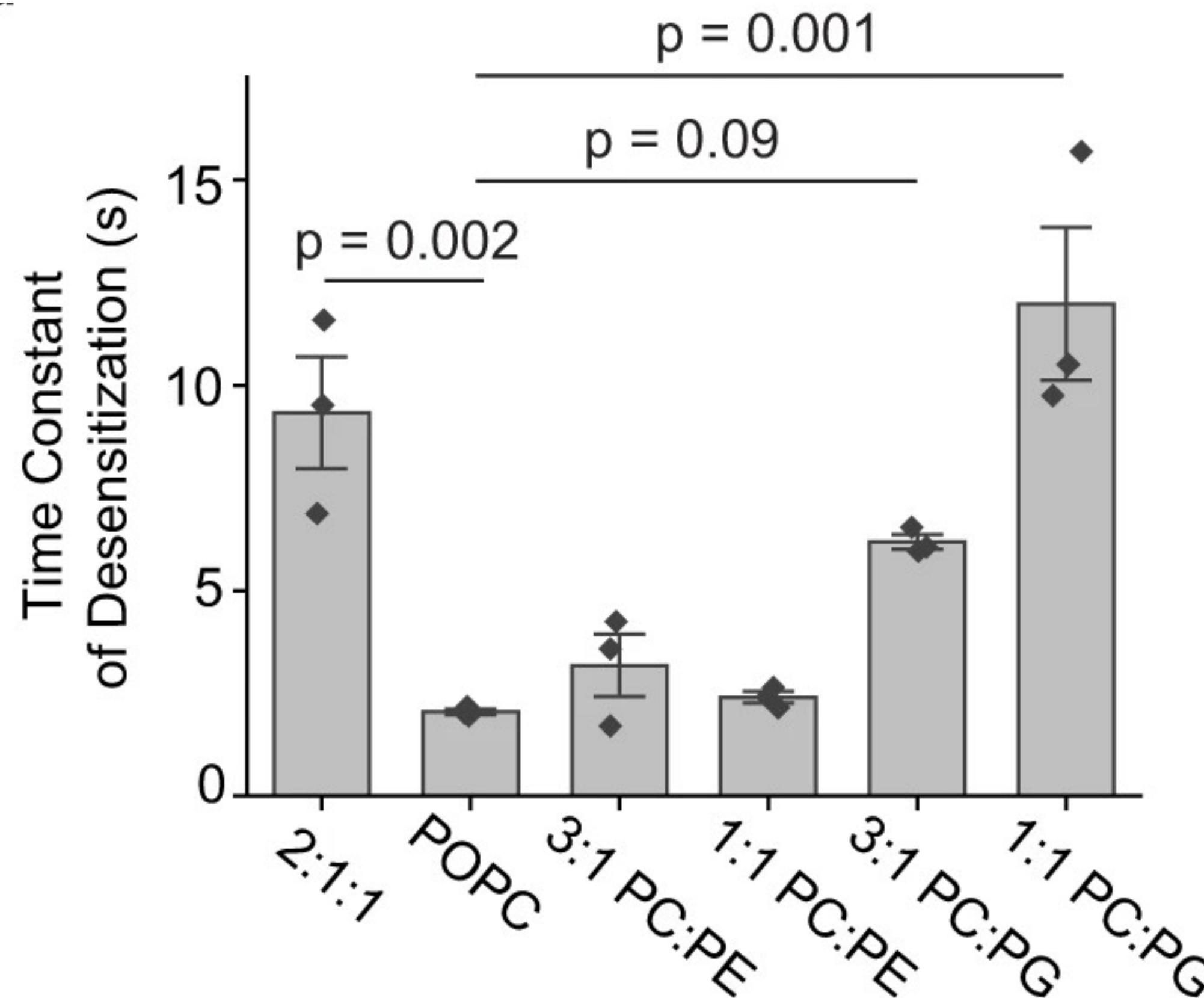
# ELIC (like many pLGICs) is Lipid Sensitive



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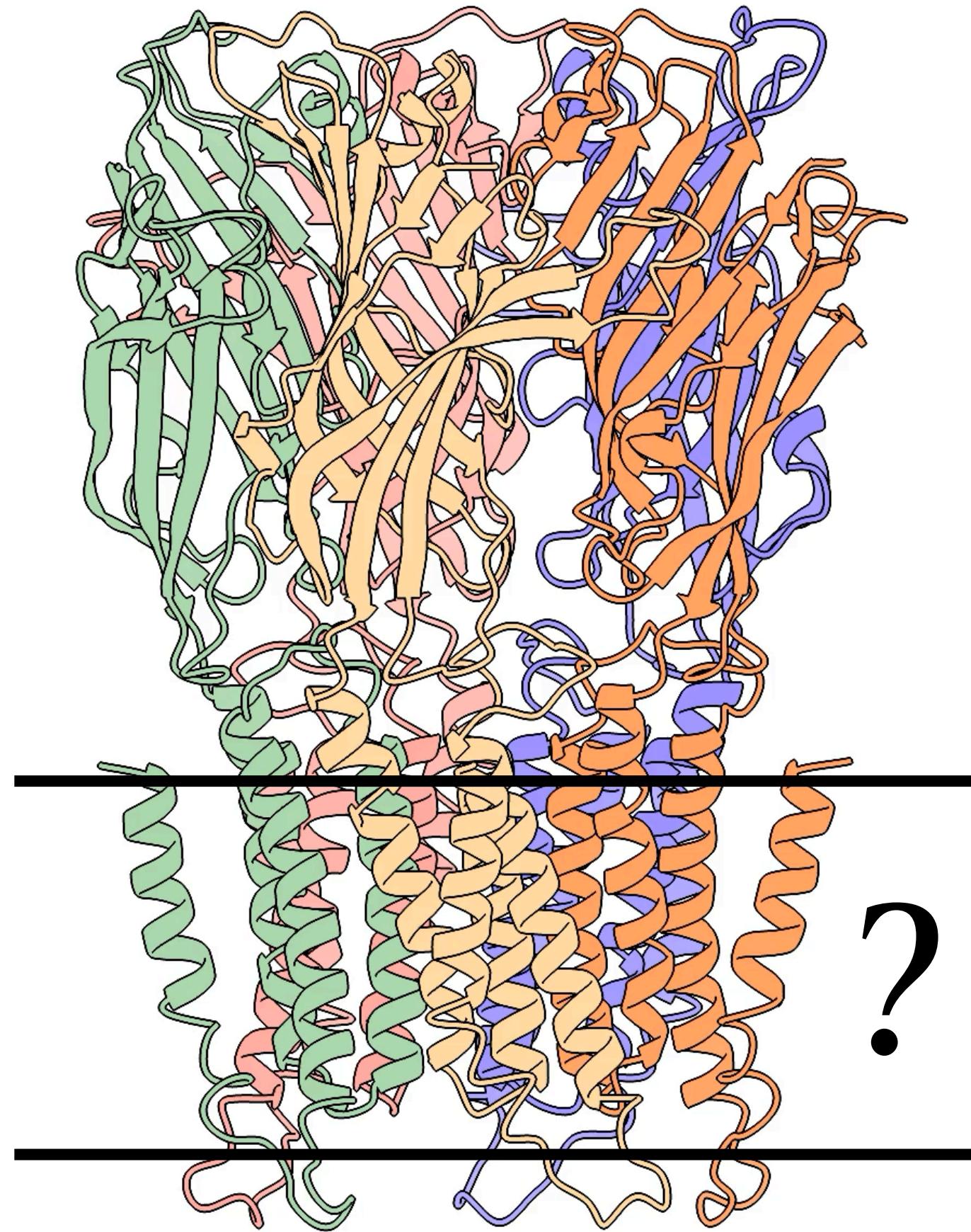


# ELIC (like many pLGICs) is Lipid Sensitive



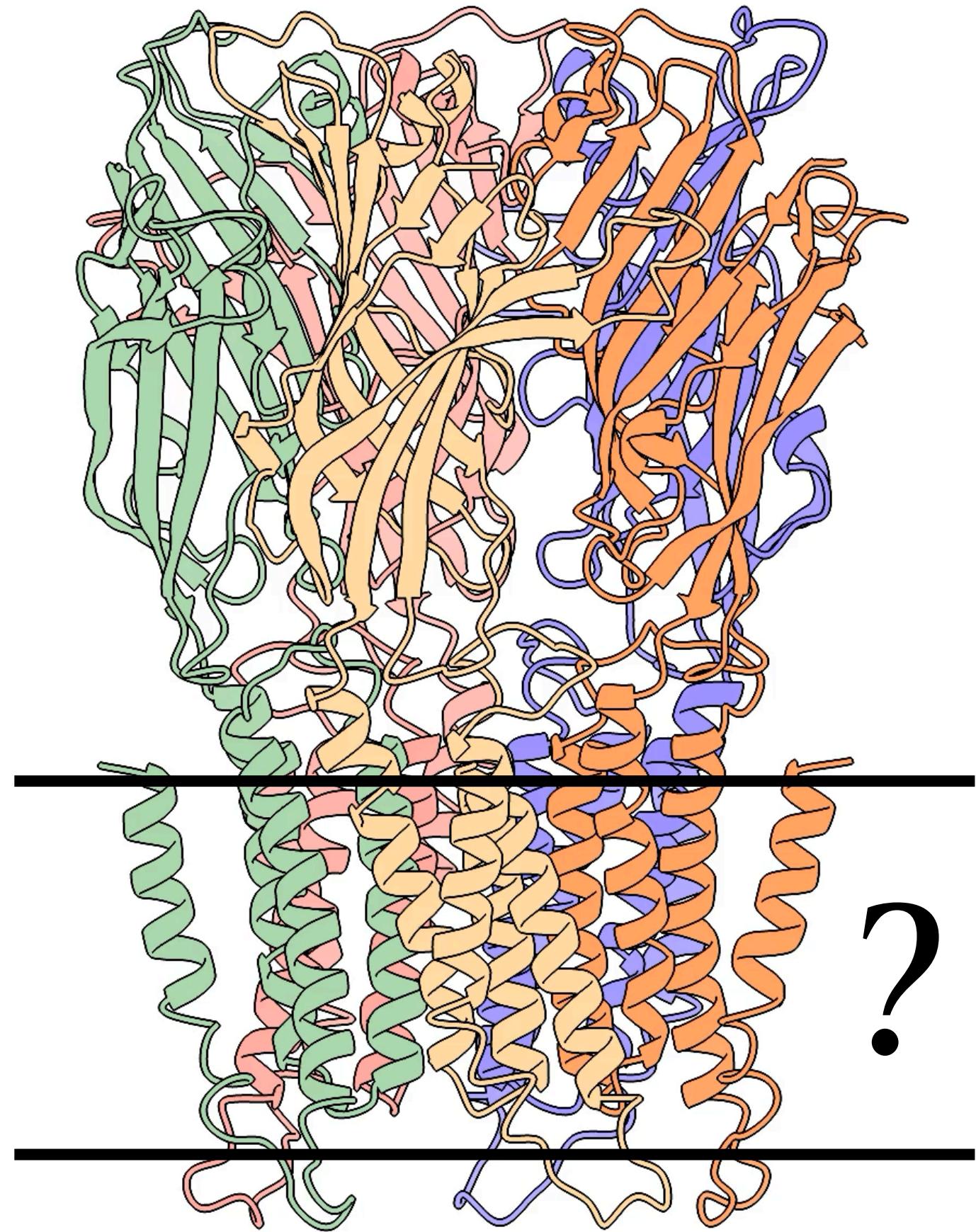
What is the  
Mechanism of this  
Effect?

# Hypothesis: Allosteric Modulation



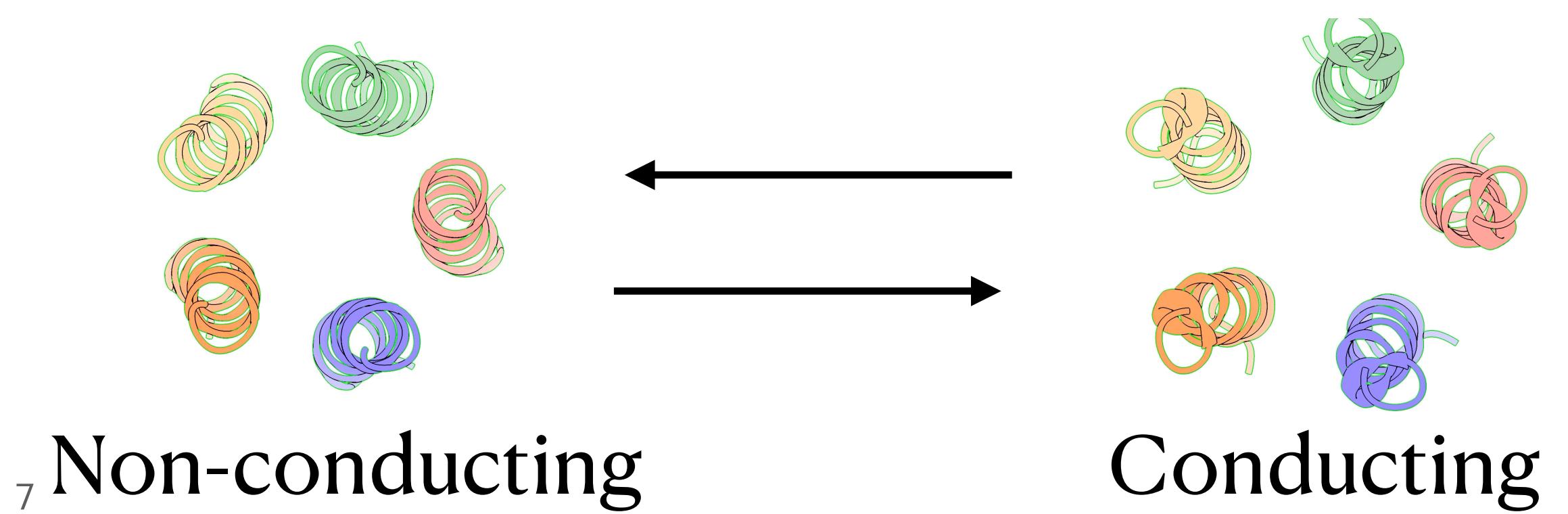
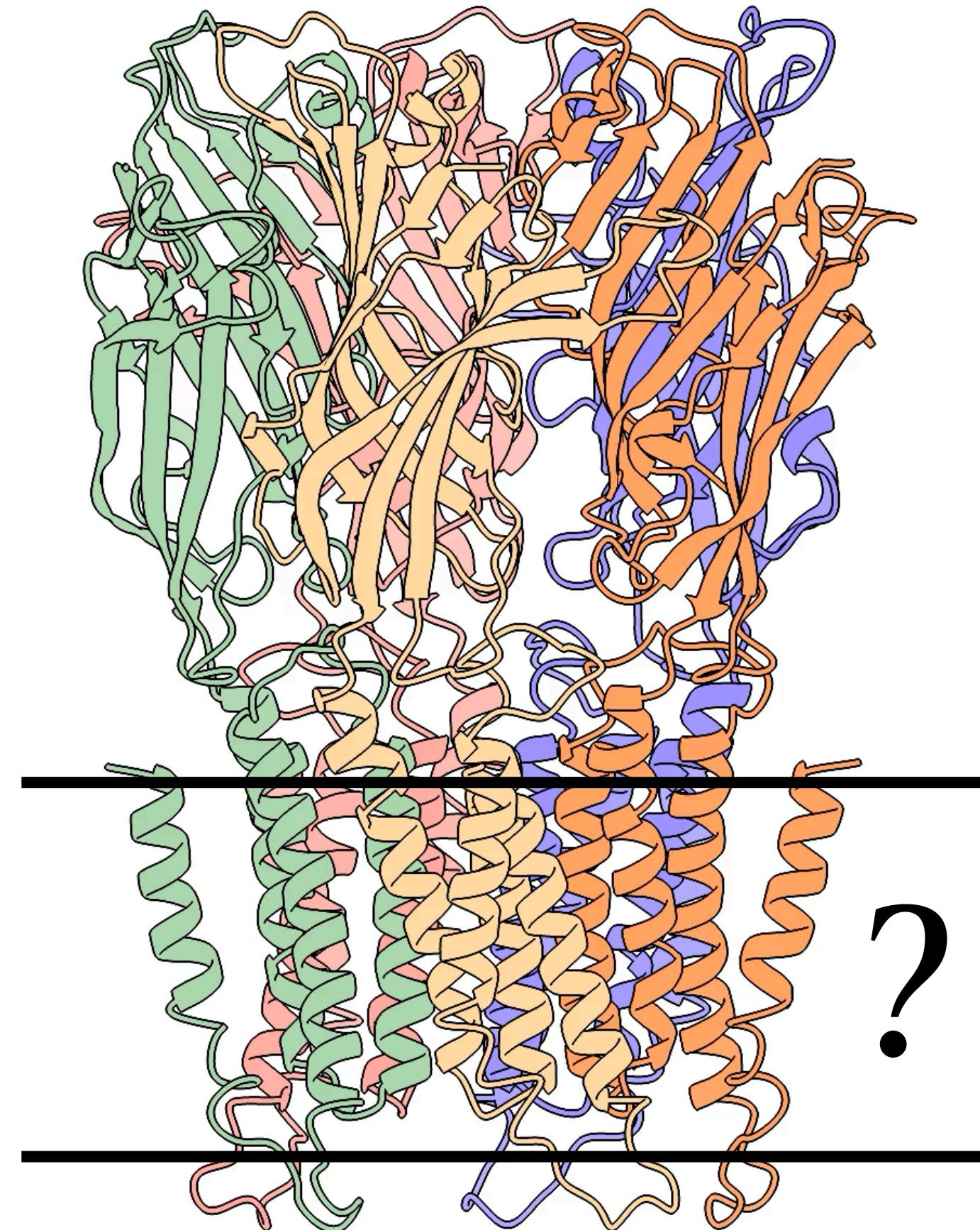
# Hypothesis: Allosteric Modulation

1. POPG Binding Site



# Hypothesis: Allosteric Modulation

1. POPG Binding Site
2. Stabilize the open conformation

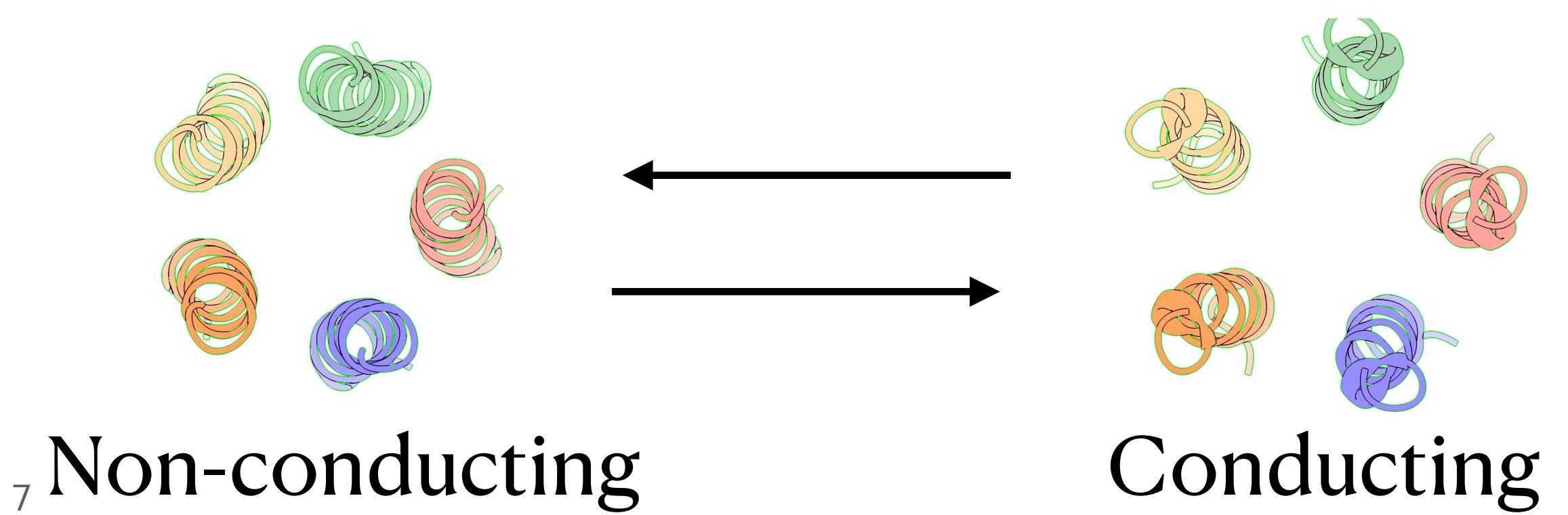
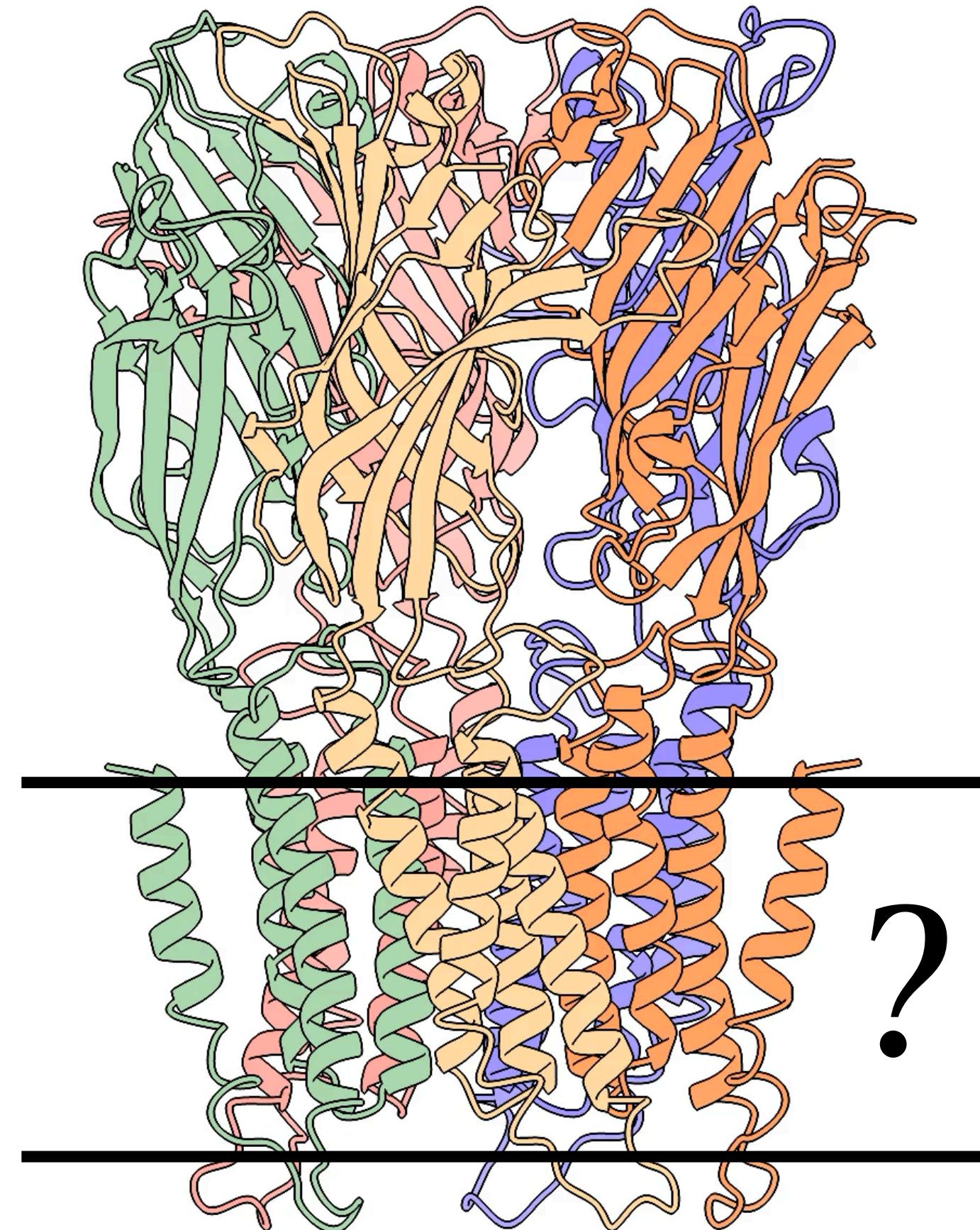


<sup>7</sup> Non-conducting

Conducting

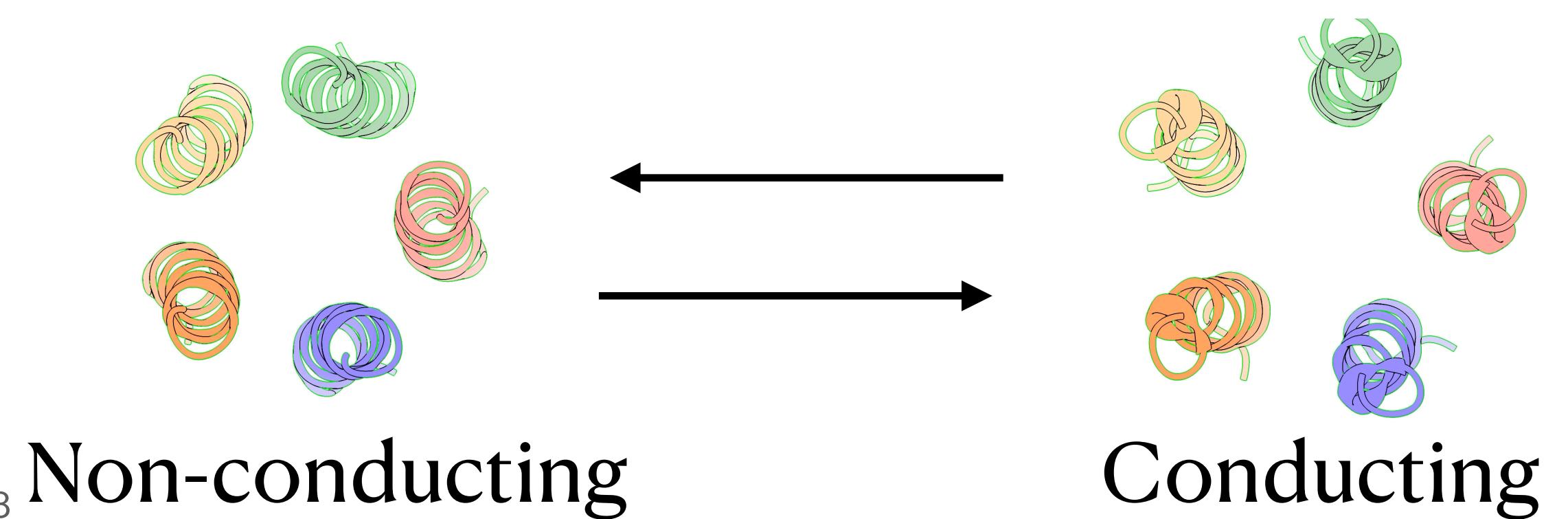
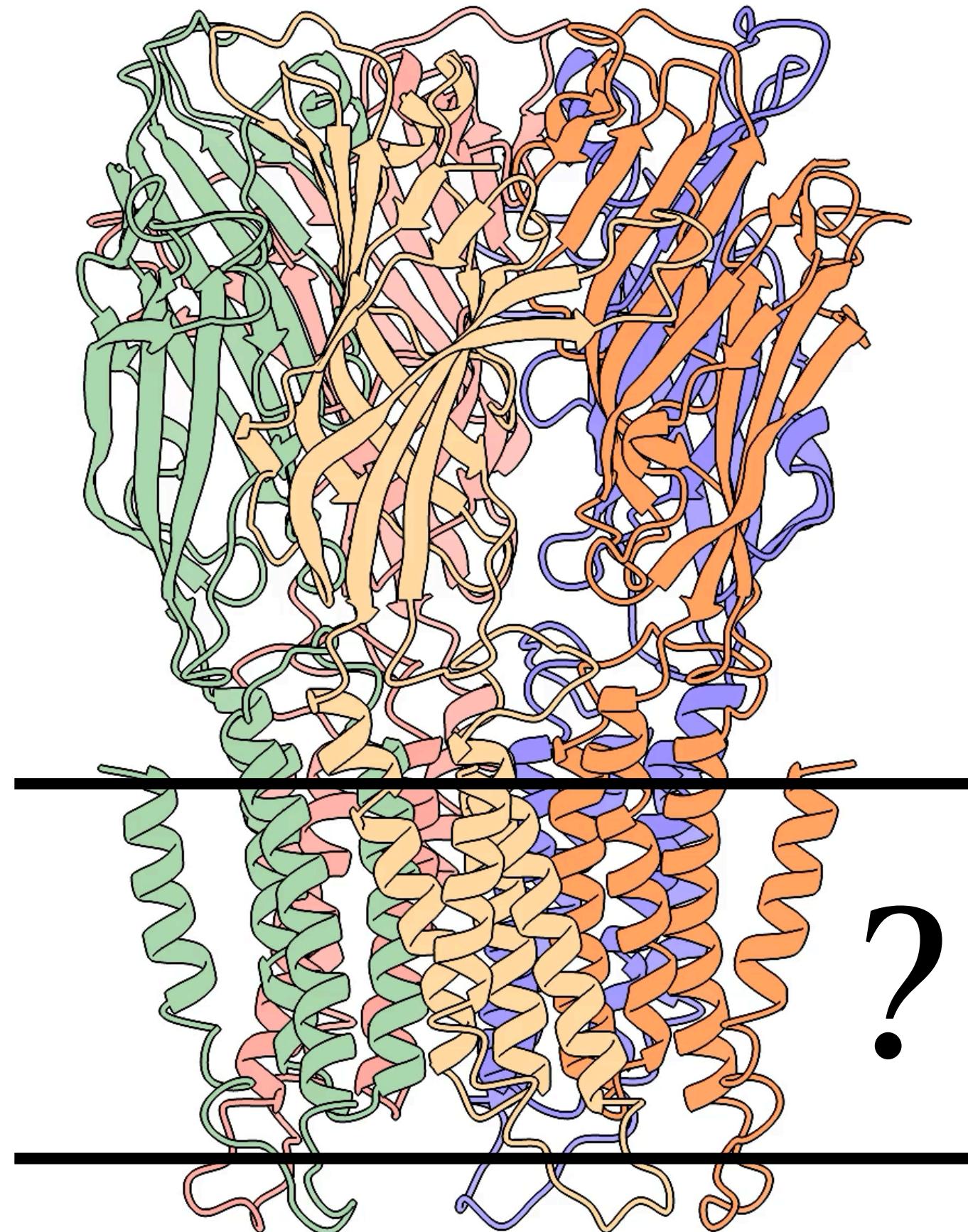
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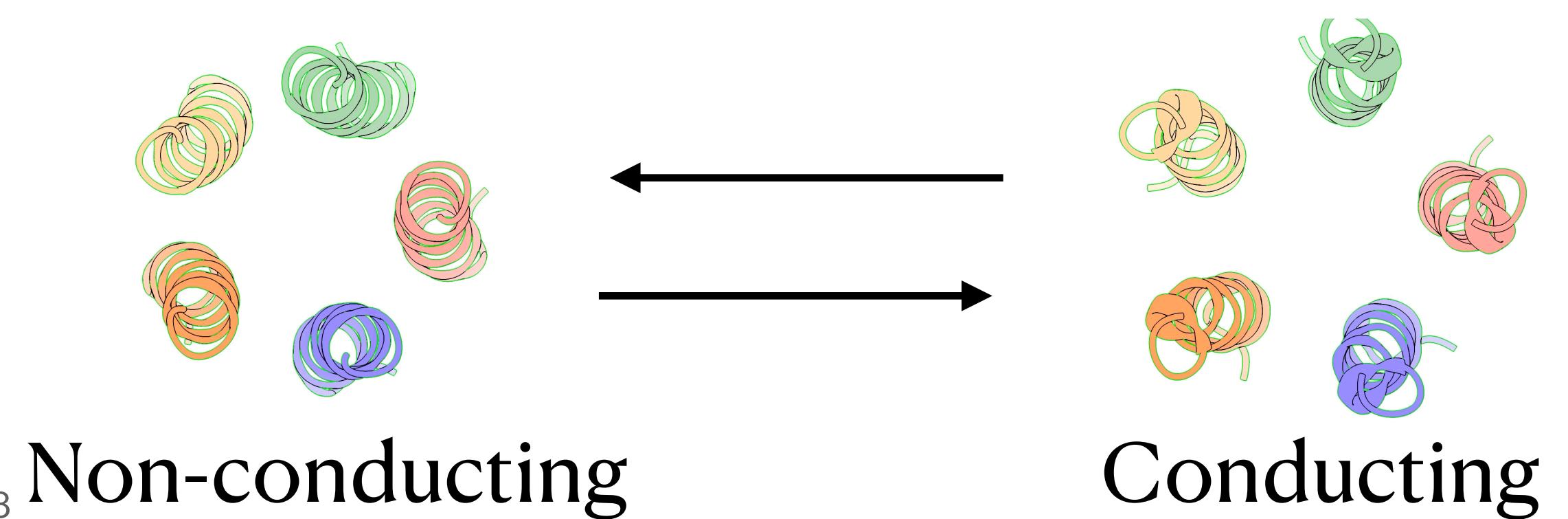
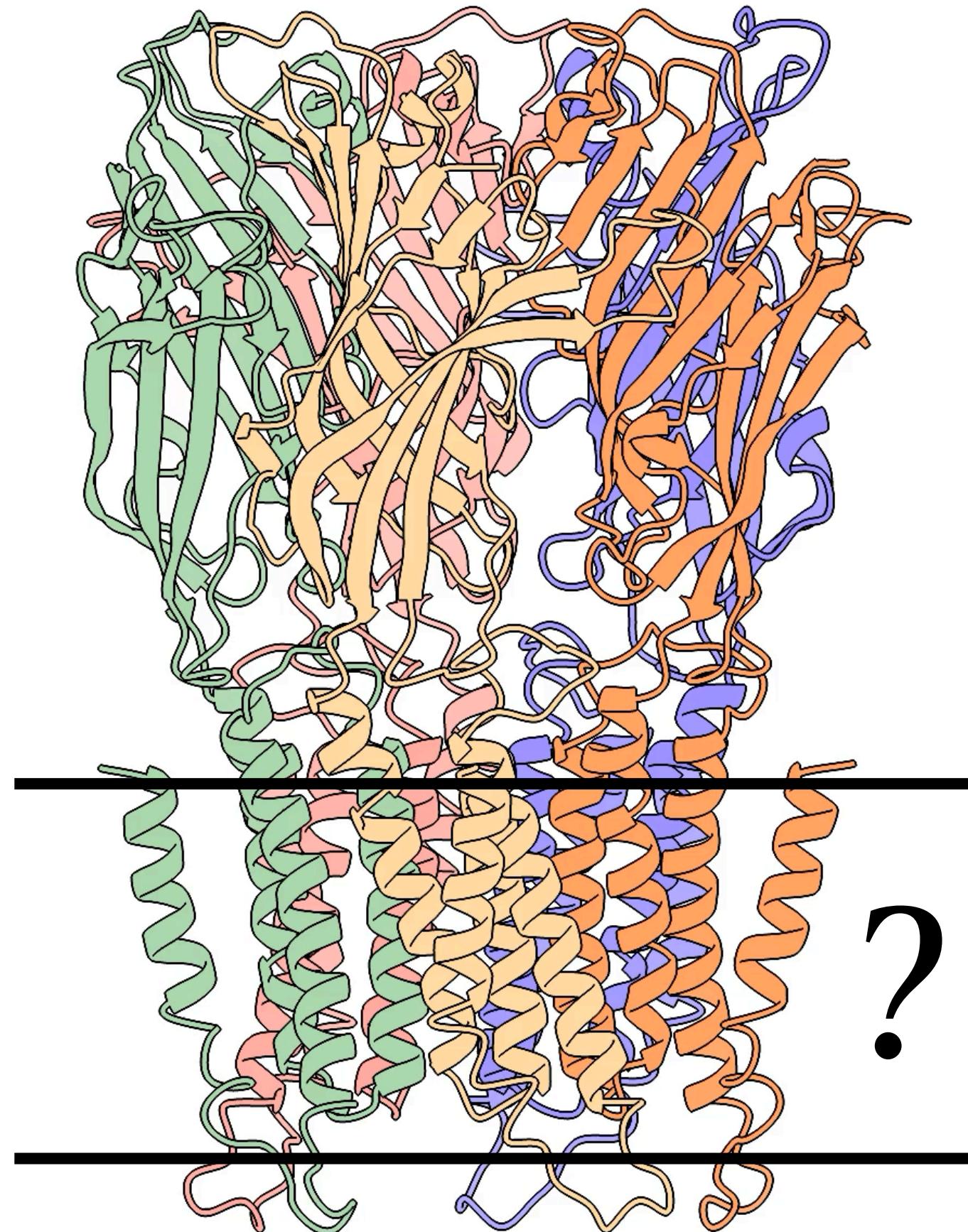


<sup>8</sup> Non-conducting

Conducting

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2. Stabilize the open conformation

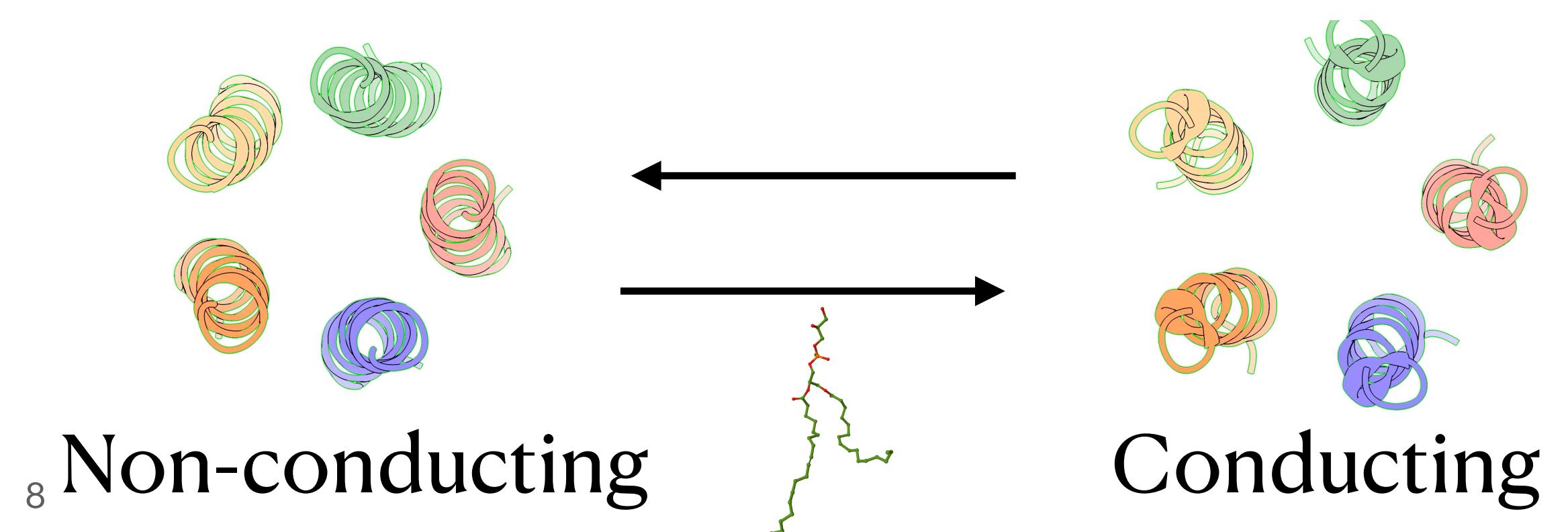
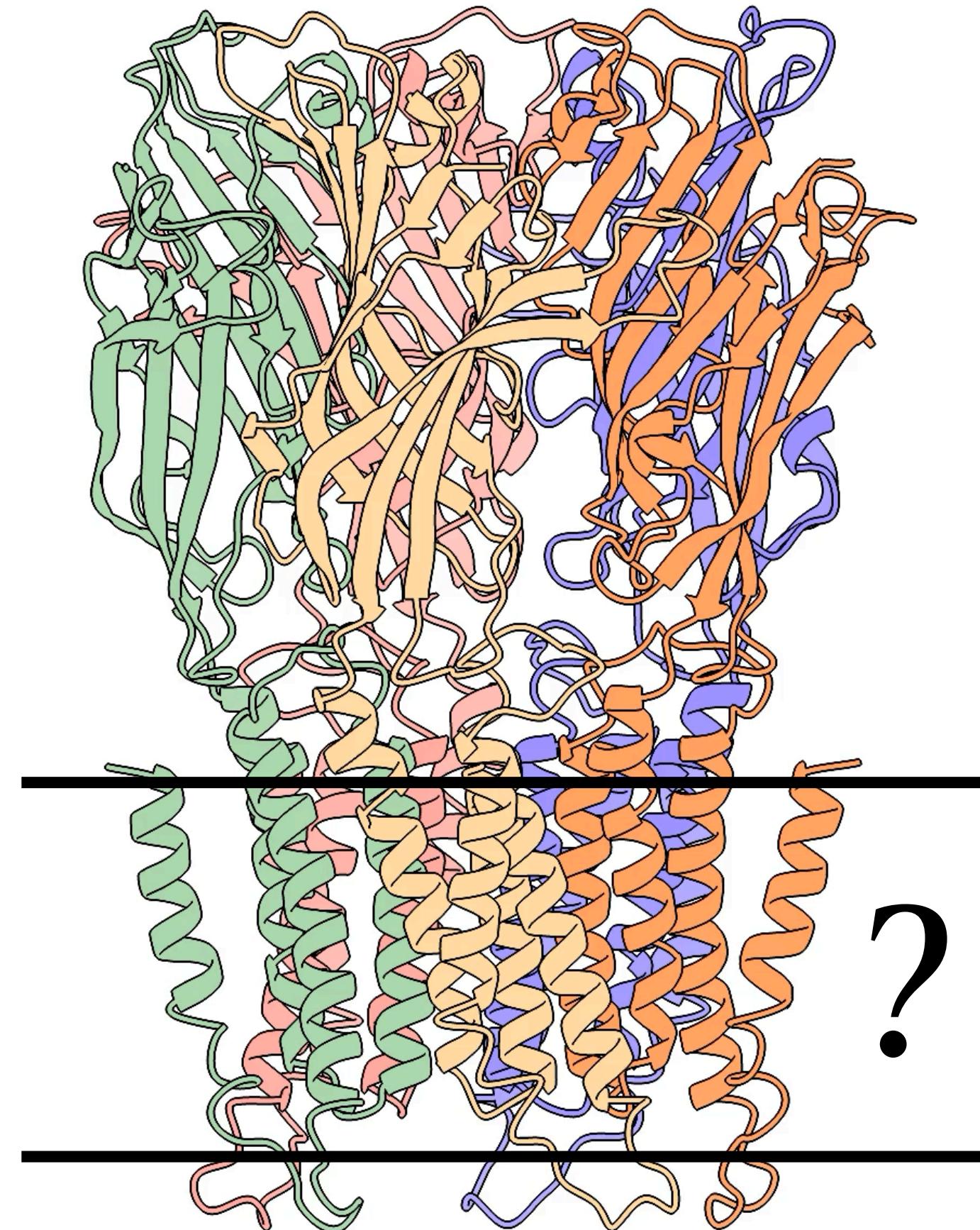


<sup>8</sup> Non-conducting

Conducting

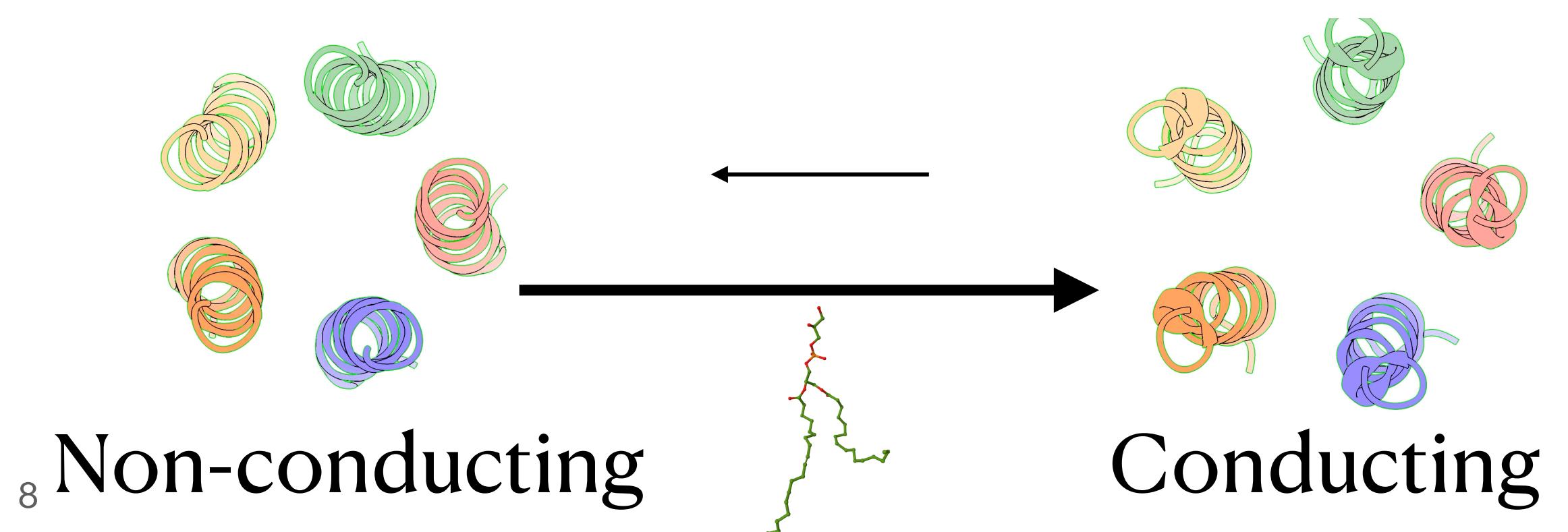
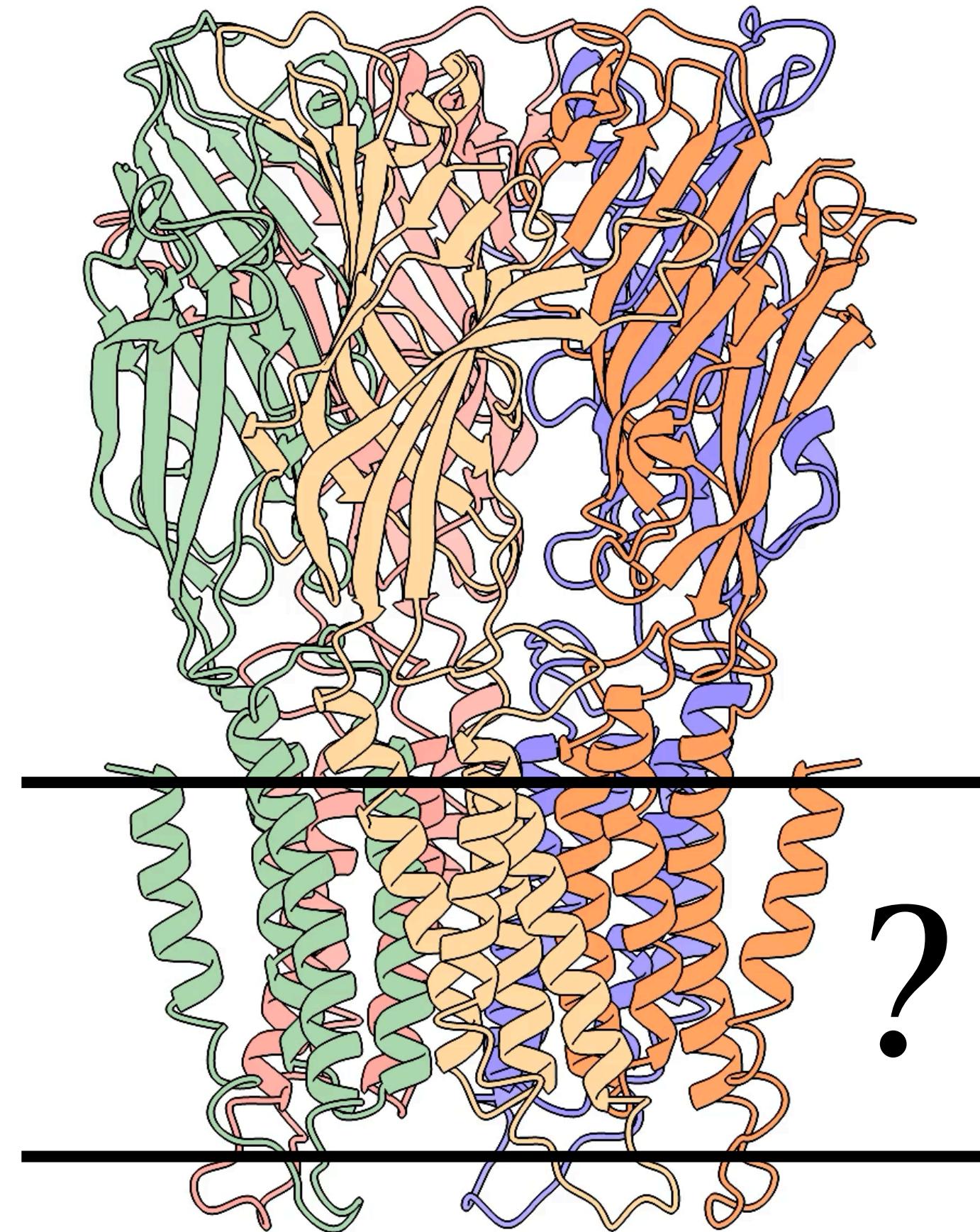
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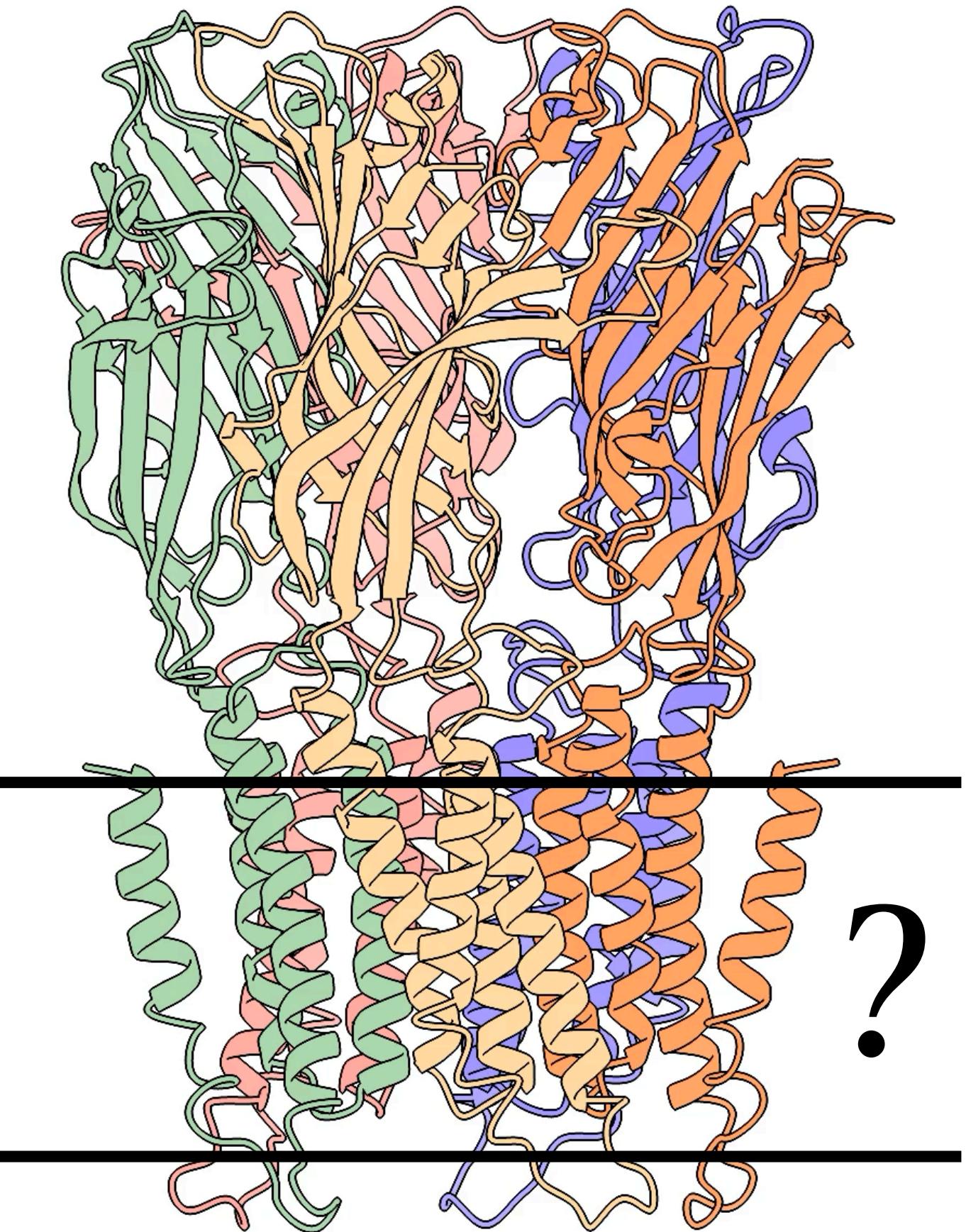
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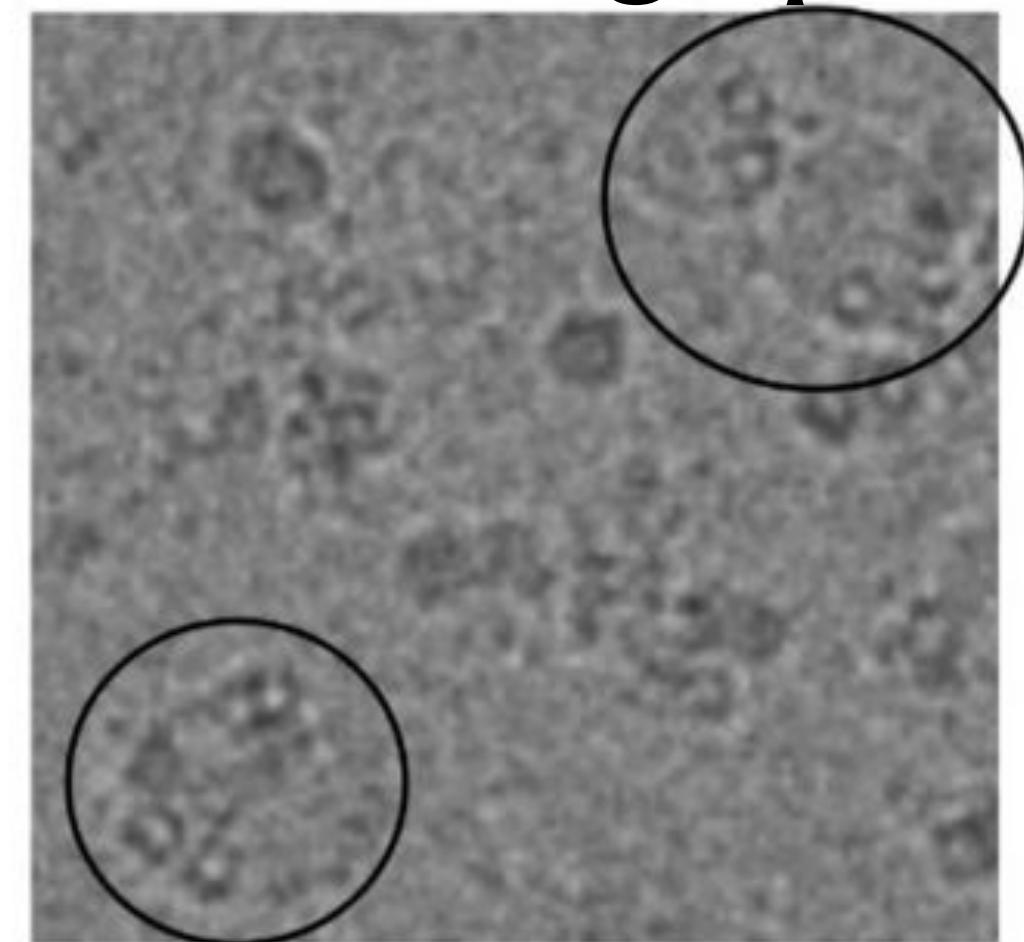
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# Cryo-EM

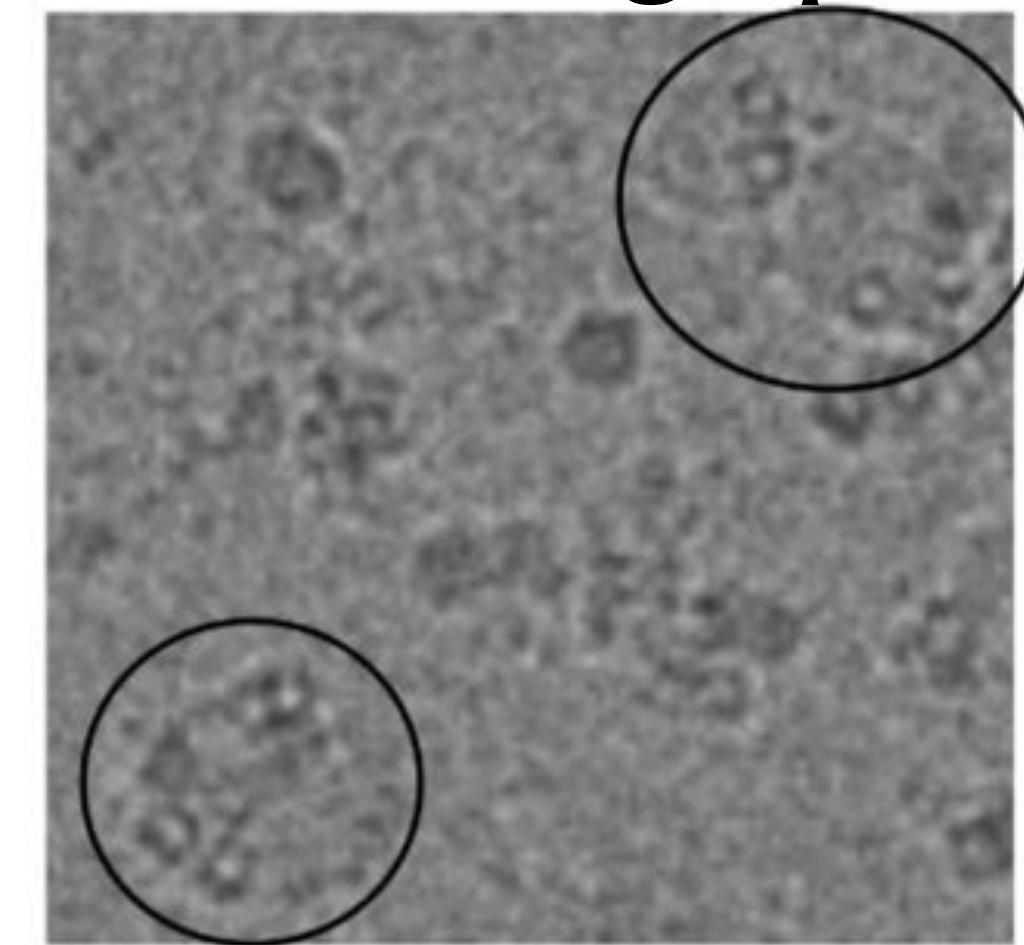
2D Micrograph



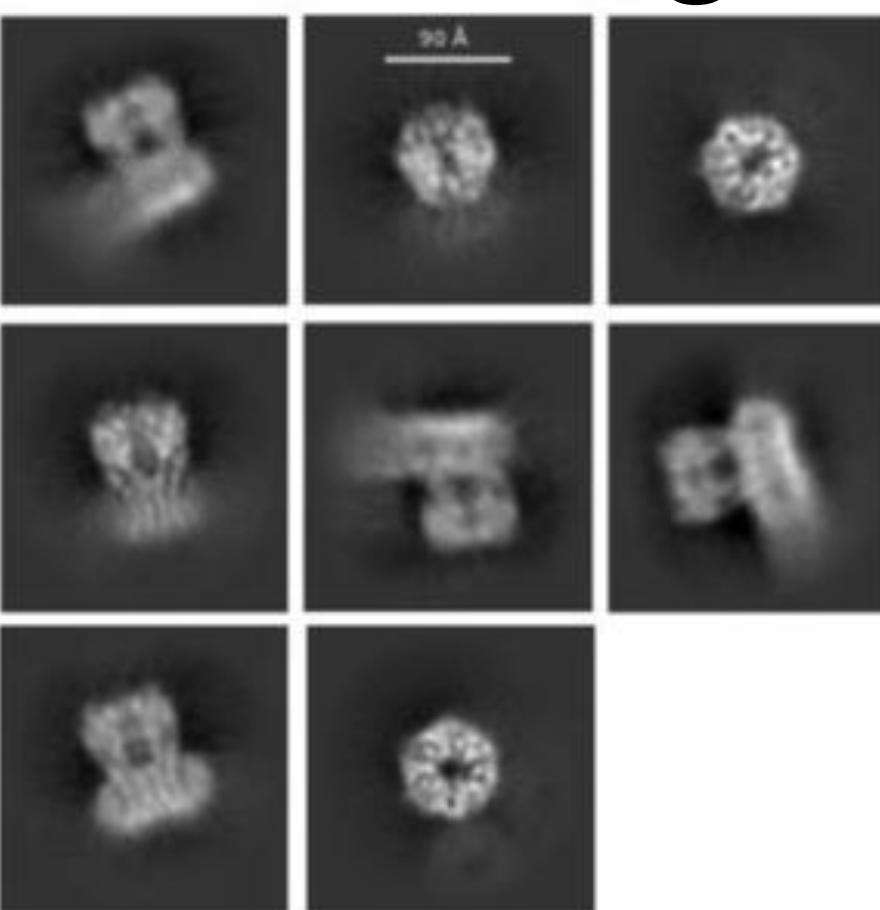
Dalal, Tan, Xu, & Wayland Cheng

# Cryo-EM

2D Micrograph



Class Averages

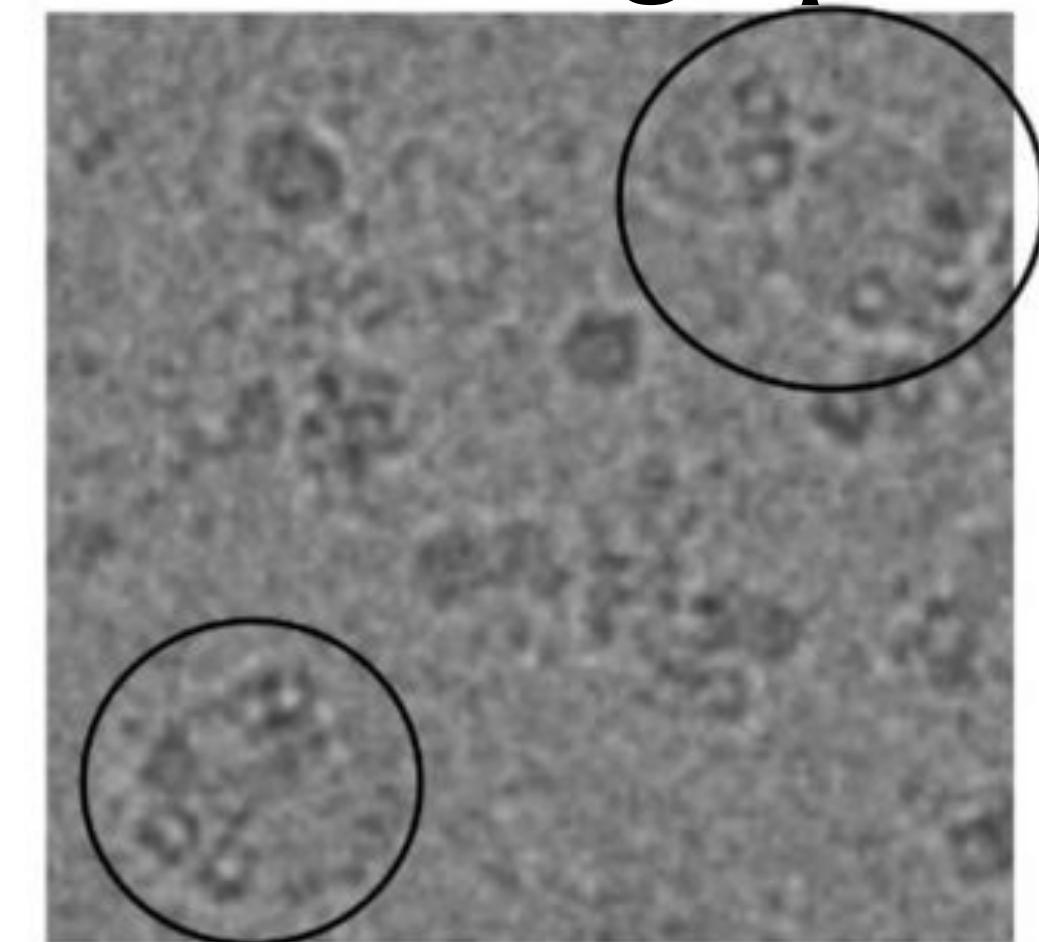


Dalal, Tan, Xu, & Wayland Cheng

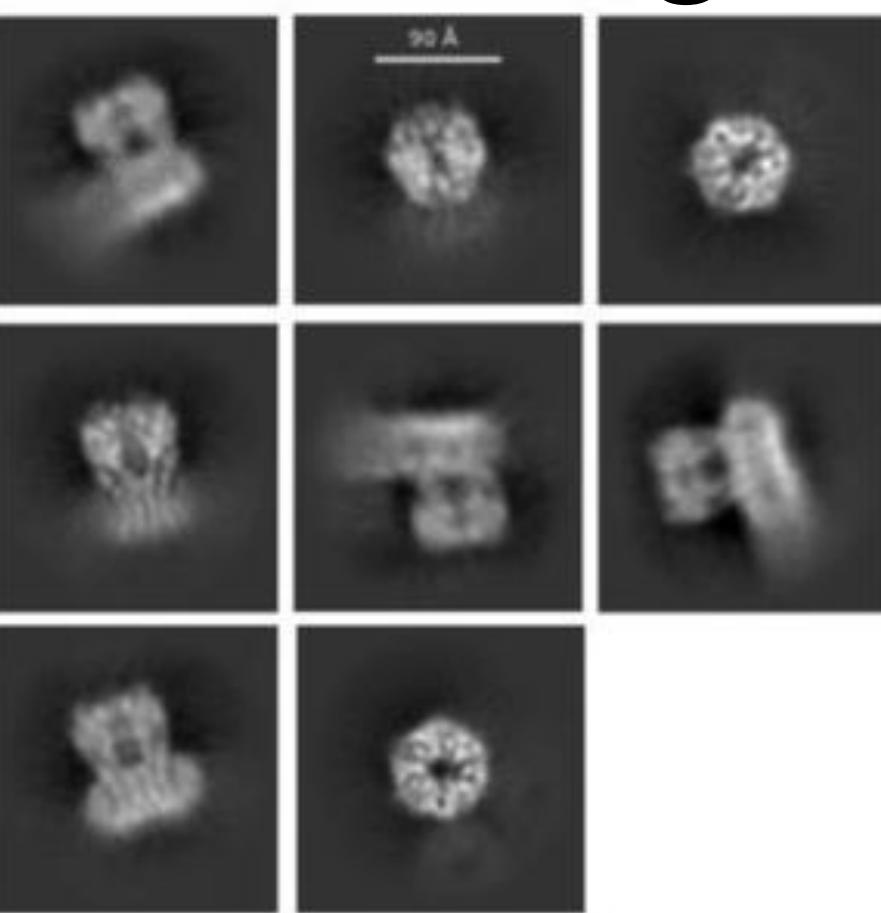
Dalal, Tan, Xu, & Wayland Cheng

# Cryo-EM

2D Micrograph

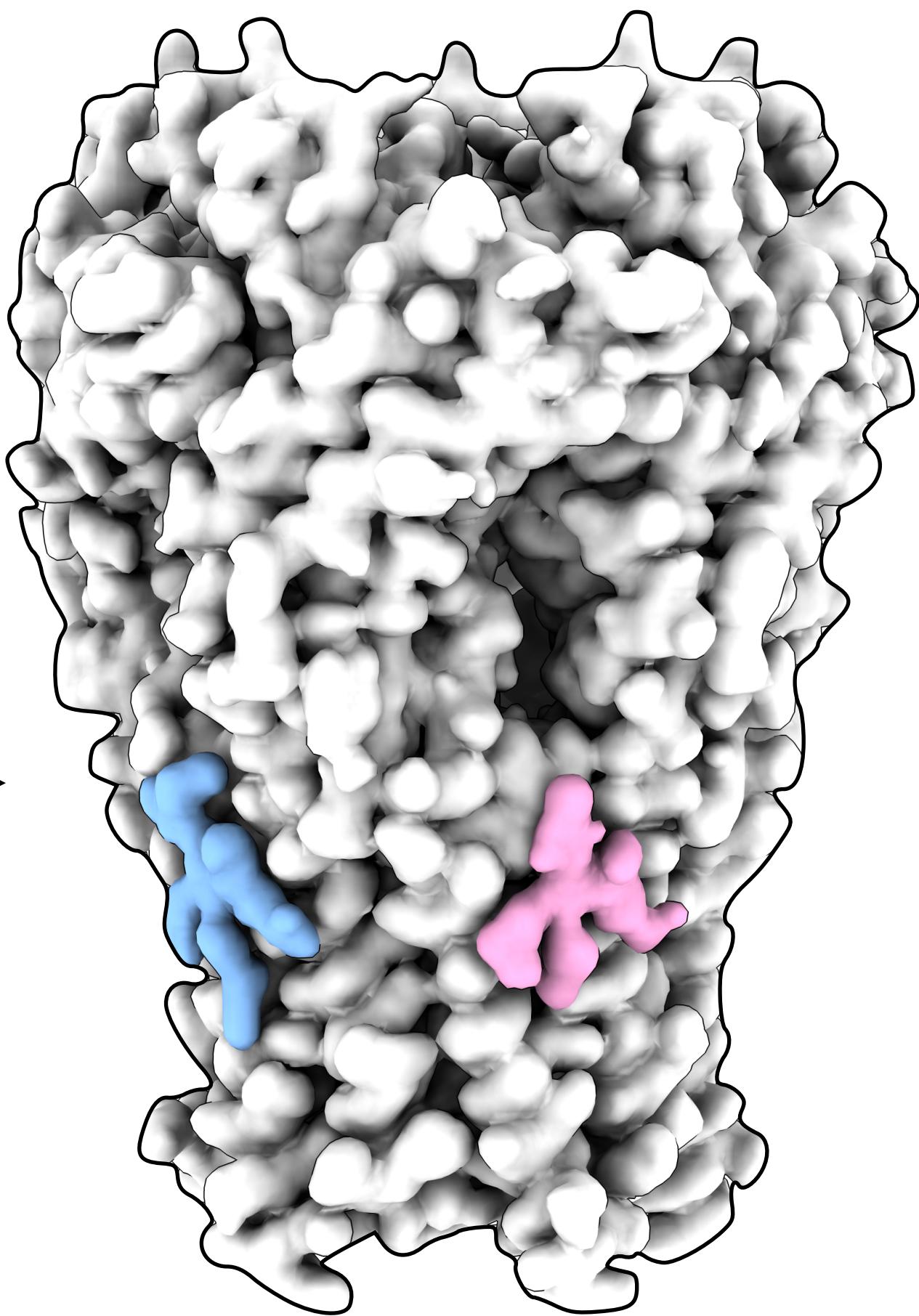


Class Averages



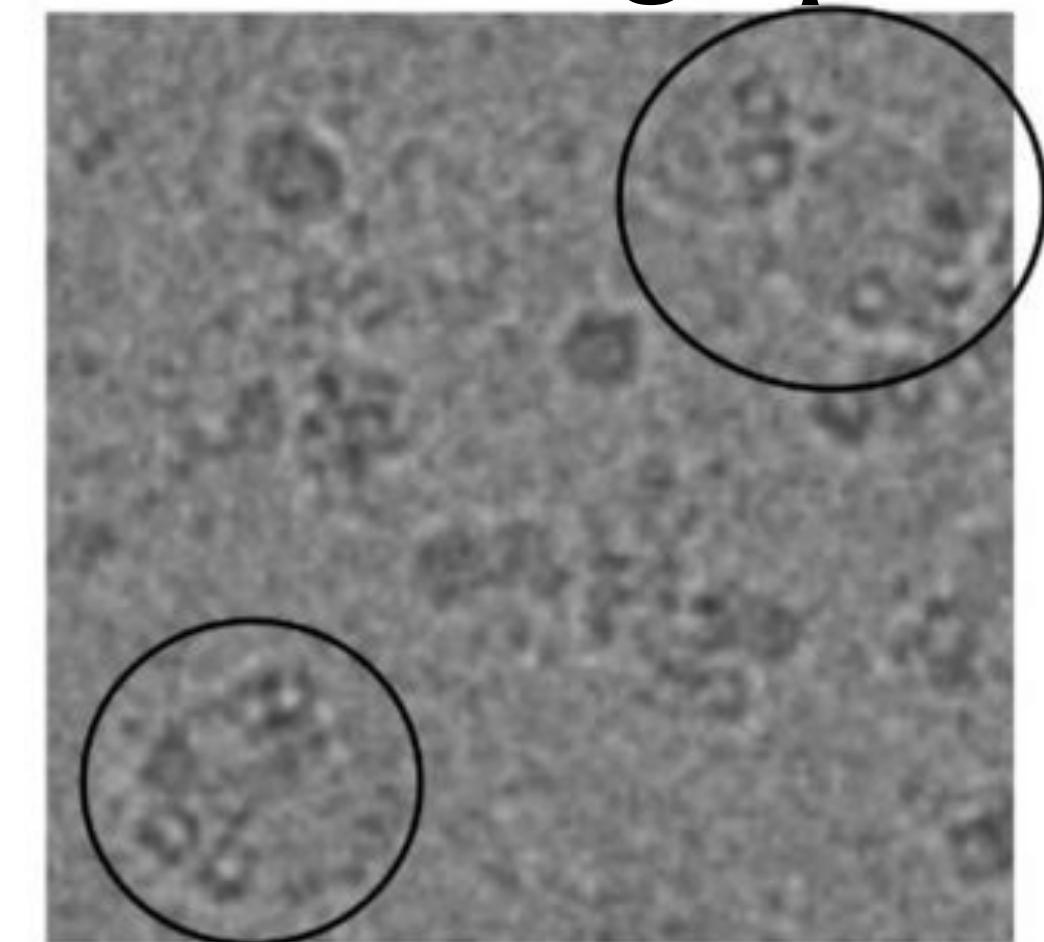
Dalal, Tan, Xu, & Wayland Cheng

Dalal, Tan, Xu, & Wayland Cheng

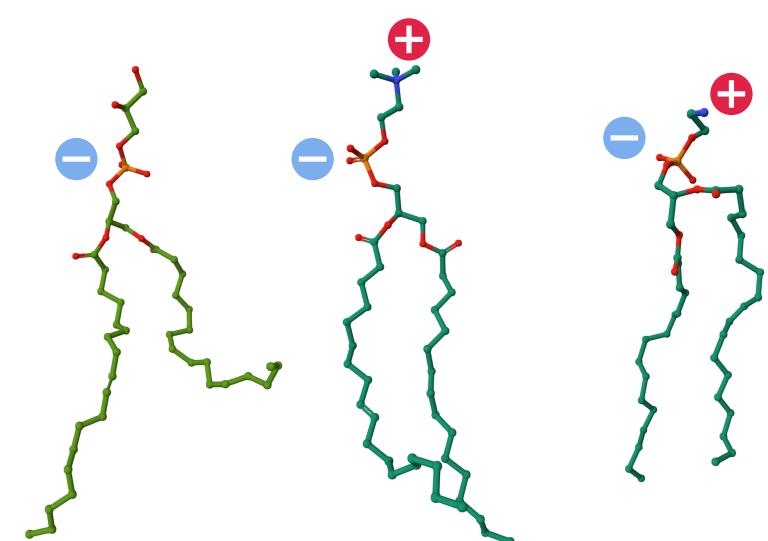
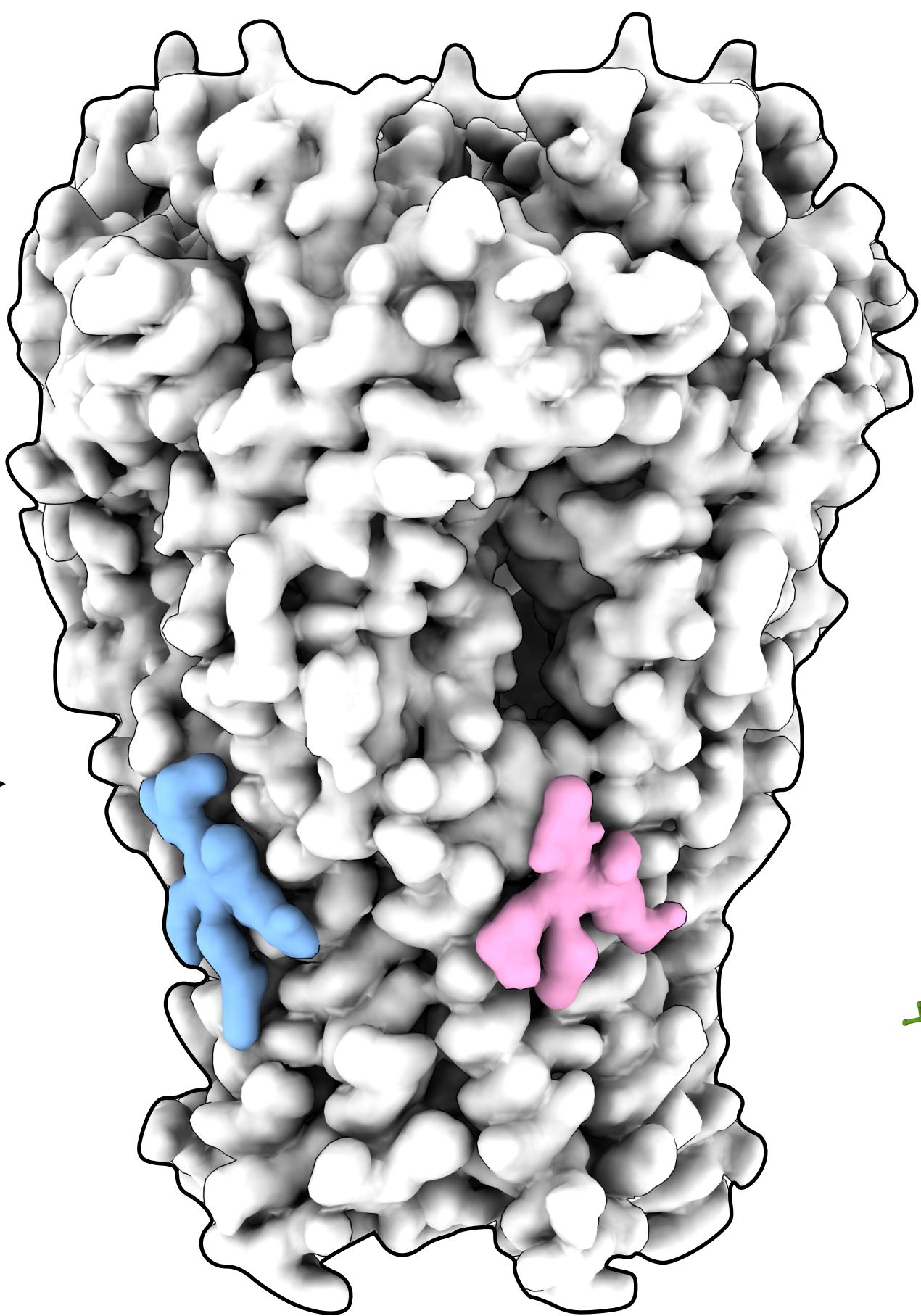
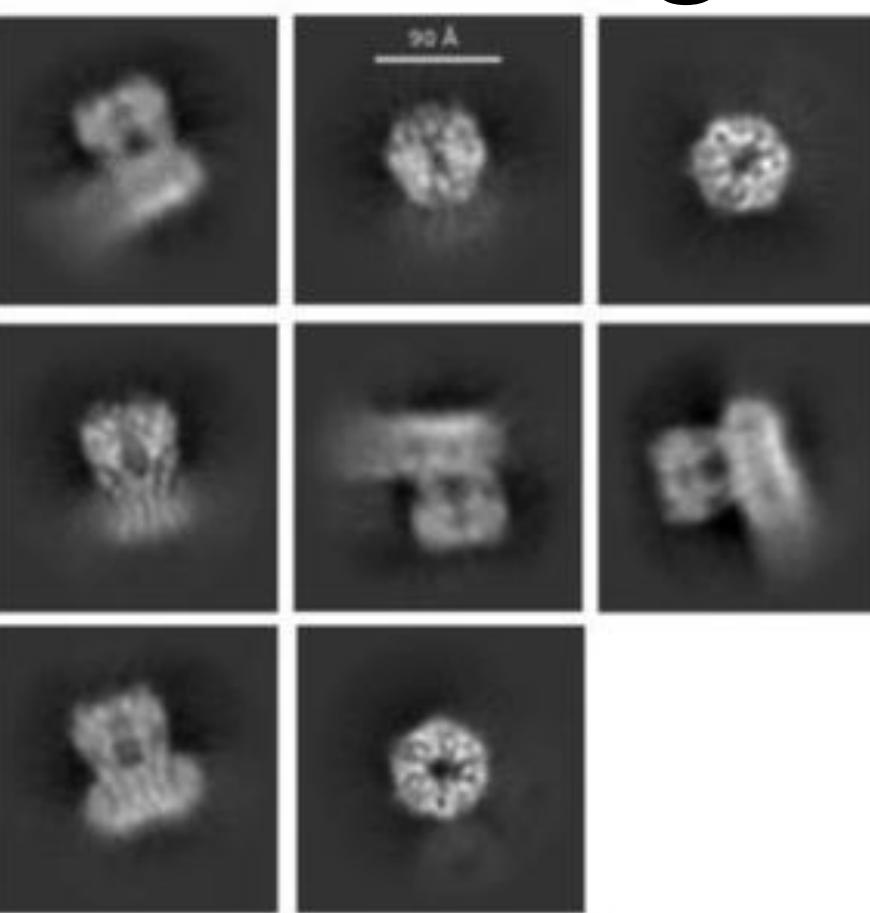


# Cryo-EM

2D Micrograph



Class Averages

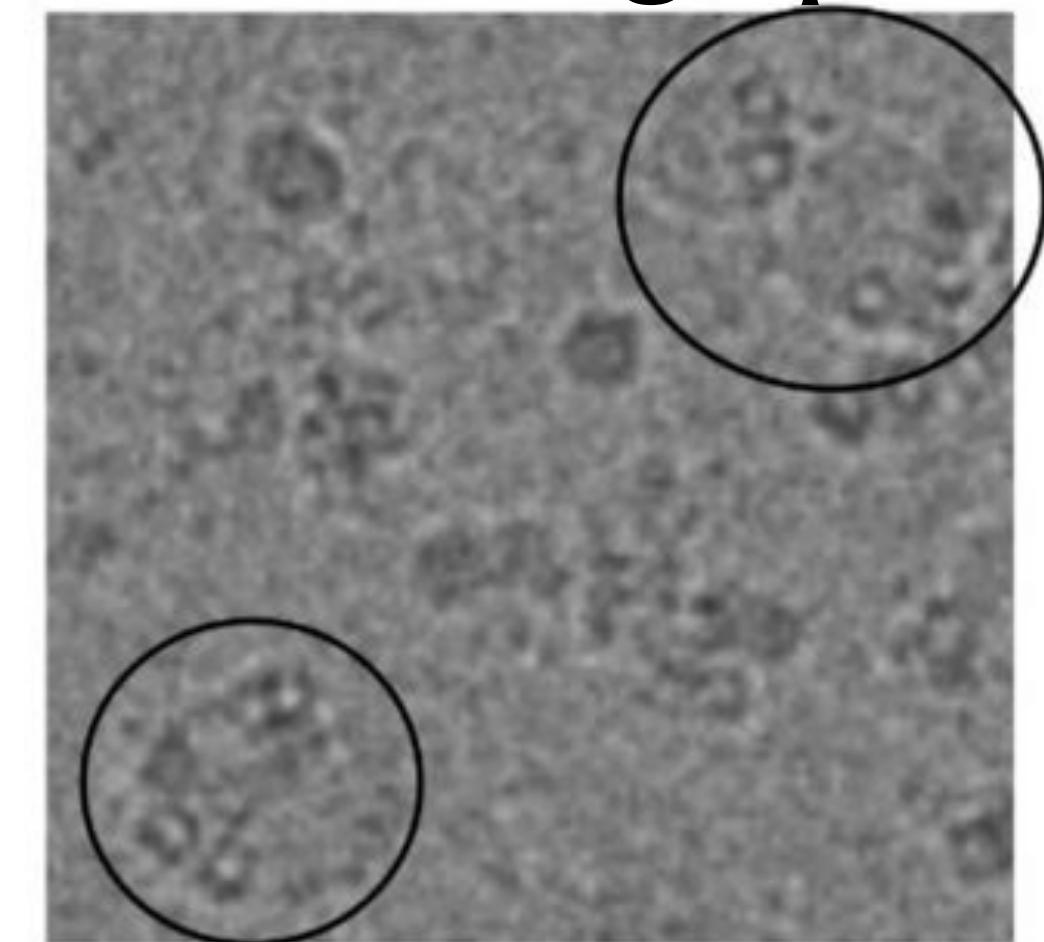


Dalal, Tan, Xu, & Wayland Cheng

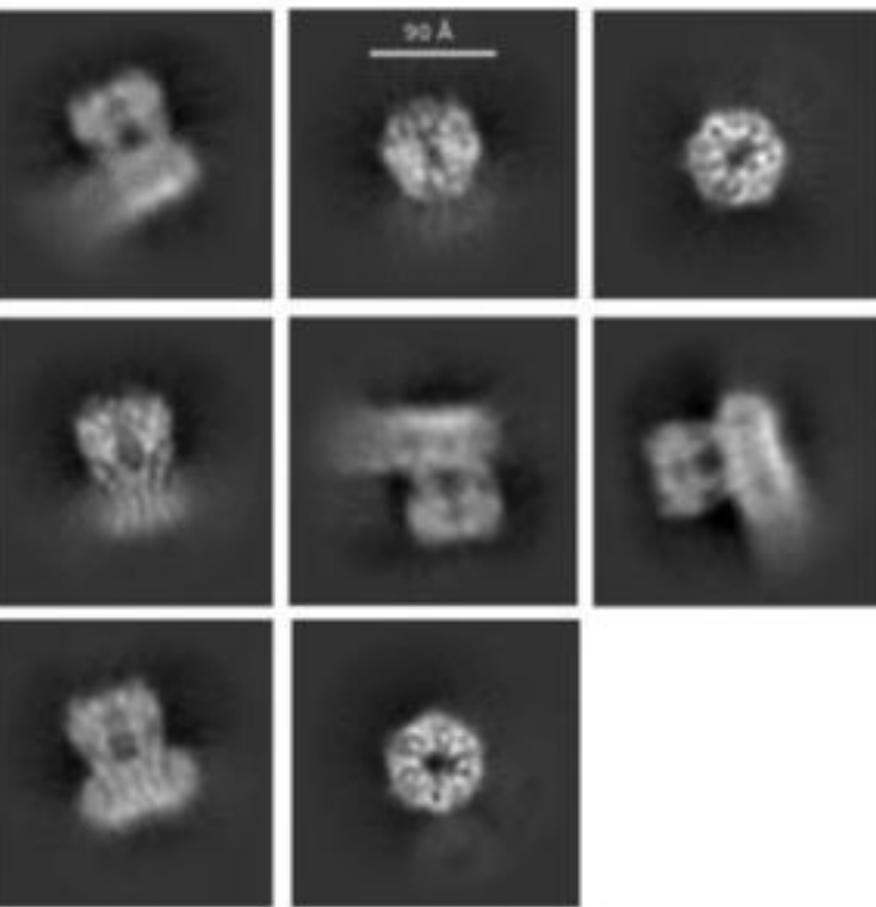
Dalal, Tan, Xu, & Wayland Cheng

# Cryo-EM

2D Micrograph

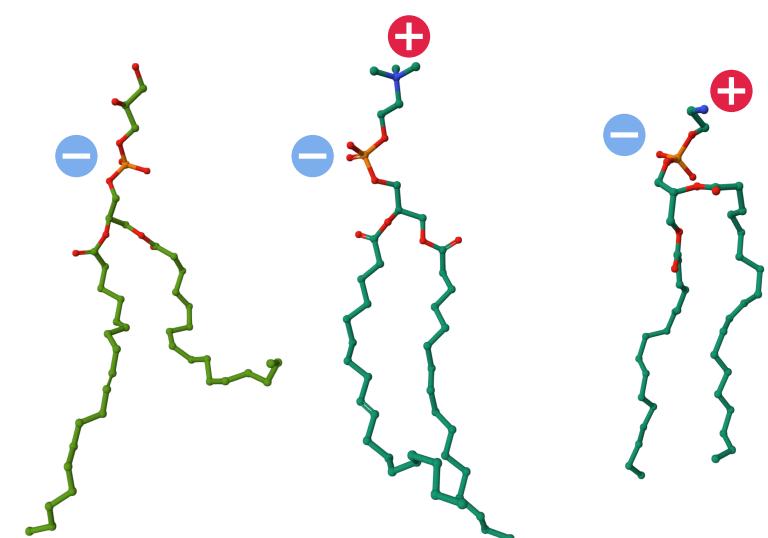
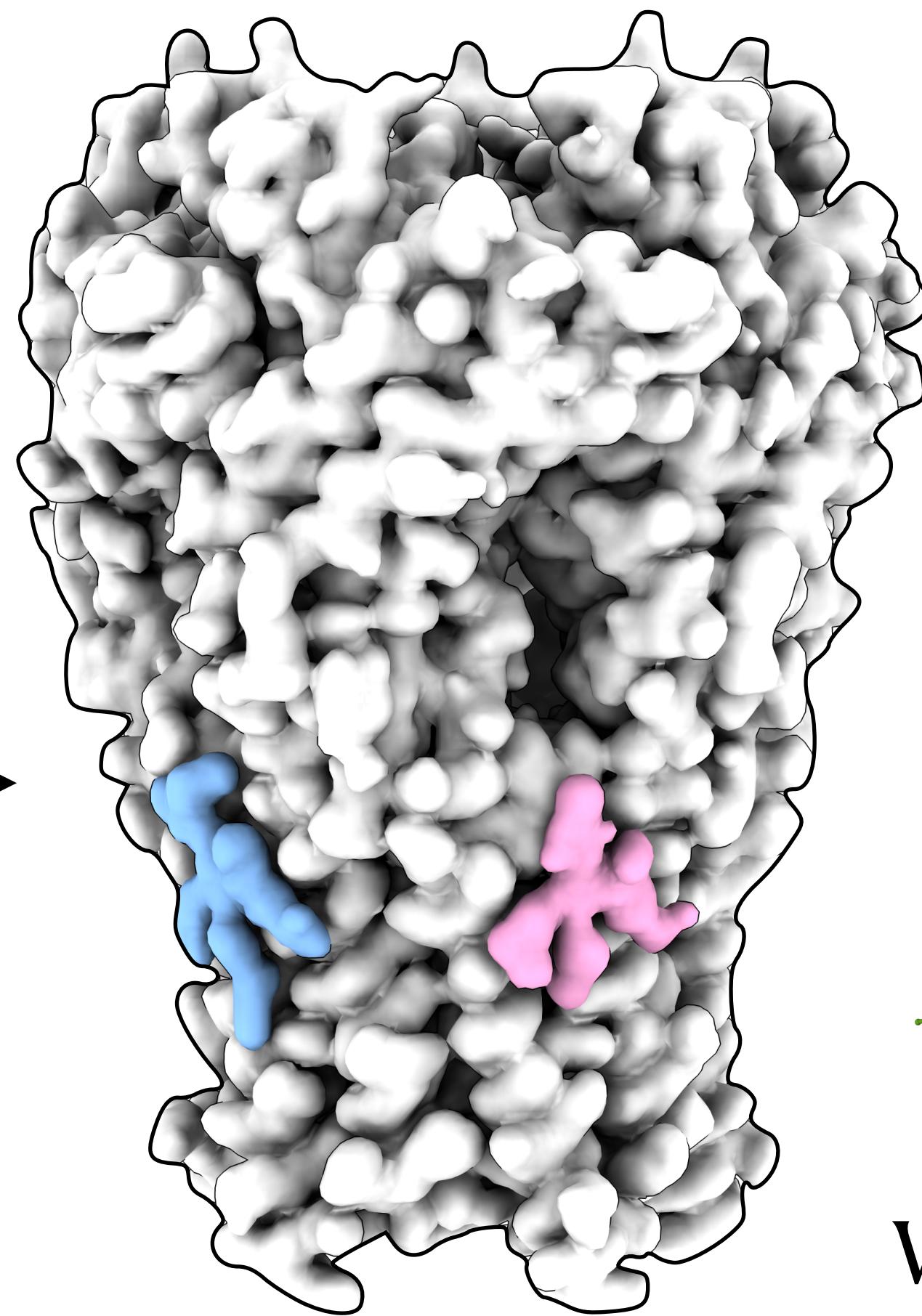


Class Averages



Dalal, Tan, Xu, & Wayland Cheng

Dalal, Tan, Xu, & Wayland Cheng

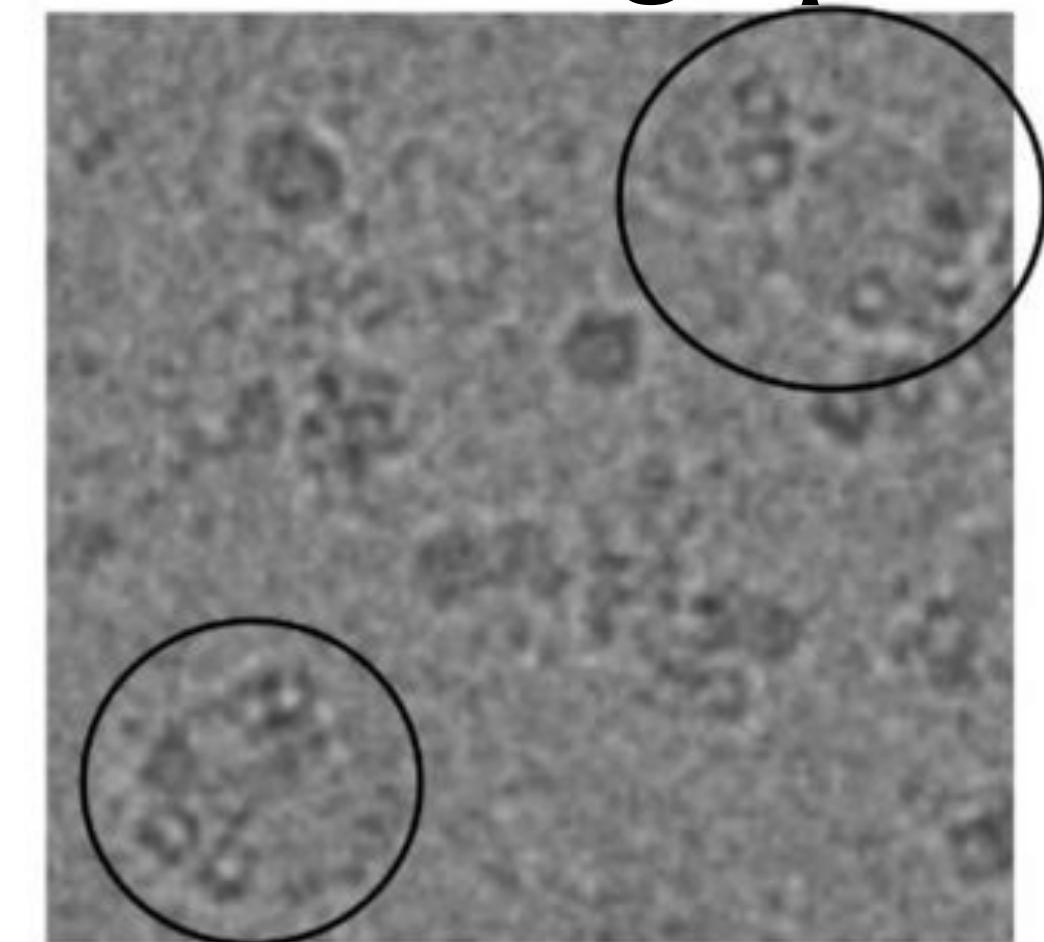


Which one?

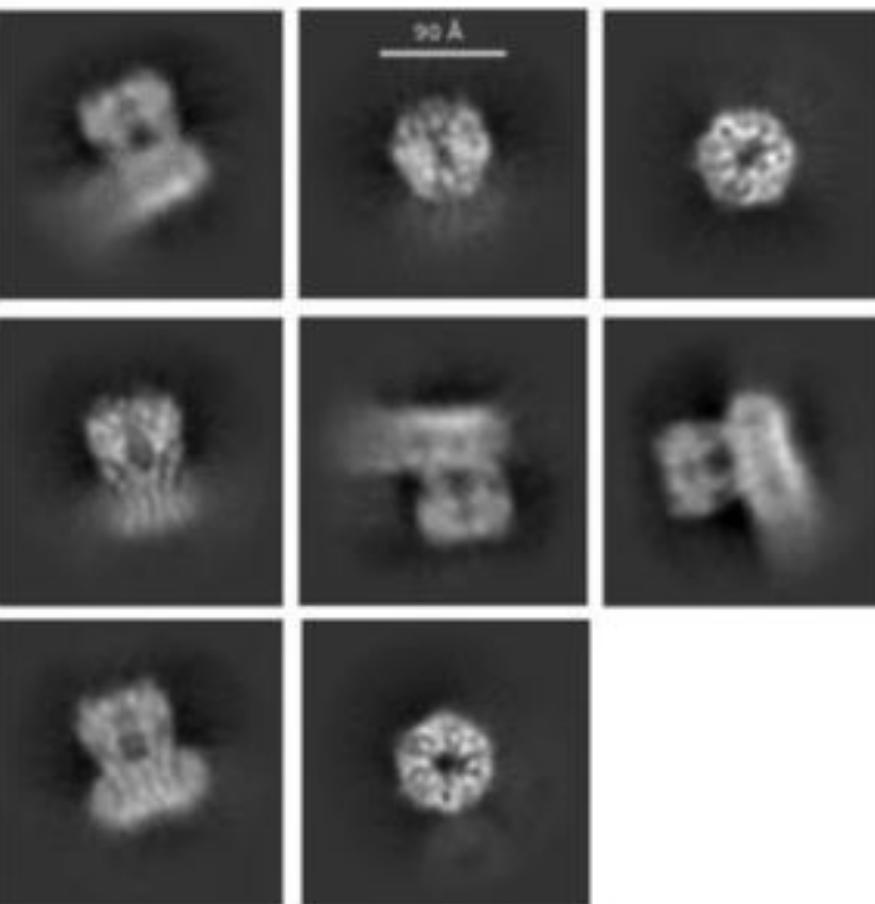
Why three tails?

# Cryo-EM

2D Micrograph

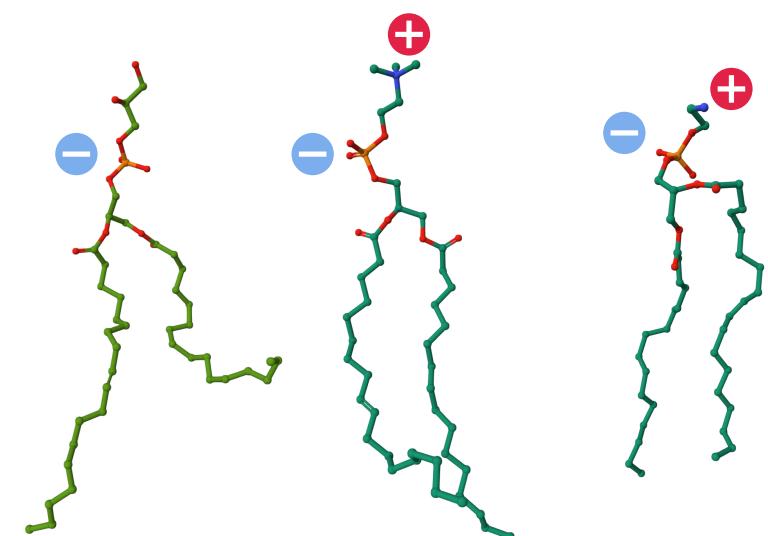
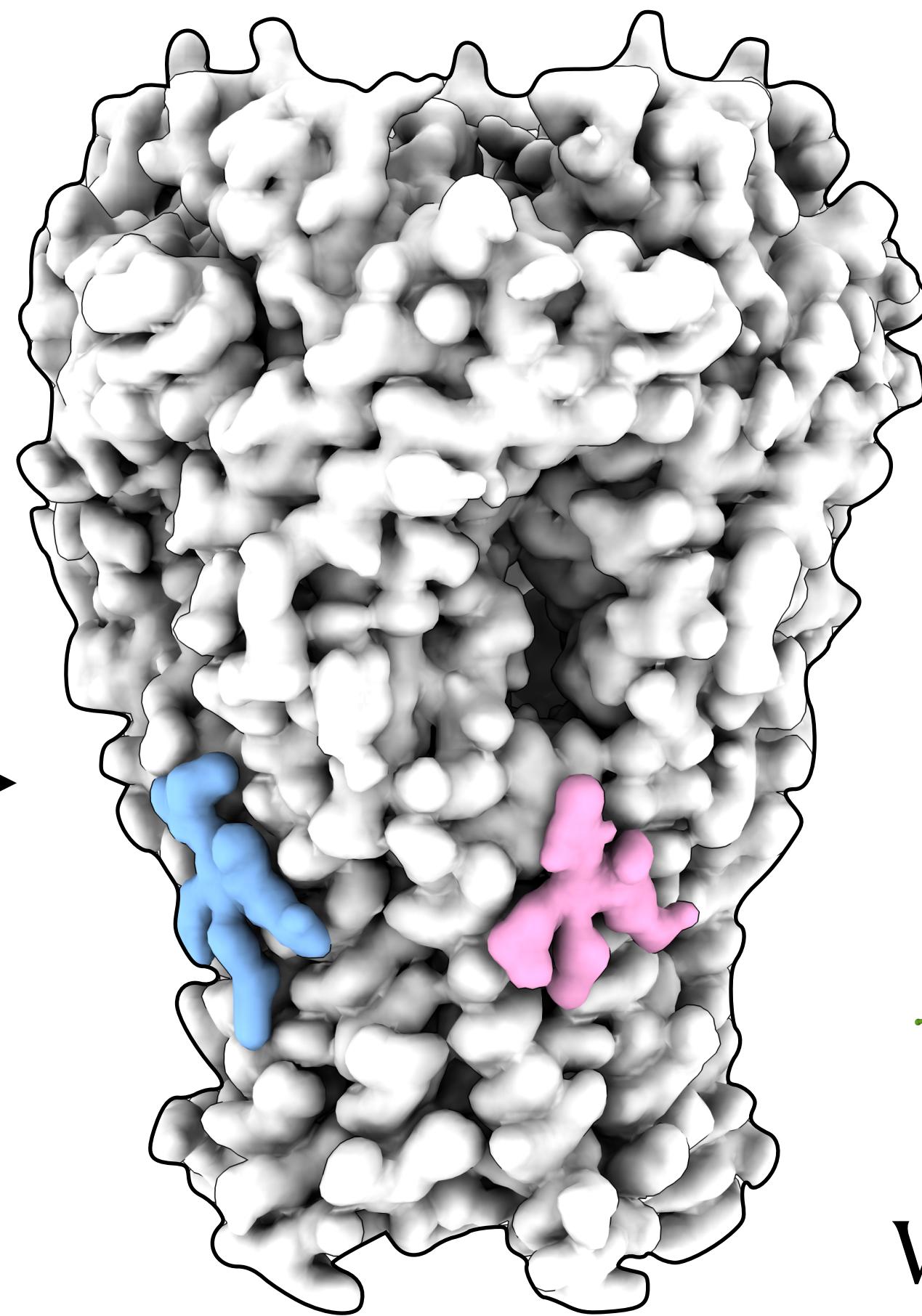


Class Averages



Dalal, Tan, Xu, & Wayland Cheng

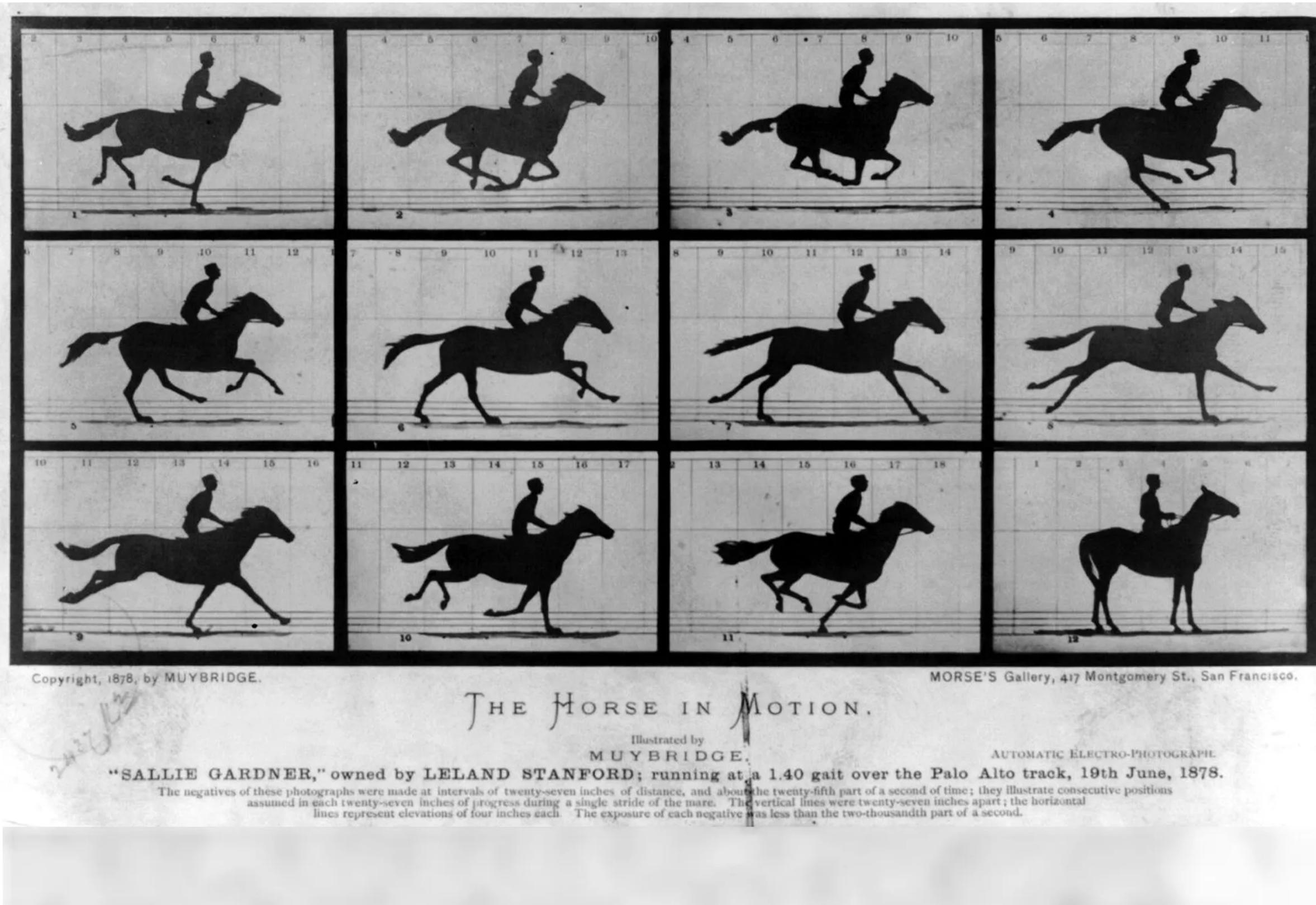
Dalal, Tan, Xu, & Wayland Cheng



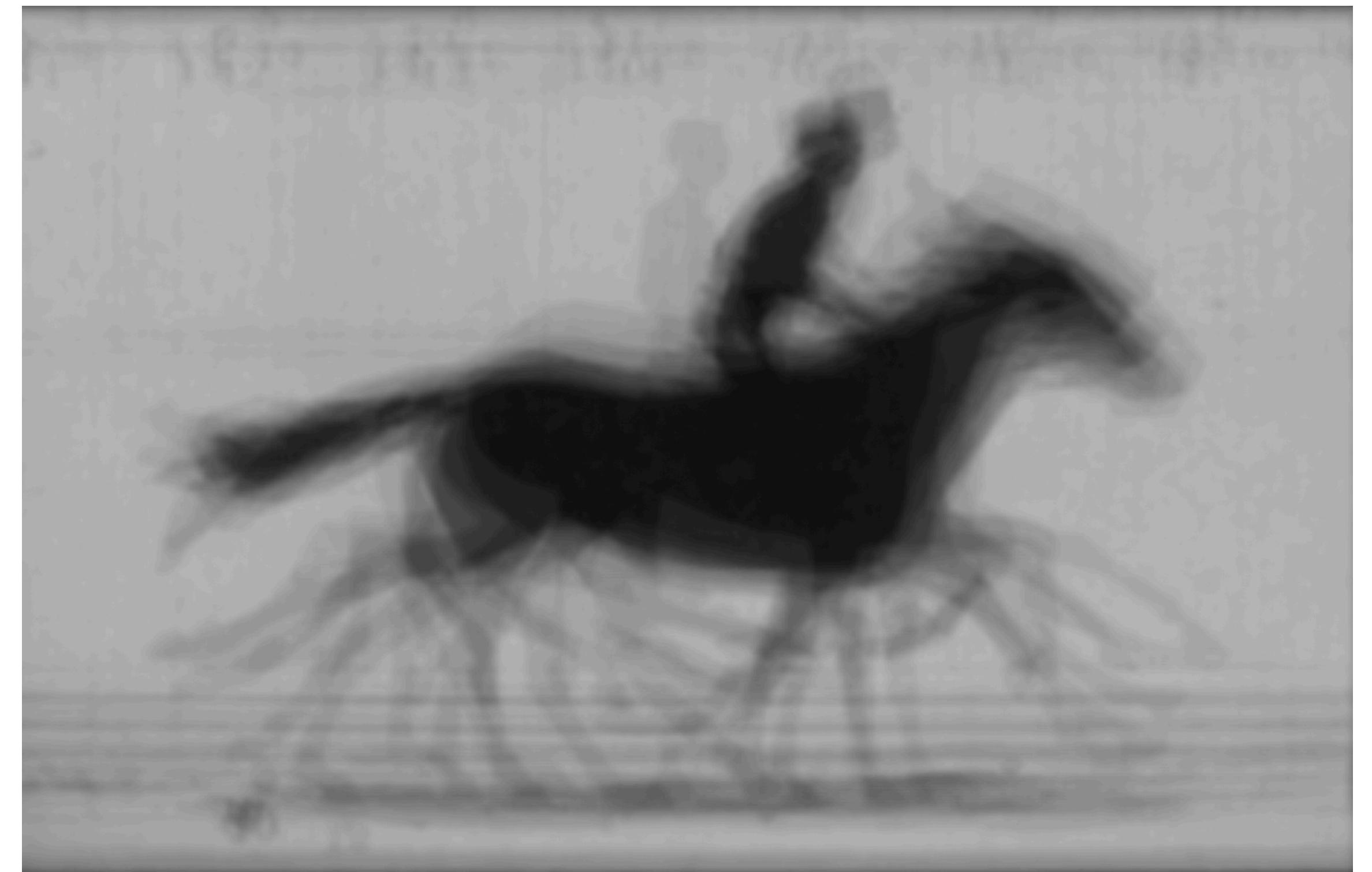
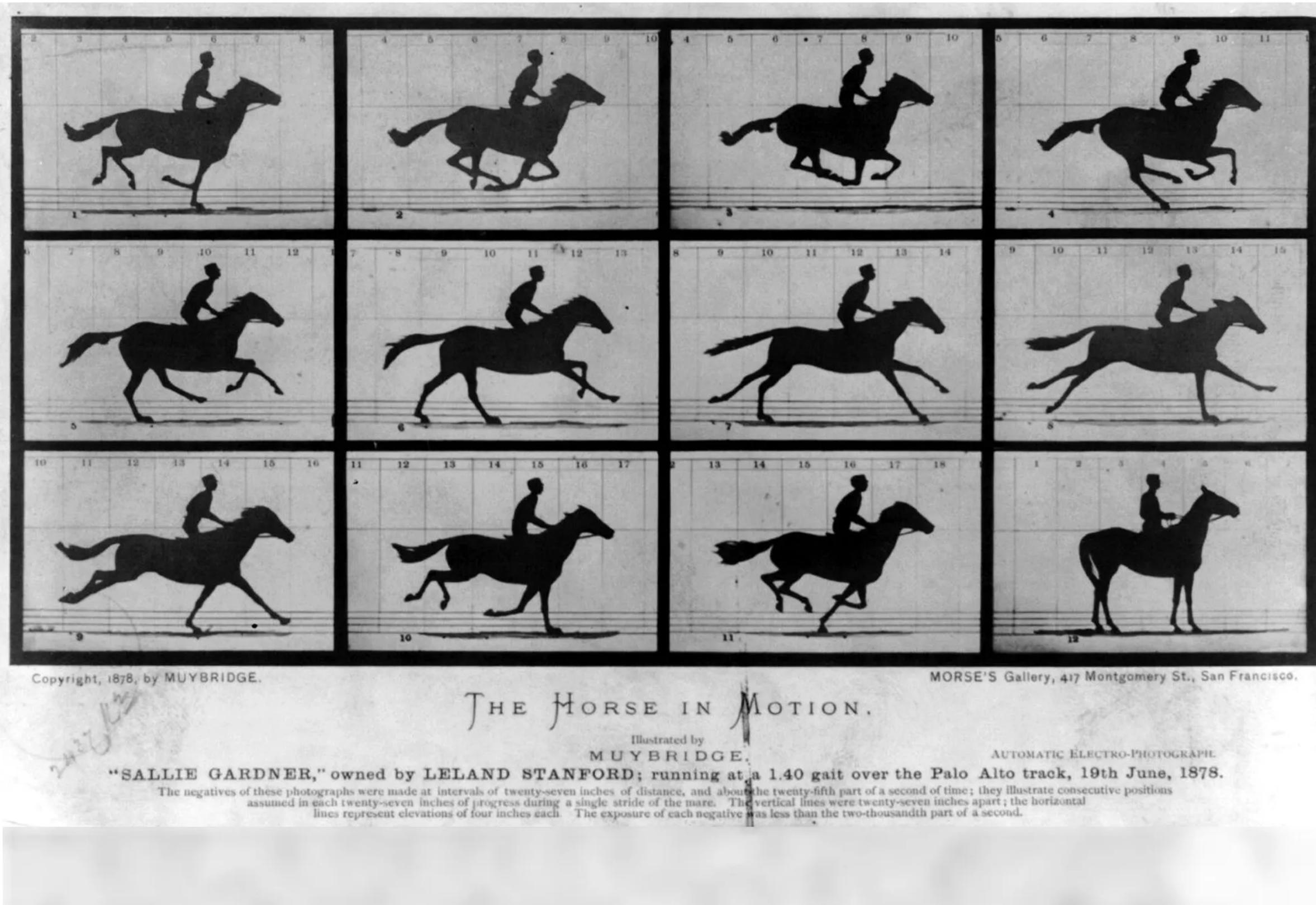
Which one?

Why three tails?

# Aside: The Problem with Averaging

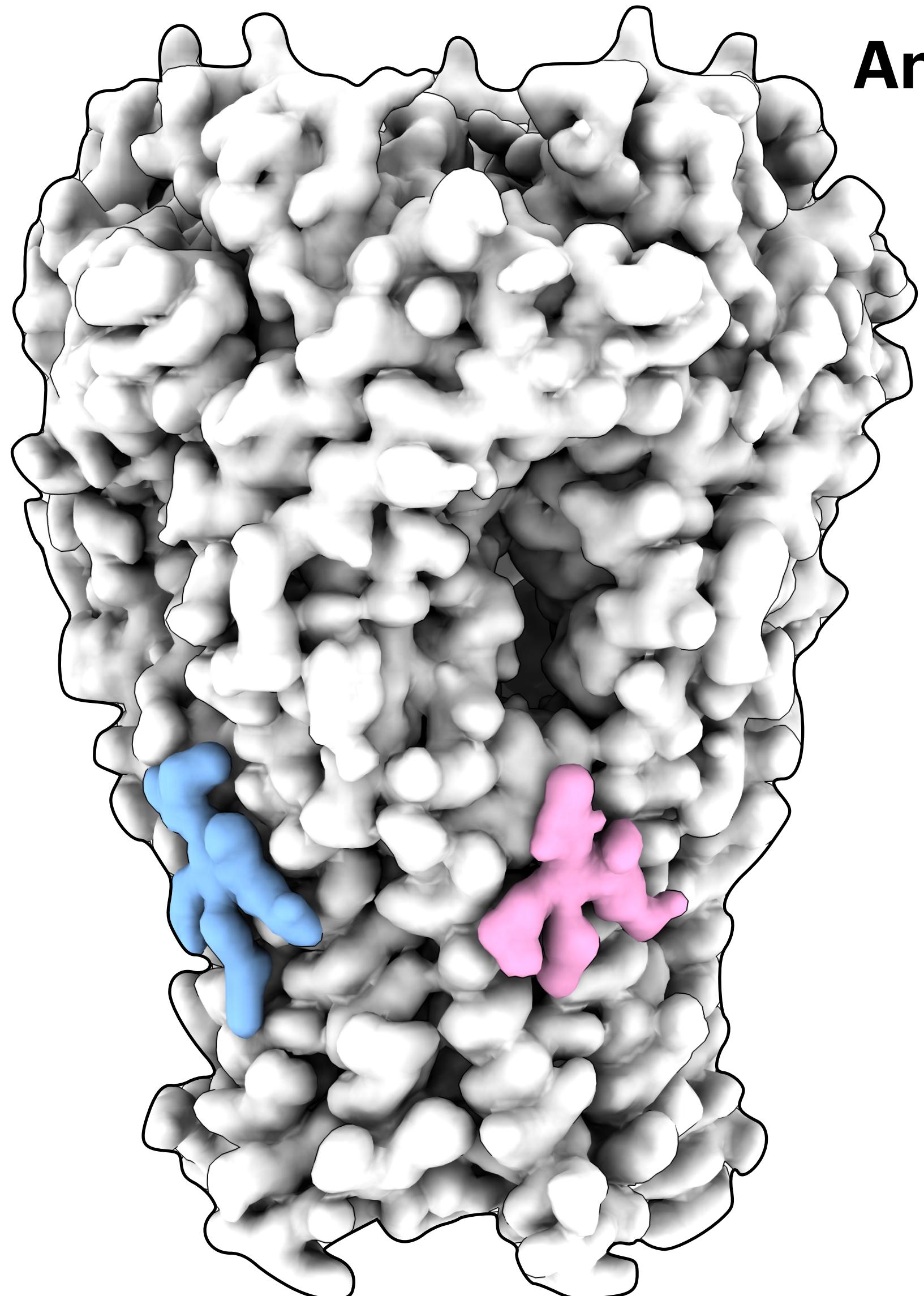


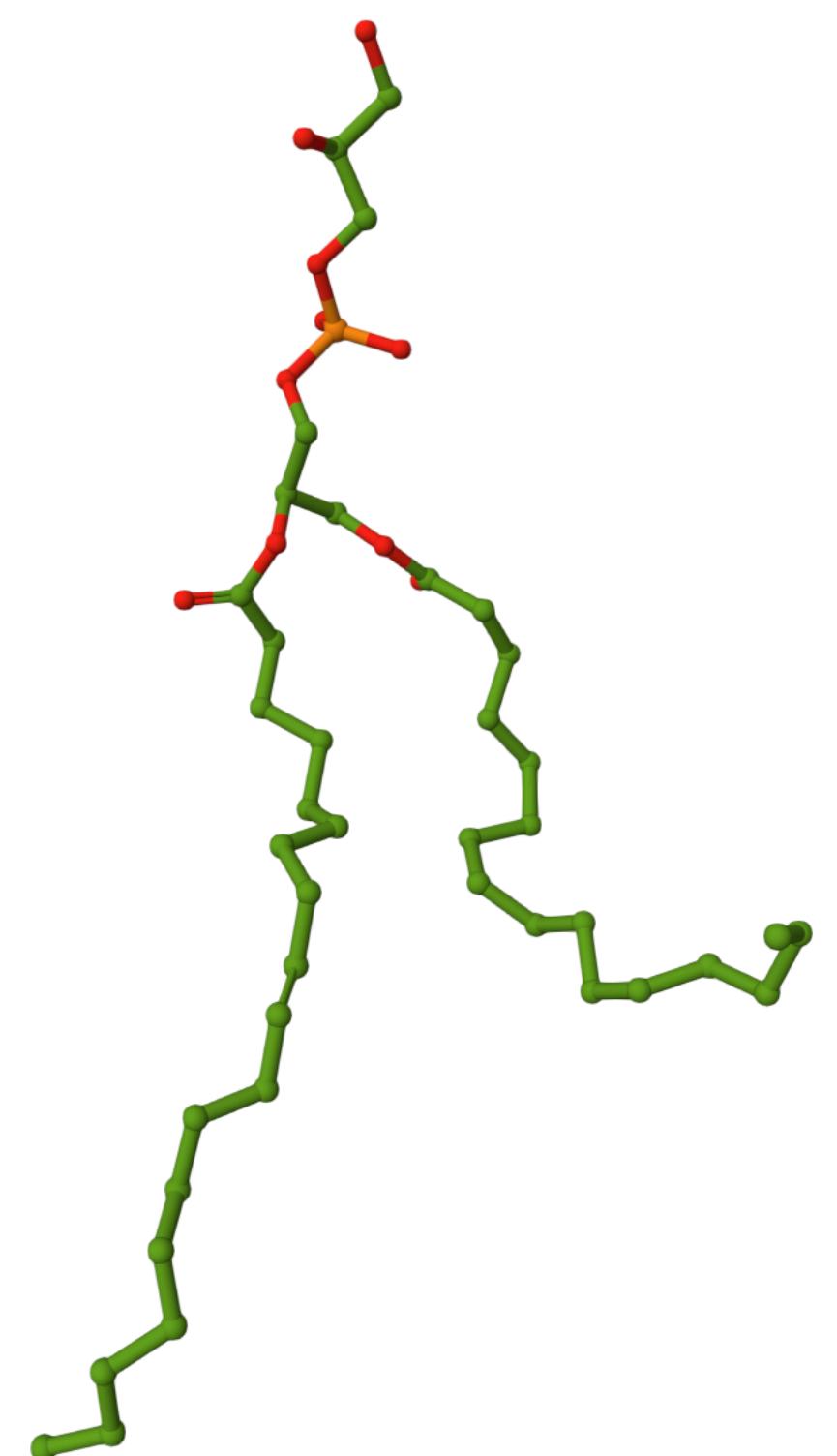
# Aside: The Problem with Averaging



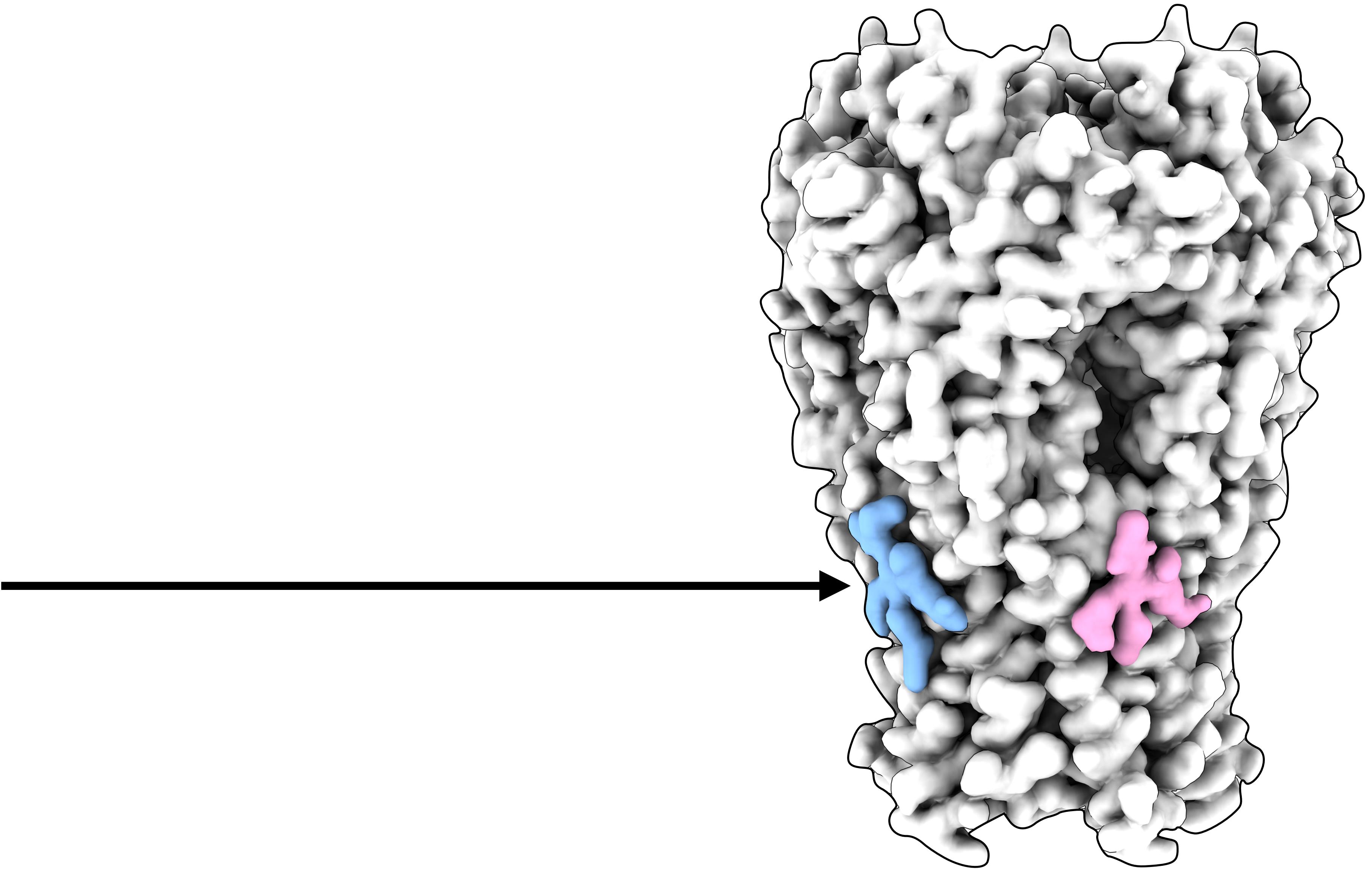
# More Legs than Expected

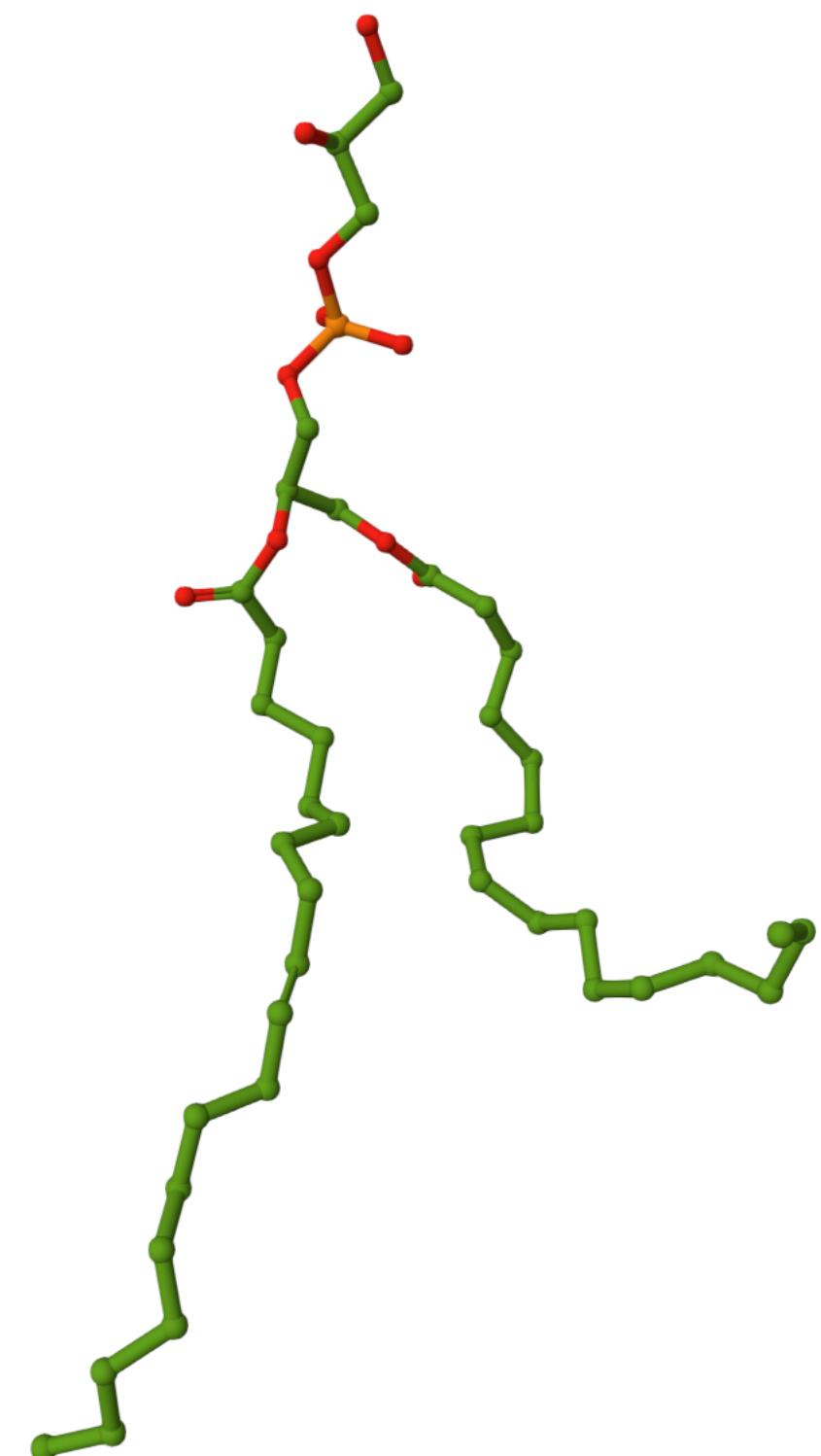
And loss of resolution





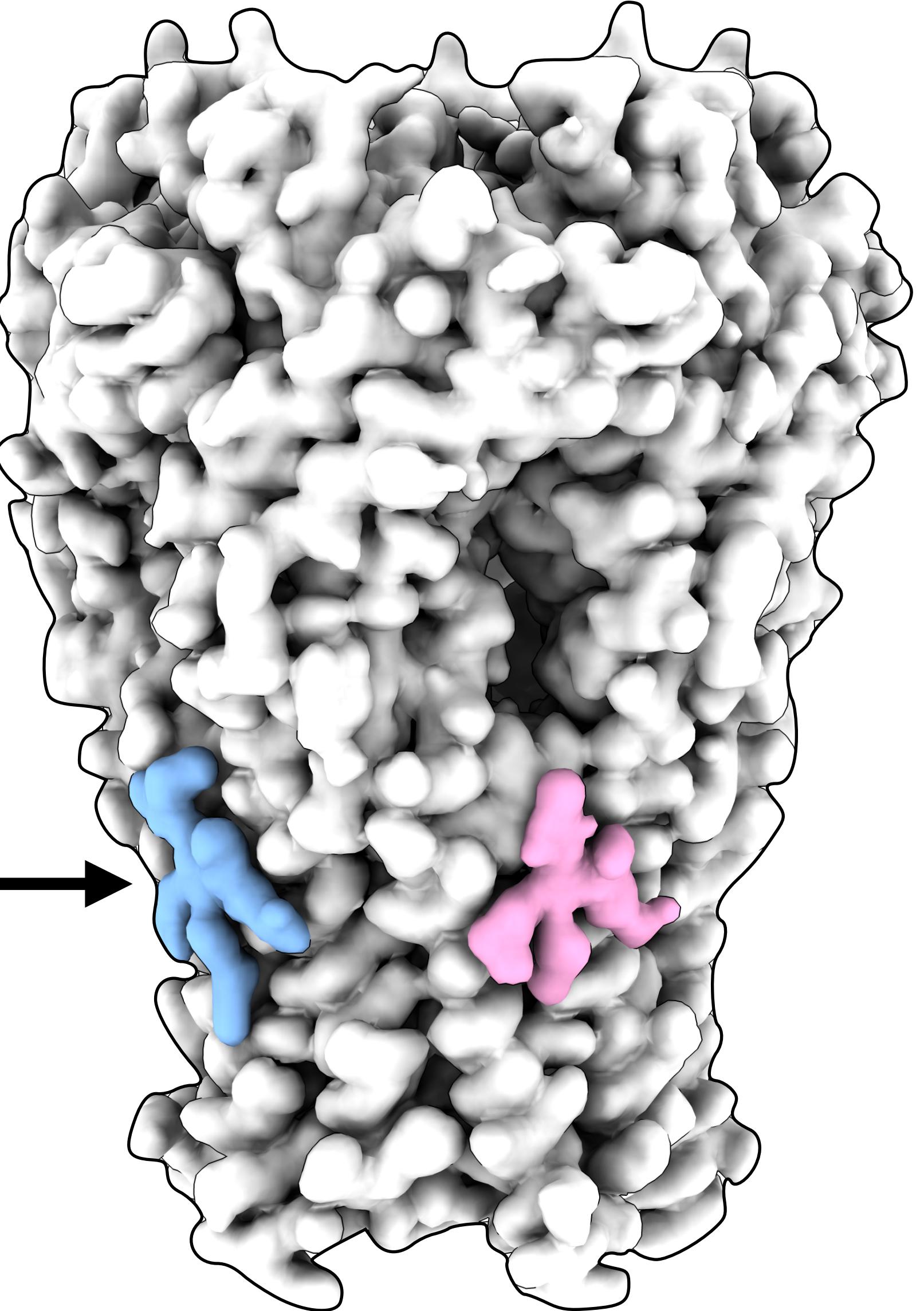
POPG



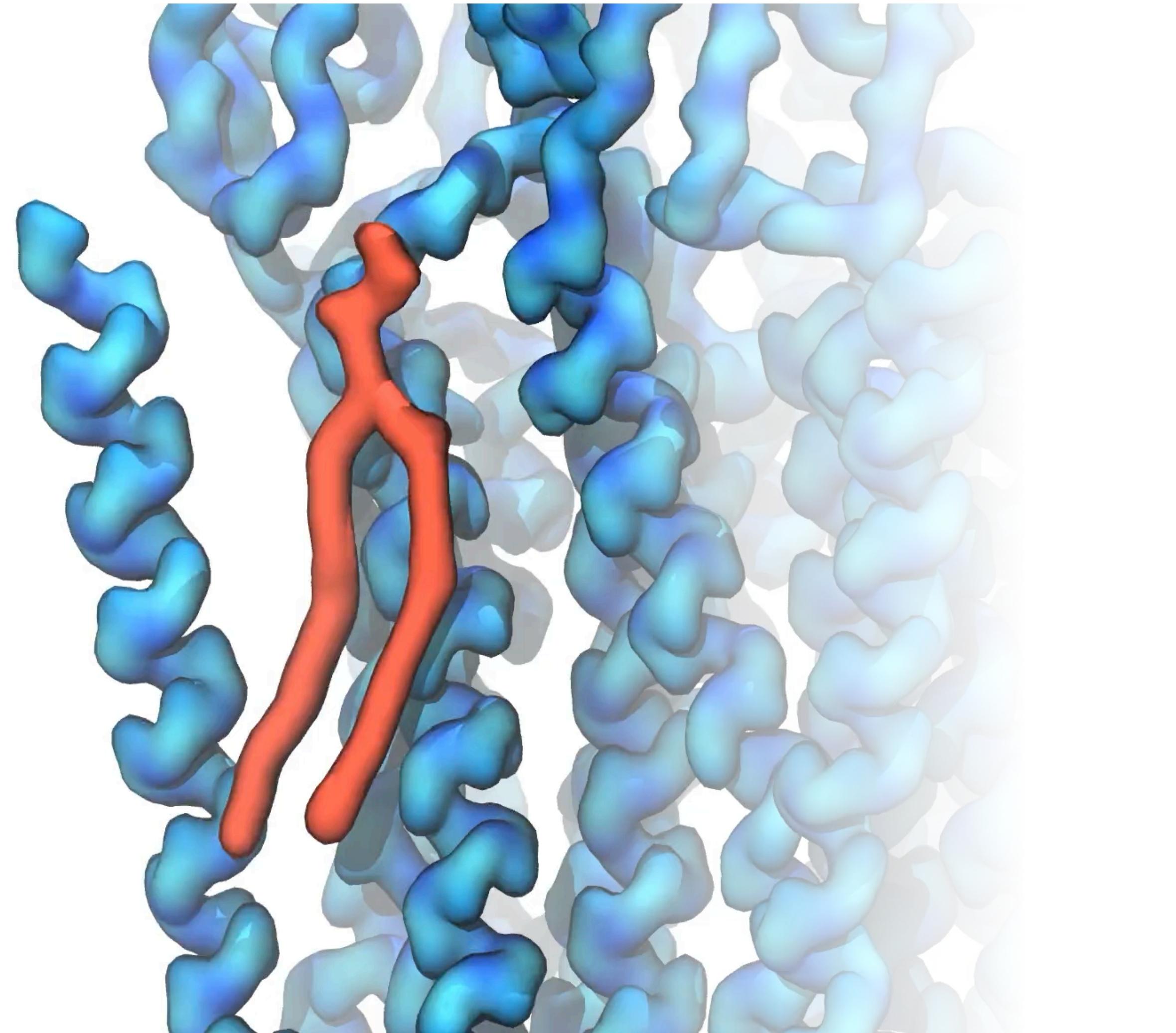


POPG

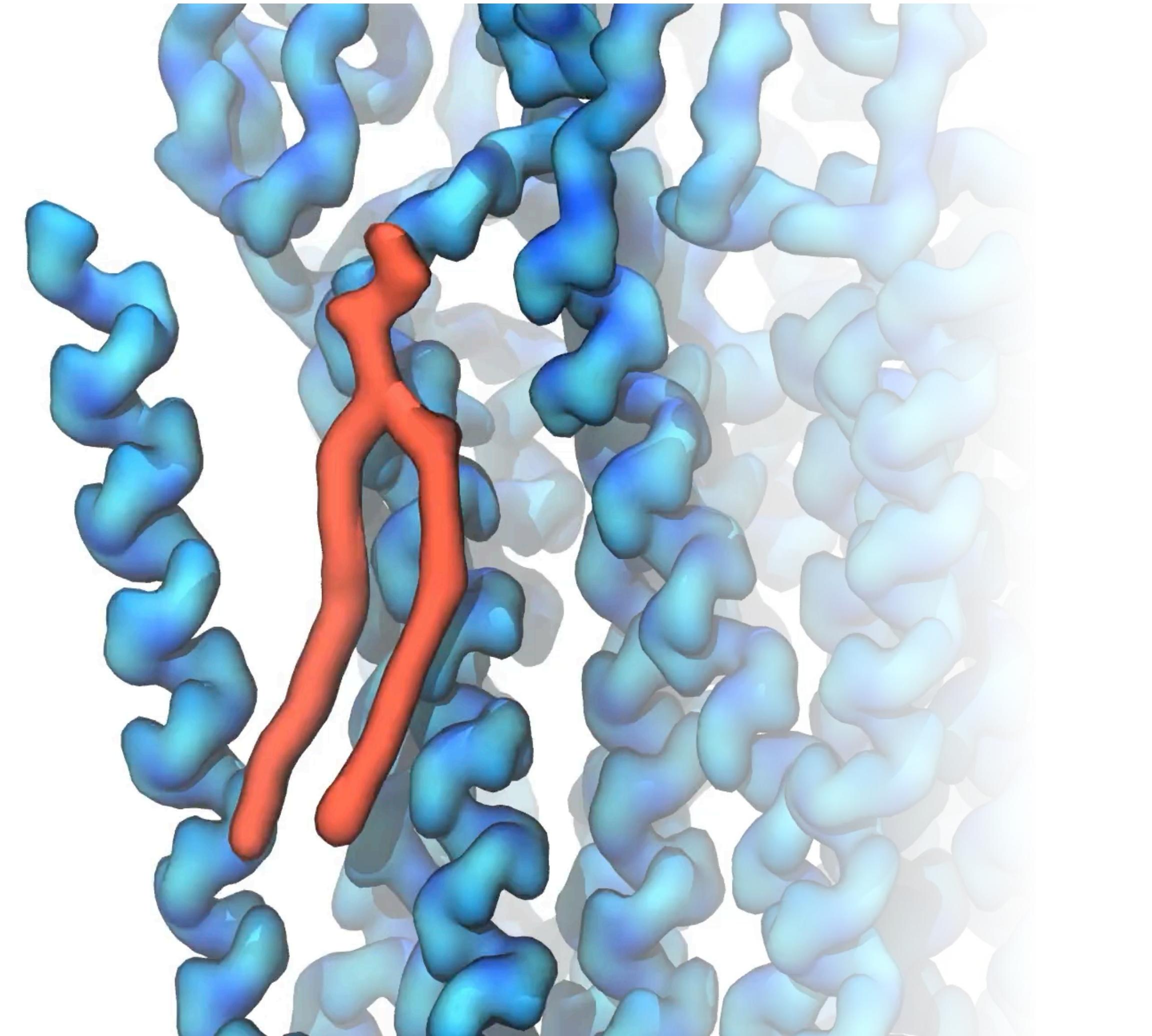
Is this POPG?



# Getting Free Energies of Binding (SAFEP)

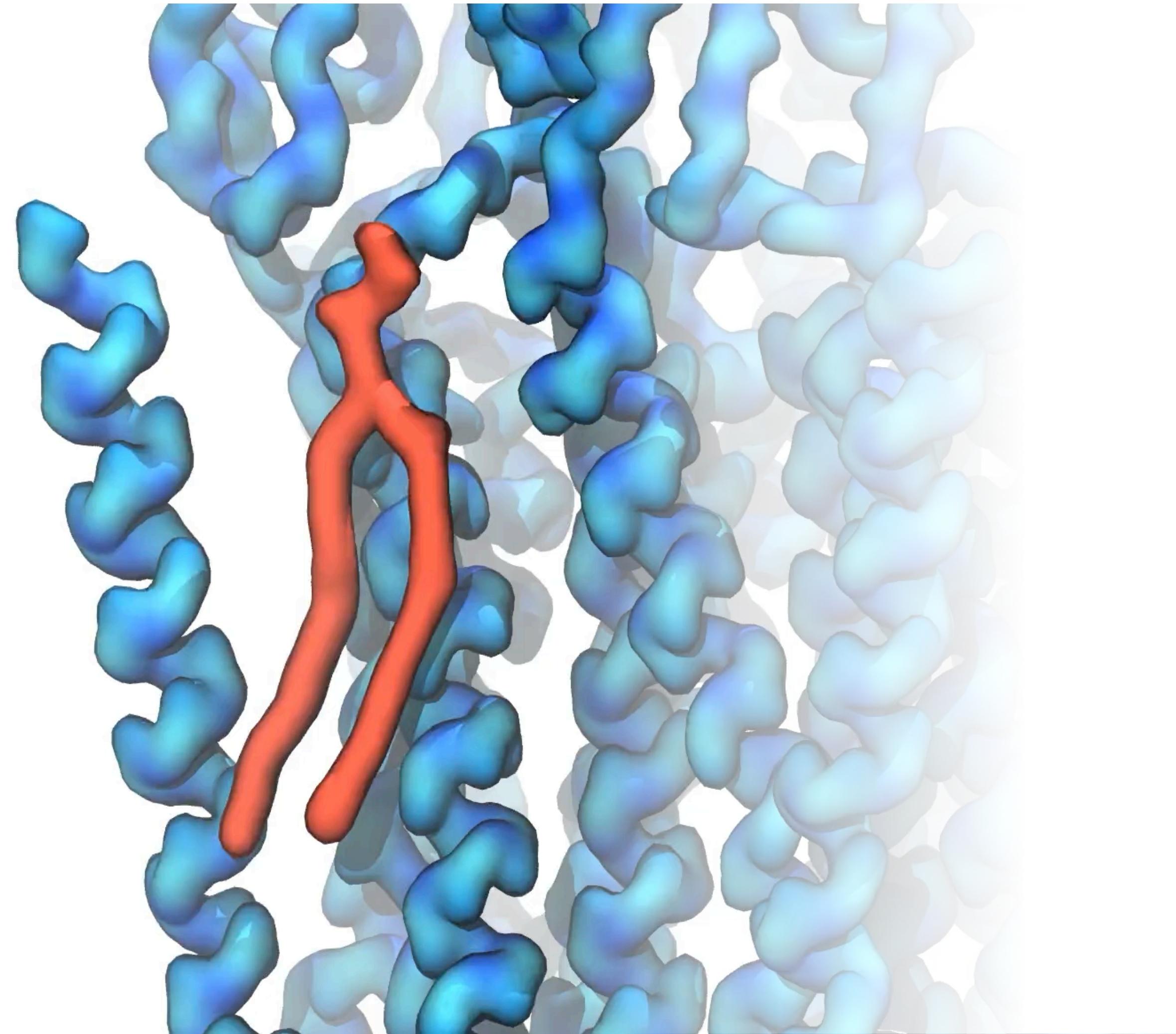


Lipid's Point of View

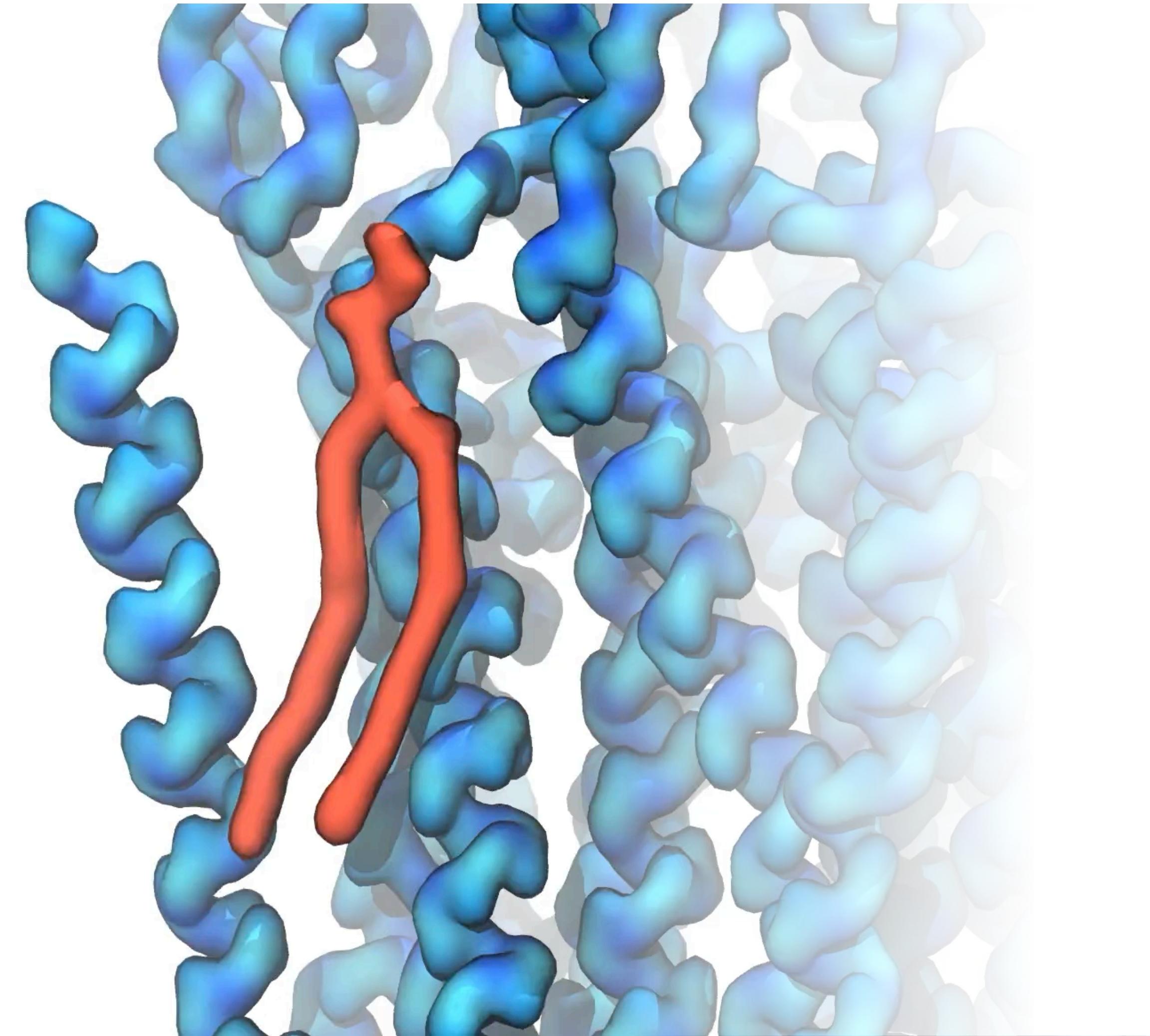


Protein's Point of View

# Getting Free Energies of Binding (SAFEP)

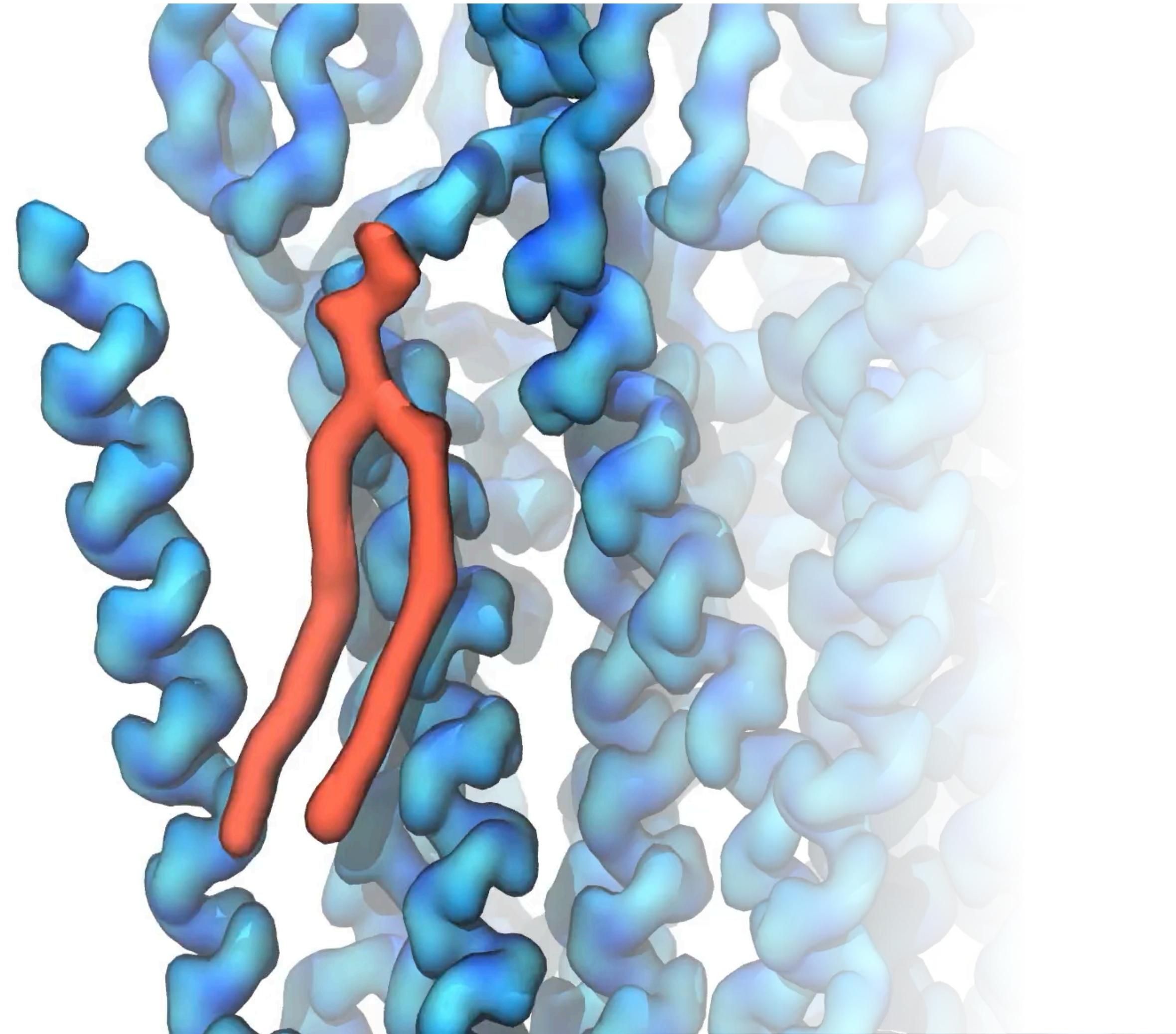


Lipid's Point of View

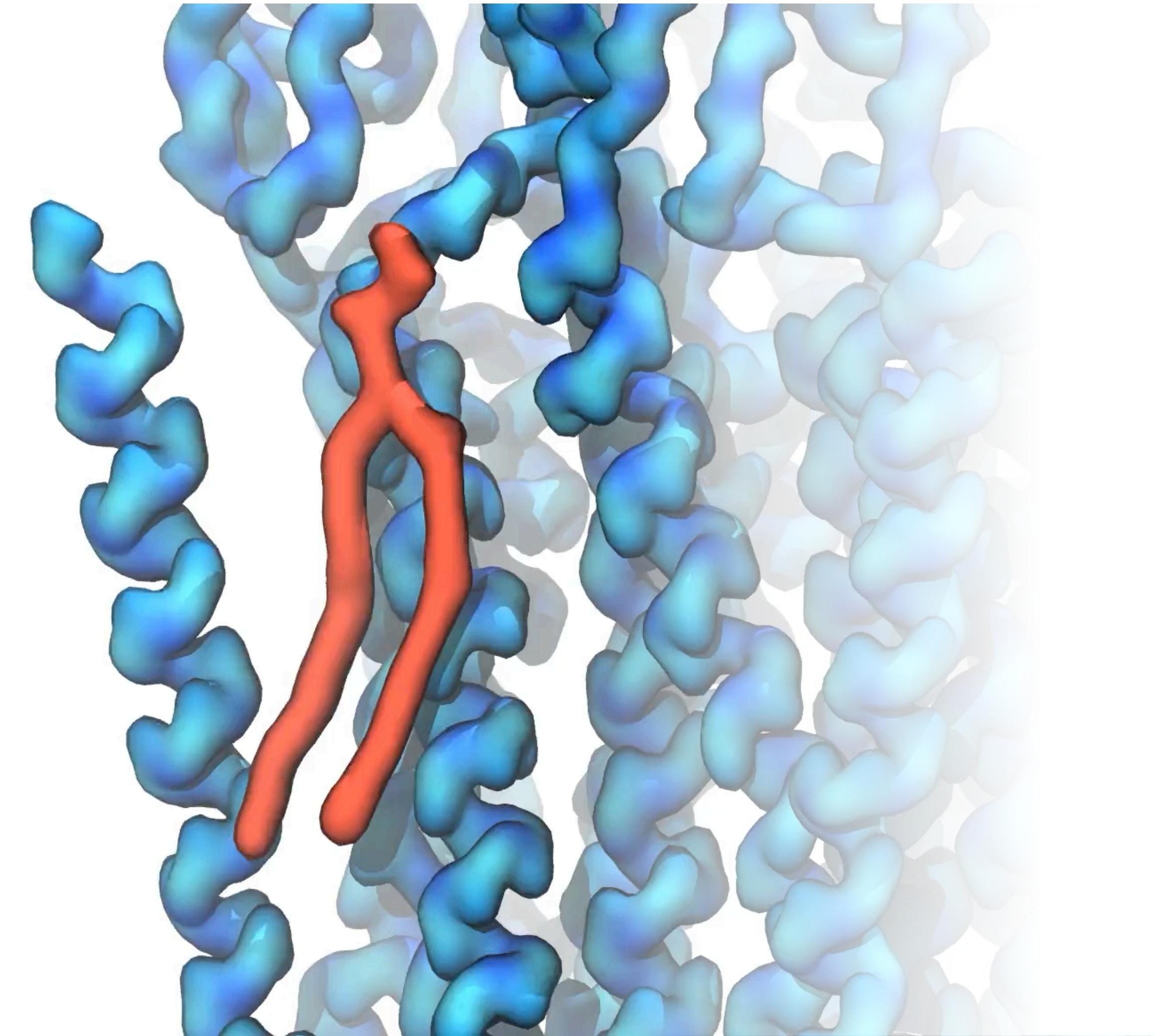


Protein's Point of View

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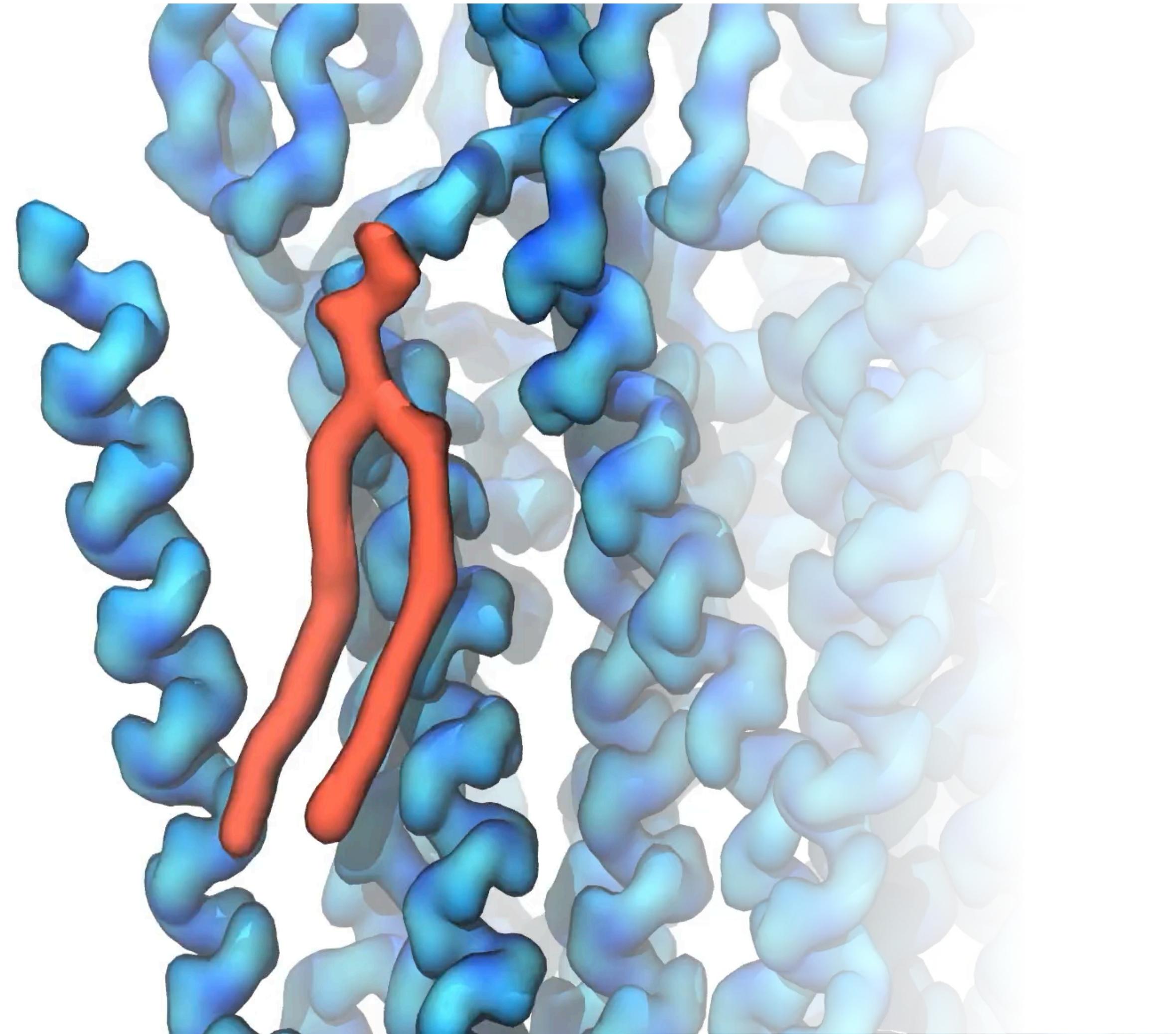


Lipid's Point of View

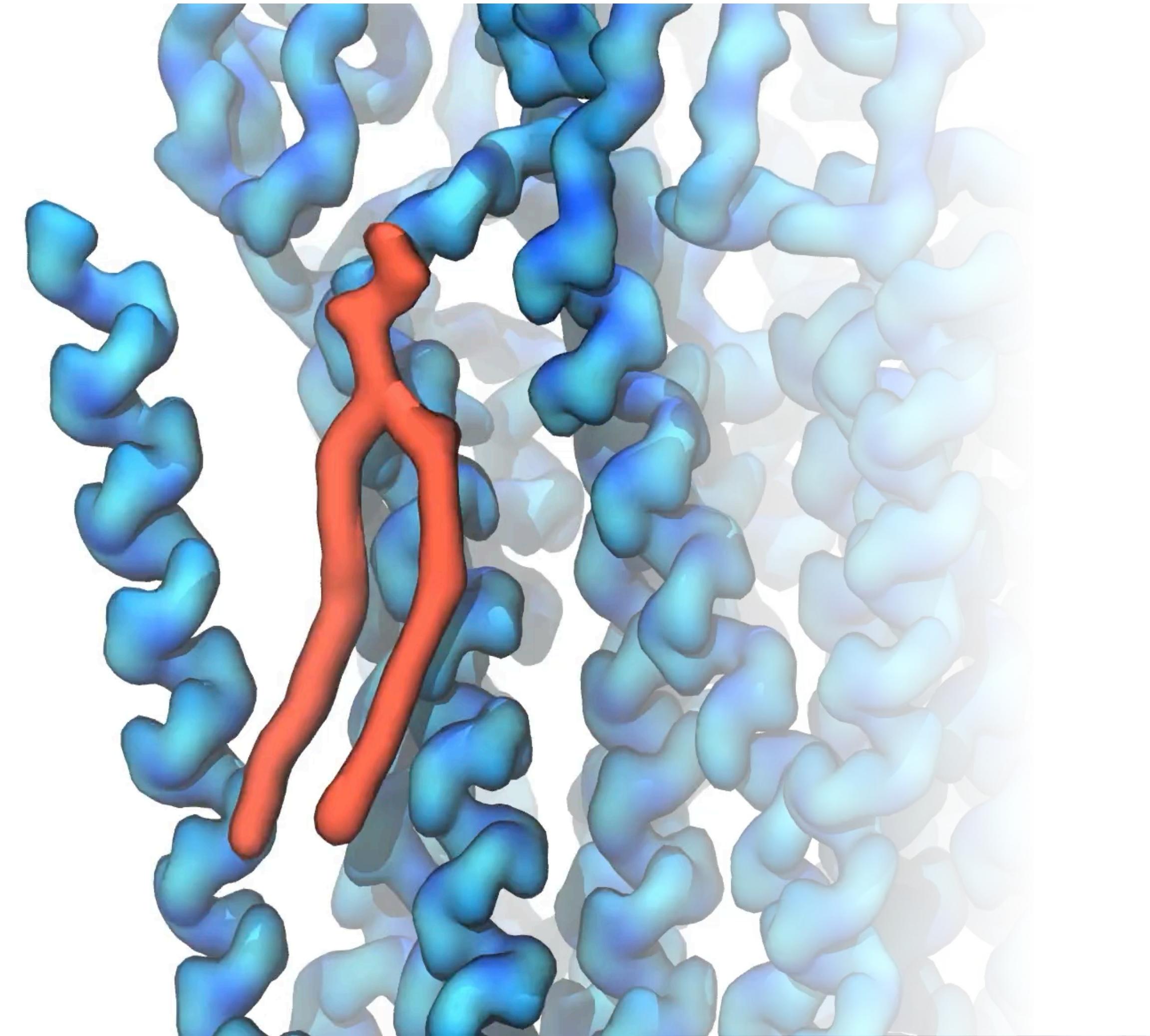


Protein's Point of View

# Getting Free Energies of Binding (SAFEP)



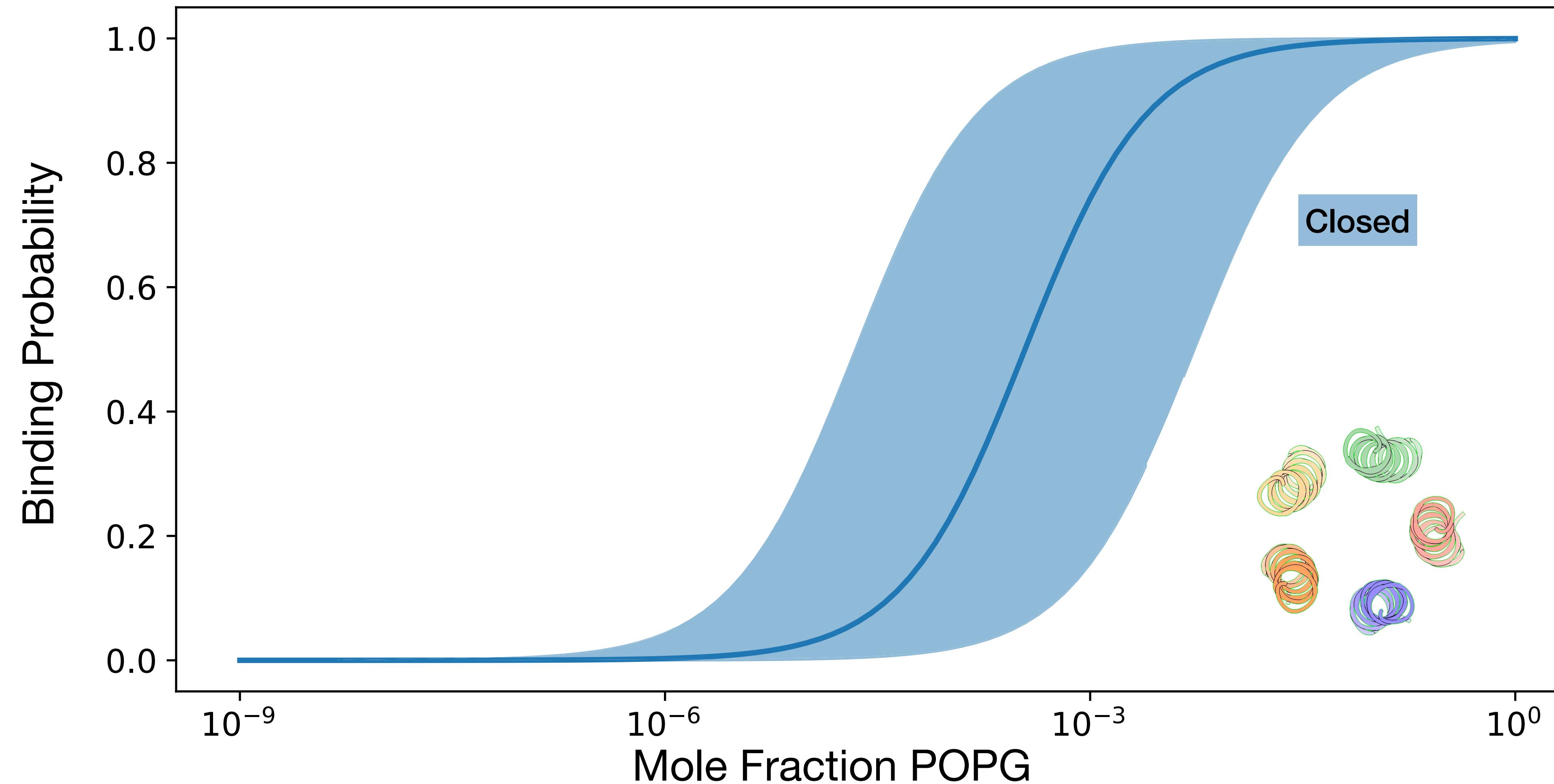
Lipid's Point of View



Protein's Point of View

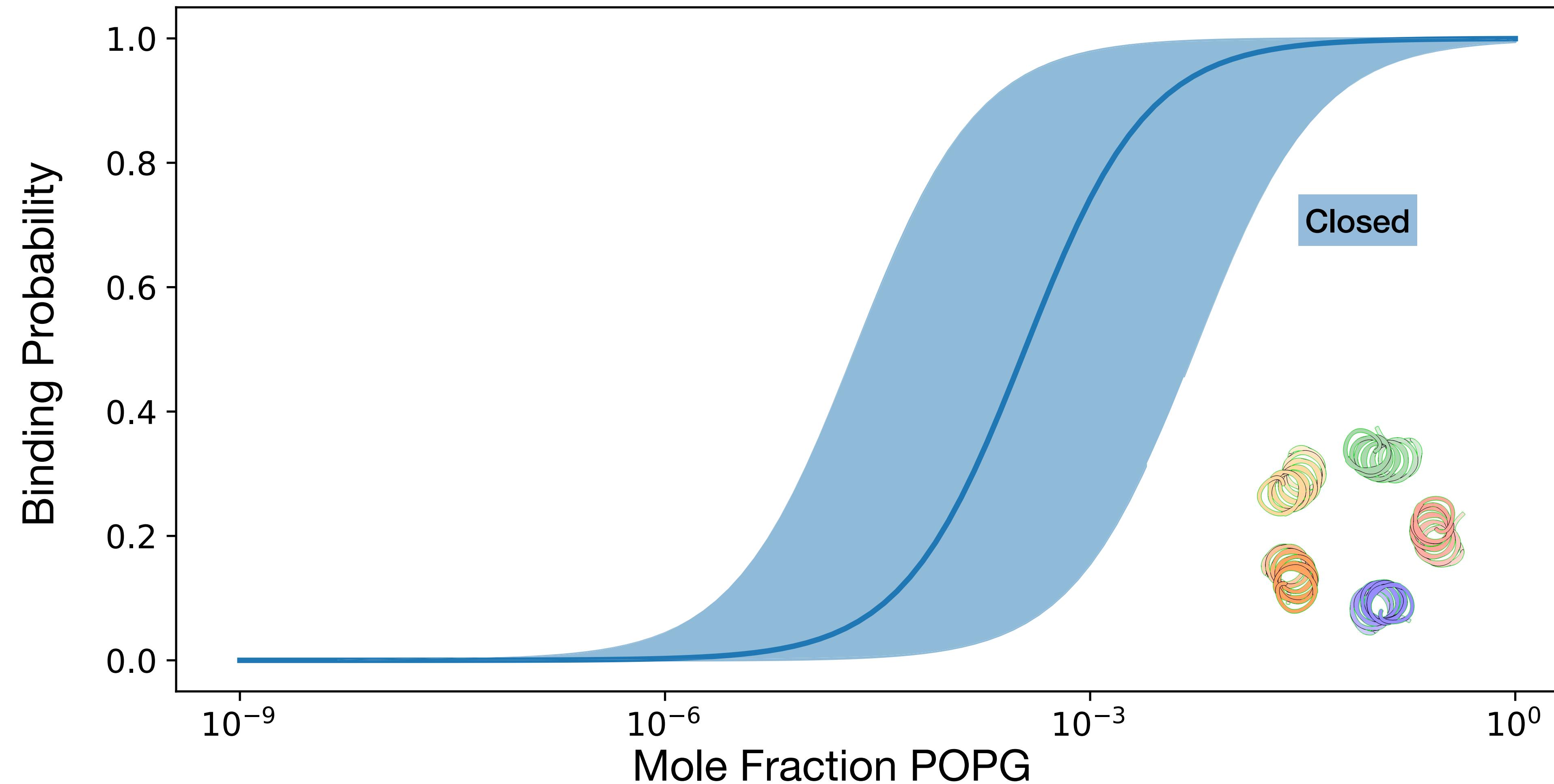
# POPG has High Affinity for the Overall Site

## Both the Open and Closed Conformations



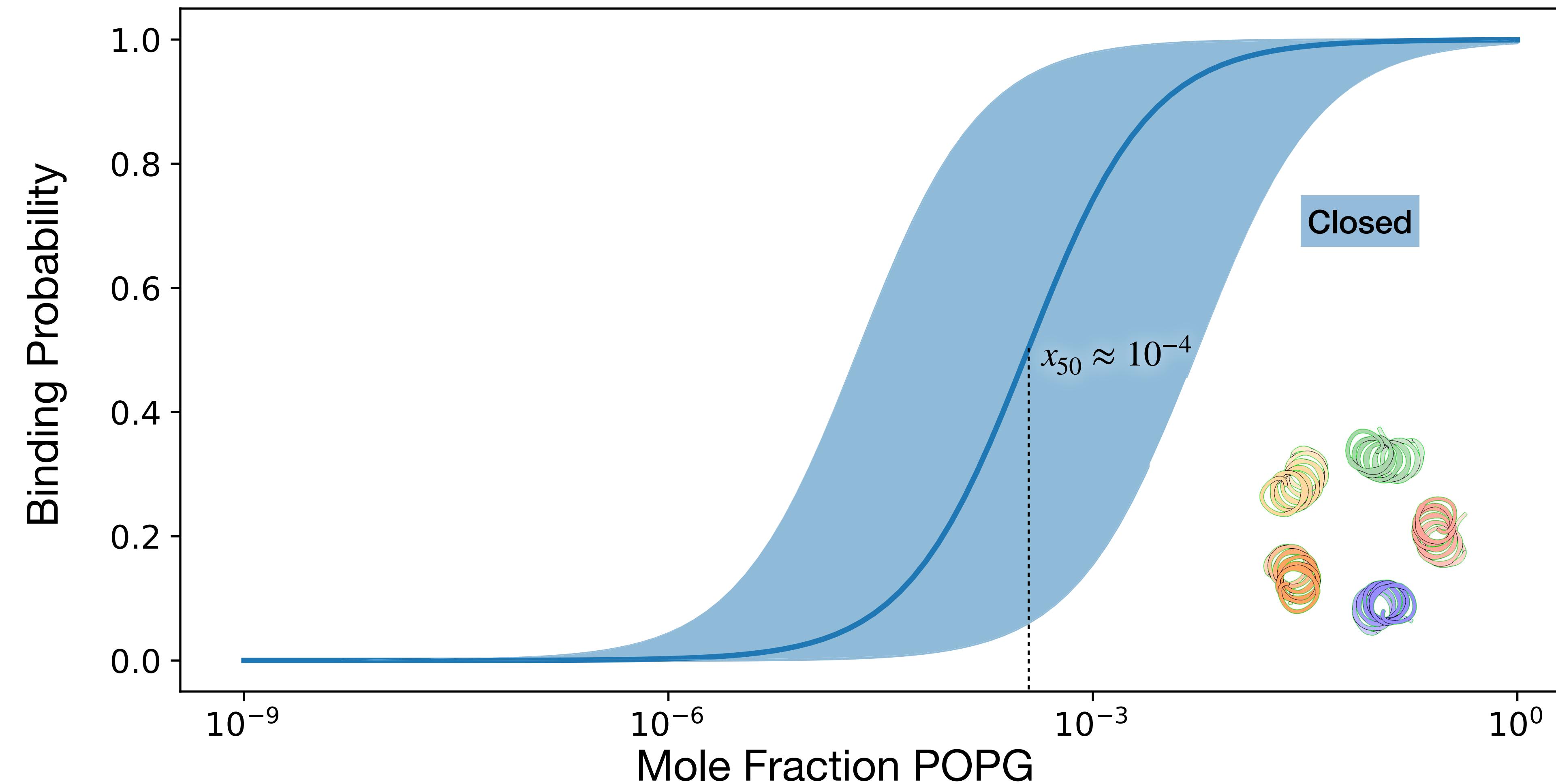
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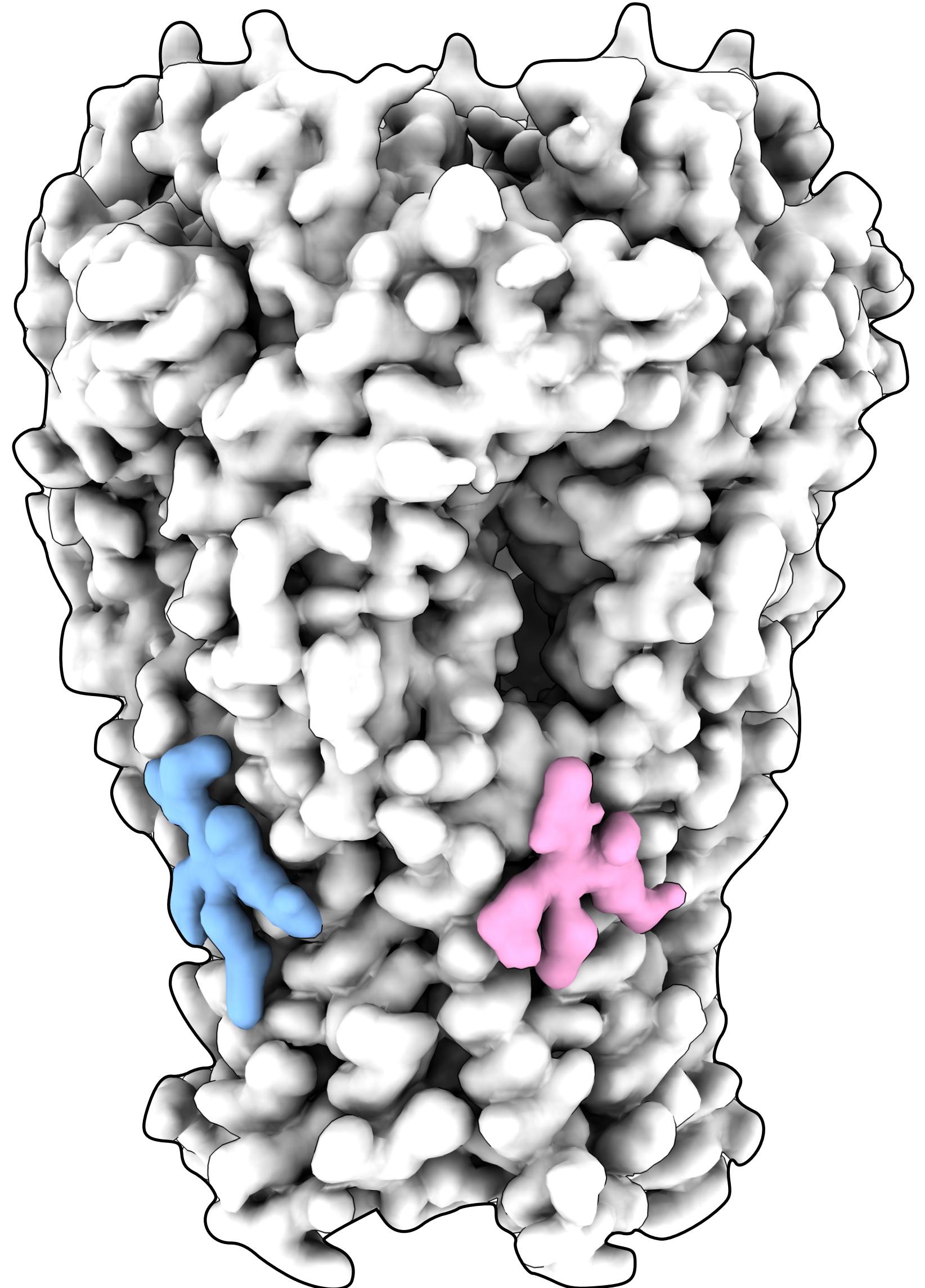
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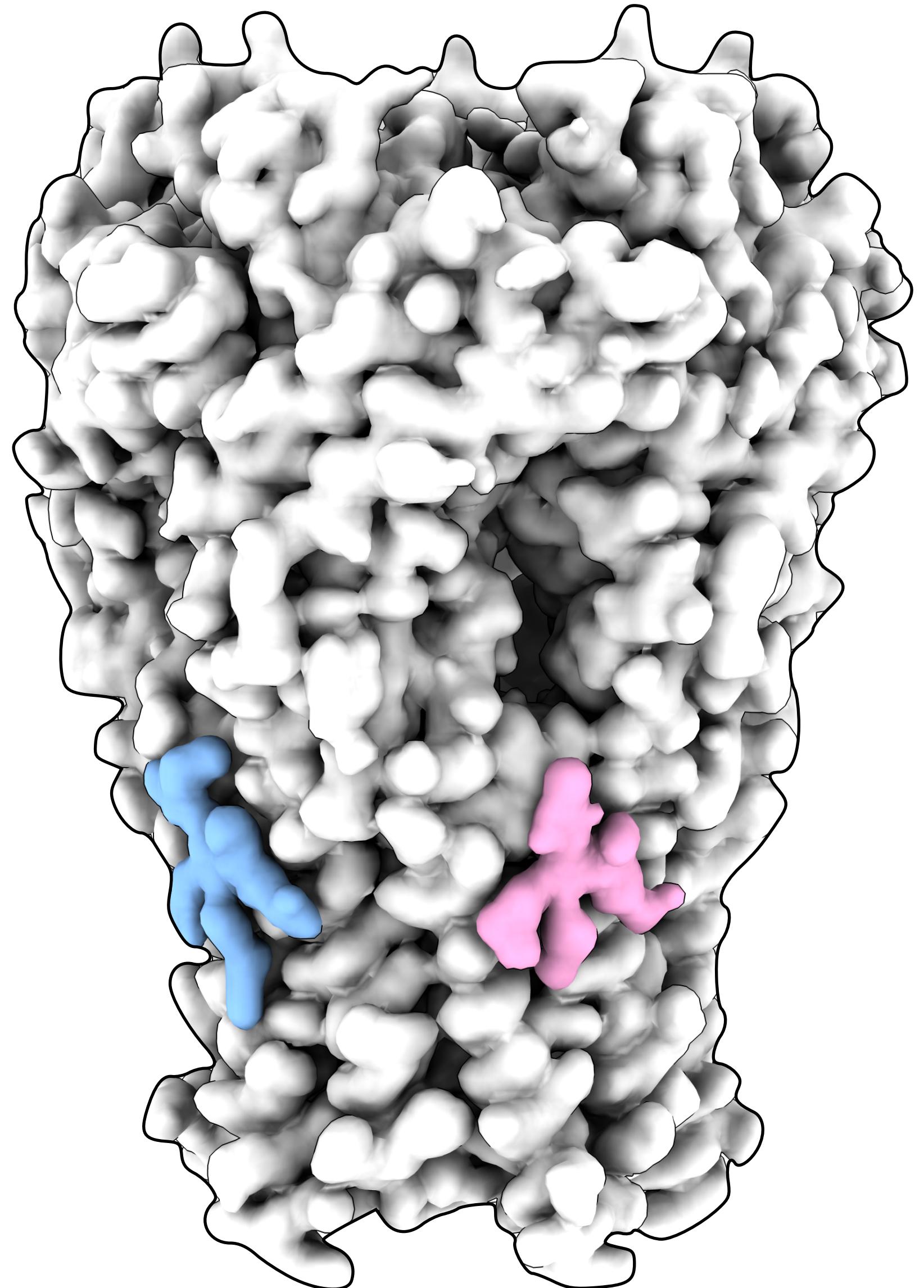
# Hypothesis: Allosteric Modulation

1. POPG Binding Site
2. Preferential binding to the open conformation



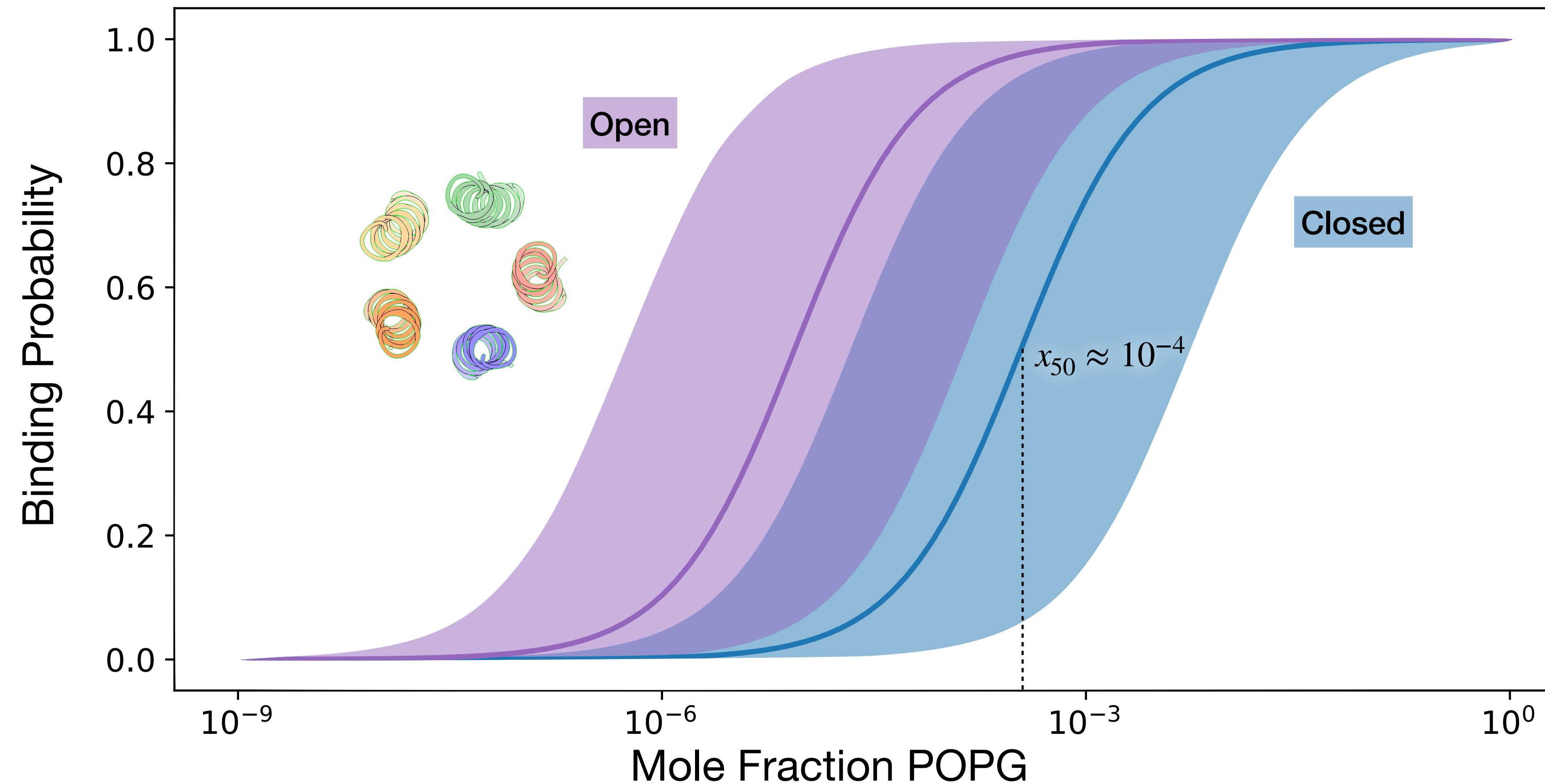
# Hypothesis: Allosteric Modulation

1. POPG Binding Site ✓
2. Preferential binding to the open conformation



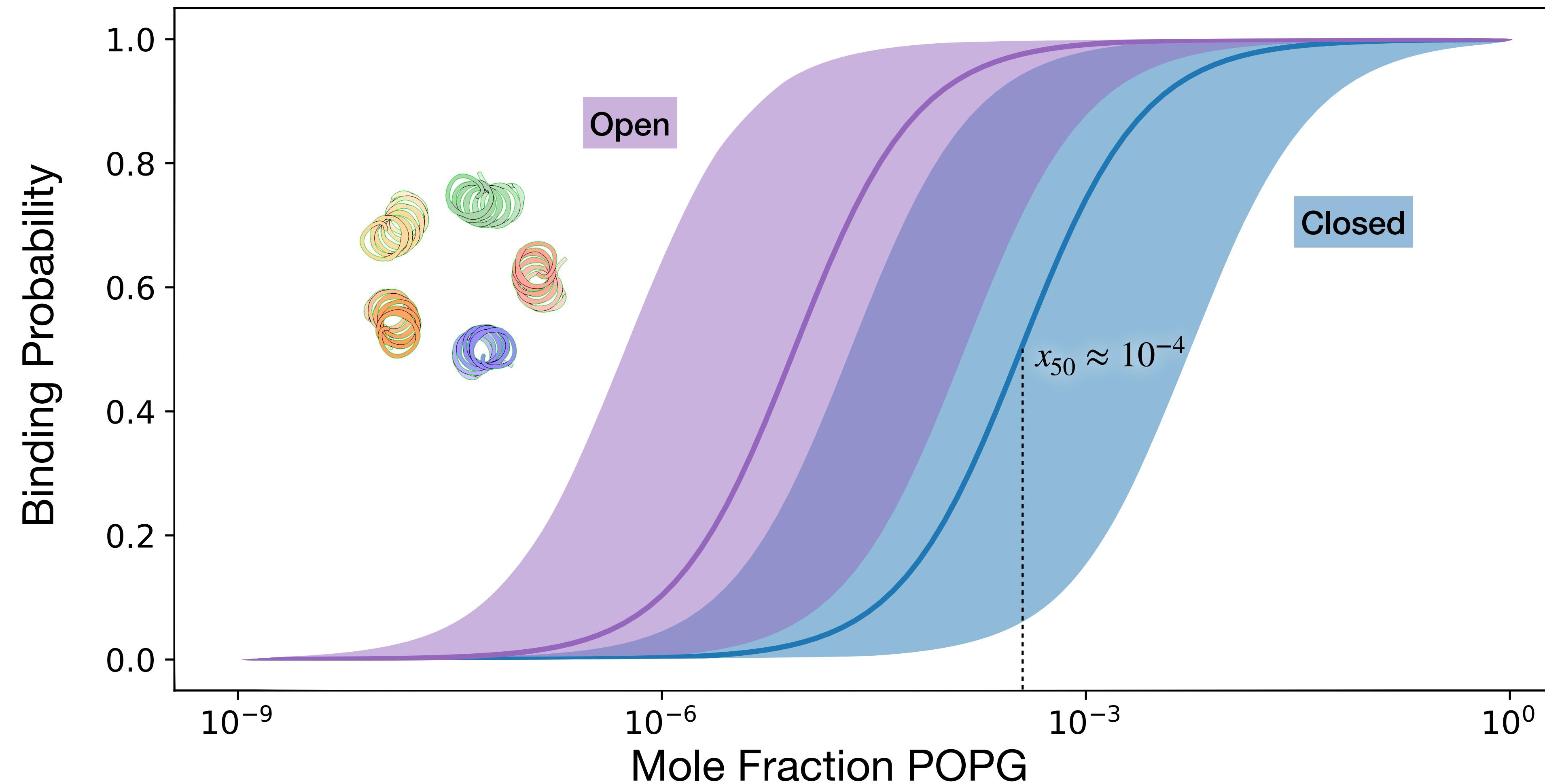
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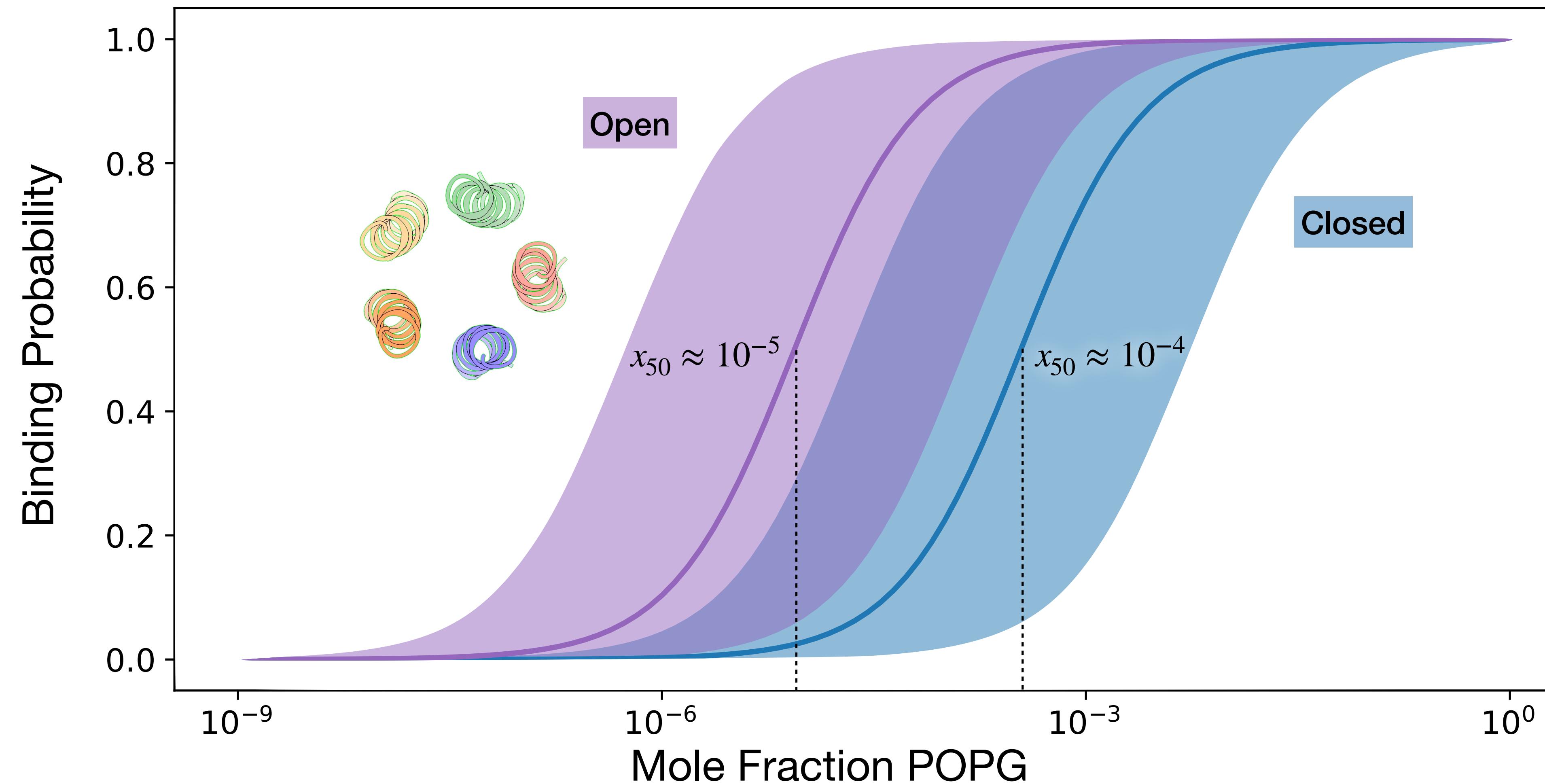
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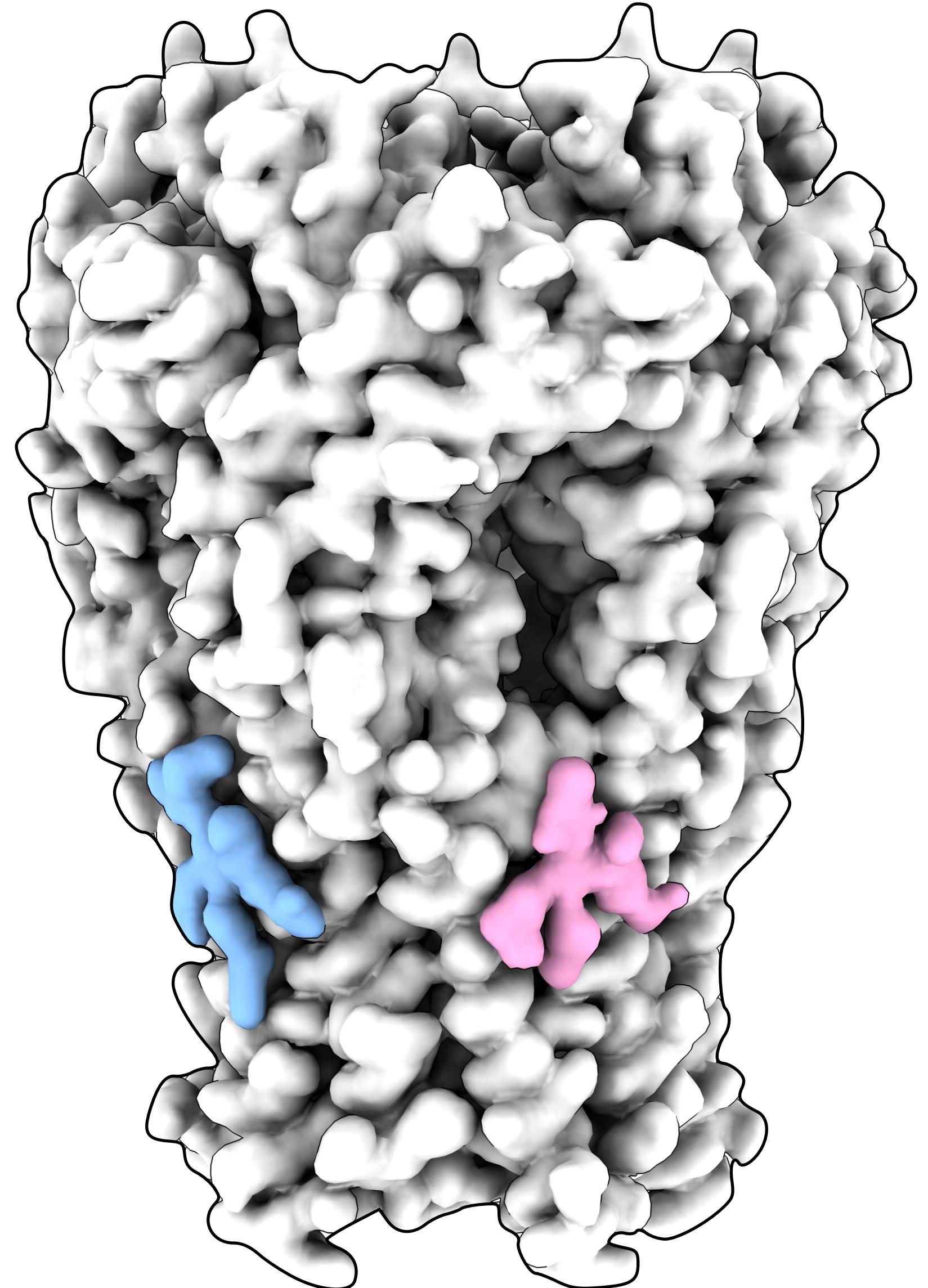
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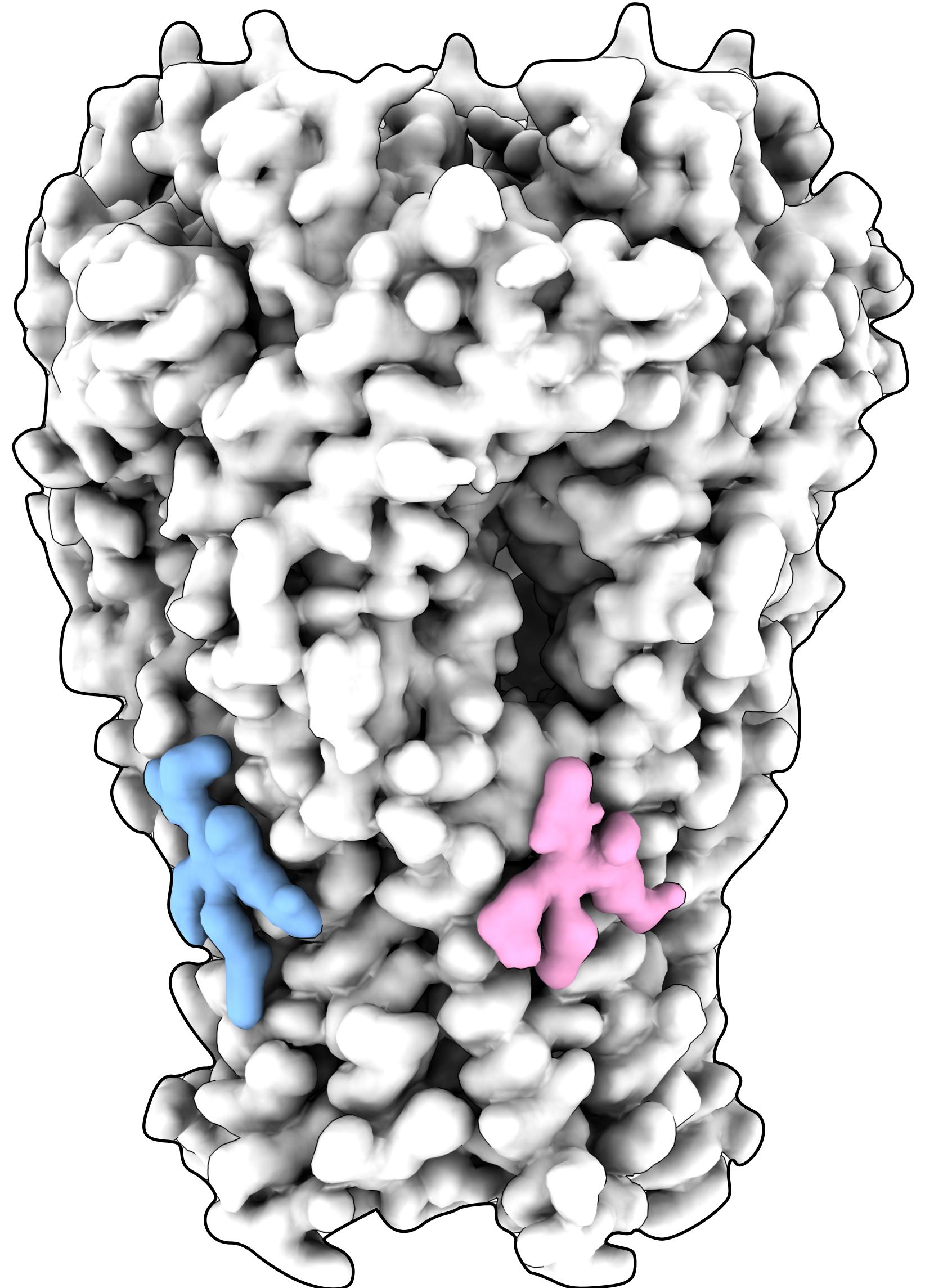
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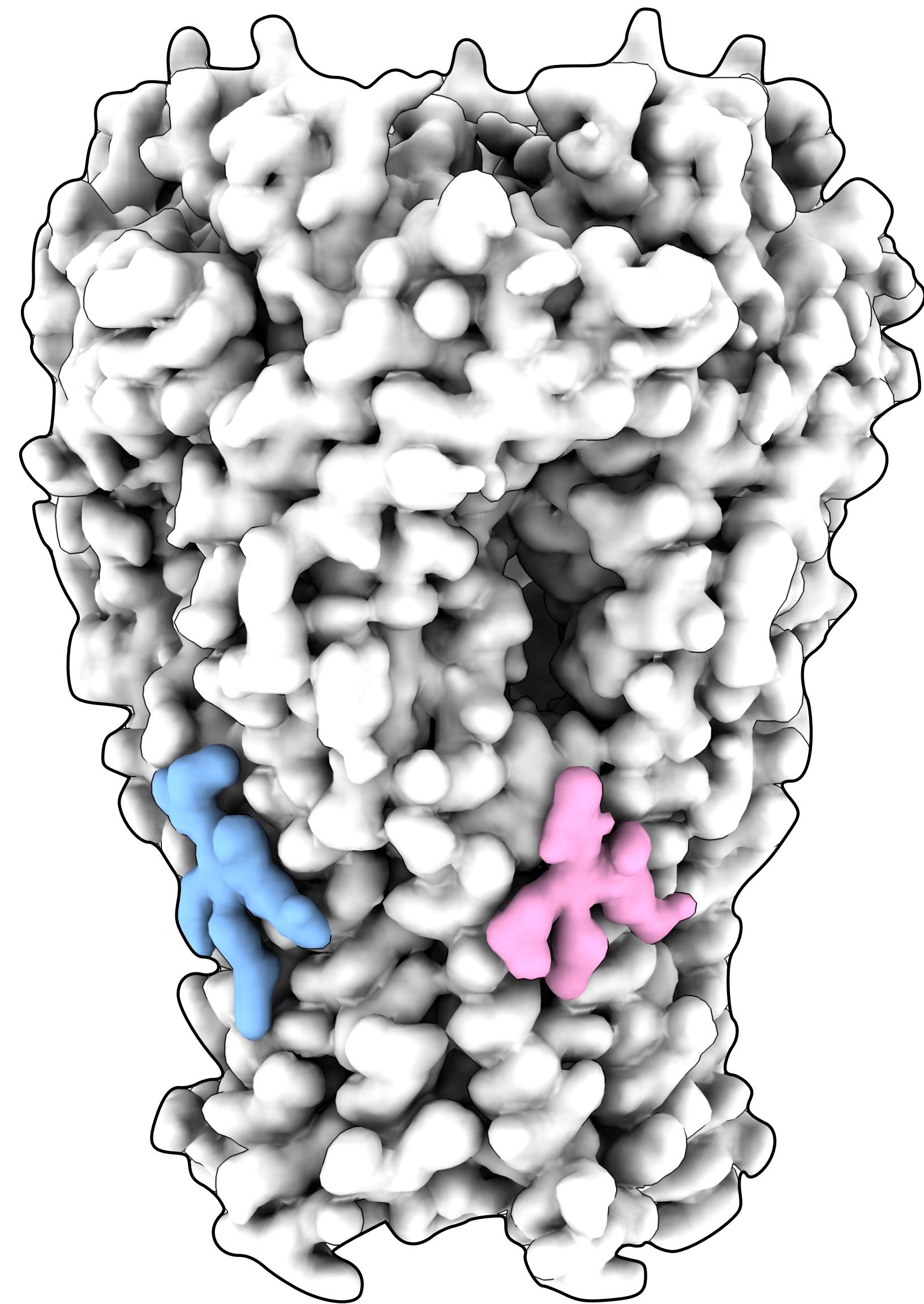


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2. Preferential binding to the open conformation ✓

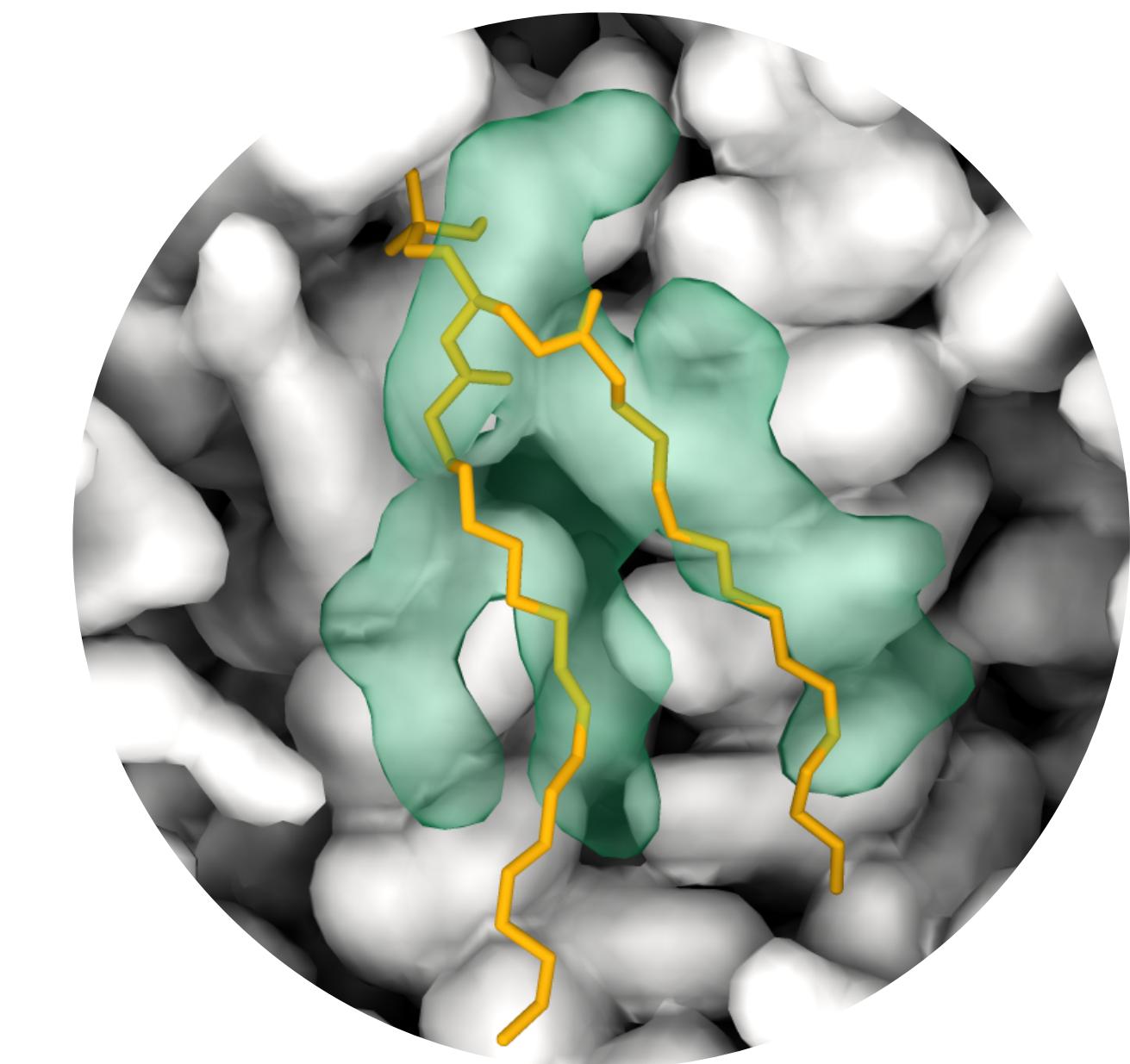
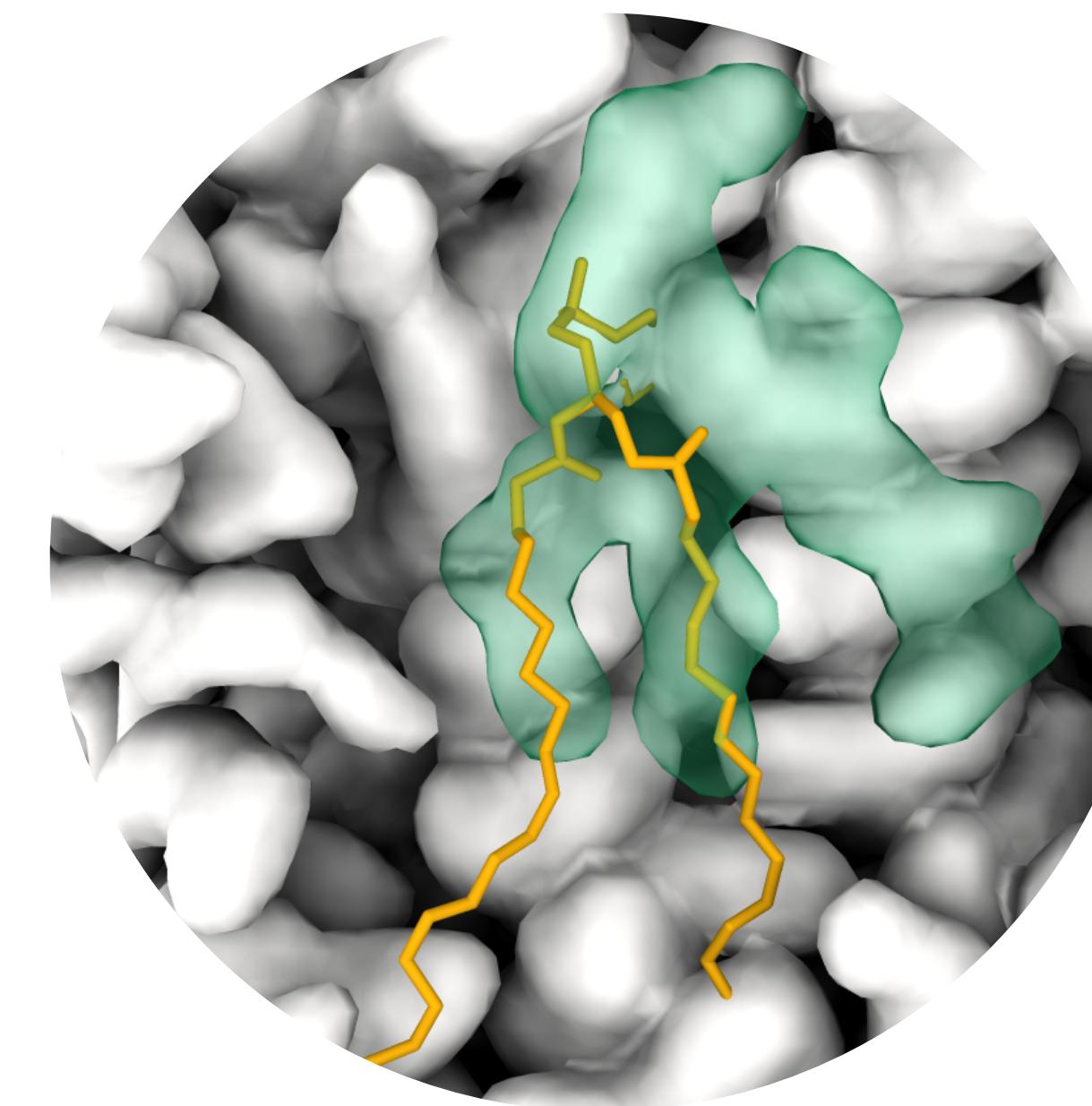
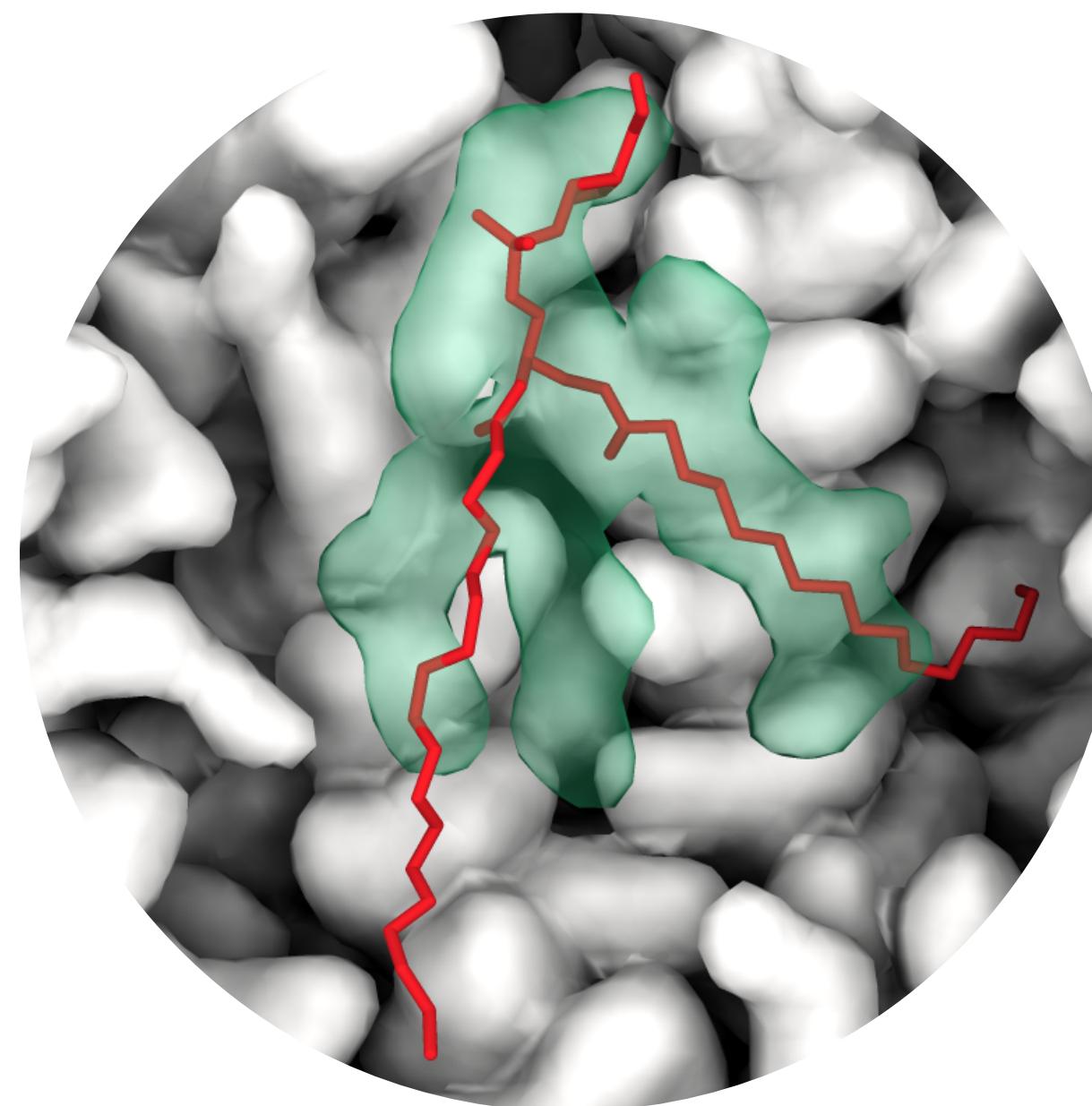


Is it *just* POPG?



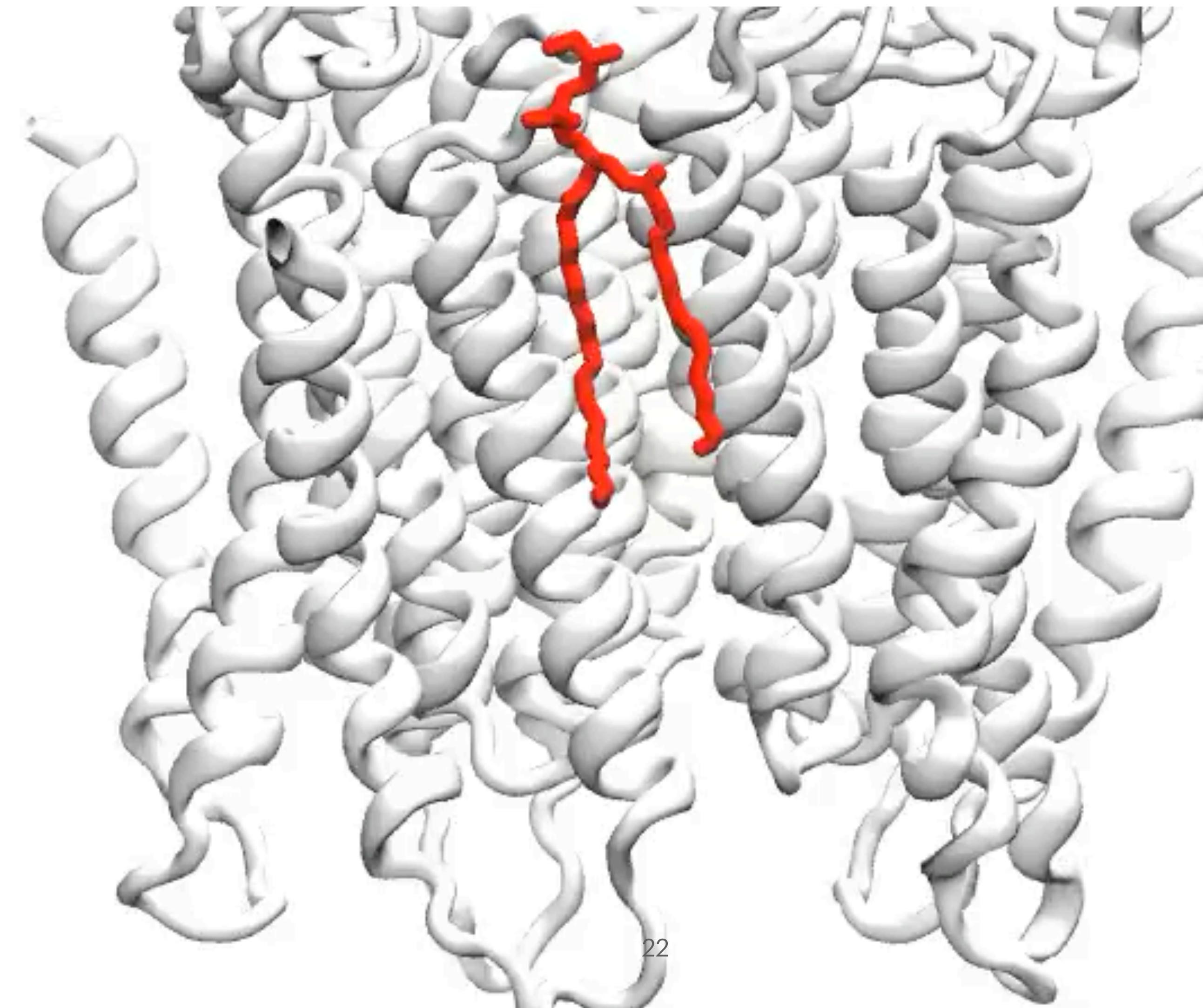
Is it *just* POPG?

# One Lipid with Three Tails?



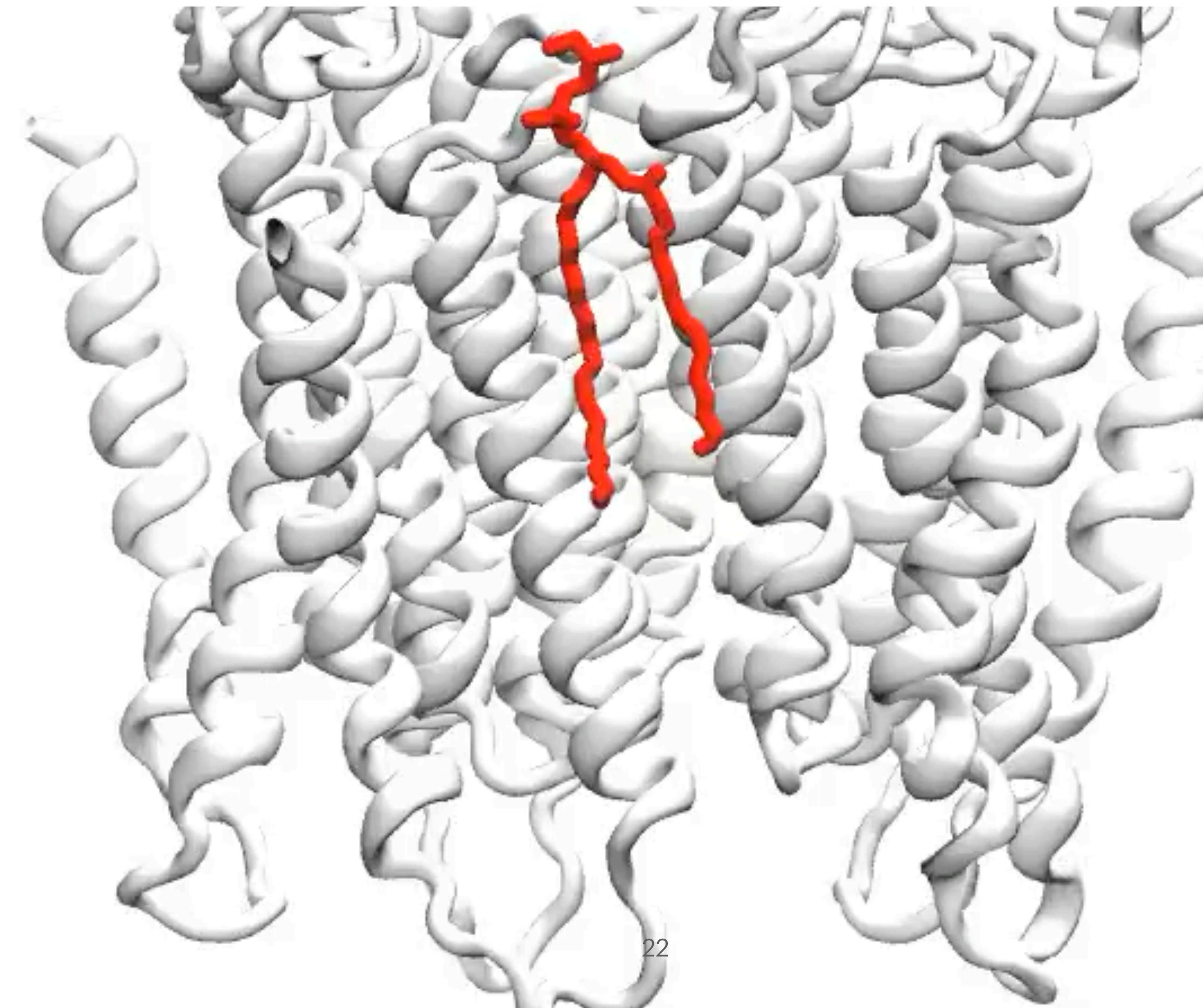
# Candidate Poses for One Lipid

Identified from  $1 \mu s$  of Unbiased MD



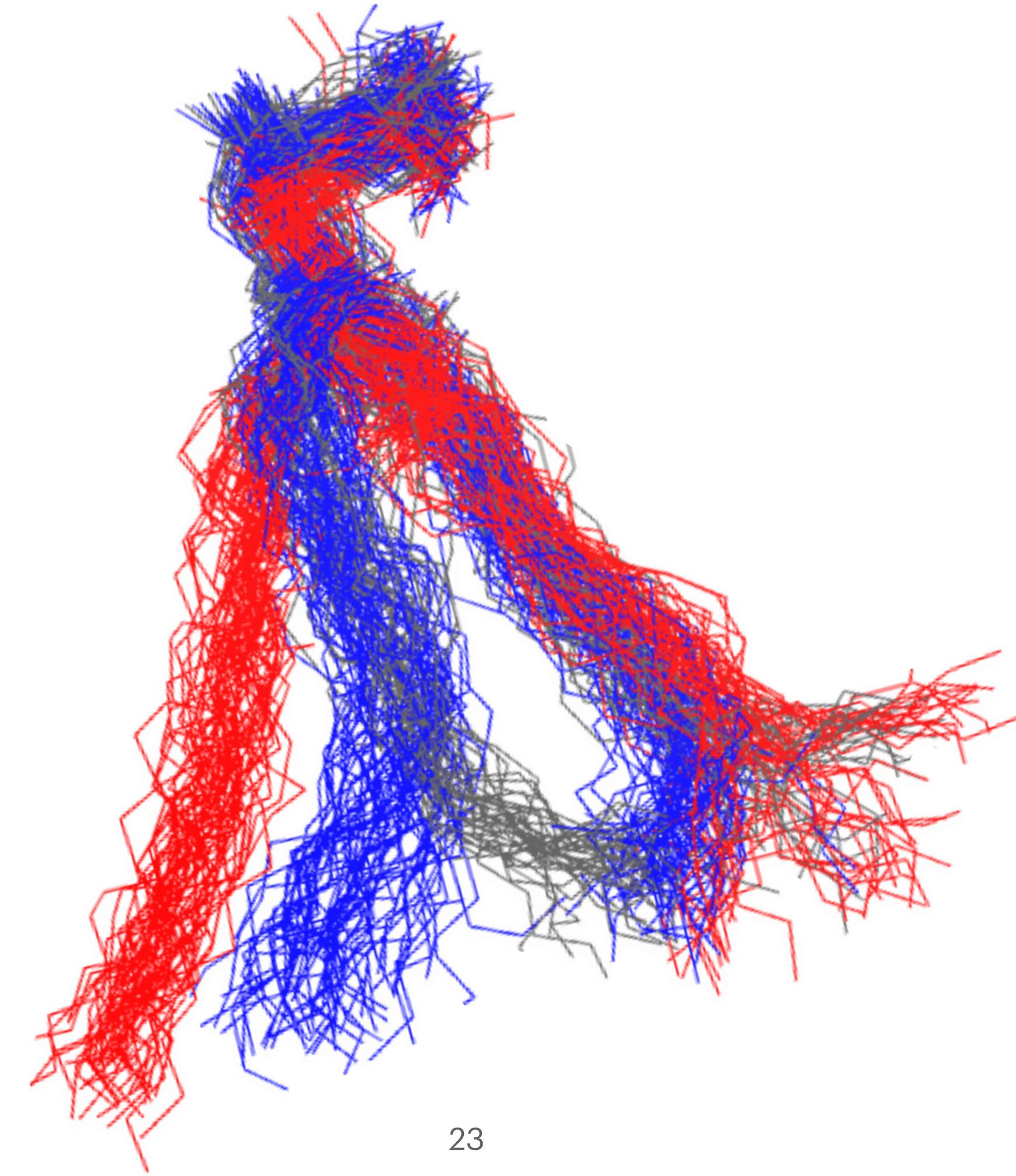
# Candidate Poses for One Lipid

Identified from  $1 \mu s$  of Unbiased MD



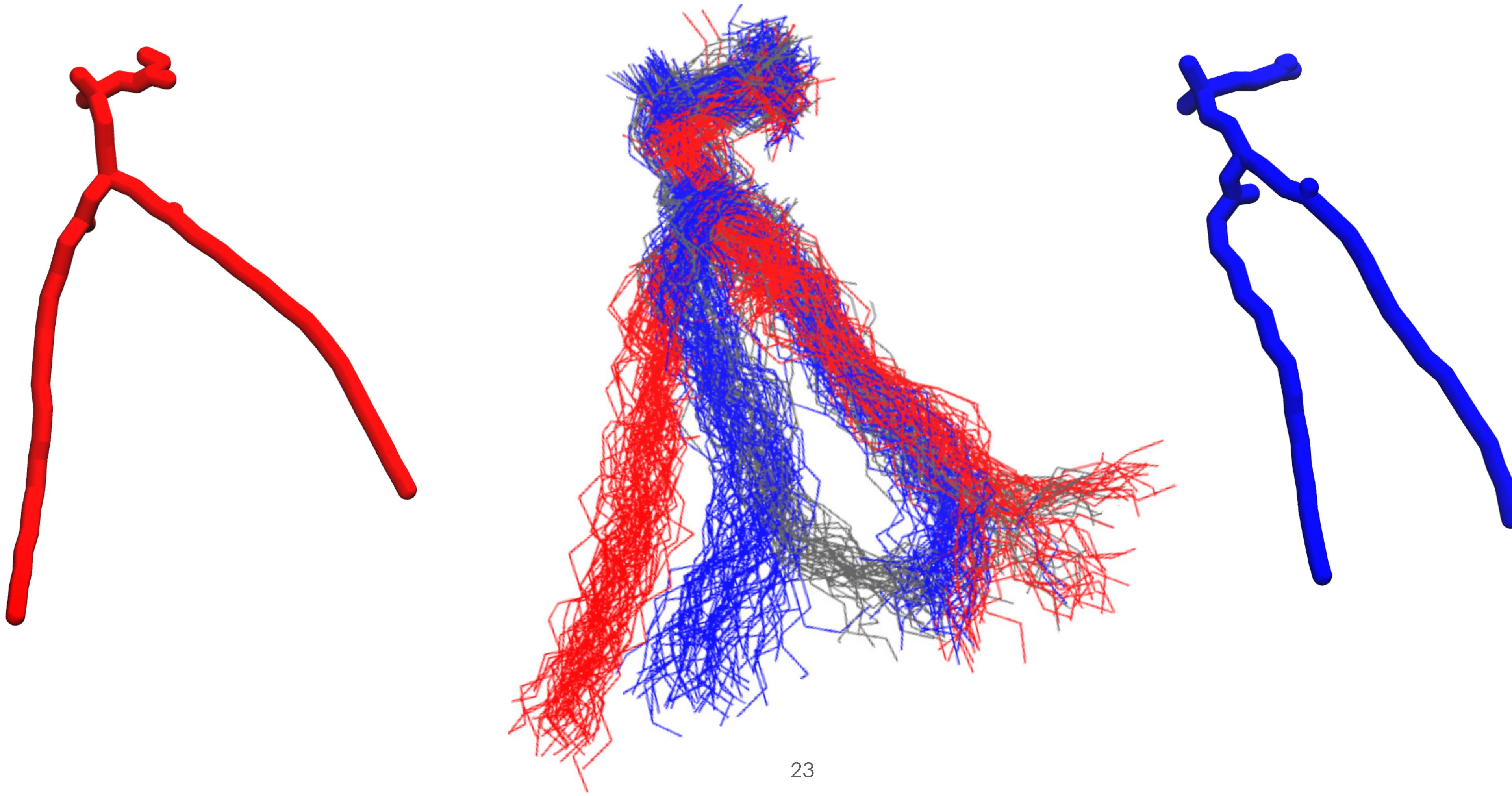
# Candidate Poses for One Lipid

Identified from  $1 \mu s$  of Unbiased MD



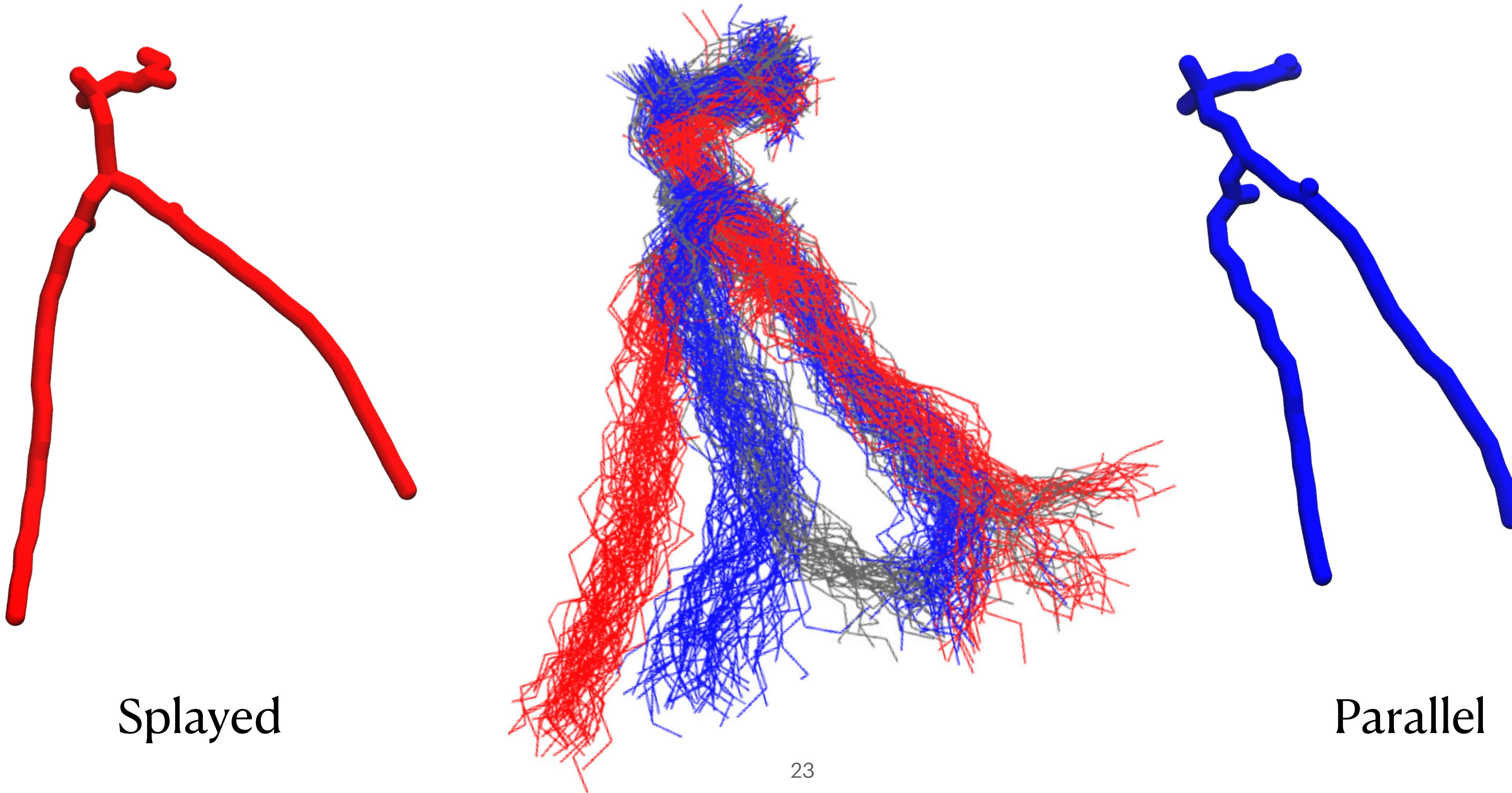
# Candidate Poses for One Lipid

Identified from  $1 \mu s$  of Unbiased MD



# Candidate Poses for One Lipid

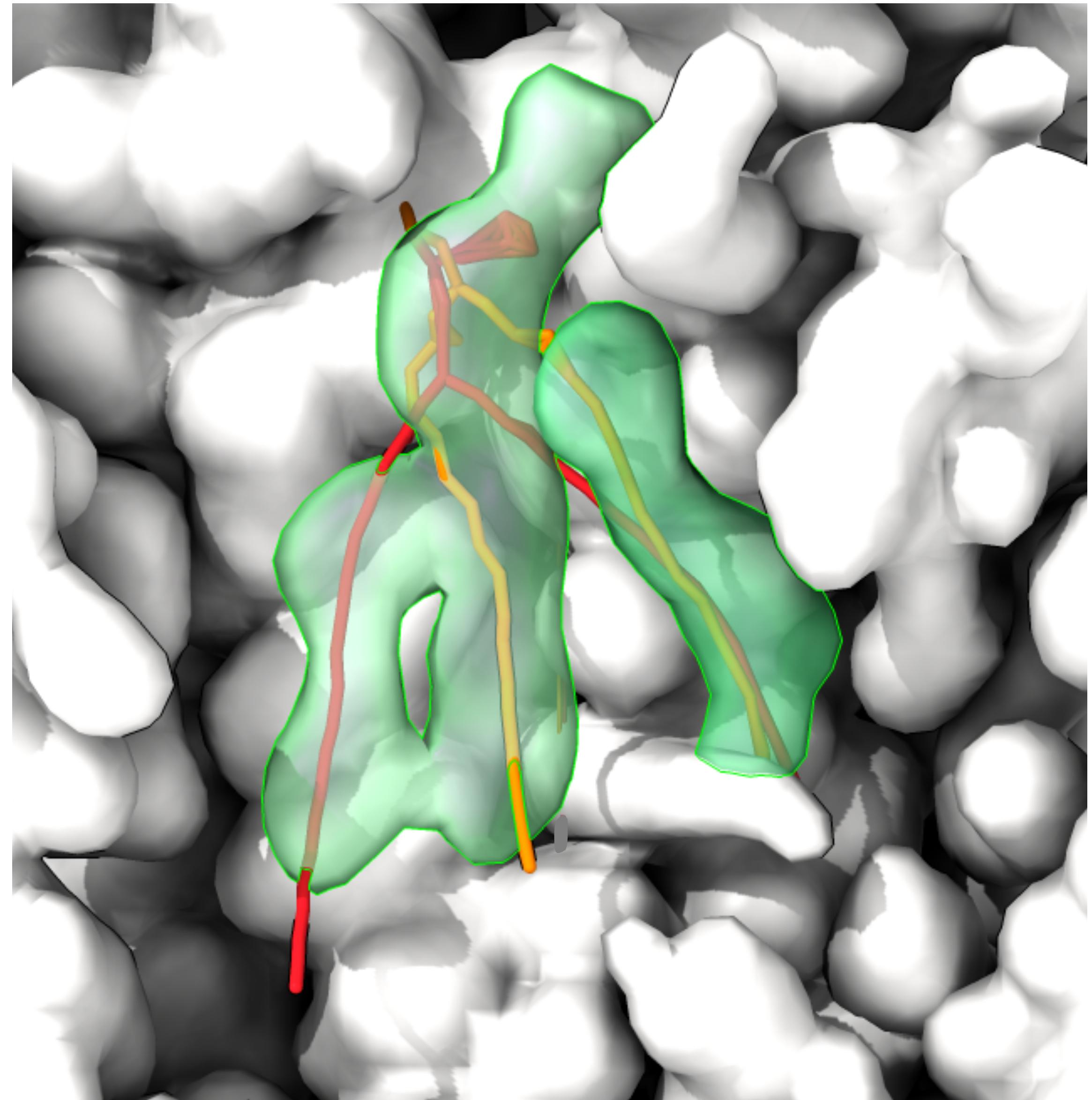
Identified from  $1 \mu s$  of Unbiased MD



# Two Poses

**Agree with the Experimental Density**

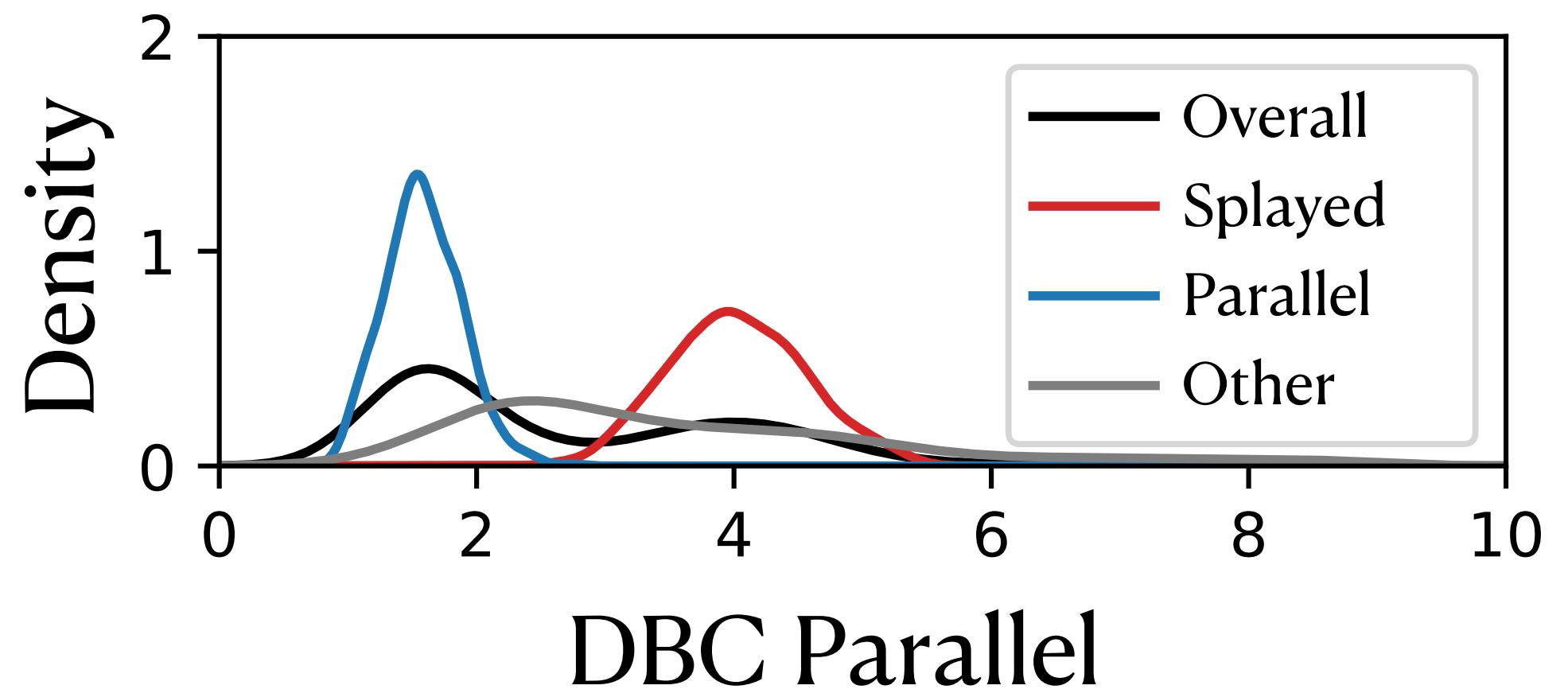
- $1\mu s$  of unbiased MD
- Quality threshold clustering
- Average clusters



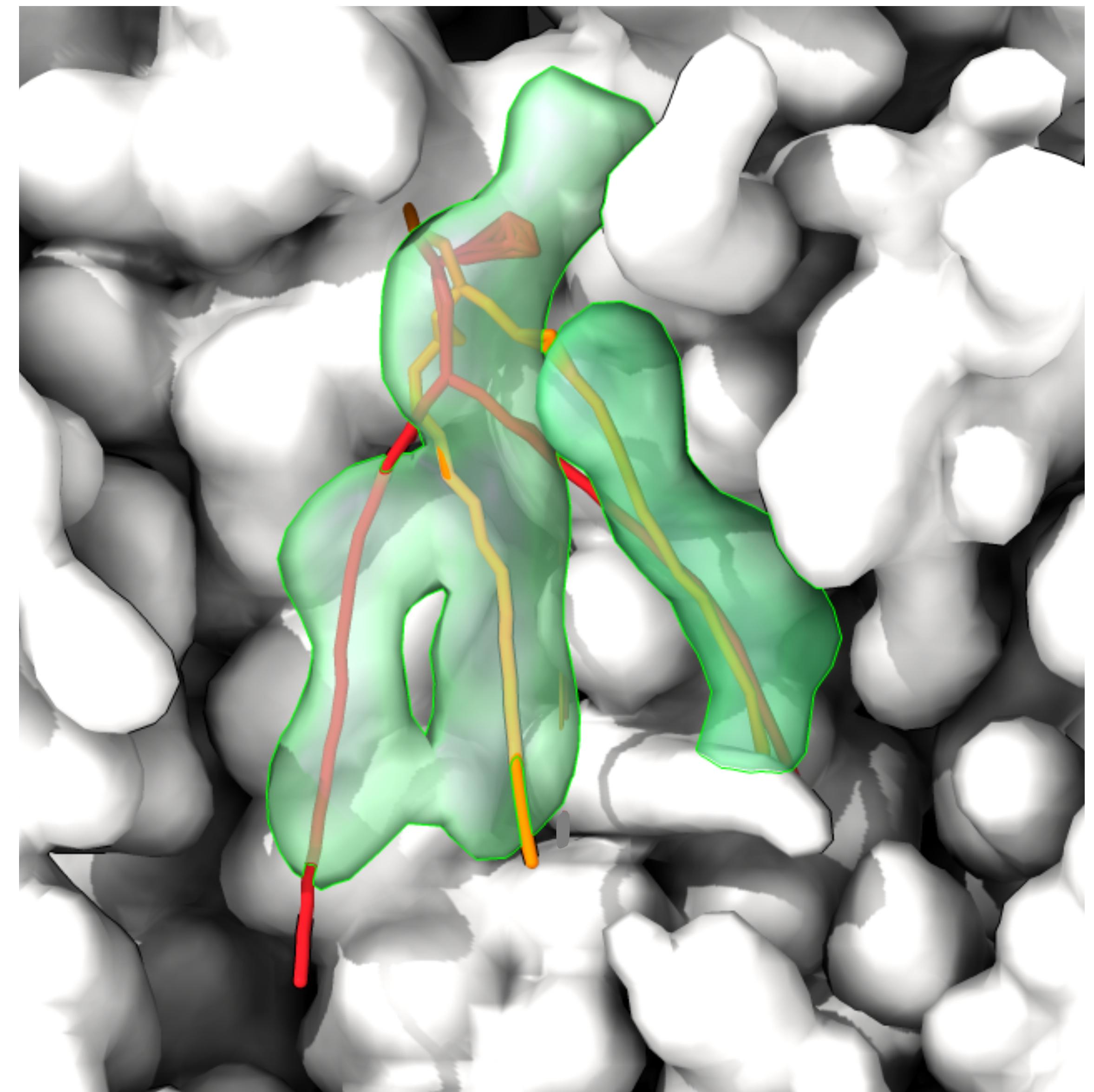
# Two Poses

Agree with the Experimental Density

- $1\mu s$  of unbiased MD
- Quality threshold clustering
- Average clusters
- Clusters are distinguishable using DBCs

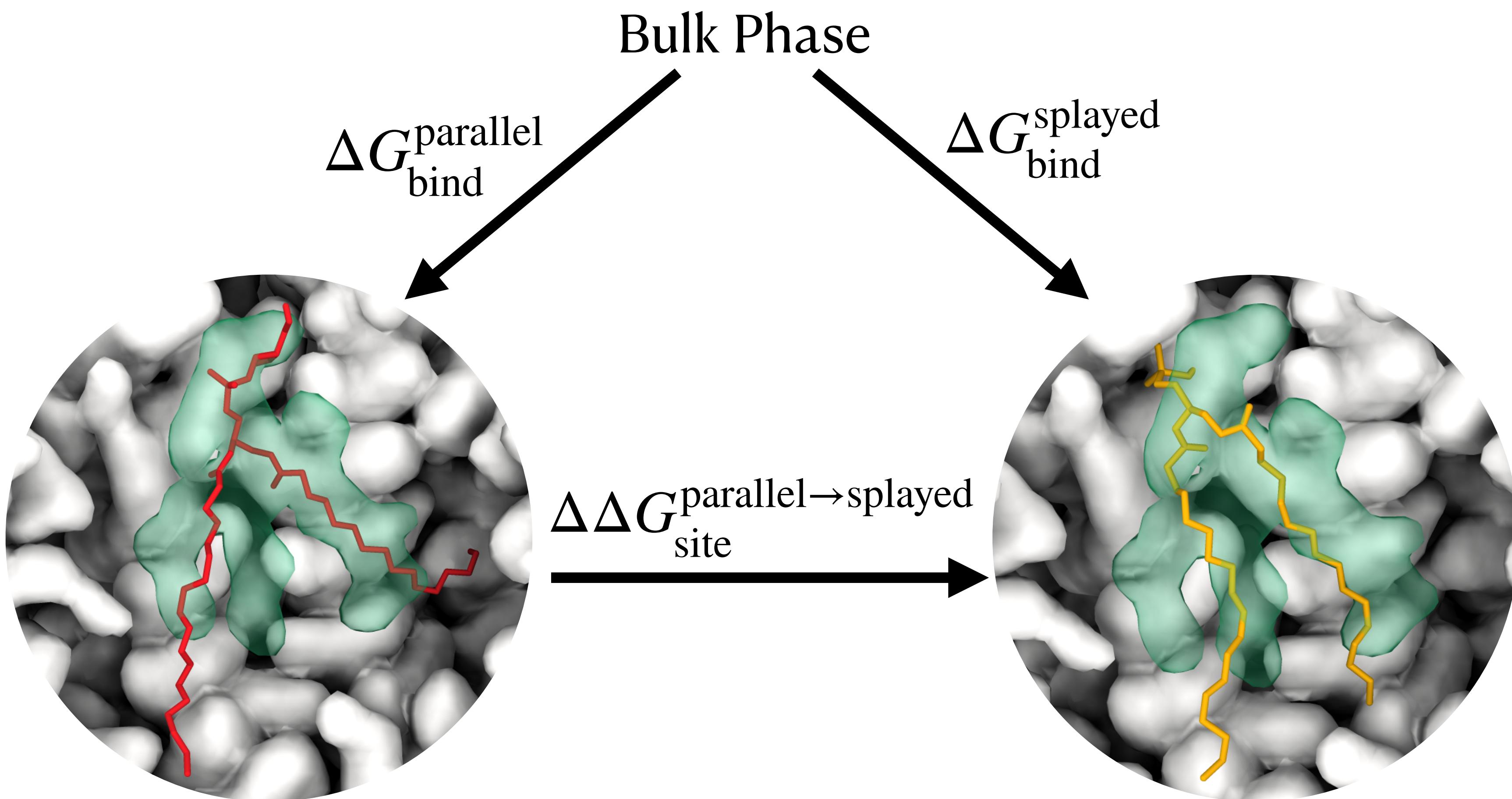


24

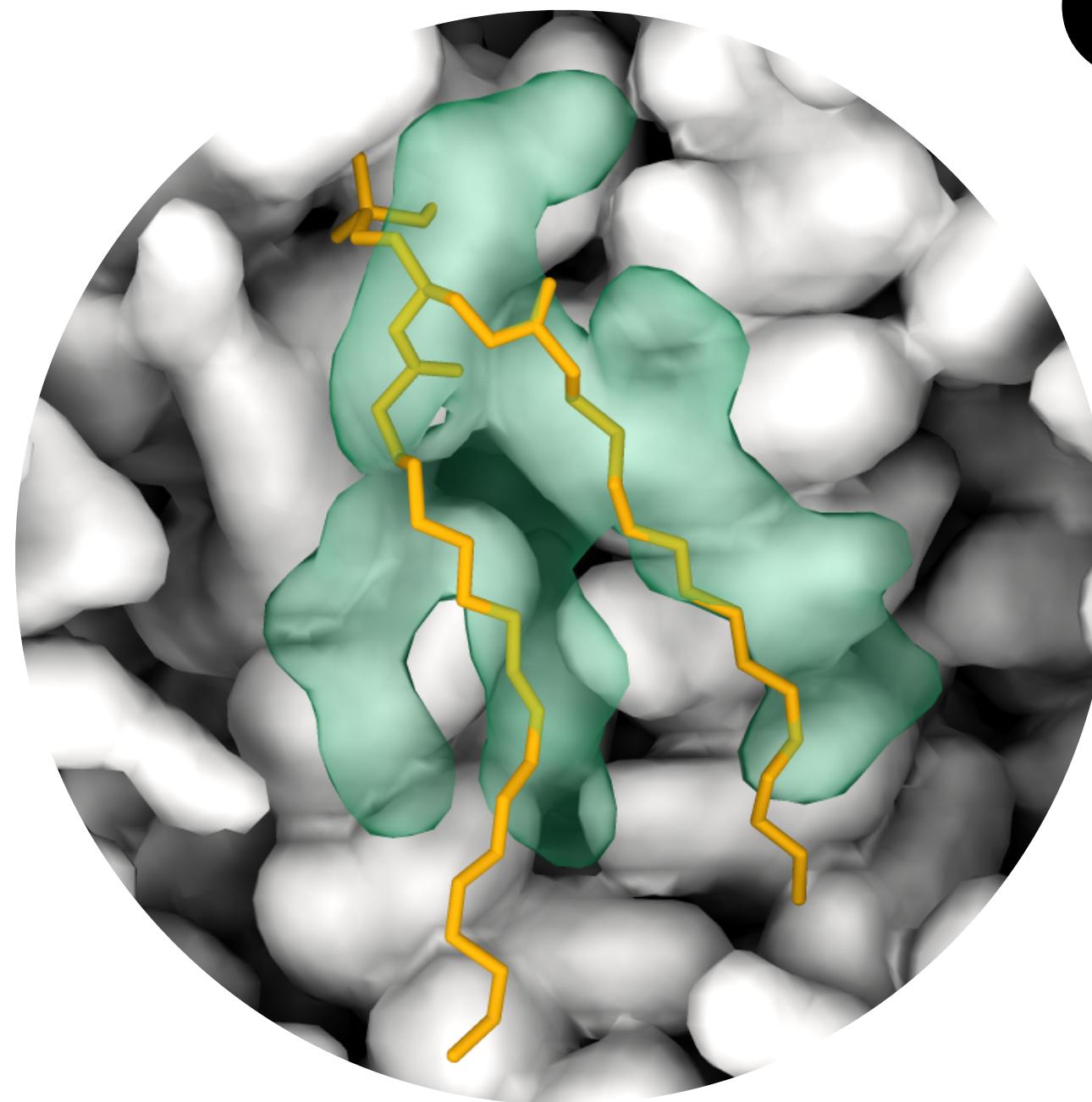


**Are Both  
Conformations Stable?**

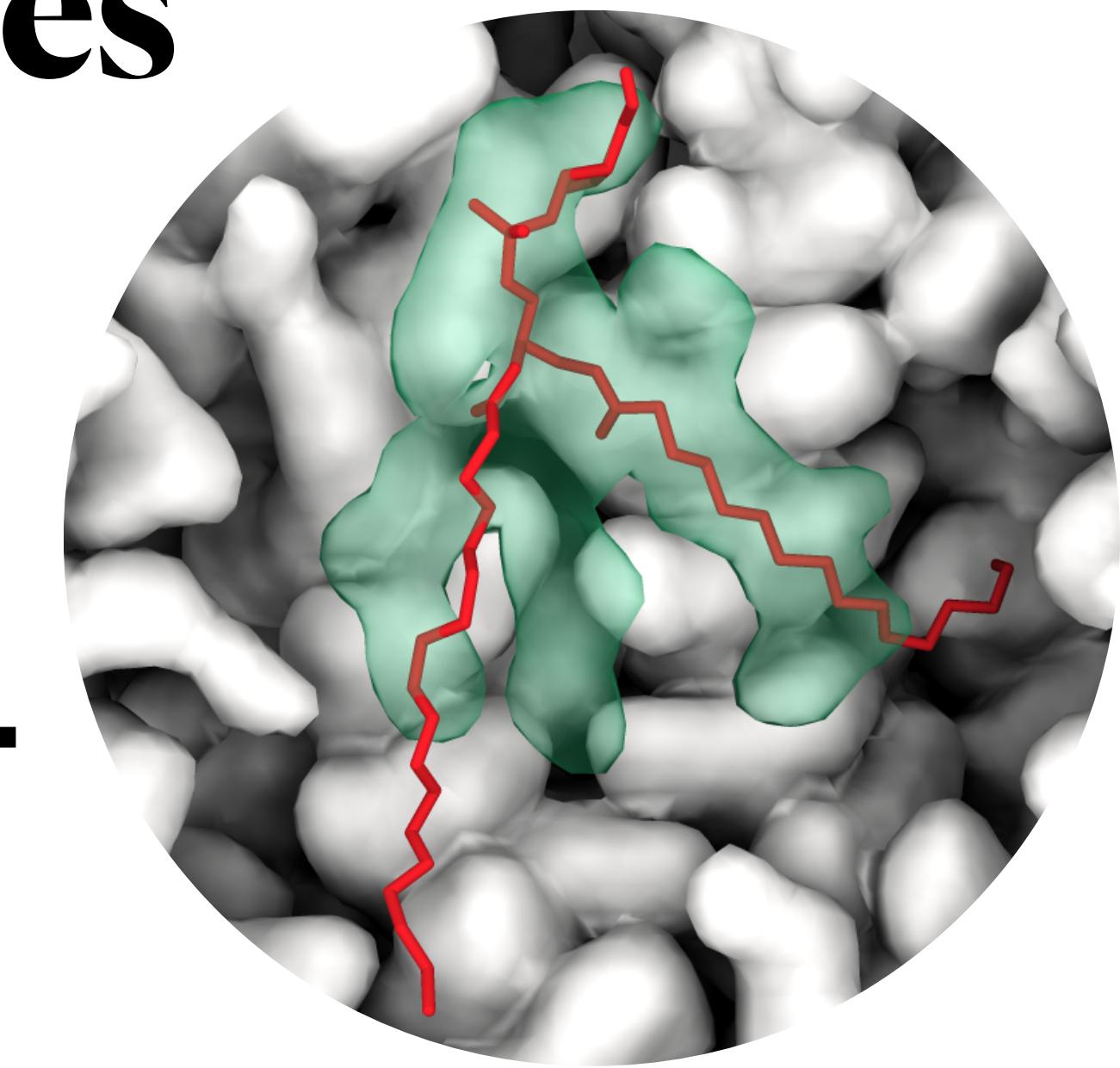
# Distinguishing Modes Using Binding Free Energies



Bound, Parallel



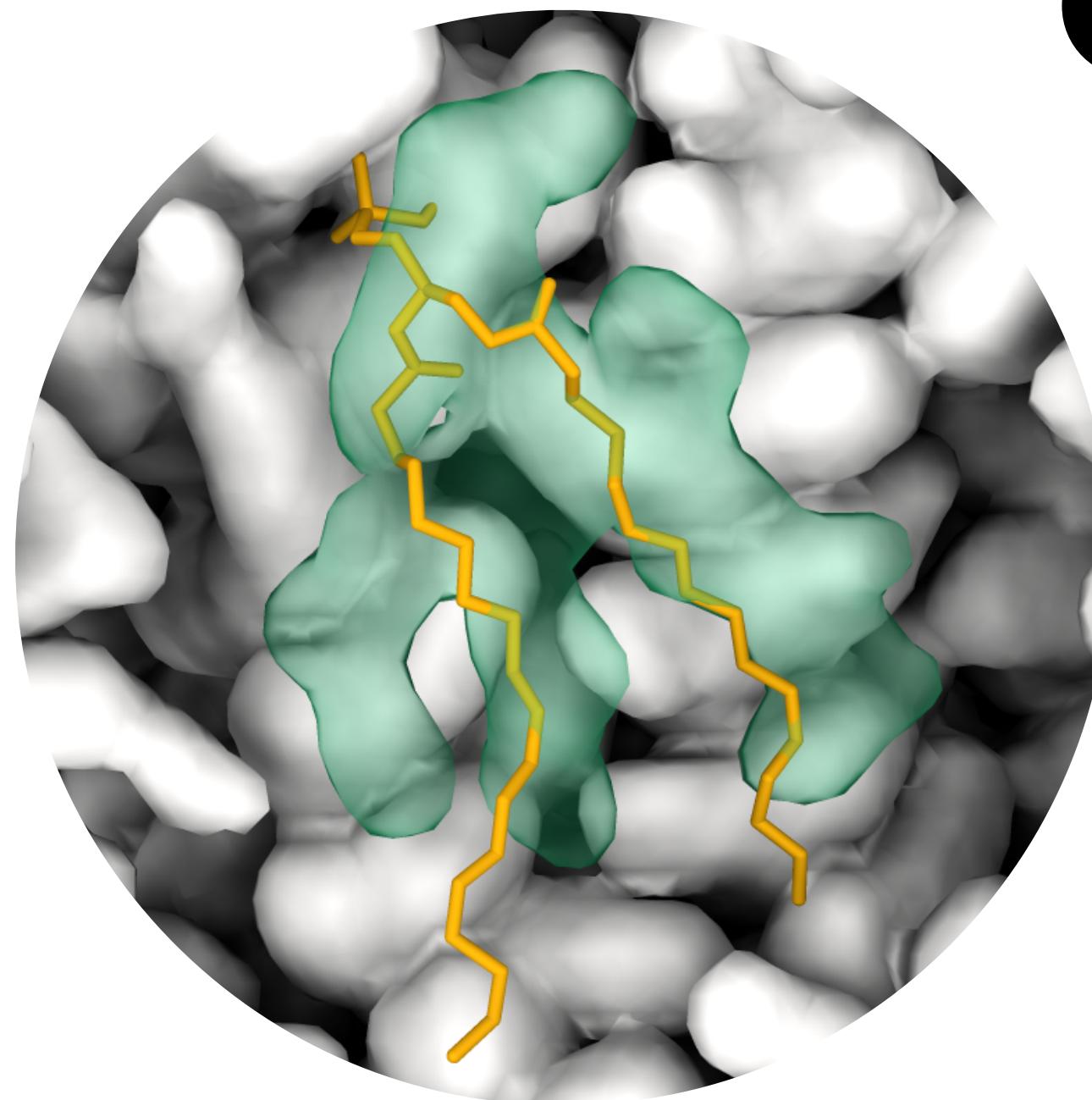
Bound, Splayed



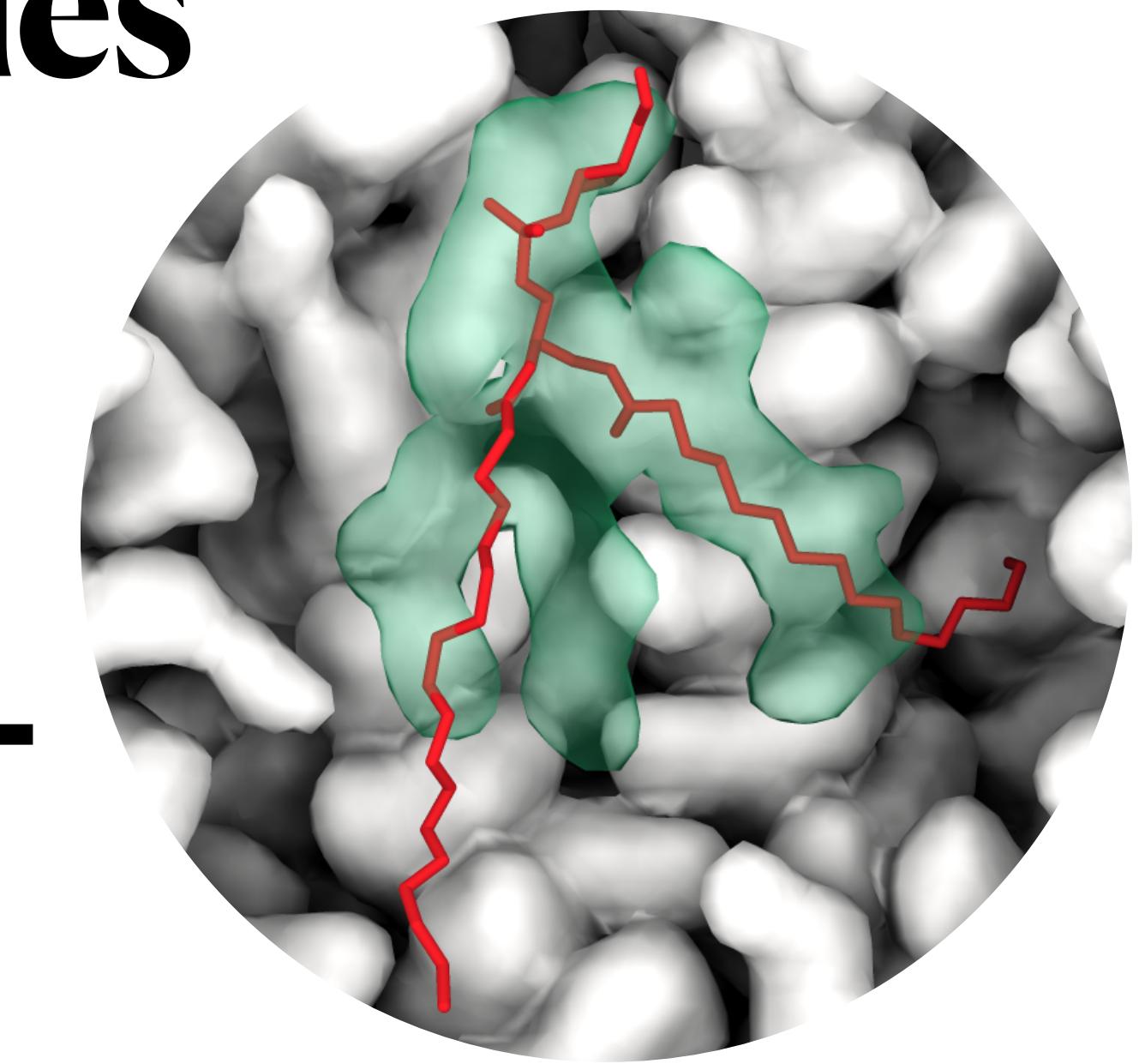
# Occupancy Probabilities



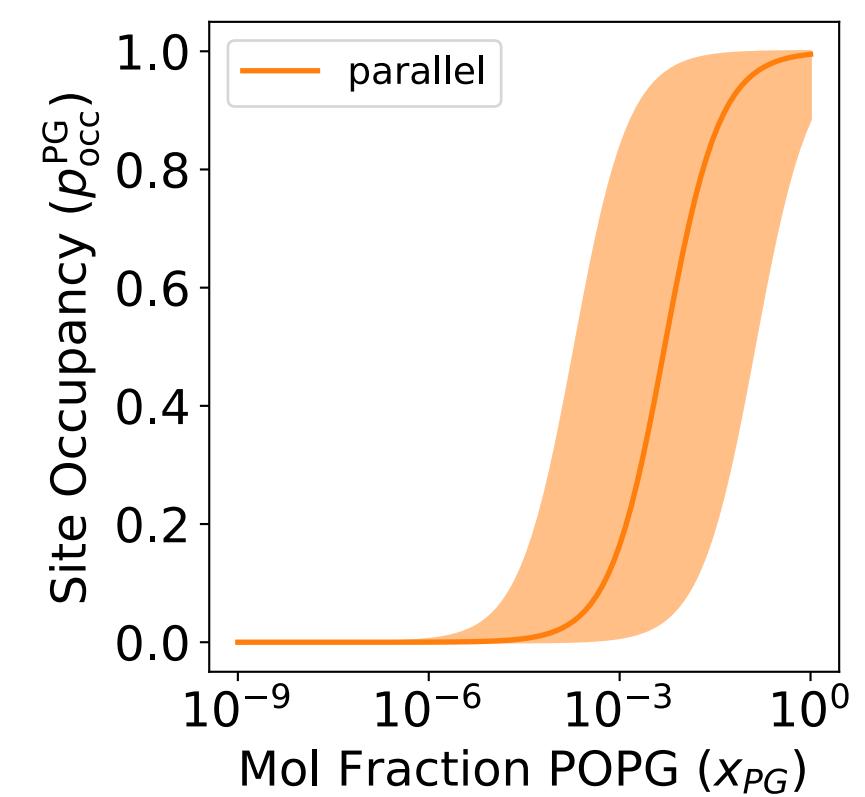
Bound, Parallel



Bound, Splayed

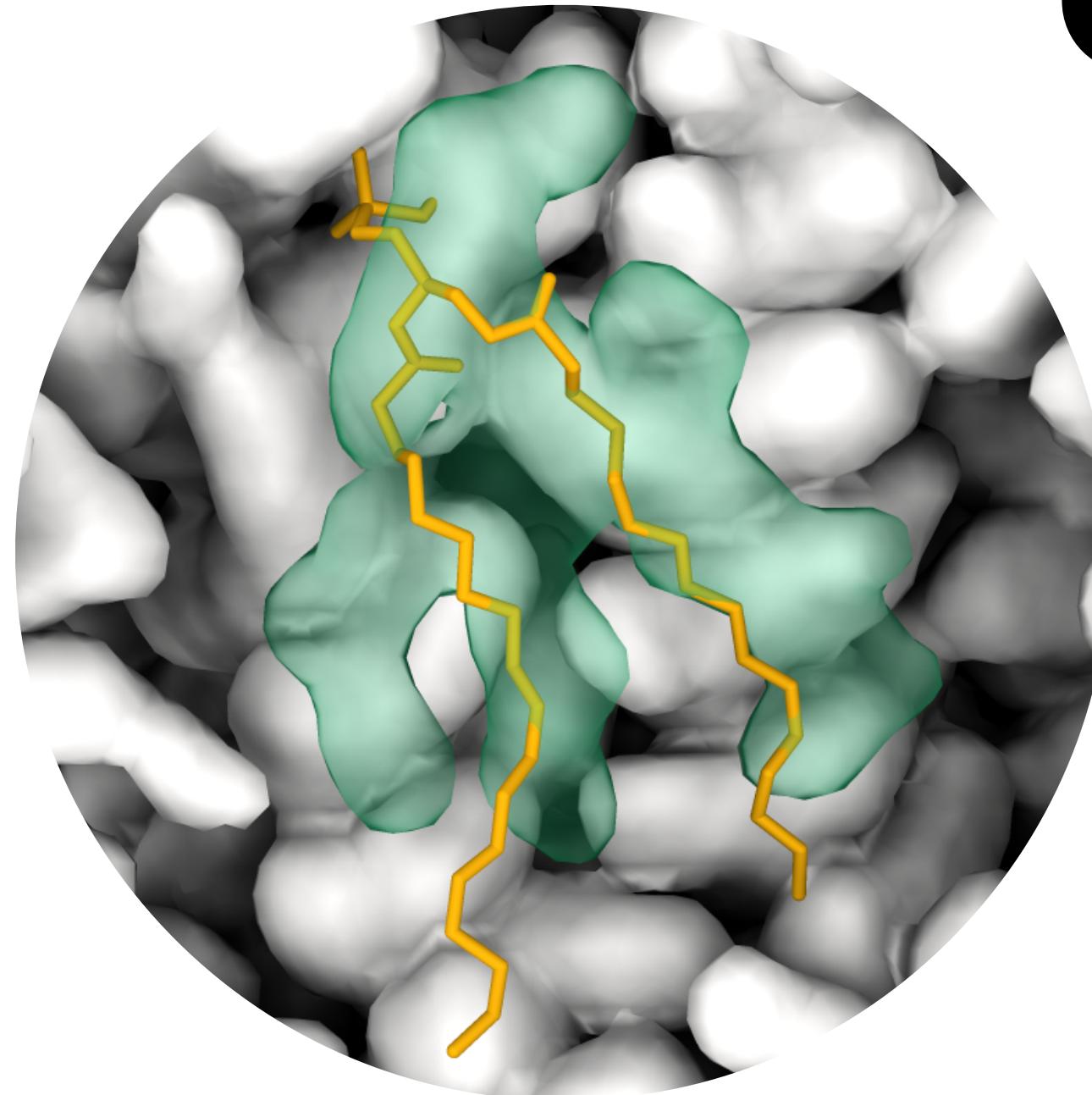


# Occupancy Probabilities



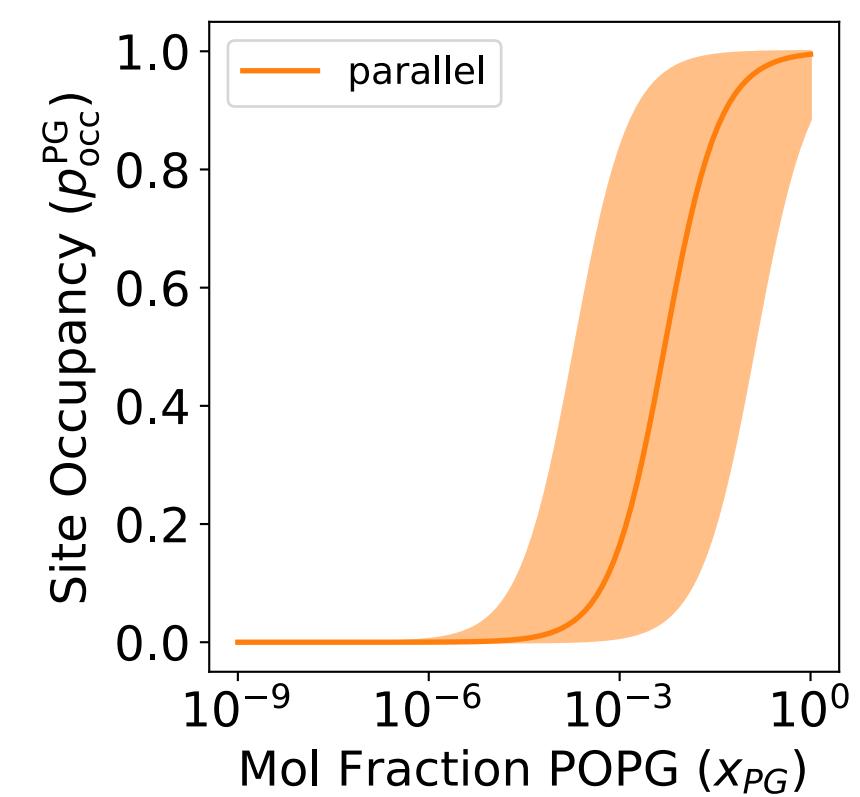
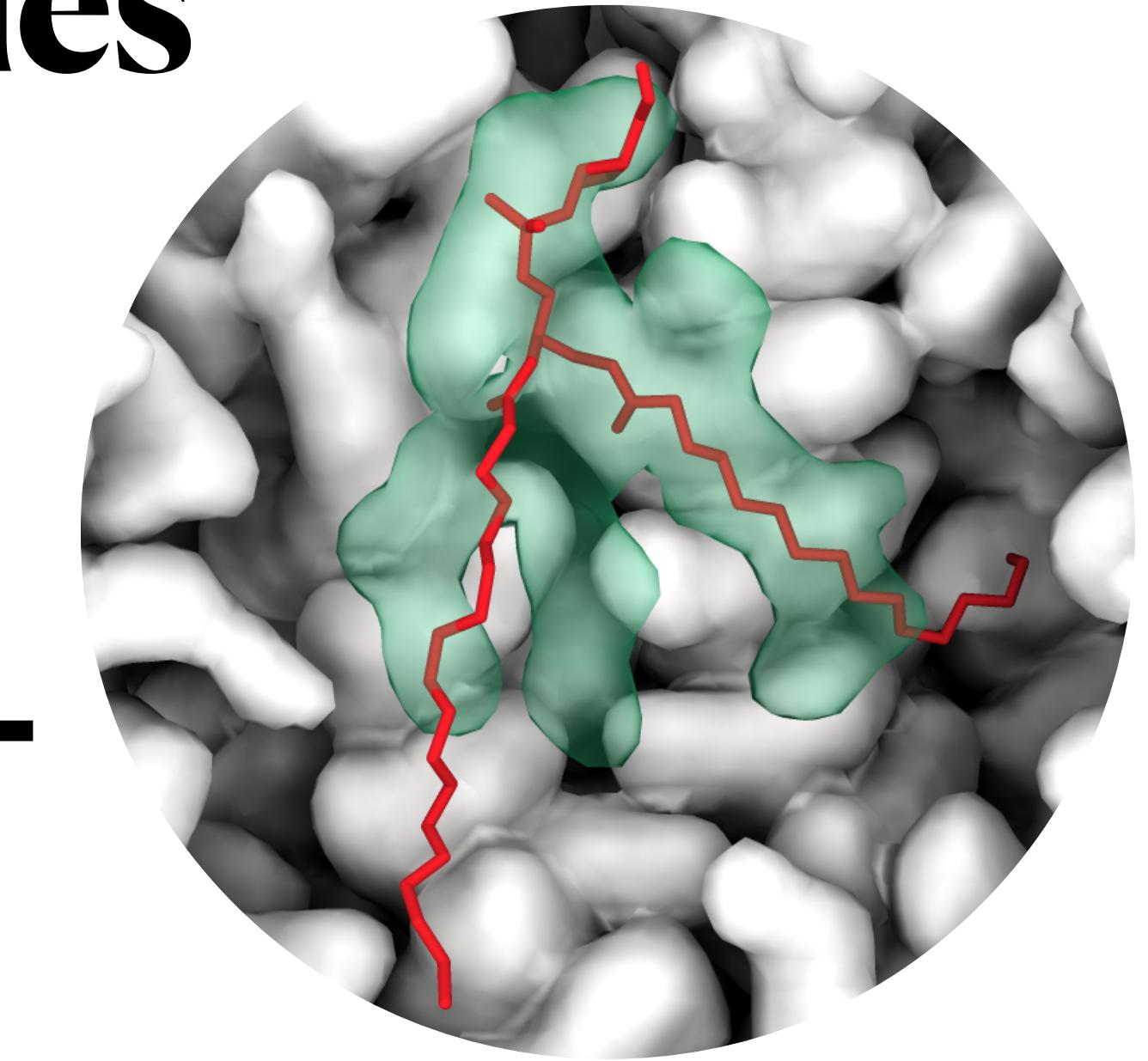
$$x_{50} = 0.5\% \pm 1\%$$

Bound, Parallel

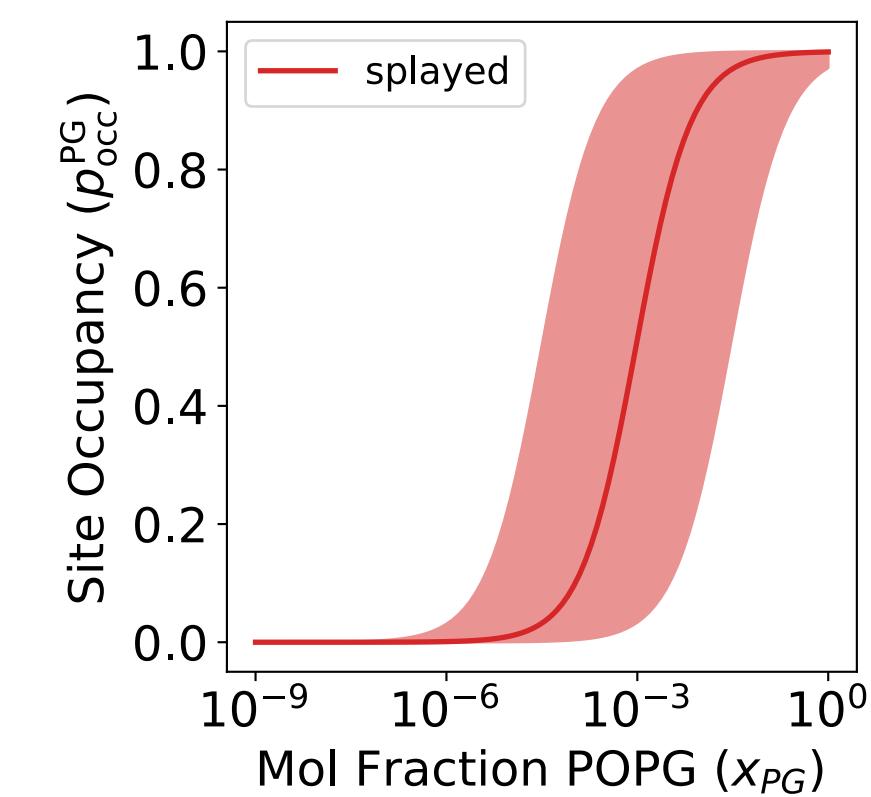


# Occupancy Probabilities

Bound, Splayed

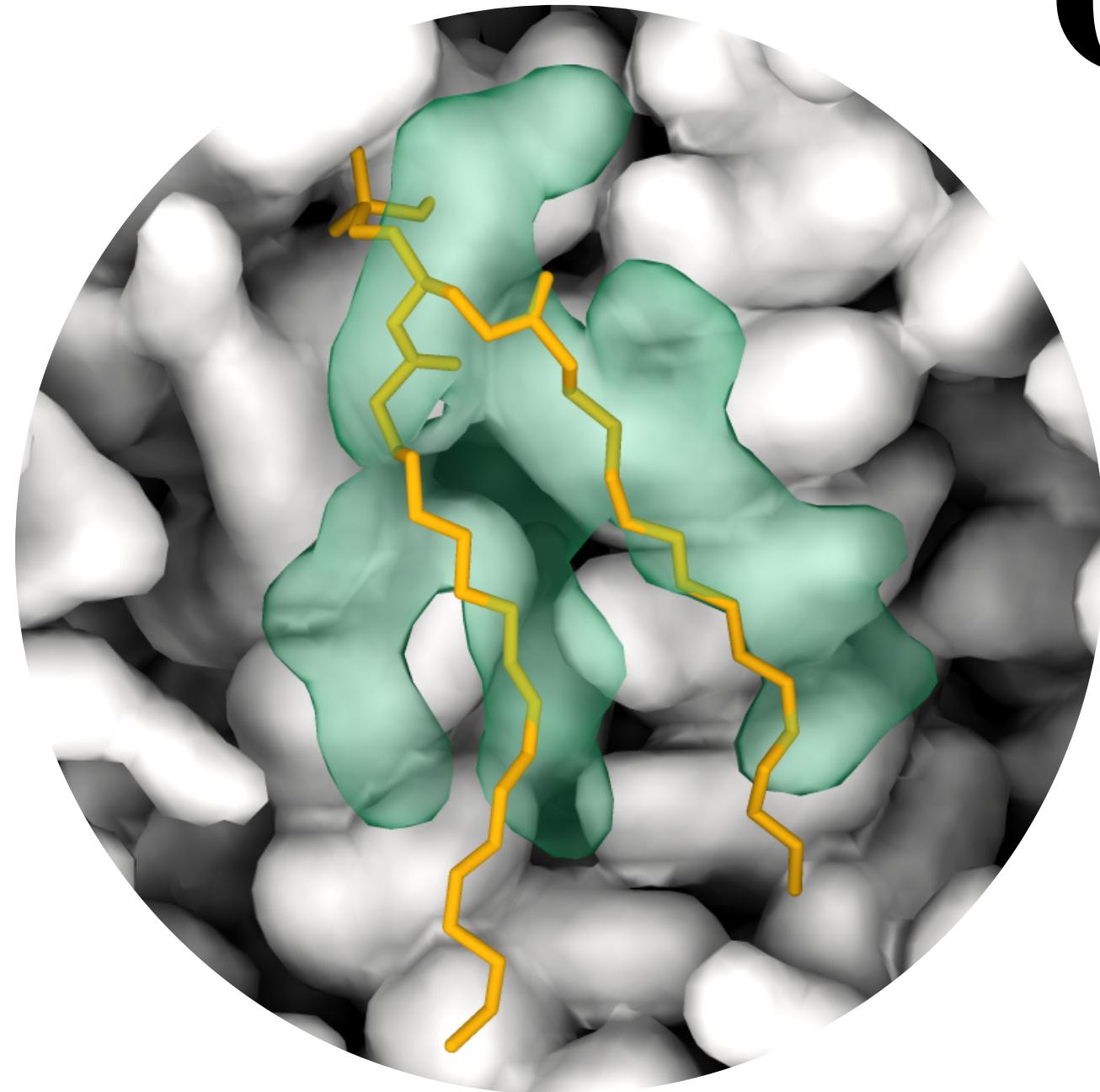


$$x_{50} = 0.5\% \pm 1\%$$

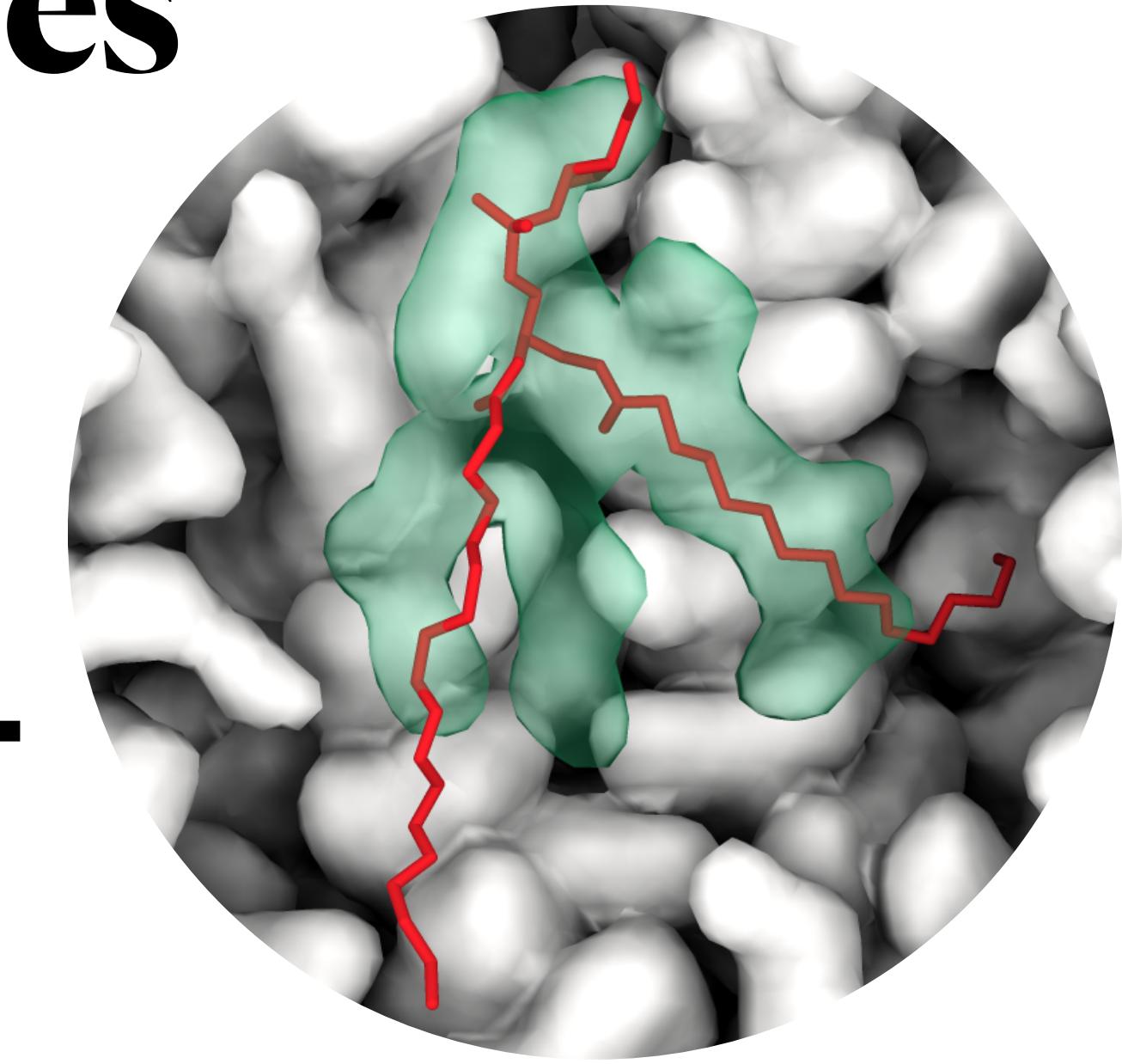


$$x_{50} = 0.1\% \pm 0.3\%$$

Bound, Parallel

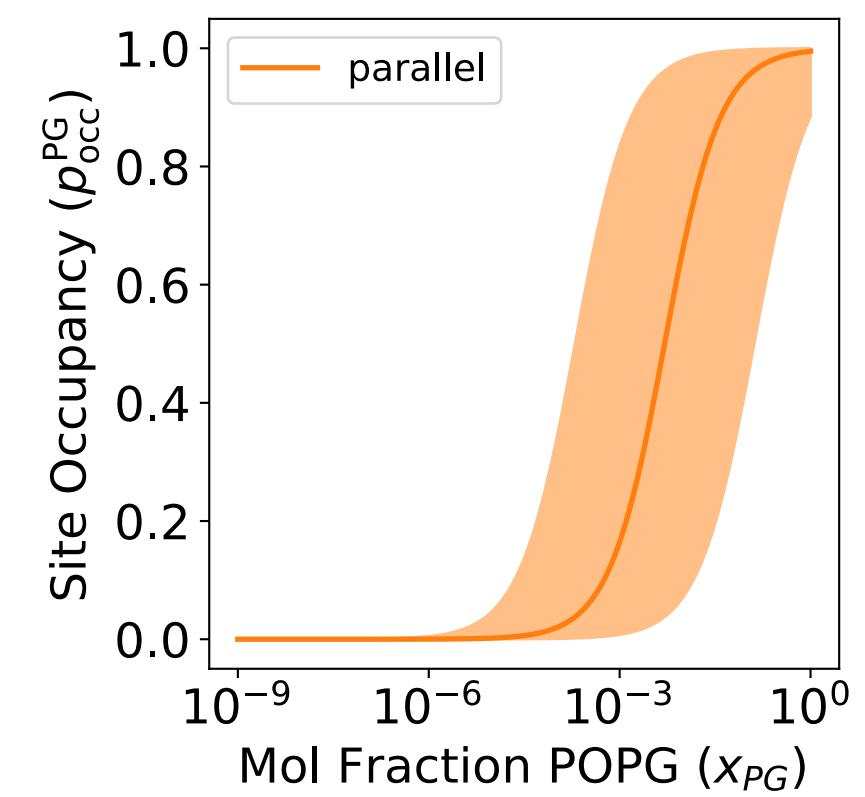


Bound, Splayed

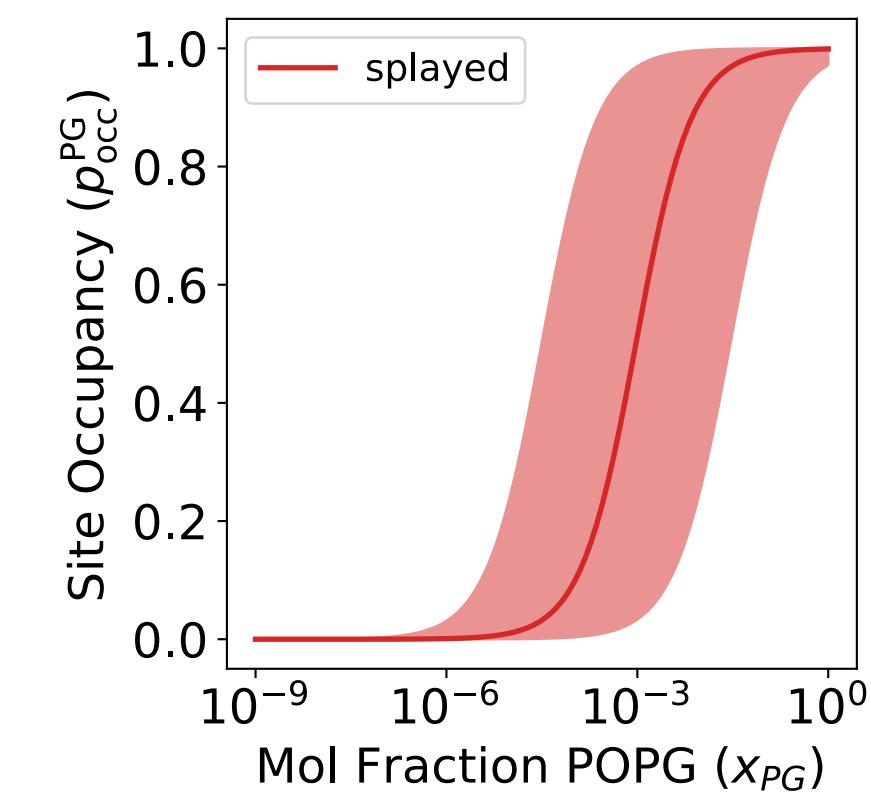


# Occupancy Probabilities

$1 \pm 1.5 \text{ kcal/mol}$

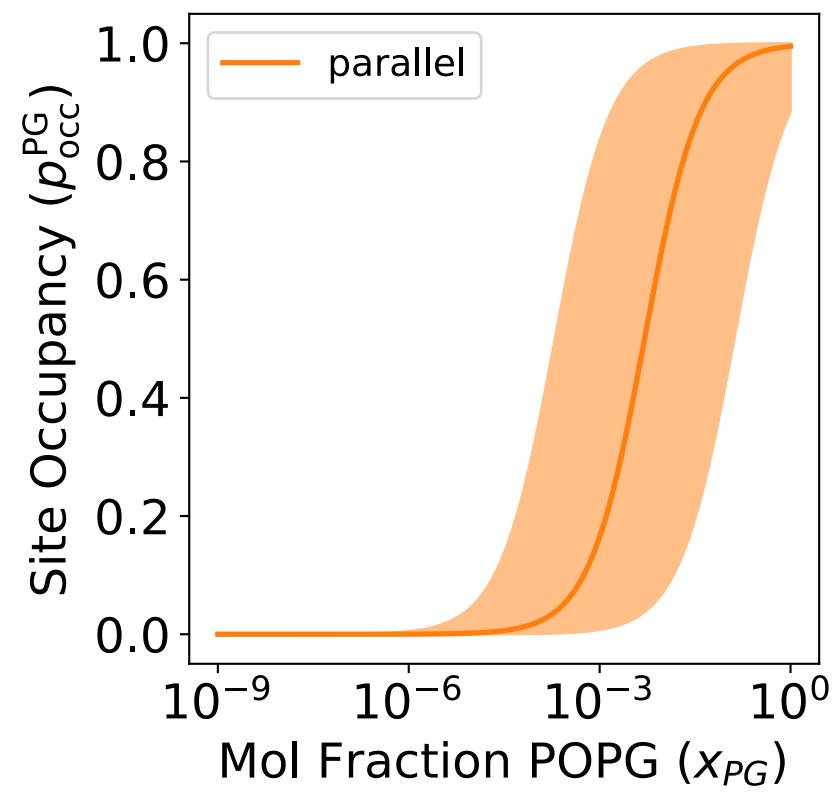
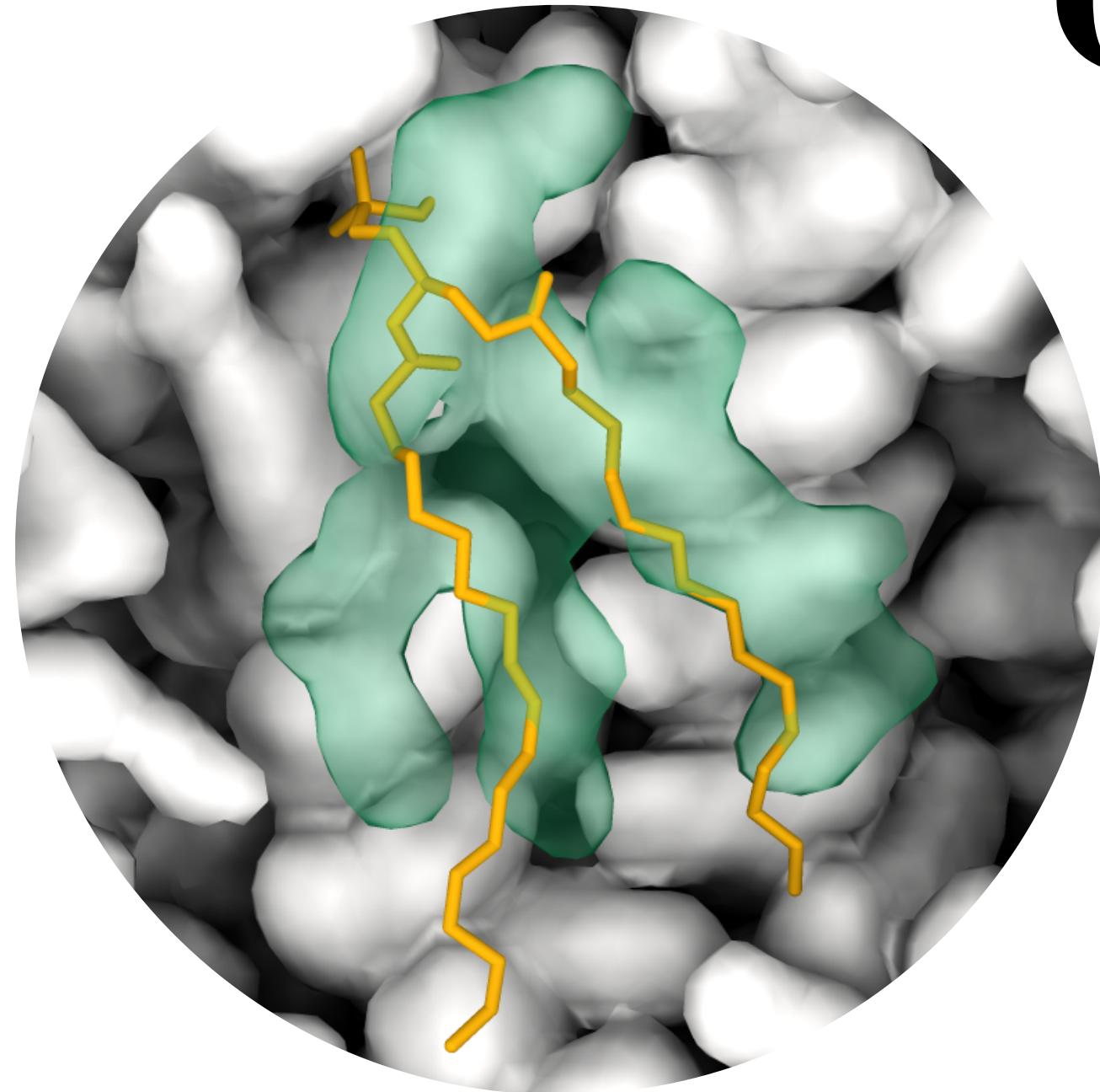


$$x_{50} = 0.5\% \pm 1\%$$

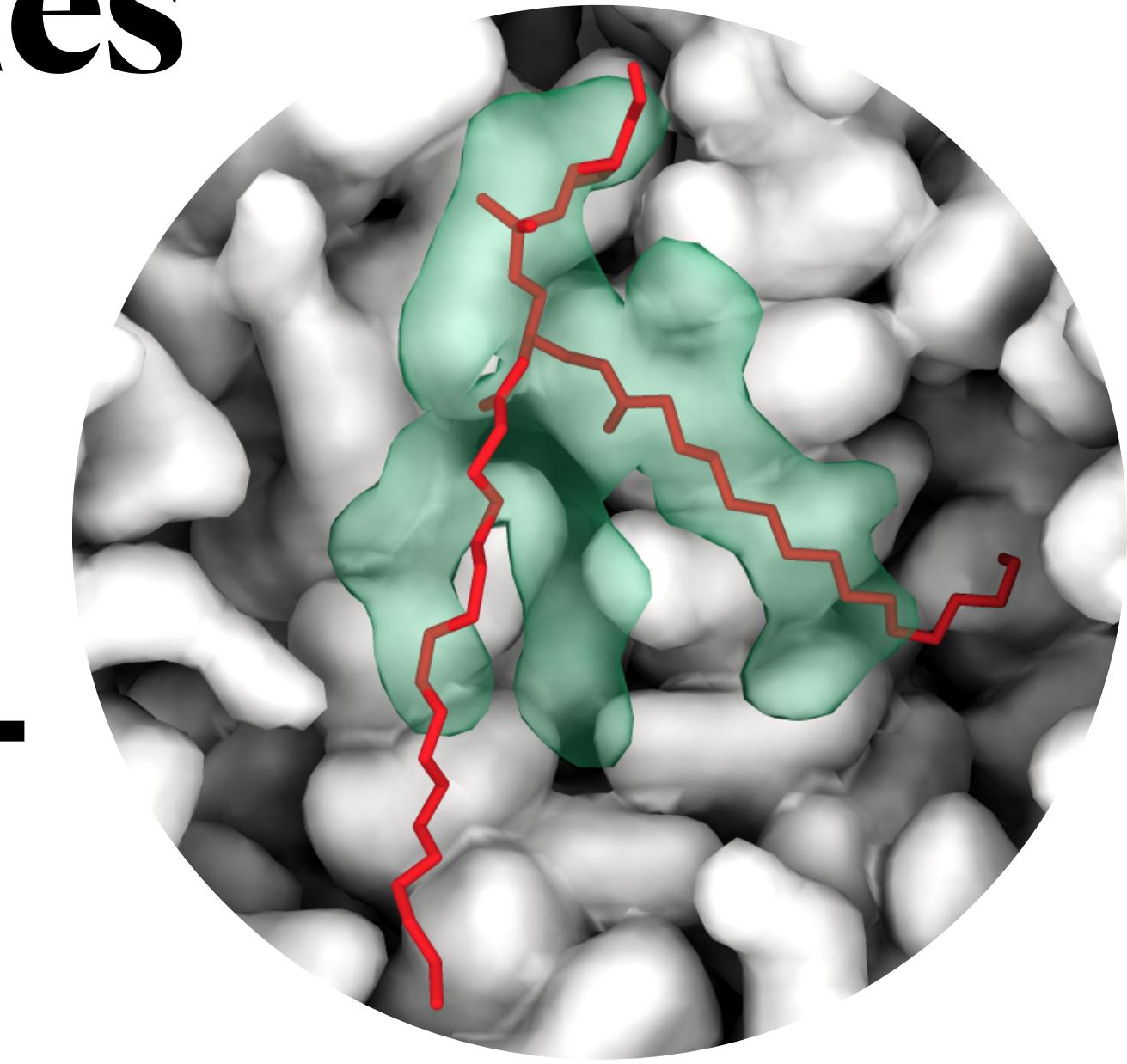


$$x_{50} = 0.1\% \pm 0.3\%$$

Bound, Parallel



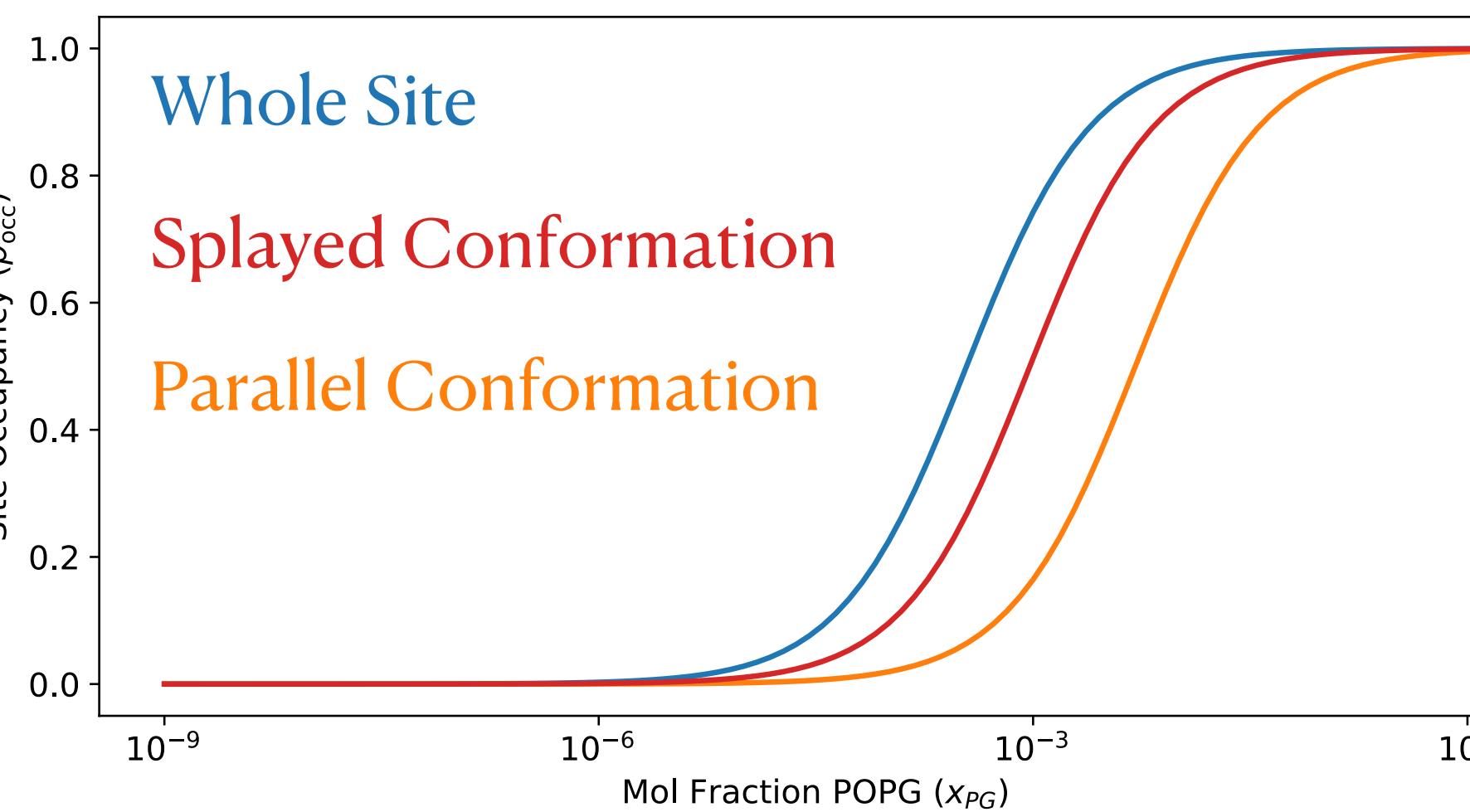
Bound, Splayed



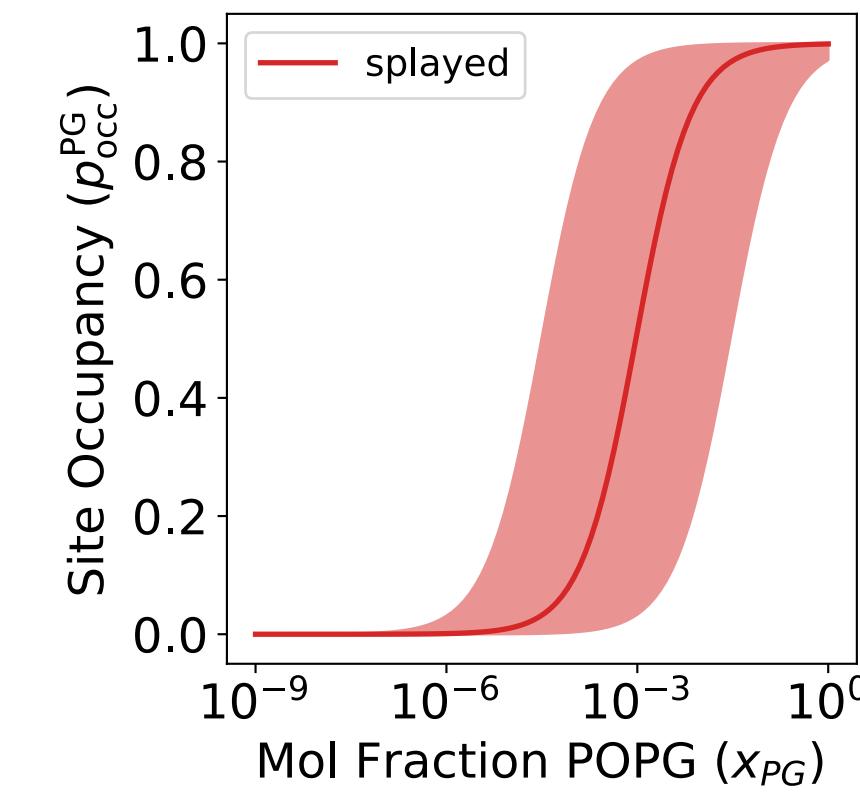
$$x_{50} = 0.1\% \pm 0.3\%$$

# Occupancy Probabilities

$$1 \pm 1.5 \text{ kcal/mol}$$

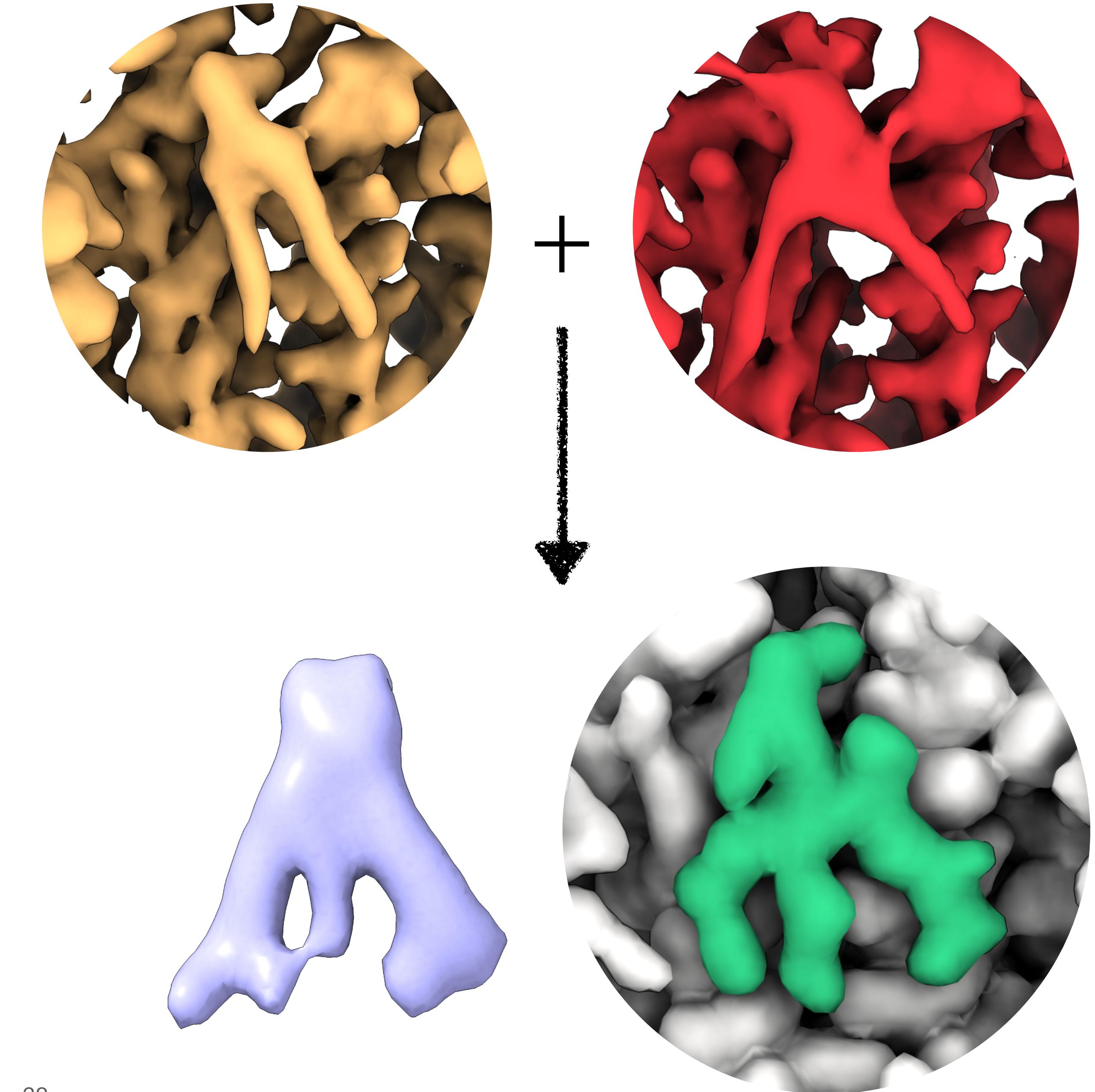


$$x_{50} = 0.5\% \pm 1\%$$



# Conclusions

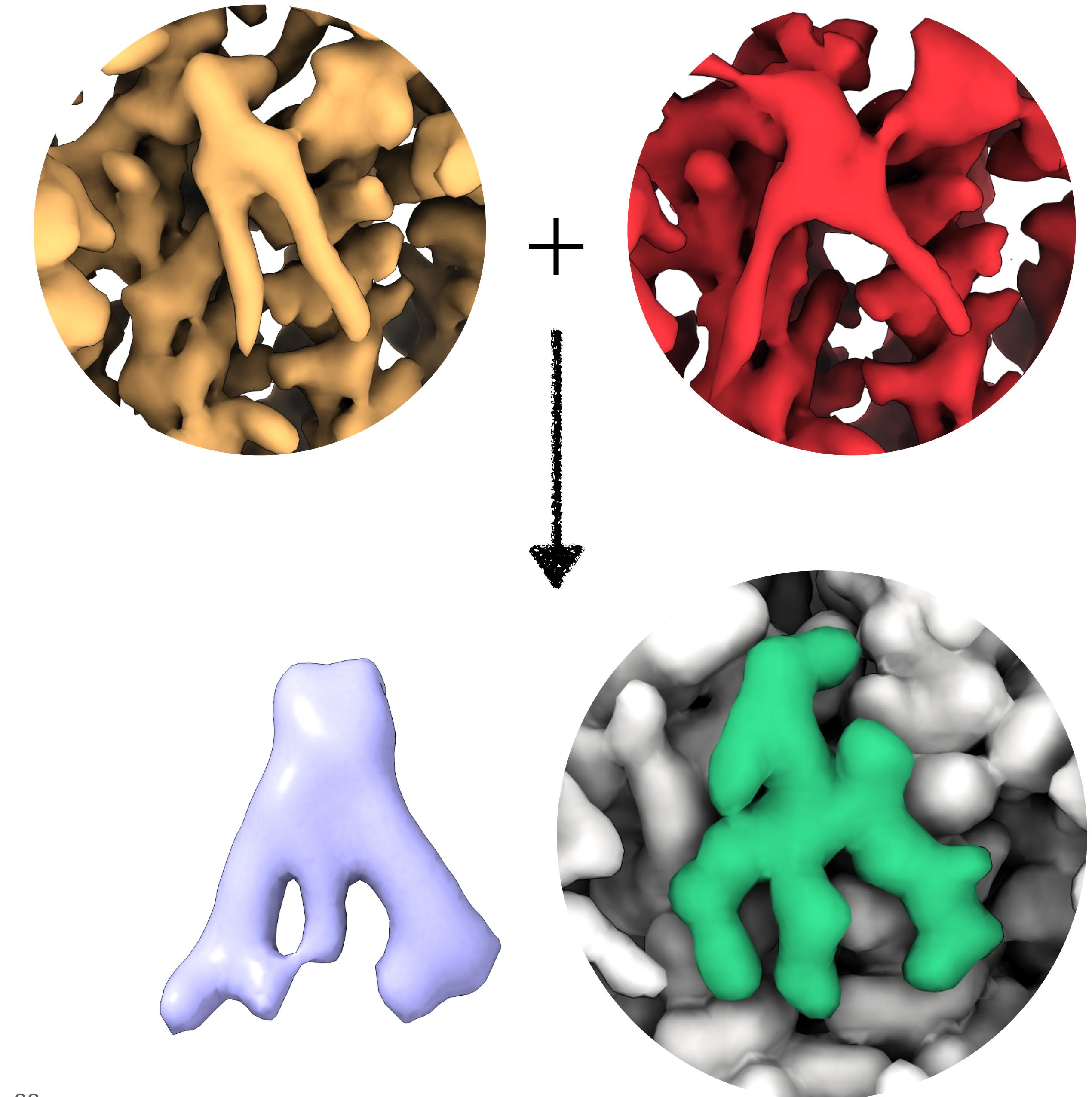
**POPG Binds ELIC**



# Conclusions

## POPG Binds ELIC

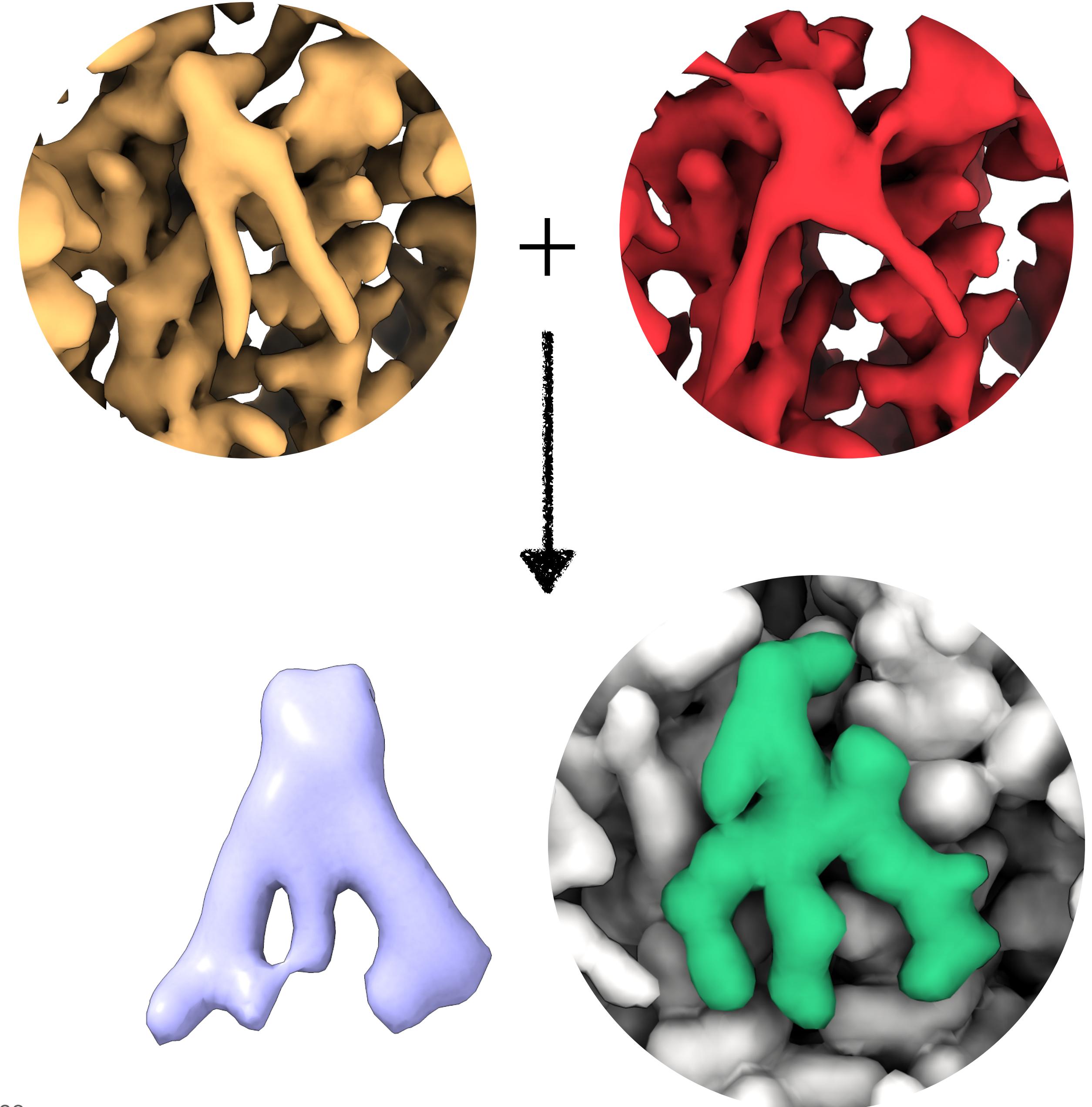
- POPG binds:
  - high affinity for the closed state
  - *higher* for the open state



# Conclusions

## POPG Binds ELIC

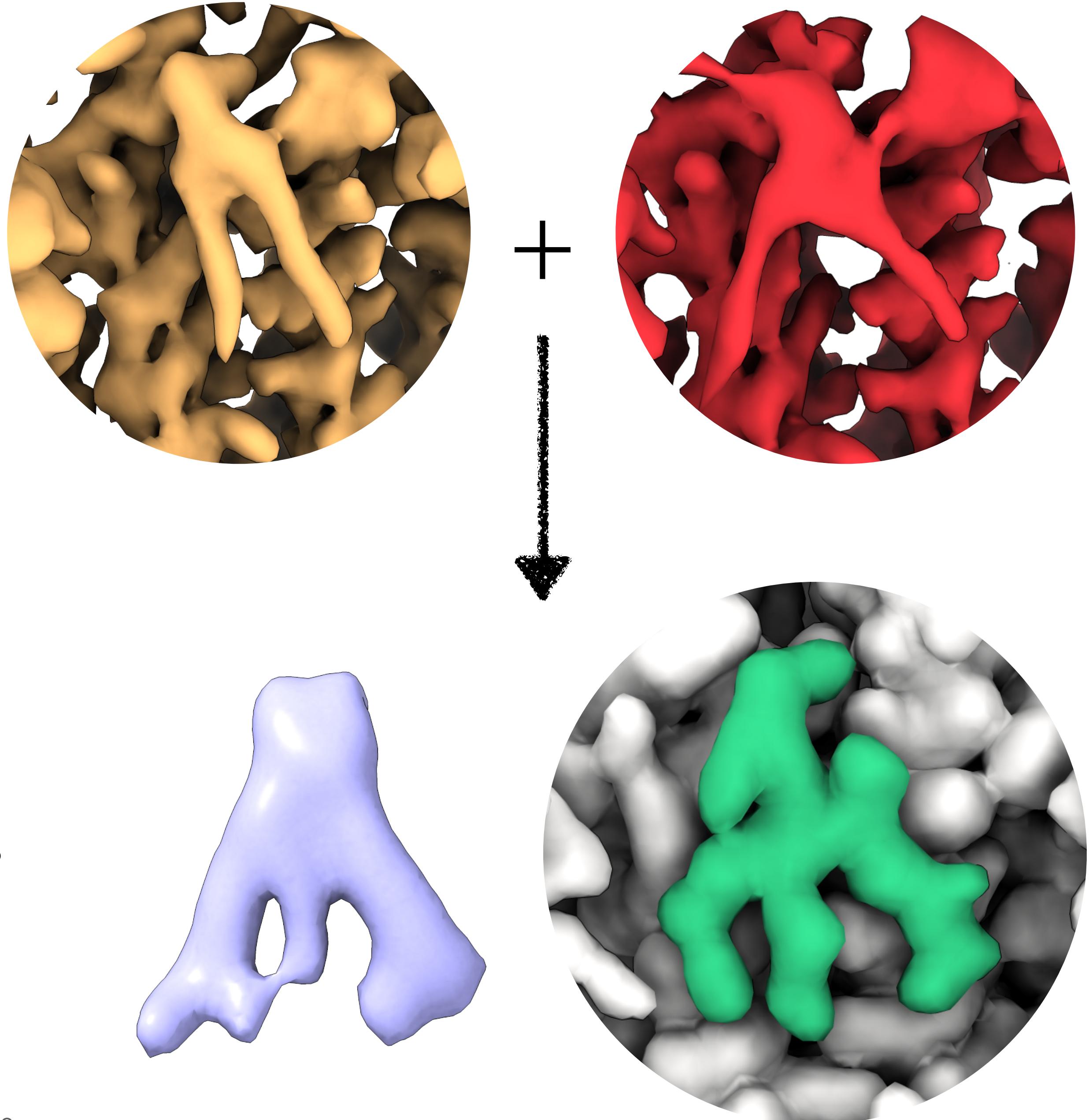
- POPG binds:
  - high affinity for the closed state
  - *higher* for the open state
- SAFEP can provide conformation-specific binding affinities



# Conclusions

## POPG Binds ELIC

- POPG binds:
  - high affinity for the closed state
  - *higher* for the open state
- SAFEP can provide conformation-specific binding affinities
- One lipid *could* produce the observed (three-legged) density
  - POPG has strong affinity for both binding modes
  - Averaging over modes can produce a three-legged density





## Brannigan Lab



R|OARC

ACCESS



## Wayland Cheng's Lab

## Collaborators

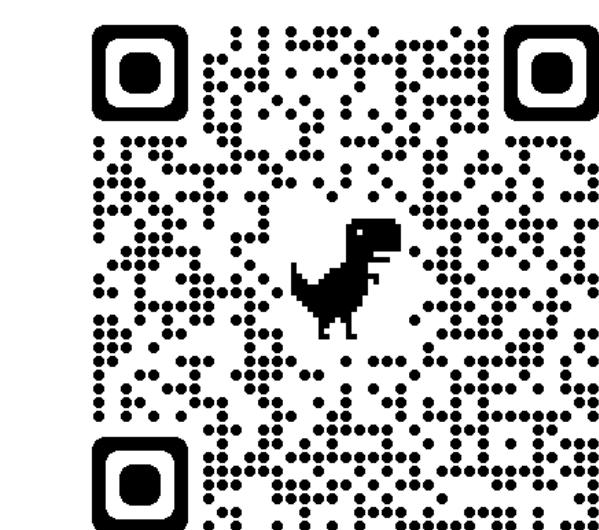


### Jérôme Hénin



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### Tom Joseph



### SAFEP Tutorial



