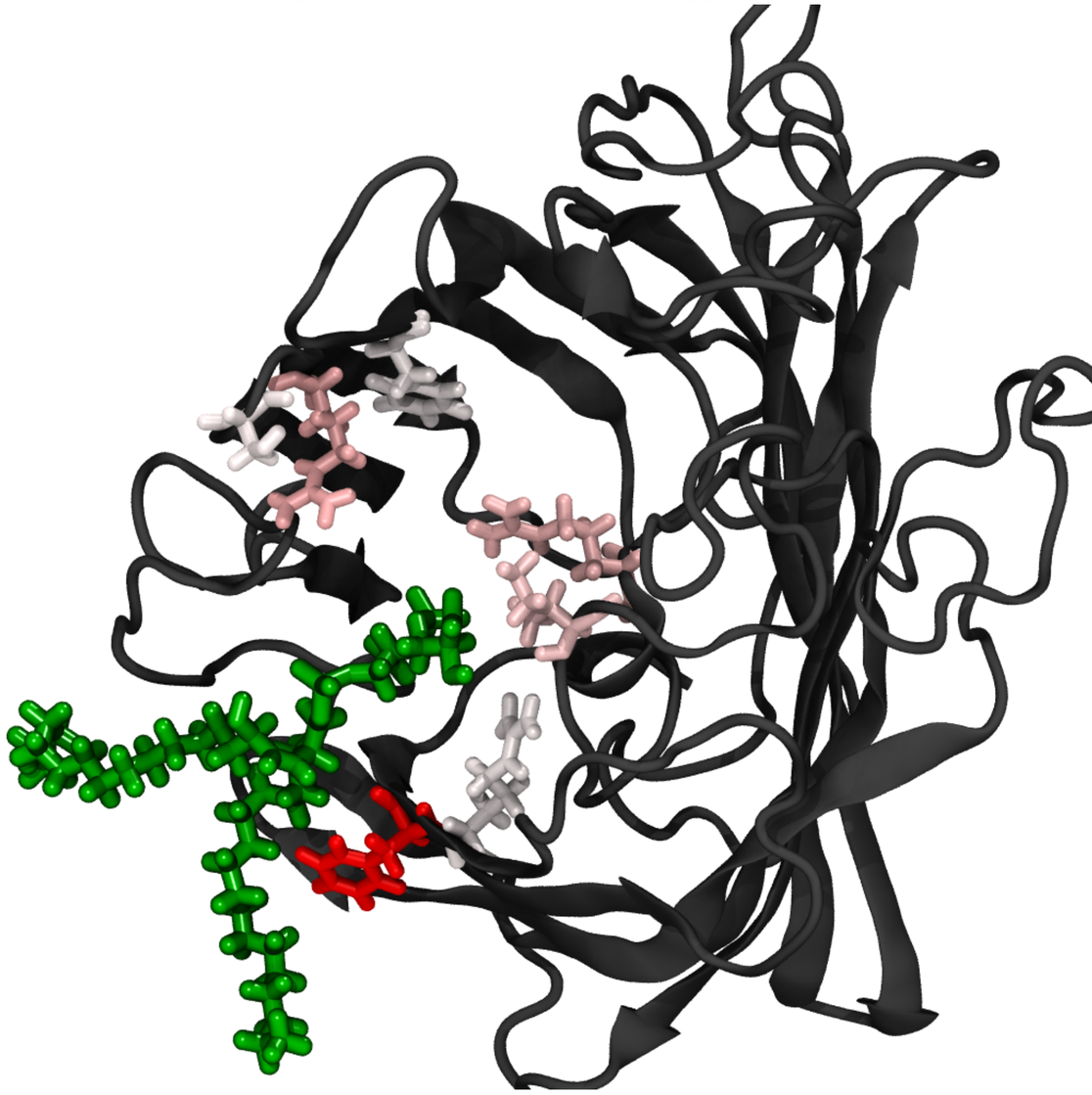
Investigating Ceramide Binding to BamA in Model Membranes



Jahmal Ennis

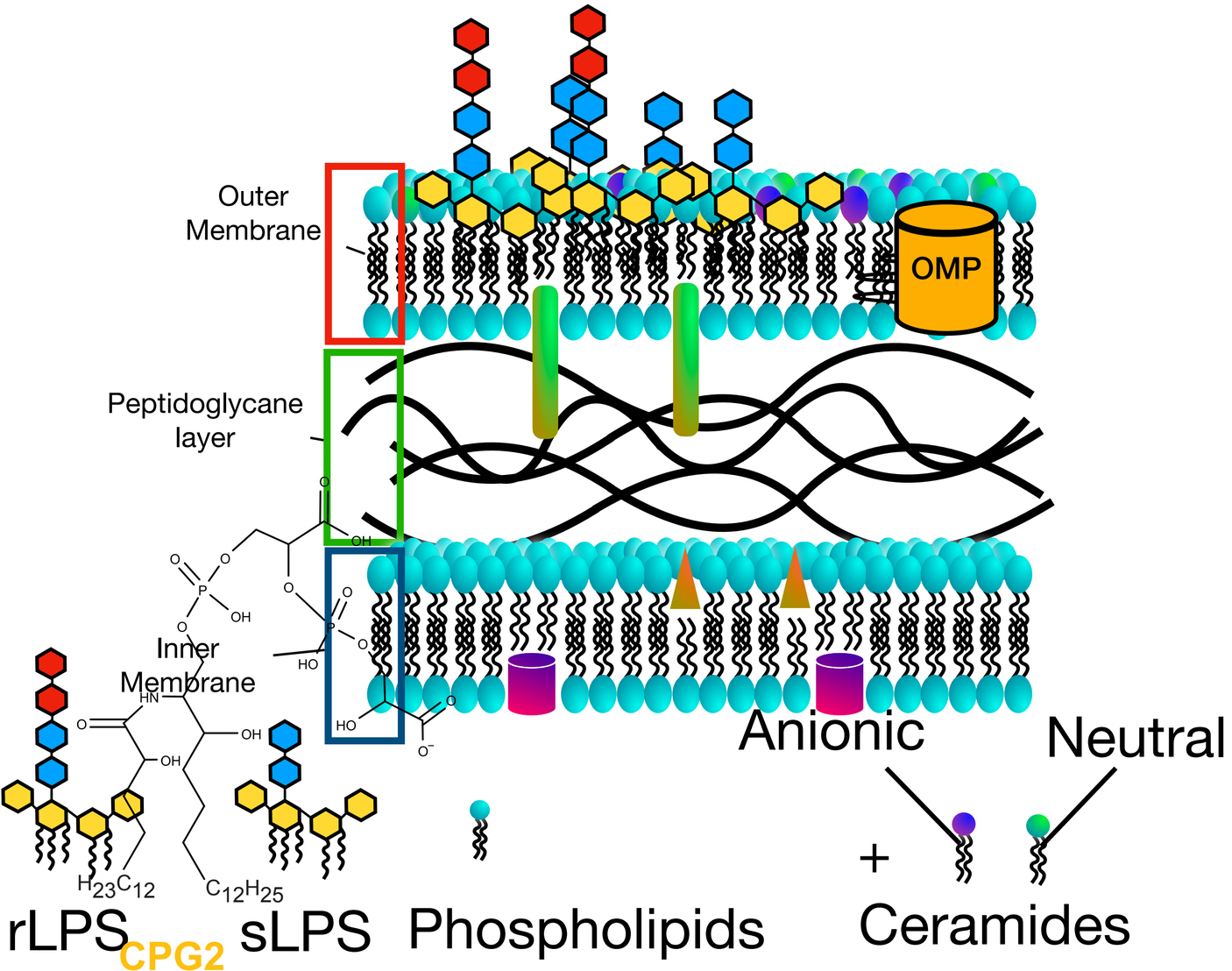
Overview

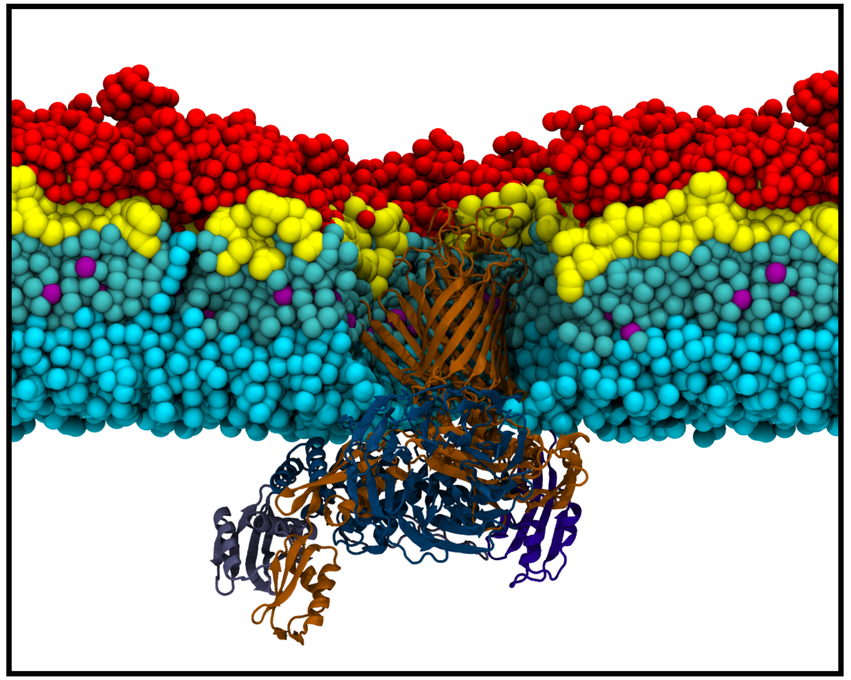
- They key players of the story (BamA and CPG2)
- Why CPG2 may be binding to BamA
- How do we plan to study lipid-protein binding
- Did we find evidence of binding?

They key players: Anionic Ceramides and BamA

Caulobacter Membrane

BamA





The Role of Ceramides in Bacterial Membranes Is Unclear

In humans ceramides play many different roles:

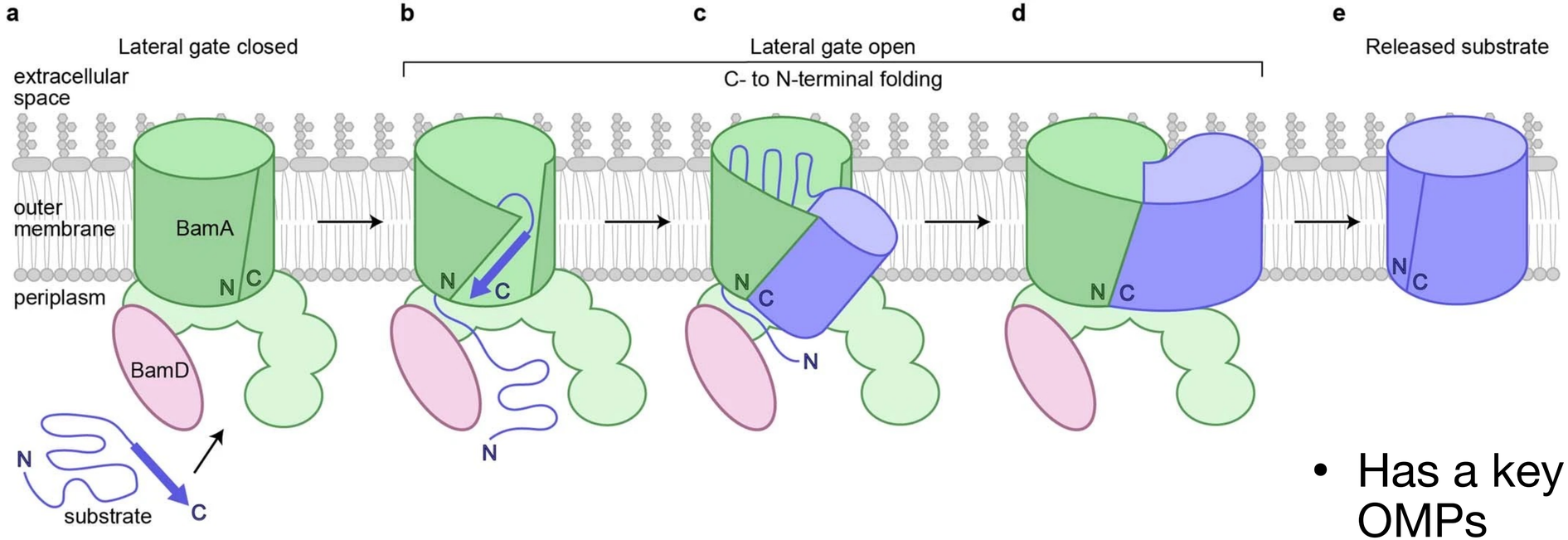
- Found in the skin
- Create tight barriers
- Reduce permeability of membrane

In Bacteria:

- Incorporated into the membrane

?

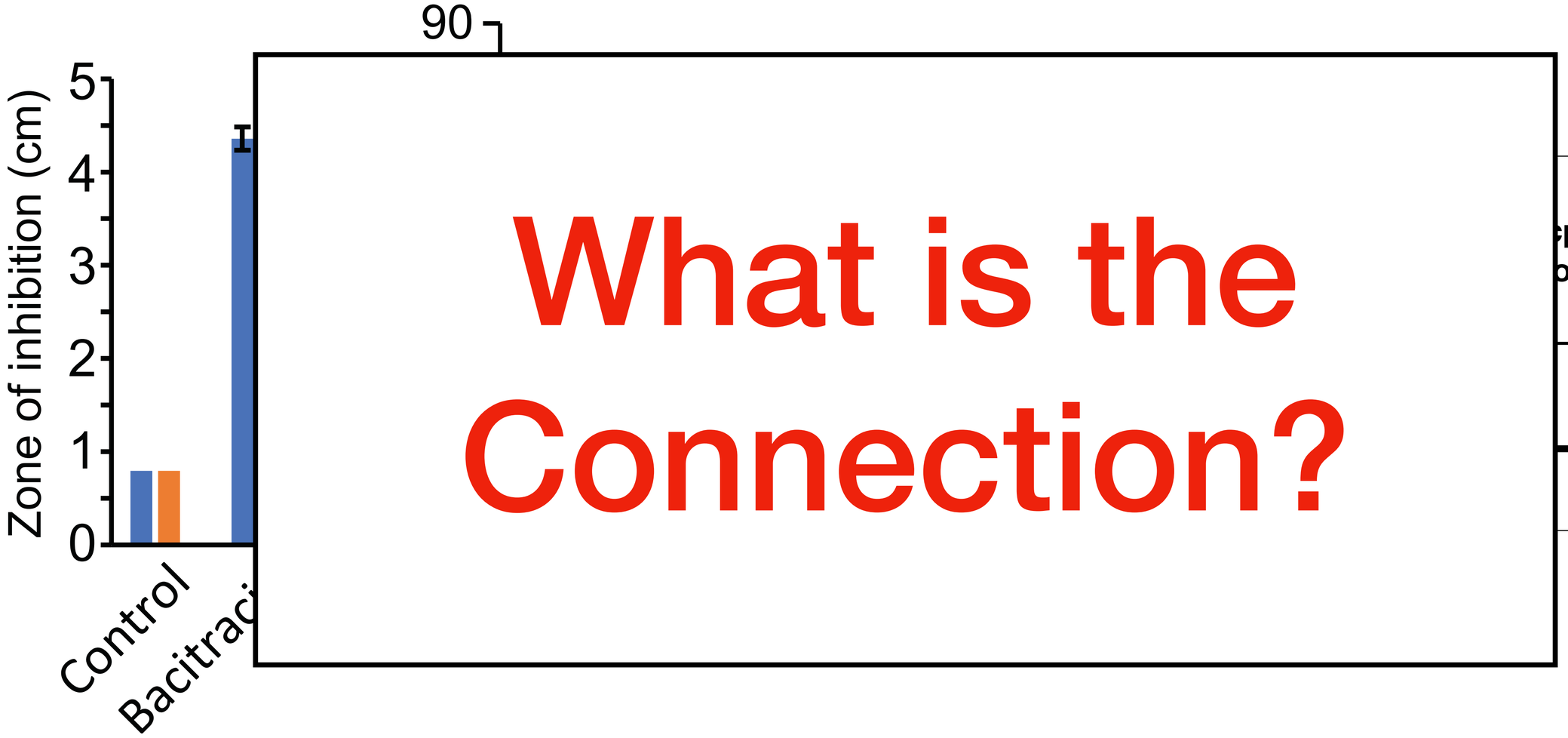
Bam Complex folds proteins in the outer membrane



- Has a key role in folding OMPs
- Functionally conserved across gram-negative bacteria
- At the cell surface making it a good therapeutic drug target

Tomasek, D., Rawson, S., Lee, J. *et al.* Structure of a nascent membrane protein as it folds on the BAM complex. *Nature* **583**, 473–478 (2020)

Ceramides may modulate BamA Function



What is the Connection?

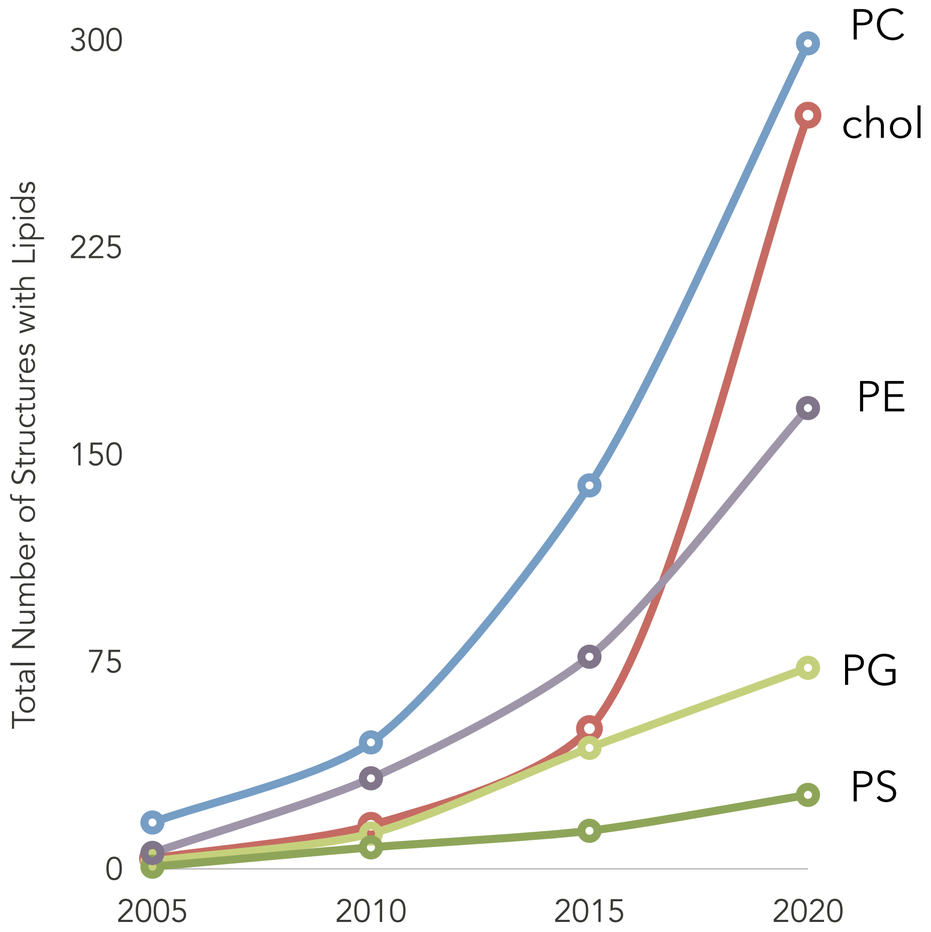
	pgA ockout	BamA Knockdown
	+	++

- *bamA* CRISPRi
- control CRISPRi

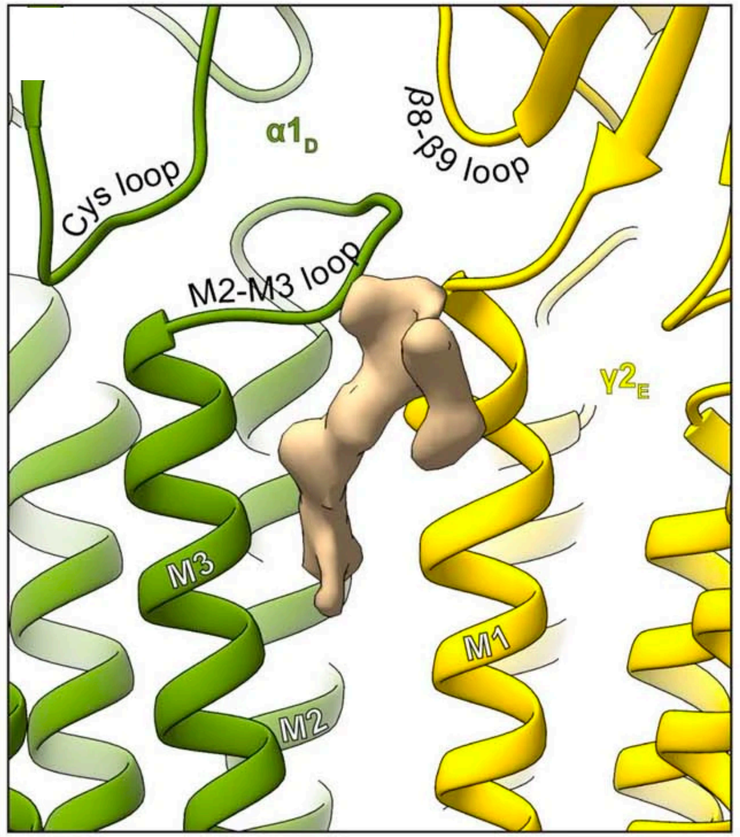
From the Klein Lab

* Bacitracin normally has little affect on gram negative bacteria

Is Lipid Binding The Connection?



values from rcsb.org



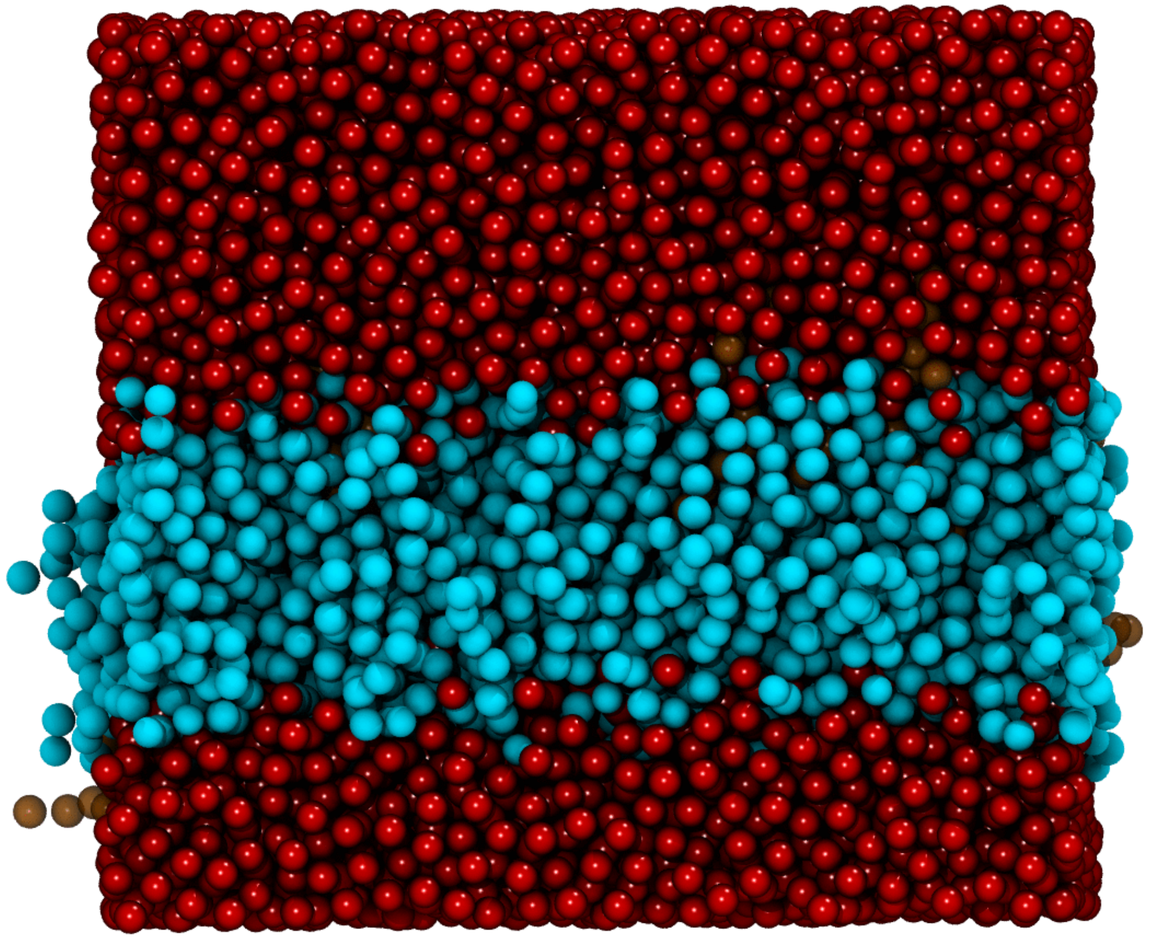
Kim...Hibbs, *Nature*, 2020

Research Question: Does Anionic Ceramide Bind To BamA?

Method & Approach

The Tool: Molecular Dynamics Simulations

Coarse-grained Molecular Dynamics



Water & Ions

Lipids

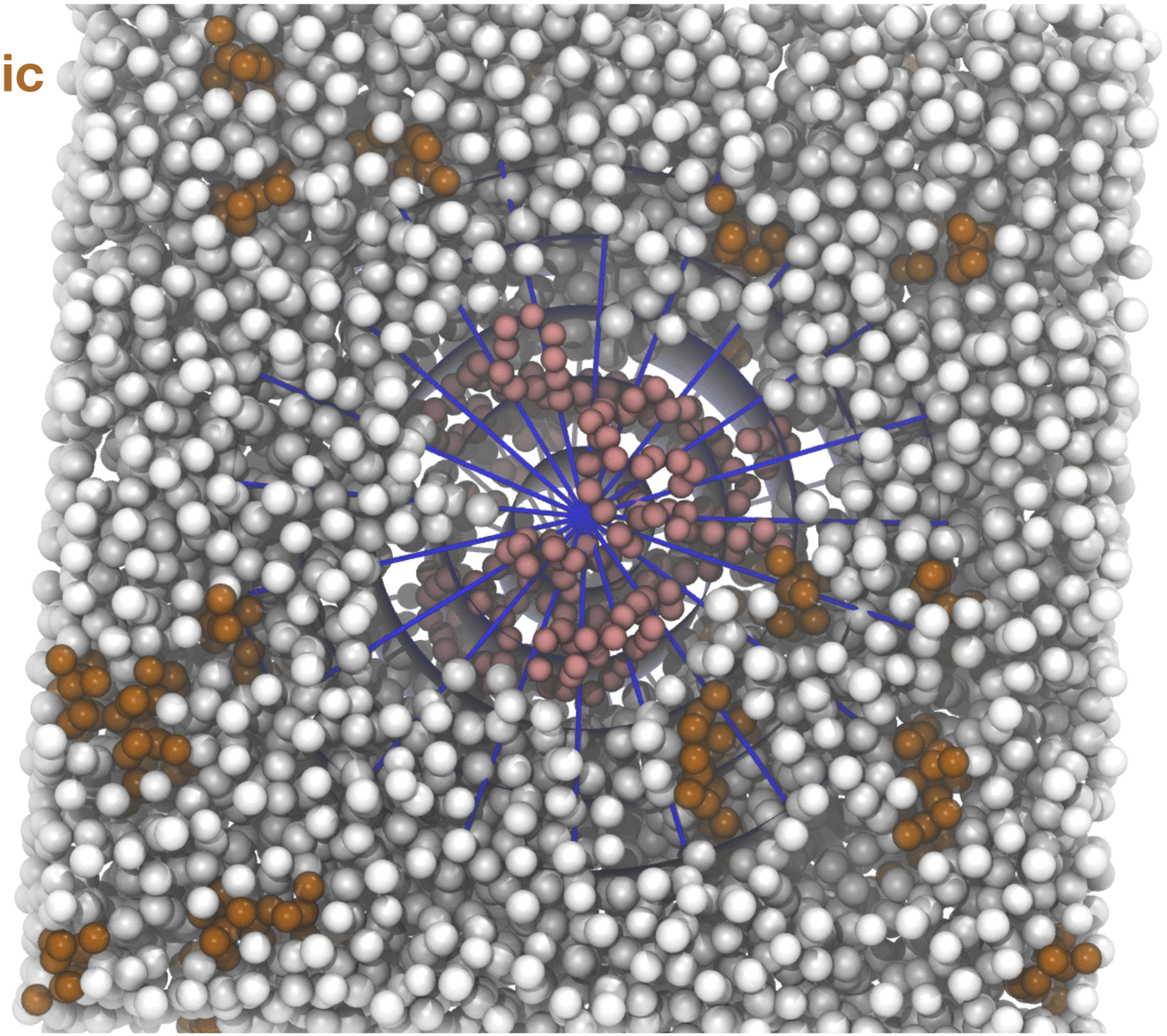
Water & Ions

The Approach: Using Densities to Find Binding Sites

Neutral

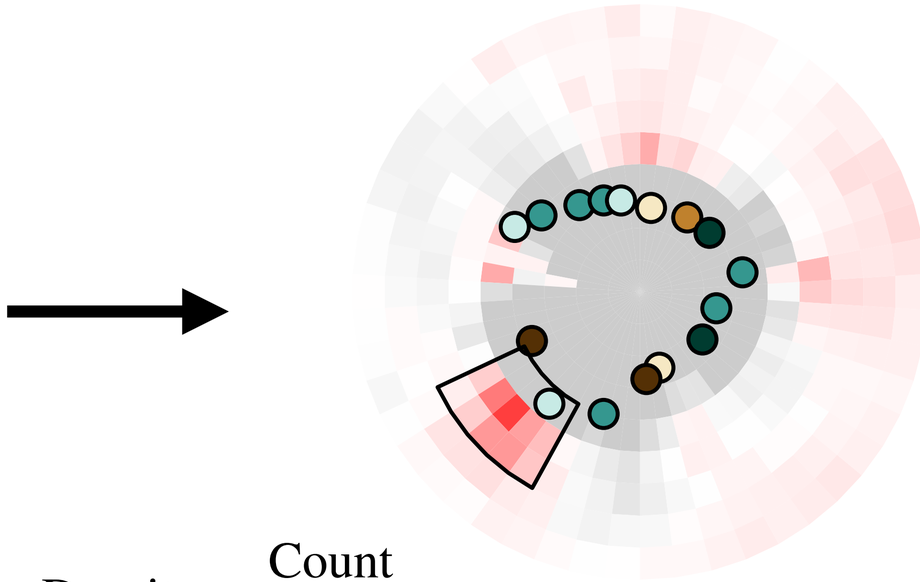
Anionic

90:10 POPC:POPG



High POPG Depletion

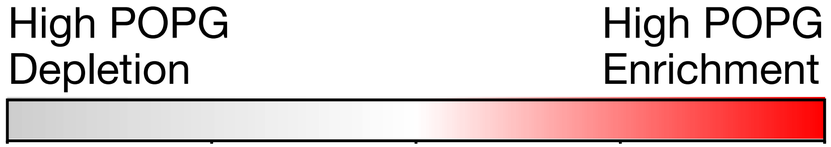
High POPG Enrichment



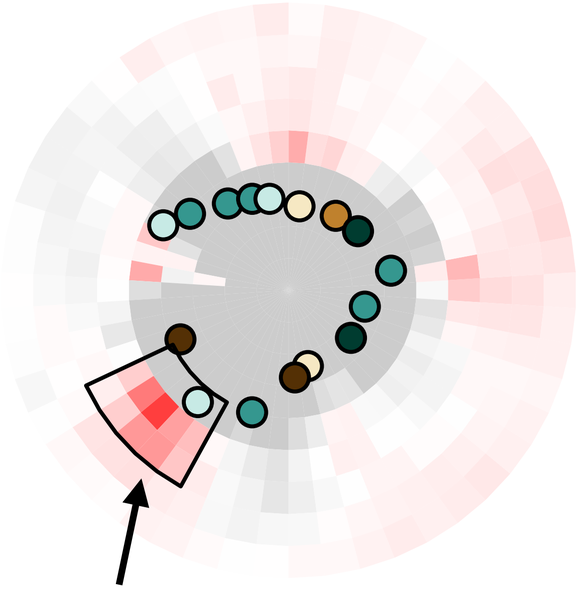
$$\text{Density} = \frac{\text{Count}}{\text{Area}}$$

$$\text{Density Enrichment} = \frac{\text{Density}}{\text{Expected Density}}$$

Lipid Binding Rationale



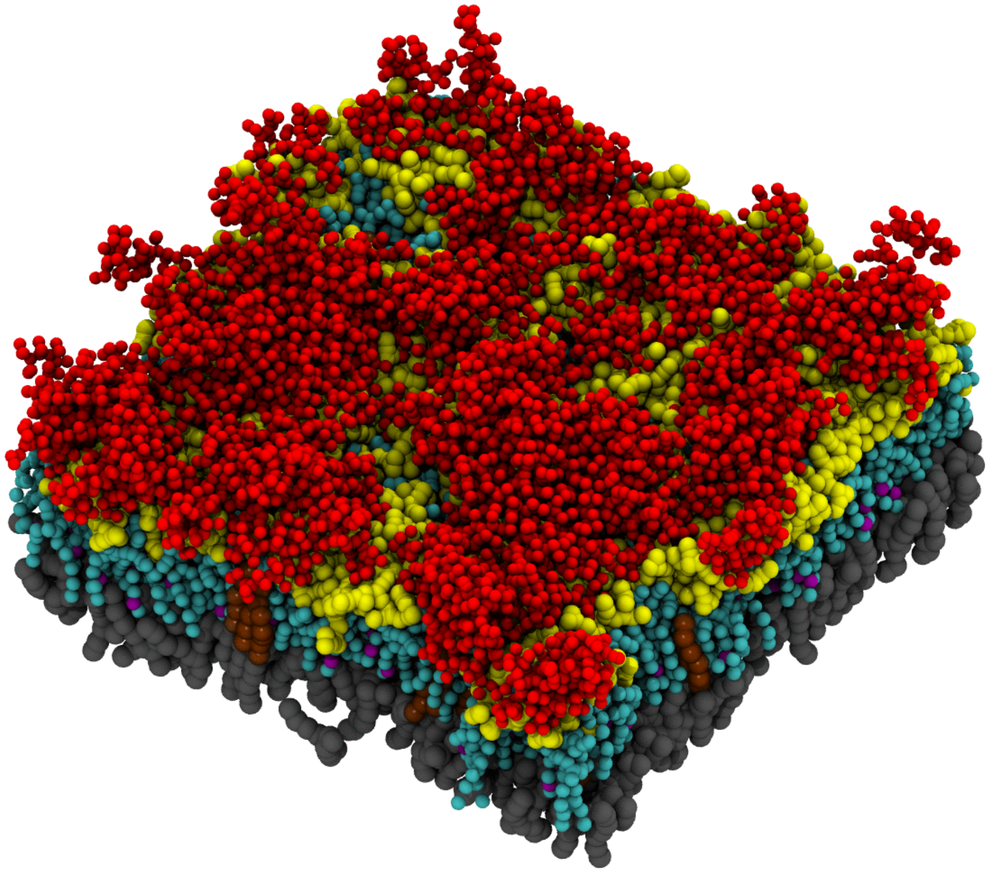
If there are regions of high density around the protein then specific lipid-protein binding is occurring



High Density Enrichment & Specificity = Lipid Binding

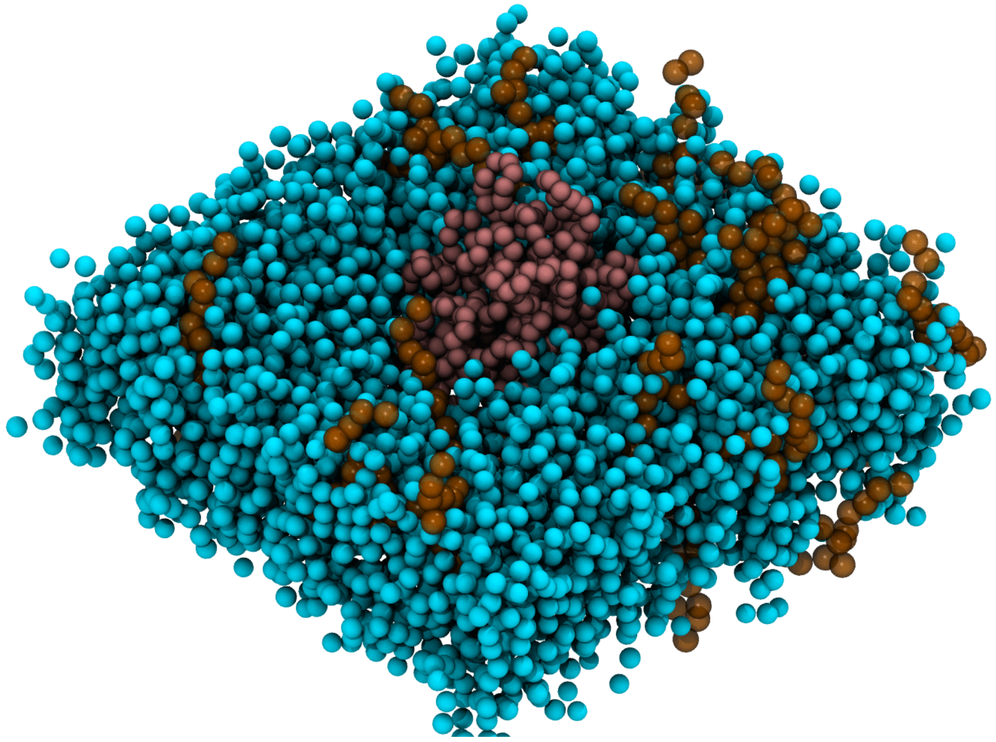
Finding Lipid Densities Requires Observable Lipid Diffusion Over Simulation Timescales

Coarse-Grained
Bacterial Lipid Diffusion



LPS/Ceramide/Phospholipids

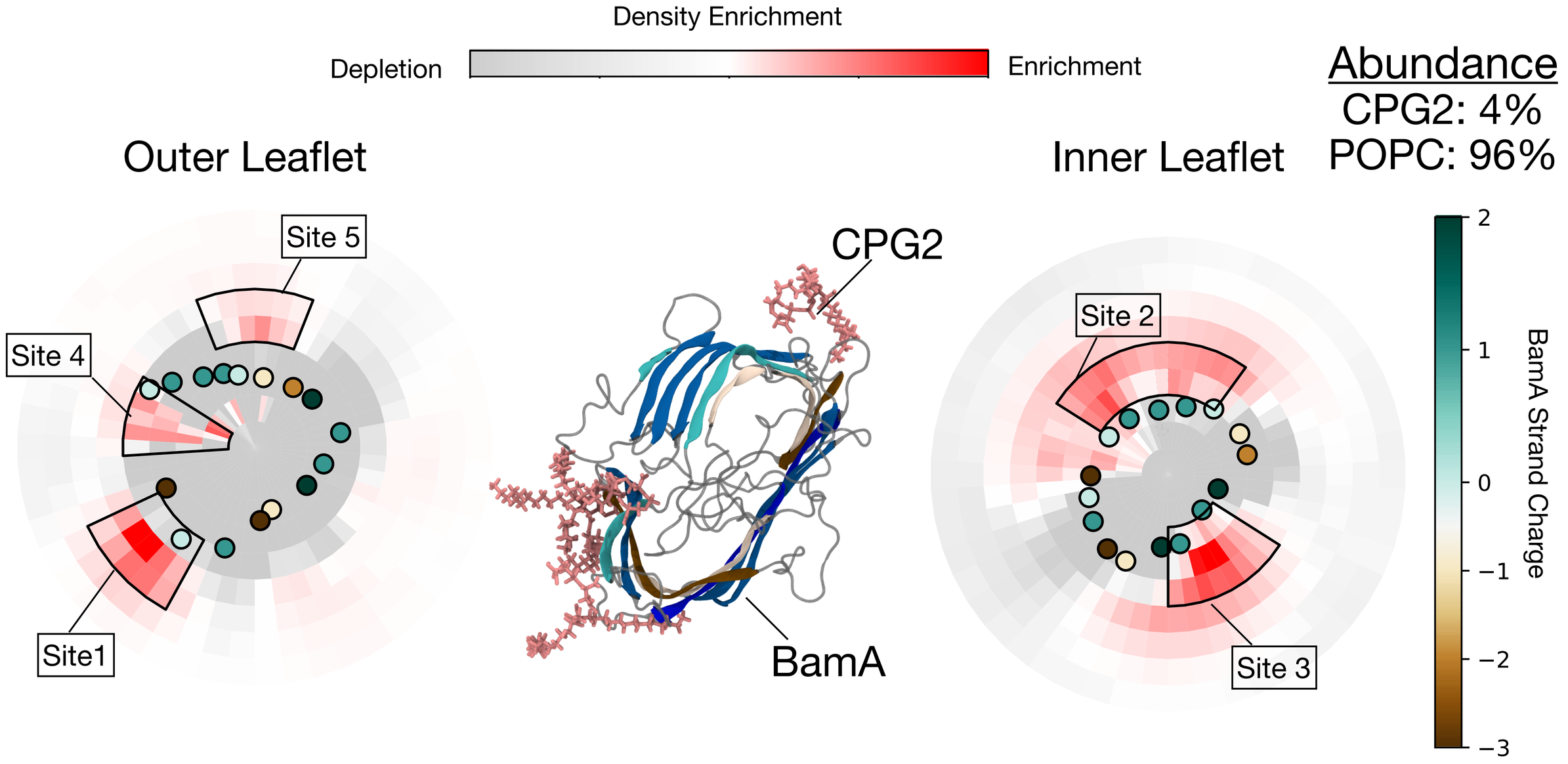
Coarse-Grained
Phospholipid
Diffusion



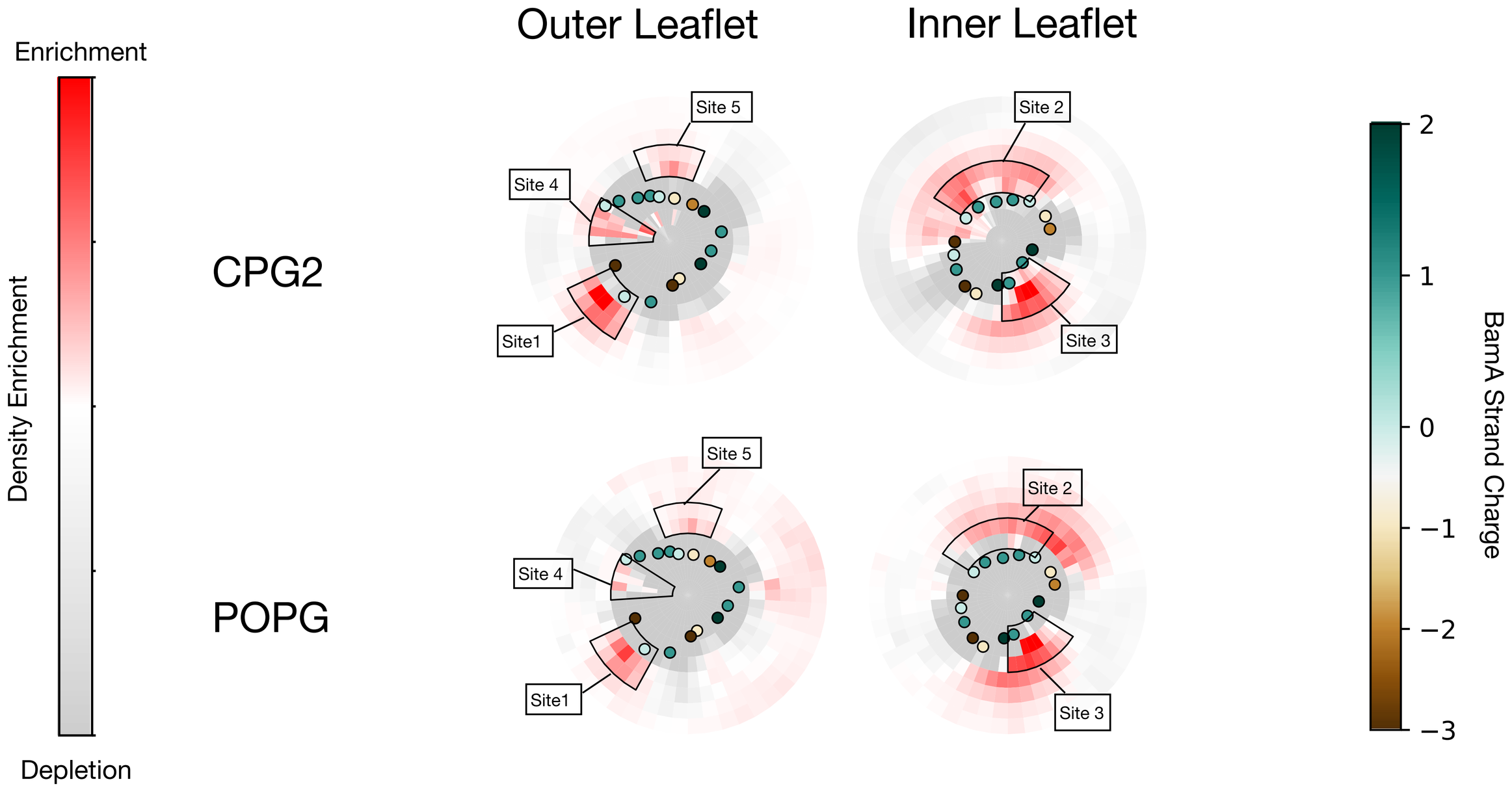
POPC/Ceramide

Preliminary Results

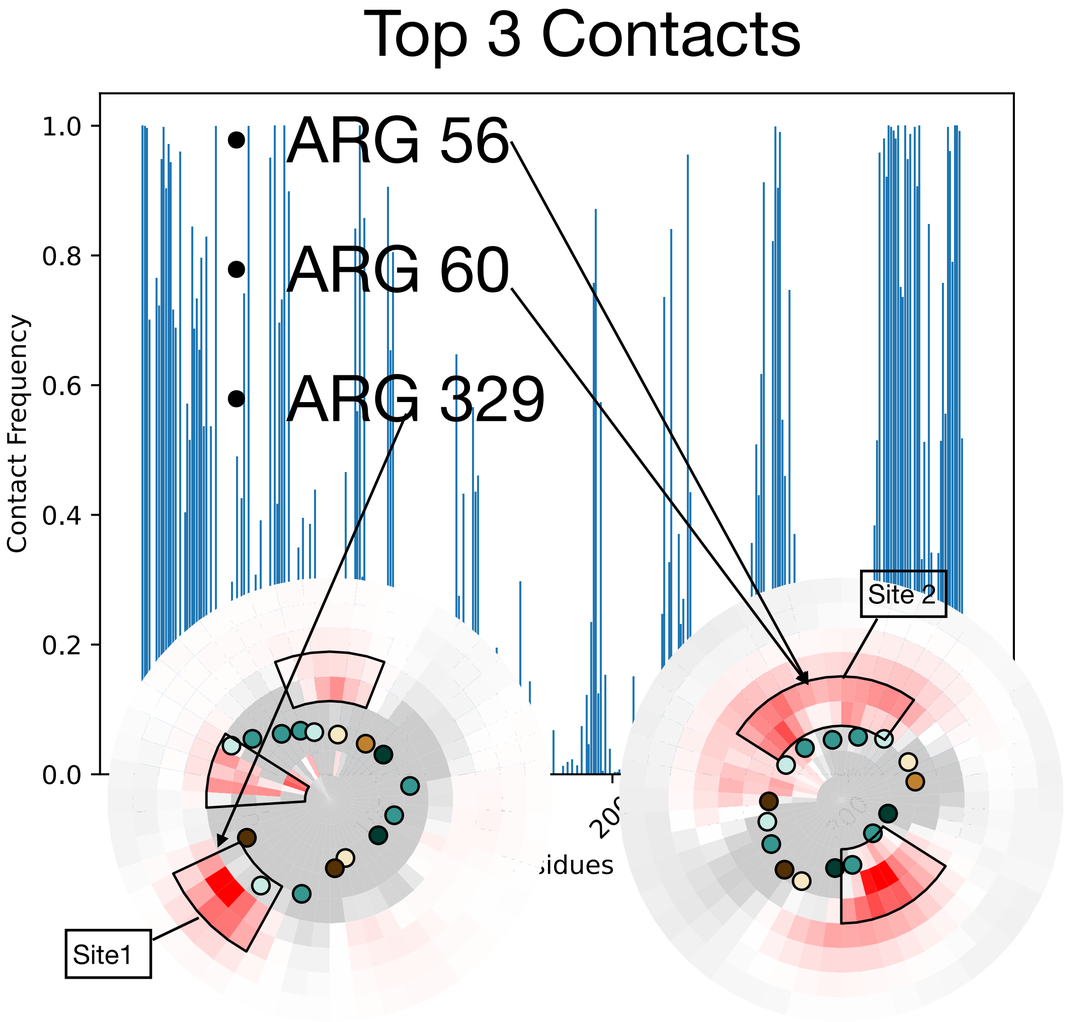
Does CPG2 Interact With BamA ?

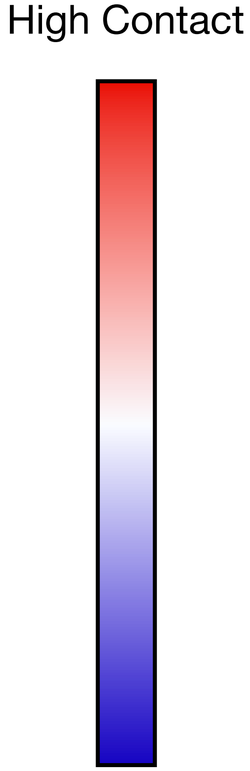


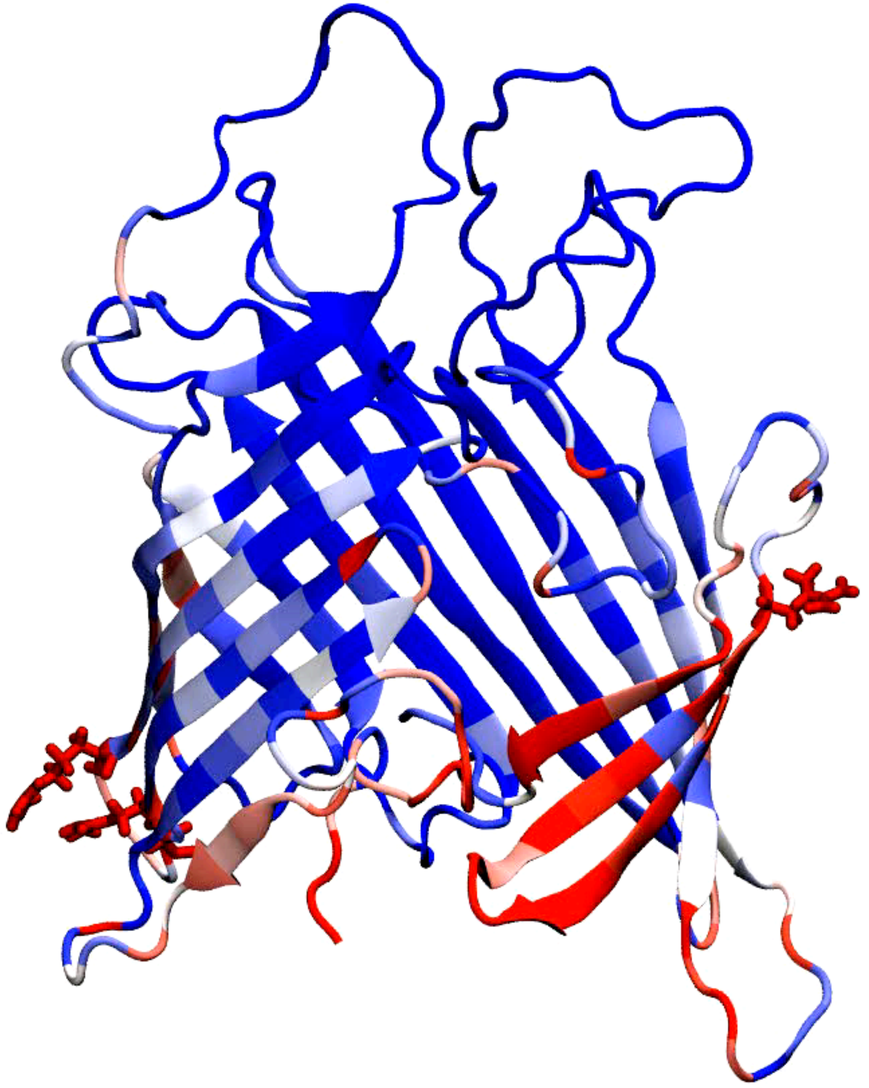
Is The Interaction CPG2 Specific?



Which Residues Does CPG2 Interact With The Most?







Summary

- Showed in model POPC-CPG2 systems that CPG2 binds to BamA
- CPG2 interacts with positively charged regions of the protein
- Identified specific residues that CPG2 binds to

Future Directions

- Use the density threshold affinity method to quantify the binding affinity
- How does the binding affinity change in a real or higher resolution system?
- Why does CPG2 bind and how it affect BamA function?

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NSF ACCESS Operations

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Questions?