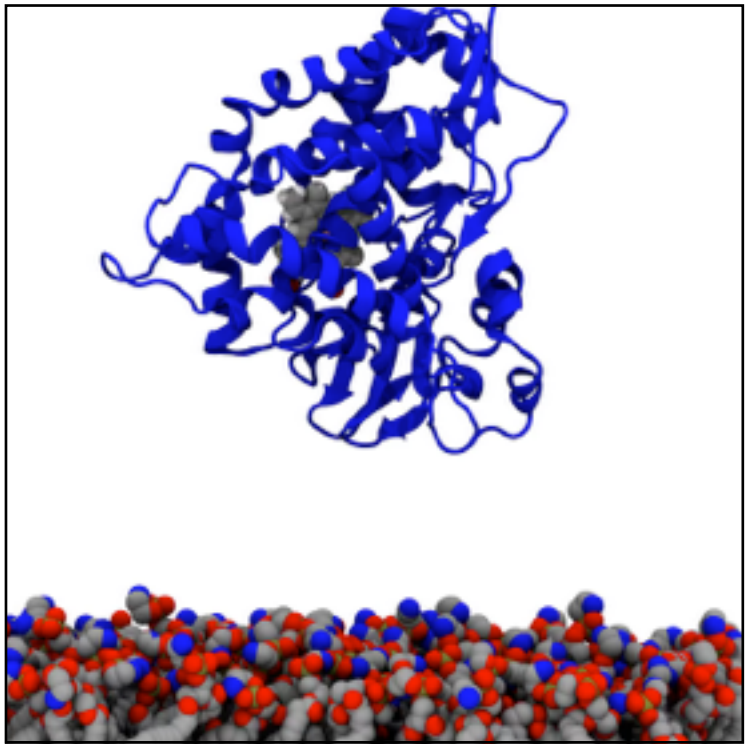

Membrane Binding of Soluble Enzymes, Explored Through Simulation of Bacterial P450s

Josh Vermaas, Gregg Beckham, and Michael Crowley

256th ACS National Meeting

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Boston, Massachusetts

NREL/PR-2700-74525

Diverse P450s Exist Throughout Metabolism

- Cytochrome P450s are a family of enzymes that use heme chemistry to oxidize a diverse complement of compounds, with drug-like molecules being of considerable interest
- 350,000 different P450 sequences identified so far, found abundantly throughout life
- Many have an associated reductase, which we won't consider here

Cytochrome P450 statistics

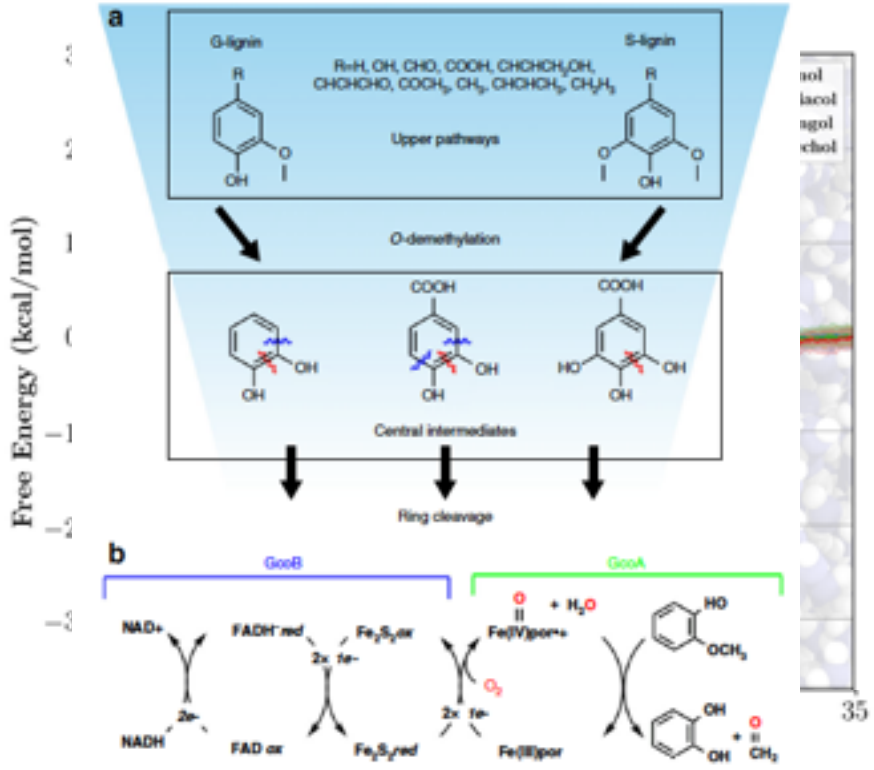
Taxon	Named P450s	Unnamed but in my possession	Total	CYP families
Animals				
Insects	7426	105	7531	208
Non-insect invertebrates	1925	0	1925	311
Mammals	2419	1334	3753	18
Other vertebrates	1461	83	1544	19
Total	13,231			
Plants	16,219	168,303	184,522	277
Fungi	7925	77,178	85,103	805
Protozoa	602	0	602	63
Bacteria	2979	59,620	62,606	591
Archaea	64	84	148	14
Viruses	28	0	28	6
Total	41,048	306,707	347,762	2252[a]

[a] 60 Families present in more than one taxon are included only once.

Nelson, BBA Proteins and Proteomics 2018

Bacterial P450 GcoA Acts on Lipophilic Substrates

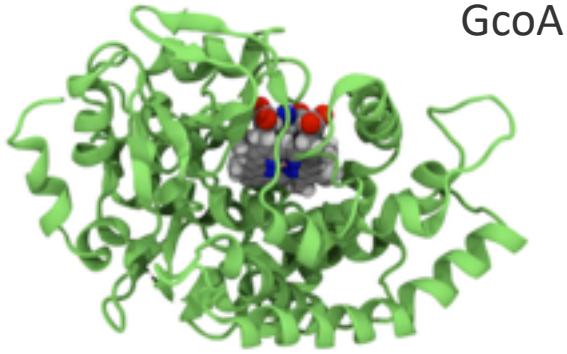
- Recently, our group has been involved with characterization of a bacterial P450, GcoA, which catalyzes the demethylation of guaiacol into catechol, a step in the lignin degradation pathway
- Parallel research has suggested that both the products and reactants will be highly concentrated in the membrane
- Does GcoA bind to the membrane?

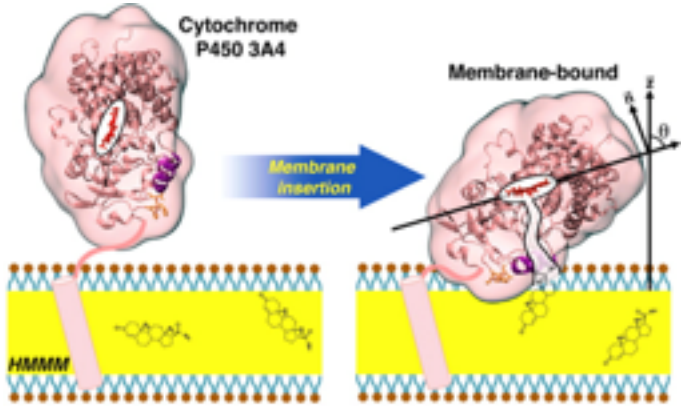


Mallinson et al., Nat. Comm. 2018

Membrane Binding in Other P450s

- Membrane-associated P450s have been previously identified, which would be useful for accessing membrane-embedded substrates
- Typically, these are eukaryotic P450s where the N-terminus is a membrane-embedded helix appended to a typical P450 architecture
- Bacterial P450s do not feature these helices. Do they bind to the membrane like truncated mammalian systems?

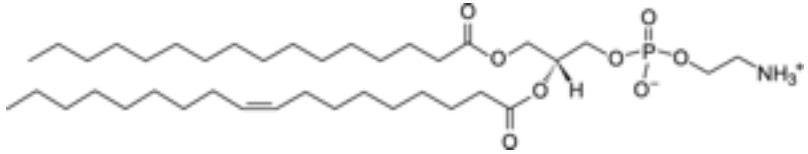


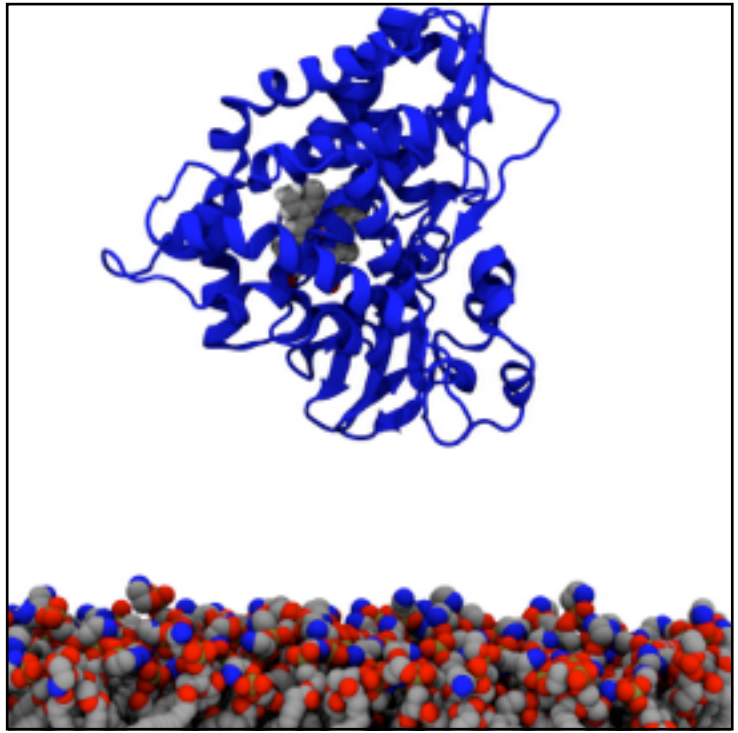


Baylon et al., J. Amer. Chem. Soc. 2013

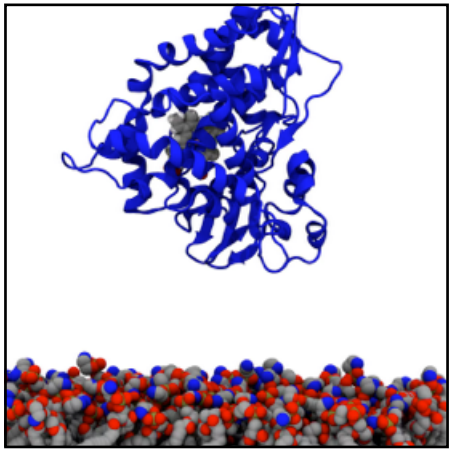
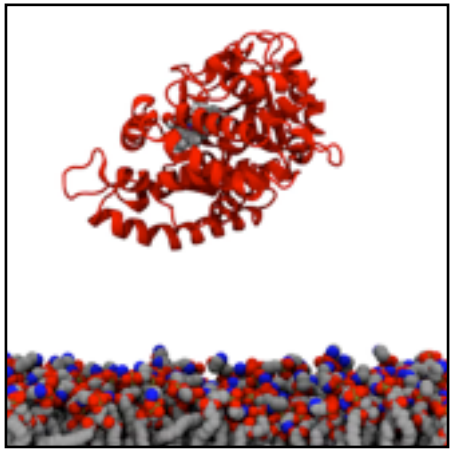
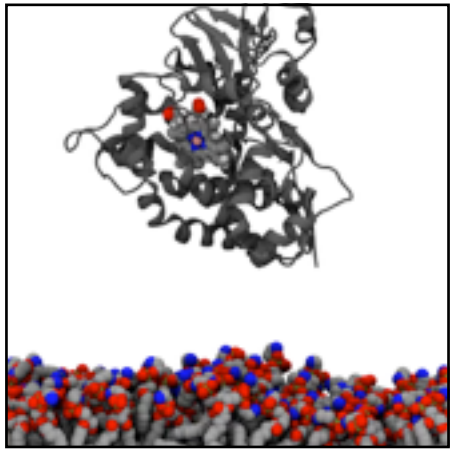
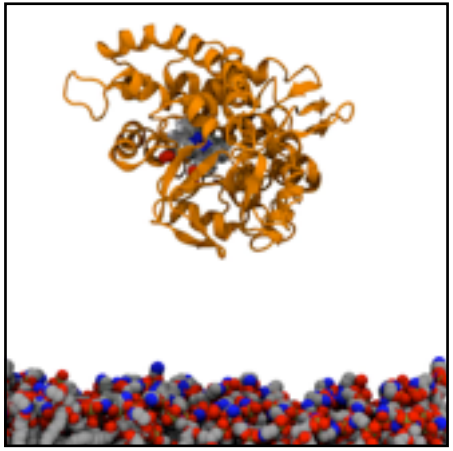
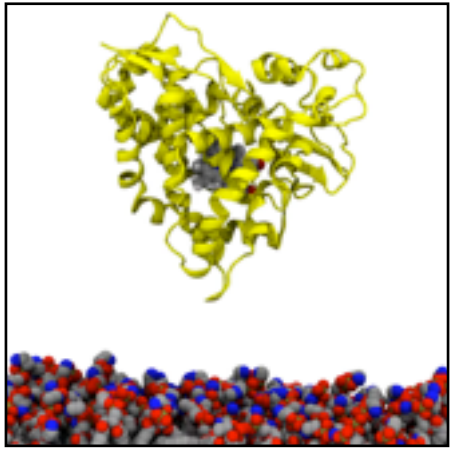
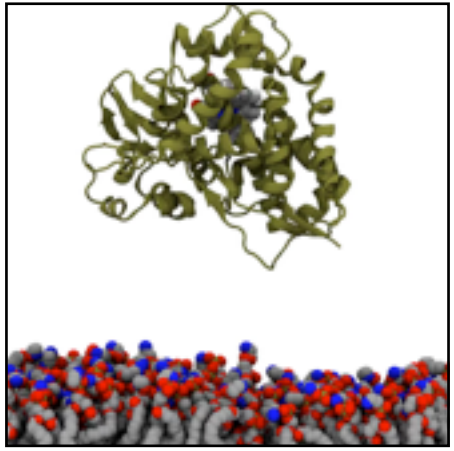
Basic Simulation Setup

- GcoA structure including heme placed above a POPE membrane constructed with CHARMM-GUI, explicit TIP3 water
- AMBER14SB force field for the protein + Lipid17 for POPE
- Rotate GcoA such that each of 6 potential faces are adjacent to the membrane
- Run each for 1 microsecond with Amber16 and observe the result





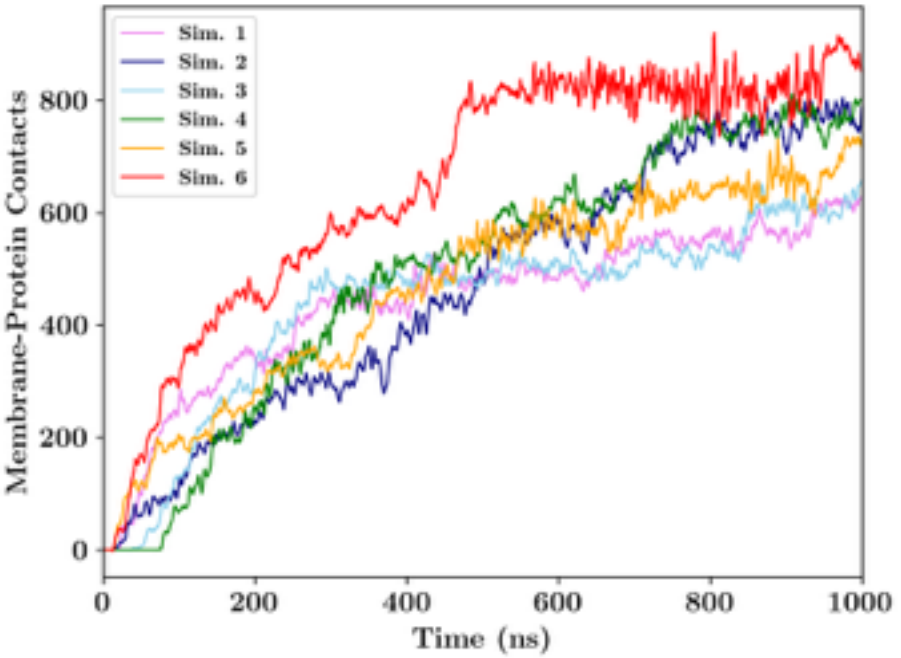
GcoA Binding Animations

Clear Membrane Binding

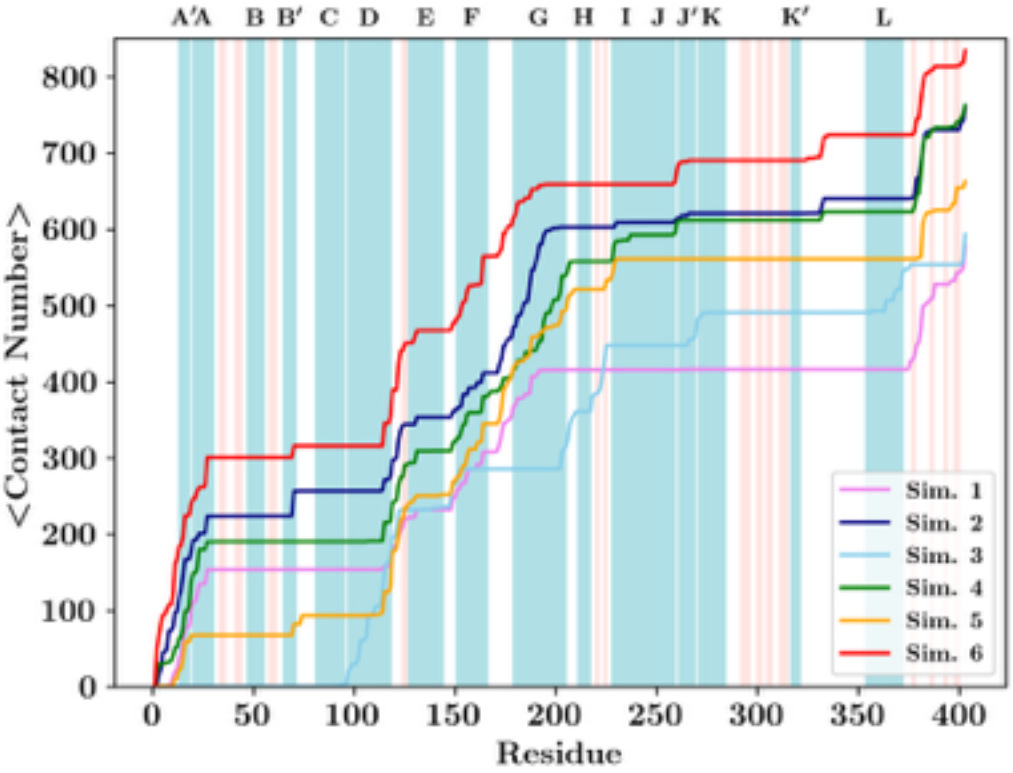
- From the animation, it is clear that membrane binding occurs rapidly, even for this “soluble” protein
- What elements consistently make contact?
- What is the strength of the interaction?
- How widely applicable is this to other P450s?

$$C_i = \sum_{j \in B} \frac{1}{1 + \exp\left(5\,\text{Å}^{-1}(d_{ij} - 4\,\text{Å})\right)}$$



Contacts, Visualized

- Lets isolate contacts after 800ns
- Major contact contributors:
  - N-terminal A helices
  - F&G helices
  - D helix and adjacent beta sheet
  - C-terminal beta sheets



F&G Helices Previously Identified as Important Membrane Binding Regions in Other P450s

- A multitude of experimental studies have identified the F&G helices to be important for membrane binding in mammalian P450s
- This feature is conserved in most of our binding simulations

A Truncation of 2B Subfamily Cytochromes P450 Yields Increased Expression Levels, Increased Solubility, and Decreased Aggregation While Retaining Function[1]

Emily E. Scott,[2] Margit Spatzenegger, and James R. Halpert
Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77555-1031

Mammalian Microsomal Cytochrome P450 Monooxygenase: Structural Adaptations for Membrane Binding and Functional Diversity

Pamela A. Williams,* Jose Cosme,† Vandana Sridhar,* Eric F. Johnson,† and Duncan E. McRee*‡
*Department of Molecular Biology MB-8
†Department of Molecular and Experimental Medicine
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Characterizing the Membrane-Bound State of Cytochrome P450 3A4: Structure, Depth of Insertion, and Orientation

Javier L. Baylon,[†,‡] Ivan L. Lenov,[§] Stephen G. Sligar,[†,‡,§] and Emad Tajkhorshid[*,†,‡,§]

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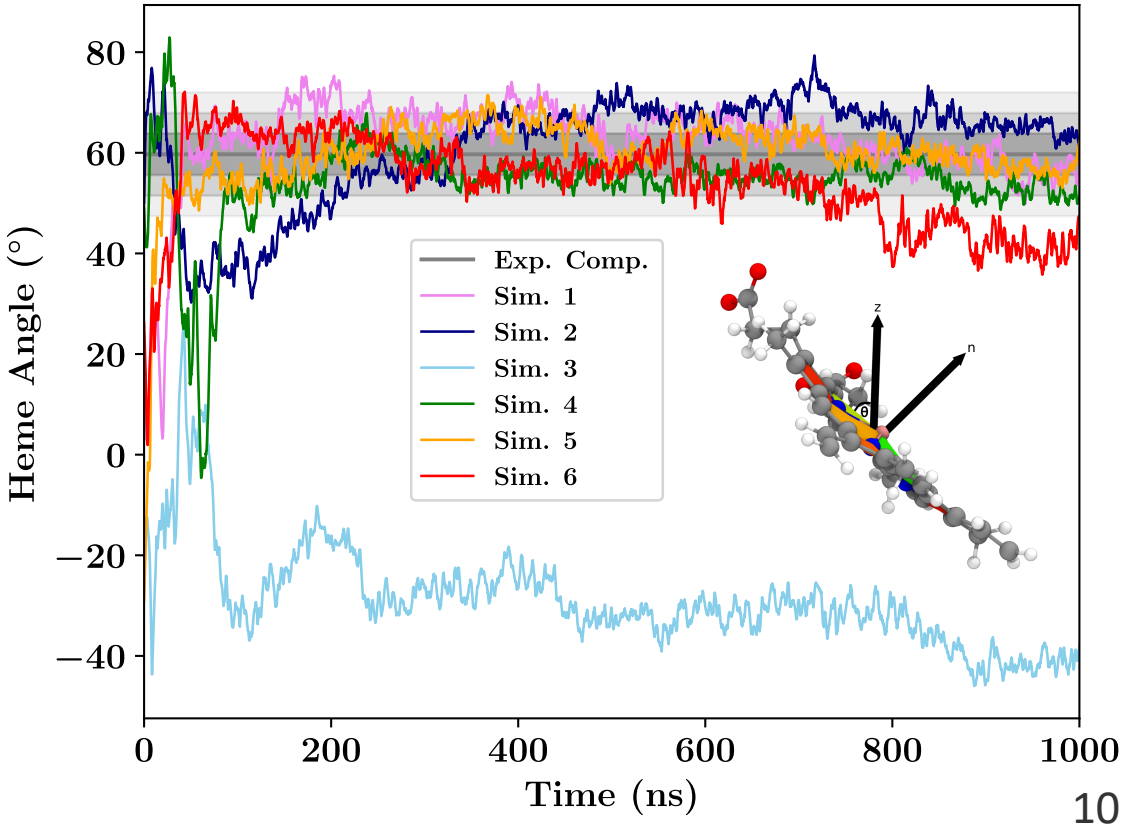
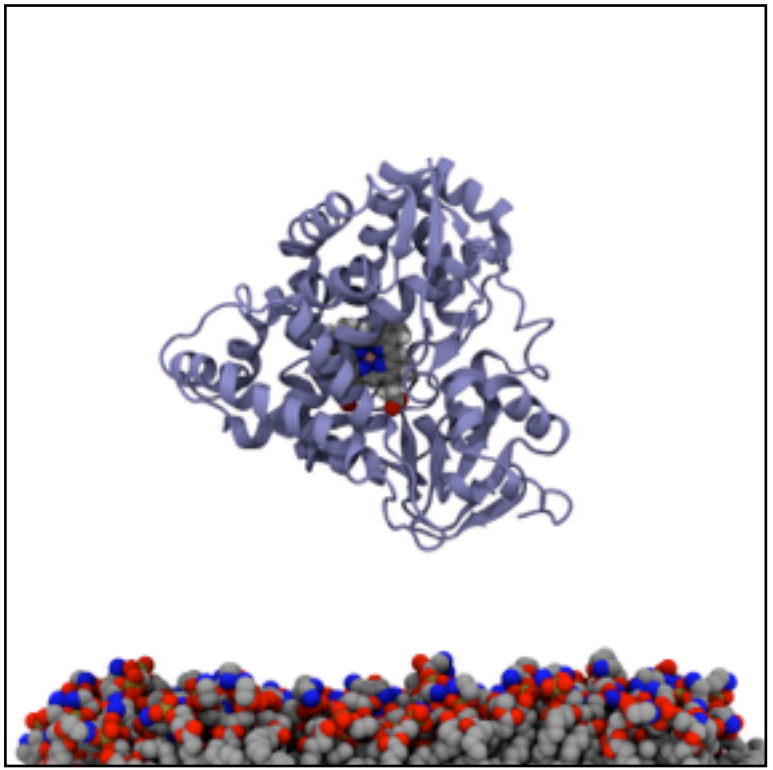
Heme Angle Comparison with Experiment

- As a further check, is our binding orientation consistent with heme angle orientations measured in experiment?
- Most trajectories match well with experiment. In another, the protein lands on a surface that would be normally covered by the reductase



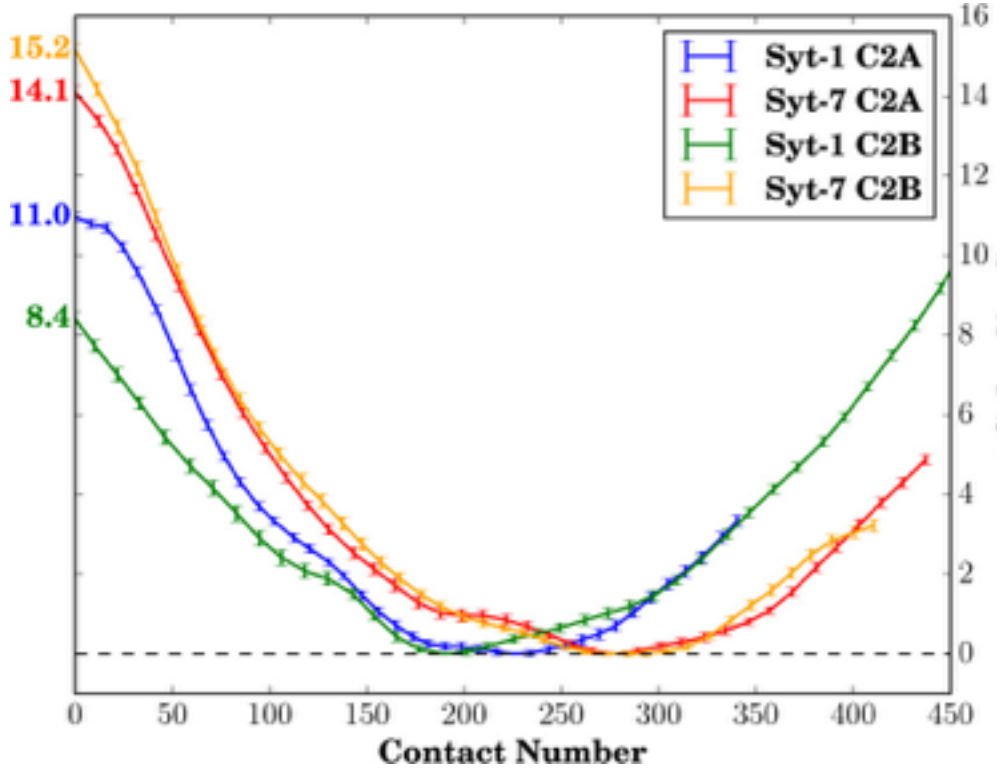
GcoA Membrane Affinity

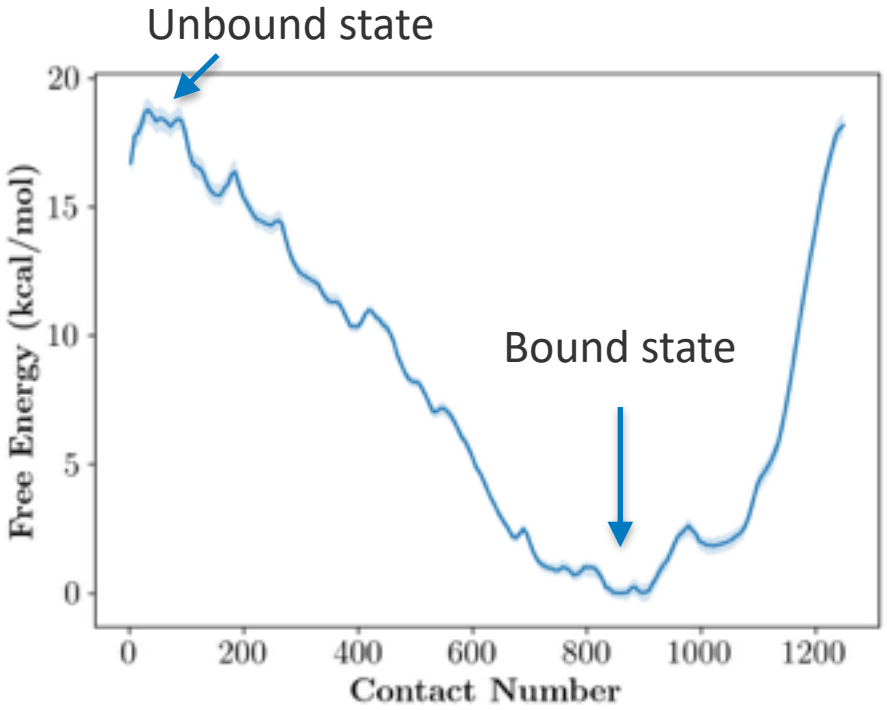
- Since GcoA binds spontaneously, we have 5 paths that lead to a bound state
- Using a contact number as a reaction coordinate, we sample along this pathway to determine the free energy difference for unbinding the GcoA from the POPE membrane
- Hamiltonian Replica Exchange Umbrella Sampling used to accelerate convergence, different contact definition used for simulation efficiency



80 starting configurations

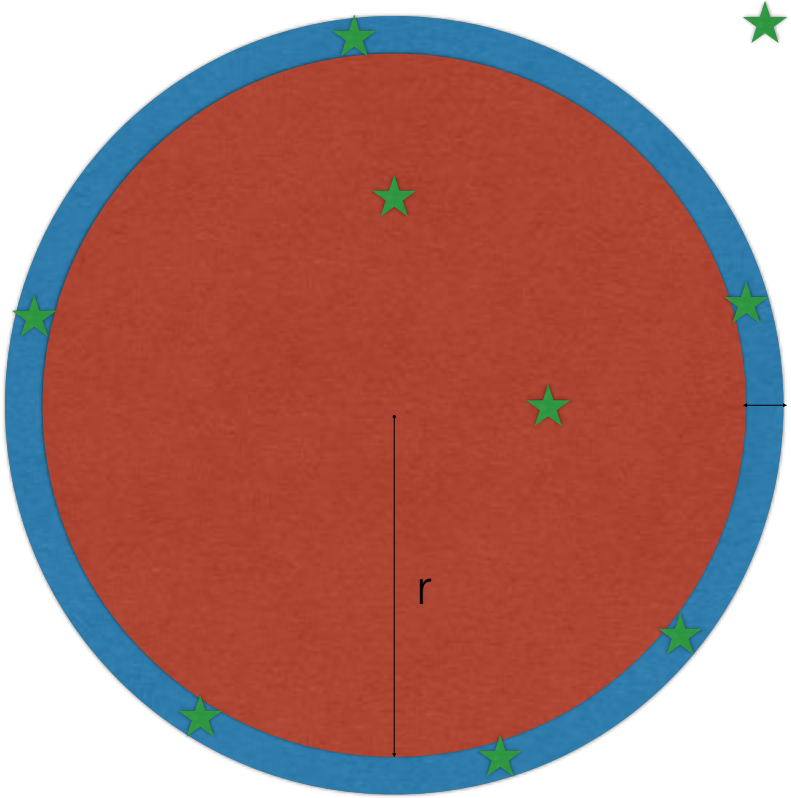
Membrane Affinity is Very Large





Vermaas & Tajkhorshid, Biochemistry 2017

Why eukaryotes added an N-terminal helix



★ P450/substrate

$$N_{interface} = \alpha N_{solution}$$

$$4\pi r^2 \Delta r c_{interface} = \alpha \frac{4}{3}\pi r^3 c_{solution}$$

$$\alpha = \frac{3\Delta r c_{interface}}{r c_{solution}}$$

$$= \frac{3\Delta r}{r} \exp\left[\frac{-\Delta G_{binding}}{RT}\right]$$

As cells get bigger, binding needs to become more favorable to keep the same ratio of interfacial to solution proteins

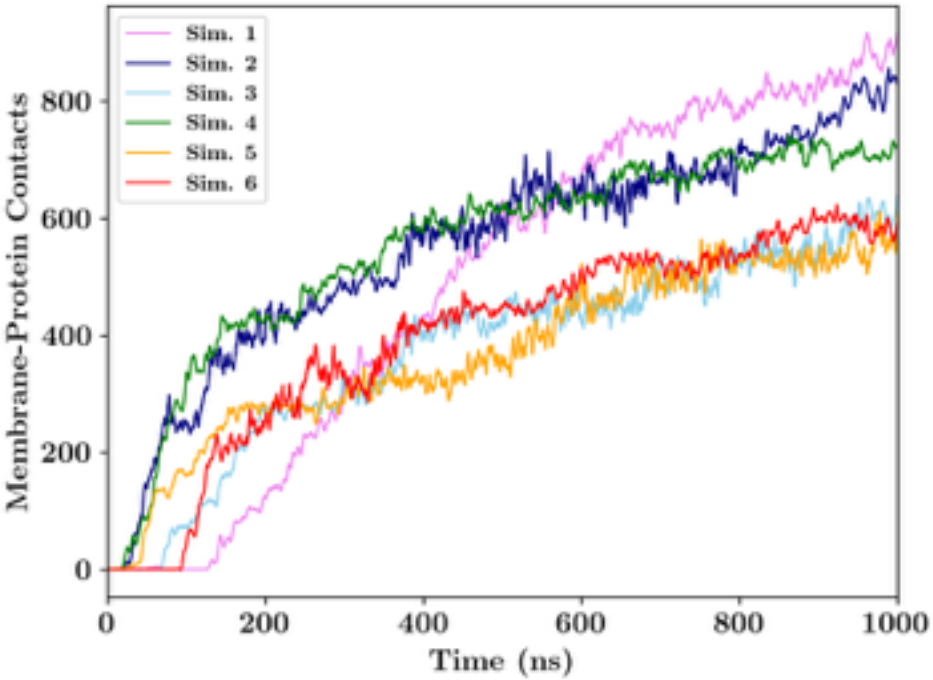
100x increase in cell radius means adding about another 3kcal/mol worth of binding strength… Or just adding an anchoring helix.

Application to other Bacterial P450s

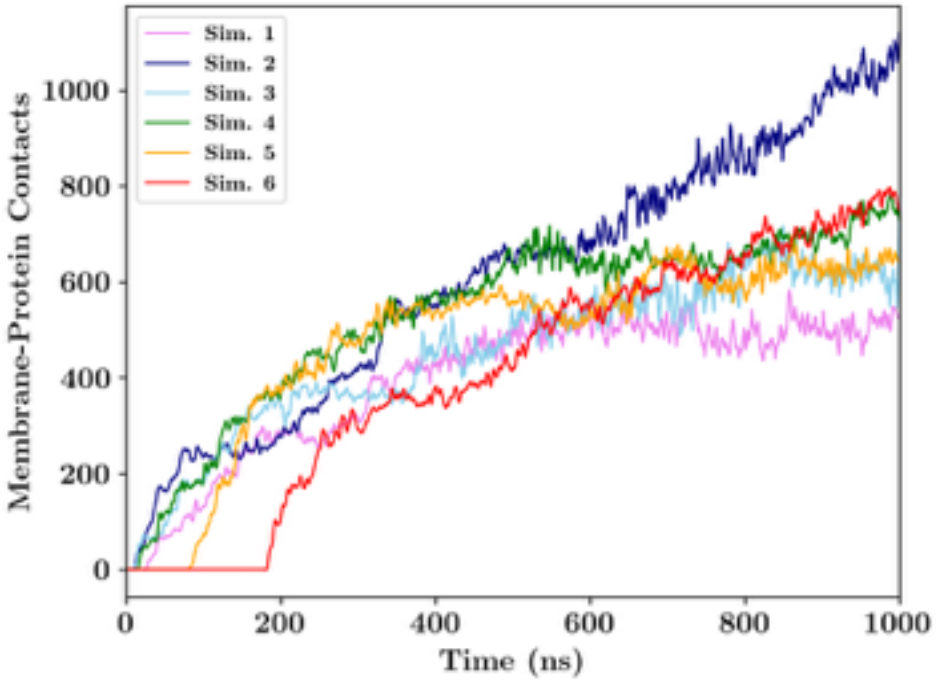
- Many other P450s lack N-terminal helices but act on lipophilic substrates
- Do they also bind to membranes?
- We consider $OleT_{JE}$ and $P450_{cam}$, both of which are P450s isolated from bacterial hosts
- $OleT_{JE}$ from *Jeotgalicoccus* oxidatively decarboxylates fatty acids to make alkanes
- $P450_{cam}$ from *Pseudomonas putida* hydroxylates lipophilic moieties
- Since their substrates are lipophillic, they should also bind to membranes

Rapid Membrane Binding…

OleT$_{JE}$

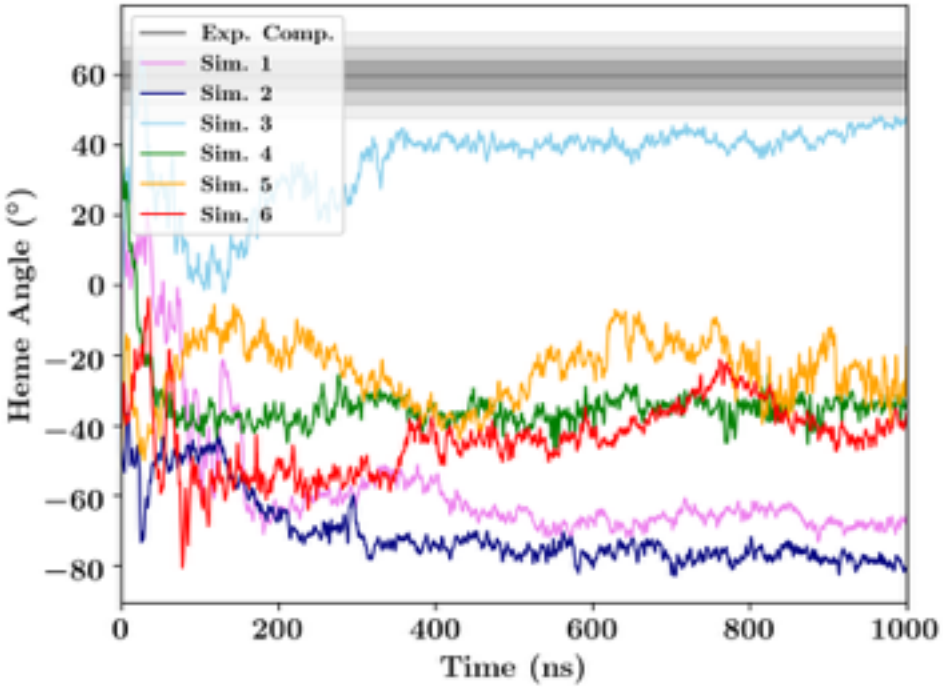


P450$_{cam}$

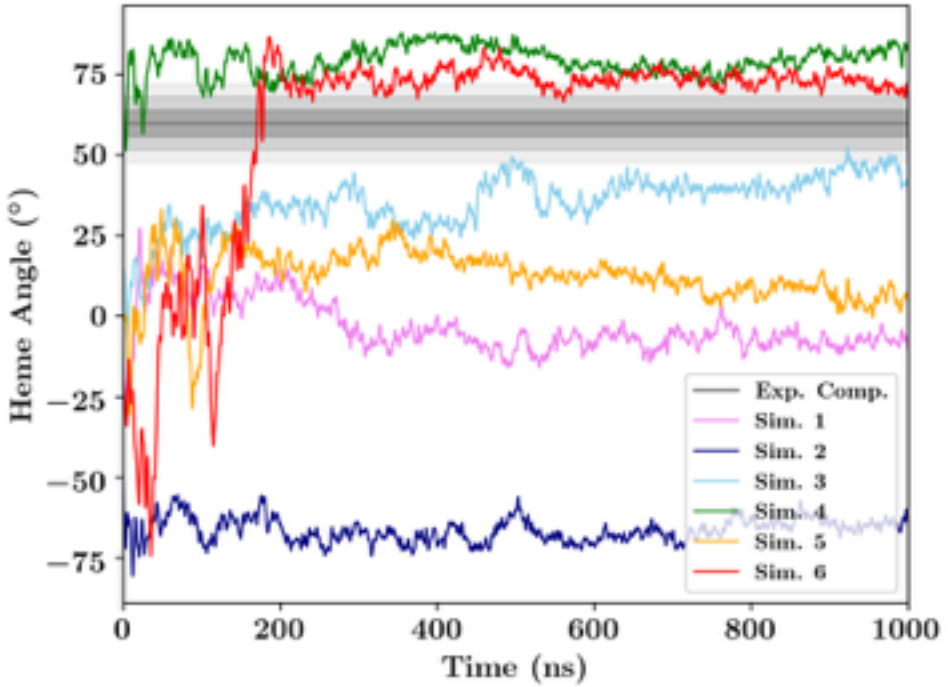


… to an Unexpected Face

OleT$_{JE}$



P450$_{cam}$



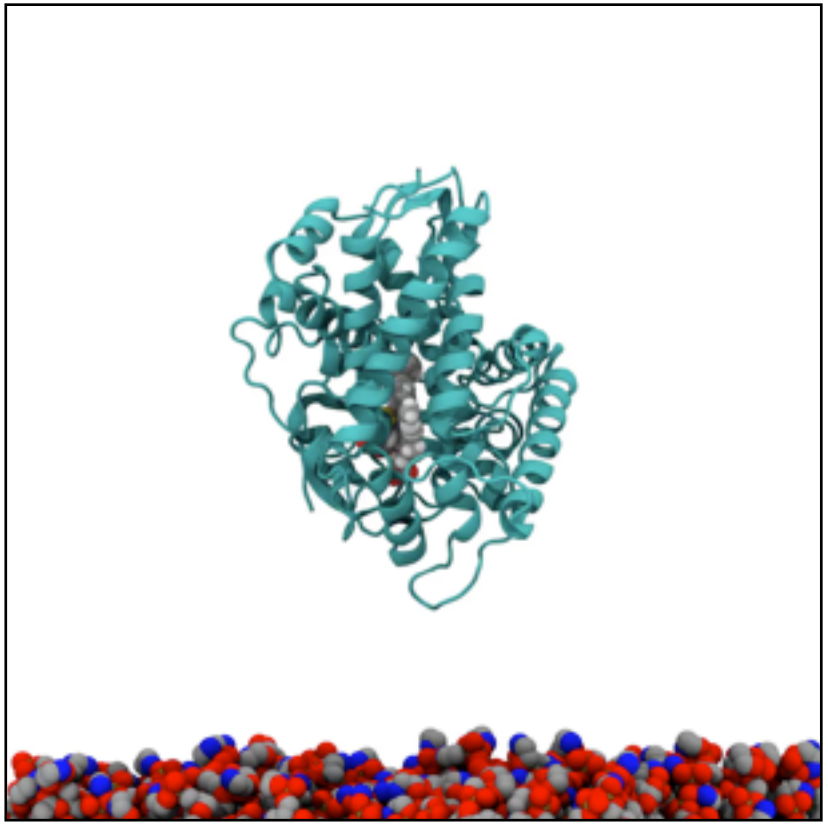
Membrane Contact & Reductase Binding Faces

- Membrane binding and reductase binding faces are likely opposite
- Reductase is on the same side as the heme-bound cysteine, minimizing the distance for electron transfer to the heme

P450cam (PDB: 4JX1)

Binding to the Reductase Face

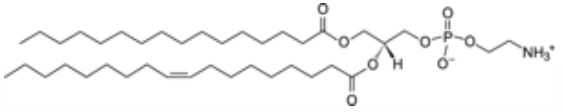
- These alternative P450s bind primarily to the face where the reductase associated to the P450 would bind
- Likely not a true membrane binding face
- What determines the binding orientation?



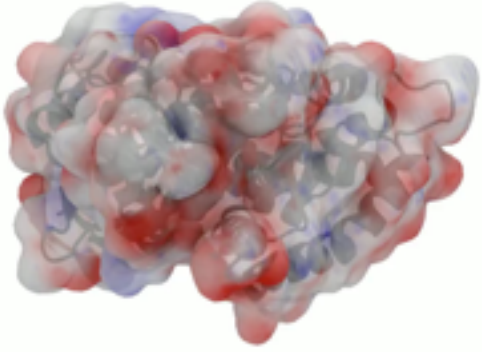
Example from $OleT_{JE}$

Electrostatics May Dictate Binding Face

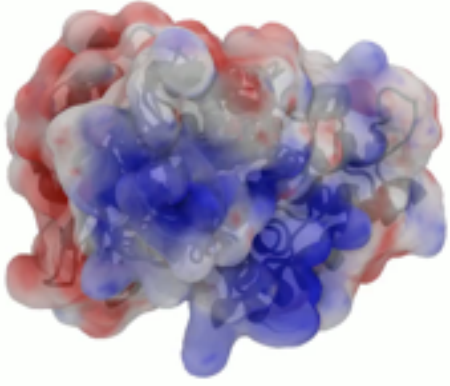
- Electrostatic potential maps are very different for the disparate P450s
- Lipid head groups for the bacterial PE lipids, while zwitterionic, have the positive charge in a more accessible position



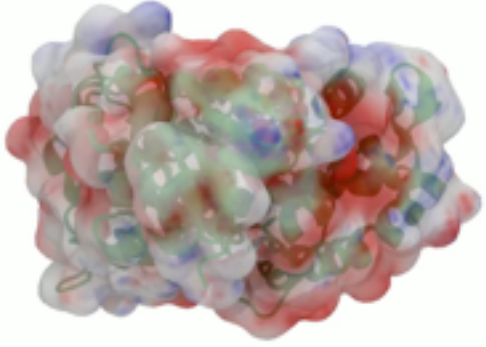
GcoA



$OleT_{JE}$



$P450_{cam}$



Negative potential

Positive potential

Summary

- “Soluble” bacterial P450 enzymes that work on lipophilic substrates conclusively bind to biological membranes
- The orientation in GcoA is more consistent than the orientation in other similar enzymes, possibly due to electrostatic interactions with the predominantly positive membrane surface
- Developed a mathematical model for why similar eukaryotic P450s frequently have a membrane-embedded N-terminal helix

Gregg Beckham

Michael Crowley

Questions?

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