Mutation of P450 Enzymes in Order to Complete Amination

Logan M. Pietrzak, Yang Tai, and Justin C. Miller
Department of Chemistry, Indiana State University, Terre Haute, IN 47809

Background

Amine functional groups are used in the synthesis of many pharmaceutical compounds making them very useful. Completing the reaction however, can be hard and time consuming, using many reagents and often requiring precursor functional groups.

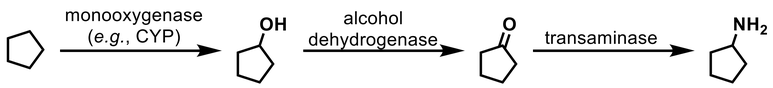


Figure 1. A bieonzymatic route for amination.

Cytochrome P450s are able to complete hydroxylation on C-H bonds with great selectivity. Reconstructing the structure of cytochrome P450 could allow for amination with high selectivity, reducing the time and reagents needed to perform the reaction.

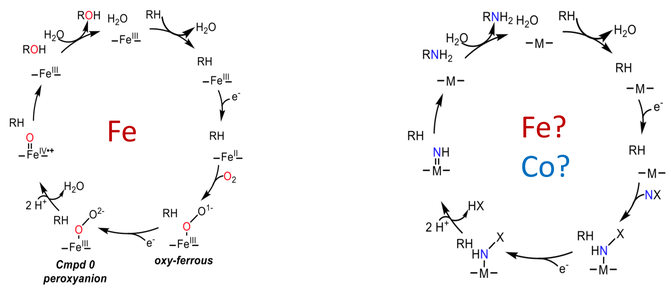
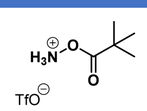
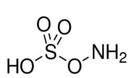
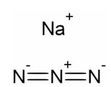
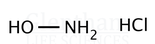


Figure 2. The native reaction cycle of cytochrome P450s (*left*) and the proposed reaction cycle of a P450 modified to perform direct C-H amination (*right*).

This type of reconstruction has been done successfully using directed evolution, a long and labor-intensive process.[1-3] Our goal is to redesign the structure of a model P450 (P450cam) to allow this reaction to take place, and develop rules that can be applied to other cytochrome P450s.

Nitrogen Source	Cost per mol	Cost per g	Structure
Hydroxylamine *O*-Pivalic Acid	$3642	$14.00	
Hydroxylamine-*O*-Sulfonic Acid	$464	$4.00	
Sodium Azide	$54	$0.83	
Hydroxylamine Hydrochloride	$29	$0.42	

Modifications to Try

We plan on changing four aspects of the P450 structure that could lead to more favorable nitrogen interactions. One such modification would be to change the amino acid ligated to the heme group.

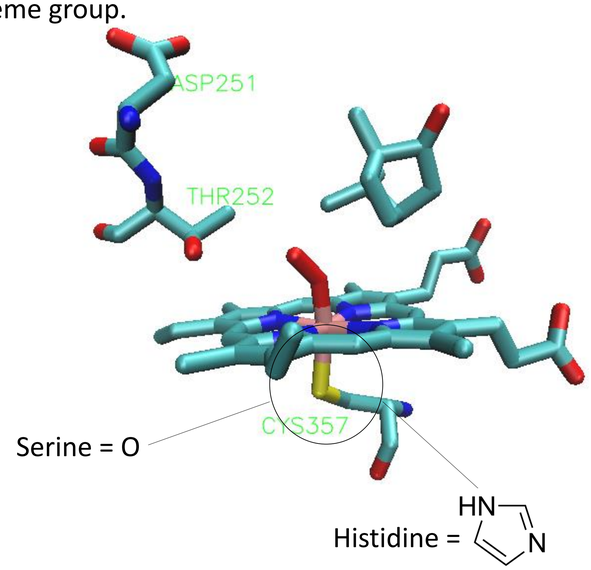


Figure 3. P450cam with different components labeled with their affect on the overall structure.

Making this modification will cause the electron density to change. Selecting the proper replacement amino acids could lead to better nitrogen interactions.

Proton Delivery

Obstructing the enzyme's ability to shuttle protons into the active site is possibly a favorable modification. The thought behind it is the nitrogen source used in the reaction may react with acidic protons, limiting its ability to yield amines. The two mutations that will cause this change are D251N and T252A.

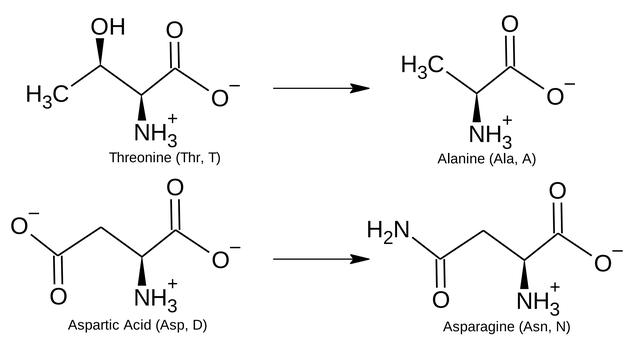
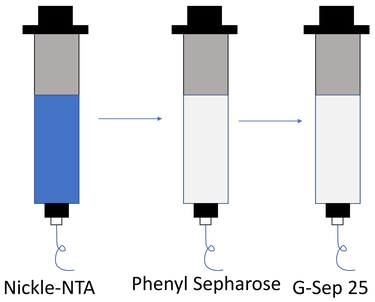


Figure 4. Amino acids for the mutations D251N and T252A

Ligated Meatal and Purification

Changing the metal ligated to the porphyrin ring will cause changes to the enzymes reactivity with the nitrogen source. We are switching Fe to Co.[4] When the enzymes contained the right mutations they were lysed and centrifuged. The supernatant containing the protein was passed through a series of columns purifying and concentrating it. A binding spectra was the done to confirm the presence of protein.



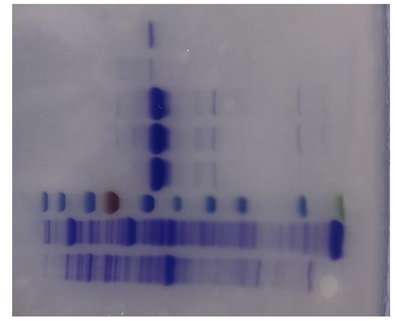


Figure 5. Purification process (*left*) and SDS-PAGE of purified proteins (*right*).

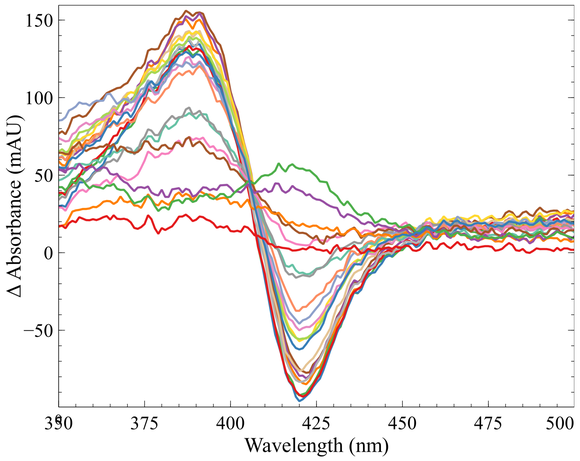


Figure 6. Binding spectra of wild type P450cam with camphor concentrations ranging from 0.25 µM to 2.00 mM.

Future

Due to instrument issues we were unable to test weather or not our mutations allowed for P450cam to complete amination. This is something that will be tested in the future, along with using different sources of nitrogen as the reactant. The cloning and purification of the serine and histidine mutations will also be completed.

Acknowledgments

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References

1) *J. Am. Chem. Soc.* **2020**, *142*, 10279-83
2) *Angew. Chem. Int. Ed.* **2021**, *60*, 24864-69
3) *J. Am. Chem. Soc.* **2022** *144*, 80-85
4) *Proc. Nat. Acad. Sci., U.S.A.* **2021**, *118*, 16