Background Information for the Catechol oxidase project

Dr. Grice

CHE321

SQ2025

Outline

- Why a CURE?
- What's the overall goal? What're the deliverables?
- Background on *catechol oxidase* chemistry
- Potential Projects within the CURE and considerations for your works

Why A CURE?

- It's hard to get research experience, as it takes extra time outside of class in addition to your coursework.

- Research is a powerful way to **learn by doing**, and allows you to use your chemistry knowledge and problem-solving skills on important problems.

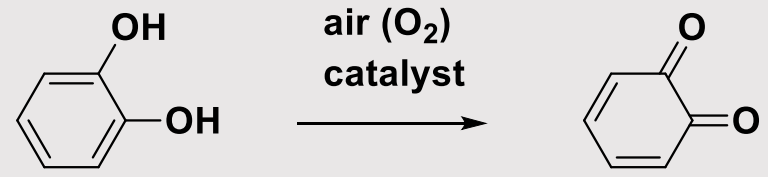
- Therefore, a **C**ourse Based **U**ndergraduate **R**esearch **E**xperience (CURE) is a way to get research experience within a course setting.

- Unlike "cookbook-style" labs, the instructor and students don't know the "expected final result" in a CURE.

- Troubleshooting and adapting is a part of a CURE, just like it is in research outside of class. *Some things won't work, and that's OK.*

What's the overall goal?

- We want to find a "good" catalyst for an oxidation reaction.



catechol **o-quinone**

- We are going to synthesize metal complexes, then will test the complexes for oxidation catalysis using UV-Vis spectroscopy.
  - Cu is what is in the enzyme, but you can do different metals (Zn, Fe, etc.!)

- We will work in groups of two, and each group will make at least two metal complex. In the end, you will report your results as a joint poster and report

Using the Literature

- In research, one of the first things you should do is read the literature and see what has been done before
  - Use Google Scholar for text searches
  - “Forward search” older papers in Google Scholar to see what has been done since

- You can use Zotero or another reference manager to put in references. This can be configured with google docs and word.

- Use ACS style! Here’s a link to the ACS Style info: ACS Style Quick Guide | The ACS Guide to Scholarly Communication | Books Gateway | ACS Publications

We are basing our starting point off of this paper (it used Ni complexes)

Exploring Michaelis–Menten Kinetics and the Inhibition of Catalysis in a Synthetic Mimic of Catechol Oxidase: An Experiment for the Inorganic Chemistry or Biochemistry Laboratory

Mark A. Chrisman * ; Michael J. Goldcamp; Alexis N. Rhodes; Jared Riffle

J. Chem. Educ. **2023**, *100 (2)*, 893–899.

https://doi.org/10.1021/acs.jchemed.9b01146

We will be doing the same oxidation, but with completely different metal complexes.

They use base to speed up the reaction, we can try it without base

Assignments

- Discussion posts – you will be given prompts to help guide your discussion with your peers, and also to do some of the work needed for the final report ahead of time.
- Project proposal/Research Plan (1 per group)
- A mid-class report summarizing what work you've done so far, and to get feedback from me to improve your final report.
- A final lab report for each group that summarizes what was done during the quarter
- A final poster for each group that summarizes what was done during the quarter

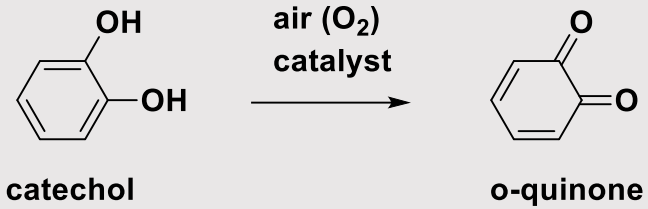
Beyond This Class

- I'm always happy to write letters of recommendation and can talk about the CURE experience (give me plenty of time to write the letters before they are due!)
- You can present your poster in the fall showcase or other venues (e.g. CAURS) if you want!
- We can discuss submitting your final research report to *DePaul Discoveries* next Feb for publication June 2026
- If you are really interested in continuing your research project beyond the course, let's chat

Catechol Oxidase Activity

- *Catechol oxidase* is a di-copper enzyme that oxidizes catechols to quinones. – Tyrosinase can be included in this category of copper-based enzymes

- It is important in the browning of fruit and the production of melanin, among other roles!



Structure of Catechol Oxidase

- *Catechol oxidase* has been crystallized and you can look at the structure of it using the PDB if you are curious. Here's a few specific catechol oxidase structures.

RCSB PDB - 1BT1: CATECHOL OXIDASE FROM IPOMOEA BATATAS (SWEET POTATOES) IN THE NATIVE CU(II)-CU(II) STATE

RCSB PDB - 1BT2: CATECHOL OXIDASE FROM IPOMOEA BATATAS (SWEET POTATOES) IN THE REDUCED CU(I)-CU(I) STATE

Catechol Oxidase vs. Tyrosinase

- If you have taken biohem lab here, you might have done some experiments with *tyrosinase*
- *Catechol oxidase* and *tyrosinase* are quite similar, but there are some key differences. If you are curious, this is a paper about it: https://doi.org/10.1002/anie.201508534
- Futher, there are other di-copper oxidation enzymes out there, such as *laccases* and others.

Mechanism

Insert image of mechanism here, such as Scheme 2 or 3 from:
https://doi.org/10.1016/j.ccr.2015.11.002

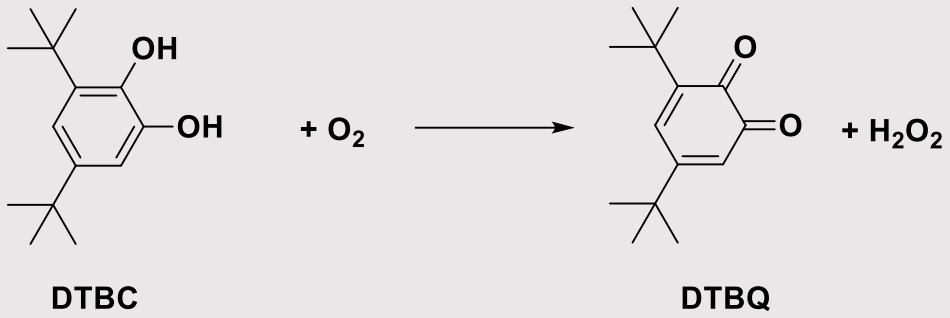
Some reviews on metal complexes for oxidation of catechol

Catechol oxidase and phenoxazinone synthase: Biomimetic functional models and mechanistic studies
Suman Kr Dey, Arindam Mukherjee

https://doi.org/10.1016/j.ccr.2015.11.002

This is a good review to skim if you need inspiration for metal compounds to propose!

Why we are using 3,5-di-tert-butylcatechol



- This particular catechol is soluble in organic solvents and makes one major product
- The reaction has been done many times, so we are confident it is reproducible.
- We have the molar absorbtivity of the product in MeOH, acetonitrile, and other solvents, so can track the reaction by UV-Vis.
- Beer's Law: $A = \varepsilon bc$

Michaelis-Menten Kinetics

- Each group will study their catalyst at a few different concentrations of substrate, and we can hopefully get Michaelis-Menten Kinetics for them!

- This allows us to find several parameters: k_{cat}, V_{max}, and K_m
  - We can then compare between systems!

- This is a good little historical review of Michaelis-Menten Kinetics, something that you will also see in Biochem:

A guide to the Michaelis–Menten equation: steady state and beyond - Srinivasan - 2022 - The FEBS Journal - Wiley Online Library

Potential Research Projects – These are Options

- 1) Synthesize a ligand that hasn't been used on this chemistry in a few steps. (ca. 0.1-1 g of ligand is plenty). Each student in a pair could make a slightly different ligand. Then test the ligand with Cu^{2+} or another metal.

- 2) Use a commercial ligand, but make a metal complex in a few steps. (ca. 100-200 mg of a metal complex is plenty to do all we need to). Each student in a pair could make a different, but related complex, then test.

- 3) Synthesize a known ligand/catalyst for this type of catalysis, but try to do it "Greener" using the methods of Green Chemistry

- Everyone will test their complexes for catechol oxidation. The goal is to get data for Michealis-Menten kinetic analysis.

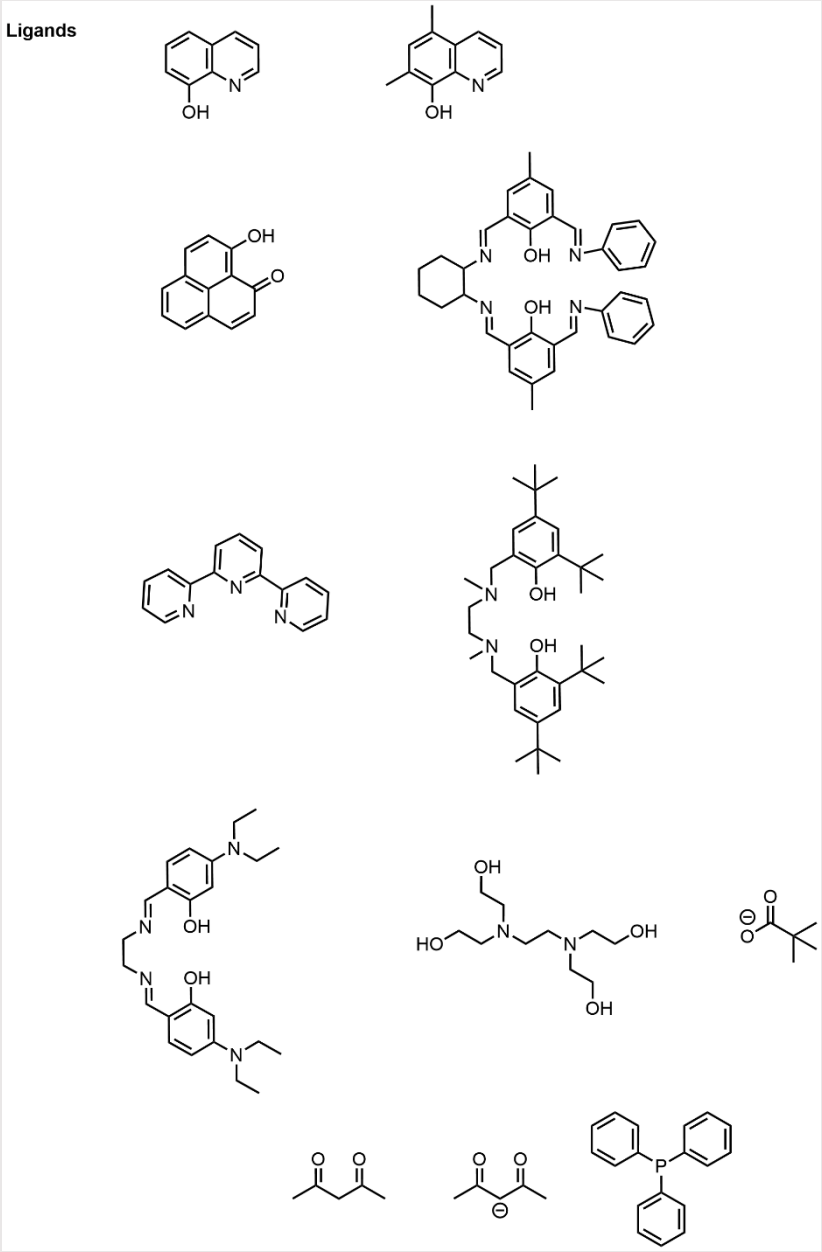
Ligand considerations

- We are going to stick with air-stable ligands (no small alkyl phosphines!)
- Is it bidentate? Tridentate? Bridging? How many do you expect to go on the metal?
- What are the X and L characteristics of the ligand? Are the donors Hard or Soft?
- Is it potentially non-innocent? (Redox-active/Hemi-labile/pendant base/etc.)
- What's it's complete formula and molar mass?
- What is it's solubility?
- How is it purified? Recrystallization is ideal. We can do columns (Combiflash!) or distillation if needed.
- Your experimental section needs to include the synthesis with a %yield if you make it

Ligands from Last year

The 8HQ ligands were explored on Mn(III) and were faster with the methylated species

A few people proposed multi-metallic clusters, which were hard to characterize/synthesize



Metal Complex Considerations

- What's the geometry?
- What's the oxidation state, d-count, and 18 electron rule count?
- Is it diamagnetic or paramagnetic? Is it high-spin or low-spin?
- What's it's complete formula and molar mass?
- How is it purified? Recrys is ideal, but some metal compounds can be columned!
- What's it's solubility in MeOH, the solvent we will do our catalysis in?
- If it's colorful, what are the transitions due to? (MLCT, LMCT, Ligand-centered orbitals, metal-centered orbitals)
- Your experimental section needs to include the synthesis with a %yield

Choosing a metal starting materials

- Copper is the metal in the enzyme, but other metal complexes show similar activity.
- First row transition metals are generally cheaper than 2nd or 3rd row. If you want to do 2nd or 3rd row, talk to me early!
- Consider the oxidation state of your compound and the anions involved. Is the complex soluble in MeOH? Is the oxidation state generally stable under air?
- Be sure to include waters of hydration in molar mass calculations if you are using hydrate salts as starting materials.
- It's a good idea to do a control experiment of just your ligand and just your mtal starting materials as catalysts to compare vs. your metal complexes

Air-stable Metal Starting materials we have already:

- Group 6: $Cr(CO)_6$, $Mo(CO)_6$, $W(CO)_6$
- Group 7: $Mn(CO)_5Br$, $Re(CO)_5Cl$, $MnSO_4$,
- Group 8: Fe salts
- Group 9: Co
- Group 10: Ni, Pd, Pt
- Group 11: CuI, $CuSO_4$ and other Cu^{2+}, AgOAc, $AgNO_3$, $(Me_2S)AuCl$
- Group 12: $Zn(O_2CR)_2 \cdot 2H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$
- Group 13: $AlCl_3$, $Al(OR)_3$
- Group 14: $SnCl_2$

- We can buy (reasonably inexpensive) other starting materials too

Monodentate ligands

- CO – generally easier/safer to start with a M-CO than make a M-CO. The group 6 and 7 species are generally made from M-CO starting materials.
- DMSO, DMF, water, methanol, THF, can be ligands through O
- Monodentate P: PPh_3, $P(OMe)_3$, $P(OPh)_3$, $P(NMe_2)_3$
- Monodentate N: NH_3, NR_3, pyridine, DMAP, imidazole, acetonitrile, benzonitrile
- Thiols/sulfides smell bad, fair warning. SEt_2, RSH

Commercial polydentate ligands we have or can buy

- Bidentate amines: en, TMEDA, Bpy, phen, dpa, bpa, 8-HQ, 8-AQ, BIAN, tren

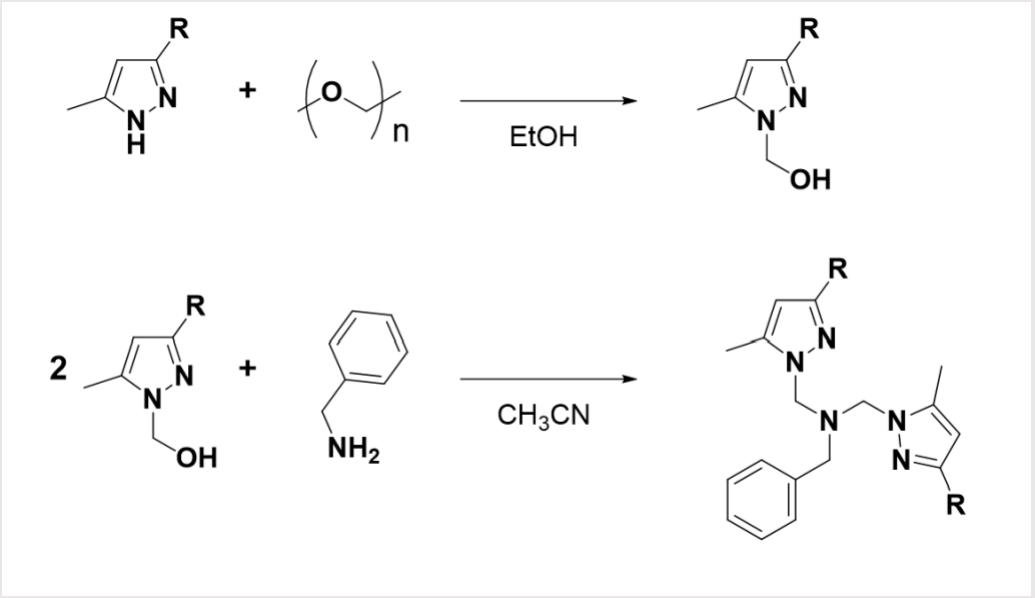
- Bidentate phosphines: Dppm, dppe, dppp, dppb, dppf, dppbz, Xantphos
  - 31P NMR is great, can see clear different in free vs. bound PR_3 if your species is diamagnetic.

- Porphyrin, phthalocyanine, cyclen, salen/salan for macrocyclic ligands

Some synthetic ligands

- Schiff base condensations to make diimines and Nacnac ligands are fairly straightforward. **Salen is easy to make**

- Pyrazoles, formaldehyde, and amines combine well to make tridentate ligands. I've made this ligand type in my lab.



12 Principles of Green Chemistry

- Prevent waste
- Atom economy
- Less hazardous synthesis
- Design benign chemicals
- Design for energy efficiency
- Use of renewable feedstocks
- Reduce derivatives
- Catalysis (vs. Stoichiometric)
- Design for degradation
- Real-time analysis for pollution prevention
- Inherently benign chemistry for accident prevention

Applying Green Chemistry Concepts

- Less energy/time/waste – Do you have to purify intermediates or can they be carried on to the next step? Can we do it hotter to make it go faster?
- Less toxic chemicals (look up info on your ligand starting materials!
- Less solvent – Ball mill or microwave
- Greener solvents: water or alcohols instead of DCM, THF, or acetonitrile.
- Less solvents on workup: Recrystallize instead of column, use Combiflash to save solvent when columning.
- Make only as much material as needed, or make things that can be used for other uses.

To Include in your report/poster

- Title
- Author list
- Abstract
- Graphical Abstract
- Introduction
- Experimental (including synthesis of your ligand/complex)
- Results and Discussion (with clear Figures)
- Conclusions
- Acknowledgements
- References
- Supporting info – full NMR spectra, etc.

Research Tools

- NMR – ^{1}H, ^{13}C, and other nuclei (^{19}F, ^{31}P, ^{11}B). If your metal complex is paramagnetic, NMR of the complex may not be possible.

- IR spectroscopy if your ligand or metal complex has strong IR-active functional groups (carbonyl, nitro, etc.)

- UV-Vis for ligand and complex if they are colorful. Fluorescence if they glow under blacklight.

- We can mail off crystals for X-ray crystallography if the ligand or complex doesn't have a X-ray structure known, but might not get the structure back in time for the final report. Can also mail out samples for elemental analysis (EA) to check purity.

- Others: DLS for nanoparticles, DFT if you have experience with computational chemistry, Cyclic voltammetry if you are interested in the redox properties of the species.

- Zotero for reference management during research and writing.

Other notes

- Take pictures of your reactions/compounds, these will be great for your paper/poster!

- This is the 2nd time trying this particular CURE, but other professors I know are also trying this general idea, and we'll share information.

- I had a student test out the catechol oxidation reaction tracking by UV-Vis, and we calculated the molar absorptivity of the 3,5-di-tert-butyl-quinone (DTBQ). It's 1630 $cm^{-1}M$ in methanol at the max absorbance (~400 nm). <u>There are some incorrect values out there!</u>

Burning Questions?