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Developing a New Water-Soluble Porphyrin as a Potential Photodynamic Cancer Therapy Agent

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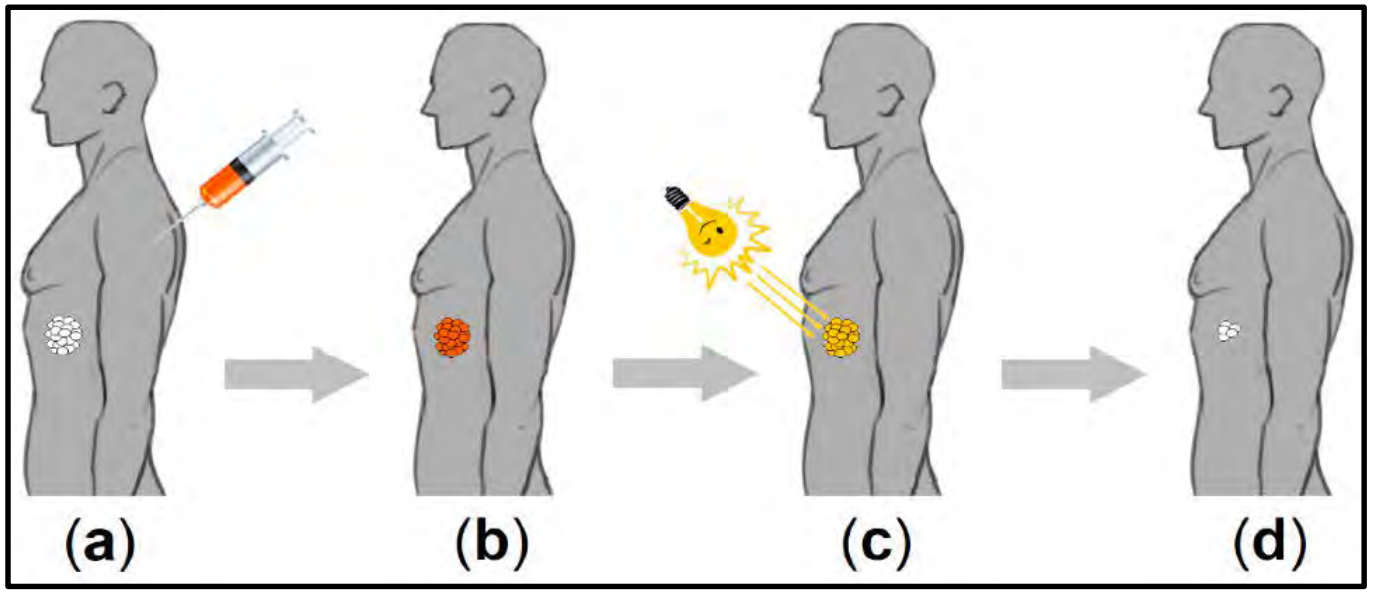
A SENIOR THESIS BY CATHERINE L SHIRLEY

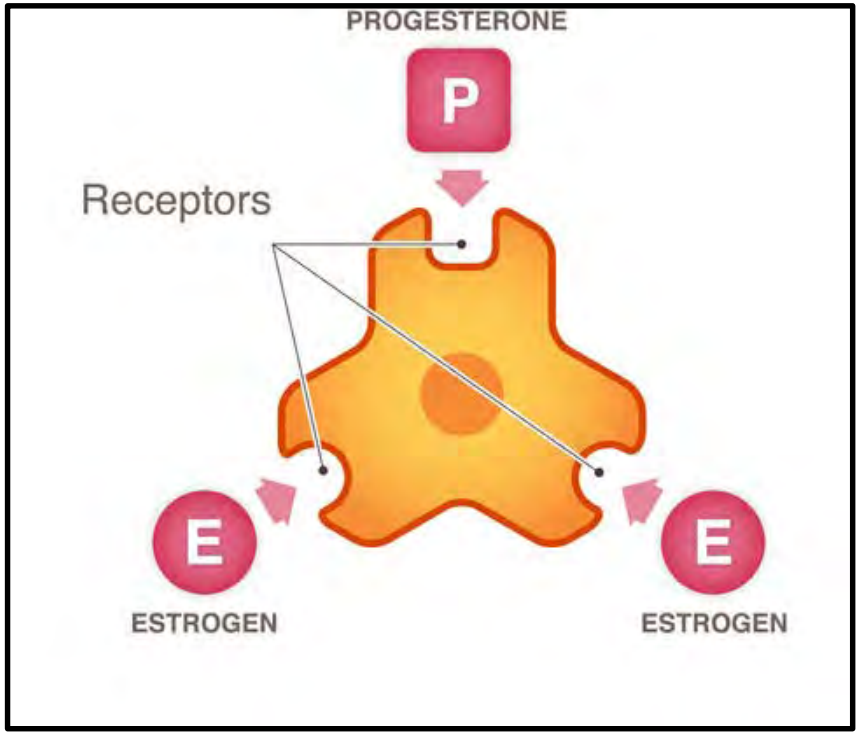
Background

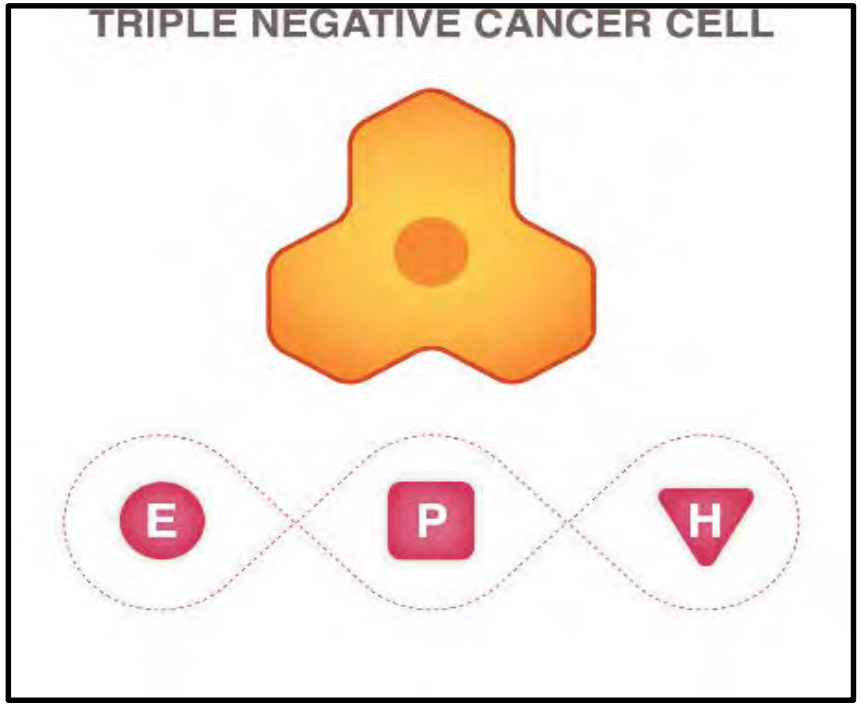
What is Photodynamic Therapy?

- Photodynamic Therapy (PDT): treating cancer with light +a photosensitive compound

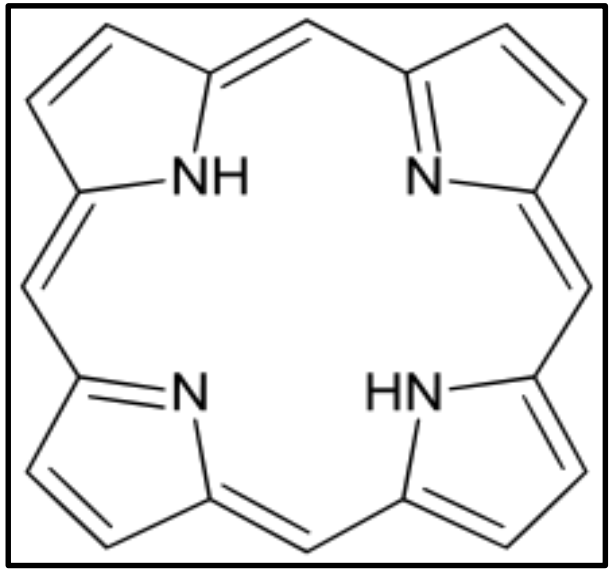


Why PDT?


PROGESTERONE
P
Receptors
E
E
ESTROGEN
ESTROGEN


TRIPLE NEGATIVE CANCER CELL
E
P
H

What are Porphyrins?



The structure of a porphyrin molecule.
Chemical formula $C_{20}H_{14}N_4$

- Known for their role in hemoglobin
- Alternating single and double bonds give aromaticity and stabilization
- Create singlet, molecular oxygen, resulting in cytotoxicity of the tumor tissue cells

How is PDT Currently Being Used?

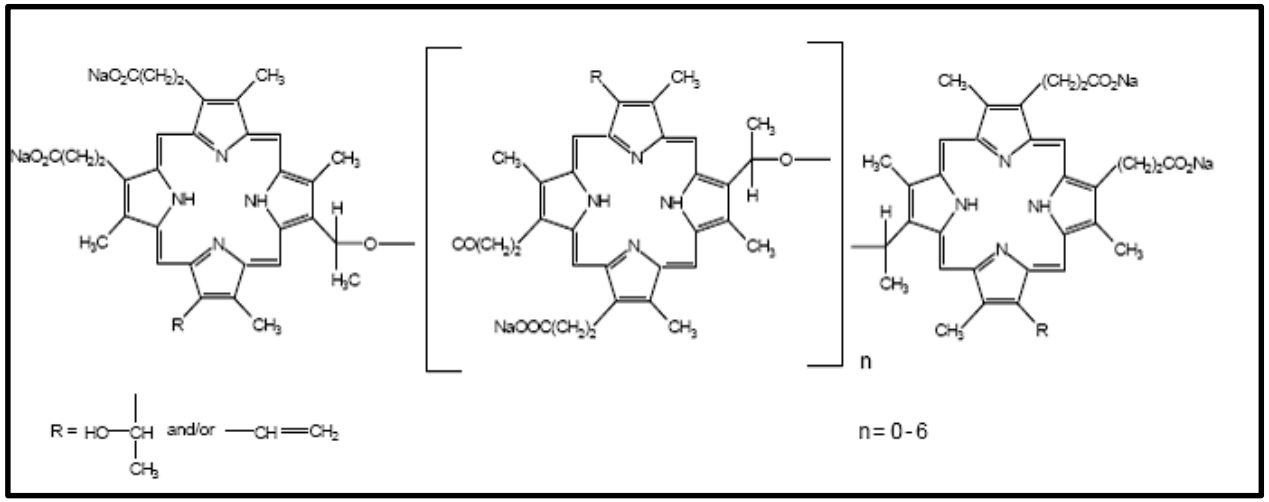
Three Photosensitizers

Photofrin®

Foscan®

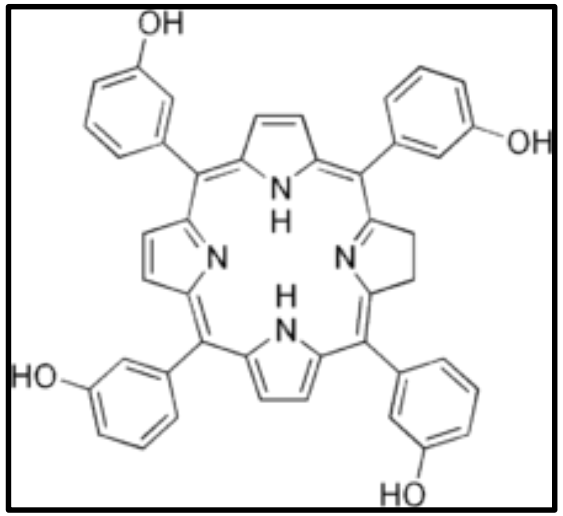
5-Aminolevulinic Acid

Photofrin®



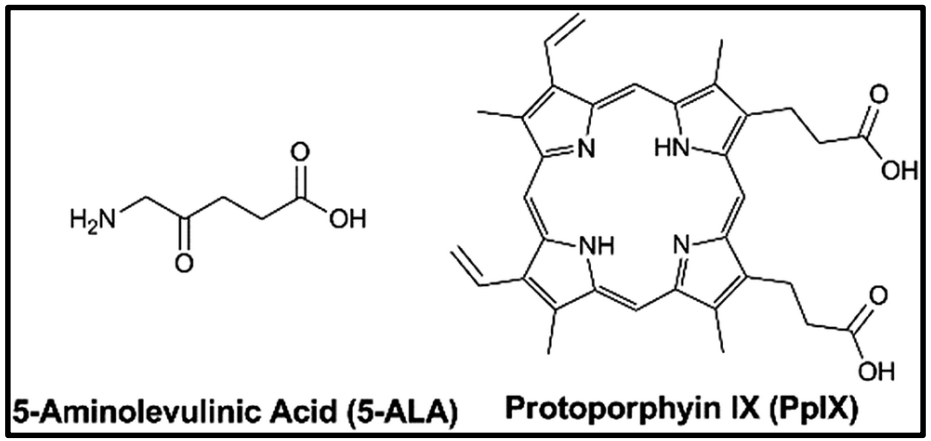
- Most studied photosensitive agent
- Reliable and easily activated
- Prolonged skin sensitivity

Foscan®



- Great tumor selectivity and deeper light penetration
- Only approved in Europe

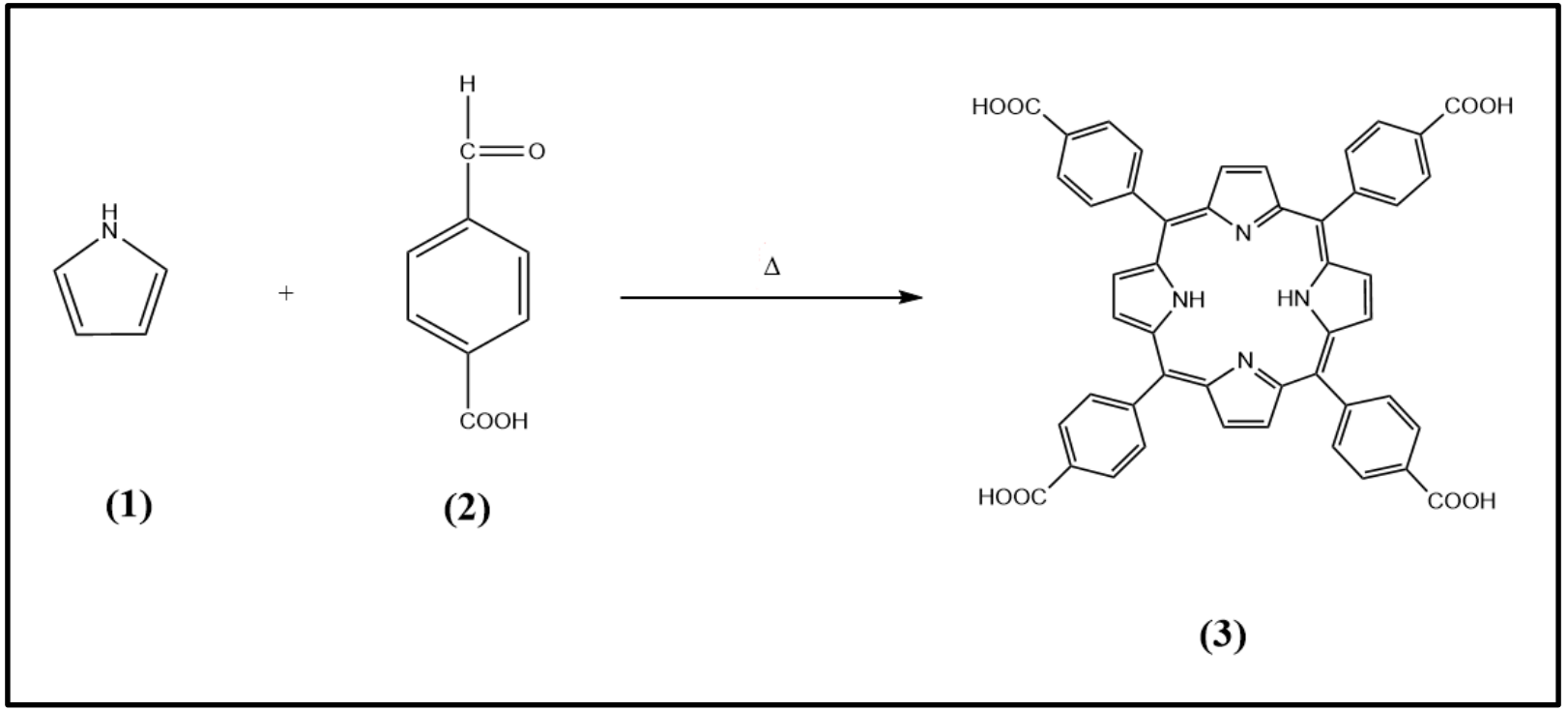
5-Aminolevulinic Acid



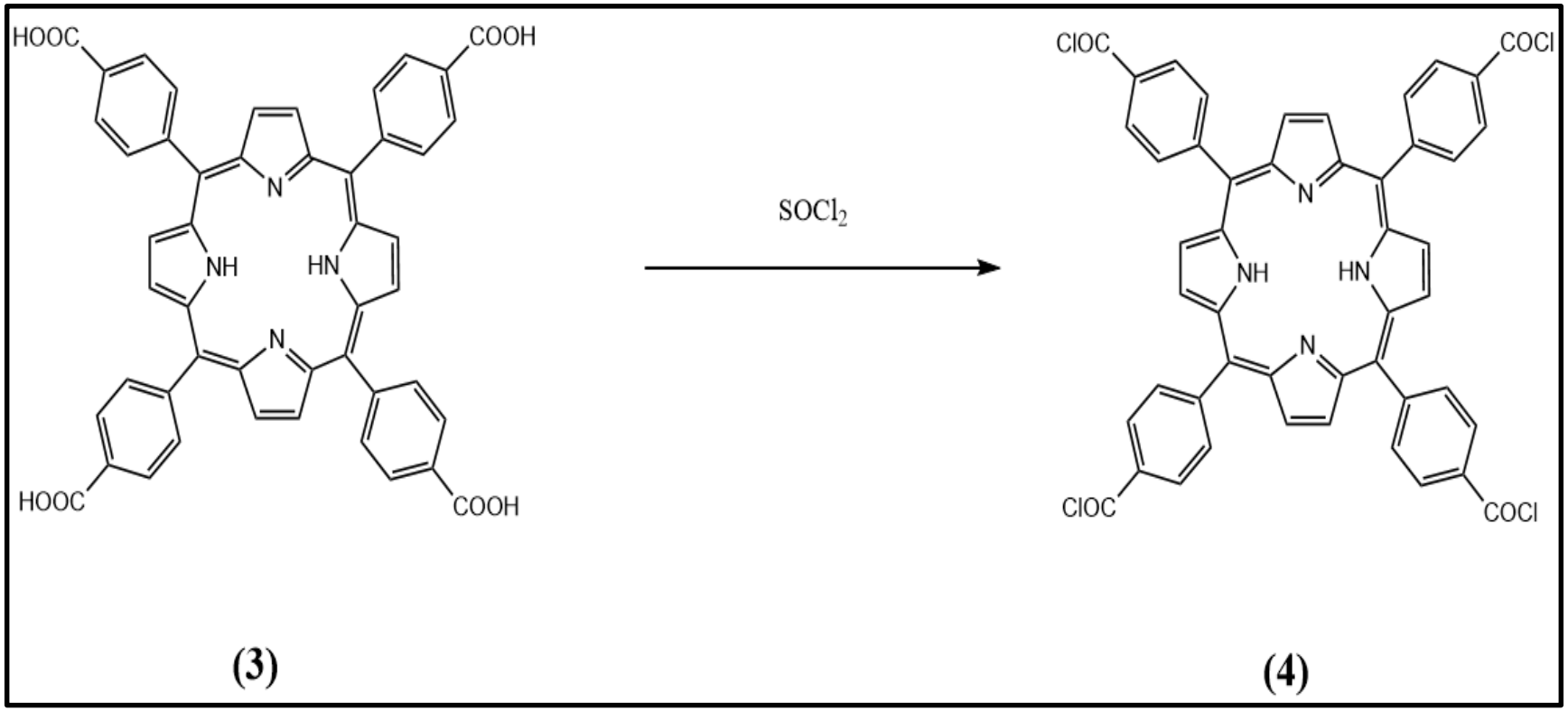
- Only for superficial lesions
- Clears from body in 48 hours
- Not effective for deep tumors

Methods:
Porphyrin Synthesis

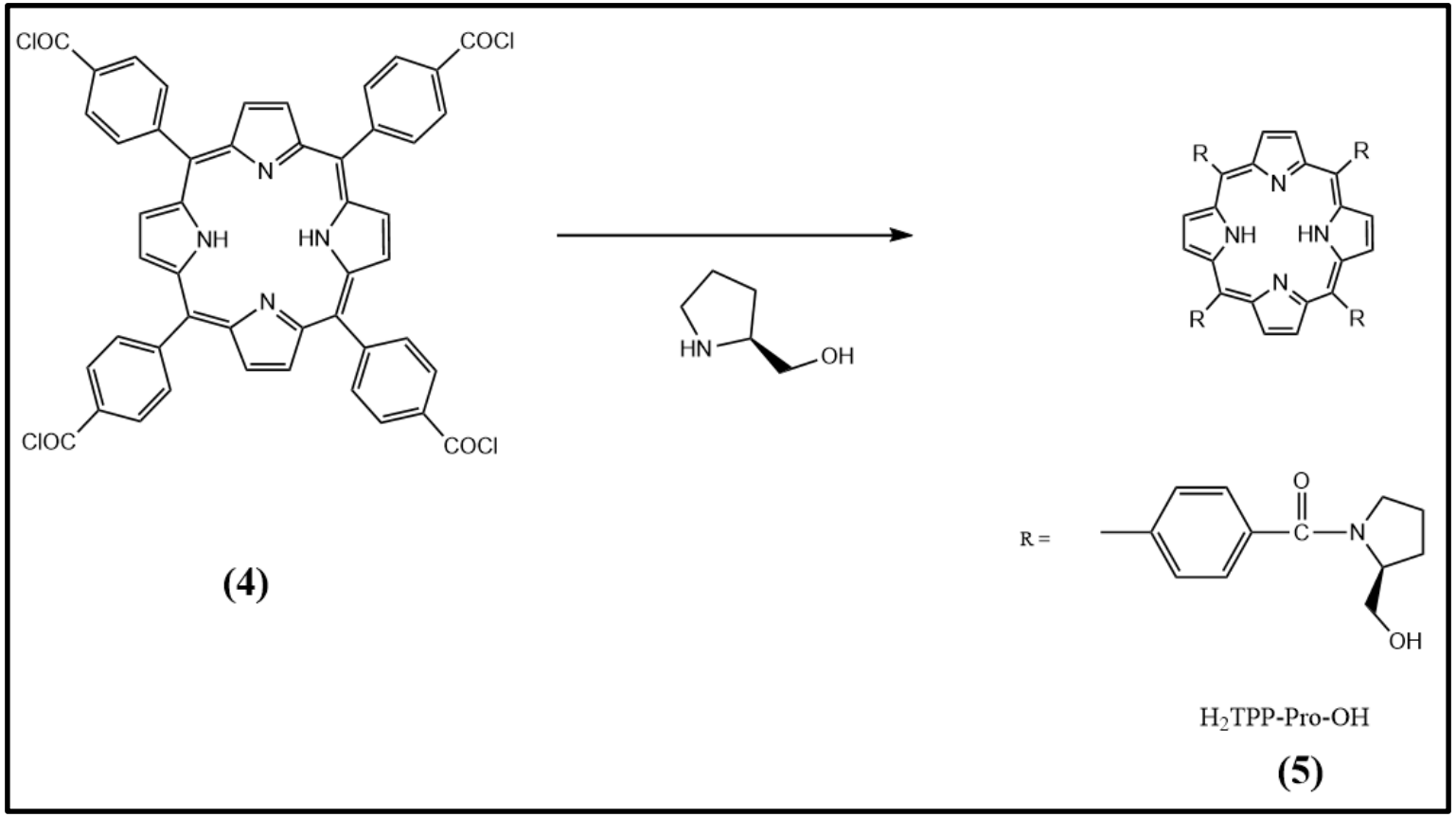
Formation of H_2TPPC



Formation of the Acid-Chloride Porphyrin



Formation of the Final Product, H_2TPP-Pro-OH

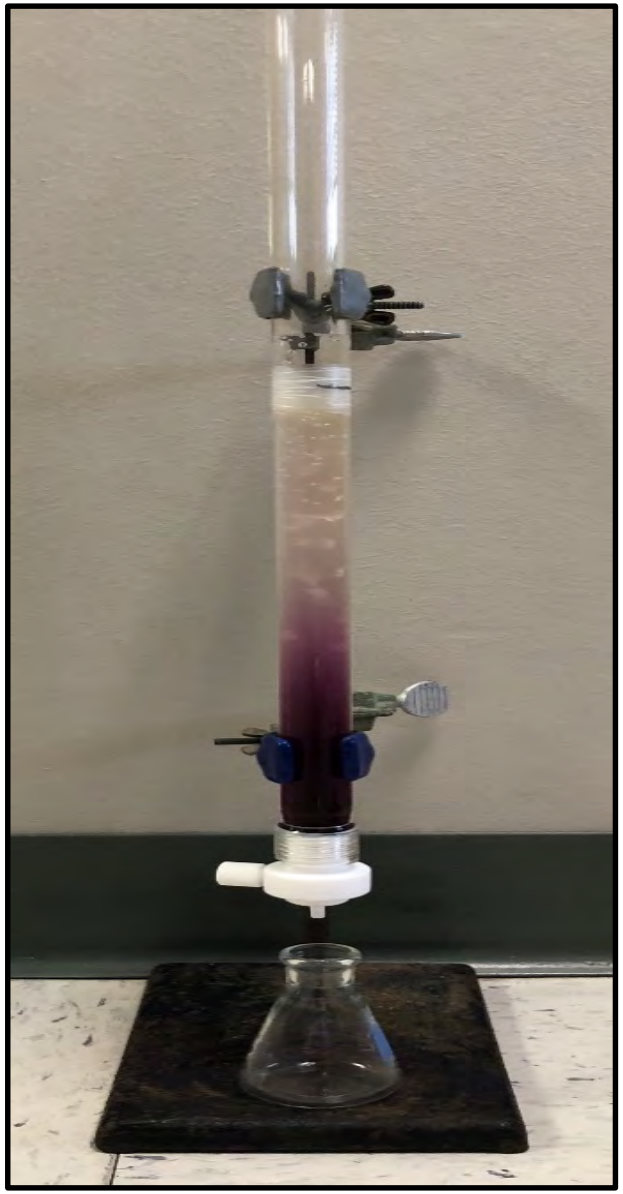


Purification

Two Methods to Purify

Sephadex LH-20

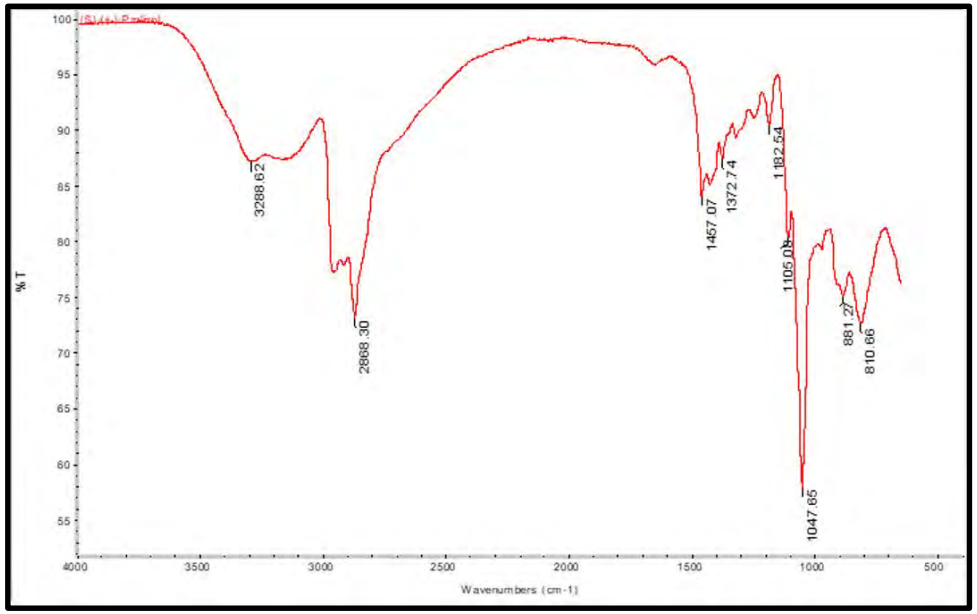
Sephadex G-50



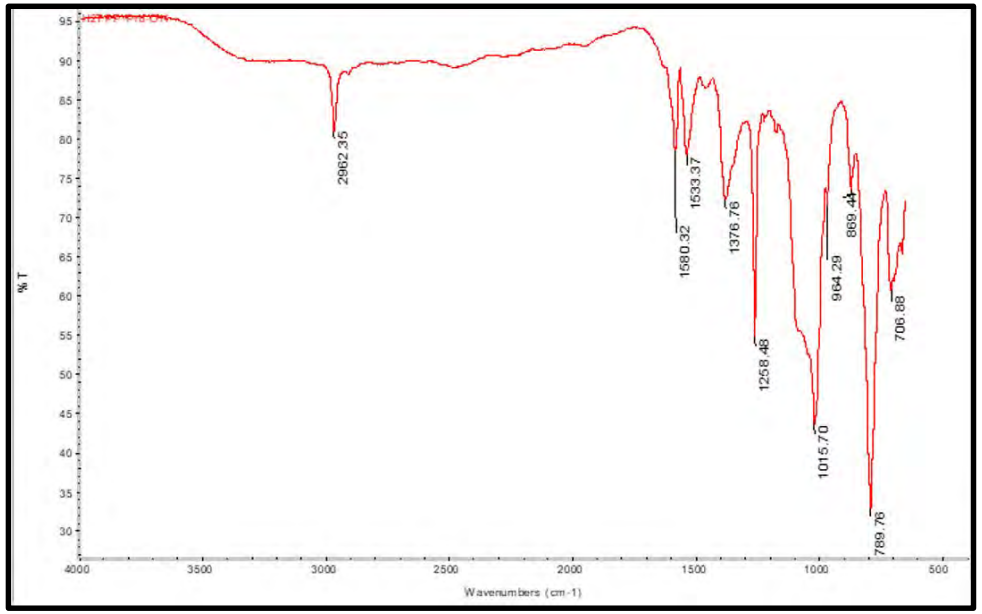
Characterization and Results

Infrared Spectroscopy

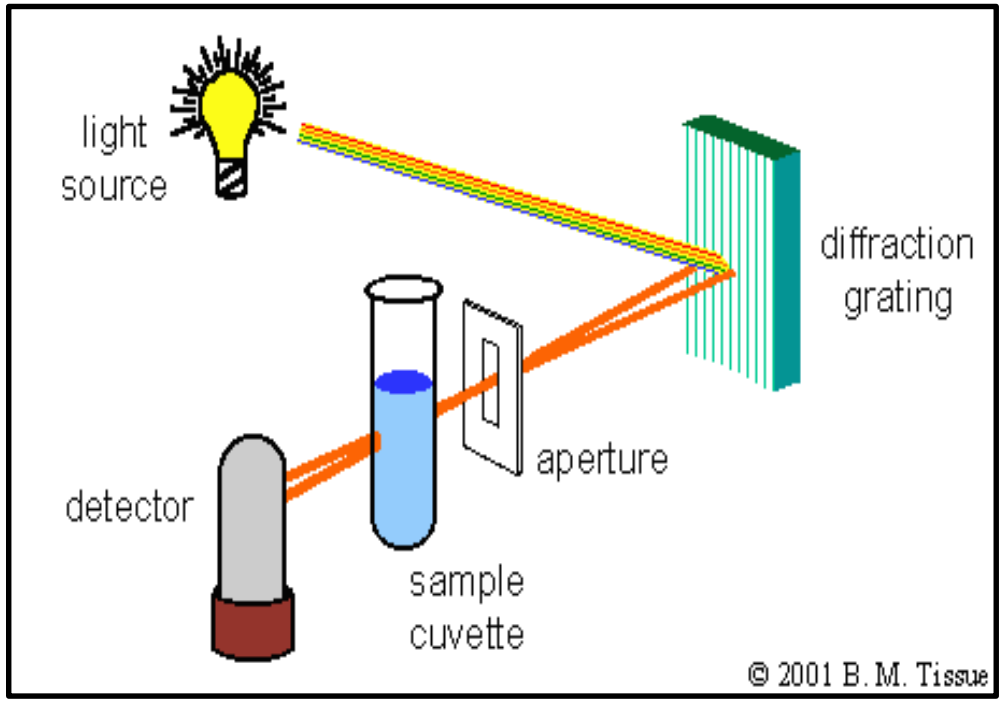
Infrared results for (S)-(+)-prolinol.



Infrared results for H_2TPP-Pro-OH.



Ultraviolet-Visible Spectroscopy

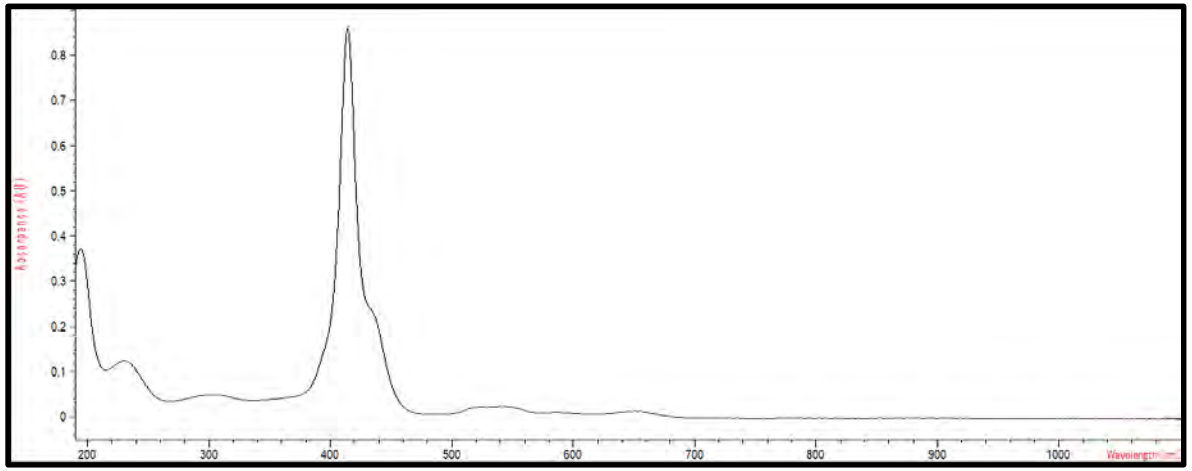


- UV-Vis Spectroscopy diagram showing how light is transmitted

Ultraviolet-Visible Spectroscopy

Top: UV-Vis results for H_2TPP-Pro-OH.

Bottom: Peaks (nm) and epsilon (ε) values for the UV-vis spectroscopy of H_2TPP-Pro-OH. Epsilon values calculated using Beer's Law: $A = \varepsilon c l$.

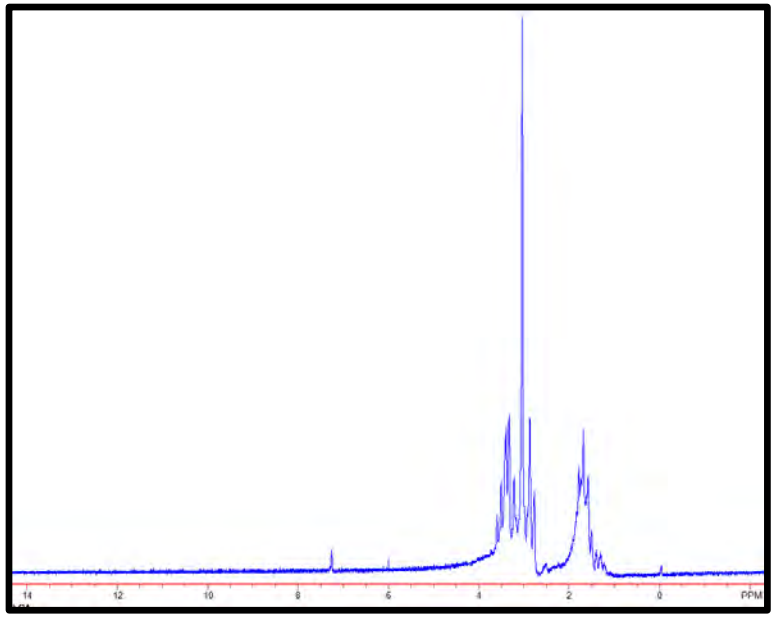


Peaks (nm)	Molar Absorptivity Coefficient, ε (cm^{-1} mM^{-1})
414	361
518	10.3
555	6.32
581	5.66
637	5.03

^{1}H Nuclear Magnetic Resonance Spectroscopy

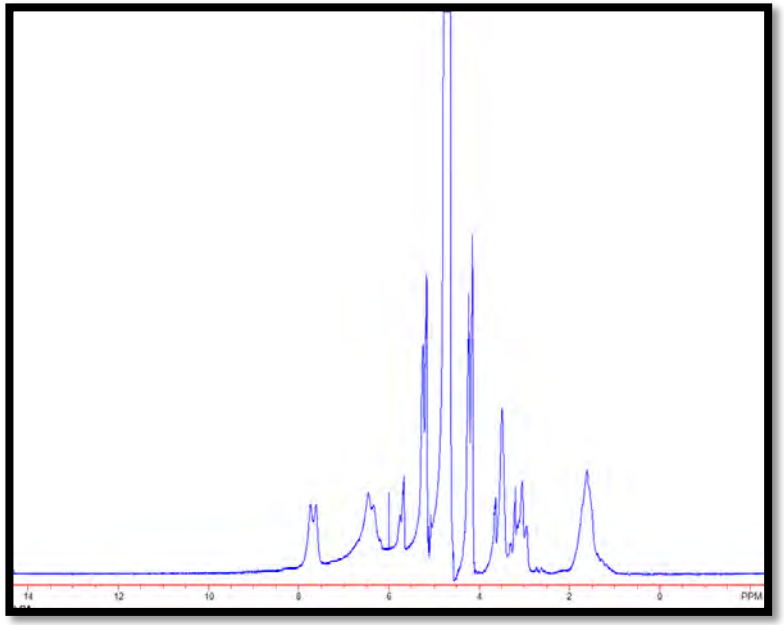
Results from ^{1}H NMR spectroscopy of the free amine,

(S)-(+)-prolinol, in $CDCl_3$.

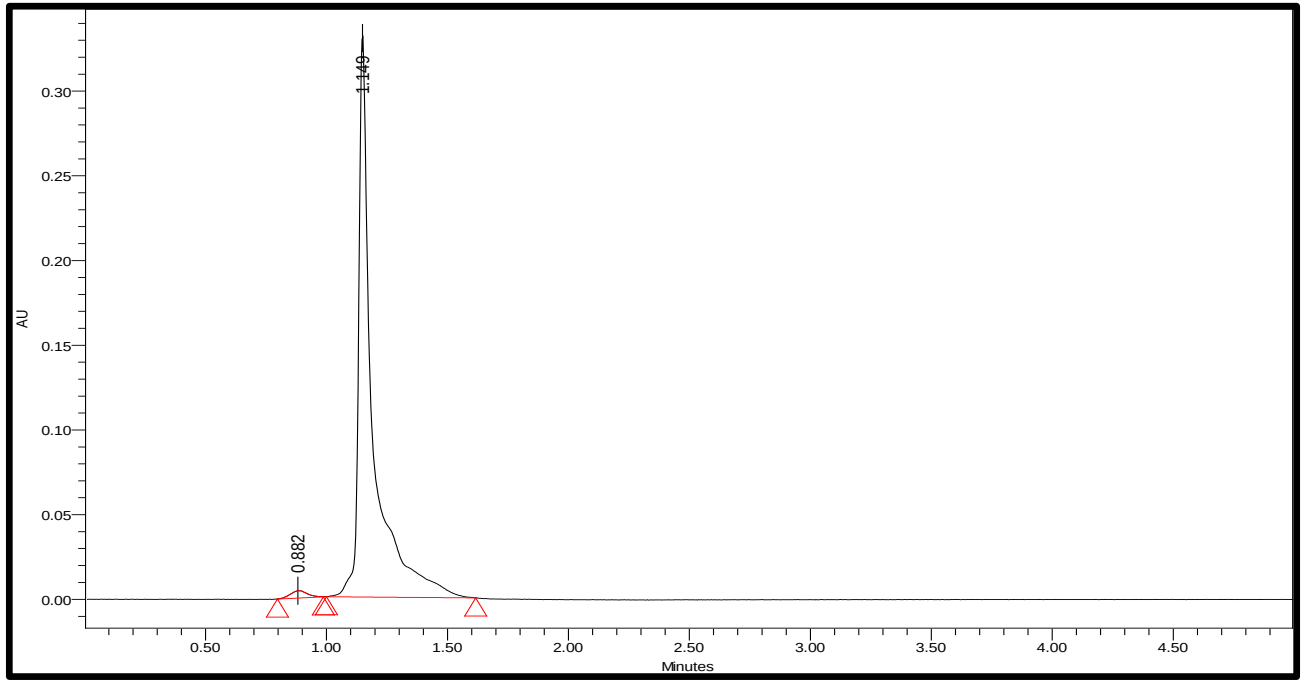


Results from ^{1}H NMR spectroscopy of final product,

H_2TPP-Pro-OH, in D_2O.



High Performance Liquid Chromatography

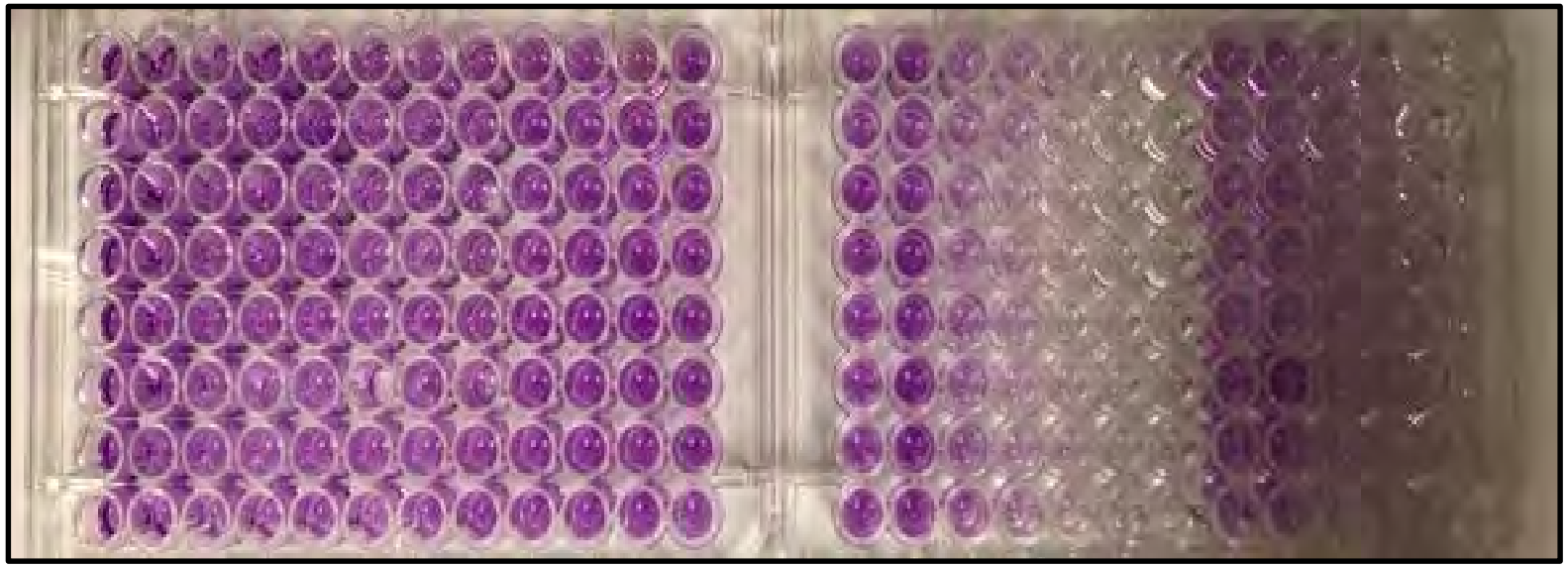


- HPLC results for H_2TPP-Pro-OH indicating 98% purity

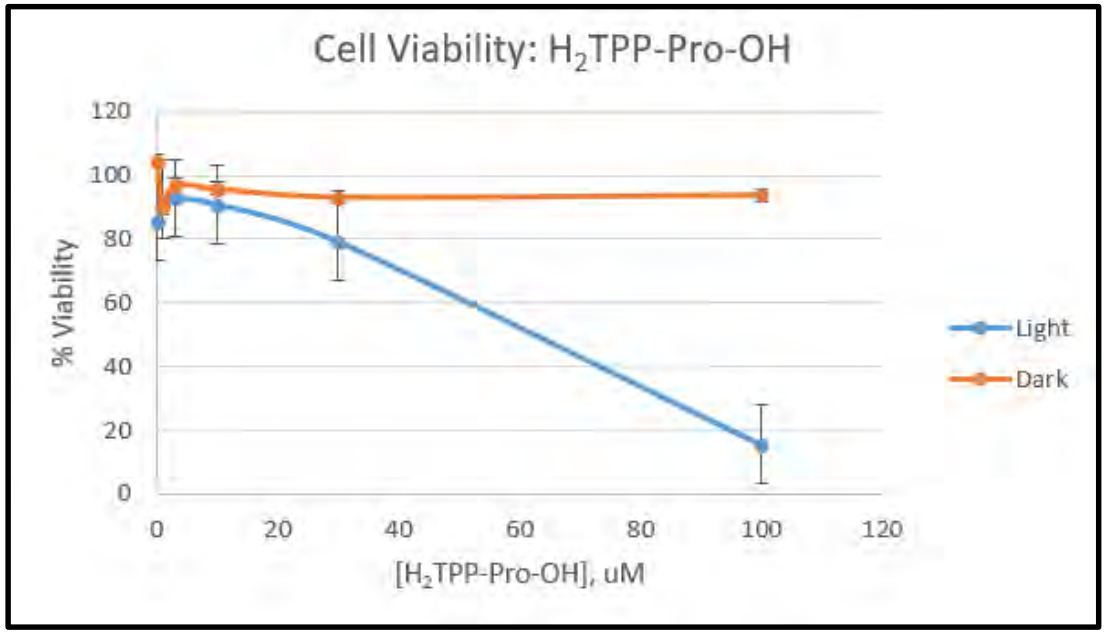
Testing

MTT Assay Procedure

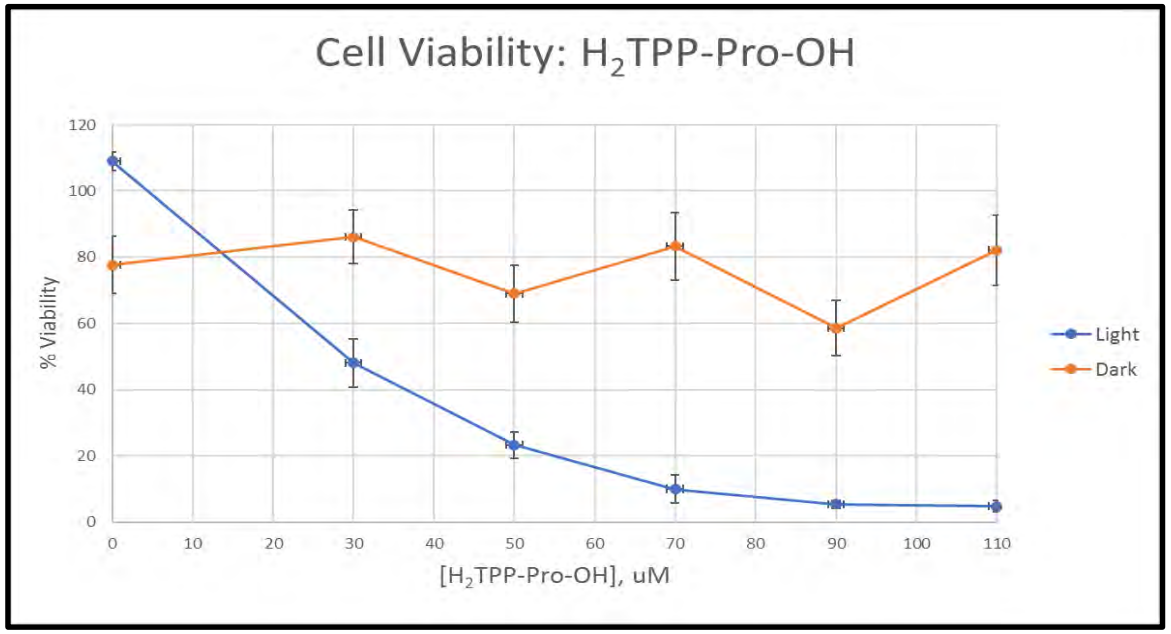
- Method of measuring cellular metabolic activity and determining cell viability
- MTT assay results after 72 hours of incubation. The left plate was entirely in dark conditions, while the right plate was exposed to light. The purple color indicates living cells.



MTT Assay Results



- Spectrophotometric MTT assay results for trial one of the light and dark 96-well plates. The porphyrin concentrations used were 1, 3, 10, 30, and 100 µM



- Spectrophotometric MTT assay results for trial two of the light and dark 96-well plates. The porphyrin concentrations used were 30, 50, 70, 90, and 110 µM

Conclusions

Future Work

Thank you!

DR. JOE BRADSHAW
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DR. TIM HAYES
DR. TERRY CARTER
TRAVIS HANKINS
ALEX PODGUZOV

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