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### The Development of a New Water-Soluble Zinc Porphyrin, ZnTPP-5AP, as a Photodynamic Therapy Ageng

Kaylee Johnson

*Ouachita Baptist University*

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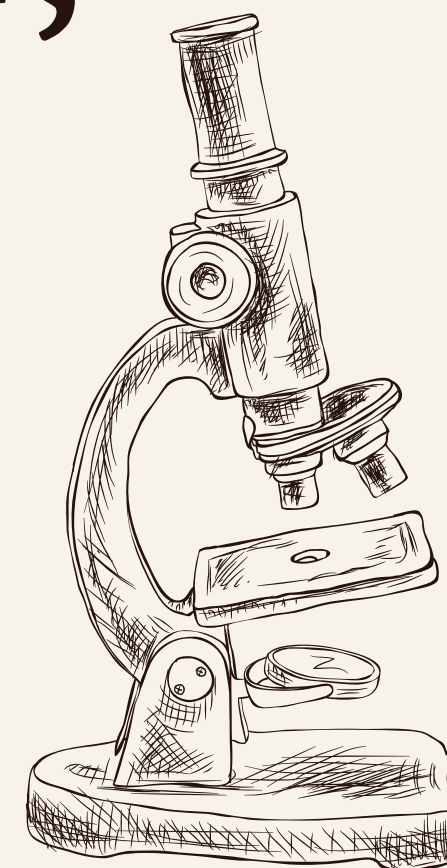
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# The Development of a New Porphyrin, ZnTPP-5AP, for Photodynamic Therapy

Senior Thesis by Kaylee Johnson



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Background

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Synthesis

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Purification and Characterization

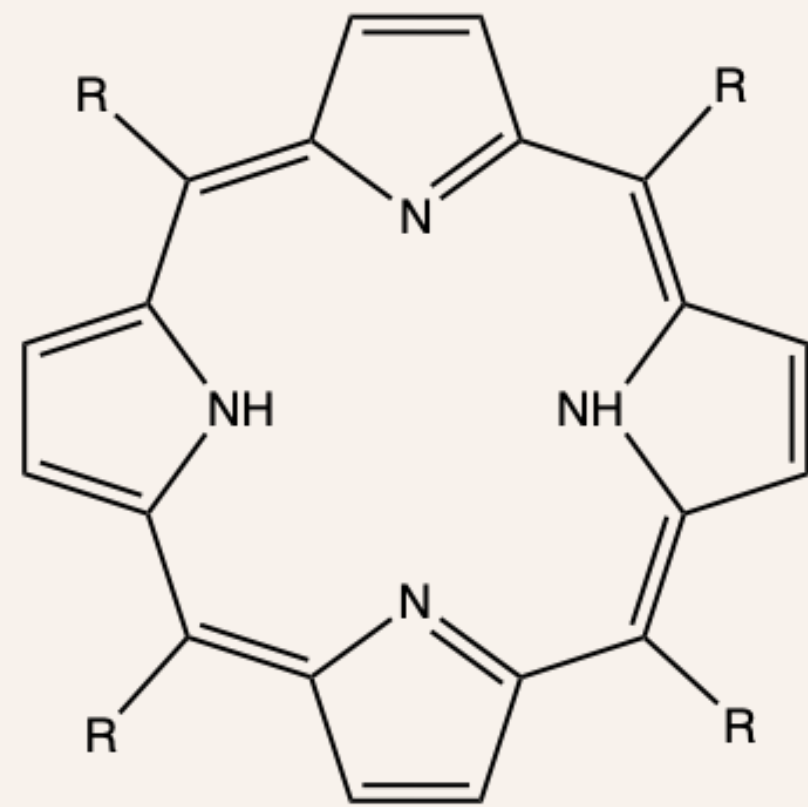
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Metabolic Analysis

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Conclusion and Future Work

# What are Porphyrins?

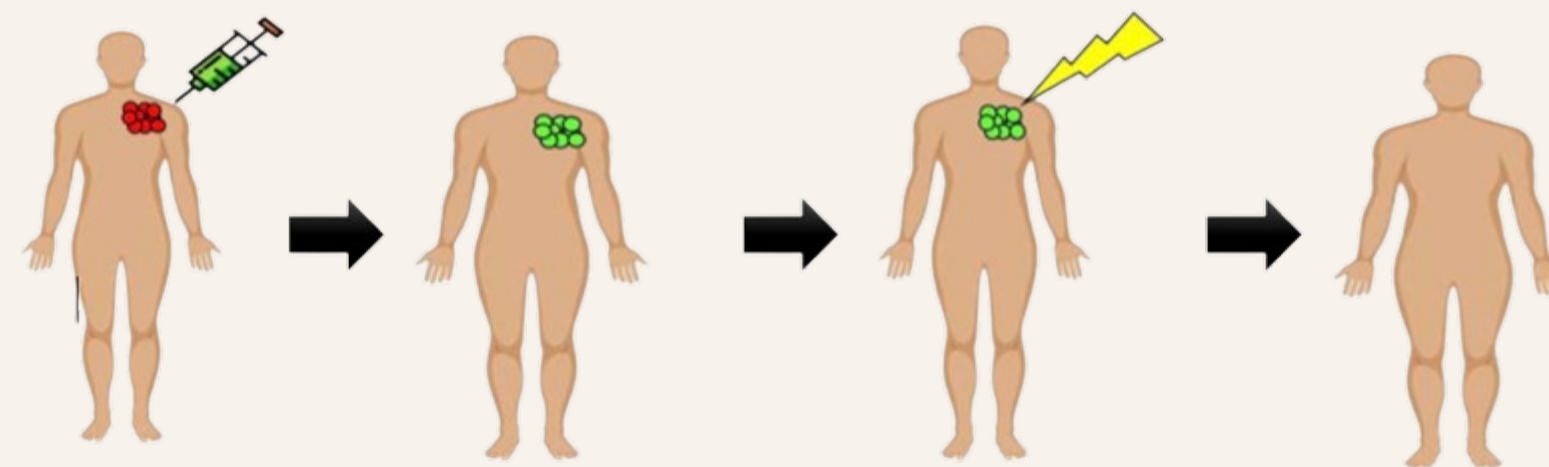


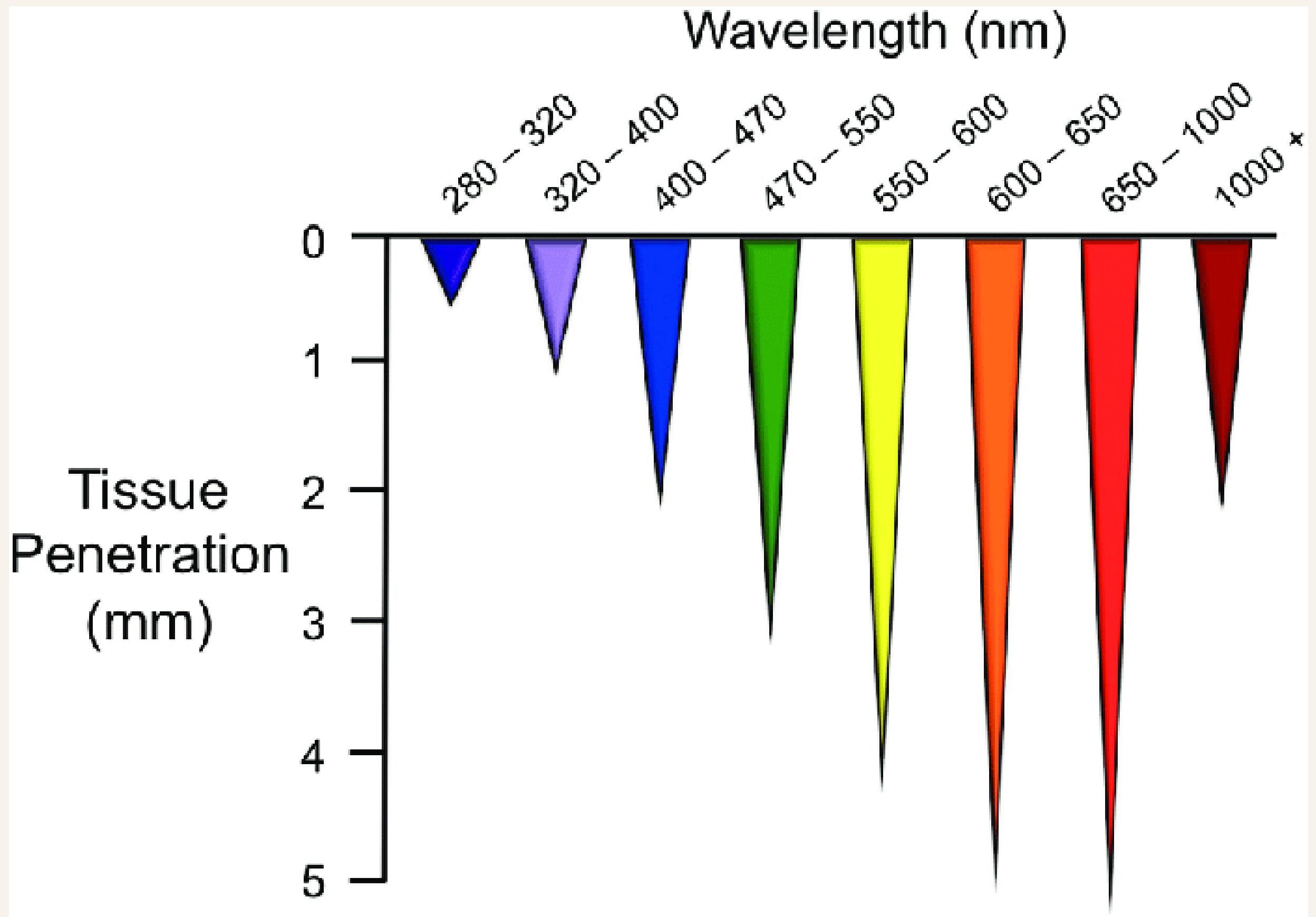
Standard Porphyrin Core

- Tetrapyrrolic molecules that contain four pyrrolic subunits linked through methine (CH) bridges
- The conjugated structure allows the porphyrin to absorb specific wavelengths
- This research specifically utilizes and analyzes the light sensitivity of the porphyrins and how they respond by resulting in cell death of lung cancer cells.
- Porphyrins have been used for several processes in the body including gene regulation, drug and iron metabolism, oxygen transport, electron transport medium, and solar cell.

# What is Photodynamic Therapy (PDT)?

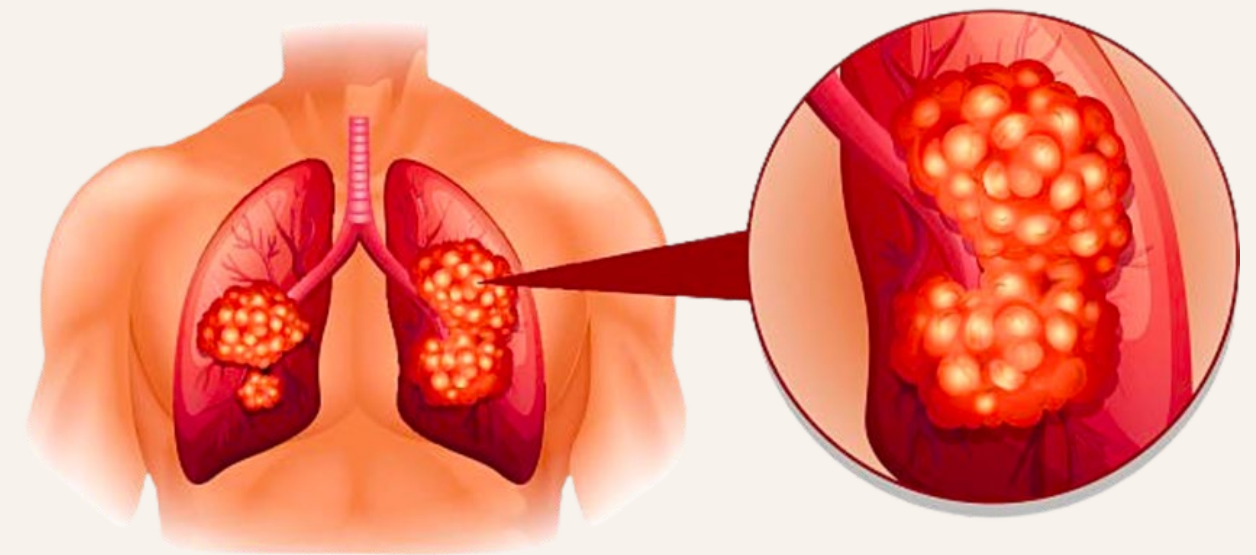
- New cancer treatment that uses light, oxygen, and a photosensitizer to kill malignant cells
- In PDT the photosensitizing agent, the porphyrin, is administered first to the patient
- The porphyrin will then concentrate at the site of the tumor
- The photosensitizing agent is then exposed to light and cell death occurs





# Why A549 NSLC Cells?

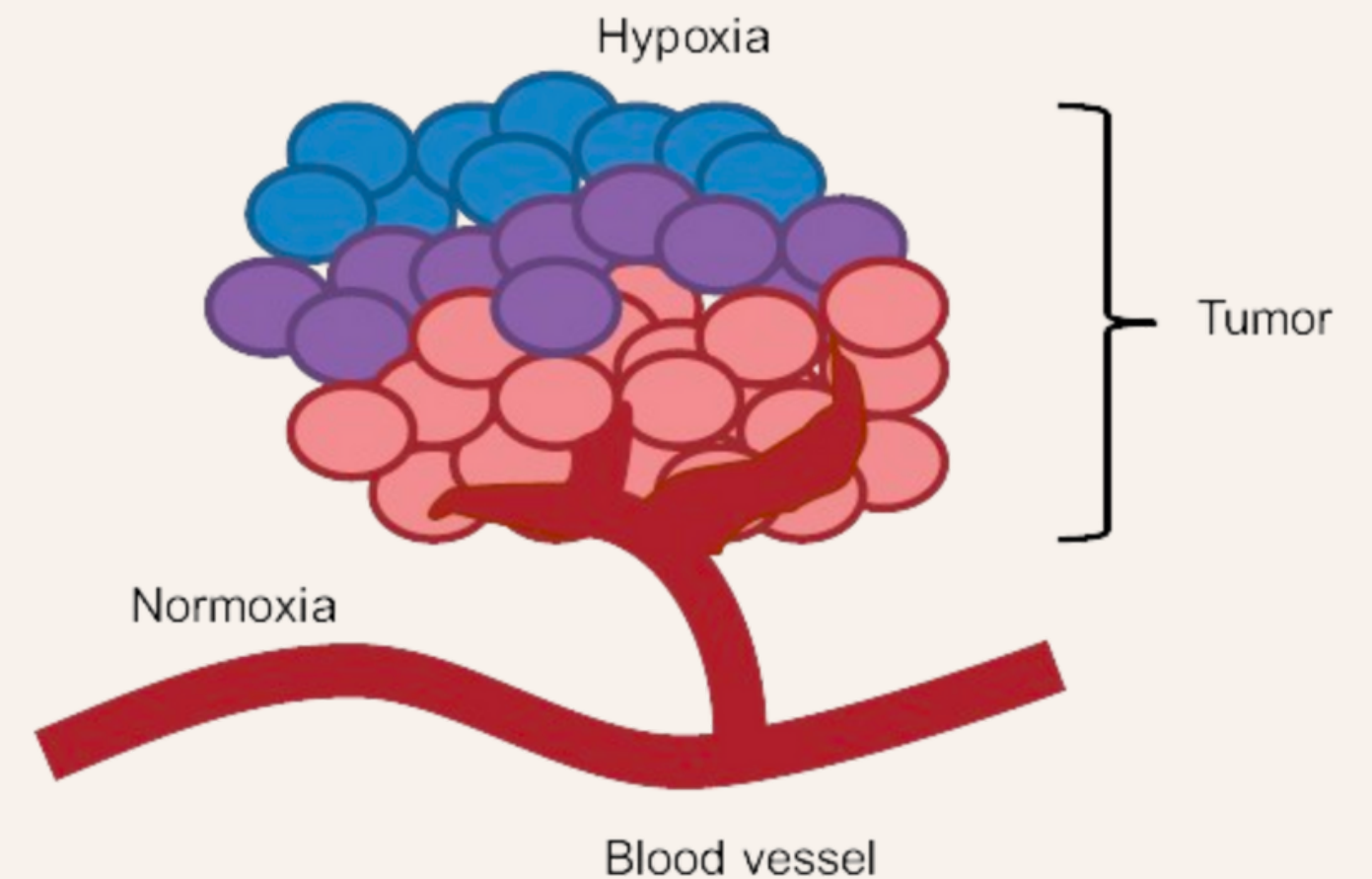
- Non-small lung cancer accounts for about 85% of all lung cancers, with lung cancer being the leading cause of cancer-related deaths in the world
- Around 2 million people each year worldwide will be diagnosed with lung cancer, affecting male and female equally
- 5-year survival rate is between 10-20%



# What is Hypoxia?

Definition: an absence of enough oxygen to sustain normal bodily functions

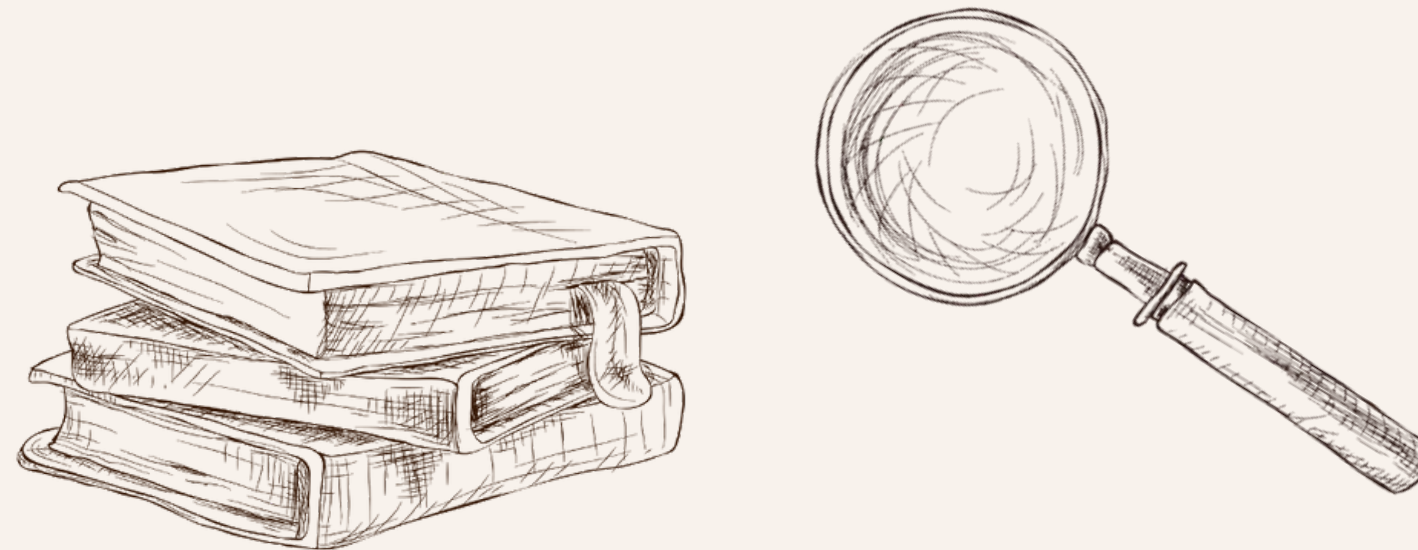
In proliferating tumors, the oxygen demand is higher than the oxygen supply. This leads to cells having low oxygen levels that are further from the vascularization. Changes in oxygen level can alter the tumor's metabolism and contribute to therapy resistance.





# Project Goals

- Synthesize, purify, characterize, and test a novel porphyrin on A549 NSLC cells
- The porphyrin should be water soluble, have a low lethal dosage (LD 50) when exposed to light, and minimal toxicity on cells when kept in the dark



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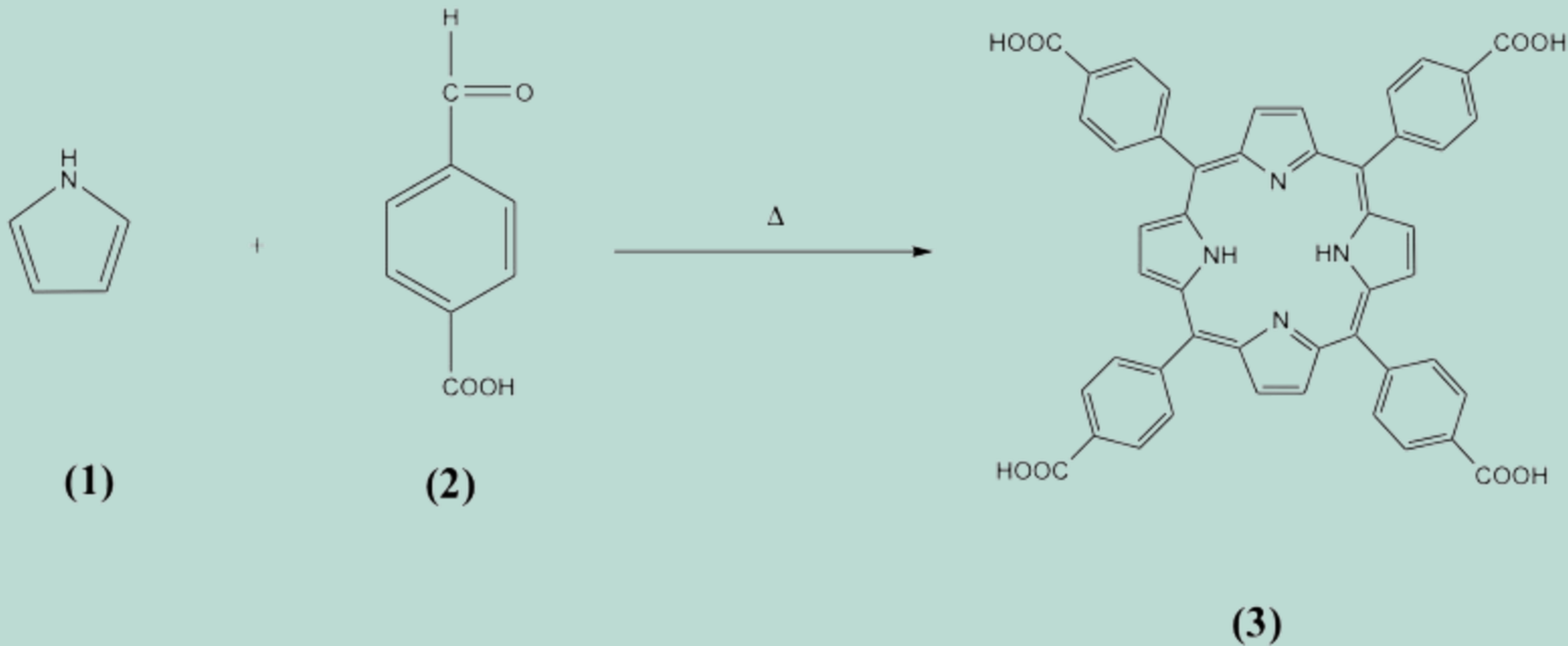
4

Metabolic Analysis

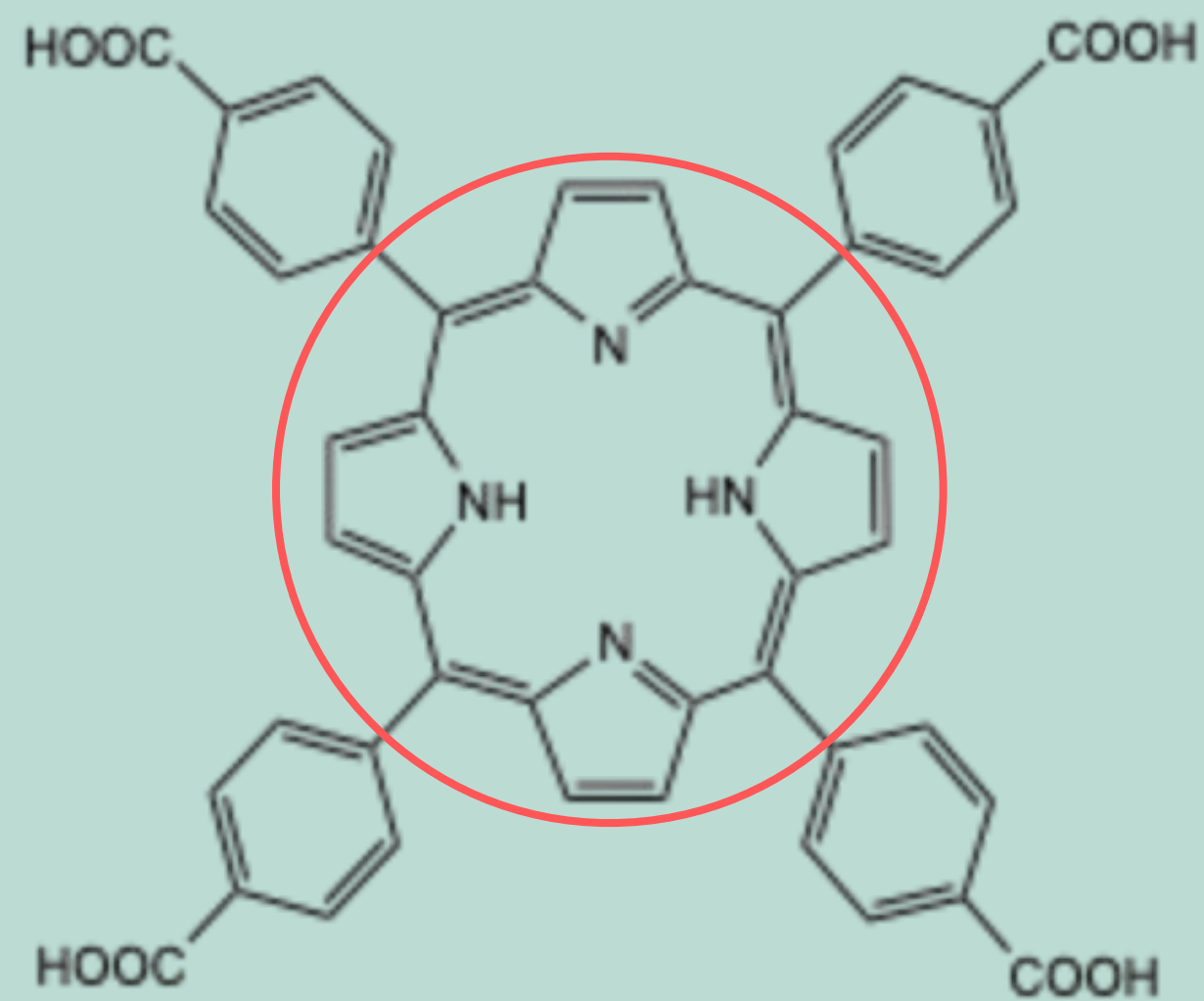
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Conclusion and Future Work

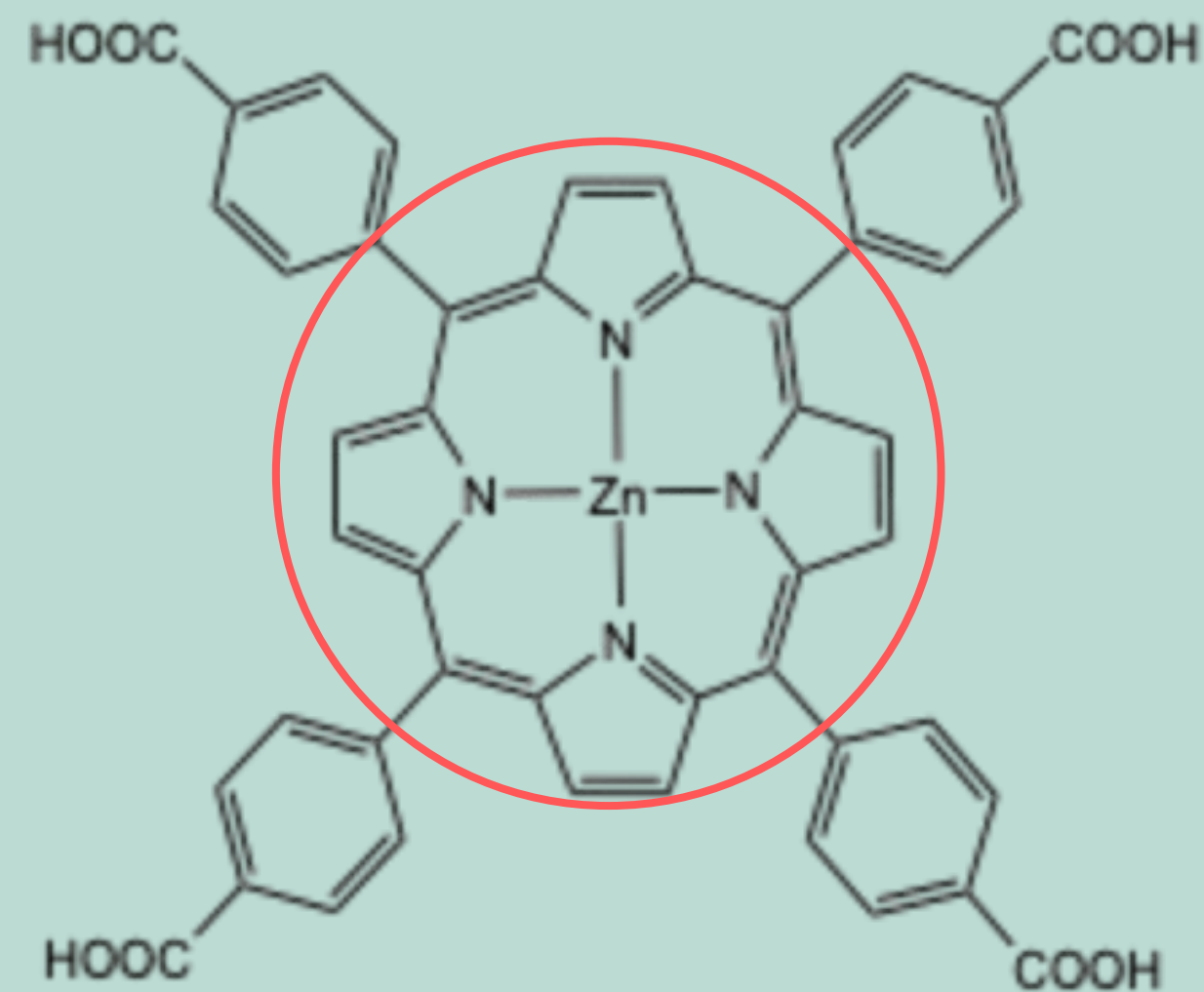
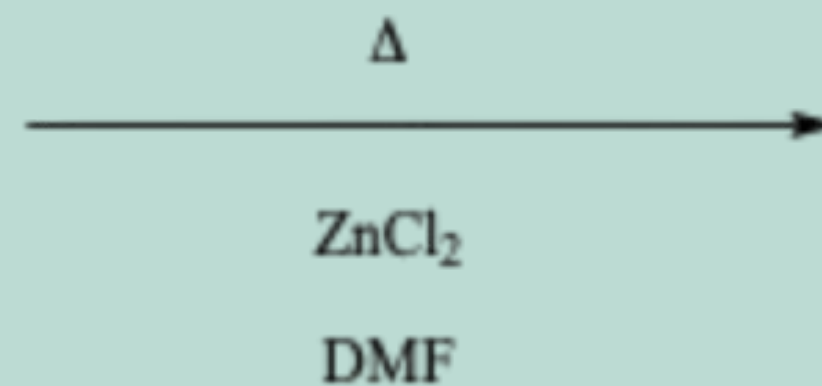
# Formation of H<sub>2</sub>TPPC



# Formation of ZnTPPC

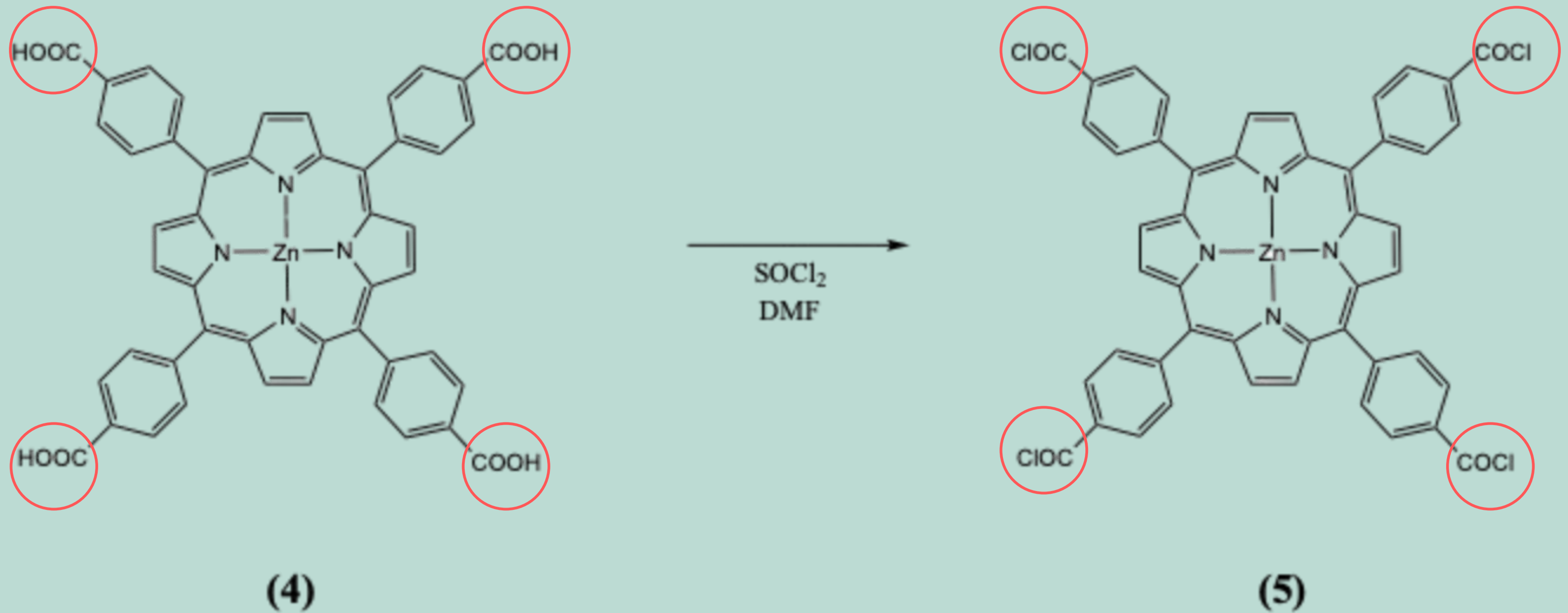


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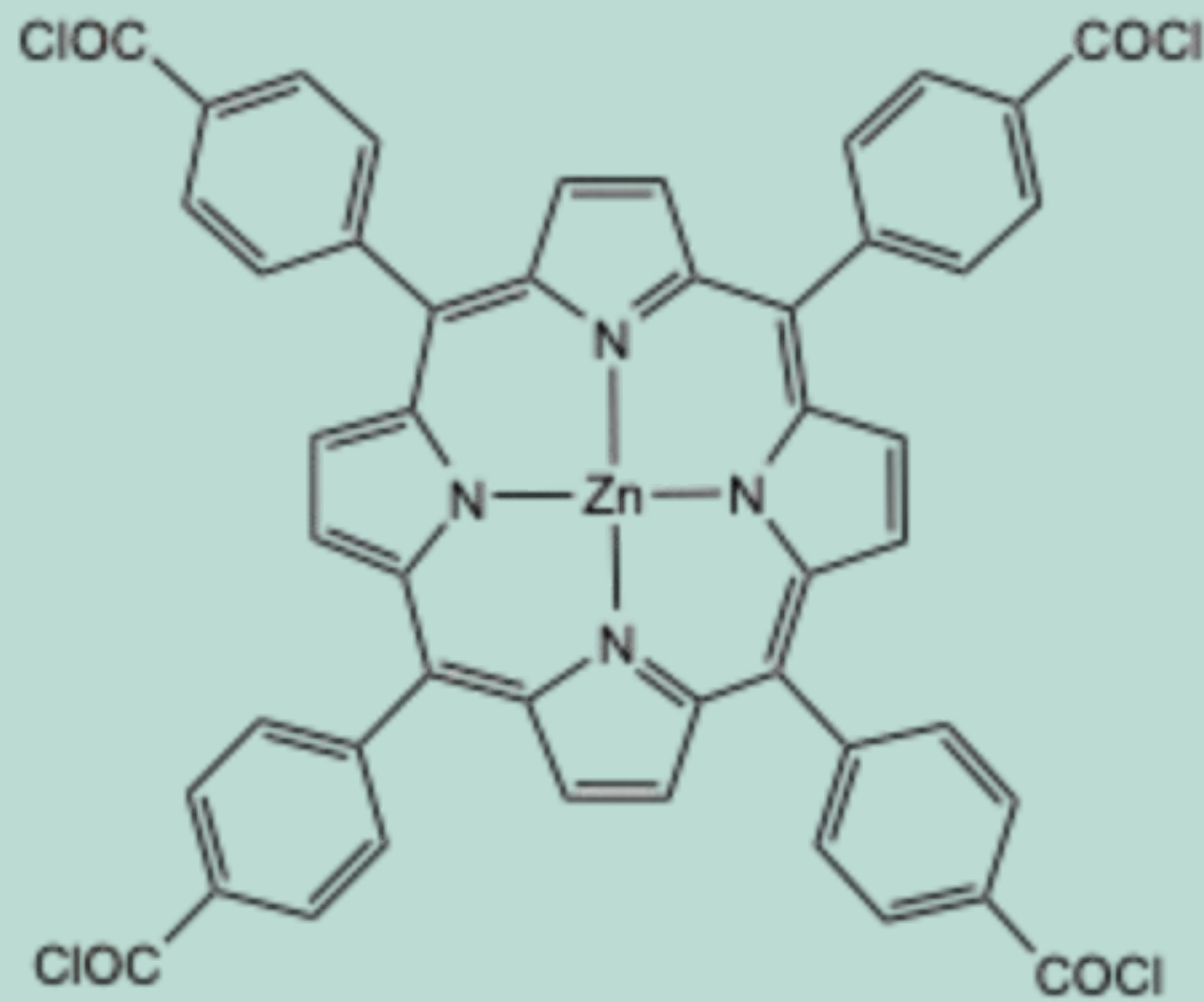


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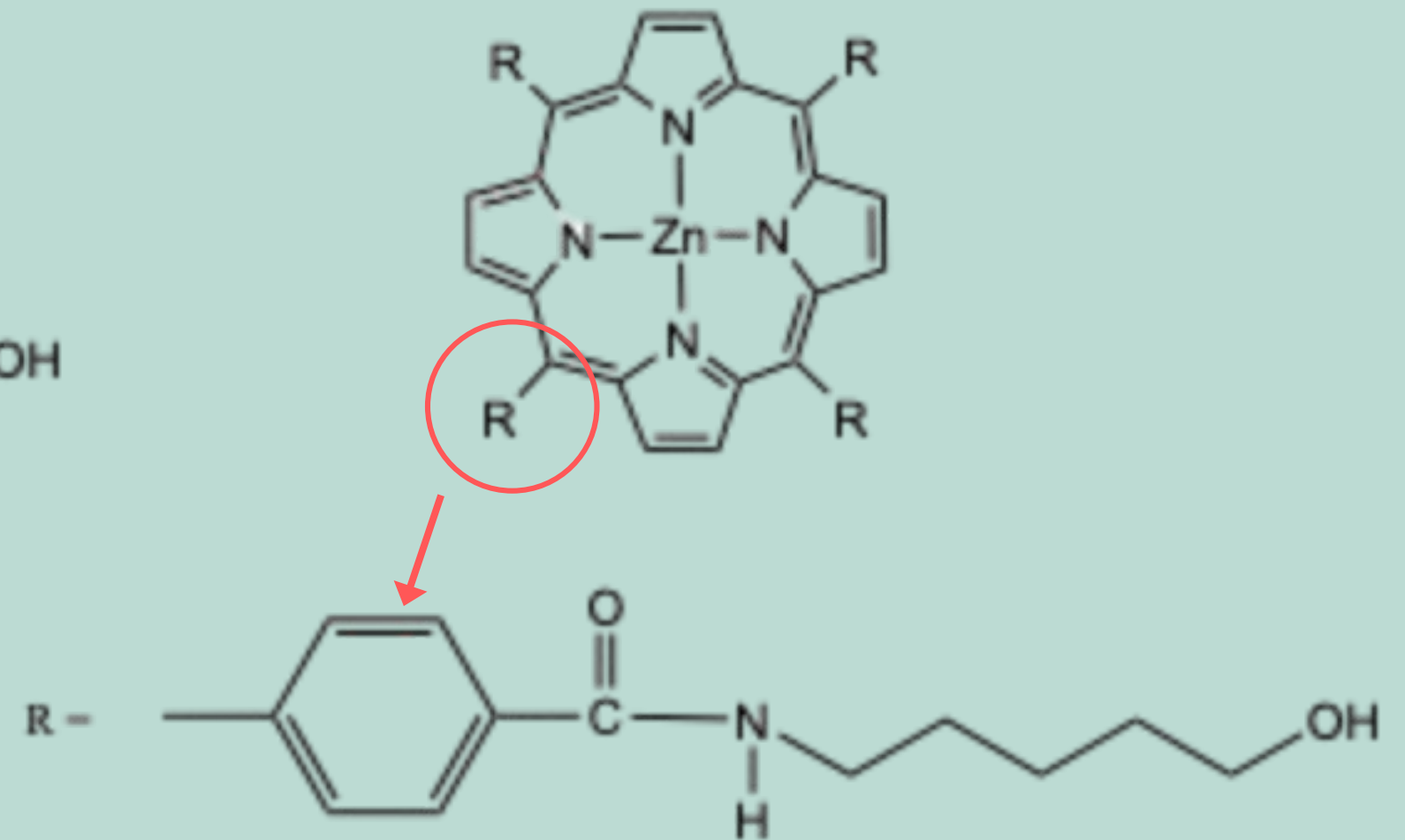
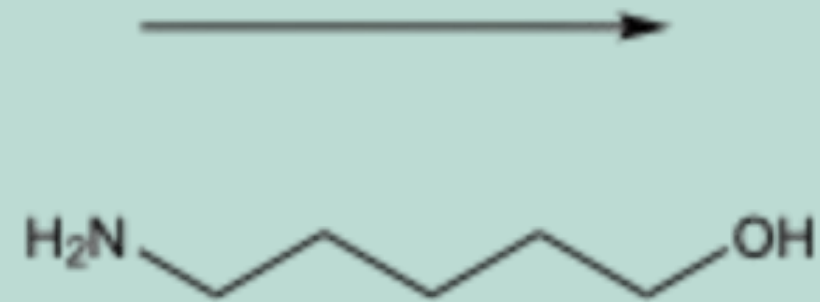
# Formation of Acid Chloride Intermediate



# Formation of ZnTPP-5AP



(5)



ZnTPP-5A

(6)

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# Purification

The final product, ZnTPP -5AP was purified with 0.45-micron nylon syringe, Sephadex LH-20, and Sephadex G-50.

0.45-micron Nylon Syringe: Filters impurities out based on size

Sephadex LH-20: Filters impurities out based on lipophilicity

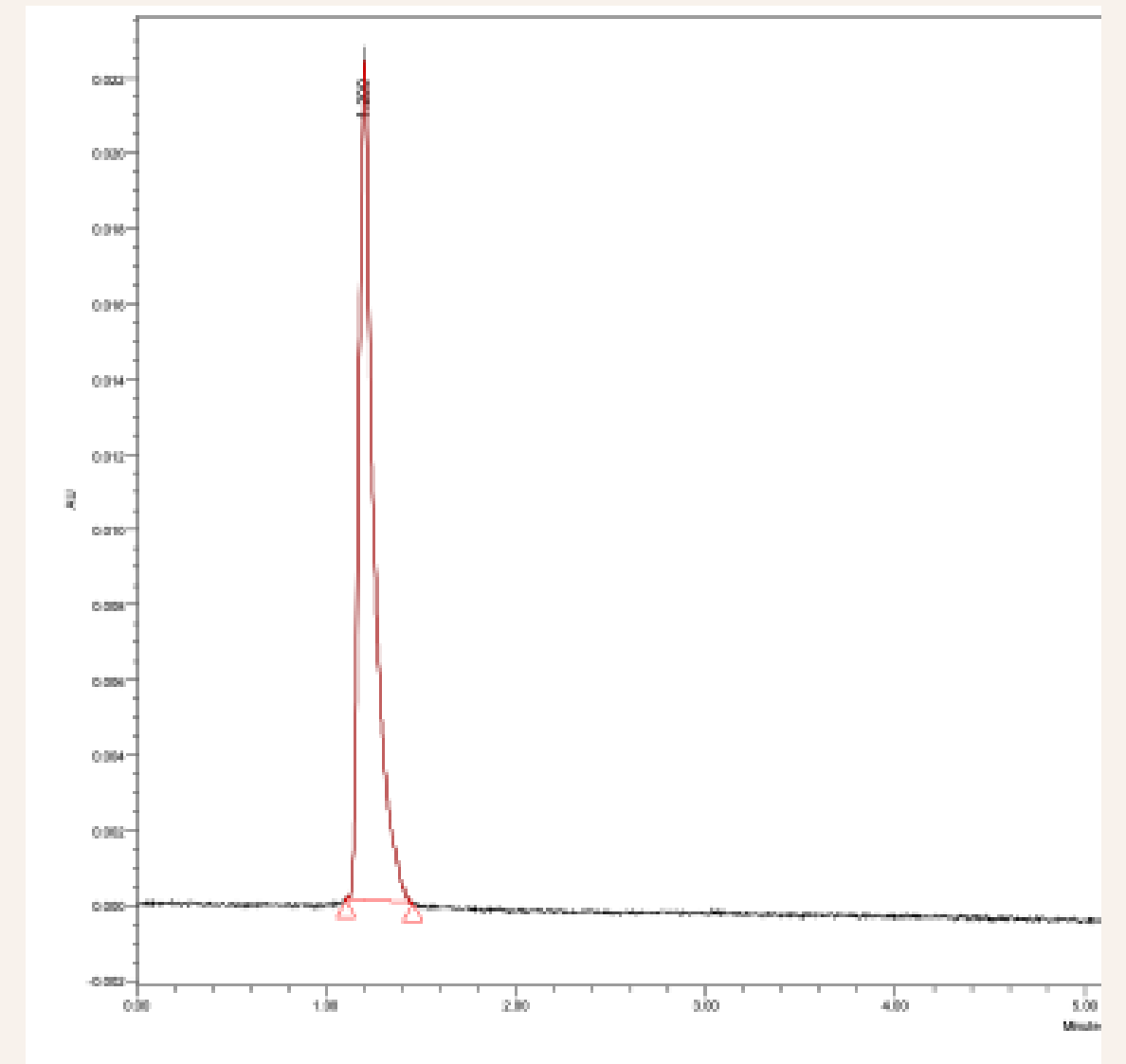
Sephadex G-50: Filters impurities out based on molecular size





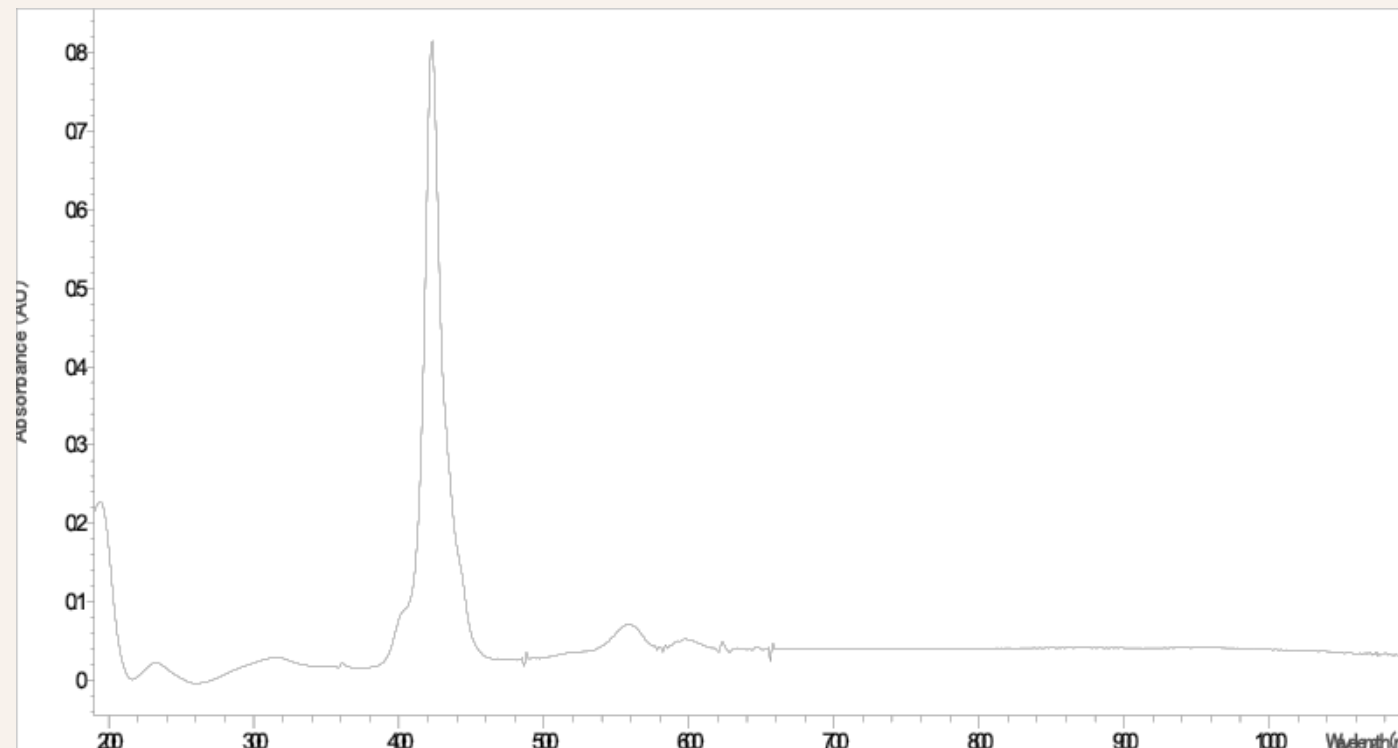
# High-Performance Liquid Chromatography (HPLC)

- Hamilton PRP -1 5 $\mu$ m column 4 x 150 mm
- Solvent: 100% acetonitrile
- Flow Rate: 1.00 mL per minute
- Sample dissolved in MQ H<sub>2</sub>O
- 99% Pure



# Ultraviolet-Visible Spectroscopy (UV-Vis)

- The peak at 423 nm confirms that the porphyrin was successfully metallated with Zn to synthesize ZnTPP -5AP.
- The two peaks in the fingerprint region, 557 and 597, are the attached substituents to the porphyrin.

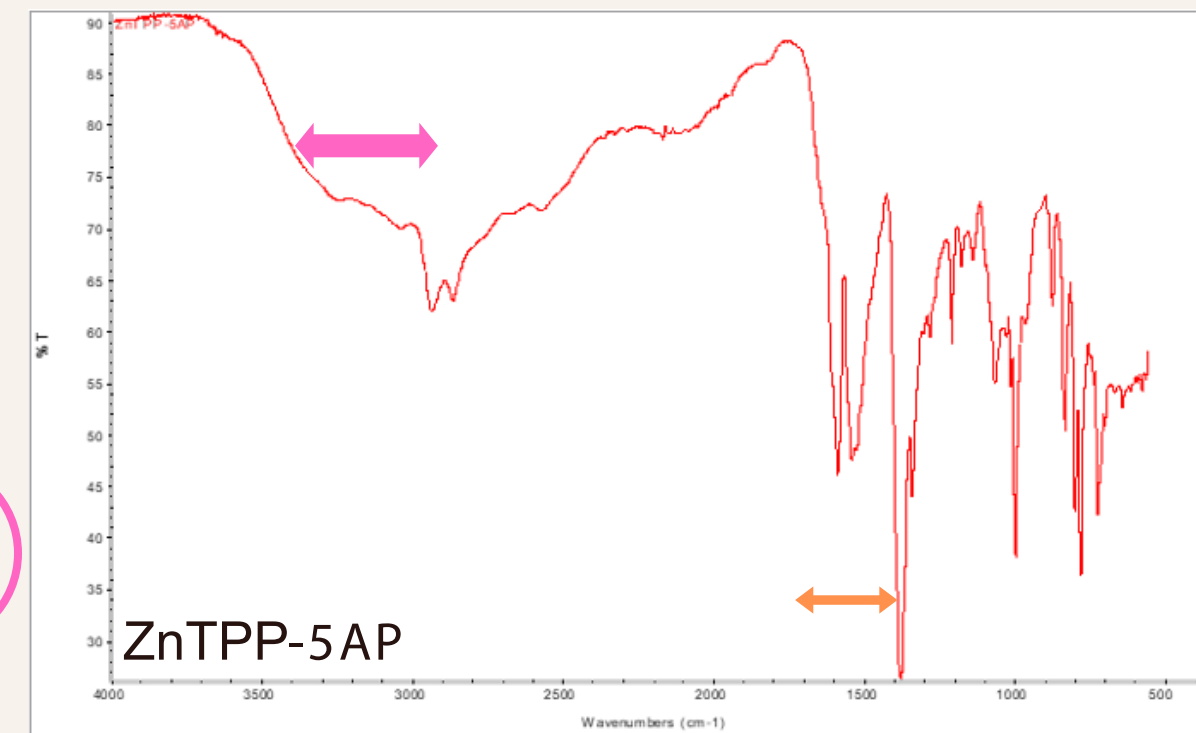
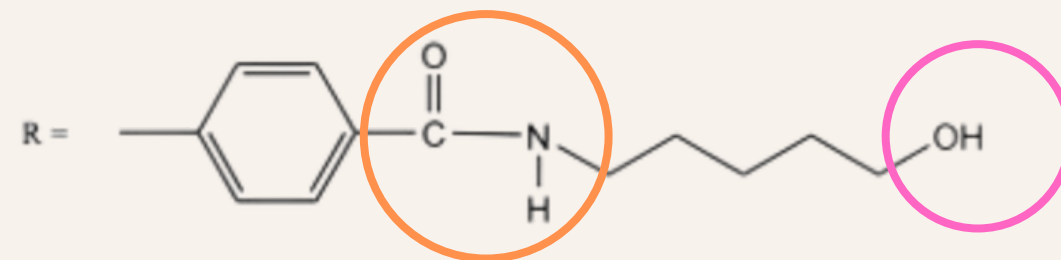
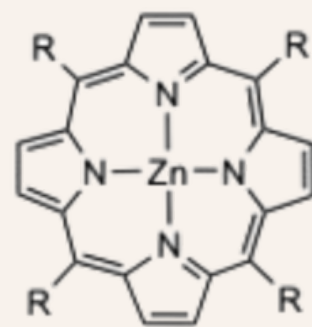
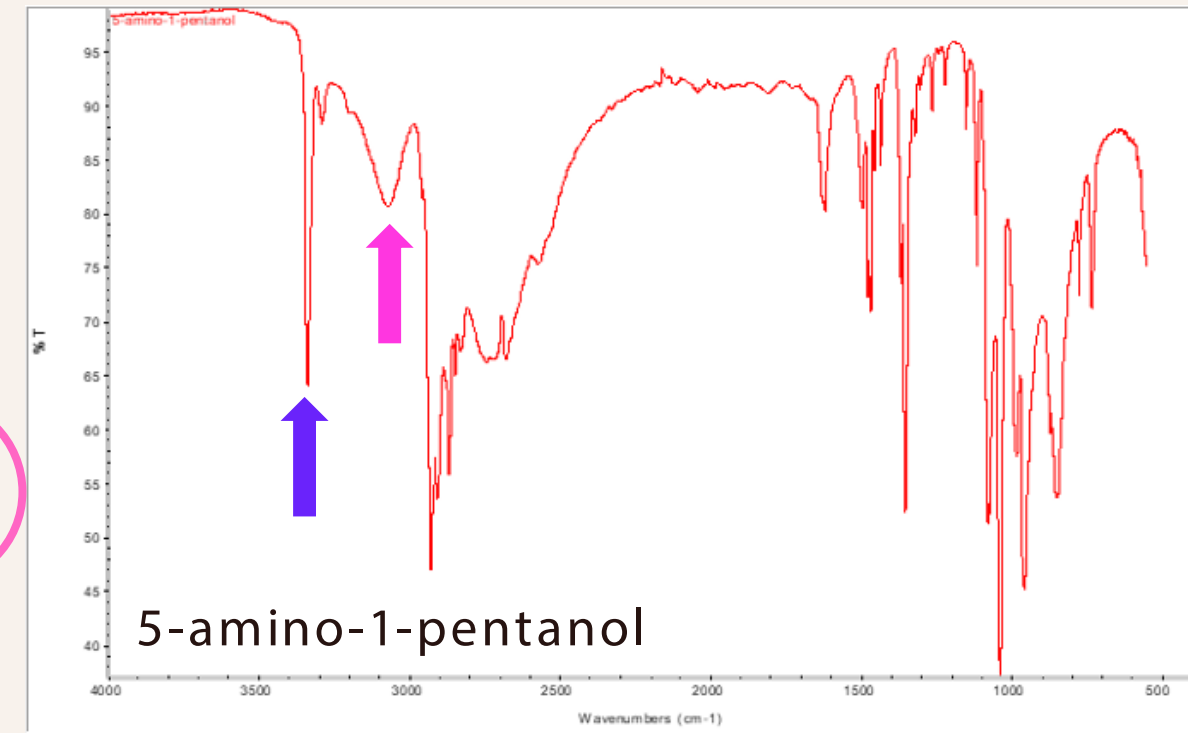
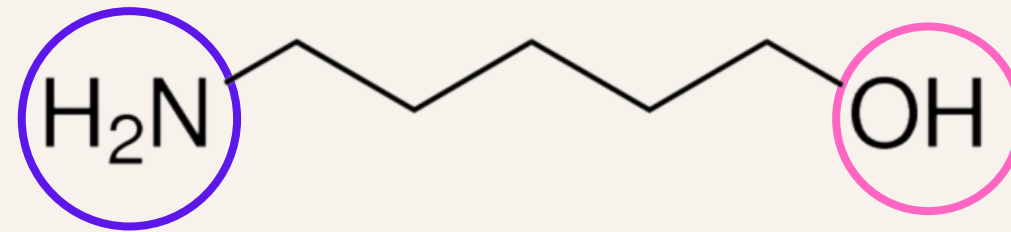


| Peaks (nm) | Molar Absorptivity Coefficient, $\epsilon$ (mM <sup>-1</sup> cm <sup>-1</sup> ) |
|------------|---|
| 423        | 497   |
| 557        | 15.5  |
| 597        | 8.67  |

Beer's law was used to calculate molar absorptivity

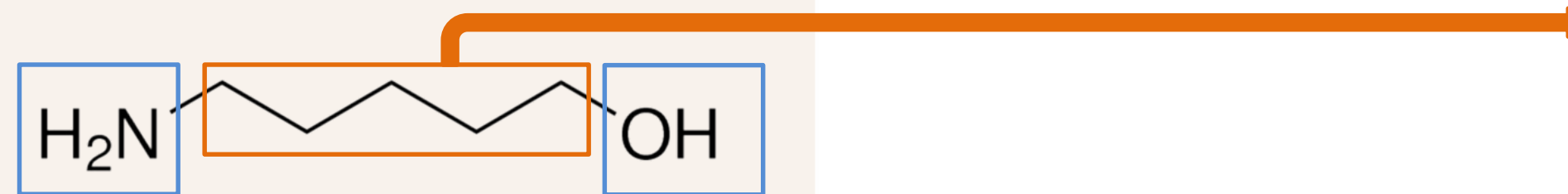
# Infrared Spectroscopy (IR)

IR uses infrared radiation to analyze molecules structures

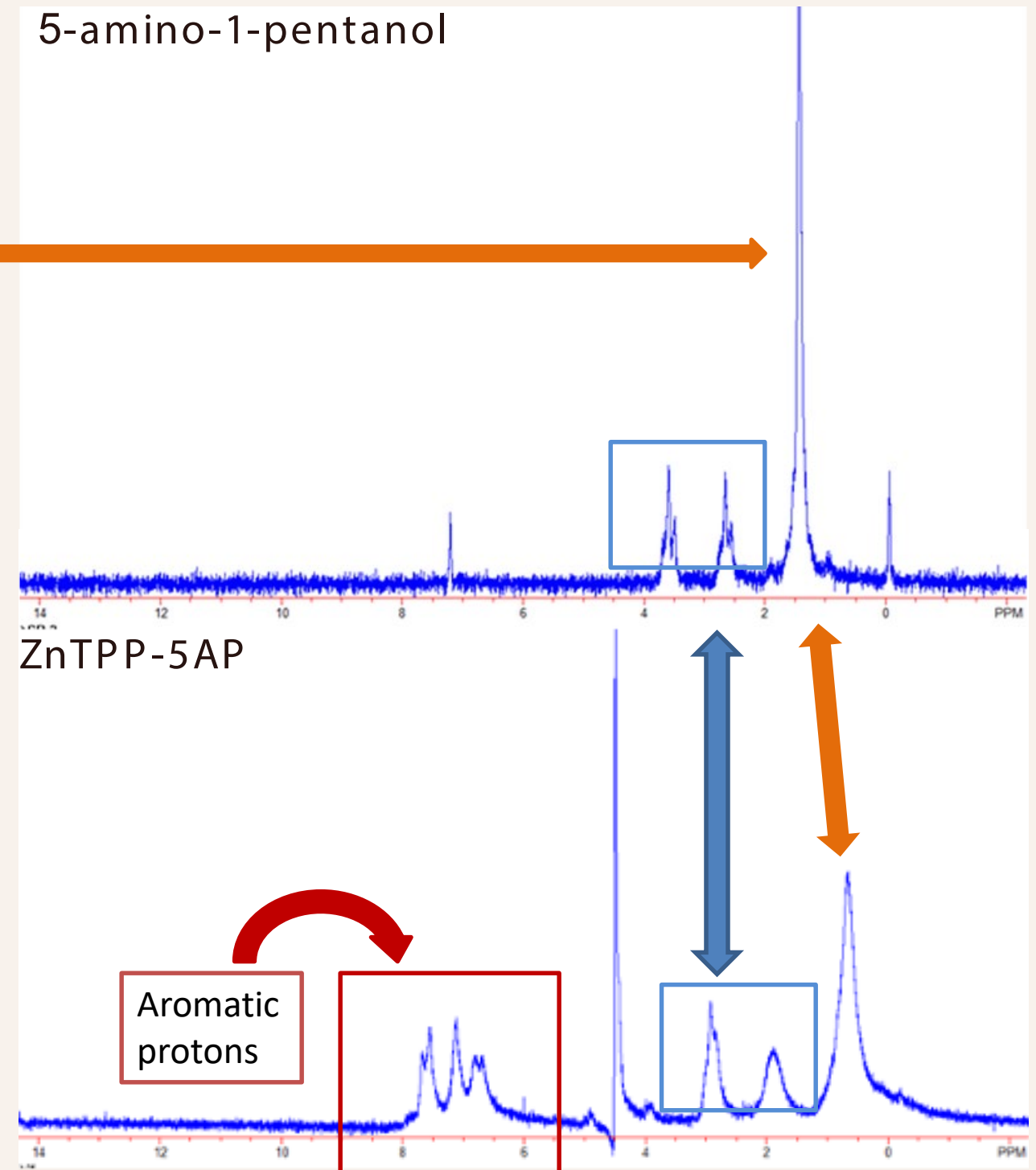
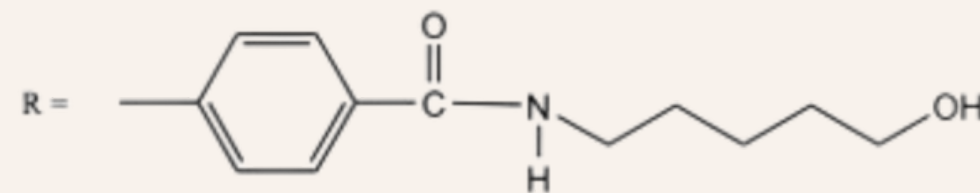
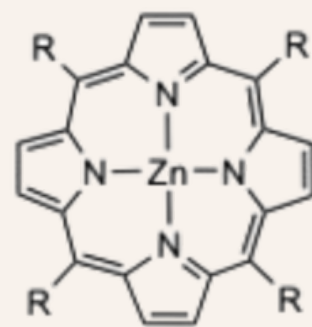


# Nuclear Magnetic Resonance (NMR) Spectroscopy

Peak at 0.0 ppm is TMS an internal reference  
Peak at 7.1 ppm is  $\text{CDCl}_3$  that was used as a solvent



The large peak at 4.2 ppm is  $\text{D}_2\text{O}$



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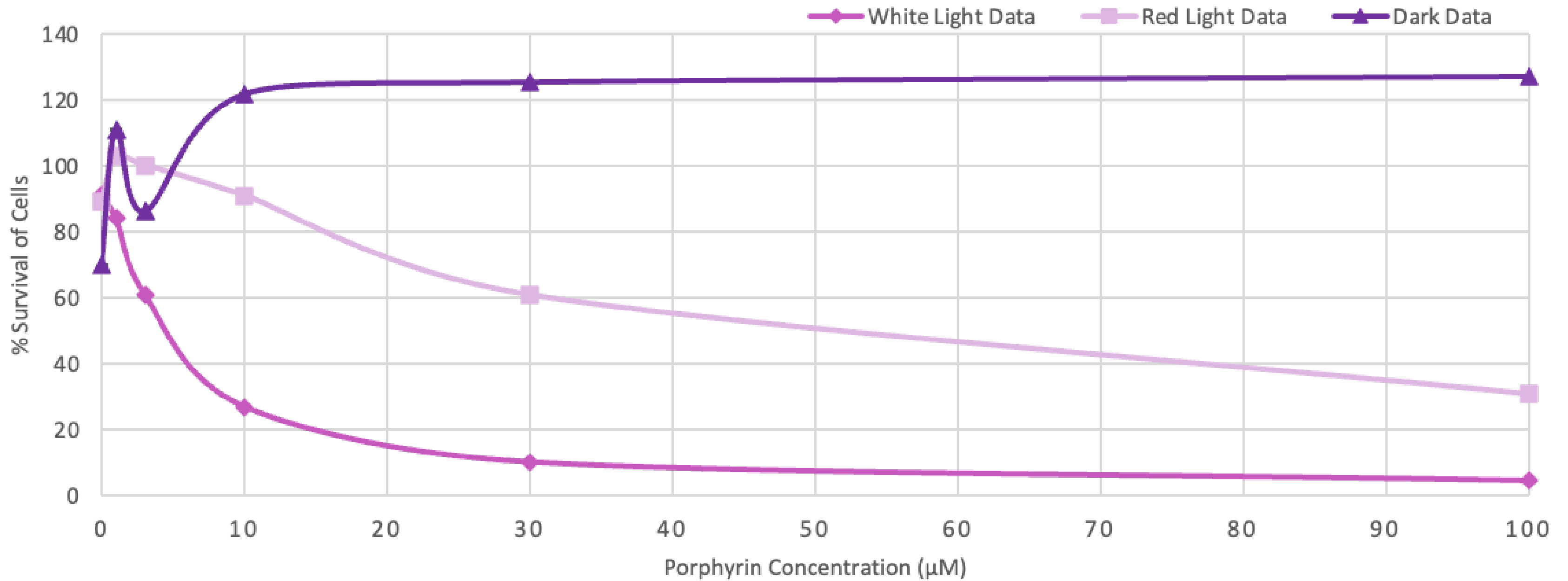
Conclusion and Future Work

# MTT Assay

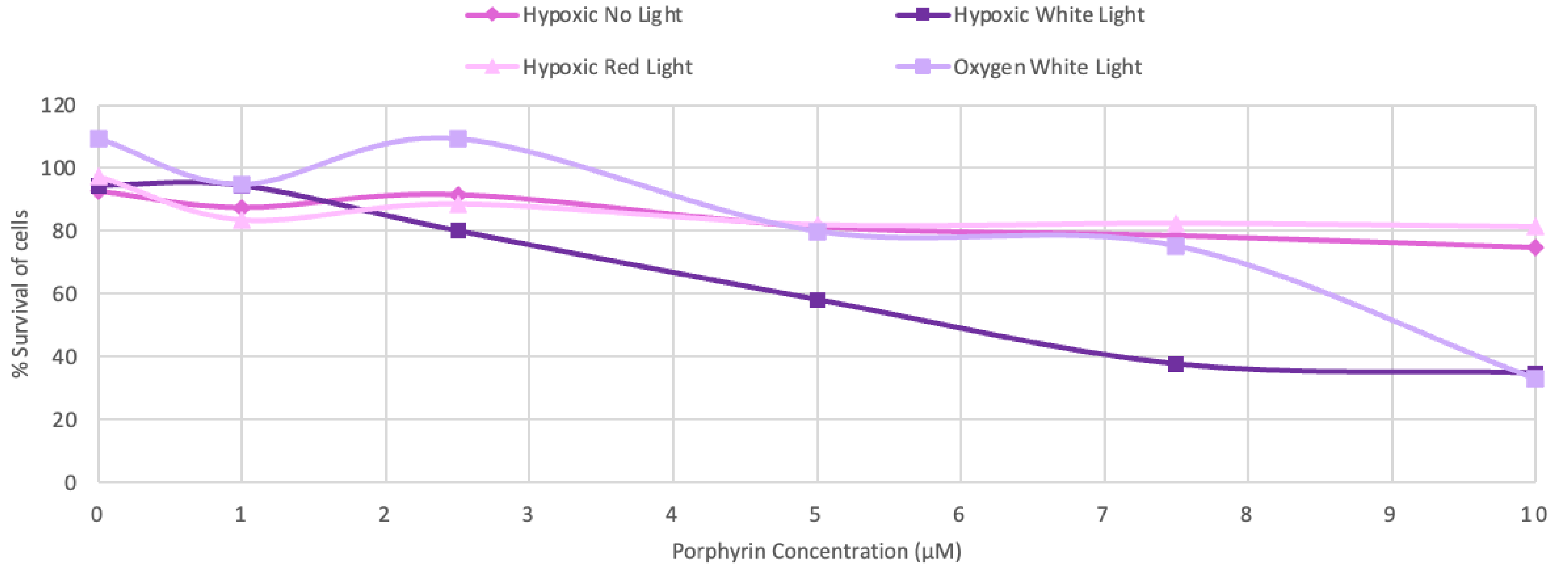
- The purple -colored wells indicates viable cells, while the clear wells indicates cell death
- The ZnTPP-5AP was added in increasing concentrations across the 96 -well plates
- The compounds efficacy was determined using LD<sub>50</sub>



# CYTOTOXICITY OF ZNTPP-5AP



## Cytotoxicity of ZnTPP-5AP under Hypoxia





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# Conclusion

- ZnTPP-5AP was able to be synthesized with a 99% purity and the structure was characterized using UV-Vis, IR, and NMR.
- Cells exposed to red light had an LD<sub>50</sub> of 50  $\mu$ M.
- Cells exposed to white light had an LD<sub>50</sub> of 5  $\mu$ M and hypoxic white light had an LD<sub>50</sub> of 6  $\mu$ M.
- There was no toxicity when the A549 cells were kept in the dark with the porphyrin.
- ZnTPP-5AP is an effective photosensitizer for A549 NSLC at low dosages of around 5  $\mu$ M with white light

# Future Work

- Test the cytotoxicity of ZnTPP-5AP with other cancer cell lines
- Perform in vivo testing using ZnTPP-5AP to see the effect on the tumor
- Synthesize an unmetalated porphyrin, H<sub>2</sub>TPP-5AP, to see if the unmetalated version is more cytotoxic.

# Acknowledgements

Dr. J. D. Patterson Summer Research Program

Dr. Joe Bradshaw

Dr. Timothy E. Hayes

Dr. Nathan Reyna

Kennedy Johnson

Keren Fernandez

Kaitlyn Montgomery

Ouachita Baptist University

# Questions?

