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Developing ZnTPP-4AB as a Potential Photodynamic Therapy Agent

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Developing ZnTPP-4AB as a Potential Photodynamic Therapy Agent

A Senior Thesis by Kennedy Johnson

Order of Presentation

01 Background

02 Methods

03 Conclusions

04 Future Directions

05 PDT in Dermatology

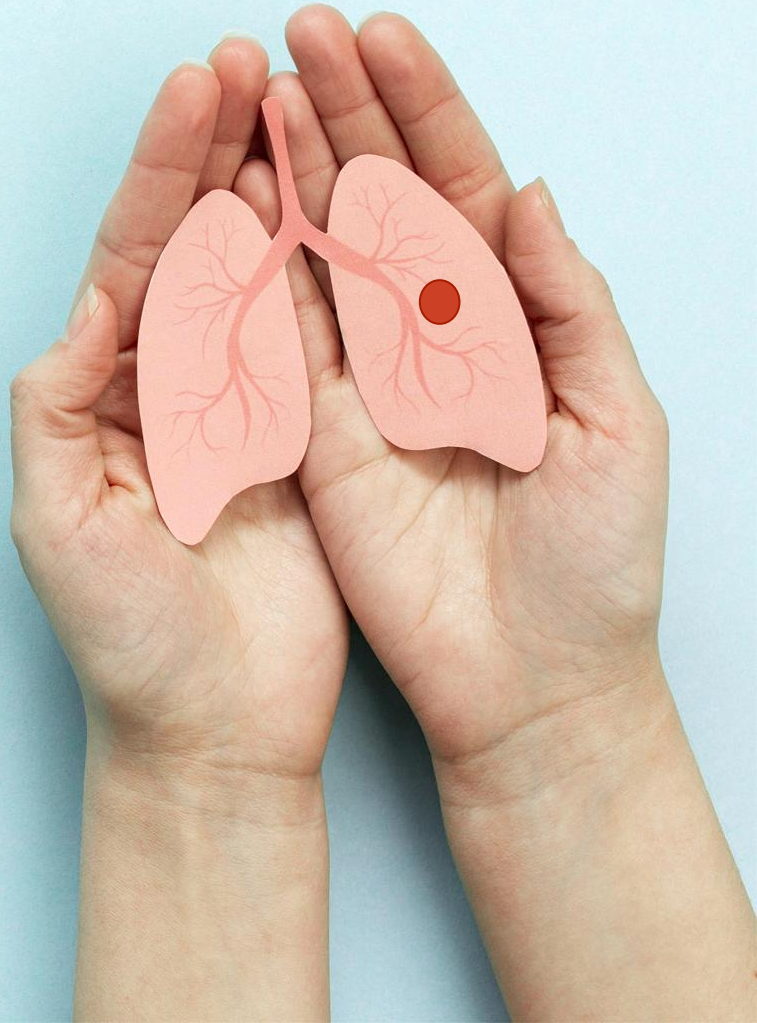
01

Background

Lung Cancer, Photodynamic Therapy, Porphyrins

Lung Cancer

- **#1 cause** of cancer-related deaths in the world
- **1 out of 17** people diagnosed
- Current methods...
- Healthy cells?

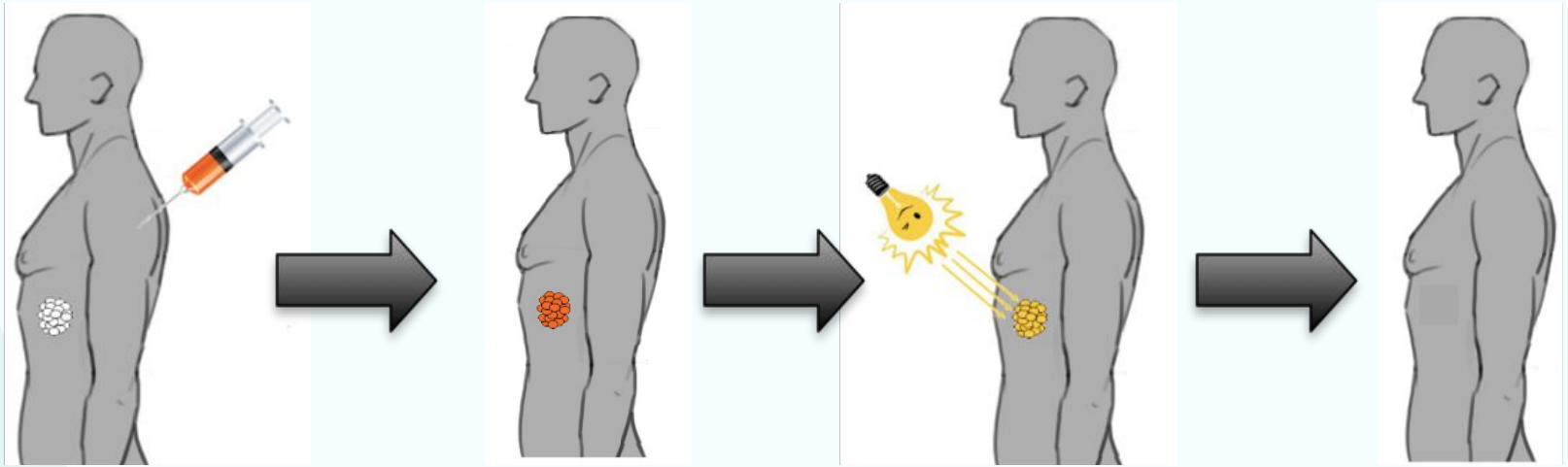


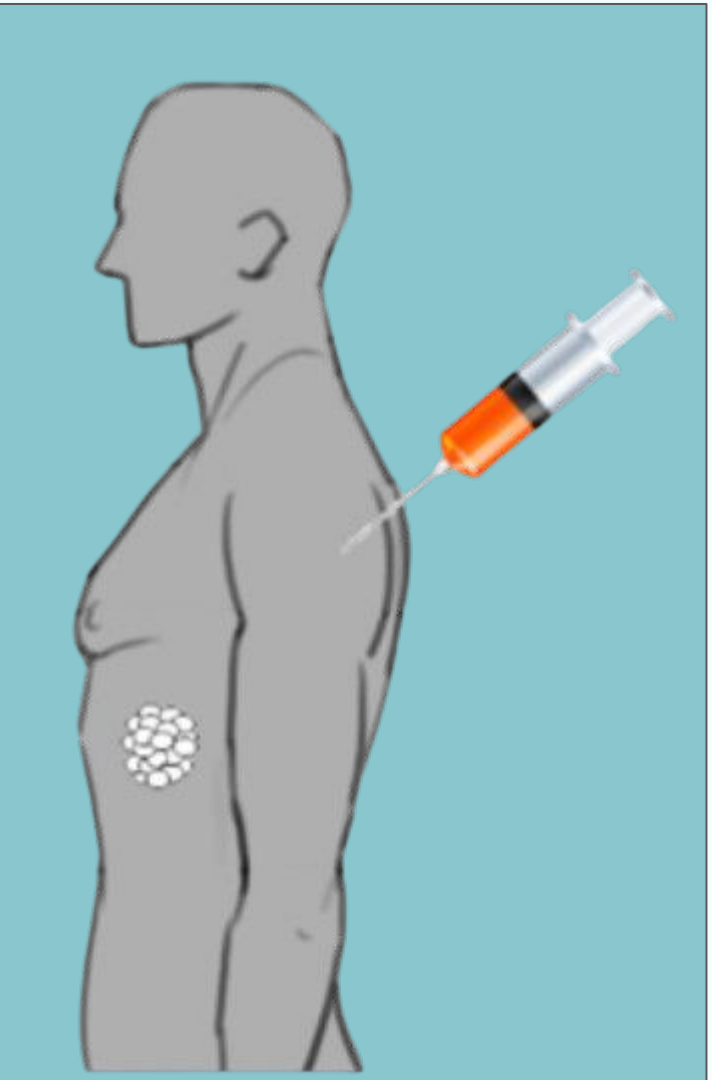
Photodynamic Therapy

- Selectively toxic
- Repeat if needed

- Accurate
- Cost Efficient

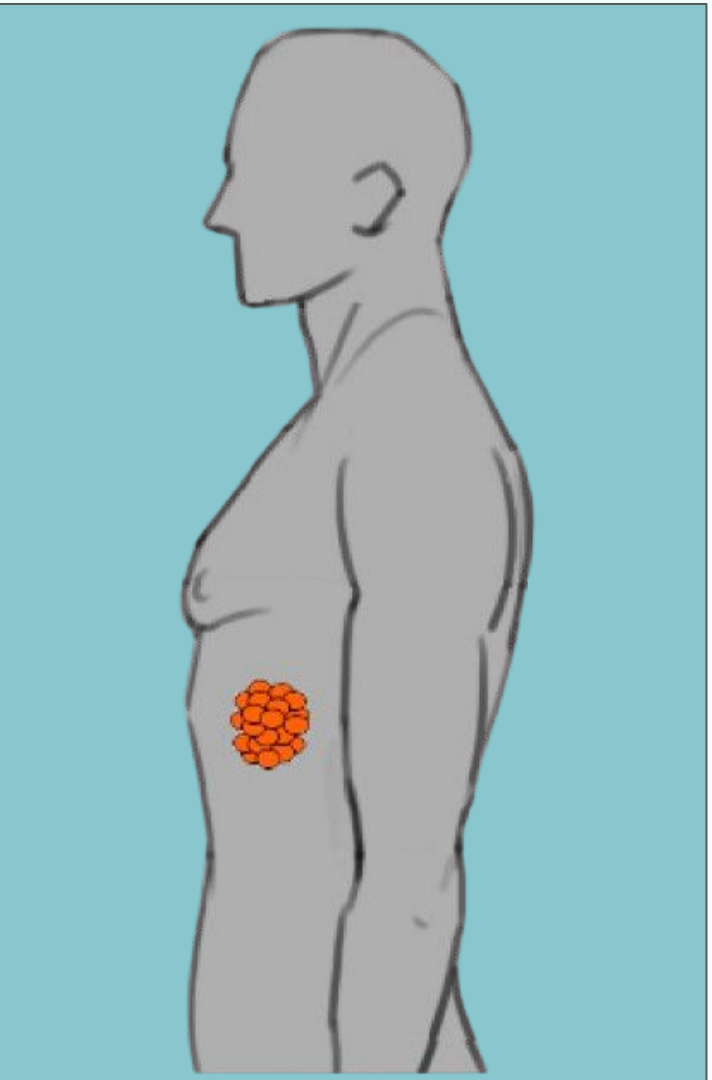
Photodynamic Therapy





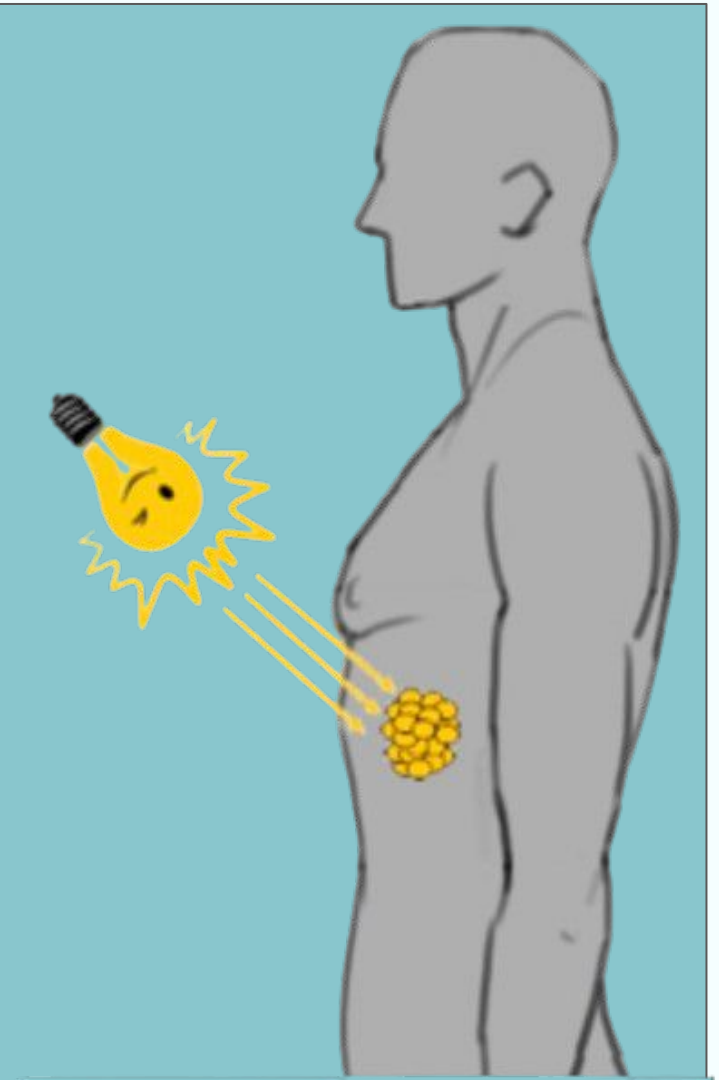
1. Injection

- At tumor site
- photosensitizer



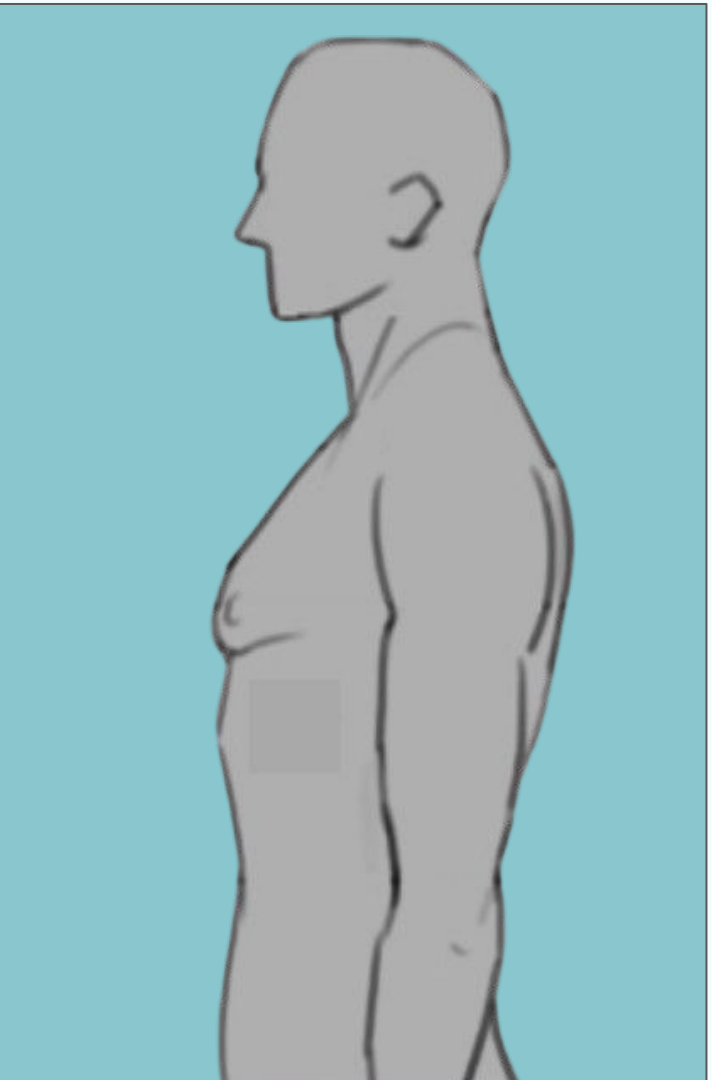
2. Incubation

- Preferential accumulation
- Cancerous cells only
- 30 mins to 4 hours



3. Light Exposure

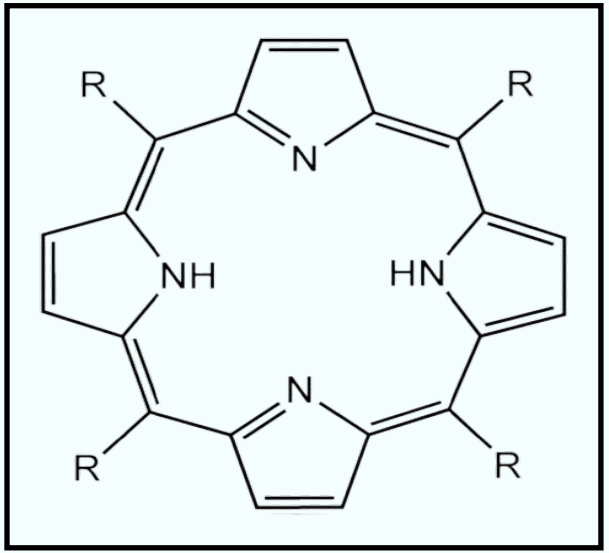
- 600 to 850 nm
- Customizable penetration depth
- Activate photosensitizer



4. Cell Death

- Excited electronic state
- Singlet oxygen= cell death
- Cancerous cells only

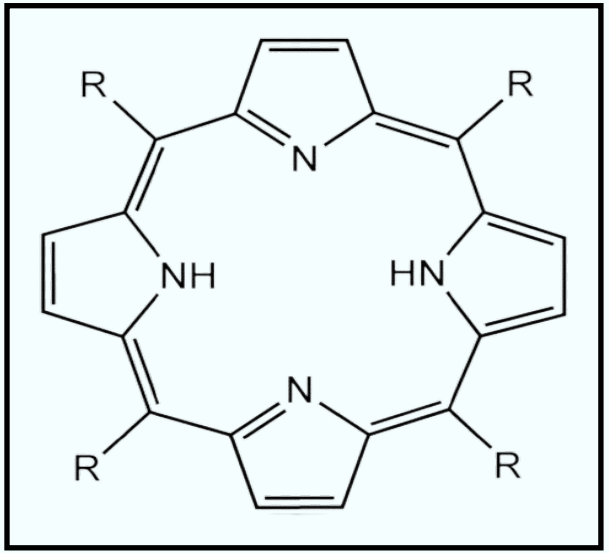
Porphyrins



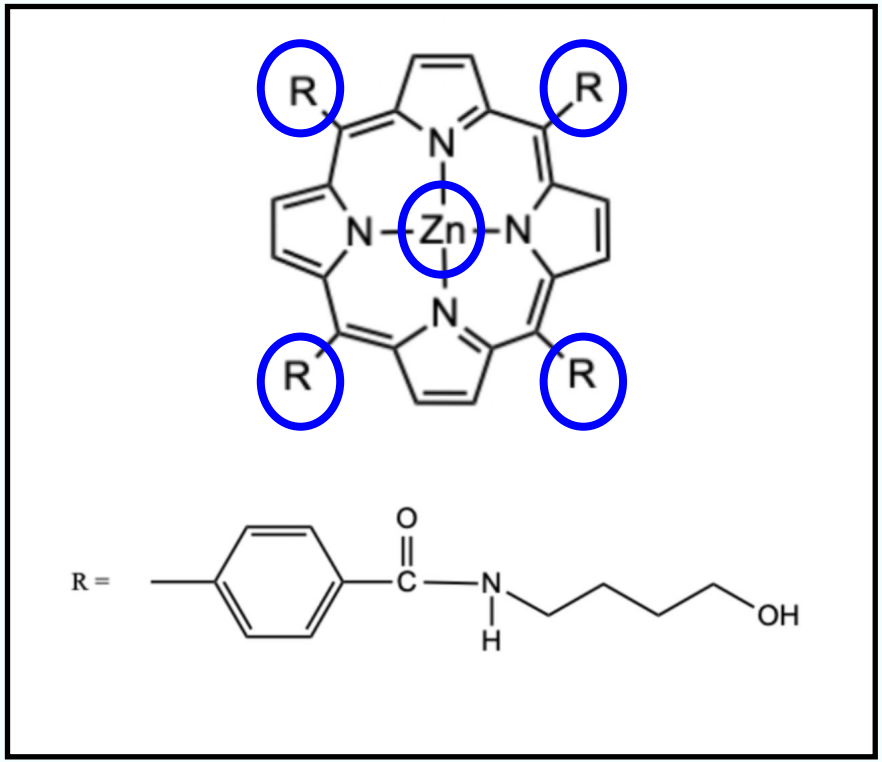
Generic Porphyrin

- Found in the human body
  - Electron transport
  - Gene regulation
  - Hormone synthesis
- Highly conjugated= good at absorbing light

Porphyrins



Generic Porphyrin



ZnTPP-4AB

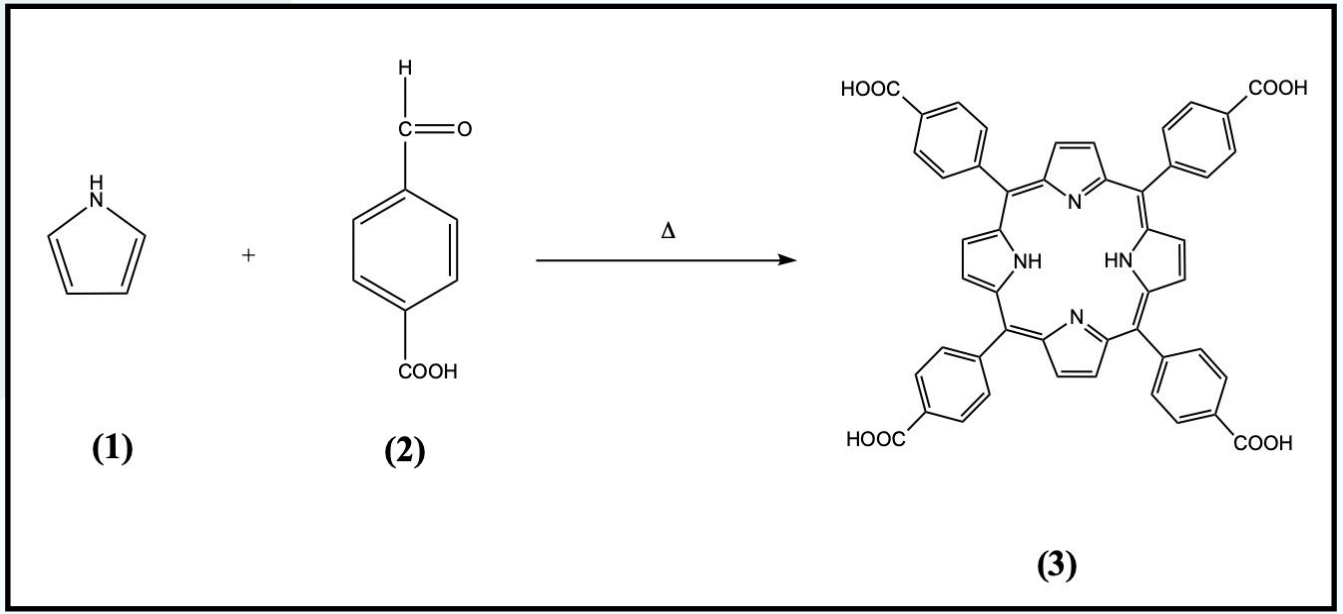
02

Methods

Synthesis, Purification, Characterization, Testing

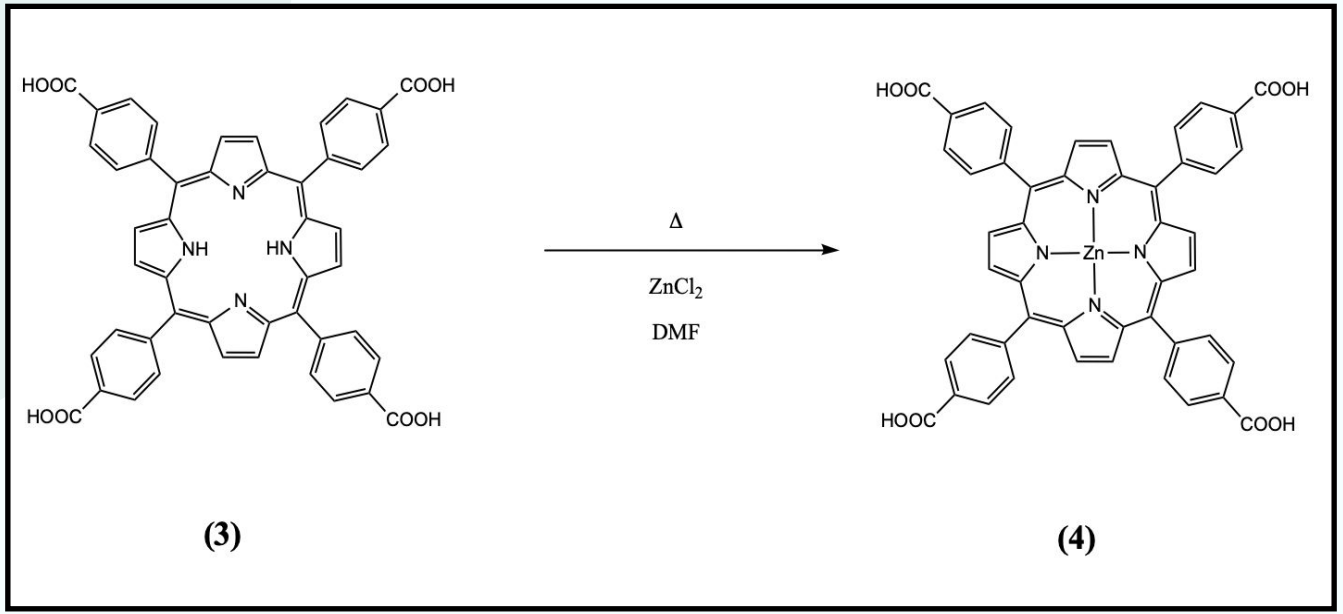
Step 1: Synthesizing ZnTPP-4AB

Reaction 1



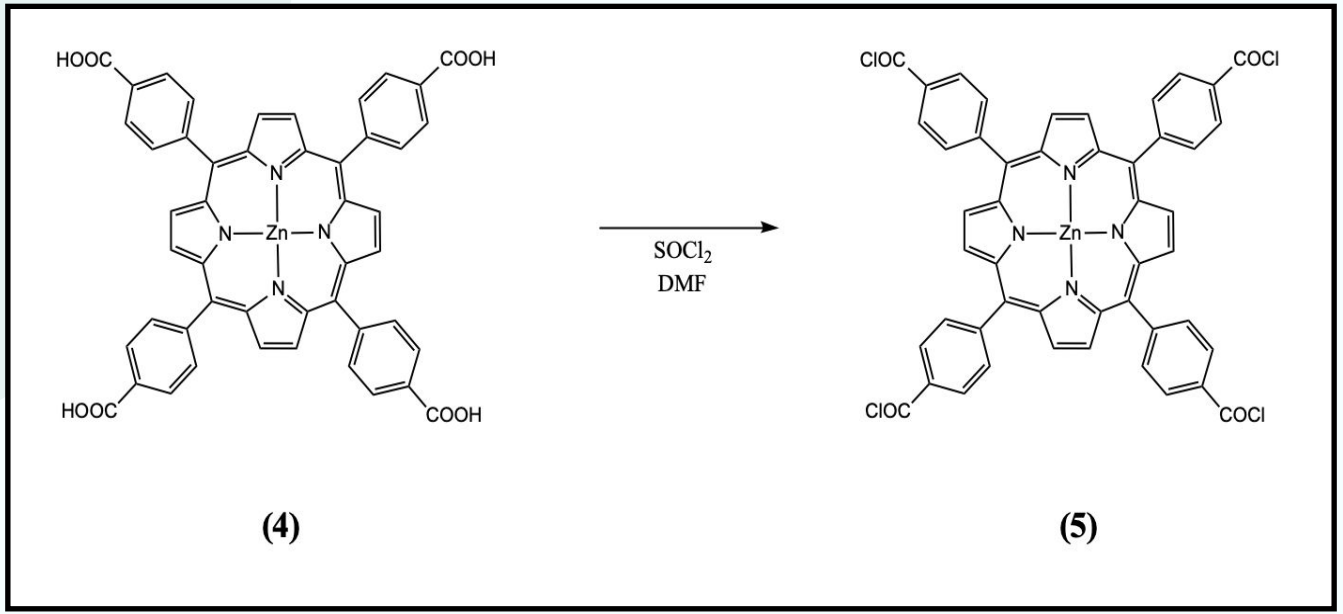
Pyrrole (1) reacted with 4-formal benzoic acid (2) in a propionic acid solution to form H_2TPPC (3).

Reaction 2



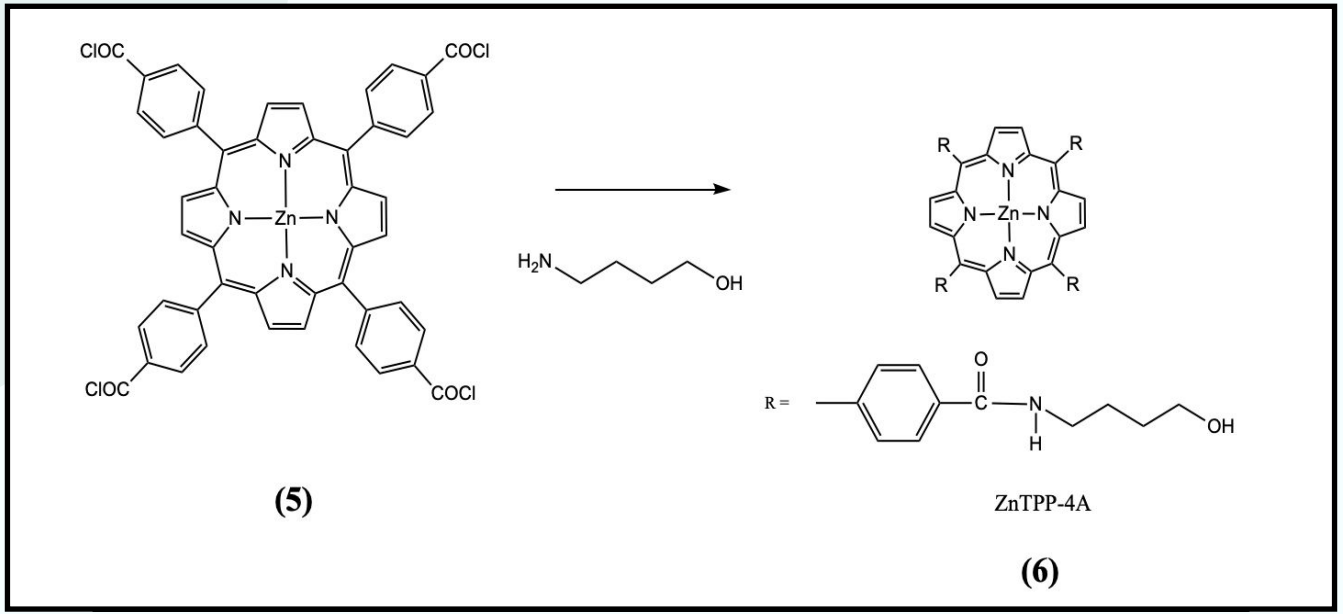
ZnCl2 reacted with H2TPPC (3) in DMF to metalate the porphyrin, creating ZnTPPC (4).

Reaction 3



The ZnTPPC (4) reacted with SOCl2 in DMF to create an acid chloride porphyrin intermediate (5).

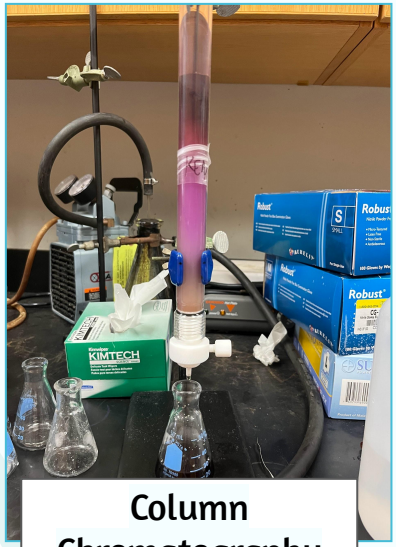
Reaction 4



The porphyrin intermediate (5) reacted with 4-amino-1-butanol in MeOH to form ZnTPP-4AB (6).

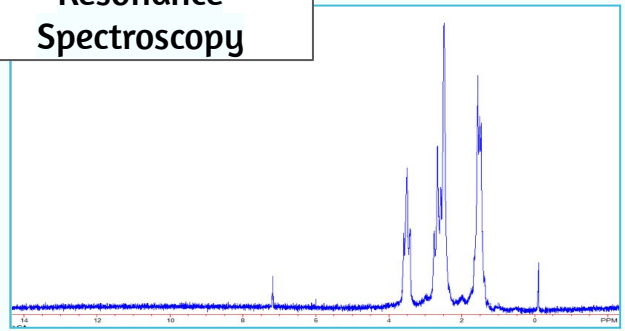
Step 2: Characterizing ZnTPP-4AB

Was pure ZnTPP-4AB synthesized?

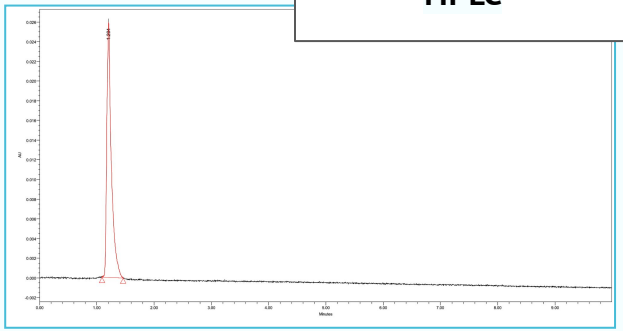


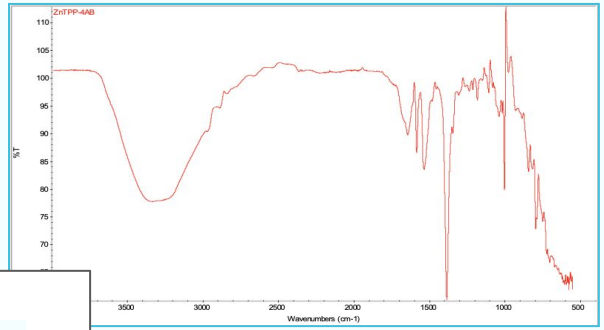
Column Chromatography

Nuclear Magnetic Resonance Spectroscopy



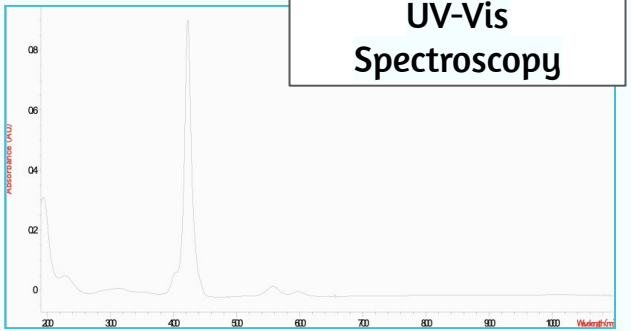
HPLC





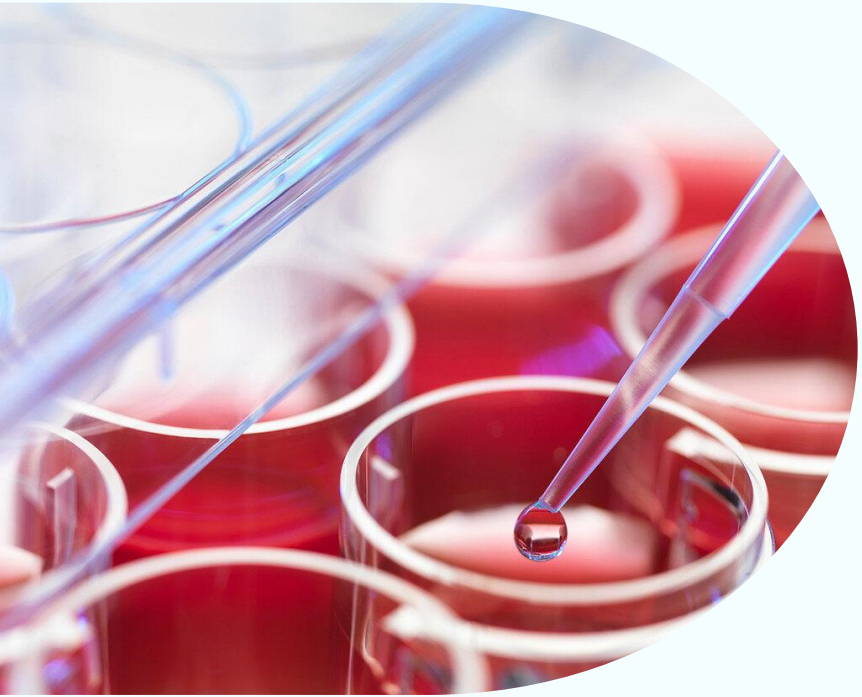
Infrared Spectroscopy

UV-Vis Spectroscopy



Step 3: Testing ZnTPP-4AB

Adding ZnTPP-4AB to Lung Cancer Cells



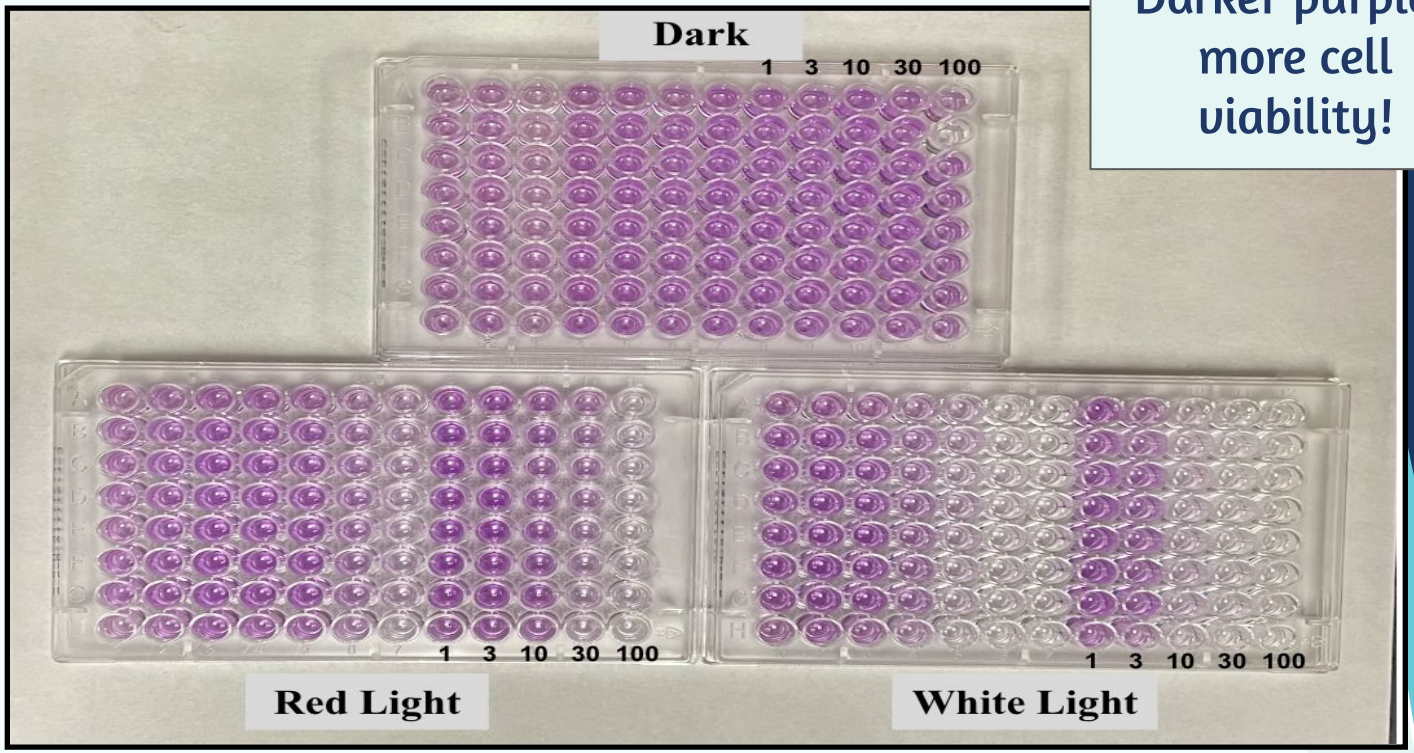
01 Add ZnTPP-4AB to cells

02 Expose cells to light

03 Determine cell death using MTT Assay

Cell Viability after MTT

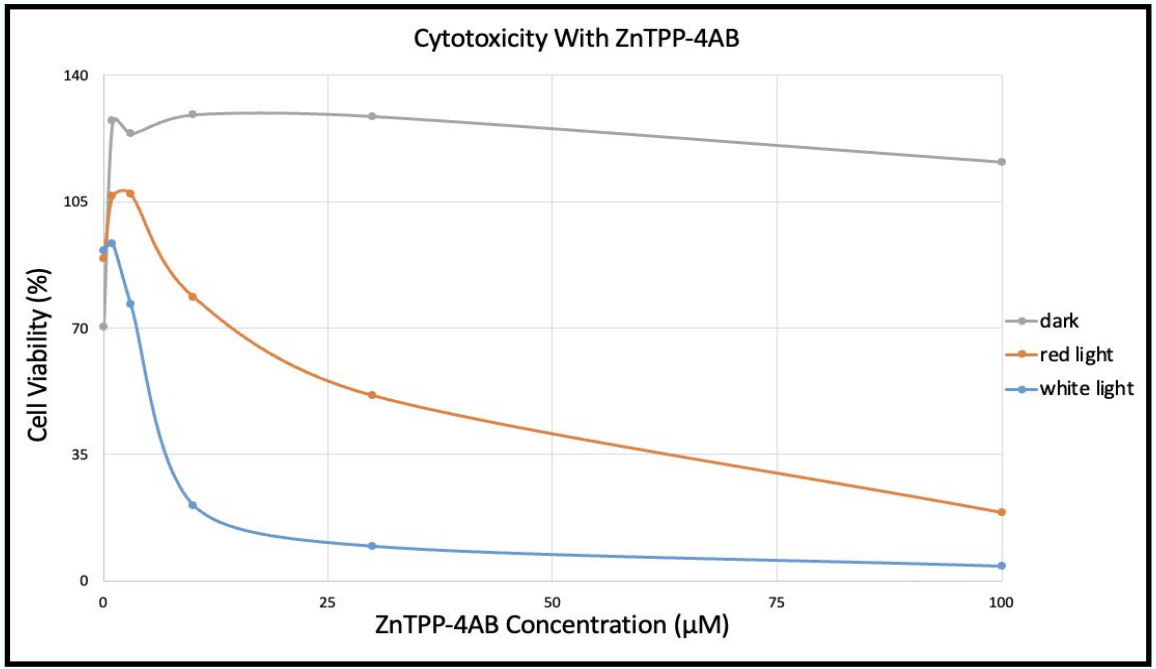
- No Light
  - 1 µM
  - 3 µM
  - 10 µM
  - 30 µM
  - 100 µM
- Red Light
  - 1 µM
  - 3 µM
  - 10 µM
  - 30 µM
  - 100 µM
- White Light
  - 1 µM
  - 3 µM
  - 10 µM
  - 30 µM
  - 100 µM



Darker purple more cell viability!

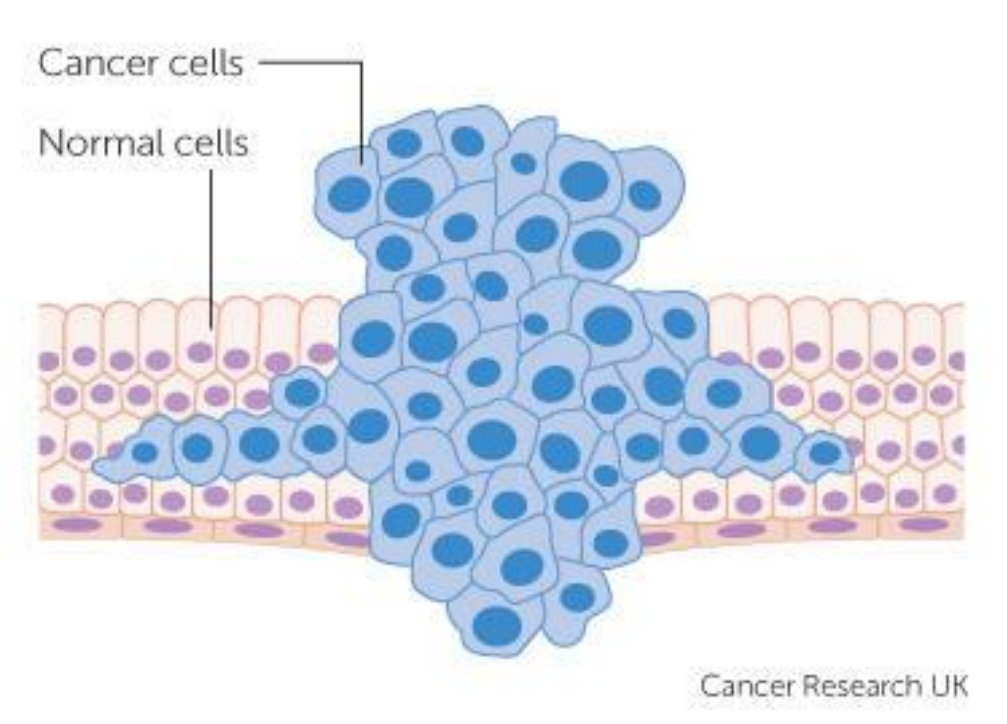
Results

- Higher concentration= less viability (more cell death)
- White light killed more cells at lower concentrations than red light
  - LD_{50} = 6 µM white light
  - LD_{50}=30 µM red light



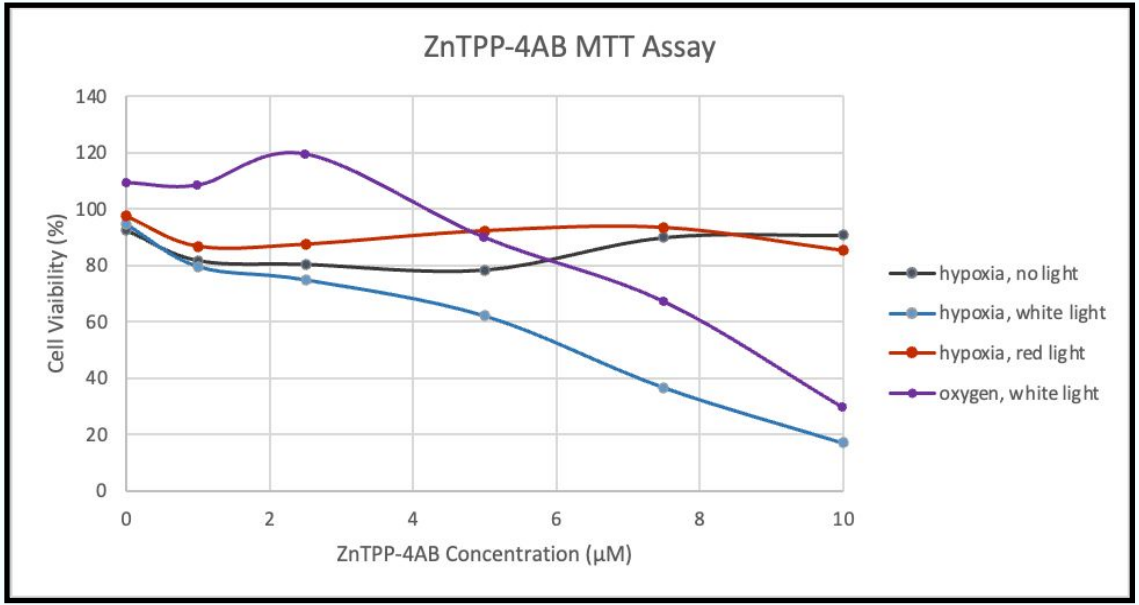
Hypoxic Environment of Tumors

- Hypoxia= no oxygen
- Hypoxic chamber to imitate this environment



Hypoxia Results

- little to no difference in cytotoxicity between no light and red light under hypoxia
- Both hypoxic and oxygen treated plates under white light had low cell viability.
- hypoxia + white light = highest cytotoxicity
    - LD_{50} : 6 µM



03

Conclusions

Did it work? What next?

Conclusions

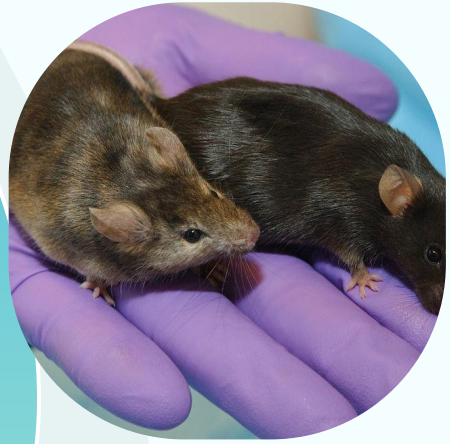
- A novel water-soluble porphyrin was successfully **synthesized**
- Characterization through UV-vis, IR, and NMR spectroscopies **validated** the porphyrin's structure.
- MTT assay **confirmed** that light exposure paired with increasing ZnTPP-4AB concentrations caused **cell viability to decrease.**

Conclusions

- **Lower** concentration of porphyrin were necessary for LD_{50} under **white light** compared to **red light.**
- **White light** in combination with hypoxia caused **more** cell death than when under normal oxygen conditions.
- ZnTPP-4AB is an **effective** photosensitizer for PDT for A549 non-small cell lung cancer cells with an LD_{50} of 6 μM.

What Next?

- Larger Range of concentrations under Hypoxia (0-100μM)
- Compare to H_2TPP-4AB (unmetallated)



- ZnTPP-4AB cytotoxicity on other cancer cell lines
- *In vivo* testing

04

PDT in dermatology

Background

- skin cancer gets diagnosed more each year than all other cancers combined
- scalp and face
- precancerous actinic keratoses (AK's)
- squamous cell carcinomas
- PDT = effective way to treat skin issues **before** they become a problem, and **before** they even appear

How it works

1. Application

Topical cream containing photosensitizer

2. Incubation

90 to 540 minutes

How it works

3. Light Exposure

16 minutes of blue light

4. Recovery

No direct sunlight or intense indoor lighting for 48 hrs

Benefits of PDT in Dermatology

Practical

Less painful than alternatives

Less invasive

Convenient

Thank you!

- Dr. Bradshaw
- J. D. Patterson Summer Research Program
- Dr. Timothy E. Hayes
- Dr. Nathan Reyna

Questions?