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Defying the Darkness: Countering Cancer with Porphyrins and Lights

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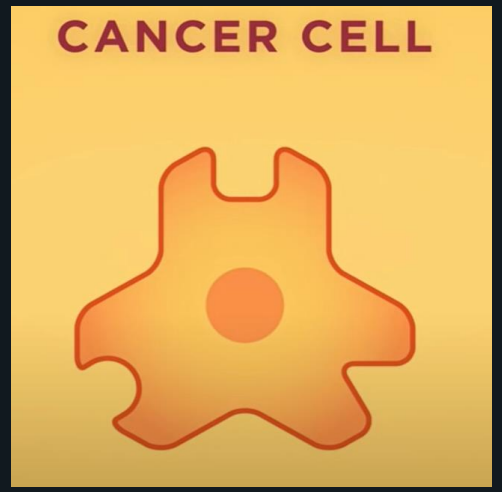
Defying the Darkness: Countering Cancer with Light

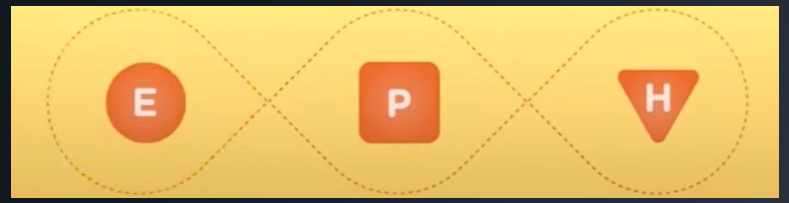
A Senior Thesis by Travis Hankins

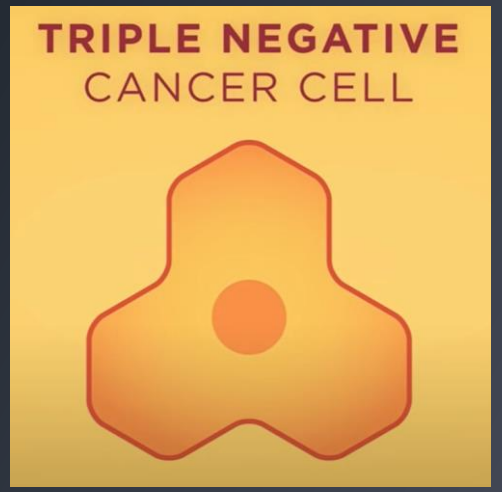
1. **Background**
2. Synthesis
3. Purification and Characterization
4. Metabolic Analysis
5. Conclusions and Future Work

What's the Focus?

- This research focused on treating Triple-Negative Breast Cancer (TNBC)
  - TNBC tests negative for normal receptors in breast cancer
  - Many current treatments rely on these receptors
- Design compound that can work without cell receptors



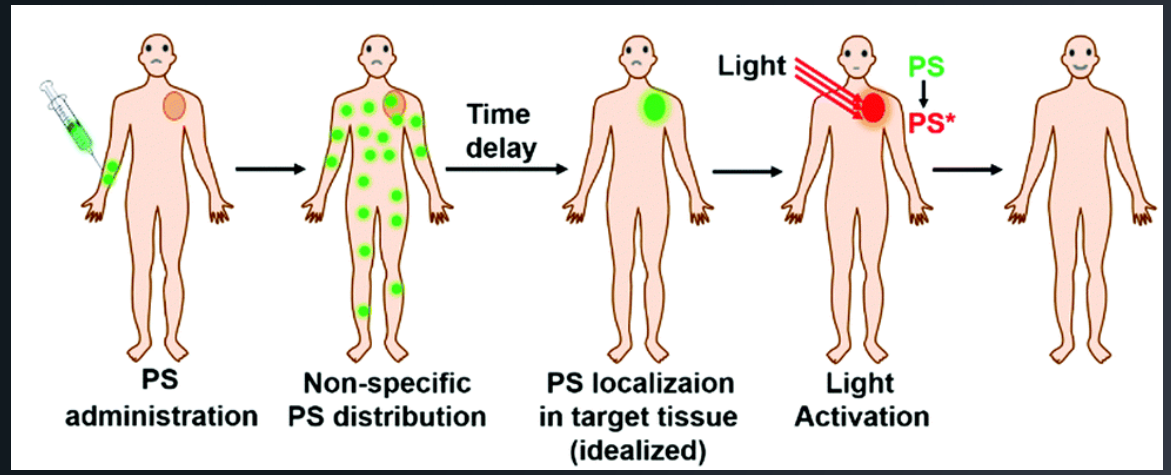




- "Triple-Negative Breast Cancer." *Centers for Disease Control and Prevention*, U.S. Department of Health and Human Services, 14 Sept. 2020, www.cdc.gov/cancer/breast/triple-negative.htm.
- "Triple-Negative Breast Cancer." *YouTube*, National Breast Cancer Foundation, Inc., 4 Sept. 2012, youtu.be/oAp4ZKbZU38.

What is Photodynamic Therapy?

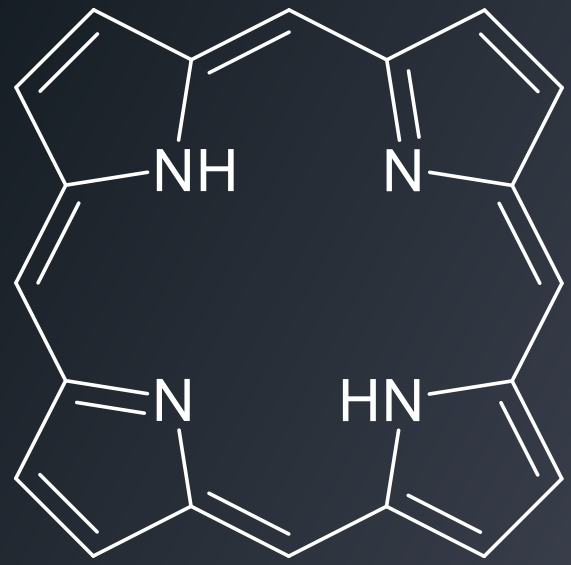
- Photodynamic Therapy (PDT): treatment of cancer using light and a photosensitive compound
  - Advantages – less invasive, fewer side effects
  - Disadvantages – relies on access to light, light sensitivity



- "Photodynamic Therapy for Cancer." *National Cancer Institute*, National Institutes of Health, 6 Sept. 2011, www.cancer.gov/about-cancer/treatment/types/surgery/photodynamic-fact-sheet.
- "Getting Photodynamic Therapy." *Radiation Therapy*, American Cancer Society, 2 Mar. 2020, www.cancer.org/treatment/treatments-and-side-effects/treatment-types/radiation/photodynamic-therapy.html.
- Reynolds, Tom. "Photodynamic Therapy Expands Its Horizons." *JNCI: Journal of the National Cancer Institute*, vol. 89, no. 2, 1997, pp. 112–114., doi:10.1093/jnci/89.2.112.

What are Porphyrins?

- Porphyrins: large, cyclic molecules
  - Most widely known is heme, a component in hemoglobin
  - Chlorophyll – Mg-containing porphyrin
- Highly versatile and stable due to bond conjugation
- Research aimed to produce a novel porphyrin that is a PDT agent



Structure of Porphin, one of the simplest porphyrins

- "Ch. 18: Porphyrins." *Journal of Chromatography Library*, by Erich Heftmann, 5th ed., Elsevier, 1992, p. 337.

Project Goals

- Synthesize, purify, characterize, and test a novel porphyrin derivative
- To be a viable photodynamic agent:
  - The porphyrin should be water soluble
  - The porphyrin should be able to kill cells in low concentrations when exposed to light
  - The porphyrin should have a minimal effect on cells when not exposed to light

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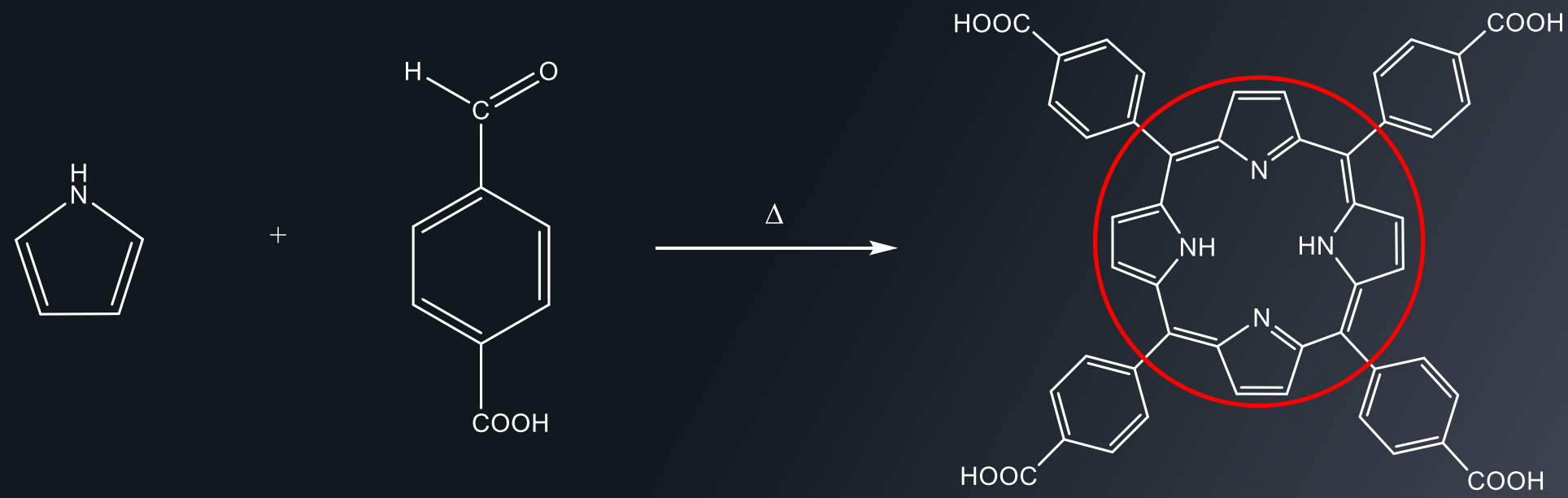
Synthesis of H_2TPPC

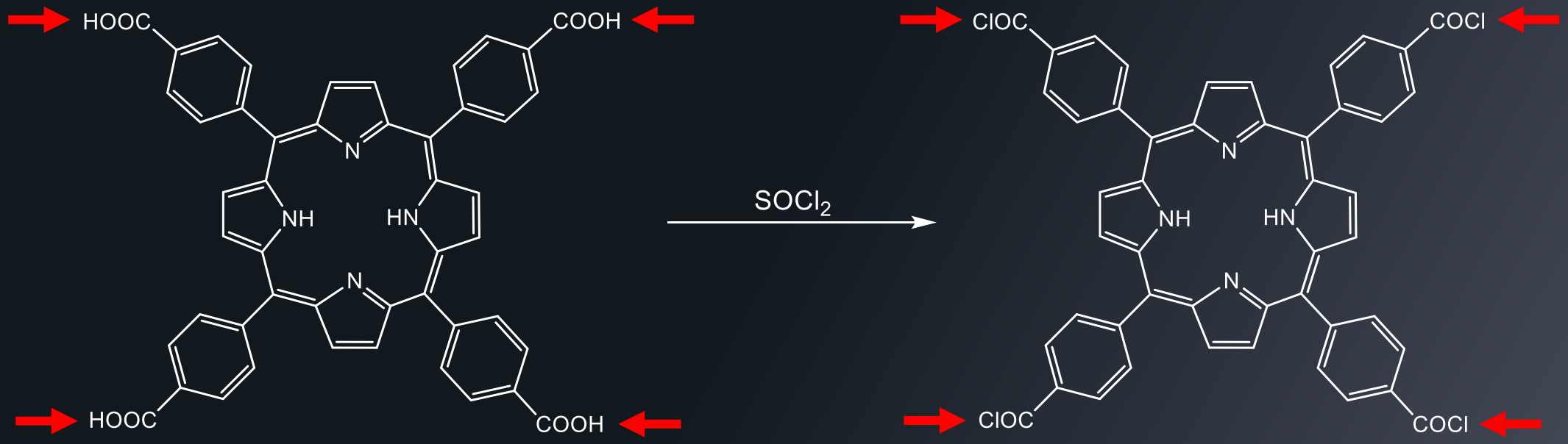


pyrrole

4-formylbenzoic acid

H_2TPPC

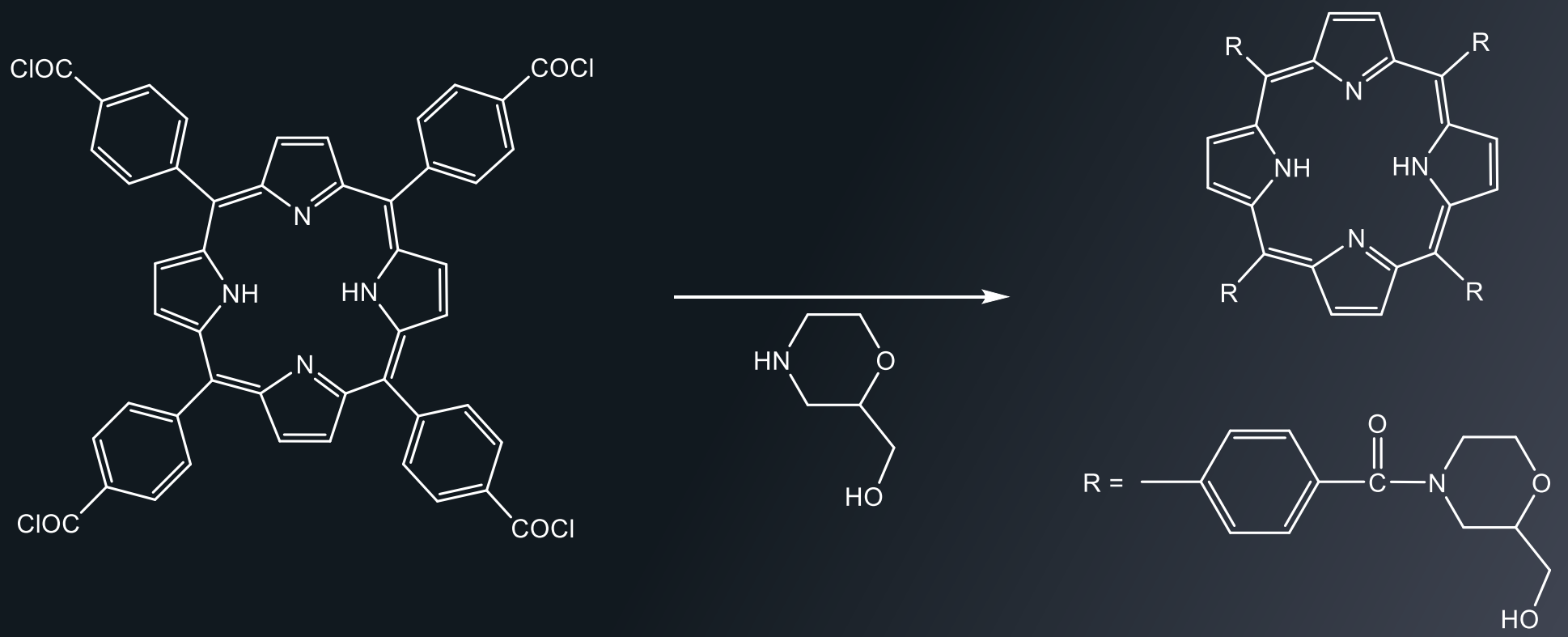
Synthesis of the Acid Chloride Porphyrin



H_2TPPC

Acid Chloride Porphyrin

Synthesis of H_2TPP-MorphMeOH



Acid Chloride Porphyrin

morpholin-2-yl methanol

H_2TPP-MorphMeOH

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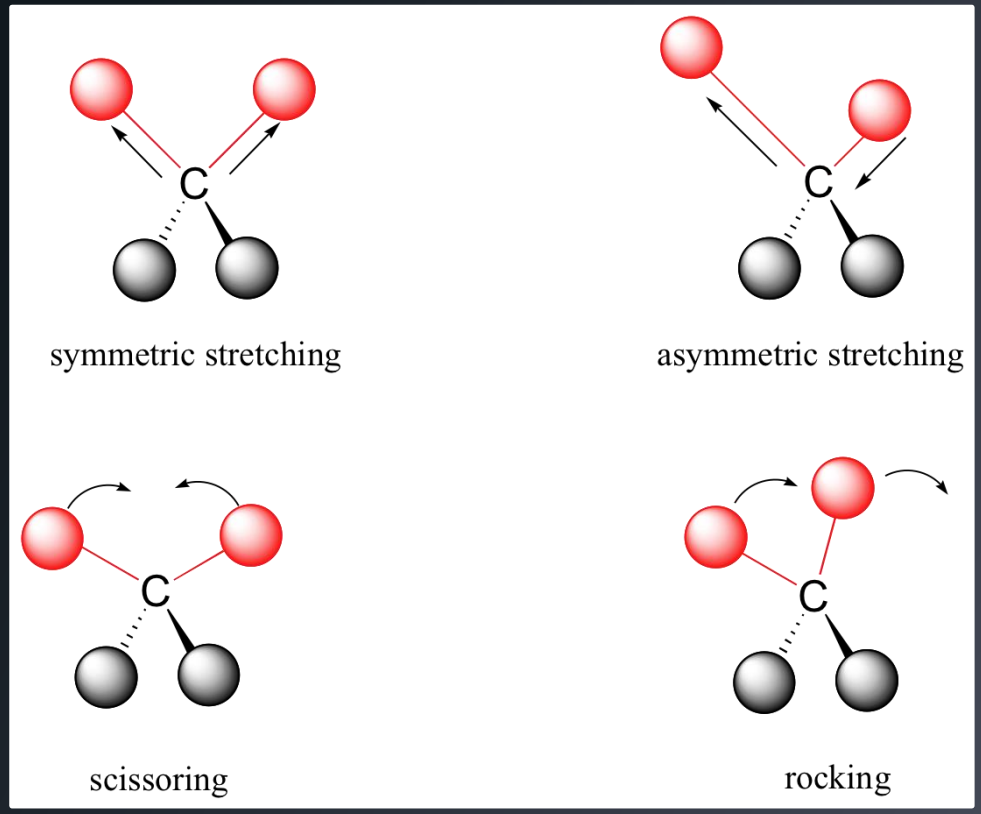
Product Purification

- The final product was purified three ways: syringe filtration, Sephadex LH-20, and Sephadex G-50
  - Syringe – eliminate coarse particulate
  - LH-20 – lipophilicity
  - G-50 – molecular size

- "Millex Syringe Filter, Nylon, Non-Sterile SLGN033." *Millipore Sigma*, Sigma Aldrich, www.sigmaaldrich.com/catalog/product/mm/slgn033?lang=en&region=US

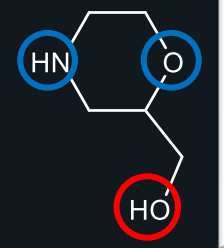
IR Spectroscopy

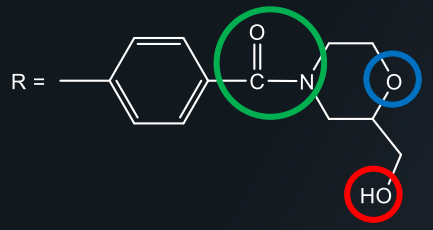
- Sample exposed to range of IR light, monitor how much of each wavelength is transmitted
- Certain bonds absorb certain wavelengths and begin vibrating in response → transmittance decreases
- Spectrum is used to identify functionalities

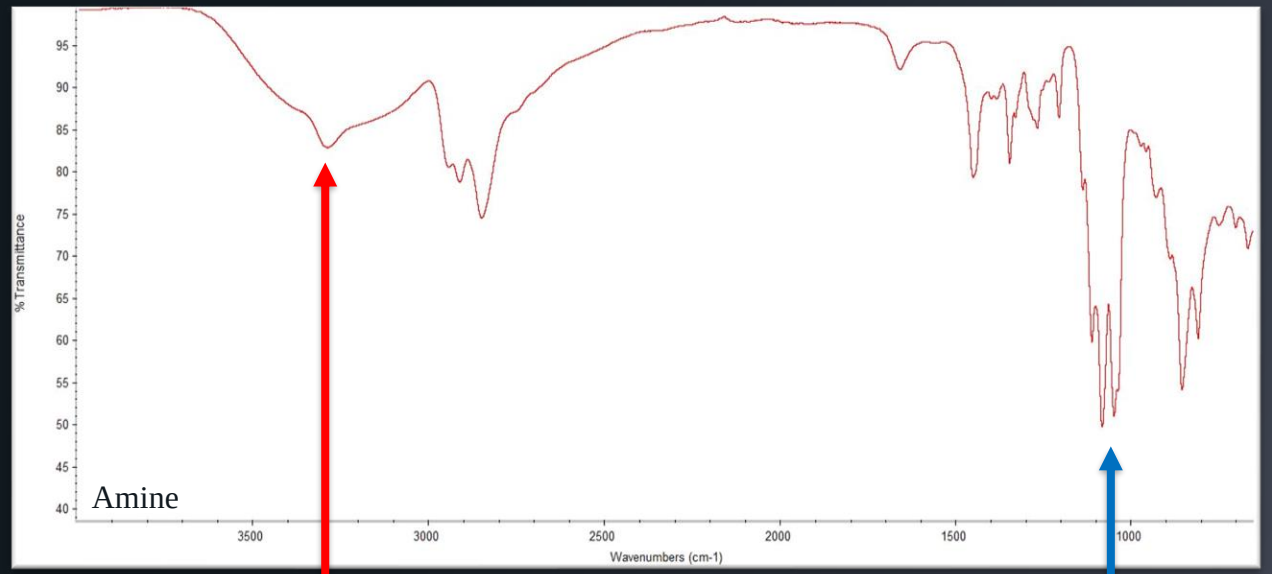


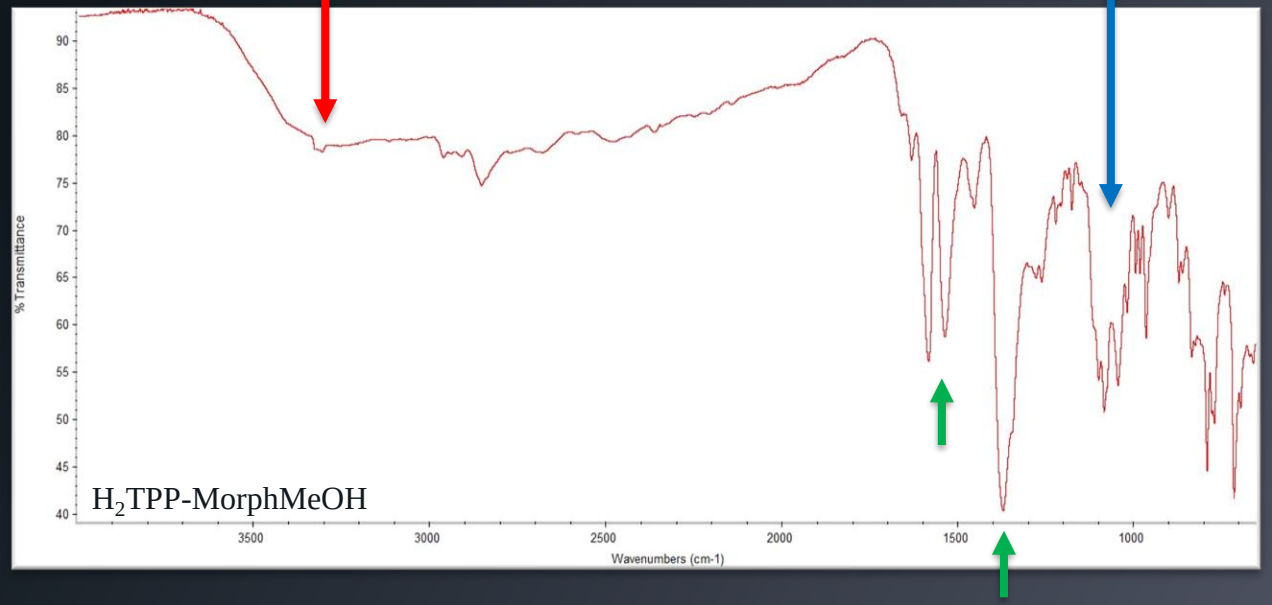
- Infrared (IR) Spectroscopy. 30 May 2020, https://chem.libretexts.org/@go/page/45256.

IR Spectroscopy



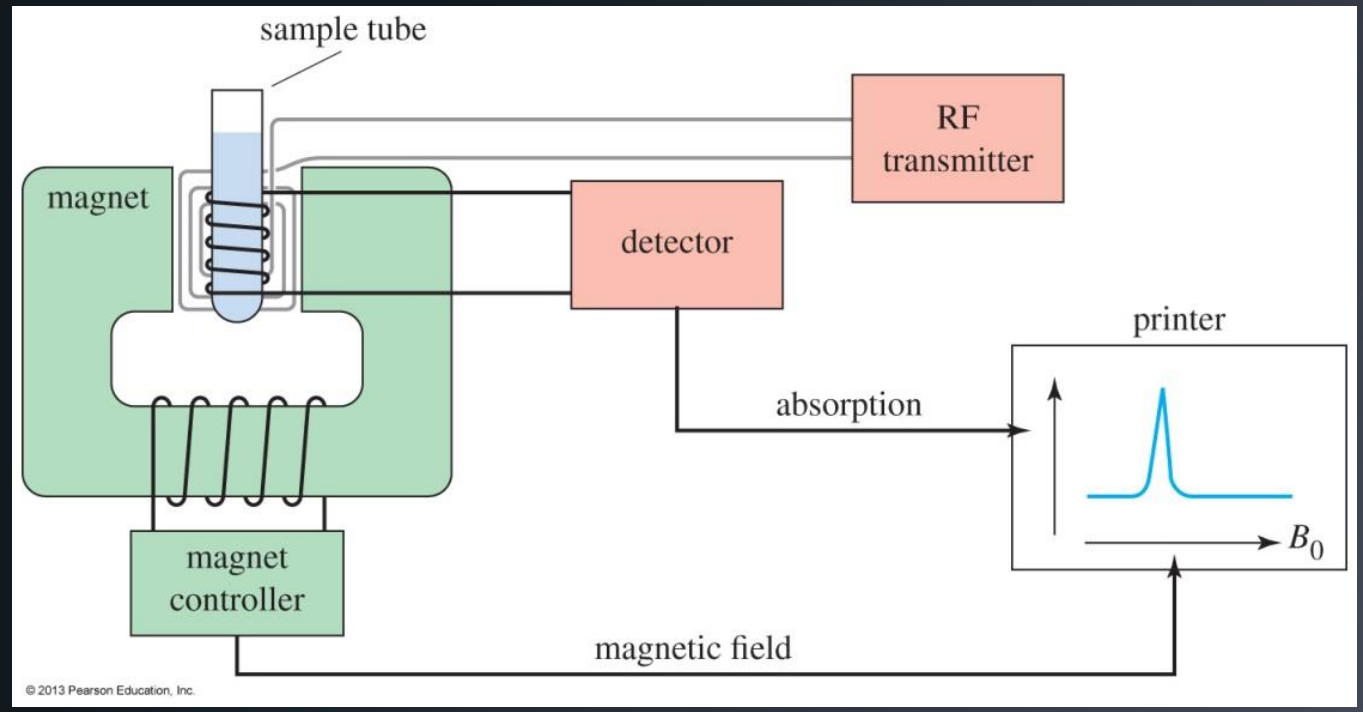




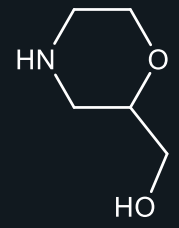


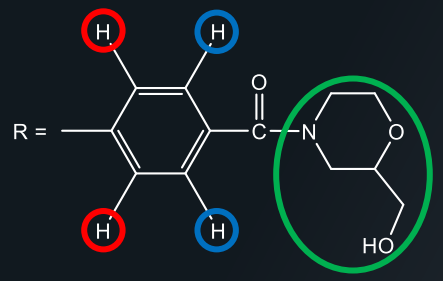
NMR Spectroscopy

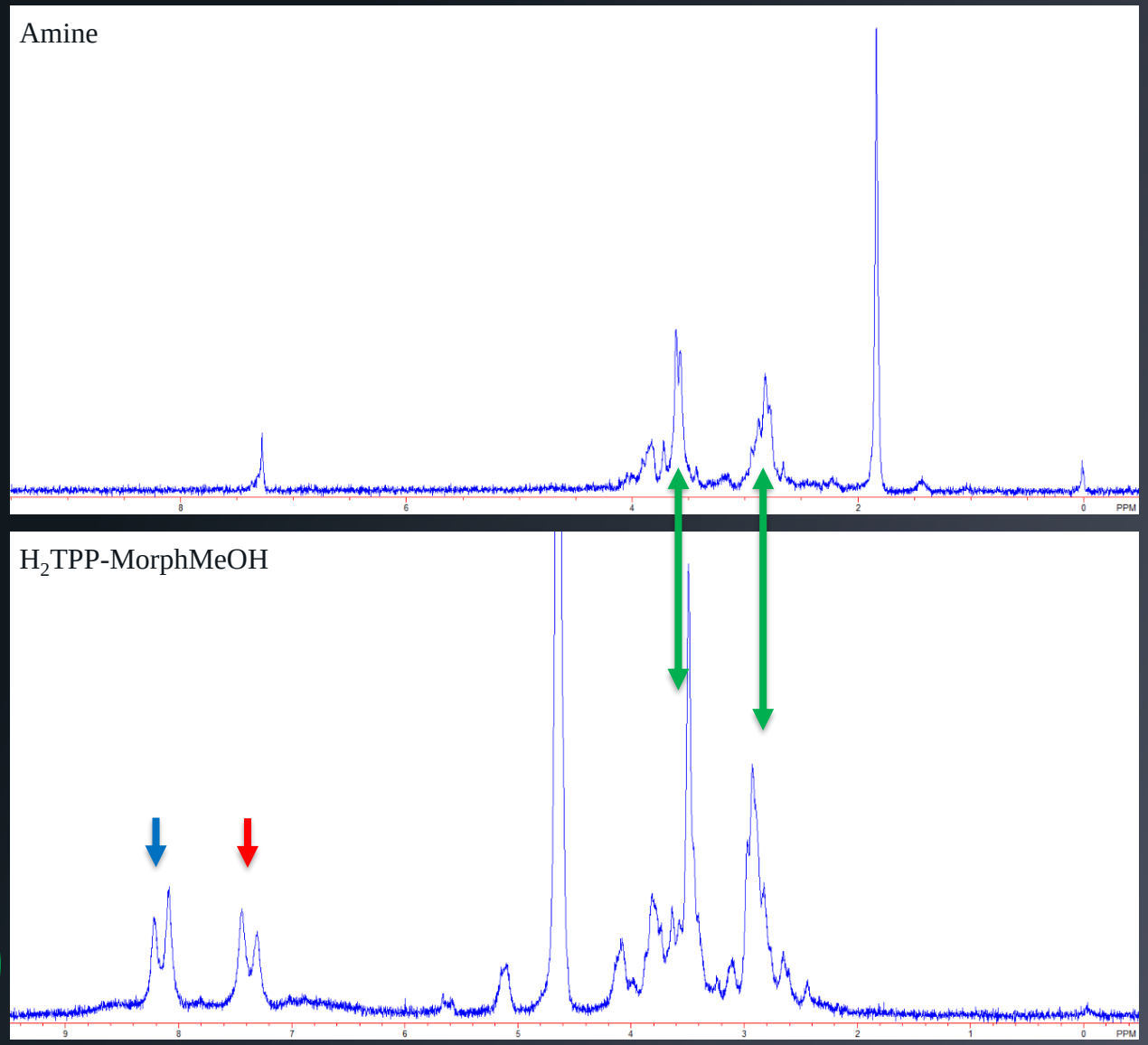
- Sample hit with an electromagnetic pulse, causing the production of signals
- Signals are interpreted by the detector and turned into a spectrum
- Spectrum is used to elucidate structures



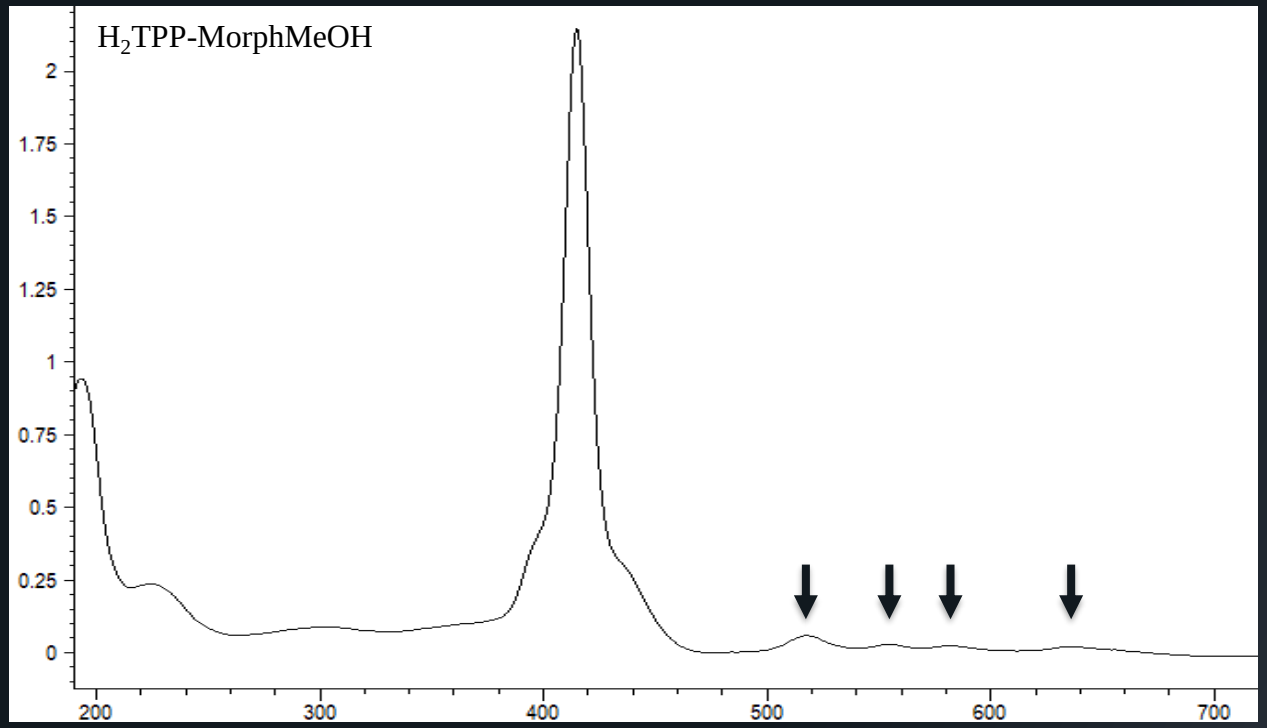
NMR Spectroscopy







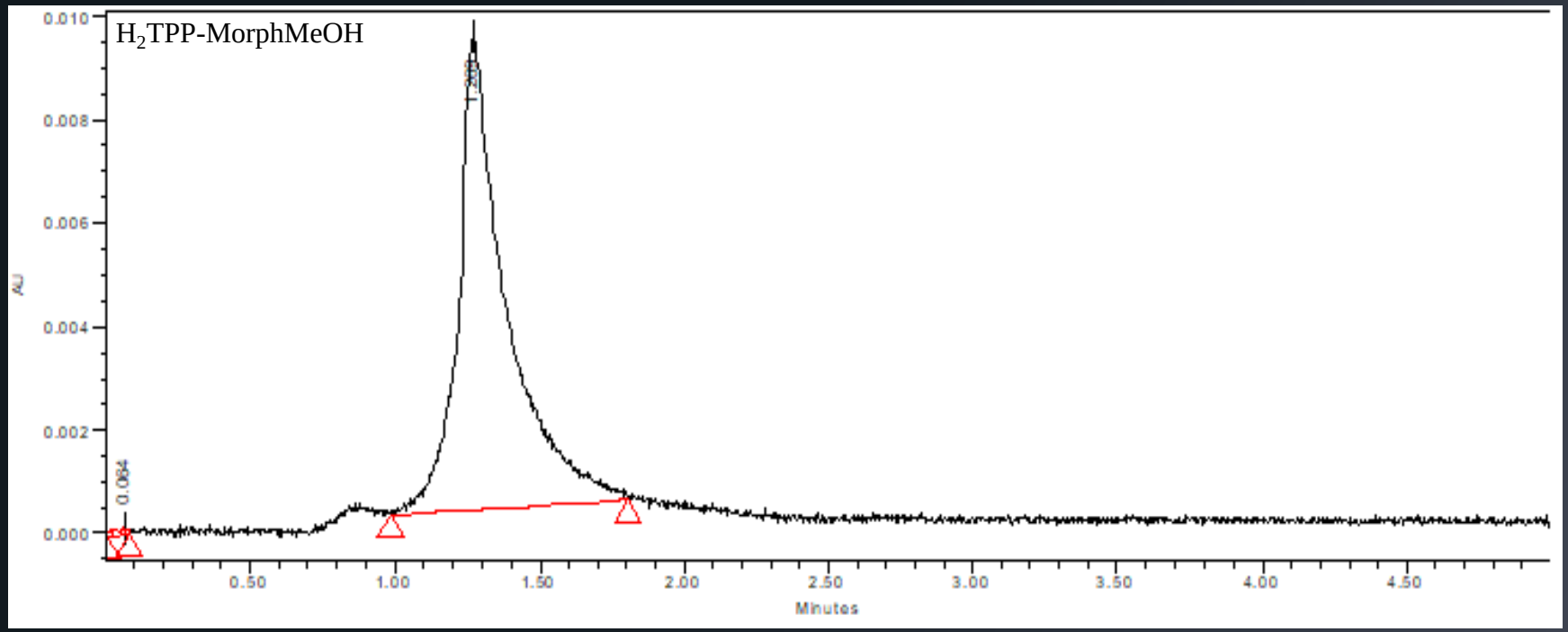
UV-Vis Spectroscopy



Peaks (nm)	Molar Absorptivity Coefficient, ε ($cm^{-1}mM^{-1}$)
413	120
518	5.35
555	2.93
581	2.45
636	1.95

High-Performance Liquid Chromatography

- Purity is based on area of chromatogram that corresponds to the compound – the purity of H_2TPP-MorphMeOH was determined to be 98%



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MTT Analysis

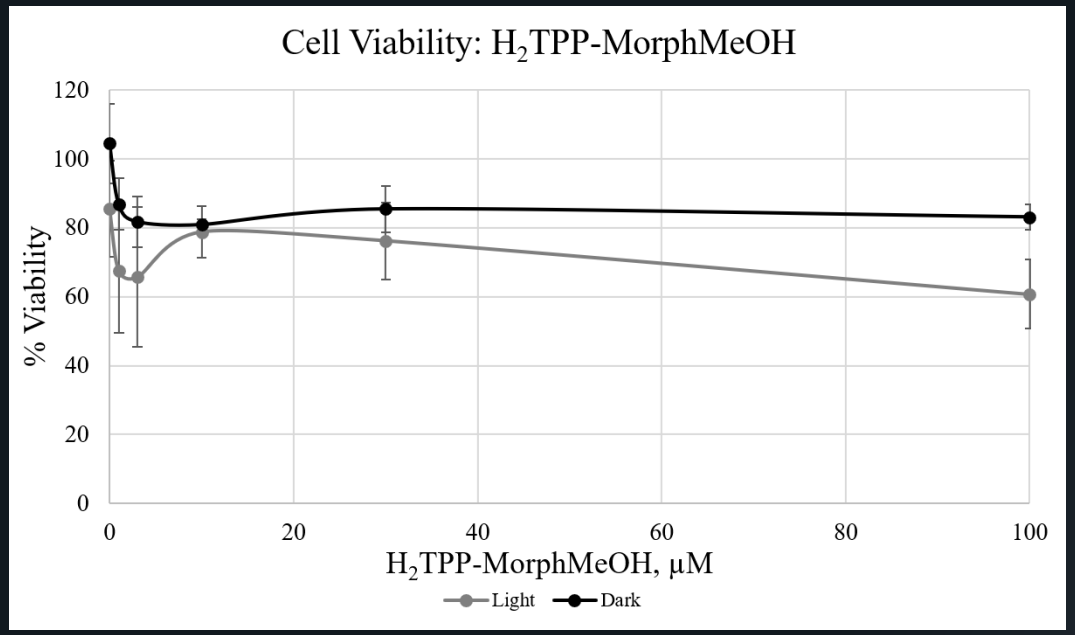
- MTT analysis – method of determining viability based on cell metabolic activity.
- Several concentrations of the compound were tested across two plates; one was kept in the dark, and the other exposed to light.
- Compound efficacy was determined using LD_{50}

Plate kept in the dark – consistent, deep purple color

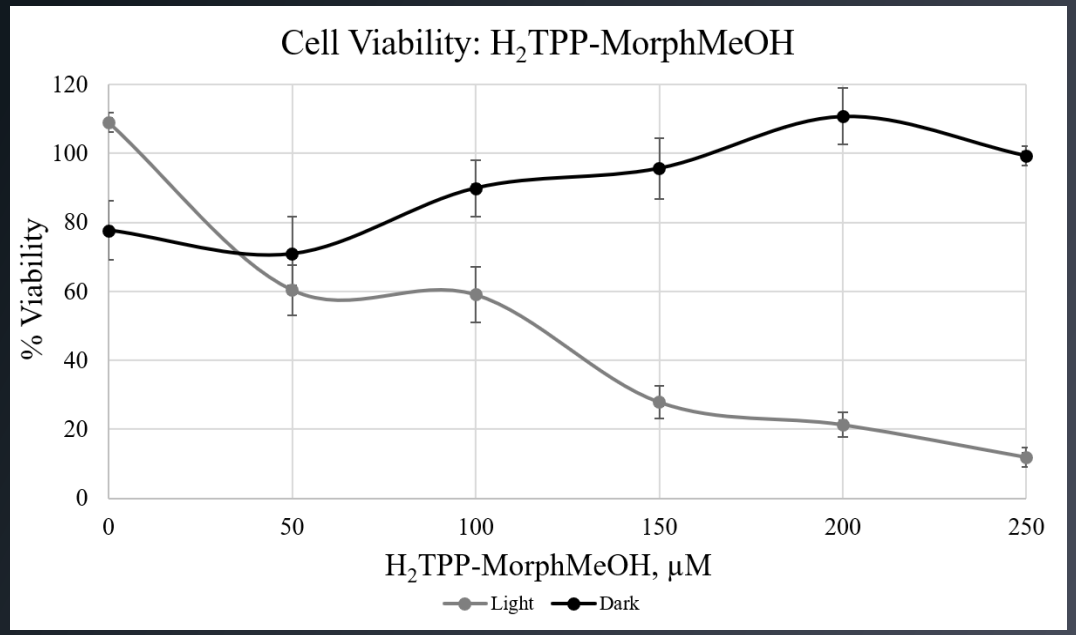
Plate exposed to light – color lessens as porphyrin concentration increases and cell death occurs

MTT Results for H_2TPP-MorphMeOH

- Plated concentrations: 0, 1, 3, 10, 30 and 100 µM



- Plated concentrations: 0, 50, 100, 150, 200, and 250 µM



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Project Review

- The novel porphyrin H_2TPP-MorphMeOH was successfully synthesized from H_2TPPC and the amine morpholin-2-yl methanol
  - The product was successfully purified (HPLC)
  - The product retained internal structure (UV-Vis, NMR)
  - The product coupled with the amine (NMR, IR)
- H_2TPP-MorphMeOH was determined to be a weak antineoplastic agent based on the results of the MTT assays

Project Goals (Revisited)

- Synthesize, purify, characterize, and test a novel porphyrin derivative
- To be a viable photodynamic therapy agent:
  - The porphyrin should be water soluble
  - ~~The porphyrin should be able to kill cells in low concentrations when exposed to light~~
  - ~~The porphyrin should have minimal effect on cells when not exposed to light~~
- Since H_2TPP-MorphMeOH doesn't satisfy all three requirements, it would not be fit as a PDT agent

Future Work

- Re-perform second MTT assay to resolve error
- Continue exploring H_2TPP-MorphMeOH's potential uses
- Continue searching for and developing other porphyrin derivatives

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Questions?