Electrochemical Proton Gradient Regulation Across Cell Membrane Mimics

Joseline Aquino, Lynn Nguyen, Cindy Mao and Dr. Hadi Tavassol
Department of Chemistry and Biochemistry
CALIFORNIA STATE UNIVERSITY LONG BEACH

BUILD CSULB

ABSTRACT

The regulation of the proton gradient present in a mitochondrion is important in preventing mitochondrial dysfunction, which affects the energy cycle of the cell. Current studies on neurodegenerative disorders, such as Parkinson's disease, have shown that exposing the mitochondrial membrane to adaptive cellular stress by using a mitochondrial uncoupler, can improve neural plasticity. 2,4-Dinitrophenol (DNP) is a synthetic mitochondrial uncoupler and proton ionophore. Our study focuses on analyzing the rate at which small decoupling molecules interact with the proton gradient and their role in regulating it. Using a bi-phasic liquid-liquid interphase allowed us to mimic the cell membrane and focus solely on the interaction of DNP with the proton gradient. UV-Visible spectroscopy and kinetic studies were employed to examine the proton transfer facilitated by DNP to determine the rate of the reaction at a specific concentration. Our study discovered that when DNP interacts with a pH higher than its pKa value of 4.09, it disrupts the proton gradient and causes the absorbance signal to decrease significantly after an hour. This study can help develop the proper dosage of DNP as a treatment for mitochondrial dysfunction.

Keywords: proton gradient regulation, mitochondrial dysfunction, UV-VIS spectroscopy, mitochondrial uncoupler

INTRODUCTION

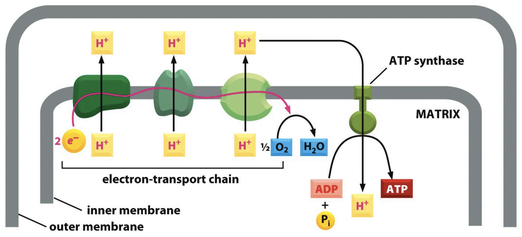
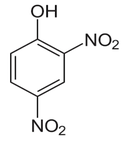


Figure 1: Oxidative phosphorylation occurring in the mitochondrial membrane.

- **Mitochondrial dysfunction** disrupts production of Adenosine Triphosphate
  - Linked to Parkinson's and Alzheimer's disease

Bi-phasic Effect of 2,4-Dinitrophenol



Low Dosage → Activates an **adaptive stress response** that **increases a cell's resistance** to environmental changes

High Dosage → Increases internal temperature, causes cardiac arrest, skin lesions and often death.[1]

METHODS

Bi-phasic Liquid-Liquid Interface:

- DNP dissolved in 1,2-Dichloroethane and Tetrabutylammonium tetraphenylborate (100 μM)
- Samples left to sit for 1 hour and 24 hours

Figure 2: Cell membrane mimics with pH 1,3,4,5,6 and 7 (left to right).

UV-Visible Spectroscopy:

- Allowed us to visualize the concentration of DNP remaining in the organic layer.
- Absorbance measured from 200-800 nm

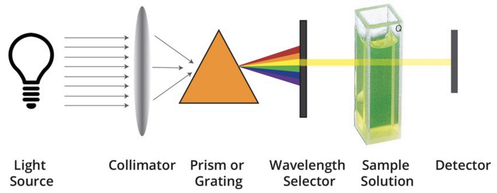


Figure 3: How UV-Vis determines the absorbance signal.

RESULTS

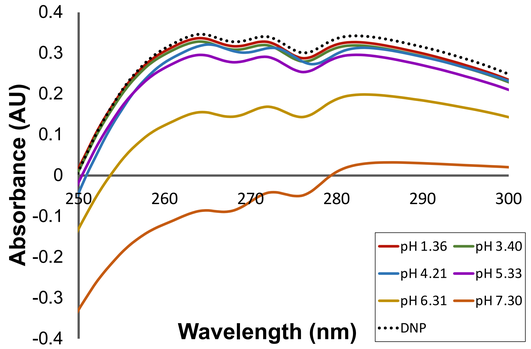


Figure 4: UV-Vis spectra showing absorbance signal of the organic layer in all the vials containing different pH's after 1 hour.

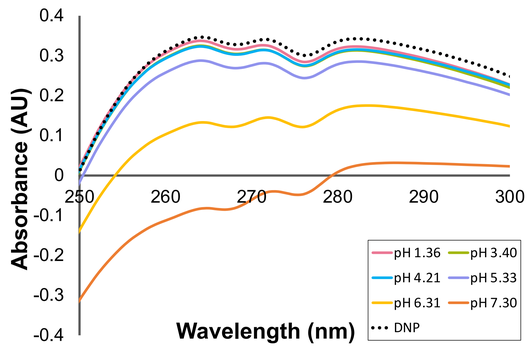


Figure 5: UV-Vis spectra showing absorbance signal of the organic layer in all the vials containing different pH's after 24 hours.

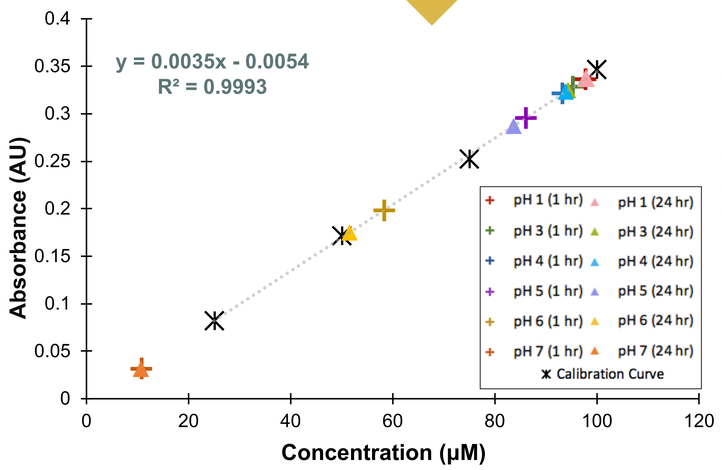


Figure 6: Highest absorbance for each pH at 1 hour and 24 hours plotted on the calibration curve to determine concentration of DNP present in organic layer.

- **pH 1-4:** 97-93 μM
- **pH 5:** 86-83 μM
- **pH 6:** 58-51 μM
- **pH 7:** 10 μM

CONCLUSION

- Previous studies found that **DNP was rapidly absorbed**
  - **Maximum effect** on metabolism **within the first hour**.[2]

pH of system increases higher than the pKa value of 4.09 → **More DNP crosses the interface and becomes protonated, decreasing the absorbance signal in 1 hour**

  - Color change in the samples indicates the protonation of DNP and the disruption of the proton gradient.
- The **reaction rate** at specific concentrations can **assist in the search for mitochondrial dysfunction treatments.**

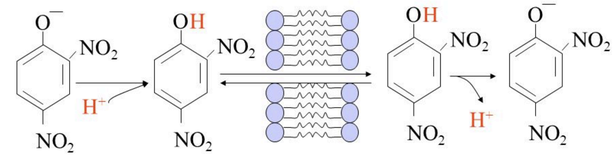


Figure 7: DNP becomes protonated and crosses the lipid bi-layer.

FUTURE WORK

- Perform kinetic studies
- Introduce phospholipids at the interface

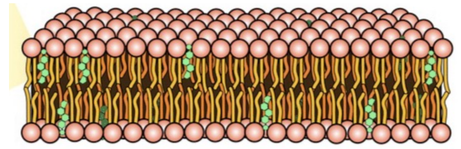


Figure 8: Phospholipid bilayer found in the mitochondria.

REFERENCES

(1) Lee, Y., Heo, G., Lee, K. M., Kim, A. H., Chung, K. W., Im, E., Chung, H. Y., & Lee, J. (2017). Neuroprotective effects of 2,4-dinitrophenol in an acute model of Parkinson's disease. *Brain research*, *1663*, 184–193.

(2) Dunlop, D. (1934). The Use Of 2:4-Dinitrophenol As A Metabolic Stimulant. *The British Medical Journal, 1*(3820), 524-527.

ACKNOWLEDGEMENTS

This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers; UL1GM118979; TL4GM118980; RL5GM118978. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.