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 the proton gradient regulation, mitochondrial dysfunction, UV-VIS spectroscopy, mitochondrial uncoupler

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

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

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

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

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 1.35: 97-93 μM
- pH 5.33: 86-83 μM
- pH 7.30: 58-51 μM
- pH 7.30: 10 μM

CONCLUSION
- Previous studies found that DNP was rapidly absorbed and caused more DNP to cross the interface and become 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.

FUTURE WORK
- Perform kinetic studies
- Introduce phospholipids at the interface

REFERENCES

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.