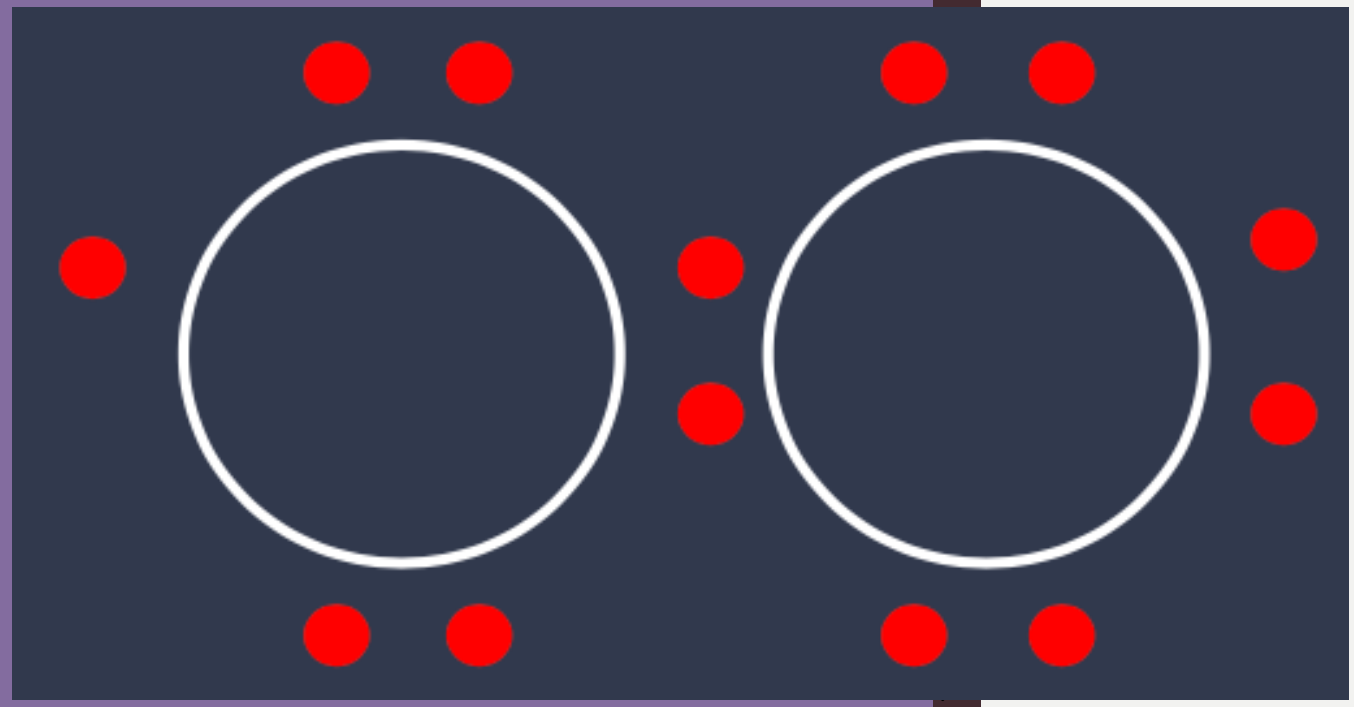
DETECTING REACTIVE OXYGEN SPECIES IN YEAST AT EARLY GROWTH POINTS.

Lottie R. Steward

2020 Linfield College Student Symposium



Reactive Oxygen Species (ROS)

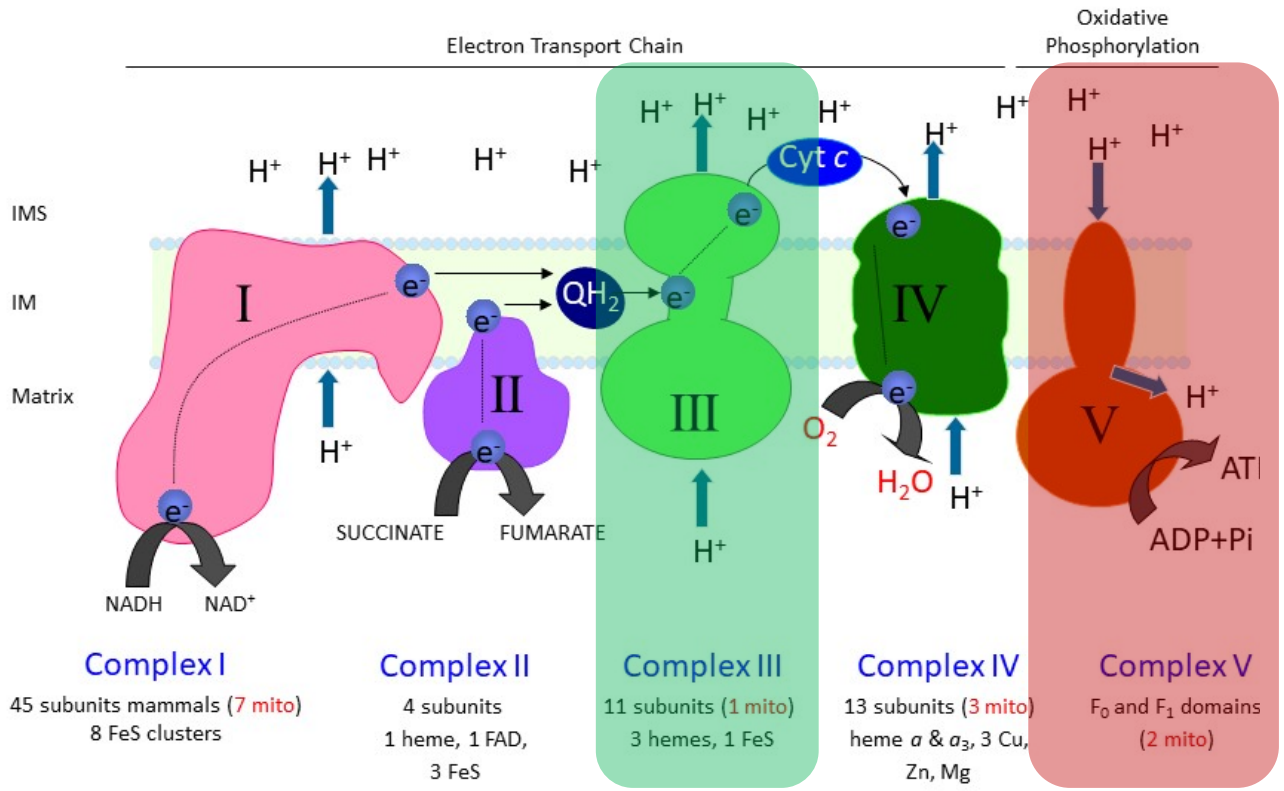
- Examples of ROS
  - Superoxide anion ($O_2^{\bullet -}$)
  - Hydrogen peroxide (H_2O_2)
  - Hydroxyl radical ($HO\bullet$)
- A radical is an atom, molecule, or ion that has an unpaired valence electron.
- ROS reacts with nuclear DNA, mitochondrial DNA (mtDNA), proteins, and lipids, creating mutations and dysfunctional molecules.

THE ELECTRON TRANSPORT CHAIN OF THE MITOCHONDRIA

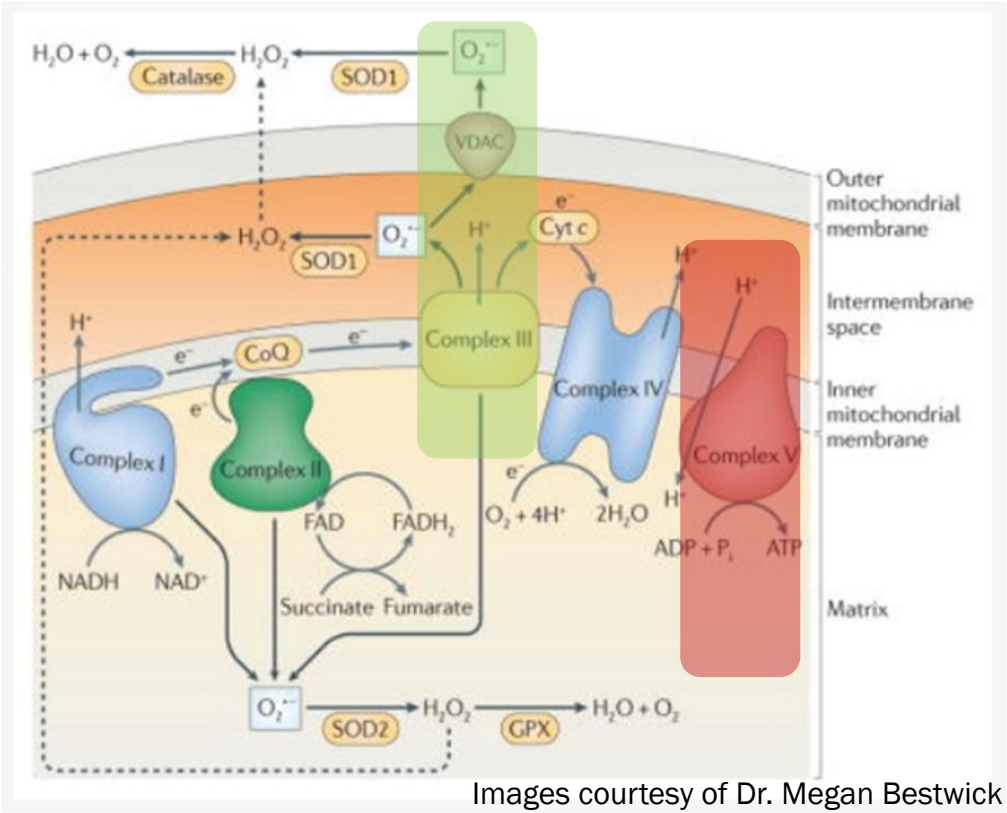
Antimycin A Inhibitor

Oligomycin A Inhibitor

The Mitochondrial Respiratory Chain: OXPHOS Complexes



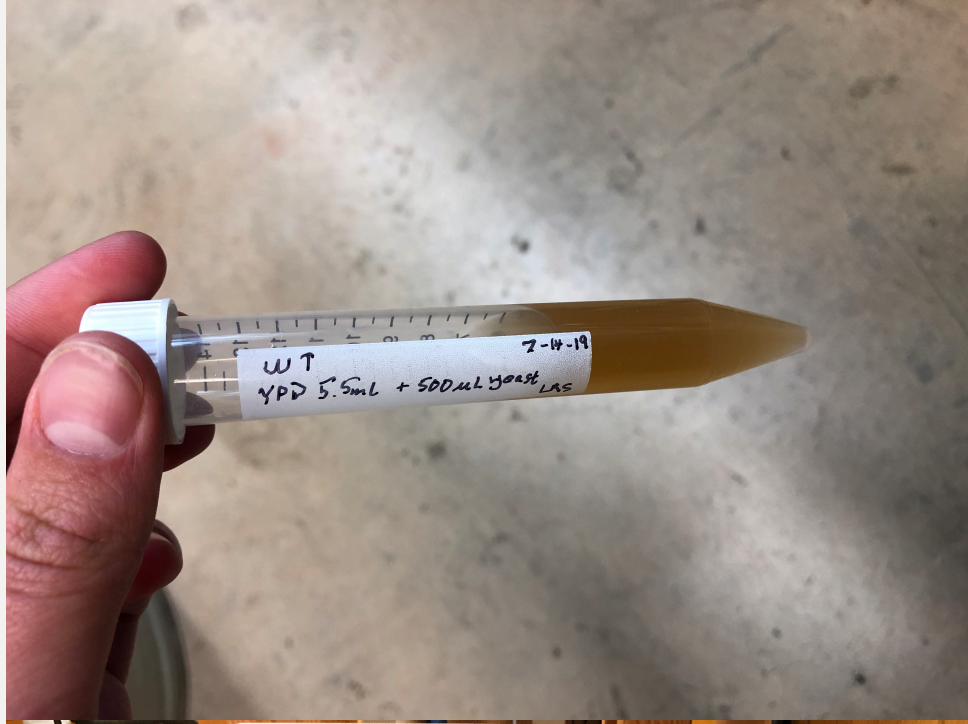
The Mitochondrial Respiratory Chain: Reactive Oxygen Species



Images courtesy of Dr. Megan Bestwick

Why is yeast a good model organism?

- Can be grown in multiple types of media
  - *liquid (tubes or flasks)*
  - *solid (plates)*
- Human and yeast mitochondria similarities, such as the Electron Transport Chain
- Particularly good for aging studies.



WHY IS THIS IMPORTANT?

Objectives

- What levels of ROS are throughout lifespan.
- How/where cells are generating ROS
  - Use inhibitors
    - Oligomycin A
    - Antimycin A

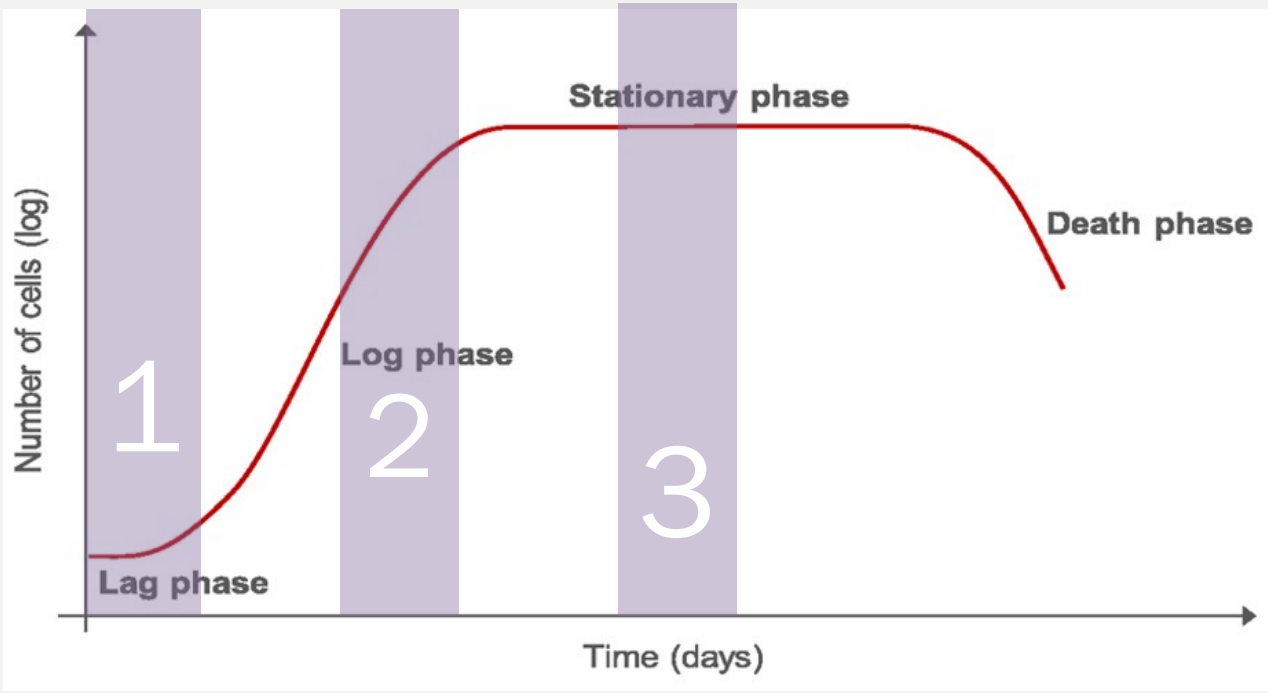
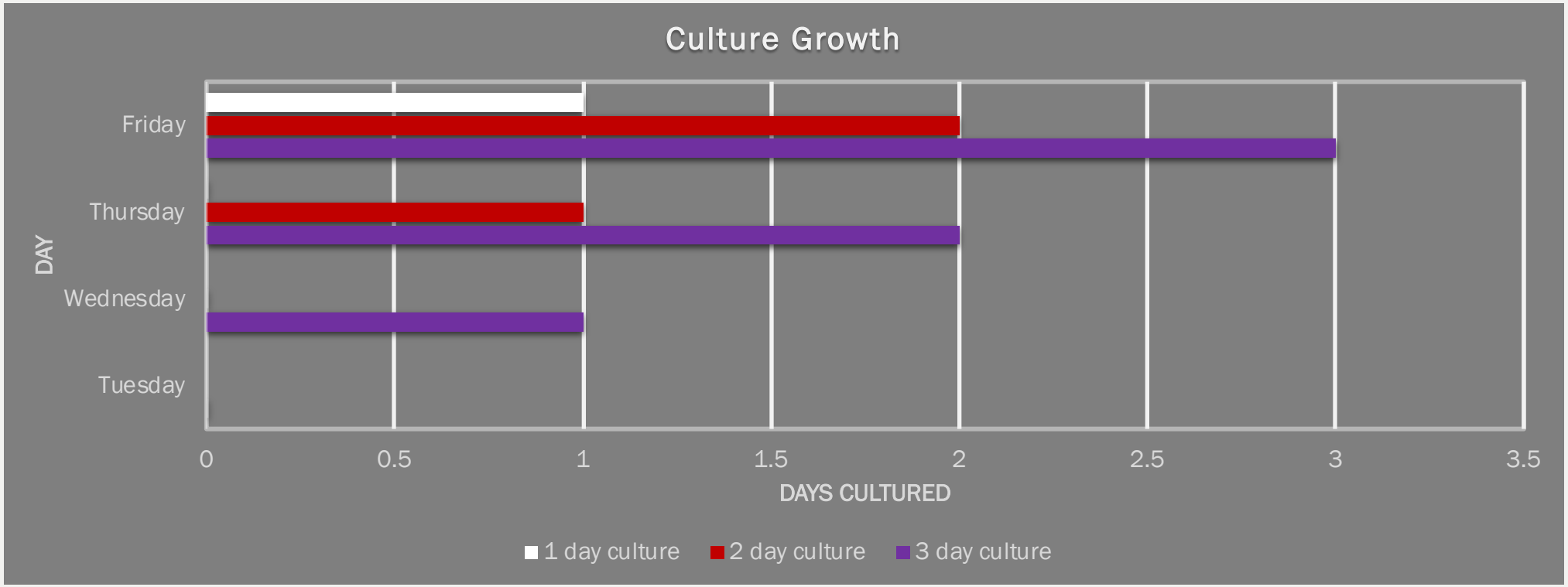


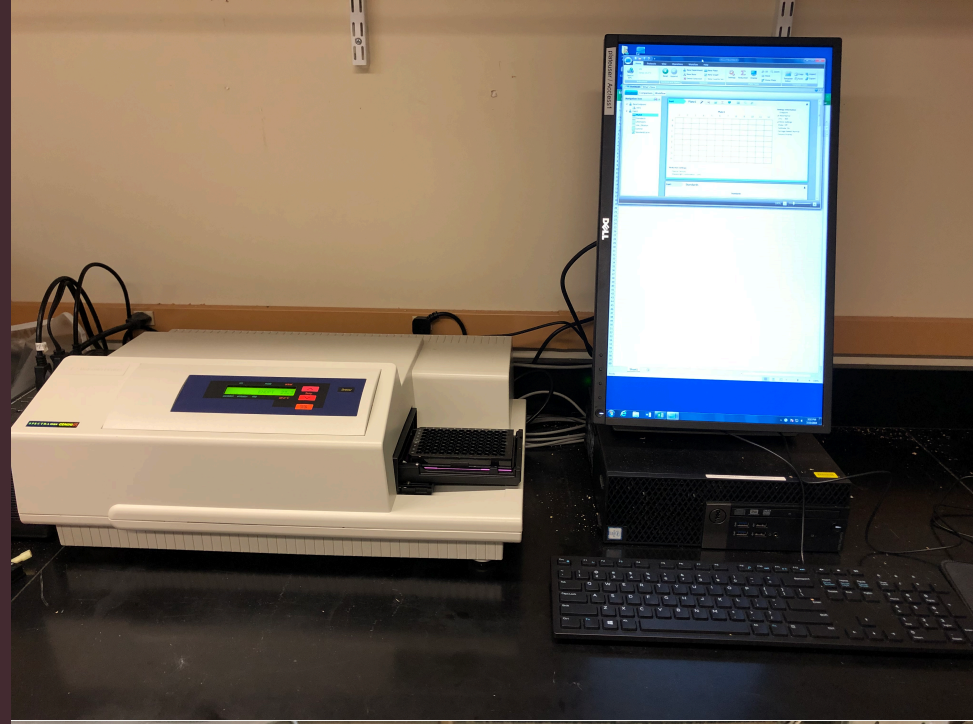
Image courtesy of Dr. Megan Bestwick

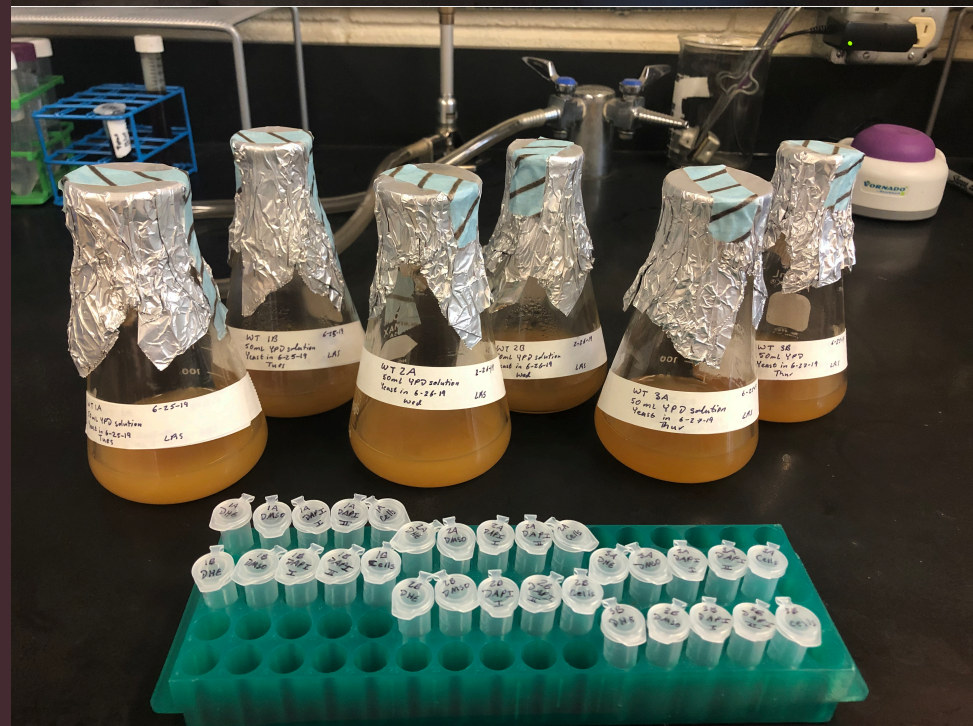
Methods
Growing Cultures



Methods
ROS determination

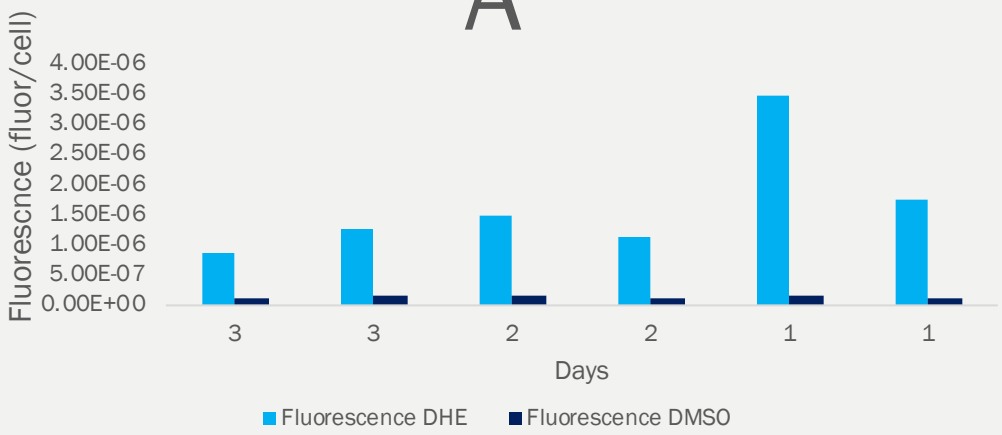
- Fluorescent Indicators
  - Dihydroethidium (DHE)
    - *Excitation 460 nm*
    - *Emission 580 nm*
  - DAPI (4′,6-diamidino-2-phenylindole)
    - *Excitation 358 nm*
    - *Emission 461 nm*
  - Dimethyl Sulfoxide (DMSO)



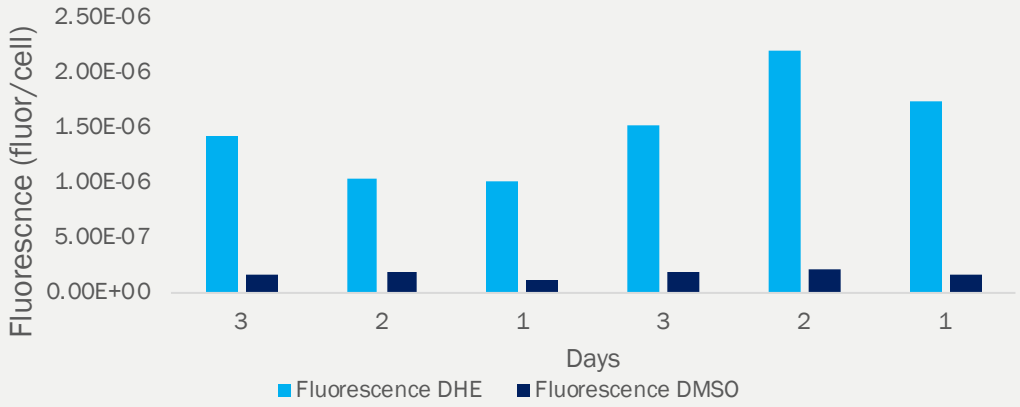


Results from Day Determination

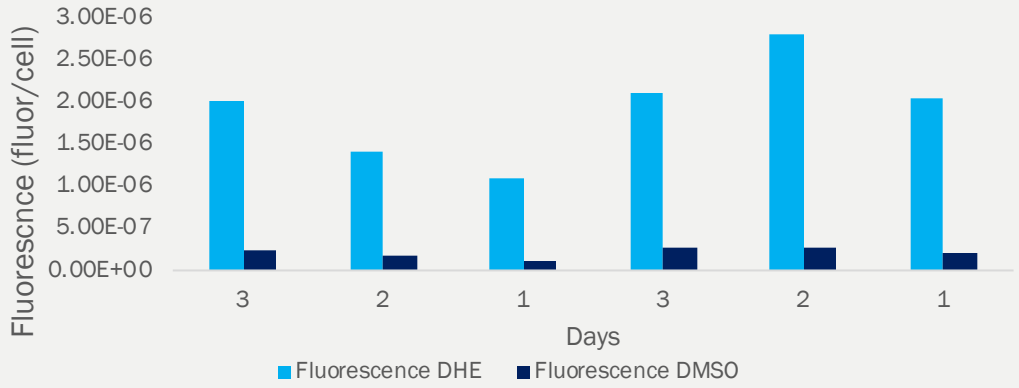
A



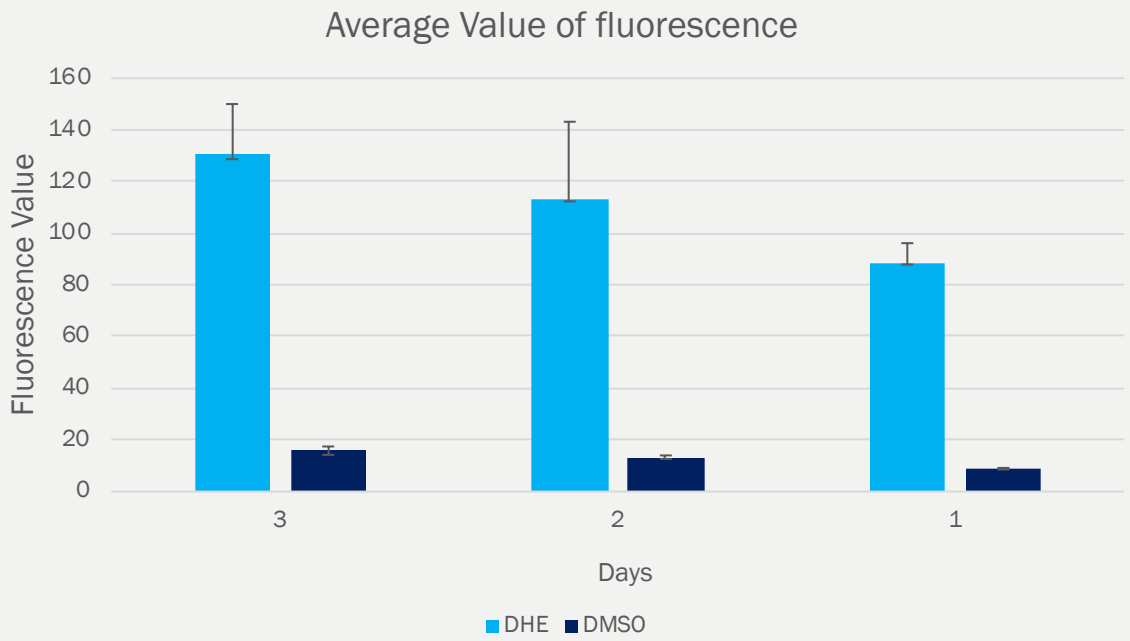
B

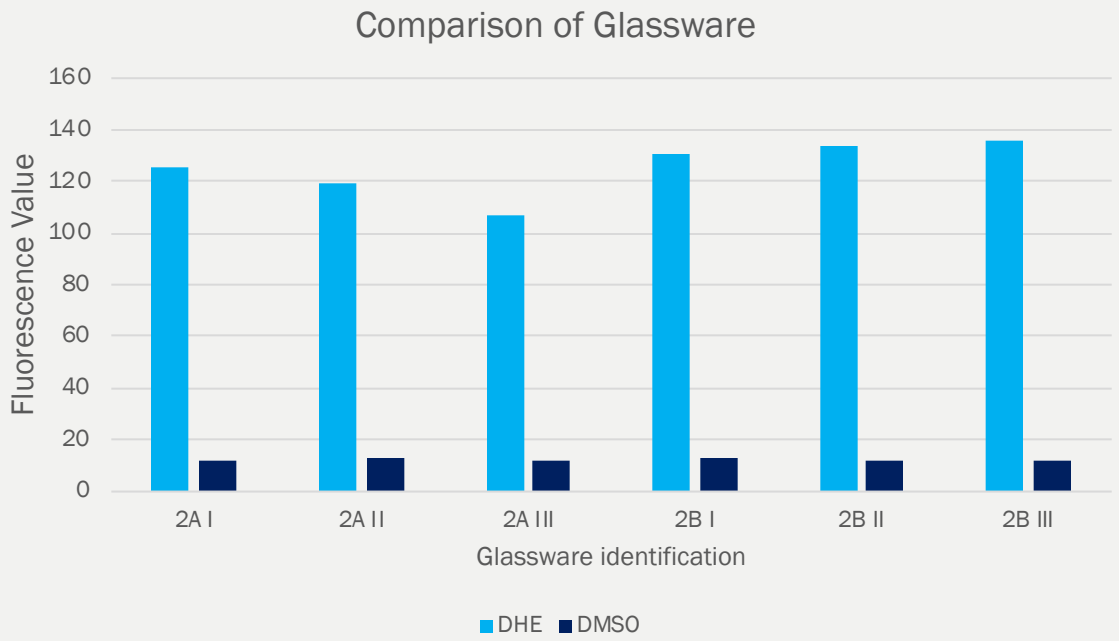


C

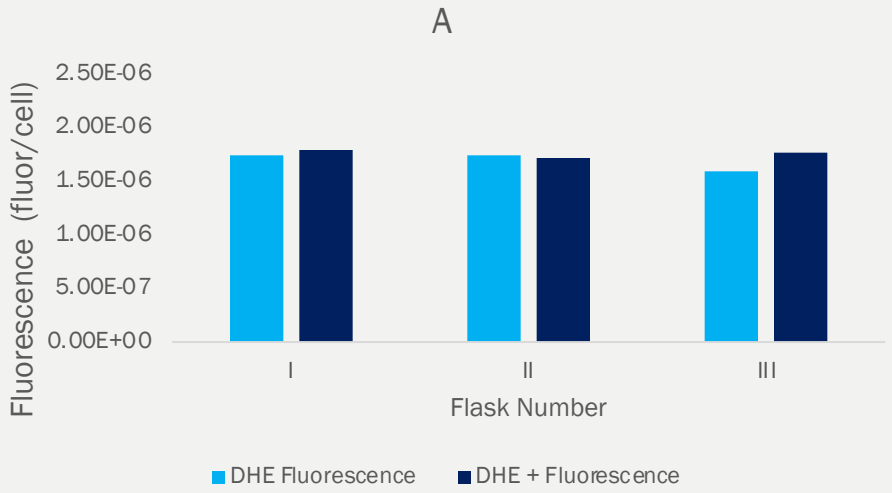


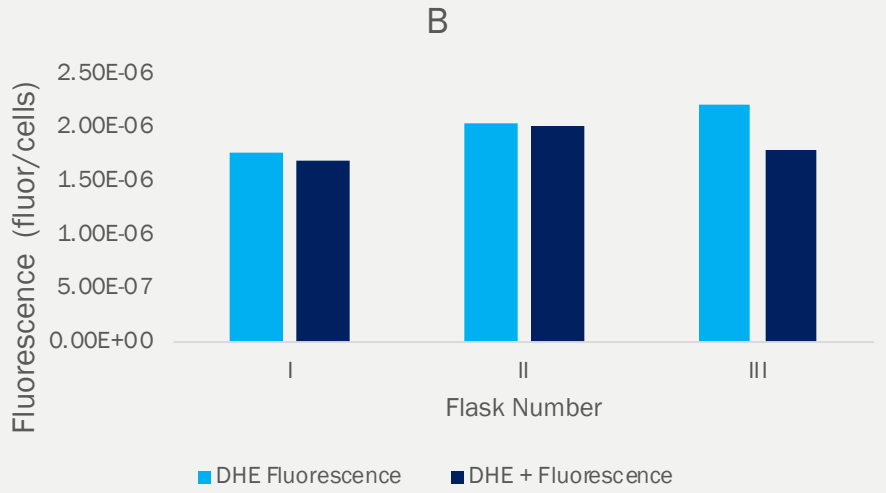
Results of Glassware Investigation

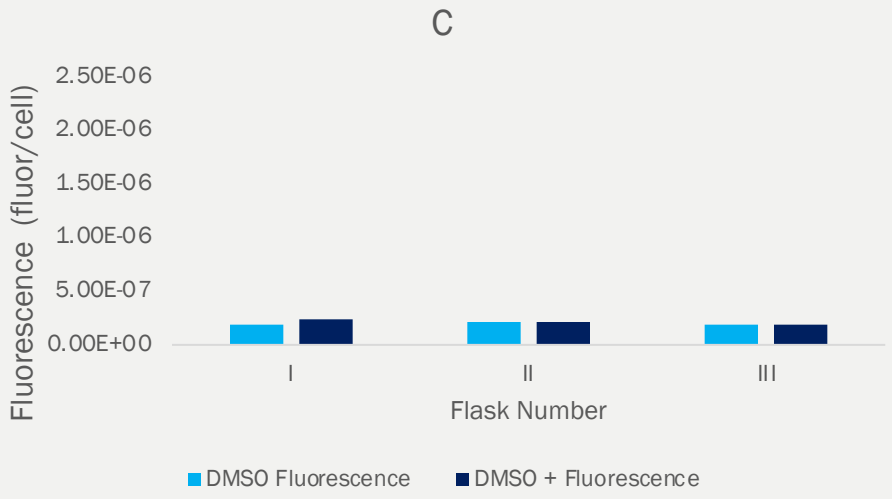


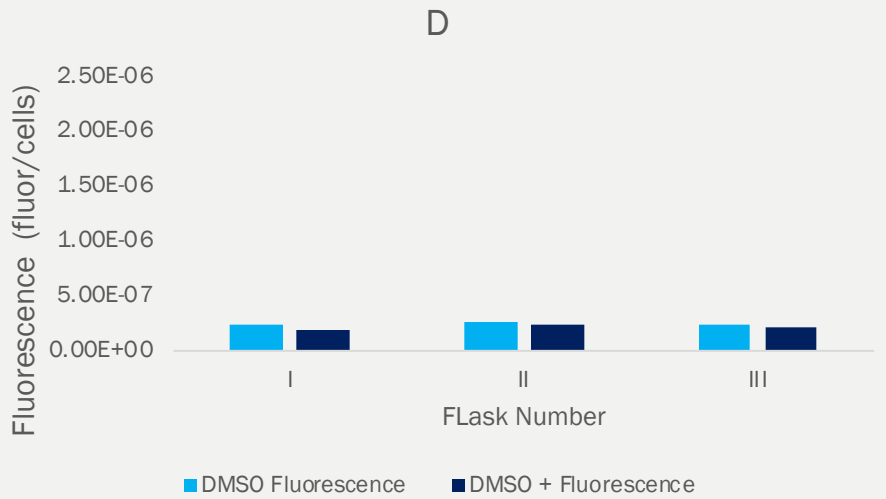


Results with Oligomycin A

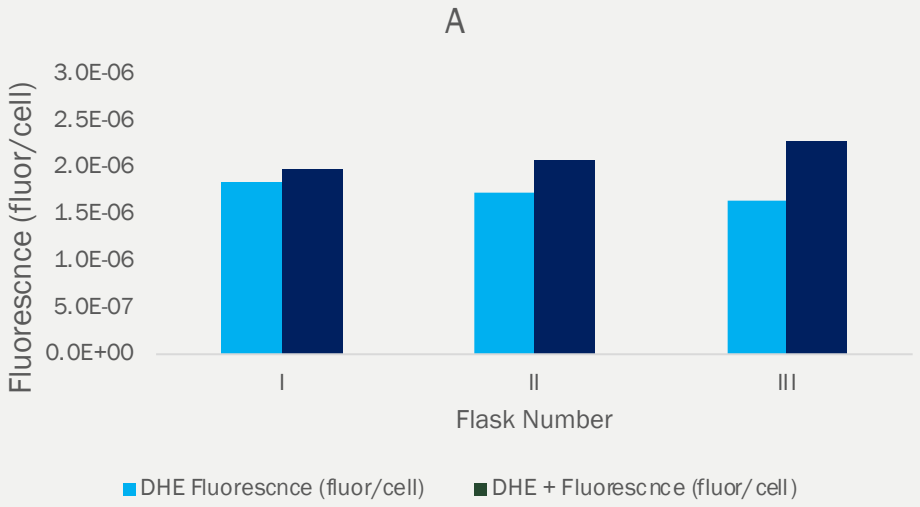


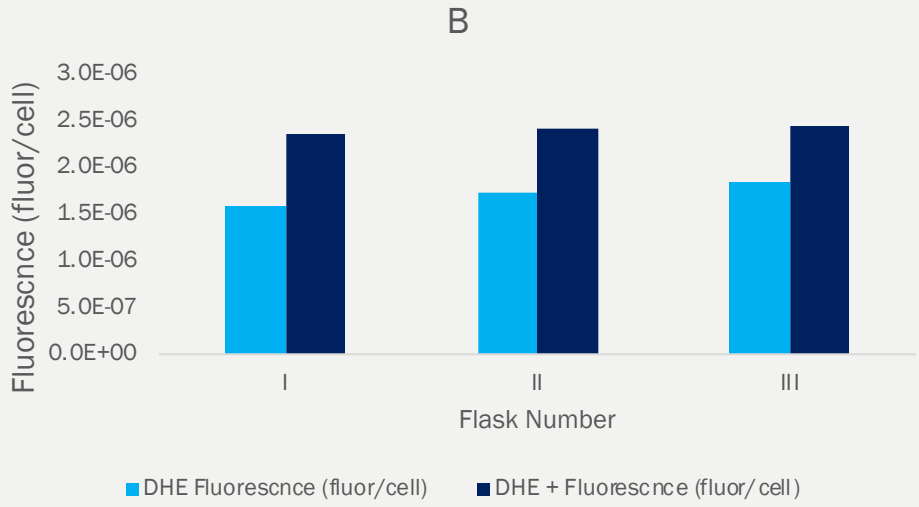


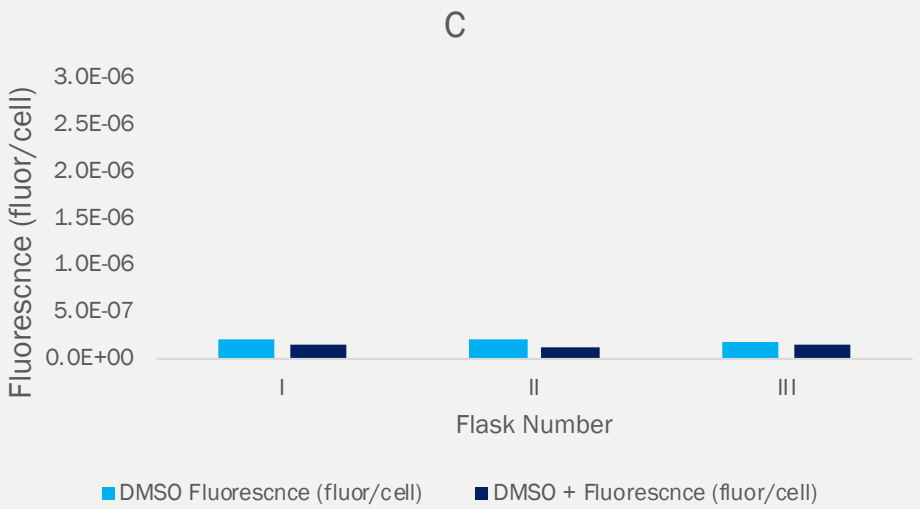


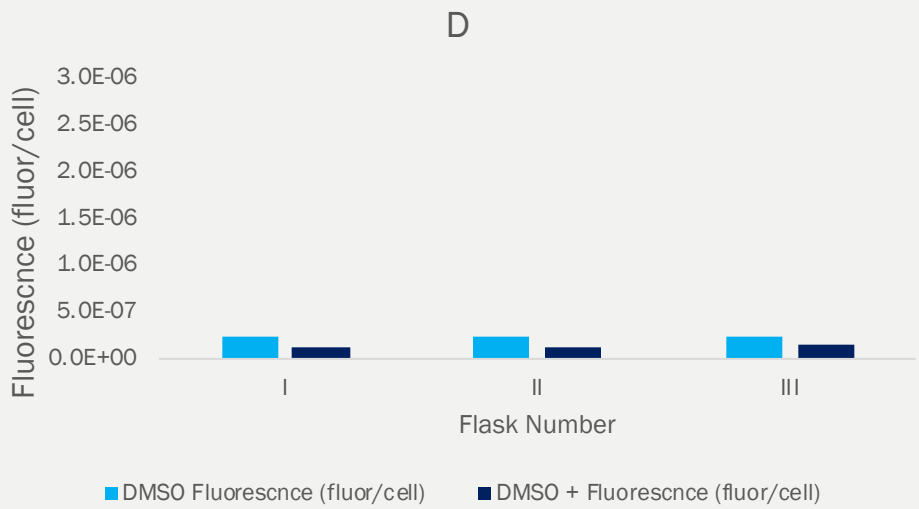


Results with Antimycin A









CONCLUSION

Acknowledgements

- Linfield Chemistry Department
- The National Science Foundation
- M.J. Murdock Charitable Trust
- Linfield College Collaborative Research Grant/Dyke Science Endowment
- Dr. Megan Bestwick
- Research Students from 2019 Summer Research
  - *Sofia Bauer*
  - *DJ Keogh*
  - *Zach McLeod*
  - Zach Sherlock
  - Hannah Waterman

References

- *Snapshot: Reactive Oxygen Intermediates (ROI).* Carl Nathan and Aihao Ding. Cell 140, March 19, 2010 ©2010 Elsevier Inc. DOI 10.1016/j.cell.2010.03.008. Weill Cornell Medical College, New York, NY 10065, USA
- *How mitochondria produce reactive oxygen species.* Michael P. Murphy.Biochem. J. (2009) 417, 1–13 (Printed in Great Britain) doi:10.1042/BJ20081386 ,MRC Dunn Human Nutrition Unit, Hills Road, Cambridge CB2 0XY, U.K.
- *Synchronized Whole Cell Oscillations in Mitochondrial Metabolism Triggered by a Local Release of Reactive Oxygen Species in Cardiac Myocytes* M.A. Aon, S. Cortassa, E. Marban, and B. O'Rourke. J. Biol. Chem. (2003), **278** (45), 44735–44744.
- *Yeast Models of Human Mitochondria1 Diseases.* Antoni Barrientos. Received 27 January 2003; accepted 7 February 2003 Address correspondence to: Antoni Barrientos, Department of Biological Sciences, Columbia University, New York, New York DepartmentofBiologicalSciences,ColumbiaUniversity,New York,NY,USA

Questions?