Exogenous Clickable Phospholipids for Imaging Hydrogen Peroxide

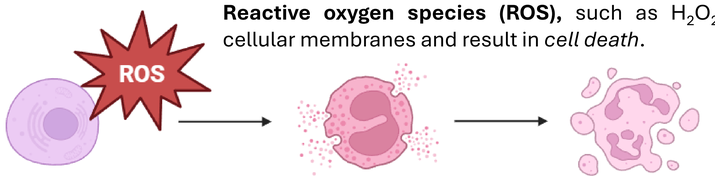
Nicholas Mixon*, Tom DePhilippo, Brittany White-Mathieu

Chemistry Department, University of New Hampshire, Durham, NH 03824

Nicholas.Mixon@unh.edu

linkedin.com/in/n-mixon/

Introduction



Reactive oxygen species (ROS), such as H_2O_2, degrade cellular membranes and result in *cell death*.

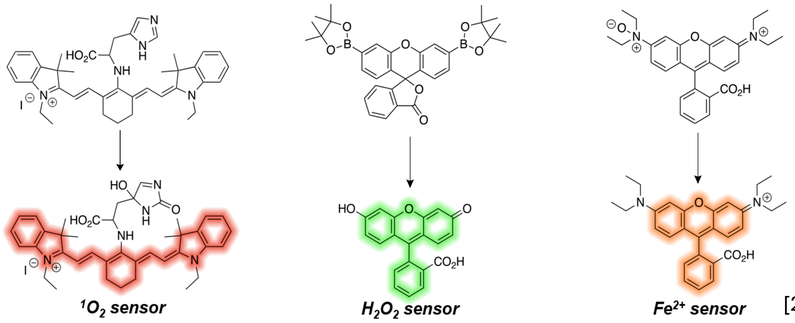
[1.]

Oxidative damage caused by H_2O_2,and other ROS are connected to:

- Aging
- Cancer
- Cardiovascular Disorders
- Neurodegenerative Diseases

Characterization of membrane degradation events is of fundamental importance for understanding disease. [2.]

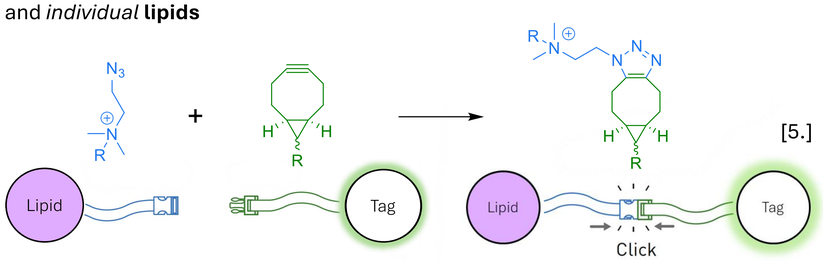
Fluorescent activity-based biosensors are uniquely poised to achieve both **detection of hazardous substrates** and **characterization of membrane structure** by illuminating areas where the analyte is present.



[2–4]

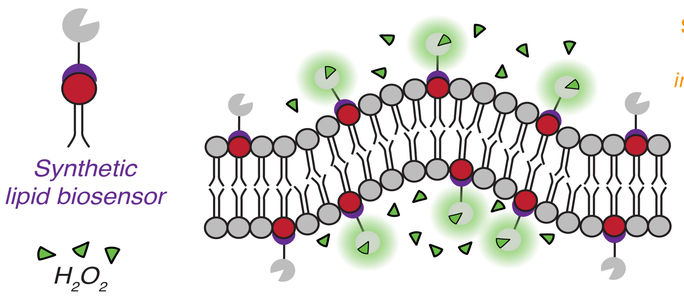
Activity-based biosensors undergo structural modification in the presence of an analyte of interest that enables the molecule to ***fluoresce***.

Bioorthogonal strain promoted azide-alkyne cycloaddition (SPAAC) click chemistry facilitates covalent bond formation between **activity-based biosensors** and *individual* **lipids**



[5.]

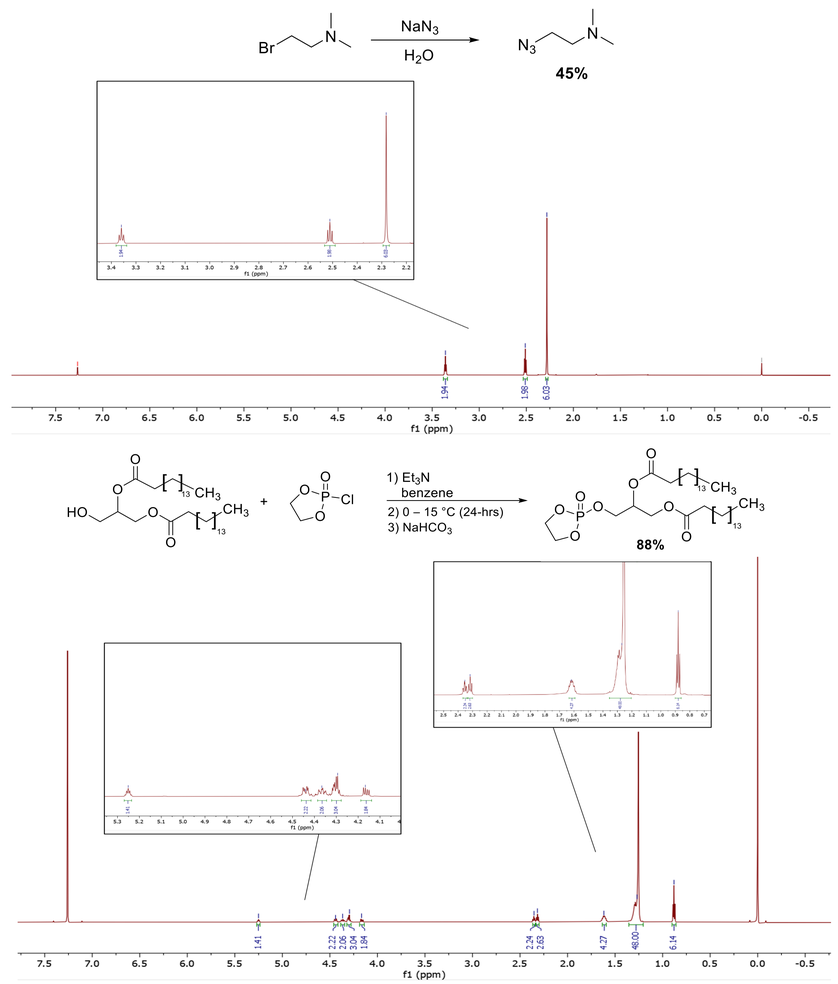
These phospholipids can then be incorporated into the membranes of **live** cells. [6-7]



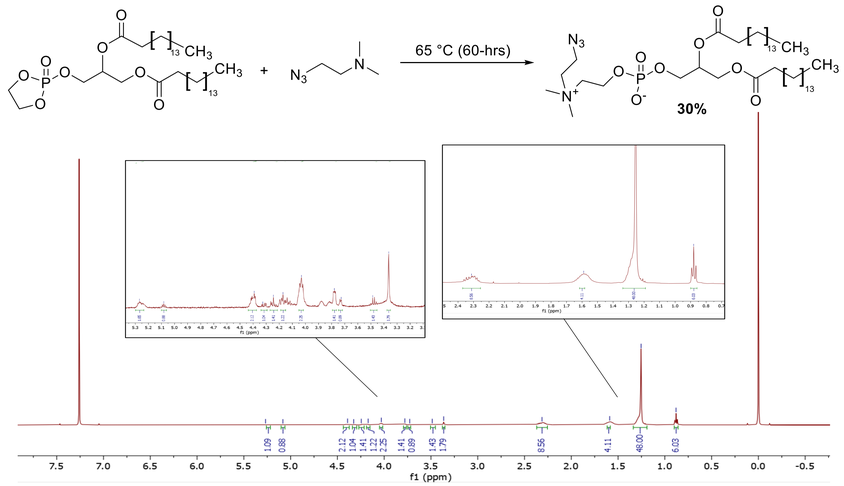
Synthetic lipid biosensors *incorporate* into natural phospholipid membranes & *react with specific analytes.*

Methods and Results[6-8]

Stage 1: Synthesis azido-amine and phospholipid.

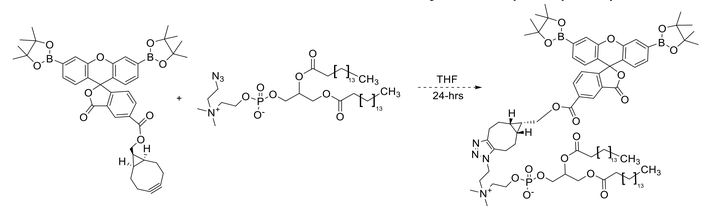


Stage 2: Synthesis of azide functionalized phospholipid.



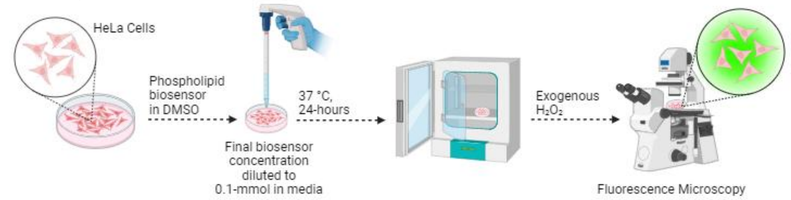
Future Directions

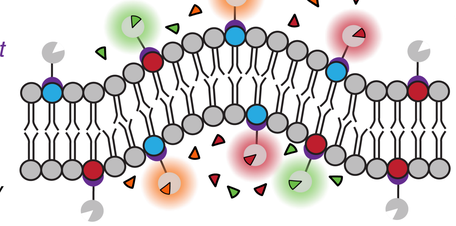
Stage 3: SPAAC “click” reaction of biosensor and synthetic phospholipid.



[1.]

Stage 4: Biosensor integration into HeLa cells, subsequent exogenous H_2O_2 activation, & characterization.





Incorporation of various sensors for characterizing membrane degradation during ***ferroptosis***...

Extensive lipid peroxidation induces cell death by ***Ferroptosis***. Iron (III) serves as an oxidant for conversion of hydrogen peroxide to a hydroxyl radical, becoming reduced to Fe^{2+}. Subsequent oxidation of this reduced species then drives peroxidation of polyunsaturated fatty acids (PUFAs), degrading membranes **cell-wide.** [9.]

We aim to **characterize** membrane decomposition with the use of these anchored **fluorescent biosensors.**

Acknowledgments

White-Mathieu Lab:
Paige Ring
Saghar Jarollahi
Tom DePhilippo
Matthew Fisk
Erin McCarthy
Taylor Stock
Kylie Armor
Muriel Lubelczyk
Nicholas Mixon
Aakriti Garg
Brittany White-Mathieu

Funding Generously Provided By…

University of New Hampshire & TRIO Ronald E. McNair Post-Baccalaureate Achievement Program

Special thanks to: Nayanthara Krishnan **&** Charles Wilson for their help in understanding key concepts.

References

[1.] *Biorender*. https://www.biorender.com

[2.] Chang, M. C. Y.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2004**, *126* (47), 15392–15393.https://doi.org/10.1021/ja0441716

[3.] Xu, K.; Wang, L.; Qiang, M.; Wang, L.; Li, P.; Tang, B. *Chem. Commun.* **2011**, *47* (26), 7386. https://doi.org/10.1039/c1cc12473k.

[4.] Hirayama, T.; Okuda, K.; Nagasawa, H. *Chem. Sci.* **2013**, *4* (3), 1250. https://doi.org/10.1039/c2sc21649c.

[5.] *Nobel Prize, Chemistry 2022. https://www.nobelprize.org/prizes/chemistry/2022/popular-information/*

[6.] Alamudi, S. H.; Satapathy, R.; Kim, J.; Su, D.; Ren, H.; Das, R.; Hu, L.; Alvarado-Martínez, E.; Lee, J. Y.; Hoppmann, C.; Peña-Cabrera, E.; Ha, H.-H.; Park, H.-S.; Wang, L.; Chang, Y.-T. *Nat Commun* **2016**, *7* (1), 11964. https://doi.org/10.1038/ncomms11964..

[7.] Gutierrez, B.; Aggarwal, T.; Erguven, H.; Stone, M. R. L.; Guo, C.; Bellomo, A.; Abramova, E.; Stevenson, E. R.; Laskin, D. L.; Gow, A. J.; Izgu, E. C. *iScience* **2023**, *26* (12), 108567. https://doi.org/10.1016/j.isci.2023.108567.

[8.] Jao, C. Y.; Roth, M.; Welti, R.; Salic, A. EuChemS, *ChemBioChem*, Volume *16* (3), pp. 472-476, January 13 2015. https://doi.org/10.1002/cbic.201402149.

[9.] Jiang, X.; Stockwell, B. R.; Conrad, M. *Nat Rev Mol Cell Biol* **2021**, *22* (4), 266–282. https://doi.org/10.1038/s41580-020-00324-8.