Literature Report 5

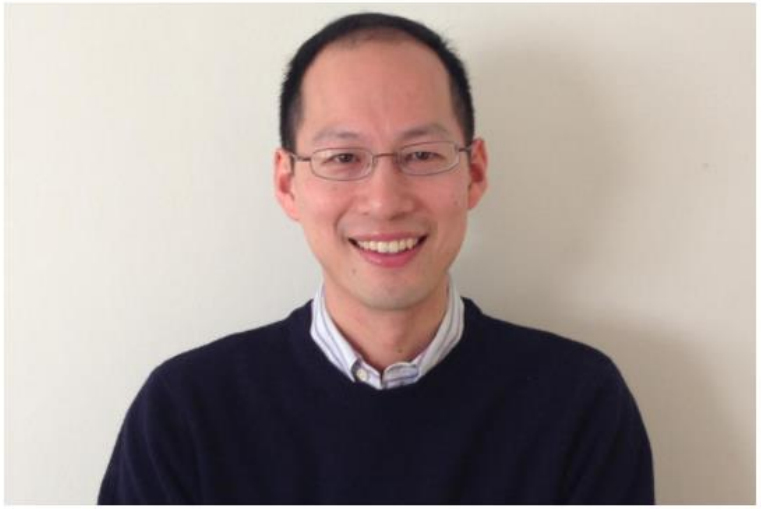
Fang Xiangning

2021.07.01

PNAS Proceedings of the National Academy of Sciences of the United States of America

A tandem activity-based sensing and labeling strategy enables imaging of transcellular hydrogen peroxide signaling

Hidefumi Iwashita[a], Erika Castillo[b], Marco S. Messina[a], Raymond A. Swanson[b,c], and Christopher J. Chang[a,d,e,1]

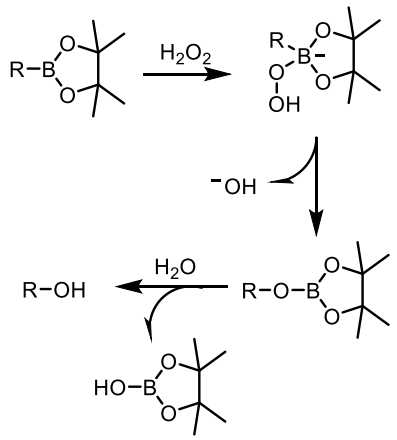


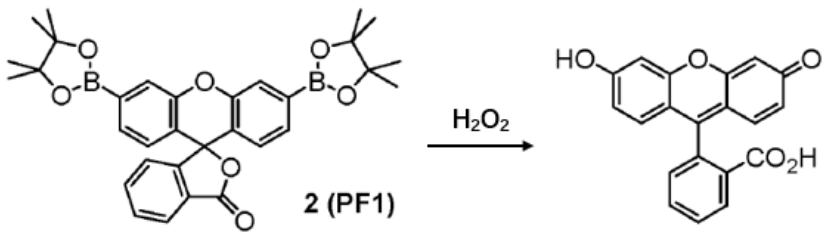
B.S./M.S. California Institute of Technology (1997)
Ph.D. Massachusetts Institute of Technology (2002)

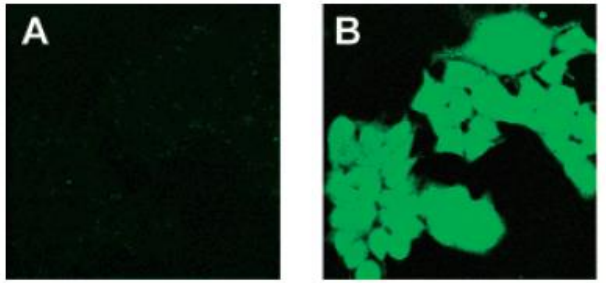
University of California, Berkeley
Class of 1942 Chair
Professor of Chemistry, Professor of Molecular and Cell Biology
Member, Helen Wills Neuroscience Institute
Adjunct Professor, UCSF

Research interest
Activity-Based Sensing: Redox and One-Carbon Signaling.
Transition Metal Signaling: Metalloallostery in the Brain and Beyond.
Artificial Photosynthesis: Catalyzing Sustainable Electrosynthesis.

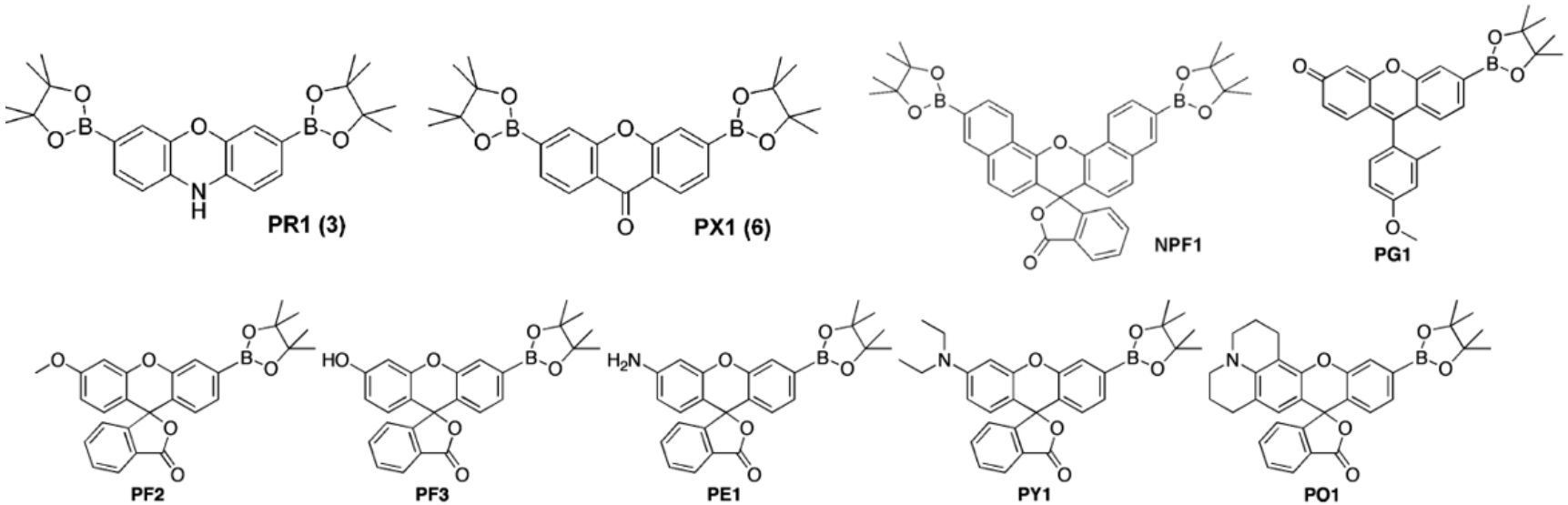
Background







J. Am. Chem. Soc., 2004, 15392

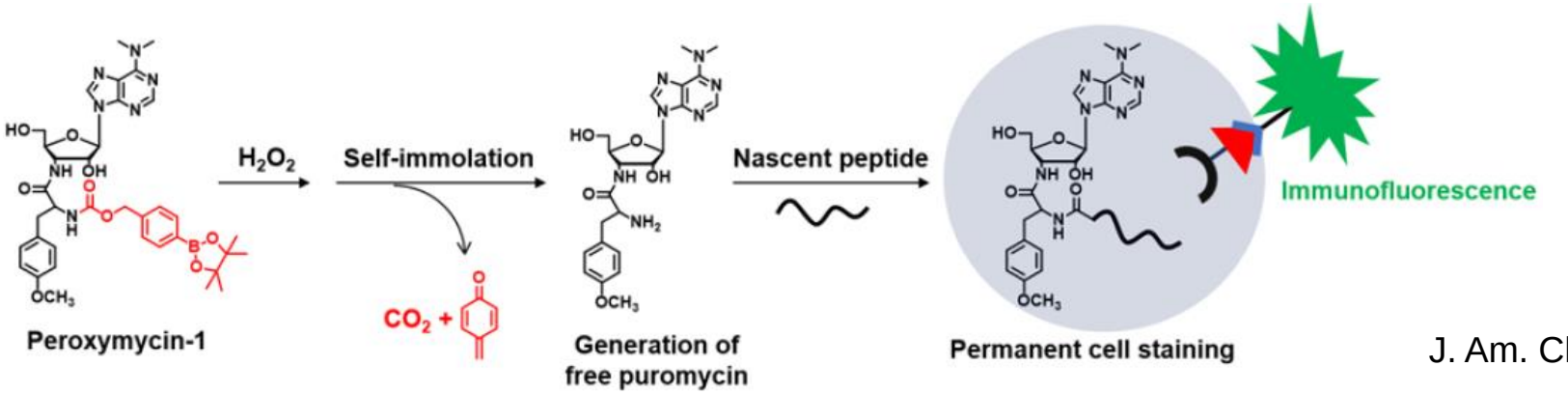


J. Am. Chem. Soc, 2005, 16652
Bioorg. Med. Chem. Lett. 2008, 5948
J. Am. Chem. Soc, 2010, 5907

无法对H_2O_2定位

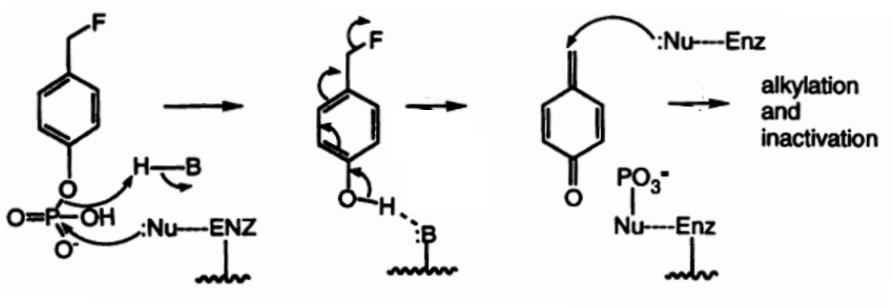
Background

免疫荧光标记H_2O_2

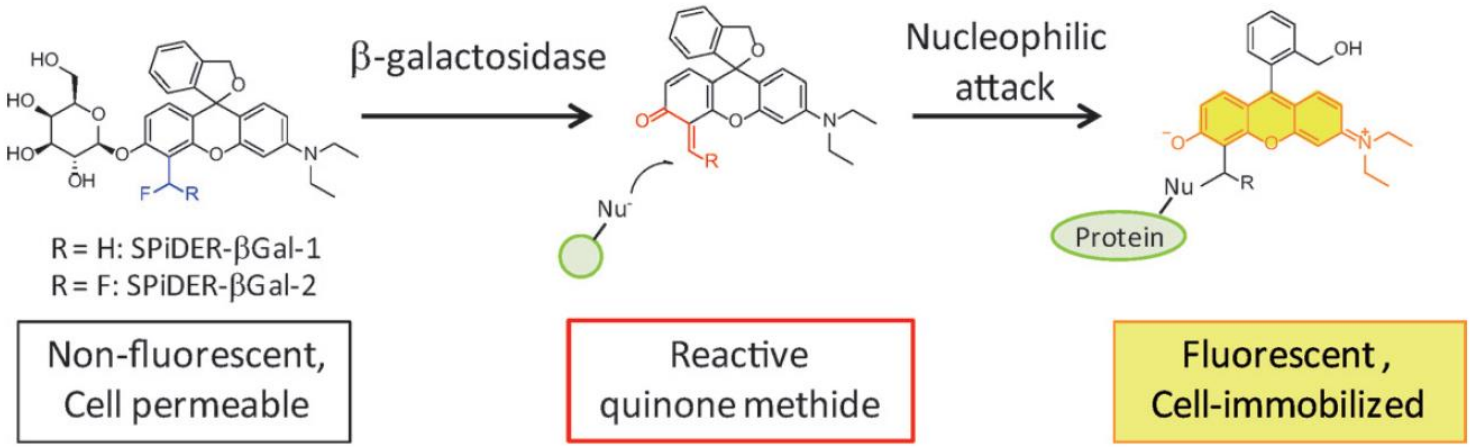


J. Am. Chem. Soc., 2018, 6109

醌甲基化策略

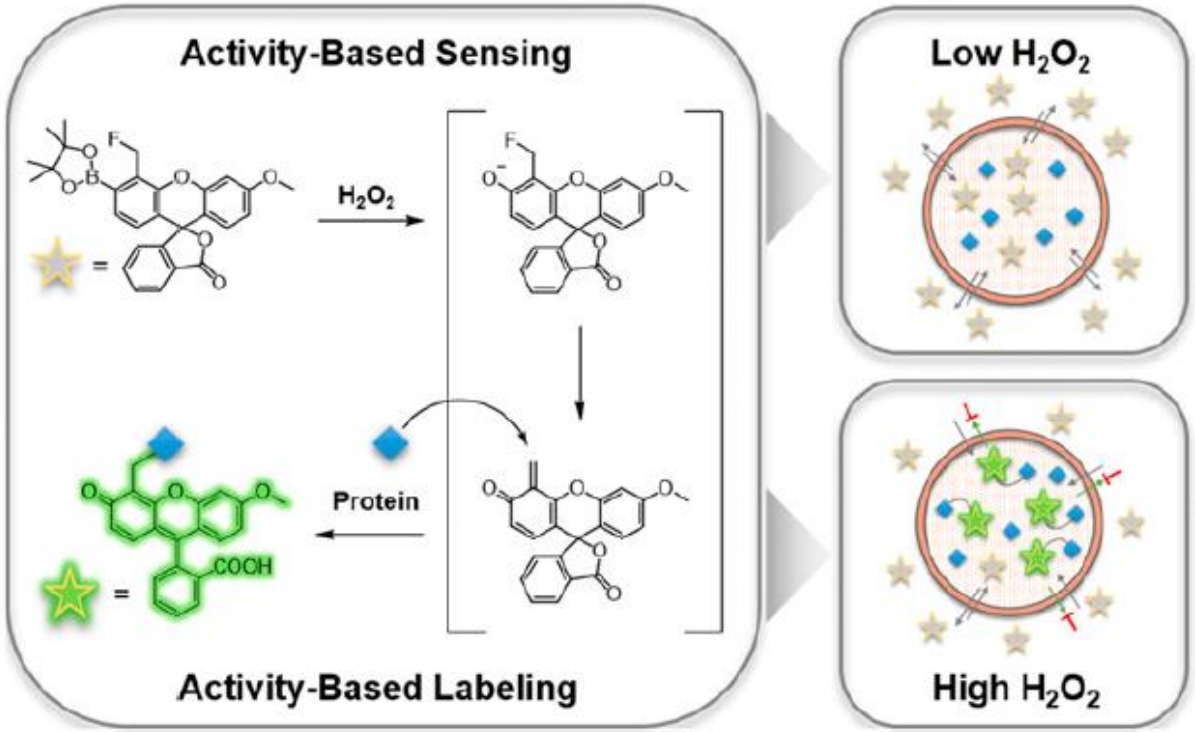


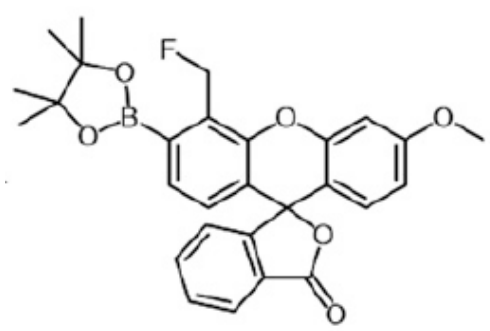
Science, 1993, 1451

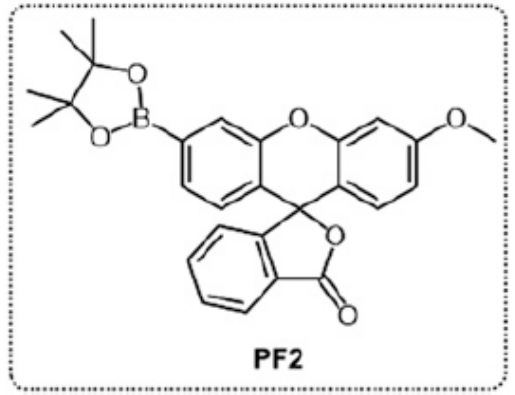


Urano Angew. Chem. Int. Ed. 2016, 9620

Probe design



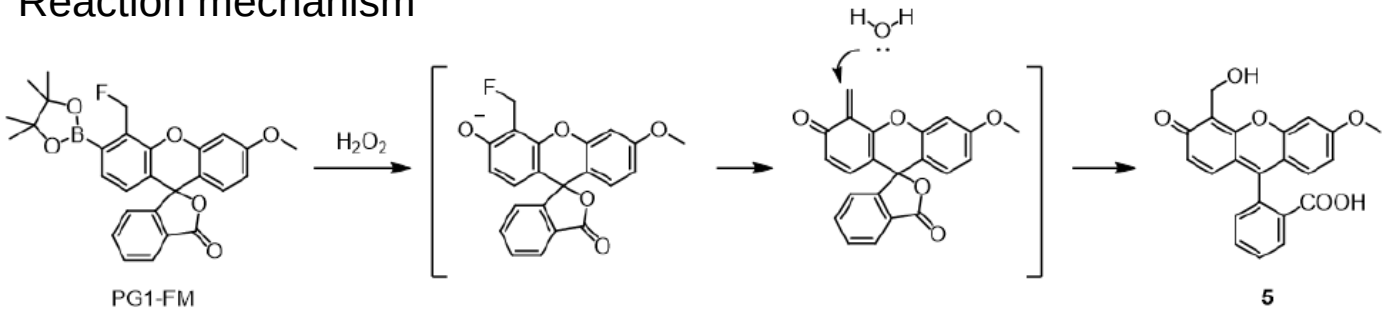

PG1-FM

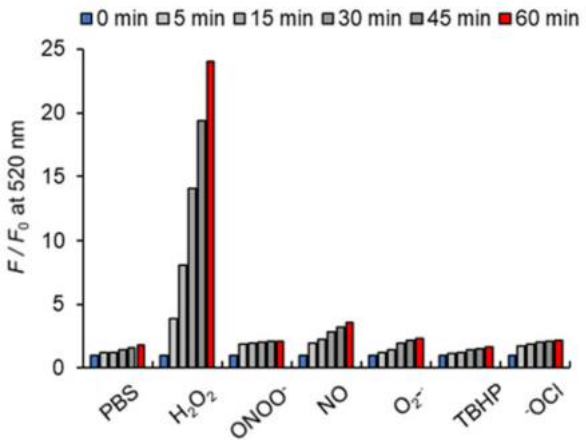

PF2

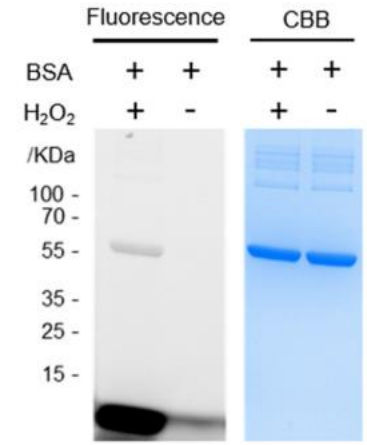
- 监测H_2O_2：硼酸酯与H_2O_2反应
- 定位：氟甲基离去后与蛋白共价偶联

Characterization

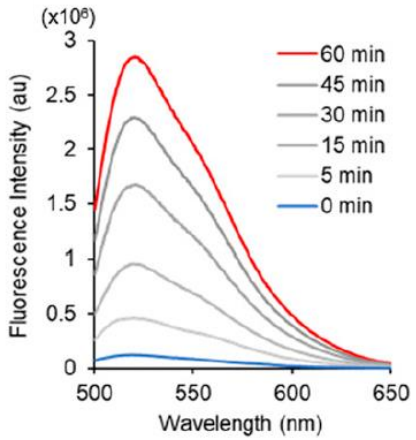
Reaction mechanism

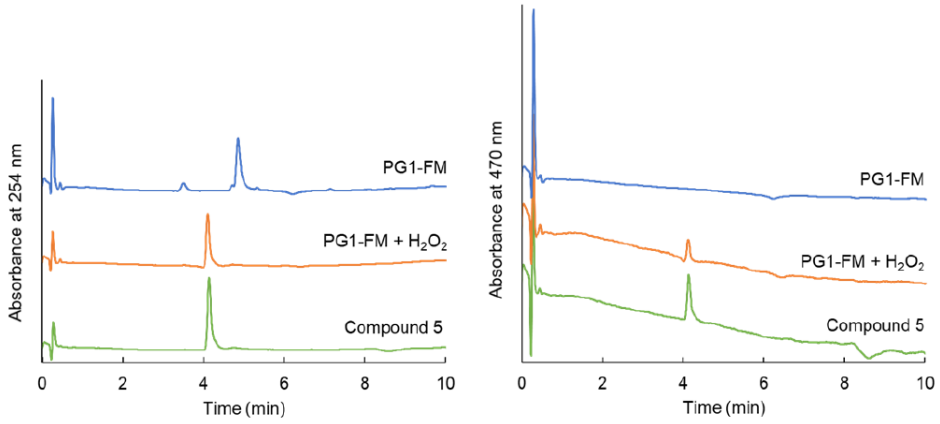






PG1-FM体外响应H_2O_2



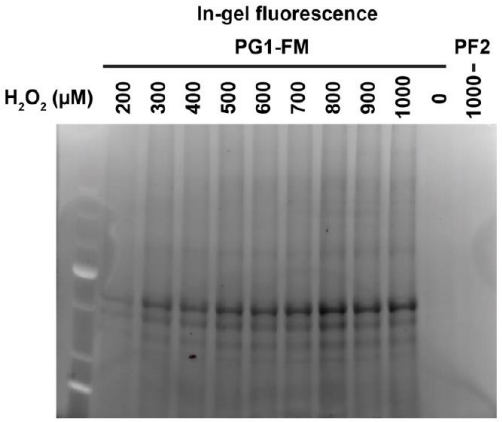


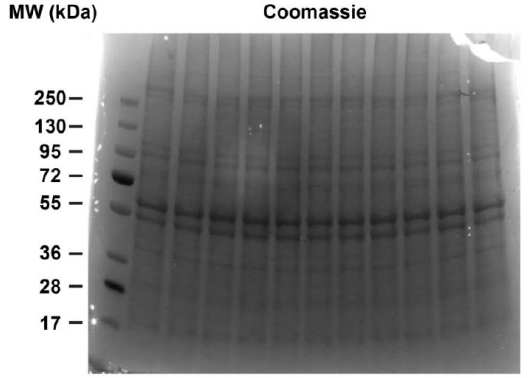
Activity-based sensing fluorescence responses of 1 μM PG1-FM to 25 μM H_2O_2.

HPLC analyses of the reaction product using PG1-FM and H_2O_2.

全细胞裂解物SDS-PAGE

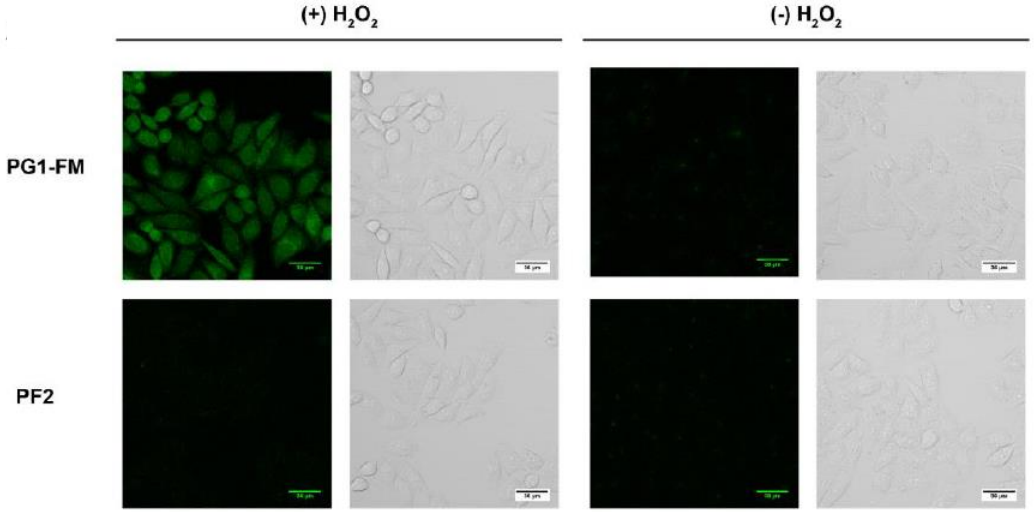
PG1-FM以非选择性方式与蛋白共价偶联



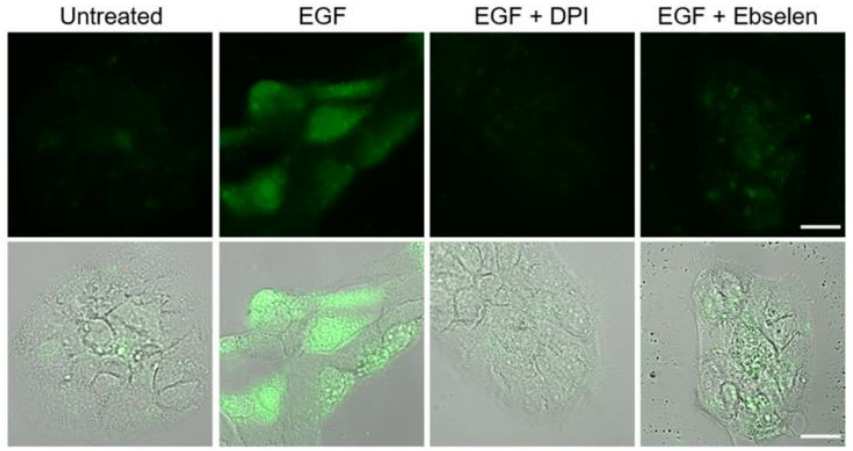


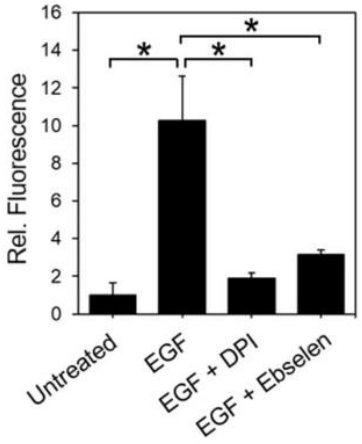
PG1-FM监测细胞中的H_2O_2信号

外源H_2O_2处理活细胞激活PG1-FM



PG1-FM监测内源性H_2O_2产生（A431 cell）



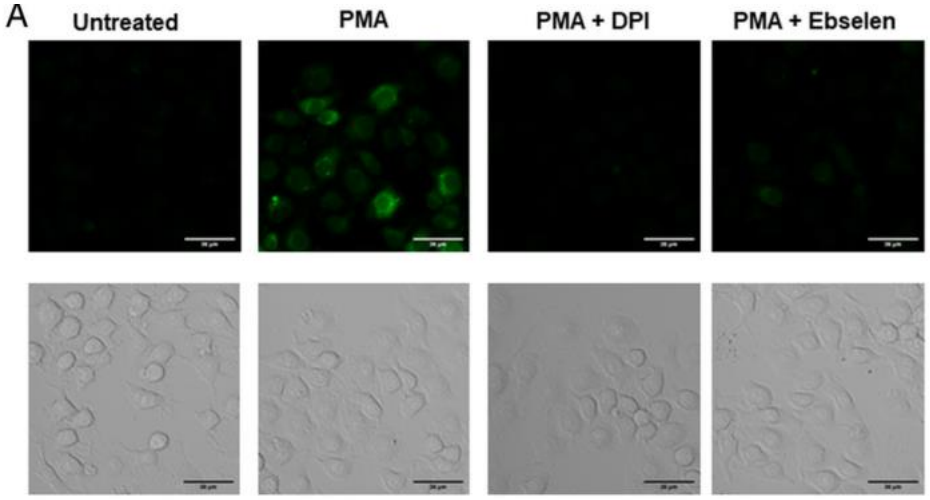


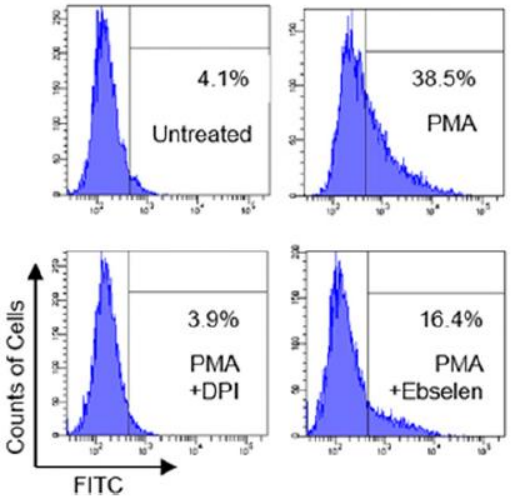
EGF
诱导细胞产生H_2O_2

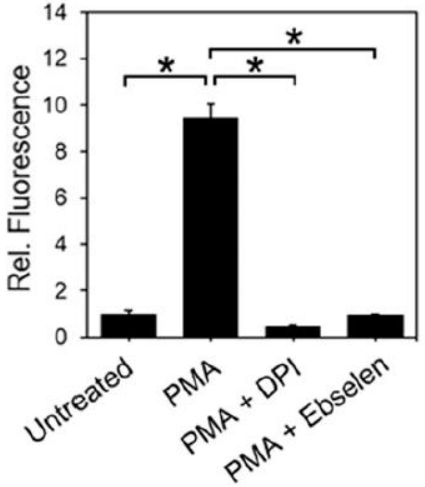
DPI
广泛的氮氧化物抑制剂

Ebselen
H_2O_2的淬灭剂

PG1-FM监测内源性H_2O_2产生（RAW 264.7巨噬细胞）



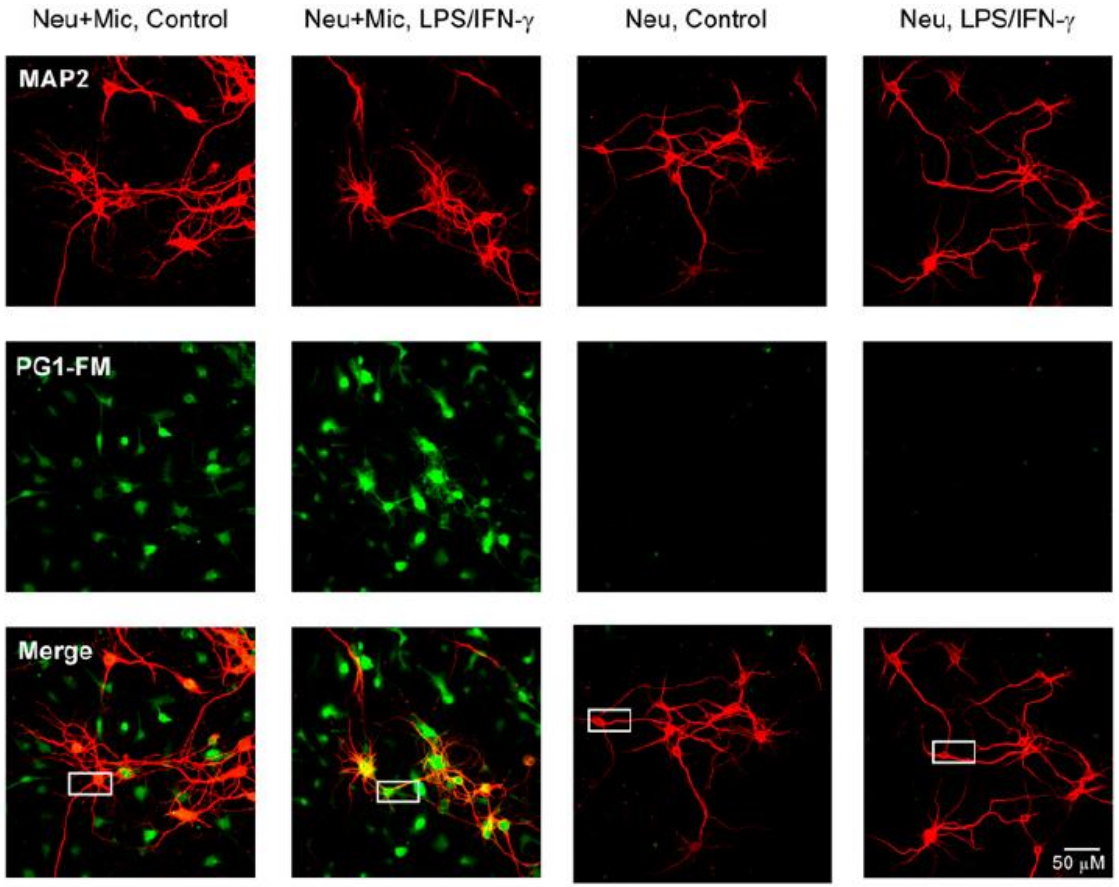


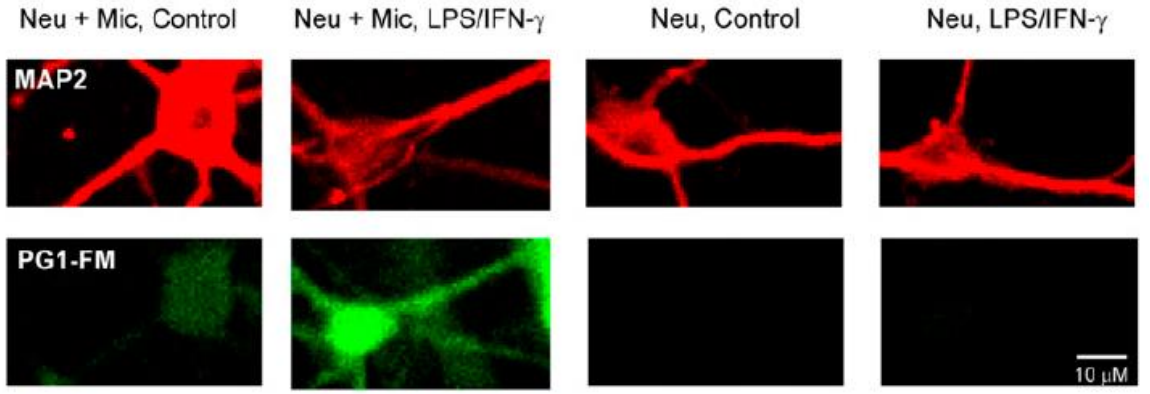


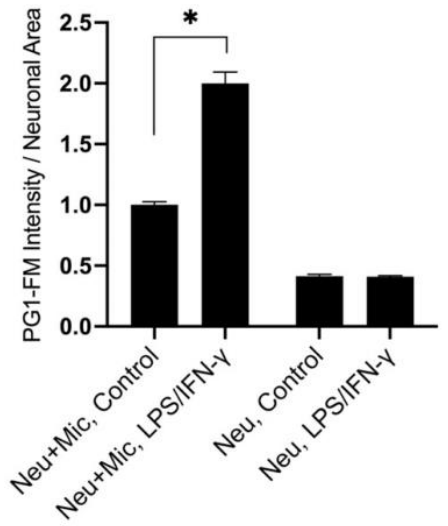
PG1-FM可视化跨细胞H_2O_2通信

小胶质细胞（Mic）- 神经元（Neu）共培养模型：模拟不同疾病过程中由小胶质细胞激活介导的神经元损伤

小胶质细胞：可以被脂多糖（LPS）和干扰素γ（IFN-γ） 刺激产生H_2O_2；神经元细胞对LPS和 IFN-γ无响应



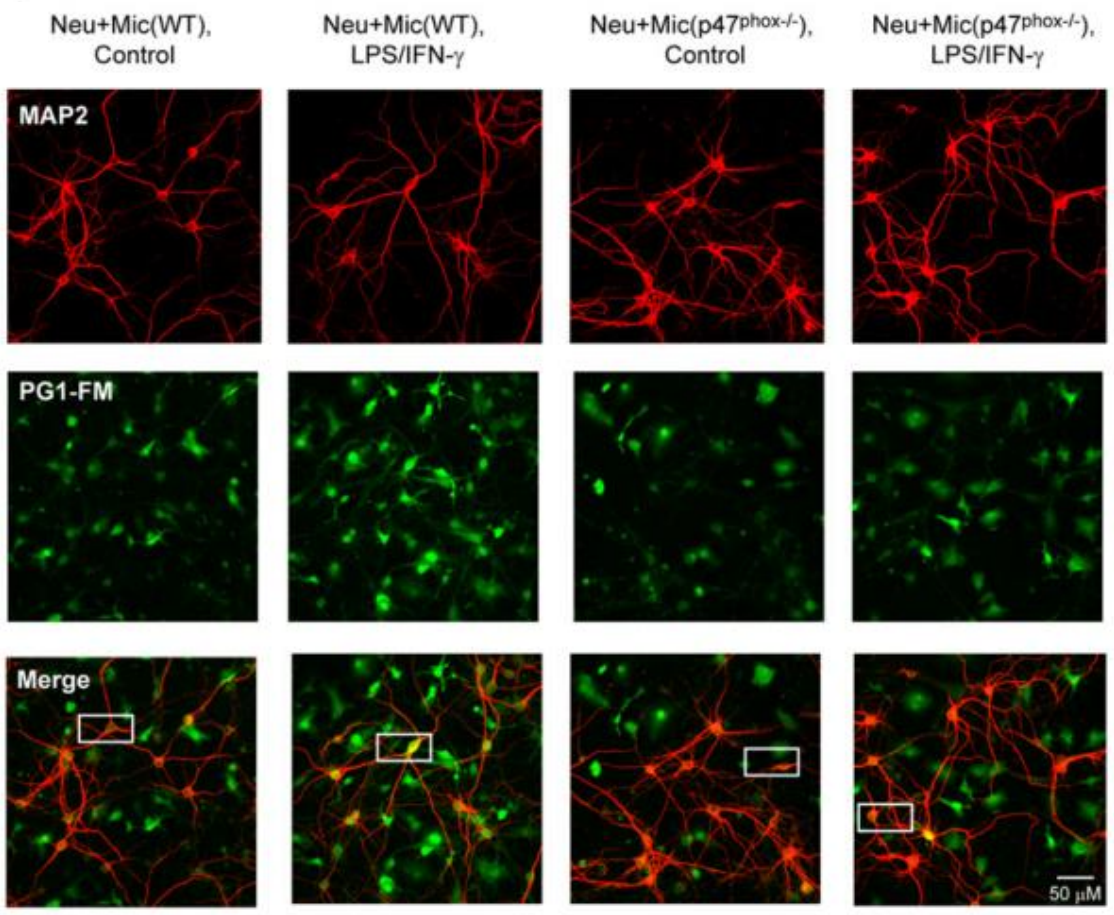


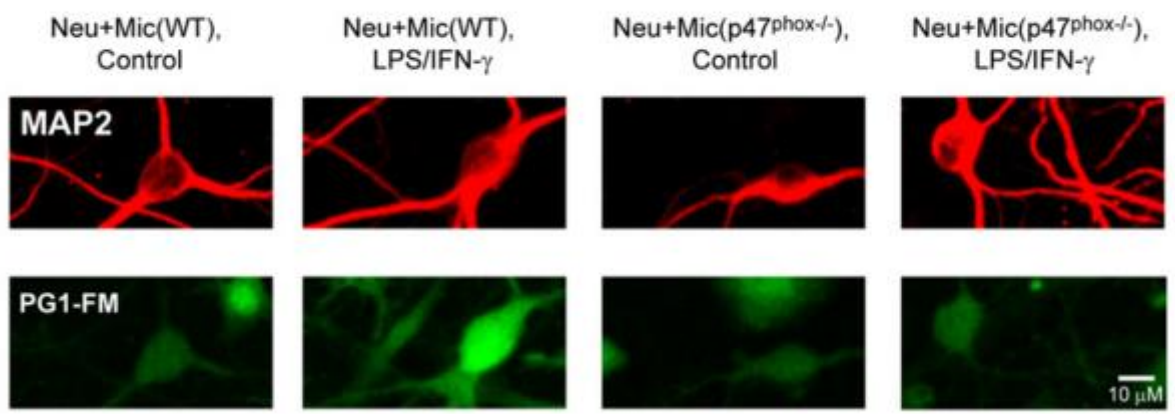


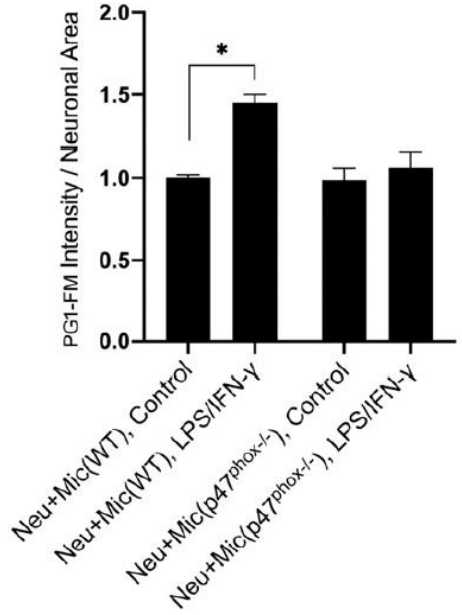
PG1-FM可视化跨细胞H_2O_2通信

p47^{phox}： 吞噬细胞氧化物酶

p47^{phox}基因敲除的小胶质细胞不会受到（LPS）和干扰素γ（IFN-γ） 刺激产生H_2O_2







Summary

- PG1-FM通过硼酸酯选择性与H_2O_2反应，氟甲基的离去导致与蛋白的共价偶联和荧光恢复。
- PG1-FM可在细胞中捕获和记录局部H_2O_2通量。
- PG1-FM可观察细胞间的H_2O_2传导。
- 蛋白共价标记不可逆，可以监测刺激前后H2O2的相对水平，但是无法监测H_2O_2动态循环。