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The Use of Boronate Probes for the Detection of Reactive Oxygen and Nitrogen Species

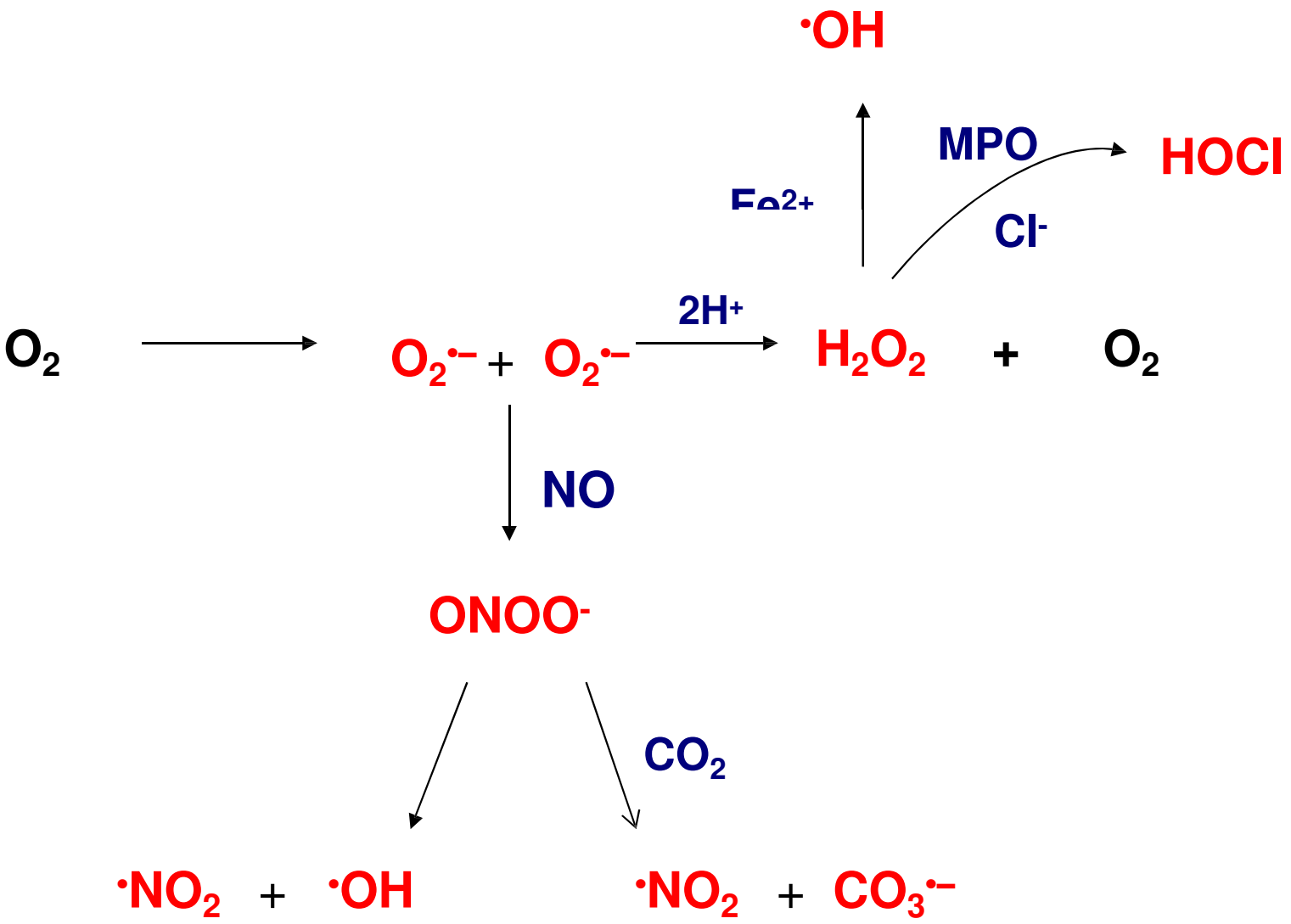
Adam Sikora, Ph.D.

Institute of Applied Radiation Chemistry
Lodz University of Technology

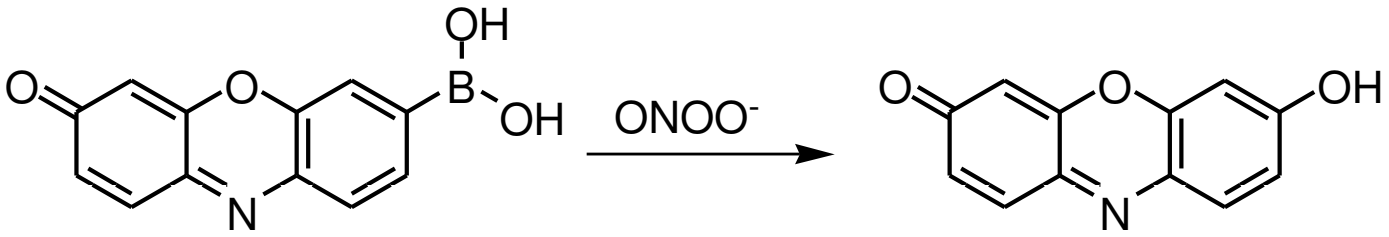
3rd International Conference and Exhibition on
Biosensors & Bioelectronics
August 11-13, 2014 San Antonio, USA

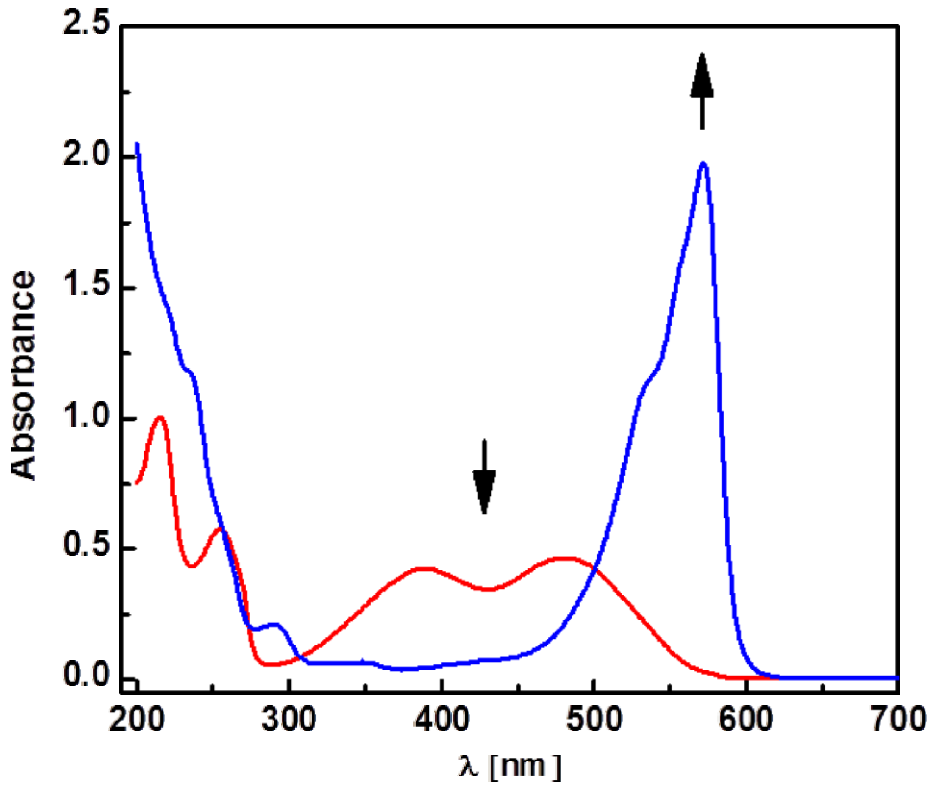
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Reactive Oxygen and Nitrogen Species (ROS/RNS)



Reaction of boronates with ROS & RNS

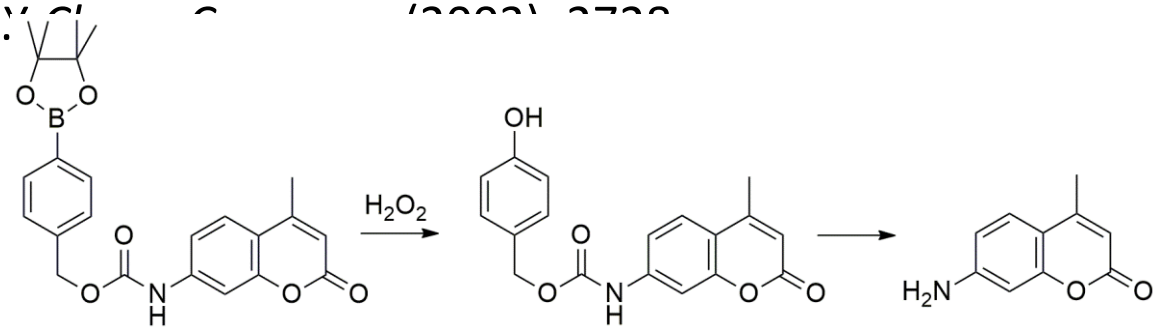




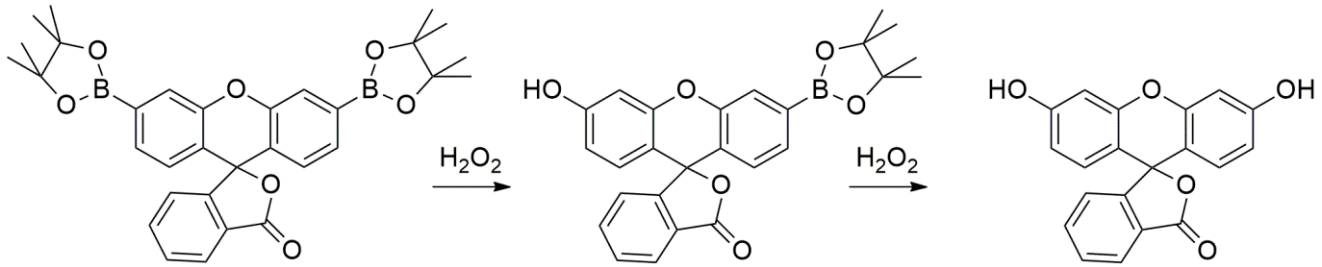
Boronate compounds as probes for H_2O_2

In the middle of the last decade of the 20th century a new class of fluorescent probes containing the boronate structure has been developed for selective detection of H_2O_2 .

1. Lo L.C., Chu C.Y. [illegible]

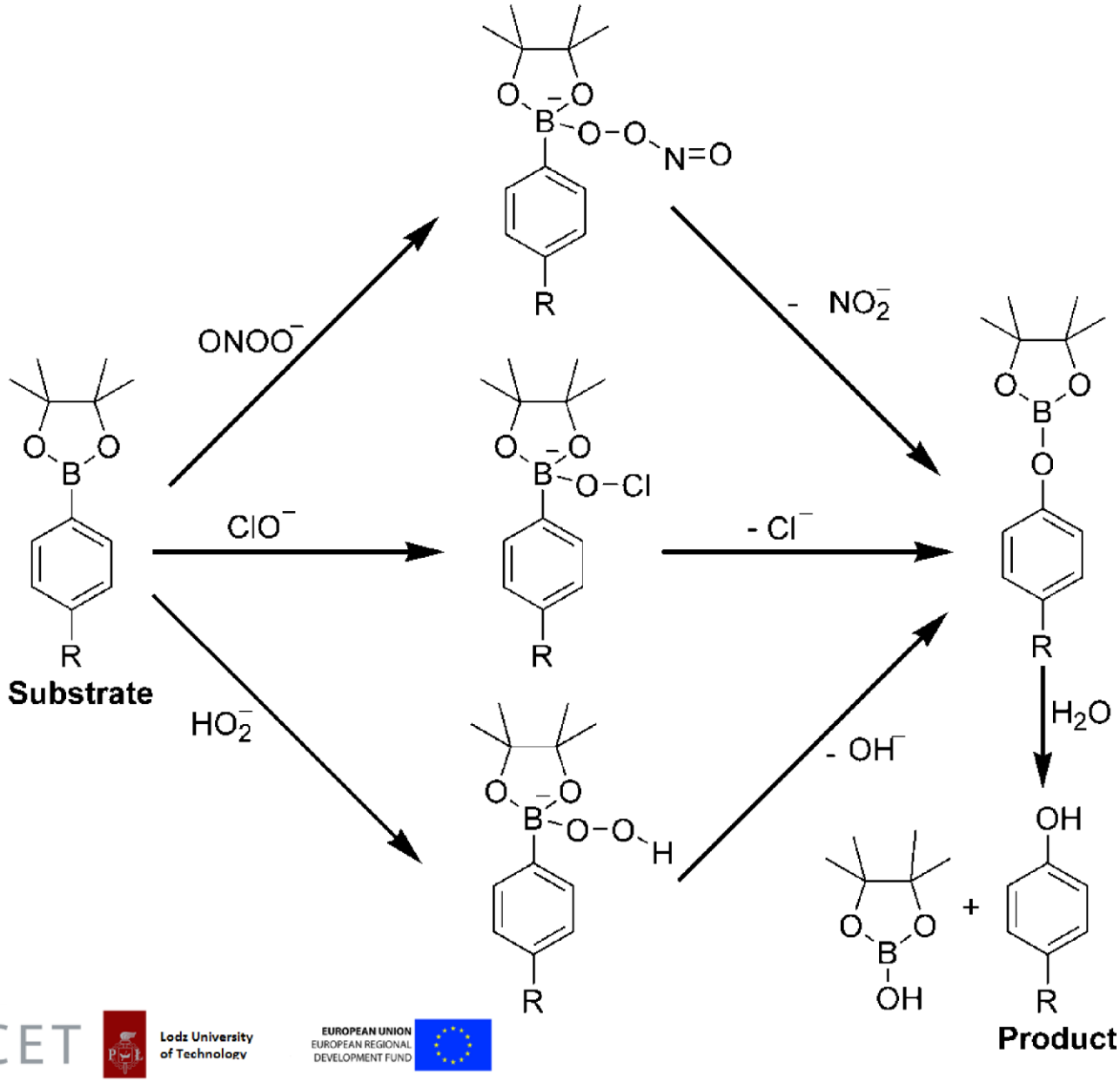


2. Chang M.C., Pralle A., Isacoff E.Y., Chang C.J. *J. Am. Chem. Soc.* (2004), **126**, 15392

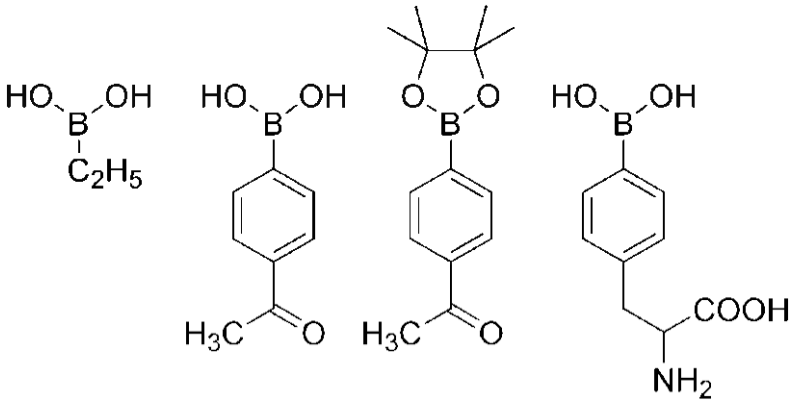


The mechanism of boronates oxidation

Sikora A, Zielonka J. *et al. FRBM* (2009), **47**, 1401



Reactions with H_2O_2, ClO^- and $ONOO^-$



EtBA PBA PBE FBA

Sikora A, Zielonka J. *et al. FRBM* (2009), **47**, 1401

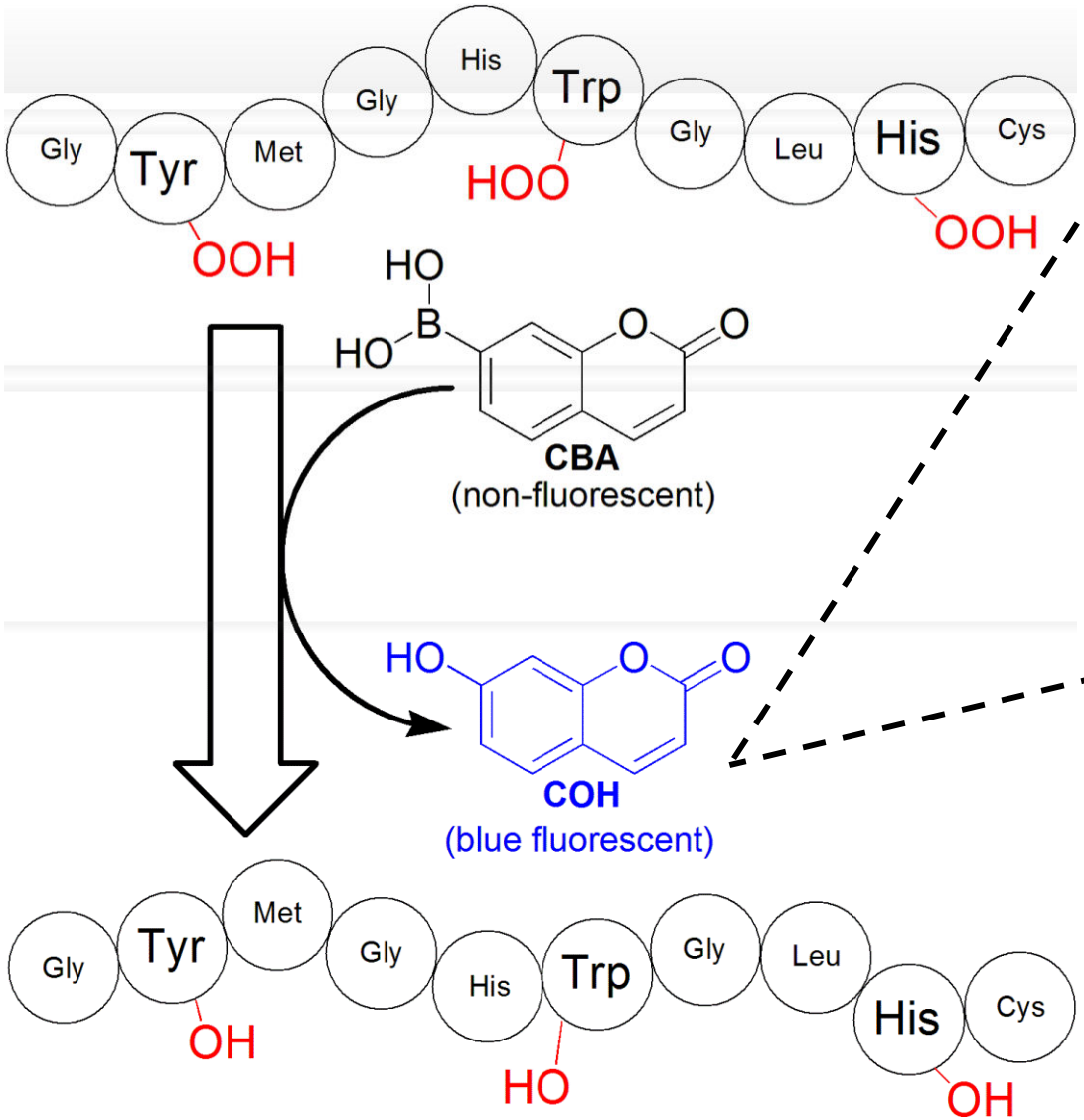
Boronate	Rate constant, k [$M^{-1}s^{-1}$] (pH = 7.4)		
	Peroxynitrite	Hypochlorite	Hydrogen peroxide
EtBA	$2.8 \cdot 10^5$	$1.2 \cdot 10^4$	-
PBA	$1.6 \cdot 10^6$	$6.2 \cdot 10^3$	2.2
FBA	$1.4 \cdot 10^6$	$2.7 \cdot 10^4$	2.5

Real-time Measurements of Amino Acid and Protein Hydroperoxides Using Coumarin Boronic Acid*

Received for publication, January 27, 2014, and in revised form, May 27, 2014 Published, JBC Papers in Press, June 13, 2014, DOI 10.1074/jbc.M114.553727

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From the [‡]Department of Biophysics and Free Radical Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin 53226 and the [§]Institute of Applied Radiation Chemistry, Lodz University of Technology, Zeromskiego 116, 90-924 Lodz, Poland



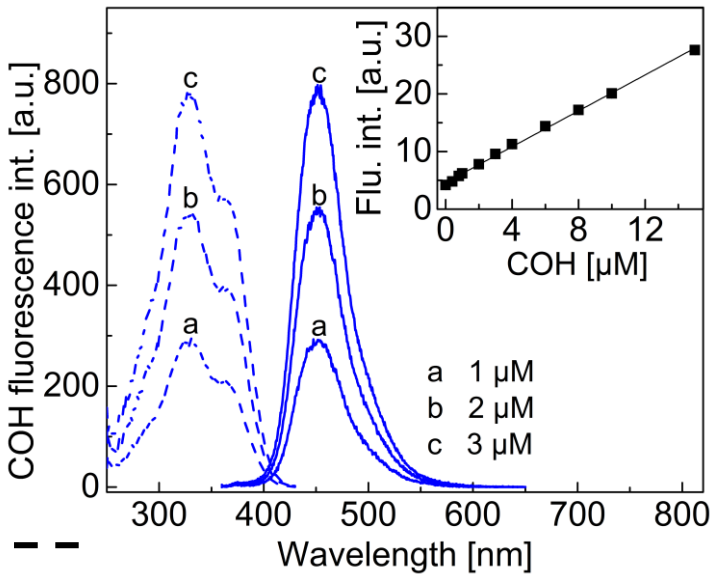
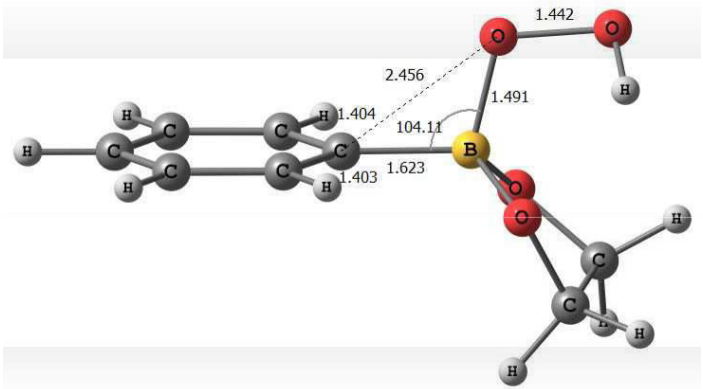


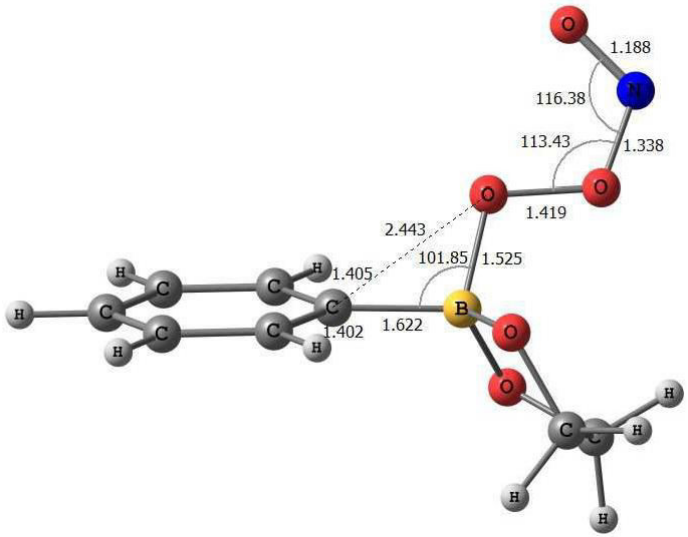
Table: Rate constants of the reactions of amino acid hydroperoxides with CBA.

Hydroperoxide	$k_{ROOH+CBA}$ [$M^{-1}s^{-1}$]
TyrOOH	23.4 ± 0.5
TrpOOH	11.4 ± 0.2
HisOOH	23.3 ± 0.7
H_2O_2	1.5± 0.2

Transition states

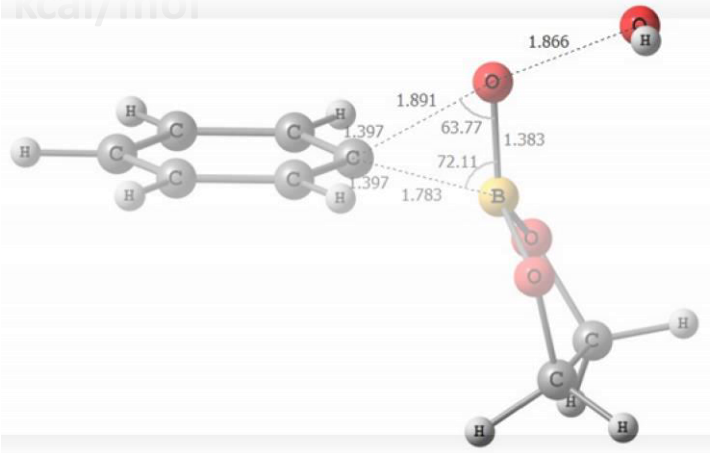
Adducts

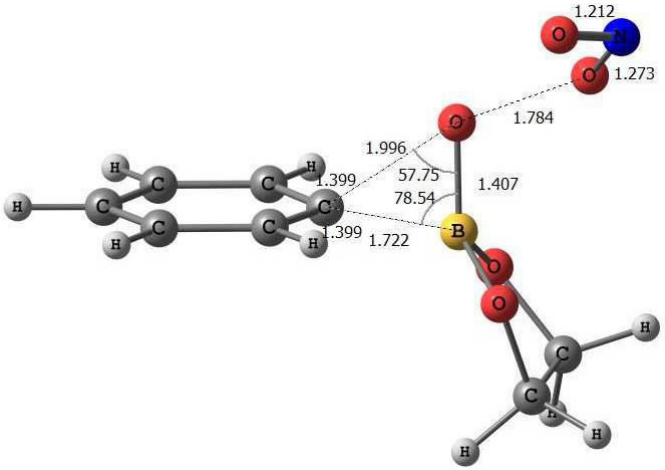




Transition states

E_a = 32.9 kcal/mol



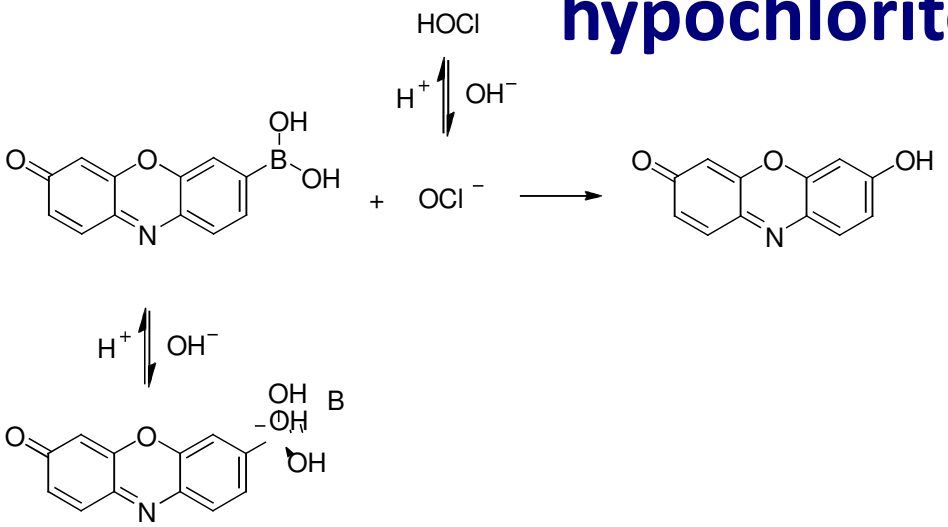


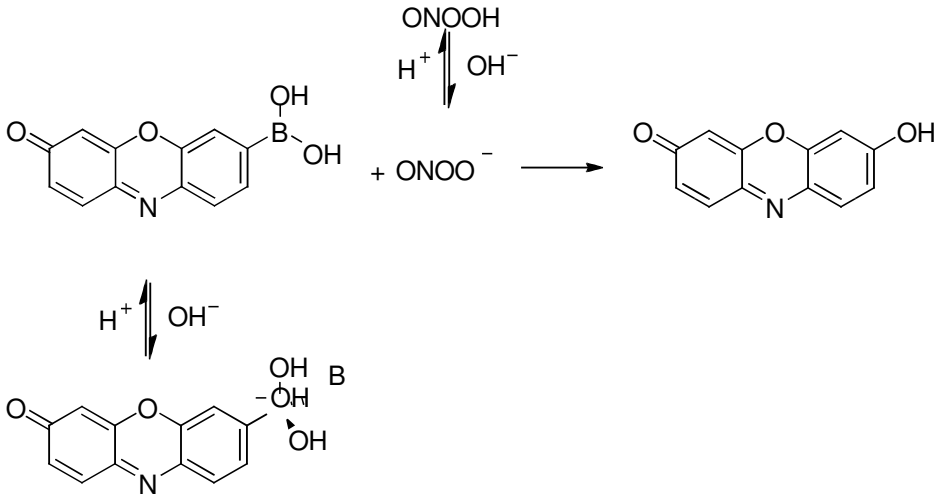
Oxidation of boronates by ONOO$^-$ and HOO$^-$ occurs with simultaneous cleavage of O-O and C-B bonds, and formation of C-O bond

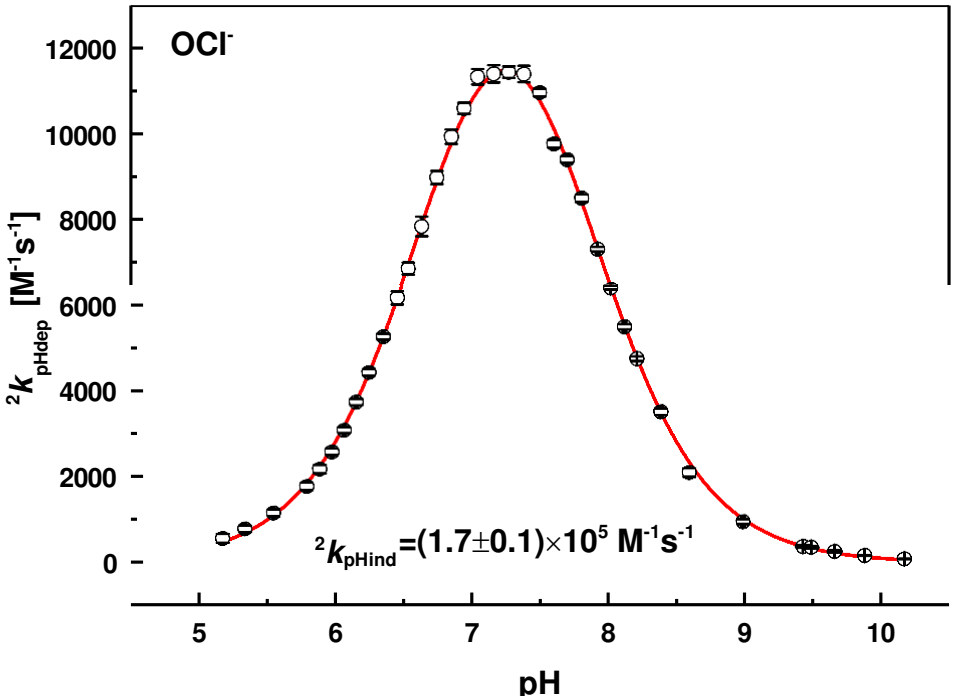
PCM/M06/6-31+G(d,p)

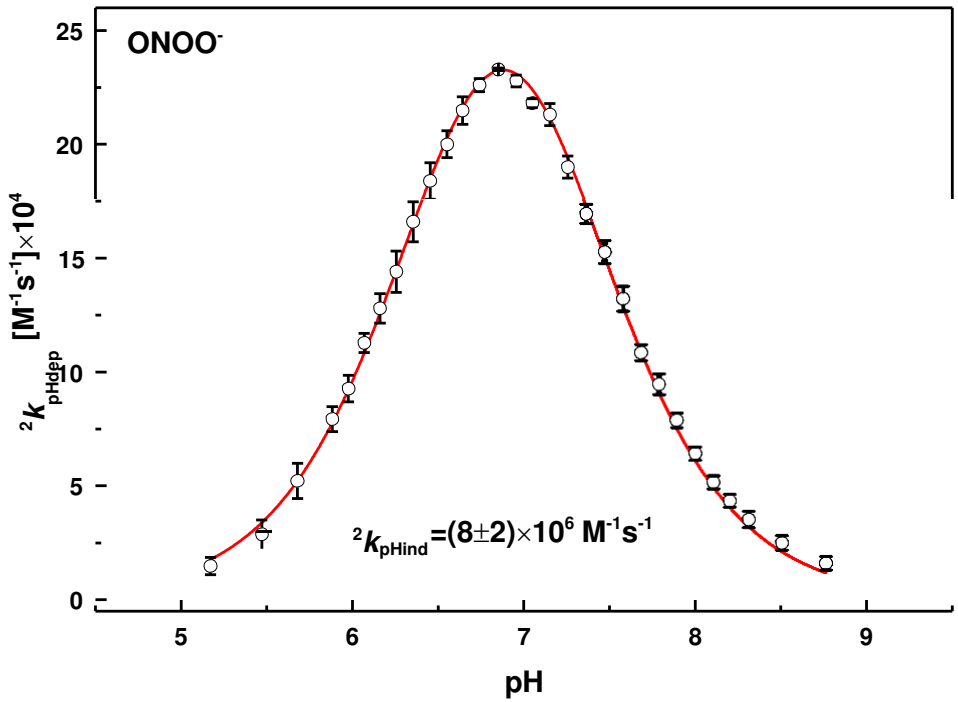
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pH-dependent reactivity of PC1 towards hypochlorite and peroxynitrite

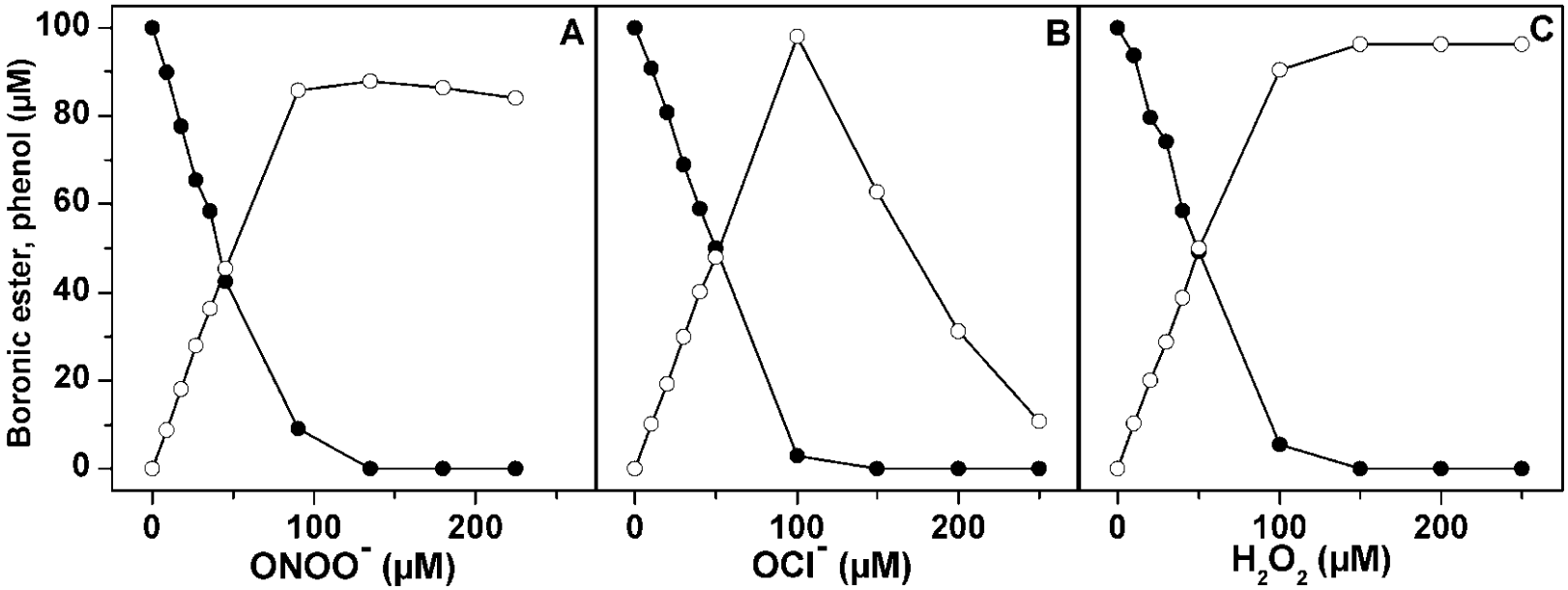








Stoichiometry of the reactions



Sikora A, Zielonka J. *et al. FRBM* (2009), **47**, 1401

Boronate consumption yield (for all oxidants) ~ 100%

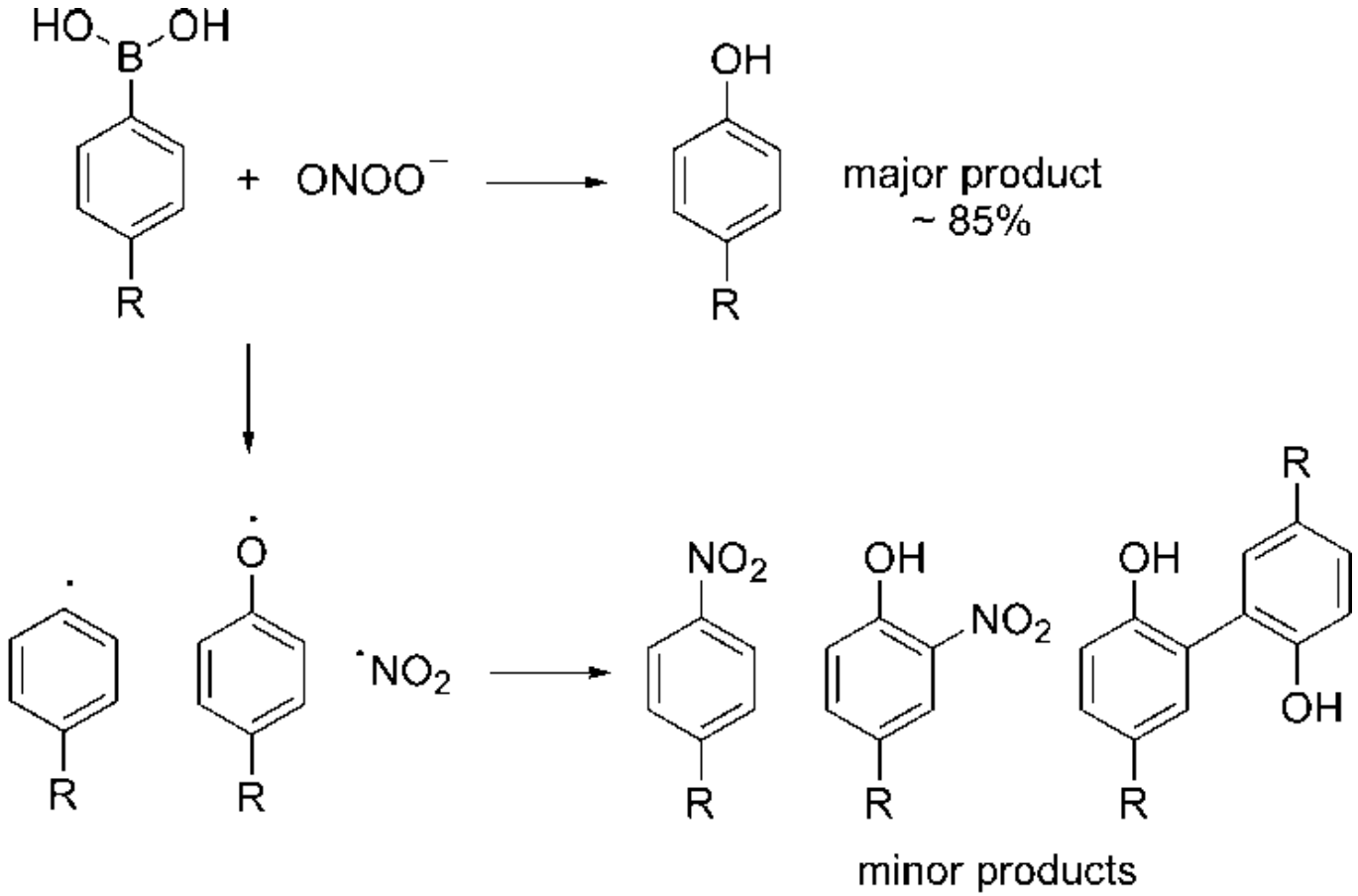
Phenol production yield:

H_2O_2 and HOCl ~ 100 %

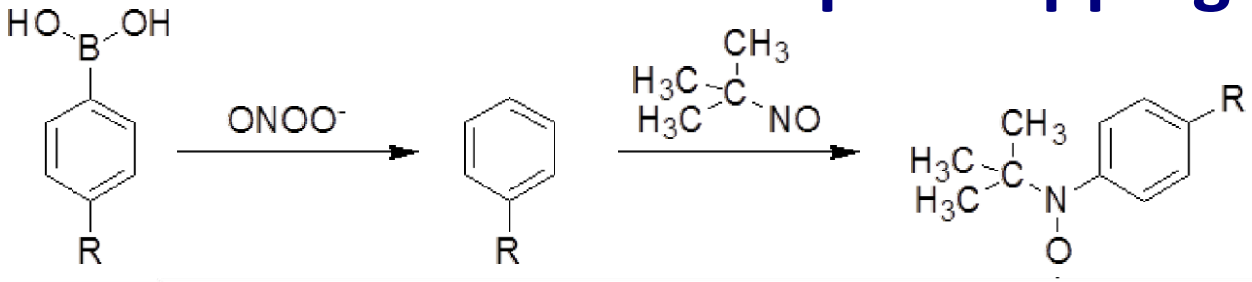
peroxynitrite ~ 85-90 %

Reaction with peroxynitrite - major and minor reaction pathways

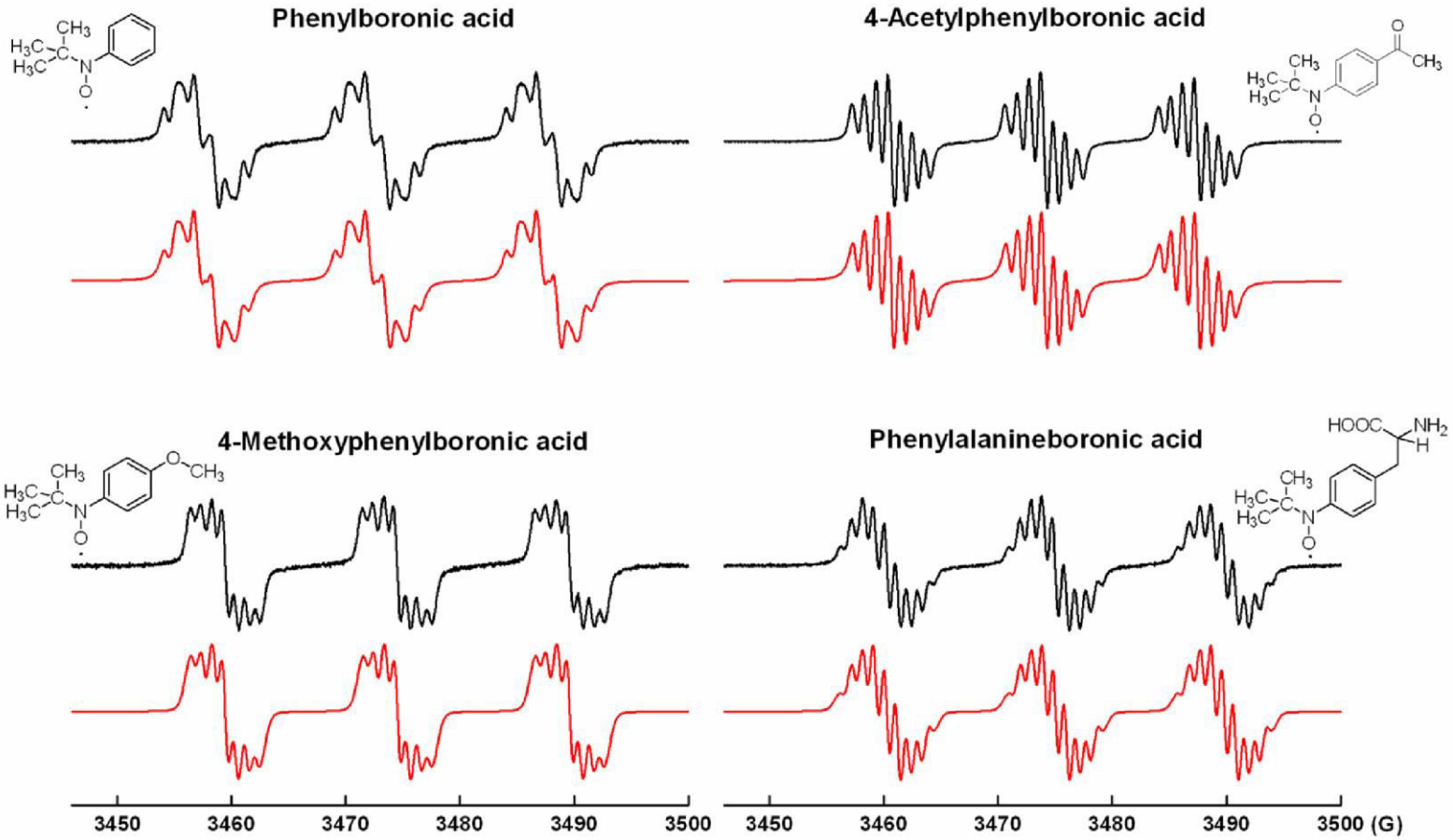
Sikora A, Zielonka J *et al. Chem. Res. Toxicol.* (2011), **24**, 687



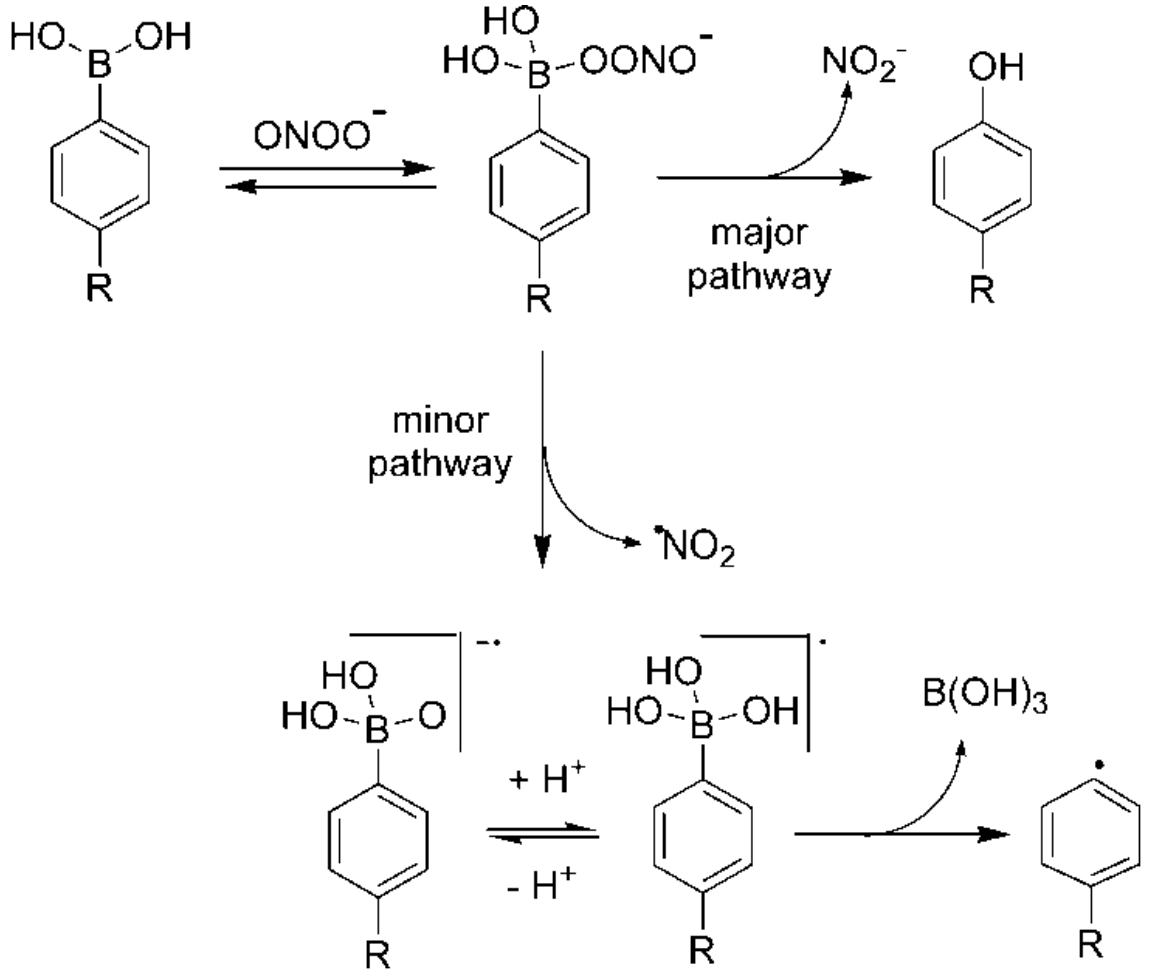
Spin trapping



Sikora A.

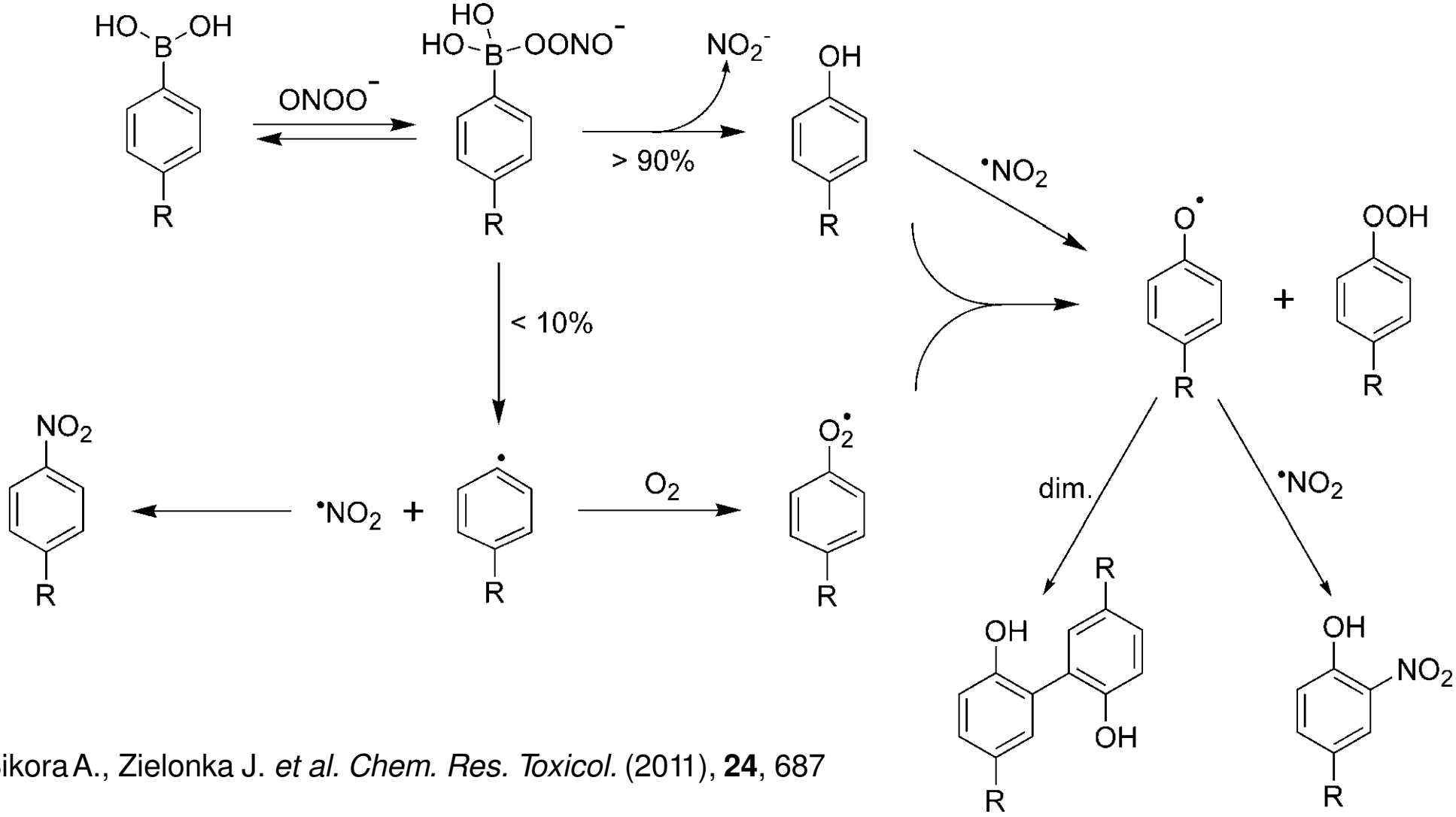


Proposed mechanism of major and minor pathway



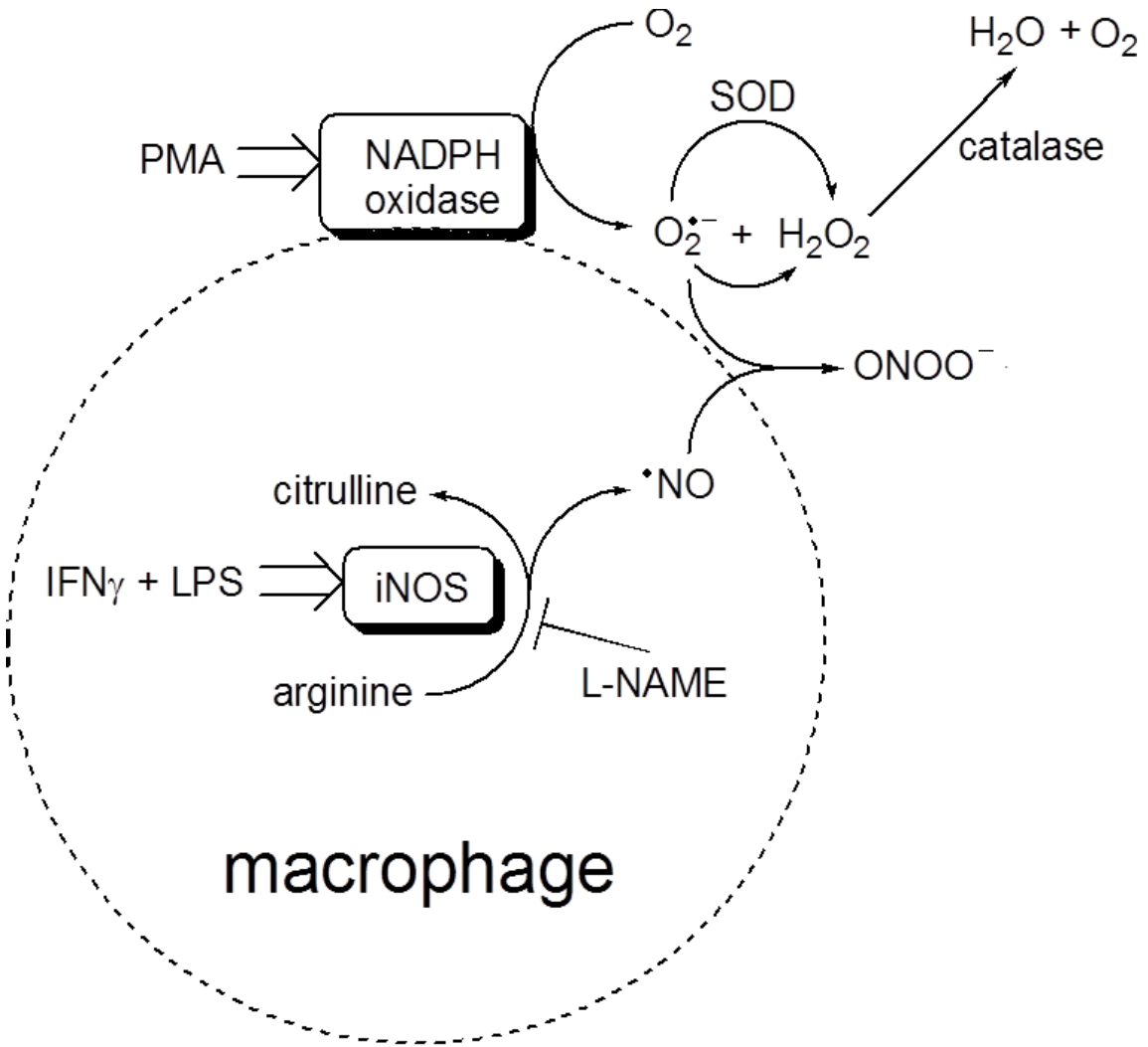
Sikora A., Zielonka J. *et al. Chem. Res. Toxicol.* (2011), **24**, 687

The mechanism of major, and minor products formation



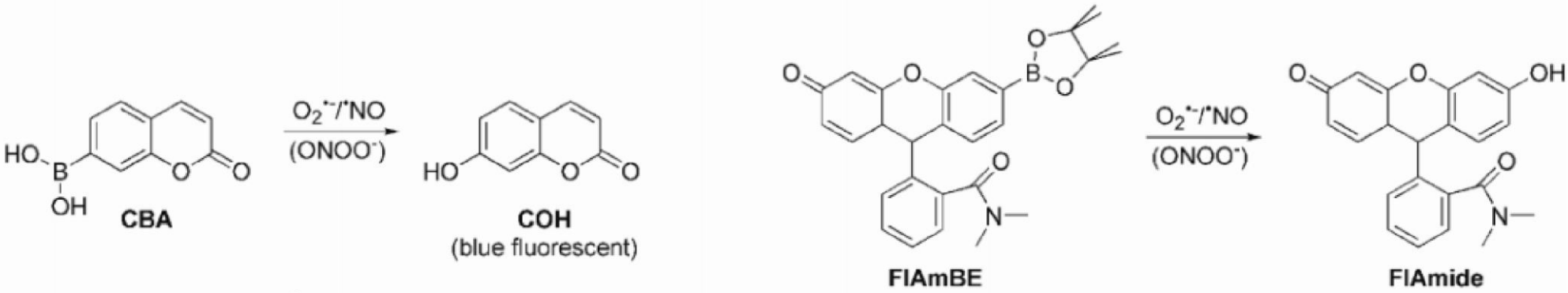
Sikora A., Zielonka J. *et al. Chem. Res. Toxicol.* (2011), **24**, 687

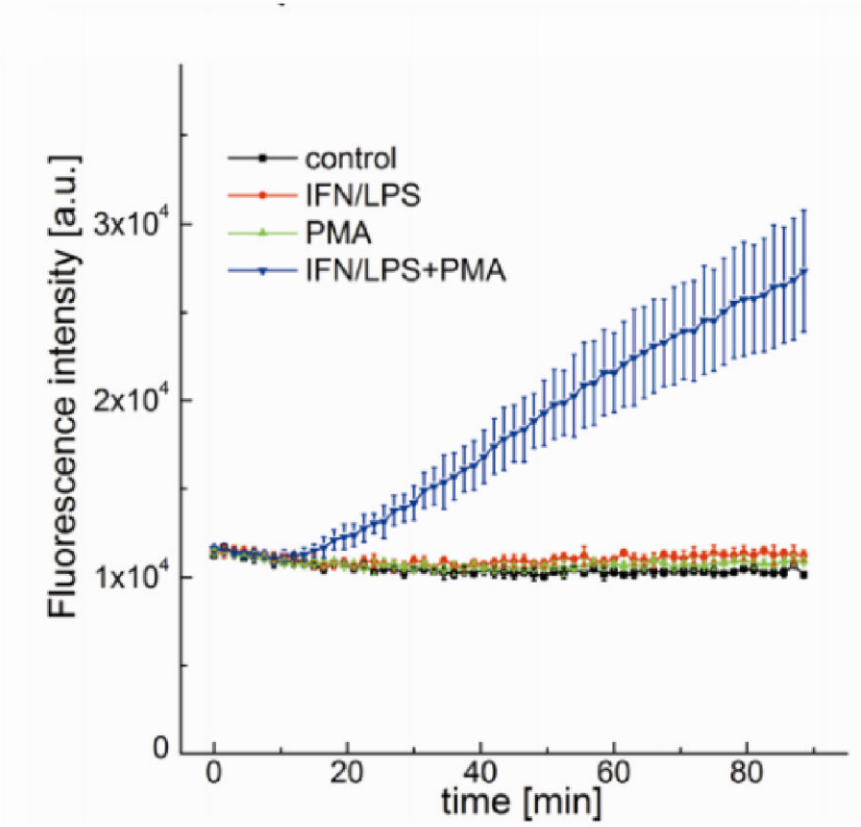
Activation of macrophages RAW 264.7 for the production of ROS/RNS

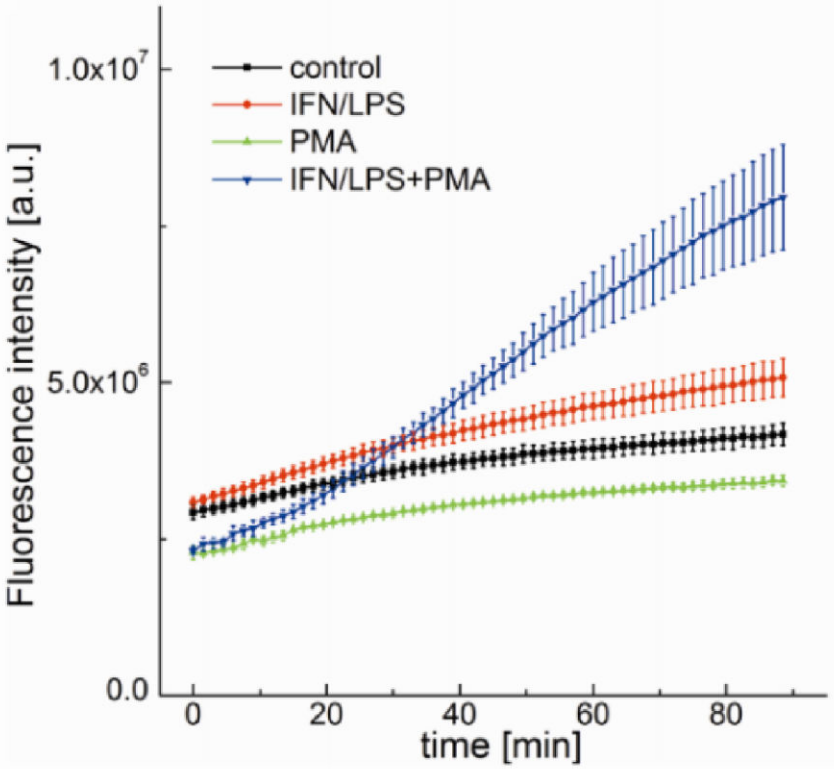


Zielonka J, Zielonka M *et al. J. Biol. Chem.* (2012), **287**, 2984

Oxidation of boronates by activated macrophages



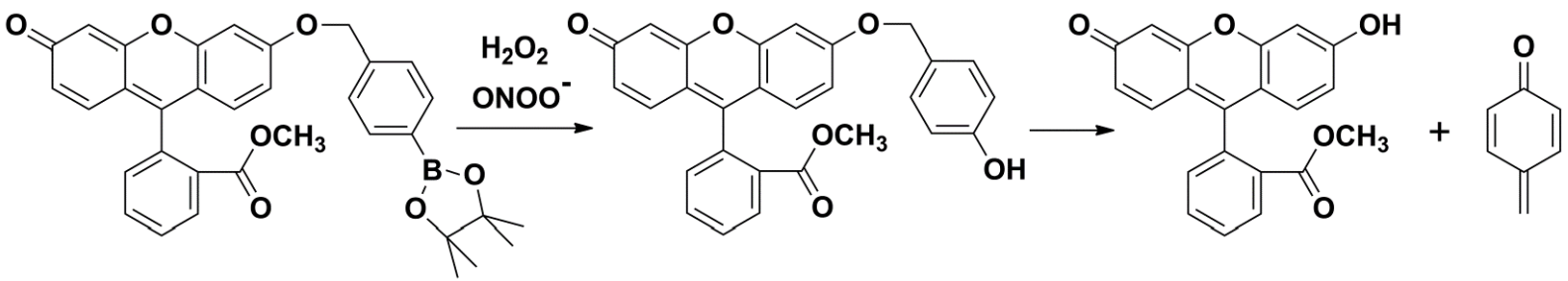


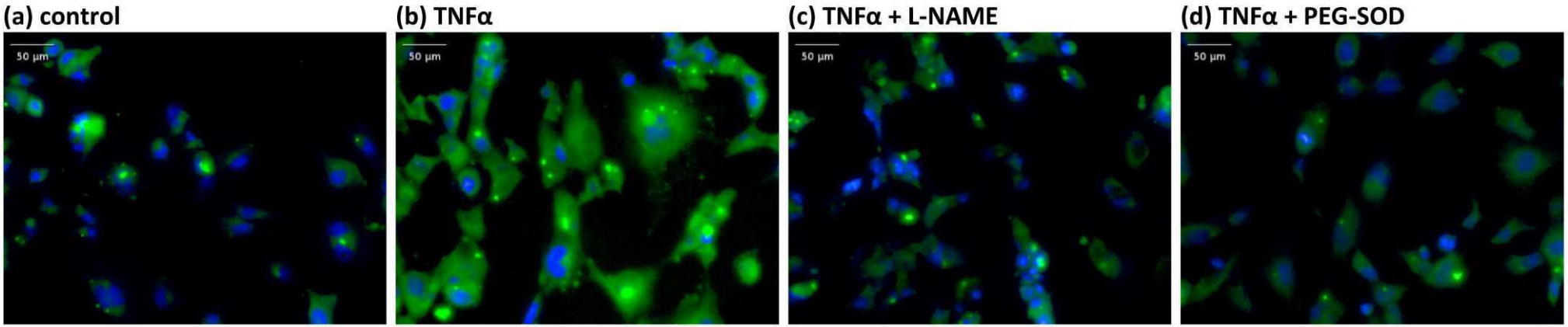


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Oxidation of boronates by endothelial cells

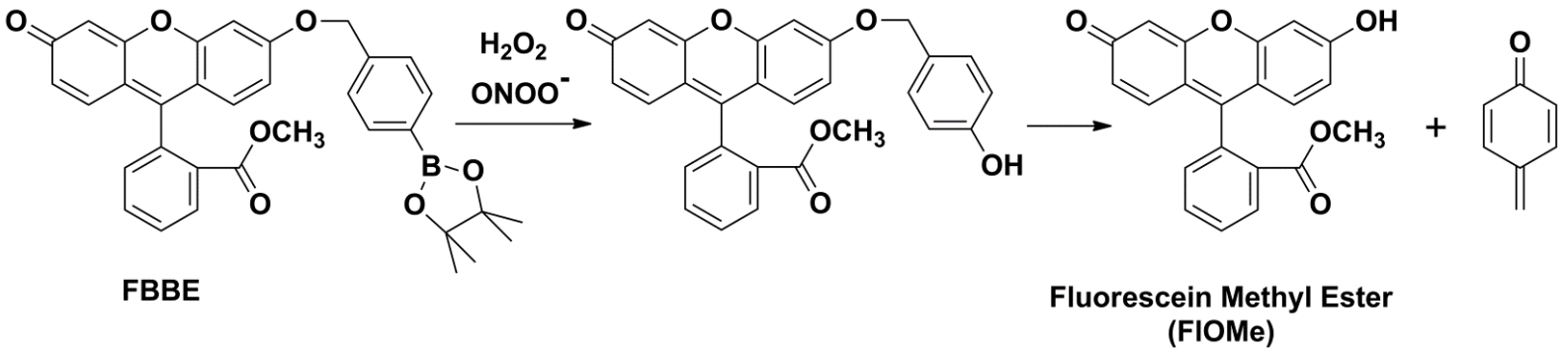




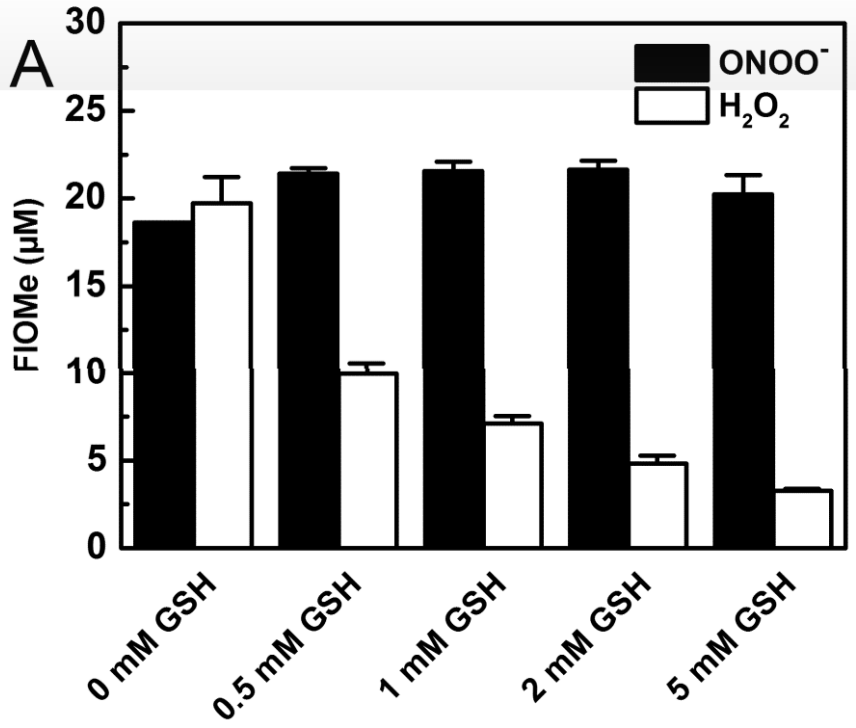
(manuscript in preparation)

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Can Boronates Detect H_2O_2 in Cells?



FBBE 25 μM, oxidant 100 μM, GSH 0-5 mM



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Piotrowski

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Tomasz Wójcik

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