Novel fluorescent probe to detect and quantify specific reactive oxygen species

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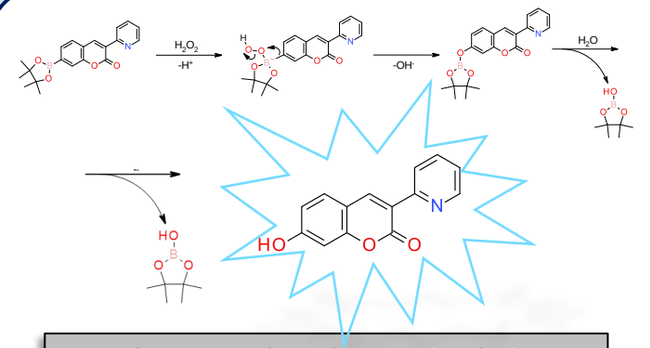
INTRODUCTION

Hydrogen peroxide (H_2O_2) plays important roles in redox signaling and oxidative stress, and its dynamic concentration is critical to human health and diseases. Here we report results for novel fluorescent probe based on pyridine-coumarin core 3-(2-pyridyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)chromen-2-one (P182) for quantitative measurement of H_2O_2. This fluorometric probe displays a fluorescence turn-on response in the process of deprotection of arylboronate to phenol in the presence of H_2O_2. It could offer good performances in terms of sensitivity and response time. Moreover, cytotoxicity studies on various model cell lines proved the non-toxic activity of the tested sensor allowing its use in vivo studies. This study provides research on molecular fluorescence probe for the detection of H_2O_2.

P182 [μM/L]	Trypan blue exclusion assay		LDH assay	
	CHO-K1	SW-620	CHO-K1	SW-620
	Cytotoxicity [% of control]			
0.010	0	0	0	0
0.1	0	0	0	0
1	2	0	3	0
10	13	5	18	8
100	20	9	27	16

Table 1. Cytotoxicity of **P182 complex** after 24 hours of incubation with normal cells (CHO-K1) and tumor cells (SW 620) as determined by the Trypan blue exclusion assay and LDH assay. Results are means ± SD (n=3)



The reaction mechanism between P182 and H_2O_2

RESULTS

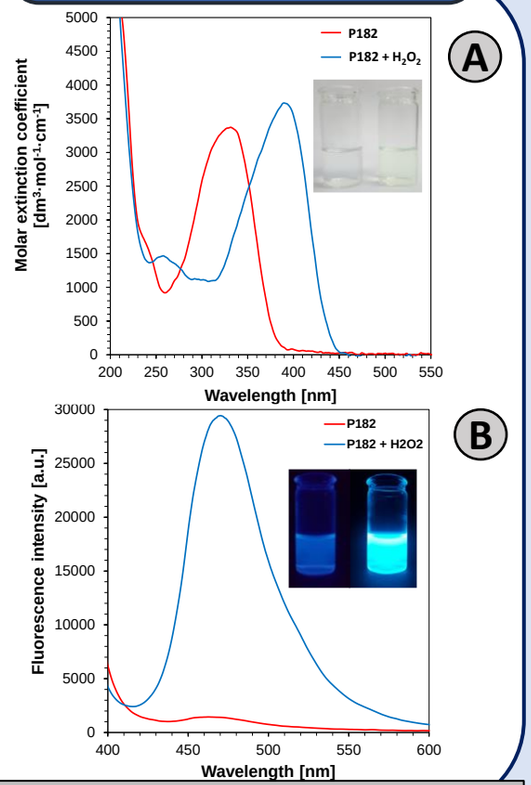


Fig 1. (A) Absorption spectra of P182 ($4.30 \cdot 10^{-5}$ μM) in the presence and absence of 1 mM H_2O_2 in PBS (pH = 7.4) **(B)** Fluorescent spectral of P182 (4.30·10-5 μM) before and after the addition of 1 mM H2O2 in PBS (pH = 7.4, λex = 365 nm)

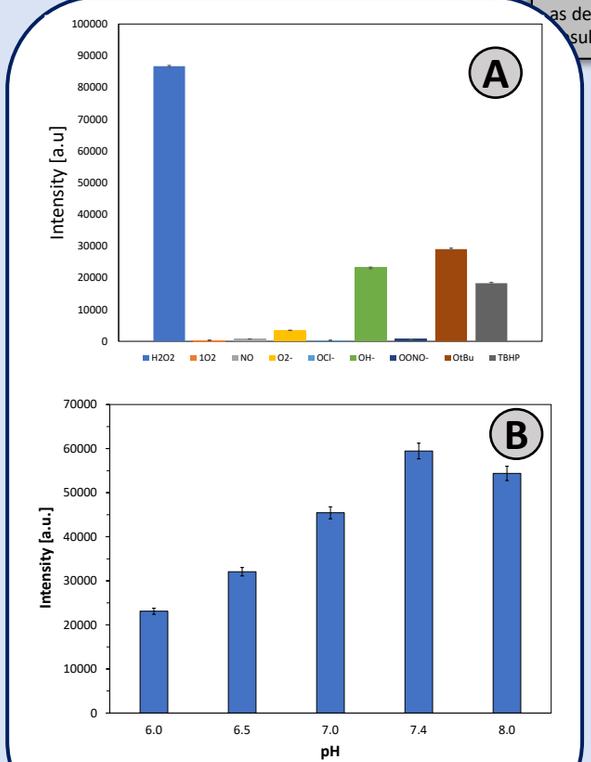


Fig. 2 (A) Correlation of fluorescence maximum of P182 sensor after incubation with different types of ROS and RNS for 60 min (PBS, pH=7.4 20 mM) **(B)** Fluorescence intensity of the sensor after 60 min incubation with 1 mM H_2O_2 at different pH

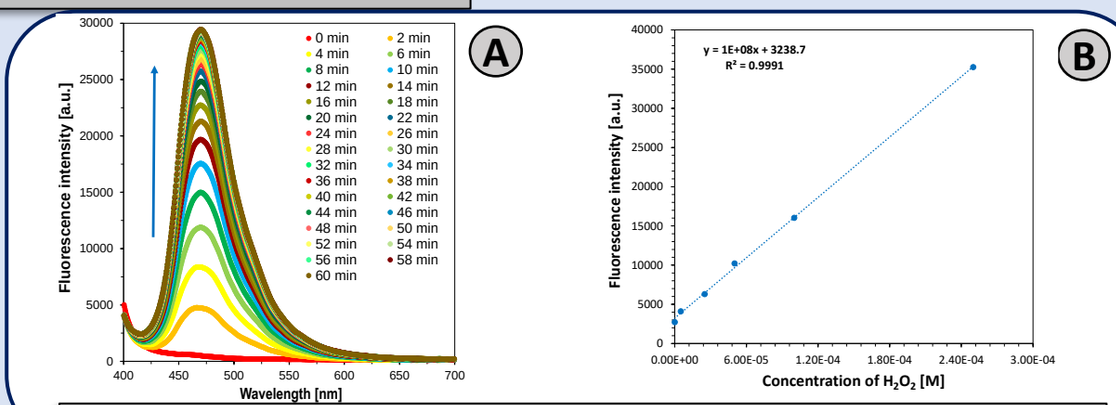


Fig. 3. (A) The wavelength dependence of fluorescence intensity for compound P182 in PBS after the addition of $1.0 \cdot 10^{-3}$ M H_2O_2 after different times; (B) Fluorescence intensity of P182 as a function of H_2O_2 concentration in the linear range

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