

**Electronic supplementary information**

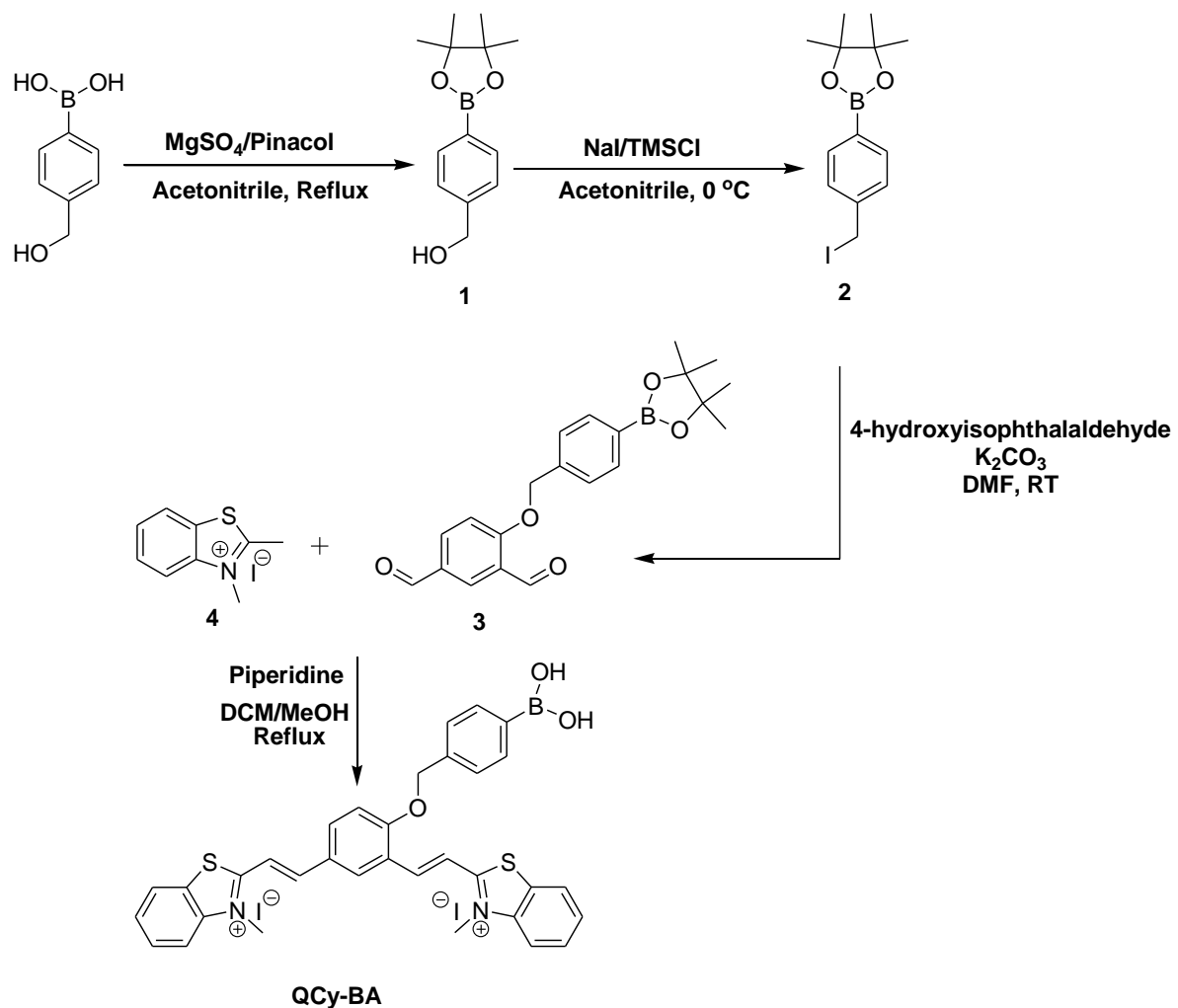
**Stimuli-responsive colorimetric and NIR fluorescence combination probe for  
selective reporting of cellular hydrogen peroxide**

Nagarjun Narayanaswamy,<sup>a</sup> Sivakrishna Narra,<sup>b</sup> Raji R. Nair,<sup>c</sup> Deepak Kumar Saini,<sup>c</sup> Paturu  
Kondaiah<sup>b</sup> and T. Govindaraju<sup>\*a</sup>

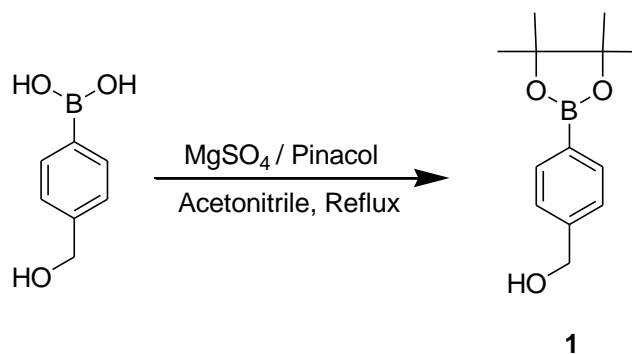
<sup>a</sup>Bioorganic Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced  
Scientific Research, Jakkur P.O., Bengaluru 560064, India.

<sup>b,c</sup>Department of Molecular Reproduction, Development and Genetics, Indian Institute of  
Science, Bengaluru 560 012, India.

### Scheme 1. Synthesis of QCy-BA

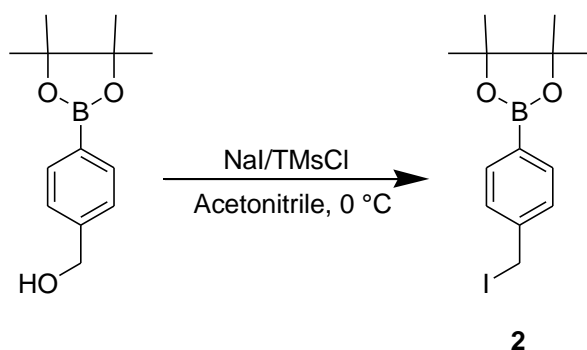


### Preparation of (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (1).



To a stirred solution of 4-(hydroxymethyl)phenylboronic acid (0.4 g, 2.63 mmol) in acetonitrile (15 mL),  $\text{MgSO}_4$  (3 g) and pinacol (0.37 g, 3.15 mmol) were added. The reaction mixture was heated up to 80 °C and allowed to reflux for 24 h. After completion of the reaction, solvent was evaporated under vacuum. The crude mixture was dissolved in dichloromethane and filtered. The obtained product (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (**1**) was used for further reaction without purification.

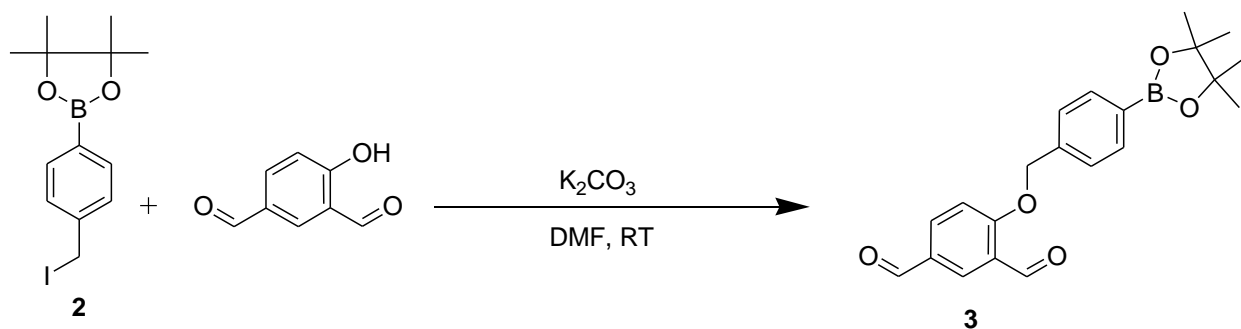
**Preparation of 2-(4-(iodomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**).**



To a stirred solution of (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)methanol (**1**) (0.55 g, 2.35 mmol) in acetonitrile (20 mL), sodium iodide (1.1 g, 7.05 mmol) and Trimethylsilyl chloride (0.65 mL, 7.05 mmol) were added at 0 °C. The reaction mixture was allowed to stir at room temperature for 1 h. After completion of the reaction solvent was evaporated under vacuum. The crude product was dissolved in saturated solution of  $\text{Na}_2\text{S}_2\text{O}_3$  to quench the unreacted iodide and the product was extracted with dichloromethane. The crude product was purified by column chromatography on silica gel using ethyl acetate/hexane (5:95) as an eluent to give product 2-(4-(iodomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**) in excellent yield (90%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{ppm}$  7.73 (d,  $J = 8$  Hz, 2H), 7.37 (d,  $J = 8$

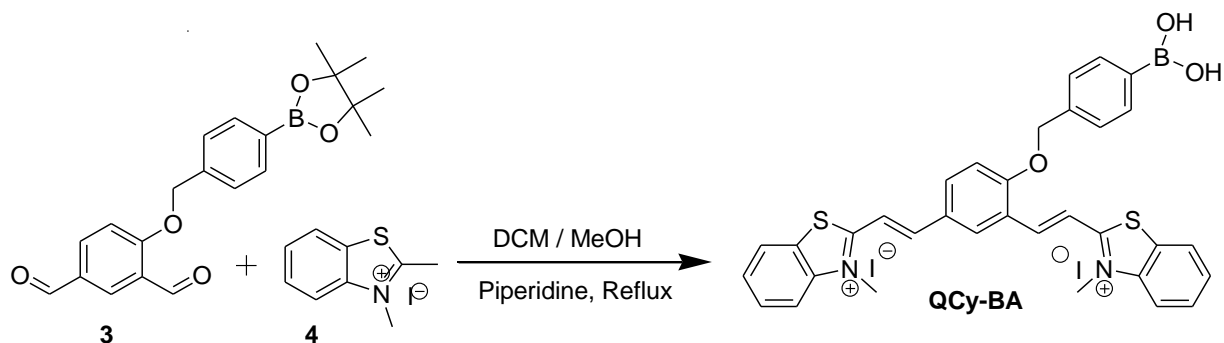
Hz, 2H), 4.45 (s, 2H), 1.34 (s, 12H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{ppm}}$  142.3, 135.3, 128.0, 24.9, 5.4

**Preparation of 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyloxy)isophthalaldehyde (3).**

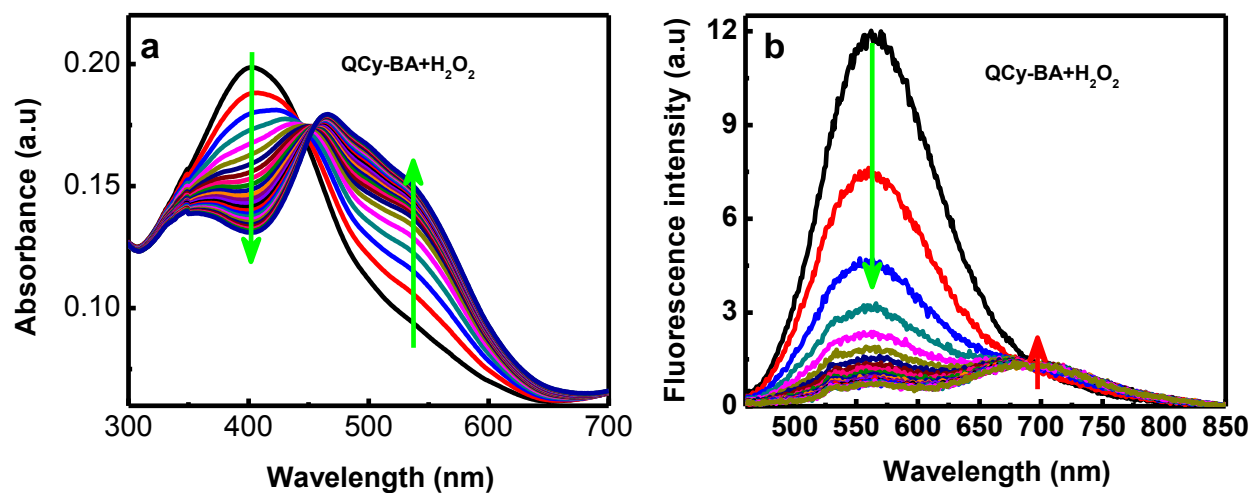


To a stirred solution of 4-hydroxyisipthaladehyde (0.11 g, 0.73 mmol) in DMF (5 mL),  $\text{K}_2\text{CO}_3$  (0.3 g, 2.17 mmol) was added and allowed to stir for 20 min. After 20 min, 2-(4-(iodomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**2**) (0.3 g, 0.87 mmol) was added and stirred overnight at room temperature (RT). The completion of reaction was monitored by TLC. After completion of the reaction, solvent was evaporated and product was extracted with diethylether ( $3 \times 100$  mL). The crude product was purified by column chromatography on silica gel using ethyl acetate\hexane (20:80) as an eluent to obtained compound **3** in good yield (60%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{ppm}}$  10.56 (s, 1H), 9.95 (s, 1H), 8.35 (d,  $J = 2.4$  Hz, 1H), 8.08 (dd,  $J = 2$  Hz, 8.8 Hz, 1H), 7.86 (d,  $J = 8$  Hz, 2H), 7.44 (d,  $J = 8$  Hz, 2H), 7.17 (d,  $J = 8$  Hz, 1H), 5.32 (s, 2H), 1.35 (s, 12H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{ppm}}$  190.1, 188.5, 164.9, 137.9, 135.5, 135.3, 131.9, 129.9, 126.5, 125.2, 113.7, 84.0, 71.0, 24.9

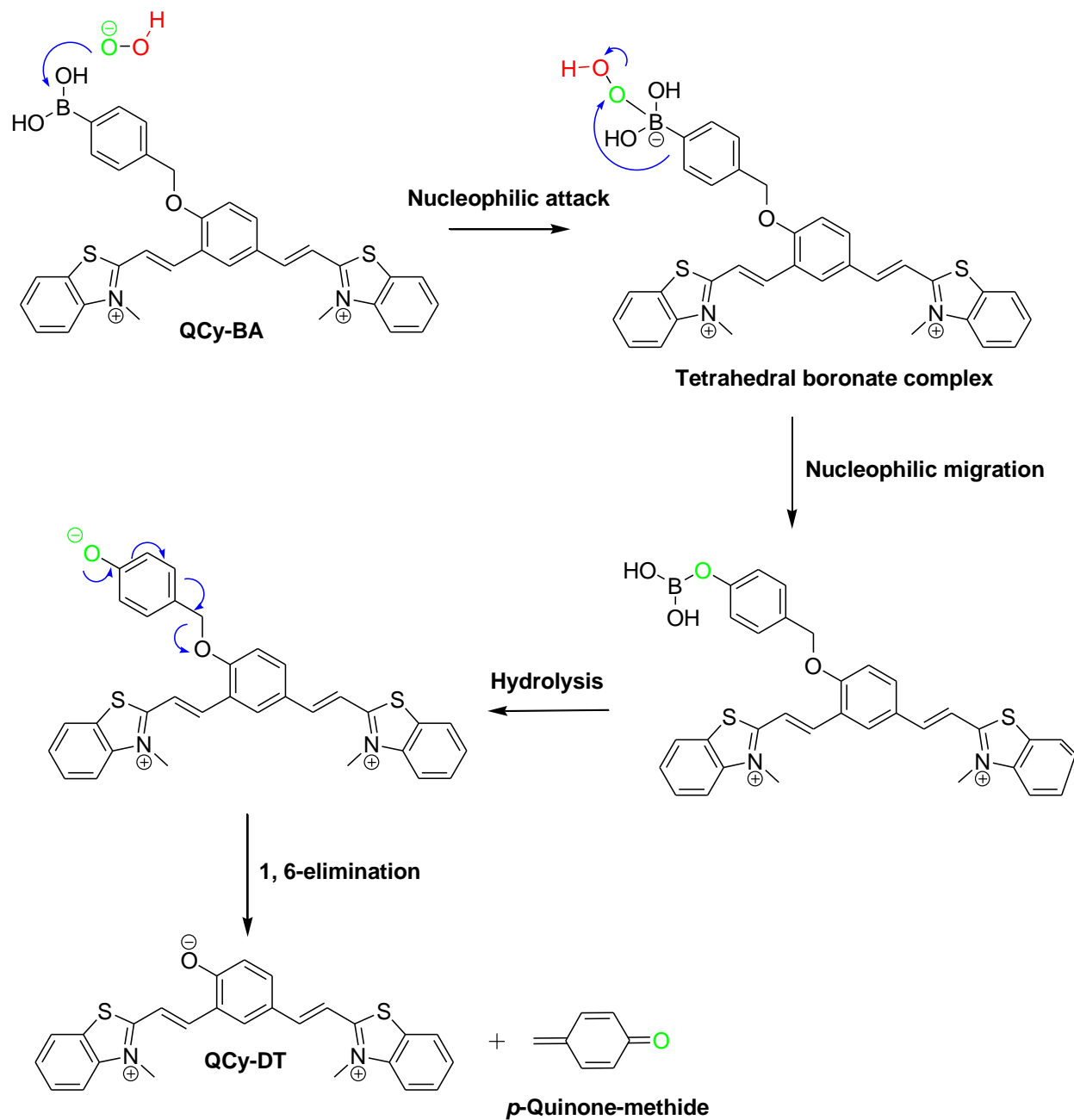
## Preparation of QCy-BA.



To a stirred solution of compound (4)<sup>1</sup> (80 mg, 0.27 mmol) in methanol (10 mL) and dichloromethane (5 mL), piperidine (8  $\mu$ L) was added and allowed to stir for 10 minutes. After 10 min, 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyloxy)isophthalaldehyde (3) (45 mg, 0.12 mmol) in DCM (2 mL) was added and heated up to 50 °C for 3 h. After the completion of reaction solvent was evaporated. The crude brown color solid was washed with diethylether (50 mL) to remove the unreacted starting materials. The brown solid was dissolved in acetonitrile/water mixture and purified by reverse phase HPLC using 0.1% trifluoroacetic acid (TFA) in water/acetonitrile (50-100%) as a mobile phase to obtained boronic acid conjugate (QCy-BA) in moderate yield 30%. <sup>1</sup>H-NMR (400 MHz, *DMSO-d*<sub>6</sub>)  $\delta_{ppm}$  8.69 (d, *J* = 2Hz, 1H), 8.44 (ddd, *J* = 2.8 Hz, *J* = 4.0 Hz, *J* = 7.6 Hz, 2H), 8.34-8.25 (m, 4H), 8.21 (d, *J* = 4.2 Hz, 1H), 8.16 (d, *J* = 12.4 Hz, 1H), 8.10 (d, *J* = 8.2 Hz, 1H), 7.92-7.88 (m, 4H), 7.81 (td, *J* = 0.8 Hz, *J* = 7.6 Hz, 2H), 7.56 (dd, *J* = 4 Hz, *J* = 8.4 Hz, 3H), 5.48 (s, 2H), 4.39 (s, 3H), 4.25 (s, 3H). <sup>13</sup>C-NMR (100 MHz, *DMSO-d*<sub>6</sub>)  $\delta_{ppm}$  171.9, 171.8, 160.6, 158.3, 158.0, 147.0, 142.1, 142.0, 141.6, 137.5, 135.0, 134.5, 132.3, 129.5, 129.4, 128.6, 128.4, 127.9, 127.8, 127.3, 127.0, 124.3, 124.2, 123.1, 118.1, 117.0, 116.9, 115.6, 115.1, 114.4, 113.0, 71.0, 36.4, 36.2. HRMS (ESI-MS): found 288.0875, calcd *m/z* = 288.0851 for C<sub>33</sub>H<sub>29</sub>BN<sub>2</sub>O<sub>3</sub>S<sub>2</sub> [M-2I]<sup>2+</sup>.

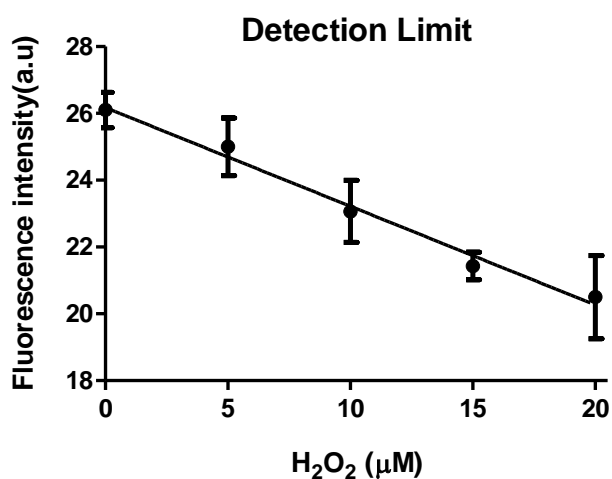


**Fig. S1** (a) Absorption spectra of **QCy-BA** (5 μM) in presence of H<sub>2</sub>O<sub>2</sub> (1 mM). (b) Emission spectra of **QCy-BA** (5 μM) in presence of H<sub>2</sub>O<sub>2</sub> (1 mM) in PBS-buffer solution as a function of time.



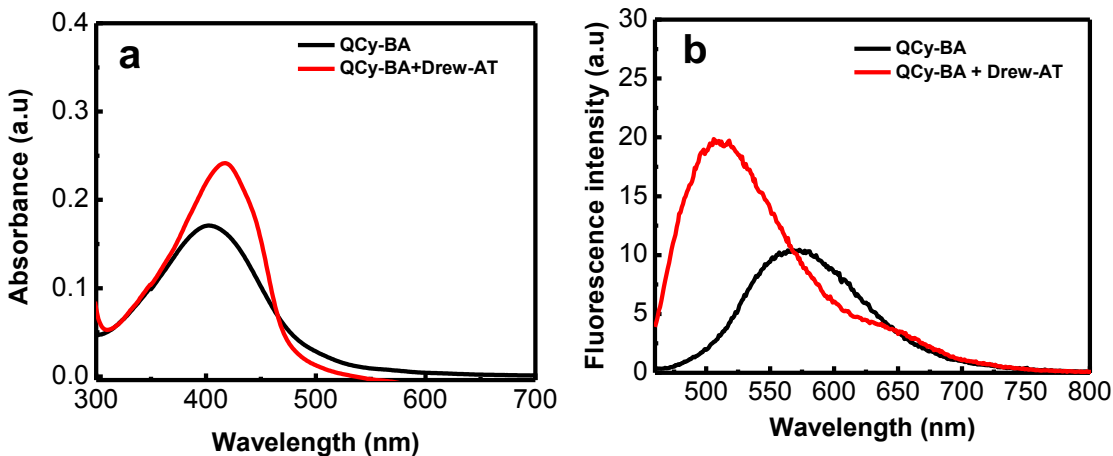
**Fig. S2** Mechanism of selective reaction of hydrogen peroxide-assisted oxidation of boronic acid in **QCy-BA** followed by hydrolysis and 1,6-elimination of p-quinone methide moiety to release DNA minor groove binder **QCy-DT**.<sup>2</sup>

**Detection limit of H<sub>2</sub>O<sub>2</sub> in presence of QCy-BA.** Concentration dependent studies were performed using microplate reader. In the well-plates, first we have taken **QCy-BA** (5 μM) in buffer solution, then we added increasing concentration of H<sub>2</sub>O<sub>2</sub> from 0 to 100 μM. Upon excitation at 400 nm, we have collected the emission at 565 nm as function time after addition of H<sub>2</sub>O<sub>2</sub>. The fluorescence intensity at 565 nm was plotted as a function of concentration of hydrogen peroxide and each experiment was done in triplicates.

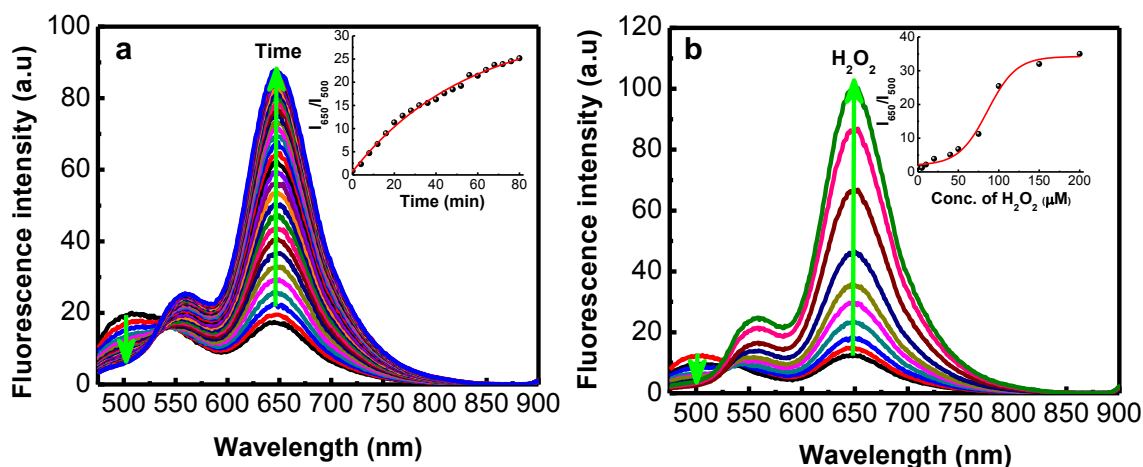


**Fig. S3** Plot of the fluorescence intensity at 565 nm against the concentration of [H<sub>2</sub>O<sub>2</sub>] PBS-buffer solution. Each data point was acquired after addition H<sub>2</sub>O<sub>2</sub> at 25 °C. The detection limit (5.3 μM) was calculated with  $3\sigma/k$ ; where  $\sigma$  is the standard deviation of blank measurement,  $k$  is the slop (-0.30).

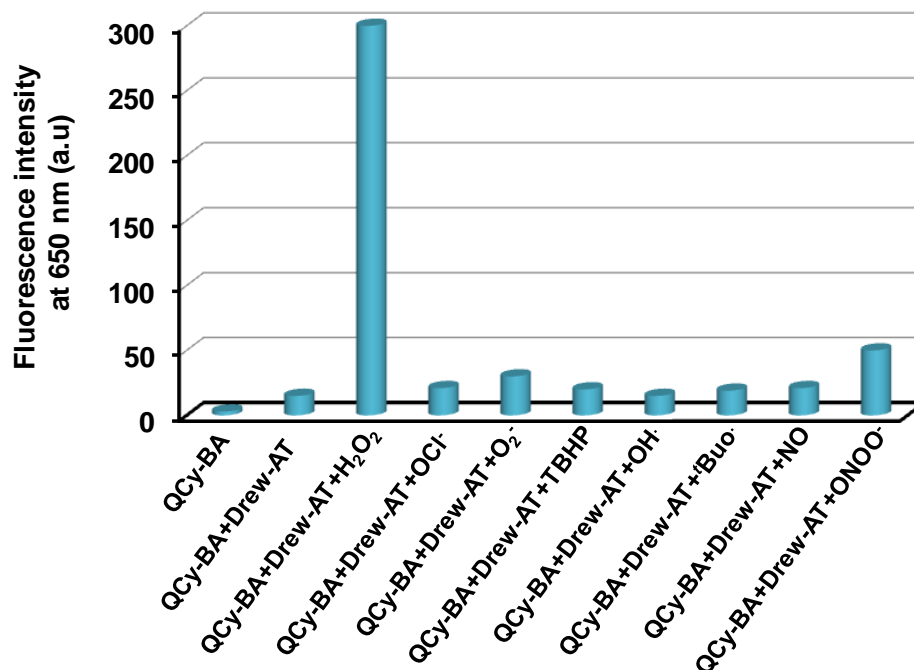




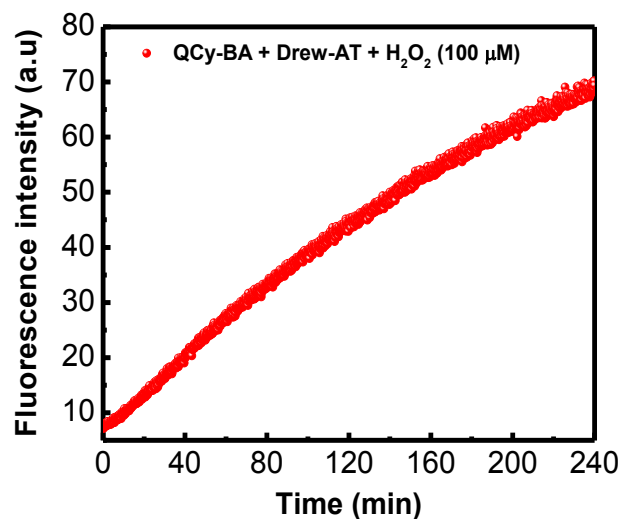
**Fig. S4** (a) Absorption spectra of **QCy-BA** (2  $\mu\text{M}$ ) in presence of **Drew-AT** (2  $\mu\text{M}$ ). (b) Emission spectra of **QCy-BA** (2  $\mu\text{M}$ ) in presence of **Drew-AT** (2  $\mu\text{M}$ ) in PBS-buffer solution.



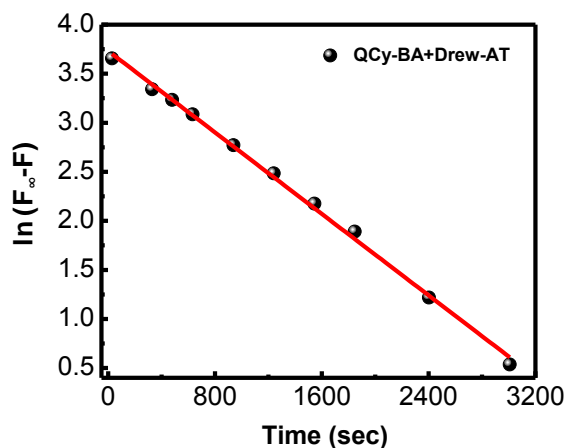
**Fig. S5** (a) Fluorescence response of the combination probe **QCy-BA@Drew-AT** (2  $\mu\text{M}$ ) in presence of  $\text{H}_2\text{O}_2$  upon excitation with wavelength corresponding to isosbestic point at 456 nm. *Inset*: Fluorescence intensity ratio of  $I_{650}/I_{500}$  as function of time (0 to 80 min). (b) Fluorescence response of the combination probe **QCy-BA@Drew-AT** (2  $\mu\text{M}$ ) with increasing concentration of  $\text{H}_2\text{O}_2$  from 0 to 200  $\mu\text{M}$  upon excitation at 456 nm. *Inset*: Fluorescence intensity ratio of  $I_{650}/I_{500}$  as function of concentration of  $\text{H}_2\text{O}_2$ .



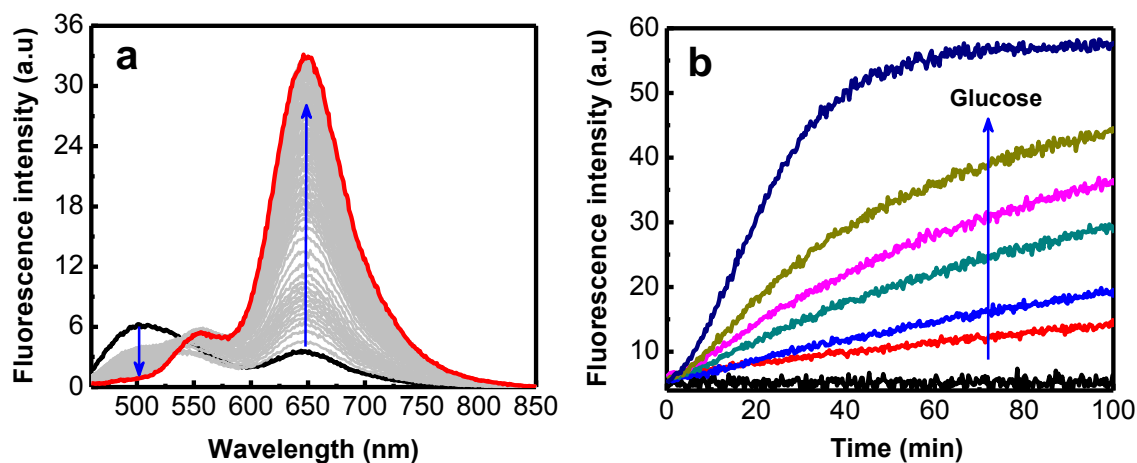
**Fig. S6** Fluorescence response of combination probe **QCy-BA** + **Drew-AT** (2  $\mu$ M) to various ROS at individual concentrations of 100  $\mu$ M upon excitation at 564 nm.



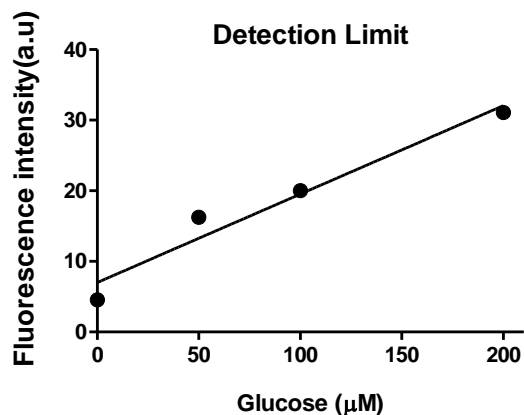
**Fig. S7** Time-dependent fluorescence spectra of **QCy-BA** (2  $\mu$ M) in presence of **Drew-AT** (2  $\mu$ M) after the addition of **H<sub>2</sub>O<sub>2</sub>** (100  $\mu$ M) upon excitation at 564 nm.



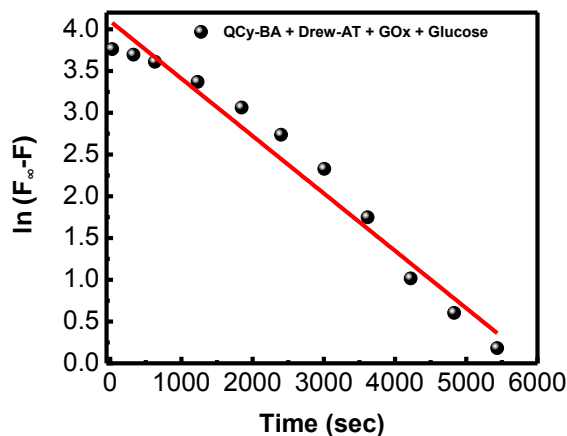
**Fig. S8** Plot of  $\ln(F_{\infty}-F)$  of combination probe **QCy-BA**+Drew-AT (2  $\mu\text{M}$ ) as a function of time at 650 nm upon addition of  $\text{H}_2\text{O}_2$  (1 mM), where  $F_{\infty}$  and  $F$  are the fluorescence intensities at 650 nm at time  $t_{\infty}$  ( $= 60$  min) and  $t$ , respectively. The  $k_{\text{obs}}$  calculated from the slope of this plot is  $1.04 \times 10^{-3} \text{ s}^{-1}$ .



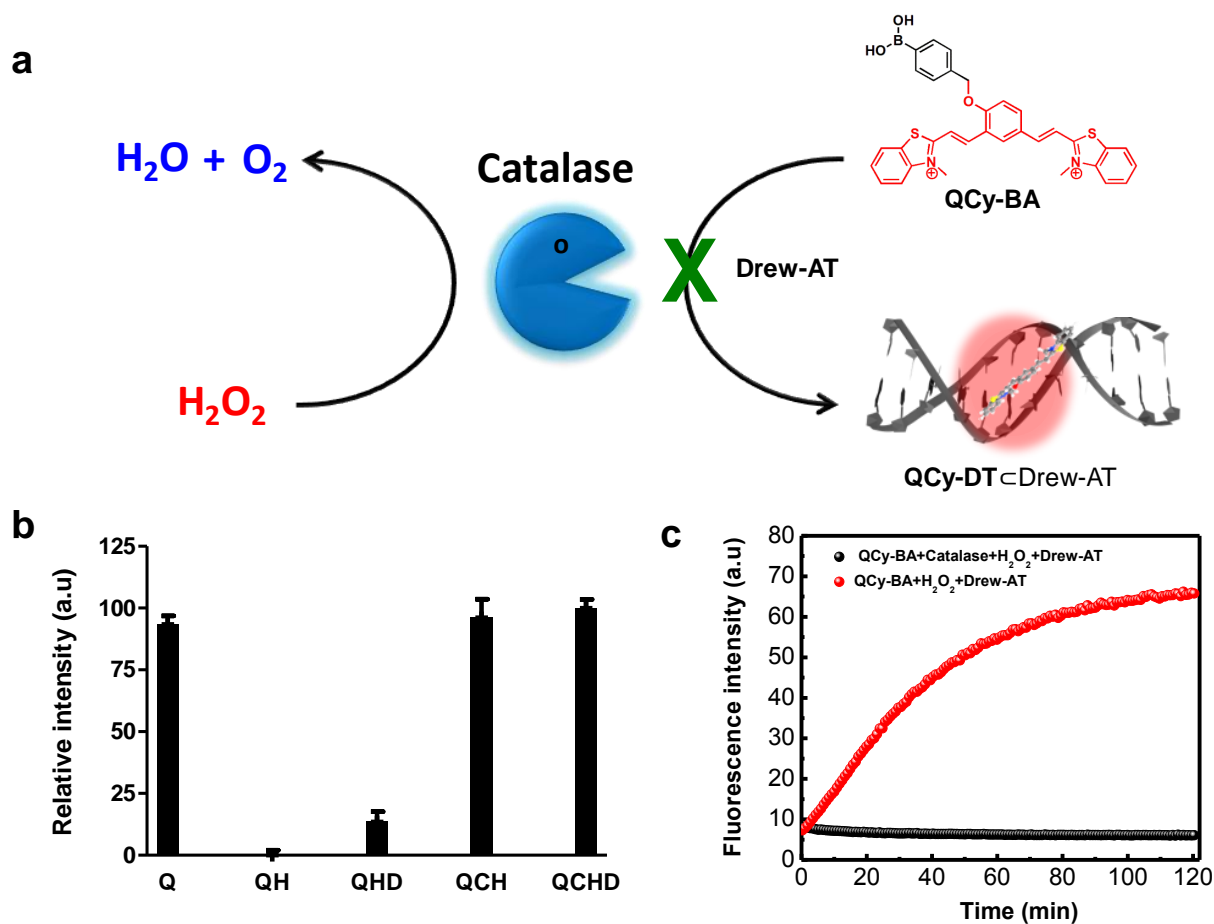
**Fig. S9** (a) Fluorescence spectra of combination probe **QCy-BA**+Drew-AT in presence of GOx (4 U/mL) and glucose (1 mM) with time, upon excitation at 400 nm. (b) Plot of fluorescence intensity of combination probe **QCy-BA**+Drew-AT at 650 nm as function of time, in presence of GOx (4 U/mL) with increasing concentration of glucose from 0 to 1 mM upon excitation at 564 nm.



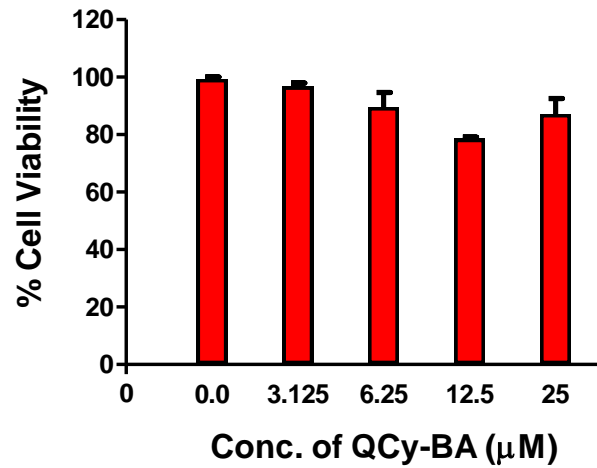
**Fig. S10** The plot of the fluorescence intensity of combination probe **QCy-BA**∩**Drew-AT** in the presence of GOx (4 U/mL) against the concentration of glucose at 650 nm. Each data point was acquired 1 h after addition of glucose and GOx at 25 °C. The detection limit (6.11 μM) was calculated by  $3\sigma/k$ , where  $\sigma$  is the standard deviation of blank measurement and  $k$  is the slope (0.12).



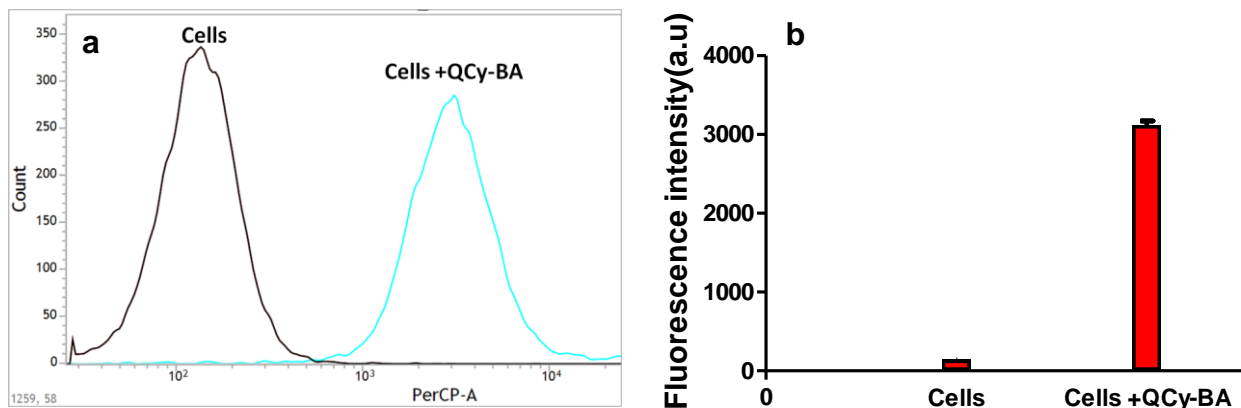
**Fig. S11** Plot of  $\ln(F_{\infty}-F)$  of combination probe **QCy-BA**∩**Drew-AT** in presence of GOx (4 U/mL) as a functions of time at 650 nm upon addition of glucose (1 mM), where  $F_{\infty}$  and  $F$  are the fluorescence intensities at 650 nm at time  $t_{\infty}$  (= 100 min) and  $t$ , respectively. The  $k_{\text{obs}}$  calculated from the slope of this plot is  $6.87 \times 10^{-4} \text{ s}^{-1}$ .



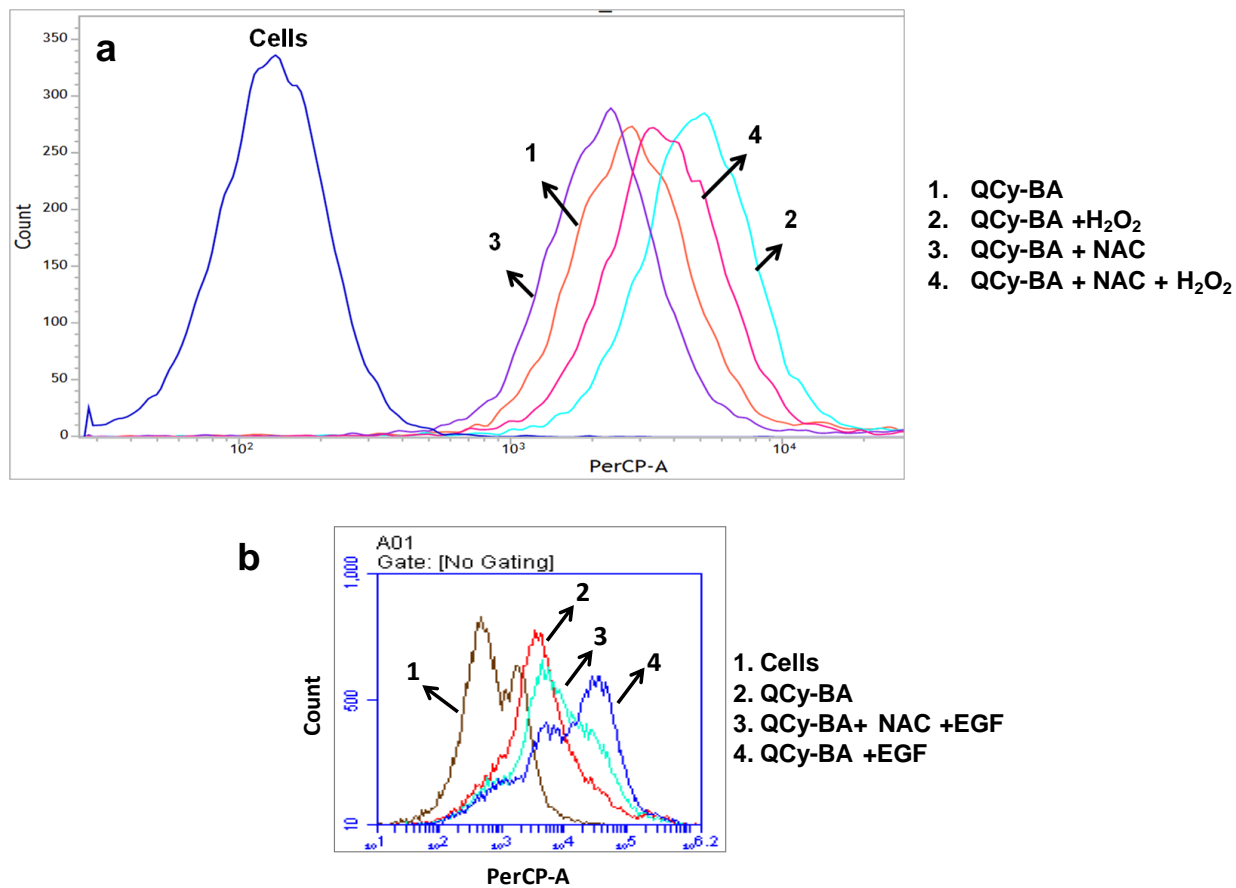
**Fig. S12** (a) Schematic diagram represents the catalase activity in presence of  $\text{H}_2\text{O}_2$  and combination probe  $\text{QCy-BA} \subset \text{Drew-AT}$ . (b) Fluorescence intensity of  $\text{QCy-BA}$  at 565 nm. Where **Q**:  $\text{QCy-BA}$ , **QH**:  $\text{QCy-BA} + \text{H}_2\text{O}_2$ , **QHD**:  $\text{QCy-BA} + \text{H}_2\text{O}_2 + \text{Drew-AT}$ , **QCH**:  $\text{QCy-BA} + \text{Catalase} + \text{H}_2\text{O}_2$ , **QCHD**:  $\text{QCy-BA} + \text{Catalase} + \text{H}_2\text{O}_2 + \text{Drew-AT}$ . (c) Time dependent fluorescence of combination probe  $\text{QCy-BA} \subset \text{Drew-AT}$  in presence and absence of catalase upon addition of  $\text{H}_2\text{O}_2$  (1 mM) upon excitation at 564 nm.



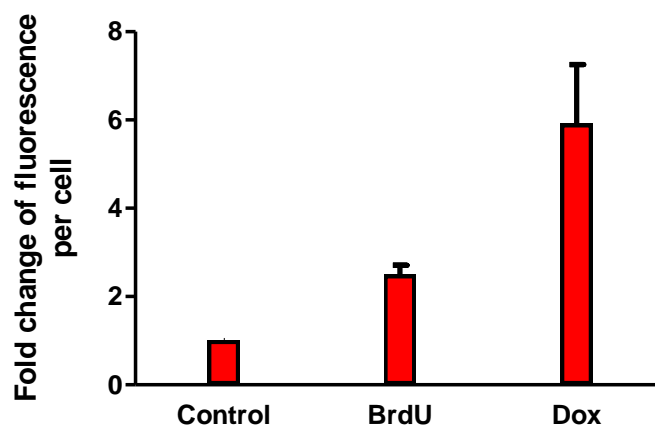
**Fig. S13** Dose dependent cell viability of HeLa cells by taking 0.0-25 µM of probe **QCy-BA** for 24 h. Error bars represent  $\pm$ standard deviation.



**Fig. S14** (a) Flow cytometry plots of HeLa cells in the absence and presence of **QCy-BA** (5 µM). (b) FACS-analysis shows the PerCP mean fluorescence intensity in HeLa cells in presence of **QCy-BA** (5 µM).



**Fig. S15** (a) Flow cytometry plots of probe **QCy-BA** (5  $\mu$ M) treated HeLa cells in the presence of H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) and NAC (8 mM). (b) Flow cytometry plots of probe **QCy-BA** (5  $\mu$ M) treated HeLa cells in the presence of epidermal growth factor (EGF) (50 ng/mL) and NAC (8 mM).



**Fig. S16** H<sub>2</sub>O<sub>2</sub> detection in attached live HeLa cells using DCFDA after treatment with BrdU (100 μM) and doxorubicin (0.1 μM) for 48 h. Fold change of fluorescence per cell is normalized to 1 for control cells (n=3).

**Table S1.** Comparison of probe **QCy-BA** with Naphtho-Peroxyfluor-1 (NPF-1).

Property	Naphtho-Peroxyfluor-1 (NPF1) <sup>a</sup>	QCy-BA <sup>b</sup>
Synthesis	Metal-catalyst required	Metal-catalyst not required
Solubility	DMSO	Water
Stokes shift	$\Delta\lambda_{\text{max}} = \sim 62 \text{ nm}$	$\Delta\lambda_{\text{max}} = \sim 280 \text{ nm}$
Detection	Fluorescence	Fluorescence as well as naked eye
Incubation time	$\sim 60\text{-}120 \text{ min}$	$\sim 15\text{-}30 \text{ min}$
Concentration required for cell imaging	$\sim 20 \mu\text{M}$	$\sim 5 \mu\text{M}$

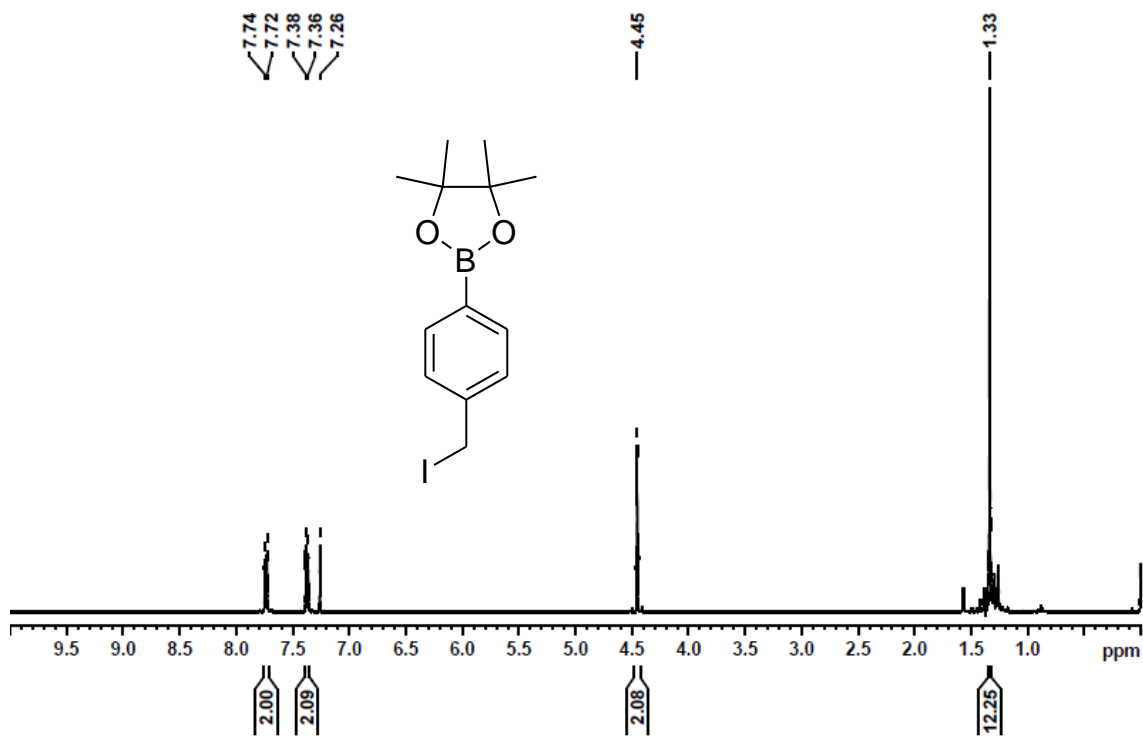
<sup>a</sup> A. E. Albers, B. C. Dickinson, E. W. Miller and C. J. Chang, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5948–5950. <sup>b</sup> Present work.



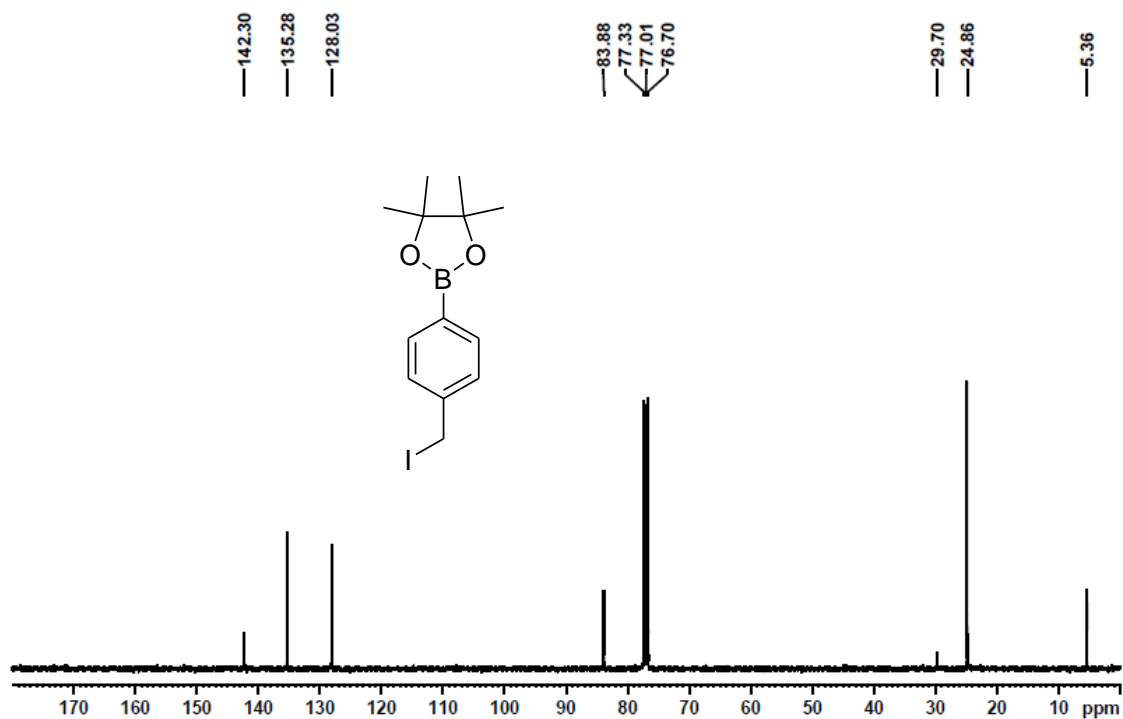
## Reference

1. N. Narayanaswamy, M. Kumar, S. Das, R. Sharma, P. K. Samanta, S. K. Pati, S. K. Dhar, T. K. Kundu and T. Govindaraju. *Sci. Rep.*, 2014, **4**, 6476.
2. A. R. Lippert, G. C. Van de Bittner and C. J. Chang, *Acc. Chem. Res.*, 2011, **44**, 793–804.

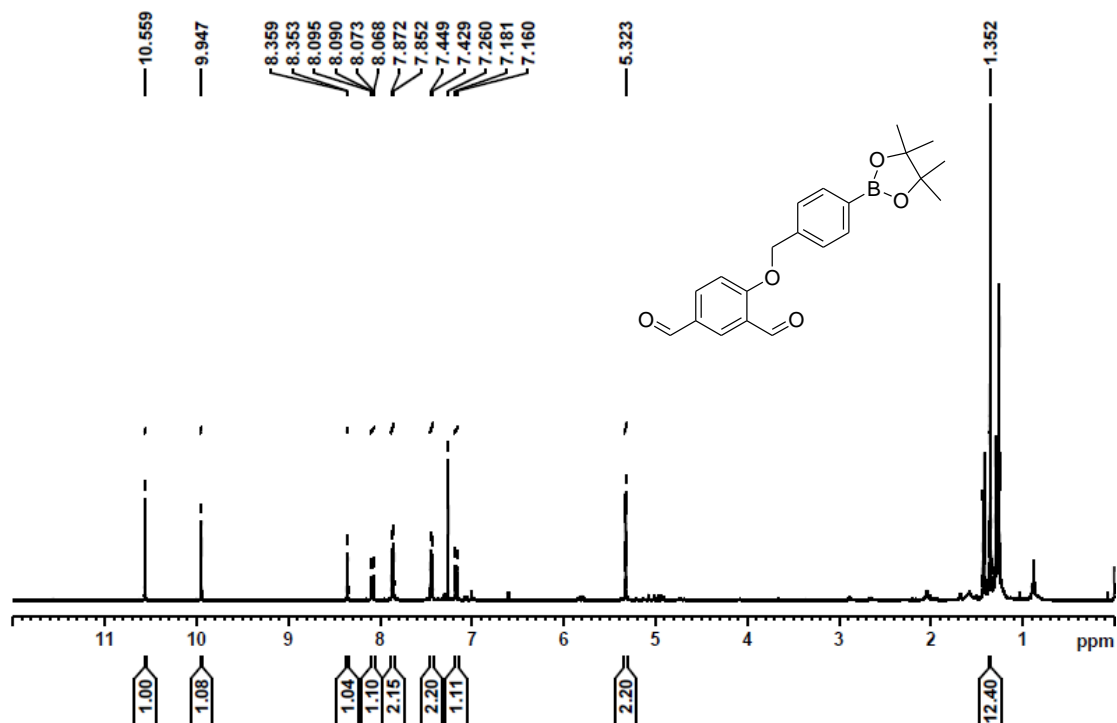
### <sup>1</sup>H NMR spectrum of compound 2



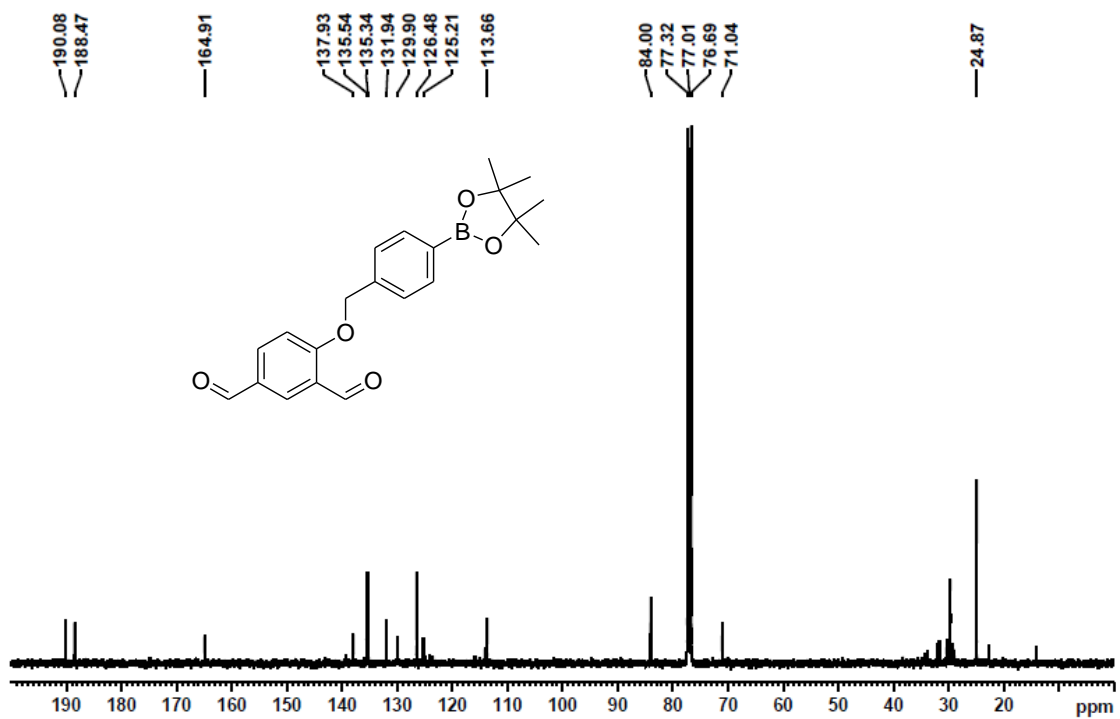
### <sup>13</sup>C NMR spectrum of Compound 2



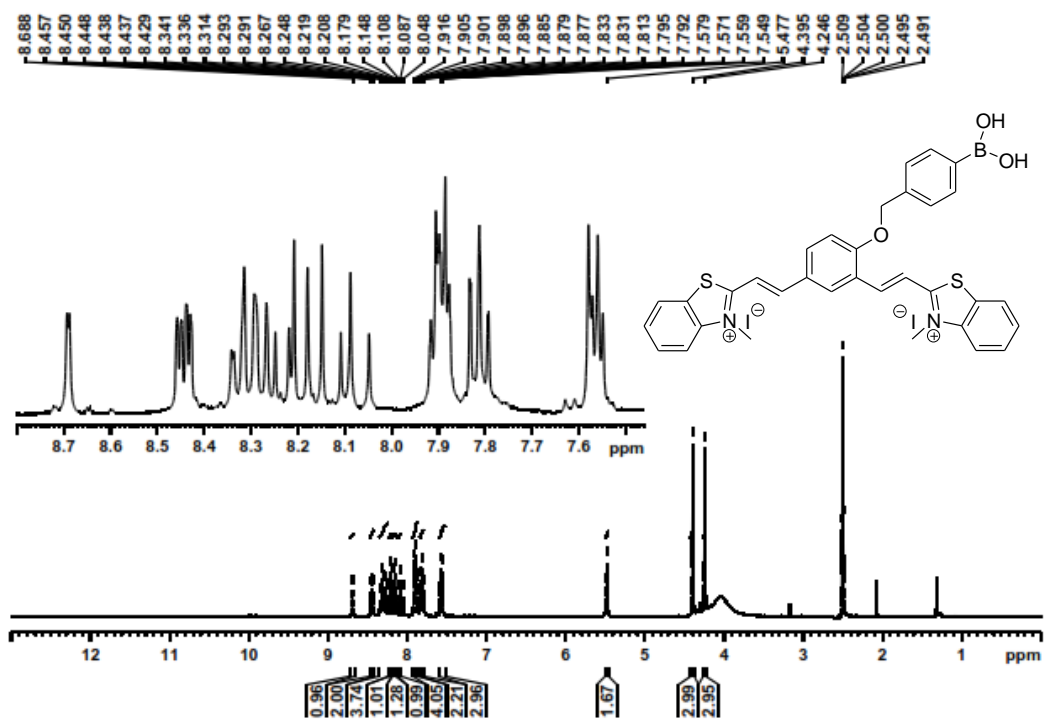
### <sup>1</sup>H NMR spectrum of Compound 3



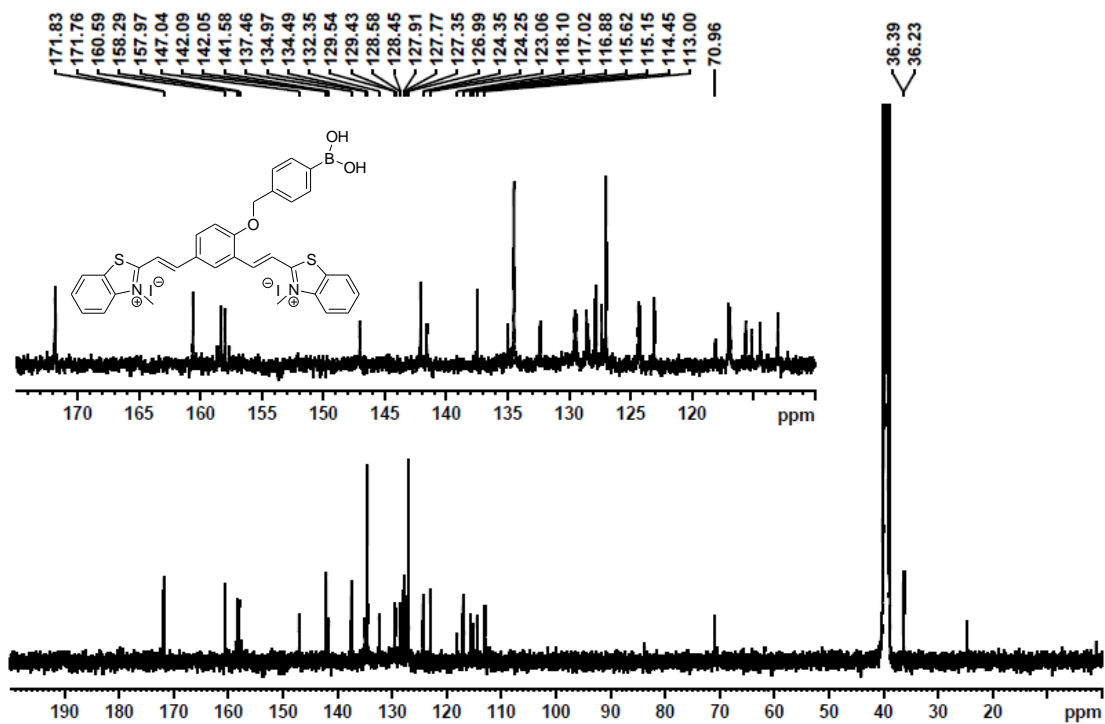
### <sup>13</sup>C NMR spectrum of Compound 3



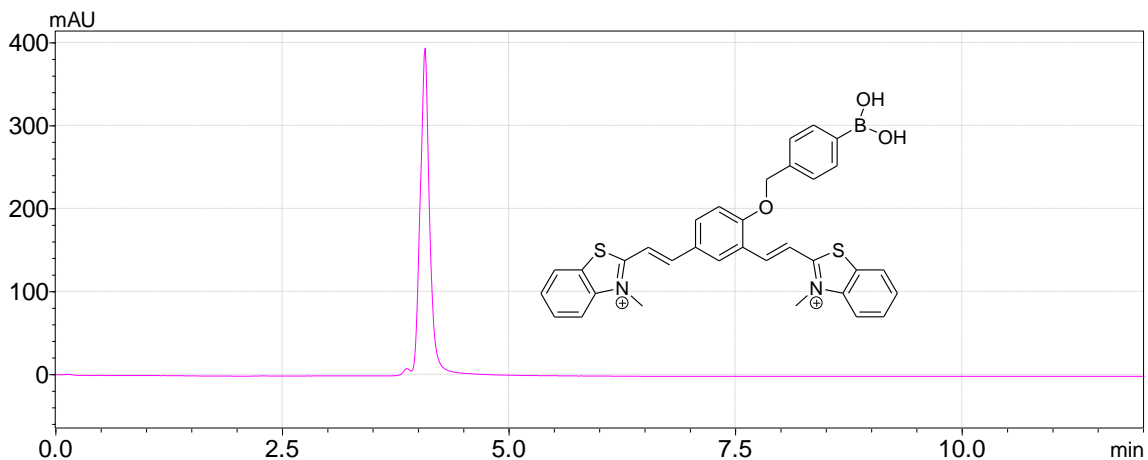
### $^1\text{H}$ NMR spectrum of QCy-BA



### $^{13}\text{C}$ NMR spectrum of QCy-BA



## HPLC trace of QCy-BA



## HRMS mass data of QCy-BA

### User Spectra

