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Electronic Supplementary Material (ESI) for

Macroscopic and fluorescent detection of reactive oxygen species by using glucose-linked tetraphenylethylene polymer gel

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Content

1. General

All reagents were commercially available unless otherwise noted. TPEBA and TPE-$(OH)_4$ was synthesized according to previous literature methods.[1, 2] All reactions were carried out under argon atmosphere and heated in an oil bath unless otherwise noted. Air and moisture sensitive liquids and solutions were transferred via syringe. All solvents were dried and distilled by standard procedures. Solutions were concentrated under reduced pressure by rotary evaporation. Chromatographic purification of products was accomplished on silica gel Si 60® (300-400 mesh).

Nuclear magnetic resonance spectra were acquired on a Bruker AMX 400 (400 MHz for ^1H) and a Bruker DRX 600 (500 MHz for ^1H). All ^1H NMR spectra are reported in parts per million (ppm) downfield of TMS and were measured relative to the signals at 7.26 ppm ($CDCl_3$) and 2.50 ppm (DMSO-D6). Data for ^1H NMR are reported as follows: chemical shift (δ in ppm), multiplicity (s = singlet; brs = broad singlet; vbs = vary broad singlet; d = doublet; t= triplet; m = multiplet), coupling constant (Hz), integration. The morphologies of the polymer were characterized by a field scanning electron microscope (SEM) of Phenom ProX. The spectroscopy (FTIR) of the monomers and polymer were measured by a spectrometer Frontier of Thermo Scientific Nicolet iS5. The UV–vis spectra was measured by UV-3600 Spectrophotometer (SHIMADZU). The fluorescence spectra was recorded by a fluorescence spectrometer of Perkinelmer FL 6500 with a 10 mm quartz cuvette. The confocal fluorescence microscopy was performed by using Olympus FV3000. High-resolution mass spectra were recorded on a Thermo Scientific Exactive Orbitrap Mass Spectrometer under Electron Spray Ionization conditions preparing sample solution in methanol. X-ray photoelectron spectroscopy (XPS) experiments were carried out on an Xray photoelectron spectrometer AXIS UltraDLD.

2. Synthesis

2.1 Preparation of polymer gels

TPEBA (10 mg, 1 equiv.) was dissolved in DMSO (10 mL) into a screw-cap tube (5 mL), followed by addition of glucose (Glu, 2 equiv.). Upon heating at certain temperature for certain time (Fig. S1), polymer gel was obtained.

2.2 Synthesis of different ROS and ions

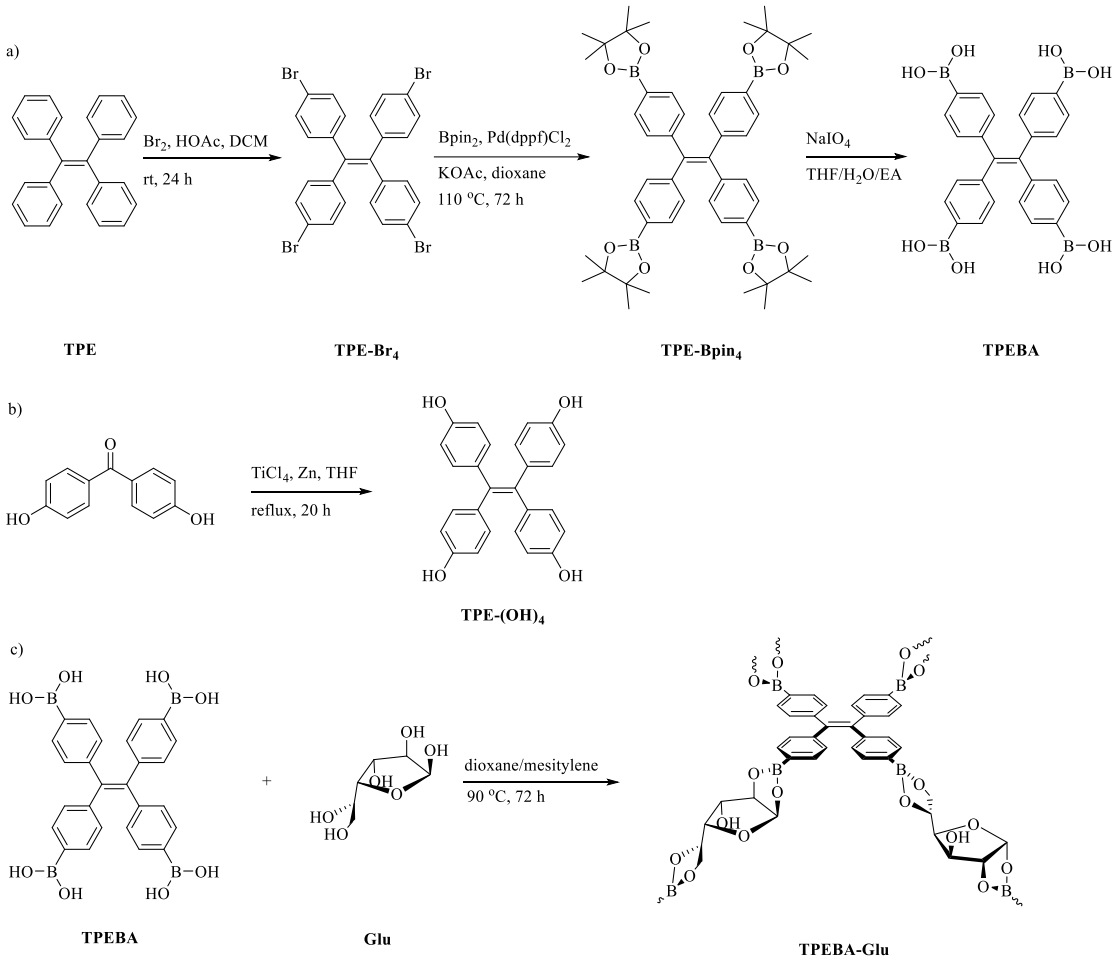
Hydrogen peroxide (10 mM) was delivered from 30% aqueous solution.

Hydroxyl radical (10 mM) was generated by Fenton reaction of 10 mM Fe^{2+} with 250 mM H_2O_2.

Hypochlorite (10 mM) was delivered from 5% aqueous solution and the concentration was determined from the absorption at 292 nm ($\varepsilon_{292\ nm}$ = 350 $cm^{-1}\cdot M^{-1}$).

The peroxynitrite alkaline stock was prepared according previous literature procedure[3] and assayed using UV-vis spectrophotometer at 302 nm ($\varepsilon_{302\ nm}$ = 1670 $cm^{-1}\cdot M^{-1}$). Peroxynitrite (10 mM) was diluted from the stock.

2.3 Synthesis of TPEBA, TPE-(OH)$_4$ and TPEBA-Glu



Scheme S1 Synthetic routes.

Tetrakis(4-bromophenyl)ethylene (TPE-Br$_4$): Molecular bromine (13.17 mL, 240 mmol) was added to a solution of 1,1,2,2-tetraphenylethylene (9.97 g, 30 mmol) in 30 mL of glacial acetic acid and 60 mL of DCM at 0 °C. Then the resulted mixture was stirred at room temperature for 24 h, and then poured into 300 mL ice water, and extracted with DCM. The organic phase was separated and washed with hydrogen sulfite, water, and brine, and dried over anhydrous Na_2SO_4. Then the organic solvent was removed under reduced pressure leading to the crude product, which was purified by recrystallization from MeOH to give 1-bromo-4-[1,2,2-tris(4-bromophenyl)ethenyl] benzene as a white solid. 17.9 gm yield: 92%.

^{1}H NMR (400 MHz $CDCl_3$): δ = 7.26 (d, J = 8.43 Hz, 8H), 6.84 (d, J = 8.44 Hz, 8H).

Tetraphenylethylenetetraboronic acid-pinacol ester (TPE-Bpin$_4$): Anhydrous dioxane (55 mL) and 2.94 g KOAc (30 mmol) in a dried 100-mL two-neck round bottle flask was N_2 bubbled for 30 min. TPE-Br$_4$ (2.05 g, 3 mmol), bis(pinacolato)diboron (3.23 g, 12.75 mmol), and [1,1'-Bis(diphenyl-phosphino)-ferrocene] palladium (II) dichloride dichloromethane adduct (122 mg, 0.15 mmol) were added subsequently under N_2. The mixture was degassed through three freeze-pump-thaw cycles and heated to reflux at 110 °C for 72 h. Then the reaction mixture was cooled to room temperature, and the dioxane was removed under reduced pressure. The residue was poured into ice-cooled water and the resulted mixture was stirred for 10 min. The precipitate was filtered, washed with water, and dried in vacuum. Pure TPE-Bpin$_4$ as a white solid was obtained after the purification by using flash column chromatograph with ethyl acetate/chloroform (1/1, v/v) as eluent and recrystallization in MeOH (100 mL). 0.92 g, yield: 36%.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.5 (d, J = 7.71 Hz, 8H), 6.98 (d, J = 7.71 Hz, 8H), 1.31 (s, 48H).

Tetraphenylethylene-tetraboronic acid (TPEBA): To a 100-mL two-neck round bottle flask equipped with magnetic stirrer reflux condenser, $NaIO_4$ (2.84 g, 13.3 mmol) was added followed by the mixture of THF/H_2O/EA (50 mL, 3/1/1, v/v/v). The reaction mixture was stirred at room temperature under continuous N_2 bubbling for 30 min. TPE-Bpin$_4$ (0.836 g, 1 mmol) was added under N_2. The reaction mixture was degassed through three freeze-pump-thaw cycles and heated to reflux at 80 °C for 24 h until the upper layer changed into a clear yellow solution. Then it was cooled down to room temperature and placed in ice-water bath. An ice cooled HCl solution (1 M, 20 mL) was added with vigorous stirring. After being stirred at 25 °C overnight, the organic solvent was evaporated in vacuum at room temperature. Water (250 mL) was added and stirred for further 30 min. The white precipitate was collected and washed with water and chloroform three times respectively, and was dried in vacuum at room temperature for 24 h leading to pure TPEBA as a white powder. 436 mg, yield: 86%.

^{1}H NMR (400 MHz, DMSO-d6): δ = 7.94 (s, 7H), 7.49 (d, J = 7.36 Hz, 8H), 6.88 (d, J

= 7.28 Hz, 8H).

Tetra(*p*-hydroxyphenyl)ethylene (TPE-$(OH)_4$): 4,4'-Dihydroxybenzophenone (2.1 g, 10 mmol) and Zn power (1.44 g, 22 mmol) were dissolved in dry THF under N_2. After being cooled to 0 ºC, $TiCl_4$ (1.3 mL, 12 mmol) was added dropwise. Then the reaction mixture was refluxed overnight. After being cooled to room temperature, 40 mL HCl solution (1 M) was added, and the aqueous solution was extracted with DCM. The combined organic phases were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was further purified by using silica gel column chromatography (MeOH/DCM 1:16) affording pure TPE-$(OH)_4$ as a white solid. 1.17 g, yield: 50%.
^{1}H NMR (400 MHz, DMSO-d6): δ = 9.22 (s, 3H), 6.70 (d, J = 8.5 Hz, 8H), 6.47 (d, J = 8.6 Hz, 8H).

TPEBA-Glu：To a 10-mL pyrex tube, TPEBA (50.9 mg, 0.1 mmol), Glu (36.7 mg, 0.2 mmol), and dioxane/mesitylene (2 mL, 1/1, v/v) were added under N_2. The mixture was sonicated for 30 seconds. Then it was sealed under vacuum, and heated at 90 °C for 3 days. After being cooled to room temperature, the yellow precipitate was collected by centrifugation, and was further washed with anhydrous THF for several times. The crude products were immersed in anhydrous acetone over 15 minutes to exchange the reaction solvents, and washed with anhydrous acetone. The desired polymer was obtained after the centrifugation, and was dried in vacuum for 24 h, affording light yellow powder. 67 mg, 78% yield.

3. Gelation test

Table S1 Gelation ability of TPEBA and Glu in various solvents

Solvent	Phase	Solvent	Phase
$CHCl_3$	I	EtOH	S
DCM	I	IPA	PG
THF	PG	DMAc	G
Dioxane	PG	THF/H_2O (1/1, v/v)	S
ACN	I	Dioxane/H_2O (1/1, v/v)	S
DMF	G	IPA/H_2O (1/1, v/v)	S
DMSO	G	DMSO/H_2O (1/1, v/v)	S
MeOH	S	H_2O	I

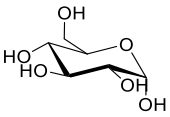
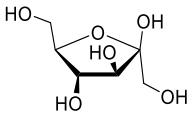
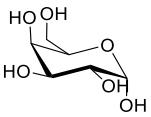
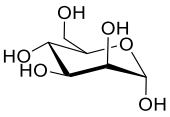
G: gel; PG: partial gel; WG: weak gel; S: soluble; I: insoluble. IPA: isopropyl alcohol.

Table S2 Gelation ability with different ratio of TPEBA and Glu in DMSO

Molar Ratio of TPEBA and Glu (n/n)	Phase
1/1	S
1/2	G
1/3	G
1/4	G

G: gel; S: soluble.

Table S3 Gelation ability of TPEBA with different monosaccharides respectively

Monosaccharide	Structure	Phase
Glucose		G
Fructose		G
Galactose		S
Mannose		WG

G: gel; WG: weak gel; S: soluble.

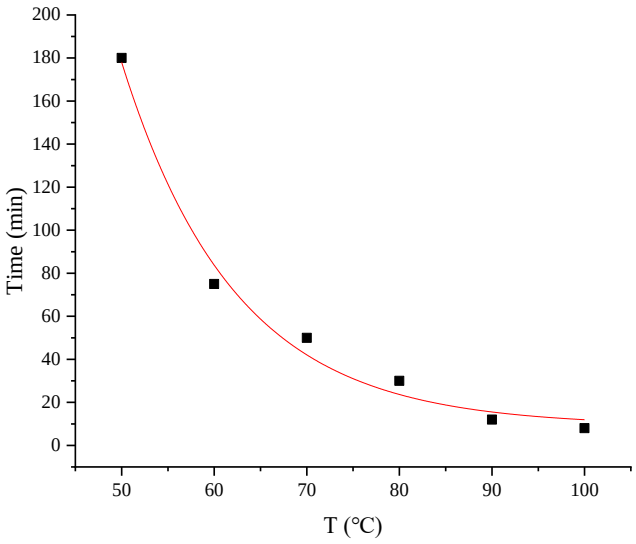


Fig. S1 Gelation time of TPEBA@Glu as a function of heating temperature.

4. UV-vis and fluorescent spectra

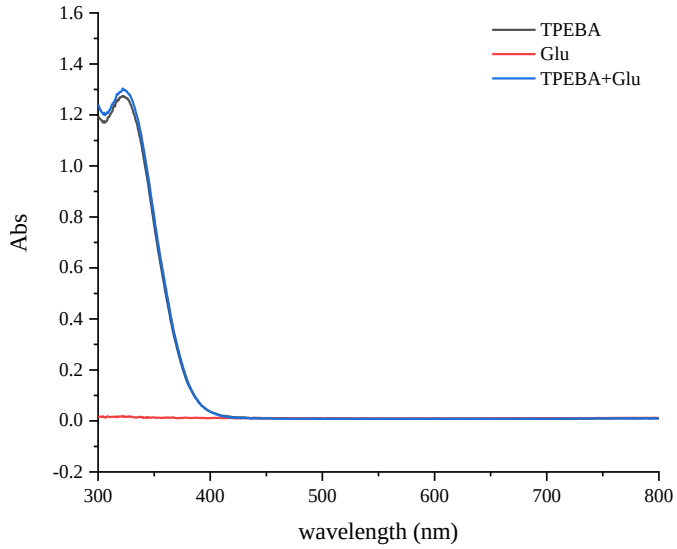


Fig. S2 UV-vis spectra of TPEBA (1×10^{-4} M), Glu (1×10^{-4} M), TPEBA + Glu (TPEBA: 1×10^{-4} M + Glu: 2×10^{-4} M) in DMSO.

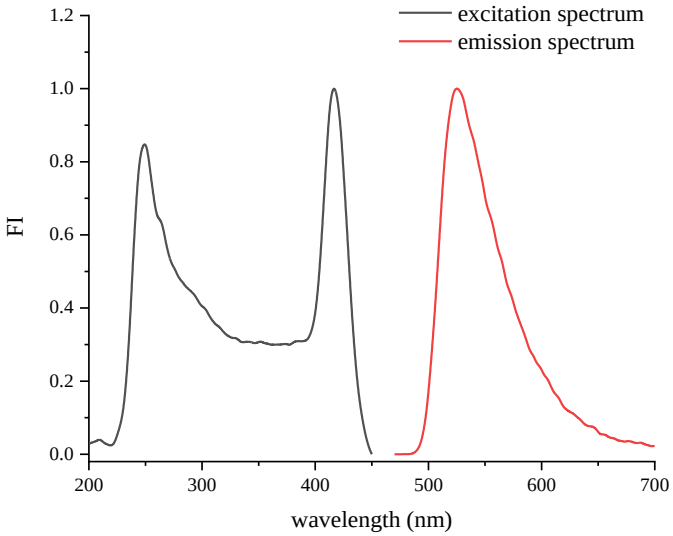


Fig. S3 The normalized fluorescence excitation and emission spectra of gel TPEBA@Glu (1.8 wt% in DMSO).

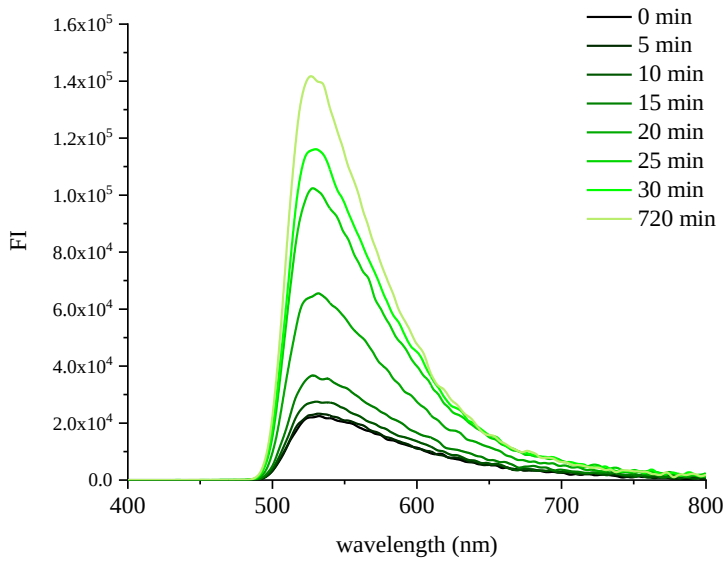


Fig. S4 Fluorescent intensity of solution of TPEBA with 2 equiv. Glu as a function of heating time at 70 °C.

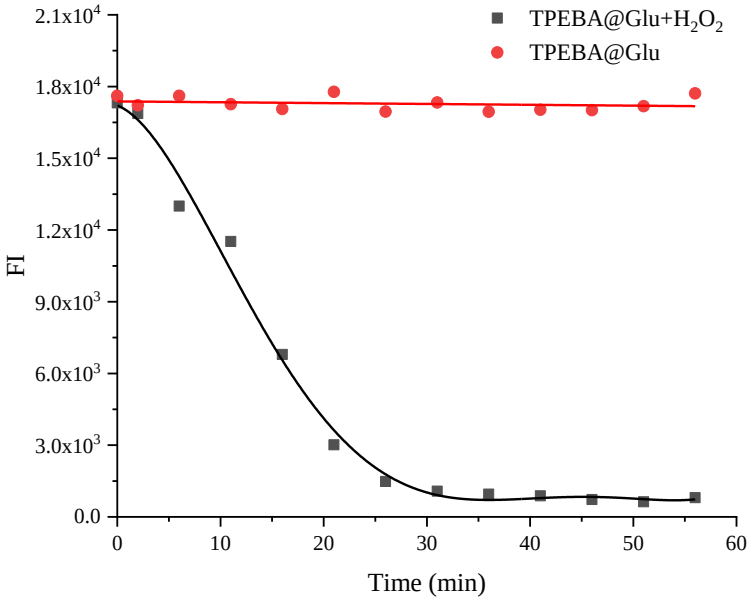


Fig. S5 Fluorescent intensity of TPEBA@Glu with and without H_2O_2 (20 mM) as a function of time.

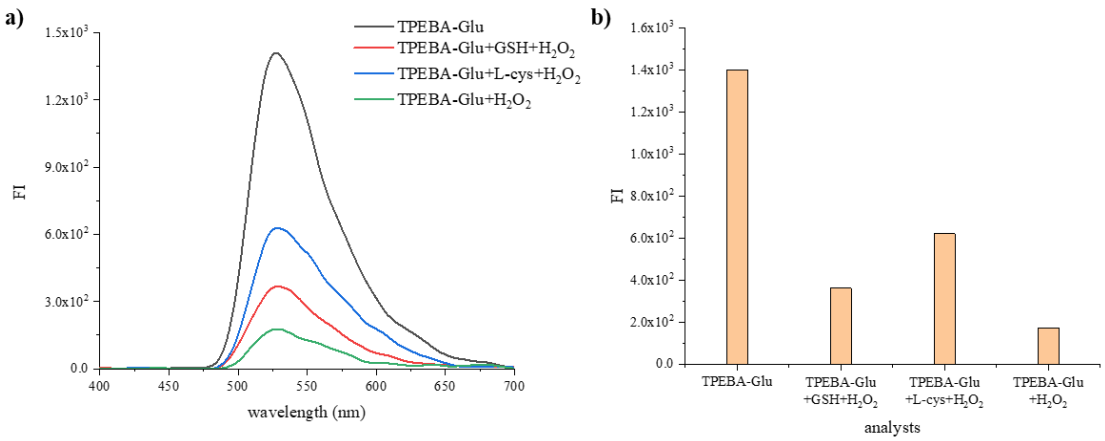


Fig. S6 a) Fluorescent intensity and b) its quantification analysis of TPEBA-Glu (6 μM in PBS buffer with 10% DMSO at pH=7.4) towards H_2O_2 (10 mM) in the presence of L-Cys (10 mM) and GSH (10 mM), respectively. λ_{ex} = 416 nm. Reaction time: 30 min.

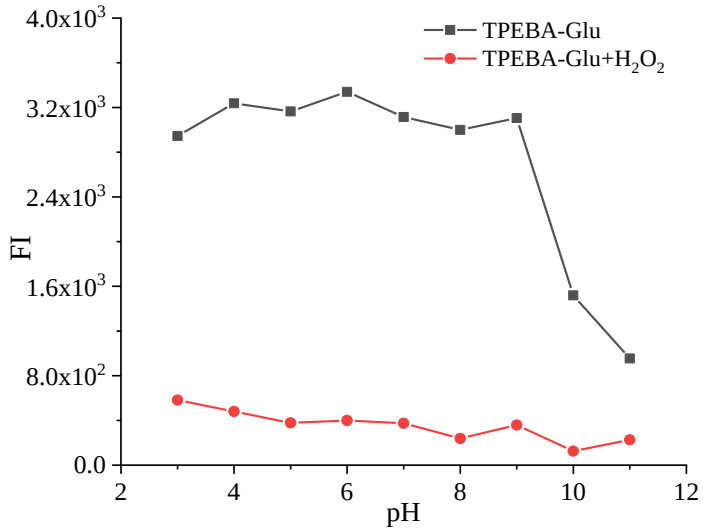


Fig. S7 pH effect to fluorescent intensity of TPEBA-Glu (6 μM) with and without H_2O_2 (10 mM).

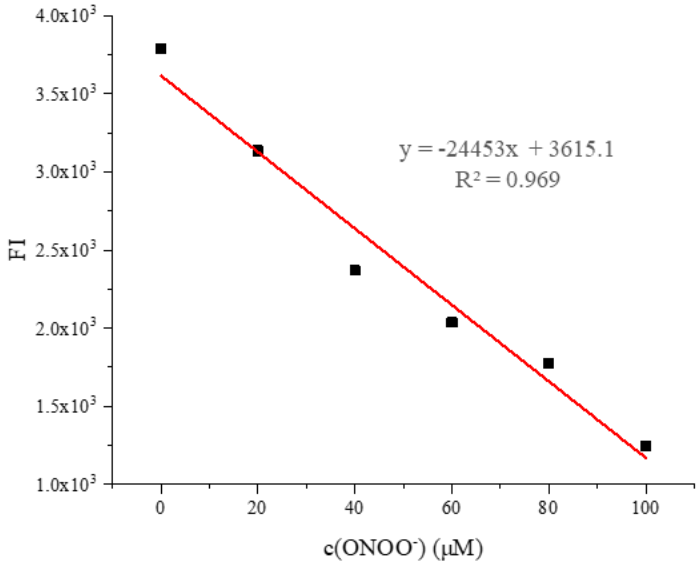


Fig. S8 Linearity of fluorescent intensity of TPEBA-Glu (6 μM in PBS buffer with 10% DMSO at pH=7.4) varied the concentration of $ONOO^-$ at 0, 20, 40, 60, 80, 100 μM. λ_{ex} = 416 nm. Reaction time: 30 min.

5. Fourier transform infrared (FTIR) spectra

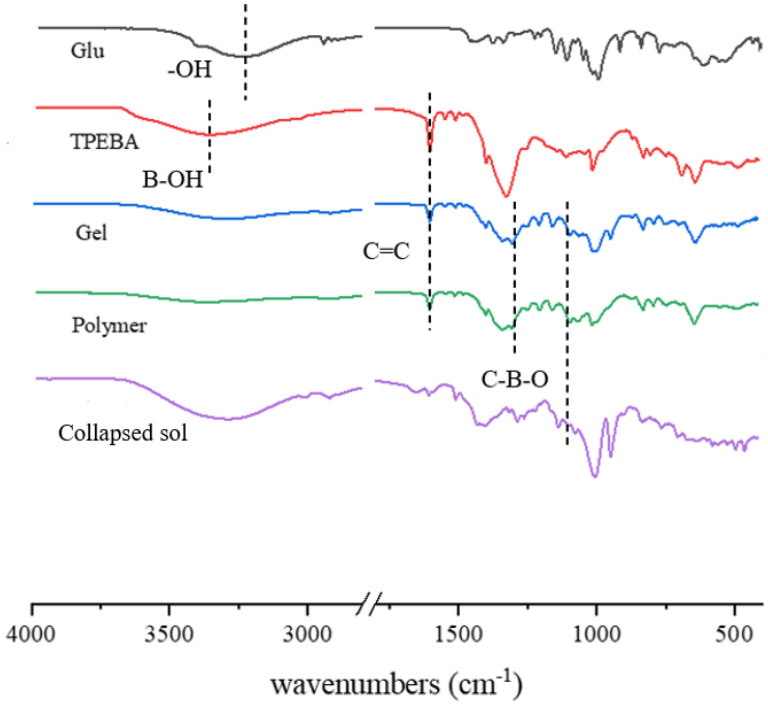


Fig. S9 FTIR spectra of Glu, TPEBA, gel TPEBA@Glu, polymer TPEBA-Glu and collapsed sol of TPEBA@Glu with excess H_2O_2.

6. ^{1}H NMR study

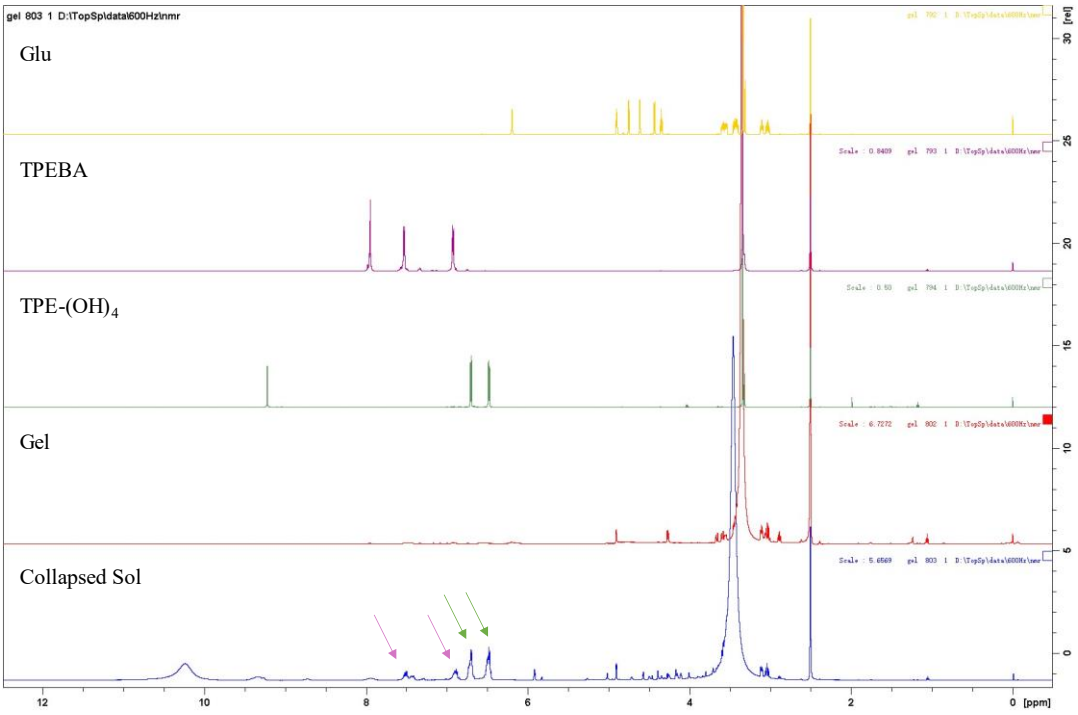


Fig. S10 ^{1}H NMR spectra of Glu (0.04 M), TPEBA (0.02 M), TPE(OH)$_4$ (0.02 M), gel TPEBA@Glu (1.8 % wt), and collapsed sol of TPEBA@Glu after adding H_2O_2 (0.2 M, reaction time: 3 h).

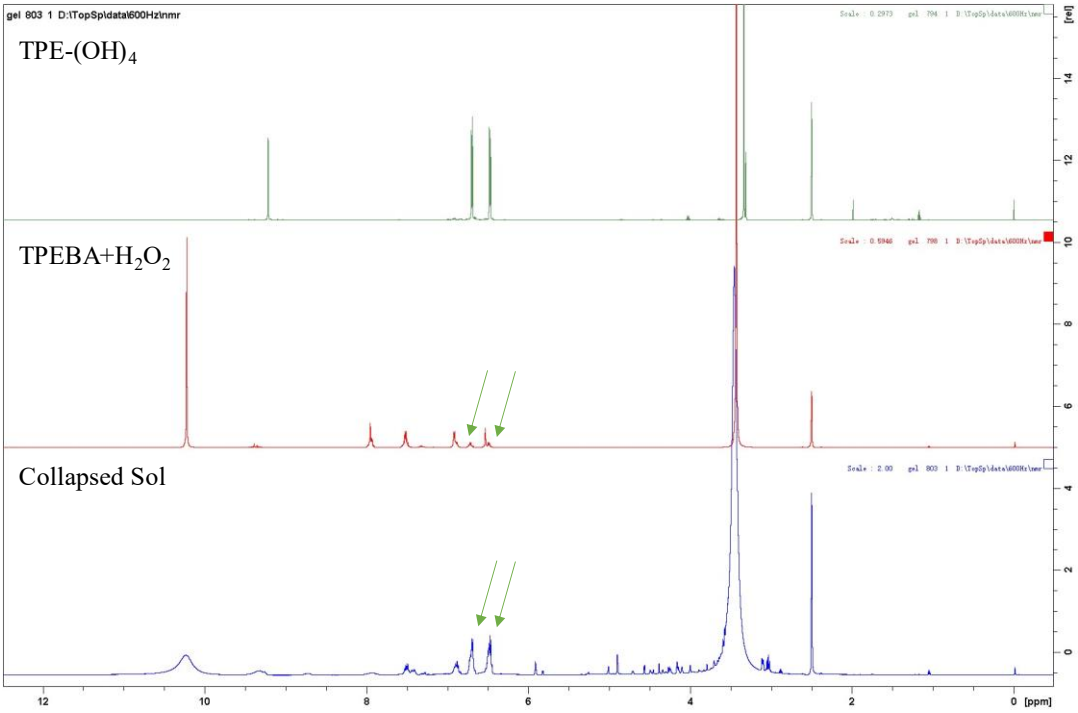


Fig. S11 ^{1}H NMR spectra of TPEBA (0.02 M), and gel TPEBA@Glu (1.8 % wt) with H_2O_2 (0.2 M, reaction time: 3 h).

7. XPS data

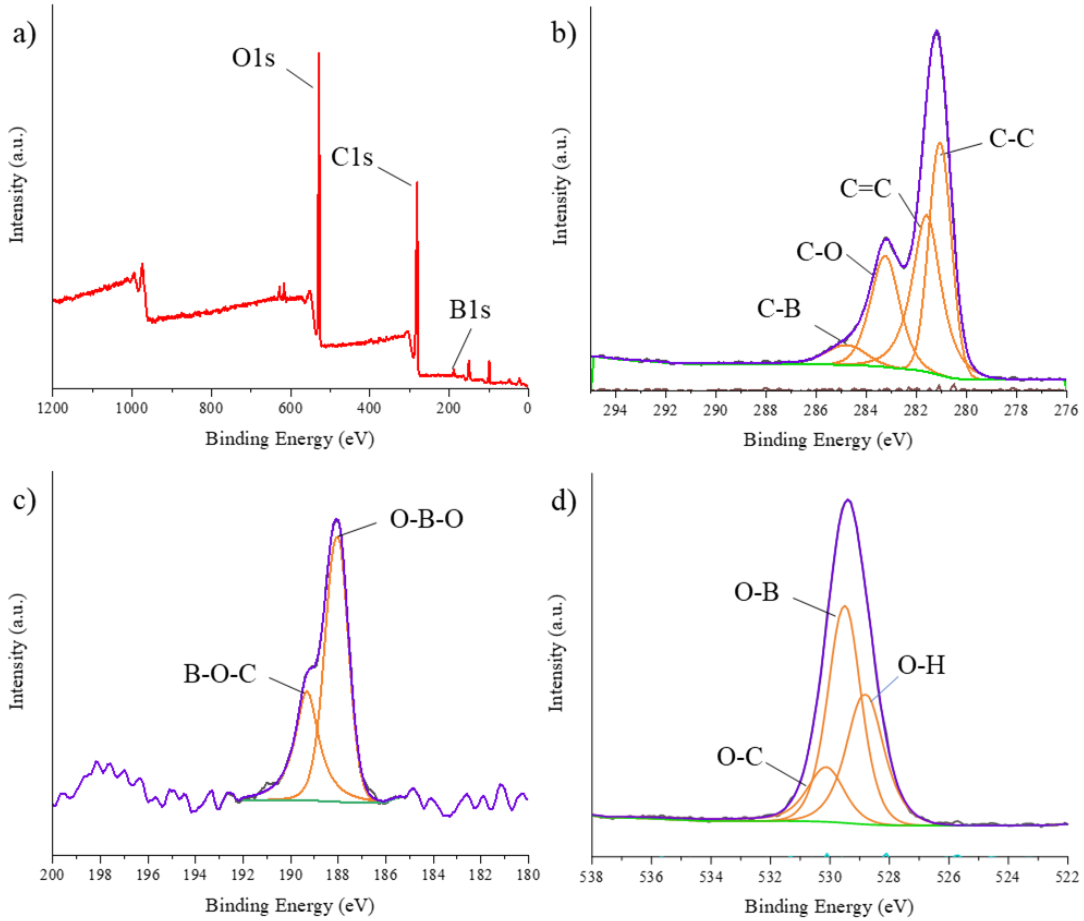


Fig. S12 XPS wide-scan spectrum of a) TPEBA@Glu xerogel. XPS narrow-scan spectra of TPEBA@Glu xerogel for b) C 1s (c) B 1s, and (d) O 1s.

The C 1s spectrum exhibited three peaks at 284.8, 283.3, 281.6 and 281.1 eV designating to C-B, C-O, C=C and C-C bond. The B 1s spectrum exhibited two peaks at 189.3 and 188 eV designating to C-B-O and O-B-O bond. From O 1s spectrum, three peaks at 530.2, 529.5 and 528.8 eV can be ascribed to three types of oxygen species, which are the C-O, B-O, and O-H bond respectively.

8. Morphology study

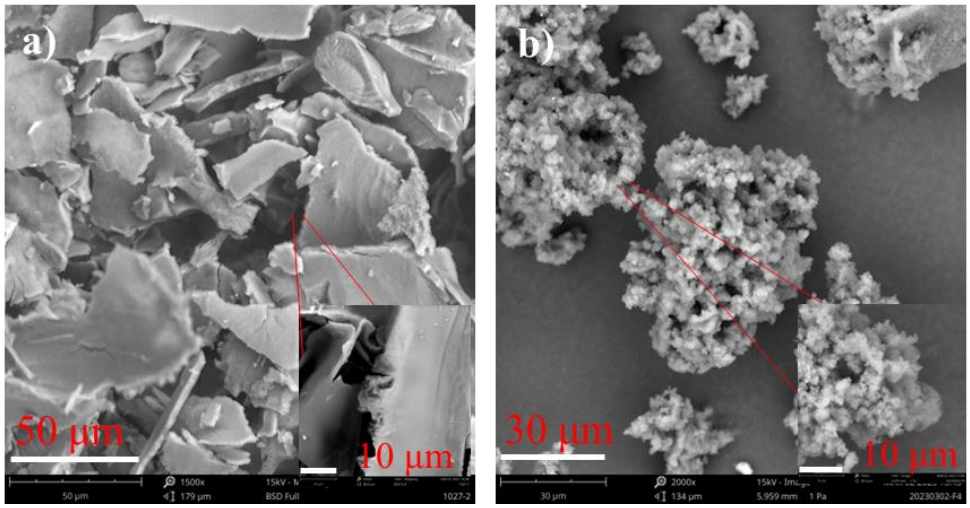
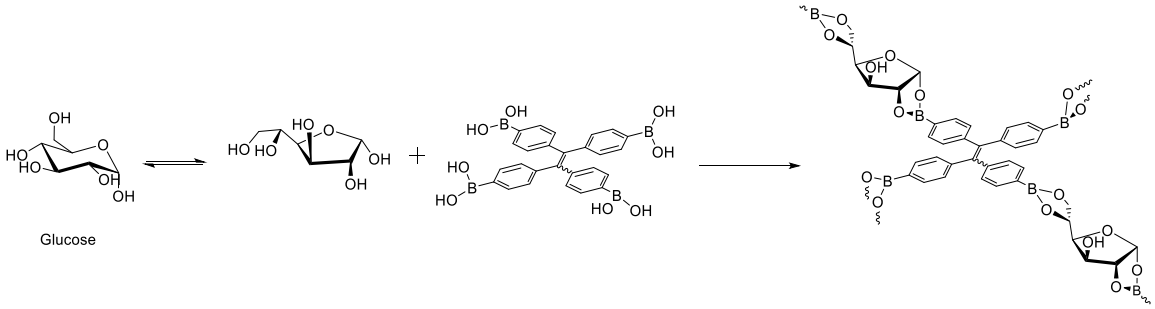


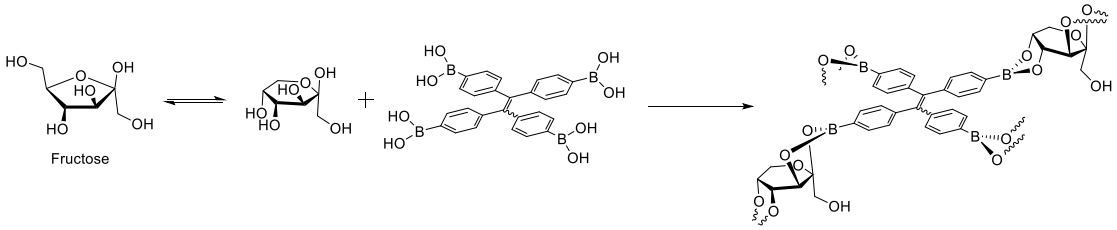
Fig. S13 SEM images of (a) xerogel TPEBA@Glu, and (b) polymer TPEBA-Glu.

9. Proposed reaction mechanism

9.1 Proposed reaction mechanism for TPEBA and monosaccharides

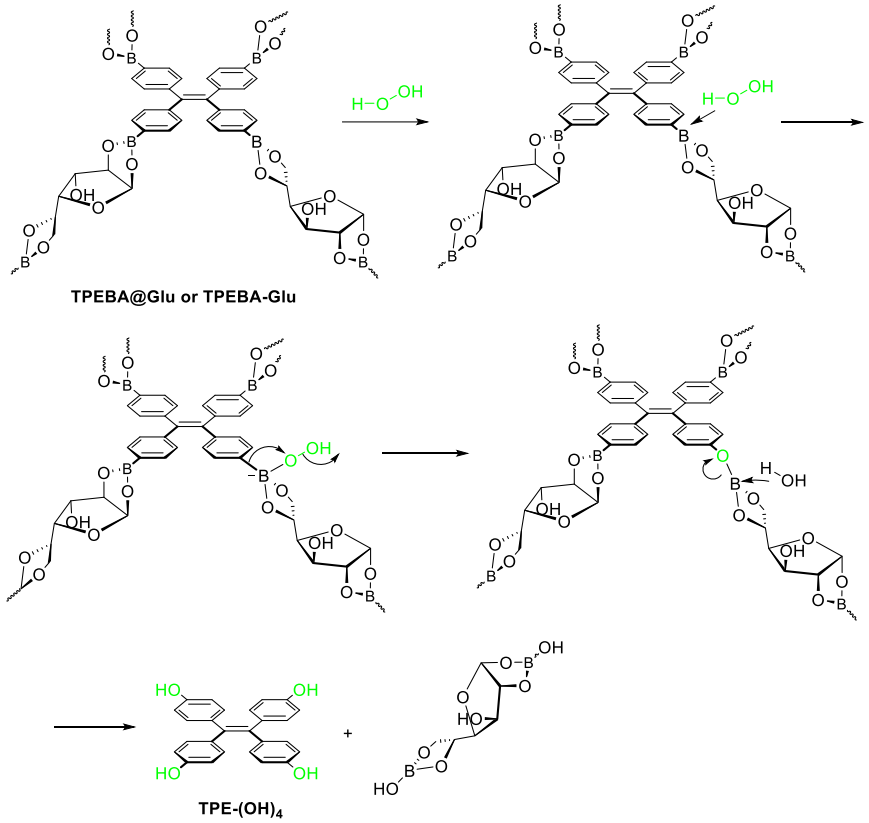


Scheme S2 Proposed reaction mechanism for TPEBA and Glucose (n/n, 1:2 in DMSO).



Scheme S3 Proposed reaction mechanism for TPEBA and Fructose (n/n, 1:2 in DMSO).

9.2 Proposed reaction mechanism for the detection process



Scheme S4 Proposed reaction mechanism for the detection of ROS (eg. H_2O_2).

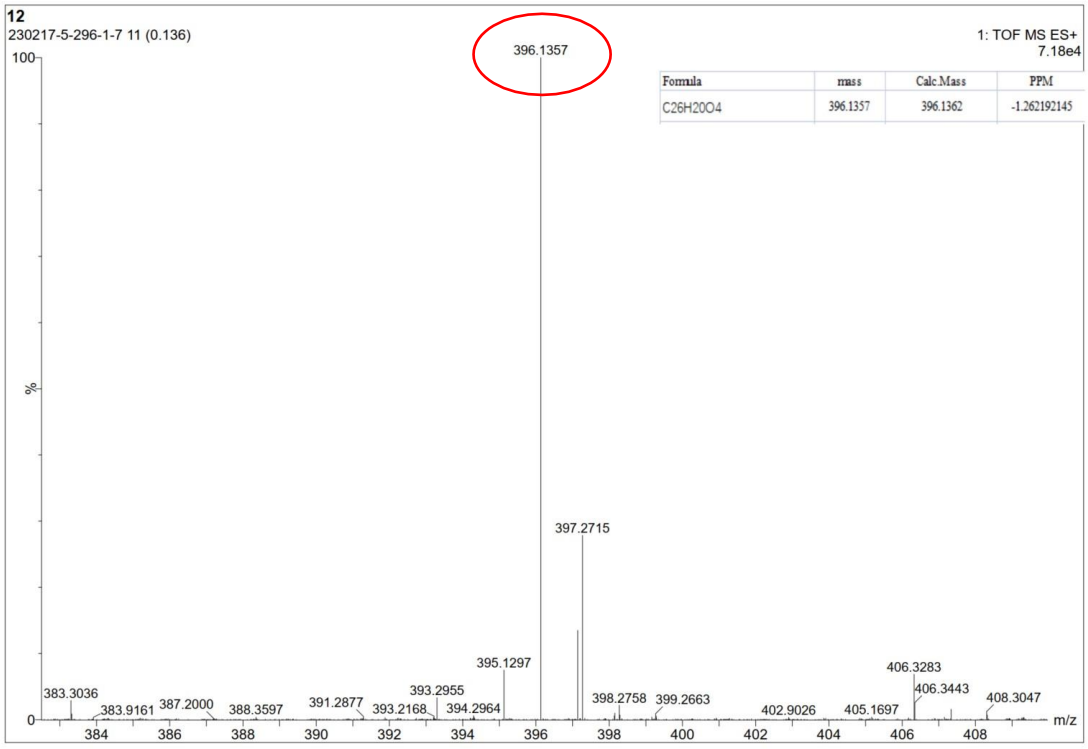


Fig. S14 HR-MS spectra of TPE-$(OH)_4$.

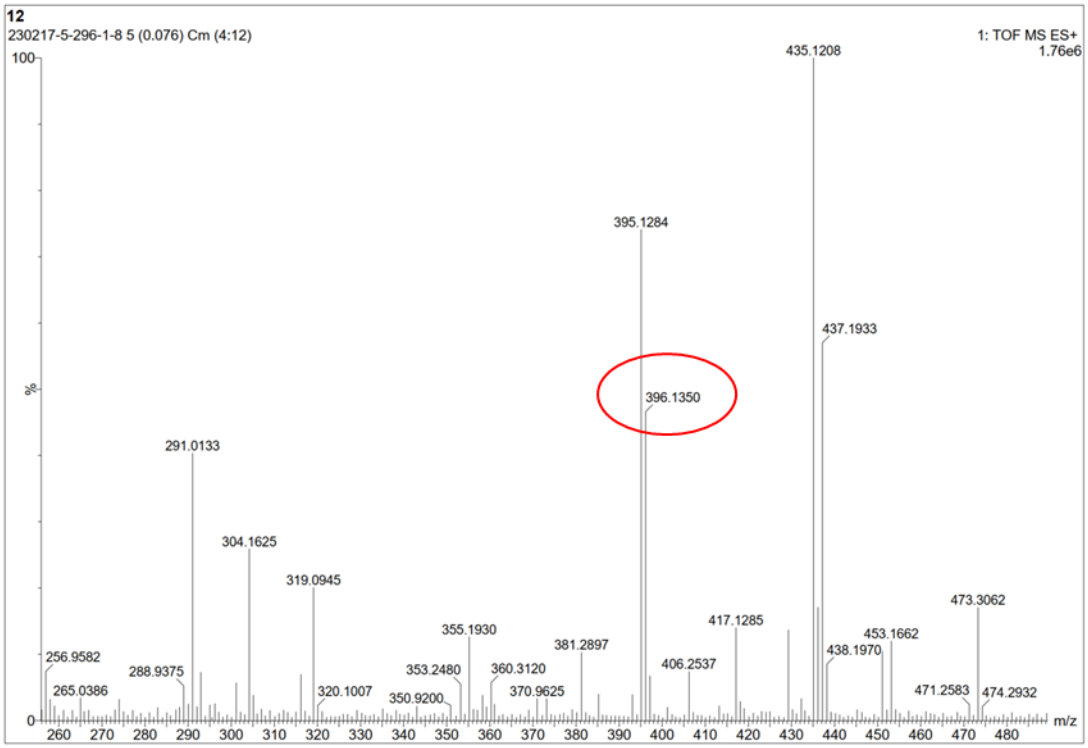


Fig. S15 HR-MS spectra of collapsed sol from the TPEBA@Glu with excess H_2O_2.

10. Cell viability

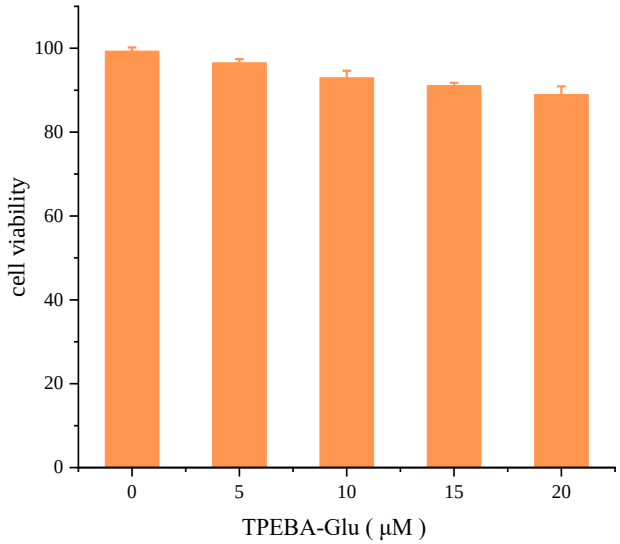


Fig. S16 MTT assay of Raw 264.7 cells with different concentrations of TPEBA-Glu at 0, 5, 10, 15, 20 μM.

11. Quantification analysis of fluorescent intensity in cell

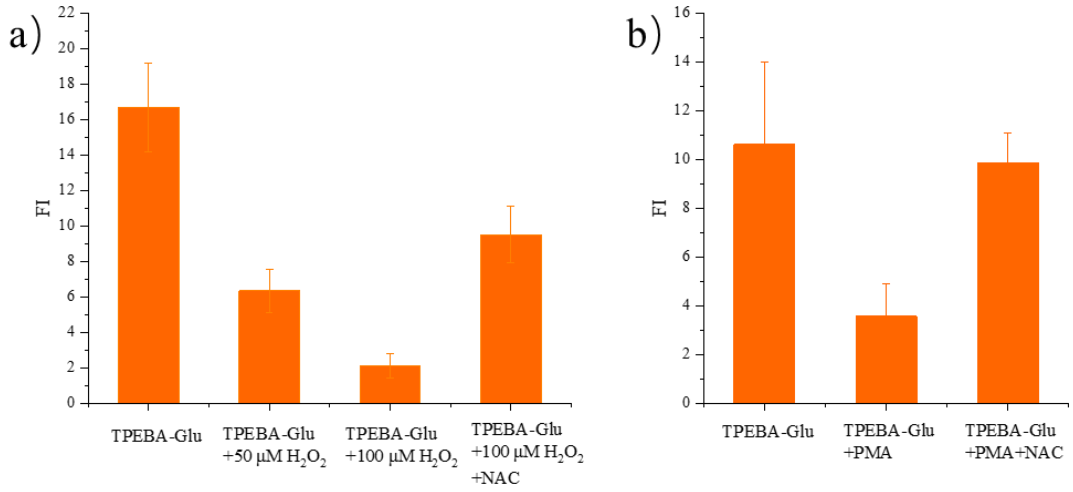
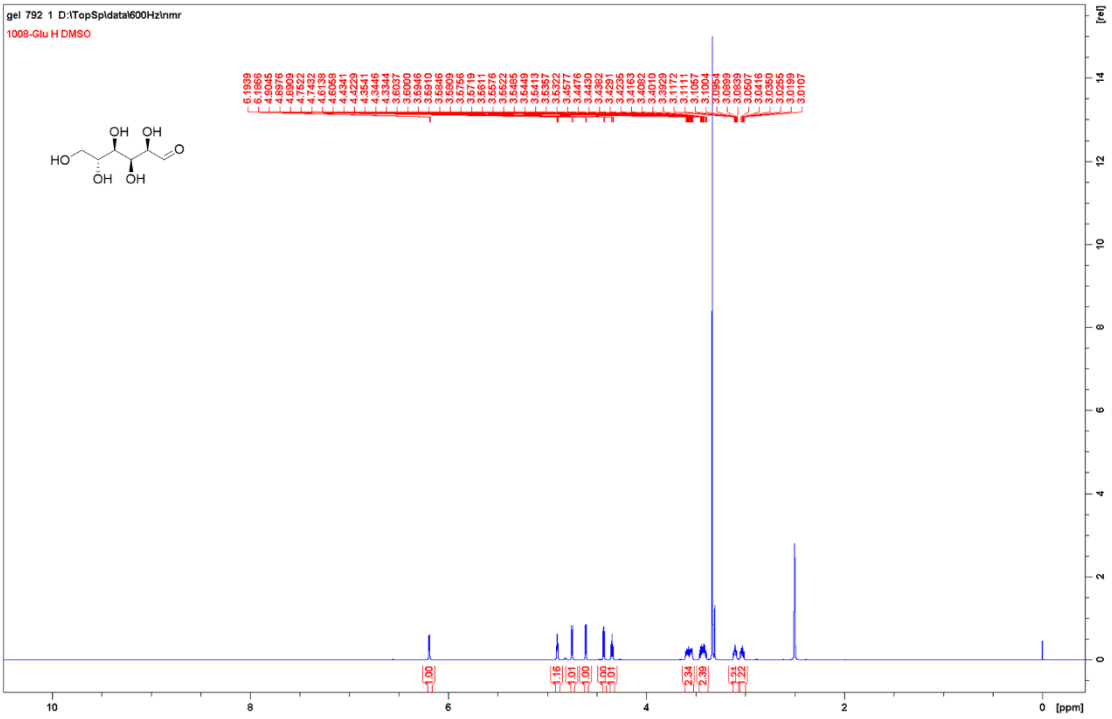
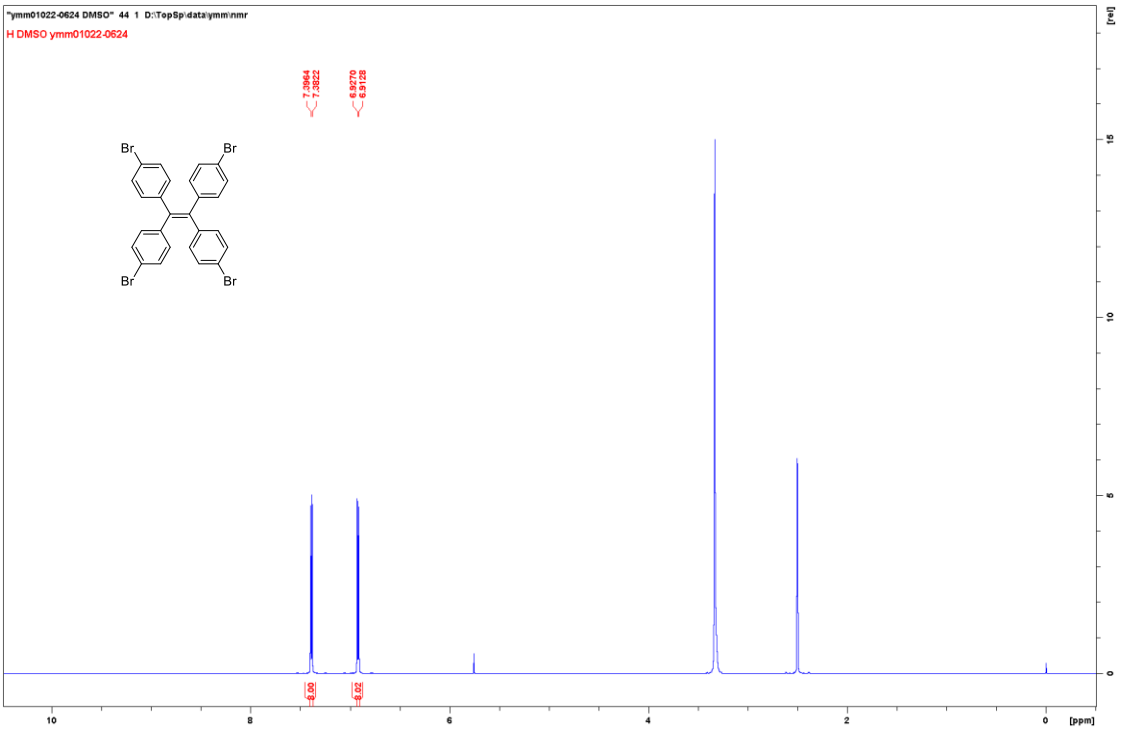


Fig. S17 Quantification analysis of fluorescent intensity of TPEBA-Glu with and without a) exogenous and b) endogenous H_2O_2 in Raw 264.7 cells.

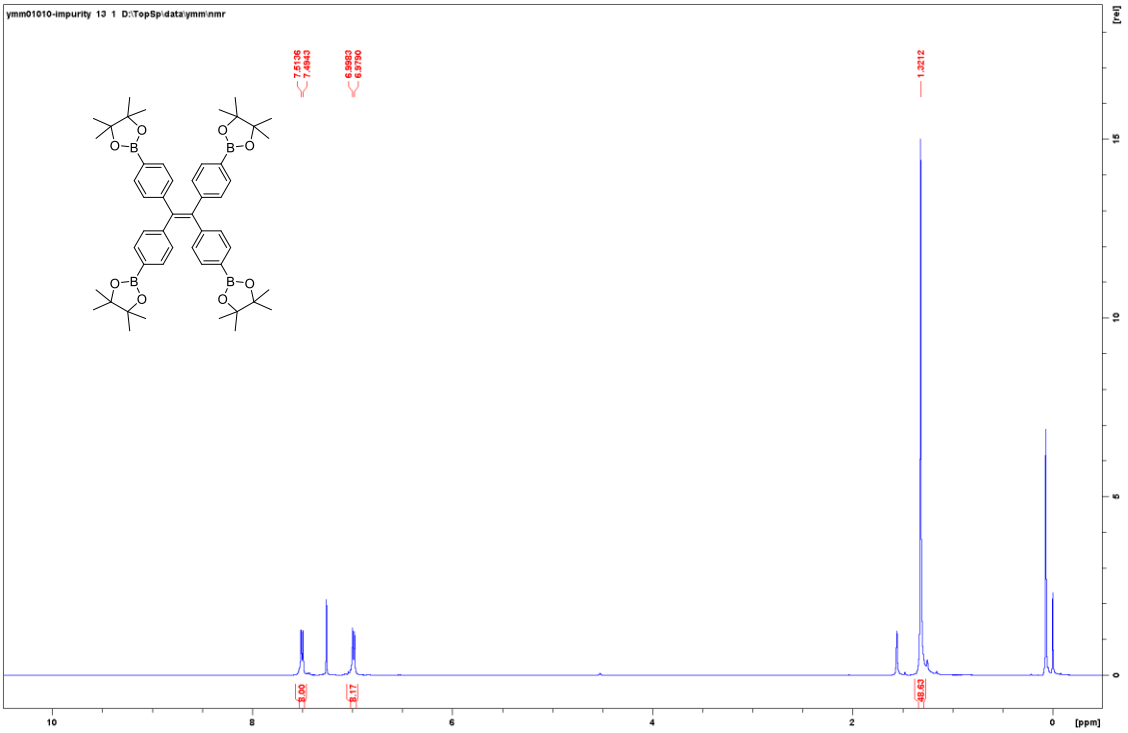
12. ^{1}H NMR spectra



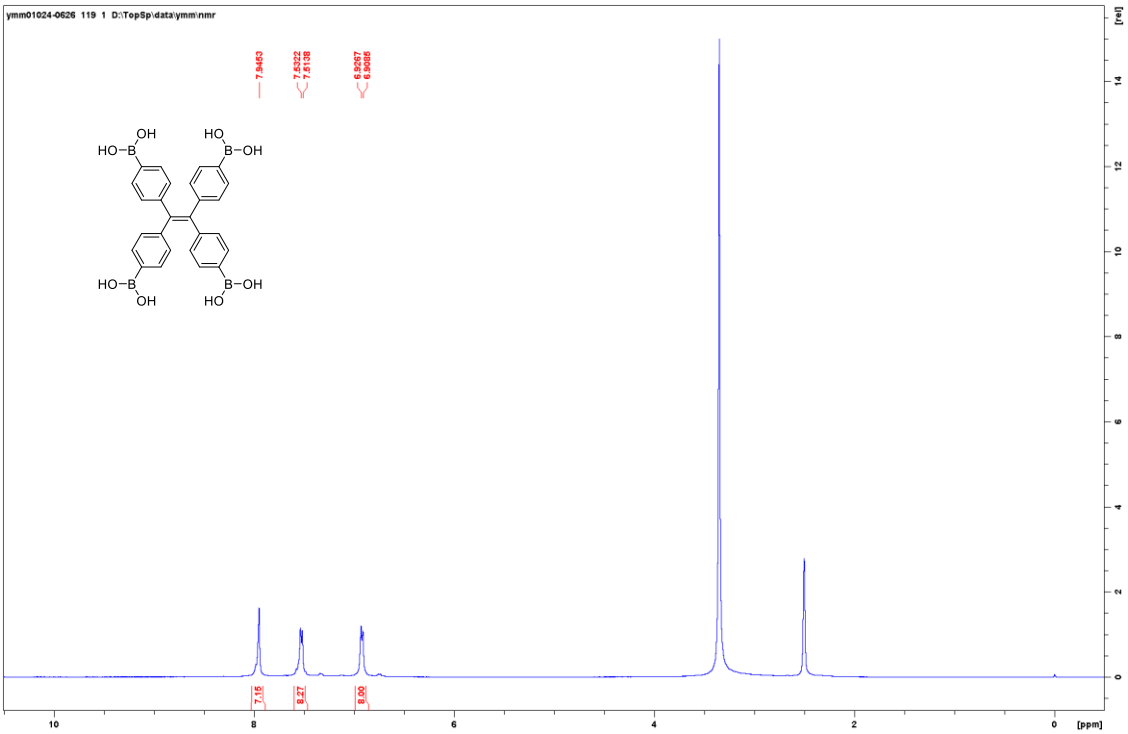
^{1}H NMR (DMSO-d6, 600 MHz) spectrum of glucose.



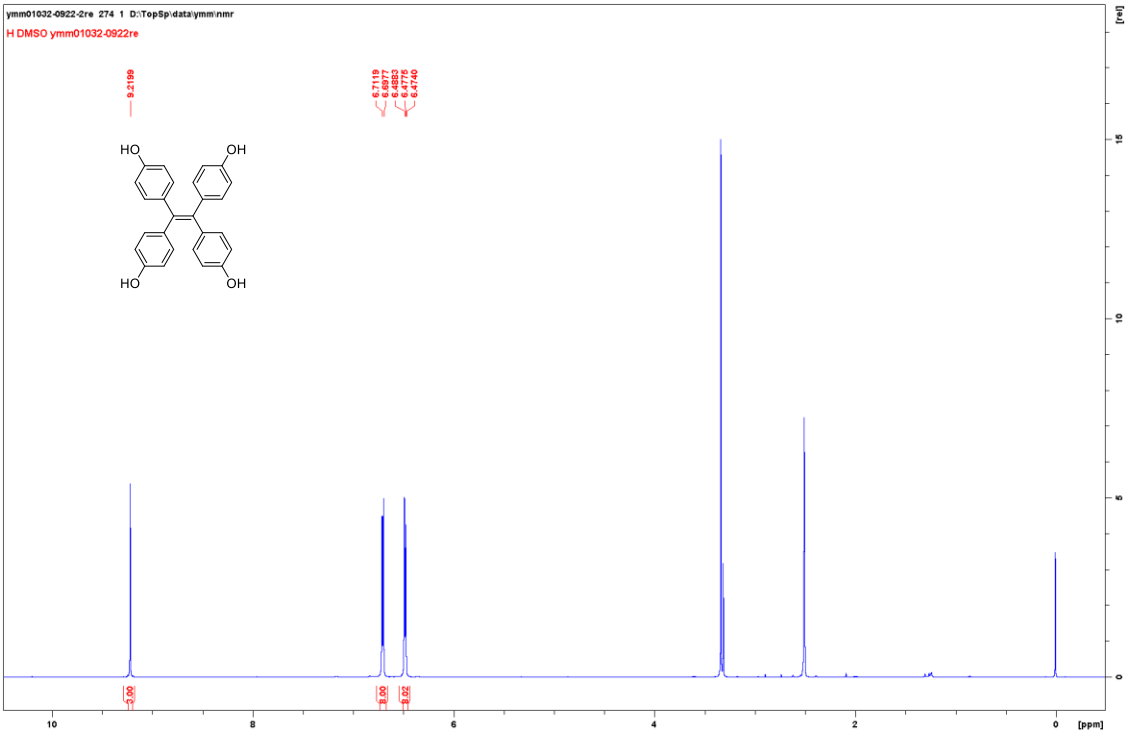
^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of TPE-Br_4.



^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of TPE-Bpin$_4$.



^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of TPEBA.



^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of TPE-$(OH)_4$.

13. Reference

1. S. Dalapati, E. Jin, M. Addicoat, T. Heine and D. Jiang, *J. Am. Chem. Soc.*, 2016, 138, 5797-5800.
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3. J. W. Reed, H. H. Ho and W. L. Jolly, *J. Am. Chem. Soc.*, 1974, 96, 1248-1249.