Supporting Information

A highly sensitive and selective antioxidant probe based on bi-modally functionalized conjugated polyelectrolyte

Dejun Pei, Jinqing Hong, Feng Lin, Zheng Shi, Zhixin Chen, Hongyuan Nie and Xiangqun Guo*

The Key Laboratory of Analytical Science and the Key Laboratory for Chemical Biology of Fujian Province, Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China. Fax: (+86)592-2188612; Tel: (+86)592-2188612; Email: xqguo@xmu.edu.cn

Chemicals and apparatus. 1, 3- propanesultone and ethyl 4-bromobutyrate were purchased from Alfa Aesar. 4-amino-2,2,6,6-tetramethylpiperidine-N-oxide free radical(4A-TEMPO), N-hydroxysuccinimide (NHS) and 1,4-diethynylbenzene were purchased from Acros Organics. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Bio Basic Inc.. Tetrakis(triphenylphosphine)palladium (Pd(PPh$_3$)$_4$) was obtained from J&K Scientific Ltd.. Chemicals used for syntheses were as received from commercially available sources of AR grade.

The emission spectra were recorded on a RF-5301PC spectrofluorophotometer (Shimadzu). Time-resolved luminescence measurements were carried out on a FluoroMax-4 TCSPC (HORIBA Jobin Yvon) spectrofluorometer, using a pulsed 457 nm diode laser (IBH NanoLED) as the excitation source. Decay data analysis was performed using the DAS6 software (HORIBA Jobin Yvon IBH). .0Absorption spectra were performed on a SP-756PC UV–vis spectrophotometer (Shanghai Spectrum Instruments). Absolute quantum yields (A-QYs) were measured using the integrating sphere approach on a FluoroMax-4 spectrofluorometer equipped with an F-3018 integrating sphere accessory (Horiba Jobin Yvon). ESR measurements were performed using a Bruker EMX X-Band ESR spectrometer. All microwave parameters were kept constant, approximately 9.8 GHz microwave frequency, 20 mW microwave power, 10 dB attenuator, 100 kHz modulation frequency, 1.0 G modulation amplitude, 0.64 ms time constant. ^{1}H NMR spectra were measured on Brucker Avance 400 NMR spectrometer at room temperature. High resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF-Q Ⅱ ESI mass spectrometer.

Synthesis of RF-PPE-SO$_3$
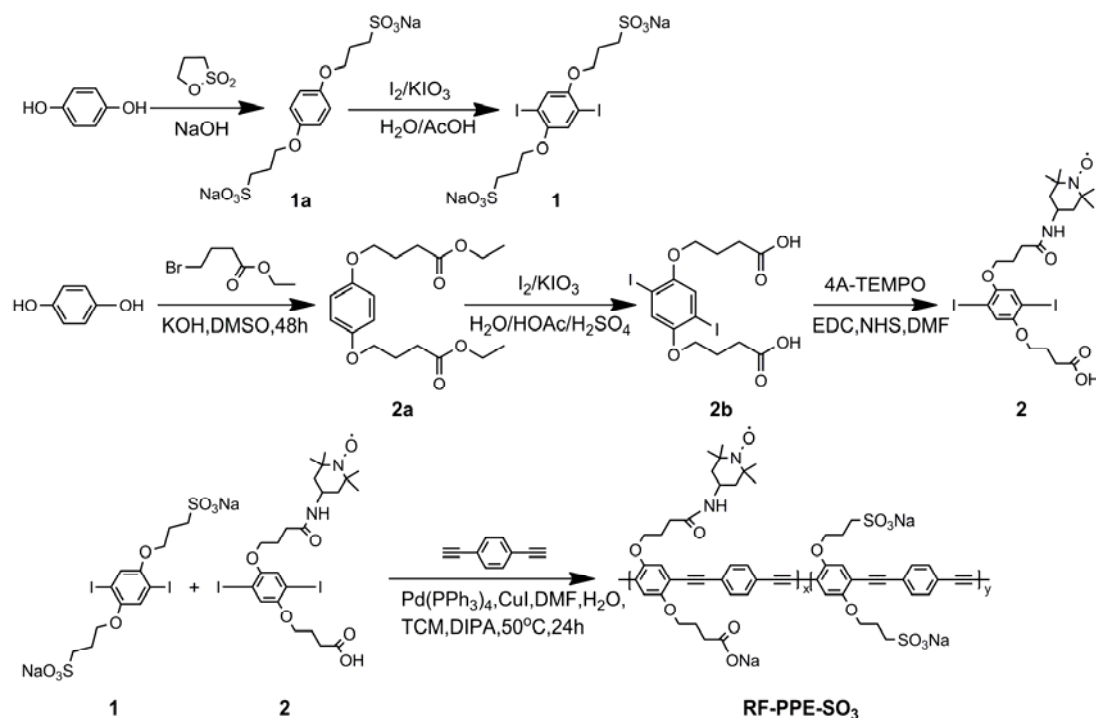
Scheme S1 Synthetic routes for the preparation of RF-PPE-SO$_3$

The synthesis procedures of monomers and RF-PPE-SO$_3$ are shown in Scheme S1. Monomer

1, 1,4-diiodo-2,5-di(propyloxysulfonate)benzene, was synthesized from *p*-hydroquinone by a two-step reaction according to the reported literature[1]. Monomer 2, 4-{4-[3-(1-hydroxy-2,2,6,6-tetramethyl-piperidinooxy-4-ylcarbamoyl)-propoxy]-2,5-diiodo-phenoxy}-butyric acid, was achieved by treating 1,4-di(4-oxy-butyric acid)-2,5-diiodobenzene (2b)[2] with 4-amino-2,2,6,6-tetramethylpiperidine-N-oxide free radical (4A-TEMPO) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). In this paper, a typical Sonogashira reaction condition was applied to the synthesis of the RF-PPE-SO$_3$ by the polymerization of 1, 2 and 1,4-diethynylbenzene.

1,4-Di(propyloxysulfonate)benzene (1a). 3.18 g of white powder of **1a** was obtained with 0.88 g (8 mmol) of *p*-hydroquinone and 2.44 g (20 mmol) of 1, 3- propanesultone as the starting reactants. The powder was characterized with 1H NMR (DMSO-d$_6$, δ$_{ppm}$): 1.96 (quintet, 4H, 2 × -CH$_2$-C*H*$_2$-CH$_2$-), 2.54 (t, 4H , 2 ×-CH$_2$-SO$_3$Na), 3.96 (t, 4H, 2 × -O-CH$_2$-), 6.82 (s, 4H, 4 × Ar-H).

Monomer 1. A fine white powder of **1** was obtained. The powder was characterized with 1H NMR (DMSO-d$_6$, δ$_{ppm}$): 1.98 (quintet, 4H, 2 × -CH$_2$-C*H*$_2$-CH$_2$-), 2.59 (t, 4H, 2 × -CH$_2$-SO$_3$Na), 4.03 (t, 4H, 2 × -O-CH$_2$-), 7.29 (s, 2H, 2 × Ar-H).

1, 4-Di (ethyl 4-oxy-butyrate)benzene (2a). A fine white powder of **2a** was obtained. 1H NMR (400MHz, DMSO-d$_6$, δ$_{ppm}$): 1.17 (t, 6H, 2 × -CH$_3$), 1.93 (quintet, 4H, 2 × -CH$_2$-C*H*$_2$-CH$_2$-), 2.43 (t, 4H, 2 × -CH$_2$-C=O), 3.93 (t, 4H, 2 × -O-C*H*$_2$-CH$_2$-), 4.06 (q, 4H, 2 × -C*H*$_2$-CH$_3$), 6.82 (s, 4H, 4 × Ar-H).

1, 4-di (4-oxy-butyric acid)-2, 5-diiodobenzene (2b). A white powder of **2b** was obtained. 1H NMR (400MHz, DMSO-d$_6$, δ$_{ppm}$): 1.91(quintet, 4H, 2 × -CH$_2$-C*H*$_2$-CH$_2$-), 2.44(t, 4H, 2 × -CH$_2$-C=O), 3.99(t, 4H, 2 × -O-C*H*$_2$-CH$_2$-), 7.33(s, 2H, 2 × Ar-H), 11.89(s, 2H, 2 × COOH).

Monomer 2. In a round-bottom flask, 2b (0.108 g, 0.2 mmol) was dissolved in DMF (6 ml) and cooled in ice-water bath for 10 min under stirring. NHS (0.07 g, 0.6 mmol) and EDC (0.116 g, 0.6 mmol) were added. The resulting mixture was stirred in ice-water bath for 2 h. Then 4A-TEMPO (0.041 g, 0.24 mmol) was added. After another 2h-stiring, the reaction mixture was dissolved in DCM (10 mL) and washed by 0.1 M of HCl (3×10 mL) and water (3×10 mL) in sequence. The organic phase was dried over Na$_2$SO$_4$ and filtered. The solvent was then removed under reduced pressure to afford an orange solid. MS (APCI) *m/z*: 688.4 [M+H]$^+$，HRMS (ESI) m/z: Found 689.0520 [M+2H]$^+$; C$_{23}$H$_{33}$I$_2$N$_2$O$_6$· requires 689.0585.

RF-PPE-SO$_3$. The copolymerization of monomer 1, monomer 2 and 1,4-diethynylbenzene was carried out in the presence of a palladium catalyst (tetrakis(triphenylphosphine)palladium, Pd(PPH$_3$)$_4$) and Cuprous iodide (CuI) at 50 ºC for 24 h. The crude RF-PPE-SO$_3$ solution was dialysed against de-ionized water using a membrane with a 14000 MWCO for 3 days. The ratio of [TEMPO] to [RF-PPE-SO$_3$] is 1:330, calculated by comparing the ESR signal of RF-PPE-SO$_3$ with a calibration curve of 4A-TEMPO (that is, ESR signals change as a function of concentrations of 4A-TEMPO. Data not shown). The weight-averaged molecular weight (M$_w$) of RF-PPE-SO$_3$ was estimated to be around 100 kD by ultrafiltration.

PPE-OBS-SO$_3$ was synthesized by the copolymerization of monomer 1, 1,4-di(ethyl 4-oxy-butyrate)-2,5-diiodo-benzene[2] and 1,4-diethynylbenzene under the similar conditions, followed by alkaline hydrolysis of the ethyl group in the mixture of NaOH, methanol and THF. The crude PPE-OBS-SO$_3$ solution was dialysed against de-ionized water using a membrane with a 14000 MWCO for 3 days. The M$_w$ of PPE-OBS-SO3 was also estimated to be around

100 kD by ultrafiltration. 1H NMR (400MHz, DMSO-d_6, δ_{ppm}): 2.15-1.94(br, 4H, -CH$_2$-CH_2-CH$_2$-) , 2.78-2.63(br, 4H, -CH$_2$-C=O/-CH$_2$-SO$_3$Na) , 4.24-4.02(br, 4H, -O-CH_2-CH$_2$-) , 7.21(s, 2H, Ar-H), 7.61(s, 4H, Ar-H).

Molecular Weight Characterization of RF-PPE-SO$_3$ and PPE-OBS-SO$_3$. Generally speaking, it is difficult to determine the molecular weight and distributions of water-soluble rigid-rod conjugated polymers[3, 4] ENREF_4. Efforts to use organic or aqueous phase gel permeation chromatography (GPC) to determine the molecular weight and polydispersity of the RF-PPE-SO$_3$ and PPE-OBS-SO$_3$ have been unsuccessful. Thus, we applied ultrafiltration method to estimate the molecular weight of the polymers according to the literature by Kirk S. Schanze[5]. Ultrafiltration experiments of the polymers in a 50:50 DMSO-water mixture were carried out using cellulose membranes with different MWCO (30kD, 50kD and 100kD). The absorption spectrum of the starting solution and the filtrate were monitored to determine the concentration of polymers. No absorbance of the filtrate of RF-PPE-SO$_3$ and PPE-OBS-SO$_3$ was observed when using 30 and 50 kD MWCO membranes. Approximately 10% of the polymers were filtrated when using 100 kD MWCO membrane. That is, the molecular weights of RF-PPE-SO$_3$ and PPE-OBS-SO$_3$ were ~100kD.
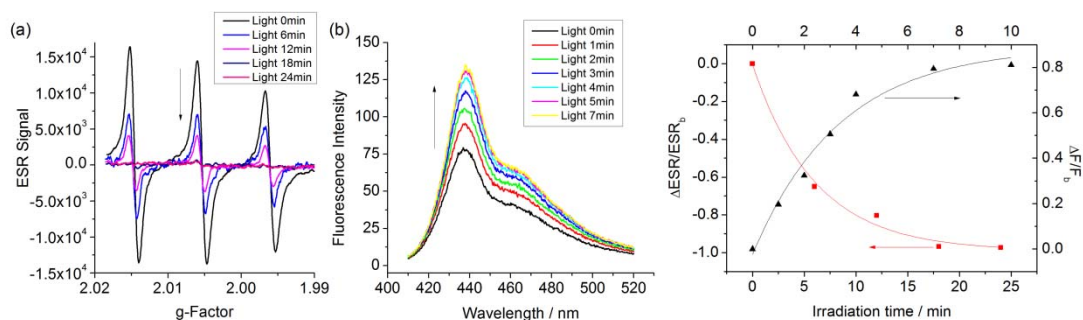

Fig.S1 ESR (a) and fluorescence (b) spectra of RF-PPE-SO$_3$ before and after trapping carbon-centered radicals. The carbon-centered radicals were resulted from the photolysis of AIBN (Scheme S2). The ESR intensity decrease was observed following irradiation of AIBN. And concomitantly, increase of fluorescence intensity was measured. (c) ΔESR/ESR$_b$ and ΔF/F$_b$ as a function of irradiation time. Where ΔESR = ESR-ESR$_b$; ΔF = F-F$_b$. ESR and F represent the ESR and fluorescence intensity of RF-PPE-SO$_3$ in the presence of AIBN, and ESR$_b$ and F$_b$ represent the ESR and fluorescence intensity of RF-PPE-SO$_3$ in the absence of 0.1M of AIBN, respectively.
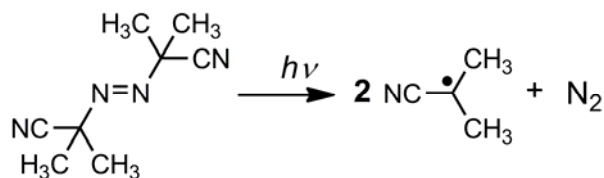
Scheme S2. The production of carbon-centered radicals from the photolysis of AIBN.
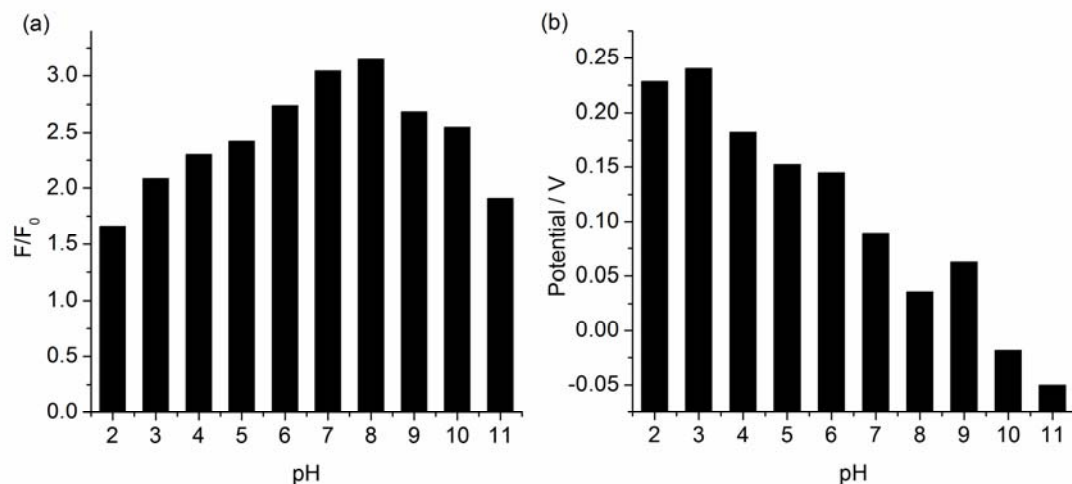
Fig.S2 Influences of pH on fluorescence recovery efficiency (F/F_0) of RF-PPE-SO$_3$ by AA (a) and potential vs. SCE of AA (b) in B-R buffer solution.
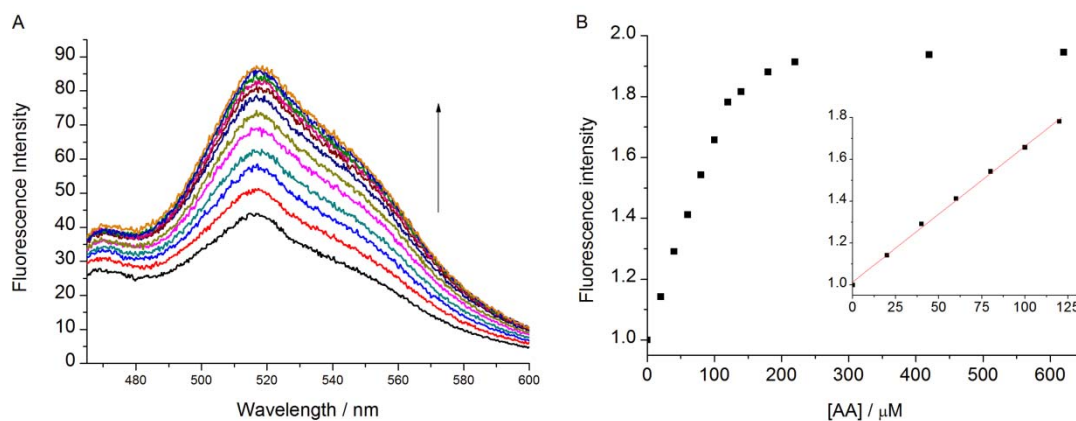

Fig.S3 (a) Fluorescence spectra of RF-PPE-SO$_3$ (0.5 µM) in the presence of various concentrations of AA (0-620 nM) (λ_{ex}=438nm, slit width: 5nm/5nm). (b) Relationship between fluorescence recovery efficiency of RF-PPE-SO$_3$ and concentration of AA
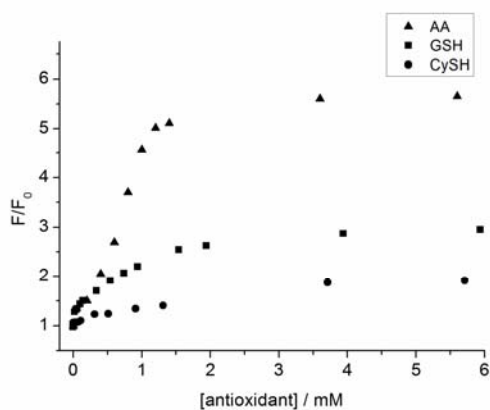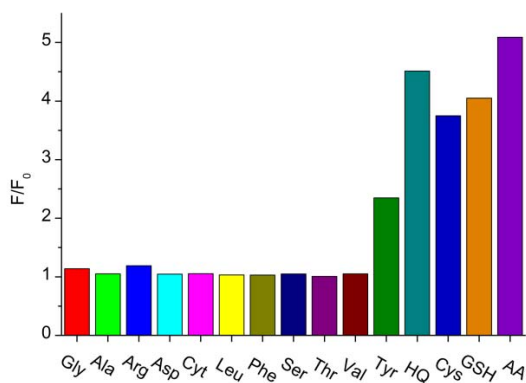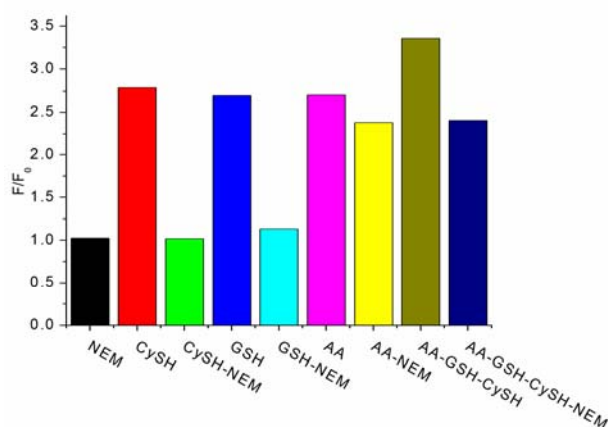

Fig.S4 Relationship between fluorescence recovery efficiency of RF-PPE-SO$_3$ (10 µM) and concentration of AA (▲), GSH (■) and CySH (●).

Fig.S5 The fluorescence response behaviors of 10 µM RF-PPE-SO$_3$ on various amino acid and antioxidant (200 µM). (λ_{ex}=438nm, slit width: 3nm/3nm)

Fig.S6 Different responses of three antioxidants toward the fluorescence of RF-PPE-SO$_3$ in the presence and absence of NEM; F represents the fluorescence intensity of RF-PPE-SO$_3$ in the presence of antioxidant and/or NEM, F$_0$ represents the fluorescence intensity of RF-PPE-SO$_3$.;[NEM] = 500µM; [CySH] = 10µM; [GSH] = 250µM; [AA] = 2.5 µM

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