Electronic Supplementary Information

Anionic conjugated polymer as a multi-action sensor for the sensitive detection of Cu²⁺, PPi, real-time ALP assaying and cell imaging

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Experimental Section

Materials and Methods: All reagents and solvents were purchased from commercial sources and were of reagent grade. Organic phosphates (AMP, GMP, UTP, ADP, TMP, GTP, CTP, TTP ATP and glucose-6-phosphate), Dulbecco's modified eagle's media (DMEM) high glucose and alkaline phosphatase from bovine intestinal mucosa was purchased from Sigma Aldrich and used as received. Breast cancer cell line MADAMB 231 and mouse macrophages J774A.1 cells were procured from NCCS, Pune. Ultravioletvisible light (UV-Vis) and photoluminescence (PL) spectra were recorded on a Perkin-Elmer Model Lambda-25 spectrophotometer and Horiba Fluoromax-4 spectrofluorometer, respectively. A 10 mm \times 10 mm quartz cuvette was used for the solution spectra and emission was collected at 90° relative to the excitation beam with a slit width of 3 nm at 298 K. Images were taken in Epi-fluorescence microscope using Nikon Eclipse 80i. Deionized water was obtained from a Milli-Q system (Millipore). ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were obtained with a Varian-AS400 NMR spectrometer. FT-IR spectra were recorded on a Perkin Elmer spectrometer with samples prepared as KBr pellets. GPC was recorded with a Waters-2414 instrument (polystyrene calibration), FESEM images were recorded on Zeiss (Sigma) microscope.

Synthesis of monomer: Synthesis of 9,9-bis(6-bromohexyl)-9H-fluorene was carried out using a previously established procedure from the literature.¹

Synthesis of Poly(9,9-bis(6-bromohexyl)-9H-fluorene) (PF): The polymer PF was prepared by a previously reported method.² In a 100 mL three-necked round-bottom flask equipped with a argon inlet, anhydrous ferric chloride (0.70g, 4.57mmol) was dissolved in 10 mL of nitrobenzene followed by the addition of 9,9-bis(6-bromohexyl)-9H-fluorene (1.0 g, 2.03 mmol) in 10 mL of nitrobenzene using a syringe. The reaction mixture was

stirred at room temperature for 36 h, followed by precipitation from methanol. The methanol solution was stirred for further 2h, centrifuged and washed thrice with methanol. The resulting polymer PF was dried under reduced pressure to obtain brown fibrous compound (0.695g, 70% yields).

¹H NMR (400 MHz, CDCl₃): δ ppm, 7.68(b), 7.33(b), 3.28(b), 2.16(b), 1.66(b), 1.19(b), 0.80 (b).

¹³C NMR (100 MHz, CDCl₃) δ (ppm): 151.70, 140.72, 127.27, 126.50, 121.51, 55.48, 45.26, 32.80, 29.88, 27.93, 23.90, 22.88.

Synthesis of Poly(tetramethyl-5,5'-(((9H-fluorene-9,9-diyl))bis(hexane-6,1diyl))bis(oxy))diisophthalate] (PFTE): PF (0.100g, 0.20mmol) and dimethyl-5hydroxyisophthalate (0.420g, 2mmol) were dissolved in dry DMF (5 mL) in the presence of K_2CO_3 (10 eq.). The reaction mixture was stirred overnight at 80 °C. After cooling to room temperature, the reaction mixture was poured into chloroform and washed with dil. HCl and brine solution. The separated organic layer was dried over anhydrous magnesium sulfate and evaporated to obtain the PFTE as light yellow powder (0.107g, 70% yields).

¹H NMR (400MHz, CDCl₃): δ ppm, 8.22(b), 7.66(b), 7.34(b), 3.89(b), 2.05(b), 1.61(b), 1.25(b), 0.88 (b).

¹³C NMR (100 MHz, CDCl₃) δ (ppm): 166.18, 159.14, 152.77, 140.43, 131.61, 127.70, 123.25, 122.69, 120.16, 119.74, 68.45, 52.32, 40.56, 29.87, 29.32, 28.92, 25.56, 23.70.
FT-IR: IR (KBr, cm⁻¹): 3418.61, 2926.59, 2852.75, 1725.85, 1595.82 1455.89,1435.33, 1338.47, 1312.13, 1242.07, 1118.52, 1045.11, 758.13..

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Synthesis of Poly[5,5'-(((9H-fluorene-9,9-diyl)bis(hexane-6,1diyl))bis(oxy))diisophthalate] sodium salt (PFT): The target conjugated polyelectrolyte was obtained through the alkali induced saponification method. PFTE (0.05g) was dissolved in THF and a solution of NaOH (0.3 g) in 3 mL water was added. The mixture was heated for 12h at 50 °C. After cooling to room temperature, THF was removed and the mixture was dialyzed against water for 3 days using a membrane with a molecular weight cut-off of 3.5 kDa. The solution was then freeze-dried to get PFT as a light brown fibrous solid (0.041g, 80%).

¹H NMR (400 MHz, CD₃OD): δ ppm, 8.53(b), 8.11(b), 7.71(b), 7.64(b), 7.53(b), 3.88(b), 2.16(b), 1.66(b), 1.19(b), 0.80(b).

FT-IR IR (KBr, cm⁻¹): 3441.61, 2928.31, 1640.37, 1571.28, 1408.39, 1387.62, 1262.92, 1125.76, 1016.88, 789.60.

UV-Vis and fluorescence titration of PFT with metal salts and anions: Stock solutions of PFT (1×10^{-3} M) and other analytes (1×10^{-2} M) were prepared in Milli-Q water. The absorption and fluorescence titrations of the polymer PFT with several metal salts and anions in water were recorded by directly adding small aliquots (typically 1µL) to 3 mL HEPES buffer (pH=7.4, 10mM) containing 16.6 µM PFT in a quartz cuvette (1 cm × 1 cm). After mixing thoroughly the absorption or fluorescence spectra were recorded at room temperature.

Enzymatic activity of ALP: From the stock solution of ALP (1.1×10^{-4} M), small aliquots (0.5μ L, 1μ L, 1.5μ L and 3μ L) were added to the cuvette containing 16.6 μ M PFT, 6.0 μ M Cu²⁺, 8.3 uM PPi in 10mM HEPES buffer (pH 7.4) separately and photoluminescence spectra was recorded at different time intervals.

Detection of PPi in urine samples: Three different urine specimens were collected from different individuals at different time intervals. To verify the insignificant effect of these samples on fluorescence emission of PFT, each specimen was added (10µL) to cuvette containing 16.6 µM PFT in 10mM HEPES buffer (pH 7.4). Individual samples were then subjected to cuvette containing 16.6 µM PFT, 6.0 µM Cu²⁺ and fluorescence changes were monitored at room temperature. Results were compared with calibration curve of PPi to acquire the possible concentration of PPi in these samples. Each urine specimen was than doped with known concentration of PPi (10⁻³ M) and aliquots were added to the cuvette containing 16.6 µM PFT, 6.0 µM Cu²⁺ and fluorescence changes were observed. Results were compared with calibration curve of PPi to estimate the recovery of PPi.



Scheme S1. Synthesis of poly[5,5'-(((9H-fluorene-9,9-diyl)bis(hexane-6,1diyl))bis(oxy))diisophthalate sodium] (PFT). (a) 1,6-dibromohexane, tetrabutyl ammonium iodide (TBAI), 50% NaOH (aq.), 70 °C, 4h. (b) Ferric chloride, nitrobenzene, 36h. (c) PFTE, dry DMF, 80 °C, overnight (d) 30% aq. NaOH, THF, 50 °C, 6h



Fig. S1 ¹H NMR (400 MHz, CDCl₃) spectrum of PF.



Fig. S2 ¹³C NMR (100 MHz, CDCl₃) spectrum of PF.



Fig. S3 ¹H NMR (400 MHz, CDCl₃) spectrum of PFTE.



Fig. S4 ¹³C NMR (100 MHz, CDCl₃) spectrum of PFTE.



Fig. S5 FT-IR (KBr) spectrum of PFTE.



Fig S6 GPC Chromatogram of PFTE.



Fig. S7 ¹H NMR (400 MHz, CD₃OD) spectrum of PFT.



Fig. S8 FT-IR spectrum of PFT.



Fig. S9 (a) Emission spectra and (b) maximum fluorescence intensity of PFT in water as a function of pH value.



Fig. S10 FESEM images of PFT in (a) acidic medium and (b) alkaline medium.



Fig. S11 UV-vis spectra of PFT in different pH conditions.



Fig. S12 Effective diameter (ED) determined by dynamic light scattering of PFT in water at **(a)** basic pH (284 nm) and **(b)** acidic pH (1685 nm).



Fig. S13 UV-vis spectra of PFT in different solvents.



Fig. S14 Emission spectra of PFT (16.6 μ M.) with different MeOH/H₂O ratios.



Fig. S15 Stern-Volmer plot of PFT upon addition of Cu²⁺ in 10mM HEPES buffer (pH 7.4, 25 °C)



Fig. S16 Calibration curve of PFT as a function of $[Cu^{2+}]$. Excitation and emission wavelength were 360 nm and 419 nm, respectively



Fig S17 UV-Vis titration spectra of PFT (33 μ M) in 10mM HEPES buffer (pH 7.4, 25 °C) with increasing concentration of Cu²⁺ in water.



Fig. S18 Bar diagram depicting fluorescence responses of PFT/Cu²⁺ (16.6 μ M/6.0 μ M) towards various organic anions (8.3 μ M) in 10mM HEPES buffer, pH 7.4, incubated at 37°C for 20 min.



Fig. S19 Fluorescence intensity of PFT-Cu²⁺ in 10 mM HEPES buffer, pH 7.4, as a function of PPi concentration.



Fig. 20 (a) UV-Vis titration spectra of PFT-Cu²⁺ in 10mM HEPES buffer (pH 7.4, 25 °C) with increasing concentration of PPi (1×10^{-4} M) in water. (b) Change in the UV-vis spectra of PFT after adding Cu²⁺ and PPi.



Fig. S21 Calibration curve of PFT-Cu²⁺ as a function of [PPi]. Excitation and emission wavelength were 360 nm and 419 nm, respectively.

References

- 1 G. Saikia and P. K. Iyer, J. Org. Chem. 2010, 75, 2714.
- 2 M. Fukuda, K. Sawada and K. Yoshino, J. Polym. Sci. Part A Polym. Chem. 1993, **31**, 2465.