A Water-soluble Conjugated Polymer with Azobenzol Side Chains Based on "Turn-on" Effect for Hypoxia Cell Imaging

Jie Li,[†] Yan Yuan,[‡] Gaoshan Zeng,[†] Xiang Li,[†] Zhen Yang,[†] Xiaozhen Li,[†] Rongcui Jiang,[†] Wenbo Hu,[†] Pengfei Sun,[†] Qi Wang,[†] Xiaomei Lu,[‡] Quli Fan,[,†] and Wei Huang[*,†,‡]*

[†] Key Laboratory for Organic Electronics & Information Displays (KLOEID) and Institute of Advanced Materials, Nanjing University of Posts & Telecommunications, Nanjing 210046, China.

[‡] Jiangsu-Singapore Joint Research Center for Organic/Bio- Electronics & Information Displays and Institute of Advanced Material, Jiangsu National Synergetic Innovation Center for Advanced Materials. Nanjing Tech University, Nanjing 211816, China.

Electronic Supplementary Information

Summary

Materials and Instrumentation

The azoreductase (DT-diaphorase human, D1315-1MG) and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich Chemical Co. and were used as received. Other chemicals were purchased from Sigma-Aldrich, Energy Chemical, and J&K and were used as received. THF was purified by distillation from sodium in the presence of benzophenone. Other organic solvents were used without any further purification. All reactions were conducted under nitrogen atmosphere. NMR spectra were recorded on a Bruker Ultra Shield Plus 400 MHz NMR. $CDCl_3$ was used as the solvent. ESI-MS of azobenzol linkage (AZO) was conducted on a Electrospray Ionization Mass Spectrometry (EI-MS) in DCM. MALDI-TOF-MS was conducted on a Bruker Autoflex without the matrix under the reflector mode for data acquisition. The UV–Vis absorption spectra were recorded on a Shimadzu UV-3600 UV–Vis–NIR Spectrophotometer. Photoluminescent spectra were recorded at RT using a fluorescence spectrophotometer with excitation and emission slit widths of 5.0 nm. ^1H NMR spectra were recorded on NMR (400 MHz) spectrometers, using tetramethylsilane as an internal standard. The particle diameters were measured by DLS using a 90 Plus particle size analyzer (Brookhaven Instruments). The MTT assay was measured by a microplate reader (BioTek, PowerWave XS/XS2, U.S.). Cell imaging was conducted by Confocal Laser Scanning Microscopy (CLSM). GPC analysis of the polymers was conducted on a Shim-pack GPC-80X column with THF as the eluent at a flow rate of 1.0 mL/min at 35 °C and polystyrene as the standard. The data were analyzed by using the software package provided by Shimadzu Instruments.

Cell culture

The human cervical cancer HeLa and MCF-7 cell line were obtained from the American Type Culture Collection and cultured in Dulbecco's modified Eagle's medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco BRL, Gaithersburg, MD, USA). Cells were cultured at 37 °C in a humidified chamber containing 5% CO_2.

Synthesis and characterization

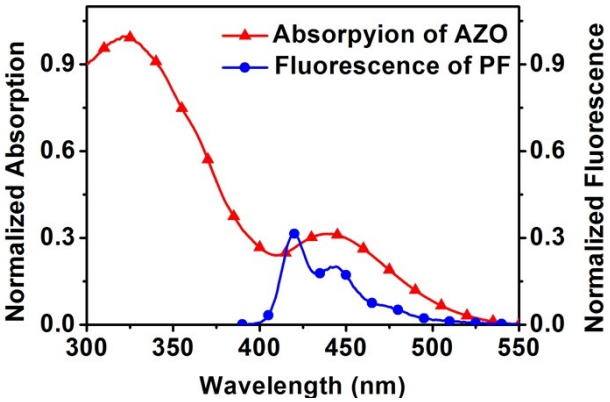


Figure S1. The spectral overlap of the normalized absorption spectra of AZO (red line, triangle) with the normalized fluorescence spectra of polyfluorene (blue line, circle).

Figure S2. The fluorescence spectra and the visualized pictures of PF-PAZO-PPEG (10 μg/mL) in 7.4 PBS buffer in dark field (λ_{ex} = 380 nm) in the absence (1) and presence (2) of azoreductase (1 μM) for 30 min.

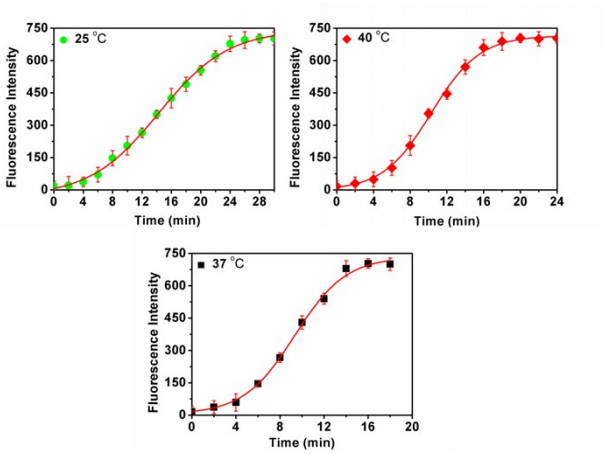


Figure S3. The fluorescence intensity of PF-PAZO-PPEG (10 μg/mL) in 7.4 PBS buffer (λ_{ex} = 380 nm) in the presence of azoreductase (1 μM) in different temperatures (circle:25 ^{o}C; square: 37 ^{o}C; diamond: 40 ^{o}C)

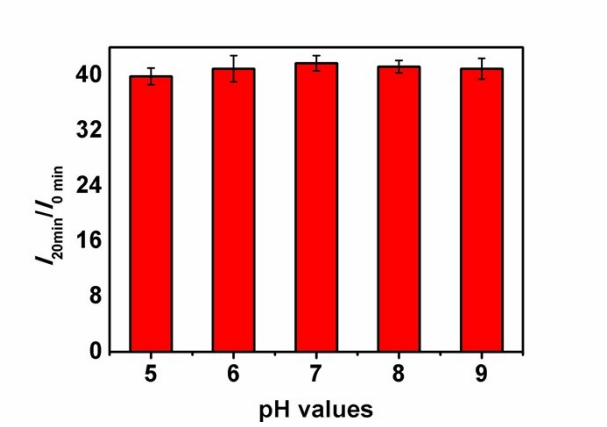


Figure S4. The ratio of fluorescence intensity of PF-PAZO-PPEG (10 μg/mL) in 7.4 PBS buffer in 20 min and 0 min with azoreductase (1 μM) in pH values from 5 to 9 at 37 ^{o}C.

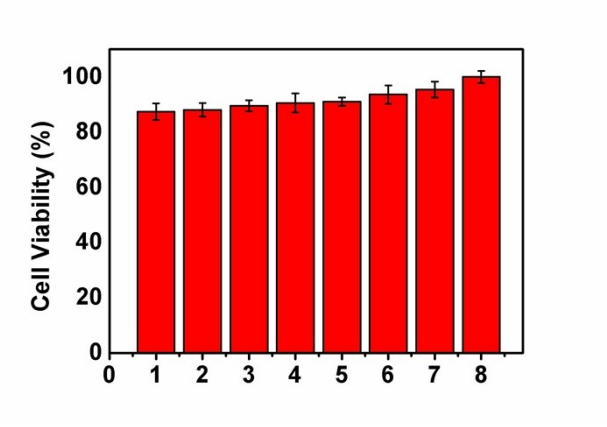
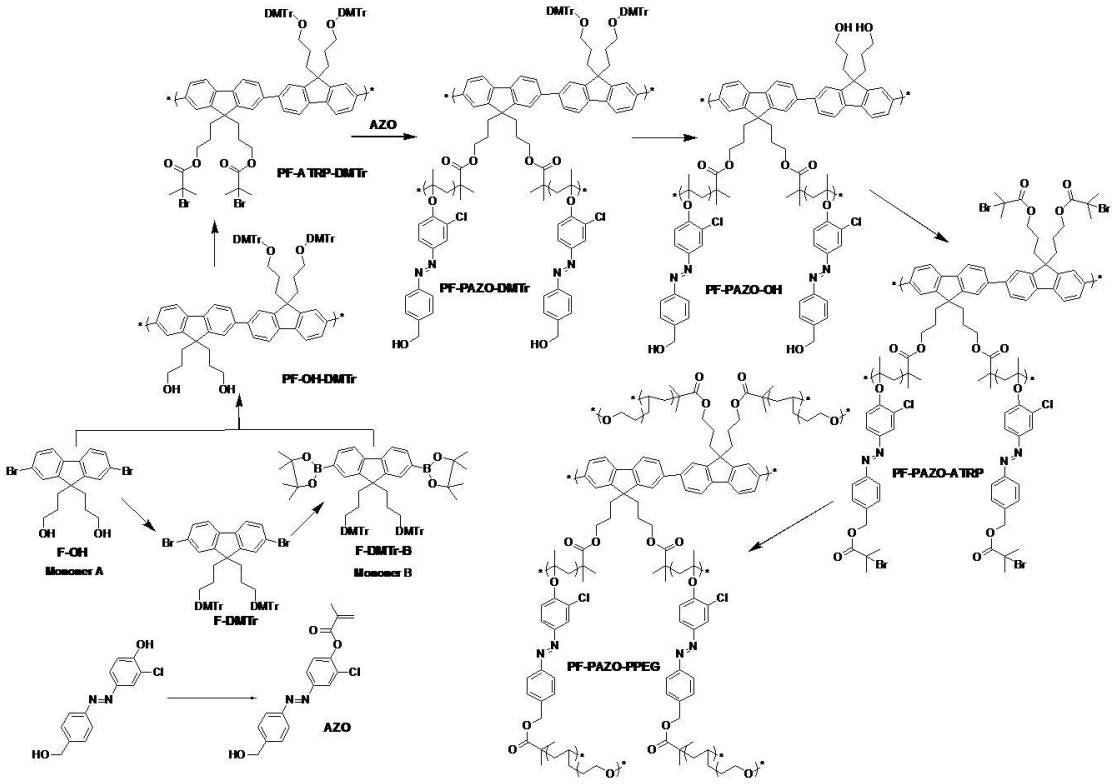


Figure S5. The cell viability with PF-PAZO-PPEG in different concentrations:(1) 1000 μg/mL; (2) 100 μg/mL; (3) 10 μg/mL; (4) 1 μg/mL; (5) 0.1 μg/mL; (6) 0.01 μg/mL; (7) 0.001 μg/mL; (8) absence of PF-PAZO-PPEG.

Synthesis



Scheme S1, the synthesis procedure of the conjugated polymer sensor for hypoxia (PF-PAZO-PPEG).

Monomer A (F-OH)

2,7-dibromo-fluorene 3.24 g (10 mmol), was dissolved in 100 mL 50% NaOH aqueous solution under nitrogen atmosphere and stilled at room temperature (rt) for 45 min. After that, 5.56 g (40 mmol) 3-bromo-1-propanol was added drop-wisely. The reaction was refluxed under 80 °C for 4h untill the color of the mixed aqueous solution turned from orange to purple. the resulting solution was extracted with ethyl acetate (EA). The organic phase was washed with HCl (2 mol/L) 3 times and dried with Na_2SO_4. The crude product was further purified by silica chromatography. The obtained product (F-OH) was recrystallized in petroleum ether (PE), and gave the yield of 85% (3.74 g). ^{1}H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm): δ = 7.55 to 7.45 (br, m, 6 H), 3.38 (s, 0.15 H), 2.05 (m, 2 H), 0.87 (m, 2 H) (Figure S). ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm): 152.26, 138.93, 129.76, 125.94, 120.88 (Figure S). Molditof-MS: [M+H]+ calculated for $C_{19}H_{20}Br_2O_2$ 440.17; found 440.502.

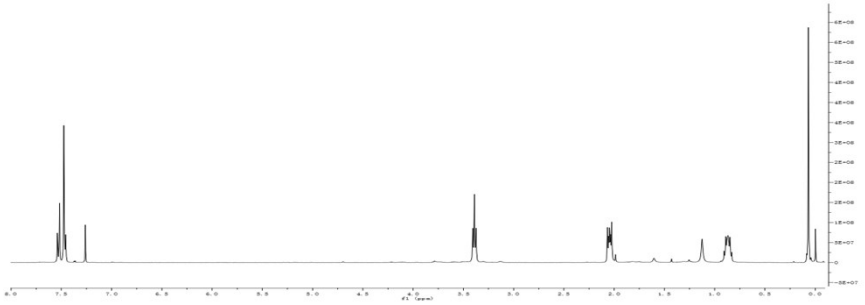


Figure S6, ^{1}H NMR of F-OH.

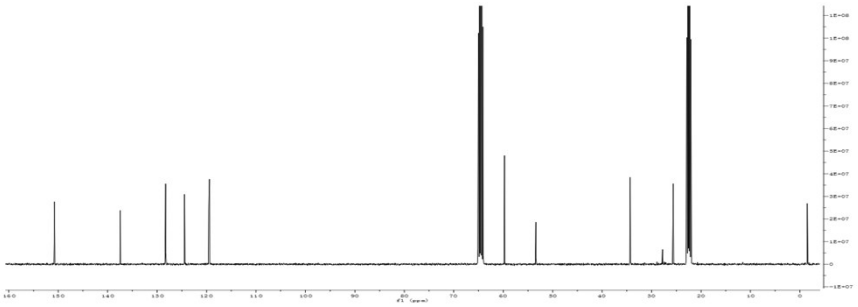


Figure S7, ^{13}C NMR of F-OH.

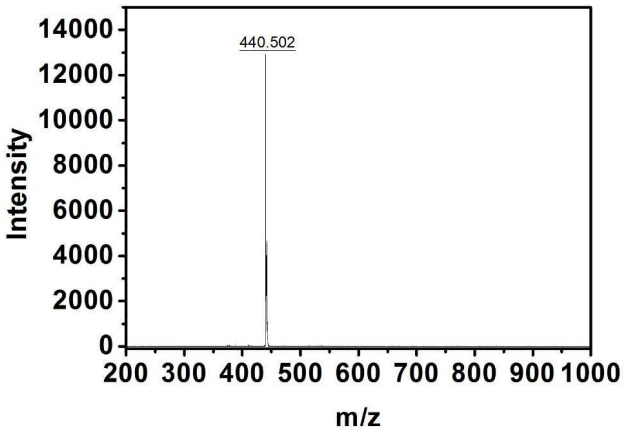


Figure S8, Molditof-MS of F-OH.

F-DMTr

F-OH 2.2 g (5 mmol) , mixed with 4,4'-Dimethoxytrityl chloride (DMTr-Cl， 6.78 g, 20 mmol) was dissolved in 100 mL pyridine, and stilled at rt overnight. The crude organic solution was concentrated and purified by silica chromatography. monomer A (F-DMTr) was obtained with yield of 95% (4.96 g).^{1}H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm): δ = 7.45 - 7.51 (br, 6H), 7.18 - 7.37 (br, 16 H), 6.78 - 6.85 (br, 10 H), 3.77 (s, 12 H), 2.75 (m, 4 H), 1.98 (m, 4 H), 0.877 (m, 4 H) (Figure S8). ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm): 171.13, 158.30, 151.78, 145.19, 129.90, 129.16, 128.11, 127.71, 126.57, 126.22, 121.69, 121.31, 113.17, 85.71, 63.38, 60.39, 55.24, 36.89, 24.63, 21.05, 14.22 (Figure S9). Molditof-MS: [M+H]+ calculated for $C_{61}H_{56}Br_2O_6$ 1044.9; found (Figure S10)

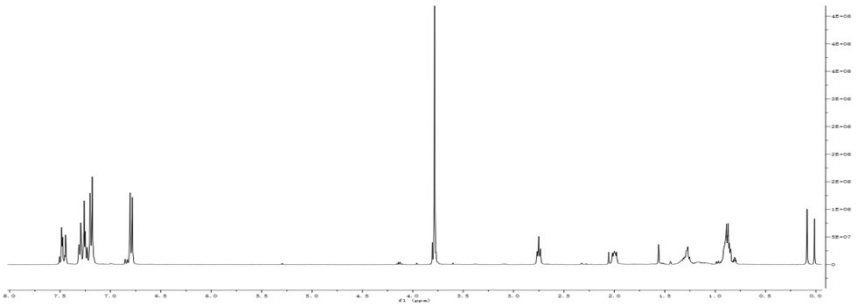


Figure S9, ^{1}H NMR F-DMTr.

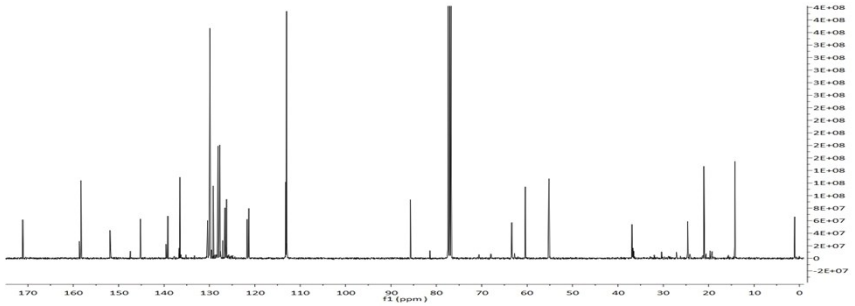


Figure S10, ^{13}C NMR F-DMTr.

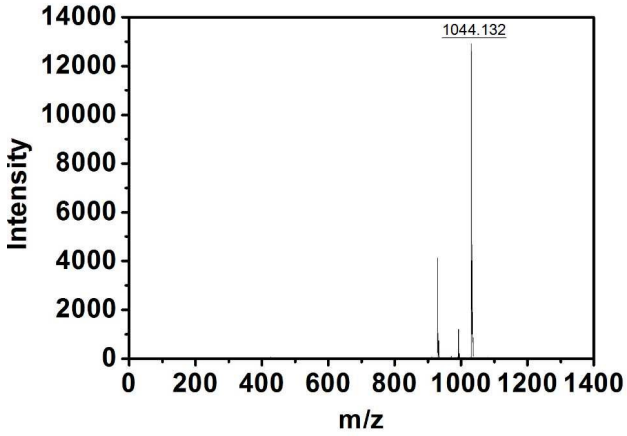


Figure S11, Molditof-MS of F-DMTr.

Monomer B (F-DMTr-B)

Monomer A (3.13 g, 3 mmol), bispinacolatodiboronmin (2.4 g, 9.5 mmol), and $Pd(dppf)_2Cl_2$ (80 mg) and KOAc (1.7 g, 17 mmol) were mixed in a round-bottomflask. Theflask was covered with aluminum foil to prevent the absorption of light. DMF was added to dissolve the reactant. The mixture was stirred and refluxed at 90 °C under N2 protection. After 12 h, the mixture was dissolved in DCM and filtered to eliminate the catalyst. Then the mixture was washed with water 3 times to remove DMF and HCl aqueous solution. $Na_2SO_4$was used to dry the organic solution. Then, the mixture was evaporated to obtain a solid and purified by silica chromatography. The final product was obtained as a white powder with the yield of 70% (2.391 g).^1H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm): δ = 7.70 - 7.83 (br, 6 H), 7.12 - 7.23 (br, 16 H), 6.74 - 6.85 (br, 10 H), 3.76 (s, 12 H), 2.70 - 2.73 (m, 4 H), 2.05 - 2.08 (m, 4 H), 1.37 (s, 12 H), 0.83 - 0.90 (m, 4H) (Figure S11). ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm): 158.10, 149.22, 145.29, 140.18, 133.37, 128.40, 126.83, 126.83, 125.59, 118.86, 112.18, 112.02, 85.17, 83.00, 79.84, 63.15, 53.92, 36.33, 29.26 (Figure S12). Molditof-MS: [M+H]+ calculated for $C_{73}H_{80}Br_2O_{10}$ 1139.03; found (Figure S13).

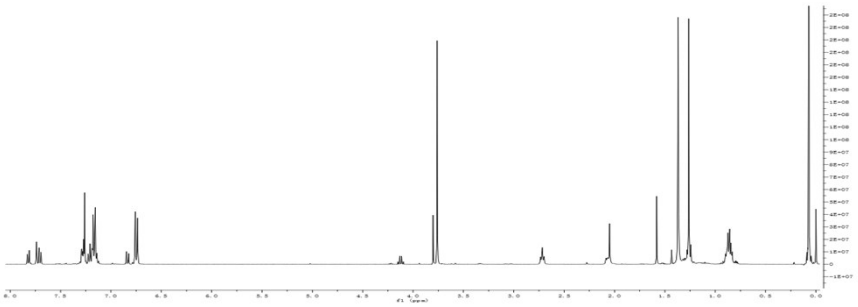


Figure S12, ^{1}H NMR of F-DMTr-B.

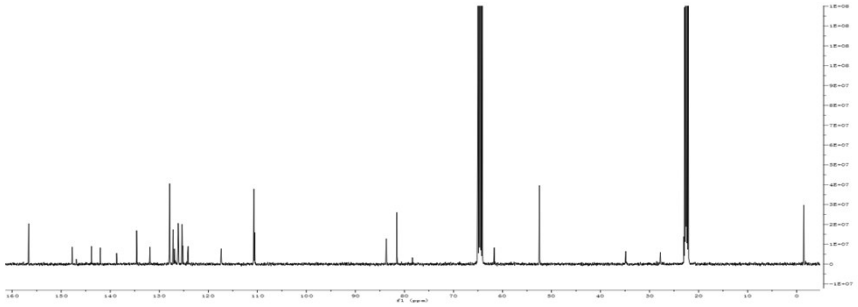


Figure S13, ^{13}C NMR of F-DMTr-B.

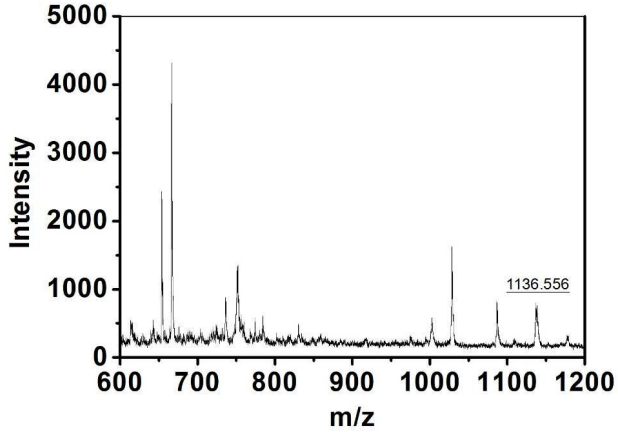


Figure S14, Molditof-MS of F-DMTr-B.

Azobenzol linkage (AZO)

4-(4-hydroxyl-3-chlorophenylazo)benzylic alcohol 2.63 g (10 mmol, synthesized following the steps in the study of Kopeček[1]) was dissolved in 50 mL pyridine, thereafter adding methacrylic anhydride 3.08 g (20 mmol) dropwise. After stilling for 8h at rt under N_2 atmosphere, the crude product was concentrated by vacuum distillation. The crude AZO was purified by silica chromatography with the yield of 90% (2.97 g).^{1}H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm): δ = 8.04 (s, 1 H), 7.89 - 7.93 (q, 3 H), 7.52 - 7.54 (d, 2 H), 7.36 - 7.38 (d, 1 H), 6.46 (s, 1 H), 5.84 (s, 1 H), 4.80 - 4.81 (d, 2 H), 2.11 (s, 3 H) (Figure S14). ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm): 163.34, 150.93, 150.37, 148.71, 146.87, 135.01, 127.31, 126.88, 126.31, 124.04, 122.77, 122.53, 122.36, 62.92, 17.02 (Figure S15). EI-MS: [M+H]+ calculated for $C_{17}H_{15}ClN_2O_3$ 330.77; found 330.0 (Figure S16).

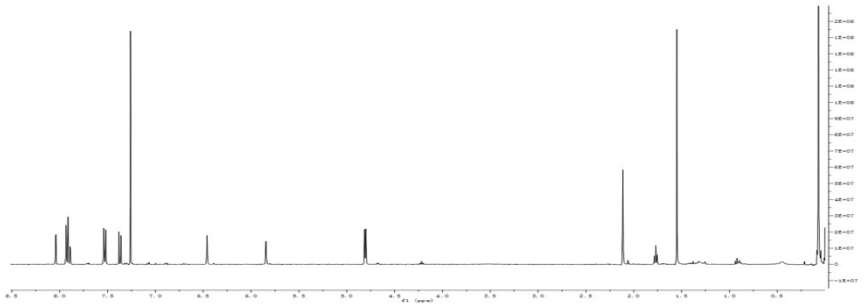


Figure S15, ^{1}H NMR of AZO.

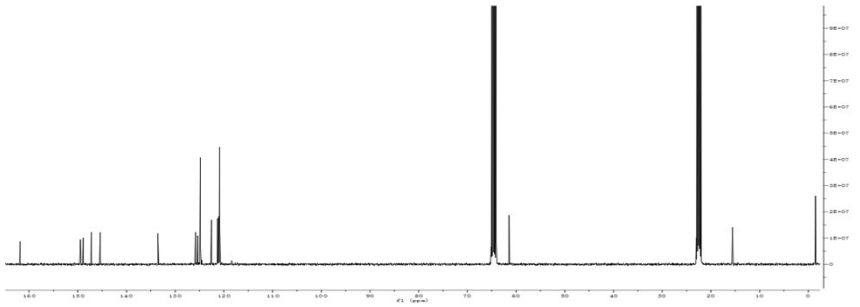


Figure S16, ^{13}C NMR of AZO.

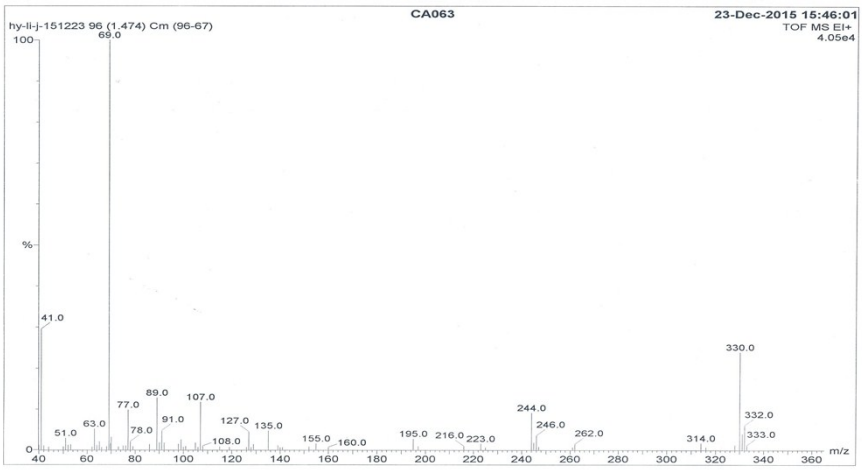


Figure S17, EI-MS of AZO.

PF-OH-DMTr

Monomer A (0.44 g, 1 mmol) and Monomer B (1.139 g, 1 mmol) were mixed with 0.05 g tetrakis(triphenylphosphine)Pd(0) under nitrogen atmosphere. Subsequently, 25 mL toluene was added as solution and 5mL K_2CO_3 (2 mol/L) was added as catalyst. The mixture was stirred at 85 °C to 90 °C for 3 days. After cooling down, the crude product was filtered through AlO_3 to remove the solid catalyst , and purified by sedimentation in methanol. The polymer, PF-OH-DMTr, was filtered to yield 0.815 g (pale yellow powder, 70 % yield).M_n = 4463, PDI =1.63 (calculated to be 4 repeat units, one momomerA and one monomer B as a repeat unit).^{1}H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm) was shown in Figure S17. ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm) was shown in Figure S18.

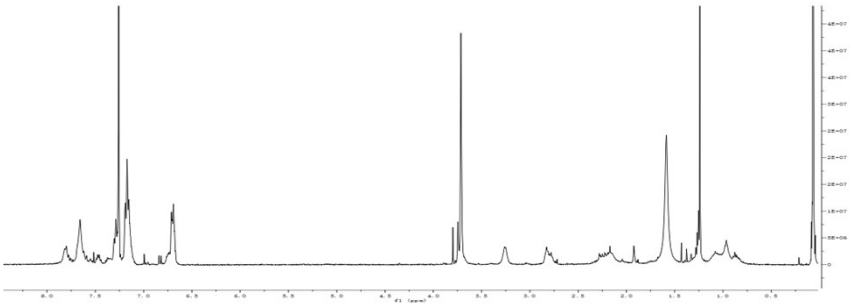


Figure S18, ^{1}H NMR of PF-OH-DMTr.

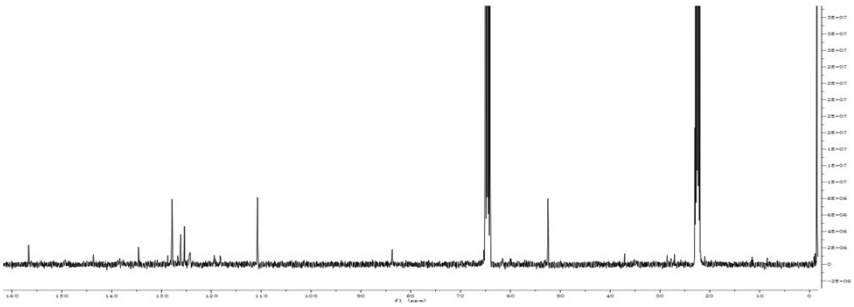


Figure S19, ^{13}C NMR of PF-OH-DMTr.

PF-ATRP-DMTr

PF-OH-DMTr 0.58 g (0.5 mmol of the repeat unit) was dissolved in 10 mL anhydrous THF with adding g 2-bromoisobutyryl bromide (0.46 g, 2 mmol) dropwise. After stilling overnight, the crude product was evaporated to remove the solvent and then purified by sedimentation in diethyl ether. Finally, PF-ATRP-DMTr was obtained with 80% yield (0.585 g). M_n = 5910, PDI = 1.65 (8 initiating groups each macromolecules). ^{1}H NMR (d-chloroform ($CDCl_3$), 400 MHz, 298 K, ppm)was shown in Figure S19. ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm)was shown in Figure S20.

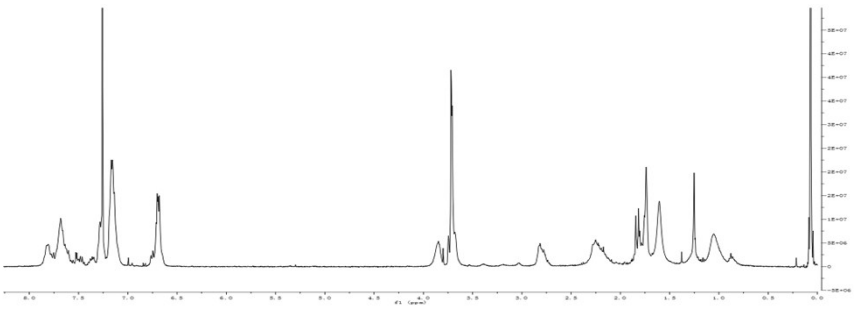


Figure S20, ^{1}H NMR of PF-ATRP-DMTr.

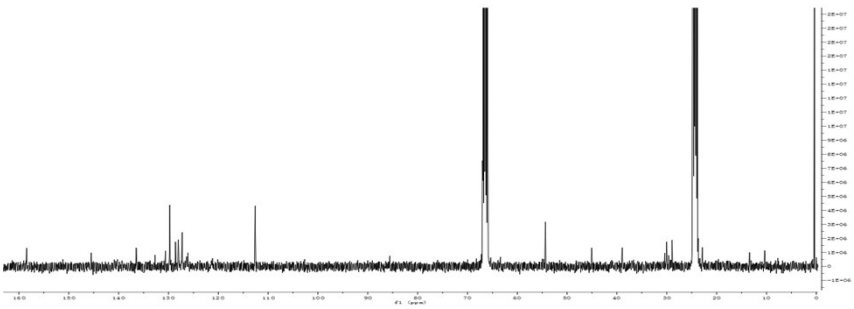


Figure S21, ^{13}C NMR PF-ATRP-DMTr.

PF-PAZO-DMTr

PF-ATRP-DMTr (0.44 g, 0.3 mmol of the repeat unit), AZO (0.99 g, 3 mmol) and CuBr (50 mg) were dissolved in 1.5 mL methyl-phenoxide. Under nitrogen protection, 50 μL pentamethyldiethylene triamine (PMDETA)was added as ligand. The solution was stilled at 80 °C for 8 h. After cooling down, the crude product was filtered by AlO_3 to remove the solid catalyst , and purified by sedimentation in methanol. PF-PAZO-DMTr was obtained with the yield of 40% (0.57 g). M_n = 14214, PDI = 1.82 (calculated to be 24 AZO each macromolecules, and 6 AZO on each repeat unit of the conjugated polymer). ^{1}H NMR (d_8-tetrahydrofuran (THF-d_8), 400 MHz, 298 K, ppm) was shown in Figure S21. ^{13}C NMR (d-chloroform ($CDCl_3$), 100 MHz, 298 K, ppm) was shown in Figure S22.

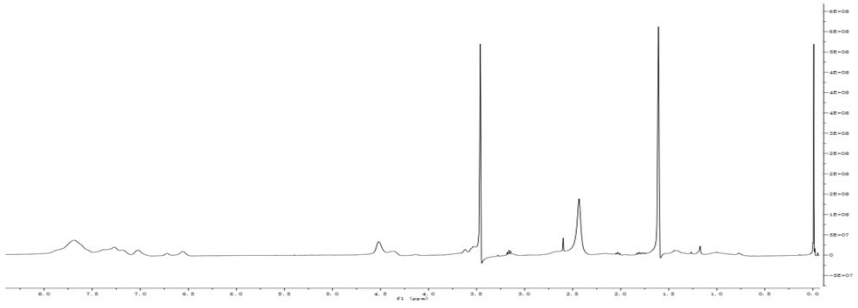


Figure S22, 1H NMR of PF-PAZO-DMTr.

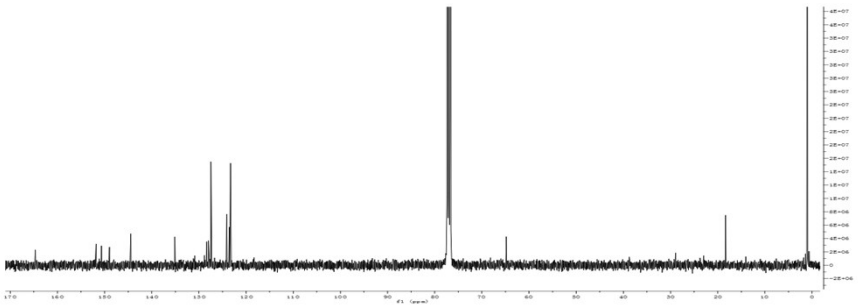


Figure S23, ^{13}C NMR of PF-PAZO-DMTr.

PF-PAZO-OH

PF-PAZO-DMTr (0.5 g) was dissolved in 10mL 5 % dichloroacetic acid/ DCM solution. Still for 30 min at rt and purifi the crude product by sedimentation in diethyl ether. PF-PAZO-OH was obtained. (0.23 g) M_n = 11782, PDI = 1.54. ^{1}H NMR (d_8-tetrahydrofuran (THF-d_8), 400 MHz, 298 K, ppm) was shown in Figure S23. ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm) was shown in Figure S24.

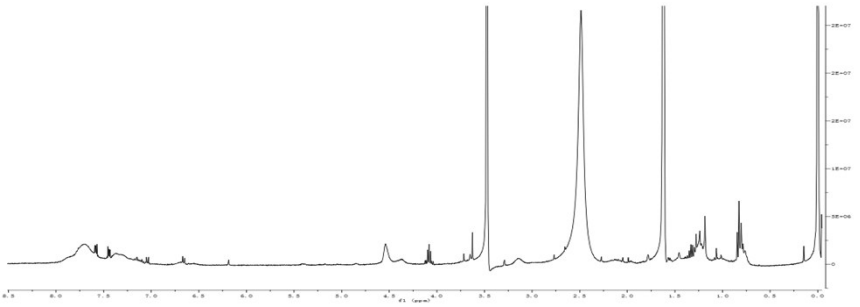


Figure S24, ^{1}H NMR PF-PAZO-OH.

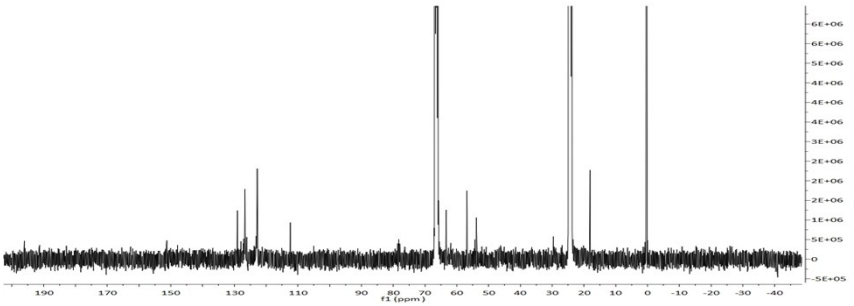


Figure S25, ^{13}C NMR PF-PAZO-OH.

PF-PAZO-ATRP

PF-PAZO-DMTr 0.2 g was dissolved in 10 mL anhydrous THF with adding g 2-bromoisobutyryl bromide (0.46 g, 2 mmol) dropwise. After stilling overnight, the crude product was evaporated to remove the solvent and then purified by sedimentation in diethyl ether. Finally, PF-PAZO-ATRP was obtained. (0.17 g). M_n = 13044, PDI = 1.74. ^{1}H NMR (d_8-tetrahydrofuran (THF-d_8), 400 MHz, 298 K, ppm) was shown in Figure S25. ^{13}C NMR (d_8-tetrahydrofuran (THF-d_8), 100 MHz, 298 K, ppm) was shown in Figure S26.

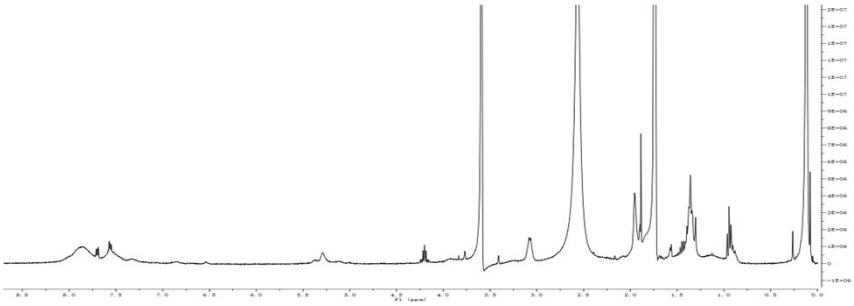


Figure S26, ^{1}H NMR of PF-PAZO-ATRP.

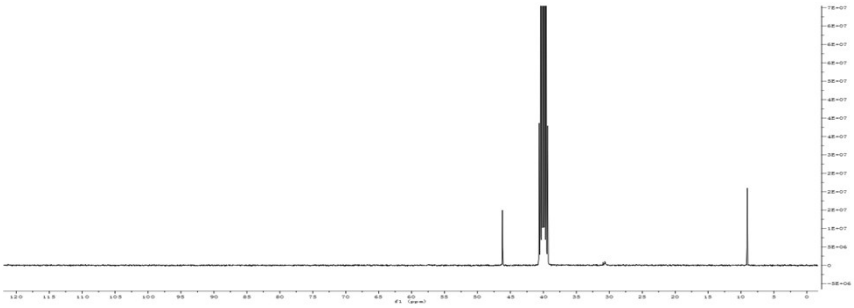


Figure S27, ^{13}C NMR of PF-PAZO-ATRP.

PF-PAZO-PPEG

PF-PAZO-ATRP (0.15 g), ethenyl polyethylene glycol (0.5 g, M_n = 950) and CuBr (50 mg) were dissolved in 1.5 mL methyl-phenoxide. Under nitrogen protection, 50 μL pentamethyldiethylene triamine (PMDETA) was added as ligand. The solution was stilled at 80 °C for 8 h. After cooling down, the crude product was filtered by AlO_3 to remove the solid catalyst, and purified by sedimentation in methanol. PF-PAZO-PPEG was obtained with the yield of 0.316 g. M_n = 39045, PDI = 1.91 (calculated to be 27 PEG units on the conjugated polymer). ^{1}H NMR (d_8-tetrahydrofuran (THF-d_8), 400 MHz, 298 K, ppm) was shown in Figure S27. ^{13}C NMR (d_6-dimethylsulfoxide (DMSO-d_6), 100 MHz, 298 K, ppm) was shown in Figure S28.

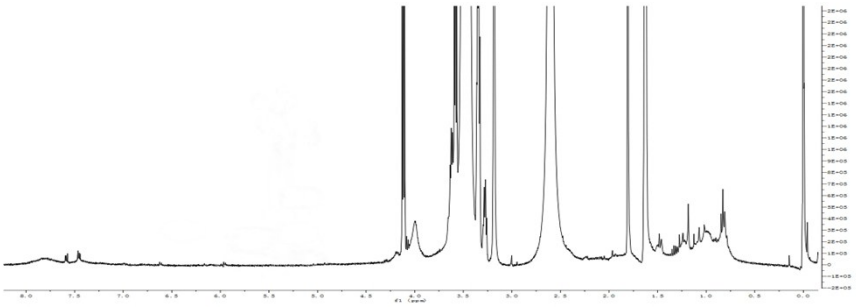


Figure S28, ^{1}H NMR of PF-PAZO-PPEG.

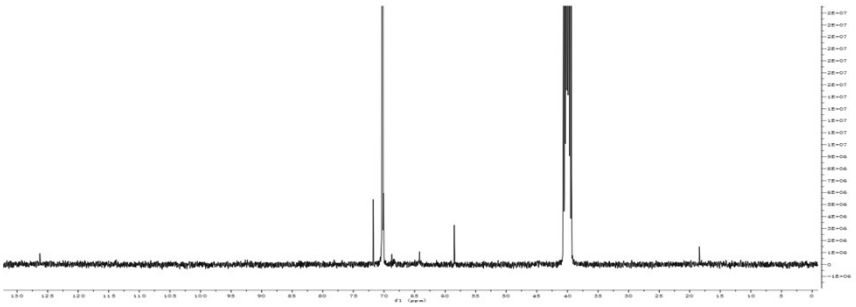


Figure S29, ^{13}C NMR of PF-PAZO-PPEG.

References

1. Gao, S. Q.; Lu, Z. R.; Petri, B.; Kopečková, P.; Kopeček, J. *Journal of Controlled Release* 2006, 110, 323.