Electronic Supplementary Material (ESI) for Journal of Materials Chemistry C.

Electronic Supplementary Information

Photoswitchable Polyfluorophores Based on Perylenemonoimide-Dithienylethene Conjugates as Super-Resolution MitoTrackers

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Contents

1. Experimental

1.1 Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on a 600 MHz Bruker AV600 with the internal standard of tetramethylsilane at ambient temperature. Mass spectra were measured on an Agilent 1100 LC/MSD Trap. UV-vis spectra were recorded on a Shimadzu UV-vis-NIR Spectrophotometer (UV-3600). Fluorescence spectra were recorded with Edinburgh instruments (FLSP920 spectrometer). The fluorescence spectra of photoswitchable cycles were recorded using a fluorescence spectrophotometer (F-7000). FT-IR were carried out on a VERTEX 70 equipped with ATP. The 302-nm UV irradiation for the “ring-closing” reaction of DTE molecular switch was operated on a trans-illuminator (UVP, LLC, US, with 3 ultraviolet tubes, each 8 W 220 V). The visible light for the “ring-opening” reaction was obtained from a common table lamp (25 W) and a long-wavelength pass filter (490 nm). The molecular weight of the polymers was determined by gel permeation chromatography （GPC PL-50） using an IR detector in THF at 40°C with monodisperse PS as the standard.

HeLa cells were seeded in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) containing 10% of fetal bovine serum (FBS) at 37 °C and 5% CO_2 in a humidified incubator. For confocal microscopy and super-resolution microscopy imaging experiments, HeLa cells were transferred into glass bottom Petri dishes. After attachment, the cells were exposed to DMEM containing P4 for 3 h, and then the monolayer of cells was washed with FBS to remove external fluorescent dyes. For Confocal Laser Scanning Microscopy imaging, we incubated the cells with P4 at 0.5 mg/mL for 3 h to label mitochondria, followed by treatment with Mito-Tracker Green at 100 nM for 30 min. Mito-Tracker Green in cells was observed by excitation at 473 nm, and fluorescence emission was collected between 480 and 540 nm using a confocal laser-scanning microscope

(Zeiss LSM710, Germany). While, the mitochondria-targeting fluorescence-switching polymeric probe P4 in cells were visualized by excitation at 514 nm and fluorescence emission was collected between 600 and 700 nm.

The super-resolution microscopy imaging was obtained by the home-built system consisting of an Olympus IX 71 inverted optical microscope, a 100x/NA1.49 oil immersion TIRF objective (UAPON 100XOTIRF, Olympus), a 473 nm solid-state laser (CNILaser, China), a 302 nm UV lamp, and an sCMOS camera (Andor iXon 897). Electronic shutters (UNIBLITZ VS14, Vincent Associates) were used to control the duration of laser irradiance and a dichroic mirror (FF509-FDi01, Semrock) and a long pass filter (BLP01−488R-25, Semrock) were used to separate the collected fluorescence from scattering laser and impurity fluorescence. ImageJ plugin written in Java was used to analyze the image. The fluorescence ON/OFF were collected at a frame rate of 20 ms and all durations were 5000 frames. When the 473 nm laser (0.65 W/cm^2) photoswitched on the probe and imparted strong fluorescence, the 302 nm UV-irradiation (0.5 mW/cm^2) was used to switch off the fluorescence. This cycle was repeated many times to collect enough data for the reconstruction of the super-resolution image. The ImageJ plugin PALMER[1] was used to analyze the images.

The ImageJ plugin PALMER was used to analyze the obtained images from sCMOS camera.[1] The PALMER algorithm is an advanced method based on SSM_BIC[2], which combines Graphics Processing Unit (GPU) parallel computation, multiple-emitter fitting, and model recommendation via Bayesian Information Criterion (BIC) together. Using the 2D Gaussian function to approach Point spread function (PSF), the observed signal can be described as

$$S_{i,j} = Poisson(\sum_{k}^{N} A_k \exp(-\frac{(x - x_{ko})^2 + (y - y_{ko})^2}{2\omega^2}) + b)$$

Here $S_{i,j}$ represents the observed signal at the pixel(i,j), A_k is the amplitude, x_{ko} and y_{ko} are the molecule positions, b is the background intensity, and ω is the width of Gaussian kernel. Poisson(x), a Poisson random number with mean value of x, is used to take the influence of photon shot noise into account. Here ω is fixed to simply the fitting process.

1.2 Materials and the general synthesis

Compounds 4, 4'-azobis(4-cyano valeric acid) (ACVA) and 4-vinylphenylboronic acid were purchased from Sigma-Aldrich Co. Other reagents and solvents for synthesis were obtained from commercial suppliers and were used without further purification unless otherwise stated. Tetrahydrofuran (THF) was dried by the way of sodium wires in a benzophenone system and distillation. Acetonitrile was dried by calcium hydride and distillation. Toluene and distilled water (18.2 MΩ/cm) used in the Suzuki cross-coupling reaction were bubbled with N_2 for than 30 min before usage.

Compounds 1 (PMI-2DTE-Br) was synthesized according to the corresponding literature [3] and the synthetic details of other compounds are given below.

Compound 2

To a 25-mL double-round flask, a mixture of compound 1 (60 mg, 0.03 mmol), compound 4-vinyl benzene boric acid (23.5 mg, 0.15 mmol), K_2CO_3 (43.9 mg, 0.30 mmol), p-tert-butyphenol (1.0 mg) and water/toluene (5 mL/10 mL). Then the tetrakis(triphenylphosphine) palladium ($Pd(PPh_3)_4$, 1.7 mg, 0.0015 mmol) was added under N_2. The reaction system was refluxed at 90 ^{o}C for 24 h. Water (20 mL) was added until the system cooled down to room temperature, and the product was extracted twice with diethyl ether. The organic layer was washed with water (30 mL×3), and then dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed on a silica-gel column using dichloromethane (DCM)/petroleum ether (PE) (1/1, v/v) as an eluent to yield a red solid (60 mg, 98%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ = 9.30 (dd, J = 10.3, 8.0 Hz, 2H), 8.37 (s, 2H), 8.06 (d, J = 8.3 Hz, 1H), 7.62-7.51 (m, 8H), 7.49 (d, J = 8.1 Hz, 2H), 7.45 (t, J = 9.7 Hz, 5H), 7.31 (d, J = 7.8 Hz, 2H), 7.22 (d, J = 2.5 Hz, 2H), 7.15 (dd, J = 12.8, 7.5 Hz, 6H), 6.89 (d, J = 8.7 Hz, 4H), 6.82 (dd, J = 17.6, 10.9 Hz, 1H), 5.87 (d, J = 17.6 Hz, 1H), 5.35 (d, J = 10.9 Hz, 1H), 3.97 (t, J = 6.6 Hz, 4H), 2.73 (dt, J = 13.6, 6.8 Hz, 2H), 1.95 (d, J = 12.1 Hz, 12H), 1.82-1.75 (m, 4H), 1.45 (dd, J = 15.3, 7.6 Hz, 4H), 1.37-1.27 (m, 16H), 1.16 (d, J = 6.8 Hz, 12H), 0.89 (t, J = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, CDCl3, ppm): δ = 163.05 (s), 159.11 (s), 152.56 (s), 145.64 (s), 142.31 (s), 141.23 (s), 136.32 (s), 130.30 (s), 129.54 (s), 127.59 (s), 126.88 (s), 126.35 (s), 125.95 (s), 123.99 (s), 121.10 (s), 118.89 (d, J = 11.6 Hz), 114.96 (s), 77.23 (s), 77.02 (s), 76.81 (s), 68.19 (s), 31.82 (s), 29.24 (dd, J = 18.5, 16.3 Hz), 26.04 (s), 24.06 (s), 22.67 (s), 14.54 (s), 14.11 (s). MS (ESI, m/z): 1908.60, found: 1931.4880 ($[M+Na]^+$).

Compound 3

To a 100-mL single-round flask, a mixture of triphenylphosphine (1.03 g, 4 mmol), hexamethylene dibromide (6.02 g, 25 mmol) and 30 mL dry acetonitrile drummed by N_2 for 30 min. The reaction system was refluxed at 90 °C for 12 h. When it cooled down to room temperature, the sovlent was evaporated under vacuum. The residue was chromatographed on a silica-gel column using DCM/methanol (8/1, v/v) as an eluent to yield a colorless oil (1.50 g, 75%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ = 7.92-7.82 (m, 6H), 7.81–7.74 (m, 3H), 7.69 (td, J = 7.8, 3.3 Hz, 6H), 6.35 (dd, J = 17.3, 1.3 Hz, 1H), 6.07 (dd, J = 17.3, 10.4 Hz, 1H), 5.78 (dd, J = 10.4, 1.3 Hz, 1H), 4.07 (t, J = 6.6 Hz, 2H), 3.96-3.88 (m, 2H), 1.69 (dd, J = 15.0, 7.8 Hz, 2H), 1.60 (td, J = 14.8, 7.3 Hz, 4H), 1.34 (dt, J = 15.3, 7.6 Hz, 2H). ^{13}C NMR (600 MHz, $CDCl_3$, ppm): δ = 135.13 (d, J = 13.2 Hz), 133.74 (dd, J = 15.9, 9.7 Hz), 130.56 (d, J = 12.7 Hz), 118.65 (s), 118.08 (s), 45.18 (s), 34.17 (s), 32.29 (s), 29.50 (d, J = 16.2 Hz), 27.71 (s), 22.75 (d, J = 49.8 Hz). MS (ESI, m/z): 506.25, found: 427.1 ($[M]^+$).

Compound 4

To a 100-mL single-round flask, a mixture of compound 3(1.52 g, 3.0 mmol), acrylic acid (1.08 g, 15 mmol), K_2CO_3 (4.15 g, 30 mmol), p-tert-butyphenol (0.033 g, 0.15 mmol) and 60 mL dry N, N-dimethylformamide (DMF). The reaction system was refluxed at 60 ^{o}C for 24 h. Then the reaction mixture was filtered, extracted by DCM and washed by water. The residue was chromatographed on a silica-gel column using DCM/methanol (7/1, v/v) as an eluent to yield a colorless oil(0.70 g, 47%). ^{1}H NMR (600 MHz, $CDCl_3$, ppm): δ = 7.92-7.82 (m, 6H), 7.81-7.74 (m, 3H), 7.69 (td, J = 7.8, 3.3 Hz, 6H), 6.35 (dd, J = 17.3, 1.3 Hz, 1H), 6.07 (dd, J = 17.3, 10.4 Hz, 1H), 5.78 (dd, J = 10.4, 1.3 Hz, 1H), 4.07 (t, J = 6.6 Hz, 2H), 3.96-3.88 (m, 2H), 1.69 (dd, J = 15.0, 7.8 Hz, 2H), 1.60 (td, J = 14.8, 7.3 Hz, 4H), 1.34 (dt, J = 15.3, 7.6 Hz, 2H). ^{13}C NMR (151 MHz, CDCl3, ppm): δ = 166.26 (s), 135.00 (d, J = 15.8 Hz), 133.64 (dd, J = 15.9, 9.9 Hz), 130.44 (d, J = 12.8 Hz), 128.49 (d, J = 15.6 Hz), 118.65 (s), 118.09 (s), 77.32 (s), 77.11 (s), 76.90 (s), 64.33 (s), 36.52 (d, J = 18.8 Hz), 31.42 (d, J = 19.8 Hz), 30.01-29.13 (m), 28.24 (s), 25.61 (s), 22.44 (d, J = 49.6 Hz). MS (APCI, m/z): 497.40, found: 417.4 ($[M]^{+}$).

Poly[(PMI-2DTE)-co-VBC-co-TPP-co-NIPAM] (P1-P4)

The radical polymerization was carried out as following way. To a 5-mL polymerization tube, monomer 2, monomer 4, vinylbenzyl chloride, N-isopropyl acrylamide, 4,4’- azobis(4-cyano valeric acid) (ACVA, 5.7 mg, 0.02 mmol), anhydrous THF and a small magnetic stirring bar were added. After undergoing three freeze-pump-thaw cycles for degasification, the tube was sealed off in vacuum and then stirred in oil bath at 70 °C for 24 h. After cooling down to room temperature, the tube was opened and the reaction system was dropped slowly into cold diethyl ether (15 mL) under slow stirring. The as-obtained red precipitate was filtered and dried in vacuum to yield a red copolymer named Poly[(PMI-2DTE)-co-VBC-co-TPP-co-NIPAM](P1-P4).

Table S1 The synthesis and characterization of photoswitchable polyfluorophores.

	NIPAM (mmol)	VBC (mmol)	4 (mmol)	2 (mmol)	M_n ($\times 10^3$)	M_w ($\times 10^3$)	PDI	Yield（%）
P1	0.5	0.2	0.2	0.06	7.83	8.33	1.23	34
P2	0.5	0.1	0.1	0.02	5.08	6.64	1.31	35
P3	2.0	0.1	0.1	0.01	11.01	14.03	1.27	87
P4	1.0	0.1	0.1	0.01	7.80	10.58	1.36	41

2. Temperature dependence of the transmittance

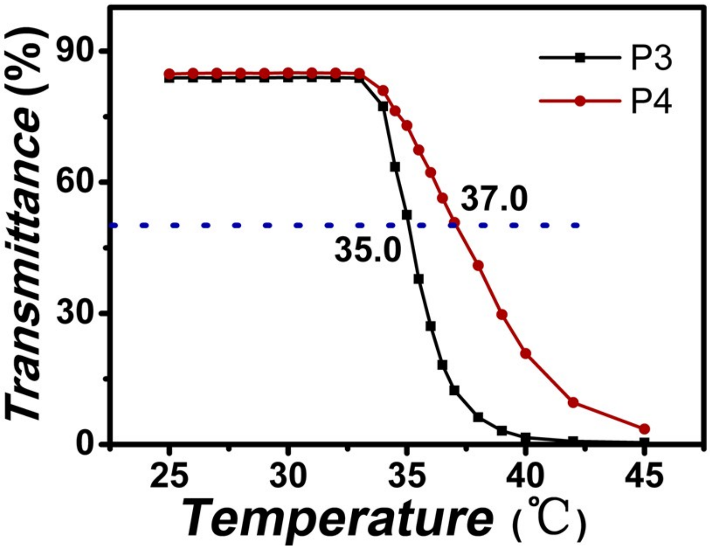


Figure S1. Temperature dependence of the transmittance at 450 nm through a 0.5 mg/mL solution of P3 and P4 in water.

3. ^{1}H NMR spectrum of P4

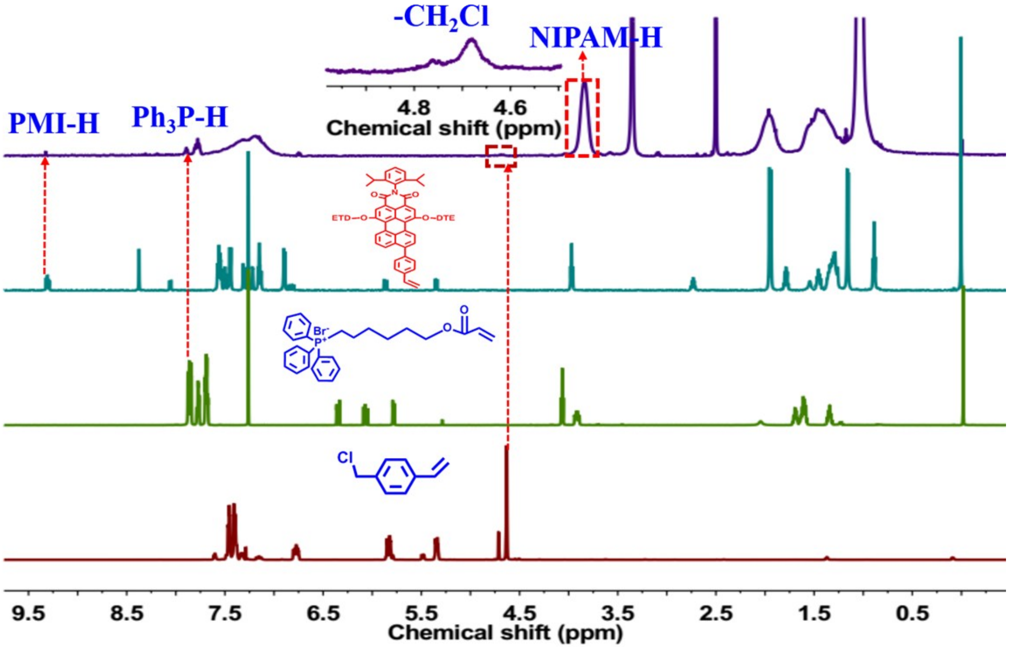


Figure S2. The ^{1}H NMR spectra of monomers and P4.

4. Optical properties

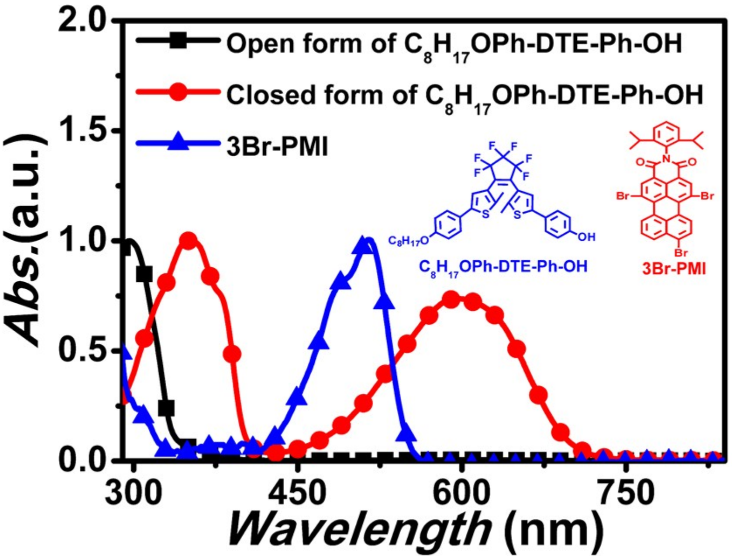


Figure S3 UV-Vis Absorption of 3Br-PMI and C_8H_{17}OPh-DTE-Ph-OH in $CHCl_3$.

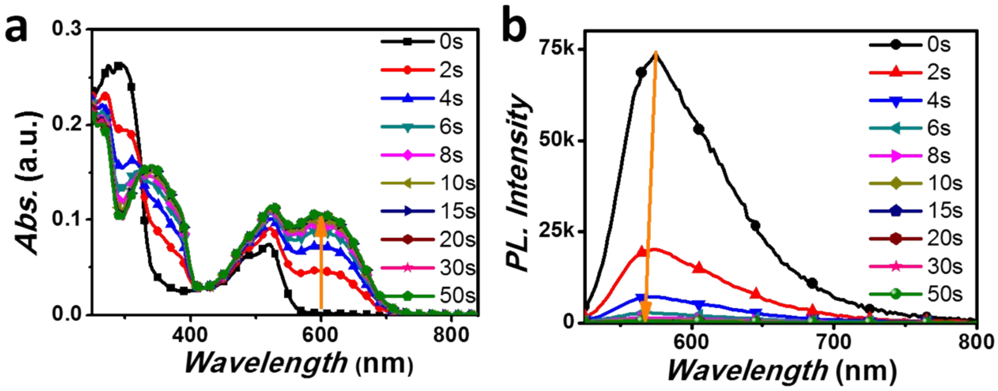


Figure S4 UV-Vis Absorption (a) and emission spectra (b) upon 302 nm irradiation of **P2** in THF. Concentration = 0.055 mg/mL. Excitation= 514 nm.

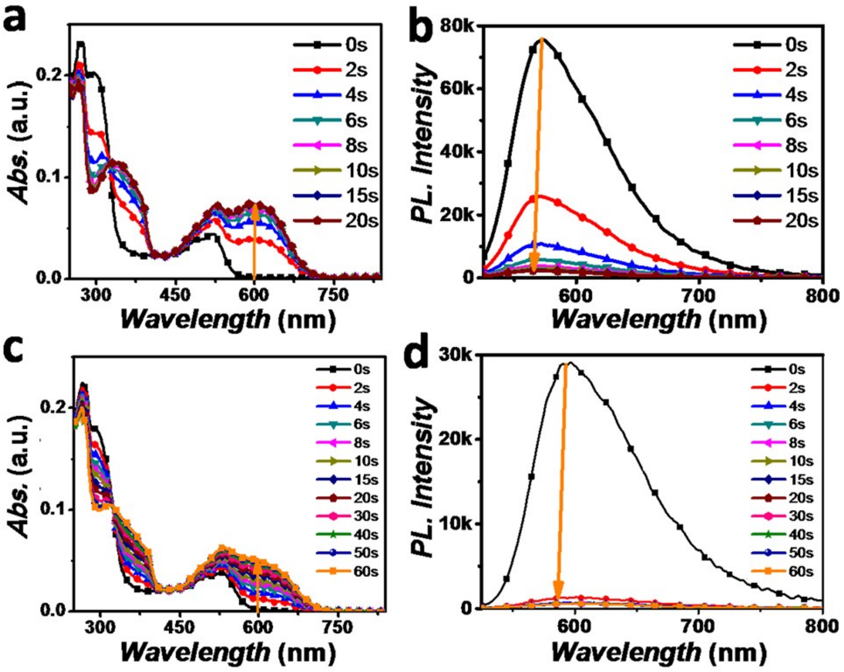
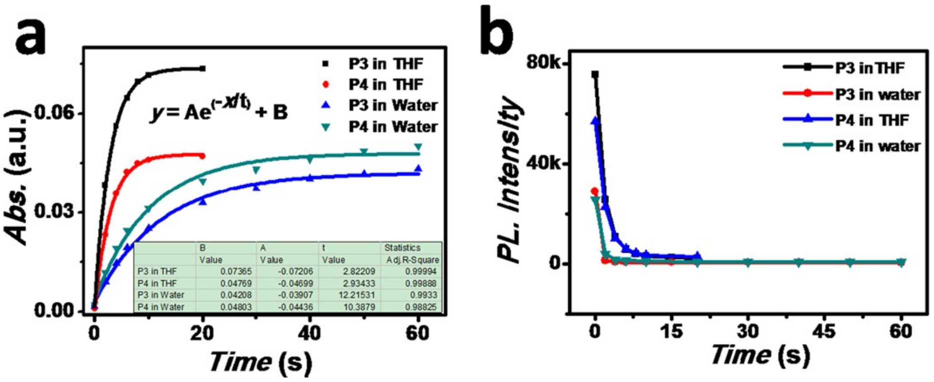


Figure S5 (a) UV-Vis absorption and (b) emission spectra upon 302 nm irradiation of **P3** in THF at the concentration of 0.16 mg/mL. (c) UV-Vis absorption and (d) emission spectra, upon 302 nm irradiation of **P3** in water at the concentration of 0.5 mg/mL. Excitation= 514 nm.

Table S2 Optical properties of probes

Sample	**Solvent**	**$\lambda_{max, abs}$ (nm) of Open form**	**$\lambda_{max, abs}$ (nm) of Closed form**	**$\lambda_{max, PL}$ (nm) of Open form**	**$\lambda_{max, PL}$ (nm) of Closed form**	**FLQY on/off [a]**	**FL on/off ratio [b]**
P2	THF	272,295, 518	262,341, 523,599	550	544	0.69/0.03	170
P3	THF	267,518	265,523, 601	573	568	0.66/0.02	23
	Water	266,532	265,540, 598	595	595	0.035/0.0036	74
P4	THF	267,518	266,528, 600	573	573	0.68/0.02	22
	Water	265,527	266,534, 600	607	596	0.0128/0.0064	30

[a]: FLQY (Fluorescence Quantum yield) were valued by using Rhodamine B (0.70 in ethanol) as the standard. [b]: FL on/off was intensity ratio at $\lambda_{em, max}$.



	B	A	t	Statistics
	Value	Value	Value	Adj.R-Square
P3 in THF	0.07365	-0.07206	2.82209	0.99994
P4 in THF	0.04769	-0.04699	2.93433	0.99888
P3 in Water	0.04208	-0.03907	12.21531	0.9933
P4 in Water	0.04803	-0.04436	10.3879	0.98825

Figure S6. (a)The photo-kinetics curves and (b) the fluorescence intensity against time plots upon 302-nm UV irradiation (2.76 mW/cm^2). The experimental absorbance data at 600 nm are fitted to a monoexponential function.

5. The photons of P4

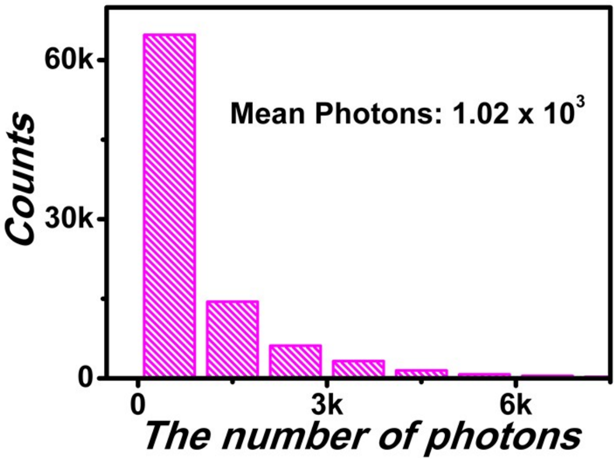
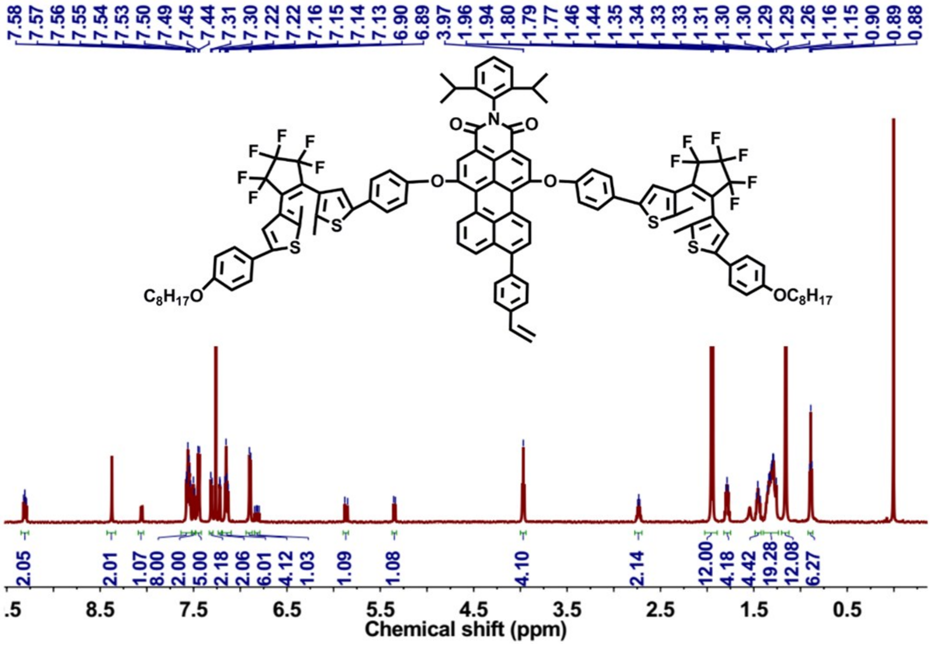
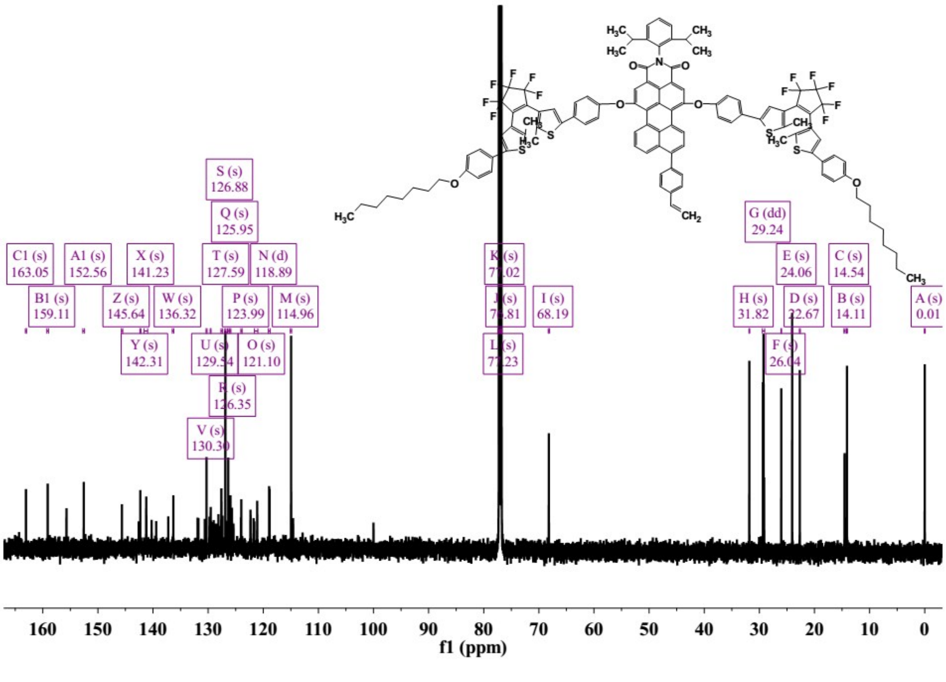


Figure S7 The histogram of photons of P4 in Hela cells per switching cycle.

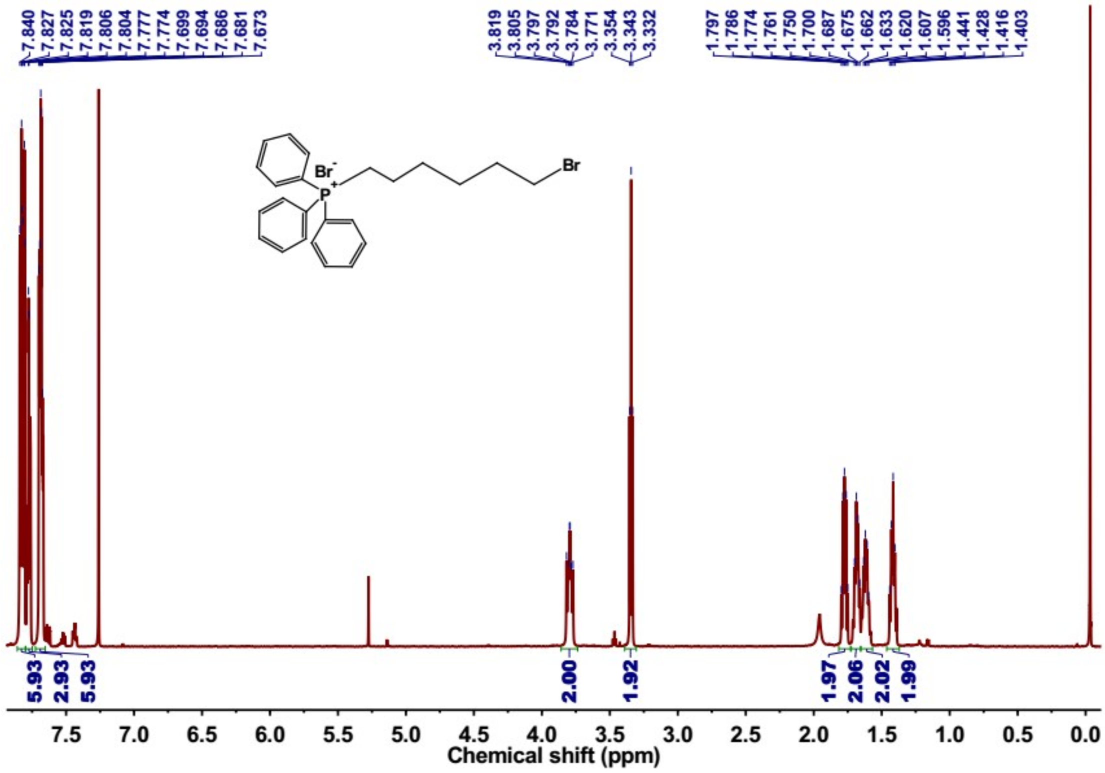
6. NMR Spectra

Compound 2





Compound 3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1.633
1.620
1.607
1.596
1.441
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1.416
1.403
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P+
Br
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2.93
5.93
2.00
1.92
1.97
2.06
2.02
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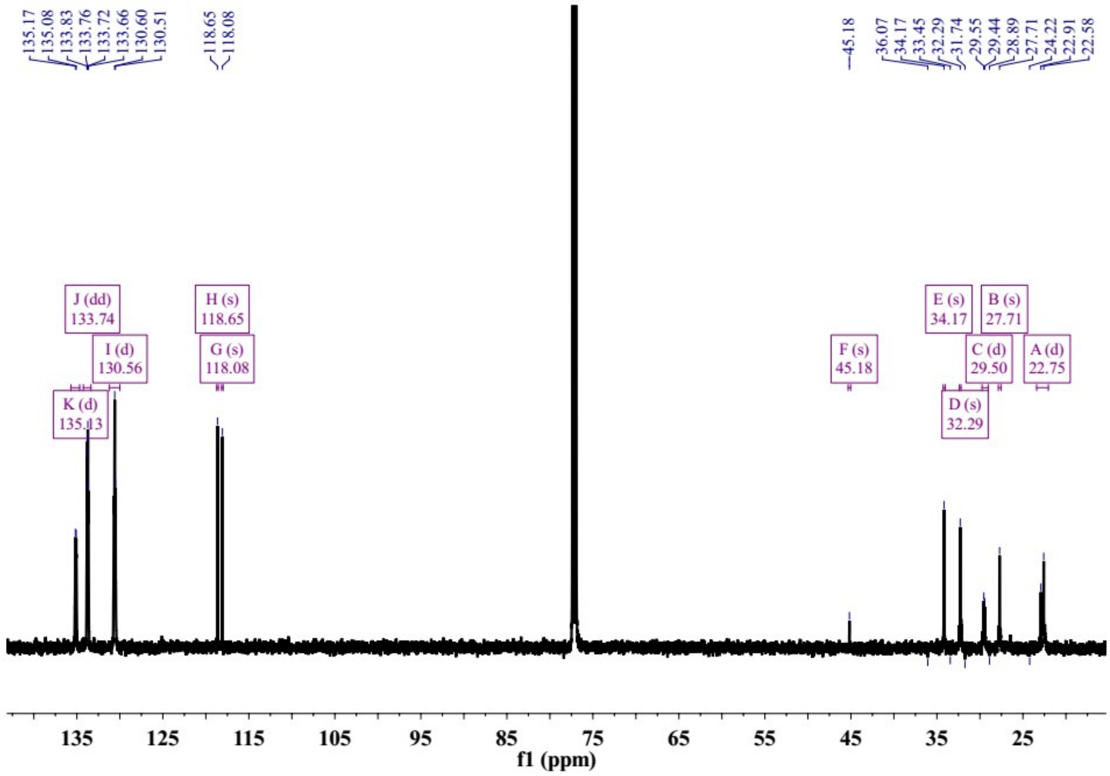
Chemical shift (ppm)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33.45
32.29
31.74
29.55
29.44
28.89
27.71
24.22
22.91
22.58
J (dd) 133.74
H (s) 118.65
E (s) 34.17
B (s) 27.71
I (d) 130.56
G (s) 118.08
F (s) 45.18
C (d) 29.50
A (d) 22.75
K (d) 135.13
D (s) 32.29
135
125
115
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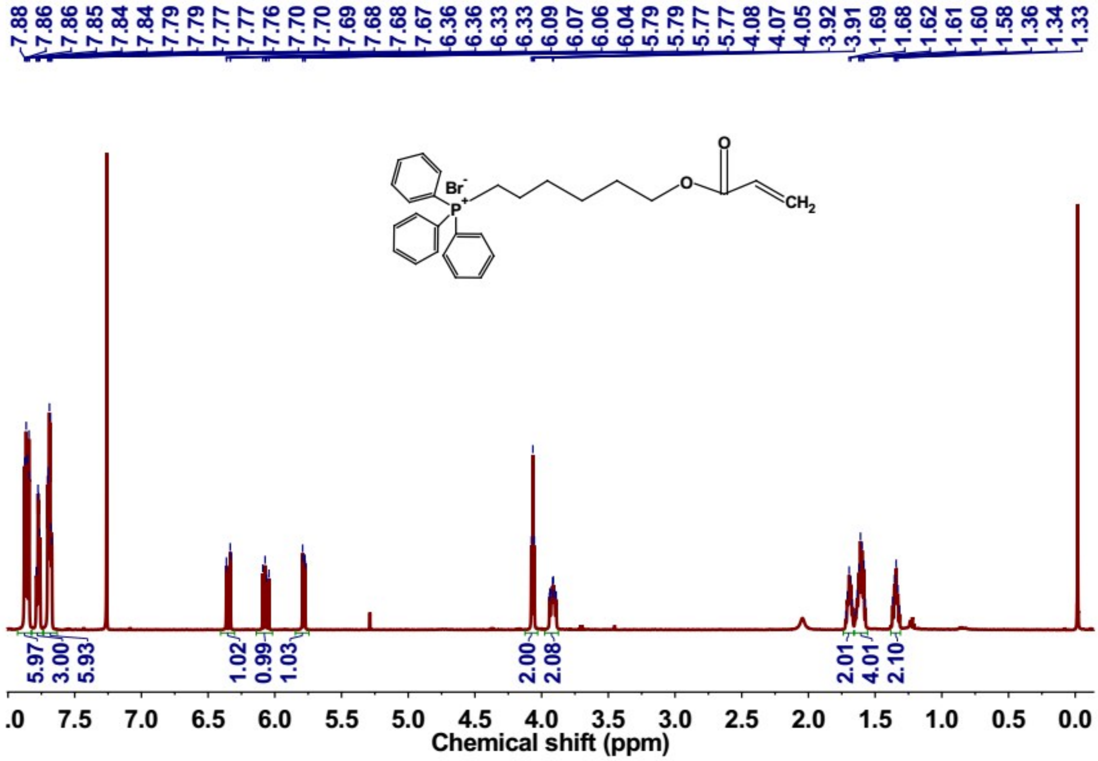
85
75
65
55
45
35
25
f1 (ppm)

Compound 4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P+
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O
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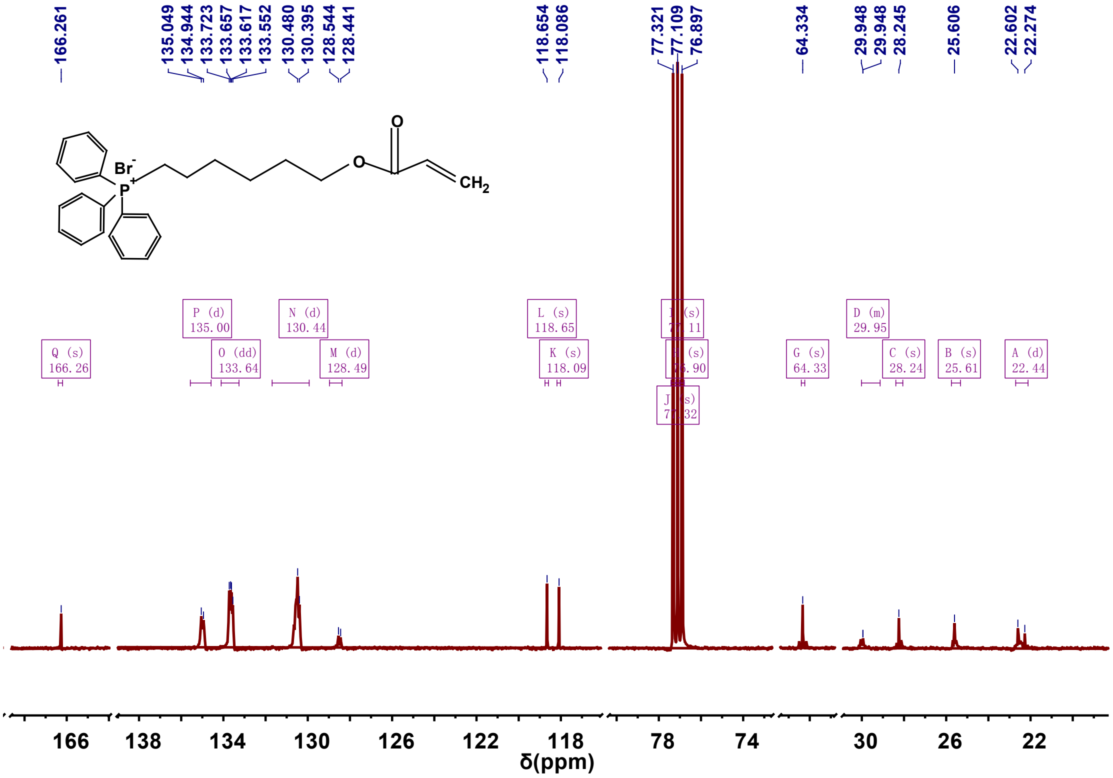
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25.606
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O
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G (s) 64.33
C (s) 28.24
B (s) 25.61
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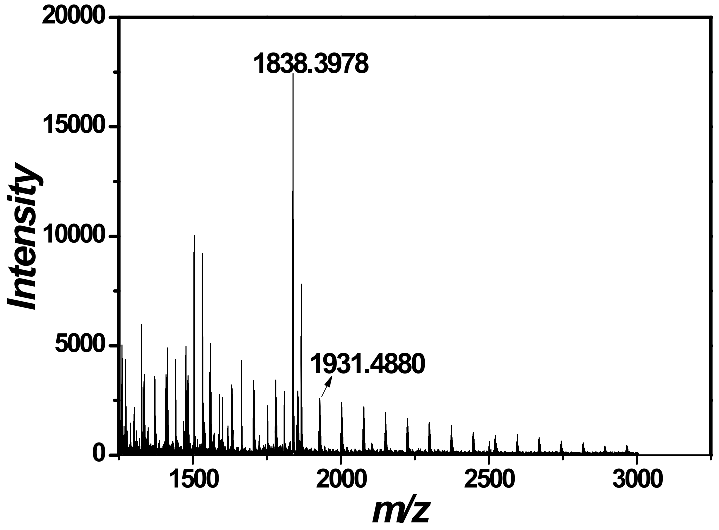
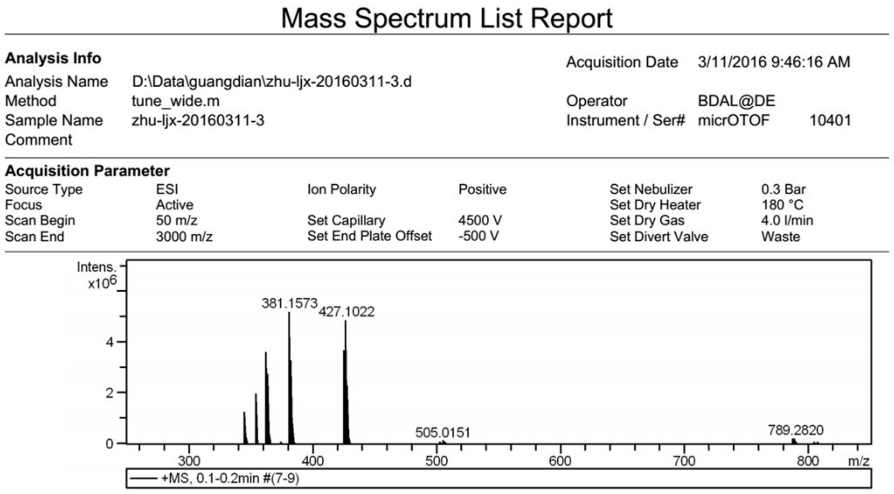
134
130
126
122
118
78
74
30
26
22
δ(ppm)

7. MS Spectra

Compound 2



Compound 3



Mass Spectrum List Report

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Operator BDAL@DE
Instrument / Ser# micrOTOF 10401

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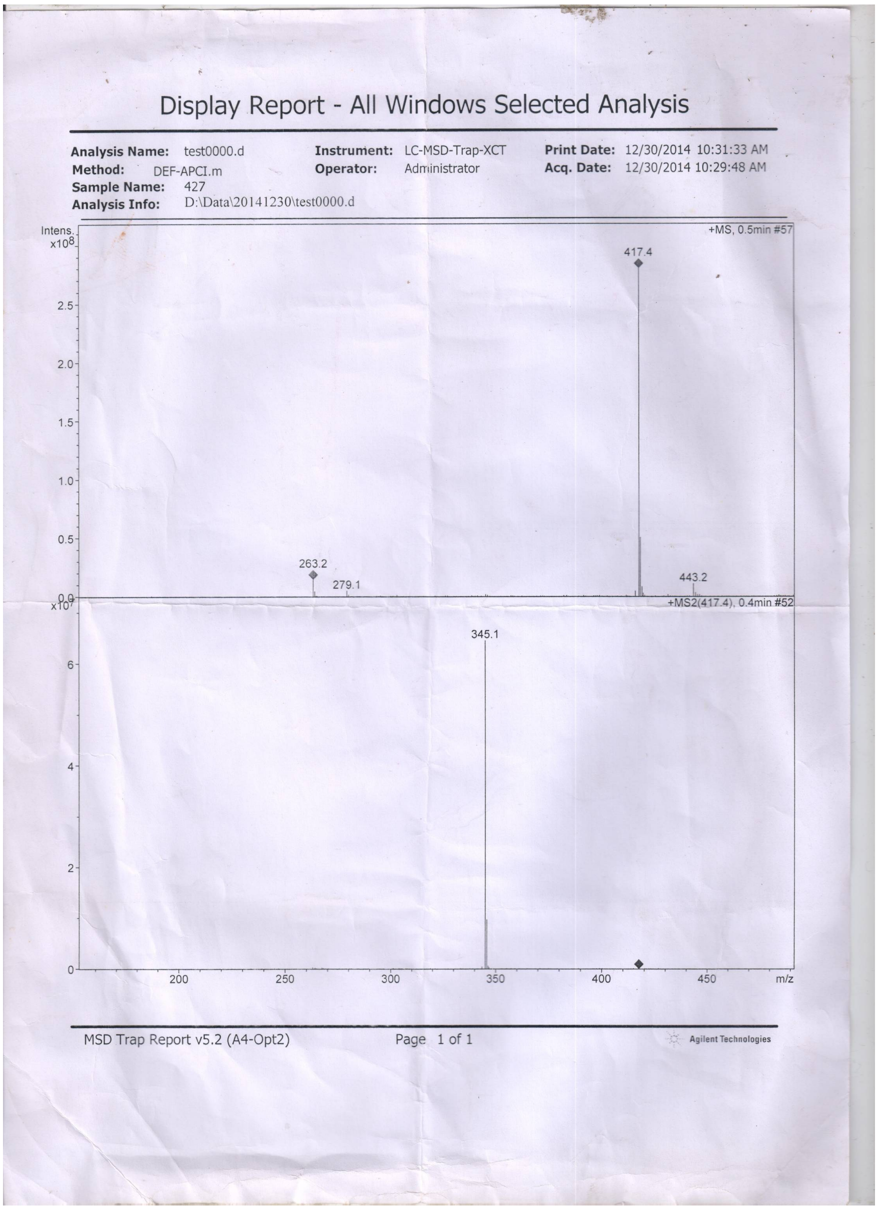
Compound 4



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Agilent Technologies

8. FT-IR properties of Polymers

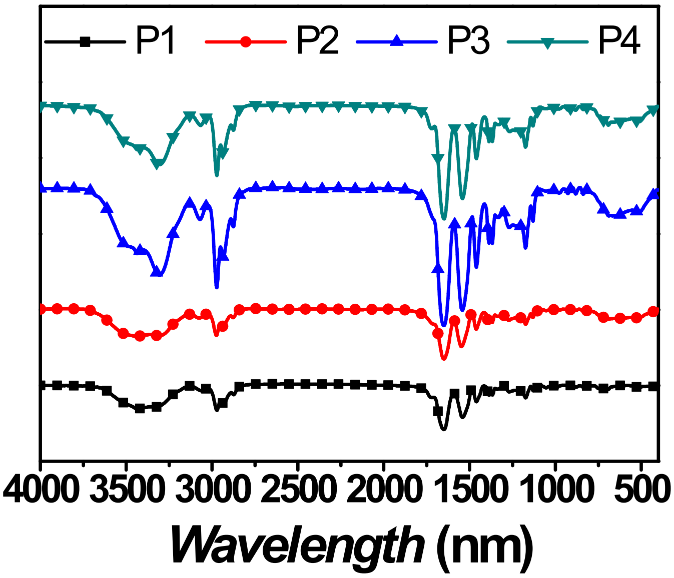

Figure S6. The FT-IR spectra of Polymers.

9. Supporting Information References

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