

Electronic Supporting Information

π -Conjugated Chromophore Incorporated Polystyrene Nanobeads as Single Optical Agent for Three Channel Fluorescent Probe in Bioimaging Application

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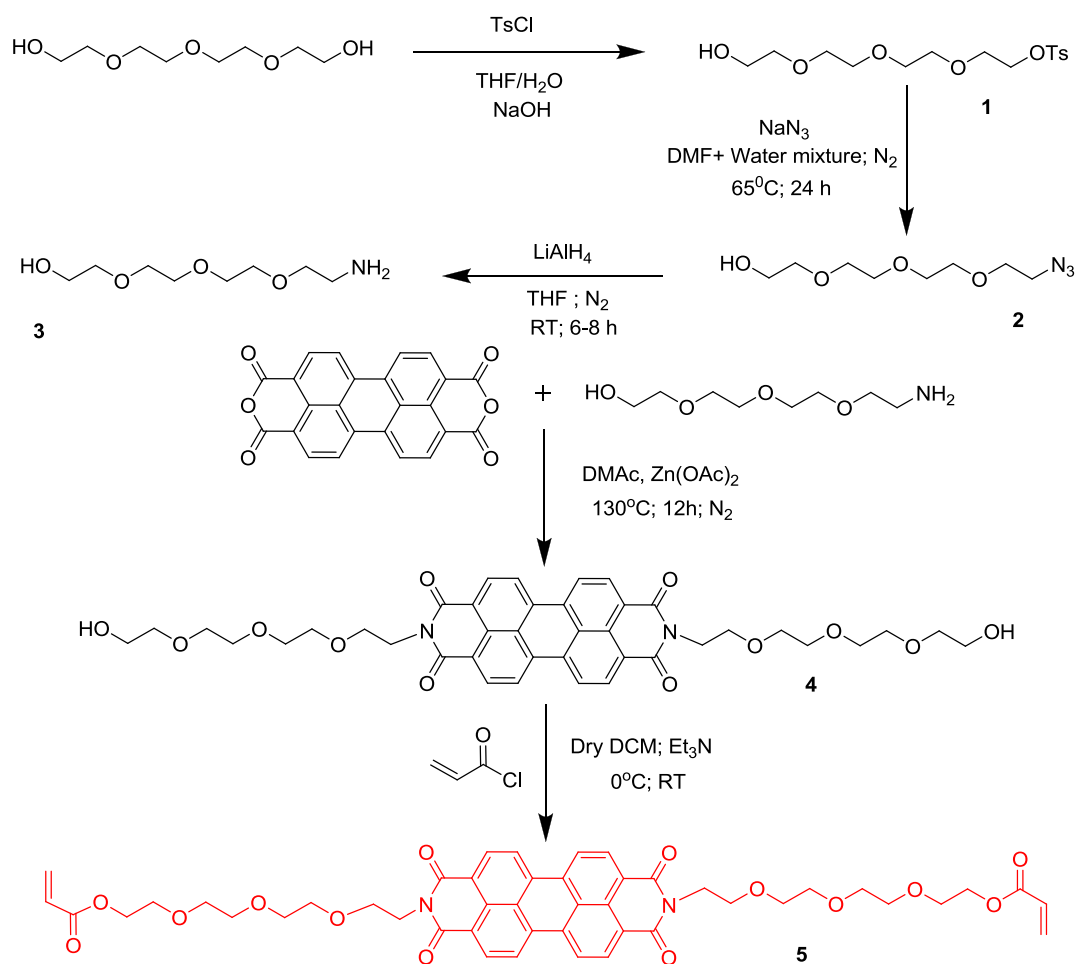
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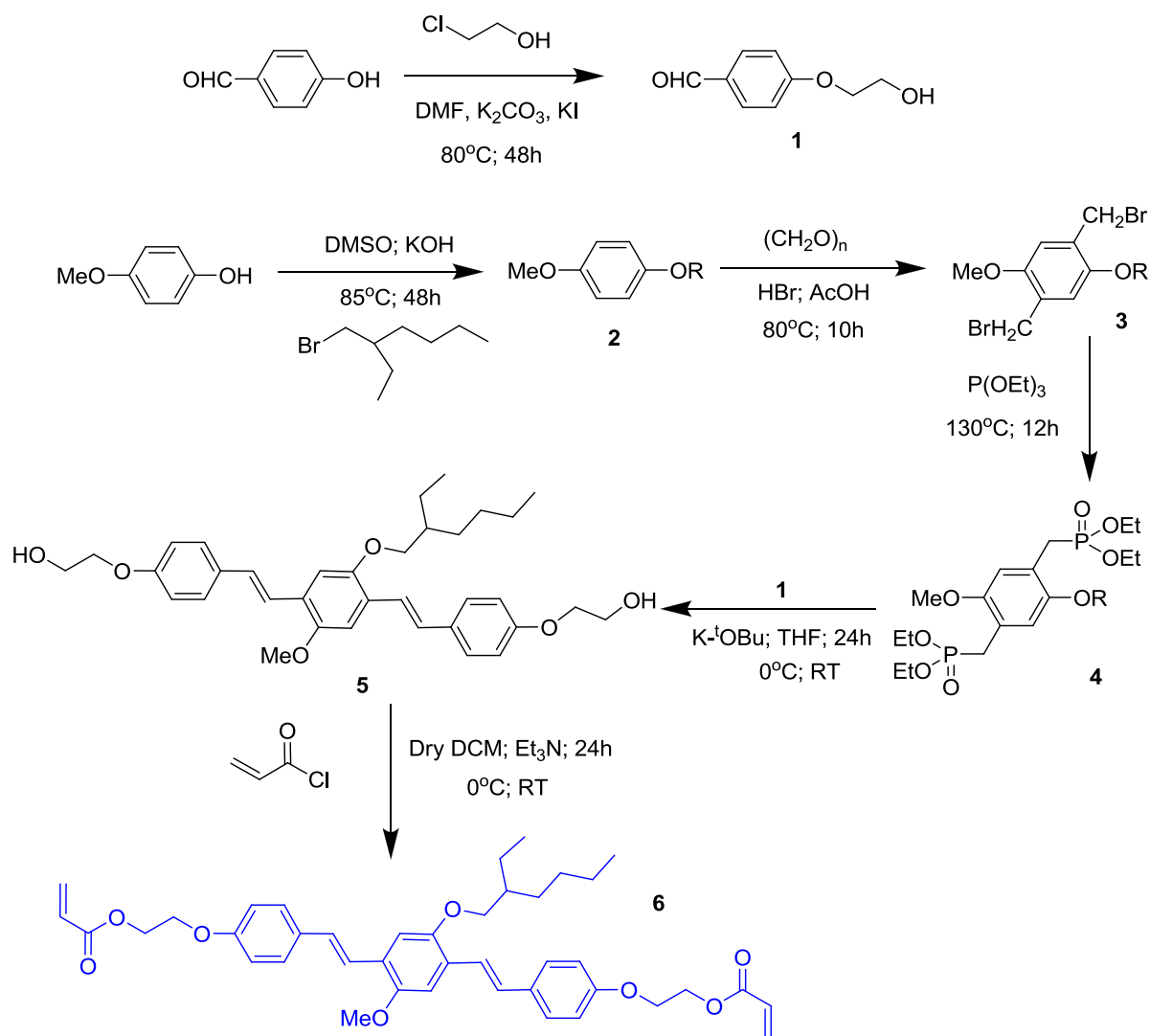
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S1 :- Synthetic Scheme of PBI Cross-linker.



S2 :- Synthetic Scheme of OPV Cross-linker.



The cross-linkers were synthesized using previously reported procedure.^{references 37, 40 main text}

Synthesis of PBI based cross-linker:-

400 mg PBI-TEG diol (5.4×10^{-4} mol) and 0.22 ml triethylamine (2.7×10^{-3} mol) were taken in 250 ml two necked round bottomed flask along with 50 ml dry DCM under nitrogen atmosphere. The flask was kept for stirring under ice cooled condition for 30 min followed by slow addition of acryloyl chloride (0.22 ml, 2.7×10^{-3} mol) over a period of 20 min at 0°C. After the addition it was brought to room temperature slowly. Then the reaction was allowed to stir for 24 h. The progress of reaction was timely monitored using TLC. The reaction was worked up by washing the organic phase with water and brine and then organic phase was evaporated off. Finally the compound was column purified using DCM/Methanol (1%) as solvent. Yield (30%). 120 mg.

¹H-NMR (400 MHz, CDCl₃, δ) :- 8.69 (m, 8H, perylene ring), 6.39 (dd, 2H, acrylic double bond), 6.13 (q, 2H, acrylic double bond), 5.84 (dd, 2H, acrylic double bond), 4.49 (t, 4H, -NCH₂), 4.30 (t, 4H, -NCH₂), 3.89 (t, 4H), 3.62 (m, 20H).

¹³C-NMR (100 MHz, CDCl₃, δ) :- 165.88, 163.10, 134.32, 131.15, 130.71, 129.09, 128.01, 126.10, 122.83, 77.33, 69.89, 69.83, 67.66, 63.42, 39.07.

LC-MS/MS for C₄₆H₄₆N₂O₁₄ (in AcCN) :- m/z calculated 850.29. Found :- 851.30 [M + H], 873.28 [M + Na].

Synthesis of OPV based cross-linker :-

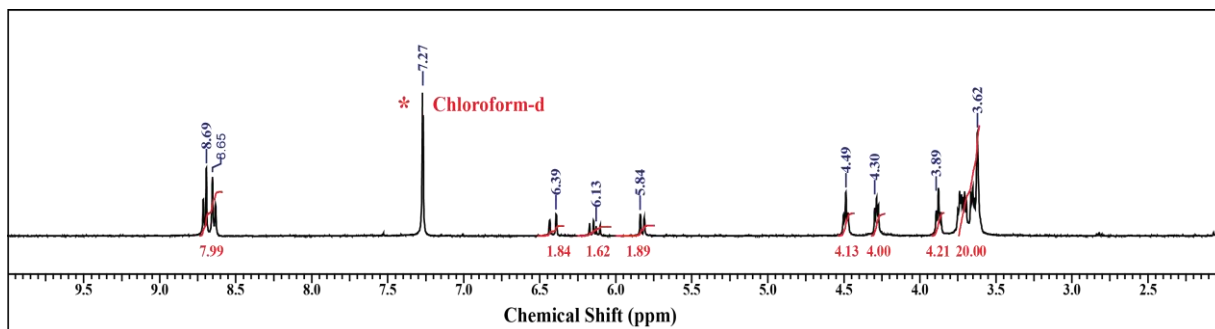
In 250 ml two- necked round-bottomed flask, 265 mg OPV diol (4.7×10^{-4} mol) and 0.33 ml of triethylamine (2.4×10^{-3} mol) were dissolved in 50 ml of dry DCM under nitrogen atmosphere. Then it was allowed to stir for 30 min at 0°C. 0.2 ml (2.4×10^{-3} mol) of acryloyl chloride was taken in DCM and added to the flask for a period of 20 min at 0°C. Reaction was allowed to stir at room temperature for 24 h and timely monitored by TLC. Crude compound was worked up by washing with water and then organic phase was dried off and it was further purified by column chromatography using Pet ether/ EtOAc (20%) as solvent system. Yield (50%). 132 mg.

¹H-NMR (400 MHz, CDCl₃, δ) :- 7.49-6.93 (3m, 14H, Ar-H, vinylic H), 6.45 (dd, 2H, acrylic double bond), 6.20 (m, 2H, acrylic double bond), 5.89 (dd, 2H, acrylic double bond), 4.54 (m, 2H, -ArOCH₂), 4.27 (m, 2H, -C(O)OCH₂), 3.92 (m, 4H), 1.83 (b, 1H, tert), 1.37 (m, 6H), 0.99 (m, 6H).

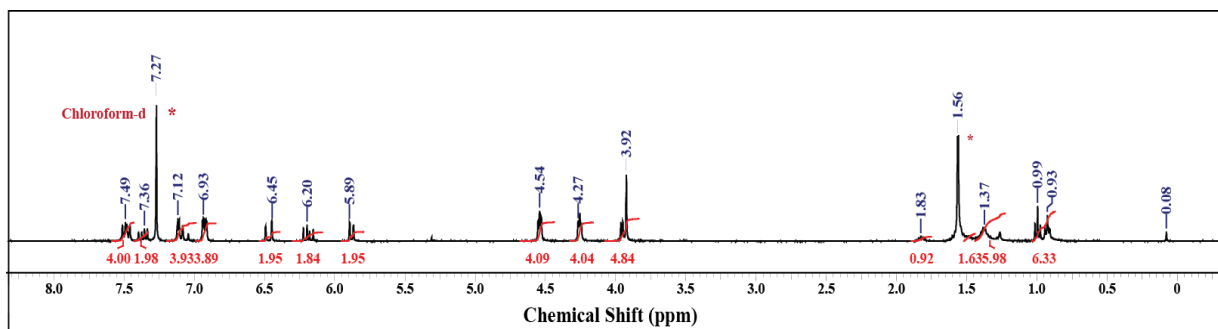
¹³C-NMR (100 MHz, CDCl₃, δ) :- 165.85, 163.14, 150.92, 131.10, 127.82, 127.56, 127.41, 121.45, 114.6, 71.56, 65.73, 62.86, 56.12, 39.54, 30.89, 30.69, 29.01, 23.98, 22.85, 13.85, 11.07.

LC-MS/MS for $C_{46}H_{46}N_2O_{14}$ (in AcCN) :- m/z calculated 668.33. Found :- 669.34 [M + H], 691.32 [M + Na].

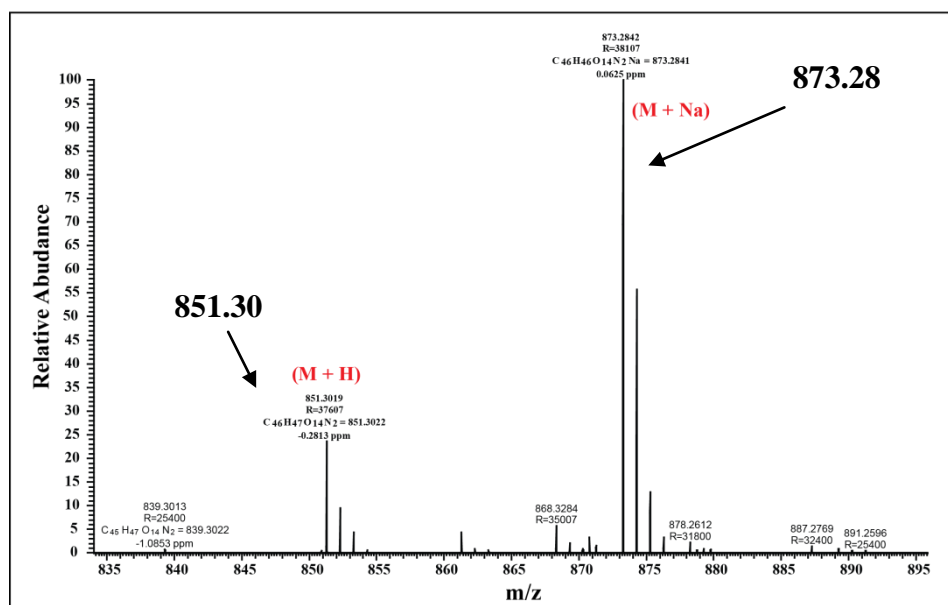
S3:- 1H -NMR spectra of PBI Cross-linker recorded in $CDCl_3$.



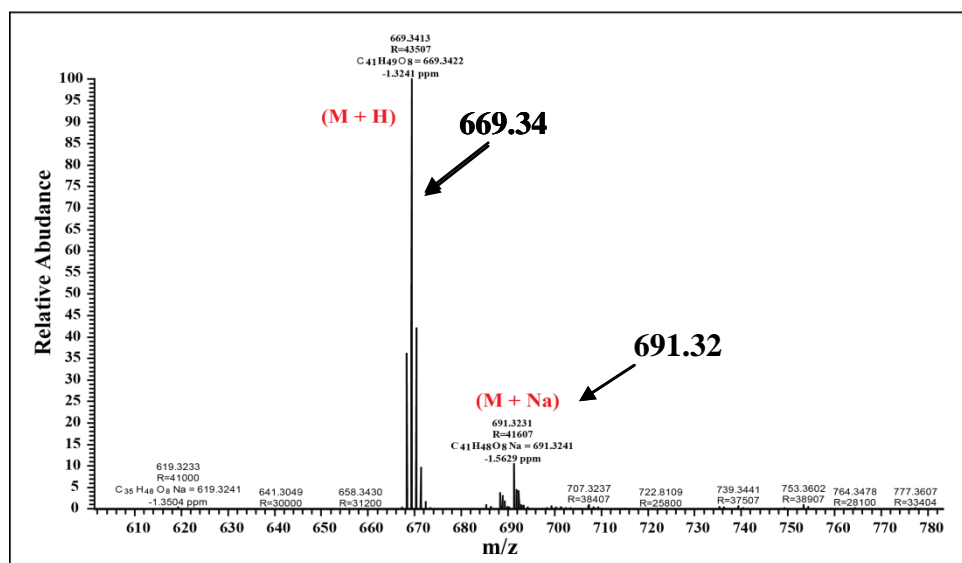
S4 :- 1H -NMR spectra of OPV Cross-linker recorded in $CDCl_3$.



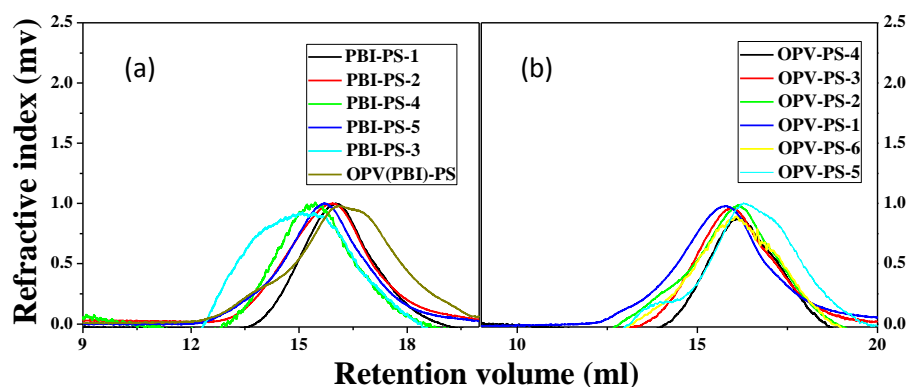
S5 :- HRMS spectra of PBI Cross-linker recorded in acetonitrile.



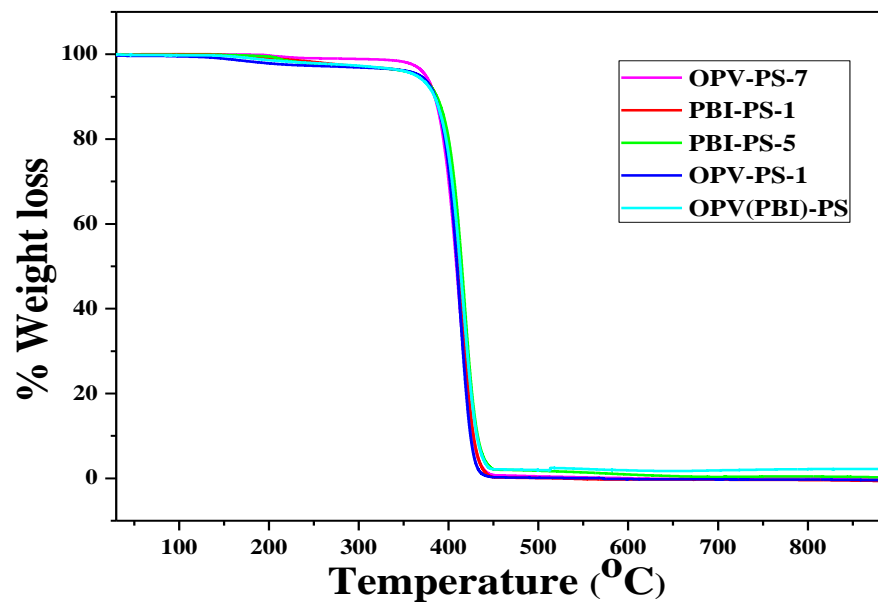
S6 :- HRMS spectra of OPV Cross-linker recorded in acetonitrile.



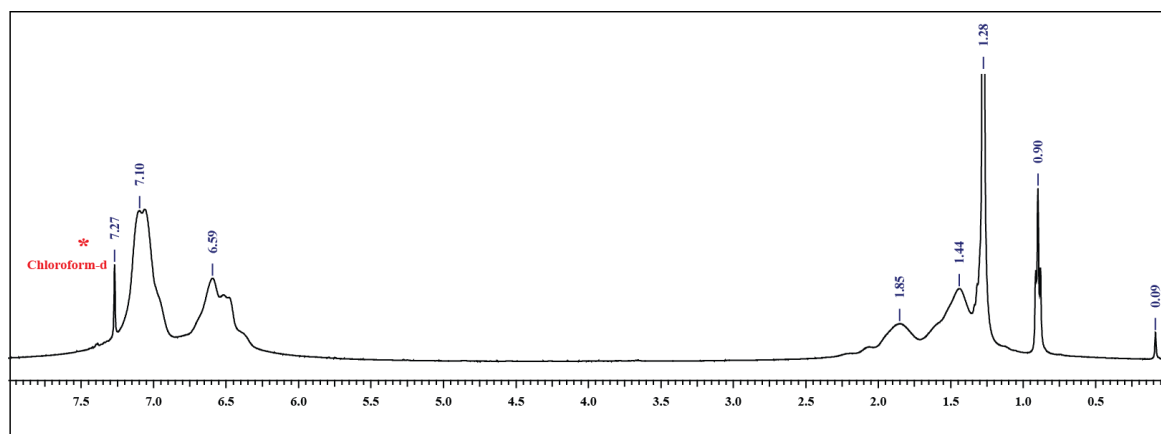
S7. GPC Chromatogram of (a) **PBI-PS-n /OPV(PBI)-PS** (left) (b) **OPV-PS-n** polymers (right) in THF with flow rate of 1ml/min.



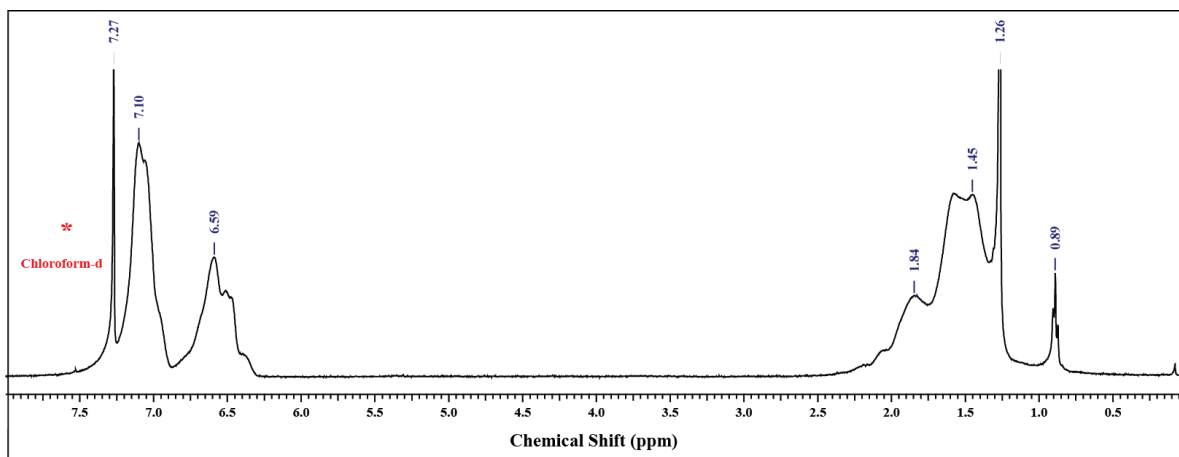
S8. TGA (Thermogravimetric analysis) of polymer samples.



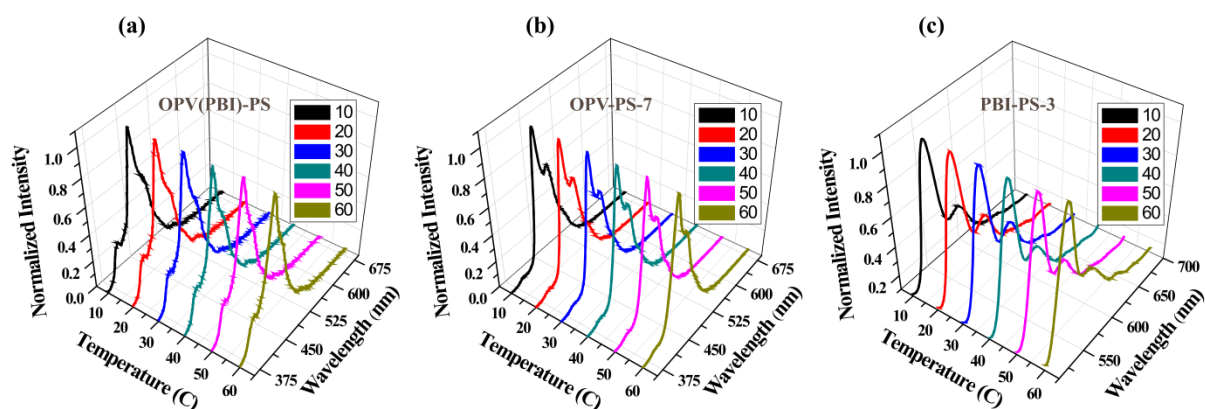
S9a. ^1H -NMR spectra of OPV-PS-7 polymer recorded in CDCl_3 .



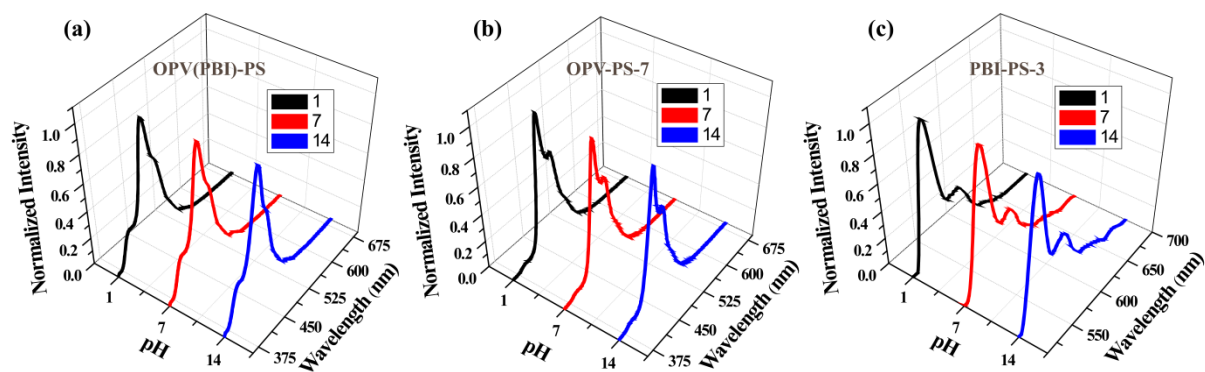
S9b. ^1H -NMR spectra of PBI-PS-5 polymer recorded in CDCl_3 .



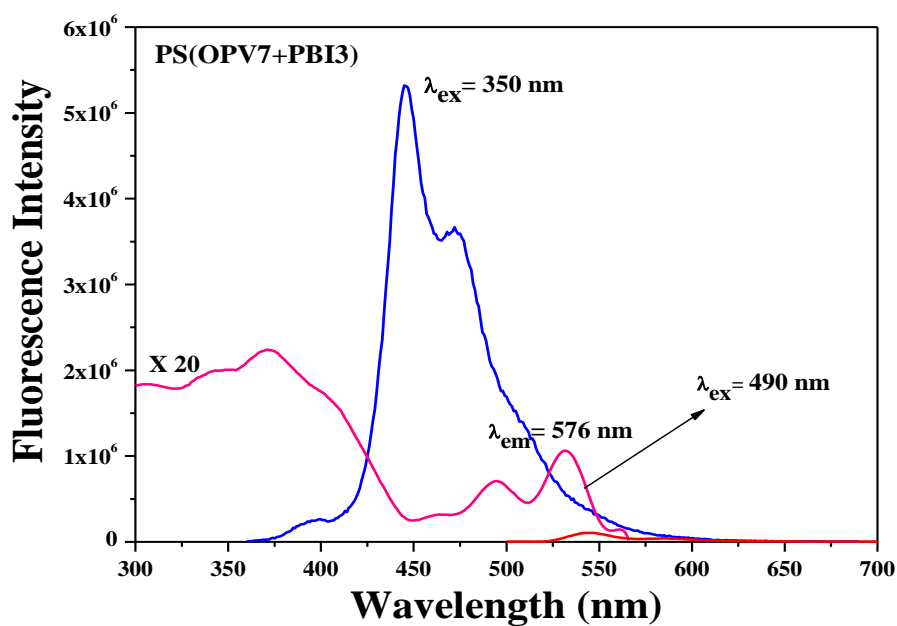
S10. Solution state emission spectra showing the dependence of fluorescence intensity on temperature varied from 0°C to 60°C for a) OPV(PBI)-PS b) OPV-PS-n (c) PBI-PS-n polymers. (0.1 mg / 3ml) in 1X PBS buffer.



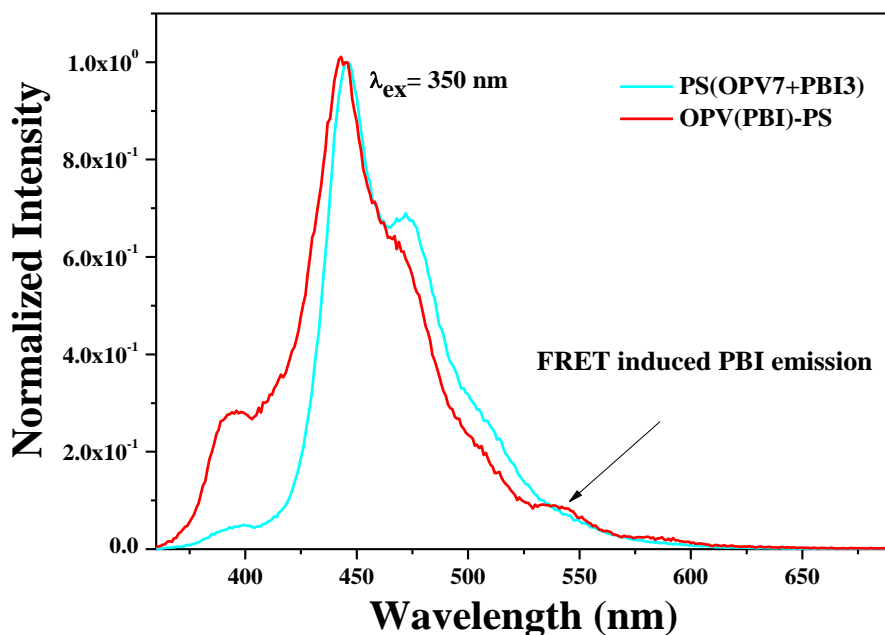
S11. Solution state emission spectra showing the dependence of fluorescence intensity on pH varied from 1 to 14 for a) OPV(PBI)-PS b) OPV-PS-n (c) PBI-PS-n polymers. (0.1 mg / 3ml) in 1X PBS buffer.



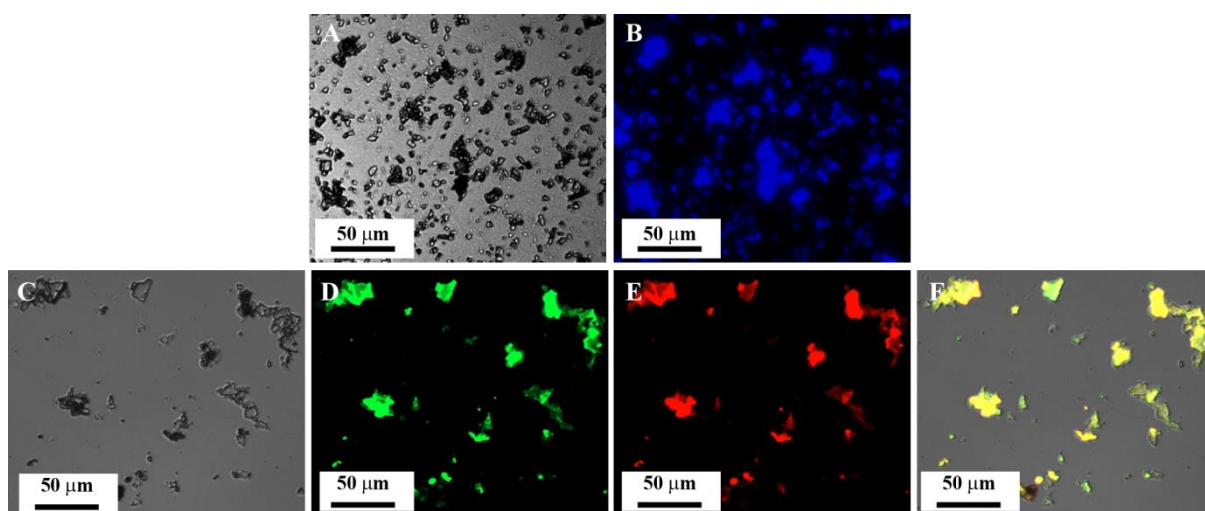
S12 . Solution state emission and excitation spectra for PS(OPV7+PBI3) (0.1 mg / 3ml) in 1X PBS buffer.



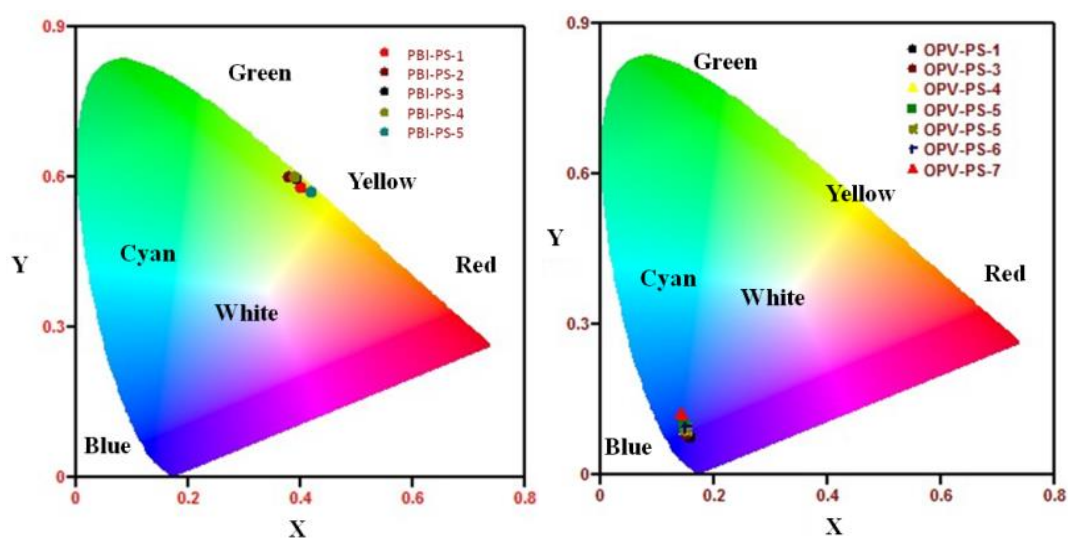
S13. Normalized emission spectra upon excitation at 350 nm for PS(OPV7+PBI3) (physical mixture) and OPV(PBI)-PS (covalent) polymer at concentration (0.1 mg / 3ml) in 1X PBS buffer showing FRET induced PBI emission in case of covalent OPV(PBI)-PS polymer.



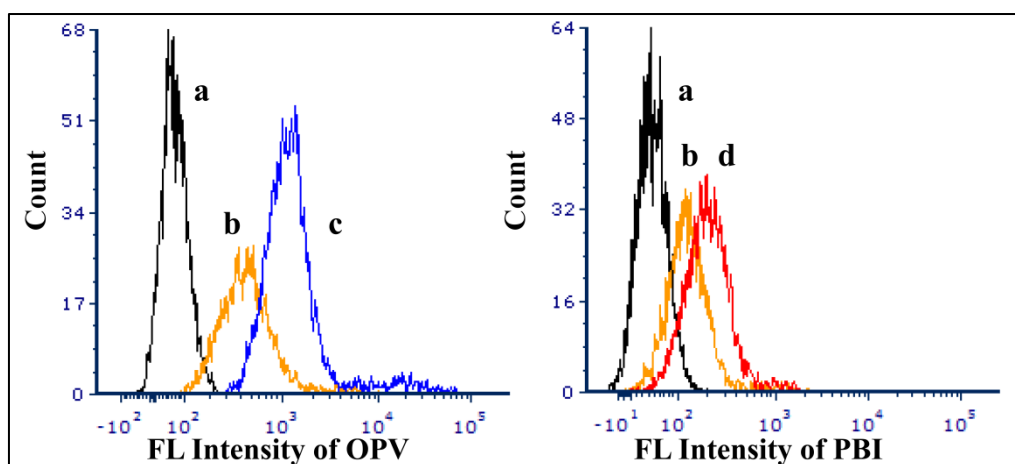
S14. Fluorescence optical microscopy images of (a) **OPV-PS-7** (A) Bright field (B) using DAPI (Blue, 350-430 nm) filter (Top) (b) **PBI-PS-3** (C) Bright Field (D) using Alexa (Green, 488-520 nm) (E) Rhodamine (Red, 480-580 nm) filters and (F) Merged Image (Bottom).



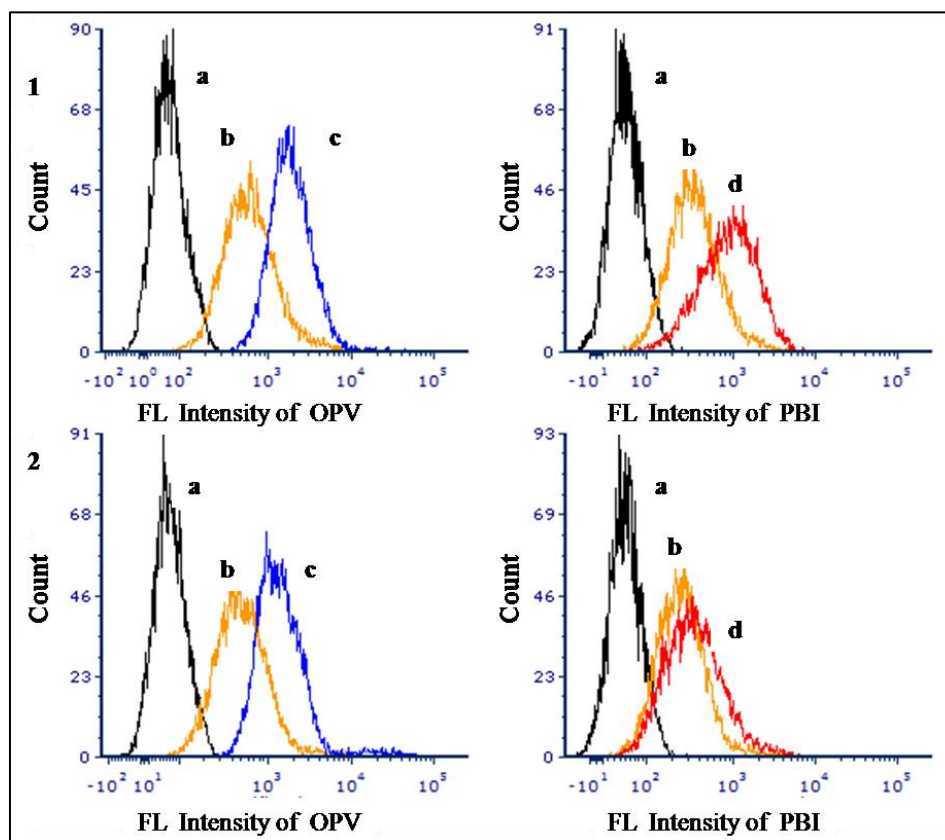
S15. CIE coordinate diagrams of **PBI-PS-n** (left), **OPV-PS-n** (right).



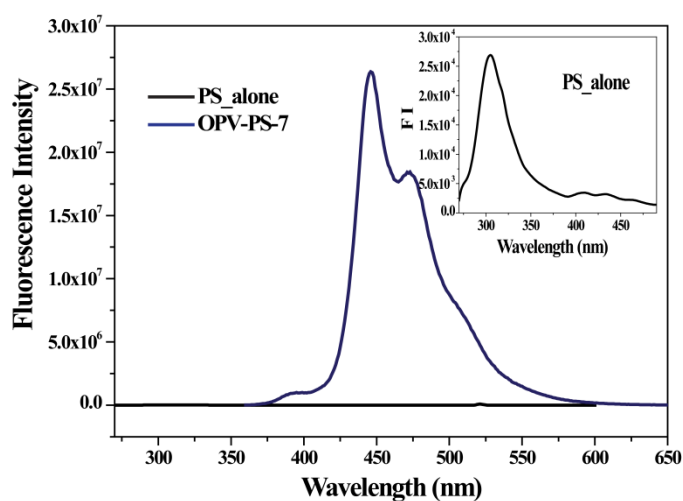
S16. Flow cytometry plots for control (a), OPV(PBI)-PS (b), OPV-PS-7 (c) and PBI-PS-3 (d) in HeLa cell lines after 9 h incubation.



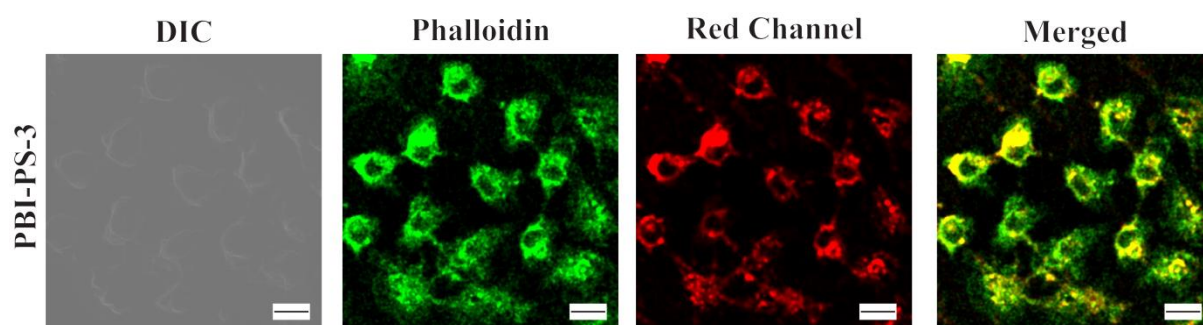
S17. Flow cytometry plots for control (a), OPV(PBI)-PS (b), OPV-PS-7 (c) and PBI-PS-3 (d) in MCF-7 cell lines after 24 h (1) and 9h (2) incubation.



S18. Comparison of solution state emission spectra for OPV-PS-7 and PS_alone in 1X PBS buffer (0.1 mg / 3ml).



S19. CLSM image of Phalloidin staining of PBI-PS-3 in HeLa cells.



S20. CLSM image of HeLa cells incubated with PS(OPV7+PBI3) (physical mixture).

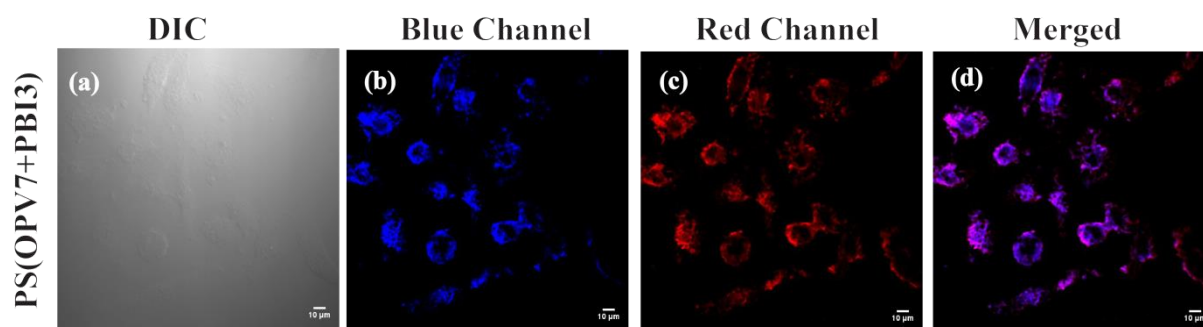


Table S1. Sample designation, Number and Weight Average Molar Mass, Polydispersity indices (\bar{M}_w/\bar{M}_n), Solid Content (%), Zeta potential (ζ)

Samples	\bar{M}_n^a	\bar{M}_w^a	PDI^a	Solid content (%)	ζ-Potential (mV)^b	TGA (T_d= 5%)
OPV-PS-1	121000	360000	2.9	12.8	-30.5	365
OPV-PS-2	94900	313000	3.3	10.5	-32.1	-
OPV-PS-3	100200	224000	2.2	13.2	-27.1	-
OPV-PS-4	82300	167000	2.0	14.2	-29.0	-
OPV-PS-5	53400	207900	3.8	20.0	-31.8	-
OPV-PS-6	88000	303200	3.4	20.4	-29.7	-
OPV-PS-7	-	-	-	19.8	-33.6	365
PBI-PS-1	74500	207300	2.7	19.3	-30.0	365
PBI-PS-2	97100	280900	2.9	18.6	-27.4	-
PBI-PS-3	152100	651900	4.2	15.3	-35.7	-
PBI-PS-4	117600	358300	3.0	18.0	-34.4	-
PBI-PS-5	126900	360300	2.8	12.7	-30.7	365
OPV(PBI)-PS	59100	242600	4.1	20.5	-31.1	365

a) Measured by Gel Permeation Chromatography (GPC) in tetrahydrofuran (THF) calibrated with linear, narrow molecular weight distribution polystyrene standards.

b) Measured at pH 7 using 0.001M KCl as background electrolyte.

Table S2: Recipe for miniemulsion polymerization.

Components	Amount
Water	4 gm
Styrene	1 gm
Cross-linker	Varied
Hexadecane	48 mg
SDS	12 -36 mg
KPS	3.3 mg

Except OPV-PS-7 and PBI-PS-3 (where SDS was taken 36 mg) the amount of SDS used for all the polymers was 12 mg. Amount of cross-linkers used were already discussed in Table 1.

Table S3: Comparison of average size of particles obtained from TEM and FESEM.

Samples	Average Size from TEM (nm)	Average Size from FESEM (nm)
OPV-PS-6	75.6±3	76.5±4
OPV-PS-2	-	97.6±9
OPV_PS-3	-	92.9±5
PBI-PS-4	72.4±3	-
PBI-PS-5	-	75.6±6
OPV(PBI)- PS	79.5±5	80.5±4

Table S4a: CIE coordinate values for **OPV(PBI)-PS/ PBI-PS-n** .

Samples	X	Y
OPV-PS-1	0.16	0.12
OPV-PS-2	0.15	0.10
OPV-PS-3	0.16	0.07
OPV-PS-4	0.15	0.09
OPV-PS-5	0.15	0.10
OPV-PS-6	0.15	0.09
OPV-PS-7	0.14	0.12
PBI-PS-1	0.40	0.58
PBI-PS-2	0.38	0.60
PBI-PS-3	0.39	0.60
PBI-PS-4	0.39	0.60
PBI-PS-5	0.42	0.57

Table S4b: CIE coordinate values for **OPV(PBI)-PS** polymer at different excitation wavelength.

Samples	X	Y
350	0.15	0.11
390	0.15	0.10
440	0.15	0.19
450	0.24	0.39
460	0.37	0.56
470	0.39	0.57
490	0.38	0.60