

# **Tuning the Emission Properties of a Fluorescent Polymer using a Polymer Microarray Approach – Identification of an Optothermo Responsive Polymer**

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## **Supporting Information**

### **Methods:**

#### **Materials and Devices**

All the monomers were purchased from Aladdin Industrial Inc and used without any purification. Other chemicals were bought from Sinopharm Chemical Reagent Co., Ltd and were used as received.

The inkjet printer was an Autodrop Compact printer from Microdrop Technologies, Germany with a nozzle diameter of 70  $\mu\text{m}$ . The inverted fluorescent microscope (GR-05DY) was from Shanghai Gold Room.

<sup>1</sup>H NMR spectra were recorded on a Bruker AVANCE III (400 MHz) using CDCl<sub>3</sub> as a solvent and tetramethylsilane (TMS) as an internal standard.

UV/Vis spectrometer and fluorescence spectrometer (LS 45) were from PerkinElmer.

#### **Preparation of Polymer Microarrays**

Polymer microarrays with 1100 polymer spots were printed with the inkjet printer. Firstly, microscopic glass slides (26×76 mm) were soaked in 1M NaOH aqueous solution for 24h before being washed with distilled water 3 times and subsequently with acetone. The cleaned glass slides were then printed with a solution of sucrose (10 wt% in water) to form a 20×55 array per slide. The distance between adjacent spots was 1 mm. The space outside the sucrose features were coated with 1H,1H,2H,2H – perfluorooctyldimethylchlorosilane (FDS) (10  $\mu$ L) and the slides were sealed inside a small plastic box for 24h before being washed with acetone ( $\times$ 3) and then water to remove the sucrose spots. The slides were dried in air and exposed glass that was coated with 3-(trimethoxysilyl) propyl methacrylate for another 24h and were ready for monomer printing after washing with acetone. The last step was the printing of monomers, crosslinker and photo-initiator onto the masked glass slide (Fig. S1a) followed with exposing to UV light (365 nm) for 30 min to make an array of copolymers (Fig.S1b).

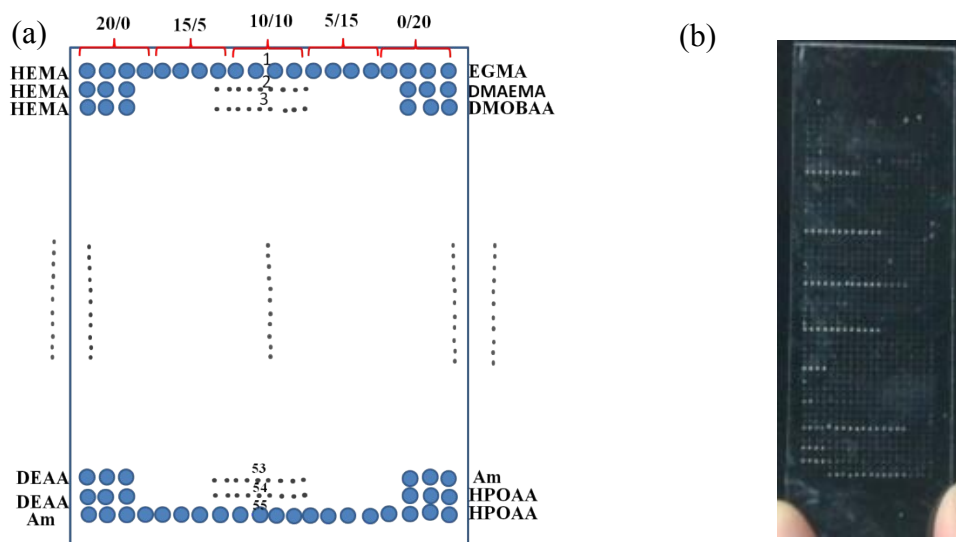


Figure S1 The fabrication of the polymer microarrays. (a) The layout of the printed array (55 rows by 20 columns). The abbreviations on the left and right sides of the slide were the monomers used to fabricate the polymers across that row (each polymer was fabricated in quadruplicate); (b) An image of the printed polymer microarray (20×55 features).

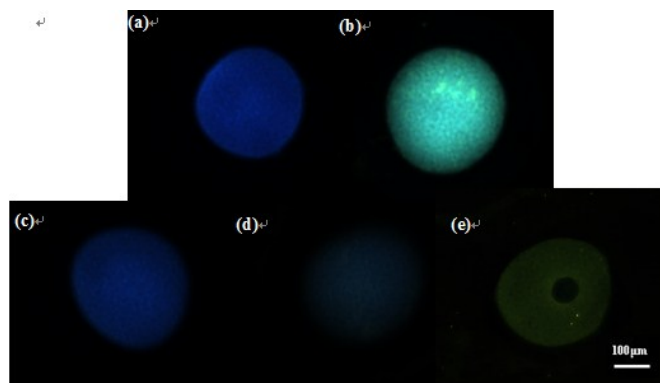


Figure S2 Images of polymer features from the fluorescent array. (a) and (c) Polymers without the conjugated fluorescent polymer; (b) and (d) Polymers with the conjugated fluorescent polymer; (e) An image of the pure dye. Images were taken on a GR-05DY inverted fluorescent microscope (Shanghai Gold Room) using UV channel with excitation wavelength of 350-370 nm. The scale bar is 100  $\mu\text{m}$ .

Table S1 The average relative fluorescent intensities (RFI) of the spots of the polymer microarray with an immobilized fluorescent polymer dye

MI	Integrated density															M2	
	0/20			5/15			10/10			15/5			20/0				
	Polymer ( $\times 10^6$ )	Polymer-A ( $\times 10^6$ )	RFI	Polymer ( $\times 10^6$ )	Polymer-A ( $\times 10^6$ )	RFI	Polymer ( $\times 10^6$ )	Polymer-A ( $\times 10^6$ )	RFI	Polymer ( $\times 10^6$ )	Polymer-A ( $\times 10^6$ )	RFI	Polymer ( $\times 10^6$ )	Polymer-A ( $\times 10^6$ )	RFI		
HEMA	3.92	6.24	0.02	31.10	151.32	1.23	64.22	111.42	0.48	13.54	37.17	0.24	6.86	9.10	0.02	EGMA	
	6.44	7.69	0.01	3.75	11.76	0.08	3.75	14.48	0.11	8.87	15.54	0.07	6.51	9.30	0.03	DMAEMA	
	3.75	4.38	0.01	3.75	16.63	0.13	3.75	9.63	0.06	3.75	7.18	0.04	5.59	9.87	0.04	DMOBAA	
	5.83	6.60	0.01	12.00	5.38	-0.07	14.41	6.56	-0.08	14.22	5.31	-0.09	6.76	9.20	0.02	NIPAA	
	4.35	10.48	0.06	6.63	8.78	0.02	4.41	9.52	0.05	3.14	10.27	0.07	6.75	9.67	0.03	CHMA	
	6.53	8.37	0.02	6.73	8.11	0.01	3.75	31.54	0.28	3.75	7.98	0.04	5.42	9.71	0.04	DMC	
	15.97	16.90	0.01	20.88	8.94	-0.12	8.81	8.48	0.00	10.72	8.47	-0.02	5.68	9.57	0.04	CEA	
	26.64	5.91	-0.21	23.15	4.01	-0.20	20.86	4.80	-0.16	7.97	6.08	-0.02	6.46	8.95	0.03	DEAA	
	24.19	31.43	0.07	13.98	46.54	0.33	11.58	24.25	0.13	1.96	11.93	0.10	6.09	9.14	0.03	Am	
28.49	20.70	-0.08	33.64	16.66	-0.17	14.10	6.40	-0.08	7.67	8.24	0.01	6.71	9.24	0.03	HPOAA		
EGMA	6.44	7.69	0.01	13.74	3.86	-0.10	7.22	4.53	-0.03	10.49	5.50	-0.05	6.68	7.28	0.01	DMAEMA	
	16.61	3.63	-0.13	1.20	9.42	0.08	1.20	8.52	0.07	4.29	13.75	0.10	6.92	7.24	0.00	DMOBAA	
	15.41	15.39	0.00	14.01	22.32	0.09	14.01	16.60	0.03	19.31	9.07	-0.10	6.90	7.85	0.01	NIPAA	
	2.73	6.28	0.04	2.73	3.64	0.01	5.51	3.67	-0.02	5.23	5.12	0.00	6.75	7.82	0.01	CHMA	
	21.83	13.82	-0.08	14.05	17.26	0.03	14.08	6.95	-0.07	21.42	7.86	-0.14	6.38	7.19	0.01	DMC	
	16.89	32.54	0.16	14.07	13.03	-0.01	18.46	21.85	0.03	16.64	14.90	-0.02	6.31	7.59	0.01	CEA	
	27.42	2.68	-0.25	10.72	1.90	-0.09	7.65	6.14	-0.02	6.42	8.88	0.03	6.19	7.25	0.01	DEAA	
	17.23	17.21	0.00	15.84	14.19	-0.02	9.89	80.18	0.72	1.67	40.54	0.40	6.16	7.78	0.02	Am	
	19.58	18.32	-0.01	20.48	16.52	-0.04	24.49	11.79	-0.13	19.70	15.27	-0.05	6.44	7.84	0.01	HPOAA	
DMAE	19.77	8.61	-0.11	20.60	7.10	-0.14	17.50	5.47	-0.12	21.08	6.73	-0.15	4.27	3.89	0.00	DMOBAA	
	11.28	39.09	0.28	13.27	45.40	0.33	6.96	42.54	0.36	3.74	8.23	0.05	3.74	2.52	-0.01	NIPAA	
	4.36	3.14	-0.01	5.64	2.70	-0.03	4.03	1.76	-0.02	9.39	4.45	-0.05	3.28	2.36	-0.01	CHMA	
	5.85	24.42	0.19	5.16	38.42	0.34	1.36	33.64	0.33	3.15	21.49	0.19	3.61	4.76	0.01	DMC	
	MA	6.08	34.65	0.29	13.16	35.10	0.22	3.74	34.23	0.31	3.74	12.20	0.09	3.74	4.75	0.01	CEA
		7.00	38.01	0.32	10.22	42.76	0.33	9.39	33.18	0.24	8.97	41.55	0.33	3.87	3.30	-0.01	DEAA
		5.09	13.52	0.09	5.16	18.39	0.14	15.07	23.20	0.08	34.61	28.42	-0.06	3.39	3.45	0.00	Am
		22.21	26.74	0.05	1.89	33.28	0.32	0.66	24.50	0.24	0.66	16.80	0.17	3.66	4.03	0.00	HPOAA
DMOBA	6.90	74.39	0.69	25.00	9.11	-0.16	14.78	10.99	-0.04	8.18	13.57	0.06	40.48	9.69	-0.32	NIPAA	
	3.74	3.22	-0.01	3.74	8.38	0.05	3.74	13.71	0.10	3.74	14.18	0.11	45.68	10.32	-0.36	CHMA	
	3.74	52.39	0.50	12.32	31.75	0.20	3.74	22.60	0.19	7.17	24.13	0.17	40.19	10.24	-0.31	DMC	
	10.98	40.30	0.30	3.74	41.34	0.38	3.74	31.13	0.28	3.74	20.44	0.17	39.59	14.57	-0.26	CEA	
	3.74	9.42	0.06	9.81	12.53	0.03	22.13	11.53	-0.11	17.59	10.03	-0.08	64.05	12.69	-0.53	DEAA	
	1.20	12.04	0.11	18.04	20.34	0.02	3.27	39.27	0.37	16.68	19.56	0.03	46.76	10.70	-0.37	Am	
	27.92	29.70	0.02	12.92	35.54	0.23	36.80	24.67	-0.12	44.77	18.59	-0.27	68.05	12.67	-0.57	HPOAA	
NIPAA	7.35	2.38	-0.05	3.74	10.37	0.07	3.74	31.90	0.29	3.74	35.31	0.32	13.74	27.62	0.14	CHMA	
	3.74	78.22	0.76	3.74	48.71	0.46	3.74	163.15	1.63	3.74	121.19	1.20	12.76	36.56	0.24	DMC	
	3.74	29.72	0.27	4.48	45.46	0.42	0.82	37.86	0.38	1.25	19.35	0.19	13.19	34.23	0.22	CEA	
	13.81	2.64	-0.11	13.81	16.21	0.02	18.36	38.67	0.21	26.79	41.98	0.16	19.87	36.75	0.17	DEAA	
	34.47	11.82	-0.23	26.78	19.61	-0.07	3.74	12.61	0.09	3.74	6.86	0.03	13.74	33.68	0.20	Am	
	23.03	27.89	0.05	3.03	35.05	0.33	81.12	25.34	-0.57	6.38	17.57	0.11	16.96	30.11	0.13	HPOAA	
CHMA	275.98	97.03	-1.83	115.41	56.00	-0.61	48.49	69.53	0.22	25.20	99.67	0.76	25.20	15.57	-0.10	DMC	
	25.20	40.68	0.16	25.20	37.30	0.12	25.20	36.63	0.12	25.20	44.32	0.20	25.20	21.17	-0.04	CEA	
	25.20	7.00	-0.19	7.88	8.20	0.00	2.95	13.52	0.11	19.79	57.50	0.39	19.79	19.87	0.00	DEAA	
	0.73	18.35	0.18	0.73	19.02	0.19	0.73	28.90	0.29	0.73	53.63	0.54	19.95	22.94	0.03	Am	
	23.74	29.06	0.05	134.51	24.68	-1.12	133.00	40.81	-0.94	171.11	18.87	-1.56	25.48	15.20	-0.11	HPOAA	
DMC	3.74	31.25	0.28	177.7	33.58	-1.48	139.81	69.14	-0.72	1.48	50.62	0.50	44.69	96.93	0.53	CEA	
	156.97	4.47	-1.56	255.7	40.00	-2.21	61.80	55.03	-0.07	125.47	45.57	-0.82	56.90	90.97	0.35	DEAA	
	133.71	20.21	-1.16	5.59	47.46	0.43	168.31	62.50	-1.08	91.05	96.23	0.05	42.55	98.36	0.57	Am	
	24.21	30.97	0.07	3.36	83.46	0.82	7.40	36.12	0.29	27.55	54.77	0.28	60.32	91.10	0.31	HPOAA	
CEA	4.31	8.26	0.04	16.75	18.32	0.02	9.55	15.73	0.06	37.06	21.91	-0.16	45.56	33.37	-0.12	DEAA	
	45.11	17.67	-0.28	15.99	32.66	0.17	3.62	31.65	0.29	4.21	48.09	0.45	49.71	32.10	-0.18	Am	
	27.94	29.11	0.01	3.91	44.92	0.42	3.76	31.84	0.29	7.60	41.44	0.35	48.57	41.47	-0.07	HPOAA	
DEAA	42.72	24.68	-0.18	3.17	35.92	0.34	3.52	28.71	0.26	100.86	29.15	-0.73	89.27	17.62	-0.73	Am	
	66.12	53.25	-0.13	25.29	28.85	0.04	84.81	17.39	-0.69	54.75	14.80	-0.41	91.66	11.69	-0.82	HPOAA	
Am	25.22	23.27	-0.02	163.3	38.71	-1.28	220.24	32.08	-1.93	163.41	44.16	-1.22	116.05	36.17	-0.82	HPOAA	

	RFI<0
	0<RFI<0.1
	0.1<RFI<1
	1<RFI

Table S1 The average relative fluorescent intensities (RFI) of the spots of the polymer microarrays with an immobilized fluorescent polymer dye. M1 and M2 are printed monomers that formed polymer spots on each line with varied combinations of the printed monomer solution drops (M1/M2 = 0/20, 5/15, 10/10, 15/5, 20/0). Polymer columns are average fluorescent intensities of 4 repeating spots on a polymer microarray without immobilized fluorescent polymer dye. Polymer-A columns are average fluorescent intensities of 4 repeating spots on a polymer microarray with immobilized fluorescent polymer dye. RFI columns are relative fluorescent intensities of each combination by using following equation:  $RFI = (FI_{\text{Polymer-A}} - FI_{\text{Polymer}}) / FI_{\text{dye}}$ . The fluorescent intensity of the fluorescent polymer dye  $FI_{\text{dye}} = 97.71 \times 10^6$  a.u./mm<sup>2</sup>.

### Preparation of Fluorescent Polymer (dye):

The fluorescent dye is a PF6-TBT-FCOOH copolymer, which was synthesized through Suzuki coupling procedure. In details, to a dry reactor equipped with a mechanical stirrer and rubber septa, was added with 9,9-dihexylfluorene -2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolane) (246 mg, 0.5mmol), 2,5-dibromothiophene (48.4 mg, 0.2 mmol), 2,7-dibromo-9,9-bis[3,3'-(carboxylic acid) propyl] fluorene (45.6 mg, 0.1 mmol), 4,7-dibromo- 2,1,3-benzothiadiazole (0.0588 mg, 0.2 mmol), Suzuki coupling catalyst Pd(OAc)<sub>2</sub> (0.05mmol) in toluene 5 mL, and base solution (K<sub>2</sub>CO<sub>3</sub>, 0.2mmol) 8 mL. The reactor with reaction reagents was evacuated and purged with nitrogen 3 times, and then heated to 95 °C to start the reaction under N<sub>2</sub> atmosphere. Three hours later, phenylboronic acid (61 mg) was added into the reaction mixture. After 2h reaction time, bromobenzene (0.12 mL) was added into the reaction. After another 2 hours, the reaction mixture was poured into 300 ml methanol to precipitate the polymer. The collected polymer was washed with methanol for 3 times. After dried under vacuum at 40 °C, the polymer was dissolved in chloroform (20 mL) and purified by using a column filled with silica gel to remove

the catalyst residue. The polymer solution was precipitated again in methanol (300 mL), and the precipitate was filtered and washed with methanol three times. After dried under vacuum, a yellowish fiber-like product was obtained (Yield 73.5%, Mn: 20900, PDI: 4.08).

Fig.S3a shows the  $^1\text{H}$ NMR spectrum of the dye in  $\text{CDCl}_3$ . The signals at 0.8 ppm are assigned to the methyl protons, signals corresponding to methylene protons connected with methyl are observed at 1.25 ppm, and the signals at 2.1 ppm are associated with methylene protons alpha to the carboxylic group. The peaks between 7.3 to 8.1 ppm are attributed to the benzenering and thiophene.

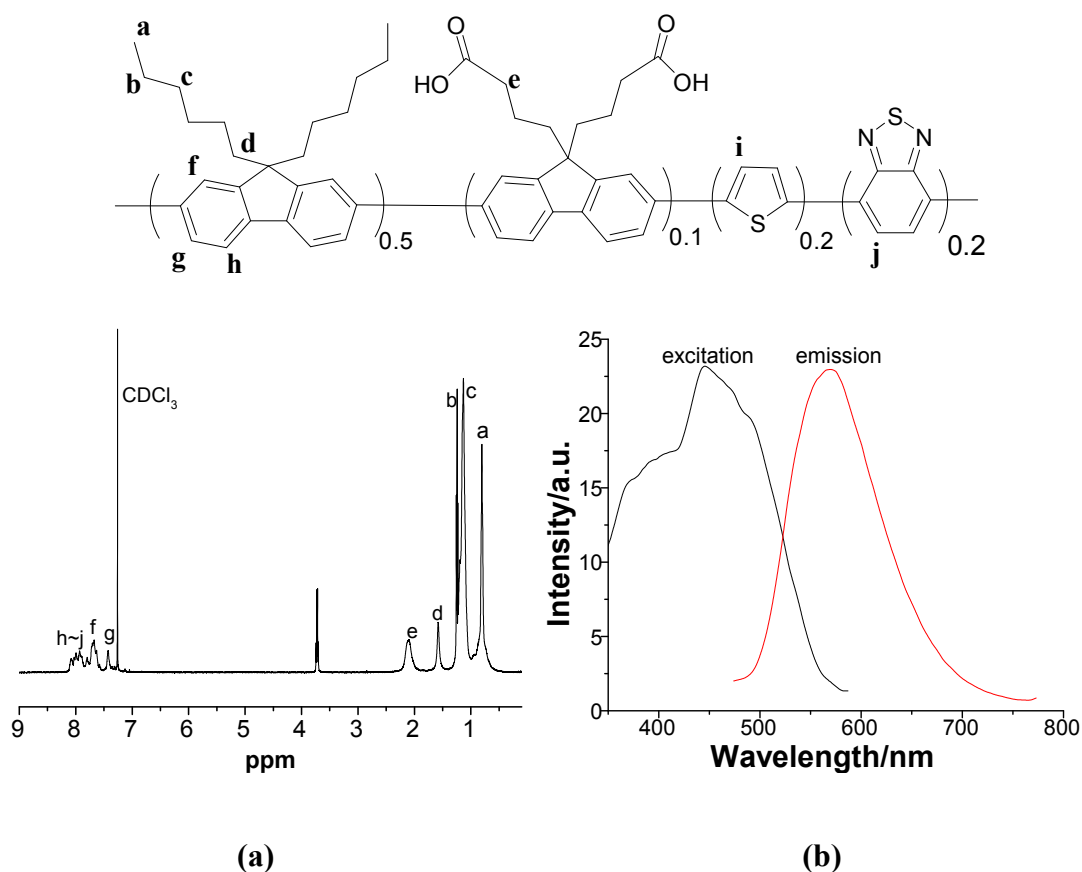
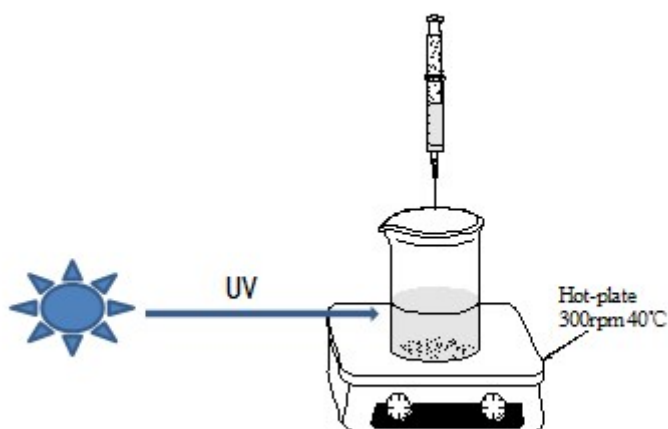


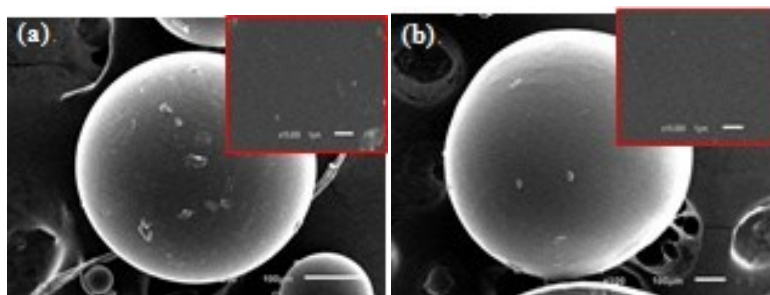
Figure S3 Characterization of the synthesized fluorescent polymer dye. (a)  $^1\text{H}$ NMR spectrum of dye A in  $\text{CDCl}_3$ . (b) Normalized absorption and emission spectra of dye A in PEG/ $\text{H}_2\text{O}$  (2:1) solution.

### Preparation and Characterization of the Polymer Beads with Entrapped Dye

A mixture of the monomers 2 g, the conjugated fluorescent polymer (0.006 g), crosslinker MBA (0.2 g) and the photoinitiator (0.12g) in NMP (2 mL) was prepared and dripped into polydimethylsiloxane (50 mL) ( $\eta=500\text{cs}$ ) with Span-80 (5 wt% with respect to the monomer) in a 100 mL beaker which was side-illuminated by UV light in 30 minutes (Scheme S1). The silicon oil was kept at 40 °C and stirred at 300 rpm to prevent the polymer beads from aggregating before hardening and the collected polymer beads were washed with toluene (20 mL) and ethanol (20 mL $\times$ 3) and dried overnight in a vacuum oven at 40 °C.



Scheme S1 Preparation of fluorescent polymer beads by dripping monomers and dye solution with a 10 mL syringe into a beaker containing silicon oil (40 °C) on a hot plate stirrer under UV light ( $\lambda=365\text{ nm}$ ).



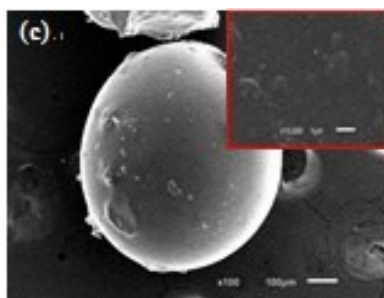


Figure S4 SEM images of the polymer beads.(a), (b) and (c) are SEM images of polymers A15K5-A (it means the polymer A15K5 with the immobilized dye), A5B15-A and E15G5-A respectively. The inserts are enlarged images of the beads. All the scale bars are 100 µm and the scale bars of inserts are 1 µm.

The beads (0.05 g) were suspended in PEG water mixture (3 mL) in a cuvette on the sample holder with a temperature control sleeve and measured under 22°C. Then increased the temperature of the cuvette and measured the fluorescent intensity again as the temperature was stable at 63 °C. The cuvette with sample was cooled down to room temperature for another measurement cycle and so on for many cycles of measurement. For a comparison, a solution of the conjugated fluorescent polymer in NMP was used as a control. The conjugated fluorescent polymer (0.3 wt%) was used for the beads preparation, so the control solution was 0.005 wt%, to be comparable with the dye in the beads.

### **Rheological Analysis of Polymers**

Polymers without the conjugated fluorescent polymer were prepared for rheological analysis. Cylindrical samples of the polymers were prepared in 20 mL plastic syringes (with sealed ends).The mixtures of monomers, cross-linker (MBA) and ammonium persulphate (APS) as the initiator in water (Table S2) were placed into the syringes and purged with nitrogen gas followed by the addition of N,N,N',N'-tetramethylethylenediamine (TEMED) solution to initiate the polymerization. The syringes were then left in a 40 °C oven for 12h. The polymer cylinders collected from the syringes were immersed in water and refreshed every 12 h for one week before use. For rheological analysis the cylinder hydrogels were cut into 3 mm thick discs with a diameter of 2 cm.



Table S2 Reagents used for the preparation of hydrogels for rheological analysis

Monomer1/Monomer2	NIPAA/DMC (15/5)	NIPAA/DMC (10/10)	NIPAA/DMC (5/15)	HEMA/EGDMA (5/15)
M1(g)	0.6	0.4	0.2	0.2
M2(g)	0.2	0.4	0.6	0.6
MBA(g)	0.12	0.12	0.12	0.12
APS(g)	0.008	0.008	0.008	0.008
TEMED( $\mu$ L)	10	10	10	10

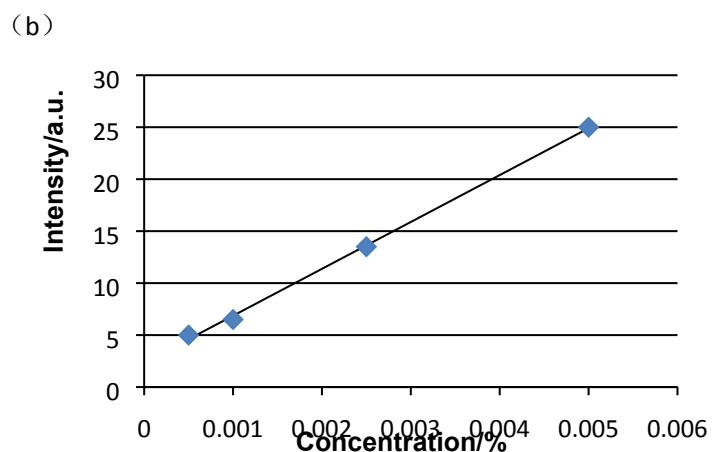
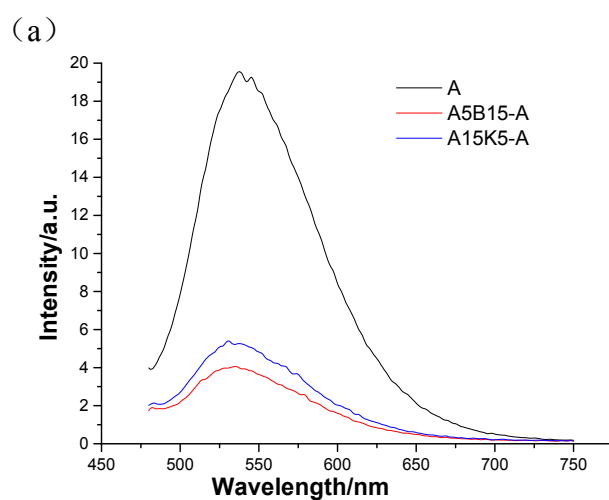


Figure S5 (a) Fluorescent intensity of the toluene solutions collected from the wash of the polymer beads immobilized with fluorescent polymer dye during preparation. The curve A is the fluorescent intensity of the dye solution in toluene with the same amount as used in the beads preparation. (b) The calibration line of the fluorescent intensity of the dye solution versus dye concentration in NMP (it was found that the intensity of the dye solution in NMP and toluene was very similar).

According to the fluorescent intensity of the washing solutions of A5B15-A and A15K5-A beads during fabrication, it was found that the immobilized dye was 90 wt% and 94 wt% of the original mass of the added dye. Therefore, the fluorescent intensity of beads and 0.005 wt% dye solution are comparable.

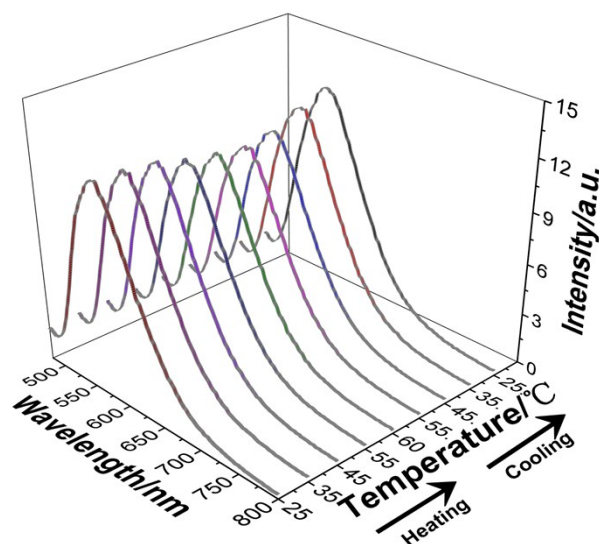


Figure S6 The influence of temperature on the fluorescence intensity of the conjugated fluorescent polymer (0.005 wt%) in a mixture of PEG400 and water with PEG:H<sub>2</sub>O =2:1. ( $\lambda_{ex}$ =450 nm) showing an emission peak at peak 575 nm when the excitation wavelength at 25 °C. Increasing the temperature had negligible effect on emission intensity.

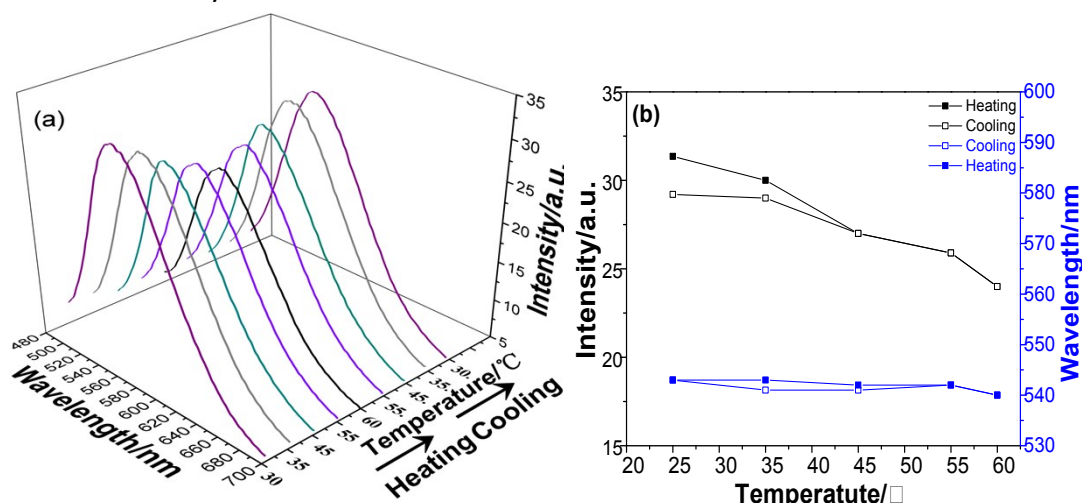


Figure S7 Fluorescence intensity change of polymer beads made from A5B15-A following the temperature rising from 25 to 60 °C and then cooled to 25 °C measured when (a)  $\lambda_{EX}$ =450 nm, (b) corresponding fluorescence intensities and emission wavelengths at various temperatures.

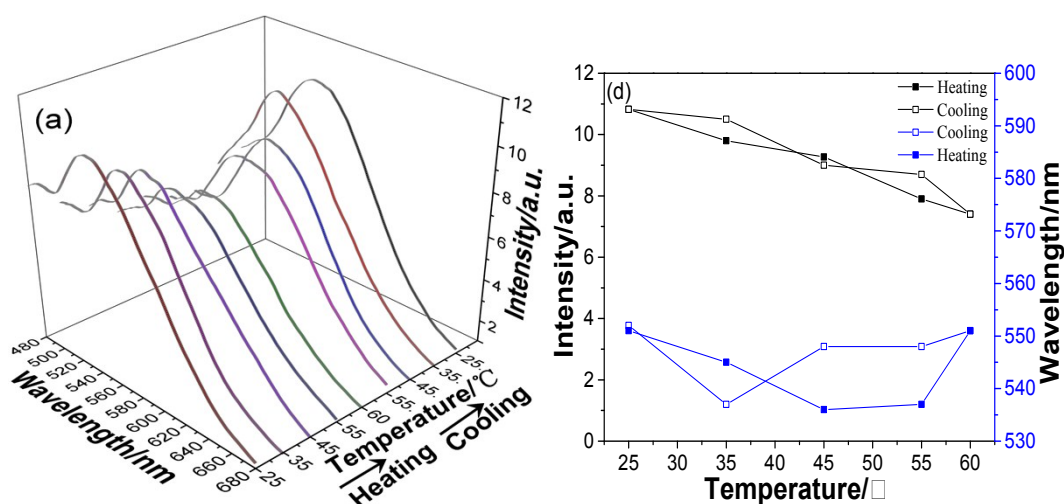


Figure S8 Fluorescent intensity change of polymer beads of E15G5-A following the temperature rising from 25 to 60 °C and then cooling to 25 °C measured when (a)  $\lambda_A=450$  nm, (b) corresponding fluorescence intensities and emission wavelengths at various temperatures.

Video S1 and S2: The videos of manipulating the fluorescence of polymer beads and membrane (E5G15-A) immobilized with dye (3‰ in weight) in PEO400/H<sub>2</sub>O (2/1) solution by change the temperature between 20 and 65 °C. Video S1 recorded the heating and cooling cycle of a bead, and S2 recorded the heating of the membrane.