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Supporting Information

Heavy-Atom Effect on Xanthene Dyes for Photopolymerization by Visible Light

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1. Materials. Fluorescein sodium salt (**1**, > 97.5%, Sigma-Aldrich), 4',5'-dibromofluorescein (**2**, >95%, Alfa Aesar), 2',4',5',7'-tetrabromo fluorescein (**3**, eosin Y, Alfa Aesar), 2',4',5',7'-tetraiodofluorescein (**4**, erythrosine B, > 95% TCI), rhodamine 6G (**5**, 99%, Sigma-Aldrich), triethanolamine (TEOA, Sigma-Aldrich), ethanol (extrapure, Daejung), rhodamine 6G (99%, Sigma-Aldrich), *N*-vinylpyrrolidone (VP, Sigma-Aldrich), poly(ethylene glycol) diacrylate (PEGDA, Sigma-Aldrich), bromine (Junsei Chemical), methanol (Alfa Aesar), diethyl ether (Alfa Aesar), agarose (low EEO, Sigma-Aldrich), slide glass (Marienfel-superior), 2-hydroxyethyl methacrylate (HEMA, Sigma-Aldrich), and deionized water (18.2 MΩ·cm, Milli-Q® Direct Water Purification System, Merck Millipore) were purchased and used without further purification. The solubility in water of (**3**) and (**4**) was 100 mg/mL at 70 °F and 70 mg/mL at 20 °C, respectively. In addition, the biological toxicity of the corresponding dyes is as follows. The data were obtained from PubChem, an open chemistry databased at the National Institutes of Health (NIH).

The biological toxicity of (**3**)

Organism	Test type	Route	Dose
Mouse	LD50	Intravenous	550 mg/kg
Rat	LDLo	Intraperitoneal	500 mg/kg
Mouse	LD50	Oral	2344mg/kg
rabbit	LDLo	Intravenous	300 mg/kg

The biological toxicity of (**4**)

Organism	Test type	Route	Dose
Rat	LD50	Oral	1840 mg/kg
Rat	LD50	Intraperitoneal	300 mg/kg
Rat	LD50	Intravenous	200 mg/kg
Rat	LD50	Unreported	1895 mg/kg
Mouse	LD50	oral	1264 mg/kg

2. Experimental details

2.1 Experimental procedures

Synthesis of 4',5'-dibromorhodamine 6G (6). Brominated rhodamine 6G was synthesized following a procedure described in the literature.[1] Briefly, bromine (11 μL, 0.213 mmol) in methanol (1 mL) was slowly added into the mixture of rhodamine 6 G (50 mg, 0.106 mmol) dissolved in methanol (3.5 mL) at room temperature. After stirring for 3 h, the solvent was removed under a reduced pressure to give a red solid. The crude product was purified by recrystallization with the mixture of methanol (7 mL) and diethyl ether (35 mL) to afford (**6**) as a green red solid (57 mg, 80% yield).

^{1}H NMR (400 MHz, CD_3OD): δ 8.34 (1H, d, J = 7.6 Hz), 7.90–7.82 (2H, m), 7.45 (1H, d, J = 7.2 Hz), 6.93 (2H, s), 4.06–3.96 (6H, m), 2.36 (6H, s), 1.36 (6H, t, J = 7.2 Hz), 0.99 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 165.1, 159.1, 155.0, 153.3, 133.0, 132.8, 131.0, 130.6, 130.4, 130.3, 129.8, 127.3, 115.0, 93.4, 61.3, 41.3, 19.4, 15.6, 12.7.

Preparation of a chip. As a chip surface, agarose-coated glass surfaces was prepared by the drop-casting technique.[2] Briefly, the slide glass (76 × 26 × 1 mm microscope slide) was cleaned by an oxygen-plasma cleaner (Harrick, PDC-32G). The agarose solution (0.2 wt% in water, 2 mL), prepared by dissolving agarose powder in hot water (100 °C), was spread evenly to all edges of the glass surfaced, and then dried it for 24 h. As the water evaporated, transparent agarose film slowly formed on the surface. Each dye of (**1**)–(**4**) was spotted on an agarose film using a SpotBot® 3 personal microarrayer (Arrayit Corporation) with a pin

(Catalog number: 946MP4) under 40% humidity at room temperature. Each chip had nine spots (3 × 3) of a dye with the same concentration, and the distance between the spots was 650 μm. The chip was characterized by a fluorescence scanner. After the characterized chip was kept in a dark room for12 h, we undertook the interfacial photopolymerization at ambient conditions. For cationic xanthene dyes, (**5**) and (**6**) were spotted on an agarose film using a micropipette. Each chip has four spots, consisting of two spots for (**5**) and two spots for (**6**) with the same concentration, on the agarose film

Interfacial photopolymerization. Before the polymerization, the mixture solution was freshly prepared and consisted of PEGDA (600 mM), VP (400 mM), triethanolamine (750 mM), and eosin Y (0.5 μM) as an enhancer in deionized water. Upon the dropping 4.5 μL of the mixture solution onto each chip, the polymerization was carried out using the InDevR ampliPOHX™ Reader (InDevR, Inc.), consisting of a photoactivation bay fitted with an array of light emitting diodes (520 ± 45 nm, approximately 1 W/cm^2) and an imaging bay equipped with white light illumination and a digital camera. After the polymerization, the chip was stained with eosin solution (50 mM in water) for 3 min, and then carefully washed with water (about 20 mL) to remove the excess staining solution. The stained chip was imaged using the digital camera, built in the equipment, after a drying process under a stream of air. For cationic xanthene dyes (**5** and **6**), we employed a green LED (power: 0.24 W, wavelength: ~520 nm, model number: KG1, SS Light Co., Ltd , Suwon, South Korea) to irradiate over a large-area.

Photopolymerization in a single-phase solution. Owing to the difficult characterization of the cross-linked hydrogel using gel permeation chromatography, two different homopolymers were synthesized by the photopolymerization. The mixture solution was freshly prepared in a

vial, containing 3 M monomer (HEMA or VP), 750 mM triethanolamine, and 50 μM each dye in deionized water. The polymerization was carried out using the photoactivation bay of InDevR ampliPOHX™ Reader.[3] Upon the irradiation over a period of time, the reaction mixture was purified by dialysis with a membrane (MWCO: 1 kDa) against 100 mL of the solvent mixtures (water: methanol = 50: 50) for 12 h. The mixture of dialysis solvent was changed two times. After dialysis, the resulting solution was concentrated with a freeze dryer (Labconco Co.).

Photobleaching. The 100 μL of mixture, containing a dye (10 μM) and triethanolamine (0.4 M) in EtOH, was put into a quartz micro-cuvette (Hellma® fluorescence cuvette). The cell was put on the photoactivation bay, and then irradiated by varying times such as 50, 100, 200, 300, 400 and 500 s. Upon the irradiation over a period of time, the solution was carefully mixed using a micropipette and was then characterized by a fluorescence spectrophotometer (Jasco spectrofluorometer, FP-6500) at 180 V of PMT. For the bleaching in an inert atmosphere, the mixture solution, including a dye, triethanolamine, and ethanol, was added into a screw-capped vial in a glove box after the triethanolamine and ethanol were thoroughly purged with argon gas for 3h. After the sealed reaction vial was taken out of the glove box, the vial put on the photoactivation bay, and then irradiation by varying time.

2.2 Characterizations

Fluorescence scanner. Fluorescence images of the chip were obtained using a SensoSpot® Fluorescence Microarray Analyzer (Sensovation AG, Radolfzell, Germany) at a wavelength of λ=528 nm (exposure time : 150 ms for (**1**)–(**4**) or 50 ms for (**5**) and (**6**)). The obtained fluorescence images were analyzed by ImageJ software (National Institutes of Health,

Bethesda, Maryland, USA).

Color intensity. All colorimetric images were obtained using an imaging bay equipped with white light illumination of the InDevR ampliPHOX™ Reader with a digital camera. The obtained images were quantitatively analyzed by ampliVIEW™ software, which calculated the mean intensity from the spots and the background.[3]

UV/Vis and fluorescence spectrophotometer. The excitation spectrum of xanthene dyes was obtained by using a UV/vis spectrophotometer (Shimadzu, UV-1800). The emission spectrum of xanthene dyes was obtained by fluorescence spectrophotometer (Jasco, FP-6500).

Gel Permeation Chromatography (GPC) Measurement. Two different GPC systems was used to analyze poly(VP) and poly(HEMA) due to the solubility of each polymer. Poly(VP) was characterized by Waters chromatograph equipped with a refractive index detector (Waters 2414) and three 5 μm Waters columns (300 mm × 7.7 mm) connected in series. Deionized water used as an eluent at 35 °C and the flow rate was 1.0 mL/min. Pullulan standards in water were used to obtain the number- and weight-average molar masses of the poly(VP). Poly(HEMA) was characterized by Agilent Infinity 1260 series equipped with an Optilab T-rEX refractive index detector and PLgel 10 uM MIXED-B columns. DMF, including LiBr, was used as an eluent at 35 °C, and the flow rate was 1.0 mL/min. Linear polystyrene standards (EasiCal) was used to measure the number- and weight-average molar masses of the poly(HEMA).

2. Supporting figures

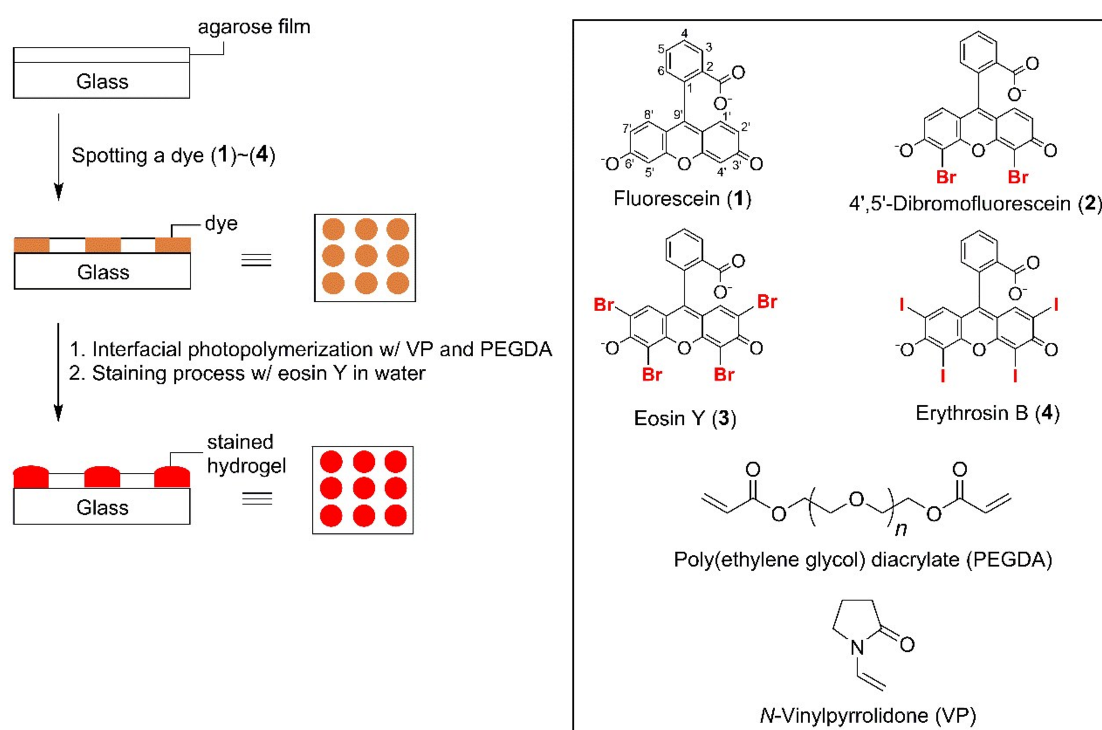


Fig. S1 Schematic depiction of the procedure for the fabrication of a chip and molecular structure of anionic xanthene dyes, employed in this work.

Table S1. The summary of the properties of (**1**)–(**4**).

Dye	**1**	**2**	**3**	**4**
λ_{max} **(nm)**	500 (500[4])	514[a]	524 (525[4])	532 (535[4])
ε (cm^{-1}·M^{-1})·	93660[4]	89600[a]	115660[4]	106460[4]
λ_{em} **(nm)**	521	538[a]	545	551
Φ_f[b]	0.87 (0.93~0.97[5,6])	0.53	0.68 (0.2~0.69[5,7])	0.16 (0.02~0.11[5,8])
Φ_{isc}	0.03[8]	N.D.	0.33[9]	0.7[9]
***p*Ka of Dye$^{\bullet}$–H**	6.1[10]	N.D.	3.8[10]	3.8[10]
$E^{T1}_{0,0}$ **(eV)**	1.94[8]	N.D.	1.84~1.91[8,9]	1.9[9]
$E^{red}_{1/2}$ **(V/SCE)**	-1.17[8]	N.D.	-1.05[9]	-1.05[9]
E^{T1}_{red} **(eV)**	0.77[8]	N.D.	0.83~0.87[8,9]	0.85[9]
ΔG_{PET} **(eV)**[c]	- 0.1[8]	N.D.	- 0.1[9]	- 0.1[9]

[a]10 mM NaOH in ethanol. [b] Rhodamine 6G was used as a standard, and data were determined from IUPAC technical report.[11] [c]ΔG_{PET} = $-[E^*_{red}(cat^*/cat^{\cdot-}) - E_{ox}(TEOA^{\cdot+}/TEOA)]$, $E^*_{red}(cat^*/cat^{\cdot-}) = E_{red}(cat^*/cat^{\cdot-}) + E^{T1}_{0,0}$, $E_{ox}(TEOA^{\cdot+}/TEOA)$, and

the data were acquired from E_{ox} of TEOA (around 0.72 eV).[12]

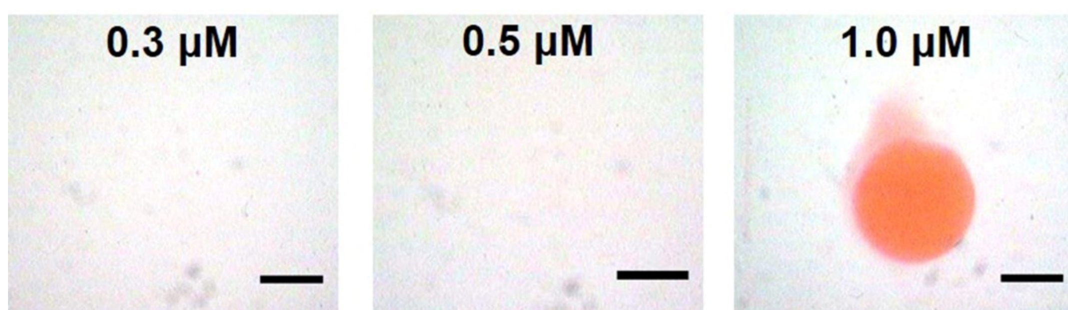
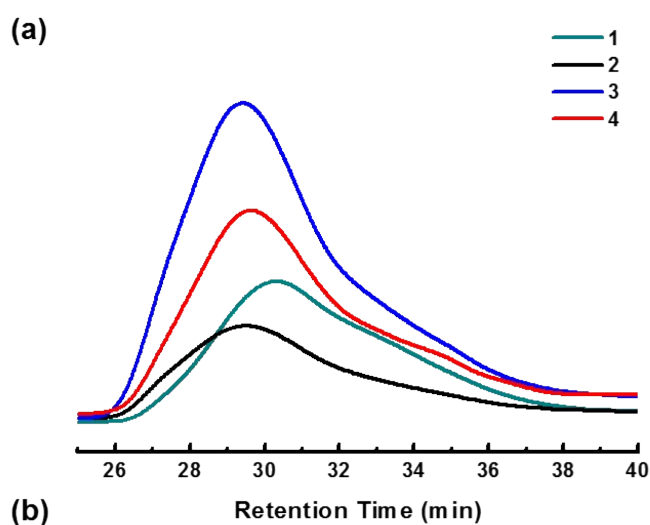


Fig. S2 The investigation of the effect for esoin Y as an enhancer. Scale bars represent 0.2 cm.



(b)

Dye	Isolated yield (%)	M_n (kDa)	PDI
1	3.98 (±0.50)	66.7	2.45
2	9.34 (±1.42)	78.7	2.57
3	10.54 (±0.55)	88.4	2.44
4	11.24 (±1.20)	108.3	1.97

Fig. S3 (a) GPC profiles of the photopolymerization in single-phase solution using (**1**)–(**4**). (b) The analysis of the poly(VP) obtained after the photopolymerization. The number-average

molar mass (M_n) and the weight-average molar mass (M_w) were calculated against pullulan standards.

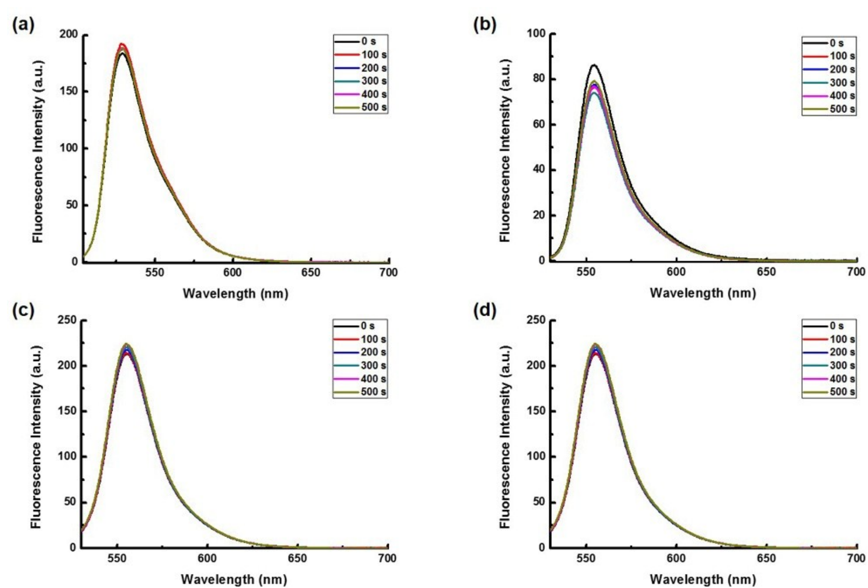


Fig. S4 The emission spectra of each for the photobleaching without employing TEOA for different irradiation time.

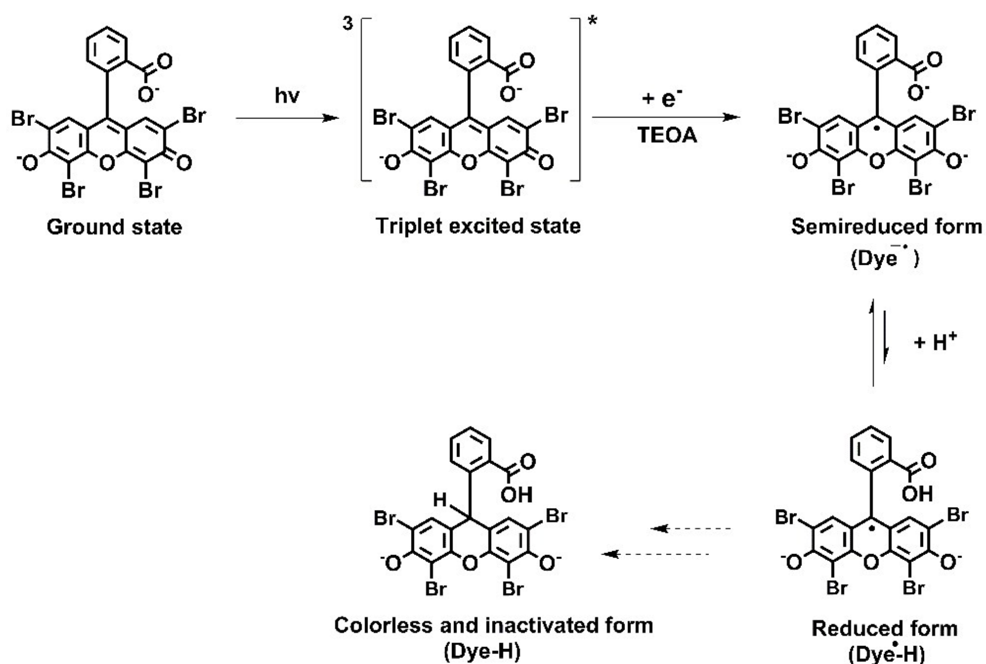
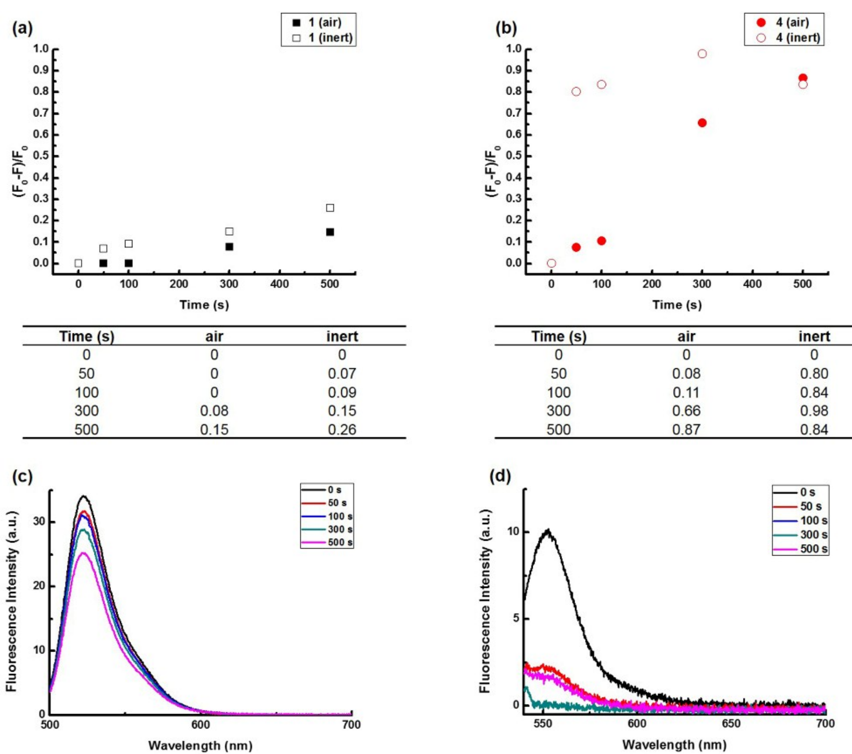


Fig. S5 Plausible mechanism of photobleaching for eosin Y as a representative example in the presence of TEOA under the visible-light-irradiation.



Time (s)	air	inert
0	0	0
50	0	0.07
100	0	0.09
300	0.08	0.15
500	0.15	0.26

Time (s)	air	inert
0	0	0
50	0.08	0.80
100	0.11	0.84
300	0.66	0.98
500	0.87	0.84

Fig. S6 The photobleaching experiments of (a) dye (**1**) and (b) dye (**4**) in the inert and air atmosphere and the corresponding emission spectra of (c) dye (**1**) and (d) dye (**4**).

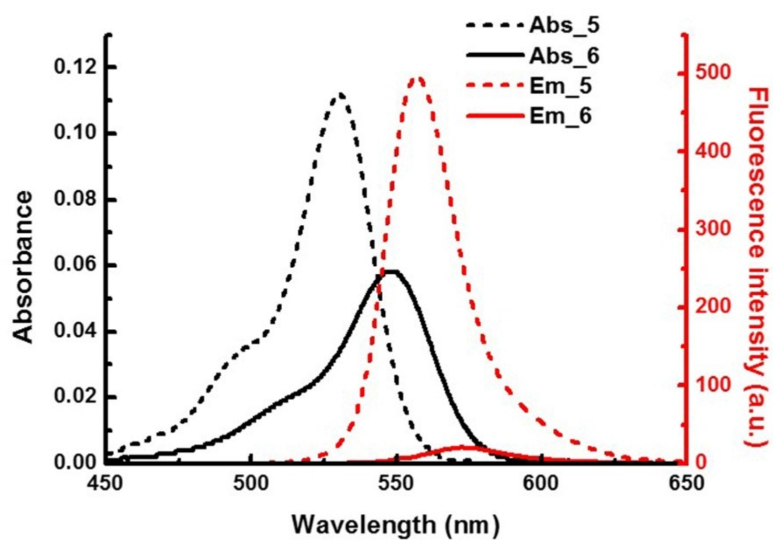
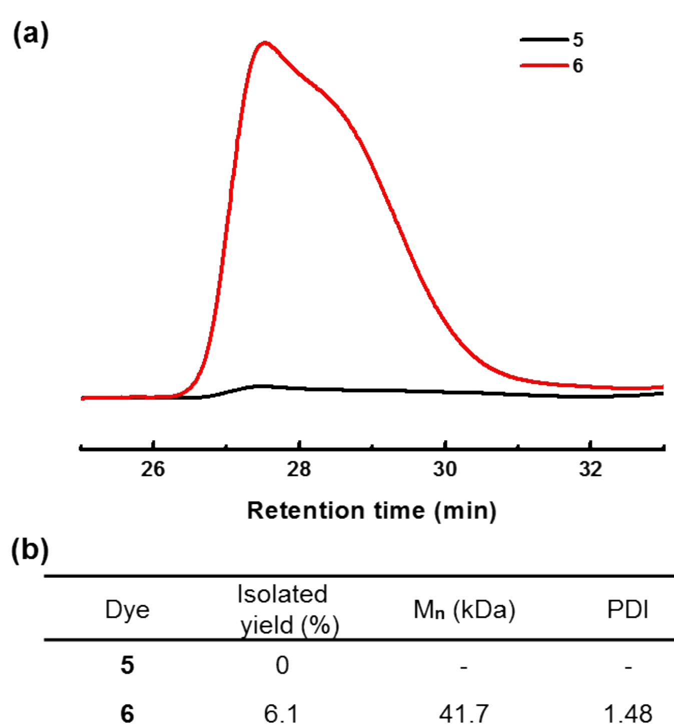


Fig. S7 Excitation and emission spectra of rhodamine 6G (**5**) and 4',5'-dibrominerhodamine 6G (**6**) in ethanol. The concentration of (**5**) and (**6**) was 1 μM.



Dye	Isolated yield (%)	M_n (kDa)	PDI
5	0	-	-
6	6.1	41.7	1.48

Fig. S8 (a) GPC profiles of the photopolymerization in single-phase solution using **(5**) and (**6**). (b) The analysis of the poly(HEMA) obtained after the photopolymerization The number-average molar mass (M_n) and the weight-average molar mass (M_w) were calculated against polystyrene standards.

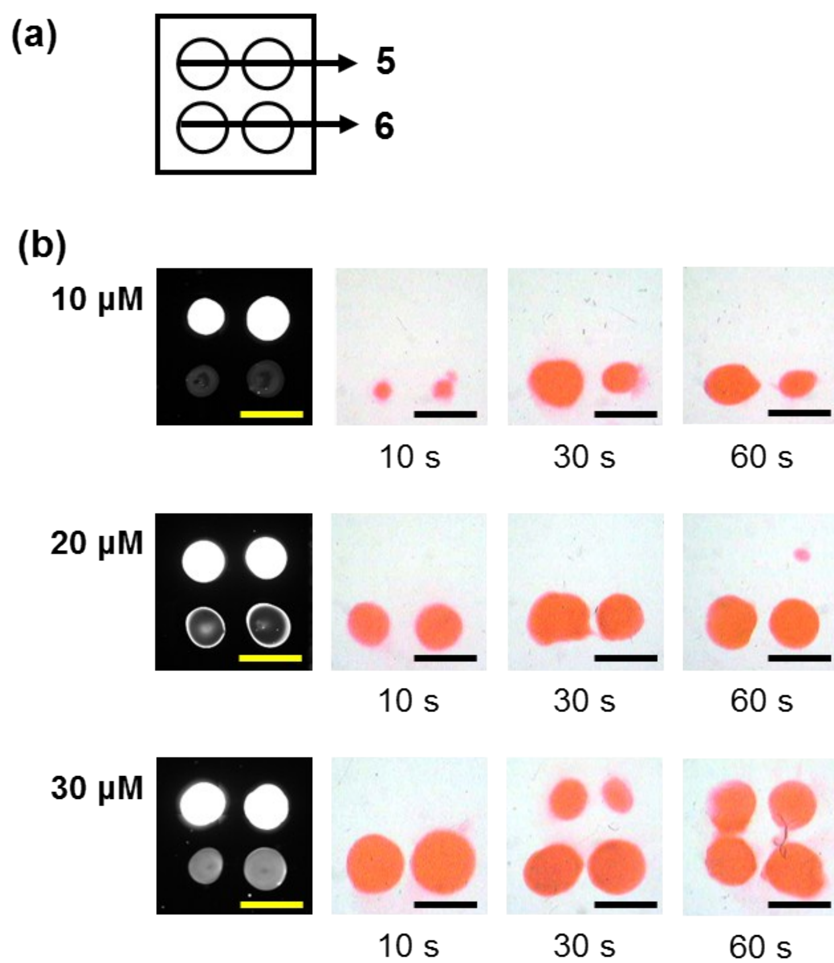


Fig. S9 (a) Schematic diagram of a chip for (**5**) and (**6**). (b) Fluorescence image (left) and colorimetric readout (2nd ~4th columns) of (**5**) and (**6**) under varied concentrations and irradiation times. Scale bars represent 0.2 cm.

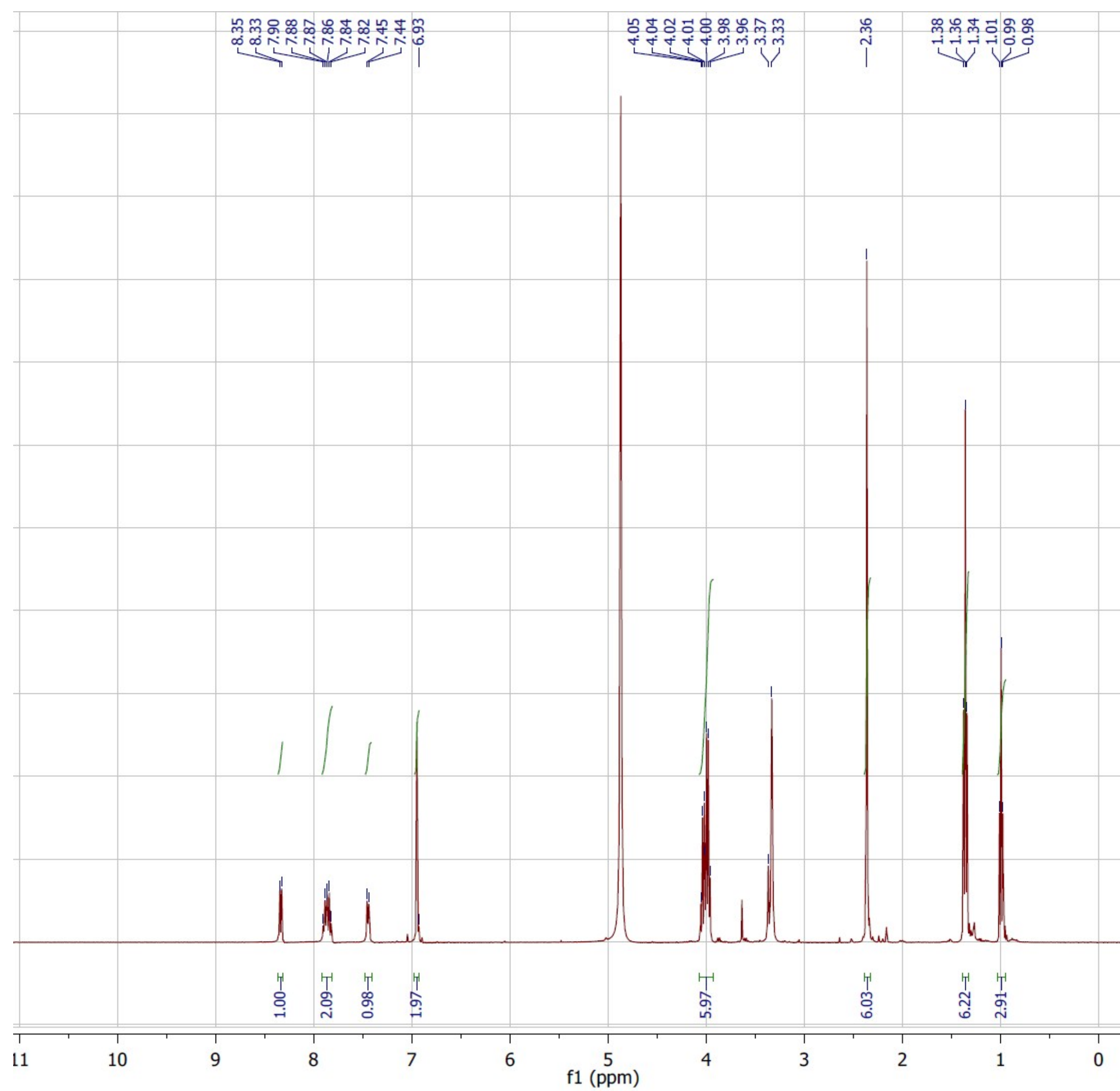
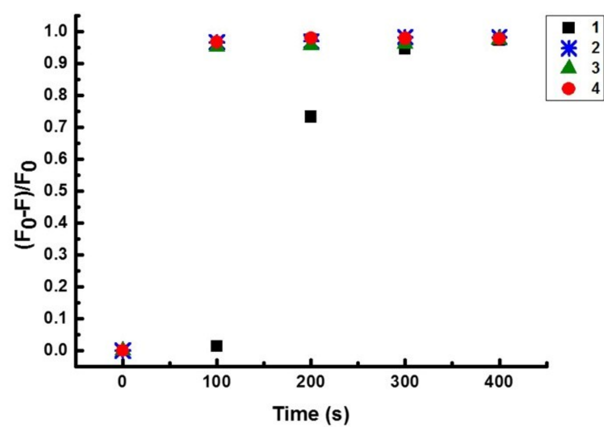


Fig. S10 ^{1}H-NMR spectrum of 4’,5’-dibromorhodamine 6G (**6**).



Time (s)	1	2	3	4
0	0	0	0	0
100	0.01	0.97	0.97	0.95
200	0.73	0.97	0.98	0.96
300	0.95	0.98	0.98	0.96
400	0.97	0.98	0.98	0.98

Fig. S11 The photobleaching of each dye under the mixture of water, VP, and TEOA for the different irradiation time.

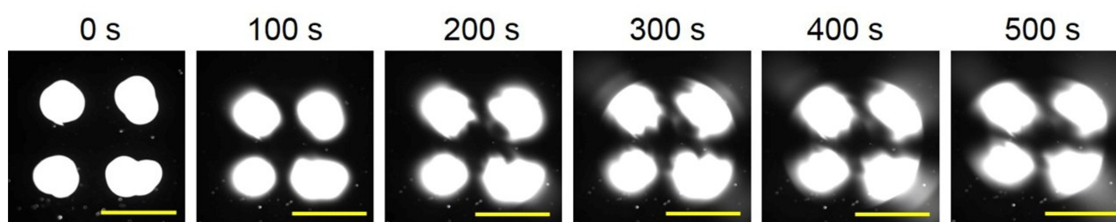


Fig. S12 Fluorescence image of fluorescein (100 uM), manually spotted on the agarose film, as a function of incubation time in the mixture solution at 100 ms of exposure. Scale bars represent 0.2 cm.

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