Supporting Information

Fluorescent Polymers via Post-Polymerization Modification of Biginelli-type Polymers for Cellular Protection Against UV Damage

Tengfei Mao[a,b,c], Xianzhe He[c], Guoqiang Liu[c], Yen Wei[c], Yanzi Gou*[b], Xingui Zhou*[b], Lei Tao*[c]

[a] State Key Laboratory of NBC Protection for Civilian, Beijing, 102205, P. R. China

[b] Science and Technology on Advanced Ceramic Fibers and Composites Laboratory, National University of Defense Technology, Changsha, 410073, P. R. China.

[c] The Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China.

EXPERIMENTAL SECTION

1. Materials

All chemicals, reagents, and solvents were purchased from commercial sources and used without further purification. 2-(acetoacetoxy) ethyl methacrylate (AEMA, Aladdin, 95%), benzaldehyde (Aladdin, > 99.5%), $MgCl_2$ (Sinopharm Chemical Reagent Co., Ltd, AR, 99%), poly(ethylene glycol) methyl ether methacrylate (PEGMA-950, average M_n~950 g·mol^{-1}, Sigma-Aldrich), 2,2'-azobisisoheptonitrile (ABVN, Energy Chemical Reagent Co., Ltd, AR), 3-phenylpropargyl chloride (Aladdin, 98%), 1-chloro-2-pentyne (J&K, 99%), 1-bromopropane (J&K, 98%), potassium persulfate (J&K, 99%), RMPI 1640 culture medium (Corning-Cellgro), phosphate buffered saline (PBS, pH~7.2-7.4, 0.01 M, Solarbio), fetal bovine serum (FBS, Gibco), penicillin-streptomycin solution (Gibco), trypsin-EDTA (Gibco, 0.25%),

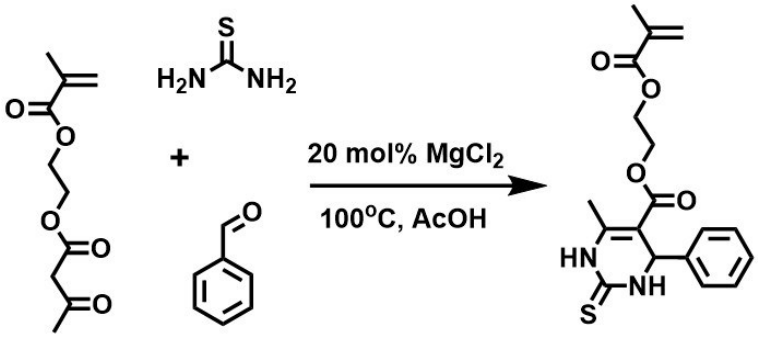
4-(3-(2-methoxy'-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetrazol-3-ium-5-yl) benzene-1,3-disulfonate (CCK-8, Solarbio), fluorescein diacetate (FDA, Sigma), propidium iodide (PI, 95%, Solarbio) were used as purchased.

2. Instruments

Gel permeation chromatography (GPC) analyses of polymers were performed using N, N-dimethyl formamide (DMF) containing 50 mM LiBr as the eluent. The GPC system was a Shimadzu LC-20AD pump system consisting of an auto injector, a MZ-Gel SDplus 10.0 μm guard column (50 × 8.0 mm, 10^2 Å) followed by a MZ-Gel SDplus 5.0 μm bead-size column (50 – 10^6 Å, linear), a Shimadzu RID-10A refractive index detector and a Shimadzu SPD-10A UV detector. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10^6 g mol^{-1}. ^{1}H NMR and ^{13}C NMR spectra were obtained using a JEOL JNM-ECA400 (400 MHz) spectrometer for all samples. The ESI-MS data were collected using a Micro TOF-QII Bruker. The FT-IR spectra were recorded in a transmission mode on a Perkin-Elmer Spectrum 100 spectrometer (Waltham, MA, USA). The fluorescence spectra were measured by a fluorescent photometer (SHIMADZU RF2000). The quantum yields were collected using a FLS920 lifetime and steady state spectrometer (Edinburgh Instruments Ltd.). Cell viability was measured via the CCK-8 assay by a VICTOR™ X3 PerkinElmer 2030 Multilabel Plate Reader. Confocal images were collected by a Zeiss LSM-780 confocal microscopic system. UV lamps (Tanon) with wavelengths of ~365 nm (6W, UV-A), ~302 nm (6W, UV-B), and ~254 nm (6W, UV-C) were used respectively to test the UV absorption ability of polymers.

3. Methods

3.1 2-(Methacryloyloxy) ethyl 6-methyl-4-phenyl-dihydropyrimidin-2(1H)-thione-5-carboxylate (M1).



The DHPM monomer was prepared via the Biginelli reaction according to the reference.[1, 2] Benzaldehyde (0.53 g, 5.0 mmol), acetoacetoxy ethyl methacrylate (AEMA) (1.07 g, 5.0 mmol), and thiourea (0.57 g, 7.5 mmol) were put in a 15 mL centrifuge tube. Then, acetic acid (5.0 mL) and magnesium chloride (190 mg, 1.0 mmol) were added. The tube was sealed and put in a shaker (100 ^{o}C) for 4 h. The mixture was simply purified by precipitation into cold water, then washed for three times by water and then diethyl ether to get the monomer as a yellowish powder (1.64 g, ~91% yield).

^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): 10.41 (s, 1H, CSNHC), 9.70 (s, 1H, CSN<u>H</u>CH), 7.30-7.10 (m, 5H, Ph), 5.95 (s, 1H, CH_2=C), 5.66 (s, 1H, CH_2=C), 5.16 (d, *J* = 3.7 Hz, 1H, CSNHC<u>H</u>), 4.30-4.10 (m, 4H, $COOCH_2CH_2$), 2.28 (s, 3H, CONHCC<u>H</u>$_3$), 1.83 (s, 3H, C<u>H</u>$_3$C=CH_2).

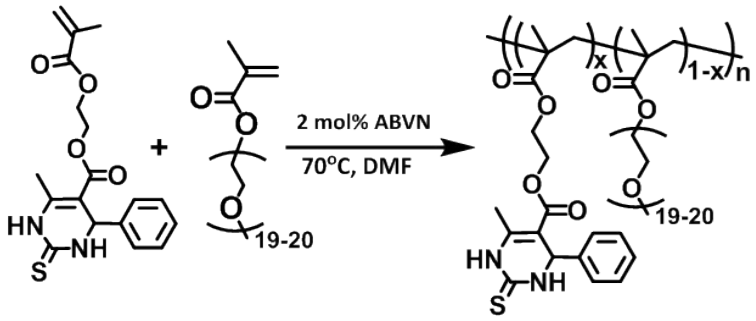
^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): 183.94, 174.36, 172.05, 166.36, 164.97, 145.78, 143.42, 135.59, 128.58, 126.20, 100.35, 62.50, 61.60, 53.96, 17.95, 17.27.

IR (ν/cm^{-1}): 3305, 3175, 2971, 1701, 1633, 1558, 1449, 1374, 1312, 1169, 1094, 943,

875, 821, 766, 698.l

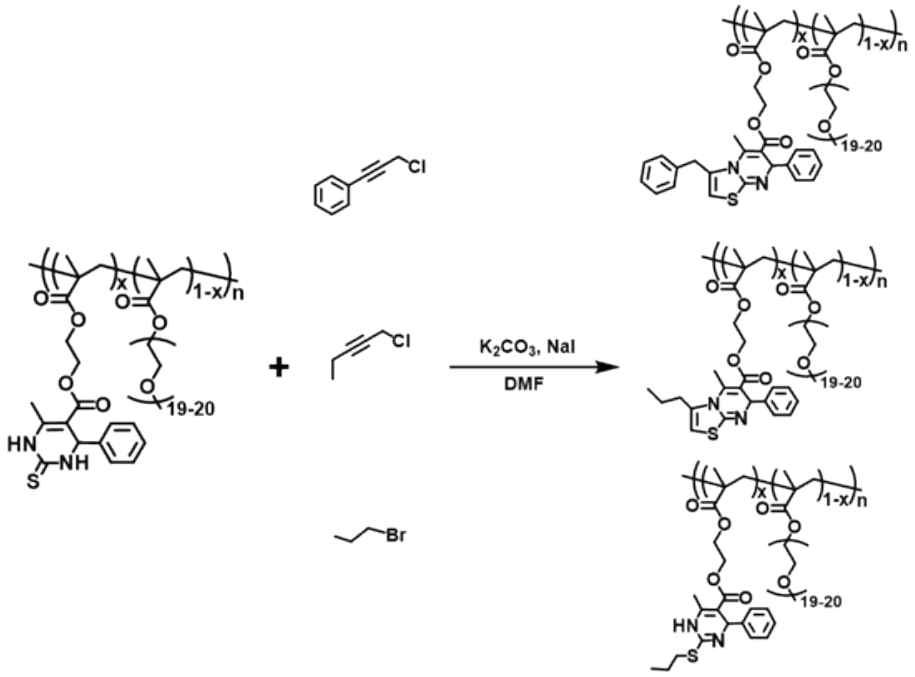
ESI-MS: observed (expected): 361.1216 (361.1217) [M + H^+].

3.2 Copolymerization of 2-(Methacryloyloxy) ethyl 6-methyl-4-phenyl-dihydropyrimidin-2(1H)-thione-5-carboxylate and PEGMA (P0).



M1 (0.72 g, 2.0 mmol), PEGMA-950 (1.90 g, 2.0 mmol), ABVN (0.02 g, 0.08 mmol) were charged into a dry Schlenk tube along with DMF (5.0 mL). The Schlenk tube was sealed with a rubber septum and purged by nitrogen flow for 15 min, then put into a 70 ^oC oil bath for 14 hrs. At the end of the polymerization, the mixture was precipitated in diethyl ether 3 times, and then dried under vacuum to obtain a viscous yellowish copolymer (P0) (2.25 g, ~ 86%).

3.3 Post-polymerization modification (PPM) of P0.



PPM of P0 using three alkyl halides was conducted according to the reference.[3] Typically, P0 (1.31 g, 1 mmol) and 3-phenylpropargyl chloride (180 mg, 1.2 mmol) were dissolved in 10 mL of dry DMF followed by adding potassium carbonate (166 mg, 1.2 mmol) and sodium iodide (223 mg, 1.2 mmol). The mixture was stirred at 80 °C for 12 h. The target polymer (P1) was simply purified by precipitation into diethyl ether, and then ultrasonic washing with diethyl ether for three times followed by filtration as a viscous brown copolymer (1.22 g, 85.8 %).

P2 (a viscous brown copolymer, 1.27 g, 93.4 %) and P3 (a viscous yellow copolymer, 1.18 g, 87.4 %) were similarly prepared using 1-chloro-2-pentyne and 1-bromopropane instead of 3-phenylpropargyl chloride for PPM, respectively.

3.4 Analysis of UV-induced CT-DNA damage.

Typically, an aqueous solutions of calf thymus DNA (CT-DNA) (50 μg/mL) and P0 (0.1 - 1.0 mg/mL) was prepared. This solution (100 μL) was added in a 96-well plates and put under a UV sterilamp (~ 254 nm, 40 W) for 15 min (300 ± 20 μw/cm^2).

Then a sample (2 μL) was taken and mixed with a loading buffer (18 μL) then dropped into a well of E-Gel® EX Agarose Gel. This gel was inserted into the power system and run for 10 minutes. The gels were recorded using gel imager under blue light (490 nm). The gray value and area of patterns were measured by Image J. Other polymers (P1, P2, P3) were parallelly analyzed, pure CT-DNA solution (50 μg/mL) with and without UV irradiation served as a control and blank, respectively.

To study the relationship between DNA break versus polymer concentrations, polymers with different concentrations were similarly analyzed.

3.5 Cell Culture.

L929 cells (a murine-derived fibroblast cell line) were purchased from ATCC (USA), they were cultured in a RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. Cells were incubated at 37°C, 5% CO_2. Culture medium was changed every two days to maintain the exponential growth of the cells.

3.6 Cytotoxicity and UV-resistant evaluation of copolymers.

The cytotoxicity and UV-resistant capability of copolymers to L929 cells was evaluated using a cell count kit-8 (CCK-8) assay. Typically, cells (~ 5×10^4/mL) were seeded in two 96-well plates (plate A and plate B) in culture medium (100 μL, 10% FBS and 1% penicillin and streptomycin). After attachment, cells were added culture medium containing different concentrated copolymers.

Cells in plate A were cultured for 24 h, washed with PBS three times, then incubated in 100 μL of culture medium containing 10% CCK-8 solution at 37^{o}C for 2

h. The plate was put into a microplate reader (VICTORTM X3 PerkinElmer 2030 Multilabel Plate Reader) to record the absorbance (450 nm). The absorbance values of cells in pure culture medium were defined as 100% viability, and culture medium without cells were defined as 0%. The data were present as mean ± SD (n = 5) to indicate the cytotoxicity of different polymers to L929 cells.

Cells in plate B were put under a UV sterilamp (~ 254 nm, 40 W) for 15 min (300 ± 20 μw/cm^2), then cultured for 24 h. The viability of cells was analyzed through a CCK-8 assay as abovementioned. The data were present as mean ± SD (n = 5) to reflect the UV-resistant capability of different polymers. The viability of cells in pure culture medium without UV irradiation was defined as 100%.

3.7 FDA/PI double staining.

FDA/PI double staining is a rapid and convenient method to simultaneously observe the living cells and dead cells. Typically, L929 cells (~5 × 10^4/mL) were seeded in a 24-well plate. After attachment, cells were added media containing different copolymers (0.5 - 10.0 mg/mL, in culture medium) and put under a UV sterilamp (~ 254 nm, 40 W) for 15 min (300 ± 20 μw/cm^2) prior to a 24-h culture. The cells were washed with PBS thrice, then incubated in a PBS–FDA–PI solution (FDA: 3 μg mL^{-1}; PI: 3 μg mL^{-1}) for 15 min (37^{o}C). A fluorescence microscope (Leica Germany) was used to observe the living and dead cells under 450-490 nm and 515-560 nm band-pass excitation filters (I3 and N2.1), respectively (100 W mercury lamp). Cells in culture medium without copolymer were used as a control, and cells in culture medium without UV irradiation were used as a blank.

3.8 Detection of P1 in cells at different time points.

Typically, L929 cells (~10^5 cells / mL) were incubated in culture media containing P1 (20 mg/mL). At different time intervals, the cells were washed three times with PBS (pH = 7.4) and then kept in PBS for observation. The fluorescent images of L929 cells were recorded by a confocal microscopy (Zeiss LSM-780) under UV (405 nm).

3.9 Colocalization analyses.

The colocalization analysis of P1 or P2 and MitoTracker Red was conducted according to previous literatures[4]. L929 cells (~10^5 cells / mL) were incubated in culture media with P1 (20 mg/mL) or P2 (20 mg/mL) for 24 h, and then incubated with Mito-Tracker Red CMXRos (0.1 μM) for 30 min. A confocal microscopy (Zeiss LSM-780) was used to observe the subcellular distribution of P1 or P2 (λ_{ex} = 405 nm) and MitoTracker Red (λ_{ex} = 543 nm).

3.10 Irradiance detection of passed UV lights.

A polymer solution (20 mg/mL in culture medium) was put in a quartz dish. This solution was exposed to a UV lamp, and a radiometer was put under the dish to record the intensity of passed UV light. An empty dish served as a blank and the culture medium was used as a control, respectively. The irradiance of blank was defined as 100%. UV lamps with different wavelengths were used, respectively. The data were present as mean ± SD (n = 5) to indicate the absorbance ability of different polymers to UV.

3.11 Statistical Analyses.

Statistically significant analyses were performed using the SPSS 24.0 statistical

software package (SPSS, Inc., Chicago, IL) by one-way analysis of variance (ANOVA) and Student's t-test (two-tailed), where differences were regarded as statistically significant with probability $p < 0.05$. Results are presented as mean ± SD.

Supporting Data

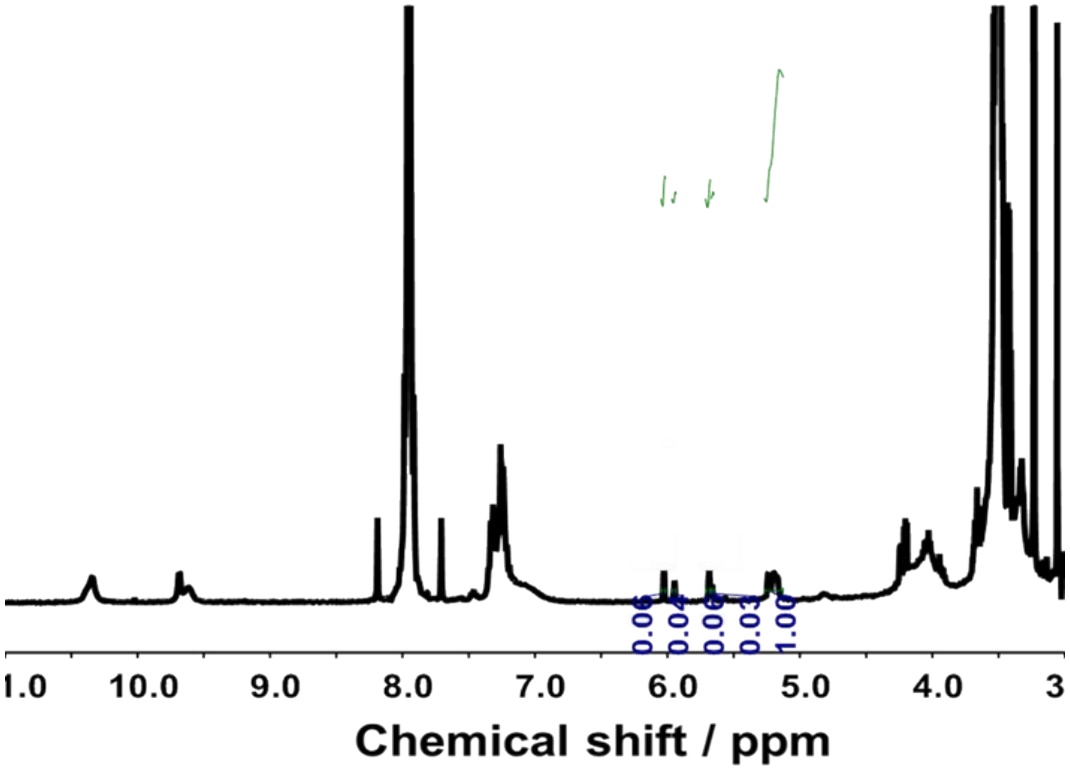


Figure S1. ^{1}H NMR spectrum (DMSO-d_6, 400M) of M1 and PEGMA after

copolymerization for conversion calculation.

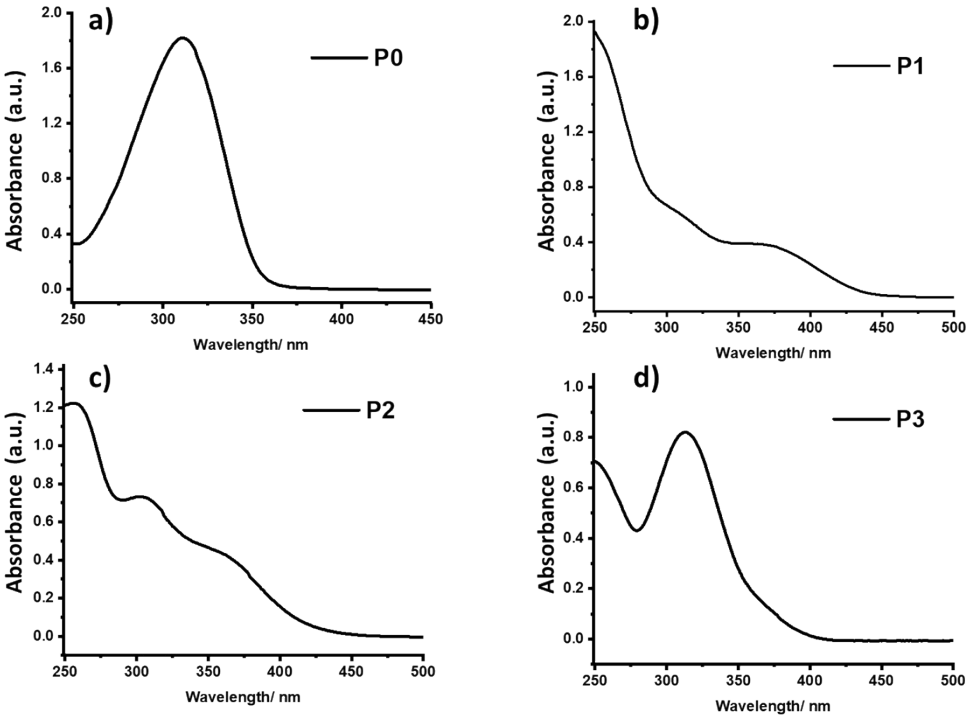


Figure S2. UV-Vis spectra of the copolymers (0.2 mg/mL, aqueous solutions): a) P0, b) P1, c) P2, d) P3.

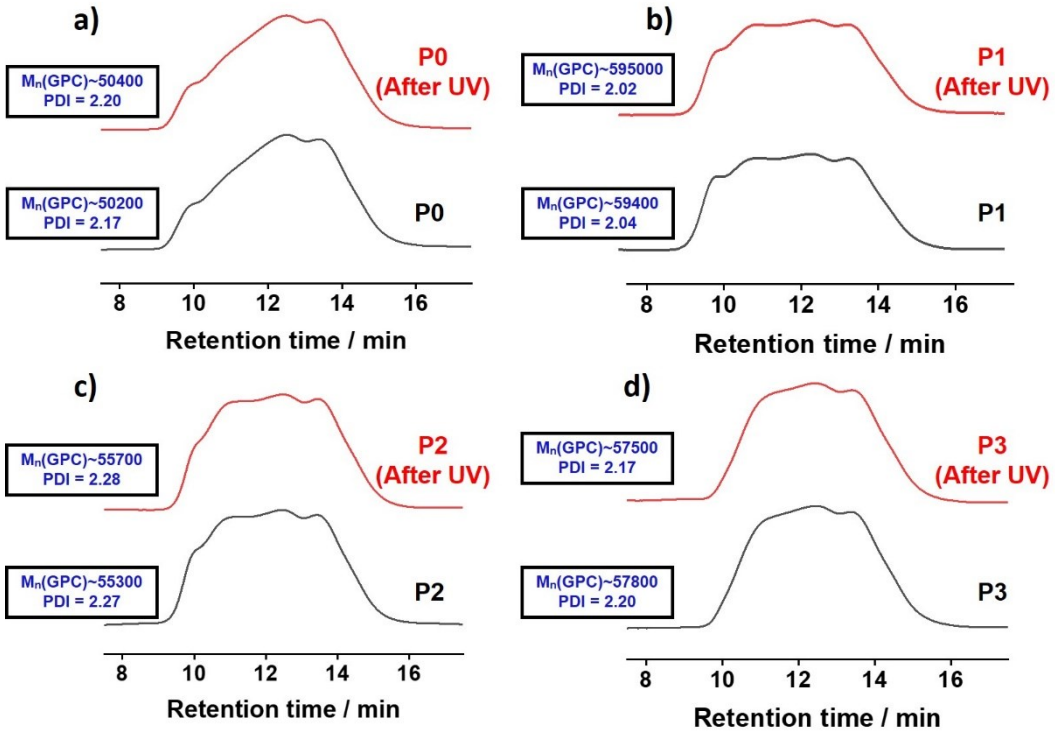


Figure S3. GPC curves of the copolymers before and after 2 h of UV irradiation. (~254 nm, 300 ± 20 μW/cm^2): (a) P0; (b) P1; (c) P2; (d) P3.

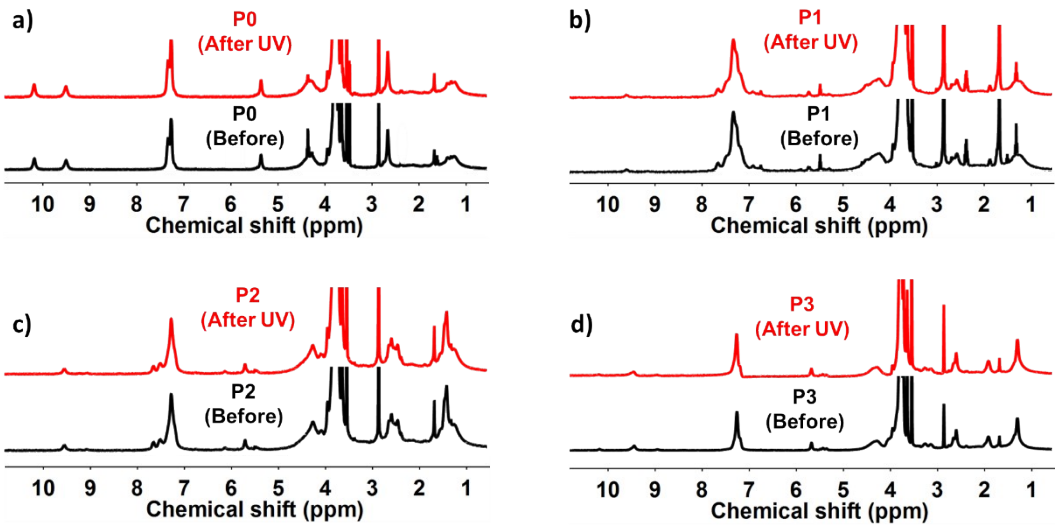


Figure S4. ^{1}H NMR spectra (DMSO-d_6, 400M) of the copolymers before and after 2 h of UV irradiation. (~254 nm, 300 ± 20 μW/cm^2): (a) P0; (b) P1; (c) P2; (d) P3.

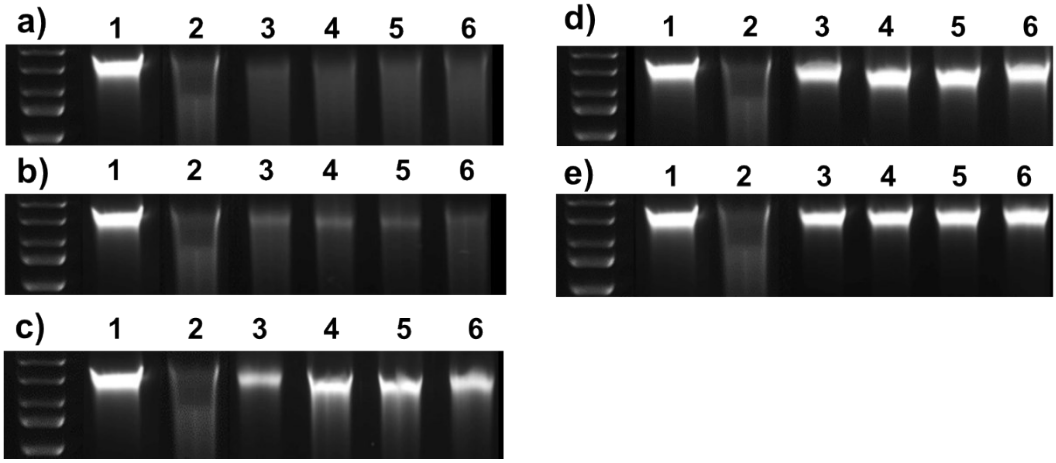


Figure S5. Agarose gel electrophoresis pattern of CT-DNA (50 μg/mL) with different concentrations of copolymers: a) 0.1 mg/mL, b) 0.2 mg/mL, c) 0.5 mg/mL, d) 0.8 mg/mL, e) 1.0 mg/mL. Samples in different lanes are: native DNA (lane 1); DNA + UV (15 min) (lane 2); DNA + P0 + UV (15 min) (lane 3); DNA + P1 + UV (15 min) (lane 4); DNA + P2 + UV (15 min) (lane 5); DNA + P3 + UV (15 min) (lane 6).

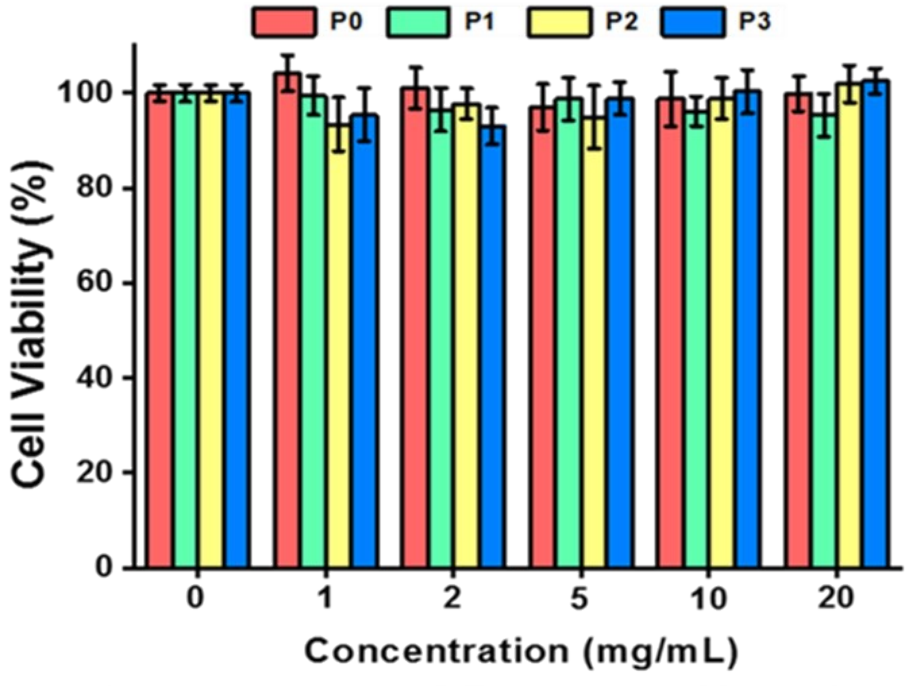


Figure S6. Cytotoxicity of copolymers to L929 cells. 24 h culture, cell viability in culture medium as the 100%. Data are represented as mean ± SD, n = 5.

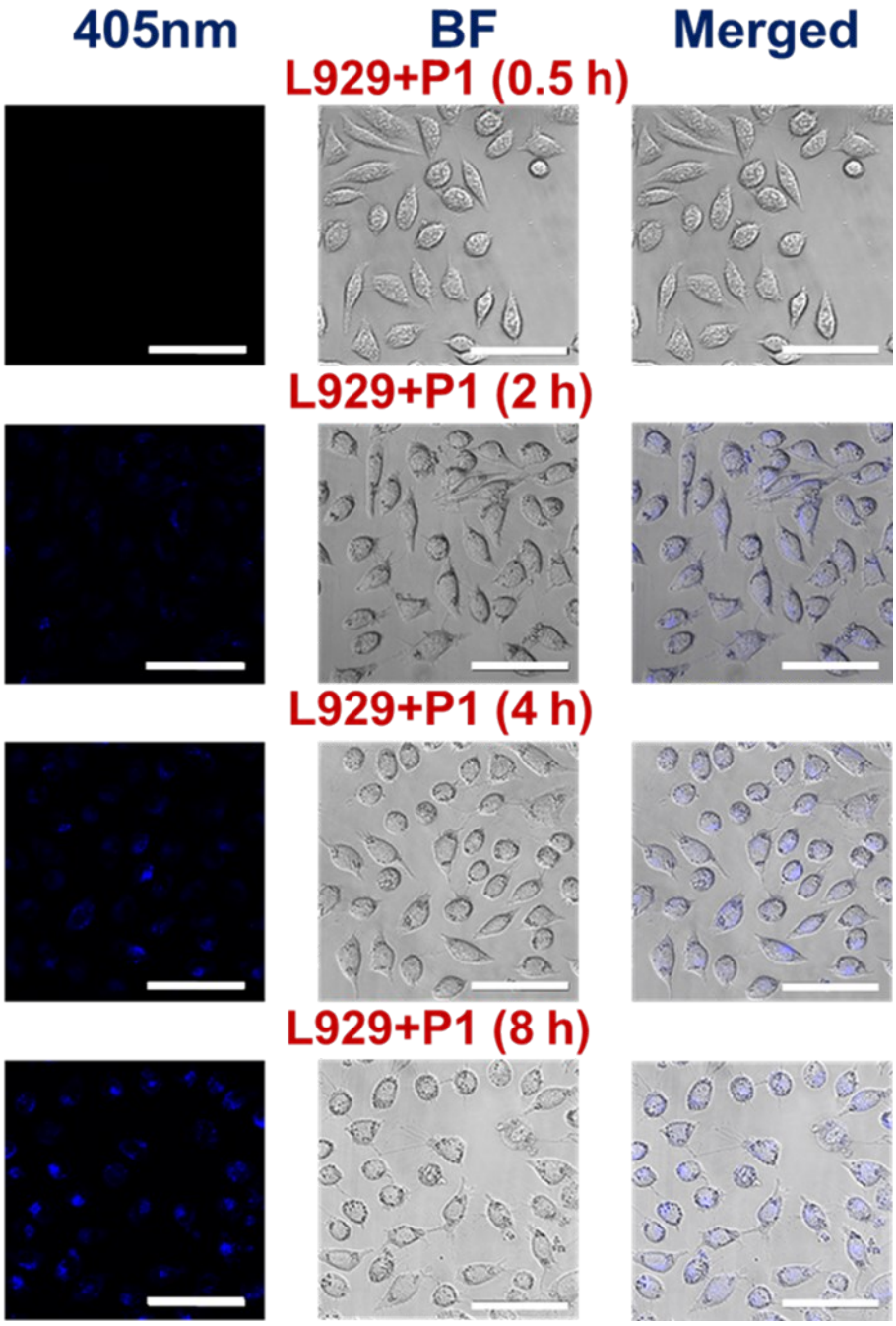


Figure S7. Confocal images of L929 cells incubated with P1(20 mg/mL) for at different

time points. Scale bar = 100 μm.

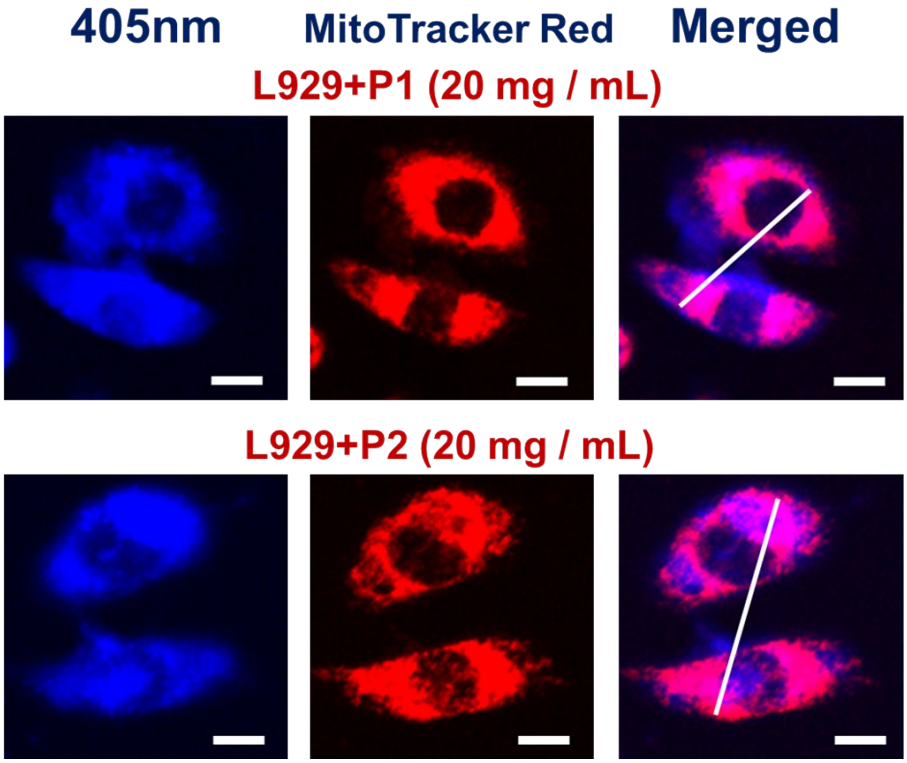


Figure S8. Confocal images of L929 cells incubated with (a) P1; (b) P2 and MitoTracker Red. Scale bar = 20 μm.

Table S1 Summary of the properties of the synthetic copolymers

Copolymer	Alkyl halide	PPM conv. (%)[a]	M_n(GPC)[b]	PDI[b]
P0	—	—	50200	2.17
P1	1-Phenyl-3-chloro-1-propyn	96.0	59400	2.04
P2	1-Chloro-2-pentyne	98.5	55300	2.27
P3	1-Bromopropane	91.5	57800	2.20

a. Calculated by ^{1}H NMR (DMSO-d_6, 400 MHz).

b. Measured by GPC using DMF as eluent (1 mL/min).

References

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4. Tang, Y.; Kong, X.; Xu, A.; Dong, B.; Lin, W., Development of a Two-Photon Fluorescent Probe for Imaging of Endogenous Formaldehyde in Living Tissues. *Angew. Chem. Int. Edit.* **2016**, *55* (10), 3356-3359.