Electronic Supplementary Material (ESI) for Soft Matter

Supporting Information

A Facile Strategy for Polymers to Achieve the Glucose-Responsive Behavior at Neutral pH

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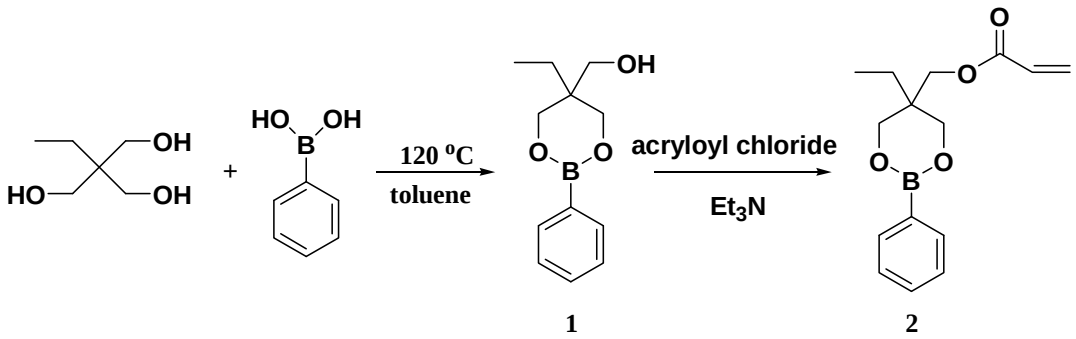
Materials

Monomethoxy poly(ethylene glycol) MPEG 2000 (M_n = 2000) purchased from Fluka, was dried by azeotropic distillation with toluene, and residual toluene was removed under high vacuum prior to use. CuBr purchased from Aldrich, was purified by stirring in acetic acid overnight, followed by washing with ethanol and diethyl ether, and dried in vacuum. Triethylamine (TEA) and methylene dichloride (CH_2Cl_2) were dehydrated with KOH and $CaCl_2$ overnight and distilled, respectively. Toluene was dried using sodium with benzophenone as color indicator. Molecular sieve was activated by muffle furnace. All of the above purified solvent and reagents were stored in solvent storage flasks prior to use. The other reagents such as 2-bromopropionyl bromide and pentamethyldiethylenetriamine (PMDETA) from Aldrich, 1,1,1-tris(hydroxymethyl) propane, phenylboronic acid(PBA), acryloyl chloride, $CuBr_2$, anisole and

twain-20 from Sinopharm Chemical (China) were used as received without further treatment.

Characterization

The ^{1}H and ^{13}C NMR spectra were carried out on a 400 MHz NMR instrument (Bruker Corporation, Germany) at room temperature using $CDCl_3$ as solvent. The chemical shifts were measured against the solvent signal of $CDCl_3$ as internal standard. The molecular weights of the polymers were determined with Waters 515-2410 gel permeation chromatograph (GPC) instrument equipped with Styragel HT6E-HT5-HT3 chromatographic column following a guard column and a differential refractive-index detector. The sample solution was filtered with a 0.45 μm syringe filter prior to ejection. The measurements were performed using THF as eluent (flow rate of 1.0 mL/ min at 30 ºC) and a series of narrow polystyrene standards for the calibration. The fluorescence spectra were recorded by a Hitachi F-4600 Fluorescence instrument (Hitachi High-Technologies Corporation, Tokyo Japan) at 37 ºC. Dynamic light scattering (DLS) measurements on polymeric micelles were performed using a "Zetaplus" Zeta Potential Analyzer (Brookhaven Instrument) equipped with ZetaPlus Particle Sizing software and with 35 mW solid state laser operated at a laser light wavelength of 660 nm. The size measurements were carried out at 37 ºC at a scattering angle of 90^{o}. The polymeric micelles were imaged on a Hitachi H800 transmission electron microscopy (TEM) (Hitachi High-Technologies Corporation, Tokyo, Japan) operated with 100 KV. For sample preparation, a drop of the sample solution was dropped on a carbon-coated copper grid, excess solution was wicked away with filter paper, and the sample was left dried in air.



1 2

Scheme 1. Synthesis route of monomer 2

Preparation of (2-phenylboronic esters-1,3-dioxane-5-ethyl) methylacrylate (PBDEMA) monomer 2 The synthesis of the monomer involving two steps was modified based on reported methods.[1] For the first step, the mixture of phenylboronic acid (5.9 g, 48.4 mmol) and 1,1,1-tris(hydroxymethyl) propane (6.5 g, 48.4 mmol) charged in one three-necked round bottle was stirred in toluene (80 mL) in the presence of small amount of molecular sieves at 120 ºC for 4 h. The reaction solution was filtered, and the crude solid precursor **1** was obtained by toluene removal in vacuum. Yield: 9.1 g (85.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.79 (d, J = 4 Hz, 2H, *o*-C_6H_5), 7.42 (m, 1H, *p*-C_6H_5), 7.32 (m, 2H, *m*-C_6H_5), 3.84-4.05 (m, 4H, CH_2OBOCH_2), 3.65 (br, 2H, CH_2OH), 1.53 (m, 2H, CH_2CH_3), 0.89 (m, 3H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 133.9, 130.9, 127.7, 66.8, 61.9, 39.2, 23.4, 7.4. IR (KBr disk): ν_{OH}, 3437 cm^{-1} (s), ν_{C-H}, 2967 cm^{-1} (m), ν_{B-Ar}, 1440 cm^{-1} (s), ν_{B-O}, 1314 cm^{-1} (s).

For the second step, the preparation of monomer **2** (PBDEMA) was carried out by the reaction of precursor **1** (10.0 g, 45.0 mmol), acryloyl chloride (4.8 g, 54.0 mmol) and triethylamine (5.4 g, 54.0 mmol) in anhydrous CH_2Cl_2 (40 mL) in an ice bath for 8 h. After filtration, the concentrated filtrate was purified by column chromatography with mixture of petroleum ether and ethyl acetate (v/v = 8/1) as eluent to afford colorless liquid. Yield: 9.7 g (78.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.77 (d, J = 8 Hz, 2H, *o*-C_6H_5), 7.42 (m, 1H, *p*-

C_6H_5), 7.34 (m, 2H, *m*-C_6H_5), 6.40 (d, *J* = 16 Hz, 1H, *CH*$_2$=CH), 6.12 (m, 1H, CH_2=*CH*), 5.84 (d, *J* = 8 Hz, 1H, *CH*$_2$=CH), 4.19 (s, 2H, *CH*$_2$OOCCH=CH_2), 4.05 (d, *J* = 8 Hz, 2H, *CH*$_2$OBOCH_2), 3.92 (d, *J* = 8 Hz, 2H, CH_2OBO*CH*$_2$), 1.50 (m, 2H, *CH*$_2$$CH_3$), 0.92 (t, *J* = 8 Hz, 3H, CH_2*CH*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.8, 133.9, 131.2, 130.8, 128.0, 127.6, 66.8, 63.7, 37.9, 23.8, 7.3. IR (KBr disk): ν_{C-H}, 2969 cm^{-1} (m), $\nu_{C=O}$, 1730 cm^{-1} (s), ν_{B-Ar}, 1440 cm^{-1} (s), ν_{B-O}, 1317 cm^{-1} (s), ν_{C-O}, 1190 cm^{-1} (s). Calcd. For $C_{15}H_{19}BO_4$: C, 65.72; H, 6.98%. Found: C, 65.94; H, 6.96%.

Synthesis of macroinitiator MPEG-Br MPEG-Br was prepared according to the reported method.[2] 2-bromopropionyl bromide (0.73 mL, 7.0 mmol) in CH_2Cl_2 (30 mL) was stepwise added into a 100 mL three-necked flask containing the mixture of MPEG 2000 (M_n = 2000 g/mol; 7.0 g, 3.5 mmol) and triethylamine (0.97 mL, 7.0 mmol) in anhydrous CH_2Cl_2 (20 mL) in an ice bath. The reaction mixture was stirred for 48 h. After filtration, the filtrate was sequentially washed with 0.1 M HCl, 0.1 M NaOH and distilled water, followed by extraction with CH_2Cl_2 twice. The combined organic phase was dried over anhydrous $MgSO_4$ and concentrated. The crude product was redissolved in $CHCl_3$ (3 mL) and precipitated in diethyl ether (100 mL) twice to obtain MPEG-Br (5.1 g) in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.39 (q, *J* = 8 Hz, 1H, CH_3*CH*Br), 4.31 (t, *J* = 4 Hz, 2H, *CH*$_2$OOC), 3.45–3.82 (m, 4n H, (O*CH*$_2$*CH*$_2$)$_n$), 3.37 (s, 3H, *CH*$_3$OCH$_2$), 1.82 (d, *J* = 4 Hz, 3H, *CH*$_3$CHBr). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.2, 71.9, 70.6, 68.7, 65.0, 59.0, 39.9, 21.6.

Synthesis of diblock polymer MPEG-*block*-PpBDEMA In a typical atom transfer radical polymerization (ATRP) procedure, a Schlenk flask with a magnetic stir bar was charged with CuBr (14.4 mg, 0.1 mmol), macroinitiator MPEG-Br (213.5 mg, 0.1 mmol) and $CuBr_2$ (1.1 mg, 0.005 mmol). The flask was degassed using three vacuum–nitrogen cycles. The liquid materials,

PMDETA ligand (20.8 mg, 0.12 mmol), monomer PBDEMA (2.73 g, 10.0 mmol) and anisole (2.5 mL), which were degassed by bubbling with nitrogen for 20 min prior to use, were introduced into the reaction flask using syringes under nitrogen atmosphere. The reaction system was further degassed using three freeze–pump–thaw cycles, and then immersed in an oil bath at 90 ºC under thermostat control. After a predetermined polymerization time, the cooled reaction solution was diluted with $CHCl_3$ (3 mL) and passed through a neutral alumina column to remove the catalyst. The concentrated reaction solution was dropwise added into the mixed solvent of hexane/diethyl ether (v/v = 4/1). The polymer was dried in vacuum at room temperature for 4 h to obtain (1.02 g) in 35% yield ^{1}H NMR(400 MHz, $CDCl_3$) δ (ppm): for PpBDEMA block: 7.75 (d, *J* = 8 Hz, 2H, *o*-C_6H_5), 7.37 (m, 1H, *p*-C_6H_5), 7.29 (m, 2H, *m*-C_6H_5), 3.76-3.99 (m, 6H, CH_2OOC, CH_2OBOCH_2 and CH_2OBOCH_2), 2.29 (br, 1H, CHCH_2), 1.60-1.91 (br, 2H, CHCH_2), 1.35 (m, 2H, CH_2CH_3), 0.79 (m, 3H, CH_2CH_3); for MPEG block: 3.64 (s, 4H, CH_2CH_2O), 3.38 (s, 3H, CH_3O). ^{13}C NMR (100 mHz, $CDCl_3$) δ (ppm): 173.6, 134.0, 132.1, 129.5, 127.7, 72.0, 70.7, 69.0, 67.9, 64.8, 59.1, 41.7, 37.7, 34.6, 23.6, 7.4.

Preparation of Fluorescein-labeled insulin (FITC-insulin) Based on the previous report,[3] the preparation method of FITC-insulin was modified. Briefly, Fluorescein isothiocyanate (FITC) (2.0 mg, 0.005 mmol) in dried DMSO (400 μL) was added dropwise to the solution containing insulin (14.8 mg, 0.0025 mmol) and 2.0 mL phosphate butter solution (0.2 mol/L, pH 7.1). The mixture was protected from light and allowed to mix for 12 h at 4 ºC under nitrogen atmosphere. After dialyzing to take away the unreacted FITC, FITC-insulin was precipitated through adjustment of the pH to 4.5-5.0 with HCl and store at 4 ºC for 48 h. The precipitation was centrifuged (10,000 rpm, 10 min) and washed with a few milliliters of cold 0.01 mol/L ammonium acetate at pH 4.5 and then lyophilized to get crude FITC-insulin.

Critical Micelle Concentration (CMC) Measurement The CMC of the amphiphilic block copolymers was determined by steady-state fluorescence spectrometry with pyrene as a fluorescent probe. The concentration of block polymer was varied from 2.0×10^{-4} to 0.8 mg/mL and the concentration of pyrene was fixed at 0.99×10^{-3} μM. The fluorescence spectra were recorded using Hitachi F-4600 Fluorescence spectrometer with the emission wavelength of 395 nm. The excitation fluorescence was monitored at 338 and 333 nm. The CMC was estimated as the cross-point when extrapolating the intensity ratio I_{338}/I_{333} at low and high concentration regions.

Encapsulation and Release of Nile Red The mixture of 50.0 mg polymer THF solution (3.0 mL) and 180 μL nile red CH_2Cl_2 solution (1.0 mM) was added into 50.0 mL deionized water with stirring at ambient temperature, followed by 3.0 mL twain-20 solution (10 mg/mL). Nile red-encapsulating micellar solution was dried in vacuum at ambient temperature for 3 h to remove complete THF. The solution was divided into three 1.5 mL samples, and adjusted to pH 7.4 and 7.8 by the addition of 0.4 mol/L phosphate buffer solution, respectively. The initial fluorescence intensity of each was measured as 100%. Then, the given amount of glucose was added at 37 ºC, and the fluorescence spectra were recorded with excitation wavelength of 490 nm and the emission intensity at 620 nm was determined at the desired time points.

Insulin Loading and Release A stock of FITC-insulin (3.0 mg) and polymer (5.0 mg) dissolved in 0.3 mL THF was added to 5.0 mL deionized water with stirring at room temperature overnight. For the complete removal of free FITC-insulin, insulin-encapsulated micellar solution was transferred into dialysis membranes (molecular weight cut off 12000) and dialyzed against distilled water until the fluorescence signal of dialysis bag inside at the emission wavelength of

519 nm was stable, during which the distilled water was changed every 6 h. The dialyzed solution was stored at 4 °C in the dark.

The *in vitro* release test of FITC-insulin from the polymeric micelles was evaluated by the dialysis method. A dialysis bag filled with 1.0 mL of purified insulin-loaded micelles was immersed in 25 mL of 0.05M PBS of pH 7.4 at glucose concentration of 4 mg/mL. The released FITC-insulin outside of the dialysis bag was sampled at determined time intervals and assayed by fluorescence spectrometry at 519 nm upon excitation at 494 nm. Cumulative release mass was calibrated according to the measured standard curve of fluorescence intensity against insulin concentration (Figure S12).

Reference

(1) (a) M. Meiland, T. Heinze, W. Guenther, T. Liebert, *Tetrahedron Lett*. 2009, **50**, 469;

(b) R. Haag, A. Hebel, *J. Org. Chem.* 2002, **67**, 9452.

(2) D. Zhang, H. Zhang, J. Nie, J. Yang, *Polym. Int.* 2010, **59**, 967.

(3) M. G. Li, W. L. Lu, J. C. Wang, X. Zhang, X. Q. Wang, A. P. Zheng, Q. Zhang, *Pharm. Nanotech.* 2007, **329**, 182.

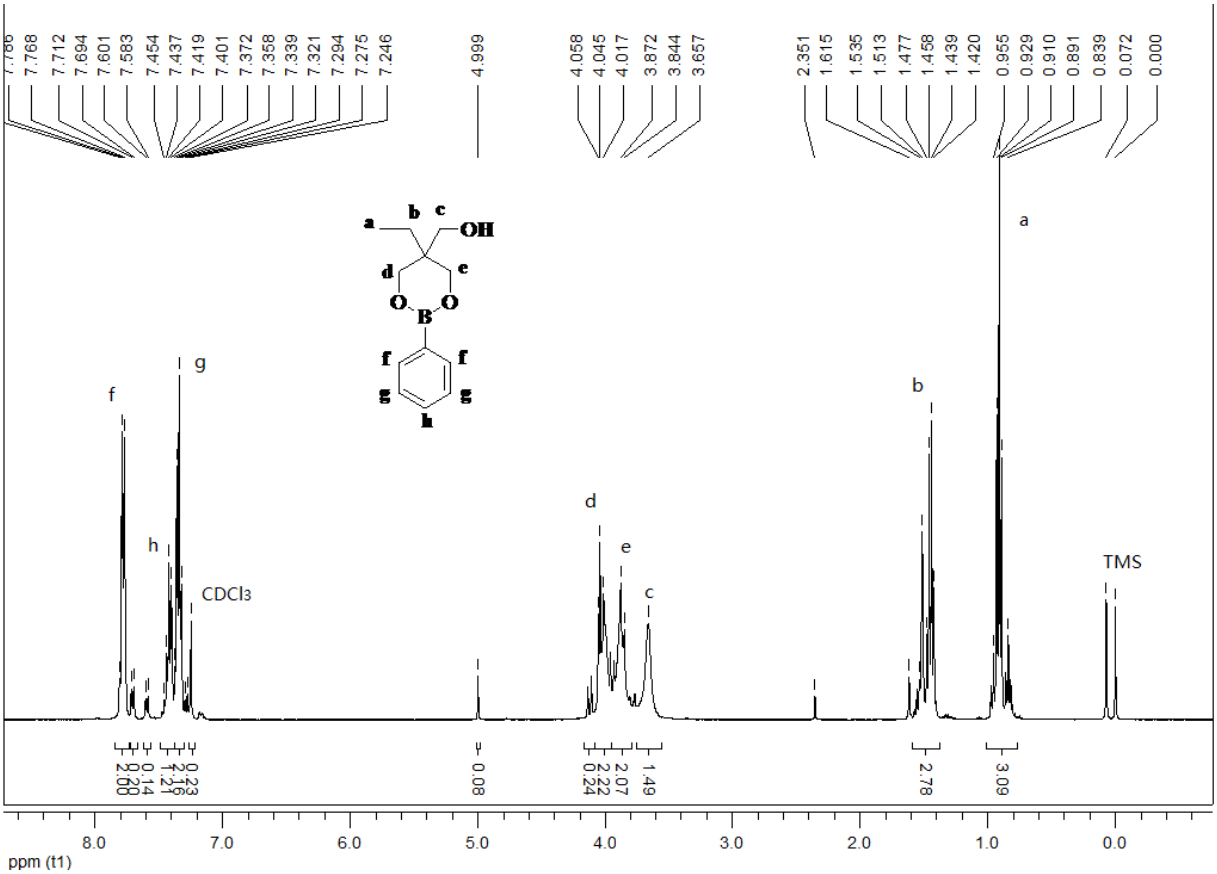


Figure S1 ^{1}H NMR spectrum of monomer precursor **1.**

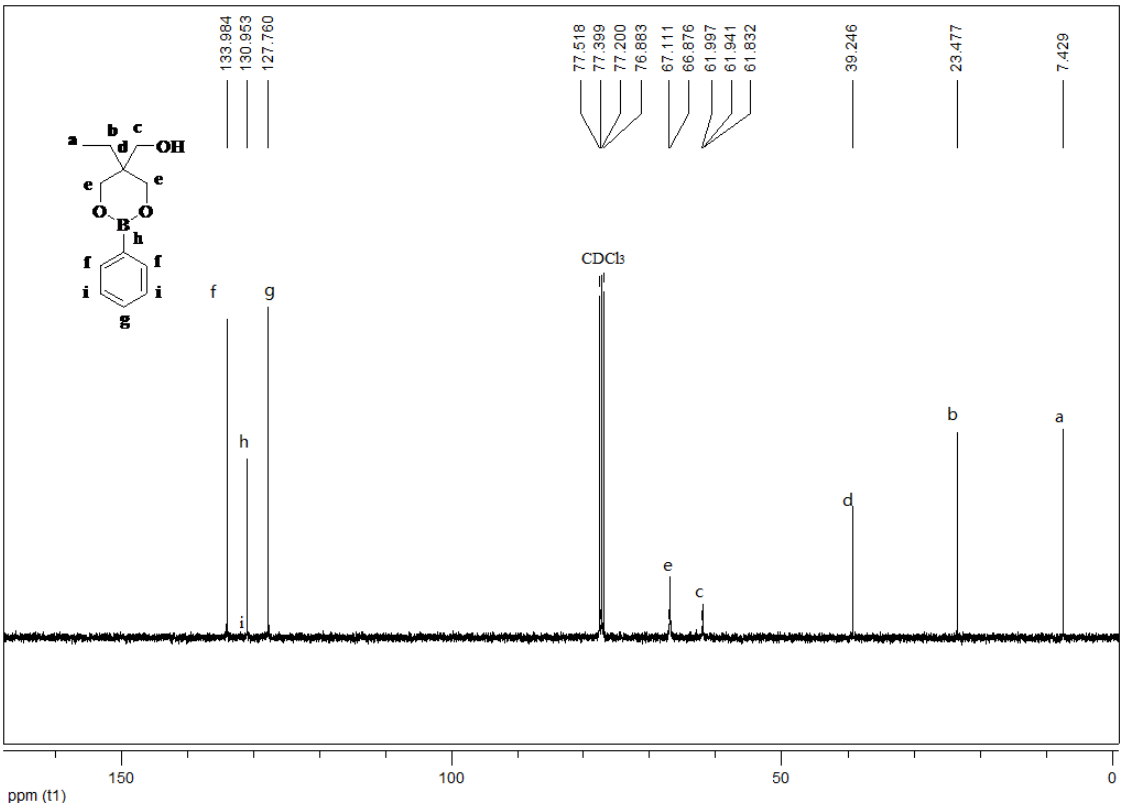


Figure S2. ^{13}C NMR spectrum of monomer precursor **1.**

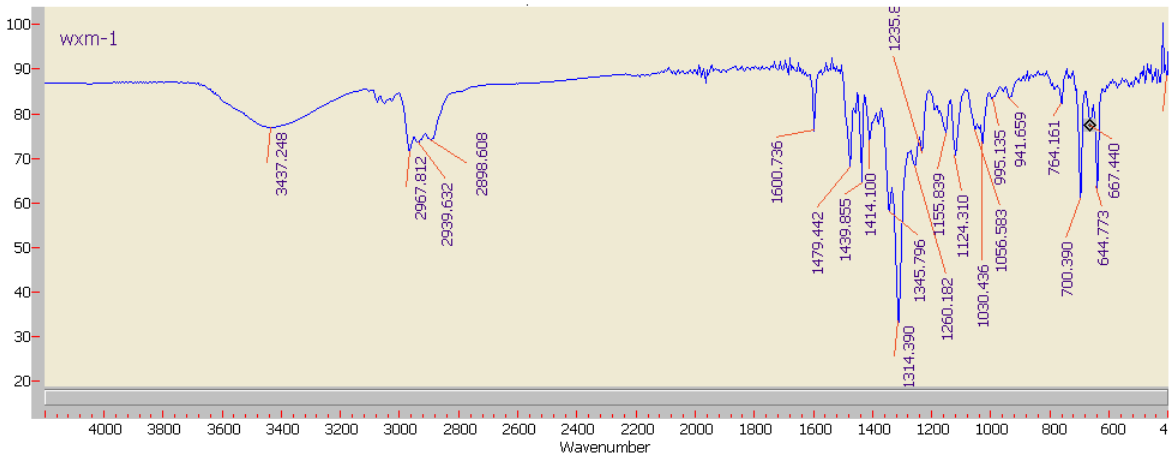


Figure S3. IR of monomer precursor **1.**

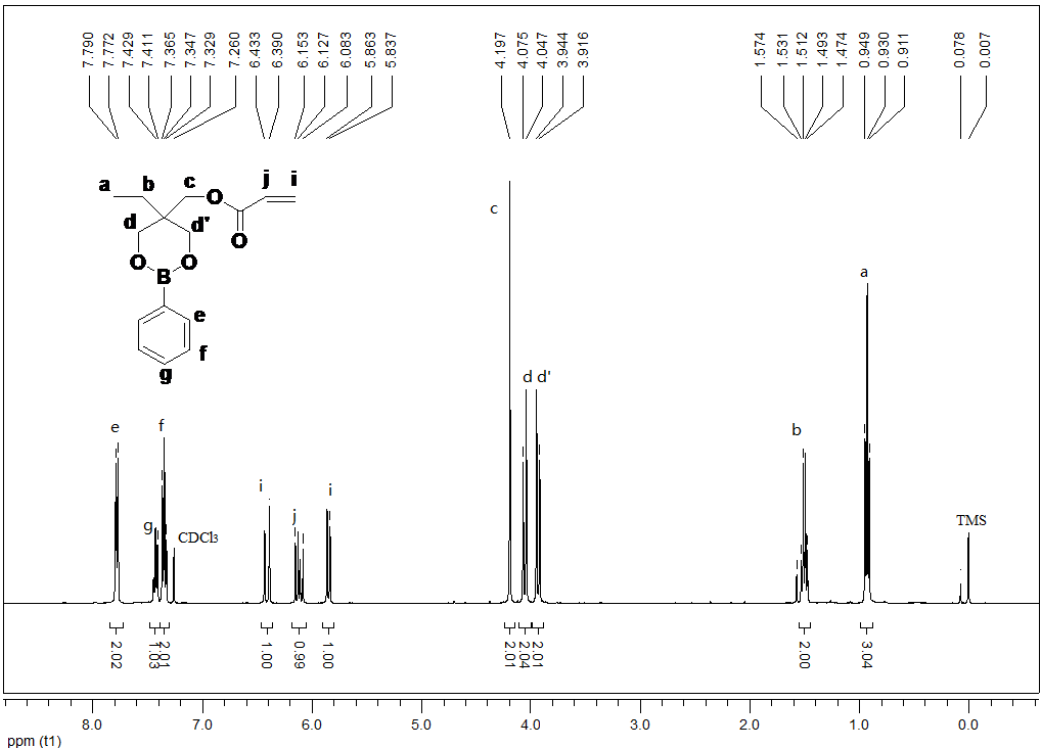


Figure S4. ^{1}H NMR spectrum of monomer PBDEMA.

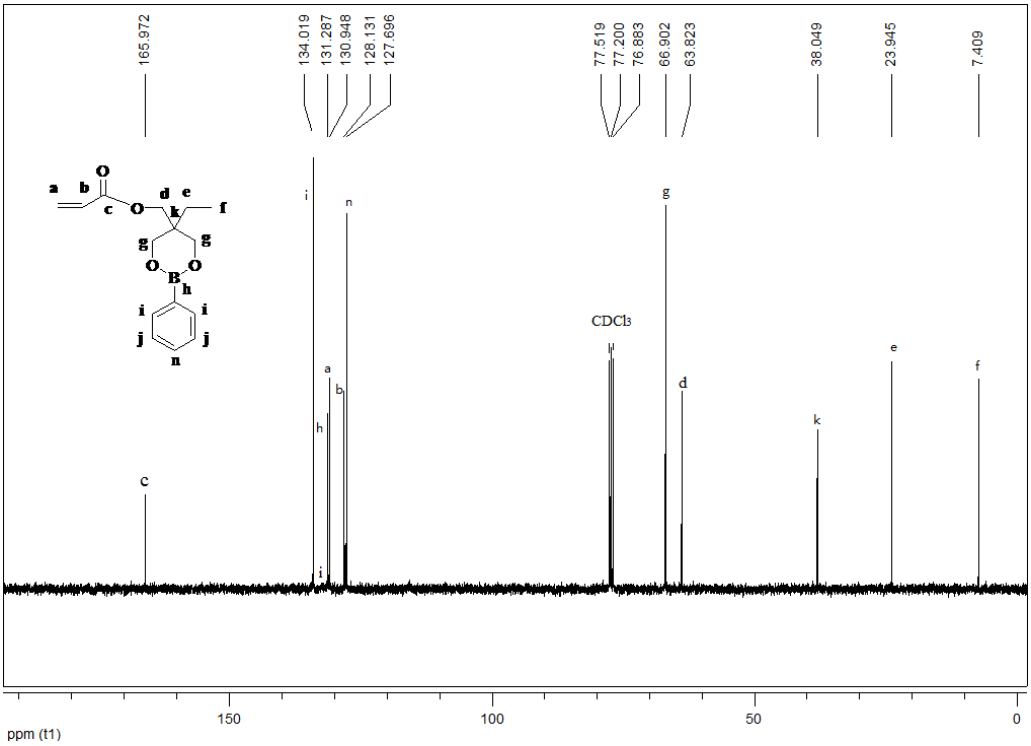


Figure S5. ^{13}C NMR spectrum of monomer PBDEMA.

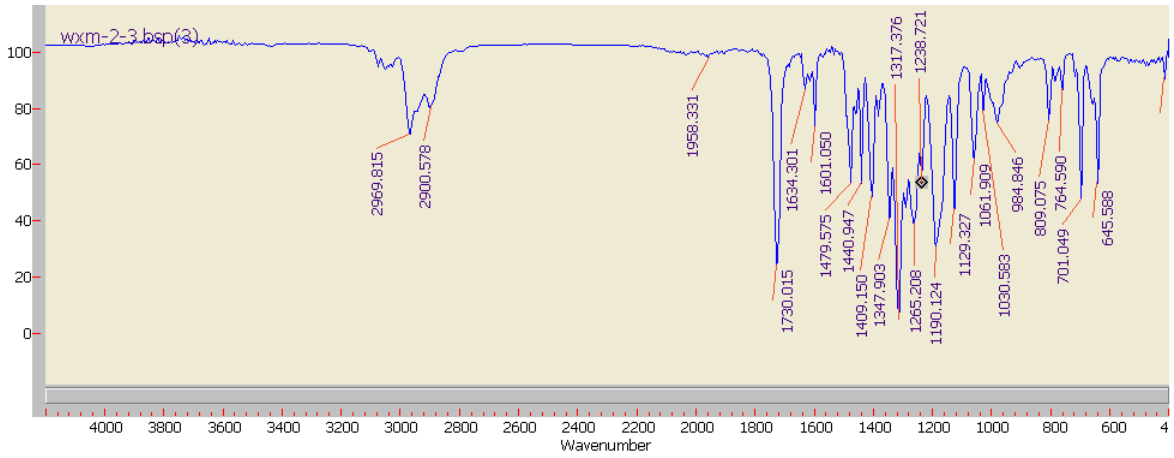


Figure S6. IR of monomer PBDEMA.

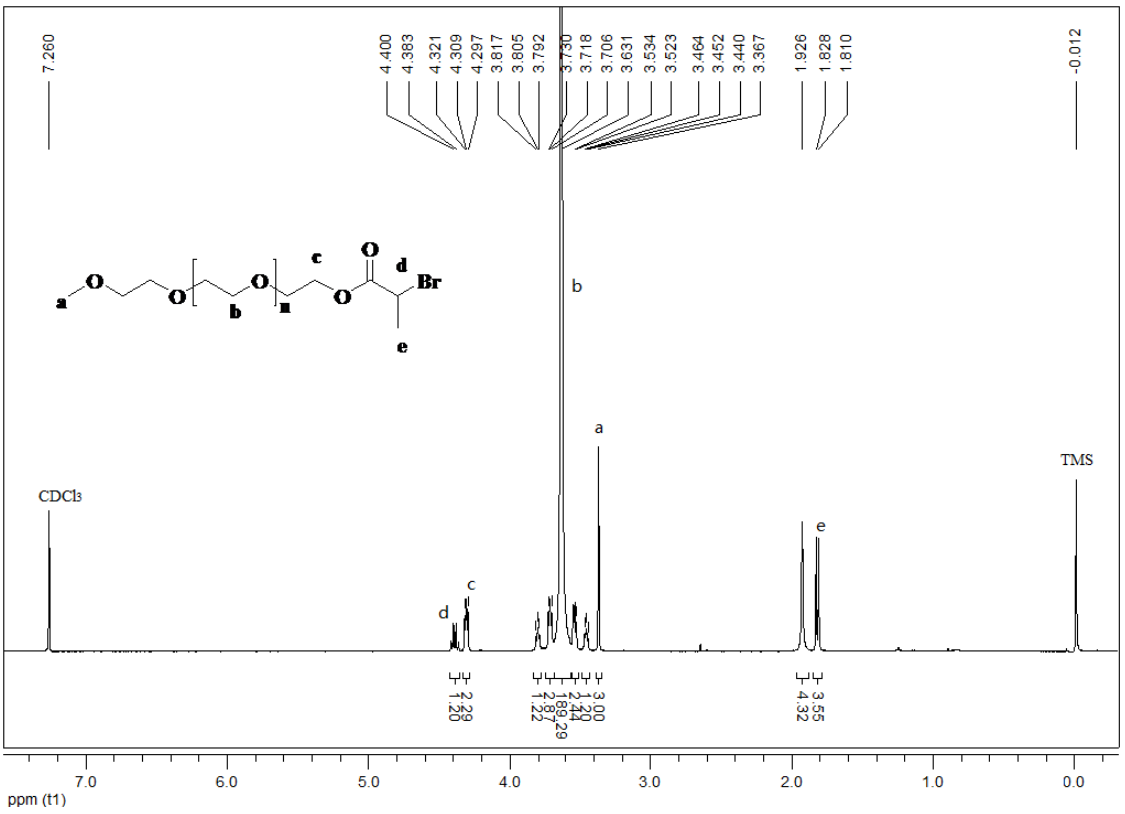


Figure S7. ^{1}H NMR spectrum of macroinitiator MPEG-Br.

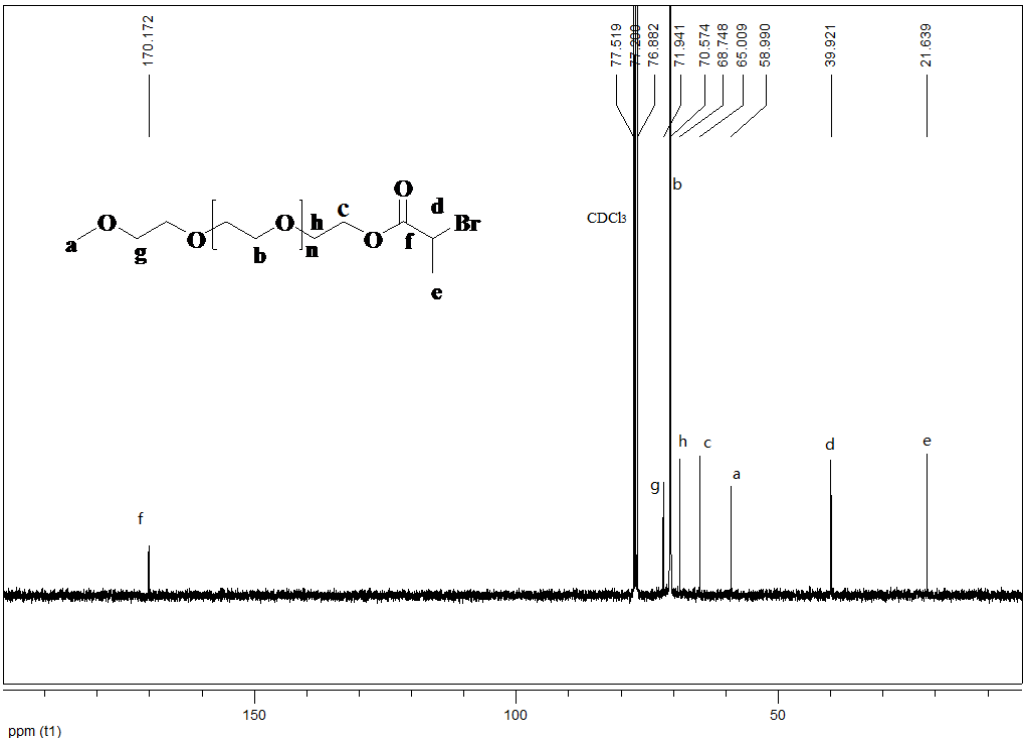


Figure S8. ^{13}C NMR spectrum of macroinitiator MPEG-Br.

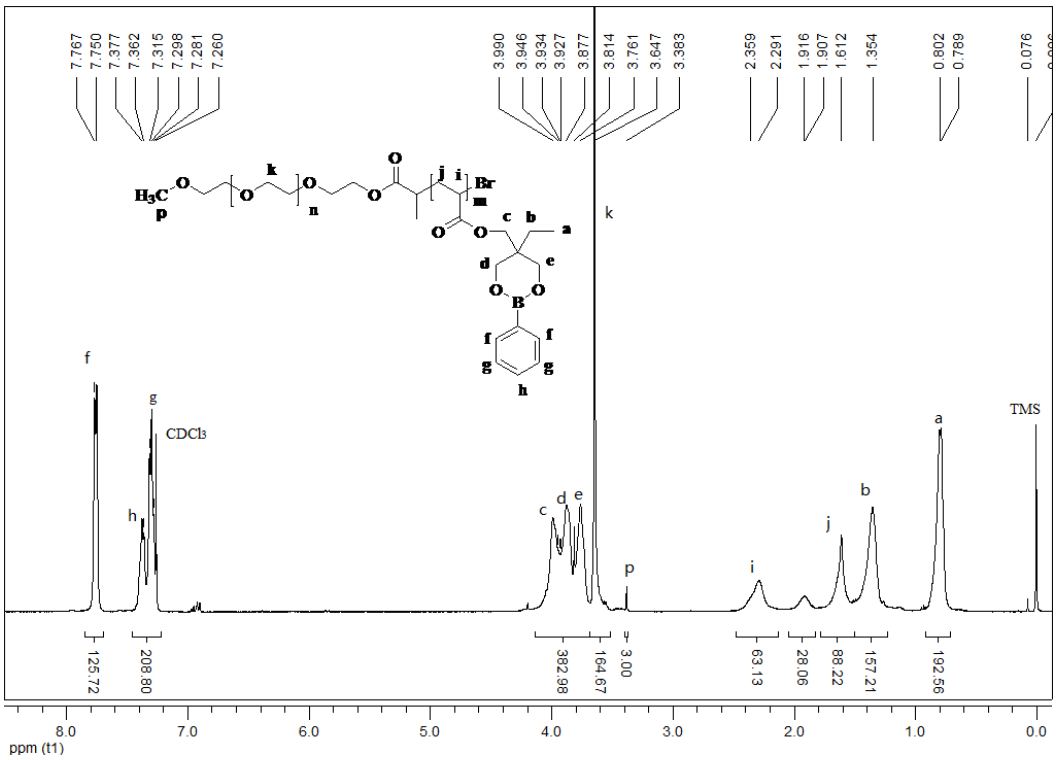


Figure S9. ^{1}H NMR spectrum of MPEG-*block*-PpBDEMA.

The repeating unit number of the hydrophobic block is calculated by ^{1}H NMR spectrum of the copolymer, depending on equation 1.

$$N = \frac{I_{2.35}}{I_{3.38}} \times 3 \qquad \textbf{Equation 1}$$

Where N stands for the repeating unit number of the hydrophobic block, $I_{2.35}$ and $I_{3.38}$ represent the integrals at 2.35 and 3.38 ppm.

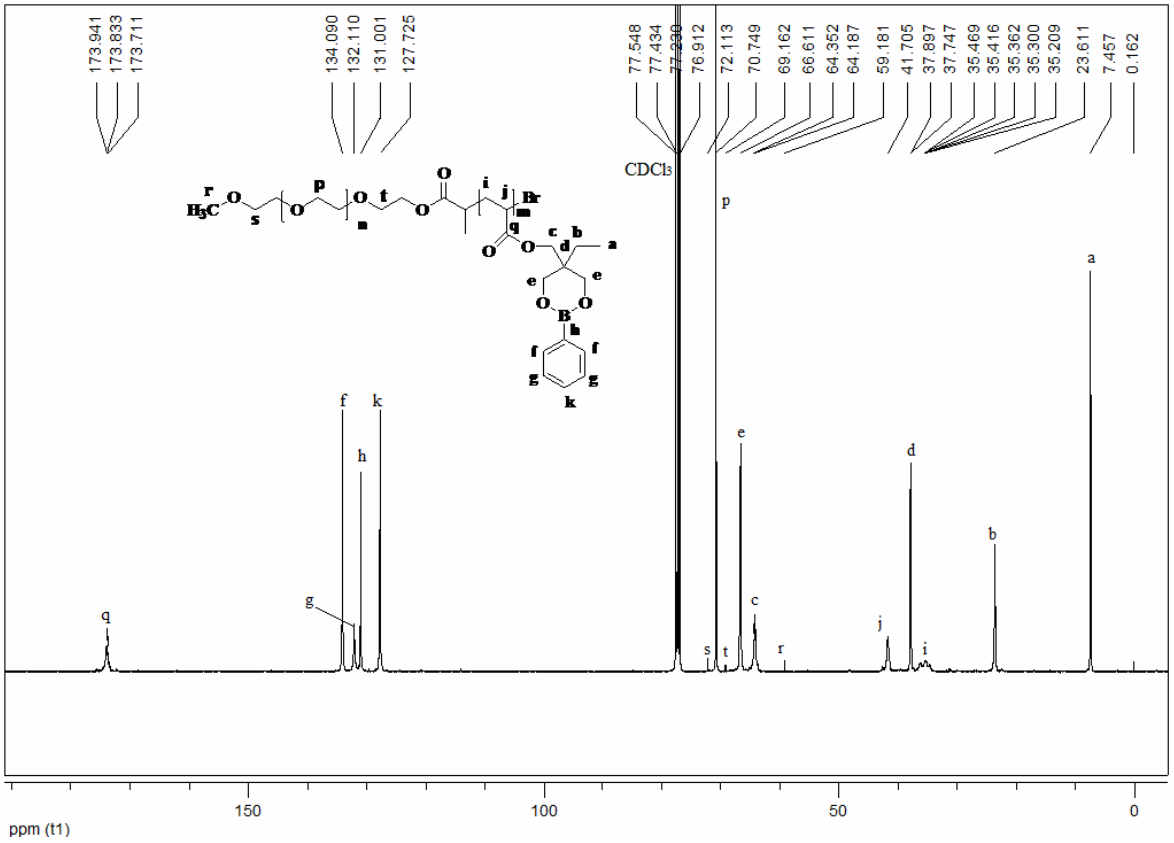


Figure S10. ^{13}C NMR spectrum of MPEG-*block*-PpBDEMA.

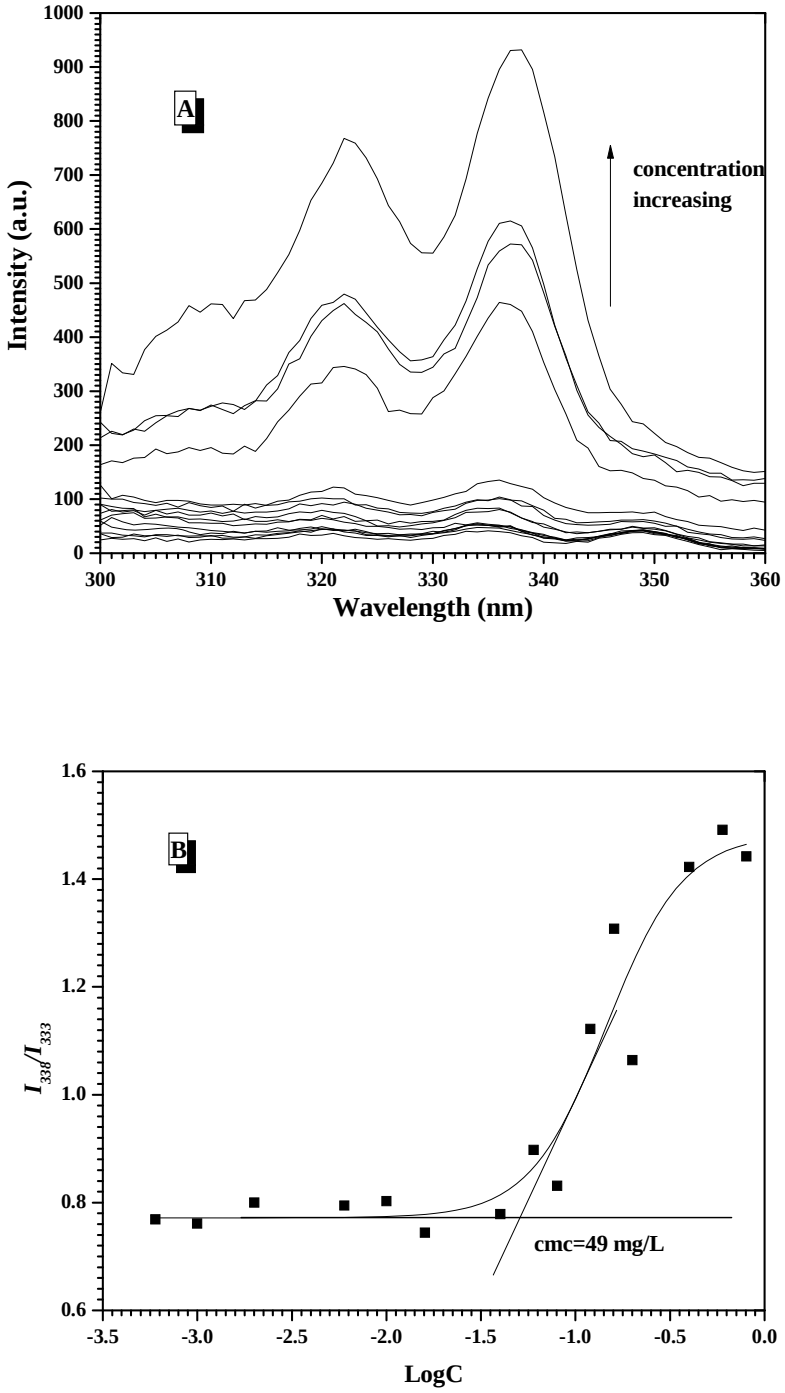


Figure S11. (A) Excitation spectra of pyrene in aqueous solution of MPEG-*block*-PpBDEMA at various concentrations. (B) Plots of I_{338}/I_{333} of pyrene excitation spectra in water as a function of the concentration of MPEG-*block*-PpBDEMA at 25 ºC.

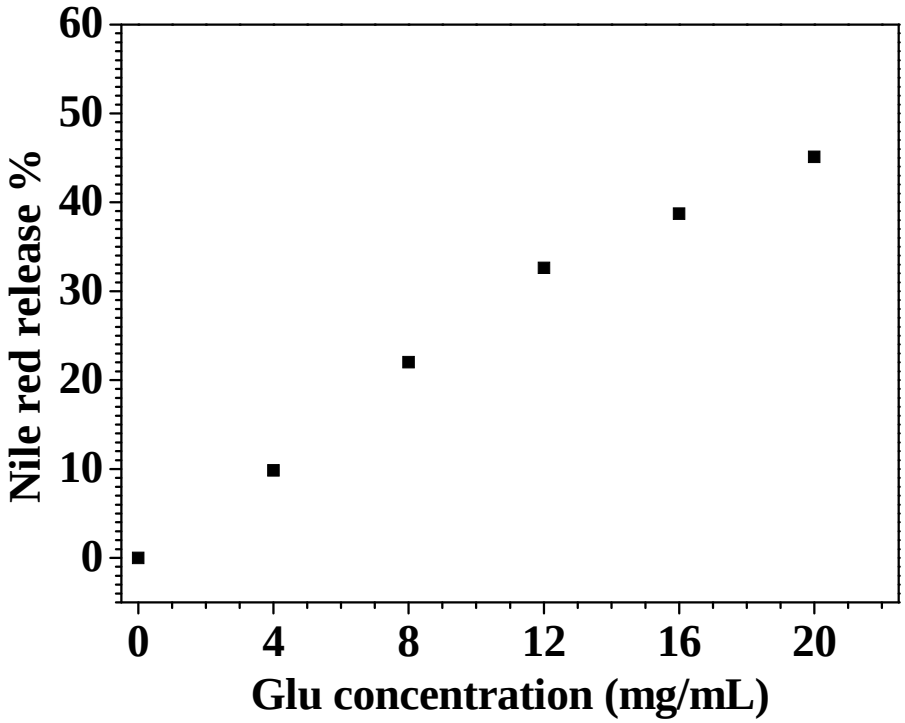


Figure S12. The relationship between the initially given glucose concentration and the final nile red release percentage at pH 7.4 and 37 ºC.

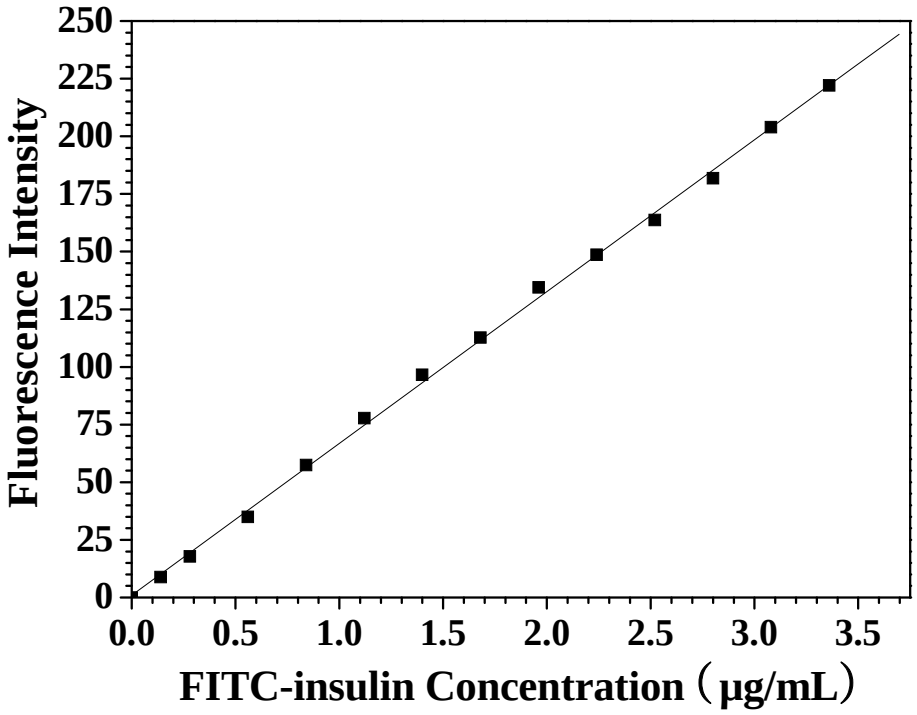


Figure S13. Fluorescence intensity at various concentration of FITC-insulin.