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for

Synthesis of PEGylated Brush-Type Copolymers for A Plurality of Plug-and-Play Functions

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Experimental Section

1.1 Materials

Poly(ethylene glycol) methacrylate (PEGMA, M_n= 360 g/mol, Sigma-Aldrich) , Poly(ethylene glycol) methyl ether methacrylate (PEGMEMA, M_n= 300 g/mol, Sigma-Aldrich) were dissolved in THF, passed through a neutral Al_2O_3 column, concentrated by a rotary evaporator, and finally dried under reduced pressure. tert-Butyl methacrylate (*t*BMA, TCI) was passed through a neutral Al_2O_3 column before use. Sodium azide (NaN_3, 99%, Sigma-Aldrich), Cyanine 3 alkyne (Cy3, Lumiprobe), 5-aminofluorescein (Sigma-Aldrich), dopamine (DA, Sigma-Aldrich), methyl-2-bromopropionate (MBP, Sigma-Aldrich), copper (I) bromide (CuBr, 99.999%, Alfa Aser), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, Sigma-Aldrich), 2-bromoisobutyryl bromide (98%, Sigma-Aldrich), tributyltin hydride (98%, Sigma-Aldrich) trifluoroacetic acid (TFA, Sigma-Aldrich), were used as received. Ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), sodium acetate (NaOAc), ethanol, ethylene glycol (EG), *N,N*-dimethylformamide (DMF), diethylene glycol (DEG), dichloromethane (CH_2Cl_2), hexanes and other reagents were purchased from Sinopharm Chemical Reagent and used as received.

1.2 Characterizations .

The chemical structures of P(PEGMA-*co*-PEGMEMA), P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA, P(PEGMA$_{-Br}$-*co*-PEGMEMA)-*b*-P*t*BMA, P(PEGMA$_{-N_3}$-*co*-PEGMEMA)-*b*-PMAA, were mainly determined by ^{1}HNMR spectra and Gel permeation chromatograph (GPC). ^{1}HNMR spectra were recorded on a 400 MHz ^{1}HNMR instrument (INOVA-400), using $CDCl_3$ and DMSO-d_6 as solvents and tetramethylsilane (TMS) as internal standard. Gel permeation chromatograph (GPC) measurements for P(PEGMA-*co*-PEGMEMA) and P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA were carried out at 30 °C using a Waters 1515 instrument with a PL gel 5.0 μm bead size guard column (50 × 7.5 mm^2), followed by two linear PL gel columns (500 Å and Mixed-C) and a differential refractive index detector. THF was used as the eluent at a flow rate of 1.0 mL/min, and calibration was performed with polystyrene standards. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker VECTOR-22 IR spectrometer. The spectra were collected at 64 scans. Transmission electron

microscopy (TEM) observations were conducted on a Hitachi H-800 electron microscope at an acceleration voltage of 200 kV. The sample for TEM observations was prepared by placing 10 μL of solutions on copper grids. The size distribution and ζ potential were gained by using dynamic light scattering equipment (Zetasizer Nano ZS, Malvern). The fluorescence spectra were obtained on a FluoroMax 4 luminescence spectrometer (HORIBA Jobin Yvon). The excitation wavelength for Cy3 and Cy3-copolymer IV was 520 nm. The excitation wavelength for 5-aminofluorescein and 5-aminofluorescein-copolymer IV was 440 nm. The fluorescent images of micro-channels were acquired by inverted fluorescence microscope (Olympus IX71) mounted with a multispectral CCD camera (Nuance, Perkin Elmer).

1.3 Synthesis of P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA

The brush-type copolymer P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA was prepared *via* the sequential ATRP reaction using MBP as the initiator under nitrogen atmosphere, the molar ratio of PEGMA and PEGMEMA is 3:7. The typical synthetic process is shown here: MBP (33.5 μL, 0.3 mmol), PEGMA (0.97 g, 2.7 mmol), PEGMEMA (1.89 g, 6.3 mmol), CuBr (43.0 mg, 0.3 mmol) and PMDETA (117.36 μL, 0.6 mmol) were dissolved in 10 mL of ethanol in a 50 mL flask, followed by three freeze-pump-thaw nitrogen cycles, then the flask was sealed and the reaction mixture was stirred at 30 °C for 5 h. An aliquot of solution was withdrawn from the mixture as the medium product and purified for GPC and ^{1}HNMR measurements. After that, a solution containing *t*BMA (3.658 g, 25.76 mmol), CuBr (36.95 mg, 0.2576 mmol), PMDETA (100.7 μL, 0.515 mmol) were added to a 25 mL flask containing 10 mL of ethanol, followed by three freeze-pump-thaw nitrogen cycles, then the solution was transferred to the reactor by a syringe. The polymerization was further carried out under nitrogen atmosphere at 30 °C for 36 h after the first step. The mixture was diluted with ethanol and passed through a column filled with neutral Al_2O_3 to remove the catalyst. The solution was evaporated and concentrated by rotary evaporation，and was precipitated in cold hexanes for three times, the product was dried under vacuum at room temperature for 24 h and white solid was collected as P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA. Besides, the purification process for P(PEGMA-*co*-PEGMEMA) was depicted here: the solution withdrawn from the solution in first step was dialyzed (MWCO 3500) against Milli-Q water for 48 h at room temperature to remove ethonal, free monomers and catalysts. The dialysis

medium was changed six times during the process. Finally, the resulting solution was freezed and collected under freeze drying for 24 h as P(PEGMA-*co*-PEGMEMA).

The bromide groups at the end of main chain were reduced using the tributyltin hydride as reducer. Typically, P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA was dissolved in 10 mL of ethanol in 25 mL of flask. 225 μL tributyltin hydride was added quickly into the solution by 1mL of syringe. The flask was purged with N_2 for three times, the polymerization was carried out at 40 °C under rigorous stirring for 10 h. The solution was purified by repeating precipitation in cold hexane twice and dried in vacuum at room temperature for 24 h. Finally, a white solid was collected as reduced P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA.

1.4 Synthesis of P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-P*t*BMA

P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-P*t*BMA was prepared between 2-bromoisobutyryl bromide and reduced P(PEGMA-*co*-PEGMEMA)-*b*-P*t*BMA by nucleophilic substitution reaction. Typically, solution containing P(PEGMA-*co*- PEGMEMA)-*b*-P*t*BMA (1.6 g, 0.0584 mmol), anhydrous TEA (0.28 mL, 2.0 mmol), 10 mL of anhydrous THF was added to the flask at 0 °C. 2-bromoisobutyryl bromide (0.46 g, 2 mmol) was then added dropwise into the THF solution at 0 °C. After stirring for 24 h at 25 °C, the mixture was filtered to remove the insoluble yellow precipitates. Then, the solution was evaporated to remove the excess THF and extracted with a mixture of dichloromethane/saturated sodium solution (2/3, *v*/*v*) for three times. The organic layer was dried over anhydrous Na_2SO_4 for 2 h and evaporated by rotary evaporation. The concentrate was dried under vacuum to a constant weight at room temperature and obtained as yellow solid, namely P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-P*t*BMA

1.5. Synthesis of P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-PMAA

A typical hydrolysis procedure was described as follows, a solution containing P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-P*t*BMA (1.0 g,0.058 mmol) in 10 mL of CH_2Cl_2 was added to a 100mL round-bottom flask, trifluoroacetic acid (TFA 1.23 mL,16.6 mmol) was slowly added into the reactor under stirring at 0 °C. After that, the reaction was carried out at 30 °C for 55 h. The solution was concentrated by rotary evaporation and precipitated with a mixture of cold hexanes/diethyl ether (10/1, *v*/*v*) for three times. Finally, it was dried in vacuum at room temperature for 24 h, and obtained as P(PEGMA$_{\text{-Br}}$-*co*-PEGMEMA)-*b*-PMAA.

1.6 Synthesis of P(PEGMA$_{\text{-N}_3}$-*co*-PEGMEMA)-*b*-PMAA

P(PEGMA$_{-N_3}$-*co*-PEGMEMA)-*b*-PMAA was synthesized between NaN_3 and bromide groups (-Br) at the end of PEG side chains with the help of nucleophilic substitution reaction. P(PEGMA$_{-Br}$-*co*-PEGMEMA)-*b*-PMAA (1.515 g, 0.2525 mmol), NaN_3 (0.1642 mg, 0.0025 mmol), and 10 mL of DMF were mixed in a flask under magnetic stirring. The flask was degassed by freeze-pump-thaw for three cycles, refilled with N_2, and the flask was sealed, and placed in an oil bath thermostat, the reaction was carried out at 45 °C for 24 h. After that, the mixture was passed through a column filled with neutral alumina to remove catalyst, the eluent was evaporated and concentrated under reduced pressure at 45 °C to remove the DMF. Then was diluted with 40 mL of CH_2Cl_2 and extracted with a mixture of dichloromethane/saturated sodium solution (4/1, *v*/*v*) twice. The organic layer was dried over anhydrous Na_2SO_4 for 2 h and concentrated by rotary evaporation to remove the excess dichloromethane. The resulting product was precipitated by a mixture of cold hexanes/cold anhydrous diethyl ether (1/1, *v*/*v*) for three times. After purification, the light yellow solid was dried under vacuum at room temperature for 48 h and collected as P(PEGMA$_{-N_3}$-*co*-PEGMEMA)-*b*-PMAA.

1.7 Assembling of P(PEGMA$_{-N_3}$-*co*-PEGMEMA)-*b*-PMAA on the surface of nanoparticles

The dopamine modified Fe_3O_4 nanoparticles (Fe_3O_4-DA) was prepared by referring to previous reports. In brief, $FeCl_3$•$6H_2O$ (1.08 g), NaOAc (4 g) were dissolved in EG (14 mL) and DEG (26 mL) with magnetic stirring, the obtained solution became earthy yellow. Then transferred this solution into three Teflon-lined stainless-steel autoclaves (20 mL) averagely, sealed and heated at 200 °C in oven. After reaction for 15 h, the autoclave was cooled to room temperature. The obtained Fe_3O_4 NPs (nearly 100 nm in diameter) were washed two times with water and ethanol respectively, and then dried under vacuum for 12 h. Afterwards, 20 mg dopamine dissolved in 2 mL water were added into 5 mL THF containing 20 mg Fe_3O_4 NPs. The mixture was sonicated for 1 h and then stirred at room temperature overnight. The product was purified by magnetic separation. The obtained Fe_3O_4-DA was dispersed in 20 mL water. The solution of copolymer P(PEGMA$_{-N_3}$-*co*-PEGMEMA)-*b*-PMAA in THF (1 mg/mL) was slowly added into Fe_3O_4-DA nanoparticles (1 mg/mL) in chloroform during ultrasonication for half an hour. The mixture was treated by ultrasound for another 60 min

and then continuously stirred for 12 h at room temperature. Excess copolymers were removed by magnetic separation and the complex was purified by repeated water washing for 5 times.

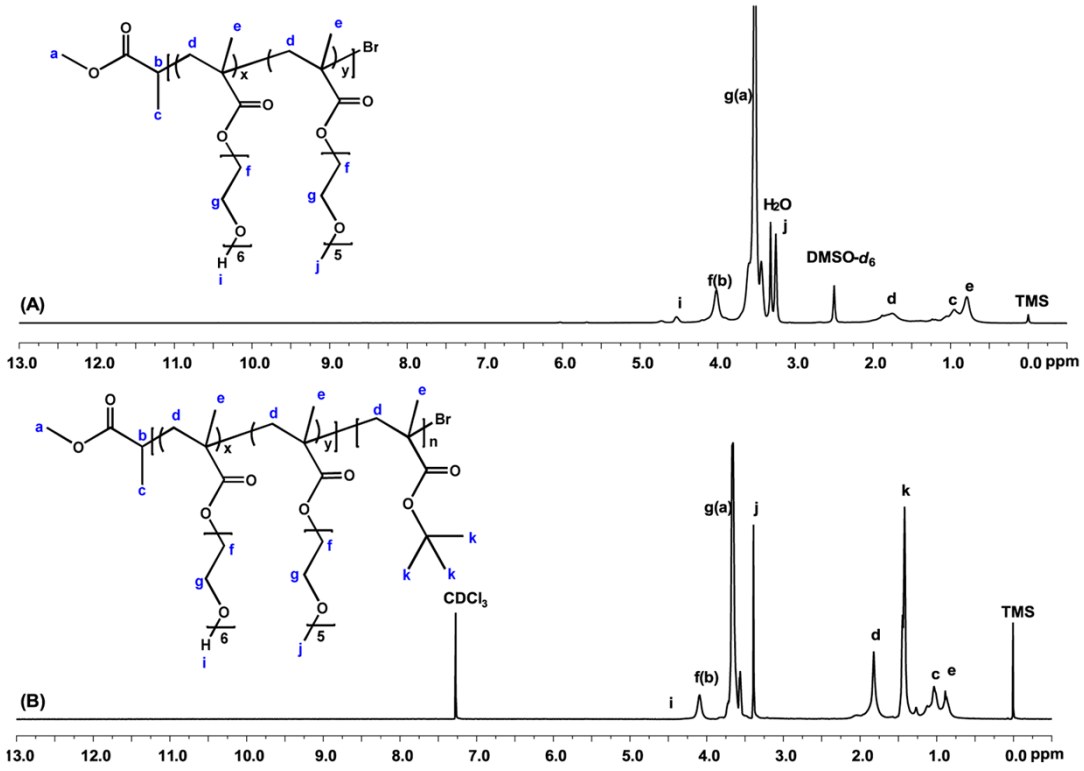

Fig. S1 ^{1}H NMR spectra of copolymer **I** (A) and copolymer **II** (B).

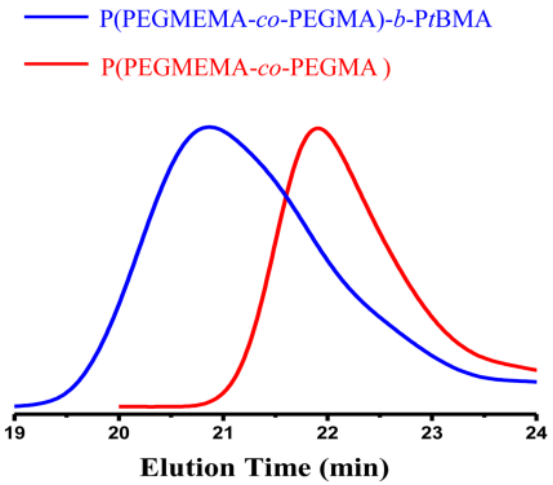

Fig. S2 GPC of copolymer I (M_n=8480 g/mol, PDI = 1.42), and copolymer II ($\overline{M_n}$= 17880 g/mol, PDI = 1.61).