SUPPORTING INFORMATION

Bioconjugation and Fluorescence Labeling of Iron Oxide Nanoparticles Grafted with Bromomaleimide-Terminal Polymers

Ruirui Qiao,[*†] *Lars Esser,*[†] *Changkui Fu,*[‡§] *Cheng Zhang,*[‡§] *Jinming Hu,*[∥] *Paulina Ramírez-García,*[†] *Yuhuan Li,*[†] *John F. Quinn,*[†] *Michael R. Whittaker,*[†] *Andrew K. Whittaker*[‡§] *and Thomas P. Davis**[†⊥]

[†]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville VIC 3052, Australia

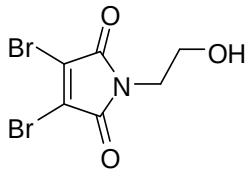
[‡]Australian Institute for Bioengineering and Nanotechnology and [§]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, The University of Queensland, Brisbane, Old 4072, Australia

[∥]CAS Key Laboratory of Soft Matter Chemistry, Department of Polymer Science and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China

[⊥]Department of Chemistry, University of Warwick, Gibbet Hill, Coventry CV4 7AL, United Kingdom

Synthetic protocols

_N_-(2-hydroxyethyl)-3,4-dibromomaleimide (1)



To a solution of 3,4-dibromomaleimide (1.00 g, 3.90 mmol) and *N*-methylmorpholine (0.43 mL, 3.90 mmol) in THF (35 mL), methyl chloroformate (0.30 mL, 3.90 mmol) was added and the mixture was stirred for 20 min at room temperature. Then dichloromethane (40 mL) was added and the organic phase was washed with H_2O, dried over $MgSO_4$ and the solvent was evaporated by vacuum. The intermediate compound *N*-methoxycarbonyl-3,4-dibromomaleimide was obtained as a pink power (1.20 g, 3.80 mmol, yield 99%).

^{1}H NMR (400 MHz, $CDCl_3$)/ppm: 4.00 (3H, s, CH_3).

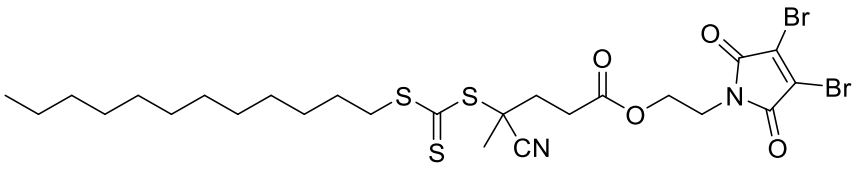
^{13}C NMR (100 MHz, $CDCl_3$)/ppm: 159.3, 147.0, 131.5, 54.9.

Next ethanolamine (0.063 mL, 1.06 mmol) was added to a stirred solution of the obtained *N*-methoxycarbonyl-3,4-dibromomaleimide (300 mg, 0.966 mmol) in dichloromethane (52 mL). After 24 h the reaction mixture was concentrated and the crude residue was purified by FLASH column chromatography (10% to 40% ethyl acetate/petroleum spirit (B.R. 40 – 60 ℃) to yield the target compound as a pale yellow solid (200 mg, 0.67 mmol, yield 66%).

^{1}H NMR (400 MHz, $CDCl_3$)/ppm: 3.80 (4H, s, NCH_2CH_2OH).

^{13}C NMR (100 MHz, $CDCl_3$)/ppm: 164.3, 129.6, 60.2, 42.2.

2-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl 4-cyano-4-(((dodecylthio)carbonothioyl)thio)pentanoate (DBM-CTA) (2)



4-Cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (161 mg, 0.4 mmol), N-(2-hydroxyethyl)-3,4-dibromomaleimide (179 mg, 0.6 mmol), and DMAP (9.76 mg, 0.08 mmol) were dissolved in anhydrous DCM (5 ml). The solution was degassed by sparging with nitrogen for 20 min in an ice bath before a solution of DCC (165 mg, 0.8 mmol) in 1 ml DCM was added dropwise. The solution was then stirred at room temperature overnight and then

was filtered to remove the while solid. The filtrate was washed with saturated $NaHCO_3$ and H_2O, dried over $MgSO_4$. After removal of the solvent by vacuum, the crude was purified by FLASH column chromatography (20% ethyl acetate/petroleum spirit) and the product was obtained after evaporation by vacuum as a yellow solid (167 mg, 0.24 mmol, yield 61%).

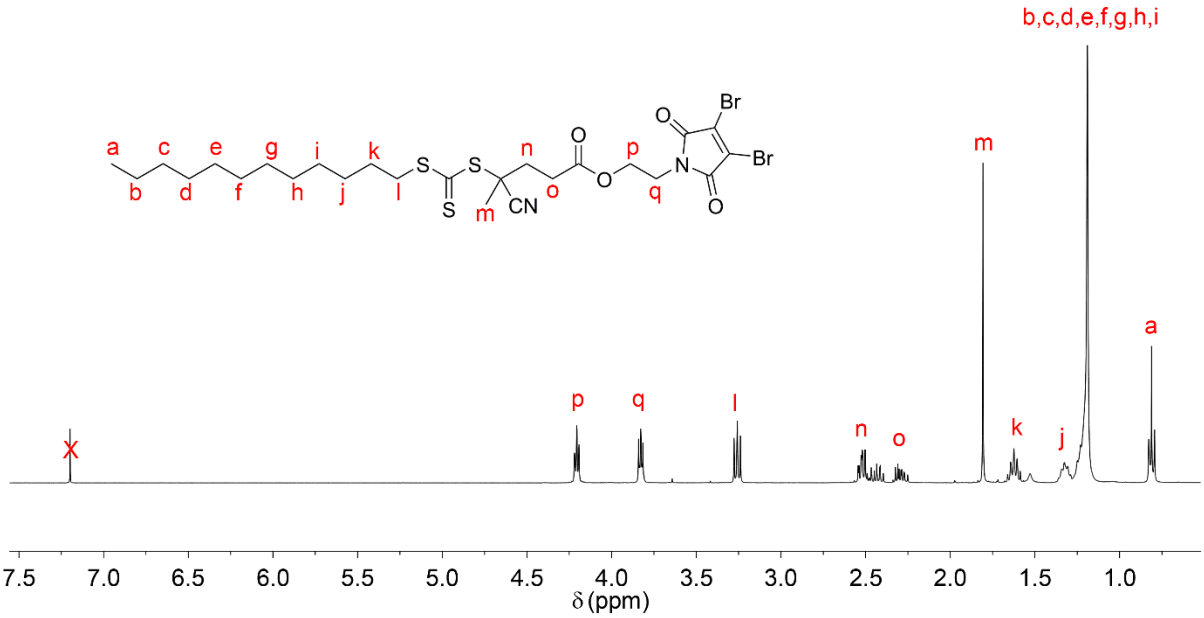
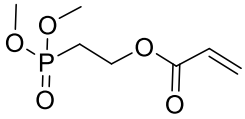


Figure S1. ^{1}HNMR spectrum ($CDCl_3$) of DBM-CTA.

General procedure for RAFT polymerisation for the synthesis of POEGA-DBM

Typically, RAFT chain transfer agent (DBM-CTA, 92 mg, 0.135 mmol), oligo(ethylene glycol) methyl ether acrylate (OEGA, 1.5 mL, 3.4 mmol) and azobisisobutyronitrile (AIBN, 2.2 mg, 0.013 mmol) were dissolved in 1,4-dioxane (2 mL) in a septa-sealed vial. The solution was degassed by sparging with nitrogen for 30 min and the reaction mixture was then stirred for 24 h at 70°C. During the polymerization, samples were taken for ^{1}H NMR spectroscopy and GPC analysis to monitor monomer conversion. After 24 h the reaction was quenched via rapid cooling and exposure to oxygen. Subsequently the reaction mixture was concentrated by partial evaporation of solvent, and the polymer was precipitated three times in a mixture of diethyl ether and petroleum spirit (B.R. 40 - 60°C) (1 : 1, v/v) to remove the small molecules. Finally the purified polymer POEGA-DBM was dried at 30°C under vacuum overnight to give 0.6 g of a yellow viscous liquid.

2-(dimethoxyphosphoryl)ethyl acrylate (PA)



As a general procedure, dimethyl (2-hydroxyethyl)phosphonate (1.54 g, 10 mmol), triethylamine (1.7 mL, 23 mmol), anhydrous DCM (4.76 mL) were introduced under an inert atmosphere in a two-neck round-bottom flask equipped with a condenser. Then acrylic anhydride (1 mL, 12 mmol) was added in a dropwise manner at 0°C. The mixture was vigorously stirred at 0°C for 1 h and then at ambient temperature for 12 h. Afterwards, the reaction mixture was washed with a saturated $NaHCO_3$ (3 times), and finally dried over $MgSO_4$. The crude residue was purified by FLASH column chromatography (5% Methanol/DCM) and the product was obtained after evaporation of the solvents by vacuum as a colourless liquid (solid (2.0 g, 9.6 mmol, yield 96%).

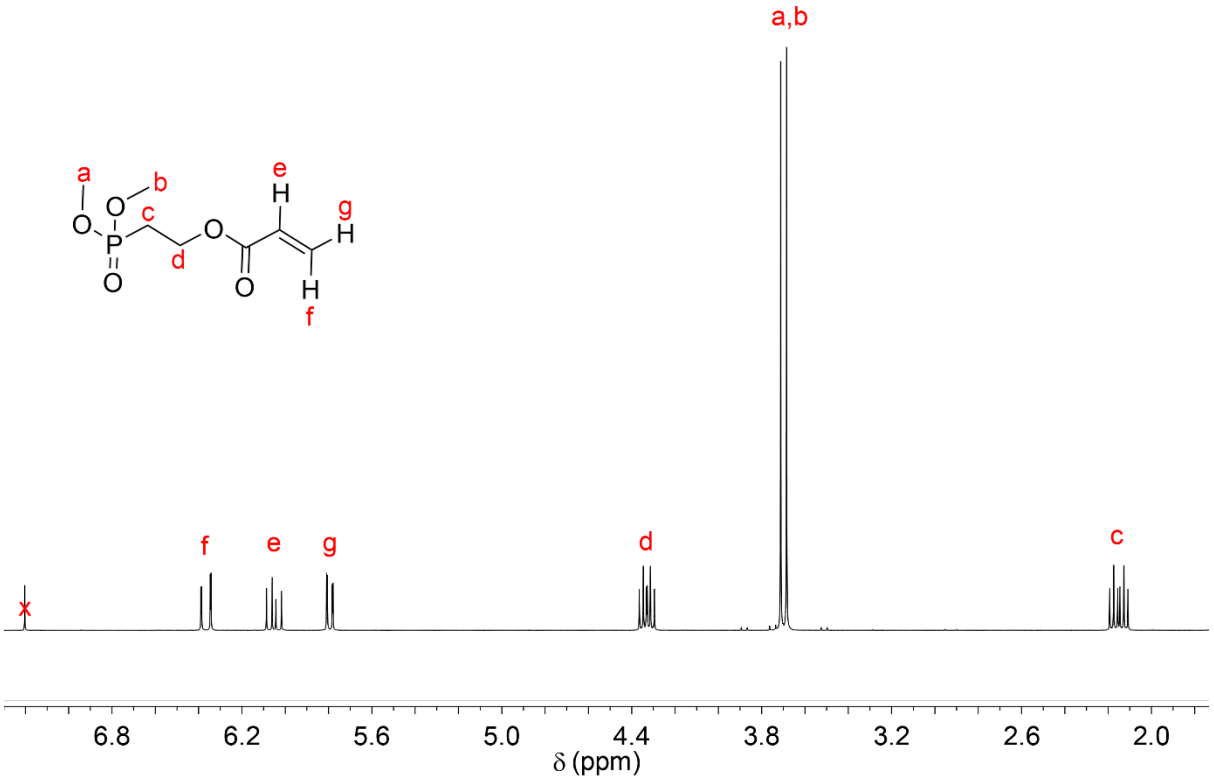


Figure S2. ^{1}HNMR spectrum ($CDCl_3$) of PA.

General procedure for chain extension of macroRAFT agent POEGA- DBM

The Macro-RAFT agent (POEGA-DBM, 458 mg, 0.05 mmol), 2-(dimethoxyphosphoryl)ethyl acrylate (105 mg, 0.5 mmol), and azobisisobutyronitrile (AIBN, 0.82 mg, 0.005 mmol) were dissolved in 1,4-dioxane (2 mL) in a septa-sealed vial. The solution was degassed by sparging with nitrogen for 30 min and the reaction mixture was then stirred at 70°C. During the

polymerization, samples were taken for ^{1}H NMR spectroscopy and GPC analysis to monitor monomer conversion. The reaction was quenched after 24 h via rapid cooling and exposure to oxygen. Subsequently the reaction mixture was concentrated by partial evaporation of solvent, and the polymer was precipitated three times in a mixture of diethyl ether and petroleum spirit (B.R. 40 - 60°C) (1 : 1 v/v) to remove the small molecules. Finally the purified polymer POEGA-*b*-PA-DBM was dried at 30°C under vacuum overnight to give 0.4 g of a yellow viscous solution. The final product was analysed by ^{1}H NMR, ^{31}P NMR (**Figure 1**) and GPC (**Figure S3**).

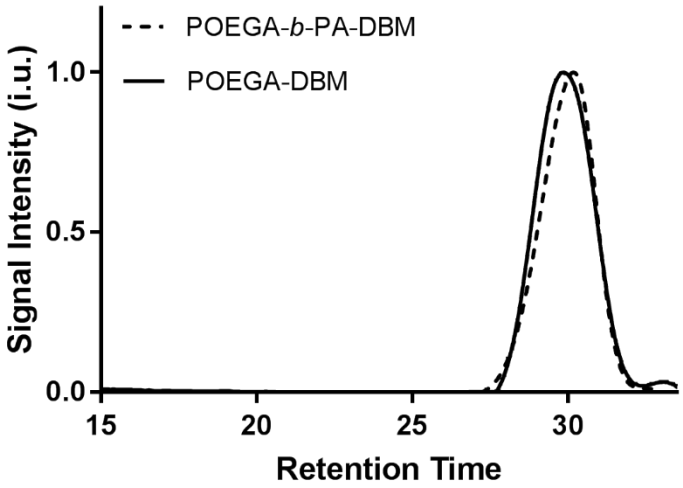


Figure S3. Retention time (as measured by GPC) of POEGA-DBM and POEGA-*b*-PA-DBM polymers.

Deprotection of the POEGA-*b*-PA-DBM

The deprotection of the phosphonate group was carried out as follows: 0.5 g (0.05 mmol) of POEGA-*b*-PA-DBM was dissolved in 3 mL of anhydrous DCM in a glass vial equipped with a magnetic stirrer bar, and sealed with a septa. The solution was stirred at 0°C under nitrogen flow and a solution of trimethylsilyl bromide (TMSBr, 0.1725 g, 147.5 µL, 1 mmol) in 2 mL of DCM was added dropwise. The reaction mixture was stirred for 24 h at room temperature. At the end of the reaction, the large excess of bromosilane and DCM were removed by evaporation under low pressure. After total elimination of bromosilane, methanol (5 mL) was added to the flask and the stirring was continued for 24 h at room temperature. Then, the final POEGA-*b*-PPA-DBM was precipitated into a large quantity of petroleum ether (B.R. 40–60°C) and washed with a 1: 1 (v : v) mixture of petroleum ether and diethyl ether. The

remaining solvent was removed in a vacuum oven overnight at 35°C and the final product was analysed by ^{1}H NMR (**Figure S4**).

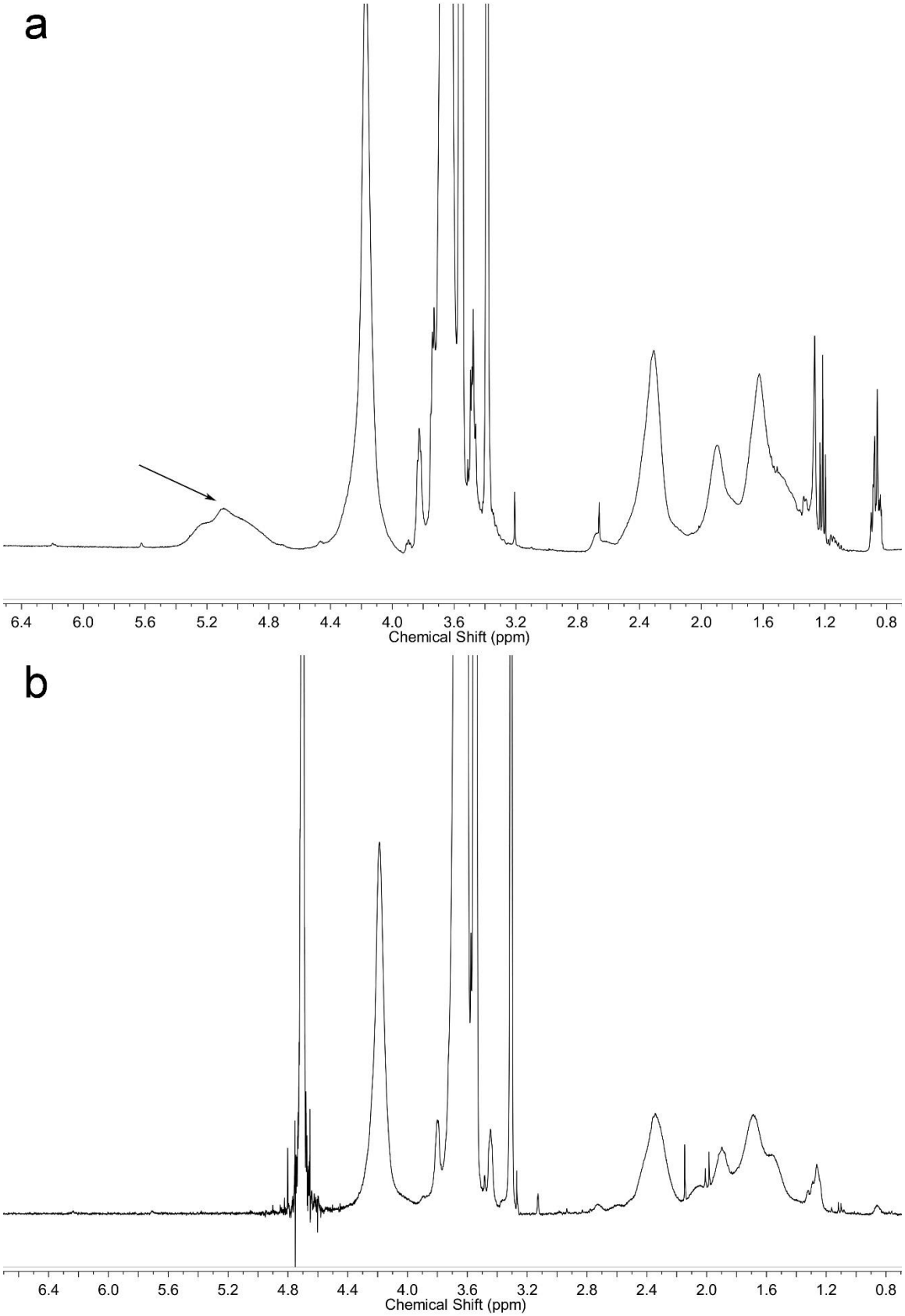


Figure S4. ^{1}NMR spectra of POEGA-*b*-PPA-DBM polymers in $CDCl_3$ (a) and D_2O (b).

Characterization of oleic acid coated iron oxide nanoparticles

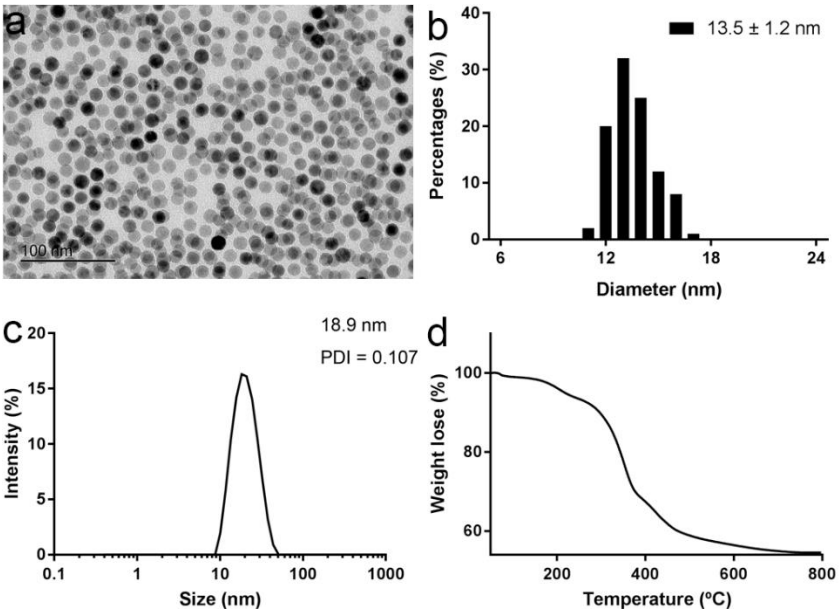


Figure S5. (a) TEM photo and (b) size histogram of oleic acid coated IONPs; (c) Hydrodynamic size and (d) TGA analysis of the particles.

General procedure of grafting of polymer on iron oxide nanoparticles

10 mg of the purified iron oxide particles capped with oleic acid and 100 mg of deprotected POEGA-*b*-PPA-DBM were dissolved in 5 mL of THF. The ligand exchange reaction took place overnight at 40°C. Then, the resulting PEGylated particles were precipitated by cyclohexane, washed with cyclohexane three times, and finally dried under vacuum at room temperature. The resultant particles was then dissolved in water and purified by ultrafiltration with an Amicon Ultra centrifugal filter (100 kD). The TGA analysis were performed by using freeze-drying powders and the result is shown in **Figure S6**.

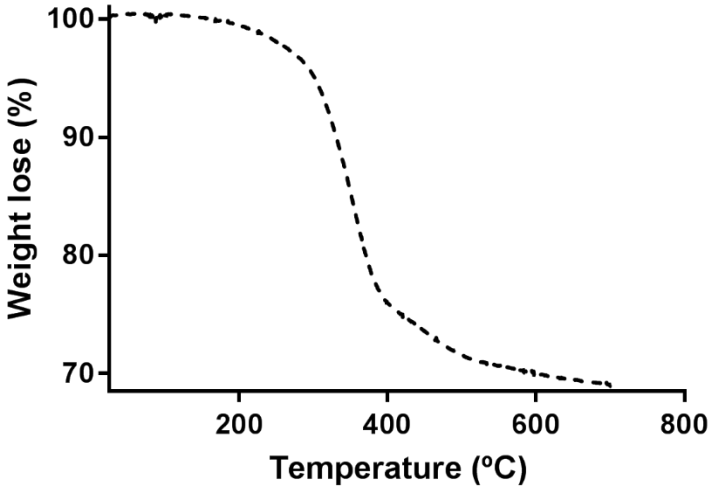


Figure S6. TGA analysis of POEGA-*b*-PPA-DBM grafted iron oxide particles

General procedure of conjugation of GRP78-binding peptide to dibromomalimide-terminated polymers

GRP78-binding peptide (CRLLDTNRPFLPY) (1.6 mg, 0.01 mmol) was added to a solution of DBM-terminated polymers (0.01 mmol, 10 mg/mL) in aqueous media with 2.5 eq. of sodium carbonate. The mixture was stirred at room temperature for 2 h and followed by dialysis in H_2O. The resulting conjugates were transferred into 1 × PBS buffer and stored at 4°C for further use.

General procedure of conjugation of dibromomalimide-terminated IONPs and amino-compound

NH_2-Cy5.5 (0.235 mg, 0.3×10^{-3} mmol) was added to IONPs (1 mg, 0.3×10^{-6} mmol) in aqueous media with 2500 eq. of sodium carbonate. The mixture was stirred at room temperature for 2 h and followed by a purification using 30 kD MWCO centrifugal devices (Amicon Ultra-0.5). The formation of the conjugates was characterised by a Shimadzu UV-3600 UV–vis-NIR spectrophotometer and Shimadzu RF-5301 pc fluorescence spectrophotometer (**Figure S7**). Similar procedure was used in conjugation between DBM-polymer coated IONPs and the GRP78-binding peptide.

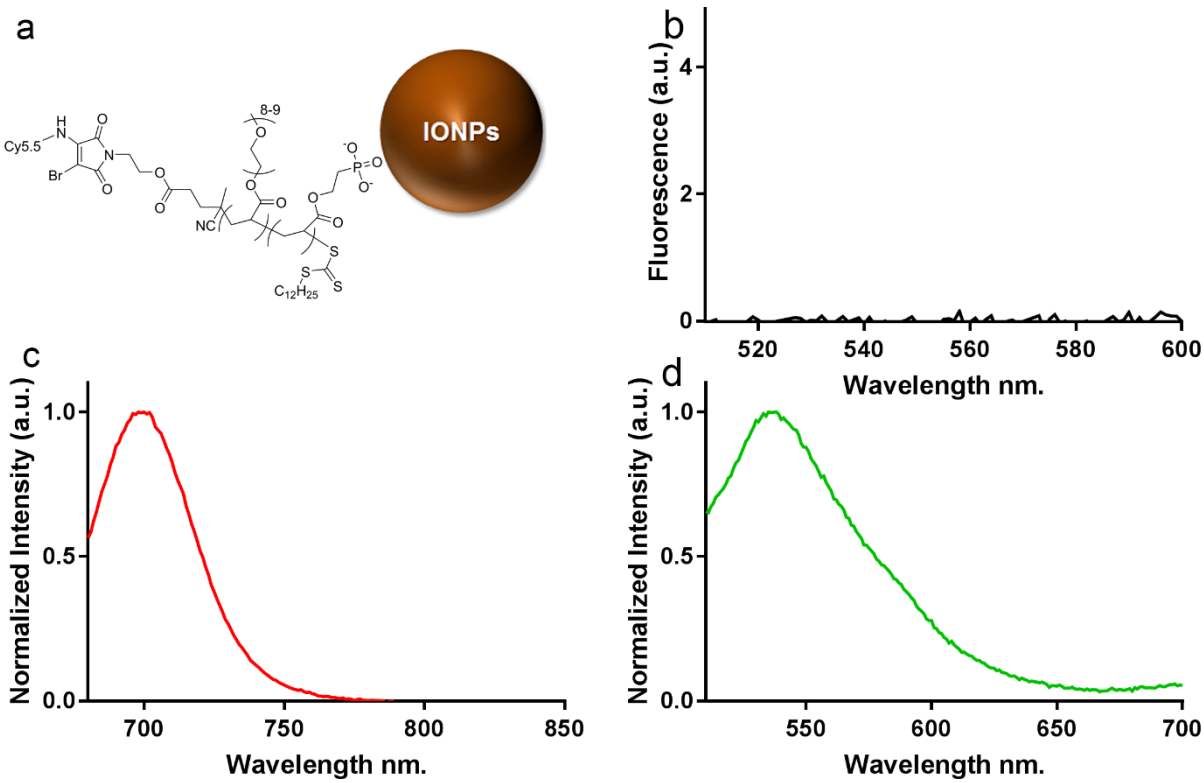


Figure S7. (a) Scheme of IONPs-Cy5.5 conjugates; (b) Fluorescence spectra of IONPs and IONPs-Cy5.5 excited at 684 nm (c) and 400 nm (d).

Cytotoxicity of POEGA-*b*-PPA-DBM

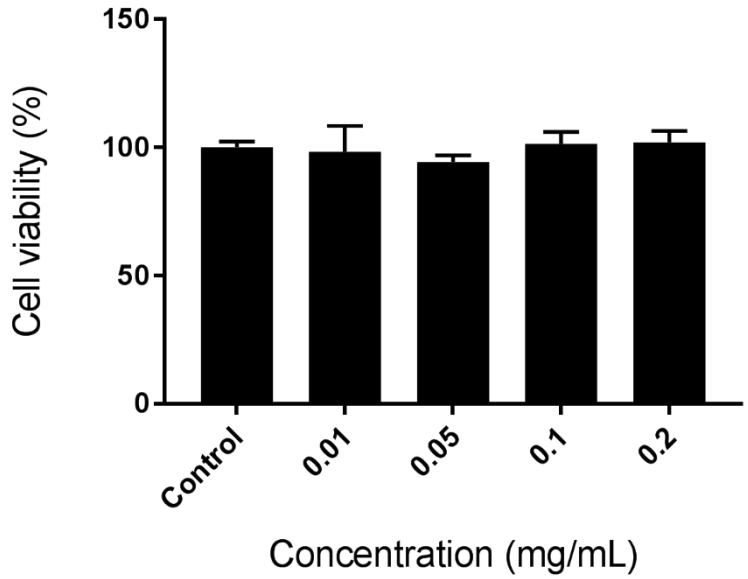


Figure S8. Cytotoxicity of POEGA-*b*-PPA-DBM polymer