Supporting Information

Synthesis of well-defined catechol polymers for surface functionalization of magnetic nanoparticles

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**Materials**

*N*-Isopropylacrylamide (NIPAM, 97%) was obtained from Sigma-Aldrich and was recrystallized from hexane to remove the inhibitor. Poly(ethylene glycol) methyl ether acrylate (PEGA$_{480}$, average $M_n$ 480, Sigma-Aldrich), 2-Hydroxyethyl acrylate (HEA, 96%, Sigma-Aldrich) and acrylamide (AM, $\geq$ 99%, Sigma-Aldrich) were used directly without removing the inhibitors prior to use. Rhodamine B piperazine and Rhodamine B piperazine acrylamide (Rhod-B AM) were synthesized for fluorescent dye monomer according to literature procedure$^{1,2}$. Mannose acrylate monomer was synthesized by a combination of Fischer glycosylation and azide-alkyne click reaction.$^{3,4}$ *Tris*(2-(dimethylamino)ethyl)amine (Me$_6$TREN) was synthesized according to literature procedure and stored under a nitrogen atmosphere prior to use.$^{5,6}$ Copper(I) bromide (CuBr, 98%, Sigma-Aldrich) was washed sequentially with acetic acid and ethanol and dried under vacuum. Iron (III) chloride hexahydrate (FeCl$_3$$\cdot$6H$_2$O, $\geq$ 99%) was purchased from Acros. Iron (II) chloride tetrahydrate (FeCl$_2$$\cdot$4H$_2$O, $\geq$99%) was purchased from Fluka. Ammonium hydroxide (NH$_4$OH, 35 vol%) and nitric acid (HNO$_3$, 1M) were purchased from Fisher Chemicals. Oleic acid (90%) were purchased from Sigma-Aldrich. HPLC grade water (H$_2$O, VWR international, LLC) was directly used as the solvent for polymerizations unless otherwise stated.

Membrane dialysis (1K MWCO) was obtained from Spectrum Laboratories. All other reagents and solvents were obtained at the highest purity available from Sigma-Aldrich and used without further purification unless otherwise stated.

**Instruments and analysis**

$^1$H and $^{13}$C NMR spectra were recorded on Bruker DPX-300, DPX-400 spectrometers using deuterated solvents obtained from Aldrich.

SEC utilizing organic solvent was conducted on Varian 390-LC system in DMF (5 mM NH$_4$BF$_4$) at 50 °C, equipped with refractive index, UV and viscometry detectors, 2 x PLgel 5 mm mixed-D columns (300 x 7.5 mm), 1 x PLgel 5 mm guard column (50 x 7.5 mm) and autosampler. Narrow linear PMMA standards in range of 200 to 1.0 x 10$^6$ g·mol$^{-1}$ were used to calibrate the system. All samples were passed through 0.45 μm PTFE filter before analysis.
Aqueous SEC was conducted on PL-GPC50 system with 0.3 M NaSO₄⁺ 0.1% NaN₃ aqueous solution as the eluent at 30 °C, equipped with refractive index detector, 2 × PL Aquagel-OH columns (300 × 8 mm), 1 × Aquagel-OH guard column (50 × 8 mm) and autosampler. Narrow linear PEO-PEG standards in range of 10⁶ to 1.3 × 10⁵ g·mol⁻¹ were used to calibrate the system. All samples were passed through 0.45 μm nylon filter before analysis.

Infrared absorption spectra were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell.

Iron oxide particle sizes and size distributions were measured by dynamic light scattering (DLS) using a Zetasizer Nano Series (Malvern Instruments Ltd.). Transition electron microscopy (TEM) analysis was performed employing a JEOL 2000FX TEM microscope with a Gatan Orius™ 11 megapixel digital camera. Samples were collected on 200-mesh Cu grids and stained with lead citrate prior to analysis. The mean diameter of the iron oxide particles, coefficient of variation and normal distribution values were calculated by analysing TEM images in ImageJ® software.

All reactions were carried out using standard Schlenk techniques under an inert atmosphere of oxygen-free nitrogen, unless otherwise stated.
Synthesis of dopamine-based initiator 2-bromo-N-(3,4-dihydroxyphenethyl)-2-methylpropanamide (DOPA-Br).

Dopamine hydrochloride (10 g, 52.7 mmol) was added to a 250 mL round-bottom three neck flask equipped with two dropping funnels and degassed with nitrogen for 30 minutes. Anhydrous MeOH (100 mL) and triethylamine (TEA, 7.35 mL, 1 eq.) were added to the flask under nitrogen protection. α-Bromoisobutyryl bromide (7.82 mL, 1.2 eq.) and anhydrous THF (5 mL) were added to the first dropping funnel. The second dropping funnel was filled with TEA (11 mL, 1.5 eq) and anhydrous MeOH (11 mL). The flask was cooled with ice bath and the content of both dropping funnels were added alternatively to keep a pH=9. After addition, the solution was allowed to stir at room temperature for 1 hour. After complete reaction, the volatiles were removed by reduced pressure and the oil residue was re-dissolved in ethyl acetate (100 mL). The organic layer was washed sequentially with 1M HCl (75 mL), saturated NaHCO$_3$ (50 mL) and brine (75 mL) and dried over Na$_2$SO$_4$. After filtration, the volatiles were removed under reduced pressure and re-dissolved in ethyl acetate prior to purification by flash column chromatography (MeOH: DCM = 5: 95, 15 mL.min$^{-1}$). The corresponding fraction were collected and dried via rotary evaporation to yield the product 2-bromo-N-(3,4-dihydroxyphenethyl)-2-methylpropanamide (DOPA-Br) as light pink oil (5.41 g, 34 % yield).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ = 6.87 (s, 1H, -NH), 6.51-5.93 (s, 2H, -OH), 6.82 (d, 1H, $J$=8.03 Hz, HOC=CH=CH$\equiv$), 6.74 (d, 1H, $J$=1.51 Hz, HOC=CH=C-R), 6.62 (dd, 1H, $J$=8.03 Hz, $J_2$=0.94 Hz, HOC=CH=C-H$\equiv$), 3.49 (t, 2H, $J$=6.27 Hz, -CH$_2$-CH$_2$-N-R), 2.72 (t, 2H, $J$=7.03 Hz, -CH$_2$-CH$_2$-N-R$^-$) and 1.91 (s, 6H, -(CH$_3$)$_2$) ppm.

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ = 172.6, 144.0, 142.7, 130.7, 120.9, 115.6, 115.3, 62.82, 41.8, 34.7, 32.5 ppm.

HRMS $m/z$: calcd for C$_{12}$H$_{16}$BrNO$_3$ (M+Na$^+$), 324.0; found, 324.1.
Figure S1. $^1$H NMR spectrum of DOPA-Br in CDCl$_3$.

Figure S2. $^{13}$C NMR spectrum of DOPA-Br in CDCl$_3$. 
Synthesis of Rhodamine B piperazine acrylamide (Rhod AM).

**Scheme S1.** Synthesis of Rhodamine B piperazine acrylamide.

The Rhodamine B piperazine acrylamide monomer was synthesized according to a modification of previous procedure.$^2$

A solution of Rhodamine B piperazine amide (2.55 g, 5 mmol), TEA (2.1 mL, 15 mmol) and anhydrous dichloromethane (100 mL) was cooled in an ice-water bath. Acryloyl chloride (1.2 mL, 15 mmol) in 15 mL DCM was then added dropwise into the solution over a period of ca. 30 min. The mixture was stirred in the ice bath for 1 h and then at ambient temperature overnight. The volatiles were removed *via* rotary evaporation and the residue product was purified *via* flash silica gel column chromatography (DCM: MeOH = 8: 1, volume ratio). Final product was obtained as purple glassy solid after removing the solvent on rotary evaporator and dried under slightly vacuum (1.2 g, 43%).

$^1$H NMR (D$_2$O, 298 K, 400 MHz): $\delta = 7.73$ (m, 2 H), 7.51 (t, 2 H, $J=7.3$ Hz), 7.02 (d, 2 H, $J=9.4$ Hz), 6.76 (dd, 2H, $J=1.7, 7.9$ Hz), 6.59 (s, 2 H), 6.41 (m, 1 H), 5.7-6.3 (overlapped, 2 H), 5.64 (d, 1 H, $J=11.6$ Hz), 2.7-3.7 (m, overlapped with MeOH, 12 H), 1.08 (t, 12 H, $J=6.9$ Hz) ppm.

$^{13}$C NMR (D$_2$O, 298 K, 100 MHz): $\delta = 11.9, 41.1, 41.3, 41.6, 41.8, 44.7, 45.0, 45.8, 46.5, 46.8, 96.2, 114.0, 126.8, 127.8, 129.1, 129.8, 130.0, 130.1, 130.5, 130.6, 131.3, 134.0, 134.1, 153.4, 153.6, 155.4, 157.0, 167.9, 169.7 ppm.

ESI-MS $m/z$: C$_{35}$H$_{41}$N$_4$O$_3$ calcd for (M-Cl): 565.3; found: 565.2.
Preparation of iron oxide particles.

Iron oxide (Fe$_3$O$_4$) nanoparticles coated with a surfactant were prepared by the method previously reported by Wooding et al.,$^7$ and further developed by Shen et al.,$^8$ and Maity et al.,$^9$ which is based on the precipitation of the Fe (II) and Fe (III) salts by NH$_4$OH in aqueous medium and consequent coating with oleic acid.

FeCl$_3$•6H$_2$O (2.35 g) and FeCl$_2$•4H$_2$O (0.86 g) were dissolved in 40 mL of deionized water and after homogenization degassed with N$_2$ for 30 min. The solution was heated to 90 °C and vigorously stirred for 1h. Subsequently NH$_4$OH (5 mL) was added to the heated solution followed by oleic acid (1 mL). The mixture was stirred for another 30 min. After cooling to the room temperature magnetite nanoparticles were flocculated by addition of acetone (200 mL) and isolated by magnetic decantation. The precipitate was washed ten times with ethanol/acetone mixture (1:1 vol. ratio) to remove an excess of oleic acid and dried at 60 °C for 8h. The obtained magnetic nanoparticles were dispersed in in water for further modification.

Synthesis of poly(AM) and poly(AM)-b-(HEA) via aqueous SET-LRP at 0 °C.

Typical polymerization procedure was shown as follow. To a Schlenk tube fitted with a magnetic stir bar and a rubber septum, H$_2$O (2 mL) and Me$_6$TREN (9.1 μL, 0.035 mmol) were charged and the mixture was bubbled with nitrogen for 10 min. CuBr (10 mg, 0.07 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for another 10 min and then the blue suspension with purple red colour copper (0) powder was allowed to stir under ice/water bath cool (0 °C) and nitrogen protection for 15 min. At the same time, to another vial fitted with a magnetic stir bar and a rubber septum, H$_2$O (3.5 mL), 2, 3-dihydroxypropyl 2-bromo-2-methylpropanoate (21 mg, 0.087 mmol) and AM (0.50 g, 7 mmol) were
charged and the mixture was bubbled with nitrogen for 15 min. After that, the
degassed monomer / initiator aqueous solution was transferred via cannula to the
Schlenk tube with Cu (0) / CuBr2 / Me6TREN catalyst. The Schlenk tube was sealed
and the mixed solution was allowed to polymerize under ice/water bath cool (0 °C)
for 0.5 h. Sample of the reaction mixture was then removed for analysis. The sample
for 1H NMR was directly diluted with D2O, which confirmed the conversion was 97%
by comparing the integral ratio of vinyl residues with relative polymer backbones.
Catalyst residues were removed by filtering through a column of neutral alumina prior
to aqueous SEC analysis.

For the polymerization of AM at other DP or with different catalyst ratios, same
amount initiator was utilized and the amounts of catalyst, monomer and solvent were
adjusted accordingly.

**Scheme S3.** Synthesis of poly(AM)-b-(HEA) via aqueous SET-LRP at 0 °C.

For the synthesis of poly(AM)-b-(HEA), the polymerization procedure is the same as
previously described. After polymerization of AM for 45 min, sampling for 1H NMR
analysis showed that the conversion reached 87%, a degassed solution of HEA (1.63g,
14 mmol) in 5 mL water was directly added to the reaction tube for block
copolymerization under ice/water bath cool. After reaction for 3 h, 1H NMR analysis
showed that the conversion reached 69% and the polymerization was then stopped.
The polymer product was purified via dialysis against water for two days and
recovered by freeze drying.
Figure S3. Molecular weight distributions of poly(AM) by aqueous SET-LRP at 0 °C characterized via aqueous SEC.

Figure S4. Molecular weight distributions of poly(AM)-b-(HEA) by aqueous SET-LRP at 0 °C characterized via aqueous SEC.
Figure S5. ¹H NMR spectrum of poly(AM)-b-(HEA).

Synthesis of dopamine-terminal poly(AM) via aqueous SET-LRP at 0 °C.

Disproportionation

SET-LRP

Scheme S4. Synthesis of dopamine-terminal poly(AM) via aqueous SET-LRP at 0 °C.
To a Schlenk tube fitted with a magnetic stir bar and a rubber septum, H$_2$O (2 mL) and Me$_6$TREN (26 μL, 0.1 mmol) were charged and the mixture was bubbled with nitrogen for 5 min. CuBr (14 mg, 0.1 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for a further 10 min and subsequently the blue suspension with purple red colour copper (0) powder was allowed to stir under ice/water bath cool for 15 min. At the same time, to another vial fitted with a magnetic stir bar and a rubber septum, H$_2$O (2 mL), i-PrOH (2 mL), DOPA-Br (75 mg, 0.25 mmol) and AM (1421 mg, 20 mmol) were charged and the mixture was bubbled with nitrogen for 15 min. After that, the degassed monomer/initiator aqueous solution was transferred via cannula to the Schlenk tube with Cu(0)/CuBr$_2$/Me$_6$TREN catalyst. The Schlenk tube was sealed and the mixed solution was allowed to polymerize at 0 °C for 1 h. Sample of the reaction mixture was then removed for analysis. The sample for $^1$H NMR was directly diluted with D$_2$O, which confirmed a conversion of >99% by comparing the integral of vinyl groups at 6.0-6.8 ppm with that of the polymer backbones at 1.2-2.5 ppm. Catalyst residues were removed by filtering through a column of neutral alumina prior to aqueous SEC analysis. The rest polymer product was purified via dialysis against water for two days and recovered by freeze drying.

For the polymerization under the ratio of [initiator]: [CuBr]: [Me$_6$TREN] = 1: 0.8: 0.4, the amount of CuBr was increased to 28 mg with same amounts of other reagents and reaction procedure. After 1 h, $^1$H NMR analysis revealed that reaction conversion is only 18% and aqueous SEC did not reveal a significant polymer peak due to the low MW of obtained polymer.
Figure S6. $^1$H NMR spectrum of dopamine-terminal poly(AM) in D$_2$O.

Figure S7. $^{13}$C NMR spectrum of dopamine functional poly(AM) in D$_2$O.
Synthesis of dopamine-terminal poly(NIPAM) via aqueous SET-LRP at 0 °C.

Scheme S5. Synthesis of dopamine functional poly(NIPAM) via aqueous SET-LRP at 0 °C.

To a vial fitted with a magnetic stir bar and a rubber septum, H₂O (2 mL) and Me₆TREN (26 μL, 0.1 mmol) were charged and the mixture was bubbled with nitrogen for 5 min. CuBr (14 mg, 0.1 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for a further 10 min and subsequently the blue suspension with purple red colour copper (0) powder was allowed to stir under ice/water bath cool for 15 min. At the same time, to another vial fitted with a magnetic stir bar and a rubber septum, H₂O (2 mL), i-PrOH (2 mL), DOPA-Br (75 mg, 0.25 mmol) and NIPAM (1140 mg, 10 mmol) were charged and the mixture was bubbled with nitrogen for 15 min. After that, the degassed monomer/initiator aqueous solution was transferred via cannula to the Schlenk tube with Cu(0)/CuBr₂/Me₆TREN catalyst.

After cannula transfer, the colour of the solution changed immediately from light blue to deep blue. After polymerization for 30 min, massive polymer precipitate was found at the bottom of the vial with mixed with red copper powders and the magnetic stir bar even cannot stir anymore. The polymerization was then stopped and sample of the reaction mixture was treated with sonication for 2 min before being removed for analysis. The sample for ¹H NMR was directly diluted with D₂O with sonication for 1 min to make sure the polymer totally soluble, which confirmed a conversion of 95% by comparing the integral of vinyl protons with that of isopropyl protons. Catalyst residues were removed by filtering through a column of neutral alumina prior to DMF SEC analysis.
The rest polymer solution was diluted with 30 mL i-PrOH and then filtered to remove the excess copper powders before dialysis against water for two days and recovered as white powder by freeze drying.

(a) (b) (c)

Figure S8. Visual observation of the poly(NIPAM) precipitate during polymerization. Conditions: a) after reaction for 30 min; b) zoom of the bottom mixture of polymer and copper of (a); c) after treated with sonication for 2 min.

Figure S9. $^1$H NMR spectrum of dopamine-terminal poly(NIPAM) in D$_2$O.
Figure S10. $^{13}$C NMR spectrum of dopamine-terminal poly(NIPAM) in D$_2$O.

Synthesis of dopamine-terminal poly(PEGA$_{480}$) via aqueous SET-LRP at 0 °C.

Scheme S6. SET-LRP of PEGA$_{480}$ in i-PrOH/H$_2$O at 0 °C via disproportionation of CuBr / Me$_6$TREN.

To a Schlenk tube fitted with a magnetic stir bar and a rubber septum, H$_2$O (2 mL) and Me$_6$TREN (26 μL, 0.1 mmol) were charged and the mixture was bubbled with nitrogen for 5 min. CuBr (14 mg, 0.1 mmol) was then carefully added under slight positive pressure of nitrogen. The nitrogen bubbling was continued for a further 10 min and subsequently the blue suspension with purple red colour copper (0) powder was allowed to stir under ice/water bath cool for 15 min. At the same time, to another vial fitted with a magnetic stir bar and a rubber septum, H$_2$O (2 mL), i-PrOH (2 mL), DOPA-Br (75 mg, 0.25 mmol) and PEGA$_{480}$ (2400 mg, 5 mmol) were charged and
the mixture was bubbled with nitrogen for 15 min. After that, the degassed monomer/initiator aqueous solution was transferred via cannula to the Schlenk tube with Cu(0)/CuBr$_2$/Me$_6$TREN catalyst. The Schlenk tube was sealed and the mixed solution was allowed to polymerize at 0 °C for 1 h. Sample of the reaction mixture was then removed for analysis. The sample for $^1$H NMR was directly diluted with D$_2$O, which confirmed a conversion of >99% by comparing the integral of vinyl groups at 6.0-6.8 ppm with that of the polymer backbones at 1.2-2.5 ppm. Catalyst residues were removed by filtering through a column of neutral alumina prior to DMF SEC analysis. The rest polymer product was purified via dialysis against water for two days and recovered by freeze drying.

**Figure S11.** $^1$H NMR spectrum of dopamine-terminal poly(PEGA$_{480}$) in D$_2$O.
Synthesis of dopamine-terminal poly(HEA) via aqueous SET-LRP at 0 °C.

Scheme S7. SET-LRP of HEA in i-PrOH/H$_2$O at 0 °C via disproportionation of CuBr / Me$_6$TREN.

The polymerization procedure is the same as previously described for the polymerization of PEGA$_{480}$. The charging amount of each component is listed as below.

Table S1. Charging amounts for SET-LRP of HEA in i-PrOH/H$_2$O with [CuBr]: [Me$_6$TREN] = 1: 1.

| Initiator / monomer system | Catalyst system |
|---------------------------|-----------------|
| Initiator     | HEA  | i-PrOH/H$_2$O | CuBr    | Me$_6$TREN | H$_2$O |
| 0.25 mmol    | 10 mmol | 2 / 2mL | 0.1 mmol | 0.1 mmol | 2 mL |
Figure S13. $^1$H NMR spectrum of dopamine-terminal poly(HEA) in D$_2$O.

Figure S14. $^{13}$C NMR spectrum of dopamine-terminal poly(HEA) in D$_2$O.
Synthesis of dopamine-terminal poly(ManA) via aqueous SET-LRP at 0 °C.

Scheme S8. SET-LRP of ManA in i-PrOH/H$_2$O at 0 °C via disproportionation of CuBr / Me$_6$TREN.

Table S2. Charging amounts for SET-LRP of MannoseA in i-PrOH/H$_2$O with [CuBr]: [Me$_6$TREN] = 1: 1.

| Initiator / monomer system | Catalyst system |
|----------------------------|-----------------|
| Initiator | MannoseA | i-PrOH/H$_2$O | CuBr | Me$_6$TREN | H$_2$O |
| 0.05 mmol | 0.54 mmol | 1 / 1mL | 0.02 mmol | 0.02 mmol | 1 mL |

Figure S15. $^1$H NMR spectrum of dopamine-terminal poly(ManA) in D$_2$O.
Synthesis of dopamine-terminal poly(ManA)-b-(Rhod AM) via aqueous SET-LRP at 0 °C.

Scheme 9. Synthesis of dopamine-terminal poly(ManA)-b-(Rhodamine B AM) copolymer by aqueous SET-LRP at 0 °C.

The polymerization procedure is the same as previously described. A degassed solution of Rhod AM / TEA (1: 1 mole ratio) in water was directly to the reaction tube for block copolymerization. The mole ratio of [Rhod AM]: [ManA] = 1: 100.
Figure 17. $^1$H NMR spectra of samples during the block copolymerization of ManA and Rhod AM in D$_2$O.

**Synthesis of dopamine-terminal poly(HEA)-b-(Rhod AM) via aqueous SET-LRP at 0 °C.**

Scheme S10. Synthesis of dopamine-terminal poly(HEA)-b-(Rhod AM) copolymer by aqueous SET-LRP at 0 °C.

The polymerization procedure is the same as previously described. A degassed solution of Rhod AM / TEA (1: 1 mole ratio) in water was directly to the reaction tube for block copolymerization. The mole ratio of [Rhod AM] : [HEA] = 1: 100.
Figure S18. $^1$H NMR spectra of samples during the block copolymerization of HEA and Rhod AM in D$_2$O.

Figure S19. Molecular weight distributions of dopamine-terminal poly(HEA) and poly(HEA)-b-(Rhod AM) by aqueous SET-LRP as measured by DMF SEC.
Figure S20. $^1$H NMR spectrum of poly(HEA)-b-(Rhod AM).

Synthesis of dopamine-terminal poly(PEGA$_{480}$)-b-(Rhod AM) via aqueous SET-LRP at 0 °C.

Scheme 11. Synthesis of dopamine-terminal poly(PEGA$_{480}$)-b-(Rhodamine B AM) copolymer by aqueous SET-LRP at 0 °C.

The polymerization procedure is the same as previously described. A degassed solution of Rhod AM / TEA (1: 1 mole ratio) in water was directly to the reaction tube for block copolymerization. The mole ratio of [Rhod AM] : [PEGA$_{480}$] = 1: 100.
Figure 21. $^1$H NMR spectra of samples during the block copolymerization of PEGA$_{480}$ and Rhod AM in D$_2$O.

Figure 22. Molecular weight distributions of dopamine-terminal poly(PEGA$_{480}$) and poly(PEGA$_{480}$)-b-(Rhod AM) by aqueous SET-LRP as measured by DMF SEC.
Modification of iron oxide nanoparticles with dopamine-terminal fluorescent labelled hydrophilic copolymers

Scheme S12. Surface modification of iron oxide nanoparticles with dopamine-terminal fluorescent labelled hydrophilic copolymers via ligand exchange reaction.

The iron oxide nanoparticles stabilized with oleic acid in water were modified with dopamine-terminal fluorescent labelled hydrophilic copolymers via ligand exchange reaction. The typical reaction and purification procedures were shown as follow.

To a 3 mL suspension of Fe₃O₄ in water (5 mg/mL), 100 mg dopamine-terminal poly(PEGA₄₈₀)-b-(Rhodamine B AM) (or 119mg poly(HEA)-b-(Rhodamine B AM) or 40 mg poly(ManA)-b-(Rhodamine B AM)) was added and the solution were stirred under nitrogen protection for one day at 40 °C and another day at ambient temperature.
After reaction, high speed centrifuge (15000 rpm x 15 min) were used to concentrate the functionalised particles at the bottom of the centrifuge tube. Up-layer solution containing free linear polymers were very carefully and slowly removed and subsequently clear water was added to disperse the bottom particles. This procedure was repeated for 6 to 10 times until the colour of up-layer solution after centrifuge changed from deep purple to very light yellow. After centrifuge, the iron oxide particles were absorbed to the wall using a strong magnetic and the aqueous solution was removed carefully. Following that the particles were dispersed in water via sonication and then passed through 0.2 µm PTFE filtration head in order to remove any insoluble materials. The final particles could be dispersed very well in water and showed red colour like red wine.

A part of the solution was lyophilized to get final solid particles with purple-red colour for further characterization. The concentration of the functionalised particles in water was calculated by measuring the weight of relative particles after lyophilisation.

**Cytotoxicity evaluation of nanoparticles**

The cellular effects of different Fe$_3$O$_4$ nanocomposites were determined using the WTS assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10$^4$ cells mL$^{-1}$ in 160 µL of respective media containing 10% FBS. After 24 h of cell attachment, the cells were incubated with 10, 20, 40, 80, 120 µg mL$^{-1}$ of different Fe$_3$O$_4$ nanocomposites for 24 h. Then nanoparticles were removed and cells were washed with PBS for three times. 10 µL of CCK-8 dye and 100 µL of Dulbecco’s Modified Eagle’s Medium (DMEM) cell culture media was added to each well and incubated for 2 h at 37 °C. Plates were then analyzed with a microplate reader (VictorIII, Perkin-Elmer). Measurements of dye absorbance were carried out at 450 nm, with the reference wavelength at 620 nm. The values were proportional to the number of live cells. The percent reduction of WST was compared to controls (cells not exposed to nanoparticles), which represented 100% WST reduction. Three
replicate wells were used for each control and test concentrations per microplate, and the experiment was repeated three times. Cell survival was expressed as absorbance relative to that of untreated controls. Results are presented as mean ± standard deviation (SD).

**Cellular uptake of Fe$_3$O$_4$ nanocomposites**

Confocal microscope images were taken to evaluate the cell uptake of Fe$_3$O$_4$ nanocomposites. Briefly, on the day prior to treatment, cells were seeded in a glass bottom dish with a density of $1 \times 10^5$ cells per dish. On the day of treatment, the cells were incubated with Fe$_3$O$_4$ nanocomposites at a final concentration of 100 µg mL$^{-1}$ (the concentration of Fe$_3$O$_4$ nanocomposites) for 3 h at 37 °C. Afterward, the cells were washed three times with PBS to remove the Fe$_3$O$_4$ nanocomposites and then fixed with 4% paraformaldehyde for 10 min at room temperature. The cell nuclei were stained with hoechst 43332. Cell images were taken with a Laser Scanning Confocal Microscopy (LCSM) Zesis 710 3-channel (Zesis, Germany) with the excitation wavelengths of 405 nm.

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