A Versatile Stable Platform for Multifunctional Applications: Synthesis of NitroDOPA-PEO-Alkyne Scaffolding for Iron Oxide Nanoparticles

EXPERIMENTAL

MATERIALS

The authors purified Tetrahydrofuran (THF; B.D.H. ACS grade) by reflux over sodium metal (Aldrich Chemistry; sodium lump in kerosene 99%) and benzophenone, and purified Naphthalene (Aldrich Chemistry ≥99%) by sublimation at 60°C under a vacuum. The following products were all used as received: bromophenol blue (Sigma Aldrich); hydrochloric acid (HCl; J.T. Baker, 1N volumetric solution); (2-(tetrahydro-2H-pyran-2-yloxy)ethanol (THP; Aldrich Chemistry, purum ≥98% (GC)); ethylene oxide (EO; Aldrich Chemistry); potassium (Aldrich Chemistry 98% in mineral oil); dichloromethane (DCM, EMD Millipore Chemicals, HPLC grade); chloroform (B.D.H.); diethyl ether (DEE; Macron Chemicals); triethyl amine (TEA; Alfa Aesar 99%), 4-(dimethylamino) pyridine (DMAP; Fluka Analytical); succinic anhydride (SA; Alfa Aesar 99%), N,N'-dicyclohexyl carbodiimide (DCC; Thermo Scientific); N-Hydroxysuccinimide (NHS; Acros Organics 98+%) and Spectrum Spectra/Por® molecular porous membrane tubing, and finally MWCO 12-14,000.

CHARACTERIZATION TECHNIQUES

NMR spectroscopy was conducted using a Joel ECX-300, followed by the preparation of polymer samples through the dissolving of 50mg in 1ml of deuterated chloroform, which was then transferred into a 5mm economy, 7", NMR sample tube via glass pipet. Each sample was then subjected to 32 scans at 20°C. Delta NMR Processing and Control Software (v 4.3.6, Windows_NT) and (v 5.0.1, Darwin-x86) were used respectively to control the NMR and analyze the spectra.

High-resolution transmission electron microscopy (TEM) images were acquired at an accelerating voltage of 300 kV on a Hitachi H-9500, and the TEM samples through the

deposition of a diluted hexane solution onto a copper grid with a carbon film. The average mean dimensions of the 181 particles under study were acquired by averaging their particle diameters using FoveaPro image analysis software.

A Horiba Jobin-Yvon MicroHR spectrometer was then used to collect both sets of Photoluminescence (PL) spectra samples, which were excited at 455nm and then collected at sizes ranging from 460nm to 600nm.

A Hi-Res TGA 2950 thermogravimetric analyzer from TA Instruments was used for the Thermogravimetric analysis (TGA) of the samples. Experiments were controlled via Thermal 67Advantage Instrument Control Software (v 1.3.0.205) and the thermogram was analyzed using a TA Universal Analysis 2000 (v 3.9A, build 3.9.0.9) system. All samples were run with platinum sample pans 100 μL in size (from TA instruments). The particles were then exposed to nitrogen for 20 minutes, followed by acceleration to 100 ℃ at a rate of 20°C min^{-1}, held isothermally for 20 minutes, and then again accelerated to 800℃ at a rate of 20 °C min^{-1}. Samples were prepared by drying 0.1ml of water-dispersed magnetic nanoparticles into platinum sample pans.

A Malvern Zetasizer Nano ZS (Model: ZEN3600) Dynamic Light Scattering (DLS)/ Zeta Potential was then used to measure the hydrodynamic radius and the surface charge of particles. Dispersion Technology Software (DTS) (v. 5.10), was then used to control and analyze measurements. Finally, a “Size & Zeta” Folded Capillary cell (DTS1060) system was used to acquire the size and zeta potential measurements of the water dispersed particles under study.

The theoretical hydrodynamic size of the polymer-particle complex was calculated based on a density distribution model.[1-4] By accounting for the Flory parameter describing the interactions between the aqueous media and the PEO brush, the polydispersity of the size of the

iron oxide core (from TEM), and the degree of surface coverage (from TGA), the theoretical hydrodynamic diameter can be determined. The calculated diameter was calculated based on an intensity weighted distribution, for ease of comparison with DLS data.

A vibrating sample magnetometry (VSM) from Quantum Designs using the physical property measurement system (PPMS) by wrapping the sample in Kapton tape and loading it onto the holder and bring it to a pressure of ~10 Torr. The sample was measured at 300 K over a field of ±3T (± 2387 kA m^{-1}) at a rate of 100 Oe s^{-1}. The background was obtained by submitting just the Kapton take to the same procedure.

METHODS

Synthesis of NitroDOPA

In a 50ml round bottom flask, equipped with a stir bar, 30ml of DI water was cooled in a brine salt bath to 0ºC before the addition of sodium nitrite (1.52g, 17.88mmol) and L-3,4-dihydroxyphenylalanine (L-DOPA, 197g, 9.99mmol). The medium was allowed to cool, followed by stirring, during which 0.92ml (90.23mmol) of sulfuric acid was added dropwise to form an orange precipitate. Once all of the acid was added, the solution was stirred overnight. The product was collected by vacuum filtration, washed with methanol, and dried in a vacuum oven (30% yield).

^{1}HNMR (300 MHz, DMSO-D_6), δ (ppm): 7.46ppm and 6.84ppm (s, CH, ring, nitroDOPA), 3.54-3.49ppm (t, J=15, -CH_2-C<u>H</u>-N-), 3.31-2.98ppm (m, -C<u>H_2</u>-CH-N-), ^{13}CNMR (300 MHz, DMSO-D_6) δ (ppm): 170.7, 153.4, 145.2, 139.2, 126.9, 119.9, 112.6, 54.8, 40.0, 35.4.

Potassium naphthalide preparation.

In a 250ml Erlenmeyer flask, 20g of naphthalene was placed under a vacuum at 60°C until all naphthalene was sublimed onto the inside walls of the flask. A stir bar was then used to

charge the sublime naphthalene to a flame dried, 250ml round bottom flask. After the solution was heavily purged with nitrogen, 100ml of THF was added via syringe. Once the naphthalene was completely dissolved, 3.96g of potassium was added. The reaction was again mixed for 12hrs at room temperature in darkness. The concentration of the solution was determined by titration using 1M HCl and bromophenol blue as an indicator. The final molar concentration was determined to be 0.9M.

Synthesis of THP-PEO-OH (1)

EO was distilled into a 300ml stainless steel Parr Reactor and cooled to -40℃ using an acetone dry ice bath, followed by the addition of 10ml of THF. A stir bar was then used, together with a nitrogen purge of the solution, to dissolve THP into 10ml of THF within a separate 50ml flame dried, round bottom flask. Next, 4.75ml of potassium naphthalene (0.9M) was added to this solution via syringe and stirred for 10 minutes. Again via syringe, the initiator solution was then charged to the reactor, followed by the addition of 50ml of THF. The reactor was brought to room temperature and the solution was then reacted for 72 hours. Polymerization was terminated with 1.90ml of 2.5M acetic acid in THF. The reactor was then purged with nitrogen for 1hr, and the solvent was removed by rotary evaporation, and dissolved in a 200ml solution of chloroform. The solution was then washed with 150ml of deionized water. The organic layer was then concentrated down and precipitated using cold diethyl ether and polymer was retrieved by filtration. The remaining polymer was dried in a vacuum oven at 25℃ and characterized by ^{1}HNMR. The molecular weight, which was 6000 g mol^{-1} , was calculated by comparing the ratio of the area of THP to the polyethylene backbone:

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): THP: 1.47-1.88 (m, CH-(C<u>H</u>$_2$)$_3$-CH_2), 3.87 (m, CH-(CH_2)$_3$-C<u>H</u>$_2$-O), 4.61 (t, J=6.9, O-C<u>H</u>-O). *PEO:* 3.64 (m, C<u>*H*</u>$_2$-C<u>*H*</u>$_2$-O).

Synthesis of THP-PEO-Alkyne (2)

A 100ml round bottom flask was equipped with a stir bar and flame dried. The flask was then charged with polymer (1) (2.0g, 0.33mmol) and sodium hydride (0.024g, 1.0mmol) and finally sealed and purged with nitrogen. THF (20ml) was then added via syringe to dissolve reactants and was brought to 0°C using a NaCl ice bath for 30mins. Propargyl bromide (0.09ml, 1.0mmol) was added slowly again via syringe over 30mins, followed by 30mins of stirring at 0°C. The reaction was removed from the ice bath and warmed to room temperature and stirred for 24hrs. The final solution was filtered via vacuum filtration, precipitated twice by DEE, and vacuum dried overnight. (81% yield)

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): 1.47-1.88 (m, CH-(C$\underline{H}_2$)$_3$-CH_2), 4.63-4.61 (t, J=6, O-C$\underline{H}$-O), 3.64 (m, C$\underline{H_2}$-C$\underline{H_2}$-O), 2.43-2.44 (t, J=3, -O-CH_2-C≡CH), 4.198-4.190 (d, J=2.4, -O-CH_2-C≡CH)

Deprotection of THP-PEO-Alkyne (3)

Polymer (2) (0.8g, 0.17mmol) was dissolved in 10ml of methanol, followed by the addition of three drops of 1N HCl n. The solution was then stirred for 4hrs. The solid product was recovered by crashing into DEE, and the solid was collected using vacuum filtration (98% yield). The product was characterized using ^{1}HNMR.

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): 3.64 (m, C$\underline{H_2}$-C$\underline{H_2}$-O), 2.42-2.44 (t, J=6, -O-CH_2-C≡C$\underline{H}$), 4.187-4.195 (d, J=2.4, -O-CH_2-C≡CH)

Synthesis of Alkyne-PEO-COOH (4)

A round bottom flask equipped with a stir bar was flame dried and charged with polymer (3) (0.8g, 0.17mmol) and placed in a vacuum oven for 30min at 80°C. SA (0.03g, 0.27mmol) and DMAP (0.002g, 0.02mmol) were added to the flask and then purged with nitrogen. Finally,

anhydrous THF (20ml) was then added to the flask via syringe and stirred for 8hrs. The reaction was then precipitated with cold DEE. The precipitant was collected and dissolved in 5ml of DCM and precipitated again with cold DEE (2X). The final precipitant was collected and dried in a vacuum oven overnight (93% yield). The polymer was characterized using ^{1}HNMR.

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): 3.64 (m, C*H*$_2$-C*H*$_2$-O), 2.58 (m,-CH_2-CH_2-C(O)-O-), 2.42-2.44 (t, J=6, -O-CH_2-C≡CH), 4.187-4.195 (d, J=2.4, -O-CH_2-C≡CH)

Synthesis of Alkyne-PEO-NHS (5)

In a flame dried, 25ml round bottom flask with a stir bar, polymer (4) (0.73g, 0.15mmol), NHS (0.02g, 0.18mmol), and DCC (0.04g, 0.18mmol) was added, capped and purged with nitrogen. THF (20ml) was added via syringe and was stirred for 4hrs at room temperature. The solution was then vacuum filtered to remove and dicyclohexylurea that precipitated during the reaction. Using rotary evaporation, the THF was removed from the filtrate and again dissolved in 5ml solution of DCM and precipitated with DEE (2x). The precipitate was collected and dried in a vacuum oven (83% yield). The product was characterized by ^{1}HNMR.

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): 3.64 (m, C*H*$_2$-C*H*$_2$-O), 4.22 (m, -CH_2-O-C(O)-), 2.72 (m,-CH_2-CH_2-C(O)-O-), 2.42-2.44 (t, J=6, -O-CH_2-C≡CH), 4.187-4.195 (d, J=2.4, -O-CH_2-C≡CH), 2.8 (s,-C(O)-CH_2-CH_2-C(O)-).

Synthesis of Alkyne-PEO-NitroDOPA(6)

A flame dried, 50ml Erlenmeyer flask equipped with a stir bar was charged with alkyne-PEO-NHS (0.69g, 0.14mmol) and nitroDOPA (0.04g, 0.17mmol) and capped with a heavy nitrogen purge. Next, 10ml of DMSO was added via syringe and stirred for 4hrs at room temperature. Finally, the resulting reaction was precipitated with DEE and again dissolved in a 200ml solution of chloroform and filtered to remove any excess nitroDOPA. The final polymer

was precipitated with cold DEE, collected, and dried in a vacuum oven (87% yield). The final product was characterized using ^{1}HNMR.

^{1}HNMR (300 MHz, $CDCl_3$), δ (ppm): 3.64 (m, C$\underline{H_2}$-C$\underline{H_2}$-O), 2.42 (s, -O-CH_2-C≡CH), 2.72 (m,-CH_2-CH_2-C(O)-O-). NitroDOPA: 6.12 and 6.70 (s, CH in ring).

Synthesis of magnetite nanoparticles **(7)**

The 7.2nm magnetic nanoparticles, synthesized using thermal decomposition of iron(III) acetylacetonate (2mmol), 1,2-hexadecanediol (10mmol), olylamine (4mmol), benzyl ether (20ml), and 6nm iron oxide seeds were added and stirred under a nitrogen flow and brought to 200°C for 1hr to purge any remaining moisture. Finally the reaction was brought to reflux for 30mins under a nitrogen head. The particles were purified by a precipitation of ethanol and characterized using TEM and DLS.

Modification of magnetite nanoparticles **(8)**

The magnetic nanoparticles were modified by first dissolving Alkyne-PEO-NitroDOPA (200 mg, 0.04mmol) into a 10ml solution of chloroform followed by the slow addition of 1ml (2mg/ml) of magnetic nanoparticles, which were also dispersed in a separate 10 ml chloroform solution. This combined nanoparticle solution was then sonicated for 30 mins, and allowed to stir overnight. The particles were then purified by precipitation with hexane, and centrifuged to separate the particles from the solvent. The particles were then dispersed in ethanol and subsequently precipitated using hexane and separated via centrifugation for collection. Finally, the particles were dispersed in deionized water and dialyzed for three days.

Functionalization of alkyne-PEO-nitroDOPA coated magnetic nanoparticles

A 0.2ml of modified particles (0.7mg/ml) was first added to a 10ml solution of DI water, followed by the addition of azido-functionalized fluorescein (0.003mmol), $CuSO_4$ (0.013mmol)

and sodium ascorbate (0.003mmol). The solution was then covered with foil and stirred at 32°C for 24hrs. The particles were then brought to room temperature and dialyzed for three days using DI water.

Next, 1ml of modified particles (4.11mg/ml), azido-fluorescein (0.67mg, 0.001mmol) and azido-carbazole (0.21mg, 0.001mmol) were added to a 50ml THF solution. While stirring, a 121 µL aqueous solution of $CuSO_4$ (15mM) and a 54µL aqueous of sodium ascorbate (11.1mM) was added to the solution and reacted for 24 hours at 32 °C. The particles were then brought to room temperature and rotary evaporation was used to remove the THF. The particles were then dispersed in 5ml of DI water and dialyzed for three days using DI water.

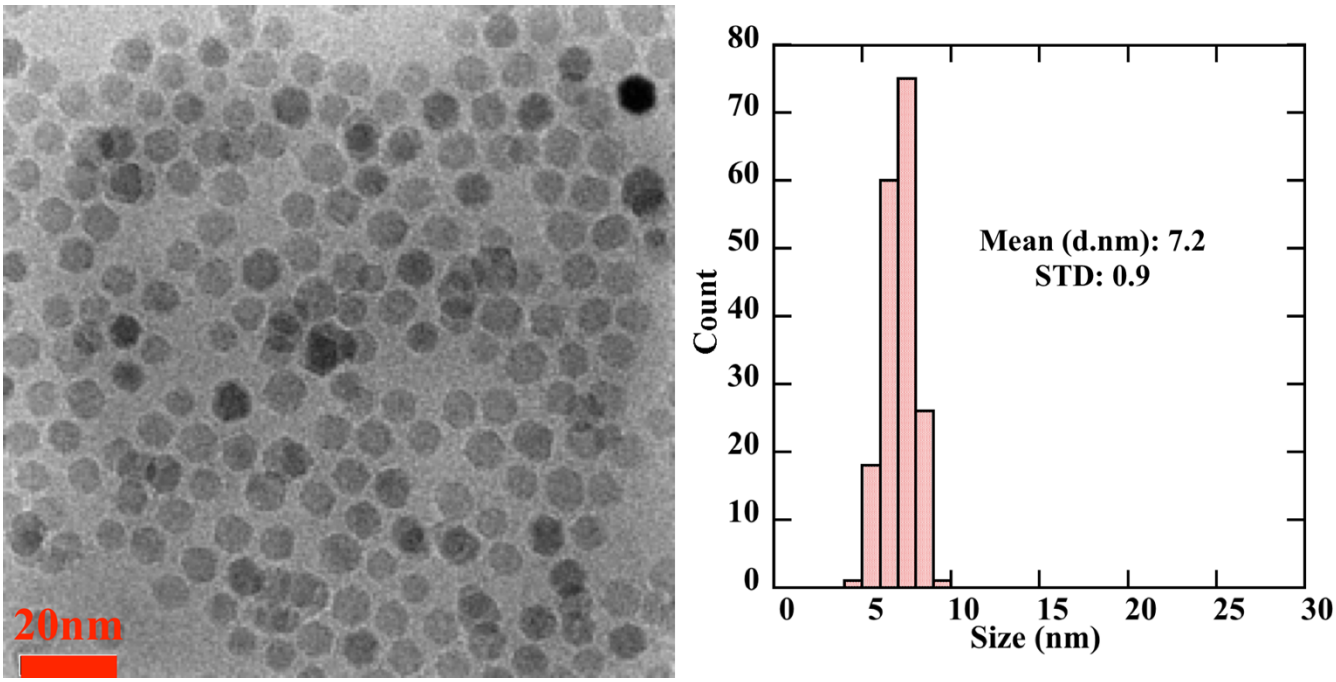

Figure S1. TEM micrograph of oleylamine coated nanoparticles (left) and a histogram of particles size distribution (right).

Table S1. DLS results of the hydrodynamic diameter of the magnetic nanoparticles before and after modification with Alkyne-PEO-NitroDOPA(6)

	Z-Avg (d.nm)	PDI	I (%, d.nm)	V (%, d.nm)	N (%, d.nm)
OA Coated	29.24	0.295	35.42±17.14	20.06±8.93	14.57±4.51
PEO-Coated	77.31	0.138	89.49±31.65	66.83±25.72	51.52±14.73

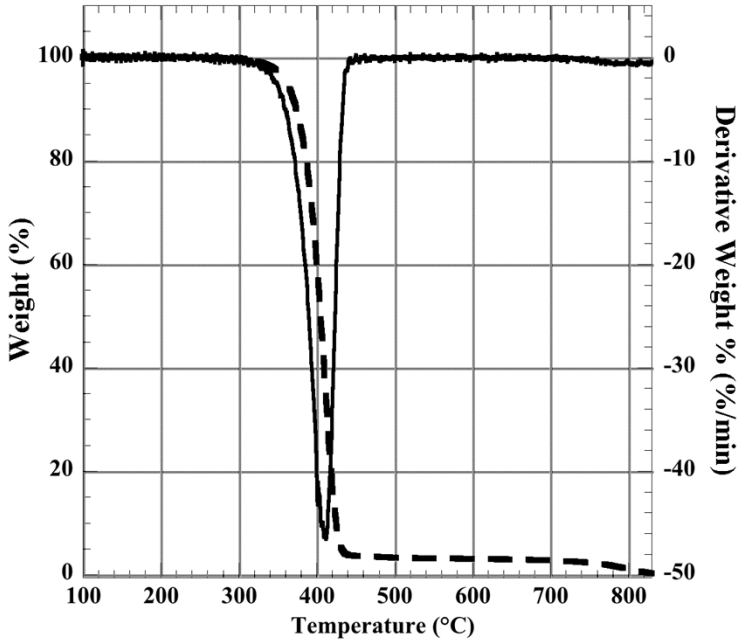

Figure S2. TGA of particles modified with Alkyne-PEO-NitroDOPA (6)

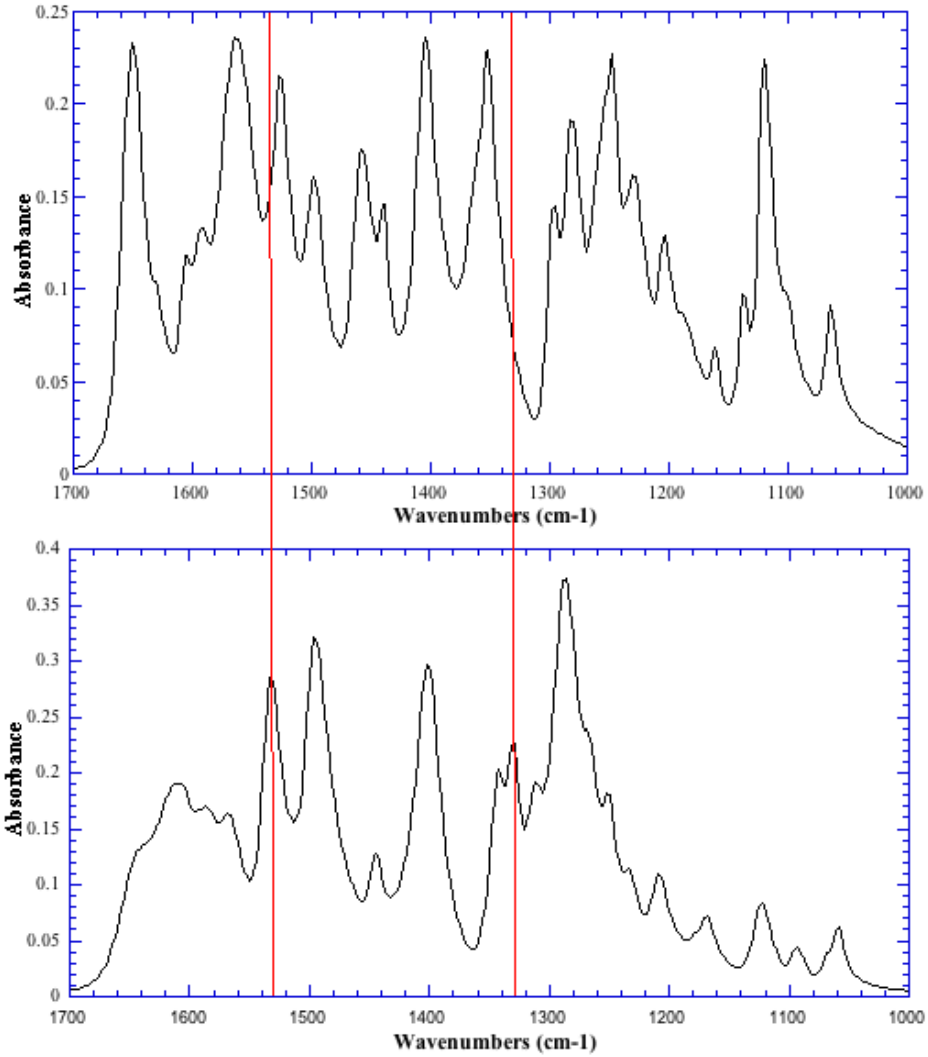

Figure S3. FTIR of DOPA (Top) and NitroDOPA (Bottom); symmetric and asymmetric stretching from the NO_2 peaks at 1330 and 1532 cm^{-1}.

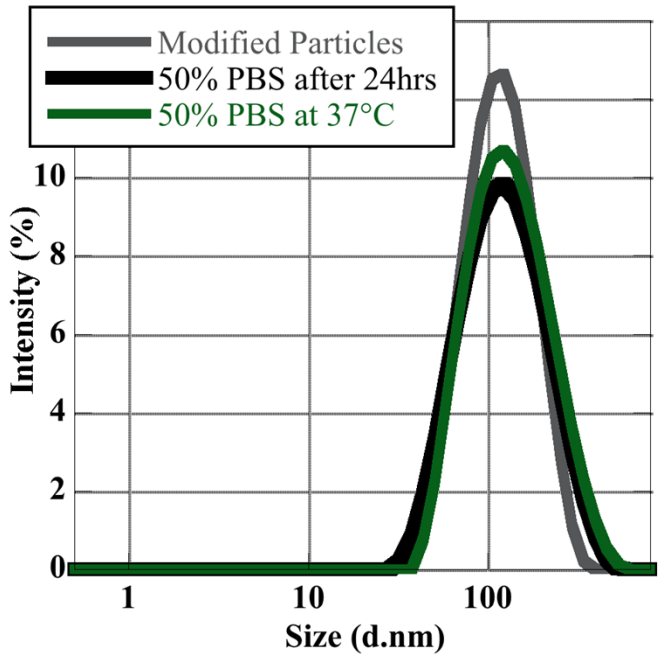

Figure S4. Hydrodynamic diameter of polymer modified magnetic nanoparticles, by intensity, in PBS after 24 hours and at a biologically relevant temperature of 37°C.

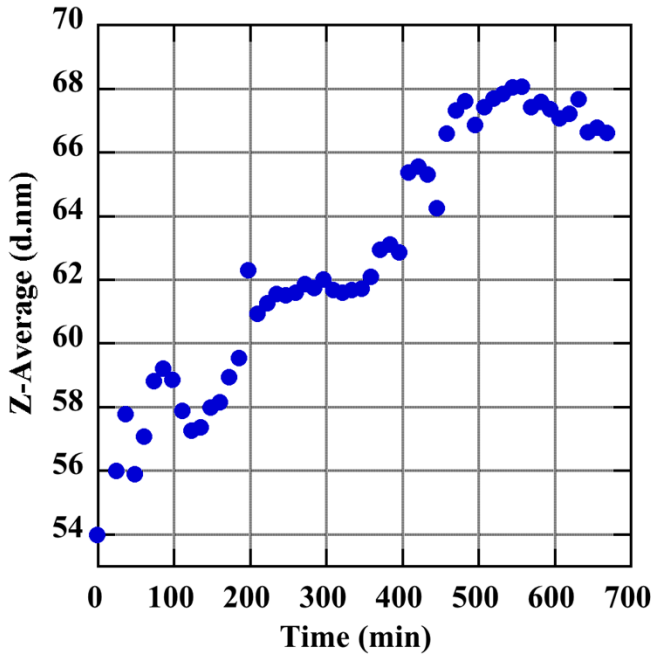

Figure S5. Time dependence study of the polymer modified magnetic nanoparticles in PBS for 24 hours at 70°C.

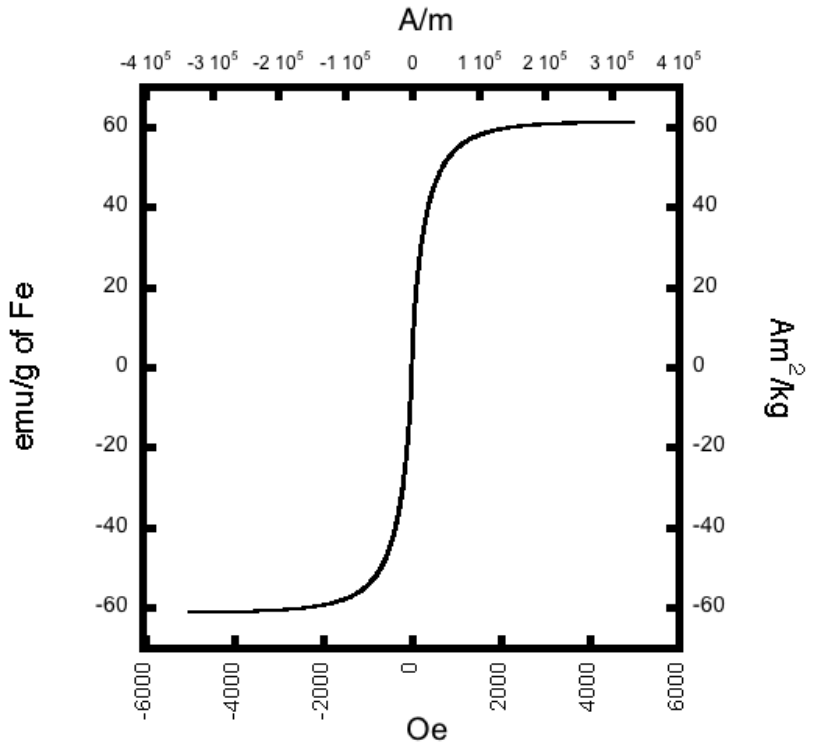

Figure S7. Magnetization curve of polymer modified particles at 300K

1. Q. Zhang, M. S. Thompson, A. Y. Carmichael-Baranauskas, B. L. Caba, M. A. Zalich, Y.-N. Lin, O. T. Mefford, R. M. Davis, and J. S. Riffle, *Langmuir*, 2007, **23**, 6927–6936.
2. O. T. Mefford, M. R. J. Carroll, M. L. Vadala, J. D. Goff, R. Mejia-Ariza, M. Saunders, R. C. Woodward, T. G. St Pierre, R. M. Davis, and J. S. Riffle, *Chem Mater*, 2008, **20**, 2184–2191.
3. S. L. Saville, R. C. Stone, B. Qi, and O. T. Mefford, *J. Mater. Chem.*, 2012, **22**, 24909.
4. S. L. Saville, R. C. Woodward, M. J. House, A. Tokarev, J. Hammers, B. Qi, J. Shaw, M. Saunders, R. R. Varsani, T. G. St Pierre, and O. T. Mefford, *Nanoscale*, 2013.