Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Versatile PEG-derivatized Phosphine Oxide Ligands for Water-Dispersible Metal Oxide Nanocrystals

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Synthesis of PO-PEGs

3 mmol of dry Poly(ethylene glycol) methyl ether was dissolved in 7 ml of anhydrous tetrahydrofuran. 1 mmol of phosphoryl chloride was added to the mPEG solution and incubated at RT for 1 day. After removing the THF by evacuation, the resulting gel was incubated at 100 ºC under vacuum for 12 h.

Synthesis of amine-functionalized PO-PEGs

2 mmol of dry Poly(ethylene glycol) methyl ether was dissolved in 5 ml of anhydrous tetrahydrofuran. 1 mmol of phosphoryl chloride was added to the mPEG solution and incubated in RT for 1 day. After removing the THF by evacuation, the resulting gel was incubated at 100 ºC under vacuum for 12 h. At RT, 5 ml of THF and 3 ml of 1,2-ethylenediamine was added. After 12 h, THF and the remaining 1,2-ethylenediamine was evacuated.

Stabilization of Fe_3O_4 nanocrystals with PO-PEGs

Fe_3O_4 nanocrystals were prepared using the reported methods.[1] 10 mg of nanocrystals were dispersed in 2 ml of THF. 20 mg of PO-PEGs was added to the solution and dissolved. After removing the THF by evacuation, the nanocrystals were incubated at 150 ºC under vacuum for 1 h. 5 ml of water was added and the dispersed nanocrystals were filtered through 220 nm syringe filter. The unbound PO-PEGs were eliminated by ultracentrifugation column chromatography, or dialysis.

Conjugation of FITC with amine-functionalized Fe_3O_4 nanocrystals

Fe_3O_4 nanocrystals were stabilized with amino-functionalized PO-PEGs, and dispersed in carbonate buffer (pH: 9.4) to make a 2 mg/ml solution. 1 ml nanocrystals were mixed with 10 μl of a FITC solution (10 mg in 1 ml of DMSO) and incubated at RT for 1 h. The FITC conjugated nanocrystals were purified by column separation or dialysis.

Cell culture

A human breast cancer cell line, SKBR-3 was maintained in DMEM/F12 medium without folate at 37 ºC in a humidified atmosphere containing 5 % of CO_2. The cell culture media were supplemented with 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were regularly passaged and reseeded 24 h before the cellular uptake experiments.

In vitro cytotoxicity against SKBR-3 cells

The SKBR-3 cells were seeded in a 96-well plate at a density of 1×10^4 cells per well, and grown in DMEM/F12 medium (without folate) supplemented with 10% (v/v) fetal bovine serum for 24 h at 37 ºC. The PO-PEGs stabilized Fe_3O_4 nanocrystals (5 nm) were added to the medium and the cells were incubated for 5 days at 37 ºC. The concentrations of Fe_3O_4 nanocrystals used were 200, 100, 50, 25, and 12.5 μg of Fe/ml, respectively. The cell viability was determined using the MTT cell viability assay. [2]

In vitro optical and MR imaging of cancer cells

SKBR-3 Cells were plated in 100 mm dishes at a density of 1×10^6 cells per dish 24 h before cellular uptake. FITC conjugated PO-PEGs stabilized Fe_3O_4 nanocrystals (5 nm) were incubated in the SKBR-3 cell culture for 1 day at 37 ºC in serum free medium. The Fe concentration was 0, 50 and 100 μM respectively. Then the cells were washed five times with cold PBS and imaged by using a fluorescent microscope. The cells were detached and centrifuged, and the resulting cell pellets were mixed with 0.5 ml of 0.8 % agarose solution. The mixture was transferred to an Eppendorf-tube for the MR measurement. The relaxation time of the labeled cells mixed with agarose were measured using a 3.0 T whole body MRI system (Philips, Achieva ver. 1.2, Philips Medical Systems, Best, The Netherlands) equipped with a 80 mT/m gradient amplitude and a 200 ms/m slew rate.

Table S1. Relaxation time of Fe_3O_4 labeled SKBR-3.

Fe concentration (μM) in growing media	**T_1 (ms)**	**T_2 (ms)**
0	1490	192
50	1460	65.5
100	1440	58.5

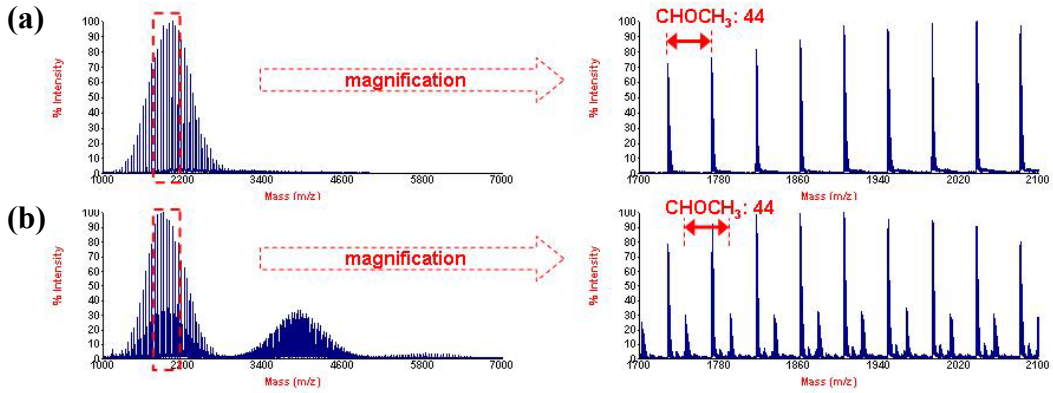

Fig. S1 MALDI-TOF mass spectra of a) reacted mPEG (Mn : 2000) and b) resulting PO-PEGs.

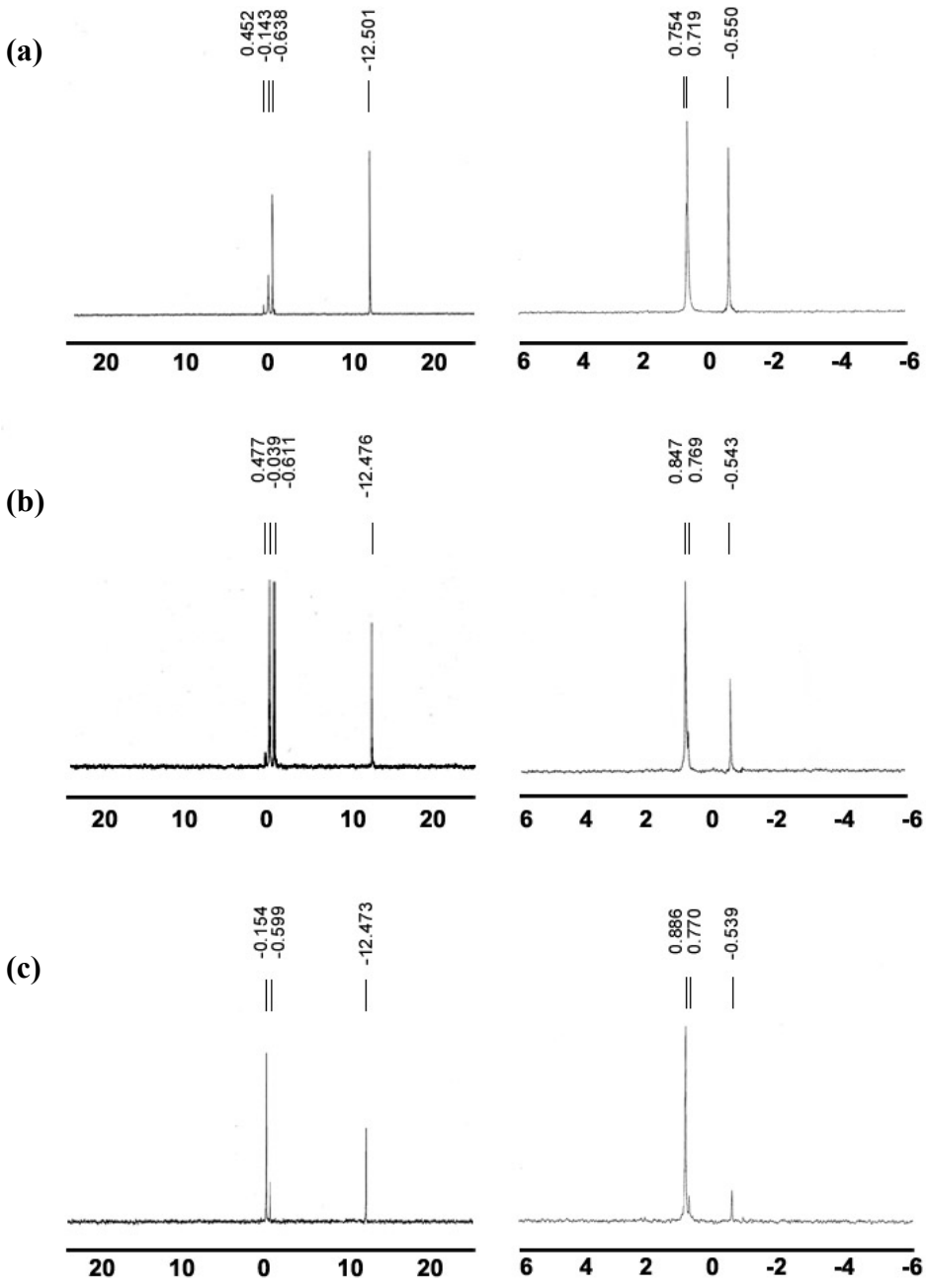

Fig. S2 ^{31}P NMR spectra of PO-PEGs by various molecular weight of mPEG (a) Mn: 550 (b) Mn: 750 (c) Mn: 2000 (left: ^{31}P NMR in $CDCl_3$ and right: ^{31}P NMR in D_2O).

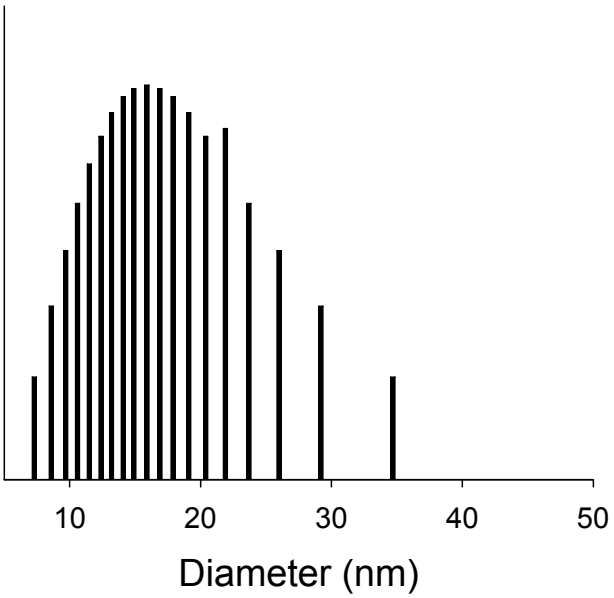

Fig. S3 Hydrodynamic diameter distribution of the PO-PEG stabilized iron oxide nanocrystals with core diameter of 8 nm. The data was obtained using light scattering method.

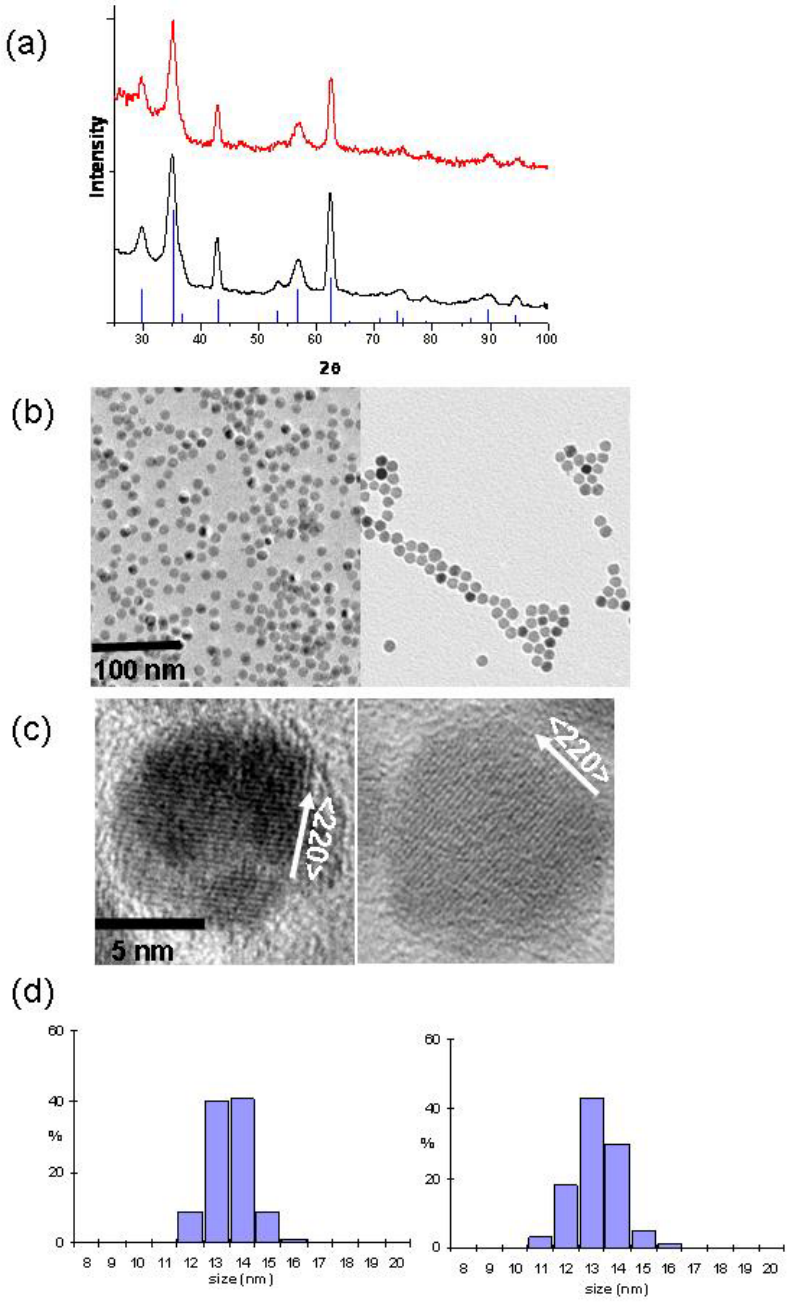

Fig. S4 Characterization of as-synthesized organic dispersible and PO-PEG stabilized Fe_3O_4 nanocrystals. (a) XRD spectra of as-synthesized organic dispersible (black) and PO-PEG stabilized Fe_3O_4 nanocrystals (red) (Blue lines: JCPDS 19-0629, magnetite). (b) TEM images of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe_3O_4 nanocrystals (right). (c) High resolution TEM images of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe_3O_4 nanocrystals (right). (d) Size distribution of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe_3O_4 nanocrystals (right).

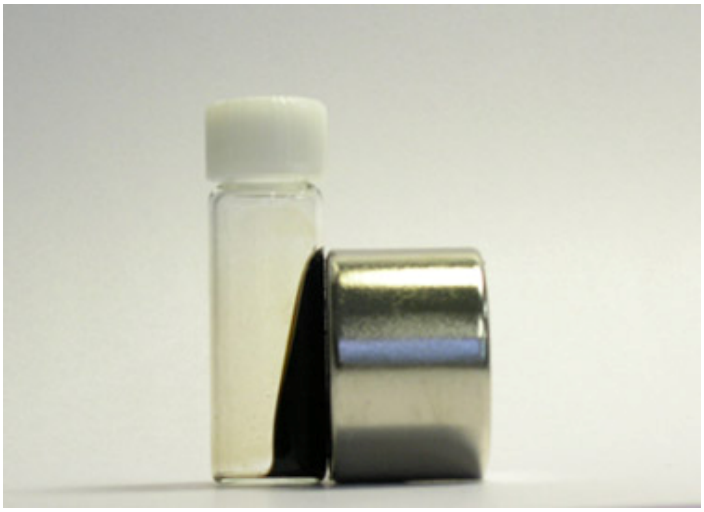

Fig. S5 Photograph of magnetically attracted Fe_3O_4 nanocrystals dispersed in water with high iron concentration of 17 mg/ml.

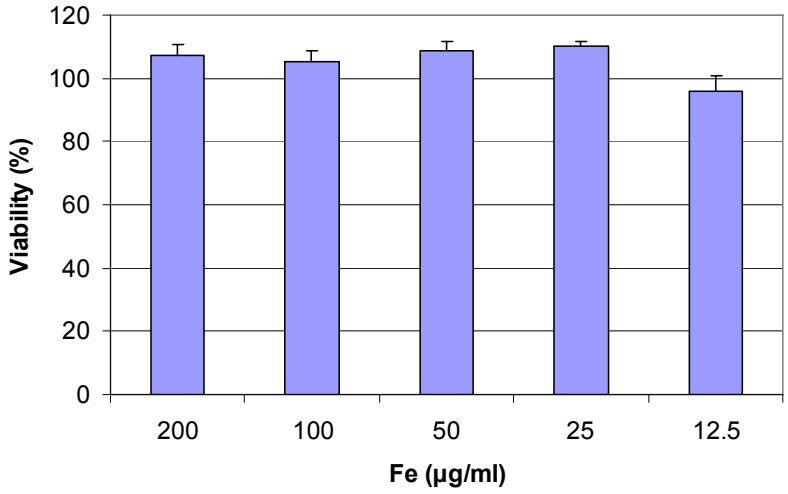

Fig. S6 In vitro cytotoxicity of the PO-PEG stabilized Fe_3O_4 nanocrystals against SKBR-3 cells.

References for the Supporting Information

1 J. Park, K. An, Y. Hwang, J.-G. Park, H.-J. Noh, J.-Y. Kim, J.-H. Park, N.-M. Hwang, T. Hyeon, *Nat. Mater.* **2004**, *3*, 891.

2 T. R. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.