Electronic Supporting Information

for

Magnetic Fe_3O_4 Nanoparticles Coupled with Fluorescent Eu Complex for Dual Imaging Applications

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Materials: α,ω-bis{2-[(3-carboxy-1-oxopropyl)amino]ethyl}polyethylene glycol Mr = 2000), other chemicals, and organic solvents were purchased from Sigma Aldrich. N-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) were from Pierce Biotechnology. All the buffers and media were from Invitrogen Corp. Water was purified by a Millipore Milli-DI water purification system. Nanosep 100kOMEGA was from Fisher. Dialysis bags were purchased from Spectrum Laboratories, Inc.

Characterization: UV-Vis absorption spectra were obtained with a PerkinElmer Lambda 35 UV-Vis spectrometer. TEM images were taken on a Philips CM 20 transition electron microscope (120 kV). Hydrodynamic sizes of the NPs were measured by Malvern Zeta Sizer S90 dynamic light scattering instrument. The fluorescence spectra were acquired on Fluoromax 4 (HORIBA JOBIN YVON Inc.) spectrofluoremeter. Optical images of SK-BR-3 cells were obtained by a Leica inverted epifluorescence/reflectance laser scanning confocal microscope. The ICP-MS measurements were carried on a JY2000 Ultrace ICP Atomic Emission Spectrometer. Transverse T_2-weighted spin echo images were acquired using a 3T Siemens Tim Trio MR Scanner.

Synthesis of the 8 nm Fe_3O_4 nanoparticles (NPs): $Fe(acac)_3$ (0.706 g, 2 mmol) was dissolved in benzyl ether (10 mL) and oleylamine (10 mL). The above mixture solution was dehydrated at 110 ^oC for 1 h under a flow of nitrogen, and under a blanket of nitrogen, quickly heated to 300 ^oC and kept at this temperature for 2 h. The black-brown mixture was cooled to room temperature. Ethanol (40 mL) was added to the mixture and precipitate was collected by centrifugation at 8000 rpm. Finally, the product was redispersed in hexane.

Fe_3O_4 NP modification: PEG(2000) diacid (40 mg), NHS (2 mg), EDC (4 mg), and dopamine (DPA) hydrochloride (1.27 mg) were dissolved in a mixture of $CHCl_3$ (2 mL), DMF (1 mL), and

anhydrous Na_2CO_3 (5 mg). The solution was stirred at room temperature for 2 h before Fe_3O_4 NPs (20 mg) were added, and the resultant solution was stirred overnight at room temperature under N_2. The modified NPs were precipitated by adding hexane (5 mL), collected by a permanent magnet and dried under N_2. The particles were then dispersed in water or PBS. The extra surfactants and other salts were removed by dialysis using a dialysis bag (MWCO 10000) for 24 h in PBS or water. Any precipitate was removed by a size-exclusion column (Sephadex G25, PD-10, GE). The final concentration of the Fe_3O_4-DPA-PEG-COOH NPs was determined by ICP-AES analysis.

Fe_3O_4 NP conjugation with Eu complex: The Fe_3O_4-DPA-PEG-COOH NPs from above procedure, NHS (2 mg) and EDC (4 mg) in water were mixed and shaken for 30 min, and then BMAP-Eu (15 mg) in 0.5 ml DMF was added dropwise into it. The solution was then shaken for 12 h at room temperature and dialyzed (dialysis bag MWCO 10000) for 24 h in PBS. Precipitates, if there were any, were removed by a size-exclusion column.

NP incubation with cells: SK-BR-3 cells were purchased from ATCC and cultured in a glass-bottom Petri dish (Mat Tek Corp.) with Dulbecco's modified Eagle Bs medium (DMEM) with 10% FBS and 1% antibiotics. Before incubation with particles, the cells were washed with PBS three times. The particle solution in DMEM was incubated with cells for 1 h. Then, those cells were washed with PBS three times and fixed by 4% paraformaldehyde solution. After 30 min fixation, the cells were washed again three times with PBS for optical imaging.

NP *in vitro* cytotoxicity study: Cytotoxicity assay was performed in 96-well plates (Fisher Inc.) with seeding density, 4000 cells per well. The cells were pre-incubated at 37 ºC for 24 h before the test substance was added. The plates were incubated with the test substance at 37 °C for 48 h in 5% CO_2. Then 5 μL 3-(4,5-dimethylthizaol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL in PBS) was added to each well to evaluate cell viability. After 2 h at 37 °C, the solution was removed. 100 μL DMSO was added to dissolve cells. After 30 min incubation at 37 °C, the viability was measured through microreader.

Cell fluorescent images: SK-BR-3 cells were cultured in glass bottom Petri dish (MatTek Corp.) with Dulbecco's Modified Eagle's Medium (DMEM) with 10% FBS and 1% antibiotics for 1 h, then were washed with PBS for 3 times and fixed by 4% paraformadehyde solution. After 30 min fixation, the cells were washed by PBS and subjected to fluorescent imaging.

MRI experiments: Transverse T_2-weighted spin echo images were acquired using a 3T Siemens Tim Trio MR Scanner. Gel preparations in 2 mL vials were placed in a holder for insertion into the eight-channel volume head resonator. The long axis of the vials was parallel to the static magnetic field, and a transverse tomographic plane orientation was used. Gradient echo acquisition was used with a repetition time of 2000 ms, an echo time of 1.8 ms, a slice thickness of 12 mm, and a flip angle of 20^o. In-plane resolution was 0.88 mm. The normal first-order shim process was applied, and the phantoms were imaged at room temperature (20^oC).

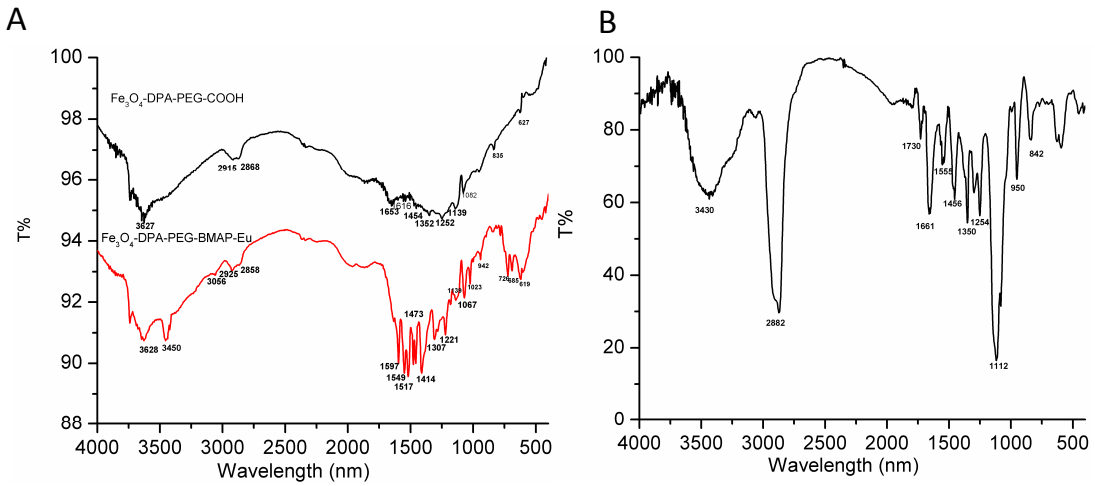

Fig. S1 IR spectra of (A) Fe_3O_4-DPA-PEG-COOH (black line), Fe_3O_4-DPA-PEG-BMAP-Eu (red line) and (B) BMAP-Eu complex.

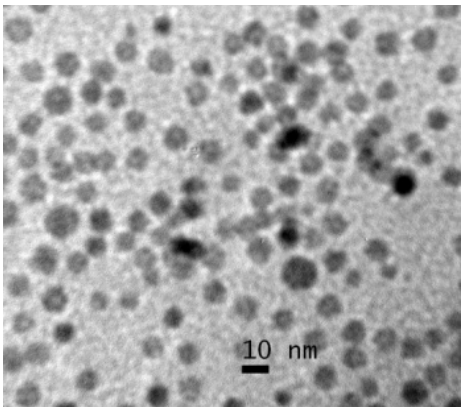

Fig. S2 TEM image of the 8 nm Fe_3O_4-DPA-PEG-COOH NPs.

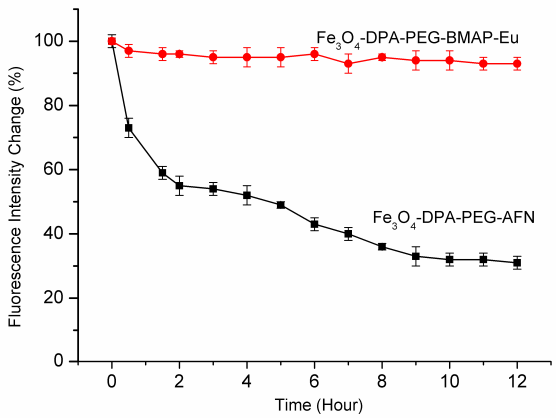

Fig. S3 Fluorescence intensity change of Fe_3O_4-DPA-PEG-BMAP-Eu and Fe_3O_4-DPA-PEG-AFN in water (100 μg Fe/ml) under UV lamp (365 nm) with time.

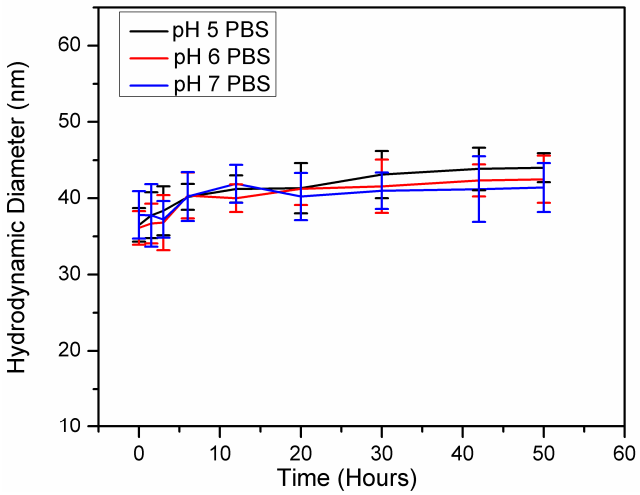

Fig. S4 Hydrodynamic diameter change of the Fe_3O_4-DPA-PEG-BMAP-Eu NPs in PBS at 37°C during the 50 h incubation period.

Table S1. Free Fe and Eu ion concentrations measured from the corresponding PBS solution outside the dialysis bag containing Fe_3O_4-DPA-PEG-BMAP-Eu NP dispersion in PBS. The PBS solution of pH = 5, 6, or 7 was incubated at 37 °C.

	pH 5		pH 6		pH 7	
Time(h)	Fe(%)	Eu(%)	Fe(%)	Eu(%)	Fe(%)	Eu(%)
1	0	0	0	0	0	0
3	0	0	0	0	0	0
5	0	0	0	0	0	0
7	0	0	0	0	0	0
21	7.58E-06	0	0	0	0	0
25	8.41E-06	0	0	0	0	0
30	1.19E-05	0	0	0	0	0