Supporting Information

Polymer Ligand-assisted Fabrication of Multifunctional and Redox-responsive Self-assembled Magnetic Nanoclusters for bimodal imaging and cancer treatment

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Methods and Experimental Details

Materials

All reagents and solvents were received without further purification. Poly(isobutylene-alt-maleic anhydride) (PIMA), iron(III) acetylacetonate ($Fe(acac)_3$), phenyl ether, triethylamine (TEA), tetrahydrofuran (THF), chloroform (CH_3CL_3), dimethyl sulfoxide (DMSO), dimethyl sulfoxide-d6 (DMSO-d6), N,N-dimethylformamide (DMF), ethyl acetate, chloroform-d6 ($CDCl_3$), dopamine hydrochloride (Dopa), cystamine hydrochloride (Cyst), phosphate buffered saline (PBS), fetal bovine serum (FBS), dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS) and diethyl ether were obtained from Sigma-Aldrich Co. (USA). Oleylamine and triphosgene were purchased from TCI Co. (Japan). Hexane, ethanol, and hydrochloric acid (HCl) were obtained from Samchun Co. (Korea). Methoxy-poly(ethylene glycol) (mPEG, Mn = 2000) was obtained from SunBio. Co (Korea). Chlorin e6 (Ce6) as a photosensitizer and singlet oxygen sensor green (SOSG) were obtained from Frontier Scientific, Inc. (USA) and Molecular Probes, Inc. (USA), respectively.

Characterization

Transmission electron microscopy (TEM) measurements were performed on a JEM-ARM 200F microscope operated at 120 kV. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were measured with a 500 NMR spectrometer (Varian Unity Inova 500NB). UV/visible absorbance was collected on a V-630 Bio UV−vis spectrophotometer. Fluorescence measurements were conducted on an FP-6200

spectrofluorometer (AMINCO, Bowman, series 2) with 5 nm slit widths for both excitation and emission, and was used to calculate the number of Ce6 molecule on the polymer ligands on the basis of the standard curve of Ce6. Elemental analysis was measured by the inductively coupled plasma mass spectrometry (ICP-MS) spectrometer (Agilent-7500i). X-ray diffractometry (XRD) structural analyses was performed on an XRD instrument (Rigaku Rotaflex D/Max) equipped with a Cu Kα radiation source (λ = 1.54056 Å). The magnetic property of material was characterized using a superconducting quantum interference device (SQUID) at 300 K. Hydrodynamic size and zeta potential were measured using a Zetasizer-ZS90 (Malvern Instrument, U.K.) at 25 °C.

Redox-dependent behavior of RMNs in vitro

To confirm the fluorescence recovery of RMNs in the reductive surrounding, the fluorescence intensities of RMNs (2 mg/mL) in PBS (pH 7.4) with 10 mM of GSH and without GSH solution were collected using a fluorescence spectrometer with excitation at 650 nm and emission at 672 nm. Free Ce6 was used as a control group.

The in vitro Ce6 release profile from RMNs was investigated in PBS (pH 7.4) with 10 mM GSH and without GSH solution at 37 °C using a dialysis method. At predetermined time intervals, 3 mL of medium was taken out and replenished with an equal volume of new medium. The mass of Ce6 released was calculated by UV−vis spectroscopy.

Redox-dependent singlet oxygen generation (SOG) evaluation

To evaluate the SOG of RMNs, RMNs (2 mg/mL) in PBS (pH 7.4) with 10 mM of

GSH and without GSH solution were mixed with singlet oxygen sensor green (SOSG, 2.0 μM). The above solution was irradiated at 5 mW cm^{-2} intensity for 5 min using a 660 nm laser source. The changes of SOSG fluorescence intensity were collected (λ_{ex} = 494 nm and λ_{em} = 534 nm) to indicate the singlet oxygen generation by comparing to the background and were plotted after 60 min.

In vitro physiological stability of RMNs

The stability of RMNs was investigated by monitoring the nanoparticle size over 5 days using a Zetasizer-ZS90 in different solution, including PBS, cell culture media (RPMI 1640) and FBS at 37 °C.

In vitro MR relaxivity measurement

For in vitro MR imaging analysis, MRI measurements were performed using a 3.0 T system (Philips, Achieva, the Netherlands). Longitudinal ($r_1 = 1/T_1$) and transverse ($r_2 = 1/T_2$) of RMNs were measured with fast spin echo sequence at on a series of dilutions of the RMNs. Linear regression analysis was carried out between relaxation rates and Fe concentration (mM) for estimating the r_1 and r_2 relaxivity values of RMNs

Cell culture

Human breast cancer (MDA-MB-231) cells were purchased from the Korea Cell Line Bank. MDA-MB-231 cells were seeded at 1×10^4 per well of 96-well plates in cell growth medium (Gibco, Grand Island, NY) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2 for 24 h.

In vitro cellular uptake

Cellular internalization of free Ce6 and RMNs was investigated using a LSM780 confocal laser scanning microscopy (CLSM, Carl Zeiss, Germany). MDA-MB-231 cells were treated with free Ce6 and RMNs at an equivalent Ce6 concentration of 20 μg/mL in fresh culture medium at 37 °C. After a 4 h incubation, the above incubated cells were washed thrice with PBS and then the cells were fixed with 4% formaldehyde for 1 h. Subsequently, the cell nuclei were stained with HOECHST 33342 for 10 min and rinsed with PBS. Fluorescent images from cell nuclei and Ce6 were observed with setting excitation at 405 nm and 650 nm, respectively. Scale bar = 20 μm.

In vitro PDT treatment

The in vitro PDT efficiency of free Ce6 and RMNs was assessed with a standard MTT method against MDA-MB-231 cells. The diluted free Ce6 and RMNs were incubated in the wells containing MDA-MB-231 cells at varying concentrations of Ce6 ranging from 5 to 0.625 μg/mL for 24 h at 37 °C. Then, cells were washed with fresh media to remove nanoparticles and irradiated with a 660 nm laser at a power density of 5 mW/ cm^2 for 5 min. After additional 24 h incubation, the cell viability was determined with the following equation:

$$\text{Cell viability } (\%) = \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100 \qquad (1)$$

A parallel set of experiments were prepared by treating cells with the respective materials to assess cytotoxicity in the dark.

In vivo studies

Balb/c nude mice (20 ± 2 g, female) aged 6 weeks were purchased from Seoul

Oriental Bio Center and used in accordance with the "Guide for the Care and Use of Laboratory Animals" provided by of the Institute of Samsung Biomedical Research. Breast cancer tumors were initiated on the right flank of Balb/c nude mice by subcutaneously implanting MDA-MB-231 cells.

In vivo MR imaging

In vivo MR imaging was observed using the mouse treated with the RMNs. The T_2-weighted MR images were observed with a 7.0 T Bruker-BioSpin MR scanner (Fallanden, Switzerland) and were collected at predetermined time points (pre, 1 h and 24 h). The fast spin echo (FSE) sequences were set the followed parameters: the field of view (FOV) = 3.56 × 2.56 × 2.56 cm^3, slice thickness (SL) = 1 mm, TR = 2000 ms, and TE = 45 ms.

In vivo NIRF imaging

To investigate in vivo biodistribution and NIRF imaging of RMNs and Ce6, RMNs (3 mg/kg body of Ce6) was injected intravenously into the tumor-bearing mice through the tail vein. NIRF images were collected using an IVIS imaging system (MA, USA) with a self-fixed excitation wavelength of 650 nm at the different time intervals (pre, 1 h and 24 h). After 24 h post-injection, the mice were sacrificed, and the tumor and other vital organs were isolated for ex vivo NIRF analysis. The fluorescence intensity was quantified by the IVIS imaging system.

In vivo PDT treatment

To investigate the photodynamic therapeutic efficacy of RMNs, group 1 (saline), group 2 (free Ce6) and group 3 (RMNs) (dose equivalent to a Ce6 of 3 mg per kg

body) were injected by the tail vein (n = 5). After 24 h post-injection, the tumor domains of group 1-3 were irradiated using a 660 nm laser at 250 mW/cm^2 for 20 min. The weights of the mice were measured over the period of post-treatment and the tumor volumes were also estimated every two days based on the following equation:

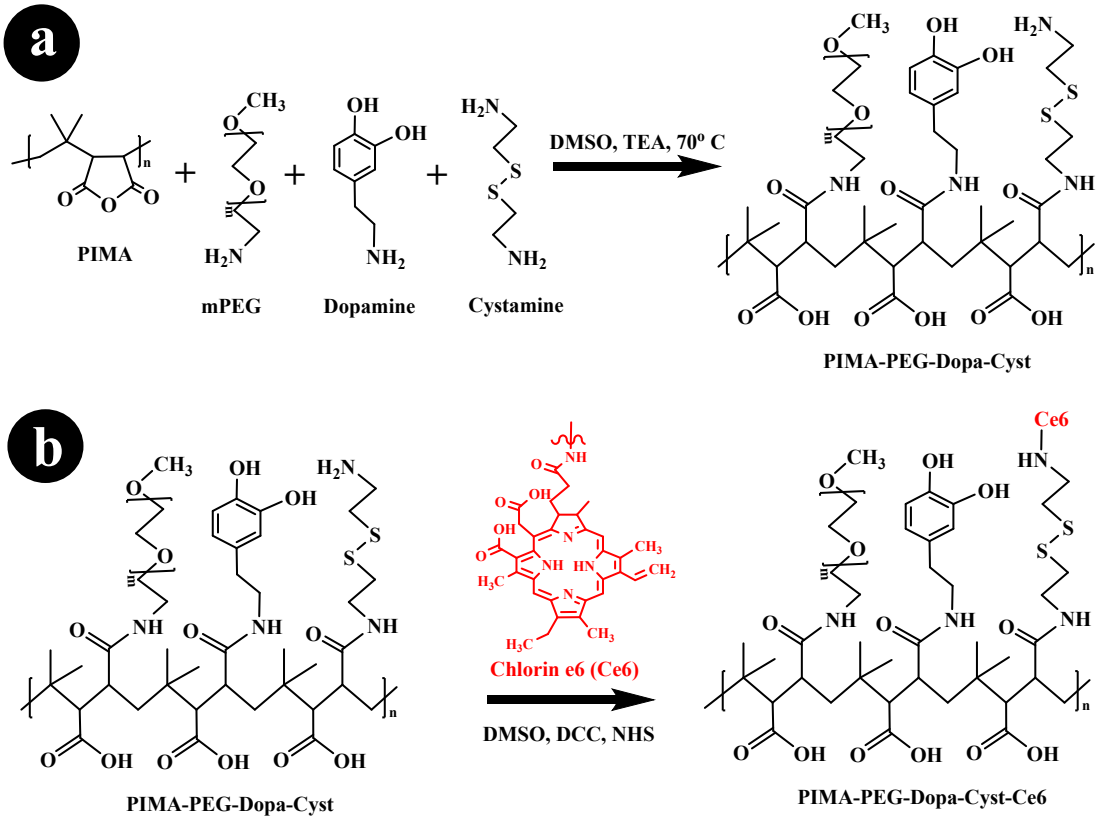
$$V = a^2 \times \frac{b}{2} \qquad (2)$$

where a and b are width and length of tumor by Vernier caliper measurements.

All mice were sacrificed after 15 days treatment and sections of tumor tissue were stained using hematoxylin and eosin (H&E) and TUNEL for histological assays.

Statistical Analysis

Quantitative data were expressed as the mean ± SD from several independent experiments. Differences between two groups were analyzed using Student's t test, and when *P < 0.05 or **P < 0.01 were considered significant.



Scheme S1. Synthetic route of polymer intermediate ligand (PIMA-PEG-Dopa-Cyst) (a) and polymeric ligands (PIMA-Dopa-Cyst-Ce6) (b)

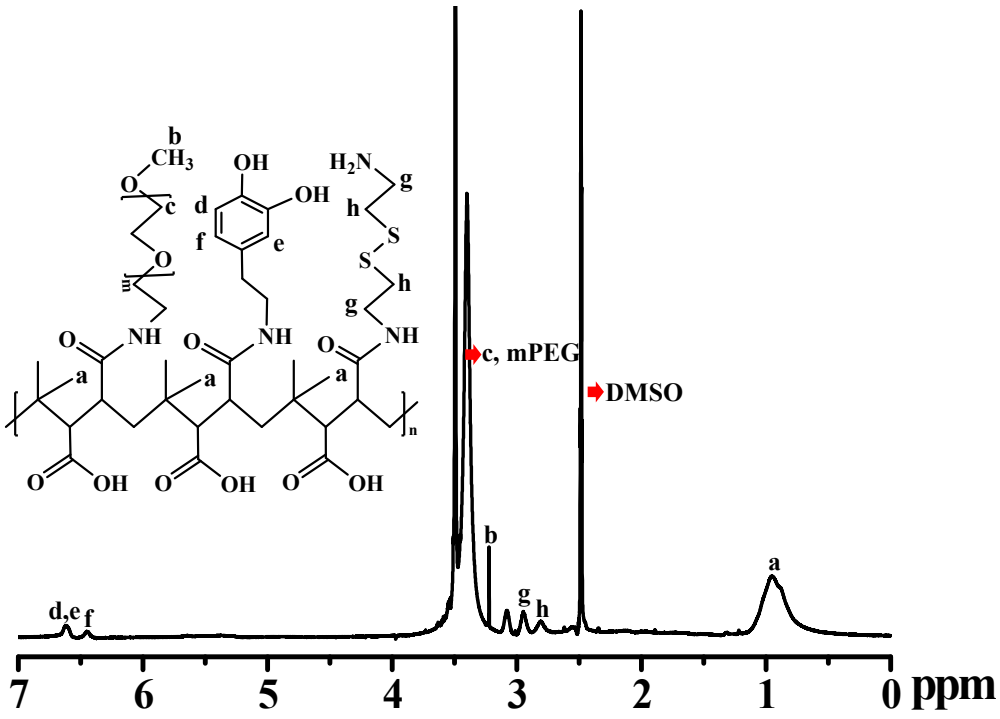


Fig. S1. ^{1}H-NMR analysis of PIMA-PEG-Dopa-Cyst polymer ligand

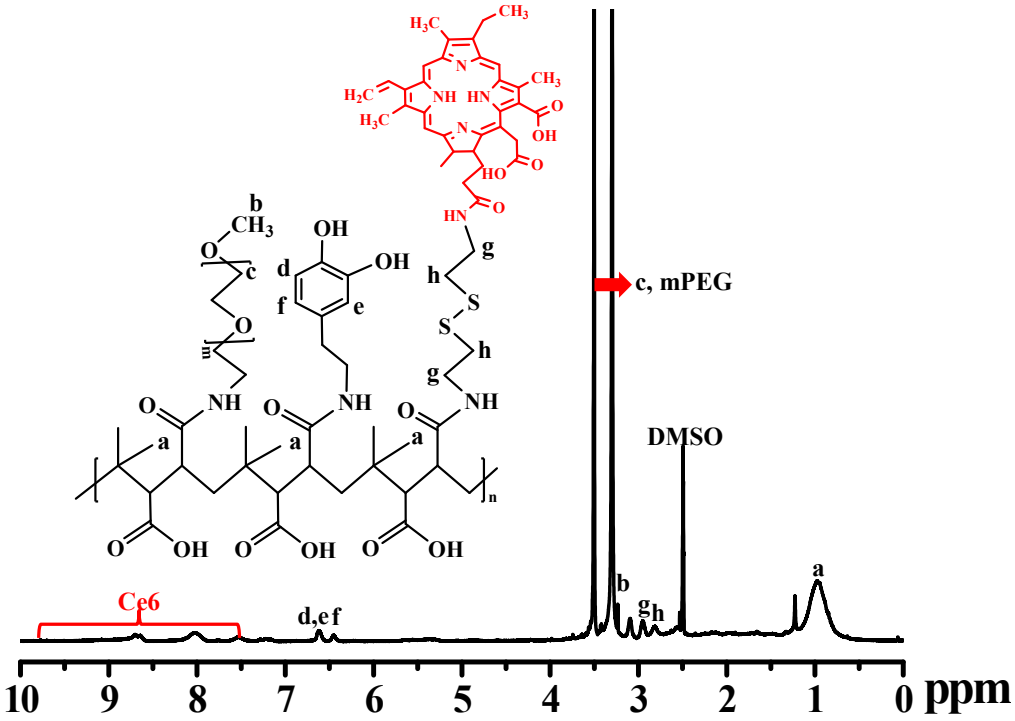


Fig. S2. ^{1}H-NMR analysis of PIMA-PEG-Dopa-Cyst-Ce6 polymer ligand

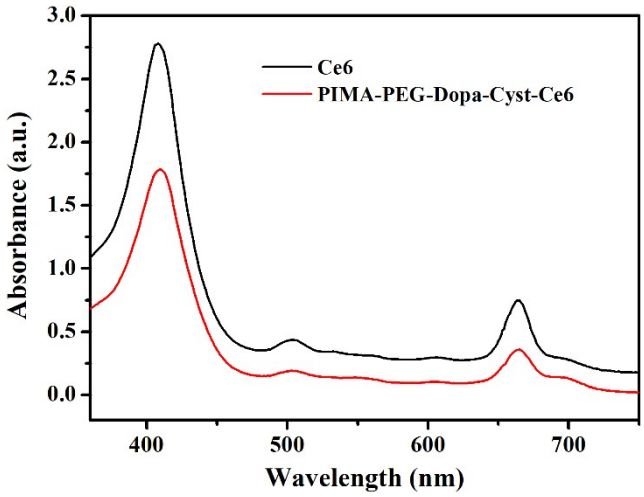


Fig. S3. UV/Vis absorption spectra of free Ce6 and PIMA-PEG-Dopa-Cyst-Ce6 polymer ligand.

Table S1 Structural characteristics of PIMA-PEG-Dopa-Cyst-Ce6 polymer ligand

Polymer ligand	Degree of Polymerization	DS (mPEG)[a]	DS (Dopa)[a]	DS Cyst[a]	DS (Ce6)[b]	Mn[c]
PIMA-PEG-Dopa-Cyst-Ce6	40	～5	～27	～8	～8	～26100

[a]Degree of substitution (DS) of mPEG, Dopa and Cyst on the basis of the ^{1}H-NMR results

[b]Degree of substitution (DS) of Ce6 on the basis of the fluorescence intensity

[c]As determined by ^{1}H-NMR

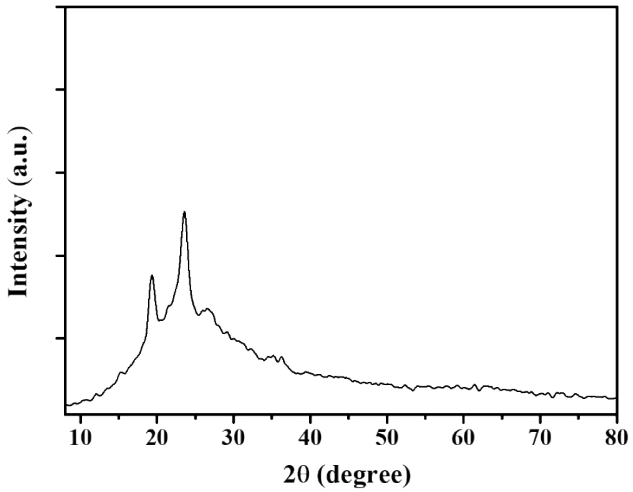


Fig. S4. XRD patterns of RMNs

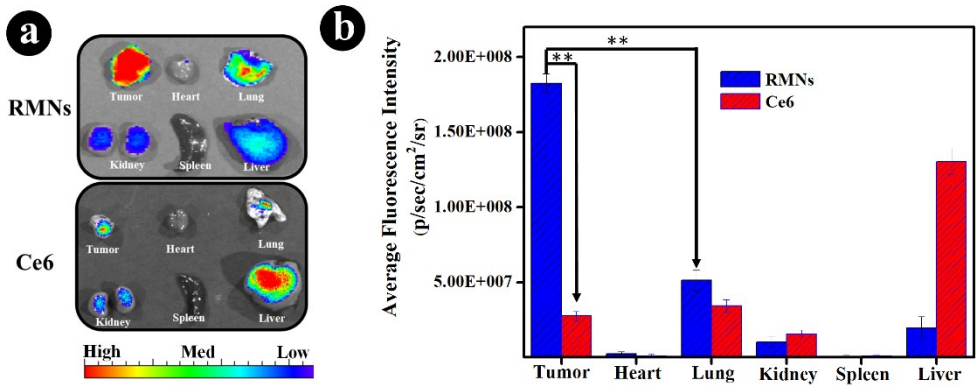


Fig. S5. Ex vivo NIR imaging of MDA-MB-231-bearing mice after intravenous injection of the RMNs and free Ce6 at 24 h after injection (including tumor, heart, lung, kidney, spleen, and liver) (a). Average fluorescence signal intensities of tumors and main major isolated from the tumor-bearing mice at 24 h after injection (b). A quantification of the ex vivo tissues and tumor were recorded as fluorescence intensity (p s^{-1} cm^{-2} sr^{-1}). All data are represented as the mean and SD (n = 3). ∗∗ is represented as significant ($P < 0.01$).