Supplementary Information

The influence of Gd-DOTA conjugating ratios to PLGA-PEG micelles
encapsulated IR-1061 in bimodal over-1000 nm near-infrared fluorescence
and magnetic resonance imaging

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Methods:

The absorption spectra of PLP-D [70], PLP, and Gd-DOTA were measured by using an ultraviolet/visible/near-infrared spectrophotometer (V770, JASCO, Inc., Japan) to determine the absorption peaks due to Gd$^{3+}$ ions. The emissions and absorptions due to Gd$^{3+}$ ions in the structures of Gd-DOTA molecules and PLP-D [70] were confirmed using a fluorescence spectrophotometer (RF-5300PC; Shimadzu Co., Kyoto, Japan). The excitation wavelength was set to the peak wavelength around 270 nm. The absorbance against emission wavelength was set to the peaks around 312 nm and 556 nm.

Fourier-transform infrared spectroscopy (FTIR): FTIR spectra were measured on an FTIR spectrometer (FT/IR-6500; JASCO, Tokyo, Japan). Samples were mixed with KBr (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) and the mixtures were ground into powder. The powder samples were pressed into pellets and set into the system for characterization.

Cell Culture: HeLa cells (human cervical cancer cells) and Colon-26 (murine colon carcinoma cells) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA); Minimum essential medium (MEM) and phosphate buffer saline (PBS) were purchased from the Thermo Fisher Scientific Inc. (Waltham, MA, USA), fetal bovine serum (FBS) was purchased from Biowest (Nuaille, FRA), penicillin-streptomycin (PS) was purchased from Sigma–Aldrich (St Louis, MO, USA). Cells were cultured in MEM supplemented with 10% (v/v) FBS, and PS (100 units/mL) at 37 °C in a humidified incubator containing 5% CO$_2$ in the air.

Cytotoxicity assays: the cell cytotoxicity of micelles was conducted by a standard WST-8 (Dojindo, Kumamoto, Japan) proliferation assay as follows: HeLa and Colon-26 cells were cultured in 96-well microtiter plates (Violamo Inc.) with a concentration of 5 × 10$^4$ cells/well by MEM incubated at 37 °C in a humidified atmosphere containing 5% CO$_2$. The cells were incubated with various
concentrations 1–100 μg/ml of PLP-D [70] for 0, 6, 24, 48 and then washed with PBS several times. The viability by WST assay was measured in a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) under the absorption at 450 nm.

Zeta (ζ) potential: the hydrodynamic size distribution of micelles was characterized by DLS using a zeta-potential & particle size analyzer ELSZ-2000 system (Otsuka Electronics, Osaka Ltd. Osaka, Japan). Micelles were prepared at 0.5 mg/mL concentration in KCl (10 mM) at various pH values for measuring ζ potential and determining isoelectric points.
Fig. S1: Gd$^{3+}$ has intrinsic absorptions around 270 nm (a), and absorption due to emissions at 312 nm and 556 nm (b), together with an emission at 312 nm from free Gd-DOTA and at 556 nm from the PLGA-PEG conjugated Gd-DOTA (c).
Fig. S2: $^1$H NMR spectrum of PLP-D [70] micelles in CDCl$_3$. 

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Fig. S3: $^1$H NMR spectrum of PLGA-PEG-COOH polymer in CDCl$_3$. 
Fig. S4: $^1$H NMR spectra of PLGA-PEG-COOH, synthesized PLP-D polymer before micelle formation and Gd-DOTA butylamine in DMSO-d$_6$. 
Fig. S5: FTIR spectra of PLP-D [70], PLGA-PEG-COOH, and Gd-DOTA.
Fig. S6: Relaxation rates of PLP-D [0-100] in water (a), saline (b), PBS (c), and albumin solution (d) at 0 min, and in water (e), saline (f), PBS (g) and albumin solution (h) at 30 min.
Fig. S7: Isoelectric points depend on pH values of the neutral surface micelles (PLP), negatively charged micelles (PLP-COOH), and micelles with Gd-DOTA on the surface (PLP-D [70]).
Fig.S8: Cytotoxicity assays of Colon-26 (a) and Hela (b) cells incubated with various PLP-D [70] micelle concentrations.