Supplementary Material (ESI) for Chemical Communications

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Multifunctional Perfluorocarbon Nanoemulsions for ^{19}F-based Magnetic Resonance and Near-Infrared Optical Imaging of Dendritic Cells

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Preparation of IRDye800-coated PFOB nanoemulsions. To fabricate perfluorooctylbromide (PFOB, Aldrich, St. Louis, MO)] nanoemulsions, PFOB liquids were emulsified in an aqueous solution using surfactant mixtures. A surfactant mixture comprising of 64 mol % lecithin (L-α-Phosphatidylcholine 95%, chicken egg, Avanti Polar Lipids, Alabaster, AL), 35 mol % cholesterol (Avanti Polar Lipids, Alabaster, AL) and 1 mol % 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG(2000) Amine, Avanti Polar Lipids, Alabaster, AL) were dissolved in chloroform, and the organic solvent was evaporated using a rotary evaporator and a vacuum oven (50°C) for 24 h. After dispersing the surfactant mixture into sterilized distilled water, the solution was sonicated. PFOB liquids (40% v/v), glycerin (2.0% w/v), surfactant mixture and distilled water were mixed for 30 s using a homogenizer. The mixture was processed through an emulsifier (M-110S, Microfluidics, Newton, MA) at 20000 psi for 3 min. The fabricated PFOB nanoemulsions were stored at 4°C. As a near-infrared fluorophore, IRDye800 NHS ester was purchased from LI-COR Biosciences (Licoln, Nebraska). For conjugation, an aliquot of PFOB nanoemulsion solutions were mixed with IRDye800 NHS ester (50 μL/mg) dissolved in water. The conjugation reaction was carried out for 2 hrs at 20 °C. IRDye800-coated PFOB nanoemulsions were separated using FPLC system.

Characterization of IRDye800-coated PFOB nanoemulsions. The mean particle diameters and zeta (ζ)-potentials of the IRDye800-coated PFOB nanoemulsions were determined using a particle size analyzer (ELS-Z, Otsuka Electronics, Japan). The intensity autocorrelations were measured at a scattering angle (θ) of 90° with electrophoretic light scattering at 25 ± 0.1°C. TEM images were obtained using a 200 kV Field Emission Transmission Electron Microscope (JEM-2100F, JEOL, LTD, Tokyo, Japan). IRDye800-coated PFOB nanoemulsions were also prepared as a thin liquid layer supported on a cryo-grid, and were immediately plunged in liquid ethane to prevent evaporation from the thinly spread sample. The frozen grids were stored in liquid nitrogen and transferred in a GATAN model 630 cryotransfer (Gatan, Inc., Warrendale, PA) in liquid nitrogen at a temperature of approximately -185°C. Direct imaging was carried out using an acceleration voltage of 120 kV and a Multiscan 600W charge-coupled device (CCD) camera (Gatan, Inc, Pleasanton, CA) at a temperature of approximately -170°C.

Fluorescence and ^{19}F MR spectroscopy of nanoemulsions. The emission spectra of IRDye800-coated PFOB nanoemulsions were measured using a fluorescence spectrometer (LS 55, PerkinElmer Instruments, Wellesley, MA). All ^{19}F spectral and imaging experiments of IRDye800-coated PFOB nanoemulsions were performed on a 600 MHz (14 T) Bruker NMR spectrometer (Avance DMX600, Bruker, Rheinstetten, Germany) equipped with a triple gradient system for microscopic imaging. The maximum gradient strength was 200 G/cm, and a 5 mm double-tuned ^{1}H/^{19}F saddle-type RF coil was used. For ^{19}F spectral measurement, IRDye800-coated PFOB nanoemulsions were inserted into standard Wilmad 5 mm NMR tubes, and fluorine spectra (30° flip angle; 2 acquisitions; 5 sec acquisition time) were acquired from the sample.

***In Vitro* and *In Vivo* Fluorescence and ^{19}F MR imaging of DCs**. DC2.4 cells (murine dendritic cells) were cultured in 10 mm dishes (1×10^{7}/dish) in Dulbecco's modified Eagle Medium (DMEM; Gibco, Invitrogen, Carlsbad, CA) supplemented with 10% heatinactivated FBS, 50 IU/ml penicillin and 50 μg/ml streptomycin. Cells were incubated with 0.63 ~ 100 μl/ml IRDye800-coated PFOB nanoemulsions for 24 h. After incubation, labeling efficiency was analyzed by flow cytometry. For fluorescence imaging, DC2.4 were incubated with 50 μl/ml IRDye800-coated PFOB nanoemulsions

for 24 h at 37 °C. After washing with PBS, the labeled cells were fixed with Cytofix/Cytoperm solution and stained with DAPI in PBS. To detect intracellular localization of nanoemulsions, DC2.4 cells were stained with the lysosomal marker, Lysosome-Associated Membrane Glycoprotein-1 (LAMP-1) by incubating with FITC-conjugated rat anti-LAMP-1 monoclonal antibody (1D4B) (BD PharMingen) for 30 min at room temperature. Fluorescence images were obtained on a Deltavision RT deconvolution microscope (Applied Precision Technologies, Issaquah, WA).

For *in vivo* fluorescence and MR imaging, DC2.4 (5×10^6 cells/ml) labeled with IRDye800-coated PFOB nanoemulsions were resuspended in 100 μl HBSS buffer and were subcutaneously injected into a mouse. All experiments involving mice were performed in accordance with the Korean NIH guidelines for the care and use of laboratory research animals. The mice were anesthetized with 300 μl of a 2.5% avertin solution (2,2,2-tribromoethanol-*tert*-amyl alcohol, Sigma). Thereafter fluorescence images (5 s exposure) were obtained home-made small animal imaging system using an excitation light source (760 nm) and an emission filter (845WB55). All images were processed with Simple PCI software (Compix Inc., Cranberry Township, PA). ^{19}F-MR images of the mouse were performed with 4.7 T Bruker scanner (BioSpec, Rheinstetten, Germany) using a double-tuned ^{1}H/^{19}F Birdcage coil design (outer/inner: 59/35 mm). For the ^{19}F-MR image, the mouse was imaged with a gradient echo sequence (128 × 128 matrix; 60 × 60 mm^2 FOV; 50.0 ms TR; 2 ms TE; 5 mm slice thickness; 20 acquisitions).

Cell viability assay. Cell viability was measured by analyzing cleavage of thiazoyl blue tetrazolium bromide (MTT; Sigma) by succinate dehydrogenases of living cells to yield formazan. After incubating DC2.4 cells (1×10^4 cells/0.1 ml) with IRDye800-coated PFOB nanoemulsions for various times in flat-bottomed 96-well plates (Corning Costar, Cambridge, MA, USA), MTT (10 μl/well of a 5 mg/ml MTT stock solution in PBS) was added directly to each well and plates were incubated at 37°C for 4 h. To assay MTT reduction colorimetrically, dimethyl sulfoxide (DMSO; Sigma) was added to solubilize formazan and absorbance was measured at 562 nm.

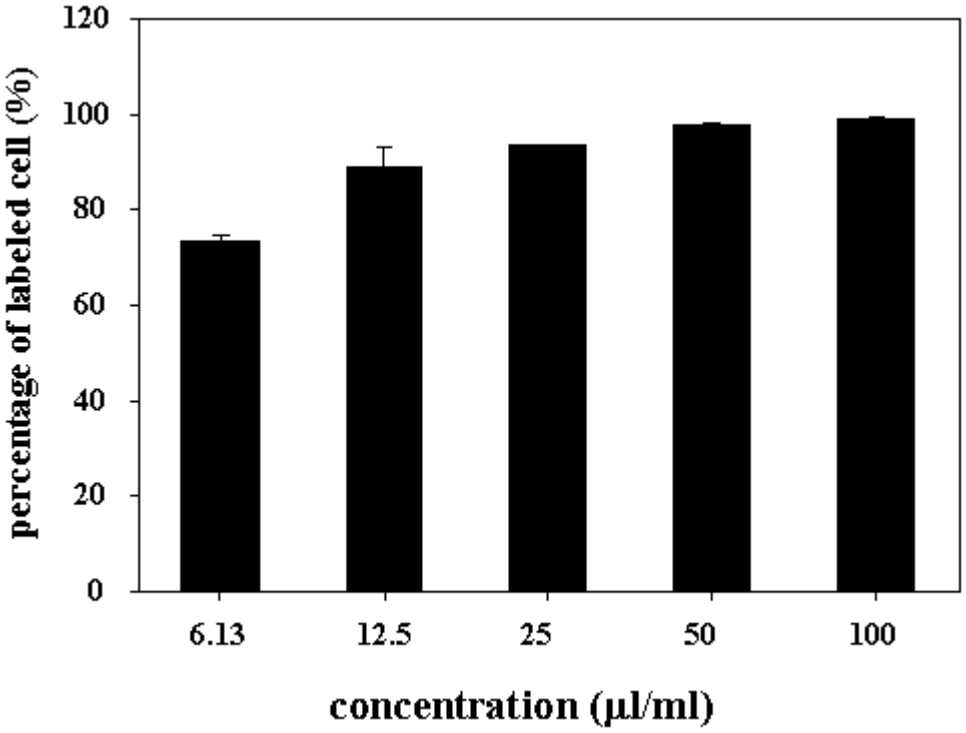

Figure S1. Labeling efficiency of dendritic cells labeled with IRDye800-coated PFOB nanoemulsions.

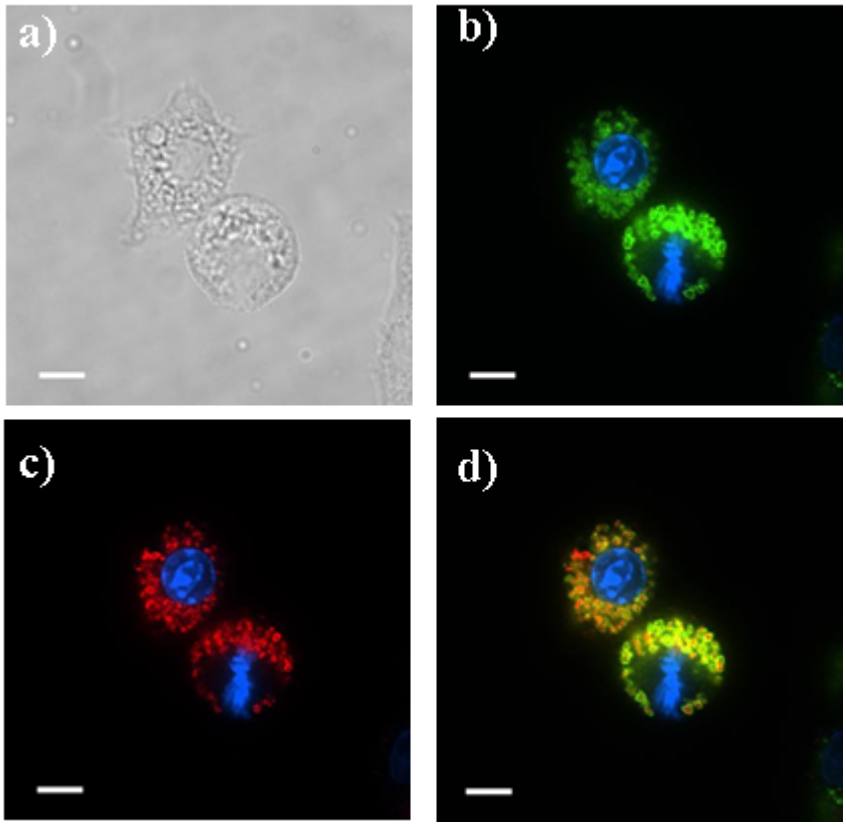

Figure S2. The localization of IRDye800-coated PFOB nanoemulsions within dendritic cells. (a) : DIC (differential interference contrast), (b) : FITC- anti-LAMP-1 stained (green; lysosome), (c) : IRDye800 (red), (d) merged. Scale bars : 10 μm. Most of IRDye800-coated PFOB nanoemulsions were localized in the lysosomes.

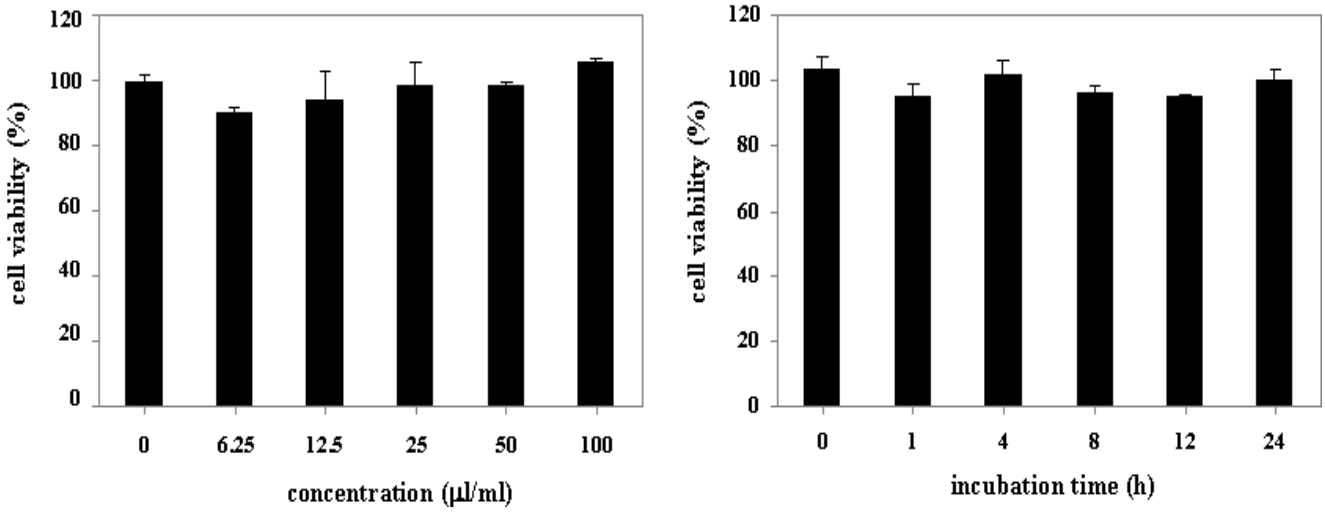

Figure S3. Viability of dendritic cells labeled with IRDye800-coated PFOB nanoemulsions