# Supplementary Information

## The impact of Gd-DOTA spatial distribution within PLGA-PEG micelles encapsulated IR-1061 on bimodal over–1000 nm near-infrared fluorescence and magnetic resonance imaging

**Thi Kim Dung Doan,*[ab] Masakazu Umezawa,[c] Kyohei Okubo,[c] Masao Kamimura,[c] Masayuki Yamaguchi,[b] Hirofumi Fujii,[b] Kohei Soga.[ac]**

*[a] Research Institute for Biomedical Science, Tokyo University of Science, 2669 Yamazaki, Noda, Chiba 278-0022 Japan.*
*[b] Division of Functional Imaging, Exploratory Oncology Research & Clinical Trial Center, National Cancer Center, 6-5-1 Kashiwanoha, Kashiwa 277-8577, Japan.*
*[c] Department of Material Science and Technology, Tokyo University of Science, 6-3-1 Niijuku, Katsushika-ku, Tokyo 125-8585, Japan.*

## Methods

### Micelle preparation

$$D[m(\%)] = \frac{m(D)}{m[(D)] + m[(PLGA - PEG)]} \times 100 \ (1)$$

*PL-D-P/PL-N-P micelles*: PLP–D were mixed with PLGA–PEG at various special concentrations (D [m (%)]) from the equation (1): where D = PL-D-P/PL-N-P, and [m (%)] = 70 % of 30 mg (total amount of polymers). 30 mg of mixed polymers were dissolved in a solution containing 996 µl ACN and 4 µl IR–1061 in ACN (5 mg/ml), and 2 mL of Milli-Q water was added into PL-D-P samples. The solutions were stirred for about 20 hours to evaporate the ACN. Ultimately, all products were purified using a gel filtration column in water.

### Micelle characterization

*OTN–NIR absorption:* An ultraviolet/ visible/ NIR spectrophotometer (V770, JASCO, Inc., Japan) was used to measure the absorption spectra.

*OTN–NIR emission:* A spectrometer (NIR–256–1.7, Avantes, Apeldoorn, The Netherlands) equipped with a fiber-coupled laser diode (SP–976–5–1015–7, Laser Components GmBH, Olching, Germany) of 980–nm excitation source was used to obtain the emission spectra. The optical set-up includes a 980–nm excitation source (0.67 W) irradiated the solution sample in a 10–mm–path–length cuvette, and the emission was collected perpendicularly to the excitation plane in a Q–pod (Quantum Northwest, WA, USA) integrated with NIR spectroscopy.

**(a)**

$$Yield\ (\%) = \frac{weight\ of\ PLP - D}{weight\ of\ PLGA - PEG - COOH + \ Gd - DOTA} \times 100\ (1.a)$$

**(b)**

$$Efficience\ (\%) = \frac{mol\ number\ of\ Gd(III)ion}{mol\ number\ of\ PLP - D} \times 100\ (1.b)$$

**(c)**

| Conjugation | Yield [a] (%) | Conjugation efficiency [b] (%) |
|---|---|---|
| PLP-D | >37 | >90 |
| PL-D-P | >30 | >95 |
| PL-N-P | >56 | >96 |

*[a] Yield = weight of polymer before and after conjugation; [b] efficiency = Gd molar number per molecular weight*

**(d)**

| *Micelles* | *Estimated Gd (III) mass (mg/mL) [a]* | *Estimated Gd (III) mass (mg/mL) [b]* | *Estimated Gd ion [%][c]* | *Actual Gd ion [%]) [d]* |
|---|---|---|---|---|
| *PLP-D* | *0.23* | *0.168±0.01* | *70* | *514* |
| *PL-D-P* | *0.23* | *0.198±0.01* | *70* | *60* |
| *PLP* | *0* | *0* | *0* | *0* |

*[a] Gd (III) mass (calculated)/10mg of micelle [b] actual Gd (III) mass (from ICP) /10mg of the micelle. [c] Gd (III) wt% (calculated)/10mg of the micelle. [d] Actual Gd (III) wt% (from ICP) /10mg of the micelle.*

Figure S.1: Yield (a) and efficiency (b) formulas and (c) actual values from ICP. (d) Gd ion estimated and the actual values from ICP.

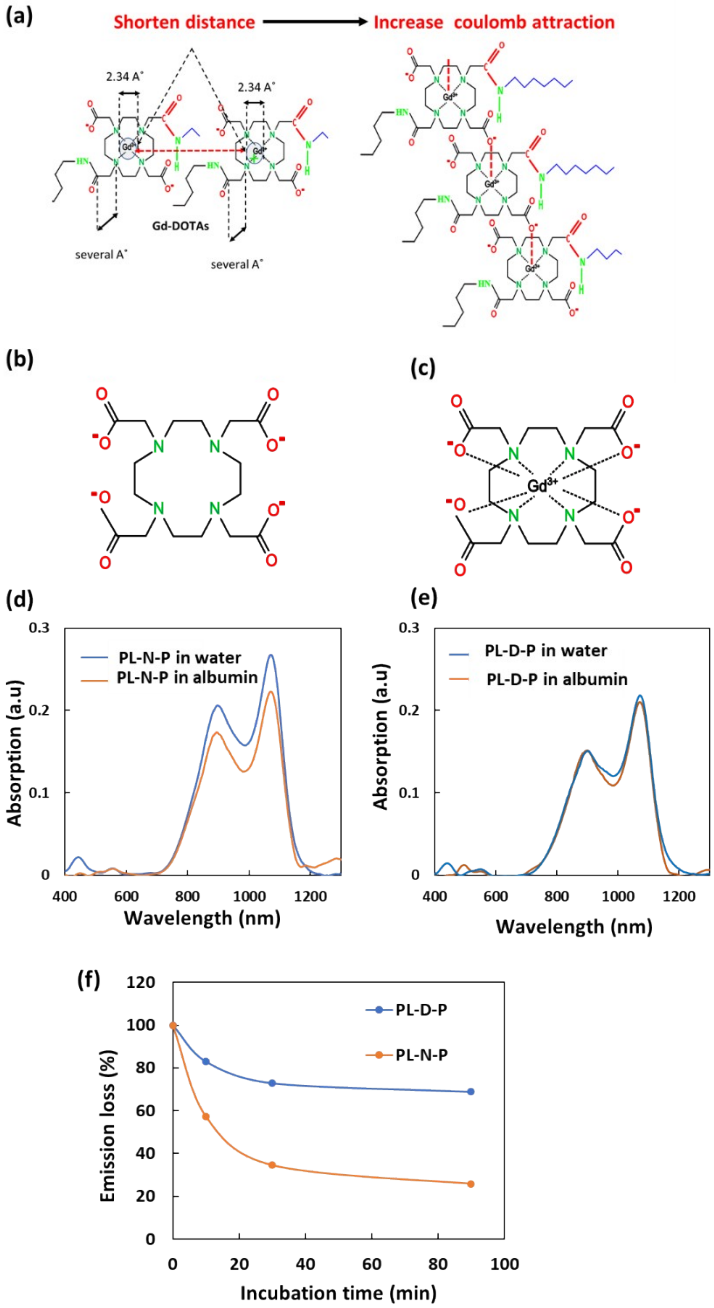


Figure S.2 (a) The cross-coordination induced by Coulomb interaction between Gd-DOTA molecules at extremely close contact. The structure of (no Gd)-DOTA (b) and Gd-DOTA (c). The absorption of PL-N-P (d) and PL-D-P (e) in water and albumin. [PL-N-P made from PLGA-PEG conjugated (no Gd) -DOTA in the middle, PL-D-P made from PLGA-PEG conjugated Gd -DOTA in the middle.] The fluorescence loss (g) difference in mouse serum between PL-N-P [70] and PL-D-P [70].

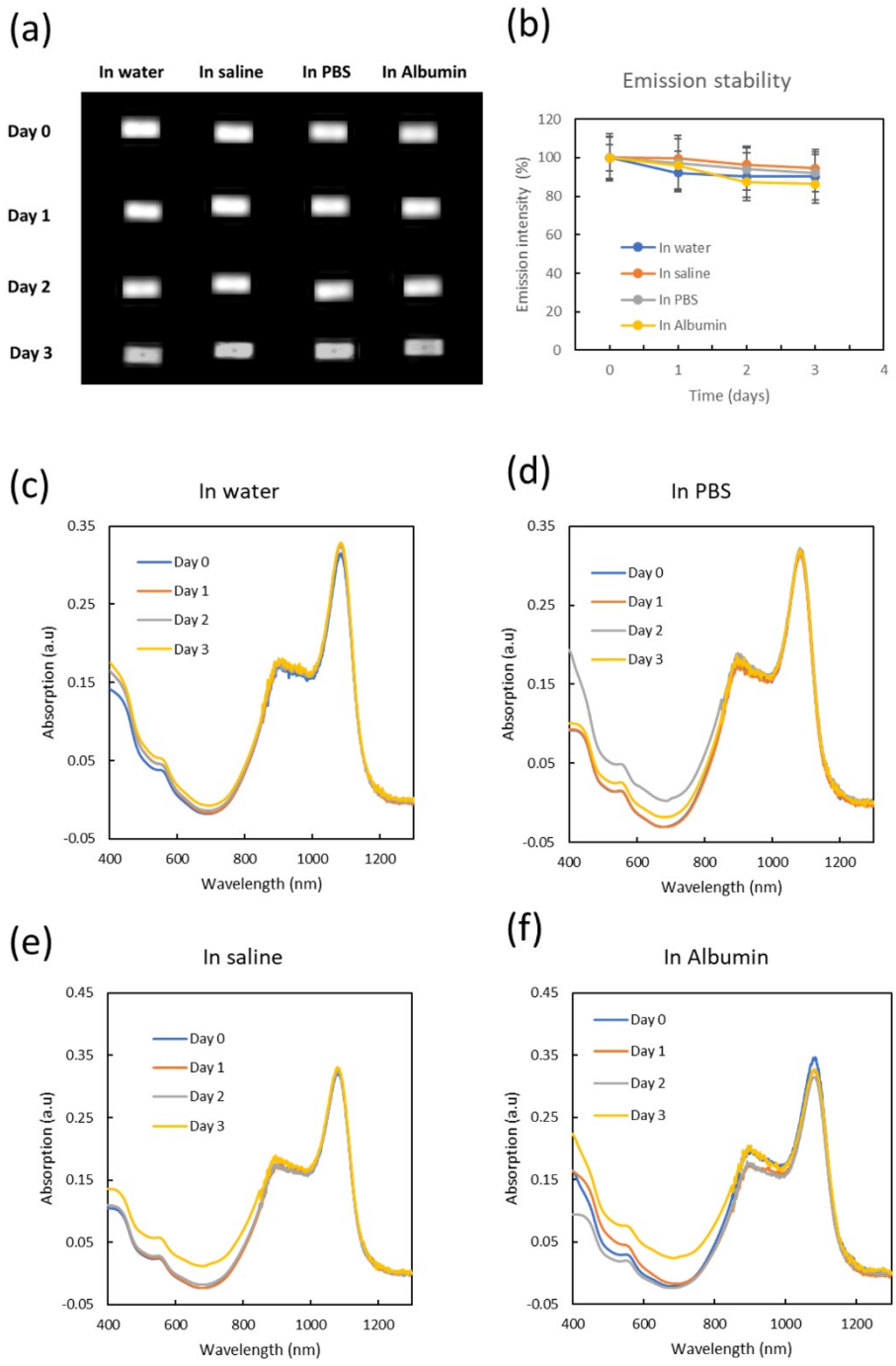


Figure S.3: (a) OTN-NIR images of PL-D-P[70] and (b) the corresponding emission intensity in four media observed within 3 days. (c-f) Absorption spectra of the probe in four media were observed within 3 days.

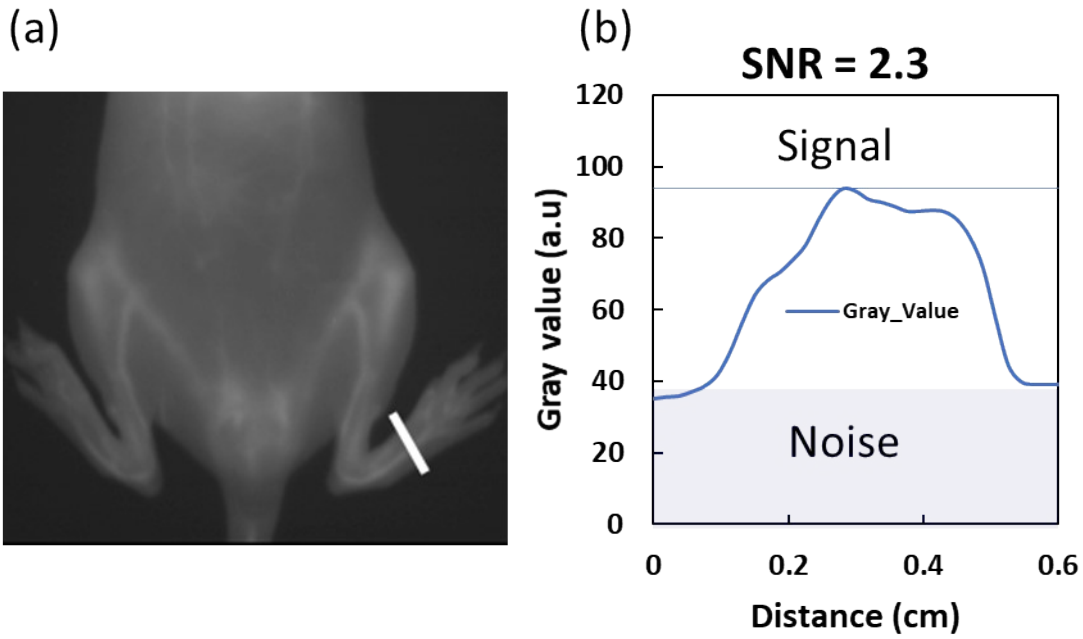


Figure S.4: (a) OTN-NIR image of the mouse injected by PL-D-P [70] at 5 min and the plot profile (b) of the blood vessel corresponding to the fluorescence image.