## **Supplementary Information**

# Near-infrared Absorbing Polymer Nano-particle as a Sensitive Contrast Agent for Photo-acoustic Imaging

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### 1. Apparatus for photo-acoustic measurements



**Figure S1.** Block diagrams for the apparatuses for the photo-acoustic measurements in an optical cell (a) and for the *in vivo* imaging of a mouse (b). In the panel (a), Laser: a dye laser, OB-401, Optical Building Blocks, pumped by a nitrogen laser, OB-4300, Optical Building Blocks; Hydrophone: H9C, Toray Engineering; Oscilloscope: TDS2022C, Tektronix. In the panel (b), Laser: LT-2211A, LOTIS TII; Hydrophone: V383, Olympus NDT; Oscilloscope: DPO3023, Tektronix; XY stage: SGAM20-35XY, Sigma Koki.

#### 2. Size distribution and photo-acoustic wave for PNP

Figure S2a shows the size distribution observed by DLS for the PNP of PS containing SiNc. This indicates that the nano-emulsion method yielded the PNP with a narrow size distribution. Figure S2b shows the PA wave from the PNP with various diameters, indicating that the waveform of the PA signal is independent of the particle size.



**Figure S2.** Size distribution (a) and PA signals (b) for PNP-PS containing SiNc with diameters of 42, 72, 95, 122, 170 nm. The PA signals were vertically shifted for visibility. The waveform of the PA signal is not dependent on the particle size.

#### 3. Photo-acoustic signal of PNP

Figure S3 shows the PA signals for the PNP prepared with various materials. The data (a)-(c) show the PA signals from the PNP of polystyrene (PS) containing SiNc, DiD, and DiD with the diameter of 100 nm. These waveforms are the same, indicating that the waveform was not dependent on the chemical structure of the light absorber. The surfactant dependence can be discussed by the comparison of the wave (a), (d), and (e), which are the PA signals from the PNPs of PS/SiNc prepared with the surfactants, Tween 20, SDS, and PEGylated phospholipid, respectively. This indicates no effect of the surfactant on the waveform. The wave (a), (f), and (g) show the PA signals from the PNP made from the different polymers: PS, poly(methyl methacrylate) (PMMA), and poly(butyl methacrylate) (PBMA). The normalized waveforms agreed with each other, indicating that the waveform is independent of the polymer.



**Figure S3.** Photo-acoustic signals from PNP prepared in various conditions. The signals a, b, and c were observed for the PNP of PS containing SiNc, DiD, and DiR, respectively, which were prepared with the surfactant of Tween 20. The signals d and e were measured for the PNP of PS containing SiNc prepared with the surfactant SDS and DSPE-PEG2000/Tween20. The signals f and g were measured for the PNP of PMMA and PiBMA, respectively. The diameters for the PNPs were 95 ± 5.0 nm. All of the waveforms were the same, indicating that the waveform of the PA signal is not dependent on the dye, surfactant, and polymers.

#### 4. Emission quantum yield of SiNc in PNP

The emission quantum yield (QY) of the PNP with SiNc was observed as the emission intensity ratio relative to rhodamine 6G, which has the QY of 0.93 [1-3]. Table S1 summarizes the emission QY of the PNP containing SiNc. The QY slightly decreased with the increase of the dye fraction in the PNP. The QY for the PNP was less than 1 %, indicating that more than 99 % of the absorbed light energy is converted to the heat.

Dye fraction / wt%	Diameter / nm	Quantum Yield
4.5	72	0.010
6.4	64	0.004
15.0	63	0.001
25.5	62	0.001
6.0	96	0.004
6.8	123	0.003

Table S1. Emission Quantum Yield of PNP Containing SiNc

#### 5. Temperature dependence of photo-acoustic signal of Au-NR

Figure S4 shows the PA signal from the Au-NR with the size of  $80 \times 25$  nm at 4 and 20 °C. No PA signal was obtained at the temperature of 4 °C. The thermal expansion factor of water at 4 °C is zero; therefore, the acoustic wave due to the volume expansion is not observed at 4 °C. This indicates that the most of the energy supplied to Au-NR diffused into the water medium.



**Figure S4.** Temperature dependence of photo-acoustic wave from Au-NR. at 4 (blue) and 20 °C (red).

#### 6. Time evolution of PA signal of Au-NR and PNP

Figure S5 shows the time evolution of the PA signal against the repeated pulse irradiation. The PA intensity of Au-NR decreased by 75% after 120 s. The Au-NR shows the structural change from a rod-like shape to a sphere [4], resulting in the decrease of the absorption coefficient at the near infrared region. On the other hand, the PA intensity was not altered after the irradiation for 600 s.



**Figure S5.** Time evolution of the photo-acoustic intensity for PNP (red) and Au-NR (blue). The laser was irradiated at a pulse energy of 40  $\mu$ J and a repetition rate of 5 Hz.

#### 7. Dispersion stability of PNP

The top panel in Figure S6a shows the size distribution of PNP-PS-104 containing SiNc. The PNP was dispersed in phosphate buffered saline (PBS, Wako Chemicals). The second panel indicates the histogram just after the dispersion in PBS, indicating that the immediate aggregation of the PNP was not observed. After the dispersion in PBS for 2 weeks, the PNP was stably dispersed in PBS as shown in the third panel of Figure S6a. The bottom two panels in Figure S6a show the size distribution of the PNP in fetal bovine serum (FBS, FB-1365, Biosera). The small peaks at 10 nm correspond to the solutes in the FBS. Also in the FBS, the size distribution was not altered after 2 weeks. This indicates that the PNP prepared here stably dispersed in a physiological condition. Figure S6b indicates the absorption spectra of the dispersion media of FBS after the removal of the PNP by a centrifugation. The black and red curves indicate the spectra after the dispersion of the PNP for 10 min and 2 weeks. There are not observed a distinct absorption peak at 780 nm corresponding to SiNc. This indicates no leakage of the dye fraction into the medium.



**Figure S6.** Size distribution of PNP-PS-104 (a) and absorption spectra of the FBS dispersing the PNP (b). In the panel a, the average diameters are shown at the right hand side of the histogram. In the panel b, the black and red curves indicate the spectra of the FBS after the dispersion of the PNP for 10 min and 2 weeks, respectively.

### 8. PA image of a mouse injected with PNP of PMMA

Figure S7 shows the PA image of a mouse injected with 20  $\mu$ L of the PNP consisting of PMMA at a concentration of 0.04 nM. The diameter and molar absorption coefficient were 180 nm and 5.7 × 10<sup>10</sup> M<sup>-1</sup>cm<sup>-1</sup>, respectively. The SNR of the obtained image was 550. The injection concentration of the PNP of PMMA was 2.5-times lower than that for PNP-PS. The SNR obtained for PMMA at 0.04 nM was similar to that for PS at 0.10 nM. This indicates that the PNP of PMMA enables the PA imaging with higher sensitivity than PNP-PS.



**Figure S7.** Photo-acoustic image of a mouse injected with the PNP consisting of PMMA. The injection concentration and amount were 0.04 nM and 20µL.

## References

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