Supporting information for "Visualizing Systemic Clearance and Cellular Level Biodistribution of Gold Nanorods by Intrinsic Two-Photon Luminescence", Tong et al.

Supporting Video

Intravital TPL imaging of PEG-NRs (gray) flowing in a blood vessel at 20 min after injection. The blood vessel was visualized by transmission illumination (blue). The image size was 105 × 105 μm^2. The video contained 60 frames (256 × 256 pixels/frame) with a scanning rate of 2 µs/pixel.

Table S1. Hydrodynamic diameter and Zeta potential of PEG-NRs.

	Hydrodynamic diameter (half width, nm)	**Zeta potential (mV)**
$mPEG_{2k}$-NR	58.0 (31.8)	0.040
$mPEG_{5k}$-NR	70.8 (41.0)	-0.029
branched PEG-NR	86.9 (51.9)	-0.099

Supporting Figures

Figure S1. Extinction spectra of gold nanorods (GNRs) before (CTAB-NR) and after PEGylation (mPEG-NR). Inset: TEM image of GNRs.

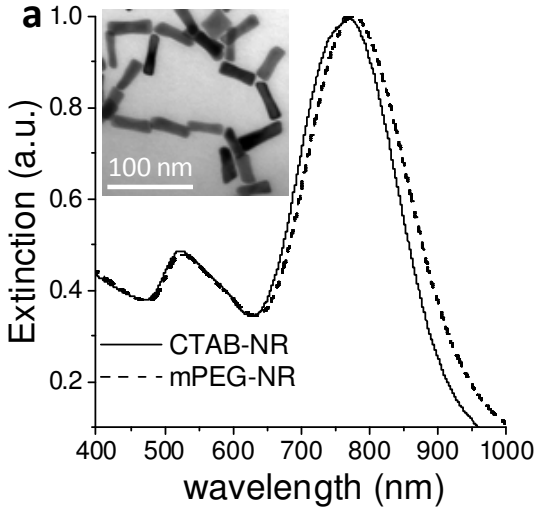

Figure S2. Hydrodynamic diameter of PEG-NRs measured by DLS.

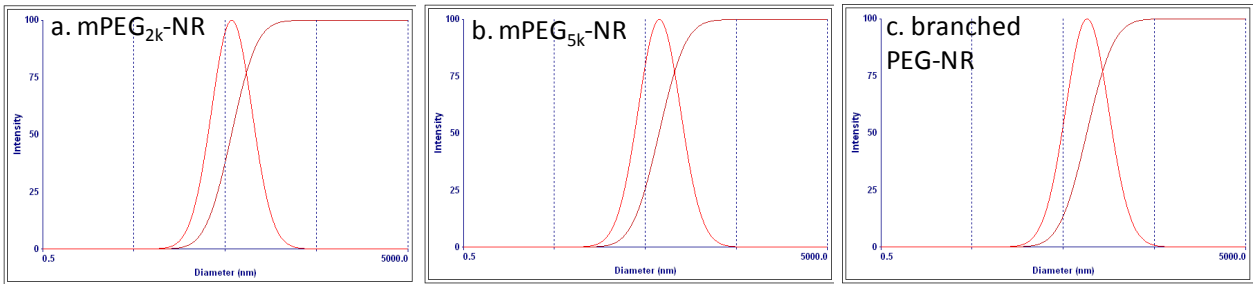

Figure S3. Laser scanning of GNRs embedded in an agarose gel loaded into the mouse ear. The imaging condition was the same to the *in vivo* experiment with a laser power of 42 mW measured after the objective and a scanning speed of 2 µs/pixel for each frame of 256×256 pixels. (a-c) Three continuous frames with acquisition time of 0.4 s/frame. Bar = 20 µm. (d) TPL intensity profile of 9 spots indicated in (a-c). Spots 1-5 represented clusters of GNRs and spots 6-9 represented single GNRs. Some of the small dots did not appear in all three frames, possibly due to the rotation of GNRs in the soft gel. Nevertheless, our result showed that these dots (e.g. 6-9) remained at the same level of TPL intensity in two sequential frames.

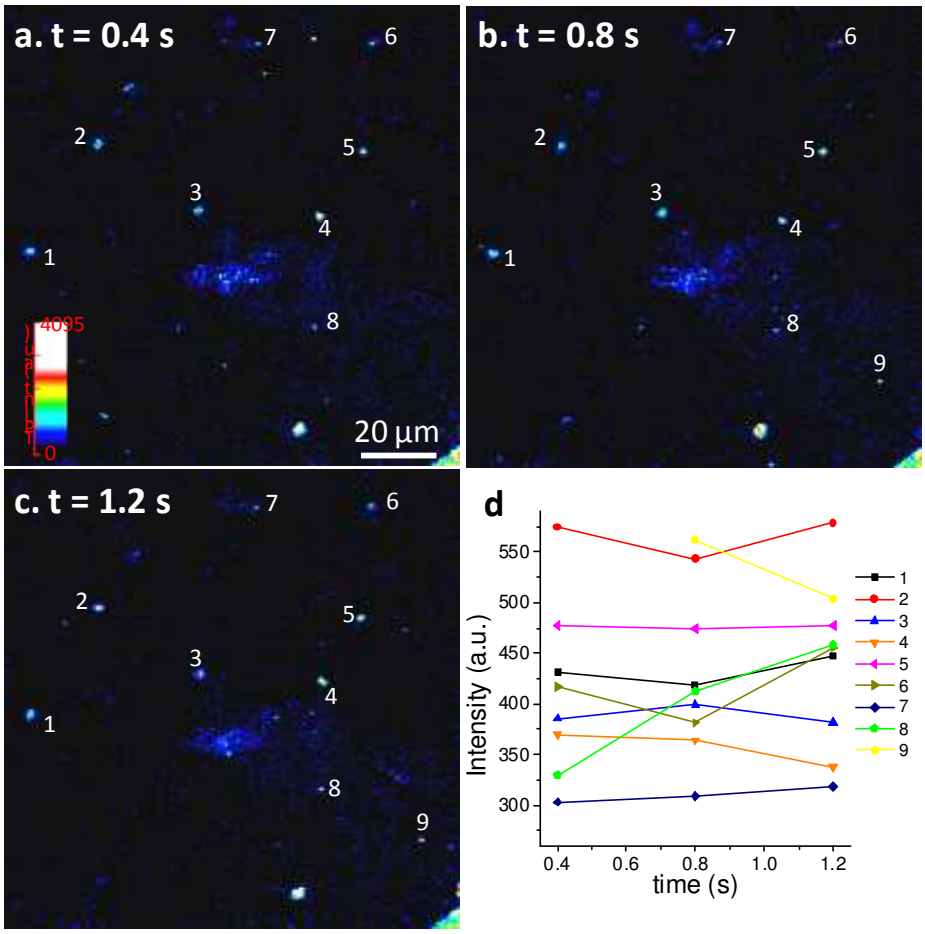

Figure S4. TPL imaging of liver explanted from BALB/c mouse without GNR injection. (a) Strong autofluorescence from liver cells. (b) No TPL signal in the 650 to 700 nm region. Bar = 10 μm.

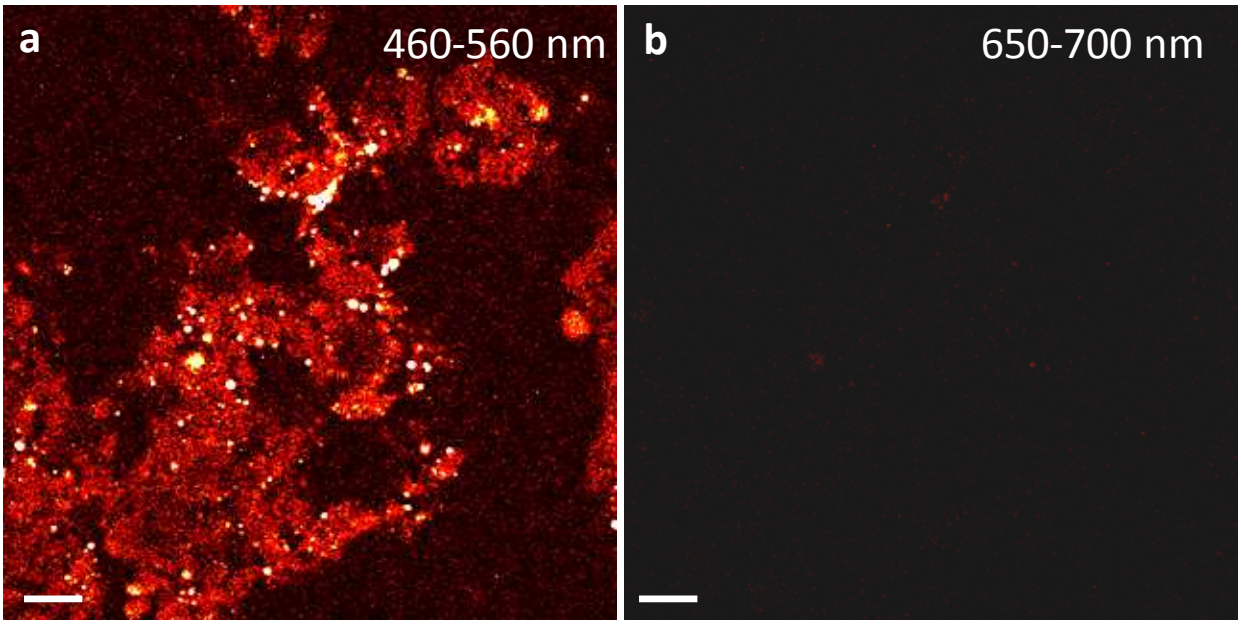

Figure S5. Confocal and TPL imaging of fresh liver explanted from a GNR-injected mouse and labeled with F4/80 antibody. (a) Confocal image of Tricolor-F4/80 antibody (green) labeled Kupffer cells in liver. (b) Spectrum from Tricolor-F4/80 antibody labeled Kupffer cell in the yellow circle in (a). (c) TPL image of GNRs (detected within 650-700 nm) in liver at the same area with (a). The TPL signals from GNRs overlapped with that from F4/80 antibody in (a), indicating that GNRs were accumulated in Kupffer cells. (d) Spectrum of GNRs in the yellow circle in (c). Bar = 10 μm.