

Supporting Information

Biodegradable Germanium Nanoparticles as Contrast Agents for Near-infrared-II Photoacoustic Imaging

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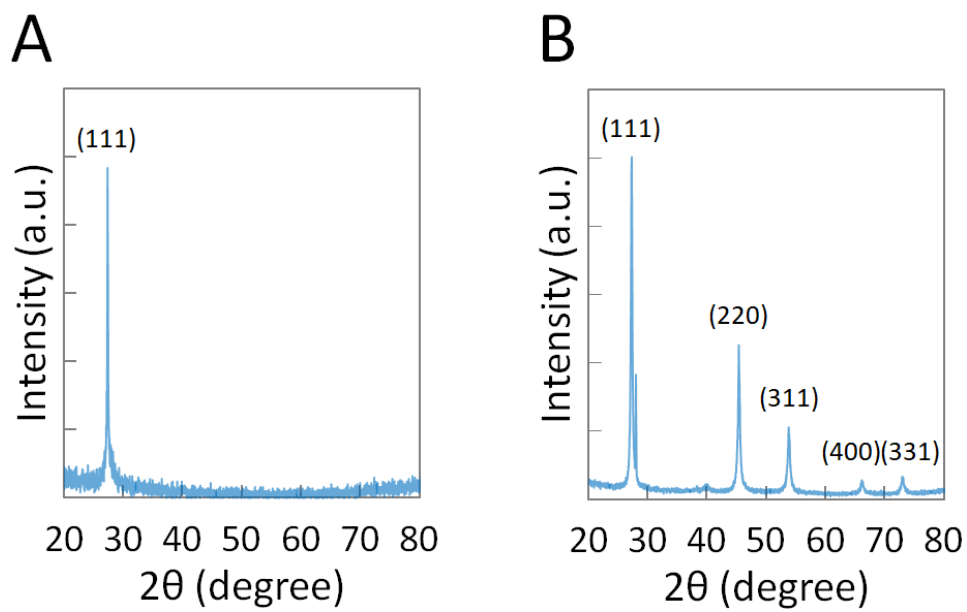


Figure S1. XRD patterns of A) the Ge wafer, which is the starting material for the GeNP synthesis, and B) the BSA-coated GeNPs.

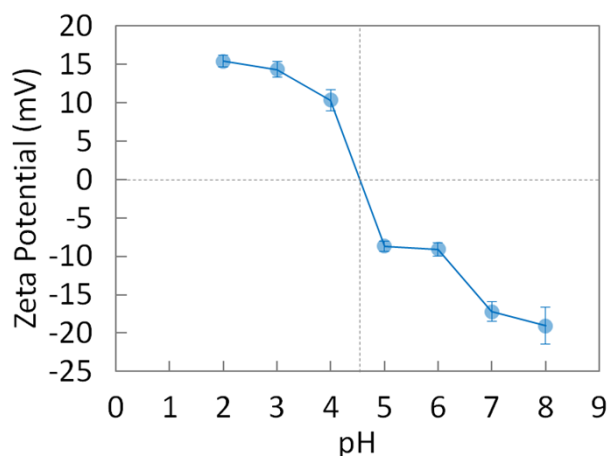


Figure S2. Zeta potential of the BSA-coated GeNPs as a function of pH. The suspensions of the BSA-coated GeNPs in water with pH = 2, 3, 4, 5, 6, 7 and 8 were prepared by using 0.1 M HCl and 0.1 M NaOH. The suspensions were sonicated for 5 min at 25°C prior to the zeta potential measurement. Each sample was measured three times and averaged. The isoelectric point of pure BSA is around pH 4.5.^[S1,S2]

[S1] A. P. Tiwari, S. S. Rohiwal, M. V. Suryavanshi, S. J. Ghosh, S. H. Pawar, Detection of the genomic DNA of pathogenic α -proteobacterium *Ochrobactrum anthropi* via magnetic DNA enrichment using pH responsive BSA@Fe₃O₄ nanoparticles prior to *in-situ* PCR and electrophoretic separation, *Microchim. Acta* **2016**, 183, 675.

[S2] S. S. Rohiwal, R. K. Satvekar, A. P. Tiwari, A. V. Raut, S. G. Kumbhar, S. H. Pawar, Investigating the influence of effective parameters on molecular characteristics of bovine serum albumin nanoparticles, *Appl. Surf. Sci.* **2015**, 334, 157.

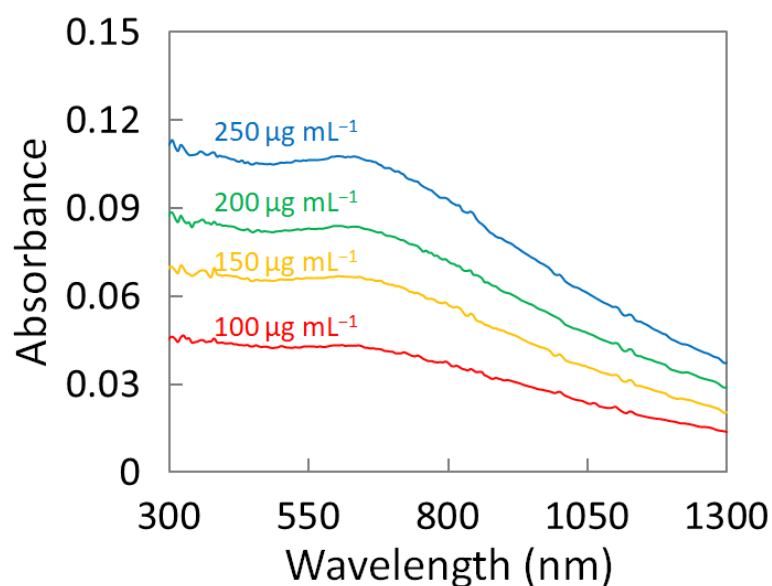


Figure S3. Absorbance spectra of 100 $\mu\text{g mL}^{-1}$ (red), 150 $\mu\text{g mL}^{-1}$ (orange), 200 $\mu\text{g mL}^{-1}$ (green) and 250 $\mu\text{g mL}^{-1}$ (blue) BSA-coated GeNP suspensions in pH 7 0.1 M PB, based on which the molar extinction coefficient spectrum in Figure 1F is derived. According to the Lamber-Beer law, the molar extinction coefficient ($\text{M}^{-1} \text{cm}^{-1}$) is equal to the absorbance / [cuvette length (1 cm) \times the GeNP molar concentration (M)]. The GeNP molar concentration can be converted from the mass concentration by applying the average GeNP diameter (204 nm), obtained from the DLS particle size distribution in Figure 1D, and the Ge density (5.35 g cm^{-3}).

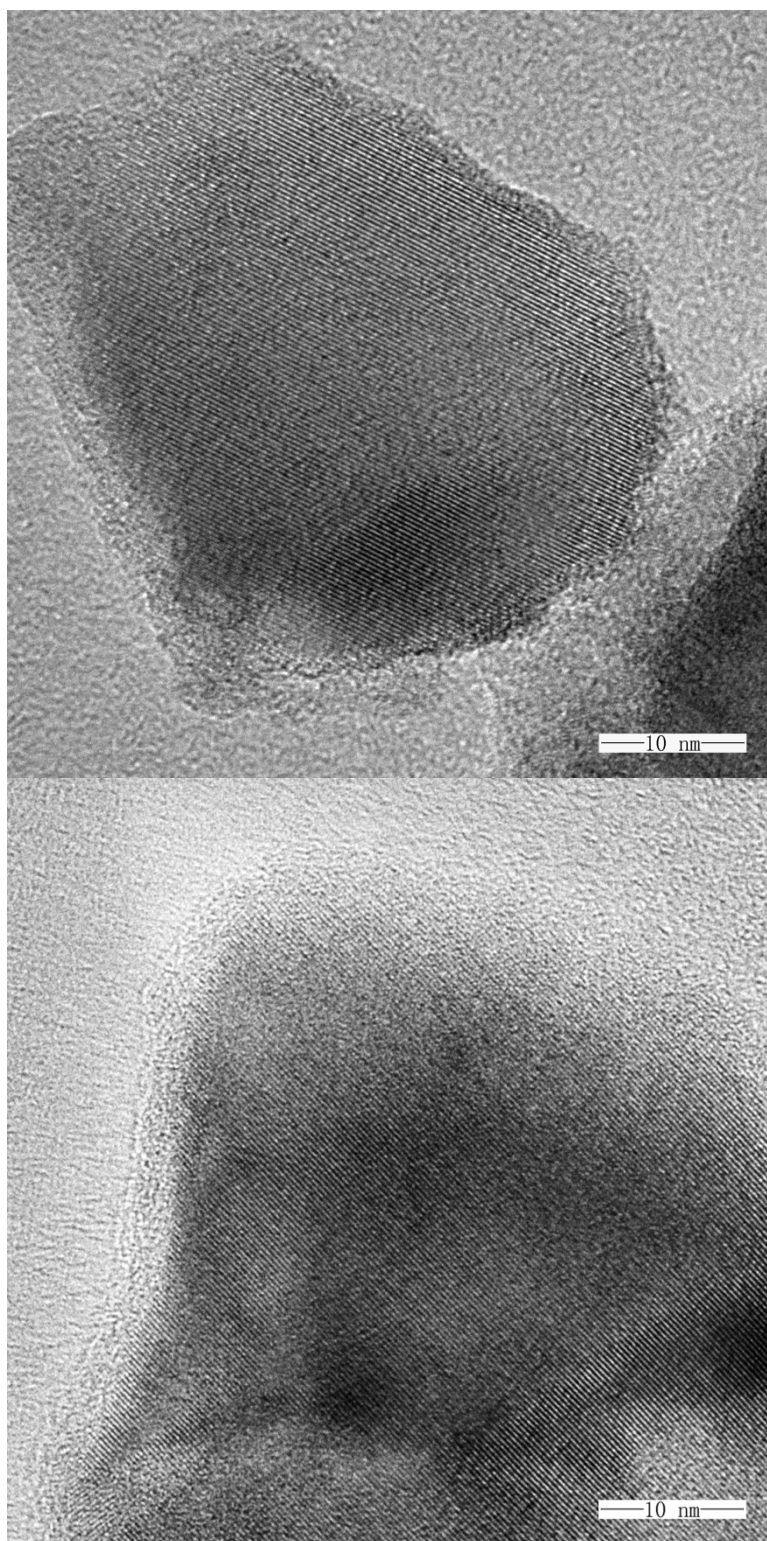


Figure S4. Two representative high-resolution TEM images of the GeNPs, showing their crystalline structures. The upper image was taken before the degradation process, while the lower image was taken at 24 h after the degradation process started.

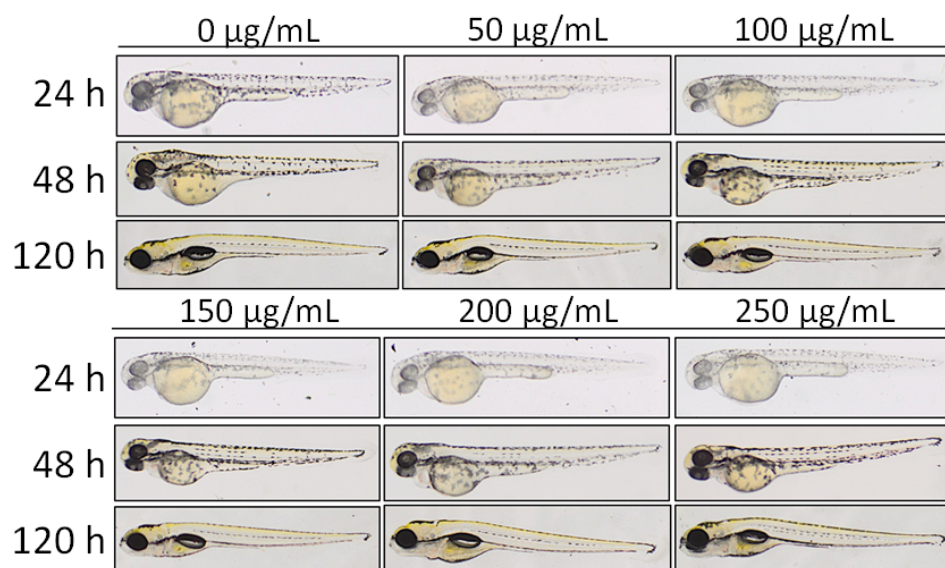


Figure S5. Representative photographs of the 26 hpf, wild-type zebrafish embryos after soaking in 0, 50, 100, 150, 200 and 250 $\mu\text{g mL}^{-1}$ BSA-coated GeNP suspensions in E3 medium for 24, 48 and 120 h.

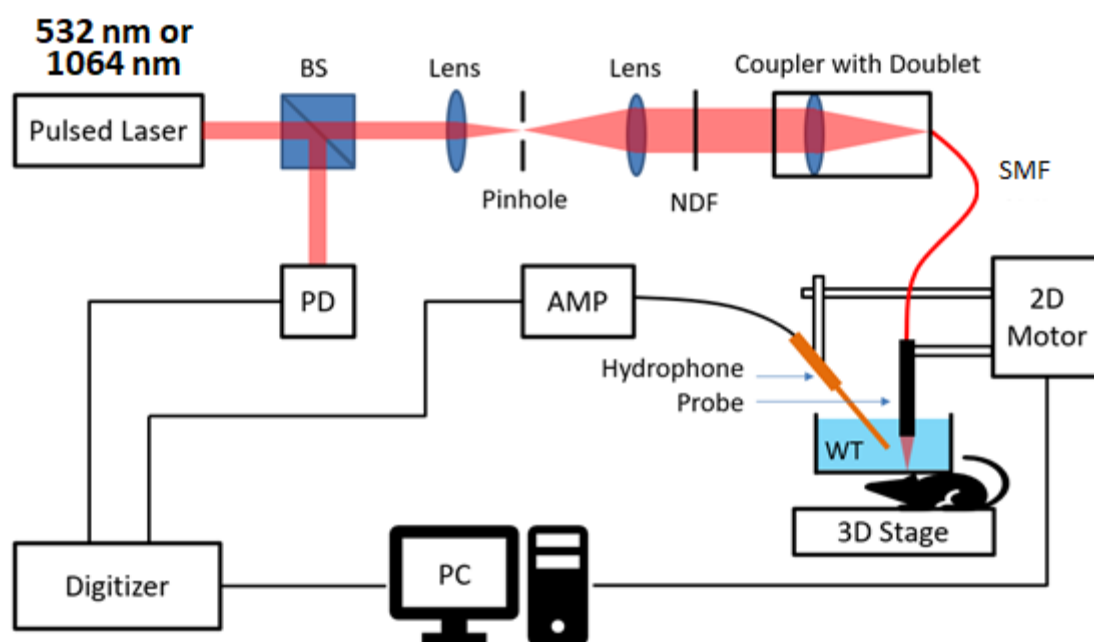


Figure S6. Schematic diagram of the OR-PAM system, in which BS represents the beam splitter, PD the photodetector, NDF the neutral density filter, AMP the preamplifier, SMF the single-mode fiber, WT the water tank and PC the personal computer.

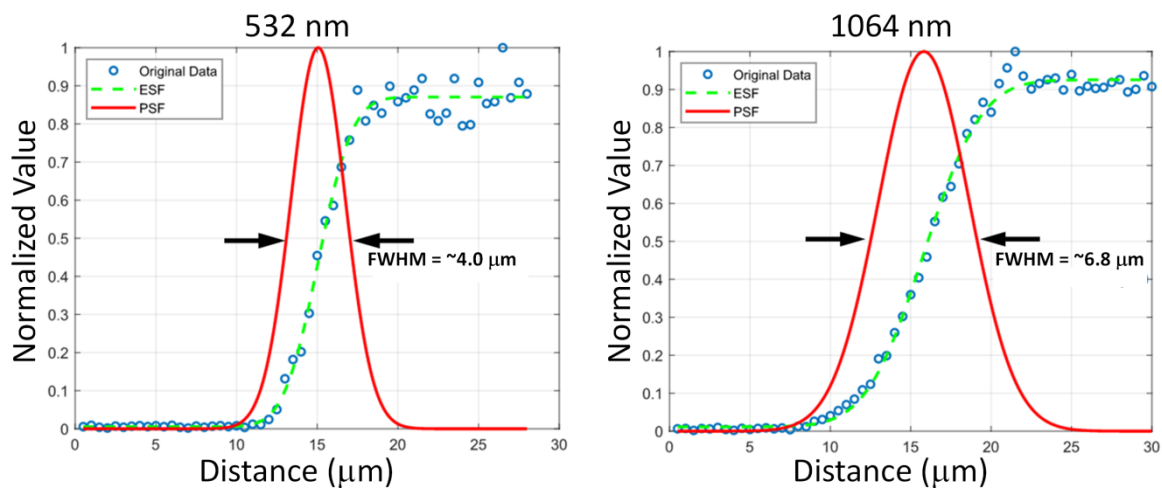


Figure S7. Lateral resolution of the OR-PAM system with 532-nm and 1064-nm excitation wavelengths. The edge of a razor blade was imaged by one-dimensional (1D) scanning, as shown by the original data (blue circles). The 1D profile is then fitted by the edge spread function (ESF). By taking the first derivative of the ESF, the point spread function (PSF) can be obtained. Finally, the full width at half maximum (FWHM) of the PSF is determined, which corresponds to the lateral resolution of the OR-PAM system.

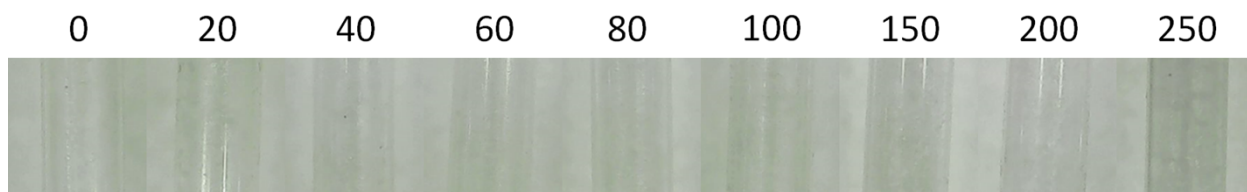


Figure S8. Bright-field images of the glass tubes (inner diameter 0.5 mm and outer diameter 0.7 mm) loaded with the BSA-coated GeNP suspensions in different concentrations (0, 20, 40, 60, 80, 100, 150, 200 and 250 $\mu\text{g mL}^{-1}$).

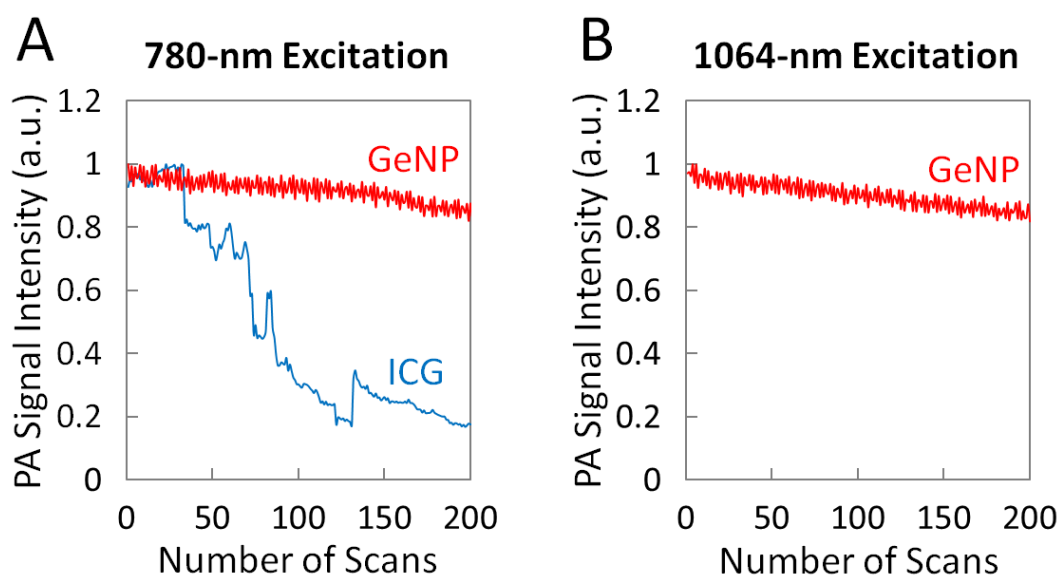


Figure S9. A) PA signal intensity of the ICG solution (blue line, 3.2×10^{-4} M in pH 7.4 PBS) and the BSA-coated GeNP suspension (red line, $100 \mu\text{g mL}^{-1}$ in pH 7 0.1 M PB) after different numbers of B-scans (along the same position) using 780-nm excitation (1.2 mJ per pulse at 100 Hz and spot size 4.7 mm^2 , which is equivalent to about 26 mJ cm^{-2}). After 200 scans, the PA signal intensity of the GeNP suspension can maintain 88% of the initial intensity, while the PA signal intensity of the ICG solution can only retain 18% of the initial intensity under the same testing condition. B) PA signal intensity of the BSA-coated GeNP suspension ($100 \mu\text{g mL}^{-1}$ in pH 7 0.1 M PB) after different numbers of B-scans (along the same position) using 1064-nm excitation (1.15 mJ per pulse at 100 Hz and spot size 4.7 mm^2 , which is equivalent to about 24 mJ cm^{-2}). After 200 scans, the PA signal intensity of the GeNP suspension can maintain 87% of the initial intensity. Note that it took less than 2 minutes for the 200 scans.

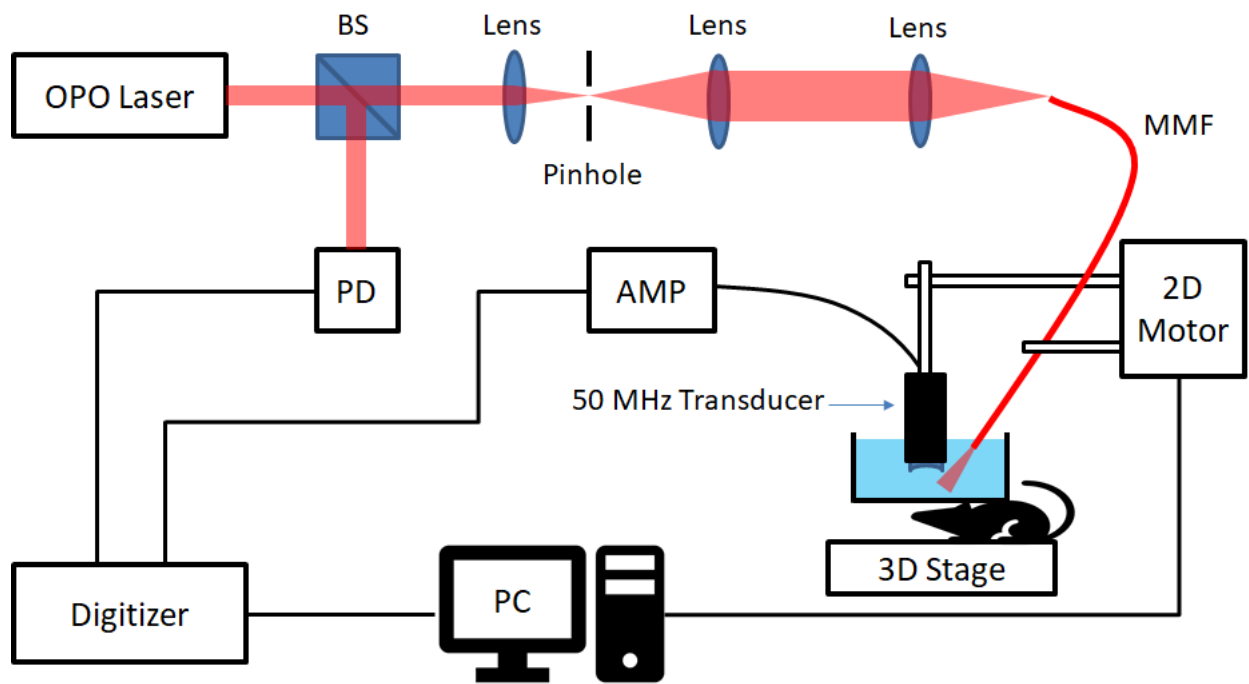


Figure S10. Schematic diagram of the AR-PAM system, in which OPO represents the optical parametric oscillator, BS the beam splitter, PD the photodetector, AMP the preamplifier, MMF the multi-mode fiber and PC the personal computer.

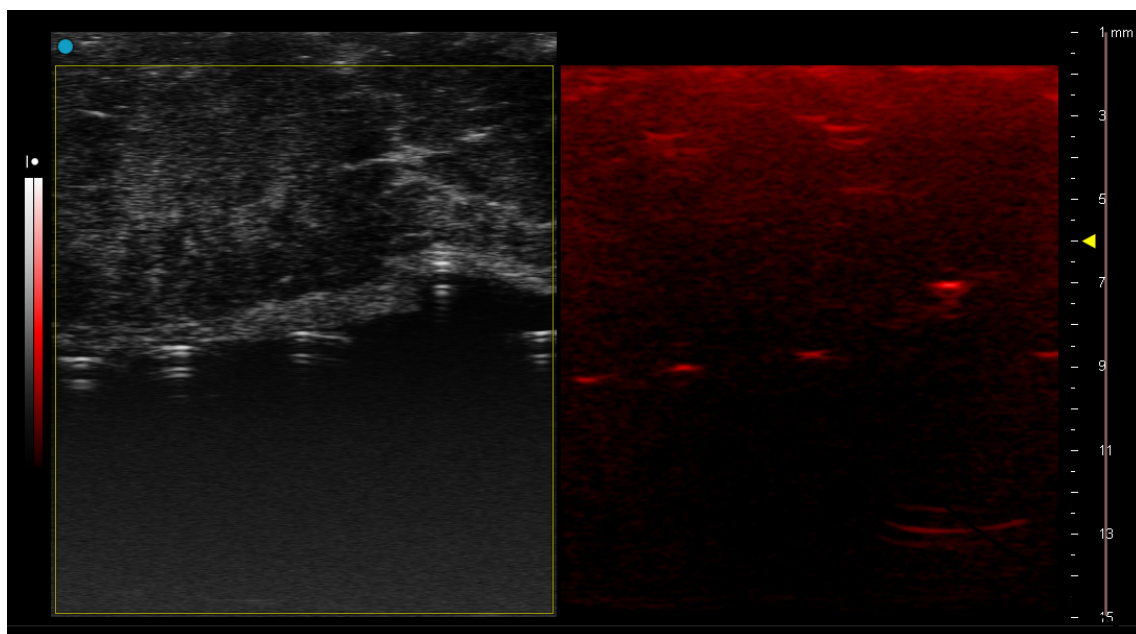


Figure S11. Ultrasound image (left) and PA image (right) of the BSA-coated GeNPs loaded in 5 plastic tubes, which were placed right under a piece of chicken breast with thickness of $\sim 7\text{--}9.3$ mm, acquired by a commercial PACT system (VEVO LAZR-X, Fujifilm VisualSonics).

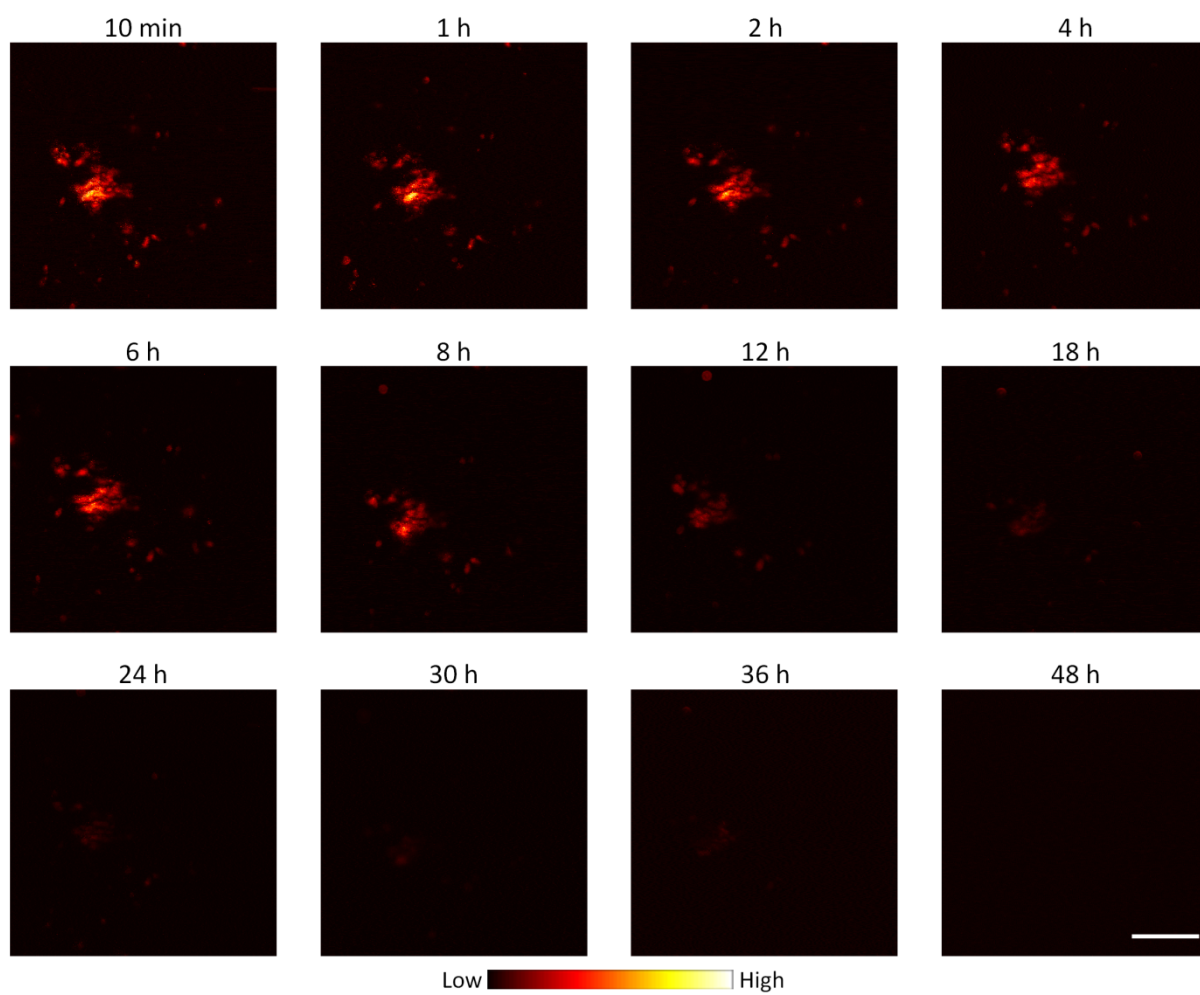


Figure S12. Time-lapse 1064-nm PA images of the mouse ear pinnae taken at 10 minutes (the same as Figure 6C), 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 24 h, 30 h, 36 h and 48 h (the same as Figure 6F) after the GeNP injection. Scale bar: 500 μm .