

## Supporting Information

### pH-induced aggregated melanin nanoparticles for photoacoustic signal amplification

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	Heat relaxation time ( $\tau_T$ )	Stress relaxation time ( $\tau_A$ )
Single spherical MelNP (130 nm)	1.7 ns	86 ps

**Fig. S1.** Stress and heat transfer time for a single spherical MelNP; Characteristic stress relaxation time ( $\tau_A$ ) and thermal relaxation time ( $\tau_T$ ) of spherical nanoparticles were calculated as followed<sup>1,2</sup>. Stress relaxation time ( $\tau_A$ ) for single spherical MelNP was calculated by

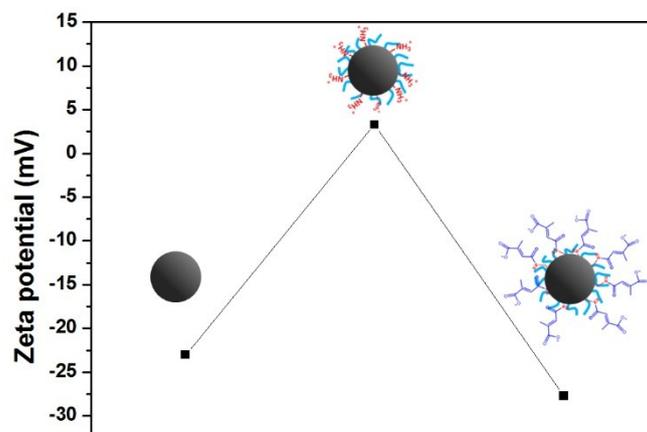
$$\tau_A \sim 2r_0/c_s,$$

where  $r_0$  is the radius of a nanoparticle,  $c_s$  is the speed of sound ( $1.5 \times 10^5$  cm/s).

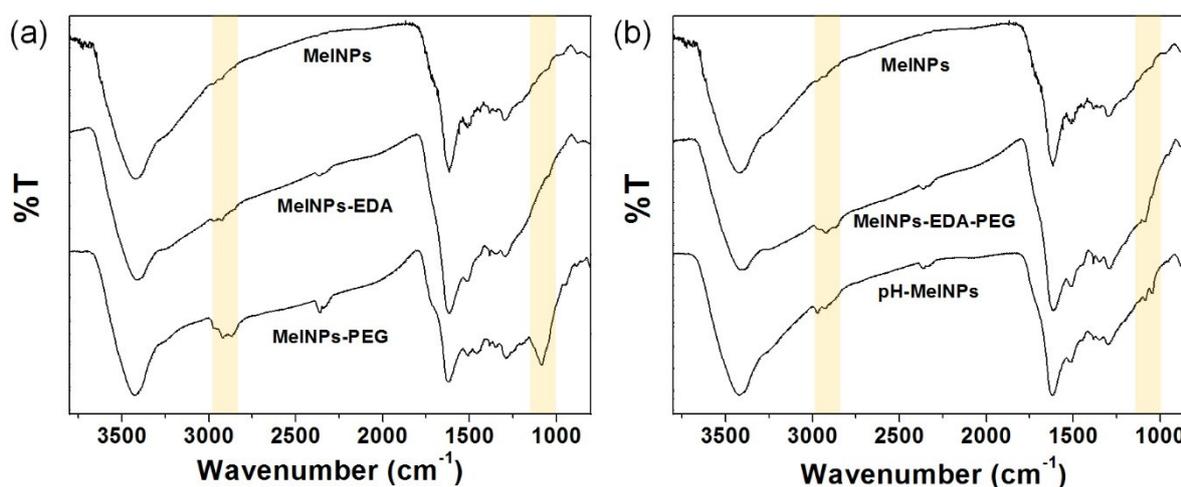
Thermal relaxation time ( $\tau_T$ ) of a spherical MelNP was calculated by

$$\tau_T \sim r_0^2 c_0 \rho_0 / 3k_\infty,$$

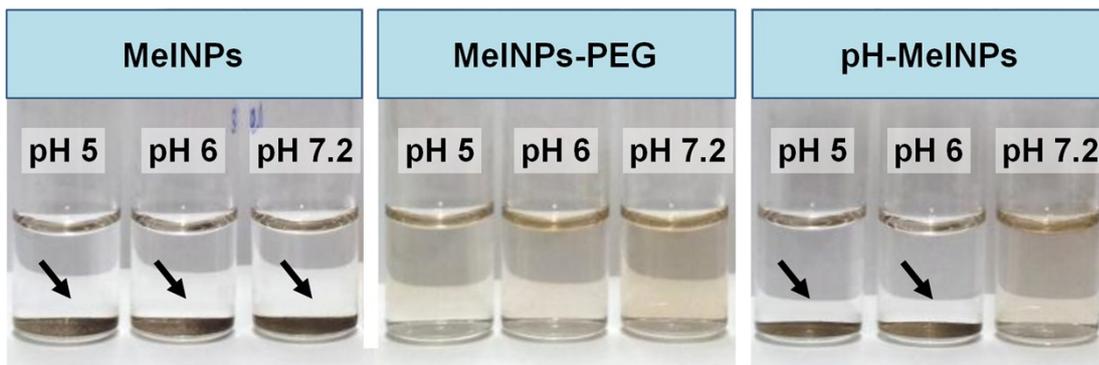
where  $r_0$  is the radius of a nanoparticle,  $c_0$  is the heat capacity of a nanoparticle,  $\rho_0$  is the density of a nanoparticle, and  $k_\infty$  is the coefficient of thermal conductivity of water ( $6 \times 10^{-3}$  W/cmK). We assumed that the heat capacity ( $c_0$ ) and density ( $\rho_0$ ) of the MelNPs were comparable to those of the synthetic melanin<sup>3</sup>, i.e.,  $c_0 = 0.25$  J/gK and  $\rho_0 = 1.43$  g/cm<sup>3</sup>.



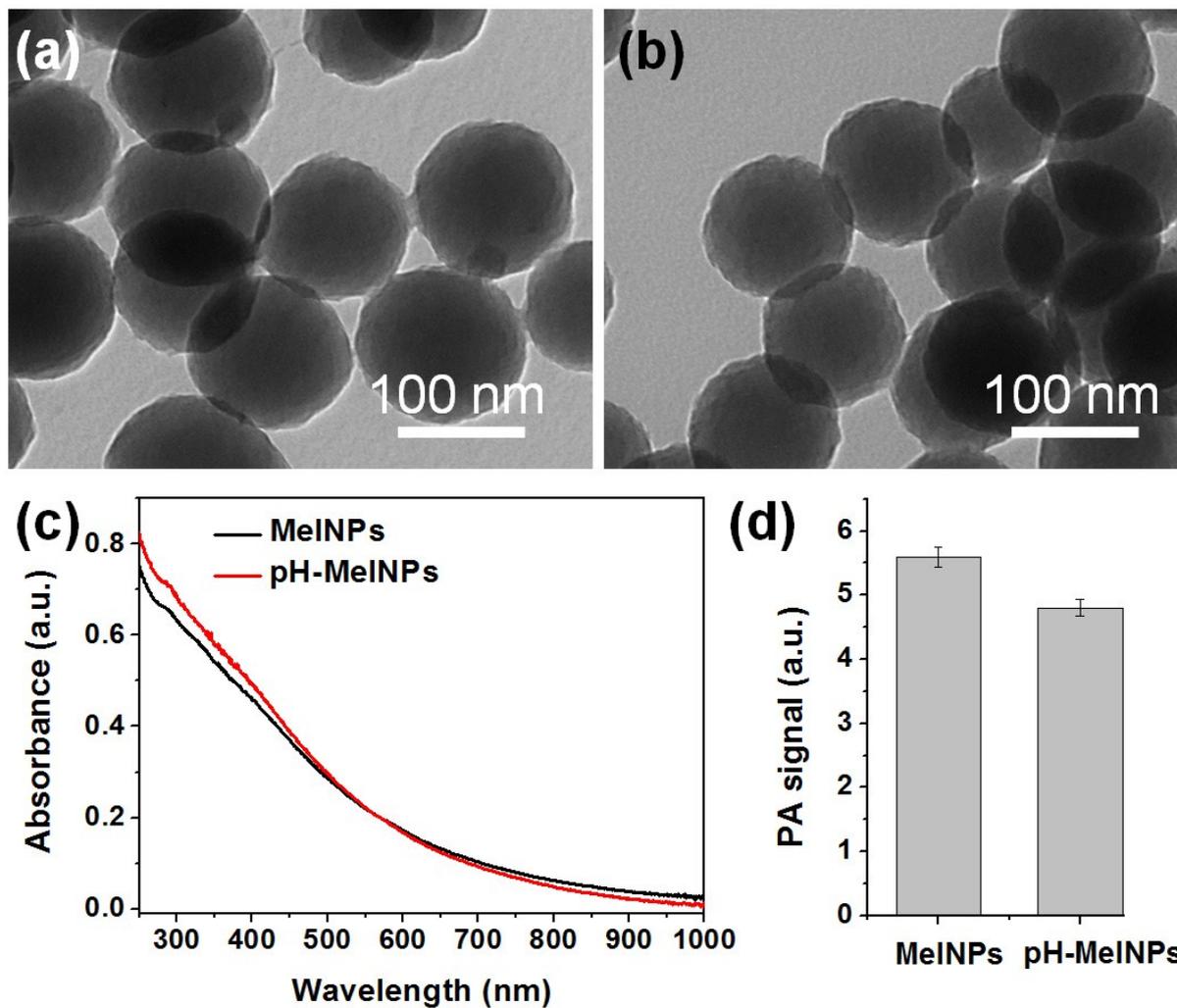
**Fig. S2.** Zeta potential of the MeINPs at each stage of the surface modification



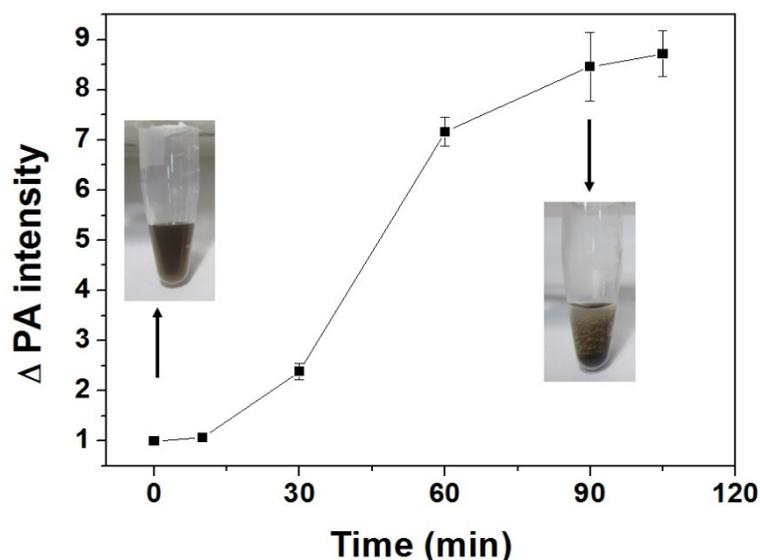
**Fig. S3.** FT-IR spectra of MeINPs and surface modified MeINPs; introduction of ethylenediamine (EDA) and mPEG-SH (PEG) onto the surface of MeINPs could be determined by FT-IR spectroscopic data. After the surface modification of MeINPs (10 mg) with mPEG-SH (15  $\mu\text{mol}$ ), they showed two characteristic bands around 2920  $\text{cm}^{-1}$  (alkyl C-H) and 1086  $\text{cm}^{-1}$  (C-O-C stretching) originated from PEG (MeINPs-PEG). After the surface modification of MeINPs (10 mg) with EDA (60  $\mu\text{mol}$ ), they showed a characteristic band around 2920  $\text{cm}^{-1}$  (alkyl C-H) (MeINPs-EDA). The Surface modification of MeINP (10 mg) with PEG (15  $\mu\text{mol}$ ) and EDA (60  $\mu\text{mol}$ ) resulted in two characteristic bands around 2920  $\text{cm}^{-1}$  and 1086  $\text{cm}^{-1}$  (MeINPs-EDA-PEG). The significant change in surface charge from negative to positive after the surface modification of MeINPs with EDA and PEG reflects the introduction of EDA onto their surface as shown in Fig. S2. Even though FT-IR data do not directly provide any additional information about further introduction of citraconic anhydride onto the MeINPs-EDA-PEG, the considerable surface charge shift from positive to negative indicates the ring opening of citraconic anhydride on the surface of MeINPs, thus causing the generation of carboxylic acid groups (Fig. S2).



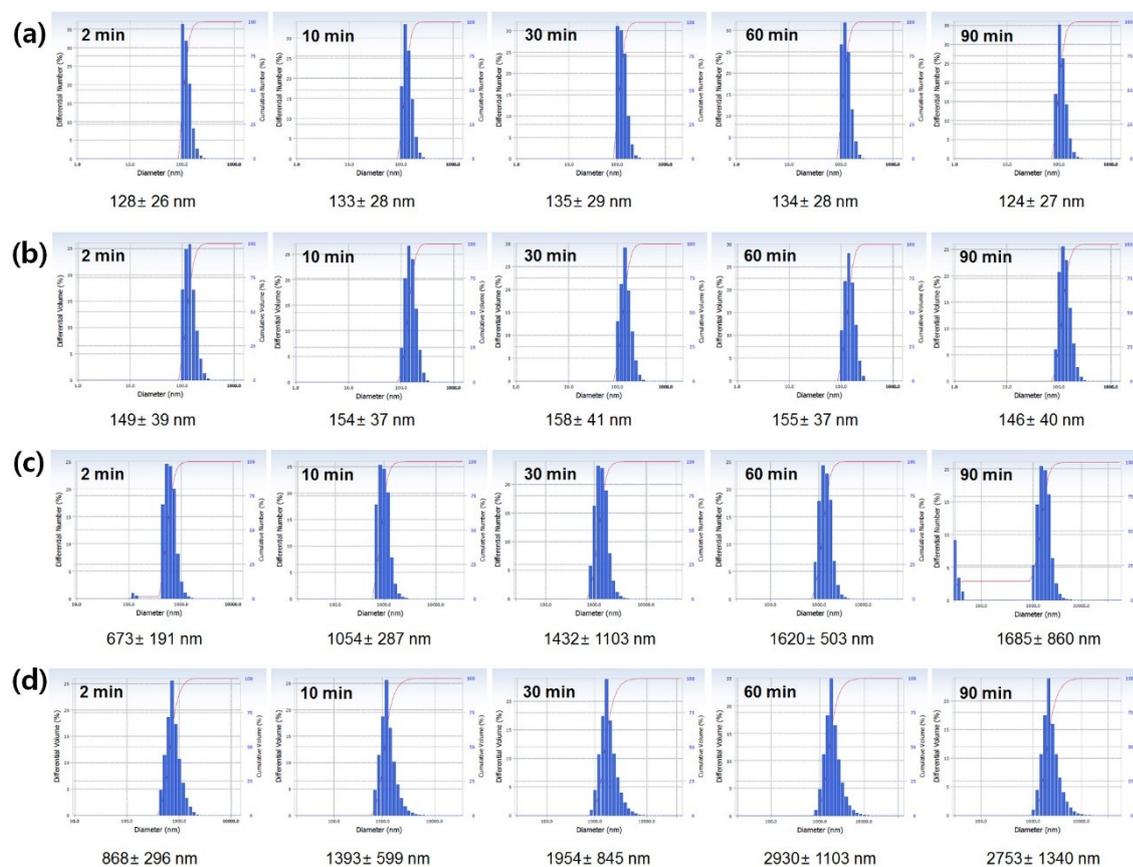
**Fig. S4.** Dispersion stability of (a) bare MeINPs, (b) PEGylated MeINPs (MeINPs-PEG) and (c) pH-MeINPs in different pH conditions; All photographs were taken 12 hours after exposure to each solution. The black arrows indicate the precipitate of the particles.



**Fig. S5.** TEM images of (a) bare MeINPs and (b) pH-MeINPs (c) UV-Vis absorption spectra of MeINPs and pH-MeINPs (d) Photoacoustic signal strength of both bare MeINPs and pH-MeINPs (0.125 mg / mL)

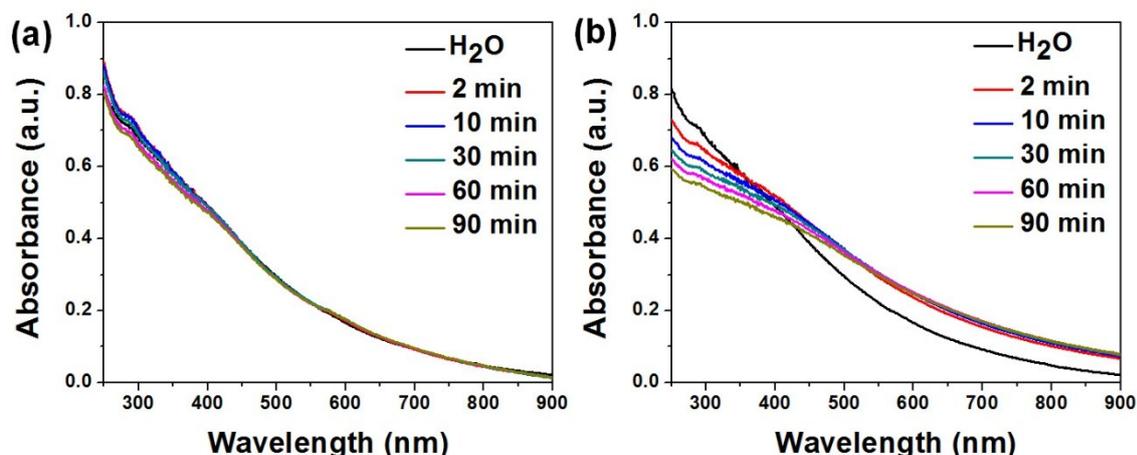


**Fig. S6.** The time course of the PA signal from the pH-MelNPs, of which weight concentration was 0.5 mg/mL, after exposure to mildly acidic condition (i.e., pH 6); the increase in the weight concentration of the pH-MelNPs from 0.125 mg/mL to 0.5 mg/mL caused the most of pH-MelNPs to be aggregated and precipitated in 90 min. Under this condition, the maximally amplified PA signals from the pH-MelNPs due to their physical aggregation were observed.

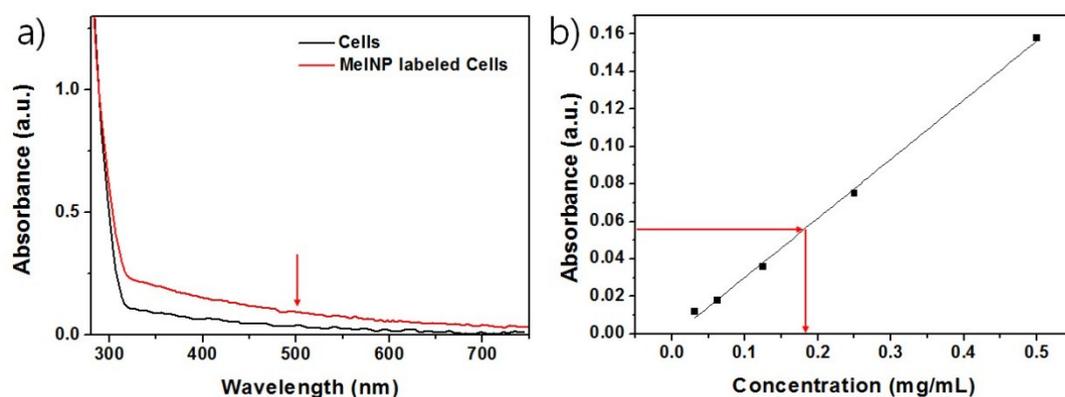


**Fig. S7.** As the pH-MelNPs are exposed to mild acidic solution and the exposure time passes, the number and the volume contribution of the large size of the particles increases. Chang in

the size distribution of the pH-MelNPs as a function of time in terms of (a) the number and (b) the volume of the particles after exposure to neutral solution (pH 7.2) and those after exposure to mild acidic solution (pH 6), i.e., (c) and (d).



**Fig. S8.** Change in the UV-Vis absorption spectra of the pH-MelNPs in (a) pH 7.2 and (b) pH 6 as the exposure time passes.



**Fig. S9.** (a) Absorption spectra of PEG-MelNP labeled SKBR3 and the same concentration of PEG-MelNP free SKBR3 after lysis; the arrow indicates the absorption wavelength (i.e., 500 nm) used for the calibration curve and quantification of MelNPs internalized in cells. (b) Calibration curve for PEG-MelNPs; the arrows indicate the absorbance of PEG-MelNPs internalized in SKBR3 cells and the corresponding weight concentration of PEG-MelNPs.

## References

- [1] Pustovalov, V. K.; Smetannikov, A. S.; Zharov, V. P. *Laser Phys. Lett.* **2008**, *5*, 775-792.
- [2] Pustovalov, V. K.; Astafyeva, L. G.; Galanzha, E.; Zharov, V. P. *Cancer nanotechnol.* **2010**, *1*, 35-46.
- [3] de Albuquerque, J. E.; Giacomantonio, C.; White, A. G.; Meredith, P. *Appl. Phys. Lett.* **2005**, *87*, 061029.