

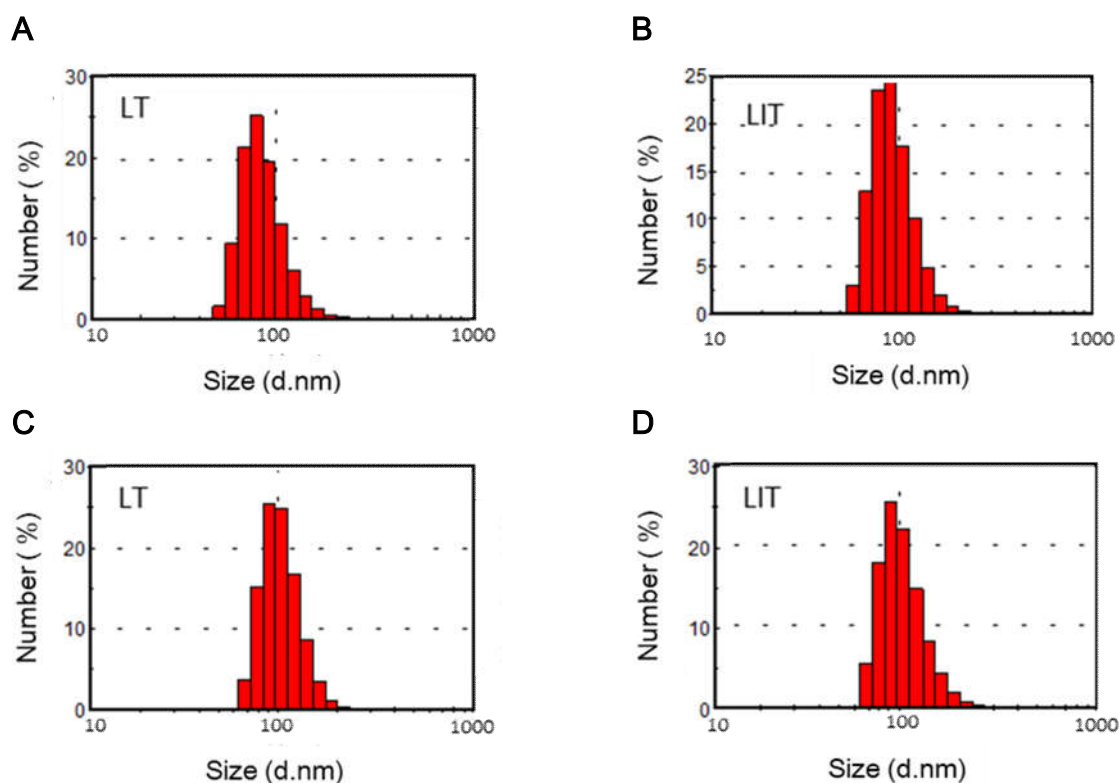
# Nanoliposomes co-encapsulating a CT imaging contrast agent and photosensitizer for enhanced, imaging guided photodynamic therapy of cancer

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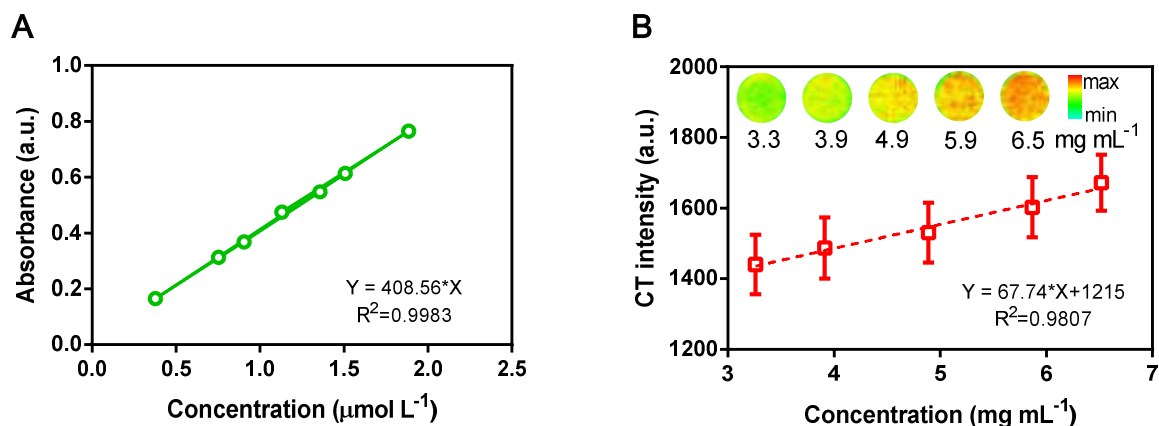
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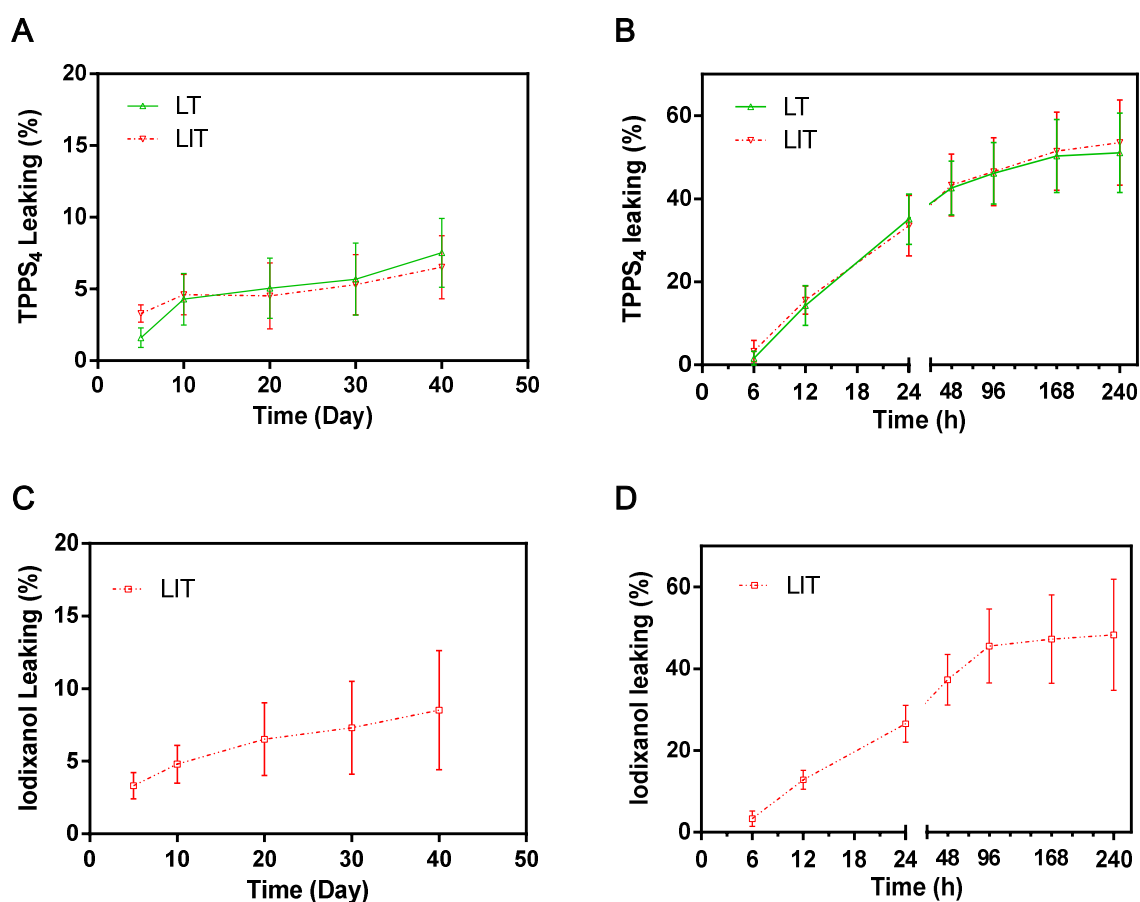
## Supplementary material



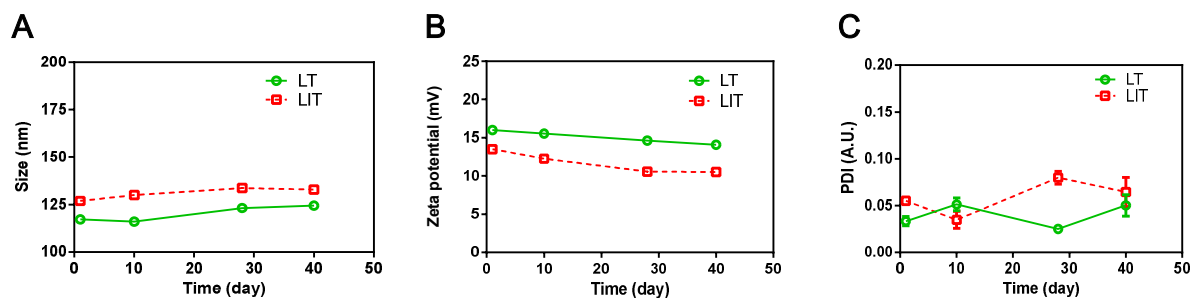
**Figure S1.** Size distribution of liposomal nanoformulations. (A) NL encapsulating TPPS<sub>4</sub> (LT) after preparation; (B) NL co-encapsulating iodixanol and TPPS<sub>4</sub> (LIT) after preparation; (C) LT after 40 days of storage. (d) LIT after 40 days of storage.



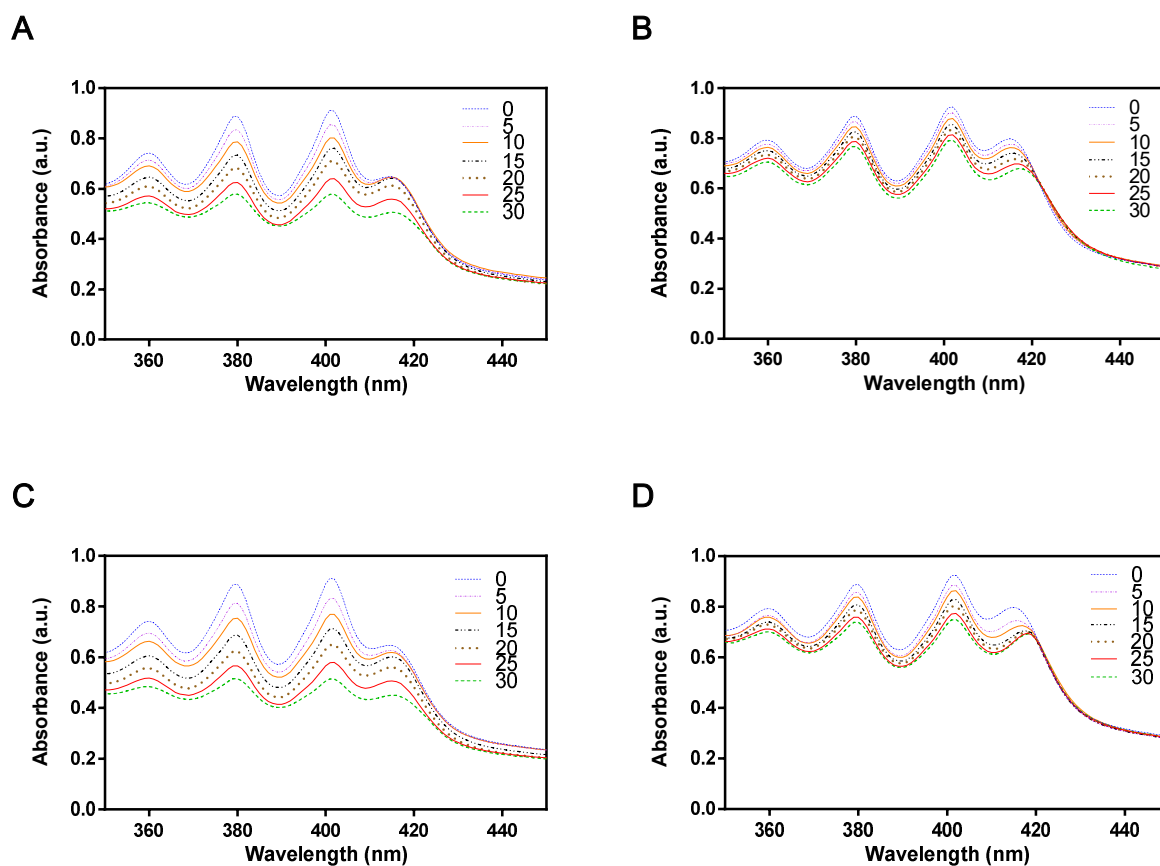
**Figure S2.** (A) Concentration dependence of TPPS<sub>4</sub> absorbance at 420 nm. (B) Phantom images and CT intensity measurements of iodixanol.



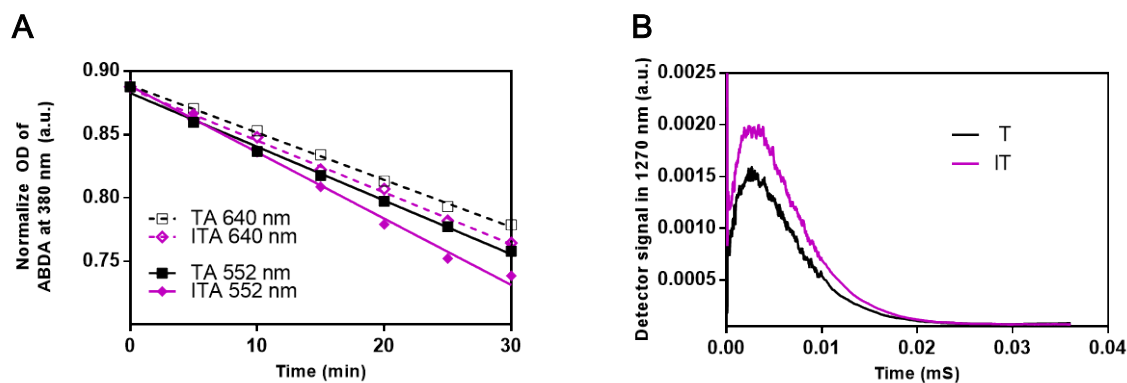
**Figure S3.** Monitoring the reagent leakage from the prepared CPNL at different storage conditions. (A) TPPS<sub>4</sub> leakage from LT and LIT (in PBS at 4 °C); (B) TPPS<sub>4</sub> leakage from LT and LIT (in PBS with 10% FBS at 37 °C); (C) Iodixanol leakage from LIT (in PBS at 4 °C); (D) Iodixanol leakage from LIT (in PBS with 10% FBS at 37 °C).



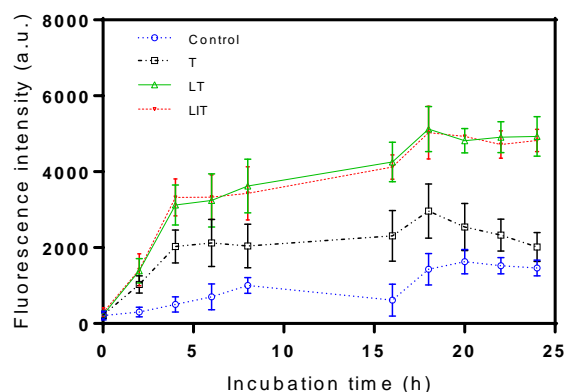
**Figure S4.** Monitoring the storage stability of size (A), zeta potential (B) and PDI (C) for the prepared NL at the storage conditions (in PBS at 4 °C).



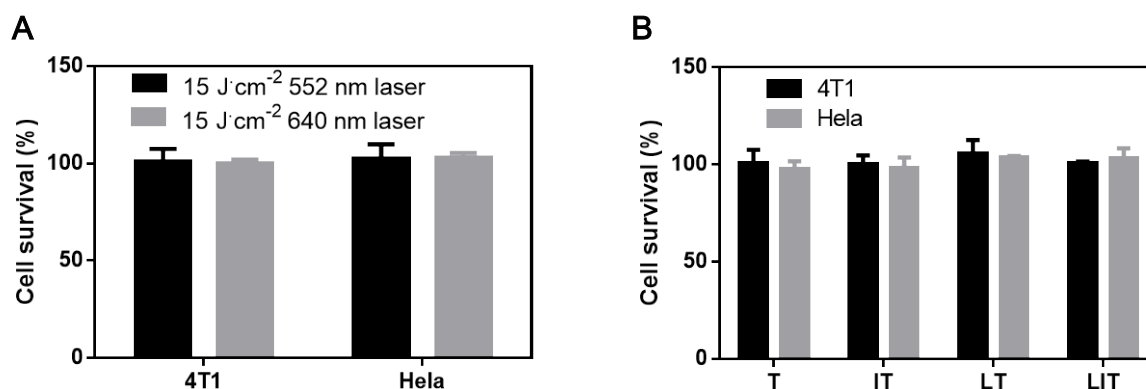
**Figure S5.** Absorption spectra of ABDA in water suspensions of TPPS<sub>4</sub> nanoformulations under 552 nm and 640 nm laser irradiation. Samples were irradiated at laser power density of 50 mW · cm<sup>-2</sup> for 0, 5, 10, 15, 20, 25 and 30 min. (A) ABDA in LIT suspension under 640 nm laser irradiation; (B) ABDA in LT suspension under 640 nm laser irradiation; (C) ABDA in LIT suspension under 552 nm laser irradiation; (D) ABDA in LT suspension under 552 nm laser irradiation.



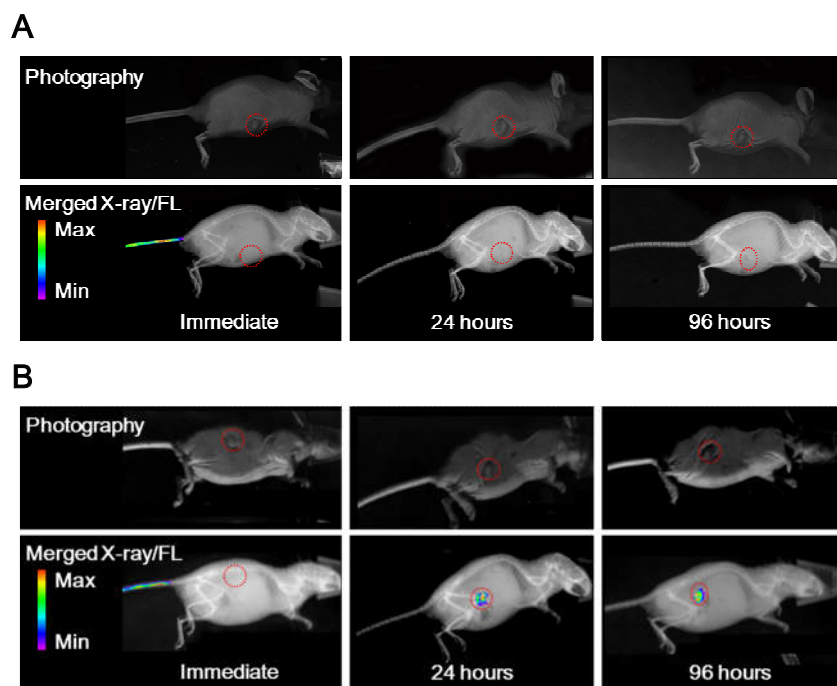
**Figure S6.** (A) Comparison of ABDA bleaching as a measure of the singlet oxygen generation in TPPS<sub>4</sub> solution (TA) and TPPS<sub>4</sub> solution win presence of iodixanol (ITA). (B) Decays of singlet oxygen phosphorescence at 1270 nm illustrating the singlet oxygen generation by free TPPS<sub>4</sub> in presence iodixanol (IT) and without it (T). PBS solutions, concentration of TPPS<sub>4</sub>  $C_{\text{TPPS}_4}=8 \mu\text{M}$ , concentration of iodixanol  $C_{\text{Iodixanol}}=7 \text{ mM}$ .



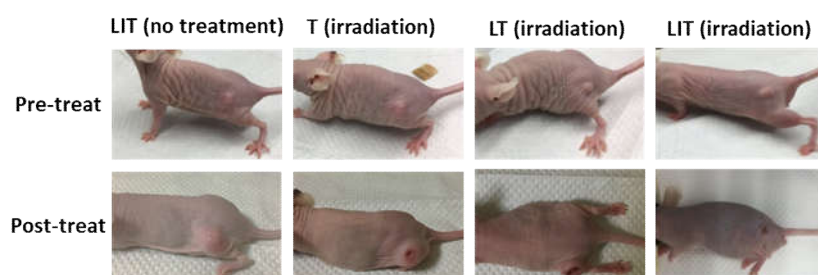
**Figure S7.** Cellular uptake of TPPS<sub>4</sub> in different formulations (free T, LT and LIT) at different incubation times. Control shows background (level of autofluorescence), when no fluorescent TPPS<sub>4</sub> was added to the cells.



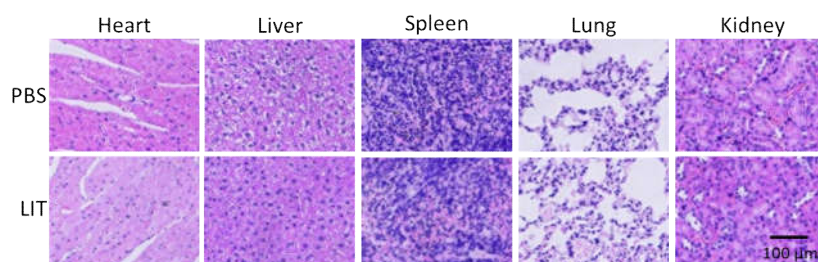
**Figure S8.** Cell viability assay performed 24 hours after treatment or irradiation. (A) irradiation with 552 nm and 640 nm lasers at the same irradiation dose ( $15 \text{ J cm}^{-2}$ ) in absence of TPPS<sub>4</sub>; (B) 4T1 and HeLa cells viability in response to the dark treatment with  $40 \mu\text{M}$  of different formulations of TPPS<sub>4</sub> (i.e., free TPPS<sub>4</sub>, IT, LT or LIT).



**Figure S9.** Photography and X-ray/fluorescence (X-ray/FL) images of the tumor-bearing nude mouse at various time points (2, 24, 96 h) post injection with (A) free TPPS<sub>4</sub> and (B) liposomal TPPS<sub>4</sub> (LT). Tumor location is shown with red circle.



**Figure S10.** Photography images of the tumor-bearing nude mice before and 30 days after treatment with different formulations and irradiation.



**Figure S11.** Histological sections of heart, liver, spleen, lungs, and kidney samples, collected after 7 days post injection with LIT formulation (lower row) and PBS (upper row). Sections were stained with H&E and observed under a light microscope.