

Supplementary Information

A dextran based redox-responsive theranostic nanoparticles for near-infrared Imaging and photothermal therapy in vitro

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Materials. Poly(ethylene glycol) methyl ether (PEG, Mn=2000), dextran (Mr=6000), Succinic
15 anhydride, N-Hydroxysuccinimide (NHS), Triethylamine (TEA), Cystamine dihydrochloride,
Ethylenediamine, Sodium cyanoborohydride and N-(3-Dimethylaminopropyl)-N'-
ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma Chemical Co. 4-Nitrophenyl
chloroformate (PNC), DL-Dithiothreitol (DTT) and Glutaraldehyde was purchased from J&K.
Indocyanine Green (ICG) was purchased from aladdin.

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Synthesis of 4-Nitrophenyl carbonate-activated PEG (PEG-PNC). PEG (0.5 g) was dissolved in
5mL of tetrahydrofuran (THF), and then TEA (140 μ L) was added. After addition of PNC (0.2 g),
the reaction mixture was stirred for 12 h at room temperature. Above reactant was centrifuged to
remove the white precipitate, and then was precipitated into 100 mL of diethyl ether two times.
25 The resultant precipitate was collected by centrifugation and dried in vacuo. The degree of PNC
substitution was 86%.

Synthesis of end-group aminated dextran (dextran-NH₂). Dextran (0.54 g) was dissolved in 15
mL of anhydrous dimethyl sulfoxide (DMSO). Sodium cyanoborohydride (0.57 g) was added and
30 the reaction mixture was stirred for 24 h at room temperature. Then 0.3 mL of ethylenediamine
was added into above mixture and stirred for another 24 h at room temperature. After the solution
was poured into excess ethanol, the resultant precipitate was collected by centrifugation and

washed with ethanol and diethyl ether, and dried in vacuo finally. The degree of amine substitution was 95%.

Synthesis of PEG-dextran block copolymer (PEG-dextran). For the PEG-dextran block copolymer, dextran-NH₂ (0.2 g) was dissolved in 4 mL of anhydrous DMSO and PEG-PNC (0.2 g) dissolved in DMSO (2 mL) was added into the dextran-NH₂ solution. After the mixture was stirred for 24 h at room temperature, it was further extensively dialyzed against distilled water using a dialysis tube (MWCO=7000) and lyophilized. The degree of substitution defined as number of PEG to the dextran was 90%.

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Synthesis of PEG-dextran(-COOH). The PEG-dextran block copolymer (0.2 g) was dissolved in 5 mL of anhydrous DMSO, and then succinic anhydride (26 mg) was added. After addition of 36 μ L of TEA, the mixture was stirred for 12 h at 50 °C. The solution was moved into dialysis tube (MWCO=7000) to dialysis against distilled water for 3 days and finally lyophilized. The degree of substitution defined as number of carboxyl group to the glucose unit was 38%.

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Synthesis of PEG-dextran(-SS-NH₂). PEG-dextran(-COOH) (50 mg) was dissolved in 5 mL of anhydrous DMSO, and NHS (85 mg), EDC (140 mg) were added. This solution was stirred for 1 h and was added dropwise into the cystamine dihydrochloride (1 g) and pyridine (200 μ L) mixture solution in 10 mL of anhydrous DMSO. The reaction proceed for 1 day at 30 °C and extensively dialyzed against distilled water using a dialysis tube (MWCO=7000) and lyophilized. The degree of substitution defined as number of amine group to the glucose unit was 30%.

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Preparation of ICG nanoparticles (ICG NPs). Each of ICG (3 mg/mL) and PEG-dextran(-SS-NH₂) (1 mg/mL) was dissolved in distilled water. The nanoparticles were obtained by mixing ICG and PEG-dextran(-SS-NH₂) solution according to the mass ratio 1:10, and the mixture solution was stirred for 20 min at room temperature. After the solution was filtered through 0.45 μ m syringe filter, glutaraldehyde was added to crosslink ICG NPs for 3 h at room temperature. The final solution was filtered through 0.45 μ m syringe filter and stored in 4 °C. The resulting solution was used without no other washing steps.

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Characterization. The $^1\text{H-NMR}$ spectra were recorded via a AVANCE 500 spectrometer operating at 500 MHz using D_2O as solvent. The MALDI-TOF MS spectroscopy was performed on a ABI MALDI-TOF-MS 4800 PLUS instrument. The absorbance and fluorescence emission spectra of free ICG and ICG NPs were obtained using a UV-Vis spectrophotometer and a fluorescence spectrophotometer, respectively. Fluorescence spectra was obtained using an excitation wavelength of 712 nm and the emission wavelength was scanned from 730 to 900 nm. The size and zeta potential of ICG NPs were measured using a Malvern ZS 90 equipped at 25 °C.

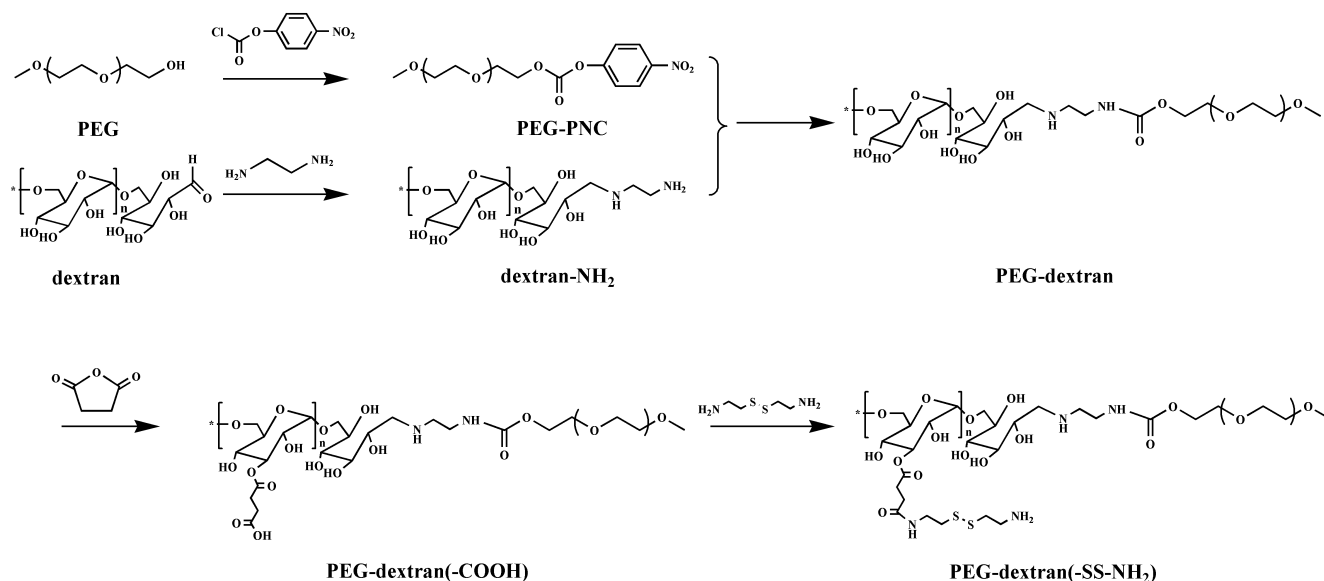
Recovery of NIR fluorescence of ICG NPs in response to DTT and esterase. ICG NPs (ICG concentration 5 $\mu\text{g/mL}$) were treated with 10 mM DTT or esterase in different buffer solution, and the fluorescence emission spectra was measured by a fluorescence spectrophotometer at the excitation of 712 nm. The size change was also measured by dynamic light scattering (DLS).

***In Vitro* Time-Dependent Fluorescence Activity.** MCF-7 cells (4×10^3 cells/well) were seeded in 100 μL of culture medium on 96-well plates. ICG NPs (ICG concentration 1 $\mu\text{g/mL}$) in serum-free DMEM were incubated with or without the attached cells at 37 °C. Near-infrared (NIR) fluorescence images were then detected with a Maestro *in vivo* imaging system. To excite ICG molecules, a 704 nm laser was used and near infrared fluorescence emission (740 to 900 nm) was detected.

***In vitro* cellular uptake.** MCF-7 cells were seeded into 8-well chambered coverglass (Lab-Tek, Nunc, USA) at a density of 2×10^4 /well (0.3 mL). After 24 h, the medium was changed to the fresh medium containing free ICG or ICG NPs (10 $\mu\text{g/mL}$ of ICG concentration). After 4 h incubation, the cells were washed thrice with phosphate buffer saline (PBS) and fixed with 4% paraformaldehyde solution for 20 min, then the nuclei were stained by 10 $\mu\text{g/mL}$ Hoechst 33258 for 8 min and washed thrice with PBS. Finally, the fixed cells were observed by confocal laser scanning microscope (CLSM, Leica TCS SP5, Germany). Excitation wavelength and emission spectrum for fluorescent indicators were as follows. Hoechst 33258 was excited at 406 nm, and the fluorescence emission was collected from 420 to 460 nm. ICG was excited at 633 nm, and emitted light was collected from 700 to 800 nm.

Temperature change measurement during NIR laser irradiation. 1 mL of ICG NPs or ICG solution with different ICG concentration were seeded onto 24-well plates and irradiated by NIR laser (808 nm) at 1 W/cm^2 . Temperature was measured in 30 s intervals with a thermocouple placed inside the solution for a total of 5 min. The thermocouple was placed outside the path of the laser beam to avoid direct exposure of the thermocouple to the laser light.

Cell viability assays. To determine cell viability under NIR laser irradiation, MCF-7 cells (4×10^3 cells/well) were seeded onto 96-well plates and incubated for 24 h. After cell stabilization, the culture medium was replaced with 100 μL of medium containing free ICG or ICG NPs (0-15 $\mu\text{g/ml}$ of ICG), followed by incubation for 4 h at 37°C . The cells were washed twice with medium and irradiated with a NIR laser (808 nm, 1 W/cm^2) for 5 min onto the 96-well plates. After 12 h incubation, cell viability was evaluated by the CCK-8 assay.



15 Fig. S1 Synthesis of PEG-dextran(-SS-NH₂) block copolymer

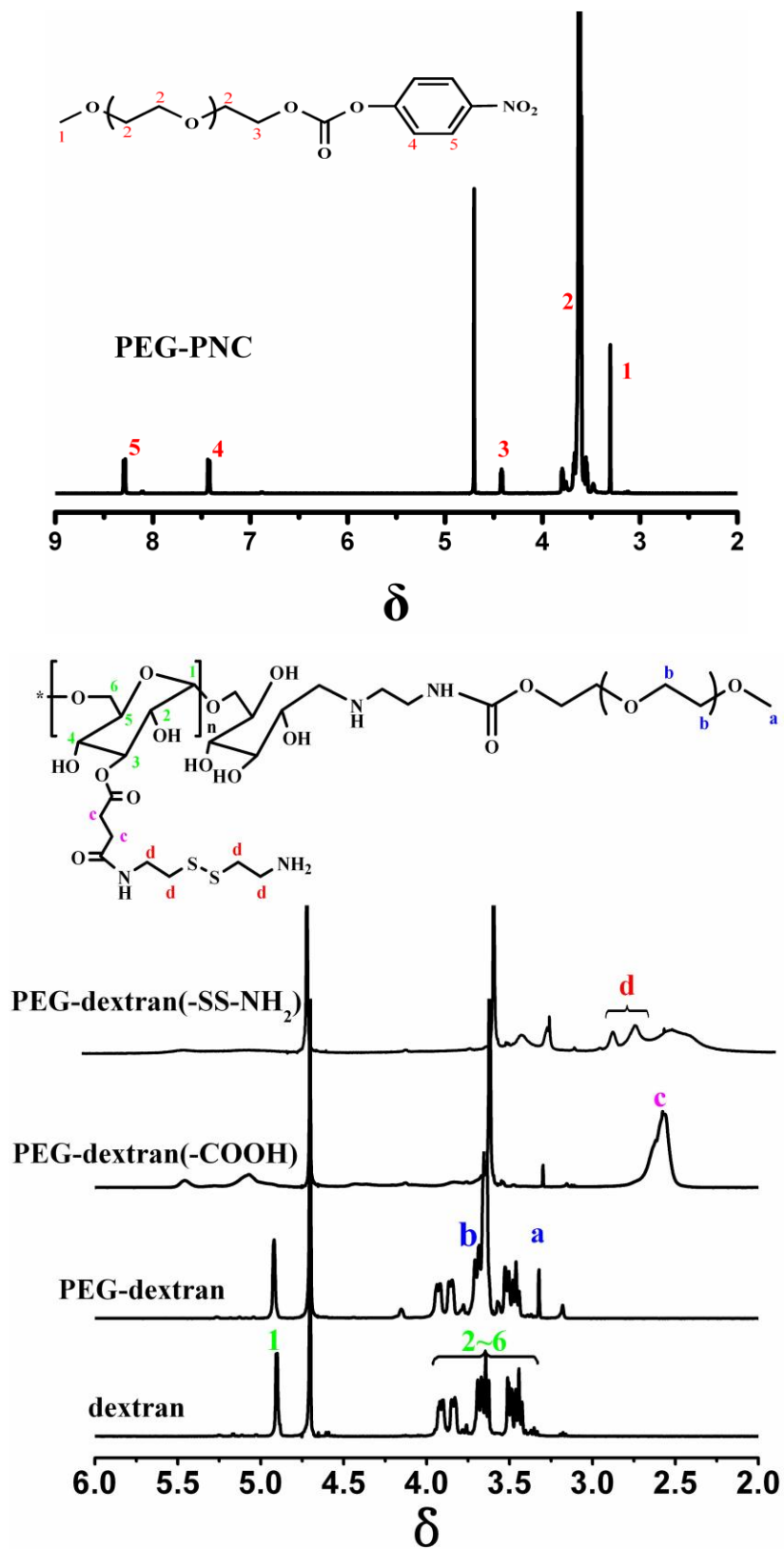


Fig. S2 ¹H-NMR spectrum of PEG-PNC, dextran, PEG-dextran, PEG-dextran(-COOH) and PEG-dextran(-SS-NH₂) in D₂O

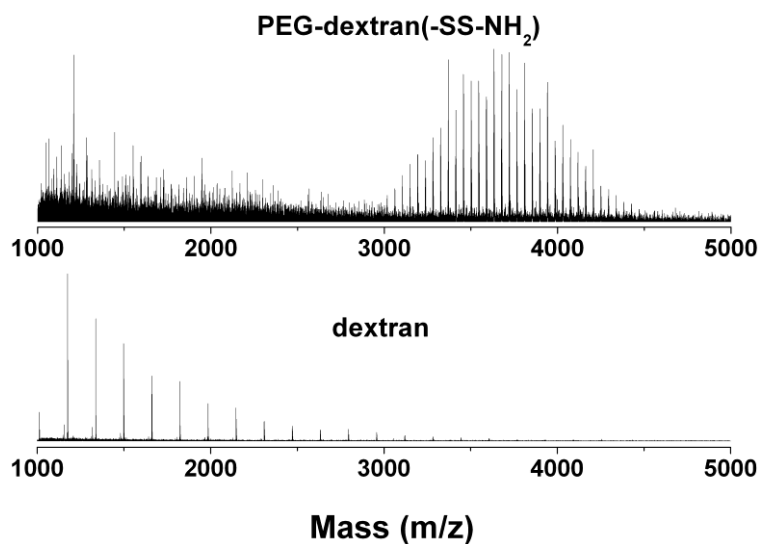
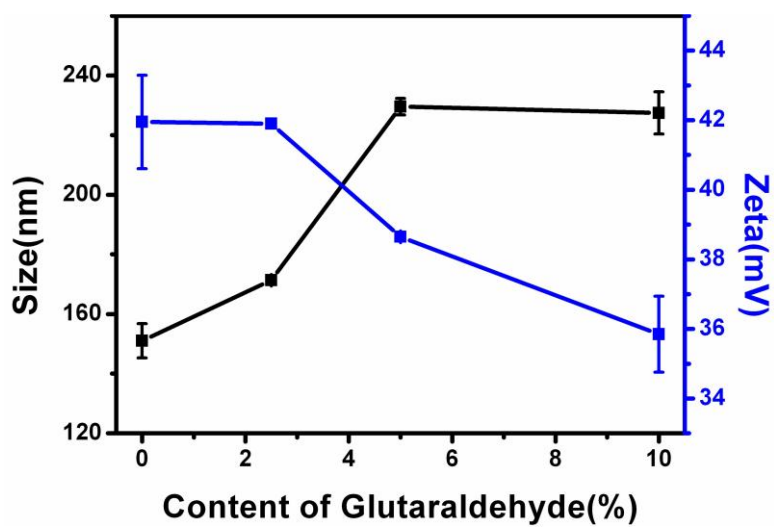


Fig. S3 MALDI-TOF-MS spectrum of the PEG-dextran(-SS-NH₂) and dextran, matrix: 2, 5-dihydroxybenzoic acid (DHB).



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Fig. S4 The size and zeta potential change of ICG NPs with different crosslinker content (w/w, %)

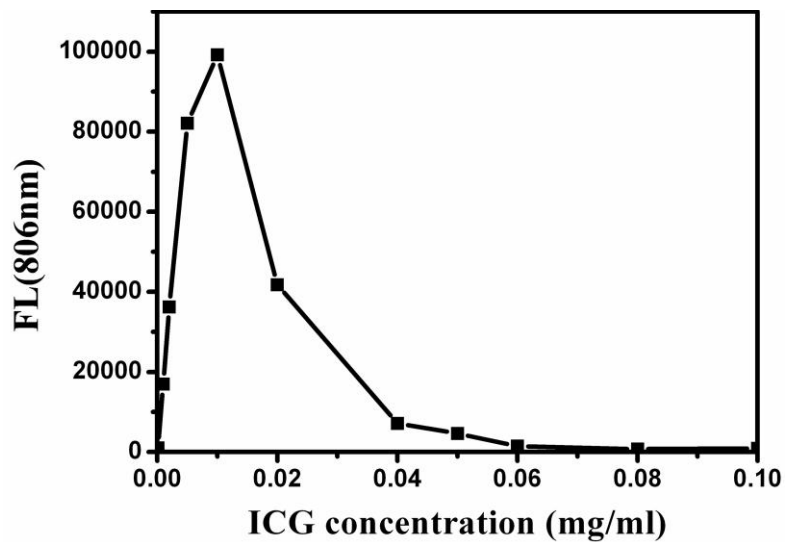


Fig. S5 Quenching of NIR fluorescence of ICG in aqueous solution

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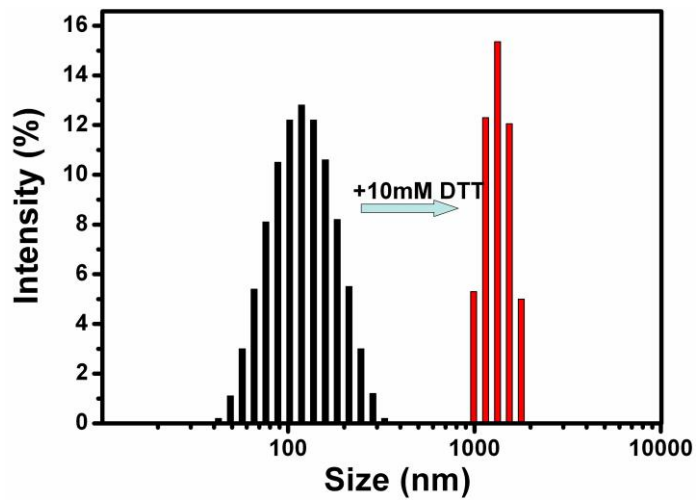


Fig. S6 Size change of ICG NPs in response to 10 mM DTT in PBS (pH7.4)

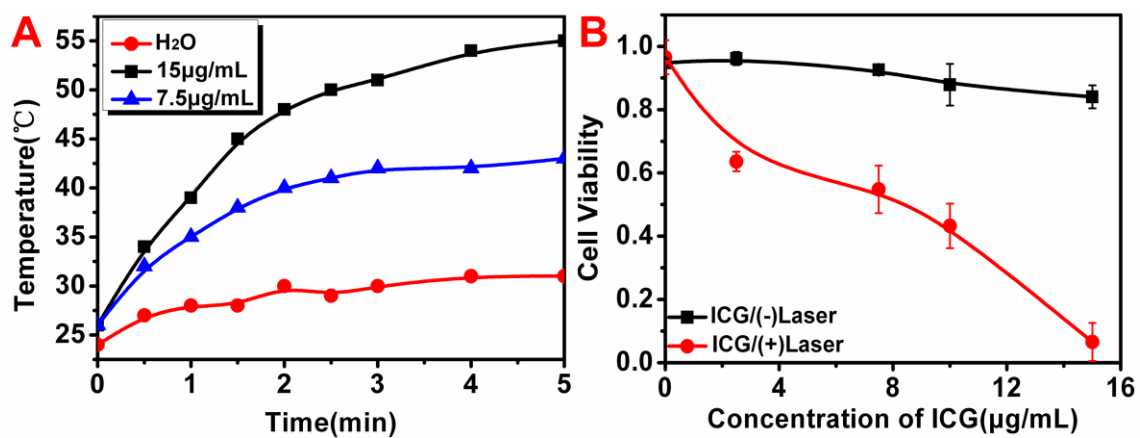


Fig. S7 (A) Temperature change of ICG in response to irradiation of a NIR laser (808 nm) with a power density of 1 W/cm² (B) Photothermal therapy efficiency of ICG with a NIR laser irradiation.