

## Supplementary information

### Experimental

#### 1. Preparation of the micellar nanoparticles with tunable levels of crosslink (LOC)

The thiolated telodendrimers (named as PEG<sup>5k</sup>-Cys<sub>4</sub>-L<sub>8</sub>-CA<sub>8</sub>, **Fig1**)<sup>1</sup> and thiol-free telodendrimer (PEG<sup>5k</sup>-CA<sub>8</sub>)<sup>2</sup> were synthesized via solution-phase condensation reactions from MeO-PEG-NH<sub>2</sub> utilizing stepwise peptide chemistry. The micellar nanoparticles with different levels of crosslink (LOC) were prepared by the solvent evaporation method as described in our previous studies<sup>1</sup>. Briefly, 20 mg total telodendrimers that contain different ratios of thiol-free telodendrimer (PEG<sup>5k</sup>-CA<sub>8</sub>) and thiolated telodendrimer (PEG<sup>5k</sup>-Cys<sub>4</sub>-L<sub>8</sub>-CA<sub>8</sub>) was dissolved in 1 mL chloroform in a 10 mL round bottom flask. The chloroform was evaporated under vacuum to form a thin film. PBS buffer (1 mL) was added to re-hydrate the thin film, followed by 30 min of sonication. The thiol groups on the telodendrimer were oxidized to form disulfide linkages via oxidation as described previously<sup>1, 3</sup>. The level of free thiol groups were monitored by Ellman's test over time. The micelle solution was used for further characterizations without dialysis after the level of free thiol groups remained at a constant low value.

#### 2. Preparation of PTX and DiD loaded micelles

PTX was loaded into the micellar nanoparticles by the solvent evaporation method as described above. The amount of drug loaded in the micelles was analyzed on a HPLC system (Waters) after releasing the drugs from the micelles by adding 9 times of acetonitrile and 10 min sonication. The drug loading was calculated according to the calibration curve between the HPLC area values and standard drug concentrations. The loading capacity is defined as the highest drug concentration that can be achieved by the micelles in aqueous solution while the loading efficiency is defined as the ratio of drug loaded into micelles to the initial drug content. DiD (a hydrophobic NIRF dye) either alone or together with PTX was loaded into the micellar nanoparticles using the same method as described above. The micelle solution was filtered with 0.22 μm filter to sterilize the sample.

#### 3. Characterizations of the micellar nanoparticles

The particle size and size distribution of the micellar nanoparticles were measured by dynamic light scattering (DLS) instruments (Microtrac). The micelle concentrations were kept at 1.0 mg/mL for DLS measurements. All measurements were performed at 25 °C, and data were analyzed by Microtrac FLEX Software 10.5.3. The morphology of the micellar nanoparticles was observed on a Philips CM-120 transmission electron microscope (TEM). The aqueous micelle solution (1.0 mg/mL) was deposited onto copper grids, stained with phosphotungstic acid, and measured at room temperature. The critical micelle concentration (CMC) of the micellar nanoparticles with tunable levels of crosslink (LOC) was measured through fluorescence spectra by using pyrene as a hydrophobic fluorescent probe as described previously<sup>1, 4</sup>. Briefly, the solutions of micellar nanoparticles were serially diluted in PBS. The stock solution of pyrene in methanol was added into the solutions with a final pyrene concentration of 2×10<sup>-6</sup> M. The solution was mildly shaken overnight. Excitation spectra were recorded ranging from 300 to 360 nm with a fixed emission at 390 nm. The ratios of the intensity at 337 to 332 nm from the excitation spectra of pyrene were plotted against the concentrations of the micellar nanoparticles. The CMC was determined from the threshold concentration, where the intensity ratio I<sub>337</sub>/I<sub>332</sub> begins to increase dramatically.

#### 4. Stability of micelles in SDS and GSH

The stability study was performed to monitor the change in particle size of the micellar nanoparticles with a variety of levels of crosslink (LOCs) in the presence of sodium dodecyl sulfate (SDS), which was reported to be able to efficiently break down polymeric micelles<sup>1</sup>. An SDS solution (7.5 mg/mL) was added to aqueous solutions of micelles (1.5 mg/mL). The final SDS concentration was 2.5 mg/mL and the micelle concentration was kept at 1.0 mg/mL. The size and size distribution of the micelle solutions was monitored at predetermined time intervals. The stability of the micellar nanoparticles was also evaluated in the presence of both GSH (10 mM) and SDS.

#### 5. Drug release study

PTX-encapsulated micellar nanoparticles with different LOCs were prepared to determine the *in vitro* drug release profile. The initial PTX concentration was 2.0 mg/mL. Aliquots of PTX-loaded micelle solution were injected into dialysis cartridges (Pierce Chemical Inc.) with a 3.5 kDa MWCO. The cartridges were dialyzed against 1 L PBS at 37 °C. In order to make an ideal sink condition, 10 g charcoal was added in the release medium. The concentration of PTX remaining in the dialysis cartridge at various time points was measured by HPLC. In some experiments, GSH (10 mM) were added to the release medium at a specific point in time (5 hrs). Values were reported as the means for each triplicate sample.

#### 6. MTT assay

SKOV-3 ovarian cancer cells were seeded in 96-well plates at a density of 10000 cells/well 24 h prior to the treatment. The cells were first treated with or without PTX-encapsulated micellar nanoparticles (final PTX concentration of 13.7 ng/mL) with LOCs at 0%, 20%, 50% and 100% for 2 hrs and then washed 3 times with PBS. The cells were incubated for another 22 or 46 hrs in a humidified 37 °C, 5% CO<sub>2</sub> incubator. MTT was added to each well and further incubated for 4 hrs. The absorbance at 570 nm and 660 nm was detected using a micro-plate ELISA reader (SpectraMax M2, Molecular Devices, USA). Untreated cells served as a control. Results were shown as the average cell viability  $[(OD_{\text{treat}} - OD_{\text{blank}})/(OD_{\text{control}} - OD_{\text{blank}}) \times 100\%]$  of triplicate wells.

#### 7. Animal model

Rats and female athymic nude mice (Nu/Nu strain), 6-8 weeks age, were purchased from Harlan (Livermore, CA). All animals were kept under pathogen-free conditions according to AAALAC guidelines and were allowed to acclimatize for at least 4 days prior to any experiments. All animal experiments were performed in compliance with institutional guidelines and according to protocol No. 07-13119 and No. 09-15584 approved by the Animal Use and Care Administrative Advisory Committee at the University of California, Davis.

#### 8. *In vivo* toxicity of the micellar nanoparticles with different LOCs

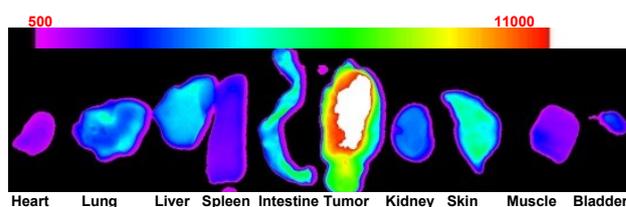
In order to investigate the potential toxicity of the drug delivery system itself, the micellar nanoparticles with different LOCs (0%, 20%, 50% and 100%) were injected in tumor free nude mice and rats via tail vein at the single dose of 200 mg/kg and 400 mg/kg, respectively. The animals were checked for possible signs of toxicity and the survival condition was monitored daily for two weeks.

#### 9. *In vivo* NIRF optical imaging

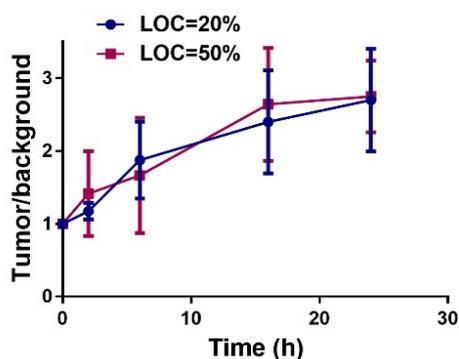
Female athymic nude mice bearing subcutaneous SKOV-3 tumor xenograft with approximate 8–10 mm in diameter were subjected to *in vivo* NIRF optical imaging. Mice were intravenously injected with 100 μL of DiD and PTX co-loaded micellar nanoparticles with different LOCs (20% and 50%). The concentrations of DiD and PTX were 0.5 and 2.0 mg/mL, respectively. At different time points post-injection, mice were scanned using a Kodak multimodal imaging system IS2000MM with an excitation bandpass filter at 625 nm and an emission at 700 nm. The mice were anaesthetized by intraperitoneal injection of pentobarbital (60 mg/kg) before each imaging. After *in vivo* imaging, animals were euthanized by CO<sub>2</sub> overdose at 24 and 48 hrs post-injection. Tumors and major organs were excised and imaged with the Kodak imaging station.

**Table S1.** The particle size and distribution of the micellar nanoparticles with tunable levels of crosslink (LOC) measured by DLS particle sizer. The PTX loading of micellar nanoparticles with different LOCs was all approximately 2.0 mg/mL as measured by HPLC.

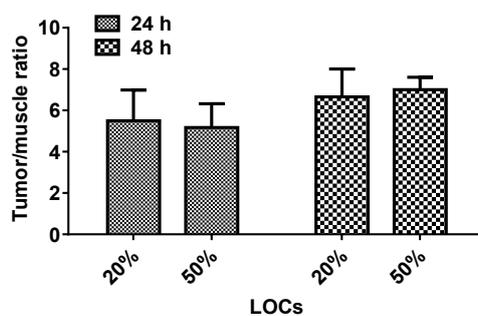
LOCs	0%	20%	30%	50%	75%	100%
Empty micelles	22±5	25±6	25±6	38±9	32±7	28±5
PTX-loaded micelles	23±4	27±5	25±4	33±7	28±6	27±6



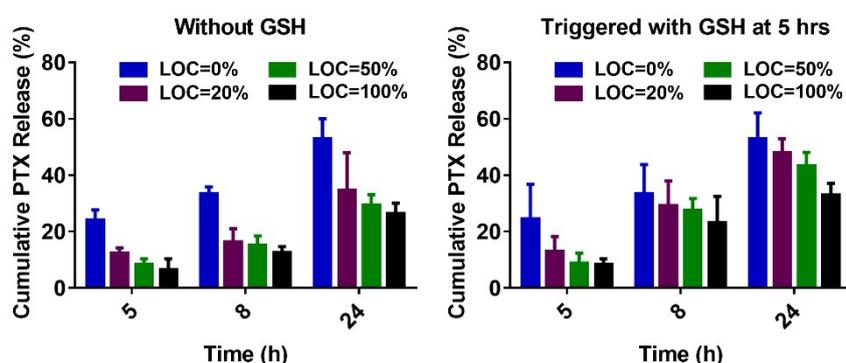
**Figure S1.** Representative *ex vivo* NIRF optical images of dissected organs and tumors from nude mice bearing SKOV-3 xenograft obtained with Kodak imaging system at 24 hrs after i.v. injection of DiD and PTX co-loaded micellar nanoparticles with a LOC at 20%; Injection volume: 100 μL, DiD: 0.5 mg/mL, PTX: 2.0 mg/mL.



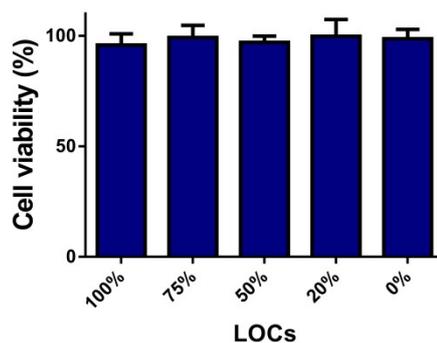
**Figure S2.** Time-dependent semi-quantitative tumor/background (normal skin) ratios of fluorescence intensity from the *in vivo* images of nude mice bearing SKOV-3 xenograft post-injection of DiD and PTX co-loaded micellar nanoparticles with LOCs at 20% and 50%. This profile was derived from the average fluorescence intensities of equal areas within tumor and normal skin tissue regions (n = 3).



**Figure S3.** Semi-quantitative tumor to muscle ratios from *ex vivo* images of nude mice bearing SKOV-3 xenograft at 24 and 48 hrs post-injection of DiD and PTX co-loaded micellar nanoparticles with LOCs at 20% and 50%, respectively. (n = 3).



**Figure S4.** Cumulative release of paclitaxel from of PTX encapsulated micellar nanoparticles with LOCs at 0%, 20%, 50% and 100% in PBS (pH 7.4) at 37°C, with and without triggered release at 5 hrs upon addition of 10 mM GSH.



**Figure S5.** MTT assays showing the viability of SKOV-3 ovarian cancer cells after 2hr incubation with empty micellar nanoparticles with LOCs at 0%, 20%, 50%, 75% and 100%, followed by washing three times with PBS and additional 46 hrs incubation (total incubation time: 48 hrs). \*:  $p < 0.05$ . Final concentration of total micelles: 274 ng/mL.

## References

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