Redox-responsive micellar-like nanoparticles can overcome intrinsic multi-drug resistance in tumour spheroids of triple negative breast cancer

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Supporting Information

1. Uptake of Cy5-labelled crosslinked and un-crosslinked micelles in 2D cell culture

The uptake of Cy5-labelled crosslinked and un-crosslinked micelles was investigated using confocal microscopy. The Cy5-labelled polymer and formulations were prepared as described previously.[1] The uptake of the Cy5-labelled micelles was firstly checked in 2D monolayers of MDA-MB-231 TNBC cells as shown in Figure S1.

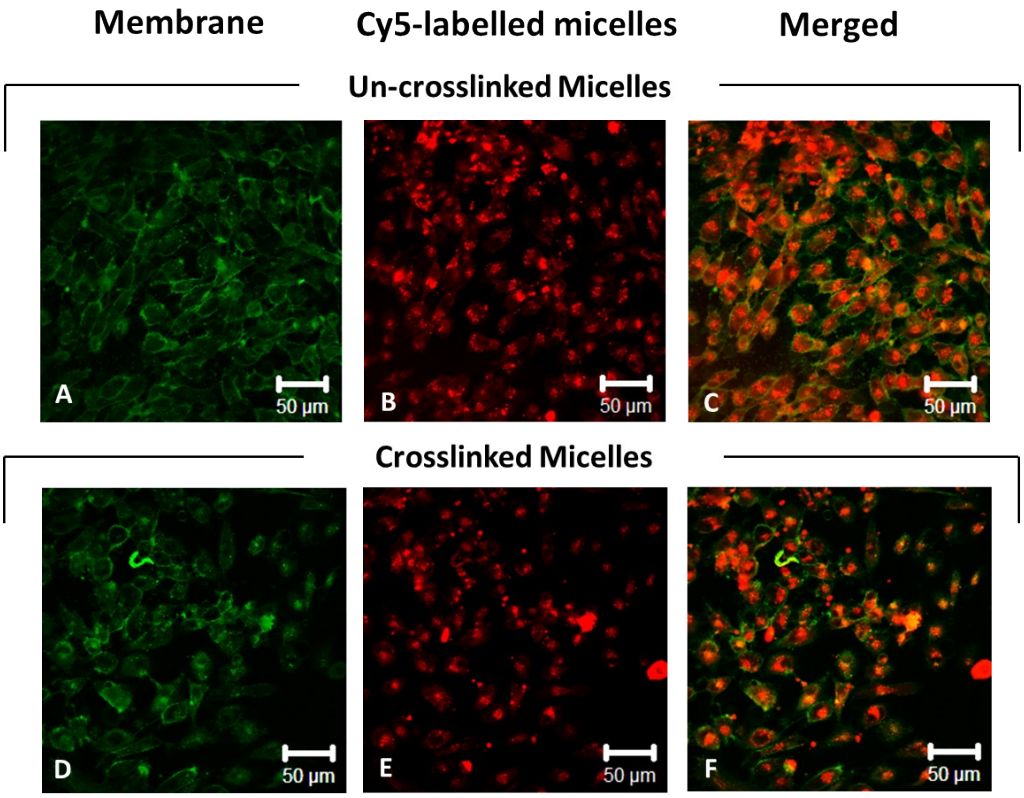

Figure S1 *Cellular uptake assessed by confocal microscopy of Cy5-labelled crosslinked and un-crosslinked micelles after 4 h of incubation. (A, D) – Green channel: showing the green fluorescence of CellMaskTM deep green plasma membrane stain. Red channel: Cy5-labelled crosslinked micelles (B) and Cy5-labelled un-crosslinked micelles. (E) – Merged: superimposition of all channels (C, F) – Scale bar 50 µm.*

MDA-MB-231 monolayers were exposed to Cy5-labelled micelles at the concentration of 50 µg/mL for 4 h (Figure S1 B,E). Afterwards, cells were washed as described previously, and cell membrane was stained with CellMask™ deep green plasma membrane stain (Figure S1 A,D).
Both Cy5-labelled crosslinked and un-crosslinked micelles were taken up by the triple negative breast cancer cells. In general, the micelles were located in the cell membranes but also around and within the nucleus.

Transport and cell internalisation of Cy5-labelled micelles was also assessed in 3D multicellular tumour spheroids of MDA-MB-231 cells. 4000 cells were seeded into a ULA 96 well-plate and the entire experiment, including the spheroids imaging, was carried out in the ULA plate. After seeding the cells, the spheroids were formed within 3 days and they were exposed to 50 µg/mL of Cy5-labelled crosslinked and un-crosslinked micelles and were incubated for 5 h. Spheroids were washed carefully and fixed.
As seen in Figure S2, spheroids nuclei were stained with Hoechst 33342 dye at concentration of 1 µg/mL (50 µL) for 30 minutes. This step for staining the nuclei was optimised by testing different concentrations of Hoechst 33342 dye and incubation time. The best results were obtained with the stated conditions.

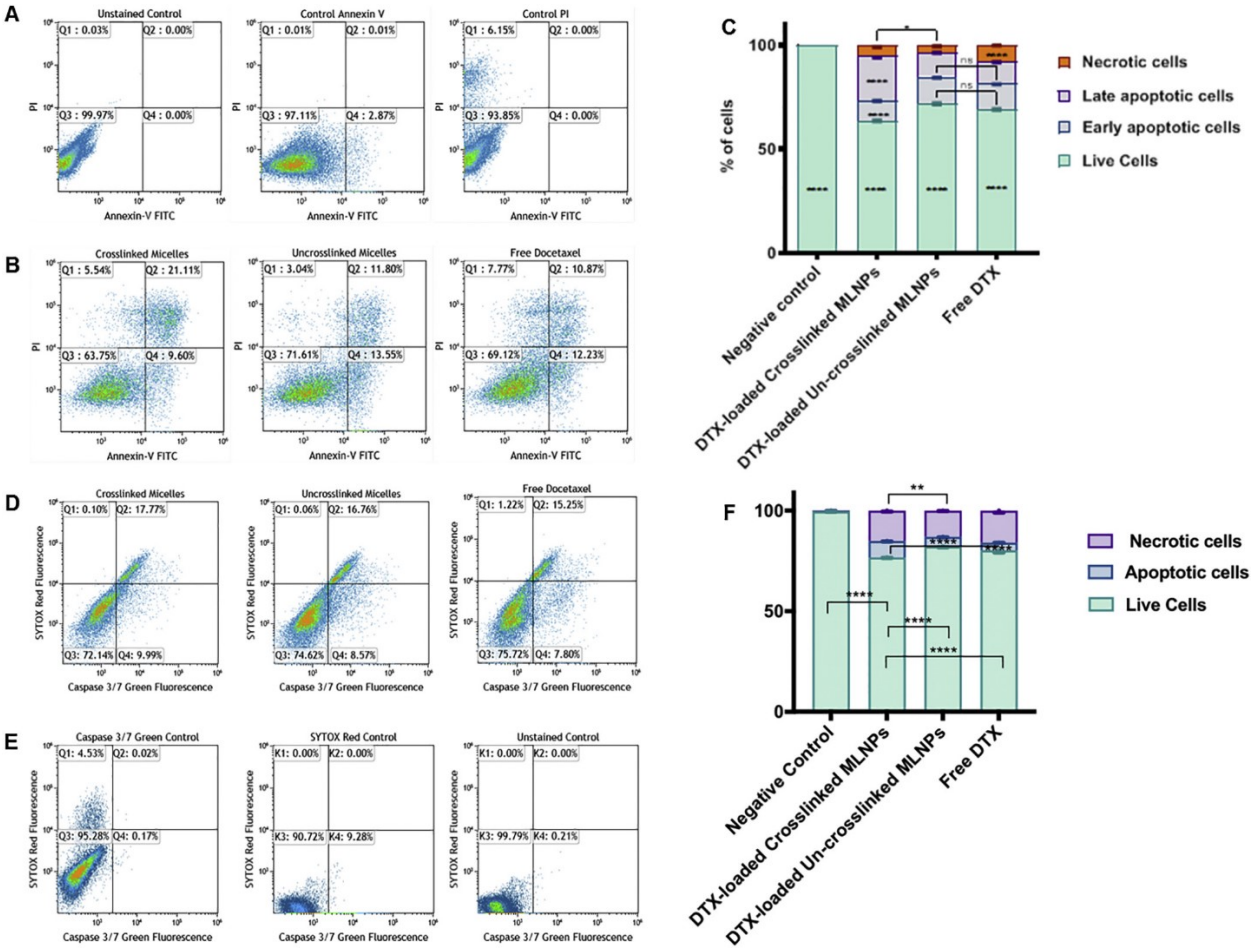

Figure S2 *Apoptosis assays annexin-V/ PI and caspase 3/7 and Sytox of the MDA-MB-231 cells treated with DTX-loaded crosslinked and un-crosslinked MLNPs assessed by FACS. (A) Controls in the panels showing unstained MDA-MB-231 breast cancer cells and controls for annexin-V FITC and PI. (B) MDA-MB-231 breast cancer cells stained with annexin-V FITC and PI, following treatment with crosslinked and un-crosslinked MLNPs encapsulated with DTX and free DTX for 24 h. (C) Proportion (%) of different cell populations after treatment with crosslinked, un-crosslinked MLNPs and free DTX using annexin/PI assay. (D) Controls in the panels showing unstained MDA-MB-231 breast cancer cells and controls for CellEvent Caspase 3/7 and Sytox. (E) MDA-MB-231 triple negative breast cancer cells were treated with crosslinked and un-crosslinked MLNPs encapsulated with DTX and free DTX for 24 h. Samples were labelled with the CellEvent Caspase 3/7 and Sytox red and analysed by FACS. (F) Cells with caspase-3/7 activation after treatment with crosslinked, un-crosslinked MLNPs and free DTX.*

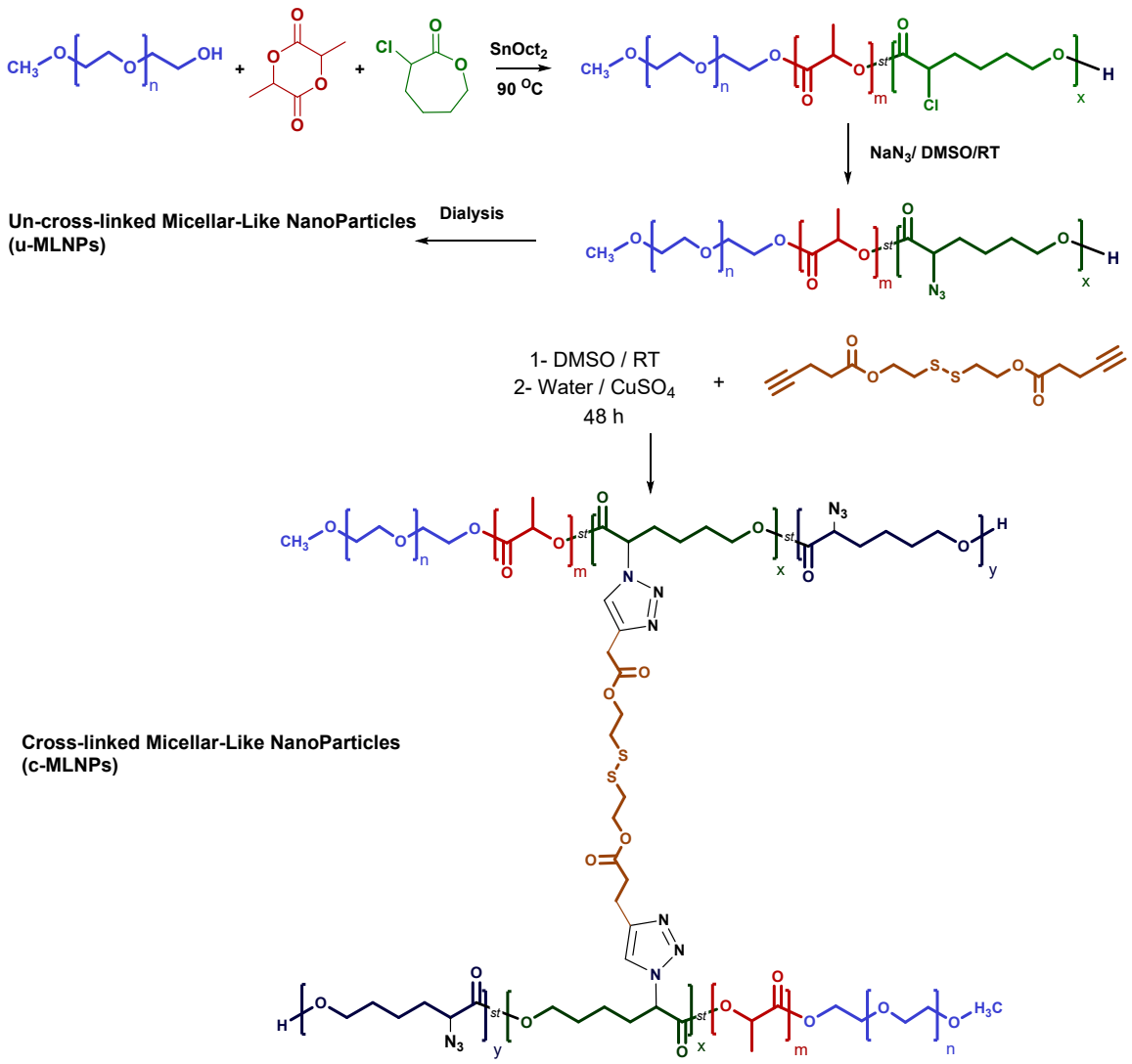

Figure S3. *Synthesis of polymers and preparation of Micellar-Like NanoParticles. Synthesis of mPEG-poly(lactide)-co-poly(chlorocaprolactone) from lactide and chlorocaprolactone with mPEG5000 and tin (II) octanoate, followed by reaction with sodium azide to form mPEG-poly(lactide)-co-poly(azidocaprolactone). Cross-linked MLNPs were prepared by reaction with disulfanediylbis(ethane-2,1-diyl) bis(pent-4-ynoate) and copper (II) sulfate in water/DMSO. Scheme adapted from reference 1.*

Table S1 *Characterisation data for polymer nanoparticles formed as empty or DTX-loaded un-crosslinked and redox responsive core crosslinked MLNPs (adapted from reference 1*

Formulation	Size (D_H, nm) ± SD	Zeta potential (mV) ± SD	Drug loading content (%) ± SD	Encapsulation efficiency (%) ± SD
Un-CC MLNPs	31.6 ± 1.0	- 5.4 ± 0.2	NA	NA
CC MLNPs	41.1 ± 0.5	-6.1 ± 0.2	NA	NA
DTX-loaded un-CC MLNPs	42.6 ± 0.5	-6.8 ± 0.3	2.3 ± 0.1	29.5 ± 1.3
DTX-loaded CC MLNPs	55.3 ± 1.2	-7.9 ± 0.5	6.6 ± 0.3	42.8 ± 0.5

n = 3 ± SD (standard deviation), NA – not applicable

[1] P.F. Monteiro, M. Gulfam, C.J. Monteiro, A. Travanut, T.F. Abelha, A.K. Pearce, C. Jerome, A.M. Grabowska, P.A. Clarke, H.M. Collins, D.M. Heery, P. Gershkovich, C. Alexander, Synthesis of micellar-like terpolymer nanoparticles with reductively-cleavable cross-links and evaluation of efficacy in 2D and 3D models of triple negative breast cancer. *J Control Release*, **2020**, 323, 549-564.