Electronic Supplementary Material (ESI) for Biomaterials Science.

Free Drug and ROS-Responsive Nanoparticle Delivery of Synergistic Doxorubicin and Olaparib Combinations to Triple Negative Breast Cancer Models

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Supplementary Information

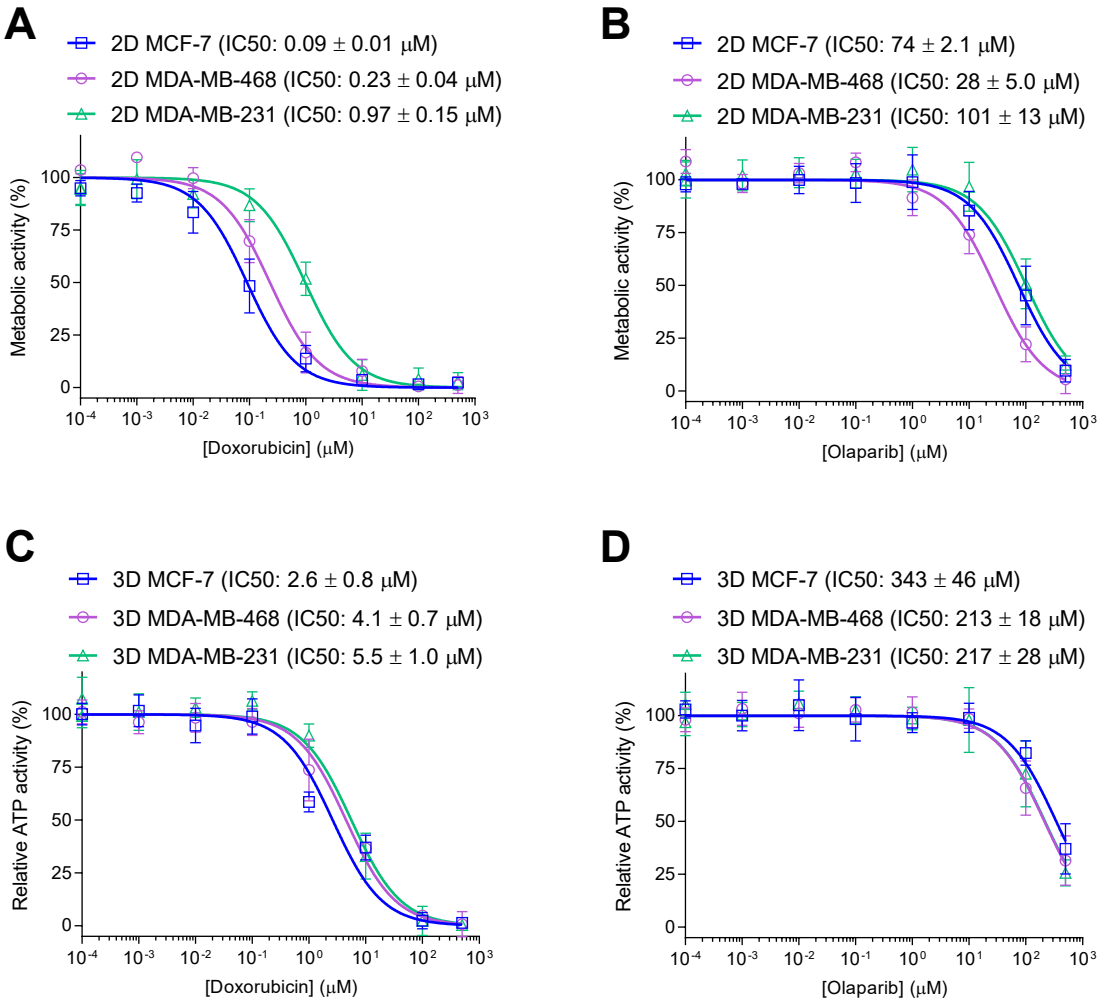

Figure S1. Potency of (A,C) doxorubicin and (B,D) olaparib in (A,B) 2D and (C,D) 3D spheroid cultured MDA-MB-231, MDA-MB-468 and MCF-7 cells. Data presented as mean ± S.D. IC50 values calculated on GraphPad Prism and are shown in key of graphs.

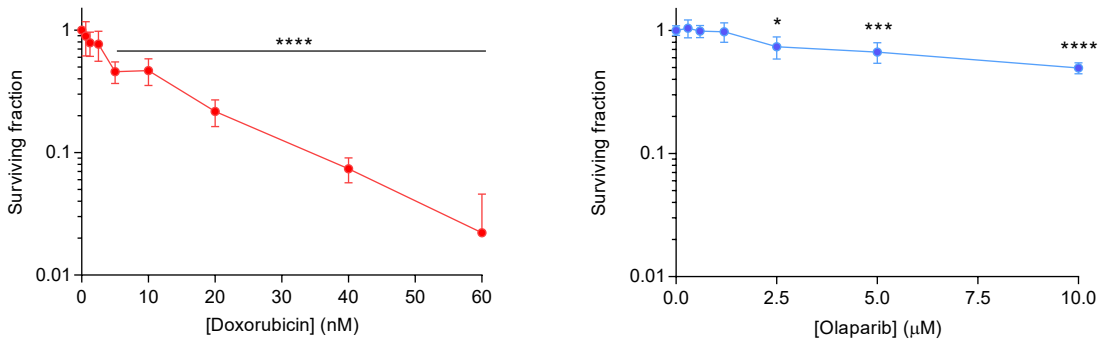

Figure S2. Clonogenic cell survival following single treatment with doxorubicin or olaparib on MDA-MB-231 cells. Data presented as mean ± S.D.

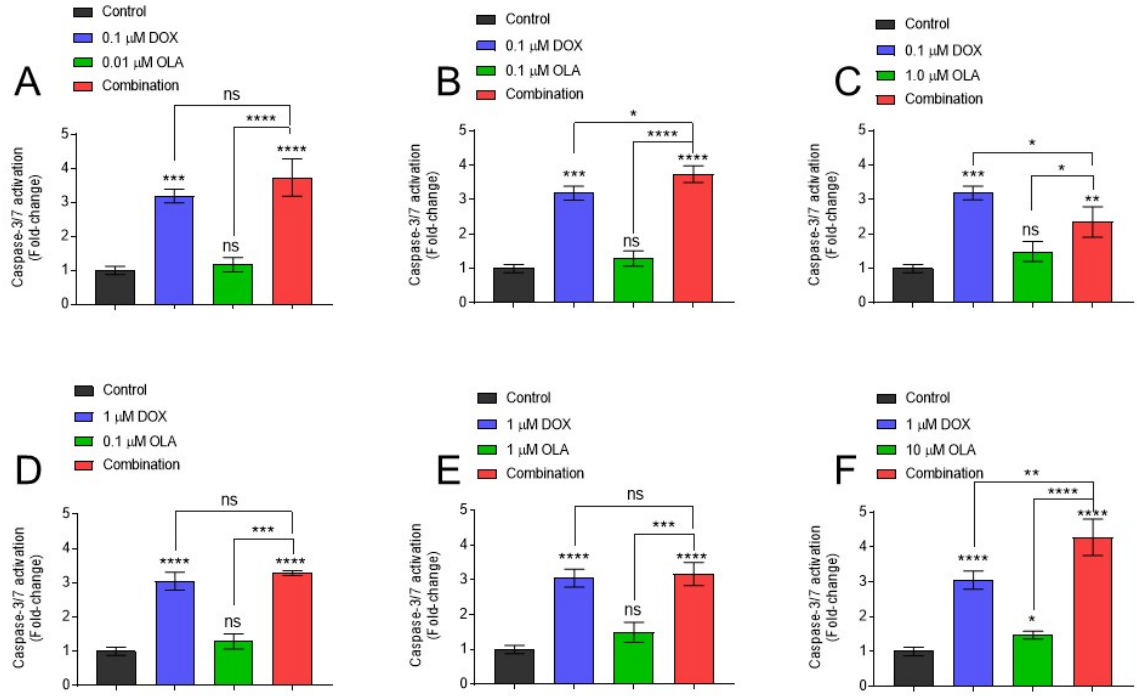

Figure S3. Detection of Caspase-3/7 activation. MDA-MB-231 cells cultured in 2D manner and incubated with single, combination or the vehicle control for 24 hours. Levels of caspase-3/7 activation then probed using CellEvent Caspase-3/7 detection reagent. Values normalised to vehicle control. Statistical analysis performed using One-way ANOVA. Data presented as mean ± S.D.

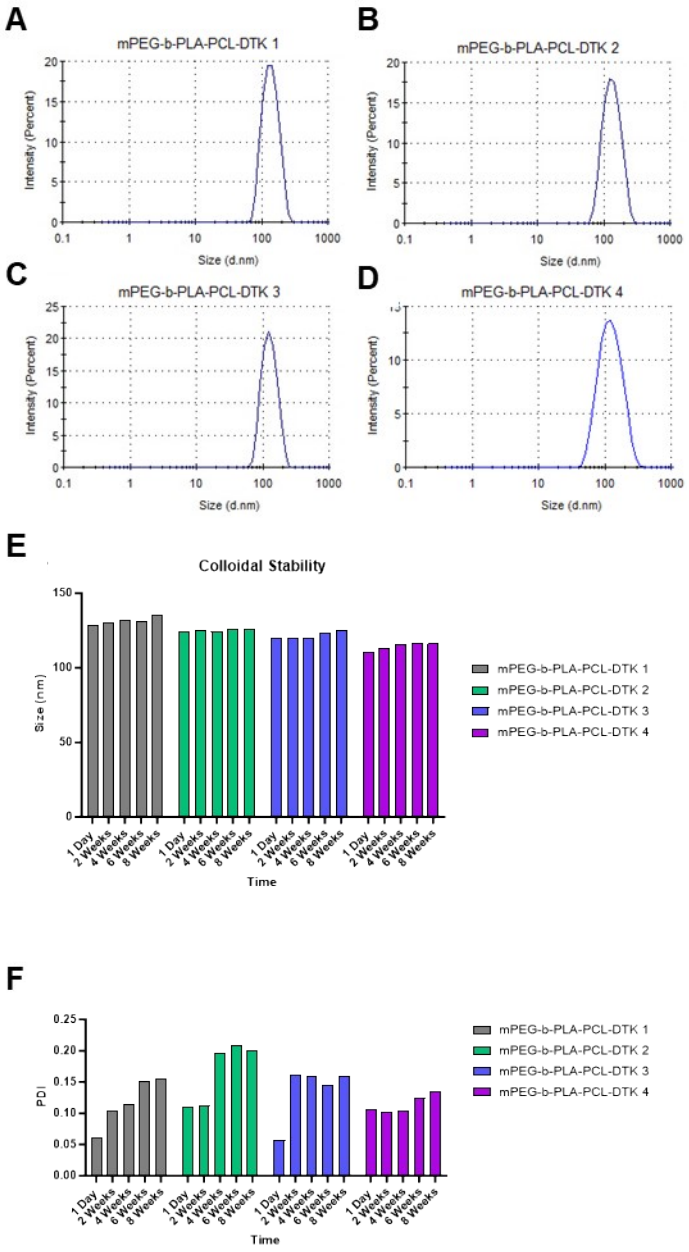

Figure S4 – (A-D) Average hydrodynamic diameter of polymer NPs (DLS intensity distribution) and (E, F), effects of storage at 4 ºC on particle size and polydispersity.

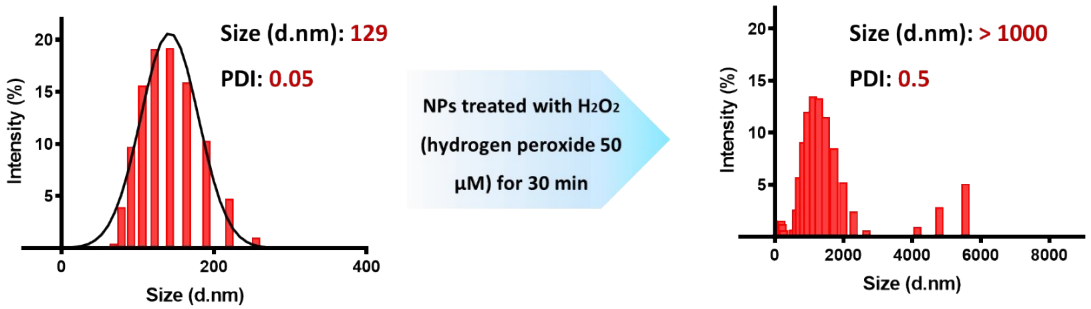

Figure S5 – Disruption of oxidation-sensitive NPs via hydrogen peroxide. Micellar-like NPs of ~ 130 nm (left) no longer form discrete stable nanoparticles resulting in the appearance of aggregates in light-scattering experiments (right).

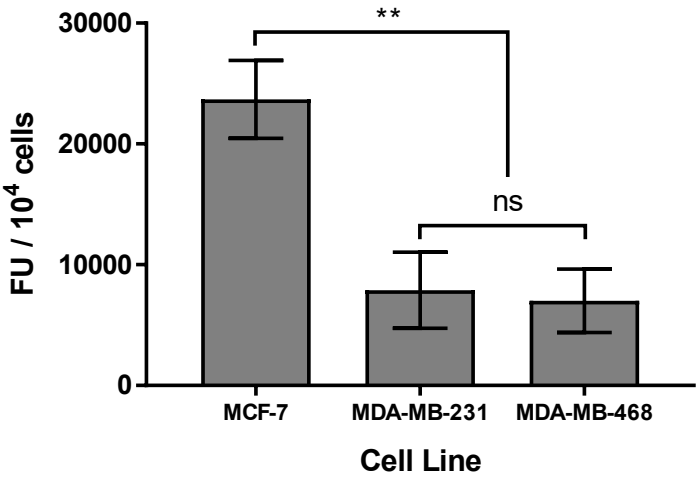

Figure S6. ROS levels in breast cancer cell lines assessed by the general oxidative stress indicator probe CM-H2DCFDA (applied in HBSS at 10 µM). Breast cancer cells (BCC) were seeded in 96 well plates at seeding density of 1x10^4 cells/well and cultured for 48h prior to assaying. Fluorescence was read at Ex 490 nm Em 520 nm and normalised to viable cell number per well following trypan blue exclusion test and counting on haemocytometer. Data presented as mean ± S.D (n=3 for MCF-7 and MDA-MB-231; n=2 for MDA-MB-468).

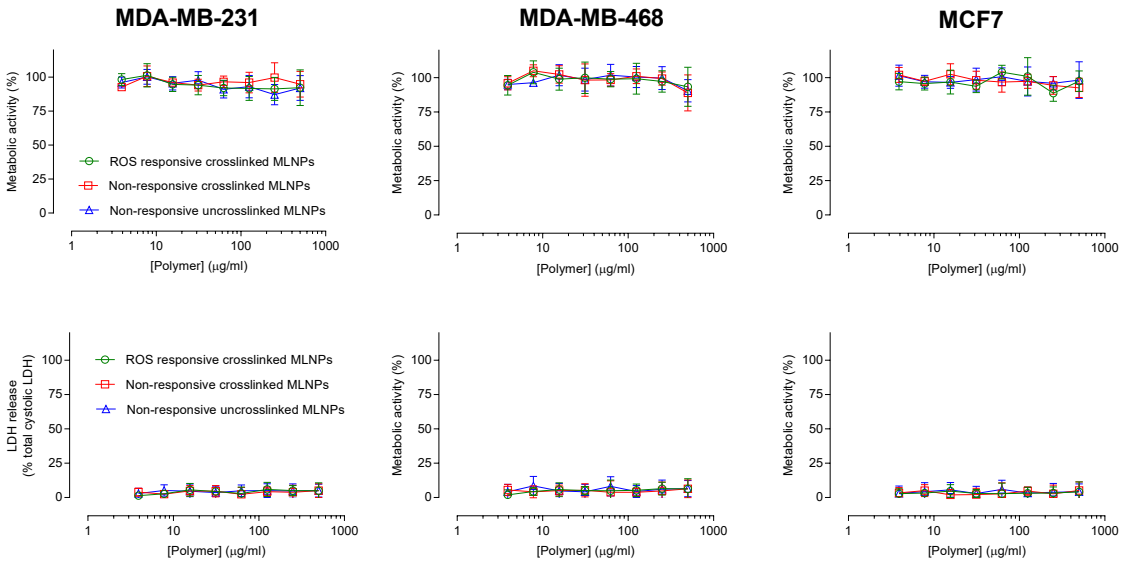

Figure S7. Effects of mPEG-b-PLA-PCL-DTK 1 micellar-like NPs (MLNPs) in MDA-MB-231, MDA-MB-468 and MCF7 breast cancer cells. Estimates of cytotoxicity were determined by **(top row)** PrestoBlue metabolic activity and (**bottom row**) LDH release as an indicator of membrane damage. Micellar-like NPs (0.39-500 µg/ml) were applied in DMEM containing 10% FBS and exposed for 48 hours to the cells. All cells were seeded at a density of 1 x 10^4 cell per well, and cultured for 24 hours prior to assaying. Data are presented as mean ± S.D (n=3, N=3)

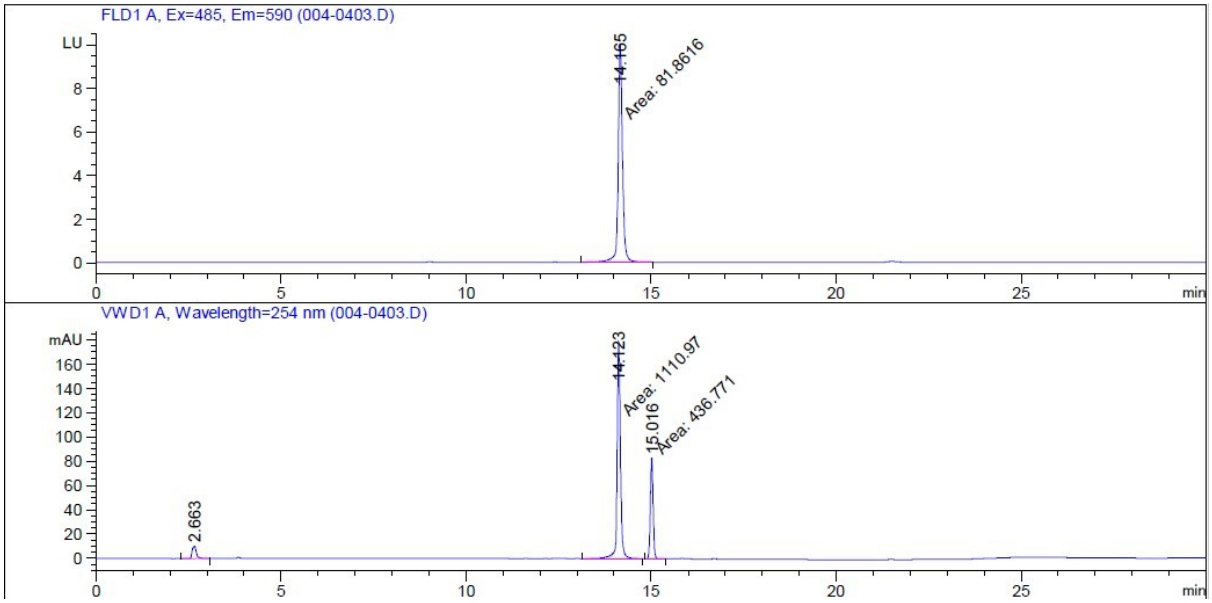

Figure S8 – Representative chromatograms for a Dox/Ola standard at 10 µg/mL. Top: FLD response at 485/590 nm (ex/em) showing Dox peak at 14.165 min. Bottom: VWD response at 254 nm showing Dox peak at 14.123 min and Ola peak at 15.016 min

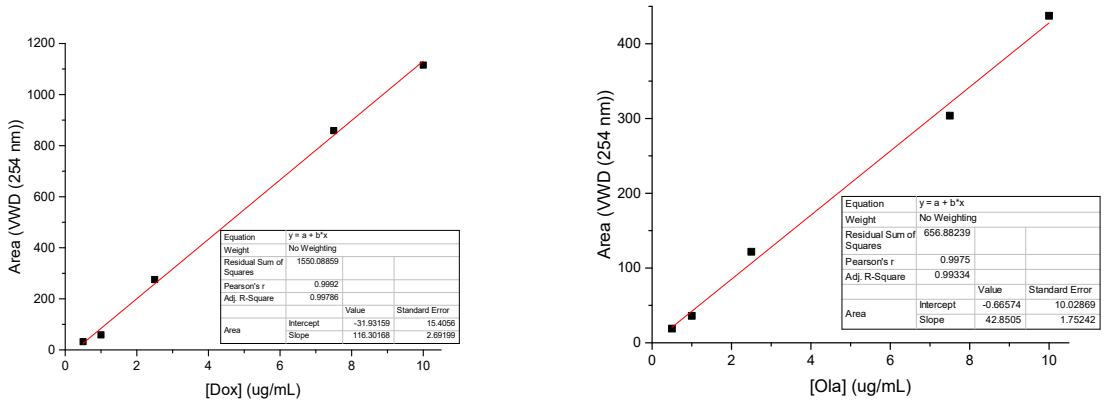

Figure S9 – Calibration curves of a) Doxorubicin and b) Olaparib using the VWD response

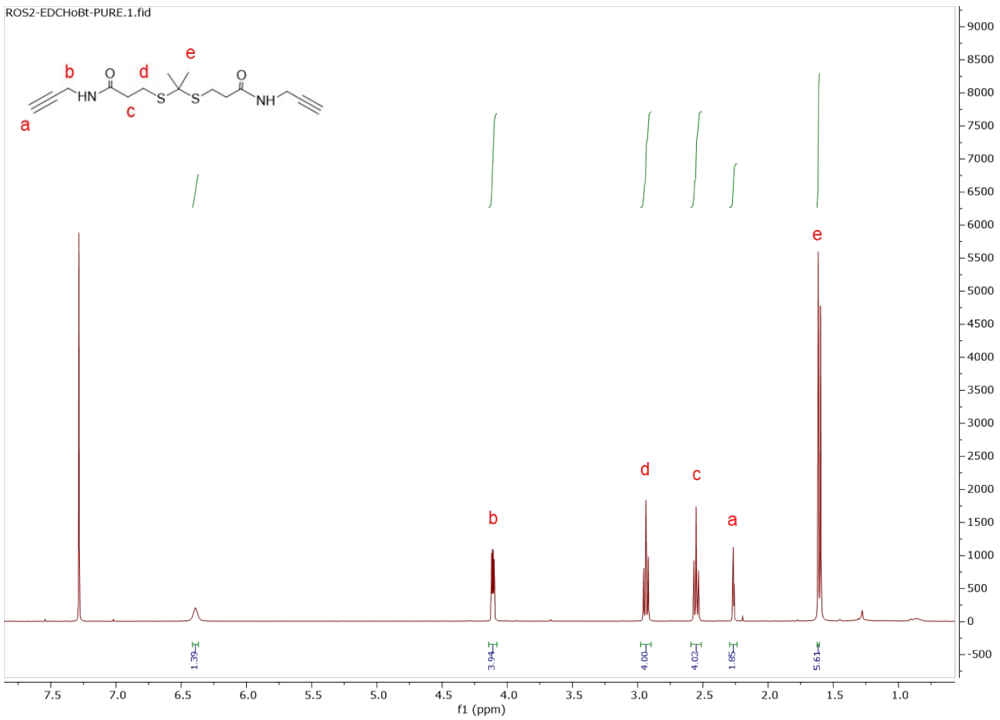

Figure S10 – ^{1}H NMR spectrum of dithioketal cross-linker (DTK)

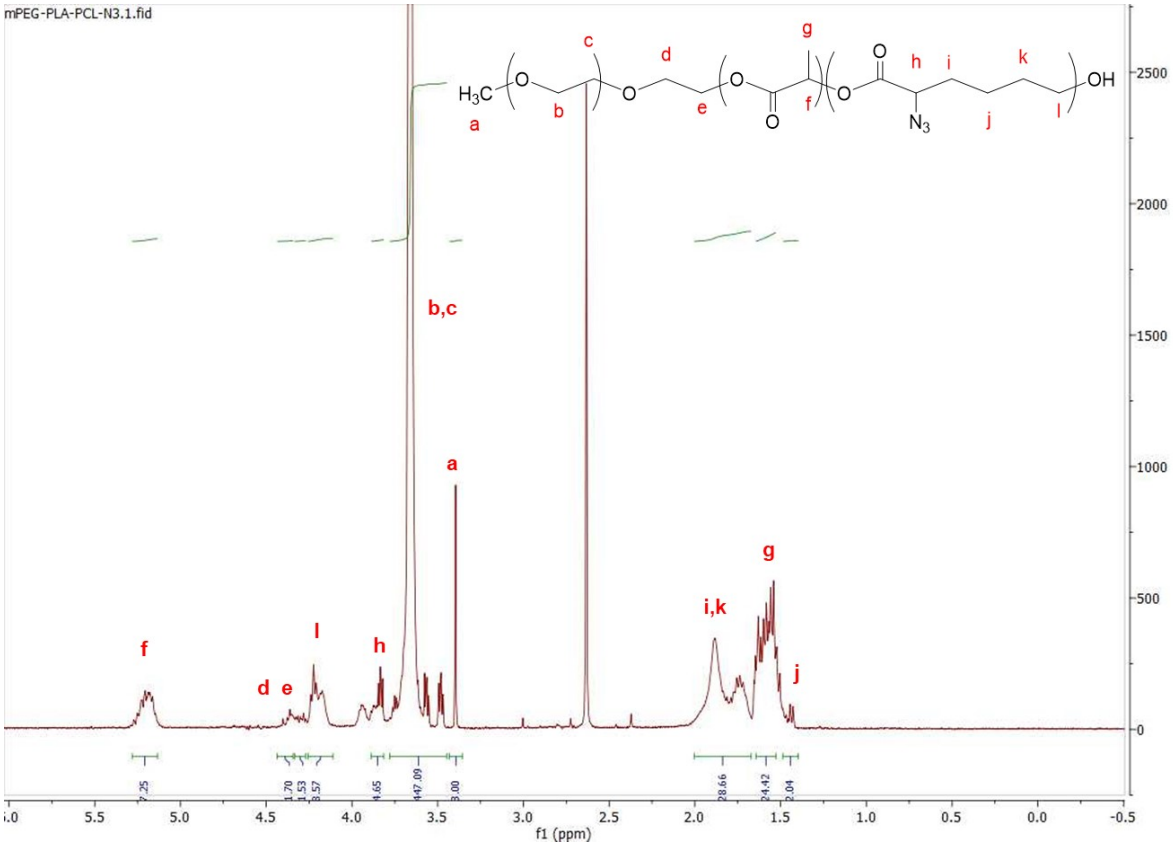

Figure S11 – 1H NMR spectrum of MeO-PEG-PLGA-PCL(N_3)

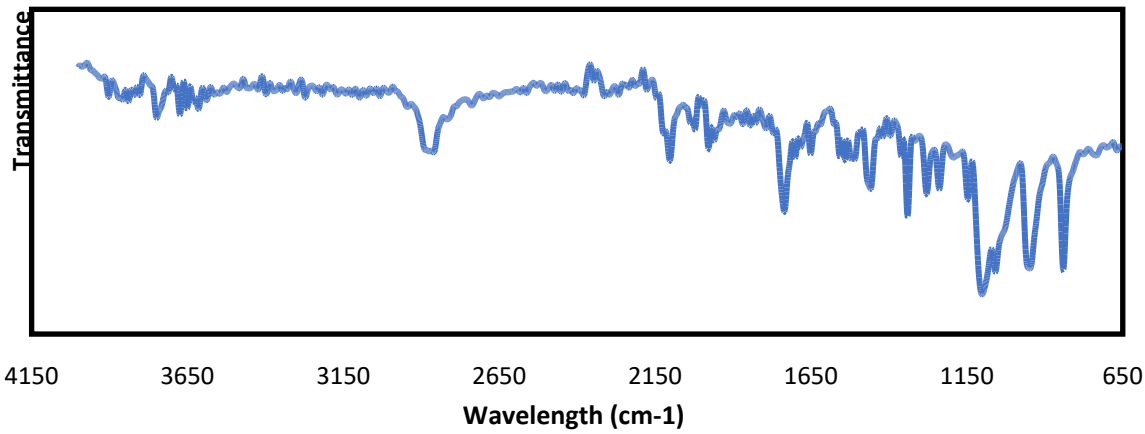

Figure S12 – IR spectrum of MeO-PEG-PLGA-PCL(N_3)