SUPPORTING INFORMATION: Polybasic Nanogels for Intracellular Co-Delivery of Paclitaxel and Carboplatin: A Novel Approach to Ovarian Cancer Therapy
by Angela M. Wagner et al.

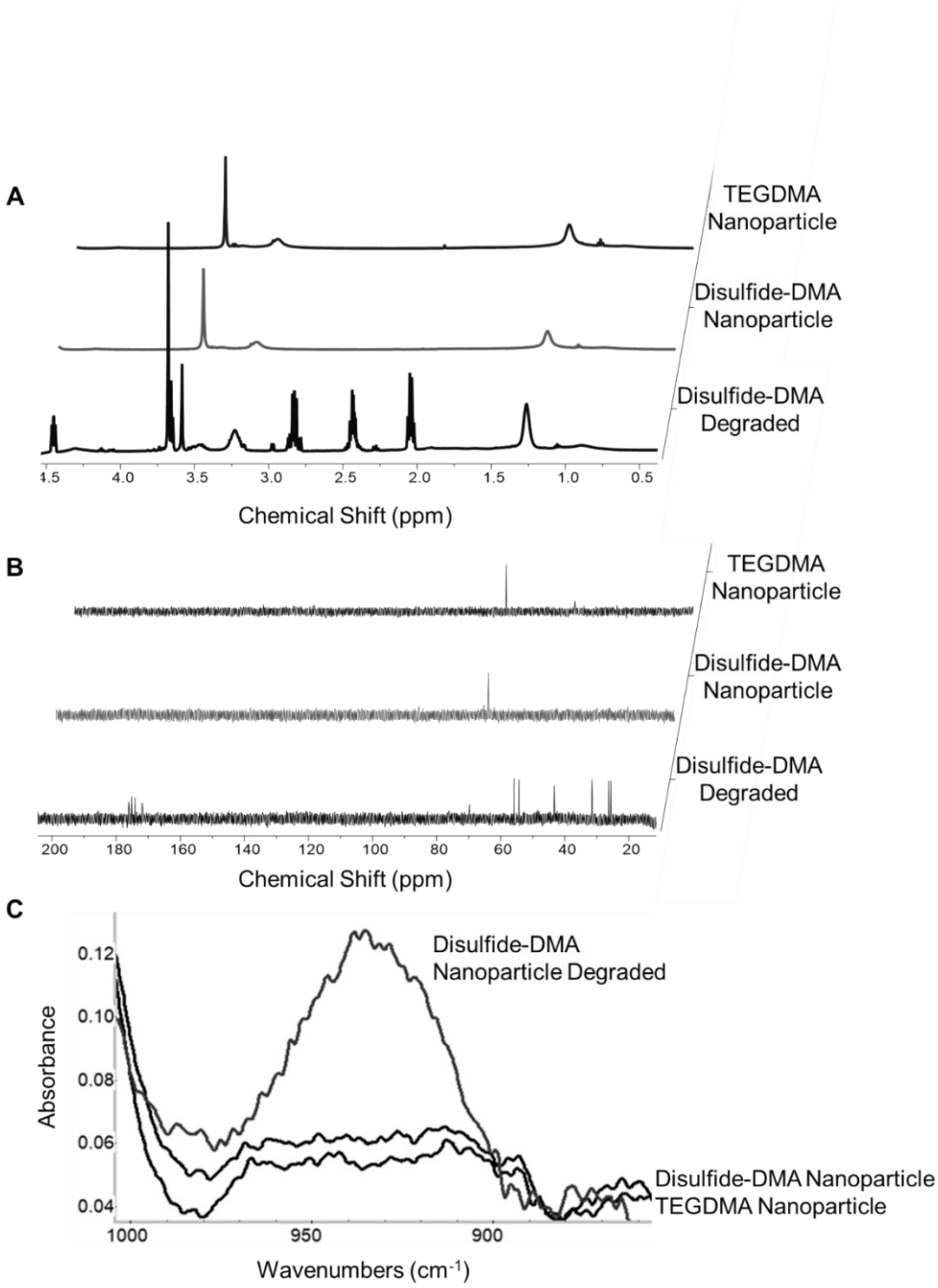

Figure S.1. (A) ^{1}H-NMR spectra, (B) ^{13}C-NMR spectra, and (C) ATR-FTIR spectra of nanoparticles synthesized with varying crosslinking agents, disulfide-DMA and TEDGMA. Nanogels were degraded in the presence of 10 mM glutathione (GSH).

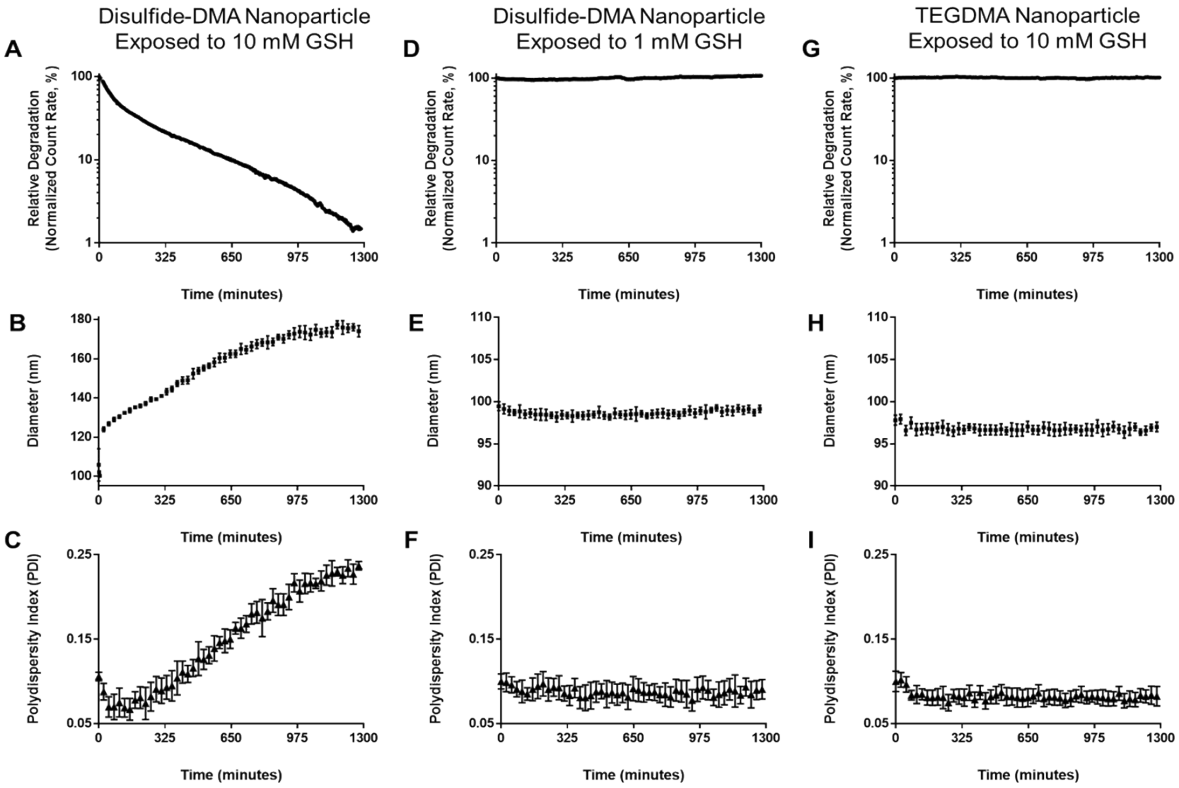

*Figure S.2 Degradation of nanoparticles synthesized with **(A-F)** disulfide-DMA and **(G-I)** TEGDMA in the presence of glutathione reducing agent (GSH), where **(A-C)** and **(G-I)** were exposed to relevant intracellular concentration and **(D-F)** were exposed to relevant extracellular concentration. Dynamic light scattering count rate was measured sequentially (approximately every 5 minutes) for nanogels at 0.5 mg/mL in 5 mM sodium phosphate pH 6.5. **(A,D,G)** Count rate normalized to the initial time point, **(B,E,H)** nanoparticle diameter, and (C,F,I) polydispersity index as a function of exposure time to GSH.*

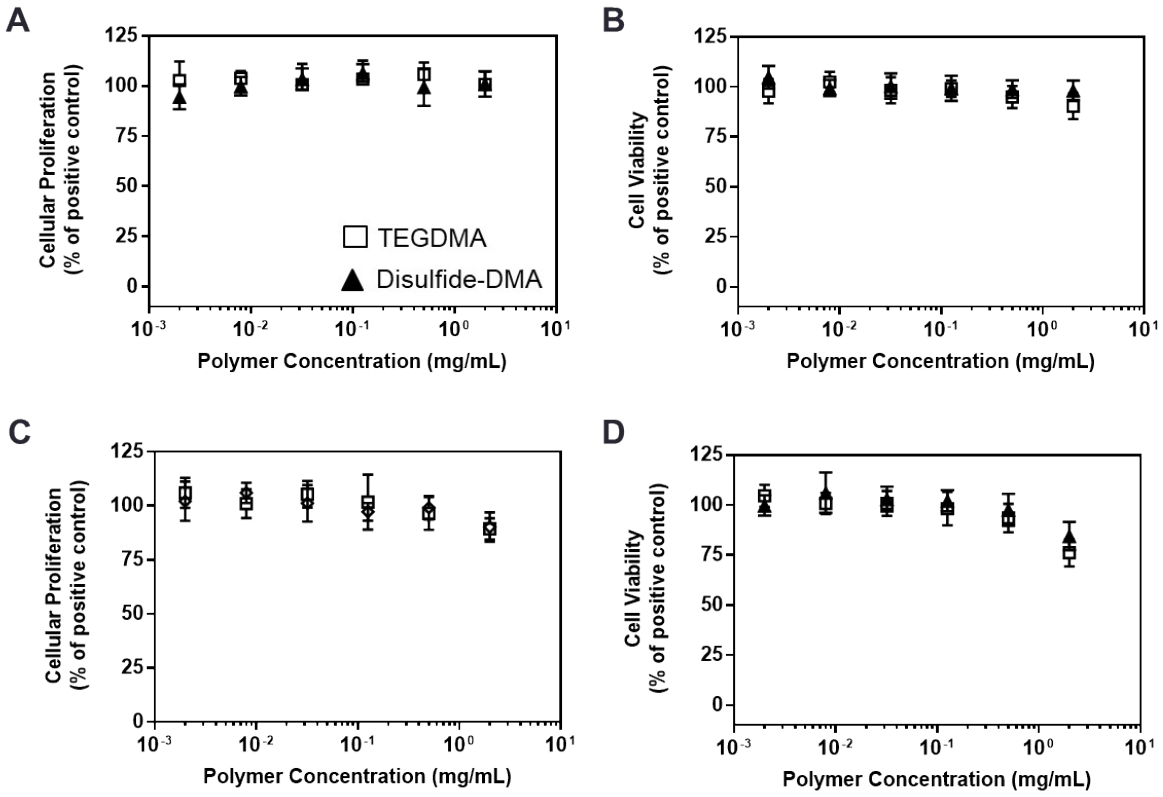

Figure S.3 The influence of crosslinking agent (disulfide-DMA versus TEGDMA), and exposure time on membrane destabilization and overall live cell health was investigated in OVCAR-3 cells using nanogel concentrations of 0.002 to 2 mg/mL. Representative results are shown for no hydrophobic monomer (λ) and cyclohexyl methacrylate monomer (•) from ***(A)*** *a 2 hour MTS assay measuring cell proliferation,* ***(B)*** *a 2 hour LDH assay measuring cell viability,* ***(C)*** *a 24 hour MTS assay measuring cell proliferation, and* ***(D)*** *a 24 hour LDH assay measuring cell viability. Data represent mean ± SEM (n=3).*

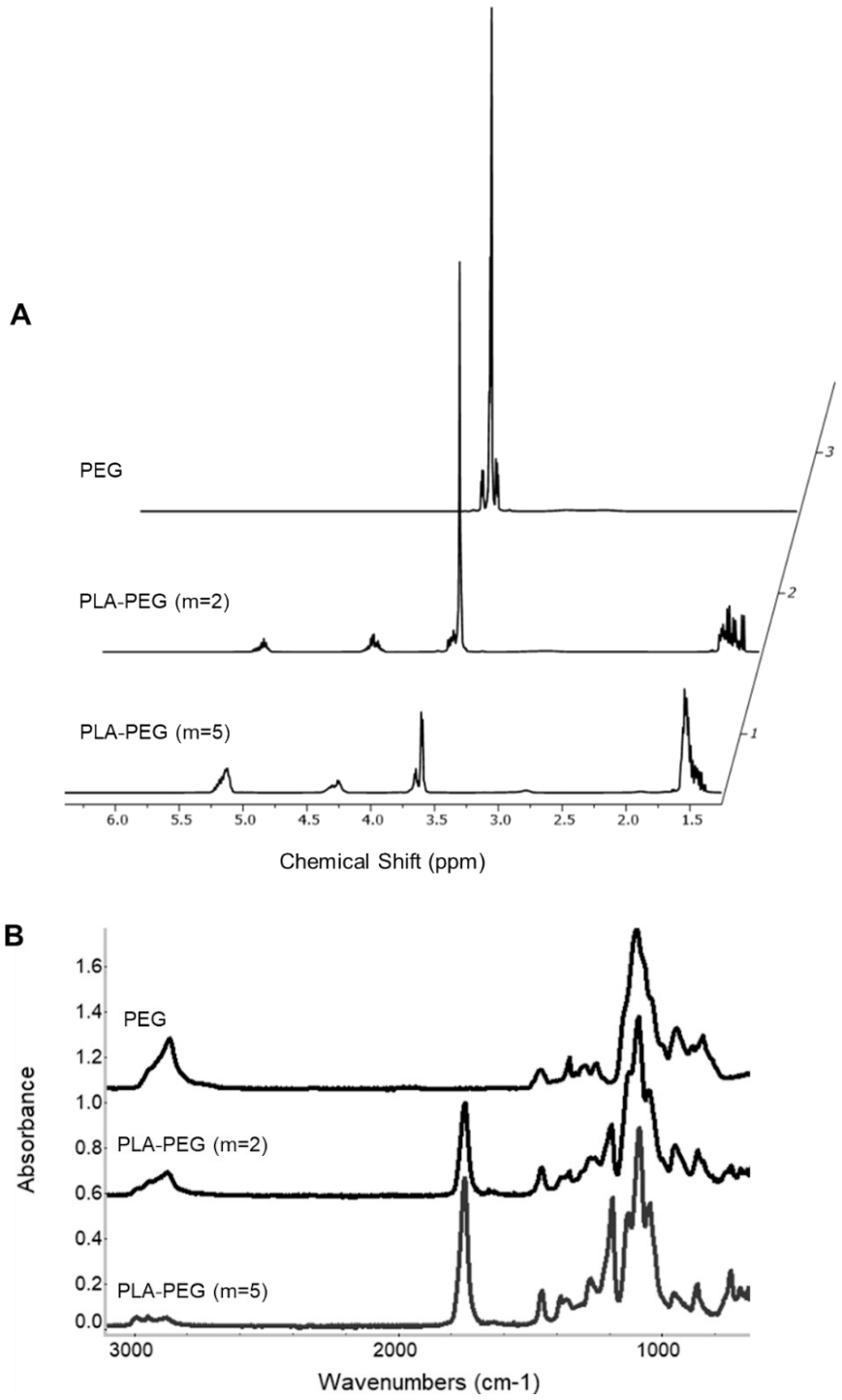

Figure S.4 ***(A)*** *^{1}H-NMR and* ***(B)*** *ATR-FTIR of confirming the synthesis of custom PLA-b-PEG-b-PLA crosslinkers with varying ratio of LA to EG units. PEG is shown for comparison.*

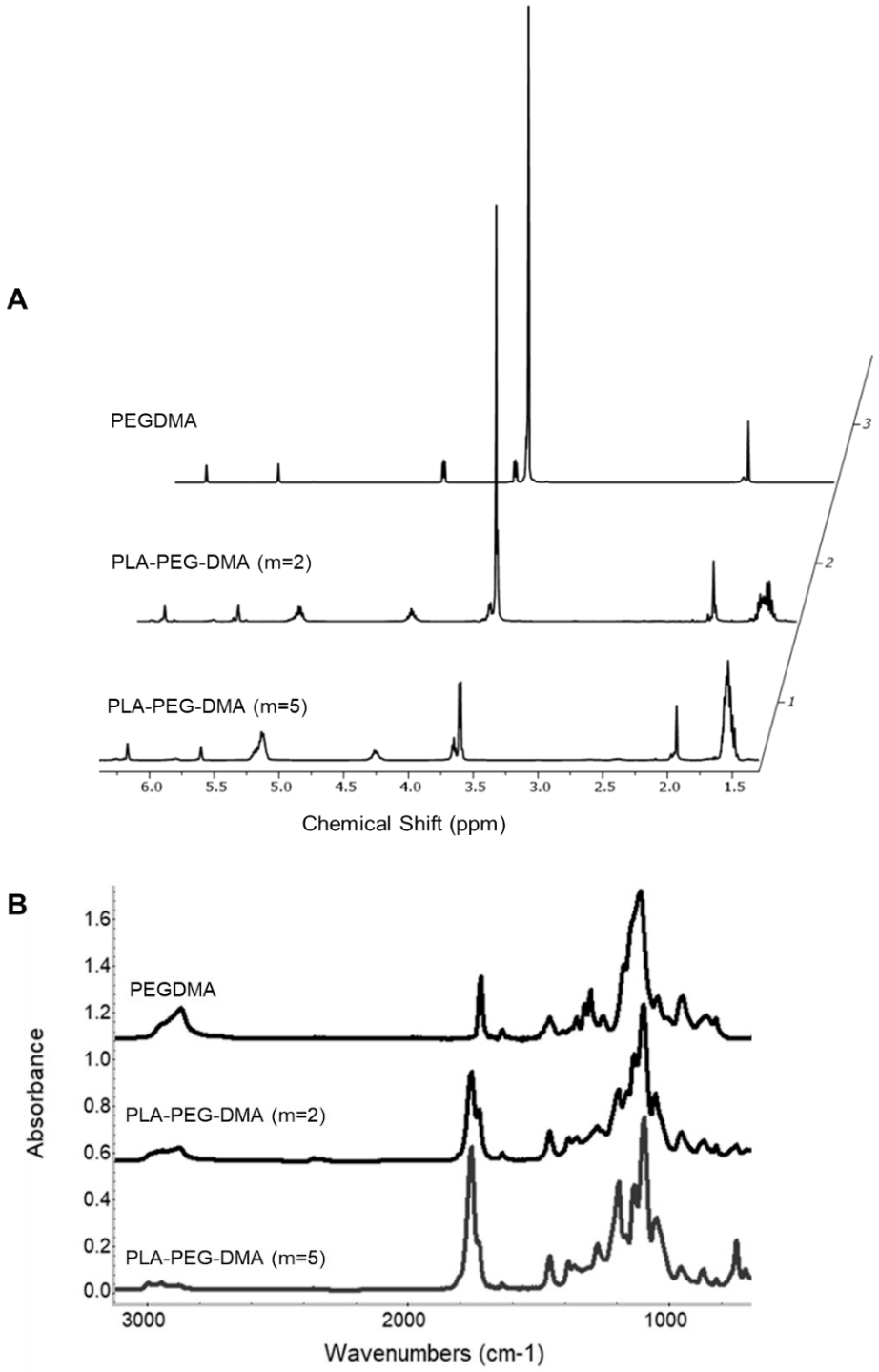

Figure S.5 **(*A*)** *^{1}H-NMR and* **(*B*)** *ATR-FTIR of confirming the dimethacrylation step of custom PLA-b-PEG-b-PLA crosslinkers. PEG-DMA is shown for comparison.*

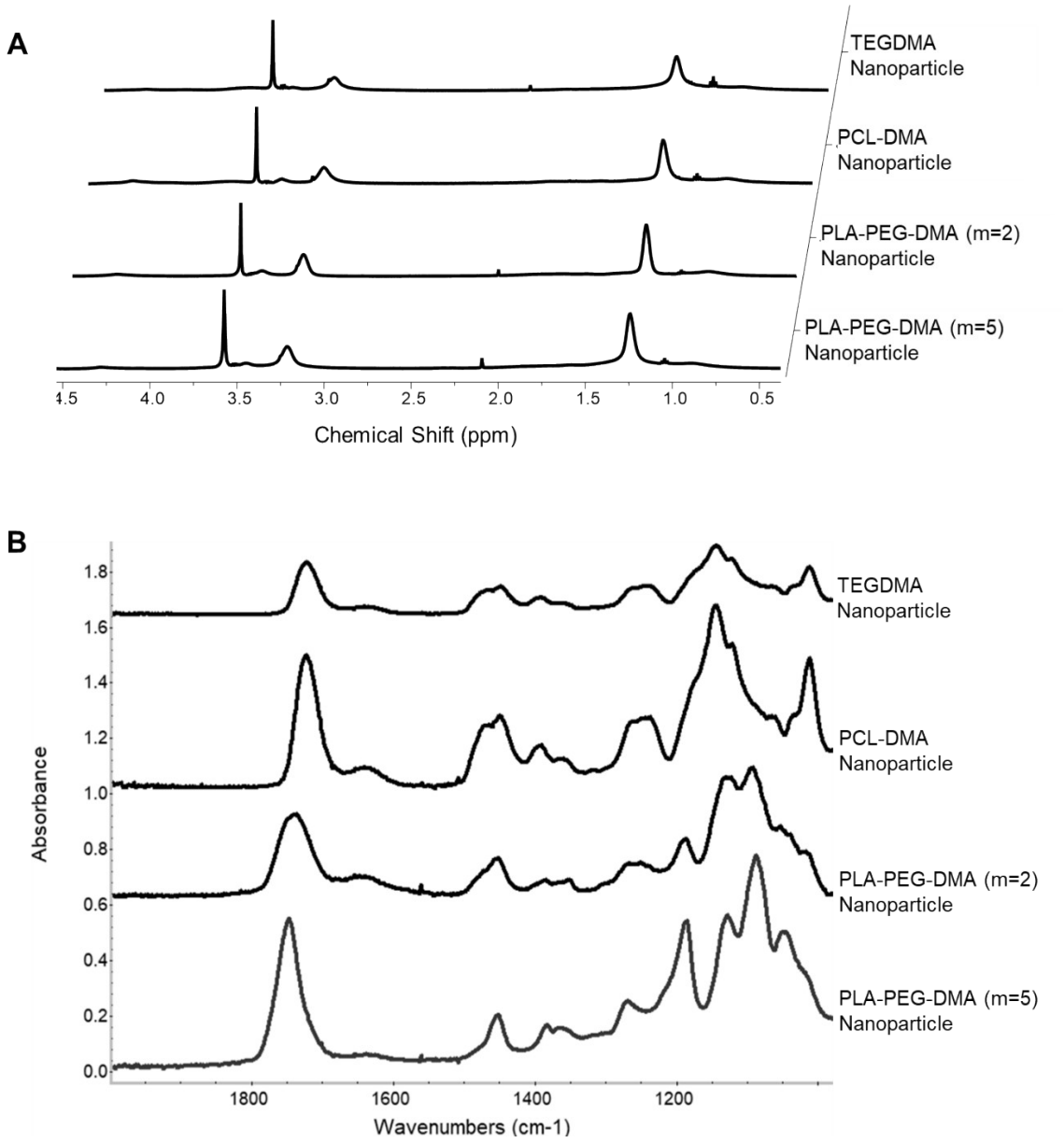

Figure S.6 (A) ^{1}H-NMR and (B) ATR-FTIR of nanoparticles synthesized with tetraethylene glycol methacrylate (TEGDMA), polycaprolactone dimethacrylate (PCL-DMA), custom poly(lactic acid)-b-poly(ethylene glycol)-b-poly(lactic acid) dimethacrylate with 4 units of lactic acid (PLA-PEG-DMA m=2), and custom crosslinker with 10 units of lactic acid (PLA-PEG-DMA m=5).

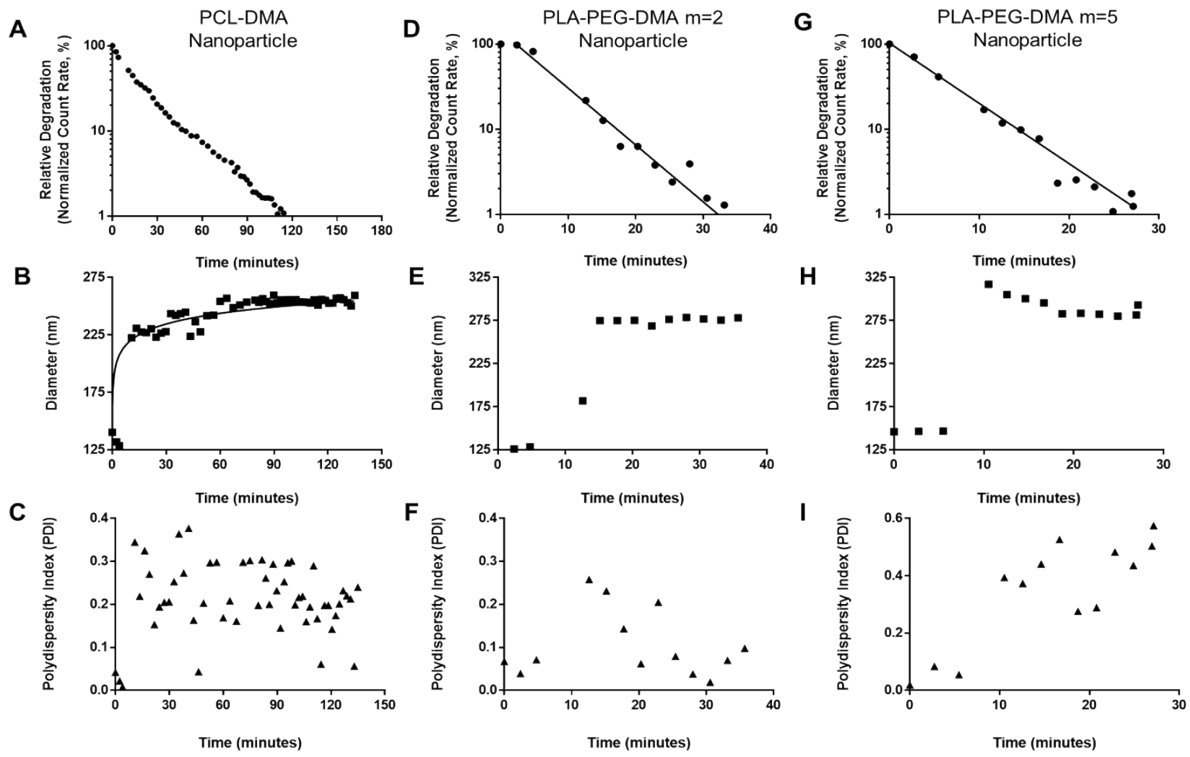

Figure S.7 Carboxylesterase-triggered degradation (10 U/mL) of nanoparticles synthesized with (A-C) polycaprolactone dimethacrylate (PCL-DMA), (D-F) custom poly(lactic acid)-b-poly(ethylene glycol)-b-poly(lactic acid) dimethacrylate with 4 units of lactic acid (PLA-PEG-DMA m=2), and (G-I) custom crosslinker with 10 units of lactic acid (PLA-PEG-DMA m=5). Degradation occurred in the presence of human carboxylesterase 2 (CES2) at a relevant intracellular conditions. Dynamic light scattering was measured sequentially (approximately every 3 minutes) for nanogels at 0.5 mg/mL in 5 mM sodium phosphate pH 6.5. (A,D,G) Count rate normalized to initial time point, (B,E,H) nanoparticle diameter, and (C,F,I) polydispersity index.

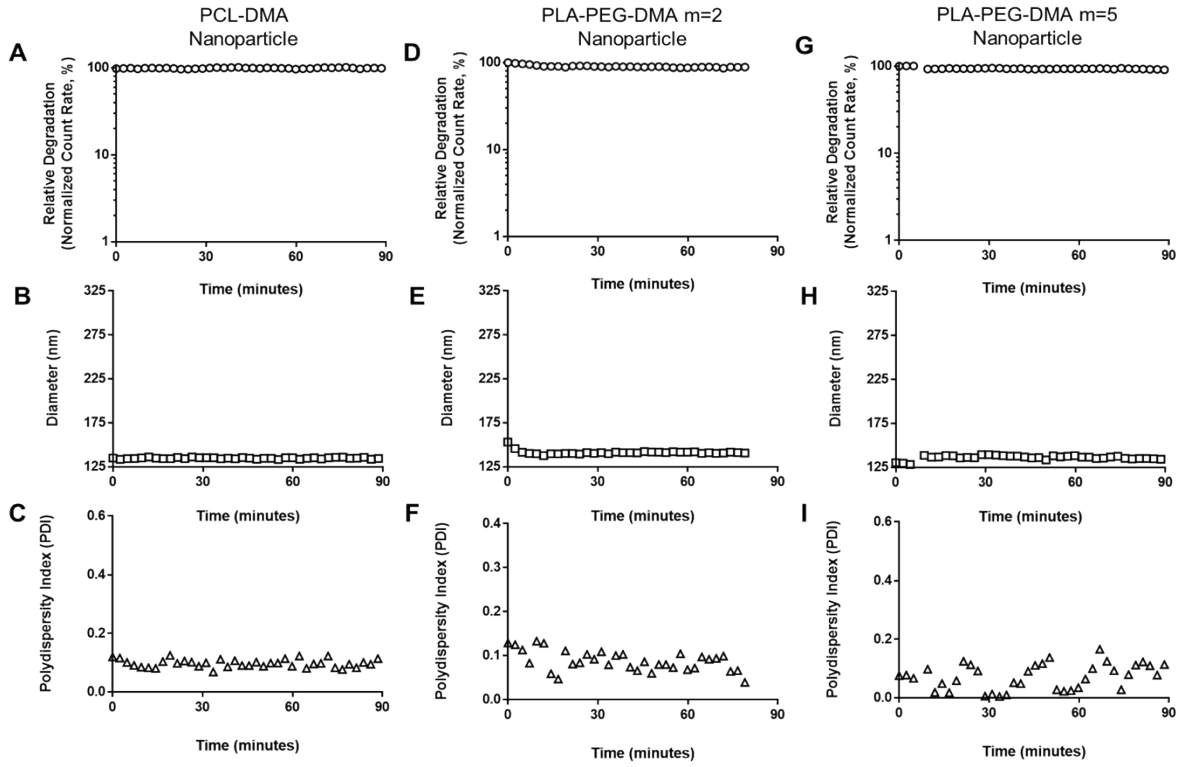

Figure S.8 Nanoparticles synthesized with (A-C) PCL-DMA, (D-F) PLA-PEG-DMA m=2, and (G-I) PLA-PEG-DMA m=5, exposed to human carboxylesterase 2 (CES2) at a relevant extracellular conditions (estimated maximum of 2.5 U/mL). Dynamic light scattering was measured sequentially (approximately every 3 minutes) for nanogels at 0.5 mg/mL in 5 mM sodium phosphate pH 6.5. (A,D,G) Count rate normalized to initial time point, (B,E,H) nanoparticle diameter, and (C,F,I) PDI.

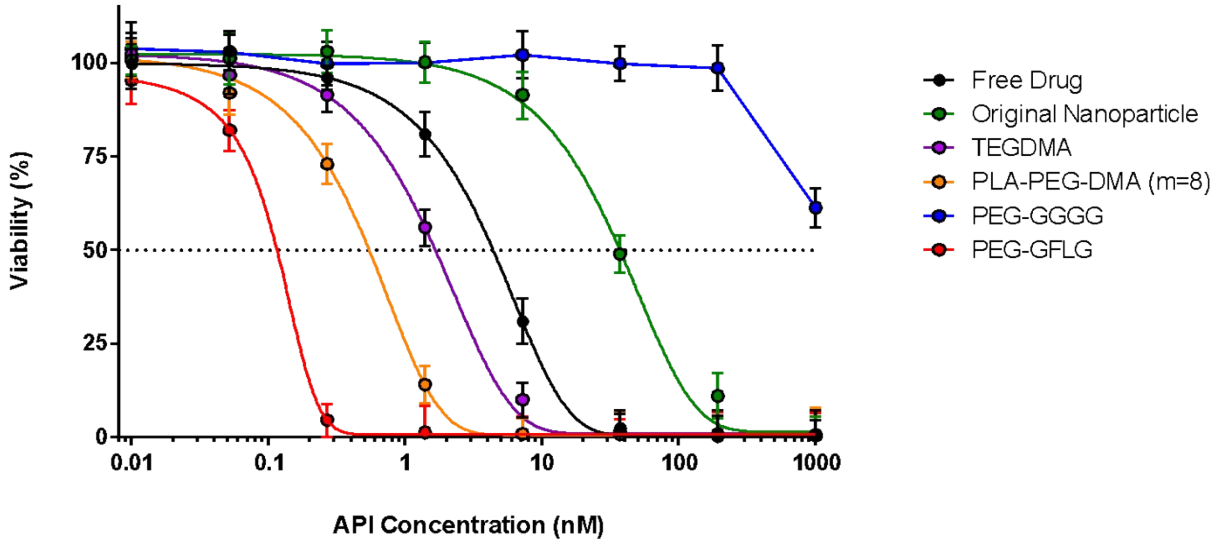

Figure S.9 In vitro efficacy of free drug compared to 5 nanoparticle formulations with paclitaxel alone using OVCAR-3 cells as determined using the MTS assay. Data compares the free paclitaxel (black); the original nanoparticle composition (green); the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with varying crosslinking agents of TEGDMA (purple) and PLA-PEG-DMA m=8 (orange); and finally the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent and either non-CathepsinB responsively linked PEG grafts (PEG-GGGG, blue) and responsive PEG grafts (PEG-GFLG, red).

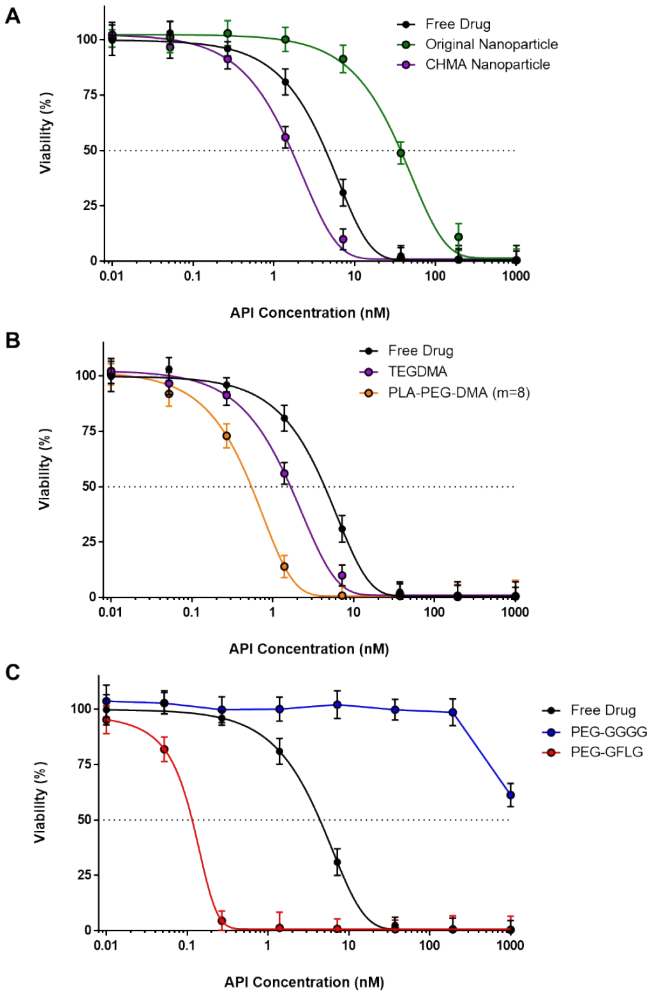

Figure S.10 In vitro efficacy of free drug compared to 5 nanoparticle formulations with paclitaxel alone using OVCAR-3 cells as determined using the MTS assay. (A) Data compares the free paclitaxel (black) with the original nanoparticle composition (green) and the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent. (B) Data compares the free paclitaxel (black) with the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with varying crosslinking agents of TEGDMA (purple) and PLA-PEG-DMA m=8 (orange). (C) Data compares the free paclitaxel (black) with the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent and either non-CathepsinB responsively linked PEG grafts (PEG-GGGG, blue) and responsive PEG grafts (PEG-GFLG, red).

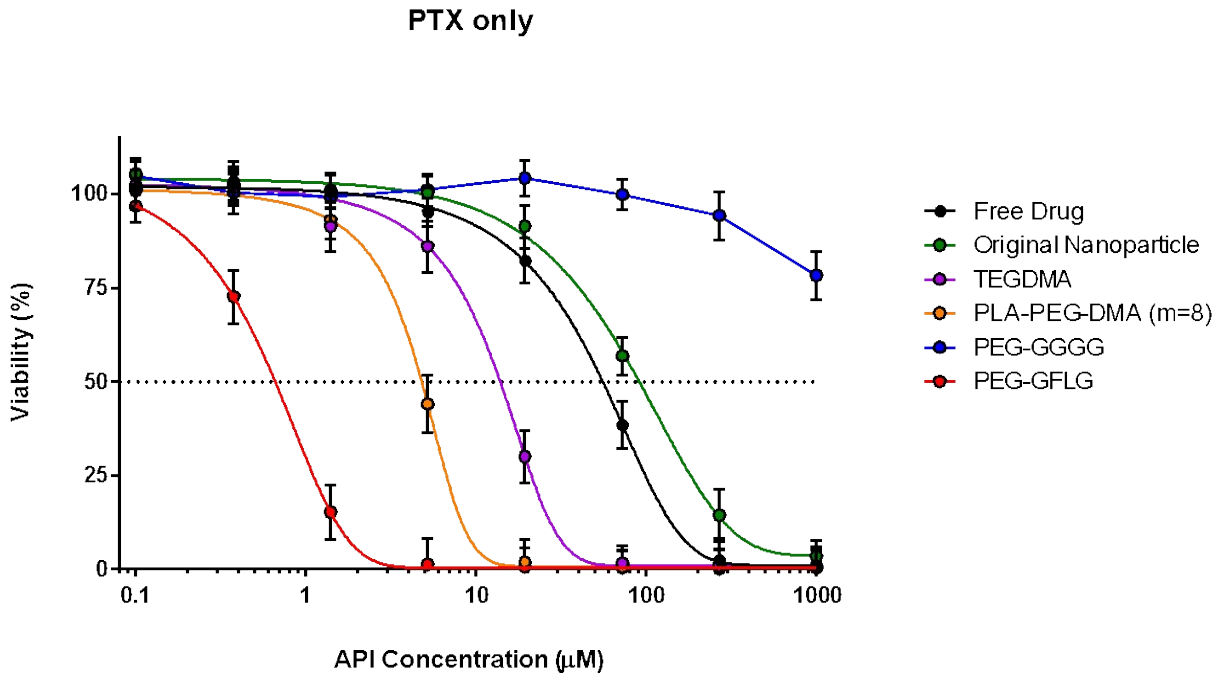

Figure S.11 In vitro efficacy of free drug compared to 5 nanoparticle formulations with carboplatin alone using OVCAR-3 cells as determined using the MTS assay. Data compares the free paclitaxel (black); the original nanoparticle composition (green); the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with varying crosslinking agents of TEGDMA (purple) and PLA-PEG-DMA m=8 (orange); and finally the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent and either non-CathepsinB responsively linked PEG grafts (PEG-GGGG, blue) and responsive PEG grafts (PEG-GFLG, red).

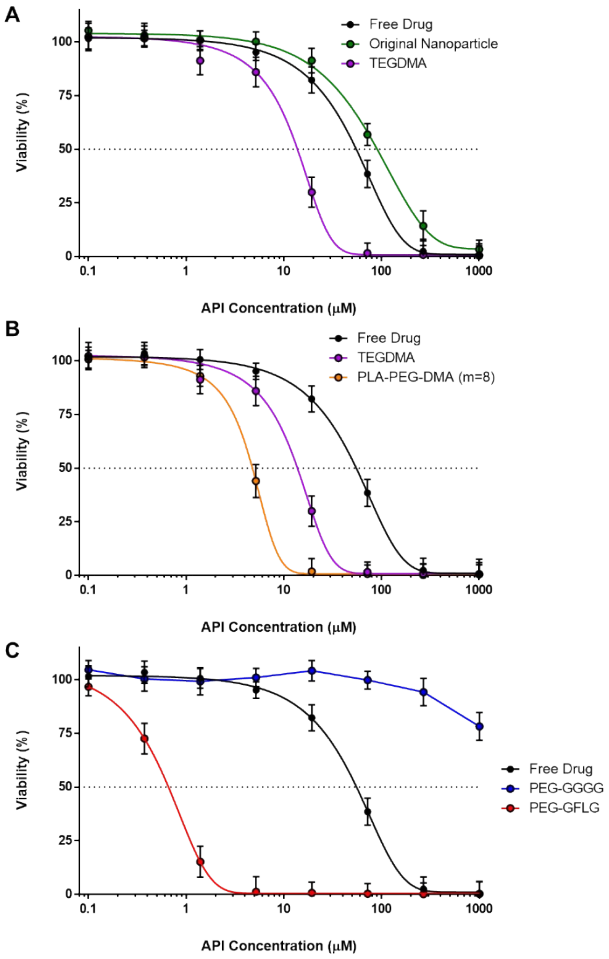

Figure S.12 In vitro efficacy of free drug compared to 5 nanoparticle formulations with carboplatin alone using OVCAR-3 cells as determined using the MTS assay. (A) Data compares the free paclitaxel (black) with the original nanoparticle composition (green) and the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent. (B) Data compares the free paclitaxel (black) with the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with varying crosslinking agents of TEGDMA (purple) and PLA-PEG-DMA m=8 (orange). (C) Data compares the free paclitaxel (black) with the optimized P(DEAEMA-co-CHMA)-g-PEGMA nanoparticles synthesized with TEGDMA crosslinking agent and either non-CathepsinB responsively linked PEG grafts (PEG-GGGG, blue) and responsive PEG grafts (PEG-GFLG, red).