Supporting information

A poly(ascorbyl acrylate)-containing nanoplatform with anticancer activity for sequential combination therapy with its loaded paclitaxel

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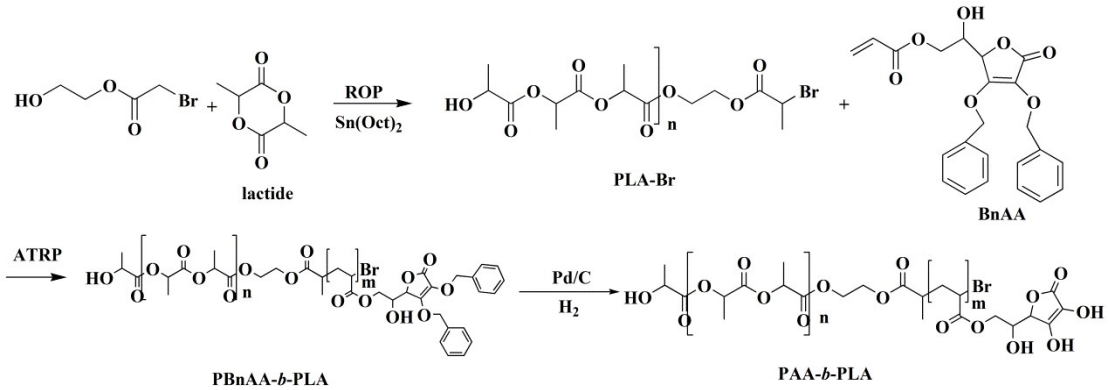
Experimental Section.

Materials. Triethylamine (TEA) and methylene dichloride (CH_2Cl_2) were dehydrated with KOH and $CaCl_2$ overnight and distilled, respectively. Toluene and tetrahydrofuran (THF) were dried using sodium with benzophenone as color indicator. All of the above purified solvent and reagents were stored in solvent storage flasks prior to use. The other reagents such as 2-bromopropionyl bromide, pentamethyl-diethylene triamine (PMDETA) and stannous octoate from Aldrich, ascorbic acid, ethylene glycol, acryloyl chloride, benzyl bromide, NaN_3, *p*-toluenesulfonic acid, triphenylphosphine, 6-amino hexanoic acid and maleic anhydride from Sinopharm Chemical (China) were used as received without further treatment.



Scheme S1. Synthesis route of PAA-*b*-PLA.

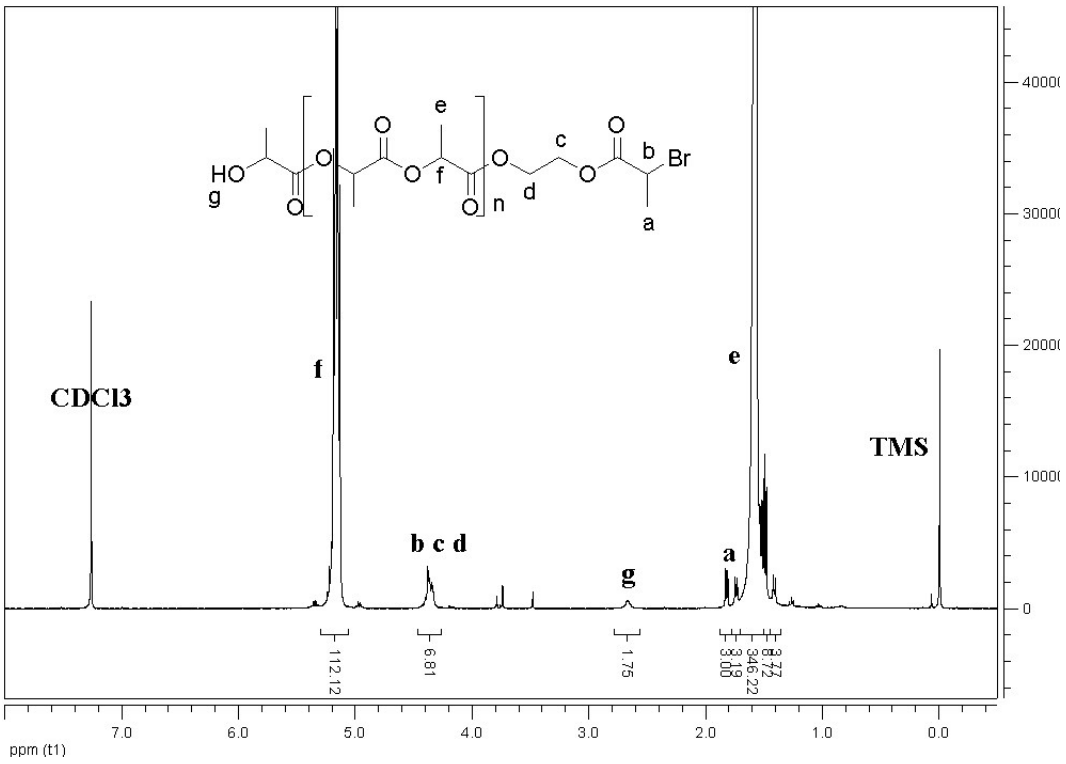


Figure S1. ^{1}H NMR spectrum of PLA-Br

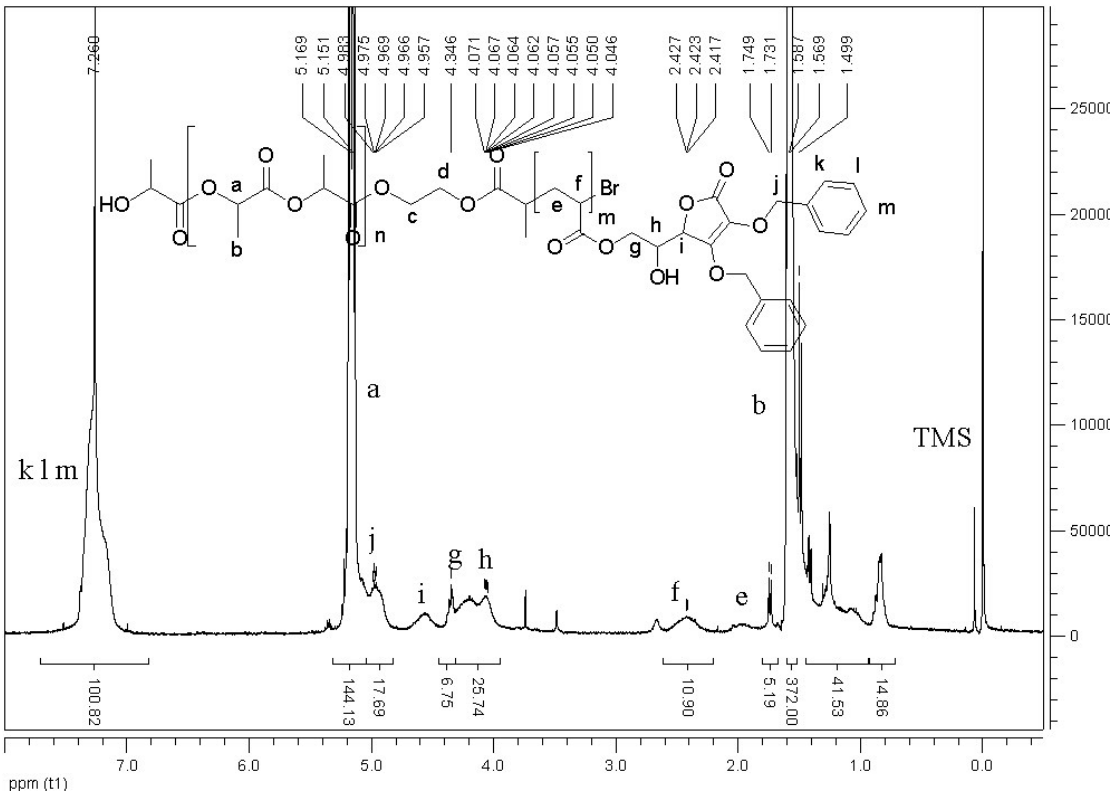


Figure S2. ^{1}H NMR spectrum of PBnAA-*b*-PLA.

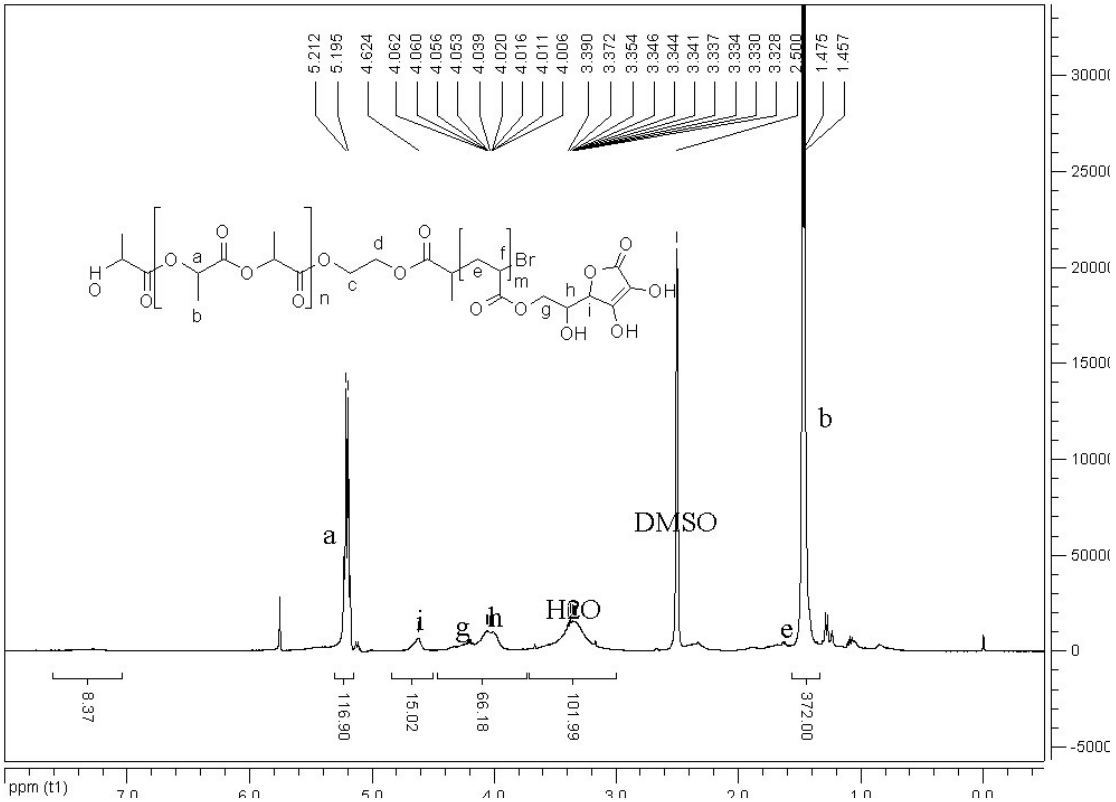
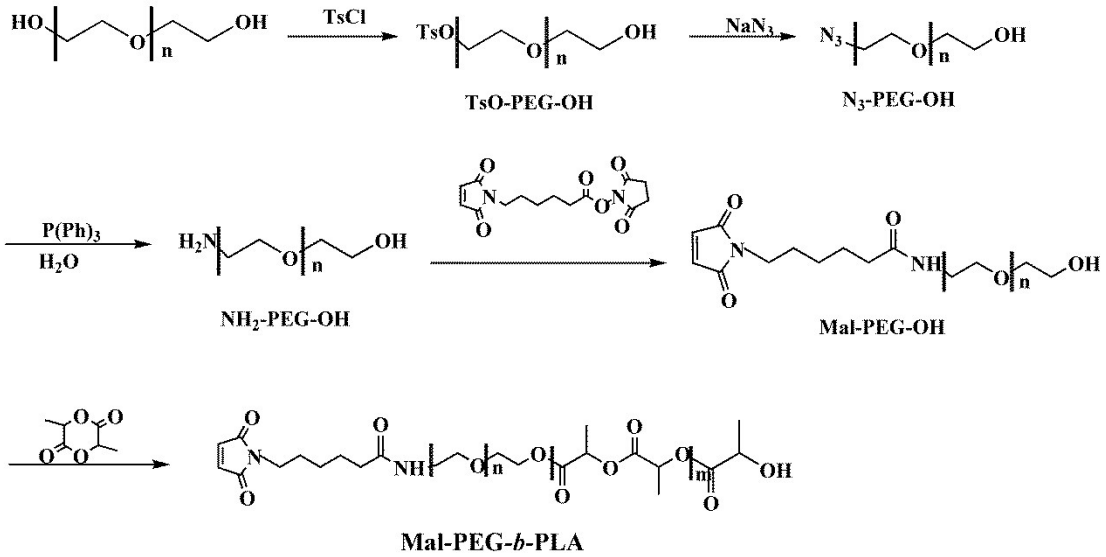


Figure S3. ^{1}H NMR spectrum of PAA-*b*-PLA.



Scheme S2. Synthesis route of Mal-PEG-*b*-PLA

Synthesis of TsO-PEG-OH. Into 250 mL three-neck flask containing PEG 4000 (5.0 g, 2.5 mmol), triethyl amine (0.35 mL, 2.5 mmol) and 2.0 mL CH_2Cl_2 was dropwise added *p*-toluenesulfonic acid (0.238 g, 1.25 mmol) in 10 mL CH_2Cl_2, followed by 36-h stirring. The reaction was washed by CH_2Cl_2 and deionized water, and dried by anhydrous $MgSO_4$. The concentrated solution was precipitated into ethyl ether and obtain white product 4.1 g in yield of 80.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.34-7.79 (dd, J = 5.6, 5.6 Hz, 4H, CH_3-$C_6\underline{H}_4$-), 4.16 (t, J = 3.2 Hz, 2H, SO_3-$C\underline{H}_2$-), 3.83-3.57 (m, nH, -O$C\underline{H}_2$-), 2.44 (S, -$C\underline{H}_3$).

Synthesis of N_3-PEG-OH. The mixture of NaN_3 (0.2 g, 3.0 mmol), TsO-PEG-OH (4.0 g, 1.0 mmol) and 50 mL CH_3CN was refluxed at 90°C for 36 h. The reaction solution was washed by brine three times, and concentrated in vacuum. The yellow product was afforded in yield of 74.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.73-3.38 (m, nH, -O$C\underline{H}_2$-), 3.37 (t, J = 5.4 Hz, 2H, N_3-$C\underline{H}_2$-).

Synthesis of NH_2-PEG-OH. The reaction solution containing Ph_3P (0.84 g, 3.0 mmol), N_3-PEG-OH (3.0 g, 0.75 mmol), 0.5 mL deionized water and 20 mL THF in 100 mL round flask was stirred at ambient temperature for 48 h. The solution was washed with 0.1 M HCl and 0.1 M NaOH, respectively. The concentrated solution was precipitated into ethyl ether and slight yellow product (2.3 g) was obtained in yield of 76.7%. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.37-3.34 (m, nH, -O$C\underline{H}_2$-), 3.33 (t, J = 3.2 Hz, 2H, H_2N-$C\underline{H}_2$-).

Synthesis of Mal-PEG-OH. The mixture of 6-maleic-hexanoyl-*N*-hydroxyl succinimide ester (0.464 g, 1.50 mmol), NH_2-PEG-OH (2.0 g, 0.5 mmol) and 25 mL CH_2Cl_2 in 100 mL round flask was stirred at room temperature for 48 h. The concentrated solution was precipitated in ethyl ether for four times. The white product was afforded in yield of 69.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.66 (s, 2H, -$NO_2\underline{H}_2C_4$), 3.37 (t, *J* = 7.2 Hz, 2H, -$C\underline{H}_2$-$NO_2H_2C_4$), 2.56 (t, *J* = 7.2 Hz, 2H, -$C\underline{H}_2$-COO-), 1.73 (m, 2H, -$C\underline{H}_2$-), 1.62 (m, 2H, -$C\underline{H}_2$-), 1.39 (m, 2H, -$C\underline{H}_2$-).

Synthesis of Mal-PEG-*b*-PLA. Into 25 mL Schlenk was added *L*-lactide (1.5 g, 10.5 mmol), Mal-PEG-OH (220 mg, 0.052 mmol), $Sn(Oct)_2$ (42.3 mg, 1.05 mmol) and 3.0 mL toluene. The reaction was maintained at 70 °C oil bath for 4 h. Subsequently, the reaction was diluted in $CHCl_3$ and precipitated in methanol three times. The product was dried in vacuum and afforded in yield of 78.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.66 (s, 2H, -$NO_2\underline{H}_2C_4$), 4.95-5.53 (m, nH, O$C\underline{H}CH_3$COO), 4.26-4.36 (m, 4H, -$\underline{H}(CH_3)$C-OH), 3.48-3.82 (m, nH, -O$C\underline{H}_2$-), 2.16 (t, *J* = 5.6 Hz, -$C\underline{H}_2$-CONH-), 1.36-1.72 (m, nH, -$C\underline{H}_3$, -$C\underline{H}_2$-,OCHC$\underline{H}_3$COO)

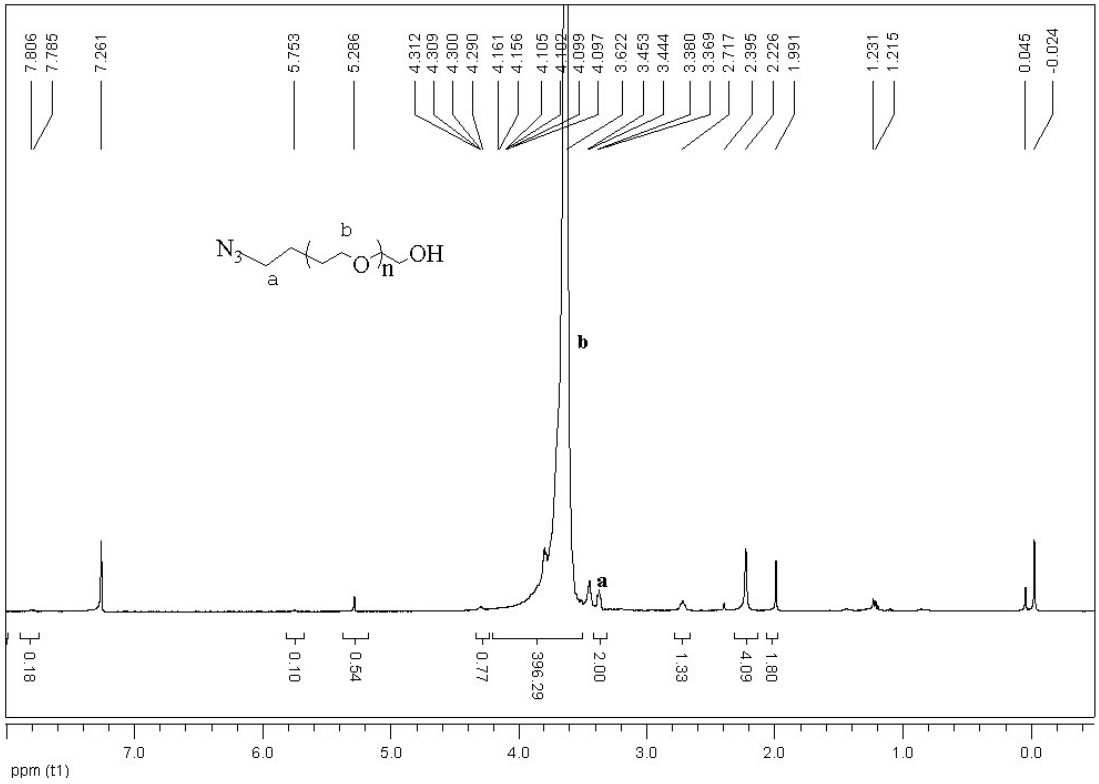


Figure S4. ^{1}H NMR spectrum of HO-PEG-N_3.

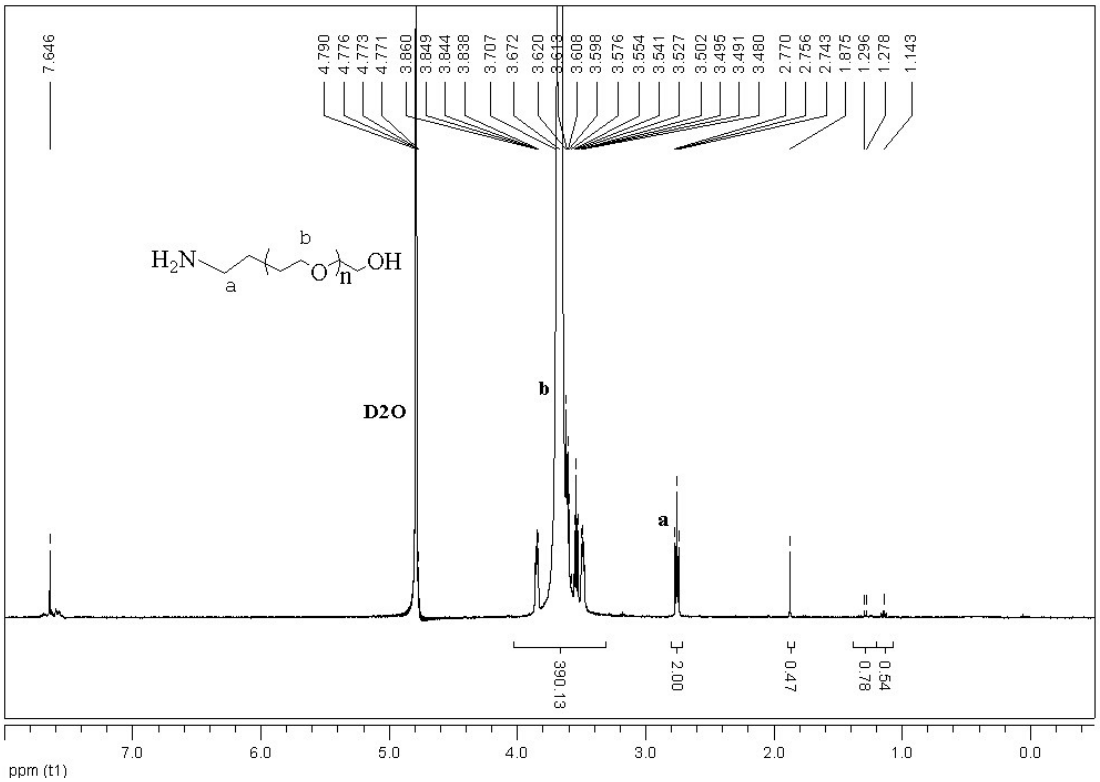


Figure S5. ^{1}H NMR spectrum of HO-PEG-NH_2.

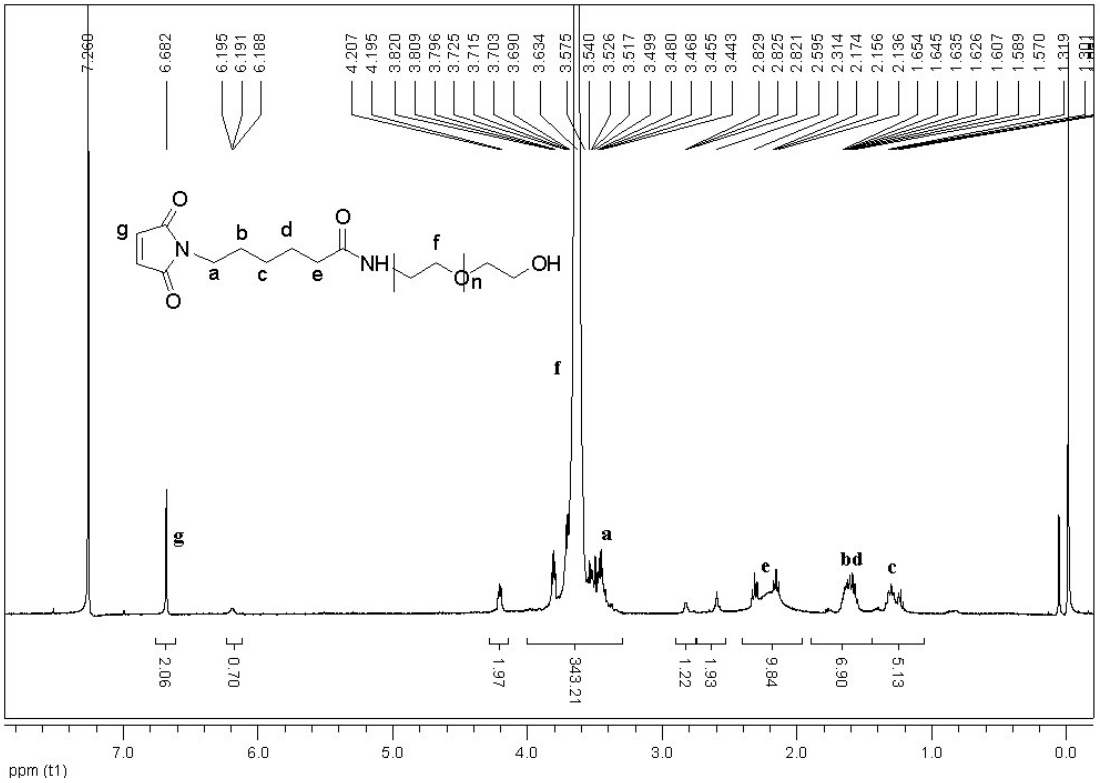


Figure S6. ^{1}H NMR spectrum of Mal-PEG-OH.

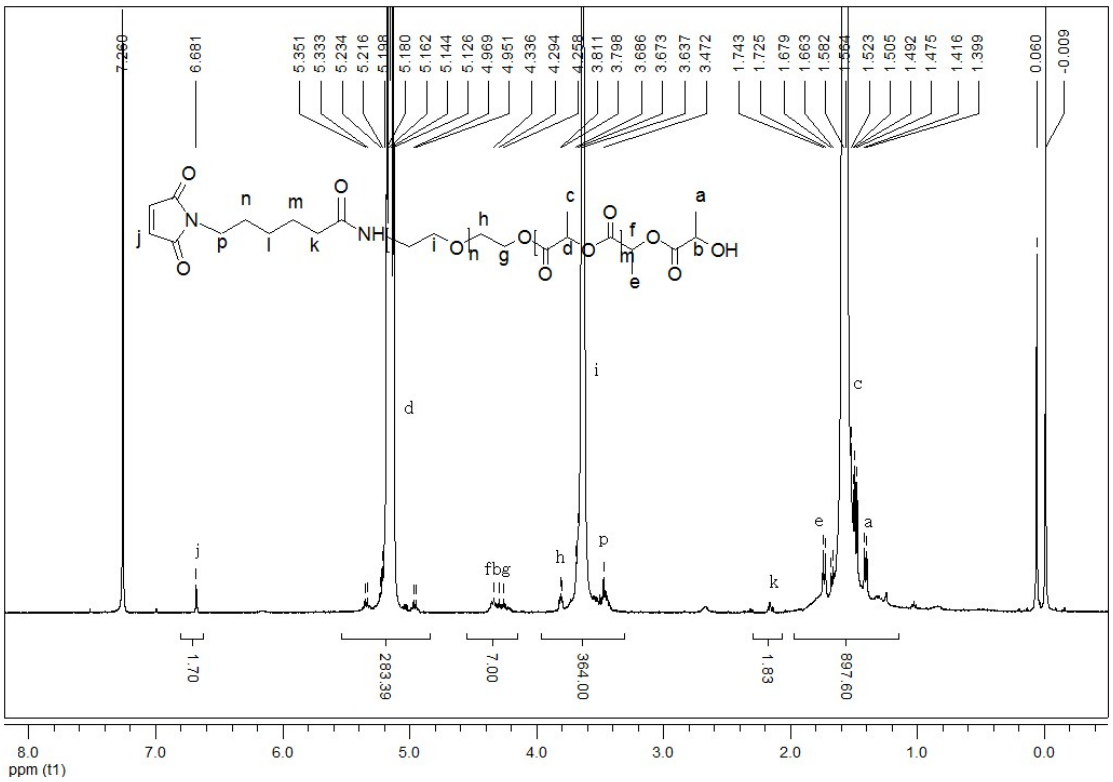


Figure S7. [1]H NMR spectrum of Mal-PEG-*b*-PLA.

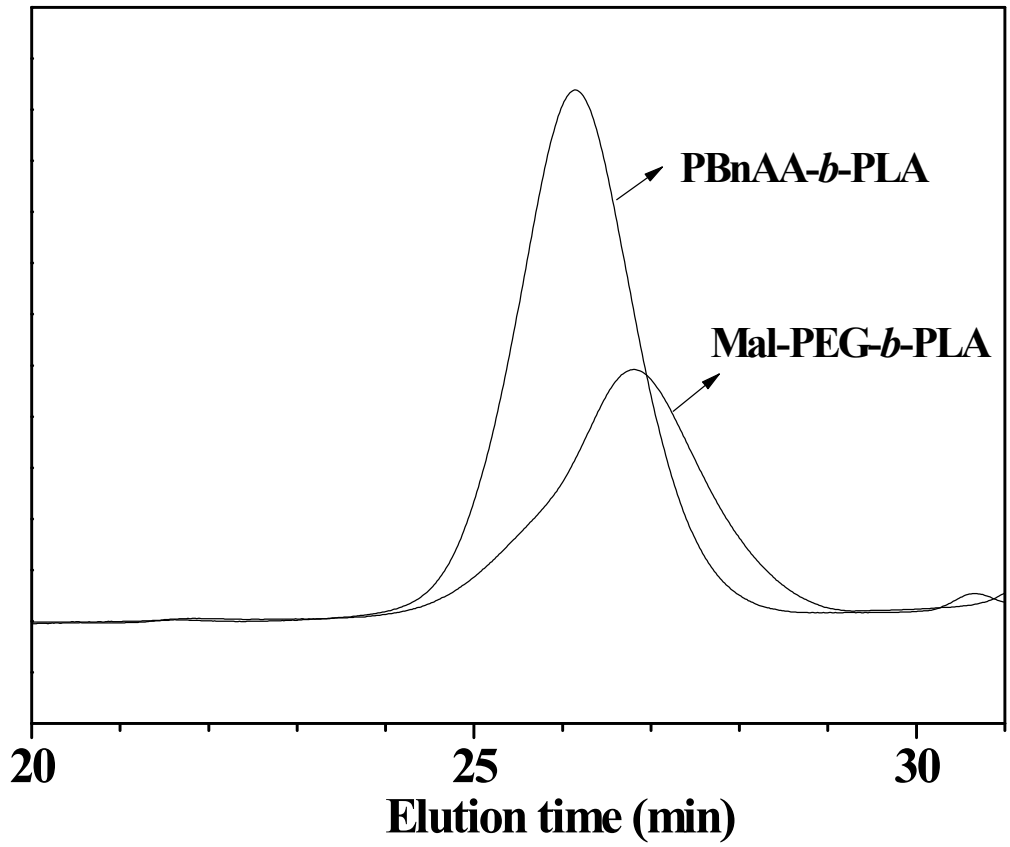


Figure S8. GPC curves of PBnAA-*b*-PLA and Mal-PEG-*b*-PLA

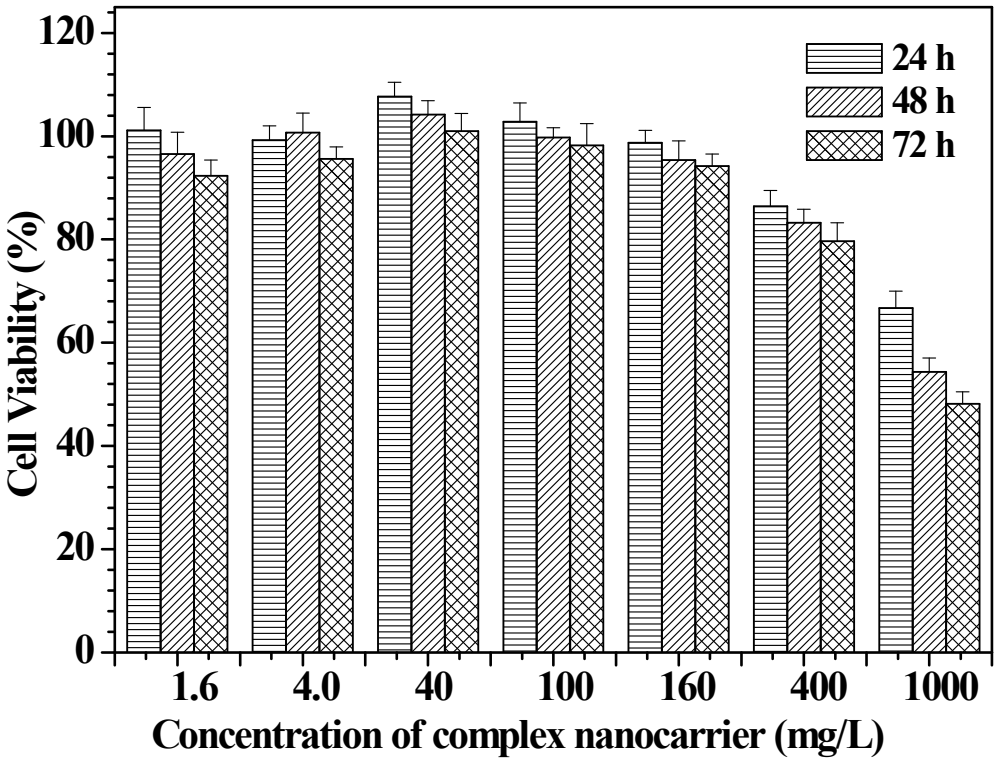


Figure S9. Cell viability of HUVEC cells treated with PAA-*b*-PLA/Mal-PEG-*b*-PLA nanoparticles at various concentrations at 37 ºC for 24, 48 and 72 h.

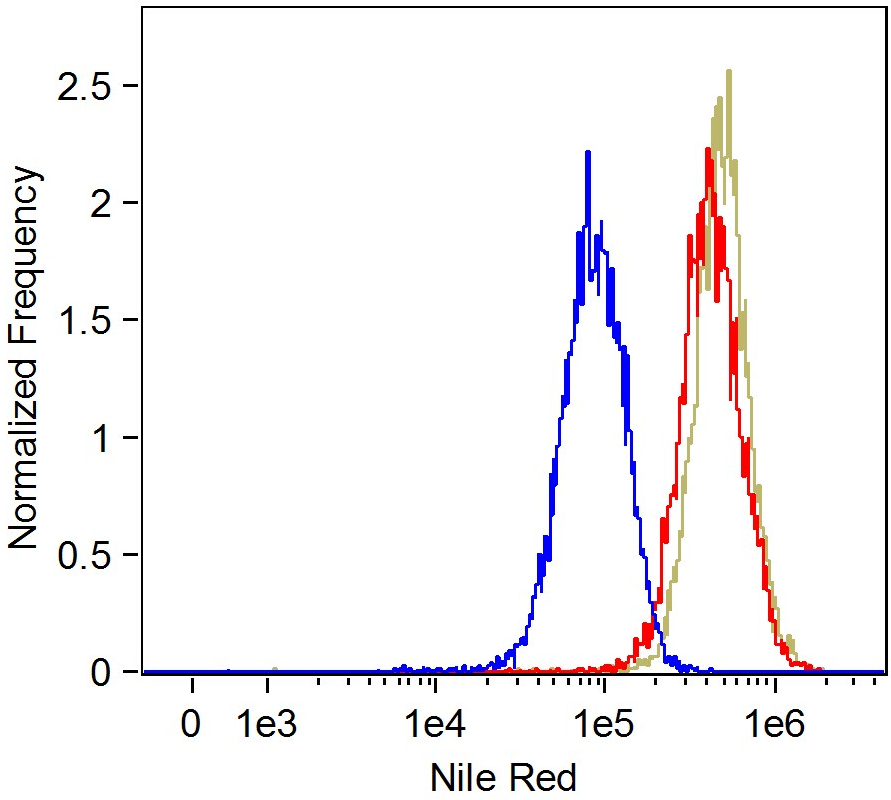
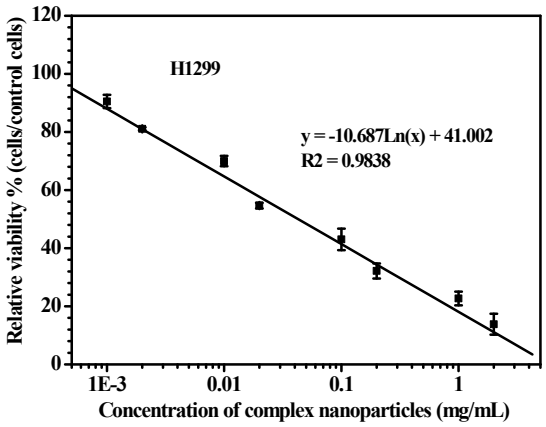
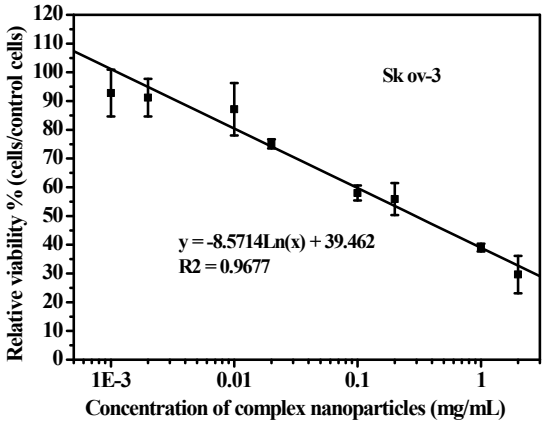
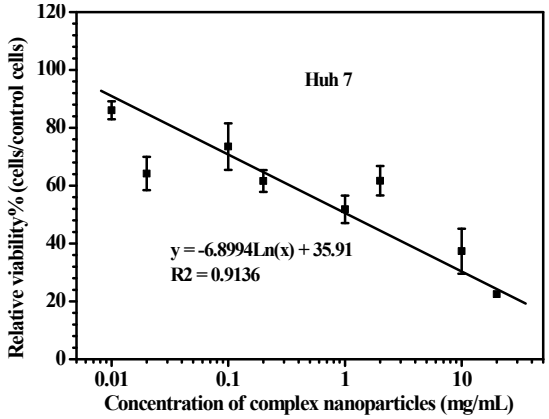
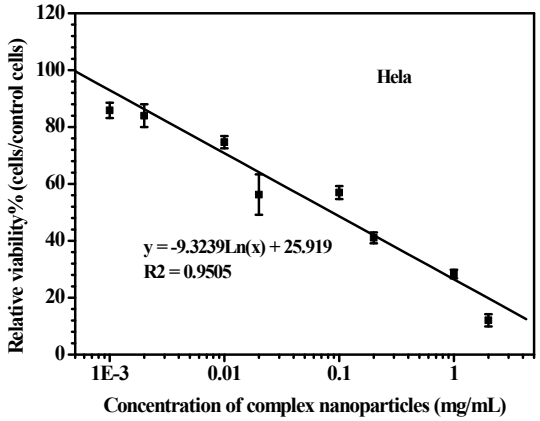


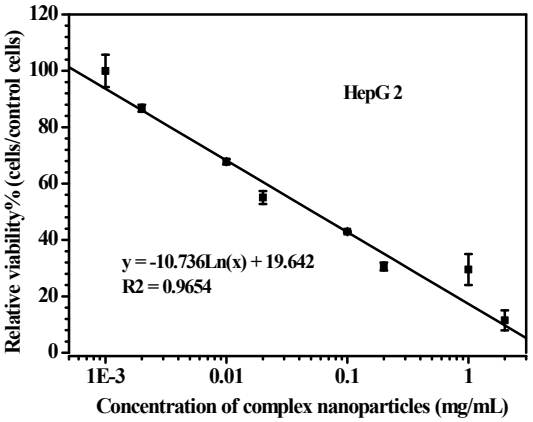
Figure S10. Flow cytometric results of MCF-7 cells incubated with PEG-*b*-PLA nanoparticles for 1 h (blue), 3 h (red) and 6 h (yellow).

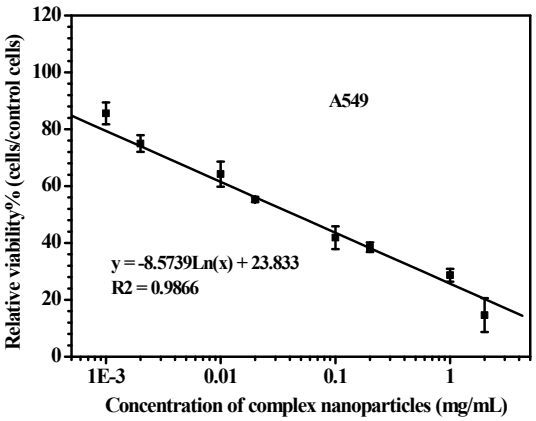

H1299
y = -10.687Ln(x) + 41.002
R2 = 0.9838
Relative viability % (cells/control cells)
Concentration of complex nanoparticles (mg/mL)

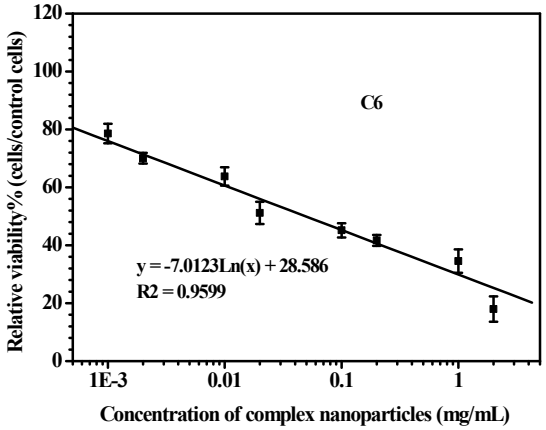

Sk ov-3
y = -8.5714Ln(x) + 39.462
R2 = 0.9677
Relative viability % (cells/control cells)
Concentration of complex nanoparticles (mg/mL)

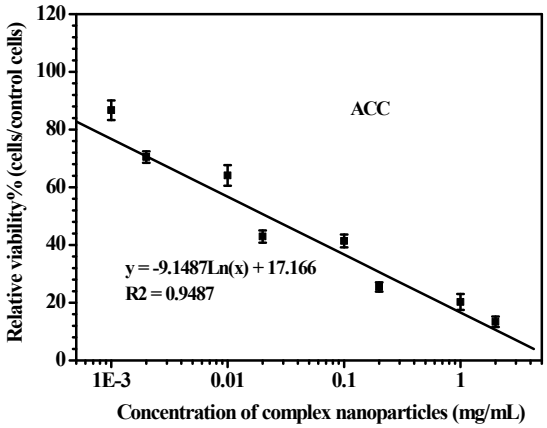

Huh 7
y = -6.8994Ln(x) + 35.91
R2 = 0.9136
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)


Hela
y = -9.3239Ln(x) + 25.919
R2 = 0.9505
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)


HepG 2
y = -10.736Ln(x) + 19.642
R2 = 0.9654
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)


A549
y = -8.5739Ln(x) + 23.833
R2 = 0.9866
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)


C6
y = -7.0123Ln(x) + 28.586
R2 = 0.9599
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)


ACC
y = -9.1487Ln(x) + 17.166
R2 = 0.9487
Relative viability% (cells/control cells)
Concentration of complex nanoparticles (mg/mL)

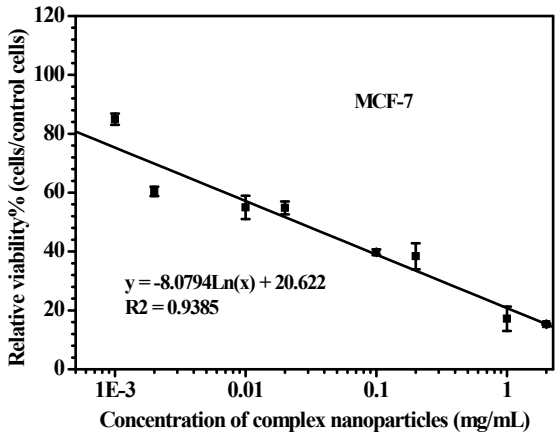


Figure S11. Sensitivity of nine cancer cells lines to PAA-*b*-PLA/Mal-PEG-*b*-PLA complex nanoparticles.

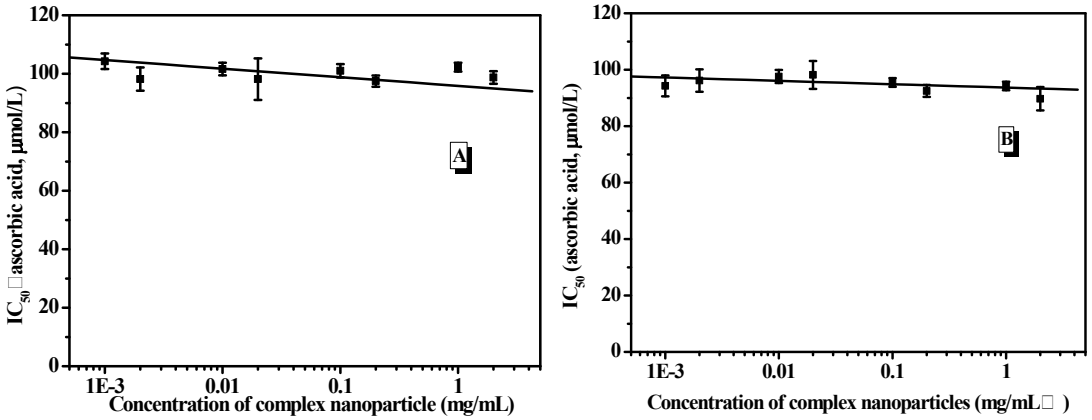


Figure S12. Sensitivity of cancer cells MCF-7 (A) and H1299 (B) to Mal-PEG-*b*-PLA nanoparticles. .

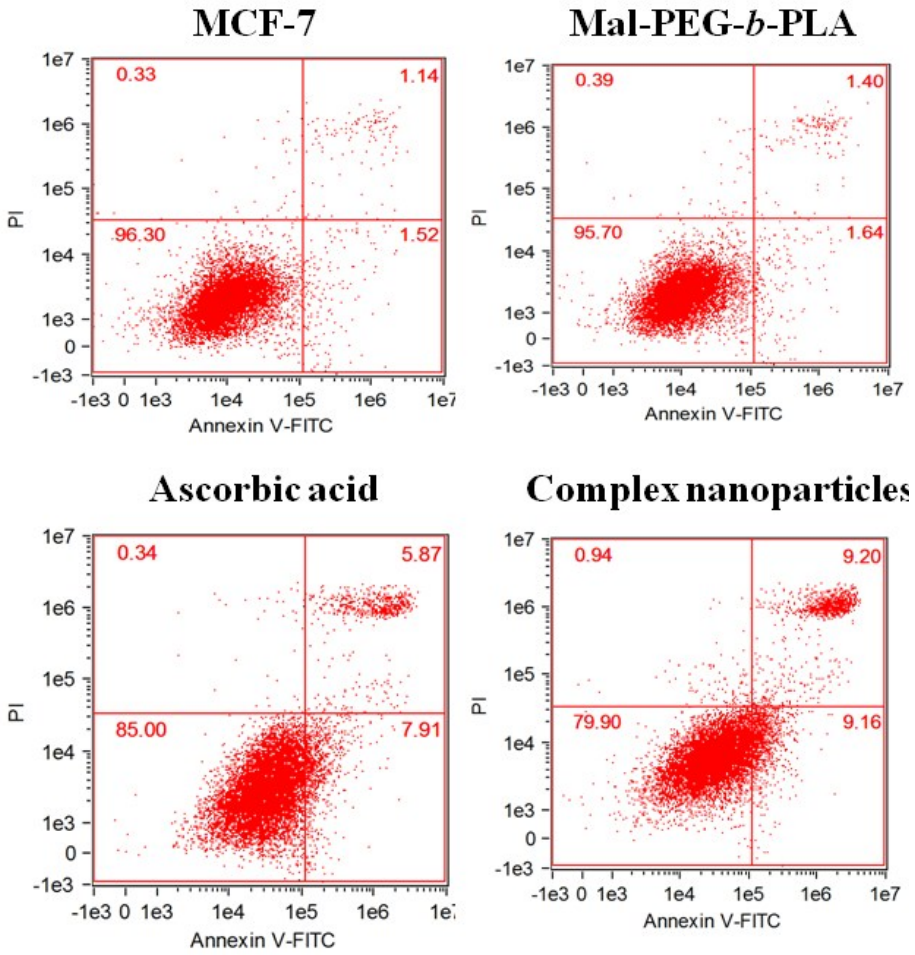


Figure S13. Apoptotic ratios of MCF-7 cells treated with Mal-PEG-*b*-PLA nanoparticles, PAA-*b*-PLA/Mal-PEG-*b*-PLA complex nanoparticles and ascorbic acid at 38 h post–incubation as measured by flow cytometry. Cells without any treatment served as the blank.

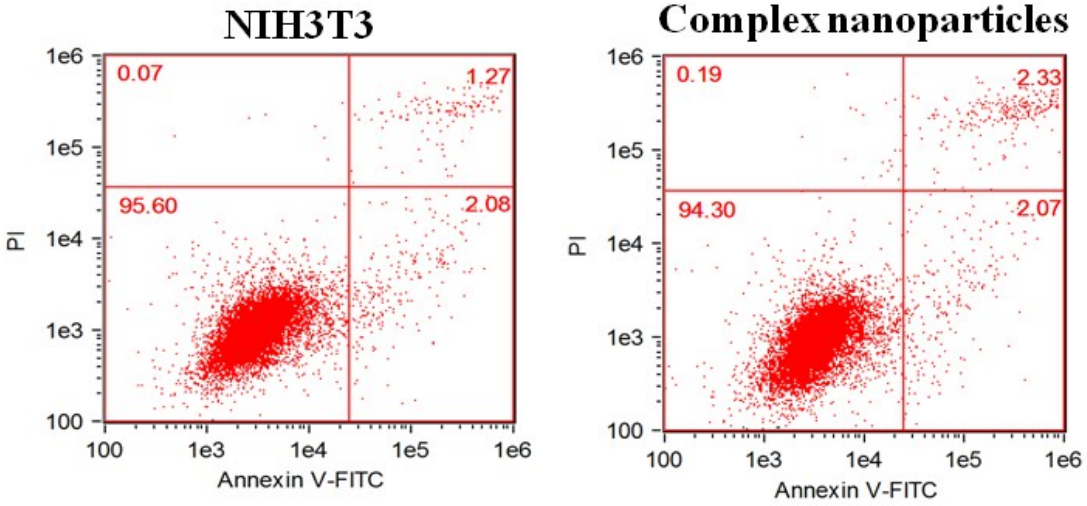


Figure S14. Apoptotic ratios of NIH 3T3 cells treated with PAA-*b*-PLA/Mal-PEG-*b*-PLA complex nanoparticles at 38 h post–incubation as measured by flow cytometry.

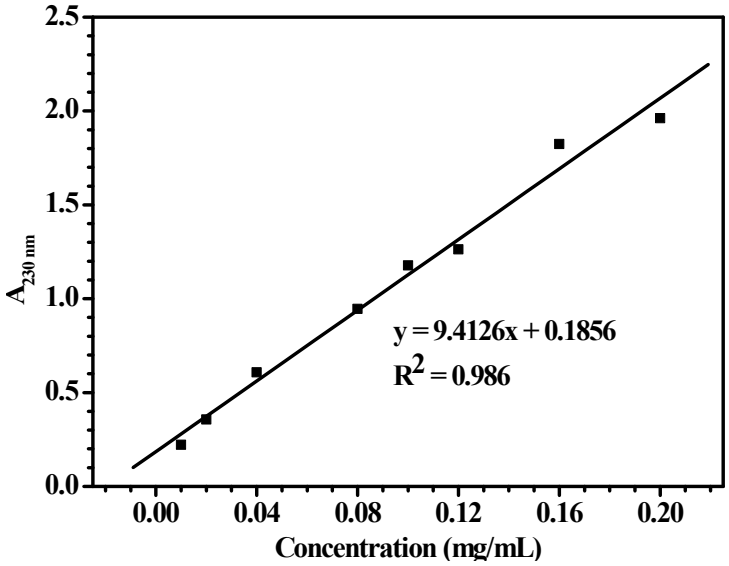


Figure S15. Standard curve of UV absorbance at 230 nm at various concentration of PTX