Supporting Information

Tumor-activatable peptide supramolecular nanoplatform for delivery of dual-gene targeted siRNA for drug-resistant cancer treatment

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Materials

Boc-Glu-OH, H-Glu(OBzl)-OBzl, 1-hydroxybenzotriazole hydrate (HOBT), H-Lys-OMe.2HCl, Boc-Cys(Trt)-OH, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl), and hexafluorophosphate (PyBop) were purchased from GL Biochem. Ltd. (Shanghai, China). Trifluoroacetic acid (TFA) and N,N-diisopropylethylamine (DIPEA) were obtained from Asta Tech Pharmaceutical (Chengdu, China). Dichloromethane (DCM) and anhydrous diethyl ether and dimethyl sulfoxide (DMSO) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Poly (ethylene glycol) (PEG, Mw = 2000 g/mol), poly(ethylene glycol) methyl ether (mPEG, Mw = 1000 g/mol) and 3,3`-dithiodipropionic acid and DL-dithiothreitol (DTT) were purchased from Aladdin Reagents Company (Shanghai, China). Terephthalaldehydic acid and triethylsilane (Et_3SiH) were purchased from Sigma-Aldrich (St. Louis, USA). The 5′-end amino modified MUC1-aptamer, 5′-end dithiol modified siRNA (hTERT-siRNA, TRF2-siRNA and scrambled sequences of siRNA NC-siRNA) and 5′-end dithiol modified siRNA labeled with a FAM dye (ex/em; 492/518 nm) or Cy3 dye (ex/em; 553/565 nm) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China) and their sequences were MUC1: 5′-C6-GCA GTT GAT CCT TTG GAT ACC CTG G-3′; The hTERT-

siRNA: sense 5′-GAGCCAGUCUCACCUUCAA-3′; antisense 5′-UUGAAGGUGAGACUGGCUC-3′; The TRF2-siRNA: sense 5′-GAGGAUGAACUGUUUCAAG-3′; antisense 5′-CUUGAAACAGUUCAUCCUC-3′; The NC-siRNA: sense 5′-UUCUCCGAACGUGUCACG U-3′; and antisense 5′-CGUGACACGUUCGGAGAA -3′ respectively.

Methods

^{1}H NMR spectra for synthetic products were obtained from 400 MHz Bruker Avance II NMR spectrometer using solvents of DMSO-d_6. The molecular weight of products was determined by matrix assisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF MS, Bruker Autoflex III). Malvern Zetasizer Nano ZS was used to measure the particle size and surface potential. Transmission electron microscope (TEM, Tecnai GF20S-TWIN, FEI, USA) and scanning electron microscopy (SEM, S-4800 Hitachi) were used to observe the morphologies of nanoparticles. Cell viability was measured using a Thermo Varioskan Flash microplate reader. The fluorescent quantitative polymerase chain reaction instrument (BIO-RAD) was used to determine the mRNA level. A western blot instrument (BIO-RAD) was used to measure the protein level. The fluorescent molecular distribution of siRNA in cells was observed under a Confocal Laser Scanning Microscopy (CLSM, Leica TCP SP5). The fluorescence imaging system (CRi Maestro EX, USA) was used to observe the distribution of siRNA in vivo. An inverted optical microscopy (Leica DMI4000B, Germany) was used to monitor treated wound healing and observe tissue slices.

Cell lines

The human non-small cell lung cancer cell line A549 and human breast cancer cell line MCF-7 were purchased from Chinese Academy of Science Cell Bank for Type Culture Collection

(Shanghai, China). The doxorubicin (DOX)-resistant human breast cancer cells (MCF-7/DOX cells) were purchased from MEIXUAN Biological Science and Technology, LTD (Shanghai, China). Drug resistance of A549/PTX cells were maintained by addition of PTX (100 ng/mL) in the medium. Drug resistance of MCF-7/DOX cells were maintained by addition of DOX.HCl (0.5 μg mL^{-1}) in the medium. The A549 cell line and A549/PTX cell line were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) at 37 °C in a humidified 5% CO_2 atmosphere. The MCF-7 cell line and MCF-7/DOX cell line were cultured in DMEM medium with 10% FBS in a humidified atmosphere containing 5% CO_2 at 37 °C.

Animals

BALB/c nude mice (about 6-week-old) were purchased from Chengdu Dashuo Experimental Animal Company (Chengdu, China). The mice were adaptively fed for one week. The feeding conditions and operations were employed following the standard of Institutional Animal Use and Care Regulations of Sichuan University.

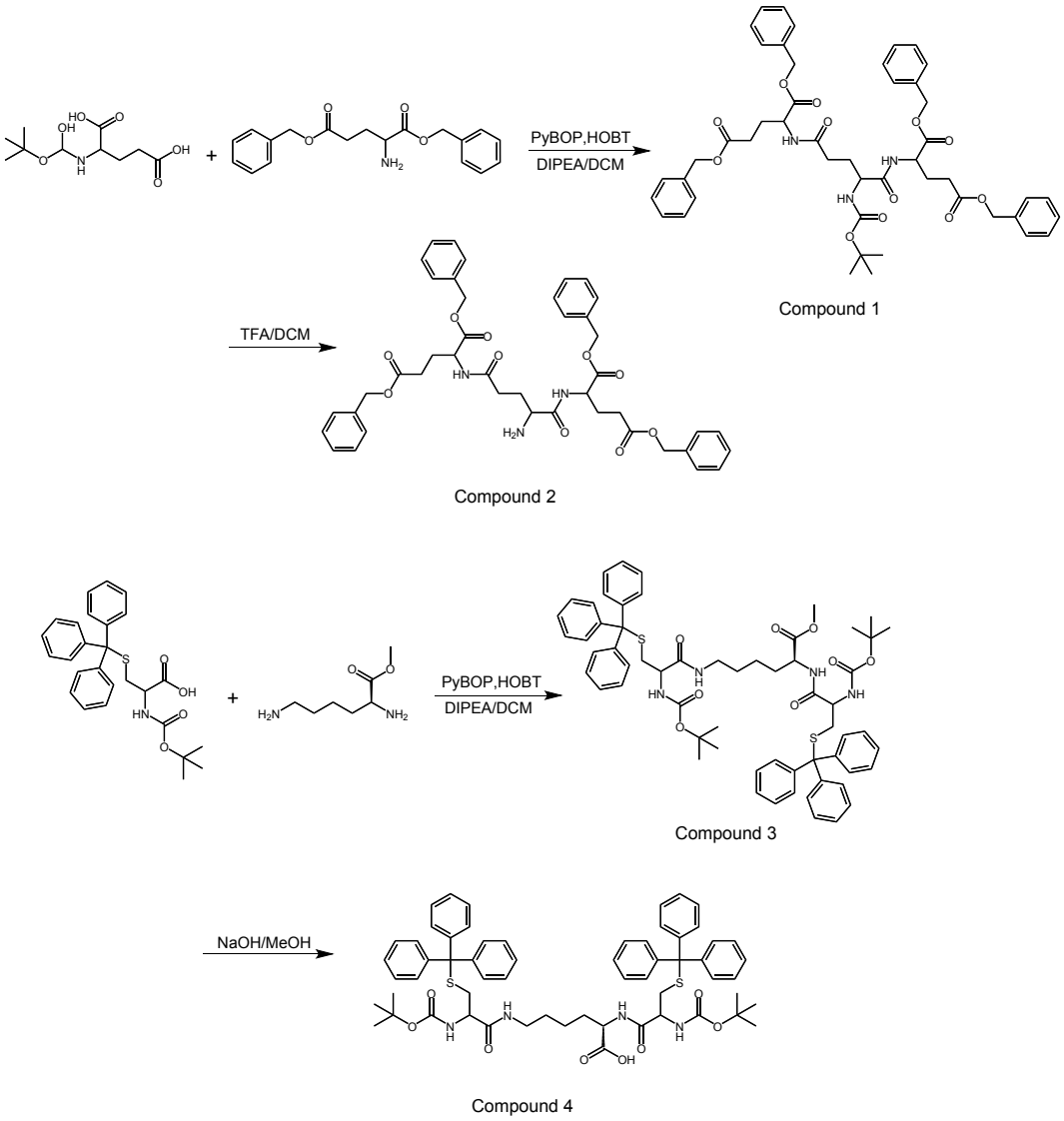
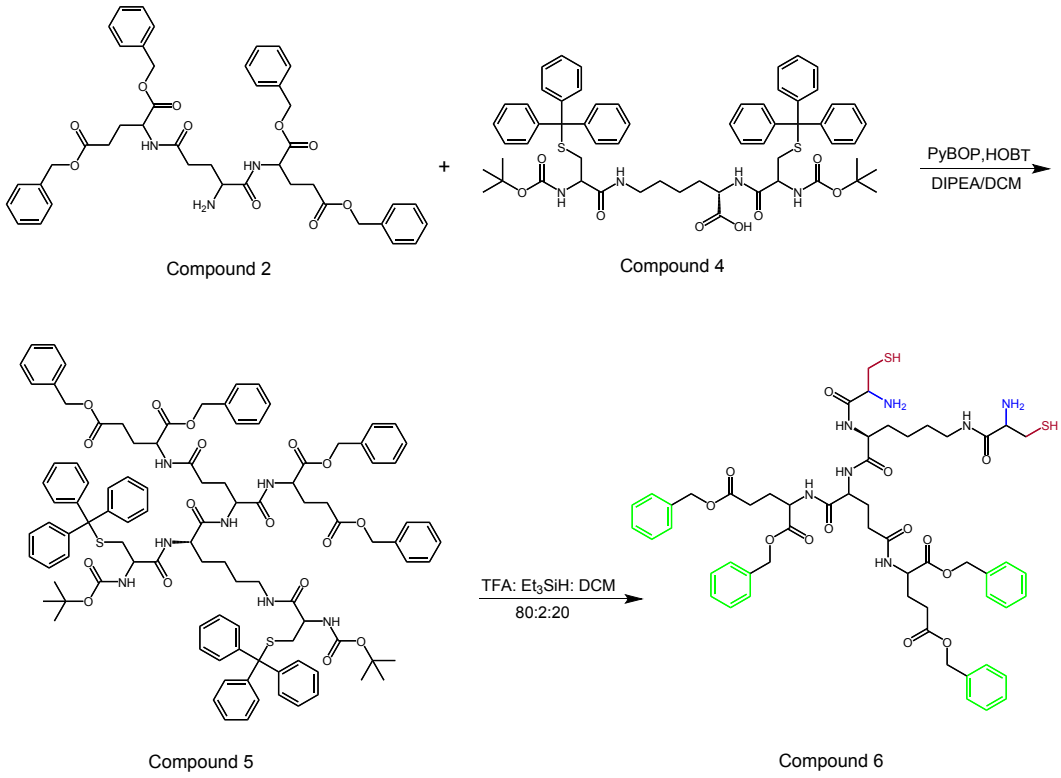
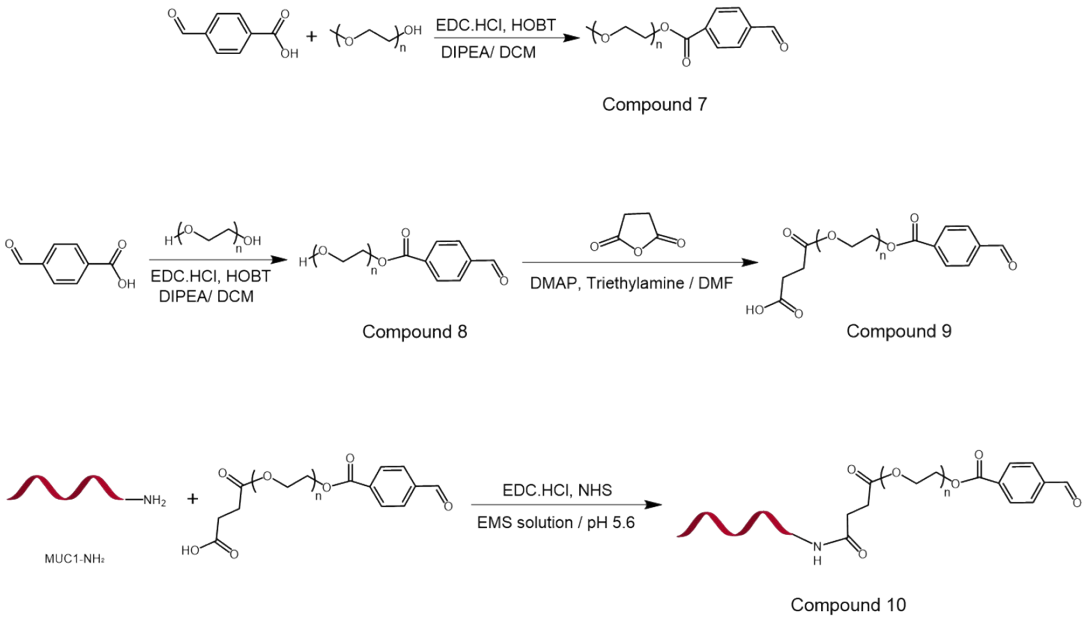


Figure S1. Synthetic route for H-Glu(G2)-Obzl and HO-lys-(Boc-Cys-Trt)$_2$



Compound 2 Compound 4 Compound 5 Compound 6

Figure S2. Synthetic route for $(H\text{-}Cys\text{-}SH)_2$-lys-Glu(G2)-Obzl



Compound 7 Compound 8 Compound 9 Compound 10

Figure S3. Synthetic route for Compound 7 and Compound 10

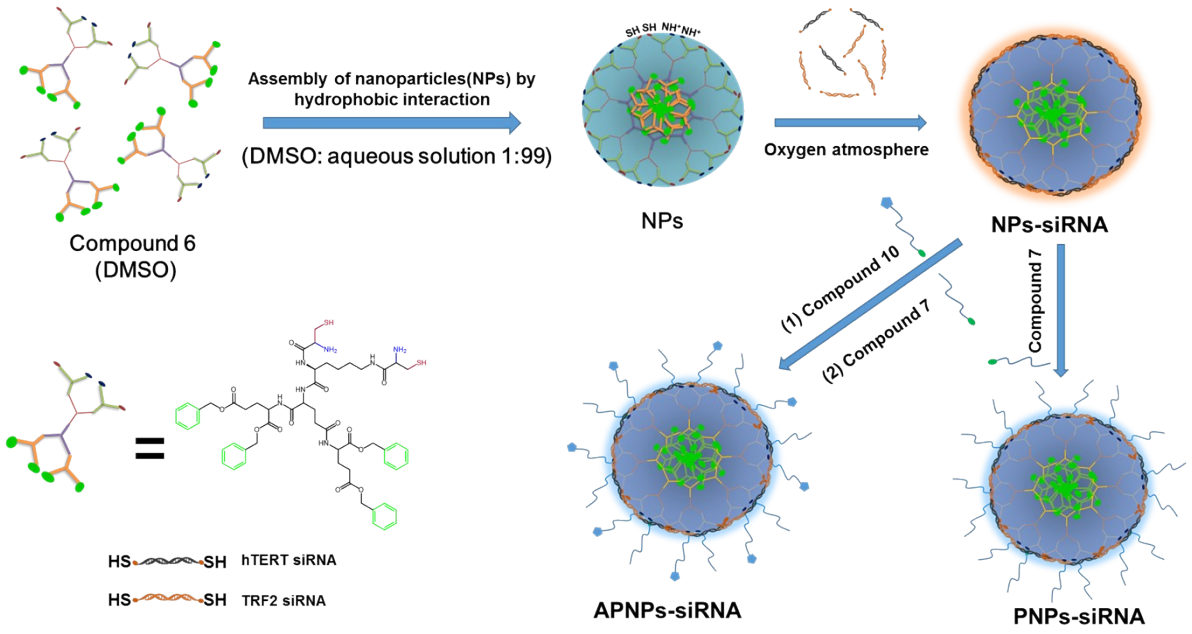


Figure S4. Synthetic route for APNPs-siRNA and PNPs-siRNA

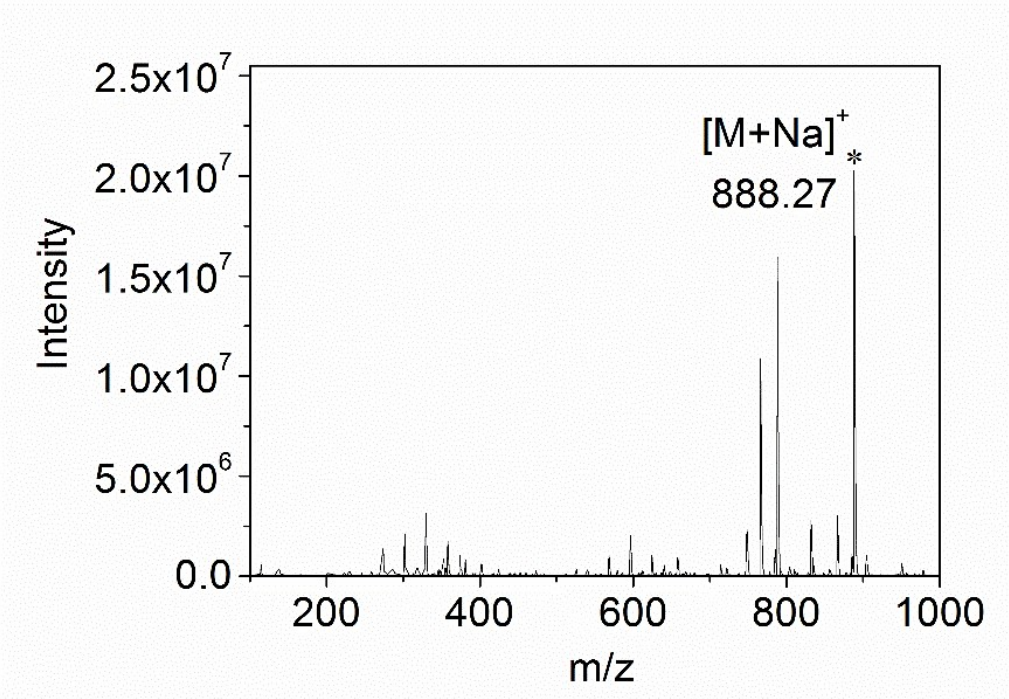


Figure S5. MALDI-TOF mass spectrum of Boc-Glu-(Glu(OBzl)-OBzl)$_2$. MS (m/z, $[M + Na]^+$): 888.27 (observed), and 888.38 (calculated).

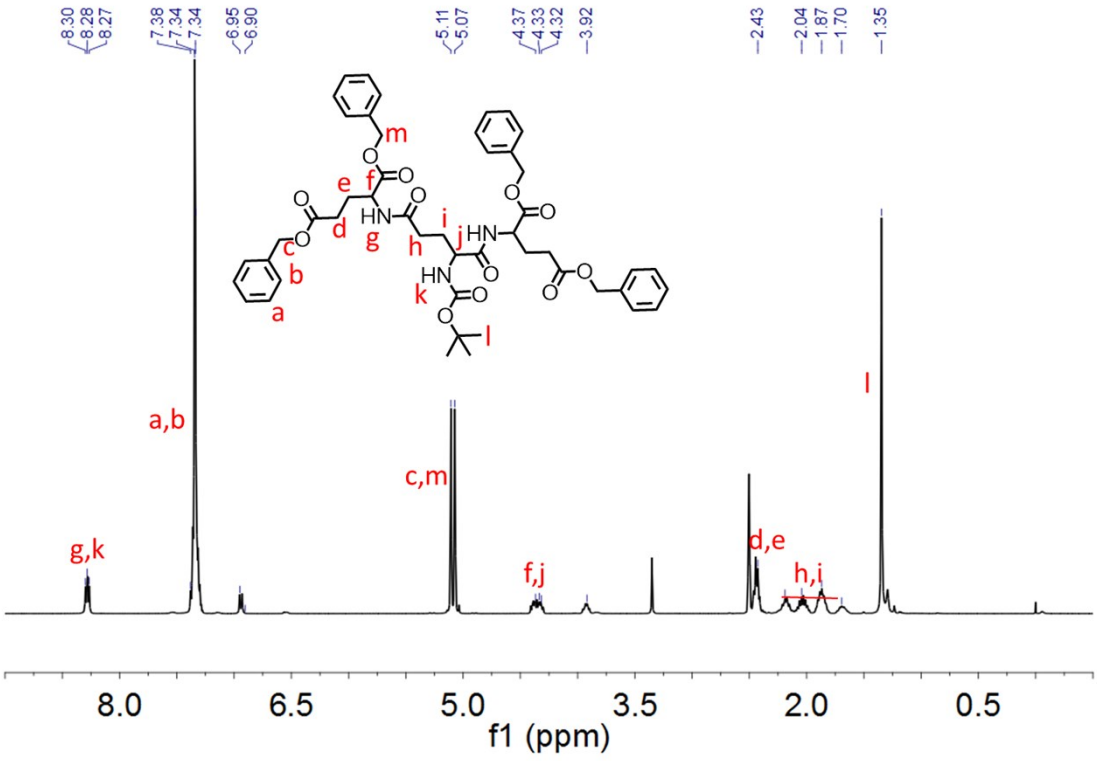


Figure S6. ^{1}H-NMR spectrum of Boc-Glu-(Glu(OBzl)-OBzl)$_2$ in DMSO-d$_6$.

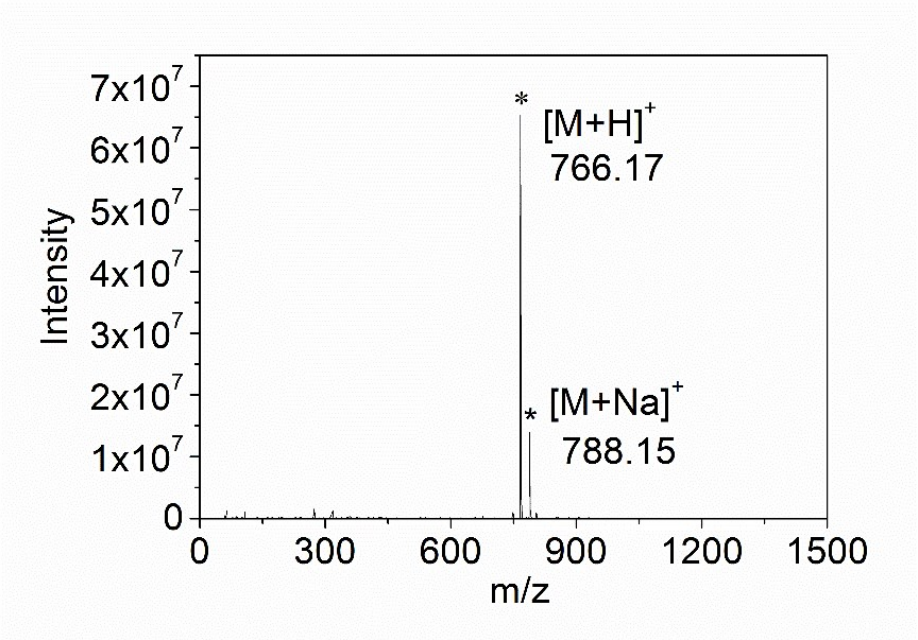


Figure S7. MALDI-TOF mass spectrum of H-Glu(G2)-Obzl. MS (m/z, [M + H]$^+$): 766.17 (observed), and 766.33 (calculated). MS (m/z, [M + Na]$^+$): 788.15 (observed), and 788.33 (calculated).

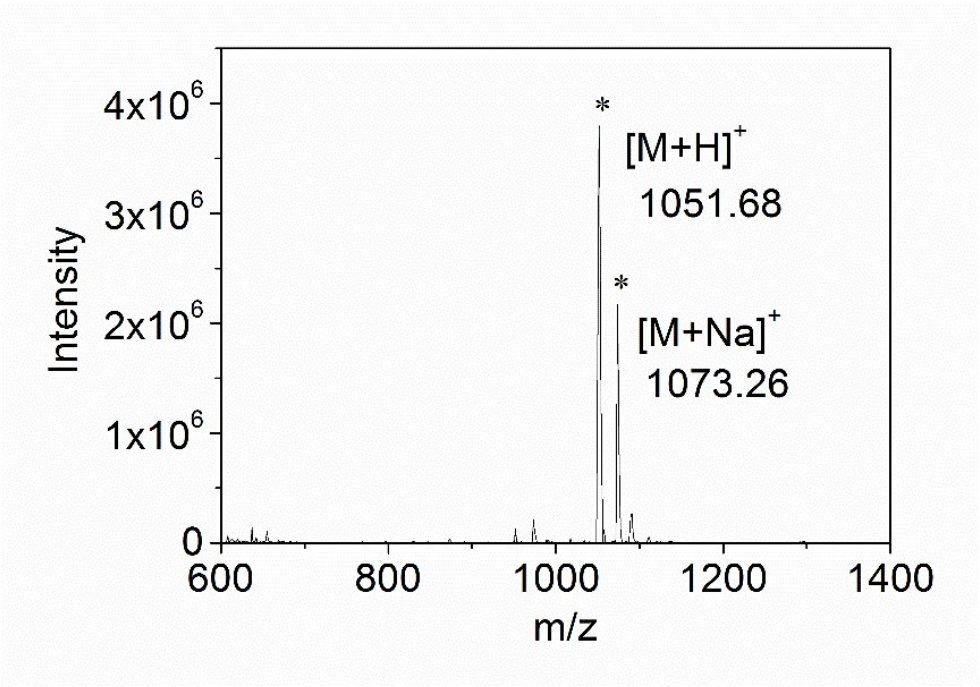


Figure S8. MALDI-TOF mass spectrum of MeO-Lys-(Cys-Boc-(Trt))$_2$. MS (m/z, [M + H]$^+$): 1051.68 (observed), and 1051.46 (calculated). MS (m/z, [M + Na]$^+$): 1073.36 (observed), and 1073.46 (calculated).

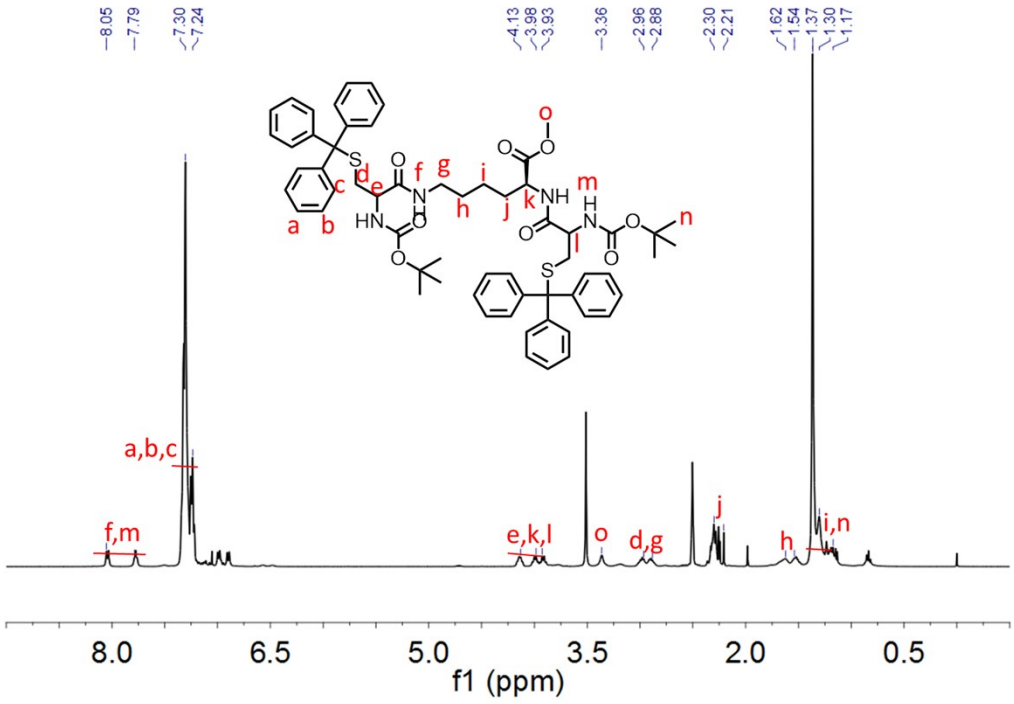


Figure S9. ^{1}H-NMR spectrum of MeO-Lys-(Cys-Boc-(Trt))$_2$ in DMSO-d$_6$.

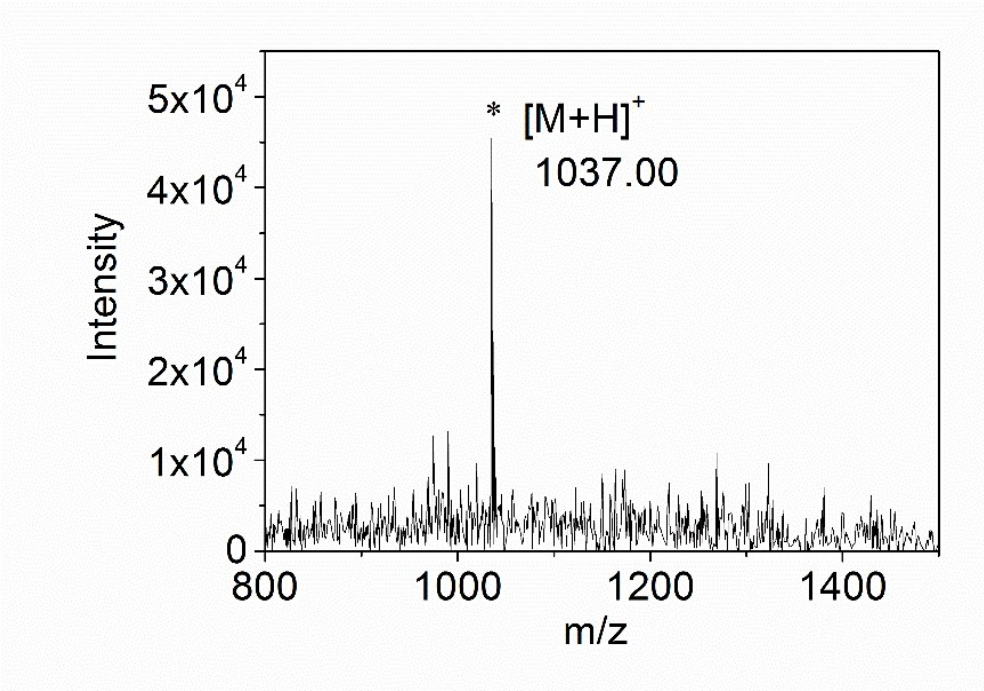


Figure S10. MALDI-TOF mass spectrum of HO-lys-(Boc-Cys-Trt)$_2$. MS (m/z, $[M + H]^+$): 1037.00 (observed), and 1037.45 (calculated).

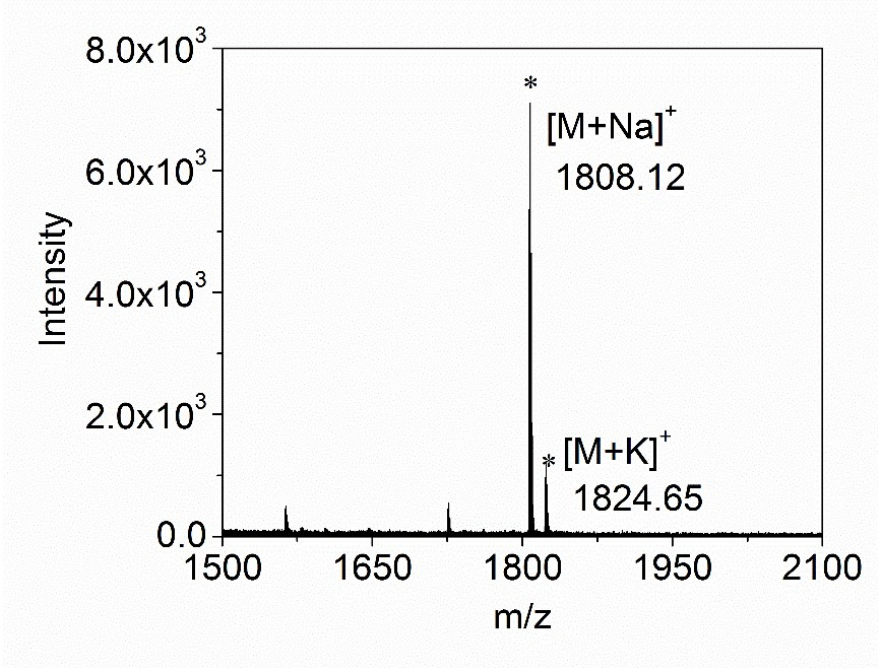


Figure S11. MALDI-TOF mass spectrum of (Boc-Cys-Trt)$_2$-lys-Glu(G_2)-Obzl. MS (m/z, $[M + Na]^+$): 1808.12 (observed), and 1806.76 (calculated). MS (m/z, $[M + K]^+$): 1824.65 (observed), and 1822.76 (calculated).

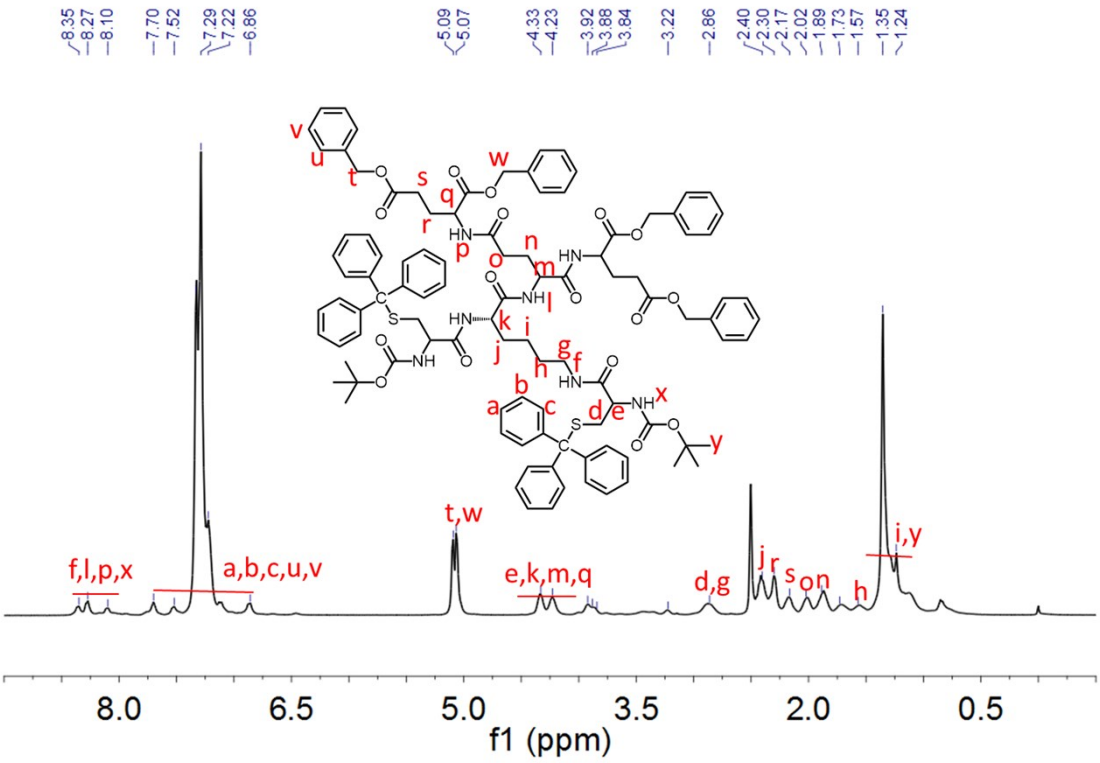


Figure S12. ^{1}H-NMR spectrum of (Boc-Cys-Trt)$_2$-lys-Glu(G$_2$)-Obzl in DMSO-d$_6$.

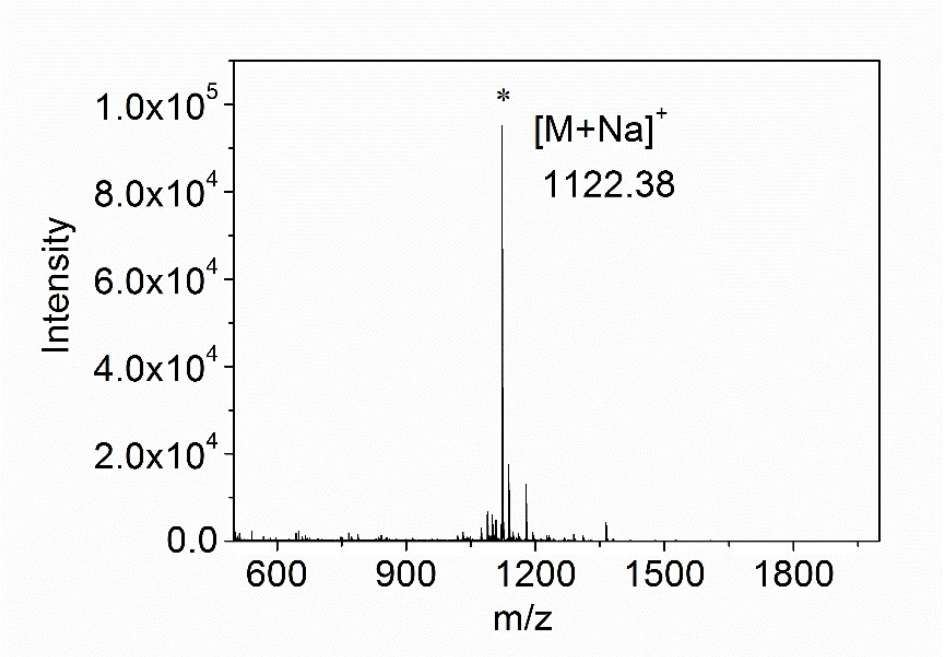


Figure S13. MALDI-TOF mass spectrum of (H-Cys-SH)$_2$-lys-Glu(G$_2$)-Obzl. MS (m/z, [M + Na]$^+$): 1122.38 (observed), and 1122.44 (calculated).

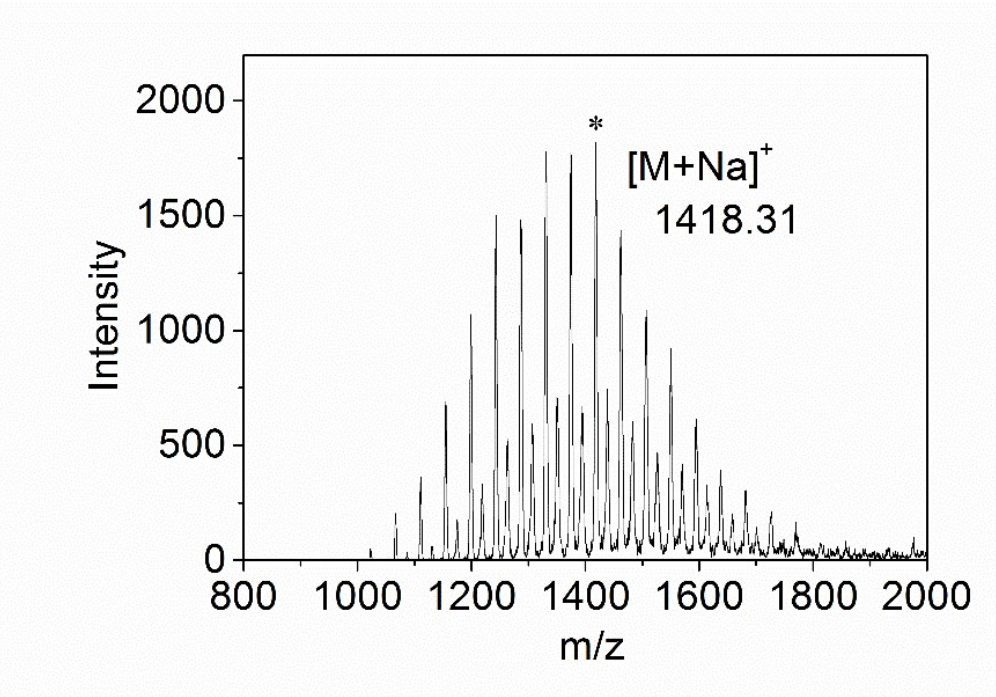


Figure S14. MALDI-TOF mass spectrum of MeO-PEG-CHO. MS (m/z, $[M + Na]^+$): 1418.31 (observed), and 1419.88 (calculated).

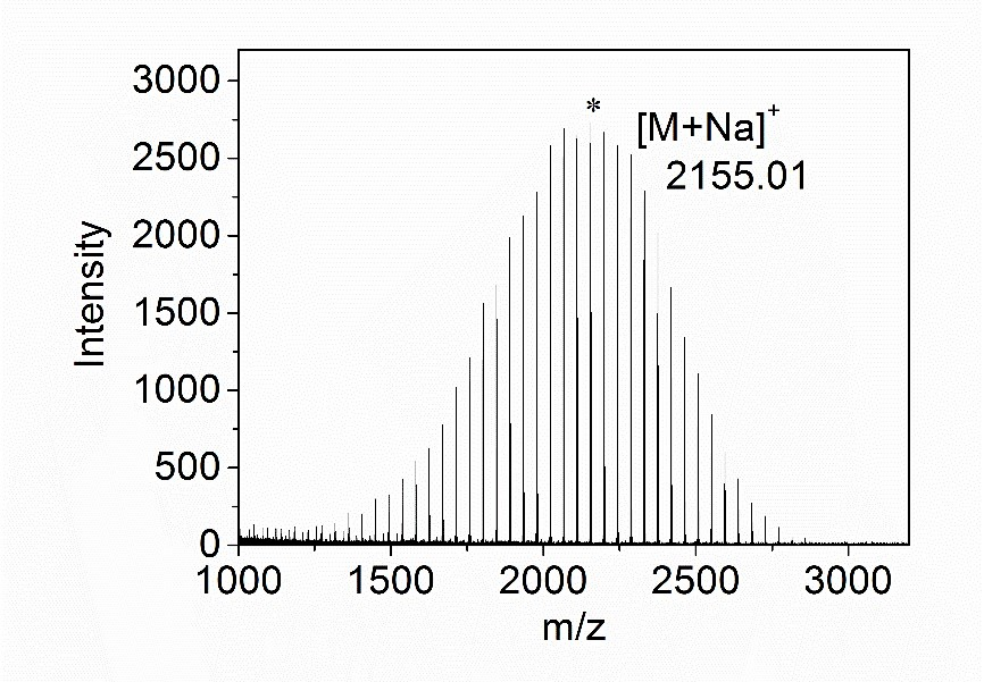


Figure S15. MALDI-TOF mass spectrum of HO-PEG-CHO. MS (m/z, $[M + Na]^+$): 2155.01 (observed), and 2154.38 (calculated).

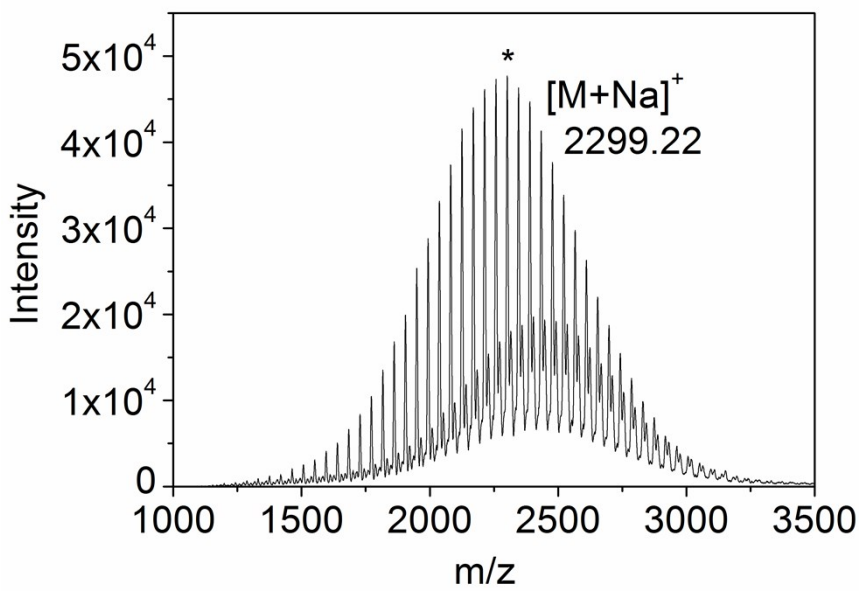


Figure S16. MALDI-TOF mass spectrum of HOOC-PEG-CHO. MS (m/z, $[M + Na]^+$): 2299.22 (observed), and 2299.42 (calculated).

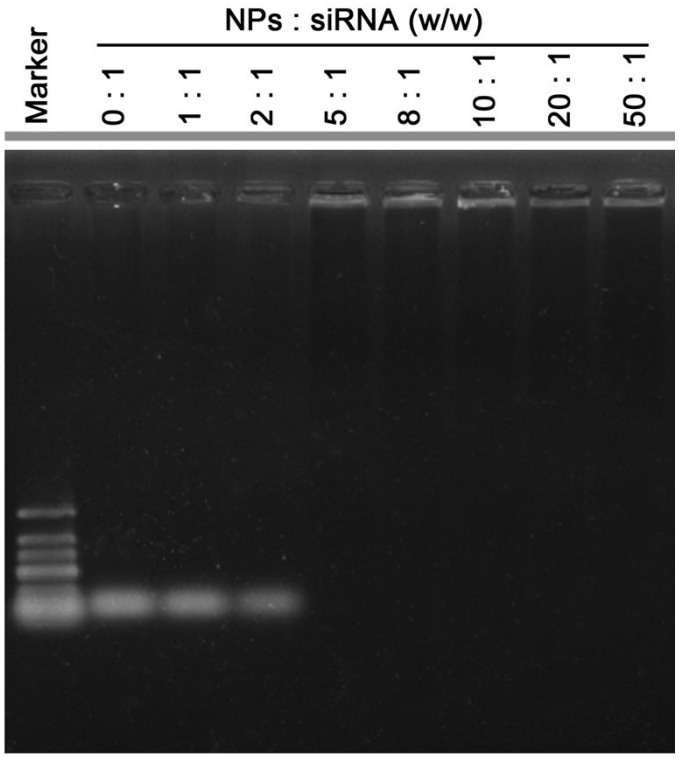


Figure S17. Gel retardation assay of NPs-siRNA complexes at the specified weight ratio.

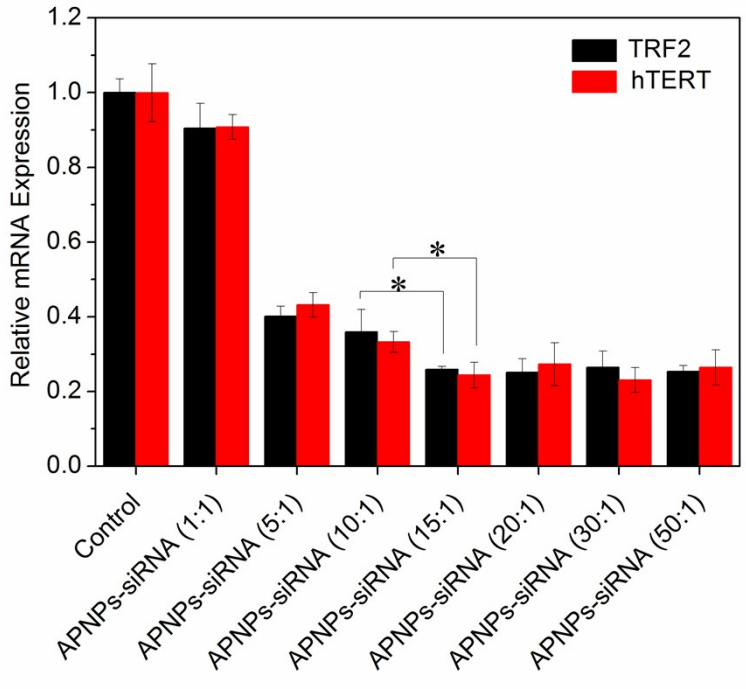


Figure S18. The relative mRNA levels in the A549/PTX cells treated with PBS (control) and APNPs-siRNA complexes at the specified weight ratio of NPs: siRNA for 24 h measured by qPCR. (n = 3).

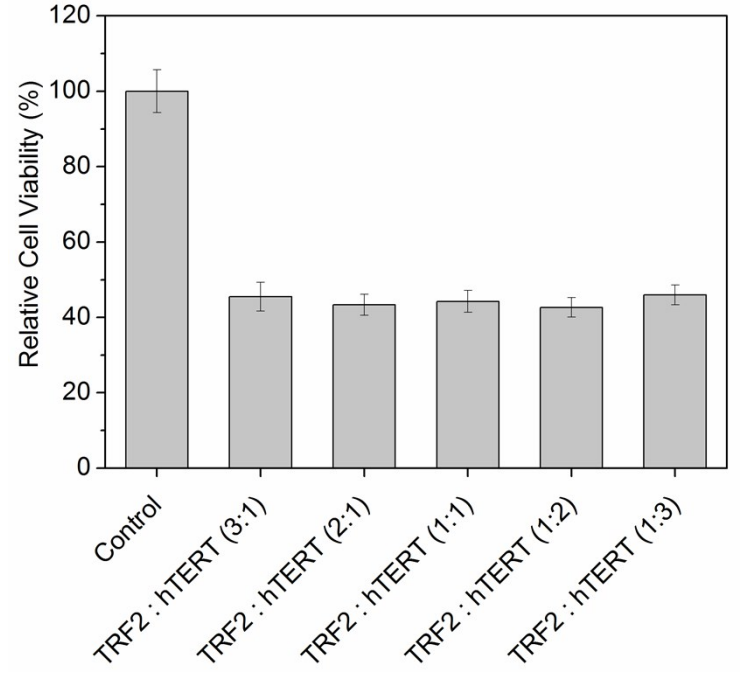


Figure S19. Relative viability of A549/PTX cells treated with APNPs-siRNA complexes containing specified ratio of the two siRNAs for 48 h (n = 6).

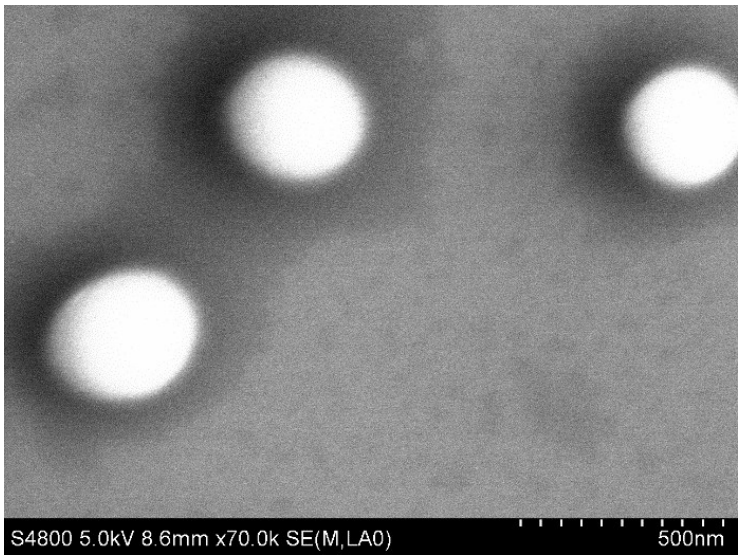


Figure S20. SEM image of APNPs-siRNA at pH 7.4.

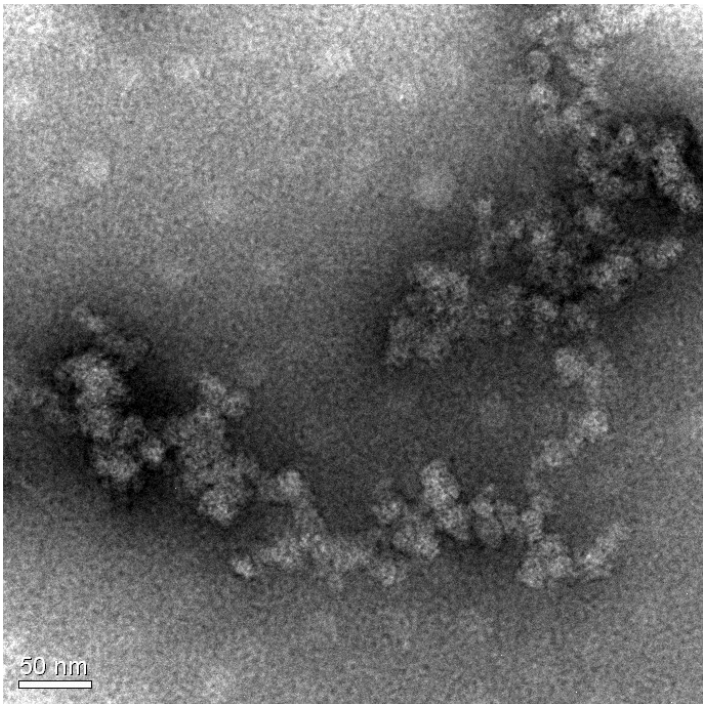


Figure S21. TEM image of APNPs-siRNA with 10 mM DTT

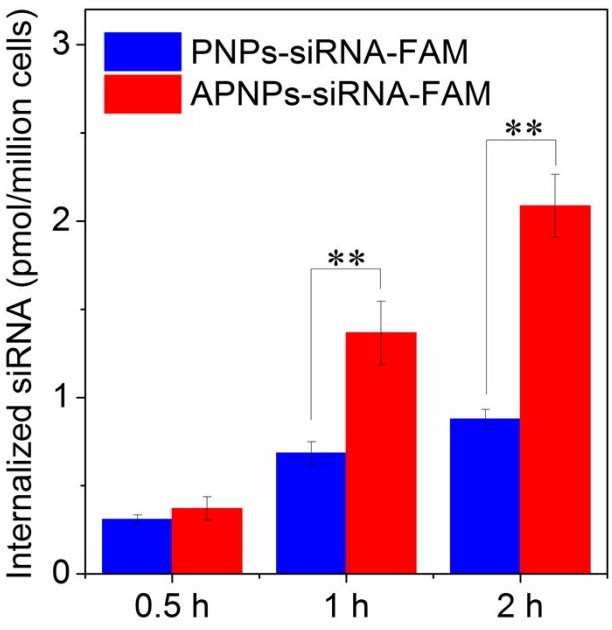


Figure S22. Internalization of APNPs-siRNA-FAM and PNPs-siRNA-FAM at pH 7.4 by A549/PTX tumor cells (means ± SD, n = 3, *p < 0.05, **p < 0.005).

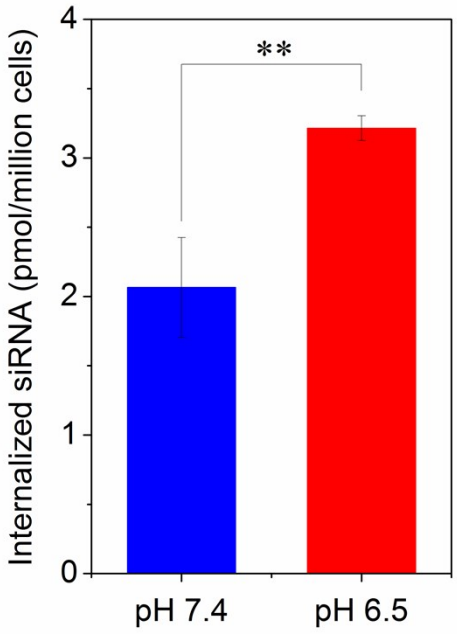


Figure S23. Internalization of APNPs-siRNA for 2 h at pH 7.4 or pH 6.5 by A549/PTX tumor cells (means ± SD, n = 3, *p < 0.05, **p < 0.005).

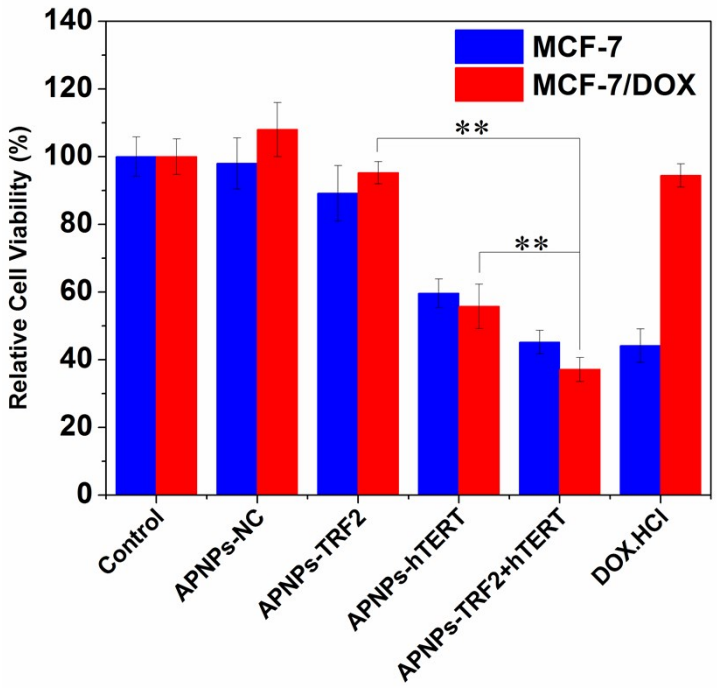


Figure S24. Relative cell viability of MCF-7/DOX and MCF-7 incubated with different formulations at pH 6.5 for 48 h (n = 6).

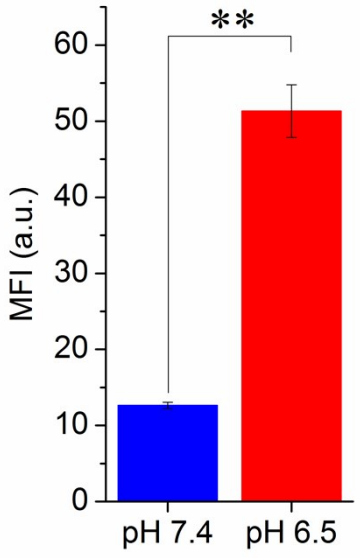


Figure S25. MFI in the white circles in spheroids at the depth of 80 μm (n = 3).

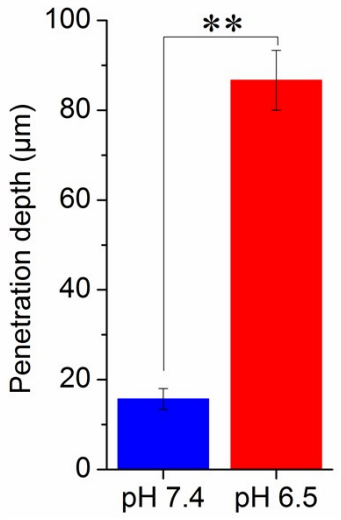


Figure S26. Penetration depth of APNPs-siRNA for 6 h in the conditions of pH 7.4 or pH 6.5 (n = 3).

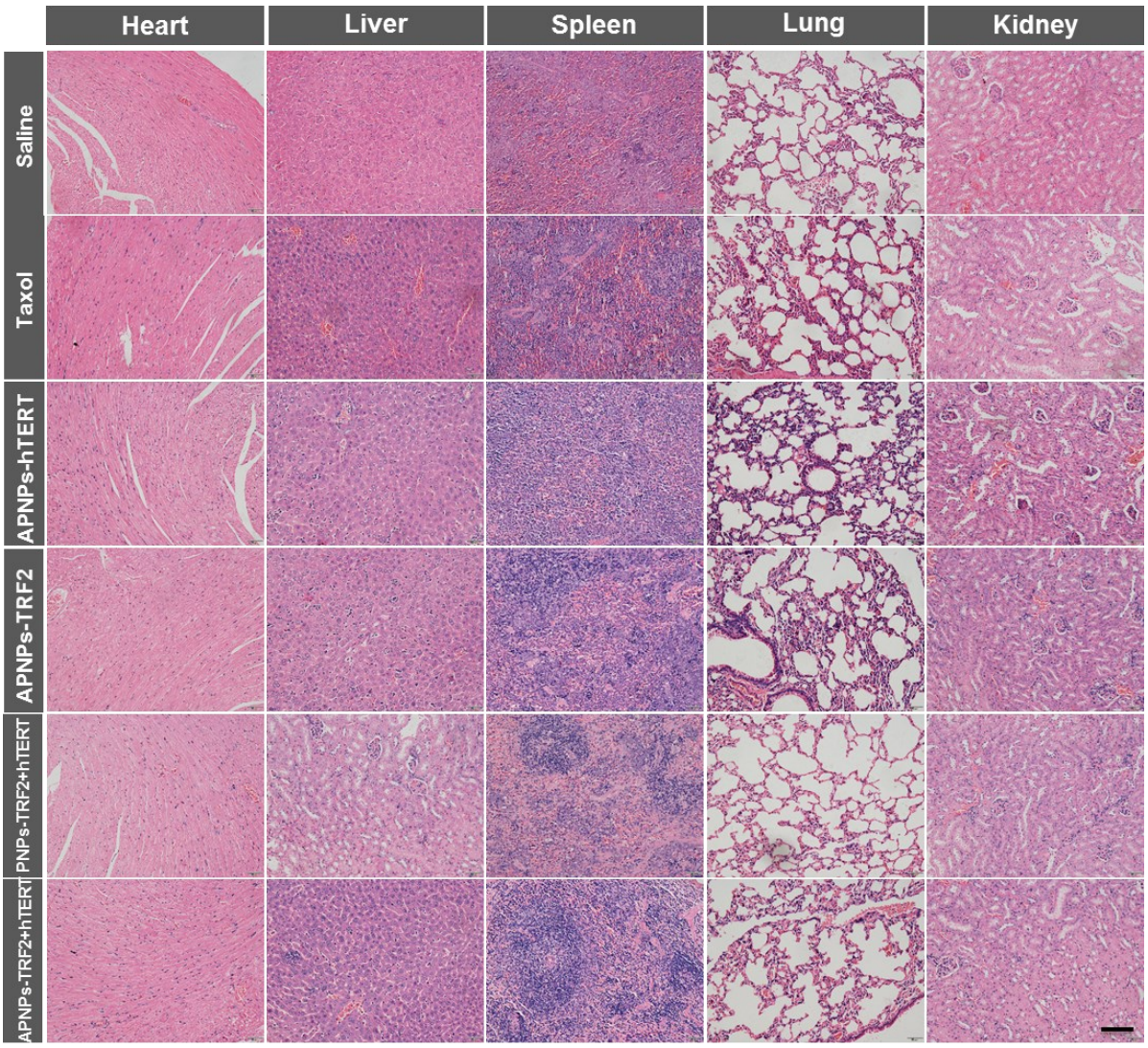


Figure S27. H&E analyses of major tissues (heart, liver, spleen, lung and kidney) treated with various formulations after 24 days (scale bar: 100 μm).

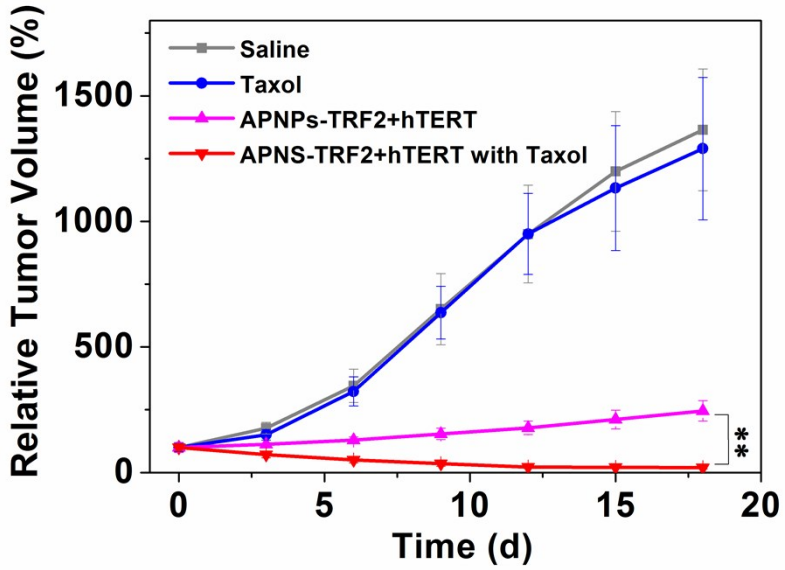


Figure S28. The relative body weight of A549/PTX tumor-bearing mice of Saline, Taxol (2mg/kg), APNPs-TRF2+hTERT, and APNPs-TRF2+hTERT with Taxol (2mg/kg) treated groups (n = 8).

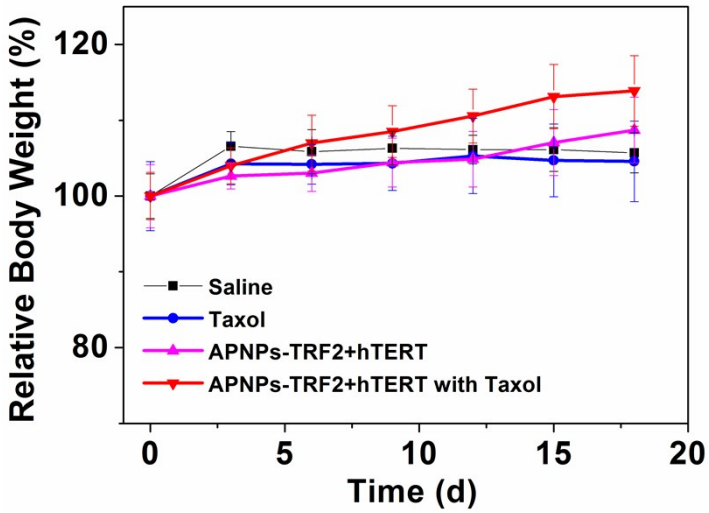


Figure S29. The relative body weight of A549/PTX tumor-bearing mice of Saline, Taxol (2mg/kg), APNPs-TRF2+hTERT, and APNPs-TRF2+hTERT with Taxol (2mg/kg) treated groups (n = 8).

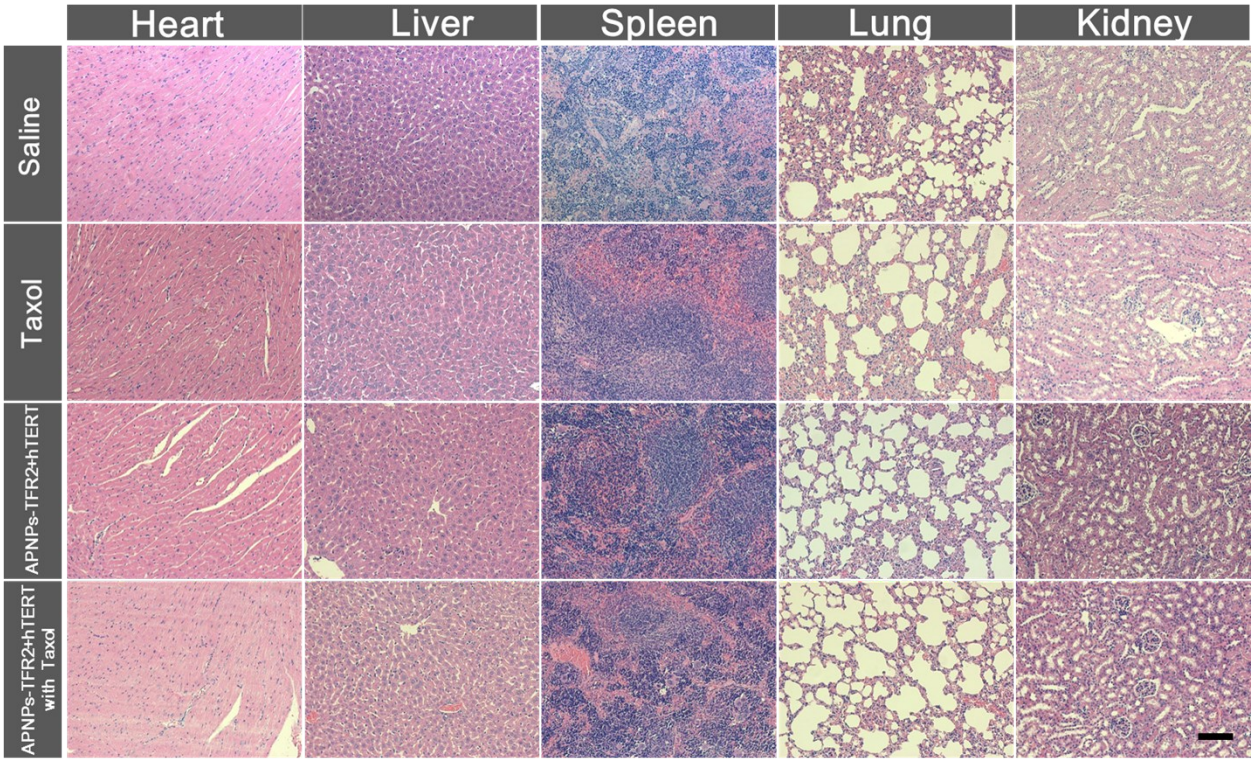


Figure S30. H&E analyses of major tissues (heart, liver, spleen, lung and kidney) treated with various formulations after 18 days (scale bar: 100 μm)