Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Pt(IV)-mediated Polymer Architecture for Facile and Stimuli-Responsive Intracellular Gene Silencing with Chemotherapy

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1. Synthesis of Pt-PA-PEG

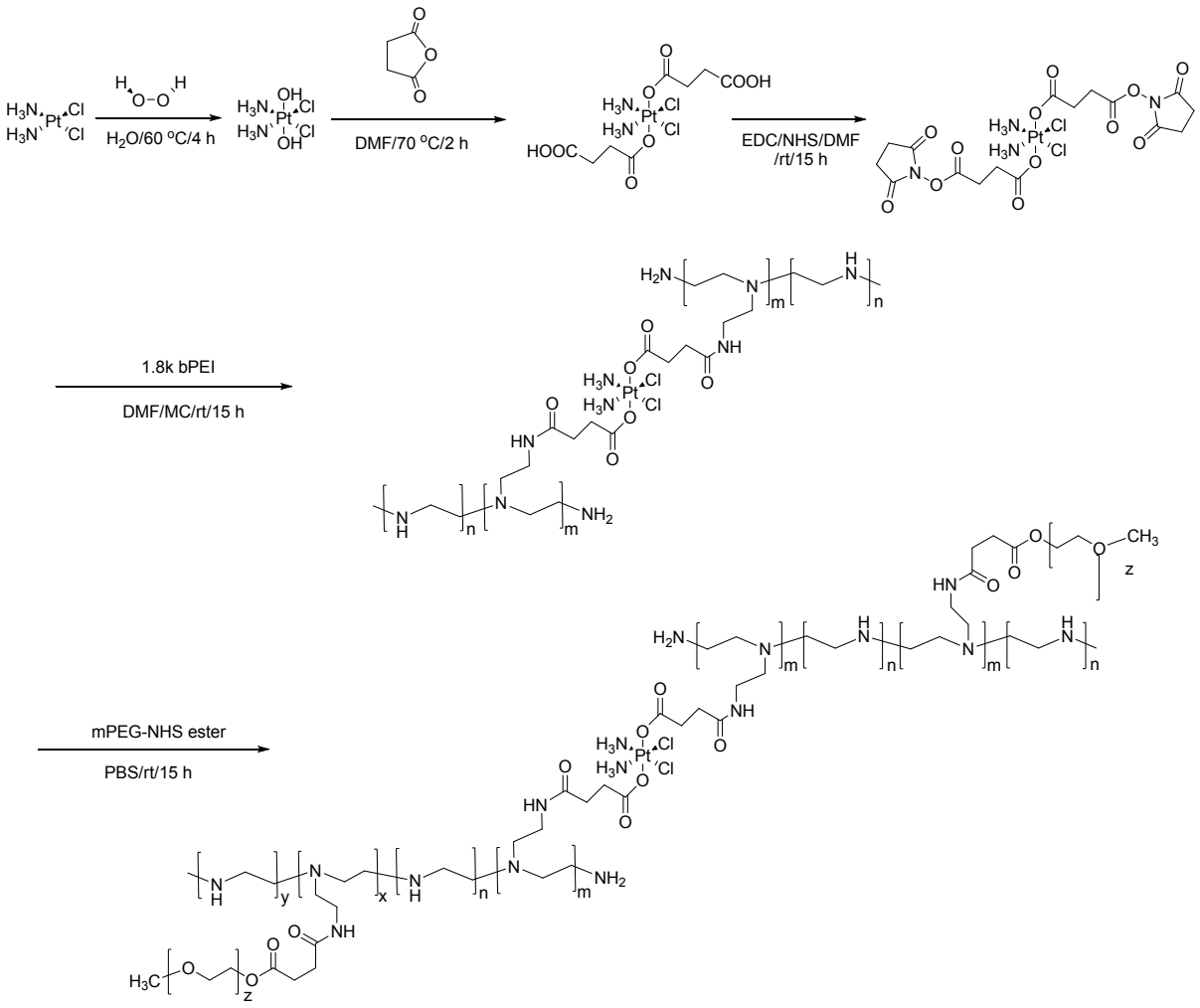

Figure S1. Synthetic scheme of Pt-PA-PEG.

2. Characterization of Pt(IV)

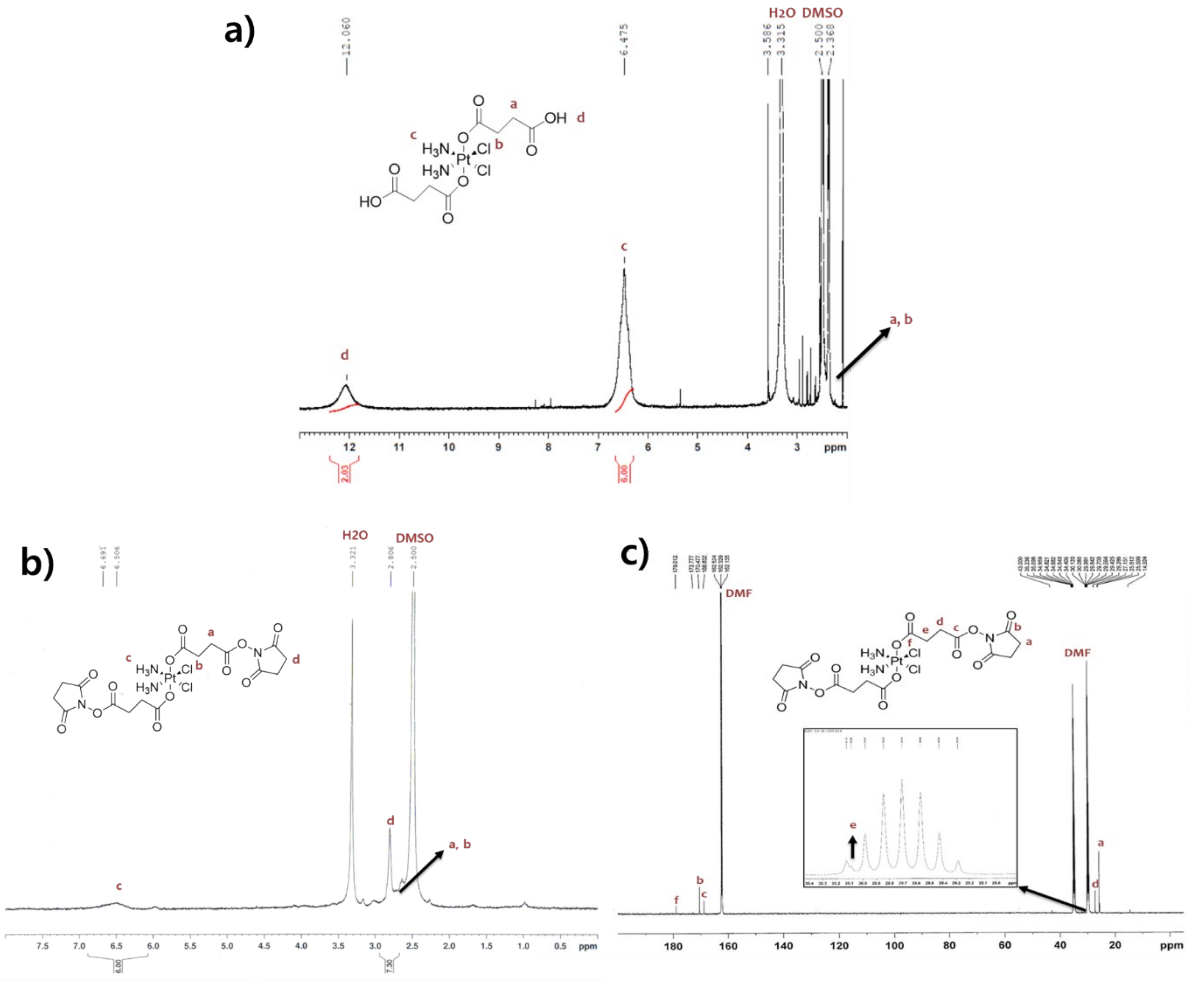

Figure S2. (a) ^{1}H NMR spectra of Pt(IV)-diCOOH, (b) ^{1}H NMR and (c) ^{13}C NMR spectra Pt(IV)-diNHS.

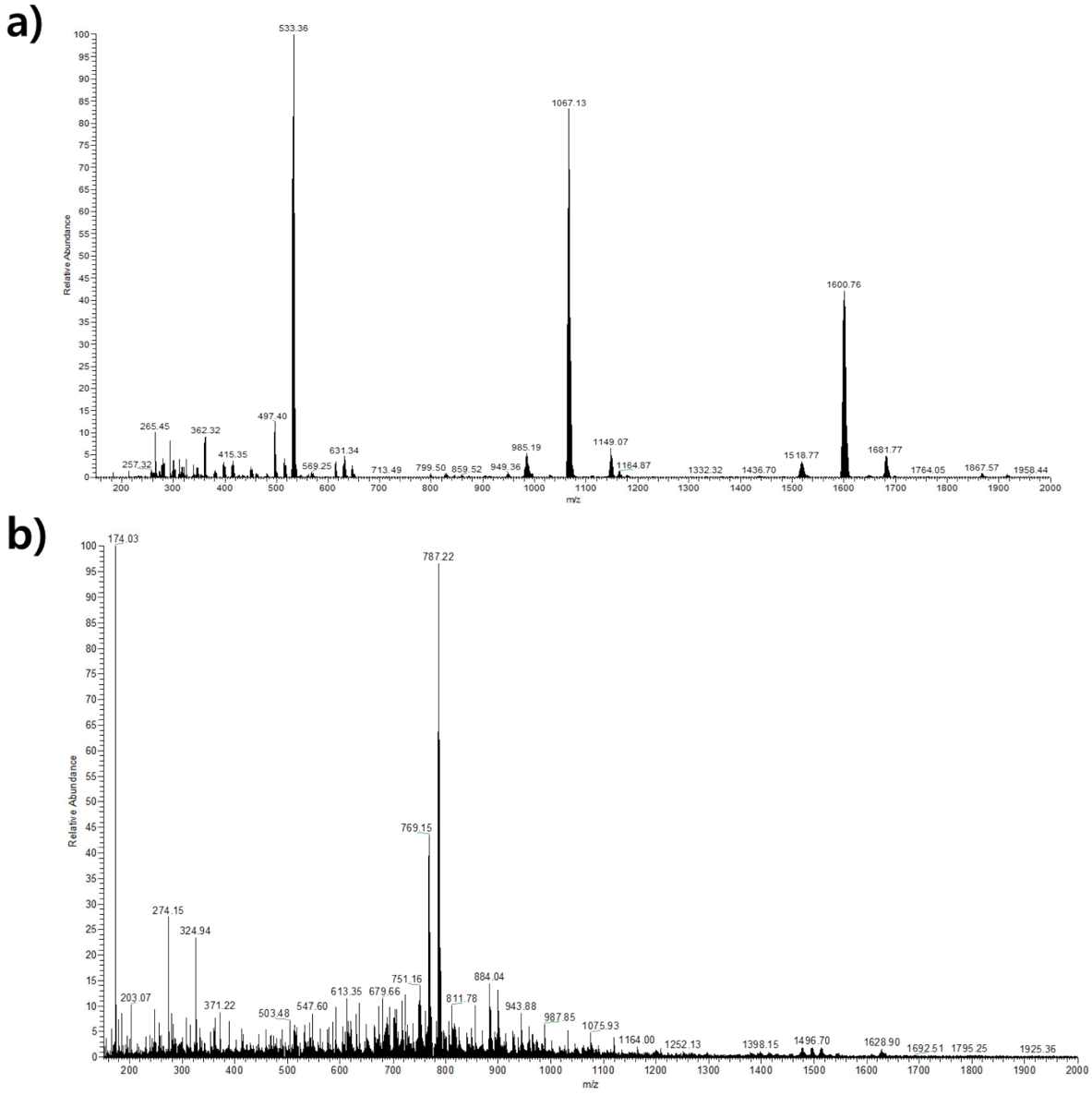

Figure S3. ESI-MS data of (a) Pt(IV)-diCOOH and (b) Pt(IV)-diNHS. Exact mass of Pt(IV)-diCOOH is 532.99 and Pt(IV)-diNHS is 727.03. The results of Pt(IV)-diCOOH showed 533.07 $[M-1]^+$, 1066.82 $[2M-1]^+$, 1600.39 $[3M-1]^+$ (negative mode) and Pt(IV)-diNHS showed adduct of Pt(IV)-diNHS ($[M+ CH_3CO_2H]^+$, 787.22, positive mode).

3. Construction and characterization of Pt-PA

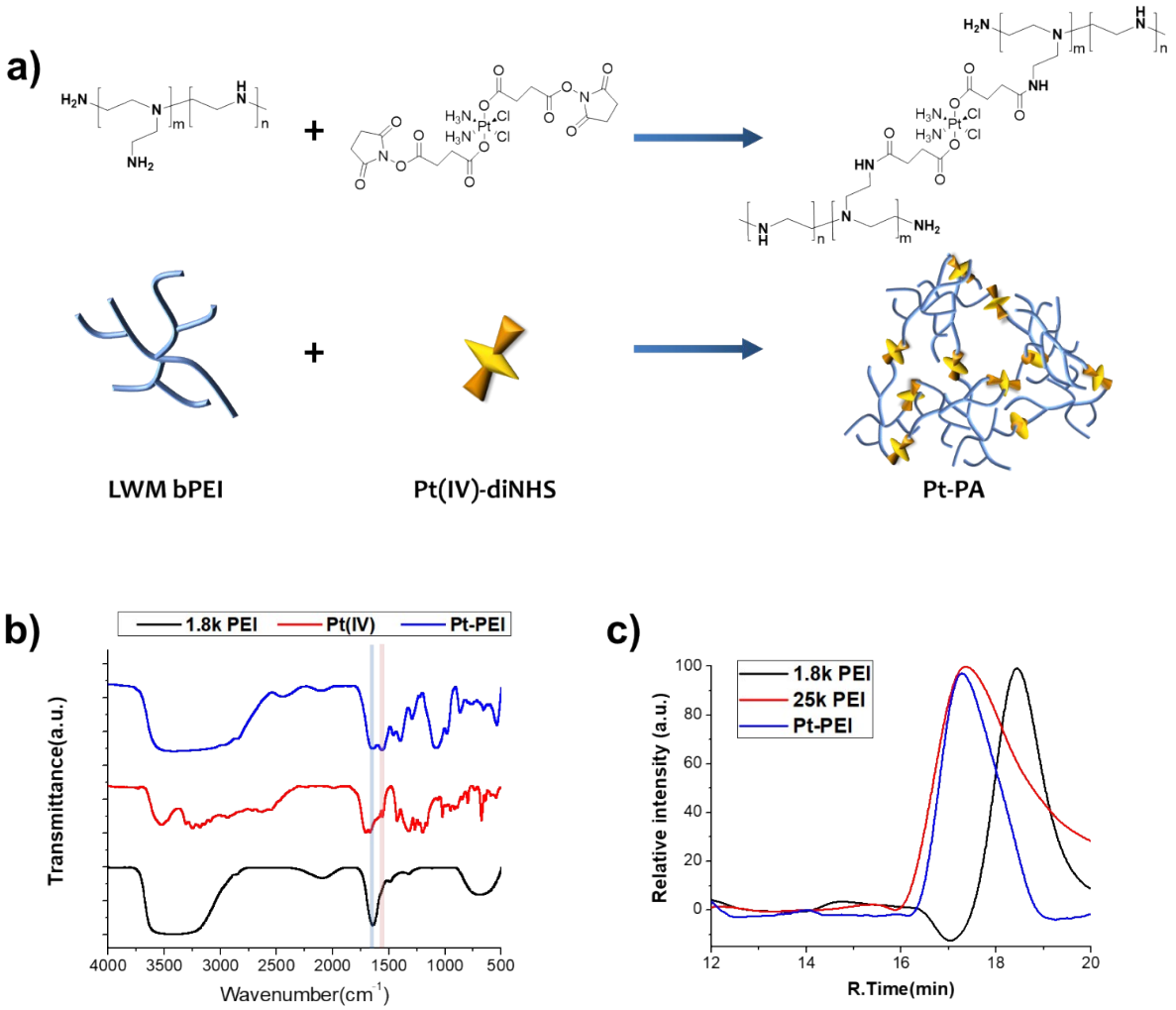

Figure S4. (a) Schematic illustration for explanation of polymer crosslinking based on Pt(IV). (b) Formation of amide bond between Pt(IV) and PEI. (c) Normalized intensity of GPC chromatogram depending on the polymer molecular weight.

4. Loading therapeutic siRNA into Pt-PA and stimuli-responsive siRNA release

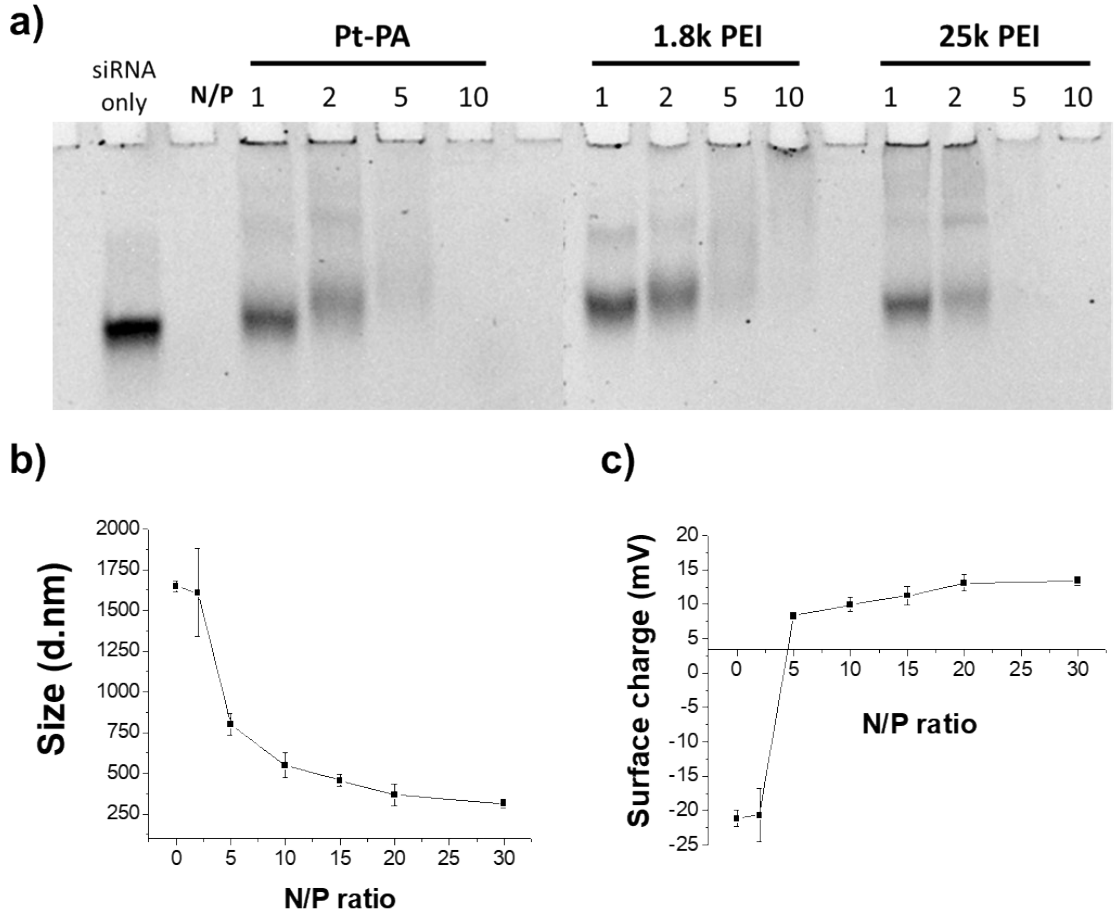

Figure S5. (a) confirmation of interaction between Pt-PEI and siRNA by polyacrylamide gel electrophoresis. (b) Hydrodynamic volume and (c) Zeta potential of polyplex depend on various N/P ratio. N/P ratio means nitrogen of PEI to phosphorous of RNA ratio.

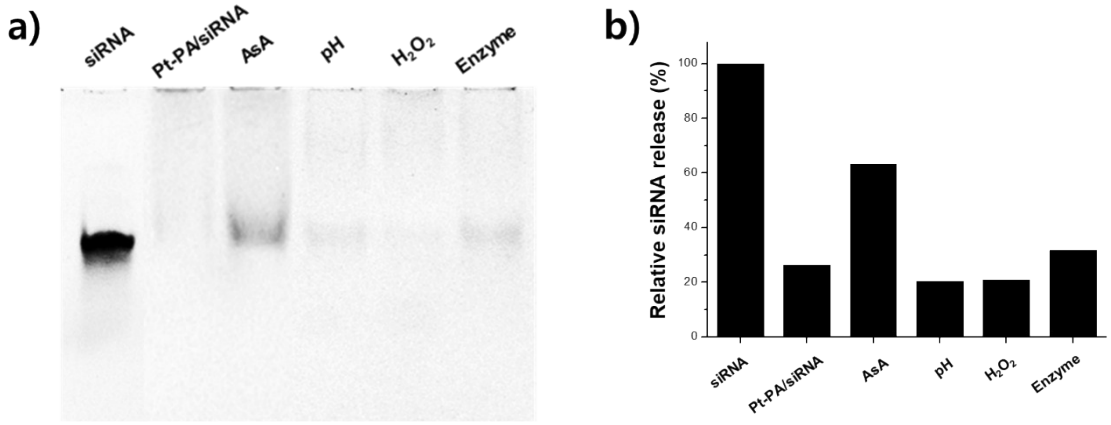

Figure S6. Release of siRNA depending on various stimuli. (a) Gel retardation and (b) its quantification of Pt-PA/siRNA nanocomplex upon various stimuli. AsA = 10mM ascorbic acid/ascorbate solution, pH = PBS at pH 5, H_2O_2 = 10 μM hydrogen peroxide and Enzyme = 10 Units of catalase, respectively.

5. Cytotoxicity studied in A549 cell

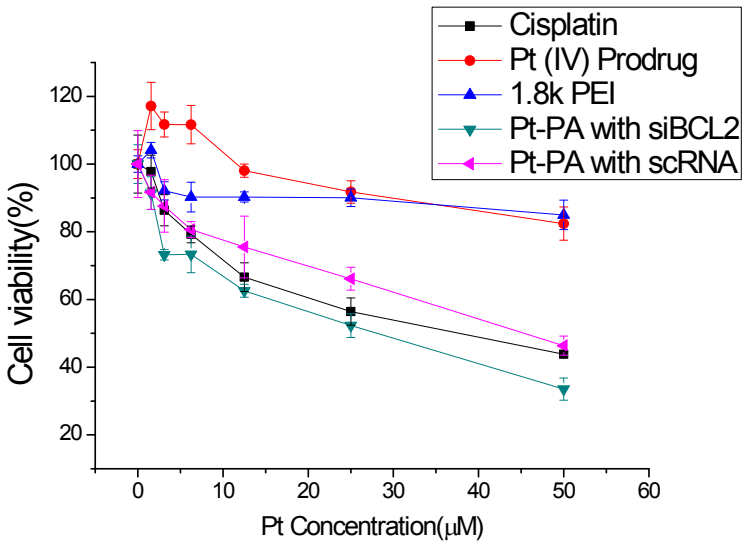

Figure S7. MTT assay results of Cisplatin, Pt(IV), 1.8k PEI, Pt-PA with siBCL2, Pt-PA with scRNA to A549 cell.

6. Cytotoxicity depend on BSO

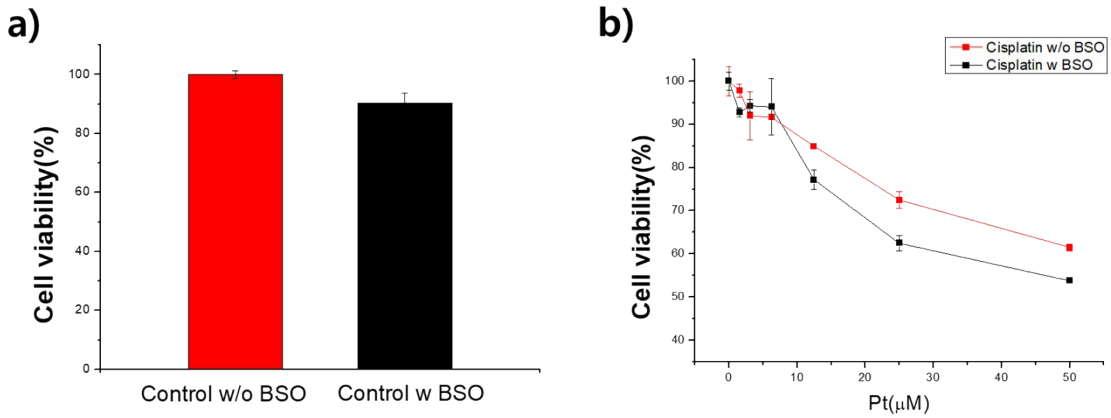

Figure S8. Cell viability depend on BSO treatment. Cell viability of control (a) and Cisplatin (b) groups.

7. Redox-facilitated siRNA release

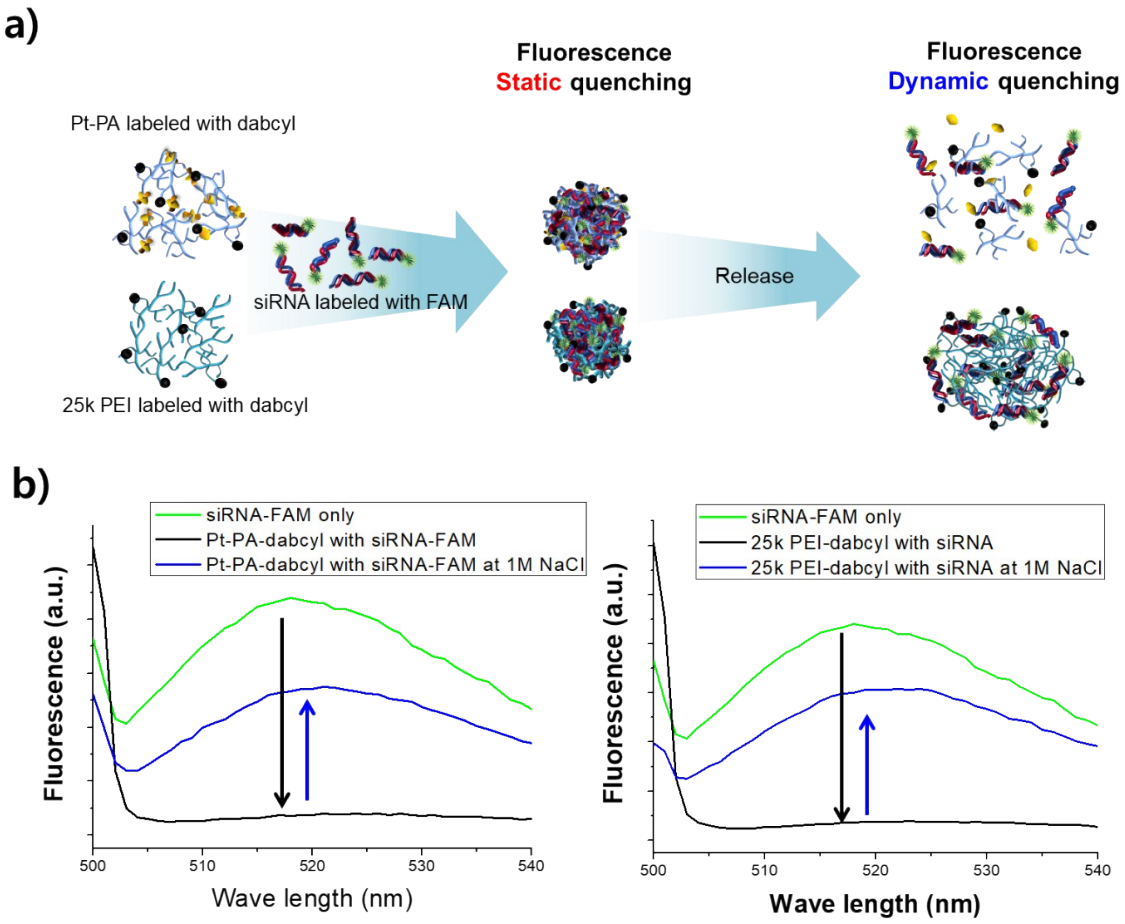

Figure S9. Redox-facilitated siRNA release using fluorescence meter. Dabcyl-NHS ester was reacted with Pt-PA and 25k PEI, and siRNA was labeled with FAM. (a) Graphical abstract of siRNA release in order to distinguish behavior between static and dynamic quenching. (b) Fluorescence meter results of each sample at static and dynamic quenching states by complexation and release via NaCl respectively.

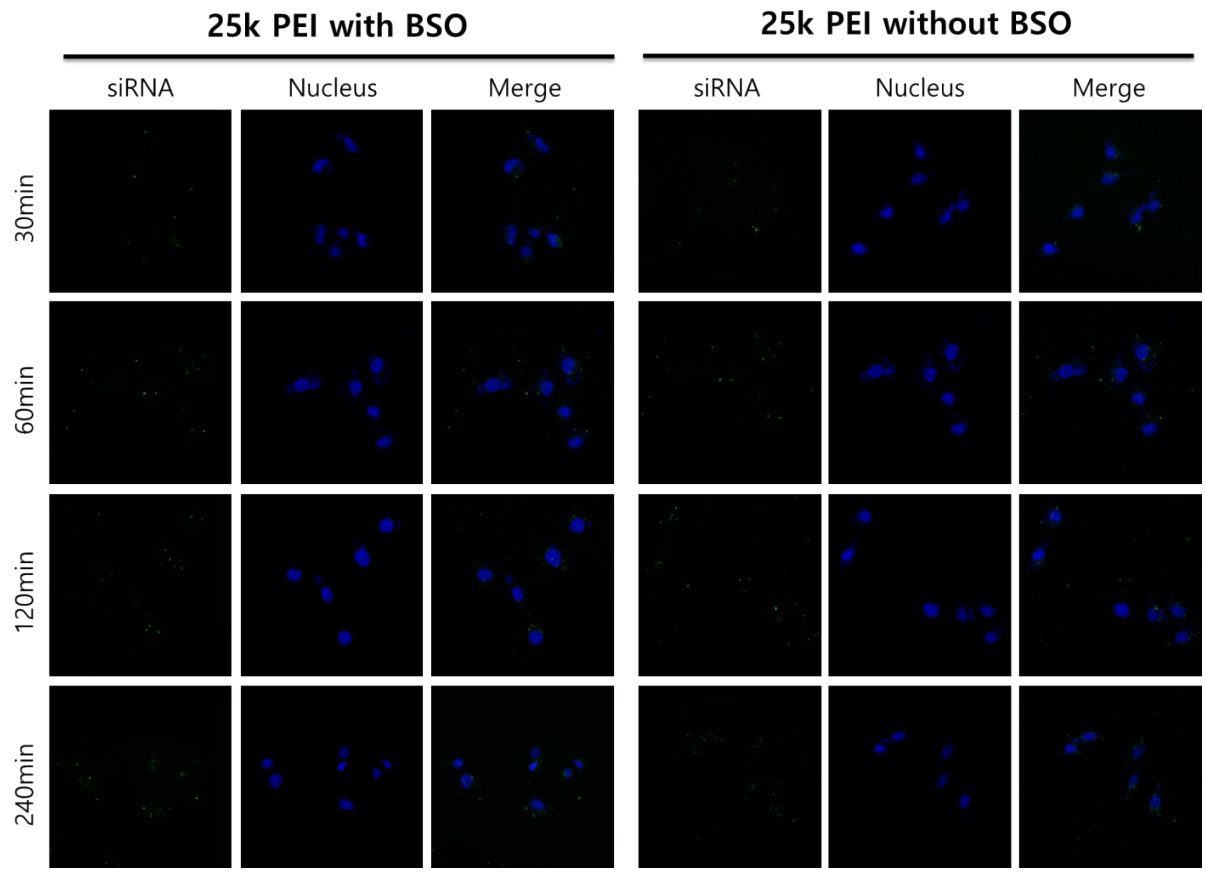

Figure S10. Intracellular siRNA release from 25k PEI using fluorescence microscopy. siRNA labeled with FAM and 25k PEI labeled with dabcyl. The nanocomplex composed with 25k PEI releases siRNA slowly regardless of BSO. Nucleus was stained with DAPI. Buthionine sulphoximine (BSO) = GSH depletion agent.

8. Characterization of Pt-PA-PEG

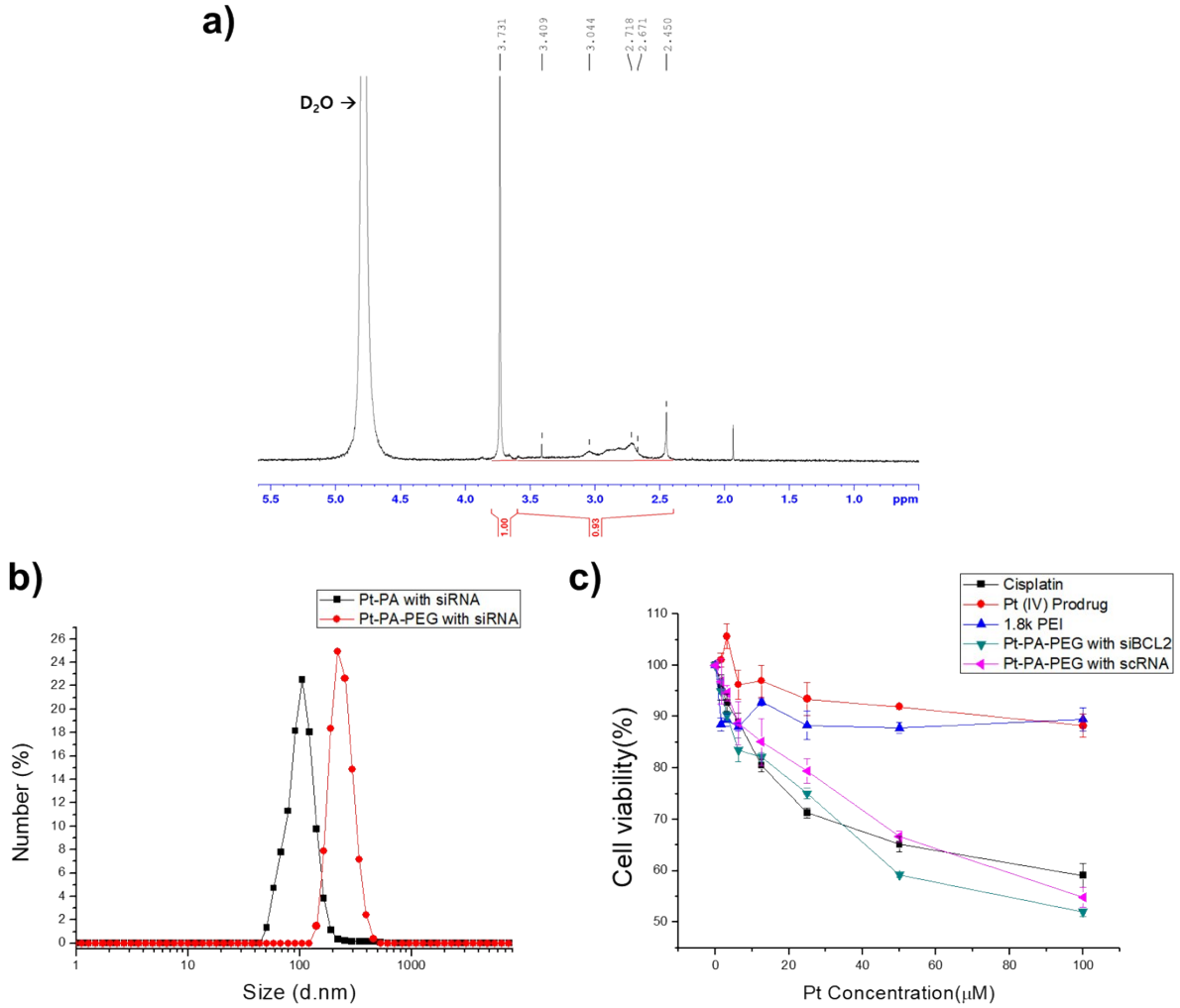

Figure S11. Characterization of Pt-PA-PEG. a) ^{1}H NMR spectra of Pt-PA-PEG. Conjugation ratio of PEI and PEG was determined by the integration of ^{1}H NMR in D_2O. From the integration of PEI (2.40-3.6 ppm, Mw= 1.8k) and PEG (3.60-3.80 ppm, Mw= 5k), PEG (5K) : PEI (1.8k) = 1:2.51 (mole). b) Size analysis of Pt-PA and Pt-PA-PEG with siRNA polyplex by DLS. c) *in vitro* cell viability of Cisplatin, Pt (IV), 1.8k PEI, Pt-PA-PEG with siBCL2, Pt-PA-PEG with scRNA to MDA-MB-231 cell. 48h incubation.

9. Accumulation of Pt-PA-PEG in tumor tissue and therapeutic effect of Pt-PA-PEG

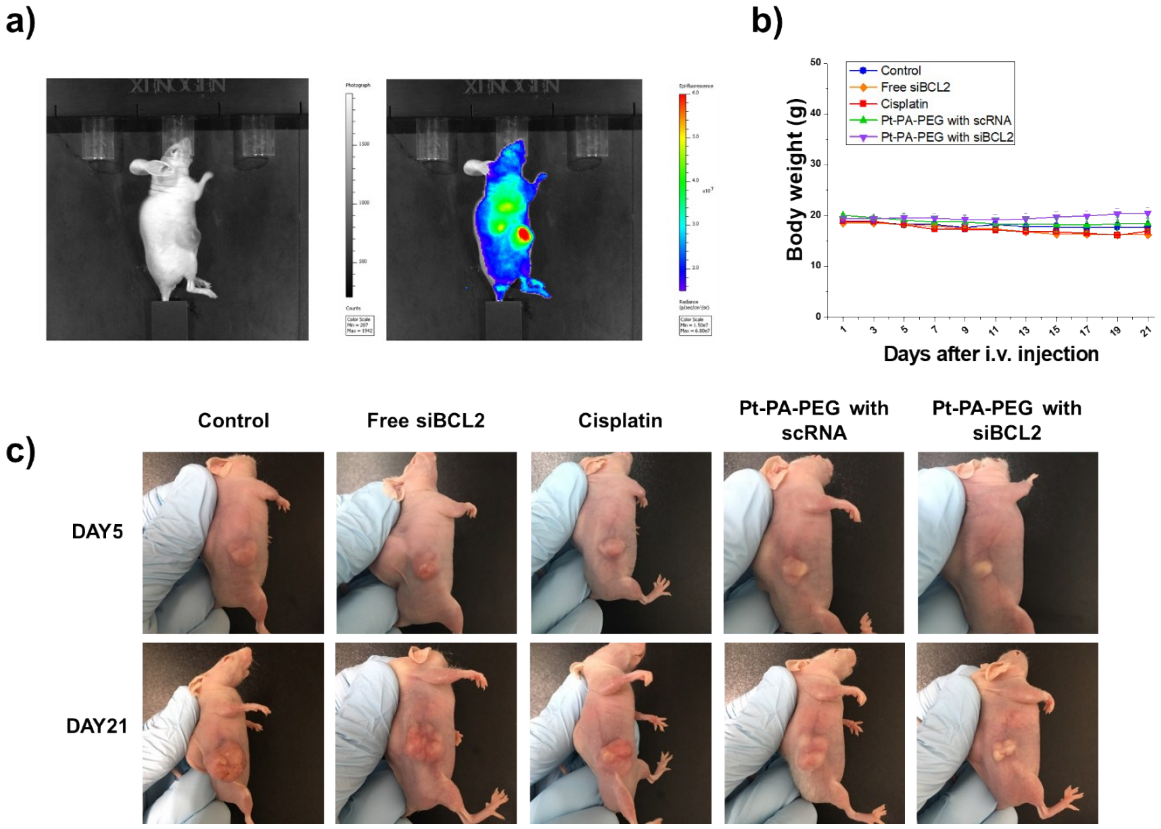

Figure S12. Accumulation study of Pt-PA-PEG-Flamma 648 with siRNA. High intensity of Flamma 648 (ex 648 nm / em 663nm) was observed at tumor site. b) Body weight measurement of balb/c-nude mice after treatment with each sample. The result represents mean ± SD (n = 5). c) Tumor bearing mouse of each group at day 5 and day 21.

All animal experiments were approved by the POSTECH Biotech Center Ethics Committee. MDA-MB-231 cells were inoculated subcutaneously (*s.c.*) at an initial density of 1 x 107 cells/mouse into the left flank of each female Balb/c-nude mice weighing 17 ± 2 g. After the average tumor volume reached 300 mm^2, the mice were treated with 200 μL of Pt-PA-PEG with siRNA complex intravenously (*i.v.*). Pt-PA-PEG was labeled with Flamma 648 dye (ex 648 / em 663, BioActs, Incheon, Korea). After 48 h, the fluorescence of mice was obtained and analyzed with an IVIS spectrum small-animal in vivo imaging system at Pohang Technopark

Biotech Center (Califer Lifescience, Hopkinton, MA).