Supplementary information

Targeted Theranostic Platinum(IV) Prodrug Containing a Luminogen with Aggregation-Induced Emission (AIE) Characteristics for In-Situ Monitoring of Drug Activation

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Experimental Section:

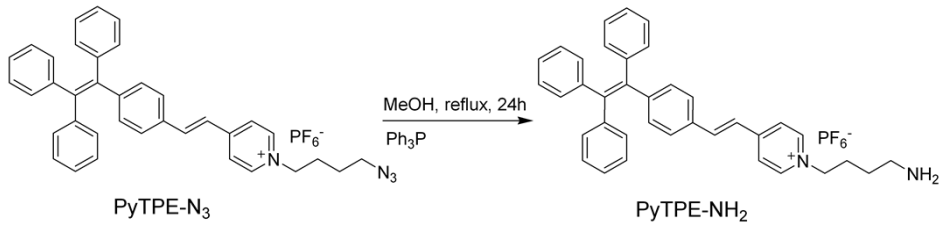
General Information. Cisplatin, *N*, *N*-diisopropylethylamine (DIEA), pentafluorophenol, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), ascorbic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), anhydrous dimethyl sulfate (DMSO), anhydrous dimethylformamide (DMF), disuccinimidyl suberate (DSS), triphenylphosphine (Ph_3P), diethyldithiocarbamate (DDTC), folate acid, glutamic acid, bovine serum albumin (BSA), lysozyme, pepsin, glutathione and other chemicals were all purchased from Sigma-Aldrich and used as received. Deuterated solvents with tetramethylsilane (TMS) as internal reference were purchased from Cambridge Isotope Laboratories, Inc.. Peptide with sequences of Asp-Asp-Asp-Asp-Asp-cyclic(Arg-Gly-Asp-D-Phe-Lys) (D5-cRGD) and Asp-Asp-Asp-Asp-Asp (D5) were customized from GL Biochem Ltd (Shanghai). 1-(4-Azidepropyl)-4-methylpyridinium hexafluorophosphate (PyTPE-N_3)[1] and *cis*, *cis*, *trans*-diamminedichlorodisuccinatoplatinum(IV)[2] were synthesized following the literature methods.

Dulbecco's Modified Essential Medium (DMEM) is a commercial product of National University Medical Institutes (Singapore). Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Breford, United States). 1× PBS contains NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), and KH_2PO_4 (1.8 mM). Fetal bovine serum (FBS) and trypsin-EDTA solution were purchased from Gibco (Life Technologies, AG, Switzerland).

Characterization. NMR spectra were measured on a Bruker ARX 400 NMR spectrometer. Chemical shifts were reported in parts per million (ppm) referenced with respect to residual solvent ($(CD_3)_2SO$ = 2.50 ppm or tetramethylsilane $Si(CH_3)_4$ = 0 ppm). The particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer

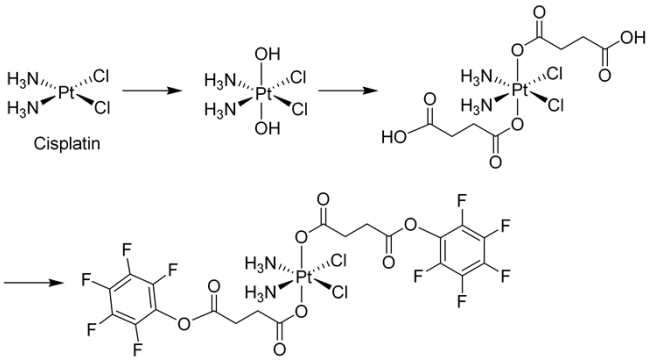
(90 Plus, Brookhaven Instruments Co., United States) at a fixed angle of 90° at room temperature. The HPLC profiles and mass spectra were acquired using a Shimadzu IT-TOF. A 0.1% TFA/H_2O and 0.1% TFA/acetonitrile were used as eluents for the HPLC experiments. High resolution mass spectra (HRMS) were recorded on a Finnigan MAT TSQ 7000 Mass Spectrometer. UV-vis absorption spectra were taken on a Milton Ray Spectronic 3000 array spectrophotometer. Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at 24 ± 1 °C.

Synthesis of 1-(4-Aminobutyl)-4-{2-[4-(1,2,2-triphenylvinyl)phenyl]vinyl}pyridinium hexafluorophosphate (PyTPE-NH_2). Solution of PyTPE-N_3 (339.1 mg, 0.5 mmol) and Ph_3P (786.9 mg, 3 mmol) in dry MeOH (30 mL) was refluxed under nitrogen for 24 h. After cooling to ambient temperature, the solvent was evaporated under reduced pressure. The residue was purified by a aluminum oxide gel column chromatography using dichloromethane and MeOH mixture (v/v = 5:1) as eluent to give PyTPE-NH_2 as a yellow solid (277 mg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.92 (d, J = 6.8 Hz, 2H), 8.18 (d, J = 6.8 Hz, 2H), 7.90 (t, J = 16.4 Hz, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.42 (d, J = 16.4 Hz, 1H), 7.11□7.18 (m, 9H), 7.06 (d, J = 8.4 Hz, 2H), 6.97-7.02 (m, 6H), 4.48 (t, J = 7.2 Hz, 2H), 2.57 (t, J = 6.8 Hz, 2H), 1.89-1.96 (m, 2H), 1.32-1.36 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 152.66, 145.39, 144.05, 142.79, 142.70, 142.55, 141.44, 140.21, 139.73, 133.08, 131.26, 130.77, 130.52, 130.42, 127.79, 127.68, 127.50, 126.68, 126.62, 126.58, 122.96, 59.35, 40.08, 28.01, 27.78. HRMS (MALDI-TOF): *m*/*z* 691.3774 [$(M+K)^+$, calcd: 691.2079].



Scheme S1. Synthetic route to PyTPE-NH_2.

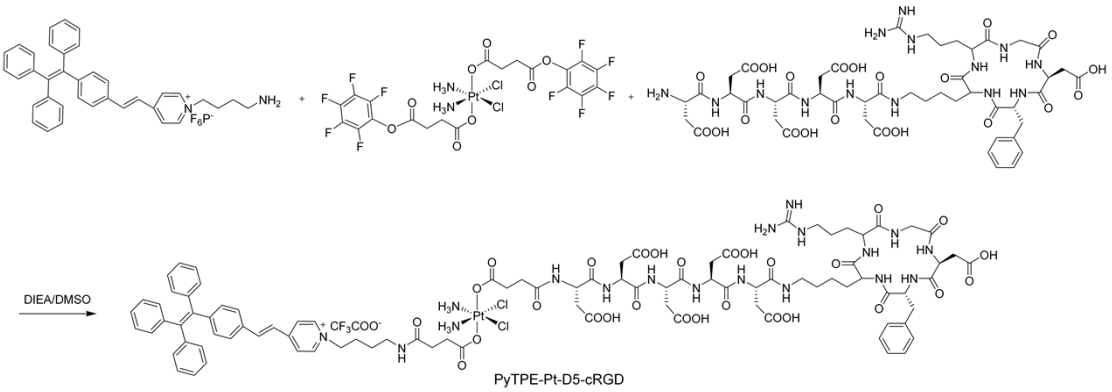
Synthesis of Pentafluorophenol-Activated Platinum(IV) Complexes. A mixture of platinum(IV) complex *cis*, *cis*, *trans*-diamminedichlorodisuccinatoplatinum(IV) (32.1 mg, 0.06 mmol), EDC (46.0 mg, 0.24 mmol) and pentafluorophenol (44.2 mg, 0.24 mmol) in anhydrous DMF (1 mL) was stirred at room temperature overnight. After that, the mixture was purified by RP-HPLC (solvent A: water with 0.1% TFA, solvent B: CH_3CN with 0.1% TFA) and lyophilized to yield the desired product as a white powder in 78% yield (34.1 mg).



Scheme S2. Synthesis of pentafluorophenol activated platinum(IV) complex.

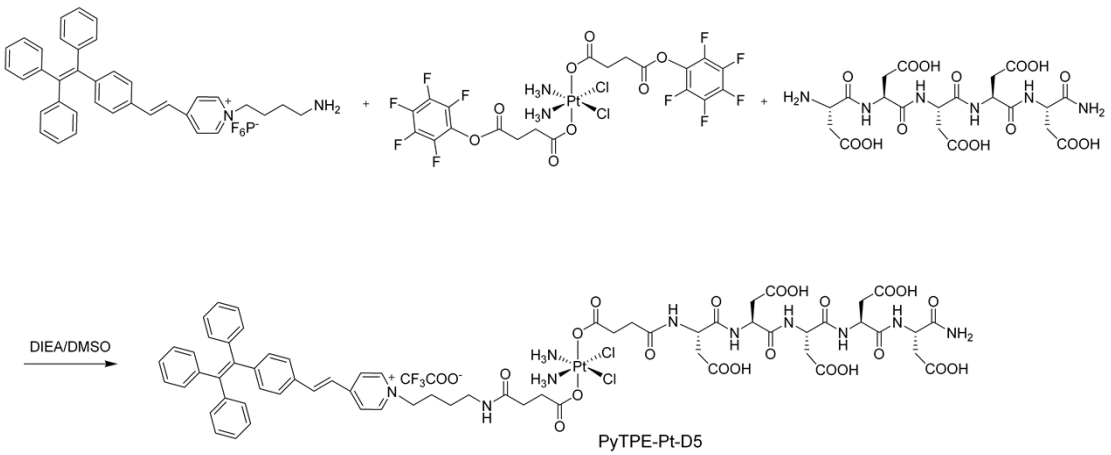
Synthesis and Purification of the Prodrug PyTPE-Pt-D5-cRGD. In a typical reaction, PyTPE-NH_2 (5.0 mg, 7.7 μmol) and amine-functionalized D5-cRGD (9.1 mg, 7.7 μmol) were dissolved in anhydrous DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then pentafluorophenol activated platinum(IV) complex (5.6 mg, 7.7 μmol) in anhydrous DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC (solvent A: water with 0.1% TFA,

solvent B: CH_3CN with 0.1% TFA) and lyophilized under vacuum to yield the prodrug as white powders in 42% yield (7.4 mg).



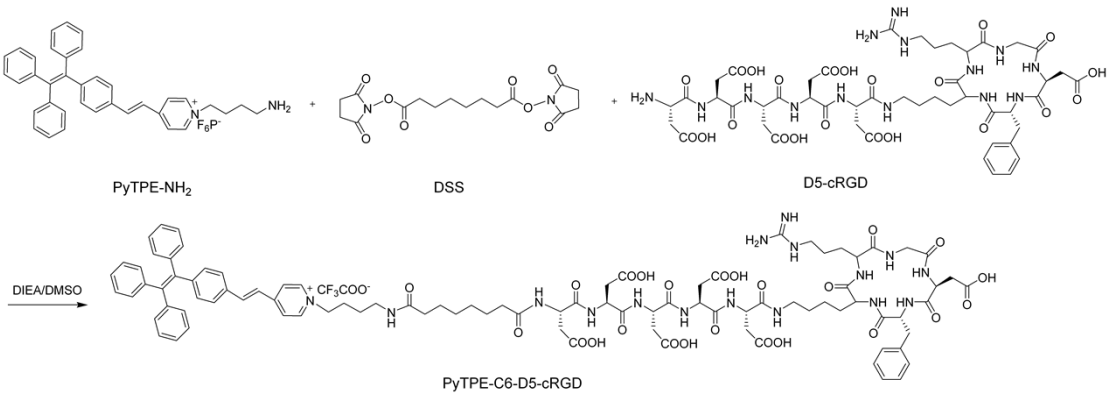
Scheme S3. Synthetic route to PyTPE-Pt-D5-cRGD.

Synthesis of Non-targetable Prodrug PyTPE-Pt-D5. Non-targetable prodrug PyTPE-Pt-D5 was prepared by a similar procedure but D5 was used instead of D5-cRGD. PyTPE-NH_2 (5.0 mg, 7.7 μmol) and D5 (4.6 mg, 7.7 μmol) were dissolved in anhydrous DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then pentafluorophenol activated platinum(IV) complex (5.6 mg, 7.7 μmol) in anhydrous DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC and lyophilized under vacuum to yield the prodrug as a white powder in 44% yield (6.1 mg).



Scheme S4. Synthetic route to PyTPE-Pt-D5.

Synthesis of the PyTPE-C6-D5-cRGD. PyTPE-C6-D5-cRGD was prepared as control by a similar procedure as PyTPE-Pt-D5-cRGD using DSS as a coupling reagent. PyTPE-NH_2 (5.0 mg, 7.7 μmol) and D5-cRGD (9.1 mg, 7.7 μmol) were dissolved in anhydrous DMSO (0.5 mL) with a catalytic amount of DIEA (1.0 μL) and the mixture was stirred at room temperature for 10 min. Then DSS (2.8 mg, 13.9 μmol) in anhydrous DMSO (0.5 mL) was added quickly to the above solution. The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by prep-HPLC and lyophilized under vacuum to yield the product as white powders in 46% yield (6.8 mg).



Scheme S5. Synthetic route to PyTPE-C6-D5-cRGD.

General Procedure for Prodrug Activation. DMSO stock solutions of PyTPE-Pt-D5-

cRGD, PyTPE-Pt-D5 and PyTPE-C6-D5-cRGD were diluted with a mixture of DMSO and PBS (v/v = 1/199). Next, each prodrug was incubated with ascorbic acid at room temperature and the change of fluorescence intensity was measured. The solution was excited at 405 nm, and the emission was collected from 525 to 750 nm.

Cell Culture. Human breast cancer cells MDA-MB-231 and MCF-7 were provided by American Type Culture Collection (ATCC). The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific) and maintained in a humidified incubator at 37 °C with 5% CO_2. Before experiment, the cells were precultured until confluence was reached.

Confocal Imaging. Human breast cancer cells MDA-MB-231 and MCF-7 were cultured in the chambers (LAB-TEK, Chambered Coverglass System) at 37 °C. After 80% confluence, the culture medium was removed and washed twice with 1× PBS buffer. The prodrug in DMSO stock solution was then added to the chamber to reach a final concentration of 10 μM. In some experiments, the cells were pre-incubated with media containing cRGD (50 μM) for 2 h prior to prodrug incubation. After incubation at 37 °C for different time, the cells were washed twice with ice-cold PBS and the cell nucleus was living stained with Hoechst 33342 (Life Technologies) following the standard protocol of the manufacturer. The cells were then imaged immediately by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany). The images were analyzed by Image J1.43 × program (developed by NIH, http://rsbweb.nih.gov/ij/).

Cytotoxicity of the Prodrug. 3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were used to assess the metabolic activity of MDA-MB-231 and MCF-7 cells.

The cells were seeded in 96-well plates (Costar, IL, United States) at an intensity of 4×10^4 cells mL^{-1} and incubated at 37 °C for 24 h. After incubating with the prodrug suspension at different concentrations for 6 h, the medium was replaced with the fresh one. After incubation for 72 h, the wells were washed twice with 1× PBS buffer, and 100 μL of freshly prepared MTT (0.5 mg mL^{-1}) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator at 37 °C. DMSO (100 μL) was then added into each well and the plate was gently shaken to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio of absorbance of the cells incubated with probe suspension to that of the cells incubated with culture medium only.

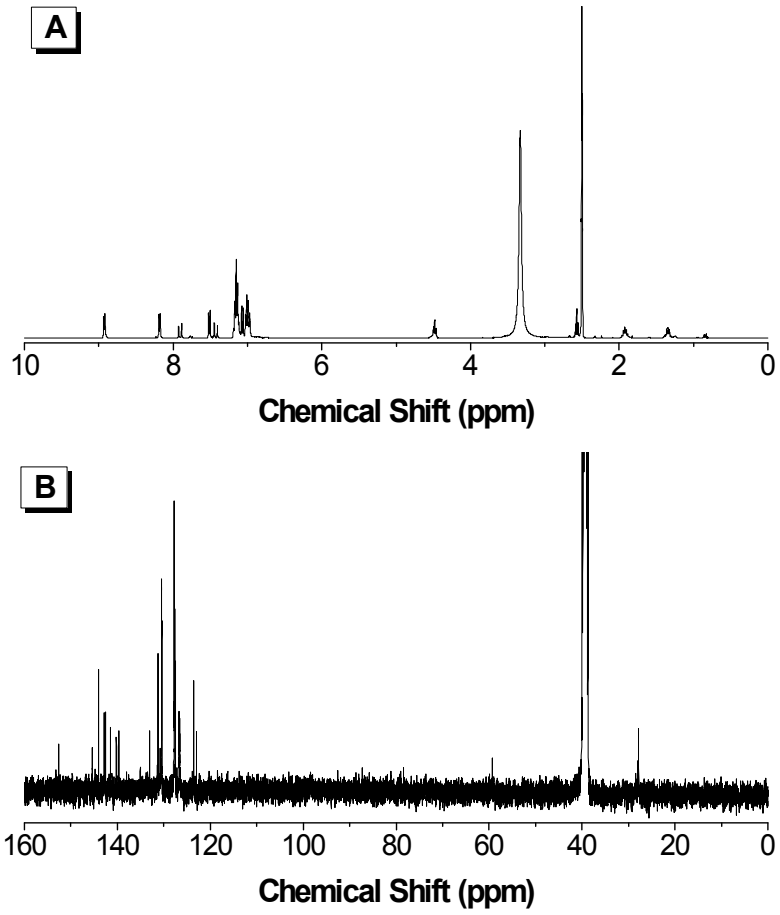


Figure S1. ^{1}H (A) and ^{13}C (B) NMR spectra of PyTPE-NH_2 in DMSO-d_6.

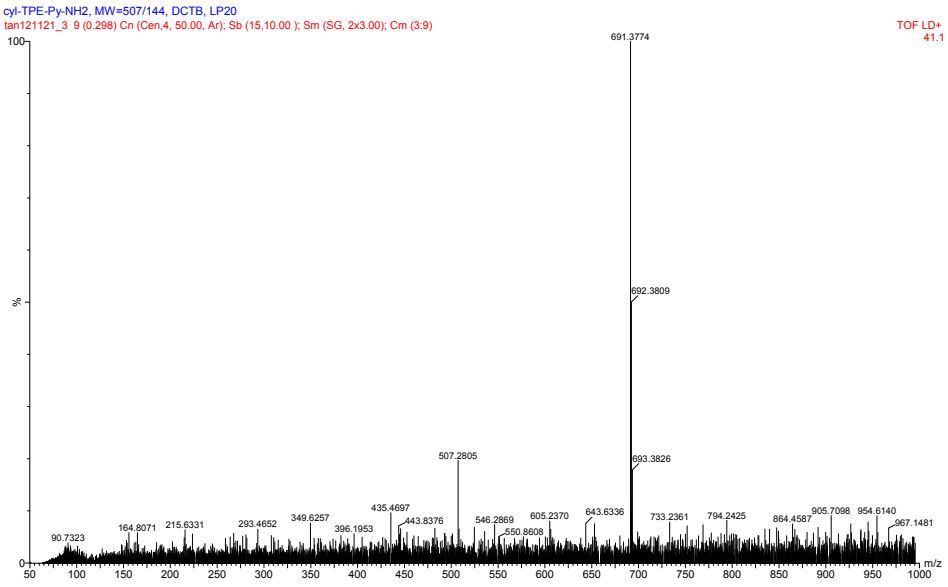


Figure S2. High resolution mass spectrum (MALDI-TOF) of PyTPE-NH_2.

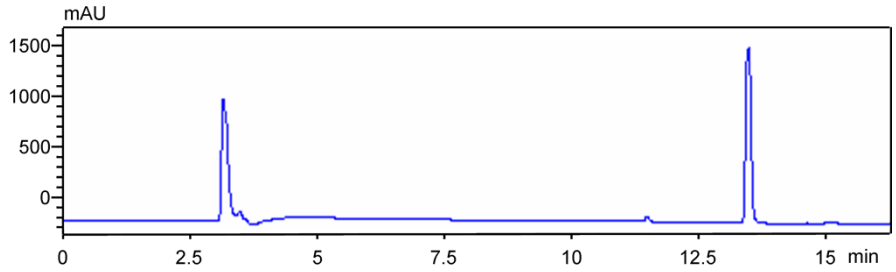


Figure S3. HPLC spectrum of pentafluorophenol activated platinum(IV) complex at absorbance of 214 nm.

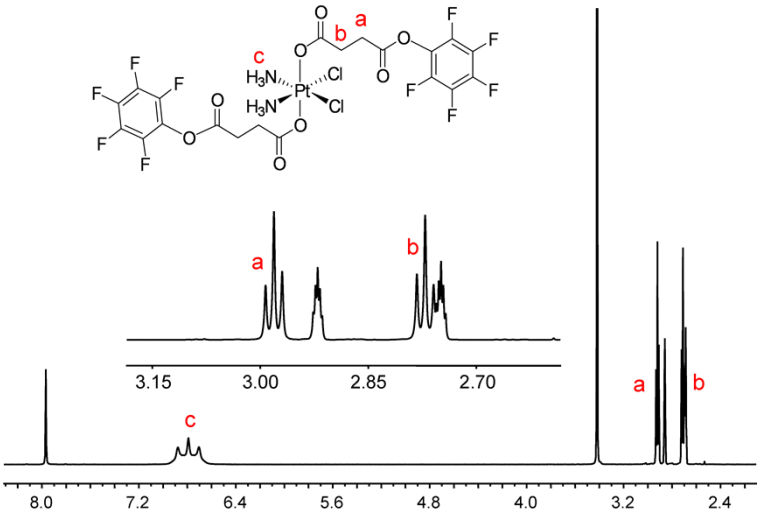


Figure S4. ^{1}H NMR spectrum of pentafluorophenol activated platinum(IV) complex in DMF-d_7. An expanded view is inserted in the figure.

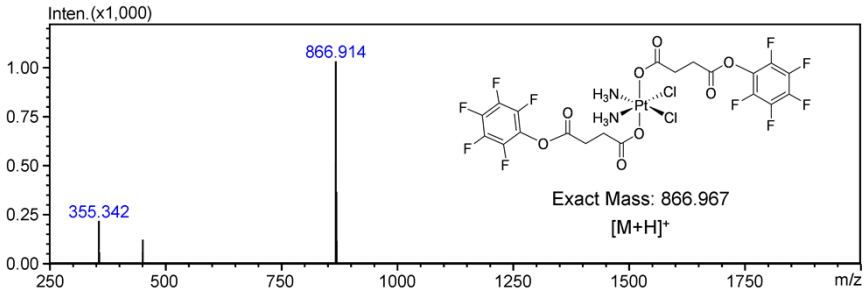


Figure S5. Mass spectrum (IT-TOF) of pentafluorophenol activated platinum(IV) complex.

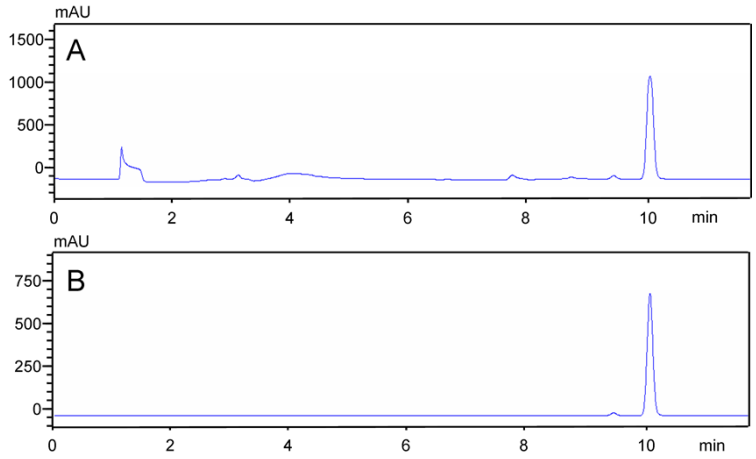


Figure S6. HPLC spectra of PyTPE-Pt-D5-cRGD monitored with absorbance at 214 nm (A) and 365 nm (B).

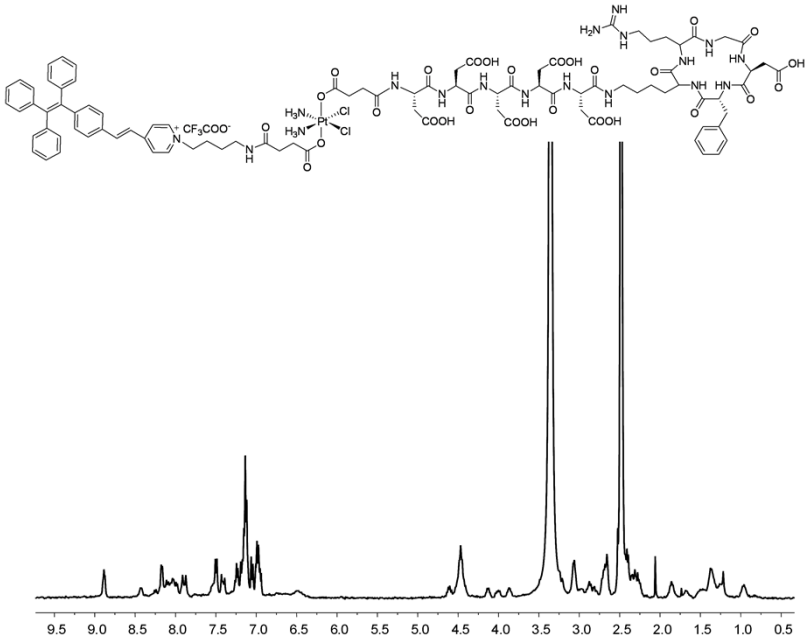


Figure S7. ^{1}H NMR spectrum of PyTPE-Pt-D5-cRGD in DMSO-d_6.

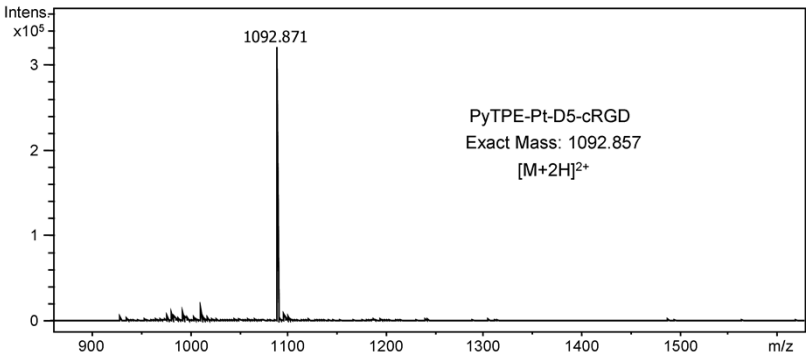


Figure S8. Mass spectrum (ESI) of PyTPE-Pt-D5-cRGD.

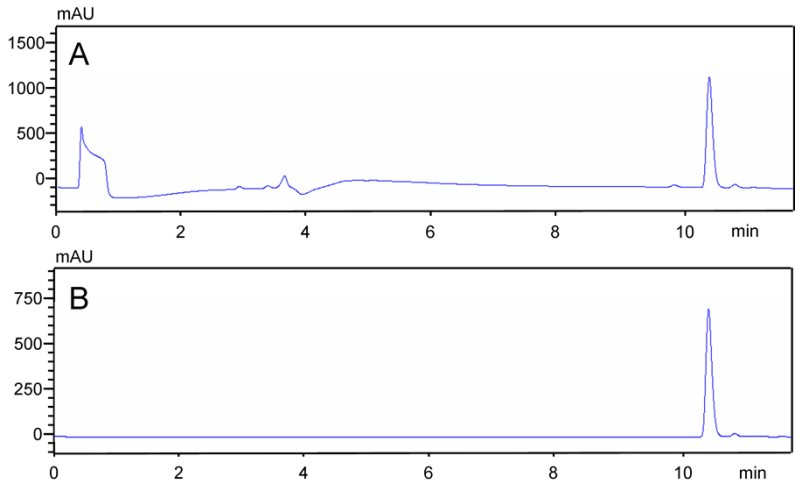


Figure S9. HPLC spectra of PyTPE-Pt-D5 monitored with absorbance at 214 nm (A) and 365 nm (B).

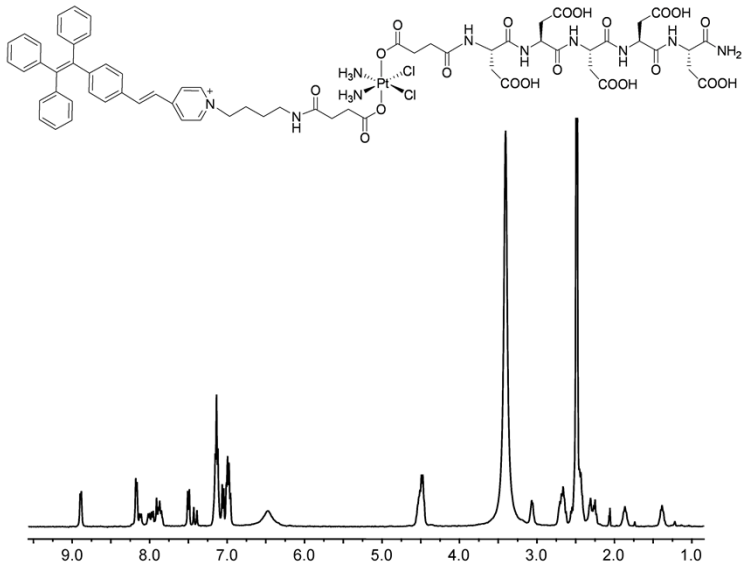


Figure S10. ^{1}H NMR spectrum of PyTPE-Pt-D5 in DMSO-d_6.

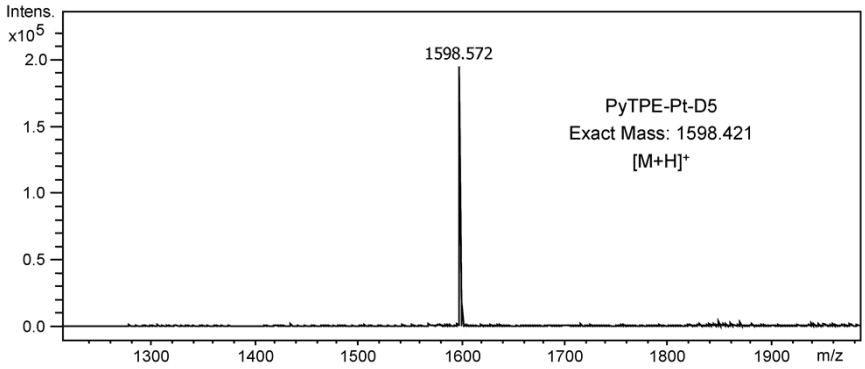


Figure S11. Mass spectrum (ESI) of PyTPE-Pt-D5.

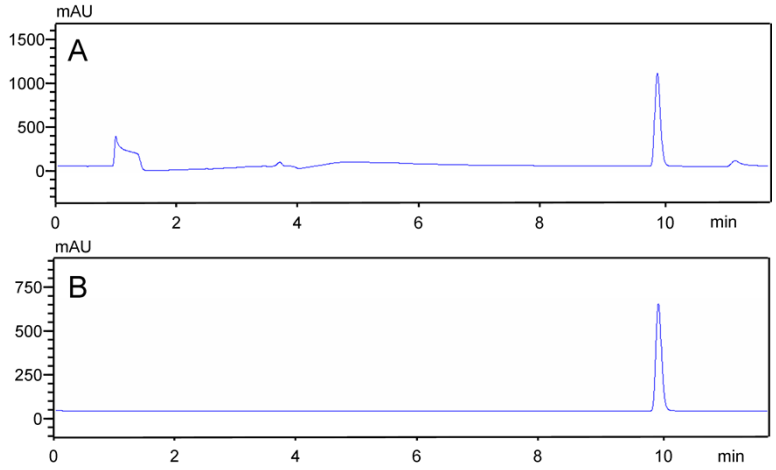


Figure S12. HPLC spectra of PyTPE-C6-D5-cRGD monitored with absorbance at 214 nm (A) and 365 nm (B).

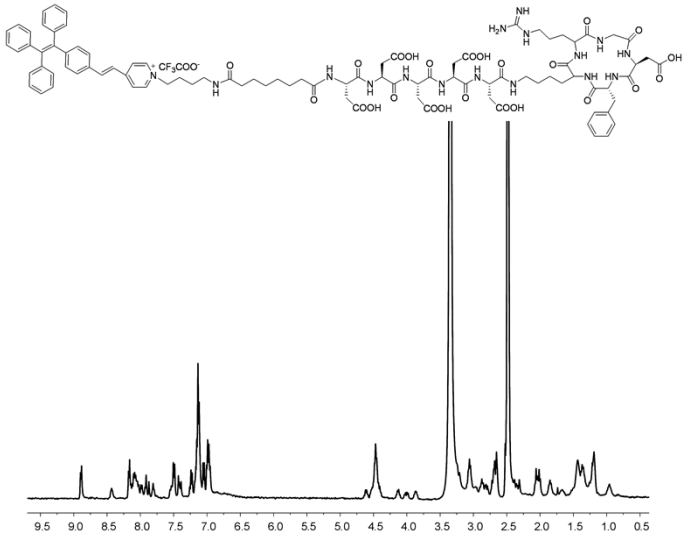


Figure S13. ^{1}H NMR spectrum of PyTPE-C6-D5-cRGD in DMSO-d_6.

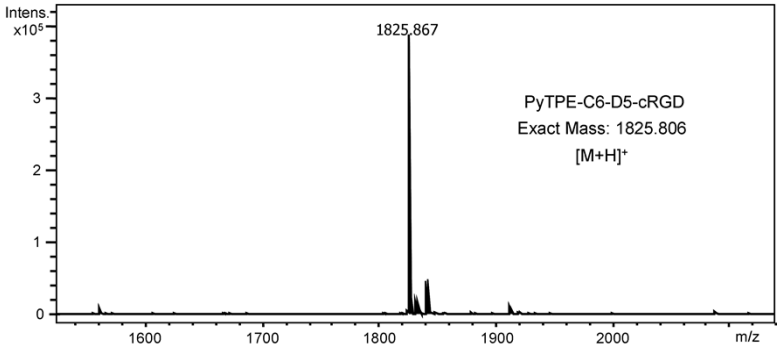


Figure S14. Mass spectrum (ESI) of PyTPE-C6-D5-cRGD.

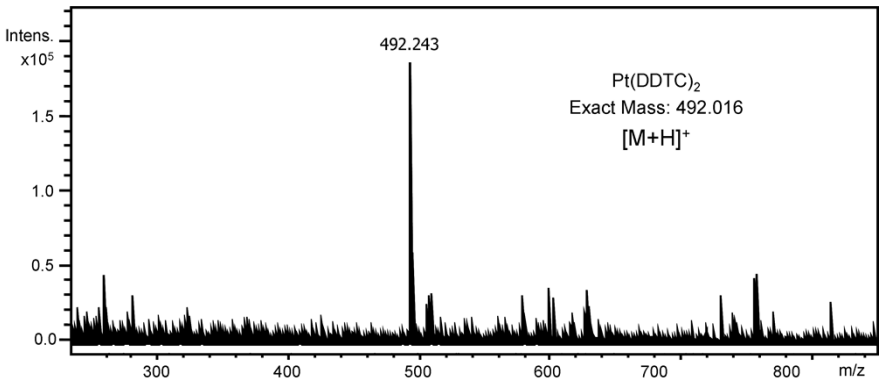
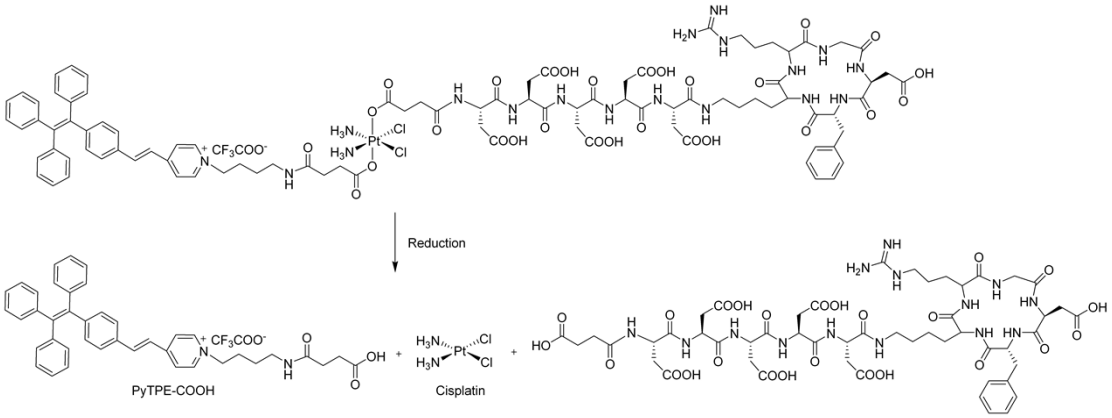


Figure S15. Mass spectrum (ESI) of $Pt(DDTC)_2$ formed from the adduction of DDTC with the reduced prodrug.



Scheme S6. The reduction mechanism of PyTPE-Pt-D5-cRGD.

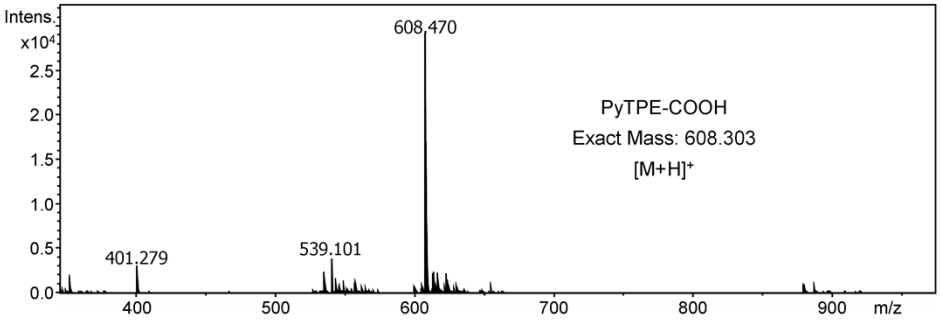


Figure S16. Mass spectrum (ESI) of PyTPE-COOH.

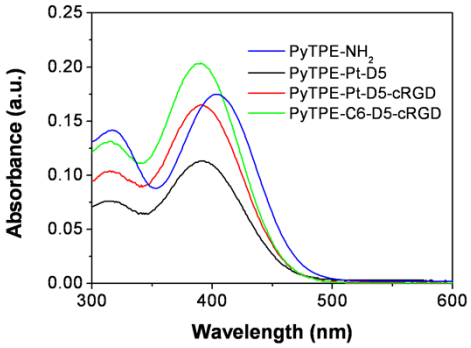


Figure S17. (A) UV-vis absorption spectra of PyTPE-NH_2 (in THF) and PyTPE-Pt-D5, PyTPE-Pt-D5-cRGD and PyTPE-C6-D5-cRGD (in DMSO/PBS (v/v = 1/199)).

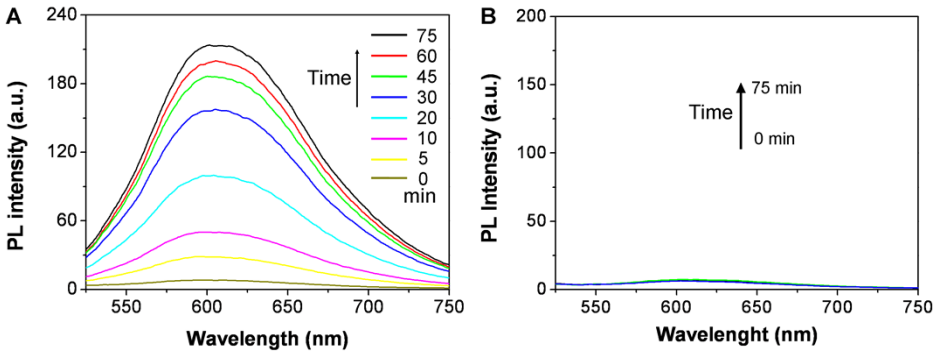


Figure S18. Time-dependent fluorescence spectra of PyTPE-Pt-D5 (A) and PyTPE-C6-D5-cRGD (B) (10 μM) upon treatment with ascorbic acid (1 mM) in DMSO/PBS (v/v = 1/199).

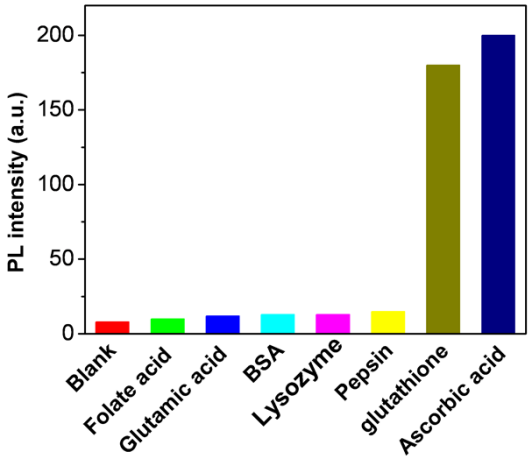


Figure S19. Fluorescence response of PyTPE-Pt-D5-cRGD (10 μM) toward 1.0 mM of folate acid, glutamic acid, bovine serum albumin (BSA), lysozyme, pepsin, glutathione or ascorbic acid in DMSO/PBS (v/v = 1/199).

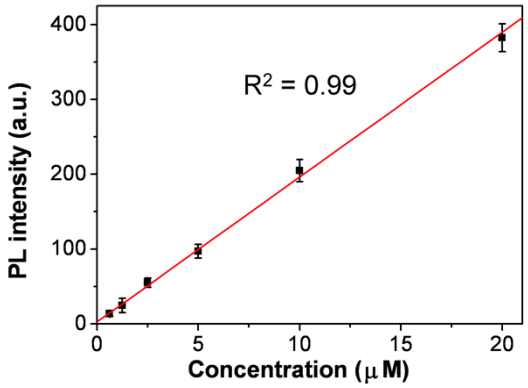


Figure S20. Plot of PL intensity at 605 nm versus concentrations of PyTPE-Pt-D5 with the incubation of ascorbic acid (1 mM) in DMSO/PBS (v/v = 1/199). Data represent mean values ± standard deviation, n = 3.

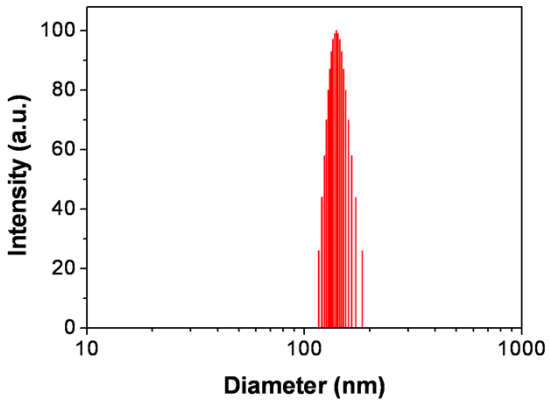


Figure S21. Hydrodynamic diameters of the residue of PyTPE-Pt-D5-cRGD (10 μM) after the reduction with ascorbic acid (1 mM) in DMSO/PBS (v/v = 1/199) obtained from LLS.

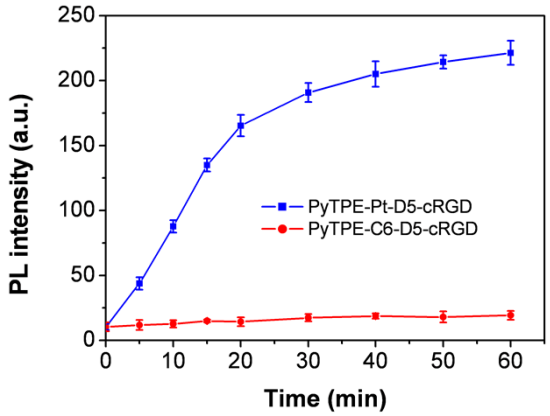


Figure S22. Time-dependent PL intensity at 605 nm of PyTPE-Pt-D5-cRGD (10 μM) incubated with MDA-MB-231 cell lysate. Data represent mean values ± standard deviation, n = 3.

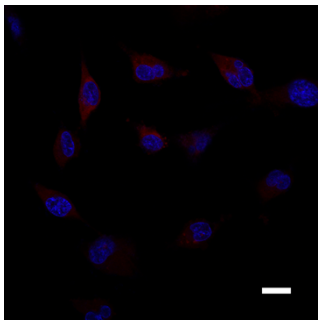


Figure S23. CLSM images of free cRGD (50 μM) pretreated MDA-MB-231 cells upon incubation with PyTPE-Pt-D5-cRGD (10 μM) for 6 h. Nuclei were stained with Hoechst 33342. The scale bar is 20 μm.

References

[1] H. B. Shi, N. Zhao, D. Ding, J. Liang, B. Z. Tang, B. Liu, *Org. Biomol. Chem.* 2013, **11**, 7289-7296.

[2] M. R. Reithofer, S. M. Valiahdi, M. A. Jakupec, V. B. Arion, A. Egger, M. Galanski, B. K. Keppler, *J. Med. Chem.* 2007, **50,** 6692−6699.