Supplementary Information for

Nanoscale Metal-organic Frameworks for the Co-delivery of Cisplatin and Pooled siRNAs to Enhance Therapeutic Efficacy in Drug-resistant Ovarian Cancer Cells

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1. Materials and cell lines

All of the starting materials were purchased from Sigma-Aldrich and Fisher (USA), unless otherwise noted, and used without further purification. The siRNA duplexes were

supplied by Dharmacon (USA) and dissolved in diethylpyrocarbonate (DEPC)-treated water before use. Survivin siRNA, Bcl-2 siRNA, and P-gp siRNA contained the anti-sense sequences of 5'-GGACCACCGCAUCUCUACAdTdT-3', 5'-UUCGGCAUUAGGCCUUCCGdTdG-3', and 5'-AGCTTATAATGGATGTACT-3', respectively. TAMRA-labeled survivin siRNA was bought from Dharmacon (USA) and used for quantification and confocal laser scanning microscopy (CLSM) studies.

SKOV-3 cells (human ovarian cancer cells) were provided by the American Type Culture Collection (Rockville, MD, USA) and were cultured in McCoy's 5a medium containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA). MCF-7 cells (human breast cancer cells) and Raw 264.7 cells (murine macrophage) were provided by the American Type Culture Collection and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS. PC-3 cells (human prostate cancer cells) and H460 cells (human lung cancer cells) were provided by the American Type Culture Collection and were cultured in RPMI 1640 medium containing 10% FBS. Cisplatin sensitive and resistant human ovarian cancer cells A2780 and A2780/CDDP were obtained from Developmental Therapeutics Core, Northwestern University and were cultured in RPMI 1640 containing 10% FBS.

2. Synthesis of UiO nanoscale metal-organic framework and drug loading

2.1 Synthesis of amino-triphenyldicarboxylic acid (amino-TPDC)

Amino-TPDC was synthesized as described previously.[1] 2,5-dibromoaniline (2.00 g, 8.0 mmol), 4-(methoxycarbonyl)-phenylboronic acid (4.40 g, 24.5 mmol) and CsF (5.82 g, 38 mmol) were suspended in 50 mL of anhydrous tetrahydrofuran (THF) under nitrogen protection in a 100 mL round-bottom flask. $Pd(OAc)_2$ (0.60 g, 2.7 mmol) and PPh_3 (1.61 g, 6.1 mmol) were then added. The mixture was heated at 50 °C for 48 h. The product was purified by water/dichloromethane extraction and silica gel column chromatography (dichloromethane: ethyl ether = 50:1 with 0.2% - 0.5% triethylamine). Yield: 58%. ^{1}H NMR (Chloroform-D): δ=8.10 (m, 4H), 7.65 (d, 2H), 7.57 (d, 2H), 7.22 (d, 1H), 7.09 (d, 1H), 7.01 (s, 1H), 3.93 (two overlapping singlets, 6H), 3.88 (s, 2H).

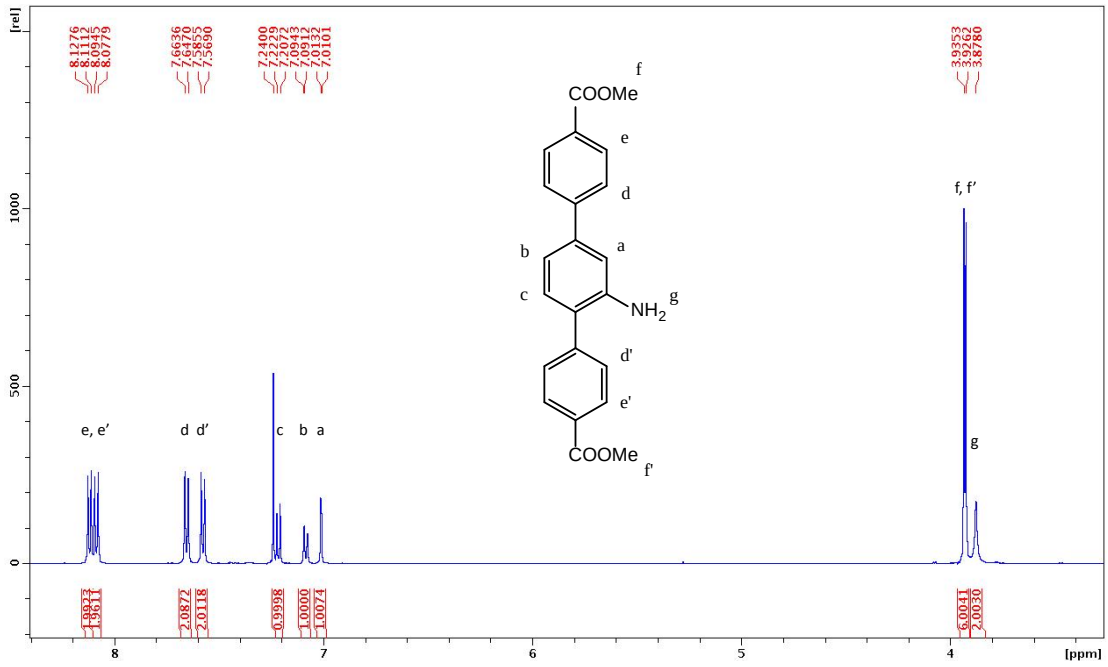


Figure S1. ^{1}H NMR spectrum of amino-triphenyldicarboxy methyl ester in Chloroform-d.

The amino-triphenyldicarboxyl methyl ester from above (1.68 g, 4.65 mmol) was suspended in 200 mL of THF and heated to 40 °C. To the suspension 100 mL of 5.5 M KOH methanol solution was added and the resulting mixture was stirred at 40 °C for 18 hours. A white solid was collected by centrifugation, and then treated with 12 mL of trifluoroacetic acid in 100 mL of THF at room temperature for 2 h. The yellow solid product (amino-TPDC) was isolated by vacuum filtration and washed with THF, methanol and ether. Yield: 80%. ^{1}H NMR (DMSO-d6): δ=12.97 (br, 2H), 8.03 (m, 4H), 7.74 (d, 2H), 7.61 (d, 2H), 7.16 (d, 2H), 7.02 (dd, 1H), 5.12 (br, 2H). ^{13}C NMR (DMSO-d6): δ=167.66, 167.63 (COOH), 146.24 ($C_{1'}$), 145.00 ($C_{1''}$), 144.25 (C_1), 139.96 ($C_{4'}$), 131.31 ($C_{6'}$), 130.40, 130.28 ($C_{3''}$, C_3), 129.98, 129.54 ($C_{4''}$, C_4), 129.19 ($C_{2''}$), 126.97 (C_2), 125.04 ($C_{2'}$), 115.96 ($C_{5'}$), 114.26 ($C_{3'}$).

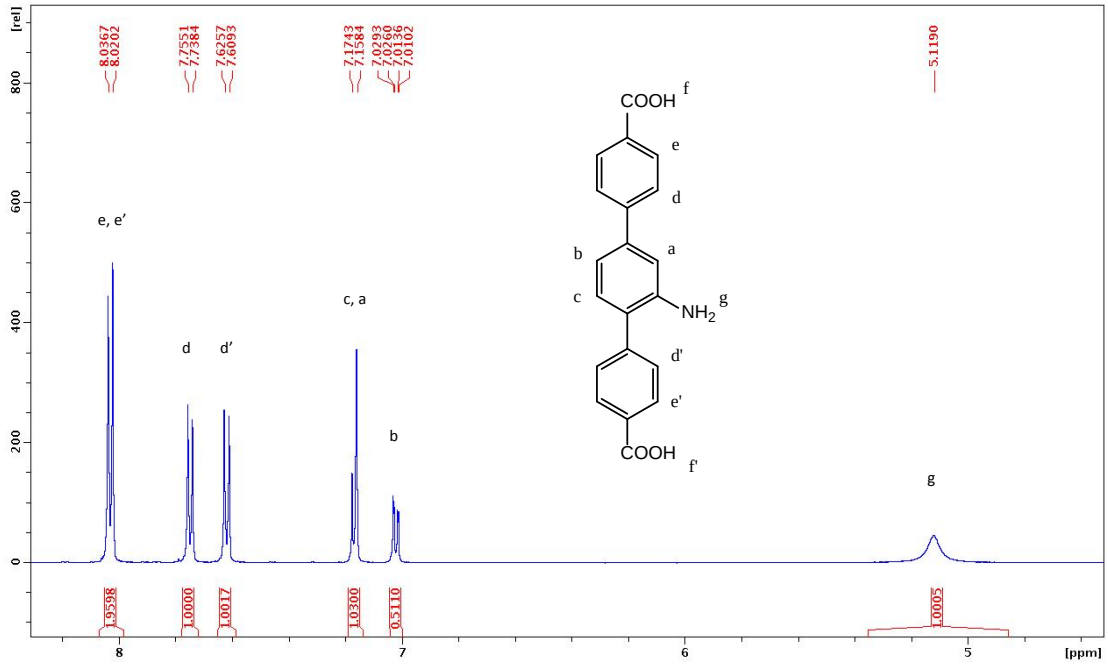


Figure S2. ^{1}H NMR spectrum of amino-TPDC in DMSO-d6.

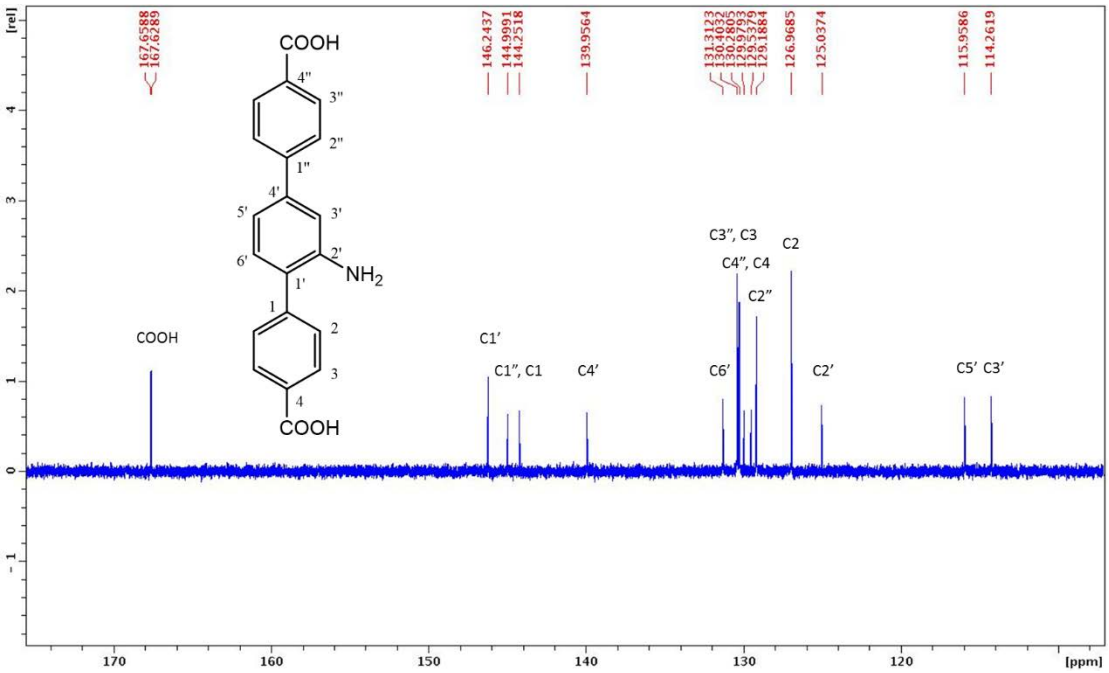
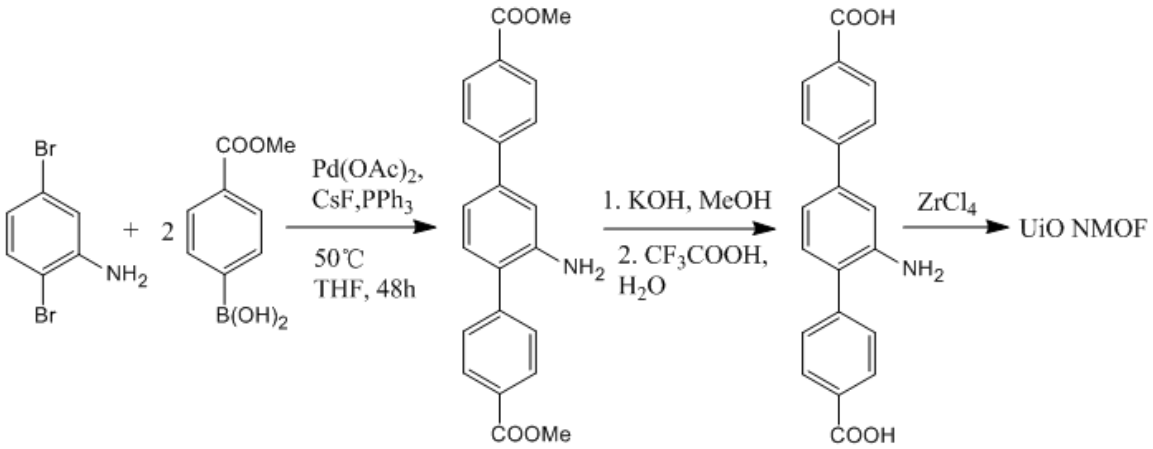
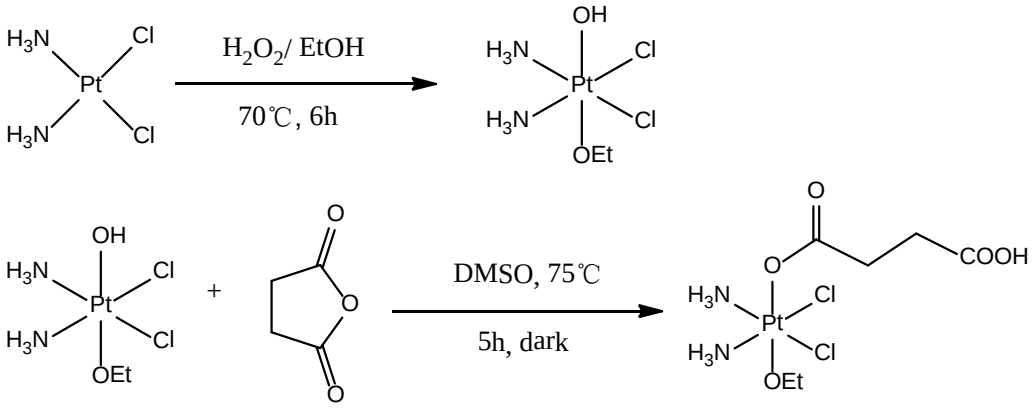


Figure S3. ^{13}C NMR spectrum of amino-TPDC in DMSO-d6.



Scheme S1. Synthesis of the amino-TPDC ligand and UiO NMOF.

2.2. Synthesis of cis, cis, trans-[$Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$]



Scheme S2. Synthesis of the cisplatin prodrug cis, cis, trans-[$Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$].

A modified procedure from a previous report was used.[2] Cisplatin (445 mg, 1.48 mmol) was suspended in 240 mL of ethanol. To the suspension 1.00 mL of 30% hydrogen peroxide solution was added. The reaction mixture was heated at 70 °C in the dark for 6 h. A yellow product of $Pt(NH_3)_2Cl_2(OH)(OEt)$ was obtained in 76% yield after removal of the solvent in vacuo. ^{1}H NMR (DMSO-d6): δ=5.55 (m, 6H), 3.50 (tetra, 2H), 1.05 (t, 3H).

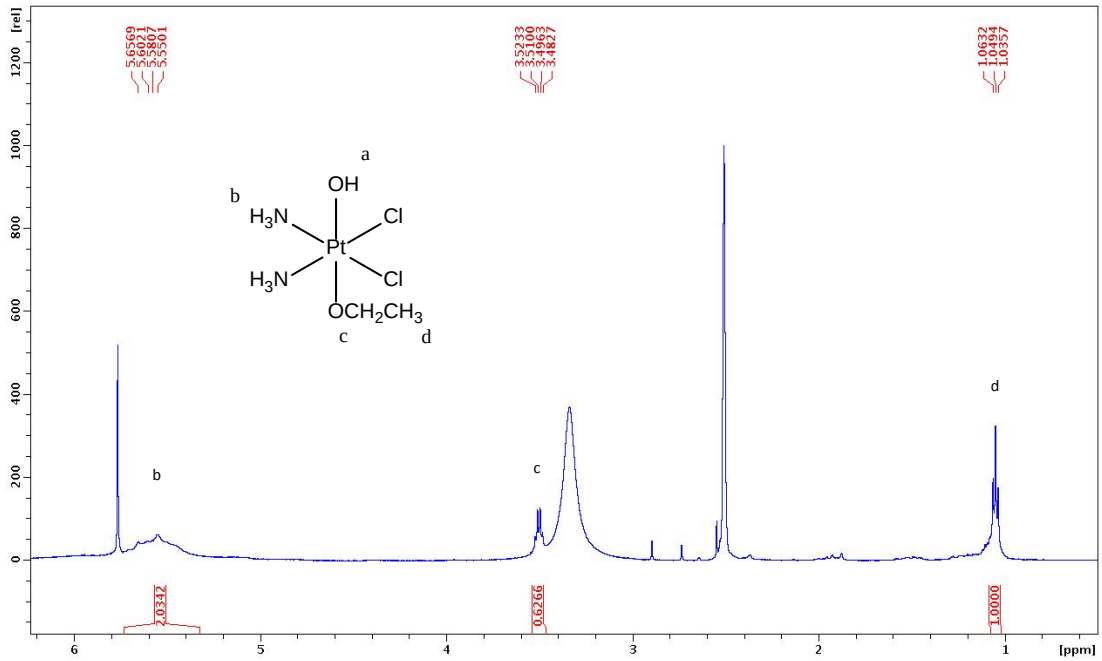
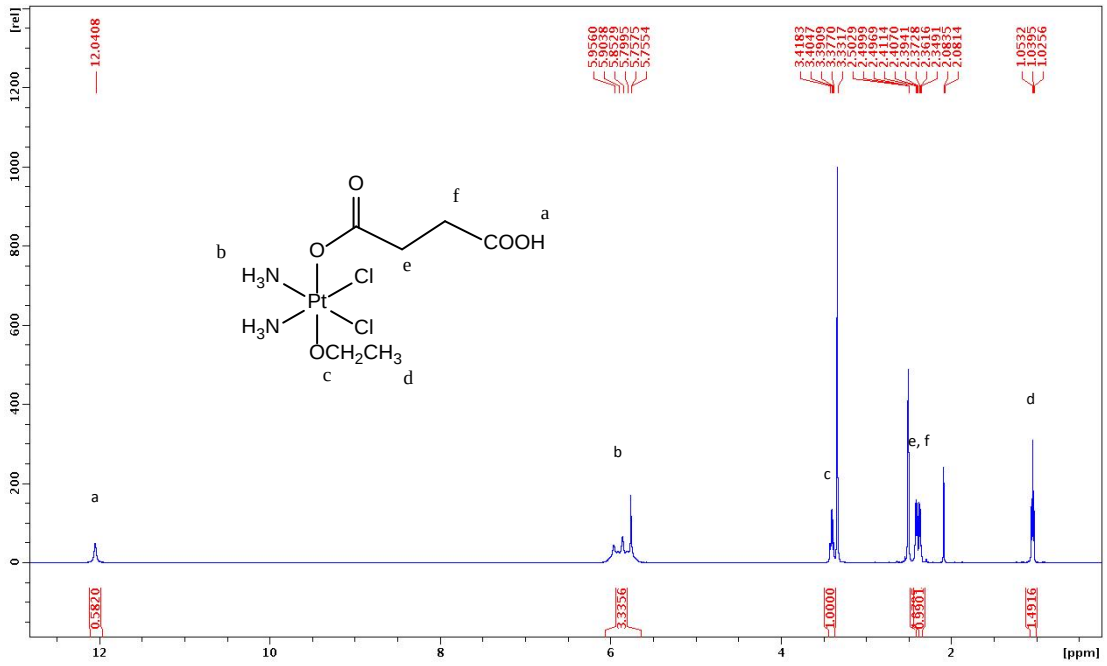


Figure S4 ^{1}H NMR spectrum of $Pt(NH_3)_2Cl_2(OH)(OEt)$ in DMSO-d6.

Succinic anhydride (298 mg, 2.98 mmol) and $Pt(NH_3)_2Cl_2(OH)(OEt)$ (107 mg, 0.30 mmol) was dissolved in 3 mL of anhydrous dimethylsulfoxide (DMSO) after drying in vacuo. The mixture was stirred in the dark at 65 °C overnight. The solution volume was reduced under vacuum and the product was precipitated by adding dichloromethane, and then recrystallized in acetone and ethyl ether. Yield: 53%. ^{1}H NMR (DMSO-d6): δ=12.04 (br, 1H), 5.86 (m, 6H), 3.40 (tetra, 2H), 2.41 (m, 2H), 2.37 (m, 2H), 1.05 (t, 3H). ^{13}C NMR (DMSO-d6): δ=180.49, 174.56 (carboxylate), 67.04 (OCH_2), 32.05, 30.67 (-CH_2CH_2-), 17.17 (CH_3). ^{195}Pt NMR (DMSO-d6): δ= 1001.7 (quintet).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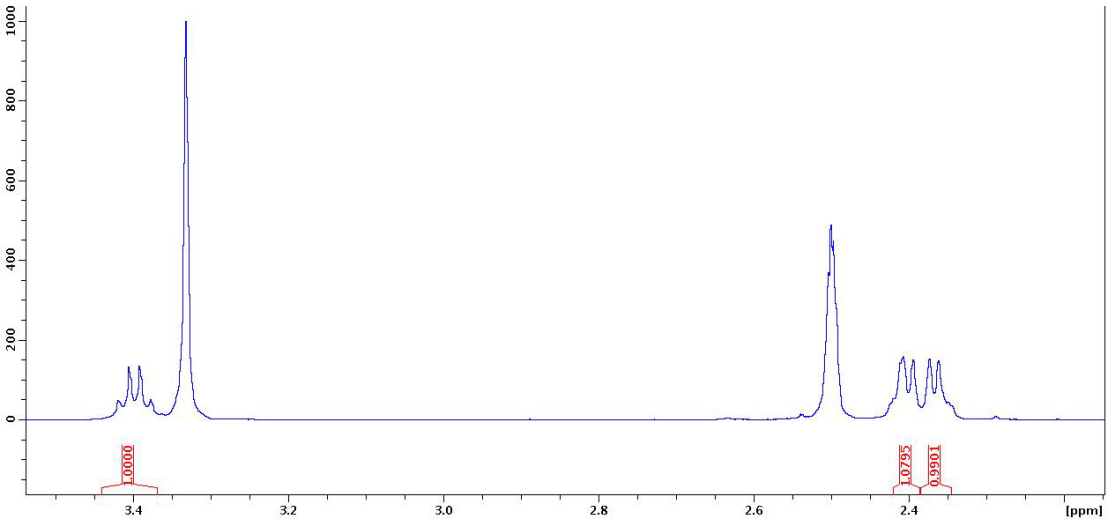
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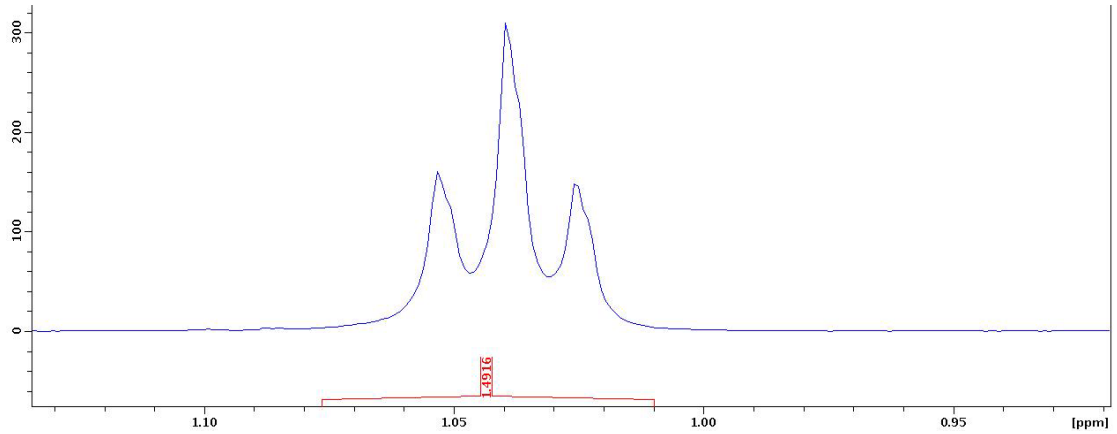

Figure S5 Top, ^{1}H NMR spectrum of $Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$ in DMSO-d6. Middle, an expanded view of a portion of the spectrum. Bottom, another expanded view of a portion of the spectrum.

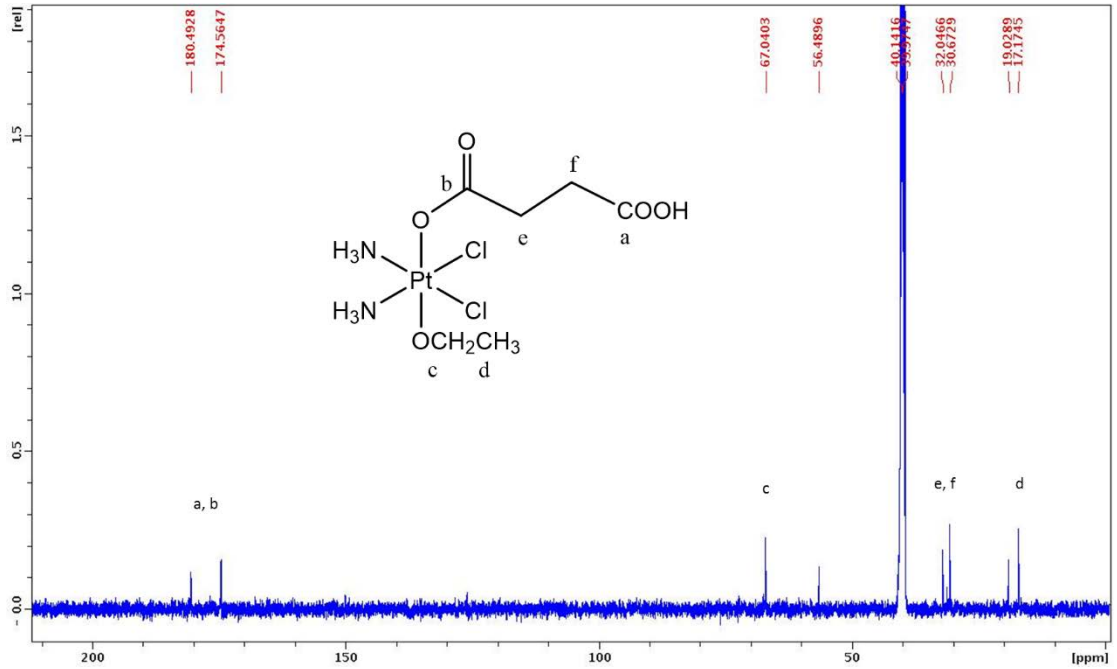

Figure S6 ^{13}C NMR spectrum of $Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$ in DMSO-d6.

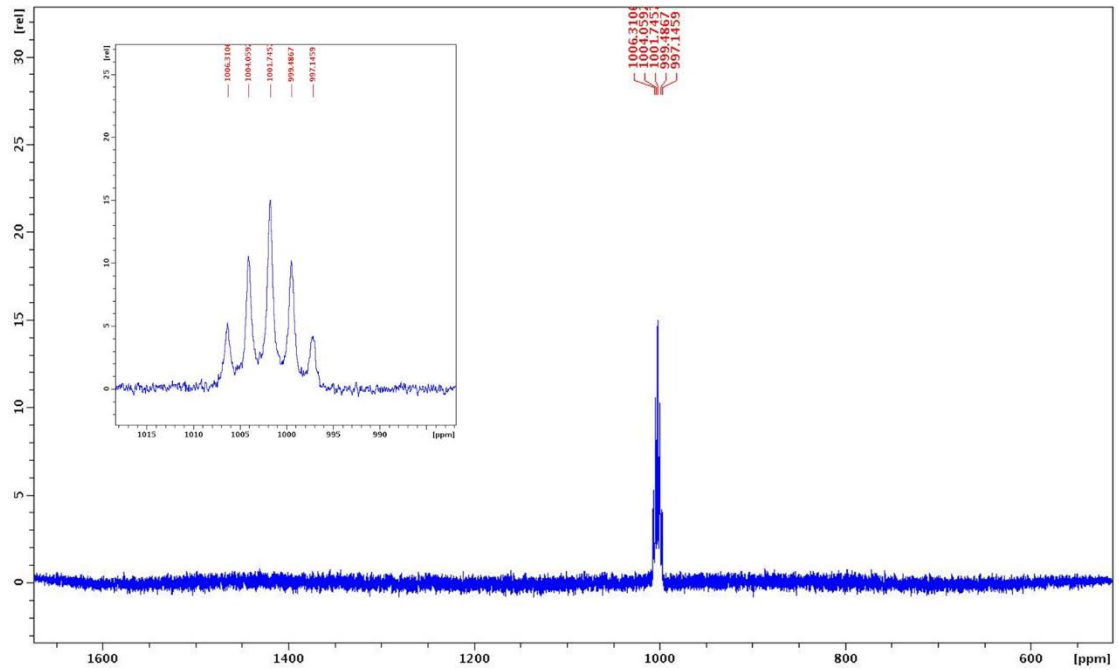

Figure S7 ^{195}Pt NMR spectrum of $Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$ in DMSO-d6. The coupling to two equivalent ^{14}N nuclei splits the peak to quintet, coupling constant $^1J(^{195}Pt\text{-}^{14}N)$=197.0.

2.3. Synthesis of the UiO nanoscale metal-organic framework (NMOF)

DMF solutions of $ZrCl_4$ (3 mL, 1.4 mg/mL, 18 μmol) and amino-TPDC (3 mL, 2 mg/mL, 18 μmol) were added to a 20 mL glass vial and the mixture was diluted to 10 mL, followed by adding 750 μL of acetic acid. The mixture was kept in an 80 °C oven for 5 days. The product was collected by centrifugation and washed with DMF, 5% triethylamine ethanolic solution and ethanol, yielding the UiO NMOF with a light yellow color (Yield: ~20%).

The particle size of UiO was determined to be 98±11 nm (PDI = 0.070) by dynamic light scattering (DLS, Nano-ZS, Malvern, UK) measurement as shown in Fig. S8.

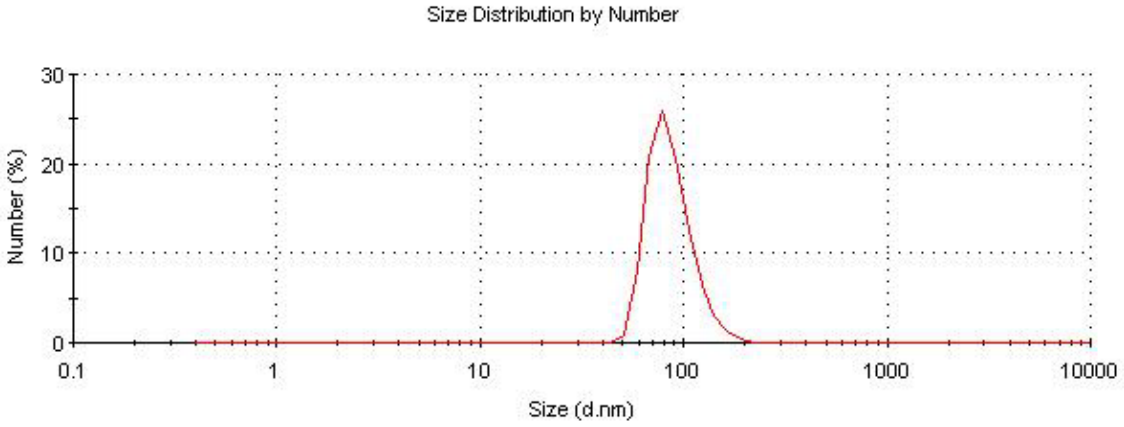

Figure S8 DLS plot showing the particle size of UiO.

2.4. Post-synthetic encapsulation of a cisplatin prodrug

Cis, cis, trans-$Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2COOH)$ (5.0 mg, 0.011 mmol) were dissolved in 2 mL of anhydrous DMF. To this solution 10.7 mg of UiO was suspended. The resulting mixture was stirred at room temperature for 36 hours. The prodrug-loaded UiO (UiO-Cis) was collected by centrifugation and washed with copious amounts of DMF and ethanol. The cisplatin loading was determined to be (12.3±1.2)% by inductively coupled plasma mass spectrometry (ICP-MS). The morphology of UiO-Cis was observed by high-resolution transmission electron microscopy (TEM, Tecnai F30, FEI, USA) (Fig. S9).

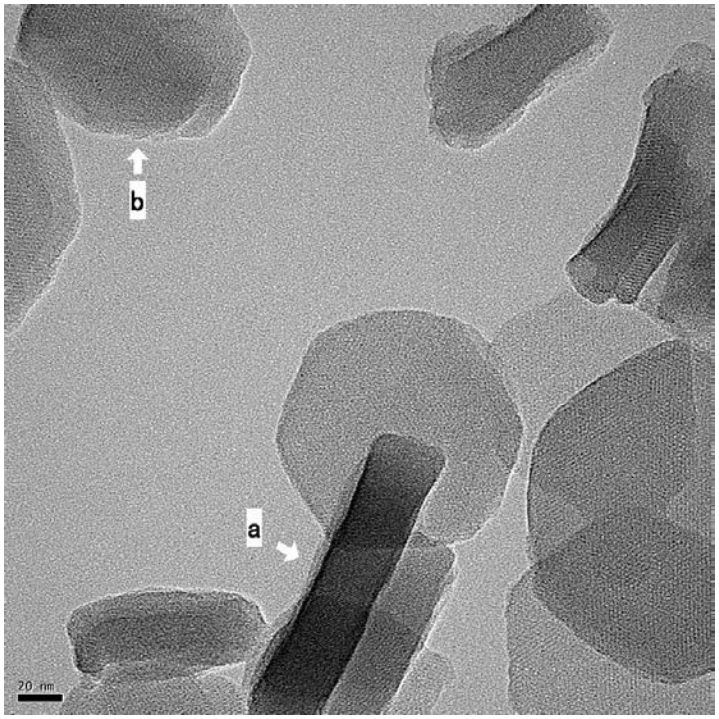

Figure S9 High-resolution TEM image of UiO-Cis. Particles "standing" perpendicular to the observation plane (a) and "leaning" on other particles (b) are observed. Bar represents 20 nm.

The particle size of UiO-Cis was determined to be 103±17 nm (PDI = 0.124) by DLS measurement as shown in Fig. S10.

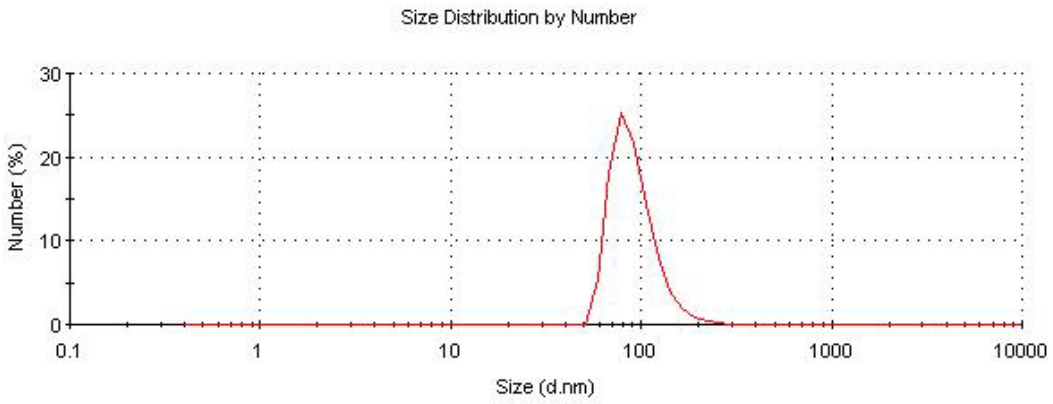

Figure S10 DLS plot showing the particle size of UiO-Cis.

The non-covalent encapsulation of cisplatin prodrug is supported by ^{1}H-NMR by digesting UiO-Cis in sodium phosphate (tribasic) saturated D_2O/DMSO-d6 (Fig. S11). The ratio of amino-TPDC ligand (δ=7.67, 7.33, 7.12, 6.88, 6.82 and 6.78) to prodrug (δ=3.19, 2.40, 2.36, 0.92) is about 3:1. Amino-TPDC may tend to be trapped by zirconium phosphate produced during digestion, which leads to a concentration bias in comparison of ICP-MS results.

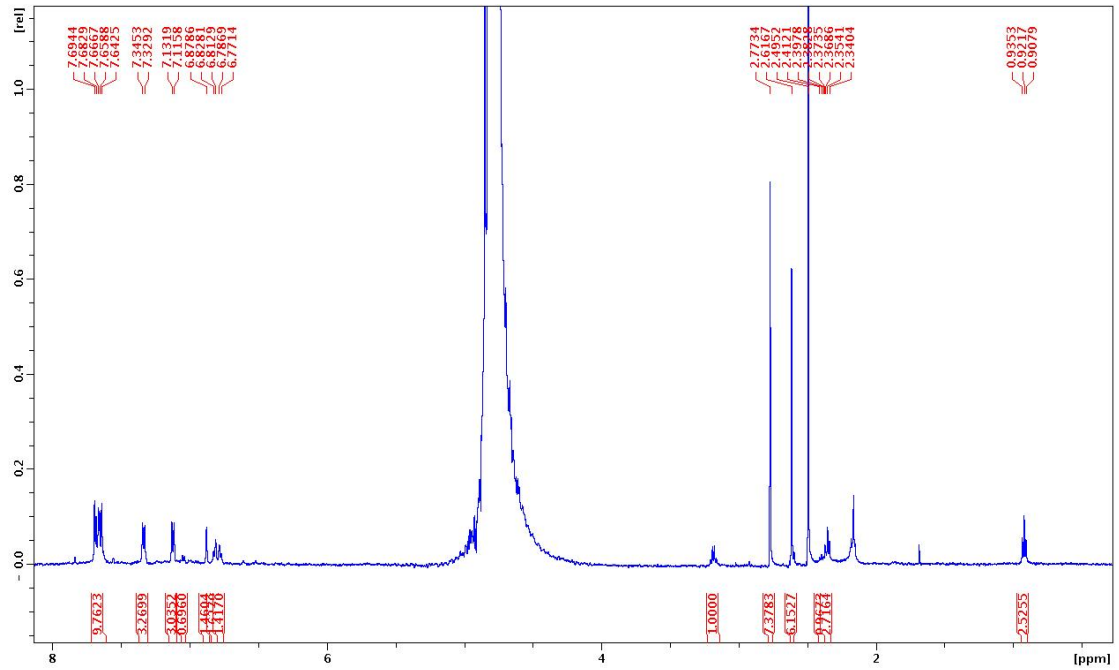

Figure S11 ^{1}H NMR spectrum of UiO-Cis after digestion, which shows the presence of both amino-TPDC and cis, cis, trans-$Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2COOH)$. This spectrum is identical to that was obtained by adding cis, cis, trans-$Pt(NH_3)_2Cl_2(OEt)$-$(O_2CCH_2CH_2COOH)$ to the digested suspension of UiO, which strongly supports the non-covalent encapsulation of the cisplatin prodrug.

The release of the cisplatin prodrug from UiO-Cis was evaluated. UiO-Cis was dialyzed in 5 mM phosphate buffer saline (PBS) in a dialysis bag (MWCO 7000) with stirring (150 rpm) at 37 °C in a beaker. Free cisplatin prodrug solution served as control. Samples were taken at 2 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 24 h to determine the release of cisplatin by ICP-MS. As shown in Fig. S12, up to 80% of the cisplatin prodrug was dialyzed out within the first 3 h for free cisplatin prodrug solution. After being encapsulated in the UiO, the cisplatin prodrug exhibited relatively slow release behavior with a total release of 34% up to 24 h. The significantly decreased release rate indicates successful encapsulation of the prodrug and can be attributed to the collapse of NMOF structure in 5 mM PBS to retard the drug release.

The released drug was confirmed to be intact cisplatin prodrug by ^{195}Pt NMR (Fig. S13). ^{195}Pt NMR (DMSO-d6): δ= 998.8.

We also studied the release of the cisplatin prodrug from UiO-Cis in water. The cisplatin prodrug was released much more slowly from the UiO-Cis in water, with less than 15% release in 24 h (as compared to 34% release in 5 mM PBS in 24 hour). The study shows that negligible amount of cisplatin prodrug was released from UiO-Cis during the siRNA loading, which was done in water in 30 min.

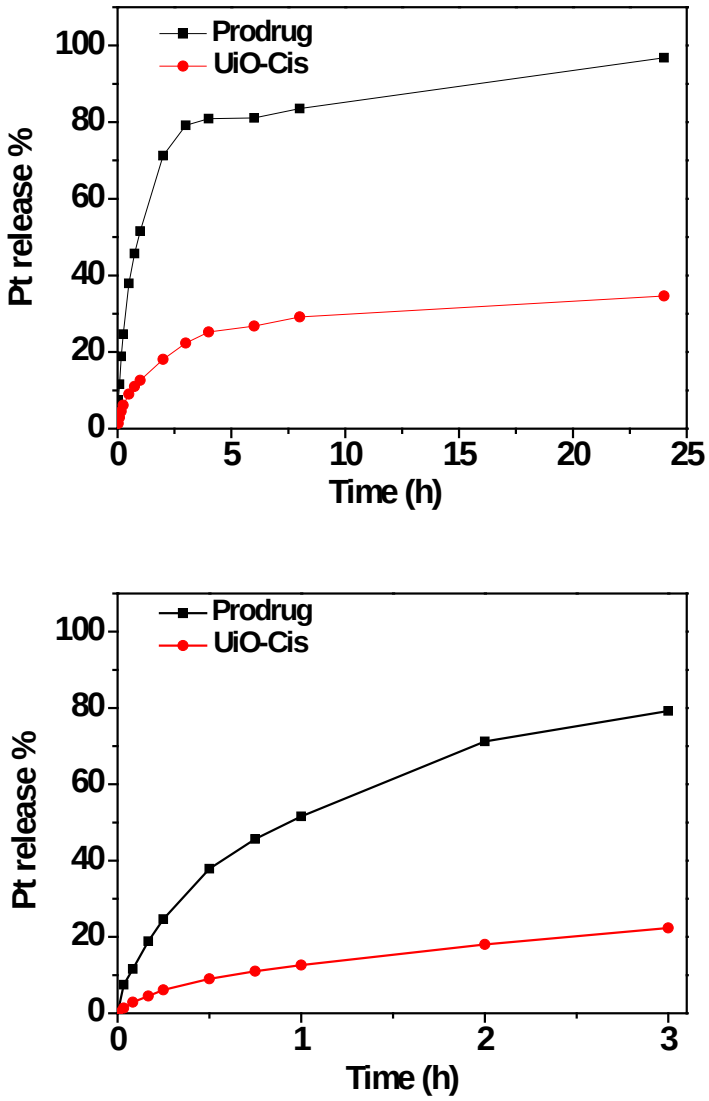

Figure S12. Top, release profiles of cisplatin prodrug from UiO-Cis in 5 mM PBS by dialysis method. Free prodrug served as control. Bottom, zoomed-in view of the release profiles in the first 3 h.

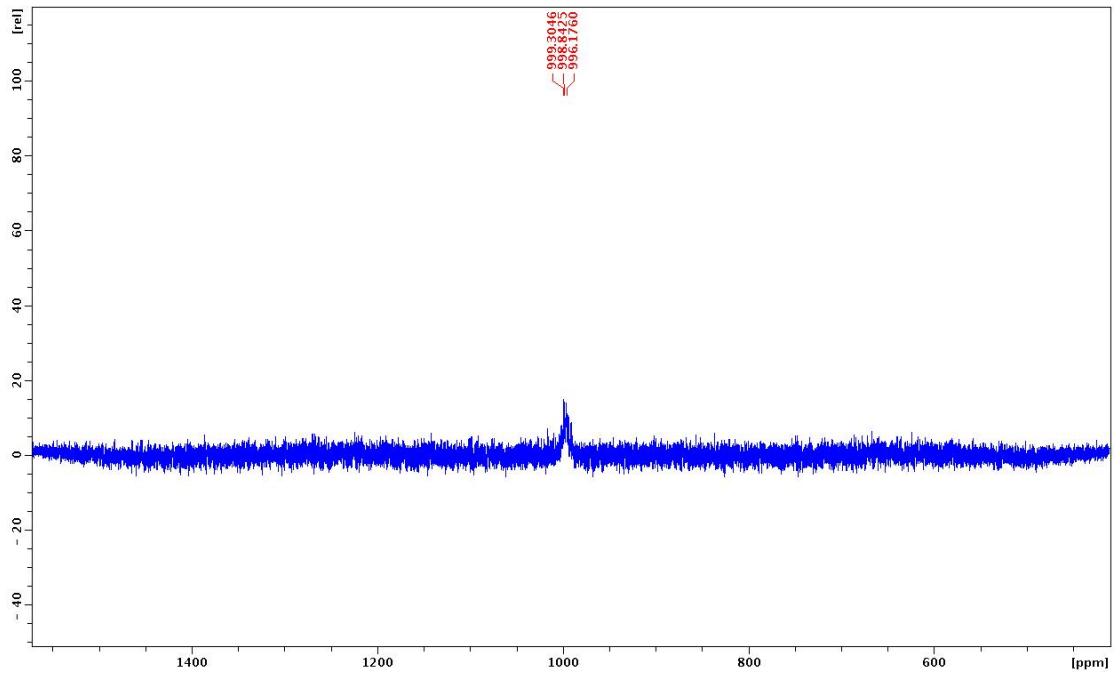

Figure S13 ^{195}Pt NMR of the released drug. The sample was concentrated, acidified with phosphoric acid and dissolved in DMSO-d6. The ^{195}Pt chemical shift is consistent to cis, cis, trans-[$Pt(NH_3)_2Cl_2(OEt)(OCOCH_2CH_2COOH)$].

3. Loading of siRNA onto the UiO-Cis NMOF

Survivin siRNA, Bcl-2 siRNA, and P-gp siRNA were dissolved in DEPC-treated water at weight ratio of 1: 1: 1 to achieve a 2 mg/mL pooled siRNA solution. UiO-Cis (1 mg/mL) dispersed in DECP-treated water was mixed with siRNA solution (2 mg/mL) at weight ratio of cisplatin : siRNA = 15 : 4, and the mixture was stirred (800 rpm) at room temperature for 30 min to afford siRNA/UiO-Cis. After siRNA loading, the NMOFs were centrifuged at 13000 rpm for 15 min. The supernatant was discarded to remove the free siRNA and the precipitate of NMOFs was collected and re-suspended in aqueous solution for subsequent evaluations.

In order to confirm that siRNA was loaded on the surface of NMOFs rather than absorbed into the pores/channels of the NMOFs, we also attempted to load siRNAs onto UiO-66, whose window/pore sizes are much smaller than those of UiO-68. The UiO-66 particles are about 500 nm in dimensions. By using the same loading methods as for UiO-68, we determined that the siRNA loading efficiency was ~ 45.1%. We believe that the slightly lower loading efficiency is due to the smaller external surface area of the much larger UiO-66 particles. When the weight ratio of cisplatin:siRNA was increased to 12:1 (from 4.5:1), the loading efficiency reached 85.7% which is higher than that of UiO-68. This control experiment strongly supports that notion that siRNAs can effectively absorb onto the surface of NMOFs through coordination.

The morphology of siRNA/UiO-Cis was observed by high-resolution TEM (Fig. S14).

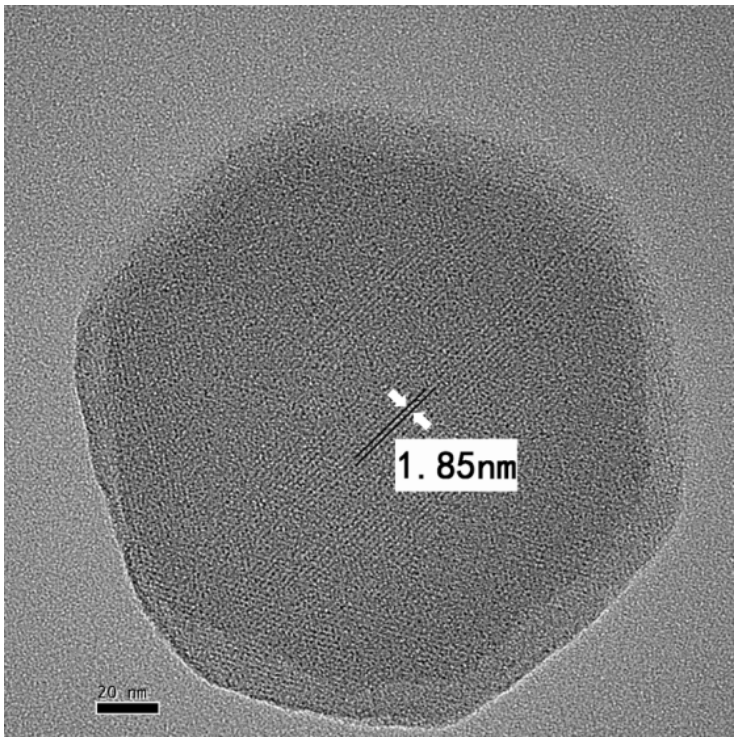

Figure S14 High-resolution TEM image of siRNA/UiO-Cis. Bar represents 20 nm.

The particle size of siRNA/UiO-Cis was determined to be 128±3 nm (PDI = 0.116) by DLS measurement as shown in Fig. S15.

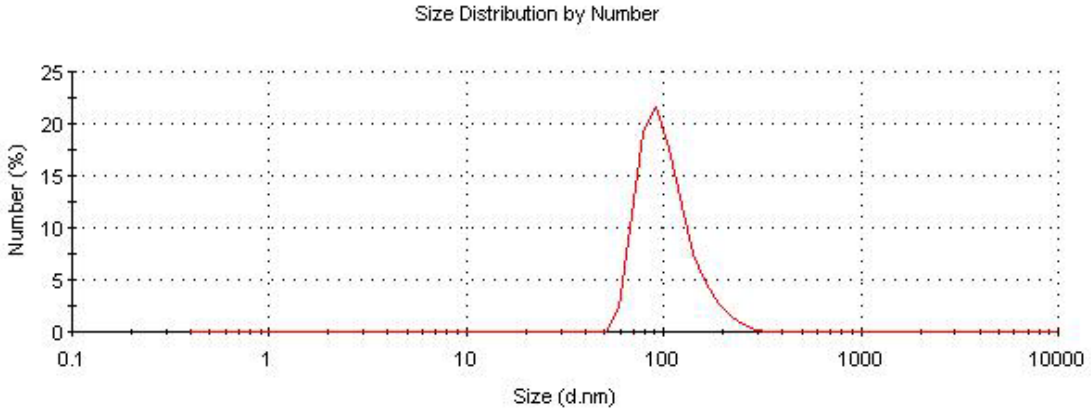

Figure S15 DLS plot showing the particle size of siRNA/UiO-Cis.

The association of siRNA with UiO-Cis was first determined by gel retardation assay on 4% (w/v) agarose gel electrophoresis containing 0.25 μg/mL of EB. The gel was imaged using the Gel Doc™ XR+ System (Biorad, USA). As shown in Fig. S16, the migration of siRNA loaded into NMOFs was completely retarded compared to naked siRNA solution, indicating the successful siRNA loading.

Figure S16 Agarose gel electrophoresis of naked siRNA (Lane 1) and siRNA/UiO-Cis (Lane 2) showing the siRNA loading into the NMOFs.

We also quantitatively determined the loading efficiency (LE) of siRNA onto UiO-Cis by fluorimetry. TAMRA-labeled siRNA was loaded onto UiO-Cis and the NMOF suspension was centrifuged at 13,000 rpm for 30 min. The amount of free TAMRA-siRNA in the supernatant was determined with fluorimetry based on the standard curve (TAMRA, λex = 565 nm, λem = 580 nm). LE was calculated from the following equation:

$$\mathrm{LE}\,(\%) = \frac{W_0 - W_1}{W_0} \times 100$$

Where W_0 and W_1 stand for the content of total siRNA and free siRNA in the supernatant, respectively.

4. siRNA protection and release

siRNA/UiO-Cis containing 1 μg of siRNA was mixed with an equal volume of FBS. After incubation for a determined period of time at 37 °C, 100 mM PBS was added to the mixture to disrupt the NMOF structure, and thus the siRNA was dissociated from NMOFs. The mixture was heated to 80 °C to inactivate the nucleases. The siRNA integrity was subsequently evaluated on 4% (w/v) agarose gel electrophoresis. Naked siRNA solution containing 1 μg of siRNA served as control.

As for the evaluation of siRNA release profiles from NMOFs, siRNA/UiO-Cis containing 1 μg of TAMRA-siRNA were incubated with 1 mL of 2 mM PBS or DEPC-treated water at 37 °C under shaking. At predetermined time intervals (0.5, 1, 2, 4, 6, and 8 h), the suspension was centrifuged at 13,000 rpm for 10 min and 0.5 mL of the supernatant was quantified for TAMRA-siRNA content by fluorimetry. An equal volume of the release medium was added, and the precipitate was re-suspended before further incubation.

5. Protein binding assay

Five hundred microliters of siRNA/UiO-Cis (1 mg/mL) and UiO-Cis (1 mg/mL) were incubated with 100, 250, 400, 750, and 1500 μg/mL of bovine serum albumin (BSA) at 37 °C for 1 h, respectively. The NMOFs were centrifuged at 13000 rpm for 10 min, and the BSA concentrations in the supernatant were determined by Pierce BCA Protein Assay Kit (Promega, USA) according to the manufacture instructions. The protein binding was expressed as the amount of protein (μg) associated to per μg NMOFs.

6. siRNA cellular uptake

SKOV-3 cells were seeded on a 24-well plate at 1×10^5 cells per well and cultured for 24 h. TAMRA-siRNA-containing NMOFs and naked TAMRA-siRNA solution (2 mg/mL) were added (0.4 μg siRNA/well). Following a 4-h incubation, cells were washed with PBS three times and then lysed with 0.5% (w/v) sodium dodecyl sulfate (SDS, pH 8.0). The lysate was quantified for TAMRA-siRNA by fluorimetry and protein content by the BCA kit. Uptake level was expressed as the amount of TAMRA-siRNA associated with 1 mg of cellular protein.

The internalization of TAMRA-siRNA/UiO-Cis was also directly visualized using confocal laser scanning microscopy (CLSM, Olympus FV1000). SKOV-3 cells were incubated with TAMRA-siRNA/UiO-Cis for 4 h at 37 °C. The cells were washed with PBS three times, fixed with 4% paraformaldehyde for 20 min at 4 °C, and stained with 4',6-diamidino-2-phenylindole (DAPI) (10 μg/mL) for 20 min at room temperature. As shown in Fig. S17, TAMRA-siRNA/UiO-Cis was efficiently taken up by SKOV-3 cells as evidenced by the large amount of red fluorescence observed in the cytoplasm.

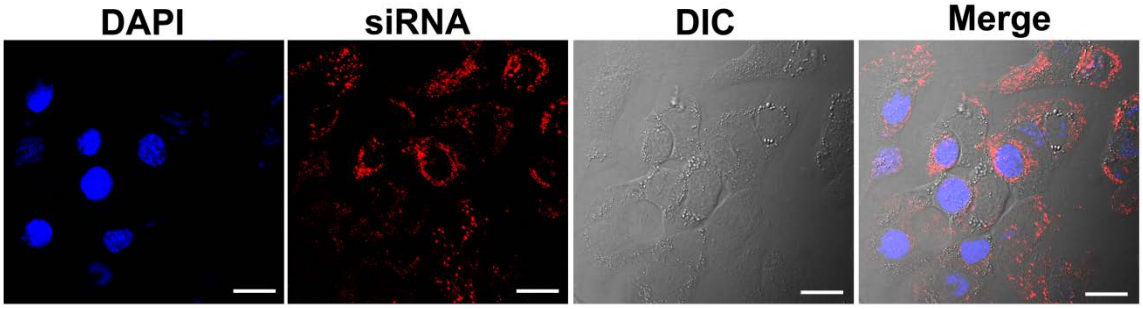

Figure S17 CLSM image showing the internalization of siRNA (TAMRA-labeled) into the cytoplasm of SKOV-3 cells. Nuclei were stained with DAPI. Bar represents 20 μm.

7. Endosomal escape

To visualize the co-localization of internalized siRNA/UiO-Cis with endosomal/lysosomal compartments, cells were incubated with NMOF containing TAMRA-siRNA for 2 h at 37 °C. The cells were washed with PBS three times, fixed with 4% paraformaldehyde for 20 min at 4 °C, and sequentially stained with Lysotracker Green (100 nM) for 2 h at room temperature and DAPI (10 μg/mL) for 20 min at room temperature before observation CLSM. As shown in Fig. S18, the green and red

fluorescence was partly separated in the cytoplasm, indicating the successful endosomal escape of siRNA/UiO-Cis.

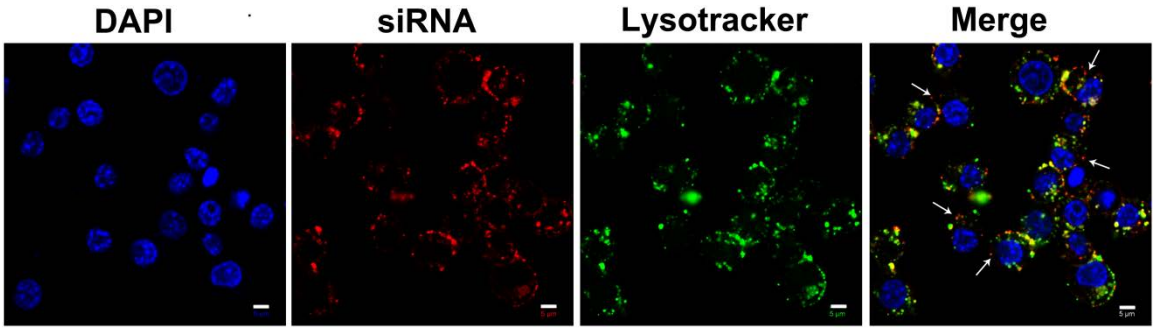

Figure S18 siRNA (TAMRA-labeled, red fluorescence) successfully escaped from endosomes as evidenced by the separation of green and red fluorescence (white arrows). Endosome/lysosome and nuclei were stained with Lysotracker Green and DAPI, respectively. Bar represented 5 μm.

A time dependent study of endosomal escape was performed by incubation UiO-Cis/siRNA with SKOV-3 cells for 10, 30, 60, 90, and 120 min followed by Lysotracker Green and DAPI staining. The time-dependent co-localization of UiO-Cis/siRNA and endosome/lysosome was observed under CLSM, and the co-localization efficiency was quantitatively determined using Image J (co-localization threshold) based on the CLSM images (Fig. S19). siRNA efficiently escaped from endosomal entrapment with time as evidenced by the decreased co-localization percent of red and green fluorescence from 78.8% to 24.3% in 2 hours (Fig. S20).

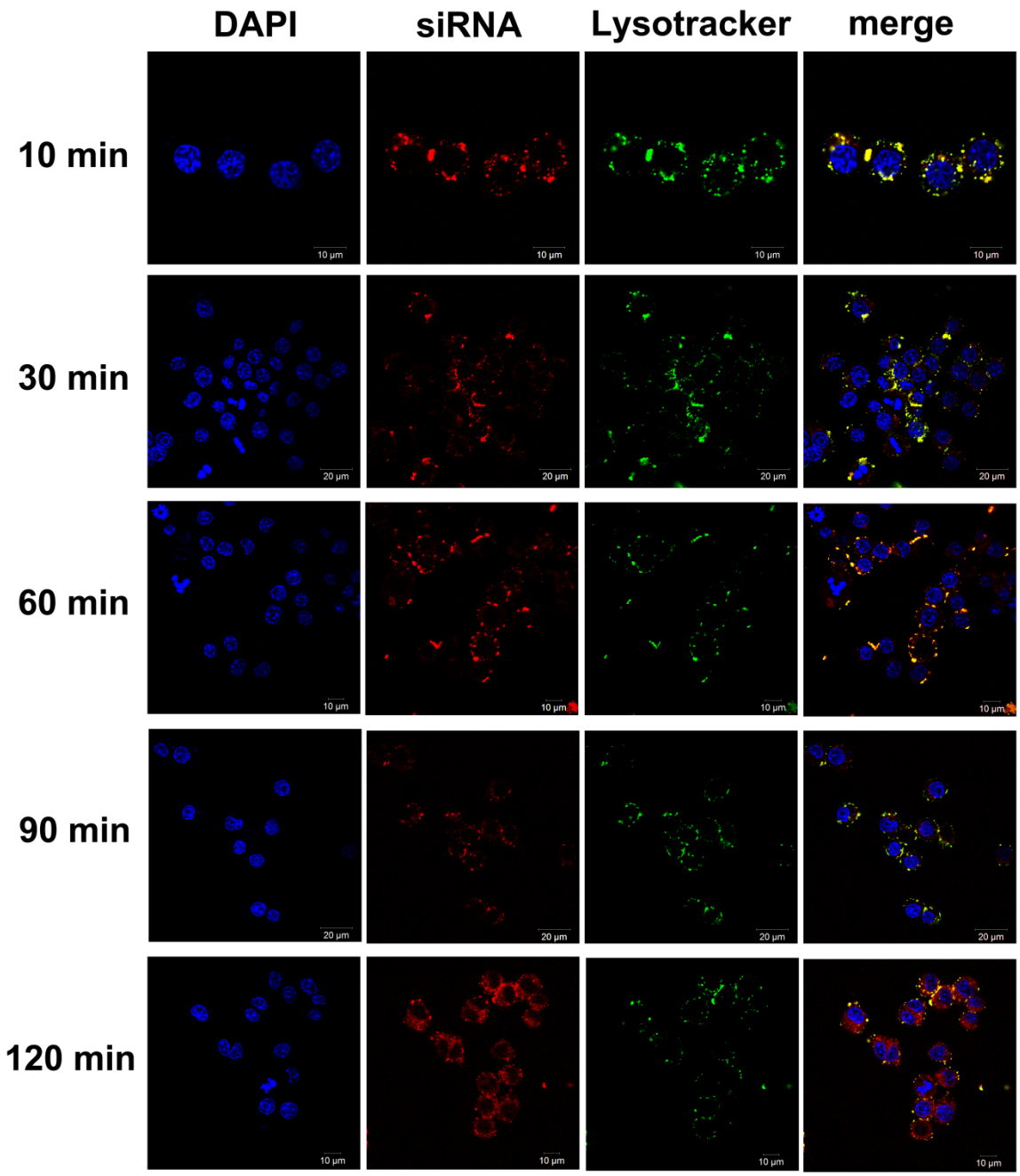

Figure S19 Time-dependent endosomal escape of siRNA in SKOV-3 cells. Endosome/lysosome and nuclei were stained with Lysotracker Green and DAPI, respectively. Bar represented 10 μm.

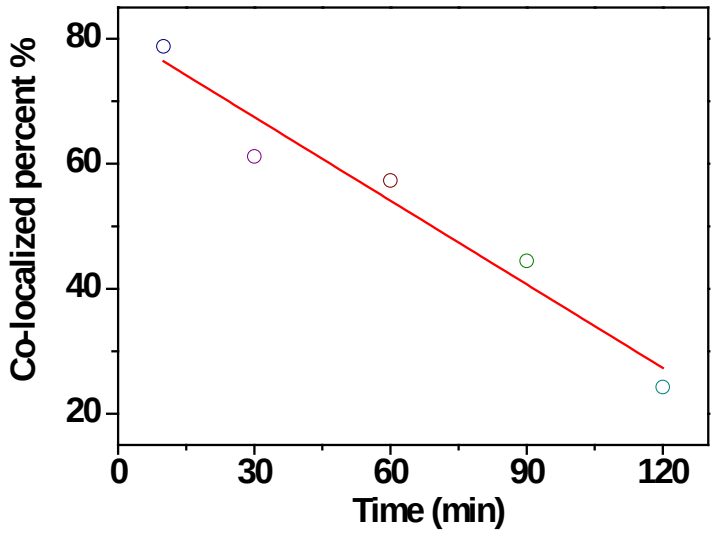

Figure S20 Percent co-localization of siRNA and endosome/lysosome quantified by Image J based on the CLSM images.

8. *In vitro* transfection efficiency of siRNA/UiO-Cis

SKOV-3 cells were seeded at 2×10^5 cells per well in 24-well plates and further cultured for 24 h. The culture media were replaced by 1 mL of pre-warmed and fresh culture media containing 10% FBS prior to the experiment. siRNA/UiO-Cis containing pooled siRNAs and single siRNA, pooled siRNAs/UiO, free siRNA solution, and UiO-Cis were added to the cells at a siRNA dose of 0.4 μg (30 nM) per well, corresponding to the cisplatin dose of 1.5 μg per well. Following incubation for 4 h, the culture media were replaced by pre-warmed and fresh culture media containing 10% FBS, and a further 20-h incubation was allowed. The supernatant of the culture media was collected for the determination of extracellular survivin and P-gp production by ELISA (R&D Systems, USA; MyBiosource, USA) following manufacture instructions. The cells were lysed, and the Bcl-2 amount in the lysate was quantified by ELISA (R&D Systems, USA).

9. *In vitro* anticancer effect

9.1. *In vitro* cytotoxicity

SKOV-3 cells were seeded at 5000 cells per well in 96-well plates and further cultured for 24 h. The culture media were replaced by 100 μL of fresh culture media containing 10% FBS. Free cisplatin solution, UiO-Cis, pooled siRNA/UiO-Cis, free cisplatin solution plus free pooled siRNA solution (weight ratio of cisplatin to siRNA = 4.5:1), free cisplatin solution plus pooled siRNAs/UiO (weight ratio of cisplatin to siRNA = 4.5:1), and pooled siRNAs/UiO at different siRNA or cisplatin doses. Following incubation for 72 h, the cell viability was determined by (3-(4,5-dimethylthiazol-2-yl)-5-

(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay (Promega, USA) according to manufacture instructions. The concentrations of cisplatin required to inhibit cell growth by 50% (IC_{50} values) were calculated.

The cytotoxicity of pooled siRNAs/UiO-Cis was evaluated on cisplatin-sensitive cancer cell lines. PC-3, MCF-7, and H460 cells were seeded at 1500, 5000, and 2500 cells per well in 96-well plates and further cultured for 24 h. The culture media were replaced by 100 μL of fresh culture media containing 10% FBS. Free cisplatin solution, UiO-Cis, pooled siRNA/UiO-Cis were added at different cisplatin doses. Following incubation for 72 h, the cell viability was determined by MTS assay. IC_{50} values were calculated accordingly. Free cisplatin, UiO-Cis, and pooled siRNAs/UiO-Cis induced effective cytotoxicity in all three cisplatin-sensitive cancer cell lines. The IC_{50} values of free cisplatin, UiO-Cis, and pooled siRNAs in terms of cisplatin dose were calculated to be (0.51±0.13), (0.54±0.13), and (0.42±0.08) μM in PC-3 cells, respectively; (8.91±0.34), (8.80±0.60), and (8.00±0.59) μM in MCF-7 cells, respectively; (7.07±0.39), (6.17±0.63), and (5.84±0.57) μM in H460 cells, respectively.

T-test was conducted to identify if significant difference exists among the IC_{50} values of free cisplatin, UiO-Cis, and pooled siRNAs/UiO-Cis. A significant difference is defined when the P value is lower than 0.05. The P values of UiO-Cis versus pooled siRNAs/UiO-Cis are 0.3658, 0.9474, and 0.7843 for H460, MCF-7, and PC-3, respectively. The P values of free cisplatin versus pooled siRNAs/UiO-Cis are 0.9139, 0.9583, and 0.8114, in H460, MCF-7, and PC-3, respectively. Consequently, the IC_{50} values of pooled siRNAs/UiO-Cis are (statistically) significantly different from those of free cisplatin and UiO-Cis in cisplatin-sensitive cell lines.

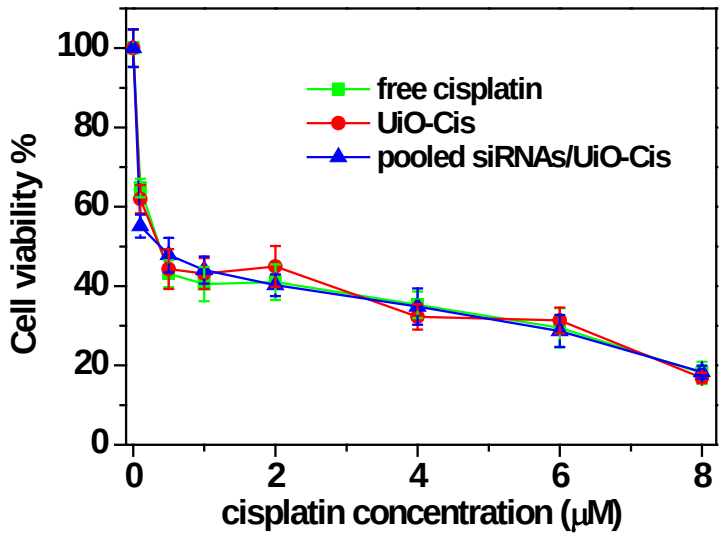

Figure S21 Cytotoxicity of pooled siRNAs/UiO-Cis in PC-3 cells. PC-3 cells were incubated with UiO-Cis, siRNA/UiO-Cis, and free cisplatin at different cisplatin concentrations for 72 h, and then the cytotoxicity was determined by MTS assay.

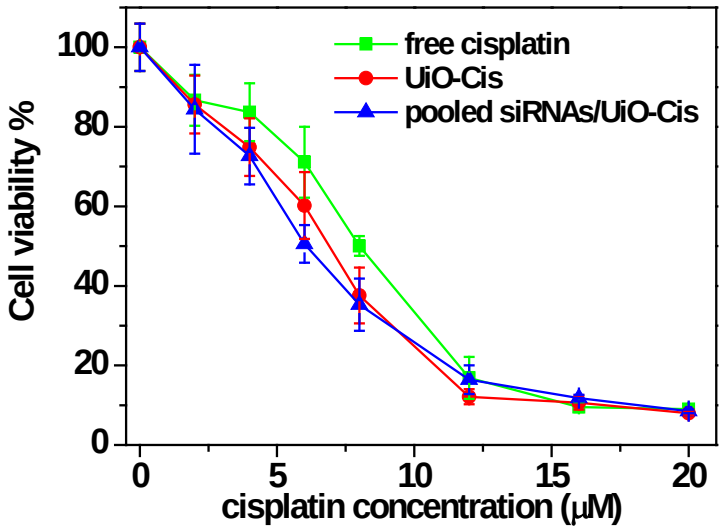

Figure S22 Cytotoxicity of pooled siRNAs/UiO-Cis in MCF-7 cells. MCF-7 cells were incubated with UiO-Cis, siRNA/UiO-Cis, and free cisplatin at different cisplatin concentrations for 72 h, and then the cytotoxicity was determined by MTS assay.

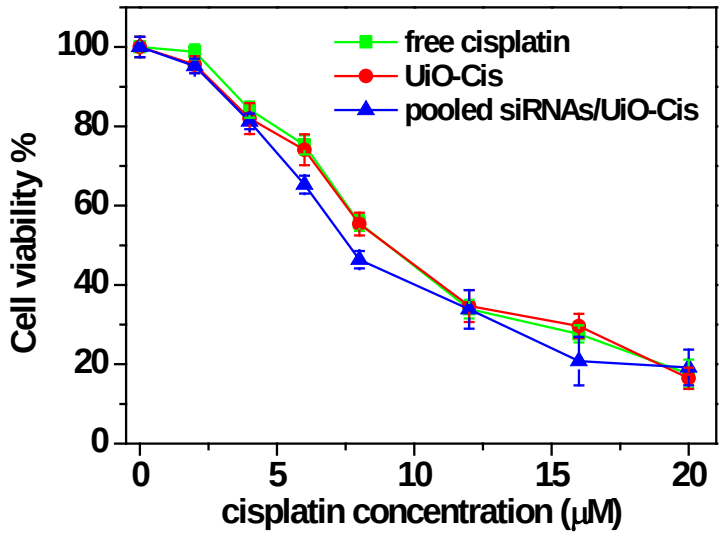

Figure S23 Cytotoxicity of pooled siRNAs/UiO-Cis in H460 cells. H460 cells were incubated with UiO-Cis, siRNA/UiO-Cis, and free cisplatin at different cisplatin concentrations for 72 h, and then the cytotoxicity was determined by MTS assay.

The enhanced *in vitro* anticancer efficacy of pooled siRNAs/UiO-Cis was further confirmed by carrying out cytotoxicity analysis on cisplatin sensitive and resistant A2780 and A2780/CDDP cells. A2780 and A2780/CDDP cells were seeded at 2500 cells per

well in 96-well plates and further cultured for 24 h. The culture media were replaced by 100 μL of RPMI 1640 containing 10% FBS. Free cisplatin solution, UiO-Cis, pooled siRNA/UiO-Cis, free cisplatin solution plus free pooled siRNA solution (weight ratio of cisplatin to siRNA = 4.5;1), free cisplatin solution plus pooled siRNAs/UiO (weight ratio of cisplatin to siRNA = 4.5:1), and pooled siRNAs/UiO were added at different cisplatin doses and equivalent siRNA doses. Following incubation for 72 h, the cell viability was determined by MTS assay. IC_{50} values were calculated accordingly. Free cisplatin, UiO-Cis, pooled siRNAs/UiO-Cis, free cisplatin solution plus free pooled siRNAs solution, free cisplatin solution plus pooled siRNAs/UiO induced effective cytotoxicity in cisplatin sensitive A2780 cells, which exhibited no significant difference (Fig. S24 and Table S1). In comparison, the IC_{50} values of pooled siRNAs/UiO-Cis and free cisplatin solution plus pooled siRNAs/UiO were decreased by 5~6 folds compared to other groups, suggesting the co-delivery of cisplatin and pooled siRNAs significantly enhanced the anticancer efficacy in cisplatin-resistant cancer cells (Fig. S24 and Table S1). The cell viability of A2780 and A2780/CDDP cells incubated with pooled siRNAs/UiO at siRNA concentrations of 100 nM and 150 nM was 96.5±3.5% and 98.2±4.9%, respectively, suggesting that pooled siRNAs alone did not exhibit effective cytotoxicity.

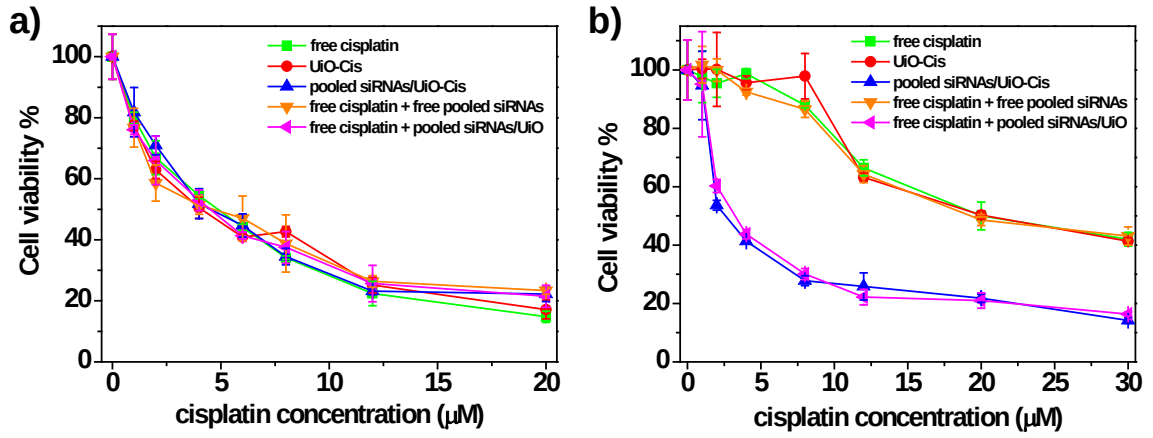

Figure S24 Cytotoxicity of pooled siRNAs/UiO-Cis in A2780 (a) and A2780/CDDP (b) cells. Cells were incubated with free cisplatin solution, UiO-Cis, pooled siRNAs/UiO-Cis, free cisplatin solution plus free pooled siRNA solution (weight ratio of cisplatin to siRNA = 4.5:1), free cisplatin solution plus pooled siRNAs/UiO (weight ratio of cisplatin to siRNA = 4.5:1) for 72 h, and then the cytotoxicity was determined by MTS assay.

Table S1 IC_{50} (μM) values in A2780 and A2780/CDDP after a 72-h incubation with free cisplatin solution, UiO-Cis, pooled siRNAs/UiO-Cis, free cisplatin solution plus free pooled siRNA solution (weight ratio of cisplatin to siRNA = 4.5:1), free cisplatin solution plus pooled siRNAs/UiO (weight ratio of cisplatin to siRNA = 4.5:1).

	A2780	A2780/CDDP
Free cisplatin	4.16±0.21	23.20±0.60
UiO-Cis	4.06±0.20	21.4±1.38
Pooled siRNAs/UiO-Cis	4.49±0.68	4.20±0.61
Free cisplatin+pooled siRNAs	4.18±0.33	22.85±2.30
Free cisplatin+pooled siRNAs/UiO	4.18±0.17	4.87±0.63

9.2. DNA ladder

SKOV-3 cells were seeded at 1×10^6 cells per well in 6-well plates and further cultured for 24 h. The culture media were replaced by 2 mL of fresh culture media containing 10% FBS. Free cisplatin solution, UiO-Cis, siRNA/UiO-Cis were added to the cells, respectively, at cisplatin concentration of 10 μM. Following incubation for 24 h, total DNA of cancer cells was extracted using DNA ladder isolation kit (Sigma-Aldrich, USA) according to the manufacture instructions and examined for DNA fragmentation on a 2% (w/v) agarose gel electrophoresis at 35 V for 3 h.

9.3. Cell apoptosis by Annexin V staining

Coverslips putting in the 6-well plates were seeded with SKOV-3 cells at the density of 1×10^6 cells per well. The cells were incubated at 37°C and 5% CO_2 for 24 h prior to nanoparticle treatment. SiRNA/UiO-Cis, siRNA/UiO solution, and UiO-Cis were incubated with cells at 37°C and 5% CO_2 for 24 h. Then, the cells were washed with PBS, stained with Alexa Fluor 488 conjugated Annexin V (Invitrogen, USA) according to the manufacture instructions for 15 min at room temperature, fixed with iced 4% paraformaldehyde for 20 min at 4 °C, and stained with 10 μg/mL of DAPI. The cells were observed using CLSM at excitation wavelength of 405 nm, 488 nm, and 546 nm to visualize nuclei (blue fluorescence), cell apoptosis (green fluorescence) and nanoparticle internalization (red fluorescence), respectively.

(a)

(b)

(c)

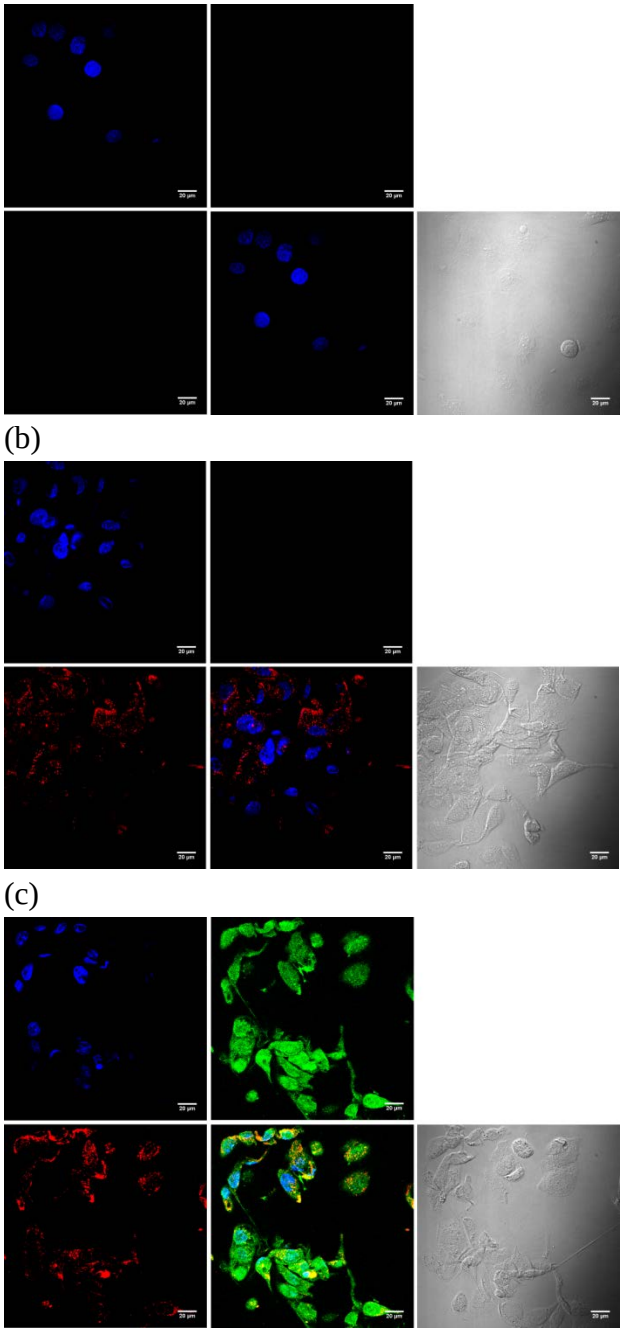

Figure S25 CLSM images showing cell apoptosis and siRNA (TAMRA-labeled, red fluorescence) internalization in SKOV-3 cells after incubation with UiO-Cis (a), siRNA/UiO (b), and siRNA/UiO-68 (c) for 24 h. The apoptotic cells were stained with Alexa Fluor 488 Annexin V conjugate, and the nuclei were stained with DAPI. Upper left: DAPI stained nuclei; upper right: Alexa Fluor 488 Annexin V conjugate stained apoptotic cells; lower left: TAMRA-labeled siRNA; lower middle: overlay of upper left, upper right, and lower left; lower right: DIC. Bar represented 20 μm.

9.4 Flow Cytometry

SKOV-3 cells were seeded at 1×10^6 cells per well in 6-well plates and further cultured for 24 h. The culture media were replaced by 2 mL of fresh culture media containing 10% FBS. Free cisplatin solution, UiO, UiO-Cis, and pooled siRNAs/UiO-Cis were added to the cells, respectively, at cisplatin concentration of 5 μM or equivalent UiO concentration of 10 μg/mL. Cells incubated with saline served as control. Following incubating for 24 h, the floating and adherent cells were collected by cell scraper and stained with Alexa Fluor 488 annexin V/dead cell apoptosis kit with Alexa Fluor 488 annexin V and PI (Invitrogen, USA) according to the manufacture instructions. The apoptosis was examined on a flow cytometer (LSRII Blue, BD, USA). No apoptosis was observed in the UiO group after a 24-h incubation (Fig. S26), suggesting its negligible cytotoxicity. The total apoptotic cell percent (including early and late apoptosis) of pooled siRNAs/UiO-Cis, UiO-Cis, and free cisplatin solution were determined to be 96.5%, 77.0%, 51.7%, respectively, suggesting that pooled siRNAs/UiO-Cis was most potent in inducing apoptosis.

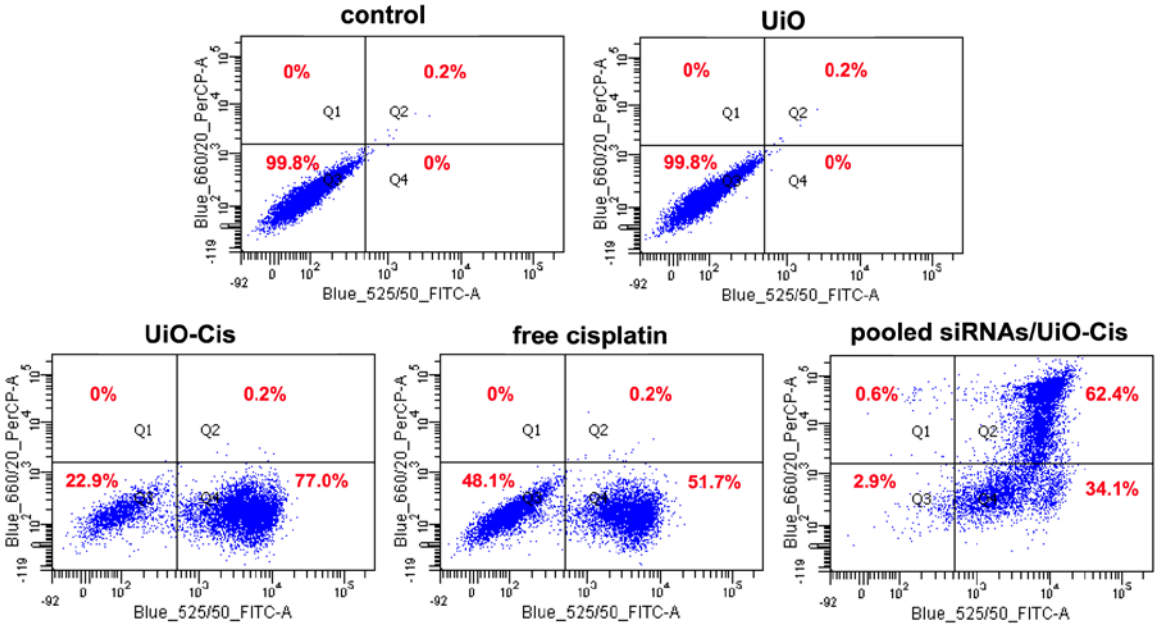

Figure S26 Annexin V/PI analysis of SKOV-3 cells after the incubation with saline (control), UiO, UiO-Cis, pooled siRNAs/UiO-Cis and free cisplatin for 24 h. The Q1-Q4 quadrants represent necrosis, late apoptotic, healthy, and early apoptotic cells, respectively. The percent of cells in each quadrant was shown on the graphs.

10. Immunogenic response

SKOV-3 cells and Raw 264.7 cells were seeded at 2×10^5 cells per well in 24-well plates and further cultured for 24 h. The culture media were replaced by 1 mL of fresh

culture media containing 10% FBS prior to the experiment. UiO and pooled siRNA/UiO-Cis were added to the cells at a siRNA dose of 0.4 μg (30 nM) per well, corresponding to the UiO dose of 10 μg per well. Following incubation for 72 h, the supernatant of the culture media was collected for the determination of TNF-α, IL-6, and IFN-γ by ELISA (R&D Systems, USA) following manufacturer's instructions. Cells treated with saline served as controls. After a 72-h incubation with UiO or pooled siRNAs/UiO-Cis, similar levels of TNF-α, IL-6, and IFN-γ were detected in SKOV-3 and Raw 264.7 cells as compared to control groups (Fig. S27). This result suggested that the blank NMOF carrier (UiO) as well as the cisplatin and pooled siRNAs loaded NMOFs (pooled siRNAs/UiO-Cis) might not evoke immunogenic response in cancer cells or macrophages.

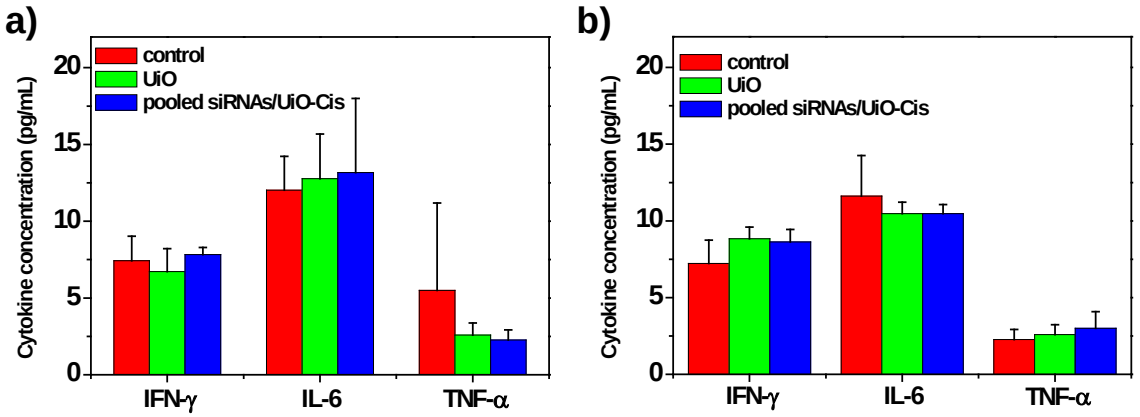

Figure S27 Immunogenic response of UiO and pooled siRNAs/UiO-Cis in SKOV-3 (a) and Raw 264.7 (b) cells. The cells were incubated with UiO or pooled siRNAs/UiO-Cis for 72 h followed by the determination of TNF-α, IL-6, and IFN-γ by ELISA.

11. References

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Full reference for **Ref 16**:
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