Electronic Supplementary Information for

Platinum(IV) prodrugs entrapped within multiwalled carbon nanotubes:
Selective release by chemical reduction and hydrophobicity reversal

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Materials and instruments

Materials

K₂PtCl₄ was purchased from Precious Metals Online. Benzoyl chloride, sodium diethyldithiocarbamate (DDTC), 5’-guanosine-2’-deoxymonophosphate (dGMP) and ascorbic acid were purchased from Sigma Aldrich. Pyridine was purchased from Merck. The reactions were performed with solvents purchased from TEDIA. Cisplatin and cis,cis,trans-Pt(NH₃)₂Cl₂(OH)₂ were prepared according to the literature procedures.¹ Ultrapure water was obtained from Sartious-Stedim biotech atrium® 611 system (18.2 MΩ-cm resistivity). SpectraPor dialysis membrane (12-14,000 MWCO) was used in the drug release experiment. Fluoropore hydrophobic PTFE membranes (0.22 µM) and Omnipore hydrophilic PTFE membranes (0.22 µM) were purchase from Millipore.
Instruments

SEM-EDX (Energy Dispersive X-ray) analysis was carried out using JSM-6700 Field Emission Scanning Electron Microscope. Thermogravimetric analysis was performed using TGA-SDT 2960 Simultaneous DTA-TGA and ICP-OES using Perkin Elmer Dual-view Optima 5300 DV Inductively Coupled Plasma Optical Emission Spectrometer by CMMAC (NUS). The chromatogram of Pt-dGMP adducts was analysed on Agilent 1200 HPLC. ICP-MS analysis was carried out using Agilent 7500A Inductively Coupled Plasma Mass Spectrometer.

Synthesis of cis,cis,trans-Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(CO\textsubscript{2}C\textsubscript{6}H\textsubscript{5})\textsubscript{2}, 1

Pyridine (0.5 mL, 6.19 mmol) was added dropwise to benzoyl chloride (0.5 mL, 3.90 mmol) in acetone (5 mL) and stirred for 15 min. cis,cis,trans-Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}(OH)\textsubscript{2} (50 mg, 0.15 mmol) was then added and the mixture was reflux for 6 h. Excess water (20 mL) was added to quench the reaction, and the reaction mixture was filtered. The precipitate was triturated in diethyl ether (3 x 10 mL), filtered and washed with more water to remove the pyridinium salt. The remaining residue was dried under reduced pressure to give complex 1 as an off-white solid. Yield: 20 mg, 25%. ¹H NMR (acetone-d\textsubscript{6}, 500 MHz): δ 7.99 (d, 4 H, Ar-H), 7.54 (t, 2 H, Ar-H), 7.41 (t, 4 H, Ar-H), 6.70 (m, 6 H, NH\textsubscript{3}, ¹J\textsubscript{HN} = 53.6 Hz, ²J\textsubscript{HPt} = 54.3 Hz). ¹⁹⁵Pt {¹H} NMR (acetone-d\textsubscript{6}, 107.6 MHz): 1110.76 (s). ESI (MS): m/z = 540.9 [M-H]-, Calculated: m/z = 541.27 [M-H]-.
Preparation of platinum complexes-containing MWCNTs

Entrapment of cisplatin in MWCNTs via nano-extraction. Cisplatin (20 mg) was dispersed by sonication in EA (8 mL). MWCNTs (4 mg) were added and the suspension was sonicated for 15 min. The mixture was stirred at r. t. for 24 h and filtered through a hydrophobic PTFE membrane. The residue was washed extensively with washing solvent (2.6:1:1 v/v EA:EtOH:water) and dried in vacuo to yield MWCNT-cDDP.

Estimation of solubility of 1 for nano-extraction. Compound 1 was weighed and added to different solvents, and sonicated for 15 min. The samples were centrifuged at 13,300 g to ensure complete solubility. The volume of solvent was increased until no observable solid remained in the solution. The solubility of 1 in EtOH, MeOH and EA was estimated to be 74, 320 and 29 µg/mL, respectively. Solubility in water and CHCl₃ was too poor to be estimated by this method.

Entrapment of 1 in MWCNTs via nano-extraction. Compound 1 (6.0 mg) was dispersed in CHCl₃ (2mL) by sonication for 10 min. MWCNTs (2.0mg) were added and the suspension was sonicated for 15 min. The mixture was stirred at r. t. for 5 days and filtered through a hydrophilic PTFE membrane. The residue was washed extensively with washing solvent (2:2.41:1 v/v CHCl₃:MeOH:water) and dried in vacuo to yield MWCNT-Pt(IV).
Quantification of encapsulated platinum complexes by TGA and ICP-OES. The samples were heated in air at a heating rate of 10°C/min until a final temperature of 1000°C was reached. The residue remaining after TGA was dissolved in *aqua regia* and diluted with 2% HNO₃ for ICP-OES determination of Pt levels.

**Figure S1.** SEM image (30 keV) MWCNT-Pt(IV).

**Figure S2.** TGA of a) MWCNTs alone, b) MWCNT-Pt(IV). TGA graphs are labeled with the wt% of metal residue after ramping the sample up to 1000 °C at a rate of 10°C/min under nitrogen.
Reactivity of platinum complexes

Reactivity with DDTC. Solutions containing cisplatin (3.3 mM, 100 μL water), 1 (1.8 mM, 100 μL 1:1 water:DMSO), or 1 (1.8 mM, 100 μL 3 mM ascorbic acid) were treated separately with 250 μL DDTC (5% in 0.2 M NaOH) and incubated at 37°C for 1 h. The solutions were extracted with EA (2 mL) and evaporated. The residue was dissolved in 1.5 mL MeCN before analysis with RP-HPLC. The column used was Phenomenex Gemini C18 column (5 μm, 100 Å, 150 mm × 4.6 mm). Mobile phase consisted of 75:25v/v MeOH:water at a flow rate of 0.60 mL/min. The column was maintained at 23°C. In sample containing cisplatin and 1 treated with 3 mM ascorbic acid, Pt(DDTC)2 was detected at Rt=13 min and was confirmed using ESI-MS on collected fractions (m/z = 492 [M+H]+).

Analysis of Pt-dGMP adduct formation. Solutions containing cisplatin (3.3 mM, 100 μL water), 1 (1.8 mM, 100 μL 1:1 water:DMSO), or 1 (1.8 mM, 100 μL 3 mM ascorbic acid) were treated separately with dGMP (2 mg/mL, 250 μL water) and incubated at 37°C for 24 h. The reaction mixture was analysed directly by RP-HPLC. The column used was Phenomenex Luna C18 column (5 μm, 100 Å, 250 mm × 4.6 mm). The detection UV was at 254nm and 280 nm. The mobile phase was 5-7% solvent B in 20 min at flow rate of 0.6 mL/min where solvent A is 10 mM ammonium acetate and solvent B is MeOH. Pt-dGMP adducts were observed only with solutions containing cisplatin and 1 treated with ascorbic acid.
Figure S3. HPLC chromatograms (280 nm wavelength) showing the activity of CDDP and reduced Pt(IV); (a) dGMP alone as control; (b) reaction of dGMP with CDDP; (c) reaction of dGMP with 1; (d) reaction of dGMP with 1 treated with 3 mM ascorbic acid; labels indicate unreacted dGMP(*), [Pt(NH₃)₂Cl(dGMP)] (♦) and [Pt(NH₃)₂(dGMP)₂] (●) as analysed by ESI-MS.

**Controlled release of Pt from platinum complexes-containing MWCNTs**

**Release from MWCNT-cDDP.** MWCNT-cDDP (containing 0.5 mg of cisplatin) was dispersed in H₂O (200µL), transferred to a dialysis button and immersed into PBS as the release media (400mL). Samples of the release medium were collected at regular intervals and their Pt content determined using ICP-OES.

**Release from MWCNT-Pt(IV).** MWCNT-Pt(IV) (containing 1.0 mg of 1) was dispersed in H₂O (400µL) and transferred to two separate dialysis buttons (200µL each). One button was immersed into PBS as the release media (400mL) while the other was immersed into PBS (400 mL) containing 3 mM ascorbic acid as the bio-reductant. Samples of the release medium were collected at regular intervals and their Pt content determined using ICP-OES.

**Binding to DNA target upon release from MWCNT-Pt(IV).** Dialysis buttons containing MWCNT-Pt(IV) (containing 1.0 mg of 1) was immersed either into PBS (40mL) containing 0.11mM dGMP or PBS (40mL) containing 3 mM ascorbic acid and 0.11mM dGMP for 24 h.
The solution was analysed directly on RP-HPLC using Phenomenex Luna C18 column (5µm, 100Å, 250mm×4.6mm). The mobile phase was 5-7% solvent B in 20 min at flow rate of 0.6 mL/min where solvent A is 10 mM ammonium acetate and solvent B is MeOH.

Pt uptake in A2780 ovarian carcinoma cells

Human ovarian carcinoma cells (A2780) were cultured in RPMI1640 medium supplemented with 10% FBS and 1% antibiotics. Three MWCNT samples were prepared: a) pristine MWCNTs as negative control, b) MWCNT-Pt(IV) which contained entrapped 1, and c) mixture of MWCNT and 1 to model a MWCNT system containing unencapsulated 1. Pt levels in MWCNT-Pt(IV) was previously ascertained using TGA/ICP-OES and used to guide the preparation of MWCNT and 1 mixture. Two different exposure conditions were prepared at two Pt concentrations of ca. 260 µg/L (condition A) and 1300 µg/L (condition B). The samples were dialysed in water for 16 h prior to treatment to simulate exposure to bodily fluids prior to arrival at targeted cell site.

A2780 cells were seeded in 100 mm cell culture dishes at a density of 1.3×10^7 cells per dish and the dishes were incubated at 37°C with 5% CO₂ for 48 h prior to MWCNT treatment. During treatment, the medium was replaced with incubation media containing either a) pristine MWCNT, b) MWCNT-Pt(IV), or c) mixture MWCNT and 1, in RPMI-1640 (5 mL, without FBS or antibiotics) at 37°C for 1 h, as well as control dishes without MWCNT exposure. After 1 h incubation, the medium in each dish was replaced with 5 mL fresh RPMI-1640 medium supplemented with 10% FBS and 1% antibiotics, and incubated for further 7 h. No obvious cell death in the treated cells was observed compared to the untreated
controls.

To collect cells, the dishes were placed on ice and cell monolayer was washed with ice-cold PBS (2 x 5 mL). PBS (1 mL) was added to each dish, and cells were collected with a scraper. The cells were pelleted by centrifugation for 15 min at 4 °C. To extract cytoplasm from cells, 100 µL lysis buffer (1% Triton/PBS) was added to each tube, and incubated on ice for 40 min. After centrifugation for 30 min at 4 °C, 100 µL supernatant was collected for analysis. Protein content of each sample was determined using the Bradford assay (Bio-Rad) in accordance to manufacturer’s instructions.

The Pt content in the cell lysate was determined using ICP-MS. Prior to Pt analysis, the cell lysate was dried in vacuum, and the remaining residue was digested in 100 µl 65% HNO₃ at r.t. for 24 h. The digested solution was diluted with MilliQ water (3 mL) prior to ICP-MS analysis.

**Table S1**: Pt content of A2780 cell extracts after treatment with a) pristine MWCNTs, b) MWCNT-Pt(IV) , or c) mixture of MWCNTs and 1.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Samples</th>
<th>Pt content (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
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<tr>
<td>low Pt exposure</td>
<td>a) pristine MWCNTs</td>
<td>(14.8 ± 0.23)×10⁻²</td>
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<tr>
<td>(ca. 260 µg/L Pt)</td>
<td>b) MWCNT-Pt(IV)</td>
<td>3.1 ± 0.32</td>
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<tr>
<td></td>
<td>c) mixture of MWCNT and 1</td>
<td>(6.8 ± 0.78)×10⁻²</td>
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<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high Pt exposure</td>
<td>a) pristine MWCNTs</td>
<td>(3.2 ± 0.07)×10⁻²</td>
</tr>
<tr>
<td>(ca. 1300 µg/L Pt)</td>
<td>b) MWCNT-Pt(IV)</td>
<td>13.7 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>c) mixture of MWCNT and 1</td>
<td>(11.1 ± 0.61)×10⁻²</td>
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<tr>
<td><strong>background</strong></td>
<td>Untreated control</td>
<td>(3.9 ± 1.00)×10⁻²</td>
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