Yangzhong Liu

Professor , University of Science and Technology of China

Obtained his master degree in 1992 from USTC

Worked in USTC between 1992-1998

Obtained his Ph.D. degree in 2002 from University of Bergen in Norway, studying the interaction between anticancer drugs and DNA.

Pursued three years postdoctoral research at University of California from 2002 -2005 at Davis, on the paramagnetic protein NMR.

In October 2006, he returned to the Department of Chemistry at USTC.

- Researches in this group focus on the bio-inorganic chemistry, more specifically, the structure of biological molecules and their interaction with inorganic compounds.
- Currently, research project in this group is about the interaction between anticancer drugs and DNA, in order to elucidate the mechanism of anticancer drugs, especially those unconventional platinum-based drugs. This study will help the design and synthesis of novel drugs.

- # Cisplatin delivery

- Copper binding promotes the interaction of cisplatin with human copper chaperone Atox1
- Cisplatin binds to human copper chaperone Cox17: the mechanistic implication of drug delivery to mitochondria

- # Novel drugs

- Combating the Drug Resistance of Cisplatin Using a Platinum Prodrug Based Delivery System
- The Ligation of Aspirin to Cisplatin Demonstrates Significantly Synergistic Effect to Tumor Cells

Cisplatin delivery

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Copper binding promotes the interaction of cisplatin with human copper chaperone Atox1†

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Background

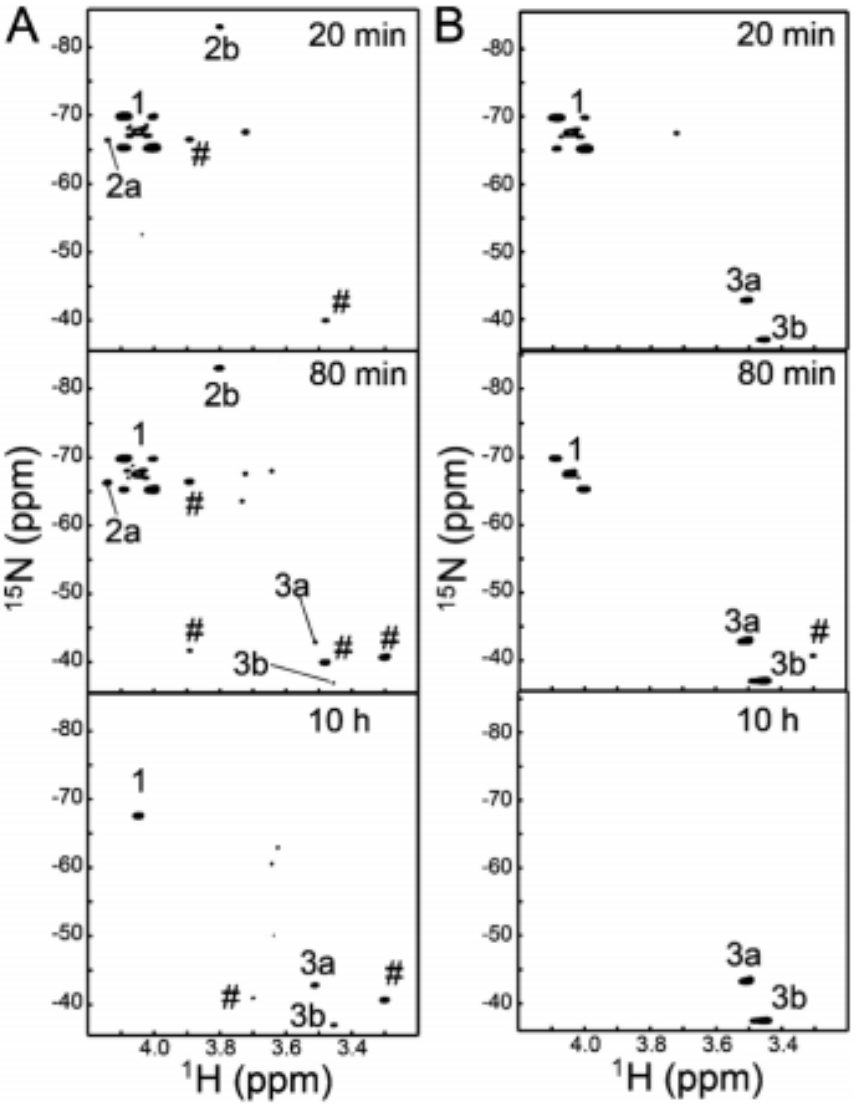
- Cisplatin is one of the most effective anticancer drugs for the treatment of several types of human malignancies.

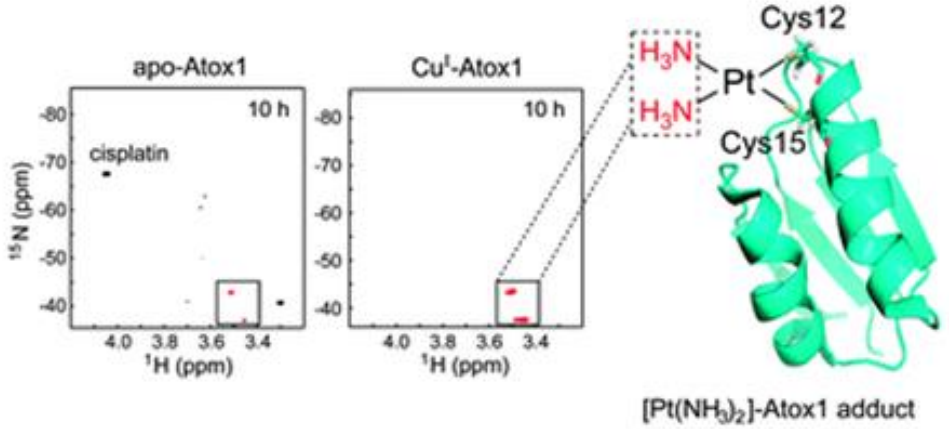
- The application of cisplatin is limited by the drug resistance. Tumor cells can acquire cisplatin resistance through several mechanisms, including reduced cellular drug uptake, drug deactivation in cells, as well as the increased DNA repair and drug efflux from the cells.

- It has been proved that copper transport proteins are also involved in the influx and efflux of cisplatin and relevant to the drug. It has been reported that Atox1 is involved in cisplatin resistance and the loss of Atox1 decreased the cell sensitivity to cisplatin.

- Copper binding increases the thermodynamic stability of Atox1 and results in detectable conformational averaging in the metal binding loop. Copper coordination significantly promotes the platinum binding to Atox1

Using NMR to elucidate





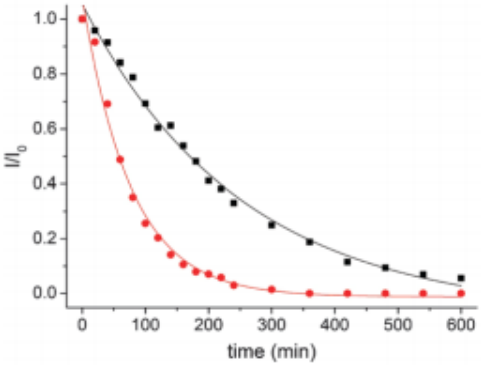


Fig. 2 Plots of the ratio of unreacted cisplatin (*I*) to initial cisplatin (I_0, 0.8 mM) *versus* time for the reactions of cisplatin with apo-Atox1 (black square and line) and Cu^{I}-Atox1 (red circle and line).

Using ESI-MS to confirm

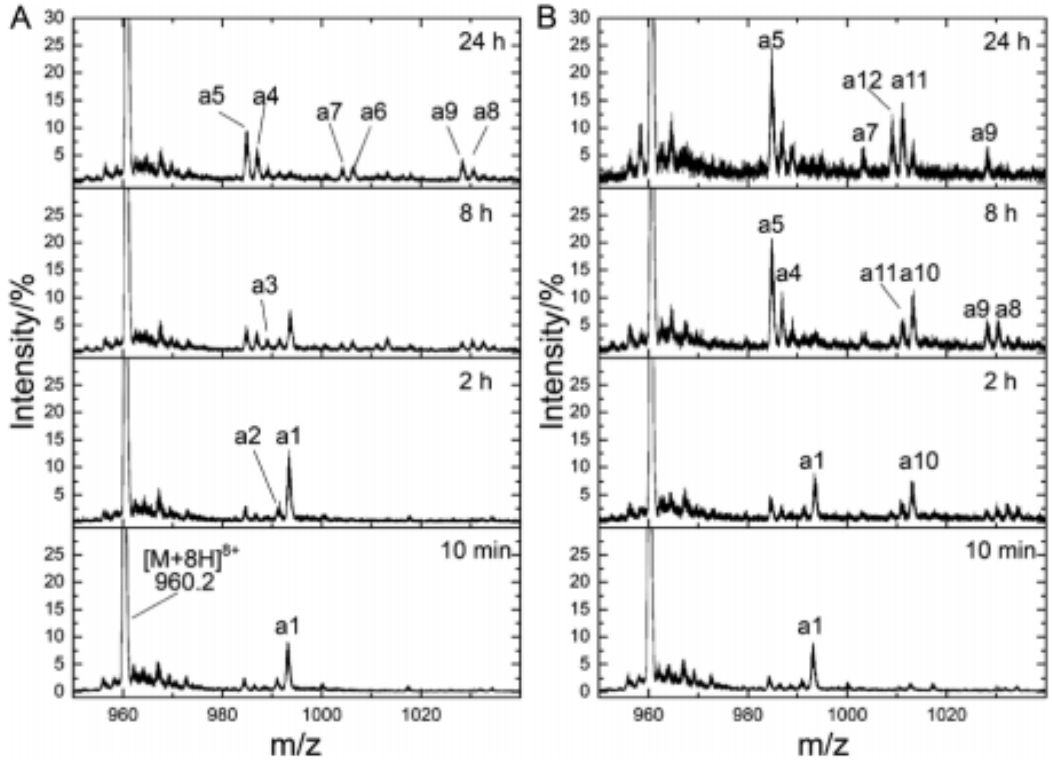


Fig. 3 Time-dependent ESI-MS spectra of Atox1 treated with equimolar cisplatin. (A) Apo-Atox1; (B) Cu^{I}-Atox1. Peaks were assigned to [Atox1 + $Pt(NH_3)_2Cl$ + 7H]$^{8+}$ (**a1**, *m/z* 993.11); [Atox1 + $Pt(NH_3)_2(H_2O)$ + 6H]$^{8+}$ (**a2**, *m/z* 990.90); [Atox1 + $Pt(NH_3)_2$ + 6H]$^{8+}$ (**a3**, *m/z* 988.63); [Atox1 + $Pt(NH_3)$ + 6H]$^{8+}$ (**a4**, *m/z* 986.50); [Atox1 + Pt + 6H]$^{8+}$ (**a5**, *m/z* 984.37); **a6–a9** are assigned to Atox1–Pt/DTT adducts; **a10–a12** correspond to Pt–Atox1 adducts with 2 : 1 stoichiometry. The relative intensity is referred to the apo-Atox1 [Atox1 + 8H]$^{8+}$ as 100%.

cisplatin binds to the copper coordination sites

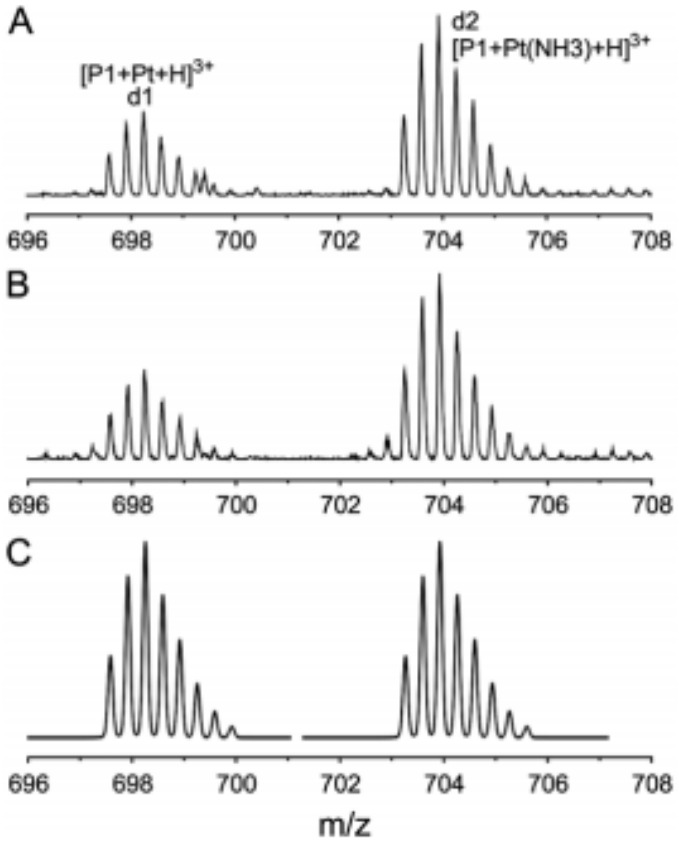


Fig. 4 Selected ESI-MS spectra of platinated peptides obtained from trypsin digestion of Atox1. Protein samples were treated with cisplatin for 4 h prior to the trypsin digestion. (A) Apo-Atox1; (B) Cu^{I}-Atox1. (C) The calculated isotopic distribution of corresponding peaks. P1 represents the peptide $H^{4}EFSVDMTC^{12}GGC^{15}AEAVSR^{21}$.

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COMMUNICATION

Cisplatin binds to human copper chaperone Cox17: the mechanistic implication of drug delivery to mitochondria†

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Background

- Mitochondria play essential roles in regulating cell apoptosis via various signaling pathways, including the change in mitochondrial transmembrane potential, the release of pro-apoptotic proteins, changes in electron transport and the alteration of cellular redox potential.

- It has been proposed that mitochondria are also associated with the mechanism of cisplatin, and the direct interaction of cisplatinwithmitochondria induces apoptosis. A recent study shows that the delivery of platinum drugs to mitochondria promotes the apoptosis of cancer cells. Indeed, platinum has been found to be highly accumulated in mitochondrial proteins.

- However, it is unknown how cisplatin was delivered to mitochondria. The copper chaperone Cox17, which is associated with copper transfer to mitochondrial proteins, could also be involved in cisplatin transfer to mitochondria

Correlation of the Cox17 with the platinum accumulation and the cytotoxicity

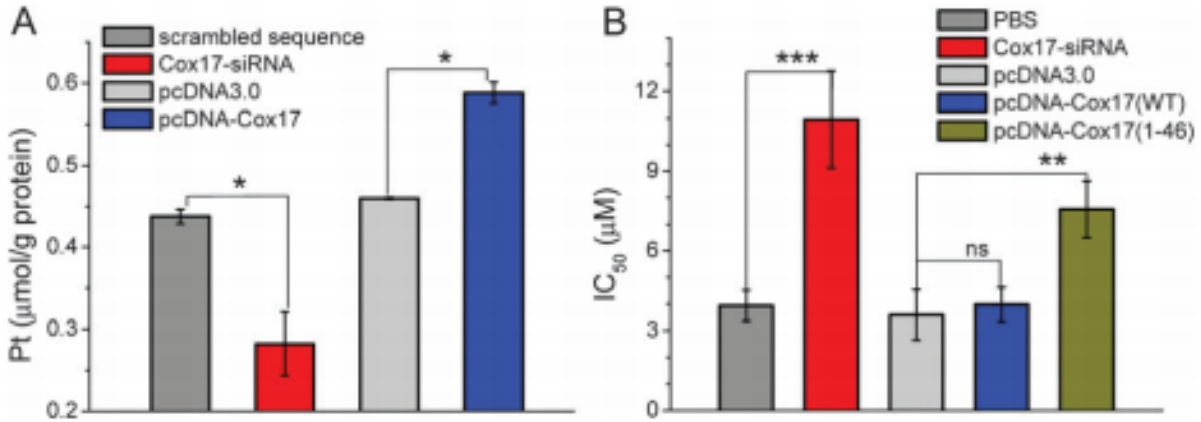
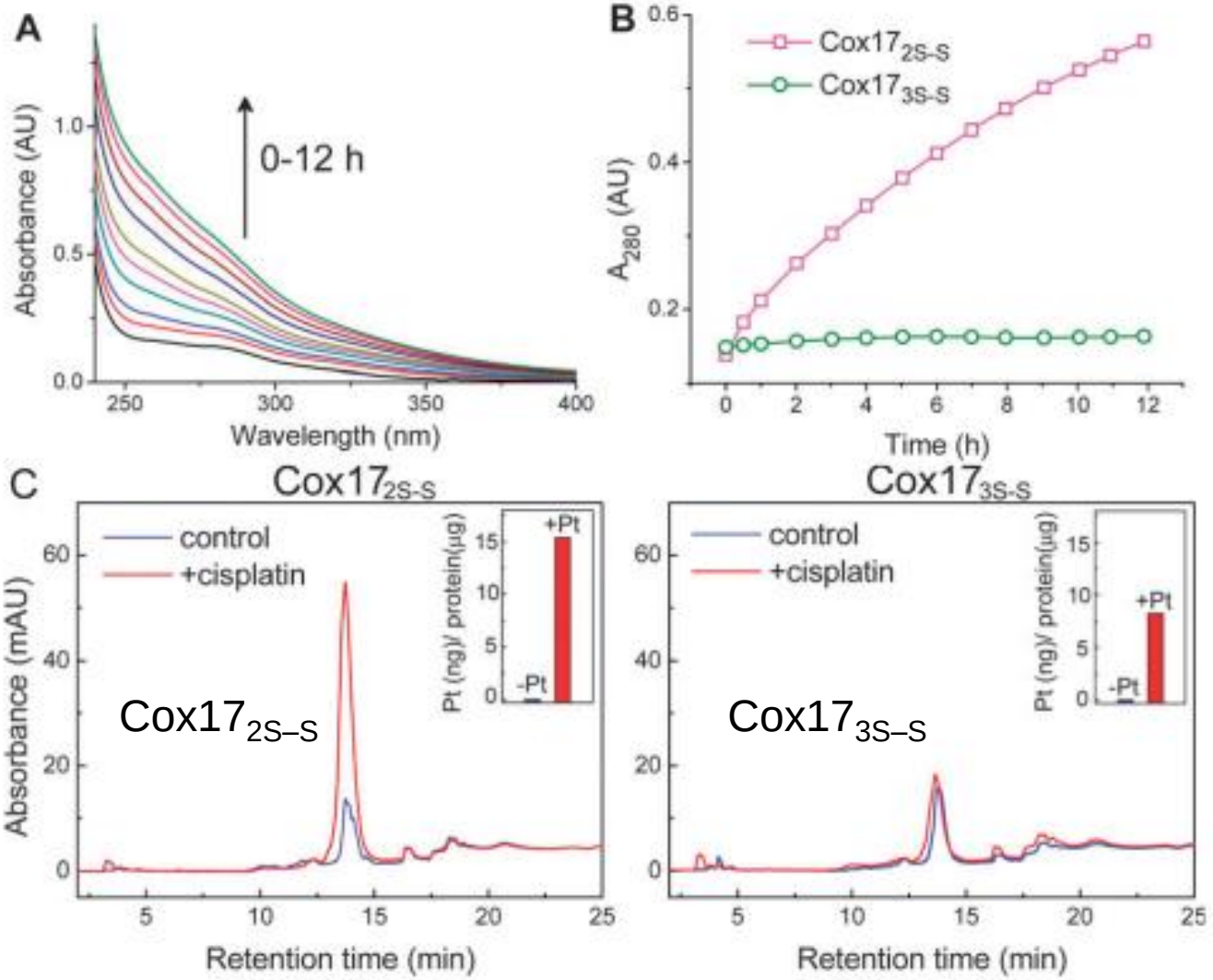


Fig. 1 Correlation of the Cox17 level with the platinum accumulation in mitochondria and the cytotoxicity of cisplatin. (A) The platinum accumulation in mitochondria of the HeLa cells with different levels of Cox17 expression. Cells were incubated with 10 μM cisplatin for 10 h prior to the measurements. (B) Effect of the Cox17 level on the cytotoxicity of cisplatin (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

roles. In addition to the delivery of cisplatin to mitochondria, the over-expressed Cox17 could also consume more cisplatin and reduce the drug interaction with the DNA target. These two opposite contributions of Cox17 caused the effect of the over-expression undetectable.

Reaction of Cox17 with cisplatin



The platination adducts of Cox17 were characterized using ESI-MS

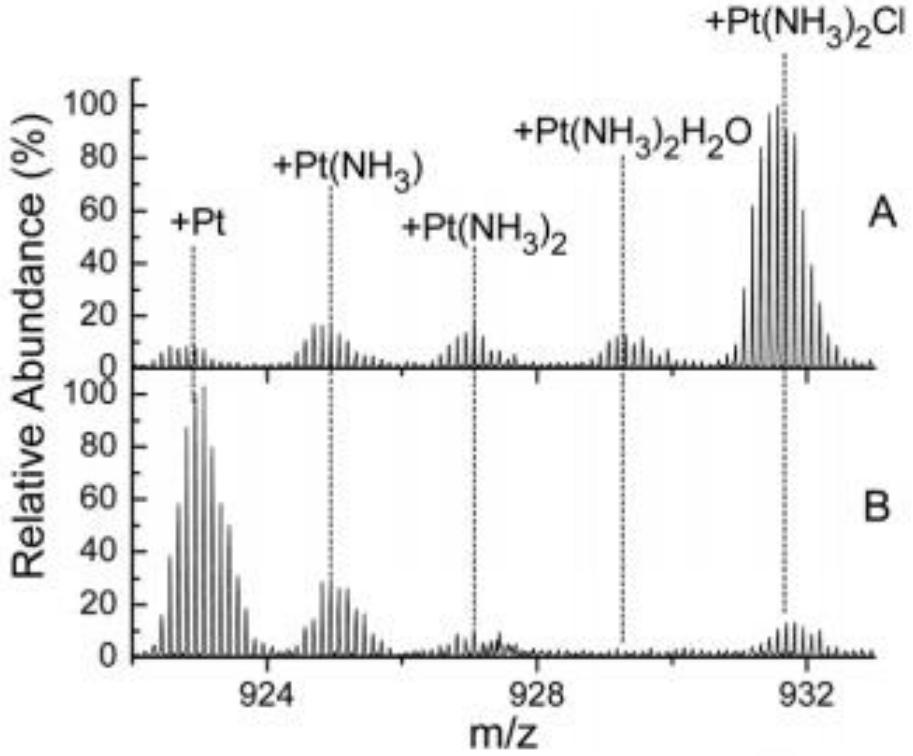


Fig. 3 ESI-MS spectra of platinated Cox17 adducts with 8+ charged peaks. (A) $Cox17_{3S-S}$; (B) $Cox17_{2S-S}$. The Cox17 proteins were incubated with 5 molar equivalent of cisplatin at 37 °C for 6 h prior to the ESI-MS measurements.

The binding of cisplatin to the copper binding site

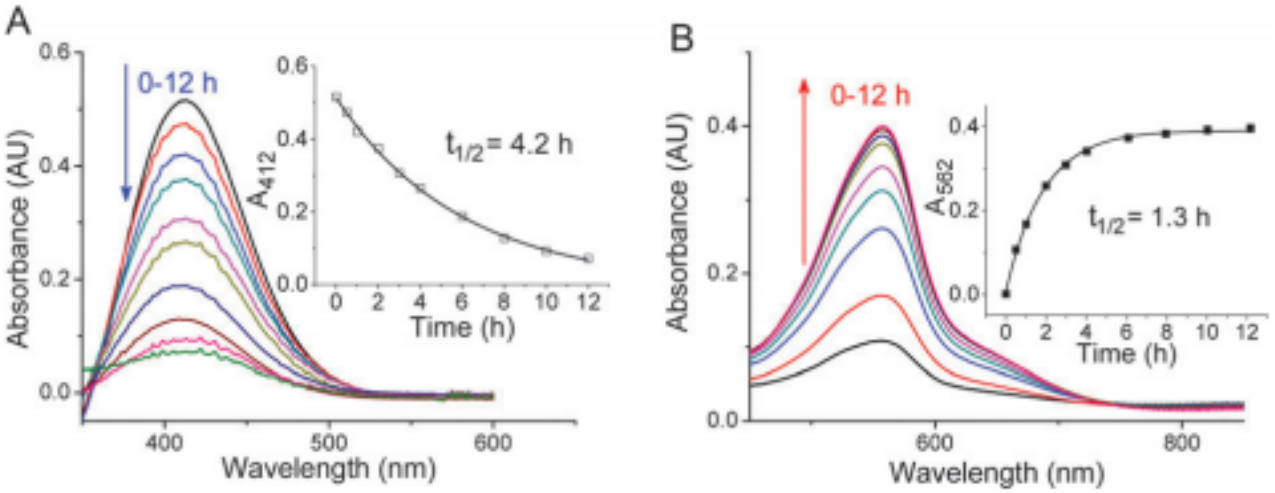


Fig. 4 (A) Time-dependent Ellman's assay on apo-$Cox17_{2S-S}$ that reacted with cisplatin. The inset shows the absorbance at 412 nm (A_{412}) *versus* reaction time. (B) Time-dependent copper release assay on Cu^{I}–$Cox17_{2S-S}$ in reaction with cisplatin. The inset shows the absorbance at 562 nm (A_{562}) *versus* reaction time. Both reactions were performed in 100 μM Cox17 and 200 μM cisplatin in 20 mM MES (pH 6.0) at 37 °C.

Novel drugs

Angewandte Communications

Platinum Drugs

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Combating the Drug Resistance of Cisplatin Using a Platinum Prodrug Based Delivery System**

Yuanzeng Min, Cheng-Qiong Mao, Siming Chen, Guolin Ma, Jun Wang, and Yangzhong Liu**

- Drug delivery systems have drawn particular attention in recent years because they can facilitate the delivery of platinum-based drugs, thus enhancing drug efficacy.

- They have reported that PEGylated gold nanorods (PEG-GNRs) can facilitate the delivery of platinum(IV) prodrugs and significantly enhance the cytotoxicity of these prodrugs in tumor cells.

- This conjugate facilitates the delivery of the platinum-based drug into cells through endocytosis. Additionally, the platinum(IV) prodrug is less susceptible to deactivation by the detoxification protein MT and the peptide GSH, which were found in high concentrations in A549R cells

The cytotoxicity assays of platinum complexes

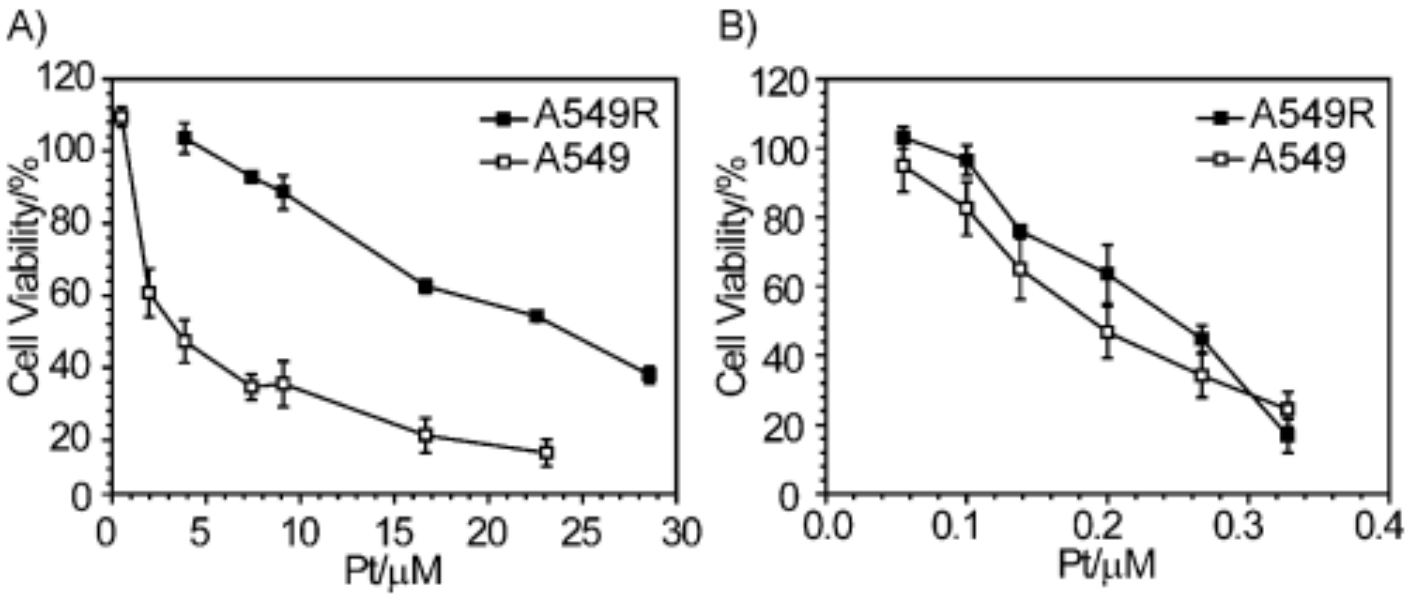
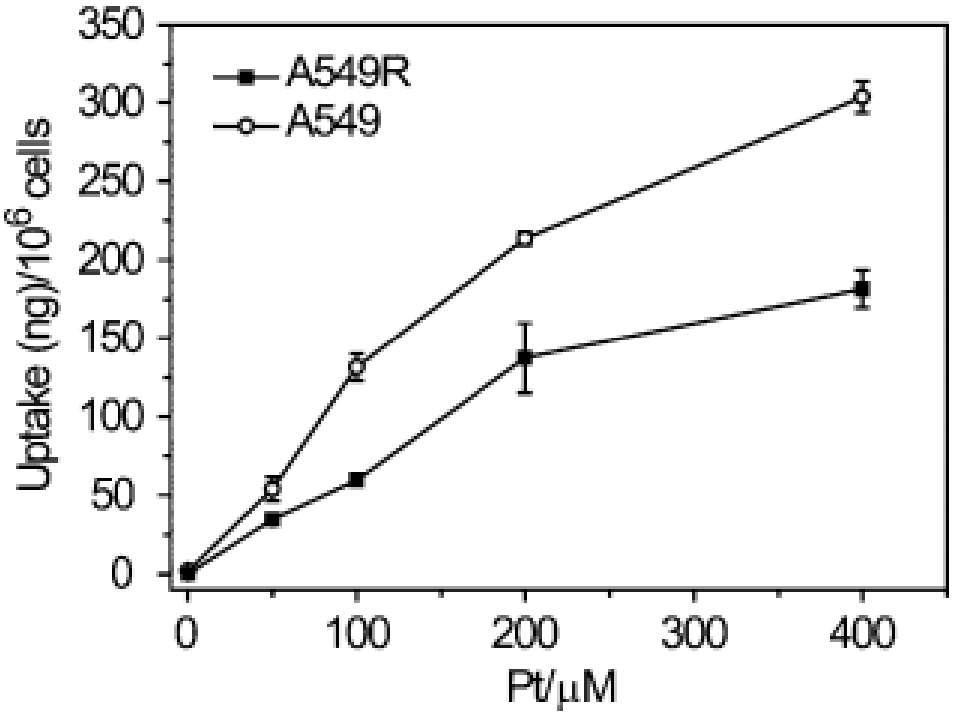


Figure 1. The cytotoxicity assays of platinum complexes. A) Cisplatin. B) Pt-PEG-GNRs conjugate. The A549 cells are denoted by (□) and the A549R cells are denoted by (■). Cells were incubated for 72 h with the platinum compounds.

The platinum uptake in A549 and A549R cells



Comparison of Ctr1 in A549 and A549R cells

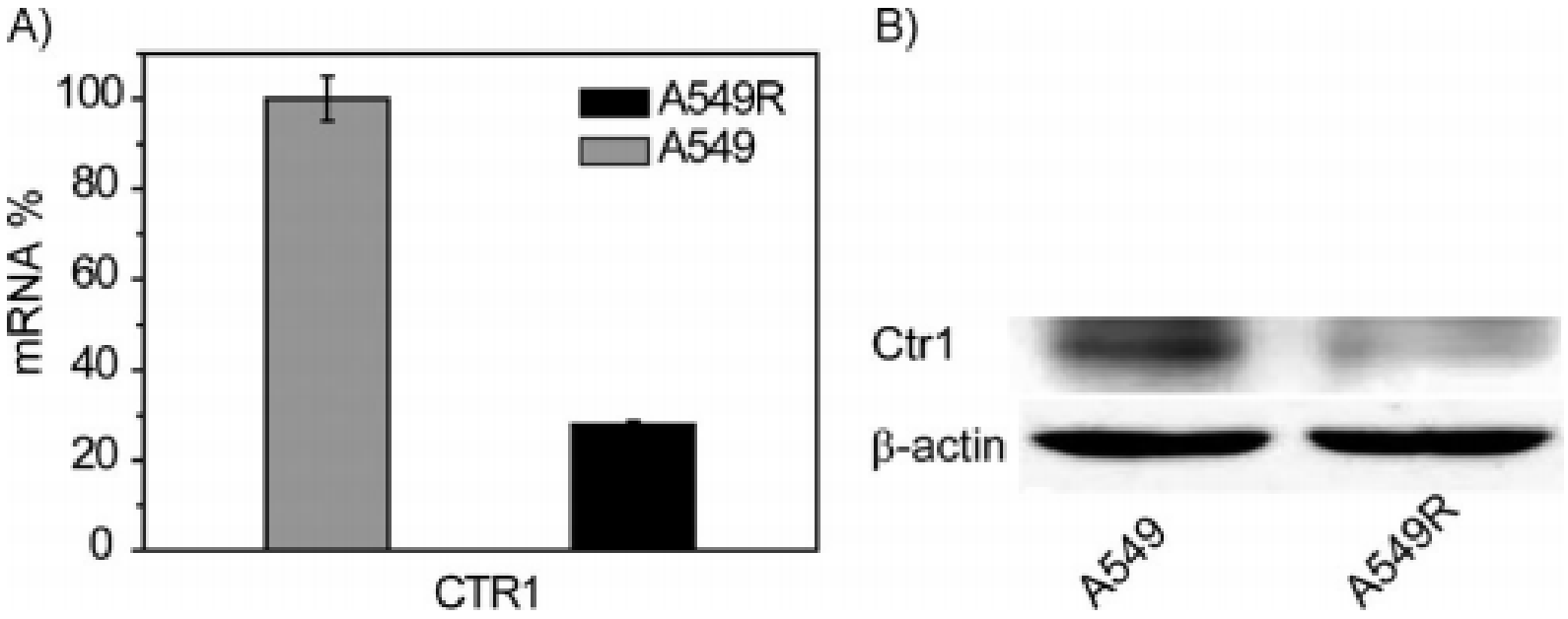


Figure 3. Comparison of Ctr1 in A549 and A549R cells. A) The mRNA level of Ctr1 measured by real-time PCR. B) Western blot analysis of the Ctr1 level.

Platinum-based drug into cells through endocytosis

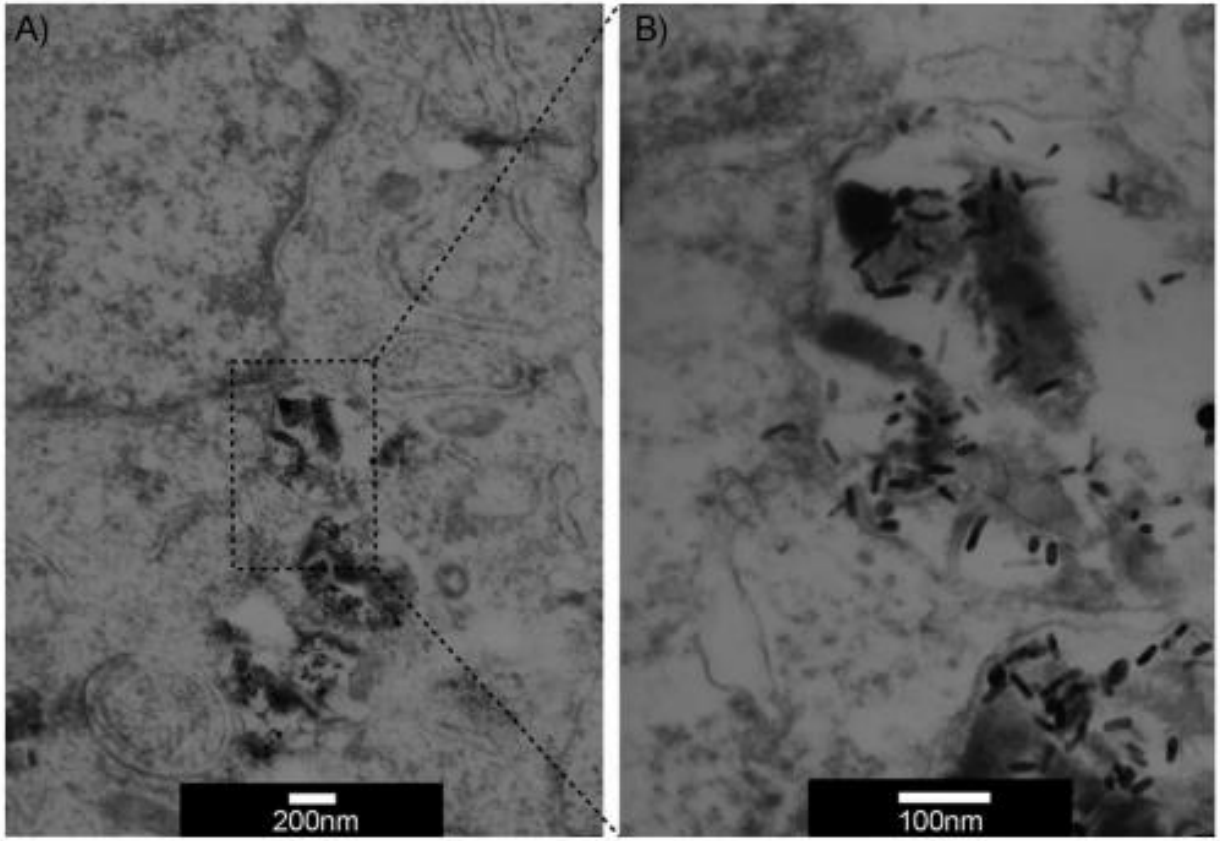


Figure 4. TEM image of endocytosis of the Pt-PEG-GNRs conjugate in A549R cells. A) Pt-PEG-GNRs conjugates trapped within endosome (scale bar:200 nm). B) Enlarged area from (A; scale bar:100 nm).

Comparison of the MT level in A549 and A549R cells

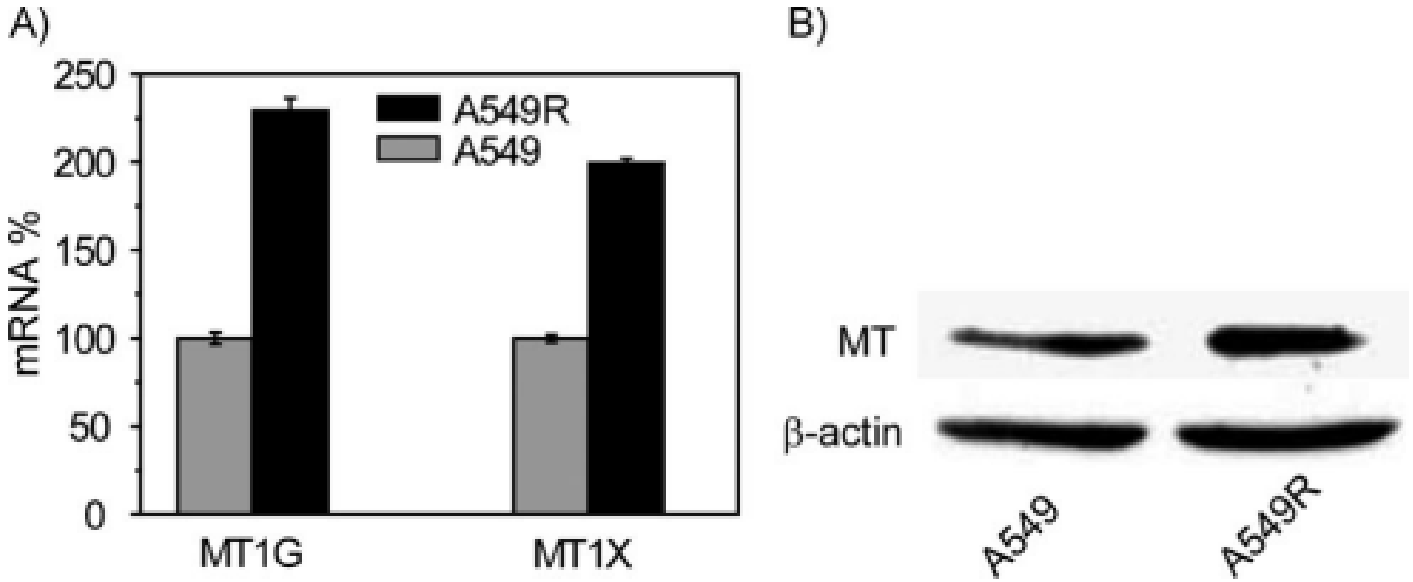


Figure 5. Comparison of the MT level in A549 and A549R cells. A) The mRNA level of MT1G and MT1X measured by real-time PCR. B) Western blot analysis of the MT level.

GSH or MT less reactive with platinum prodrug

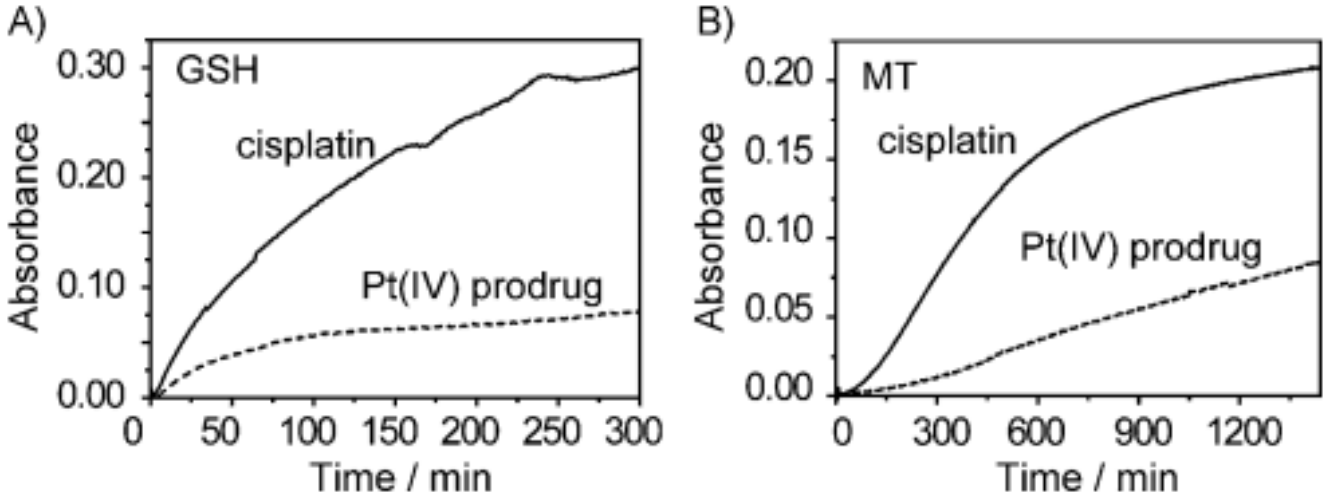


Figure 6. Time course of reactions of platinum complexes with GSH or MT at 37 °C, pH 7.0. A) Reaction of GSH (16 mM) with cisplatin (32 μM, solid line) and the platinum(IV) prodrug (*c*,*c*,*t*-[Pt-$(NH_3)_2Cl_2(O_2CCH_2CH_2CO_2H)_2$]; 32 μM, dashed line). B) Reaction of MT (10 μM) with cisplatin (20 μM, solid line) and the platinum(IV) prodrug (20 μM, dashed line). Reactions were monitored by observing the increase in the absorption at 260 nm for the GSH reaction (A) and at 285 nm for the MT reaction (B).

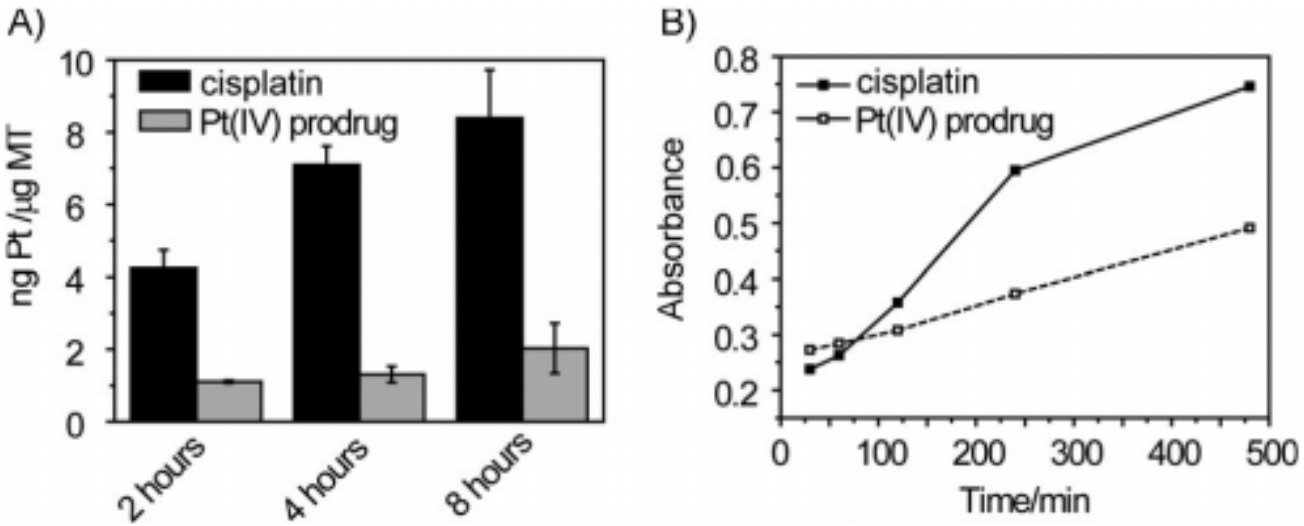


Figure 7. Time-dependent platination of MT by cisplatin and the platinum(IV) prodrug. A) The amount of platinum bound to MT after different reaction times using cisplatin (black bars) and the platinum(IV) prodrug (grey bars). The platinated MT was purified with an ÄKTA purifier equipped with a HiTrap desalting column, and the platinum content was measured by ICP-MS. B) The amount of Zn^{II} released by the platination of MT was recorded for different reaction times. The reactions between MT (10 μM) and both cisplatin (20 μM, solid line) and the platinum(IV) prodrug (20 μM, dashed line), in the presence of PAR (0.1 mM), were monitored by observing the absorption at 500 nm, which is a measure of the amount of $Zn(PAR)_2$ formed. The control experiment confirmed that no reaction occurred between MT and PAR in the absence of platinum complexes.

Journal Name

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COMMUNICATION

The Ligation of Aspirin to Cisplatin Demonstrates Significantly Synergistic Effect to Tumor Cells

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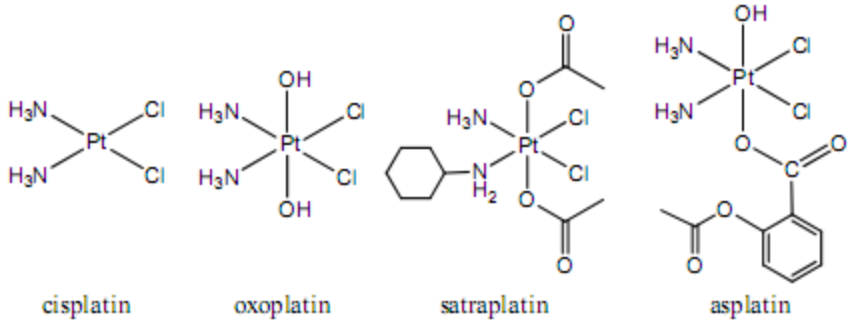
Background

- Aspirin, also known as acetylsalicylic acid, is one of the most widely used medicines in the world. It possesses antipyretic, analgesic and anti-inflammatory activities.

- The Pt(IV) prodrugs possess two additional axial ligands, which offer an unique approach for the drug design to modify the pharmacokinetic effects of prodrugs, including lipophilicity, redox potential and biological activity.

- In this work, we designed an aspirin ligated Pt(IV) complex by tethering aspirin to oxoplatin to generate a satraplatin-like prodrug, asplatin.



Scheme 1. The structure of cisplatin, satraplatin, oxoplatin and asplatin.

Inhibitory effect of asplatin on cancer cells

	HeLa	MCF-7	HepG2	A549	A549R	RF
Cisplatin	4.51 ± 1.70	10.10 ± 1.91	8.25 ± 0.7	9.65 ± 0.66	51.92 ± 6.65	5.38
Oxoplatin	21.45 ± 3.08	78.64± 14.89	36.03 ± 6.34	51.16 ± 4.99	203.8 ± 32.5	3.98
Oxoplatin + Aspirin	23.01 ± 5.12	78.19± 12.02	36.78 ± 6.61	52.00 ± 7.02	169.5 ± 26.0	3.26
Asplatin	0.45 ± 0.16	3.10 ± 0.3	1.49 ± 0.24	8.53 ± 0.42	9.48 ± 1.31	1.11
Fold increase[#]	10.02	3.26	5.56	1.13	5.48	

IC_{50} ratio of cisplatin/asplatin

Reactions of asplatin with DNA upon the reduction

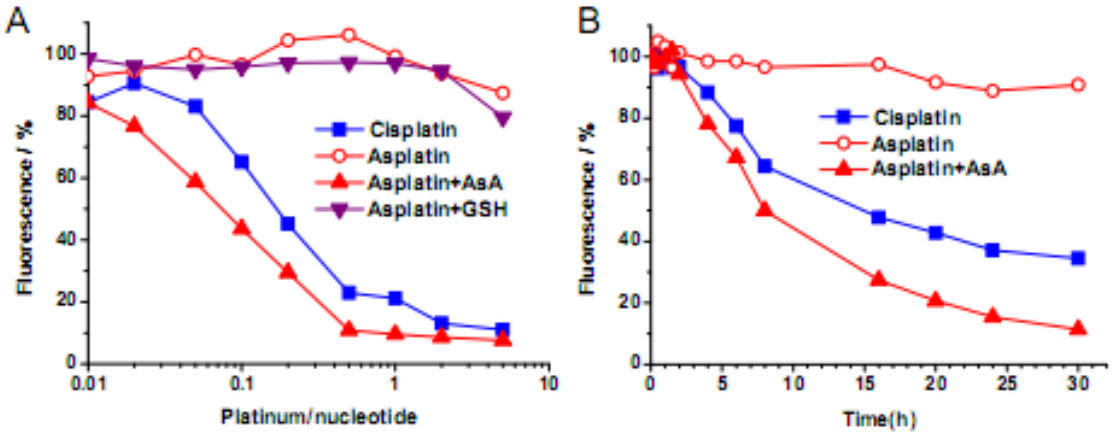


Fig. 1. Reactions of asplatin with DNA upon the reduction. (A) The platination of DNA with different ratio of Pt/nucleotide ratio (r_i). (B) Time dependent platination of DNA with the r_i ratio of 0.2. All reactions were performed on 0.01 mg/ml DNA in 10 mM $NaClO_4$ and 10 mM phosphate buffer (pH=7.4) at 37 °C for 36 h. EtBr (0.04 mg/mL) was added before the fluorescence measurement. The excitation wavelength was 530 nm and the emission was recorded at 592 nm.

similar to that of cisplatin (Fig. S6). Taken together, these results indicate that asplatin functions as the prodrug of cisplatin upon the activation with the reductant AsA.

Cellular Drug Uptake and DNA platination

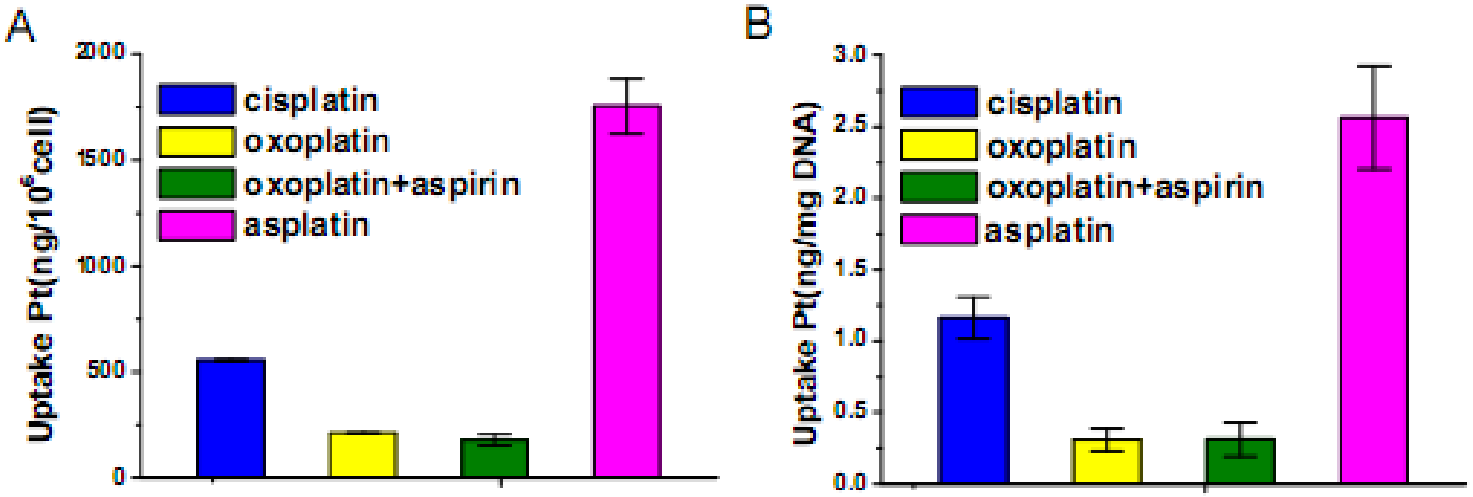


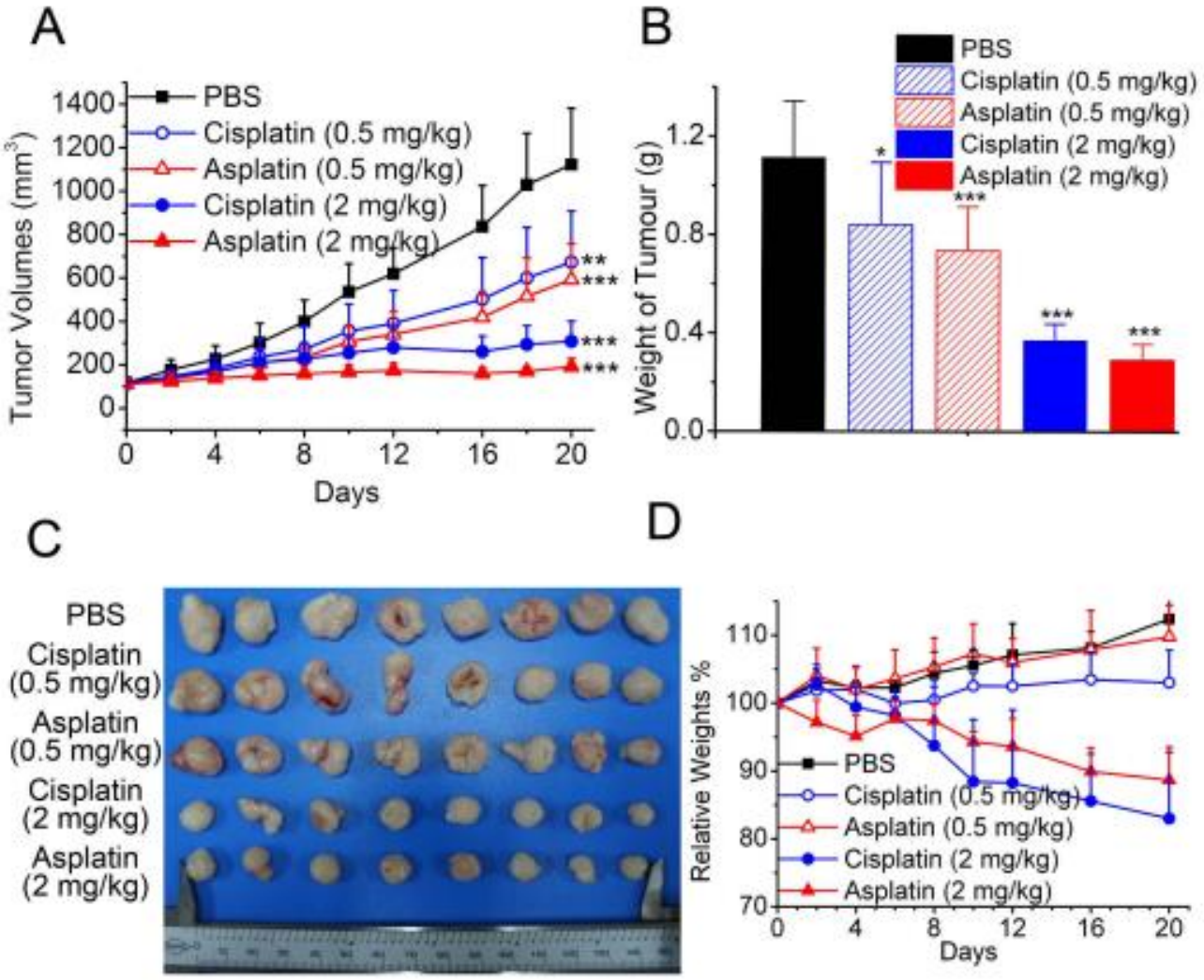
Fig. 2. Cellular Drug Uptake and DNA platination. Platinum in HeLa cells was determined using ICP-MS after the 3 h treatment of 100 μM platinum complex. (A) Platinum in whole cells. (B) Platinum in the DNA.

Quantification of apoptosis in HeLa cells

	Early apoptosis, %	Late apoptosis, %	Necrosis, %	Sum, %
Aspirin[#]	3.37	1.17	0.39	4.93
Cisplatin	7.98	4.38	1.34	13.7
Oxoplatin	5.87	1.63	0.63	8.03
Oxoplatin + Aspirin	6.24	1.86	0.26	8.36
Asplatin	27.8	13.6	2.36	43.76

All compounds are in 1 μM and the incubation time is 30 h.

In vivo antitumor activity of asplatin and cisplatin



Thank You!