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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Exafs Study of Pt anticancer drugs in cells and tissues.	**Experiment number**: MD 425
Beamline:	**Date of experiment**: from: 08.04.2009 to: 12.04.2009	**Date of report**:
Shifts:	**Local contact(s)**: Sergey Nikitenko	*Received at ESRF:*

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Report:

Aims of the experiment and scientific background

Cisplatin is among the most important chemotherapeutic agents ever developed. It is a critical component of therapeutic regimens in a broad range of malignancies [1]. However, more than three decades after its clinical introduction, the exact mechanism of cisplatin action on tumor cells is not fully understood. It is commonly accepted that cisplatin kills the cancer cell by binding covalently and essentially irreversibly to the nuclear DNA. The binding occurs in the major groove of the DNA where the two cis-chloride ligands are replaced by two N7 atoms of two adjacent guanines on the same strand of the DNA. This distortion of the DNA triggers cellular responses that in favorable cases end in the induction of apoptosis [2]. It is noteworthy that only approximately 1% of the cellular platinum ends up bound to the nuclear DNA. Yet, nearly 99% of the research efforts have been directed to studying all aspects of Pt-DNA lesions.

Pt-DNA adducts formed in the different cancer cells are expected to be the same, but why is cisplatin very effective only against some types of cancer (testicular, ovarian). It seems, the Pt-non-DNA adducts are responsible for that. Very little is known about these interactions and most of the information pertaining to the mode of action is derived from simplistic aqueous models. Based on model studies, and basic inorganic chemistry, it is expected that most of the cellular cisplatin will bind to sulfur containing ligands and especially to glutathione (GSH), a thiol containing tripeptide that exists in mM concentrations. There is one report claiming that 60% of the cellular platinum is bound to GSH [3]. Yet, in recent [^{1}H,^{15}N] 2D-NMR studies that we conducted by labeling cisplatin with ^{15}N ammine ligands, and monitoring the interactions with aqueous extracts of cancer cells, contrary to expectations, we were unable to detect any sulfur binding to cisplatin. This might be due to certain limitation of NMR spectroscopy, which would make it impossible to observe signals of cisplatin bound to large biopolymers.

The interaction of cellular sulfur with Pt anticancer agents is believed to play a major role, and could serve to either inactivate the Pt drugs (by binding to thiols) or to activate drugs such as carboplatin (by binding to thioether).

Results

EXAFS L_3 Pt-edge were collected on all samples at BM26A beamline (ESRF). Samples of cells, cell extracts and mice treated tumors were measured. Samples were placed in Kapton sealed holders and kept frozen at all times. Measurements were performed at 15 K using a cryostat and data was collected using a multielement Ge solid-state detector.

The April 2009 beamtime has been dedicated on two projects:

1) In March 2008 we monitored the cellular reduction of Pt(IV) complexes in mouse cancer cells, the cells were treated with the Pt(IV) complexes: ct-[$Pt(NH_3)_2(OH)_2$(oxal)] and ct-[$Pt(NH_3)_2(OAc)_2$(oxal)] at a concentration of 100 mM. In generell a reduction of Pt(IV) compounds is seen over time.

2) Hall et. al. [4] showed that the XANES spectra of Pt(IV) and Pt(II) complexes have absorption profiles that differ primarily in the height of their edges, with the former displaying a much higher edge. The authors also showed that the ratio of a/b (Fig. 1 right) is characteristic of the oxidation state and that this ratio correlated linearly with percentage of each of the oxidation states in mixtures. We decided to employ XANES to check whether the intermediates in the reduction of *ctc*-[$PtCl_2(^{13}CH_3CO_2)_2(^{15}NH_3)$(NBA)] (NBA = n-butylamine) by ascorbate, peak 2 in fig. 1 left, is a Pt(II) or Pt(IV) complex. The reduction was monitored by [$^1H,^{13}C$] HSQC and was allowed to proceed until the peak of the Pt(IV) starting material comprised approximately 10-15 % of the total HSQC signal. At this point the strongest peak in the spectrum was that of the intermediate (peak 2 fig. 1 left). The sample was quickly frozen in liquid nitrogen and lyophilized, and a boronitride pellet was analysed by XANES. The XANES spectra showed that approximately 77 % of the platinum is in the form of Pt(II) and 23 % is in the form of Pt(IV). These results support the NMR data that suggest that the intermediate is a Pt(II) complex [11].

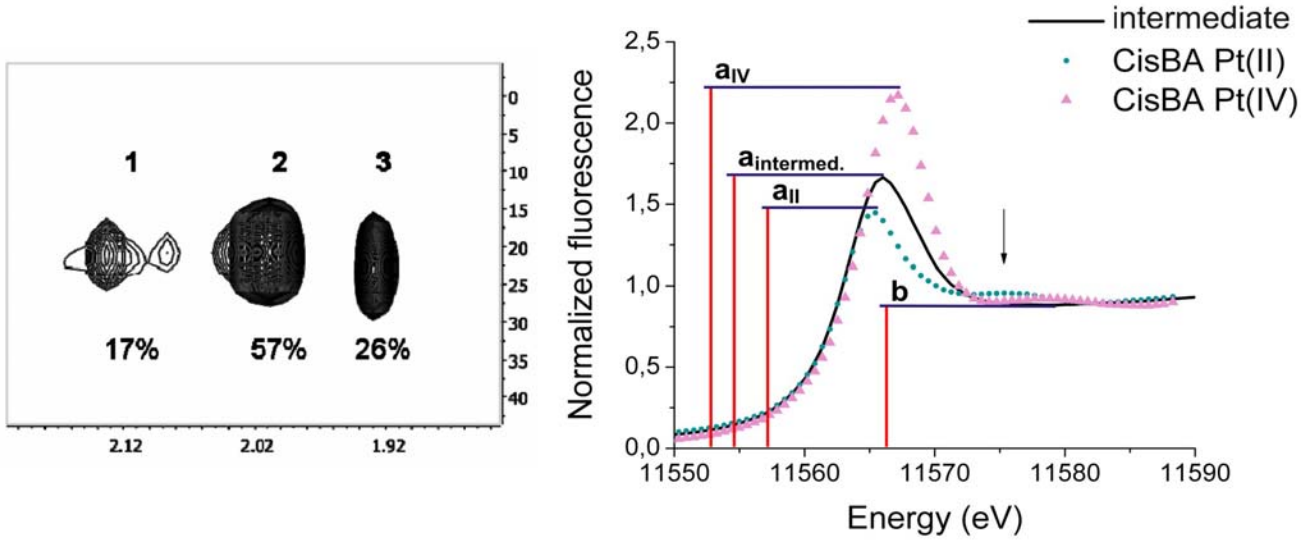

Fig. 1 left: The [$^1H,^{13}C$] HSQC spectrum of the reduction of *ctc*-[$PtCl_2(^{13}CH_3CO_2)_2(^{15}NH_3)$(NBA)] by 10 eqiv. of sodium ascorbate in phosphate buffer at 37 ºC showing around 17% starting material and primarily the Pt intermediate.

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