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ARTICLE

Novel *in situ* methodology to observe the interactions of chemotherapeutical Pt drugs with DNA under physiological conditions

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The binding of the antitumor drug cisplatin with DNA was determined by means of *in situ* resonant inelastic X-ray scattering (RIXS) spectroscopy. Because of the penetrating properties of hard X-rays, we could determine, under physiological conditions, the identity and number of platinum complexes present. *In situ* RIXS revealed that under physiological conditions, water molecules replace chloride ligands owing to drug hydration. The subsequent interaction with DNA, led to the bonding of the aqua complexes into the DNA structure with simultaneous loss of the coordinating water and chloride ion. The data analysis reveals that Pt is coordinated by two adjacent guanines giving *cis*-[Pt(NH$_3$)$_2$\{d(GpG)-N7(1),-N7(2)\}] upon losing its coordinating water or chloride ligands.

Introduction

Platinum(II) drugs and in particular *cis*-diamminedichloroplatinum(II) (cisplatin) are used routinely in oncology to treat testicular, ovarian, head and neck tumors,[1] and most recently bladder, cervical, and small cell lung tumors.[2] Other platinum drugs have been developed and marketed, such as carboplatin, [*cis*-diammine-1,1'-cyclobutanedicarboxylatoplatinum (II)], which is less toxic than cisplatin but possesses the same spectrum of antitumor activity and oxaplatin, used in combination therapy with 5-fluorouracil for colon cancer.[3] Satraplatin, picoplatin, nedaplatin, and triplatin have been developed to varying extents.[4]

The understanding of the regio-, stereochemistry, and specificity of the platinum binding site is essential to develop as well as to improve efficiency of platinum drugs and in overcoming resistance. This requires a combined effort from molecular biology, cell biology, immunology and bioinorganic chemistry. The molecular mechanism of action provides considerable information on how drugs, and in particular cisplatin, induces the death of a tumor. The main target of cisplatin in the tumor cell is DNA,[5] and the most common binding modes of DNA to cisplatin include DNA interstrand crosslinks,[6] DNA intrastrand crosslinks,[7] and protein–DNA crosslinks.[8] The most common binding mode of cisplatin to DNA involves the loss of two chloride ions and formation of two Pt-N bonds with N(7) atoms of two adjacent guanosine on the same DNA strand, i.e., intrastrand crosslink.[9] For stereochemical reasons, the trans isomer cannot form intrastrand crosslinks. The X-ray structure of the adduct of cisplatin bonded to the dinucleotide d(GpG) was published in 1985,[10a] and later studies confirmed the formation of intrastrand crosslinks (*cis*-[Pt(NH$_3$)$_2$\{d(GpG)-N7(1),-N7(2)\}]) in interactions with DNA.[10b-d]

Binding of DNA to platinum drugs, particularly cisplatin, has been studied by a plethora of methods,[11] including indirect methods such as nuclear magnetic resonance (NMR),[12] circular dichroism,[13] biochemical methods,[14] theoretical calculations[15a,b] and kinetic measurements,[15c,d] or directly by crystal structure determination of isolated adducts.[10] However, *the direct observation* of the coordination of platinum(II) compounds to DNA under physiological conditions is rare.

^{195}Pt NMR spectroscopy gives direct information about the platinum coordination but requires enrichment of the ^{195}Pt isotope thereby modifying the drug composition.[16] Atom-based analytical techniques, such as electron microscopy, X-ray analysis, synchrotron radiation induced X-ray emission, micro-X-ray absorption near edge structure spectroscopy, and X-ray fluorescence, are able to map the cellular distribution of the elements with high accuracy but are limited in respect to chemical speciation, e.g. unable to distinguish between intact and reacted drug.[17] Extended X-ray absorption fine structure (EXAFS) is a technique able to probe local environment of the platinum ion in solution. However, it requires concentrated samples and/or relatively long acquisition times, from several hours to days, in order to attain statistically meaningful

results.[16,18] The very low solubility of cisplatin (1-2 mg/mL at room temperature)[11] prevents the use of concentrated solutions. Furthermore, EXAFS is a structural technique with relatively low chemical resolution, i.e., unable to resolve between Pt bonded to O or C or N due to similarity in bond distance and atomic number.[19]

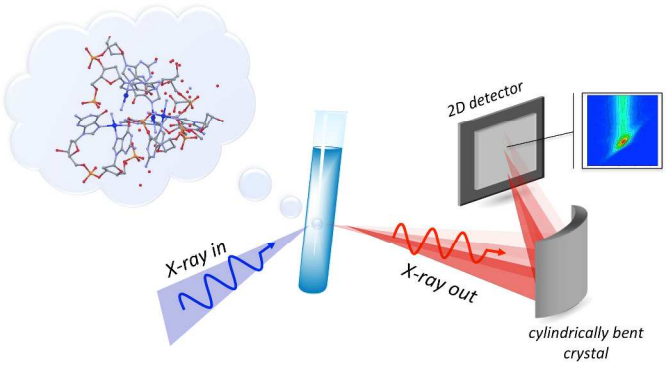

Fig. 1 Schematic representation of the experimental setup used to acquire *in situ* RIXS maps.

Herein, we report a strategy to follow the coordination of platinum-based antitumor drugs by DNA under physiological conditions, namely by means of *in situ* resonant inelastic X-ray scattering (RIXS) spectroscopy. RIXS is an atom specific photon-in photon-out scattering technique where the incoming photon excites the atom to an intermediate state, which decays to its final state by the emission of a photon. RIXS combines X-ray absorption, which reflects the unoccupied density of states of an atom and X-ray emission processes, which characterize its occupied density of states. The technique has been applied to a variety of systems, enabling a detailed investigation of the local electronic structure of the metal of interest.[20] Furthermore, because of the hard X-ray light penetration, RIXS can be performed *in situ*, thus providing information about occupied and unoccupied electronic states under ambient/operational conditions, i.e., direct observation of species at the molecular level in low (biological) concentrations without the need for preconcentration, extraction or crystallization. [21]

Recently, Sá et al. demonstrated that RIXS is extremely sensitive to changes in metal coordination,[22] and it can be carried out with enough time-resolution (up to second) to follow chemical transformations in real time without loss of chemical sensitivity.[23] This was primarily due to the implementation of high-resolution dispersive type spectrometers, such as the von Hamos at fast scanning monochromator beam lines like the SuperXAS at the Swiss Light Source. By means of *in situ* RIXS measurements at the Pt L_3-edge, we were able to determine reaction products from cisplatin hydration and the subsequent binding with DNA. Now that the technique can be validated with a well understood system it can be adapted to the study of any other platinum-based drug, making it an important complementary and validating technique to the methods currently used. Figure 1 shows the experimental concept used to perform *in situ* RIXS measurements.

Results and discussion

Figure 2 shows the RIXS maps measured *in situ* around the Pt L_3-edge for cisplatin in water, buffer, and with DNA. The central resonance, located at incident beam energy of 11565eV, reflects the density of unoccupied Pt *5d* electronic states. It should be mentioned that RIXS spectrum of solid cisplatin was as the same as cisplatin in deionized water. We note that higher intensities in the presented RIXS planes correspond to higher un-occupancy values in the *5d* orbitals, which are sensitive to the type of coordinating ligand, bonding strength and angle. According to Nørskov's model,[24] the availability of valence orbitals to form chemical bonds depends on their electron occupancy and energy. Consequently, a change in the platinum binding site can be easily detected by measuring its density of states in the *5d* orbital. To better understand the experimental RIXS maps, FEFF9.0 calculations[25] were performed. Crystal structure data were used as input files for the calculations when available, such for the cases *cis*-$[PtCl_2(NH_3)_2]$ (CUKRAB) and *cis*-$[Pt(NH_3)_2\{d(GpG)$-N7(1),-N7(2)$\}]$ (CCDC 562451). The structures were retrieved from the Cambridge Crystallographic Data Centre.[26] Since we were unable to retrieve the crystal structure of the cisplatin hydration products, a planar structure containing a water molecule $\{$*cis*-$[PtCl(NH_3)_2(H_2O)]^+\}$ or two equidistant water molecules $\{$*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}\}$ instead of the chloride ions was optimized *in silico*.

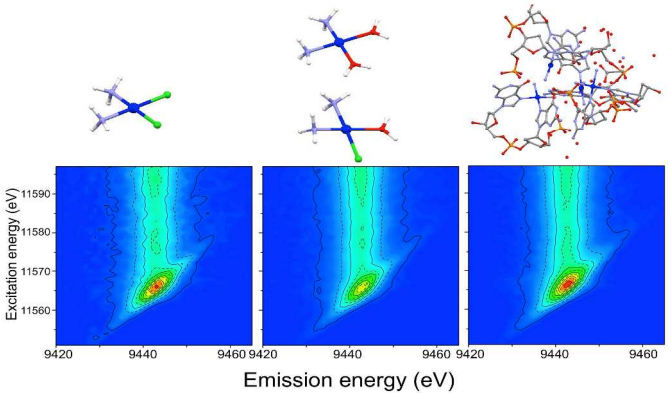

Fig. 2 *In situ* RIXS maps measured around Pt L3-edge of (*left*) cisplatin in deionized water; (*centre*) cisplatin in buffer solution; and (*right*) cisplatin in buffer solution + DNA.

Figure 3 shows the RIXS map difference (Δ-RIXS) resulting from hydration of cisplatin in a buffer solution (physiological serum). The experimental result could be fitted with two possible structures, namely *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ with a chloride ion at 2.30±0.05 Å from the platinum center, and a diaqua complex (*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$) with water molecules located at 2.20±0.05 Å from the platinum center. The optimized structure related to the loss of a single chloride, has the remaining water molecule at around 2.21 Å from the platinum center.

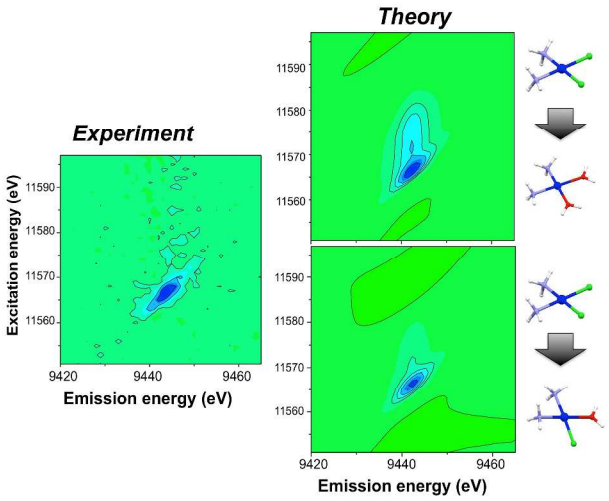

Fig. 3 ΔRIXS maps resulting from cisplatin hydration in buffer solution. The result is compared with theoretical predictions (*top*) loss of two chloride ions; and (*bottom*) loss of a chloride ion.

A comparison with reported structural data and calculations favour interpretation as *cis*-$[PtCl(NH_3)_2(OH_2)]^+$. Thus, the Pt-Cl bond length corresponds with those (2.292(4)-2.3599(4) Å)[27] of a range of square planar Pt^{II} complexes with a *cis*-$PtCl(OH_2)$ unit, Pt-Cl of cisplatin (2.3088(10); 2.313(2) Å), calculated values for $[PtCl(NH_3)_2(OH)_2]^+$ (2.289-2.325 Å), and is consistent with EXAFS data (but which has large errors) for cisplatin in a hydrated liposome. The Pt-OH_2 bond length is more deviant from values (2.07(1)-2.129(2) Å)[27] for the same *cis*-$PtCl(OH_2)$ complexes and that calculated for $[PtCl(NH_3)_2(OH_2)]^+$ (2.124-2.129 Å). However, if assigned as $[Pt(NH_3)_2(OH_2)_2]^{2+}$, agreement with Pt-O (2.036(5)-2.052(5) Å) of square planar complexes with a *cis*-$Pt(OH_2)_2$ array is worse.[28] A calculated value for $[PtCl(NH_3)_2(OH)_2]^{2+}$ (2.121-2.128 Å) is closer, but does not account for effects of solvation by water.

Since mono- and diaqua complexes induce similar effects on Pt *5d* density of states it is difficult to judge which of the structures is prevalent and/or more reactive. Baik et al.[29a] and Cepeda et al.[29b] mentioned that both structures are likely to play a role in the reaction with DNA but kinetic evidence favours *cis*-$[PtCl(NH_3)_2(H_2O)]^+$ as the key intermediate.[16a,16b] The present results show that RIXS measurements coupled with theoretical calculations can provide structural data for platinum drugs under physiological conditions with high precision and at much lower concentrations than is possible with EXAFS. The approach is particularly valuable when crystal structures are available, as is the case for $[PtCl(NH_3)_2)]^+$ and $[Pt(NH_3)_2(OH_2)_2]^{2+}$.

Figure 4 shows the Δ-RIXS resulting from the addition of DNA to the aquated cisplatin. It should be mentioned that DNA is the primary intracellular target and therefore the reason to use it as the model system.[17] RIXS analysis shows a clear change in the unoccupied Pt *5d* density of states, consistent with bond formation. The difference in the resultant RIXS map can be assigned to a single species. The species is related to hydrated $Pt(NH_3)_2$ units bond to the DNA via the N(7) position of adjacent guanines, according to FEFF9.0 calculations, a result which is consistent with data published for isolated crystals.[10] It should be noted that the difference observed in the measured RIXS map could not be fitted with hydrated cisplatin bonded to a single N(7) position. The single bonding site was modeled by cleaving one of the Pt-N(7) bond and replacing it with either Cl or H_2O located at different bond distances.

If all the hydrated cisplatin species is bonded to the DNA, a +145% signal change at an excitation energy of 11568 eV is expected, and since we detected only one species after DNA addition, the effectiveness of drug bonding after 24 h can be estimated by simply dividing the measured signal difference (in this case +95%) by the theoretical expectation (145 %), which equates to 65% of the drug being bonded to the DNA after 24 h incubation. Similar values were reported by Gonnet et al. [29a] who applied HPLC, and also by van der Veer et al.,[29b,c] who used NMR to determine the drug binding sites. The calculation assumes that an insignificant amount of drug was lost due to beam damage, a conclusion that is reasonable because we stirred the solution, and there is an excess of bonding sites because we used an excess of DNA. The remaining 35% relates to residual aquated cisplatin complex.

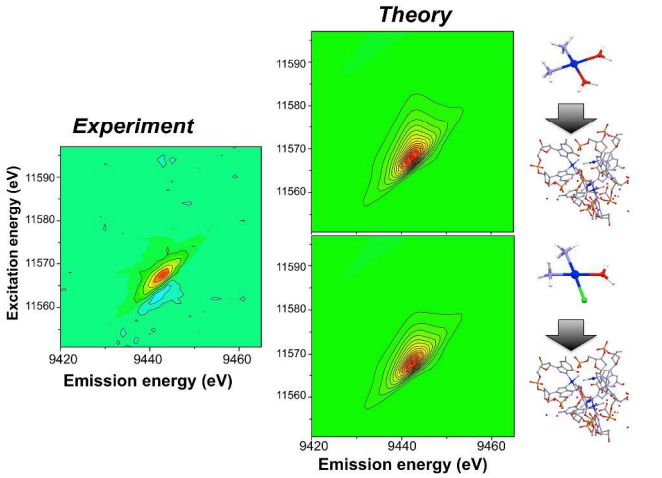

Fig. 4 ΔRIXS maps resulting from the bonding of the aquated cisplatin with DNA. The result is compared with theoretical predictions (*top*) diaqua complex + DNA; and (*bottom*) monoaqua complex + DNA.

High-resolution X-ray absorption spectroscopy (HR-XAS) measures the unoccupied Pt *5d* density of states and can be extracted from RIXS by cutting the plane across the most intense emission energy (~ 9443 eV).[30] Figure 5 shows the changes in HR-XAS resulting from hydration and the subsequent coordination by DNA (*top panel*), along with the orbital contributions responsible for the measured HR-XAS (*subsequent panels*).

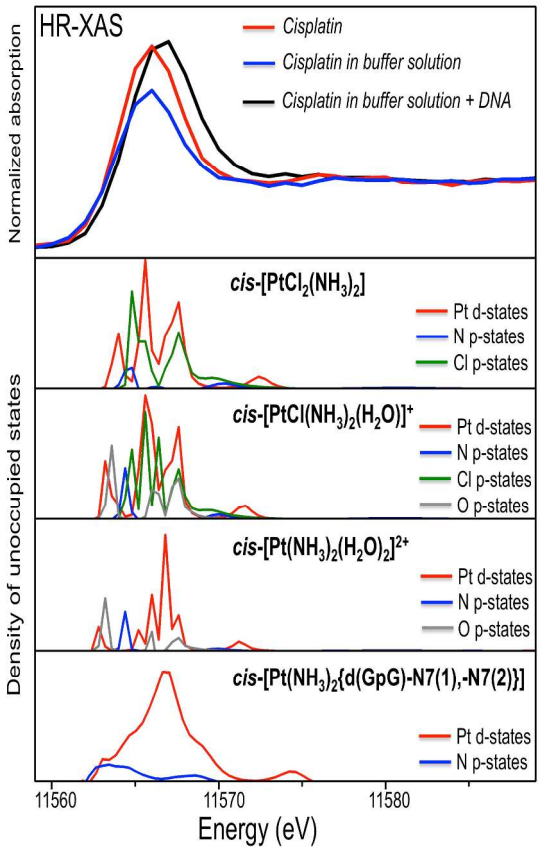

Fig. 5 HR-XAS extracted from the RIXS maps of (*top panel*) cisplatin in deionized water (*red*); cisplatin in buffer solution (*blue*); and cisplatin in buffer solution + DNA. (*black*). Pt *5d*-density of states orbital contribution computed with FEFF9.0 (*subsequent panels*).

The hydration of cisplatin and consequent formation of mono- and/or di-aqua complexes led to a striking decrease in the unoccupied *5d* states of Pt, reflected in Figure 3 by a negative signal difference and a significant decrease in the whiteline intensity (direct measure of *5d* empty states) in respect to cisplatin (Figure 5 (*top panel*)). The change can be rationalized in terms of orbital contributions. In the case of cisplatin, the whiteline is primarily composed from a hybridization of Pt *d*-orbitals with Cl *p*-orbitals with a minor contribution of N *p*-orbitals from the amine groups. Substitution of the chloride ligands by water led to a drastic decrease of whiteline intensity because of the removal of the Cl *p*-orbital contribution and the appearance of a contribution due to the O *p*-orbitals from the water molecules. There seems to be a weaker hybridization between Pt *d*-orbitals and O *p*-orbitals compared with Pt *d*-orbitals and Cl *p*-orbitals, i.e., less overlap of the orbitals. This suggests that water molecules are not strongly bonded to Pt and therefore can be replaced, justifying the highly reactive nature of the aqua complexes toward nucleophile centers of biomolecules. [30a,31] It should be mentioned that hydration of cisplatin occurred under physiological conditions but not in demineralized water.

Incubation of the hydrolyzed complex in physiological solution with DNA led to an increase of the *5d* empty states of Pt and a shift to higher energy of the main resonance (Figure 5 (*top panel*)). The result is consistent with the binding of the drug, confirmed by FEFF9.0 theoretical calculations (Figure 4). The N(7) atoms of the imidazole rings of guanine and adenine located in the major groove of the DNA double helix are the most accessible and reactive nucleophilic sites.[32] Therefore it is understandable that aquaplatinum complexes coordinate preferentially to the N(7) position of guanines adjacent to each other.[10] In the process its two coordinating water molecules, and/or coordinating water and chloride ions are released. The absence of other discernible species confirms *cis*-$[Pt(NH_3)_2\{d(GpG)\text{-}N7(1),\text{-}N7(2)\}]$ as the main adduct. Electronically speaking, the coordination to N(7) atoms resulted in a significant increase in the empty states of Pt and a strong hybridization of the N *p*-orbitals of the ligands (Figure 5 (*bottom panel*)) since there is a clear overlap between Pt *d*-orbitals and N *p*-orbitals. The significant increase in empty states of Pt suggests strong electronic donation from Pt, i.e., strong involvement of Pt valence states in the Pt-N bond. Consequently, the newly formed bonds are significantly stronger that Pt-Cl (cisplatin) and Pt-O (mono- and diaqua complexes), leading to the shift to higher energy.

Conclusions

In conclusion, the present work shows the applicability of *in situ* RIXS combined with FEFF9.0 theoretical calculations to validate and/or elucidate the mechanism of action of cisplatin under physiological conditions. The reported RIXS study suggested that cisplatin aquates in a buffer solution leading to the formation of a mono- and a diaqua complex. The aqua complexes are substituted by the N(7) position of guanines adjacent to each other, in an equal manner. We estimate that 65% of the drug bonded to the DNA after 24 h incubation. The bond strength and identity of the coordinating group could be interpreted on the basis of electronic orbital contributions.

In our view, RIXS and HR-XAS methodology is a welcome addition to the plethora of strategies used to establish reaction mechanisms of antitumor drugs since it can be carried out *in situ* and even *in vivo* due to the large penetration depth of hard X-ray without the need for concentration, extraction or crystallization of reaction products. These properties allow not only the determination of structural considerations in equilibrium but more importantly, enables time-resolved studies to monitor reaction kinetics and the amount of drug bonded, in real time, which is important in the estimation of drug dosage. It should be mentioned, that the emphasis of this work is to demonstrate that the technique possesses sufficient sensitivity and resolution to perform chemical speciation under *in situ* conditions before embarking on time-resolved studies. The methodology can be used to elucidate potential competitive and/or promoting factors along with the influence of the DNA-type, and drug selectivity. Finally, the method should be very useful in assessing if new metal containing drugs, including Pt ones, target DNA.

Experimental section

Calf Thymus DNA (25 mg/mL), in an aqueous buffer (20 mM HEPES solution), was incubated with 4mM of cisplatin at 37 °C for 24 hours before the measurement. The same concentrations of cisplatin in 20 mM HEPES buffer solution and on deionized water

were studied as references. Sigma Aldrich supplied DNA and HEPES. Institute of Drug Technology, Victoria, Australia, provided a sample of commercial cisplatin.

The Pt L_3-edge RIXS maps were performed at the SuperXAS beamline at the Swiss Light Source, Paul Scherrer Institute, Switzerland. The X-ray beam delivered by the 2.9 Tesla super-cooled bending magnet was collimated by an spherically bent Rh mirror. The collimated X-rays were monochromatized by means of a double Si (111) crystal monochromator and focused by a toroidal bent Rh mirror. The beam was focused down to 100 μm^2 and the photon flux on the sample was 7–8 Å~ 10^{11} photons/s. A 4 μm thick Pt foil was used to calibrate the energy. X-ray detection was performed using a wavelength-dispersive spectrometer, in the von Hamos geometry.[33] The X-rays emitted from the sample were diffracted by a Ge (660) cylindrically bent crystal with a radius of curvature of 25 cm, and detected via a 2D Pilatus detector. The spectrometer was operated in the vertical scattering geometry. The acquisition time per map was ca. 10 min, and 6 maps were collected per sample, equating to 60 min total acquisition time. The sample was placed in a polyvinyl sealed tube, which was stirred continuously to prevent beam damage and sample homogeneity throughout.

Theoretical calculations of the RIXS and HR-XAS spectra were performed with FEFF9.0, which is an *ab initio* self-consistent multiple-scattering code able to simulate simultaneously the excitation spectra and electronic structure. Crystal structure data were used as input files either from previously published data *cis*-$[PtCl_2(NH_3)_2]$ (CUKRAB)[34] and [*cis*-$[Pt(NH_3)_2$\{d(GpG)-N7(1),-N7(2)\}, CCDC 562451] or optimized *in silico*.

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Notes and references

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1. P. J. Loehrer, L. H. Einhorn, *Ann. Intern. Med.* 1984, **100**, 704.
2. M. A.Fuertes, C. Alonso, J. M. Pérez, *Chem. Rev* 2003 **103**, 645.
3. a) I. Hudson, L. R. Kelland, *Drugs* 2000, **59** (suppl. 4), 29; b) L. Shi, H. L. Sings, J. T. Bryan, B. Wang, Y. Wang, H. Mach, M. Kosinski, M. W. Washabaugh, R. Sitrin, E. Barr, *Clin. Pharmacol. Ther.* 2007, **81**, 259; c) M. R. McLemore, *Clin. J. Oncol. Nursing* 2006, **10**, 559; d) L. B. Harrison, M. Chadha, R. J. Hill, K. Hu, D. Shasha, D. *Oncologist*. 2002, **7**, 492.
4. a) L. Kelland, *Nat. Rev. Cancer* 2007, **7**, 573; b) N. P. Farrell, *Current Top. Med. Chem.* 20011, **11**, 2623; c) X. Wang, Z. Guo, Ch. 4, pg. 97 in Bioinorganic Medicinal Chemistry, Ed. E. Alessio, Wiley VCH, Weinheim, 2011; d) E. Wong, C. M. Giandomenico, *Chem. Rev.* 1999, **99**, 2451.
5. J. J. Roberts, M. F. Pera Jr., in Platinum, Gold, and Other Metal Chemotherapeutic Agents, Sj. J. Lippard, Ed. (ACS Symposium Series No. 209, America Chemical Society, Washington D.C., 1983), 3-25.
6. J. J. Roberts, J. M. Pascoe, *Nature* 1972, **235**, 282.
7. a) P. J. Stone, A. D. Kelman, F. M. Sinex, *Nature* 1974, **251**, 282; b) G. L. Cohen, J. A. Ledner, W. R. Bauer, H. M. Ushay, C. Caravana, S. J. Lippard, *J. Am. Chem. Soc.* 1980, **102**, 2487.
8. L. A. Zwelling, K. W. Kohn, W. E. Ross, R. A. G. Ewig, T. Anderson, *Cancer Res.* 1978, **38**, 1762.
9. A. L. Pinto, S. J. Lippard, *Biochim. Biophys. Acta* 1984, **780**, 167.
10. a) S. E. Sherman, D. Gibson, A. H.-J. Wang, S. J. Lippard, *Science* 1985, **230**, 412; b) P. M. Takahara, C. A. Frederick, S. J. Lippard, *J. Am. Chem. Soc.* 1996, **118**, 12309; c) E. R. Jamieson, S. J. Lippard, *Chem. Rev.* 1999, **99**, 2467; d) P. M. Takahara, A. C. Rosenzweig, C. A. Frederick, S. J. Lippard, *Nature* 1995, **377**, 649.
11. S. E. Sherman, S. J. Lippard, *Chem. Rev.* 1987, **87**, 1153-1181.
12. *for example:* A. Gelasco, S. J. Lippard, *Biochemistry* 1998, **37**, 9230.
13. J. Arpalahti, R. Sillanpää, K. J. Barnham, P. J. Sadler, *Acta Chem. Scand.* 1996, **50**, 181.
14. *for example:* a) H. T. Q. Nguyen, A. M. Galea, V. Murray, *Bioorg. Med. Chem. Lett.* 2013, **23**, 1041; b) U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, S. J. Lippard, *Nature* 1999, **399**, 708.
15. *for example:* a) J. Kozelka, G. A. Petsko, S. J. Lippard, G. J. Quigley, *J. Am. Chem. Soc.* 1985, **107**, 4079; b) H. Heiberg, L. Johansson, O. Groppen, O. B. Ryan, O. Swang, M. Tilset, *J. Am. Chem. Soc.* 2000, **122**, 10831; c) D. P. Bancroft, C. A. Lepre, S. J. Lippard, *J. Am. Chem. Soc.* 1990, **112**, 6860; d) M. S. Davies, S. J. Berners-Price, T. W. Hambley, *Inorg. Chem.* 2000, **39**, 5603.
16. E. Curis, K. Provost, I. Nicolis, D. Bouvet, S. Bénazeth, S. Crauste-Manciet, F. Brion, D. Brossard, *New J. Chem.* 2000, **24**, 1003.
17. A. V. Klein, T. W. Hambley, Chem. Rev. 2009, 109, 4911.
18. a) E. Curis, K. Provost, D. Bouvet, I. Nicolis, S. Crauste-Manciet, D. Brossard, S. Bénazeth, *J. Synchrotron Rad.* 2001, **8**, 716; b) I. Arčon, A. Kodre, R. M. Abra, A. Huang, J. J. Vallner, D. D. Lasič, *Coll. Surf. B*

2004, **33**, 199; c) D. Bouvet, A. Michalowicz, S. Crauste-Manciet, E. Curis, I. Nicolis, L. Olivi, G. Vlaic, D. Brossard, K. Provost, *J. Synchrotron Rad.* 2006, **13**, 477; d) D. Bouvet, A. Michalowicz, S. Crauste-Manciet, D. Brossard, K. Provost, *Inorg. Chem.* 2006, **45**, 3393; e) E. C. Beret, K. Provost, D. Müller, E. S. Marcos, *J. Phys. Chem. B* 2009, **113**, 12343; f) M. Obata, M. Harada, H. Ohi, S. Hirohara, M. Gottchaldt, S. Yano, *Chem. Pharm. Bull.* 2009, **57**, 1107.

19. B.-K. Teo, P. Eisenberger, J. Reed, J. K. Barton, S. J. Lippard, *J. Am. Chem. Soc.* 1978, **100**, 3225.
20. *Progress in Resonant Inelastic X-Ray Scattering*, Ed. M. Simon, T. Schmitt, special issue *J. Electron. Spectrosc. Rel Phenom*. 2013, **188**, 1-182.
21. *for example*: a) P. Glatzel, J. Singh, K. O. Kvashnina, J. A. van Bokhoven, *J. Am. Chem. Soc.* 2010, **132**, 2555; b) J. Sá, J. Szlachetko, M. Sikora, M. Kavčič, O. V. Safonova, M. Nachtegaal, *Nanoscale* 2013, **5**, 8462; c) M. Bauer, C. Gastl, *Phys. Chem. Chem. Phys.* 2010, **12**, 5575.
22. J. Sá, J. Szlachetko, C. Lothschütz, E. Kleymenov, M. Ranocchiari, M. Servalli, O. V. Safonova, G. Smolentsev, M. Nachtegaal, J. A. van Bokhoven, *RSC Advances* 2013, **3**, 12043.
23. a) J. Szlachetko, J. Sá, M. Nachtegaal, U. Hartfelder, J.-C. Dousse, J. Hoszowska, D. L. A. Fernandes, H. Shi, C. Stampfl, *J. Phys. Chem. Lett.* 2014, **5**, 80; b) J. Sá, Y. Kayser, C. J. Milne, D. L. A. Fernandes, J. Szlachetko, *Phys. Chem. Chem. Phys.* 2014, DOI:10.1039/C3CP54622E.
24. a) B. Hammer, J. K. Nørskov, *Nature* 1995, **376**, 238; b) T. Blingaard, J. K. Nørskov, S. Dahl, J. Matthiesen, C. H. Christensen, J. Shested, *J. Catal.* 2004, **224**, 206.
25. a) A. L. Ankudinov, B. Ravel, J. J. Rehr, S. D. Conradson, *Phys. Rev B* 1998, **58**, 7565; b) J. J. Rehr, R. C. Albers, *Rev. Mod. Phys.* 2000, **72**, 621; c) A. L. Ankudinov, J. J. Rehr, J. Low, S. R. Bare, *Phys. Rev. Lett.* 2001, **86**, 1642.
26. http://www.ccdc.cam.ac.uk/pages/Home.aspx
27. a) W. Chen and K. Matsumoto, *Bull. Chem. Soc. Japan*, 2002, **75**, 1561; b) I.A. Baldini, G.I. Zharkova and S.A. Gramilov, *J. Struct. Chem.,* 1998, **59**, 549; c) F.D. Rochon, P.C. Kong and R. Melanson, *Inorg. Chem.*, 1990, **29**, 2708; d) N.P. Rath, K.A. Fallis and G.K. Anderson, *Acta Crystallogr., Sect. C. Cryst. Struct. Comm.,* 1993, **49**, 2079.
28. a) A. Singh, U. Anandhi, M.A. Cinello and P.A. Sharp, *Dalton Trans*., 2008, 2314; b) K.J. Bonnington, F. Zhang, M.M.A.R. Moustafa, B.F.T. Cooper, M.C. Jennings and R.J. Puddephatt, *Organometallics*, 2012, **31**, 306; c) F. Zhang, E.M. Prokopchuk, M.E. Broczkowski, M.C. Jennings and R.J. Puddephatt, *Organometallics*, 2006, **25**, 1583.
29. a) F. Gonnet, J. Kozelka, J.-C. Chottard, *Angew. Chem. Int. Ed.* 1992, **31**, 1483; b) J. L. van der Veer, J. Reedijk, *J. Chem. Brit.* 1988, **20**, 775; c) A. M. J. Fichtinger-Schepman, J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman, J. Reedijk, *Biochem.* 1985, **24**, 707.
30. *for example*: a) D. Friebel, V. Viswanathan, D. J. Miller, T. Anniyev, H. Ogasawara, A. H. Larsen, C. P. O'Grady, J. K. Nørskov, A. Nilsson, *J. Am. Chem. Soc.* 2012, **134**, 9664; b) D. Friebel, D. J. Miller, D. Nordlund, H. Ogasawara, A. Nilsson, *Angew. Chem. Int. Ed.* 2011, **50**, 10190; c) A, Iglesias-Juez, A. M. Beale, K. Maaijen, T. C. Weng, P. Glatzel, B. M. Weckhuysen, *J. Catal.* 2010, **276**, 268; d) J. Szlachetko, M. Nachtegaal, J. Sá, J.-C. Dousse, J. Hoszowska, E. Kleymenov, M. Janousch, O. V. Safonova, J. A. van Bokhoven, *Chem. Commun.* 2012, **48**, 10898; e) H. G. Manyar, R. Morgan, K. Morgan, B. Yang, P. Hu, J. Szlachetko, J. Sá, C. Hardacre, *Catal. Sci. Technol.* 2013, **3**, 1497.
31. a) J. Reedijk, *Pure & Appl. Chem.* 1987, **59**, 181; b) S. J. Lippard, *Pure & Appl. Chem.* 1987, **59**, 731; c) S. E. Miller, D. A. House, *Inorg. Chim. Acta.* 1991, **187**, 125.
32. A. Ummat, O. Rechkoblit, R. Jain, J. R. Choudhury, R. E. Johnson, T. D. Silverstein, A. Buku, S. Lone, L. Prakash, S. Prakash, A. K. Aggarwal, *Nat. Struct. Mol. Biol.* 2012, **19**, 628.
33. a) J. Szlachetko, M. Nachtegaal, E. de Boni, M. Willimann, O. Safonova, J. Sá, G. Smolentsev, M. Szlachetko, J. A. van Bokhoven, J.-C. Dousse, J. Hoszowska, Y. Kayser, P. Jagodzinski, A. Bergamaschi, B. Schmitt, C. David, A. Lu□cke, *Rev. Sci. Instrum.* 2012, **83**, 1031105; b) J. Szlachetko, J. Sá, O. V. Safonova, G. Smolentsev, M. Szlachetko, J. A. van Bokhoven, M. Nachtegaal, *J. Electron. Spectrosc. Relat. Phenom.* 2013, **188**, 161.
34. G. Raudaschl, B. Lippert, J. D. Hoeschele, H. E. Howard-Lock, C. J. L. Lock, P. Pilon, *Inorg. Chim. Acta* 1985, **106**, 141.