ESRF	Experiment title: **Excited state investigation of photoactivable Ru anticancer complexes**	**Experiment number**: **CH-2719**
Beamline: ID09B	**Date of experiment**: from: 16/11/ 2008 to: 20/11/2008	**Date of report**: 25 /02/ 2009
Shifts: 9	Local contact(s)**: M. Wulff and M. Cammarata**	*Received at ESRF:*

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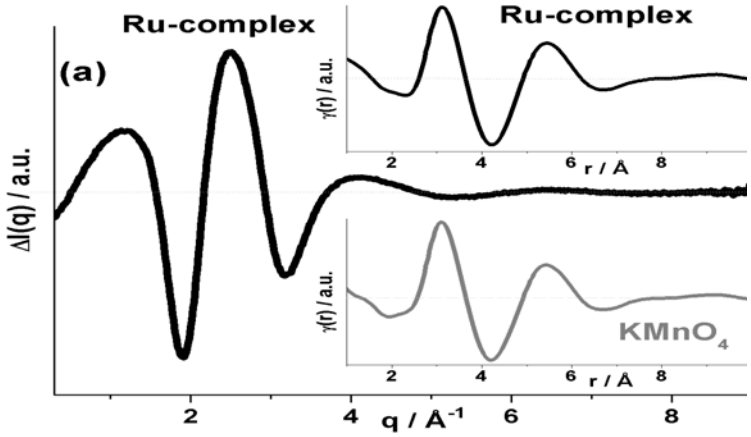
Transient X-ray liquidography experiment at the ID09B (CH2719) probed dissociation of a Py ligand from the complex $[Ru(bpy)_2(Py)_2]Cl_2$ (**1**) (where bpy = 2,2'-bipyridine and Py = pyridine). This and other similar metal-based photoactivable complexes have become recently extremely interesting in the medical field as anticancer drugs; such structures, when irradiated with light, can undergo chemical transformation (such as metal redox reactions or ligand dissociation) and produces reactive species able to target DNA and proteins causing cell death [1]. The action of the drug is then localized to the irradiated area.

This experiment was performed first using a 510 nm laser in order to excite the ^{3}MLCT band of **1**; the complex was diluted in pure water solution, at the concentration of 20 mM. With this set up 300, 500, 1000 and 5000 ns delays between laser pump and X-ray probe was measured using as reference for non excited ground state the signal acquired at negative delay -2000 ns.

We benefit of the new fast FReLoN area detector just installed on the beamline few days before the experiment. The raw images have been corrected in order to take into account several unavoidable effects: i) pixel/optical fibers connection; ii) fluorescent screen non-homogeneity; iii) X-ray beam polarization (in plane, linear); iv) X-ray to visible photon conversion efficiency due to the θ-dependence of the optical path through the fluorescent screen; X-ray extinction due to sampleθ-dependence absorption (again θ-dependent). Afterwards, the azimuthal averaging procedure was performed, to obtain the I(θ) and thus I(q) curves using the center of mass of the narrow λ distribution of the spectrum emitted by the U17 undulator. This procedure is repeated for each time delays τ. Differences $\Delta I(q, \tau) = I(q, \tau) - I(q, \tau = -2000\ ns)$ curves are calculated between adjacent acquisitions and then averaged over 500 acquisitions as reported in Figure 1a for τ = 300 ns. This curve has an excellent S/N



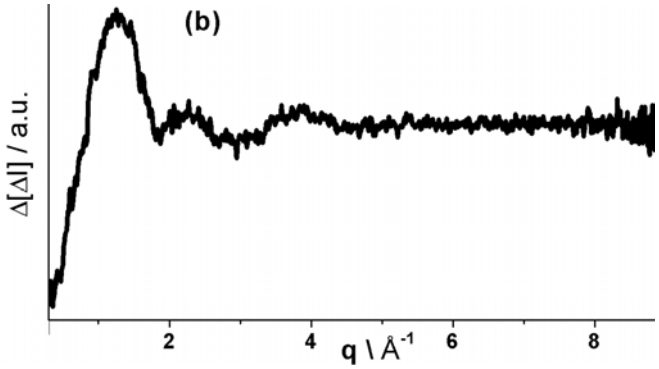


Figure 1. (a): ΔI(q, 300) of **1** and its FT (top inset). The bottom inset reports the corresponding ΔI(q, 300) for the $KMnO_4$ chromofore. (b) Difference of the two ΔI(q, 300) signals. Pump at λ = 510 nm

ratio, but mainly contains the modification of the radial distribution of the solvent molecules around the photo-dissociated complex due to local thermalization process. This is proved by the same experiment repeated for the for the $KMnO_4$ chromofore, that absorbs photons in the same range than **1** without undergoing any dissociation; compare the FT of the two $\Delta I(q, \tau)$ curves reported in the insets of Figure 1a. The thermal effect on the solvent can be subtracted by our signal using the blank $KMnO_4$ experiment, as shown in Figure 1b. This curve, much

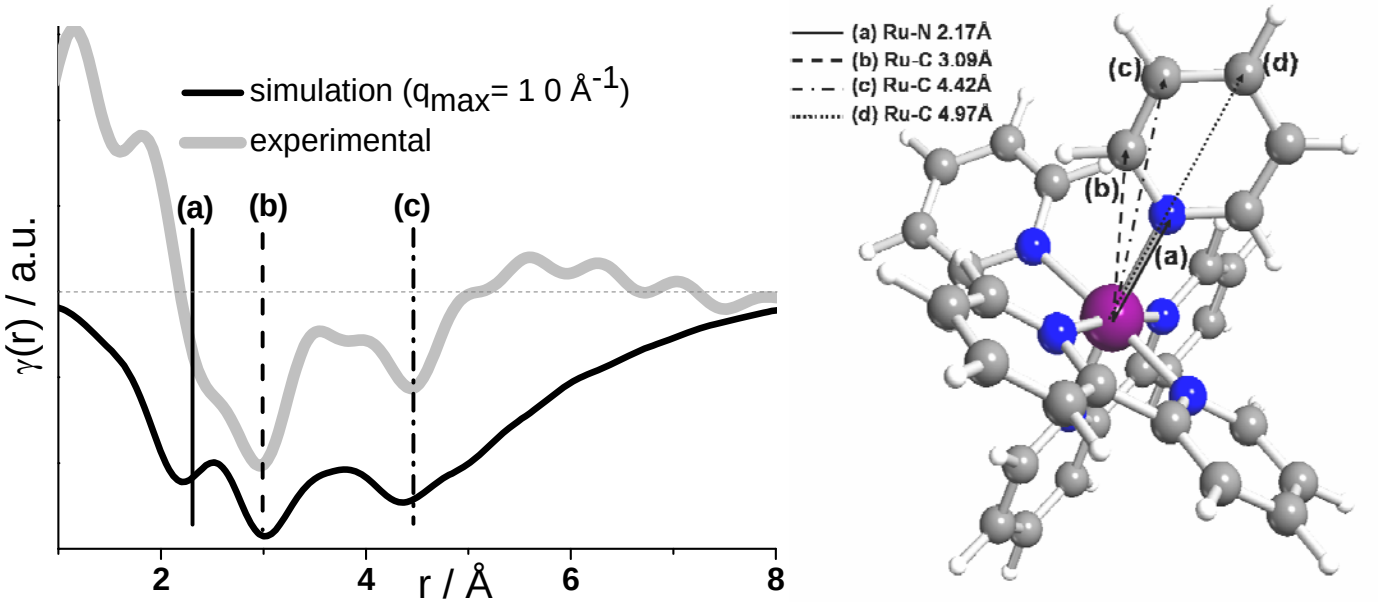


Figure 2. Left. Experimental (grey, from pump and probe at ID09B: 300 ns delay minus negative delay, λ = 510 nm) and simulated (black, DFT) difference radial distribution function of **1**. All instrumental corrections and the effect of solvent heating have been taken into account, see report. Right. Optimized structure of **1**, showing the distances where it is expected to observe a decrease in the radial distribution function upon Py ligand loss by photodissociation.

more noisy, contains only the information we are looking for. Its FT, reported as gray curve in Figure 2 left, exhibits three clear negative components at 2.2 Å (shoulder) and at 2.94 Å and 4.40 Å (well defined peaks). To understand the experimental datum, theoretical I(q) curves have been obtained from the structures optimized at DFT level for $[Ru(bpy)_2Py(H_2O)]^+$ and $[Ru(bpy)_2(Py)_2]^{2+}$ complexes, representing our model for the photodissociated and the unperturbed complex, respectively. q-broadening due to the small non monchromaticity of the U17 undulator has been taken into account. The validity of the DFT structures has been confirmed by EXAFS experiments kindly performed in static condition by S. Nikitenko at BM26A. Successively, the theoretical $\Delta I(q, \tau = +\infty)$ has been calculated and its FT reported in the left part of Figure 2 as black curve. Beside the critical low R-region, the agreement between experimental and theoretical curves is remarkable for this kind of experiment. This simulation allows us to assign the three negative peaks to the dislodgement of a Py molecule: a N atom at 2.17 Å , two C atoms at 3.09 Å and other two C atoms at 4.42 Å of Py. The contribution of the last C atom around 5 Å is below the S/N ratio. The invariance of $I(q, \tau)$, and thus of $\Delta I(q, \tau)$, in the 300-5000 ns τ-interval implies that the photodissociation process is completed before 300 ns, in agreement with our TD-DFT calculations.

Consequently we used the faster laser. The $\Delta I(q, \tau)$ measured in the 0.1-5 ns interval (Figure 3), clearly show a signal that progressively increases with τ, indicating that now we are really in the time scale of the photodissociation process. Unfortunately, a first data analysis suggests that we just observed modification of the radial distribution of the solvent molecules around the photo-dissociated complex due to local thermalization process.

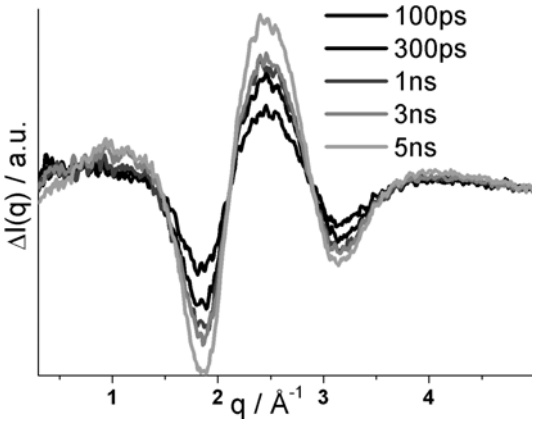


Figure 3. $\Delta I(q, \tau)$ curves obtained with the fast laser pump (λ = 390 nm).

[1] P. J. Sadler et al., *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 20743.