EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON



## **Experiment Report Form**

ESRF	Experiment title: Probing the light induced electron transfer and structural dynamics of cob(III)alamin compounds in solution	Experiment number: SC-3994
Beamline:	Date of experiment:	Date of report:
	from: 27.11.2014 to: 03.12.2014	05.03.2015
<b>Shifts:</b> 18	Local contact(s): Gemma Newby	Received at ESRF:
Names and affiliations of applicants (* indicates experimentalists): Dmitry Khakhulin* (European XFEL, Hamburg) Sophie Canton*, Sreevidya Thekku Veedu*, Simone Techert (MPI fur Biophysikalische Chemie, Göttingen)		

**Report:** This is a preliminary report on the SC-3994 experiment to support our future applications.

We performed a combined time-resolved liquid wide-angle x-ray scattering (WAXS) and x-ray emission spectroscopy (XES) measurement (setup on **Fig.1**) on vitamin B12 derivatives, namely CN-Cbl and MeCbl, in solution. The WAXS result shows the presence of fast kinetics component which can be attributed to the solute response however there was no observable change in the Co K $\beta$  emission spectra. Considering the excitation was performed only at one wavelength, i.e. 520nm, the measurement preliminary confirms the expected mechanism of the reaction.

The goal of the experiment was to measure the structural dynamics on the vitamin B12 molecule and the electronic state evolution of its Co centre during the photo-induced homolisys process. Originally being in the 3+ oxidation stage the Co<sup>III</sup> centre is reduced to 2+ upon homolytical cleavage that should result in the spin change. Assuming the Cobalt is in quasi-octahedral coordination the Co<sup>III</sup> would imply the low spin (LS) state (S=0) whereas upon the photo-reduction the Co<sup>II</sup> is in the high spin (HS) with S=3/2. It was proposed in literature that for the Me-Cbl derivative the photo-homolysis only happens by 400 nm radiation whereas irradiation by green light, e.g. at 520 nm, does not cleave the bond. However at 520 nm irradiation an MLCT state is being formed with lifetime of the order

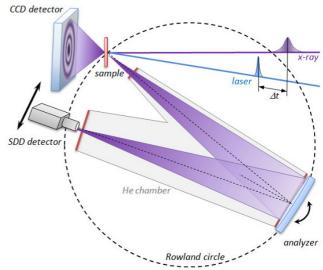
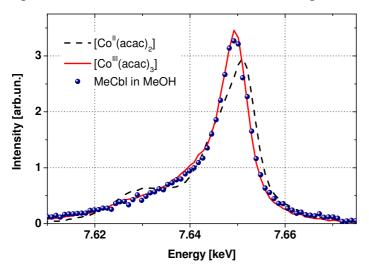
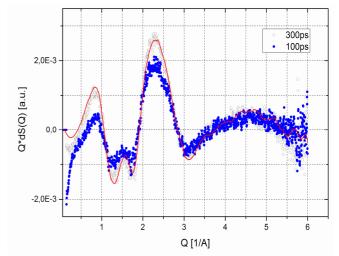


Fig.1 Experimental setup for combined WAXS and XES

of 1ns.

To be able to observed the transient spin or oxidation state change of Co we initially collected K $\beta$  emission spectra (with Si(620) analyser crystal) of the reference samples, namely Co<sup>II</sup>(acac)<sub>2</sub> and Co<sup>III</sup>(acac)<sub>3</sub> in powder. The result as expected demonstrated the HS (S=3/2) and the LS (S=0) states as reflected in the lineshaped (**Fig.2**). Additionally we measured the MeCbl in MeOH solution (5mM) spectrum which also as expected looks identical to the LS Co<sup>III</sup>(acac)<sub>3</sub> spectrum.



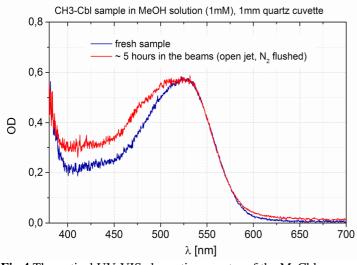


**Fig.2** Static Co Kb lineshapes for the reference powders and Me-Cob(III)alamin sample in MeOH solution.

**Fig.3** Difference WAXS signal of MeCbl in MeOH solution at 100ps and 300 ps delays; along with the neat MeOH response signal at 100ps (red solid line).

The optimization of the transient signal is usually much taster to perform using the time-resolved WAXS, therefore first we collected the difference WAXS signals at a set of pump-probe delays (**Fig.3**). The previously measured methanol response signal to the laser heating is also plotted on the sample figure. As can be seen the MeCbl/MeOH solution (5mM) signal at 300 ps is very similar to the neat solvent. However at 100 ps delay the difference WAXS signal is very different which suggests a quickly relaxing structural change in the molecule. We tried to fit this signal using our simulated curves for a dissociated or photo-cleaved molecule but could not produce a good fit. A transient K $\beta$  lineshape at the 100 ps delay was identical to the unexcited samples, that most likely means the bond is not cleaved. Moreover the very fast recovery of the WAXS signal supports the assumption since the bond formation, or recombination, is a rather long process for this molecule. So as suggested in the literature we do not observe the bond cleavage, but we see a large structural change which could be attributed to the short living MLCT state. Molecular structure in this MLCT state is interesting result which we are trying to extract from the data.

Similar measurements and analysis was done on CN-Cbl derivative but both the transient WAXS and XES produced signals identical to solvent response and the unexcited sample K $\beta$  lineshape, respectively. That is



**Fig.4** The optical UV-VIS absorption spectra of the MeCbl sample before and after the laser exposure of 5h.

excited sample Kβ lineshape, respectively. That is also in line with the literature reports showing that the CN-Cbl is in general more resistant to irradiation and cannot be easily excited. More measurements are required using this protocol, e.g. we would like to perform excitation at 400 nm and also test another B12 derivative Ado-Cbl, as well as perform studies in different solvents It is worth mentioning that the experimental

program was not fully completed for this beamtime. The main reason is huge difficulties with handling of the B12 samples. It's known the B12 is air sensitive therefore the sample preparation had to be done in a plastic glove box and the experiment itself at the anaerobic conditions. Initially we started the measurement with an HPLC pump cycling the sample through a 1 mm diameter capillary but the pressure in the pump loop was growing very fast (up to 30 bar) to lead finally to the damage of the loop. Preparation of new sample and the pump loop repair was very time consuming. Half way through the beamtime it was decided to switch to the standard open flat jet system and flushing the N2 through the sample cell. Comparison of the sample degradation rate and the transient WAXS signals demonstrated that the switch to the open jet experiment did not change the signals, which was reassuring. However every few hours the MeOH solutions have to the changed because of the degradation (see **Fig.4**). Our efforts to measure on water solutions of MeCbl were not successful since the sample was degraded in few tens of minutes. Overall, important lessons learned on sample handling and excitation conditions.

Following technical issues were encountered during the beamtime:

- Crash of the WAXS data reduction PC, LAUE, which complicated the start of the experiment in the absence of fast feedback on the transient signal.
- Crash of the main control PC EWALD, the recovery took at least 2 shifts thanks to the efforts of the beamline staff and ESRF IT support (the crash happened on the weekend).
- The high speed chopper was occasionally overheated especially when the XES spectra were collected with photon energies lower than typical for the beamline, thus higher heatload. The commissioning of the new heatload chopper will greatly help to deal with the issue.