EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** **Biomineralisation and speciation of gold in individual cells of the metallophilic bacterium *Cupriavidus metallidurans* incubated with three gold(I)-complexes. Part I: XANES**	**Experiment number**: SC - 3094
Beamline: ID22	**Date of experiment**: from: 10 Feb 11 to: 13 Feb 11	**Date of report**: 02 March 11
Shifts: 9	**Local contact(s)**: Gema + Jamie	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Frank Reith, Adelaide Univeristy, Joel Brugger, Adelaide University, Barbara Etschmann, Adelaide University

Gema, Jamie

Report: Aim

Understanding gold (Au) biomineralisation in metallophillic bacteria such as *C. metallidurans* will allow us to use these organisms for the production of gold nano-particles, as bioprocessing tools and as whole cell biosensors in gold exploration. While Au(III)-complexes are easy to work with (esp. with respect µXRF- and µXANES method development in 3 previous experiments) and occur in highly oxidising chloride rich environments, our recent research suggests that Au(I)-complexes such as Au(I)-thiosulfate and Au(I)-cyanide are the most likely to occur complexes in surface soil environments [4]; in addition cyanidation of Au is the most common process used in Au ore processing [1]. In recent experiments we have shown that Au(I)-complexes are readily accumulated by *C. metallidurans* cells and the apparent formation of nano-particulate Au was observed in Au(I)-thiosulfate amended cells but not in cells amended with Au(I)-cyanide, yet the mechanisms are not understood [5]. Thus before we use this organism for any type of biotechnological application such as biosorption for ore processing or nano-particle formation, we need to understand the Au accumulation process including the formation of the Au(I)-complexes .
Preliminary data on Pt uptake in cells were also measured.

Experimental

Two series of data were collected at the Au L_3 (11.919 keV) and the Pt L_3 (11.564) edges at the ID22 beam line: a kintetic series (1/5 mins to 24 hours) and another series investigting other effects (cell status, medium, concentration).

Update 29/2/2016. Publication of the data was delayed because we developed key ideas in a Nature Geoscience paper that was just accepted (17/2/2016):

1. Reith, F., Zammit, C., Said, S.S., Etschmann, B., Botrill, R., Southam, G., Oberthür, T., Ball, A. and Brugger, J. Biotransformation of platinoid nuggets. Nature Geosciences.

A paper based mainly on the ESRF data has been submitted following the acceptance by Nature Geoscience (22/2/2016):

Etschmann, B.E., Brugger, J., Fairbrother, L., Grosse, C., Nies, D.H., Marinez-Criado, G. and Reith, F. Applying the Midas touch: Differing toxicity of mobile gold and platinum complexes drives biomineralization in the bacterium Cupriavidus metallidurans. Geochimica Cosmochimica Acta.

Au-Thiomalate (2-14)				3 hours	
Au-Thiomalate (2-16)				24 hours	67% Au-CN + 33% Au
Au-Thiomalate (22118)	Tris MM	Live	500 uM	6 days	46% Au-CN + 54% Au
Au-Thiomalate (22119)	Tris MM	Live	500 uM	18 days	31% Au-CN + 69% Au
Au-Thiomalate (22129)	Tris MM	Live	50 uM	18 days	25% Au-CN + 75% Au
Au-Thiomalate (22218)	Tris MM	Inactive	500 uM	6 days	59% Au-CN + 41% Au
Au-Thiomalate (22318)	Tris MM	Dead	500 uM	6 days	67% Au-CN + 33% Au
Au-S_2O_3 (1-1)				1 min	100% Au-CN + 0% Au
Au-S_2O_3 (1-3)				3 min	
Au-S_2O_3 (1-14)				6 hours	93% Au-CN + 7% Au
Au-S_2O_3 (11129)	PME	Live	50 uM	18 days	52% Au-CN + 11% Au+ 37% Au_2S

Au-S_2O_3 (21118)	Tris MM	Live	500 uM	6 days	25% Au-CN + 21% Au+ 54% Au_2S
Au-S_2O_3 (21218)	Tris MM	Inactive	500 uM	6 days	52% Au-CN + 9% Au+ 39% Au_2S
Au-S_2O_3 (21318)	Tris MM	Dead	500 uM	6 days	52% Au-CN + 6% Au+ 42% Au_2S
Pt-cisplatin (5-1)				1 min	
Pt-cisplatin (5-14)				6 hours	
Pt-cisplatin (5-16)				24 hours	
Pt-cisplatin (15119)	PME	Live	500 uM	18 days	
Pt-cisplatin (25119)	Tris MM	Live	500 uM	18 days	
Pt-cisplatin (25128)	Tris MM	Live	50 uM	6 days	
Pt-Cl (4-1)				1 min	
Pt-Cl (4-14)				6 hours	
Pt-Cl (4-16)				24 hours	
Pt-Cl (14119)	PME	Live	500 uM	18 days	
Pt-Cl (24119)	Tris MM	Live	500 uM	18 days	
Pt-Cl (24128)	Tris MM	Live	50 uM	6 days	
Pt-Cl (24129)	Tris MM	Live	50 uM	18 days	
Pt-Cl (24228)	Tris MM	Inactive	50 uM	6 days	
Pt-Cl (24328)	Tris MM	Dead	50 uM	6 days	

General observations (ligand, time, medium)

Au-CN: Unsurprisingly these spectra resemble the non-bacterial Au-CN standard collected previously. There is a slight decrease of the pre-edge, which occurred for (i) samples that had longer incubation times (18 days), and (ii) those cells that were inactive or dead. For the kinetic series, the 24 hour sample (Au-CN 3-16) resembled that of Au-23118 (6 days); the Au concentrations in the other samples were low and the spectra very noisy.

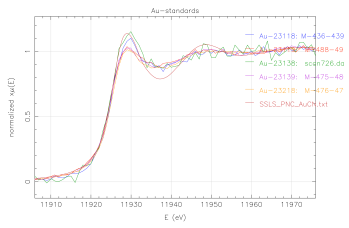
Au-thiomalate: The kinetic data showed some Au up-take, but the concentrations were low and the spectra very noisy. However, the spectra had a small edge peak, indicating that the Au was not metallic (sample 2-16 can be described by a linear combination of 67% Au-CN + 33% metallic Au; spectrum 2-14 is similar to 2-16). For the other series, again the samples with the longer incubation time (18 days) had a smaller pre-edge. However this time the dead and inactive cells had a higher pre-edge than the live cells (all 6 days).

Au-S_2O_3: The kinetic data showed a decrease in the pre-edge peak with increasing incubation time. The pre-edge in the 6 day data was the same for the inactive and dead cells (21218 & 21318), and was higher than that for the live cells (21118). The 18 day data (11129) was similar to the 6-day data for inactive or dead cells.

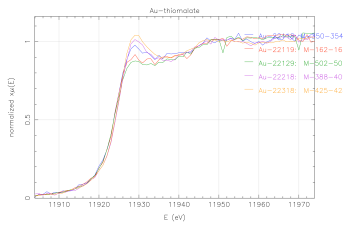
Pt-cisplatin: There is little variation in these spectra. There is a small increase in the white line with increasing incubation time for the kinetic series, but such small changes could be experimental artefacts. The spectra for the other series are almost identical.

Pt-Cl: The kinetic series spectra were identical. The spectra for the other series are similar – there is a small decrease in the white line with increasing incubation time (6 to 18 days).

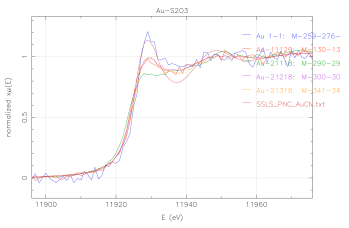
1. It should be noted that some of the variation noted in the spectra could also be artefacts due to noisy spectra induced by low elemental concentrations. This needs to be explored further.
2. It is interesting that while the Au-CN and Au-thiomalate spectra could be well matched by using a linear combination of the Au-CN and metallic Au standard spectra, a third standard was required to get a good match for the Au-thiosulfate spectra.



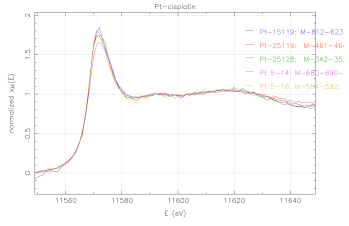
Au-CN



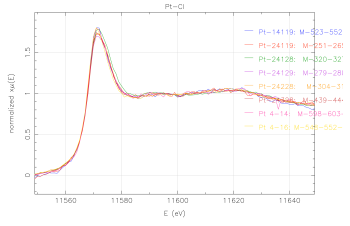
Au-thiomalate



Au-thiosulfate



Pt-cisplatin



Pt-Cl