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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

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- 1st March for experiments carried out up until June of the previous year;
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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:** X-ray absorption spectroscopy studies on gold nanoparticles formed by bacteria and their surface layer proteins	**Experiment number:** 20 _01_ 638
Beamline: BM20	**Date of experiment:** from: 05 March 2005 to: 06 March 2005	**Date of report:** 29.08.06
Shifts: 3	**Local contact(s):** Dr. Andre Rossberg	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

ABSTRACT. Cells and S-layer sheets of *B. sphaericus* JG-A12 were used as templates for the deposition of metallic gold nanoclusters using dimethyl amino borane (DMAB) as reducing agent . Gold L_{III}-edge XAS measurements confirmed the formation of Au(0) nanoclusters in both cases.

A combination of X-ray absorption spectroscopy (XAS) and Iterative Target Test Factor Analysis /1/ was used previously to characterize the gold nanoparticles formed on the cells and S-layer protein of *B. sphaericus* JG-A12 using H_2 as reducing agent. The results demonstrated that only 67% and 37% of the Au(III) was reduced to Au(0) on the cells and S-layer protein of this bacterium, respectively /2/. The present study was undertaken in order to optimize the Au reduction process using another reducing agent dimethyl amino borane (DMAB).

EXPERIMENTAL. *B. sphaericus* JG-A12 cells were grown in a batch culture to mid exponential phase and harvested by centrifugation. The preparation of S-layer protein was performed as described in /3/. For sorption of Au(III), 15 mg of dialysed protein and 20 mg of cells, were incubated in 150 ml and 30 ml of a solution of 2 mM $HAuCl_4$ x $3H_2O$, respectively. After 3 hours incubation at room temperature under shaking in the darkness the samples were centrifuged (20 min, 10000 $\times$ *g*) and the pellets were resuspended in H_2O. Residual salts were removed by dialysis of the metallized proteins and cells against H_2O. Au(III) was reduced by the addition of DMAB to produce Au(0)-nanoparticles. The metallized cells and protein samples were centrifuged (20 min, 10000 $\times$ *g*) and dried in a vacuum oven (48h, 80°C).

RESULTS. Figure 1 shows the XANES regions of the EXAFS spectra obtained with Au-treated S-layer and cells after addition of DMAB and for reference compounds containing two oxidation states of gold: Au(III) (solution of 2 mM $HAuCl_4$ x $3H_2O$) and metallic Au (gold foil). Comparison of the experimental spectra to the reference spectra clearly shows that Au is present mainly as metallic Au in the Au-treated S-layer and cells. To determine the relative amounts of Au(0) and Au(III) present in the biological samples, we applied Iterative Transformation Factor Analysis. The calculation revealed a mixture of 89% metallic Au and 11% Au(III) for the Au-loaded cells sample and 81% metallic Au and 19% Au(III) for the S-layer sample.

Gold L_{III}-edge EXAFS spectra of cells and S-layers of *B. sphaericus* JG-A12 in presence of DMAB are shown together with Au foil in Fig. 2. In these samples Au is present mainly as metallic phase where the interatomic distances found are comparable with the one of metallic foil. The coordination numbers (N) are diffrent from the bulk ones, showing the presence of small metal particles. The reduction of the coordination number of the first shell is used to estimate the average particle. The coordination number value of the Au-Au found in this work (8.7 ± 0.3 for the S-layer and 6.3 ± 0.4 for the cells sample) is not the real coordination number of the Au per nanoparticles. This value correspond to the coordination number of Au-Au per sample. The coordination number of later bond per nanoparticle is weighted by the atomic percentage of Pd atoms in the metallic phase. Using the cubic (FM3-M) structure of elemental gold, the average cluster size was estimated. It was found that the nanoparticles deposited on the cells and S-layer protein have a mean diameter of about 0.6 –0.8 nm and 1.5-2.5 nm, respectively.

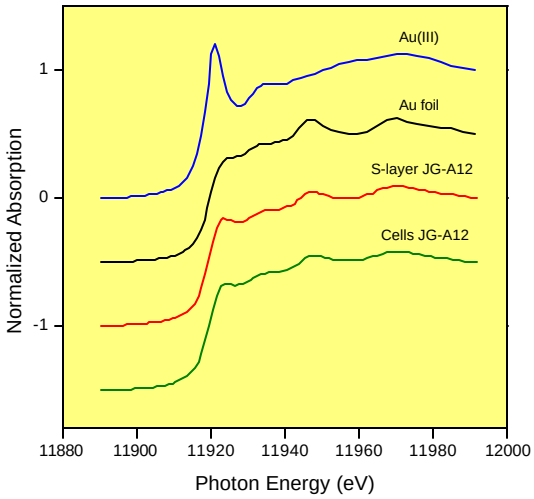

Fig.1: XANES region of EXAFS spectra of the Au L_{III}-edge in reference compounds and for Au-loaded cells ad S-layer of *B. sphaericus* JG-A12.

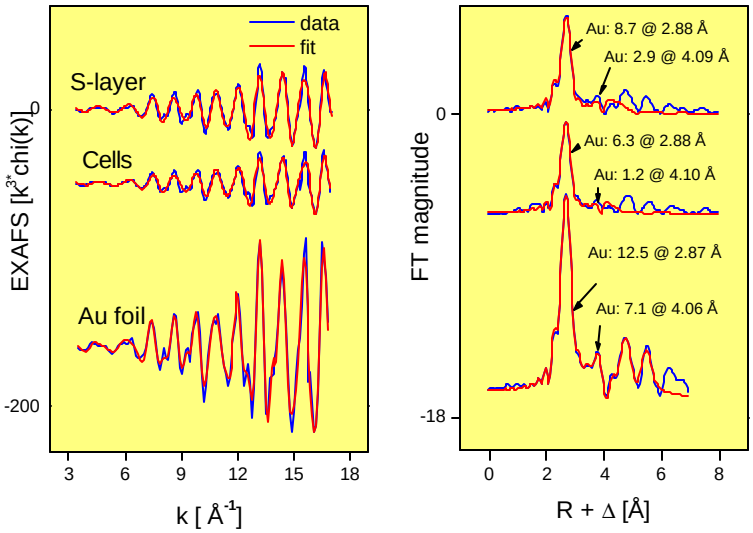

Fig.2: Au L_{III}-edge EXAFS spectra and their FT of Au- treated cells and S-layer of *B. sphaericus* JG-A12 and gold foil.

REFERENCES

[1] Rossberg, A. *et al.* (2003) *Anal. Bioanal. Chem.376*, 631-638.
[2] Merroun et al. Mater. Sci. Engin C (in press)
[3] Raff, J. Ph. D. Thesis, FZR-358 (2002)