Report for the Experiment EC – 231 (22 - 26 February 2008)

Title: Mechanisms of gold accumulation by the metallophillic bacterium Ralstonia metallidurans

F. Reith, J. Brugger, B. Etschmann

Aim:

The aim of this study is to use synchrotron X-ray spectroscopy (μXRFs) to elucidate fundamental mechanisms of gold bioaccumulation by *Cupriavidus* (formerly *Ralstonia*) *metallidurans*. The aim was to use X-ray fluorescence (μ-XRF) to assess the distribution of gold and other metals in individual *C. metallidurans* cells. This data is important to understand how the bacterium deals with toxic gold complexes, which functional groups are involved in binding the gold, and how ultimately the reduction of gold complexes to metallic gold is mediated.

Results:

To establish the uptake kinetics and distribution of Au in *C. metallidurans* after uptake, experiments (exposure of cells to 50 μM Au(III)-chloride in solution from 1 minute to 144 h at pH 7 in PME medium) confirmed a two-stage reduction mechanism for Au(III)-chloride complexes. The distribution of Au and other elements was mapped in individual cells using synchrotron μ-X-ray fluorescence (μXRF; Fig. 1, 2). A spot size as small as 120 nm x 150 nm enabled the collection of more than 30 full element spectra per cell, and heavier elements expected in bacterial cells (*i.e.*, S, Ca, Fe, Cu, Zn, Mn; Fig.2) were successfully mapped.

After 1 minute of exposure to Au(III)-chloride, *C. metallidurans* cells had taken up 1.82 ± 0.19 ng cm^{-2} of Au (Fig. 2). The concentrations of accumulated Au in the cells increased to 2.79 ± 0.31 ng cm^{-2} after 6 h and to 12.2 ± 1.3 ng cm^{-2} after 72 h (Fig.2). Similarly to Ca, Cu, Fe, S and Zn, accumulated Au was distributed throughout the cells (Fig. 1). However, after 72 h of incubation a number of zones containing up to 34.6 ± 2.4 ng cm^{-2} Au were detected (Figs.2, 3). These 'hot spots' were associated with cell envelopes and were

not present in the earlier samples. After 144 h of exposure, Au concentrations in the cell cytoplasm had decreased to 2.25 ± 0.79 ng cm^{-2}, whereas regions of the cell envelope containing high Au concentrations appeared more numerous and often >200 nm in diameter. This suggests that cells actively removed Au from the cytoplasm and precipitated it as Au^0 in the periplasm.

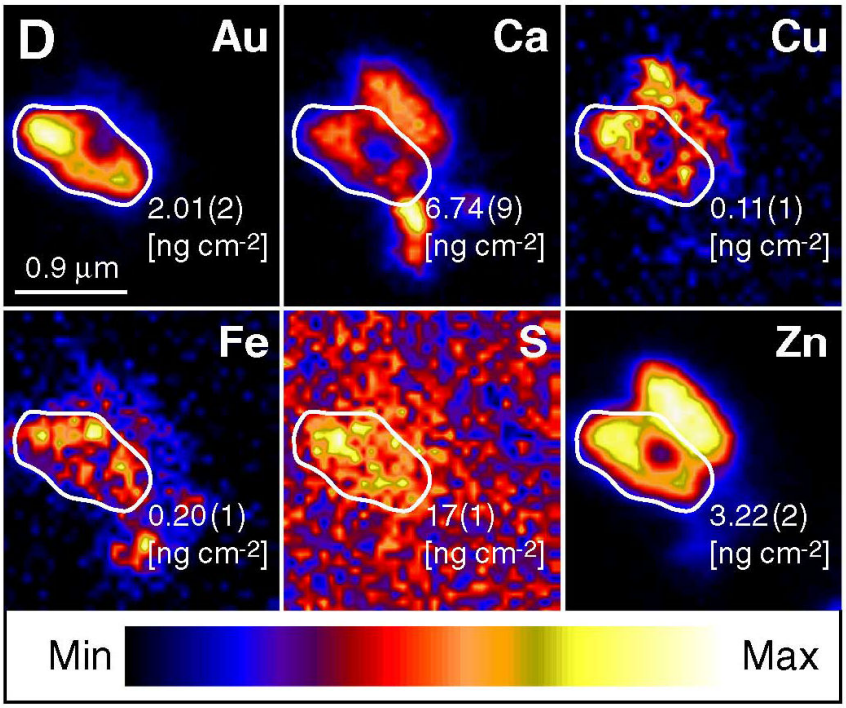

Figure 1:
Quantitative µ-XRF-maps showing the distribution of Au, Ca, Cu, Fe, S, and Zn in an individual cell after 1 min exposure to Au(III)-chloride at pH 7 (the quantified area is marked in the image, and concentrations (± calculated errors) are given in the image, concentration ranges for elements are Au: 0-4.16, Ca: 0-18.78, Cu: 0-0.29, Fe: 0-0.44, S: 0-60.52 and Zn: 0-24.57 [ng cm^{-2}])

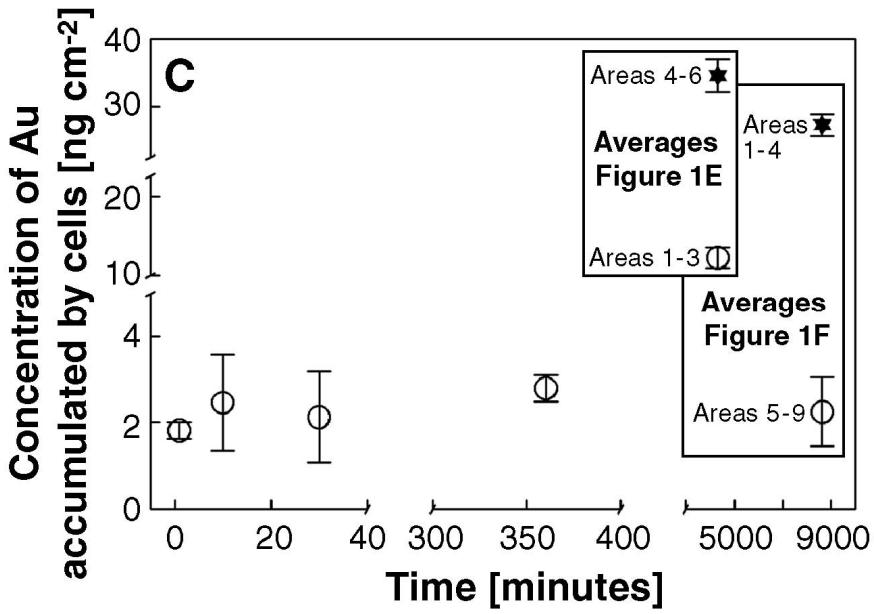

Figure 2

Concentrations of Au in individual *C. metallidurans* cells (○) and particles associated with cells (✶) in [ng cm^{-2} based on quantitative µ-XRF maps, error bars represent the standard deviation of replicate samples.

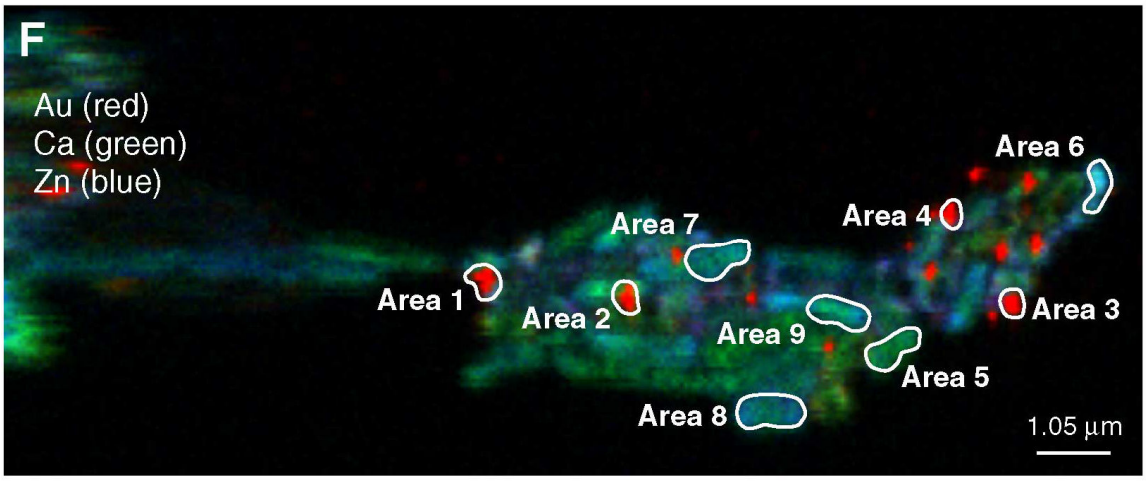

Figure 3

Overlay false color quantitative µ-XRF-maps of the distribution of Au (red), Zn (blue) and Ca (green) in cell clusters after 144 h of incubation at pH 7.

Publications:

Brugger J, Pring A, Reith F, Ryan C, Etschmann B, Liu W, O'Neill Band Ngo Y (2009) Probing ore deposits formation: New insights and challenges from synchrotron and neutron studies. Rad. Phys. Chem. doi:10.1016/j.radphyschem.2009.03.071.

Reith F., Etschmann B., Grosse C, Moors H., Benotmane M.A., Monsieurs P., Grass G., Doonan C., Vogt S., Lai B, Martinez-Criado G, George GN, Nies DH, Mergeay M, Pring A, Southam G, Brugger J (2009, in press) Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans* (Proceedings of the National Academy of Sciences, PNAS).

Reith, F., Etschmann B., Brugger J. 2009. Understanding gold biomineralisation using micro analyses techniques. AMAS Annual Confernce Adelaide Feb. 2009.