ESRF	**Experiment title:** Imaging modern and antique microbe-mediated processes in mineralized environments	**Experiment number**: ME-824
Beamline: ID21	**Date of experiment**: 28 Avril – 3 Mai 2004, 16 March -21 March 2005 and 25 to 29 November 2005	**Date of report**: December 2005
Shifts: 15+17 +12 = 44	**Local contact(s)**: M. Salomé, M. Cotte	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

P. Philippot*[1], J. Cauzid*[1,2], S. Dupraz*[1], J. Foriel*[1], E. Gérard*[1], B. Ménez*[1], C. Rommevaux*[1]

[1] Laboratoire Géobiosphère Actuelle et Primitive, IPGP Paris, case 89, 4 place Jussieu, 75252 Paris 05

The aim of this Long Term Project was to bring together a variety of specialists of different scientific horizons to image and quantify living and fossil biomaterial in mineralized environment and fluid inclusions using a variety of tracers such as sulfur, lipids, selenium, arsenic and iron. By exploring the full spectrum of microbial ecology in extreme environments, this has provided important new insights into life-sustaining processes in the rock record and contribute to the understanding of the environmental conditions and the origin of life on the Primitive Earth.

We have obtained a total of 44 shifts on ID21 and 82 shifts on ID 22 from April 2004 to December 2005.

On ID 21, the 44 shifts were dedicated to:

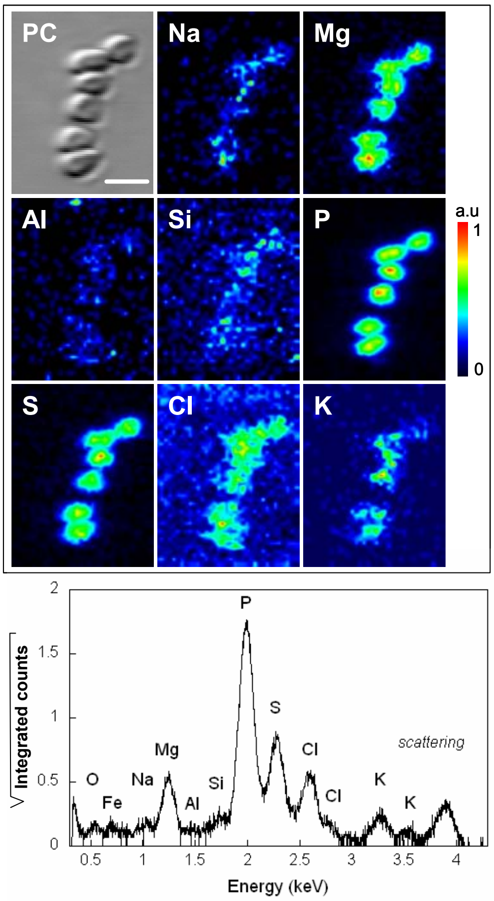
I) the characterisation and imaging of individual microbes placed on different mineral substrates using the SR µ-XRF and µ-XANES set up.

II) the characterization of kerogeneous material collected in pristine drilled core samples from the oldest hydrothermal system on Earth, namelly the Dresser Formation in Western Australia dated at 3.525 billion years old (6 shifts from 12 to 15 November 2005).

I -1. 2D imaging of microorganisms on mineralized samples (28 Avril – 3 Mai 2004 & 16 March -21 March 2005)

One major goal of this long term proposal was to develop techniques for the in situ study of microbial activity in mineralized samples from several extreme environments. Accordingly, there is a strong need of the scientific community for appropriate methodologies allowing to localize and identify prokaryotic cells in mineralized environments. We evaluated here, the possibility of associating scanning X-ray microscopy available on ID21 to a newly protocol developped by our team and based on fluorescent in situ hybridization (FISH) coupled to ultra-small immunogold detection [Gérard et al., 2005, J. Microbiol. Meth., 63, 20-28]. Practically it implies associating heavy metal fluorescence signals emerging specifically from gold nanoparticles enhanced with silver, to the location of microbes, highlighted either by phase contrast imaging or light element fluorescence (e. g. P, S, Cl, K). To estimate spatial resolution and microprobe sensitivity, samples, which consist of bacteria and archaea deposited on different substrates (i.e. polymer films, slides, minerals including quartz, calcite, siderite, basalt) were mapped in fluorescence mode using the scanning transmission X-ray microscope at ID21 beamline. Figure 1 and 2 present some of the results obtained on bacteria deposited on ultralene and siderite, respectively. Note that individual cells are very precisely and undubitatively localized and identified. Images at this resolution level were rarely obtained by other groups.

Results show that this analytical protocol alllow the in situ imaging of the occurrence and nature of microorganisms and potentially their metabolism in rock samples. The possibility of associating simultaneously, complementary methods (i. e. X-ray microdiffraction and X-ray absorption microspectroscopy) for the chemical and structural characterization of the associated mineral phases (i. e. the substratum as the nutrient mineral and the biomineralisations), offers great interest for understanding how mineral and microbe are interacting. 27 shifts on beamline ID21 were dedicated to this work. The results were presented at 5 international and 2 national meetings. A paper willl be submitted in the near future to Proceedings of the National Academy of Sciences. A second one is also in preparation for Geomicrobiology Journal.



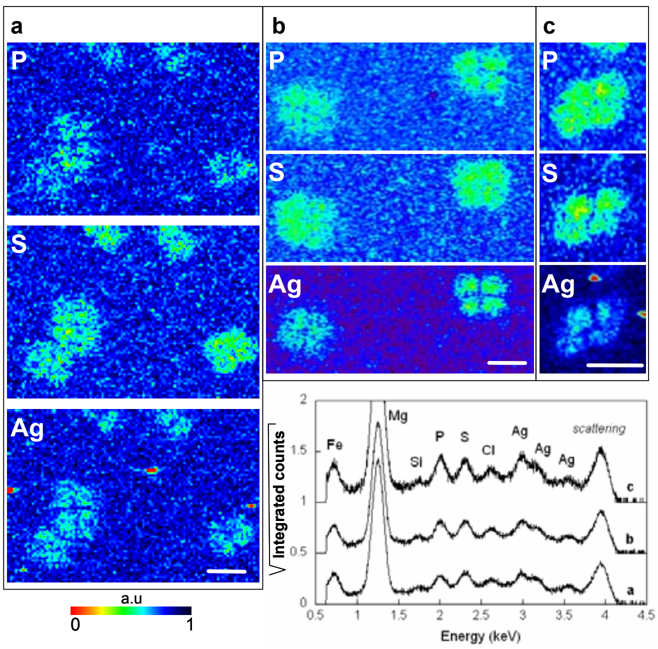


Figure 1 - *D. radiodurans* cells on polypropylen film as shown by X-ray microscopy in transmission and fluorescence modes and associated X-ray fluorescence spectrum. Phase contrast image (PC) and two-dimensional elemental distributions were obtained using 70 and 2000 ms/pixel integration time with an associated spatial resolution of 0.1 * 0.1 and 0.2 * 0.2 µm², respectively. The scale bar corresponds to 2 µm.

Figure 2 - Silver-enhanced ultrasmall immunogold particles in hybridized cells of *D. radiodurans* cells on siderite (with the fluorescein-labelled probe universal for Bacteria EUB338) and associated X-ray fluorescence spectra. Cells position is underlined by S, P elemental distributions allowing to distinguish silver-enhanced ultrasmall immunogold particles in hybridized cells from Ag grains belonging to the surrounding noise. Two-dimensional elemental maps were obtained using 2 s/pixel integration time with an associated spatial resolution of 0.2 * 0.2 µm². The scale bar corresponds to 5 µm.

I-2 - Indicators of microbe-mediated processes (6 shifts from 12 to 15 November 2005)

During the last experiment in november 2005, several shifts were dedicated to the study of (bio)carbonates, as potential indicators of microbe-mediated processes. Accordingly, microorganisms interact with minerals and/or terrestrial fluids and process new material preserving a signature pertaining to this past microbial activity. Calcium carbonates together with calcium phosphates are commonly associated with microbial activity and are also among the most prevalent minerals involved in microbial fossilization. Characterization of both the organic and mineral components in biomineralized samples is, however, usually difficult at the appropriate spatial resolution (i.e. at the submicrometer scale). Here we aimed at identifying if carbonates can constitute new indicators of microbe-mediated processes in the rock record by using X ray Absorption Spectroscopy (mainly X-ray Absorption Near Edge Structure). Ca K-edge XANES spectra of calcium-containing minerals common in biomineralization processes and of samples from biomineralization experiments by model microorganisms, were recorded. Measurements by micro-infrared spectroscopy were also performed on the same samples. Results are currently under evaluation but appear to be very promising. In particular, Ca K-edge for several carbonates are unique and can be used as probes to detect these different mineral phases. Consequently, dedicated proposals will be submitted in the upcoming months to obtain beamtime on line ID21 for this topic.

II – Kereogneous material and Early life on Earth - Synchrotron Radiation Infrared Spectroscopy (6 shifts during 12 to 15 November 2005)

The residual six shifts were used to characterize the nature and occurrence of tiny clots of organic matter preserved in the North Pole dome dated at 3.525 billion years (oldest hydrothermal systems preserved on Earth) and the carbonaceous material from the Tumbiana Formation (2.729 billion years old), which correspond to the oldest occurrence of carbonaceous material associated with anaerobic methanotrophs that produced $\delta^{13}C$ values down -55 ‰. The sample studied are from pristine diamond drill cores (Pilbara Drilling Project) recently collected by our team as part of a collaboration between the Institut de Physique du Globe de Paris and the Geological Survey of Waestern Australia. Results presented here are from the North Pole samples

The North Pole consists of shallow-water, variably silicified micritic Fe-carbonate, pyrite laminates with stratiform (stromatolitic?) forms, that are pervasively hydrothermally-altered by a feeder vein network composed of black to grey silica. Both the hydrothermal feeder dikes and sedimentary stratified horizons contain abundant carbonaceous material occurring either as dispersed clots throughout individual layers or dikes, or as minute films at interfaces between sedimentary beds and interstratified hydrothermal precipitates. Carbonaceous films resembles typical "microbial mats" described in the literature in similar types of environments. However, it is known from modern day environements that abundant organic matter of abiotic origin can form as a result of Fischer-Tropsch Type (FTT) synthesis due to extensive interaction of CO2-bearing aqueous fluids with underlying Mg-rich metabasalts at high temperature.

High resolution in situ analysis of carbonaceous material using the newly installed SR-IRS device combined with bulk carbon stable isotope analysis of organic carbon allowed reporting degree of structural order and composition of carbonaceous matter as well as $\delta^{13}C$ values of carbonaceous material in individual sedimentary and hydrothermal layers/veins. We show that most of the carbonaceous matter displays $\delta^{13}C_{PDB}$ values between -28.2 and -31.5 ‰ in agreement with previous studies. This organic matter is most likely abiotic in origin as suggested by abundant H_2O-CO_2 ± H_2S ± CH_4 ± hydrocarbon fluid inclusions in the underlying metabasalt and chert-barite veins and Cr-bearing minerals at the top of the hydrothermal system supports an abiotic origin. In contrast, however, some horizons contain low fractionated carbonaceous matter showing important aliphatic C-H stretching bands in the 2900 cm^{-1} region (Figure 3) and two broad bands at 1604 and 1350 cm^{-1} indicative of weak structural organization and low degree of thermal alteration. This later carbonaceous matter could be truly biotic in origin, hence representing a new window for investigating some of the oldest prokaryote metabolic pathways.

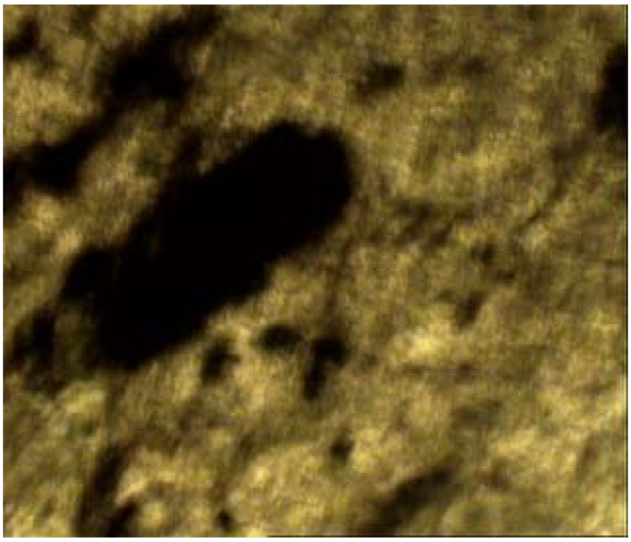
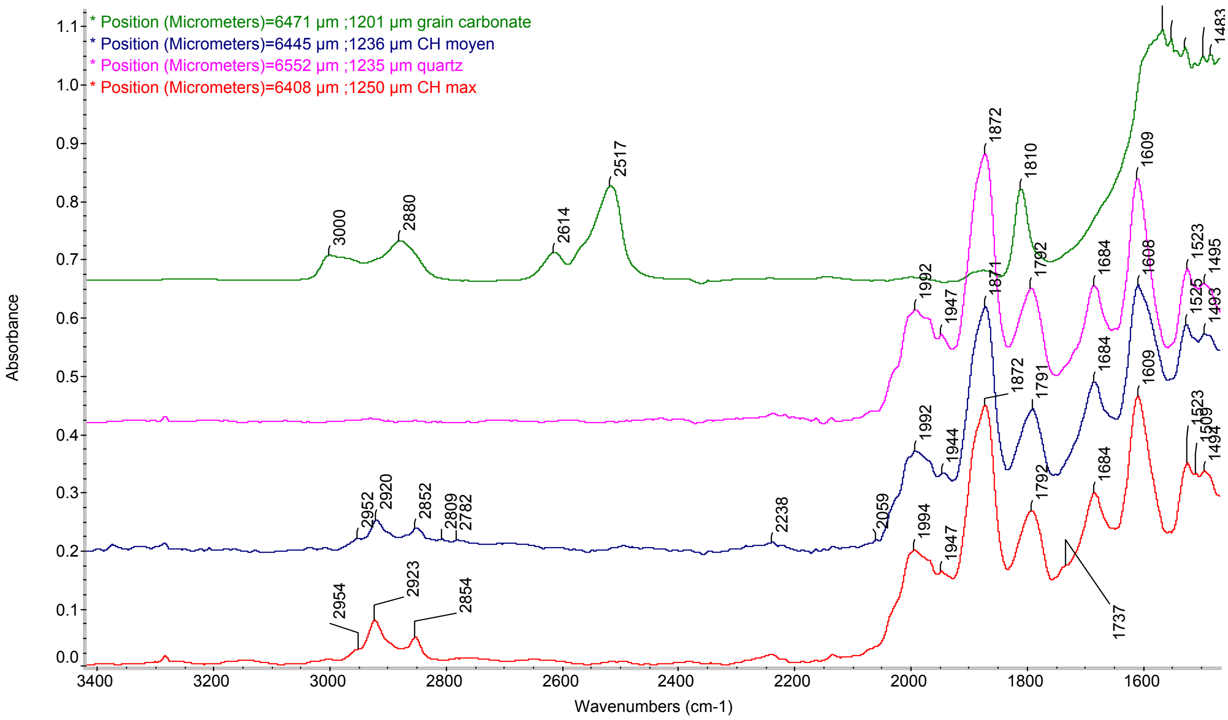


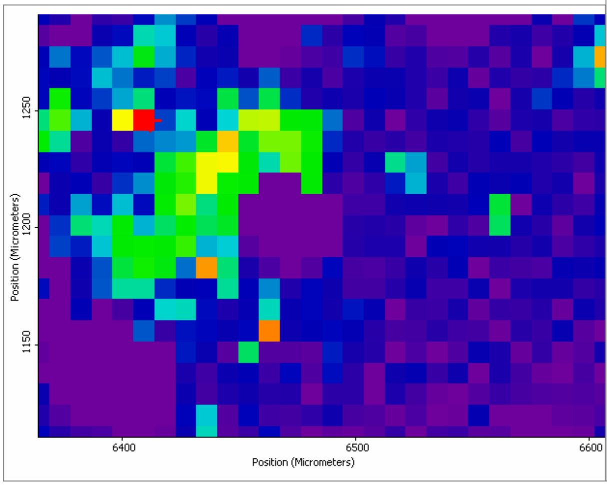
Figure 3. Microphotographs of a 50 µm large organic matter clots preserved in a sedimentary horizon of the 3.525 billion years old North Pole Dome (Western Australia). Question remains concerning the nature of this aggreagate. Is it abiotic and formed as a result of Fischer-Tropsch Type (FTT) synthesis due to extensive interaction of CO2-bearing aqueous fluids with the underlying Mg-rich metabasalts at high temperature or biotic and associated with the Calvin-Benson CO_2 carbon fixation pathway?

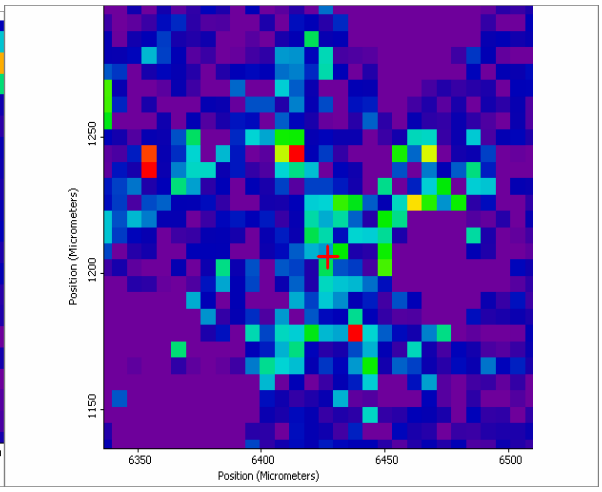
PDP2B F84.7 - map1sr-9µm



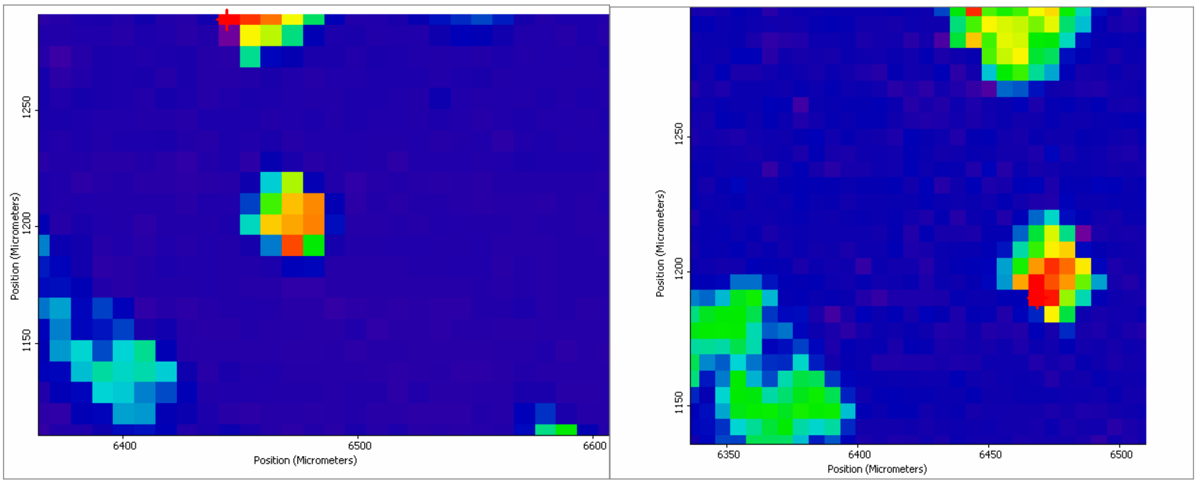
SR-µIRS analysis yielded crucial information of the composition of the kerogenous clots shown above, with marked aliphatic C-H stretching bands in the 2900 cm^{-1} region (redand blue spectra) and CH map (below) compared to carbonate (green spectrum) and quartz (pink spectrum) host matrix mineral forming the chert-carbonate drill core sample F84.7.

CH (2937-2900, ldb 2946-2884)

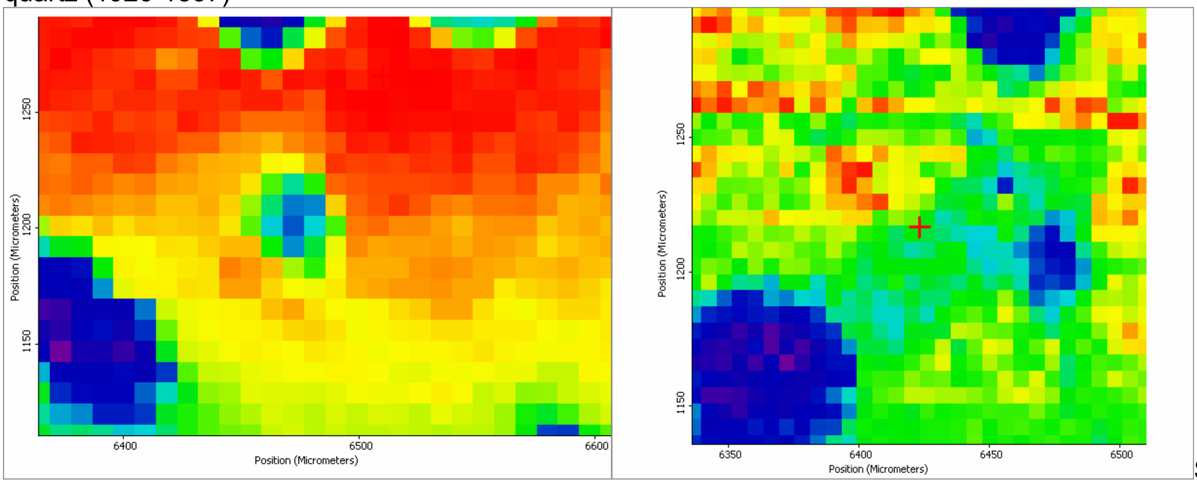




carbonate (2635-2424)



quartz (1926-1837)



S

Publications

Foriel V., Philippot P., Cauzid, J., Susini J., Dumas P., Khodja H, Ménez, B., Somogyi, A., Moreira D., Fouquet Y. and Lopez-Garcia P. 2004. High resolution synchrotron-based imaging of trace element, sulfur oxidation states and organic molecule distribution in individual microfossils and contemporary microbial filaments. Geochim. Cosmochim. Acta. 68, 1561-1569.

Gérard E., Guyot F., Philippot P., López-García P., 2005, Fluorescence in situ hybridisation coupled to ultra-small immunogold detection to identify prokaryotic–cells using transmission and scanning electron microscopy. Journal of Microbiological Methods, 63, 20-28.

Gérard E., Guyot F., Ménez B., Rommevaux-Jestin C., Wang Y., Salome M., Philippot P., López-García P., 2005. Fluorescence in situ hybridisation coupled to ultra-small immunogold detection to identify prokaryotic cells on minerals by electron microscopy and synchrotron radiation, Geophysical Research Abstracts, Vol. 7, 07872, *European Geoscience Union, General Assembly,* Vienne, Austria.

Lepot, K., P. Philippot, K. Benzerara, M. Cotte, 2006. In-situ structural and chemical characterisation of biogenic organic matter in 2.72 Ga stromatolites, Tumbiana Formation, Western Australia. EGU 6 meeting, special symposium on Environments of Life on Early Earth, Philippot and Van Kranendonk, co-covenors, Vienna, April 2006.

Ménez B., Rommevaux-Jestin C., Salomé M., Philippot P., Wang Y., Gérard E., *In situ* identification of labelled prokaryotic cells on mineral surfaces using Scanning X-Ray Microscopy, submitted to *PNAS*

Ménez B., Gérard E., Rommevaux-Jestin C., Salomé M., Wang Y., P. Bleuet, P. Philippot, 2005. Contribution of Scanning X-Ray Microscopy for unravelling prokaryotes and mineral interactions in the deep biosphere, *Joint International Symposia for Subsurface Microbiology and Environmental Biogeochemistry*, Jackson Hole, Wyoming, USA.

Ménez B., Rommevaux-Jestin C., Guyot F., López-García P., Salomé M., Wang Y., Philippot P., Gérard E., 2005. Fluorescence in situ hybridisation coupled to ultra-small immunogold detection to identify prokaryotic cells on minerals by electron and X-ray microscopy, *ESF Joint Initiative: Investigating Life in Extreme Environments Workshop,* Sant Feliu de Guixols, Spain.

Rommevaux-Jestin C., Ménez B., Gérard E., 2005. Microorganisms/minerals interactions in oceanic hydrothermal environments: new methods for chemical and structural imaging. *3rd International Symposium on Seep and Hydrothermal Vent Biology*, La Jolla, California.

Rommevaux-Jestin C., Ménez B., Gérard E., 2005. Microorganisms/minerals interactions in oceanic hydrothermal environments: new methods for chemical and structural imaging, *International MoMAR Implementation Workshop,* Lisbonne.

Philippot, P., K. Lepot, C. Thomazo, M. Van Kranendonk, M. Cotte, 2006. Abiotic *vs* biotic carbonaceous matter in 3,525-Myr-old hydrothermally-altered subseafloor sediments (Pilbara Craton, Western Australia), EGU 6 meeting, special symposium on Environments of Life on Early Earth, Philippot and Van Kranendonk, co-covenors, Vienna, April 2006.