ESRF	**In situ XANES mapping of the organic compounds that drive the carbohydrate crystallization and environment recording process in invertebrate shell layers**	**Experi numb** EC-2 ESRF
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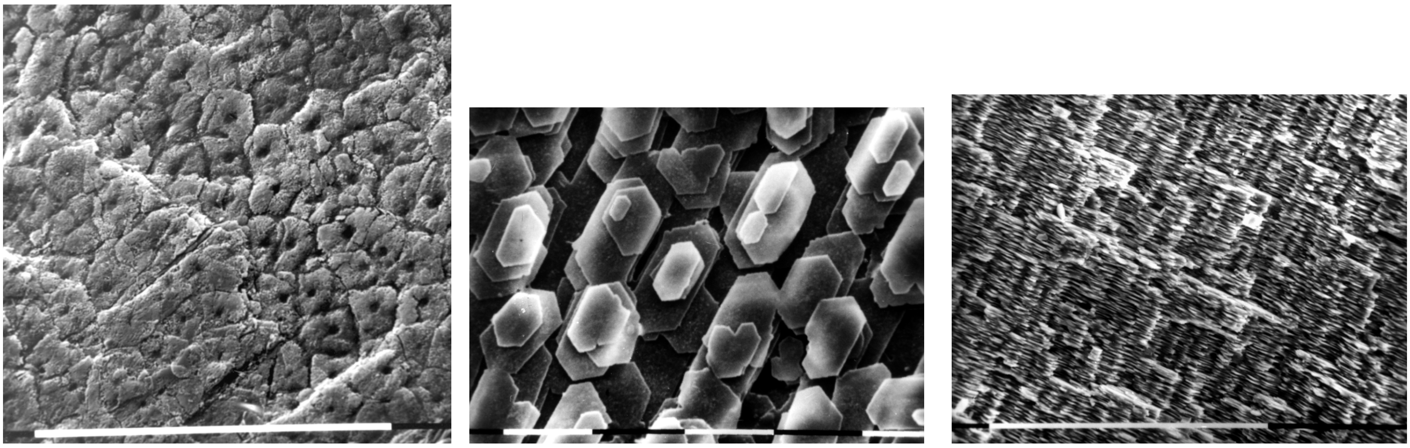
Report:

It is well known that invertebrate calcareous skeletons are composed of a mineral part and organic components. Previous experiments on carbonate skeletons (Mollusk shells, Coral skeletons) had shown that sulphur is a good marker of the organic matrix in biogenic aragonites and calcites. HPLC, infrared spectra and electrophoresis analyses have shown that the quantity, the composition and the structure of the organic matrix extracted from these skeletons are taxonomically controlled. XANES spectra of the dried soluble and insoluble organic matrices extracted from these samples have confirmed these results. In most samples, the importance of the organic sulfate has been evidenced, and these results confirm the presence of acidic and organic sulfate linked to polysaccharides previously observed by electrophoresis. The role of the sulphated sugars in the biomineralization processes had been shown by Wada (1964, 1980), but the main studies deal only with the proteins. Moreover, in situ spectra and maps have shown that the growing process of carbonate skeletons is incremental: growth lines of 1-3 µm thick are not only topographic, they are also compositional.

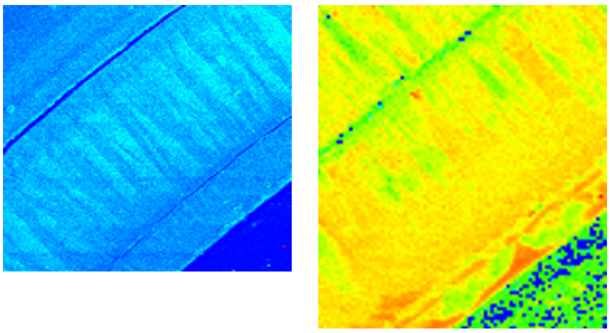
EC-208 was dedicated to biochemical *in-situ* characterization at the ID21 line where micro-XANES maps and spectra at the sulphur K-edge in focussed mode have been made to assess the speciation of S in modern Invertebrate shells. The nacreous layer of the Mollusk shell is the best-known structure, and the base for all the biomineralization models. The nacre has a simple geometry, with inter-crystalline organic membranes that are easy to isolate, and thus were chosen for this first experiment. Such experiments have been done on polished sections, but for the first time we have tried to map fixed and isolated the inter-crystalline membranes of these skeletons to determine what is due to the inter- and intra-crystalline organic matrices.

Differences in the structures of the brick-and-mortar nacre of bivalve and cephalopod mollusks were shown by Wise (1972) and Erben(1972), so that an example of each taxa was selected. Because of the low S contents of the interlamellar organic membranes, maps are not decisive. However, strong similarities between spectra obtained on fixed membranes and lyophilized organic matrices of *Nautilus* were observed. Some peaks in the lyophilized organic membranes were not interpreted (Dauphin 2006) because it could be suggested they were artifacts. These peaks were not detected with in situ spectra of the non decalcified shell layer (Dauphin 2006). However, the same peaks were observed in the EC206, despite another *Nautilus* species was used, and despite different preparative process. Interestingly, the spectra obtained from the nacre of the cephalopod *Nautilus* and the bivalve *Atrina* showed different the sulphur speciation, which indicates that there are differences in their organic matrix compositions. Further experiments on these matrices are now in preparation at the department of Structural biology (Weizmann Institute, Israel). Thus, these results may contribute to develop a new approach of the biomineralization processe.



SEM images of the nacreous layer of *Nautilus*, showing the heterogeneous complex structure of the tablets (left, scale bar 100 µm), the shape of the tablets (center, scale bar 10 µm) and the pseudo-prismatic structure at low magnifications. The centers of the tablets are superimposed (scale bar 100µm).

EC208 was also used to perform in situ spectra and maps of larva of *Pinctada*, a bivalve used for the production of pearls, black pearls of Polynesia, brachiopods (to increase the range of studied samples at EC24). Detailed spectra of lyophylised organic matrices (soluble and insoluble matrices) extracted from the same samples were also done.



XANES maps of a black pearl from Polynesia.
Left : prismatic layer (p) and nacreous layer (n) at 2.482 keV for sulfate (scan field 750x690 µm); Right: detail of the prismatic layer at 2.473 kev for S amino acids (scan field 180x200 µm). The sulfate contents fo the prismatic and nacreous layer seem similar, an unusual feature in *Pinctada* shells.

The first experiments on isolated organic membranes are promising, despite the fact that maps are not conclusive due to the low S contents. However, the spectra on these samples lead to a re-examination of previous results obtained on lyophyilised matrices.

Submitted papers:

Y. Dauphin, J.P. Cuif, C.T. Williams, **Soluble organic matrices of aragonitic skeletons of Merulinidae (Cnidaria, Anthozoa)** - Comp. Physiol. Biochem.

Y. Dauphin, A. D. Ball, M. Cotte, J.P. Cuif, A. Meibom, M. Salomé, J. Susini, C. T. Williams, **Structure and composition of the nacre - prisms transition in the shell of *Pinctada margaritifera* (Mollusca, Bivalvia)** - Analyt. Biochem. Chem.