



HAL
open science

Nano-Composite Structure of Nacre Biocrystal.

Xavier Bourrat, Marthe Rousseau, Evelyne Lopez, Philippe Stempflié

► **To cite this version:**

Xavier Bourrat, Marthe Rousseau, Evelyne Lopez, Philippe Stempflié. Nano-Composite Structure of Nacre Biocrystal.. 2005, 6p. <hal-00092027>

HAL Id: hal-00092027

<https://insu.hal.science/hal-00092027v1>

Submitted on 8 Sep 2006

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Nano-Composite Structure of Nacre Biocrystal.

Xavier Bourrat*¹, Marthe Rousseau², Evelyne Lopez², Philippe Stempflié³.

¹*Institut des Sciences de la Terre d'Orleans ISTO, Orleans University, Geosciences BP 6759, 45 067
Orleans cedex 2, FRANCE Xavier.Bourrat@univ-orleans.fr*

²*Museum National d'Histoire Naturelle, Département des Milieux et Peuplements Aquatiques UMR 5178 :
CNRS-MNHN : « Biologie des Organismes Marins et Ecosystèmes »7, rue Cuvier 75005 Paris France
rousseam@gmx.net, lopez@mnhn.fr*

³*Ecole Nationale d'Ingénieurs de Tarbes, Laboratoire Génie de Production, 47 avenue d'Azereix, 65016
Tarbes, France philippe.stempflié@enit.fr*

Abstract

Intermittent-Contact Atomic Force Microscopy with phase detection imaging reveals a nanostructure within the tablet (*Pinctada maxima*). A continuous organic framework divides each tablet into nanograins. Their mean extension is 45nm. Transmission electron microscopy performed in the darkfield mode evidences that intracrystalline matrix is highly crystallized and responds like a 'single crystal'. The organic matrix is continuous inside the tablet, mineral phase is thus finely divided but behaves in the same time as a single crystal. It is proposed that each tablet results from the coherent aggregation of nanograins keeping strictly the same crystallographic orientation thanks to an hetero-epitaxy mechanism.

1. Introduction

Structure of nacre is already well documented till the works of Wada, 1961, Wise, 1970, Grégoire, 1972 and Mutvei, 1979. A long tradition of structural analysis is existing in this field, often conducted by electron microscopy. Grégoire *et al.* already used this technique in 1955 to evidence the interlaminar sheet of organic matter. Bevelander and Nakahara, 1969, later brought decisive insights to establish the compartment theory. Erben and Watanabe, 1974 meanwhile discussed some aspect of this theory. Concomitantly, Mutvei, 1969, 1970 and 1979 also cleverly played with preparation procedures to highlight certain fine details related to biocrystal growth. Very recently, the sophisticated method of cryo-TEM was employed to cross-check the fine structure usually observed by dehydration and staining techniques with the same matrix but hydrated and vitrified at low temperature (Levy-Kalisman *et al.*, 2001). The composite structure was also studied, particularly the role of the organic matrix by a bio-chemistry approach (Weiner and Straub, 1980; idem, 1984; Weiner and Addadi, 1991; Mann, 1989; Mann *et al.*, 1984; Falini *et al.*, 1996; Belcher *et al.*, 1996; Feng *et al.*, 1999; Thompson *et al.*, 2000). It resulted in different models proposed by Schäffer *et al.*, 1997 or Levi-Kalisman *et al.* 2001).

In a recent paper (Rousseau *et al.*, 2005) we arrived to the conclusion that mineralization of the compartment follows a Voronoi tiling model. Here, we propose to study the structure of the organic matrix using techniques such as darkfield TEM or intermittent-contact atomic force microscopy coupled with phase imaging (AFM in tapping mode).

2. Materials and methods

2.1. Atomic Force Microscopy (AFM)

The microscope used was a *Dimension 3000* connected to an electronic controller, the *Nanoscope IIIa*TM produced by *Digital Instruments* (USA). The spatial and vertical resolutions are less than 1 nm and the field is between 100nm and 100 μ m. The images recorded in this work were taken at high resolution (512 X 512 pixels) by using an intermittent-contact mode (called Tapping ModeTM) coupled with phase detection imaging (PDI). The tapping-mode makes it possible to minimize the interactions between the probe and the surface during acquisition and considerably improves the resolution compared to the contact mode (Aimé *et al.*, 2001). This PDI mode, in addition to the topographic images, provides a map characterizing the variations of the mechanical properties of the scanned surface in the present work (Magonov *et al.*, 1997). The probe is in SiNi with a round tip of between 5 and 10 nm. The work frequency, the stiffness and the amplitude of cantilever were respectively: 270 kHz, 42 N.m⁻¹ and 25 nm. The scanning rate was 1.2 μ m.s⁻¹. Data were collected in air and at room temperature after polishing parallel to the nacre surface.

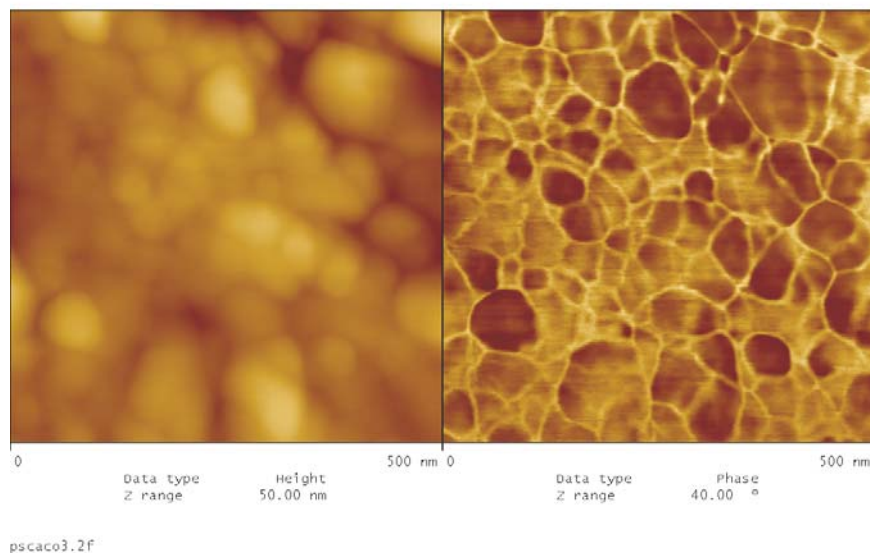


Fig.3. AFM images of the polished surface of nacre tablets in tapping-mode (polished with tablets flat-on) : a) Topography of the surface (Height) b) Picture in Phase Contrast showing the foam-like structure of the intracrystalline organic matrix.

2.2. Transmission electron microscopy

Two types of samples were selected: (i) nacre cut from the recent shell of *Pinctada maxima*; and (ii) from old and dry nacre from collections. First, a mechanical thinning was obtained on small slides, 3mm in diameter. At a thickness of about 80 μ m, both sides were polished and then glued on to a single-hole copper grid. Ion milling was then performed using a Gatan DuoMill at room temperature. Thinning was continued until electron transparency was obtained. Artifacts due to sampling appeared to be reduced and limited to the edge of the hole. For beam stability reasons, the old nacre was preferred for the preparation of high resolution images.

Numerous TEM studies have been carried out on nacre over the last 50 years. Both traditional sample preparations of ultramicrotomy or ion-beam milling have been used. Both techniques are known to introduce specific artifacts. Ultramicrotomy generally breaks the samples, producing splinters. Within each splinter the structures remain, enabling full analysis (Gunnison *et al.*, 1992). In this case the interfaces are destroyed. It is hard to work on the interlaminar matrix with this technique. Ion beam milling carries the danger of carbonization of the organic matrix. Using thermal gravimetric analysis, we have measured that nacre can in fact sustain

temperatures as high as 230°C without any mass change under air. Several groups have used this technique initiated by Towe and Hamilton, 1968, in this field. Aksay's group in particular, fully discussed this point, reaching the conclusion that radiolysis is probably very minor (Sarikaya *et al.*, 1995).

Observations were conducted in a Philips CM30ST at 300kV with a point resolution of $\delta=0.2\text{nm}$. Darkfield technique was performed using an objective opening of 2.15nm^{-1} . Once the stage is tilted in order to get parallel to the axes, the beam is tilted near the transmitted spot avoiding any mineral reflection. In these conditions the contrast is provided by the crystallized organic matrix (mineral spots are eliminated by the small objective aperture).

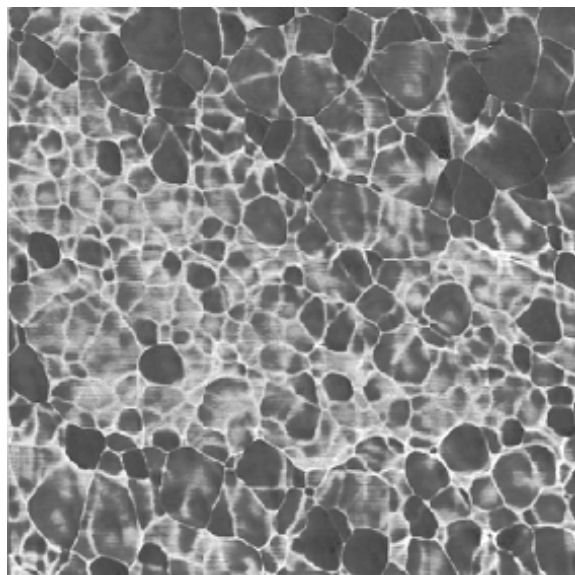


Fig. 4. AFM picture in Phase Contrast ($1 \times 1 \mu\text{m}^2$).

3. Results and discussion

3.1. Nanostructure of the platelet by AFM

In *Tapping Mode*TM coupled with phase detection imaging, two types of determination are simultaneously obtained : a Height Map (Fig. 1a) enabling monitoring of the flatness of the sample (polished parallel to tablets) and the Phase Contrast Map (Fig. 1b) is enabling the detection of variations in composition, adhesion, friction, viscoelasticity or other properties, including the detection of the different composite components (Magonov *et al.*, 1999). Technically, the images in Figs. 1b and 2 are obtained by measuring the phase difference between the excitation signal and the cantilever response. This information can be related to the dissipation of energy during the interaction of the tip onto the surface. These data are related to local variations of the material. The resulting phase contrasts are often difficult to interpret because of the complex interactions of chemical and physical effects (Cleveland *et al.*, 1998; Tamayo and Garcia, 1998). Here, the interactions can be related to the drastic difference of the elastic properties of the intracrystalline organic matrix regarding those of the mineral phase. The NiSi tip of the AFM is chemically non-reactive : chemical interactions with the sample are more than likely negligible with respect to the contrast expected with the elastic modulus variations between mineral and organic phases (Lafon *et al.*, 2000).

It has long been demonstrated that intracrystalline matrix is present within the tablets (Schmidt, 1924) and located at the micrometer scale (Mutvei, 1970; Mutvei, 1979) but its relationship with the mineral has never been shown at the nanometer scale.

Mineral nanograins can be evidenced in Figures 1a and b. They are *surrounded by an organic phase*, evidenced by means of the strong elastic modulus contrast between proteins and aragonite. Figure 2 is another phase-contrast picture recorded on a wider field of $1 \times 1 \mu\text{m}^2$. The organic matrix is organized as in the form of "foam" with very thin walls and closed cells. The mineral nanograins are thus encapsulated inside the organic framework (vesicle). Post processing treatment was applied to the different images (grain analysis using the software

*SPIP*TM by *Image Metrology ApS*). This makes it possible to determine the morphology (size and shape) of the nanocrystals. A mean nanograin size of 44nm (± 23) was found. The tablet responds as a single-crystal by electron diffraction (not shown here; see e.g. Liu *et al.*, 1992). *Tablet is in fact made up of a coherent aggregation of nanograins.*

Finally, figures 1b and 2 clearly show that *the organic framework is continuous within the tablet; nothing evidences that the mineral is also continuous.*

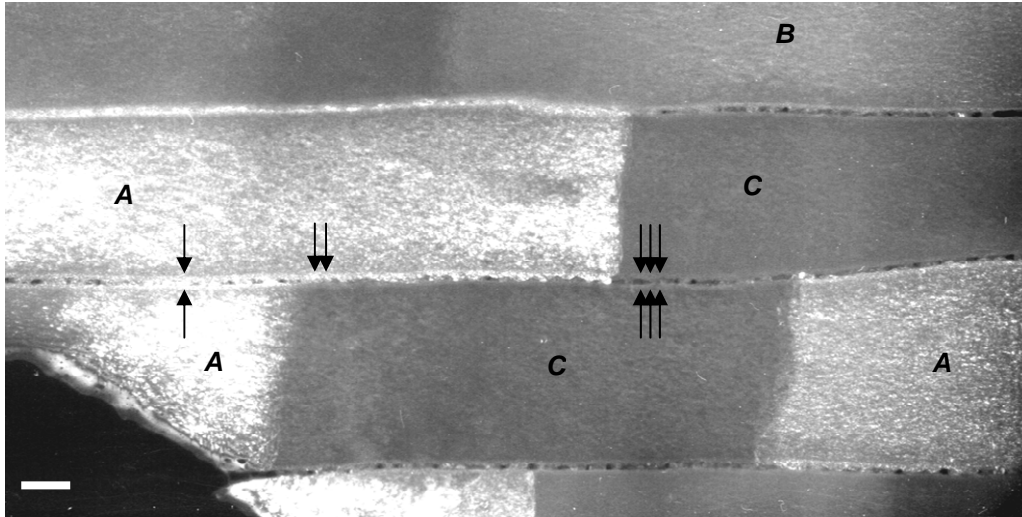


Fig. 2. Darkfield TEM image of nacre evidencing the crystalline structure of the organic matrix (bar is 100nm) . Organic matter is in contrast when under Bragg conditions whilst the mineral phase remains systematically extinguished.

3.2. Continuity of the organic framework in the tablet (TEM)

Chitin or proteins have ordered structures which diffract (Nakahara *et al.*, 1982; Weiner *et al.*, 1983). Their diffusion coefficient and preferred orientation are supposed to be weaker, compared to those of the mineral lattice. The contrast related to the organic and mineral phases is anyway difficult to distinguish in most cases. By using filtering in the reciprocal space, known as darkfield TEM, it is possible to select one of the spatial frequencies ($s=\lambda/\theta$) related to the organic matrix by means of a small objective aperture and have it in contrast, despite the presence of the mineral phase which remains extinguished. That is the way the organic framework can be studied, *in situ*.

Figure 3 is a darkfield image obtained by filtering the reciprocal space with a very small objective aperture, avoiding the aragonite spots. Thus, *only the organic matrix is in contrast (part of it only)*. First, the highest contrast comes from the organic matrix of the interlaminar region.

Second and most importantly, this technique evidences the presence of the intracrystalline organic matrix in a different way as compared to AFM. In each tablet, the intracrystalline matrix is seen to diffract under the form of tinny spots : all the spots get in contrast together in the tablet. The variation of the contrast from one tablet to the other in Fig. 3 suggests that *the intracrystalline organic matrix reacts as a 'single crystal' network*. If the organic matrix is oriented under the Bragg angle it brightens all over the tablet (case of tablet A). If it is just slightly misoriented, only a faint contrast appears (tablet B). This is related to a Bragg tolerance phenomenon classically observed in the case of small coherent domains such as these. In other tablets (as C) the intracrystalline organic network is extinguished because not selected in this direction or not under the Bragg angle. It is to note that distortions appear in tablet A. Are they related to sampling artefacts or real? More work is needed in this sense.

Finally, the relationships between the intracrystalline and interlaminar matrix are worthy of note. The interlaminar matrix brightens substantially when the intracrystalline matrix also brightens. If the neighbor is also in contrast, both sides of the interlaminar sandwich are bright (single arrows), but only one side is bright if the neighbor is extinguished (double arrow). When the two neighbors are extinguished, the interlaminar matrix is also extinguished or faintly in contrast (triple arrow). The median layer of the interlaminar sequence is formed

by a porous layer appearing as a succession of bridges in cross section (studied by TEM high resolution in next part) and imaged flat-on by SEM (Fig. 8) in a previous work (Rousseau *et al.*, 2005).

4. Concluding remarks

1. The tablet of nacre, the biocrystal, does diffract as a single crystal but is made up of a continuous organic matrix (intracrystalline organic matrix) which breaks the mineral up into coherent nanograins (~45nm mean size).
2. The single crystal-like orientation of the tablet is supposedly provided by the heteroepitaxy of the intracrystalline matrix. This is a strong hypothesis since in the same time, this intracrystalline matrix appears well crystallized and diffracts as a “single crystal”.
3. Finally, this work provides an interesting hypothesis to explain why the tablets are first growing with a cylindrical shape and then turning polygonal when contacting each other (Rousseau *et al.*, 2005). The existence of nanograins encapsulated in an organic vesicle raises the hypothesis of an aggregation-like control of the extension of the tablet by the organic template and not directly by the atomic forces.

References

- Aimé JP, Boigard L, Nony G, Couturier G. Influence of nanocontact dissipation in Tapping-mode: attempt to extract quantitative information on the surface properties with the local force probe method. *J. Chem. Physics* 2001; 114:4945-4954.
- Belcher AM, Wu XH, Christensen RJ, Hansma PK, Stucky GD, Morse DE. Control of crystal phase switching and orientation by soluble mollusc-shell proteins. *Nature* 1996; 381:56-58.
- Bevelander G, Nakahara H. An electron microscope study of the formation of nacreous layer in the shell of certain bivalve molluscs. *Calc. Tiss. Res.* 1969; 3:84-92.
- Cleveland JP, Schmid AE, Elings VB. Energy dissipation in tapping-mode atomic force microscopy. *Appl. Phys. Lett.* 1998; 72:2613-2615.
- Erben HK, Watabe N. Crystal formation and growth in bivalve nacre. *Nature*. 1974; 248: 128-130.
- Falini G, Albeck S, Weiner S, Addadi L. Control of aragonite or calcite polymorphism by mollusk shell macromolecules. *Science* 1996; 271:67-69.
- Feng QL, Li HB, Cui FZ, Li HD. Crystal orientation domains found in the single lamina in nacre of the *Mytilus edulis* shell. *J. Mater. Sci. Lett.* 1999; 18:1547-1549.
- Grégoire C, Duchateau G, Florin M. La trame protidique des nacres et perles. *Ann. Océano.* 1955; Tome XXXI:1-36.
- Grégoire C. Structure of the molluscan shell. In Florin M, Scheer BT editors, *Chemical Zoology*, vol. II, New-York, 1972:45-102.
- Gunnison K, Sarikaya M, Liu J, Aksay IA. Structure-mechanical property relationships in a biological ceramic-polymer composite: nacre. In Aksay IA, Baer E, Sarikaya M, Tirrel DA editors, *Hierarchically structured materials*, Proc. MRS 255, Pittsburg USA : MRS, 1992 :171-183.
- Lafon V, Paris E, Henriot A. The use of AFM to atmospheric particles, case of car exhaust particles. *Digital Instrument Applications Note*, 2000:2-3.
- Levi-Kalishman Y, Falini G, Addadi L, Weiner S. Structure of the nacreous organic matrix of a bivalve mollusk shell examined in the hydrated state using Cryo-TEM. *J. Struct. Biol.* 2001; 135:8-17.
- Liu J, Sarikaya M, Aksay IA. A hierarchically structured model composite: a TEM study of the hard tissue of red abalone. *Mat. Res. Soc. Symp. Proc.* 1992; 255:9-17.
- Magonov SN, Elings V, Whangbo M-H. Phase imaging and stiffness in tapping-mode atomic force microscopy. *Surf. Sci.* 1997; 375:385-391.
- Mann S. In Mann S, Williams RJP editors, *Biomineralization : Chemical and Biological Perspectives*, Weinheim (Germany) VCH Verlags gesellschaft 1989:146-148.
- Mann S, Archibald DD, Didymus JM, Douglas T, Heywood BR, Meldum TC, Reeves NJ. Crystallization at inorganic-organic interfaces: biominerals and biominetics synthesis. *Science* 1993:261.
- Mutvei H. On the micro and ultrastructure of conchiolin in the nacreous layer of some recent and fossil molluscs. *Stockholm Centr. Geol.* 1969; 20:1-17.
- Mutvei H. Ultrastructure of the mineral and organic components of molluscan nacreous layers. *Biomineralization research report.* 1970; 2: 48-72.

- Mutvei H. On the internal structures of the nacreous tablets in molluscan shells. *Scan. Electr. Micros.* 1979; II:457-462.
- Nakahara H., Bevelander G, Kakei M, Electron microscopic and amino acid studies on the outer and inner shell layers of *Haliotis rufescens*. *Venus*, Japan. *J. Malacol.* 1982; 41:33-46.
- Rousseau M, Lopez E, Couté A, Mascarel G, Smith DC, Naslain R, Bourrat X. Sheet nacre growth mechanism : a Voronoi model. *J Struc Biol* 2005; 149: 149-157.
- Sarikaya M, Liu J, Aksay IA. Nacre: properties, crystallography, morphology and formation, In Sarikaya M, Aksay IA, editors, *Biomimetics: Design and Processing of Materials*. USA AIP Press, 1995:35.
- Schäffer TE, Ionescu-Zanetti C, Proksch R, Fritz M, Walters DA, Almqvist N, Zaremba CM, Belcher AM, Smith BL, Stucky GD, Morse DE, Hansma PK. Does abalone nacre form by heteroepitaxial nucleation or by growth through mineral bridges? *Chem. Mater.* 1997; 9: 1731-1740.
- Schmidt N J. *Die Bausteine des Tierkörpers in Polarisierten Lichte*, Bonn : Cohen, 1924.
- Tamayo J, Garcia R. Relationship between phase shift and energy dissipation in Tapping-mode scanning force microscopy. *Appl. Phys. Lett.* 1998; 73:2926-2928.
- Thompson JB, Poloczi JT, Kindt JH, Michenfelder M, Smith BL, Stucky G, Morse DE, Hansma PK. Direct observation of the transition from calcite to aragonite growth as induced by abalone shell proteins. *Biophys. J.* 2000; 79(6):3307-3312.
- Towe KM, Hamilton GH. Ultrastructure and inferred calcification of the mature and developing nacre in bivalve mollusks. *Calc. Tiss. Res.* 1968; 1:306-318.
- Wada K. Crystal growth of molluscan shells. *Bull. Natl. Pearl Res. Lab.* 1961; 7:703-828.
- Weiner S, Addadi L. Acidic molecules of mineralized tissues: the controllers of crystal formation. *TIBS* 1991; 16:252-256.
- Weiner S, Talmon Y, Traub W. Electron diffraction of mollusc shell organic matrices and their relationship to the mineral phase. *Int. J. Biol. Macromol.* 1983; 5:325-328.
- Weiner S, Traub W. X-diffraction study of the insoluble organic matrix of mollusc shells. *Eur. Biochem. Soc.* 1980; 111:311-316.
- Weiner S, Traub W. Macromolecules in mollusc shells and their functions in biomineralization. *Phil. Trans. R. Soc. Lond.* 1984; B 304:425-434.
- Wise SW. Microarchitecture and mode of formation of nacre (mother of pearl) in Pelecypods, Gastropods, and Cephalopods. *Eclogae geol. Helv.* 1970; 63:775-797.