

# Chapter 36

## Mollusk Shells: Does the Nacro-prismatic “Model” Exist?



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**Abstract** The “nacro-prismatic” shells are the most studied mollusks, and they are often said to be “the” model to unravel the biomineralization mechanisms. Nevertheless, the nacro-prismatic structure is not unique, despite most data are provided by only three genera. The aragonitic nacre is taxon dependent: in cephalopods and gastropods, nacre is columnar, whereas bivalves have a spiral or sheet nacre. The inner structure of gastropod and cephalopod columnar nacre differs. The shape of the tablets is specific of the taxa. Calcitic and aragonitic prisms exist. The composition of the organic matrices extracted from calcitic prisms with a similar shape and mineralogy strongly differs. The inner structure of aragonite prisms is complex, with a central zone and divergent elongated crystallites at the periphery. Additionally, the relationships between nacre and prisms are also taxonomically related. From these data, whatever the scale at which they are studied, every component of the “nacro-prismatic” model – nacre, prisms, and prism–nacre topographic relations – is highly variable, so that this “model” does not exist; it is a structure.

**Keywords** Mollusks · Nacre · Prismatic layer · Model

### 36.1 Introduction

The most common structure in mollusk shells is the aragonite crossed-lamellar layer, but the most studied is the “nacro-prismatic” arrangement. Almost all data about mollusks are from three bivalve genera with flat large shells: *Atrina*, *Pinna*, and *Pinctada*. These genera are taxonomically related (Pteriomorpha), with

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large polygonal prismatic units and an inner nacreous layer, so that separating the two layers for detailed analyses is not difficult. They are often used as “the” model to understand the biomineralization processes. Sometimes, *Unio* and *Mytilus*, both with a nacro-prismatic structure, are also used as a model. The concept of model to describe this structure suggests that all these nacro-prismatic shells are identical in terms of structure and composition. The examination of the structure and composition of the layers and of the prismatic and nacreous units, as well as their relationships, demonstrates that the nacro-prismatic arrangement is not unique. It is impossible to enter into the detailed description of all mollusk shells. So, the present article will concentrate largely on the differences between the nacro-prismatic shells.

## 36.2 Materials and Methods

Details about the origin of the samples and preparative process and setup of the diverse used techniques are given in the relevant publications listed in the References.

### 36.2.1 Materials

Bivalves (*Pinctada*, *Pinna*, *Nucula*, *Neotrigonia*, *Unio*), gastropods (*Haliotis*, *Trochus*, *Turbo*), and cephalopods (*Nautilus*, *Sepia*, *Spirula*) were used. Depending on the genera, several species were studied (*Pinna*, *Sepia*, *Haliotis*, among others).

### 36.2.2 Methods

Micro- and nanostructures were studied using thin sections, fractures, and polished etched surfaces for the scanning electron microscope (secondary and backscattered electron modes, Philips SEM XL30, FEI Phenom) and atomic force microscope (Veeco Nanoscope Dimension 3100). Electron microprobes (energy- and wavelength-dispersive spectrometry) (Link AN10000, CAMECA SX50, SX100) were used for quantitative elemental chemical composition and distribution maps. Chemical distribution maps were also performed using NanoSIMS (CAMECA N50), TOF-SIMS (TOF-SIMS IV Ion-Tof GmbH), and XANES (ID21, ESRF). Thermogravimetric analyses allow to quantify the organic matrix content. Infrared and Raman spectrometry were used on both bulk samples and extracted organic matrices. Liquid chromatography and electrophoresis were used for molecular weights and acidity of the soluble matrices. Lipid content was known using thin-layer chromatography. Amino acid analyses were done on both soluble and insoluble matrices.

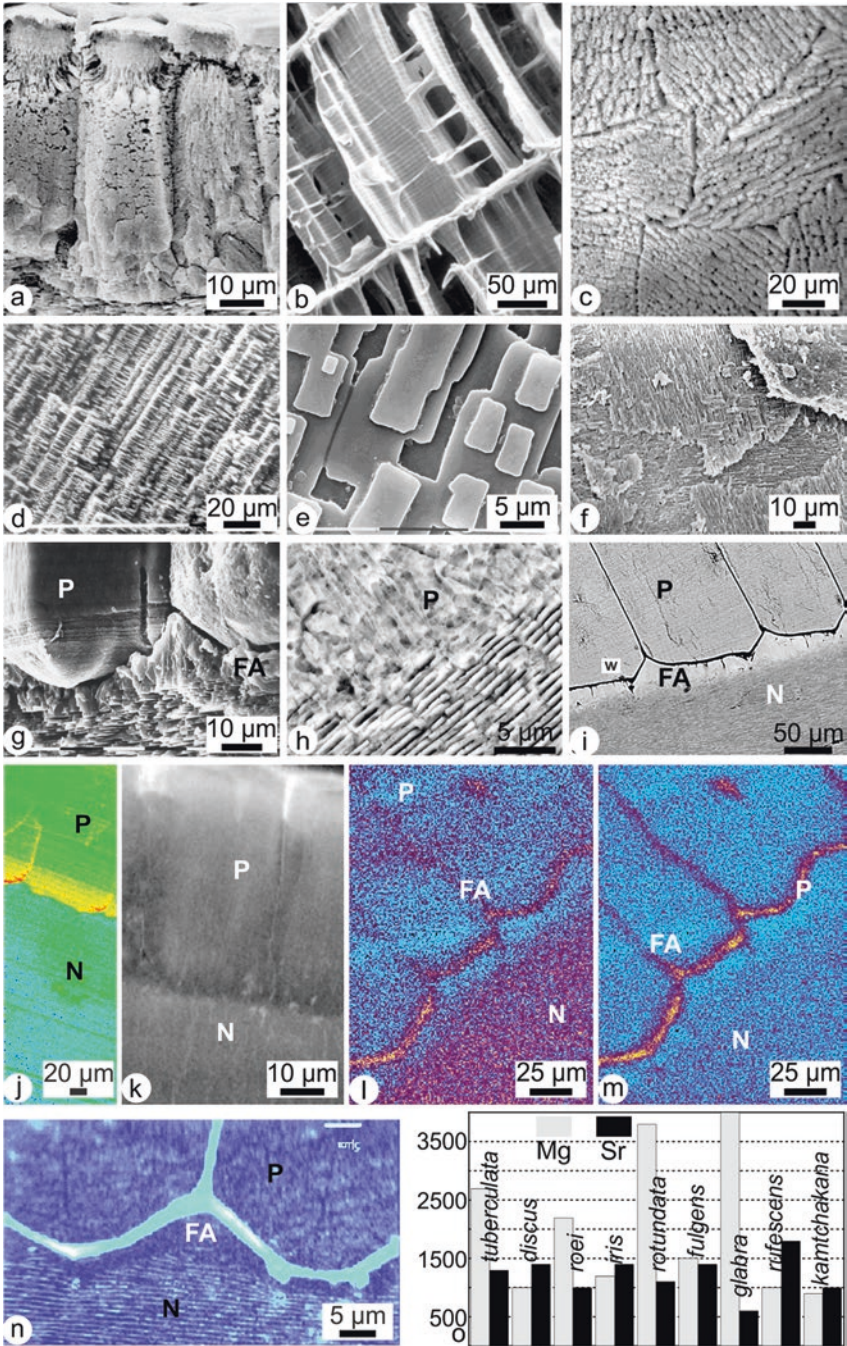
## 36.3 Results

### 36.3.1 Microstructures

Prisms are aragonite (*Neotrigonia*, Unionidae, Cephalopoda) or calcite (Pteriomorphia) (Fig. 36.1a–c) (Boggild 1930; Taylor et al. 1969, 1973; Ben Mlih 1983; Checa et al. 2014; Cuif et al. 2011). In some species, calcitic and aragonitic prisms coexist (Dauphin et al. 1989). The inner structure of the prisms is also variable, but the morphological and microstructural diversity is not related to the mineralogy (*Sepia*, Fig. 36.1b; *Haliotis*, Fig. 36.1c) but to the taxa. The inner structure of aragonite prisms is complex, with a central zone and divergent elongated crystal-lites at the periphery. Calcitic prisms are mono- or polycrystalline. The nacre is aragonite, but the tablets are deposited in vertical columns in gastropods and cephalopods, whereas they are in lenses in bivalves (Wise 1970). The inner structure of gastropod and cephalopod columnar nacre differs. Moreover, the shape and the inner structure of the tablets are species dependent (*Nautilus*, Fig. 36.1d; *Pinna*, Fig. 36.1e; *Sepia*, Fig. 36.1f) (Mutvei 1978, 1979). In coleoid cephalopods, the nacreous layer has no tablets (Mutvei 1963) (Fig. 36.1f).

Not only the shape, mineralogy, and inner structure of the prisms or tablets differ, but the transition between the two layers is also taxonomically dependent. When the prisms are calcite, there is no direct contact between the calcite and the nacre (Cuif et al. 2011). Both layers are separated by a thick organic membrane and an irregular layer of fibrous aragonite (*Pinctada*, Fig. 36.1g). In shells with aragonitic prisms, the transition is smooth, without an organic membrane (*Neotrigonia*, Fig. 36.1h) (Dauphin et al. 2014). Chemical differences also exist in the transition zone. Backscattered electron image of the calcitic–aragonitic transition demonstrates that the first aragonitic deposits are not nacre (*Pinctada*, Fig. 36.1i) (Dauphin et al. 2008). XANES map shows that the chemical composition of the end of the calcitic prisms is modified (*Pinctada*, Fig. 36.1j) (Dauphin et al. 2003), so that it cannot be said that the termination of prisms is “abrupt” (Hovden et al. 2015). No organic membrane exists between aragonitic prisms and nacre (*Neotrigonia*, Fig. 36.1k) (Checa and Rodriguez-Navarro 2001; Dauphin et al. 2008, 2014). The amino acid content of the fibrous aragonite differs from that of the nacreous layer, as shown by TOF-SIMS maps (*Pinctada*, Fig. 36.1l, m) (Farre et al. 2011), and the N map confirms the difference between the nacre and the fibrous aragonite in calcitic–aragonitic shells (*Pinctada*, Fig. 36.1n) (Dauphin et al. 2008).

It must be added that the elemental chemical composition of a given structure (nacre, calcitic or aragonitic prisms) is species dependent (Fig. 36.1o) (Dauphin et al. 1989).



**Fig. 36.1** (a) Unetched fracture showing the complex aragonitic prisms of *Neotrigonia*. (b) Aragonitic prismatic layer of the dorsal shield of *Sepia* – unetched fracture. (c) Calcitic prisms of *Haliotis rufescens*, polished and etched fracture. Formic acid 5%, 7 s, 20 °C. (d) Columnar nacreous layer of *Nautilus* – unetched fracture. (e) Rectangular nacreous tablets of *Pinna* – unetched sample. (f) Type 2 nacre: layered structure without tablets in a lamella of the ventral part of *Sepia* – unetched sample. (g) Unetched fracture showing the calcitic prismatic – aragonitic nacreous

### 36.3.2 Organic Components

It is now well-known that mollusk shells are organo-mineral biocomposites. For a given structure, the quantity and nature of the organic matrices differ and depend on the taxa as shown by TGA data of the nacre in *Nautilus* (Cephalopoda), *Trochus* (Gastropoda), and *Pinctada* (Bivalvia) (Fig. 36.2a, b). Insoluble matrices comprise proteins and lipids. It must be noted that the results differ following the sample preparation (decalcification or lipid extraction using organic solvents, Farre and Dauphin 2009). Using the same preparative process, the lipidic composition of the calcitic prisms of *Pinna* and *Pinctada* differs (Fig. 36.2c). The molecular weights of the soluble matrices of these prisms also differ (Fig. 36.1d) (Dauphin 2003). As for the insoluble matrices, most analyses are dedicated to the protein contents, mainly amino acid analyses (*Pinctada*, *Nautilus*, Fig. 36.2e). Nevertheless, infrared spectrometry of the insoluble matrices shows the presence of lipids and sugars in the insoluble matrices of nacreous layers (*Nautilus*, *Pinctada*, Fig. 36.2f). Despite the similarity of shape and mineralogy of the prisms of *Pinna* and *Pinctada*, the acidity (pI) and aliphatic index (indicative of the thermal stability for globular proteins) of the insoluble matrices differ (Fig. 36.2g).

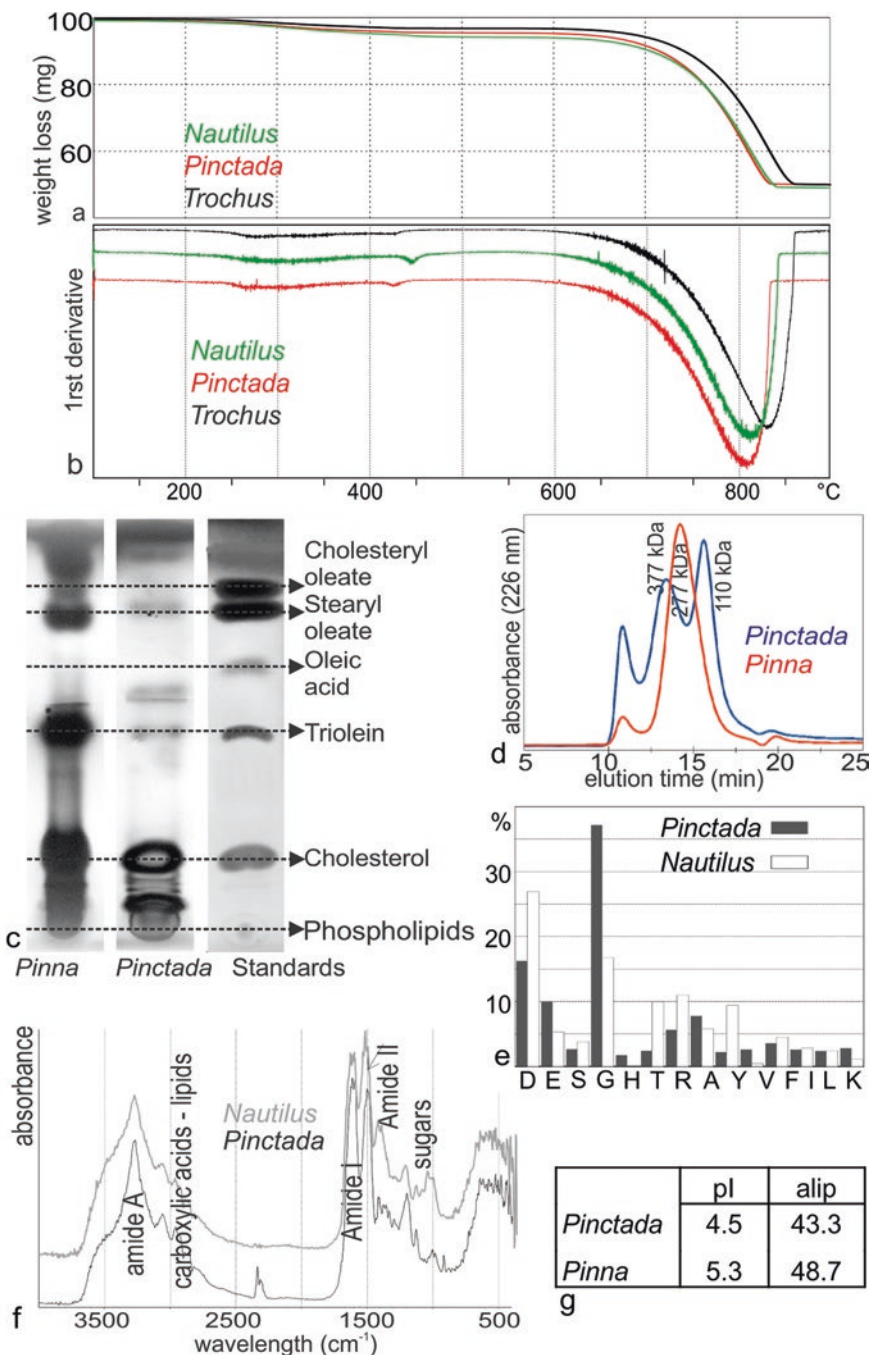
## 36.4 Discussion and Conclusion

There is a strong contrast between the small number of studied taxa with a nacro-prismatic structure and the diversity of their shells. The examination of the shape, inner structure, mineralogy, and composition of both mineral and organic components of these shells show the large diversity of these characteristics (Samata 1990), despite some superficial similarities. The relationships between the two layers are also variable and controlled by the organism. However, the diversity is not hazardous: every characteristic is taxonomically dependent, usually at a specific level. Most often, the presence and role of acidic proteins in the biomineralization process is emphasized, but the role of sugars and lipids is neglected (Kocot et al. 2016). Up to now, proteomics and genomics data have not permitted to select the possible mechanisms of the secretion (Suzuki and Nagasawa 2013; Simkiss 2016).

Thus, not only the structure and composition of the nacre and prisms are heterogeneous, they are also dependent on the species, so that this heterogeneity does not

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**Fig. 36.1** (continued) transition in *Pinctada*, with a thick organic membrane and an irregular layer of fibrous aragonite. **(h)** Aragonitic prism – nacre transition in *Neotrigonia* – BSE image of a polished and etched section (HCl 1% 10 s). **(i)** BSE map showing the organic membrane and the fibrous aragonite between the calcitic prisms and the nacre in *Pinctada*. **(j)** XANES map of S in amino acids in the shell of *Pinctada*. **(k)** XANES map of sulfate in *Neotrigonia*. **(l)** TOF-SIMS map of alanine in the shell of *Pinctada*. **(m)** TOF-SIMS map of glycine of the same section. **(n)** NanoSIMS map of N in *Pinctada*. **(o)**: Mg and Sr contents of the prismatic calcitic layers in some species of *Haliotis*



**Fig. 36.2** (a, b) Thermogravimetric profiles showing the differences in the quantity and composition of the organic matrices in three nautilus layers. (c) Thin-layer chromatography showing the lipidic composition of the calcitic prisms in two bivalve shells. (d) Liquid chromatography of the soluble organic matrices of calcitic prisms. (e) Amino acid composition of the insoluble matrix of nautilus layers. (f) Infrared spectrometry of the insoluble organic matrix of nautilus layers. (g) Isoelectric point (pI) and aliphatic index (alip) of the insoluble organic matrix of calcitic prisms

suit the usual characteristics of a model. They are neither simple nor unique, so that the nacro-prismatic model concept cannot be sustained.

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