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Polarization-dependent Imaging Contrast (PIC) mapping reveals nanocrystal orientation patterns in carbonate biominerals

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ABSTRACT

Carbonate biominerals are one of the most interesting systems a physicist can study. They play a major role in the CO_2 cycle, they master templation, self-assembly, nanofabrication, phase transitions, space filling, crystal nucleation and growth mechanisms. A new imaging modality was introduced in the last 5 years that enables direct observation of the orientation of carbonate single crystals, at the nano- and micro-scale. This is Polarization-dependent Imaging Contrast (PIC) mapping, which is based on X-ray linear dichroism, and uses PhotoElectron Emission spectroMicroscopy (PEEM). Here we present PIC-mapping results from biominerals, including the nacre and prismatic layers of mollusk shells, and sea urchin teeth. We describe various PIC-mapping approaches, and show that these lead to fundamental discoveries on the formation mechanisms of biominerals.

1. Introduction to biomineralization

Bones, teeth, mollusk and crustacean shells, eggshells and otoconia are examples of biominerals formed under direct control of eukaryotic organisms [1]. Biominerals provide skeletal support, locomotion, protection from predators, weapons, biting and mastication, magnetic and gravitational field sensing, balance, and myriads of other functions that afford competitive advantages to biomineralizing animals and plants. The incorporation of biominerals within organisms coincided with and perhaps enabled the Cambrian explosion of diversity 530 million years ago [2]. Since then, biominerals have been refined by natural selection, leading to the fantastic forms and functions we observe today. From a materials perspective, biominerals consistently outperform the sum of their parts with respect to toughness, hardness, multi-scale ordering, architecture, and other properties. From a physics perspective forming biominerals fill space and undergo controlled phase transitions, resulting in arrays of co-oriented nanocrystals across different length-scales, far exceeding human control at present [3]. From a biology perspective, eukaryotes have evolved fine-tuning their biominerals for their functions. This control is enacted at the molecular level by proteins *directly* interacting with minerals, and regulating the physical and chemical aspects of biomineral formation to an accuracy and extent that are unimaginable in synthetic systems, and unparalleled in living systems.

Organisms adapt their biominerals for specific functions, and thus gain competitive evolutionary advantages. By controlling the size, composition and interfacial chemistry of nanoscale mineral particles, they are able to control the crystal phase and morphology. By controlling the spatial organization and connectivity of nanoparticle and protein assemblies, they are able to control the propagation of crystallinity, the transport of materials within growing structures, and the properties of the final composite materials. Forming biominerals can show us how to harness and control the properties of nanophase materials, while using molecular-scale organic-mineral interactions to control materials properties that emerge at the mesoscopic scale (10 nm–10 μm). By understanding these processes, it will be possible to synthesize complex materials with structures and functions that parallel or exceed those found in the natural world. Using a broader materials palette and the lessons from biomineral systems, truly novel composite materials can be conceived that are urgently needed for society's pressing challenges. The nanocrystalline building blocks could be light absorbing, while the molecular scaffolding could be electron or hole conducting, in a self-assembled solar panel [4]. Moreover, the formation of oceanic carbonate ($CaCO_3$) biominerals is a major component of the global carbon cycle, and could inspire methods for the removal of CO_2 from the carbon cycle and its storage into

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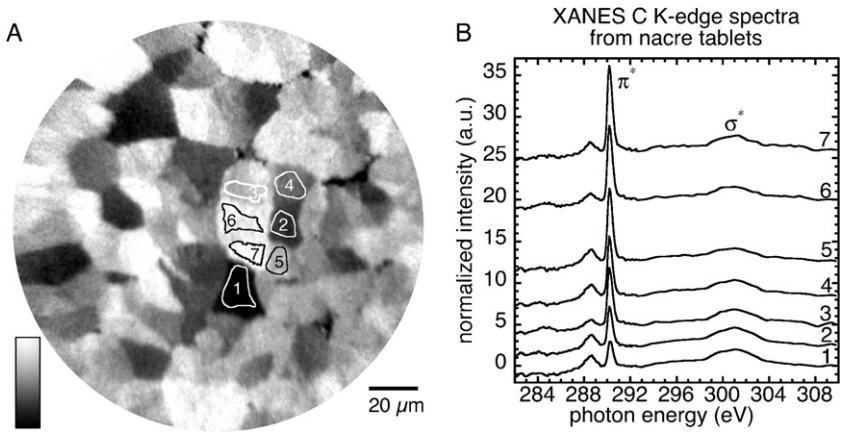

Fig. 1. First observed imaging contrast across red abalone tablets, polished approximately in the plane of one nacre layer, thus the tablets appear as irregular polygons. (A) This carbon π* map was obtained by ratio of 290.3 eV and 280 eV images (π* peak and pre-edge, respectively). (B) XANES spectra extracted from the correspondingly labeled regions of interest in (A). Notice the dramatic difference in π* peak intensity, indicating that the aragonite crystal *c*-axes are spread by tens of degrees.
Unpublished data, acquired by Bradley H. Frazer in 2005, using the SPHINX spectromicroscope [35] on the HERMON beamline at SRC.

minerals or biominerals stable over the geologic timescale. In order to achieve this, we must truly understand how biominerals form.

Many marine biominerals are composites of a few weight-percent organic molecules and calcium carbonate ($CaCO_3$) [5,6]. Anhydrous calcium carbonate can occur in three crystal polymorphs: calcite, aragonite, and vaterite, distinct by their crystal structures, which are trigonal, orthorhombic, and hexagonal, respectively [7]. In calcite each Ca atom is coordinated by 6 oxygen atoms, in aragonite by 9 oxygen atoms [8], in vaterite by 6, 7, or 8 oxygen atoms [9]. The calcium X-ray Absorption Near-Edge Structure (XANES) spectra [10] of these three polymorphs, therefore, are dramatically different from one another. The combination of X-ray Absorption Near-Edge Structure (XANES) spectroscopy [10], Photo-Electron Emission spectroMicroscopy (XANES–PEEM) [11] and the strong linear dichroism [12] effect occurring in aragonite and calcite crystals [13] make XANES–PEEM an ideal method to observe the mutual arrangement of nanocrystals at the mesoscopic scale (10 nm–10 μm). Here we describe the evolution of this method in the last 5 years and the specialized data-taking strategies, then we present a review of recent results and their significance in biomineralization. All presented results could not be obtained with other methods and were breakthrough-discoveries when first observed.

2. Introduction to linear dichroism

X-ray linear dichroism [12] has been studied extensively theoretically [10,12] and used experimentally to characterize man-made systems such as magnetic materials [14–18], organic molecules [19], molecular monolayers [12,20] and liquid crystals [21]. Recently, Metzler et al. showed that there is a strong X-ray linear dichroism effect in natural calcium carbonate minerals and biominerals [13,22]. In calcite and aragonite crystals the carbonate ions (CO_3^{2-}) are planar triangles, all co-oriented with one another and perpendicular to the crystallographic *c*-axis. The π-bonded p orbitals of O and C atoms are perpendicular to the carbonate planes, and parallel to the *c*-axis. In the dipole interaction with linearly polarized X-ray photons, therefore, both C and O spectra exhibit π* peak intensities strongly dependent on the relative orientation of the polarization and the π molecular orbitals. When these are parallel ($\theta = 0^\circ$) or perpendicular ($\theta = 90^\circ$) the π* peaks in XANES spectra have maximum or minimum intensity, respectively. This effect is well-known as X-ray linear dichroism [12]. Based on this effect, Polarization-dependent Imaging Contrast (PIC) mapping done using X-ray PhotoElectron Emission spectroMicroscopy (X-PEEM) revealed the gradual ordering mechanisms in mollusk shell nacre [23], the existence of mineral blocks alternating in orientation in sea urchin teeth [24], and the mechanism of co-orientation via secondary nucleation also in sea urchin teeth [25].

Elliptically polarizing undulators (EPUs) [26] are available at most synchrotrons around the world [27–31], and they also produce linearly polarized X-ray beams, in which the electric field vector (hereafter referred to as linear polarization vector) can be rotated at will, in the plane perpendicular to the X-ray propagation direction. Using an EPU, the PIC-mapping method has recently become semi-quantitative, to measure the orientations of individual nanocrystals distinct from one another in adjacent pixels with size 10 nm [32]. It is possible to make this method fully quantitative, as described below, and this final development is currently underway.

The linear dichroism methods described here have similarities to those used to determine the magnetization direction in magnetic materials, and can be applied to any crystal with uniaxial symmetry in which dipole transitions are dominant [17,33], including calcite and aragonite, two common crystalline forms in marine biominerals [32].

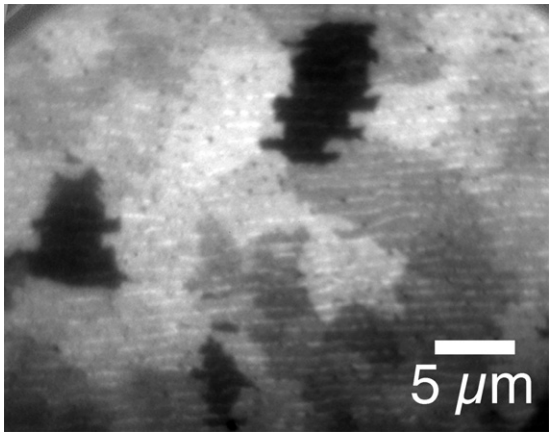

Fig. 2. Polarization-dependent Imaging Contrast (PIC) map of red abalone nacre polished cross-section. This oxygen π* map was obtained by ratio of 534 eV and 518 eV images (π* peak and pre-edge, respectively). The angle between the growth direction and the polarization vector was ~61°. The growth direction in this sample was 60° from the normal of the polished nacre surface, so the layer thickness appears to be greater than 400 nm.
Data acquired using the SPHINX spectromicroscope [35] on the VLS-PGM beamline at SRC, and published in Ref. [13].