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Automated crystal harvesting, freezing and X-ray diffraction

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This system is an evolution of the G-Rob systems. G-Rob system was developed on protein crystallography beamline FIP-BM30A at the ESRF. G-Rob, A 6-axis robotic arm based system, is a fully integrated device for crystallography beamlines and laboratories. G-Rob is an "all in one" system. Thanks to its tool changer, it goes automatically from one application to another.

A new tool for the robot and an adapted environment has been developed to allow G-Rob *in situ* crystal manipulation from crystallization plates. The harvesting step allows mounting the crystal on the robot's fishing tool with X-ray transparent terminal organs. Once the crystal has been fished, an automated cycle prepares the crystal for diffraction trough cryoprotection and flash cooling steps. Thus the sample is ready for X-ray diffraction without dismounting and human manipulation. At this point, the classical G-Rob's goniometer function [1] is used for data collection.

A visualization bench with an inverted 90° angled microscope and an image processing programs has been developed to offer the ability to evaluate crystals positions in Greiner CrystalQuick™ X plates. The video acquired from the microscope is processed to find three corners of the square well containing the crystallization drop. Accordingly, a click on the centre of an interested crystal will save the coordinates of the crystal in the well frame. The same coordinates are used in situ X-ray analysis of the crystal, in its crystallization drop using G-Rob [2], prior to harvesting.

With this new function, G-Rob can go from *in situ* analysis to data collection on frozen crystal with no need of manual manipulation. All the process can then be operated remotely.

[1] Jacquamet *et al.*, *Structure,* **2004** *12*, 1219. [2] Jacquamet *et al.*, *Acta Cryst.,* **2004** *D60*, 888.

Keywords: crystal_fishing, robot goniometer, x-ray screening automation

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Biomimetic carbonate-apatite nanoparticles functionalized with doxorubicin for applications in nanomedicine

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Inorganic nanosized drug carriers are one of the most promising fields in nanomedicine applied to cancer therapies. These materials must be non-toxic, highly biocompatible and biodegradable. Hydroxyapatite (HA), which is the major inorganic component in hard tissues of vertebrates, may be an attractive biomaterial as nanocarrier for drugs, proteins and genes delivery [1]. HA presents the following advantages: favourable biodegradability, biocompatibility and pH-dependent dissolution; soluble and less toxic than silica, quantum dots, carbon nanotubes, or magnetic particles; low production costs and excellent storage abilities (not easily subjected to microbial degradation); in particular they are dissolved at low pH, e.g. in lysosomes after the cellular intake or in the environment of solid tumours, thereby releasing incorporated drugs or biomolecules. In addition, most of the synthetic micro- and nanocrystalline HA find important biomedical applications such as osteologic implant coatings, grafts and scaffolds for bone cavity fillings [1]. For these reasons, nanocrystalline apatites have been the object of extensive research in several interdisciplinary areas with objectives ranging from better understanding of the formation mechanisms in natural mineralization processes to its applicability as a biomedical or industrial material [2].

In this work we present two different methods for the synthesis of carbonate-HA (cHA) nanocarriers to be functionalized with a chemotherapeutic drug. Firstly, we have employed batch precipitation based on thermal decomplexing of Ca/citrate/phosphate solutions [3]. Secondly, the HA has been synthesized by dropping a solution of H_3PO_4 into a $Ca(CH_3COO)_2$ suspension, keeping the pH at a constant value of 10 by addition of $(NH_4)OH$ solution. The nanoparticles have been characterized by HREM, XRD, FTIR and Raman and subsequently functionalized with Doxorubicin hydrochloride (Doxo), a drug commonly used in cancer chemotherapy.

The adsorption isotherms of this drug onto the different cHA nanocarriers plot the adsorbed amount, Γ_{Doxo} (mg/mg_{HA}), calculated from the difference between the concentrations of the Doxo solutions before and after adsorption on cHA, against the drug concentration remaining after adsorption, C_{Doxo} (mg/mL). An initial slope related with the drug affinity for the cHA surface characterizes all the isotherms. The amount of drug adsorbed on cHA increases with the concentration of Doxo in the solution until it reaches the saturation concentration. Both the affinity and the saturation concentration are much higher for Doxo adsorbed on cHA synthesized by the thermal decomplexing batch method. A model describing the interaction between Doxo molecules and cHA surface is proposed from Raman and FTIR data.

This work has been carried out within the framework of the Spanish-Italian Integrated Action ref. IT2009-0028.

[1] M. Iafisco, B. Palazzo, M. Marchetti, et al.. *J. Mater. Chem.* **2009**, *19*, 8385-8392. [2] M. Iafisco, J. Gómez-Morales, M. A. Hernández-Hernández et al. *Adv. Eng. Mater.* **2010**,*12*, B218-B223. [3] A. López-Macipe, J. Gómez-Morales, R. Rodríguez-Clemente. *Adv. Mater.* **1998**, *10*, 49.

Keywords: HA nanoparticles, Doxorubicin, nanomedicine.

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Evolution of microstructure and crystallographic orientation during shell growth

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We have studied the evolution of the microstructure and crystallographic orientation of mollusc shells of different species, specifically, shells of *Nautilus belauensis* (Cephalopoda) and *Psilunio littoralis* (Bivalvia) using Scanning Electron Microscopy and X-Ray synchrotron diffraction. These mollusk shells are composites of aragonite ($CaCO_3$) crystals which are disposed in superimposed layers with different three-dimensional arrangements or microstructure types

[1], [2].

A sequence of bidimensional X-ray diffraction patterns were acquired in transmission mode at regular intervals across the shell thickness. The scattering or degree of preferential orientation of crystals was calculated from the intensity profile along the selected Debye-Scherrer rings (χ-scans).

The degree of crystal orientation was determined from the angular length of the arcs displayed in the Debye rings on the 2D X-ray diffraction patterns. The values of reflection intensities, degree of orientation and crystallinity show progressive variations within during the same shell layer as well as abrupt changes at the transitions between layers with different microstructural organizations. This study provides useful insights into both the mechanisms that control the development of order in mollusc shell microstructures and those that determine the switch between layers with different microstructural organizations.

This information could be of interest to understand the processes of self-assembly that happen in these biomaterials and may be applied to the design of bio-inspired advanced ceramic materials.

[1] J.D. Taylor, J.D. Kennedy, A. Hall, *Bull. Br. Museum* **1969**, *3*; 1-125. [2] A.G. Checa, A.B. Rodríguez-Navarro, *P. Roy. Soc. Lond. B. Bio.* **2001,** *268*, 771-778.

Keywords: mollusk shell, crystallographic orientation, X-ray diffraction

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Oriented nucleation of hemozoin at the food vacuole membrane in *P. falciparum*

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The nucleation of hemozoin (HZ) in the digestive vacuole (DV) of *Plasmodium falciparum* in malaria-infected red blood cells (RBCs) is a topic of current interest. HZ crystals have been reported encased within neutral lipid nanospheres in the DV, which appears inconsistent with the concepts of catalyzed nucleation of HZ at a lipid surface and inhibition of nucleation of HZ via antimalarials that target the HZ crystal surface. To resolve this conundrum, we probed the orientation of HZ crystals in the DV, their position, the site and mechanism of nucleation. HZ crystal clusters in the RBCs were detected and their amount estimated by microfocus X-ray Fe-fluorescence, and their orientations determined by microfocus X-ray diffraction. The diffraction patterns were interpreted in terms of HZ crystals aligned along their needle axes, arranged on a curved surface, exposing their {100} side faces. Using various microscopy techniques, including stain-free cryogenic soft X-ray tomography, freeze-fracture SEM and thin section TEM, we find that nucleation occurs in proximity to the DV inner membrane, where furthermore we find a thickened lipid coating. Morphological evidence supports the {100} orientation facing the lipid, consistent with interpretation of X-ray diffraction results (mentioned above) and *in vitro* nucleation of synthetic hemozoin at various interfaces.

Keywords: biomineralization, malaria, hemozoin

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Single crystal growth and characterization of lead hydroxyapatite

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Calcium hydroxyapatite (CaHAP, $Ca_5(PO_4)_3(OH)$) is the dominant component of human enamel, dentin, and bone. Its structure belongs to space group $P6_3/m$ and is susceptible to ionic substitution in both anion and cation sites. Pb^{2+} can replace Ca^{2+} in the apatite structure resulting in lead hydroxyapatite (PbHAP) which is isostructural with CaHAP. This work reports products from a gel crystallization method used for preparation of larger crystals of PbHAP by controlling nucleation and crystal growth rate by changing the density of the gel medium. Crystals obtained in milli-scale on top of the gel exhibit equant morphology while crystals inside the gel layer exhibit pennant morphology. FT-IR spectra of the products exhibit asymmetric (PO) stretching, symmetric (PO) stretching, and (OPO) bending in the 1002-1047 cm^{-1}, 924-956 cm^{-1}, and 518-600 cm^{-1} regions, respectively, and OH stretching at 3555 cm^{-1}. The FT-IR spectrum of the product on top of the gel also showed NO_3^- bands (NO_3^- from lead nitrate starting material) which are not present in the product formed inside the gel layer.

Keywords: hydroxyapatite, apatite, gel crystallization

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Self-assembly of metallated TPP porphyrin by external dipyridyl ligands

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Supramolecular entities based on self-assembly of metalloporphyrins are paradigmatic examples of the great efficiency of the nanodevices used by natural systems in photosynthesis, oxygen transport, electron transfer and catalysis [1]. Therefore, they constitute reference models for the development of new materials that make these, and other yet unexplored, functions.

While metalloporphyrin biosystems operate in solution, the preparation of materials based on these macrocycles moves the problem to the solid state synthesis. Thus, obtaining supramolecular entities may be approached by different strategies of synthetic design. One of them consists on the use of external dipyridyl ligands to assemble the metallated porphyrin units. In this aspect, the range of compounds that can be used is endless. In this context, our research group is working with different combinations of organic ligands and metalloporphyrins, and the work herein presented corresponds to the compound [FeTPP(bipy)] (TPP=meso-tetraphenylporphyrin and bipy=4,4′-bipyridine), obtained by solvothermal synthesis.

The crystal structure of [FeTPP(bipy)] consists of 1D chains of alternating FeTPP and bipy molecules bonded to the axial positions of the coordination sphere. These chains are sustained by π-π stacking between the phenyl rings at about 5 Å.

So far, very few compounds with TPP and bipy have been described, of which only one [2] is really a 1D coordination polymer, being all others isolated dimers. It is also remarkable that, as far as we know,