crystals

MDPI

Editorial

Biological Crystallization

Jaime Gómez-Morales [1,*], Giuseppe Falini [2] and Juan Manuel García-Ruiz [1]

1 Laboratorio de Estudios Cristalográficos, IACT, CSIC-UGR. Avda. Las Palmeras 4, 18100 Armilla, Granada, Spain
2 Dipartimento di Chimica "Giacomo Ciamician", Alma Mater Studiorum Università di Bologna, via Selmi 2, 40126 Bologna, Italy
* Correspondence: jaime@lec.csic.es

Received: 2 August 2019; Accepted: 3 August 2019; Published: 6 August 2019

check for updates

Keywords: biomineralization; biomimetic materials; biomorphs; calcium carbonate; nanoapatites; nucleation; growth; crystallization of macromolecules; bioremediation; materials science; biomedicine

---

"Biological Crystallization" is today a very wide topic that includes biomineralization, but also the laboratory crystallization of biological compounds such as macromolecules, carbohydrates or lipids, and the synthesis and fabrication of biomimetic materials by different routes. In this Special Issue, special attention is paid to the fundamental phenomena of crystallization (nucleation and growth), and the potential applications of the crystals in environmental science, materials science and biomedicine.

This issue collects 15 contributions, starting with the paper of Torres-Aravena et al. [1]. This paper reviews the main characteristics of a microbially induced precipitation process (MICP), which promotes calcium carbonate (calcite) precipitation. The authors propose to consider this method for heavy metal removal from wastewater/waters.

In the second article, Opel et al. [2] present a method to convert silica/carbonate biomorphs into hybrid organic/carbonate composite materials similar to biominerals. It is worth highlighting that silica/carbonate biomorphs are a class of biomimetic materials named so since they resemble primitive living organisms and their inner textures mimic biominerals. However, compared to biominerals, which are hybrid inorganic-organic materials, the biomorphs are purely inorganic composite materials, the structuring role of organic compounds being taken over by amorphous silica.

Calcium carbonate ($CaCO_3$) is considered a key mineral by many organisms to build its exoskeletons for protecting and supporting purposes. The most common crystal habit of the thermodynamically stable polymorph of calcium carbonate, calcite, is the rhombohedral one, which exposes {10.4} faces. However, in presence of Li^+ the tabular {00.1} faces appear together with the {10.4}, thus generating truncated rhombohedrons. The paper of Matijaković et al. [3] explores the morphological aspects and adsorbing properties of model organic substances of the {10.4} versus {00.1} faces, which are relevant for the understanding of biomineralization processes, in which the {00.1} faces often interact with organic macromolecules and open new routes for the usage of calcite as adsorbing substrate with applications in the environment.

In biomineralization the interactions between organic macromolecules and the nascent inorganic solids play a pivotal role in controlling the shape, size distribution, polymorphism, orientation and even assembly of the formed crystals. At the laboratory scale, it is not easy to carry out high-throughput experiments with only a few macromolecule reagents using conventional experimental methods. In the fourth paper, He et al. [4] explore the surface-tension-confined droplet arrays technique to fabricate $CaCO_3$ using polyacrylic acid as a modified organic molecule control. These authors prove the possibility of performing biomimetic crystallization and biomineralization experiments using this technique.

Nanocrystalline calcium phosphates apatites are a class of biomimetic materials displaying morphological and crystalline properties close to those of bone and dentine apatites. Due to their excellent biocompatibility, osteoconductivity and osteoinductivity, these nanoparticles find application in the field of biomaterials. In the fifth paper, Gómez Morales et al. [5] explore the nucleation of apatite nanoparticles on exfoliated graphene flakes to yield graphene/apatite nanocomposites with applications as bone grafts.

Crystallization of biological macromolecules is the largest part of this Special Issue (papers 6–15 fall in this section). Crystallization is a crucial step in the pathway to determine the three-dimensional structure of macromolecules by X-ray diffraction techniques, and also to obtain crystals for specific applications in environment, industry or medicine. Blackburn et al. [6] present a simple technique, based on gel exclusion of nucleation inducing elements, for generating large well-diffracting crystals from conditions that yield microcrystals when using other techniques. This method is successfully applied to generate diffraction quality crystals of lysozyme, cubic insulin, proteinase k, and ferritin.

Nanev tackles the fundamental aspects of nucleation and growth of protein in the successive articles nº 7 and 8. In his first paper [7], he presents a study that establishes the supersaturation dependence of the protein crystal nucleus size of arbitrary lattice structures. His approach is compared to the classical one of Stranski and Kaischew, which is applied merely for the so-called Kossel-crystal and vapor grown crystals. In his second paper [8], Nanev reviews investigations on protein crystallization and aims to present a comprehensive rather than complete account of recent studies and efforts to elucidate the mechanisms of protein crystal nucleation, and the importance of both physical and biochemical factors in these mechanisms.

An interesting feature of protein crystals is that they are usually colorless. However, they can be stained a variety of hues by saturating them with dyes or by co-crystallization. The colors assumed by dyes are a function of chemical factors, particularly pH and redox potential. In paper nº 9, McPherson [9] presents a number of experiments using pH or redox sensitive dye-saturated protein crystals, and some experiments using double dye, sequential redox–pH changes.

In this book, membrane proteins are also represented. Human carbonic anhydrase IX is a multi-domain membrane protein that is, therefore, difficult to express or crystallize. In paper nº 10, Koruza et al. [10] present successful crystallization results of the catalytic domain SV of the human carbonic anhydrase IX by using the microseed matrix screening technique. The crystals were employed as a case-study for neutron protein crystallography.

In paper nº 11, Sun et al. [11] report a new strategy that allows for the removal of cadmium and chromium from wastewater by using fusion crystals of a Cry protein and a low molecular weight cysteine-rich protein (SmtA) known to bind heavy metals. These fusion crystals were microbially grown on *Bacillus thuringiensis* (Bt). The authors suggest the potential uses of these types of crystals for bioremediation of heavy metals.

Park et al. [12] authored paper nº 12. They report crystallization and preliminary X-ray crystallographic data of frog (*Xenopus tropicalis*) ependymin, obtained in a synchrotron facility. Ependymin is a glycoprotein of the extracellular fluid of brain fish and it has been suggested to have various roles related to learning behavior. Ependymin-related proteins also exist in other animals such as sea urchins, frogs, and even mammals. In the same line of crystal structure determination, paper nº 13, authored by Liu et al. [13], presents results of the catalytic domain of the phosphoethanolamine transferase MCR-1 (cMCR-1) co-crystallized with d-Xylose. This study is of great interest to fight drug-resistant enterobacteria. Paper nº 14, authored by Rahman and coworkers, reports the purification, crystallization by hanging drop vapor diffusion, and preliminary X-ray crystallographic studies on plasmid-encoded *Campylobacter concisus*-secreted protein 1 (Csep1p) [14]. This plasmid was recently identified as a putative pathogenicity marker associated with active Crohn's disease, a clinical form of the inflammatory bowel disease. Finally, in paper nº 15 Prudnikova and coworkers report the crystallization and crystal structure determination of the double mutant (Ile44Leu + Gln102His) of the haloalkane dehalogenase DbeA from *Bradyrhizobium elkanii* USDA94 (DbeAΔCl [15]. Haloalkane dehalogenases are a very important class of microbial enzymes for environmental detoxification of halogenated pollutants.

In summary, the articles presented in this Special Issue are representative of some of the lines of a topic as broad as biological crystallization as well as of its importance in different scientific fields, and cover aspects ranging from biomineralization and biomimetic crystallization to crystallization of biological macromolecules and its applications in bioremediation and biomedicine.

Funding: Grant number PGC2018-102047-B-I00 (MCIU/AEI/FEDER, UE).

Acknowledgments: The Guest Editors thank all the authors contributing in this Special Issue and the Editorial staff of *Crystals* for their priceless support.

References

1. Torres-Aravena, Á.E.; Duarte-Nass, C.; Azócar, L.; Mella-Herrera, R.; Rivas, M.; Jeison, D. Can microbially induced calcite precipitation (MICP) through a ureolytic pathway be successfully applied for removing heavy metals from wastewaters? *Crystals* **2018**, *8*, 438. [CrossRef]
2. Opel, J.; Unglaube, N.; Wörner, M.; Kellermeier, M.; Cölfen, H.; García-Ruiz, J.M. Hybrid biomimetic materials from silica/carbonate biomorphs. *Crystals* **2019**, *9*, 157. [CrossRef]
3. Matijaković, N.; Magnabosco, G.; Scarpino, F.; Fermani, S.; Falini, G.; Kralj, D. Synthesis and adsorbing properties of tabular {001} calcite crystals. *Crystals* **2019**, *9*, 16. [CrossRef]
4. He, Z.; Xia, Z.; Zhang, M.; Wu, J.; Wen, W. Calcium carbonate mineralization in a surface-tension-confined droplets array. *Crystals* **2019**, *9*, 284. [CrossRef]
5. Gómez-Morales, J.; González-Ramírez, L.A.; Verdugo-Escamilla, C.; Fernández Penas, R.; Oltolina, F.; Prat, M.; Falini, G. Induced nucleation of biomimetic nanoapatites on exfoliated graphene biomolecule flakes by vapor diffusion in microdroplets. *Crystals* **2019**, *9*, 341. [CrossRef]
6. Blackburn, A.; Partowmah, S.H.; Brennan, H.M.; Mestizo, K.E.; Stivala, C.D.; Petreczky, J.; Perez, A.; Horn, A.; McSweeney, S.; Soares, A.S. A simple technique to improve microcrystals using gel exclusion of nucleation inducing elements. *Crystals* **2018**, *8*, 464. [CrossRef]
7. Nanev, C.N. Recent insights into protein crystal nucleation. *Crystals* **2018**, *8*, 219. [CrossRef]
8. Nanev, C.N. Peculiarities of protein crystal nucleation and growth. *Crystals* **2018**, *8*, 422. [CrossRef]
9. McPherson, A. pH and redox induced color changes in protein crystals suffused with dyes. *Crystals* **2019**, *9*, 126. [CrossRef]
10. Koruza, K.; Lafumat, B.; Nyblom, M.; Knecht, W.; Fisher, Z. From initial hit to crystal optimization with microseeding of human carbonic anhydrase IX—A case study for neutron protein crystallography. *Crystals* **2018**, *8*, 434. [CrossRef]
11. Sun, Q.; Cheng, S.W.; Cheung, K.; Lee, M.M.; Chan, M.K. Cry protein crystal-immobilized metallothioneins for bioremediation of heavy metals from water. *Crystals* **2019**, *9*, 287. [CrossRef]
12. Park, J.K.; Sim, Y.W.; Park, S. Over-expression, secondary structure characterization, and preliminary X-ray crystallographic analysis of xenopus tropicalis ependymin. *Crystals* **2018**, *8*, 284. [CrossRef]
13. Liu, Z.-X.; Han, Z.; Yu, X.-L.; Wen, G.; Zeng, C. Crystal structure of the catalytic domain of MCR-1 (cMCR-1) in complex with d-Xylose. *Crystals* **2018**, *8*, 172. [CrossRef]
14. Rahman, M.M.; Goff, B.; Zhang, L.; Roujeinikova, A. Refolding, characterization, and preliminary X-ray crystallographic studies on the campylobacter concisus plasmid-encoded secreted protein Csep1p associated with Crohn's disease. *Crystals* **2018**, *8*, 391. [CrossRef]
15. Prudnikova, T.; Kascakova, B.; Mesters, J.R.; Grinkevich, P.; Havlickova, P.; Mazur, A.; Shaposhnikova, A.; Chaloupkova, R.; Damborsky, J.; Kuty, M.; et al. Crystallization and crystallographic analysis of a bradyrhizobium elkanii USDA94 haloalkane dehalogenase variant with an eliminated halide-binding site. *Crystals* **2019**, *9*, 375. [CrossRef]