MASTER 2 Internship Proposal 2020

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Internship period: 5 mois from February 2020
Salary: Yes

Title

Syntheses of $CaCO_3$ minerals colored by bio-based organic pigments extracted from sea urchins

Scientific Project:

1. Project

Sea urchin spines show intense and diverse colors (from purple to green) that are due to a family of organic molecules, the polyhydroxylated-naphthoquinone (PHNQ) [1] (Figure A and B). Although the majority of synthetic organic pigments (non-water soluble) hardly disperse in the medium to be colored and fade rapidly under sunlight, PHNQ mass-color and provide long-lasting colors to sea urchin spines.

These remarkable properties are likely due to the encapsulation of the PHNQ molecules within the crystalline biogenic calcite, which protects the organic pigments from the external environment. The PHNQ encapsulation takes place during the coupled pigmentation and biomineralization processes. PHNQ molecules are first localized in the so-called red-spherule cells (Figure C) and progressively integrate the growing mineral (=biomineralization) (Figure D). Biomineralization in sea urchin involves the presence of amorphous calcium carbonate (ACC) precursors phases [2,3], which are thermodynamically unstable under ambient conditions but can be temporarily stabilized by inorganic ions such as Mg^{2+} and macromolecules, mostly N-glycosylated proteins [4]. During ACC crystallization, Mg^{2+} as well as diverse macromolecules (proteins, lipids, polysaccharides, metabolites) and PHNQ are incorporated into the biogenic calcite.

This project aims at synthetizing colored hybrid $CaCO_3$ based materials bio-inspired by the pigmentation and biomineralization processes occurring in sea urchin spines and therefore via the synthesis of ACC-PHNQ samples.

2. Specific techniques and methods

PHNQ molecules will be extracted from the red-spherules cells located in the coelomic fluid (≈blood) of the sea urchins by centrifugation techniques and osmotic shock approaches. The molecular structure of the PHNQ molecules will be elucidated by $^{1}H/^{13}C$ solution state NMR.

Based on the literature, two strategies will be employed to synthetize colored $CaCO_3$ crystals with occluded PHNQ via the formation of ACC particles. 1) Spontaneous precipitation by direct mixing of $CaCl_2$ into Na_2CO_3 solution [5] containing water soluble PHNQ will be performed (Figure E) with a dedicated automatic device that allows the simultaneous measurement of pH and Ca^{2+} activity during the ACC synthesis. 2) Slow carbonation diffusion methods [6] will be also performed in ethanol with the ethanol-soluble PHNQ fractions (Figure F). Crystallization of the samples will be induced under heating and/or controlled humidity. Due to the complexity of the natural PHNQ mixture, simplified and commercially available molecules (lawsone and naphthazarin) will also be considered.

The PHNQ-ACC hybrid samples will be characterized by ATR-FTIR and the incorporation of the PHNQ within the $CaCO_3$ crystals will be studied by TG analyses and observed by optical microscopy. The likely stabilization effect of the PHNQ on the ACC phases will be determined by DSC. Finally, ACC nanoparticles and faceted $CaCO_3$ crystals will be observed by SEM and the nature of the crystalline polymorphs (calcite, vaterite, aragonite) will be determined by XRD.

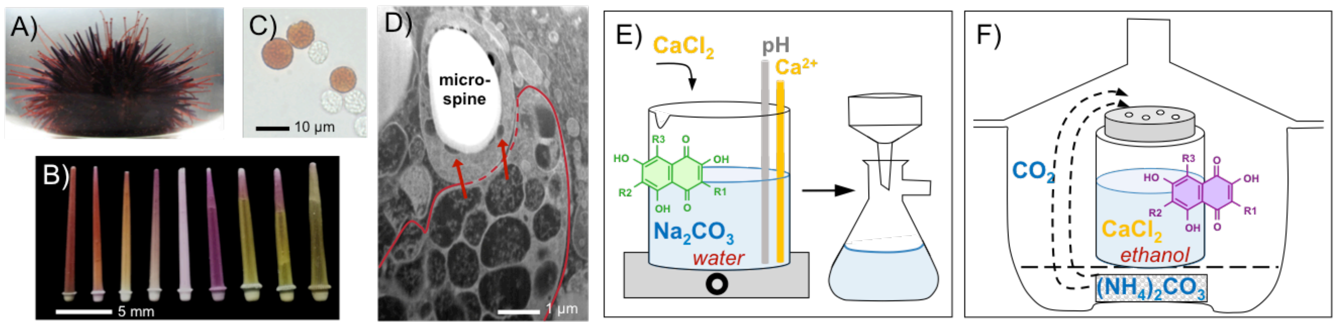

Figure. A) *Paracentrotus lividus* sea urchin, B) spines of different colors from different sea urchins, C) red-spherule cells, D) incorporation of the PHNQ from a cell to the growing mineral, E) spontaneous precipitation of ACC and F) slow carbonation diffusion.

3. References

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[3] M. Albéric, C. Stifler, Z. Zou, C. Sun, C. Killian, P. Gilbert, S. Valencia, M. Mawass, L. Bertinetti, Y. Politi, Growth an regrowth of adult sea urchin psines involve hydrated and anhydrous amorphous calcium carbonate precursors, Journal of structural biology in press (2019).
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