

Résumé du projet de thèse

Cyanobacteria are crucial photosynthetic organisms, utilizing solar energy to sequester atmospheric CO₂, constituting a significant portion of Earth's biomass and supporting the global food chain. Some cyanobacteria accumulate high amounts of calcium and other alkaline earth elements (AEEs) like strontium, barium, or radium within intracellular amorphous calcium carbonate (iACC), making them exceptional scavengers¹. This unique trait extends to radioactive isotopes, suggesting potential for innovative water decontamination method².

Numerous cyanobacterial strains, including those from the Pasteur Cultures of Cyanobacteria (PCC) collection, exhibit iACC formation capabilities, even in environments where thermodynamics suggests it is unfavorable, implying some energy investment³. Despite the potential importance and of this process, how iACC form and what is their function remain poorly understood. iACCs may serve as pH buffers or storage for calcium and carbon, but the rationale behind storing costly amounts of AEEs requires clarification. Noteworthy, ACC is much more reactive (i.e. dissolves or forms faster) than its crystalline counterparts. Therefore, its disordered structure may aid in its calcium and/or inorganic storage function. However, an additional issue is that abiotic ACC is notoriously unstable and spontaneously transforms into crystalline solids. Were it to happen inside cells, this may alter its function and be deleterious, since it may disrupt cellular structures. Conversely, bacterial iACC remains amorphous throughout cell life. The origin of such a stability (involvement of proteins within iACC and/or chemical impurities) remains to be determined. Last, the calcyanin protein family, encoded by the *ccyA* gene found uniquely in iACC-forming cyanobacteria, has been implicated in iACC formation⁴. However, other genes likely cooperate in iACC formation, as evidenced by a recent study where we showed co-expression patterns of neighboring genes in one cyanobacterium (Bruley et al., subm). The involvement of these additional genes remains to be ascertained.

The overarching objective of this PhD project is to integrate microbiology, microscopy, and spectroscopy to elucidate the environmental conditions, genetic determinants, and structural characteristics of iACC. Specific aims include assessing conditions favoring iACC formation, identifying associated genes through transcriptomics, and characterizing iACC structure and chemistry at nanoscale resolution.

In the first phase, cyanobacterial growth under varying pCO₂ and calcium concentrations will be monitored alongside iACC quantification. Strains harboring different *ccyA* types will be examined, and experiments will employ ultrahigh-density photobioreactors for precise environmental control. The controlled growth of cyanobacteria will be conducted in Pasteur, and characterization of iACC content at IMPMC.

The second phase involves transcriptomic analysis under favorable and unfavorable iACC formation conditions to identify candidate genes involved. Bioinformatics tools will assist in data analysis, and both experiments and analyses will be conducted in Pasteur.

Lastly, nanoscale investigation of iACC and associated organic content will employ advanced spectroscopies and microscopy techniques. Analyses of synthetic ACC models will precede analyses of extracted iACC, utilizing methods such as transmission electron microscopy (TEM) and electron energy loss spectroscopy (EELS) for structural insights. These are cutting-edge techniques developed and conducted at IMPMC.

This comprehensive approach aims to unravel the complex interplay of environmental cues, genetic factors, and molecular mechanisms governing iACC formation in cyanobacteria. By advancing our understanding of iACC, this research holds promise for innovative bioremediation strategies and sheds light on fundamental biological processes. The advisors have successfully collaborated in the past years. They have complementary expertise relevant to this project: the PALM group at IMPMC masters the use of analytical TEM to characterize biominerals (Menguy). The PCC is unique worldwide for its collection of well characterized cyanobacteria strains and expert in the cultures and gene expression analyses (Gugger). Overall, this PhD project will be a unique opportunity for a student to develop advanced expertise in the field of biomineralization, at an interdisciplinary frontier between microbiology and physics.

Références

¹De Wever A., (...), **Gugger M.** (2019) Evidence of high Ca uptake by cyanobacteria forming intracellular CaCO₃ and impact on their growth. *Geobiology*, 17, 676-690

²Mehta N et al. (2022) Cyanobacteria accumulate radium (²²⁶Ra) within intracellular amorphous calcium carbonate inclusions. *ACS EST&T Water*, 2, 616-623

³Benzerara K., (...), **Gugger M.**, (...), **N Menguy** (...) (2014) Intracellular Ca-carbonate biomineralization is widespread in cyanobacteria. *Proc. Natl. Acad. Sci. USA* 111, 10933-10938

⁴Benzerara K, (...) **Gugger M.**, (...) Callebaut I (2022) A new gene family diagnostic for intracellular biomineralization of amorphous Ca-carbonates by cyanobacteria. *Genome Biol Evol*, 14:evac026