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Confocal Micro-Raman spectroscopy investigation of calcite biomineral phase: The case of terrestrial isopod Porcellio scaber

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The exceptional properties of biological composites are based on a complex hierarchical architecture of inorganic and organic components that are organized at different structural levels ranging from the nano- to the meso-scale. Nevertheless, only a few biological composites have been thoroughly studied from a materials science perspective. Besides bone or nacre the mineralized exoskeleton formed by the cuticle of crustaceans is an excellent model to study biological composite materials. The exoskeleton not only provides mechanical stability but also offers the attachment for muscles, environmental protection, and a water barrier. In spite of the diversity of crustacean species they share a similar structural principle for their cuticle: An organic matrix composed of chitin-protein fibers associated with various amounts of crystalline and amorphous calcium carbonate (ACC).
The investigation of calcium carbonate forms in living organisms is important in order to understand how these biominerals are formed. Therefore structural and developmental biologists extensively studied the early and intermediate stages of the biomineralisation process and chemists and materials scientists also analyzed the final products of the process. These study indicate that the differentiation of calcium carbonate biominerals by comparing morphological forms are rather difficult because biomineral morphology may significantly differ from crystal habit of both crystalline synthetic and mineral analogues. Applying scanning confocal Raman microscopy (SCRM) to study biominerals and biomimetic systems enables not only to determine the chemical composition and to allocate the distribution of organic and inorganic components but also to discriminate between different crystalline modifications.
The terrestrial isopod Porcellio scaber was used as a model system to study the local compositional changes within the anterior and posterior tergite cuticle for various moulting stages. Spectral Raman images taken for different moulting stages show that mineral phases have a layered arrangement where calcite is restricted to the outer area of the cuticle and amorphous calcium carbonate (ACC) is localized in the middle having only little overlap with the crystalline layer. ACC is preferentially mobilized and stored probably due to its higher solubility compared to calcite. High amounts of proteins present within the first hour of formation of the cuticle give strong indication that these molecules attribute to the hardening of the new chitin matrix. Concurrently, the deposition of ACC precedes the formation of calcite in distal layers of the cuticle forming the outer protective layer. Although this crystalline layer appears homogeneous in SEM and AFM images SCRM investigations reveal the oriented growth of nanocrystalline calcite within the outer part of the crystalline layer of the cuticle.