Exploring biomineral chemistry at the nanometer scale

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Biominerals are naturally occurring biomaterials combining organic and inorganic compounds organized in a multi-scale structure, ranging from the macroscopic level down to nanometer. They are found to constitute physiological hard tissues (bones and teeth of vertebrates and shells of invertebrates) but also pathological calcifications. To understand the early stages of the biomineral genesis one needs data on their chemical composition and structure at the nanoscale. Of particular relevance is the characterisation of the interface between the mineral and the organic compounds. Conventional TEM cannot achieve this, since it cannot easily distinguish the different compounds present. Compared to other spectroscopic approaches, EELS spectro-microscopy offers the advantage of an outstanding spatial resolution in both imaging and chemical analysis. By coupling STEM imaging to EELS (electron energy-loss spectroscopy), one can map the elements composing the sample with a resolution defined by the diameter of the electron probe (typically better than a nanometer). Beyond elemental identification, the fingerprint of the elemental edges provides information on the chemical bonding and makes possible the identification of the chemical compounds. For sensitive samples such as organic materials, it is essential to limit the radiation damage induced by the electron beam. The measurements are obtained using a nitrogen-cooled sample stage and with the minimum electron dose. Under these conditions, EELS signals are extremely noisy and difficult to analyze using the raw data. The use of multivariate analysis methods (principal component analysis, PCA) significantly improves the detection limit and the signal-to-noise ratio.
To illustrate the interest of this approach for biomaterial studies, we will present selected results concerning some biominerals (kidney calcifications, bone, shells). For kidney calcifications, the composition was analyzed on resin sections prepared from human kidneys. In order to identify the chemical compounds present in calcifications, the elemental edges were compared with mineral and organic references. The compounds forming the biological tissue were clearly distinguished from the embedding resin. Nitrogen maps reveal that organic compounds are intimately associated with the mineral at the nanometer scale and constitute the matrix embedding the nano-calcifications to form larger structures (Figure A and B). The mineral phase was found in objects with very diverse morphologies and sizes, and nanoparticles with sizes down to one or two nanometers were detected (Figure A). Two main compounds were found in the mineral particles: calcium phosphate and calcium carbonate (Figure C). These results shed new light on the mechanisms involved during the early stages of kidney stone genesis.

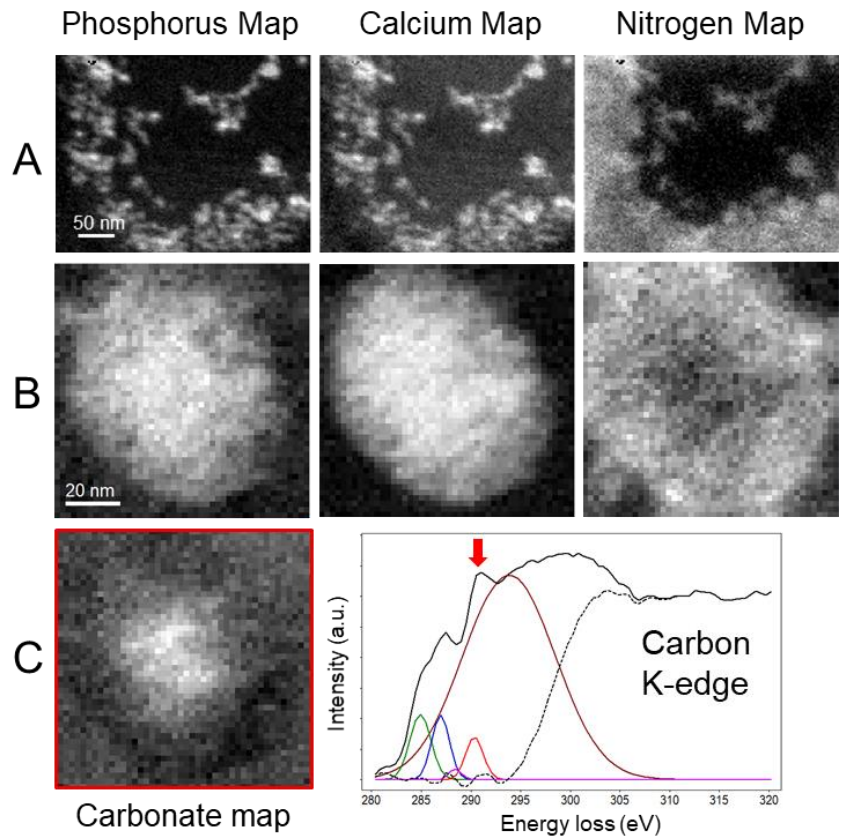

Figure: (A) and (B) Elemental maps corresponding to the presence of calcium, phosphorus and nitrogen in two kidney nanocalcifications. The resolution is equal to 1 nm and particles with sizes ~1-2nm are detected. (C) The spectrum corresponds to carbon K-edge measured on the nanocalcification presented in figure B. The different peaks correspond to the presence of different chemical groups. For instance, the peak at 290 eV (red arrow) reveals the presence of carbonate. The carbonate abundance can be mapped by a gaussian fitting (red curve) of this peak.