Orientation-dependent crystallographic properties in osteocyte-associated bone revealed by nano-scanning X-ray diffraction and fluorescence with a sub-50 nm X-ray beam

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Bone has a complex hierarchical structure built mainly from mineralized collagen fibrils [1]. Bone is traversed by a cellular network of osteocytes that are housed in lacunae interconnected by a few-hundred nm diameter canaliculi [1-2]. It is known that the bone matrix close to osteocyte lacunae has a different protein composition than that further away, yet it has hitherto been assumed that bone biomineral has uniform crystallographic properties on sufficiently small length scales. Herein, we show the existence of orientation-dependent differences in bone biomineral crystallographic properties within a few µm from an osteocyte. Since the osteocyte is about 3-10 µm in diameter and the perilacunar matrix is also in the micron range, ultrahigh spatial resolution is required.

Previously, combined small angle X-ray scattering and wide angle X-ray scattering tensor tomography with a 1 µm voxel size indicated differences in orientation distributions between the biomineral crystallographic c-axis and bone nanostructure in localized areas of human lamellar bone even if most of the sampled material followed the expected co-alignment of the two [3]. X-ray diffraction and fluorescence tomography revealed spatially varying crystallographic properties on the ~10 µm length scale in human bone [4]. These results suggest that indeed, bone biomineralization may locally be more heterogeneous than hitherto believed.

To obtain sufficient spatial resolution, we conducted X-ray diffraction and fluorescence experiments with a sub 50 nm diameter X-ray beam building on previous work [5]. The data were collected at ID13 of the ESRF. We studied a ~4 µm thick human bone specimen that we raster scanned through the beam in two different projective views, i.e. sampling two different orientations of the sample with respect to the beam. Due to the preferred orientation of the bone crystals, each view provides information on different crystal populations from the same bone volume. We analysed each diffraction pattern by Rietveld refinement. Surprisingly, we find systematic differences in lattice constants and apparent crystallite sizes between the two views. Furthermore, we find differences between bone close to and further away from the osteocyte lacuna. This indicates that, on a sub 100 nm length scale, bone mineral crystallographic properties are dependent on the crystal orientation. We expect that this most likely reflect their relation to the collagen network and other non-collagenous proteins.


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