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# Mapping Crystallographic (Dis)Order and Crystal Properties in Human Enamel

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Dental enamel is a principal component of our teeth, providing protection to the underlying dentin. It has evolved to bear large masticatory forces, resist mechanical fatigue, and withstand wear over decades of use. However, as an acellular tissue, it lacks some of the sophisticated self-repair capabilities of other mineralized tissues such as bone. Functional impairment or loss of enamel, for instance as a consequence of developmental defects or tooth decay (caries), has a dramatic impact on health and quality of life, and causes significant costs to society.[1] The ability to characterize the chemically complex microstructure of enamel is fundamentally enabling research targeted at improving caries prophylaxis and early/non-invasive intervention, understanding developmental mechanisms, and developing novel and/or bio-inspired materials.

Enamel covers the entire crown of human teeth and can reach a thickness of several millimeters (**Figure 1**). A characteristic microstructural element is the enamel rod (**Figure 1C**), which in turn is comprised of of lath-like hydroxyapatite ($Ca_5(PO_4)_3(OH)$) crystallites (**Figure 1D**).[2] Crystallites sectioned normal to their long axis appear as oblong polygons with an edge length of 20-50 nm in the short and 70-170 nm in the long direction (**Figure 1E**).[3] On the order of $10^4$ crystallites are bundled into rods that that in human enamel have keyhole-shaped cross-sections with typical dimensions of 9 µm x 5 µm. Within each rod, the *c*-axes of the crystallites are roughly aligned to the rod axis. However, toward the rod boundaries and in the rod 'tail,' the c-axes rotate significantly and take a wider range of orientations.[4,5] It has been challenging to systematically assess orientation relationships at the length scale of individual rods. Shorter length scales, on the order of small groups of crystallites, have been probed with (S)TEM.[3,6] X-ray diffraction has been used to establish the crystalline phase of enamel, extract lattice parameters, map bulk crystallographic orientation and approximate crystalline domain size, but invariably at length scales larger than individual rods. As a consequence, there is currently no clear picture of sub-rod length-scale variation of crystallite populations' dimensions and crystallographic characteristics, for instance lattice parameters, crystallographic orientation distribution, coherent domain size, or micro-strain.

Here, we report on closing this knowledge gap using a beam of monochromatic X-rays with ~500 nm diameter.Sections of human inner enamel (18 µm x 8 µm x 1 µm) were prepared in three orthogonal directions using focused ion beam lift out technique (**Figure 2A-D**). Diffraction maps recorded provide detailed sub-rod structural information. Each pattern in the map consists of incomplete diffraction rings reflecting texture and preferred orientation. By computing an azimuthal autocorrelation for the quadruplet reflections ({121},{112},{030},{022}), differences in local crystallographic order could be quantified. This allows us to distinguish rod and interrod sample points. C-axis orientation and spatial divergence were extracted by fitting the {002} reflection, revealing the local variation of the crystallite orientation across multiple rods for the first time. Remarkably, analysis of integrated 1D diffraction patterns revealed that crystallographic order is correlated with crystallite size, lattice parameters, and microstrain (**Figure 2E-F**). Specifically, we find that there are systematic variations in these parameters for rod and interrod crystallite populations that suggest differences in local composition and imply that there are distinct crystallization environments during amelogenesis.[8]

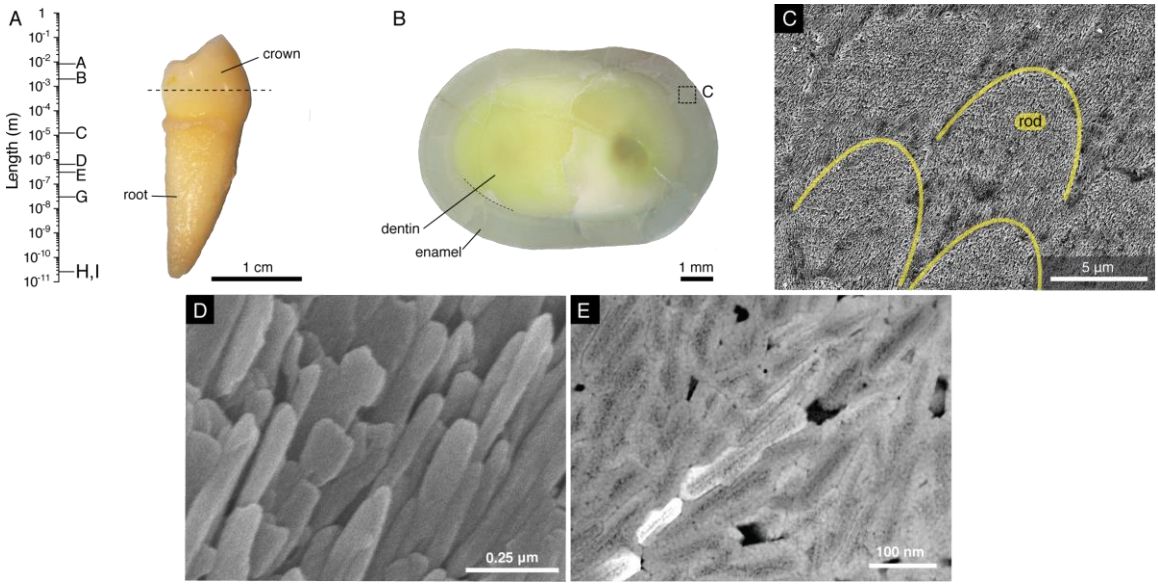


**Figure 1.** Microstructure of human enamel. A. Human premolar. B. Section parallel to the mid-coronal cervical plane. C. SEM image showing enamel rods and inter-rod enamel in lactic acid-etched outer enamel. D. SEM image of enamel crystallites. E. STEM-ADF image of enamel crystallites in cross section.

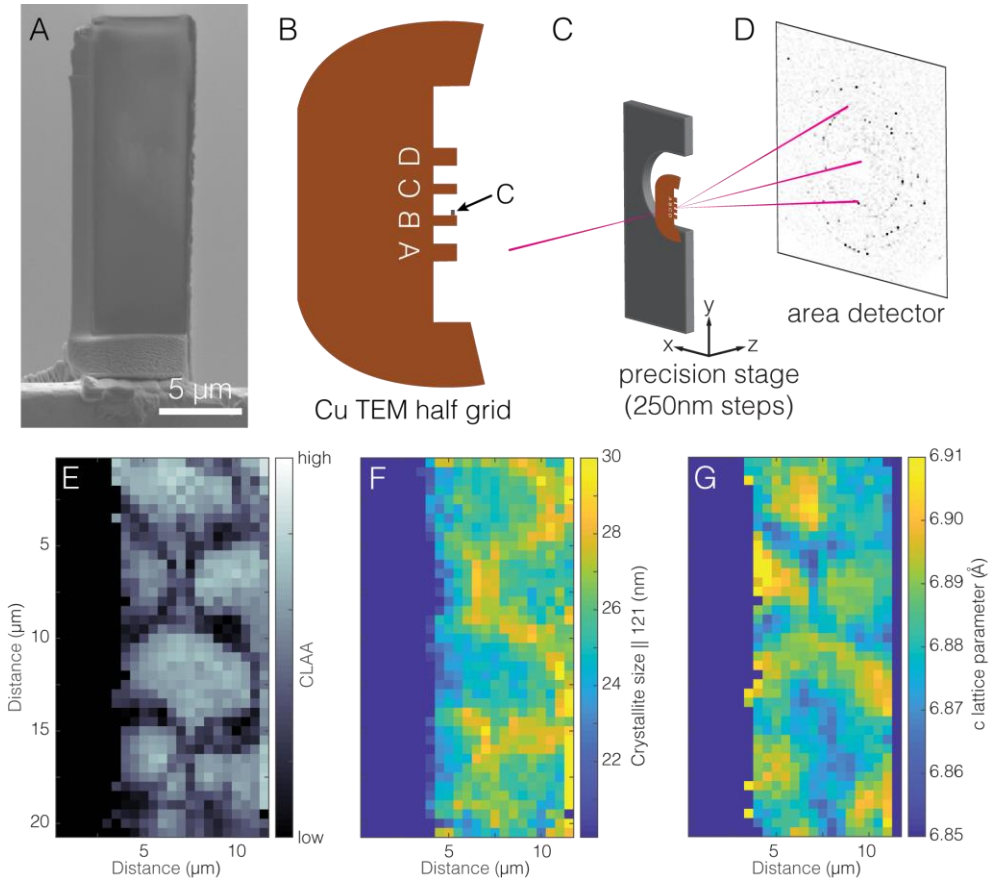


**Figure 2.** Mapping Crystallite Properties. A. FIB-prepared lift-out of human enamel. B. Schematic drawing of half-grid that liftoff was counted on. C. Halfgrid with liftoff is scanned through beam with 500 nm spot size using high-precision stage to record WAXS patterns. D. Autocorrelation analysis of

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azimuthally unwrapped WAXS patterns reveals rod/interrod structure. E-F. Analysis of radially integrated WAXS patterns reveals systematic changes of crystallite size and lattice parameters in rod vs interrod enamel.

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