



## X-Ray Diffraction Topography of Protein Crystals and Charge-Density Waves

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**One of the most important applications of X-ray diffraction techniques is to characterize disorder in crystals and other periodic structures. In many cases one is interested not only in an overall assessment of the disorder but also in how the disorder varies with position inside the sample.**

X-ray diffraction topography provides a simple, fast, and direct technique for imaging crystal disorder. In conventional single-crystal diffraction (e.g., as performed by macromolecular crystallographers), the sample is illuminated using a beam with a finite angular divergence and the diffraction pattern is recorded using a detector placed  $\sim 0.1$ - $1.0$  m from the sample. Under these conditions, the finite beam divergence and large sample-to-detector distance produce largely featureless diffraction spots. But if the incident beam has a very small angular divergence and a high spatial resolution detector is placed very close to the sample, the diffracted beams from different parts of the crystal are spatially resolved and the diffraction spots provide images of the crystal. Contrast in these images arises due to variations in lattice orientation (mosaicity) and lattice spacing (strain) that cause variations in diffracted intensity from point to point in the crystal. Contrary to what its name suggests, X-ray topography is a bulk imaging technique: the “topography” is of the diffracting planes within the crystal. The images that it produces can be crudely viewed as the “dark-field” counterpart to the “bright-field” images produced by the direct transmitted beam in conventional radiography.

X-ray topography is particularly easy to perform at synchrotron sources, where the X-ray beams are intense, broad, and have very low angular divergence. In our experiments on CHESS stations B-2 and C-1 we illuminate the entire sample with the monochromatic beam from a double-bounce monochromator and record the diffraction spots using fine-grain film placed a few cm from the sample and normal to the diffracted beam direction. Typical exposure times range from seconds to minutes, and the resulting diffraction spots are enlarged and photographed using a microscope. More sophisticated versions of the technique exist, but much can be learned even using this very simple approach, especially when topographs are combined with other diffraction measurements such as high-resolution lineshape (mosaic and  $\theta$ - $2\theta$ ) scans.

X-ray topography has been used since the 1960's to image defects in single crystals including dislocations, twins, grain boundaries, stacking faults, inclusions, precipitates and growth bands. It has also been applied to study martensitic and incommensurate-commensurate structural phase transitions, domain structures in ferroelectric and magnetic materials, and strains in integrated circuits and artificial semiconductor superlattices. In recent years it has received far less attention than imaging techniques based on scanned probes such as scanning tunneling microscopy (STM) and atomic force microscopy (AFM). X-ray topography is in many ways complementary to these techniques, providing a bulk rather than surface probe with far greater sensitivity to lattice strains and mosaicity at the expense of far worse (but still respectable) spatial resolution.

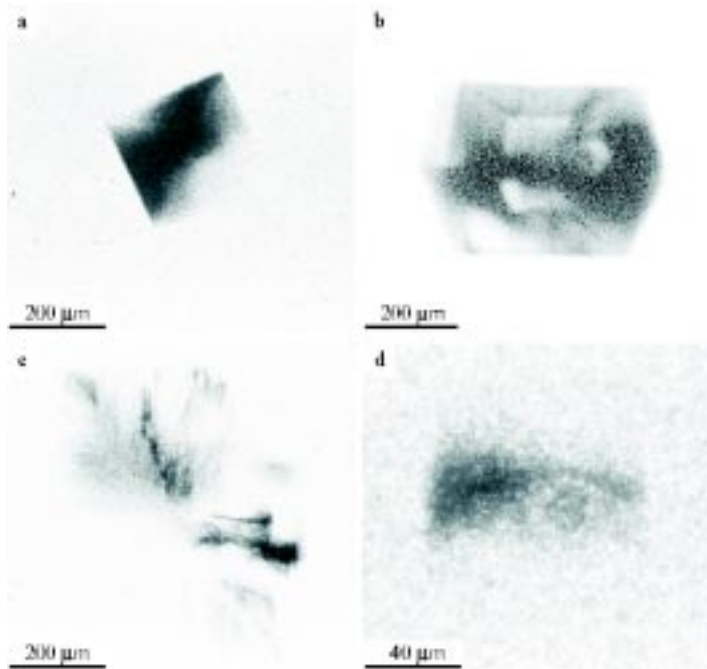
In the last three years we have applied X-ray topography to two very different systems - protein crystals and charge-density waves - where spatially-resolved diffraction information has been essential in understanding the nature of disorder. A more detailed account of this work can be found elsewhere [1,2].

### Protein Crystals

One of the most important factors limiting the rate at which protein and virus structures are determined by X-ray crystallography is the difficulty of obtaining high-quality crystals. The diffraction from typical crystals shows abundant evidence of disorder including broad mosaic widths and a rapid fade-out of diffracted intensity with increasing scattering angle  $2\theta$ . However, the precise nature of the disorder is in most cases unknown. For example, broad mosaicity can result from short-length-scale variations of the lattice orientation associated with dislocations or from long-length-scale orientation variations associated with bending and cracks. Minimizing each type of disorder will require distinct crystal growth and handling strategies, so distinguishing between them is essential.

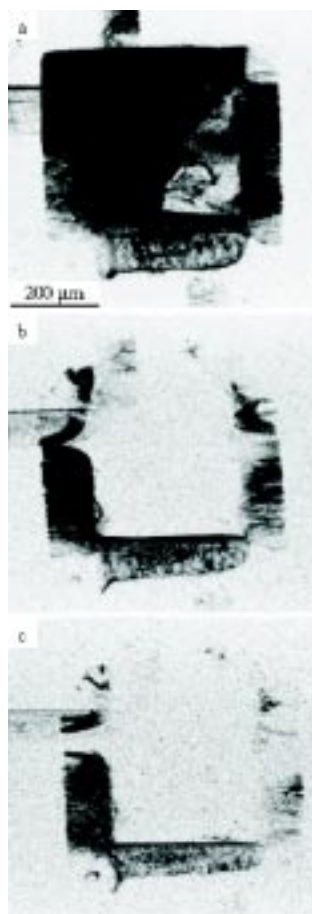
We have used X-ray topography to image protein crystal defects, their spatial distribution, and how they arise during and after growth [1]. Figures 1 and 2 show some examples of the topographs we have acquired. Figures 1(a) and (b) show topographs of hen egg white lysozyme (HEWL) crystals grown from a nominally pure solution and from a

solution containing 20% w/w turkey egg white lysozyme (TEWL), respectively. The crystal grown from the pure solution shows little contrast, whereas the crystal in (b) shows significant contrast between inequivalent growth sectors (i.e., between regions grown by addition of molecules to inequivalent faces) and enhanced contrast along the growth sector boundary. The contrast pattern in (b) indicates that the impurity incorporates with different densities in inequivalent growth sectors, creating stresses that drive cracking and mosaic broadening. Figure 1(c) shows a topograph of a concanavalin crystal, exhibiting dislocations that radiate in characteristic directions and that are responsible for the broad mosaic width of this protein's crystals. Figure 1(d) shows a needle-like catalase crystal that contains an incorporated microcrystal.



**Figure 1:** Topographs of protein crystals.

Figure 2 shows in more detail how topography can be used to diagnose the cause of mosaic broadening. The HEWL crystal shown was subjected to an abrupt change in salt concentration midway during growth, not unlike that which can occur during macroseeding. The mosaicity of this crystal is substantially broadened relative to crystals grown under uniform conditions. The topographs in Figure 2 acquired at different crystal orientations within the mosaic width show that the pre-change crystal core is well ordered and has a narrow mosaic width. However, the post-change growth region has dislocations radiating from inclusions formed at the boundary and these are responsible for the mosaic broadening. Our real-time observations of crystals undergoing dehydration suggest that topography should be especially well suited for understanding mosaic broadening caused by heavy atom compound soaks, flash freezing, and other post-growth treatments.



**Figure 2:** Topographs of a lysozyme crystal acquired at rocking angles  $\Delta\theta$  of (a)  $0.01^\circ$ , (b)  $0.03^\circ$  and (c)  $0.05^\circ$ .

### Sliding Charge-Density Waves

When many quasi-one-dimensional metals are cooled, they develop a superlattice characterized by a single wavevector  $Q=2k_F$ , where  $k_F$  is the Fermi wavevector. This superlattice consists of coupled modulations of the conduction electron density and the host lattice, and is known as a charge-density wave (CDW). Unlike most other superlattices, the CDW superlattice can “slide” relative to the host lattice when electric fields greater than a threshold field  $E_T$  are applied, and this motion produces an electric current. The CDW interacts strongly with impurities that are randomly distributed in the host lattice, producing the “depinning” threshold  $E_T$  - which increases with impurity concentration and (for more subtle reasons) decreasing crystal thickness - and very complex dynamics of the superlattice for  $E > E_T$ . The ingredients that describe this dynamics - damping, disorder, and elasticity - are essential in describing a variety of other systems from vortex lattices in type-II superconductors and Wigner crystals of electrons in semiconductor heterostructures to fluid interfaces in porous media and earthquakes.

A fundamental issue in most of these systems is the role played by plasticity in the dynamics. Crudely, a CDW moving in the presence of impurities is analogous to a corrugated rubber sheet moving over a random bed of nails. Although the sheet deforms as it moves, does it ever tear, creating dislocations of the superlattice? Recent theoretical predictions have said that it does, and that this should produce broadening of the CDW superlattice diffraction peak transverse to the direction of motion that is largest just when the CDW begins moving and becomes small when it moves very quickly.

Experimentally, CDWs in quasi-one-dimensional conductors like  $NbSe_3$  and  $K_{0.3}MoO_3$  show abundant evidence of plasticity including field-dependent transverse diffraction peak broadening. Similar predictions have been made for

vortex lattices in type II superconductors, and neutron diffraction peak broadenings have been observed in the moving vortex lattice. However, as in the case of protein crystal mosaic widths, there are many effects that could produce transverse peak broadening, and it has been unclear whether a given broadening is due to homogeneous intrinsic plasticity or instead arises from extrinsic plasticity associated with chemical or structural inhomogeneities of the host lattice.

To resolve this issue for CDWs, we have acquired X-ray topographs of both the main lattice reflections and the CDW superlattice reflections in NbSe<sub>3</sub> as a function of applied electric field [2]. NbSe<sub>3</sub> crystals grow as long, ribbon-like whiskers, and CDW motion occurs along the whisker axis. Essentially all crystals of this and several related CDW materials exhibit steps in thickness across their width that run along the whisker axis, and these produce corresponding variations in the depinning field  $E_T$  within the crystal cross-section. Topographs acquired at different angles in the mosaic

curve of main lattice reflections show that these steps are associated with small angle grain boundaries. Figure 3 shows topographs of a CDW superlattice reflection as a function of electric field for a crystal with one large step. Just above  $E_T$  the CDW on the thicker side of the step shears from the CDW on the thinner side and begins sliding, and the resulting shear strains on the thicker side reduce the diffracted intensity there. At high fields the shear strains diminish and transverse correlations recover. The resulting broadening of the CDW mosaic width shows the predicted variation with field, but the origin of this variation is extrinsic plasticity associated with the step rather than predicted intrinsic plasticity. Based on these results and related electrical transport measurements we have concluded that extrinsic sources dominate the plasticity observed in CDW conductors. The results in Figure 3 also demonstrate X-ray topography (usually thought of as a probe of static structure) as a powerful probe of superlattice structure and dynamics.

**Figure 3:** X-ray topographs of a NbSe<sub>3</sub> crystal obtained using a CDW superlattice reflection as a function of electric field.

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## References

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