

Protein Crystals are Twisted Even with Dislocation-Free and of High Quality

High-quality or dislocation-free protein crystals have been grown by various methods so far. From the assessment of crystal imperfection using digital X-ray topography, we found that protein crystals are slightly twisted even with dislocation-free and of high-quality. The twisting clearly appears in small crystals or in the initial stage of crystal growth. It uniformly relaxes with the crystal growth and becomes smaller in larger crystals. Such behavior might be explained by a “geometric frustration” assembly proposed for twisting in organic molecular crystals. We suggest that the slight twisting observed in this study is intrinsic phenomena in many protein crystals composed of asymmetry or chiral molecules.

High-quality protein crystals are a prerequisite for the structure analysis of proteins by X-ray diffraction and the understanding of their intrinsic physical properties. Generally, it is difficult to obtain high-quality protein crystals because of the complexity of the shape of protein molecules and intermolecular interaction, compared with those of simple inorganic and organic crystals [1]. To date, dislocation-free perfect crystals such as silicon and diamond have been limited to only two kinds of protein crystals, such as glucose isomerase and ferritin crystals [2, 3]. It is expected that many other high-quality or dislocation-free protein crystals still exhibit some imperfection.

In this work, we explore that the protein crystals have slight twisting even with dislocation-free by digital X-ray topography [4]. Fig. 1(A) shows an optical micro-

scope image and schematics of typical tetragonal hen egg-white lysozyme (HEWL) crystal, which is the well-defined shape. Fig. 1(B) shows the results of digital X-ray topographic images (slice images) in $\bar{1}10$ reflection. The slice images were obtained with the rotation of the crystal. The white band corresponding to the region of Bragg diffraction is shifted by the crystal rotation. This phenomenon can be explained by not the X-ray optics in this experimental configuration, but the left-handed twisting of the crystals along the c-axis as seen in Fig. 1(C).

Figure 1(D, E) show the digital X-ray topographic image and the profiles of local rocking curve, respectively. There is no dislocation in the crystal and the profile of the rocking curve shows the single peak, which means the high quality of the crystal. Figure 1(F) shows

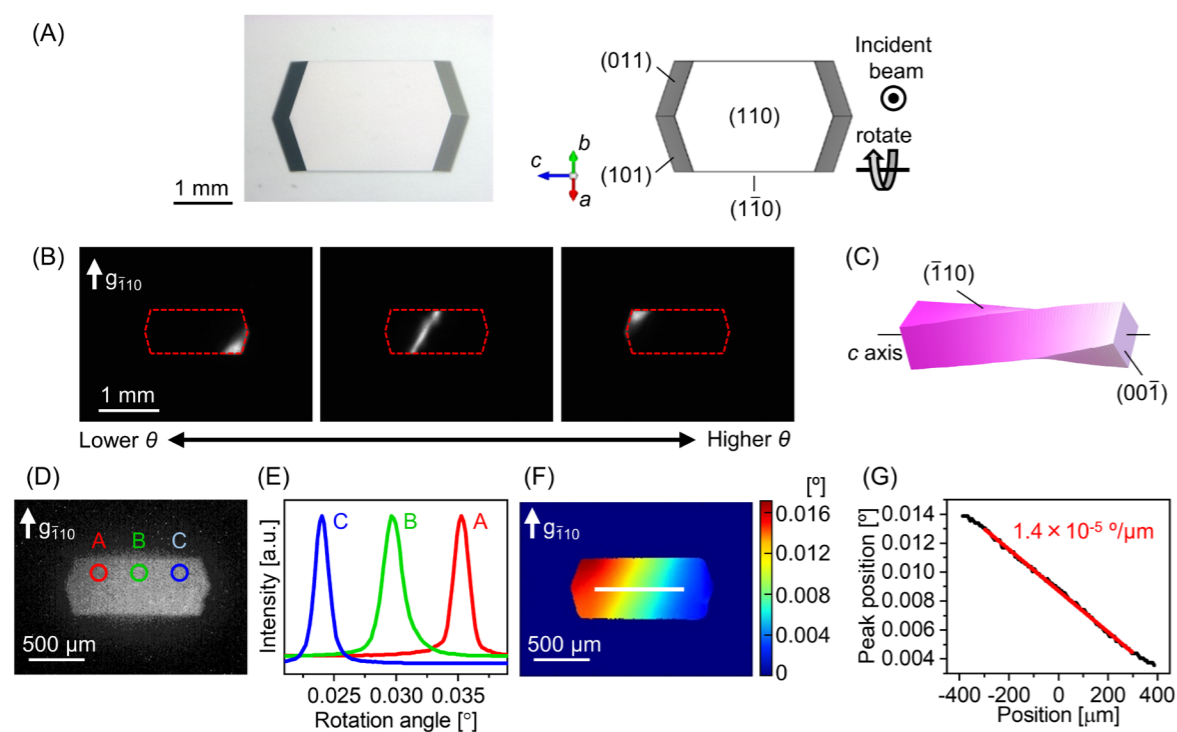


Figure 1: (A) Optical micrograph and corresponding schematics of typical tetragonal HEWL crystal, (B) digital X-ray topographic images (slice images) taken in $\bar{1}10$ reflection, (C) schematic model for twisting crystal, (D) digital X-ray topographic image, (E) local rocking curves in selected regions in (D), (F) angular position of maximum intensity (peak position) for local rocking curves, (G) peak position of local rocking curve as a function of the location along the straight line in the map in (F) [4].

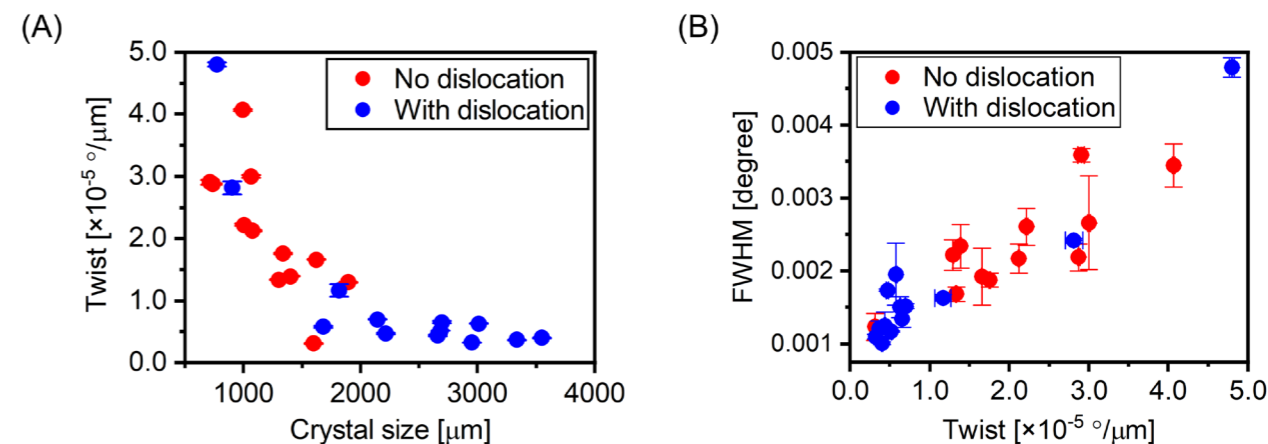


Figure 2: Correlation of (A) the magnitude of twisting with the crystal size, (B) the magnitude of twisting with the FWHM, excluding (red) and including (blue) dislocations [4].

the map of angular position of the maximum diffraction intensity. The Bragg diffraction occurs in the crystal from blue to red region with the rotation along the c-axis in the crystal. Figure 1(G) shows the peak position of local rocking curve as a function of the location along the line in the map of Fig. 1(F). The value of the slope corresponds to the magnitude of twisting and is almost constant in the whole region of the crystal, indicating uniform twisting. The magnitude of twisting is evaluated to be $1.4 \times 10^{-5} \text{ } ^\circ/\mu\text{m}$. This value is more than two orders of magnitude smaller than those in various twisted crystals reported so far [5]. We demonstrate that digital X-ray topography is a useful technique for evaluating the slight twisting in single crystals.

Similar measurements were carried out for the different-sized crystals. Some of the crystals are dislocation-free, and the others exhibit a few dislocations. It should be noted that all crystals exhibit only left-handed twisting with or without dislocations. This means that the twisting is not related to dislocation-associated Eshelby twist that is one of main causes of twisting. Figure 2(A) shows the correlation of the magnitude of twisting with the crystal size. Note that the crystal size means the length of the crystal along $[1\bar{1}0]$. It shows that the smaller the crystal size is, the larger is the magnitude of twisting. Furthermore, it is found that the magnitude of twisting is correlated with the full width at half maximum (FWHM) exhibiting imperfection as seen in Fig. 2(B). The FWHM increases with increasing the magnitude of twisting in crystals. This implies that the imperfection in high-quality protein crystals is primarily attributed to twisting.

In this study, the measurements have been carried out not only tetragonal form but also orthorhombic, monoclinic and triclinic HEWL crystals. As results, all samples of tetragonal, orthorhombic, and triclinic forms are left-handed twisting, on the other hand, only monoclinic forms are right-handed twisting crystals. The

handedness of twisting depends on the crystal form although all crystals are composed of identical HEWL chiral molecules. Moreover, the twisting cannot be explained by the crystallographic screw axis. Here we suggest that the handedness of twisting may depend on the local molecular interaction related to the asymmetric units in the crystal. Only monoclinic HEWL crystals have asymmetric units composed of two molecules, whereas asymmetric units in other forms comprise a single molecule. The handedness of twisting is ascribed to the local anisotropic interaction of chiral protein molecules associated with the asymmetric units in the crystal forms. This mechanism of twisting may correspond to the geometric frustration proposed as a primary mechanism of twisting in molecular crystals [6]. Our finding provides insights for the understanding of growth mechanism and the growth control of high-quality crystals.

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BEAMLINES

BL-14B and BL-20B

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