

5.1. CRYSTAL MORPHOLOGY, OPTICAL PROPERTIES AND MOUNTING

in a crystal, [001] being the c axial direction. Thus, information about several faces of a crystal is contained in information about one face if the relevant symmetry of the crystal is known. If the atomic structure of the crystal is known, it is possible to determine which aspects of the macromolecule lie on the various crystal faces.

5.1.1.2.2. *Measurement of crystal habit*

The habit of a crystal may be described in detail by measurements, particularly of the angles between adjacent faces. This can be done with an optical goniometer, in which a collimated visible beam of light is reflected from different faces as the crystal is rotated, and the angles between positions of high light intensity are noted. Such studies can be combined with X-ray diffraction measurements of the crystal orientation of the unit cell with respect to the various crystal faces (Oki *et al.*, 1999).

5.1.1.3. *Optical properties*

Crystals interact with light in a manner which depends on the arrangement of atoms in the crystal structure, any symmetry in this arrangement and the chemical nature of the atoms involved. Refraction is seen as a change in the direction of a beam of light when it passes from one medium to another (such as the apparent bend in a pencil placed in a beaker of water). The refractive index is the ratio of the velocity of light in a vacuum to that in the material under investigation and is greater than unity. It is measured by the extent to which the direction of a beam of light changes on entering a medium.

If a protein crystal has grown in the cubic system, its refractive index will be the same in all directions and the crystal is described as optically isotropic. Most protein crystals, however, form crystals with anisotropic properties, that is, their properties vary with the direction of measurement of the crystal. For example, some crystals appear differently coloured when viewed in different directions, and are described as pleochroic. The absorption of light is greatest when light is vibrating along bonds of chromophoric groups in the molecules in the crystals rather than perpendicular to them, and therefore they show interesting effects in plane-polarized light (in which the electric vectors lie in one plane only) (Bunn, 1945; Wahlstrom, 1979).

Anisotropy of the refractive index of a protein crystal, that is, its birefringence, can be used by biochemists to determine whether or not a protein has been crystallized. If the protein preparation in a test tube is held up to the light and shaken, birefringent protein microcrystals are revealed by light streaks, a schlieren effect. This occurs because the crystals have a different refractive index from the bulk of the liquid. Birefringence implies that there is double refraction as light passes through a crystal, and the light is split into two components (the ordinary and extraordinary rays) that travel with different velocities and have different properties (those of the ordinary ray being normal). Iceland spar (calcite) provides the ideal example of double refraction. Birefringence is measured as the difference between the refractive indices for the ordinary and extraordinary rays, and a crystal is described as positively birefringent if the refractive index is greater for the extraordinary ray. If a crystal is positively birefringent ($n_E > n_O$), it can be assumed to contain rod-like bodies lying parallel to the single vibration direction of greatest refractive index (n_E). If a crystal is negatively birefringent ($n_O > n_E$), it can be assumed to contain plate-like bodies lying perpendicular to the single vibration direction of least refractive index (n_E). For example, in crystalline naphthalene, the highest refractive index is in the direction of the highest density of atoms, along the long axis of the molecule. Similar arguments can be applied to crystalline macromolecules, such as haemoglobin, which is strongly pleochroic, appearing dark red and opaque in two extinction directions, and light red and

transparent in the third (Perutz, 1939). Thus, the coefficient of absorption is high when the electric vibration of plane-polarized light is parallel to the haem groups, but is low in other directions. Similarly, a specific carotenoid protein has been found to appear orange or clear depending on the orientation of the crystal relative to the direction of polarization of the light hitting it (Kerfeld *et al.*, 1997). The darkest orange colour, corresponding to a maximum absorbance of the carotenoid cofactor, is found when the polarizer is aligned along the a axis (the long axis of the crystals). This suggests that all the carotenoid cofactor molecules in this crystal structure lie nearly parallel to the a axis.

The directions along which double refraction is observed can be used to give some information on the crystal class. Some crystals are found to have one, and only one, direction (the optic axis) along which there is no double refraction. Crystals with this property are called uniaxial. They have two principal refractive axes and are tetragonal, hexagonal or rhombohedral. Other crystals are found to have two directions along which there is no double refraction (two optic axes), and these are called biaxial. Such crystals are either orthorhombic, monoclinic or triclinic and have three principal refractive indices.

5.1.1.3.1. *Crystals between crossed polarizers*

Most protein crystals are birefringent and are brightly coloured in polarized light. In order to view these effects, crystals (in their mother liquor) are set on a microscope stage with a Nicol prism (polarizing material) between the light source and the microscope slide (the polarizer). Another Nicol prism is set between the crystal and the eyepiece (the analyser). The crystal should not be in a plastic container, since this would produce too many colours. If the vibration plane of the analyser is set perpendicular to that of the polarizer (to give 'crossed Nicols'), no light will pass through in the absence of crystals, and the background will be dark. If the crystal is isotropic, the image will remain dark as the crossed Nicol prisms are rotated. If, however, the crystal is birefringent (with two refractive indices), the crystal will appear coloured except at four rotation positions (90° apart) of the crossed Nicol prisms, where the crystal and background will be dark (extinguished). At these positions, the vibration directions of the Nicol prisms coincide with those of the crystal. If one is looking exactly down a symmetry axis of a crystal that is centrosymmetric in projection (such as a tetragonal or hexagonal crystal), the crystal will not appear birefringent, but dark. By noting the external morphology of the crystal with respect to its angle of rotation, one can often deduce the directions of the unit-cell axes in the crystal (Hartshorne & Stuart, 1960). Examination of a crystal under crossed Nicol prisms can also provide information on crystal quality. For example, sometimes the components of a twinned crystal extinguish plane-polarized light independently. Other methods of examining crystals include Raman spectroscopy (Kudryavtsev *et al.*, 1998).

5.1.1.3.2. *Refractive indices and what they tell us about structure*

The refractive index of a crystal can be measured by immersing it in a mixture of liquids of a known refractive index in which the crystal is insoluble. The liquid composition is then varied until the crystal appears invisible. At this point, the refractive indices of the crystal and the liquid are the same. If the refractive index is the same in all directions, the crystal is optically isotropic, but most protein crystals are optically anisotropic and have more than one refractive index. For example, tetragonal crystals have different refractive indices for light vibrating parallel to the fourfold axis and for light vibrating perpendicular to it. These refractive indices are measured by the use of plane-polarized light.