5. CRYSTAL PROPERTIES AND HANDLING

5.1. Crystal morphology, optical properties of crystals and crystal mounting

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5.1.1. Crystal morphology and optical properties

When crystals of a biological macromolecule are grown, they are first examined under the microscope. This can show the crystal quality and may reveal crystal symmetry. Some external properties of macromolecular crystals are described here, including information on shape, habit, polymorphism, twinning and the indexing of crystal faces. Optical properties are also described. Every crystal form, however, has to be treated individually; only by working with it can the crystallographer discover its physical properties. The diffractionist aims to be able to mount a macromolecular crystal in a totally stable manner so that it does not deteriorate or slip in position during the data collection. Some methods for mounting such macromolecular crystals for X-ray diffraction studies are described here together with the necessary tools. For general information on purchasing supplies to do this see the list at http://www.hamptonresearch.com.

5.1.1.1. *Crystal growth habits*

5.1.1.1.1. *The shape of a crystal – growth habits*

Morphology is the general study of the overall shape of a crystal, that is, the arrangement of faces of a crystal. It can often provide useful information about the internal symmetry of the arrangement of atoms within the crystal (Mighell *et al.*, 1993). The periodicity of the arrangement of molecules or ions in a crystal can be represented by three non-collinear vectors, **a**, **b** and **c**, which give a unit cell in the form of a parallelepiped with axial edges a, b and c, and interaxial angles α, β and γ (α between **b** and **c**, *etc.*). The vectors **a**, **b** and **c** from the chosen origin of the unit cell are, by convention, selected in a right-handed system. Since there may be several possible choices of unit cell, the simplest, with the smallest possible repeats and with interaxial angles nearest to 90°, is the best choice. One method used to highlight the periodicity of the atomic arrangement within a crystal is to replace each unit cell by a point; this mathematical construction gives the crystal lattice. The entire crystal structure is the convolution of the unit-cell contents with the crystal lattice.

Biological macromolecules are, in general, chiral and can only crystallize in those space groups that do not contain symmetry operations that would convert a left-handed molecule into a right-handed one (improper symmetry operations). Proper symmetry operations involve translations, rotation axes and screw axes. These maintain the chirality of the molecule and hence are appropriate for crystals of biological macromolecules. The number of possible space groups is therefore reduced by this constraint on the types of symmetry operations allowed from the usual 230 for molecules in general down to 65 for chiral molecules.

The appearance of a crystal that has grown under a particular set of experimental conditions is called its habit. It is a result of the different relative growth rates of various crystal faces, and these rates, in turn, depend on the nature of the interactions between the molecules in the crystal, the degree of supersaturation of the solution and the presence of any impurities which may affect the growth rates of specific crystal faces. The term 'habit' is only used to describe the various appearances of crystals that are composed of identical material and maintain the same unit-cell dimensions and space group. The faces that have developed on these crystals are various subsets of those implied in the overall morphological description of the crystal. Any change in the experimental conditions under which a crystal is grown may alter its habit; a judicious selection of experimental conditions may permit formation of crystals with a chunky habit that are more suitable for X-ray diffraction analysis than thin plates or needle-like crystals. Examples of the crystalline forms of haemoglobins are provided by Reichert & Brown (1909).

Various descriptions of crystal habits appear in the literature. These include terms such as 'tabular', 'platy' or 'acicular' crystals, 'hexagonal rods' and 'truncated tetragonal bipyramids', among others. Some crystal habits are not very appropriate for X-ray diffraction analyses; these include spherulites, which are polycrystalline aggregates of fine needles with an approximately radial symmetry, and dendrites, which have a tree-like structure. The habit of a crystal can sometimes give information on the molecular arrangement within it. For example, flat molecules that stack readily upon each other produce long crystalline needles, because interactions in the stacking direction are stronger than those in other directions.

A crystal is bounded by those faces that have grown most slowly. Fast-growing faces quickly disappear as more and more molecules are deposited on them, constrained by surrounding faces that are growing more slowly. Any factor that changes the relative rates of growth of crystal faces, such as impurities in the crystallizing solution, will affect the overall habit. Different faces of protein crystals have different arrangements of side chains on their surfaces; thus, an impurity may bind to certain faces rather than others. Adsorption of an impurity on a particular face of a crystal may retard the growth of that face, causing it to become more prominent than normal in the growing crystal.

5.1.1.1.2. *Quality of protein crystals*

Protein and nucleic acid crystals contain a high proportion of water in each unit cell and are therefore fragile. The proportion of solvent to macromolecule in the crystal can be expressed, as described by Matthews (1968), as V_m in $\text{Å}^3\ \text{Da}^{-1}$ for the asymmetric unit. Values in the range 1.7 to 4.0 are usual for proteins, but nucleic acid crystals generally have a higher water content. Crystal fragility due to water content may be used to determine whether or not a crystal contains protein or buffer salt. Pressure with a fine probe will settle this question because a protein crystal will shatter, while a salt crystal, which is much sturdier, will generally withstand such treatment. If crystals have grown into one another, or appear as clumps, it is sometimes possible to split off a single crystal by prodding the clump gently at the junction point between the crystals with a scalpel or a glass fibre.

5.1.1.1.3. *Polymorphism*

Intermolecular contacts between protein molecules in the crystalline state determine the mechanical stability of the crystal. If the conditions used for crystallization vary, the number and identity of these contacts may be changed, and polymorphs will result. Polymorphism is the existence of two or more crystalline forms of a given material. Polymorphs have different unit-cell dimensions and hence different molecular arrangements within

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