

3.1. PREPARATION, SELECTION, AND INVESTIGATION OF SPECIMENS

Table 3.1.1.3. Crystallization matrix parameters for sparse-matrix sampling, adapted from Jancarik & Kim (1991)

Precipitating agents			
Non-volatile	Salts	Volatile	Mixture
2-Methyl-2,4-pentanediol (MPD) Poly(ethylene glycol)(PEG) 400 PEG 4000 PEG 8000	Na, K tartrate NH ₄ phosphate NH ₄ sulfate Na acetate Li sulfate Na formate Na, K phosphate Na citrate Mg formate	2-Propanol	NH ₄ sulfate + PEG 2-Propanol + PEG
Range of pH: 4.6, 5.6, 7.5, 8.5			
Salts, additives:		Ca chloride, Na citrate, Mg chloride, NH ₄ acetate, NH ₄ sulfate, Mg acetate, Zn acetate, Ca acetate	

used, since they minimize the loss of protein integrity. The several classes of detergent employed tend to be non-ionic or zwitterionic at the pH used, have a maximum hydrocarbon chain length of 12 carbon atoms, and possess a critical micelle concentration. The key to crystallizing membrane protein-detergent complexes appears to be the attainment of conditions in which the protein surfaces are moderately supersaturated and, in addition, the detergent micellar collar is at, or near, its solubility limit (Scarborough, 1994). Most successful integral membrane protein crystallizations are near the micellar aggregation point of the detergent (Garavito & Picot, 1990).

3.1.2. Selection of single crystals

3.1.2.1. Introduction

The final results of a structure analysis cannot be better than the imperfections of the crystal allow, and effort invested in producing crystals giving a clearly defined, high-resolution diffraction pattern is rarely wasted. The selection of twinned crystals, aggregates, or those with highly irregular shapes can lead to poor diffraction data and may prohibit a structure solution. There are many properties of crystals that can be examined prior, or in addition, to an X-ray or neutron diffraction study. These are summarized in Table 3.1.2.1. Many of these properties can yield useful information about the crystal packing and the overall molecular shape. For example, the shape and orientation of the optical indicatrix may be used to find the orientation of large atomic groups that possess shapes such as flat discs or rods and therefore also have strong anisotropic polarizability. A morphological examination can reveal information not only about the crystal quality but also in many cases about the crystal system, whilst identification of extinction directions can assist in crystal mounting. It is regrettable that many modern practitioners of the science of crystallography give little more than a cursory optical examination to their specimens before commencing data collection and a structure analysis.

3.1.2.2. Size, shape, and quality

A frequently occurring question involves the size and shape of single crystals required for successful diffraction studies. Among other factors, the intensity of diffraction is dependent on the volume of the crystal specimen bathed by the X-ray or neutron

beam and is inversely proportional to the square of the unit-cell volume (see Chapter 6.4). Hence, small crystals with large unit cells will tend to give rise to weak diffraction patterns. This can be compensated for by increasing the incident intensity, *e.g.* using a synchrotron-radiation source in the case of X-rays. How large should a crystal be, and what is the smallest crystal size that can be accommodated? X-ray collimators, or slit systems, with diameters in the range 0.1 to 0.8 mm are commonly employed for single-crystal diffraction studies. For many diffractometers, the primary beam is arranged to have a plateau of uniform intensity with dimensions 0.5×0.5 mm. For most small inorganic and organic compounds, crystals with dimensions slightly smaller than this will suffice, depending on the strength of diffraction, although successful structure determinations have been reported on very small crystals (0.1 mm and less) with both conventional and synchrotron X-ray sources (Helliwell *et al.*, 1993). Microfocus beam lines at the third generation of synchrotron sources such as ESRF are designed to examine crystals routinely in the 10 μ m range (Riekell, 1993). In the case of a biological macromolecule of molecular weight 50 kDa and using a conventional X-ray source (a rotating-anode generator), a crystal of 0.1 mm in all dimensions will probably give diffraction patterns from which the basic crystal system and unit-cell parameters can be deduced, but a crystal of 0.3 mm in each dimension, *i.e.* roughly 30 times the volume, would be required for the collection of high-resolution data (Blundell & Johnson, 1976). The higher intensity and smaller beam divergence inherent in a synchrotron X-ray source mean that high-resolution data of good quality could be obtained with the smaller crystal. Indeed, useful intensity data have been obtained with crystals with a maximum dimension of 50 μ m (Subsection 3.4.1.5). At cryogenic temperatures, radiation damage is greatly reduced, and increased exposure times can be utilized (at the expense of increased background) to compensate for a small crystal volume. In the case of neutrons, the sample size is generally larger than for X-rays, owing to lower neutron flux and higher beam divergence. For a steady-state high-flux reactor such as that at the Institut Laue-Langevin (France), a crystal volume of 6 mm³ or larger is recommended for biological samples. Unfortunately, crystals of this size are not readily obtainable in most cases.

The shape or habit of a single crystal is normally determined by the internal crystal structure and the growth