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 μm range (Riekel, 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 μm (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

Table 3.1.1.4. *Reservoir solutions for sparse-matrix sampling (Jancarik & Kim 1991)*

No.	Salt	Buffer	Precipitant (% by mass)
1	0.02 M Ca chloride	0.1 M Acetate	30% MPD
2			0.4 M Na, K tartrate
3			0.4 M NH$_4$ phosphate
4		0.1 M Tris	2.0 M NH$_4$ sulfate
5	0.2 M Na citrate	0.1 M Hepes	40% MPD
6	0.2 M Mg chloride	0.1 M Tris	30% PEG 4000
7		0.1 M Cacodylate	1.4 M Na acetate
8	0.2 M Na citrate	0.1 M Cacodylate	30% 2-Propanol
9	0.2 M NH$_4$ acetate	0.1 M Citrate	30% PEG 4000
10	0.2 M NH$_4$ acetate	0.1 M Acetate	30% PEG 4000
11		0.1 M Citrate	1.0 M NH$_4$ phosphate
12	0.2 M Mg chloride	0.1 M Hepes	30% 2-Propanol
13	0.2 M Na citrate	0.1 M Tris	30% PEG 400
14	0.2 M Ca chloride	0.1 M Hepes	28% PEG 400
15	0.2 M NH$_4$ sulfate	0.1 M Cacodylate	30% PEG 8000
16		0.1 M Hepes	1.5 M Li sulfate
17	0.2 M Li sulfate	0.1 M Tris	30% PEG 4000
18	0.2 M Mg acetate	0.1 M Cacodylate	20% PEG 8000
19	0.2 M NH$_4$ acetate	0.1 M Tris	30% 2-Propanol
20	0.2 M NH$_4$ sulfate	0.1 M Acetate	25% PEG 4000
21	0.2 M Mg acetate	0.1 M Cacodylate	30% MPD
22	0.2 M Na acetate	0.1 M Tris	30% PEG 4000
23	0.2 M Mg chloride	0.1 M Hepes	30% PEG 400
24	0.2 M Ca chloride	0.1 M Acetate	20% 2-Propanol
25		0.1 M Imidazole	1.0 M Na acetate
26	0.2 M NH$_4$ acetate	0.1 M Citrate	30% MPD
27	0.2 M Na citrate	0.1 M Hepes	20% 2-Propanol
28	0.2 M Na acetate	0.1 M Cacodylate	30% PEG 8000
29		0.1 M Hepes	0.8 M Na, K tartrate
30	0.2 M NH$_4$ sulfate		30% PEG 8000
31	0.2 M NH$_4$ sulfate		30% PEG 4000
32			2.0 M NH$_4$ sulfate
33			4.0 M Na formate
34		0.1 M Acetate	2.0 M Na formate
35		0.1 M Hepes	1.6 M Na, K phosphate
36		0.1 M Tris	8% PEG 8000
37		0.1 M Acetate	8% PEG 4000
38		0.1 M Hepes	1.4 M Na citrate
39		0.1 M Hepes	2% PEG 400, 2.0 M Na sulfate
40		0.1 M Citrate	20% 2-Propanol + 20% PEG 4000
41		0.1 M Hepes	10% 2-Propanol + 20% PEG 4000
42	0.05 M K phosphate		20% PEG 8000
43			30% PEG 1500
44			0.2 M Mg formate
45	0.2 M Zn acetate	0.1 M Cacodylate	18% PEG 8000
46	0.2 M Ca acetate	0.1 M Cacodylate	18% PEG 8000
47		0.1 M Acetate	2.0 M NH$_4$ sulfate
48		0.1 M Tris	2.0 M NH$_4$ sulfate
49	1.0 M Li sulfate		2% PEG 8000
50	1.0 M Li sulfate		15% PEG 8000

Abbreviations: tris: 2-amino-2-(hydroxymethyl)-1,3-propanediol; hepes: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Buffers: Na acetate buffer, pH = 4.6; Na citrate buffer, pH = 5.6; Na cacodylate buffer, pH = 6.5; Na hepes buffer, pH = 7.5; tris/HCl buffer, pH = 8.5.

conditions. For diffractometry purposes, it is customary to bathe the crystal in the X-ray beam, so that elongated crystals may require cutting with a razor blade in order to trim them to an appropriate size. Large crystals of hard materials can be ground into spheres or cylinders (Jeffery, 1977), so that corrections can be readily made to the observed intensities for systematic errors in absorption (see Chapter 6.3). Crystals that have elongated prismatic or needle shapes are often useful if data are collected using oscillation geometry, since the crystal can be translated in the X-ray beam at intervals during data collection to minimize radiation damage (Subsection 3.4.1.5). In general, all shapes can be accommodated, but those that are grossly asymmetric (*e.g.* very thin plates) may give elongated or distorted

Table 3.1.2.1. *Use of crystal properties for selection and preliminary study of crystals, adapted from MacGillavry & Henry (1962); morphological, optical, and mechanical properties*

Crystal property	Uses and comments	Relation with structure
Morphological properties		
Crystal habit	Setting crystal parallel to edge, or to symmetry axis, derived from goniometric measurement Habit can be influenced by solvent, crystallization conditions, trace impurities Well formed crystals can be accurately measured for analytical corrections for absorption	Morphological determination of crystal class may narrow down choice of space group Best-developed faces correspond to net planes with large density of lattice or pseudo-lattice nodes (Bravais' law, extended by Donnay & Harker) Prominent faces tend to be parallel to important bond systems Face development correlates inversely with surface free energy
Twinning	Twins may be hard to detect by morphological or diffraction methods. Investigate under the polarizing microscope: optical anomalies strongly indicate mimetic twinning, stacking faults, *etc*. Mechanical twinning may occur when a single crystal is cut or ground. In such cases, the crystal should be shaped by use of a solvent	May indicate hemimorphy or pseudo-hemimorphy of the cell or supercell; see Chapter 1.3 Pseudo-symmetrical stacking
Etch figures; epitaxy	See *IT* A (2002), Section 10.2.3 (pp. 805–806), and chemical properties below	
Optical properties		
Refractive index; birefringence (see IT *A*, Section 10.5.4, p. 790)	Checking quality of crystal: homogeneous extinction, interference figures Extinction direction is used for setting badly formed or ground crystals Magnitude of refractive index may be used for identification of crystal orientation	High refractive index may indicate close packing Shape and orientation of indicatrix may be useful for finding orientation of large atomic or ionic groups with strongly anisotropic polarizability (*e.g.* flat or rod-shaped groups)
Optical activity	Distinguishes between optical antipodes in studies of absolute configuration	Difficult to measure, or even detect, in optically biaxial crystals. No obvious relation with structure
Pleochroism	Identification of crystal orientation through dependence of colour on direction of light vibration	Extended conjugated-bond systems have strong absorption of light vibrating parallel to system; weak absorption perpendicular to system String-like arrangement of some atoms [*e.g.* iodine in poly(vinyl alcohol)] produces strong absorption parallel to string In inorganic compounds, absorption is greatest for light vibrating along directions in which ions are distorted
Reflection of light		Opaque substances contain loosely bound electrons
Raman effect		May give information on the orientation and symmetry of scattering groups
Mechanical properties		
Cleavage	Useful for obtaining good surfaces for crystal setting Useful for improving crystal shape	Correlates with bond-strength anisotropy
Hardness	Anisotropy of hardness may produce ellipsoids instead of spheres when an abrasion chamber is used	Hardness gives an indication of bond strength and bond density Hardness may be very sensitive to impurities, changes in texture through ageing or heat treatment, *etc*.
Plasticity	Single crystals: avoid cutting or grinding Polycrystalline material: plastic deformation is often strongly anisotropic, and may then be used to produce single or double orientation	Non-directive bonding between large strongly bonded units (long-chain paraffins, layer structures) Plastic flow may also be associated with mechanical twinning or lattice imperfections

Table 3.1.2.1. *Use of crystal properties for selection and preliminary study of crystals (cont.)*

Crystal property	Relation with structure
Magnetic properties	
Paramagnetism; diamagnetism	In an isomorphous series of paramagnetic salts, the values of the average susceptibility and of magnetic anisotropy are dependent on the nature of the paramagnetic ion. The shape of the coordination polyhedron may be found from the crystal anisotropies
	In aliphatic non-conjugated organic crystals, the numerically largest diamagnetic susceptibility is along the direction in which lie the largest molecular directions
	In crystals containing aromatic compounds or molecules with coplanar conjugated bonds, the numerically largest molecular diamagnetic susceptibility is normal to the plane of the molecular orbitals, and may thus indicate the molecular orientations
Ferromagnetism; antiferromagnetism; ferrimagnetism	Neutron diffraction by magnetic compounds may give information about the directions of the resultant spin and orbital moments. X-ray diffraction effects are usually unimportant
	In magnetic materials, the interatomic distances, and, in antiferromagnetic oxides, the valency angles at the oxygen ions are related to the diameter of the electron shell
Nuclear magnetic resonance	The line width in NMR spectra is related to the distances between the nuclei with magnetic moments
Electrical properties	
Ferroelectricity; pyroelectricity	See *IT* A (2002), Section 10.2.5, p. 807. Ferroelectricity indicates (i) a structure of polar symmetry, and (ii) the probability of another high-symmetry structure of nearly equal energy, derivable from the ferroelectric by a displacive transition. Often there are several related structures, some ferroelectric and some antiferroelectric
	Pyroelectricity indicates noncentrosymmetry. Second-harmonic generation is ordinarily a more sensitive test
Piezoelectricity	Piezoelectricity gives information on symmetry; it occurs only in ten crystal classes. See *IT* A, Section 10.2.6
Thermodynamic properties	
Heat capacity ('specific heat')	Anomalies indicate polymorphic transitions, disorder, approach to melting point, and temperature variation gives Einstein and/or Debye characteristic temperatures
Melting point	Atoms in crystals with a low melting point often have large thermal movements; diffraction experiments should preferably be carried out at low temperatures
	Anomalies in the variation of melting point in a series of homologues indicate a change in packing or bond type
Density	For measurement, see Chapter 3.2. Necessary for determination of number of formula weights per cell. May indicate liquid of crystallization, isomorphous replacement, degree of approach to close packing, first-order transitions with change of temperature or pressure
Thermal expansion	Thermal expansion is usually greatest in directions normal to layers or chains. Abrupt variation with change of temperature or pressure indicates a second-order transition
Chemical properties	
Chemical analysis	Gives kinds of atoms in the structure and (in conjunction with the density) the number of each kind in the unit cell
Attack of surface	May be used to shape crystals
	Etch figures are sensitive indicators of point-group symmetry (see *IT* A, Section 10.2.3). Change of orientation of etch figures on a face may reveal twinning. Rows of etch pits may reveal grain or sub-grain boundaries
Oriented growth on parent crystal	Epitaxy often reveals similarity of lattice parameters and even of atomic arrangement in the interface
	Grain boundaries and twinning orientations may be marked by epitaxic growth, or by oriented growth of crystals or reaction products on the mother crystal ('topotaxy')

reflections, leading to poor data quality in certain regions of the diffraction pattern.

The ultimate test of the quality of a crystal and its suitability for a structure analysis is the quality of the diffraction pattern. Ideally, the reflections should appear in the case of monochromatic radiation as single spots without satellites, tails, or streaks between the spots. The diffraction pattern should be indexable in terms of a single lattice.

3.1.2.3. *Optical examination [see IT A (2002), Section 10.2.4]*

Optical examination of a crystal under a polarizing microscope should be a prerequisite before mounting the specimen for a diffraction experiment. The presence of satellite crystals, inclusions, and other crystal imperfections will degrade the data quality, indicating the selection of a better specimen. The external morphology can often give a strong indication regarding