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9. MONOCHROMATIC DATA COLLECTION

9.1. Principles of monochromatic data collection

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9.1.1. Introduction

X-ray data collection is the central experiment in a crystal structure analysis. For small-molecule structures, the availability of intensity data to atomic resolution, usually around 0.8 Å, means that the phase problem can be solved directly and the atomic positions refined with a full anisotropic model. This results in a truly automatic structure solution for most small molecules.

Macromolecular crystals pose much greater problems with regard to data collection. The first arise from the size of the unit cell, resulting in lower average intensities of individual reflections coupled with a much greater number of reflections (Table 9.1.1.1). Secondly, the crystals usually contain considerable proportions of disordered aqueous solvent, giving further reduction in intensity at high resolution and, in the majority of cases, restricting the resolution to be much less than atomic. Thirdly, again mostly owing to the solvent content, the crystals are sensitive to radiation damage. Such problems have severe implications for all subsequent steps in a structure analysis. Solution of the phase problem is generally not possible through direct methods, except for a small number of exceptionally well diffracting proteins. The refined models require the imposition of stereochemical constraints or restraints to maintain an acceptable geometry. Recent advances, such as the use of synchrotron beamlines, cryogenic cooling and high-efficiency two-dimensional (2D) detectors, have made data collection technically easier, but it remains a fundamental scientific procedure underpinning the whole structural analysis. Therefore, it is essential to take the greatest care over this key step. The aim of this chapter is to indicate procedures for optimizing data acquisition. Overviews on several issues related to this topic have been published recently (Carter & Sweet, 1997; Turkenburg *et al.*, 1999).

9.1.2. The components of a monochromatic X-ray experiment

To collect X-ray data from single crystals, the following elements are required:

(1) a source of X-rays;
(2) optical elements to focus the X-rays onto the sample;
(3) a monochromator to select a single wavelength;
(4) a collimator to produce a beam of defined dimension;
(5) a shutter to limit the exposure of the sample to X-rays;
(6) a goniostat with associated sample holder to allow rotation of the crystal; and
(7) the crystalline sample itself.

Other desirable elements are:

(1) a cryogenic cooling device for frozen crystals;
(2) an efficient, generally 2D, detector system;
(3) software to control the experiment and store and display the X-ray images;
(4) data-processing software to extract intensities and associated standard uncertainties for the Bragg reflections in the images.

Many of these are discussed elsewhere in this volume. This chapter aims to provide guidance in those areas where choices are to be made by the experimenter and is concerned with the interrelations between parameters and how they conspire for or against different strategies of data collection.

Table 9.1.1.1. *Size of the unit cell and number of reflections*

Compound	Unit cell		Reflections	Average intensity
	Edge (Å)	Volume (Å^3)		
Small organic	10	1000	2000	1
Supramolecule	30	25000	30000	1/25000
Protein	100	1000000	100000	1/1000000
Virus	400	100000000	1000000	1/100000000

9.1.3. Data completeness

The advantage of diffraction methods over spectroscopy is that they provide a full 3D view of the object. Diffraction methods are theoretically limited by the wavelength of the radiation used, but, in practice, every diffraction experiment is further limited by the aperture and quality of the lens. In the X-ray experiment, the aperture corresponds to the resolution limit and the quality of the 'lens' to the completeness and accuracy of the measured Bragg reflection intensities.

In this context, completeness has two components, the first of which is geometric and hence quantitative. It is necessary to rotate the crystal so that all unique reciprocal-lattice points pass through the Ewald sphere and the associated intensities are recorded on the detector. Ideally, the intensities of 100% of the unique Bragg reflections should be measured. The second component is qualitative and statistical: for each hkl, the intensity, I_{hkl}, should be significant, with its accuracy correctly estimated in the form of an associated standard uncertainty, $\sigma(I)$. The data should be significant in terms of the $I/\sigma(I)$ ratio throughout the resolution range. This point will be returned to below, but it is especially important that the data at low resolution are complete and not overloaded on the detector, and that there is not an extensive set of essentially zero-level intensities in the higher-resolution shells.

9.1.4. X-ray sources

There are two principal sources of X-rays appropriate for macromolecular data collection: rotating anodes and synchrotron storage rings. These are discussed briefly here and in more detail in Chapters 6.1 and 8.1.

9.1.4.1. *Conventional sources*

Rotating anodes were initially developed for biological scattering experiments on muscle samples and have the advantage of higher intensity compared to sealed-tube generators. They usually have a copper target providing radiation at a fixed wavelength of 1.542 Å. Alternative targets, such as silver or molybdenum, provide lower intensities at short wavelengths, but have not found general applications to macromolecules. Historically, rotating anodes were first used with nickel filters to give monochromatic Cu $K\alpha$ radiation. Current systems are equipped with either graphite

monochromators, a focusing mirror, or multilayer optics. The latter provide substantially enhanced intensity. Rotating anodes remain the source of choice in most structural biology laboratories. An important choice for the user is in the selection of optimal collimator aperture: this should roughly match the crystal sample dimensions. For large crystals, especially if the cell dimensions are also large, it may be preferable to use collimator settings smaller than the crystal in order to resolve the diffraction spots on the detector. The fine-focus tubes currently being developed may affect the choice of home source over the next years (Arndt, Duncumb *et al.*, 1998; Arndt, Long & Duncumb, 1998).

9.1.4.2. *Synchrotron storage rings*

The radiation intensity available from rotating anodes is limited by the heat load per unit area on the target. In the early 1970s, it was realized that synchrotron storage rings produced X-radiation in the necessary spectral range for studies in structural molecular biology (Rosenbaum *et al.*, 1971), and the last three decades have seen great advances in their application to macromolecular crystallography (Helliwell, 1992). Synchrotron radiation (SR) is now used for more than 70% of newly determined protein-crystal structures.

The general advantages of SR are:

(1) High intensity: third-generation sources provide more than 1000 times the intensity of a conventional source.

(2) A highly parallel beam allowing the resolution of closely spaced spots from large unit cells.

(3) Short wavelengths, less than 1 Å, essentially eliminating the problems of correcting for absorption.

(4) Tunability of the wavelength, allowing its optimization for single- or multiple-wavelength applications; this is simply not possible with a conventional source.

(5) The ability to use a white, non-monochromated beam, the so-called Laue technique discussed in Chapter 8.2.

(6) Collection of complete images generated from a single circulating bunch of particles in the ring, only relevant for time-resolved experiments (Chapter 8.2).

SR beamlines take a number of forms. The source may be a bending magnet or an insertion device, such as a wiggler or an undulator. The properties of different beamlines thus vary considerably, and it is vital to choose an appropriate beamline for any particular application. The beamline capabilities are, of course, affected by the detector as well as the source itself. As far as the user is concerned, the primary questions regard the intensity, the size of the focal spot, the wavelength tunability and the detector system.

The present consensus for new synchrotron beamlines for macromolecular crystallography is that they should be on sources with an energy of at least 3 GeV and should receive radiation from tunable undulators. Together, these provide high and tunable intensity over the range required for most crystallographic experiments, including multiwavelength anomalous dispersion (MAD). The impact of free-electron lasers, which are likely to be built within the next decade, is not yet possible to assess.

Present beamlines produce radiation of extremely high quality for macromolecular data collection. At third-generation sources, such as the European Synchrotron Radiation Facility (ESRF) or the Advanced Photon Source (APS), complete data sets can be collected from cryogenically frozen single crystals in minutes.

9.1.5. Goniostat geometry

9.1.5.1. *Overview*

The diffraction condition for a particular reflection is fulfilled when the corresponding reciprocal-lattice point lies on the surface of the Ewald sphere. If a stationary crystal is irradiated by the X-ray beam, only a few reflections will lie in the diffracting position. To record intensities of a larger number of reflections, either the size of the Ewald sphere or the crystal orientation has to be changed. The first option, with the use of non-monochromatic, or 'white', radiation, is the basis of the Laue method (Chapter 8.2). If the radiation is monochromatic, with a selected wavelength, the crystal has to be rotated during exposure to bring successive reflections into the diffraction condition.

Several different ways of rotating the crystal have been used in crystallographic practice. These range from rotation about a single axis to use of a three-axis cradle, depending on the detector and application.

9.1.5.2. *Film methods: the precession and Weissenberg methods*

The first data-collection techniques involved photographic methods with visual estimation of the intensities, and the geometry of the original cameras involved simple rotation of the crystal. The basis of the screenless rotation method is discussed in Section 9.1.6 and Chapter 11.1. Two further developments of film methods involved rotation coupled to translation of the film (the Weissenberg technique) or precession photography, with more complex coupling of parallel precession of the crystal and film. Both methods involved isolating the diffraction from single layers of reflections through the use of screens. The intensities from the films were estimated by eye. This was an extremely time-consuming and inaccurate procedure and was only applicable for small cells. The original Weissenberg camera was not extensively used for protein data.

A key feature of the precession camera (Buerger, 1964) was that it provided an undistorted representation of individual layers of the reciprocal lattice, which were easy to index by eye, and it was an excellent tool for teaching prospective crystallographers. A disadvantage was that it required extremely accurate orientation of the crystal on the goniometer. The precession camera became an important tool for many years in most structural biology laboratories for defining the symmetry and lattice dimensions of new crystals and for screening derivatives, but it has largely been superseded by 2D detectors.

Volume C of *International Tables for Crystallography* (1999) presents a full and proper discussion of the precession and Weissenberg geometries.

9.1.5.3. *Single-counter diffractometers*

A great advance in automation came with the development of single- and later three- and five-counter diffractometers. The most common type was the four-circle diffractometer (Arndt & Willis, 1966). Single-scintillation-counter detectors are capable of measuring the intensity of only one individual reflection at a time. Therefore, in this technique, it is necessary to set the counter at the appropriate 2θ angle and to orient the diffracting plane so that the vector normal to it bisects the angle between the source and the detector. This can be achieved by the use of three axes of the Eulerian ω, χ, φ cradle or of the ω, κ, φ cradle. Such systems lent themselves readily to automated computer control, with accurate intensities and standard uncertainties output directly to storage devices at the rate of one reflection every one to five minutes. A full discussion of four-circle diffractometers and their associated geometry is given in *IT* C (1999).

Single-counter diffractometers are still widely used for small molecules. They were also applied in the 1960s and 1970s to the first protein structures, albeit at limited resolution. Their use is

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