PART 9. X-RAY DATA COLLECTION

Chapter 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, usually around 0.8 Å, resolution 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, the crystals are sensitive to radiation damage (see Section 9.1.12). 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.

At modern 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 (Carter & Sweet, 1997; Evans & Walsh, 2005; Dauter, 2005).

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;
(7) the crystalline sample itself;
(8) a cryogenic cooling device for vitrified crystals;
(9) an efficient, generally 2D, detector system;
(10) software to control the experiment and store and display the X-ray images;
(11) data-processing software to extract intensities and associated standard uncertainties for the Bragg reflections in the images.

On a number of beamlines, automated procedures have been implemented for sample changing, automatic crystal centring, evaluating the diffraction and proposing a strategy for data collection. These allow rapid and more effective selection of the best sample and optimal parameters.

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.

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 a significant fraction 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 not discussed in detail here as they are described in Chapters 6.1 and 8.1.

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

references