19. OTHER EXPERIMENTAL TECHNIQUES

19.1. Neutron crystallography: methods and information content

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19.1.1. Introduction

Neutron and X-ray crystallography are similar in both their experimental methodologies and in the resulting information content. The principal difference between the two methods is brought about by the characteristic scattering potential of the atom types. The scattering of neutrons by material is not proportional to the atomic number, as is the case in X-ray scattering, but rather depends on the individual nuclear characteristics of each atom type. As seen in Table 19.1.1.1, these characteristics show considerably less deviation and systematic trend among the different atom types. For instance, the heavy atoms in biological material – carbon, oxygen and nitrogen – scatter with about the same magnitude as a lead or uranium atom. In addition, neutrons are scattered by the atomic nuclei, which are essentially point sources, producing diffracted intensity not attenuated by a form-factor fall-off at increasingly higher scattering angles, as is the case in X-ray diffraction (Bacon, 1975).

There are a few atomic nuclei that induce a phase change of 180° in the scattered neutron, which results in negative peaks in a neutron density map. An extremely important example of this is the hydrogen nucleus, with a scattering length of −3.7 f ($1\ \text{f} = 10^{-13}$ cm). Its isotope, deuterium, on the other hand, scatters to give positive peaks (+6.7 f). The fact that H and D atoms can be so clearly distinguished from one another has very important implications for assessing biophysical parameters, as will be discussed below.

The application of the neutron-diffraction technique, which assigns H-atom positions in proteins and differentiates between H and D atoms, has been mainly focused on structural issues in three research areas: (1) protein reaction mechanisms; (2) protein dynamics; and (3) protein–water interactions (Kossiakoff, 1985, and references therein). It must be pointed out that recent advances in nuclear magnetic resonance have made protein dynamics investigations using H/D exchange procedures much easier than similar experiments by neutron diffraction. Additionally, the advances in ultra-high-resolution X-ray crystallography, which have allowed some level of experimental determination of hydrogen atoms in proteins, have further limited the uniqueness of the neutron method. Nevertheless, a number of important structural issues that are best approached by neutron crystallography remain.

Table 19.1.1.1. *Scattering lengths for atom types*

Element	Atomic No.	Scattering length (f; 1 f = 10^{-13} cm)
H	1	−3.7
D	1	6.7
C	6	6.6
N	7	9.4
O	8	5.8
Mg	12	5.2
S	16	2.8
Ca	20	4.7
Hg	80	12.7
Pb	82	9.4
U	92	8.5

19.1.2. Diffraction geometries

The general experimental setup involves use of a monochromated beam, employing normal-beam (Caine *et al.*, 1976) or flat-cone geometry (Prince *et al.*, 1978). Both approaches use flat detector surfaces, and thus there is a distortion inherent in all the diffraction phenomena that increases as a function of layer line along the axis of rotation. The extent of this effect can be calculated from the experimental parameters, but, in the case of a linear detector, there is only a moderate amount of flexibility available to make the necessary adjustments. The flat-cone geometry is well suited for a linear detector, since upper-level data fall on an undistorted plane. However, such a scheme requires that the detector be adjusted to different orientations with respect to the spectrometer axis (Prince *et al.*, 1978). In the normal-beam configuration, the crystal is usually mounted on a four-circle goniometer, allowing independent rotations around the φ, χ and ω axes to cover a full sphere of reciprocal space. This method can be efficient when used with a two-dimensional area detector because of the distortion of the diffraction pattern.

19.1.2.1. *Quasi-Laue diffractometry*

A significant advance in neutron crystallography has been the development and use of modified Laue methods to collect data (Wilkinson & Lehmann, 1991; Wilkinson *et al.*, 1992; Niimura *et al.*, 1997). These methods greatly increase the available neutron flux by using the white neutron spectrum. The full white radiation cannot be used due to very high background scattering and overlap between the diffraction peaks. A reasonable compromise between maximizing intensity while minimizing the experimental problems is to limit the white radiation component to about a 20% wavelength band by employing Ti–Ni multiple-spacing multilayers (Niimura *et al.*, 1997). In practice, the use of the Laue method in X-ray diffraction allows most of the reciprocal space to be recorded in one crystal setting. The quasi-Laue application requires several settings, depending on the neutron intensity distribution $I(\lambda)$ and the crystal symmetry. Data processing can be done using Laue software modified for neutron data.

19.1.3. Neutron density maps – information content

Fig. 19.1.3.1 illustrates several types of structural information derived from neutron density maps. Fig. 19.1.3.1(*a*) shows a well ordered tyrosine ring in the 1.4 Å structure of the protein crambin (Teeter & Kossiakoff, 1984). It can be seen that the ring hydrogen-atom locations are in positions of negative density. These peaks appear to be slightly displaced from their true positions, because the map is not at atomic resolution. At 1.4 Å, a portion of the negative peak of the hydrogen overlaps the positive peak of the ring carbon, effectively cancelling density between the atoms and giving the

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