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# Fibre diffraction studies of biological macromolecules

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ABSTRACT

Two fundamental structures in molecular biology, DNA and the α-helix, were determined using X-ray fibre diffraction data, and yet fibre diffraction occupies an obscure niche in structural biology. Relatively few structures are appropriate for the technique, and it seldom supplies data of the quality common in protein crystallography; however, it has proven indispensable in some cases. Here we outline some aspects of helix diffraction mathematics, and then illustrate the application of fibre diffraction by three case studies: DNA, filamentous bacterial viruses, and bacterial pili. These examples are illustrative, not exhaustive, and reviews of other important structures such as plant viruses, polysaccharides and amyloids are also cited, as appropriate. Finally we describe in more detail the methods currently used to obtain and analyze fibre diffraction patterns of biological macromolecules, to give a technique-oriented tutorial which may be useful to researchers who find that they require fibre diffraction for their work.


**Contents**



*Abbreviations:* CC, correlation coefficient; cryo-EM, cryo electron microscopy; MAS, magic angle spinning; M/L, mass per length; $M_r$, molecular mass; NCC, non-equatorial CC; PDB, Protein Data Bank; $Pf1^H$, higher-temperature symmetry of phage Pf1; $Pf1^L$, lower-temperature symmetry of phage Pf1; STEM, scanning transmission electron microscopy; TMV, tobacco mosaic virus.

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## 1. Introduction

Long thin polymers were among the first biological macromolecules to be studied by X-ray diffraction (Astbury and Street, 1932), in part because the experiment is simple. Although elongated molecules seldom form true three-dimensional crystals, they are often found in nature as bundles or fibres (for instance hair, wool, or cotton) in which the long axes of the molecules are roughly parallel. Fibres of elongated molecules can be formed more readily than three-dimensional crystals of more globular molecules, but improving the order within the fibre often requires substantial further experimentation. An elongated assembly of identical subunits is likely to form a helical array, because in a helix all subunits have the same environment. The diffraction pattern of a helix has features that simplify the interpretation of the structure.

Here we present a revised and expanded version of the article on X-ray fibre diffraction by Marvin and Nave (1982). We approach this subject from three different directions. In Section 2, we outline the mathematics of diffraction from fibres of helical structures, since some aspects may be unfamiliar to some readers. In Section 3, we present case studies that illustrate practical aspects of structure determination by fibre diffraction. Finally in Section 4 we review current fibre diffraction methods.

## 2. Diffraction by a helix

A fibre of elongated molecules in a collimated monochromatic X-ray beam gives a pattern of diffracted X-rays that can be imaged on a detector placed behind the fibre (Fig. 1). When viewing a diffraction pattern, it is important to keep in mind the reciprocal relation between the spacings on the diffraction pattern and the spacings in the structure being studied: diffraction at large angles (far from the centre of the pattern) gives information about small details of structure, and *vice versa*. A simple illustration of the principles of diffraction was given by Bragg (1913). The well-known Bragg equation

$$\lambda = 2dsin\theta \quad (1)$$

gives the relationship between the spacing $d$ between rows of

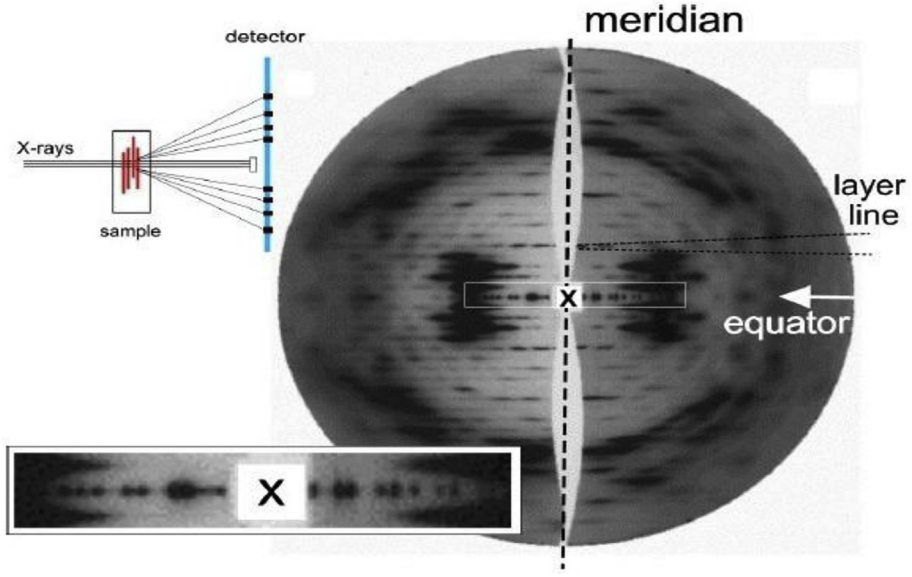


**Fig. 1.** X-ray fibre diffraction pattern geometry. A collimated monochromatic X-ray beam is diffracted by a sample (top left). If the sample is perpendicular to the incident X-ray beam, diffraction is symmetrical above and below the beam. Discussions of fibre diffraction patterns often mention the meridian, an imaginary vertical line through the centre of the pattern parallel to the direction of the long axes of the molecules in the fibre; and the equator, an imaginary horizontal line through the centre of the pattern perpendicular to the meridian. The diffraction pattern (right) shows a series of lines of intensity, the layer lines, extending horizontally from the meridian. The central layer line or equator may show crystalline reflections even when the other layer lines do not (enlarged box at lower left). The diffraction patterns shown here are from $Pf1^{H}$ (left) and Pf3 (right) filamentous phage (discussed further in Section 3.2.2), and are shown paired only for illustrative purposes; in most experiments the observed diffraction pattern is mirrored across the meridian. In the jargon of diffraction studies, the term "real space" refers to the space occupied by the molecule under study; "reciprocal space" refers to the space occupied by the Fourier transform of the molecule; and "detector space" refers to the space in which the experimental data are collected. The distribution of diffracted data in detector space can be converted to the distribution in reciprocal space by well-defined geometric relations. In this figure, the experimental diffraction patterns have been mapped from detector to reciprocal space, quadrant averaged, and mirrored across the equator. The outer edges of these diffraction patterns are at about 3.1 Å. Diffraction patterns reproduced from Fig. 1 of Welsh et al. (1998b). Figure prepared by Dr M. F. Symmons.