This highly illustrated monograph provides a comprehensive treatment of the study of the structure and function of proteins, nucleic acids and viruses using synchrotron radiation and crystallography.

Synchrotron radiation is intense, polychromatic and finely collimated, and is highly effective for probing the structure of macromolecules. This is a fast-expanding field, and this timely monograph gives a complete introduction to the technique and its uses. Beginning with chapters on the fundamentals of macromolecular crystallography and macromolecular structure, the book goes on to review the sources and properties of synchrotron radiation, instrumentation and data collection. There are chapters on the Laue method, on diffuse X-ray scattering and on variable wavelength dispersion methods. The book concludes with a description and survey of applications including studies at high resolution, the use of small crystals, the study of large unit cells, and time-resolved crystallography (particularly of enzymes). Appendices are provided which present essential information for the synchrotron user as well as information about synchrotron facilities currently available and planned. A detailed bibliography and reference section completes the volume. Many tables, diagrams and photographs are included.

This book is aimed at crystallographers, physicists, chemists and biochemists in universities, research institutes and in the pharmaceutical industry.
MACROMOLECULAR CRYSTALLOGRAPHY WITH SYNCHROTRON RADIATION
Macromolecular crystallography with synchrotron radiation

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To

my Mother and the memory of my Father
and

Madeleine, James, Nicholas and Katherine
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Preface

The scope of this book covers the use of synchrotron radiation in the X-ray analysis of single crystals of proteins, nucleic acids and viruses. The impact of this new X-ray source with its polychromatic nature and associated high intensity and fine collimation has brought important advances in the field of macromolecular crystallography. It has extended structure determinations to higher resolution, allowed use of smaller samples and larger, more complex, unit cells. Several new methods have come to the fore and some old methods have been revived. Firstly, the Laue method is being developed and used now for quantitative, time resolved analysis of structure. Secondly, variable wavelength methods are being developed and used for phase determination for metallo-proteins or derivatised proteins. Thirdly, the diffuse scattering is being measured more easily and procedures for analysing it are being developed in order to study molecular flexibility; hopefully its use will be increasingly widespread but at present it is the least developed of these three methods. The availability of the synchrotron is a very modern development but it has reopened fundamental questions of which crystallographic method to use. It is interesting to wonder what von Laue, W. H. and W. L. Bragg and the other early pioneers would have made of the synchrotron instead of starting with the X-ray emission tube. Certainly the Braggs were advocates of the monochromatic rotating crystal method. Wyckoff and Pauling used the Laue method although the weakness of the Bremsstrahlung continuum argued against it. With these difficulties it was fine then to be dismissive, as the Braggs were, of this method. The Braggs raised other fundamental objections to Laue geometry. These were the ‘multiplicity problem’, the ‘wavelength normalisation problem’ and the problem of determining absolute cell parameters from Laue data. Only the last of these three is limiting although progress is being made even there.

Variable wavelength approaches to phase determination using anomalous dispersion were discussed in the late 1950s by Okaya and Pepinsky as well as Mitchell, in the 1960s by Herzenberg and Lau and Karle and pioneered by Hoppe and Jakubowski. Technically these methods and others have only become really feasible with the synchrotron.
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Diffuse scattering was pioneered as a technique by Lonsdale and others. One imagines that these investigators would have been delighted to see the diversity of the diffuse scattering in the single crystal diffraction patterns recorded from macromolecular crystals at the synchrotron. As station master for the two instruments for protein crystallography at the SERC Synchrotron Radiation Source in Daresbury, England from 1980 to 1985 I saw hundreds of samples and their diffraction patterns during routine data collection runs. The uniqueness and diversity of the diffuse scattering was most striking.

This book will, I hope, serve as a source of information on the properties of synchrotron radiation from storage rings, diffraction instrumentation (such as optics and detectors) as well as diffraction methods and applications. To help the newcomer to the field or to assist experts from other disciplines, there are two chapters covering the fundamentals. The basics of macromolecular crystallography are described. Also, the principles of macromolecular structure are covered and various aspects of biological functions are discussed including oxygen transport and storage, enzyme catalysis, the ribosome and protein synthesis and virus structure. Applications in biotechnology such as protein engineering and drug design are mentioned. There is a wealth of results where synchrotron radiation has been critical to a given structure determination or definition of molecular function. The role of synchrotron radiation in furthering particular crystallographic analyses is therefore addressed and tabulated in detail.

The underlying importance of structure in defining function has meant that a wide range of structure determining methods have been developed in the last few decades. These other methods, such as extended X-ray absorption fine structure (EXAFS) spectroscopy (see Appendix 4), fibre diffraction and nuclear magnetic resonance (NMR), have been brought to bear in various studies as complementary tools to X-ray crystallography. In the Bibliography therefore I have given references to texts on these other techniques. However, macromolecular crystallography is the main method for precisely determining three-dimensional molecular structure over a huge range of molecular weights (up to several million in the case of viruses).

Synchrotron radiation is used when a conventional, home, X-ray source with electronic or image plate area detector proves inadequate. This is not to belittle the X-ray tube or rotating anode. On the contrary, I have also endeavoured to develop conventional X-ray source data collection capabilities based on area detectors. It is fair to say that a synchro-
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tron radiation source is a national and international resource for tackling the most technically demanding problems and an investigator may turn to it when necessary. The usefulness of synchrotron radiation in this research has been recognised by pharmaceutical companies who are now participating actively at synchrotron radiation facilities in the building and use of instruments dedicated to macromolecular crystallography.

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John R. Helliwell
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A note on units

Currently accepted units of measurement are used in this book. This generally means the SI system. An exception is the use of ångstrom (Å) rather than nanometre (nm). Although the latter is the SI standard the Å is the unit in common use. Inevitably therefore the Å is adopted here also. Indeed this is a reasonable unit of length because a carbon–hydrogen bond length is of the order of 1 Å (10⁻¹⁰ m).