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Macromolecular Crystallization and Crystal Perfection

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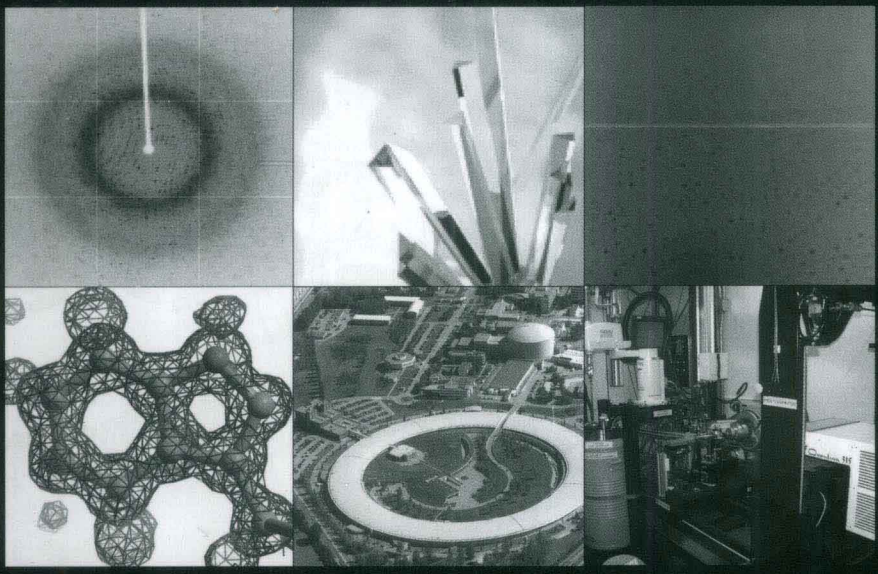

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Macromolecular Crystallization and Crystal Perfection

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Preface

Structural crystallography provides key information to understand biological mechanisms. To this end the technique requires high-quality crystals which are necessary for determining the structure and dynamics of biological macromolecules.

These structural objectives have required a concerted action across the field of macromolecular crystallography over the last several decades and embraced various science disciplines. Notably these have included molecular biology for sample preparation and molecular engineering; a science and understanding for macromolecular crystal-growth conditions; applied physics for improved X-ray and neutron sources as well as detectors and for a characterization of the crystal perfection and harnessing white-beam diffraction and anomalous scattering in data collection; software for numerous stages of calculation; structural chemistry and biology for the basis of enzymatic function and the mechanisms of disease; and finally systems biology to gather these results into a unified genomic framework of interacting proteins and nucleic acids.

The methods today have a sense of maturity; after all, the Protein Data Bank is on target for 100 000 macromolecular crystal structures within a few years from now. However, the motivation for this book stems from the fact that the field is still afresh with change which arises for various reasons:

- the field continues to expand and new investigators and students are turning to macromolecular crystallography for tackling critical biological and chemical problems, often without training in physics in general or methods in particular, and who we aim to serve in providing in one volume the key topics of macromolecular crystallization and crystal use in diffraction structure determination;
- the sources of X-ray and neutrons, and associated apparatus, are still changing radically, bringing new capabilities;
- the understanding of crystal perfection as well as types of disorders and how to deal with them is improving; the types of twinning and lattice disorders of crystals is an excellent example and where protocols for remediation in the most awkward cases are becoming known; there are more adventurous diffraction geometries for protein structure determination being developed including protein powder X-ray diffraction and single-molecule structure determination envisaged with the X-ray lasers that are coming on line.
- synchrotron X-ray microbeams will soon be routinely available to be applied to micrometre and probably sub-micrometre crystals.
- the pixellated area detectors have recently arrived after many years of development and will allow shutterless X-ray data collection as the norm;

- consideration is now being made to archive raw diffraction images. This would provide the most complete record for a given crystal's diffraction characteristics rather than the merged, unique, Bragg diffraction 'spot intensities or amplitudes' alone. An added benefit of this approach is that the information on the continuum diffuse scattering would be retained until such time as its interpretation might become fully possible thereby giving further insight into molecular flexibility and motion;
- this book interfaces with the complementary techniques of structure determination which include solution small-angle X-ray scattering (SAXS), and cryo–electron crystallography, microscopy and tomography applied to large multi-macromolecular complexes.

We hope we have provided the reader with an overview of the complex topics involved in structural crystallography together with some of the more esoteric areas that can become very critical in key cases.

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Cover picture. A montage showing clockwise from top left; a typical monochromatic X-ray diffraction pattern from a macromolecular crystal, crystals of the C1 Domain of Cardiac Myosin Binding Protein-C (Govada and Chayen, 2009) (grown by Dr Lata Govada, Imperial College London), Laue neutron diffraction pattern (also from a macromolecular crystal), the hutch at beamline 11-1 at SSRL (courtesy of Dr Aina Cohen, SSRL, Stanford), the ESRF and ILL facilities in Grenoble France (courtesy Denis Morel, ESRF, Grenoble), and an example of the ultimate quality data that can be achieved, an ultrahigh-resolution electron-density image.

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PART I

INTRODUCTION AND OVERVIEW

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Introduction

Biological macromolecules are the machinery of life; visualizing their three-dimensional structure helps us to fully understand their function. Visual observation with a light microscope is not possible as their sizes are well below the wavelength of visible light. While X-rays and neutrons allow visualization, they cannot be focused, so diffraction techniques have to be used. An understanding of three-dimensional macromolecular structure gives us a deeper understanding of basic biological concepts and processes, reveals the causes of diseases, assists rational pharmaceutical design and can lead to the design of macromolecules with novel properties. Visualizing these macromolecules is a complex ballet involving diverse but interrelated fields of endeavour. In this book, we aim to describe in some detail these complementary techniques, which include crystallization, diffraction and analysis of the data to obtain atomic structure from crystals of macromolecules. We cover areas where problems can occur and potential solutions to those problems. Finally, we touch on some of the developments in the not so distant future.

When we use the term crystal in the context of a biological macromolecule, we are describing an ordered array of macromolecules in an environment that keeps them stable. Biological macromolecules are predominantly made up of low atomic weight atoms, including hydrogen, carbon, nitrogen, oxygen, sulphur and phosphorous. Unlike inorganic crystals, a significant proportion (30–70%) of a macromolecular crystal is water (Matthews, 1968). This makes biological crystallography challenging; the process of crystallization is very complex and the crystals themselves diffract very weakly in comparison to inorganic crystals due to the low atomic weight content and disordered water making up a large proportion of the crystal volume. The biochemical and biophysical process of crystallization is still a largely empirical process. High-throughput technologies have been employed to speed up the crystallization process, allowing for more experiments to be set up using less sample, but their greater power may be realized if we achieve the array of experiments needed to better understand this complex process to develop crystallization.

Once we get the crystals, technologies and methods have advanced significantly and transformed our capabilities for structure analysis. In the X-ray field, highlights of the last three decades have included synchrotron radiation, detectors and cryo-crystallography. In a synergistic development neutron Laue methods, along with very large area neutron-sensitive image plates, and new spallation source

developments, are enhancing the potential for complete, i.e. with-H atoms (as deuteriums), structures. Complementary to these are developments in molecular biology techniques that go beyond the simple cloning and expression (production) of the target sample. Rational mutagenesis is providing functional information and being used to improve crystal quality, while fully perdeuterated protein production is enabling neutron studies.

1.1 Crystal Growth

The history of crystallization has been described by McPherson (1991; 1999a). The first published observation of crystallization, haemoglobin, was noted by Hünefeld (1840) when the blood of an earthworm was pressed between two microscope slides. This was followed by a slow growth in a number of other crystallized macromolecules until the 1930s when the pace quickened. Crystallization was initially used as a method of isolation with Northrop, Sumner and Stanley sharing the 1946 Nobel Prize for chemistry for the isolation and crystallization of proteins and viruses. The first biomolecular crystal-structure determined was that of vitamin B-12 in 1957 by Dorothy Crowfoot Hodgkin (1957) who subsequently won the Nobel prize for chemistry as a result of the work. Initially there were no set rules or recipes as to where to start to crystallize a macromolecule. Macromolecules were solubilized and then treated with arrays of precipitants in order to find favourable conditions for crystallization.

Crystallization is still largely empirical with many experiments sampling a large range of possible crystallization conditions. This idea of a designed sampling of many conditions, or screening, was introduced in 1979 (Carter and Carter). The numerous experiments required repetitive pipetting, a laborious, time-consuming and tedious task but the principal difficulty was attributing a quantitative score to the results to enable a meaningful mathematical analysis. In the late 1980s and early 1990s, the development of automatic means of dispensing crystallization trials (Chayen *et al.*, 1992; Chayen *et al.*, 1994; Chayen *et al.*, 1990; Cox and Wever, 1987; Oldfield *et al.*, 1991; Rubin *et al.*, 1991; Sadaoui *et al.*, 1994; Soriano and Fontecillacamps, 1993; Ward *et al.*, 1988) showed the promise of designed screening. However, it was not until the commercialization of a crystallization screen developed by Jancarik and Kim (1991) that systematic screening became a standard laboratory technique.

In the 1980s an effort was initiated to turn crystallization from an art into a science; the first of a continuing series of international conferences on the subject occurred (McPherson and Giege, 2007) and the term 'crystallogenesis' was coined (Giege *et al.*, 1986). The purpose of crystallogenesis was to understand the fundamental principles of the crystallization process, to quantitatively measure the biophysical and chemical parameters that are involved in crystal growth, and to use that knowledge to design experiments for obtaining better diffracting crystals. Systematic studies were performed mostly on lysozyme and other model proteins that crystallized with ease.

The aim was to extrapolate that information to target proteins that were proving difficult to crystallize. Progress was slow due to lack of suitable equipment for monitoring the crystallization process and to the complexity of the problem. Even the crystallization process for model proteins was not straightforward given the number of variables that were involved. Considerable development effort has been ongoing in the last 20 years on crystal-growth methods as well as more sophisticated monitoring and characterization, such as use of light scattering, interferometry and other techniques.

Much of the success of the last decade has come by way of automating and miniaturizing crystallization trials (Kuhn *et al.*, 2002; Luft *et al.*, 2003; Walter *et al.*, 2003) and by way of the development of diagnostic apparatus to study the crystallogenesis aspect of crystallization (Dierks *et al.*, 2008; Yeh and Beale, 2007). The ability to dispense trials consisting of nanolitre volumes in a high-throughput mode has cut the time of setting up experiments from weeks to minutes, and reduced sample requirements by an order of magnitude, a scenario that was unimaginable even in the recent past. While high-throughput approaches and miniaturization do not elicit a better understanding of crystallization, the analysis of these systematic and highly reproducible trials will improve our comprehension of the crystallization process, and application of these methods to specifically understand this process will enable us to answer and then ask many more questions.

Having a well-diffracting single crystal is a first step, but is not necessarily sufficient to solve the macromolecular structure. Detectors are able to measure the position and intensity (amplitude) of scattered reflections, but do not record phase information. In order to use a Fourier transform to go from a diffraction pattern, to an interpretable electron-density map that can be used to model the structure, we need phase information. We can use several approaches to provide an initial set of phases. If the protein has fewer than 1,000 non-hydrogen atoms, and the resolution of the diffraction data is near atomic resolution, we can use *ab initio* phasing (direct methods) to solve the structure (Hauptman, 1997; Uson and Sheldrick, 1999), or if there is significant sequence homology to other known structures, then molecular replacement may be used to solve the structure without modifying the sample.

If not, sample modification will likely be required. This can be accomplished by soaking, or co-crystallizing the sample with heavy atoms to provide phase information for a sub-structure (Islam *et al.*, 1998). Another approach, exploits differences in diffraction intensities caused by anomalous scattering, absorbance of X-rays by elements at wavelengths at, or near a particular element's absorption edge. The most common application of this method uses molecular biology to replace naturally occurring sulphur atoms in the protein's methionine residues with selenium atoms, creating a selenomethionine variant (Hendrickson *et al.*, 1990). The derivative is crystallized, and diffraction data collected at several wavelengths near the selenium absorption edge; this is an example of MAD (multiple anomalous dispersion) phasing. SAD (single anomalous dispersion) phasing is similar, but uses only one wavelength, making this a better-suited method for radiation-sensitive crystals (Gonzalez, 2007).