FIBROUS PROTEIN STRUCTURE

Edited by
John M. Squire
and **Peter J. Vibert**

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A volume dedicated to Dr Arthur Elliott

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FOREWORD

At the end of World War II, Francis Crick, then a physicist in his late twenties, decided to go into biology. On the advice of the physiologist A. V. Hill, who told him to familiarize himself with living cells, Crick took up work with the late Honor Fell and Arthur Hughes at the Strangeways Laboratory in Cambridge. That was a time when J. B. S. Haldane defined protoplasm as "a name which is a mere repository of our ignorance". Hughes and Fell thought that its viscosity might reveal the shape of the molecules it contains. They therefore asked Crick to let cells explanted from the frontal bone of chick embryos phagocytose iron filings and to measure the response of the filings to a magnetic field. The results of such measurements would allow Crick to calculate the viscosity of cytoplasm. Crick found that the behaviour of the filings was complex, and he summarized his results by likening cytoplasm to *Mother's Workbasket*, a heterogeneous mixture of particles of all shapes and sizes.[1,2] This volume introduces some of its bits and pieces and describes better physical methods, such as x-ray diffraction and electron microscopy, that are nowadays used to study them. Brief mention in Chapter 5 is made of infrared microscopy, the method that Arthur Elliott himself brought to bear on the structure of proteins and synthetic polypeptides in the late 1940s.[3,4] That was an exciting time. W. T. Astbury had found protein fibres to give three types of x-ray pattern, characteristic of α- and β-keratin and of collagen, and he proposed atomic structures for each of them. But were they correct? The information content of Astbury's x-ray diffraction patterns was minimal and the ratio of variable parameters to observations was 10^n:1, where n was quite a large number. Besides, keratin consisted of more than one chemical species of unknown amino acid composition and sequence. C. H. Bamford, Arthur Elliott and their colleagues at the Courtauld Research Laboratories decided that a study of synthetic homopolymeric peptides was more likely to lead to a clear answer. Their hopes were fulfilled when fibres or films of poly-γ-benzyl-L-glutamate and other polymers of single amino acids gave x-ray diffraction patterns with a wealth of sharp reflections in place of the two or three diffuse smudges found on x-ray pictures of wool, yet showing clear resemblances to the patterns given by keratin. The x-ray pattern of poly-γ-benzyl-L-glutamate

was to play a prominent part in the history of molecular biology. When Linus Pauling saw it, he recognized that it fitted the α-helix that he had first built at Oxford the previous year. When I read Pauling and Corey's paper on the α-helix, it occurred to me that the x-ray pattern of poly-γ-benzyl-L-glutamate should show a prominent meridional reflection at 0.15 nm, corresponding to the axial repeat of successive residues along the α-helix. Arthur Elliott generously gave me a small piece of poly-γ-benzyl-L-glutamate with which to test my idea, and I did indeed find that reflection, which excluded all structures previously proposed, including some of my own, and was consistent only with the α-helix.[5] However, interpretation of the rest of the diffraction pattern defeated me and I kept nagging the two theoreticians at the Cavendish Laboratory, Francis Crick and Bill Cochran, to help me. One day I brought them a draft paper by someone else who had tried and got it wrong. That stung them into action. Next morning each of them came into the lab with the theory of diffraction from helical chains, developed independently by different methods, but with the same answer. Cochran's was the more elegant that was later published.[6,7] Afterwards Crick's familiarity with that theory was crucial for the solution of the structure of DNA. It follows that without the work on synthetic polypeptides by Elliott and his colleagues, the α-helix would have continued to languish in Pauling's drawer and the double-helix of DNA might not have been discovered, at least not in March 1953.

Since those bad old days when biophysicists studied composite protein fibres of unknown sequence by inadequate physical methods, enormous progress has been made. Gene technology has leap-frogged protein chemistry: amino acid sequences that took protein chemists tens of man-years to determine can now be worked out by one individual in a few months, and cloning of genes allows the synthesis of homogeneous protein that used to be difficult or impossible to isolate in pure form. X-ray diffraction pictures that took many hours' exposure can now be obtained in milliseconds, allowing structural changes in muscle to be followed as a function of tension during a succession of single twitches. Cryo-electron microscopy provides more faithful pictures at higher resolution than any of the staining methods used in the past.

Yet the great biological problems still stare at us mockingly, tantalizingly unsolved. By what mechanism do the crossbridges that span from myosin to actin generate tension? How do microtubules function? How does mitosis work? How do single cells move? This book comes to grips with such aspects of these forbiddingly complex mechanisms as have been isolated for study so far, and it shows us how much has been achieved and how much more still remains to be done.

M. F. Perutz

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EDITORS' PREFACE

Remarkable progress has been made in the last few years in the elucidation of the structures of that enormously diverse group of biological materials known as the fibrous proteins. However, there are few up-to-date and comprehensive review texts on these structures. An excellent early book is the detailed monograph by R. D. B. Fraser and T. P. MacRae on *The conformation of fibrous proteins*. This is an invaluable source of information on the relatively early work on this subject. More recently, a number of specialist books have been published, including the two volumes edited by D. A. D. Parry and L. K. Creamer on *Fibrous proteins: scientific, industrial and medical aspects* that were based on the proceedings of the Fourth International Conference on Fibrous Proteins held at Massey University, New Zealand in 1979, and the 1981 monograph on *The structural basis of muscular contraction* by J. M. Squire.

Although there existed a clear need for a good new textbook on fibrous proteins, the present volume came into existence for quite another reason. Dr. Arthur Elliott, in his 82nd year at the time of writing, is now thinking of retiring from his research on fibrous proteins at King's College, London, and many of his past and present colleagues and collaborators thought that it would be appropriate to pay tribute in some way to his friendship, his fine example and his considerable scientific achievements. It became apparent that a review book on fibrous proteins, written by his friends and colleagues and in part by Dr. Elliott himself, would be both a fitting tribute to him and a very useful and up-to-date textbook in its own right. With the help of Academic Press, this idea developed into the present volume dedicated to Dr. Elliott.

In assessing at what level this book should be written, it became clear to us that the field as a whole had reached a sufficient level of maturity and had seen so many important advances in recent years that a broad text, suitable as an introduction to the present state of the art and accessible to newcomers to the field, would be extremely useful. However, it was thought that the treatment should also be sufficiently deep to be of interest to those already working on fibrous proteins or related subjects and who might wish to be brought up to date on progress in other areas of their field. Each chapter is

therefore a concise review of what is known either about the structure and function of different proteins or about the methods used to study them.

For this volume to be as useful as possible as a textbook, we have asked the authors of the various chapters to provide citations only to the most important references, but to provide the reference in full, including the title of each article. This makes the reference list itself much more informative, but inevitably means that some references to original work or in some cases to alternative views may have been omitted to keep the length of the book within bounds. The editors and authors express here their apologies to any who may feel that their work has been described without being directly cited. Efforts have been made in such cases to provide other references, possibly to published reviews, that would cite all of the original sources.

As editors, we acknowledge our gratitude to all those who have readily allowed some of their published figures to be reproduced here. But, above all, we thank the various authors, many of whom are internationally recognized leaders in their chosen fields, for contributing so successfully towards our goal of producing not only a useful textbook on fibrous proteins but also a fitting monument to the work of Arthur Elliott.

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CONTENTS

Arthur Elliott

In the world of fibrous proteins there is only one "Arthur". He was born on 4 November 1904 in Cleadon village in County Durham. His father, who was an office clerk, died of tuberculosis when Arthur was five years old; to support her only son, his mother first became a dressmaker and then, during World War I, took over the local Post Office, which she ran very successfully. Many troops were stationed nearby during the Great War, and Arthur has vivid recollections of their visits to enjoy the hospitality of the Cleadon villagers.

Arthur was educated at local schools and was clearly an able child: although youngest in his class, he was always within the top three in academic achievement. But those who know his great talent for designing and making his own equipment, always using exactly the right materials, will recognize in these formative years the influence first of his two grandfathers and later of his uncle "Tot" Brown. One grandfather was the local joiner, the other—father of Tot, who succeeded him—was the local blacksmith. Arthur used to watch with fascination the processes used by these masters of their profession and he learnt a great deal from them about the properties and handling of different materials.

Having gained his Higher School Certificate, Arthur became an undergraduate at Armstrong College in Newcastle upon Tyne, a college which at that time was part of the University of Durham. He graduated with a physics degree in 1925, obtained a diploma in the theory and practice of teaching in 1926 and then, in 1927, started his research career at Armstrong College, first as a student demonstrator in physics, and then as a College Fellow. It was at this time that he became interested in spectroscopy, especially of diatomic molecules, an interest kindled by Professor William Curtis, later FRS. But a major stroke of luck was the fact that nearby there was a wealthy amateur scientist who indulged in spectroscopy as a hobby. The amateur was a Tynemouth shipowner, one Wilfred Hall, who had an engineering degree but whose passion was spectroscopy. He had just purchased a 21-foot Rowland diffraction grating. By good fortune Arthur heard of this and was in fact the first to use it. Wilfred Hall lived comfortably and was looked after by his

butler-cum-chauffeur Robert. In true tradition of the versatility of British butlers, Robert used to act during the day, while his employer was running his shipping concern, as Arthur's laboratory assistant. Every evening Arthur would be entertained to dinner by Wilfred Hall.

In 1930 Arthur won a Rockefeller Fellowship to work on spectroscopy at the University of Utrecht, but he returned briefly to Newcastle after a few months for his PhD oral examination. In this single year Arthur not only gained his PhD, he also obtained a DSc in Utrecht. He had to defend the Utrecht dissertation publicly in an oral examination. Fortunately, this dissertation could be written in English and an agreement was reached whereby he was questioned at his oral in Dutch but could reply in English—only, of course, after addressing the questioner appropriately with full Dutch honours. Despite the problems this must have caused, Arthur gained his Utrecht DSc *cum laude*. In this memorable year he also met a Dutch girl, Lies, whom he married in 1931. They have just celebrated 55 years of marriage.

In 1931 Arthur became an assistant lecturer in physics at the University of Sheffield, where he continued his spectroscopy work [5–12] mainly with W. H. B. Cameron, an Ulsterman of Scottish descent who tragically died in the late 1930s from a neglected septic ulcer.

During World War II, Arthur was keen to put his scientific skills to good use and from 1940 to 1946 he was Temporary Scientific Officer at the Admiralty Research Laboratory, Teddington. It was during this time that his interest in infrared radiation developed. At Teddington, Arthur's group leader was Ernest G. Hill (Plate I) who is now over 90 and still going strong. Later in the war Arthur himself became group leader. His brief was to develop infrared systems for tracking night bombers and for making possible night-time manoeuvres of, for example, tanks and armoured cars. At the suggestion of Hill and working closely with J. D. McGee (later FRS) at EMI, an "image converter" was developed. This consisted of a thin infrared-sensitive photo-electric surface on a glass plate positioned 2–3 mm from a fluorescent screen. With 2000–4000 V applied across this assembly, electrons from the front plate, stimulated by incident infrared radiation, were accelerated across the gap between the plates and thus gave a strong visible image when they bombarded the fluorescent screen. Although the initial idea was to follow the exhausts of enemy bombers, the Germans soon caught up with this possibility and fitted screens behind their engines. However, the system became useful in combination with infrared torches in small commando raids, especially in Norway.

Clearly, to be portable, such systems had to contain a voltage source of up to 4000 V that was not too cumbersome. Very little current was required (less than 10^{-9} A). It happened that one of Arthur's colleagues was Bill Merton (Plate I), son of Sir Thomas Merton, the inventor of the Merton "nut"

used, among other things, in the preparation of diffraction gratings and in accurate measuring devices relying on uniform translation along screw threads. Bill Merton remembered seeing in the Clarendon Laboratory in Oxford what is known as a Zamboni Pile. This was a battery invented in about 1812 by de Lue and improved by G. Zamboni in about 1815. It consisted of paper layers coated on one side with a mixture of thin starch paste (later with gelatine), manganese dioxide and a trace of zinc chloride, and on the other side with tin foil. Each paper layer had a potential difference between the two faces of about 0.6–0.8 V and suitable stacks of such wafers were found to give the required 3000 V. Arthur redeveloped the Zamboni pile on a laboratory scale and, with the help of the papermakers John Dickinson at their Home Park Mills at Kings Langley and at their Basildon Works in Tottenham, one of Arthur's colleagues, E. W. Jackson, devised a system for coating large paper sheets that were then punched out to give large numbers of small circular disks. The disk size was chosen to fit into the cylindrical shafts of bicycle pumps (since this tubing was already available) and a 6-inch stack of disks gave the required high voltage with very little weight. These piles were incorporated into the handles of the infrared image converter to form a compact system. After the war, Arthur published a fascinating account of Zamboni piles and their manufacture [15].

Arthur's spectroscopy before the war was concerned with molecules containing only two atoms. After the war he moved to a new research laboratory being set up at Maidenhead by Courtaulds Ltd. His new brief was to study infrared spectra of molecules containing thousands of atoms—the new polymers that were then being developed. A great interest at that time was to develop a true artificial silk (a polypeptide) to replace the cellulose-based "silk" then used. Many of the polymers being synthesized by Arthur's colleagues at Courtaulds (C. H. Bamford, W. E. Hanby and D. G. H Ballard: Plate II) were synthetic polypeptides. Well-known examples are poly-L-alanine, poly-γ-methyl-L-glutamate (PMLG), which proved to be very silk-like but not commercial, poly-γ-benzyl-L-glutamate (PBLG) and poly-β-benzyl-L-aspartate (PBA). The chemical formulae of these long-chain polymers were well known, but almost nothing was known at that time about the 3-dimensional organization of their chains. One approach to this problem was to use polarized infrared spectroscopy to define the orientations of specific groups characteristic of polypeptides. The fact that a good infrared polarizer was not available did not deter Arthur: he designed and made one. He produced thin films of selenium on a polystyrene base that was later dissolved away. Stacks of about six such films, tilted at the Brewster angle to the incoming beam, formed a "selenium polarizer" [13,14], which, owing to the high refractive index of selenium, would gradually reflect away the unwanted polarization-component at successive surfaces. This was a great

improvement over the rolled-silver-chloride polarizers then available. By this means Arthur and E. J. Ambrose [16–18] obtained the first direct evidence for chain-folding in polypeptides. An extended structure would have the C=O and N—H groups directed almost perpendicular to the fibre axis, as was found to occur in β-silks and in some similar polypeptide forms (see Chapter 5). But many polypeptide preparations were found to have these groups oriented parallel to the fibre axis, meaning that the polypeptide backbone must be oriented approximately across this axis and must probably be folded. Other evidence of chain-folding was coming from x-ray diffraction studies carried out at Coventry first by F. Happey and later by L. Brown, using oriented samples provided by Arthur and his colleagues.

A theoretical study of polypeptide chain-folding into integral helices by W. L. Bragg, J. C. Kendrew and M. Perutz in 1950 [*Proc. R. Soc. Lond. Ser. A*, **203,** 321] had suggested that, among other possibilities, helices with either three or four amino acids per turn might be favoured. These are the 3_{10} and ω-helix respectively. A short time later (1951), L. Pauling, R. B. Corey and H. R. Branson [*Proc. Natl Acad. Sci. USA* **37,** 205] incorporated their chemical knowledge of the planarity of the amide group and the linearity of hydrogen bonds and proposed a non-integral helical polypeptide structure with 3·6 amino acids per turn: the well-known α-helix. Who was right? As it happens poly-β-benzyl-L-aspartate under certain conditions does produce an ω-helix with four amino acids per turn. If this had been the first polypeptide structure to be studied extensively by x-ray diffraction, it could well have taken much longer for the α-helix model to be substantiated. Fortunately, PMLG and PBLG were much more readily available and their diffraction patterns out to 5 Å seemed consistent with the α-helix. The clinching observation was obtained by Max Perutz [1951: *Nature* **167,** 1053] when, using an oriented specimen of PBLG provided by Arthur, he observed the meridional x-ray diffraction peak at 1.5 Å characteristic of only the α-helix.

In those pioneering years at Courtaulds, Arthur was involved with infrared and x-ray diffraction studies of synthetic polypeptides and of fibrous proteins that helped to establish and characterize not only the α-helix but also the β-sheet and the ω-helix [20,23–46]. In addition, polarized infrared work was also carried out on globular proteins [19,21,22]. One of the most notable achievements of the work on polypeptides was the determination, with B. R. (Ben) Malcolm (Plate II), of the structure of the α-helical form of poly-L-alanine. This showed, among other things, the handedness of the helix of L-amino acids. In this work they modelled the observed x-ray fibre diffraction patterns from oriented poly-L-alanine using the then-novel technique of optical diffraction. As usual, Arthur made his own very high-quality diffractometer. He used a concave, spherical mirror 20 cm in diameter, 10 m radius of curvature, which he made himself. He originally set up his

diffractometer in the main Courtaulds laboratory. He used a pinhole in front of a mercury arc lamp as the source 10 m from the mirror, placed the mask of holes (representing the model to be investigated) almost in contact with the mirror, and observed the diffraction pattern at a position very close to the source. (The mask contained holes in positions appropriate to a side-on projection of the molecule being modelled: one hole for each atom (unless they overlapped) and larger holes for bigger atoms.) The mercury source was very weak (this was before the advent of lasers) and long exposures (10–20 min) were required to record the diffraction patterns. Unfortunately, diffractometers of this kind are very sensitive to movements of air and therefore to temperature fluctuations and these proved to be very troublesome. What could be done? As it happens, Courtaulds had taken over for their laboratory a country house on the Thames known as "The Islet", which had previously been the home of a wealthy businessman named Wagg. At some point Wagg was in dispute with the local water authority, and he therefore decided to build his own water supply, a large tank containing thousands of gallons of water and raised 30 feet off the ground to provide a sufficient head of pressure. The enclosed space beneath this huge volume of water enjoyed remarkable thermal stability, so Arthur decided to move his diffractometer there. He located the mirror face-up on the floor and the source and viewing positions were at the top of a flight of stairs up to the tank. The system worked perfectly. By testing a variety of models, Elliott and Malcolm [35] unambiguously confirmed the earlier suggestion of Huggins [1943: *Chem. Rev.* **32,** 195] that L-amino acids form right-handed α-helices. They also showed that in poly-L-alanine there is a random distribution of "up" and "down" molecules.

Among Arthur's many notable colleagues at Courtaulds were two scientists who are very well known today, R. D. B. (Bruce) Fraser (now Chief of the Division of Protein Chemistry at CSIRO, Melbourne) and E. M. (Morton) Bradbury (now Professor and Chairman of the Department of Biological Chemistry in the University of California at Davis, USA). This was an extremely productive laboratory with a galaxy of experts brought together in the right place at the right time to make a very considerable contribution to the study of polypeptide and protein structure. But in 1962 it closed. Courtaulds were fighting off a take-over bid by ICI and the Research Laboratory at Maidenhead had to go to reduce their costs. Towards the end of 1962, then, Arthur moved briefly to London, where he worked in the laboratory of his son, Jim, at the London Hospital. In early 1963 he visited his old friend Bruce Fraser in Melbourne, Australia [45,47].

Arthur returned to England in late 1963 and took up a post as Lecturer in the new Biophysics Department at King's College, London, being set up by Professor Sir J. T. Randall, FRS. One of his first enterprises was to design and build, with the help of his very able and well liked workshop technician, the