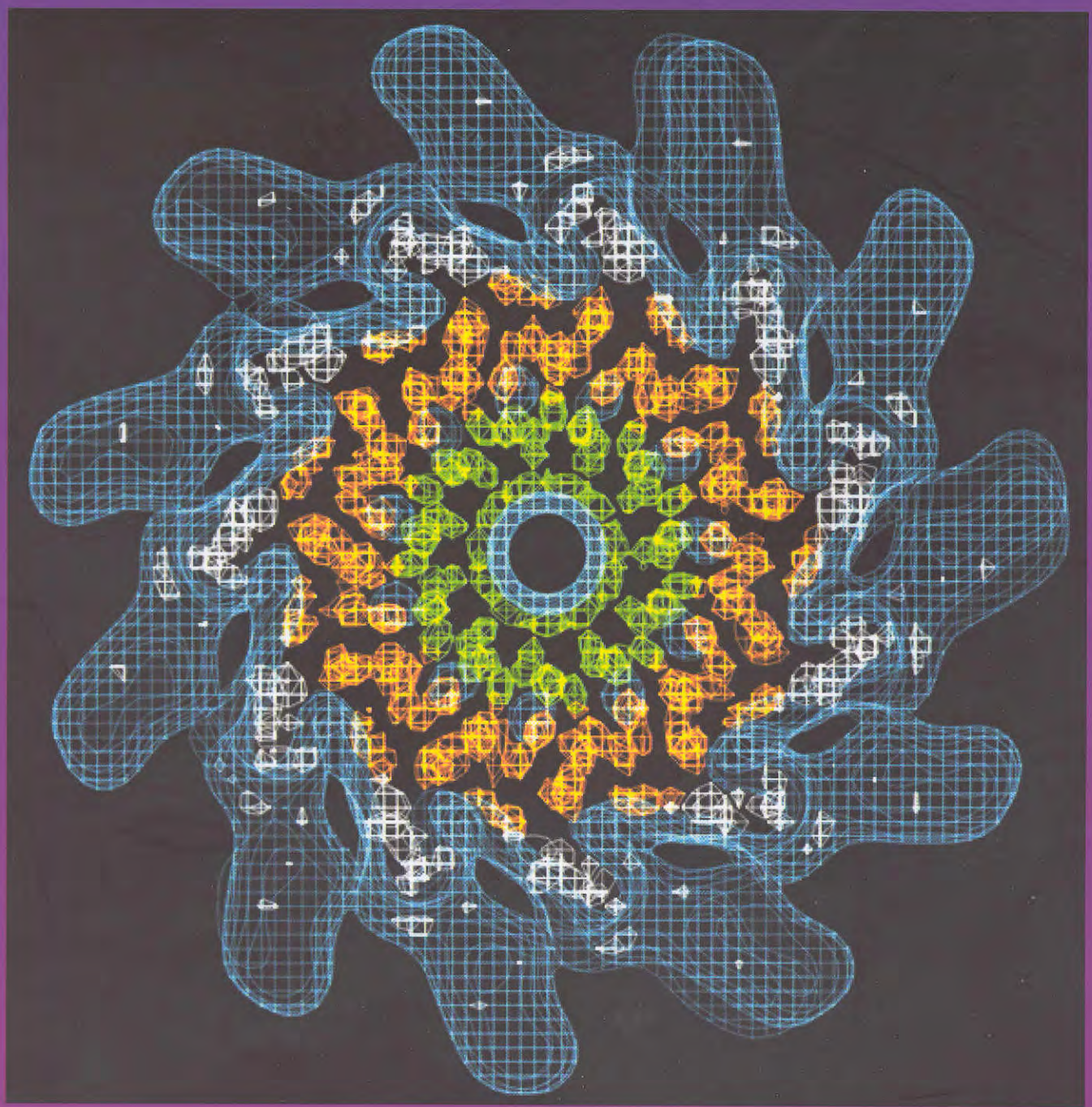
FIBRE DIFFRACTION REVIEW

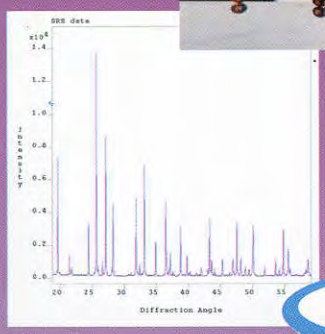
THE CCP13 NEWSLETTER

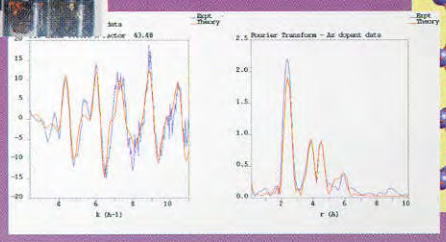
Software Development for Fibre Diffraction

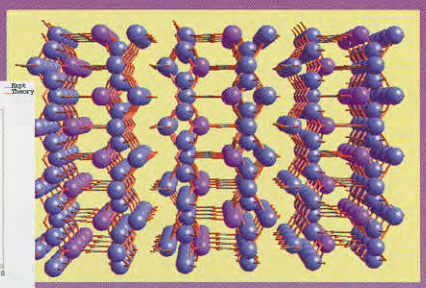


Issue 7 December 1998

DARTS
The National Synchrotron Radiation Service







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Dr. Sarah Stevens
DARTS
CLRC Daresbury Laboratory
Warrington WA4 4AD, UK

phone +44 (0)1925 603141
FAX +44 (0)1925 603124
e-mail darts@dl.ac.uk

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CLRC

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Front Cover Image

Electron density map of the R-type flagellar filament at 9 Å resolution. A cross section of a thickness of 50 Å is viewed down the axis. The smooth contours coloured white/blue represent the molecular envelope of the filament, and the detailed contours in green and brown colours are for the inner and outer tubes, respectively.

Newsletter Production
Editor: Prof. J. Squire, Biophysics Section, Blackett Laboratory, Imperial College, London SW7 2BZ
Production: Dr M.W. Shotton, Dr R. Denny and Dr G. Mant, CLRC Daresbury Laboratory, Warrington WA4 4AD
Printer: Eaton Press Ltd, Westfield Road, Wallasey, Merseyside L44 7JB. (Tel. 051-652 9111, Fax 051-653 5936)

The CCP13 Committee Members (1998)

Chairman Prof. John Squire (to 1999)
Biophysics Section, Blackett Laboratory, Imperial College, London SW7 2BZ
Phone 0171 594 7691 **Messages** 0171 581 5864 **Fax** 0171 589 0191 **Email** j.squire@ic.ac.uk

Secretary Dr Richard Denny (to 2001)
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603169 **Email** r.denny@dl.ac.uk

Research Assistant (Ex Officio) Dr Mark Shotton
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603626 **Email** m.shotton@dl.ac.uk

Members

Chairman Elect Dr Trevor Forsyth (1999 to 2002)
Institut Laue-Langevin, BP 156 F-38042, Grenoble Cedex 9, France
Phone +33 4 (0) 76207158 **Fax** +33 4 (0) 76483906 **Email** tforsyth@ill.fr

Dr Mike Ferenczi (to 2001)
National Institute for Medical Research, Ridgeway, Mill Hill, London NW7 1AA
Phone 0181 959 3666 **Email** m-ferenc@nimr.mrc.ac.uk

Dr Manolis Pantos (to 1999)
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603275 **Fax** 01925 603275 **Email** e.pantos@dl.ac.uk

Prof. Tony Ryan (to 2000)
Department of Chemistry, The University of Sheffield, Dainton Building, Brookhill, Sheffield, S3 7HF
Phone 01142 229409 **Fax** 01142 738673 **Email** tony.ryan@sheffield.ac.uk

Dr Tim Wess (to 1999)
The University of Stirling, Department of Biological and Molecular Sciences, Stirling, FK9 4LA
Phone 01786 467775 **Fax** 01786 464994 **Email** tjw3@stir.ac.uk

Dr Steve King (to 2001)
CLRC Rutherford Appleton Laboratory, Chilton, Didcot, OX11 0QX
Phone 01235 446437 **Fax** 01235 445720 **Email** s.m.king@rl.ac.uk

Members (Co-opted)

Dr Greg Diakun
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603343 **Email** g.diakun@dl.ac.uk

Dr Rob Lewis
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603544 **Email** r.a.lewis@dl.ac.uk

Dr Geoff Mant
CLRC Daresbury Laboratory, Keckwick Lane, Daresbury, Warrington WA4 4AD
Phone 01925 603169 **Email** g.r.mant@dl.ac.uk

Chairman's Message

CCP13 News

As in previous years, 1998 has seen the Collaborative Computational Project in Fibre Diffraction (CCP13) go from strength to strength. As detailed elsewhere in this, the 7th issue of *Fibre Diffraction Review*, we had an excellent Annual Workshop in May, there were several visits to outstanding meetings and conferences by people sponsored by or associated with CCP13 and there were many interesting publications of work in which CCP13 software has played a central part. *Fibre Diffraction Review* is being incorporated as part of the CCP13 World Wide Web pages (as with the previous Newsletters: details elsewhere: see http://www.dl.ac.uk/SRS/CCP13) and it is also now recognised by libraries by having an allocated ISSN number (ISSN 1463-8401).

The Annual Workshop this year saw some changes in CCP13 personnel. Dr. Richard Denny, who was the CCP13 Research Assistant from its early days, was offered and accepted a permanent position at the CLRC Daresbury Laboratory. Richard has been absolutely central to the development and collation of the CCP13 software suite and, as many of you will know, his input and expertise have contributed a large part to the undoubted success of CCP13. Our congratulations go to him as do our very best wishes for success in his new job. However, I am delighted to say that Richard will still remain associated with CCP13. Dr. Geoff Mant has decided to step down as CCP13 Secretary, once again a role that he has carried out with great enthusiasm and skill since the start of CCP13 and to whom our thanks are due, and Richard Denny has agreed to take over from Geoff as the new CCP13 Secretary. Geoff will also remain in touch with CCP13 as a member of the CCP13 Committee ex officio. The loss of Richard Denny as the CCP13 RA obviously left a vacancy to be filled. We were fortunate in being able to recruit another recent PhD from Keele University, Dr. Mark Shotton. Elsewhere in this issue Mark gives a short biography of himself so that you can all get to know him and love him as in the case of Richard Denny! You can contact him at m.shotton@dl.ac.uk.

The original appointment of Mark Shotton was for a few months to the end of September 1998, because that was the termination date of the CCP13 grant from the BBSRC. An application to the BBSRC for renewal of the grant for another three years was greatly aided by the decision of CLRC Daresbury Laboratory to part sponsor the Research Assistant position. The activities of CCP13 and its association with the Non-Crystalline Diffraction (NCD) community at Daresbury are closely linked and this sponsorship by Daresbury is a clear recognition of that. Fortunately the BBSRC application was successful and CCP13 is funded for another three years to September 2001 with Mark Shotton's appointment being extended to that date. As well as funding the Research Assistant and some much needed computing equipment, the new BBSRC grant helps to cover the costs of the Workshops and Newsletters.

At the Annual General Meeting of CCP13, formal elections to the Committee were made. The terms of office of Dr. Trevor Forsyth and Dr. Mike Ferenczi both concluded at the Annual Workshop this year but both members indicated their willingness to serve again if elected.

At the Annual General Meeting of CCP13 at the 1998 Workshop, I announced that I thought it to be timely and appropriate for me to step down as Chairman of CCP13 in the near future. I suggested that to make a smooth transition a new Chairman should be elected with a view to him/ her taking over from the time of the Annual Workshop in 1999. The CCP13 Committee considered the choice of successor and it was unanimously agreed that the name of Dr. Trevor Forsyth should be put forward to the AGM. This was done and at the AGM Trevor was duly appointed as Chairman-elect of CCP13. I have no doubt that Trevor's abilities and infectious enthusiasm for fibre diffraction research will ensure that CCP13 is in good hands and will fare well in the coming years. But no, you haven't seen the last of me yet! For the time being, at least, I will still be running the BBSRC grant and I will still edit *Fibre Diffraction Review*. Also at the AGM, Dr. Mike Ferenczi was elected to serve on the CCP13 Committee for another three year term.

We very much hope that you will come along to the 1999 Workshop (June 15-17, 1999) at St. Andrews University - details of which are given elsewhere in this issue. Among the eminent participants at the Workshop will be our host, the Principal of St. Andrews, Professor Struther Arnott, and also the

Principal of Stirling University, Professor Andrew Miller, both well-known fibre diffractionists. Remember not only that your poster could win a large cash prize (1st Prize - £100; 2nd Prize - £50), but also that abstracts will be included in the 1999 *Fibre Diffraction Review* - your work will automatically be available to a worldwide audience on the web. As usual, there will be bursaries available for students and young scientists to attend the 1999 Workshop. Details of all these are given at the end of the Newsletter.

CCP13, its Newsletter and its Friends Overseas

Your Contribution

Interested groups or individuals are invited to contact any of the officers of CCP13 to obtain information about CCP13 Workshops, software developments, software standards and so on. Offers of home-written software that could be incorporated into the new CCP13 suite of programs would be much appreciated and will, of course, permanently carry the author's attribution. Make sure that you are on the CCP13 mailing list and you will be kept informed.

International Cooperation

Although these CCPs are UK funded projects, there is a very strong interest in making them international through cooperation with interested scientists in other countries. A natural link for CCP13, for example, exists with the Special Interest Group (SIG) in Fibre Diffraction of the American Crystallographic Association and possibly with some American synchrotron users (CHESS). Others exist with the ESRF at Grenoble and with the Photon Factory in Japan.

Newsletter Editorial Policy

Articles for inclusion in *Fibre Diffraction Review* are welcome by the Editor at any time, but preferably items for the December 1999 issue should arrive before the end of November 1999. It is hoped that *Fibre Diffraction Review* will become an annual 'essential' for fibre diffractionists. This is the place to advertise your fibre diffraction or NCD meetings, to report on new software or 'hot' results obtained using the CCP13 or other fibre pattern processing suites and to provide reports of meetings of interest, preferably together with one or two photographs. All technical articles will be scrutinised both for scientific content and presentational style by the Editor (or his nominee) together with at least one other member of the CCP13 Steering Panel. In this way we hope to maintain high standards. Remember that the Newsletter not only goes to other fibre diffractionists, but also to various members of the Research Council Secretariats and to other funding agencies.

Fibre Diffraction Featured on Covers of IUCr Publications

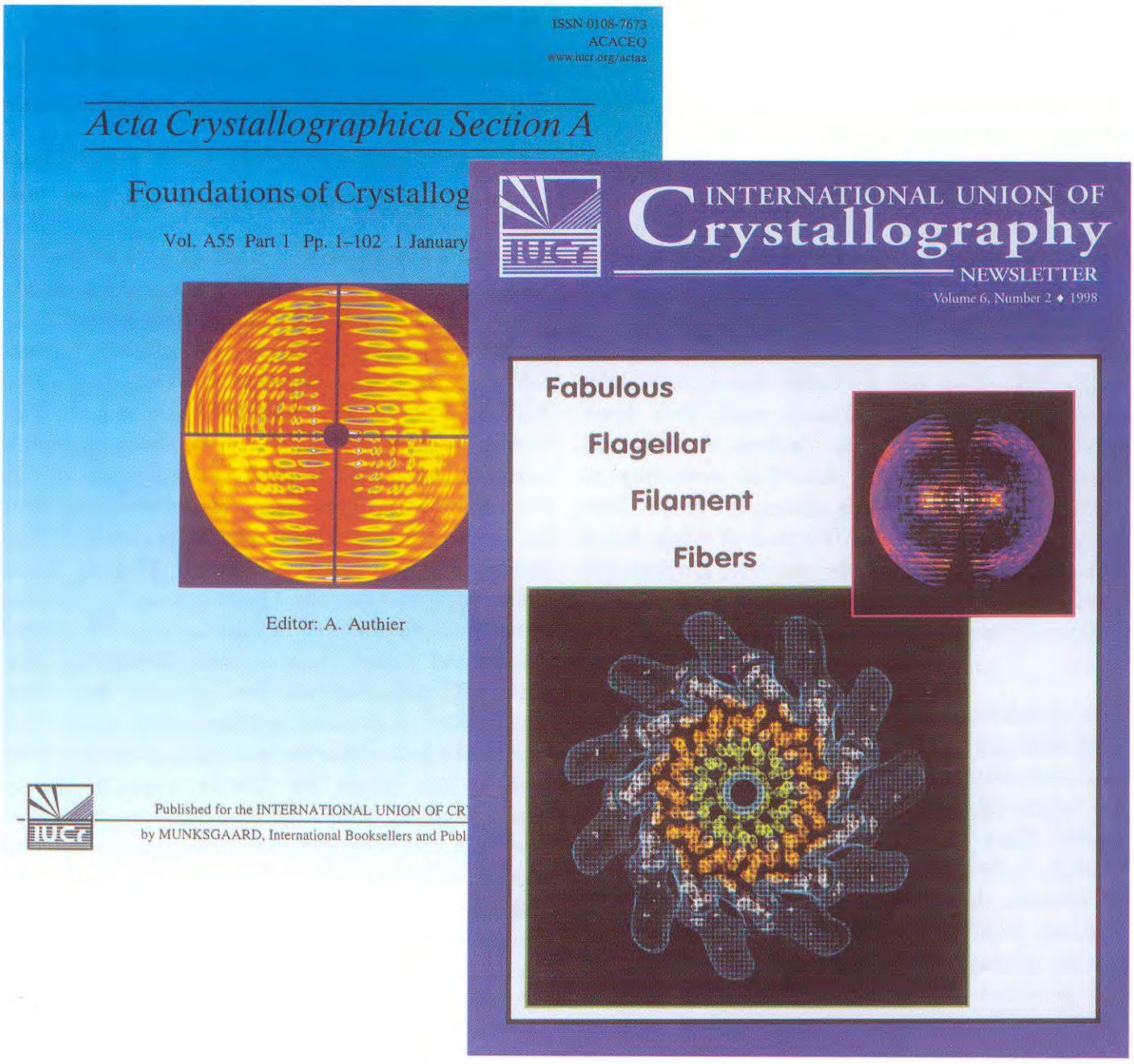
Fibre diffraction has recently received increased exposure in the crystallographic community, being featured on the covers of two IUCr (International Union of Crystallography) publications.

The May (Vol. 6, No. 2) 1998 issue of the IUCr Newsletter featured work from Keiichi Namba's laboratory (International Institute for Advanced Research, Japan) on the structure of bacterial flagellar filaments. This work (see Nature Structural Biology, 5, 125-129, 1998) describes X-ray fibre diffraction data from the left and right supercoiled states of the flagellar filament, and the 9Å structure of the R-type filament. Bacteria swim using the rotating filaments, and bacterial motility involves switching between the left and right states. An editorial on fibre diffraction, as well as a report on the Third Fibre Diffraction Workshop, held in Kentucky in October 1998, appeared in the same issue.

The 1999 issues (Vol. 55) of Acta Crystallographica Section A feature work from Rick Millane's (Purdue University, USA) laboratory on diffraction by disordered polymer fibres. This work (see Acta Cryst. A52, 812-829, 1996) describes theory and

methods for calculating fibre diffraction patterns from polycrystalline fibres in which there are quite general and complicated forms of disorder. The methods are used to analyse the disorder present in two polynucleotide fibres.

Rick Millane, Purdue University



American Crystallographic Association Annual Meeting, Arlington, Virginia, 23rd July 1998

The Special Interest Group session, *The state of the art in fibre diffraction* was held on the final day of the ACA annual meeting in Arlington, Virginia. The session was organised and introduced by Gerald Stubbs (Vanderbilt), who described the range of fibre diffraction experiments and current difficulties with weakly scattering samples, the short data collection times required for time-resolved experiments and the

need for improved resolution. He illustrated these problems with reference to his own work on tobamoviruses and other filamentous viruses. Tom Irving (IIT) described the facilities available at BioCAT at the Advanced Photon Source with careful use of TLA's (three-letter acronyms). Shyam Baskaran (Purdue) gave an account of progress in producing optimal Fourier difference maps from fibre diffraction data. Dan Kirschner (Boston) talked about the approaches he has employed to elucidate the folding and molecular organisation of amyloid proteins. A combination of magnetic alignment, small and wide-angle data collection and the use of known beta-pleated sheet atomic coordinates is proving fruitful in accounting for the observed polymorphism of amyloid. David Grubb (Cornell) gave an entertaining description of his work on spider silk. This has included the simultaneous collection of small and wide-angle data from dragline silk from Nephila clavipes. Rengaswami Chandrasekaran (Purdue) described modelling of triple-stranded nucleic acids against continuous fibre diffraction data and Lee Makowski (Florida State) took the audience through the processes employed to study variants of M13 bacteriophage from data reduction to molecular modelling.

Also of interest for fibre diffraction users was the symposium on *New Directions in Neutron Scattering Instrumentation for Structural Biology*. The session was organised by Benno Schoenborn and Bob von Dreele (Los Alamos National Laboratory) to promote technical developments for the application of neutron diffraction in structural biology. It included contributions from instrument scientists, protein crystallographers, and fibre diffractionists and provided numerous examples of important aspects of macromolecular structure that are best tackled using neutron diffraction rather than X-ray diffraction methods. There is now considerable interest in the use of image plates in neutron diffraction experiments since they offer a cheaper alternative to gas detectors and can easily record over a large solid angle. Such detectors have huge potential both in crystallography and in neutron fibre diffraction. Experiments that have been carried out on the new LADI diffractometer at the Institut Laue Langevin (ILL) on the location of solvent and hydrogen positions in lysozyme (Nimura, JAERI), concanavalin A (Helliwell, Manchester) and cob(II)alamin (Langan, LANL) have demonstrated the scope of image plate technology in neutron diffraction. Although the main limitation for this technology remains the sensitivity of the plates to gamma radiation, the ongoing development of new phosphors with low gamma sensitivity is likely to be extremely important in the future. The progress that has been made in this area to date was reflected in some extremely interesting presentations. Nobuo Nimura (JAERI) gave an exciting talk on the development of neutron image plate technology, both on LADI at the ILL and on BIX1 and BIX 2 at JAERI in Japan. John Helliwell (Manchester University) described his recent work on concanavalin A. Paul Langan described the design of a structural biology station at LANL – this will allow both single crystal and fibre diffraction experiments to be carried out. After a presentation by Bob von Dreele on the application of neutron powder diffraction in protein structure determination, Peter Timmins from the ILL described how low resolution neutron diffraction, in parallel with contrast variation methods, were used to locate detergent in various porin structures. There were two presentations from the fibre diffraction community in this session. One was from Magdalena Ivanova (Florida State University) who described how a model for the coat protein of filamentous bacteriophage M13 was constructed using neutron fibre diffraction data collected from magnetically oriented gels with specifically deuterated residues (this work was carried out at Brookhaven National Laboratory). The second was given by Trevor Forsyth (Keele University/ILL) who described neutron diffraction work that had been performed with deuterated fibre samples of DNA. The experiments were carried out at the ILL on instrument D19. D19 currently operates with a thin "banana" detector that captures only a limited amount of the available data at a given time. However, the upgrade that is planned for D19 involves the replacement of this detector by an array of 9 area detectors. This will improve the efficiency of the instrument by a factor of 15-20 and will consequently have an enormous impact on the quality, throughput and scope of neutron fibre diffraction experiments.

Richard Denny (Daresbury Laboratory) and Trevor Forsyth (ILL/Keele).

CCP13 visit to the Experimental Station at the DuPont R & D plant (July 19-20th 1998)

On July 19-20th 1998 Richard Denny and I (both wearing CCP13 hats) were invited to present seminars at the Experimental Station at the DuPont R & D plant near Wilmington, in Delaware. The visit was hosted by Kenn Gardner and Roger Leach of DuPont so that we could both present our work to the company and also heighten awareness amongst DuPont scientists of the CCP13 mission and of the software that is currently available in the CCP13 suite. Naturally the visit also allowed the two of us an opportunity to see the facilities that are available to research scientists working at DuPont. On the morning of the 20th July, Richard and I were shown around numerous laboratories devoted to structural work and in particular establishing the relationship between structure and physical properties using diffraction and other techniques. We also spent some time discussing with Kenn his own work on materials including the ether-ketone polymers, Kevlar, Nylon, as well as the unique properties of spider silk.

Richard's talk described the application of the CCP13 suite to typical (and non-typical!) fibre diffraction problems. He outlined the methods used in tackling these problems and the software that he had written to implement them. Included in this was a summary of the latest version of LSQINT and also of a recently developed version of F-XPLOR. F-XPLOR was derived from X-PLOR (Brünger *et al.*, *Science* (1987) **235**, 458) and was modified by Hong Wang and Gerald Stubbs (Vanderbilt) to cater for continuous fibre diffraction. Richard's modification now allows the treatment of polycrystalline diffraction data (see Denny, Shotton & Forsyth in last year's edition of *Fibre Diffraction Review*).
In my own talk I gave a description of the application of both X-ray and neutron fibre diffraction techniques in the study of nucleic acids, of the analytical methods used, and of the results that have been obtained. Part of this work illustrates the advantages that arise from availability of specifically and non-specifically deuterated polymer analogues in neutron diffraction. Although nucleic acids are somewhat outside DuPont's mainstream interests, the methodology that was used in this work is of general interest for structural studies of synthetic polymers as well as biological polymers. The isotopic replacement methods that have been used in neutron diffraction studies of DNA hydration can also be used to study the location of aromatic groups and of isolated hydrogen atoms in a wide variety of industrial polymers. Later on the three of us were able to discuss future collaborations in this area and it is pleasing that the first neutron experiments based on this collaboration have just been scheduled.

Before we left for Washington, Kenn treated us to a meal at his favourite restaurant. We ended up eating a large quantity of quite ferocious looking (although fortunately dead) crabs. You thought fibre diffraction was difficult? Just try eating a crab from first principles! There can have been few more entertaining sights than the two of us trying to work out the edible parts of the crustacean anatomy. I am by no means certain that I got it right.....

Trevor Forsyth
Institut Laue-Langevin & Keele University

7th Annual Fibre Diffraction/NCD Workshop

The 1998 joint Collaborative Computational Project for Fibre Diffraction and Non-Crystalline Diffraction Workshop was again held at CLRC Daresbury Laboratory from 12th - 14th May.

The first talk by Keiichi Namba (Matsushita Electric Industrial Co., Ltd.) got the meeting off to a fascinating start. Keiichi described the techniques involved in producing specimens of bacterial flagellum oriented to better than a spread of 1°, involving stages of liquid crystallisation, slow centrifugation and magnetic orientation. The excellent fibre diffraction data collected from these samples combined with phases from electron

microscopy gave electron density maps to 9Å resolution which proved crucial to the understanding of the conformational switching in the filaments which takes place when bacteria cease to swim and start to tumble. Manolis Pantos (CLRC Daresbury Laboratory) then gave a description of a genetic algorithm used to determine the shapes of proteins from solution scattering data. Ian Hamley (Leeds) described the effect of shear on block copolymer gels and Kenn Gardner (DuPont CR&D) gave an account of the difficulties encountered processing fibre diffraction data from the triclinic cell of polyethylene terephthalate. Wim Bras (ESRF) rounded off the first session of talks by reporting on the canSAS workshop on small-angle scattering data file formats held in February at the ILL. The evening's poster session was accompanied by sherry and a buffet dinner, with hands-on demonstrations of software on three Sun workstations provided by Esteem Computers PLC.

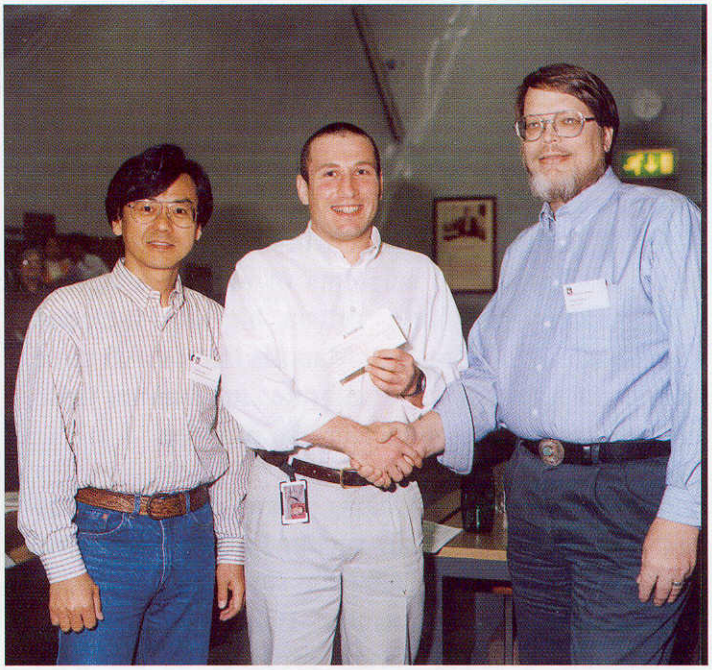
Wim Bras enlivened the Wednesday morning audience with a description of a SAXS/WAXS beamline being built at the ESRF by a Dutch-Belgian consortium. In particular, Wim described the design of a new detector capable of coping with the extremely high count rates expected on his station. Nick Terrill (CLRC Daresbury Laboratory) described plans for creating a variety of sample environments on NCD stations and the design of an automatic sample changer. He also announced the incorporation of station 16.2 into the NCD group and solicited for proposals from those interested in the structures of surfaces and interfaces. Richard Denny (CLRC Daresbury Laboratory) concluded the first session by introducing some modifications made to Axel Brünger's X-PLOR program. The modifications, following on from the work of Gerald Stubbs and Hong Wang, allow the program to be used for optimising molecular models against polycrystalline fibre diffraction data.

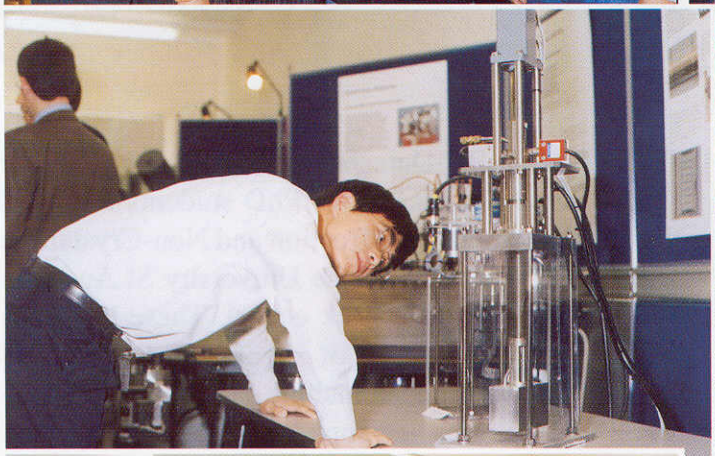
Geoff Mitchell (Reading) began the afternoon by describing novel equipment which allows time-resolved X-ray scattering measurements to be performed on samples subjected to shear flow and explained the power of this technique to illuminate the complex flow behaviour of liquid crystal polymer systems. Trevor Forsyth (Keele) highlighted the uses of neutron fibre diffraction experiments with particular reference to instrument D19 at the ILL, illustrating the technique with examples from his own work on ordered water around the DNA double-helix. Boris Shekunov (Bradford) described studies of the structural transition phenomena in a hydrogel saturated with an active compound using SAXS/WAXS techniques at the SRS. The experiments showed the pronounced influence of drug concentrations on the mechanical properties of the hydrogel.

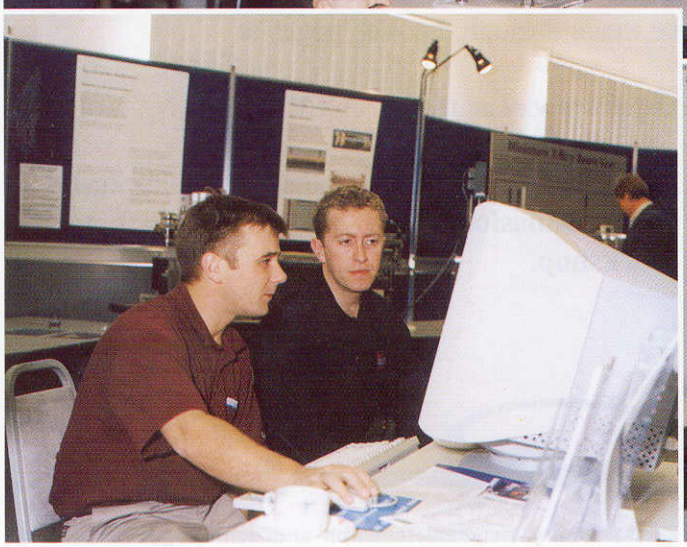
After lunch, Kell Mortensen (Risø) described experiments on triblock copolymers where the solvent is matched to the middle block. Kell explained how the microscopic structure and macroscopic mechanical behaviour can be correlated using simultaneous rheometric and neutron scattering measurements. The power of neutron diffraction was further reinforced in the minds of the audience by Steve King (CLRC Rutherford Appleton Laboratory) who described the recent upgrade to the SANS time-of-flight instrument LOQ at ISIS.

The final session of the day commenced with Andy Hammersley (ESRF), who stressed the importance of performing distortion corrections on data collected with area detectors. Andy described how his program FIT2D can be used to correct spatial and intensity distortions and discussed some of the many graphical and analytical tools available therein. Anne Terry (Bristol) discussed the properties of surfactant liquid crystalline phases under the effects of flow and Tony Ryan (Sheffield) concluded the talks for this session by instructing us on how to make molecular hairpins from rings and chains. The CCP13/NCD business meeting followed in which several retirements from the CCP13 steering committee were announced. John Squire stated his intention not to stand for re-election as chairperson in 1999 and put forward the committee's recommended candidate, Trevor Forsyth as his replacement. The assembled masses seemed content to be guided by the committee's suggestion. John also announced that Richard Denny would be resigning as research assistant to take up a permanent position at Daresbury. The committee had concluded that a sensible re-arrangement would be for Geoff Mant to step down as secretary but take up a position as a co-opted member in his role as the NCD group software engineer. Richard Denny could then take over as secretary with Mark Shotton from Keele taking over as research assistant for the remainder of the period of the current grant, thereby ensuring some continuity in the development of the software suite. Again those present allowed the committee's view to hold sway, possibly bewildered by the intricate machinations of the committee and


Looking at Crystals








Light
Infrared
Microwaves

pre-occupied with the imminent arrival of the conference dinner.

The Daresbury Park Hotel again provided an excellent venue for the conference meal. After dinner, some impromptu presentations were made by Alvina Vazina from the Russian Academy of Sciences. The lucky recipients of awards allegedly sanctioned by President Yeltsin himself, included John Squire, Wim Bras and Manolis Pantos. All three retired to the bar to celebrate in manner also allegedly favoured by the Russian premier.

Randall Richards (Durham) began the Thursday morning session with an account of reflectometric small-angle neutron scattering studies of polymer "brushes". David Norman (CLRC Daresbury Laboratory) outlined recent progress at the SRS including a description of the planned upgrades and the status of DIAMOND. Günter Grossmann (CLRC Daresbury Laboratory) described the way in which a molecular envelope, determined by solution scattering measurements of proteins, could be used as a starting point for phase extension methods. After coffee, Patrick Fairclough (Sheffield) highlighted the use of station 16.2 at the SRS in studies of thin films of diblock copolymers and Neville Boden (Leeds) concluded the talks with a fascinating description of the self-assembly of peptides to form biopolymer tapes and their similarity to the fibrils found in Alzheimer's or Parkinson's disease.

John Squire closed the meeting by asking Kenn Gardner and Keiichi Namba to present cheques to the winners of the poster competition. Shao-Min Mai (Manchester) and Simon Turner (Sheffield) jointly accepted the award for the best poster in the polymer category while Richard Denny accepted a cheque on behalf of Jeff Harford (Imperial and King's College) for the best poster in the biology category.

Richard Denny

Photographs of the meeting on page 9:

Richard Denny gleefully accepts a cheque on behalf of Jeff Harford (now you know, Jeff). Siao-Min Mai and Simon Turner share a prize. A "hands-on" training session for CCP13 and NCD software takes place during the poster session.

Forthcoming meetings

8th Annual Fibre Diffraction and Non-Crystalline Diffraction Workshop

15-17 June 1999
University of St Andrews

For further information and registarion, see the web pages at

http://www.dl.ac.uk/SRS/CCP13

or contact
a.mutch@dl.ac.uk

DARTS bursaries

DARTS at CLRC Daresbury Laboratory has kindly funded several bursaries for PhD students to attend the 8th Annual Fibre Diffraction and Non-Crystalline Diffraction Workshop at the University St Andrews (see above and inside back cover). These bursaries will cover the cost of accommodation and registration and may include a contribution to travelling expenses. An application for a bursary can be made through the web pages at

http://www.dl.ac.uk/SRS/CCP13

All bursary applications must be accompanied by the submission of a poster abstract to the Workshop.

DARTS

Daresbury Analytical Research and Technology Service

A Profile of the CCP13 R.A., Dr Mark Shotton

Mark Shotton graduated in physics from Imperial College, University of London, in 1992, and then went on to spend 18 months as an accountant before coming to his senses and commencing postgraduate work in the Keele University Physics Department in 1994. His time at Keele was spent in the molecular biophysics group, studying DNA structure and hydration by complementary neutron and X-ray fibre diffraction, under the guidance of Dr Trevor Forsyth and Professor W. Fuller.

Mark's main research interest was in analysing the specific patterns of ordered water that stabilise the various conformations adopted by the DNA double helix. Ordered water networks surrounding DNA were identified through high-angle neutron fibre diffraction experiments conducted using instrument D19 at the Institut Laue-Langevin with a monochromatic beam of neutrons of wavelength ≈ 2.4Å (the D19 instrument is described in detail on pages 17 to 24). These experiments all utilised the ability to isotopically replace H_2O by D_2O in the sample, leading to decreased sample absorption and incoherent scattering and making use of the large coherent neutron scattering length of deuterium in order to image ordered water sites. The A and B conformations of natural DNA [1-3] and the A and D conformations of poly[d(A-T)].poly[d(A-T)] were all studied using this technique. Figures 3 and 4 on pages 20 and 21 show the ordered water sites identified around the A conformation of perdeuterated *E. Coli* DNA and the B conformation of hydrogenated calf thymus DNA.

Mark began work as the CCP13 R.A. in May 1998. A description of the software that has been developed since then is included on pages 40 to 44. Additionally, in response to increasing user demand, it has been possible to release Linux versions of all programs in the CCP13 suite and it is in Mark's brief to support Linux versions of all future CCP13 software. The news of software upgrades is e-mailed to the CCP13 bulletin board (to subscribe, send "subscribe ccp13bb" to majordomo@dl.ac.uk) and is included in the *Latest News* section of the new-look CCP13 web pages at

http://www.dl.ac.uk/SRS/CCP13.

If you encounter any difficulties with CCP13 software, or have any suggestions for possible improvements, please contact Mark by e-mail to m.shotton@dl.ac.uk, or phone (01925) 603626.

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Summary of Available CCP13/NCD Software

Program	Description
XOTOKO	1-D data manipulation
BSL	2-D data manipulation
V2A	vax to unix data conversion
A2V	unix to vax data conversion
OTCON	ascii to otoko data conversion
RECONV	otoko to ascii data conversion
TIFF2BSL	image plate (tiff) to bsl conversion
BSL2TIFF	bsl to tiff conversion
I2A	ieee to ansi data conversion (DEC only)
XCONV	file format conversion (GUI-driven)
XFIT	1-D fitting and plotting (GUI-driven)
XFIX	fibre pattern analysis (GUI-driven)
CONV	file format conversion (command line)
FTOREC	reciprocal space transformation
LSQINT	2-D integration and background fitting
CORFUNC	correlation function
SAMPLE	Fourier-Bessel smoothing
FDSCALE	scaling and merging of intensities
FD2BSL	intensity to bsl conversion

The tables list the currently distributed CCP13/NCD programs, available as executable modules. The dates refer to the last creation of the executable.

A LOQ2BSL conversion program, for ISIS neutron data to BSL format, is also available for Solaris platforms.

Program	Solaris 2.6	Irix 6.2	OSF 3.2	Linux
XOTOKO	28/11/97	30/05/96	29/04/97	-
BSL	02/05/97	21/03/97	27/04/97	-
V2A	19/05/95	-	-	-
A2V	19/05/95	-	-	-
OTCON	06/06/95	08/07/94	-	08/05/97
RECONV	06/06/95	31/10/94	-	08/05/97
TIFF2BSL	17/03/97	-	-	-
BSL2TIFF	21/03/97	-	-	-
I2A	n/a	n/a	29/04/97	02/05/97
XCONV	07/09/98	07/09/98	-	07/09/98 *
XFIT	10/07/98	10/07/98	10/07/98	10/07/98 *
XFIX	12/11/98	12/11/98	12/11/98	12/11/98 *
CONV	10/06/97	10/06/97	10/06/97	10/06/97
FTOREC	05/11/96	04/11/96	04/11/96	04/11/96
LSQINT	24/03/98	24/03/98	24/03/98	24/03/98
CORFUNC	26/10/95	26/10/95	-	26/10/95
SAMPLE	05/11/96	04/11/96	04/11/96	04/11/96
FDSCALE	05/11/96	04/11/96	04/11/96	04/11/96
FD2BSL	05/11/96	04/11/96	04/11/96	04/11/96

* These programs have been tested on Slackware 3.4, SuSE 5.3 and RedHat 5.0 distributions of Linux.

The mechanisms of self-assembly and polymorphic switching of the bacterial flagellar filament

K. Namba[1,2], K. Hasegawa[1], I. Yamashita[1], Y. Mimori-Kiyosue[1], H. Suzuki[2] and F. Vonderviszt[1,3]

1 International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd.
2 Protonic NanoMachine Project, ERATO, JST, Seika, Kyoto 619-0237, Japan.
3 Department of Physics, University of Veszprém, Veszprém H-8201, Hungary.

Introduction

Bacterial flagellum is a helical filament by means of which bacteria swim. Each filament is rotated by the motor at its base, working as a screw that propels the cell, but it is not simply a rigid propeller. In wild-type strains of Salmonella and Escherichia coli, the filament is normally in a left-handed supercoiled form and several of them form a bundle behind the cell when bacteria swim. But, the filament switches its supercoiled form into a right-handed one upon quick reversal of the motor rotation. This makes the filament bundle fall apart quickly and smoothly, enabling the cell to tumble for a fraction of a second. Alternative repeat of the straight swimming and tumbling plays an essential role in the tactic behavior of bacteria.

The filament is a tubular structure formed by helical assembly of single protein, flagellin. The supercoiling of the filament is thought to involve regular arrays of two distinct subunit conformations and/or packing [1,2] in a filament structure, whose mechanism is interesting in terms of conformational distinctness and adaptability of flagellin. To understand the mechanisms of self-assembly and polymorphism of the filament, X-ray fibre diffraction and electron cryomicroscopy (EM) have been used to analyze the structures of various straight filaments. This report describes how these two methods have been combined in a complementary way to deduce the structures of the filaments, and the molecular mechanism of polymorphic supercoiling based on the deduced structure.

Materials and Methods

Two types of the straight filaments, which have distinct helical symmetries called L and R-types, were isolated from two mutant strains of Salmonella typhimurium. Electron cryomicrographs of frozen hydrated filaments were collected by using an electron microscope equipped with a field emission electron source and a specimen stage that can be cooled down to 1.5 K with liquid helium (JEOL JEM3000SFF). Helical image analysis was carried out for selected images of the filaments that show sharp layer lines in their Fourier transforms. After correcting for the contrast transfer function, 15 to 20 filament images were aligned to one another and averaged to produce a three dimensional density map [3]. For X-ray fibre diffraction, we developed a new method to orient liquid crystalline sols of filamentous assemblies of macromolecules [4]. The method involves sequential steps of liquid crystallization, slow centrifugation, and magnetic orientation. The flagellar filaments were well aligned with their angular distribution of 0.6 degree. X-ray fibre diffraction patterns from such well oriented sols allowed us to measure the layer-line spacings, helical symmetries, and layer-line amplitudes very accurately. These amplitude data were combined with phases from the EM analysis and the phases were refined by the solvent flattening procedure to obtain an electron density map [5].

Results and Discussion

In the density maps obtained by cryoEM analyses, the L and R-type filaments were found to have very similar structure as expected from direct comparison of their X-ray diffraction patterns. Both filaments have a densely packed core region out to a radius of 60 Å with a central channel 30 Å in diameter. The outer parts of the subunits are well separated from each other, and there are two domains of vertical and horizontal extension in each subunit. The outer radii of the filaments are 115 Å. The core region is formed by a concentric double-tubular structure (the inner and outer tubes) connected by radial spoke-like features. The structures of two straight filaments, the L- and R-type, which are thought to represent two states of the flagellin subunit that coexist in supercoiled filament structures, show only a small difference in the subunit packing and orientation, and

no appreciable differences in the overall subunit shapes were observed. These indicate that the structural changes involved in polymorphism are very small. The helical symmetries and repeat distances of the two types of the filaments were accurately determined by X-ray fibre diffraction. The intersubunit distances along the 11-stranded protofilaments were calculated from these structural parameters. They are 52.7 Å and 51.9 Å for the L- and R-type filament, respectively. The L-type is longer than the R-type by 0.8 Å, which quantitatively explains observed curvatures of various supercoils based on a two-state subunit model.

The electron density map at 9 Å resolution obtained for the R-type straight filament shows detailed molecular features of the flagellin subunit within the core of the filament (Figure 1). Many rod-like densities are observed in the inner and outer tubes and are aligned almost parallel to the filament axis with lateral distances of about 10 Å. These densities most likely represent α-helices that are predicted in the amino acid sequence of both terminal regions. The structures of straight filaments reconstituted from flagellin fragments that were produced by removing 30 to 40 residues from both termini clearly show removal of the inner tube portion but almost

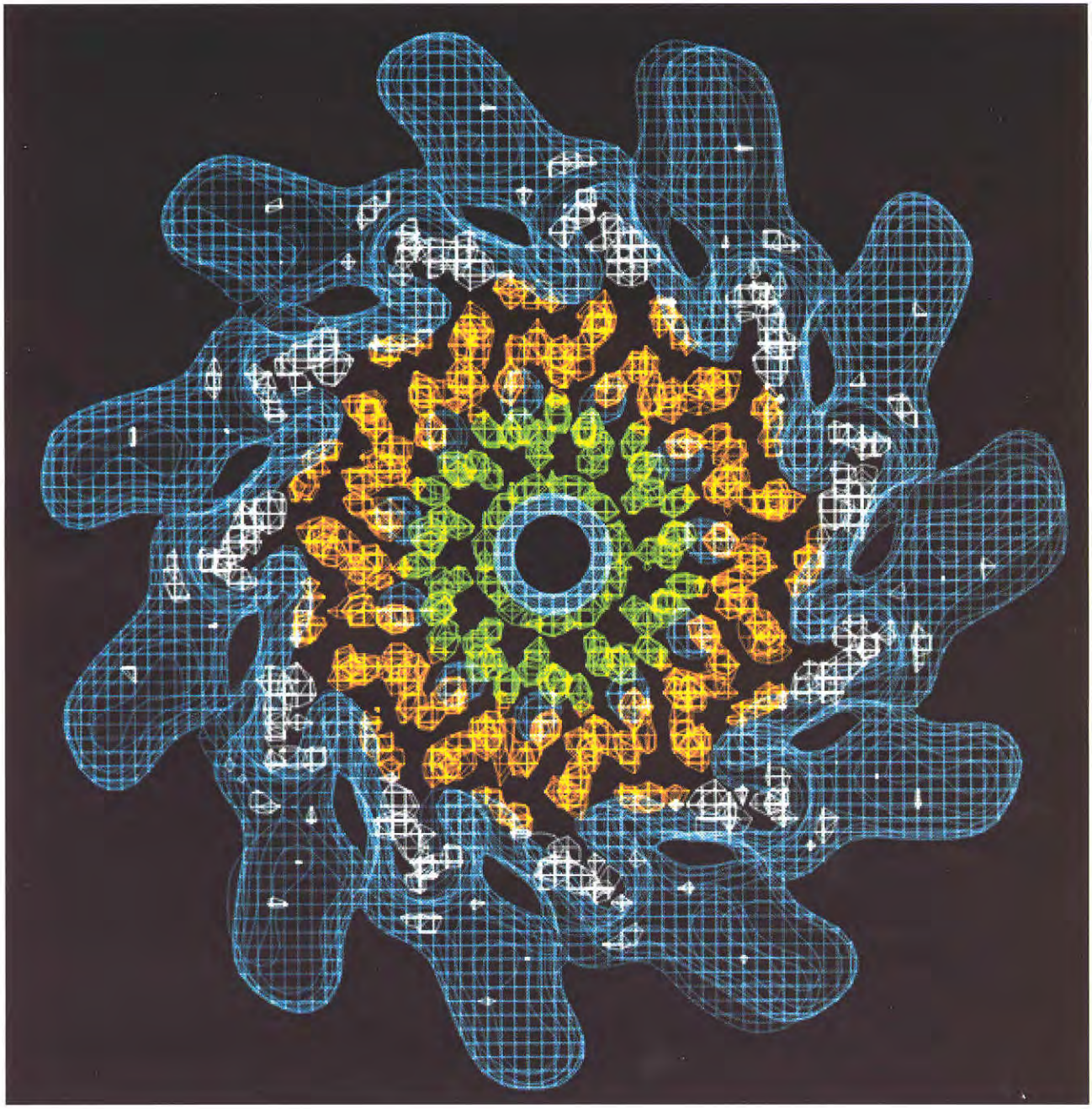

Figure 1: Electron density map of the R-type flagellar filament at 9 Å resolution. A cross section of a thickness of 50 Å is viewed down the axis. The smooth contours coloured white/blue represent the molecular envelope of the filament, and the detailed contours in green and brown colours are for the inner and outer tubes, respectively.

intact local subunit packing in the outer tube. This indicates that the intersubunit interactions in the outer tube are mainly responsible for filament formation and structural switching. The inner tube structure appears to be the base structure against which the subunit interactions in the outer-tube switch from one state to the other.

About 65 NH_2-terminal and 45 COOH-terminal residues are known to be in a flexible and disordered conformation in the monomeric form of flagellin in solution. Under physiological conditions, flagellin monomers alone do not form the filament; the structure of the distal end of the filament is required as a template on to which monomers assemble either in vivo or in vitro. Together with the location of the terminal regions in the filament structure, these can be interpreted such that the disordered terminal regions play essential roles in regulating the self-assembly process, preventing spontaneous filament formation in the absence of the distal end structure of the filament.

The observed difference of the local subunit lattice between the L and R-type and the axially aligned α-helices in the outer tube region indicates that the switching consists of two distinct mutual sliding movements of the subunits at the 11-stranded joints of the protofilaments: one of them, between subunits neighboring along the 6-start helix, is a sliding of 2.6 Å; the other, between subunits neighboring along the -5-start helix, is a sliding of 1.8 Å. These movements result in a shortening of the intersubunit distance along the strand joints by 0.8 Å upon switching from the L to the R-type lattice (Figure 2).

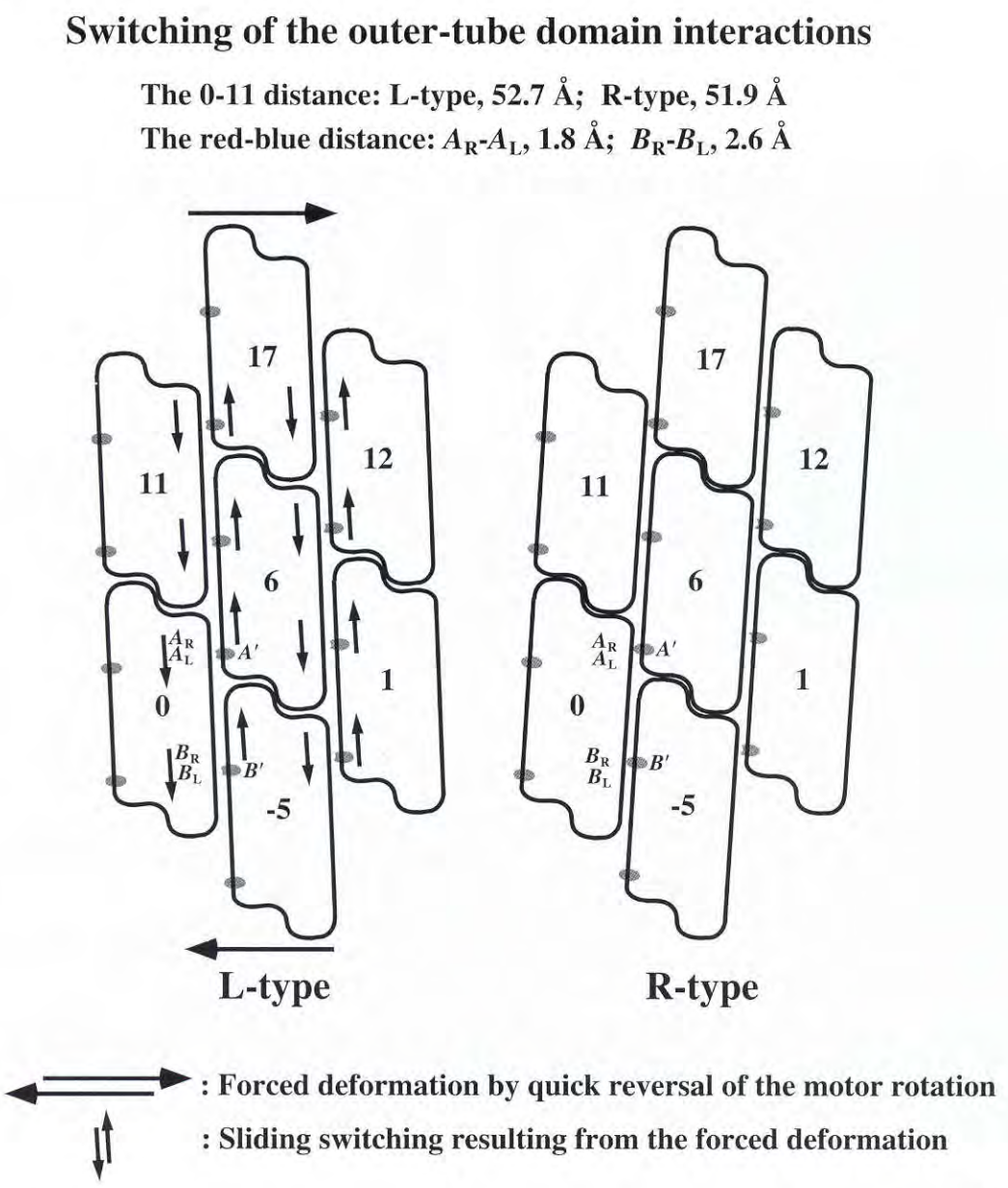

Figure 2: Model of switching at the strand joints explaining how the L- and R-type lattices are formed by the two distinct modes of subunit interactions.

A mutual coaxial disposition of the inner- and outer-tube lattices restrains the strand joint lattice to be twisted against the filament axis by a specific angle, resulting in a mismatch in the subunit interactions at a strand joint closing the tubular structure. Closure of the tube then requires a certain mixture of the two types of lattice, which in turn limits the curvature and twist of the filament to specific values associated with a specific type of supercoil. Thus, 10 distinct types of supercoil can be predicted using the lattice parameters of the L- and R-type straight filaments, and these predicted supercoils have curvatures and twists in good agreement with those of actual supercoiled filaments observed by dark field microscopy (Figure 3).

The twisting force produced at the base of the filament by quick reversal of the high speed motor rotation is converted by the strand lattice of the filament into a shear force at strand joints, and the shear force turns some of the L-state strand joints into the R-state, which results in a macroscopic switching of the supercoiled filament from a left handed one called normal to a right handed one called curly. This switching in the helical handedness makes the bundle of filaments fall apart and this is how the reversal of the motor rotation makes the cell tumble.

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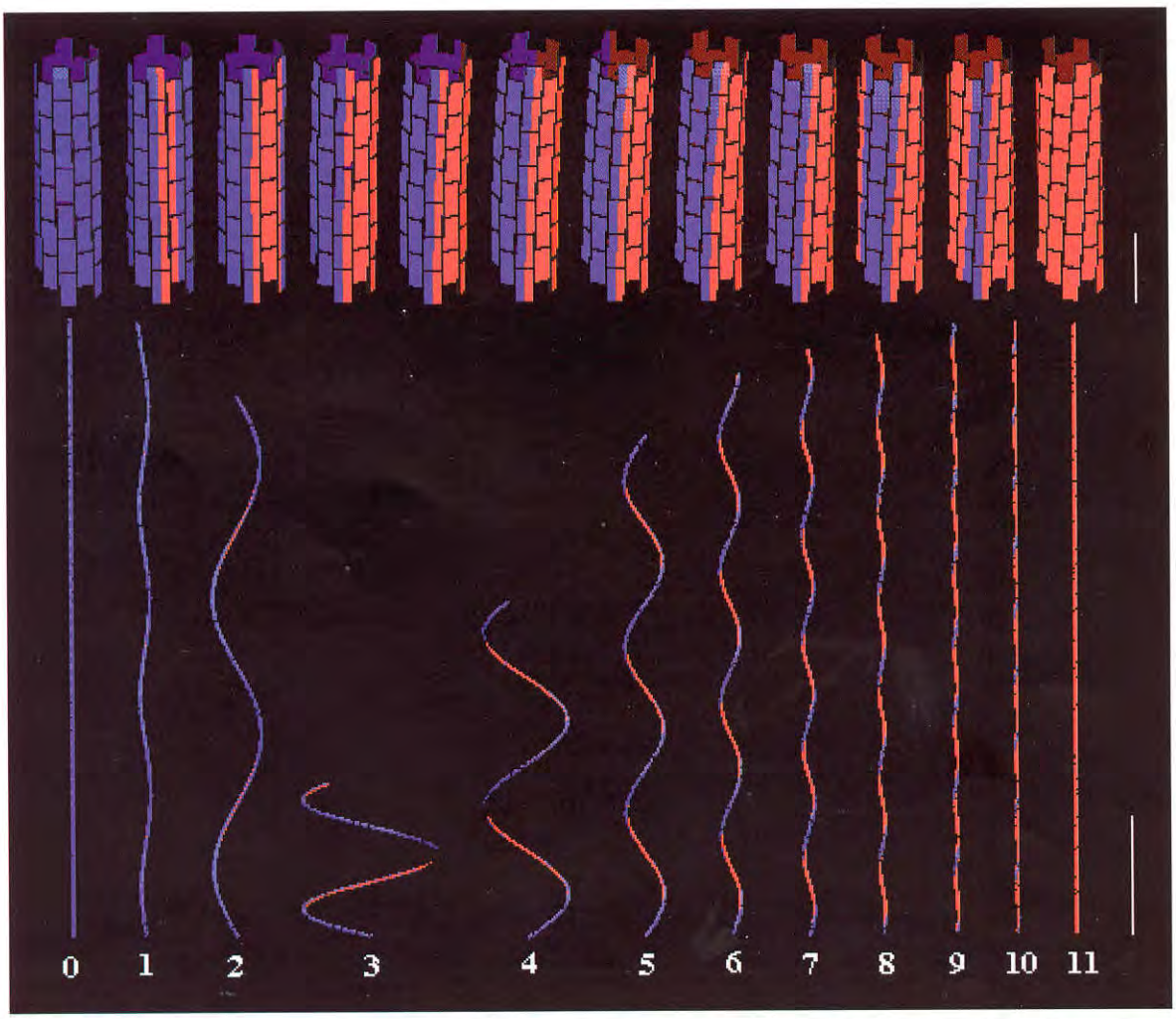


Figure 3: Supercoils predicted from the lattice parameters of the L- and R-type filaments obtained by X-ray fibre diffraction. The upper panel shows subunit lattices of a short filament segment and the lower panel shows the overall morphology of supercoils. The L- and R-type strand joints are coloured blue and red, respectively. The numbers at the bottom indicate the number of the R-type strand joints.

Instrument D19 at the Institut Laue-Langevin: A High Resolution Diffractometer for Single Crystal and Fibre Diffraction Studies

V.T.Forsyth[2,1], M.W. Shotton[1], H. Ye[1], C. Boote[1], P. Langan[2*], L.H. Pope[1], R.C. Denny[3]

1 Physics Department, Keele University, Staffordshire ST5 5BG, U.K.
2 Institut Laue-Langevin, Avenue des Martyrs, BP 156 F-38042, Grenoble Cedex 9, France.
3 Daresbury Laboratory, Warrington, Cheshire, WA4 4AD, U.K.
* Current adress: MS-M888, Life Science Division, Los Alamos National Laboratory, Los Alamos, NM 87545, U.S.A. (http://lsdo.lanl.gov/nsb)

Contact tforsyth@ill.fr for further information or see http://www.ill.fr/YellowBook/D19/help

The diffractometer D19 at the Institut Laue Langevin (ILL) is currently available to the fibre diffraction community for high-resolution neutron diffraction experiments. Such experiments offer a unique complementarity to X-ray diffraction studies and in the past the instrument has been used to carry out definitive structural work on nucleic acids, filamentous viruses, cellulose, hyaluronic acid and a number of industrial polymers. This review describes the configuration of the instrument and the facilities that are available for fibre diffraction users. These are illustrated by results that have been obtained using both hydrogenated and perdeuterated DNA. D19 is currently about to be upgraded to provide a large array of detectors to replace the single narrow detector currently available. This development and the huge impact that it will have for the scope and quality of neutron fibre diffraction work are discussed.

1. Introduction

Fibre diffraction is a powerful method of molecular structure analysis that has played a vital role in the determination of a large number of important biological and synthetic structures. These systems are typically ones that are either impossible to crystallise or ones where doubt about the significance of information from single crystal studies arises because of limited chain length or of packing effects.

X-ray diffraction has been and will remain a key method of structure determination in high resolution studies of both single crystals and fibres. However it should be noted that there is some information that is either difficult or impossible to obtain using X-ray diffraction methods alone. Hydrogen atoms and water positions are only reliably obtained by X-ray diffraction studies of biological macromolecules when the resolution of the study is very high (usually 1.2Å or less). This imposes a serious limitation since the vast majority of such crystals do not provide this sort of resolution and on occasions, attempts to locate these groups have led to serious errors. Such information is, however, absolutely crucial in understanding the activity and interactions of biological macromolecules where, for example, enzyme substrate interactions involving hydrogen atoms and water molecules play a vital role in biological function. Neutron diffraction studies allow such information to be obtained at lower resolution. Moreover, neutron crystallography has the unique ability to distinguish between hydrogen and deuterium exchanged positions in the crystal. Analysis of the pattern and extent of H/D exchange in an otherwise 'static' crystal structure can then provide a direct and elegant probe of group accessibility, of mobility and of exchange dynamics. Facilities that have been installed for neutron protein crystallography on the LADI diffractometer at the Institut Laue Langevin (ILL) are now starting to produce this type of information (Nimura *et al.*, 1997; Habash *et al.*, 1997; Langan *et al.*, 1999) and developments are also occurring at the LANSCE (Los Alamos) and Oak Ridge National Laboratory (ORNL) sources that are likely to yield important results in the future.

For fibre studies, the resolution obviously varies from one system to another, but a fairly typical diffraction pattern recorded from a sample having a moderately large unit cell would diffract to approximately 3Å. Attempts have been made to determine hydration structure in such systems using X-ray data alone but these studies have not proved decisive and the best that can usually be done is to optimise the modelling of the rest of the structure by attempting to make bulk water corrections to the recorded X-ray data. Although these corrections have been quite successful, they do not permit an analysis of the location of structured water. The availability of neutron diffraction data in such cases adds a powerful dimension to a fibre diffraction analysis. Neutron diffraction results obtained where the solvent is H_2O are of little analytical value on their own since H_2O (with two hydrogens having a

negative scattering length and an oxygen with a large positive one) is essentially self cancelling and barely visible in density maps. However, it is usually possible to record data from a system in which all the water around the molecule has been replaced by D_2O, and this can be used to provide a strong image of the location of structured water (Forsyth *et al.*, 1989; Langan *et al.*, 1992; Shotton *et al.*, 1997). The principle behind this work is that the large difference in the neutron scattering powers of the two isotopes results in clearly significant changes in the observed diffraction patterns that can be used to image the location and occupancy of ordered water around the double helix by Fourier synthesis and difference Fourier methods. Furthermore, if it is possible to produce samples in which the hydrogen atoms that are covalently linked to carbons are replaced by deuterium, a very large increase in data quality can be obtained by minimising the level of hydrogen spin incoherence that contributes to the background of the diffraction pattern (Shotton *et al.*, 1997). If such substitutions can be made selectively then it is straightforward to devise experiments aimed at determining the location of specific parts of the molecular structure.

2. The D19 diffractometer

The Keele fibre diffraction group developed high-angle neutron fibre diffraction as a method that proved to be well suited to the investigation of the location of water around polymeric DNA and other fibrous systems. Experiments of this type require an area detector that would ideally allow all available diffraction data to be recorded in one exposure. Although such a system does not currently exist, the D19 diffractometer allows the collection of fibre diffraction datasets as a series of (4° x 64°) 'strips' that can be merged together to form a continuous image that can be processed using CCP13 routines in the normal way. Figure 1 shows a picture of the experimental arrangement on D19.

All of the software that is required for processing D19 datasets is available under the CCP13 suite. The program ILLD19 (Shotton and Forsyth, unpublished) contains routines that allow the assembly and correction of the recorded data that can then be measured using LSQINT (Denny *et al.*, in preparation). Fourier maps can be generated using any of a number of programs such as O (Jones *et al.*, 1991) or XtalView (McRee, 1992). Routines are also available that allow the refinement of position and occupancy parameters derived from the density maps.

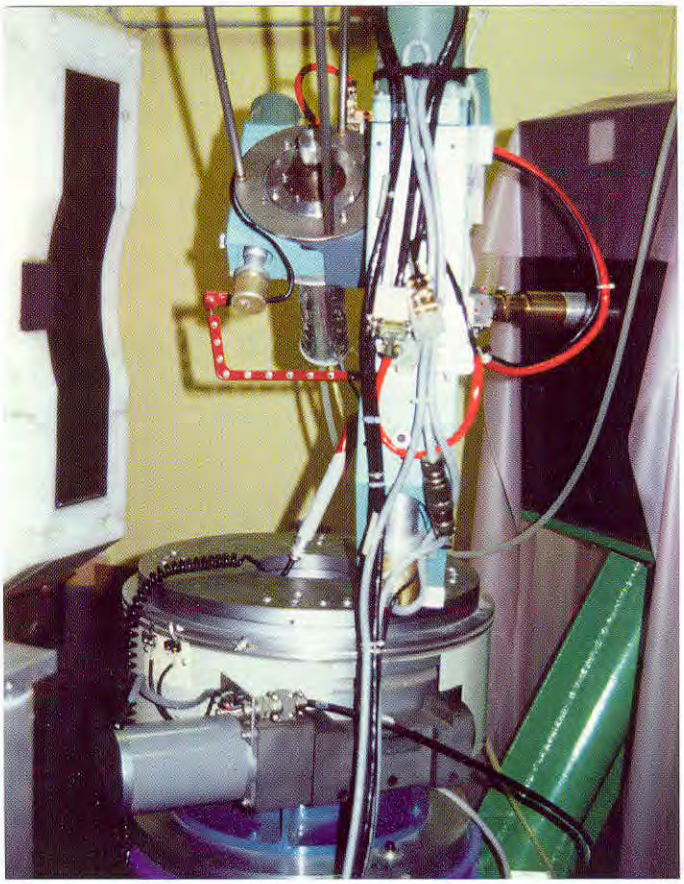
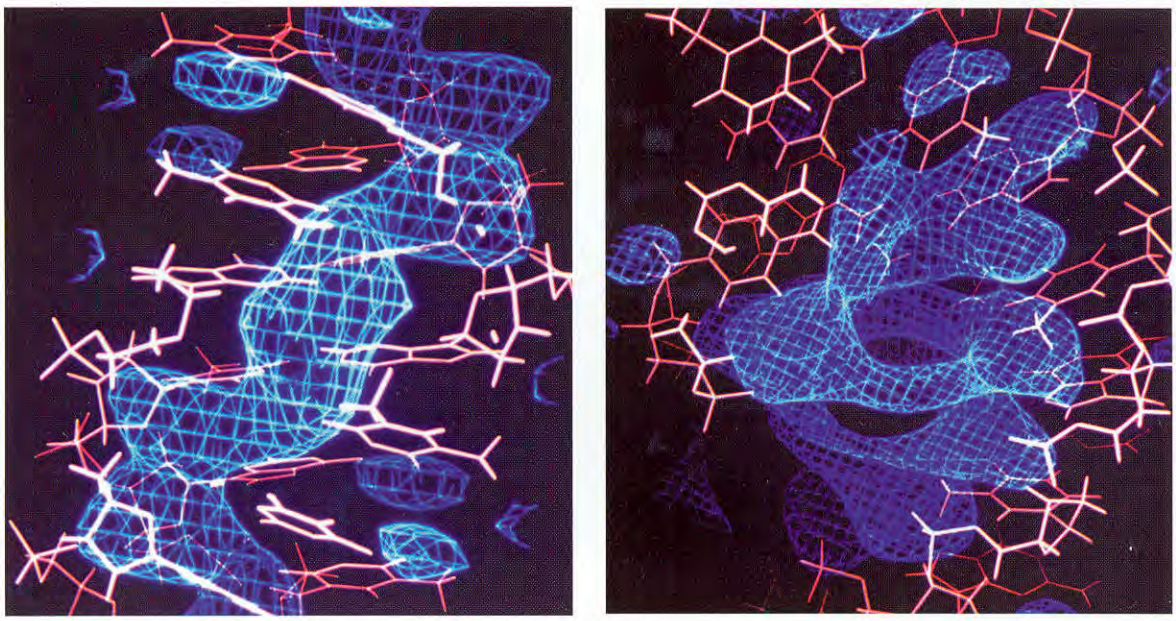


Figure 1: The D19 diffractometer. The beam enters at the right of the picture; the detector is visible at the left. The sample enclosure (in this case a humidity cell) is located at the centre of the Eulerian cradle (seen more or less edge-on in this picture). Good sample environment facilities exist at the ILL so that in addition to a humidity-controlled environment it is possible to perform experiments over a wide range of temperatures and pressures.

3. The first high-angle neutron fibre diffraction studies at the ILL

In our first combined neutron and X-ray high-angle fibre diffraction experiments, we studied the location of ordered water around the D form of poly[d(A-T)].poly[d(A-T)] (Forsyth *et al.*, 1989). This study revealed well-defined regions of water associated with both grooves of the double helix and in stereochemically reasonable positions relative to the DNA and alkali metal ions that were positioned on the basis of the X-ray isomorphous replacement experiments (Forsyth *et al.*, 1990). In later experiments, similar methods were used to study the A form of natural DNA from E. Coli. The neutron Fourier difference maps showed a water distribution with 11-fold helical symmetry following that of the DNA (Langan *et al.*, 1992). The main interactions



(a) (b)

Figure 2: Fourier difference synthesis images of **(a)** D-DNA (Forsyth *et al.*, 1989) showing minor groove hydration and **(b)** A-DNA showing major groove hydration (Langan *et al.*, 1992).

observed in this analysis were water molecules linking charged O1 oxygens in successive phosphate groups along each strand of the DNA and water molecules deep in the major groove of the DNA which, because of the large base displacement, forms a core located on the helix axis. X-ray isomorphous replacement studies of A-DNA yielded two types of cation sites: one which zig-zags across the opening of the major groove between charged phosphates on opposite strands and a second family running down the centre of the groove.

4. The location of water around the A conformation of deuterated DNA

More recently, instrument D19 has been used to study the A conformation of deuterated DNA. This experiment was based around the use of deuterated DNA that had been obtained by bacterial culture during the course of an EMBO fellowship (to VTF) held at the EMBL Outstation in Grenoble. In common with our previous neutron fibre diffraction studies of DNA, this work exploited the ability to isotopically replace H_2O around the DNA by D_2O. However, this study benefitted additionally from the fact that the hydrogen atoms that are covalently bonded to carbon atoms in the DNA sugars and bases were replaced by deuterium, so that incoherent scattering and absorption effects were minimised. Successive cycles of Fourier synthesis and Fourier difference synthesis allowed water peaks to be identified and their positional and occupancy parameters refined against the observed diffraction data using the downhill simplex method of Nelder and Mead (1965). The results confirmed the main hydration features noted in our earlier studies with a clear network of water running along the inside edge of the major groove linking successive O1 phosphate oxygen atoms. However, the central core running along the axis of the double helix was very much clearer in this work; additionally this study showed chains of ordered water lying in the centre of the major groove.

These results have been described in detail (Shotton *et al.* (1997), Pope *et al.* (1998)) who considered the four water sites in terms of possible hydrogen bonding interactions with the DNA. Since random sequence DNA was used for this, sequence dependent features were averaged and would be expected to be observed at lower than unit occupancy. The occupancy of site 1 suggests that the intrastrand phosphate O1 bridges may also participate in sequence dependent interactions with the major groove base edge atoms. Site 2 is likely to be independent of base pair sequence and may be associated with a water-cationic network involved in the interstrand bridging of backbone oxygen atoms. The distances between successive site 3 positions and between site 3 and site 1 positions are within the

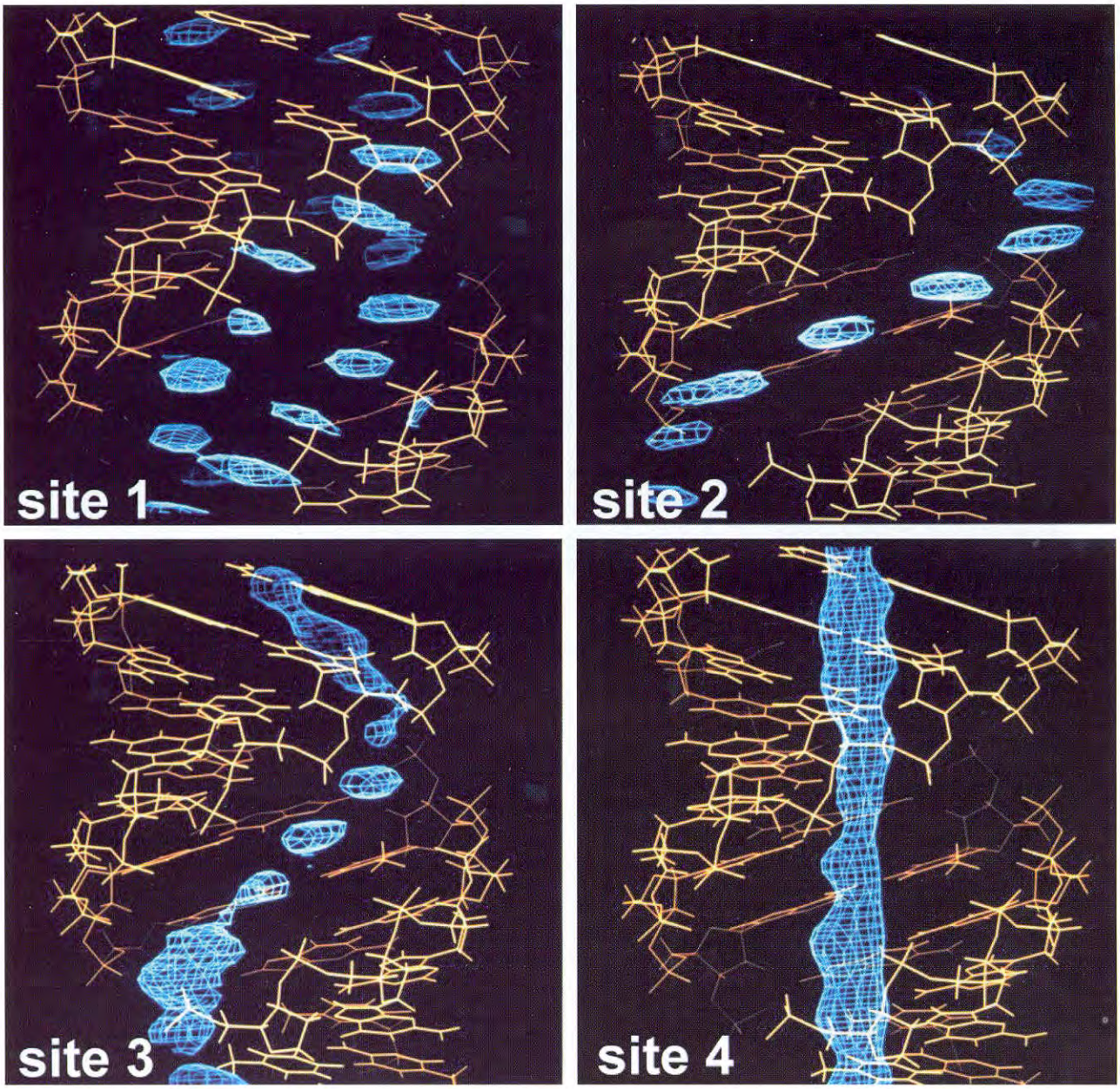
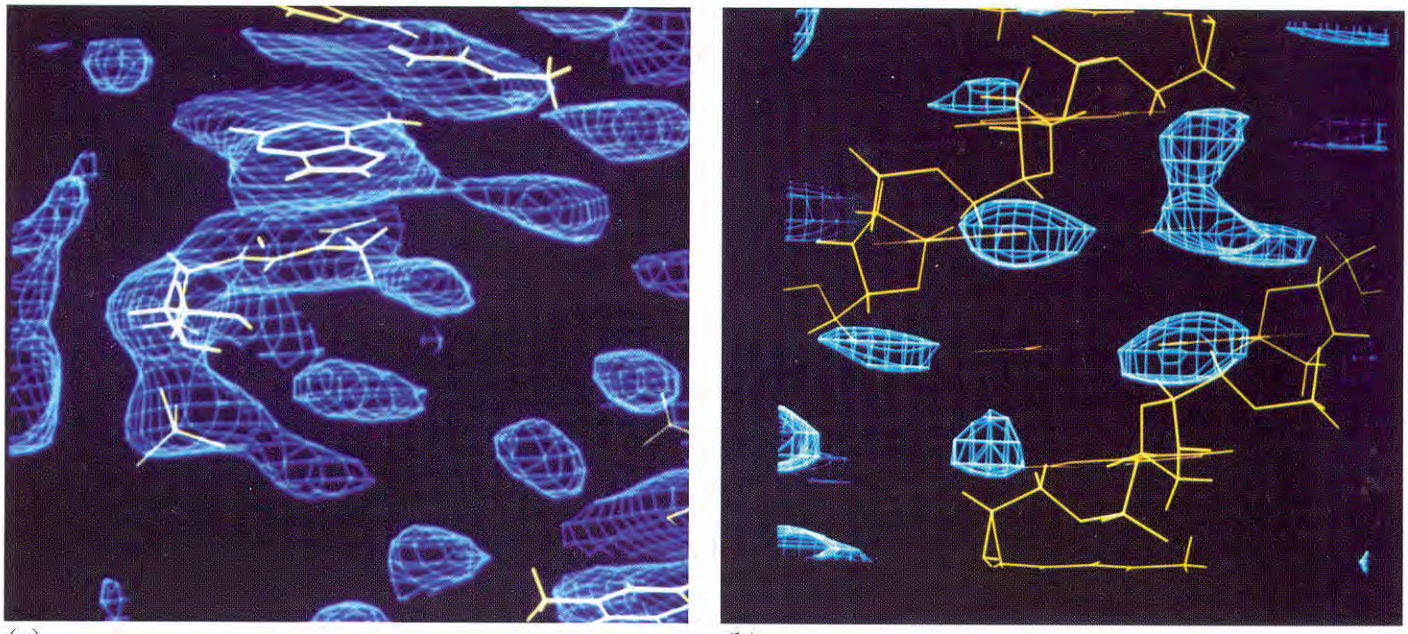


Figure 3: The four ordered water networks surrounding the A-DNA double helix (Shotton *et al.*, 1997).

range of hydrogen bonding interactions. Site 4, the continuous core of density, is within hydrogen bonding distance of adenine NH_2, thymine O4, cytosine NH_2 and guanine O6 in the major groove. Due to the sequence averaging within this dataset it was not possible to determine the exact nature of this water network. Work involving fibres prepared from DNA of repetitive sequence such as poly[d(A-T)].poly[d(A-T)], poly[d(G-C)].poly[d(G-C)] and poly[d(G)].poly[d(C)] is currently in progress and should enable sequence dependent features to be elucidated.

5. Neutron fibre diffraction studies of wet-spun sheet samples of DNA

Neutron fibre diffraction studies of the hydration of the B and the A conformations of DNA were performed on instrument D19 using wet-spun sheet samples of DNA prepared by Rupprecht (1970). These samples were of a size that allowed the collection of data with good counting statistics and a shape that enabled simple procedures to account for effective absorption due particularly to the presence of hydrogen in the sample. However, during these experiments, data collection and analysis methods were complicated by the fact that some of these samples have double orientation with crystallites being aligned not only in the direction of the fibre axis but also in a direction perpendicular to this axis. Such samples are not cylindrically averaged and data collection procedures have to accommodate the fact that the samples are in many ways analogous to a single crystal. Figure 4 shows Fourier maps



(a) (b)

Figure 4: **(a)** Fourier synthesis map obtained for the sheet samples of A-DNA; **(b)** Fourier difference synthesis obtained for the crystalline B conformation.

computed from data recorded from these samples (a) for the A conformation and (b) for the crystalline B conformation.

The results for the A-DNA sheet samples are fully consistent with those that have been obtained for hydrogenated and deuterated samples of A-DNA prepared using arrays of fibres, as can be seen from Figure 3(a) which clearly shows the water chain linking successive phosphates long the backbone inside the major groove. The results obtained for the crystalline B conformation (Shotton *et al.*, 1998) are consistent with those observed in single crystal X-ray diffraction studies of B-type oligomers in which a 'double ribbon' of hydration was identified in regions of relatively large minor groove width (Leonard *et al.*, 1993) and suggest that this is the dominant minor groove hydration feature in long polymeric natural DNA. There was also evidence in this study of two additional chains of peaks running along either side of the major groove, also possessing 10-fold screw symmetry. The ordered water in the major groove may interact with cations located in the centre of the groove as were observed in an X-ray fibre diffraction study of the location of caesium cations around B-DNA (Bartenev *et al.*, 1983) in which it was suggested that these cations were separated from the phosphate groups by a hydration layer one or two water molecules thick.

6. Neutron fibre diffraction studies of structural transitions

During neutron fibre diffraction experiments involving biological samples, the requirement for accurate and reproducible control of the relative humidity of the sample environment is critical. In the case of DNA work, both the conformation of the DNA and the degree of crystallinity of the sample are extremely sensitive to hydration and for 'static' experiments it is desirable to be able to control the humidity to within ~1% of a selected value. In order to optimise this, a programmable humidity control system was designed and built for us on D19 (Shotton & Langan, 1995; Shotton *et al.*, 1998). During trial experiments Langan (1997) subsequently showed that it is possible to "trap" conformational intermediates that occur in DNA during structural transitions. This led to the first full scale experiments of this type when D19 was used to record high angle fibre diffraction data at four different stages during the D to B structural transition (Figure 5).

The quality of the diffraction data recorded during this study was outstanding. The analysis of this work centres around the availability from previous work of (a) detailed information from time-resolved X-ray fibre diffraction studies of the D to B transition, and (b) detailed knowledge from high-angle neutron work of the location of water around the D

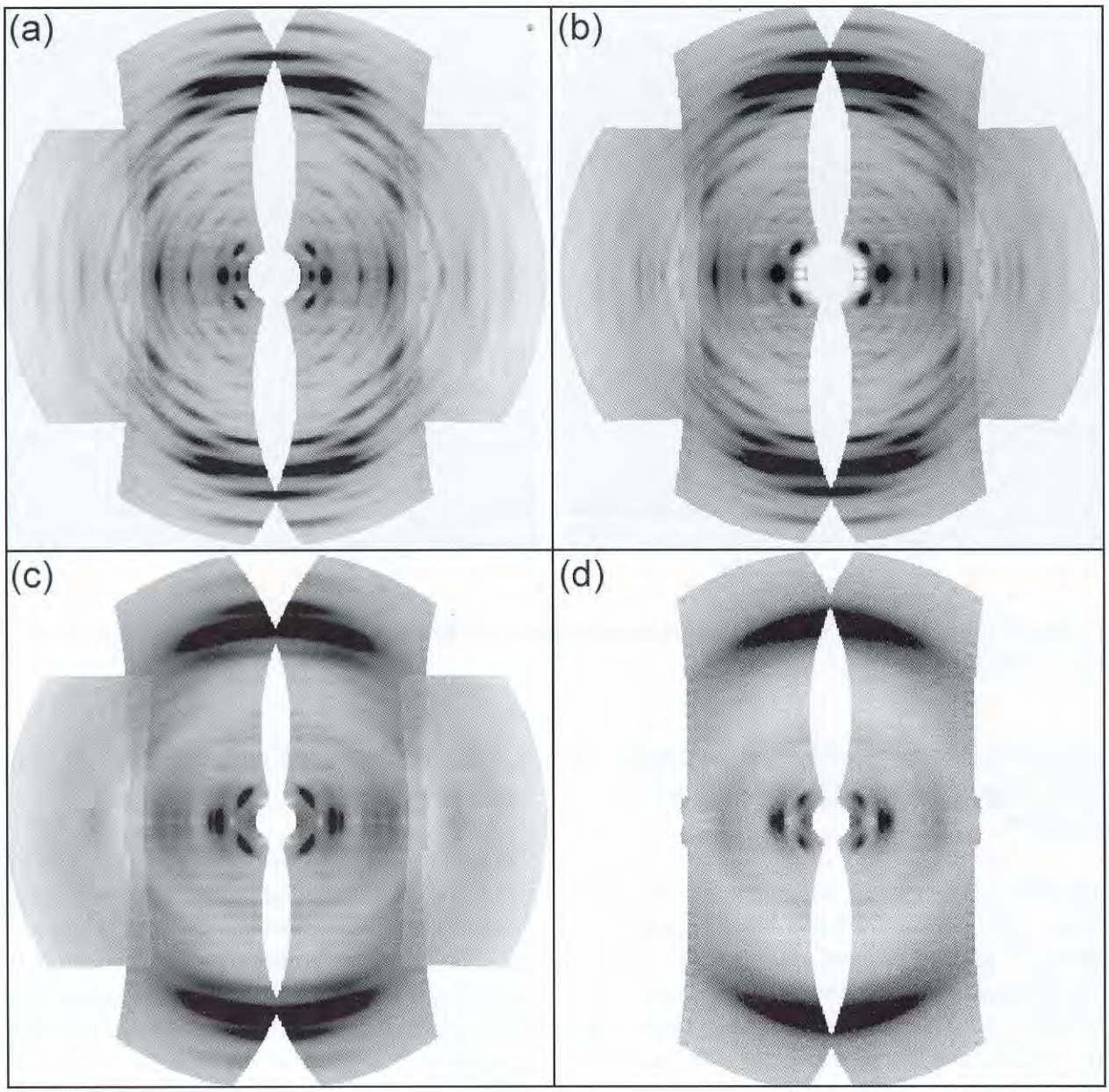


Figure 5: High angle neutron fibre diffraction patterns recorded during the D to B transition in the poly[d(A-T)].poly[d(A-T)] double helix at four relative humidities: (a) 58%, (b) 74%, (c) 78%, (d) 90%.

conformation [5,6] and the B conformation [26]. A two pronged approach involving both a Fourier analysis of the crystalline diffraction data and modelling of the continuous data is currently in progress. A version of X-PLOR that has been modified for fibre diffraction data is being used to refine the molecular structures generated during this analysis (Denny, 1998).

7. The upgrade of the D19 diffractometer

D19 is an excellent instrument and indeed there is no other instrument in the world that is capable of producing neutron fibre diffraction data of comparable quality. In addition to the work on nucleic acids that has been reviewed here the instrument has also been used to study filamentous bacteria (Mitsch, 1996), hyaluronic acid (Deriu *et al.*, 1997), cellulose (Langan *et al.*, 1996) and a range of polymers that are of industrial significance. Some of the more recent experiments on cellulose have produced exceptionally good datasets (Nishiyama *et al.*, 1999) - the results are currently being prepared for detailed publication (Langan *et al.*, in preparation).

However, in the current configuration, diffraction patterns are recorded as a series of 'strips' of data, each having angular dimensions of 64° x 4°. During a typical experiment, approximately 20 such slices may be needed to cover the required region of reciprocal space which means that at any given instant in time approximately 95% of the diffraction pattern is unrecorded. This problem is illustrated in Figure 6 which shows a comparison of neutron fibre

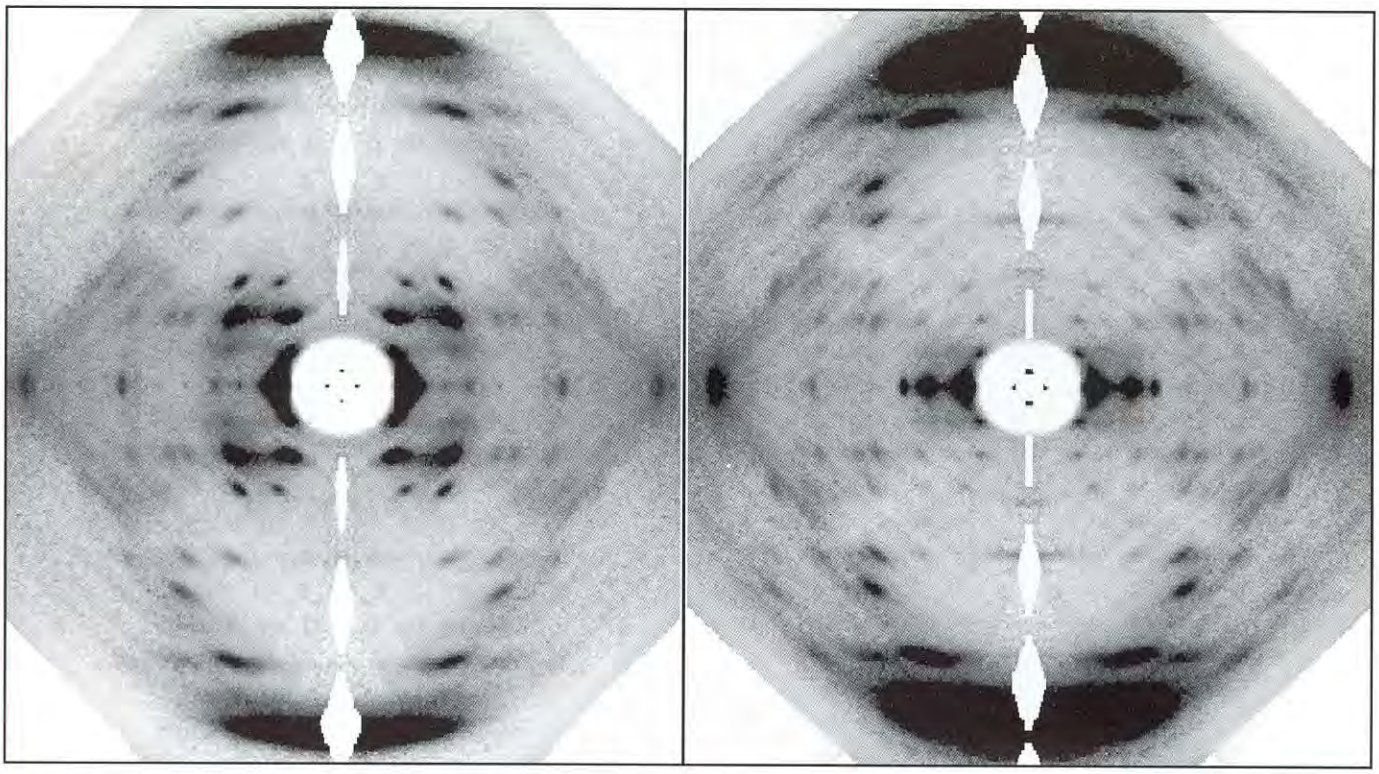


Figure 6: Neutron fibre diffraction patterns recorded from the crystalline B conformation of calf thymus DNA (**a**) when the sample was hydrated with H_2O and (**b**) when the sample was hydrated with D_2O.

diffraction data recorded from the lithium salt of B-DNA (a) hydrated with H_2O and (b) hydrated with D_2O. It is possible to see the individual detector strips making up the image, thus clearly emphasising the ratio between the amount of data required for an experiment and the amount that is recorded in a single strip.

It is very clear therefore that a massive improvement is available in terms of data quality, throughput of experiments on the instrument, and also in terms of the types of project that can be tackled using this instrument. To address this, it is planned that D19 is upgraded with an array of area detectors as illustrated in Figure 7.

It is estimated that this development will produce a factor of between 15 and 20 in data collection efficiency and that this will have a very substantial effect on the quality, scope and throughput of neutron fibre diffraction experiments at the ILL.

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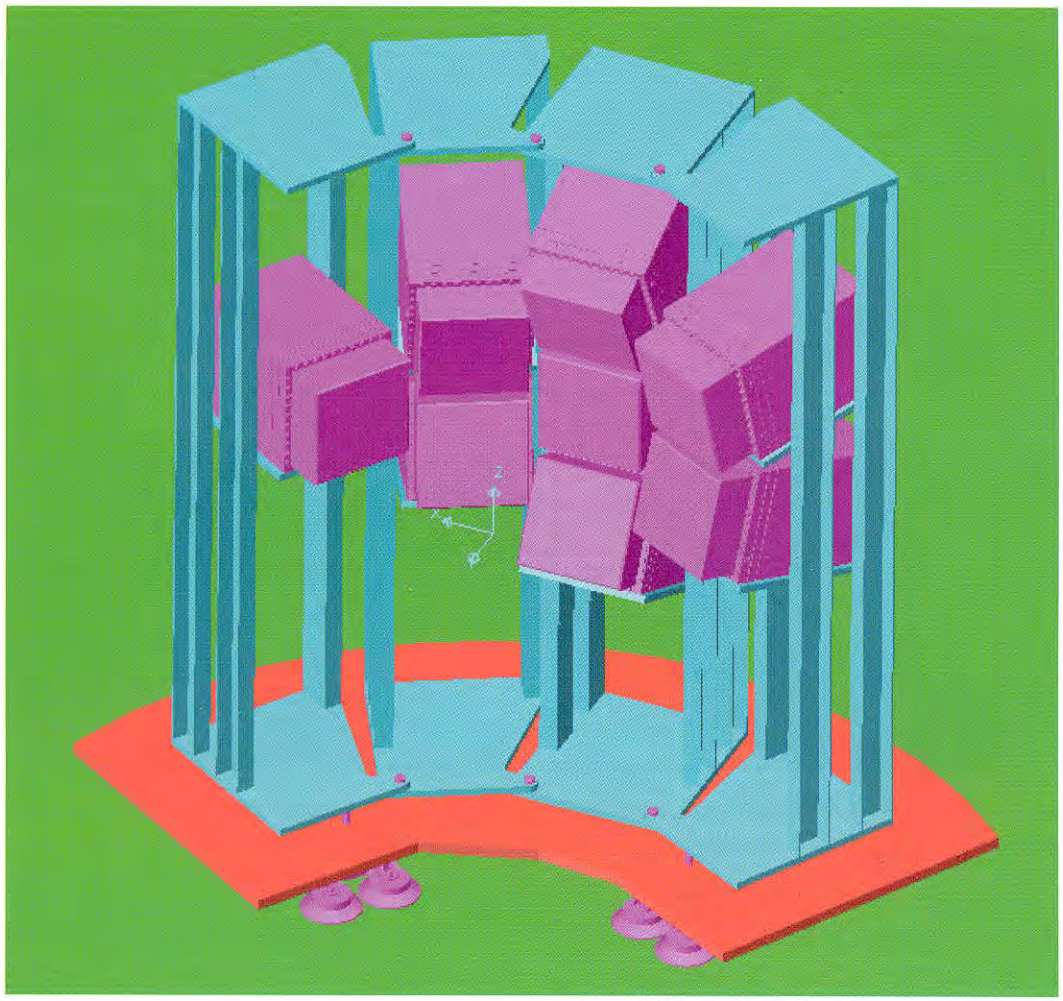

Figure 7: The array of area detectors that is planned as part of the D19 upgrade. Each individual detector will capture an angular range of 18.2° x 18.2°.

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Block copolymer micelles, micellar networks and mesophases

K. Mortensen

Condensed Matter Physics and Chemistry Department, Risø National Laboratory, DK-4000 Roskilde, Denmark.
E-mail: kell.mortensen@risoe.dk

Block copolymers aggregate in selective solvents into micelles of various form and size depending on molecular architecture and interaction parameters. The micelles constitute the basis for a variety of novel mesophases, including bicontinuous phases and networks of ordered micelles. Small-angle scattering of X-rays and neutrons are among the most central tools to investigate the structural properties, and thereby thermodynamics and dynamics of such complex block copolymer micellar systems.

Introduction

The physical properties of amphiphilic macromolecules constitute a rich topic which in recent years has attracted interest within both applied and basic science [1-7].

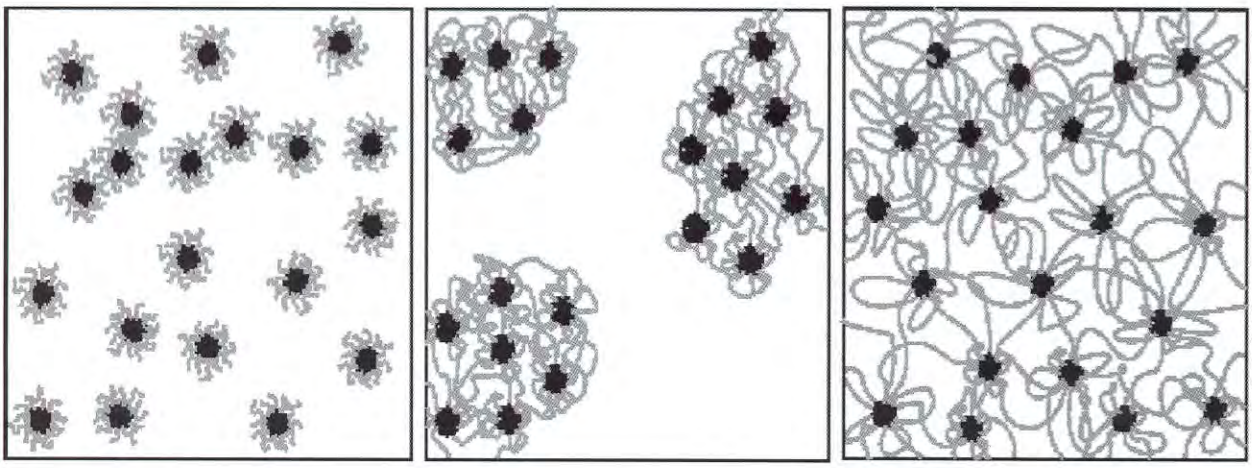
When block-copolymers are mixed in a solvent which dissolves only one of the blocks, the molecules self-associate into specific structures to avoid direct contact between solvent and the blocks which are insoluble. This self-association gives rise to a wide range of phase behavior, including the formation of micelles of various form and size, complex structured microemulsions, and liquid crystalline phases. A variety of block copolymers, have in this context been studied when dissolved in selective solvents of both polar and non-polar type. In aqueous systems, particular interests have concerned block copolymers based on poly(ethylene oxide), PEO, as the water soluble block.

Block copolymer self-association into micellar aggregates

It is well established that di- and triblock copolymers of AB or ABA type, respectively, typically form micellar aggregates in solvents which are thermodynamically good for the A-block and precipitants for the B-block. Such micelles constitute a liquid suspension of hard sphere interacting units, as illustrated in Figure 1a. Triblock copolymers of BAB architectures may also form individual micelles, but this implies that all polymer chains start and end in the same micelle having the middle A-block dispersed into the liquid, the so-called flower type micelles. More likely, such micelles form interconnected networks, where cores are connected by the soluble A-polymer block, as shown schematically in Figure 1b and c.

Critical micellization temperature and concentration

In general, micellization of block copolymers assumes equilibrium between molecularly dispersed copolymers (unimers) and multi-molecular aggregates (micelles). Aqueous systems of block



(a) (b) (c)

Figure 1: Schematic representation of spherical micelles. **(a)** Micelles of AB or ABA type of block copolymers, resulting in independent hard-sphere interacting aggregates. **(b)** and **(c)** Micelles of BAB type of block copolymers, resulting in domains of interconnected networks of spherical aggregates.

copolymer composed of PEO with either PPO or PBO constitute very good model systems for studying the micellization process and micellar interactions. At low temperature all these polymers are hydrophilic, while at higher temperatures PPO and PBO become hydrophobic.

PEO-PPO-PEO and PEO-PBO-PEO copolymers therefore appear as unimers when mixed with water at low temperatures. Structural studies based on neutron scattering [5] indicate, however, that the unimers have the form of uni-molecular micelles presumably with the PPO-blocks at the centre, rather than Gaussian chains. The temperature-induced change in hydrophobicity leads to a temperature above which micellar aggregates are formed with a core dominated by PPO (respectively PBO) and surrounded by a corona of hydrated PEO sub-chains. Depending on the molecular architecture and the interaction parameters, various micellar forms can appear, including spherical, rod-like and disc shaped. In the PEO-PPO-PEO-systems, thermodynamic changes from spherical to rod-like, discs and possibly bicontinuous microemulsion [8] can be induced by changing temperature or concentration.

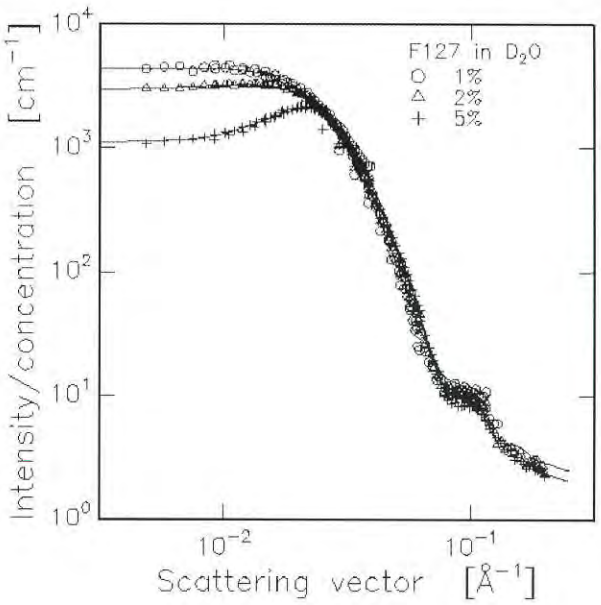


Figure 2: Examples of scattering functions, $I(q)$, of different concentrations of spherical ABA triblock copolymer micelles ($EO_{97}PO_{65}EO_{97}$) obtained at T=35°C. The solid lines represent the best fit of micellar form factor and structure factor with a hard-sphere interacting potential.

Typical scattering functions of $EO_{99}PO_{65}EO_{99}$-micelles are shown in Figure 2 [9]. The characteritics of the scattering functions are the concentration-dependent correlation hole at low-q (q being the scattering vector), the side maximum near q=0.1Å^{-1} and the limiting I~q^{-2} behavior at high q-values, reflecting to first order respectively the inter-micellar correlations, the micellar core and the dispersed PEO-chains.

The micellar form factor can be expressed analytically assuming a dense core and Gaussian chains in the corona [10]. The solid lines in Figure 2 represent best fits to this formula, including instrumental smearing and inter-micellar correlations based on hard sphere interactions in the Percus-Yevick approximations [11].

The spherical micellar conformations have been confirmed by direct imaging, using cryo-TEM [9]. The micellar characteristics as obtained from SANS may also be compared to the hydrodynamic radius, R_h, as obtained using dynamic light scattering [12,13]. Generally, the hydrodynamic radius is larger than the core and smaller than the interaction-radius.

One of the important parameters obtained from the experimental scattering data and other indirect techniques is the micellar volume fraction, ϕ. Figure 3 shows a contour plot of ϕ-data of $EO_{25}PO_{40}EO_{25}$ [5,14]. The variation in ϕ separates into four regimes. At low temperatures and concentrations, all polymers are dissolved as the unimers. Above a line of critical micellization temperatures (T_{cm1}) and concentrations (cmc_1), a regime of coexisting micelles and unimers appears. The dispersion is totally dominated by micelles in the regime above T~30°C. For copolymer concentration more than approximately 20%, the micelle volume fraction reaches a limiting value of the order of $\phi_c \sim 0.53$. On crossing this $\phi_c \sim 0.53$ line, the suspension undergoes a transition from a low-T / low-concentration liquid to a high-T / high-concentration solid.

Micellar Size and Aggregation Number

The micellar core-radius, R_c, of PEO-PPO-PEO copolymers is roughly independent of copolymer concentration, but shows temperature dependence reflecting changes in aggregation number [5,15]. The change in micellar radius for different PEO-PPO-PEO copolymers shows very similar characteristics. The core size follows an empirical scaling relation

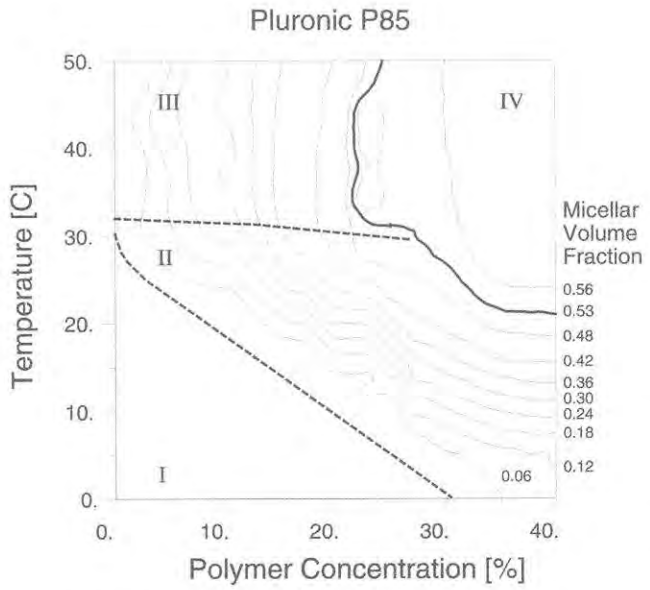


Figure 3: Temperature/polymer-concentration contour plot showing the experimental micellar volume fraction ϕ of aqueous solutions of $EO_{97}PO_{65}EO_{97}$. The solid line represents ϕ=0.53 separating the micellar liquid and cubic ordered phase. The other lines are guides separating the characteristic regimes of pure unimers (phase I); unimers + micelles (phase II); and spherical micelles (phase III (liquid) and phase IV (solid)).

relative to the reduced temperature: $R_c \sim(T\text{-}T_{cm1})^{0.2}$. The aggregation number N_{agg} can be calculated both from the core dimension and, independently of, based on the limiting micellar volume fraction, with very good agreement [16]. The values range from less than 10 to more than 200, depending on temperature.

Rod and worm-like micelles

Depending on the block copolymer design and depending on the specific interaction parameters between solvent and polymer blocks, other forms than spherical aggregates may occur. In the $EO_mPO_nEO_m$-copolymers it is possible thermodynamically to follow such transitions from spherical to rod and disc shapes by changing the temperature and/or changing the size of the PEO-blocks. At elevated temperatures, aqueous solutions of PEO-PPO-PEO show form-transformation from sphere to rod-like micelles [5,15]. The origin of the sphere-to-rod transition is related to the size of the spherical aggregates. Close to the sphere-to-rod transition the core-radius is large relative to the polymer backbone, resulting in either highly stretched PPO-chains, or major mixing of EO and PO inside the core, which respectively is entropically costly and causes an increase in chemical potential [5,15].

The rod-like micelles form potentially a nematic phase above some concentration limits. In steady shear it was shown that the PEO-PPO-PEO rod-like micelles align to various degrees depending on polymer concentration and shear-rate, as shown in Figure 4 [5,14].

Cubic phase of spherical ABA and AB block copolymer micelles

In the contour plot of the micellar volume fraction, ϕ, shown in Figure 3, we see that ϕ saturates at a limiting value of the order of ϕ_c=0.53. When the ϕ_c border-line is crossed the micellar liquid undergoes a first order phase transition to a cubic crystal [17,18], in agreement with simple hard-sphere crystallization. Both bcc [5,14] and fcc [19] phases have been reported in the PEO-PPO-PEO and related micellar systems [20].

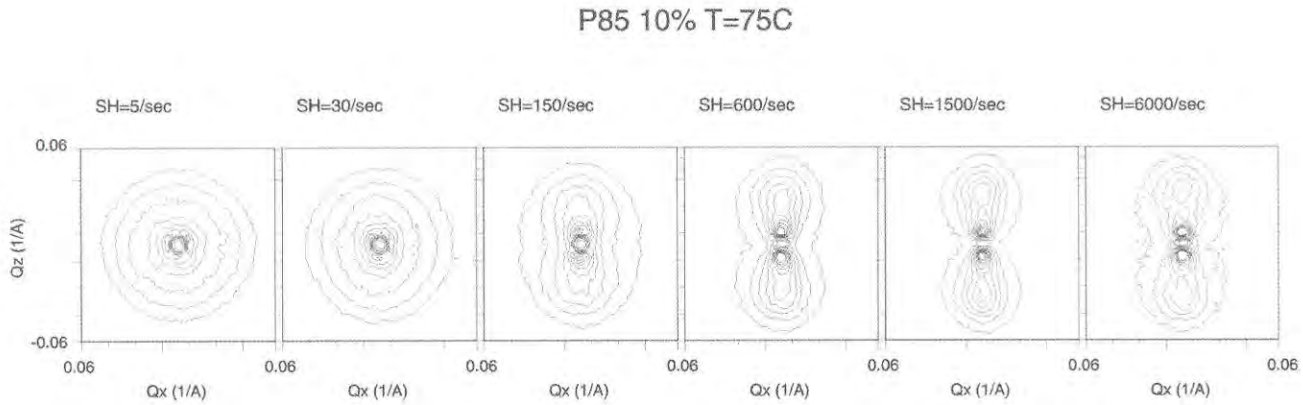


Figure 4: Shear induced alignment of rod-like micelles into a nematic state.

Shear has a marked effect on the cubic crystalline texture, but seems to depend critically on the specific copolymer architecture. While original studies showed only minor shear dependence [5], more recent experiments have shown shear thinning and structural dislocation [21-23] analogous to the results of Gast and coworkers on PS-PI [20]. In static flow conditions, the shear aligned texture of bcc-crystals is most often found corresponding to a mono-domain with $q_\upsilon \parallel [112]$ and $q_\nabla \parallel [111]$, where q_υ and q_∇ are the q-vector parallel to shear flow and shear gradient, respectively. In large amplitude oscillatory shear, on the other hand, the texture is usually a twin structure with $q_\upsilon \parallel [111]$ and $q_\nabla \parallel [110]$.

The mono-domain cubic phase of PEO-PPO-PEO micelles typically has a mosaicity of the order of 10° [5,24]. With shear-oriented crystals, it is possible to perform crystallographic studies and indexing of the observed Bragg-reflections [5,24]. In Figure 5 is shown the two-dimensional scattering pattern of $EO_{96}PO_{39}EO_{96}$ with the beam along the shear gradient and when the sample is rotated 35° around the primary [110]-reflection. The scattering pattern is in agreement with a bcc-lattice, as indicated by the associated Miller indices.

In the limit of high temperature and/or high polymer concentration, the cubic phase of the $EO_mPO_nEO_m$ micelles melts near the transition from a spherical to a rod-like form.

Micelles with glassy cores.

A number of micelles are composed of block copolymers where the non-soluble block is a glass at relevant temperatures. Any dynamics involving molecules jumping from one micelle to another or micellar shape transformations are frozen out. Block copolymers of PS are examples of such systems. Mixing PS-PEO block copolymers with water at ambient temperatures leads to large plate or rod-like micelles given by the original lamellar sheets of the bulk PS-PEO. Only when annealed above the glass transition of the PS-block do the micelles relax to the spherical equilibrium structure [25]. In triblock copolymers of the BAB type, the glassy cores have a particularly strong influence, since these make up a permanent physical network structure.

BAB block copolymer architecture

The phase behavior of the BAB-type of triblock copolymer in a solvent selective for the mid-block, is to form quite different structures relative to the AB and ABA types. These polymers may also form micellar aggregates. Frequently, however, the dilute-concentration causes loose structures of less well defined aggregates [7,26].

In the micellar phase, the A-midblocks of the BAB copolymers form either loops or bridges between micelles. The inter-micellar bridging gives rise to

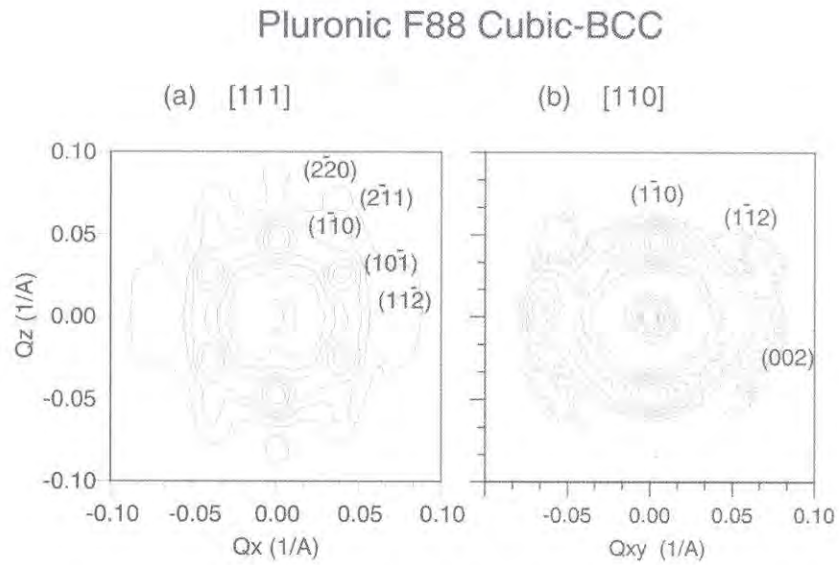


Figure 5: Two dimensional scattering function of $EO_{96}PO_{40}EO_{96}$, **(a)** as obtained with the shear axis parallel to the beam, and **(b)** as observed when the sample is rotated by 35° around the vertical axis.

clusters of highly interconnected micelles. For a certain copolymer concentration, the micellar networks extend over the whole sample volume, thus providing a macroscopically isotropic physical gel. In $PO_nEO_mPO_n$ and $BO_nEO_mBO_n$-systems, cubic ordered structure is observed in the isotropic phase at low temperatures [26-30].

The usual Pluronics, $EO_mPO_nEO_m$, might be of the BAB-type if dispersed in nonpolar solvents. Alexandridis and coworkers and Chu and coworkers have studied the aggregation behavior of $EO_mPO_nEO_m$ in xylene and found micelles with predominantly individual flower type micelles [31-34].

Micellar networks

Depending on the lifetime of the polymer blocks associated to a given core, BAB-material may show a finite elastic response. Systems with glassy micellar cores are ideal systems for such elastomers crosslinked by self-association. A number of studies have focused on the PS-type of block copolymers, including PS-PI-PS [35,36], PS-PEP-PS [37] and PS-PEB-PS [38,39]. Scattering experiments as well as electron microscopy imaging have clearly revealed the spherical PS-cores with effectively hard-sphere interaction.

The microscopic response to macroscopic deformation of the three-dimensional network was studied by neutron scattering. Upon stretching up to about 100%, additional correlations appear in specific directions, resembling induced para-crystalline order, as shown in Figure 6 [40]. Further stretching gives rise to an anisotropic pattern of the butterfly type, indicating non-homogeneous connectivity [41].

Shear alignment of BAB copolymer ordered networks

For polymer concentrations of 15% or more, ordered cubic structures are observed above $T \approx 55°C$ [42]. As in the AB and ABA systems, the BAB ordered gels might align into a mono-domain cubic structure upon application of shear [27,43]. In BAB-networks of PS-PEB-PS micelles, the scattering pattern resembles a twinned body centreed cubic morphology with lattice constants of the order of 400Å as shown in Figure 7. Upon cooling, preliminary measurements indicate a transition to

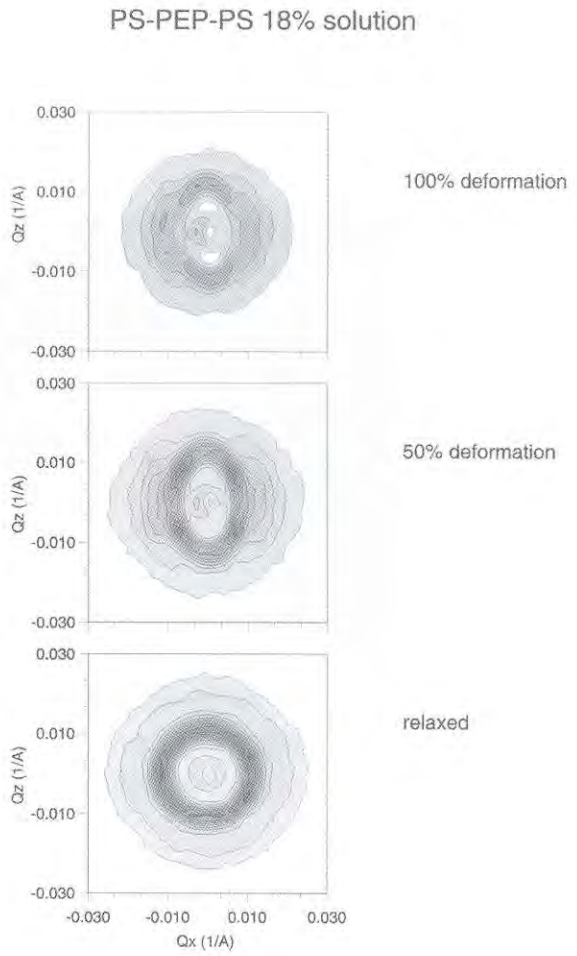


Figure 6: Two dimensional scattering function of PS-PEP-PS when stretched between 2 and 10 times.

another ordered morphology, presumably an fcc-structure.

Conclusions

It is clear from the present review that the field of block-copolymer micelles is an active research field with a large variety of materials. The unique structural characteristics provide novel attractive properties. X-ray and neutron small angle scattering are among the central tools for basic studies of this class of materials.

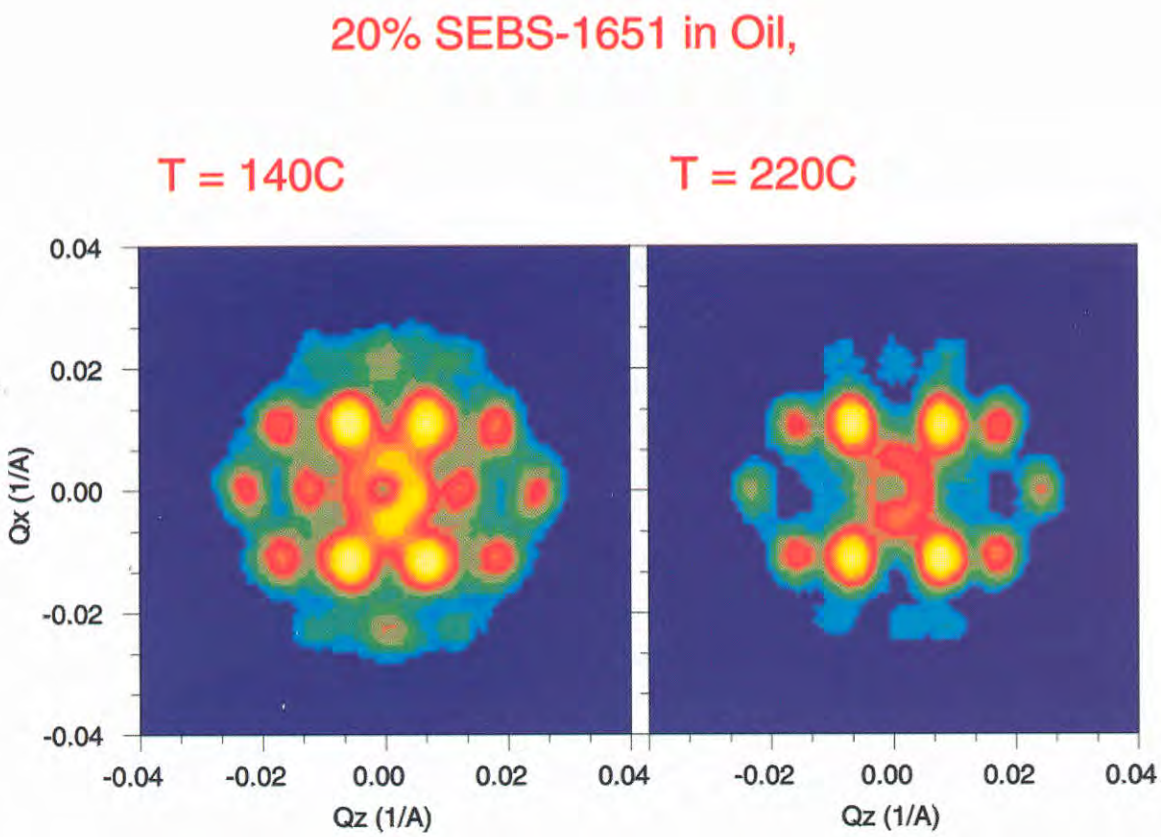


Figure 7: Two-dimensional structure of PS-PEB-PS micellar network structure in two ordered phases: Twinned bcc, and possibly fcc.

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Microtubule biopolymers: fibre diffraction and effects of interparticle scattering

W. Bras[1], G.P. Diakun[2], R.C. Denny[2], A. Gleeson[2], C. Ferrero[3], Y.K. Levine[4] and J. F. Díaz[5]

1 Netherlands Organisation for Scientific research (NWO) DUBBLE CRG/ESRF BP 220 F38043 Grenoble Cedex France
2 CLRC Daresbury Laboratory, Daresbury, Warrington, Cheshire WA4 4AD, U.K.
3 European Synchrotron Radiation Facility BP 220 F38043 Grenoble Cedex France
4 Utrecht University Molecular Biophysics PO Box 20000 Utrecht Netherlands
5 KU Leuven, Laboratory for Chemical and Biological Dynamics, Celestijnenlaan 200 D, Leuven, Belgium

Introduction

Microtubules play several important roles in the cell [1]. They consist of long chains of tubulin protein dimers, called protofilaments, which connect laterally to form a hollow cylindrical structure with an external diameter of approximately 30 nm and a length which can extend to microns. The number of protofilaments can vary, but the predominant number in a normal functioning cell is 13. The persistence length of these polymers is reported to be between 2000 and 5200 μm [2,3], so that for most experiments they can be considered to be a monodisperse rigid rod system with a large polydispersity in the length. In the experiments described here the average length is approximately 5 μm [4] giving an average molecular weight of 10^9 Dalton.

The preparation of hydrated microtubule samples suitable for small angle X-ray fibre diffraction is not trivial. A reasonably successful method has been centrifugation over extended lengths of time (>24 hours) and subsequent rehydration [5]. We have developed a less invasive method using the cooperative effect of the diamagnetic moment of the tubulin dimers in combination with strong magnetic fields to induce orientation on microtubules in diluted solutions. For rigid rod molecules with an axial ratio of 50 - 200 without any constraint applied to them, Flory has predicted that the angular distribution of the long axis would be at most 10.2 -

11° [6,7]. This prediction has been confirmed by our earlier experiments [8]. The analysis of the fibre diffraction pattern is therefore rather difficult since the degree of alignment is such that at higher q-values the reflections belonging to the layer lines can start to overlap with the diffraction arcs from the equator or other layer lines. In Figure 1 the diffracted intensity on the equator and the first layer line is shown. To obtain these curves it was necessary to correct for the disorientation and several geometrical parameters. For this the CCP13 suite of software was used [8, 9, 10]. Due to the longitudinal staggering of the different protofilaments, the surfaces of microtubules have shallow helical grooves running over them so that the diffraction pattern has to be explained in terms of helical diffraction theory [11,12].

In order to increase the accuracy and to avoid software induced systematic errors for the intermediate order diffraction peaks, a different method has been tried. In this case the microtubules were aligned with their axis parallel to the X-ray beam. This means that one only observes the scattering pattern from the equatorial plane and thus avoids the overlap between the different reflections at least until the resolution where higher order layer lines start to overlap as a consequence of the curvature of the Ewald sphere. An additional advantage is that it is also possible in this case to perform a radial integration over the full area of the two dimensional detector, thus considerably increasing the statistics. This allows one to study the low angle range where (concentration dependent) interparticle scattering weakly contributes to the scattering intensity.

Results

The protein tubulin was purified from pig brains and prepared for experiments as described elsewhere [13]. The samples were assembled and aligned in a 9 T magnetic field before being transported to the SAXS beamline. For these experiments, station 2.1

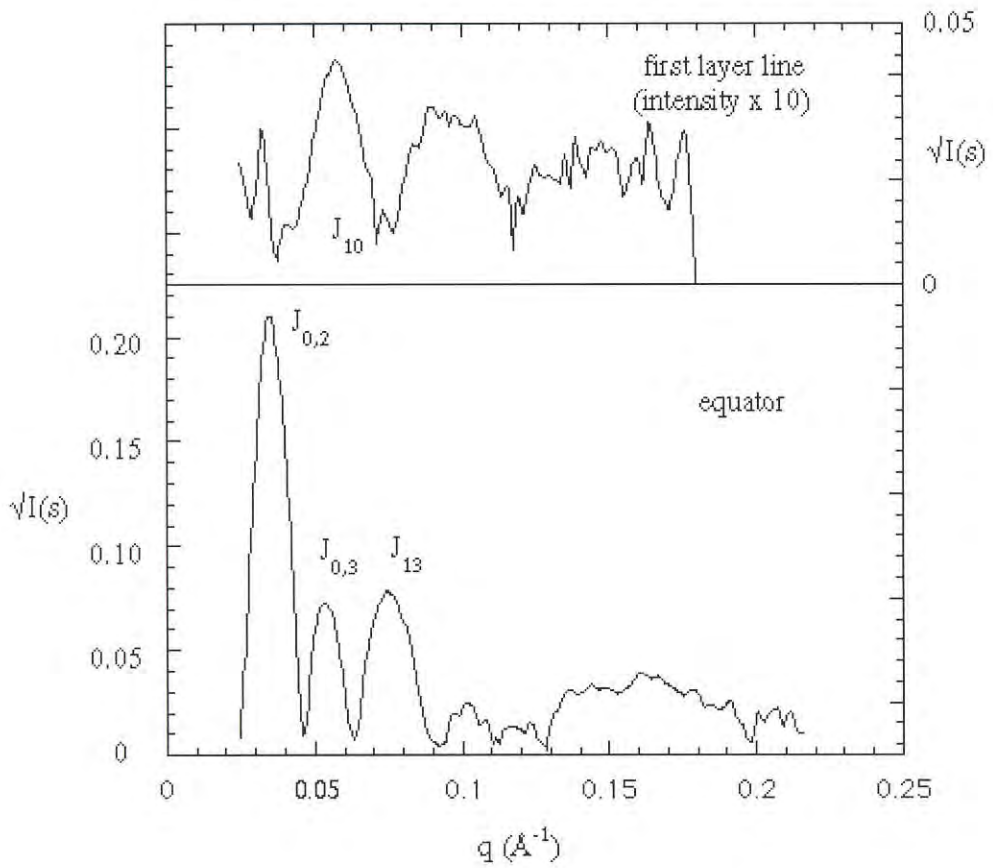

Figure 1: Equatorial and first layer line intensities of the microtubule fibre diffraction pattern. The vertical intensity axis is in arbitrary intensity units. The layer line intensity is weak compared to the equatorial intensity which makes the mathematical correction for the spread in the angular orientation very difficult. For this reason an alternative method of data collection and interpretation was used as described in the text.