Fibre Diffraction Review 13, 52 - 75, 2005 doi: 10.1382/fdr.2005.1310

13th Annual Workshop Abstracts

Talk Abstracts

Joint use of small-angle X-ray and neutron scattering from biomacromolecular solutions

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Small-angle scattering of X-rays and neutrons (SAS) is a fundamental tool in the study of biological macromolecules [1]. SAS allows to study the structure of native particles in nearly physiological solutions and to analyse structural changes in response to variations in external conditions. The method is applicable to a broad range of sizes, from individual macromolecules to multi-domain proteins and large macromolecular assemblies. The scattering data bear information about the overall shape and internal structure at a resolution of 1-2 nm. Recently developed advanced methods of data analysis significantly enhance resolution and reliability of structural models provided by the technique and make solution scattering a useful complementary tool to high resolution methods.

Advanced methods to analyze SAS data from solutions of biological macromolecules will be presented including *ab initio* low resolution shape and domain structure determination and modeling of quaternary structure by rigid body refinement. Special emphasis will be put to the joint use of X-rays and neutrons combined with contrast variation by hydrogen/deuterium exchange. Practical applications of the methods will be illustrated by recent examples.

Why are joint X-ray and neutron scattering studies useful for studying antibodies?

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Classical X-ray and neutron solution scattering studies each provide information at low structural resolutions of between 2-4 nm. Analytical ultracentrifugation provides complementary structural information from sedimentation velocity experiments. In recent years, the structural detail from these experiments has been improved by the use of constrained molecular modelling based on known crystal structures in order to yield structures at medium resolutions. The advantages of a joint X-ray/ neutron/ ultracentrifugation strategy include the use of (1) more reliable experimental data acquisition; (2) a wide range of solute-solvent contrasts; (3) combination of different instrumental conditions; (4) complementary hydrated and unhydrated views of the macromolecule; (5) comparison of different modelling algorithms. The combination of all three experimental approaches has yielded insight into the solution structures of antibodies and their biological function. Examples taken from scattering projects with the five human and mouse antibody classes will illustrate these points.

The use of hydrogenated and deuterated DNA in neutron studies of DNA-membrane interactions

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Vesicles formed from natural phospholipids have been overlooked as DNA delivery vectors because of the low affinity shown by zwitterionic lipids for DNA. Recent studies however have suggested that the interaction of DNA with zwitterionic lipids can be enhanced by the addition of divalent ions such as calcium. The aim of the work was to establish the existence and extent of such interactions using the technique of neutron reflectivity, examining the effects of DNA, in the presence and absence of calcium, on a monolayer formed at the air-water interface by the zwitterionic phospholipids 1, 2,-distearoyl-sn-glycero-3-phosphocholine (DSPC). Deuterated and protonated forms of DMPC and DNA were used in the study.

Analysis of the reflectivity data indicated that structural changes to the phospholipid monolayers were observed in the presence of calcium and were the direct result of interaction with DNA. The DNA appeared to insert itself into the phospholipid monolayer and/or cause a structural rearrangement of the phospholipid molecules within the layer to occupy a greater interfacial area. Changes of a similar nature but of a much smaller magnitude and slower time were observed in the absence of calcium. On the basis of these findings, it has been ossible to propose a mechanism for how calcium mediates the interaction of phospholipid with DNA.

It is clear from these studies that calcium does promote interactions between zwitterionic phospholipids and DNA, and that these systems might thus make suitable (non-toxic) vectors for gene delivery.

ILL's instrument suite for biology

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Introduction
ILL has a large range of instruments for the study of biomolecular structure and dynamics. In general the instruments are not dedicated to biological studies but are used over a wide range of condensed matter studies. Broadly speaking the instruments may be divided into five categories:

High resolution single crystal and fibre diffraction
LADI is a diffractometer for high resolution neutron crystallography of biological macromolecules, run jointly by ILL/EMBL. It is based on the quasi-Laue principle, which exploits a wavelength of band of some 2 - 4 Å exploiting an image plate as the detecting device. An upgrade of LADI (called LADI-3) is one of the Millenium projects.
D19 is a diffractometer optimised for fibre diffraction and small single crystal systems. Major modifications, funded by the EPSRC, yielding an overall efficiency gain of ~25, will be implemented in the coming 12-18 months.

Low resolution diffraction
D16 is a two axis spectrometer suitable for the study of membrane systems. Over the past 5 years the instrument has been sited on a dedicated 20 cm high guide and been installed with a focussing pyrolytic graphite monochromator. A new multi-wire detector, covering a larger angular range than previously, has been installed.
DB21 is a cold neutron diffractometer jointly run with EMBL and is dedicated to the study of large molecular complexes at low (~10Å) resolution. The H_2O/D_2O contrast variation technique is exploited to look at single components of, for example, protein-nucleic acid complexes.

Small angle scattering
D11 and **D22** are the world's highest flux small angle neutron scattering instruments. D11 has recently been refurbished with a new collimation system allowing and optimal balance between beam divergence and sample-detector distance. D22 has a slightly higher flux than D22 and thanks to it's 1 metre square multidetector which can be laterally offset, a wider dynamic Q-range. A new detector consisting of an array of tubes with individual wires allowing count rates of up to 2 x 10^6 neutrons per second has recently been installed.

Reflectometry
D17 is a time-of-flight reflectometer working with samples in vertical geometry. Its applications in biology are in the study of model membranes and the penetration of peptides or small proteins into the lipid bilayer.

Inelastic scattering
IN5, IN6, IN10, IN13 and **IN16** are instruments for inelastic scattering, each of which carries out some studies of the dynamics of macromolecules. The energy and Q resolutions of the instruments vary and therefore any particular study may require the use of a number of these instruments. In general they exploit the incoherent scattering from hydrogen atoms and therefore "reverse" labelling (i.e. the substitution of specific hydrogens into a fully deuterated molecule) is required.

The ILL-EMBL Deuteration Facility: A platform for labeling of biological macromolecules for Neutron Studies

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The ability to deuterate samples has for a long time been a key issue for biological neutron scattering. As part of the expansion of its life sciences programme the ILL, in collaboration with the EMBL-Grenoble, has established a joint laboratory to support the deuteration of biological molecules for neutron scattering experiments. This initiative as part of the Partnership for Structural Biology Programme will provide the tools and facilities required for the specific and selective isotopic-labelling of complex bio-molecules such as proteins, nucleic acids and lipids. The provision of these deuterated molecules should greatly enhance both the quality and quantity of

neutron experiments that can be done at ILL and in many cases will make feasible new more sophisticated experiments than can presently be performed.

The laboratory has been totally refurbished and fulfils L2 standard. A young and vigorous research program aims at developing procedures for deuterium labelling and high density cell culture techniques as well as applying these methods to provide material for in-house studies. Last year 3 EPSRC funded post-docs have been recruited to work on UK based projects involving deuteration and neutron diffraction. We are optimizing labelling strategies for over-expressed recombinant proteins and DNA, primarily in *E. coli* and are testing the feasibility of alternative labeling strategies.

As part of the in-house research activity we aim to characterise and evaluate the response of *E.coli* to deuterated environments using proteomic techniques.

Via a peer review procedure external users have access to the facilities, equipment and in-house expertise necessary to support and develop their own deuteration projects.

Likely Future Directions in User Software

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The past and present state of scientific reduction, analysis, and visualisation software will be briefly reviewed. Some possible future directions will be suggested, and a joint ESRF / CCP13 "proto" project for data acquisition, on-line visualisation, and first look data reduction will be presented.

In situ X-ray studies of the mechanism of muscle myosin

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The regular repeat of myosin motors in each half of a bipolar filament produces an X-ray interference effect that allows their axial motions to be followed in an intact muscle fibre with a precision of about 1Å. We used this effect to measure the unitary working stroke of myosin motors *in situ*, as they pull the actin filaments towards the centre of the myosin filament during muscle shortening (Reconditi *et al. Nature* **428**, 578, 2004). To eliminate axial motions associated with the compliance of the filaments, the load was held constant. At low load (0.25 times the isometric force) the average working stroke was 12 nm, consistent with crystallographic studies. The working stroke was smaller and slower at higher load.

Low-angle X-ray Diffraction Studies of Muscle using the SRS, ESRF and APS

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The highly ordered but different striated muscles found in certain vertebrates, such as bony fish, and in some invertebrates, such as the insect asynchronous flight muscles in the giant water bug *Lethocerus*, provide enormously rich low-angle X-ray diffraction patterns which in some cases can be fully 'solved' to yield the dispositions of some of the component molecules in the muscle sarcomeres (Hudson et al., 1997; Squire et al., 1998, 2003(a-d); AL-Khayat et al., 2003). Analysis of the diffraction patterns from resting muscle has already shown that the myosin head shapes and distributions on the myosin filament surfaces in these two muscle types are different, but they both contrive to place the motor (catalytic) domains of some of the outermost myosin heads in a configuration that is almost what is needed for direct attachment of the heads to the adjacent actin filaments. New results from bony fish muscle using the very small and highly collimated X-ray beam on line ID-02 at the ESRF in Grenoble (Squire et al., 2004) reveal the presence of numerous hitherto unseen X-ray reflections. These promise to provide information not just about the myosin heads, but also

about other components of the muscle A-band, namely C-protein (MyBP-C; Squire et al., 2003c) and titin (Connectin) on the myosin filaments, and troponin on the actin filaments. In addition, time-resolved X-ray diffraction studies can help to reveal the sequence of events that takes place on the onset of activation of an intact muscle and the nature of the structural changes that may be involved in force generation. The latest X-ray diffraction results on these two muscle systems will be reported and preliminary interpretations given.

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SANS studies of the subunit structure of type I restriction-modification (R-M) enzymes

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Type I restriction-modification (R-M) systems comprise 3 genes, one for each of the subunits (S, M and R) that are responsible for specificity, methylation and restriction respectively. Two genes (M and S) are required to form the trimeric 160kDa methyltransferase (MTase), M_2S, that methylates a specific base within the recognition sequence and protects the DNA from cleavage by the endonuclease.

The way in which the S domains are organised to interact with the two M subunits, and with the DNA recognition sequence, is of considerable interest. A model has been proposed in which the domains adopt a novel circular arrangement with the N- and C-termini in close proximity, leading to a pseudo-two fold rotation axis in the Mtase.

An expression system has now been established to produce the cloned subunits of the DNA methyltransferase M.AhdI, and have found conditions whereby the subunits can be combined to form an active enzyme. Deuteration of individual subunits has now been successfully performed at the Deuteration Laboratory at the ILL. Reconstitution of the methyltransferase with selectively perdeuterated subunits enables us to carry out small angle neutron scattering experiments, making use of contrast variation, to observe the movement of the labelled subunits following the interaction with its specific DNA recognition sequence.

"Report from CanSAS-IV"

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The 4th CanSAS (Collective Action for Nomadic Small-Angle Scatterers) Workshop took place at the Rutherford

Appleton Laboratory, UK, May 12 - 14, 2004.

CanSAS is an "umbrella forum" under which SAS beam line scientists and technical support staff, scientific software developers, and interested SAS experimentalists can meet and exchange views and ideas on the many and varied aspects of small-angle scattering; from experiment design and control to data reduction and analysis. A particular focus of CanSAS is the development of a standardised SAS data file format.

This presentation will give an overview of what was presented and discussed at CanSAS-IV, and the implications for CCP13.

Flexible filamentous virus structures from fiber diffraction

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Potexviruses and potyviruses are flexible filamentous plant viruses, of great importance in virology, biotechnology, and agriculture. Fiber diffraction patterns had been obtained from oriented sols and dried fibers of potexviruses by earlier researchers, but no fiber diffraction patterns have been reported for potyviruses, and the published potexvirus diffraction patterns have been far less ordered than, for example, the well-known patterns from the rigid rod-shaped tobacco mosaic virus.

We have been able to obtain very good fiber diffraction patterns from oriented sols of the potexviruses potato virus X (PVX) and narcissus mosaic virus (NMV), and we have obtained diffraction from the potyvirus wheat streak mosaic virus (WSMV), which, although limited, is nevertheless much better than anything previously observed.

Oriented sols are prepared by slow centrifugation in capillary tubes, followed by exposure to strong (up to 18.8 Tesla) magnetic fields. This procedure has produced excellent specimens of PVX and NMV. Diffraction data collected at the BioCAT beamline at APS, Argonne, have enabled us to determine the symmetry of PVX, and to demonstrate the presence of deep intersecting grooves in the viral surface. Because of small differences in the symmetry of the different potexviruses, data from NMV have allowed us to determine the inner and outer radii of the virion and a probable location of the genomic RNA. Diffraction from WSMV sols has allowed us to determine a probable symmetry for this virus.

Detailed structure determination of the intact viruses will probably require both the fiber diffraction data and high-resolution crystallographic structure determination of isolated coat proteins. Experiments directed toward these goals are in progress.

Virus structure research supported by NSF grant MCB-0235653 and USDA grant 2003-01178. Fiber diffraction methods research supported by NSF Research Coordination Network grant MCB-0234001. Use of the APS supported by the U.S. Department of Energy under contract W-31-109-ENG-38. BioCAT is a NIH-supported Research Center RR-08630.

Time-resolved X-ray Diffraction Experiments on Muscles using "High-flux" Beamline at SPring-8

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SPring-8/JASRI

A beamline for very high flux (BL40XU) has been built at SPring-8, which is based on a helical undulator and two focusing mirrors. This beamline provides a flux more than ten times higher than any other beamline that has been used for muscle diffraction experiments. At present, three different types of muscle experiments are carried out at this beamline. (1) Experiments on whole frog skeletal muscle, which are made at a time resolution of up to 0.5 msec using a CCD-based framing X-ray detector. (2) Experiments using caged compounds: an array of single skinned fibers is used as a specimen. (3) Time-resolved diffraction experiments on a whole mammalian heart.

The X-ray flux is high enough to record an intensity change of the third-order meridional reflection from the thick filament in frog skeletal muscle at 0.5-msec time resolution in a single quick-release experiment. Time course of intensity changes in weak layer-lines from myosin heads was also measured successfully. On the other hand, radiation damage due to the high flux is severe. A hole is found in a muscle after an exposure of 5 msec. Thus, it is necessary to move the muscle rapidly during an exposure in experiments using the high flux.

Hierarchical Deformation Mechanisms in Collagen and Wood

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Collagen and wood are fully polymeric biological tissues with remarkable mechanical properties. Details on the

deformation mechanisms can be determined by tensile testing and simultaneous synchrotron X-ray diffraction. This in-situ methodology allows to follow deformation processes at several levels of the tissues hierarchical structures. It turns out that collagen fibrils are stretching more than the molecules they are composed of. The whole tissue stretches even more than the fibrils, by an amount depending on the strain rate. This indicates a viscoelastic shear coupling between fibrils mediated by a matrix of mostly proteoglycans and water. In wood, cellulose microfibrils are wound helically around the lumen of tube-like cells, which are shown to react to tension like elastic springs. Again, the stiff cellulose fibrils are shear coupled via a matrix, which in this case consists of hemicelluloses reinforced with lignin.

Analysis of the myosin superlattice in striated muscles

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Luther and Squire observed many years ago that vertebrates possess two classes of underlying muscle ultrastructure in which the packing of the myosin filaments is different. In bony fish muscles the filaments are in rotational register in each myofibril, whereas in most other vertebrate muscles they adopt one of two opposite orientations in a semi-random arrangement. This latter arrangement is refereed to as the myosin superlattice. The two different kinds of myosin packing likely lead to functional differences in myosin-actin interactions, but of particular importance in the context of fibre diffraction analysis of muscle structure is the effect of the superlattice packing on fibre diffraction patterns.

In order to model X-ray diffraction from muscle specimens, an accurate statistical description of the superlattice disorder is required. Fortunately, the superlattice can be observed directly using electron microscopy methods developed by Luther and Squire. Methods for automated analysis of the myosin orientations in such micrographs will be described. Data from images of a number of muscle samples have been used to parameterise the disorder in these samples.

Fibre Diffraction from Poly-Alanine and Poly-Glutamine Assemblies

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Pathological accumulation of fibrillar, proteinaceous amyloid deposits can occur in a variety of neurodegenerative diseases, two well-known ones of which are Alzheimer's disease and the transmissible spongiform encephalopathies. In these two examples the misfolded protein or protein fragment (amyloid β and prion, respectively) has a heterogeneous sequence. By contrast, in two additional examples where fibrillar amyloid can accumulate-Huntington's disease (HD) and oculopharyngeal muscular dystrophy (OPMD)-polypeptide homopolymers underlie the fibrillar assembly. In HD, glutamine trinucleotide expansion to >$(CAG)_{35\text{-}40}$ results in misfolded proteins (poly-Glutamine$_n$, or polyGln$_n$) that may manifest a toxic gain-of-function. Besides HD, there are at least eight other inherited neurodegenerative diseases caused by polyGln expansions. In OPMD, which is an adult-onset disorder, alanine trinucleotide expansion from $(GCG)_6$ to $(GCG)_{8\text{-}13}$ in the poly(A) binding protein 2 (PABP2) gene leads to accumulation of intranuclear, 85 Å-wide filaments. To explore how polyGln aggregate structure may depend on expansion length, and how fibril formation may form from expanded polyAla sequences, we are investigating with X-ray fiber diffraction the structures of assemblies formed by synthetic acetyl-Gln_8-amide, $Asp_2Gln_{15}Lys_2$, $Lys_2Gln_{28}Lys_2$, and $Lys_2Gln_{45}Lys_2$, and by peptides having 7, 11, 13 and 20 Ala residues.

Structural Studies at ESRF on Silk Formation by SAXS/WAXS techniques

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Silk fibres are produced in nature by spiders or silk worms from liquid crystalline protein solution. Details of the fibre formation by in-situ experiments are currently largely unknown. I will review in my talk the present sta-

tus of SAXS/WAXS experiments on silk formation using the ID02 and ID13 beamlines of the ESRF. In-situ micro-SAXS/WAXS experiments during silking of live spiders at the ID13 beamline showed the presence of a thread in the immediate vicinity of the spigots but not the onset of fibre formation. Complimentary SAXS/WAXS experiments have therefore been performed during shearing of regenerated Bombyx mori silk using Couette cell geometry at the ID02 beamline. These experiments show the evolution of fibroin molecular shape during shearing and an aggregation into an amorphous material at the highest shearing rates. The aggregated material is capable of crystallizing into β-sheet containing silk II during drying. Scanning micro-SAXS/WAXS at ID13 on partially dried material shows the coexistence of silk II with an intermediary hydrated silk I type phase.

Non Crystalline Diffraction on Diamond - I22 and beyond

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Non-Crystalline Diffraction is an important method for studying the structural properties of non- or semi-crystalline states of matter. These include biological macromolecules e.g. fibres or proteins and their complexes in solution, synthetic polymers, gels, liquid crystals, oils, paints, ceramics and environmental aggregates. The technique yields information on the shape and size of these molecular assemblies and is particularly sensitive to phase changes or conformational rearrangements on a length scale ranging from 10 to 10000 Å.

Diamond is the new third generation synchrotron source that is currently being designed and constructed for the UK Science community. The 3GeV machine will begin operations in 2006 with commissioning of the first seven beamlines to follow.

The requirements to meet the scientific and technological challenges of the next decade are for a high resolution, high brightness beamline. These can only be provided by an undulator insertion device on a 3rd generation synchrotron radiation light source. I22, served by a U25 in vacuum undulator will deliver high photon flux (1 x 10^{14} ph/s/0.1%b.w.) into a focused 300 x 75 μm spot (1 x 1 μm with microfocussing) in the energy range 4-20keV. The End Station with associated linear and area detectors for static and time resolved measurements will be capable of recording the scattered radiation from samples contained in purpose designed specialised environmental cells. The end station's modular arrangement will allow a choice between small angle scattering for large fibrous structures or microfocus illumination each with a wide angle scattering option for materials studies. The latest developments in design for the beamline will be described.

The main points of the interim report on a "SAXS Roadmap" for Diamond will also be discussed.

Micro-scale Polymer Processing: using scattering techniques to investigate polymer processing

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Following crystallization in semi-crystalline polymers such as polyethylene and polypropylene, can be achieved by using simultaneous Small- and Wide -Angle X-ray scattering (SAXS/WAXS) techniques. Here, SAXS probes the long-range order or macrostructure providing details of the lamellar spacing and WAXS gives information concerning the atomic ordering or microstructure of the system. Following the structure development (crystallization) in polymers is particularly important as this process leads to the stabilisation of the final product, influencing the aesthetic and mechanical properties of the material. Thus, to produce useful materials it is essential to understand and predict the crystallization process, which can be explored with a variety of scattering experiments.

Several, experiments, which allow us to follow the crystallization in polymers have been performed using synchrotron radiation sources. Quiescent and shear -induced crystallization of samples have been studied using time resolved SAXS/WAXS on various beamlines at the Daresbury SRS and the ESRF. Here, we show how recent beamline developments with improved experimental techniques, can give an insight in to mechanisms of early crystallization kinetics as well as the final crystalline morphology. For example, quiescent crystallization experiments have been performed using an in-situ DSC

instrument on the Dubble -CRG station at the ESRF and 8.2 plus the new MPW station 6.2 at Daresbury SRS. This has allowed comparisons of SAXS/WAXS data to be made specifically, concerning new improvements made in detector technology, which have revealed information on the very early crystallization process. Shear-induced crystallization has also been investigated using SAXS/WAXS again on Dubble and station 16.1 at the Daresbury laboratory. An *in-situ* shearing devices and an online extruder, has provided an insight into crystallization under the influence of flow. Finally, experiments involving simultaneous SAXS/WAXS and rheology have been performed on station ID2 at the ESRF. This unique set-up allows time resolved SAXS/WAXS data along with the rheological responses of the polymer to be obtained throughout the crystallization process.

All these techniques are currently being used to investigate the crystallization of commercial and novel polymer systems. The use of time resolved synchrotron radiation is an invaluable technique in probing such processes and his will enable us to expand our understanding of crystallization kinetics with relevance to industrial processing procedures. This is envisaged to give a better understanding of polymer processing towards improved materials for the future.

Real-time investigation of protein kinetics using small-angle neutron scattering on instrument D22 at the ILL

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The high neutron flux (10^8 neutrons/cm^2 s) and the specific sample environment equipment available at the small-angle scattering instrument D22 of the Institute Laue-Langevin allow one to perform time-resolved measurements down to frame lengths of the order of 10 ms. Overall times per frame of about 10 to 100 s are required to achieve sufficient counting statistics per frame. These are obtained by repeating the measurement cycles a sufficient number of times.

Examples of experiments performed so far include the observation of the formation of complexes between DNA and cationic vesicles with a stopped-flow device, chasing experiments with chaperones (E. coli GroEL), and light-flash activation of photo-active yellow protein.

We expect that with the installation of a new time-resolving high-count-rate (> 2MHz at 10% dead time) detector, investigations in particular of smaller proteins will be further facilitated.

Time-resolved X-ray diffraction patterns from indirect flight muscles in living Drosophila during tethered flight

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The indirect flight muscles (IFM) of insects provide a particularly well-ordered system for structural studies directed at understanding the molecular mechanisms underlying muscle physiology. The fruit fly, Drosophila, with its rich set of tools for genetic manipulation, is a widely used model system for integrative biology. Here I report the results of time-resolved x-ray diffraction experiments on the dorsal longitudinal flight muscles (DLM) of adult flies during tethered flight at the BioCAT beam line at the Advanced Photon Source. Detailed 2D x-ray diffraction patterns were collected at rest and at 8 350 microsecond time frames equally spaced in the ~5 ms wing beat cycle. The results provide convincing evidence for cyclical attachment and detachment of force producing myosin molecules to its binding sites on actin in a living organism. Implications and future directions will be discussed.

X-ray Fibre Diffraction Analyses of Myosin Filaments in Resting and Contracting Skeletal Muscles

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X-ray fibre diffraction has for many years played a key role in studying the molecular mechanism of muscle contraction because it has an ability to investigate muscle structures under physiological conditions at the relevant timescale.

X-ray fibre diffraction analyses of the thick myosin filament have been made to elucidate the structure of the two-headed crossbridges in the resting and isometrically contracting states of live skeletal muscle. In the resting state of skeletal muscle, there are rich of layer-line reflections as well as meridional reflections arising from thick

myosin filaments, and during contraction, the most of the layer lines are much weakened but the principal meridional reflections are still retained with strong intensity. These myosin-based reflections are partially affected by the lattice structure of a hexagonal filament array in a sarcomere. In the analysis of the resting structure, the cylindrically symmetrical Patterson function $\Delta Q(r,z)$ based on only the layer-line intensity data except those on the equator (Namba, Wakabayashi & Mitsui: JMB, 138, 1-26 (1980)) was used. By removing the interfilament vectors, the $\Delta Q(r,z)$ was inversely transformed to get the interference-free intensity function I(R). The modelling of the two-headed myosin crossbridge was performed to obtain the best-fit to the obtained single-filament intensities.

In the second approach, the meridional reflections that correspond to the mass density projected onto the fibre axis were analysed. The meridional reflections are also heavily sampled by the hexagonal filament array. The sampling effect was markedly reduced during contraction and the reflection widths are radially broadened. The intensity data in the contracting pattern were corrected for such a radial broadening. It has been suggested that the existence of an axial perturbation of the crossbridge repeat generates a series of the reflections indexed to a basic repeat of 43 nm on the meridian, forming a triplet structure of the crossbridge arrangement (14.3 nm x 3). Modelling analysis of the myosin-based meridional reflections was made to derive the more precise crossbridge periodicity and the axial disposition of the two-headed crossbridges.

In the first approach, we could successfully remove the contibutions of interfilament vectors from the $\Delta Q(r,z)$ function obtained from the observed intensity data. The resulting I(R) functions were very similar to those obtained from the muscle at non-overlap length of the thick and thin filaments in the sarcomere, where the interference effect was greatly reduced. Modeling results indicated that in the resting state the two heads of a myosin crossbridge are axially separated by about 8 nm and are helically arranged at the radius of 13.5 nm with their curved surface surrounding the filament backbone. The second approach revealed that the myosin filament has a mixed structure of two different periodicities and axial dispositions of the two-headed crossbridges. These features were altered when muscle went from the resting to the contracting state. The crossbridge arrangement consisted of the two regions: in the resting state the crossbridge repeat in the regular region was 14.3 nm and in the perturbed region the repeating unit consisted of three crossbridge repeats slightly deviated from a 14.3 nm-separation. In both the regular and perturbed regions, two myosin heads of a crossbridge were axially separated and tilted against the plane perpendicular to the filament axis. The arrangement of two heads of a crossbridge was different in the regular and perturbed regions. In the contracting state, the disposition of the two-headed crossbridge was altered towards a more perpendicular orientation to the filament axis in the regular and perturbed regions. In the regular region the crossbridge repeat was 14.5 nm and in the perturbed region the basic repeat was 43.5 nm with different triplet repeats.

Reorientation behaviour of smectic liquid crystals due to external fields

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The reorientation behaviour of smectic liquid crystals due to externally applied forces is at present not very well understood. The interest in these systems lies in the fact that for several classes of smectic liquid crystals it is not necessary to achieve a full 90 degree rotation in order to achieve a 90 degree rotation of the optical axes. This could lead to faster switching displays.

Time-resolved SAXS experiments are a tool that can be used to study such systems. The conventional method of applying electric fields to induce and change orientation is less suitable for diffraction experiments since the alignment axis is not necessarily parallel to the magnetic field nor does the induced electric dipole have a linear relation with the applied field. To overcome these problems we have decided to utilise magnetic fields. Samples were rotated in a 7 Tesla field. The reorientation mechanism that follows is rather complicated and it is shown that there are several competing pathways for the molecules to realign themselves with the field.

Analytical Model of Fibre Diffraction Layerlines with Bent Baselines

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An analytical model for Fourier transformation of a filament with bent baseline was developed. The transformed fibre diffraction layerlines were derived with multiple convolution theory. The theoretical diffraction patterns were calculated for continuous bent helix, discontinuous bent helix, and real filamentous molecules. The method was applied to refine the atomic model of F-actin against the experimental fiber diffraction data. The results were compared with those refined using long-range normal modes in our previous study (Biophys. J. 86, 116 (2004)). Decreases of R-factors were observed and the agreement between the analytical and normal-mode-based models indicates the feasibility of the analytic model. Finally, the

advantages of the analytical model and the potential future improvements were discussed.

Fibre Diffraction from Carbon Nanotubes

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We are interested in using fibre diffraction techniques to study ordered assemblies of carbon nanotubes. To this end I am writing computer programs to predict X-ray fibre diffraction patterns from such structures, looking at packing and the effects of misorientation in the first instance. The calculations use helical diffraction therory to calculate the structure factor of carbon nanotubes as reported by Lucas et al (Scanning Microscopy Vol. 12, No. 3, 1998, 415-436).

Crystal structure of native cellulose - Complementary X-ray and neutron experiments

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Native cellulose displays an allomorphism of a monoclinic and a triclinic form. Most native cellulose in plants are a mixture of the two allomorphs. Growth stress might favour one of the two forms. In tension wood fibres of poplar trees the monoclinic crystal form is dominant. Thehigh orientation of the cellulose microfibrils in tension wood fibresmakes it possible to collect diffraction data with a resolution of 1.1 Angström. Complementary X-ray and neutron data can be used to determine the hydrogen atom positions and, thus, the hydrogen bond network in native cellulose.

The 'roll and lock' mechanism of force generation in muscle

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In the lever arm model, muscle force results from a tilting of the 'neck' domain of the myosin head while its catalytic domain is fixed on actin. Involvement of a change in the actin-myosin interface, namely a locking transition from a non-stereo to a stereo-specific binding was also proposed. The X-ray diffraction pattern obtained from contracting muscle fibers provides information about the protein structures responsible for force generation. We show here that when a temperature jump (T-jump, 5 °C to 30 °C) causes a trebling of the developed, isometric force, the diffraction pattern reveals a simultaneous enhancement of the helix of the actin filaments by actin-attached myosin cross-bridges. As these changes occur at constant number of myosin heads bound to actin, the observations show that a stereo-specific 'locking' of initially weakly attached myosin heads is an intrinsic part of force generation. The T-jump also induces a two-phase change in intensity of the myosin M3 reflection which is sensitive to axial head movement: a quick drop followed by a larger rise with a time course similar to that of tension. These observations, together with measurements of the axial head displacement using X-ray interferometry suggest that the actin-myosin motor works in two steps: a 'roll and lock' transition to a stereo-specifically bound state followed by lever arm tilting. A kinetic and structural model based on the two-step mechanism of force-generation quantitatively explains these observations.

Real-Time SANS Study of Transient Phases in Polymer Crystallization

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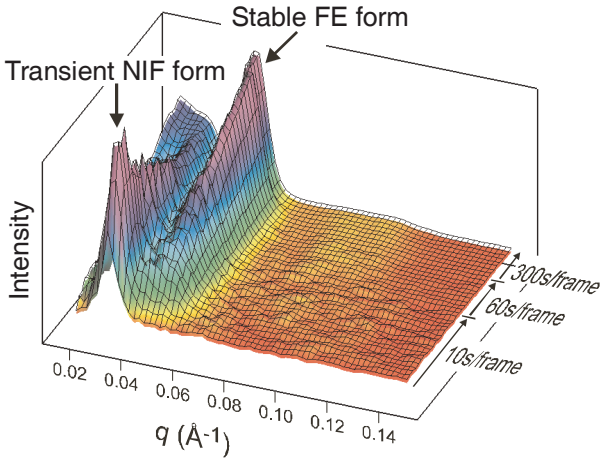
Small angle neutron and X-ray scattering techniques

(SANS and SAXS) are a powerful combination in studying structural details of semicrystalline polymer structure using ultralong linear alkanes with deuterated chain ends as models.[1]

Currently, SAXS data with a reasonable signal-to-noise ratio can be collected in sub-second frames by using synchrotron radiation. This makes real-time SAXS suitable for studies of the crystallization process, transient phases, and solid-state transformation in polymer and low-molecular systems. It would be desirable to carry out SANS experiments in real time as well. Although SANS normally requires exposure that are considerably longer (usually hours), in this study it is demonstrated that good quality data can be collected in no more than 10 seconds (see figure below), provided a rational approach is adopted to selected deuteration. We present here the first real-time SANS study of the crystallization process of a model polymer system. The compounds were selectively deuterated so as to maximize the SANS contrast. A T-jump cell is used, capable of rapid cooling of a large sample from melt to the crystallization temperature.

This development opens a new way of using neutron scattering in studies of materials under industrial processing conditions.



Real-time SANS spectra recorded during isothermal crystallization of a monodisperse long n-alkane with deuterated chain ends: $C_{12}D_{25}C_{192}H_{384}CHDC_{11}D_{23}$.

Reference

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Poster Abstracts

SAXS/WAXS data acquisition and reduction programs at ESRF beamline ID02 for time-resolved experiments

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Data rates from two dimensional detectors at synchrotron radiation beamlines are steadily increasing. An overview of SAXS/WAXS data treatment on the High-brilliance Beamline (ESRF ID02) is presented.

CCD detectors with 1024 x 1024 pixels and 16 bit data are in routine use for time resolved experiments. Today, up to 14 images per second can be taken. To facilitate automatic data processing each scattering image is saved together with a descriptive set of parameters. The data storage is done inside the framework of the ESRF data format. The use of the bsl format is disliked because it does not provide enough possibilities to include all required information. The data reduction can either be done offline, using the traditional command line based programs of ID02 ("saxs"), or online, using a newly developed program package ("online-saxs").

All detectors require various corrections before the measured intensities are available in absolute differential scattering cross-sections. The data reduction includes the correction of detector artifacts such as dark current, image distortion and spatial inhomogeneities, as well as normalization of intensities to incident photon flux, sample transmission and spherical angle.

The programs are also used on other ESRF beamlines, e.g. at the anomalous scattering beamline ID01. They can read bsl data and are available to users. Versions for a number of different platforms (UNIX, LINUX, WINDOWS) are available.

Spatial mapping of collagen fibril organisation in primate cornea

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WAXS methods were used to map the fibrillar arrangement and distribution of collagen over 3 common marmoset corneas, at 0.5 X 0.5 mm resolution. The maps provide new insight into corneal structure in the normal mar

moset eye, and should aid the interpretation of data from pathological corneas or primate models of refractive surgery. The results indicate the presence of a circumcorneal annulus of highly aligned collagen, 0.5 %96 1.5 mm wide, where the cornea and sclera fuse at the limbus; a feature similar to that observed in human tissue. As in humans, the annulus varies in width, fibril angular spread and collagen density around its circumference. Implications for the mechanical properties of the cornea are discussed. More centrally, the marmoset cornea features a preferred lamella orientation in which proportionally more fibrils are oriented along the superior-inferior corneal meridian. This observation is in striking contrast with the situation in the central human cornea, where an orthogonal arrangement of preferentially aligned fibrils dominates. Investigation of a further 16 corneas confirmed that approximately 33% (+/- 1%) (n 76) of fibrils in the central marmoset cornea lie within a 45-degree sector of the superior-inferior meridian. These observations may help to explain the difference in corneal birefringence between humans and monkeys.

Time course of formation of myosin cross-bridges and rise of a parallel elasticity measured by X-ray diffraction in tetanized single fibres from frog muscle

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Two-dimensional X-ray patterns from intact skeletal muscle fibers of *Rana temporaria* (2.1µm sarcomere length, 4°C) were recorded with 5ms time resolution using a gas-filled detector (RAPID) at beam line ID02, ESRF, Grenoble, France. Isometric force development was accompanied by an increase of the spacing of the third order myosin meridional reflection (S_{M3}) from 14.34 nm to 14.56 nm (Piazzesi et al., J. Physiol. 514:305, 1999). When unloaded shortening (velocity V_0) was imposed 5ms after the start of stimulation, S_{M3} decreased to 14.30 nm during shortening, then increased to 14.56 nm during force development after shortening. When shortening at 1/4 V_0 was imposed at 55 ms, to maintain a force of half the isometric plateau force (T_0), S_{M3} continued to increase from 14.45 nm to 14.56 nm during shortening, with the same time course as in an isometric tetanus. In contrast, the intensity of the M3 reflection (I_{M3}) during such a shortening period was less than during the same period of an isometric contraction, and was similar to the value observed when shortening at 1/4 V_0 was imposed from the tetanus plateau. These results suggest that during muscle activation shortening at V_0, but not at 1/4 V_0, prevents the formation of force-generating cross-bridges, revealing the rise of an elasticity joining the Z-line with the thick filament (Bagni et al., *Biophys. J.* **82**:3118, 2002). This elasticity exerts a compressive force on the thick filament.

Supported by MIUR (Italy), MRC (UK), ESRF, EMBL

SANS studies of the subunit structure of type I restriction-modification (R-M) enzymes

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Type I restriction-modification (R-M) systems comprise 3 genes, one for each of the subunits (S, M and R) that are responsible for specificity, methylation and restriction respectively. Two genes (M and S) are required to form the trimeric 160kDa methyltransferase (MTase), M2S, that methylates a specific base within the recognition sequence and protects the DNA from cleavage by the endonuclease.

The way in which the S domains are organised to interact with the two M subunits, and with the DNA recognition sequence, is of considerable interest. A model has been proposed in which the domains adopt a novel circular arrangement with the N- and C-termini in close proximity, leading to a pseudo-two fold rotation axis in the Mtase.

An expression system has now been established to produce the cloned subunits of the DNA methyltransferase M.AhdI, and have found conditions whereby the subunits can be combined to form an active enzyme. Deuteration of individual subunits has now been successfully performed at the Deuteration Laboratory at the ILL. Reconstitution of the methyltransferase with selectively perdeuterated subunits enables us to carry out small angle neutron scattering experiments, making use of contrast variation, to observe the movement of the labelled sub-

units following the interaction with its specific DNA recognition sequence.

The Fibrillar Arrangement and Distribution of Collagen in Normal and Keratoconus Corneas

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Purpose: To examine the distribution and the preferred fibril orientation of collagen in the normal human cornea and in corneas with the disease keratoconus and to relate any observed variations to features on corneal surface topography maps.

Methods: Keratoconus corneal buttons of 8mm diameter (n 4) and normal human corneas (n 3) were tagged with a nylon suture at the 12 o%27clock position, before being preserved in formaldehyde. For each cornea a videokeratographic image of surface dioptric power was recorded(in vivo for keratoconus corneas and in vitro for the normal controls). Wide angle x-ray scattering (WAXS) patterns were obtained at 0.4mm intervals over the entire area of each sample using a computer operated translation stage on Station 14.1 at the Daresbury Synchrotron Radiation Source, UK. Each WAXS pattern was analysed to produce quantitative information regarding the total amount of collagen (aligned and isotropic), the preferred orientation of aligned collagen and the ratio of aligned to total collagen at a known corneal location. By arranging the data onto a grid of corneal position various maps were produced to illustrate the distribution and preferential orientation of collagen. The relationship between collagen arrangement and surface topography was examined in detail for both the normal and keratoconus corneas.

Results: In the cone region of keratoconus corneas the orthoganol preferred orientation of collagen fibrils that is seen in the normal human cornea, is absent. In the normal cornea there is a gradual symmetrical increase of collagen from the central region to the periphery. However, in keratoconus corneas maximal thinning occurs at the apex of the cone. Outside the cone region the increase in collagen occurs asymetrically and is less gradual than in the normal cornea. The distribution of aligned collagen is especially altered in keratoconus corneas and appears to correlate closely with cone shape.

Conclusion: The results indicate a redistribution of collagen in keratoconus corneas, which supports the theory that corneal thinning in keratoconus occurs as result of lamella sliding away from the cone region. The existence of this mechanism would also help to explain the altered orientation of collagen fibrils in this region.

Time Resolved X-ray Diffraction Studies of Active Bony Fish Muscle

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X-ray diffraction is a particularly useful tool for studying the mechanisms of muscle contraction. Not only does is provide a wealth of structural data but it can also be used on live intact muscle preparations allowing data to be obtained whilst the muscle undergoes contraction. Bony fish muscle is an extremely good preparation to use for this work because it is very well ordered compared to other vertebrate muscles such as frog, showing long range order and a simple 3-dimensional crossbridge lattice.

Previous work carried out on bony fish muscle has looked at the changes which occur in the X-ray pattern during contraction in conjunction with the tension time course produced by the muscle [Harford & Squire, 1990]. Up until now the effects of any sarcomere length change which might occur during muscle contraction have been neglected. There is evidence for a small, but very fast, change in lattice spacing during contraction and it has been previously argued that, therefore, the sarcomere length change may be small [Harford & Squire, 1992]. However, this needs to be confirmed and if, in fact, the tension time-course is affected by maintaining sarcomere length, then the sarcomere length will need to be controlled.

This poster describes the laser and detector system which has been developed to control the sarcomere length of plaice fin muscles whilst collecting X-ray patterns. It also presents some of the preliminary time-resolved low-angle X-ray diffraction data obtained using this feedback system in experiments on beamline 16.1 at the CLRC Daresbury Synchrotron Radiation Source.

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New Ionic Polymer Materials for Films. Physico-Chemical Behavior of Carboxyl Containing Acrylic Copolymers: Conformation, Dynamics and Structure Development in Mixed Solvents.

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Introduction

The formation of films from latex materials is well understood and has been explained to take place in several steps [1, 2], whereas film formation from ionic polymer solutions has been less frequently studied. In particular, the film formation process from aqueous solutions of charged polymers needs to be revealed, and the effect of co-solvents on the rheology and final film properties of charged polymers. These phenomena have an impact on performance in hair fixative polymer formulations, water-soluble polymers for non-wovens, solution polymers for glass fiber sizing etc., but also likely starch solutions that film form during application. During the film formation process, water acts as a suspending and stabilizing medium for the macromolecules that allows for thermal motion of the system that allows intermolecular interactions and close packing. Upon drying, water and co-solvent evaporate, leading to concentration of the solute and eventually film formation. The present work is focused on the initial step of structure development, i.e. intermolecular interactions in dilute and concentrated polymer solutions in mixed solvents.

Results and Discussion

Dynamic light scattering (DLS) and small-angle X-ray scattering were employed to study the properties of random copolymers of methacrylic acid (MAA) and butylmethacrylate (BMA) (Mw approx. 38,000) at various degrees of acidity (MAA content) and at various solvent compositions of isopropanol (IPA) and water. DLS measurements revealed the presence of mainly two dynamic structure factors, one fast diffusive and one slow non-diffusive. The fast mode is attributed to the translational motion of the copolymer chain while the slow mode relates to interpolyion solution correlations that are strongly dependent on the long-range electrostatic forces between anionic MAA units. SAXS results showed that the copolymer chain conformation is elongated. Ionized MAA is soluble in 100% H_2O but insoluble in 100% IPA, while BMA is soluble in 100% IPA but not in 100% H_2O. Thus, these random copolymers are soluble only in mixtures of IPA and H_2O, and only at certain compositions of the two solvents. This delicate feature gives rise to some sophisticated micro- and macroscopic solution properties. The existence of inter-particle aggregation can be controlled by varying the IPA-to-water ratio, an initial step for the understanding of structure development during film formation.

The interaction potentials between copolymer chains and solvent - in dilute solution - are reported in terms of the hydrodynamic virial coefficient k_D. In line with the enhanced intermolecular association when the IPA/H_2O ratio is decreased, there is a simultaneous reduction in k_D. Scattering experiments give two results that indicate an elongated macromolecular conformation: *i*) The shape of the pair-distance distribution functions (PDDF) [3]; *ii*) The high values of the particle-shape and internal-density parameter (= $\langle R_G \rangle_Z$ / $\langle R_H \rangle_Z$). The PDDFs at certain IPA/H_2O compositions are similar to those of cylindrical molecules and give information about a cross-sectional radius R_{CS} and length L (Figure 1).

Figure 2 shows the concentration dependence of the apparent radius of gyration $\langle R_G \rangle_Z$, at different solvent compositions of IPA and H_2O. It is obvious that conformational changes take place as the mass fraction of H_2O is increased, and eventually macroscopic phase-separation occurs. Combination of intra- and intermolecular interactions in selective solvents leads to diverse possible structures of associates, see for instance ref. [4, 5].

Conclusions

When the solvent mass ratio IPA/H_2O is decreased, the overall solvent quality decreases. There is no pronounced dependence of the mutual diffusion coefficient D_m on copolymer concentration. When present, the dependence is slightly inverse, especially at low IPA/H_2O ratios. DLS shows mainly two relaxation modes, one fast diffusive mode and one slow non-diffusive describing long-range electrostatic interaction. The slow non-diffusive structure factor vanishes in the presence of salt, supporting the interpretation of electrostatic correlation. When the solvent mass ratio IPA/H_2O is decreased, the shape factor increases, suggesting a decreased 'surface' stability of the sphere-equivalent macromolecules.

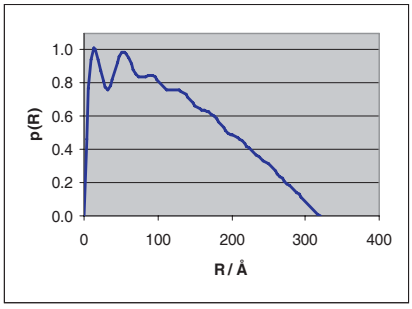

Figure 1. Pair-distance distribution function (PDDF) for 3 wt% MAA-BMA (20/80) in IPA/H_2O 80/20.

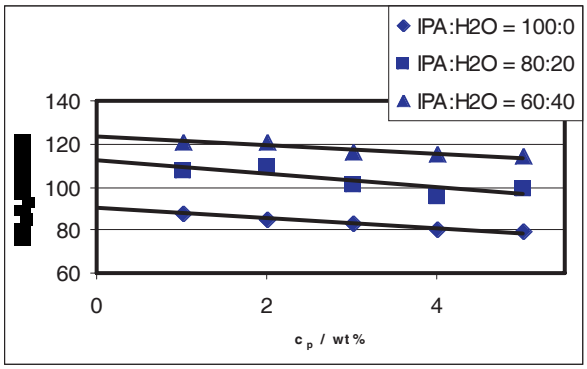

Figure 2. The concentration dependence of $\langle R_G \rangle_Z$, app for various IPA/H_2O compositions.

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An Investigation into the Crystallisation of PET using AFM and X-Ray Scattering Techniques

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Polymer properties are generally customised by changing the monomer structure, however by modifying the manufacturing process more subtle variations or improvements in properties can be achieved. The aim of this investigation is to determine the effect of molecular orientation on the properties of Polyethylene Terephthalate using x-ray scattering and atomic force microscopy.

DSC and x-ray scattering techniques on commercially produced cast PET show that higher annealing temperatures induced a faster crystallisation, with the crystallites having a lower dimensionality of growth. Friction force microscopy has established a non-linear relationship between load and the friction force suggesting Johnson, Kendall and Roberts contact mechanics.

The effect of molecular orientation will be investigated using the Keele drawing camera looking at the extent and rate of the draw. Drawing the polymer aligns the polymer chains and orients the crystallites, while increasing the overall crystallinity. The extent of these effects will be studied in situ with time resolved x-ray scattering and the polymers produced will be analysed using AFM. The extent of shear will be indicated by the effect this has on the friction force across the surface.

By gaining a thorough understanding of the structure-property relationships of various polymers, there is a possibility in the future for most links in the processing chain to be modelled by mathematical methods. The eventual aim is to use these models to predict the structure and properties of the final product, allowing engineering design experiments to be performed before expensive real materials and machinery are used and produced.

SANS at the ISIS second target station

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Construction of a 10Hz "cold neutron" target station for ISIS is under way. An enhanced flux of long wavelength neutrons from an optimised lower power target will enable world class pulsed source instruments to be built. The first SANS instrument will have two 1m square detectors in a 3m diameter, 12m long, vacuum tank. Neutrons of 1.5 to 12 angstrom wavelength will be used by time of flight. This will enable an unsurpassed simultaneous Q range, having good overlap with wide angle diffraction, suitable for a wide range of science.

Continuous Flow SAXS To Resolve Curved Guinier Plots Of Aggregating Or Self-Associating Proteins

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We have combined Small Angle X-ray scattering and Gel Filtration Chromatography within a unified experimental set up to obtain molecular size information. Besides providing simultaneous corroborative data from two distinct experimental techniques bearing on the same question, passing the samples over a gel filtration column immediately prior to illumination by X-rays provides both a more homogeneous sample as well as providing a continuous set of data as the concentration is extrapolated to zero. This greatly facilitates analysis of data from even mildly aggregating proteins.

Laser Cleaning of Parchment: X-ray Diffraction Analysis

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Laser cleaning of parchment is a novel technique that has the potential to provide contactless, chemical-free cleaning of historically important documents. However, the effect of laser cleaning on the collagenous structure of parchment is still poorly understood, as is the effect of the wavelength or the energy density (fluence level) used to clean parchment. In this study, small angle X-ray scattering (SAXS) of parchment samples after laser cleaning reveal the effect of cleaning to the structural characteristics of parchment. The effect of cleaning at infrared (1064nm), green (532nm) and ultraviolet (266nm) wavelengths at a range of fluence levels is investigated. Laser cleaning at IR or green wavelengths appears not to alter the collagen diffraction pattern from SAXS, but parchments cleaned at the ultraviolet wavelength display low-coherence structural damage. SAXS is used to investigate the removal of dirt from parchment by laser cleaning, and shows that laser cleaning preferentially removes larger dirt particles.

Microfocus Small Angle X-Ray Scattering Study of Avian Eggshell Structure

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The eggshell is a highly ordered bioceramic composite, but currently little is known about the structure at the nanometer scale. Microfocus small angle X-ray scattering (mSAXS) allows us to probe the fine structural alterations, which may arise from subtle changes in the eggshell matrix. This paper describes the use of mSAXS to resolve different aspects of the shell growth process and to investigate textural changes at different layers within the eggshell. Microfocus analysis was carried out on beamline ID18F at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, using 300-micron thick sections of eggshell embedded within resin. A compound reflective lens was used to generate a microbeam of 1.5 microns by 15 microns. This powerful technology has not been used before to examine eggshell structure; therefore, our results are novel in the field of bioceramics.

Our previous results using X-ray diffraction and SAXS have given indications of the size and shape of the crystals. A comparison of these two methods have shown that variations exist in both sets of data, which could be attributed to the nanoinclusions and distortions in the eggshell that may be due to the organic matrix proteins. mSAXS has allowed us to produce two-dimensional maps that revealed the structural changes on a micron length scale due to the focussed beam size. Using the microfocus beam, eggshell sections were scanned over 500- by 300-micron size areas, and two-dimensional maps of crystallite characteristics were generated. The results clearly show changes in size and shape of mineral composition at different layers of the eggshell. This technique has allowed us to generate conclusions about the textural changes between different quality eggshells. Variables such as bird housing, nutrition and environmental stresses can alter the form and texture of the eggshell through alterations in crystal morphology. The eggshell matrix is thought to influence nucleation and control crystal growth and shape.

This investigation considers the influence of the eggshell matrix on calcite crystal formation, namely whether this will produce localised variations in coherence. Furthermore, organic/inorganic interfaces within the eggshell are studied; both of these characteristics are ideal for mSAXS examination. The two-dimensional maps provide evidence that microtextural changes, which are manifest at the macroscopic level, can influence the overall appearance of the eggshell. Our overall conclusions from examining the results produced by mSAXS suggest that there are subtle textural variations, which may be due to the presence of the organic matrix embedded within the calcium carbonate crystals.

Study of crystal reorganisation during unfolding transitions and molecular length dependance of the pressure-temperature phase diagram in ultra-long alkane crystals

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Ultra-long, strictly monodisperse alkanes with chain length between 100 and 400 carbons, synthesised by Dr. G. Brooke et al [1], crystallize into extremely regular lamellae with a thickness that is an integer fraction of the extended chain length, and have been investigated as model systems for polymer crystallisation, crystal perfection and melting.

High resolution time resolved wide angle X-ray scattering on ID11, ESRF, Grenoble has been used to determine high precision changes in lattice parameters as crystal perfection, related to crystal thickness, occurs. It has also been identified that as the lamellae thickens due to the chains unfolding from one integer folded state to another, a contraction in the lattice occurs.

The impact of un-folding on lattice parameters was further studied using in situ temperature modulation pseudo differential scanning calorimetry experiments to compliment TMDSC studies of the samples, to determine directly the reversible and irreversible parts of the transition between different crystal thicknesses.

The alkanes also provide an opportunity to carry out fundamental studies of the temperature-pressure phase diagram but on a truly pure system. The same approach of following contractions in the crystal lattice was used to obtain a high precision map of the effects of temperature and pressure on the lattice parameters of extended chain crystals, providing a link between the phase behavour of high polymers, such as polyethylene and its oligomers, and of small molecular systems, such as the n-parraffins.

High precision lattice parameters obtained were compaired to those obtained at ambient pressure, to study the effect of constraining chain motion on crystal thickening.

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Investigations of the crack tip deformation zone in Polyethylene pipe materials using Scanning Small Angle X-Ray Scattering (sSAXS)

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Polyethylene (PE) is the mostly used synthetic polymer. By varying the molecular and morphological architecture and by adding filling particles, the mechanical properties of the material can be optimized for many applications. In pipe applications, PE has been successfully used for about 40 years. The mechanical properties of pipe materials and especially the long term behavior are important for manufacturers and users of pipes. For that, the change in morphology during deformation and fracture can give hints for the production of new materials and lead to a better understanding of the dominant failure mechanism in this class of materials. We used scanning small-angle x-ray scattering (scanning-SAXS) methods to study deformation near a crack tip during quasi brittle crack growth. SAXS is particularly useful to investigate the lamellar properties of polymers and, therefore, changes in the morphology occurring during a fracture test. In most polymers at high deformation levels, two processes occur at the same stage: deformation of the lamellar structure, often together with an induced orientation, and the formation of cavities. The measured SAXS pattern (IM) is a summation of the contribution from lamellae scatter (IL) and cavity scatter (IC). The contributions are often superimposed and must be separated for further investigations. With the assumptions that the size of cavities is large compared to the long period of the polymer and that the scattering contribution from cavities decreases according to Porods law, IC can be described as ICbq-4. We show that this assumption is compatible with the data obtained near the crack tip and use it to separate Ic and IM. In this way, maps of the deformation in the region of the crack tip can be deduced from the scanning-SAXS analysis despite rather large volume fractions of deformation-induced cavities.

Skin to Historical Parchment: X-ray Scattering studies on collagen

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Parchment contains important historical information, both within the text and in its structural composition. Made of animal skin, they are delicate materials that should be protected from environmental pressures such as UV irradiation, air pollution and bacterial degradation.

The main focus of this interdisciplinary project is to use the synergy between structural biophysics and proteomics to give an understanding of the effects on collagen during the process of parchment manufacture and its predisposition to degradative pathways.

The alignment and packing of collagen gives mechanical strength and its interactions with the macromolecules, hyaluronic acid, dermatan sulphide and lipids, contributes to a stabilising association.

The use of a synchrotron radiation source for small/wide angle x-ray scattering has given important information on the nanostructural texture of collagen. Our results show that the liming of wet skin causes a 1.9nm shortening of the axial packing repeat distance, and a 0.09nm widening of the intermolecular lateral packing in the fibrils. The drying of skin reduces the intermolecular lateral packing by 0.173nm, but drying limed skin has a reduction of 0.306nm. Biochemical techniques are being used to characterise lipid and proteoglycans affected due to the processing from skin to parchment to explain these effects on collagen.

Structure and Blood Flow in Normal and diseased Equine Metacarpophalangeal Joint

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Osteoarthiritis, the degeneration of the articular cartilage and the underlying bone of synovial joints is a very common disease, causing considerable distress and disability both in man. It is relatively uncommon in animals, but occurs frequently in horses, where it causes similar suffering.

Despite its wide-spread effects, and many years of research, the factors influencing the development and progression of arthritis are still poorly understood, and this is a barrier to the normal development of therapeutic strategies.

The present research was designed to investigate some of the fundamental processes that are thought to be involved in the development of arthritis. The disease clearly involves changes in the structure of both fibrous and inorganic phases of bone and cartilage. The structure and organisation of both components has been characterised largely by means of x-ray diffraction methods. The first aim of this work was to take advantage of recent methodological advances largely associated with the availability of intense synchrotron radiation, to investigate structure with unprecedented spatial resolution. We sought to characterise the structure of the interfacial region between bone and cartilage and the structural changes in the focal lesions of the equine metacarpo-phalangeal joint, which shares many of the structural features of human lesions.

The second theme of the research was to explore the role of disturbances in blood flow in the development of the disease. The normal function of the cells of cartilage is dependent on the ability of the microcirculation to maintain the supply of nutrients and to remove metabolites, and a number of theories link the failure of this system to the development of disease. Testing these hypotheses, however, presents a technical challenge, because many standard methods of measuring microcirculatory flow are unsuitable for use in bone. We therefore sought to refine a recently reported method using the detection of metal microspheres using X-ray fluorescence. We were particularly anxious to develop an x-ray based technique since this enables us, for the first time to locally relate blood flow to tissue structure.

Modelling Analysis of Myosin-based Meridional Reflections from Skeletal Muscles in Relaxed and Contracting States

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Intensity analysis of the myosin-based meridional reflections in the X-ray diffraction patterns of live frog skeletal muscles was performed to propose a more detailed structural model for the myosin crown periodicity and the axial disposition of two-headed crossbridges along the

thick filament in a sarcomere.

In the X-ray patterns with high angular resolution from relaxed and contracting muscles, myosin-based meridional reflections are sampled axially by the closely-spaced diffraction peaks arising from interference between the two symmetrical halves of thick filament centered on the M-line in a sarcomere as well as radially by the lateral filament array. The axial sampling period on the so-called forbidden reflections (M1, M2, M4….) corresponds to the separation between two regions with a 42.9-nm basic period in which the crown levels are systematically perturbed (the perturbed regions). Detailed analysis of the fine splitting periods of the second and fifth order meridional reflections showed that the average period was ca. 820±40 nm in the relaxed state, while it was ca. 1010±40 nm in the contracting state. This finding indicates that the perturbed regions of crossbridge crown levels occupy the central zone of crossbridge arrays in the relaxed state and shift towards the Z-band along the thick filament in the contracting state.

For modeling the crossbridges structure along the thick filament in the relaxed and contracting states, we used the observed intensities of the second to eleventh order meridional reflections. The intensities were corrected for the effect of lattice disordering. In the model, the crown regions with a regular repeat of 14.3 nm were assumed to be located on both sides of the perturbed region. The intensities of the meridional reflections from the assumed models were calculated by using eleven independent parameters; the shift of the crown level from the 14.3-nm repeat in the triplets, the width of projected density of each myosin head onto the thick filament axis and the distance between two myosin heads of a crossbridge and the number of the crossbridges in each region. The most probable values of these parameters were determined by searching the best fit of the calculated reflection intensities to those observed.

In the relaxed state, the perturbed regions are ca. 560-nm long with 13 triplet levels of the 42.9-nm repeat. The lengths of the inner (towards the M-line) and outer (towards the Z-band) regular regions on either side of the perturbed regions are ca. 57 nm and ca. 86 nm, respectively. In the contracting state, the perturbed regions become shorter than in the relaxed state and the lengths are ca. 480 nm with 11 triplet levels of the 43.5-nm repeat. The inner regular regions are ca. 160-nm long and the outer regular regions are ca. 73-nm long. The orientation of two-headed myosin crossbridges along the thick

Comparison of Breast Cancer Diagnosis Using Small angle X-ray Scattering from Synchrotron Radiation and a Conventional X-ray Source

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It has been indicated previously that breast tissue diagnosis could be performed using small angle X-ray scattering with synchrotron radiation. The use of conventional X-ray sources would allow widespread implementation of this technique as a laboratory based system. As a way to investigate this possibility both synchrotron radiation and a conventional X-ray source were used to collect data from core biopsy breast tissue. The biopsies were collected from tumor samples, as well as control tissue from cosmetic reduction procedures. As expected the laboratory based system produced lower angular resolution data sets and required longer exposure times than with synchrotron radiation. However consistent and reliable differences in amorphous scatter could be observed between normal and tumor samples using both systems. Thus indicating that it may be possible to develop a laboratory based diagnosis technique.

pH Responsive Polymer Systems for use in Molecular Motors, Pumps and Valves

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The ability of individual polymer molecules to respond to changes in temperature and chemical environment with drastic changes in size and conformation has been appreciated for many years. The volume transition of responsive gels represents a direct, macroscopic manifestation of the conformational response of the individual molecules making up the gel. Responsive gels have been enthusiastically greeted as candidates for a new generation of intelligent materials with sensor, processor and actuator functions.

In our program, we concentrate on weak polyacids and polybases, which respond to changes in pH. At the macroscopic level, these systems, when coupled with a chemical reaction that cause spontaneous pH oscillations, allow us to make a free-running chemical motor. We explicitly link the macroscopic behaviour to the microscale behaviour by studying the response of triblock copolymers to pH oscillations. The active chains are the mid-blocks and are effectively crosslinked by the domains of the hydrophobic end block. The expansion and contraction of

the grafted polyelectrolyte chains causes the distance between hydrophobic domains to change, this separation can be monitored by SAXS and the molecular and macroscopic effects correlated.

Shaft peptides from the beta-structured adenovirus fibre protein self-assemble into amyloid fibrils in the absence of a trimerization motif

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Viral fibrous proteins have recently been shown to contain novel beta-folding motifs composed of repetitive structural elements. We have been studying folding and assembly of such beta-structures using the fibre protein from adenovirus as a model system. Adenovirus fibres are trimeric proteins consisting of three parts: an N-terminal tail, a fibrous shaft in a triple beta-spiral conformation, and a C-terminal globular head domain. We have previously reported studies on a 41 amino acid peptide derived from the shaft sequences; this peptide failed to assemble into its native triple beta-spiral conformation and formed amyloid-type fibrils. In this work we have synthesized five shorter peptides (25, 12, 8, 6 and 4 amino acids) derived from the original 41 amino acid peptide and studied their potential for self-assembly using electron microscopy, Congo red binding, FTIR spectroscopy, and X-ray fibre diffraction. We find that formation of amyloid fibrils is a general propensity of shaft sequences when isolated from their native context. The formation of amyloid by these sequences may occur as a result of out-of register interactions in the absence of the globular head. In the context of the native fibre, the head domain may act as a natural registration signal that directs correct folding and assembly. We discuss the possible structure and arrangement of these sequences within the amyloid fibril, as compared to the one adopted within the native structure. These results are relevant to understanding amyloid formation by repetitive sequences from disease-associated proteins. The adenovirus shaft sequences can provide a model system to study folding, assembly and registration of beta-type structures both in native and in amyloid contexts.

Collagen interfibrillar spacing in the developing chick cornea and the link with keratan sulphate

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Proteoglycans in the avian corneal stroma are seen as potential modulators of corneal structure in the latter stages of development as the tissue becomes transparent. Previous chemical quantification of glycosaminoglycans from corneal isolates has indicated no change in the amount, molecular size, or degree of sulphation between developmental days 10 and 14 (Hart, J Biol Chem 1976;251:6513-21). After this time keratan sulphate becomes more highly sulphated, with the proteoglycan lumican likely bearing most of these chains (Cornuet, et al. IOVS 1994;35:870-7; Dunlevy, et al. Exp Eye Res 2000;70:349-62). The current study was designed to ascertain whether or not changes in the levels of sulphated KS in the corneal stroma correlate with alterations in collagen fibril spacing.

To do this we first carried out a SAXS analysis on Daresbury Station 2.1 of 78 isolated corneas from chicken embryos obtained daily from developmental day 12 to day 18 (n10 to 12 at each timepoint). This provided us with a measure of the average centre-to-centre collagen fibril Bragg spacing. Next, quantification by ELISA of papain digests of the same corneas was performed using the monoclonal antibody 5/D/4 that recognises highly sulphated epitopes on the KS chain.

Antigenic KS in the developing chick cornea (given as ug/mg wet weight of tissue (+/-SD)) measured 2.7+/-1.5 (day 12), 3.1+/-0.7 (day 13), 2.0+/-0.8 (day 14), 2.6+/-1.5 (day 15), 11.6+/-4.4 (day 16), 16.1+/-8.4 (day 17), and 24.1+/-7.9 (day 18). At the same time the fibril spacing in these same corneas dropped from 65nm to 53nm, and mainly this occurred after day 15. For the data set of 78 individual corneas interfibrillar Bragg spacing and antigenic KS levels showed an inverse correlation (p0.001; R*20.501).

As the chick cornea develops and becomes transparent in the week before hatching the compaction of stromal collagen fibrils is accompanied by an increase in tissue levels of sulphated KS.

Structural aspects of force generation by myosin heads probed by X-ray interference in single frog muscle fibres

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Force generation in muscle is thought to be due to transition between states with different degree of tilting of the light chain domain of the myosin head (Rayment et al., *Science* **261**, 58, 1993). We tested this idea by using X-ray diffraction interference to measure the changes in axial position of the heads associated to changes in isometric force with temperature, a factor that is known to change the force per myosin head (Piazzesi *et al., J. Physiol*., **549**, 93, 2003 and references therein). During the isometric contraction of single muscle fibres of *Rana temporaria*, X-ray interference between the two arrays of myosin heads in each myosin filament splits the M3 reflection from the 14.5 nm axial repeat of the heads into two main peaks (Linari *et al., PNAS* **97**, 7226, 2000). In the experiments reported here patterns were collected at ID2 (ESRF) on the image intensified FReLoN CCD detector placed at either 10 m (to collect intensity and fine structure of the low order meridional reflections) or 3 m (to collect intensity of the higher order meridional reflections and of the actin layer lines). Increasing the temperature from 0°C to 17°C increased the isometric tetanic force (T_0) by ~40% (43±2%), decreased the relative intensity between the high angle peak and the low angle peak (R) of the M3 reflection from 0.93 ± 0.02 (mean ± SE, 5 fibres) to 0.77 ± 0.02 and increased the spacing of the M3 and M6 reflections linearly with the isometric force by the same amount as that estimated with step length changes (0.26%/T_0, Reconditi *et al., Nature* **428**, 578, 2004). The intensity of the 1st actin layer line, measured in the same range of temperatures, increased by 57 ± 18% (5 fibres). The extension of the actin filament measured by the spacing changes of the 5.1/5.9 nm actin-based layer lines increases linearly with the force, with a slope 2.5 times that obtained from length step experiments (0.74 ± 18%/T_0 instead of 0.2-0.3%/T_0, Huxley et al., *Biophys*. J. **67**, 2411, 1994; Wakabayashi et al. *Biophys*. J. **67**, 2422, 1994) indicating that the actin filament is viscoelastic. Changes in the fine structure of M3 reflection that accompany temperature dependent changes in the isometric force show that a step forward in the working stroke can be entropically driven. A simulation of the changes in R, with the tilting lever arm model of the myosin head and a mechano-structural model of the sarcomere, indicates that the axial movement of the heads accounts for the increased instantaneous stress-strain relation of the myofilaments.

Supported by MIUR (Italy), MRC (UK), ESRF, EMBL

SAXS study of lobster aortal microfibrils

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We have obtained for the first time X-ray diffraction images of lobster aortal microfibrils. Lobsters have highly developed circulatory systems, but little is known about the exact tissue structure and biomechanical properties of their aorta. Microfibrils are essential components of the extracellular matrix, and are thought to impart force-bearing and elastic biomechanical properties to dynamic connective tissues. The ultrastructure of microfibrils is rather complex, due to their non-crystalline, variable, heterogeneous nature. Multidisciplinary studies of mammalian tissues have revealed that microfibrils form linear structures with a characteristic beaded appearance of an average periodicity of 56nm in the relaxed state. Our preliminary SAXS investigations under stretching conditions gave us some ultrastuctural information that can be compared with that of mammalian microfibrils. The tentative fundamental axial periodicities we have obtained in relaxed and tensed states are smaller than those for mammalian. The understanding of the organisation of microfibrils across various species is a fundamental step towards biomimetics. Interestingly, previous workers (Shadwick, 1992) have shown that elastin,

which is a major component of mammalian tissue, is not found in lobster aorta. However, lobster microfibrils do exhibit a modulus of elasticity similar to that of elastin, implying a unique elastic role for the lobster aortal microfibrils. Our SAXS study addresses important questions about elasticity mechanisms, for example whether or not extension of the aortal tissue is due to the deformation of microfibrils themselves.

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I22 - an NCD beamline for Diamond

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The requirements to meet the scientific and technological challenges of the next decade are for a high resolution, high brightness beamline. These can only be provided by an undulator insertion device on a 3rd generation synchrotron radiation light source. I22, served by a U25 in vacuum undulator will deliver high photon flux (1 x 10^{14} ph/s/0.1%b.w.) into a focused 300 x 75 m spot (1 x 1 m with microfocussing) in the energy range 4-20keV. The End Station with associated linear and area detectors for static and time resolved measurements will be capable of recording the scattered radiation from samples contained in purpose designed specialised environmental cells. The end station's modular arrangement will allow a choice between small angle scattering for large fibrous structures or microfocus illumination each with a wide angle scattering option for materials studies. The latest developments in design for the beamline will be described.

Medical Application of Diffraction Imaging

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Life sciences exploitation of X-rays has followed two distinct disciplines to date, medical imaging and X-ray diffraction. Each field has developed independently of one other. Analysis of X-ray diffraction data enables effects of structures at molecular and supramolecular scales to be determined. Until now the more conventional X-ray absorption has relied upon the removal of scattering effects that occur whenever X-rays traverse matter in order to produce high quality images be it conventional absorption or sophisticated 3-dimensional imaging such as computer assisted tomography.

Diagnostic methods rely on the detection of abnormalities either by appearance or chemical/histopathalogical differences that can be recognised by a clinician. Diagnoses can be highly subjective and any new method that adds to diagnostic armoury, preferably one that has a more direct way of measuring biochemical and structural changes, would be valuable. X-ray diffraction will gather information regarding the molecular structure of the tissue. The big advavantage of using X-ray diffraction is that tissue specific components (e.g. collagen, lipids etc.) can be isolated and used to form images.

An initial application of this technique that is being explored is that of breast cancer diagnosis. Breast cancer has been chosen as an initial application as it is a particularly accessible connective tissue. Scattered X-rays are discarded as a mater of coarse as they would degrade standard mammography scans which accounts for 60% of the incident X-rays. These scattered X-rays contain a wealth of information about the molecular structure which may well provide earlier diagnosis. There is evidence that cancers affect the molecular structure of the connective tissue there is an opportunity for early identification of cancers using a non-invasive method. Rather than simply a number from a site biopsy, this method will provide a visual method of identification which will work well with current problematic cases from young women where breast density (i.e. greater collagen content).

The current stage of research has software in place ready to create images from series of wide beam diffraction patterns from phantoms and test tissues. Here methods of image construction are discussed which provide a relatively automated way of producing diffraction images.

In conjunction with histological methods and analysis of narrow beam diffraction patterns, core cut samples will be characterised to determine whether clinically useful information can be derived. Once 2D has been fully explored then 3D molecular imaging will also be used.

X-ray diffraction experiments on heart

N. Yagi

SPring-8/JASRI

Heart is a physiologically and medically important organ. However, there have been only a very limited number of x-ray diffraction studies on cardiac muscles, especially on intact ones. At SPring-8, small-angle diffraction experiments are carried out on both isolated papillary muscle and whole heart. In both cases, the specimen is kept

under a physiological condition and beating continuously. Although the amount of information available from the diffraction pattern, which is dominated by equatorial reflections, is not abundant, the feasibility to study molecular aspects of cardiac function under different physiological and pathological conditions makes these experiments attractive to the medical society.

Use of size exclusion chromatography for Improved SAXS measurements

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We have combined small angle X-ray scattering and size exclusion chromatography within a unified experimental set up to obtain molecular size information Besides providing simultaneous corroborative data bearing on the same question from two distinct experimental techniques, passing the samples over a gel filtration column immediately prior to illumination by X-rays provides both a more homogeneous sample and a continuous set of data as the concentration is extrapolated to zero. This greatly facilitates analysis of data from oligomerizing or aggregating proteins and increases the reliability of the results.

Age-related changes in collagen fibril diameters in the mouse tail tendon

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The extra-cellular matrix (ECM) in tendons contains collagen-rich fibrils which provide reinforcement for the tissue. Changes in the size and composition of these fibrils, e.g. during the process of ageing, will affect the overall mechanical properties of the tissue. We have characterized collagen fibril diameters in tail tendons from young to old mice to complement an on-going ageing study on the composition of the ECM. Collagen fibril diameters and the frequency distributions of these diameters were determined for tail tendons from young to old mice using transmission electron microscopy (TEM). We have also derived the fibril packing fraction from TEM images. For each age group small angle x-ray scattering (SAXS) pattern of a tendon from the same tail was acquired; the intensity along the equatorial region of the SAXS pattern was compared with calculated values obtained from a mathematical model of the form factor using fibril diameter data from TEM. This approach provides a way for assessing how representative is the population of fibrils selected for diameter analysis. Additionally, the goodness-of-fit between the two sets of results also provide a check for the packing density derived from TEM. Our findings show that fibril diameters are distributed over a range of values. The spread of values is skewed in young mice and broadens, with increasingly larger diameters observed, as mice mature. Consequently, the mean fibril diameter in the tail tendons increases from 6 week to 4 months. From 4 to 30 months, no appreciable increase was observed. The packing fraction of fibrils in the tendon also exhibits similar trend with age. The information derived from this study, in combination with those from a separate study on ECM composition, may then help provide insights into the mechanisms governing the changes in the mechanical properties of the tendon.

PROP - Bridging the Gorge between Unix and Windows

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The calculations performed in programs originating on Unix are usually easy to port to PC-Windows. The Windows user however is little used to the command line style of Unix, and is faced with constructing batch files to present a correct environment for the ported program. He then has to face the problem of printing the results on a Windows printer as well as dealing with PostScript graphics files.

PROP is a graphical program written in Tcl/Tk which aims to reduce this trauma by providing an environment (often set up in the Unix login scripts) for installing, running programs, and viewing and printing results. To simplify installation it has been packaged together with the Tk interpreter, and also basic graphical libraries and tools for Tim Pearson%27s PGplot graphics for both MinGW and Visual Studo compilers. These latter include a Windows graphics server from Tsuguhiro Tamaribuchi which again resolves many problems of printing on Windows. The source code of programs using PGplot libraries remains identical on Unix and Windows.

PROP has a program directory defined; programs therein

appear in a list box, and can be started by selection. Console programs are given a temporary console window; GUI applications are launched separately. Results are accumulated in PROP%27s working directory, and again these can be listed, and selection allows results to be viewed in an editor and printed. A re-usable PGplot graphics window is used for all graphics, and there are options to print from this.

A practical example of use is in federating together the set of SANS data treatment programs at the ILL. The MinGW compiled programs for Windows now share code with Unix, Linux and Macintosh OS-X programs.

More information on the program and graphical tools may be found at http://www.ill.fr/data_treat.

In-situ WAXS studies of structural changes in wood foils and in individual cells during microtensile tests

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The mechanical behaviour of wood is closely related to the cell wall architecture and to the magnitude of microfibril angle (MFA). Though there have been a significant effort to chacterize both the structural properties of wood cell wall and the mechanical performance of the tissue, systematic in-situ investigations of wood microstructure under external forces are rare. In this contribution, structural changes in wood foils and in individual wood cells upon mechanical loading are studied.

Three different compression wood types were chosen, namely *Ginkgo biloba* L., *Juniperus virginiana* L. and *Picea abies* [L.] Karst, for wide-angle x-ray scattering (WAXS) combined with tensile tests. The experimental setup allowed to monitor simultaneously the structural development in foils (or in individual cells in the case of single cell experiments) using a 2D CCD detector and relate this information to the actual magnitude of stress and strain.

The results indicate that the MFA magnitude decisively influences the mechanical performance of the tissue. The stress-strain dependence in foils from various tissues as well as the MFA dependence on the strain can be in some respect derived from the initial MFA magnitude at the beginning of the tensile experiment. Generally, the magnitude of MFA decreases linear with the increasing strain though, in individual cells, the decrease is inhomogeneous. The WAXS and mechanical data moreover indicate that the hierarchical architecture with specific MFA predefine also the maximal stress and strain magnitudes applicable. A qualitatively novel information was obtained from tensile experiments with interrupted straining or with loading and unloading cycles. The results indicate a stiffness recovery phenomena beyond the yield point even for strain values very close to fracture. This behavior was interpreted by a molecular stick-slip mechanism in the cell wall between the cellulose microfibrils which allow a reorganization of the amorphous cell wall constituents during the tensile experiment [1].

Reference

[1] J. Keckes, I. Burgert, K. Frühmann, M. Müller, K. Kölln, M. Hamilton, M. Burghammer, S.V. Roth, S. Stanzl-Tschegg & P. Fratzl (2003). Cell-wall recovery after irreversible deformation of wood, *Nature Materials* **2**, 811-814.