

12th Annual Workshop Abstracts

MYOSIN HEAD CONFIGURATION IN RELAXED INSECT FLIGHT MUSCLE: X-RAY MODELLED RESTING CROSS-BRIDGES IN A PRE-POWERSTROKE STATE ARE POISED FOR ACTIN BINDING

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Low-angle X-ray diffraction patterns from relaxed insect flight muscle recorded on the BioCat beamline at the Argonne APS have been modelled to 6.5 nm resolution (R-factor 9.7%, 65 reflections) using the known myosin head atomic coordinates, a hinge between the motor (catalytic) domain and the light chain-binding (neck) region (lever arm), together with a simulated annealing procedure. The best head conformation angles around the hinge gave a head shape that was close to that typical of relaxed M·ADP·Pi heads; a head shape never before demonstrated in intact muscle. The best packing constrained the 8 heads per crown within a compact crown shelf projecting at ~90° to the filament axis. The two heads of each myosin molecule assume non-equivalent positions, one head projecting outward while the other curves round the thick filament surface to nose against the proximal neck of the projecting head of the neighbouring molecule. The projecting heads immediately suggest a possible cross-bridge cycle. The relaxed projecting head, oriented almost as needed for actin attachment, will attach, then release Pi followed by ADP, as the lever arm with a purely axial change in tilt drives ~10 nm of actin filament sliding on the way to the nucleotide-free limit of its working stroke. The overall arrangement appears well-designed to support precision cycling for the myogenic oscillatory mode of contraction with its enhanced stretch-activation response used in flight by insects equipped with asynchronous fibrillar flight muscles.

Order-order transitions in block copolymer solutions: epitaxy, mechanisms, and kinetics

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Block copolymer solutions exhibit a rich variety of thermotropic and lyotropic order-order transitions. Many of these transitions are epitaxial, although quite complicated in

detail. The use of shear-orientation in combination with SAXS is crucial in understanding the transition mechanisms, as will be illustrated for the transformation of close-packed to body-centred lattices of spherical micelles. A combination of SANS and SAXS has been used to quantify the temperature dependence of the micellar characteristics, and thus to establish exactly how changes in solvent selectivity determine the choice between close-packed and body-centered packings. Finally, transition kinetics can be followed by a variety of techniques, but the use of polarized optical microscopy is particularly helpful in characterization nucleation density and individual grain growth rates, as will be demonstrated for the formation of the gyroid phase.

Astringency - A Molecular Approach

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Astringency is generally described as a feeling of dryness, puckering or roughness in the mouth and is associated with a number of beverages, including tea, coffee, beer and red wine. All those beverages contain tannins which are water-soluble, polyphenolic compounds with molecular weights between 500 and 5000 Da. The sensation of astringency is the result of the binding of plant polyphenols to salivary proline-rich-proteins (PRP). The main binding interaction is a hydrophobic stacking of a phenolic ring belonging to a polyphenol over a prolyl ring of a salivary PRP. The PRPs lack any secondary structure and exist as random coils in the saliva. The studies presented suggest that upon binding of polyphenols the molecular dimensions of the proline-rich proteins decrease; this may be due to multiple binding of one protein to one polyphenol. The proline-rich protein (PRP) is able to wrap around and bind very tightly to the phenolic compounds, the random coiled structure becomes therefore more dense and compact.

In this project dephosphorylated b-casein is used as a model for salivary proline-rich protein and (-)-epigallocatechin gallate (EGCG) as a representative for tannins. The effect of the binding event on the structure of b-casein is investigated using small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) pulsed gradient spin echo methods, analytical ultracentrifugation, dynamic laser light scattering (DLS) and transmission electron microscopy (TEM).

Kinetics of Phase separation in Non-ionic Systems

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We will report on studies of the phase separation in non-ionic block copolymer systems. The structure formation in these systems evolves rapidly from a disordered mixed state to a microphase separated state, with a length scale ideal for SAXS and SANS studies. We will present recent results on the evolution of structure within these systems after a temperature quench.

Using Hydrogenated Polybutadienes with Novel Architectures to Learn about Polymer Crystallisation

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During processing, crystallisation is the essential step that determines the quality of the final product (*i.e.* toughness, smooth surface, elasticity, *etc.*). Thus, in order to develop more useful, job specific, materials, the crystallisation process has to be controlled. To do this, model compounds with well-defined architectures are required. They can facilitate the understanding and prediction of the relationships between polymer properties and their structure.

Polyethylene-like materials from hydrogenated polybutadienes with well-controlled molecular architecture, a range of molecular weights and very narrow polydispersities have been made, using anionic polymerisation techniques. Small- and Wide-angle X-ray Scattering (SAXS/WAXS) are excellent tools to look at the macrostructure development or long range ordering (lamellae stacking) (SAXS) and the microstructure development or crystalline atomic ordering (WAXS). Here we investigated the quiescent crystallization of hydrogenated polybutadienes by means of time-resolved, simultaneous SAXS/WAXS/DSC techniques at synchrotron sources (SRS CLRC Daresbury Laboratory, UK and at ESRF, Grenoble, France). These allowed the kinetics of crystallisation to be analysed and the morphological parameters of the samples to be determined (using correlation function analysis).

Results obtained from scattering were also confirmed by other laboratory techniques, such as Differential Scanning Calorimetry (DSC) and polarised optical microscopy. Avrami exponents, which are a measure of the dimensionality of growth, were obtained and compared.

Future work will involve using shear regimes during scattering and optical microscopy experiments. Also, rheology measurements will be performed.

References

1. G. Ungar, Xiang-bing Zeng, *Chem. Rev.* 2001, **101**, 4157-4188
2. B. Chu, B.S. Hsiao, *Chem. Rev.* 2001, **101**, 1727-1761

Progress in the evaluation of SAXS data from dense and partially ordered systems

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During the last years we have developed the so-called generalized indirect Fourier transformation method. It allows the separation of the form factor and structure factor from SAXS and SANS data from interacting dense systems. The most recent development concerns the evaluation of scattering data from lamellar systems. The basics of the method will be explained and some applications will be demonstrated.

Beamline 11 on Diamond for the Non-Crystalline Diffraction Materials and Life Sciences Communities

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Beamline I22 has been approved for year 2 on DIAMOND. The beamline is to include features for standard Small Angle scattering and fibre diffraction as well as a microfocus capability. The latest technical design ideas for the beamline will be presented.

Nucleation of thermodynamically stable structures for polyglutamine amyloid fibres

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It has been recently suggested [1] that the formation of amyloid plaques, which are implicated in the pathology of a wide range of neurodegenerative disorders, may be related to nucleation of protein aggregates. In one particular case, Huntington's disease, it is known that the age of onset depends exponentially on the length of a sequence of glutamine residues in the Huntingtin protein. This can be explained if the rate-determining step in the disease process is the formation of a stable nucleus for a fibrillar amyloid structure. In order to test this hypothesis, we have examined a number of proposed structural models for polyglutamine fibres using canonical molecular dynamics

simulations [2]. We discuss the thermodynamic stability of these models as a function of time, temperature and glutamine repeat length, and use these results to parameterise a simple model for the aggregation process. We also present simulated X-ray fibre diffraction patterns from the polyglutamine amyloid nuclei that show similar features to experimental patterns.

References

- [1] M. F. Perutz and A. H. Windle *Nature*, **412**, 143-144, 2001.
 [2] J. A. Elliott, J. Starikow, J. Crawshaw, P.R. Claiden and A.H. Windle, in *Nucleation Control* (eds. G. W. Greenwood, A. L. Greer, D. M. Herlach, K. F. Kelton), CUP, Cambridge, in press 2003.

SAXS ANALYSIS OF POLYURETHANE MORPHOLOGY AND ITS RESPONSE TO DEFORMATION.

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Thermoplastic polyurethanes (TPU) are usually copolymers, composed of alternating urethane-containing hard segments (HS) and more flexible soft segments (SS). As a result of immiscibility between the HS and SS parts of the polymer, microphase-separated morphologies can develop, with characteristic lengths on the nanometre scale, which depend on the overall polymer composition and processing history. In this structure, the dispersed, HS-enriched, microdomains act as physical cross-links within the surrounding SS-enriched matrix, which gives rise to the elastomeric properties of TPU.

In the present work, the morphological responses of several TPU formulations were studied during deformation, using real-time measurements of two-dimensional small-angle X-ray scattering (2D-SAXS). The results were analysed on the basis of the model proposed recently by Blundell *et al.* [1,2], in which the 2D-SAXS patterns were interpreted as arising from rigid particles on a deformable statistical lattice. By curve-fitting this model to the experimental data, it was found that the morphological responses involved a combination of HS-enriched microdomain rotation, fragmentation and approximately affine deformation of the SS-enriched matrix.

This work forms part of a larger project to develop polyurethanes for medical applications. A thorough understanding of the mechanical behaviour of TPUs is, clearly, of key importance. Moreover, the morphology may also affect the biostability of these polymers.

References

- [1] Blundell, D.J. Eeckhaut, G. Fuller, W. Mahendrasingam, A. and Martin, C. *Polymer* 2002; **43**: 5197-5207.
 [2] Blundell, D.J. Martin, C. Mahendrasingam, A. Fuller, W. and Eeckhaut, G. *Fibre Diffraction Review* **2002**; 10: 50-56.

A North American Research Coordination Network for Fiber Diffraction

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A network of fibre diffraction groups has been established to coordinate activities in the USA. The goal of the network is to develop biological fibre diffraction methods, particularly computational methods. This will be done through a program of software development, and through a series of retreats and workshops. The network software will be complementary to CCP13 software; together, they will eventually cover all aspects of biological fiber diffraction.

A major focus of activity will be cooperation between fibre diffractionists in the USA and others, particularly those in Britain. A second focus will be the coordinated use of the BioCAT X-ray beamline facility at the Advanced Photon Source, Argonne National Laboratory. Network meetings will include three types of activity: retreats that will formalize a successful informal series held sporadically since 1989, workshops at BioCAT, and partial sponsorship of sessions organized by the fiber diffraction Special Interest Group of the American Crystallographic Association.

Towards the complete atomic structure of the bacterial flagellum

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The bacterial flagellum is made of a rotary motor and a long helical filament by means of which bacteria swim. The flagellar motor at its base rotates at around 300 Hz and drives the rapid rotation of each flagellum to propel the cell's movements in viscous environments. The filament is a tubular structure with a diameter of about 20 nm and made of 20,000 to 30,000 copies of a single protein flagellin, and yet the filament can form left-handed or right-handed helical forms and switch between these two in response to the twisting force produced by quick reversal of the motor rotation. This allows bacteria to alternate their swimming pattern between run and tumble for taxis. The filament is connected to the motor through a highly curved short segment called the hook. Its bending flexibility makes it function as a universal joint, while the filament is relatively

more rigid to work as a propeller. A very short segment called the hook-filament junction, made of HAP1 and HAP3, is a mechanical buffer to connect these two mechanically distinct structures. The flagellum is constructed through various self-assembly processes, in which all the axial structures growing in the cell exterior are constructed by proteins translocated from the cytoplasm to the distal end of the growing structure, where three different cap complexes help efficient self-assembly of these proteins in different stages.

We have been trying to visualize the structure of the flagellum in atomic detail to understand how it self-assembles and works. We solved the crystal structures of core fragments of the flagellar axial component proteins by X-ray crystallography. X-ray fibre diffraction gave high-resolution structural information. Electron cryomicroscopy also visualized the structures of the filament, cap and cap-filament complex, and recently enabled us to build an atomic model of the filament based on a density map at 4 Å resolution. All these structures have given interesting implications for the function of each molecule, demonstrating the importance of the dual nature of protein molecules, flexibility and precision.

Examining the structure of amyloid fibrils

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Amyloid fibrils deposited in the tissue in a number of diseases are known collectively as the Amyloidoses. This group of diseases includes Alzheimer's disease as well as the Spongiform encephalopathies. Each disease is characterised by a different protein that aggregates to form amyloid fibrils. The normally functional protein undergoes considerable conformational change associated with the fibrillogenesis.

We have examined the structure of amyloid fibrils using X-ray diffraction as well as electron microscopy. Using short peptides to make ordered amyloid fibrils in vitro, we have been able to collect ordered diffraction patterns and electron microscopy images to enable us to construct a model for the beta-sheet core structure of the amyloid fibril.

Liquid crystalline aspects of starch structure

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Small Angle X-ray Scattering (SAXS) and Small Angle Neutron Scattering (SANS) have been used to study the internal supramolecular packing within starch granules. These small angle scattering techniques are able to provide information on the packing of the semicrystalline lamellae and the amorphous growth rings. Rationalising the changes in the lamellae that occur under different conditions (such as temperature and extent of plasticisation) it is useful to consider the constituent highly branched amylopectin molecule within the framework of side chain liquid crystalline polymers. The side chains are

known to form double helices which then order laterally, and these double helices are considered as the mesogens. In unplasticised starches the mesogens are disordered forming a nematic phase, but upon solvent ingress their packing improves and a smectic phase is formed. Furthermore, by comparing the SAXS from different species of starch, it becomes apparent that the nature of the lamellae themselves varies between species. Those which have a significant length of flexible spacer can form rather flat lamellae, but undulations of the lamellae are present if the antagonistic effects of side chain and backbone organisation dominate.

Structures of classical and therapeutic antibodies by constrained solution scattering modelling

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Highly constrained scattering curve modelling potentially offers many advantages for determining solution structures for antibodies. Crystal structures for the antibody Fab and Fc fragments are often known for scattering modelling purposes. The main unknown is the averaged structure of the hinge peptide that connects each Fab fragment to the Fc fragment. A trial and error strategy based on the generation of as many as 10,000 random conformations of the hinge peptide generally results in the identification of a few structures that fit the scattering data. The results are biologically significant in explaining the several unique features of the antibody in question. Two examples will be discussed. (1) Two classic 12-domain antibody structures are exemplified by the monomeric forms of the IgA1 and IgA2 subclasses. The applications and adaptations of the above strategy to determine their solution structures will be discussed. IgA1 is notable for possessing a long glycosylated hinge, while IgA2 possesses a short hinge and a disulphide bridge connecting the two Fab fragments. (2) Antibodies designed for therapeutic treatments have their Fab fragments replaced by an active protein reagent, thereby doubling the concentration of the reagent, and coupling this with antibody effector function. The activity of the chimeric antibodies thus created will depend on its solution structure. The case of the five-domain rodent complement-related receptor protein/gene γ (Crry) will be discussed. The determination of the solution structure for the Crry chimera with an Fc fragment was able to account for its reactivity.

Grazing-incidence X-ray diffraction studies of polyfluorenes

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We report on grazing-incidence X-ray diffraction and reflectivity studies of aligned pi-conjugated polymers. Since both the applications and basic research of pi-conjugated polymers are concentrated on thin films, the thin film structures instead of the fibre structures are the focus of interest here. Among them, we have studied the important polyfluorene class of materials. We present excellent data concerning the film quality and we find interesting and novel results on the molecular ordering in the films with highly aligned molecules forming different phases in different orientations. These properties are dependent on the processing conditions.

Rheology and structure of hydrophobically modified polyacrylamide solutions in the semidilute regime

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The ordering of hydrophobically modified polyacrylamide polymers, with disubstituted acrylamides di-n-propylacrylamide and di-n-octylacrylamide used as hydrophobic comonomers, has been studied by small angle scattering (SAXS) and rheology. The overall content of hydrophobe and length of hydrophobic sequences in the random copolymer was varied using a micellar copolymerization technique. Changes in the correlation length of the semidilute solutions as a function of the temperature are analyzed, and evidence is presented for cluster formation due to an increasing association between hydrophobic groups at high temperature. Information on the solution from SAXS is compared to that extracted from rheology experiments, and comparisons are made to scaling theories for associating polymers.

Two-dimensional maps of archaeological bone nanotexture: variation across bone features.

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The prediction of the survival of biomolecular material in retrievable form over archaeological time-scales has been a long-standing problem for archaeology and paleontology alike. Recent studies have shown that an intact mineral phase in bone can shelter biomolecules such as DNA and osteocalcin that can subsequently be extracted for analysis. We describe here a method of visualizing bone mineral nanotexture in modern and archaeological bone using microfocus small-angle X-ray scattering, in an attempt to determine areas of mineral alteration. Two-dimensional maps of bone crystallite shape and thickness have been produced here. These show both the variation present in modern bone due to histological features and the changes to the biogenic mineral structure following processes of diagenesis.

High Resolution Wide Angle X-ray Scattering of Protein Solutions

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Wide angle X-ray scattering patterns from proteins in solution contain information relevant to the determination of protein fold. But at relevant scattering angles these data are weak, and the degree to which they might be used to categorize the fold of a protein is unknown. Preliminary work has been performed at the BioCAT insertion device beamline at the Advanced Photon Source which demonstrates that one can collect X-ray scattering data from proteins in solution to spacings of 0.22 nm. These data are sensitive to protein conformational states, and are in good agreement with data modelled using the program CRY SOL and the known three-dimensional atomic coordinates of the protein. An important issue in the exploitation of this technique as a useful tool for structural genomics is the extent to which the high-intensity X-rays of a third generation synchrotron source chemically or structurally

damage proteins. Various data collection protocols have been investigated demonstrating conditions under which structural degradation of even sensitive proteins can be minimized, making this technique a viable tool for protein fold categorization, the study of protein folding, unfolding, protein-ligand interactions and domain movement.

Further Research Into Breast Cancer Diagnosis Using Small Angle X-Ray Scattering SAXS

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Preliminary research investigating the SAXS pattern obtained from breast tissue with a view to using the information as a marker for cancer, is present in the literature (K. D. Rogers *et al* 1999, R.A. Lewis *et al* 2000 and M. Fernandez *et al* 2002). These works compared the subtle changes in the SAXS pattern of normal, malignant and benign samples using synchrotron radiation. Changes are correlated with the disease state of the sample, which is determined by standard histo-pathological analysis. Our findings suggest that the structural organisation of the collagen within the connective tissue of the breast is disrupted in areas of tumour genesis. These changes were evident at some distance from the centre of the tumour. However, before this technique could be considered for diagnosis further work is required to ensure accuracy, repeatability, sensitivity and specificity.

The supramolecular characteristics and mechanical properties of elastic fibrillin-rich tissues

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Fibrillin-rich microfibrils are essential elastic structures contained within the extracellular matrix of a wide variety of connective tissues. Microfibrils have been characterised as macromolecular structures with a regular beaded appearance and fundamental axial periodicity of approximately 56nm in the untensioned state. Small-angle X-ray scattering studies have proven to be pivotal in the understanding of these structures.

Despite extensive investigation, however, the basis of this elasticity remains unknown. This study combined small-angle X-ray scattering and Raman microscopy for the first time to investigate the packing of microfibrils within the intact tissue and to determine the role of molecular reorganisation in the elasticity of microfibrils.

The application of relatively small strains produced no overall change in either molecular or macromolecular microfibrillar structure. In contrast, the application of large forces to the tissue resulted in a markedly different structure, as observed by both small-angle X-ray scattering and Raman microscopy. These changes occurred at different levels of architecture and are interpreted as ranging from alterations in peptide bond conformation to domain rearrangement. This study demonstrates the importance of molecular elasticity in the mechanical properties of fibrillin-rich microfibrils in the intact tissue.

Molecular packing interactions in type I collagen as revealed by X-ray diffraction of cryo-cooled tendon

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Regular crystalline-like three-dimensional (lateral) packing interactions between type I collagen molecules in rat tail tendon produce discrete Bragg peaks that intersect the equatorial plane of the X-ray diffraction pattern as a series of row-lines. These rowlines are overlain by a slowly varying layer of diffuse scatter that is thought to arise from liquid-like thermal and static molecular disorder. We show here that cryo-cooling the sample during data collection alters the profile of this diffuse scatter without disrupting the triclinic lattice of type I collagen. There is a lower overall intensity of diffraction in the cryo-cooled samples, both in the diffuse scatter and Bragg peak profiles and particularly in the near-equatorial region, where there are strong contributions from the gap region. Any diffuse scatter remaining after cryo-cooling may arise from a combination of static (positional) molecular disorder and residual thermal movement. Results are also shown of progress made towards successfully diffracting a single fibril of collagen, of typical diameter 100-500 nm. This is a technically challenging project, but one that will present a unique opportunity to track molecular paths through the gap and overlap regions at a more fundamental level of structure.

Collagen Organisation in Adult and Foetal Marmoset Cornea

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Purpose: The precise arrangement of fibrillar collagen in the corneal stroma influences the tissue's transparency and dioptric power. The purpose of this study was to examine fibril organisation in adult and foetal marmoset cornea in order to assess their potential as animal models for exploring corneal structure/function relationships.

Methods: WAXS methods were used to map the orientations and proportions of aligned collagen fibrils over 3 whole adult and 2 whole (age-matched) foetal corneas at a maximum spatial resolution of 0.5 X 0.5 mm. SAXS methods also provided information on the average diameter and separation of the fibrils at 0.5 mm intervals along 2 orthogonal corneal diameters.

Results: Collagen orientation maps of all adult and 130 day (of a 144 day gestation term) foetal marmoset corneas clearly showed a circumferential annulus of fibrils at the limbus, similar to that observed previously in humans and some other vertebrate species. More centrally, the foetal corneas exhibited a predominantly orthogonal arrangement of fibrils reminiscent of that seen in mature human cornea and in immature tissue from some lower vertebrates. The current data suggest that the preferred orientation in the foetal marmoset cornea may be preserved in the adult tissue; a situation which has thus far only been seen in humans. An index of X-ray scattering intensity from aligned collagen as a fraction of total scattering intensity from all fibrillar collagen showed that in the foetal tissue approximately 20% of fibrils were preferentially aligned in the central cornea, compared to 45% at the limbus. For the adults the corresponding values were 19% and 38%. SAXS results revealed fibril separations and diameters for the adult and foetal tissues in the normal range for monkey cornea. Both parameters remained fairly constant across the cornea before increasing sharply at the limbus; a pattern similar to that observed in human cornea.

Conclusions: This work represents the most comprehensive study of collagen organisation so far performed across whole, intact non-human corneas. The results suggest that, structurally, the human cornea may mirror that of the marmoset more closely than that of any other animal so far studied. By this token, the results reconcile with the established view, gained from optical and retinal studies, that the marmoset eye is well represented as a scaled-down version of the human eye. The marmoset could potentially make a useful animal model for corneal pathologies.

Diffraction from Small Crystals

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Recently, much attention has been focused on spinodal decomposition as a possible mechanism for nucleation in a number of semi-crystalline polymer systems. In many cases, the data presented are from simultaneous small and wide-angle X-ray scattering (SAXS and WAXS) experiments carried out during isothermal crystallization. A common feature of these measurements is that a build-up of small-angle intensity is observed some time before any wide-angle trace is discernible. On this basis it has been suggested that long-range density fluctuations are present, in the melt, before the onset of crystallisation, and which may promote the nucleation of crystals.

We have calculated the SAXS and WAXS patterns from randomly oriented stacks of ideal lamella crystals of iPP, at various stages during a hypothetical growth process. It can be shown that, when the crystals have a small lateral width, it is possible to detect a long-period peak in the SAXS pattern under conditions when the crystalline WAXS trace is too weak to be detected above the background noise. This finding has important implications for the interpretation of simultaneous SAXS and WAXS data, and in particular, it casts doubt on suggestions that such measurements, alone, can provide conclusive evidence for nucleation induced by spinodal decomposition.

Modelling based on Monte Carlo simulations

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SANS and SAXS studies of block copolymer micelles:

Block copolymers dissolved in a selective solvent form micelles with the insoluble blocks in the core and the soluble blocks in a solvated corona. Such micelles can be studied by small-angle neutron and X-ray scattering (SANS and SAXS, respectively). The data can be analysed by scattering functions recently derived from Monte Carlo simulations for a model with a spherical core and a corona of semi-flexible chains interacting with a hard-core potential. Least-squares fit to the data of this model gives very detailed information. It provides information on aggregation number, polydispersity, core size, core solvation, corona shape/size, intermicellar interactions, and on the interactions between the chains in the corona. Micelles of PS-PI (polystyrene-polyisoprene) of relatively high molecular weight in *n*-decane have been studied. Contrast variation SANS was performed using mixtures of hydrogenated and deuterated decane and by inclusion of SAXS data on the same samples. The results are in very good

agreement with simulation results on the same systems. In another study micelles of a Brij surfactant with a C18-chain and a poly(ethylene oxide) (PEO) block with 100 EO units have been studied in water as a function of temperature and concentration. In this study SANS and SAXS were done on the same sample to provide two different contrast conditions. The variation of the temperature results in a variation of the solvent quality of the PEO blocks and it is clearly observed that the interactions are reduced at elevated temperatures.

Simultaneous determination of structure and tensile properties of wood

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The purpose of this work was to investigate the behaviour of normally grown wood when subjected to tensile deformation. Small pieces of Norway spruce (*Picea abies* [L.] Karst.) were stretched and in situ WAXS (Wide Angle X-ray Scattering) measurements were made. This was done in order to simultaneously determine both the structure and the tensile properties of this material. The tensile properties of the samples can be determined from the stress/strain -curves while X-ray diffraction gives information on cellulose crystallisation, crystal structure and fibre texture. Diffraction patterns recorded before stretching and right before fracture were compared and from these patterns the reflections *004* and *200* of cellulose were investigated. The reflection *004* gives information on the dimension of the cellulose unit cell parallel to the cellulose chains, whereas the reflection *200* gives dimensional information perpendicular to the chains.

In order to achieve sufficient time resolution for the tensile test, a high intensity X-ray beam was needed. Therefore the use of synchrotron radiation was essential. Measurements were carried out at beamline A2 of HASYLAB, Hamburg. Samples were stretched with a constant rate of $2 \cdot 10^{-4}$ mm/s or $4 \cdot 10^{-4}$ mm/s and diffraction patterns were measured simultaneously using a MAR CCD detector. The exposure time was either 24 or 34 seconds per sample, depending on the scattered intensity. When the dead time of the detector is taken into account, the time resolution of the tests was 30 or 40 seconds, respectively. The results presented are from two early wood samples from different stems grown in southern Finland. Sample A is from the 22nd annual ring of stem 1 and sample B is from the 23rd annual ring of stem 2. Both samples represent wood material in mature phase. In stem 1, the average density was 341 kg/m^3 and in stem 2 it was 392 kg/m^3 , the average width of annual rings was 5.16 mm in stem 1 and 3.07 mm in stem 2.

To obtain more information on the structure of the samples in a non-strained state, other WAXS measurements were made using a reference sample set of the same material at the University of Helsinki using a diffractometer with Bragg-Brentano geometry and $\text{CuK}\alpha_1$ radiation. The orientation of cellulose microfibrils (Microfibril Angle, MFA) was determined by using X-ray diffraction arising from the lattice planes *200* and *004* of cellulose, the crystallinity of the samples was determined both with symmetrical reflection and symmetrical transmission geometry [1]. The mean MFA was determined to be (6 ± 2) degrees in sample A and (10 ± 2) degrees in sample B. Crystallinity of the samples was (34 ± 4) per cent in sample A and (33 ± 4) per cent in sample B.

In the tensile experiments, the maximum stress of sample A was (95 ± 5) MPa, while with sample B it was (47 ± 2) MPa. Fracture occurred at a strain of 6.7 per cent in sample A and at 1.4 per cent in sample B. Sample A was stretched with a rate of $2 \cdot 10^{-4}$ mm/sec, sample B with a rate of $4 \cdot 10^{-4}$ mm/sec. The intensities of the reflections became lower as a consequence of the applied strain, but the effect was not uniform in the samples. Changes in the shapes of the reflections occurred as well, but the changes were not unambiguous among the samples. Upon stretching, the reflection *004* shifted towards smaller and *200* towards larger scattering angles, which indicates changes in the dimensions of the unit cell. In the direction of the cellulose chains the unit cell elongated by (0.4 ± 0.1) per cent in sample A and by (0.1 ± 0.1) per cent in sample B. Perpendicular to chain direction the unit cell became thinner in sample A and remained unchanged in sample B, but the observed changes were much smaller in magnitude than in the direction parallel to the chains. No significant effect on the orientation of cellulose microfibrils was observed during stretching. A similar type of shift of the reflection *004* towards smaller scattering angles has been reported earlier on thin foils of wood [2], but in that study the microfibril angle was also affected. One reason for the difference can be that the wood foils used in [2] had much larger MFA than the samples used in this study.

References

- [1] Andersson, S., R. Serimaa and P. Saranpaa. 2003. The crystallinity of wood and the size of cellulose crystallites in Norway spruce. *J. Wood Sci.*, in press
- [2] Keckes, J., P. Fratzl and M. Hamilton. 2002. *ESRF Newsletter* 36: 13.

X-RAY AND NEUTRON DIFFRACTION STUDIES OF FIBROUS BIOLOGICAL MOLECULES

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Fibre diffraction is an extremely powerful technique for investigating the structure of biological polymer systems. The work described in this poster illustrates how X-ray and neutron diffraction methods can be used to provide highly complementary information that is not accessible to either method alone.

X-ray fibre diffraction methods have provided a major contribution to our understanding of a wide variety of biological polymers. However they are less effective for study of location of water and hydrogen atoms. Here neutron methods can provide vital information. The ability to deuterate the biopolymers either throughout the entire molecule or in a more specific way adds a powerful dimension to work aimed at investigating hydration patterns or hydrogen positions and changes that occur during water-driven transitions.

This poster also describes new facilities for sample preparation that are being developed between the ILL and the EMBL Grenoble Outstation. These facilities include a Deuteration Laboratory that will be dedicated to the production of deuterium labelled molecules for use in biological neutron scattering experiments.

New or updated versions of XCONV, XFIX, FTOREC & LSQINT for Windows and Unix platforms

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During the past decade many CCP13 related computer programs have been developed for stripping and analysing fibre diffraction patterns. However, most of this software was implemented on Unix platforms. Because of the increasing popularity of Microsoft Windows, we have recently redeveloped these programs, which include XCONV, XFIX, FTOREC and LSQINT, for Windows platforms. The

presentation will show how the four programs mentioned above have been redeveloped and how they work under Microsoft Windows.

Basically, XCONV is used to provide for the conversion of various image data files supported by the scanner to either a common format, BSL, that is used by other CCP13 software, or to TIFF format. XFIX is designed to display BSL files and to help to determine such things as the pattern centre, detector orientation and fibre tilt of a fibre diffraction pattern. It also allows the use of three different background subtraction tools. FTOREC is designed to transform image data from detector space to reciprocal space once provided with the specimen to film distance, the orientation of the image, the wavelength and the tilt of the specimen as determined in XFIX. LSQINT provides an automatic method for the integration of intensities for fibre diffraction data. It also allows four background subtraction options.

As well as implementation for Windows, several modifications and improvements have been made to existing programs to enhance the performance and capability of the CCP13 suite across different computer platforms.

Heavy water inhibits the super-aggregation process of α -crystallin in response to increasing temperature

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In our previous X-ray diffraction studies we have observed the super-aggregation of α -crystallin with increasing temperature both in the solution and the gel state (Regini *et al.*, 2003, *J. Mol. Biol.*, in press; Regini & Grossmann, 2003, *Fibre Diffraction Review*, 11, 95-101). The aim of our subsequent study was to determine possible changes in the hydration shell surrounding these super-aggregates during heating using both neutron and X-ray solution scattering techniques. Surprisingly, we found that in the presence of D₂O the super-aggregation process is significantly reduced with increasing temperature. This finding was established using both scattering techniques.

Fibre Diffraction Review On-line

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Fibre Diffraction Review (FDR) is a scientific journal with a specific interest in the fibre diffraction and non-crystalline diffraction fields. It includes original papers, technical reports, reviews, comments/letters and meeting reports. To disseminate FDR as widely as possible, an on-line version of Fibre Diffraction Review has been developed. From now on, all the published FDR papers, including papers in back issues, are being made available in PDF format for downloading from the CCP13 websites. To further maximise broad publicity and accessibility for authors and readers, the journal has joined CrossRef, which is the citation-linking backbone for on-line publications. Established in 2000 by scholarly publishers as an independent, non-profit entity, CrossRef enables researchers to navigate electronic journals across publishers, based on open-standards technology. Each document is tagged with a Digital Object Identifier (DOI). Here we report the progress on the preparation of DOI metadata. As soon as this has been done, each article in FDR will have a DOI for cross-referencing throughout the on-line environment. Finally, an example of how to search an article by DOI is also presented.

Development of a CCP13 Front-End GUI ProgramAndrew He¹, Ganeshalingam Rajkumar¹, David Dover¹, Trevor Forsyth², John Squire¹

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CCP13 is the Collaborative Computational Project for fibre diffraction started on 1st January, 1992. Its primary interests are to develop software used to strip, analyse and model data from fibre diffraction patterns. Since then, CCP13 has made a set of programs available. What we present here is the development of a Front-end graphical user interface (GUI) program called CCP13 Front-End (now known as ICE) as an attempt to integrate these programs together. Apart from the standard windows menus, this program consists of a program start-up panel, a file display panel and a file and directory panel. For example, the program start-up panel can dynamically load a number of CCP13 programs and launch any of them by clicking the loaded program image icon. The file display panel is a client windows container that holds the CCP13 program editor and common image display windows. When we click on a text file or an image file on the file and directory panel, a CCP13 program editor or common image display window will be launched as a separate client window inside the container. The main purpose of developing this program is to increase user productivity and to provide a user-friendly environment to the CCP13 software user.

NEW OPPORTUNITIES IN NEUTRON SINGLE CRYSTAL AND FIBRE DIFFRACTION ON D19 AT THE INSTITUT LAUE LANGEVINV.T. Forsyth^{1,4}, S.A. Mason¹, J.A.K Howard², M. Davidson³, W. Fuller⁴, D.A.A. Myles⁵

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Neutron diffraction has proven applications in biology, chemistry, physics, materials science and polymer science [1,2]. D19 is the only instrument at the ILL that can record monochromatic single crystal diffraction data to atomic resolution from small samples with relatively large unit cells; it is also without doubt the best instrument in the world for neutron fibre diffraction. Until now the limited size of its detector has restricted applications in the most challenging fields.

D19 is currently undergoing a complete rebuild that will yield a factor of ~25 in performance. This upgrade, which is funded in large part by a major EPSRC award, will have a real impact on the quality of experiments, combining all the advantages of monochromatic data with new opportunities arising from multiparametric experiments, smaller samples and the ability to study larger unit cells. It will considerably widen the scope of both single crystal and fibre diffraction experiments [3].

In chemical crystallography, new fields will be opened up in charge density analysis [4], accurate structure determination for crystal engineering [5], studies of transition metal catalysis [6,7], structural studies of new organic materials [8], characterisation of weak intermolecular interactions involving hydrogen [9], and structure-property studies of optoelectronic/magnetic materials [1]. In biological crystallography, there will also be strong impact for the study of oligonucleotides and small proteins. Of particular interest are situations where data at moderate resolution (~2Å) can yield definitive information on H atom and water positions, or even where high resolution data are available but where large thermal displacement parameters (>10Å²) make their visualisation by X-ray diffraction very difficult. Other applications include fibre diffraction studies of biopolymers (nucleic acids, filamentous viruses, cellulose & other polysaccharides, chitin, amyloid fibres etc) [10,11,12].

References

- [1] Wilson, C.C., *Single Crystal Neutron Diffraction from Molecular Materials*, World Scientific Publishing (2000);
 [2] G. Stubbs, *Current Opinions in Structural Biology* **9**, 615-619 (1999);

- [3] Forsyth, V. T., Mason, S. A., Howard, J. A. K., Davidson, M. G., Fuller, W. and Myles, D. A. A., *Neutron News* **12** (4), 10 (2001);
- [4] Coppens, P., *X-ray Charge Densities and Chemical Bonding* IUCr/Oxford University Press (1997);
5. Desiraju, G., and Steiner, T., *The Weak Hydrogen Bond* OUP (1999);
6. Howard, Johnson, Koetzler, Spencer, *Inorg. Chem* **26**, 2930 (1987);
7. Bakhmutov, Howard, Keen, Kuzmina, Leech, Nikonov, Vorontsov and Wilson, *J. Chem. Soc., Dalton Trans.* 1631-1635 (2000);
8. Mackenzie Gravett, Howard, Astin and Tomlins, *J. Chem. Soc., Perkin Trans.* **2**, 1233-1242 (1996);
9. Davidson, Goeta, Howard, Lamb and Mason, *New J. Chem.* **24**, 477-479 (2000);
10. M. W. Shotton, L.H. Pope, V.T. Forsyth, R.C. Denny, J. Archer, P. Langan, H. Ye, C. Boote, *J. Appl. Cryst.* **31** (5), 758-766 (1998);
11. M.W. Shotton, L.H. Pope, T. Forsyth, P. Langan, R.C. Denny, U. Giesen, M.-Th. Dauvergne, W. Fuller, *Biophysical Chemistry* **69** (1), 85-96 (1997);
12. Y. Nishiyama, T. Okano, P. Langan, H. Chanzy, *Int. J. Biol. Macr.* **26** (4), 279-283 (1999).