Collagen Orientation During Development of the Embryonic Avian Cornea.

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Purpose: During the latter stages of development, the secondary chick corneal stroma undergoes significant structural and compositional changes. The current study was designed to provide information about the orientation of collagen molecules (and thus fibrils) as the cornea matures before hatching. Collagen orientation is an important biomechanical parameter given that collagen lamallae consisting of parallel fibrils are thought to be strongest axially. Methods: Forty-eight corneas were obtained from fertilised chick embryos at developmental days 13 through 18 (n8 at each timepoint), stored frozen and later examined at the Synchrotron Radiation Source, Cheshire UK, where high-angle X-ray diffraction patterns were obtained. From the X-ray patterns the amount of X-ray scatter from aligned versus non-aligned (i.e. isotropic) collagen molecules was measured. Given that collagen molecules run approximately axially within fibrils, molecular orientation was taken to represent fibrillar orientation. Results: X-ray scatter from fibrillar stromal collagen (aligned and isotropic) as a proportion of X-ray scatter from all matrix elements measured 0.018 (day 13), 0.018 (day 14), 0.018 (day 15), 0.020 (day 16), 0.027 (day 17), and 0.033 (day 18). The increase after day 16 is indicative of increased deposition of fibrillar collagen. The amount of X-ray scatter from aligned fibrillar collagen as a proportion of scatter from all matrix elements (including aligned and isotropic collagen) measured 0.51 (day 13), 0.51 (day 14), 0.50 (day 15), 0.48 (day 16), 0.39 (day 17) and 0.30 (day 18). The decrease after day 16 points to lower levels proportionally of aligned collagen. Conclusions: The stromal matrix of the secondary chick cornea at day 13-16 of development contains a sizeable proportion of collagen fibrils that are preferentially aligned, often in a four-fold, orthogonal manner. Thereafter, this preferential alignment recedes, presumably because new collagen fibrils are being deposited in an non-orthogonal array, thereby masking the initial orthogonal template.

A Wide Angle Fibre Diffraction Camera for station 14.1 of The SRS

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Collecting the best possible data from a wide variety of samples on a single synchrotron station can be difficult to achieve. Requirements include easy optimisation of the X-ray beam size, optimisation of intensity, accommodating sample mounts varying in size and shape, easy sample alignment, providing a variety of sample environment conditions, providing both transitional and rotational degrees of freedom to sample movement. A wide angle diffraction camera designed to meet these demanding requirements is being used on station 14.1 of The SRS at Daresbury Laboratory.

Changes in cellulose crystal structure under tensile stress

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Cellulose is the most abundant structural biopolymer found on earth. It is organised as a fibrous composite material made up of small crystals (microfibrils) and disordered regions. The crystalline structure of the cellulose microfibrils itself is well-known [1]. On the contrary, there are still open questions about the morphology and the tensile properties of cellulose as composite material. As a first step in understanding this material a determination of the mechanical properties of the individual microfibrils is required. We investigated the tensile properties of flax cellulose fibres using microfocus wide-angle X-ray diffraction (WAXS) at the ESRF (ID13) and standard WAXS at HASYLAB (A2). Single flax fibres (at ID13) and small bundles of flax and ramie fibres (at A2) were mounted in a stretching device. Tensile load was applied along the fibre direction. The

measurement of the displacement of the jaws and the force onto the fibre direction yielded a stress--strain curve of the fibre as a whole. In addition, the stress--strain relationship for the microfibrils could be monitored by the change in lattice spacing using the recorded WAXS pattern. Assuming an isotropic distribution of the stress within the composite material, Young's modulus can then be calculated for the entire fibre as well as for the microfibrils. Furthermore parameters such as microfibril angle, crystallinity, and defects of the crystals could be deduced, thus, leading to a better understanding of the morphology of cellulose. An unexpected increase of the lattice spacing perpendicular to the applied force was also observed. This can be explained by radiation damage to the intermolecular hydrogen bonds, leading to an increase of the chain-chain distance of the cellulose chains within the crystals. This effect has already been observed for electron radiation [2].

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Structure of native wood cellulose

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The models for the crystal structure of native cellulose (cellulose I) are more than 25 years old [1,2]. Since cellulose single crystals are not available, fibre diffraction is the only access to the structure. Cellulose nanocrystals (microfibrils) are long (about 100 nm) but very thin (2.5 to 25 nm, depending on the origin). We investigated the highly oriented cellulose poplar tension wood fibres. In order to profit from the high orientation, single wood fibres (2 mm long, 30 microns wide) were investigated with an X-ray microbeam (ID13, ESRF). The fibre was cooled to 100 K and thus stable in the intense beam for several minutes. Fibre diffraction data with a resolution of about 0.11 nm were obtained as a basis for structure determination.

Structural changes of alpha-crystallin during heating observed with small and wide angle X-ray scattering

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Whole eye lens and alpha-crystallin gels and solutions were investigated using X-ray scattering techniques at temperatures ranging from 20 to 70ºC. In whole lens the spacing of the single X-ray reflection seen with small angle scattering was constant from 20 to 45ºC, increasing at 50ºC to 165 Å. These results indicate that in whole lens alpha-crystallin is capable of protecting other lens proteins against superaggregation up to 50ºC. In alpha-crystallin gels a moderate increase in both the spacing and intensity of the reflection was observed from 20 to 45ºC, followed by a dramatic increase from 45 to 70ºC. Upon cooling, this effect was found to be irreversible over an eleven-hour period. Qualitatively similar results were observed for alpha-crystallin solutions at a variety of concentrations. Wide angle scattering reflections from the alpha-crystallin gel arise primarily from the beta-sheet organization of the alpha-core, and appear to be essentially preserved throughout the temperature range. These results confirm earlier observations at low concentrations of alpha-crystallin in vitro of a major temperature transition around 50 ºC, and can be extrapolated to physiological concentrations.

Quantitative interpretation of the 2D X-ray diffraction patterns from skeletal muscle using direct modelling

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A 3D structural model of the actin-myosin super-lattice based on available atomic structures of F-actin, myosin subfragment-1 and their complex was developed. To reduce the number of parameters in

the model, a principle of 'minimal elastic distortion energy' was employed. According to this principle, a myosin head chooses that actin monomer on one of six surrounding thin filaments for which binding requires the least elastic distortion energy. In our model binding of up to 270 myosin heads to actin in a unit cell is determined by two parameters only: fraction of stereo-specifically attached myosin heads, n, and the ratio of the axial and transversal cross-bridge stiffness, l. Calculated 2D X-ray diffraction patterns were compared with the low angle X-ray diffraction data from skeletal muscle. The model provides good fit of the diffraction pattern from rabbit muscle fibres in rigor without a global parameter search. The stiffness ratio, l, and parameters of disorder in the actin-myosin lattice can be estimated separately from individual layer lines. The total off-meridional intensity of the 1-st actin layer line, A1, was found to be independent of lattice sampling and tilting of the light chain domain of the myosin head. Radial distribution of the intensity along the A6 and A7 actin layer lines appeared to be very sensitive to the shape of bound heads. We also tested how azimuthal disorder of attached myosin heads affects the intensity of the actin layer lines. The A6 and A7 layer lines were found to be almost insensitive to such disorder, while the intensity of A1 significantly decreases when the disorder increases. The fraction of stereo-specifically bound heads, n, during isometric muscle contraction at different temperatures was estimated from the time-resolved X-ray diffraction data obtained in T-jump experiments with small (3-5 fibres) bundles from rabbit muscle. The T-jumps from ~6ºC to ~36ºC induced 2.6-fold tension rise. Estimated n increased nearly proportionally to tension: from ~18% at ~6ºC to ~45% at ~36ºC. X-ray diffraction data was collected with RAPID 2D gas-filled detector on beamline 16.1, SRS, Daresbury Laboratory. Supported by grants from INTAS, HHMI and RFBR and by MRC and Daresbury Laboratory, UK.

BS - 2D X-ray diffraction data processing program

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A program was written for the treatment of 2D low angle X-ray diffraction data as MS Windows version of BSL. It is currently used for the analysis of the X-ray diffraction patterns from skeletal muscle, but like BSL can deal with any fibre diffraction data. Windows interface makes the program user friendly and open codes provide greater flexibility to adopt to the ever changing requirements. Colour graphics and export to standard graphic formats (BMP, TIFF) is implemented. Mouse selection of integration limits is available for horizontal, vertical and 2D integration. 1D plots can be displayed in a separate window and saved in ASCII format. A set of mirroring functions contains horizontal and vertical mirroring as well as standard four quadrants mirroring operation, the centre of the pattern can be defined with a half pixel precision. A scrpit with the set of instructions can be written as the text file. The program is available at http://www.imec.msu.ru/~natalia/bs_form.htm. Supported by grants from INTAS, HHMI and RFBR.

Fibre Diffraction Studies of Potato Virus X on the BioCAT Beamline at the Advanced Photon Source.

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Fibre diffraction from flexible filamentous viruses has been limited by disorientation in the fibres, and by the close spacing of the layer lines in the diffraction patterns. Potato virus X (PVX) is the most important member of a major group of filamentous viruses, the potexviruses, and has great potential significance for basic virology, agriculture, and biotechnology. There have been reports of fibre diffraction from potexviruses for many years, but the diffraction patterns have been highly disordered. We have adapted methods originally developed for bacterial flagella by Namba's group to orient sols of PVX as fibre diffraction specimens. A combination of centrifugation in glass capillaries to form liquid crystals and exposure to strong magnetic fields (up to 18 Tesla) has produced greatly improved specimens. We hae constructed a camera for high resolution fibre diffraction on the BioCAT beamline at the APS,

Argonne, Il, USA allowing small (35 x 60 micron), high intensity (~2 x 10^{12} photons/s) X-ray beams to examine small ordered domains in the sols. Data so obtained have enabled us to determine accurately the symmetry of the helical virus, and to demonstrate the presence of deep intersecting helical grooves running longitudinally and azimuthally in the surface of the virus. Detailed structure determination will depend on further development of the specimen preparation methods, and will probably require high-resolution crystallographic structure determination of the isolated coat protein. Supported by grants MCB-9809879, DBI-9604789, and INT-9602486 from the National Science Foundation, and by Vanderbilt University. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Basic Energy Sciences, Office of Science, under contract No. W-31-109- ENG-38. BioCAT is a National Institutes of Health-supported Research Center RR-08630.

Type-4 bacterial pili: molecular models and their simulated diffraction patterns

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Bacterial pili are long thin assemblies of pilin protein subunits, that extend outwards from the surface of bacteria and are involved in interaction of bacteria with their environment, notably attachment of pathogenic bacteria to their host and "twitching motility" of bacteria along surfaces. Pili are about one-third the diameter of bacterial flagella. Some types of pili are the adsorption sites of filamentous bacteriophage. X-ray fibre diffraction patterns of type-4 pili are classical alpha-helix patterns, with strong intensity in the equatorial direction at about 10 Å and in the meridional direction at about 5 Å [1]. The crystal structure of the type-4 pilin subunit has a highly conserved ~50-residue N-terminal alpha-helix and a less conserved ~100-residue globular C-terminal domain, and this subunit structure has been used to construct models of type-4 pili in which the N-terminal alpha-helix of the pilin forms the central core of the pilus [2-4]. We find that the calculated fibre diffraction patterns predicted for these models are less similar to the observed diffraction patterns than diffraction patterns predicted for models built from only the N-terminal alpha-helix portion of the subunit. Disorder in the globular domain may be one explanation for this effect, and the globular domain of pilin in pili may be "intrinsically unstructured" as found for binding regions of some globular proteins [5]. Twitching motility and phage infection proceed by retraction of pili [6], probably involving dissolution of pilin at the base of the pili into the host cell membrane, reminiscent of the process of filamentous phage infection by disassembly of the phage subunits into the membrane.

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MYOSIN CROSSBRIDGE DYNAMICS IN FISH MUSCLE

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The crossbridge power stroke on actin appears to involve a change in angle between the actin-attached motor domain and the neck region of the myosin heads. Fast (1ms) time-resolved low-angle X-ray diffraction from contracting fish muscle during the rising phase of an isometric tetanus has been used to study the kinetics of the crossbridge cycle. Fish muscle is particularly advantageous for such studies because of its high degree of 3D order (Harford and Squire, 1986; Harford and Squire 1993; Hudson *et al*, 1997; Squire 2000). The data recorded at Daresbury (line 16.1) using the rapid detector are explained by five different kinetic states and three different structural states for the myosin heads: A resting state (structurally indistinguishable from an "off" and "reset" state), a weakly bound state and a strongly bound state. Weak and strong binding states are presumably related by a change in angle between myosin neck and motor domain. The myosin head starts the cycle in the resting state (solved by Hudson *et al*, 1997) and becomes mobile on activation going through an "off" state to a "weak" initial binding state on actin. The power stroke (weak to strong binding states) occurs, the head then resets and restarts the contractile cycle again in the "off" position. Time-resolved X-ray diffraction is proving effective in providing reasonable values for the kinetic constants and in separating the weak and strong crossbridge states in the contractile cycle.

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MPW 6.2 - A station dedicated to investigating Materials for the Third Millenium

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Station 6.2 has entered its commissioning phase haven taken 'first light' in December 2001. The station brings together three techniques optimised for materials processing. The beamline's optics comprise a vertically focussing collimating mirror followed by a double crystal sagittal bent monochromator and then a plane focusing mirror. It is designed for very rapid, tuneable, combined SAXS/WAXS experiments, X-ray diffraction and XAS (X-ray absorption spectroscopy). The station is equipped with state of the art detectors constructed along similar lines to the RAPID 2D detector for station 16.1. These will enable count rates of ca 10 MHz for both 1D small and wide angle experiments exceeding the existing capabilities of the detectors on station 8.2 by ca. 40 and 500 respectively. This has huge advantages as in many experiments it is necessary to attenuate the X-ray beam to avoid overloading the detectors. In addition because of the variable wavelength it will also be possible to carry out anomalous scattering experiments, something which is new to the repertoire of facilities offered to the non-crystalline community.

MYOSIN HEAD DISPOSITION MODELLED FROM LOW-ANGLE X-RAY DIFFRACTION OF RELAXED LETHOCERUS INSECT FLIGHT MUSCLE

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Superbly ordered sarcomere structures in fibrillar insect flight muscle (IFM) and bony fish skeletal muscle favor detailed structural studies. We seek to picture the 3D molecular structures and actions of actin and myosin filaments in IFM by computer-modeling of low-angle X-ray patterns. Initially we are modeling IFM myosin head disposition to 5 nm resolution using the 1.43 nm resolution versions of both Rayment and Dominguez myosin heads crystal structures, and using 105 myosin reflections from MgATP-relaxed fibres of glycerinated IFM. We use the simulated annealing and local refinement approaches shown by Hudson *et al*. (J. Mol. Biol. 273: 440, 1997) to yield a 3% R-factor in their final best-fit-to-X-rays model of myosin head disposition on relaxed fish thick filaments. Our IFM model so far, based on layer lines 10, 16, 22, 26 and 32 (orders of 232 nm) has all crowns alike, all 8 [Rayment-type] heads per crown projecting at ~90° in a square shelf of density that rotates 33.75° for every 14.5 nm axial repeat. R-factor is 11.96%. Polarity re: Z- vs. M-band is clear from a unique fit to negative stained IFM thick filaments (Morris *et al*., J. Struct. Biol. 107: 237, 1991). Later, we propose to model the full unit cell, including thin filaments, against X-rays from relaxed, rigor and active IFM, ultimately to 1 nm resolution and study the interactions between the different proteins in the different muscle states.

MPW 6.2 - A station dedicated to investigating Materials for the Third Millennium

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A new X-ray instrument, MPW6.2 is being commissioned for the study of non-crystalline and polycrystalline materials at the SRS, Daresbury Laboratory. The beamline is optimised to receive synchrotron X-rays of 5-18 keV from a multi-pole (x11) wiggler (B2 tesla). The optics - two mirrors and a Daresbury sagittal monochromator - have been arranged to focus the beam vertically and horizontally respectively to deliver an estimated flux of 1011-10^12 photons/mm2/s/0.01% onto the sample under investigation, at least 100 times more intense than any other XRD source on the SRS. At the heart of this development are two state-of-the-art detectors. The first of these is a new wide-angle curved position-sensitive detector to be mounted on a heavy-duty two-circle diffractometer (theta, 2theta). This high angular resolution (0.06degrees) detector system is based on the novel micro-gap multi-wire (RAPID) technology for fast data acquisition [1], and will allow an entire X-ray diffraction pattern (XRD) to be recorded in a very short time (microseconds to seconds). Utilising the same technology, a quadrant style detector will also be installed for small angle X-ray scattering (SAXS). Together, these two installations constitute an exceptionally powerful instrument for combined small/wide-angle scattering (SAXS/WAXS). Additionally, X-ray absorption apparatus will be provided for combining these techniques with parallel spectroscopy measurements (EXAFS). Purposely designed for kinetic studies, to follow structural changes in solid and liquid state reactions and materials processing, the combination of tuneable wavelength, high flux and rapid data collection make this new beamline a world-class facility for carrying out simultaneous measurements by researchers in the field of materials science.

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Ultrastructural Changes in Keratoconic-like Mice Corneas

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Purpose: Corneas from transgenic mice showing a form of keratoconus (conical cornea) were investigated by X-ray fibre diffraction to see if structural collagen alterations were responsible for the misshapen tissue. Methods: Low-angle (station 2.1) fibre diffraction was used to measure collagen interfibrillar spacing and fibril diameter in normal and keratoconus corneas. Also, the intermolecular spacing and the degree of preferred collagen fibril orientation was measured at 200 micron intervals across four keratoconus (two males and two females) and two normal mice corneas (one male and one female) using high-angle X-ray diffraction on station 14.1. Results: Collagen interfibrillar spacing and fibril diameter was greater in male (n8) and female (n6) keratoconus than in normal (n8) mice corneas. Variation in the amount of preferred collagen fibril orientation across mice corneas is similar to that seen in humans, with strong alignment occurring at both edges of the cornea suggesting the presence of an annulus at the limbus. Differences were observed between normal and keratoconus mice in the amount of preferred collagen fibril orientation across the cornea, with the differences being most marked in one of the male keratoconus mice which showed no evidence of an annulus. Conclusion: Changes in the corneal fibrillar array are possibly responsible for biomechanical and shape changes in mice corneas with a form of the human disease keratoconus.

The modelling of strain induced molecular changes in type I collagen in rat tail tendon

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Type I collagen is one of the most important stress bearing proteins in vertebrates, being predominant in tendon, skin and bone. Tendon is composed of almost 100 percent type I collagen, and displays outstanding mechanical properties which have been extensively studied. However, as yet, no satisfactory explanation has been proposed to describe the changes in conformation at the molecular level. These changes are evident in the diffraction pattern of tendon revealed during mechanical testing, collected in a time-resolved manner utilising synchrotron radiation. This work shows the attempts made to explain the changes in the profile of the meridional diffraction pattern of rat tail tendon which occur when the tissue is stretched. When observing the diffraction pattern of stretched tendon the changes in the intensity profile can be summarised as: 1)The attenuation of the higher orders in the diffraction series, 2)The modulation of the lower order diffraction terms apparent in the change in the ratio of the even to odd orders, most apparent in the 2nd and 3rd orders, The attenuation of the higher orders were interpreted as incoherence in the structure produced through a microfibrilar shearing process. The modulation in the even to odd orders was modelled as molecular slippage causing a change in the electron density profile. This has produced the most satisfactory models as yet proposed to describe these events.

Challenges Facing DNA Fibre Crystallography

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Initial theoretical studies of alternative helical DNA structural models have been published recently and matched against fibre diffraction patterns [1,2]. These models arise from the wide range of kinetic, mechanistic and other experimental results for duplex DNA which have accumulated since 1950 [3,4,5]. Variety in DNA structure remains a topic of great interest [6]. Apart from a rare, exceptional polynucleotide fibre diffraction pattern of poly(d(AT)) at very high resolution derived from an yet unreported atomic structure [7], DNA fibre diffraction has too low a resolution to allow certain competing helical structures to be distinguished from each other without taking cognisance of results from other fields. Results from studies of oligodeoxynucleotide diffraction from true crystals are compromised by a convention that imposes algorithmic constraints, thus ensuring that there is only a restricted range of permitted structural solutions [8].

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The three dimensional packing of type I collagen molecules in rat tail tendon

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The axial packing of type I collagen molecules is well described, but the lateral or three-dimensional packing topology is less clear. It is generally accepted that the molecules pack on a triclinic lattice in a microfibrillar manner; however, the precise long- and short-range packing interactions remain unclear. This work aims to define these through a combination of X-ray diffraction analysis and computer modelling. Lateral packing interactions between type I collagen molecules in rat tail tendon give rise to a series of Bragg peaks overlain with diffuse scatter in the equatorial region of the X-ray diffraction pattern. The diffuse scatter may partly arise from thermal molecular disorder and we show that it is possible to reduce the degree of diffuse scatter, and thus facilitate analysis of the underlying Bragg intensities, by cryo-cooling the sample to 90-100 Kelvin during data collection. We also report on various methods used to distinguish the Bragg peaks from the diffuse scatter in XRD patterns collected from cryo-cooled and ambient samples. We describe

a computer model that simulates the lateral packing of type I collagen molecules. In this model, a unit cell slice of five molecular segments with triclinic lattice coordinates is energy minimised to a global minimum using the discriminatory procedure of simulated annealing which finds the optimal, lowest energy, three dimensional packing conformation. For the first time, this model incorporates amino acid interactions such as charge and Van der Vaal radii and accounts for interactions in the gap, overlap and telopeptide regions.

The Conjugative Pili of The RP4 Plasmid

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Conjugative pili are long filaments, consisting of multiple copies of a single pilin subunit. The pili protrude from the surface of bacteria and are involved in DNA transfer. They are also the sites of attachment of many filamentous bacteriophages. The RP4 plasmid codes for the conjugative RP4 pilus, which is the initial attachment site for filamentous bacteriophage Pf3. This pilus is involved in the transfer of RP4 plasmid DNA between Gram-negative bacteria and to a wide range of organisms such as Gram-positive bacteria and even yeasts. The RP4 pilin subunit, TrbC, has a molecular weight of 8 kDa and in contrast to the pilin of most other conjugative pili, is circular, with N- and C- termini joined by a peptide bond. We are studying Pf3 bacteriophage / RP4 pilus interaction and fibre diffraction studies of RP4 pilus structure are in progress.

A Bayesian approach to phase extension

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A Bayesian approach to the heavy-atom method for solving crystal will be presented. It will be shown that, in contrast to conventional procedures, probability theory makes full use of the information inherent in a known fragment since both the related phase and amplitude play a central role. This property is particularly important for powder data, where peak overlap makes it difficult to infer the intensities of individual reflections reliably. A covariance matrix is also shown to be essential, in the latter case, for capturing the constraints imposed by the diffraction measurements in the space of the structure factors. Prior knowledge about the positivity of the underlying electron density, at least for X-ray diffraction, can be encoded through the use of an entropic prior, which further enhances the quality of the results.

Photonic Structures from Amphiphilic Block Copolymers

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The order-disorder transition (ODT), microdomain structures and the phase diagram for ternary blends of low molecular weight PS, PI and PS-PI have been determined by a combination of X-ray scattering and transmission electron microscopy. The distribution of the homopolymer within the layers of a related PS/PB/PS-PB system has also been determined by a combination of X-ray and neutron reflectivity. A series of nearly symmetric, ternary blends of polystyrene (PS), polyisoprene (PI) and polystyrene-block-polyisoprene (PS-PI) have been studied by small angle X-ray scattering, static light scattering and transmission electron microscopy. The molecular weight of the homopolymers and block copolymer were in the ratio NH / NBCP 0.19, which gave a block copolymer ODT and a homopolymer blend TC that were similar (TC / TODT ~ 1.1). The block copolymer and its blends showed a weakly first-order transition from a lamellar phase to a fluctuating disordered phase in the volume fraction range FH 0.77. A bicontinuous microemulsion was found between FH 0.79 and FH 0.93, and for FH 0.93 macrophase separation was observed. In a similar PS, polybutadiene (PB) and polystyrene-block-polybutadiene (PS-PI) system the distribution of the homopolymer diluent was studied by X-ray and neutron reflectivity with deuterium labelled PS. The initial microstructure formed on spin coating had a dry-brush structure with the homopolymer concentrated in the centre of the domains and on subsequent annealing a wet-brush morphology was observed with the homopolymers uniformly distributed. The potential applications of self-assembled block copolymers as photonic structures will be highlighted.

The Preparation and Characterisation of Polyisocyanurates with Graded Modulus.

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The preparation of polyisocyanurate elastomers for use as damping coatings on mechanical parts, with a range of glass transition temperature, Tg, through the depth of the sample has been achieved. This was accomplished by varying the density of cross-links in the sample. Material properties were characterised using a variety of techniques including FT-IR Microscopy, Laser Ablation Mass Spectroscopy, Small Angle X-ray Scattering and Nano-indentaion. SAXS was used to monitor the phase separation of the material under different processing conditions. Phase separation was seen in all materials made from polyisocyanurates with polyether segments with Mw greater than 2200, regardless of the proportion of diisocyanate used. The degree of microphase separation, obtained from SAXS results is important in the determination of the mechanical properties of the elastomer. The kinetics of phase separation could be studied by performing the curing in the X-ray beam. FT-IR Microscopy and Laser Ablation Mass Spectroscopy were used to characterise the changing chemical structure through the depth of the material. This was possible due to the chemical difference between the isocyanurate cross-linking units and the polyether soft segments. The Nano-indentor proved to be a powerful tool for investigating the mechanical properties of the material, as it makes use of dynamic and static indentation under thermally controlled conditions. These elastomers were also used to aid the understanding of the nano-indentation technique for 'soft' materials.

Structural Changes in Muscle Correlated with ATP Hydrolysis

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Two dimensional X-ray diffraction was obtained from skinned rabbit psoas muscle fibres. The goal is to correlate structures of the cross-bridge population with various intermediate states in the ATP hydrolysis cycle. By systematically using analogs, or variations in temperature and ionic strength, the distribution and disposition of myosin heads in eight of the intermediate states have been characterized. Some highlights of the findings: in the state of A.M.ATP, before ATP is hydrolyzed, the binding of myosin to actin is characterized by random orientations, and the binding site on actin differs from that for rigor binding. When myosin is not bound to actin, only the state with the hydrolysis products ADP.Pi bound at the active site (i.e. M.ADP.Pi) exhibits an ordered helical arrangement on the myosin filament. Myosins in other states (M.ATP, A.ADP, M) are disordered. Recently, Malnasi-Csizmadia, *et al*. (Biochemistry (2000), 39:16135-16146; and (2001) 40:12727-12737) studied the effects of temperature and ligands on the open - closed conformational transition in Dictyostelium myosin. The enthalpy change for the open - closed transition is identical for the disorder - order transition found in the myosin filament by X-ray diffraction. The close correlation strongly suggests that the open - closed conformational change and the disorder - order transition are the same process. It also suggests that helical order may be used as a signature for the closed conformation of myosin in relaxed muscle.

SAXS studies of sheared nanophase separated copolymers

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Recent work in our group has focussed on the effect of shear on nanophase separated block copolymers in solution, melt and crystalline states. The main focus of this talk will be synchrotron SAXS studies of shear-induced alignment of poly(oxyethyelene)-poly(oxybutylene) diblock copolymers in solution [1]. The orientation of twinned face-centred and body-centred cubic crystals has been studied following steady shear in a Couette cell, or oscillatory shear using a modified Rheometrics rheometer. The latter has recently been modified to allow WAXS as well as SAXS with simultaneous rheology - a powerful combination to examine the effect of shear on crystallization in polymers. Preliminary results from experiments on

crystallization in block copolymers containing a semicrystalline PEO block will be presented [2]. We have also constructed a small goniometer stage to fit inside the rheometer oven, and this allows "mesoscopic crystallography" experiments to be performed, where samples are first sheare and then mounted on the goniometer which is rotated with respect to the shear axis, allowing the 3-dimensional diffraction pattern of shear-aligned copolymer mesophases to be mapped out. Time (and results) permitting, SAXS studies of shear-induced orientation of cross-linked gels formed by hydrophobically-modified poly (N-isopropy-lacryalmide) in water will also be discussed. ([1] Work done in collaboration with group of C.Booth (Dept of Chemistry, University of Manchester). [2] Work done in collaboration with Prof G. Floudas (University of Ioannina, Greece), and Dr F.Schipper (FORTH-IESL, Crete, Greece))

The Liquid-Solid Transition in a Micellar Solution of a Diblock Copolymer in Water

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The structure of a diblock copolymer solution in the vicinity of the transition between micellar liquid and solid phases was investigated using small-angle X-ray scattering. An amphiphilic poly(oxyethylene)-poly(oxybutylene) diblock was studied in water. Static and dynamic light scattering techniques were used to provide an independent measure of micelle dimensions and aggregation numbers. Dynamic shear rheometry and mobility measurements were used to locate phase transitions. A micellar liquid phase was identified at low concentration and a cubic micellar phase at higher concentration, the transition between the two occuring at higher temperature as the concentration increased. The cubic micellar phase behaves rheologically as a solid and SAXS confirmed a face-centered cubic structure. Intermediate between these two phases, a viscoelastic solft solid was observed, with finite yield stress but with a much lower dynamic modulus than the crystalline solid. Several distinct suggestions have been put forward for the structure of the solution in this region. In a poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene) Pluronic triblock, small-angle neutron scattering and rheology provided evidence for a percolation transition between micellar liquid and solid phases [L.Lobry *et al.*, Phys Rev E 1999, 60, 7076], indicating a fractal structure of micelles aggregated due to attractive interactions. Alternatively, a defective solid phase has been proposed. We analysed the structure of solutions of our diblock copolymer via detailed model fits to the SAXS data for concentrations spanning the liquid- solid transition. The micellar form factor was modelled as a homogeneous micellar core with attached Gaussian chains; and the intermicellar structure factor could be described using the hard sphere model. Thus there is no evidence for percolation induced by effective attractive interactions between micelles in our system. In contrast SAXS data indicates there is a coexistence region between hard sphere fluid and solid crystal phases, in which small grains of close-packed crystal coexist with fluid. It is apparent that block copolymer micelles act as model colloidal systems in which it is possible to investigate the influence of attractive and repusive interactions between spherical particles by varying the copolymer composition.

The crystal structures and hydrogen bonding in cellulose polymorphs

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Although the crystalline nature of cellulose has been one of the most studied problems in polymer science there remain many open questions. We are determining the precise crystal structures and hydrogen-bonding systems of cellulose polymorphs by fibre diffraction. X-rays are used to determine the positions of carbon and oxygen atoms. Neutrons, in combination with isotopic substitution of labile hydrogen atoms in the fibres by deuterium, are used to determine the positions of hydrogen atoms involved in hydrogen bonding. Methods have been developed for obtaining oriented polycrystalline fibres that diffraction X-rays and neutrons to atomic resolution [1,2]. For the first time data have been collected from natural occurring pure cellulose I-alpha isolated from Glaucocystis and from pure cellulose I-beta isolated from Tunicate. We have also collected data from the polymorphs resulting from cellulose processing; II(both mercerized and regenerated), III(I) and III(II). Our initial results on regenerated cellulose II, mercerized cellulose II[3,4] and cellulose I-beta[5], have lead to new crystal structures, direct identification of hydrogen bonding systems, and new insights into the factors that determine the structure and properties of cellulose.

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Transthyretin Amyloid Fibres

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Familial Amyloidotic Polyneuropathy (FAP) is characterized by deposits of insoluble amyloid fibres in which transthyretin (TTR) is the major protein component. TTR is a tetramer with identical 127 aminoacid sub-units and an extensive β-sheet structure. How this structure is related to the molecular organization of the FAP amyloid fibre is one of the major questions in understanding the pathophysiology of the disease. The mechanism by which the soluble protein converts to insoluble fibre can only be conveniently established when the structure of the fibres is elucidated. Amyloid fibres constitute a high molecular weight, insoluble material and therefore the atomic structure cannot be investigated by conventional X-ray crystallography or nuclear magnetic resonance (NMR). However, information about the fibrillar structure can be obtained by X-ray fibre diffraction from very well oriented samples. Using this approach it was shown that they exhibit a cross β-sheet structure [1] and are formed by a continuous β-sheet helix [2] or an association of units with a structure close to the TTR monomer [3]. Aiming at improving our knowledge about the molecular structure of amyloid fibrils, new X-ray experiments were performed at European Synchrotron Radiation Facility (ESRF), beam line ID13. The fibrillar Leu55Pro TTR samples were drawn up into siliconized glass capillary tubes, sealed at the top, then placed into a 2-T magnet and allowed to dry at ambient conditions. X-ray diffraction patterns from these samples indicated the presence of the cross-b pattern, characterized by a meridional reflection at 4.65 Å and a broad equatorial reflection at 10.3 Å. Interpretation of the data shows that the protofilament building blocks are monomers composed of a pair of β-sheets with an intersheet distance of 10 Å. End-to-end association of four monomers in the fibre direction and related by some kind of axial rotation constitute a repeating unit of 116 Å. The estimated protofilament diameter is 42 Å and the calculated mass-per-length is 0.47 kDa / Å. These values are in good agreement with results obtained by scanning transmission electron microscopy (STEM) [4]. Future work concerning the alignment of the amyloid fibres and their interaction with fibre disrupters will be performed.

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The Compaction and Delivery of DNA.

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The medical profession increasingly desires the ability to deliver treatment, by a drug, to a specific organ or area of the body. Examples include the targeting of anti-tumour drugs for the treatment of cancer, or genetic material for gene therapy. One particularly effective strategy is to use a carrier particle to house the drug while in vivo. Viruses have been investigated for this purpose, but this can provoke an immune response. Therefore bio-compatible polymers are being designed to circumvent these problems. We investigate a simplification of the drug molecule - carrier particle relationship by examining the association between a charged spherical particle (representing a polyelectrolyte micelle) and an oppositely charged polyelectrolyte, in this case DNA. Dynamic light scattering, Raman spectroscopy and small-angle X-ray scattering are used to examine the conformational behaviour of the complexes.

A solution structural model of human IgA2

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There are two subclasses of human immunoglobulin A, IgA1 and IgA2. Human IgA2 has a short hinge region of 8 amino acids linking the Fab arms and the Fc region, while IgA1 possesses a much more extended hinge of 23 residues. X-ray and neutron scattering analysis of recombinant monomeric IgA2 (of IgA2m(1) allotype) revealed a radius of gyration RG of 5.1 nm, which is significantly smaller than that of IgA1 at 6.1-6.2 nm. For IgA2, the distance distribution function P(r) comprised a single peak and a maximum dimension of 17 nm, while IgA1 gave two peaks and a 21 nm maximum dimension. An automated curve fit search analysis of a homology model for IgA2 was conducted, in which random IgA2 hinge structures connect the Fab and Fc fragments in any orientation. Around 50 out of 10,000 models fitted the scattering and ultracentrifugation data. This approach produced an IgA2 structure that is significantly more compact than that of IgA1. The IgA2 Fab and Fc arrangement resembles that in the crystal structure of the hinge-deleted human IgG1 Mcg, which shares the characteristic of an inter-light chain disulphide bond.

Synchrotron studies of exotic smectic liquid crystals.

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Liquid crystals are ordered fluids that are well-known for their use in display devices. One family of these materials, the smectic phases, have the potential to exhibit ferroelectricity, antiferroelectricity and ferrielectricity through subtle variations in their structure. All such liquid crystals have titled molecules that pack in layers with an overlying helicoidal structure. They differ in the interlayer periodicity. Ferroelectric liquid crystals change little from one layer to the next, antiferroelectrics alternate in tilt direction (so have a two-layer periodicity), while the structures of the intermediate phases have proven to be a considerable challenge to solve, but are known with both 3- and 4-layer periodicities. As well as deducing the structures that these materials adopt, it is also important to understand the structural modifications that occur within devices both with and without the application of electric fields. This paper describes the structures and properties of this novel class of fluids in devices. Static and time-resolved small angle X-ray scattering have been used to probe ferroelectric switching, while resonant X-ray scattering experiments have provided unique information on the layer structures of the antiferroelectric and intermediate phases, and their modification using switching fields. The experiments and results are summarised.

A-DNA before, during and after Watson Fuller's PhD thesis

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With retrospective wisdom we can allocate some of the confusion in interpreting the earliest (1930s) fibre diffraction patterns of DNA to the fact that the specimens were mixtures of the two commonest allomorphs of the DNA duplex. These were labelled A and B only after uncontaminated patterns of each were obtained by Maurice Wilkins and his associates in the early 1950s. From the beginning of their studies they found that all of their A-DNA specimens were not only uniaxially oriented but also polycrystalline. Despite this presumed advantage which attracted much of Rosalind Franklin's analytical effort, more attention came to be focussed on the apparently more hydrated B allomorph that was expected to be the more relevant biological form especially after oriented and polycrystalline specimens were obtained for it also. The A-DNA duplex has 2 111 symmetry with the pitch of its helical chains 2.86nm. The duplexes are packed in a C-face-centered monoclinic (space group C2) unit cell (a 2.17, b 3.99, and c 2.80nm, b 96.80) which approximates to the close packing of cylinders of about 2.25nm diameter. One consequence of this is that although there are many Bragg reflexions on each layer line they are often in partially overlapping clumps. Even today a convincingly scrupulous extraction of structure factors from such a complicated fibre diffraction pattern would be non-trivial. In the late 1950s the task was herculean and had to be followed by equally meticulous rounds of manual model-building and repeated Fourier transform calculations. It is to Watson Fuller's great credit that his efforts and 1960 conclusions about this structure and its details have not been improved upon significantly since its belated publication in 1965 (Fuller *et al*. J. Mol. Biol. 12, 60). What revived interest in A-DNA itself was the discovery that ribonuclease-resistant ribosomal RNA fragments and the lengthier nucleic acid components of certain RNA viruses consisted of Watson-Crick base-paired duplexes with A-DNA-like secondary structures. Subsequent studies of DNA-RNA hybrid helices, and of the Watson-Crick base-paired duplex regions in t-RNA and other RNAs with complex tertiary structures, and even of polynucleotide duplexes and triplexes with non-Watson-Crick base-pairings have confirmed the importance of A-DNA-like secondary structures whose C3'-endo furanose ring conformations are associated with a morphology quite different from that in double helices like the B form where all the furanoses have C2'-endo puckering. More surprising has been the discovery that some helical duplexes with purine-purine or pyrimide-pyrimidine base-pairs have structures in which the the polynucleotide chains are not just similar to those of A-DNA but identical with them. To understand why this DNA chain conformation is so special as to persist with no modification in so many environments one has to look to the geometry of the water molecules that are associated with this unique polynucleotide fold. Crystal structures of oligonucleotides have provide the needed insight but it would not be surprising if Watson Fuller's good data could not be persuaded to confirm this directly for the eponymous structure itself.

An Investigation into the Phase Behaviour of Block Copolymers in Solution - Phases Formed and their Transition Kinetics

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Various block copolymers in aqueous solution have been studied to show how the chain architecture effects the phase behaviour and the kinetics of the phase transitions. This has many biological applications such as artificial lung surfactants in premature babies(1). The block copolymers under investigation are diblock PEO-PI copolymers, triblock PEO-PBO-PEO copolymers, and triblock PBO-PEO-PBO copolymers. Micelles are formed when the concentration of polymer in water reaches a critical value known as the CMC. As the concentration is increased further the micelles pack together to form ordered arrays of specific geometry such as Cubic, Hexagonal or Lamella structures. PI-PEO and PEO-PBO-PEO block copolymers form spherical micelles rather than "flower like" micelles (PBO-PEO-PBO) due to the positioning of the hydrophobic component within the polymer. These stuctures have been deduced by Simultaneous SAXS/WAXS/DSC on Beamline 8.2 of the CCLRC

Daresbury Laboratory, Warrington, U.K and at the CRG BM26 at the European Synchrotron Radiation Facility, (ESRF), Grenoble, France. The positioning of the SAXS peak-to-peak ratio is indicative of the structure formed. The samples are heated and the structure changes due to a change in the peak ratio. This can be monitored by using the CCP13 Program XFIT. From this we can deduce the whereabouts of the phase transition, and consequently map the phase morphologies. The kinetic parameters of the phase transition can be studied by performing SAXS/WAXS/DSC. The sample is heated to a point in the phase diagram where it displays a certain structure. It is then rapidly quenched to another point in the phase diagram of a different structure. Here a peak can be seen to grow. This will give us information on the kinetics of the phase transition. These experiments have been performed at various quench depths. In addition, Polarised Optical Microscopy experiments have been performed in a similar way. Here the number of coloured pixels emerging from an image are counted. Howver this technique only works from an isotropic phase (Cubic or Disorder) to an Anistropic phase (Hexagonal or Lamellae). Avrami kinetics are performed on the SAXS and Microscopy data to achieve values for the rate consatnt and the Avrami exponent, which tells us information abut the mechanism of the phase transition. (1)- W. R. Perkins, R. B. Dause, R. A. Pareante, S. R. Minchey, K. C. Neuman, S. M. Gruner, T. F. Taraschi, A. S. Janoff.; Science, 273, 1996, 330.

The Structrue of type I and type III collagen heterotypic fibrils: An X-ray diffreaction study

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The molecular packing arrangement within collagen fibrils has a significant effect on the tensile properties of tissues. To date, most studies have focused on homotypic fibrils composed of type I collagen. This study investigates the packing of type I/III collagen molecules in heterotypic fibrils of colonic submucosa using a combination of X-ray diffraction data, molecular model building and simulated X-ray diffraction fibre diagrams. A model comprising a 70 nm diameter D-(~65nm) axial periodic structure containing type I and type III collagen chains was constructed from amino acid scattering factors organised in a liquid-like lateral packing arrangement simulated using a classical Lennard-Jones potential. The models that gave the most accurate correspondence with diffraction data revealed that the structure of the fibril involves liquid-like lateral packing combined with a constant helical inclination angle for molecules throughout the fibril. Combinations of type I:type III scattering factors in a ratio of 4:1 gave a reasonable correspondence with the meridional diffraction series. The attenuation of the meridional intensities may be explained by a blurring of the electron density profile of the D period caused by non-specific or random interactions between collagen types I and III in the heterotypic fibril.

Effect of Chain Architecture on the Crystallisation of Polyolefins

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Advances in understanding and predicting relationships between polymer properties (such as their rheology) and their structure require the availability of macromolecules with well-defined architectures and narrow molecular weight distributions. Polybutadiene with controlled molecular weight and polidispersity close to 1.0 was synthesised using high vacuum techniques. It was then hydrogenated using the diimine method, which is the most direct route to obtain near-monodisperse linear low-density polyethylene. These and some industrial polyethylenes were subjected to simultaneous Small Angle X-Ray Scattering (SAXS)/ Wide Angle X-Ray Scattering (WAXS) / Differential Scanning Calorimetry (DSC) experiments performed at SRS CLRC Daresbury Laboratory, UK and at ESRF, Grenoble, France to analyse their crystallisation kinetics. Rheology (arm retraction mechanisms) were also investigated.

Small Angle X-ray Scattering Analysis of Historic Parchment

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Small Angle X-ray Scattering (SAXS) is a powerful yet non-destructive technique that is capable of providing information about the level of deterioration of collagen fibres within historic parchment. A great deal of cultural heritage is stored on historic parchment throughout the world. Over time and subject to the conditions that the parchment is stored in, the parchment deteriorates. This can be brought about by the effects of pollution, humidity, chemical modification and conservation techniques. As the parchment deteriorates the long-range order of the collagen fibres is degraded. SAXS reveals the level of this degradation. SAXS can provide data concerning the molecular detail of the deterioration of historic parchment in a non-destructive fashion that can be utilised in a conservation context.

Using SAXS/WAXS to follow Shear-Induced Crystallization

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Investigations of shear-induced crystallization have been performed on several commercial and laboratory synthesized polyethylene (PE) samples. A Linkam CSS450 shear cell has been used to shear PE samples providing a pulse of shear at shallow under-coolings from the melt where, quiescent crystallization would normally take several hours. Small- and Wide- Angle X-ray Scattering (SAXS/WAXS) has been used to follow the crystallization kinetics: SAXS giving long range structural ordering, WAXS giving atomic ordering. Time resolved simultaneous SAXS was recorded at the Dubble station ESRF, France and SAXS/WAXS at station 16.1 Daresbury SRS, UK. From the data obtained molecular orientation from a pulse of pre-shear, increases crystallization kinetics up to two orders of magnitude greater than that compared with quiescent crystallization at the same temperature. The SAXS shows that crystalline lamellae structures grow perpendicular to the initial flow direction giving oriented meridional scattering peaks. The WAXS shows similar development of Bragg peaks with some orientation being concentrated in the equatorial direction. During pre-sheared crystallization, the formation of a stacked lamella structure is said to follow a route in which oriented molecular chains act as 'orientation-inducted nuclei'. These then facilitate crystalline lamellae to grow outwards from these sites perpendicular to the direction of flow and the stacked lamellae structure. This is termed as the 'shish-kebab' morphology (the 'shish' being the oriented molecular chains (nuclei) and the 'kebabs' the overgrowth of lamellae). The formation of shear induced nuclei, depends greatly on the molecular weigh, polydispersity and molecular architecture of the sample along with temperature. Critical shear rates are also required to provide oriented chains to form the nuclei. From the experimental investigation, the high molecular weigh chains are seen to give shear-induced nuclei however, low molecular weight chains (and high polydispersity) do not give such well defined oriented lamella morphologies.

An Investigation into the phase behaviour of block copolymers in solution - phases formed and their transition kinetics

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Various block copolymers in aqueous solution have been studied to show how the chain architecture effects the phase behaviour and the kinetics of the phase transitions. This has many biological applications such as artificial lung surfactants in premature babies(1). The block copolymers under investigation are diblock PEO-PI copolymers, triblock PEO-PBO-PEO copolymers, and triblock PBO-PEO-PBO copolymers. Micelles are formed when the concentration of polymer in water reaches a critical value known as the CMC. As the concentration is increased further the micelles pack together to form ordered arrays of specific geometry such as Cubic, Hexagonal or Lamella structures. The structure is deduced from simultaneous SAXS/WAXS/DSC experiments performed on beamlines 8.2 of the SRS Daresbury Laboratory and CRG DUBBLE at the European Synchrotron Radiation Facility (ESRF), Grenoble. Slow heating ramps are employed whilst SAXS/WAXS is collected. The postions of the peak-to-peak ratios determined the geometry that the micellar array adopts. Such experiments are repeated for different block copolymer concentration in order to map the morphology. From the phase diagrams the position of the Order-Order and Order-Disorder Transitions are located. These positions can be located more accurately by monitoring the intensity of the various reflectiojs from the SAXS pattern. This is done using the CCP13 program XFIT. The kinetics of the phase transitions has initially been studied using Polarised Light Microscopy. The sample has been heated to a point where the structure is disordered, and isotropic. Under crossed polars we observe a black image. The sample is rapidly quenched using a temperature jump apparatus to a point where the sample will form a hexagonal or lamellae structure. Coloured images are formed at this point, since the sample is becoming more and more birefringent, thus visible under crossed polars. The degree of birefringence is monitored with time, and Avrami kinetic theory(2) is employed to determine the exponent, which gives us information on the mechanism of the transforamtion, and the rate constant for the phase transition. Simliar experiments have been carried out with SAXS/WAXS techniques. In this case the evolution of a peak is monitored with time.

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Polymers : SAXS/WAXS techniques at DUBBLE(CRG-ESRF)

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The Dutch-Belgian beamline (DUBBLE) is a collaborative research group (CRG) beamline at the European Synchrotron Radiation Facility (ESRF). 30% of its time is available to ESRF users. The SAXS/WAXS station has been operational since January 2000. Most of the research is concentrated in the soft condensed matter area for which the beamline was designed, but it is also possible to perform biological molecule solution scattering experiments. Up to now a considerable range of sample environments has been implemented and made available to the users in order to accommodate the requirements for their specific projects. Complicated sample environments are available like magnets, high temperature heating cell (1500ºC) and the users can combine their SAXS/WAXS experiments with others techniques (FTIR, Raman). Results obtained on polymers with different sample environments will be shown.

Simultaneous SAXS and WAXS study of the precipitation of calcium carbonate

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In this work, we report the study of crystallization of calcium carbonate from aqueous solutions of calcium nitrate and sodium carbonate using simultaneous Wide Angle X-ray Scattering (WAXS) and Small Angle X-ray Scattering (SAXS) techniques. By means of a novel flow cell, in-situ monitoring of the crystallization processes as a function of initial supersaturations has been made on a time-resolved basis. The WAXS results show that the precipitation under the condition of high initial supersaturations proceeds by the production of an amorphous phase, and is then followed by the transformation of this phase to a crystalline phase of which calcite is predominant. The use of the Scherrer Formula and the Full Width at Half Maximum (FWHM) of the Gaussian symmetrical profile function fitted to the calcite (104) Bragg peak has yielded results showing the change in crystallite size with time. The change in integrated intensity of the (104) peak is used to follow the change in mass fraction of crystals in solution with time. The SAXS results show a corresponding change with time. This work has significant relevance to the industry particularly because the on-line observation of the crystallization phenomena has been done using an experimental set-up that mimics industrial crystallizers. It has been shown that WAXS and SAXS techniques are informative, in-situ experimental methods that allow simultaneous observations of time-resolved processes such as those taking place in real, industrial processes.

CRYDAM - From atomic coordinates of macromolecules to scattering intensity

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Computation of scattering patterns from atomic models of macromolecules permits one to validate theoretical models, to analyse similarities between the quaternary structure in the crystal and in solution and to perform rigid body refinement. A program for evaluating the X-ray or neutron scattering intensity from proteins and/or nucleic acids with known atomic structure (e.g. from the Protein Data Bank) is presented. The program is a further development of the programs CRYSOL [1] and CRYSON [2] but uses a more robust way to compute the scattering from its excluded volume and its hydration shell. A grid of densely packed dummy solvent atoms with the radius 0.15 nm is built enclosing the particle. The excluded volume is built from the dummy atoms contacting an atom in the particle. Subsequently, the dummy atoms contacting the excluded volume atoms are considered as potentially belonging to the hydration shell. The program can either predict theoretical scattering intensity or fit experimental curves by varying two parameters, the excluded volume and the contrast of the hydration layer. The program runs on IBM PCs and on the major UNIX platforms.

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