

attenuation at all. In contrast, the delay line detector required a 9x attenuator to be inserted in front of it, to reduce the count rate to a level which it could handle. The image plate of course required no attenuation.

Figure 3 shows diffraction images of dry rat tail collagen taken on the three different detector systems at 3 exposure times. The signal to noise in the RAPID images is clearly superior to that from the other 2 detectors with the 3rd, 6th and 9th order reflections being visible even from a 10ms single shot. The noise level of the image plate obliterates everything in the 10ms exposure whilst the 9x attenuator in front of the delay line detector means that there are insufficient photon statistics to make out any reflections.

During the tests, various problems with the RAPID system were identified. One of these problems can be seen in the intense part of the images where some structure is apparent which is not seen in the image plate data. This structure is caused by an under optimization of the interpolation algorithm which was used for these tests because there was insufficient information to perform the optimization. Now that we have a fully working system, we are confident that this can be corrected.

Conclusions

RAPID works and produces high quality 2-D images at a significantly higher rate than is possible with existing photon counting detectors. It is capable of handling unattenuated diffraction from the SRS from both polymers and biological samples and its noise performance is very much better than an image plate. The electronics commissioning is almost complete and the racks and cooling system are working well. The prototype MicroGap detector with active area of 128 x 128 mm is fully commissioned and working satisfactorily and a new 200 x 200mm active area detector with 128 x 128 electrodes is under construction.

Several difficulties have been identified and further work remains to be done. The calibration and interpolation algorithms must be optimized and system performance tests and characterisation performed. The 4 way striping of the memory system has to be implemented and the user software needs to be completed and debugged.

After completion of the system, integration with the

large area high pressure [3] technology will produce a high rate parallax reduced system for high angle diffraction.

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Neutron Diffraction Study of B-DNA Hydration

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A high angle neutron fibre diffraction study of the distribution of water around the B conformation of DNA has been carried out using the D19 diffractometer at the ILL, Grenoble. Datasets were recorded for DNA in both a D₂O and H₂O environment. Various data analysis techniques involving CCP13 software have been exploited to generate Fourier maps which show the water distribution around the DNA. An ordered water network has been observed in both the major and minor grooves. Refinement of these water positions is revealing a detailed picture of the hydration of this particular conformation.

Introduction

Understanding the structure and hydration of DNA is biologically extremely important. For example it has been shown that water molecules within the groove of the double helix stabilise protein-DNA interactions involved in the regulation of gene

expression. The conformations that the DNA double helix can adopt depend primarily on base pair sequence, hydration and ionic environment. Determination of the hydration network of a particular DNA conformation will hence reveal important information about the role that H_2O plays in structure stabilisation.

Determining DNA structures and the location of interacting cations has been successfully achieved using X-ray fibre diffraction techniques. However these techniques have proved to be less suitable for locating positions of water molecules involved in the stabilisation of DNA conformations. Neutron fibre diffraction was developed as a technique to locate these water positions. The technique is based on the ability to isotopically replace H_2O by D_2O . The large difference in scattering powers of the two hydrogen isotopes can be used to generate difference Fourier maps showing the locations of the water molecules.

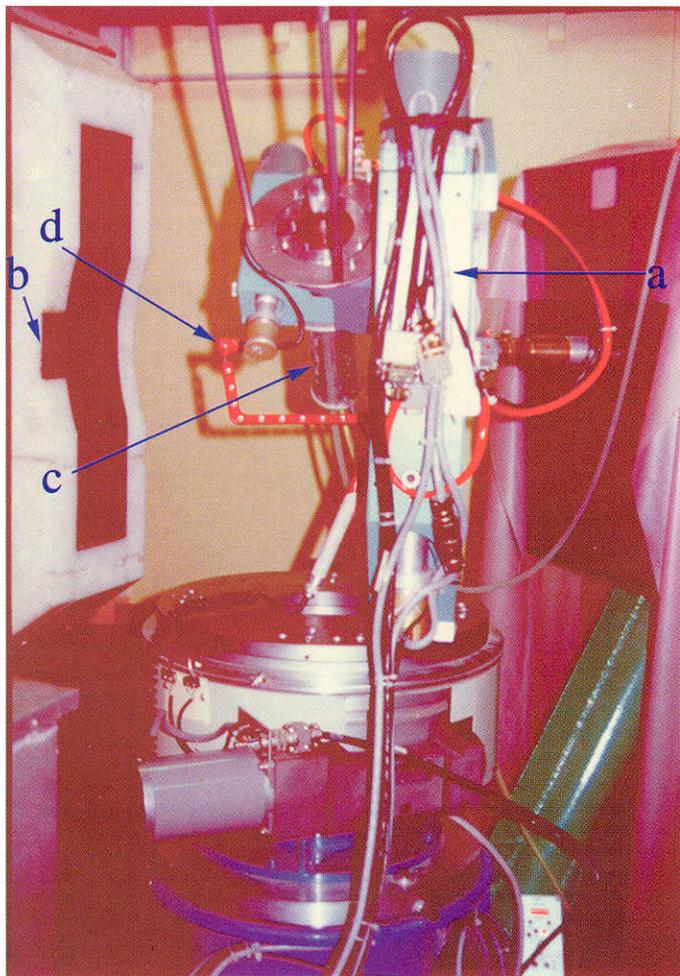


Figure 1: A photograph of D19 showing the four circle diffractometer, the gas filled curved position sensitive detector and the sample can designed to obtain either a H_2O or D_2O sample environment at the required humidity. The sample is positioned inside the can and is attached to the diffractometer using a goniometer mount. The components are indicated as follows: (a) four circle diffractometer, (b) detector, (c) sample can and (d) backstop

Fourier synthesis maps of the DNA + D_2O data also successfully reveal water positions and have the added advantage that they are computed from data where incoherent background scattering from hydrogen is minimised. Previous neutron diffraction experiments have used isotopic difference Fourier techniques to locate the water positions around the A-DNA and D-DNA helices.

In contrast to previous DNA neutron fibre diffraction experiments, there were two new features concerning data acquisition.

(1) A wet spun film sample, with dimensions $\sim 2\text{cm} \times 2\text{cm} \times 0.75\text{mm}$ was used. The quality of the sample was excellent in both orientation and crystallinity. Previous experiments were carried out using samples prepared from single fibres arranged in a parallel array.

(2) Fibre precession geometry was used. This method was devised in order to optimise the efficiency of the data collection process and also to fill in the "blind region" parallel to the fibre axis [1]. D19's 4 circle diffractometer was used to vary the geometry of the sample position relative to both the neutron beam and the gas-filled position-sensitive detector. Figure 1 shows a photograph of the experimental set up on the D19 instrument.

Sample Preparation

The film sample used was prepared using the "wet spinning" method as devised by A. Rupprecht [2]. The wet spinning technique involves the extrusion of DNA, using an automated syringe, through a spinnerette into an alcoholic solution. This causes the formation of threads of DNA which are then wound onto a teflon coated cylinder which has both axial rotation and translation movement. The DNA is then cut from the cylinder and the sheet bathed in ethanol with the required salt content. For the crystalline B conformation the sheet was prepared using calf thymus DNA in a high concentration lithium salt environment.

Data Collection

The data were collected using a curved position sensitive detector, with an aperture of 64° by 4° . Two datasets were recorded, firstly with the DNA in a H_2O environment and then in a D_2O environment. The diffraction patterns obtained consisted of a series of $64^\circ \times 4^\circ$ strips, each strip with a 1° overlap in order

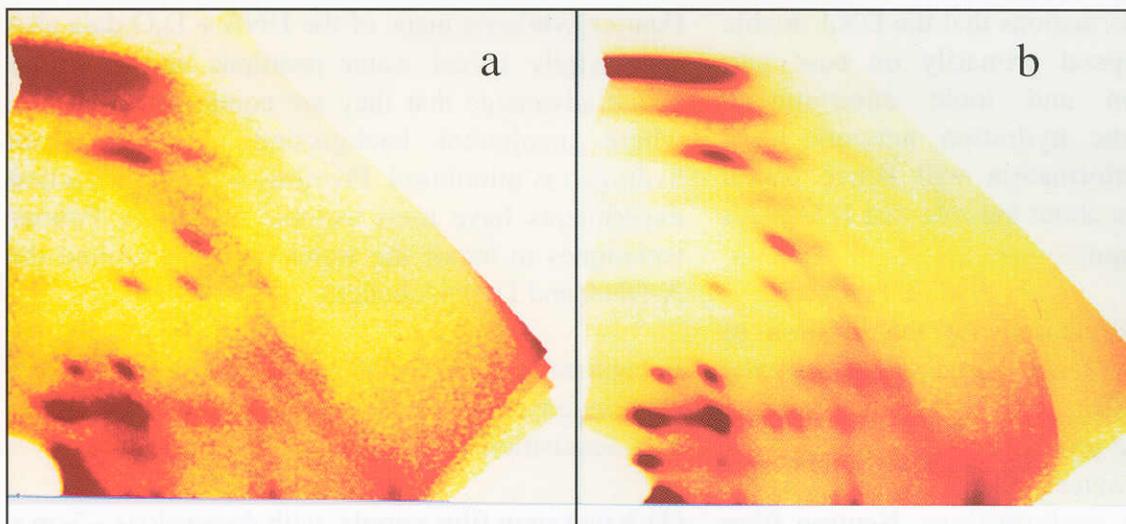


Figure 2 (a) The H₂O data set, comprised of four different sample orientations, mapped into reciprocal space. (b) A continuous LSQINT fit to the data.

to avoid edge effect errors in the final image. The fibre precession technique was used which involved the collection of data with the film sample at four unique orientations relative to the incident beam and the detector. These orientations correspond to four separate fibre tilt angles and therefore allowed the blind region around the (0,0,10) to be filled in.

Data Analysis

Software developed for D19 was used to correct for detector response and map the data into reciprocal space. A correction was applied to the data to account for the effects of absorption by the sample. CCP13 software was next used in order to measure the relative intensities of the diffraction maxima [3]. For comparison two independent methods were used:

(1) LSQINT with the Bragg fitting option and the roving window method for background calculation was used to measure the data. Due to the poor statistics of neutron data, generating a good fit was difficult. In order to eliminate the generation of additional peaks, which were not present in the raw data, LSQINT was modified so that it only fitted peaks to given h,k,l values.

(2) A continuous LSQINT fit to the data was generated and measured using a program which had FIT incorporated within it. This enabled us to interactively measure the intensities of the peaks and assign the intensity to the desired lattice indices. It can be seen from figure 2 that the method provided an excellent fit to the observed data.

The B form of DNA packs into an orthorhombic lattice in space group $P2_12_12_1$ having parameters $a = 30.8\text{\AA}$, $b = 22.5\text{\AA}$ and $c = 33.8\text{\AA}$. A molecular model

was generated using the atomic coordinates published by Arnott and Hukins [4] from which structure factor amplitudes and phases were calculated. The phases for the B model with exchangeable hydrogens replaced by deuterium were

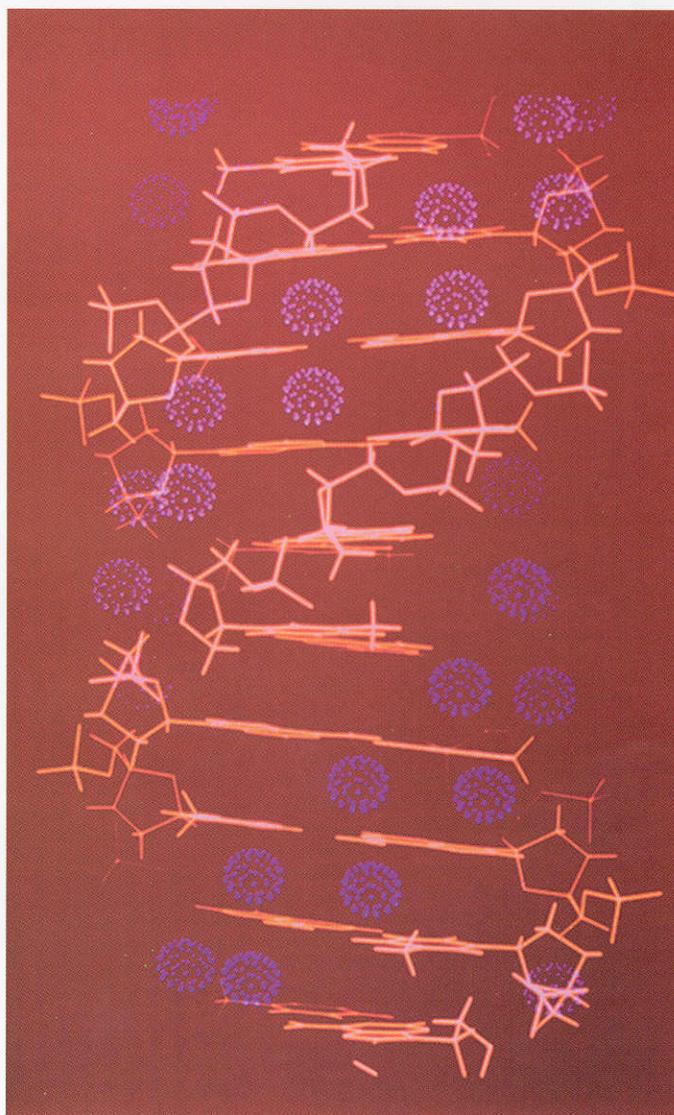


Figure 3: The above photograph shows the B-form model with refined water positions in the major and minor grooves. The major groove positions can be seen as blue stippled spheres and the minor groove positions as purple spheres.

used as starting phases for the D₂O dataset, which enabled the generation of a Fourier synthesis map. In addition to the density associated with the DNA model itself, peaks were identified with D₂O around the molecule. These positions were refined and then included in the model and hence used to generate a new set of phases and a new synthesis map. Figure 3 illustrates the initial refined positions that were chosen in both the major and minor grooves. Repeating this procedure of phase and position refinement is allowing an accurate model of the B-DNA plus hydration to be constructed. A difference Fourier map of $F_{\text{DNA+D}_2\text{O}} - F_{\text{DNA+H}_2\text{O}}$ amplitudes was used as a comparison to the DNA + D₂O Fourier synthesis.

Results

The two Fourier maps generated from the continuous and Bragg fit to the data were virtually identical. This was encouraging as using a continuous fit to measure Bragg data could be useful for measurement of poorly observed data in the future. The Fourier difference map was found to be in good agreement with the Fourier synthesis map. Both maps showed extensive major and minor groove hydration. In

particular close interactions of the water between the sugar oxygen and the N3 of purine bases or the O2 of pyrimidine bases in the minor groove were observed.

Discussion

Neutron diffraction experiments of DNA require very large volume samples. Samples containing individually prepared fibres or those obtained by wet spinning are ideal. Results from neutron high angle fibre diffraction studies can be compared with results from single crystal X-ray diffraction experiments on short oligonucleotide duplexes. Whilst these single crystal experiments typically offer atomic resolution, care must be taken in extrapolating the results to the continuous polymer, since the influence of end effects in the oligonucleotide and crystal packing forces must be considered.

The neutron fibre diffraction study described clearly provides information relating to the extended polymer. However, in this case the data are sequence averaged since random sequence calf thymus DNA was used. The positions which have so far been identified occur in an environment which is relatively independent of base sequence. Any sequence-dependent water interactions are expected to be observed at much lower intensity due to averaging. For example, single crystal studies of the hydration of B-DNA have revealed a minor groove spine of hydration which is disrupted by G-C sequences [5]. Future neutron fibre experiments involving the study of the B conformation of specific base sequences will be of significant interest. Of particular interest will be the effect that extended G-C sequences have on the minor groove spine of hydration.

Future Work

Recent x-ray diffraction studies revealed that the sample exhibited double orientation and so in order to collect a much more detailed diffraction dataset the sample had to be rotated about its meridian and data collected at each sample rotation. This experiment has been performed on instrument D19 at the ILL, Grenoble. The sample was mounted on the diffractometer with its meridian aligned with the diffractometer phi axis. Phi was then incremented to provide sample rotations from -60° to +60° in steps of 10°, with data being collected at each phi. The 10° increment was considered an adequate sampling interval for the Bragg reflections which exhibited

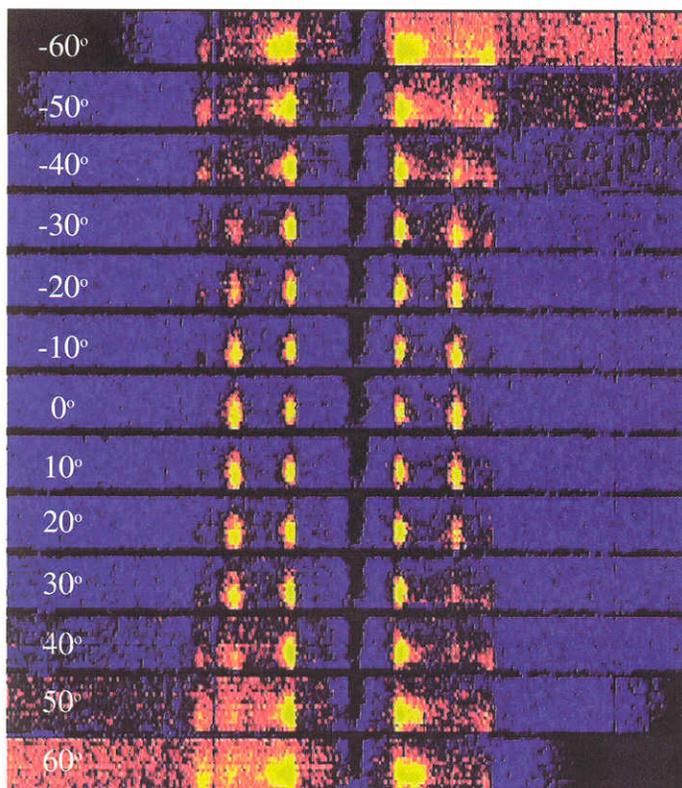


Figure 4: Recent equatorial data collected on D19 illustrating the double orientation of the sample. Each frame corresponds to a different phi rotation about the c axis as labelled above. A number of reflections can be seen coming on and off the Ewald sphere.

angular widths of approximately 60° . In order to collect a complete diffraction dataset a range of rotation of 180° was required. Physical sample restrictions limited data collection to a range of 120° but due to the large angular width of the reflections, reciprocal lattice points occurring in the 'physical blind region' had associated reflections which were visible within our range of rotation and could be fitted by extrapolation of the recorded data. Figure 4 shows data collected on the equator at sample rotations ranging from -60° to $+60^\circ$ in increments of 10° . The data recorded during this neutron fibre diffraction experiment are in the process of analysis at the present time. The data recorded are expected to reveal a more detailed picture of B-DNA hydration.

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Chain Conformations in Polyurethanes : A SAXS & SANS Study

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Segmented polyurethanes are statistical block copolymers comprising incompatible hard and soft blocks. A range of poly(ether-urethane) copolymers have been synthesised in order to verify which is the primary driving force behind microphase separation: block incompatibility or crystallisation of the segments. Two classes of copolymer have been synthesised: one class contained a semi-crystalline 4,4'-methylene diphenyl diisocyanate (4,4'-MDI) and butanediol (BDO) based hard segment, the other contained an amorphous hard segment based on a blend of 2,4- and 4,4'-methylene diphenyl diisocyanate (2,4/4,4'-MDI) and BDO. The soft segment in both types of copolymer was polypropylene oxide (PPO). The materials were synthesised with various hard segment contents: 25, 50 and 75 % by weight. The PPO samples had

molecular weights that ranged from 400 to 4000. Characterisation of the materials involved heating the materials until homogeneous in order to remove all traces of prior thermal history, and then annealing the samples at different temperatures. Quenching in liquid nitrogen was used to "freeze-in" the resultant morphologies. These samples were then analysed using separate differential thermal analysis experiments (DTA) and X-ray scattering experiments. Both the 4,4'-MDI and 2,4/4,4'-MDI copolymers which contained low hard segment contents or low molecular weight soft segments were shown to be homogeneous after annealing. Annealing had little effect on these materials. However, annealing the higher molecular weight 4,4'-MDI copolymers promoted crystallisation and produced larger microdomains with sharper interfaces. As a consequence of this, the PPO chains were stretched more in the 4,4'-MDI copolymers than in the 2,4/4,4'-MDI materials. In contrast, annealing had little effect on the 2,4 / 4,4'-MDI copolymers, evidence that crystallisation plays an

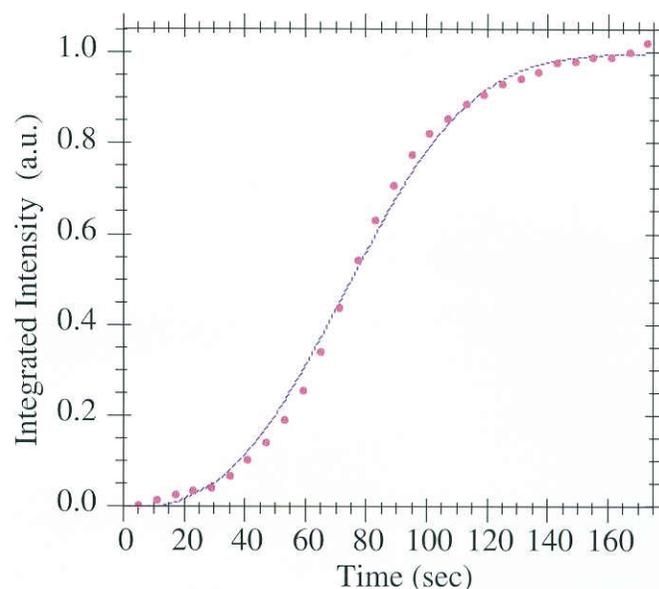


Figure 1 Integrated intensities during the isothermal microphase separation of a polyurethane. The line is a fit of the Avrami model to the data.

important part in the microphase separation process. The kinetics of microphase separation and crystallisation were investigated using time-resolved X-ray scattering experiments. Several models were used, including Avrami and time-dependent Ginzburg-Landau (TDGL) analysis. Aspects of the microphase separation process fitted both the spinodal decomposition (SD), and nucleation and growth (NG) models. However, the latter was favoured because the Avrami equation was seen to be in excellent agreement with the data (see Figure 1),