Experimental report

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Proposal: 9-13-625 **Council:** 4/2015

Title: The structure and the melting transition of fibre DNA in solution

Research area: Physics

This proposal is a new proposal

Main proposer: Adrian GONZALEZ RODRIGUEZ

Experimental team: Andrew WILDES
Adrian GONZALEZ RODRIGUEZ
Mark Robert JOHNSON
Trevor FORSYTH
Nikos THEODORAKOPOULOS
Santiago CUESTA LOPEZ
Marta MARTY RODA
Michel PEYRARD
Jean Luc GARDEN

Local contacts: Estelle MOSSOU
Viviana CRISTIGLIO
Bruno DEME

Samples: Deoxyribonucleic Acid (DNA) from salmon testes

Instrument	Requested days	Allocated days	From	To
D16	3	3	30/11/2015	03/12/2015
D19	6	5	03/12/2015	08/12/2015

Abstract:

We have previously used neutron scattering to study the melting transition in fibre DNA, measuring the change in the intensity and shape of Bragg peaks as a function of temperature to determine the spatial correlations along the molecule and comparing the results to statistical mechanics models. Our work did not account for the effects of confinement, imposed by the fibre structure, on the melting transition. We now wish to study those effects by immersing fibre DNA in solutions that will allow the samples to swell, but not to dissolve. The solutions will be water containing either polyethylene glycol (PEG), thereby applying osmotic pressure to the samples, or ethanol of sufficient concentration that the DNA is immiscible.

The experiment attempted to study the melting transition of DNA fibers submerged in different aqueous media. The Bragg peak that arises at 1.87 A^{-1} in crystalline samples of DNA is related to the correlation among close base pairs (it is often called 10^{th} layer peak). It allows to study the evolution of the correlation length in the molecules through the transition.

Several samples were studied. All of them consisted of Na-DNA fibers sealed inside aluminum cassettes under different environments. Among others: One ‘’dry’’ sample which was sealed under a 92% humidified atmosphere. Three PEG samples; each one with a solution of polyethylene glycol of 6000 MW in D_2O with concentrations 17, 20 and 40 % (w/w). Two ethanol samples; sealed with mixtures of deuterated ethanol and D_2O at 60% and a 66% D-ethanol concentration (v/v).

The experiment had two parts. The first one was carried out in D16 and its purpose was to get a structural characterization of the samples as well as to gauge the intermolecular distance in order to quantify the confinement of the molecules. The second one was the melting experiment, performed in D19, in which the evolution of the 10^{th} layer peak in function of temperature was recorded.

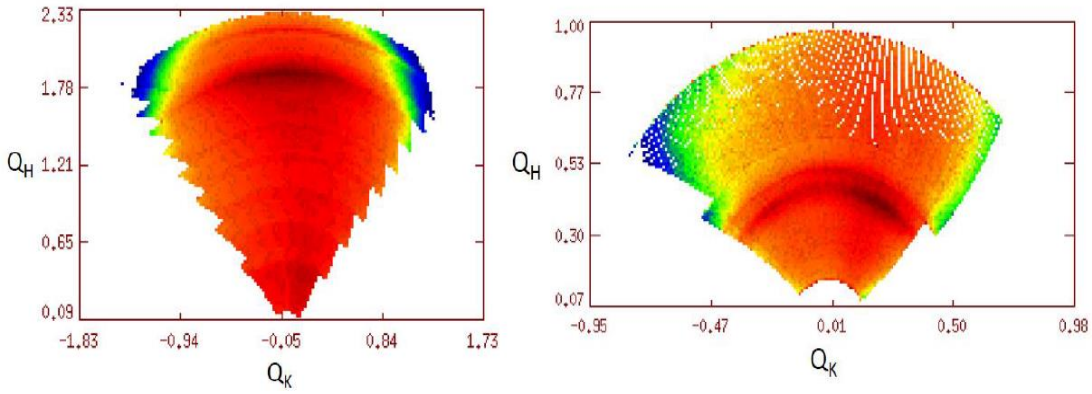
At D16 reciprocal space maps (RSM) were recorded. This procedure was repeated for two sample orientations. These orientations were: horizontal (fiber axis in the scattering plane) and vertical (fiber axis perpendicular to the scattering plane). An example of the results can be seen in Fig 1.

The RSM in horizontal orientation (example in Fig. 1a) proved all PEG samples were in crystalline B form (due to the broad Bragg peak placed at 1.87 Q_H). Ethanol samples were mostly B form also but they showed some degree of A form contamination.

From the shift in the ring features located in the low Q region of the vertical reciprocal map (Fig. 1b) a change in the inter-axial distance of the samples with respect to the ‘’dry’’ sample can be deduced (in the dry sample the confinement is maximum). For better gauging this shift we performed a radial integration and plotted the integrated intensity versus Q (Fig. 3). For the samples in which the peaks shifted to lower Q the intermolecular distance increased. The distance became bigger for all the submerged samples except for the PEG 40% in which the peaks are roughly at the same position. The increases in the PEG samples reached up to 12% whiles in the ethanol samples were around 2% at most.

On D19 three samples were studied: the 17% PEG sample, the 60% and the 66% ethanol samples. Here the focus was to get good quality scans along the fiber axis, this is a scan in which we record the intensity along Q parallel to the fiber axis (Q_H) when Q perpendicular to the fiber axis (Q_K) is zero, see Fig. 2. These scans were recorded at temperatures between room temperature and around 100 °C. An example of the evolution of the peak is shown in Fig 3.

Figure 1: Reciprocal space maps in horizontal (a) and vertical (b) orientations for the PEG 17% sample.



(a) (b)

Figure 2: Schematic of the DNA fiber sample inside the aluminum sample holder. The DNA reciprocal coordinate system with respect to the sample cassette has been represented.

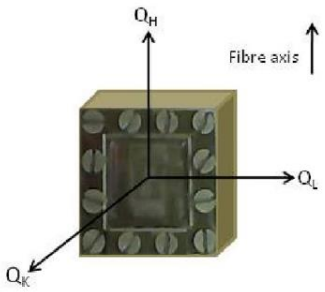
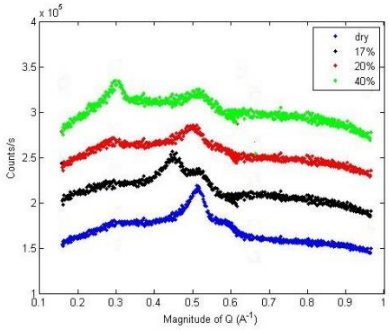


Figure 3: Radial integrated intensity in function of the modulus of Q.



During the melting study improvements were achieved with respect to previous experiences. It was noticed that the 10th layer peak of the samples was relatively symmetric which may allow an easier fit of the data.

At ANSTO we were afraid of boiling the solution in the sample and we stopped the heating below 100 °C. A small but detectable peak was still recorded at the highest temperature and upon cooling most of the peak signal was recovered. In this new experiment, as it could be noticed in Fig 2., at 112 °C the only features remaining seem to be scattering from the buffer solution and the signal was not recovered at all during cooling. Most possibly in our first trial we did not complete the melting so the structure inside the fibers was still ordered and it could recover. Oppositely we think this time we were able to observed the whole transition which is an asset.

Figure 4: Evolution of the base-pair peak (10th layer peak) of the PEG 17% sample with temperature. Black corresponds to 25 °C, red to 90 °C and blue to 112 °C. Cyan data was collected at 100 °C from a control sample: a cassette with a PEG solution (no DNA) identical to the solution used in the DNA sample. Error bars have been removed for the shake of clarity.

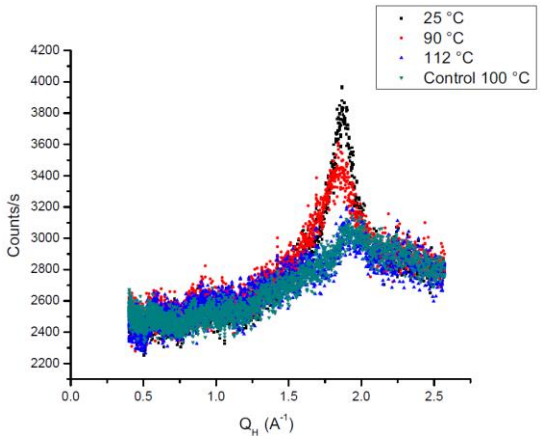
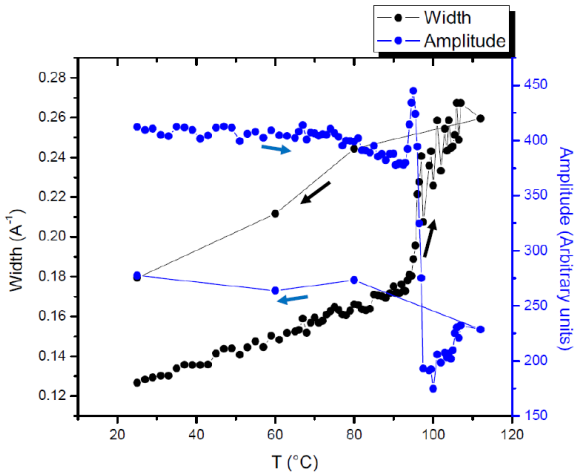


Figure 5: Amplitude and Width of the 1.87 A^{-1} peak in function of temperature. The curves were fitted to a lorentzian function with a sloping background.



In Fig. 4 one can observed the amplitude and width of the peak (calculated by using a lorentzian fit) in function of temperature. The amplitude starts to decrease steadily at around 70 °C and drops sharply at 90 °C which is coherent with the onset of the melting transition (the intensity of the peak is proportional to the number of closed base pairs which is decreasing as the transition advances). In the same temperature interval the width of the peak undergoes a sudden increase which is as well coherent with the melting and the decrease in the average size of the closed domains in the ADN molecule. These data can be used for calculating the evolution of the correlation length along the molecule in function of temperature and percentage of open base pairs.

These experiences proved that studying the melting transition in aqueous environments with scattering techniques is feasible and that the background in the PEG samples can be reduced to manageable levels. Also, this is the first time the melting transition of DNA is studied in this fashion in an ethanol/water solution.