Experimental report

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Proposal: 9-13-751 **Council:** 4/2017

Title: The melting of fiber DNA submergedin D-ethanol/D2O mixtures.

Research area: Physics

This proposal is a new proposal

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Samples: Li-DNA submerge in D-ethanol/water mixture

Instrument	Requested days	Allocated days	From	To
D19	4	6	05/09/2018	11/09/2018
D16	1	1	04/09/2018	05/09/2018

Abstract:

We are focused on finishing an ongoing project about studying the melting transition (or thermal denaturation) of DNA fibers submerged in different solutions. Namely solutions of polyethylene glycol in D2O and mixtures of D-ethanol and D2O. The peak that appears in the scattering pattern of this samples at Q=1.87 reciprocal Angstroms is related with the coherent scattering from closed base pairs and can be used to follow the evolution of the average size of the closed domainds during the transition. This average size is related to the coherence lenght of the distribution of closed basepairs which is a fundamental parameter for characterizing the transition. The part of the project focused on polymer solutions is completed and a publication is under preparation. We request now beam time in order to collect important missing data in the D-ethanol/D2O samples. These D-ethanol samples have showed a higher signal-to-noise ratio in comparison with the samples with long-chain polymers. The data suggest the ethanol mixtures affects the structure of the fibers differently from the polymer solutions.

The melting transition of DNA has been studied for decades but there is still little structural information on how the base pairs open as the temperature increases. We have previously probed how osmotic pressure exerted by an aqueous environment affects the transition [1]. This new experiment aimed to study how the changes in the hydrophobic and electrostatic forces (caused by different deuterated ethanol/water mixtures) affect the melting transition as both ethanol and heat-induced partial/total opening of DNA sequences are ubiquitous in biotechnology labs.

The experiment consisted of two parts. In the first one we used D16 to characterize the samples at room temperature which allowed to assert the crystalline quality of each sample, measured by the signal to noise ratio or representative Bragg peaks. This data also allowed to estimate the average distance between dsDNA molecules in our samples. The intermolecular distance is an important factor to understand the transition as the intermolecular interactions are strongly dependent on this distance and affect the evolution of the transition [1]. Figure 1 shows the Bragg peaks arising from the strong correlation between pararelly oriented molecules in the fibres which are distributed forming an orthorhombic crystalline lattice [2]. By indexing these peaks, the intermolecular distance for each sample can be estimated. Table 1 summarizes the results. The Interaxial distance decreases as the fraction of ethanol increases as expected by the decrease of the water activity of the mixture and previous works [3].

Figure 1: Intensity versus the magnitude of the scattering vector for DNA fibres submerged in deuterated ethanol/water mixtures in v/v % and equilibrated at 75% relative humidity. For collecting these data, the fibre axis was perpendicular to the incident beam. The red line represents the fit of several gaussians over sloping backgrounds to extract the centre of the peaks which is used in the intermolecular distance calculation.

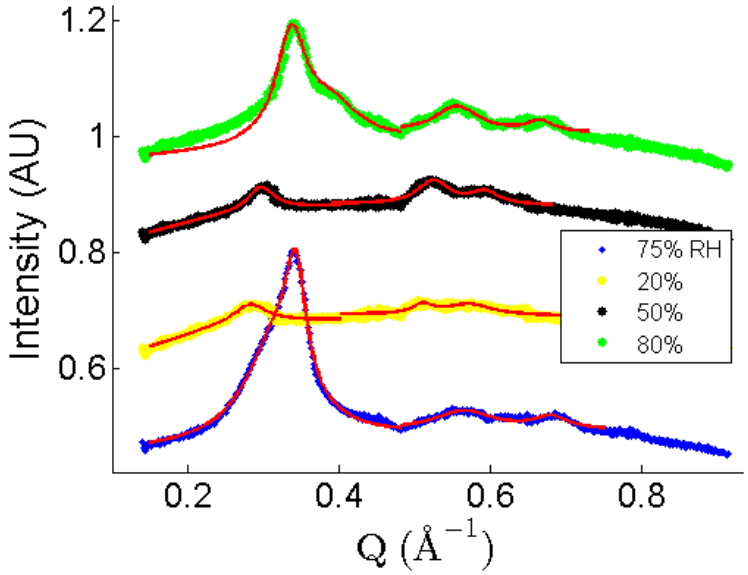


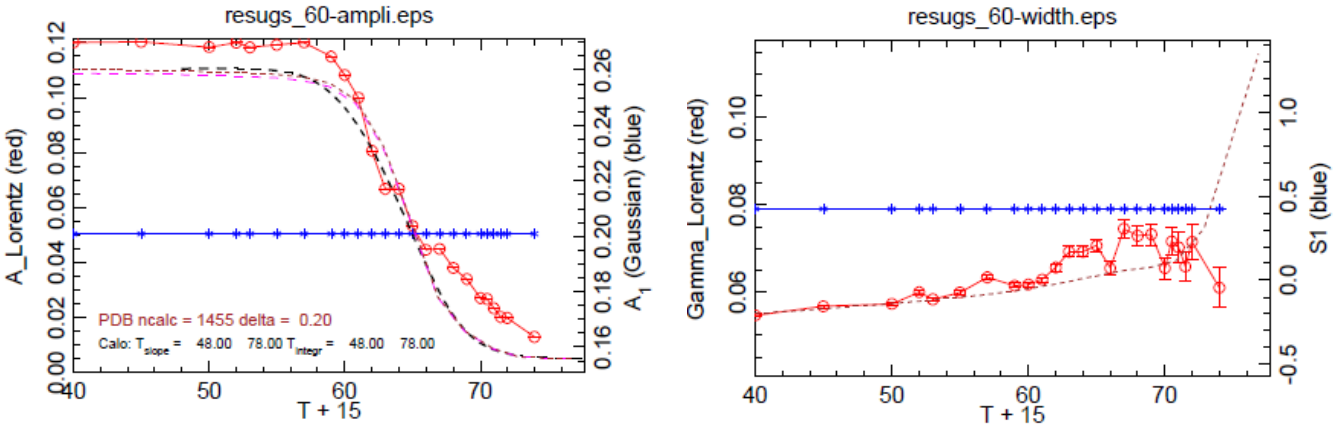
Table 1: Interaxial distance for each sample and percentage interaxial distance increase with respect to the humidified fibres.

Sample	I_d (Å)	$\frac{\Delta I_d}{I_{d,Hu}}$ (%)
75% RH (Humid)	17.62	0
20%	20.96	18.96
50%	20.49	16.28
80%	17.58	-0.22

The second part of the experiment was carried on in D19. The Bragg peak with centre at $Q = 1.87$ Å^{-1} which arises from the strong correlation between closed base pairs in the direction of the fibre axis was monitored as a function of temperature for the different samples. The integrated intensity of this peak is proportional to the number of closed base pairs so it can be used to compare this measurement to calorimetry data. The width of the peak is inversely related to the correlation length of the average DNA molecule which relates to the distribution of open and closed base pairs along the molecule. In order to interpret the data, these quantities were modelled with a mesoscopic statistical mechanical model, the Peyrard-Bishop-Dauxious model (PBD model) [1]. Figure 2 shows a compassion of experiment and theory for a representative sample. The overall agreement between theory and experiment validates the theory behind the PBD model. The fact that the background does not seem to change significantly with temperature suggest that it does not affect the extraction of the melting data. The model describes the behaviour of the base stacking (inter-base pair interactions) and Morse potential (intra-base pair interaction) as the ethanol concentration increases which adequately quantifies the effect of the ethanol on the transition.

The complete results of this experiment will be extensively discussed in a paper which is now in preparation.

Figure 2: A) Integrated intensity of the 1.87 Å^{-1} Bragg peak given by a Lorentzian fit of the neutron scattering data (red open circles) versus temperature. The dashed black line is proportional to the fraction of closed base pairs found by performing calorimetry in identical samples. The dashed pink line is the prediction of the fraction of closed base pairs given by the model and the blue dotes related to the amplitude of a gaussian used to described phenomenologically the diffuse scattering background around the Bragg peak during the fitting of the data. B) Width of the same Bragg peak in panel A given by the fit as a function of temperature (red open circles). The dashed pink line is the prediction by the model and the blue dots are the slope of the sloping background used during the fit.



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[1]- Melting Transition of Oriented DNA Fibers Submerged in Poly(ethylene glycol) Solutions Studied by Neutron Scattering and Calorimetry; J. Phys. Chem. B 2018, 122, 2504−2515.

[2]- The Molecular Con_guration of Deoxyribonucleic Acid.I, J. Mol. Biol., 1960, 2, 19-37.

[3]- R. Franklin and R. Gosling. The structure of sodium thymonucleate fibers i: The influence of water content., Act. Crys., 1953, 6, 673.