Experimental Report

Proposal: **9-13-467** **Council:** 4/2012

Title: The measurement of the persistencelength in DNA as a function of temperature

This proposal is a new proposal

Researh Area: Physics

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Samples: DNA

Instrument	Req. Days	All. Days	From	To
D22	4	3	29/11/2012	02/12/2012

Abstract:

The persistence length of DNA is a quantity used to define the flexibility of the molecule. It is a key parameter to describing the conformation of DNA and, as a result, many biological functions of the molecule including how it packs and its interaction with proteins and membranes. The persistence length varies in the presence of denatured regions, called "bubbles", which can be created using temperature as a parameter. We propose to use SANS to follow the shape of short-length DNA with predefined sequences as a function of temperature, thus correlating the persistence length to the formation of bubbles. We plan to follow the scattering up to the temperature where the two chains of the molecule completely denature, known as the "melting" temperature, and will support our findings with quantitative modeling using the Peyrard-Bishop-Dauxois (PBD) model.

The experiment attempted to use SANS to measure the persistence length of synthetic DNA as a function of temperature. The ultimate aim of the project is to investigate the statistical dynamics of the molecule. The dynamic fluctuations of DNA will increase as the temperature increases. The fluctuations influence the flexibility of DNA and will eventually lead to the bonds between the base pairs being broken at a sufficiently high temperature, with the double helix separating into two single-chain DNA molecules in the so-called "melting transition". The transition can be modelled using non-linear statistical mechanics, and can be explicitly modelled when the DNA sequence is known [1]. Our intention was to test the theory in a well-defined experiment.

The sample consisted of short-chain synthetic DNA, having 200 base pairs, in a dilute solution of deuterated water. The DNA was GC-rich at the ends with an AT-rich sequence in the middle. AT base pairs, having two hydrogen bonds, are more weakly bound than GC base pairs with three hydrogen bonds, hence the middle of the molecule was expected to become more flexible than the ends as temperature was increased, separating to form a "bubble" at a lower temperature than the melting transition.

A specialized sample environment was constructed to enable the melting transition to be followed using *in-situ* UV absorption spectroscopy. There were numerous technical issues with the UV equipment, namely synchronizing the measurements with the neutron data collection, and this was unsurprising given that this was the first time that the equipment was used. The ILL instrument control service are now addressing the problem of incorporating the control of the UV equipment inside the NOMAD software package.

The experiment was a qualified success, as we were able to measure the SANS and UV signals as a function of temperature. SANS data are shown in Figure 1 and UV absorption data are shown in Figure 2. The data show a clear change with temperature, reflecting the expected change in the persistence length and in the eventual melting of the sample.

Some issues during the experiment meant that the program could not be completed, and further measurements will be needed. The issues may be summarized as:

- The experiment required that the SANS data represented the scattering in the dilute limit. Data analysis showed the possibility that coagulation was an issue in our sample, due to the DNA not having been left in solution for a sufficiently long time. This issue may be rectified by improved sample preparation protocols, as shown in a subsequent SAXS experiment.
- The experiment appeared to show that effects from the formation of DNA bubbles were present even at the lowest temperatures (25°C). This issue may be rectified by measuring from lower initial temperatures, which is entirely feasible on D22.
- Some ambiguity in the analysed data suggested that the AT-rich part of the sequence may have been somewhat too large. This issue may be rectified by refining the sequence for the artificial DNA.

We plan to address these issues, along with improving the synchronization of the SANS measurements with the UV measurements, in the future.

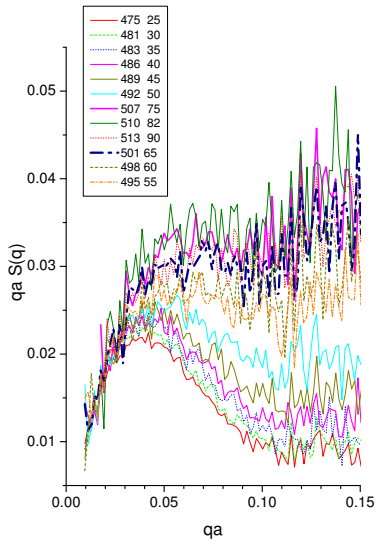

Figure 1: Initial D22 data from the melting of short-chain DNA given in dimensionless units where *a* is the mean distance between base pairs. The legend shows the temperature of each curve in Kelvin (left) and Celsius (right). The sample was first heated, then cooled.

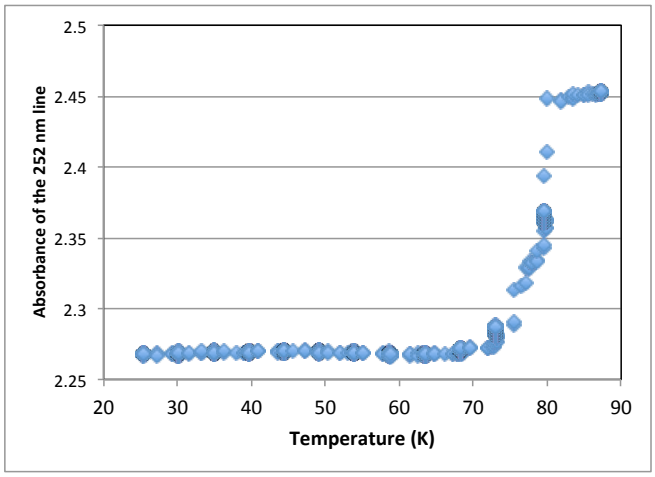

Figure 2: *In-situ* UV absorption data for 252 nm light measured during the data collection in Figure 1. The melting transition is apparent from the shape increase at ~ 78°C.

References:

[1] N Theodorakopoulos and M. Peyrard, Phys. Rev. Lett. **108** (2012) 078104