Experimental report

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Title: The spatial variation of the persistence length along Widom-601 DNA

Research area: Biology

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Samples: Widom-601 DNA
RF III DNA

Instrument	Requested days	Allocated days	From	To
D22	4	2	03/08/2015	05/08/2015

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 is probably not constant along the length of the molecule, however. One biological function that may be strongly influenced is histone positioning, which may be driven by local variations in the persistence length. The commonly investigated Widom-601 DNA molecule is known to have a strong histone positioning effect. Spatial variations in the persistence length will result in changes in the conformational structure of DNA, and these will be observable in scattering experiments. We propose to use SANS to measure the conformational structure of Widom-601 in solution as a function of temperature to determine the spatial variation in the persistence length along its helical axis.

The experiment attempted to use SANS to gauge the persistence length of synthetic DNA as a function of temperature. The ultimate aim of the project is to investigate the effect of the local openings of the base-pairs (so called bubbles) in the flexibility of DNA and so the potential impact of the sequence in the biological functionalities of the molecule. The dynamic fluctuations of DNA will increase as the temperature increases. The fluctuations will eventually lead to the bonds among the base pairs being broken at a sufficiently high temperature, with the double helix separating into two single strands 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.

The sample was a solution of a synthetic DNA commonly known as the widom-601 sequence at 2.2 mg/ml in tris buffer with deuterated water, EDTA and NaCl. It has 145 bp and a rich AT-region in one end.

The experiment proceeded in the following way: we stabilized the sample temperature at 15 °C, we performed two SANS measurements with a detector distances of 5 and 17 m. Then, we set a new temperature for the sample environment using a water bath and we ran another measurement of SANS during the time the sample temperature took to equilibrate (15 min). Then we repeated the procedure. During each SANS measurement a set of 20 U-VIS spectra were taken through the two sample position. One position contained the sample and the second position contained a similar cell filled with the buffer (no DNA). These measurements were equally distributed in time over the duration of the SANS measurement. The maximum temperature reached in the heating process was 92 °C (approximate sample temperature).

After the DNA sample we recorded SANS curves at different temperature of a similar sample container filled with the buffer used for the solvation of the DNA.

A contrast of a DNA curve and a buffer curve recorded at room temperature can be seen in Fig. 1a. Thus it can be assured that we detected a signal from the DNA in the low Q region.

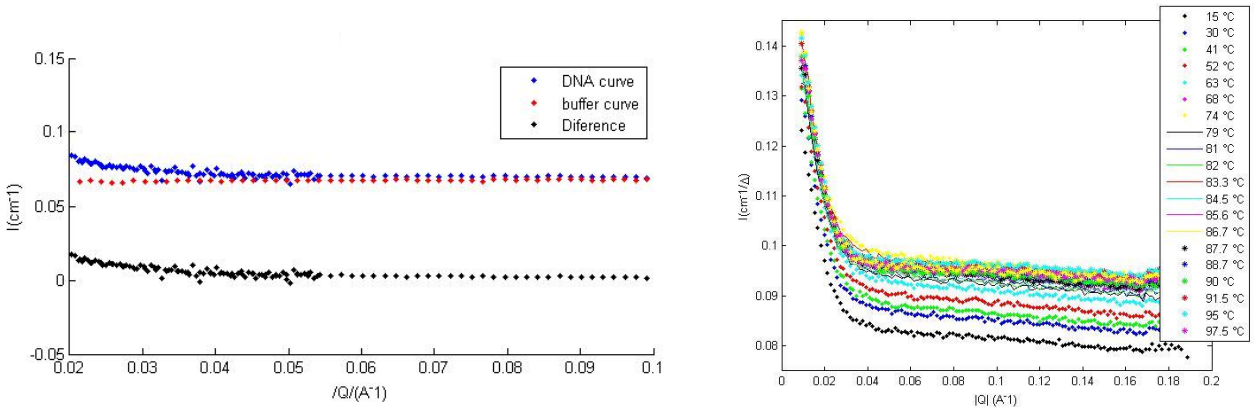
In Fig. 1b we present the different SANS curves in function of temperature. There is an obvious increase of the general intensity of the curves (a non Q dependent shift of the intensity of the curves to higher values) as temperature increases. This effect cannot be related with the DNA since it appears in the buffer solution, thus it is due to some temperature related changes in the solution but not in the DNA. This effect is still unexplained.

A potential issue is the fact that apart from this escalating factor in intensity the curves do not seem to undergo extreme changes. Once they are normalized to zero baseline by subtraction of the buffer they appear to be pretty much identical. Not only the curves are identical to the naked eye but the our analysis was not able to detect significant changes in the form factor and so in the persistence length of the molecules. We think that since the DNA molecules use had a length of about 32 nm and the persistence length of DNA is nominally 50 nm further decrease in the persistence length did not generated a dramatic change in the shape of the molecules and therefore the scattering pattern also remain stable with temperature. Another reason for not detecting dramatic changes is that the melting transition was not completed, we can assure such a thing since the U-Vis absorption (Fig. 2) proves that at the highest temperature we could reach with our set up the percentage of open base pairs is 82%.

There are changes nonetheless. Normalizing the temperature-dependent data to those measured at 15 °C resulted in Q-dependent data that could be fitted with a straight line (Fig. 3a). The slope of the normalized curves increases with temperature (Fig. 3b) which implies there is an increase in the signal at high Q as temperature raises.

This temperature dependent evolution points out to an increased flexibility of DNA, associated to a "folding" in smaller spatial scales, hence an increased signal in the high Q region, but the present data are not sufficient to allow a quantitative measurement of the persistence length versus temperature, so further experiments are required.

Figure 1: Comparison of a SANS curve of the DNA sample and of the buffer at room temperature (a) and SANS curves of the DNA sample at different temperatures.



(a) (b)

Figure 2: U-Vis absorption in function of temperature for the DNA sample.

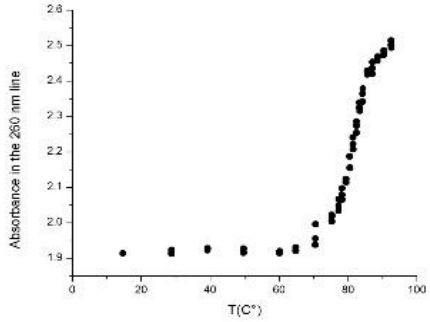
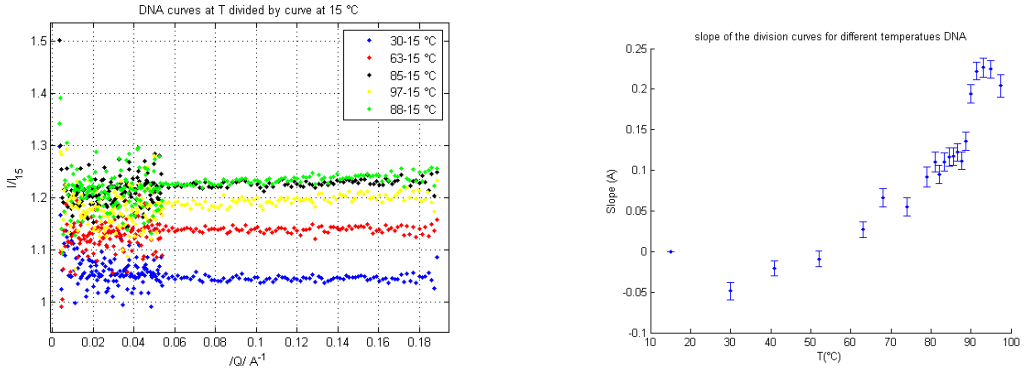


Figure 3: Normalization of the SANS curves at T by the curve at 15 °C (a) and slope of the linear fit to the normalized curves in function of temperature (b).



(a) (b)