# An experimental study of the temperature-dependent DNA elasticity using optical tweezers.

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Temperature plays a key role in all biological processes. Slight changes of temperature may lead to completely different behaviors of biological systems. In fact, living matter carry out its function in a small range of temperature. Therefore, it is interesting to study and understand what is the effect of temperature in biological systems. Single-stranded DNA (ssDNA) is one of the most relevant molecules in biological processes, providing us an excellent scenario to understand how the temperature affect its properties. In this project we aim to understand and characterize the elastic response of ssDNA at different temperatures. We have used the Laser Optical Tweezers (LOT) technique in order to measure the Force-Distance Curves (FDC) of ssDNA under the effect of a mechanical force at different temperatures. By fitting the stretching response of ssDNA to two semiflexible polymer models we have obtained the temperature dependence of the elastic parameters. We have found that persistence length and Kuhn length increase with temperature while the stretching modulus seems to be insensitive to temperature changes. Finally, we have found that the persistence length is proportional to the Debye screening length and that it varies with temperature according to a power law with exponent $< 1$.

## I. INTRODUCTION

DNA (DeoxyriboNucleic Acid) is one of the most important biomolecules due to its major role in many biological processes: DNA encodes the genetic information in living organisms. At the beginning of the 20th century, Phoebus Levene suggested that DNA is made up by individual units called nucleotides [1]. Each nucleotide is composed by a nitrogenous base, a sugar and a phosphate group. The nucleotides are often identified by their nitrogenous base. In the case of DNA, there are four possible bases: cytosine, guanine, adenine and thymine (C,G,A,T respectively).

It was not until the 50s when Watson and Crick proposed the tridimensional structure of DNA molecules [2]. They showed that DNA consists on two polymer strands coiled around each other forming a double helix. Such strand is called a polynucleotide. The double helix structure is stabilized by hydrogen bonds between the bases of the two strands. Base pairing obeys the following rules: A pairs up with T via two hydrogen bonds and G pairs up with C via three hydrogen bonds.

As we mentioned before, DNA is involved in a large number of processes and biological reactions. For example, important processes affecting DNA are replication and transcription. Nowadays we know that the cell replicates its DNA before dividing. In order to copy the DNA, the double helix structure has to be unwound under the action of the helicase enzyme. In other words, DNA undergoes a structural transition from the double stranded form (dsDNA) to the single stranded form (ssDNA). Each ssDNA molecule will act as a template to assemble a new DNA molecule.

In the last years, there has been a great interest in the mechanical properties of single DNA molecules. Thanks to recent developments of single molecule experiments (SME) it is possible to study some molecular reactions manipulating one single molecule at a time. We can explore the behavior of one molecule at once, in contrast to bulk experiments, where only the average behavior of many molecules is measured [3]. By applying mechanical forces to the ends of dsDNA molecules it is possible to extract precise information about its elastic response.

On the other hand, we know that temperature has a great impact in metabolic reactions or kinetic rates. In this study we are interested in the temperature dependence of the mechanical response of ssDNA molecules.

In this project we aim to understand the elastic behavior of ssDNA molecules performing stretching experiments under different thermal conditions. We mechanically pulled DNA hairpins until ssDNA molecules are obtained. By flushing an oligonucleotide solution designed to bind in a specific region of the hairpin, it is possible to inhibit the refolding of the hairpin, allowing us to carry out pulling experiments of ssDNA molecules in a wide temperature and force window.

This work contributes to a better understanding of the elastic response of ssDNA at different temperature conditions, which is of fundamental importance to extract the base pairing free energies of dsDNA at different temperatures.

The report is organized at follows: in section II we introduce two of the most well-known elastic models that characterize the mechanical response of ssDNA under the action of a mechanical force. Then, the factors that can affect such elastic response are briefly discussed (Section III). In the following section we introduce the experi-

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mental setup and the measuring protocol that we have used. In section V we present the main results that we have obtained and, finally, we discuss the main conclusions of our work in section VI and explain some of future perspectives of the study in section VII.

## II. ELASTIC MODELS

The word polymer comes from ancient Greek. It results from mixing the prefix *poly-*, which means "many", and the suffix *-mer*, which means "parts". It is a widely used word in biochemistry and physics to refer to long molecules composed of many repeated sequence units (monomers). The total number of subunits that compose a particular sequence is the so-called degree of polymerization. A molecule is usually called a polymer if the degree of polymerization exceeds the number of 100. In such context, nucleic acids are a good example of biological polymers.

Polymer molecules can have a large number of degrees of freedom. For instance, consider the polyethylene molecule. It is composed by repeating a monomer unit, the ethylene: $C_2H_4$. Each C-C bond can rotate in an independent way from the others. It allows the molecule to explore many different configurations. Then, how do we describe a polymer from a physical point of view?

We will focus in the two most used elastic models that describe the behavior of long molecules when a mechanical force is applied to their ends.

### A. Freely Jointed Chain

In the Freely Jointed Chain (FJC) model we can imagine our polymer under study as a succession of $N$ bonds of fixed length $b$ (the so-called Kuhn length). Each bond is assumed to be rigid and the bond junctions can take all possible angles with equal probability independently of each other (i.e. their relative orientations are completely random). Hence, the directions of neighboring bonds are completely uncorrelated.

When the chain is subjected to an external force $f$, the bonds tend to align along the direction of the external force. Just as in the case of a dipole in an electric field, we may write the energy of our chain under tension as:

$$\mathcal{H}_{FJC} = -fb\sum_{i=1}^{N}\cos\theta_i \tag{1}$$

The external force is opposed by the thermal agitation, that tends to disorder the bonds of the polymer. Thus, the force changes the equilibrium end-to-end distance, forcing the polymer to adopt less probable conformations. Therefore, the elastic response of the polymer is mainly entropic. The mean end-to-end distance for the FJC model is given by:

$$\langle x\rangle = L_c\left(\coth\frac{fb}{k_{\mathrm{B}}T} - \frac{k_{\mathrm{B}}T}{fb}\right)\left(1+\frac{f}{S}\right) \tag{2}$$

Where $L_c$ is the contour length of the molecule, $k_{\mathrm{B}}$ is the Boltzmann constant, $T$ is the absolute temperature and $S$ is the stretching modulus of the polymer. The last term is an enthalpic correction for high forces.

### B. Worm-Like Chain

The Worm-Like Chain (WLC) model is more sophisticated than FJC model. Instead of imagining the polymer as a succession of rigid rods of fixed length, in the WLC model the polymer can be visualized as a continuous string of length $L$ without sharp bends. The description of the chain involves a set of unit vectors, $\hat{\mathbf{t}}(\mathbf{s})$, that are tangent to each point of the curve $\mathbf{s}$. The energy for the WLC model when the molecule is subjected to an external force $f$ can be written as follows:

$$\mathcal{H}_{WLC} = \frac{\kappa}{2}\int_0^L ds\left(\frac{\partial\hat{t}}{\partial s}\right)^2 - f\int_0^L\cos\theta(s)ds \tag{3}$$

$\kappa$ accounts for the resistance of the chain to bending (in fact, it is called bending constant) and $\theta$ is the angle between the tangent vector at $s$ and the z-axis (we consider that the applied force is parallel to the z-axis). The main difference between WLC model and FJC model is that the orientations of the elements of the chain are now correlated. That is:

$$\left\langle\hat{t}(s)\cdot\hat{t}(s')\right\rangle = e^{-|s-s'|/L_p} \tag{4}$$

$L_p$ is called persistence length and represents the characteristic distance above which the tangent vectors become uncorrelated. It is possible to show that, in ideal chains, persistence length equals one half of Kuhn length.

The force required to introduce and end-to-end distance $x$ in a flexible chain of length $L$ is given by the following interpolating formula [4]:

$$f = \frac{k_{\mathrm{B}}T}{4L_p}\left(\frac{1}{\left(1-\frac{x}{L}\right)^2} + 4\frac{x}{L} - 1\right) \tag{5}$$

It is important to mention that even if the last equation is only an approximation, WLC model works very well and provides an excellent description of molecular elasticity at low and intermediate forces. However, it is possible to introduce an additional correction (in terms of a seventh-order polynomial) to the previous formula that improve the calculation of the force for any given extension [5].

## III. FACTORS THAT AFFECT ELASTIC RESPONSE OF DNA

### A. Temperature

Direct measurements of force and extension of single molecules (see the next section) allow us to address the study of the elastic response of individual molecules, such as ssDNA under tension.

As we mentioned before, the elastic response of polymers is mainly entropic. A flexible polymer in solution adopts the shape of a random coil: its average end-to-end distance is shorter than the total contour length of the polymer. Entropy dominates this process. Thus, it is straigthforward to figure out that temperature (that is the temperature of the medium surrounding the molecules) will play a key role in the elastic response of molecules in solution.

From equation 3 we can see that the average extension for a given force decreases when the temperature grows, with the smallest decrease occurring at the largest forces. Nevertheless, such theoretical prediction is not consistent with experimental results.

Recent studies have shown that the extension for a given force of ssDNA depends on the temperature in a complex way [6]. For instance, it has been reported that in some force ranges the extension increases with temperature and in other force ranges the extension is insensitive of temperature until a certain temperature, where the expected theoretical behavior is observed.

The fact that experimental results do not match with theoretical predictions makes us think that elastic behavior (i.e. elastic parameters) of ssDNA has a dependence on the temperature that can not be neglected. As we will show in the following section, it is possible to use the power of single-molecule manipulation to study the elastic response of ssDNA molecules.

### B. Ionic effects

Experiments with DNA are carried out in the presence of a buffer solution (i.e. an aqueous solution to prevent changes of the pH). Experiments with ssDNA using Laser Optical Tweezers (LOT) instruments have shown that the force-distance curve (hereafter referred to as FDC) of ssDNA has a strongly dependence on the ionic strength [7].

For instance, large deviations from ideal chain model are observed at high salt concentrations. Such effect is associated to the formation of secondary structures (i.e. different structures than the native at zero force) when the ssDNA folds.

Ionic effects on DNA can be understood using the Debye-Hückel theory of polyelectrolytes. It is known that DNA has a net charge along its backbone due to the presence of phosphate groups [8]. It causes an electrostatic interaction between the DNA and the counterions of the surrounding medium. However, in polyelectrolytes charges do not interact via a long range interaction (i.e. the Coulomb potential). Such interaction can be thought of using Yukawa potential. Because of charge screening, electrostatic interactions have an effective characteristic length: the so-called Debye screening length.

At low salt concentrations (i.e. low number of counterions) the screening length increases. This means that the effective range of electrostatic interactions increases. On the other hand, when the ionic strength grows up, there are a large number of counterions that are able to screen Coulomb potential.

But, how does this affect the elasticity of ssDNA?

As we explained before, persistence length is the distance at which the orientation of the elements of a polymer (i.e. the tangent vectors) become uncorrelated. Such correlation is due to the electrostatic interaction between the net charges of the monomers. Then, within distances shorter than the Debye length, charges will feel the effect of the full Coulomb potential, which causes an electrostatic repulsion between the charges of the backbone. This fact has an enormous impact on the persistence length.

Hence, the persistence length may be seen as the sum of two terms,

$$L_p = L_p^0 + L_p^{el} \tag{6}$$

The first term is called the intrinsic persistence length and the second term, called electrostatic persistence length accounts for ionic effects on DNA persistence length. Regarding the ssDNA as a solid cylinder of radius $r$, the intrinsic persistence length can be computed using classical elasticity [9]:

$$L_p^0 = \frac{Sr^2}{4k_{\mathrm{B}}T} \tag{7}$$

Where $S$ is the stretching modulus of ssDNA (see equation 2). From this last equation it is straightforward to see that the intrinsic persistence length decreases when the temperature increases.

However, the behavior of electrostatic persistence length is not clear. The theories of Odijk [10] and Skolnick and Fixmann [11], (referred as OSF from now) predict that: $L_p^{el} \sim r_D^2$ ($r_D$ is the Debye screening length). Nevertheless, all variational theories give: $L_p^{el} \sim r_D$ [12]. Going further, some studies have suggested that persistence length depends on Debye length in a more complex way, via a power law: $L_p \sim r_D^{\nu}$ [13].

On the other hand, from mean field calculations it is possible find that Debye length depends on the square root of the temperature. In this project we will try to find out which is the behavior of persistence length with temperature.

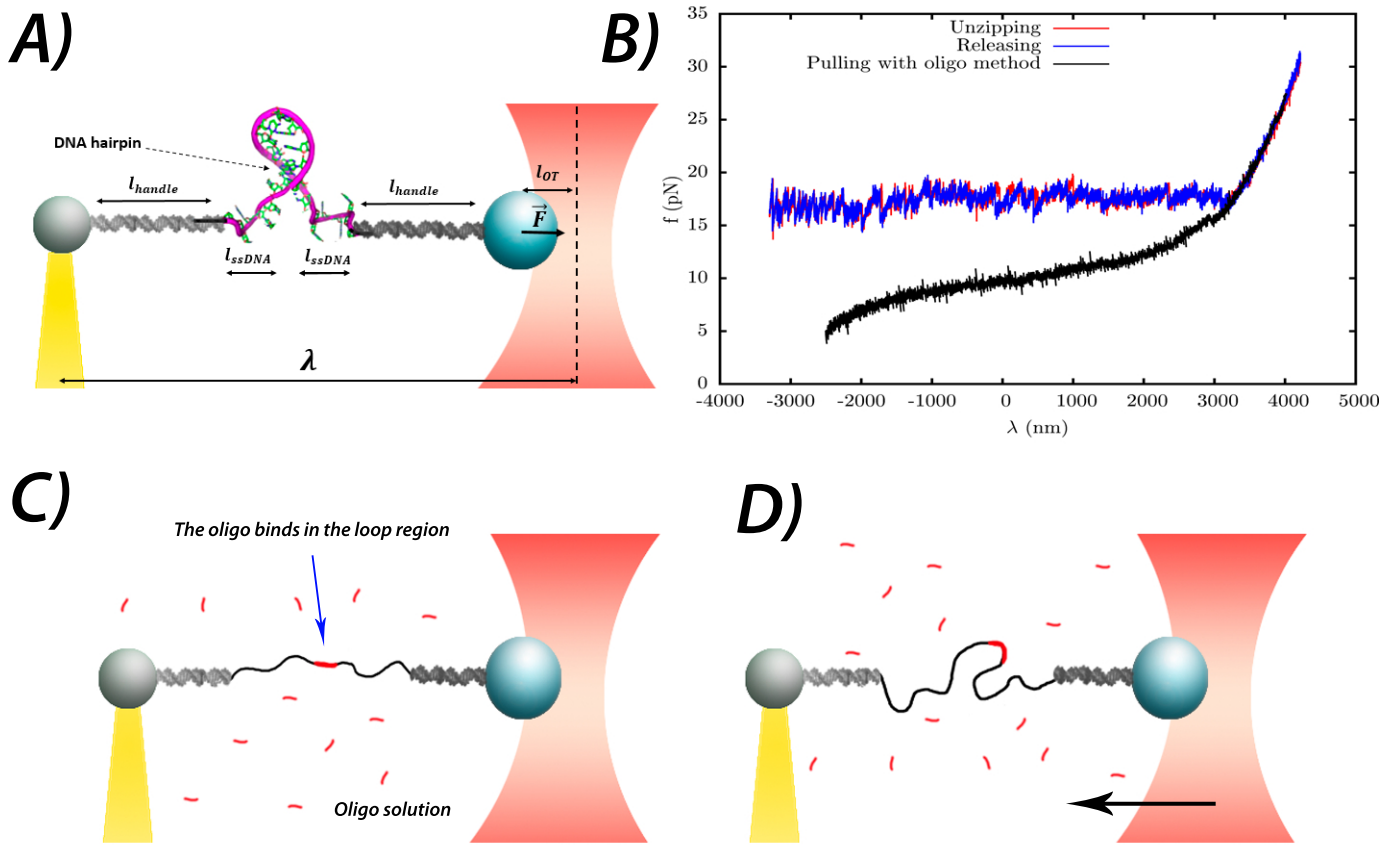


FIG. 1: **Molecular construction, FDC and schematic oligo method a.** Molecular construction. **b.** Comparison of the obtained FDC during unzipping process (red curve), refolding process (blue curve) and re-pulling with the presence of the oligo (black curve). **c.** Schematic illustration of the action of the oligo. **d.** Schematic illustration of refolding process with the presence of the oligo solution.

## IV. METHODS

### A. Single Molecule Experiments (SME). Optical Tweezers.

SME provide a brand new scenario to study and investigate the properties of individual molecules. A key advantage of SMEs is that they make possible the measurement of deviations from the average (bulk) behavior and probability distributions. For instance, biophysicists have been able to measure the energy consumption of enzymes, the kinetics of biochemical reactions, thermodynamic quantities and elastic properties of molecules such as DNA or RNA.

In the early 1970s, Arthur Ashkin pioneered the field of laser-optical trapping. While he was working at Bell Laboratories he observed experimentally the interaction between a laser beam and a tiny dielectric particle. He could accelerate small particles along the direction of propagation of light. Moreover, Ashkin realized that it is possible to trap microscopic dielectric particles using two counter propagating lasers [14]. LOTs are based on the fact that gradient force due to the focused laser beam is higher than the scattering force, which causes the trapping of a particle with a refractive index higher than that of the surrounding medium [15].

The effect of optical trapping can be visualized as a potential well. A very good approximation is to consider that the trapping potential is harmonic. Thus, the optical trap acts like a spring.

### B. Unzipping experiments and oligo method.

As we mentioned before, during cell replication, DNA molecule has to be unwound (i.e. the double helix structure must be disrupted). When the DNA molecule is completely unwound, two complementary strands of ssDNA are obtained. Thanks to SMEs we can carry out protocols in which the DNA is mechanically unwound by applying a force to one of its ends. Such process is called unzipping.

In this project we have carried out SME using LOT technique. Such experiments are performed inside a mi-

crofluidics chamber. The experimental setup used to perform unzipping experiments is shown in figure 1A. The molecular construction is composed by the molecule under study (a DNA hairpin of 6838 base pairs in the stem ending in a tetraloop) connected to two short dsDNA (29 bp) molecules, called handles. The handles are used to attach the hairpin to two beads at each end. One bead is held by air suction on the tip of a glass micropipette and the other one is confined in the optical trap generated by the laser beams. The molecular construction is synthesized as explained in [17]. Buffer conditions were TE pH 7.5 (Tris-HCl 10mM, ethylenediaminetetraacetic acid 1mM) NaCl 1M.

Considering that our control parameter is the distance between the center of the optical trap and the tip of the micropipette (distance $\lambda$ in figure 1A ), by moving the optical trap we can modify the distance $\lambda$ and, in consequence, apply forces to the molecule. By pulling the molecule (i.e. increasing $\lambda$), one can completely unzip the DNA hairpin. Recording the relative trap-pipette distance and the force exerted on the hairpin, we can obtain the force-distance curve (FDC) of the unzipping process. The FDC shows a characteristic sawtooth pattern (see red curve in figure 1B) with several force rips. Such force rips are closely related to the sequence of the DNA hairpin under study and correspond to the releasing of a group of basepairs. When the hairpin is completely unzipped, a ssDNA molecule is obtained and the force increases monotonically with the distance $\lambda$.

However, if now we begin to approach the optical trap to the micropipette, the ssDNA molecule forms the hairpin once again (blue curve in figure 1B). In order to prevent the formation of the hairpin in the refolding process, we use the blocking oligo method [18]. When the hairpin is completely unzipped and the ssDNA molecule is formed, a solution containing an specificic oligonucleotide is flowed in the fluidics chamber. Such oligonucleotide is designed to bind to the loop region of the hairpin (see scheme on figure 1C), inhibiting the refolding of the hairpin when the tension is released (see figure 1D). In fact, the effect of the oligo is to create a large kinetic barrier that avoids the formation of the native structure. Thanks to the oligo method we can study the stretching response of the ssDNA (black curve in figure 1B) over a wide range of forces and temperature conditions.

## V. RESULTS

In this project we measured, over a range of 5 to 50 °C, the elastic response of ssDNA molecules under tension. As we said before, force increases monotonically with the distance. At low forces, ($< 10$ pN) we observe a characteristic force plateau related to the formation of secondary structure (i.e. the hairpin folds in a non-native conformation).

In order to study the elastic behavior of the ssDNA molecules we have performed a nonlinear least squares fit of the stretching curve to an elastic model (see section II) implementing the Levenberg-Marquardt algorithm [19]. The fit was carried out in the range of 18 - 35 pN to avoid the effects of the secondary structure. Recalling figure 1A we see that:

$$\lambda = \lambda_0 + 2l_{\text{ssDNA}} + 2l_{\text{handles}} + l_{\text{OT}} \tag{8}$$

Where $\lambda_0$ is a position offset that has to be determined for each molecule. On the one hand, the term $l_{\text{ssDNA}}$ can be computed depending on the elastic model. On the other hand, we can consider as force-independent the length of the handles because of they are short dsDNA molecules stiffer than the DNA hairpin. As a consequence, that term can be absorbed in the contribution of $\lambda_0$. Finally, regarding the OT as an ideal spring, we may write the previous equation as follows:

$$\lambda(f) = \lambda_0 + 2l_{\text{ssDNA}} + \frac{f}{k} \tag{9}$$

Where $f$ is the force and $k$ is the stiffness of the OT (the measured value is: $k = 0.077 \pm 0.005$ pN/nm). Thus, knowing $\lambda$ and $f$ we can carry out the fit. The fit procedure may be summarized in the following steps:

1. Selection of the model: FJC or WLC model
   (a) If the selected model is the FJC model, the fitting parameters will be $\lambda_0$, $b$ and $S$.
   (b) If the selected model is the WLC model, the fitting parameters will be $\lambda_0$ and $L_p$.
2. Select an initial value for the fitting parameters.
3. Find the values of the fitting parameters that minimize the quantity: $\mathcal{S}^2 = \sum_{i=1}^{N_{\text{points}}} (\lambda_{\text{exp}} - \lambda_{\text{teor}})^2$
4. Repeat selecting new initial points to ensure that the fit converges to the same result.

### A. Analysis with FJC model

In order to quantify the elastic properties of ssDNA, we have followed the fitting recipe explained before using the FJC model. It is important to mention that we have fixed the distance between base pairs to $l = 0.57$ nm, as in [20]. It means that the contour length is: $L_c = Nl$, where $N$ is the number of base pairs of the molecule.

In figure 2 we can observe the result of the fit for a representative molecule at different temperatures. We note that the characteristic force plateau related to the formation of secondary structure disappears at high temperature.

In the following table we report the obtained values from our fits of the Kuhn length and the stretching modulus for each temperature.

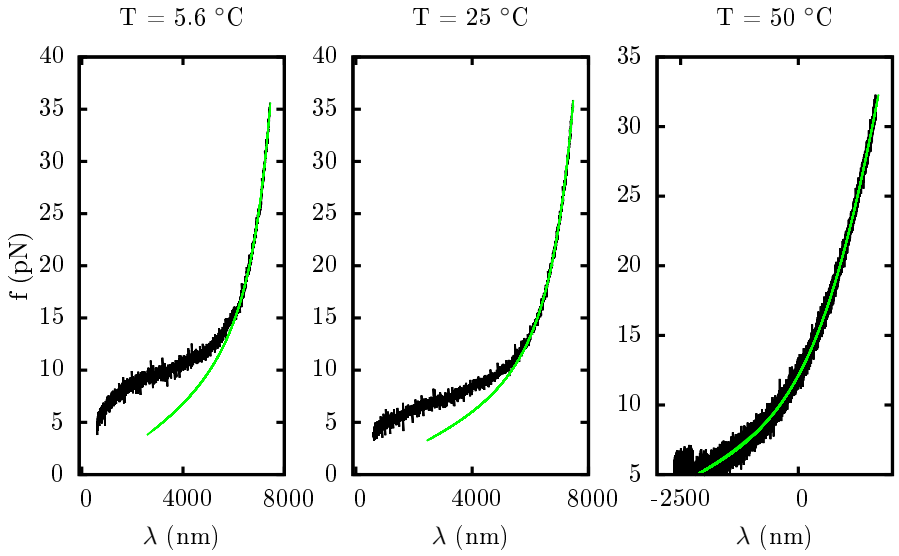


FIG. 2: **Comparison between the experimental FDC (black) and the fits in the range of 15 - 35 pN using FJC model (green) for different temperatures.**

TABLE I: Reported values of the Kuhn length and the Stretching modulus with their respective standard errors

| T (°C) | $b$ (nm) | $S$ (pN) |
|---|---|---|
| 5.5 | 1.18(6) | 490(20) |
| 15.3 | 1.27(6) | 590(30) |
| 21.5 | 1.34(7) | 780(40) |
| 25.5 | 1.33(2) | 840(50) |
| 27.1 | 1.30(3) | 544(12) |
| 32.1 | 1.34(1) | 670(20) |
| 41.5 | 1.38(2) | 830(60) |
| 43.8 | 1.39(7) | 910(60) |
| 50.7 | 1.39(7) | 640(30) |

Figures 3 and 4 summarize the results obtained in our fits by plotting the Kuhn length in front of the temperature and the stretching modulus versus the temperature, respectively.

From figure 3 can we see that the Kuhn length increases in a linear way with temperature. We note that our results agree with the accepted value at 25° and 1M of NaCl buffer: 1.31 ± 0.03 nm (the accepted value is: 1.31 ± 0.06 nm) [17].

On the other hand, we note that the stretching modulus does not show any clear trend in the range of temperature under study (see figure 4). Hence, we can consider that the stretching modulus is independent of the temperature at which the ssDNA is. The average value of our fit results is $S = 700 \pm 50$ pN.

This result is compatible with the reported at 25° and 1M of NaCl buffer: 850 ± 100 pN [17].

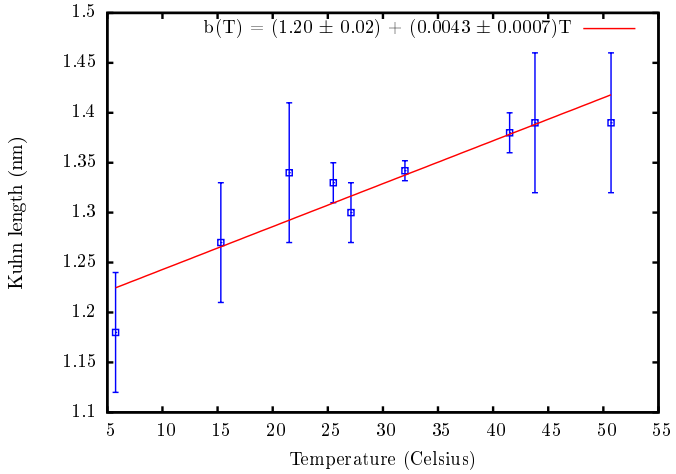


FIG. 3: **Kuhn length values at different temperatures. Solid line corresponds to a linear fit on the data.**

### B. Analysis with WLC model

We have also used the WLC model in order to extract the behavior of persistence length with the temperature. In that case, we have fixed the interphosphate distance (distance between base pairs) to the value reported for 25 °C and a 6838 bp molecule, $l = 0.70$ nm [17].

In figure 6 we show the result of the fits for different

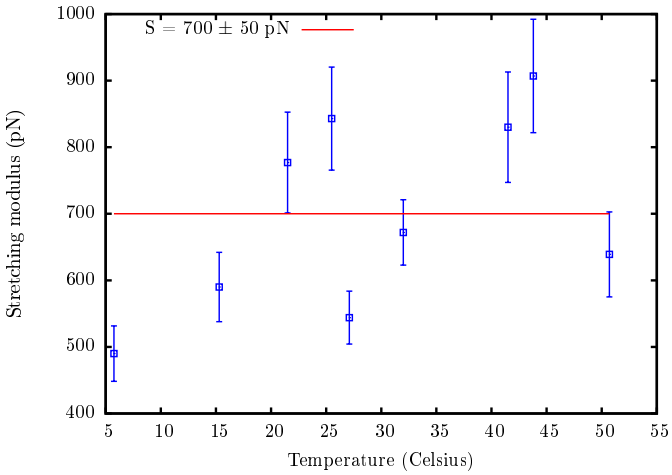


FIG. 4: **Evolution of stretching modulus within our temperature range. The solid line corresponds to the average of the obtained values.**

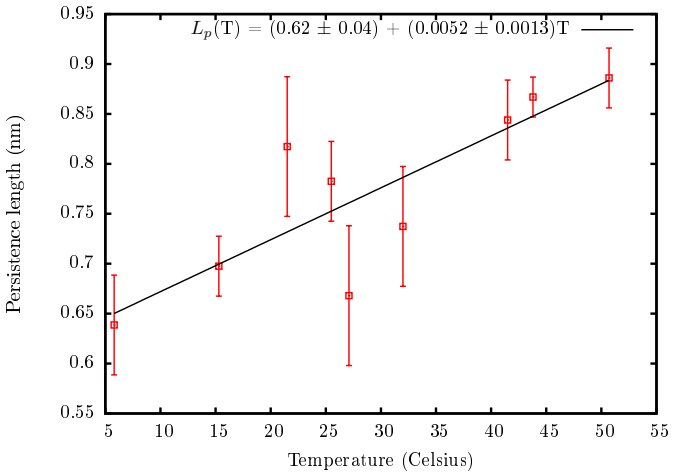


FIG. 5: **Persistence length values at different temperatures. Solid line corresponds to a linear fit on the data.**

thermal conditions. Results are shown for a representative molecule.

In the following table we collect the fit results for the WLC model.

TABLE II: Reported values of the persistence length with its standard error

| T (°C) | $L_p$ |
|---|---|
| 5.5 | 0.64(5) |
| 15.3 | 0.70(3) |
| 21.5 | 0.82(7) |
| 25.5 | 0.78(4) |
| 27.1 | 0.68(8) |
| 32.1 | 0.74(6) |
| 41.5 | 0.84(4) |
| 43.8 | 0.87(2) |
| 50.7 | 0.89(3) |

The behavior of the persistence length with the temperature is summarized in figure 5.

As we observed with Kuhn length, persistence length also increases with temperature following a linear trend.

By comparing the results corresponding to the variation of Kuhn length and persistence length with temperature we see that for temperatures below 32 °, the relation $b = 2L_p$ is approximately satisfied. Such relation is valid for semiflexible chain models.

Besides, we note that fits using FJC model are more accurate at low temperatures than at high temperatures (i.e. higher than room temperature). The case of WLC model shows the opposite behavior, at high temperatures the fits are more accurate than at low temperatures.

### C. Verification of the dependence of Debye length on the temperature

As we explained in section III B, persistence length has two main contributions: the intrinsic persistence length and the electrostatic persistence length. We have already seen that intrinsic persistence length decreases with temperature as $T^{-1}$ and the electrostatic persistence length has not a well known theoretical behavior.

Accepting that Debye length depends on the temperature as predicted in mean field theory: $r_{\mathrm{D}} \sim T^{1/2}$ [21], we have tried to check if this prediction matches with our experimental data. In order to do that, we have tried to fit our data to a function like:

$$L_p(T) = A + \alpha\sqrt{T} \tag{10}$$

Where $A$ is a fitting parameter and $\alpha$ is a temperature independent coefficient.

$\alpha$ has been measured in [17]: $\alpha = \frac{2.19\mathrm{nm}}{\sqrt{298\mathrm{K}}}$

Then, the fitting function becomes ($T$ is the temperature in Celsius):

$$L_p(T) = A + 2.19\sqrt{\frac{T + 273.15}{298}} \tag{11}$$

The fitting parameter $A$ is related to the persistence length at 25°C as follows:

$$L_p(T = 25°\mathrm{C}) = A(\mathrm{nm}) - 2.19(\mathrm{nm}) \tag{12}$$

Thus, it allows us to check the validity of our fit by obtaining the persistence length at 25°, which has a reported value of 0.76 ± 0.05 nm [17] for 1M of NaCl (see black solid line in figure 7).

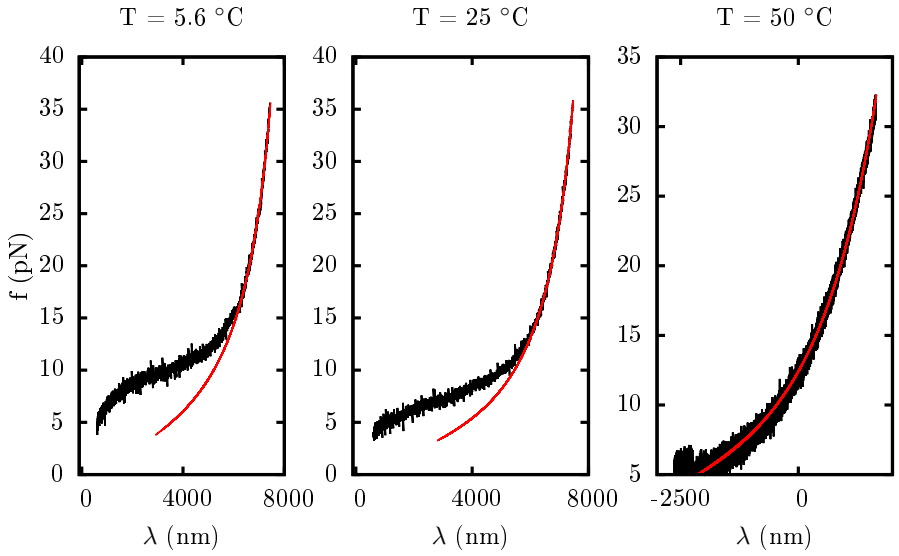


FIG. 6: **Comparison between the experimental FDC (black) and the fits in the range of 15 - 35 pN using WLC model (red) for different temperatures.**

The obtained value of the persistence length at room temperature is: $L_p(T = 25°\text{C}) = 0.76 \pm 0.02$ nm. This result is in very good agreement with the reported value.

On the other hand, as we explained in section III B, the behavior of the electrostatic persistence length on the Debye length is not fully understood yet. As a consequence, we have tried to figure out if another behavior different from the square root fits well to our experimental data. In order to do so, we do not fix the exponent of the temperature dependence. Then, recalling that:

$$L_p^{el} \sim r_D^{\nu} \sim T^{\nu/2} \tag{13}$$

Thus, we can suggest a new fitting function like:

$$L_p(T) = A + 2.19\left(\frac{T + 273.15}{298}\right)^{\nu/2} \tag{14}$$

Where $A$ is defined as before. The obtained exponent in this latter case is: $\nu/2 = 0.71 \pm 0.17$. This result does not agree with the theoretical predictions [10–13]. It supports the idea that the behavior of the persistence length is still unknown.

On the other hand, as we did before, we have obtained the value of the persistence length at room temperature: $L_p(T = 25°\text{C}) = 0.75 \pm 0.02$. In figure 7 we compare the obtained results for both fits.

In the following figure we see that the behavior of the fit with an exponent $\nu/2 > 1$ (solid blue line) is more accurate to our experimental data than the predicted by mean field theory (solid black line).

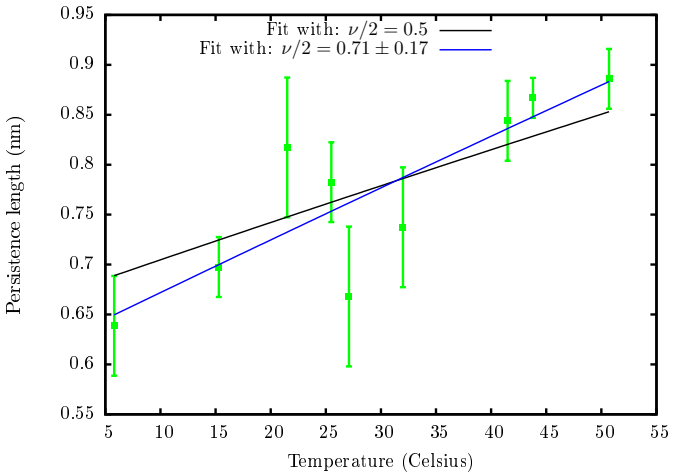


FIG. 7: **Comparison between the results of the fits using a square root dependence (black solid line) and a power law dependence (blue solid line).**

## VI. CONCLUSIONS

In this project we have investigated the temperature dependence of the elastic properties of ssDNA molecules. By using LOT technique, we have carried out unzipping experiments on dsDNA until ssDNA molecules were obtained. ssDNA molecules were stretched in a wide range of forces and their stretching response (i.e. the FDC) was studied in a temperature window of 5 to 50 °C. By fitting the experimental data to two of the most known

elastic models (WLC and FJC) we obtained the temperature dependence of the elastic parameters of the ssDNA molecules.

First of all, we studied how the Kuhn length depends on the temperature. Kuhn length represents the typical length of the monomers in an ideal chain (a freely jointed chain). Each segment in a freely jointed chain can randomly orient in any direction without the influence of any forces, independent of the directions taken by other segments. We found that Kuhn length increases in a linear way with the temperature. It means that, considering our polymer as ideal, the typical distance at which the monomers are randomly oriented increases with temperature.

In a similar way, the behavior of persistence length follows the same trend. It also increases with temperature in a linear way. Nevertheless, the persistence length has a deeper physical meaning. Imagining our polymer as a flexible rod, the persistence length arises from considering the characteristic distance at which the correlations between the orientation of the elements of the chain become independent.

Besides, we found that the relation $b = 2L_p$, valid for ideal chains, is satisfied at low temperatures (below 32°C). In a similar way, we observed that FJC model is more accurate at low temperatures, while the WLC model shows the opposite behavior.

On the other hand, we know that persistence length depends on the typical length of electrostatic interactions. Such typical length is called the Debye length and also depends on the temperature. However, its behavior is still unknown. We have shown that persistence length depends on the Debye length via a power law with an exponent >1. Besides, it is important to mention that within our temperature range it is very difficult to figure out which is the correct behavior of the persistence length with Debye length. Thus, the determination of the exponent of the power law may not be unique.

Finally, we have shown that the stretching modulus of ssDNA does not show a clear trend in our temperature range. In consequence, we assume that it does not change within the temperature range under study (5 - 50°C). Such assumption is in good agreement with the reported value.

## VII. FUTURE PERSPECTIVES

In this project we learnt how the elastic parameters depend on the temperature, but it would be interesting to find out how they depend on the salt concentration and temperature in order to to obtain a full dependence of the elastic parameters on ionic strength and temperature. For instance, we know that persistence length has an important contribution due to electrostatic interactions. It means that the number of charges in solution play a key role in the flexibility of the ssDNA. Hence, how does ssDNA behave at different salt and temperature conditions? Is it possible to observe a compensation effect between ionic strength and temperature?

On the other hand, thanks to the power of LOT we can analyze the elastic behavior by performing unzipping experiments on different relevant biomolecules. For example, we could study the dependence on the temperature of the elastic response of RNA (RiboNucleic Acid) or some proteins. Does the temperature have the same effect as on ssDNA?

Those are just a few of the interesting questions that we may be able to answer going further in this study using the power of SMEs.

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