Chapter 4

Melting of DNA in confined geometry

4.1 Introduction

The stability of DNA molecules during the encapsulation process is a topic of intense research. In this chapter, we investigate the thermal stability of the double-stranded DNA (dsDNA) molecule of different lengths in a confined space. Deoxyribonucleic acid (DNA) is one of the interesting and complex biomolecules. The importance of this molecule lies in its wide applications in the molecular motor, DNA computer, origami formation, DNA chip, and biomedicine. To perform gene therapy [85], nanorobotics [181], diagnostics [182] performance, DNA degradation is a major obstacle for the effective and efficient use of DNA. The DNA degradation eventuates in chemical breakdown [85] or sometimes through mechanical forces [183]. In the gene therapy technique, DNA is protected by a physical barrier. There are many different techniques that have been using to get better results like complexation with polycations [85], charged copolymers of different architectures, cationic lipids or liposomes [86]. As other options, DNA can be confined within gel [87, 88], polymeric nanocapsules (micelles) [89, 90], microparticles. There are many elegant and versatile approach for DNA encapsulation [91].

In DNA encapsulation, the ability to preserve the DNA and efficiently release it, are the processes which are inversely related. The objective of good DNA encapsulation is to find an optimal balance between these two issues. Researchers are trying to balance it through numerous ways; one of them is short DNA encapsulated by a spherical inorganic nanoshell with an overall thickness of $\sim$10 nm [184]. Carbon nanotubes also have been proved themself as a potential candidates

for DNA encapsulation [92]. The thermodynamical spontaneity of DNA encapsulation of carbon nanotube under different conditions is still a significant area of research. The threshold diameter of this tube is also a vital issue to investigate since below the threshold encapsulation is inhibited [93]. Many sensitive parameters are involved in DNA encapsulation technique like the medium and topology of the carrier, thermodynamic parameters, etc.

In vivo, DNA molecule is confined in a limited space such as the cell chamber or a channel and is in highly dense solvent conditions [68–71]. This confinement restricts the conformation and movement of DNA molecules in the cell. The thermodynamic properties of DNA molecules highly depend on the confined space [72]. It is known that conformational properties of biopolymers under confinement are the crucial relevance in living systems like DNA packing in eukaryotic chromosomes, viral capsids, etc. [73].

In most of the studies, either rectangular or spherical geometry [68] is considered as confinement. Despite recent progress, still little is known about the profiles of confinement nature of DNA [79]. In the present chapter, motivated by all these different kinds of experiments in different geometries, we attempt to understand the thermal stability of DNA molecule that is confined in different geometries through a statistical model.

4.2 Introducing the Confinement in The Model

The interactions in the DNA, containing N base pairs, are represented as,

$$H = \sum_{i=1}^{N} \left[\frac{p_i^2}{2m} + V_M(y_i) \right] + \sum_{i=1}^{N-1} \left[W_S(y_i, y_{i+1}) \right], \tag{4.1}$$

The stacking interaction, is represented by,

$$W_S(y_i, y_{i+1}) = \frac{k}{2}(y_i - y_{i+1})^2[1 + \rho e^{-b(y_i + y_{i+1})}]. \tag{4.2}$$

For our studies, we find these model parameters as $k = 0.015$ eVÅ^{-2} , $\rho = 50.0$ as suitable values for the current investigation. The hydrogen bonding between the two bases in the i^{th} pair is represented by the Morse potential as,

$$V_M(y_i) = D_i(e^{-a_i y_i} - 1)^2. \tag{4.3}$$

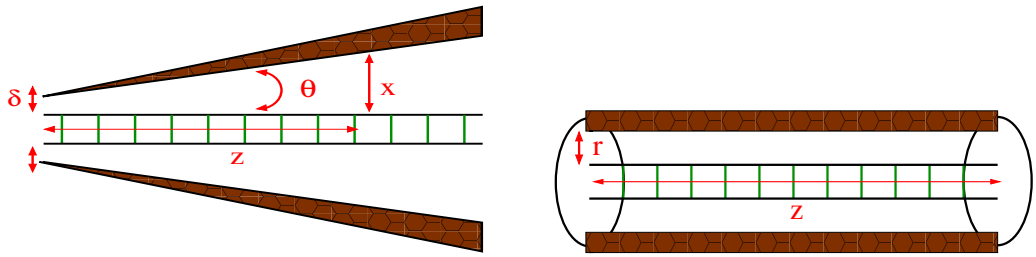

Figure 4.1: (a) The schematic representation of the DNA molecule in a confined cell of conical shape. The distance x represents the distance between a pair and the confining wall while θ is the angle at which the cellular wall is suppose to confine the DNA.Here $z = 3.4 \times i$, the distance along x-axis. (b) The schematic representation of the DNA molecule in a confined cell of cylindrical geometry. The r is the distance of the confined wall from the DNA strand. So the diameter of the cylinder becomes r+DNA diameter.

The potential parameters are taken as $a_{AT} = 4.2$ Å^{-1}, $a_{GC} = 1.5 \times a_{AT}$ and D_{AT} = 0.075 eV while, $D_{GC} = 1.5 \times D_{AT}$. We can study the thermodynamics of the transition by evaluating the partition function.

$$Z = \int \prod_{i=1}^{N} \{dy_i dp_i \exp(-\beta H)\} = Z_p Z_c, \tag{4.4}$$

In this work, we study the effect of confined geometries on the melting profile of $phage - \lambda$(GenBank: J02459.1) DNA chain of first 120bp sequence. Some interesting studies that reveal many interesting features of the melting of the DNA molecule that is confined in a rectangular geometry [78, 185]. Our focus is mainly on the stability of the DNA molecule that is confined in a conical as well as cylindrical geometry as shown in Fig.4.1. How the confinement can be realized in the PBD model? We adopt the following scheme. Due to surrounding cellular environment, the movement of base pairs will be restricted which in turn affect the overall movement of the individual base pairs. We restrict the configuration space of the system as shown in Fig. 4.1. There is no change in the lower limit of integral for both the geometry, however, the upper limit of integral for each base pair is modified according to the distinct geometry. For conical confined geometry, the upper limit $x = 3.4 \times i \times \tan(\theta)$ where i is the site index ($i = 1, 2, 3, ..., N$), and N is the total number of base pairs in the chain. The term θ is the angular separation between the confining wall and the DNA strand. Since two base pairs are approximately 3.4 Å distance apart we take this as the multiplication factor in calculation. Thus, the configurational space for our calculation extends from -5

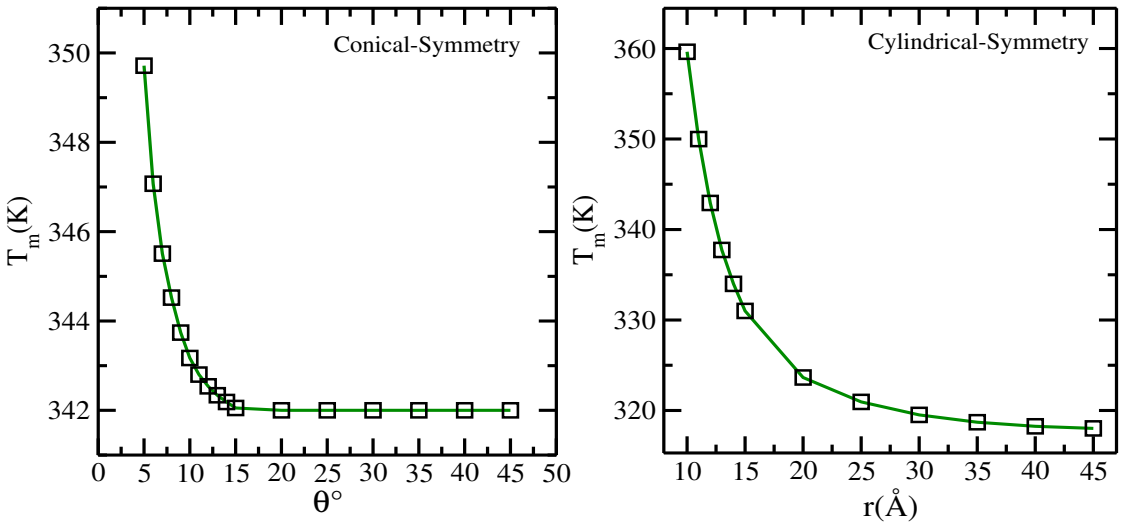

Figure 4.2: (on left)The change in the melting temperature with the change in the angular separation between the confining wall and DNA molecule. The pore width $\delta = 0$ Å. Figure shows that beyond a certain angle, θ_c there is no effect of confining wall on the melting temperature, T_m of the molecule. (on right) The DNA is confined in a cylinder and the plot shows the change in melting temperature with radius of the cylinder.

Å to x Å. There is another important factor that affects the melting transition is the cross section of the pore. The cross section of the confining wall (or pore width) is represented by the term, δ. The value of $\delta = 0$ Å means the distance between the vortex and the DNA strand is 0 Å (as shown in Fig. 4.1a). Now for cylindrical geometry the upper limit is defined as r. The r is the distance of the confined wall from the DNA strand. Using the modified scheme we calculate the partition function and hence evaluate all the thermodynamical properties of the system for both geometries.

4.3 Results

We consider the first 120 bp of the phage-λ DNA chain sequence and restrict it according to the confinement geometry. By evaluating the partition function, we calculate free energy per base pair hence specific heat as a function of temperature. The melting temperature T_m of chain at various angular separations(θ) for conical confined geometry and in different diameter of the cylinder for cylindrical confined geometry are calculated through the peak in specific heat as a function of temperature [140, 186]. The results are plotted in Fig. 4.2. The obtained re-

sults clearly show that the confinement has a strong effect on the stability of the molecule. We find that the melting temperature (T_m) of dsDNA increases as the confinement becomes stronger. We vary the anglular separation and found that T_m decreases as the angle changes from 5^0 to 6^0. The melting temperature for an angular separation of 5^0 is found to be 349.7 K while it reduces to 347 K for an angle of 6^0, *i.e.* a reduction of approximately 2.7 K in the melting temperature. The melting temperature further reduces for the higher angular separations and get saturated at an angle of $\approx 15^0$(342K). The stability of DNA confinement in a cylindrical symmetric wall has been investigated by various researchers using Poland Scheraga model [185, 187].

To investigate the effect of different geometry on the stability of the DNA molecule, we consider a DNA chain confined in a cylinder. We change the radius of the cylinder and calculate the change in the melting temperature of the system. The results are plotted in Fig.4.2. When the DNA is confined in a cylinder at $r = 10$ Å, we find the melting temperature of the system as ~ 359.6 K. The melting temperature reduces drastically to a value of ~ 350 K for the radial separation of 11 Å(reduce to 9.6K). On further increasing the separation the T_m reduces and almost get saturated at value of ~ 318 K. Since the two geometries supress the entropy of the system in a different way, one can believe that DNA which is confined in cylindrical geometry is more stable than the DNA that is confined in conical geometry upto a certain separation. The point to note that while for shorter separation stability is higher for cylindrical confinement but it is lower at higher separation (*please see Fig. 4.2* for a detail comparision).

To understand the microscopic details of opening of the DNA chain in a confined space, we calculate the average separation of the chain, $\langle y_i \rangle$, with the change in the angular separation (θ). From the opening profile, the effect of confinement angle is more apparent. When the angular separation is 5^0, the effect lasts for ∼25 base pairs while for the 10^0 angular separation, the effect lasts for $\sim$ base pairs. How the different sections of the chain open below the melting temperature is also the matter of interest here. From the plot (Fig. 4.3), the sequence and effect of conical confinement are visible. The weaker section of the chain consists of AT pairs is more entropic than the section containing GC pairs.While the opening of the homogeneous chain is very smooth, the opening of the heterogeneous chain has bubbles. One can see that how these bubbles disappear above the melting temperature of the chain when the DNA is in the denatured state.

Depending on the geometry the opening profile differs. The opening profile of

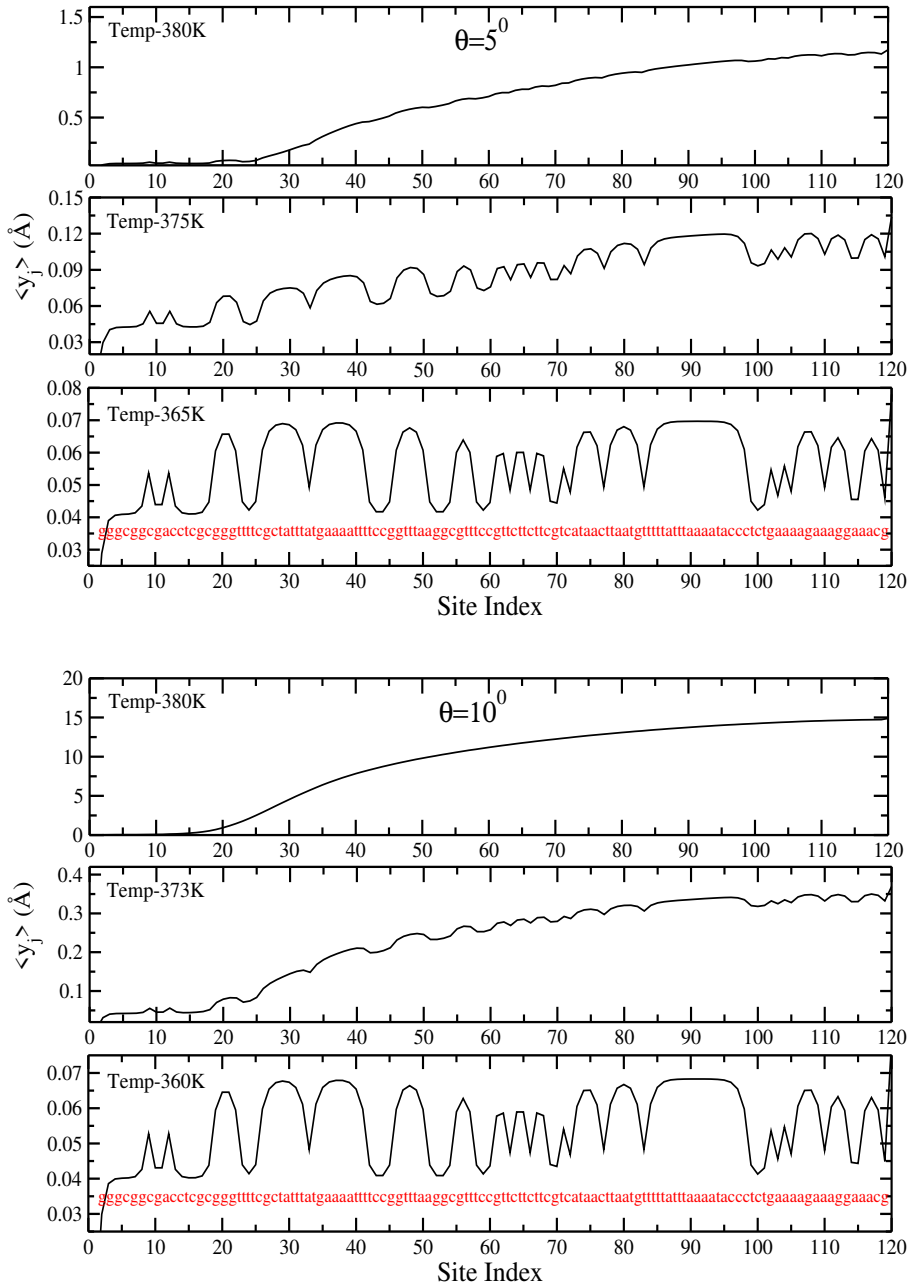

Figure 4.3: The opening of the chain in different angular separation(θ=5^0& 10^0). When the angular separation is 5^0, the effect lasts for ∼25 base pairs while for the 10^0 angular separation, the effect lasts for ∼20 base pairs at 380K.

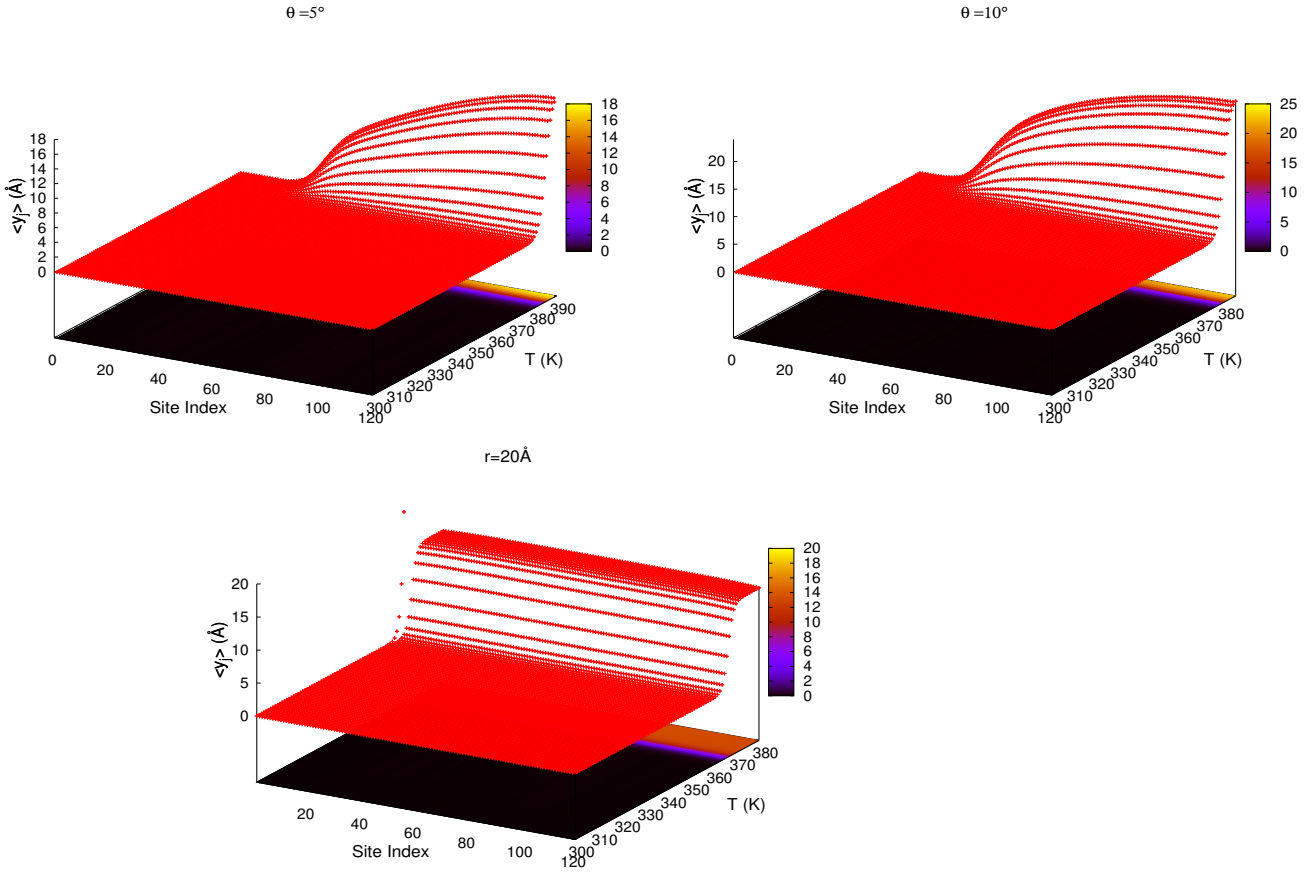

Figure 4.4: The opening profile of the chain is shown through the 3D plots which shows a variation in $\langle y_j \rangle$ for the 120bp chain. Here we show the profile for two different angular separation, $\theta = 5^0$ and $\theta = 10^0$ (for pore width $\delta = 0$ Å) and for the cylindrical geometry r=20 Å. The x-axis is site index while y-axis is temperature (in K) and z-axis denotes the average separation, $\langle y_j \rangle$ in Å.

the chain in these two different geometry are shown through 3D plots in Fig.4.4 which shows the variation of $\langle y_i \rangle$ with temperature and base pair.

We also investigate the effect of confinement on different sized DNA molecule by varying the chain size. We consider the chain sequence of 120, 180, 240,& 300 base pairs of the phage-λ DNA sequence and investigate the variation in melting temperature with varying angular separation. The resulting plots are shown in Fig. 4.5.The qualitative feature of all the plots are same, however, the decrease in melting temperature for all the chains is not same. For the chain of 120 bp, the T_m is 349.7 K for an angular separation of 5^0 which reduces to 347 K for and angle of 6^0. The asympototic value of T_m is 342 K which occur at $\approx 15^0$ of angular separation. This means for a span of 10^0 ($15^0 - 5^0 = 10^0$) the variation in melting temperature vanishes and the decrease in T_m is $\approx$5 K($349.7 - 342 = 7.7$ K). For

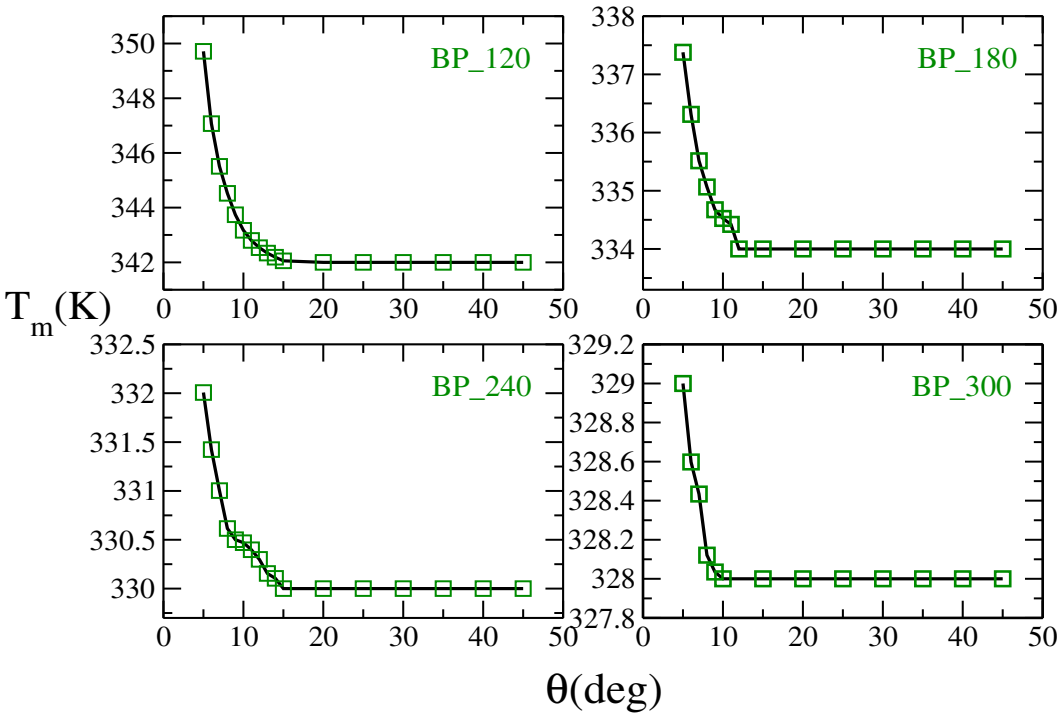

Figure 4.5: How the effect of confinement changes with the linear size of the molecule is shown here. We consider the phage-lambda DNA molecule sequence of different lengths as 120, 180, 240 and 300. The pore diameter $\delta = 0$ Å.

the 180 bp chain,this shift is about 3.3 K ($337.3 - 334 = 3.3K$) for a span of 7^0 ($12^0 - 5^0 = 7^0$) as it gets the asympototic value of T_m at 12^0, while, for 300 bp chain the melting temperature reduces from 329 K (at 5^0) to 328 K (at 9^0) so the shift of T_m is 1 K ($329 - 328 = 1$ K). One can clearly see that as the length of the chain increases the change in melting temperature with angle is reducing. For chain of length more than 350 base pairs, we observe that there is no change in the melting temperature with the change in the angular separation. The probable arugement for the small variation is the entropy of the system that varies with size. The smaller the chain, the stronger the suppression of entropy. For a bigger chain, a large section of the chain might not be seeing the confining wall because the distance between the wall and the DNA chain increases (please refer to Fig.01 a). Next, we study the thermal stability of the system with the change in the pore width,δ for conical geometry. Here we calculate the change in the melting temperature with the change in the pore width, δ for a fix value of θ (the angular separation). The results are shown in Fig.4.6. From the Fig.4.6 it is clear that with the increase in pore width (δ) for a same value of angular separation (θ), the melting temperature decreases. This is due to fact that when the pore width

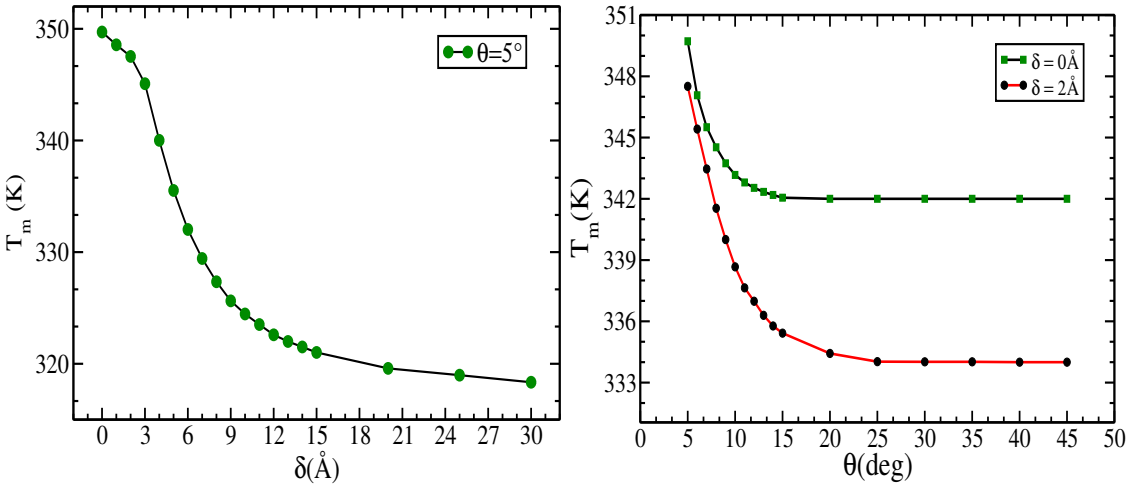

Figure 4.6: (On left) The change in the melting temperature (T_m) with the pore width (δ) for a fix value of angular separation ($\theta = 5^0$). (On right) The change in melting temperature (T_m) with the confinement angle (θ) for two values of the pore width (δ).

increases the end at the vortex of the cone gets more space which increases the end entropy. Due to more entropy, DNA opens at lower temperature.

4.4 Summary

In this chapter, we have studied, theoretically, the stability of phage-λ DNA molecule sequence of different base pair length that is confined in conical as well as cylindrical geometry. Probably this is the first model based study that studies the impact of angular confinement on the stability of the DNA molecule in a thermal ensemble. Our studies show that not only the confinement but also the geometry of the confinement plays a crucial role in the stability as well the overall activity of the DNA molecule.

When the confining wall is closer, the configuration space for the DNA molecule, in cylindrical geometry, is restricted which suppresses the entropy of the chain. Hence the melting temperature of the system in cylindrical geometry is more than the melting temperature of the DNA that is confined in conical geometry upto a certain extends. If we compare two cases of the confinement (conical and cylindrical) and find the rate of decrease in the T_m, then we see that the DNA confined in cylindrical geometry has higher decrease rate. Through simulation of the short DNA molecule that is confined in a cylindrical as well as in spherical geometry, Huaping*et al.* [188] has found that the melting temperature of the system de-

creases with the increasing diameter of the cylinder/sphere. That designates that for the cylindrical geometry our finding are consistent with with the findings of Huaping *et al.*. In case of cylindrical confinement, the complete chain is restricted while in the case of conical confinement, there is a segment of the chain which might not be seeing the confining wall as the separation is angular.