DNA denaturation in the rodlike polyelectrolyte model

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Abstract

The denaturation of the DNA is analyzed using an analytic model. The DNA molecules are described in the Primitive Model of Polyelectrolytes (PMP), where the polyelectrolyte molecules are cylinders with charged sites. We show that the DNA stabilization arises as the result of the competition between the electrostatic repulsion of the phosphate groups and the attractive forces of the H-bonds. We also show that the addition of salt in the system screens the electrostatic interactions and favors the double strand configuration.

Keywords: polyelectrolytes, DNA denaturation, charged systems, complex fluids

1. Introduction

Complexation of DNA with ions and macromolecules [1–5] are important not only for understanding biological processes, but also for applications as solubilization in chemical compounds and drug delivery [6, 7]. The addition of surfactant [8–10], multivalent ions or polyelectrolytes [11–13] leads to complex and layered structures in which the negative DNA has it charge decreased or even reverted [14], leading to effects such as like-charge attraction [15].

In addition to the complexation a number of biological processes depend if the DNA is double or single stranded. Therefore important phenomena associated to DNA systems are related to thermal denaturation. This melting is the transition from the native double helix B-DNA to a new structure in which the two strands separate from each other [16, 17]. As the temperature is increased the double stranded DNA gains entropic energy by forming two

strands. The transition can in principle be modeled by two states systems [18–26].

In addition to the temperature, the presence of salt and other macromolecules also affects the transition. Cations in high enough concentration stabilize the duplex DNA [1, 2, 27, 28]. Intercalators as ethidium bromide and daunomycin in the presence of monovalent salt induce the denaturation [29, 30] what suggests that the denaturation depends not only on the electrostatic interaction. As result, the balancing between salt concentration and the melting temperature (T_m) of duplex DNA is being proposed as the mechanism for controlling the denaturation process [31–34].

A number of models have been focused in the local process of the denaturation as the result from the local electrostatic and entropic energies [35–37]. In some cases [38, 39] the mechanism is related to the flexibility and specific knowledge of the DNA structure where salt enters as a local screening of the spring interactions. In these models the counterions and the salt condensation to the DNA as a function of temperature do not play a relevant hole in the process.

In this work we introduce a model for the DNA denaturation as a function of temperature and salt concentration where the ion condensation is relevant to the process. Our model also includes the influence of Guanine-Cytosine binding energies and how it is screened by the salt [1, 2]. The DNA is treated in the context of the Primitive Model of Polyelectrolytes [4, 40] within this theory the DNA denaturation arises from the competition between the electrostatic effects due to the DNA salt interaction and the Guanine-Cytosine binding interaction and entropic effects. The melting temperature is obtained as a function of salt concentration and number of Guanine-Cytosine groups.

This paper is organized as follow: in Sec. 2 we introduce the model; in Sec. 3 we present our Helmholtz free energy that describes the system we are modeling; in Sec. 4 the results are given, conclusions end this section.

2. The model

We study a mixture of single stranded and double stranded DNA. In salt solution the DNA becomes ionized with charges distributed along the DNA. The charged groups are modeled as the monomers in a long polymeric chain. Depending on the amount of salt in the solution and on the temperature the DNA can be in a double or single stranded configuration. The DNA molecules are therefore represented as charged cylinders for both single and

double stranded molecules as shown in Figs. 1(a) and 1(b). The single helix, the single stranded DNA (ssDNA), is shown as a rigid cylinder with the phosphate groups uniformly distributed separated by a distance $2b$. The double helix, the double stranded DNA (dsDNA), appears as a two rigid cylinders connected by hydrogen bonds. In this case the phosphate groups are also separated by a distance b.

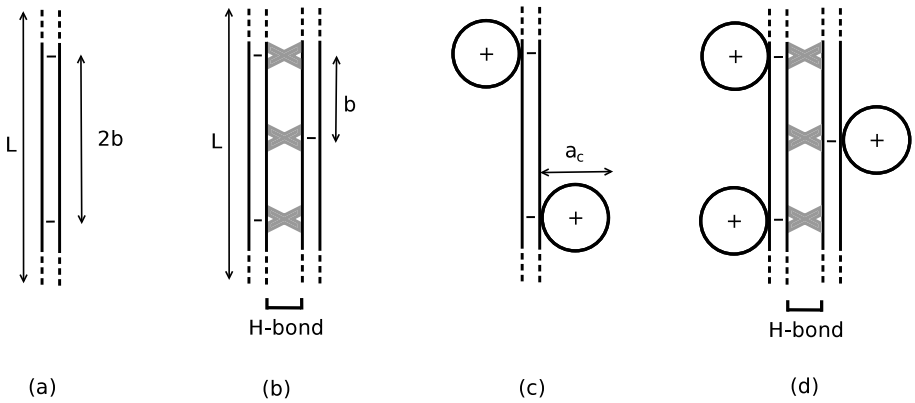

Figure 1: Schematic representation of (a) double stranded DNA; (b) single stranded DNA; (c) single stranded complex; (d) double stranded complex

The relevant parameters represented in Fig. 1 are the spacing b between charged monomers in the double stranded and $2\,b$ in the single stranded DNA ($b = 1.7\mathring{\text{A}}$), the number Z and $Z/2$ of charged sites in the double strand and in the single strand, respectively, and the DNA length L. In addition to the concentration ρ_p of DNA full molecules, the solution also has a concentration ρ_s of salt and $Z\,\rho_p$ counterions. Both ions of salt and counterions have diameter a_c. For simplicity the diameter of DNA single or double strand is a_p. The use of the same diameter for double and single stranded is a simplification based in the closest proximity between the phosphate groups and the counterions that will be the same in both cases. We expect that this simplification will not affect the final results. The two strands repel each other due to the electrostatic interaction but are bounded by H-bonds that are stronger for the Guanine-Cytosine groups. Here this attractive energy is represented by χ. The overall solution is neutral.

The strong electrostatic interaction between DNA molecules and ions in solution leads to the association and formation of complexes [41–44]. In addition to the electrostatic energy, we also consider the energy between

single stranded molecules that leads to the formation of the stable double stranded molecule. The corresponding polyelectrolytes number density is $\rho_p = N_p/V$. The number density of monovalent salt is denoted by ρ_s. In the denaturation process each double stranded molecule of DNA dissociates in two single stranded molecules, hence we have at chemical equilibrium,

$$\rho_p = \rho_2 + \frac{\rho_1}{2}\,, \tag{1}$$

where

$$\rho_1 = \frac{N_1}{V}\,, \tag{2}$$

and

$$\rho_2 = \frac{N_2}{V}\,, \tag{3}$$

are the number densities of single stranded and double stranded polymers, respectively. We have then

$$N_p = N_2 + \frac{N_1}{2}\,. \tag{4}$$

The two kinds of molecules have the same total length, but the charge spacing and monomers number are different. Due to charge neutrality, we have that the positive and negative free ions in solution are given by

$$\rho_+ = Z\,\rho_p + \rho_s - \frac{Z}{2}\,m_1\,\rho_1 - Z\,m_2\,\rho_2\,, \tag{5}$$

$$\rho_- = \rho_s\,, \tag{6}$$

where $m_1 = 2N_1/Z$ and $m_2 = N_2/Z$ are the association fractions for the ssDNA and dsDNA, respectively. ρ_+ and ρ_- are the densities of free positive and negative ions. Since ρ_1 and ρ_2 are not independent we may define using Eq. (1) the dissociation degree α,

$$\rho_1 = 2\,\alpha\,\rho_p\,, \tag{7}$$
$$\rho_2 = (1-\alpha)\,\rho_p\,. \tag{8}$$

For $\alpha = 0$, $\rho_1 = 0$ and $\rho_2 = \rho_p$, corresponding to a system in which all the polymers are double stranded. All chains remain in the double stranded form. For $\alpha = 1$ we have $\rho_1 = 2\rho_p$, and $\rho_2 = 0$, corresponding to complete dissociation, all chains are in the single stranded form. We choose to call the system in denaturation state if $\alpha \geq 1/2$. We choose this value because according melting profiles obtained by absorbance experiments, the fraction of broken base pairs was calculated in way that reported transition (melting) temperatures are temperatures at the midpoint of the transitions where half of the base pairs are broken (melted) [1].

3. The Helmholtz free energy

The system in equilibrium has complexes of double stranded DNA associated with salt, complexes of single strand DNA associated with salt and free ions as illustrated in Figure 2. Our model consists in constructing a Helmholtz free energy. This free energy is approximated as a sum of relevant contributions [45],

$$\begin{aligned} F_{tot}(m_1, m_2, \alpha) &= F_{id}(m_1, m_2, \alpha) + F_a(m_1, m_2, \alpha) \\ &\quad + F_{int}(m_1, m_2, \alpha) + F_{elet}(m_1, m_2, \alpha)\,, \end{aligned} \tag{9}$$

where the first term accounts for the entropic contribution of all the species: complexes of double stranded, complexes of single stranded and free ions. The second term accounts for the energy interaction between two strands. The third term is the internal free energy of each complex and the last contribution is the electrostatic energy between the complexes and the free ions. There are a number of additional energy contributions that we are ignoring here since we believe they play no relevant role in the denaturation. For instance we are not taken into account the interaction between two DNA molecules because we are analyzing low densities solutions. The flexibility was not explicitly incorporated in the model. The influence of the flexibility is taken into account in an effective way in the χ parameter.

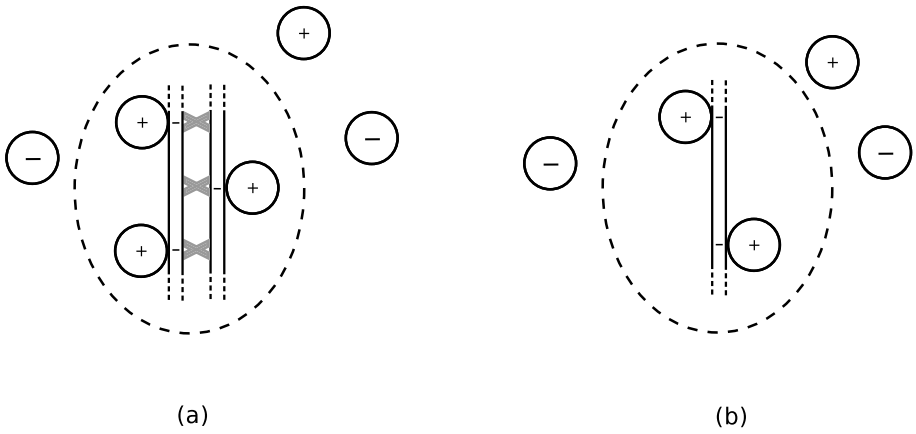

Figure 2: Schematic representation of complex formed by (a) double stranded DNA and salt ions (b) single stranded DNA and salt particles.

The first term in Eq. 9 is the ideal gas approximation for the different species of free ions [46, 47], namely

$$\beta F_{id}(m_1, m_2, \alpha) = \sum N_j \left[\ln \rho_j^* - 1\right], \tag{10}$$

where $\beta \equiv (k_{\rm B}T)^{-1}$, $\rho_j^* = \rho_j \, \sigma^3$ with $\sigma = (\sigma_c + \sigma_p)/2$. The sum is over all the species present in solution: complexes with ssDNA, dsDNA, free positive ions and free negative ions. The other terms are related to interaction between the different species. F_a in Eq. 9 is the association of single chains to form the double chains. This is described by an effective energy interaction χ between monomers. The corresponding free energy is proposed to be in a mean field approximation

$$\beta F_a(m_1, m_2, \alpha) = -m_2 \, \frac{Z^2}{2} \chi \, . \tag{11}$$

The free energy describing the interaction between the particles within the complex, F_{int} in Eq. 9, includes entropic and electrostatic contributions, namely

$$F_{int}(m_1, m_2, \alpha) = F_{ent}(m_1, m_2, \alpha) + F_{ion}(m_1, m_2, \alpha) \, . \tag{12}$$

The entropic free energy inside the complexes, given by F_{ent} in Eq. 12, is given by the ideal gas of single and double strand complexes [47],

$$\begin{aligned}\beta F_{ent}(m_1, m_2, \alpha) &= 2\, m_1\, Z^2\, [m_1 \ln m_1 + (1 - m_1) \ln(1 - m_1)] \\ &\quad + m_2\, Z^2\, [m_2 \ln m_2 + (1 - m_2) \ln(1 - m_2)]\,. \quad (13)\end{aligned}$$

F_{ion} in Eq. 12 describes the electrostatic interaction between the ions within the complex. Figure 3 illustrates that when a salt particle is associated to the complex it cancels the local charge. Only naked charges will contribute to this electrostatic energy [9, 10], namely

$$\beta F_{ion}(m_1, m_2, \alpha) = 2\, Z\, m_1\, \frac{\lambda_{\rm B}}{b_1}\, p_1^2\, S_1 + Z\, m_2\, \frac{\lambda_{\rm B}}{b_2}\, p_2^2\, S_2\,, \quad (14)$$

where the net valences on a monomer in the two types of chains are $p_1 = -1 + m_1$ e $p_2 = -1 + m_2$,

$$S_1 = Z_1\, [\psi(Z_1) - \psi(1)] - Z_1 + 1\,, \quad (15)$$

$$S_2 = Z_2\, [\psi(Z_2) - \psi(1)] - Z_2 + 1\,, \quad (16)$$

The digama function $\psi(n)$ is defined as

$$\psi(n+1) = -C + \sum_{k=1}^{n} \frac{1}{k}\,, C = 0.577215...\,, \quad (17)$$

and $\lambda_{\rm B} = \beta q^2/4\pi\varepsilon_s$ is the Bjerrum length. We have denoted the permittivity of solvent by ε_s.

The electrostatic free energy between the complex and the ionic solution [43, 44] is given by (see the Fig. 2)

$$\beta F_{elec}(m_1, m_2, \alpha) = \left\{ Z^2\, m_1\, p_1^2\, \frac{\lambda_{\rm B}}{L_1} + Z^2\, m_2\, p_2^2\, \frac{\lambda_{\rm B}}{L_2} \right\} \frac{K_0\,(\kappa R)}{\kappa R\, K_1(\kappa R)}\,, \quad (18)$$

where the inverse Debye screening length κ is given by

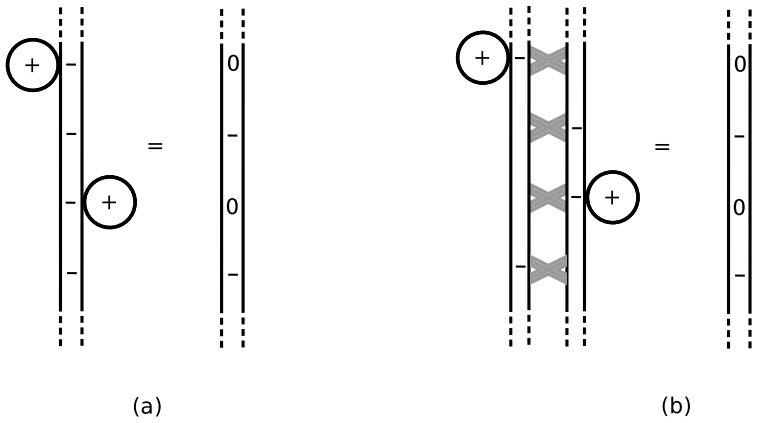

Figure 3: Schematic representation of the association between the charged groups at the DNA and the salt particles. At the site in which the salt associates the effective charge becomes zero.

$$\kappa = \sqrt{4\,\pi\,\lambda_{\rm B}\,(\rho_+ + \rho_-)}\,. \tag{19}$$

The complete free energy is therefore a function of three variables: the fraction of double strand DNA, α, the fraction of cation associated to single strand DNA, m_1, and the fraction of cation associated to double strand DNA, m_2, for a fixed set of densities and temperature. The equilibrium configuration for different temperatures and salt concentrations is therefore obtained by the minimization of the free energy in terms of these three quantities.

Before minimizing this free energy and finding the denaturation temperature it is important to define the parameter χ. Considering that the melting temperature is a function of the density of salt and of the fraction of GC base pairs (f_{GC}), we propose the following expression for χ,

$$\chi(n_{GC}, \rho_s) = f_1(n_{GC}) - f_2(n_{GC})\,\log \rho_s^*\,, \tag{20}$$

where n_{GC} indicates the fraction of GC base pairs in the DNA segment and $\rho_s^* = \rho_s\,a_c^3$ is the salt density in reduced units. This expression, even though empirical, is based in the effect of addition of salt in the electrostatic energy of a line of charges. The two functions $f_1(n_{GC})$ and $f_2(n_{GC})$ are assumed to be linear in terms of n_{GC}, namely

$$f_1(n_{GC}) = a_{11} - a_{12}\, n_{GC}\,, \tag{21}$$
$$f_2(n_{GC}) = a_{21} + a_{22}\, n_{GC}\,. \tag{22}$$

The coefficients a_{11}, a_{12}, a_{21} and a_{22} will be computed in the next section by adjusting the result for the melting of a number of systems for three different $n_{GC} = N_{GC}/Z$ base pairs densities.

Our hypothesis is that the attractive interaction between the aminoacids decreases as the amount of salt in the solution is increased. The salt due to the electrostatic interactions will be located also between the strands, increasing the distance between them, making the polarization of the H-bonds less effective.

We assume that this interaction acts as a "zipper effect", therefore f_1 and f_2 are linear functions of n_{GC}. If more Guanine-Cytosine interactions are present, stronger are the local Guanine-Cytosine interactions. This would be qualitatively explained by the dipole-dipole effects present in the H-bonds.

4. Results and Discussion

In the previous section we did construct a free energy, in the Eq. (9), as a function particles of salt that associate to the double and single stranded DNA, m_2 and m_1 respectively. In addition, F_{tot} is also a function of the fraction of DNA molecules that are single $(2\,\alpha)$ and double stranded $(1-\alpha)$. The equilibrium configuration of our system is obtained by minimizing F_{tot} in terms of m_1, m_2 and α named

$$\begin{aligned}
\left.\frac{\partial F_{tot}(m_1,m_2,\alpha)}{\partial m_1}\right|_{m_1=m_1^*,m_2=m_2^*,\alpha=\alpha^*} &= 0\,,\\
\left.\frac{\partial F_{tot}(m_1,m_2,\alpha)}{\partial m_2}\right|_{m_1=m_1^*,m_2=m_2^*,\alpha=\alpha^*} &= 0\,,\\
\left.\frac{\partial F_{tot}(m_1,m_2,\alpha)}{\partial \alpha}\right|_{m_1=m_1^*,m_2=m_2^*,\alpha=\alpha^*} &= 0\,.
\end{aligned} \tag{23}$$

In order to check if our assumption for the behavior of χ given by Eq. 20 is a good approximation, we proceed as follows. For a fixed salt concentration $\rho_s = 220m\mathrm{M}$ and $\rho_{\mathrm{DNA}} = 2\mu\mathrm{M}$ the equation for minimization (Eq. 23) and for

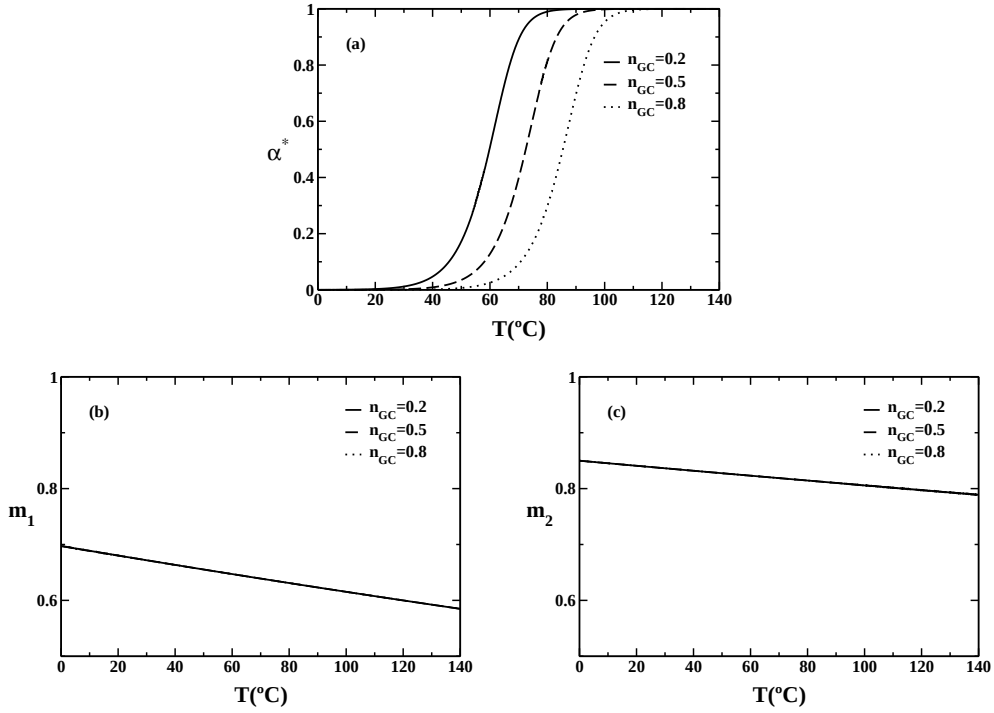

Figure 4: (a) α^*, (b) m_1 and (c) m_2 as a temperature function for different n_{GC} base pairs for salt density $\rho_s = 220m$M and $\rho_{\rm DNA} = 2\mu$M.

$n_{GC} = 0.2, 0.5, 0.8$ base pairs densities χ is fitted to give the melting transition temperatures $T = 77.8$°C, 81.6°C and 84.6°C, respectively, presented at [1]. The minimization of the free energy is insured by checking the free energy stability.

Figures 4(a), 4(b) and 4(c) show the behavior of α, m_1 and m_2 for these three base pairs densities. They show a very smooth behavior and m_1 and m_2 that are fraction of electrolytes associated to the single and double stranded DNA show no effect with changing $n_{GC} = 0.2, 0.5, 0.8$. The values of the melting temperature increase with n_{GC} as observed experimentally.

Then in order to check Eq. 20 the salt concentration was varied as indicated in the Table 1. In this case the values for the GC base pairs were $n_{GC} = 0.2, 0.5, 0.8$ and the density of DNA used was $\rho_{\rm DNA} = 2\mu$M. The value of χ was again fitted to give the experimental values shown in the Table 1 [1, 2]. Figure 5(a) illustrates the behavior of χ versus salt density showing a logarithmic behavior as suggested in Eq. 20. In this case the

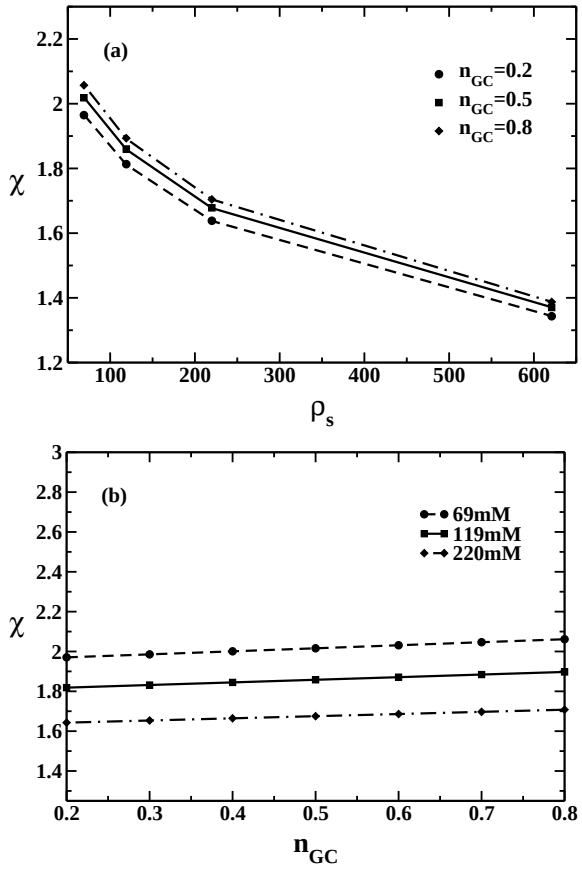

Figure 5: (a) χ as a function of salt concentration for fixed $n_{GC} = 0.2, 0.5, 0.8$ and (b) χ as a function of n_{GC} for fixed salt concentrations $\rho_s = 69m$M, $\rho_s = 119m$M, $\rho_s = 220m$M and $\rho_s = 621m$M. These plots result from fitting the theoretical denaturation theory with experiment in [1]

melting temperature increases with the density of salt.

Next, the same fitting procedure was performed for fixed salt concentrations $\rho_s = 69\mu$M, 119μM and 220μM but varying n_{GC} following the values in Table 1 and Table 2. Figure 5(b) shows that χ varies linearly with n_{GC} as proposed in Eq. 20.

Now that the expression for χ was validated in order to use it in general cases, it is necessary to find the coefficients a_{11}, a_{12}, a_{21} and a_{22}. For that purpose we adjust χ with the melting values of Table 1 for different values of salt and n_{GC} what leads to the coefficients:

$$
\begin{aligned}
a_{11} &= 0.021412\,, \\
a_{12} &= 0.00096406\,, \\
a_{21} &= 0.27611\,, \\
a_{22} &= 0.000368\,.
\end{aligned} \tag{24}
$$

In order to confirm if the fitting in Eq. (24) obtained using the systems summarized in Table 1 is robust, we test the values in Eq. (24) in new systems given by the experimental parameters in Table 2.

Figure 6 illustrates the behavior of α^* versus the temperature obtained by the minimization of Eq. (23) with the expression for χ given by Eq. (20) and the coefficients given in Eq. 24. α^* is computed for $n_{GC} = 0.3, 0.4, 0.6, 0.7$ and $\rho_s = 69m$M, $\rho_s = 119m$M, $\rho_s = 220m$M and $\rho_s = 621m$M. The curve shows a denaturation temperature T that agrees with experimental result from Ref.[1] shown in Table 2. Here no fitting was employed.

Table 1: Experimental denaturation temperatures for the systems A, B, C and D. The systems differ by the salt concentration, ρ_s, and by the base pairs density, n_{GC} [1].

	ρ_s (mM)	n_{GC}	T_{DEN} (° C)
System A	69	0.2	50.7
		0.5	55.0
		0.8	59.3
System B	119	0.2	66.3
		0.5	70.4
		0.8	74.5
System C	220	0.2	77.8
		0.5	81.6
		0.8	84.6
System D	621	0.2	65.1
		0.5	78.8
		0.8	87.7

Table 2: Experimental denaturation temperatures for the systems E, F, G and H. The systems differ by the values of salt concentration, ρ_s, and by the density of base pairs, n_{GC} [1].

	ρ_s (mM)	n_{GC}	T_{DEN} ($^\circ$ C)
System E	69	0.3	58.3
		0.4	62.7
		0.6	71.3
		0.7	74.4
System F	119	0.3	61.9
		0.4	66.8
		0.6	74.7
		0.7	78.4
System G	220	0.3	66.1
		0.4	70.8
		0.6	78.5
		0.7	81.5
System H	621	0.3	71.3
		0.4	75.9
		0.6	82.7
		0.7	85.2

5. Conclusion

In this work we analysed the denaturation process as result of the competition between the electrostatic contribution, the H-bonds and the entropy using a mean field analytic approach. Using the minimization of a free energy with respect to the fraction of double stranded, single stranded DNA and fraction of salt ions associated to them, the melting temperature was computed.

For a fixed value of the attractive parameter χ and salt concentration our model shows that the melting temperature increases with the density of n_{GC} base pairs. In addition, the temperature increases with the increase of salt concentration if n_{GC} is kept fixed.

Our free parameter, the attractive energy between the two strands, was

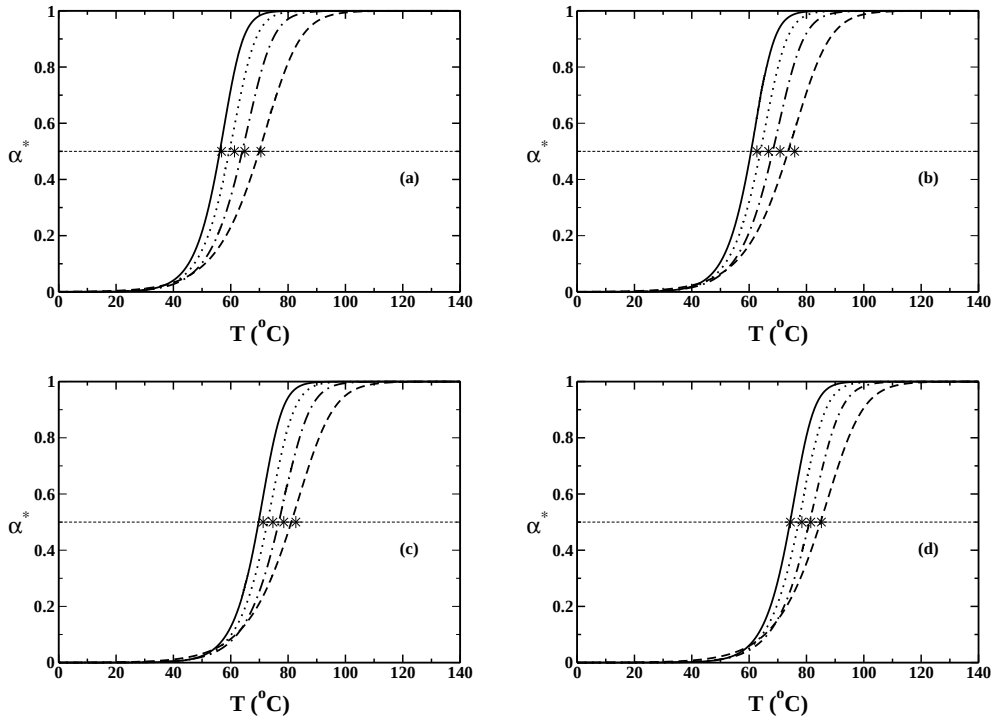

Figure 6: α^* as a function of n_{GC} for different salt concentrations: $\rho_s = 69m$M (solid line), $\rho_s = 119m$M (dotted line), $\rho_s = 220m$M (dot-dashed line), $\rho_s = 621m$M (dashed line), $Z\,\rho_p = 2\mu$M and (a) $n_{GC} = 0.3$, (b) $n_{GC} = 0.4$, (c) $n_{GC} = 0.6$ and $n_{GC} = 0.7$.

adjusted using a set of experimental results for the denaturation temperature. After that our free energy functional was tested with another set of experiments and showed a good agreement.

Even though we have tested our model only against experimental data for short DNA segments, we hope that our approach can be used without additional fitting parameters to compute the denaturation for other systems regardless the length of the DNA, concentration of salt and number of GC base pairs.

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