Onset of DNA Aggregation in Presence of Monovalent and Multivalent Counterions

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We address theoretically aggregation of DNA segments by multivalent polyamines such as spermine and spermidine. In experiments, the aggregation occurs above a certain threshold concentration of multivalent ions. We demonstrate that the dependence of this threshold on the concentration of DNA has a simple form. When the DNA concentration $c_{\text{DNA}}$ is smaller than the monovalent salt concentration, the threshold multivalent ion concentration depends linearly on $c_{\text{DNA}}$, having the form $\alpha c_{\text{DNA}} + \beta$. The coefficients $\alpha$ and $\beta$ are related to the density profile of multivalent counterions around isolated DNA chains, at the onset of their aggregation. This analysis agrees extremely well with recent detailed measurements on DNA aggregation in the presence of spermine. From the fit to the experimental data, the number of condensed multivalent counterions per DNA chain can be deduced. A few other conclusions can then be reached: i) the number of condensed spermine ions at the onset of aggregation decreases with the addition of monovalent salt; ii) the Poisson-Boltzmann theory over-estimates the number of condensed multivalent ions at high monovalent salt concentrations; iii) our analysis of the data indicates that the DNA charge is not over-compensated by spermine at the onset of aggregation.

INTRODUCTION

Condensation and aggregation of DNA, induced by multivalent counterions, have been extensively studied in the past two decades (for a review, see Bloomfield et al. (2000) and references therein). The term condensation usually refers to the collapse of a single, long DNA chain. Condensation plays an important role in storage and packing of DNA; for example, in viral capsids (Gelbart et al., 2000). Aggregation of DNA is a closely related phenomenon, where multiple chains attract each other and form a variety of condensed mesophases of complex structure (Pelta et al., 1996). In both phenomena multivalent counterions play a crucial role, screening the electrostatic repulsion between charged strands of DNA and mediating an effective attraction.

A variety of tri- and tetra-valent ions can induce aggregation and condensation, among them the polyamines spermidine ($3^+$) and spermine ($4^+$) (Chattoraj et al., 1978; Gosule and Schellman, 1978; Tabor and Tabor, 1984), as well as cobalt-hexamine (Widom and Baldwin, 1980; 1983). In typical experiments on aggregation (Pelta et al., 1996; Raspaud et al., 1998; Saminathan et al., 1999) multivalent ions are gradually added to a solution with fixed concentration of DNA segments and monovalent salt. Two such examples for spermine and spermidine are reproduced in Fig. 1 (Pelta et al., 1996). As the multivalent ion concentration is raised above a certain threshold, DNA segments begin to aggregate, and precipitate from the solution. Above the aggregation threshold, the DNA concentration decreases gradually or abruptly, depending on various parameters such as the monovalent salt concentration and total DNA concentration. Further addition of multivalent ions at higher concentrations reverses the aggregation. Above a second, re-dissolution threshold, $c_{\text{redissol}}$, all the DNA is dissolved. The data is adapted from Pelta et al. (1996).

FIG. 1 Percent of solubilized DNA, as function of polyamine concentration: square symbols - spermine, circles - spermidine. The solid and dashed lines are guides for the eye. DNA and NaCl concentrations are 3 mM and 25 mM, respectively. Below the aggregation threshold, $c_{\text{aggr}}$, and above the re-dissolution threshold, $c_{\text{redissol}}$, all the DNA is dissolved. The data is adapted from Pelta et al. (1996).
of DNA concentration. Furthermore, for $c_{\text{DNA}}$ smaller than the monovalent salt concentration we show that this dependence is linear: $c_z,\text{aggr} = \alpha c_{\text{DNA}} + \beta$. The coefficient $\beta$ is the multivalent counterion concentration far away from the DNA chains, while $\alpha$ accounts for the excess of multivalent ions around each chain. These quantities can be extracted, e.g., from the four experimental curves of Fig. 2. Several further conclusions are then drawn on the onset of DNA aggregation and on the counterion distribution around each double-stranded DNA.

**THEORETICAL CONSIDERATIONS**

Consider an aqueous solution containing monovalent (1:1) salt, multivalent (z:1) salt and DNA segments below their threshold for aggregation. Throughout this paper, the DNA solution is assumed to be dilute enough such that the DNA segments do not overlap. We also assume that these DNA segments can be regarded as rigid rods.

The concentrations of added monovalent salt, multivalent salt and DNA monomers are denoted by $c_s$, $c_z$ and $c_{\text{DNA}}$, respectively. These are the solute concentrations per unit volume as controlled and adjusted in experiments. We will assume that the monovalent and multivalent salts have the same type of co-ion, so that altogether there are three ion species in the solution:

1. A multivalent counterion contributed from the z:1 multivalent salt, of concentration $c_z$.

2. A monovalent counterion contributed by multivalent (z:1) salt and DNA segments below their threshold for aggregation. Throughout this paper, the DNA solution is assumed to be dilute enough such that the DNA segments do not overlap. We also assume that these DNA segments can be regarded as rigid rods.

3. Co-ions coming from both z:1 and 1:1 salts, of concentration $c_s + z c_z$.

Each DNA segment attracts a layer of oppositely charged counterions referred to as the condensed counterions. As long as the typical distance between segments is large compared to the electrostatic screening length $\kappa^{-1}$, the electrostatic potential decays exponentially to zero far away from the DNA segments. In turn, the concentrations of the three ion species decay to well-defined *bulk* values denoted by $c_z^\infty$ for the monovalent ions and $c_s^\infty$ for the z-valent ones. These concentrations should be distinguished from the concentrations $c_s$ and $c_z$ introduced above, which are the average concentrations of added salts regulated experimentally.

The Debye screening length, $\kappa^{-1}$, characterizing the exponential decay of the electrostatic potential, is determined by the bulk concentrations of all three ionic species:

$$\kappa^2 = 4\pi l_B \left[ c_s^\infty + z^2 c_z^\infty + (c_s^\infty + z c_z^\infty) \right].$$  \hspace{1cm} (1)

where the third term is the co-ion concentration. It is equal to $c_s^\infty + z c_z^\infty$ due to charge neutrality far from...
the DNA where the potential decays to zero. The
above equation makes use of the Bjerrum length, \( l_B = \varepsilon^2/(\varepsilon k_B T) \), equal to about 7 Å in aqueous solution at
room temperature, \( k_B T \) is the thermal energy, \( \varepsilon \) is the
electron charge and \( \varepsilon = 80 \) is the dielectric constant of
water. The Debye length as well as \( c_\infty^z \) are shown schematically in Fig. 3. Other quantities that will be
defined below are also indicated in this figure.

In dilute solutions different DNA segments do not overlap. Following previous works, we introduce a cell model
also shown schematically in Fig. 3. Note that the model serves to illustrate the subsequent derivations but is not essential for the validity of our main results. In the cell model, each segment, of a cylindrical cross-section, is at the center of a cylindrical cell of radius \( R \) and area
\( A = \pi R^2 \) such that

\[
c_{\text{DNA}} = 1/(aA).
\]

Namely, each DNA monomer occupies a specific volume
\( aA \), where \( a \simeq 1.7 \) Å is the average charge separation
on the chain taken hereafter as the monomer length.

We will assume below that the DNA solution is dilute enough so that \( R \) is large compared to the Debye length \( \kappa^{-1} \). This assumption is essential for our derivation and can be verified for all the experimental data considered
in this paper. Density profiles of the three ion species are then practically identical to those near an isolated DNA
segment with the same bulk concentrations \( c_1^\infty \), \( c_2^\infty \). In other words, the profiles are determined uniquely by \( c_1^\infty \)
and \( c_2^\infty \), with practically no dependence (or, more precisely, an exponentially small dependence) on the DNA
monomer concentration. A demonstration of this claim is presented in Fig. 4, using the Poisson-Boltzmann theory
in a cell model. For two very different values of \( R \) corresponding to different \( c_{\text{DNA}} \), the counterion profiles
match perfectly when the values of \( c_1^\infty \) and \( c_2^\infty \) are the same. Note that the average concentrations of added
salts, \( c_s \) and \( c_{s_2} \), have different values in the two cells because of the contribution of condensed ions.

The total number of \( z \)-valent counterions, per cell unit
length, is given by:

\[
A\zeta = A\zeta^\infty + \rho_z (c_1^\infty, c_2^\infty)
\]

where \( \rho_z \) is the excess number of \( z \)-valent ions per unit
length near the DNA. Throughout the paper we use the
symbol \( e \) to denote concentrations per unit volume and
\( \rho \) for concentrations per DNA unit length. The excess
\( \rho_z \) can be evaluated in the limit of infinite cell radius,
corresponding to an isolated chain:

\[
\rho_z = 2\pi \int_0^\infty r dr \left[ n_z(r) - c_z^\infty \right],
\]
where \( n_z(r) \) is the \( z \)-valent local counterion concentration at distance \( r \) from the axis of symmetry, and \( n_z(\infty) = c_z^\infty \). Following the discussion in the previous paragraph, the excess \( \rho_z \) is determined uniquely by \( c_z^1 \) and \( c_z^\infty \). Its exact functional dependence on these variables is generally not known, although it can be evaluated approximately, e.g., using the Poisson-Boltzmann equation or in computer simulations.

For monovalent counterions we have, in a similar fashion:

\[
Ac_s + Ac_{DNA} = Ac_1^1 + \rho_1 (c_1^1, c_1^\infty),
\]

where \( \rho_1 \), the excess of monovalent counterions per unit length, is defined as in Eq. 4 and \( Ac_{DNA} = 1/a \) is the DNA charge density per unit length. The extra term in the left-hand-side of Eq. 5 originates from monovalent counterions contributed by the DNA monomers. Using Eq. 2 we can rewrite Eqs. 3 and 5 as:

\[
c_z = c_z^\infty + a \rho_z (c_z^\infty, c_z^\infty) c_{DNA}
\]

and

\[
c_s = c_s^\infty + [a \rho_1 (c_s^\infty, c_s^\infty) - 1] c_{DNA}.
\]

These two equations relate the experimentally adjustable \( c_s, c_z \) and \( c_{DNA} \) to the bulk densities \( c_z^\infty, c_s^\infty \) that in turn, are important because they determine the ion density profiles.

In the limit of infinite DNA dilution, \( c_{DNA} = 0 \), and therefore \( c_z = c_z^\infty \) and \( c_s = c_s^\infty \). At any finite DNA concentration \( c_z \) and \( c_s \) are not equal to \( c_z^\infty \) and \( c_s^\infty \), respectively, because each segment captures some of the multivalent ions and releases a number of monovalent ones. Equations 6 and 7 express the correction to \( c_z, c_s \) at given \( c_z^\infty, c_s^\infty \) for both mono- and multi-valent counterion species. The dimensionless quantities \( a \rho_1, a \rho_z \) are the excess of the mono- and multi-valent counterion species, respectively, per DNA monomer.

We would like to emphasize the generality of Eqs. 6 and 7. They do not depend on the assumption of parallel DNA residing in the middle of oriented cylindrical unit cells, or on any mean-field approximation for the distribution of counterions. The only assumption required to derive Eqs. 6 and 7 is that the average distance between DNA segments is large compared with the Debye length. Although Eqs. 6 and 7 are correct for any \( c_s, c_z \) and \( c_{DNA} \) below the onset of DNA aggregation, we will be interested below specifically in the aggregation onset.

### Onset of aggregation

Our aim now is to find how the value of \( c_z \) at the onset of aggregation, \( c_{z, aggr} \), depends on \( c_{DNA} \). We will assume that this aggregation onset depends on \( c_1^\infty \) and \( c_1^\infty \), but not on the average distance between DNA chains. We motivate this assumption by the fact that \( c_1^\infty \) and \( c_1^\infty \) determine the density profile of multivalent counterions around the DNA chains, which, in turn, mediate the attraction necessary for aggregation. Before discussing this assumption in more detail, let us first consider its consequences. We can imagine an experiment where \( c_z^\infty \) is gradually increased while \( c_z^\infty \) is kept fixed. Aggregation will start, in this experiment, above a certain threshold value of \( c_z^\infty \). Our assumption is that this threshold does not depend on \( c_{DNA} \). In real experiments, however, \( c_z \) is adjusted rather than \( c_z^\infty \), and \( c_s \) is kept fixed rather than \( c_s^\infty \). In order to find the threshold value in terms of the experimentally available \( c_z \) we need to map \( c_z^\infty, c_s^\infty \) onto \( c_s, c_z \). This mapping is described by Eqs. 6-7 and involves \( c_{DNA} \). It is only through this mapping that \( c_{DNA} \) will affect the threshold of aggregation.

**The limit of \( c_{DNA} \ll c_z \):**

The limit \( c_{DNA} \ll c_z \) offers a particularly simple dependence of \( c_{z, aggr} \) on \( c_{DNA} \) and is considered first. Most models and experiments indicate that monovalent counterions cannot overcharge DNA segments. Hence the monovalent excess, \( a \rho_1 \), in Eq. 7 is a number between zero and one, because the excess monovalent charge is smaller than that of DNA. From Eq. 7 \( |c_z - c_1^\infty| \ll c_z \) as long as \( c_{DNA} \ll c_z \). It is then possible to replace \( c_1^\infty \) by \( c_z \), leading to a simplification of Eq. 6:

\[
c_z = c_z^\infty + a \rho_z (c_z, c_z^\infty) c_{DNA}.
\]

Note that \( c_{DNA} \) is indeed smaller than \( c_z \) in most of the experimental points in Fig. 2. However a similar simplification cannot be applied for \( c_s \) because it is typically much smaller than \( c_z \), and often smaller than \( c_{DNA} \).

According to our principal assumption, aggregation starts at a threshold value \( c_z^\infty = c_z^* \), which does not depend on \( c_{DNA} \) (while \( c_{z, aggr} \), the average multivalent salt concentration does depend on \( c_{DNA} \) through Eq. 5). Similarly, the density profile at the threshold does not depend on \( c_{DNA} \), because it is determined by \( c_z^\infty = c_z^* \) and \( c_s^* \). The excess of \( z \)-valent counterions, as determined from this profile, is equal to:

\[
\rho_z^* = \rho_z (c_s, c_z^*),
\]

with no dependence on \( c_{DNA} \). Using the threshold values \( c_z^* \) and \( \rho_z^* \) in Eq. 8 we find that the average concentration of \( z \)-valent ions at the onset of aggregation is:

\[
c_{z, aggr} (c_{DNA}) = c_z^* + a \rho_z^* c_{DNA}.
\]

This is the threshold concentration that was measured experimentally in [Raspaud et al. (1998)]. Note that in Eq. 10 \( c_z^* \) as well as \( \rho_z^* \) depend on the monovalent salt concentration, \( c_s \), but the explicit dependence is omitted for clarity.

The simple relationship expressed by Eq. 10 is one of our main results. As a visualization of this result we refer
again to Fig. 3. The quantities $\rho_z$, $c_z^\infty$ and the density profile $n_z(r)$ are indicated in this figure. At the onset of aggregation $c_z^\infty$ is equal to $c_z^*$ and does not depend on $c_{DNA}$ (or equivalently, on the spacing between DNA segments, $R$). As $c_{DNA}$ is increased the distance between DNA strands decreases. The onset values of $c_z^\infty$ and $\rho_z$ do not change, but the contribution of $\rho_z$ to the average concentration increases, leading to an increase in $c_{z,agr}$.

The coefficients $a\rho_z^*$ and $c_z^*$ of the linear dependence in Eq. 10 are the coefficients $a$ and $\beta$ defined in the introduction section. They can be easily found from the experimental data: $c_z^*$ is the value of $c_{z,agr}$ in the limit of infinite DNA dilution, $c_{DNA} \rightarrow 0$, since in this limit $c_z = c_z^\infty = c_z^*$. The excess at the onset, $\rho_z^*$, can be found from the slope of $c_{z,agr}$ as function of $c_{DNA}$. Before presenting a detailed comparison with experiments, we generalize the treatment for small $c_{DNA}$ to arbitrary values.

The case of $c_{DNA} \geq c_s$:

When $c_{DNA}$ is of the same order as $c_s$ or larger, corrections to $c_z^\infty$ must be taken into account, as expressed by Eq. 9 and the linear relation of Eq. 10 no longer holds. The ion density profiles as well as $c_z^\infty$ are now determined by the two variables $c_z^\infty$ and $c_z^\infty$. The relation between $c_z^\infty$ and $c_z^\infty$ and the experimentally controlled $c_s, c_z, c_{DNA}$ is given by Eqs. 10 and 11. In terms of $c_z^\infty$, $c_z^\infty$ the criterion for aggregation remains the same as in the previous case:

$$c_z^\infty = c_z^*(c_z^\infty).$$

The three equations [6] and [11] with the three unknowns $c_z^\infty$, $c_z^\infty$ and $c_z^*$ lead to a unique solution for $c_{z,agr}$. Note that $c_z^\infty$ is larger than $c_s$ because of counterions coming from the DNA as can be seen in Eq. 7 where $a\rho_1 - 1$ is negative. In Eq. 10 $c_s$ is replaced by $c_z^\infty$, which is larger than $c_s$ for large $c_{DNA}$. Hence, increasing $c_{DNA}$ has an effect similar to addition of monovalent salt. As noted above, this effect is significant only for $c_{DNA} > c_s$.

**COMPARISON WITH EXPERIMENT**

Raspaud et al. [1998] measured the spermine ($z = 4$) concentration $c_z$ at the onset of aggregation as a function of $c_{DNA}$ for four values of $c_s$ and with $c_{DNA}$ ranging over four orders of magnitude — from $10^{-2}$ to $10^2\text{mM}$. We fitted the data (E. Raspaud and J.-L. Sikorav, private communication) for each $c_s$ to a straight line according to Eq. 10. The least square fit presented in Fig. 5 takes into account the experimental error bars and data points up to $c_{DNA} = 10\text{mM}$. In Fig. 5 a the fit is shown using a linear scale which covers the range of $c_{DNA}$ only up to $c_{DNA} = 1.5\text{mM}$ for clarity purposes. Due to the large range of $c_{DNA}$ it is impossible to show all the data on the linear scale of Fig. 5 a. Instead, the same data and linear lines are shown in Fig. 5 b on a log-log scale over the full experimental range of $c_{DNA}$.

The linear fit is very good for all four values of monovalent salt concentration $c_s$. Note that for $c_s = 88\text{mM}$ the fit is very good up to the largest value of $c_{DNA} = 48\text{mM}$ reported in the experiment, although our fit takes into account only data points up to $c_{DNA} = 10\text{mM}$. It was previously suggested [Raspaud et al. 1998] that a sepa-
rate regime exists for $c_{\text{DNA}} \gtrsim 10 \text{mM}$, characterized by a power law relation between $c_s$ and $c_{\text{DNA}}$ with an exponent smaller than unity. Our analysis suggests a different conclusion. The fit clearly demonstrates that the relation is linear all the way up to $c_{\text{DNA}} = 48 \text{mM}$, as predicted by Eq. [10]. Note also that even at $c_{\text{DNA}} = 48 \text{mM}$ we have $c_{\text{DNA}} < c_s$ so the assumptions leading to Eq. [10] are still valid.

The only points in Fig. 5 b that deviate significantly from the fit are the three points where $c_s = 13 \text{mM}$ (triangles) and $c_{\text{DNA}} > 20 \text{mM}$ (two of these points coincide with points having $c_s = 88 \text{mM}$, shown using square symbols.) This deviation is easily explained by the fact that $c_{\text{DNA}} \gg c_s$ so that corrections to $c_s^\infty$ must be taken into account. For example, at $c_{\text{DNA}} = 90 \text{mM}$ the nominal monovalent counterion concentration is $103 \text{mM}$, taking into account counterions contributed by the DNA. In order to find $c_s^\infty$ we need to subtract the condensed counterions, as determined by $\rho_1$. We can estimate $\rho_1$ at this point by solving the Poisson-Boltzmann equation in a unit cell with the appropriate radius. The chemical potentials of the three ion species are tuned such that their concentrations match the known values of $c_s$ and $c_s$. This leads to an estimate: $c_s^\infty \approx 68 \text{mM}$. Hence, $c_s$ at the onset of aggregation should lie a little below the continuation of the $c_s = 88 \text{mM}$ line which is, indeed, where it is found. The trend for $c_s = 13 \text{mM}$ can probably be seen already at the point $c_{\text{DNA}} = 15 \text{mM}$, although the deviation at this point is still within the range of experimental error. The few other experimental points with $c_{\text{DNA}} \approx c_s$ deviate slightly from the straight line as well (still within experimental error bars). In all these cases the deviation is in the direction corresponding to a higher value of $c_s$, as expected.

A linear relation of the form $c_{z,\text{aggr}} = \alpha c_{\text{DNA}} + \beta$, was previously suggested on empirical basis for aggregation induced by spermidine ($3^+$), on a smaller range of DNA concentrations [Osland and Kleppe, 1977; Pelta et al., 1996]. Although this result looks similar to our prediction on the onset of aggregation, it is not directly related to our analysis because $c_{z,\text{aggr}}$ was taken in those works to be the transition midpoint. This is the point where half of the maximal precipitation of DNA is reached. Our analysis does not apply at the transition midpoint since it requires all the DNA segments to be well separated from each other. Indeed, the coefficient $\alpha$, related to the transition midpoint, was found in [Osland and Kleppe, 1977] and [Pelta et al., 1996] to be of order $10^2$, much larger than unity. Such a value of $\alpha$ cannot be interpreted as the excess of spermidine ions per monomer near isolated chains.

The parameters of the linear fit in Fig. 5 are summarized in Table 1 for the four experimentally used values of $c_s$.

| $c_s[\text{mM}]$ | $c_s^*[\text{mM}]$ | $a \rho_z^*$ |
|-----------------|--------------------|---------------|
| 2               | $0 \pm 0.0003$     | 0.194 $\pm 0.020$ |
| 13              | 0.011 $\pm 0.002$  | 0.191 $\pm 0.013$ |
| 23              | 0.031 $\pm 0.005$  | 0.173 $\pm 0.025$ |
| 88              | 0.52 $\pm 0.05$    | 0.135 $\pm 0.026$ |

Crossover in the log-log plot

For presentation purposes we plot in Fig 5 b, $c_{z,\text{aggr}}$ vs. $c_{\text{DNA}}$ on a log-log scale, as appeared in [Raspaud et al., 1998]. The linear relation that was found between these two quantities is not clearly manifested on the log-log plot, because a linear dependence of the form $y = \alpha x + \beta$ is not easily recognized in such a plot. Furthermore, such a linear relation appears on a log-log plot to be artificially characterized by two distinct behaviors, at low and high values of the independent variable. These two behaviors were mentioned in [Raspaud et al., 1998] and can be seen in Fig. 5 b. However, they do not represent in our opinion two real physical regimes and can be understood by taking the logarithm of Eq. [10]. For small $c_{\text{DNA}}$ (large $R$):

$$\log c_z \simeq \log c_z^*$$

i.e., $c_z$ does not depend on $c_{\text{DNA}}$ as is seen in Fig. 5 b in the small $c_{\text{DNA}}$ limit. In the opposite limit of large $c_{\text{DNA}}$ (small $R$):

$$\log c_z \simeq \log c_{\text{DNA}} + \log a \rho_z^*$$

Here, the linear dependence of $c_z$ on $c_{\text{DNA}}$ yields a line of slope 1 in the same figure.

The crossover between these apparent behaviors occurs when the number of bulk and excess ions are the same:

$$c_{\text{DNA}} = \frac{c_z^*}{a \rho_z^*}$$

When $c_{\text{DNA}}$ is much smaller than this crossover value, the number of excess multivalent ions near DNA segments is negligible compared to their total number. In the other extreme of $c_{\text{DNA}}$ much larger than the crossover value, the number of free multivalent ions is negligible compared to the excess ions, and nearly all multivalent ions are bound to the DNA.

For the experimental data in Fig. 5 the crossover value is equal to 0.06, 0.18 and 3.9 mM for $c_s = 13$, 23 and 88 mM, respectively, and smaller than $1.5 \times 10^{-3}$ mM for $c_s = 2$ mM. The first three crossover points are indicated by arrows in Fig. 5 b.

**DNA AGGREGATION AND COUNTERION CONDENSATION**

We separate the discussion following our results in three parts. The first addresses the conditions required...
for DNA aggregation. The coefficients of the linear relation in Eq. \[10\] \(c_s^*\) and \(c_z^*\) have a definite physical meaning. Their values, as extracted from the experimental data provide insight on these conditions. The second part deals with condensation of counterions on DNA (to be distinguished from condensation of DNA chains). The general relation \(\rho_z = \rho_z(c_s^\infty, c_z^\infty)\) that was introduced in Eqs. \[3\]-\[4\] is a property of counterion condensation on isolated chains. By extracting the values of \(\rho_z\), \(c_s^\infty\) and \(c_z^\infty\) at the onset of DNA aggregation, we can learn about exact density profiles of spermine around DNA, and compare our findings with approximations such as Poisson-Boltzmann theory. Finally, we comment on our main assumption, which was used in the theoretical considerations section.

**Conditions at the onset of aggregation**

Most of the proposed theoretical models for inter-chain attraction and aggregation (see, for example, Arenzon et al. (1999); Borukhov et al. (2001, 2002); Ha and Liu (1997); Nguyen et al. (2000); Olvera de la Cruz et al. (1995); Raspaud et al. (1998); Wittmer et al. (1995)) regard the charged chain as surrounded by a layer of condensed ions which is usually modeled as a one-dimensional gas. This layer mediates an inter-chain attraction, and the models predict the number of condensed ions required to initiate aggregation of the chains. In the current work we do not address this theoretical problem, but rather concentrate on what can be inferred from the experimental results using the analysis presented in the previous section. This analysis provides insight on the conditions prevailing at the onset of aggregation. In particular, the excess \(\rho_z^*\) characterizes the number of condensed multivalent counterions that are present near each chain at the onset. Although in general the notion of condensed counterions is somewhat ill-defined, as it depends on which ions are regarded as bound to the DNA, we show in the Appendix that in our case it does have a reasonably well defined meaning. Furthermore, the number of condensed multivalent ions per monomer is practically the same as \(a\rho_z^*\).

The excess of multivalent counterions per monomer, \(a\rho_z^*\), is shown in Fig. 6 as function of \(c_s\). All values are taken from Table 1, as extracted from the experimental data. The dashed line is a linear fit. Two different axis scales are used on the left and right of the plot. The left axis shows the value of \(a\rho_z^*\). The right one shows the part of DNA charge that is compensated by condensed multivalent ions, \(z a\rho_z^*\), where \(z = 4\) for spermine. From the plot we deduce the following two conclusions:

1. The number of condensed multivalent ions (per DNA monomer) \(a\rho_z^*\) at the onset decreases as the monovalent salt concentration increases, with variation between 0.19 and 0.14. A possible reason for this trend may be that the bare electrostatic repulsion between chains is decreased due to increased screening. Hence a smaller number of multivalent ions is required in order to overcome this repulsion. The change in \(\rho_z^*\) may also be related to the competition between monovalent and multivalent ions in the aggregated DNA state.

2. The data indicates that there is no over-charging of the DNA by spermine at the onset (see also Nguyen et al. (2000)) since \(za\rho_z^* < 1\). At higher concentration of spermine, beyond the threshold, we do not rule out the possibility of DNA over-charging, as was suggested by Nguyen et al. (2000).

Although \(\rho_z^*\) decreases with increase of \(c_s\), it is of the same order of magnitude for all the \(c_s\) values in Table 1. In contrast, \(c_s^*\) varies in Table 1 over more than three orders of magnitude. As was previously suggested (Olvera de la Cruz et al. 1995; Raspaud et al. 1998), this large variation in \(c_s^*\) is a result of competition between monovalent and multivalent counterions. We discuss the relation between \(\rho_z^*\) and \(c_s^*\) to some extent in the following subsection. A more detailed analysis of this relation, emphasizing the role of competition between the two counterion species, will be presented in a separate publication (see also Belloni et al. (1984); Wilson and Bloomfield (1979); Wilson et al. (1980)).
The most simple model to consider is the Poisson-Boltzmann (PB) theory (see Andelman [1994]; Guéron and Weisbuch [1980]; Le Bret and Zimm [1984]; Oosawa [1971]). In Table 2 we compare the excess predicted by PB theory with the experimental result, by solving the PB equation such that $c_z^\infty$ and $c_z^\ast$ match the experimental values of $c_s$ and $c_z^\ast$ from Table 1. The excess is then calculated from the PB density profile, and compared with the experimental value of $a\rho_z$ (equal to $a\rho_z^\ast$ of Table 1). The DNA is modeled as a uniformly charged cylinder of radius $d = 10\;\text{Å}$.

Inspection of the results in Table 2 shows that there is a reasonable agreement with experiment (within the error bars) for the three smaller values of $c_s = 2, 13, 23\;\text{mM}$. However, for $c_s = 88\;\text{mM}$ there is a 30% deviation. The two data points with $c_{\text{DNA}} > 10\;\text{mM}$ that were not taken into account in the linear fit of Fig. 5 suggest that $\rho_z$ is closer to the lower bound of the experimental error range, whereas the PB value is larger than the upper bound.

Overall, the agreement with PB theory (Table 2) is surprisingly good considering that PB theory does not work so well for bulky multivalent ions. Deviations from PB theory have several sources. One of these sources is specific molecular details such as the geometrical shape of ions, DNA structure and short-range interactions. Another source for deviations are ion-ion correlations between spermine molecules, computed in theories which go beyond the mean-field approximation. However, these correlations tend to increase the number of bound multivalent counterions (Lyubartsev and Nordenskål [1997]), while for $c_s = 88\;\text{mM}$, the number of bound multivalent counterions is decreased. We conclude that correlation effects by themselves are not the main source of the deviations seen in Table 2. In addition the data analysis does not indicate over-charging of the DNA. Such an effect may be expected if correlation effects are strong (Nguyen et al. [2000]).

In Fig. 7 we compare the DNA aggregation data with PB predictions at finite DNA concentrations. For each DNA concentration the PB equation is solved in a cylindrical cell of the appropriate radius. The multivalent counterion concentration $c_z$ is gradually increased until the onset is reached, and its onset value, $c_{z,\text{onset}}$, is plotted as function of $c_{\text{DNA}}$. Two different criteria are used to determine the onset $c_{z,\text{onset}}$. In Fig. 7a it is chosen as the point where $c_z^\infty$ is equal to the experimental value $c_z^\ast$ of Table 1; whereas in Fig. 7b the onset is chosen the point where $\rho_z = \rho_z^\ast$. In order to span all the data range we use for convenience a log-log plot, as in Fig. 5b.

On a linear scale all the lines in Fig. 7a and b are straight lines. This fact serves as additional confirmation of our general analysis in the theoretical considerations section. In accordance with our analysis, both $c_z^\ast$ and $\rho_z$ are constant along each line, and the slope of each line is equal to $a\rho_z$. Note that the relation between $c_z^\ast$ and $\rho_z$ is determined in Fig. 7 within the PB approximation, while in Fig. 5 both of these coefficients are related to the actual counterion density profiles in the experimental system. The use of the PB equation is the source of deviations from experimental data in Fig. 7.

On first inspection the match with experiment in Fig. 7a is very good, whereas the match in Fig. 7b is not as good. On closer inspection it is seen that the fit in Fig. 7b is not good for small values of $c_{\text{DNA}}$, while it is actually better than in Fig. 7a for large $c_{\text{DNA}}$. With the PB equation it is not possible to obtain a perfect fit for both small and large $c_{\text{DNA}}$ because the values of $c_z$ and $\rho_z$ are not independent. Fixing $c_z^\infty = c_z^\ast$ (as in Fig. 7a) sets a value of $\rho_z$ that is different from $\rho_z^\ast$; and the opposite happens in Fig. 7b. The fit in Fig. 7a is quite good even for large $c_{\text{DNA}}$ because the values of $\rho_z^\ast$ are of similar order of magnitude for all four lines.

Deviations as in Fig. 7 are inevitable if any approximations are used to model the distribution of counterions around DNA. Note however that within such approximate models our general theoretical considerations should apply, as long as the total number of ions in the system is counted properly. Such a model that goes beyond PB was proposed in (Nguyen and Shklovskii [2001]). Indeed, within this model a linear relationship similar to Eq. [11] was found.

The experimental results analyzed in this section may be influenced, to a certain degree, by the fact that there was more than one type of monovalent counterion in the system. For the three higher salt concentrations, except for $c_s = 2\;\text{mM}$, the solution contained 10 mM of TrisH+ ions in addition to Na+. (Raspaud et al. [1993]). For the largest salt concentration, 88 mM, where significant deviations from PB theory are found, this effect is probably negligible. Another detail regarding the TE buffer is that the Tris ions may be only partly ionized. If only 80% of Tris is ionized, as suggested in (Tang et al. [1997]), the concentrations $c_s = 13\;\text{mM}, 23\;\text{mM}$ and $88\;\text{mM}$ should be reduced by 2 mM. Although this will have only a small effect on our results, it will improve both the comparison with PB and the fit with the dashed line in Fig. 6, for the point $c_s = 13\;\text{mM}$. For the two other concentrations

| $c_z^\infty$ [mM] | $c_z^\ast$ [mM] | $a\rho_z$ (exp) | $a\rho_z$ (PB) |
|------------------|-----------------|----------------|----------------|
| 2                | $0 \pm 0.0003$ | 0.194 ± 0.020 | 0.186 ± 0.005 |
| 13               | 0.011 ± 0.002  | 0.191 ± 0.013 | 0.178 ± 0.002 |
| 23               | 0.031 ± 0.005  | 0.173 ± 0.025 | 0.172 ± 0.002 |
| 88               | 0.52 ± 0.05    | 0.135 ± 0.026 | 0.164 ± 0.002 |

**Counterion condensation**

We now turn to analyze the condensation of monovalent and multivalent ions around DNA. Each line in Table 1 provides a measurement of the excess $\rho_z$ at certain values of $c_z^\infty$ and $c_z^\ast$. The general relation $\rho_z(c_z^\infty, c_z^\ast)$ is a property of counterion density profiles around isolated DNA segments. Hence, the data in Table 1 can be used to test any particular theory used to calculate such ion distributions.

Deviations as in Fig. 7 are inevitable if any approximations are used to model the distribution of counterions around DNA. Note however that within such approximate models our general theoretical considerations should apply, as long as the total number of ions in the system is counted properly. Such a model that goes beyond PB was proposed in (Nguyen and Shklovskii [2001]). Indeed, within this model a linear relationship similar to Eq. [11] was found.
of 23 mM and 88 mM the effect will be negligible.

Further comments on underlying model assumption

Our underlying assumption, that the onset of aggregation depends uniquely on \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \) (but not on \( c_{\text{DNA}} \)), is an approximation that can be justified on several different levels but deserves further and more thorough investigation. The most simple motivation for this assumption is that multivalent ions, in the vicinity of the chains, mediate the attraction necessary for aggregation. In turn, the number of condensed multivalent ions near each chain is determined by \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \).

Let us first suppose that aggregation starts when a net attraction appears between two chains. This assumption may be justified if chains are sufficiently long and their translational entropy can be neglected. In order to find the onset of two-chain attraction the free energy of a two-chain complex should be calculated as a function of the distance between the two chains. This free energy represents the effective interaction between the two chains, mediated by the ionic solution. The counterion distribution near each chain will not be the same for close-by and for isolated chains. However in both cases the concentrations must decay to their bulk values throughout the solution, \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \). This requirement serves as a boundary condition, imposed at a large distance from the two chains. It will determine uniquely the counterion distribution between the chains, as well as the free energy associated with the two-chain complex. Hence \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \) determine the effective interaction between chains, and in particular whether an attraction occurs at a certain range of inter-chain separations; in terms of these variables the onset of two-chain attraction does not depend on \( c_{\text{DNA}} \).

Strictly speaking, the onset of aggregation and the onset of two-chain attraction are not the same. The aggregate phase involves interactions between multiple chains, whereas chains in the dilute phase interact very weakly with each other. Aggregation starts when the free energy per chain is equal in the dilute and aggregate phases. Note that the chemical potential of each ion species must be the same in the two phases, and that in the dilute phase these chemical potentials are directly related to \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \). Hence \( c_{1}^{\infty} \) and \( c_{2}^{\infty} \) determine the free energy per chain in the two phases. The approximation of independence on \( c_{\text{DNA}} \) neglects the translational entropy of DNA segments, which can be justified for long enough and rigid segments. It also neglects contributions from interactions between chains in the dilute phase, which are assumed to be small compared to the free energy of the single DNA-counterion complexes.

SUMMARY

We have shown that the onset of aggregation at finite (non-zero) DNA concentration, \( c_{z,\text{onset}} \), is determined by the onset in the limit of infinite DNA dilution. For DNA monomer concentration smaller than that of monovalent salt, \( c_{\text{DNA}} \lesssim c_{z} \), the multivalent counterion concentration at the onset, \( c_{z,\text{onset}} \), depends linearly on \( c_{\text{DNA}} \). The coefficients of this linear dependence are the bulk concentration of multivalent counterions and their excess relative to the bulk near each DNA segment. Both of these coefficients are of theoretical interest and can be extracted from the available experimental data.
Our main assumption is that the onset of aggregation can be related to the ion density profiles around each chain. Hence, it is uniquely determined by \( c^\infty_z \) and \( c^\infty_1 \), the bulk concentrations of the two counterion species, respectively. Our results and fit to experiment strongly support this assumption. Nevertheless, we believe that more detailed theoretical and experimental investigations are needed in order to fully understand its range of validity. For example, it will be of interest to test experimentally the equilibration of a DNA solution through a dialysis membrane, with a cell containing only count-erions (Braunlin et al., 1982; Plum and Bloomfield, 1988; Subirana and Vives, 1981). This procedure allows a direct control of the ionic bulk concentrations.

In order to predict precisely the onset of aggregation, the structure of the aggregated phase must be considered. Nevertheless, it is instructive to focus only on single chains at the onset, as is often done. At the aggregation onset the electrostatic repulsion between isolated chains in solution must be overcome by a sufficiently strong attraction mediated by multivalent counterions. This number of counterions is expected to depend only weakly on physical parameters such as the monovalent salt concentration. Our analysis does not address directly the question of the onset origin, but merely supports the fact that the number of condensed multivalent ions at the onset, \( a \rho^*_z \), is of the same order of magnitude, regardless of the \( c_z \) value. A more refined result of our analysis is that \( a \rho^*_z \) is not constant but decreases with increase of \( c_z \). On the other hand \( c^*_1 \), the value of \( c^\infty_1 \) at the onset, depends strongly on \( c_z \). This is mainly a result of the competition between monovalent and multivalent ions, as will be addressed in a separate publication.

Our analysis also sheds light on counterion condensation on DNA, which is independent on the criterion for DNA aggregation. The experimental data indicates that for high \( c_z \) the number of spermine ions in the vicinity of DNA is smaller than the prediction of Poisson-Boltzmann theory. A similar trend was observed in computer simulations (Lyubartsev and Nordenskiöld, 1997) of spermidine (3\(^+\)) and NaCl in contact with DNA. Spermidine binding was affected by addition of monovalent salt more strongly than the Poisson-Boltzmann prediction. For high salt concentrations spermidine binding was considerably smaller. In the computer simulations both molecular specific interactions, the geometrical shape of the constituents and inter-ion correlations were taken into account. All these effects, and in particular the geometry of the spermidine molecule, which is similar to that of spermine, were found to play an important role.

The above analysis demonstrates that specific interactions play an important role in determining the threshold of aggregation. In the dilute phase these interactions strongly influence the competition between monovalent and multivalent ions and the free energy of DNA-counterion complexes. Similarly, specific interactions play a prominent role in the dense phase (Strev et al., 1998). Force measurements under osmotic stress (Rau et al., 1984; Rau and Parsegian, 1992) provide a wealth of information on these interactions.

In conclusion, the physical parameters extracted here from experiment on the onset of DNA aggregation provide insight on the conditions required for aggregation, and on condensation of ions around DNA. These parameters may turn out to be of great value in assessment of various theoretical models. Additional detailed experiments may further deepen our understanding of these complex phenomena.

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**APPENDIX**

In this appendix we discuss the relation between the excess and the number of condensed ions. The latter quantity is not as well defined as the former, but relates more naturally to the aggregation mechanism. The notion of condensed ions suggests that some ions are bound to the charged chain while others are free. In reality there is a density profile that extends all the way from \( r = d \) to \( r = R \) with no definite separation between condensed and free ions. In the following we define condensed ions rather loosely as the number of ions up to a certain characteristic distance from the chain (Belloni et al., 1984; Wilson et al., 1980). We show that for multivalent ions this number does not depend strongly on the choice of this characteristic distance. Hence, the number of condensed ions is reasonably well defined. Moreover, the excess number of multivalent counterions, which can be directly calculated from the experimental data, is nearly identical to this quantity. This point will be further explained below.

Fig. 8 shows the excess of 4-valent counterions \( \delta \rho_4(r) \) up to a distance \( r \) from the DNA axis, as a function of \( r \):

\[
\delta \rho_4(r) = 2\pi \int_0^r r' \, dr' \left[ n_z(r') - c^\infty_z \right] \tag{A1}
\]

with the limit \( \delta \rho_4(\infty) = \rho_4 \) of Eq. [4]. The density profile was calculated using the Poisson-Boltzmann equation, with the radius of DNA taken as \( d = 10 \, \text{Å} \) and
with bulk densities of ions as in the last line of Table 1: \( c_s = c_1^\infty = 88 \text{ mM} \), \( c_z^\infty = 0.52 \text{ mM} \).

Three observations can be made. First, most but not all of the excess z-valent ions are localized very close to the DNA, at a distance of order \( \lambda/z \), where \( \lambda \) is the Gouy-Chapman length (see Andelman (1994)):

\[
\lambda = \frac{1}{2\pi B \sigma} = \frac{d}{l_B \rho_{\text{DNA}}}
\]

where \( \sigma \) is the average charge per unit area on the cylinder surface, \( \sigma = \rho_{\text{DNA}}/2\pi d \), and \( \rho_{\text{DNA}} = 1/a \) is the DNA charge per unit length. At room temperature the Bjerrum length \( l_B \simeq 7 \text{ Å} \) and for DNA with 4-valent counterions \( \lambda/z \approx 0.6 \text{ Å} \). Second, the counterions within a layer of few times the Debye length (\( \kappa^{-1} = 10.0 \text{ Å} \) in Fig. 8) neutralize the DNA charge. Nearly all the excess distribution is in this layer. Third, in order to estimate the total amount of counterions in the condensed layer of thickness \( \alpha \kappa^{-1} \), where \( \alpha \) is a number of order unity, we need to add \( \delta \rho_z \), to the bulk contribution, \( \pi \alpha^2 \kappa^{-2} c_z^\infty \).

Using \( \kappa \) from Eq. 4 the latter is equal to:

\[
\left( \frac{\alpha^2}{4l_B^2} \right) \frac{c_z^\infty}{2c_1^\infty + z(z+1)c_z^\infty}
\]

In experiment, \( c_z^\infty \) is much smaller than \( c_1^\infty \) at the onset, and the bulk contribution of Eq. A3 can be neglected relative to \( \rho_z \), for \( \alpha \) of order unity. This can be seen specifically in Fig. 8 by comparing the solid and dashed lines.

The outcome of the above discussion is that \( \rho_z \), defined in Eq. 4 as the excess of counterions throughout the cell, can be regarded, to a good approximation, as the total number of counterions within a condensation layer whose thickness is approximately the Debye length. For typical concentration ranges as considered here we do not expect that this outcome will change, even for models going beyond Poisson-Boltzmann theory.

As a further demonstration, the number of multivalent counterions up to several different distances from the DNA is shown in Table 3, as calculated in a unit cell using the Poisson-Boltzmann equation. For each \( c_s \) in Table 1 we find the Poisson-Boltzmann density profile such that \( c_1^\infty = c_s \) and \( \rho_z = \rho_z^* \), and then calculate the number of multivalent ions (per DNA monomer) up to the following distances from the DNA radius: 10 Å, 20 Å, \( \kappa^{-1} \) and 2\( \kappa^{-1} \). The values of \( \kappa^{-1} \), as obtained from Eq. 4, are equal to 68, 26, 20 and 10 Å for \( c_s = 2, 13, 23 \) and 88 mM, respectively. These numbers are compared with \( a\rho_z^* \). All the different measures in Table 3 yield results that are very close to each other.

### Table 3: Number of z-valent counterions, per DNA monomer, up to several different distances from the DNA axis, compared with \( a\rho_z \).

| \( c_s [\text{mM}] \) | d + 10 Å | d + 20 Å | d + \( \kappa^{-1} \) | d + 2\( \kappa^{-1} \) | \( a\rho_z^* \) |
|---------------------|---------|---------|-----------------|-----------------|----------------|
| 2                   | 0.191   | 0.193   | 0.194           | 0.194           | 0.194          |
| 13                  | 0.187   | 0.190   | 0.191           | 0.191           | 0.191          |
| 23                  | 0.171   | 0.172   | 0.172           | 0.173           | 0.173          |
| 88                  | 0.134   | 0.135   | 0.134           | 0.135           | 0.135          |

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