Coil-globule Coexistence and Compaction of DNA Chains

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Abstract In this work we discuss different factors governing coil-globule coexistence in the compaction process of DNA. We initially analyse the role played by fluctuations in the degree of binding of an external compacting agent in the conformational behavior of the chain backbone. The analysis relies both on Monte Carlo simulation results and simple statistical approaches. Compacting agents of various binding characteristics are taken into consideration and the degree of charge neutralization upon the chain is related to conformational indicators. Selected model systems comprising stiff chains in the presence of multivalent ions are employed to assess intrinsic single-chain conformational fluctuation, in the presence of external agents but not resulting from differences in binding. It is shown that trends found for a variety of compacting agents, including the extension of the coil-globule coexistence regions, can be rationalised on the basis of this analysis.

Key words DNA · coil-globule coexistence · compaction · condensation

1 Introduction

The folding of macromolecules has always attracted a considerable interest from a biological and physico-chemical point of view, much of it being related to the tight packaging of DNA in living cells or viral capsids.

Fluorescence microscopy is a technique that allows for the visualization of large single DNA molecules in solution. It has therefore been widely used to study the

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interactions between DNA and several co-solutes, such as polycations [1–3], surfactants [4, 5], catanionic vesicles [6], and metal ions [7, 8]. This technique has put in evidence a number of interesting aspects concerning these systems. One is the fact that highly charged cationic species and aggregates induce the collapse of the DNA molecules from an extended coil conformation to a compacted globular state. Intermediate states are not usually found and, for intermediate concentrations of the compaction agent, the two populations coexist in solution.

The compaction of DNA is driven by attractive interactions between different parts of the molecule due to electrostatic correlation effects arising from the presence of multivalent species [3, 11] or a solvent with a low dielectric constant [12].

It should be noted that, even though the concentrations of condensing agent required to compact DNA are usually very low, 10 μM of spermine for example, this corresponds to a large excess of positive charges when compared to the concentration of DNA nucleotides, 0.1 μM [2]. Furthermore, the polycation concentration required to compact DNA increases by an order of magnitude from spermine, with four charges, to spermidine, with three charges [2, 3]. In the presence of an excess of cationic species, both experimental [9] and simulation [10] results indicate the possibility of redissolution.

There is one other interesting aspect that has not, to our knowledge, been addressed before. Indeed, as mentioned above, the amount of spermidine required to start compacting DNA is much larger than that of spermine, and the coexistence region for the first system spans a much larger interval of concentrations. However, if we proceed to a normalisation by taking the ratio between the concentration for which the last coils compact and the one, C , at which the first globules are formed, we obtain similar values. The end of the coexistence region is achieved in both cases for 2.5 C . Furthermore, when other condensing agents are considered, the ratio does not vary significantly, being 1.3 for cobalt hexamine, a trivalent ion [8], 3 for cetyltrimethylammonium bromide, a cationic surfactant [5], and 5 for poly-(L-arginine), a polycation [1]. This is somewhat surprising since it is valid for such different species such as metal ions, surfactants and polymers, displaying different condensation and compacting mechanisms, and very different values of C .

Due to the interest in these systems, a number of simulation studies have been performed regarding the condensation or compaction of polyelectrolytes by addition of multivalent ions [3, 10, 13–19], or oppositely charged polyelectrolytes [15, 20–24]. Experimental trends have been, in general, successfully demonstrated. It was shown that, by increasing the valence of the counterions, the polyelectrolyte becomes more compact due to the accumulation of ions in the vicinity of the chain [3, 25], and that the chain is more compacted in the presence of multivalent ions than a corresponding Gaussian chain [25]. Accordingly, it was shown that multivalent ions of valence of at least three are typically required for the polyelectrolyte compaction [14]. Interestingly, it was also found that an excess of trivalent salt is required to attain the most compact and spherical-like structures [10].

When considering oppositely charged small polyions as condensing agents it was shown that these are not as efficient condensing agents as simple ions with the same valency [3]. However, polycations with a larger number of monomers, and with appropriate spatial distribution of charges, can induce compaction by promoting bridging between different sites of the negatively charged polymer. Larger polycations are therefore more efficient in compaction, and complete charge neutralization is not

required for obtaining an appreciable degree of compaction [21], in accordance with the experimental observations [1].

A number of models has been suggested to quantitatively understand the condensation phenomena (see [26] and [27] for a detailed account of such approaches). The Ψ -condensation was interpreted in the light of a coil-to-globule transition [28, 29], as observed in poor solvent for flexible chains. The approach is based on the extension of the Flory lattice model to accommodate one more term which includes a third virial coefficient. Therefore, when the potential between the molecule segments is attractive, the new term gives rise to a new minimum in the free energy, a collapsed state. However, the large persistence length of DNA implies orientational order [30], and coupling between the orientational and translational degrees of freedom [26].

Further developments have been suggested by Yoshikawa and co-workers [31, 32]. In a series of papers [2, 7, 8, 33–35] the free energy of the system has been described essentially by the sum of four different contributions: an elastic free energy of the chain, F_{ela} ; a free energy of interaction between the segments of the chain that takes into account the second and third virial coefficients, F_{int} ; the free energy corresponding to the translational entropy of small ions, such as the counterions and multivalent ions; and, finally, the electrostatic contribution. The last is often disregarded since it is claimed to be much less significant than the translational and elastic contributions. This theoretical treatment allows us to obtain free energy profiles for single chains and, on the basis of analysis of the competition between F_{ela} and F_{int} within the framework of mean field approximation, it is obvious that there are double minima in the free-energy profile of a sufficiently long polymer [7]. It is therefore relatively easy to describe a region of coexistence between coil and globule states that depends on a single intensive variable, associated with some measure of the dimension of the conformation. On the other hand, Bloomfield [36] has reviewed the various forces that are important in DNA condensation. The bending and mixing entropy are costly and resist condensation, and the dominant contributions arise accordingly from the fluctuating counterion electrostatics, or correlated fluctuations, and hydration forces.

Most of these contributions can be introduced and directly assessed in model systems currently used in coarse-grain simulations, especially when an effective attraction between the segments is replaced by the explicit presence of the compacting agent. However, few simulation studies involving condensing agents have suggested the presence of a coexistence region. We have observed, using a semi-flexible chain and trivalent ions, that both more extended structures and more compact ones were obtained for certain concentration range of the condensing agent [10], but without a clear separation between the two structures. Recently, Khan and Chan have showed double maxima in the end-to-end distribution function of stiff chains in the presence of tetravalent ions, corresponding to compacted toroid and extended structures [19]. The role of such structures in DNA nucleation has been experimentally analysed from a kinetic perspective [37].

In this work, we discuss some aspects related to coil-globule coexistence in solutions containing DNA and different compacting agents. We start by analysing the effect of binding fluctuations on the coil-globule coexistence, and illustrate it by Monte Carlo simulations and statistical calculations. Several condensing agents with different binding characteristics are considered and the main aspects of DNA compaction and coil-globule coexistence will be discussed in this light.

Conformational fluctuations are intrinsic to the behavior of polyelectrolytes in solution, even without considering variations in the degree of binding of an external agent. This type of fluctuation is also analysed, resorting to model systems consisting of tetravalent ions and stiff polyelectrolytes.

2 Simulation and Statistical Analysis

2.1 Simulation Aspects

The systems studied in this work were modelled using a coarse-grain approach. Each particle present is represented as a charged hard sphere interacting with every other particle through an electrostatic potential

$$U(r_{ij}) = \begin{cases} \infty, & r_{ij} < R_i + R_j \\ \frac{z_i z_j e^2}{4\pi \varepsilon_0 \varepsilon_r} \frac{1}{r_{ij}}, & r_{ij} \geq R_i + R_j \end{cases}, \quad (1)$$

where r_{ij} is the distance between particles i and j , R_i the radius of particle i , z_i the charge of particle i , ε_0 the dielectric permittivity of the vacuum, ε_r the relative dielectric permittivity of water at 298 K.

The chains are composed by a number of particles, N_{seg} , that are sequentially bound by a harmonic potential, U_{bond}

$$U_{bond,(i,i+1)} = \frac{k_{bond}}{2} (r_{i,i+1} - r_0)^2, \quad (2)$$

where k_{bond} is the force constant, r_0 is the reference bond length, and $r_{i,i+1}$ is the distance between segments i and $i + 1$. The chain particle components also interact through an angular potential U_{ang} ,

$$U_{ang,i} = \frac{k_{ang}}{2} (\alpha_i - \alpha_0)^2, \quad (3)$$

which defines the intrinsic chain stiffness. In Eq. 3, α_i is the angle between the vectors $\mathbf{r}_{i+1} - \mathbf{r}_i$ and $\mathbf{r}_i - \mathbf{r}_{i-1}$, with $\mathbf{r}_i$ denoting the position of segment i , k_{ang} the angular force and $\alpha_0 = 180^\circ$ the reference angle.

The systems are enclosed in a spherical cell with radius R_{cell} corresponding to a segment concentration of 1 mM. The amount of compacting agent is defined by the charge fraction β

$$\beta = \frac{Z_{comp}}{N_{seg} \times z_{seg}}, \quad (4)$$

where Z_{comp} is the total amount of charges in compacting agent elements present in a system. All systems are electroneutral and all counterions and coions to the polyelectrolyte are considered explicitly.

The simulations presented in this work involved two sets of systems corresponding to two different compacting agents. In one of these sets the compacting agent used is a 4:1 salt and, in the second set, chains, composed of $N_{seg} = 25$ particles, were used. In both cases, polyanion chains contained 100 segments, with negative elementary charge, $k_{bond} = 0.4 \text{ N m}^{-1}$, $k_{ang} = 1.7 \times 10^{-24} \text{ J deg}^{-2}$, in the presence of polycations, and $k_{ang} = 1.7 \times 10^{-23} \text{ J deg}^{-2}$, for a stiffer chain in the presence of tetravalent ions, yielding intrinsic persistence lengths, for the corresponding neutral chains, of 21 and 81 Å, respectively. $r_0 = 5 \text{ Å}$ for the polyanions and 5.6 Å for the polycations. All monovalent ions and segments are represented by hard spheres with a radius of 2 Å and tetravalent ions with a radius of 4 Å.

The solvent is considered as a continuum with a relative dielectric permittivity $\varepsilon = 78.4$.

The systems were solved by Monte Carlo simulation at 298 K, using the Molsim package [38]. Besides single particle moves, other trial moves (chain slithering, pivot rotation and chain translation) were used so as to produce a more efficient sampling of chain conformations. The efficiency of the pivot rotations and chain translations was also increased by using a cluster move technique [39]. In this case, all small ions within a distance 8 Å from any displaced segment were also subject to a displacement. The use of cluster moves is very important in these systems since the strong attraction between the polyanion and the surrounding multivalent ions would otherwise prevent an efficient sampling. Equilibration was performed with no less than 1×10^6 Monte Carlo (MC) steps for systems with tetravalent ions and 2×10^6 MC steps for systems with polycations; production runs involved 5×10^6 MC steps. One step encompasses N trial moves, where N is the total number of particles in the system.

2.2 Statistical Analysis

Simulation results are obtained for short polyanions with a contour length of $\approx 600 \text{ Å}$. We note that increasing the number of beads also results in increased computational effort. It is presently not feasible to approach the dimensions of DNA molecules consisting of a few kbase pairs. As such, to analyse the situation in longer chains and assess the degree of fluctuation in the binding of the compacting agent molecules or aggregates, we resort to a simple random distribution analysis. The chain ensemble is considered as a very large number of boxes into which compacting elements are randomly distributed. Every box has a maximum occupancy of N_c compacting elements, so that N_c multiplied by “the charge of a compacting agent”, z_+ , corresponds to the “total charge of the chain”.

The random assignment of elements to the chain is done by groups of “elementary charges” with a size corresponding to z_+ . For instance, when studying the condensation fluctuation of trivalent ions, each attribution involves three “elementary charges” at a time while in the condensation fluctuation of macroions with charge $z_+ = 60$, 60 charges are placed at the same time. During the process of assignment, if the addition of one more compacting agent exceeds the maximum occupation of the box to which it is being attributed, this element is not assigned and is considered as having stayed in bulk solution.

3 Results and Discussion

3.1 Fluctuations in the Binding of the Compacting Agent

In a previous work [21], it was shown that the degree of compaction of a polyelectrolyte, induced by shorter, oppositely-charged polyelectrolytes, depends on the size and number of the latter. It was observed that the compaction can occur abruptly with the addition of one more chain, if sufficiently long.

In view of this, it is easy to picture a model system consisting of two polyanion chains presenting a coexistence of extended and compacted structures, when the number of oppositely charged chains suffices to induce compaction in one of the backbones, but not the other. This is clearly shown in the snapshot of Figure 1. In this light, the coexistence region observed for DNA can be explained by a fluctuation in the concentration or number of compacting agents that are condensed in the polyelectrolyte chains (binding fluctuations).

Polycations bind strongly to the oppositely charged longer chain. It is difficult to induce exchange of polycations between the two chains. It is, however, possible to initiate different simulations so that we obtain different combinations. For four polycation chains we have three possibilities: four chains in a longer one (4,0), (3,1) and (2,2). Only the first combination, with a probability of ~ 0.125 results in coexistence, the other two corresponding to situations of fairly extended coils. For five chains, the probability of coexistence increases to 0.375, (5,0) and (4,1), and, for six, the probability is very high, 0.6875, and corresponds to (6,0), (5,1) and (4,2). A binomial distribution was used to obtain the probability values, assuming random assignment of the polycation chains.

Figure 1 Snapshot of a model system composed of two chains of 100 segments and six polycations of 25 segments each. One of the chains has condensed four polycations, while only two are condensed to the second chain.

Figure 2 Radius of gyration probability distributions for systems where the chain is in the presence of a different number of polycation chains. The total number of polycations, and the number of polycations condensed to each larger polyanion chain is indicated in each curve.

Some of the above situations are presented in the distributions of Figure 2. In the case for four polycations (3,1) none of the chains is compacted, although the differential condensation results in two slightly different conformations, with one chain displaying a very large electrostatic repulsion between segments. The addition of six polycations corresponds e.g. to the (3,3) situation, in which the number of condensed polycations is the same in both chains, which are still fairly extended. For seven chains, we have clear coexistence in the (4,3) result. Total compaction is found for eight chains (4,4). It is to note the resemblance between Figure 2 and the histograms of the long-axis length of DNA molecules often presented in connection with fluorescence microscopy experiments on DNA compaction [7]. However, it should be stressed that the latter is built from the direct observation of a larger number of chains, essentially kinetically trapped.

The compaction of the chain by using rather large polycations as compacting agents presents other aspects beyond what is observed by using small ions. The latter are much more efficient in achieving a large degree of compaction of the chain. The large dimension of the polycation is a variable that adds new aspects to the compaction mechanism. A very large polycation not only condenses extensively to the chain, due to the large overall charge, but also has the ability to bridge strongly distant regions of the chain and may introduce steric restrictions to chain movement. Direct inspection of the snapshots of the system shows that it is rather common to obtain a large overcharge of some chains with dangling ends of the polycation chains being maintained in the equilibrium “complexes.” Again, as shown above, DNA in solution complexed with a varying number of oppositely charged chains is a distinct possibility. It is unlikely that such a coexistence phenomenon may be associated with dynamical exchange of polycations, at least for a relevant timescale.

3.2 Long DNA Chains

Each polycation chain in the preceding example carries 25% of the charge of the anionic backbone, and so any fluctuation yields drastic conformational changes.

Naturally it is difficult to extrapolate the above results directly to more realistic situations in which longer chains and relatively fewer charged agents are involved. When, for example, spermidine is used as a compacting agent over a several kbase pair DNA chain, does the number of bound molecules vary in such a way that it may induce a coexistence behavior?

We have used the random attribution model described in Subsection 2.2 to estimate the degree of fluctuation of binding. We recall that an aspect of this simple model is that when a compacting agent is attributed to a chain that is already neutralized, the co-solute remains, nevertheless, in the vicinity of that chain and it is not attributed to a different one. This assumption contrasts somewhat with the strong binding induced in the long polycation systems, since these can overcharge the polyanion, but is typical of less charged agents. We validate this assumption by resorting to an independent algorithm [40], that is applied to a specific configuration from a Monte Carlo simulation of a two-chain system, of 50 beads each, with excess tetravalent counterions. The algorithm consists of a modified hierarchical cluster analysis, that allows both the identification of groups and objects not belonging to groups. It is seen that two groups are formed, but a number of ions are not assigned to either of the chains (Figure 3). This is also consistent with previous systematic discrimination between bound and bulk counterions [16], which is omnipresent when the concept of condensation is addressed.

Some results are depicted in Figure 4, corresponding to “condensing agents” of different charge, from $z_+ = +3$ to $z_+ = +60$, over 1,000 “oppositely charged chains” of 100,000 bases each, possessing a total charge corresponding to the same number of negative elementary charges. The dispersion in the charge of the chains is shown for different points, corresponding to various concentrations of the compacting agents. A similar result would be obtained resorting to a Poisson distribution, in which the number of bound elements correspond to events. The model starts to diverge from the Poisson distribution as the first chains become saturated.

It can also be seen in Figure 4 that, as the charge of the compacting agent is increased, the fluctuations in the number of elements attributed to a chain are larger. For systems where trivalent ions are added, the fluctuation in the condensed charge

Figure 3 Configuration represented in Cartesian space and used for the classification of the tetravalent ions into groups in a two-chain system via hierarchical cluster analysis. Symbols indicate two groups of bound ions (*circles* and *squares*), one for each chain, and a number of free ions ($\times$) that cannot be ascribed to either of the chains.

Figure 4 Dispersion in binding of charged elements to a long polyanion chain. The charge of these elements is indicated for each curve. Dispersion is given as one standard deviation.

on the chain is very small, with a range of approximately 1% of the total charge of the chain. Note also that for this case the fluctuations are essentially similar for the different concentrations of compacting agents. However, for highly charged compacting agents the binding fluctuation increases with the amount of condensing agents. This very large fluctuation is in agreement to the results obtained by simulation of the compaction of the chain by polycations. When the chain is considered to occupy only a part of the corresponding box, the amount of condensing agents necessary to obtain any degree of neutralization of the chain is naturally larger, but the fluctuations in charge are the same as in the previous case. An increase in the amount of agent necessary to compact DNA is characteristic of those that bind less strongly to the backbone than, for instance, long polycations. In the former case, an exchange of ions between the molecule and the bulk is expected. Due to the large amount of ions in solution it is perhaps counterintuitive that there could be binding fluctuations, that is, that different macromolecules experience a slightly different degree of condensation. What this random assignment model shows is that the fluctuation is small, but discernible.

In a previous communication [16] we have analysed how the fraction of condensed ions is related to the compaction of the chain. The decrease of the effective charge of the chain as more ions are condensed corresponds to a decrease of the average dimensions of the chain. The effect is not very pronounced for monovalent ions but increases significantly with the valence of the salt ions. For trivalent ions, normally said to possess the minimal charge necessary to compact DNA chains [41], the difference in the average dimensions of the chains is substantial when compared to the chain in the absence of salt and with a charge ratio $\beta = 1$. When the charge ratio is further increased from 1 to 3, the change in the average radius of gyration is not very significant, but the number of condensed ions is also not substantially altered.

It has been shown elsewhere [42] that the process of condensation of small ions (e.g., cobalt hexamine) to a chain presents two regimes. When a small amount of salt has been added (low charge fraction), the condensation is very efficient and the slope of the plot corresponding to the fraction of charge neutralized in the chain vs. the number of condensed ions is very steep. Once the average neutralized fraction

of the chain reaches $\sim 90\%$, the slope suddenly changes and it is almost invariant for larger amounts of added multivalent salt. That is, to increase, in certain situations, the neutralization of the chain from 90 to 95%, the value of β needs to be increased by a factor of 3. This explains why such an excess of positive species is required to compact DNA molecules, as observed in the fluorescence microscopy experiments. It is obvious that there is a substantial difference between the ion bulk concentration and the concentration in the vicinity of the DNA molecules resulting from ion condensation. We note that, at these higher degrees of neutralisation, electrostatic effects, including charge correlation, should be complemented by, e.g., van der Waals forces. These effects are not included, however, in our simple models.

Cationic surfactants, such as cetyltrimethylammonium bromide, present a somewhat different binding mechanism due to the amphiphilic nature of these molecules. As they have only one charge per molecule there will be an initial concentration where the surfactant molecules will replace the counterions to the polyanion but will have no effect on the conformation of the macromolecules. However, above a certain surfactant concentration, called the critical association concentration [43], micelles will be formed in the vicinity of the DNA, thus inducing the compaction of the macromolecules. Therefore, the compaction of DNA by cationic surfactants depends on the hydrophobicity of the amphiphile molecules, and the longer the hydrocarbon chain the less surfactant is required to compact DNA [5]. When the system evolves according to this double cooperativity mechanism, a small fluctuation may be amplified by the subsequent cooperative binding. For instance, when a sufficient number of surfactant molecules are aggregated to a polyion, this may induce an almost complete neutralization of the latter, due to the formation of larger micelles.

It was already mentioned that the normalisation of the coexistence region by taking the ratio between the concentration for which the last coils suffer compaction and the one at which the first globules appear, leads to similar values for very different agents and for coexistence regions characterized by very different concentrations of the agent. A possible explanation is the following: on one hand, large polycations or macroions, such as micelles, bind more strongly, and induce compaction by bridging different parts of DNA. However, the fluctuation in the number of bound species is large, which has the effect of enlarging the coexistence region. Also, there is a minute exchange with the bulk. On the other hand, less efficient compacting agents, such as trivalent ions, require a greater concentration to bind to DNA above a certain ratio of charge neutralization. The increase in the degree of neutralization with concentration is slow, and the mobility of ions is large. However, the fluctuations in terms of binding in these systems are smaller, which contributes to a relatively narrow coexistence region, probably also corresponding to an abrupt conformational change with the degree of charge neutralization.

3.3 Conformational Entropy

Simulations performed on a model system involving a rigid chain and tetravalent salt are used to focus on yet another contribution to a coexistence situation.

It is possible to extract a free energy profile from the probability distribution of some variable characterizing the chain conformational behavior, such as the radius of gyration

$$F/RT = -\ln[P(R_g)] \quad (5)$$

Figure 5 Free energy profiles for the chain in the presence of tetravalent ions.

where the symbols have their usual meaning. The profile can be subsequently used to assess the existence of barriers separating different conformations.

Although a detailed analysis of the degree of binding has shown no significant difference for compact and extended structures, such a system displays double maxima in the R_g probability distribution for certain charge ratios, originating from corresponding double minima in the free energy profile, as seen in Figure 5. The neutral chain free energy profile may be regarded as the chain “elastic” component of the free energy of the system since all characteristics of the chain, with the exception of the electrostatic interactions, are considered. It is observed that the region of the profile for extended conformations ($\beta = 0.8$) matches approximately the corresponding region in the profile for the neutral chain (see Figure 6a for an illustration of such conformations). However, a second minimum appears for $\beta = 0.8$, compatible with the existence of compact structures, separated from the random coil zone by a free energy barrier. These compact structures can be attributed, by

Figure 6 Snapshots of characteristic structures observed upon addition of tetravalent salt to the polymer system. **(a)** random coil conformation observed mainly in $\beta = 0.8$ system, **(b)** a hairpin conformation observed both in $\beta = 0.8$ and 1 and **(c)** toroidal conformation characteristic of the $\beta = 1$ system.

visual inspection of snapshots of the system, to hairpin conformations (Figure 6b). When β is further increased, the minimum at larger R_g becomes less pronounced and an increase in the populations for smaller R_g , corresponding to toroids (Figure 6c), is observed. These are clearly coexistence situations. Coexistence between random chains and compact structures ceases with the disappearance of the free energy maximum between compact and extended conformations, but a different barrier appears, separating two different kinds of compact structures, toroids and hairpins.

The barriers in the free energy profiles for certain concentration values of the compacting agent can be explained by the opposing action of the effective attraction of the multivalent ions and chain beads, and the chain conformational entropy. Coexistence is a phenomenon that may be achieved by controlling the chain and compaction agent properties. A very small flexible chain does not possess a coexistence region since the difference in the average dimensions of the compacted chain and the coil are not sufficiently different to determine a clear cut separation in the energy profiles. Instead, a smooth transition between extended and compact conformations is observed [10]. As the size of the chain or its intrinsic rigidity are increased, the value of R_g where the minimum in free energy for the reference neutral chain is located will naturally increase. By using the same compacting agent, the dimensions of the compacted chain will not depend significantly on the size of the chain [42, 44] and a separation between random coils and compact structures will be more clearly defined. This could also be observed experimentally using dynamic light scattering [45]. Note, however, that the efficiency of the compacting agent is also important for the control the width of the well corresponding to the “effective attraction” between chain segments, giving rise to structures with a varying degree of compaction (see [10] and [21]).

4 Conclusion

We have shown that fluctuations in the degree of binding of various types of compacting agents are responsible, at least partially, for the coexistence between DNA in the form of extended coils and compact globules. These fluctuations tend to increase with the charge of the compacting agent and are clearly significant for long polycations and large macroions. For less charged agents, binding fluctuations must be associated to abrupt conformational changes induced by changes in the degree of charge neutralization of the chain backbone. Also, this degree of charge neutralization tends to increase very slowly for higher concentrations of the compacting agent.

A polyelectrolyte in solution possesses, additionally, an intrinsic conformational fluctuation that does not derive from the action of an external agent. This variation in conformation also leads to coexistence when coils and globules significantly differ in size, being visible in simulation results of stiff chains in the presence of tetravalent ions. It is apparent that the separation between these two types of structures results from the conflicting action of the conformational entropy (elastic energy) and the effective Coulomb attraction involving ions and chain beads.

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