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How are small ions involved in the compaction of DNA molecules?

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Abstract

DNA is a genetic material found in all life on Earth. DNA is composed of four types of nucleotide subunits, and forms a double-helical one-dimensional polyelectrolyte chain. If we focus on the microscopic molecular structure, DNA is a rigid rod-like molecule. On the other hand, with coarse graining, a long-chain DNA exhibits fluctuating behavior over the whole molecule due to thermal fluctuation. Owe to its semiflexible nature, individual giant DNA molecule undergoes a large discrete transition in the higher-order structure. In this folding transition into a compact state, small ions in the solution have a critical effect, since DNA is highly charged. In the present article, we interpret the characteristic features of DNA compaction while paying special attention to the role of small ions, in relation to a variety of single-chain morphologies generated as a result of compaction.

Key words: DNA, folding transitions, single-chain conformations, salt effect, polyelectrolyte
PACS: 87.14.Gg, 87.15.By, 82.35.Rs, 64.70.Nd, 82.45.Gj

1. Introduction

Organisms that are amazingly well-integrated systems consist of various biopolymer molecules. The systems of such biopolymer molecules are known to exhibit a characteristic synaptic behavior even if they have rather simple compositions. For example, amphiphilic lipid molecules form micelles and lamellae of various shapes according to the physicochemical environment. Moreover, such a synaptic behavior is not an exclusive feature of an aggregate of polymer.

For example, one can see it in the process of protein folding. A new protein molecule is simply a linear heteropolymer without any functions. Once a protein is folded properly, it exhibits crucial activity to enable a complex biological function. Such a folding into a specific structure occurs naturally in cells. Thus, the folding of protein molecules has attracted considerable attention from biophysicists and physical chemists [1, 2]. A single protein molecule actually have huge dimension of state space, and it is influenced by endless thermal fluctuations in an aqueous solution. Nevertheless, its natural form is found with a rather

ordered structure. It seems important to understand this kind of phenomenon from the viewpoint of a collective phenomenon resulting from interactions with various small molecules existing in the environment.

Another example that is a main interest in the present paper is the folding of giant DNA, the genetic material in all life on Earth. The folding of DNA is a fascinating problem because this change in the structure of the molecule itself plays a significant role in the life cycle. For example, in a eukaryote cell, DNA usually forms a nucleosome (a complex with histone proteins) and the nucleosome forms a large-scale folded structure called a chromatin. This chromatin structure is uncoiled during the process of cell division for the purpose of replication. After replication, DNA is folded into a tightly compacted structure known as a chromosome in preparation for partition upon cell division.

As a simple model system in vitro, “DNA condensation” or the coil-globule transition has been studied extensively over the past several decades [3–7]. In polymer physics, it is well-known that polymeric chains condense through a multi-chain process with a change in the solvent quality from good to poor [8, 9]. Theoretically, the application of poor solvent conditions to an isolated chain results in folding of the single chain. For this transition at the level of an individual chain, the term “coil-globule transi-
"folding transition" is often used, instead of "DNA condensation", to emphasize the "individual chain". Since this transition can be well-reproduced with an experimental methodology of single-molecule observation [11], it has been studied as a physical model to understand the complicated behavior of DNA in vivo. In this paper, we describe recent developments in the study of this fascinating problem.

2. Coil-Globule Transition: DNA Compaction

In a dilute DNA solution, an individual long-chain DNA molecule undergoes a transition between an elongated, fluctuating coiled state and a compact folded globule state. Typical images of a coil and globule by fluorescent microscopy are shown in Fig. 1. Up to the past decade, it has been believed that this coil-globule transition is continuous [4, 10], due to a finite transition width obtained from, e.g., light scattering experiments.

Quite recently, fluorescence microscopy revealed the bimodality in the size distribution of individual DNA molecules [6]. Successive studies have come to establish the concept of a first-order phase transition, under the Landau's symmetry argument, in the single-chain conformation [11-16]. It is natural to expect that the ensemble average of a characteristic quantity changes continuously due to a gradual shift in the relative population between coils and globules.

As shown in Fig. 2, the conformation of an unfolded DNA chain is highly influenced by thermal fluctuation [18, 19]. Such a fluctuating conformation is well-described by the random walk model. Without segmental interactions, this model polymer is considered an ideal chain.

For a stiff chain like DNA, a single step in the random walk is not a monomer unit because of significant correlation with neighbors. The distance at which an orientational correlation disappears is called the persistence length, and this is regarded as a segmental unit.

Besides the persistence length, the Kuhn length is defined from the spatial size of an ideal chain, $R_0$, such as the end-to-end distance and the radius of gyration. $R_0$ has a scaling relation with the unit length per segment, $l$, as $<R>_0 \sim \sqrt{l}$, where $L = NL$ is the contour length of the whole chain, and $N$ is the number of segments. Thus, an experimentally measured coil size gives the Kuhn length: the effective segmental length. The Kuhn length of DNA is about 100 nm in physiological condition. Consequently, a DNA chain with a few dozen kilo base pairs, for example, consists of about 100 effective segments.

The conformation of a polymeric chain is influenced by the solution environment. The actual dimensions of the chain can be characterized by the expansion factor, $\alpha$, as $<R> = \alpha <R>_0$. The free energy of a single-chain polymer can be described as a function of the expansion factor as follows [in $k_B T$ units] (8, 9).

$$ F = \frac{3}{2} (\alpha^2 + \alpha^{-2}) + B N^{1/2} \alpha^{-3} + C \alpha^{-6} \quad (1) $$

where the first term represents an elastic entropy and the rests are virial expansion for dissected segments. When hard-core repulsion exists, the second virial coefficient, $B$, is greater than 0. Within the first-order estimation, there are two classes of solutions for $\alpha$ at the free energy minimum.
If \( N \gg B^{-2} \), \( \alpha \) is \( B^{1/3}N^{1/10} \) at the free energy minimum and the chain size, \( R \), scales to \( N^{3/5} \). This rough result corresponds to the long-chain limit of a self-avoiding random walk [9]. On the other hand, if \( N \ll B^{-2} \), \( \alpha \) is independent of \( N \) and the polymeric chain behaves as an ideal chain.

The coil-globule transition of polymeric chains generally occurs when the solvent quality is changed from good to poor. There are various ways to modify the solvent quality. For a DNA molecule, the following experimental parameters are known to induce the folding transition: the addition of a chemical agent such as a surfactant [13, 14], flexible polymer [3, 6], or multivalent counter-ions [15, 20]; an increase in temperature [16, 21]; and a decrease in the dielectric constant of the solvent [22]. In a general theoretical treatment of a polymer chain, the solvent quality is introduced by changing the second virial coefficient. If \( B \) becomes 0 as a result of a change in the solvent quality (called \( \theta \)-point), a polymeric chain follows the scaling behavior of an ideal chain.

When \( B \) decreases further and takes a enough negative value, the first-order estimation leads to three classes of possible stable solutions, 1. \((2C/|B|)^{1/3}N^{1/6} \) and \((2C)^{1/6} \). For a semiflexible polymer \(( C \ll 1 )\), the solution, \((2C)^{1/6} \), is not realized. In addition, within the range \( B^{-2} \gg N \gg B^{-2}(2C)^{1/2} \), the semiflexible polymer undergoes a first-order phase transition between \( \alpha = 1 \) and \( \alpha = (2C/|B|)^{1/3}N^{1/6} \). The scaling behaviors of the two free energy minima are \( R \sim N^{1/2} \) (coil) and \( R \sim N^{1/3} \) (condensate), respectively. Note that polymer folding is a first-order phase transition only if the polymeric chain is sufficiently stiff \(( C \ll 1 )\) and long \(( N \gg 1 )\). Otherwise, the potential barrier between the two phases becomes lower than the thermal energy, and the distribution of individual chains becomes continuous. As for more discussion on the mean-field treatment on the semiflexible polymer, see [23].

The scaling relation for the condensate, \( R \sim N^{1/3} \), indicates that the condensate has a densely packed morphology. In fact, the volume ratio of coil to globule for T4 DNA (166 kbp) is on the order of \( 10^4 \)-\( 10^5 \), and the estimated radius of the globule indicates that it has a very densely packed structure. However, the structure of such a globule is not necessary to be amorphous, or liquid-like, as assumed in the above framework of coil-globule transition. Experimentally, a toroidal and rod-like T4 DNA globules have been observed [4, 7, 24, 25]. Figure 3 shows photographs of these folded structures by electron microscopy. A characteristic difference between an amorphous globule and other forms is the appearance of parallel ordering. In this sense, the toroidal and rod-like condensates are considered to have a crystalline structure. On the other hand, a real-time observation of plasmid DNA-polymer condensates indicated that these two conformations are not necessarily in a solid state [27].

In studies on polymer folding, computer simulation is a powerful tool for investigating all possible structures that appear as thermodynamically stable conformations. For example, the appearance of a disk-like globule is predicted for a short-chain semiflexible polymer [28]. Figure 4 shows a schematic phase diagram of a single polymeric chain, deduced from multi-canonical Monte Carlo simulations [29]. The spherical globule is stabilized thermodynamically when the attractive interaction overwhelms the bending energy in simulations as well as in actual experiments [30]. On the other hand, since a deep potential well leads to rapid folding under large nonequilibrium, it is possible that the observed spherical morphology of T4 DNA is a kind of frozen structure kinetically trapped.

In addition, the effect of the helicity or supercoiling [31-34] of double-stranded DNA was tested for a circular chain where the total helicity is topologically preserved [35, 36]. As a more adequate model for biological systems, the formation of a nucleosome [37-39] and chromatin [40] has also been studied by computer simulations. In fact, the nucle-
osome structures seem to be governed by a little bit complicated topological effect \cite{41,42}, and the investigation of equilibrium would require an excellent simulation study.

The histone protein which forms a nucleosome complex with DNA is a positively charged polymer with a charge opposite to that of DNA, and the formation of a nucleosome arises from the electrostatic interaction between DNA and histones \cite{43,44}. Since a DNA molecule carries a large number of electric charges, ionic species in solution greatly influence the behavior of DNA. In the following section, we overview the characteristics of DNA compaction with regard to ionic species based on the result of single chain observation, and discuss the underlying physical model.

3. Effects of ionic species in DNA compaction

Small ionic species affect DNA compaction in a rather complex manner. Figure 5 shows two contrasting phase diagrams for a monomolecular DNA chain. As seen in Fig. 5(a), monovalent salt and nonionic flexible polymer work complementarily to fold DNA. On the other hand, Fig. 5(b) indicates that the monovalent salt interferes with the folding transition induced by multivalent counter-ions. These apparently opposite behaviors can be explained as follows by considering the nature of the counter-ions.

First, the mechanism of DNA folding induced by a flexible polymer is explained by the so-called depletion force, i.e., entropic attraction between giant molecules. Compared to a DNA molecule, a flexible polymer behaves as a relatively small colloidal particle. When giant molecular structures approach each other, their exclusive volumes overlap, and this leads to an entropic advantage because of the additional free volume for the flexible polymer. However, this attractive force competes with electrostatic repulsion between the DNA segments.

The effect of salt in this process can be introduced \cite{45,46} simply by adding the electrostatic self-energy of the polyelectrolyte ball and the difference in the translational entropy of salt ions between the bulk and the ball. In a dilute monovalent salt solution, these terms would roughly obey the following scaling relation \cite{46}:

\[
F_{\text{ele}} \sim \frac{N^{3/2}(1-f)^2}{\alpha} \tag{2}
\]

\[
F_{\text{tra}} \sim N|f| \ln \left( A \frac{|f|}{N^{1/2} \alpha c_0} \right) \tag{3}
\]

where \(N\) is the number of segments, \(f\) is the fractional neutralization of the polyelectrolyte charge, \(A\) is a numerical constant, and \(c_0\) is the monovalent salt concentration in the bulk. The value of \(f\) is determined by competition between the two terms. In principle, the compaction of a polyelectrolyte chain requires significant charge neutralization of a polyelectrolyte chain. This neutralization can be accomplished more easily when \(c_0\) increases. This is why the addition of a monovalent salt results in the compaction of DNA.

In contrast to a nonionic flexible polymer, the compaction induced by multivalent cations occurs through the mediation of DNA segments. It is difficult to formulate this type of compaction precisely because the effective attraction between polyelectrolyte segments is now a function of the concentrations of all ionic species. Like-charge attraction mediated by counter-ions has been extensively studied \cite{47-55}. The detailed discussion of this problem is found in other literatures \cite{56,57}.

The inhibitory effect of a monovalent salt in DNA condensation can be effectively explained \cite{16,21} with a two-state model, instead of continuous model. Such a treatment is rationalized by the fact that the change in single-chain conformation between the unfolded and folded structures is generally discrete as discussed in the previous section. When the folding transition occurs, the ionic struc-
ture of the chain changes dramatically during the folding process [38]. Such a dynamical change is also supported by some simulation studies [59, 60]. In an aqueous solution, a highly-charged polyelectrolyte like DNA usually carries lots of counter-ions by the effect of counter-ion condensation [61, 62]. Since an ionic folding agent also acts as a counter-ion, the addition of a folding agent results in competition between the folding agent and monovalent counter-ions on the polyelectrolyte chain.

Figure 6 shows a schematic representation of the ionic structure of a polyelectrolyte chain in the folded and unfolded states. The equilibrium between the two states is controlled by competition between multivalent and monovalent cations. When monovalent cations are added, they try to replace the folding agents on DNA entropically. This ion-exchange stabilizes the unfolded DNA chain. In fact, this competition is influenced by short-range interactions and molecular detail of salt ions [63]. It leads us to consider the role of co-ions in the folding phenomenon.

Generally, the addition of a monovalent salt increases monovalent anions. Some of these co-ions should electrostatically stick to the counter-ions condensed on the polyelectrolyte chain. Such binding should be advantageous with regard to enthalpy and disadvantageous with regard to entropy for an unfolded polyelectrolyte chain. For a compact form, these co-ions should be replaced by each segment of the polyelectrolyte chain itself. Thus, it is anticipated that an increase in monovalent co-ions should reduce the entropic disadvantage of the dispersed structure, and induce unfolding of the DNA chain.

Such an ionic process should simultaneously explain the non-intuitive thermodynamic behavior of DNA folding induced by multivalent counter-ions. In a system where two phases coexist, the heat is consumed by both the phase transition and the rise in temperature. For coil-globule transition, direct observation of the ensemble of DNA chains enables us to obtain the net difference in the Gibbs potential between the two phases by calculating the logarithm of the ratio of the two phases. The latent heat, i.e., the difference in enthalpy can be obtained by studying the temperature dependence of the histogram of coils and globules [16, 21]. The obtained latent heat that accompanied the transition from coil to globule had a negative value. In other words, the formation of the globule was caused by an advantage in entropy. Thus, the ordered compact state of a globule has greater entropy than the disordered fluctuating state of a coil.

This result can be associated with the release of monovalent counter-ions accompanied with the folding transition. In addition, the behavior of monovalent co-ions should play a key role in this thermodynamic behavior. The electrostatic binding of monovalent co-ions to condensed counter-ions on an unfolded DNA chain gives the unfolded state an advantage in enthalpy and a disadvantage in entropy. In the compact state, DNA itself would replace these co-ions, and their release into the solution should bring an entropic advantage to the folded DNA chain.

While many simulations of polyelectrolytes examining the explicit behavior of counter-ions simultaneously includes co-ions explicitly [60, 64], the effect of co-ions in the folding phenomena has not necessary been shown clearly. At present, the role of co-ions seems to be emphasized...
only in a charge inversion phenomenon [65–68]. In order to construct an appropriate thermodynamics of the folding transition of a polyelectrolyte chain, an analysis from the perspective of the co-ion distribution would be required (See also discussion about a protein binding on DNA in Ref. [56]).

Finally, the term “DNA condensation” includes two types of phenomena: single-chain folding and aggregation (precipitation) as a multi-chain process. The physicochemical nature of aggregation [69–72] is similar to that of folding, and electrostatic effects should also strongly influence the aggregation of polyelectrolyte chains. If we ignore the electrostatic effect, it leads to the inaccurate interpretation that single-chain folding can be observed only in an extremely dilute DNA solution.

For example, consider a lattice model where lattice points are spaced by the Kuhn length, $l (\approx 100\text{nm})$. The toroidal condensation can be modeled as a shuttling between two neighboring lattice points. The decrease in entropy accompanied with folding the ideal chain into the toroidal condensate is given by $-N \ln 6$ where $N$ is the number of segments. For T4 DNA, $N$ is approximately 570. Next, we describe the energetic gain per segment inside the toroid as $\mu_s$. The energetic gain by the segment on the surface can be estimated as a half of $\mu_s$. The number of segments on the surface of a toroid is approximately $4\pi \sqrt{285}/\pi \approx 120$. Thus, the total energetic gain to form toroid is $51\mu_s$.

In the region of coexistence of coils and globules, the loss of entropy balances the energetic gain, and it leads to the value of $\mu_s$ to be $-2.0k_B T$. In order to derive a necessary condition for globules not to assemble each other, we consider the concentration of DNA at which the loss of entropy accompanied with the assemblage balances the corresponding energetic gain. The energetic gain by the coalition of two toroids arises from the binding between segments on the surface. We estimate that a quarter of the surface (30 segments per toroid) contributes to the coalition of two toroids. Thus, the energetic gain is $30\mu_s$. On the other hand, the loss of entropy by this coalition takes the form $-\ln(V/F^2)$, where $V$ is the inverse of the chain concentration. If the two terms balance each other, $V$ is the order of $10^{26}F^2$. In other words, the average distance of neighboring chains is about $5 \times 10^7$ times the gyration radius of the ideal chain. Within this framework, the coil-globule transition at the level of the single chain can be observed only in a more dilute solution than this extremely small concentration. DNA assemblage (aggregation) does not occur even at a DNA concentration much greater than this value without an excess addition of condensing agent.

Folding and an aggregation need neutralization of remaining charge on DNA. In single-chain folding, the chain is rolled up from its end. Thus, cascade charge neutralization is sufficient to forward the transition process. Such cascade neutralization proceeds rapidly across successive small potential barriers. On the other hand, in an aggregation of globules, most surface charges have to be neutralized at once. This kind of large fluctuation requires a rather high density of cations to appear with a realistic frequency. Thus, aggregation rarely occurs at a dilute salt concentration due to a large potential barrier. In fact, in an actual experiment, the clustering of globules is not observed when they collide with each other as the result of Brownian motion. Moreover, it has been confirmed that, the stable cluster formation of aggregates does not occur even if two globules are held in contact with each other using laser tweezers [73]. After the laser irradiation is turned off, the globules diffuse apart.

4. Role of ionic species in intra-chain phase segregations

Recently, it has been revealed that the so-called pearl-necklace structure appears in a single polyelectrolyte chain in experiments on DNA [74–76], synthesized polyelectrolytes [77], and simulations of single-chain polyelectrolytes [78–82]. In general, these structures consist of several densely packed structures linked by fluctuating elongated parts. To explain the appearance of this structure, one possible mechanism was proposed on the basis of Rayleigh instability for a charged colloidal material [83]. When charged materials associate and grow to a larger colloidal ball, the electrostatic energy grows in scale to $N^{2/3}$, where $N$ is the number of segments that form the polyelectrolyte ball. When the interaction energy of the condensing force scales to $N$, the growth of condensates is terminated at a certain point where the condensing force balances the electrostatic repulsion.

Usually, the solution condition in DNA experiments is not salt-free, and thus charge neutralization by counter-ions cannot be ignored. Moreover, the ratio of charge neutralization can shift according to the structure of the polyelectrolytes. This ratio should be determined by electroosmosis, and directly influences the instability of charged condensates. For example, a theoretical analysis based on the Poisson-Boltzmann equation suggested that there is a critical Bjerrum length above which the equilibrium size of a charged droplet goes to infinity due to charge neutralization by counter-ions [84].

The effect above was discussed in terms of the scaling property in the condensation of a semiflexible polyelectrolyte chain [46]. At a realistic salt concentration, charge neutralization by counter-ions significantly suppresses the increase in electrostatic energy. Here, $N$ is the number of segments in a folded region, and $f$ is the fractional neutralization of the polyelectrolyte charge. In a large condensate, the electrostatic interaction dominates the first-order behavior of the system. Consequently, the charge of the condensate is almost neutralized by its counter-ions as $f \approx 1$. Interestingly, the resultant electrostatic energy behaves as a second-order term in the total free energy. On the other hand, the translational entropy of salt in the folded region scales to $N f$. This term is divided into the first-order term ($\sim N$) and the second-order term ($\sim N(1 - f)$).
The second-order term of the translational entropy balances the electrostatic contribution, which leads to the following scaling equation:

\[ N^{5/3}(1 - f)^2 \sim N(1 - f) \]  

The fractional neutralization scales as \( (1 - f) \sim N^{-2/3} \). The electrostatic self-energy then "increases" more slowly than the mass of the folded part. Thus, the electrostatic term acts as a potential barrier between the folded and unfolded states. On the other hand, the "decrease" in the osmotic term of salt is slow, as with the electrostatic term. In this case, the osmotic term acts as the potential well in contrast to the electrostatic term. At a lower salt solution, the osmotic term becomes dominant compared to the electrostatic term. Consequently, the intermediate state (partially folded chain) appears as a stable state. In addition, this osmotic instability occurs more easily at a higher temperature, as opposed to the result from simple Rayleigh instability.

The morphology of a folded part is another factor in the scaling behavior. From the experiment with T4 DNA, a rings-on-string structure was observed as a segregated chain (Fig. 7). In general, the size of a torus is characterized by two parameters: the radius, \( R \), and thickness, \( r \). In a densely packed torus, these two parameters have a scaling relation as \( r^2 R \sim N \), and are not determined uniquely. Thus, even if neutralization by counter-ions is ignored, electrostatic instability can be inhibited by transformation of the torus. For example, a balance equation between the surface and electrostatic energies of the torus was proposed [85]:

\[ \sqrt{N} R \sim N^2 / R \]  

This relation results in the relation, \( R \sim N \), and consequently, both the surface and electrostatic energies become linear with regard to the torus mass. If the bending energy of the chain acts as a second-order term, its scaling behavior is determined from the above scaling relation:

\[ E_{\text{bend}} \sim N/R^2 \sim 1/N \]  

Within this framework, a single torus is the most stable conformation. Nevertheless, the appearance of a multi-tori structure is anticipated [76] if the entropy arising from the variety of the multi-tori is taken into account.

Finally, a low salt concentration was premised through the above discussions. Interestingly, the opposite case was also found in actual experiments [75] on DNA folding induced by multivalent cations. Intra-chain phase segregation in a dense salt solution can be difficult to understand because the size effect of electrostatic interaction is hardly expected due to a significant electrostatic screening effect. Moreover, segregation does not occur at all at a low salt condition where the size effect of electrostatic interaction should be strong.

This intra-chain phase segregation may be associated with the critical state of a polyelectrolyte chain. In a low-salt solution, the elongated chain segments would electrostatically repulse each other. On the other hand, this electrostatic repulsion is significantly screened in a high-salt solution. Thus, the presence of salt lowers the free energy barrier between the elongated and folded states, in terms of thermodynamics. In this sense, a polyelectrolyte chain in a high-salt solution may be regarded as being near the critical state. In such a case, hidden nonlinearity becomes important in the folding process.

5. Summary

A double-stranded DNA chain is a stiff molecule at an atomic scale. However, if it is sufficiently long, it appears to be flexible because of the accumulation of thermal fluctuation. Thus, a DNA chain in an aqueous solution shows an elongated, randomly fluctuating conformation. According to the solution environment, this fluctuating chain is folded into a compact state in a discrete transition. This compact structure can be classified into several conformations. In particular, the appearance of parallel ordering is quite remarkable in this phenomenon, and this enables us to regard the transition as a first-order phase transition between a gaseous state and a crystalline solid state. Moreover, a computer simulation of a single semiflexible chain revealed that such various phases in DNA can be derived from a very simple physical mechanism.

However, to observe a physicochemical property of an actual process, it is important that we understand the physical details. In particular, the role of counter-ions and co-ions has attracted increasing attention in the study of single DNA chains since their effect is not monotonic. For example, a monovalent salt assists neutral polymers in folding DNA. In contrast, in the presence of multivalent cations, the addition of monovalent salt induces the unfolding of a DNA chain. These apparently opposite results can be ex-
plained by considering the change in the association equilibrium of salt ions on polyelectrolyte chains. Moreover, such an ionic effect can effectively explain both the characteristic temperature-dependence of the transition degree, and the stability of a single folded chain in a semi-dilute DNA solution.

The most fascinating structure in a single-chain morphology is the pearl-necklace/multi-tori structure. The scaling argument plays a critical role in understanding this phenomenon, and particularly in association with the electrostatic instability known as Rayleigh instability. It is notable that the presence of salt ions changes the framework of Rayleigh instability. Likewise, the distribution of small ionic species would be a fundamental factor in understanding the intra-chain phase segregation of a polyelectrolyte chain. The study of a single-chain conformation actually represents a new field of research. In addition, the complex coupling of several components tends to give unpredictable results. A further variety of higher-order structures of a single-chain polyelectrolyte may be still hidden beyond our present knowledge.

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