Journal of the Iranian Chemical Society, Vol. 3, No. 2, June 2006, pp. 161-167.

JOURNAL OF THE
Iranian
Chemical Society

Nanoclusteration of DNA with Dendronized Polymer in Terms of Free Energy

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(Received 8 October 2005, Accepted 15 February 2006)

A thermodynamic cycle for the nanoclusteration between DNA and dendronized polymer is proposed and electrostatic free energies of a series of DNA- dendronized polymer nanoclusteration processes are calculated. The free energies for assembling fixed charges and mobile ions, and the bending energies of the DNA chain wrapped around the dendronized polymer are taken into consideration. The free energies of nanoclusteration are calculated for a number of nanoclusters with different conformations at constant temperature and constant ionic strength. The effects of temperature and ionic strength on the free energy of nanoclusteration and stability of their conformations are also investigated.

Keywords: Nanoclusteration, Dendronized polymer, DNA, Free energy, Nano-cylinder, Gene therapy

INTRODUCTION

The investigation of nanoclusteration between flexible chain DNA with surface negative charges and macro-ions with opposite charges is an important subject in nanobiotechnology and genetic science [1]. There has been great interest in the aggregation of oppositely charged macro-ions in solution, experimentally [2]. For example, the nanoclusteration of DNA with oppositely charged liposomes has been intensely studied in the context of gene therapy [1-3]. DNA delivery has also been pivotal in developing new approaches (*e.g.*, gene therapy and DNA vaccination) for treating and controlling diseases that are likely to impact clinical medicine and nanobiotechnology over the next few years.

The main problem is to introduce DNA with negative surface charges into the living cell which has a negatively charged membrane of lipoprotein. One approach pioneered by Felgner and Ringold [4] relies on associating anionic nucleic acids and cationic lipid liposomes, at which point the neutralized DNA-lipid complex can approach the negatively charged phospholipid membrane. In addition, there are a number of synthetic vectors, including polylysins, cationic polymers and, recently, dendrimers, which have been used for this purpose [5-7].

Dendrimers are a new class of synthetic, highly branched polymers, which have a structural advantage for gene transfer. These molecules are uniform in size with a high density of charged primary amino groups restricted to the surface and are highly soluble and stable in aqueous solution. Recent studies have shown that starburst dendrimers are non-immunogenic and can mediate the enhanced delivery of diverse nucleic acids, including single-stranded, double-stranded, natural, or synthetic DNA or RNA [8,9].

Despite of extensive theoretical and experimental research

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on the polyelectrolyte (PE) solutions [10], DNA solutions are relatively poorly understood compared to their importance. To simulate DNA-dendrimer nanoclusters, which are flexible polyelectrolyte chain systems, the effects of bending, twisting and the electrostatic potential of DNA in solution have to be considered. The aspects of bending, twisting and stretching under different ranges of forces (*i.e*. elastic DNA conformation) [11,12] have been analyzed for both closed circular and open linear configurations by using Lagrangian mechanics [13-16], numerical molecular dynamics [17] and statistical mechanics [18,19].

The electrostatic potential models are mainly based on the Poisson-Boltzmann (PB) equation [10]. The density of charges is applied and electrostatic potentials are calculated by solving the linear or nonlinear differential PB equation [20].

In our previous works [21-26], the electrostatic potential and then electrostatic free energies, entropic free energies and distribution of counter ions were determined for a series of DNA-dendronized polymer nanoclusters with different DNA pitches by application of the finite difference numerical method and solving the nonlinear PB equation at various ionic strengths and different temperatures. It was shown that in a series of conformations at constant ionic strength, the electrostatic free energies and entropic free energies primarily decrease sharply and then smoothly increase with the increase in DNA pitch size. It was also shown that, with the decrease in the ionic strength, electrostatic free energies and entropic free energies will decrease and the conformation with the least electrostatic free energy corresponds to the smallest DNA pitch. The effect of temperature on the most stable conformation of DNA-dendronized polymer nanocluster was also investigated.

In the present work we propose a thermodynamic cycle for the calculation of electrostatic free energies for the process of nanoclusteration between DNA and dendronized polymer at five different ionic strengths, 0.005, 0.05, 0.1, 0.15, and 0.2 M, at 298.15 K. The electrostatic effects of DNA, dendronized polymer and DNA-dendronized polymer nanocluster are modeled and the elastic effects were investigated by considering the bending and twisting aspects of DNA.

Theory and Method

The free energy of nanoclusteration, ΔG_{NC}, is produced by two major effects, known as electrostatic and elastic effects, which have to be modeled and used in the nanoclusteration process.

Electrostatic model. The electrostatic potential of the nanocluster system can be modeled by the nonlinear PB equation, which is derived from the Poisson equation [10].

$$\nabla \cdot \varepsilon(\bar{r}) \nabla \phi(\bar{r}) = \frac{-e}{kT} \left\{ \sum_j q_j^{f-} \delta(r - r_j) + \sum_i q_i^{f+} \delta(r - r_i) \right\} + \varepsilon \kappa^2 \mathrm{Sinh} \phi(\bar{r}) \tag{1}$$

where ε is the dielectric constant (= $\varepsilon_r \varepsilon_u = 78 \times 8.854 \times 10^{-12}$ C^2 m^{-2} N^{-1} (in water) and Ψ (volt) and $\phi = \Psi e/kT$ are the electrostatic potential and dimensionless electrostatic potential, respectively. At any point in the system, q_i^{f+} and q_j^{f-} are positive and negative fixed charges, respectively. κ is the modified Debye-Hückel parameter, $\kappa^2 = 1/\lambda^2 = 2e^2I/\varepsilon kT$, λ is the Debye length, I is the ionic strength of the bulk solution and $\bar{r}$ (m) is the position vector of the charges. The analytical solution of this equation is only possible for simple and symmetrical systems. Since the DNA-dendronized polymer nanocluster is a complicated system and the analytical solution of the PB equation is impossible, the finite difference method is used to obtain the electrostatic potential of system [21].

Electrostatic free energies, which are produced in the process of nanoclusteration, result from the electrostatic potential [27-28] of the DNA, dendronized polymer and DNA-dendronized polymer nanoclusters.

Elastic model. The first elastic model for DNA supposes that the molecule is a slender cylindrical elastic rod [29] that is parameterized by arc length, denoted by *s*. We describe the rod by relating its local coordinate frame L to its frame L_0 rigidly embedded in the curve in its relaxed configuration at each point of the arc length. The configurations are specified by each cross-section, described by a frame of three orthonormal unit vectors {**u**(s), **n**(s), **t**(s)} along the chain, where **t**(s) is the unit tangent vector determining the shape of the backbone, **u**(s) is a perpendicular vector of **t**(s) related to the bending of the chain, and **n**(s) = **t**(s) × **u**(s) is a unit vector that keeps track of the twist. The relationship between local frames is specified by the Eüler angles α(s), β(s) and γ(s), which are needed to rotate L_0 into L [30].

$$\mathbf{u}(s) = (-\mathrm{Sin}\gamma\,\mathrm{Cos}\beta\,\mathrm{Cos}\alpha - \mathrm{Cos}\gamma\mathrm{Sin}\alpha)\,\mathbf{i} + (-\mathrm{Sin}\gamma\,\mathrm{Cos}\beta\,\mathrm{Sin}\alpha + \mathrm{Cos}\gamma\,\mathrm{Cos}\alpha)\,\mathbf{j} + (\mathrm{Sin}\gamma\mathrm{Sin}\beta)\,\mathbf{k} \quad (2)$$

$$\mathbf{n}(s) = (\mathrm{Cos}\,\gamma\,\mathrm{Cos}\beta\,\mathrm{Cos}\alpha - \mathrm{Sin}\gamma\,\mathrm{Sin}\alpha)\,\mathbf{i} + (\mathrm{Cos}\,\gamma\,\mathrm{Cos}\beta\,\mathrm{Sin}\alpha + \mathrm{Sin}\,\gamma\,\mathrm{Cos}\alpha)\,\mathbf{j} + (-\mathrm{Cos}\gamma\,\mathrm{Sin}\beta)\,\mathbf{k} \quad (3)$$

$$\mathbf{t}(s) = (\mathrm{Sin}\beta\,\mathrm{Cos}\alpha)\,\mathbf{i} + (\mathrm{Sin}\beta\,\mathrm{Sin}\alpha)\,\mathbf{j} + (\mathrm{Cos}\beta)\,\mathbf{k} \quad (4)$$

The elastic constants of bending and torsional stiffness are denoted by A and C, respectively. The bending and twisting free energies for a rod with the length ℓ have been defined [29] by:

$$\Delta G_{elas} = \Delta G_{bend} + \Delta G_{twis} = \frac{A}{2}\int_0^{\ell} \frac{\partial \mathbf{t}}{\partial s}\cdot\frac{\partial \mathbf{t}}{\partial s}\,ds + \frac{C}{2}\int_0^{\ell} [(\mathbf{n}\times\frac{\partial \mathbf{n}}{\partial s})\cdot\mathbf{t}]^2\,ds \quad (5)$$

By introducing Eqs. (2) and (3) into Eq. (5) the elastic energy is expressed by Eüler angles

$$\Delta G_{elas} = \frac{A}{2}\int_0^{\ell}\left[(\frac{\partial\alpha}{\partial s})^2 Sin^2(\beta) + \left(\frac{\partial\beta}{\partial s}\right)^2\right]ds + \frac{C}{2}\int_0^{\ell}\left[\frac{\partial\alpha}{\partial s}Cos(\beta) + \left(\frac{\partial\gamma}{\partial s}\right)\right]^2 ds \quad (6)$$

The nanocluster is formed by wrapping DNA around the cylindrical dendronized polymer (Fig. 1) and the DNA's configuration is then described by the helical space curve, which is parameterized [31] as:

$$\mathbf{r}(s) = \begin{pmatrix} RCos(\frac{2\pi s}{\eta\sigma}) \\ RSin(\frac{2\pi s}{\eta\sigma}) \\ \frac{s}{\eta} \end{pmatrix} \quad (7)$$

where R is the radius of wrapping, η is the length of DNA per unit length of dendronized polymer, and σ is the height per turn. The **t**(s) is expressed by derivation of **r**(s) with respect to *s*. The Eüler angles which are obtained by using Eqs. (2), (3), and (4), are shown by

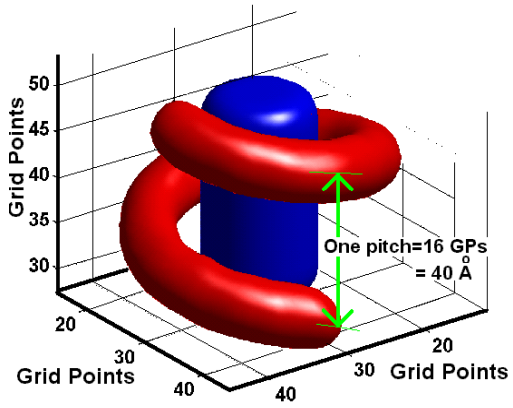


Fig. 1. DNA wraps around the dendronized polymer resulting in 40 *Å* pitch (single helix characterized by the height per turn). On the distance axis any number must be multiplied by 2.5.

$$\alpha = k\pi + \frac{2\pi s}{\eta\sigma} \quad (8)$$

$$\beta = ArcCos\frac{1}{\eta} \quad (9)$$

$$\gamma = k\pi \quad (10)$$

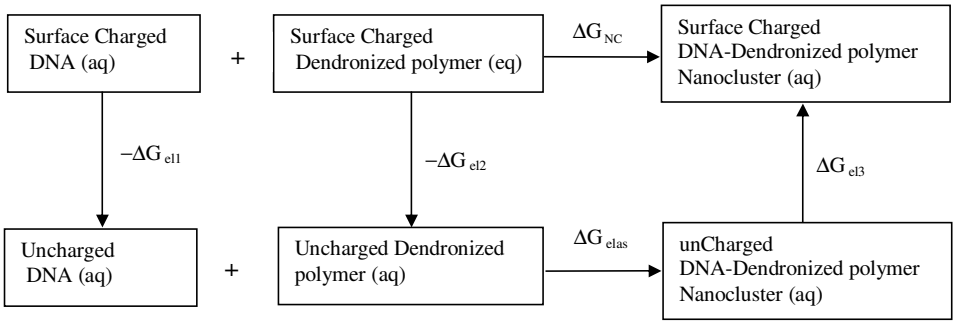
The bending and twisting free energies of DNA are given by the substitution of Eüler angles into Eq. (6), and integration along the chain as follows:

$$\Delta G_{bend} = \frac{A}{2}\ell R^2\left(\frac{2\pi}{\eta\sigma}\right)^4 \quad (11)$$

$$\Delta G_{twis} = \frac{C}{2}\frac{\ell}{\eta^2}\left(\frac{2\pi}{\eta\sigma}\right)^2 \quad (12)$$

Since the free energy is a state function, the following cycle can be designed to calculate the free energy of nanoclusteration, ΔG_{NC}.

In order to calculate the total free energy of nanoclusteration, the reagents, *i.e.* DNA and dendronized polymer, are assumed to be in separate solutions of 1:1 salt (such as NaCl) with the same ionic strength, and their functional surface groups are completely dissociated so that the contribution of chemical free energy is eliminated from



Scheme 1. Cycle of free energies of nanoclusteration

our calculation. The surface charge densities of the DNA and dendronized polymer produce an electrostatic field in the solution, which causes the mobile ions and solvent (water) molecules to rearrange around the polyelectrolyte. Consequently the system will have a new free energy. The DNA in solution is assumed to be neither bent nor twisted. Since DNA has negative surface charges (negative electrostatic potential), it is also flexible. Once mixed with the dendronized polymer solution, DNA is adsorbed by the dendronized polymer and wraps around it, resulting in the formation of a nanocluster with the lowest free energy. The free energy of nanoclusteration can then be calculated by using the following equation.

$$\Delta_{NC}G = \sum \Delta G_{(products)} - \sum \Delta G_{(Reactants)} \tag{13}$$

where

$$\sum \Delta G_{products} = \Delta G_{el} + \Delta G_{bend} + \Delta G_{twis} \tag{14}$$

and

$$\sum \Delta G_{Reactant} = \Delta G_{el(DNA)} + \Delta G_{el(dendrimer)} \tag{15}$$

The new arrangements of ions and water molecules around the nanocluster, the initial conformations of DNA and dendronized polymer and the resistance elasticity of DNA are included in this equation. The resistance elasticity is responsible for the bending and twisting free energies and is a characteristic property of the structure of DNA.

RESULTS AND DISCUSSION

The bending free energies of different conformations of DNA in different nanoclusters, calculated by using Eq. (11), are represented in Fig. 2. Note that the free energy of bending decreases as the size of the pitch increases. This occurs, because the length of the wrapped part of DNA and the radius of the curvature of the DNA decreases. Equation (11) includes these two contributions and shows that the free energy of bending is proportional to the length of the wrapped part of DNA and to the fourth power of the inverse of the length per turn.

The twisting free energies, obtained by using Eq. (12) for different conformations of DNA, versus the size of the pitch are plotted in Fig. 3. This figure illustrates that the twisting free energy is increased with increasing pitch. Equation (9) includes the following three factors responsible for this observation:

1) The length of the wrapped part of DNA, ℓ.
2) The length of DNA per turn, $\eta\sigma$.
3) The length of DNA per unit of cylinder, η.

The twisting free energy decreases with an increase in the first and second factors but the third factor has the inverse effect. Not only does this factor increase the twisting free energy but it also compensates for the effect of the two primary factors.

The electrostatic free energies of DNA and dendronized polymer in their solutions at constant temperature and different ionic strengths are shown in Fig. 4. However, DNA has 255 negative charges and the dendronized polymer has

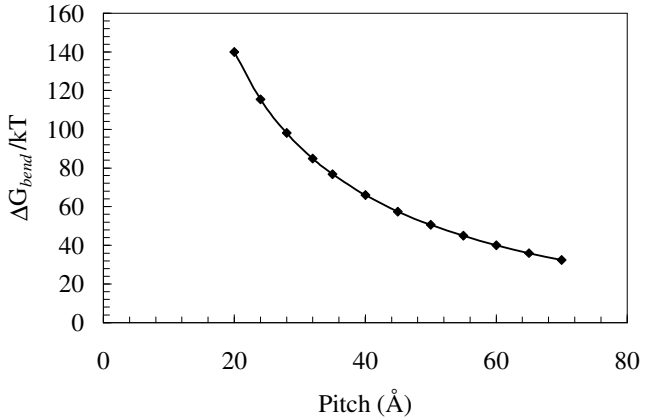


Fig. 2. Bending free energy of wrapped DNA *vs*. the pitch of DNA for different conformations of nanoclusters.

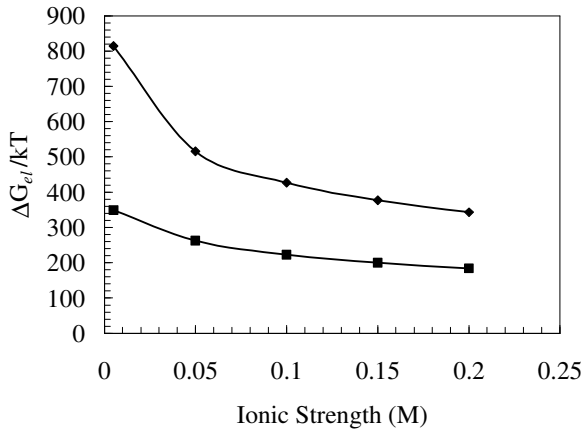


Fig. 4. Electrostatic free energy of reactants DNA (◆) and dendronized polymer (■) at various ionic strengths at constant temperature (T = 298 K).

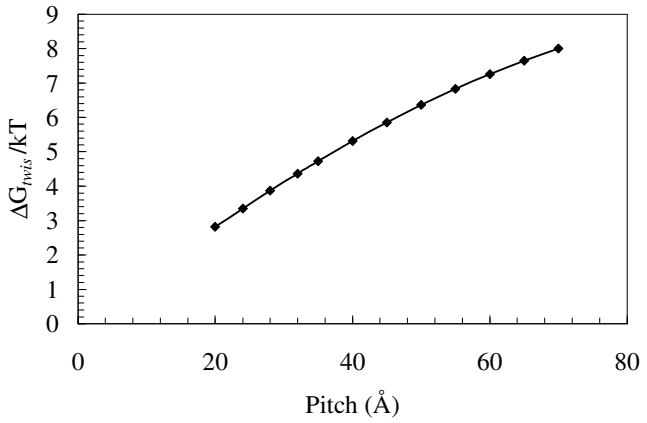


Fig. 3. Twisting free energy of wrapped DNA *vs*. the pitch of DNA for different conformations of nanoclusters.

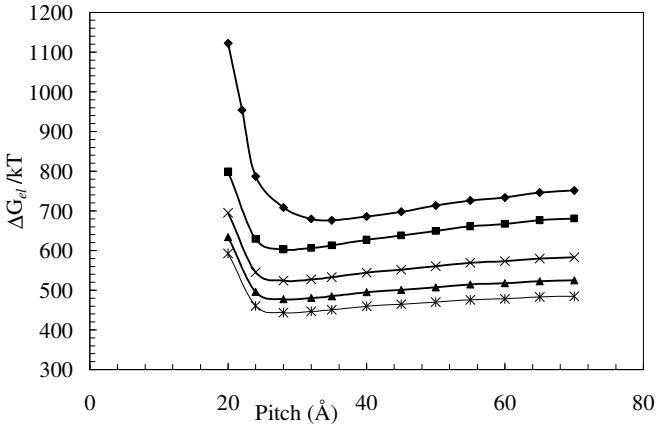


Fig. 5. Total electrostatic free energy for different conformations of nanoclusters *vs*. the pitch of DNA at 298 K and various ionic strengths: (◆) 0.006 M, (■) 0.06 M, (▲) 0.1 M, (×) 0.16 M, (∗) 0.2 M.

only 80 positive charges but the electrostatic free energy of the dendronized polymer is larger than the electrostatic free energy of DNA. These values are reasonable when we compare the surface charge density of the dendronized polymer (0.0159 e/Å^2) and DNA (0.0094 e/Å^2). When the ionic strength of the solution increases, the electrostatic free energies of the DNA and the dendrimer decrease and their difference also decreases.

The electrostatic free energies of nanoclusters are shown in Fig. 5. Like that of the reagents, the electrostatic free energy of the nanocluster decreases incrementally with the ionic strength of solution. Moreover, the higher the ionic strength, the more stable the nanoclusters with smaller pitch. This reveals that when the density of the mobile ions is large, the permeability of the electrostatic field in the solution decreases and different parts of the macroion is neutralized within a tiny space. Therefore, the repulsion force between the parts of DNA wrapped around the dendronized polymer decreases and the condensed DNA occupies a tiny space so that the smaller

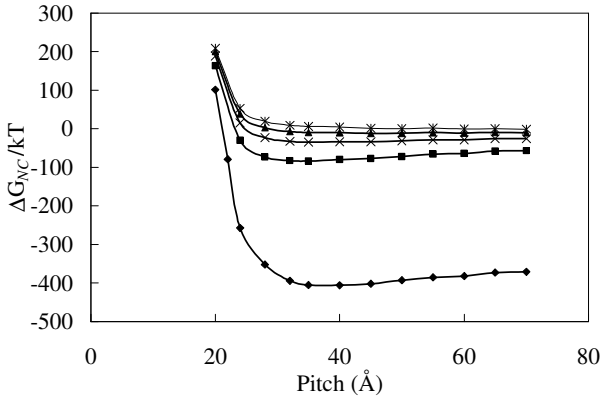


Fig. 6. Total free energy for different conformations of nano Clusters *vs*. the pitch of DNA at 298 K and various ionic strengths: (◆) 0.005 M, (■) 0.05 M, (▲) 0.15 M, (×) 0.1 M, (∗) 0.2 M.

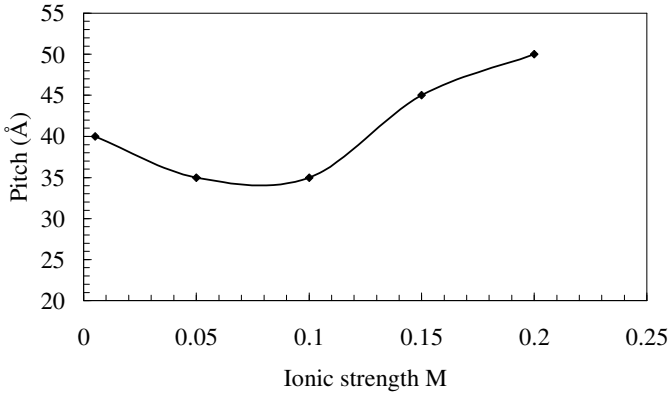


Fig. 7. The most stable conformation of nano-cluster *vs*. ionic strength at constant temperature (T = 298.15 K).

pitched conformations will be more stable.

A comparison between the values of the three contributions of free energies, *i.e*. electrostatic free energies ($\Delta G_{el(DNA)}$, $\Delta G_{el(dendrimer)}$, $\Delta G_{el(nano\text{-}cluster)}$), bending free energy ($\Delta G_{bend}$) and twisting free energy (ΔG_{twis}) (Figs. 2-4), in the nanoclusteration process reveals that the electrostatic free energies of the dendronized polymer, DNA, and nanocluster solutions have major contributions on the free energy of nanoclusteration.

Figure 6 represents the free energies of nanoclusterations in which nanoclusters with different conformations are obtained at different ionic strengths. It can be seen that the free energy of nanoclusteration at 0.005 M is the most negative, which means that nanoclusteration occurs spontaneously when the free energy of nanoclusteration at 0.2 M is the most positive. This can be related to the role of electrostatic free energy plays in nanoclusteration (see Eqs. 13-15), which is negative and predominant in the calculation of the free energy of nanoclusteration. It was also found that the size of the optimum pitch (the pitch with the minimum free energy value) depends on the ionic strength. Figure 7 indicates that the size of the optimum pitch decreases from 40 Å to 35 Å, while the ionic strength increases from 0.005 M to 2 M. It should be mentioned that the free energies of bending and twisting are both positive, independent of ionic strength and decrease with increasing pitch size.

CONCLUSIONS

A method for the calculation of the total free energy of nanoclusteration between DNA and dendronized polymer was demonstrated and elastic, electrostatic and chemical free energies, three major contributors in the total free energy of the nanoclusteration process, were then calculated.

We found that both bending and twisting free energies are positive at any ionic strength in the range of 0.005-0.2 M, the optimum pitch depends on the ionic strength and the free energy of nanoclusteration is negative in solutions of I < 0.2 M.

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SYMBOLS

ε	Dielectric constant
$(= \varepsilon_r \varepsilon_u = 78 \times 8.854 \times 10^{-12}\ C^2\ m^{-2}\ N^{-1}$ (in water)	
Ψ	Electrostatic potential [volt]
$\bar{r}$	Position vector
q_i^{f+}	Positive fixed charge
q_j^{f-}	Negative fixed charge
e	Charge of electron
k	Boltzmann constant
T	Absolute temperature
$\phi = \Psi e/kT$	Dimensionless electrostatic potential
κ	Modified Debye-Hückel parameter
$\delta(r - r_i)$	Kronecker Delta
λ	Debye length
I	Ionic strength of the bulk solution
ΔG_{el}	Electrostatic free energy
ΔG_{elas}	Elastic free energy of DNA
ΔG_{bend}	Bending free energy of DNA
ΔG_{twist}	Twisting free energy of DNA
$\alpha(s), \beta(s), \gamma(s)$	Eüler angles
l	Length of DNA
L	Local coordinate frame
L_0	Rigidly local coordinate frame
A	Elastic constant of bending
C	Elastic constant of twisting
s	Arc length
η	Length of DNA per unit length of dendrimer
σ	Height per turn
R	Radius of wrapping