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Linker Formation in an Overcharged Complex of Two Dendrimers and Linear Polyelectrolyte

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The complexes formed by two dendrimers with charged terminal groups and oppositely charged long linear polyelectrolyte (LPE) have been studied using Brownian dynamics simulations. The structural properties of the complexes and their dependence on the LPE chain length were investigated. It was observed that dendrimers in the considered complexes are sufficiently overcharged; i.e., the number of adsorbed LPE monomers is larger than required for the neutralization. The degree of overcharging increases with the increase of the LPE length and is accompanied by the linker appearance until saturation in overcharging is reached. Nonmonotonic dependence of the linker size on the LPE length was observed. To describe the structural properties of the complexes formed by two macroions and a polyelectrolyte chain, the correlation theory has been developed.

1. Introduction

Dendrimers, or regularly hyperbranched macromolecules, attract great attention nowadays. Due to their exceptional architecture and high monodispersity, dendrimers have found various applications.^{1–4} One of the most promising ways to use dendrimers is utilizing them as carrier agents for drug and gene delivery.^{5–8} In this case, dendrimers and “guest” molecules form complexes that often are stabilized by the electrostatic interactions.^{5,9,10} In order to control the drug delivery and release processes, deep insight into the physical properties of such complexes is required.

The nature and structure of “guest” molecules can be different. Complexes containing linear polyelectrolyte (LPE) molecule as a “guest” are the most interesting objects, because they correspond to the complexes formed, for example, by DNA. The study of the DNA complexation by different transport agents is hugely important for many biomedical applications.^{11–14}

In our previous publications, we have already considered complexes formed by the flexible LPE chain and branched polymers (macroions) of different topology including star-like polymers,¹⁵ dendrimers,^{16–19} and irregular hyperbranched polymers.²⁰ For all of those complexes, the overcharging effect has been observed; i.e., the charge of the adsorbed part of a LPE exceeds that necessary for the neutralization of the macroion charge.

The overcharging plays an important role in gene delivery. It is known that the cell surface is charged negatively and the complex of charged carriers with an oppositely charged DNA has to be charged positively to reach the cell. The overcharging phenomenon attracted large attention in the past and was intensively studied experimentally,^{21–23} theoretically,^{24–32} and by computer simulations.^{33–36}

The correlation theory for the overcharging effect in complexes formed by a spherical impenetrable macroion and oppositely charged flexible LPE has been developed by Nguyen, Grosberg, and Shklovskii.^{24–27} Nguyen et al. assume the formation of a regular structure of adsorbed LPE (solenoid-like configuration) onto the macroion surface with properties resembling those for a Wigner crystal. The theory predicts a significant degree of overcharging in such complexes and nonmonotonic dependence of the adsorbed charge on the LPE length: when the LPE length reaches some critical value N_{ch}^* , the adsorbed charge decreases sharply and a free tail releases.²⁴ The simulation results for complexes formed by a single dendrimer with charged terminal groups and a long flexible LPE agree qualitatively with theoretical predictions.^{16–19}

Our previous studies dealt with the complexes comprised by one macroion and one LPE chain.^{15–20} However, one LPE can form a complex with several macroions. Such complexes do exist, and are formed, for example, by PAMAM dendrimers of $g = 4$ generations and salmon sperm DNA.^{37,38} The overcharging of each dendrimer in a complex formed by DNA and a number of similar oppositely charged dendrimers is established both theoretically and experimentally.³⁸ This situation may be realized when the dendrimer concentration in solution increases at constant LPE concentration. Kabanov et al.³⁹ experimentally found that the conformation of LPE in complexes formed by several poly(propyleneimine) dendrimers and linear polyanion influences the complex solubility. In the present study, we consider for the first time complexes formed by two dendrimers and one oppositely charged LPE in the framework of a simple coarse-grained model to investigate the most general structural properties of this system.

There are three main questions which arise in this case and which we deal with in the present study:

- What is the configuration of the complex (i.e., the size of the adsorbed and unadsorbed LPE parts, and distribution of LPE monomers between tails and linker)?

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- What is the value of the overcharging for each dendrimer as compared to the corresponding value for a complex formed by a single dendrimer?
- What is the dependence of the size of the adsorbed part on the overall LPE length?

Nguyen and Shklovskii have developed the correlation theory for the complexes formed by the infinite amount of spherical macroions and one LPE.²⁶ They show that such complexes form a periodic necklace-like structure consisting of macroions with winded LPE which are separated by linkers. However, as we will show below, this theory does not take into account the edge effects and, in principle, is not applicable to the complexes formed by the finite (say, only two) number of macroions. Therefore, we have modified the existing correlation theory^{24–27} for the case of two spherical macroions and have made comparisons with the simulation results for the complexes formed by two dendrimers and one LPE.

The rest of the paper is organized as follows. In section 2, the model and simulation algorithm are described. The results of the simulations and some comparison with the developed correlation theory are discussed in section 3. In the Appendix, the modification of the correlation theory for complexes formed by two spherical macroions and a LPE chain is discussed.

2. Model and Simulation Algorithm

Model. Models for dendrimers and LPE are chosen similar to those used already in our previous simulations of a complex formed by a single dendrimer. Dendrimers and an LPE chain are represented by a set of beads with a friction coefficient ζ connected by rigid bonds of length l . For simplicity, no valence- and torsion-angle potentials are taken into consideration. LPE chain is composed of N_{ch} “beads” all possessing the negative charge $-e$. Dendrimers with a three-functional core, three-functional groups, and a spacer length of $s = 1$ are studied. The total number N of beads in a generation g dendrimer is calculated as $N = 3s(2^{g+1} - 1) + 1$. All terminal beads of the dendrimers possess an equal positive charge $+e$.

As long as we are interested in a study of the overcharging effect, we have to consider LPE with a total charge exceeding that of two dendrimers in the simulated complexes. In the current paper, we consider complexes formed by two generation $g = 3$ dendrimers. It was shown that small dendrimers of low ($g = 3-4$) generation numbers have also a low toxicity^{5,40–44} and, therefore, they could be the most suitable objects to be used as carrier agents in drug delivery applications. The total charge of each of the simulated dendrimers is equal to 24, and the total charge of two macroions in all considered complexes defines the minimal simulated LPE length, $N_{\text{ch}} = 48$. The relaxation of the LPE is one of the slowest processes in the complexes,^{17,45} and it determines the equilibration time of a total complex. Therefore, the maximum LPE length is limited by the required computational efficacy. The total charge of two $g = 3$ dendrimers is equal to the charge of a single $g = 4$ dendrimer. For complexes formed by such a single dendrimer, we have simulated LPE in the range $N_{\text{ch}} = 48-100$.¹⁶ Therefore, in the present study, we vary the LPE length in a similar range, $N_{\text{ch}} = 48-110$.

As in our previous studies,^{15–19} the excluded-volume interactions between nonbonded i th and j th monomers are described by the modified Lennard-Jones potential (without an attractive part) corresponding to the case of an athermal solvent:⁴⁶

$$\tilde{U}_{\text{LJ}}(r_{ij}) = \begin{cases} 4\epsilon_{\text{LJ}} \left[\left(\frac{\sigma}{r_{ij}} \right)^{12} - \left(\frac{\sigma}{r_{\text{cut}}} \right)^{12} \right], & r_{ij} \leq r_{\text{cut}} \\ 0, & r_{ij} > r_{\text{cut}} \end{cases} \quad (1)$$

r_{ij} is a distance between two monomers, and $r_{\text{cut}} = 2.5\sigma$ is a cutoff radius of the potential; $\sigma = 0.8l$ and $\epsilon_{\text{LJ}} = 0.3k_{\text{B}}T$.

Electrostatic interactions between j th and i th charged monomers are described by the Debye–Hückel potential:

$$\frac{U_j^{\text{C}}}{k_{\text{B}}T} = \lambda_{\text{B}} \sum_i \frac{\exp(-r_{ij}/r_{\text{D}})}{r_{ij}} \quad (2)$$

where λ_{B} is a Bjerrum length characterizing the strength of the electrostatic interactions in the medium with dielectric constant $\tilde{\epsilon}$:

$$\lambda_{\text{B}} = \frac{e^2}{4\pi\tilde{\epsilon}\epsilon_0 k_{\text{B}}T} \quad (3)$$

The value of λ_{B} in water at room temperature is equal to 7.14 Å that is close to the length of a segment of a common flexible linear polymer. Thus, in the present study, we consider $\lambda_{\text{B}} = l$ without loss of generality. The Debye radius r_{D} in eq 2 describes a screening of electrostatic interactions due to the presence of salt in the solution. We choose $r_{\text{D}} = 8.96l$ which was also used in our previous studies.^{16–18} For the complexes formed by a single dendrimer which have been simulated by us earlier, this value of r_{D} is larger than the size of a dendrimer; therefore, the effect of the electrostatic screening on the complex structure is small; almost all electrostatic contacts are taken into account. As we will show below for a complex formed by two dendrimers and a long LPE chain with large N_{ch} , the distance between dendrimers may be larger than this value of r_{D} , and screening effects could be, in principle, more pronounced. To test the effect of the electrostatic screening on the complex structure, the additional simulations of complexes with large N_{ch} at $r_{\text{D}} = 100l$ have been performed.

We must notice at this point that the Debye–Hückel approximation is valid only for the uniform distribution of charged particles. Strictly speaking, in order to take into account the electrostatic interactions properly, the computer simulations should be performed with explicit counterions. Indeed, in the condensed polyelectrolyte systems, significant counterion condensation is possible which can strongly influence the structural properties of the considered molecules. However, we remind you that in the present simulations only the dilute solutions have been considered. For a single LPE at such low concentrations, the effect of counterion condensation is expected to be small, LPE counterions are far away from the macroion. As for the dendrimer counterions, first of all, for a single charged dendrimer, the majority of its own counterions are condensed inside even in a very dilute solution. Due to entropic reasons, the dendrimer counterions leave the complex when the LPE charge exceeds that of two dendrimers, and the cloud of condensed dendrimer counterions is replaced by the adsorbed LPE charges. It means that the dendrimer counterions are located far away from the complex as well. This effect for short LPE chains was recently confirmed by Lyulin et al.⁴⁷ Then, as was shown by us recently^{47,48} using molecular dynamics simulations of similar systems with explicit counterions, the essential counterion condensation occurs only at $\lambda_{\text{B}} > l$ which is larger than the value of the Bjerrum length in the present study ($\lambda_{\text{B}} = l$). Finally, one of

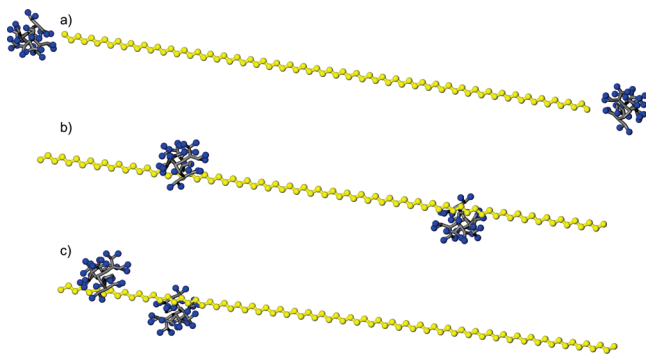


Figure 1. Various initial configurations of a complex formed by two $g = 3$ dendrimers and one LPE with $N_{\text{ch}} = 80$ monomers.

the goals of the present study is to compare these simulation results with the predictions of the modified correlation theory of Nguyen and Shklovskii.²⁴ This theory deals only with energetical aspects of the overcharging, and does not take into account entropic effects related to the counterion release during the complexation of a macroion with LPE. Taking all of these arguments into account, in the present study, the counterions have not been simulated explicitly.

Simulation Algorithm. We perform Brownian dynamics simulations using the algorithms developed in our previous studies.^{15–19} We chose a free-draining model both for dendrimers and LPE neglecting the hydrodynamic interactions between beads, as the main focus of our study is the equilibrium structural properties only.

The finite-difference numerical scheme implemented here is based on the Ermak–McCammon equation.^{49,50} The total force \mathbf{F}_j^0 acting on a j th bead in the system is given by

$$\mathbf{F}_j^0 = - \sum_{k=1}^N \mu_k \left(\frac{\partial v_k}{\partial \mathbf{r}_j} \right)_{r^0} - \partial \tilde{U}_{\text{L}} / \partial \mathbf{r}_j^0 - \partial U_j^C / \partial \mathbf{r}_j^0$$

where $v_k = 1/2(\mathbf{r}_{k+1} - \mathbf{r}_k)^2 - l^2 = 0$ is the equation for the k th rigid constraint, μ_k is the corresponding Lagrange multiplier, and \mathbf{r}_j^0 is the position vector for the j th bead before an integration time step Δt .

In the present study, we use dimensionless quantities where length l , energy $k_B T$, time $\zeta l^2 / k_B T$, and the translational friction of a single bead ζ are set to unity. The dimensionless integration step is equal to $\Delta t = 10^{-4}$. This value of Δt was chosen in order to have the maximum displacement of a bead during this time less than 10% of the bond length. The SHAKE algorithm⁵¹ with a relative tolerance of 2×10^{-6} is used to maintain a fixed bond length.

The initial conformations of the LPE chain and dendrimers were obtained as follows. First, two dendrimers and a single LPE in the trans conformation were generated using a procedure described in detail elsewhere.^{16–18} Dendrimers were placed near the ends of LPE (at a single bond length distance) (Figure 1a). For some complexes, additional simulations have been carried out starting from the different initial configurations (Figure 1b and c). No difference has been found in the simulated characteristics.

Each of the generated initial configurations has been equilibrated during 1.4×10^7 to 2.8×10^7 simulation steps. The length of the equilibration procedure depends on the LPE length and is determined by the relaxation time of the LPE size. Equilibrium was considered to be achieved when the size of the complex as a whole and the size of its components are not changed further

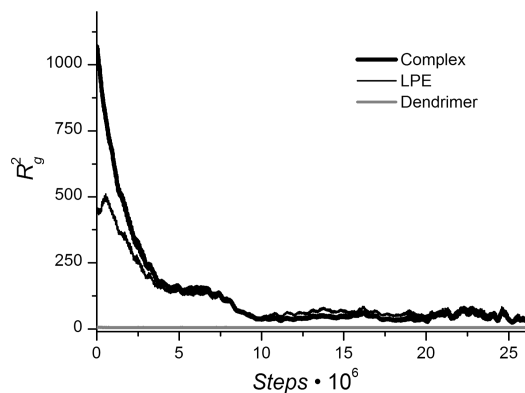


Figure 2. Instant values of the squared gyration radius R_g^2 of the complex and its components (dendrimer and LPE) during the equilibration runs for the complex formed by two $g = 3$ dendrimers and LPE with $N_{\text{ch}} = 90$. Equilibration of the complex was performed during 2.6×10^7 simulation steps.

and only small size fluctuations occur near some average values. The squared gyration radius R_g^2 was used as a measure of the size of complex constituents

$$R_g^2 = \frac{1}{N} \sum_{n=1}^N (\mathbf{r}_n - \mathbf{r}_C)^2 \quad (4)$$

where \mathbf{r}_C is the radius-vector corresponding to the center of mass of the component, \mathbf{r}_n is the radius-vector of the n th bead, and N is the number of beads. As an example, the instant values of R_g^2 for a total complex and its components during the equilibration are shown in Figure 2 for $N_{\text{ch}} = 90$.

The production run of 1.4×10^7 simulation steps has been performed after this equilibration procedure; all simulated characteristics of complexes have been averaged over this production run.

The structural properties of complexes formed by two $g = 3$ dendrimers (referred to here as 2g3 complexes) will be compared below with those for complexes formed by a single $g = 3$ dendrimer (referred to as the 1g3 complex) or $g = 4$ dendrimer (referred to as the 1g4 complex), both studied previously.^{16–18} Note that a $g = 4$ dendrimer has exactly the same charge as two dendrimers of $g = 3$ generations; at the same time, a $g = 4$ dendrimer is larger than a $g = 3$ dendrimer and it preserves almost spherical shape in complexes. Thus, the comparison of structural properties and configurations for 2g3 complexes and complexes formed by a single dendrimer, 1g3 and 1g4, allows us to reveal the influence of a size and a shape of a macroion on a structure of complexes with LPE.

3. Results

Stable complexes formed by two dendrimers have been simulated for different values of the LPE length N_{ch} ; typical snapshots are shown in Figure 3. In all cases, the LPE charge exceeds that of two dendrimers. It is seen that the configuration of a complex strongly depends on N_{ch} . We recognize in Figure 3 the region of chain lengths where dendrimers are close to each other and form a joint macroion without linker (Figure 3a). At large N_{ch} , the linker between dendrimers exists (Figure 3b and c). Below, we will consider the properties of these complexes in a more quantitative manner.

Mean-Squared Radius of Gyration. We calculated the mean-squared radius of gyration $\langle R_g^2 \rangle$ separately for dendrimers

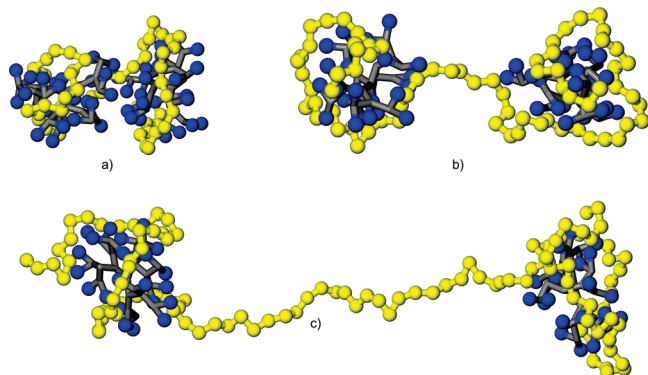


Figure 3. Snapshots for equilibrated 2g3 complexes with $N_{ch} = 55$ (a), 70 (b), and 110 (c). LPE monomers are shown by yellow spheres, blue spheres represent charged terminal groups of the dendrimers, and gray rods correspond to the uncharged inner groups of the dendrimers.

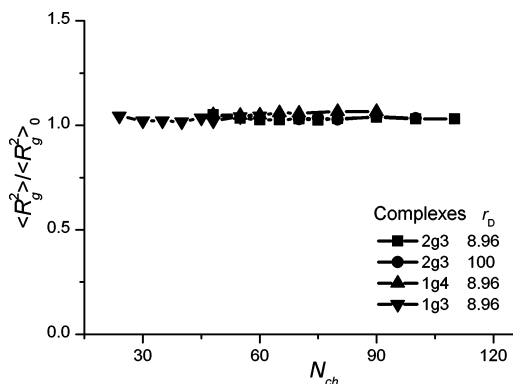


Figure 4. Ratio of $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ for a single dendrimer in different complexes as a function of the LPE length. $\langle R_g^2 \rangle_0$ is a mean-squared gyration radius for a corresponding single neutral dendrimer. Here and below, the results for single dendrimers are taken from our previous studies.^{16–18}

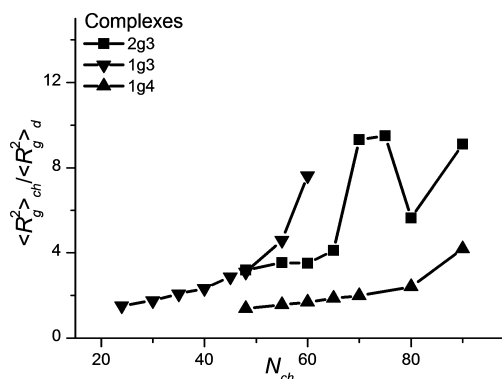


Figure 5. Ratio of the mean-squared gyration radii $\langle R_g^2 \rangle_{ch}$ of a LPE and a single dendrimer $\langle R_g^2 \rangle_d$ in different complexes at $r_D = 8.96$. In the case of complexes comprised by two dendrimers (2g3), $\langle R_g^2 \rangle_d$ is a mean-squared gyration radius of one dendrimer.

(Figure 4) and for a LPE (Figure 5) in a complex. In Figure 4, the ratio $\langle R_g^2 \rangle / \langle R_g^2 \rangle_0$ of the size of a dendrimer in a complex to the size of the corresponding neutral dendrimer is plotted as a function of the LPE length. The size of each dendrimer in a simulated complex is close to that of a neutral dendrimer with the same number of generations. Note also that the size of a dendrimer in complexes with LPE does not depend on the LPE length (the same effect has been observed previously for 1g3 and 1g4 complexes^{16,17}) and on the Debye screening length r_D (Figure 4). The results obtained earlier for the complexes

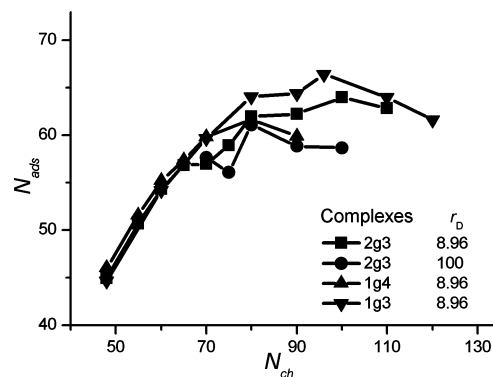


Figure 6. Number of the adsorbed LPE monomers N_{ads} as a function of the LPE length N_{ch} . The “local” criterion of adsorption has been used. The numerical data for 1g3 complexes is multiplied by a factor of 2 (doubled) in order to facilitate the comparison with the complexes of two dendrimers.

comprised by a single dendrimer^{16–18} are shown in Figure 4 as well. These results clearly show that the oppositely charged adsorbed LPE monomers effectively neutralize dendrimer charged groups.

After the formation of a complex, the mean-squared gyration radius of a LPE decreases significantly (as compared to the individual chain). Its dependence on the LPE length N_{ch} (Figure 5) is similar qualitatively to that for complexes formed by a single dendrimer. In this dependence, we distinguish two different regimes. First, $\langle R_g^2 \rangle$ slowly increases with an increase of N_{ch} until some value of $N_{ch} \sim 65$ is reached. In this regime, almost all LPE monomers are adsorbed onto the dendrimers. At large N_{ch} (above $N_{ch} \sim 80$), the faster growth of the LPE gyration radius is observed. For complexes formed by a single dendrimer, such a growth is connected with the release of the LPE tail. In the case of two simulated dendrimers, the size of the LPE chain increases due to the appearance of a linker between dendrimers. Dendrimers start to separate from each other. It is interesting to note that there is an intermediate region, $65 < N_{ch} < 80$, where the short linker appears and then disappears again. In this region, the dependence of the LPE gyration radius on the LPE length is nonmonotonic, with some initial increase and a subsequent decrease of the LPE chain size. This nontrivial behavior will be discussed in more details below.

Overcharging. In the present simulations, it has been found that the number of LPE monomers adsorbed on the dendrimers in 2g3 complexes is larger than that required to neutralize the charge of macroions. This overcharging effect was also observed earlier in the complexes comprised by a single dendrimer.^{16–18}

Following the correlation theory of Nguyen et al.,^{24–27} the overcharging of a macroion by the LPE chain has an energetic origin, and results from the correlation between “exceeds” of a charge of the adsorbed parts of the LPE on the macroion surface.

For quantitative characterization of the overcharging effect, it is necessary to define the criterion of the monomer adsorption. We use the same “local” criterion^{15–20} which was used by us earlier for complexes comprised by a single dendrimer. LPE monomer is considered as adsorbed if the distance between this monomer and the nearest monomer of a dendrimer is less than some value r_c , $r_c = l + \sigma = 1.8\sigma$.¹⁶ The chain-length dependence of the average number of adsorbed LPE monomers N_{ads} is shown in Figure 6, together with the results obtained earlier^{16–18} for the 1g3 and 1g4 complexes.

The number of adsorbed LPE monomers is also influenced by the value of the Debye radius. It is well-known that the increase of a salt concentration in solution leads to the growth

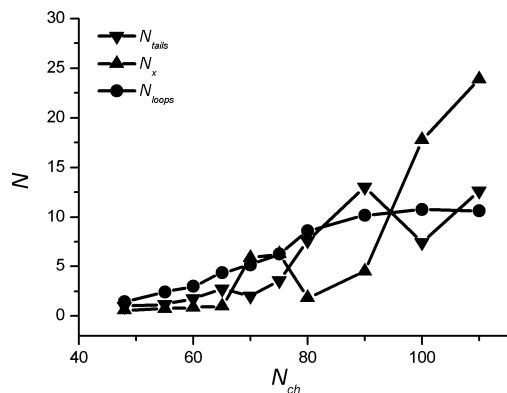


Figure 7. Average number of LPE monomers in a linker (N_x), tails (N_{tails}), and loops (N_{loops}) calculated using “local” adsorption criterion ($r_D = 8.96$).

of LPE adsorption on macroions.^{24,33} Lowering the value of r_D or increasing the concentration of salt leads to the increase of N_{ads} , Figure 6. In the case of a lower value of r_D ($r_D = 8.96$), a slightly larger amount of monomers is adsorbed on a dendrimer in the complexes with LPE of the same length.

The nonadsorbed parts of LPE can be distributed between tails, loops, and a linker. We define the tail as an unadsorbed part of LPE which has only one adsorbed end. Both ends of loops are adsorbed on the same dendrimer. Ends of a linker are adsorbed on different dendrimers. Figure 7 shows the average number of LPE monomers in these different nonadsorbed parts. In the 2g3 complexes formed by a short (up to $N_{ch} = 65$) LPE, almost all LPE monomers are adsorbed on the dendrimers and the corresponding lengths of the linker, tails, and loops are negligible. In the case of a long LPE ($N_{ch} > 80$), the stable linker is formed. In the intermediate region ($65 < N_{ch} < 80$), some interplay between linker and tail is observed. First, the short linker is formed when the LPE length is equal to $N_{ch} = 70$. This short linker almost vanishes and tails are released with a further increase of the chain length. Note that the average number of monomers in loops N_{loops} increases monotonically and saturates with a further increase of the LPE length N_{ch} . The value of N_{loops} is comparable with the total length of tails N_{tails} . Nevertheless, the average number of monomers in a single loop N_L is small (Figure 8b); i.e., with an increase of the LPE length, the number of loops in complexes increases.

The average numbers of loops and average loop length for the 2g3 complexes have almost a similar dependence on the length of LPE as in the 1g3 and 1g4 complexes,¹⁸ Figure 8. The number of loops and their average length in complexes reach saturation when transition from compact complexes to complexes with long nonadsorbed parts of the LPE (tails or linker) occurs, i.e., at $N_{ch} > N_{ch}^c$.

It is seen that loops contain a small number of LPE monomers; the average length of a loop is about two monomers. The same value was obtained for complexes formed by a single dendrimer.¹⁸

As was mentioned above, the enhancing of the electrostatic screening results in some increase of the LPE adsorption, also in agreement with another studies.^{24,33} This increase of the number of adsorbed monomers should lead to the decrease of unadsorbed parts of LPE, viz., linker and tails. Indeed, the comparison of the simulation results at different values of r_D shows that the average length of a linker and tails decreases with the increase of screening, i.e., with a decrease of r_D (Figure 9).

The correlation theory²⁴ for a spherical impenetrable macroion predicts that at some critical value of the LPE length N_{ch}^c the

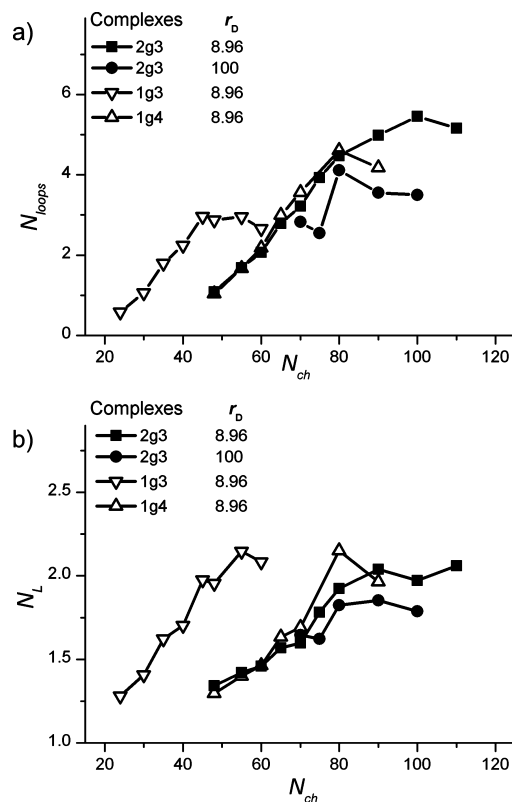


Figure 8. Overall number N_{loops} of LPE monomers in loops per one dendrimer (a) and the average number N_L of monomers in a single loop (b) in different complexes.

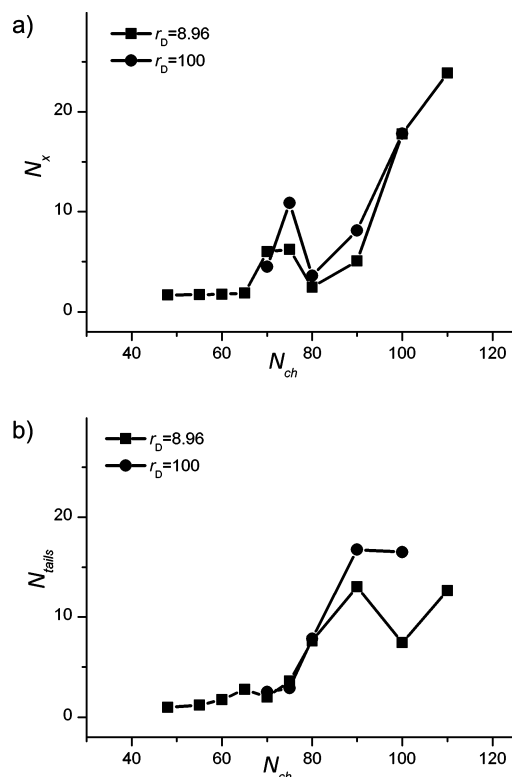


Figure 9. Number of LPE monomers in a linker (a) and in tails (b) in 2g3 complexes at different values of the Debye screening radius r_D .

first-order phase transition occurs and the unadsorbed LPE tail releases. For the complex formed by a single charged dendrimer and an LPE, such behavior has also been detected by BD simulations.¹⁸ In the present study for the complexes formed

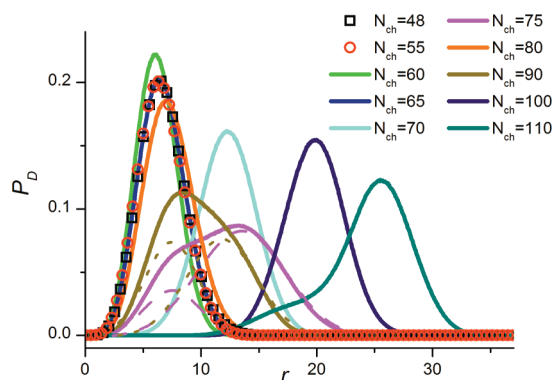


Figure 10. Pair distribution functions $P_D(r)$ for the complexes formed by LPE of different lengths N_{ch} . For all complexes, $P_D(r)$ resembles a Gaussian distribution except for $N_{ch} = 75$ and 90 , where $P_D(r)$ can be represented as a superposition of two Gaussian distributions that are shown by dashed lines of the corresponding colors and the position of the first peak is close to that for $N_{ch} = 48$ – 65 , 80 .

by two dendrimers, we observe the region of the LPE length ($65 < N_{ch} < 80$) where complex configuration is unstable. In this region, the mean-squared deviation of the number of adsorbed LPE monomers is around 4.8, which is comparable with the average linker length in the complexes with $N_{ch} = 70$ – 75 . Therefore, we discuss now the release and subsequent vanishing of the linker in this region in some more detail.

First of all, twice longer simulations have been performed for this region. The analysis of the produced trajectories did not lead to any changes in the observed nonmonotonic behavior. We also checked the influence of the initial complex configuration on the final results. To do that, we performed the full simulation procedures described above starting from the different initial configurations shown in Figure 1b and c for chain lengths of $N_{ch} = 75$ (the region of the first linker appearance) and $N_{ch} = 80$ (the region of the linker disappearance). The dendrimers were slightly shifted along the LPE chain from its ends closer to the LPE center or both dendrimers were placed close to the same end of the chain, and the equilibration was started all over again. Such a change of the initial complex configuration did not change the final results. The averaging over all initial configurations only slightly increases the nonmonotonic linker behavior in the region $65 < N_{ch} < 80$.

To obtain even more evidence that this nonmonotonic behavior of the linker size does exist and is not the result of statistical fluctuations, the pair distribution function $P_D(r)$ of monomers of one dendrimer relative to the monomers of another dendrimer has been calculated (Figure 10). This function represents an average probability to find any bead of the first dendrimer at a distance r from the bead of the second dendrimer.

The pair distribution functions for $N_{ch} = 48, 55, 60, 65$, and 80 can be represented as a Gaussian distribution with the same maximum position at $r_m^0 \sim 6.5$. According to the results shown in Figure 7, no linker exists in these complexes. For $N_{ch} = 70$ (as well as for $N_{ch} = 100$), $P_D(r)$ also resembles a Gaussian distribution with a single peak located at $r_m > r_m^0$ that confirms linker existence.

For the complexes with $N_{ch} = 75$ and 90 , the maximum of $P_D(r)$ is shifted to higher values of r ; the distribution function $P_D(r)$ has clearly asymmetric shape, and can be represented as a sum of two Gaussian distributions shown by dashed lines of the same colors in Figure 10. The position of the first Gaussian peak is close to r_m^0 . This behavior indicates the coexistence of configurations with and without a linker in these complexes.

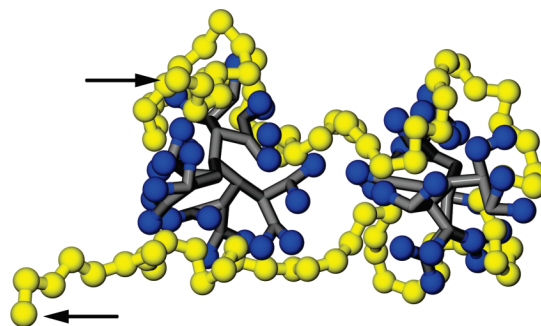


Figure 11. Snapshot of the 2g3 complex with $N_{ch} = 80$ where LPE is in a “hairpin” conformation. Ends of the LPE chain are denoted by arrows. LPE monomers are shown by yellow spheres, blue spheres represent charged terminal groups of the dendrimers, and gray rods correspond to the uncharged inner groups of the dendrimers.

We suppose that the formation of a linker in these complexes is related to the separation of the overcharged dendrimers due to the electrostatic repulsion. This effect is similar to the increase of the shape anisotropy of irregular hyperbranched polymer with “open” core (referred to as HBP_{max} polymer in ref 20) before the maximum of LPE adsorption is reached.

The vanishing of the linker in complexes with $N_{ch} = 80$ is due to changes in conformation of the LPE chain. In these complexes, the chain often forms a “hairpin” conformation where LPE is bent around one dendrimer and both ends of the LPE are located close to another dendrimer (Figure 11).

Separation of dendrimers in the complexes where LPE is in the “hairpin” conformation results in the formation of two parallel linkers of negligible length between dendrimers. Due to the repulsion between two parallel linkers at some linker length, such a conformation is unfavorable as compared to the conformations with a tail or a single linker. Thus, in complexes where LPE has a “hairpin” conformation, no linker formation is observed.

To verify in which complexes LPE forms a “hairpin”, the conformation function P_L was calculated as follows. First, the distances between ends of the LPE and both dendrimers were calculated and were used to determine which dendrimer (either the first or second) is closer to the LPE end. If both ends of the chain are close to the same dendrimer, P_L was considered to be equal to zero. In the other case, when LPE ends are located near different dendrimers, $P_L = 1$. The “hairpin” conformation corresponds to the case $P_L = 0$, and $P_L = 1$ corresponds to conformations with extended LPE including conformations with a single linker formed. The averaged value $\langle P_L \rangle$ over all simulations characterizes the predominant conformation of the LPE in a complex with given N_{ch} . As can be seen from Figure 12, in the complexes where linker is formed ($N_{ch} = 70, 75$; $N_{ch} \geq 90$), $\langle P_L \rangle \approx 1$, i.e., LPE has extended conformations. However, for the complex formed by two dendrimers and LPE with $N_{ch} = 80$, where linker vanishing was observed, $\langle P_L \rangle \approx 0.4$ and LPE is often in a “hairpin” conformation.

It should be noted that the process of linker formation may be sensitive to the presence of the explicit counterions in the simulations. This effect will be checked in our future studies.

Comparison with the Correlation Theory for a Complex Comprised by Two Spherical Macroions. For the case of a complex formed by a single dendrimer and LPE, a semiquantitative agreement of simulation results with predictions of the correlation theory^{24–27} developed for the complex formed by one spherical solid macroion has been observed in our previous simulations.^{16–18} We have modified this theory for the complex of LPE with two spherical macroions (see the Appendix).

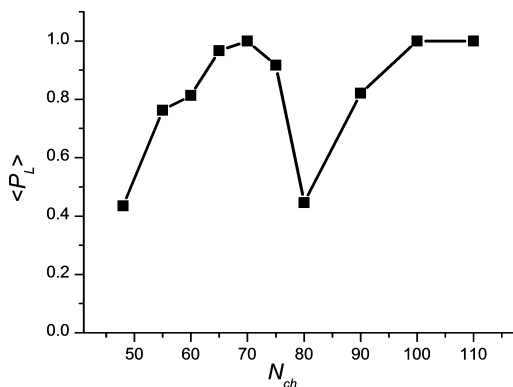


Figure 12. Average conformation function $\langle P_L \rangle$ as a function of LPE length N_{ch} for complexes formed by two dendrimers $g = 3$ and LPE ($r_D = 8.96$).

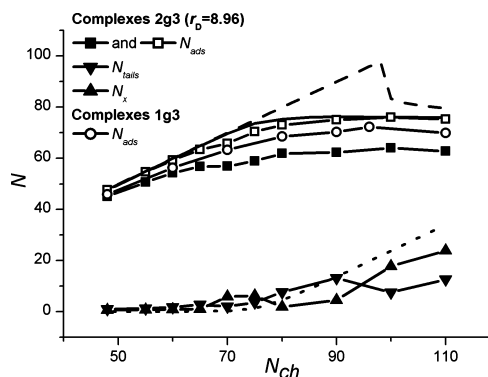


Figure 13. Comparison of the simulated amount of the adsorbed LPE monomers (N_{ads}) and numbers of LPE monomers in tails (N_{tails}) and linker (N_x) with theoretical predictions. N_{ads} , N_{tails} , and N_x for the 2g3 complexes were calculated using “local” (closed symbols) and “rough” (open symbols) adsorption criteria. Solid and dotted lines are theoretically predicted dependences for N_{ads} (solid line) and N_x (dotted line) for a complex formed by LPE and two spherical macroions (see the Appendix). The dashed line represents the dependence $N_{ads}(N_{ch})$ obtained using the theoretical approach of Nguyen and Shklovskii.²⁴ Simulation results for the 1g3 complexes are multiplied by a factor of 2 in order to facilitate the comparison with the case of 2g3 complexes.

Developed theory predicts that there are two regimes for a complex configuration as a function of the LPE length. Below some critical length, the chain is wound around two macroions which are close to each other. The overcharging increases with the lengthening of LPE. When the maximum overcharging degree is achieved, the linker appears and macroions start to separate. All nonadsorbed LPE monomers are in the linker, and the tail appearance is unfavorable. For the complex formed by a single macroion, the tail appearance is a first-order phase transition and is accompanied by a sharp decrease of the amount of adsorbed monomers. In contrast to it, in the case of two macroions, the appearance of a linker does not change the length of the adsorbed part. Figure 13 compares the theoretical predictions for the overcharging and the linker length with the simulation results for the 2g3 complexes. For theoretical calculations, the values of $R = (\frac{5}{3}\langle R_g^2 \rangle)^{1/2}$ (where $\langle R_g^2 \rangle$ is the gyration radius of a dendrimer) have been used as the radius of a spherical macroion. The charge of spherical macroions was equal to the charge of the $g = 3$ dendrimer with charged terminal groups ($N_Q = 24$). The radius of a LPE chain a was taken to be equal to the Lennard-Jones radius of a bead $a = 0.5\sigma$. The charge density is $\eta = 1$.

It is seen that the local criterion of adsorption gives qualitative agreement with the theory predictions but the degree of the

overcharging is lower than the theoretical value. To interpret these results correctly, we have to remind you that the theory assumes the ideal solenoid-like packing of the LPE monomers on the surface of macroions. However, computer simulations of complexes of flexible LPE with spherical macroion have shown⁵² that a LPE chain does not fit the sphere surface tightly but can form short loops instead. By including these monomers in the number of adsorbed ones, the authors of ref 52 obtained rather good agreement with the theory predictions for the overcharging. For dendrimers with not that strongly defined solid surface, the formation of loops should be even more probable than for spherical macroions. In fact, our results show the existence of short loops (Figure 8). Thus, we can introduce another “rough” criterion of adsorption where monomers of LPE are considered as adsorbed not only if they satisfy the local criterion but if they belong to loops as well as to short tails and a linker. Figure 8 shows that the average number of monomers per loop is less than three. Therefore, it would be reasonable to consider tails and linker as adsorbed if their length does not exceed three monomers. As it follows from Figure 13, such a “rough” criterion gives very good quantitative agreement with theoretical predictions for dependence of $N_{ads}(N_{ch})$. At the same time, the dependences $N_x(N_{ch})$ and $N_{tail}(N_{ch})$ do not differ significantly, being calculated using different adsorption criteria.

The developed theory predicts also that all nonadsorbed LPE monomers belong to the linker. Its length increases monotonously after the maximum of adsorption is achieved. However, our simulations show the release of a remarkable tail which coexists with the linker. Therefore, the length of the linker occurs to be smaller than the theoretical one in this region of the LPE length.

Conclusions

We have studied the structure of the complexes formed by two dendrimers and an oppositely charged LPE. In these systems, dendrimers are strongly overcharged by the adsorbed LPE monomers and the maximum degree of the overcharging is close to that in complexes formed by one dendrimer and LPE. In the 2g3 complexes, the overcharging degree does not decrease significantly after the maximum of chain adsorption is reached, in contrast to the complexes comprised by a single dendrimer and LPE. The increase of the LPE in the complexes leads to the linker appearance between dendrimers. Surprisingly, the linker size depends on the LPE length nonmonotonously. In spite of the rather narrow interval of simulated parameters and model potentials (absence of hydrogen bonding, Debye–Hückel approximation for the electrostatic interactions, etc.) which may be far from the realistic biological situation, we believe that our findings provide new insights for dendrimer–LPE complexation. The obtained simulation results are in qualitative agreement with the predictions of the developed correlation theory for complexes formed by two spherical macroions and a flexible polyelectrolyte chain.

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Appendix

We consider a complexation of a flexible LPE with the linear charge density η , and radius a with two equal spheres with radius R and positive charge Q . The model is similar to that used in ref 24 with only the difference being that we have two

spheres instead of one. As in ref 24, it is assumed that the LPE length L is greater than the neutralizing length $\Lambda = 2Q/\eta$. The part of a LPE $L_1 = 2L_{S_i}$ is tightly wound around spheres. The rest of the chain with length $L_2 = L - L_1$ can form a linker with the length L_x and two tails with length L_{T_i} (Figure 14). Our goal is to calculate the charge adsorbed on spheres ηL_1 and the charge inversion ratio $(\eta L_1 - \Lambda)/2Q$ as well as the average length of a linker and tails. We consider the salt-free solution.

Similar to ref 24, the total energy of the system is presented as a sum of self-energies of nonadsorbed parts of LPE and spherical complexes (macroion with winded LPE) and interaction energies of complex constituents with each other

$$E = E_{\text{self}} + E_{\text{int}} \quad (1A)$$

where

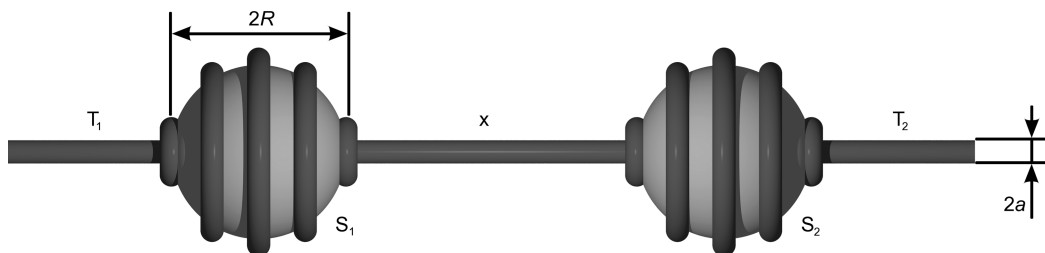


Figure 14. LPE winds around two spherical macroions. The nonadsorbed part of LPE forms a linker and two tails.

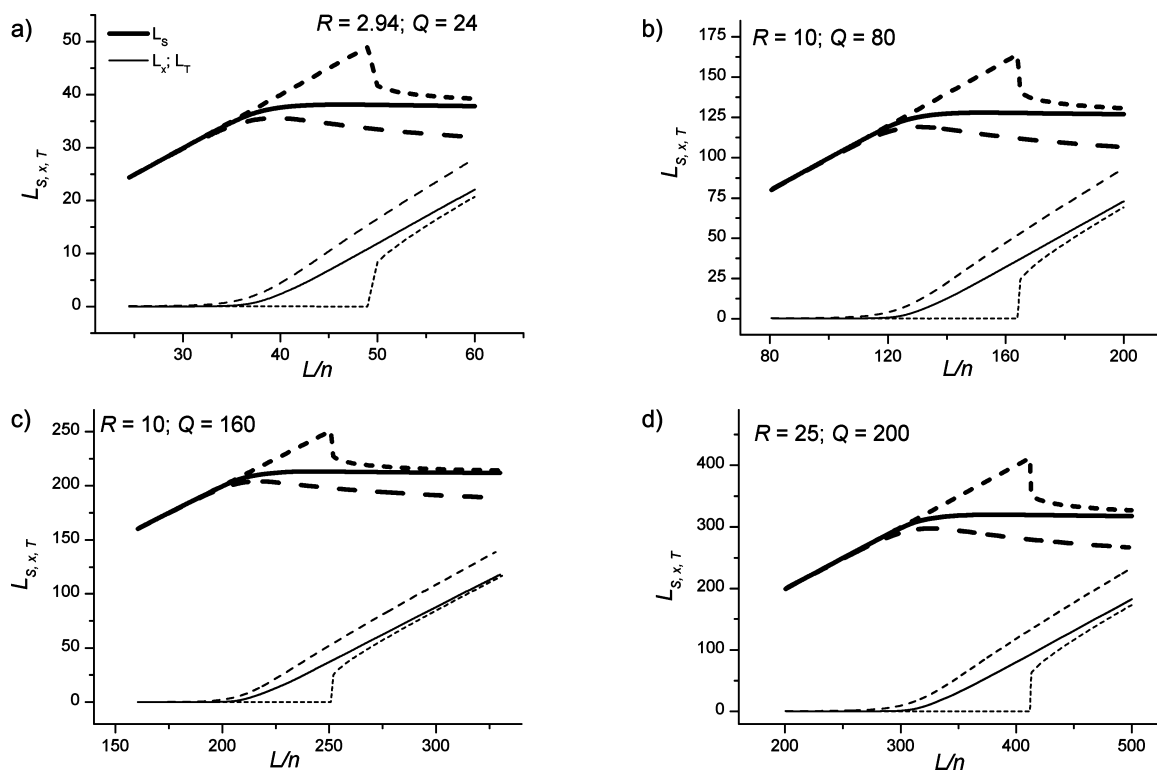


Figure 15. Dependence of the length of adsorbed LPE L_s and length of the linker L_x or of the tail L_T (per one macroion; n , number of macroions in a complex) on the LPE length for the complex formed by two spherical macroions and flexible LPE (solid lines correspond to the present theory), for the necklace model²⁶ for the number of macroions $n = 2$ (dashed lines); for a complex comprised by one macroion and LPE²⁴ (dotted lines). Macroions in complexes have the radius $R = 2.94$ (a), 10 (b and c), and 25 (d). The charge of macroions is $Q = 24$ (a), 80 (b), 160 (c), and 200 (d).

$$E_{\text{self}} = E_{S_1} + E_{S_2} + E_{T_1} + E_{T_2} + E_x \quad (2A)$$

E_{S_1} and E_{S_2} are the self-energies of two spherical macroions with wound LPE, E_{T_1} and E_{T_2} are the self-energies of two straight tails, and E_x is the self-energy of the linker.

The interaction energy E_{int} is a sum of energies of interaction of different parts of a complex

$$E_{\text{int}} = E_{S_1 S_2} + E_{T_1 S_1} + E_{T_1 S_2} + E_{T_2 S_2} + E_{T_2 S_1} + E_{x S_1} + E_{x S_2} + E_{T_1 T_2} + E_{T_1 x} + E_{T_2 x} \quad (3A)$$

The first term in eq 3A is the energy of interaction of two macroions with LPE wound around them. The next six terms correspond to interaction energies of tails or linker with spherical macroions wound by LPE. The last three terms are energies of interaction of tails with each other or with a linker.

As in refs 24 and 26, the energy is written in units of η^2/D where D is a dielectric constant of water. Thus, energy has a dimensionality of length.

Expressions for the self-energies of different parts of the complex are similar to those used in ref 24. The self-energy E_{S_i} of a spherical macroion with adsorbed LPE is given by

$$E_{S_i} = L_{S_i} \ln(R/a) - L_{S_i} \ln(L_{S_i}/R) + (L_{S_i} - \Lambda)^2/2R, \quad i = 1, 2 \quad (4A)$$

We have to remind you that the second term in eq 4A is a correlation energy. This term results in the lowering of the energy due to formation of the ordered solenoid-like conformation of LPE on the surface of a macroion.²⁴

The self-energies of straight nonadsorbed parts of LPE (E_{T_i} for tails and E_x for linker) are written as follows:

$$E_{T_{ix}} = L_{T_{ix}} \ln(L_{T_{ix}}/a) \quad (5A)$$

The energy of interaction of two spherical complexes $E_{S_1S_2}$ is equal to

$$E_{S_1S_2} = \frac{(L_{S_1} - \Lambda)(L_{S_2} - \Lambda)}{2R + L_x} \quad (6A)$$

Here, $(L_{S_i} - \Lambda)$ is the net charge of the i th macroion with adsorbed LPE ($i = 1$ or 2) and $2R + L_x$ is a distance between centers of the spheres.

The energy of interaction $E_{T_jS_j}$ of the nonadsorbed parts of LPE with a spherical complex is given by

$$E_{T_jS_j} = (L_{S_j} - \Lambda) \ln\left(\frac{L_{T_j} + Z}{Z}\right); \quad i = 1, 2; j = 1, 2 \quad (7A)$$

We put $Z = R$ and $Z = 3R + L_x$ for the interaction of a tail with the nearest complex ($i = j$) or a linker with any complex and of a tail with a distant complex ($i \neq j$), correspondingly.

Expressions for the energy of interaction of nonadsorbed parts of the LPE with each other ($E_{T_iT_j}$ for the interaction of tails or E_{T_ix} for the interaction of a tail with a linker) are written as

$$E_{T_iT_j} = (L_{T_i} + L_{T_j} + Z) \ln(L_{T_i} + L_{T_j} + Z) - (L_{T_i} + Z) \ln(L_{T_i} + Z) - (L_{T_j} + Z) \ln(L_{T_j} + Z) + Z \ln Z \quad (8A)$$

where $Z = 2R$ for the interaction of a tail with a linker and $Z = 4R + L_x$ for the interaction of two tails.

The value of the adsorbed charge and the lengths of unadsorbed LPE parts have been estimated by using numerical minimization of theoretical expression for the energy of the complex (1A). Calculations show that the most probable complex configuration is that with two macroions connected by a linker without tails. Chain length dependence of the number of adsorbed LPE monomers L_s and number of monomers in the linker L_x obtained from the numerical minimization of eq 1A are presented in Figure 15. For short LPE, the macroions are close to each other. LPE remains to be adsorbed on macroions up to some critical value of L where the linker appears and macroions start to separate. All nonadsorbed LPE part goes into a linker. The value of the adsorbed charge does not change with the linker appearance and remains almost

constant by further increase of the LPE length. Such a behavior is different from that for a complex comprised by one spherical macroion where the adsorbed charge sharply decreases with the tail appearance (Figure 15). The absence of the sharp change of the adsorbed charge after the linker formation for the complex formed by two macroions shows that, in contrast to the case of one macroion, the first-order phase transition is absent.

It can also be seen that the maximum degree of the overcharging is observed in the complex comprised by a single macroion. Addition of another macroion decreases the value of the adsorbed charge per one macroion due to the electrostatic repulsion between them. Correspondingly, the linker appears at lower values of LPE length (per one macroion).

The predictions of the theory for a necklace model²⁶ for the complex of two macroions with one long LPE have been shown as well, Figure 15. After the linker formation, the adsorbed charge decreases monotonously with an increase of L in this case, and this is the main difference from the modified theory.

The variation of radius and charge of the macroion in the complexes does not change the obtained dependences.

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