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ARTICLE

Packaging of a Polyelectrolyte into a Neutral Spherical Cavity

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ABSTRACT: Using molecular dynamics simulations, we studied how salt valency and concentration affect the forces required to tightly package a charged chain into a neutral, salt-permeable spherical shell. We find that in the presence of multivalent salt the packaging energy can be lowered to a value smaller than what is required to confine a neutral, fully flexible chain in the same cavity. In addition, we find that at high chain packing fractions there exists a threshold salt concentration above which the packaging free energy, ΔF, saturates, and we show how ΔF is completely independent of salt concentration when the salt is monovalent.

Understanding how polymers behave when constrained into tight spaces is a problem whose relevance stretches well beyond the field of polymer physics. Besides the several industrial applications in which this phenomenon plays a crucial role—colloidal stabilization,[1] filtration, drug delivery,[2] and flow injection[3]—the confinement of biopolymers is also a ubiquitous phenomenon in nature. Numerous biological processes rely on confinement to perform a diverse set of fundamental tasks, including the release of DNA prepackaged by molecular motors inside virus capsids[4−6] and bacterial gene swapping.[7]

The free energy barrier required to insert a flexible neutral chain with radius of gyration R_G into a spherical cavity of radius R ($< R_G$) can be obtained by a simple scaling argument that accounts for the for the excluded volume interactions among segments. The result is $\beta\Delta F \sim (R_G/R)^{3/(3\nu-1)}$,[8−10] where ν is the size exponent of the polymer. This expression is accurate even at very large densities, provided that the polymer structure is fluid-like.[11,12]

Although the physics concerning the confinement of neutral flexible polymers is well understood, such a claim cannot be made for charged chains. While the bulk properties of a charged chain in the presence of monovalent salt can be properly described within the framework of the Poisson−Boltzmann theory—the interaction between the charged monomers can be encoded into an effective chain bending rigidity, κ_e, whose value decreases as the electrostatic interactions are screened by addition of salt in solution—the phenomenology is much more complex either when the chain is confined within a small region[13] or when multivalent salt is present in solution. For instance, it is known[14−17] that the size of a charged chain in the presence of multivalent salt presents a nonmonotonic behavior as a function of salt concentration ρ_S, resulting in a conformational collapse when multivalent counterions neutralize the bare charge of the chain, and then a subsequent re-expansion, due to the inversion of the chain net charge, at larger values of ρ_S.[17]

A large body of literature has been devoted to unravel the behavior of a charged chain under confinement (see ref 13 and references therein), and most of it focuses on the problem of DNA packaging inside a viral capsid (see refs 18 and 19 for a short survey of modeling approaches). However, apart from a few exceptions,[20−22] most published works have not considered the explicit role of the salt.

The goal of this paper is not to provide a quantitative estimate of the DNA packaging energy, which would require a much more detailed description of the DNA, accounting for its double-stranded nature, its intrinsic bending rigidity, etc., but to understand, at a qualitative level, how the presence of multivalent salt in solution affects the process of packaging of a charged chain at very high densities and to show how the physical properties of polyelectrolytes may be altered by the presence of a confining agent.

In this paper we report the first direct molecular dynamics simulation where the effect of salt valency and concentration is explicitly accounted for when evaluating the energy required to densely package a charged chain inside a neutral, salt-permeable, spherical cavity.

We performed molecular dynamics simulations using the large-scale atomic molecular massively parallel simulator, LAMMPS,[23] with a Langevin thermostat. Following Stevens and Kremer,[24] the polyelectrolyte is described with a bead−spring model and the solvent is represented by a continuous medium with a uniform dielectric constant, ε. The CPU time for all simulations was ~800 days on an Intel Xeon X5355 2.66 GHz processor.

The chain is composed of $N = 324$ monomers of diameter σ, each carrying a charge e. Connections between adjacent monomers are described via a Fene potential with a maximum extension of 2σ and a spring constant $k = 7\varepsilon_{LJ}/2$. Included in the continuous medium are monovalent counterions of diameter σ and z-valent salt molecules with salt concentration, ρ_S, ranging between 0 and 100 mM. Excluded volume interactions between any two charged particles in the system are enforced via a shifted-truncated Lennard-Jones (LJ) potential with coupling constant $\varepsilon_{LJ} = k_BT/1.2$, where k_B is the Boltzmann constant and T the absolute temperature. Finally, the electrostatic interaction between charged particles, $E_c = k_BT\lambda_B z_i z_j/r$, is evaluated by means of the Ewald summation, where z_i and z_j are the valences of the particles and λ_B is the Bjerrum length. λ_B has been set to 3σ, as this is typical of many flexible polyelectrolytes.

We have selected this particular coarse-grained model to describe the polyelectrolyte because its physical properties, and specifically its condensation and resolubilization in the presence of multivalent salt, have recently been the subject of a careful study.[17] This model thus represents a good starting point for our analysis. The choice of the ions size is also important as the phenomenon of charge inversion is quite sensitive to it.[17] In most

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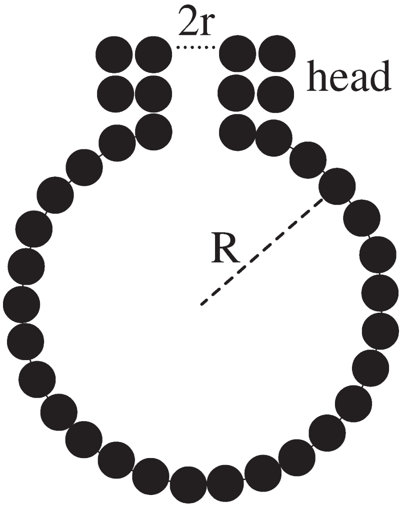

Figure 1. Sketch of the cavity where we insert the polyelectrolyte. The cavity is built by placing 482 neutral in a (4,4) icosadeltahedral configuration.[25]

of our simulations we have chosen ions of diameter σ because under these conditions the effect is maximized.

We modeled the spherical cavity with 482 neutral particles of diameter σ arranged into a densely packed spherical crystal configuration.[25] The position of the cavity particles is held constant throughout the simulations. The cavity is permeable to both salt molecules and counterions, but it is impermeable to the polyelectrolyte. The repulsion between cavity particles and polyelectrolyte is imposed via the shifted-truncated Lennard-Jones potential described above. The radius of the spherical cavity is fixed to $R = 5\sigma$. This particular value enables the inserted polyelectrolyte to reach sufficiently large chain packing fractions $\eta \simeq 0.44$, while allowing for the full insertion of the chain. The polyelectrolyte is inserted into the cavity through an opening (denoted the head of the shell) of radius $r = 1.5\sigma$, defined by a double-layered hollow cylinder of height 2σ as sketched in Figure 1. The radius of the opening has been selected to minimize the formation of configurations containing hairpins that could potentially slow down the equilibration of the system. The two layers of monomers on top of the head have been added to prevent the chain from achieving unphysical configurations by sliding away from the opening and becoming trapped with part of the chain inside the cavity and part of the chain outside. We performed our simulations according to the following scheme. First, the polyelectrolyte with one of its extremities pinned to the head of the shell and the rest of the monomers outside the cavity is allowed to thermalize in the presence of the salt. Subsequently, we pull the chain into the cavity by applying a local constant force, f, at the shell's opening. For a given force we find the average chain fraction inside the cavity x/N. To guarantee chain equilibrium at all inserted lengths, each simulation at a given force, f_i, begins with the thermalized configuration obtained by a simulation at the slightly smaller force f_{i-1}. From a set of simulations at different values of the applied force, we can extract the functional dependence of the force on the average packed chain fraction. To determine the total free energy of packaging ΔF, i.e., the free energy difference between the packaged and unpackaged chain at a given salt concentration and valency, we go one step further and fit the force to a polynomial function which we integrate over the inserted chain fraction. The whole process is repeated for different salt concentrations and valency.

To understand the role of electrostatic interactions in the packaging of a charged chain, we analyzed how the force and free energy required to insert the polyelectrolyte into the cavity depend on salt concentration and valency. Specifically, we analyzed salts with valency $z = 1$, 2, and 3 at various concentrations and compared the results to those relative to a fully flexible neutral polymer ($z = 0$).

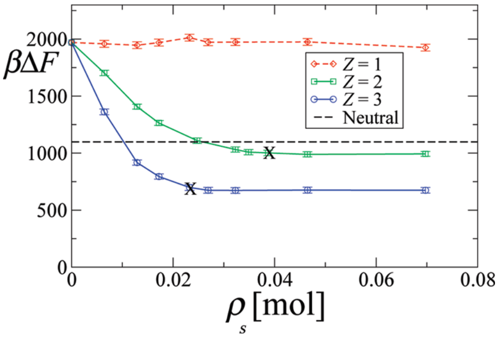

Figure 2. Free energy required to *fully* insert the chain inside the cavity as a function of salt concentration and valency. The dashed line indicates $\beta\Delta F$ for a neutral chain in the presence of 108 neutral particles. The X symbols indicate the location of the neutralization concentrations for divalent ($\rho_S = 0.039$ mol) and trivalent ($\rho_S = 0.023$ mol) salt.

Figure 2 displays our findings. The work required to insert the whole chain inside the cavity, $\beta\Delta F$, is plotted against salt concentrations for different values of z.

There are several aspects of our results that are quite interesting, as they clearly uncover a nontrivial dependence of the packaging energy on salt concentration and valency. Notice that for monovalent salt $\beta\Delta F$ is independent of ρ_S. This is quite surprising because screening the electrostatic interactions leads to a more flexible chain, which should result in an easier insertion. The phenomenology is richer for multivalent salt. Figure 2 reports the total packaging energy in the presence of divalent and trivalent salt molecules. In this case we observe a strong dependence on salt concentration up to the chain neutralization point,[16,17] i.e., that salt concentration for which the charge carried by the multivalent ions bound to the chain equals the total bare charge of the chain.

At higher values of ρ_S, $\beta\Delta F$ plateaus to a constant value. The low density behavior is consistent with the picture of the chain collapse in the presence of multivalent ions as observed in both experiments[14] and numerical simulations,[17] but we see no effect of chain charge inversion in the packaging energy at large ρ_S.

Finally, we computed the total work required to insert a flexible neutral chain into the cavity. To compare the results of the charged chains with those of neutral chains in a manner that properly accounts for the excluded volume interactions, when computing $\beta\Delta F$ for the neutral chain, we forced $N_0 = 108$ neutral mobile particles inside the cavity. N_0 corresponds to the number of counterions that surrounds the confined charged chain in the presence of trivalent salt at the neutralization point. The dashed line in Figure 2 represents $\beta\Delta F$ for the neutral flexible chain, which turns out to be substantially larger than $\beta\Delta F$ for $z = 3$. This is an important results of our simulations: the work required to insert a charged chain into a cavity not only decreases dramatically in the presence of multivalent ions, but it is even smaller than what is required to package a neutral chain.

Let us now turn our attention to the unexpected dependence of our results on salt concentration. We report in Figure 3 the explicit dependence of the insertion force as a function of the fraction of the chain inserted into the cavity, x/N, at different salt concentrations, for the case of $z = 3$. As expected, the force increases as the chain is inserted inside the cavity; however, for salt concentrations above the neutralization point, the curves are indistinguishable. That means that adding extra salt into the system has no effect throughout the insertion process. This is quite surprising, as one would naively expect the chain to have a

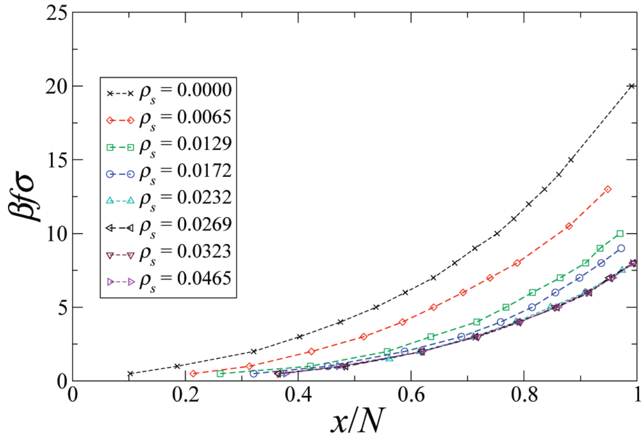

Figure 3. Force required to insert a fraction of monomers x/L when $z = 3$ for different values of salt molar concentrations.

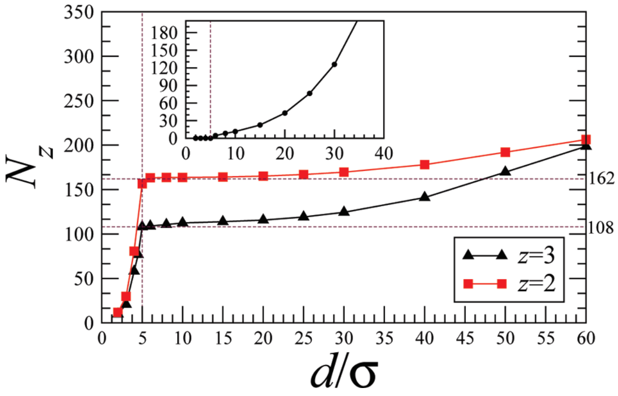

Figure 4. Number of multivalent counterions, N_z, within a distance d from the center of the cavity for $z=2$ and $z=3$. The inset shows the total number of monovalent charges (excluding the chain) as a function of d, for $z = 3$. The horizontal dashed line indicates the number of multivalent counterions required to screen the bare charge of the chain, and the vertical dashed line indicates the radius of the cavity. In both cases we selected a salt density above the chain neutralization point of 0.0465 mol.

radius of gyration which re-expands at salt concentrations above the neutralization point, making it harder for the chain to be inserted. The problem with this argument is that it assumes that the behavior of the chain inside and outside the cavity is identical, while actually the confining shell imposes a strong bound on the number of charges allowed around the polyelectrolyte. Indeed, the mechanism behind the re-expansion of the chain is quite complicated, and for this model it has been shown[17] to be related to the ability of extra multivalent counterions to bind to the chain and subsequently attract co-ions in solution, effectively building up an a second layer of charges along the chain. Such a mechanism seems to be strongly suppressed inside the cavity, where excluded volume effects become large.

To investigate this possibility, we measured the number of multivalent ions, N_z, inside a sphere concentric with the cavity as a function of its radius d, for salt concentrations larger than those required to simply neutralize the chain bare charge.

The results are shown in Figure 4. As the radius of the sphere approaches the size of the shell $R=5\sigma$, N_z increases quite sharply, indicating the presence of densely packed multivalent ions. N_z plateaus at $d=R$ to a constant value equal to N/z (108 for $z=3$ and 162 for $z=2$), the neutralization charge. That means that the shell contains only as many ions as they are necessary to neutralize the chain and that any extra multivalent counterion in solution remains outside the shell. Finally, N_z slowly increases for $d \gg R$ as it detects all the remaining ions in solution (216 for $z=2$ and $z=3$). The inset of Figure 4 shows a similar analysis for $z=3$, where N_z now accounts for the total number of monovalent free ions in solution, independent of their charge sign. Clearly, apart from the chain and the trivalent ions, no other charges are present inside the cavity.

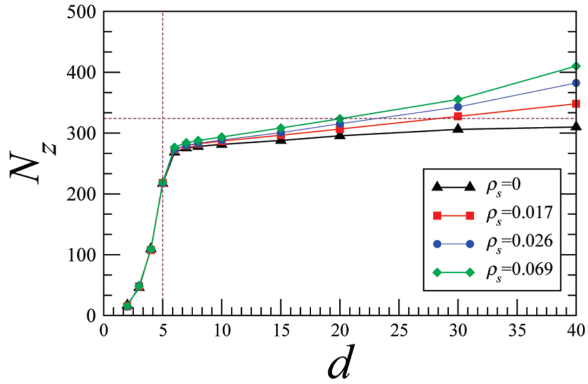

Figure 5. Number of counterions, N_z, within a distance d from the center of the cavity for different values of monovalent salt concentration. The horizontal dashed line indicates the bare charge of the chain, and the vertical dashed line indicates the radius of the cavity.

These results suggest that confinement alters the delicate entropic/energetic balance which favors the overcharging/re-expansion mechanism of the chain in the bulk. In other words, the large energetic cost that would be associated with the re-expansion of the charged chain inside the cavity makes the overcharging of the chain unfavorable.

Figure 5 shows N_z for a chain in the presence of monovalent salt as a function of ρ_S. The result is quite illuminating. Although the trend is similar to what we found for the multivalent salt, there are crucial quantitative differences. First of all, the total number of counterions inside the shell does not account for the bare charge of the chain ($-324e$), leaving unscreened electrostatic interactions between the monomers of the polyelectrolyte inside the shell. Not surprisingly, in this last case the insertion energy is the greatest. Another fundamental difference is that N_z inside the shell ($d \le 5\sigma$) is independent of ρ_S. Notice how the dense regime begins to plateau at $d=6\sigma=(R + 1\sigma)$, indicating the presence of a layer of counterions surrounding the sphere. These results suggest that we can think of the cavity as a spherical rigid volume containing the net unscreened charge Q of the chain and that any amount of monovalent salt added to the system only has the effect of screening such an excess charge by forming external layers of counterions around the cavity. Such layering will obviously not affect the internal energy of the chain that is rigidly confined within the cavity and explains why ΔF, as shown in Figure 2, is independent of salt concentration for monovalent salt.

To ensure that the latter result is not an artifact of our model, specifically of our choice of ion size, we repeated our measures in the presence of counterions and monovalent salt with size $\sigma/2$ and $\sigma/4$ for three different salt concentrations—this leads simultaneously to a larger binding energy between ions and chain. The results are shown in Figure 6 and clearly support our previous findings: the packaging energy is independent of salt concentration. The large drop in ΔF is due to the corresponding decrease in total volume fraction inside the cavity.

Clearly, as we know that the polyelectrolyte under investigation does present a nonmonotonic behavior of the radius of gyration as a function of salt concentration for multivalent ions in the bulk, we can expect the existence of a threshold chain packing fraction below which the constrained imposed by the confining cavity is negligible and bulk properties are recovered.

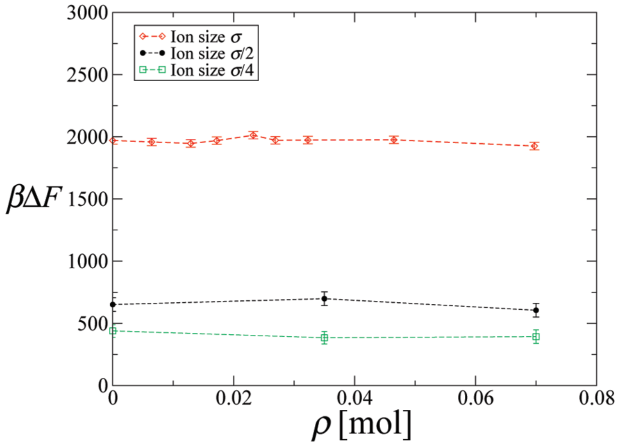

Figure 6. Packaging energy of a polyelectrolyte as a function of monovalent salt concentration for three different ion sizes, σ, $\sigma/2$, and $\sigma/4$.

Our results hold for chain packing fractions as large as $\eta \sim 0.44$. It is possible that at extreme densities all counterions are expelled out of the cavity to accommodate the full size of the chain. To test this scenario, we forced a chain of size $N = 500$ into the same cavity in the presence of monovalent salt, which is the one that has the weakest binding energy to the chain. We find that the number of bound counterions does not decline, but rather increases, as more monomers are forced in by pushing their way against the Lennard-Jones repulsion of the neutral cavity particles. We have therefore no reason to expect a different phenomenology in this extreme density regime.

In conclusion, we have shown how the explicit presence of salt in solution has a fundamental impact on the behavior of a charged chain under confinement. We have shown how multivalent salt can compensate for the electrostatic repulsion among the chain monomers, leading to a dramatic decrease of the packaging energy. We have also found that monovalent salt plays no role in the packaging energy when the cavity is neutral and salt-permeable. Although the quantitative aspects of our results are dependent on the specific choices we have made in selecting the model for the polyelectrolyte and for the salt ions, the qualitative aspect of our results are nevertheless quite general and should apply to any polyelectrolyte under comparable conditions.

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