## Article

# Role of Charge Regulation and Fluctuations in the Conformational and Mechanical Properties of Weak Flexible Polyelectrolytes

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Abstract: This work addresses the role of charge regulation (CR) and the associated fluctuations in 1 the conformational and mechanical properties of weak polyelectrolytes. Due to CR, changes in the 2 pH-value modifies the average macromolecular charge and conformational e quilibria. A second 3 effect is that, for a given average charge per site, fluctuations can alter the intensity of the interactions Δ by means of correlation between binding sites. We investigate both effects by means of Monte Carlo 5 simulations at constant pH-value, so that the charge is a fluctuating quantity. Once the average charge 6 per site is available, we turn off the fluctuations by assigning the same average charge to every site. 7 A constant charge MC simulation is then performed. We make use of a model which accounts for 8 the main fundamental aspects of a linear flexible polyelectrolyte *i.e.* proton binding, angle internal a rotation, bond stretching and bending. Steric excluded volume and differentiated treatment for 10 short-range and long-range interactions are also included in the model. It can be regarded as a kind of 11 "minimal" model in the sense that contains a minimum number of parameters but still preserving the 12 atomistic detail. It is shown that, if fluctuations are activated, gauche state bond probabilities increase, 13 and the persistence length decreases, so that the polymer becomes more folded. Macromolecular 14 stretching is also analyzed in presence of CR (the charge depends on the applied force) and without 15 CR (the charge is fixed to the value at zero f orce). The analysis of the low force scaling behavior 16 concludes that Pincus exponent becomes pH-dependent. Both with and without CR, a transition from 17 1/2 at high pH-values (phantom chain) to 3/5 to low pH-values (Pincus regime), is observed. Finally, 18 the intermediate force stretching regime is investigated. It is found that CR induces a moderate 19 influence in the force-extension curves and persistence length (which in this force regime becomes 20 force-dependent). It is thus concluded that the effect of CR on the stretching curves is mainly due to 21 changes in the average charge at zero force. It is also found that, for the cases studied, the effect of 22 steric excluded volume is almost irrelevant compared to electrostatic interactions. 23

- <sup>24</sup> Keywords: polyelectrolytes; charge regulation; charge fluctuations; weak polyelectrolyte; annealed
- <sup>25</sup> polyelectrolyte; Monte Carlo simulation; semi-grand canonical ensemble; binding equilibria;
- <sup>26</sup> conformational equilibria; constant pH ensamble; stretching; scaling law

(c) (i)

Charge regulation (CR) is defined as the capability of ionizable macromolecules, nano-particles

# 27 1. Introduction

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and surfaces to modify their ionization state as a response to external physico-chemical perturbations. 29 In contrast to strong polyelectrolytes, such as DNA or RNA, whose phosphate groups are charged in 30 very different environmental conditions, weak polyelectrolytes are specially sensitive to changes in 31 the pH-value or the ionic strength, solvent composition, interactions with metal ions or other charged 32 molecules. The paradigmatic mechanism of CR is the binding of small ions present in solution, and, 33 in particular, acid-base equilibria, due to the ubiquitous presence of protons in aqueous solutions 34 [1,2]. In a wide range of situations, the physicochemical behaviour of charged polymers cannot be 35 understood without the presence of CR. A few examples would be the stability of colloidal systems [3], 36 protein-surface [4,5], protein-protein [6,7] and protein-polyelectrolyte [8,9] interactions, nano-particle 37 coating [10], supramolecular chemistry [11,12], ligand-receptor binding in biochemistry [13–15], drug 38 delivery [16], protein folding [17], among many others. 39 Besides the ability to modulate the electric charge to external changes, there are two relevant aspects of CR which make the difference compared to systems with fixed charge. Firstly, although CR can also take place in rigid structures, weak polyelectrolytes use to be very flexible. That means that, 42 due to the presence of electrostatic interactions between charged groups, the system tries to minimize 43 the electrostatic repulsion (or maximize the attraction) between charged groups by means of changes 44 in the conformational structure. In the same way, modifications in the conformational structure affect 45 the interactions between charged sites and thus their ionization state. Conformational and ionization degrees of freedom are thus highly coupled [18,19]. The natural mechanism for this fact is the rotation 47 of the chemical bonds. It has been recently shown that one can even build effective rotational potentials 48 which explicitly depend on the pH-value [20,21]. A natural conclusion of this fact is that the stretching 49 properties of weak-polyelectrolytes should depend up to some extent on the ionization state of the 50 macromolecule (i.e. the pH-value and the ionic strength), since the application of an external force modifies the conformational structure of the macromolecule. 52 In the last two-decades, the development of single-molecule force spectroscopy has lead to a 53 huge expansion of the field of mechano-chemistry [22]. Mechanically induced chemical reactions or 54 conformational transitions have been recently described both in neutral and charged macromolecules 55 [23–29]. The stretching of strong polyelectrolytes such as DNA and RNA, two strong polyelectrolytes, have also been the subject of a number of studies, which results to be extremely dependent on the 57 valence and concentration of the counterions [30-32]. It has been also shown that self-avoiding 58 electrostatic repulsion forces produce new elastic regimes and scaling behaviors [33–35]. In a recent 59 paper, our group has explored the possibility of induced charge regulation by means of the application 60 of an external force to a weak polyelectrolyte [36]. Mechanical stretching leads to an increase in the 61 distance between charged groups and significant changes in the degree of protonation are observed 62 at certain pH and ionic-strength conditions. In the same way, changes in the pH-value affect the 63 extension/force curves. 64 A second aspect involved in CR is that the charge is no longer a fixed quantity, but a fluctuating 65 one, which can lead to surprising effects. For instance, it is well known that, under certain conditions, 66 charge fluctuations (CF) can produce attraction between two macromolecules with the same average 67 charge. This phenomenon was firstly predicted in a classical work by Kirwood and Shumaker [37], 68 who used statistical mechanics perturbation methods, and since then has been verified in a number 69 of simulation and experimental studies on protein-ligand, protein-protein and protein-membrane 70 interactions [2]. Simulation methods have proved to be specially useful in the quantification of this 71 effect by turning on and off the CF [6]. However, the influence of CF in the conformational and 72

<sup>73</sup> mechanical properties of flexible weak polyelectrolytes has been so far hardly addressed. Most the

studies aimed to determine the conformational properties (end-to-end distance, radius of gyration,

persistence length, etc) the charge is taken as a constant, sometimes by considering the polymer a
 string with constant charge density [38,39]. In a number of works, CF are taken explicitly into account

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by regarding the polymer as a set of punctual protonating sites liked by rigid or harmonic bonds, so
that rotational conformations are not taken into account [40–43].

The present work addresses the role of charge regulation and the associated fluctuations in

the conformational and mechanical properties of weak polyelectrolytes by means of Monte Carlo

simulations at constant pH-value, so that the charge is a fluctuating quantity. Once the average

<sup>82</sup> charge *per* site is available, we turn off the fluctuations by assigning the same average charge to every

site. A constant charge MC (ccMC) simulation is then performed. With this aim we make use of a

<sup>84</sup> model, described in section 2, which accounts for the main fundamental aspects of a linear flexible

polyelectrolyte *i.e.* proton binding, dihedral angle rotation, bond stretching and bending. Steric
 excluded volume and differentiated treatment of short range (SR) and long-range (LR) interactions are

<sup>87</sup> also included. It can be regarded as a kind of "minimal" model in the sense that contains a minimum

number of parameters but still preserving the atomistic detail. In section 3 the effect of CF in the

<sup>89</sup> conformational properties of a weak polyelectrolyte at zero force is analyzed. Section 4 focuses on the

<sup>90</sup> influence of CR and CF in the scaling behavior at low forces, *i.e.* linear and Pincus regime. Finally,

<sup>91</sup> section 5 is devoted to the effect of CR and CF in the extension-force curves.

## 92 2. Charge regulation and stretching of weak polyelectrolytes

#### 93 2.1. Minimal model of a weak flexible polyelectrolyte

Let us consider a model, depicted in Figure 1, which captures the most relevant aspects of a 94 weak flexible polyelectrolyte (proton binding, dihedral angle rotation, bond stretching and bending) 95 but involving a minimum number of parameters. Bond rotation represents the main mechanism of 96 stretching at moderate and even relatively high forces. Bond stretching and bond angle bending are 97 only relevant at very high forces (typically larger than 500 pN) for which the stretching regime is fully 98 independent of the ionization state, as shown in a recent publication [36]. Assuming that the polymer 99 is symmetric (it presents planar symmetry when it is fully extended) complications due to tacticity 100 can be avoided. As usual, the ionization state of the macromolecule is described by a set of variables 10:  $s = \{s_i\}$  with  $i=1 \dots N$ , where  $s_i = 1$  indicates that the site is protonated and  $s_i = 0$  otherwise. This is 102 the basis of the Site Binding (SB) model [1]. The conformational state is determined by the set  $c = \{\phi_i\}$ 103 ,  $j=1 \dots M$ , where  $\phi_j$  is the rotational angle of the bond j. It will be assume that only the rotational states 104 corresponding to energy minima (typically *trans* (t), gauche+  $(g^+)$  and gauche-  $(g^-)$ ) are significantly 105 populated. This is the central assumption of the Rotational Isomeric State (RIS) model, firstly proposed 106 by Flory in order to calculate conformational properties of neutral chain molecules [?]. Combining 107 RIS and SB models, we obtain the SBRIS model, previously proposed in order to deal with ionization 108 and conformational degrees of freedom on the same foot [18,19,21]. The SBRIS free energy can be 109 expressed as 110

$$F(s,c) = \mathscr{F}_{rot} + \mathscr{F}_{p} + \mathscr{F}_{E} + \mathscr{F}_{lenght} + \mathscr{F}_{angle} + \mathscr{F}_{SEV} + W$$
(1)

and

$$\mathscr{F}_{\rm rot} = \sum_{j=1}^{M} \epsilon_{{\rm rot},j} \left( \phi_j \right) \tag{2}$$

represents the RIS free energy contribution which is the sum of the torsional energies  $\epsilon_{\text{rot},j}(\phi_j)$  of the *M* rotating bonds. Under the RIS approximation, we choose for  $\phi_j$  the possible values  $\phi_1 = 0$  (*t*),  $\phi_j = +2\pi/3 \ (g^+)$ , and  $\phi_j = -2\pi/3 \ (g^-)$ . Since the chain is symmetric,  $\epsilon_{\text{rot}} (+2\pi/3) = \epsilon_{\text{rot}} (-2\pi/3)$ . In this work we will assume that the three rotational states have the same energy so that  $\epsilon_{\text{rot},j}(\phi_j) = 0$ .

The term  $\mathscr{F}_{p}$  represents the contribution of proton binding to the free energy

$$\mathscr{F}_{p} = \sum_{i=1}^{N} \mu_{i} s_{i} = \sum_{i=1}^{N} \ln (10) (pH - pK_{i}) s_{i}$$
(3)

4

where  $\beta = 1/k_BT$  and  $\bar{k}_i = \ln(K_i a_H)$  is the reduced chemical potential corresponding to the proton activity  $a_H$  and the protonation constant of site i,  $K_i$ . A site is positively charged when it is protonated. The chain contains a protonating site every three chain positions so that it can be regarded as a simplified version of linear poly-ethilene imine (LPEI), for which a complete conformational study is reported in ref. [19]. For the particular case of identical sites and bonds  $pK_i = pK$ .

The electrostatic interaction energy  $\mathscr{F}_E$  between charged sites is split into short range and long range contributions

$$\mathscr{F}_{\mathrm{E}} = \mathscr{F}_{\mathrm{SR}} + \mathscr{F}_{\mathrm{LR}}.$$

This distinction is necessary due to the fundamental differences in the physical mechanism of SR and LR interactions. LR interactions are chemically unspecific and mediated by the solvent and they can be reasonably described by simple pair-potentials. In this work the Debye-Hückel (DH) potential has been chosen

$$\beta \mathscr{F}_{LR} = \sum_{i=1}^{N} \sum_{j=i+2}^{N} \frac{l_B}{d_{ij}} e^{-\kappa d_{ij}} s_i s_j \tag{4}$$

where  $d_{ij}$  is the distance between the sites *i* and *j*,  $\ell_{\rm B} \simeq 0.7$  nm and  $\kappa^{-1}$  (nm) = 0.304/ $\sqrt{I({\rm M})}$ represent, respectively, the Bjerrum and the Debye lengths in water at 298.15 K and ionic strength *I*. SR interactions between neighboring sites, however, are mediated by the macromolecular skeleton and they strongly depend on the chemical environment of the interacting sites [1,19]. As a consequence, they need specific parameters to be described.  $\mathscr{F}_{\rm SR}$  reads

$$\beta \mathscr{F}_{SR} = \ln (10) \sum_{i=1}^{N-1} \epsilon_{int,3i-1} (\phi_{3i-1}) s_i s_{i+1}$$
(5)

where  $\epsilon_{\text{int},3i-1}(\phi_{3i-1})$  corresponds to the interaction energy between two neighboring sites linked by a bond in a given rotational state  $\phi_{3i-1}$ . This term clearly couples the ionization and the conformational degrees of freedom, a characteristic feature of the SBRIS model.

The elasticity of the bond length  $l_i$  and bond angle  $\alpha_i$  are included *via* the harmonic potentials

$$\mathscr{F}_{\text{length}} = \sum_{j=1}^{M} \frac{k_{\text{length},j}}{2} \left( l_j - l_{j,o} \right)^2, \tag{6}$$

and

$$\mathscr{F}_{\text{angle}} = \sum_{j=1}^{M-1} \frac{k_{\text{length},j}}{2} \left( \alpha_j - \alpha_{j,o} \right)^2,\tag{7}$$

where  $k_{\text{length},j}$  and  $k_{\text{angle},j}$  are the bond stretching and bending force constants and  $l_{j,o}$  and  $\alpha_{j,o}$  denote the equilibrium length and the equilibrium bending angle of bond *j*, respectively. The stretching of the chain exerted by the the applied force *F* is quantified by the mechanical work

$$W = -Fr \tag{8}$$

where *r* is the end-to-end vector. Finally  $\mathscr{F}_{SEV}$  accounts for the steric excluded volume (SEV) effects, due to the finite size of the sites and chemical groups composing the chain. They are implemented by means of a hard-sphere potential

$$\mathscr{F}_{\text{SEV}} = \sum_{i,j=i+4}^{M+1} \begin{cases} \infty & d_{ij} \le R_i + R_j \\ 0 & d_{ij} > R_i + R_j \end{cases}$$
(9)

where  $R_i$  is the hard-sphere radius of site *i*. They will be turned on and off in the computations throughout this work in order to quantify their importance in the conformational and stretching properties. More details about the model and its approximations can be found in ref. [36].



**Figure 1.** Outline of the two different kind of simulations of a weak flexible polyelectrolyte performed in this study: Semi-Grand Canonical Monte Carlo (SGCMC) and constant charge Monte Carlo (ccMC) simulations. In SGCMC the pH-value is kept constant and the charge is free to fluctuate by means of proton equilibria (blue and cyan circles depict protonated and deprotonated sites, respectively). Conversely, in ccMC simulations the charge of a site is fixed to its average value (purple circles). Grey circles represent inert sites. The bonds holding two ionizable sites are allowed to rotate. Bond stretching and angle bending and the stretching due to the action of an external force are also included in the model.

#### 135

#### 136 2.2. Constant charge versus constant-pH simulations

In order to explore the effect of CR and CF on the structural and stretching properties of a single linear weak polyelectrolyte, we make use of a Semi-Grand Canonical Monte Carlo (SGCMC) code previously developed by our group [19,21]. This program has been recently used to study the possibility of inducing CR by means of mechanical stretching [36]. Since in the present work we are particularly interested in the behavior of the system when CR and CF are turned on and off, the code has been modified in order to perform both constant-pH (SGCMC) and constant charge Monte Carlo (ccMC) simulations, as outlined in Figure1. In SGCMC simulations, the pH is the control variable and CR is explicitly taken into account. Statistical averages of the conformational, stretching and binding properties are computed and they are pH-dependent. The average charge per monomer  $\theta$  is defined as

$$\theta = \frac{\langle N_+ \rangle}{N} = \langle s_i \rangle , \qquad (10)$$

where  $\langle N_+ \rangle$  is the average number of protonated sites. Note that since the simulations are performed at constant pH,  $N_+$  is a fluctuating quantity. The macroscopic quantity which measures the intensity of the fluctuations is the Binding Capacitance [2,13] which is defined as the variance of the probability distribution of  $N_+$ 

$$C = \left\langle \left(N_{+} - N\theta\right)^{2} \right\rangle = N \left(\frac{\partial\theta}{\partial\mu}\right)_{F}$$
(11)

<sup>137</sup> The second identity can be proved by means of elementary statistical mechanics. As commented in

the introduction, CF are the responsible of interesting phenomena such as the effective attraction ofmacromolecules with the same charge sign, under certain conditions [37].

In order to assess the relevance of CF we will compare the same conformational property of interest obtained from ccMC and SGCMC simulations for the same average charge. Unlike SGCMC, in ccMC simulations CR and CF are switched off. The charge state of each site is imposed beforehand and kept constant at the value  $\theta$  obtained from the equivalent SGCMC simulation, which means that  $\theta$  is an output for SGCMC and an input for ccMC. Since correlations and CF are absent, the ionization and conformational degrees of freedom are now decoupled. The ionization states of two different sites become independent too. As a result, the average electrostatic interaction energy reads

$$\langle \epsilon_{int,i,j}(c) s_i s_j \rangle \simeq \epsilon_{int,i,j}(c) \langle s_i s_j \rangle \simeq \epsilon_{int,i,j}(c) \theta^2$$

which is exact only in the limit of long enough distances between sites. This is equivalent to take  $s_i = \theta$ 

in Eqns. 4 and 5. As a consequence, only conformational degrees of freedom are free to change and  $\mathscr{F}_p$ 

in Eqns. 4 and 5. As a consequence, only confor does not contribute to the free energy.

Concerning the conformational properties, special attention will be paid to the *gauche* state probability

$$P(g) = \frac{\langle M_g \rangle}{M}$$

where  $\langle M_g \rangle$  is the average number of bonds in the *gauche* state, and to the persistence length  $l_p$ , defined as the average sum of the projections of all the bonds  $j \ge i$  on a given bond i in an infinitely long chain

$$l_{\rm p}/l_0 = \sum_{j\geq i} \left< \mathbf{b}_i \cdot \mathbf{b}_j \right> \tag{12}$$

 $\mathbf{b}_i$  denote unitary vectors pointing to the direction of the bonds.  $l_p$  is related to the average square end-to-end distance  $\langle \mathbf{r}^2 \rangle$  by the relationship [44]

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$$l_{\rm p} = \frac{\langle \mathbf{r}^2 \rangle}{2Ml_o} + \frac{l_0}{2} = \frac{\langle (\mathbf{r}_{M+1} - \mathbf{r}_1)^2 \rangle}{2Ml_o} + \frac{l_0}{2}$$
(13)

## 147 2.3. Parameters used in the simulations

We consider a chain with N = 50 identical ionizable sites with pK = 9, a large enough number 148 to avoid end effects. The interactions parameters are  $\varepsilon_{int}(t) = 1$  and  $\varepsilon_{int}(g^+) = \varepsilon_{int}(g^-) = 3$ , which 149 means that a bond holding two neighbor charged sites have a very small probability of being in the 150 gauche state. As in LPEI, the chain consists of an ionisable site every two inert sites (i.e. 148 nodes or 147 bonds), as shown in Fig 1. When excluded volume interactions are included, the sites are 152 treated as hard spheres with radii R = 1.7 Å and R = 1.55 Å for inert and ionisable sites, respectively. 153 These values were used in a previous study on the conformational and binding properties of LPEI 154 [19]. For simplicity, only the bonds with pending ionisable sites are allowed to exhibit free internal 155 angle rotation ( $\epsilon_{\sigma}(t) = \epsilon_{\sigma}(g^+) = \epsilon_{\sigma}(g^-) = 0$ ) whereas the rest of bonds are forced to remain in 156 trans conformation. All bonds are considered to have equal bond stretching and bending parameters 157  $l_0 = 1.5 \text{ Å}, \alpha_0 = 120^{\circ}, k_{bond} = 300 \text{ kcal mol}^{-1 - 2} \text{ and } k_{ang} = 0.01 \text{ kcal mol}^{-1} \text{ deg}^{-2}$ , which are the 158 typical values used in Molecular Dynamics for C-C bonds [45]. The simulations are performed at room 159 temperature T = 298.15 K. The results presented represent the average over 8 to 16 different SGCMC 160 simulations, which have been equilibrated in the first  $5 \times 10^{7}$  configurations. The thermal averages 161 have been computed in the following  $1 \times 10^9$  realizations. More computational details about the used algorithm, such as trial probabilities, can be found in ref. [36]. 163

#### <sup>104</sup> 3. Effect of charge regulation in the binding and conformational properties at zero force

Let us first analyze the influence of charge regulation on the conformational properties of the polyelectrolyte when no mechanical force is applied. Since the average degree of protonation  $\theta$  is at once output from the SGCMC and input for ccMC simulations, we firstly discuss the dependence of this quantity on the pH-value. The resulting titration curves are shown in Figure 2a for four different ionic strengths ranging from 1 to 0.001 M (from top to bottom). As a general trend, it is observed that  $\theta$ decreases in lowering the ionic strength, since electrostatic interactions get stronger and more energy is needed to protonate a site.

It is important to recall that in our model nearest neighbor (SR) interactions and LR interactions are treated in a different way. SR are described by chemically specific parameters ( $\varepsilon_{int}$ ) which, in accordance with experiments [1,19], are taken as independent of the ionic strength. This fact implies that SR interactions tend to create correlations between the protonation of neighboring sites. At  $\theta \simeq 1/2$ , which corresponds to the plateau observed in the titration curve, an ordered structure consisting of alternated protonated and deprotonated sites is formed [46]. Due to the interaction with the two neighbors, the empty sites now bind protons with a different effective pK-value (roughly  $pK - 2\varepsilon_{int}$ ). As a result, the titration curve resembles that of a system with two different pK-values.

Conversely to SR, LR interactions are described by the DH potential, which becomes long ranged 180 at low ionic strengths. That means that LR interactions tend to destroy binding correlation between neighboring sites, and it is more amenable to mean-field treatments. This is reflected in the shape of 182 the titration curves when the ionic strength decreases, which become flatter and the plateau at  $\theta \simeq 1/2$ 183 progressively disappears. The differences between SR and LR interaction can be better understood by 184 computing the capacitance C as a function of the pH-value, which is shown in Figure 2b. As expressed 185 by eqn. 11, C allows to directly quantify the intensity of charge fluctuations. It can be observed that, for 186 187 large ionic strength, C present two maxima at pH  $\simeq$  6 and pH  $\simeq$  9, which correspond to the inflections points in the titration curve and a maximum in the charge fluctuation. On the other hand, a minimum 188 value is observed at pH  $\simeq$  7.5, related to the presence of the "ordered" alternating state at  $\theta \simeq 1/2$ . 189 When the ionic strength decreases, C becomes progressively wider and flatter until the maxima and 190 the minimum disappear as result of the decorrelation introduced by LR interactions. 191

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Let us turn CR and CF off and discuss the effect in the conformational properties of the 192 probabilities. The  $\theta$ -value obtained from SGCMC is now used as an input in the ccMC simulations, 193 in which all the sites have a fixed charge equal to  $\theta$ . Unfortunately, up to our knowledge, there is no established theory for the role of CF in the intra-molecular interactions and the consequences on the 195 conformational structure of flexible weak polyelectrolytes. However, the contribution of CF in the 196 force between two polyelectrolytes have been the object of a number of previous works, both from the 197 theoretical and experimental point of view [6]. The interaction energy U(R) between two identical 198 macromolecules with average charge Q separated by a distance R depend not only on their average charge but also on their capacitances. In absence of counterions, it is given by [2,37] 200

$$\beta U(R) = \frac{l_{\rm B}Q^2}{R} - \frac{l_{\rm B}^2}{R^2}QC - \frac{l_{\rm B}^2}{2R^2}C^2 \tag{14}$$

Probably the most striking and counter-intuitive consequence of this theory is the presence of negative, attractive, terms in (14), despite the charge of both interacting molecules has the same sign. The first
term corresponds to the usual coulombic repulsion. The second and third term in the r.h.s. of (14) are
direct consequence of CR and CF.

One of the objectives of this work is clarify whether a similar effect can play a role, not only for 205 inter-molecular interactions between charged macromolecules, but in the intra-molecular interactions 206 between different regions of a polyelectrolyte. If this was the case, one could expect the polymer to be 207 more folded than in absence of CR due to the attractive contribution of CF. In order to put some light 208 on this point, we have investigated the influence of CR in two quantities: the probability of a bond to 209 be in gauche state, P(g), and the persistence length  $l_p$ . P(g) is a 'local' conformational property, in the 210 sense that it refers to the behavior of a single bond. The persistence length,  $l_p$ , however, is a 'global' 211 quantity, closely connected with the end-to-end distance by eqn. (13), and its value results from the 212 behavior of many coupled bonds and sites. 213

In Figure 3 P(g) (a) and  $l_p$  (b) are plotted as a function of  $\theta$  with CR (filled markers) and without CR 214 *i.e.* fixed charge (empty markers) for ionic strengths ranging from 1 to 0.001 M. We have also included 215 the results of SGCMC simulations if the SEV effects are also present (star-shaped markers). Clearly, 216 in all the studied cases, SEV are very weak and are almost irrelevant compared to the self-avoiding 217 electrostatic repulsions. The corresponding capacitance versus  $\theta$  is also reported in 3c. First of all, 218 it is clear in Figure 3a that in both cases, with CR and without CR, P(g) decreases when the ionic 219 strength decreases, since electrostatic screening is weaker and LR interactions are stronger and the 220 macromolecule swells by forming more *trans* states. Moreover, two limiting behaviors for P(g) can 221 be observed, which are also common to SMGMC and ccMC simulations. For low  $\theta$ -values, the 222 polyelectrolyte is uncharged so that  $P(g) \rightarrow 2/3$  since the three conformational states have the same 223 energy. When excluded volume is included, the asymptotic value is slightly smaller since some 224 combinations involving gauche conformations are forbidden. For  $\theta$  close to unity, the chain is fully 225 extended in order to minimize the electrostatic repulsion, which implies that all bonds are in *trans* state and  $P(g) \rightarrow 0$ . Clearly there are no fluctuations in this two limiting situations and the capacitance is 227 very small. 228

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**Figure 2.** (a) Average degree of protonation  $\theta$  and (b) capacitance *C* at ionic strengths (from top to bottom) 1 M, 0.1 M, 0.01 M and 0.001 M obtained by means of pH-constant, SGCMC simulations. Steric excluded volume contribution is not shown because it resulted to be irrelevant in the obtained  $\theta$ -values

However, at intermediate  $\theta$ -values CR and CF are important, as the values of the capacitance 229 indicate, and clear differences arise between ccMC than for SGCMC simulations. P(g) is significantly 230 smaller without CR than with CR, independently of the  $\theta$ -value and ionic strength. This fact can 231 be explained because fluctuations allow to create uncharged regions in the chain which allow the 232 chain to fold (through forming gauche states) while the extended regions preserve the total average 233 charge. By assuming that all the sites have the same average charge, the possibility of the interplay 234 between conformation and charge equilibria is lost and, as a result, the polyelectrolyte chain gets 235 stiffer. This effect is confirmed by the behavior of the persistent length in Figure 3c. For intermediate 236  $\theta$ -values (0.3 <  $\theta$  < 0.8), ccMC clearly overestimates the  $l_{\rm P}$  value with specially significant deviations 237 at high ionic strengths, *i.e.* when CF are larger. As a conclusion, CR allows the chain get more folded 238 while absence of CR makes it stiffer. Probably, a mechano-statistical theory based on first principles, 239 similar to Kirkwood theory for interacting macromolecules [37], would be desirable in order to better 240 understand this point. 241



**Figure 3.** (a) *gauche* state probability, P(g) and (b) persistence length  $l_P$  and (c) capacitance *C* vs. the average degree of protonation  $\theta$  at ionic strengths (from top to bottom) 1 M, 0.1 M, 0.01 M and 0.001 M.  $l_P$  is normalized to the equilibrium bond length  $l_0 = 1.5$  Å. Filled markers correspond to SGCMC without excluded volume, while empty markers correspond to ccMC simulations. In Figs. a and b, star-shaped markers denote results obtained with SGCMC but including steric excluded volume effects.

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## <sup>242</sup> 4. Scaling properties of mechanical stretching in the low force regime

Let us firstly discuss the role of charge fluctuations in the stretching properties at the low force regime, *i.e.* when  $F < k_{\rm B}T/l_{\rm P} \simeq 1$  pN, under which the chain can be seen as a set of freely joined fragments with characteristic length equal to the Kuhn length  $l_{\rm K}$  [47]. This regime can be in turn divided into two different sub-regimes. For very low forces (F < 0.3 pN), the extension  $L_z$  responds linearly to force

$$L_{\rm z}/Ml_0 = \beta F \frac{l_{\rm K}}{3} = \beta F \frac{2l_{\rm P} - l_0}{3}$$
(15)

which is a direct consequence of the fluctuation-dissipation theorem [48]. The Kuhn length  $l_{\rm K}$  is related to the persistence length as  $l_{\rm K} = 2l_{\rm P} - l_0$  [44]. It is important to note that  $l_{\rm P}$ , as it will be shown in the next section, can only be considered constant in the low force regime. This fact is due to the activation of the rotational degrees of freedom at intermediate forces [36]. For forces ranging 0.3 < F < 1 pN, the action of electrostatic self-avoiding interactions makes the force/extension curve to follow the Pincus scaling law [33,48,49]

$$L_{\rm z} \propto F^{1/\nu - 1} \tag{16}$$

which indicates the existence of a second low force sub-regime.  $\nu = 3/5$  corresponds to strong polyelectrolytes such as DNA. For  $\nu = 1/2$  the linear behavior of a phanton chain is recovered.

The extension/force curves resulting from SGCMC simulations (markers) are shown in Figure 4 245 for pH-values ranging from 2 to 10 (from top to bottom). The best fit of linear Eq. 15 (dashed lines) 246 and Pincus scaling law Eq. 16 (continuous lines) are also plotted. The linear eqn. (15) is fitted to data 24 for which F < 0.3 pN while the scaling law (16) is fitted to the points corresponding to 0.3 < F < 1248 pN, the respective ranges of validity. Two ionic strengths are considered: I = 1 M (Figure 4a) and 249 I = 0.001 M (Figure 4b). For large pH-values *i.e.* when the polyelectrolyte is neutral,  $\nu = 1/2$  and 250 linear behavior is found for all the force-values. In the other limiting situation, for very low pH-values, 251 when the polyelectrolyte is fully charged, simulations deviate from the linear behavior and they follow 252 the Pincus scaling law (Eq. 16) with  $\nu \simeq 3/5$ , which nicely matches with the theoretical predictions 253 [49]. The presence of SEV does not affect this conclusion, as shown in the Supplementary Information. 254 For intermediate pH-values and charges, a preliminary study [36] suggested that weak 255 polyelectrolytes exhibit intermediate  $\nu$ -values between the two limiting cases. It is not clear, however, 256 whether such a transition is consequence of the CF, or it is a direct consequence of the change in the 257 average charge induced by modifying the pH-value. With this aim, we perform ccMC simulations 258 using as input the average degree of protonation  $\theta$  obtained from SGCMC at F = 0 (Figure 2a). 259 The scaling exponents  $\nu$  resulting from SGCMC without SEV (filled markers), SGCMC with SEV 260 (star-shaped markers) and ccMC simulations (empty markers) versus  $\theta$  are reported in Figure 5 for two 261 ionic strengths I = 1 M (green squares) and I = 0.001 M (blue circles). 262

12



**Figure 4.** Normalized chain extension *versus* force curves in the low force regime for pH-values ranging from 2 to 10 (from top to bottom) obtained from SMCMC simulations without SEV at two ionic strengths 1 M (a) and 0.001 M (b). Dashed and continuous lines represent the best fit linear and Pincus scaling law, respectively. Chain extension is normalized to the contour length  $L_{\rm C} = Nl_0 \cos((\pi - \alpha_0)/2)$ .

Comparing the results obtained from SGCMC and ccMC simulations, it is observed that, in effect,  $\nu$  presents a transition between linear and Pincus behavior both in presence or in absence of CR. Curiously, for I = 0.001 M the transition between the two limiting  $\nu$  -values is found to be linear with  $\theta$ 

$$\nu = m\theta + n \tag{17}$$

where  $m = 0.129 \pm 0.004$  and  $n = 0.494 \pm 0.001 \simeq 0.5$  are fitted parameters to the SGCMC points. Best 263 fitted Eqn. (17) is depicted as a black dashed line in Figure 5. The situation, however, becomes more 264 complex for I = 1 M, for which no significant deviation from the limiting value v = 1/2 is observed 265 for  $\theta < 0.5$ . For  $\theta > 0.5$ , a drastic increase in the  $\nu$ -value is observed when CR is taken into account. 266 It is also worth mentioning that the limiting value  $\nu = 3/5$  is not observed even at  $\theta = 1$ , when the 267 polyelectrolyte is fully charged, independently of the presence of CF. This fact suggests that this could 268 be also the case for strong polyelectrolytes at high ionic strengths. Again, SEV does not produces any 269 significant effect in  $\nu$ , even when the polyelectrolyte is almost uncharged ( $\theta \simeq 0$ ). 270



**Figure 5.** Pincus scaling exponent v versus  $\theta$  at two ionic strengths: 1 M (green markers) and 0.001M (blue markers). The results of three different kind of simulations are plotted: SGCMC without SEV (filled markers), SGCMC with SEV (star-shaped markers) and ccMC (empty markers).

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# <sup>272</sup> 5. Influence of Charge Fluctuations in the intermediate force regime

It is well-established that conformational and binding degrees of freedom in weak polyelectrolytes 273 are highly correlated because of charge regulation. Since, in flexible polyelectrolytes, mechanical 274 stretching dramatically changes the distance within the macromolecule, changes in  $\theta$  are expected 275 when an external force is applied. This effect has been extensively discussed in a recent publication [36]. 276  $\theta$  versus force is plotted in Figure 6 at different pH-values (4, 6, 8 and 10) and for two ionic strengths 27 I = 1 M (6a) and I = 0.001 M (6b). For intermediate pH-values and low ionic strengths, moderate 278 CR is observed. This effect is significantly enhanced if the *gauche* bond state is favored, for instance, 279 because of the formation of a hydrogen bond, although this issue is out of the scope of this work. 280 Although this point would probably require a more intensive study, we present here some preliminary 281 results. 282

The influence of CR and CF in the extension-force curves is evaluated by again comparing the 283 results obtained by SGCMC and ccMC. They are shown in Figure 7 for the same pH-values and ionic 284 strengths as in Figure 6. It is observed that for I = 1 M clear differences can be appreciated for pH=8 285 but specially for pH=6. It is interesting the fact that those are the conditions under which the binding 286 capacitance and thus the binding fluctuations are larger (see Figure 2b). As expected, no effect is 287 observed when the polymer is fully charged (pH = 4) or fully uncharged (pH = 10). For I = 0.001M fluctuations are weaker and the observed differences are smaller than for I = 1 M. Although not 289 shown in the figure, we find that SEV does not affect significantly the extension/force curves. Similar 290 trends are found in the persistent length, depicted in Figure 8. Note that, again, the persistence length 291 is a function of the pH-value for intermediate forces, due to the activation of the rotational degrees of 292 freedom. The main differences between SGCMC and ccMC also take place for pH = 6 and I = 1293 M, when correlation effects are larger. 294



**Figure 6.**  $\theta$  *versus* force at pH-values (from top to bottom) 4, 6, 8 and 10 and ionic strengths 1 M (a) and 0.001 M (b), the simulation results correspond to SGCMC without excluded volume.



**Figure 7.** Normalized chain extension *versus* force at pH-values (from top to bottom) 4, 6, 8 and 10, and ionic strengths 1 M (a) and 0.001 M (b). Filled markers correspond to SGCMC while empty markers refer to ccMC. The chain extension  $L_z$  is normalized to the contour length  $L_C = Nl_0 \cos((\pi - \alpha_0)/2)$ .

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16



**Figure 8.** Normalized persistence length  $l_P$  *versus* force at pH-values (from top to bottom) 4, 6, 8 and 10, and ionic strengths 1 M (a) and 0.001 M (b). Filled markers correspond to SGCMC while empty markers refer to ccMC.  $l_P$  is normalized to the equilibrium bond length  $l_0 = 1.5$  Å.

# 295 6. Conclusions

This work addresses the role of charge regulation (CR) and the resulting fluctuations in the 296 conformational and mechanical properties of weak polyelectrolytes, and it is motivated by recent 297 findings which suggest that charge regulation can be induced by mechanical stretching in weak 298 polyelectrolytes [36]. Due to CR, changes in the pH-value modifies the average macromolecular 299 charge and conformational equilibria. A second effect is that, for a given average charge per site, 300 fluctuations can alter the intensity of the interactions by means of the correlation between binding 301 sites. We investigate both effects by means of Semi-Grand Canonical Monte Carlo (SGCM) simulations 302 at constant pH-value, so that the charge is a fluctuating quantity. Once the average charge per site is 303 available, we turn off the fluctuations by assigning the same average charge to every site. The molecule 304 is now in a "frozen" ionization state, and a MC simulation at constant charge is performed. The main 305 conformational and stretching properties with and without CR are then compared. 306

We make use of a model which accounts for the main fundamental aspects of a linear flexible polyelectrolyte *i.e.* proton binding, angle internal rotation, bond stretching and bending. Steric excluded volume and specific treatment of short range and long-range interactions are also included in the model. It can be regarded as a kind of "minimal" model in the sense that contains a minimum number of parameters but still preserving the atomistic detail. We firstly study the case when no external force is applied. It is shown that, if fluctuations are activated, *gauche* state probabilities become larger, and the persistence length smaller, so that the polymer becomes more folded. Electrostatic

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repulsion is thus enhanced if the charge is fixed and weakened when charge fluctuations, which arequantified by means of the binding capacitance, are present.

In the presence of an applied force, macromolecular stretching is also analyzed in the presence of CR (the charge depends on the applied force) and without CR (the charge is fixed to the value at zero 317 force). The analysis of the scaling behavior at the low force concludes that Pincus exponent becomes 318 pH-dependent. Both with and without CR, a transition from 1/2 at high pH-values (phantom chain) to 319 3/5 to low pH-values (Pincus regime), is observed. This fact suggests than Pincus regime is essentially 320 driven by the average charge and that CR plays a minor role. Finally, the intermediate force stretching regime is investigated. It is found that CR induces a moderate influence in the force-extension curves 322 and in the persistence length (which in this force regime becomes force-dependent). It is thus concluded 323 that the effect of CR on the stretching curves is mainly due to changes in the average charge at zero 324 force, although some differences arise at intermediate pH-values. It is also found that the effect of 325 steric excluded volume is almost irrelevant compared to electrostatic self-avoiding interactions for all 326 the cases studied. 327

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## 340 Abbreviations

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<sup>341</sup> The following abbreviations are used in this manuscript:

ccMC	constant charge Monte Carlo
CF	Charge Fluctuation
CR	Charge Regulation
LPEI	Linear PolyEthylenImine
LR	Long Range
MC	Monte Carlo
RIS	Rotational Isomeric State
SB	Site Binding
SBRIS	Site Binding Rotational Isomeric State
SEV	Steric Excluded Volume
SGCMC	Semi-Grand Canonical Monte Carlo
SR	Short Range

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