The many origins of charge inversion in electrolyte solutions: effects of discrete interfacial charges

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Abstract

We show that charge inversion, i.e. interfacial charges attracting counterions in excess of their own nominal charge, is a general effect that takes place in most charged systems next to aqueous solutions with multivalent ions and identify three different electrostatic origins for this effect 1) counterion-counterion correlations, 2) correlations between counterions and interfacial charges and 3) complexation. We briefly describe the first two regimes and provide a detailed characterization of the complexation regime from united atom molecular dynamics simulation of a phospholipid domain in contact with an aqueous solution. We examine the expected conditions where each regime should apply and describe a representative experimental example to illustrate each case. We point out that our results provide a characterization of ionic distributions irrespectively of whether charge inversion takes place and show that processes such as proton release and transfer are also linked to ionic correlations. We conclude with a discussion of further experimental and theoretical implications. Key words: Charge inversion; electrostatics; ion binding; molecular dynamics simulations; divalent ions.

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I. INTRODUCTION

The electrostatics of molecules in aqueous media is key for understanding fundamental biological or physico-chemical processes and exhibits a fascinatingly diverse range of phenomena that remains the subject of intense theoretical and experimental work [1, 2, 3]. A relevant example is charge inversion, where interfacial charges attract counterions in excess of their own nominal charge, thus leading to an interface whose effective charge is opposite in sign. Experimental examples of charge inversion have been observed on a wide range of systems such as lipid vesicles, solid interfaces, colloids or Langmuir monolayers among others, in contact with an aqueous solution containing multivalent ions [4, 5, 6, 7, 8, 9]. At the theoretical level, although there is consensus that the origin of charge inversion lies in the presence of correlations among charged objects in solution [2], it is still unclear to what extent the different proposed theories describe the experimental data.

With few exceptions (see [10, 11, 12, 13, 14]), previous theoretical studies have assumed that interfacial charges can be smeared to a uniform distribution. In this paper, we investigate effects related to the discrete nature of interfacial charges and their possible conformational degrees of freedom and further elucidate how they lead to charge inversion. Although, as stated, the subject of this paper is mainly charge inversion, this effect is a consequence of the spatial distribution of charges in solution, so an elucidation of the former provides a detailed understanding for the latter. Therefore, the results presented in this paper provide a precise characterization of ionic distributions next to charged interfaces, which applies also in those cases where charge inversion does not take place.

The organization of the paper is as follows. In Sect. II we discuss the different correlation regimes and describe their main features. A comparison of new simulation results with the different theories is provided in Sect. III, which also provides a detailed example for the newly introduced complexation regime. The discussion of the expected range of validity of the different correlation regimes and its relation to existing experiments is provided in Sect. IV. We finish with some general conclusions in Sect. V.
II. CORRELATION REGIMES AND CHARGE INVERSION.

In the analysis of the electrostatics of charged interfaces in contact with electrolyte solutions it is customary to divide the aqueous solution into two regions, the so-called Stern and diffuse layers \[7\]. The Stern layer corresponds to the region immediate to the interface and may include bound counterions whereas the diffuse layer consists of an atmosphere of ions in rapid thermal motion. A common phenomenological approach describes the Stern layer as a Langmuir isotherm with several phenomenological parameters (such as binding constants, interfacial dielectric constant for water, etc..) coupled with the classical Poisson-Boltzmann (PB) \[15\] theory describing the diffuse layer. While the description of the diffuse layer by PB theory is usually quite satisfactory (almost exact in the dilute regime), a more rigorous description of the Stern layer without resorting to empirical parameters presents considerable theoretical difficulties, but this description is necessary for an unambiguous description of the correlations that lead to charge inversion.

Let us illustrate in some detail how correlations induce charge inversion \[2\]. We will assume solutions with approximately unit activity, so that the chemical potential of the counterions within the diffuse layer is simply $\mu_{\text{diff}} = k_B T \ln(n_C v_0)$, where $n_C$ is the salt number density of the solution and $v_0$ can be taken as the volume of a single counterion (we note that more concentrated regimes can be described from the results in \[16\]). The chemical potential within the stern layer is given by $\mu_{\text{Stern}} = k_B T \ln(n_C^s s_0) + q_C e \phi_0 + \mu_{\text{corr}}$, where $n_C^s$ is the two dimensional number density of counterions with cross-sectional area $s_0$ and ionic valence $q_C$, while $\phi_0$ is the contact value potential and $\mu_{\text{corr}}$ is the contribution arising from correlation effects. The onset of charge inversion takes place at a salt concentration $c_{\text{inv}}$, where the contact value potential is zero ($\phi(0) = 0$) and the Stern layer number density satisfies the neutrality condition $n_C^s = -\sigma_0 / q_C e$, where $\sigma_0$ is the “bare” interfacial charge density. This leads to the equation

$$c_{\text{inv}} = \frac{|\sigma_0|}{2 r_C q_C e} \exp \left( \frac{\mu_{\text{corr}}}{k_B T} \right),$$

where $r_C$ is the counterion radius. For charge inversion to occur within the dilute regime (ionic strengths of the order of 0.1M or lower) a significant favorable correlation energy is necessary (more precisely, the correlation chemical potential must satisfy $\mu_{\text{corr}} \ll -k_B T$).

Where the favorable correlation energy comes from? if electric charges are smeared into a continuum, we revert back to the standard PB description where $\mu_{\text{corr}} = 0$ and no charge
inversion can take place. In other words, a favorable correlation energy arises from effects that are related to the underlying discreteness of electric charges. We next discuss different explicit scenarios that lead to $\mu_{\text{corr}} \ll -k_B T$ and give raise to charge inversion.

## A. Counterion-counterion or lateral Correlation (LC) regime

This regime is dominated by counterion-counterion correlations within the Stern layer, consequently approximating interfacial charges as a smeared uniform charge density. This regime will be designated as lateral correlation (LC) regime herein, and has been studied quite extensively, so we refer to Refs. [1, 2, 16, 17] for detailed presentations. Here we just review the most salient features.

The magnitude of the counterion-counterion correlations is quantified by the coupling parameter (or plasma parameter) $\Gamma$ defined as:

$$\Gamma = \frac{q_{\text{C}}^2 e^2}{\varepsilon a_C k_B T} = \frac{q_{\text{C}}^2 l_B}{a_C}, \quad (2)$$

where $q_{\text{C}}$ is the valence of the counterions, $a_C$ is the typical lateral counterion separation within the Stern layer and $l_B = 7.1 \AA$ is the Bjerrum length [15]. At strong coupling $\Gamma >> 1$, the counterions form a strongly correlated two dimensional liquid (the one component plasma (OCP)) that provides the favorable free energy [18] required for charge inversion. The most relevant predictions for LC theories are:

- The correlation chemical potential $\mu_{\text{corr}}$ is given by the chemical potential of the OCP, quoted for example in [18].

- The counterion pair distribution function within the Stern layer is described by the OCP (quoted in [18]).

- Counterion distributions from the interface fall of exponentially [17, 19]

  $$g_{\text{CI}} \approx \exp\left(-\frac{z}{\lambda_{\text{GC}}^\text{G}}\right), \quad (3)$$

  where $\lambda_{\text{GC}}$ is the Guoy-Chapman length, which characterizes the counterion separation from the interface [15].

- Charge inversion occurs for large values of the parameter $\zeta$ defined as [20]:

  $$\zeta = \frac{q_+ e}{\pi \sigma_0 \lambda_D^2}, \quad (4)$$
where $\lambda_D$ is the Debye length.

**B. Counterion-interfacial charge or transverse correlation (TC) regime**

This regime is dominated by correlations between interfacial charges and single counterions bound to the interfacial groups with a fixed stoichiometric ratio. Herein, it will be designated as the transverse correlation (TC) regime.

The description of the TC regime consists of an interface providing a number of binding sites (with a binding constant $K_I$) for counterions. In its simplest version, the Stern layer is described as a Langmuir adsorption theory \[14, 21, 22\]. As an illustrative example, we consider divalent cations $C^{++}$ with bulk number density $n_C^B$ binding to an interface containing a surface concentration $[P^-]$ of singly charged molecules. Assuming that each interfacial molecule provides a binding site for each counterion (that is a 1:1 stoichiometric ratio) the interface consists of a mixture of $[P^-]$ and $[C^{++}P^-]$ species in equilibrium

$$
[C^{++}P^-]/[P^-] = K_I n_C(0) = K_I n_C^B e^{-2\phi_0/k_B T},
$$

where $n_C(0)$ and $\phi_0$ are respectively the contact value counterion concentration and the contact value potential. The surface charge density $\sigma$ at the Stern layer, which includes the bound counterions, is given by

$$
\frac{\sigma}{\sigma_0} = \frac{1 - K_I n_C(0)}{1 + K_I n_C(0)} = \frac{1 - K_I n_C^B e^{-2\phi_0/k_B T}}{1 + K_I n_C^B e^{-2\phi_0/k_B T}},
$$

where $\sigma_0$ has already been defined as the bare surface charge of the interface. This equation serves as a boundary condition to the Poisson-Boltzmann equation \[15\], thus providing a self-consistent solution with $K_I$ as a free parameter.

The condition for charge inversion is $\sigma/\sigma_0 < 0$, which implies (see Eq. (6)) $n^B > 1/K_I$. As an specific example, we consider an interface consisting of singly charged groups with a molecular area of 80 Å$^2$ (typical of a charged phospholipid such as Phosphatidylserine) in contact with a solution containing divalent counterions. The binding constant is taken as $K_I = 100$ M$^{-1}$ (A discussion of the experimental relevant values is provided in Sect. [IV]). The interfacial charge $\sigma$ is shown in Fig. 1 as a function of salt concentration. The interface is neutralized at counterion concentrations $c_{inv} = 1/K_I = 10^{-2}$M, and the charge is reversed for concentrations larger than $c_{inv}$ (becoming completely reversed, $\sigma = -\sigma_0$ for large enough counterion concentrations).
FIG. 1: Plot of the ratio of the effective charge of the interface $\sigma$ to its bare charge $\sigma_0$ as a function of bulk concentration. The results are for $\sigma_0 = -e/80$ Å$^{-2}$ and divalent counterions, with $K_L = 100$ M$^{-1}$. The onset of charge inversion $\sigma = 0$ is at $c_{inv} = 1/K_L$. Complete reversal ($\sigma = -\sigma_0$) takes place at large concentrations provided that counterion-counterion repulsion can be ignored. The inset shows the free energy per particle as a function of bulk concentration.

If only electrostatic interactions are involved the binding constant $K_I$ can be computed by generalizing the Bjerrum pairing theory of electrolytes \[23\]

$$K_I^{TC} = c_g 4\pi (|q_+ q_-| l_B)^3 G \left( \frac{|q_+ q_-| l_B}{r_0} \right),$$  \(7\)

where $G(x) = \int_0^x dz e^{-4z^2}$, $r_0$ is the sum of the crystallographic radius of the bound ions and the interfacial charge and $q_+, q_-$ their valences. The factor $c_g < 1$ takes into account steric constrains, with $c_g = 1/2$ being a reasonable value \[14\]. The binding constant Eq. 7 reflects the discrete nature of both individual interfacial charges and counterions and encodes their mutual correlations. The main predictions of TC theories are \[14\]:

- Counterions bind with a fixed stoichiometric ratio to interfacial charges.
- The binding constant are electrostatic in origin and given by Eq. 7 (in some situations, corrections need to be included \[14\]).
- The pair distribution functions satisfy the following relations for $r << l_B|Q_I q_+^C/2$, where C stands for counterion charge and I for interfacial charge:

$$g_{CI}(r) \approx \exp(-q_+^C Q_I l_B r)$$  \(8\)

$$g_{CC}(r) \approx 0$$  \(9\)
$Q_I$ is assumed to be the charge of the interfacial molecule to which the counterion is bound (for example, $Q_I = -1$ for PS$^-$ membranes). We stress that the condition $r < < l_B|Q_I|q_C^2/2$ is an upper bound to the validity of the correlation functions, see [14] for a more detailed discussion.

In the language of Eq. 11, the above description corresponds to a free energy gain $\mu_{\text{corr}} = -k_B T \ln(K_I v)$, where $v$ is a volume that depends on the units on which the binding constant $K_I$ is defined.

C. Electrostatic complexes or complexation correlation (CC) regime

In this regime the Stern layer consists of electrostatic complexes formed by several counterions, interfacial charges and water molecules in equilibrium with the bulk solution. This regime will be denoted as the complexation correlation (CC) regime herein and will be illustrated with a concrete example in the next section. It is characterized by:

- Counterions generally bind to many interfacial charges.
- There are correlations among counterions and with interfacial charges, that is, the pair distribution function for both counterion-interfacial groups and bound counterion-counterion ions show peaks.

- The pair distribution function $g_{CI}$, where I is an interfacial atom and C a counterion bound to it has a peak at $r_0$, the sum of the crystallographic radius for C and I atoms, with a width $l_0$ ($l_0/r_0 < < 1$) reflecting strong binding. Also, for $r < r_0 + r_C$ (where $r_C$ is the crystallographic radius of the counterion C) it is $g_{CC}(r) \approx 0$, reflecting that the complex staggers positive and negative charges.

Several approximations enable quantitative estimates within the CC regime. In the general case, the complexes consist of a distribution of patches with different sizes and compositions, thus resulting in a distribution of correlation free energies for counterions. We make the approximation that an average potential of mean force $V_{MF}(r)$ for binding of a counterion to the complex can be defined. From this potential of mean force, an average binding constant is obtained by generalizing the binding constant of the TC case, yielding the expression

$$K_I^{CC} = c_g \int_{r_0}^{d_{Max}} dr 4\pi r^2 \exp(-\beta V_{MF}(r)) ,$$

(10)
where and $d_{Max}$ is a cut-off, which can be chosen as the minimum of density $n(r) \sim r^2 \exp(-\beta V_{MF}(r))$, following the same prescription as Bjerrum [23]. In general, a derivation of $V_{MF}$ requires a knowledge of the chemical structure of the interfacial molecules, which makes its evaluation a difficult task. We estimate the potential of mean force from the observation that the binding to the complex is electrostatic so we approximate the potential of mean force as

$$V_{MF}(r) \approx \frac{q_C^+ Q_{I}^{eff}}{\varepsilon r}$$

where $r \geq r_0$ is the distance from the counterion to the nearest neighbor interfacial groups and $q_C^+$ is the charge of the counterion. The only parameter in this potential is $Q_{I}^{eff}$, the effective charge of the binding site. Combining Eq.(11) with Eq.(10) provides the generalization of Eq.(7) to the CC regime [41]

$$K_{CC} = c_g 4\pi (|q_C^+ Q_{I}^{eff} l_B|) G(\frac{|q_C^+ Q_{I}^{eff} l_B|}{r_0}).$$

For this expression to be predictive, a prescription to compute $Q_{I}^{eff}$ is required. This is obtained from the self-consistent equation (which generalizes expressions Eq. (8) and (9))

$$g_{CI}(r) \approx \exp(-\beta V_{MF}) \approx \exp(-q_C^+ Q_{I}^{eff} l_B)$$

$$g_{CC}(r) \approx 0,$$

expected to be valid for $r < d_{Max}$. The value of $Q_{I}^{eff}$ can be obtained, for example, from MD simulations where the simulated pair distribution function is fitted to the form Eq.(13) leaving $Q_{I}^{eff}$ as the only fitting parameter. An example illustrating this case is discussed next.

**III. THE CC REGIME: THE CASE OF PHOSPHATIDIC ACID**

In this section we describe new MD simulation results (technical details have been provided in [24, 25]) of an interface consisting of a phosphatidic acid lipid domain in contact with a ionic solution containing BaCl$_2$. This system provides a concrete realization of the CC regime and will be used to discuss its most salient features.
FIG. 2: Chemical structure of the DMPA molecule and its assignment of electrical charges. OA, OS, O2, OB label different oxygen atoms and CH2A and CH2A different hydrocarbon groups. These distinctions are useful to characterize the different binding sites for mobile ions for DMPA molecules.

A. Description of simulations

In our MD simulations, we have considered a monolayer with 100 DMPA$^{2-}$ phospholipids at close packing (molecular area $\approx$ 41 Å$^2$) in contact with 50 divalent counterions (Ba$^{2+}$) and added salt (100 BaCl$_2$). This system is particularly suited for this study because experimental studies report charge inversion [8] and DMPA and other lipids with the phosphatidic acid head group play a fundamental role in a wide range of biological processes [26].

The structure of the DMPA$^{2-}$ (1,2-dimyristoyl-sn-glycero-3-phosphatidic acid) phospholipid molecule is given in Figure 2 together with its charge attributions following the AMBER force fields as described in [28]. Water was included explicitly within the SPC/E model and all simulations were carried with the DLPOLY2 simulation package [27]. Technical details and a more extended analysis on other aspects of the simulations can be found in [24]. In this paper, we just provide the results related to charge inversion and ion distributions.

B. Complex formation and charge correlations within the Stern layer

As shown in Fig. 3, the diffuse layer shows an excess of negative charge (Cl$^-$) in the immediate vicinity of the interface and a depletion of bulk counterions, which provides conclusive evidence that the Stern layer has an overall positive charge. Defining a counterion as bound if it has a DMPA oxygen within its first coordination shell, we obtain for the number of bound Ba$^{2+}$ $N_{Ba}^{bound} = 1.065(10)N_{DMPA}$. Hence, the negative charge of the DMPA$^{2-}$ is overcompensated by the counterions leaving a positive interfacial charge of $q = 0.13e$ per
FIG. 3: (color online:) Number density distribution $\rho(z)$ of Cl$^-$ (squares), Ba$^{2+}$ (circles), P (triangles) as a function of the distance from the interface $z$. For the sake of clarity, we show only a partial interval of the diffuse layer. The Stern layer number density distributions are shown on the inset. The position $z = 0$ is defined so that it corresponds to the maximum of the phosphate number density distribution.

FIG. 4: (Color online:) Snapshot showing a Ba$^{2+}$ ion bound to 8 oxygens and 3 DMPA molecules.

The snapshot in Fig. 4 shows binding of a given counterion to several DMPA molecules, forming a complex involving the interfacial oxygens, phosphates, additional barium counterions and water molecules (not shown in the snapshot). These complexes induce inhomogeneities within the surface charge distribution. In Fig. 5 the cumulative number of atoms of a particular type $N_{\text{total}}$ within a distance $r$ of a given bound Ba$^{2+}$ ion is shown. If the interfacial charge could be approximated as a uniform charge density $N_{\text{total}}^{a} / 40 \equiv n_{a} cr^2$ where $n_{a}$ is the number of atoms of type $a$ per DMPA molecule (from Fig. 2) $n_{\text{OS}} = 3$, $n_{P} = 1$, $n_{\text{Mg}} = 1$, $n_{\text{Ba}} = 1$. 

FIG. 5: (Color online:) Cumulative number of atoms of a particular type $N_{\text{total}}^{a}$ within a distance $r$ of a given bound Ba$^{2+}$ ion.
FIG. 5: (Color online:) Total number of atoms \( N_{\text{total}} \) within a distance \( r \) from a given \( \text{Ba}^{2+} \) ion. The coefficient is \( c = \frac{\pi}{40} \). The symbols are atom types, defined according to Fig. 2. The dashed and solid lines are \( N_{\text{total}} \) for an equivalent smeared uniform distribution. The inset shows an enlargement of the region comprising 2.5 – 4 Å.

etc.). The actual distribution for \( N^{a}_{\text{total}}(r) \) is shown in Fig. 5. Large deviations from uniform distribution are found for small distances \( r < 6.5 \) Å, which implies strong correlations among \( \text{Ba}^{2+} \) ions and interfacial groups. The inset in Fig. 5 shows an enlargement of the region between 2.5 – 4 Å, where deviations from the uniform density are the largest. At larger distances, small deviations are still visible up to 10Å.

C. Pair distribution functions within the Stern layer

Results for the pair distribution functions \( g_{\text{OBa}^{2+}} \), where O is one of the different oxygen atoms from DMPA\(^2\) (see Fig. 2) are shown in Fig. 6. The most salient feature is a sharp peak located at a distance around 3 Å, which corresponds to the sum of the crystallographic radius of oxygen and Barium (see Ref. 7), confirming the strong binding among \( \text{Ba}^{2+} \) and O. The second peak and the barely visible third peak reflect correlations among nearest neighbors phospholipids, which define a near crystalline structure with lattice constant 6.8 Å.

The simulations results for \( g_{\text{OBa}^{2+}} \) allow to obtain \( Q_{I}^{\text{eff}} \) as described previously with the fit shown in Fig. 6 (CC case), leading to an effective charge \( Q_{I}^{\text{eff}} \approx -4 \). Bound counterions are tightly bound to 6-8 DMPA oxygens, so the charge in the immediate vicinity of a \( \text{Ba}^{2+} \) ion is between \(-4.8e \) and \(-6.4e \), which is significantly lower than the obtained value \( Q_{I}^{\text{eff}} \approx -4e \).
We regard this difference as an screening effect due to neighboring positive charges. The value $Q_{\text{eff}}^{\text{I}}$ differs substantially from the nominal charge of a single DMPA phospholipid (which is $-2e$), thus implying that binding sites with an effective local charge more negative than that of the single molecule have been created at the interface.

Further understanding on the magnitude of the screening effects is obtained from the analysis of the position of the first peak (located at the sum of the crystallographic radius of Ba$^{2+}$ and O). A rough estimate for this peak position is obtained by assuming that a Ba$^{2+}$ ion is bound to an O atom and ignoring any other further away charges, leading to $d_0 = \left(\frac{48\sigma\varepsilon}{2q_-|e|}\right)^{1/11} \sigma \approx 2.7\,\text{Å}$, where $\varepsilon$ and $\sigma$ are the values for the Lennard-Jones contribution of the O-Ba interaction used in simulations (see [24]). This value is smaller by 10% from the one obtained in the simulations because of the screening effects of nearby positive charges, which are ignored in the estimate and repel the Ba$^{2+}$ from the O center. We include those by assuming that there is a positive charge of valence $q_+$ at a distance $d$ behind the Ba-O pair, which brings a correction to the distance $d_0$ by $\Delta d_0 = \frac{2q_+|e|^2}{\kappa(r_0+d)^2}$, where $\kappa = 11(2q_-|e|^2)^{3/11}\frac{2q_-|e|^2}{\sigma^3}$. For the cases where the ‘repelling’ charge is a P, $d = 2\,\text{Å}$ and $q_+ = 1.3$, while if it is a pair of protons, $d = 0.58\,\text{Å}$ and $q_+ = 0.84$, both cases giving the 3Å quoted above. This calculation shows that the leading contribution to the first peak comes from the nearest neighbor O atoms, while the further away positive charges can be included as a perturbation, very similarly as in the discussion of the value of $Q_{\text{eff}}^{\text{I}}$.

The Ba-Ba pair distribution function is shown in the inset of Fig. 6. It is quite apparent that Ba$^{2+}$ ions strongly repel each other, with the first nearest neighbor Ba$^{2+}$ being as far as 5 Å away. The peaks of the $g_{\text{BaBa}}(r)$ distribution function can be qualitatively predicted from the structure of the DMPA molecules. The average distance among P groups in DMPA at molecular area $A_M = 40\,\text{Å}^2$ is given by $d_{PA} = \sqrt{\frac{2A_M}{\sqrt{3}}} = 6.8\,\text{Å}$. Therefore, we expect a peak for at distances $< d_{PA}$, which reflects binding of two counterions to the same head group and other weaker (as counterions become less correlated) peaks at distances $> d_{PA}$ reflecting Ba$^{2+}$ ions bound to a nearby DMPA. The position of the first peak can be estimated more precisely from the observation that two Ba$^{2+}$ ions bound to the same DMPA must have O atoms, and have to be separated by the phosphate group, thus providing a distance $d_0 \approx d' \cos(109/2) \approx 5\,\text{Å}$, where $d'$ is the Ba-P distance on binding. Furthermore, this first peak implies a second peak in the $g_{\text{BaO2}}$ function at about the same position, as the O2
FIG. 6: (Color online:) Plot of the pair distribution functions $g_{0Ba^{2+}}(r)$ as a function of $r$, for O2 and OS oxygens (see Fig. 2). The inset shows the $g_{Ba^{2+}Ba^{2+}}(r)$ pair distribution. The result of CC predictions with $Q_{eff}^{I} \approx -4$ Eq. (13) is shown as a solid line (CC) and LC predictions as thinner solid lines (LC).

oxygens are covalently attached to the P groups.

D. PA domains provide an example of the CC regime

From the previous discussion it is quite apparent that PA domains provide an explicit example of charge inversion by CC. We now analyze whether such results can be described as either LC or TC regimes.

The results from the MD simulations show the critical role played by the interfacial oxygens in providing binding sites for counterions, as revealed by the snapshot shown in Fig. 4 and more quantitatively by Fig. 5 which shows the inaccuracies of approximating the surface charge by a continuum. Therefore, the basic hypothesis for LC theories do not hold in this case.

The pair distribution of the OCP describes $g_{Ba^{2+}Ba^{2+}}$ within LC and is tabulated in Ref. [18] and shown in Fig. 6. OCP predicts that the probability of finding $Ba^{2+}$ ions in proximity is much higher than it is found in our simulations. Furthermore, counterions at the interface are not in a liquid state, but are bound to the interface with occasional exchange with the bulk. The ion distribution within LC is described by Eq. (3), which is in disagreement with MD results as shown in Fig. 6. In conclusions, the results of the MD simulations are not consistent with charge inversion as described within LC theories.
The snapshot in Fig. 4 shows that a typical counterion is bound to several DMPA molecules, and that counterions may share the same DMPA as binding sites. Therefore, there is no clear evidence of binding with a fixed stoichiometric ratio, which is one of the key assumptions within TC theories. The pair distribution between Ba\(^{2+}\) ions should satisfy Eq. 9 and as shown in Fig. 6 this equation is satisfied within the first 4.5 Å, but a strong peak, indicating correlations among nearby bound Ba\(^{2+}\) ions is observed at a distance of 5 Å. Furthermore, according to TC theories, the first peak of the pair distribution between O and Ba\(^{2+}\) should be described by Eq. 8 with the nominal charge of DMPA (\(Q_{I} = -2\)) while as described in the simulations, a value \(Q_{eff}^{I} \approx -4\) is obtained. Although the predictions within TC models are able to reproduce some of the features observed, particularly at short distances < 4.5Å, several predictions are in clear disagreement and TC theories do not provide a satisfactory description of the MD simulations.

E. Properties of PA domains as a CC regime

From the previous discussion we can now compute the potential of mean force Eq.(11) and the average binding constant from Eq. 10. Assuming values for the steric coefficient \(c_{g}\) between 1 (no steric effects) and 1/10 (strong steric reduction) we obtain a binding constant in the range \(K_{eff}^{I} \approx 10^6 \text{ to } 10^7 \text{ M}^{-1}\). This very large value shows a strong affinity of DMPA\(^{2-}\) for divalent cations.

Implicit in all our simulations is that DMPA\(^{2-}\) is doubly charged. We discuss this point in more detail, as DMPA can exist as DMPA\(^{-}\) or even as a neutral entity. We assume that DMPA has the pK\(_{a}\) values of phosphoric acid, a first pK\(_{a}^{1}\) = 2.1 and a second pK\(_{a}^{2}\) = 7.1 \[29\]. The validity of using bulk pK\(_{a}\) values to interfaces has been shown in \[14, 30, 31\]. The critical Ba\(^{2+}\) concentration, \(c_{crit}\) where DMPA becomes doubly deprotonated is obtained when the free energy for Ba\(^{2+}\) binding is the same as the free energy of a proton being transferred to DMPA\(^{2-}\). This leads to \(\ln(K_{B}c_{crit}) = \ln(10^{pH-pK_{2}^{2}})\) or \(c_{crit} \sim 10^{-7}/c_{g} \text{ M}\). Even if the geometric correction \(c_{g}\) increases \(c_{crit}\) by an order of magnitude, the presence of divalent ions should doubly-deprotonate DMPA already in extremely dilute regimes, and charge inversion should immediately follow (\(c_{inv} \sim 1/K_{I}\)). For DMPA domains, deprotonation and charge inversion occur simultaneously. These results illustrate how correlations affect many other processes such as proton release and transfer.
F. PA domains with monovalent ions

We now briefly discuss how results are modified in the presence of monovalent ions. In this case, the binding constants Eq. 9 give $c_{inv} > 1M$ (where the dilute regime is no longer valid). Generally, the resulting binding constant predict a small binding fraction of monovalent ions, even more so for larger ions such as Cs$^+$. For the case of DMPA, the monovalent ions fully release the first proton (becoming DMPA$^-$) only at concentrations larger than $\sim 10$ (30, 31) mM but contrary to the situation with divalent ions, the balance of free energies does not strip off the second proton. In general, we expect that solutions of monovalent ions are systems without LC regimes and weak or negligible TC or CC regimes, thus following mean field theory very accurately in dilute regimes, as shown recently from X-ray experiments (30, 31, 32) and emphasized earlier by McLaughlin (4).

IV. COMPARISON OF THE DIFFERENT REGIMES WITH EXPERIMENTAL RESULTS

In previous sections we identified three correlation regimes and provided a detailed description of the CC regime by analyzing a particular case. We now provide specific criteria to identify the expected range of validity for each regime and discuss a representative experimental example.

The appearance of the different regimes depends on whether the charges at the interface are fixed or have conformational degrees of freedom and whether the bound counterions “see” the discrete interfacial charges or a smeared interface, which is controlled by the parameter

$$f_r = 0.35a_L/d_{ap}.$$  \hspace{1cm} (15)

The prefactor 0.35 corresponds to a hypothetical situation where both interfacial charges and counterions are forming crystalline structures (14), but we use this factor in any other situation (a factor of 1 severally overestimates the role of discrete charges, while a factor of $1/(2\pi)$, proposed in, for example (7), underestimates discreteness effects). In the above formula $a_L$ is the distance among interfacial charges and $d_{ap}$ is the distance between a counterion and a charged interfacial group (see Fig. 7). For $f_r << 1$, the counterions “see” the smeared interface, while for $f_r > 1$ they “see” the discrete interfacial charges (We emphasize that as shown in (14), discrete charge effects are already present for $f_r$ not much
FIG. 7: Summary of the range of validity of the different theories. With interfacial molecules without conformational degrees of freedom, the transition from LC to TC depends on the parameter $f_r$. When the interfacial molecules have conformational degrees of freedom, complexation (CC regime) may occur.

larger than 1, so we keep the condition as $f_r > 1$, instead of $f_r >> 1$). We now discuss each regime in turn.

A. LC theories

For LC theories to apply it is basically necessary that interfacial charges may be approximated as a continuum. This can be achieved if 1) the interfacial charges are fixed (do not have conformational degrees of freedom) and 2) the condition $f_r << 1$ (see Eq. (15)) is satisfied.

A strong experimental candidate for the LC regime is provided by solid surfaces in contact with different trivalent and tetravalent ions with large radius ($r_+ \sim 4 - 4.5\text{Å}$) [5, 6]. In those experiments, condition 1) was trivially satisfied by the nature of the interface, while from the values reported for the bare surface charge, it is found $f_r \sim 0.08 - 0.4 << 1$, so condition 2) is also met. Indeed, the detailed experimental results show general agreement with LC theories of Shklovskii and collaborators [17, 20]. For example, the concentration at which charge inversion appears ($c_{inv}$) is very well predicted from Eq. (11) and the correlation energy described in Sect. II A. Recent experiments [33] show that charge inversion disappears at higher trivalent ion concentrations, in marked disagreement with the predictions in [20], but in agreement with the LC theory of Pianegonda et al. [16], who include the effect of bulk Bjerrum pairing in the chemical potential of the bulk solution.
It is possible that the results in [6] can be described by a TC theory? The general trends of \( c_{inv} \) regarding its dependence on dielectric constant and multivalent concentration are in agreement with the binding constant Eq. 7. The strongest evidence against charge inversion as described within the TC regime comes from the observed dependence of \( c_{inv} \) with increasing bare surface charge, which according to the TC regime is given by \( c_{inv} \sim 1/K_I \), that is, independent of surface charge. Understanding the observed dependence of \( c_{inv} \) on bare surface charge within the TC regime could only be explained by appealing to complex binding scenarios where counterions bind to several interfacial charges, which in view of the good agreement within LC theories, does not seem justified. We point out, however, that similar experiments for divalent (Ca\(^{2+}\) and Mg\(^{2+}\)) ions show that \( c_{inv} \sim 0.3M \), in disagreement with LC theories, which predict \( c_{inv} \sim 0.01M \). We point out that Ca\(^{2+}\) and Mg\(^{2+}\) have a small radius leading to \( f_r \sim 2 \), and indeed, in this case the TC regime predicts \( c_{inv} \sim 1/K_I^{TC} \approx 0.1M \). It is quite possible that the experiments reported in [33] for divalent ions provide an example for the TC regime. Further experiments or simulations will be required to establish this point.

B. TC theories

TC theories require that 1) that interfacial charges do not have conformational degrees of freedom and 2) \( f_r = 0.35a_L/d_{app} > 1 \).

A clear experimental candidate for charge inversion for the TC regime is provided by PS membranes in a solution containing Ca\(^{2+}\) ions [34]. It is found that \( f_r \sim 4 \) so condition 2) is satisfied. It is not entirely obvious that condition 1) is satisfied, as PS lipids have significant conformational degrees of freedom. The experiments in [34] where done with brain PS, which contain different degrees of unsaturated hydrocarbon chains that might leave PS head groups sufficiently separated to prevent complexation from taking place. More detailed analysis, which can be provided from accurate MD simulations will be required to assess the degree of complexation found in these systems. The binding constant computed from Eq. 7 for Ca\(^{2+}\) give \( K_I^{TC} \approx 10M^{-1} \), which allows to reproduce the values of contact value potential extracted from electrophoretic measurements over five decades in salt concentration [34] without any fitting parameters. Furthermore, results for Na\(^{+}\) are also reproduced from Eq. 7 without fitting parameters. It is therefore quite apparent that the experimental results of [34] are
described by TC theories \[14\] with surprising accuracy. It is remarkable that the same binding constants can be used to predict ionic distributions at higher surface density, such as in Langmuir monolayers \[14, 35\] (with some corrections, as discussed in \[14\]) and in diluted PS membranes \[36\] with considerably lower surface charge density. These observations are in full agreement with the expectations from the TC regime, since Eq. (7) predicts that the binding constant does not depend on the surface charge density $\sigma_0$.

Is it possible that the PS membranes are described by LC theories? LC theories would predict a much lower $c_{inv}$ (in the mM regime) and $\zeta$ potential values should exhibit some corrections due to the presence of the two dimensional correlated liquid. Furthermore, the fact that binding constants are independent of surface charge provides strong evidence against PS membranes being described by LC theories. In general, amphiphilic interfaces show a strong dependence on the nature of the interfacial molecule, which makes it generally incompatible with the LC regime. For example, DMPA monolayers with a surface charge $\sigma_0 \simeq -e/20\text{Å}^2$ show charge inversion in presence of $\mu\text{M}$ concentrations of $\text{BaCl}_2$ \[8\] but fatty acids, with basically the same surface charge, do not exhibit charge inversion \[37\].

Regarding the description of PS membranes within CC theories, in \[34\] it was assumed that divalent ions bind in a 1:1 stoichiometric ratio, while in \[36\], a 2:1 ratio was assumed, both experiments giving good results. Furthermore, the results in \[34\] show some specificity in ionic type, which is slightly larger than predicted from TC theories. Although TC describe the experiments with surprising accuracy, it is therefore likely that more detailed studies will reveal some degree of complexation.

C. CC theories

Complexation requires that interfacial groups have conformational degrees of freedom. X-ray reflectivity experiments with monolayers of DMPA at molecular area $A_M = 40\text{Å}^2$ report charge inversion \[8\] at the mM regime for $\text{Ba}^{2+}$, and some indications that it may extend at least up to the $\mu\text{M}$ range \[38\]. Recent results provide charge inversion within the $\mu\text{M}$ range for $\text{La}^{3+}$ \[9\]. If these results were described by TC, $c_{inv}$ would be in the $10^{-3}\text{M}$ range for divalent ions and in the $5\cdot10^{-4}\text{M}$ for the trivalent ions. Clearly, the observed charge inversion takes place at much lower concentrations and is consistent with the predictions of CC discussed in the previous section.
A more stringent test to assess the prediction of the CC theory described in the previous section would require 1) to assess the number of interfacial O surrounding a given Ba$^{2+}$ and 2) establishing the presence of a diffuse layer of co-ions (Cl$^-$, etc.) next to the surface. Both predictions can be realistically validated from X-ray studies. X-ray spectroscopy from recently developed surface sensitive EXAFS [39] has provided a detailed description of the oxygen atoms bound to Cs$^+$ ions next to charged interface, so the same technique could be applied to Ba$^{2+}$ solutions in order to elucidate point 1). Regarding point 2), if the amount of charge inversion is substantial, use of heavy ions such as I$^-$ (as opposed to Cl$^-$), which have resonances in the X-ray region, could be detected by anomalous X-ray surface sensitive scattering [8] combined with fluorescence methods.

V. CONCLUSIONS

Charge inversion always results from a favorable correlation free energy (Eq. 1). We identified three different regimes leading to charge inversion, schematically illustrated in Fig. 1) and provided specific criteria to theoretically identify each different regime (also shown in Fig. 1), further illustrating it with a representative experimental example.

We have provided a detailed discussion of the CC regime using recent MD simulations of phosphatidic acid domains [24] and showed how processes such as proton transfer and release are intimately related to correlations. Furthermore, our results show the critical role played by strongly electronegative atoms (the oxygens), which create binding sites, as shown in Fig. 4, leading to the formation of highly cohesive complexes. This observation emphasizes the necessity of including atomic details in investigations of charged monolayers and membranes. Future ab-initio calculations, with more rigorous inclusion of polarizability effects, may provide more precise insights into these issues. Our results show possible pitfalls of excessive coarse-grained theories and simulations. Considering a model where DMPA consists of single divalent point charge at the head group would be unable to generate the kind of complexes shown in Fig. 4. In this hypothetical coarse-grained model, divalent counterions would most likely bind by TC in a stoichiometric ratio of 1:1 and charge inversion could not follow.

Although our MD simulations have only discussed the case of DMPA, our study can be used to predict ion distribution and charge inversion in many other systems. Fatty acids,
for example, with a surface charge very close to DMPA do not exhibit charge inversion [37]. Fatty acids only have two oxygens per molecule available for binding and binding constants become on the order 1M$^{-1}$ [14]. A similar situation is observed for DHDP (Di-hexadecyl hydrogen-phosphate) [31], which does not exhibit charge inversion. On the other hand, we predict that charge inversion should be a common effect in membranes of biologically relevant charged phospholipids such as phosphatidyl serine (PS), phosphatidylinositol or glycolipids such as the gangliosides, which contain more than 10 oxygens per molecule and can become multiply charged. Detailed quantitative predictions for these lipids will be presented elsewhere.

The role of hydration sheaths on binding has not been discussed in this paper. Although a detailed analysis is reported elsewhere [24], our simulation results show that Ba$^{2+}$ ions lose roughly half of their hydration sheaths upon binding, so that the distance between bound ions and oxygens is given by the sum of their crystallographic radius. It is expected that this is a general result, as with very few exceptions, all negatively charged interfaces consist of oxygen atoms, and as exemplified from Fig. 2, oxygen charges are basically the same whether the oxygen is from water or within an interfacial group, and therefore trading a water for an interfacial oxygen is entropically favored, similarly as in mechanisms for ion selectivity in ion channels [40]. Our results also extend to other ions such as Ca$^{2+}$ or Sr$^{2+}$, which share the same electronic structure with Ba$^{2+}$ but with a considerably smaller crystallographic radius, which should enhance charge inversion by allowing the ions to get closer to the O atoms and increase their binding. Other divalent ions like Cd$^{2+}$, which do not have the electronic structure of a noble gas, bind more strongly than Ba$^{2+}$ or Ca$^{2+}$, but this binding is mainly covalent [14]. This situation is usually referred as specific binding [6] and can be described by a phenomenological binding constant encoding the free energy of the covalent bond. In this situation, $K_L$ cannot be estimated theoretically (with a formula such as Eq. 7) and it is independent of environmental variables such as the dielectric constant of the solution, the charge surface density of the interface, etc..

In conclusion, our results show that charge inversion is a common effect in charged interfaces in contact with a solution of multivalent ions, but it may have many different origins. How our results may extend to other charged systems such as proteins or biopolymers like DNA or actin remains the subject for future work.

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