

## Discrete charge patterns, Coulomb correlations and interactions in protein solutions

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**Abstract.** – The effective Coulomb interaction between globular proteins is calculated as a function of monovalent salt concentration  $c_s$ , by explicit Molecular Dynamics simulations of pairs of model proteins in the presence of microscopic co and counterions. For discrete charge patterns of monovalent sites on the surface, the resulting osmotic virial coefficient  $B_2$  is found to be a strikingly non-monotonic function of  $c_s$ . The non-monotonicity follows from a subtle Coulomb correlation effect which is completely missed by conventional non-linear Poisson-Boltzmann theory and explains various experimental findings.

A more fundamental understanding of the interactions between nano-sized biomolecules is critical to the long-term advance of modern biomedical research [1]. The best strategy for a predictive calculation is to study simple coarse-grained models where effects can be clearly separated and approximations can be systematically tested. While for micron-sized colloidal particles such coarse-grained models have led to a quantitative understanding of the effective interactions [2], the challenging question is how far this concept can be transferred to nano-particles.

A particular issue is the aggregation and crystallization of globular proteins in solution, driven by their mutual interactions, including steric repulsion, van der Waals attraction, Coulombic interactions, hydration forces, hydrophobic attraction and depletion forces [2]. Most of these are effective interactions which depend sensitively on solution conditions. In particular Coulombic forces are functions of  $pH$  (which determines the total charge of the proteins) and of electrolyte concentration, which controls the Debye screening length  $\lambda_D$ , and hence the effective range of Coulombic interactions. This dependence on solution conditions is exploited in “salting-out” experiments where large salt concentrations are used to trigger protein crystallization, a crucial step towards the determination of their structure by X-ray diffraction [3]. While the forces acting between micro-sized colloidal particles are dominated by generic interactions, and are directly measurable by optical means [4–6], the interactions between globular proteins are highly specific at short range, and are less directly accessible.

One possible indirect determination of the total force between two proteins may be achieved via measurements of the osmotic equation of state by static light scattering, which in the low protein concentration regime yields the value of the second osmotic virial coefficient  $B_2$  [7,8]. The variation of  $B_2$  with solution conditions yields valuable information on the underlying effective protein pair interactions. Moreover, it has been shown empirically that there is a strong correlation between the measured values of  $B_2$  and the range of solution conditions under which protein crystallization is achieved [7–10]. This letter focuses on the effective interactions between globular proteins mediated by the microscopic co and counterions, and on the resulting  $B_2$ . The conventional Derjaguin-Landau-Verwey-Overbeek approach [11], borrowed from colloid science, leads one to expect that  $B_2$  will monotonically decrease as the concentration of salt increases, since higher salt concentrations lead to enhanced screening (*i.e.* reduction of  $\lambda_D$ ), and hence to a decrease of the effective protein diameter. This behavior rests on the standard “coarse-grained” model of uniformly charged colloids and smoothed local densities of the microions. We show that the discrete nature of the protein surface charge distribution, together with the Coulomb correlations between all charges involved, lead to a striking non-monotonic variation of  $B_2$  with salt concentration  $c_s$ . The occurrence of a minimum of  $B_2$  as a function of  $c_s$  has recently been reported in lysozyme solutions for  $c_s = 0.3$  M [12] and in apoferritin solutions for  $c_s = 0.15$  M [13]. Related experimental findings are non-monotonic variations of other quantities which strongly correlate with  $B_2$  [12,14] such as the interaction parameter [15,16], the cloud point temperature [17,18], and the solubility [19]. All these trends can be qualitatively understood by our calculation.

We consider two spherical proteins of diameter  $\sigma_p$ , each carrying a total charge  $Ze$  (where  $Z$  depends on pH), surrounded by monovalent co and counterions, assumed to have identical diameters  $\sigma_c$ . The solvent (water) is assumed to be a dielectric continuum of permittivity  $\epsilon$ ; this simplification, which ignores the molecular granularity of the solvent, amounts to the standard “primitive” model of ionic solutions [20].

In the case of highly charged colloidal particles, the total negative charge  $-Ze$  is usually assumed to be uniformly distributed on the surface, a situation which will be referred to as the “smeared charge model” (SCM). This simplification is much less justified for the smaller, weakly charged proteins (where  $Z \simeq 10$ ). We have hence adopted a second, discrete charge model (DCM) where  $Z$  monovalent discrete point charges are distributed over the surface of a sphere of diameter  $\sigma_p - 2\Delta = 0.96\sigma_p$  (*i.e.* slightly inside the surface of the protein), in such a way as to minimize the electrostatic energy of the distribution; the resulting pattern kept fixed in the following does not correspond to the real charge distribution on any specific protein, but does provide a well-defined discrete model for comparison with the SCM, and between different values of  $Z$ . At this stage the two models (SCM and DCM) involve only excluded volume and bare Coulombic interactions (reduced by a factor  $1/\epsilon$  to account for the solvent) between all particles (proteins and microions). However, in view of the large-size asymmetry, the effective force between the proteins, which ultimately determines the second virial coefficient, involves a statistical average over microion configurations in the field of two fixed proteins [21]. For distances  $r > \sigma_p$  between the centres of proteins 1 and 2, the total force  $\vec{F}_1 = -\vec{F}_2$  acting on each of the proteins is the sum of three contributions,  $\vec{F}_1 = \vec{F}_1^{(1)} + \vec{F}_1^{(2)} + \vec{F}_1^{(3)}$ , where  $\vec{F}_1^{(1)}$  is the direct Coulomb repulsion between the charges on the two proteins,  $\vec{F}_1^{(2)}$  is the microion-induced electrostatic force and  $\vec{F}_1^{(3)}$  is the depletion force due to the imbalance of the microion osmotic pressure acting on opposite sides of the proteins [21]. Both  $\vec{F}_1^{(2)}$  and  $\vec{F}_1^{(3)}$  are averages over microion configurations; according to the contact theorem  $\vec{F}_1^{(3)}$  is directly related to the integral of the microion contact density over the surface of the protein [22,23].

The statistical averages leading to  $\vec{F}_1^{(2)}$  and  $\vec{F}_1^{(3)}$  were computed using Molecular Dynamics (MD) simulations. The two proteins were placed symmetrically with respect to the centre along the body diagonal of a cubic simulation cell of length  $L = 4\sigma_p$ , which also contained monovalent co and counterions in numbers determined by their bulk concentrations; periodic boundary conditions were adopted. The choice of  $L$  was made to ensure that the box length is much larger than the range of the total (effective) protein-protein interaction, so that the results would be independent of  $L$ . For our model to be a rough representation of lysozyme, we chose  $\sigma_p = 4$  nm, and  $Z = 6, 10$  and  $15$ , corresponding to three different values of the solution pH. The microion diameter is  $\sigma_c = 0.267$  nm. Note that the SCM always implies vanishing multipole moments, whereas within the present DCM, the only charge pattern with a non-vanishing dipole moment is that for  $Z = 15$ . A snapshot of a typical equilibrium microion configuration around two proteins is shown in fig. 1, for the case  $Z = 15$ . Note that the dimensionless Coulomb coupling parameter for a protein-counterion contact, namely  $\Gamma = e^2/[\epsilon k_B T(\Delta + \sigma_c/2)]$  for the DCM, and  $\Gamma = 2Ze^2/[\epsilon k_B T(\sigma_p + \sigma_c)]$  for the SCM, are comparable and of the order of  $\Gamma = 3$  at room temperature. For the DCM, the total force  $\vec{F}_1 = \vec{F}_1(\vec{r}, \vec{\omega}_1, \vec{\omega}_2)$  depends on the centre-to-centre separation vector  $\vec{r} = \vec{r}_1 - \vec{r}_2$  and on the unit vectors  $\vec{\omega}_1, \vec{\omega}_2$  describing the orientations of the charge patterns. However, by comparing data for 100 different orientations, fixed during the simulation, the anisotropy turns out to be weak. The standard definition of the second virial coefficient is  $B_2 = \frac{1}{2} \int d^3r [1 - b(r)]$  with

$$b(r) = \frac{1}{16\pi^2} \int d^2\omega_1 \int d^2\omega_2 \exp[-V_{\text{eff}}(\vec{r}, \vec{\omega}_1, \vec{\omega}_2)/k_B T]. \quad (1)$$

Here,  $V_{\text{eff}}$  is the effective potential such that  $\partial V_{\text{eff}}(\vec{r}, \vec{\omega}_1, \vec{\omega}_2)/\partial \vec{r} = \vec{F}_1(\vec{r}, \vec{\omega}_1, \vec{\omega}_2)$ . Using the identity  $b(r) = \exp[\int_{-\infty}^r dr' \frac{d}{dr'} [\ln b(r')]]$ ,  $B_2$  can be expressed—in formal analogy with the spherically symmetric case of the SCM—as

$$B_2 = \frac{1}{2} \int d^3r [1 - \exp[-V(r)/k_B T]], \quad (2)$$

involving the potential of the orientationally averaged projected force

$$V(r) = \int_r^\infty dr' \left\langle \frac{\vec{r}}{|\vec{r}'|} \cdot \vec{F}_1(\vec{r}', \vec{\omega}_1, \vec{\omega}_2) \right\rangle_{\omega_1 \omega_2}. \quad (3)$$

where  $\langle \dots \rangle_{\omega_1 \omega_2}$  refers to a canonical statistical average over mutual orientations of the two proteins [24]. Results for the second virial coefficient in units of its value  $2\pi\sigma_p^3/3$  for hard spheres of diameter  $\sigma_p$ ,  $B_2^* = B_2/B_2^{(\text{HS})}$ , are shown in fig. 2 for the SCM and DCM models, with three values of the total protein charge. The second virial coefficient in units of its value  $2\pi\sigma_p^3/3$  for hard spheres of diameter  $\sigma_p$ ,  $B_2^* = B_2/B_2^{(\text{HS})}$ , can then be proven to be given by

$$B_2^* = 1 + \frac{3}{\sigma_p^3} \int_{\sigma_p}^\infty dr r^2 [1 - \exp[-V(r)/k_B T]], \quad (4)$$

a result formally identical to that valid for spherically symmetric forces. Results for  $B_2^*$  as a function of salt concentration are shown in fig. 2 for the SCM and DCM models, with three values of the total protein charge. In order to obtain values of  $B_2$  comparable to measured virial coefficients, we have taken short-range attractions between proteins into account, by adding to the effective Coulomb potential in eq. (4) a “sticky” hard-sphere potential of the Baxter form [25], with potential parameters  $\delta = 0.02\sigma_p$  and  $\tau = 0.12$ , which are known to

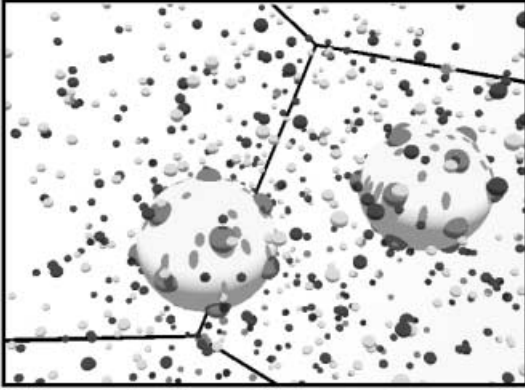


Fig. 1

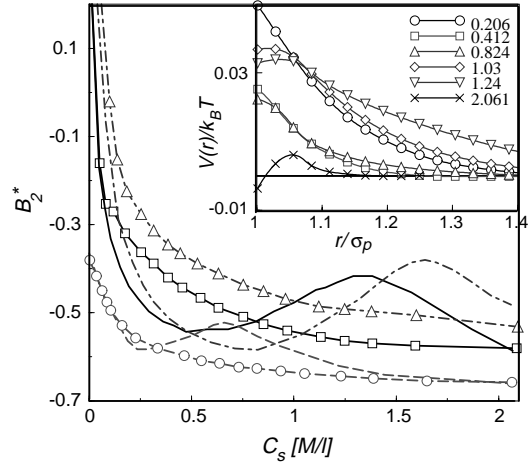


Fig. 2

Fig. 1 – Snapshot of a typical MD-generated microion configuration around two proteins, separated by  $r = 1.7\sigma_p$ . The proteins carry 15 discrete charges  $e$ ; monovalent salt molarity is  $c_s = 0.206$  M/l, the system temperature is  $T = 298$  K. The globular protein molecules are shown as two large gray spheres. The embedded small dark spheres on their surface mimic the discrete protein charges in the DCM model. The small gray spheres are counterions, while the black spheres are coions.

Fig. 2 – Normalized second virial coefficient  $B_2^* = B_2/B_2^{(\text{HS})}$  of a protein solution *vs.* added salt molarity. Results are shown for protein charges  $Z = 6$  (dashed lines),  $Z = 10$  (solid lines) and  $Z = 15$  (dot-dashed lines). The lines with (without) symbols correspond to the SCM (DCM) model. The inset shows the effective protein-protein interaction  $V(r)$  in the DCM model *vs.* separation distance  $r$  for  $Z = 10$ . Various symbols in the inset relate to the different added salt molarities, indicated in the legend.

yield reasonable osmotic data for lysozyme solutions [9, 26] in the high salt concentration regime, where Coulombic interactions are essentially screened out. The key result, illustrated in fig. 2, lies in the considerable *qualitative* difference between the predictions of the SCM and the DCM models for the variation of  $B_2^*$  with monovalent salt concentration  $c_s$ , irrespective of the total protein charge  $Ze$ . While the SCM (dashed curves) predicts a monotonic decay of  $B_2^*$  with  $c_s$ , the DCM leads to a markedly non-monotonic variation, involving an initial decay towards a minimum followed by a subsequent increase to a maximum and a final decrease towards a high  $c_s$  value similar to that predicted by the SCM. The location of the minimum and of the maximum shift to higher values of  $c_s$  for larger protein charges  $Z$ .

The origin of the non-monotonic variation of  $B_2^*$  with  $c_s$  can be traced back to the dependence of the effective (screened) Coulomb interaction on salt concentration as shown in the inset of fig. 2 for  $Z = 10$ . While the spherically averaged, repulsive effective potential  $V(r)$  of the DCM is initially strongly reduced as  $c_s$  is increased, its amplitude and range increase very significantly at intermediate concentrations ( $c_s \simeq 1$  M/l), before it nearly vanishes at the highest salt concentrations. Note that  $V(r)$  becomes even slightly attractive at contact ( $r = \sigma_p$ ) for  $c_s \simeq 2$  M/l. The enhanced effective Coulomb repulsion at intermediate salt concentrations cannot be rationalized in terms of simple mean-field screening arguments; it is caused by a subtle correlation effect which leads to the non-monotonic behavior of  $B_2$  within the DCM. The protein-microion correlations are of a sufficiently different nature in the SCM, to lead to

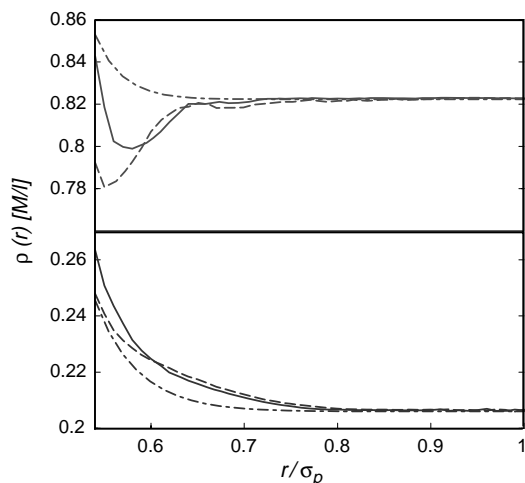


Fig. 3

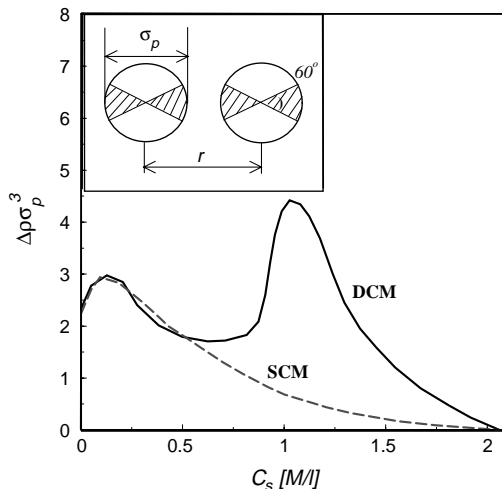


Fig. 4

Fig. 3 – Total density profiles  $\rho(r) = \rho_+(r) - \rho_-(r)$  of small ions around a single protein, for salt molarities  $c_s = 0.206$  (bottom set of curves) and  $c_s = 0.824$  (upper set of curves). The solid and dashed lines are simulation results for DCM and SCM models, respectively, while the dot-dashed lines are predictions of non-linear Poisson-Boltzmann theory.

Fig. 4 – Microion density imbalance  $\Delta\rho$  vs. salt molarity for protein charge  $Z = 10$  and separation  $r = 1.2\sigma_p$ . The solid and dashed lines correspond to the DCM and SCM models, respectively. The inset shows the angular range over which  $\Delta\rho$  is averaged (see text).

a much more conventional, monotonic decay of  $B_2$  with  $c_s$ , similar to that expected from a linear screening picture.

Even though the effective Coulomb potential between proteins is of small amplitude, only a few percent of the thermal energy  $k_B T$ , the effect on  $B_2$  is dramatically enhanced by the presence of the strong short-range attractive component due to van der Waals and hydrophobic interactions, which we have included in the form of the Baxter “sticky” sphere potential. This potential is independent of salt-concentration, and has no influence on the qualitative dependence of  $B_2$  on  $c_s$ .

In order to gain further insight into the physical mechanism responsible for the unusual variation of the effective Coulomb potential and of  $B_2$  with salt concentration, we have investigated in detail the local microion density in the immediate vicinity of the protein surfaces. First, as a reference case, the radial microion density profile  $\rho(r) = \rho_+(r) + \rho_-(r)$  around a *single* isolated protein is shown in fig. 3, for  $Z = 10$ , and two salt concentrations (the profiles are orientationally averaged in the case of the DCM). At the lower salt concentration ( $c_s = 0.206$  M/l) the SCM and DCM models both yield an accumulation of the microion density near contact, in semi-quantitative agreement with the prediction of standard Poisson-Boltzmann (PB) theory. At the higher salt concentration, however, there is a marked depletion in the microion density, signaled by a minimum of  $\rho(r)$  well below the asymptotic bulk value. This correlation effect is of course absent in the (non-linear) PB theory, which always predicts a monotonically decreasing density profile  $\rho(r)$ . Note however a significant difference between the SCM and DCM profiles. While the latter predicts a contact value  $\rho_c(r = (\sigma_p + \sigma_c)/2)$  larger than the bulk value, SCM predicts a much stronger microion depletion near contact.

This finding illustrates the sensitivity of correlation effects to the assumed charge pattern at the surface of a protein: taking into account the discreteness of the surface charges leads to a significant reduction of microion depletion at contact, compared to the simplified picture of a uniformly smeared charge (SCM).

Next, consider the influence of a second near-by protein on the microion distribution near contact. We have computed the difference between “inner” and “outer” shell microion contact densities, as schematically illustrated in the inset to fig. 4. The local microion density is no longer spherically symmetric, due to the interference of the electric double-layers associated with the two proteins. The difference  $\Delta\rho = \rho_{\text{in}} - \rho_{\text{out}}$  between the mean number of microions within a fraction of a spherical shell of radius  $R = 0.6\sigma_p$  subtended by opposite  $60^\circ$  cones is plotted in fig. 4 *vs.* salt concentration.  $\Delta\rho$  is always positive, indicating that microions (in fact mostly counterions) tend to cluster in the region between the proteins, rather than on the opposite sides, as one might expect due to the enhanced lowering of the electrostatic energy for counterions shared between the two proteins. However, there is a very significant difference in the variation of  $\Delta\rho$  with  $c_s$ , between the SCM and the DCM models. Both exhibit similar behavior for  $c_s \leq 0.5$  M/l, with a small maximum around 0.2 M/l. Beyond 0.5 M/l, however, the SCM predicts a monotonic decrease of  $\Delta\rho$ , while the DCM leads to a sharp peak in  $\Delta\rho$  for  $c_s \simeq 1$  M/l. This highly non-monotonic behavior clearly correlates with the non-monotonicity of  $B_2$  evident from fig. 2. The excess number of microions between the two proteins leads to an imbalance in osmotic pressure, which is the origin of the increased *repulsion* between proteins around  $c_s = 1$  M/l, as shown in the inset of fig. 2. The main finding of the present work is that the second osmotic virial coefficient of protein solutions has a non-monotonic dependence on salt concentration if the charge pattern on the protein surface is discrete (as is the case for real proteins) rather than uniformly smeared out, as usually assumed in the related case of charge-stabilized colloidal dispersions, involving much larger particles. The lesson to be learned from this finding is that one must be cautious in attempting to extend coarse-graining concepts and approximations, developed and routinely used on the colloidal scale, to the nanometric scale of proteins. The discreteness of the charge pattern is crucial to obtain non-monotonic behavior of  $B_2$ , which is a subtle Coulomb correlation effect [27], totally missed by non-linear PB theory.

We chose our simple models to help highlight and separate the effects of discrete charge patterns and Coulomb correlations. Extending our MD calculations to the more complex (*pH*-dependent) charge patterns of realistic proteins [28] is technically straightforward. We expect that the physical mechanism leading to enhanced protein repulsion at intermediate salt concentration, which is illustrated by the microion density imbalance shown in fig. 4, will carry over. Since the second osmotic virial coefficient determines much of the excess (non-ideal) part of the chemical potential of semi-dilute protein solutions, it is anticipated that the non-monotonicity of  $B_2$  may have a significant influence on protein crystallization from such solutions in the course of a “salting-out” process. The non-monotonic behavior also suggests the possibility of an inverse, “salting-in” effect, whereby a reduction of salt concentration may bring  $B_2$  into the “crystallization slot” [8].

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