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How the excluded volume architecture influences ion-mediated forces between proteins

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The effective interactions between model proteins of various shapes are computed by means of Monte Carlo simulations. In particular, we determine how the modification of the excluded volume architecture influences both entropic and purely electrostatic ion-mediated forces between proteins. We find that interprotein interactions are strongly affected by protein shape, which results in a high decrease of electrostatic screening for typical active site geometries. Effective interactions are then closer to the direct Coulombic interactions, and both affinity and selectivity are enhanced by several orders of magnitude.

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Protein recognition and binding lie at the heart of many biological phenomena, but also result in many human diseases. Deciphering the role of protein architecture in the association process is crucial to design better pharmalogical inhibitors [1,2]. The specificity of molecular recognition is often explained by a "lock and key" mechanism, i.e., by shape and charge complementarities of the molecular partners. Such a rigid-body model lacks the plasticity required to describe the molecular events during the binding process, but it is sufficient to compare the energy of different complex structures [3,4]. Although the influence of shape complementarity on the interaction between proteins in vacuum can be easily understood and estimated, it becomes less intuitive as far as water-mediated and salt-mediated interactions are concerned. In this Rapid Communication, we focus on the role of salt ions, which screen the direct Coulombic interactions between charged proteins. So far, shape has never been regarded as a factor of screening optimization. Most theoretical works have been devoted to suspensions of spherical or rodlike particles [5,6], showing that ion-induced interactions influence both their static [7,8] and dynamical properties [9–11]. Nonspecific interprotein potentials deduced from small-angle neutron scattering experiments are well described by screened Coulombic potentials [12], which indicates the relevance of considering electrostatic screening as a key phenomenon in protein solutions.

In this work, we use explicit-ion, continuum-dielectric Monte Carlo simulations to derive exact ion-averaged potentials of mean force (PMFs) between proteins, whose level of modeling is chosen to unravel the role of the protein shape. Although calculations at the Poisson-Boltzmann level are frequently used, explicit-ion simulations are required for nanometric particles, since correlations between ions are not negligible [13]. The primitive model that describes ions as charged spheres is used for its ability to explain the finest trends of ion-mediated electrostatic interactions, such as the attraction between like-charged particles [14,15]. We extend this model to nonspherical particles. For proteins that differ only by their shape, we quantify the intensity of ion-mediated forces for like-charged (+10 and +10) and oppositely charged proteins (+10 and −10). The charges of the model proteins are located at a single point. The impact of charge distribution within the protein has been considered elsewhere (see Ref. [16]). We stress that, in all cases, the direct force between the proteins in vacuum is the same, so that we can isolate ion-induced effects from any other forces. We first present the results for two spherical nanoparticles. This enables us to justify the choice of the protein models designed to investigate the effect of shape on the ion-mediated PMFs. We find that ion-averaged forces dramatically affect both the affinity between oppositely charged partners and the repulsion between like-charged ones when the shape changes: the architecture of the excluded volume can be optimized to amplify the magnitude of Coulombic forces. This is due to an important variation of the electrostatic effective force, which is not compensated by an entropic counterpart.

The species interact through the pair potential $V_{ij}(r_{ij}) = (Z_i Z_j e^2/4\pi\epsilon_0\epsilon_r)(1/r_{ij}) + V_H(r_{ij})$, where ϵ_0 is the permittivity of the vacuum, ϵ_r is the relative permittivity of water (taken equal to 78.25), e is the elementary charge, Z_i is the charge of particle i, and r_{ij} is the distance between particles i and j. The hard potential $V_H(r_{ij})$ is infinite in the particles and zero outside. The salt ions are hard spheres of radius $a_m = 0.15$ nm. For this model of interactions, the mean force includes two contributions in addition to the direct Coulombic interaction between the two proteins: the electrostatic interactions of the proteins with the surrounding ions, plus an osmotic term or collision force. Both forces are functionals of ion density: collision forces are due to hard-core repulsions, and are thus functionals of surface density; electrostatic forces are due to long-range Coulombic interactions, and are thus functionals of volume density. More precisely, we denote as $\mathbf{F}^1_{\mathrm{dir}}$ the direct electrostatic force exerted by the protein 2 (P_2) on the protein 1 (P_1). $\mathbf{F}^1_{\mathrm{el}}$ is the electrostatic force exerted by ions on P_1. It reads

$$\mathbf{F}^1_{\mathrm{el}} = \frac{Z_{P_1} e^2}{4\pi\epsilon_0\epsilon_r} \left\langle \sum_{\alpha=1}^{2} \left(\sum_{j=1}^{N_\alpha} \nabla_{\mathbf{r}_1} \frac{Z_\alpha}{(|\mathbf{r}_j^\alpha - \mathbf{r}_1|)} \right) \right\rangle, \qquad (1)$$

where $\mathbf{r}_1$ is the position of P_1, $\mathbf{r}_j^\alpha$ is the position of ion j of type α and charge Z_α, and N_α is the number of ions of type α. The angular brackets denote the canonical ensemble average. The third contribution $\mathbf{F}^1_{\mathrm{coll}}$ is entropic, i.e., it scales with the thermal energy $k_B T$. This collision force results from the asymmetry of the ion density at the surface of the proteins. It may be expressed as [17]

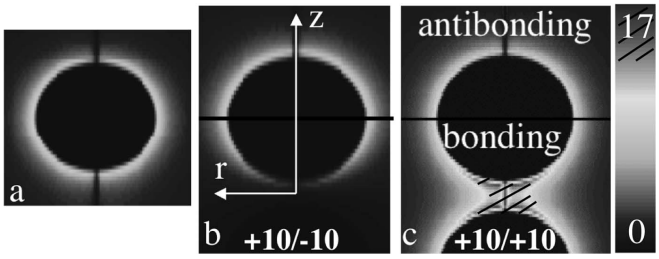

FIG. 1. Anion density field $\rho/\rho(R=\infty)$ around spherical proteins (a) in the case of a protein alone, and in both cases of (b) two oppositely charged proteins, and (c) two like-charged proteins, for the reduced interprotein distance $R/R_c=1.5$. The bonding and antibonding regions are separated by a line, and the coordinates (r,z) are indicated.

$$\mathbf{F}^1_{\text{coll}} = -k_B T\left(\lim_{\delta z\to 0+}\frac{\langle N_c\rangle}{\delta z} + \lim_{\delta z\to 0-}\frac{\langle N_c\rangle}{\delta z}\right)\frac{\mathbf{r}_{12}}{R}, \qquad (2)$$

where N_c is the number of ions that are located in a volume that has the same shape and orientation as P_1, whose coordinate on the z axis carrying the two proteins is $z_1+\delta z$. $\mathbf{r}_{12}=\mathbf{r}_1-\mathbf{r}_2$ is the interprotein vector, and $R=|\mathbf{r}_{12}|$ is the interprotein distance.

Our simulations are carried out in the canonical (*NVT*) ensemble with the temperature set to 298 K. The forces are averaged over the ion configurations for a set of interprotein distances. The proteins are symmetrically placed along the room diagonal of a cubic simulation box with periodic boundaries. Their concentration is sufficiently low that the ion distributions are not influenced by the periodic images of the proteins [9,16]. In such conditions, a cutoff of electrostatic interactions is justified, yielding identical results with bigger box lengths. The force is averaged over $(4\times10^8)-10^9$ steps, so that the uncertainty of the calculations never exceeds 2%. The proteins are immersed in a monovalent salt whose Debye length κ_D^{-1} is 1 nm [18], which roughly corresponds to the physiological case.

In what follows, the discussion focuses on F^1_{dir}, F^1_{el}, and F^1_{coll}, defined by $\mathbf{F}^1(R)=F^1\mathbf{r}_{12}/R$, and on the mean or effective force $F_{\text{eff}}=F^1_{\text{dir}}+F^1_{\text{el}}+F^1_{\text{coll}}$. We integrate the effective force with distance to obtain the potential of mean force. For each run, we check that Newton's third law is obeyed, i.e., that the effective force exerted on protein 1 is equal to the one exerted on protein 2.

We first ask how ion-mediated interactions between two spherical proteins may be explained by the structure of the ionic cloud. A structural analysis is achieved by computing the anion density field $\rho(R,r,z)$, depicted in Fig. 1. (r,z) are the coordinates on a cylindrical frame, and the coordinates of the charges of both proteins are $(0,R/2)$ and $(0,-R/2)$. Let us consider a protein of charge $Z=10$, located at $(0,R/2)$. The space around this protein may be divided into two areas: for $z<R/2$, anions are located in *bonding* areas, causing an effective electrostatic force that pushes this protein toward the other one, and, conversely, for $z>R/2$, anions are located in *antibonding* areas. One must note that anion bonding zones correspond to cation antibonding zones. When two proteins are getting closer to each other, the response of the ionic cloud critically differs, depending on whether the proteins are oppositely charged or not. As shown in Fig. 1(c), when like-charged proteins are close, oppositely charged ions predominantly condense in bonding regions, where the electric field is the most intense. This induces an electrostatic attraction, but also a repulsive collision force. Conversely, Fig. 1(b) shows that, with oppositely charged proteins, it is an unequal depletion around the proteins that leads to the screening effect: the ions are less condensed in bonding areas than in antibonding ones, which causes an electrostatic repulsion of both proteins, and an attractive collision force. Ion-induced electrostatics dominates over collision forces, and thus ions reduce the direct Coulombic interactions.

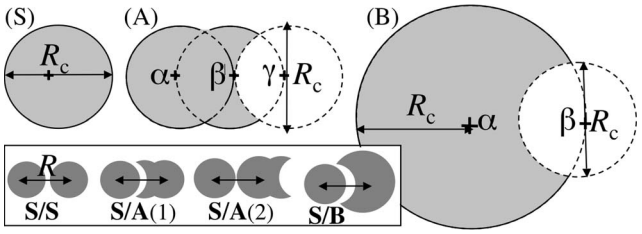

FIG. 2. Schematic view of the different model proteins. The charges of the proteins are placed on α. The interactions are computed as a function of the distance R between the charges, for the geometries sketched in the box.

The previous trends lead us to design protein models as presented in Fig. 2: the complexes *S*-*S*, *A*-*S*, and *B*-*S* differ by their volume and by the area of the interface. Indeed, what should influence ion-mediated forces in protein-protein complexes is the accessibility of the protein surface and of the surrounding volume to ions. The biological representativity of those models is also taken into account: in most biological complexes, such as the much studied barnase-barstar complex, a ligand links into the cavity of a protein, the so-called active site. The chosen shapes could model an enzyme and its substrate, or a hormone and its receptor. The proteins *A* include three aligned sites: α and β are the centers of hard spheres of radius $0.5R_c$ and γ is the center of a cavity of radius $0.5R_c$. An ion i is excluded if $r_{i\alpha}<0.5R_c+a_m$ or $r_{i\beta}<0.5R_c+a_m$ and $r_{i\gamma}>0.5R_c-a_m$. The proteins *B* include two sites α and β: $V_H=\infty$ if $r_{i\alpha}<R_c+a_m$ and $r_{i\beta}>0.5R_c-a_m$. In every case, the distance between the charges of both proteins at contact is $R_c=2$ nm, and the absolute charge of both proteins is 10.

To compare the different cases, we focus on the modification of the interaction exerted on the unchanged protein *S*. We emphasize that the orientation-averaged mean force is not computed because our main focus is to investigate whether the lock-and-key orientation (1) is electrostatically favorized. Indeed, the rotational diffusion that enables the active sites to line up is a crucial step of the recognition process. The mean force between proteins *S* and *A* is derived for the two orientations depicted in Fig. 2. The study of orientation (2) yields the same results as with two spheres (the deviations are less than 1%), and thus we focus on orientation (1). Figure 3 shows the different contributions to the effective force exerted on a spherical protein *S* as a function

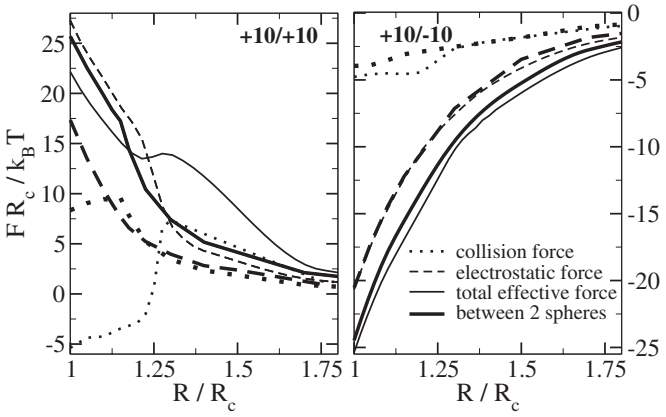

FIG. 3. Different contributions to the scaled effective force $F_{\text{eff}}R_c/k_BT$ exerted on a protein S as functions of the reduced distance R/R_c between two proteins S and A, which are either like charged (+10 and +10) or oppositely charged (+10 and −10). The same is plotted for two spherical proteins S (the three thickest curves of each graph).

of the distance from a protein A in orientation (1) (thin curves), and as a function of the distance from another protein S (thick curves). When the two proteins carry the same charge ($Z=10$), two behaviors are observed. At the largest distances, the force between the proteins S and A is more repulsive than the one between two proteins S, but below a distance of about $1.25R_c$ the force between two spherical proteins becomes more repulsive.

Since ion-averaged forces are density functionals, we present an analysis of the result based on a comparison of the density fields depicted in Fig. 4, for $R/R_c=1.4$. Around a protein S in the presence of a protein A, there is a large deficit of anions in the bonding zone, and thus a large decrease of the screening intensity. Furthermore, this deficit is counterbalanced by an enhancement of anion density around the excluded zone, which enhances collisions at the surface of the spherical protein. This is a particular case where the mean variations of surface and volume densities are opposite. Therefore, both electrostatic and collision forces between the proteins S and A are more repulsive than those between two spheres.

A similar structural analysis would show that, below $R/R_c=1.25$, both surface and volume densities decrease. Indeed, the excluded volumes around both proteins overlap, and thus ions are excluded from the surface of the spherical

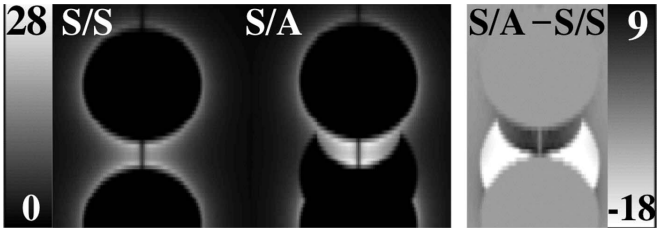

FIG. 4. Anion density field $\rho/\rho(R=\infty)$ for the reduced distance between like-charged proteins $R/R_c=1.4$, for a system with two spherical proteins S (left) and a system with proteins A and S. On the right, the difference of those fields is presented.

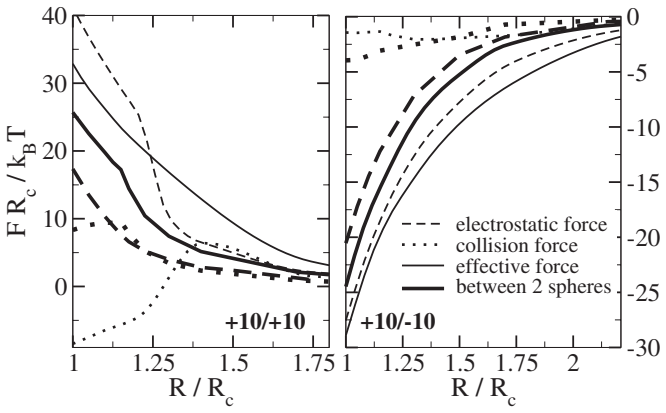

FIG. 5. Same as Fig. 3, but now the two proteins are of types S and B.

protein. That is why the collision force becomes attractive. Such behavior has not been reported so far to our knowledge for like-charged particles in a monovalent salt. Indeed, when like-charged particles get close, there is an ion compression, which decreases the total entropy of the system. However, in the present case, this compression mostly happens for $r > 0.5R_c$, where there is no accessible protein surface on which ions can exert a pressure. There is thus an ion-mediated depletion force that dominates in that range, so that the effective force is less repulsive than the one between two spheres.

As expected, the effect of shape change from S to A is much less pronounced for oppositely charged proteins, for which the additional excluded volume is in a region of low ion density. What is more unexpected is that the effective force is slightly more attractive between proteins A and S than between two spherical proteins for all interprotein distances, as shown in Fig. 3.

Finally, an analysis of the interaction between proteins S and B is achieved. The forces are shown in Fig. 5. In both S-A and S-B cases, the area of the interface between the proteins is the same (see Fig. 2). What changes is the excluded volume far from the interface, in bonding zones. Thus, in the case of like-charged proteins, the collision forces on a protein S are similar with both proteins A and B, while electrostatic forces are much more perturbed by a pro-

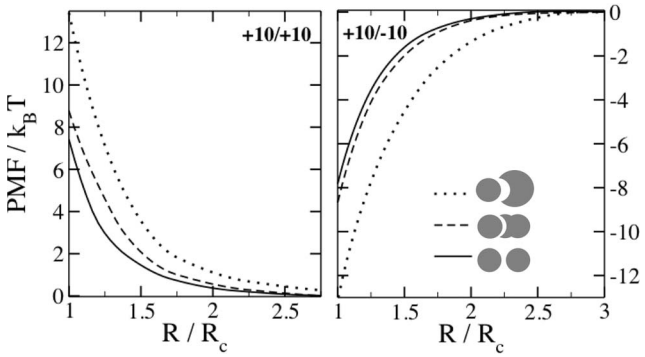

FIG. 6. Scaled potentials of mean force PMF (in units of k_BT) as a function of the reduced interprotein distance R/R_c for different protein shapes.

tein B. The loss of entropic repulsion is dominated by the loss of electrostatic attraction, and thus the force between proteins S and B stays more repulsive than the force between spheres for all distances. Similarly, the electrostatic interaction between oppositely charged proteins S and B is much less screened than the one between proteins S and A. This is also due to the exclusion of ions near the protein B, but in the present case those ions are predominantly antibonding cations.

In order to quantify the thermodynamic consequences of shape effects, the potentials of mean force are computed (see Fig. 6). For oppositely charged proteins, the depth of the potential well is increased by about $6k_BT$ for the interactions between proteins S and B, which increases the thermodynamic association constant by more than two orders of magnitude. In the meantime, the height of the potential wall between like-charged particles is enhanced by the same amount. Interestingly, those effects are not only present at short distances: their range is comparable to κ_D^{-1}.

A couple of remarks may be added. First, additional attractive ion-induced interactions, such as van der Waals forces [19], may enhance the trends observed here. Second, water-induced interactions [20,21] may also present the same features: dielectric screening depends on the volume of the proteins, like ion-mediated screening, while hydrophobic forces depend on the area of the interface, like collision forces. It would be of interest to repeat such a systematic study of shape effects with an explicit description of the solvent.

In conclusion, we have generalized the study of ion-induced forces within the primitive model of electrolyte to nonspherical particles such as interacting proteins. The topology of the volume excluded to salt ions influences the effective interactions between proteins both quantitatively and qualitatively. In particular, the sign of ion-induced entropic forces may change. When the shape of the particles recalls the usual protein-protein interfaces, the modification of the effective interaction leads to a greater affinity between oppositely charged nanoparticles. Moreover, our findings suggest that the decrease of ion-induced forces amplifies the impact of charge mutation (replacement of a basic residue by an acidic one). The unique structural optimization of proteins is thus linked not only to the place of their charges: in all studies of interprotein association or even in *in silico* inhibitor design, the architecture of the volume excluded to ions must be considered as a key factor influencing the interaction.

[1] D. P. Ryan and J. M. Matthews, Curr. Opin. Struct. Biol. **15**, 441 (2005).
[2] D. B. Kitchen, H. Decornez, J. R. Furr, and J. Bajorath, Nat. Rev. Drug Discovery **3**, 935 (2004).
[3] B. Honig and F. B. Sheinerman, J. Mol. Biol. **318**, 161 (2002).
[4] O. Schueler-Furman *et al.*, Science **310**, 638 (2005).
[5] J.-P. Hansen and H. Löwen, Annu. Rev. Phys. Chem. **51**, 209 (2000).
[6] R. R. Netz, Eur. Phys. J. E **5**, 557 (2001).
[7] J.-F. Dufrêche, T. O. White, and J. P. Hansen, Mol. Phys. **101**, 1741 (2003).
[8] N. Asherie, A. Lomakin, and G. B. Benedek, Phys. Rev. Lett. **77**, 4832 (1996).
[9] V. Dahirel, M. Jardat, J.-F. Dufrêche, and P. Turq, J. Chem. Phys. **126**, 114108 (2007).
[10] M. Jardat *et al.*, Mol. Phys. **104**, 3667 (2006).
[11] J.-F. Dufrêche, O. Bernard, P. Turq, A. Mukherjee, and B. Bagchi, Phys. Rev. Lett. **88**, 095902 (2002).
[12] A. Tardieu *et al.*, J. Cryst. Growth **196**, 193 (1999).
[13] V. Vlachy, Annu. Rev. Phys. Chem. **50**, 145 (1999).
[14] E. Allahyarov, I. D'Amico, and H. Löwen, Phys. Rev. Lett. **81**, 1334 (1998).
[15] K. C. Lee, I. Borukhov, W. M. Gelbart, A. J. Liu, and M. J. Stevens, Phys. Rev. Lett. **93**, 128101 (2004).
[16] V. Dahirel, M. Jardat, J.-F. Dufrêche, and P. Turq, J. Chem. Phys. **127**, 095101 (2007).
[17] J. Z. Wu, D. Bratko, and J. M. Prausnitz, Proc. Natl. Acad. Sci. U.S.A. **95**, 15169 (1998).
[18] κ_D^{-1} is defined by $\kappa_D^2=(e^2/\epsilon_0\epsilon_r k_BT)\Sigma_{i=1}^2\rho_i z_i^2$, where ρ_i is the concentration of microion i.
[19] S. A. Edwards and D. R. M. Williams, Phys. Rev. Lett. **92**, 248303 (2004).
[20] J. Dzubiella and J. P. Hansen, J. Chem. Phys. **121**, 5514 (2004).
[21] D. Chandler, Nature (London) **437**, 640 (2005).