Competitive adsorption in model charged protein mixtures: Equilibrium isotherms and kinetics behavior

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The competitive adsorption of proteins of different sizes and charges is studied using a molecular theory. The theory enables the study of charged systems explicitly including the size, shape, and charge distributions in all the molecular species in the mixture. Thus, this approach goes beyond the commonly used Poisson–Boltzmann approximation. The adsorption isotherms of the protein mixtures are studied for mixtures of two proteins of different size and charge. The amount of proteins adsorbed and the fraction of each protein is calculated as a function of the bulk composition of the solution and the amount of salt in the system. It is found that the total amount of proteins adsorbed is a monotonically decreasing function of the fraction of large proteins on the bulk solution and for fixed protein composition of the salt concentration. However, the composition of the adsorbed layer is a complicated function of the bulk composition and solution ionic strength. The structure of the adsorb layer depends upon the bulk composition and salt concentration. In general, there are multilayers adsorbed due to the long-range character of the electrostatic interactions. When the composition of large proteins in bulk is in very large excess it is found that the structure of the adsorb multilayer is such that the layer in contact with the surface is composed by a mixture of large and small proteins. However, the second and third layers are almost exclusively composed of large proteins. The theory is also generalized to study the time-dependent adsorption. The approach is based on separation of time scales into fast modes for the ions from the salt and the solvent and slow for the proteins. The dynamic equations are written for the slow modes, while the fast ones are obtained from the condition of equilibrium constrained to the distribution of proteins given by the slow modes. Two different processes are presented: the adsorption from a homogeneous solution to a charged surface at low salt concentration, and large excess of the large proteins in bulk. The second process is the kinetics of structural and adsorption change by changing the salt concentration of the bulk solution from low to high. The first process shows a large overshoot of the large proteins on the surface due to their excess in solution, followed by a surface replacement by the smaller molecules. The second process shows a very fast desorption of the large proteins followed by adsorption at latter stages. This process is found to be driven by large electrostatic repulsions induced by the fast ions from the salt approaching the surface. The relevance of the theoretical predictions to experimental system and possible directions for improvements of the theory are discussed. © 2003 American Institute of Physics. [DOI: 10.1063/1.1578992]

I. INTRODUCTION

Protein adsorption is a complex process determined by the interplay between large competing interactions, including van der Waals (vdw), electrostatic, and short-range, steric, repulsions. Furthermore, the specific thermodynamic state of the protein solution in contact with the surface also affects the adsorption process. One of the reasons for the complexity is due to the fact that the interactions are in general large compared to the thermal energy. This is due to the colloidal character of the proteins. An additional complexity arises when the proteins are charged.1–5 Most proteins contain amino acids with side chains that can be charged, depending on the pH of the solution. The relative importance of the electrostatic interactions on the adsorption process can change depending upon the ionic strength of the solution, since the screening of the electrostatic interactions is a function of the amount of ions in the solution. There is another important complexity in the adsorption of proteins and that is due to the ability of the proteins to undergo conformational changes upon adsorption.6–13

Protein adsorption plays an important role in many biological processes14,15 and is one of the primary methods used in separation of proteins, e.g., chromatography.5,16–18 Thus, it is important to understand the parameters that determine the competition in protein adsorption from mixtures. Further, the factors that determine the equilibrium adsorption isotherms are not necessarily the same that are responsible for the time-dependent adsorption. For example, it is found experimentally that in a mixture of albumin, immunoglobulin-G (IgG) and fibrinogen (Fgn) in contact with a polystyrene surface,19,20 the initial adsorption is dominated by the smaller protein (albumin), which is also at larger concentrations in the bulk, to be later replaced by the larger proteins like IgG and Fgn.

It is clear that the understanding of the adsorption process, both equilibrium and kinetics, requires the understanding of the role of each and all of the interactions involved in
+the process. In this paper we present systematic theoretical studies of the adsorption isotherms and kinetics of adsorption for model charged binary protein mixtures. The theoretical approach is a generalization of the approach that we have used to study the thermodynamics and kinetics of protein adsorption in the absence of electrostatic interactions.\textsuperscript{21–25} The work presented here studies the role of electrostatic interactions, coupled to the nonelectrostatic interactions, in determining the competitive adsorption.

Electrostatic interactions are different than vdw or steric repulsions in that their range and strength can be manipulated by adding electrolyte in a solution.\textsuperscript{26,27} In general, the range of the electrostatic interactions is determined by the Debye length, which is inversely proportional to the square root of the salt concentration. Thus, in the case of mixtures one can use the salt concentration to selectively adsorb one charged species over another. As we will see, these type of arguments are useful but are not enough to describe the adsorption behavior. However, salt will be very useful in manipulating the amount and type of protein that will adsorb as the result of the interplay between electrostatic, vdw, and packing interactions.

In the next section we present the theory for the equilibrium and kinetics of adsorption. The theory will be derived specifically for the systems for which the calculations will be presented in the Results section. The last part of the paper presents concluding remarks with a discussion of the potential and limitations of the methodology we use.

II. THEORY

The theoretical approach that we use is the same one that we have developed to study the adsorption of proteins on surfaces with and without grafted polymers.\textsuperscript{21,24} The predictions of this molecular theory have been shown to be in excellent quantitative agreement for the adsorption isotherms of lysozyme and fibrinogen on surface modified with short\textsuperscript{23} and long\textsuperscript{22} grafted polyethylene oxide. Therefore, we are confident of the quality of the theoretical predictions. Further, we have recently presented an extensive study on the equilibrium and kinetics of competitive adsorption without electrostatic interactions.\textsuperscript{24} There, we presented a thorough derivation of our theoretical approach for the most general case. Our objective in the work presented here is to see how the addition of electrostatic interactions influences the adsorption behavior. Therefore, the presentation of the theory is done specifically for the system of interest and the reader is referred to Ref. 24 for the generalization of the theory to other cases.

A. Equilibrium approach

The system of interest is composed of a surface of area $A$ that spans the $x$, $y$ plane at the origin of the $z$ axis. The surface is in contact with a solution containing a binary mixture of charged proteins modeled as spheres of radius $R_s$ and $R_l$ for the small and large particles, respectively; see the schematic representation of the system in Fig. 1. The density of proteins in the bulk solution is $\rho_{pro,s}$ and $\rho_{pro,l}$; thus, we define the composition of small proteins by $y_s = 1 - y_l = \rho_{pro,s}/(\rho_{pro,s} + \rho_{pro,l})$. Equivalently, we can define the solution properties by the protein chemical potentials, $\mu_{pro,s}$ and $\mu_{pro,l}$ for the small and large protein, respectively. The solvent molecules have volume $v_w$ and the salt is at concentration $c_{salt}$ and it is assumed to be completely ionized into monovalent cations and anions, each assumed to be spherical and of equal radius $r_{salt}$. The bulk chemical potentials of the cations and anions are defined as $\mu_+$ and $\mu_-$, respectively. We will use the solvent volume $v_w$ as the unit of volume throughout.

The starting point of the theory is to write the free-energy functional of the system. For simplicity we will consider that all intermolecular (but not surface–molecule) vdw interactions are equal. Therefore, we need to consider a system with electrostatic interactions, short-range (hard-core) steric repulsive interactions, and protein–surface vdw interactions. The addition of the intermolecular vdw interactions in the theory has been shown elsewhere.\textsuperscript{24}

We assume that the direction perpendicular to the surface is the only inhomogeneous one. Therefore, for the system defined above we can write the free-energy density (per unit area) in the form

\[
\frac{\beta BW}{A} = \int_0^\infty \rho_{pro,l}(z)[\ln \rho_{pro,s}(z)v_w - 1 + \beta U_{s,s}(z) - \beta \mu_{pro,l}]dz + \int_0^\infty \rho_{pro,s}(z)[\ln \rho_{pro,l}(z)v_w - 1 + \beta U_{s,s}(z) - \beta \mu_{pro,s}]dz
\]

\[
\times dz + \int_0^\infty \rho_+(z)[\ln \rho_+(z)v_w - 1 - \beta \mu_+]dz + \int_0^\infty \rho_-(z)[\ln \rho_-(z)v_w - 1 - \beta \mu_-]dz + \int_0^\infty \rho_{w}(z)
\]

\[
\times [\ln \rho_{w}(z)v_w - 1]dz + \frac{1}{2} \beta \int_0^\infty Q_{sol}(z)\psi(z)dz. \tag{1}
\]

FIG. 1. Schematic representation of the system containing a binary mixture of charged proteins dissolved in a low molecular weight solvent and dissociated salt in contact with a charged surface. The filled charged circles are protein molecules with different sizes and the empty charged circles are the ions from the salt. The protein molecules have charges that are opposite to that of the surface. The $z$ direction is defined perpendicular to the surface. The proteins at position $z'$ represent the molecules with their point of shortest distance with the surface being $z$.
where \( \beta = 1/k_B T \), where \( k_B \) is the Boltzmann constant and \( T \) is the absolute temperature. All the densities are now explicit functions of the distance from the surface, e.g., \( \rho_{\text{pro},i}(z) \) is the density of small proteins at a distance \( z \) from the surface. The distance from the surface is defined as the point of closest approach to the surface; see Fig. 1.

The first two terms in Eq. (1) represent the contribution for the large and small proteins, respectively. They include the \( z \)-dependent entropy of mixing; the protein–surface vdw attraction, with \( U_{1,s}(z) \) the strength of the vdw interaction between protein \( i \) at \( z \) and the surface; and the chemical potential term that ensures thermodynamic equilibrium at all \( z \). The next two terms are for the cations and anions and include mixing entropy and chemical potential. The fifth term is the mixing entropy of the solvent, and the last one is the total electrostatic energy, with \( \psi(z) \) being the electrostatic potential at \( z \), which represents the \( z \) component of the Coulombic potential

\[
\psi(r) = \frac{1}{4\pi\epsilon\epsilon_0} \int \frac{Q_{\text{tot}}(r')}{r-r'} \, dr',
\]

where \( \epsilon \) is the dielectric constant of the solvent. The total charge density at \( z \) is

\[
Q_{\text{tot}}(z) = \int_{z-2R_j}^z \rho_{\text{pro},i}(z') q_{\text{pro},j}(z;z') \, dz' + \int_{z-2R_j}^z \rho_{\text{pro},s}(z') q_{\text{pro},s}(z;z') \, dz' + \int_{z-2R_{\text{salt}}}^z \rho_+ (z') q_+(z;z') \, dz' + \int_{z-2R_{\text{salt}}}^z \rho_- (z') q_-(z;z') \, dz',
\]

with \( q_i(z;z') \) representing the charge that particle of type \( i \) at \( z' \) contributes to \( z \), i.e., it is the particle charge distribution.

The free-energy density, Eq. (1), does not include the excluded volume interactions. These are accounted for by packing constraints. Namely, at each distance \( z \) from the surface the available volume is filled up by large and small proteins, cations, anions, and solvent molecules. This is written in the form

\[
\int_{z-2R_j}^z \rho_{\text{pro},i}(z') v_{\text{pro},i}(z;z') \, dz' + \int_{z-2R_j}^z \rho_{\text{pro},s}(z') v_{\text{pro},s}(z;z') \, dz' + \int_{z-2R_{\text{salt}}}^z \rho_+ (z') v_+(z;z') \, dz' + \int_{z-2R_{\text{salt}}}^z \rho_- (z') v_-(z;z') \, dz' + \phi_w(z) = 1; \quad 0 \leq z \leq \infty,
\]

where \( v_i(z;z') \) is the volume that a molecule of type \( i \) at \( z' \) contributes to \( z \), i.e., it is the volume distribution of molecule \( i \) and it contains all the information on the size and shape of the molecules, also \( \int_{z-2R_j}^z \rho_i(z,z') \, dz' = V_i \) the total volume of molecule \( i \). \( \phi_w(z) = \rho_w(z) v_w \) is the volume fraction of water at \( z \).

We can find the density profiles of each component by minimizing the free-energy density, (1), subject to the packing constraints, Eq. (4). This is done by introducing Lagrange multipliers, \( \beta \pi(z) \), to yield

\[
\rho_{\text{pro},i}(z) v_w = \exp \left[ \beta \mu_{\text{pro},i} - \beta U_{1,i}(z) \right] - \int_{z}^{z+2R_j} \beta \pi(z') v_{\text{pro},i}(z';z) \, dz' - \int_{z}^{z+2R_j} \beta \psi(z') q_{\text{pro},i}(z';z) \, dz',
\]

\[
\rho_{\text{pro},s}(z) v_w = \exp \left[ \beta \mu_{\text{pro},s} - \beta U_{1,s}(z) \right] - \int_{z}^{z+2R_j} \beta \pi(z') v_{\text{pro},s}(z';z) \, dz' - \int_{z}^{z+2R_j} \beta \psi(z') q_{\text{pro},s}(z';z) \, dz',
\]

\[
\rho_+(z) v_w = \exp \left[ \beta \mu_+ - \beta U_+(z) \right] - \int_{z}^{z+2R_{\text{salt}}} \beta \pi(z') v_+(z';z) \, dz' - \int_{z}^{z+2R_{\text{salt}}} \beta \psi(z') q_+(z';z) \, dz',
\]

\[
\rho_-(z) v_w = \exp \left[ \beta \mu_- - \beta U_-(z) \right] - \int_{z}^{z+2R_{\text{salt}}} \beta \pi(z') v_-(z';z) \, dz' - \int_{z}^{z+2R_{\text{salt}}} \beta \psi(z') q_-(z';z) \, dz',
\]

\[
\phi_w(z) = \exp \left[ - \beta \pi(z) v_w \right],
\]

for the large protein, small protein, cation, anion, and solvent, respectively. In order to derive Eqs. (5)–(9) we have used the explicit dependence of the electrostatic potential on the density, as shown in Eqs. (2) and (3).

The Lagrange multipliers associated with the packing constraints are related to the osmotic pressure necessary to keep the chemical potentials of each molecular entity constant at all \( z \). In other words, \( \pi(z) \) is the mean-field repulsive field responsible for fulfilling the packing constraints.\(^{21,24}\)

The determination of the density profiles requires the knowledge of the electrostatic potential, \( \phi(z) \), and the repulsive fields, \( \pi(z) \). They are obtained by replacing the explicit expressions for the densities, Eqs. (5)–(9), in the constraints equation, Eq. (4), and considering that the electrostatic potential is the solution of the Poisson equation, which for the one-dimensional inhomogeneities considered here can be written as

\[
\frac{\partial^2 \psi(z)}{\partial z^2} = -\frac{1}{\epsilon} Q_{\text{tot}}(z),
\]
with the total charge density \( Q_{\text{tot}}(z) \) given by Eq. (3), with the corresponding densities from Eqs. (5)–(9).

A close look at the Poisson equation, Eq. (10), with the charge density replaced by (3) and the densities in it from (5)–(9), shows that this theory goes well beyond the Poisson–Boltzmann approach\(^{26,27} \) commonly used to treat electrostatic interactions in complex systems. As it can be seen in the expressions derived here, our approach explicitly includes the size, shape, and charge distributions of the molecules. These molecular quantities clearly affect the interactions in the environment and the resulting balance of forces that will determine the adsorption behavior. Furthermore, it is straightforward to include conformational degrees of freedom to the theory in general\(^{21} \) and for the case of charged systems in particular.\(^{28} \)

The boundary conditions that we use in this work for the Poisson equation is that the electrostatic potential in the bulk, homogeneous, solution is zero and that the surface charge density, \( \sigma \), is fixed. From the electroneutrality condition then, the boundary condition for the electrostatic potential becomes

\[
\sigma = -\epsilon \left( \frac{\partial \psi}{\partial z} \right)_{z=0}.
\]

We have now the complete sets of equations necessary to determine the repulsive and electrostatic fields. More explicitly, we replace the expressions for all \( \rho_i(z) \) from Eqs. (5)–(9) into both the constraint equation, (4), and into the total charge, Eq. (3), that is needed in the Poisson equation, Eq. (10). The resulting two sets of equations are discretized in the \( z \) directions and thus converted into a set of nonlinear coupled equations that are solved by standard numerical methods to obtain the coupled fields \( \pi(z) \) and \( \psi(z) \). See the Appendix for details on the numerical methodology.

In order to solve the equations we need to define the systems that we will consider. For all the calculations presented below we study mixtures of two spherical proteins; the large has a radius of \( R_l = 15 \) Å, while the small one has \( R_s = 9 \) Å. The charge on the proteins is varied, and thus it will be provided explicitly for each case. In all cases we only study proteins, in which it is assumed that the charge is homogeneously distributed on the surface of the protein. The cations and anions are modeled as spherical with radius \( r_s = 3 \) Å; they are monovalent and their charge is localized at their center. The solvent volume is \( V_w = 30 \) Å\(^3 \), and its dielectric constant is \( \epsilon = 80 \). The surface charge density in all cases is \( \sigma = 0.5 e/\text{nm}^2 \).

The protein–surface vdw interaction is assumed to follow the same functional form as that obtained by Lee and Park\(^{29} \) for the interaction between lysozyme and polymeric surfaces. However, the strength of the interaction is changed so that upon contact with the surface the large proteins gain 20\( k_BT \). The specific potentials used in the calculations are shown in Fig. 2. We see that the potentials are strong for distances of the order of the size of the proteins. At longer distances there is a weak attraction. For all distances \( U_{l,s}(z)/U_{s,s}(z) = R_l/R_s \), as expected for vdw interactions in colloidal systems.\(^{26,27} \)

**B. Dynamic approach**

Once the equilibrium is understood we are interested in studying what determines the kinetics of the adsorption process. In the case of charged systems there are two main questions of interest. The first is how do the proteins from the bulk homogeneous solution adsorb to the surface, and what is the surface rearrangement for the cases in which the bulk protein composition is different than that of the equilibrium adsorbed layer. The second is the kinetic process in which the adsorbed layer changes its composition and structure due to a change in the bulk solution properties, e.g., the bulk salt concentration.

The theoretical approach is a generalization of the theory we developed to study the kinetics of adsorption in uncharged systems.\(^{23,24} \) The basic idea of this theory is the separation of time scales into fast and slow. The fast degrees of freedom are then assumed to equilibrate in the time scale of the motion of the slow modes. Therefore, we can minimize a constrained free energy to determine the fast modes. We will consider the motion of the small cations, anions, and that of the solvent to be the fast modes. Therefore, we write equations of motion for the slow modes, i.e., for the two proteins. This is done with generalized diffusion equations of the form

\[
\frac{\partial \rho_i(z,t)}{\partial t} = D_i \frac{\partial}{\partial z} \left( \frac{\partial \mu_i(z,t)}{\partial z} \right),
\]

\[
\frac{\partial \rho_{\text{tot}}(z,t)}{\partial t} = D_t \frac{\partial}{\partial z} \left( \frac{\partial \mu_{\text{tot}}(z,t)}{\partial z} \right),
\]

where we have defined the time-dependent chemical potential of protein \( i \) as an extension of the equilibrium definition, i.e.,

\[
\mu_i(z,t) = \frac{\delta W(t)/A}{\delta \rho_i(z,t)},
\]

where \( W(t)/A \) is the free energy as given by Eq. (1) minimized with respect to the density of cations, anion, and solvent, but with the values of \( \rho_i(z,t) \) and \( \rho_s(z,t) \) given by the
dynamic equations, Eq. (12), and there are no chemical potential terms for the proteins contributions in Eq. (1).

The explicit form of the time-dependent chemical potential is given by

$$\beta \mu_i(z;t) = \ln p_i(z;t) + \beta U_i + s(z)$$

$$+ \int_z^{z+2R} \beta \pi(z';t) v_i(z';z) dz'$$

$$+ \int_z^{z+2R} \beta \psi(z';t) q_i(z';z) dz', \quad \text{(14)}$$

where the time-dependent potentials $\pi(z;t)$ and $\psi(z;t)$ are determined by the minimization of the free energy with respect to the fast modes, for fixed distribution of the slow modes, i.e., for $p_i(z;t)$ given by (12). In other words, at each time step the constraint and the Poisson equations are solved for fixed distributions of large and small proteins.

Our dynamic approach can be thought as a generalization of the Poisson–Nernst–Planck (PNP) equation. This is along the same lines that our equilibrium approach is a generalization of the Poisson–Boltzmann equation since it includes the size, shape, and charge distributions of all the particles in the system. Our kinetic theory is at the same level of molecular detail as our equilibrium approach. Therefore, since the PNP equation is the dynamical version of the PB equation, our dynamic approach is a generalization of the PNP theory in the sense that it includes the size, shape, and charge distribution of the molecules involved.

To solve the diffusion equation we need to specify the boundary conditions. They depend upon the specific dynamic process that we want to study. We study the system from one equilibrium state, $\mu_i(z) = \text{constant}$ for all $z$, in which a perturbation is made at time $t=0$, such that $\mu_i(z,t=0)$ is not the same for all $z$ anymore, to a final equilibrium state where the chemical potentials are constant at all $z$. The details of how the dynamic simulations are carried out can be found in Ref. 24, with the only addition that now we also include the electrostatic interactions and thus, the Poisson equation is also solved at each time step. In all the calculations we scale the time by the diffusion constant of the small protein $D_t = D_{t,i}$. Further, we assume that the protein diffusion constants follow the Stokes–Einstein relations, and thus $D_t/D_{t,i} = R_s/R_{t,i} = 3/5$. The explicit equations that are solved numerically are shown in the Appendix.

III. RESULTS

There are two important components of the adsorption in the mixtures that need to be studied in order to understand competitive adsorption in charged systems. One is the structure of the adsorbed layer, and the second is the amount of material and the composition of the adsorbed layer. We define the adsorption as the excess of material over the bulk value, i.e., the adsorption of protein $i$ is

$$\Gamma_i = \int_0^\infty \left[ p_i(z) - \rho_{\text{bulk},i} \right] dz,$$

and thus, the fraction of adsorbed large protein is given by

$$y_{\text{ads},i} = \Gamma_i / (\Gamma_i + \Gamma_s).$$

Figure 3 shows the fraction of large proteins adsorbed as a function of the fraction of large proteins in bulk. There are results for uncharged mixtures and charged systems with different salt concentrations, as denoted in the figure. The inset shows the total adsorption as a function of the fraction of large proteins in bulk for the same cases. The charge on the large proteins is $Q_i = +12e$ and the charge on the small proteins is $Q_s = +4e$. The total volume fraction of protein in bulk is $\phi_{\text{bulk}} = 0.001$. $\Gamma_\text{total}$ is dimensionless, $\Gamma_\text{total} = \sum_i \left[ \rho(i) v_i - \rho_{\text{bulk}} v_i \right].$

For the charged proteins we see in Fig. 3 that at all salt concentrations the adsorption of the smaller proteins is even more favored than in the uncharged case, for almost all values of $y_{\text{bulk},i}$. This may seem surprising since the larger proteins have more charges than the small ones. The charge on the large proteins is such that the surface charge density on the large proteins is larger than in the small ones, thus a stronger contribution for the surface–protein electrostatic attraction. However, the smaller proteins can accommodate overall more charges closer to the surface, and therefore gain in overall electrostatic energy. Further, the adsorption of a large number of the larger proteins will result in larger electrostatic and steric interprotein repulsions, as discussed in more detail below.

The effect of bulk composition on the overall adsorption is shown in the inset of Fig. 3. The total amount of adsorbed proteins, $\Gamma_\text{total} = \Gamma_i + \Gamma_s$, is a monotonic decreasing function of the bulk composition of large proteins. Further, the curves also show the important quantitative effect of electrostatic interactions on the total adsorption. Namely, as the salt concentration decreases the adsorption of the charged proteins increases. The charging of the proteins results in a very large
increase of the amount of adsorbed proteins, even in the case of relatively high salt concentration.

The adsorption isotherms in Fig. 3 show the complexity involved in the balance of forces that determines the adsorption behavior. Each of the curves shows that there is a monotonic increase in the fraction of large adsorbed proteins as a function of the fraction of large protein in bulk. However, the different salt concentrations (and also the uncharged proteins) show different slopes at different $y_{\text{bulk},l}$. To gain a better understanding of the role of salt for different values of the bulk composition, Fig. 4 shows the isotherms in a different plane than the one presented in Fig. 3. This is the variation of the fraction of large proteins adsorbed as a function of salt concentration, for a variety of values of the bulk fraction of large proteins. It is clear that the effect of salt depends on the value of $y_{\text{bulk},l}$. First, the inset shows the total adsorption as a function of salt concentration and, as expected, the total amount of proteins adsorbed decreases as the salt concentration increases, due to the screening of the electrostatic interactions. It is important to emphasize that the salt screens both the surface–protein electrostatic attractions as well as the protein–protein electrostatic repulsions. The inset shows that in all the cases studied here there is a monotonic decrease of the adsorption with salt concentration; therefore, the effect of salt is stronger on the attractions than on the repulsions; see also Fig. 7 below and discussion thereafter.

The effect of salt on the fraction of adsorbed proteins is rather complex and depends on the bulk composition. Figure 4 shows that when the bulk composition has an excess of small proteins the composition of large proteins adsorbed decreases with salt concentration. However, for $0.9 \leq y_{\text{bulk},l} \leq 0.5$ the fraction of large proteins adsorbed shows a minimum. For higher values of $y_{\text{bulk},l}$ the fraction of large proteins adsorbed becomes a monotonic increasing function of the salt concentration. This behavior is the result of the balance between the different thermodynamic forces determining the adsorption including the electrostatic, vdw, excluded volume, and entropy of mixing. To gain a better understanding of these properties we now look at the structure of the adsorbed layers for different conditions.

Figure 5 shows the structure of the adsorbed layer for four different salt conditions, all for $y_{\text{bulk},l}=0.995$. The reason for choosing this particular composition is twofold. On the one hand, it is a relevant composition for understanding of separations processes in nature, e.g., blood proteins. On the other hand, it is an interesting case that shows no trivial behavior both for equilibrium and kinetics, as will be shown below. Furthermore, for the parameters studied here and in all the cases where the fraction of large particles in bulk is smaller (see Fig. 4), the small protein completely dominates the adsorption behavior.

Figure 5 shows that in the absence of added salt there is a large adsorption of the small proteins as a monolayer on the surface. There is a small number of large proteins adsorbed at the surface, followed by two more layers that contain only large proteins. The multilayer structure is the result of the long-range electrostatic interactions. Before we compare with the cases in which salt is added, it is important to quantify the range of the electrostatic interactions. Even though there is no added salt, screening is present due to the finite bulk concentration of the proteins and their counterions. We can quantify the screening by the Debye length, defined by

$$
\frac{1}{\kappa} = \left( \frac{\varepsilon_0 \varepsilon k T}{\sum q_i^2 |\rho_i|} \right)^{1/2},
$$

where the sum runs over all the charged species. For low salt concentration the dominant term comes from the bulk proteins concentration. For the large $y_{\text{bulk},l}$ considered in Fig. 5, the most dominant term is the one containing the concentration of large proteins due to the $q_i^2$ term. To get a quantitative idea of the effect, the largest Debye screening length for the cases shown in the figure is the one of the salt-free case, $1/\kappa=1.42$ nm, which is almost the same as the radius of the large protein. Therefore, the range of the electrostatic inter-
actions in all the cases shown in Fig. 5 is of the order of the size of the large protein. More precisely, it is within a few protein diameters.

A quantitative determination of the electrostatic interactions can be obtained by looking at the electrostatic potential as a function of the distance from the surface. This is shown in Fig. 6 for the four cases shown in Fig. 5. The strength of the electrostatic potentials at the surface is smaller (in absolute value) as the bulk salt concentration increases. Furthermore, the range of the interactions decreases with increasing salt. Note the finite range of the potential for the salt-free case, as discussed above. However, note that the Debye length is not enough to determine the structure of the adsorbed layer, or quantitatively the range of the interactions, and it should be taken as a guideline only, particularly in the region close to the surface.

The range of the electrostatic interactions explains the multilayer structure of the adsorbed proteins; compare Figs. 5 and 6. The four cases show clearly the formation of two layers for the large proteins but only one for the small one. The reason for only one layer of the small one is its very low bulk concentration and the relatively small charge that will induce a weak (long-range) electrostatic attraction with the surface. Furthermore, the first layer of large proteins adsorbed presents a very large steric barrier for a second layer of the small ones. Note that the second adsorbed layer of small proteins will be located close to \( z = R_l / 2 \), which is the position of the maximal volume fraction of large adsorbed proteins.

As the salt concentration increases there are two main changes on the adsorbed layer. First, the total amount of adsorbed proteins decreases, as also shown in the inset of Fig. 4. Second, there is a change in the relative amounts of large and small adsorbed proteins in the first layer. The source for this change is that as the concentration of ions from the salt increases, the relative importance of the electrostatic surface–protein attractions as the main driving force for adsorption diminishes. Thus, the vdw attractions become more dominant and there is an increase in the fraction of large proteins adsorbed.

It is important to emphasize that the changes in the dominant role of the electrostatic interactions as salt concentration increases are not due only to the change in the Debye length, as seen in the decay of the electrostatic potential in Fig. 6. More importantly for the adsorption is the fact that more cations from the salt adsorb on the surface replacing the proteins. To quantify this point, and to further show the important changes in the structure of the adsorbed layer due to the presence of the small proteins, Fig. 7 shows the volume fraction of proteins adsorbed and that of salt for two salt concentrations. In both cases the bulk solution contains only large proteins and salt. The very large number of cations adsorbed makes the effective surface charge for the proteins rather small, and therefore a smaller number of proteins are on the surface (left panel). Thus, the role of the cations is to compete for adsorption sites with the larger proteins. This may seem counterintuitive since the salt cations are much smaller that the proteins and are monovalent. Therefore, replacing proteins with the cations on the surface results in a decrease in the entropy of the system. However, in the cases treated here one needs to consider also the repulsive interactions associated with packing particles on the surface. The repulsive interactions are a strong function of the sizes of the molecules, and thus it is the competition between the electrostatic, packing, and the entropy of mixing that determines which molecules will adsorb. Therefore, the adsorption of the small cations is effectively the result of the competitive adsorption in the same way that the smaller proteins are in general preferred on the surface, as discussed in relation to Figs. 3–5.

To gain a better understanding of the relative role of the electrostatic interactions in determining the competition between the two proteins, we show in Fig. 8 the structure of the adsorbed layers at low salt concentration in four cases. The difference between the graphs is the charge on the small proteins, while the large proteins are identical in all cases. The four cases are shown in the same scale and thus, the volume fraction profiles show that in the four cases the total amount of proteins adsorbed is similar. However, the partition between the large and small proteins shows a very strong dependence on the charge of the small proteins. For low charge on the small proteins almost all the multilayer
adsorbed is composed by the large proteins. There are very well-defined layers adsorbed whose periodicity is determined by the size of the large proteins. The structure in this case is very similar to that obtained in the case of adsorption from a solution containing only the large proteins; see Fig. 7.

Adding one more charge to the small proteins results in a replacement of some of the large proteins adsorbed in the layer in contact with the surface by small proteins. The second layer, formed exclusively by large proteins, is identical to the one shown in the upper left graph of Fig. 8 and to the pure large protein second layer, Fig. 7. For the charges in the proteins corresponding to Figs. 3–5 above, there are enough small proteins adsorbed on the first layer resulting in a displacement of the layers of large proteins adsorbed. Further increase on the charge of the small protein results in a total replacement of the first layer of adsorbed proteins by the small ones.

In all cases shown in Fig. 8 the second layer of adsorbed proteins is exclusively formed by the large protein. The amplitude of the second layer and its position depends upon the partition of large and small proteins in the first layer. The amplitude is determined by the effective charge that the proteins see from the surface, while the position is a function of the excluded volume repulsions exerted by the number and type of proteins adsorbed on the first layer. The replacement of large proteins by small ones in the first layer, and the related changes in the overall adsorption is qualitatively the same for other bulk salt concentrations.

The results presented in Figs. 3–8 reflect how the interplay between the different interactions in the system determines the structure, amount, and type of proteins adsorbed. The complexity of the observed behavior and the nature of the sensitive interplay between the electrostatic, vdw, excluded volume, and the bulk conditions shows the need for a molecular description of the systems that is sensitive to all the relevant interactions. Further, the rich behavior and the ability to predict the preferential adsorption can be used as a design tool to obtain optimal desired adsorption by manipulating the experimental accessible variables, e.g., bulk salt concentration, bulk composition, and/or surface charge. For example, consider the case in which the adsorption of the large proteins is desired. We find that the conditions that are favorable are those in which the large proteins are in excess in the bulk phase, the charge of the small proteins is small, and then low salt concentration results in a large amount of large proteins adsorbed. However, for larger charges on the small proteins the salt concentration needs to be large, resulting in a much smaller overall adsorption with an excess of large proteins.

The discussion so far concentrated on the equilibrium isotherms of the mixture. The next question that we would like to address is how does the adsorption process take place. Namely, what is the time dependence of the adsorption. We will study two different processes. First, we will look at the problem of a bulk system at time zero is put in contact with a charged surface. The molecules, which at \( t = 0 \) are distributed isotropically in the absence of the surface, see a sudden large gradient of chemical potential due to the presence of the attractive surface. This is seen at the initial steps of the adsorption as shown in Fig. 9. The plot shows the density of proteins in contact with the surface as a function of time. There is a very sharp increase of the amount of large proteins adsorbed at the initial steps of the adsorption. The density of large proteins shows a maximum and then as the small proteins start to adsorb on the surface the large proteins desorb to leave enough room for the smaller ones.

The reason for the very large overshoot is that \( y_{\text{bulk}, I} = 0.995 \). Namely, 99.5% of the proteins in solution are the large ones and therefore reach the surface in larger numbers faster than the smaller ones. Actually, the maximum in the adsorption overshoot corresponds to the density of large proteins that would adsorb from a solution containing just the large proteins (at the same salt concentration). Once the small proteins start to reach the surface there is surface exchange. This process is found in experimental systems that study proteins adsorption from mixtures. We had previously discussed in detail the different possible scenarios for un-
charged systems. The addition of electrostatic interactions does not affect the basic physical behavior of the system in this respect, under the conditions of large protein excess in the bulk solution.

We have seen in the equilibrium isotherms that changing the bulk salt concentration has a very dramatic effect on the total amount of proteins adsorbed as well as in the composition of the adsorbed layer. Consider a system that has reached equilibrium at a given salt concentration, e.g., the case shown in Fig. 9, and after the system reaches equilibrium the solution is changed by one with the same bulk concentration of proteins but a different salt concentration. For example, consider the cases of Fig. 5 for the bulk salt concentration $c_{\text{salt}} = 0.001 \text{ M}$ and $c_{\text{salt}} = 0.1 \text{ M}$. In changing from the equilibrium at the low salt concentration to the high salt content, the amount of large proteins adsorbed increases while that of the small proteins decreases. Further, the structure of the adsorbed layer shows important structural changes, in particular for the position of the primary adsorption of the large proteins. The question, though, is how this process takes place.

Figure 10 shows the time-dependent changes of the density of large and small proteins in contact with the surface after changes in the bulk concentration of salt. Immediately after the increase of salt concentration, which occurs at $t = 0$, there is a very fast desorption of both the large and small proteins from the surface. Only after there is an almost complete desorption of the large proteins, and a large desorption of the small ones; the large particles start to adsorb again with a continuing desorption of the small proteins to finally reach the equilibrium state. The question that arises is what is the source of the initial fast desorption of the large proteins. To answer this question we need to consider the structure of the whole adsorbed layer and not only the proteins in contact with the surface. To this end, we show a three-dimensional presentation of the volume fraction profiles of the two proteins as a function of time in Figs. 11 and 12.

The dynamics of desorption of the small proteins is rather simple (Fig. 12). There is a fast desorption of small proteins adsorbed on the surface. The large proteins, however (see Fig. 11), show a more complex dynamic behavior. At very short times it is observed that the two layers of proteins close to the surface desorb. Then, after a short period of depletion of the proteins from the surface there is a relatively fast growth of a large first layer and a rather small secondary layer. To gain a better understanding of the fast desorption at short times, Fig. 13 shows the early stages of the volume fraction profile as a function of time for the large proteins.
There is a very fast leakage of proteins towards the bulk solution (large $z$). This is the only case where one can observe the presence of proteins at all $z$. This is because in a very short time the large amount of proteins adsorbed to the surface is forced to leave towards the bulk as the result of the sudden change in local environment. After the very fast desorption there is a period of time where the large proteins start slowly to adsorb again. It is interesting to note that the fast desorption is larger for the layer in direct contact with the surface. There is a minimum in the first layer of proteins as function of time (see also Fig. 10); however, that is not the case for the second layer. In the latter case there is a monotonic decrease towards the low final equilibrium value, even though the position of this layer shifts toward larger values of $z$; see Fig. 5 and the discussion thereafter.

Figures 10–13 allow for a description of the kinetic behavior, but they do not offer a complete explanation. There are two main questions that need to be addressed. First, why does the large protein desorb at such a fast rate upon change of the salt concentration. Second, why is there a variation in the amount of proteins at all $z$ only for the large proteins and further only during the short period of time of the fast desorption. To answer the first question we recall that our kinetic approach assumes that the salt ions and the solvent are much faster than the proteins. What we find is that upon change in the salt concentration there is a very fast migration of ions from the bulk solution towards the surface. These ions adsorb and thus there is an excess of charge on the surface. The large proteins were adsorbed on the surface mostly due to the electrostatic interactions. The sudden change in the local environment close to the surface, due to the small ions from the salt, induces a very large driving force for desorption driven almost exclusively by the electrostatic contribution. This is quantitatively obtained by looking at the separate contributions to the potential of mean force, where we learn that it is the gradient of the last term in expression (14) that is responsible for the desorption of the large particles.

The answer to the second question is that only for the very large desorption close to the beginning of the process shown in Figs. 12 and 13 is the rate of proteins leaving the surface faster than the rate to reach the bulk solution. Note that the bulk concentration is very low. In all the other stages of the kinetic processes that we have studied the flow of proteins towards the surface (or from the surface out as shown for the small protein in Fig. 11) is such that the local concentration change is small enough that it cannot be seen in the scales shown in Figs. 11 and 13. However, we can also see the presence of a small, temporary, second layer of small proteins at the beginning of the desorption process; see Fig. 12. This layer is formed by the proteins desorbing from the surface but it is very small in amplitude and short lived.

It is important to emphasize that the kinetics of structural change driven by a change in the ionic strength of the solution seems to be a generic feature of systems with fast, small charged particles (ions from the salt) and large slow particles. We found this behavior in all the cases that we studied, e.g., for the different charges on the small proteins as shown in Fig. 8 a change in salt from 0.001 to 0.1 M results in the same qualitative kinetic behavior as the one presented above. Further, as explained above, the fast adsorption of the ions from the salt results in a large electrostatic imbalance that drives the large proteins out of the surface with a very large force, resulting in a complete desorption followed by a much slower adsorption to reach the final equilibrium state.

IV. CONCLUSIONS

We have presented a systematic study of the thermodynamics and kinetics of adsorption of a binary mixture of charged model proteins. The studies were carried out using a molecular mean-field theory that treats the electrostatic interactions well beyond the commonly used Poisson–Boltzmann approach. The theory incorporates the size, shape, and charge distributions of all the molecular species in the solution. As the results show, the effect of the molecular parameters in determining the adsorption behavior is very important, even in the cases where electrostatic interactions are the dominant driving force for the adsorption. Therefore, the molecular theory with its possible generalizations (see below) provides us with an approach that allows for the systematic study of molecular charged systems.

In the cases studied here, we looked at the behavior of mixed spherical proteins of different size and charge in a solution containing dissociated small salt ions. The equilibrium theory is formulated by writing the free-energy functional of the system in terms of the densities of each molecular species. The repulsive interactions are included through packing constraints. The density profiles resulting from the minimization of the free energy are a function of two fields, a pressure field associated with the packing constraints and the electrostatic potential arising from the charges. The coupled fields are determined through the packing constraints and the solution of the Poisson equation. The resulting sets of coupled equations are solved numerically for the different conditions.

The kinetic approach is based on writing a generalized diffusion equation for each of the proteins. The driving force for the time-dependent behavior is the gradients of chemical potentials that arise when there is a change in some of the external variables in the system. This approach assumes that there is a separation of time scales for the motion of the proteins (slow) and that of the small ions from the salt and the solvent (fast). The fast modes are assumed to equilibrate at all times for the motion of the slow modes. The gradients of chemical potentials are then obtained from a constrained minimization of the system’s free energy. Namely, the free energy is minimized with respect to the fast modes for fixed configuration of the slow ones. Thus, at each time step the time-dependent pressure and electrostatic fields are obtained by solving the coupled packing constraints (for fixed protein distributions) and the Poisson equation.

We have previously developed the equilibrium and kinetic approaches to study protein adsorption on surfaces and without grafted polymers without electrostatic interactions. We found that the predictions of the theory are in excellent agreement with experimental observations and thus we expect the predictions pre-

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sented here, which include electrostatic interactions, to be reliable.

We found that the equilibrium adsorption isotherms and the structure of the adsorbed layers depend upon the composition of the bulk solution. Due to the strong effect of the electrostatic interactions in determining the adsorption behavior, the fraction of adsorbed proteins depends upon the bulk molar fraction and the solution ionic strength. Furthermore, we have found that the structure of the adsorbed layer is strongly dependent on the bulk salt concentration.

All of our studies were carried out for mixtures containing a large and a small protein. The large proteins have a larger number of charges (and larger charge surface density) than the small proteins, and also a stronger vdw surface-protein attraction. However, we find that in most cases the adsorption is dominated by the small proteins. This is due to the weaker steric repulsions (which are proportional to the proteins’ volume) between the small proteins, and in most cases a larger concentration of small proteins allows for an overall larger number of charges close to the adsorbing surface. Clearly, if the charge of the small proteins is decreased below a certain value, the larger (highly charged) protein dominates the adsorption.

For very high molar fractions of the large proteins, changing the salt concentration from low to high results in a change of the dominant protein adsorbed on the surface. At low salt concentrations the adsorption is dominated by electrostatic interactions and thus there are very large amounts of proteins adsorbed. The layer in contact with the surface is mostly dominated by the small proteins. However, the adsorption is multilayered and beyond the first layer the large protein is the only one adsorbed. At very high salt concentrations the overall amount of adsorbed proteins decreases, but the adsorption is dominated by the larger protein, including in the first layer. This is an interesting finding because the salt becomes an important degree of freedom that can be used for separation purposes. The main conclusion, though, is that the resulting competition depends upon the details of the protein mixture.

The kinetics of protein adsorption shows a very interesting phenomenon. First, we found, as it is the case of uncharged proteins, that there is an overshoot in the adsorption of the protein that is in large excess in solution, followed by the replacement of the more dilute protein. Note that in the cases that the small protein is in excess there is no overshoot, since the smaller proteins dominates the equilibrium and the kinetic behavior. Once the mixture adsorption reaches equilibrium at low salt concentration, changing the solution in equilibrium with the surface to one with high ionic strength results in a very fast complete desorption of the large proteins, followed by a new adsorption from solution. The small proteins, however, show a monotonic decrease on the amount of proteins adsorbed from one equilibrium state to the new one. The reason for the very fast desorption of the large proteins is that the ions from the salt are very fast in reaching the surface upon change in the solution. The large proteins are at the surface mostly due to the electrostatic interactions, and the sudden presence of many cations results in a very strong electrostatic repulsion that expels the protein from the surface. Once the surface is free of large proteins there is a relatively slow adsorption to reach the new equilibrium state, which has a different structure and amount of proteins adsorbed. This process, of explosive desorption followed by slow adsorption, is generic and we found it in all the mixtures that we have studied.

The theoretical study that we have presented has several important limitations: First, we have only considered inhomogeneities in the direction perpendicular to the surface. It is important to look at what will be the effect of including lateral correlations. In particular, this will enable the introduction of structural features of the adsorbing surface, e.g., discrete surface charges. In principle, the theory can be formulated for this problem, as it has been shown for other systems. The main limitation is that is not clear how feasible systematic calculations are, due to the very large computational needs. However, we are starting efforts in this direction with the idea to find under what conditions the full three-dimensional solution is necessary and under what conditions the one-dimensional inhomogeneities are sufficient. Second, we have used very simple models for the protein. While the model contains the basic ingredients for the understanding of the forces that control protein adsorption, it is well known that proteins are highly inhomogeneous molecules. Therefore, the next step is to include the explicit structure of the proteins. This can be done for proteins with known structure, e.g., by downloading the structure from the protein data bank, since the structure of the protein is an input for the theory. Third, we have not considered the ability of the proteins to undergo conformational changes upon adsorption. We have shown earlier, for noncharged proteins, that conformational changes upon adsorption are very important. In the work presented here, the motivation is to see what is the effect of charges, and the results show that they are very important. It is clear that conformational changes have associated with them changes in the charge distribution. Preliminary results (not shown here) show that the adsorption may change for different protein charge distributions. More work in this direction is certainly necessary. Fourth, we have assumed that the charges on the protein do not change under all the different conditions studied. The charge on the proteins is a function of pH. Therefore, charge regulation due to the anisotropic distribution of protons and local ionic strength needs to be included. Furthermore, we have not considered the possibility of charge condensation on the protein surfaces. We expect that this will not change the qualitative features of our findings. However, we are planning to include this feature in future studies to confirm under what conditions this statement is correct. Fifth, in the kinetics studies we have assumed a separation of time scales between the fast (salt and solvent) and slow (modes). While this is a reasonable approximation on physical grounds, it is not clear whether or not it introduces artifacts to the kinetic behavior. Again, further study is necessary to check the validity of this approximation.

The last limitation that we would like to mention is that the theory is effectively a mean-field approach. Therefore, there are certain level of correlations that cannot be included. Optimally, one would like to study the adsorption with ato-
mistic resolution using molecular dynamic simulations. However, that is out of the question since the time scale for adsorption is many orders of magnitude beyond what can be done with atomistic descriptions. Further, we have shown that the theory can predict the adsorption isotherms of proteins accurately when a good model of the protein is included. Thus, we believe that our theoretical approach can be systematically improved, and it presents a very good molecular method to study the complexity involved in protein adsorption. Clearly, it is very important to also keep in perspective the approximations used in the theory so that the predictive power of the approach is balanced with its intrinsic limitations.

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APPENDIX: NUMERICAL METHODOLOGY

In this Appendix we show the discrete equations that are used in the numerical implementation of the theory.

1. Equilibrium approach

Since the system is assumed inhomogeneous only in the $z$ direction, the continued space is discretized along $z$ into layers of thickness $\delta$. Layer $i$ is defined as the region between $(i-1)\delta<z<i\delta$. In the discretized space, all the integrals along $z$ are transformed into sums over $i$.

The packing constraint equations, Eq. (4), in discrete form at layer $i$ is given by

\[
\sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{\text{pro},i}(i-j+1)v_w \right] \frac{v_{\text{pro},i}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{\text{pro},i}(i-j+1) v_w \right] \frac{v_{\text{pro},i}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{+}(i-j+1) v_w \right] \frac{v_{+}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{-}(i-j+1) v_w \right] \frac{v_{-}(j)}{v_w} + \phi_w(i) = 1 \tag{A1}
\]

where $M_{\text{type}}=2R_{\text{type}}/\delta(\text{type}=l,s,salt)$; $\{i<M_{\text{type}}:i,M_{\text{type}}\}$ means if $i<M_{\text{type}}$, the upper limit for $j$ is $i$; otherwise it is $M_{\text{type}}$; $v_{\text{type}}(j)$ is the partial volume of the sphere type that is bound between two parallel planes at $(j-1)\delta$ and $j\delta$.

The Poisson equation is discretized as

\[
\frac{\psi(i+1)-2\psi(i)+\psi(i-1)}{\delta^2} = -\frac{1}{\epsilon} Q_{\text{tot}}(i) \quad i \neq 1; \tag{A2}
\]

and

\[
\frac{\psi(2)-\psi(1)+\delta\sigma/\epsilon}{\delta^2} = -\frac{1}{\epsilon} Q_{\text{tot}}(i) \quad i = 1; \tag{A3}
\]

with

\[
Q_{\text{tot}}(i) = \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{\text{pro},i}(i-j+1)v_w \right] \frac{q_{\text{pro},i}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{\text{pro},i}(i-j+1)v_w \right] \frac{q_{\text{pro},i}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{+}(i-j+1)v_w \right] \frac{q_{+}(j)}{v_w} + \sum_{j=1}^{i<M_{i}:i:M_{j}} \left[ \rho_{-}(i-j+1)v_w \right] \frac{q_{-}(j)}{v_w}, \tag{A4}
\]

where $q_{\text{type}}(j)$ is the charge that molecule type contributes to layer $j$. For example, for the proteins the charge is assumed to be homogeneously distributed on the surface of the proteins. For the spherical proteins and the equipotential planes of the discretization, this results in $q_{\text{type}}(j) = Q_{\text{type}}(j)/2R_{\text{type}}$.

The discretized density profiles are

\[
\rho_{\text{pro},i}(i)v_w = \exp \left[ \beta \mu_{\text{pro},i} - \beta U_{\text{pro},i}(i) - \sum_{j=1}^{M_{i}} \beta \pi(i-j) \right], \tag{A5}
\]

\[
\rho_{\text{pro},i}(i)v_w = \exp \left[ \beta \mu_{\text{pro},i} - \beta U_{\text{pro},i}(i) - \sum_{j=1}^{M_{i}} \beta \pi(i-j) \right], \tag{A6}
\]

\[
\rho_{+}(i)v_w = \exp \left[ \beta \mu_{+} - \sum_{j=1}^{M_{\text{salt}}} \beta \psi(i-j)q_{+}(j) \right], \tag{A7}
\]

\[
\rho_{-}(i)v_w = \exp \left[ \beta \mu_{-} - \sum_{j=1}^{M_{\text{salt}}} \beta \psi(i-j)q_{-}(j) \right], \tag{A8}
\]

\[
\phi_w(i) = \exp \left[ -\beta \pi(i)v_w \right]. \tag{A9}
\]

Replacing the density profiles shown in Eqs. (A5)–(A9) into both the constraint equation [Eq. (A1)] and the total charge [Eq. (A4)], that is needed in the Poisson equation [Eqs. (A2), (A3)], results in two sets of coupled nonlinear equations that are solved by standard numerical methods to obtain $\pi(i)$ and $\psi(i)$ for all $i$, with the appropriate boundary conditions mentioned in the Theory section.

2. Kinetic approach

To solve the diffusion equations in the slow modes, both the space ($z$) and the time ($t$) are discretized. The space is discretized equally into layers of thickness $\delta$ as shown
above. The time is not discretized equally. An adaptive method is used to adjust the time step $\Delta t$ as shown in Ref. 24.

Expanding the diffusion equation for large proteins [Eq. (12)] gives

$$\frac{\partial \rho_i(z,t)}{\partial t} = D_i \frac{\partial^2 \rho_i(z,t)}{\partial z^2} + D_i \rho_i(z,t) \frac{\partial^2 \mu_i(z,t)}{\partial z^2}.$$

(A10)

Applying finite differences in Eq. (A10) gives

$$\frac{\rho_i(t+\Delta t) - \rho_i(t)}{\Delta t} = f_i(t),$$

(A11)

with

$$f_i(t) = D_i \left[ \frac{\rho_i(t+1,t) - \rho_i(t)}{\delta} \right] \left[ \beta \mu_i(t+1,t) - \beta \mu_i(t) \right]$$

$$+ D_i \rho_i(t) \left[ \beta \mu_i(t+1,t) - \beta \mu_i(t) \right]$$

for $i = 1$,  (A12)

and

$$f_i(t) = D_i \left[ \frac{\rho_i(t+1,t) - \rho_i(t)}{\delta} \right] \left[ \beta \mu_i(t+1,t) - \beta \mu_i(t) \right]$$

$$+ D_i \rho_i(t) \left[ \beta \mu_i(t+1,t) - 2 \beta \mu_i(t) + \beta \mu_i(t-1,t) \right]$$

for $1 < i$,  (A13)

where the densities are now $\rho_i(t) = \rho_i(t)v_w$. Namely, we have used dimensionless densities. Equation (A12) is obtained by imposing the boundary condition in Eq. (12).

The chemical potential for large proteins in the discretized space is

$$\beta \mu_i(t) = \ln[\rho_i(t)v_w] + \beta U_i(t) + \sum_{j=1}^{M_i} \beta \pi(i+j-1,t)$$

$$\times v_{\text{pro},i}(j) + \sum_{j=1}^{M_i} \beta \psi(i+j-1,t)q_{\text{pro},i}(j).$$

(A14)

The initial distribution of proteins, $\rho_i(0,0)$ and $\rho_i(0,0)$, will be given as inputs. At each time step $t$, with the distributions of proteins known, we can solve $\pi(i,t)$ and $\psi(i,t)$ by minimizing the constrained free energy. The chemical potentials for proteins at each time step $t$ are then determined. The distributions of proteins at the next time step $t + \Delta t$ are obtained from

$$\rho_i(t+\Delta t) = \rho_i(t) + f_i(t) \Delta t,$$

(A15)

and

$$\rho_i(t+\Delta t) = \rho_i(t) + f_i(t) \Delta t.$$

(A16)

The cycle is repeated, with the appropriate time discretization, until the new equilibrium state is reached.