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Model for evaluating patterned charge-regulation contributions to electrostatic interactions between low-dielectric spheres

Dawn Hollenbeck^{1,*}, K. Michael Martini¹, Andreas Langner², Anthony Harkin³, David S. Ross³, and George M. Thurston^{1,†}

¹Department of Physics, Rochester Institute of Technology, Rochester, New York 14623-5603, USA

²Department of Chemistry, Rochester Institute of Technology, Rochester, New York 14623-5603, USA

³School of Mathematical Sciences, Rochester Institute of Technology, Rochester, New York 14623-5603, USA

Abstract

We study the electrostatic contribution to the effective potential between two spherical low-dielectric particles that carry proton-titratable sites within a linearized setting. To evaluate the needed work of charging for each possible proton occupancy configuration, together with its crucial dependence on sphere separation, we numerically solve a coarse-grained linear Debye-Hückel model that incorporates nonuniform dielectric and ionic solution properties at a series of intersphere separations and for chosen titratable charge locations on each sphere. We combine the resulting work-of-charging matrix with site-specific chemical potentials of proton binding to construct the Boltzmann-weighted probabilities of each possible occupancy pattern of the titratable sites as functions of intersphere separation. With the use of these probabilities we find that a nonmonotonic average electrostatic potential can result that is repulsive at larger sphere separations but attractive at close separations. The nonmonotonic potential corresponds to particular choices of site-specific unoccupied charge values and their corresponding proton affinities, and its occurrence is dependent on pH in relation to the pK_a values of the titratable groups. For the chosen titratable groups, we identify the particular change from repulsive to attractive proton occupancy patterns with decreasing intersphere separation that gives rise to the modeled nonmonotonic dependence and derive more general conditions under which such a nonmonotonic dependence can occur. Within the present model we find that stationary points of the charge-regulated average electrostatic potential, considered as a function of intersphere separation, occur when a normalized Boltzmann-averaged intersphere charge number product equals its covariance with an average free energy of charging divided by $k_B T$. We derive more general conditions for the location and nature of critical points in the electrostatic intersphere potential, which are not dependent on the validity of the present linear model. Analysis of the present simple prototype model can be a helpful step toward developing a framework for predicting when (i) patterned charge-regulated occupancy patterns, (ii) orientation-dependent attractions due to relatively fixed heterogeneous charging patterns, and (iii) screened net protein

* dmhsps@rit.edu. † georgemthurston@gmail.com.

charge could separately dominate the electrostatic portion of the interactions between model biological macromolecules and other nanoparticles.

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I. INTRODUCTION

The close approach of biological macromolecules can involve a dynamic multiple chemical equilibrium, in which protons and other species, such as other ions and water, bind and unbind partially in response to local potentials and in so doing alter potentials that affect their binding probabilities. For example, even a fairly small protein can have 50 chemically heterogeneous acidic and basic sites near its surface, each of which can, in principle, donate or accept protons from the surroundings, thereby changing the site charge and the nearby partially screened electrostatic potential. While their site specific varying proton affinities typically have the consequence that only a tiny fraction of the 2^{50} possible configurations will occur, there can nevertheless be a large number of probable configurations.

As protons come on and off the protein, the resulting change in electrostatic potential will also change the probabilities that neighboring titratable sites are occupied with protons in the process of charge regulation [1–6]. The affected sites can be on the same molecule (see, for example, [5]) or on neighboring molecules [6]. The probabilities of the most common proton occupancy configurations can therefore change in response to relative motion of neighboring macromolecules. Such fluctuations between probable configurations have long been recognized to contribute to the effective forces between the macromolecules [7]. At the same time, the charges of certain titratable sites or appropriate combinations of titratable sites [5] may instead be quite robust with respect to the typical magnitudes of change in the electrostatic potential in the pH range of interest, and these charge patterns that are essentially fixed on each molecule also contribute to the intermolecular potential.

In view of these possibilities it is important to quantitatively address the question of how and when (i) patterned charge-regulated occupancy patterns, (ii) orientation-dependent attractions due to relatively fixed heterogeneous charging patterns, and (iii) screened net protein charge combine to determine the electrostatic portion of the interactions between model biological macromolecules and other nanoparticles.

The present investigation examines a simple prototype model system for helping to identify principles involved in addressing these questions, an example of which is depicted in Fig. 1(a). We place groups of proton acceptor sites near the surfaces of two low-dielectric model spheres that are immersed in a salt solution. Groups 1 and 3 have bare charge of -1 and bare pK_a values of 6.5 and 7.0, respectively, while groups 2 and 4 have bare charges of 0 and bare pK_a values of 6.8 and 7.3, respectively. We numerically solve the linearized Poisson-Boltzmann equation to model the influence of each charge configuration on the chemical equilibrium as a function of sphere separation. To generate the plot shown in Fig. 1(b) we set the static dielectric coefficient of the spheres, relative to vacuum, to be 8, the dielectric

coefficient of the surrounding medium to be 80, the Debye screening length to be 6 Å, and a dielectric smoothing parameter s (see Appendix A) to be 0.2 Å. The spheres have radii of 17 Å and each titratable group is located 16.5 Å from the surface. The Debye screening length chosen corresponds to an ionic strength close to 0.26M. Through the use of the resulting binding polynomial [8], we show that due to the response of the chemical equilibrium to sphere separation, three classes of effective electrostatic interactions occur, as shown in Fig. 1(b): (i) interactions that are repulsive at long and short ranges, (ii) interactions that are repulsive at long range but attractive at short range, and (iii) interactions that are attractive at all ranges.

While the dielectric and ionic properties relevant to a particular modeling situation will, of course, vary from the ones chosen above, we find that the occurrence of nonmonotonic effective electrostatic interactions can be obtained with many other choices of these parameters, as can changes in proton occupancy patterns with sphere separation that are quite similar to those studied below. For example, it is not necessary to assume that the interior and exterior dielectric coefficients differ, and it is not necessary to have mobile ions present. The present example is therefore sufficient to illustrate the principles we wish to study.

We use the terminology “effective electrostatic interactions” since there will be many other factors that affect the overall interactions in physical systems, including the van der Waals forces, solvation, ion binding, and realms in which the linearized Poisson-Boltzmann equation does not provide a sufficiently accurate model for determining the electrostatic potential [9–11].

A number of other works have addressed charge-regulation interactions between neighboring particles but have typically focused on interactions between models that include continuously distributed regulated surface charge [3, 12–15]. The present approach differs in its focus on model particles with specific site placements of titratable groups. The work of Lund and Jönsson [6] also addresses the issue of charge-regulated interaction between neighboring particles, with the use of a different approach from that used here, which involves a multipole expansion of the electrostatic energy and the evaluation of statistics of fluctuations in overall protein charge and its moments, in combination with the Monte Carlo simulation of the surrounding mobile ion distributions. They have applied their approach to the study of interactions between proteins that contain a large number of protonation sites. The model investigated here focuses instead on few sites and a simple model geometry, without the use of a multipole expansion, as a step in studying site-specific aspects of charge regulation that could, in principle, depend on the statistics of each joint occupancy pattern involving the interacting larger particles.

In this paper we identify the particular changes from repulsive to attractive proton occupancy patterns with decreasing sphere separation that give rise to the modeled nonmonotonic dependence and derive more general conditions under which such a nonmonotonic dependence can occur. An outline of the paper is as follows. We summarize a theoretical framework, in part to set notation, which essentially follows tracks originated by Kirkwood and colleagues [7, 16], and study what general conditions it implies about the

possibility of nonmonotonic effective potentials or more generally about critical points of the effective potential with respect to parameters of interest. We then specify our numerical implementation and its validation and proceed to analyze the sphere-separation dependent chemical equilibrium corresponding to the situation depicted in Fig. 1 in detail by calculating the probabilities of all proton occupancy configurations as a function of sphere separation and pH . By this method we identify distinct proton occupancy configurations that dominate the potential at large and small intersphere separations. We then discuss implications and steps needed to extend this work toward quantitative study of orientational aspects of patterned charge regulation before concluding.

II. THEORY

A. Overview

To work toward a quantitative framework for studying pattern-specific charge regulation, we first model the screened electrostatic potential in the vicinity of titratable groups on particles using the linearized Poisson-Boltzmann equation for a medium with a dielectric coefficient and ionic strength that can both vary in space. After presenting some general considerations, we treat the case of two neighboring low-dielectric spheres with interiors that do not contain mobile solvent ions but which do carry acidic and basic sites that can change their charges through protonation or deprotonation. As a function of the possible geometric relationships between these site-decorated spheres, we numerically solve the linearized Poisson-Boltzmann equation to determine the influence of the charge of each possibly protonated site on the electrostatic potential at every other possibly charged site. In this linearized context, each such calculation yields a row (or the corresponding column) of a work-of-charging matrix. The work-of-charging matrix is then used in combination with site-specific proton affinities to construct a binding polynomial that incorporates the response of the Boltzmann-averaged probability of each possible proton-charging pattern to changes in intersphere separation and other parameters. The dependence of the average free energy of charging on intersphere separation then gives the modeled charge-regulated electrostatic contribution to the potential of mean force between the spheres, which we analyze further.

B. Screened electrostatic model

We model the response of the electrostatic potential ϕ to a specified distribution of fixed charge per unit volume $\rho(x, y, z)$ through the use of the linearized Poisson-Boltzmann equation written here for a medium with spatially varying dielectric coefficient $\epsilon(x, y, z)$ and Debye screening parameter $\kappa(x, y, z)$,

$$\nabla \cdot [\epsilon(x, y, z) \nabla \phi] = \kappa^2(x, y, z) \phi - \rho(x, y, z). \quad (1)$$

In order that κ corresponds to its usual definition, in Eq. (1) we take $\epsilon(x, y, z)$ to be the local dielectric coefficient divided by the dielectric coefficient of the solvent. Although more sophisticated models of electrolyte solutions will be needed in order to incorporate important physical effects such as finite ion size, ion-binding, polarizability-related interactions, solvation, nonlocal, and nonlinear dielectric response, Eq. (1) is nevertheless a

useful starting point for investigating patterned charge-regulation-mediated electrostatic interactions for a number of reasons. First, the linearity of Eq. (1) has the consequence that, as detailed below, the work of charging a given configuration of titratable sites may be expressed as a symmetric quadratic form in the vectors of site charges. Second, the simplicity of Eq. (1) is a benefit for helping to identify principles in the face of complexity. For example, in the case of the interaction between two small proteins that each carries 50 sites, to construct the needed work-of-charging matrix for a given separation and relative orientation of the proteins, even within this linear model, in principle requires solving Eq. (1) with 100 different functions $\rho(x, y, z)$, one for a test charge at each of the possibly charged sites.

C. Work of charging

In the present model we presume that the dielectric response of the medium is not only local but also linear in the electric field, specifically, that $\epsilon(x, y, z)$ is not itself a function of $\nabla\phi$. Under these conditions the electrostatic portion of the work of assembling a set of free

charges $\{q_i\}$ at locations $\{\vec{r}_i\}$ is given by $W_{el} = \frac{1}{2} \sum_{ij} q_i \phi_{ij}$, in which ϕ_{ij} is the potential at $\vec{r}_i$ that results when Poisson's equation is solved with the use of a free charge $\{q_j\}$ placed at $\vec{r}_j$ [17]. However, in the case of the mobile ions, one of the conditions for the derivation of the Poisson-Boltzmann equation is that the chemical potential of each type of mobile ion is not a function of position within the solution [9]. As a result mobile ions should not be included in the indices i and j entering into W_{el} above in the context of assessing free energy changes associated with changing protonation states since the mobile ion free-energy contributions are set by their fixed chemical potentials [18]. Nevertheless, since the ϕ_{ij} entering the work of charging should, in principle, include contributions produced by all the other charges, it is worth noting that when Eq. (1) is used to calculate the ϕ_{ij} , the contributions to $\phi(\vec{r}_i)$ due to (i) the altered spatial distributions of mobile ions and (ii) the spatial and surface polarization charges associated with the spatially nonuniform dielectric, which both occur in response to the presence of q_j at $\vec{r}_j$, will be included automatically. Since such altered distributions also affect the potential at $\vec{r}_j$, there is a “self-term” ϕ_{jj} that requires careful consideration.

We define the quantity w_{ij} to equal ϕ_{ij} at $\vec{r}_i$ for the case of a positive unit elementary charge placed at $\vec{r}_j$, found by solving Eq. (1), and express the $\{q_i\}$ in units of elementary charges e , so that the modeled contribution of the work of charging to the free energy becomes

$$W_{el} = \frac{1}{2} e^2 (q_1, q_2, \dots, q_n) \cdot \mathbf{W} \cdot (q_1, q_2, \dots, q_n) = \frac{1}{2} e^2 (\vec{Q}_b + \vec{\theta}) \cdot \mathbf{W} \cdot (\vec{Q}_b + \vec{\theta}), \quad (2)$$

in which $\mathbf{W}$ denotes the matrix of the w_{ij} and $\vec{Q}_b$ denotes the vector of signed bare charge numbers of the titratable groups, for example, -1 for unoccupied carboxylates and 0 for an amine or a histidine side chain. The vector $\vec{\theta}$ specifies the proton occupancy of each

titratable site and consists of 1's and 0's, depending on whether each specific site is occupied (1) or vacant (0), for example, $\vec{\theta} = \{1, 1, 0, 0, 1, 0, \dots, 1\}$. In Appendix B it is shown that the use of Eq. (1) and appropriate boundary conditions to calculate the w_{ij} implies that $w_{ij} = w_{ji}$ and in Appendix C it is shown that the use of Eq. (1) also implies that for $i \neq j$ one has $w_{ij} > 0$. The symmetry of $\mathbf{W}$ can be useful for helping to check code and shorten numerical computation. For the present purpose of evaluating the effective Boltzmann-averaged potential between the spheres, it is important to note that each of the w_{ij} is a function of both the separation and the relative orientation of the charged sites on the spheres at least in principle.

D. Chemical equilibrium, binding polynomial, and effective electrostatic potential

To find the effective electrostatic potential between the spheres we need to average the sphere-configuration dependent free energies of formation $G_{\alpha}(\mathbf{x})$ of each *joint* proton occupancy pattern $\vec{\theta}_{\alpha}$ of the two-sphere combination, weighted by their probabilities $P_{\alpha}(\mathbf{x})$, where $\mathbf{x}$ denotes the relative positions of the spheres, including the relative orientations of their charged sites. We model the $G_{\alpha}(\mathbf{x})$ by adding to $W_{el,\alpha}(\mathbf{x})$ the pH-dependent site-specific free-energy changes of proton-binding, $\mu_i(z)$, corresponding to each group that is occupied in configuration $\vec{\theta}_{\alpha}$. Here, $z=10^{-\text{pH}}$ denotes the proton activity a_p and we assume that each $\mu_i(z)$ is independent of both α and $(\mathbf{x})$. With the proton-binding affinity vector $\Delta\vec{\mu} \equiv (\Delta\mu_1, \Delta\mu_2, \dots, \Delta\mu_N)$, the model reads

$$\Delta G_{\alpha}(\mathbf{x}, z, \kappa) = \Delta\vec{\mu}(z) \cdot \vec{\theta}_{\alpha} + \frac{1}{2}e^2 \left(\vec{Q}_b + \vec{\theta}_{\alpha} \right) \cdot \mathbf{W}(\mathbf{x}, \kappa) \cdot \left(\vec{Q}_b + \vec{\theta}_{\alpha} \right), \quad (3)$$

with

$$P_{\alpha}(\mathbf{x}) = \frac{e^{-\beta \Delta G_{\alpha}(\mathbf{x})}}{\mathcal{Z}(\mathbf{x})}, \quad (4)$$

where $\beta=1/k_B T$. The quantity $\mathcal{Z}(\mathbf{x}) \equiv \sum_{\alpha} e^{-\beta \Delta G_{\alpha}(\mathbf{x})}$ is called the binding polynomial since it can be written as a polynomial in powers of the proton activity [5, 8, 19], as will be evident from Eq. (11).

The charge-regulated average effective potential due to electrostatics, $U_{el}(\mathbf{x}, z, \kappa)$, is then given by the difference between the average free energy of proton binding at a given relative configuration $\mathbf{x}$ of the particles and its average value at large particle separations,

$$U_{el}(\mathbf{x}, z, \kappa) = \sum_{\alpha} \{ \Delta G_{\alpha}(\mathbf{x}, z, \kappa) P_{\alpha}(\mathbf{x}, z, \kappa) - \Delta G_{\alpha}(\infty, z, \kappa) P_{\alpha}(\infty, z, \kappa) \}, \quad (5)$$

in which $G_{\alpha}(\infty, z, \kappa)$ and $P_{\alpha}(\infty, z, \kappa)$ refer to their values at large particle separations.

In Eq. 5, note that since $\mathbf{W}(\mathbf{x}, \boldsymbol{\kappa})$ is independent of z and $\Delta \vec{\mu}(z)$ is independent of $\mathbf{x}$ and $\boldsymbol{\kappa}$, numerical computation of $\mathbf{W}(\mathbf{x}, \boldsymbol{\kappa})$ for a given fixed value of $\boldsymbol{\kappa}$ can be used to predict $U_e(\mathbf{x})$ over a range of pH values, as is done below. We note that the average potential defined in Eq. (5) differs from that employed by Kirkwood and Shumaker [7], who instead made the assumption that the occupancy configurations on the different interacting particles were independent.

It is instructive to rewrite Eq. (5) in the following manner, in which we have suppressed the dependence on z and $\boldsymbol{\kappa}$ for clarity:

$$U_{el}(\mathbf{x}, z, \boldsymbol{\kappa}) = \sum_{\alpha} \{ \Delta G_{\alpha}(\mathbf{x}) [P_{\alpha}(\mathbf{x}) - P_{\alpha}(\infty)] + P_{\alpha}(\infty) [\Delta G_{\alpha}(\mathbf{x}) - \Delta G_{\alpha}(\infty)] \}. \quad (6)$$

For each proton occupancy configuration α , the two contributions to each summand given in Eq. (6) indicate that the free-energy changes associated with each configuration as well as the changes in their probabilities enter into the intersphere distance dependence of the effective potential. Equation (6) will be helpful for examining the contributions of individual summands to the effective nonmonotonic potentials below (Fig. 8 and text).

We note that Eq. (5) makes no mention of the intended application to just two particles and could also be applied in the case of more than two particles that carry titratable sites.

It is convenient to further identify the terms entering into the proton affinity contribution to the free energy, $\Delta \vec{\mu}(z) \cdot \vec{\theta}_{\alpha}$ by considering what the consequences of the equilibrium between proton configuration α and the unoccupied configuration u would be in a presumed absence of charge interactions.

$$\mu_u^o + k_B T \ln \rho_u + k_{\alpha} \mu_{H^+} = \mu_{\alpha}^o + k_B T \ln \rho_{\alpha}, \quad (7)$$

in which μ_u^o and μ_{α}^o are standard chemical potentials of states u and α , respectively, ρ_u and ρ_{α} are their corresponding number densities, μ_{H^+} is the proton chemical potential, and $k_{\alpha} = \vec{\theta}_{\alpha} \cdot \vec{\theta}_{\alpha}$ is the total number of protons bound to the combined two sphere system in configuration α . Equation (7) gives

$$\rho_{\alpha} = \rho_u e^{\left(k_{\alpha} \mu_{H^+} / k_B T \right)} e^{-(\mu_{\alpha}^o - \mu_u^o) / k_B T} = \rho_u z^{k_{\alpha}} e^{-(\Delta \mu_1^o, \Delta \mu_2^o, \dots, \Delta \mu_N^o) \cdot \vec{\theta}_{\alpha} / k_B T}. \quad (8)$$

Thus, we take

$$\Delta \vec{\mu}(z) \cdot \vec{\theta} = - \left(\Delta \mu_1^o, \Delta \mu_2^o, \dots, \Delta \mu_N^o \right) \cdot \vec{\theta}_{\alpha} + k_{\alpha} \ln z, \quad (9)$$

and we note that

$$\exp\left(-\frac{\Delta\mu_i^0}{k_B T}\right) = 10^{-pK_{a,i}}. \quad (10)$$

Assembling these relationships, the polynomial nature of $\mathcal{Q}(\mathbf{x})$ becomes more apparent when it is written in the following form from which like powers of z can be combined to identify each coefficient:

$$\mathcal{Q}(\mathbf{x}) = \sum_{\alpha} e^{-\beta \Delta G_{\alpha}(\mathbf{x})} = \sum_{\alpha} z^{k_{\alpha}} e^{-\beta (\Delta \vec{\mu}^0 \cdot \vec{\theta}_{\alpha})} e^{-\beta W_{el,\alpha}(\mathbf{x})}. \quad (11)$$

E. Further properties of the effective interparticle potential

An alternate form of U_{el} that can be useful results from combining Eqs. (4) and (5) and (11),

$$U_{el}(\mathbf{x}, z, k) = - \left(\frac{\partial \ln \mathcal{Q}(\mathbf{x}, z, k)}{\partial \beta^*} - \frac{\partial \ln \mathcal{Q}(\infty, z, k)}{\partial \beta^*} \right). \quad (12)$$

In this equation, the $*$ represents differentiation only with respect to the explicit β appearing in Eq. (11) since the terms in the exponent aside from β may depend on temperature, so that a differentiation with respect to $1/k_B T$ would not yield Eq. (5).

We are now in a position to further identify conditions in which the potential will be nonmonotonic by finding the derivative of U_{el} with respect to r and setting it to zero. With the use of Eq. (5) one can now account for the fact that work-of-charging entries change with r and thereby affect the derivative of the effective potential directly, but also that these same changes affect the relative occupancies of various proton configuration change through Eq. (4). Thus, noting that $\mathcal{Q}(\infty, z, k)$ is independent of r , we obtain

$$\begin{aligned} \frac{\partial U_{el}(\mathbf{x}, z, k)}{\partial r} &= - \left(\frac{\partial}{\partial \beta^*} \right) \frac{\partial \ln \mathcal{Q}}{\partial r} = \left(\frac{\partial}{\partial \beta^*} \right) \frac{\beta \sum_{\alpha} \frac{\partial \Delta G_{\alpha}}{\partial r} \exp[-\beta \Delta G_{\alpha}]}{\sum_{\alpha} \exp[-\beta \Delta G_{\alpha}]} \\ &= \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \right\rangle + \beta \left\langle -\frac{\partial \Delta G_{\alpha}}{\partial r} \Delta G_{\alpha} \right\rangle \\ &\quad - \beta \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \right\rangle \langle -\Delta G_{\alpha} \rangle \\ &= \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \right\rangle - \left\{ \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \beta \Delta G_{\alpha} \right\rangle - \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \right\rangle \times \langle \beta \Delta G_{\alpha} \rangle \right\} \\ &= \left\langle \frac{\partial \Delta G_{\alpha}}{\partial r} \right\rangle - \text{cov} \left(\frac{\partial \Delta G_{\alpha}}{\partial r}, \beta \Delta G_{\alpha} \right), \end{aligned} \quad (13)$$

where $\langle \rangle$ denotes the average over all proton configurations at fixed $\mathbf{x}$. We note that an expression exactly like Eq. (13) would apply in the case of differentiation of averages of other properties, P_{α} , of interest in place of G_{α} , where the differentiation could be taken with respect to any parameter of interest (for instance, orientational degrees of freedom, pH , or ionic strength). Thus, while we do not make use of it in this work, Eq. (13) also provides an approach for identifying critical points of the effective potential in more general settings. The second derivatives follow from similar considerations.

We now specialize to the case of two particles such as those depicted in Fig. 1. It is convenient to partition $W_{el,\alpha}$ into the parts that pertain to each particle as follows:

$$W_{el,\alpha}(\mathbf{x}) = \frac{1}{2} \frac{e^2}{k_B T} (\vec{Q}_{1\alpha}; \vec{Q}_{2\alpha}) \cdot \begin{pmatrix} \mathbf{W}_{11} & \mathbf{W}_{12} \\ \mathbf{W}_{12} & \mathbf{W}_{22} \end{pmatrix} \cdot (\vec{Q}_{1\alpha}; \vec{Q}_{2\alpha}), \quad (14)$$

where $\vec{Q}_{1\alpha} = \vec{Q}_{b1} + \vec{Q}_{\alpha1}$ and $\vec{Q}_{2\alpha} = \vec{Q}_{b2} + \vec{Q}_{\alpha2}$. Expanding Eq. (14) we have

$$W_{el,\alpha}(\mathbf{x}) = \frac{1}{2} \frac{e^2}{k_B T} \left[\vec{Q}_{b1} \cdot \mathbf{W}_{11}(\mathbf{x}) \cdot \vec{Q}_{1\alpha} + \vec{Q}_{2\alpha} \cdot \mathbf{W}_{22}(\mathbf{x}) \cdot \vec{Q}_{2\alpha} + 2 \vec{Q}_{1\alpha} \cdot \mathbf{W}_{12}(\mathbf{x}) \cdot \vec{Q}_{2\alpha} \right], \quad (15)$$

giving

$$\frac{\partial W_{el,\alpha}(\mathbf{x})}{\partial r} = \frac{1}{2} \frac{e^2}{k_B T} \left[\vec{Q}_{b1} \cdot \frac{\partial \mathbf{W}_{11}(\mathbf{x})}{\partial r} \cdot \vec{Q}_{1\alpha} + \vec{Q}_{2\alpha} \cdot \frac{\partial \mathbf{W}_{22}(\mathbf{x})}{\partial r} \cdot \vec{Q}_{2\alpha} + 2 \vec{Q}_{1\alpha} \cdot \frac{\partial \mathbf{W}_{12}(\mathbf{x})}{\partial r} \cdot \vec{Q}_{2\alpha} \right]. \quad (16)$$

We now make an approximation that is suggested by our numerical results. The changing spatial relationships of both dielectric inhomogeneities and the salt solution imply that both $\mathbf{W}_{11}$ and $\mathbf{W}_{22}$ will, in principle, depend on the relative position of the spheres. However, in numerical work we find that while these dependences are indeed present, the changes in $\mathbf{W}_{11}$ and $\mathbf{W}_{22}$ with r are generally quite small compared to changes in the entries of $\mathbf{W}_{12}$. We note that to evaluate the effect of the changing dielectric and salt geometry for the diagonal terms in the work-of-charging matrix requires considerations that are not as simple as those for the off-diagonal terms. However, the result is still that the changes are small compared to the changes in the interparticle terms, and we therefore make the approximations that $\mathbf{W}_{11}(\mathbf{x})/r=0$ and $\mathbf{W}_{22}(\mathbf{x})/r=0$. With these approximations and noting that $\Delta \vec{\mu}_{\alpha}^0 \cdot \vec{Q}_{\alpha}$ is independent of r , we find by substituting Eq. (16) into Eq. (13) that

$$\frac{\partial U_{el}(\mathbf{x}, z, k)}{\partial r} \simeq \frac{1}{2} e^2 \left[\left\langle \sum'_{(ij)} q_{i,\alpha} \frac{\partial w_{ij}}{\partial r} q_{j,\alpha} \right\rangle - \text{cov} \left(\sum'_{(ij)} q_{i,\alpha} \frac{\partial w_{ij}}{\partial r} q_{j,\alpha}, \beta \Delta G_{\alpha} \right) \right], \quad (17)$$

where $\sum'_{(ij)}$ means that i and j are sites on different particles. Equation (17) further delineates the electrostatic potential derivative and chemical affinity-based particle properties that combine to determine the effective force. In the first term the derivatives of each interparticle work-of-charging element enter directly and are averaged over all configurations α , weighted by P_{α} . However, as the particle separation is changed, the probabilities P_{α} also change, and the corresponding changes in occupancy will require protons to come off or on each particle. The changes in free energy associated with these occupancy changes must also be accounted for. From the derivation leading to Eq. (13), it can be seen that the second term in Eq. (17) originates from the latter consideration.

In the present work, we make a further approximation that permits us to focus attention on the interplay between the electrostatic interactions and the acid-base equilibria. In particular, we make the approximation that for all (ij) pairs on different particles,

$$\frac{\partial w_{ij}}{\partial r} = \frac{\partial w}{\partial r}, \quad (18)$$

giving

$$\frac{\partial U_{el}}{\partial r}(\mathbf{x}, z, k) = \frac{1}{2} e^2 \frac{\partial w}{\partial r} [\langle Q_{1\alpha} Q_{2\alpha} \rangle - \text{cov}(Q_{1\alpha} Q_{2\alpha}, \beta \Delta G_{\alpha})], \quad (19)$$

where $Q_{1\alpha}$ and $Q_{2\alpha}$ are the net charges on particles 1 and 2 in their joint proton occupancy configuration α .

Within the present approximation, Eq. (19) states that the potential can have a stationary point with respect to sphere separation r when the average intersphere charge number product $\langle Q_{1\alpha} Q_{2\alpha} \rangle$ equals its covariance with the normalized configurational free energy $\beta \Delta G_{\alpha}$.

III. VALIDATION OF NUMERICAL MODEL

In order to validate our finite difference method we compared our modeled solution to the analytic solutions given by Tanford and Kirkwood [16] for inside and outside a low-dielectric sphere, surrounded by an ionic solution. As can be seen in Fig. 2, we achieved strong agreement between our model and the analytical solution. For the case of the charge at the center of a 17 Å low-dielectric sphere as shown in Fig. 2(a), the potential inside the sphere follows a $1/r$ decay, while outside there is a decay proportional to $e^{-r/\lambda_D}/r$, where

λ_D is the Debye length. For the case illustrated in Fig. 2(b), where the charge is placed off center at 15 Å it is clear that the computed solution is in strong agreement with the analytical solution; however, it is necessary to note that the analytic solution assumes a discontinuous jump in the dielectric constant at the edge of the sphere, whereas our model has a smoothed change in dielectric constant. The effect of the smoothing parameter s on the dielectric coefficient, as noted in Fig. 2, is given in Eq. (A1). The match to the analytic solution is strong for both cases shown. At the same time, it is clear that the correlation is stronger for the case of $s=0.1$. This is to be expected since for smaller smoothing parameter values the model is closer to the situation modeled analytically.

We have also applied this numerical model to find potentials in more complex cases such as a pair of neighboring proteins each with a large number of protonation sites, on the order of 50, with site placement based on experimentally determined protein structures. These calculations have allowed us to confirm that the numerically calculated work-of-charging matrix, as discussed in Sec. II C, is symmetric, as required in Eq. (1) (see Appendix B). To do so we used a single test charge placed at one site, numerically calculated the potential at another site in the system, and then interchanged the test charge and evaluation sites. For all cases examined we found the calculated potentials to agree.

These comparisons of our potential with analytically computed solutions, together with the symmetry of the calculated work-of-charging matrix, have given us confidence that our numerical implementation accurately represents the potential functions needed to consider charge regulation, as modeled by Eq. (1), namely, those due to a charge or charges placed fixed at a variety of locations within possibly disjoint low-dielectrics of varying geometries, in the presence of an ionic solvent medium.

IV. RESULTS

A. Work of charging including alteration of potential by low-dielectric spheres

Figure 3 presents xy -plane cross sections of the electrostatic potential, numerically calculated with the use of Eqs. (A3)–(A5), due to an on-axis elementary charge that is 2 Å inside the lower sphere. Comparison of the cross sections shown in (a) and (b) clearly demonstrates the extent to which proximity of the upper low-dielectric sphere to the test charge distorts and extends the spatial range of ionically screened electrostatic potential contours. Thus, as expected for low-dielectric particles, altered proton occupancy of sites on the two spheres will occur at larger particle separations than would be inferred from estimates based solely on the Debye screening length. This is also shown in Fig. 3(c) by the outward movement of the $1k_B T$ on-axis potential contour in response to close sphere proximity.

For comparison, Fig. 4 presents the corresponding xy -plane cross sections of the electrostatic potential, numerically calculated with the use of Eqs. (A3)–(A5), for a Debye length of 12 Å, showing further extension of the contours. Notably, the $0.5k_B T$ contour, which can give rise to significant changes in proton occupancy, extends into the upper sphere even for sphere separations of 10 Å. It is interesting to note that the numerically calculated potential contours within the upper sphere are very close to spherical, with a sphere

separation dependent center, as if an effective but screened image of the charge on the lower sphere was to be placed slightly below the upper sphere. We do not analyze that situation further here but restrict ourselves to the most basic implications of on-axis potential curves such as those depicted in Fig. 3(c), consistent with the approximation stated in Eq. (18).

B. Changes in proton occupancy patterns with sphere separation

From the results shown in Figs. 3 and 4 it is to be expected that the titration curves of proton occupancy vs pH for the combined two-sphere model system will show noticeable changes from their infinite-separation values even at sphere separations that somewhat exceed the Debye length. That this is the case shown in Fig. 5, which presents the overall proton occupancy as well as those of each of the four sites as a function of pH , at intersphere surface separations of (a) 10 Å and (b) 2 Å for a Debye length of 6 Å. In order to evaluate the binding polynomial and its consequences so as to generate Fig. 5, we fixed the intrasphere work-of-charging entries to be $1k_B T$, calculated the intersphere work-of-charging entries by parametrizing the on-axis results from a series of numerical solutions of Eq. (1) for different sphere separations, and used those numerical solutions that corresponded to placing each fixed charge 2 Å into the interior of each sphere from its surface.

On each axis of Fig. 5, values corresponding to the roots of the binding polynomial at infinite separation (small dots) and at finite separation (large dots) are also shown. The small dots would correspond closely to the pK_a values of the four titratable groups, though altered somewhat by the presence of the assumed fixed intrasphere work-of-charging value. The large dots correspond to the *apparent* pK_a values of the overall titration curve and correspond to the *apparent* pK_a values analyzed and related to corresponding proton occupancy patterns by Onufriev *et al.* [5]. Specifically, the large dots are each the negatives of the base-10 logarithms of the negatives of the roots of the binding polynomial, which are, in turn, expressed in molarity units. That the roots have started to split from their infinite separation values is already apparent at an intersphere separation of 10 Å and is much more clear at 2 Å. That the roots are real has also been noted from numerical analysis in the literature [5]. We have found a proof that this is implied by positive off-diagonal work-of-charging entries, which will be published separately.

The titration curves of the specific individual sites are shown by the thin distinctly dashed curves for infinite separation and by the thick curves that correspond in dashing to each thin curve for closer separations. For clarity the sites that become negatively charged at high pH are shown by the curves that rise with increasing pH ; that is, what is plotted on the vertical axis for an individual site is the absolute value of its average charge vs pH . As analyzed in the literature [5], the individual titration curves do not have the classic Henderson-Hasselbalch forms. This can be seen especially in Fig. 5(b). Note, for example, the long persistence of the thick dotted-dashed curve at low but nonzero charge, up to pH values that are high above the pH near 5 where this curve passes through average occupancy of 0.5.

Figure 5 clearly indicates that the site occupancies change dramatically in response to sphere proximity and therefore also indicates that the intersphere potential will change as a consequence. However, while the individual titration curves may serve as a very useful guide

to the qualitative phenomena to be expected, the very fact that there are nondiagonal elements of the work-of-charging matrix dictates that the occupancies of the sites are decidedly not independent random variables. Thus, it becomes natural to investigate the occupancies of each of the possible configurations. Since there are only 16 in this simple setup, we can readily display all of them.

In view of the need to consider the interdependence of sites, even on the two different spheres, we present in Fig. 6 the titration surfaces of all 16 configurations, together with small- and large-separation cross sections. Figure 6(a) shows that occupancies of all 16 possible proton configurations change dramatically as sphere separation is decreased. The view from the close-separation side, shown in the upper right (b), shows a dominant configuration over a large pH range. The rise of the average occupancy of the dominant close-range configuration as the spheres approach is shown in the constant sphere separation plots (c), (d), and (e) for successively smaller separations. The dominant close-range proton configuration turns out to correspond to intersphere attraction, and its specific proton occupancy pattern corresponds to the dashed curve shown in Fig. 8 below for which the two spheres have opposite charges.

C. Nonmonotonic effective electrostatic potentials

As noted in Sec. I, certain configurations of titratable sites lead to the pH -dependent possibility of nonmonotonic effective potentials between the spheres. Figure 7 shows the charge-regulated electrostatic potential at two pH values: one for which a nonmonotonic curve results (pH 8.5) and one for which there is monotonic attraction (pH 7.0). In each case, the figure shows that, at small sphere separations, the charge-regulated potentials, shown by the solid curves, can become considerably more attractive than the potentials that would have resulted if the proton occupancy probabilities had been assumed to remain fixed at their large separation values, as shown by the corresponding dashed curves. Similar increases in attraction due to charge regulation may be found, for example, in [6]. It is also interesting to note that Fig. 7(b) also indicates that the charge-regulated potential can also slightly exceed these fixed-occupancy curves at larger separations.

D. Occupancy pattern origins of nonmonotonic effective electrostatic potentials

Figure 8 presents the origin of the modeled nonmonotonic potential in somewhat more detail by plotting the probabilities vs separation for the three occupancy configurations that give the most prominent contributions to the model potential at pH 8.5. In particular, the surfaces in Fig. 8(a) and the curves in Fig. 8(b) show a transition from large-separation dominance of a repulsive proton configuration having positive $\langle Q_1 Q_2 \rangle$, through intermediate-separation dominance of configurations for which $\langle Q_1 Q_2 \rangle$ vanishes, to attractive small-separation configurations having negative $\langle Q_1 Q_2 \rangle$. This transition provides a straightforward rationale for the nonmonotonic potential curve. Briefly, repulsive configurations favored by their proton affinities at large separation become unoccupied at small separations due to their increasingly positive work of charging, and less repulsive or attractive configurations are available to replace them.

Figure 8(c) presents the intersphere separation dependences of the key summands in the effective charge-regulated potential, which show that more subtle aspects of the transition are also present. Each curve corresponds to a summand in Eq. (5) that is the product of a configuration's probability and its free energy. To understand the mathematical origins of the corresponding curves, it is helpful to consult the form of U_{el} presented in Eq. (5). In particular, note from Eq. (5) that even if a configuration's free energy of formation does not change as intersphere separation is varied, it may nevertheless correspond to a summand whose contribution to U_{el} does change with intersphere separation since the probability of the corresponding configuration may change dramatically as it becomes either more prominent or crowded out by the other configurations.

The forms of the individual curves in Fig. 8(c) may be described qualitatively as follows. The summand in U_{el} for the key repulsive configuration at pH 8.5, $\{-1,0\}$, $\{-1,0\}$, first grows with decreasing intersphere separation due to its increasingly positive work of charging but then falls with continued decrease in intersphere separation because of its rapidly decreasing probability. The summands corresponding to the key attractive configuration, $\{-1,0\}$, $\{0,1\}$, and the principal configuration having zero intersphere charge product, $\{-1,1\}$, $\{-1,0\}$, first grow with decreasing intersphere separation due to their increasing probabilities. The attractive configuration summand then gets large and negative once its negative work of charging and its large probability dominate the corresponding terms from the other configurations. Note that the configuration having zero intersphere charge product gives a negative contribution at low separation due to its reduced probability there, relative to its probability at large intersphere separations, consistent with Eq. (5).

V. DISCUSSION

The present model examines a framework for quantifying how charge-regulated electrostatic interactions enter into the average potential between particles having specific patterns of chemically distinct sites. With suitable elaborations this model can be applied to help study interactions between biological macromolecules and many other types of nanoparticles for which charge regulation is thought to play an important role.

The observation of nonmonotonic electrostatic potentials, together with the identification of specific criteria for their occurrence, leads to the natural question as to whether other selections of titratable groups could also lead to nonmonotonic potentials. In fact, we also find nonmonotonic potentials for other model sets of sites, not presented here, including three site models with one site on one particle and two on the other. Inspired by the occupancy transition found above, such combinations can be made quite simply by choosing site pK_a values and bare charges to favor repulsive contributions at long range while making available an attractive configuration at short range. It would be interesting to construct a parameter-space phase diagram detailing conditions for obtaining nonmonotonic potentials and to see whether such conditions can be realized experimentally.

The present model has only treated contributions to the effective potential for sites that are assumed to have the same interparticle work-of-charging coupling as for sites on the center-to-center axis joining the spheres. Clearly, more detailed consideration of the geometry and

the orientation dependence of the interaction is needed. Because of the large space of relative orientations to be investigated, it will clearly be useful to find reliable quick approximations to speed the numerical evaluation of the work-of-charging matrix. In this regard, the spherical cross sections of the potential within the upper sphere shown in Figs. 3 and 4 suggest the possibility of developing simple approximations for the contours within the upper sphere.

It would clearly be of interest to extend the present results to the context of more sophisticated models of electrolyte solutions, which may include finite-ion size [11], interactions dependent on varying ionic polarizabilities [20], fluctuation contributions to the free energy [21], explicit ions and/or solvent [9, 22], nonlinear dielectric response [23], nonlocal dielectric response [24], solvation forces [25], van der Waals forces [26], and other issues [27]. However, it should be noted that with certain types of elaborations, the work of charging will no longer take the relatively simple quadratic form considered above, and this may vastly complicate the numerical and theoretical analyses. As one example, certain models, notably the nonlinear version of the Poisson-Boltzmann equation, result in work-of-charging entries that do not obey the Maxwell relations [9, 28]. As another example,

$W_{el} = \frac{1}{2} \sum_{ij} q_i \phi_{ij}$ does not, in principle, hold in the presence of nonlinear dielectric response [17], while at the same time nonlinear response may indeed be expected within the enormous electric fields present near ions in solution [23]. It should be noted that models of the present type also neglect quantum-mechanical considerations that could link the local properties of the potential and the corresponding field with proton affinity changes [29], one of the possible reasons that the first term in Eq. (3) would no longer be independent of $\mathbf{x}$ (see also [30, 31]).

The nonmonotonic contributions to the effective potential, described above, result in part from the interdependence of proton occupancy patterns between neighboring portions of the two particles. As explained above, the alteration of the potential is contingent on separation-dependent biasing of occupancy configurations, but the same biasing is also manifest in the titration curves. Figure 5 suggests that factors which change the proportion of particle pairs with small interparticle separations should also alter the proton-binding curves of a solution of those particles. Since particle concentration is one such factor, it would be of interest to measure the particle concentration dependence of overall proton-binding curves vs pH for suitable model particles. In a more detailed setting the NMR-titration curves of individual protonation sites could also be studied as functions of particle concentration [19, 32], as tests of the model.

Finally, we note that the possibility of a nonmonotonic effective electrostatic potential may influence quantitative evaluation of the relative importance of electrostatic and van der Waals forces in determining the potential of mean force in solutions of colloids and biological macromolecules [18, 25, 26]. Clearly, the possibility of such a contribution depends on the susceptibility of probable patterns of proton occupancy to the local electrostatic potential. In principle, these susceptibilities need to be evaluated with consideration of the interdependence of charge states on one or both particles.

VI. CONCLUSIONS

The present work uses a simple model to help investigate charge-regulated forces between particles in solution. It identifies the possibility of nonmonotonic effective charge-regulated electrostatic potentials between particles that depend on the proton affinities of titratable sites, the dielectric and ionic parameters in the screened electrostatic interaction potential model, the positions of titratable sites in the particles, and pH . It will be of interest to extend these results to the consideration of orientation-dependent interactions, to see how they change within more sophisticated models of screened electrostatic interactions, and to test them experimentally with the use of suitable model particles, biological macromolecules, and spectroscopic techniques.

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APPENDIX A: DETAILS OF THE NUMERICAL METHOD

In order to solve Eq. 1 for the case of two low-dielectric spheres placed at different separations, we used a successive over-relaxation method. We chose this method for its execution speed, accuracy, and implementation accessibility. In particular, we sought a method that would provide for the needed flexibility in calculating the $N(N+1)/2$ different elements of the work-of-charging matrix for each intersphere separation, which at each separation needs N separate calculations for the off-diagonal terms and additional calculations for the on-diagonal terms, on which we do not focus in the present work, as explained in the text. We chose to use a grid-based method for simplicity in the face of the needed varying geometry of the low-dielectric regions. In an effort to create a robust numerical model and a physically grounded model, we assumed that our dielectric coefficient was not discontinuous at the sphere boundaries but made a gradual transition from its value inside the sphere to the dielectric coefficient of the surrounding medium. The model for this transition, similar to a model in the literature [33], is given by

$$\epsilon = \epsilon_m - (\epsilon_m - \epsilon_i) \frac{e^{(|r|-R)/s}}{1 + e^{(|r|-R)/s}}, \quad (A1)$$

where R is the radius of the sphere, s is the characteristic distance over which the transition takes place, and r is the distance a point is away from the center of the sphere. This transition function was used for both dielectric spheres with the distance r being calculated from the closest center. In addition to having a slowly varying dielectric coefficient, our model also has a distributed charge distribution instead of the Dirac delta function-based point charges. In so doing we avoided a variety of numerical accuracy and stability problems and created a physical robust model.

In our model we also interleaved the points at which the potential and dielectric coefficients are evaluated, allowing us to approximate the varying dielectric over three times more points

than a traditional method in which the potential and dielectric coefficient are evaluated at the same points [34,35]. An illustration of this grid can be seen in Fig. 9, where ϵ^x is the dielectric coefficient defined on the face normal to the yz plane of the computational cell, ϵ^y is the dielectric coefficient on the face normal to the xz plane of the computational cell, and ϵ^z is the dielectric coefficient defined on the face normal to the xy plane of the computational cell.

The finite difference version of the differential equation [Eq. (1)] is

$$\begin{aligned} & \epsilon_{i-1,j,k}^x [\phi_{i,j,k} \\ & - \phi_{i-1,j,k}] + \epsilon_{i,j,k}^x [\phi_{i,j,k} \\ & - \phi_{i+1,j,k}] + \epsilon_{i,j-1,k}^y [\phi_{i,j,k} \\ & - \phi_{i,j-1,k}] + \epsilon_{i,j,k}^y [\phi_{i,j,k} \\ & - \phi_{i,j+1,k}] + \epsilon_{i,j,k}^z [\phi_{i,j,k} \\ & - \phi_{i,j,k-1}] + \epsilon_{i,j,k}^z [\phi_{i,j,k} \\ & - \phi_{i,j,k+1}] + \kappa_{i,j,k}^2 \epsilon_m h^2 \phi_{i,j,k} \\ & = q_{i,j,k} / h, \end{aligned} \quad (\text{A2})$$

where $\phi_{i,j,k}$ is the electrostatic potential at the center of cell ijk , h is the grid size, q is the charge distributed over the computational cell, κ is the Debye screening parameter defined for the cell of interest, and ϵ_m is the dielectric constant outside the spheres considered from a point far enough from the boundaries of the spheres that we can take it as a constant.

Solving for ϕ and introducing the relaxation parameter w the equation to iterate becomes

$$\phi_{i,j,k} = (1 - w) \phi_{i,j,k} + \frac{w(\sigma + q_{i,j,k}/h)}{\epsilon_{i,j,k} + h^2 \epsilon_m / \lambda_D^2}, \quad (\text{A3})$$

where

$$\sigma = \epsilon_{i-1,j,k}^x \phi_{i-1,j,k} + \epsilon_{i,j,k}^x \phi_{i+1,j,k} + \epsilon_{i,j-1,k}^y \phi_{i,j-1,k} + \epsilon_{i,j,k}^y \phi_{i,j+1,k} + \epsilon_{i,j,k-1}^z \phi_{i,j,k-1} + \epsilon_{i,j,k}^z \phi_{i,j,k+1} \quad (\text{A4})$$

and where

$$\epsilon_{i,j,k} = \epsilon_{i-1,j,k}^x + \epsilon_{i,j,k}^x + \epsilon_{i,j-1,k}^y + \epsilon_{i,j,k}^y + \epsilon_{i,j,k-1}^z + \epsilon_{i,j,k}^z. \quad (\text{A5})$$

For our model we chose to implement a set of boundary conditions by calculating the potential at what would be a point externally adjacent to the edge of the box and forcing the

value at the edge to have the same value. This method essentially sets the normal component of the electric field to zero at the boundary.

As discussed in Sec. III the procedure we have used is among the many known procedures that can provide an accurate model for the electrostatics of low-dielectric regions without salt, placed in ionic solution having a different dielectric constant.

APPENDIX B: PROOF OF SYMMETRY

The work-of-charging matrix should be symmetric if interactions are linear; that is, the potential at site one due to a charge at site two should be the same as the potential at site two due to a charge at site one. This is shown to be true for the linearized Poisson-Boltzmann equation [Eq. (1)] in the following manner. The equations for the potential due to point charges are written as follows:

$$\nabla \cdot [\epsilon(\vec{r}) \nabla \phi_i] = \kappa^2(\vec{r}) \phi_i(\vec{r}) + \delta^3(\vec{r} - \vec{r}_i), \quad (\text{B1})$$

$$\nabla \cdot [\epsilon(\vec{r}) \nabla \phi_j] = \kappa^2(\vec{r}) \phi_j(\vec{r}) + \delta^3(\vec{r} - \vec{r}_j). \quad (\text{B2})$$

Now the question is whether $\phi_i(\vec{r}_j) = \phi_j(\vec{r}_i)$ or equivalently whether $\phi_j(\vec{r}_i) - \phi_i(\vec{r}_j) = 0$. To address this question, multiply Eq. (B2) by the potential due to charge site i and subtract it from Eq. (B1) multiplied by the potential due to charge site j . Integrate the resulting difference over all space. We are left with the following results:

$$\begin{aligned} & \phi_j(\vec{r}_i) - \phi_i(\vec{r}_j) \\ &= \int \int \int \phi_j \delta^3(\vec{r} - \vec{r}_i) - \phi_i \delta^3(\vec{r} - \vec{r}_j) d^3r \\ &= \int \int \int (\{\nabla \cdot [\epsilon(\vec{r}) \nabla \phi_i]\} - \{\nabla \cdot [\epsilon(\vec{r}) \nabla \phi_j]\}) d^3r = \int \int \int \nabla \cdot [\epsilon(\phi_j \nabla \phi_i - \phi_i \nabla \phi_j)] d^3r. \end{aligned} \quad (\text{B3})$$

In order for the work-of-charging matrix to be symmetric $\int \int \int \nabla \cdot [\epsilon(\phi_j \nabla \phi_i - \phi_i \nabla \phi_j)] d^3r$ must be equal to zero. This is straightforward to show by using the divergence theorem to convert the integral to a surface integral. Once the integral has been converted to a surface integral, recognize the boundary conditions used for calculating the potential: the normal E field is zero, $\nabla \phi_j \cdot \hat{n} = 0$, and $\nabla \phi_i \cdot \hat{n} = 0$. Thus, the equation becomes

$$\oint \int [\epsilon(\phi_j \nabla \phi_i - \phi_i \nabla \phi_j)] \cdot \hat{n} d^2r = 0, \quad (\text{B4})$$

showing that $\phi_j(\vec{r}_i) - \phi_i(\vec{r}_j) = 0$.

APPENDIX C: POSITIVITY

Here, we show that off-diagonal work-of-charging entries, as defined above for positive test charges, must themselves be positive. To do so we consider the possible sign of ϕ as follows. Consider the governing equation

$$\nabla \cdot [\epsilon(\mathbf{r}) \nabla \phi(\mathbf{r})] = \kappa^2(\mathbf{r}) \phi(\mathbf{r}) + f. \quad (\text{C1})$$

We now show that if $\kappa \in \mathbb{R}$, then if $f \geq 0$ everywhere, it follows that $\phi \geq 0$, given that $\phi \rightarrow 0$, as $|\mathbf{r}| \rightarrow \infty$.

If ϕ is well defined everywhere and approaches zero at infinity and if $\phi < 0$ at some point, then ϕ would be bounded and have a negative local minimum value. At that local minimum

$$\kappa^2(\mathbf{r}) \phi(\mathbf{r}) + f(\mathbf{r}) < 0 \quad (\text{C2})$$

since $f \geq 0$ and $\phi \leq 0$ at the minimum. But,

$$\nabla \cdot (\epsilon \nabla \phi) = \nabla \epsilon \cdot \nabla \phi + \epsilon \nabla^2 \phi. \quad (\text{C3})$$

Given that at the minimum $\nabla \phi = 0$ and $\nabla^2 \phi > 0$ this is a contradiction. So if $f \geq 0$ then $\phi \geq 0$. Note that $f \geq 0$ corresponds to an arbitrary distribution of fixed positive charges.

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FIG. 1.

The Boltzmann-averaged response of proton occupancy patterns to proximity of two spheres carrying titratable groups can result in nonmonotonic electrostatic contributions, U_{el} , to their potentials of mean force. (a) Model arrangement of protonatable groups that gives rise to the U_{el} function shown at right. (b) Attractive effective potentials near neutral pH are flanked by potentials that are repulsive for larger separations but attractive at close separation.

Potentials are repulsive at all separations at still higher and lower pH values. The vertical axis shows U_{el} in $k_B T$, as a function of both pH and the smallest separation between the sphere surfaces. Groups 1 and 3 have bare charge of -1 and bare pK_a values of 6.5 and 7.0, respectively, while groups 2 and 4 have bare charges of 0 and bare pK_a values of 6.8 and 7.3, respectively. Other parameters are described in the text.

FIG. 2.

(Color online) Validation of finite difference method used. The calculated potential agrees with the analytic potential for a low-dielectric sphere in a high dielectric medium with salt (a) for a point charge placed at the center of the sphere and (b) for one located 15 $\text{\AA}$ out from the center.

FIG. 3.

Proximity of low-dielectric spheres distorts and extends the spatial range of ionically screened electrostatic potential contours, important for calculating proton occupancy and charge of heterogeneous titratable sites. In each of the upper plots, all distances are measured in angstroms on the left-hand scales, and potential contour values and shading are shown on the right-hand scales, in units of $k_B T/e$. (a) and (b) xy -plane cross sections of a potential map due to an on-axis elementary charge that is 2 Å inside the lower sphere. The spheres have surface-to-surface separations of (a) 10 Å and (b) 3 Å, the Debye length is 6 Å, the inner dielectric coefficient is 8, and the outer dielectric coefficient is 80. Contour lines are plotted at $0.5k_B T/e$, $1k_B T/e$, $1.5k_B T/e$, $2k_B T/e$, $2.5k_B T/e$, and $3k_B T/e$. (c) Changing the sphere separation from 10 to 3 Å extends the $1k_B T$ contour radially outward by more than 50%. The on-axis potentials are plotted for the conditions in (a) and (b). Thus, for low-dielectric particles, charge regulation occurs at larger particle separations than would be inferred solely from the use of the Debye screening length.

FIG. 4.

xy -plane cross sections of a potential map due to an on-axis elementary charge that is 2 Å inside the lower sphere for a Debye length of 12 Å . Other parameters, scales, and shading are as given in the caption of Fig. 3. (a) shows that the $0.5k_B T$ contour, which can yield significant changes in proton occupancy, extends into the upper sphere even for sphere separations of 10 Å . (b) At 3 Å sphere separation, more contours enter the upper sphere than for a Debye length of 6 Å as expected.

FIG. 5.

At a Debye length of 6 Å, proximity of low-dielectric spheres changes proton occupancy and splits the roots of the binding polynomial at an intersphere surface separation of 10 Å, as shown in (a), and much more so at 2 Å, as shown in (b). Each type of curve, described below, is thin for infinite separation and thick for the specified surface separation. Overall proton occupancies of the two-sphere four-group system vs pH are plotted vertically as the solid curves. Amine group charges are shown by the two dashed-dotted curves that approach 1 at low pH . Absolute values of carboxyl group charges given by the dashed and dotted curves that approach 1 at high pH . Small dots on the pH axis correspond to the values of the roots of the binding polynomial at infinite intersphere separation, while large dots give the roots at the specified surface separations.

FIG. 6.

The average occupancies vs pH of all 16 possible proton configurations of the two-sphere four-site model change dramatically as sphere separation is decreased and over a substantial pH range become dominated by an attractive configuration for which the net charges of the two spheres have opposite signs. Each surface in the upper figures gives the average occupancy of one of the 16 possible proton occupancy configurations vs both pH and intersphere surface separation. The same surfaces are shown, viewed from (a) large and (b) small intersphere surface separations. (c)–(e) show cross sections of the upper figures that illustrate the growth in occupancy of the two attractive configurations (thick solid curves) at successively smaller separations, (c) 10 Å, (d) 5 Å, and (e) 2 Å, at the expense of the repulsive configuration that is dominant at large separations at pH 8.5 (dashed) and other configurations that dominate at intermediate separation (thin curves). 15 surfaces appear in (a) since two configurations have the same surface within the plot resolution. At the front face of (b) some configurations have surfaces below the lower cutoff, e^{-7} .

FIG. 7.

Plots (b) and (c) show pH 8.5 and pH 7.0 cross sections of Fig. 1(b) [repeated at left as (a) for convenient reference], showing nonmonotonic and monotonic dependences of the charge-regulated electrostatic contribution to the potential of mean force on sphere separation, as the solid curves. The dashed curves are the potentials that would have resulted if the proton occupancy probabilities had been assumed to remain fixed at their large separation values. The dotted-dashed curves show the difference between those potentials and the charge-regulation potentials (solid curves).

FIG. 8.

The nonmonotonic charge-regulated electrostatic portion of the potential involves a gradual transition from repulsive to attractive joint proton occupancy patterns of the two model spheres. In (b) and (c) particular occupancy patterns are denoted by their corresponding site charges $\{q_1, q_2\}$, $\{q_3, q_4\}$, with sets in the first and second brackets referring to the site pairs on a given sphere. As intersphere separation is decreased, there is a transition from (i) large-separation predominance of repulsive proton configurations [upper right surface in (a) and solid curves in (b) and (c)] through (ii) intermediate-separation dominance of configurations for which $\langle Q_1 Q_2 \rangle$ vanishes [example shown by middle surface in (a) and dotted curve on right], and finally to (iii) attractive small-separation configurations [leftmost surface in (a) and dashed curves on right]. Plot (c) shows the intersphere separation dependence of each summand in U_{el} for the principal configurations and is discussed further in the text. Of the 16 possible configurations, only the principal configurations that combine to determine the effective potential at pH 8.5 are shown in (a) and its pH 8.5 cross section (b).

FIG. 9.

An illustration x - y plane of the finite difference method grid. The potential is defined at the center of a grid cell and the dielectric is defined at the faces of the grid cell. Charges are treated as if they are spread out over the volume of the grid cell containing them.