Charge optimization leads to favorable electrostatic binding free energy

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Variational optimization of molecular electrostatic charge distributions is a tool for the study of association reactions of molecules in solution. In principle, this method can be used in drug design and protein folding to analyze and improve molecular interactions and to provide electrostatic templates for molecular design. This optimization problem reduces to an inverse source problem in classical electrostatics, where the sources are determined by a combination of external and self-polarization potentials. In this paper, we show that the electrostatic portion of the free energy of association for electrostatically optimized molecules has an upper bound of zero in many situations of physical interest. That is, variational optimization provides a ligand-charge distribution that contributes favorably to the energetics of binding, even in a strongly polar medium. This stabilizing effect on association reactions is contrary to the usual role of electrostatics in aqueous complexes, in which desolvation effects generally dominate. We also show the existence and nonuniqueness of the variational solution and make a connection to the electrostatic image charge problem.
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Electrostatics play an important role in the association of molecules in solvent. Complementary electrostatic interactions are determinants of specificity [1–4], but are currently believed to contribute unfavorably to the free energy of binding near room temperature (see [5], and references therein). Recent work has shown that a carefully designed charge distribution for one reactant can lead to an electrostatic contribution to the binding free energy that is optimal and favorable (negative) [5–8]. While some theoretical details of this variational optimization process have been elucidated [6,7], many properties of the resulting charge distributions, including their implicit utility for molecular design, remain unaddressed. In particular, no theoretical bound has been placed on the electrostatic binding free energy of the optimum. Herein we demonstrate that optimization leads to a favorable electrostatic contribution to binding; that is, variational optimization guarantees successful electrostatic charge distributions for use in molecular design. We also demonstrate the existence and nonuniqueness of optimal charge distributions and make a connection between variational optimization and the method of electrostatic images.

THEORETICAL BACKGROUND

Figure 1 depicts an example of an association reaction in which two reactant molecules, a ligand (l) and receptor (r), associate rigidly to form a complex (c). The free energy change of the solution due to binding in the standard state can be separated into electrostatic, $\Delta G^0_{\rm es}$, and nonpolar, $\Delta G^0_{\rm np}$, contributions [9,10], where $\Delta G^0_{\rm np}$ represents the standard binding free energy of the reactants when their charge distributions are everywhere zero. $\Delta G^0_{\rm es}$ can be obtained in the continuum electrostatic approximation, wherein the solvent is treated as a dielectric continuum ϵ_s, the molecules (i) as rigid dielectric cavities ϵ_m with embedded charge distributions $Q_i(\mathbf{x})$, and the system obeys the Poisson equation. Here we restrict the molecular cavities to closed, bounded regions V_i with regular bounding surfaces S_i (note that V_i may contain continuum-solvent cavities bounded by regular surfaces). Denoting by $\mathcal{G}_i(\mathbf{x},\mathbf{y})$ the Green function for the Poisson equation satisfying the boundary conditions for molecular cavity (i) alone in solvent, the electrostatic free energy of molecule (i) is $\frac{1}{2}\int d\mathbf{x}\, d\mathbf{y}\, Q_i(\mathbf{x})\mathcal{G}_i(\mathbf{x},\mathbf{y})Q_i(\mathbf{y})$ [11,12]. $\Delta G^0_{\rm es}$ is the difference in the electrostatic free energies of the product and reactants,

$$\Delta G^0_{\rm es}=\frac{1}{2}\int d\mathbf{x}\, d\mathbf{y}\{Q_r(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y})-\mathcal{G}_r(\mathbf{x},\mathbf{y})]Q_r(\mathbf{y}) + 2Q_r(\mathbf{x})\mathcal{G}_c(\mathbf{x},\mathbf{y})Q_l(\mathbf{y}) + Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y})-\mathcal{G}_l(\mathbf{x},\mathbf{y})]Q_l(\mathbf{y})\}. \quad (1)$$

The molecular coordinate systems have been chosen, without loss of generality, such that the charge distribution and cavity of the complex is the superposition of the charge distri-

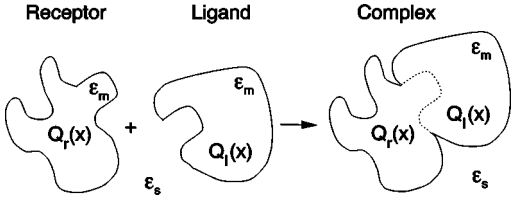

FIG. 1. Example binding geometry showing rigid ligand and receptor molecules associating in a unique arrangement to form a complex.

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butions and cavities of the reactant molecules, $Q_c(\mathbf{x}) = Q_r(\mathbf{x}) + Q_l(\mathbf{x})$, when their unbound-state coordinate axes are aligned. Note that the cavity of the complex V_c is the union of the ligand and receptor cavities, which have at most a set of regular surface elements in common (i.e., their volumes do not overlap in the bound state).

We have shown in other work [6,7] that Eq. (1) can be variationally extremized with respect to $Q_l(\mathbf{x})$ for a fixed receptor-charge distribution $Q_r(\mathbf{x})$. The resulting ''complementary'' ligand-charge distribution, $Q_l^{\mathrm{comp}}(\mathbf{x})$, is given by a solution to the Fredholm integral equation of the first kind

$$\int_{V_l} d\mathbf{x}\, Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_l(\mathbf{x},\mathbf{y})] = -\int_{V_r} d\mathbf{x}\, Q_r(\mathbf{x})\mathcal{G}_c(\mathbf{x},\mathbf{y}) \tag{2}$$

$\forall \mathbf{y} \in V_l$.

In the following sections we show that a complementary ligand exists that represents a nonunique minimum to the electrostatic binding free energy. The perhaps surprising result of nonuniqueness arises from the fact that the optimization condition (2) defines properties of the potential of the complementary ligand, and a family of related charge distributions can create the necessary potential. With this formalism, we then demonstrate that the optimized electrostatic contribution to the binding free energy is favorable when the receptor and ligand do not both contain buried solvent cavities. The implications are discussed in the conclusion.

EXTREMIZATION OF ΔG^0_{es}

For the extremum of Eq. (2) to be a minimum, the ligand desolvation (dehydration) penalty must always be nonnegative. It represents the cost of changing the dielectric constant from ϵ_s to ϵ_m in the region V_r adjacent to the unbound ligand,

$$\Delta G_l^{\mathrm{hyd}} = \frac{1}{2}\int_{V_l} d\mathbf{x}\, d\mathbf{y}\, Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_l(\mathbf{x},\mathbf{y})]Q_l(\mathbf{y})$$

$$= \frac{-(\epsilon_m - \epsilon_s)}{8\pi}\int_{V_r} d\mathbf{x}\, \mathbf{E}_l(\mathbf{x})\cdot\mathbf{E}_l^0(\mathbf{x}), \tag{3}$$

where $\mathbf{E}_l^0(\mathbf{x})$ is the unbound ligand's electric field and $\mathbf{E}_l(\mathbf{x})$ is the electric field after alteration of the dielectric constant [11,12]. This can be approximated in terms of a sum of n perturbations to the dielectric constant,

$$\Delta G_l^{\mathrm{hyd}} \approx \frac{-\delta\epsilon}{8\pi}\sum_{j=0}^{n-1}\int_{V_r} d\mathbf{x}|\mathbf{E}_l^j(\mathbf{x})|^2, \tag{4}$$

where $\mathbf{E}_l^j(\mathbf{x})$ is the electric field around the ligand when the dielectric constant in V_r is $\epsilon_s + j\delta\epsilon$ and $\delta\epsilon = (\epsilon_m - \epsilon_s)/n$. This approximation becomes increasingly exact as $n \to \infty$ and shows that the desolvation penalty is non-negative for $\epsilon_m < \epsilon_s$; therefore, the extremum minimizes ΔG^0_{es} for $\epsilon_m < \epsilon_s$ and $Q_l^{\mathrm{comp}}(\mathbf{x})$ binds the receptor with the most favorable electrostatic binding free energy, $\Delta G^{0,\mathrm{opt}}_{\mathrm{es}}$, for the stated geometry [7].

The desolvation penalty arises from a change in the dielectric constant of a region, such as V_r, altering the electrostatic potential throughout space and changing the free energy of the system. However, a charge distribution placed in V_r such that the potential in the region exterior to V_r, denoted $\overline{V_r}$, has the same form it did before the dielectric constant was modified, is commonly referred to as an image charge distribution [11,12]; the original charge distribution in $\overline{V_r}$ is the inverse-image charge distribution. When Eq. (2) is satisfied, $Q_r(\mathbf{x})$ causes the potential in V_l (where all charges external to V_r are located) to be the same before and after binding; however, the potential is not necessarily unchanged in $\overline{V_c}$. In this case, we refer to $Q_r(\mathbf{x})$ as a generalized image charge distribution for $Q_l^{\mathrm{comp}}(\mathbf{x})$, and $Q_l^{\mathrm{comp}}(\mathbf{x})$ as a generalized inverse-image charge distribution for $Q_r(\mathbf{x})$. The term ''generalized'' may be dropped in cases where the potential in $\overline{V_c}$ is unaltered upon binding.

THE COMPLEMENTARY CHARGE DISTRIBUTION IS NONUNIQUE

It suffices to demonstrate the existence of a nonzero $Q_l(\mathbf{x})$ in the null space of Eq. (2), satisfying

$$\int_{V_l} d\mathbf{x}\, Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_l(\mathbf{x},\mathbf{y})] = 0 \tag{5}$$

$\forall \mathbf{y} \in V_l$. A solution to Eq. (5) shall be denoted a ''null charge distribution.'' Any spherically symmetric distribution of zero total charge located entirely within V_l produces a zero potential exterior to itself by Gauss's law and the convention that the potential vanish at infinity (the interior potential is entirely coulombic). Any superposition of such charge distributions is a solution to Eq. (5), Q.E.D. This nonuniqueness implies a family of solutions to Eq. (2). In practice, when solving Eq. (2) with a finite basis set (e.g., point charges, multipoles, or chemical groups) representing $Q_l(\mathbf{x})$, the inherent degeneracy reduces to a space spanned by at most a finite basis set, which defines a set of different but useful optimized charge distributions.

THE COMPLEMENTARY CHARGE DISTRIBUTION EXISTS

We show the existence of a $Q_l^{\mathrm{comp}}(\mathbf{x})$ for any $Q_r(\mathbf{x})$ under the condition that the receptor cavity is not totally encapsulated by the ligand cavity. First, consider the set of functions $\mathcal{D} = \{\int_{V_l} d\mathbf{x}\, Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_l(\mathbf{x},\mathbf{y})]\}$ defined by $Q_l(\mathbf{x})$ taking on all possible values. $\mathcal{D}$ is a closed space of harmonic functions on V_l because its elements satisfy the Laplace equation, the set of all $Q_l(\mathbf{x})$ is closed, and the integral over the Green functions is a continuous mapping from charge distributions to potentials [13]. Next, consider the set $\mathcal{F}$ of all harmonic functions on V_l with the inner product $\langle f(\mathbf{x})|g(\mathbf{x})\rangle = \int_{V_l} d\mathbf{x}\, f(\mathbf{x})g(\mathbf{x})$. This space is complete because any Cauchy sequence from $\mathcal{F}$ converges uniformly to some function [13] on V_l and therefore also on S_l; additionally, because V_l is a closed region of space, the sequence converges to a harmonic function [14], an element of $\mathcal{F}$. It can further be shown that $\mathcal{F}$ is a Hilbert space [13].

Now, suppose that a receptor-charge distribution $Q_r(\mathbf{x})$ exists for which there is no solution to Eq. (2). Without loss

of generality, we can additionally assume that the harmonic function $f(\mathbf{y})=-\int_{V_r}d\mathbf{x}\,Q_r(\mathbf{x})\mathcal{G}_c(\mathbf{x},\mathbf{y})$ over V_l is in the orthogonal complement $\mathcal{D}^{\perp}$ of $\mathcal{D}$ because if $f(\mathbf{y})\in\mathcal{D}$ then there would be a solution to Eq. (2), and $\mathcal{D}$ is a closed subset of $\mathcal{F}$, so $\mathcal{D}\oplus\mathcal{D}^{\perp}=\mathcal{F}$ [13]. Then, due to the orthogonality of $f(\mathbf{y})$ to the elements of $\mathcal{D}$, we must have

$$\int_{V_l}d\mathbf{x}\,d\mathbf{y}\,Q_l(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y})-\mathcal{G}_l(\mathbf{x},\mathbf{y})]f(\mathbf{y})=0 \qquad (6)$$

$\forall Q_l(\mathbf{x})$. Interpreting $\alpha f(\mathbf{y})$ as a ligand-charge distribution, where α merely converts units of potential to units of charge density, it is clear from Eq. (6) that $\alpha f(\mathbf{y})$ satisfies Eq. (5) and is thus null and has a zero desolvation penalty. By Eqs. (3) and (4), the electric field in V_r due to $\alpha f(\mathbf{y})$ in the bound and unbound states must be zero and the potential a constant. Using the harmonic continuation theorem [14] in the bound state coupled with the condition that V_r not be encapsulated by V_l, it can be shown that the potential in V_r due to the ligand-charge distribution is zero, so $\int_{V_l}d\mathbf{x}\,\alpha f(\mathbf{x})\int_{V_r}d\mathbf{y}\,\mathcal{G}_c(\mathbf{x},\mathbf{y})Q_r(\mathbf{y})=0=-\alpha\int_{V_l}d\mathbf{x}\,f^2(\mathbf{x})$. Consequently, $f(\mathbf{x})=0$ because $f(\mathbf{x})$ is a harmonic function; however, if this were actually so, then $Q_l(\mathbf{x})=0$ would solve Eq. (2), contradicting the supposition of nonexistence, Q.E.D.

$\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ FOR $\epsilon_s\to\infty$

When $\epsilon_s\to\infty$, the potential in each solvent region is constant; exterior to all molecules it is taken to be zero. Because for the unbound receptor the conducting solvent in V_l may be insulated from the exterior solvent, the potential in V_l may be nonzero. However, grounding the solvent in this cavity zeros its potential, lowering the free energy of the unbound receptor. The potential on S_r is now the same as it will be in the bound state because the potential on S_l for a complementary ligand will be zero in both the bound and unbound ligand states. Therefore, the potential at all ligand-receptor interfacial surface elements remains zero in the bound and grounded unbound states. The potentials acting on $Q_l(\mathbf{x})$ and $Q_r(\mathbf{x})$ are also unchanged due to the uniqueness theorem for Dirichlet boundary conditions [11,12], so the change in free energy for binding the grounded receptor to the ligand is zero and $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ with $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}$ being the (necessarily favorable) free energy change for grounding the ligand cavity.

CASES WHEN $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ FOR $\epsilon_s<\infty$

Consider the change in $\Delta G_{\mathrm{es}}^{0}$ when the solvent dielectric constant is perturbed by the quantity $\delta\epsilon_s$. This is given by the change in solvation free energy of the complex minus the change in solvation free energy of the two solute molecules. Because the perturbation is infinitesimal, Eq. (3) can be approximated by the first term in the sum, so the total change in the binding free energy is

$$\Delta\Delta G_{\mathrm{es}}^{0}\approx\frac{-\delta\epsilon_s}{8\pi}\left[\int_{\overline{V}_c}d\mathbf{x}|\mathbf{E}_c^0(\mathbf{x})|^2-\int_{\overline{V}_l}d\mathbf{x}|\mathbf{E}_l^0(\mathbf{x})|^2-\int_{\overline{V}_r}d\mathbf{x}|\mathbf{E}_r^0(\mathbf{x})|^2\right], \qquad (7)$$

where $\mathbf{E}_i^0(\mathbf{x})$ is the total electric field for molecule (i) alone in solvent before the perturbation.

The first set of geometries for which we show that $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ for finite ϵ_s requires that the unbound receptor be free of buried solvent regions (i.e., interior regions of solvent entirely disconnected from exterior solvent by the receptor cavity). Generally, unless a buried solvent cavity is very large, any water molecules in the cavity are likely to be ordered and might be treated as part of the low-dielectric receptor. The lack of buried solvent in the unbound receptor implies that all bound-state solvent regions have common surface elements with the ligand. Let $\Phi(\mathbf{x})=\Phi_c(\mathbf{x})-\Phi_l(\mathbf{x})$ be the difference in the total potentials of the bound complex and unbound complementary ligand states. Clearly, $\Phi(\mathbf{x})=0\ \ \forall\mathbf{x}\in V_l$ from Eq. (2). Therefore, $\Phi(\mathbf{x})$ and its normal derivative are zero on S_l because they are continuous across S_l. By the harmonic continuation theorem [14], the potential $\Phi(\mathbf{x})$ in $\overline{V_c}$ is a harmonic continuation of $\Phi(\mathbf{x})$ in V_l and so is zero. This implies that $\Phi_c(\mathbf{x})=\Phi_l(\mathbf{x})$ in $\overline{V_r}$ and that $Q_r(\mathbf{x})$ and $Q_l^{\mathrm{comp}}(\mathbf{x})$ are image and inverse-image charge distributions, respectively. The result of this is that the first two integrals of Eq. (7) partially cancel, leaving

$$\Delta\Delta G_{\mathrm{es}}^{0}\approx\frac{\delta\epsilon_s}{8\pi}\left[\int_{\overline{V}_r}d\mathbf{x}|\mathbf{E}_r^0(\mathbf{x})|^2+\int_{V_r}d\mathbf{x}|\mathbf{E}_l^0(\mathbf{x})|^2\right], \qquad (8)$$

which is negative for reducing the solvent dielectric constant. Furthermore, reoptimizing the ligand-charge distribution after the perturbation can only make $\Delta\Delta G_{\mathrm{es}}^{0}$ more negative. Thus, $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}$ becomes monotonically more favorable as ϵ_s is reduced. Together with the fact that $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ for $\epsilon_s\to\infty$, we have that $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ for this set of geometries, even with ϵ_s finite.

The second set of geometries for which we show that $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}\leqslant 0$ for finite ϵ_s requires that the ligand have no buried solvent cavities (although the receptor may). In this case one could variationally optimize the receptor-charge distribution with respect to a particular ligand-charge distribution by solving Eq. (9). Then, by the previous result, $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}$ would be favorable; however, we really want to specify the receptor-charge distribution, not find it. So, if one can show that the target receptor-charge distribution is complementary to some ligand-charge distribution, then the complementary ligand-charge distribution must bind at least as well as this nonoptimal ligand-charge distribution and $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}}$ will be favorable.

This reduces to showing that for any $Q_r(\mathbf{x})$, there exists a generalized image charge distribution $Q_l(\mathbf{x})$ satisfying

$$\int_{V_r}d\mathbf{x}\,Q_r(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y})-\mathcal{G}_r(\mathbf{x},\mathbf{y})]=-\int_{V_l}d\mathbf{x}\,Q_l(\mathbf{x})\mathcal{G}_c(\mathbf{x},\mathbf{y}) \qquad (9)$$

$\forall\mathbf{y}\in V_r$. The existence proof proceeds similarly to that for the complementary charge distribution. We first note that the set $\mathcal{E}=\{-\int_{V_l}d\mathbf{x}\,Q_l(\mathbf{x})\mathcal{G}_c(\mathbf{x},\mathbf{y})\}$, defined as $Q_l(\mathbf{x})$, takes on all values, is a closed space of harmonic functions over V_r, and we assume the existence of a $Q_r(\mathbf{x})$ such that there is no solution $Q_l(\mathbf{x})$ to Eq. (9). Without loss of generality, we assume that $g(\mathbf{y})=\int_{V_r}d\mathbf{x}\,Q_r(\mathbf{x})[\mathcal{G}_c(\mathbf{x},\mathbf{y})-\mathcal{G}_r(\mathbf{x},\mathbf{y})]$ over V_r is in the orthogonal complement $\mathcal{E}^{\perp}$ of $\mathcal{E}$, implying

$\int_{V_l} d\mathbf{x} \int_{V_r} d\mathbf{y}\, g(\mathbf{y}) \mathcal{G}_c(\mathbf{y},\mathbf{x}) Q_l(\mathbf{x}) = 0 \quad \forall Q_l(\mathbf{x})$. As before, $\alpha g(\mathbf{y})$ is treated as a receptor-charge distribution. Because the bound-state potential in V_l due to $\alpha g(\mathbf{y})$ is zero, the electric field there must also be zero. Generalizing Eqs. (3) and (4) for the receptor, we find that its desolvation penalty is zero and that the electric field and potential in V_l in the unbound state must also be zero. Because the potential in V_l is zero in both states, application of the electrostatic boundary conditions implies that the potential throughout space is the same before and after desolvation. Thus, $\alpha g(\mathbf{y})$ is null, satisfying

$$\int_{V_r} d\mathbf{x}\, \alpha g(\mathbf{x}) [\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_r(\mathbf{x},\mathbf{y})] = 0 \tag{10}$$

$\forall \mathbf{y} \in V_r$. Assuming that $\alpha g(\mathbf{x}) \neq 0$ [for if it were zero, then $Q_l(\mathbf{x}) = 0$ would be a solution to Eq. (9)], we must have $\alpha^2 \int_{V_r} d\mathbf{x}\, g^2(\mathbf{x}) \neq 0$ because $g(\mathbf{x})$ is a harmonic function. This implies that

$$\alpha^2 \int_{V_r} d\mathbf{x}\, d\mathbf{y}\, g(\mathbf{x}) [\mathcal{G}_c(\mathbf{x},\mathbf{y}) - \mathcal{G}_r(\mathbf{x},\mathbf{y})] Q_r(\mathbf{y}) \neq 0, \tag{11}$$

contradicting Eq. (10). Therefore, the existence of the generalized image charge distribution is assured and $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}} \leqslant 0$ for these geometries as well.

MUTUAL COMPLEMENTARITY

The ligand- and receptor-charge distributions are mutually complementary when $Q_r(\mathbf{x})$ is null or when $\epsilon_s \to \infty$ and neither the unbound receptor nor the unbound ligand have buried solvent cavities. In these cases the potential in the solvent is always zero and in V_l it is the same in both the bound and unbound complementary ligand states. Uniqueness and boundary conditions indicate that the potential in V_r is also the same, so $Q_r(\mathbf{x})$ and $Q_l^{\mathrm{comp}}(\mathbf{x})$ satisfy Eq. (9), implying that $Q_r(\mathbf{x})$ is complementary to $Q_l^{\mathrm{comp}}(\mathbf{x})$. In this case, both the receptor- and complementary ligand-charge distributions are images and inverse images of each other, $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}} = 0$, and nonunique image and inverse-image charge distributions exist.

CONCLUSION

We have shown that charge optimization leads to a favorable electrostatic contribution to the binding free energy for many cases of biophysical interest. The current proof is limited to cases in which the receptor and ligand do not both have buried solvent cavities and to cases in which the solvent ionic strength is negligible. Generalizations to other cases may be possible.

Inclusion of other effects not considered here leads to further enhancements to binding electrostatics. For example, if the ligand and receptor cavities overlap in the bound state, then the optimal electrostatic binding free energy will be even more favorable. Likewise, inclusion of conformational change in the complex will improve the free energy of binding if it is the unbound configurations that are modeled, as all relaxation must be favorable. Note that if the molecular surfaces are defined through use of a solvent probe molecule, the complex cavity may be larger than the union of the ligand and receptor cavities and these theorems will not strictly hold. However, when the additional volume is not large or is distant from regions of high charge density, the binding free energy is expected to remain favorable.

In natural complexes, $\Delta G_{\mathrm{es}}^{0}$ is usually unfavorable (positive), suggesting that nature may not generally employ electrostatics to enhance binding affinity (see [5], and references therein). However, because optimization provides $\Delta G_{\mathrm{es}}^{0,\mathrm{opt}} \leqslant 0$, electrostatics could, in principle, be used to improve affinity. In fact, it seems that significant gains in binding free energy may be obtained through the application of electrostatic charge optimization [5,7,8,15].

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