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Molecular thermodynamics for charged biomacromolecules

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Abstract

Significant progress has been made over the past 25–30 years toward understanding the molecular basis of stability and biological functions of proteins and nucleic acids. Electrostatic calculations hold a prominent place among most advanced and popular computational methods to describe the influence of solution conditions on and the precise role played by individual amino acids or atoms in stability, folding and molecular recognition. In this article, we present a tutorial overview of coarse-grained and atomistic thermodynamic methods commonly used in modeling electrostatic interactions that are prevalent among biological processes including structural transitions of biomacromolecules and ligand–receptor associations. Illustrative examples are discussed on applications of these thermodynamics models to computational design of proteins with improved function and stability, to charge-transfer equilibria, to structure transitions of nucleic acids and proteins, to protein–ligand interactions, and to ion transport through lipid membranes. Some perspectives are also given on the future trends of computational modeling in biological thermodynamics.

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1. Introduction

In a departmental colloquium presented at the University of California at Riverside in November 2004, Professor John Prausnitz urged an audience of chemical and environmental engineers, the authors of this article in particular, to be like the bee instead of the ant or spider. The analogy comes from a quotation of Sir Francis Bacon discussed in the preface of the 2nd ed. of Molecular Thermodynamics of Fluid-Phase Equilibria [1]. Experimenters are like ants; they only collect and use. Theoreticians resemble spiders that make cobwebs out of their own substance. But bees gather materials from the flowers and digest them by their own power. Professor Prausnitz insists (and exemplifies by himself) that a molecular thermodynamicist should be like the bee, seeking useful solutions to real world problems by a delicate combination of rigorous statistical–mechanical theories with adequate semi-empirical approximations.

In traditional chemical engineering, molecular thermodynamics is concerned primarily with a single problem, i.e., to find the compositions of two (or occasionally, more) coexisting phases at various combinations of known and unknown thermodynamic variables. Toward that end, concepts from molecular physics, statistical mechanics, and more recently molecular simulations, have been integrated into equations of state and excess Gibbs-energy models that provide effectual correlations/predictions of the thermodynamic properties of fluid mixtures, fugacity coefficients in particular. Because phase-equilibrium calculations are essential in optimization of separation processes that are routinely used in the petrochemical industry, the fugacity-coefficient models consist of a cornerstone of traditional chemical engineering.

In recent years, however, conventional vapor–liquid and liquid–liquid equilibria have faded away from the center stage of chemical engineering, yielding to burgeoning fields mostly labeled with bio or nano. Instead of reactivity and phase behavior of petroleum fluids and natural gases, chemical engineers today are mostly concerned with the microscopic structures and thermophysical properties of complex fluids, i.e., solutions of polymers, surfactants, nanoparticles, and biomacromolecules, that can be deployed for synthesis of advanced materials, for design and delivery of therapeutic drugs, for fabrication of smart sensors, and for development of various environmentally friendly and energy-efficient chemical and biological products and processes. Applications of thermodynamics are not lim-
2. Simple electrostatic models

Under physiological conditions, virtually all biomacromolecules are charged and their properties and biological functions are closely related to the surface electrostatic potential and interactions with surrounding ions [5]. For example, nucleic acids bear a negative charge (PO₄⁻) for each base, making them among the strongest natural polyelectrolytes. More than 20% of amino-acid residues in a globular protein are ionizable in an aqueous solution; these include contributions from the carboxyl groups in the side chains of aspartic and glutamic acids and from the basic groups located at lysine, arginine and histidine residues. The vast majority of biological membranes entail certain percentages of negatively charged phospholipids. Furthermore, a typical biological solution contains ample ions such as Na⁺, K⁺, Mg²⁺, Cl⁻, CH₃COO⁻, PO₄⁻ and derivatives of adenosine such as ATP and ADP. Electrostatic interactions are crucial to the structure and function of biomacromolecules, ranging from enzyme catalysis, ligand binding and the fine-tuning of redox potentials, to the stability of folded proteins and the translocon-mediated integration of transmembrane segments into the endoplasmic reticulum [6].

Coarse-grained models provide a first-step toward understanding electrostatic interactions in biological systems. In these highly simplified models, biomacromolecules are depicted as uniformly charged objects of simple geometry, namely proteins as spheres or spheroids, nucleic acids as cylinders, and lipid membranes as planar surfaces; small ions are represented by point charges or charged hard spheres, and the solvent by a dielectric continuum [7]. Within the “primitive” model, the electrostatic potential and ionic distributions near a biomolecular surface can be described by the Poisson–Boltzmann (PB) equation. Specifically, the Poisson equation connects the electrostatic potential ψ(r) to the local charge density ρ(r):

\[ \nabla^2 \psi(r) = -\frac{Q(r)}{\varepsilon_0 \varepsilon_r} \]

(1)

where \( \varepsilon_0 = 8.854 \times 10^{-12} \text{ C}^2 \text{ J}^{-1} \text{ m}^{-1} \) is the permittivity of free space, and \( \varepsilon_r \) is the dielectric coefficient or relative permittivity of the solvent. The local charge density, \( Q(r) = \sum_i Z_i \varepsilon_0 \varepsilon_r \rho_i(r) \), is in turn determined by the Boltzmann law for the spatial distributions of ions:

\[ \rho_i(r) = \rho_i^0 \exp[-Z_i e \beta \psi(r)] \]

(2)

where \( \rho_i^0 \) is the ion concentration at zero electrostatic potential, and \( \beta = 1/k_B T \). While the Poisson equation is exact in the framework of classical electrostatics, the Boltzmann law for the distribution of small ions is only approximate; it neglects the ion sizes, non-Coulomb interactions, and correlations in charge distributions [8,9]. In conjunction with an appropriate boundary condition for the surface electrostatic potential or charge density, the electrostatic potential \( \psi(r) \) and ionic distribution function \( \rho_i(r) \) can be solved from Eqs. (1) and (2) by using various numerical procedures [10].

Despite the simplicity of the PB equation, an analytical solution is limited to a few special circumstances, such as systems containing only one-type of ions or if the exponential function in the Boltzmann law is approximated by a linear expansion. In the latter case, the PB equation becomes

\[ \nabla^2 \psi(r) = \kappa^2 \psi(r), \]

(3)

where \( \kappa = \left[ \sum_i \rho_i^0 Z_i^2 e^2 \beta / (\varepsilon_0 \varepsilon_r) \right]^{1/2} \) is the Debye-screening parameter [11]. Table 1 presents asymptotic solutions to the linearized PB equation in planar, spherical, and cylindrical geometries [12]. In Appendix A, we give analytical solutions to Eq. (3) in more details and discuss important length scales affiliated with electrostatic interactions [7,13].

With the advent of modern computers, the PB equation and its variations have been scrutinized by extensive comparison with molecular simulations [14–16]. Regrettably, the comparison is often unsatisfactory because the PB equation ignores the excluded volumes of ions and correlations in ionic distributions. The situation is most contentious for systems containing multivalent counterions. For example, the PB equation is unable to describe attraction between similar charges and

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Here both the electrostatic potential and the surface distance are expressed in dimensionless units, \( \psi = e \beta \psi/k_B T \) and \( x = \kappa r \). The proportionality constants are determined by the boundary conditions.
charge inversion\(^2\) that have been revealed by both molecular simulations and experiments [17–20]. In recent years, a number of more sophisticated statistical–mechanical methods have been proposed that yield much improved results in comparison with simulations [21,22]. The classical density functional theory (DFT) represents a powerful alternative to the Boltzmann equation for ionic distributions [23–27]. In addition to the electrostatic potential, DFT is able to account for non-Coulomb interactions and intermolecular correlations in terms of an excess Helmholtz energy \(F^\text{ex}\). In general, the distribution of mobile ions is given by [24]:

\[
\rho_i(r) = \rho_i^0 \exp\left(-Z_i e\psi(r) - \beta F^\text{ex}\right),
\]

(4)

Eq. (4) reduces to the Boltzmann law if the excess Helmholtz energy due to ion size and charge correlations is neglected. As a result, the difference between DFT and the PB equation is most significant for systems with strong electrostatic interactions that lead to charge localization and subsequently magnified excluded-volume effects [24]. Fig. 1 shows, for example, the surface electrostatic potential of a charged sphere as a function of the surface charge density predicted from the PB equation, from an integral-equation theory, and from DFT [25]. While the predictions of DFT agree well with the simulation results, the performance of the PB equation is unsatisfactory, in particular in the presence of dense counterions.

The discrete nature of water molecules and non-Coulombic interactions of small ions play an important role in biological specificities of ion-binding proteins, nucleic acids, ion channels, and biomembranes [28,29]. In such cases, more accurate molecular models are required to incorporate the atomic details of solvent and ions. Toward that end, DFT holds major advantages over the PB equation not only because it is able to account for ion size and density correlations that are ignored in the PB equation but more important, because it provides a systematic approach to incorporating the specific properties of ions and solvent molecules beyond the primitive model [30].

3. Cell model

The cell model was originally proposed for predicting the osmotic coefficients of synthetic or natural polyelectrolyte solutions and the potentiometric titrations of weakly ionizable polyelectrolytes [31,32]. In recent years, it has also been used to describe structural transitions of biomacromolecules, particularly DNA duplex [33–37].

Within the cell model, a DNA molecule is depicted as an infinitely long, rigid rod of uniform surface charge density.\(^3\) A DNA solution is represented by uniformly distributed, cylindrical cells containing individual DNA molecules coaxially placed at the cell centers. The electrostatic potential and ionic distributions within each cell are described by the PB equation, which, in the cylindrical geometry, depends only on the radial distance:

\[
1 \frac{\partial}{\partial r} \left( r \frac{\partial \psi}{\partial r} \right) = -4\pi N_0 \sum_i \rho_i^0 Z_i e^{-Z_i \psi(r)},
\]

(5)

where \(\psi = e\psi/k_B T\) is the reduced electrostatic potential, \(k_B = e^2/(4\pi\varepsilon_0\varepsilon k_B T)\) is the Bjerrum length,\(^4\) and \(\rho_i^0\) is the number density of ion \(i\) at the cell boundary where a zero electrostatic potential is selected. As discussed later, \(\rho_i^0\) can be determined from the electrostatic potential and average concentration of DNA molecules in the solution.

With the boundary conditions for the electrostatic potential at the DNA surface,\(^5\) \(\psi(\alpha) = 2\xi/\alpha\), and at the cell boundary \(\psi(R) = 0\), Eq. (5) can be formally integrated

\[
\psi(r) = 2\xi \ln(r/R) - 4\pi N_0 \sum_i Z_i \left[ \ln \left( \frac{r}{R} \right) \int_0^r r' \rho_i(r') \, dr' \right] + \int_r^R r' \rho_i(r') \ln \left( \frac{r'}{R} \right) \, dr'.
\]

(6)

As shown in Fig. 2, \(a\) is the radius of the cylindrical rod, \(b\) the cylindrical length per unit charge, i.e., the spacing between nearest-neighbor charges along the axis of the cylindrical rod. The parameter \(\xi = l_B/b\) stands for a linear charge density in dimensionless units; it has a pronounced effect on the radial distribution of small ions and on the salt-dependence of the electrostatic potential. With the Boltzmann equation for ionic

\(^2\) Charge inversion is a non-intuitive electrostatic phenomenon in which a macroion adsorbs an excess amount of counterions such that the surface electrostatic potential is opposite to that of the bare charge.

\(^3\) At physiological conditions, the persistence length of a double-strand (ds)-DNA is about 50 nm and that for a single-strand (ss)-DNA is about 1 nm.

\(^4\) The Bjerrum length provides a measure of the distance at which the electrostatic potential two elementary charges is equal to the thermal energy \((k_B T)\). For water at 25 °C, the Bjerrum length is 0.714 nm.

\(^5\) Gauss’ law states \(\nabla \psi = 0\) at \(r = r_{\text{ion}}\) where \(\psi = -e/(2\pi\varepsilon ab)\) represents the surface charge density for a negatively charged polyelectrolyte.
distributions, \( \rho_i(r) = \rho_i^0 e^{-Z_i \varphi(r)} \), the electrostatic potential can be numerically solved using an iteration method [38].

For a DNA solution free of salt, the PB equation can be solved analytically. In that case, the reduced electrostatic potential is given by [32]:

\[
\varphi(r) = \ln \left( \kappa_c^2 r^2 \sinh \frac{B \ln(Ar)}{2B^2} \right),
\]

where \( \kappa_c = \sqrt{4 \pi \rho_c^0 \epsilon_0 Z_c^2 Z_e} \), subscript “c” denotes counterions (i.e., those ions with charges opposite to that of the DNA), and \( A \) and \( B \) are parameters obtained from \( 1 + B \coth(B \ln(AR)) = 0 \) and \( 1 + B \coth(B \ln(Aa)) = \xi \). From the boundary condition \( \varphi(R) = 0 \), we find the concentration of counterions at the cell boundary:

\[
\frac{\rho_0^c}{\rho_c} = \frac{1 - B^2}{2\xi},
\]

where \( \rho_c \) is the average concentration of the counterions in the salt-free DNA solution.

### 3.1. Osmotic pressure

The osmotic pressure of a DNA solution can be approximated by the summation of that for a salt-free DNA and that for the simple electrolyte solution [32]. The former can be calculated from the osmotic pressure at the cell boundary where the electric field vanishes, i.e., \( \Pi/k_B T = \rho_c^0 = \rho_c(1 - B^2)/2\xi \); the latter can be expressed in terms of the average salt concentration \( \rho_s \) and the osmotic coefficient \( \phi_s \) for a simple electrolyte. The overall osmotic pressure is given by

\[
\frac{\Pi}{k_B T} = \frac{(1 - B^2)\rho_c^0}{2\xi} + 2\rho_s \phi_s.
\]

The osmotic coefficient is defined as the osmotic pressure of the DNA solution divided by that of an ideal solution:

\[
\phi = \frac{\Pi}{\Pi^\text{id}} \approx \frac{(1 - B^2)\rho_c^0}{2\xi} + 2\rho_s \phi_s.
\]

At low DNA concentration and free of salt, \( a/R \) and subsequently \( B \) vanishes. The osmotic coefficient is reduced to \( \phi^\infty = 1/(2\xi) \), which is the same as that predicted by the counterion-condensation theory discussed in Appendix A [39].

Fig. 3 shows the osmotic coefficients predicted by the cell model in comparison with experimental data [40]. In these calculations, both the DNA radius \( (a = 1 \text{ nm}) \) and the spacing between two nearest phosphate charges \( (b = 0.17 \text{ nm}) \) are estimated from independent measurements. The solid lines shown in Fig. 3 are calculated from Eq. (12). The dashed lines, providing slightly improved results, are from a refined cell model where the DNA duplex chains are treated as hollow cylinders, taking into account

\[\text{[...]}\]

\[\text{[...]}\]

\[\text{[...]}\]

\[\text{[...]}\]
the major and minor grooves in the α-helical structure. Over a wide range of DNA concentrations, both the original and modified cell models predict the osmotic coefficients in good agreement with experimental data.

### 3.2. Stability of DNA duplex

In development of new medicinal strategy to target nucleic acids, thermal denaturation experiments are commonly carried to access the influence of drugs on the stability of the double-strand structure [41]. The denaturation curve reflects the double helix to single-strand equilibrium and is generally analyzed using a “two-state” model. Each nucleic acid is considered to be either totally in the double helix form or totally dissociated. The melting temperature ($T_m$) is defined as the temperature at which half of the nucleic acids are in the single-strand form. Experimental studies show that the melting temperature is mainly determined by the charge densities of double-strand (ds) and single-strand (ss) DNA chains. Furthermore, this transition is highly sensitive to the salt concentration ($C$), or more precisely, the mean activity ($a_+$) and charges of cations in the solution.

Electrostatic models have been used to account for the variation of DNA duplex stability with respect to solution conditions, in particular the types and concentrations of salts. The Gibbs energy of the helix-coil transition for a ds-DNA can be effectively expressed in terms of the electrostatic and non-electrostatic contributions:

$$\Delta G_m = \Delta G^0 + \Delta G^C,$$  \hspace{1cm} (13)

where $\Delta G^0$ accounts for all non-electrostatic contributions, and $\Delta G^C$ accounts for the electrostatic contributions. At the melting point, ds-DNA chains are in equilibrium with the ss-DNA chains, i.e., $\Delta G_m = 0$. Because the non-electrostatic free energy is relatively insensitive to electrolyte conditions, $\Delta G^0$ can be obtained from calorimetric data for denaturation of a ds-DNA at a particular salt condition (e.g., NaCl). With an empirical correlation for the non-electrostatic free energy, the cell model then provides a predictive tool for describing the variation of the melting point temperature due to addition of a salt.

To a good approximation, one may ignore the change in volume due to the helix-coil transition, i.e., $\Delta G_C \approx \Delta U_C - T\Delta S_C$. The electrostatic energy and entropy for both ds- and ss-DNA polynucleotides can be calculated from the electrostatic potential and ionic distributions within the cell model [37]:

\[
\frac{U^C}{bk_B} = \frac{\psi(a)}{2b} + \frac{1}{2} \sum_i \int_a^R \rho_i(r') Z_i \psi'(r') 2\pi r' \, dr',
\]

\[
\frac{S^C}{bk_B} = -\sum_i \int_a^R \left\{ \rho_i(r') \ln \left( \frac{\rho_i(r')}{\rho_i^0} \right) - \rho_i(r') + \rho_i^0 \right\} 2\pi r' \, dr',
\]

where the electrostatic potential is given by Eq. (6). Fig. 4 shows a comparison of the theoretical predictions with experiments when the transition occurs in the presence of magnesium ions. While the cell model slightly underpredicts the melting temperature, the opposite is true for the counterion-condensation theory (see Appendix A). Similar results have been obtained when Eqs. (13)–(15) are applied to DNA helix-coil transition in the presence of the natural polyamines such as putrescine$^{2+}$, spermidine$^{3+}$, spermine$^{4+}$, and their synthetic homologs with different spacing between the charged amino groups [42].

### 4. Generalized Poisson–Boltzmann equation

Computational modeling of biomacromolecules from an atomic or molecular perspective often starts with an atomic resolution of the biomacromolecular structure that can be deter-

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7 The electrostatic potential according to this refined cell model is given by $\psi(r) = 2 \ln[r \cos(2 \ln(r/R_{M}))] - \ln(2 \xi_1)$ for $a < r < R$ and $\psi(r) = \psi_0 + 2 \ln(1 + c_4 r^2)$ for $r < a$. The integration constants $c_1$, $R_{M}$, $c_2$ and $\psi_0$ are obtained from the boundary conditions $\psi(0) = \psi/R = 0$, the Gauss law $\psi'' - \psi' = 2\xi/a$ and the continuity condition $\psi_c = \psi_0$. 

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mined by experimental or computational means. Each atom from a biomacromolecule is represented by a sphere embedded in a medium with the dielectric coefficient in the range of 2–20, depending on the computational protocol. The atomic radius is set to the van der Waals radius and when the atom belongs to a polar group, it carries a partial charge. As in simple electrostatic models, the aqueous environment is represented by the primitive model.

Near the surface of a biomacromolecule, the electrostatic potential satisfies the generalized Poisson equation:

$$\nabla \cdot \left[ \varepsilon(r) \nabla \psi(r) \right] = -Q(r),$$  \hspace{1cm} (16)

where $\varepsilon(r)$ stands for the local dielectric coefficient, the symbol “$\nabla$” denotes gradient when applied to a scalar and divergence when applied to a vector. The inhomogeneous dielectric coefficient $\varepsilon(r)$ is fully determined by the 3D-structure of the biomacromolecule and its value in the protein interior is different from that of the solvent. Conversely, the local charge density $Q(r)$ consists of contributions from the biomacromolecule and from small ions. Assuming that the biomacromolecule has a fixed charge distribution denoted by $q_M(r)$, we may express the generalized PB equation as

$$\nabla \cdot \left[ \varepsilon(r) \nabla \psi(r) \right] = -q_M(r) - \sum_i Z_i \varepsilon \Gamma_i(r) \rho_i^0 e^{-Z_i e \psi(r) k_BT},$$  \hspace{1cm} (17)

where $\Gamma_i(r)$ represents the accessibility to ion $i$ at position $r$, typically defined by the van der Waals radii of the surface atoms and a probe sphere of fixed radius (e.g., of 2 Å for monovalent ions) representing the ion. The PB equation can be linearized if the reduced electrostatic potential is small. Nevertheless, it has been shown that in terms of practical applications, the difference between the linear and non-linear PB equations is not appreciable even at large value of the reduced electrostatic potential [43]. As a result, the linear PB equation is popular in modeling biomacromolecules and biological processes including those described below [12,43–47].

Numerical solutions of the generalized PB equation are computationally demanding due to the complex shapes and charge distributions of biomacromolecules. Typically, the values for charges and dielectric coefficients are assigned on a 3D-grid surrounding the biomacromolecule. The electrostatic potential for each grid point is then numerically solved by using space discretization methods [48,49]. The accuracy of the numerical solution depends on the scheme used for the space discretization and on the size of the computational grid. Popular software packages include APBS [50], UHBD [51], DELPHI [52], MEAD [53], WHAT IF [54], and CHARMM [55]; most of these algorithms are based on different versions of finite difference or finite element methods for the solution of the PB equation.

The electrostatic potentials of biomacromolecules can be intuitively visualized using software packages such as GRASP [56], MOLMOL [57], SWISS PDB VIEWER [58], and INSIGHT II (Accelrys Software Inc., San Diego, CA). Visualization of the electrostatic properties is a standard tool for analysis of protein structures. The electrostatic potential is projected on the biomolecular surface or alternatively, its spatial distribution is viewed in the form of iso-potential surfaces or lines at a fixed potential value (in units of $kT/e$). Such presentations are popular in the publications of newly determined protein structures and complement other types of analyses for structure specificity and stability, and for binding either at a molecular level, such as surface complementarity, or at the individual amino acid level, such as van der Waals interactions, hydrogen bond, and salt bridge formations.

Electrostatic calculations are helpful to analyze the electrostatic character of surfaces, volumes, and surrounding space for proteins and nucleic acids, biomacromolecular complexes and assemblies, and binding and active sites [44,50]. For example, a combined molecular dynamics (MD) and electrostatics study has provided a rational explanation for the high catalytic rate of the enzyme acetylcholinesterase [59]. It was shown that acetylcholinesterase generates a strong electrostatic field capable to attract the positively charged substrate acetylcholine to the active site; however, the high catalytic rate of the enzyme was inconsistent with the long and narrow gorge at the active site observed in the crystal structure. The MD/electrostatics study revealed an additional transient opening of a “back door” channel for substrate entry [59]. It was proposed that the dual substrate entry is responsible for the high catalytic rate of the enzyme, which could be tested by specific mutations. Another example is provided by the interaction of the immune system protein C3d with its B and T cell receptor CR2. Site-directed mutagenesis studies proposed a binding site based on loss or enhancement of the binding ability by selected mutants involving charged amino acids. The selection of charged amino acids for mutations was based on several earlier experimental studies which had shown pH and ionic strength dependence for the C3d–CR2 association. However, when the crystal structure of the C3d–CR2 complex became available, the binding site was found elsewhere. This ambiguity was resolved by electrostatic calculations which lead to the proposal of a general model for association driven by electrostatic macro-dipoles (recognition process) and short range interactions (binding process) [60]. This model provides a rational explanation of all available experimental data, including those which were previously thought to be contradictory. Fig. 5 shows the iso-potential contour surfaces for native C3d, two of its mutants with variable binding ability, and CR2. The single mutation K162A alters the balance in favor of the negative electrostatic potential, which correlates with the significant increase of binding ability observed in experiment. Conversely, the triple mutation D36A/E37A/E39A alters the balance in favor of the positive electrostatic potential, which correlates with the significant decrease of binding ability. Application of electrostatic calculations can also be illustrated with the analysis of the highly homologous pox viral proteins VCP (of the vaccinia virus) and SPICE (of the variola virus). The electrostatic potentials were
used to explain the up to 1000-fold difference in their activities against the immune system protein C3b [61,62]. These studies provided the theoretical basis for the design of several mutants of VCP/SPICE with predicted variable activities. A combination of MD simulations and electrostatic calculations for VCP/SPICE and selected mutants was used to demonstrate the effect of correlated inter-modular motions on the spatial distribution of the electrostatic potential [62]. Knowledge of the role of dynamics and electrostatics in VCP-C3b interaction is essential for the development of efficient vaccines against the evasion of the immune system by the pox viruses. Fig. 6 shows projections of calculated electrostatic potentials on the surface of VCP, using structure snapshots from an MD simulation. The figure shows the effect of dynamics (flexibility and mobility) in the variability of the surface electrostatic potential. The electrostatic potentials are also useful to study the regulation of protein–membrane association by ions, for proteins involved in signal transduction and vesicle trafficking. It was shown that Ca\textsuperscript{2+}-mediated non-specific electrostatic interactions contribute to the association of C2 protein domains with different phospholipid membrane surfaces [63]. This is possible by the binding of Ca\textsuperscript{2+} to clusters of aspartic acids in a C2 loop which interacts with the membrane.

Some efforts have been made to quantitate electrostatic potentials by using a single parameter that provides an easy comparison of molecular interaction fields across homologous proteins or mutants. For example, similarity indices are useful to distinguish molecular properties, such as sequences or electrostatic potentials. The electrostatic similarity index (ESI) between proteins ‘a’ and ‘b’ is defined as $\text{ESI}_{a,b} = 2(P_a \cdot P_b)/(P^2_a + P^2_b)$ where $(P_a, P_b)$, $P^2_a$, and $P^2_b$ are the scalar products of the protein electrostatic potentials at the grid-points $(i, j, k)$, within a specified “skin region” in the space surrounding the proteins, e.g., $(P_a, P_b) = \sum_{i,j,k} \psi_a(i, j, k) \psi_b(i, j, k)$ [64]. Pairwise comparisons of electrostatic similarity indices are possible using principal component analysis or clustering methods, as was the case for the classification of 104 proteins of the Pleckstrin homology domain family [65] and 33 homologous blue copper proteins [66]. A charge density probability function has been introduced to predict conservation of electrostatic properties and enzymatic function within four different enzyme families and one super-family [67]. Long-range electrostatic interactions are responsible for steering the substrate from the solvent toward the catalytic site and the short-range electrostatic interactions are responsible for local proton sharing and transfer during the catalytic process. Such analyses are useful to predict common functional properties of homologous proteins and phylogenetic trees of proteins.

5. Ionization of biomacromolecules

Acid–base equilibria, ion-binding, and electron-transfer redox equilibria are important for the functions of biomacromolecules in many biological processes. All these charge-transfer equilibria are critically dependent on electrostatic interactions and can be modeled in essentially the same manner [68–77].

![Fig. 6. Projections of electrostatic potentials on molecular surfaces for three MD snapshots of the VCP structure. VCP is made of four modules connected with short and flexible loops. The figure depicts the surface variation of the electrostatic potentials owed to the spatial mobility of the four VCP modules and the internal flexibility of each individual VCP module. The snapshots represent structures at 1, 5, and 8 ns (shown at the bottom panels in ribbon representation) [62]. Negative and positive potentials are colored in red and blue, respectively.](image-url)
We consider first the dissociation of a proton from a single acidic group isolated in the solution (e.g., an aspartic acid):
\[
\text{AH} \overset{K_a}{\longleftrightarrow} \text{A}^- + \text{H}^+,
\]
(18)
where \( K_a \) is the dissociation constant, usually expressed as \( pK_a = -\log K_a = 0.434 \Delta G/k_B T \), \( \Delta G \) is the free energy of protonation. The Henderson–Hasselbalch equation relates the pH with the \( pK_a \):
\[
\text{pH} = pK_a + \log \frac{[\text{A}^-]}{[\text{AH}]}
\]
(19)
and shows that the \( pK_a \) is the pH where the concentration of protonated state is equal to that of the deprotonated state. The fractional protonation or protonation probability, \( f = [\text{AH}]/([\text{AH}] + [\text{A}^-]) \), is related to \( pK_a \) by
\[
\ln \frac{f}{1 - f} = 2.303(pK_a - \text{pH}).
\]
(20)
We will proceed with our analysis for acid–base equilibria but similar schemes can be applied to ion-binding or electron-transfer redox reactions. For example, in a redox reaction:
\[
\text{A}_{\text{ox}} + e^- \overset{\text{ET}}{\longleftrightarrow} \text{A}_{\text{red}},
\]
(21)
the redox potential is given by the Nernst equation:
\[
\Phi = \Phi^0 + \frac{k_BT}{F} \ln \frac{[\text{A}_{\text{ox}}]}{[\text{A}_{\text{red}}]},
\]
(22)
where \( \Phi^0 = (k_BT/F) \ln K_{\text{ET}} \) is the potential in which there is an equal concentration of oxidized and reduced states, \( F \) is the Faraday constant, and \( K_{\text{ET}} \) is the electron transfer equilibrium constant. Apparently, the Nernst equation is analogous to the Henderson–Hasselbalch equation.

We now consider protonation of an ionizable group in a biomacromolecule that bears permanent charges and also other ionizable groups. The \( pK_a \) for the isolated ionisable group is referred to as the model \( pK_a \) or \( pK_a^0 \), and its value in the biomacromolecular environment is referred to as the apparent \( pK_a \) or \( pK_a^{\text{app}} \). To a good approximation, \( pK_a^0 \) is the same as that for the protonation of a single acid in solution as discussed earlier. In other words, the values for \( pK_a^0 \) are known from experimental measurements for single ionizable groups (e.g., \( pK_a^0 = 4.0 \) for aspartic acid in water). Because of the permanent background charges (or partial charges), desolvation effects, and other ionizable groups of the biomacromolecule, \( pK_a^{\text{app}} \) is in general different from \( pK_a^0 \).

The presence of other ionizable groups in a biomacromolecule makes the calculation of \( pK_a^{\text{app}} \) complicated because at a given pH, the ionization states of neighboring ionizable groups are interdependent. To circumvent this problem, a pH-independent hypothetical quantity has been proposed, called intrinsic \( pK_a \) or \( pK_a^{\text{intr}} \) [78]. The intrinsic \( pK_a \) describes the ionization process of a specific ionizable group when all other ionizable groups are in their neutral states. The apparent \( pK_a \) for an acid is related to \( pK_a^{\text{intr}} \) and a free energy reflecting the pH-dependent interactions of this ionizable group with all other ionizable groups of the biomacromolecule:
\[
pK_a^{\text{app}} = pK_a^{\text{intr}} + \frac{\Delta G^{\text{inter}}}{2.303k_B T},
\]
(23)
Once \( pK_a^{\text{intr}} \) values are calculated, they are used to determine the pairwise interactions among all ionizable groups, \( \Delta G^{\text{inter}} \), using statistical–mechanical methods as discussed below to account for all possible ionization states of the biomacromolecule. In general, evaluation of \( pK_a^{\text{app}} \) requires the titration curve of the system rather than direct use of Eq. (23).

Fig. 7 shows a thermodynamic cycle that allows us to compute the \( pK_a^{\text{intr}} \) relative to \( pK_a^0 \) of the same group isolated in a solution. The upper horizontal process describes the dissociation of an isolated ionisable group (e.g., an aspartic acid in solution) in the high dielectric aqueous solution, whereas the lower horizontal process describes the dissociation of the same ionizable group in the biomacromolecular environment. The vertical processes describe “desolvation” effects, i.e., the transfer of the protonated and deprotonated states from an aqueous solution to a biomolecular environment. The lower horizontal process in Fig. 7 provides the \( pK_a^{\text{intr}} \):
\[
pK_a^{\text{intr}} = pK_a^0 - \frac{Z \Delta G^{\text{env}}}{2.303k_B T},
\]
(24)
where \( Z \) takes \(-1\) for acids and \(+1\) for bases. The term \( \Delta G^{\text{env}} \) describes the interactions with the environment, i.e., solvent and background charges, for the ionizable group when all other ionizable groups are in their neutral states
\[
\Delta \Delta G^{\text{env}} = \Delta G_A - \Delta G_B = \Delta G_A^- - \Delta G_{AH}.
\]
(25)
The free energy double difference (Fig. 7 and Eq. (25)) consists of two contributions:
\[
\Delta \Delta G^{\text{env}} = \Delta \Delta G_B + \Delta \Delta G_C,
\]
(26)
where \( \Delta \Delta G_B = \Delta G_B^A - \Delta G_{AH}^B \) with \( \Delta G_B^A \) being due to the change of the dielectric environment for \( i = A^- \) or \( \text{AH} \), and similarly \( \Delta \Delta G_C \) is the corresponding change due to the interactions with the background charges. For either the protonated or deprotonated state, the single-difference term \( \Delta G_B \) is the change in
reaction field (or Born) energy:

\[ \Delta G^B = \frac{Ze^2}{2} (\psi_m^B - \psi_m^B), \]  

(27)

where \( \psi_m^B \) and \( \psi_m \) are the reaction field potentials, owed to induced polarization of charges in the surrounding environment, at the position of the charged group. The term \( \Delta G^C \) is the change in the Coulombic interactions with background charges:

\[ \Delta G^C = Ze(\psi_m^C - \psi_m^C), \]  

(28)

where \( \psi_m^C \) and \( \psi_m^C \) are the Coulombic potentials at the position of the charged group. Because of the background charges, the changes of the electrostatic energies apply to both the protonated and deprotonated states of the ionizable group.

In Eq. (23), \( \Delta G^{\text{inter}} \) is not calculated directly. Instead, it is estimated by the interaction potential between different ionic groups at all possible ionization states. The ionization microstates of a biomacromolecule with \( N \) ionizable groups can be described by \( n = 2^N \) vectors, \( \delta^n = \{\delta_1^n, \delta_2^n, \ldots, \delta_N^n\} \), with each element \( \delta_i^n \) taking value 0 or 1 for the protonated or deprotonated ionizable group \( i \), respectively. The free energy difference of the \( n \)th microstate from a fictitious microstate with all the ionizable groups in their neutral state is given by [77]:

\[ \Delta G^n = \sum_{i=1}^{N} \left\{ 2.303 k_B T \delta_i^n (pH - pK_{i}^{\text{init}}) + \delta_i^n \sum_{1 \leq j < i} \delta_j^n \Delta G^{ij} \right\}, \]  

(29)

where \( \Delta G^{ij} \) is the pairwise interaction free energy between ionizable groups \( i \) and \( j \) in their charged states, calculated from the electrostatic potential at group \( j \) due to group \( i \) in the biomacromolecule (\( Z_i \) and \( Z_j \) are incorporated in \( \Delta G^{ij} \)). The charge of group \( i \) in microstate \( n \) is \( q_i = Z_i \delta_i^n \) and the net charge of the biomacromolecule in microstate \( n \) is \( Q = \sum_{i=1}^{N} q_i \). The total free energy for all ionization microstates is given by

\[ \Delta G^{\text{ion}} = -k_B T \ln \Omega, \]  

(30)

where \( \Omega \) is the partition function:

\[ \Omega = \sum_{n=1}^{2^N} e^{-\left(\Delta G^n / k_B T\right)}, \]  

(31)

The mean charge \( \langle q_i \rangle \) of group \( i \) as a function of \( pH \) is given by the Boltzmann-weighted sum divided by the partition function:

\[ \langle q_i \rangle = \frac{\sum_{n=1}^{2^N} Z_i \delta_i^n e^{-\left(\Delta G^n / k_B T\right)}}{\Omega}, \]  

(32)

and the net mean charge of the biomacromolecule as a function of \( pH \) is given by

\[ \langle Q \rangle = \sum_{i=1}^{N} \langle q_i \rangle. \]  

(33)

Plots of \( \langle Q \rangle \) and \( \langle q_i \rangle \) as a function of \( pH \), could reproduce the titration properties of a biomacromolecule with multiple ionizable groups.

A small protein of 100 ionizable amino acids can assume \( 2^{100} \) ionization states, which makes an exact calculation of Eqs. (29)–(33) computationally impossible. To overcome the \( 2^N \) computational challenge, several approximations have been proposed to reduce the number of meaningful interactions, such as the reduced site approximation [74], the hybrid statistical mechanical/Tanford–Roxby approximation [77], the clustering method [75], and a Monte Carlo method [76]. The titration curve for each ionizable group is described by plotting the approximated \( \langle q_i \rangle \) as a function of \( pH \) and for the whole biomacromolecule by plotting the approximated \( \langle Q \rangle \) as a function of \( pH \). The apparent \( pK_a \) value for ionizable group \( i \) is equal to the \( pH \) value at which the group \( i \) is 50% charged. The \( pK_a \) values predicted by these methods have been successfully tested with experimental results [71–73,77,79–83].

Analysis of \( pK_a^{\text{app}} \) values has been used to identify electrostatic interactions which contribute to the structural stability of D/D PKA RIIα [84], the catalytic mechanism of the protein GART [85], the coil-helix transition of GART [86], and the association of C3d with CR2 [60]. Fig. 8 illustrates the effect of the protein environment on \( pK_a^{\text{app}} \) values. It shows the shifts in the \( pK_a^{\text{app}} \) values of ionizable amino acids compared to model
Fig. 9. Theoretical titration curves for several ionizable groups in or in the proximity of the catalytic site of the enzyme GART. The figure demonstrates the quality of the calculated sigmoidal titration curves for basic groups (A) and acidic groups (B). When more than one proton is involved (e.g., from an another ionizable group in the vicinity) there is either a slight deviation from sigmoidal shape or the transition occurs over a wider pH range. The apparent $pK_a$ values correspond to half protonation or partial charge 0.5 for basic residues and −0.5 for acidic residues. Significant shifts from model $pK_a$ values (not shown) are present for the strongly interacting ionizable groups of the catalytic site [85].

Fig. 10. (A) Theoretical titration curves for the catalytic triad of GART, comprised of a histidine (squares), a basic substrate group (circles), and an aspartic acid (triangles). Another histidine that modulates the catalytic process is also shown (diamonds). The unusual non-sigmoid shape of the titration curve, significant apparent $pK_a$ shifts from the model $pK_a$, and wide pH range of partial charge during titration reflect the electrostatic inter-dependence of these groups. (B) The effect of 180°-flip for the side chain ring ($\chi_2$-torsion angle) of the catalytic histidine on the titration curves of the other catalytic residues (the symbols are the same as in panel A). The flip of the catalytic histidine ring altered the local electrostatic interactions in the catalytic site, which is demonstrated by the highly perturbed titration curves compared to those of panel (A). Histidine flip states are difficult to be determined by the X-ray diffraction methods used to solve protein structures because both the 0°- and 180°-flip states have nearly identical electron densities. This ambiguity makes necessary a global hydrogen-bonding optimization calculation to determine the preferred side chain conformation of histidines for a specific protein structure [85].

$pK_a$ values, for an NMR ensemble of structures of D/D PKA RII$\alpha$ [84]. The lower $pK_a^{app}$ values for acids and higher $pK_a^{app}$ values for bases in the protein environment depict favorable Coulombic interactions. However, there are also opposite compensatory effects from the unfavorable desolvation. The arrows depict examples of secondary, tertiary, and quaternary structure interactions, which are shown with molecular graphics in the top panels. Fig. 9 shows the predicted titration curves for several amino acids of the protein GART [85]. Most titration curves follow the expected sigmoid shape for non-interacting ionizable groups. The apparent $pK_a$ values, expressed as the midpoint of the sigmoid, show variable shifts from their model $pK_a$ values, depending on their local environment within the protein. Fig. 10 shows irregular titration curves for catalytic residues of the protein GART [85]. Some of these curves include more than one inflection points corresponding to multiple apparent $pK_a$ values. This unusual behavior demonstrates the presence of strong electrostatic interactions, such as proton proximity, sharing, or double protonation for histidines, which are important for proton transfer and for the catalytic process. Other studies have utilized perturbed titration curves and unusual $pK_a^{app}$ values to identify catalytic sites of enzymes [82,87]. Also, predicted $pK_a^{app}$ values for bacteriorhodopsin in the presence and absence of membrane models have been compared [88–90]. Finally, an example for the coupling between protonation and reduction in proteins with multiple redox centers has been presented in the case of cytochrome c3 [70].

6. Biomacromolecular stability

The charge-transfer protocols described above are also useful for predicting the effects of pH and ionic strength on biomacromolecular stability [71,91]. Specifically, electrostatic calculations have been used to study global protein folding-unfolding transitions, local helix-coil transitions within otherwise folded proteins, and protein association or ligand binding.

The effect of pH on protein stability can be examined by considering the net mean charges of the unfolded and folded states [92,93]:

$$\frac{\partial \Delta G^\text{unfold}(pH)}{\partial pH} = 2.303k_B T (\langle Q^U \rangle - \langle Q^F \rangle),$$  \(34\)

where $\langle Q^U \rangle$ and $\langle Q^F \rangle$ are the net mean charges of the biomacromolecule in its unfolded and folded states, respectively. Integration of Eq. (34) yields an expression for relative $\Delta G^\text{unfold}(pH)$ with respect to a reference state at pH0:

$$\Delta G^\text{unfold}(pH) - \Delta G^\text{unfold}(pH_0) = 2.303k_B T \int_{pH_0}^{pH} (\langle Q^U \rangle - \langle Q^F \rangle) \, dpH,$$  \(35\)

which can be evaluated numerically. The net mean charges are calculated according to Eqs. (32) and (33) using available biomacromolecular structures and the free energies of the ionizable group in the folded and unfolded states. Eq. (35) provides the relative free energy of the folding-unfolding transition. For the folded state, $\Delta G^U_F$ used for calculation of $\langle Q^F \rangle$ is given...
by Eq. (29). For the unfolded state, however, $\Delta G_F^{\text{ion}}$ required for calculation of $\langle Q_i \rangle$ is difficult to obtain because of lack of biomacromolecular structures of unfolded proteins. To circumvent this problem, typically the ionizable groups of the unfolded state are assigned with pK_a values equivalent to those of isolated groups in solution (model pK_a values), mimicking an environment of non-interacting ionizable groups. The free energy of the unfolded state is then given by a simplified version of Eq. (29) which involves only the model pK_a:

$$
\Delta G_U^{\text{ion}} = \sum_{i=1}^{N} 2.303 k_B T \delta_1^{\text{ion}}(pH - pK_a^{\text{ion}}).
$$

(36)

Because residual electrostatic interactions may also be present in the unfolded states, alternative methods have been proposed based on the “explosion” of the structure of the folded state by systematically altering its van der Waals interactions [94]. Typically, pH_0 is chosen such that all ionizable groups are either in their neutral or charged states in both folded and unfolded states [i.e., $\langle Q_i \rangle = \langle Q_i^{\text{F}} \rangle$ and $\Delta G_{\text{unfold}}^{\text{ion}}(pH) = \Delta G_{\text{unfold}}^{\text{ion}}(pH_0)]$. A comparison with experimental data gives $\Delta G_{\text{unfold}}^{\text{ion}}(pH)$ by a simple scaling of $\Delta \Delta G_{\text{ion}}(pH)$ at or close to pH_0.

The free energy of the folding-unfolding transition, $\Delta G_{\text{unf}}^{\text{ion}}(pH)$, can also be calculated using the thermodynamic cycle of Fig. 11, which decomposes the folding-unfolding transition into pH dependent ($\Delta G_{F}^{\text{ion}}$, $\Delta G_{U}^{\text{ion}}$, $\Delta G_{\text{unf}}^{\text{ion}}$) and pH independent ($\Delta G_{\text{unf}}^{\text{neutral}}$) processes:

$$
\Delta G_{\text{unf}}^{\text{ion}}(pH) = \Delta G_{\text{unf}}^{\text{neutral}} = \Delta G_{F}^{\text{ion}}(pH) - \Delta G_{F}^{\text{ion}}(pH_0).
$$

(37)

$\Delta G_{\text{unf}}^{\text{neutral}}$ is the unfolding energy of a biomacromolecule with all ionizable groups in a hypothetical neutral state; it includes contributions from non-polar effects, from hydrogen bonds, from desolvation of non-charged polar groups, and from configurational entropy. A comparison of the predicted $\Delta G_{\text{unf}}^{\text{ion}}(pH)$ using Eq. (36) with experimental data gives $\Delta G_{\text{unf}}^{\text{ion}}(pH_0)$. We notice that $\Delta G_{\text{unf}}^{\text{neutral}}$ is different from $\Delta G_{\text{unf}}^{\text{ion}}(pH_0)$ (Eq. (35)) because the former contains only a pH-independent contribution whereas the latter contains both the pH-independent and ionization contribution at pH_0.

Characteristic examples for the prediction of the pH dependence of stability are studies of hyperthermophilic proteins [95,96] and helix-coil transitions of polylysine peptides [97].

Fig. 12. Plot of the free energy difference $\Delta G_{\text{unf}}^{\text{ion}} - \Delta G_{\text{neutral}}^{\text{ion}}$ using Eq. (37) as a function of pH for C3d (A) and CR2 (B) at ionic strengths corresponding to 0 mM (solid line) and 150 mM (dotted line). The plot depicts the ionization contribution to the free energy of unfolding (Zhang and Morikis, unpublished data).

For example, Yang and Honig reported the effect of pH and ionic strength on the sequential unfolding process of myoglobin in both the presence and absence of the heme [98]. Myoglobin is the standard example for protein folding–unfolding studies, which have implicated the ionization properties of histidines in modulating the transition. A study has been reported on the relation of the optimum pH for maximal protein stability to the protein isoelectric point, base/acid ratio, and average pK_a shift, by comparing computational and experimental data [99]. Fig. 12 shows the pH dependence of the predicted free energy of unfolding according to Eq. (36) for free C3d and free CR2 at two ionic strengths. Both proteins are stable at physiologically relevant pHs. In the pH range 4–10 where most acids and bases are charged, thus amenable to favorable Coulombic interactions, we have maximum stability. At extreme pHs where either acids become neutral (low pH) or bases become neutral (high pH), we have low stability. The electrostatic calculations at ionic strengths corresponding to 0 mM concentration and the physiologically relevant 150 mM concentration reveal the screening effect of small ions on the electrostatic interactions which contribute to protein stability (Fig. 12). For example, the curves corresponding to 0 mM ionic strength depict the full strength of the Coulombic effects between acid–base pairs, whereas the curves at 150 mM ionic strength demonstrate the reduction of acid–base Coulombic effects because of competition from Coulombic interactions with solvated ions.
7. Biomacromolecular association

Association of biomacromolecules A and B to form a complex AB can be schematically described by a two-step scheme:

$$ \text{A} + \text{B} \xleftrightarrow{K_{AB}} \text{A} : \text{B} \xrightarrow{K_{AB}} \text{AB}. \quad (38) $$

The first step describes the “recognition” between the two components to form a complex and the second step describes the actual “binding” after local rearrangements take place at the binding interface. The recognition step involves long-range electrostatic interactions between biomacromolecules and the binding step involves short-range electrostatic interactions, such as salt bridges and hydrogen bonds, van der Waals interactions, hydrophobic interactions, and entropic effects related to the exclusion of solvent and local structural changes.

Electrostatic calculations based on the generalized PB equation have been used to explain the effects of mutations at and away from the association interface in modulating the binding of C3d with CR2 [60] and TEM1-β-lactamase with its protein inhibitor BLIP [100]. Also, electrostatic potentials have been used to design proteins with variable activities or association constants, e.g., for the interaction of the TEM1-β-lactamase with BLIP [101] and of the viral protein VCP with C3b [61]. In both cases the protein design involved minimal number of surface mutations forming hotspots for association and the predicted properties were found in good agreement with experimental data.

The electrostatic contribution to the binding free energy [46,102] for a single ionization state is given by

$$ \Delta G_{\text{electr}} = \Delta G_{\text{electr}}(\text{complex}) - \left[ \Delta G_{\text{electr}}(\text{component 1}) + \Delta G_{\text{electr}}(\text{component 2}) \right]. \quad (39) $$

This equation assumes fixed conformational states. The structure of the complex should be known and the structures of individual components are taken after separation from the complex. Although Eq. (39) is sufficient to calculate the binding free energy, the thermodynamic cycle of Fig. 13 is typically used to separate the Coulombic interactions from the solvation effects and to assess the magnitude of their contributions:

$$ \Delta G_{\text{electr}} = \Delta G_{\text{electr}}(\text{Coulomb}) + \Delta G_{\text{electr}}(\text{solv}) = (\Delta G_{\text{solv}}^A + \Delta G_{\text{solv}}^B). \quad (40) $$

The upper horizontal process assumes the same dielectric coefficient for the biomacromolecule ($\varepsilon_m$) and the solvent ($\varepsilon_s$), typically $\varepsilon_m = \varepsilon_s = 2–20$ or the dielectric of the protein interior, and zero ionic strength. The lower horizontal process assumes dielectric discontinuity between the biomacromolecule, $\varepsilon_m = 2–20$, and the solvent, $\varepsilon_s \sim 80$. The vertical processes describe solvation (or desolvation) effects. Because of the uncertainty in the choice of the internal protein dielectric coefficient, usually a relative change in the free energy of association is calculated owed to some perturbation, e.g., changes in ionic strength, rather than an absolute free energy of association.

Non-polar contributions to the free energy of association are typically described in the form of differences in the free energies of hydration for the complex and the sum of its components. The hydration free energy of a biomacromolecule, treated as a cavity/van der Waals system, is represented as proportional to the biomacromolecular solvent accessible surface area (SASA). The non-polar free energy of association, $\Delta G_{\text{non-polar}}$, is given by

$$ \Delta G_{\text{non-polar}} = \gamma \Delta \text{SASA(\text{complex})} - \gamma \Delta \text{SASA(\text{component 1})} + \Delta \text{SASA(\text{component 2})}. \quad (41) $$

The surface tension coefficient $\gamma$ can be determined by fitting experimental data. Variable values of $\gamma$ have been reported depending on the biomacromolecular process. For small non-polar molecules, $\gamma$ is about $\sim 5 \text{cal/(mol ˚A}^2\text{)}$ [103]. Typically, the sum of the van der Waals radii of surface-exposed atoms and a probe sphere of 1.4 ˚A radius, representing a water molecule, are used to define SASA.

The effect of pH on biomacromolecular association can be studied using a protocol similar to that used in studying the pH dependence of biomacromolecular stability. In this case, Eq. (35) can be rewritten as

$$ \Delta G_{\text{assoc}}(\text{pH}) = \Delta G_{\text{assoc}}(\text{pH}_0) = 2.303 k_B T \int_{\text{pH}_0}^{\text{pH}} \left( \langle Q^A \rangle - \langle Q^A \rangle - \langle Q^B \rangle \right) \text{d pH}, \quad (42) $$

where $\langle Q^A \rangle$ and $\langle Q^B \rangle$ are the net mean charges of components A and B, and $\langle Q^{AB} \rangle$ is the net mean charge of the complex AB.

A thermodynamics cycle similar to that in Fig. 11 describes the protein–protein association:

$$ \Delta G_{\text{assoc}}(\text{pH}) = \Delta G_{\text{neutral}} $$

$$ = \Delta G_{\text{ion}}^{\text{AB}(\text{pH})} - \Delta G_{\text{ion}}^{\text{A}(\text{pH})} - \Delta G_{\text{ion}}^{\text{B}(\text{pH})}. \quad (43) $$

Characteristic examples of electrostatic modeling for protein–protein or protein–ligand association include the binding of major histocompatibility complex (MHC), proteins with several peptides [104], the dimerization of the GCN4 leucine zipper [105], and the binding of several low-molecular mass inhibitors to HIV-1 protease [106]. The latter example uses combination of MD and electrostatic calculations. A combination of MD simulations and electrostatic calculations has also been applied to asparagine and aspartic acid binding of aspartyl-tRNA synthetase as a computationally more efficient alternative of MD-based free energy calculations [107,108]. An analysis of various contributions to the electrostatic free energy from charged and polar amino acids, salt bridges, hydrogen bonds and their networks, and desolvation effects indicates
that, depending on the complementarity of charge distributions, electrostatic interactions may oppose, favor or have a zero net effect on protein–protein interactions [109]. Electrostatic calculations have also been used to examine the effect of ionic strength on the stability of ligand–DNA and protein–DNA association [110,111], and on the homo-multimeric equilibria of halophilic proteins [112]. The interaction between complement component C3d and its receptor CR2 has been known to be pH and ionic strength dependent by several different experimental and computational studies [60,113]. In the case of C3d–CR2 interaction, the use of molecular dynamics simulations helps generate the relaxed three-dimensional structures (from crystal packing effects) and improves the agreement between theoretical and experimental data compared to the use of raw crystal structures [113].

8. Ion channels

Potassium (K\textsuperscript{+}) channels are tetrameric membrane-spanning proteins that provide a selective pore for the conductance of K\textsuperscript{+} across the cell membranes [114]. These channels are important for brain cell communication, heat rate regulation, and hormone secretion. One remarkable property of K\textsuperscript{+} channels is their ability to discriminate K\textsuperscript{+} from Na\textsuperscript{+} by more than a thousand fold while conduct ions at a throughput rate near the diffusion limit. MD simulations reveal that in the KcsA potassium channel, a single file of K\textsuperscript{+} separated by water molecules dynamically move through the narrowest region of the pore in a highly correlated fashion [115]. As illustrated in Fig. 14, the high microscopic event underlying ion conduction is the concerted transition between the two stable low-energy configurations with three confined K\textsuperscript{+}. The calculated maximum electric conductance of K\textsuperscript{+} through the selectivity filter of KcsA is on the order of 300–500 ps, in good agreement with experimental measurements [116]. The free energy perturbation (FEP) computations based on atomic models show that occupancy of Na\textsuperscript{+} in the KcsA channel is thermodynamically unfavorable, which explains the high selectivity of K\textsuperscript{+} over Na\textsuperscript{+} [117].

9. Future perspectives

In recent years, more and more 3D-structures of biomacromolecules become available. These include the structures of large biomacromolecular complexes and supramolecular assemblies. The progress is owed to methodological improvements for high resolution structure determination using crystallographic or NMR methods, as well as availability of synchrotron radiation sources for efficient X-ray diffraction data collection, robotic improvements for sample preparation and handling, and computational improvements for experimental data analysis and structure refinement. Electrostatic calculations have kept up with this pace by employing more accurate multi-grid numerical methods and by using more efficient computer parallelization, which enable the study of larger biological systems. For example, the program APBS [50] has been used to calculate the electrostatic potentials of a working module of microtubules (1.35 million atoms) and two crystallographically determined subunits of the ribosome (88,000 and 95,000 atoms) [50]. We expect more biomacromolecular studies at the nanoscale in the near future, aiming to understand important and complex biological processes.

Another area of promise is the study of membrane–protein and membrane–protein–inhibitor complexes and ion and water membrane channels [118]. In ion channels, the charges of ionizable side chains control single-channel conductance, ion selectivity, open-channel block, gating and voltage sensing. Knowledge of the protonation state of these residues is required for a thorough understanding of these electrostatically controlled phenomena in quantitative detail, but the \( pK_a \) values of ionizable side chains in ion channels, as well as in most other large membrane proteins, continue to elude experimental determination [119]. We also expect more studies that incorporate combination of MD simulations and electrostatic calculations to study biomacromolecular complexes [120]. The protein docking problem is of interest to experimentalists who want rapid computational methods to locate the interface of protein–protein complexes or to scan numerous compounds for binding in drug discovery projects [121–123]. Incorporation of dynamics in docking is challenging but is necessary for more accurate representation of recognition and binding. Several studies have used Brownian dynamics to calculate binding rates for processes dominated by electrostatics [124]. Methodological improvements for the use of more detailed dynamics to predict binding rates may be another area of promise. Structural bioinformatics analyses, based on structural homology and electrostatic potentials, may be capable for predicting functional similarities or dissimilarities for phylogenetic trees [64]. The effect of salts on the folding and stability of nucleic acids is also an important field for computational studies [136,137]. Finally, combination of coarse-grained methods for rapid evaluation of electrostatic properties of biological nano-systems, e.g., viruses, followed by atomic resolution methods for localized specific functional areas will be of future interest.

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List of symbols

- \(a\) polyion radius
- \(A, B\) dimensionless parameters used in the cell model
- \(b\) axial distance between successive charges
- \(c\) integration constants
- \(C\) molar concentration
- \(e\) unit charge
- \(E\) electric field
- \(F\) Helmholtz energy
- \(F\) Faraday constant
- \(G\) Gibbs energy
- \(g(r)\) radial distribution function
- \(h_B\) Boltzmann constant
- \(K_a\) dissociation constant
- \(K_{ET}\) electron transfer equilibrium constant
- \(l_B\) Bjerrum length
- \(P\) a set of electrostatic potentials at the grid-points
- \(q_M(r)\) charge density of a biomacromolecule
- \(Q(r)\) local charge density or instantaneous charge
- \(r\) distance
- \(R\) cell radius
- \(S\) entropy
- \(T\) absolute temperature
- \(u(r)\) pair interaction potential
- \(U\) internal energy
- \(V\) volume
- \(W(r)\) potential of mean force
- \(x\) reduced distance
- \(Z_i\) ion valence

Greek letters

- \(\gamma\) surface tension constant
- \(\Gamma(r)\) ion surface accessibility
- \(\varepsilon_r\) dielectric coefficient or relative permittivity
- \(\varepsilon_0\) permittivity of free space
- \(\varepsilon\) surface charge density
- \(\kappa\) Debye screening parameter
- \(\lambda\) a dimensionless parameter used in the cell model
- \(\lambda_{GC}\) Gouy–Chapman length
- \(\lambda_i\) thermal wavelength
- \(\mu\) chemical potential
- \(\xi\) number of unit charges per Bjerrum length
- \(\rho_i(r)\) local number density
- \(\rho_i^0\) bulk ionic density
- \(\varphi\) surface charge density
- \(\Phi\) redox potential
- \(\psi(r)\) reduced electrostatic potential
- \(\phi\) osmotic coefficient
- \(\psi(r)\) electrostatic potential
- \(\Omega\) partition function

Appendix A. Analytical solutions of the Poisson–Boltzmann equation

The Poisson–Boltzmann (PB) equation can be solved analytically in a number of circumstances. Because of their numerical efficiency, not only these analytical solutions are most widely used in practical applications, probably more important, they also become a tenet of various phenomenological theories for understanding electrostatic phenomena at conditions even when the underlying assumptions are not truly valid. In this appendix, we discuss three popular electrostatic theories in the literature, namely, the Debye–Hückel theory, Gouy–Chapman theory, and Manning’s counterion-condensation theory. Although we discuss the solutions of the PB equation only in spherical, planar and cylindrical geometries, analytical solutions to the linear PB equation can also be found near spheroidal surfaces [127,128] in an infinitely long hollow cylinder\(^9\) or in a cylindrical pore across a charged membrane [129].

A.1. Debye–Hückel theory

The Debye–Hückel (DH) theory is derived in the limit of low salt concentration where the reduced electrostatic potential around each ion is very small \(\varphi \equiv e\varphi/k_BT \ll 1\). In this case, the linearized PB equation becomes

\[
\nabla^2 \varphi = \frac{1}{r^2} \frac{d}{dr} r^2 \frac{d\varphi}{dr} = \kappa^2 \varphi, \quad (A1)
\]

where \(\kappa^2 = 4\pi\lambda_i \sum \rho_i^0 Z_i^2\) is the Debye screening parameter, \(l_B\) the Bjerrum length, \(Z_i\) the ion valence, and \(\rho_i^0\) is the ion density in the bulk. At the surface of a tagged ion, Gauss’s law requires

\[
\frac{d\varphi}{dr} \bigg|_{r=a} = -\frac{Z_i e}{4\pi a^2 \varepsilon_r \varepsilon_0}, \quad (A2)
\]

where \(a\) is the ion radius and \(Z_i e\) is the charge. With an additional boundary condition \(\varphi(\infty) = 0\), integration of Eq. (A1) yields:

\[
\varphi(r) = \frac{Z_i l_B e^{-\kappa r}}{1 + \kappa a r} \approx Z_i l_B e^{-\kappa r}, \quad (A3)
\]

The approximation in Eq. (A3) is justified by a small value of \(\kappa a\) in a dilute electrolyte solution. From Eq. (A3) and the linearized Boltzmann equation, we can derive the density distributions near the tagged ion or the radial distribution functions (RDF):

\[
g_{ij}(r) \equiv \frac{\rho_{i,j}(r)}{\rho_{i,0}} = 1 - Z_i Z_j l_B e^{-\kappa r}, \quad (A4)
\]

The RDF can then be substituted into the energy equation which gives\(^10\):

\[
\frac{U^e}{V} = -\frac{\kappa^3 l_B k_B T}{8\pi}. \quad (A5)
\]

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\(^9\) According to the linearized PB equation, the reduced electrostatic potential in a hollow cylinder containing electrolyte is given by \(\varphi(r) = \phi(r) \Omega_1 / \Omega_0 (\kappa a)\) where \(\kappa\) is the Debye screening parameter, \(l_0\) the zero-order modified Bessel function of the first kind and \(r\) is the radial distance from the axis of the cylinder whose radius is \(a\).

\(^{10}\) The excess Helmholtz energy is defined relative to that of an ideal gas at the same temperature and number density.
The excess Helmholtz energy is obtained from the Gibbs–Helmholtz equation:

\[
\frac{F^{ex}}{V} = -\frac{k^3 k_B T}{12\pi}
\]

and the ionic chemical potential is

\[
\mu_i = \left( \frac{\partial F}{\partial N_i} \right)_V = k_B T \left[ \ln \rho_i \Lambda_i^3 - \frac{k_B Z_i^2}{2} \right],
\]

where \( \Lambda_i \) represents the thermal wavelength of ion \( i \). The osmotic pressure is obtained from the difference in the Gibbs and Helmholtz energies:

\[
\Pi = k_B T \left[ \sum_i \rho_{i,0} - \frac{k^3}{24\pi} \right].
\]

Eq. (A8) suggests that at sufficiently low temperature, an electrolyte solution may undergo a vapor–liquid-like phase transition. This phase transition arises from the strong positional correlations between the oppositely charged ions, which is very different from that predicted by the van der Waals theory where the thermodynamic instability is a consequence of the competition between the intermolecular attraction and repulsion. Because the DH theory is valid only in the limit of low concentration, the thermodynamic properties directly derived from the DH theory are rarely in good agreement with experimental results. Nevertheless, the DH theory forms the theoretical basis for a number of engineering-oriented semi-empirical theories [130].

According to the DH theory, the overall interaction between ionic species or the potential of mean force follows a “screened” Coulomb potential:

\[
W_{ij}(r) = -k_B T \ln g_{ij}(r) \approx Z_i Z_j k_B T \frac{e^{-\kappa T r}}{r}.
\]

Eq. (A9) suggest that the contribution of surrounding ions to the potential mean force can be understood as if the charge of the tagged ion is “screened” by an exponential factor \( e^{-\kappa T r} \). When \( r = 1/\kappa \), the potential of mean force is about 37% of the “bare” Coulomb potential. For \( r > 1/\kappa \), the overall electrostatic interaction may be considered unimportant from a practical point of view. A conventional wisdom is that the electrostatic interactions between charged species are insignificant when their separation is beyond the Debye screening length. Eq. (A9) provides the essential basis of the DLVO\(^{11}\) theory for describing the electrostatic contribution to colloidal forces.

A.2. Electric double layer

When a charged plate is surrounded only by the counterions, the non-linear PB equation can be solved analytically. In this case, the PB equation in reduced units is expressed as

\[
\frac{\partial^2 \psi}{\partial r^2} = -4\pi \rho c Z c e^{-Z e \psi(r)}.
\]

where \( r \) is the perpendicular distance from the surface, \( \rho c \) the counterion density at the surface, and \( Z c \) is the counterion valence. The density of counterions at contact can be obtained from the contact-value theorem [22]:

\[
k_B T \rho c = \frac{\varepsilon^0 \varepsilon_0 E^2}{2},
\]

where \( E = -\psi'(0) \) is the electric field at the surface. Using Gauss’ law \( \psi'(r=0) = \sigma / \varepsilon_0 \), we have \( \rho c = 2\pi \lambda^2 (\sigma / \varepsilon)^2 \) with \( \sigma \) standing for the surface charge density. With the boundary conditions \( \psi(0) = 0 \) and \( \psi'(r = 0) = \sigma / \varepsilon_0 \), Eq. (A10) can be solved analytically:

\[
\psi(r) = \frac{2 \ln \left( \frac{r}{\lambda_{GC}} + 1 \right)}{Z c},
\]

where \( \lambda_{GC} = (2\pi Z c k_B \sigma / \varepsilon)^{-1} \) is the Gouy–Chapman length. Because the counterion–wall electrostatic energy is given by \( u(r) = k_B T \lambda^2 \lambda_{GC} \), the Gouy–Chapman length provides a measure of the distance at which the thermal energy equals the counterion–wall interaction energy. Substituting Eq. (A12) into the Boltzmann equation for counterion distribution gives

\[
\rho(r) = \frac{\rho c}{\left( \frac{r}{\lambda_{GC}} + 1 \right)^2}.
\]

From Eq. (A13), one can find that the Gouy–Chapman length also corresponds to a distance from the charged surface that contains half of the counterions.

A.3. Counterion-condensation (CC) theory

The concept of counterion condensation stems from consideration of an infinitely long rod with a linear charge density \( \xi = l_0 / b \) surrounded by counterions of valence \( Z_i \). The system serves as a limiting condition for a salt-free polyelectrolyte at infinite dilution. The unscreened Coulomb interaction between the charged rod and a counterion at a distance \( r \) from the rod is

\[
\beta u(r) = 2|Z_i|\xi \ln \left( \frac{r}{R} \right)
\]

where \( R \) is a reference position where the electrostatic potential is defined as zero. With the assumptions that the rod has negligible radius and all counterions are independent to each other, the partition function of each counterion within a cylindrical cell of radius \( R \) is given by

\[
\Omega = \frac{1}{\pi R^2} \int_0^R e^{-u(r)/k_B T} 2\pi r \, dr = \frac{2}{R^2 - 2|Z_i|\xi} \int_0^R r^{1-2|Z_i|\xi} \, dr.
\]

Because the integration starts from zero, \( \Omega \) diverges when \(|Z_i|\xi \geq 1\). In order to avoid such divergence, Manning argued
that in this case, the ion atmosphere is unstable and some counterions must condense to the poliony such that its linear density is reduced to $|Z_i|\xi = 1$ [39]. As a result, a fraction of the fixed charges, $1 - (|Z_i|\xi)^3$, becomes completely neutralized by the counterions. Manning’s argument coincides with an independent work by Oosawa who proposed a two-state model for the distribution of counterions around a charged rod, i.e., a bound state and a free state [131]. In the PB equation, the distribution of counterions is continuous and there is no bound state. Although the instability condition is valid only for an infinitely thin line charge in the limit of zero concentration, the counterion-condensation (CC) theory presumes the prevalent applicability of the “limiting law”, i.e., $|Z_i|\xi \leq 1$ for all linear polyelectrolytes. It further assumes that the distributions of uncondensed ions and electrostatic potential can be calculated from the linear PB equation.

Clearly the divergence of the partition function can be avoided if a more realistic model of polyelectrolyte is considered [132]. For this reason, the CC theory has been controversial from the outset and subjected to a number of modifications, in particular on the spatial distribution of condensed counterions and their division from the uncondensed ions [133–135]. Nevertheless, it remains a popular choice in practical applications not only because of its simplicity but also, probably more important, because of its good agreement with experiments for a number of important properties of polyelectrolyte solutions including those containing DNA.

References


