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Direct measurements of biomolecular electrostatics through experiments

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Abstract

Biomolecular electrostatics has been a subject of computational investigations based on 3D structures. This situation is changing because emerging experimental tools allow us to quantitatively investigate biomolecular electrostatics without any use of structure information. Now, electrostatic potentials around biomolecules can directly be measured for many residues simultaneously by nuclear magnetic resonance (NMR) spectroscopy. This NMR method can be used to study electrostatic aspects of various processes, including macromolecular association and liquid-liquid phase separation. Applications to structurally flexible biomolecules such as intrinsically disordered proteins are particularly useful. The new tools also facilitate examination of theoretical models and methods for biomolecular electrostatics.

Introduction

In life, complex networks of molecular interactions involve electrostatic forces that influence structure and function of biological macromolecules. Electrostatic interactions are crucial for many biomolecular processes such as protein-nucleic acid binding, enzymatic catalysis, and liquid-liquid phase separation [1-6]. Accurate electrostatic information is also key to success in protein engineering [7] and drug design [8]. Thus, electrostatics is important for our fundamental understanding of biomolecular functions as well as for biotechnological development.

Computation of electrostatic potentials in solution from 3D structures using the Poisson-Boltzmann equation solver programs (e.g., APBS [9] and DelPhi [10]) is common in structural biology. However, their validity range should be examined more extensively because the computational method is approximate and uses empirical parameters. Structure-based assessment of electrostatics may also be challenging for structurally flexible biomolecules such as intrinsically disordered proteins (IDPs) and single-stranded RNA.

Recently, it has become possible to directly measure local electrostatic potentials for individual residues of biomolecules by nuclear magnetic resonance (NMR) spectroscopy

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[11] (Figure 1A). Since this method provides effective near-surface electrostatic potentials (ϕ_{ENS}), we referred to it as the ϕ_{ENS} method. This NMR method can be used to examine theoretical electrostatic models, because ϕ_{ENS} potentials can also be predicted from 3D structures [11-15]. Owing to the *de novo* nature requiring no structural information, the ϕ_{ENS} method also enables electrostatic investigations of conformationally disordered biomolecules [15-19]. In this minireview, we introduce the principle and applications of the ϕ_{ENS} method and compare it with other electrostatic methods.

Challenges in computational assessment of electrostatics

In the past, biomolecular electrostatics has been a subject of computational investigations. Quantitative assessment of biomolecular electrostatics is not as straightforward as it may appear. Biomolecules in solutions are surrounded by mobile ions (e.g., Na^+, K^+, Cl^-), which electrostatically influence the thermodynamic and kinetic properties of macromolecules [20,21]. Distribution of mobile ions around charged biomolecules are nonuniform [22,23]. Electrostatic potentials around biomolecules depend not only on their charged moieties but also on the concentrations and spatial distributions of the surrounding mobile ions. Mobile ions also cause screening, which dampens electric fields [24].

The Poisson-Boltzmann equation-based computations take mobile ions into consideration and calculate electrostatic potentials. Programs such as APBS and DelPhi compute electrostatic potentials on grid points in a sufficiently large 3D space containing a biomolecular structure [9,10] (see Figure 1B for example). The computation utilizes a continuum dielectric approximation and requires a point charge for each atom of the biomolecule. Typically, the charge of each atom in titratable groups is calculated from a specified pH and a pK_a predicted from the structure. However, even advanced pK_a prediction methods give root mean square errors as large as 0.8 [25,26], which may cause considerable errors in electric charges. Structural flexibility of biomolecules adds another layer of complexity in assessment of electrostatics [27].

Experimental measurement of near-surface electrostatic potentials

The ϕ_{ENS} method allows us to directly measure local electrostatic potentials for many residues of biomolecule (Figure 1C). This NMR method provides the effective near-surface electrostatic potentials (ϕ_{ENS}) that represent average electrostatics in exterior space near the molecular surface close to the observed ^{1}H nuclei. The ϕ_{ENS} method is useful to validate theoretical electrostatic models [11-14] and to investigate electrostatic impacts of inter- and intra-molecular interactions [11,15-17]. Table I summarizes applications of the ϕ_{ENS} methods for biophysical research.

Paramagnetic relaxation enhancement (PRE) arising from two analogous paramagnetic cosolutes with different charges are used to determine ϕ_{ENS} potentials. PRE arising from paramagnetic cosolutes has been referred to as solvent PRE [28,29]. Solvent PRE rates (Γ_2) for ^{1}H transverse magnetizations can readily be measured with various methods using paramagnetic and corresponding non-paramagnetic samples [18,29]. The ϕ_{ENS} potential is defined as [11]:

$$\phi_{ENS} = \frac{k_B T}{(z_b - z_a)e} \ln\left(\frac{\Gamma_{2,a}}{\Gamma_{2,b}}\right), \quad [1]$$

where $\Gamma_{2,a}$ and $\Gamma_{2,b}$ are the solvent PRE rates for the two paramagnetic cosolutes a and b at an identical concentration; z_a and z_b are their charge valence; e is the elementary charge; k_B is the Boltzmann constant; and T is temperature. The spatial distributions of the charged cosolutes around a biomolecule are related to local electrostatic potentials. This allows one to obtain electrostatic information. The PRE rates are governed by the paramagnetic cosolutes within a zone proximal to the observed ^{1}H nucleus, which we refer to as the effective near-surface (ENS) zone. The ϕ_{ENS} potential represents an average electrostatic potential within the ENS zone [11] (Figure 1D).

PROXYL or TEMPO derivatives have been used in the ϕ_{ENS} methods [11-19]. For example, aminomethyl-PROXYL, carboxy-PROXYL, and carbamoyl-PROXYL ($z = +1$, -1, and 0, respectively, at neutral pH) were used (Figure 2). Combination of positively and negatively charged paramagnetic probes is ideal for accurate ϕ_{ENS} measurements [19]. For strongly charged systems (e.g., nucleic acids), PRE arising from one of the charged probes may be too small for ϕ_{ENS} determination. In such a case, a pair of analogous neutral and charged probes can be used [14,15,18]. Tetramethyl nitroxide is known to interact with hydrophobic surfaces of proteins [30-32]. If the hydrophobic or other non-electrostatic interactions are the same for the two analogous cosolutes, the contributions of such interactions are canceled in the ratio $\Gamma_{2a} / \Gamma_{2b}$ [11,13]. Consequently, $\Gamma_{2a} / \Gamma_{2b}$ in Eq. 1 illuminates electrostatic components.

Prediction of ϕ_{ENS} potentials from 3D structure

A convenient feature of ϕ_{ENS} potential data is that they can be predicted from 3D structures. Owing to this feature, ϕ_{ENS} data are useful for evaluation of theoretical models and methods (see Figure 1C for example). A simple way to predict ϕ_{ENS} potential is to use the following equation together with electrostatic potentials ϕ_i at individual grid points around the molecule [11,12]:

$$\phi_{ENS}^{PB} = -\frac{k_B T}{2e} \ln\left(\sum_i \rho_i r_i^{-6} \exp[-\frac{e\phi_i}{k_B T}] / \sum_i \rho_i r_i^{-6} \exp[\frac{e\phi_i}{k_B T}]\right), \quad [2]$$

in which r_i is the distance from the ^{1}H nucleus to the grid point i; and ρ_i is a factor that represents the accessibility of the grid point i and is either 1 (accessible) or 0 (inaccessible). Predictions with Eq. 2 typically yield the root mean squared difference of ~5 mV compared with the experimental ϕ_{ENS} potentials [11,12,14].

Eq. 2 assumes the same spatial distribution for the charge and the unpaired electron. This assumption may be simplistic because the charged moiety and the unpaired electron of the

PROXYL derivatives are on the opposite sides of the molecules. Angular correlations for the paramagnetic probes with respective to the biomolecule are not taken into account in Eq. 2 either. Chen et al. demonstrated that ϕ_{ENS} potentials can be predicted more accurately by using atomistic models of the paramagnetic cosolutes along with appropriate treatment of the angular correlations [13], which is computationally more expensive.

Applications to intrinsically disordered proteins

Because it does not require structural information, the ϕ_{ENS} method is particularly useful for electrostatic investigations of IDPs. Liquid-liquid phase separation (LLPS) play various regulatory roles in biology [33]. Recently, Kay and coworkers applied the ϕ_{ENS} method to map per-residue surface electrostatic potential of the CAPRIN1 protein along its trajectory of LLPS [16,17] (Figure 3). In the absence of ATP, where this IDP was not phase-separated, ϕ_{ENS} potentials around the Arg-rich regions were positive. However, as ATP was added and the protein transitioned into phase separation, ϕ_{ENS} potentials of the region decreased and became close to zero [16]. The decrease in ϕ_{ENS} potentials reflect electrostatic interactions between the positively charged Arg-rich region and the negatively charged ATP molecules. When the ATP concentration was as high as 90 mM, the ϕ_{ENS} potentials became even negative, leading to re-entrance into a mixed state. The studies on CAPRIN1 demonstrated the effectiveness of the ϕ_{ENS} method for investigations of LLPS. Electrostatics are crucial for LLPS of some proteins. For example, co-partitioning of transcription regulators through LLPS is governed by patterned charge blocks in IDRs [34]. We anticipate that the ϕ_{ENS} method will reveal more about the electrostatic aspects of LLPS.

Clore and coworkers applied the ϕ_{ENS} method to both the folded and unfolded states of the drkN SH3 domain [15]. The variation of ϕ_{ENS} potentials among different residues was smaller in the unfolded state, presumably due to averaging of various structures. Using snapshots of a replica-exchange molecular dynamics (MD) trajectory on the unfolded state of this protein [35], ϕ_{ENS} potentials were predicted and found to agree well with experimental ϕ_{ENS} potentials. This seems to support the validity of the MD ensemble for the unfolded state. In conjunction with other experimental data (e.g., NMR chemical shifts, residual dipolar couplings), ϕ_{ENS} potential data may facilitate experimental assessment of structural ensembles of IDPs/IDRs.

Advantages over other methods for electrostatic potentials

Electron-electron double resonance (ELDOR) can also provide near-surface electrostatic potentials using an extrinsic probe attached to a biomolecule in the presence of charged or neutral paramagnetic cosolutes [36]. Cysteine modification kinetics or diffusion-enhanced fluorescence energy transfer data have also been used to estimate electrostatic potentials [37,38]. However, these methods provide only one electrostatic potential for each sample, requiring preparation of multiple samples to measure electrostatic potentials at multiple sites. In contrast, the ϕ_{ENS} method provides electrostatic potentials at many sites simultaneously. For example, ϕ_{ENS} potentials were measured for > 150 different sites for

ubiquitin, a 76-residue protein [11,12]. Furthermore, the ϕ_{ENS} method does not require any chemical modification of biomolecules.

In principle, cryogenic electron microscopy (cryo-EM) can directly provide electrostatic potential relevant to atomic scattering factor [39,40]. However, cryo-EM electrostatic potential maps do not provide quantitative information of electrostatic potentials in the exterior space around the biomolecules. Radiation damage also makes it practically difficult to quantitatively analyze cryo-EM electrostatic potential maps [39]. In contrast, the ϕ_{ENS} method provides quantitative information of electrostatic potentials in exterior space around the biomolecules in solutions under physiological conditions. Thus, the ϕ_{ENS} method is currently the most powerful experimental method for quantitative investigations of electrostatic potentials.

Comparison with vibrational spectroscopy methods for electric fields

Vibrational Stark effect (VSE) spectroscopy has been used to investigate external electric fields that perturb the energies for vibrational transitions of covalent bonds [41]. Since covalent bonds that exhibit unique infrared (IR) signals are desirable for VSE spectroscopy, nitrile C≡N or aldehyde/ketone C=O bonds, which are absent in natural proteins, are often introduced through chemical modifications, amber suppression, or ligand binding. Site-specific ^{13}C-labeling and ^{12}C — ^{13}C difference spectra were also used to analyze the VSE for specific sites [42,43]. For aldehyde groups, electric field orientations can be extracted using two directional vibrational probes by exploiting the VSE of C=O and C-D bonds [44]. IR signal intensities of nitrile C≡N bonds were also found to be useful for analyzing electric fields [45]. Using VSE spectroscopy along with enzyme kinetics for wild-type and mutant enzymes, Boxer and coworkers demonstrated the role of electrostatics in enzymatic catalysis [43,46,47].

Information from VSE data differs from that from ϕ_{ENS} data. VSE data provide electric fields at the observed bonds inside a molecule of interest, whereas ϕ_{ENS} data provide average electrostatic potentials in a local exterior space close to the observed ^{1}H nuclei. The electric field $\boldsymbol{F}$ is the gradient $(\partial/\partial x, \partial/\partial y, \partial/\partial z)$ of the electrostatic potential ϕ multiplied by −1. With a charge valence z, the electric field gives the electric force $ze\boldsymbol{F}$, whereas the electrostatic potential gives the electrostatic energy $ze\phi$. The VSE and ϕ_{ENS} methods are complementary regarding biomolecular electrostatics.

Conclusions

Recent methodological advances have enabled direct electrostatic measurements for many sites on biological macromolecules through experiments. Direct measurements of electrostatic potentials facilitate investigations of inter- or intra-molecular electrostatic interactions, particularly for those involving IDPs/IDRs. Electrostatic interactions involving IDRs impact thermodynamic and kinetic properties of some proteins [48-52]. Direct measurements of electrostatics may greatly facilitate quantitative investigations of such electrostatic interactions. The emerging experimental tools for electrostatics can be applied to a wide variety of biomolecular processes such as electrostatic steering, post-translational

modifications, and co-partitioning in LLPS. Experimental measurements of electrostatic potentials also allow for examination of theoretical electrostatic methods.

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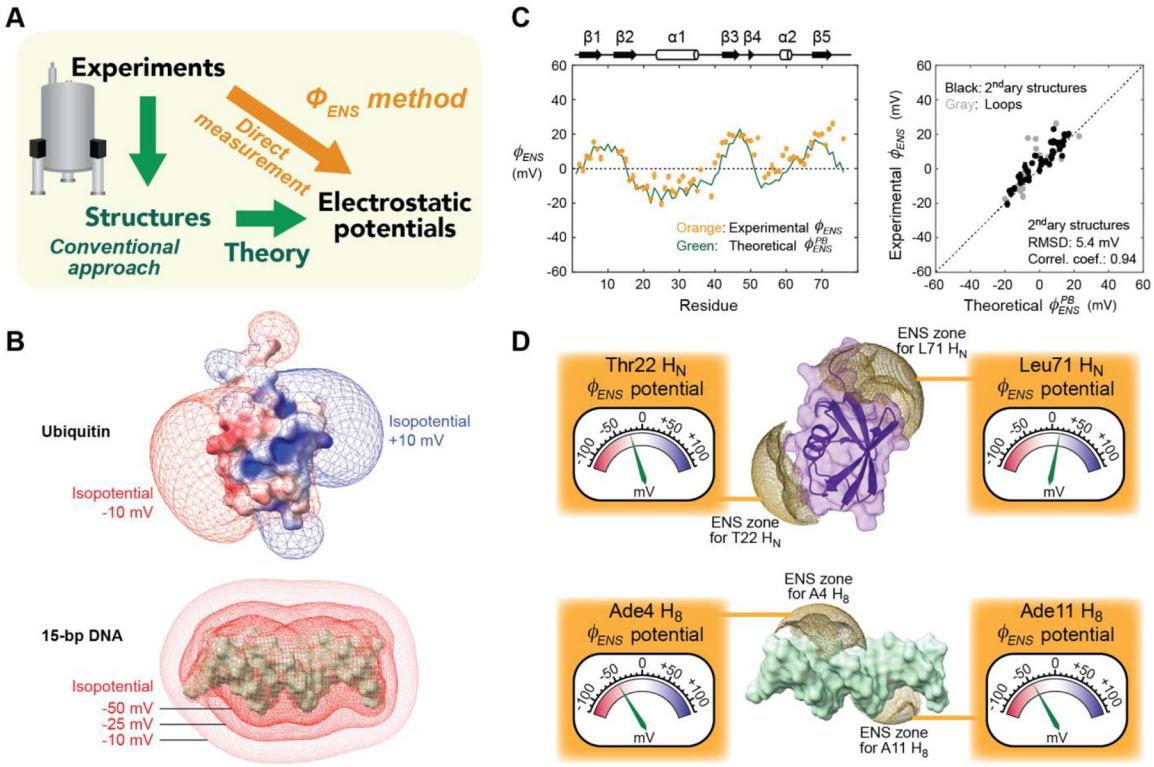

Figure 1.

Direct measurement of electrostatic potentials by the ϕ_{ENS} method. (**A**) Conventional (green) vs. new (orange) approaches to analyze electrostatic potentials around biomolecules. (**B**) Electrostatic potentials around ubiquitin and 15-bp DNA computed with the Adaptive Poisson-Boltzmann Solver (APBS) software. (**C**) ϕ_{ENS} potentials measured for $^{1}H_{N}$ nuclei of ubiquitin. The experimental data are compared with predictions using Eq. 2. Adapted from Ref. [11]. (**D**) Physical meanings of ϕ_{ENS} potentials. Each ϕ_{ENS} potential represents an average electrostatic potential within the effective near-surface (ENS) zone (brown) for the observed ^{1}H nucleus. Some examples for ubiquitin and 15-bp DNA are shown. The structures were drawn with ChimeraX [53]. [Double-column figure]

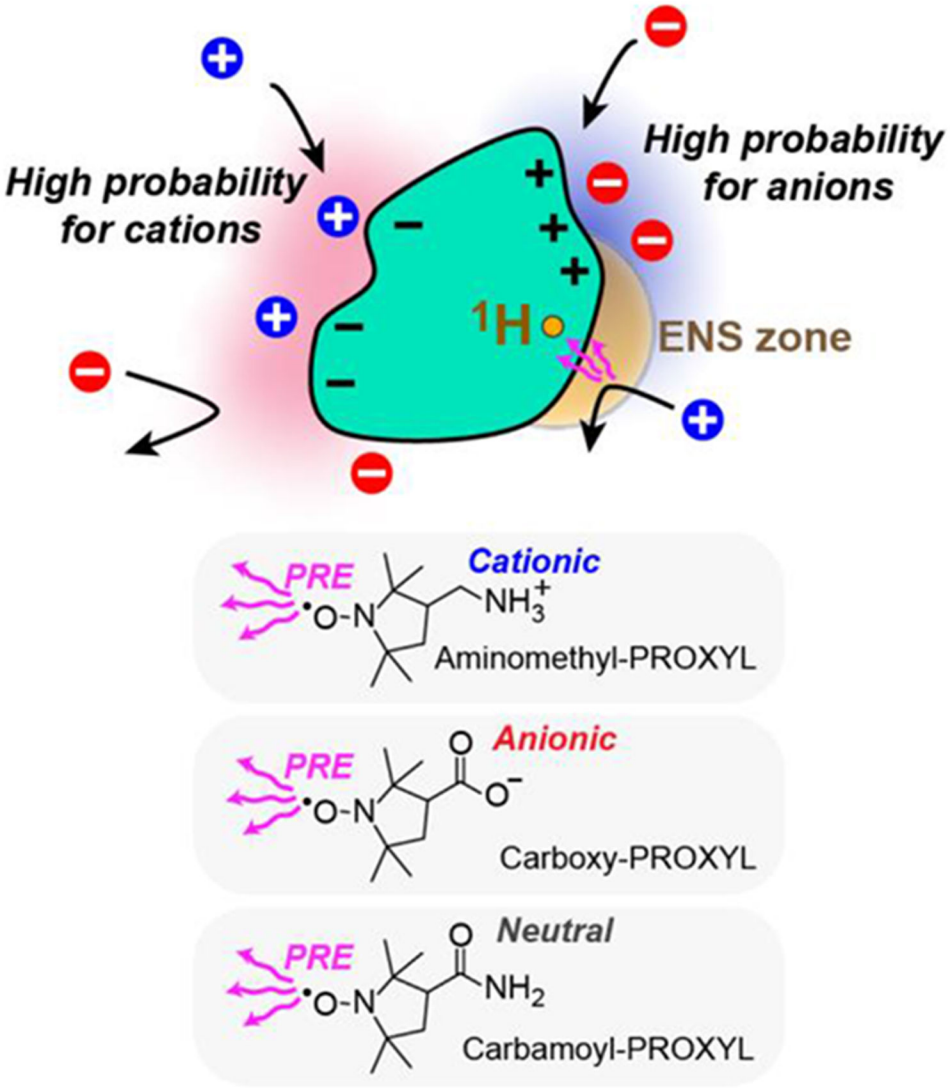

Figure 2.

Examples of paramagnetic cosolutes for the ϕ_{ENS} method. It is crucial that electrostatic interactions between the biomolecule and the cosolutes governs differences in the spatial distributions of the cosolutes around the biomolecule. Hydrophobic interactions at the tetramethyl nitroxide moiety common to the cosolutes does not affect the ϕ_{ENS} method [11,13]. [Single-column figure]

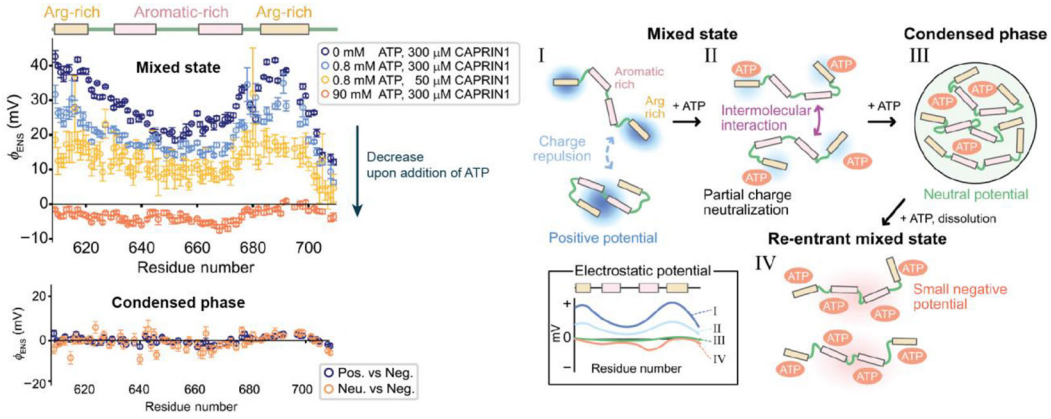

Figure 3.

Mapping of ϕ_{ENS} potentials along the adenosine triphosphate (ATP)-induced phase-separation trajectory for CAPRIN1 [16]. As ATP was added, the ϕ_{ENS} potentials around CAPRIN1 progressively decreased. Upon phase separation, the ϕ_{ENS} potentials became close to zero, with ~5 ATP molecules associated with each CAPRIN1 chain. Increasing the ATP concentration further inverted the ϕ_{ENS} potentials, leading to re-entrance into a mixed phase. Adapted from Ref. 16. [Double-column figure]

Table I.

Biophysical applications of the ϕ_{ENS} method.

Applications	References
• Examination of theoretical electrostatic models	11-15
• Electrostatic aspects of molecular binding	11, 16, 17
• Impact of salt on biomolecular electrostatics	11, 13, 17
• Role of electrostatics in LLPS	16, 17
• Electrostatics of disordered proteins	15-19
• Electrostatics of nucleic acids	14
• Structural ensemble	11,15