

## ELECTRONIC SUPPLEMENTARY INFORMATION

### Myoglobin Molecule Charging in Electrolyte Solutions

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## 1. Formulation of the Poisson-Boltzmann equation

The Poisson equation governing the electric potential distribution where there appears a space charge has the following form [1]

$$\nabla \cdot (\varepsilon \nabla \psi) = -\rho_e \quad (\text{S1})$$

where  $\varepsilon$  is the electric permittivity,  $\rho_e$  electric charge density, and  $\psi$  is the electric potential.

The net charge density consists of the space (immobile) charge density  $\rho_{ef}$  due to the dissociation of ionogenic groups and the mobile charge density  $\rho_{em}$  due to the presence of ions and reacting to the local value of the electric potential

$$\rho_e = \rho_{ef} + \rho_{em} \quad (\text{S2})$$

Within the framework of the Poisson-Boltzmann approach it is assumed that the mobile (ionogenic) charge is given by

$$\rho_{em} = e \sum_i z_i n_i^b e^{-z_i e \psi / kT} \quad (\text{S3})$$

where  $e$  is the elementary (proton) charge,  $z_i$  is the valence of the ion,  $n_i^b$  is the bulk concentration and of the ion,  $k$  is the Boltzmann constant and  $T$  is the absolute temperature.

One should mention that in Eq.(S3) the specific ion interaction with interfaces and other ions, as well the steric effects due to finite ion sizes are neglected.

Substituting Eq.(S3) into Eq. (S1), one obtains the general form of the PB equation

$$\nabla(\varepsilon \nabla \psi) = -e \sum_i z_i n_i^b e^{-z_i e \psi / kT} - \rho_{ef} \quad (\text{S4})$$

It is useful to consider some limiting forms of the above PB equation. For example, in the case of a radially symmetric fixed charge distribution and no mobile charge, Eq.(S4) expressed in the spherical coordinate system assumes the form

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \varepsilon \frac{d\psi}{dr} \right) = -\rho_{ef}(r) \quad (\text{S5})$$

where  $r$  is the radial coordinate.

One should mention that Eq.(S5) is valid for an arbitrary magnitude of the charge, and in consequence for arbitrary electric potential value.

On the other hand, for a radially symmetric fixed charge distribution, 1:1 electrolyte (e.g., NaCl) and a position independent electric permittivity Eq.(S4) assumes the form

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi}{dr} \right) = (kT/e) \kappa^2 \sinh \left( \frac{e\psi}{kT} \right) - \rho_{ef}(r) / \varepsilon \quad (\text{S6})$$

where

$$\kappa^{-1} = \left( \frac{\varepsilon kT}{2e^2 I} \right)^{1/2} \quad (\text{S7})$$

is the Debye screening length,  $I = z^2 n_b$  is the ionic strength of the electrolyte solution and  $n_b$  is the bulk concentration of the electrolyte.

Another frequently used form of the PB equation can be derived by applying the linearization procedure, which is justified if the maximum term  $|e\psi/kT|$  does not considerably exceed unity. In this case one obtains the linear form of the PB equation

$$\nabla^2 \psi = \kappa^2 \psi - \rho_{ef}(r) / \varepsilon \quad (\text{S8})$$

It is useful to derive some limiting analytical solutions using the above forms of the PB equation. Accordingly, integration of Eq.(yields the following expression for the electric potential distribution inside the sphere

$$\psi_s = \psi_r - \int \frac{dr}{\varepsilon(r)r^2} \int \rho_{ef}(r)r^2 dr = \psi_r - \int \frac{q(r)}{\varepsilon(r)} dr \quad (\text{S9})$$

$$\text{where } q(r) = \frac{1}{r^2} \int \rho_{ef}(r)r^2 dr \quad (\text{S10})$$

and  $\psi_r$  is the reference potential.

For a position independent electric permittivity and a uniform charge distribution Eq.(S9) simplifies to

$$\psi_s = \psi_r - \frac{\rho_{ef} r^2}{6\varepsilon_s} = \psi_r - \frac{Q r^2}{8\pi\varepsilon_s a^3} \quad (\text{S11})$$

where

$$Q = \frac{4}{3} \pi a^3 \rho_{ef} \quad (\text{S12})$$

is the net charge within the sphere and  $\epsilon_s$  is the constant electric permittivity of the sphere.

Thus, the uniform potential at the sphere surface is given by

$$\psi_{s0} = \psi_r - \frac{Q}{8\pi\epsilon_s a} \quad (\text{S13})$$

Assuming that outside the sphere there are no charges, the potential vanishes according to the formula

$$\psi = \psi_{s0} \frac{a}{r} \quad (\text{S14})$$

Using the boundary condition  $\epsilon_s \left( \frac{d\psi_s}{dr} \right) = \epsilon \left( \frac{d\psi}{dr} \right)$  at  $r = a$  one obtains

$$\psi_r = \frac{Q}{4\pi\epsilon a} + \frac{Q}{8\pi\epsilon_s a} = \left(1 + \frac{\epsilon}{2\epsilon_s}\right) \psi_0 \quad (\text{S15})$$

where

$$\psi_0 = \frac{Q}{4\pi\epsilon a} \quad (\text{S16})$$

Thus, the electric potential distribution within the sphere is explicitly given by

$$\psi_s = \left(1 + \frac{\epsilon}{2\epsilon_s}\right) \psi_0 - \frac{\epsilon r^2}{2\epsilon_s a^2} \psi_0 \quad (\text{S17})$$

The average potential in the sphere is given by

$$\langle \psi_s \rangle = \frac{1}{v_s} \int_0^a 4\pi r^2 \psi(r) dr = \psi_0 + \frac{\epsilon}{5\epsilon_s} \psi_0 \quad (\text{S18})$$

where  $v_s = \frac{4}{3} \pi a^3$  is the sphere volume.

It is interesting to calculate the potential for a sphere of the size corresponding to the myoglobin molecule. Hence assuming  $a = 2$  nm,  $Q = 1 e$  and the dielectric permittivity of the pure water at 298 K, i.e.,  $78.6 \times 8.85 \times 10^{-12}$  C (V m)<sup>-1</sup> one obtains  $\psi_0 = 9.18$  mV. Accordingly, for  $Q=24 e$  (this corresponds to the nominal PROPKA charge at pH 3.5) one has  $\psi_0 = 220$  mV at the sphere surface, whereas the average potential is equal to 260 mV. This potential would produce an enormous electric field at the sphere surface equal to  $1.4 \times 10^8$  V m<sup>-1</sup>. Therefore, one can deduce that such a strong field would exert considerable electric forces on free ions present inside the sphere (mainly protons or OH<sup>-</sup> groups) and on the ions present in the

electrolyte solution. This result in the formation of an electric double-layer and an significant compensation of the primary surface charge of the sphere.

Moreover, the electric potential in the sphere should affect the local pH value shifting it toward larger values for positively charged spheres and toward smaller values in the opposite case of negatively charged spheres. These effects are quantitatively analyzed in the following section.

## 2. Calculating the Electric Potential Distribution within and around the Myoglobin Molecule

### 2.1 Linear Models

We first consider a uniformly charged sphere immersed in an electrolyte solution where the mobile ions cannot penetrate into its interior. The non-linear PB equation describing the electric potential distribution within the sphere is as follows (see Fig.S1)

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi_s}{dr} \right) = -\rho_{ef}(r) / \varepsilon \quad \text{for } a < r \leq a \quad (\text{S19})$$

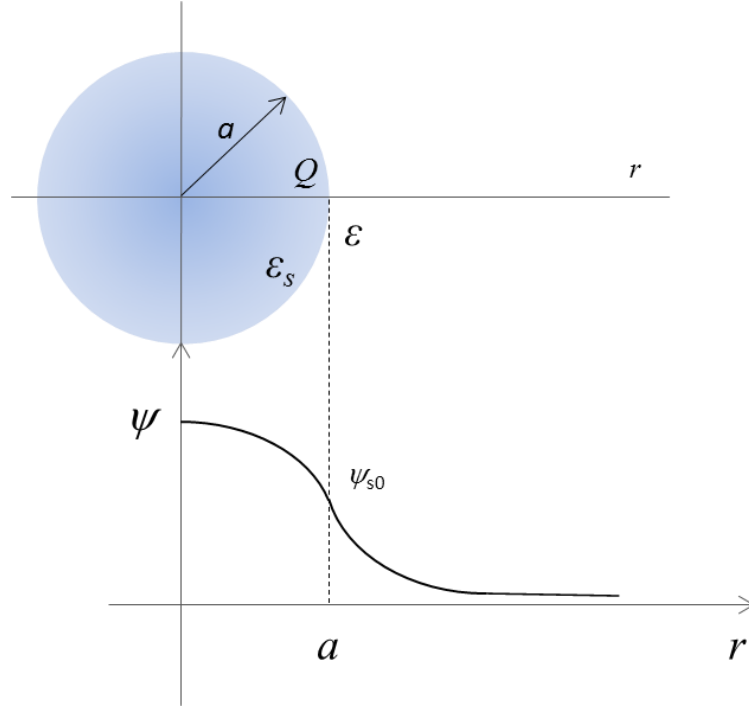
On the other hand, the electric potential distribution within the electrolyte is described by the linear form of the PB equation

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi}{dr} \right) = \kappa^2 \psi \quad \text{for } a < r < \infty \quad (\text{S20})$$

with the following boundary conditions

$$\varepsilon_s \left( \frac{d\psi_s}{dr} \right) = \varepsilon \left( \frac{d\psi}{dr} \right) \quad \text{at } r = a \quad (\text{S21})$$

where  $\varepsilon$  is the electric permittivity of the electrolyte.



**Fig. S1.** Potential distribution within and outside a charged sphere in electrolyte solution – no ion penetration into the sphere.

Assuming a uniform charge density within the sphere the potential distributions inside and outside (within the double-layer) region are (se Fig. S1)

$$\psi_s(r) = \psi_r - \frac{1}{2} \psi_0 \frac{\varepsilon}{\varepsilon_s} \frac{r^2}{a^2} \quad (\text{S22})$$

$$\psi = \psi_{s0} \frac{a}{r} e^{-\kappa(r-a)}$$

Using the above boundary conditions, Eq.(S21), one can derive the following formula for  $\psi_{s0}$

$$\psi_{s0} = \frac{1}{(1+\kappa a)} \psi_0 = \frac{Q}{4\pi\varepsilon a(1+\kappa a)} \quad (\text{S23})$$

Accordingly, the reference potential at the sphere center is

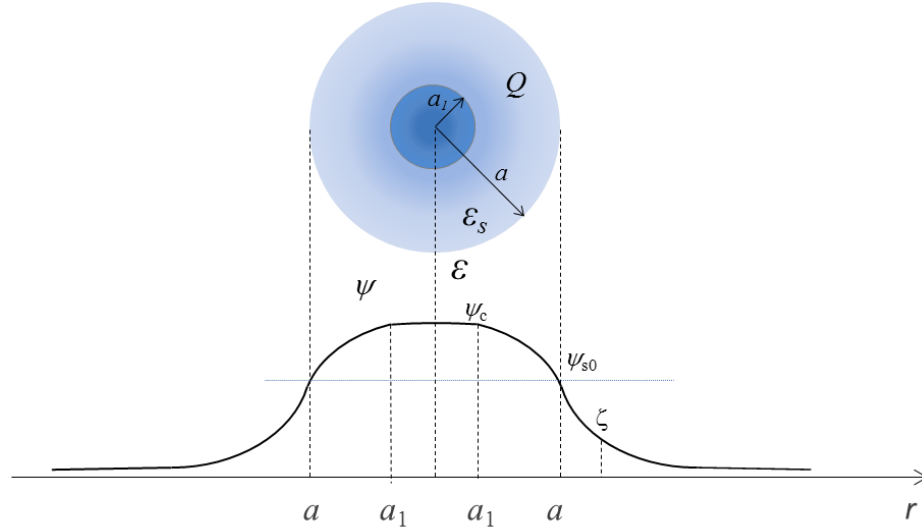
$$\psi_r = \psi_{s0} + \frac{1}{2} \frac{\varepsilon}{\varepsilon_s} \psi_0 \quad (\text{S24})$$

One can infer from Eq.(S22) observe that the  $\psi_{s0}$  potential exponentially vanishes with the distance from the sphere surface, proportionally to  $(\kappa a)^{-1}$

Analogously, the average potential in the sphere is given by

$$\langle \psi_s \rangle = \psi_r - \frac{1}{2} \frac{\varepsilon}{\varepsilon_s} \psi_0 \frac{4\pi}{v_s a^2} \int_0^a r^4 dr = \psi_{s0} + \frac{1}{5} \frac{\varepsilon}{\varepsilon_s} \psi_0 \quad (\text{S25})$$

We also consider a more general case where the penetration of ions into the sphere becomes possible. In this model it is also assumed that there is an uncharged core part within the sphere having the radius equal to  $a_1$ , (see Fig. S2), impenetrable to the electrolyte ions. On the other hand, the region within the sphere  $a_1 < r < a$  is accessible for ions and it bears a uniformly distributed electric charge of the net magnitude equal to  $Q$ .



**Fig. S2.** Schematic representation of the electric potential distribution within and outside an ion-penetrable sphere with the impenetrable core part of the radius  $a_1$ .

In consequence, the PB equation assumes the following form within the ion penetrable region

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi_s}{dr} \right) = \kappa_s^2 \psi - \rho_{ef} / \varepsilon_s \quad (\text{S26})$$

where  $\kappa_s$  is the reciprocal double-layer parameter for  $a_1 < r \leq a$ .

The PB equation in the electrolyte has the form

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \frac{d\psi}{dr} \right) = \kappa^2 \psi \quad (\text{S27})$$

The boundary conditions for Eq. (S26) are

$$\begin{aligned} \frac{d\psi_s}{dr} &= 0 & \text{at } r &= a_1 \\ \psi_s &= \psi_{s0} & \text{at } r &= a \end{aligned} \quad (\text{S28})$$

On the other hand, the general boundary condition for the PB equation in the bulk, Eq.(S27) is as follows

$$\frac{d\psi}{dr} = 0 \quad \text{at } r = R_b \text{ (stagnant solutions)} \quad (\text{S29})$$

where,  $R_b$  is the external boundary radius.

Eq.(S29) is applicable for the crowding environment conditions, i.e., for concentrated protein suspensions. For infinitely diluted suspension where  $R_b \gg a$ , it simplifies to the usual form

$$\psi = 0 \quad \text{at } r \rightarrow \infty \quad (\text{S30})$$

The PB inside the molecule and in the electrolyte are coupled by the boundary condition

$$\varepsilon_s \left( \frac{d\psi_s}{dr} \right) = \varepsilon \left( \frac{d\psi}{dr} \right) \quad \text{at } r = a \quad (\text{S31})$$

The electric potential distribution within the sphere, which fulfills the first boundary condition given by Eq.(S28) has the form

$$\psi_s(r) = \psi_r - \frac{a}{r} \left[ C_1 \sinh \kappa_s (r - a_1) + C_2 \cosh \kappa_s (r - a_1) \right] \quad (\text{S32})$$

where  $C_1$  and  $C_2$  are the constants of integration given by

$$C_1 = \frac{\psi_r - \psi_{s0}}{\sinh \kappa_s (a - a_1) + \cosh \kappa_s (a - a_1)} \quad (\text{S33})$$

$$C_2 = \kappa_s a_1 C_1 \quad (\text{S34})$$

and

$$\psi_r = \frac{\rho_{ef}}{\varepsilon_s \kappa_s^2} = \frac{3 \varepsilon}{\varepsilon_s (\kappa_s a)^2 (1 - (a_1/a)^3)} \psi_0 \quad (\text{S35})$$



On the other hand, the potential distribution in the electrolyte for  $R_b/a \gg 1$  is given by

$$\psi(r) = \psi_{s0} \frac{a}{r} e^{-\kappa(r-a)} \quad (\text{S40})$$

Exploiting the third boundary condition, Eq.(S31), one obtains the explicit formula for the surface potential

$$\psi_{s0} = \psi_r f_2(\kappa a, \kappa_s a, a_1/a, \varepsilon/\varepsilon_s) \quad (\text{S36})$$

where the  $f_2$  function is given by

$$f_2 = \psi_r \frac{f_3}{f_3 + \frac{\varepsilon}{\varepsilon_s} (1 + \kappa a) [\sinh \kappa_s (a - a_1) + \kappa_s a \cosh \kappa_s (a - a_1)]} \quad (\text{S37})$$

where

$$f_3 = \kappa_s (a - a_1) \cosh \kappa_s (a - a_1) + (\kappa_s a_1 \kappa_s a - 1) \sinh \kappa_s (a - a_1) \quad (\text{S38})$$

The potential distribution within the sphere is given by

$$\psi_s(r) = \psi_r - (\psi_r - \psi_{s0}) \frac{a}{r} \frac{\sinh \kappa_s (r - a_1) + \kappa_s a_1 \cosh \kappa_s (r - a_1)}{\sinh \kappa_s (a - a_1) + \kappa_s a_1 \cosh \kappa_s (a - a_1)} \quad (\text{S39})$$

The average potential in the ion-penetration region can be calculated as

$$\langle \psi_s \rangle = \psi_r - \frac{4\pi a}{v_s} \int_{a_1}^a r (C_1 \sinh \kappa_s r + C_2 \cosh \kappa_s r) dr = f_a \psi_0 \quad (\text{S41})$$

where  $v_s = \frac{4}{3} \pi (a^3 - a_1^3)$  and the constant  $f_a$  is given by

$$f_a = \psi_r - I_1 - I_2 \quad (\text{S42})$$

$$I_1 = \frac{4\pi a^3}{v_s \kappa_s a} C_1 \left[ \cosh \kappa_s a - (a_1/a) \cosh \kappa_s a_1 - (1/\kappa_s a) \sinh \kappa_s a + (1/\kappa_s a) \sinh \kappa_s a_1 \right] \quad (\text{S43})$$

$$I_2 = \frac{4\pi a^3}{v_s \kappa_s a} C_2 \left[ \sinh \kappa_s a - (a_1/a) \sinh \kappa_s a_1 - (1/\kappa_s a) \cosh \kappa_s a + (1/\kappa_s a) \cosh \kappa_s a_1 \right] \quad (\text{S44})$$

The average potential within the molecule is needed for evaluating the pH shift according to the method described later on.

## 2.2 The Non-Linear Model

For the non-linear models, the general PB equation formulated in spherical coordinates was applied

$$\frac{1}{r^2} \frac{d}{dr} \left( r^2 \varepsilon(r) \frac{d\psi}{dr} \right) = -e \sum_i z_i n_i^b e^{-z_i e \psi / kT} - \rho_{ef}(r) \quad (\text{S45})$$

To facilitate its numerical solution this equation was transformed to a set of two non-linear ordinary differential equations

$$\begin{aligned} \frac{d\psi}{dr} &= -E \\ \frac{dE}{dr} &= \left[ \left( -e \sum_i z_i n_i^b e^{-z_i e \psi / kT} - \rho_{ef}(r) \right) / \varepsilon(r) - \frac{2}{r} E - \frac{1}{\varepsilon(r)} \frac{d\varepsilon(r)}{dr} E \right] \end{aligned} \quad (\text{S46})$$

where  $E$  is the electric field.

Eq.(S46) was expressed in the dimensionless form using the following scaling

$$\begin{aligned} \bar{u} &= \bar{\psi} = \psi / \psi_{ch} \\ \bar{w} &= \bar{E} = E / (\psi_{ch} \kappa) \\ \bar{r} &= r / a \\ \psi_{ch} &= kT / e \end{aligned} \quad (\text{S47})$$

where  $\bar{\psi}$  is the scaled (dimensionless) potential and  $\bar{E}$  is the scaled electric field.

In this way, Eq.(S46) is transformed to the following equation system

$$\begin{aligned} \frac{d\bar{u}}{d\bar{r}} &= -\kappa a \bar{w} \\ \frac{d\bar{w}}{d\bar{r}} &= \bar{\rho}_f(\bar{r}) / \varepsilon(\bar{r}) - \frac{e^2 a}{\varepsilon k T \kappa a} \sum_i z_i n_i^b e^{-\bar{u}} - \frac{2}{\bar{r}} \bar{w} - \frac{1}{\varepsilon} \frac{d\varepsilon}{d\bar{r}} \bar{w} \end{aligned} \quad (\text{S48})$$

where

$$\bar{\rho}_f(\bar{r}) = \frac{e a^2}{\varepsilon k T \kappa a} \rho_{ef}(r) = \frac{a^2}{\varepsilon \psi_{ch} \kappa a} \rho_{ef}(r) \quad (\text{S49})$$

is the dimensionless fixed charge density

One should mention that Eq.(S49) is valid for an arbitrary magnitude of the charge, and in consequence for arbitrary electric potential value.

For a symmetric electrolyte where  $z_1 = -z_a = z$ , Eq.(S48) assumes the form

$$\frac{du}{dr} = -\kappa a w$$

$$\frac{dw}{dr} = \bar{\rho}_f(\bar{r}) / \varepsilon - \kappa a \sinh(z\bar{\psi}) - \frac{2}{r} w - \frac{1}{\varepsilon} \frac{d\varepsilon}{dr} w \quad (\text{S50})$$

For a uniform charge density, one has

$$\bar{\rho}_f(\bar{r}) = \frac{3 Q}{4\pi\varepsilon a \psi_{ch} \kappa a} \left[ 1 - (a_1/a)^3 \right] \quad (\text{S51})$$

The boundary condition at the surface of the core region within the sphere (see Fig. S2) can be formulated postulating the vanishing of the electric field  $\bar{E}$

$$w = \bar{E} = 0 \quad \text{at} \quad \bar{r} = a_1/a \quad (\text{S52})$$

However, the electric potential at the core is not known and should be determined from the boundary condition at the external cell given by Eq.(S29) expressed in the dimensionless form

$$\frac{du}{dr} = 0 \quad \text{at} \quad \bar{r} = R_b/a \quad (\text{S53})$$

Accordingly, the calculation algorithm was the following. Initially, the equation system given by Eq.(S50) was numerically solved by a precise fourth order Runge-Kutta method using the boundary condition Eq.(S53) and a guessed potential at the core  $\bar{\psi}_c$ .

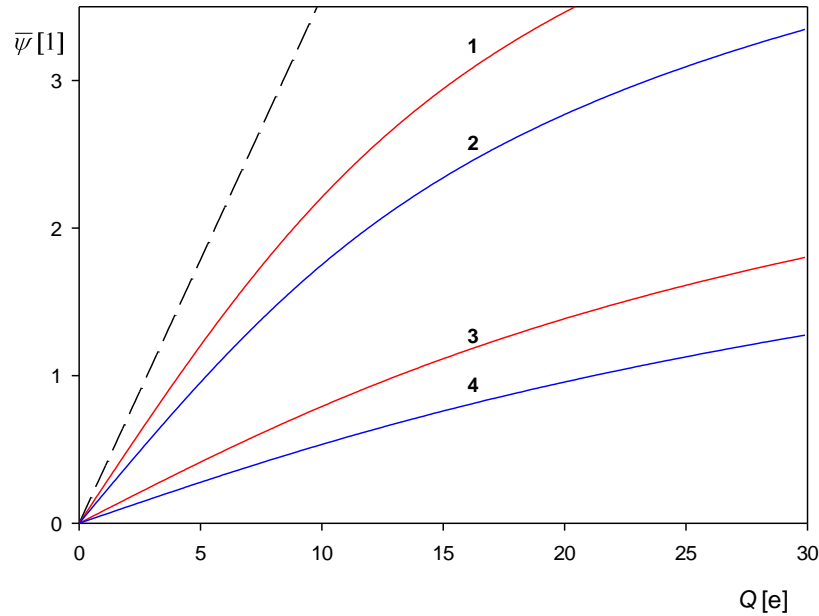
Because of a large instability of the equation system, Eq.(S50) especially for  $\kappa a > 1$  the calculations were started from low fixed charge using the guessed potential value derived from the linear model, given by Eq.(S39). An efficient iteration scheme was applied in order to derive the core potential with a relative precision of  $10^{-6}$ . Using this value, the potential at the sphere surface  $\bar{\psi}_{s_0}$ , i.e., at  $r = a$  was calculated by solving once again the initial equation system, Eq.(S50) within the domain  $a_1/a < r/a < 1$ . The potential obtained for the initial charge is applied as a useful guessed value for larger charges systematically increased by a fixed increment.

Primary, in these calculations the dependencies of the surface and the average potential on the total fixed charge were obtained for a set of the dimensionless parameters, i.e.,

$$\bar{\psi}_{s_0} = f_2(Q, \kappa a, \kappa_s a, a_1/a, R_b/a, \varepsilon/\varepsilon_s) \quad (\text{S54})$$

where  $\bar{\psi}_{s_0} = \psi_{s_0} / \psi_{ch}$  is the normalized surface potential at the sphere.

Using this algorithm the dependencies of the normalized surface and the average potentials on the charge are calculated. They are shown in Fig. 3 for the set of parameters corresponding the myoglobin molecule of the diameter 4 nm immersed in a 0.15 and 0.01 M, 1:1 electrolyte solution with the core size of 2 nm.



**Fig. S3.** The dependence on the dimensionless potentials on the charge  $Q$  calculated for the following set parameters:  $a_1/a = 0.5$ ,  $R_b/a = \infty$ ,  $\varepsilon/\varepsilon_s = 1$

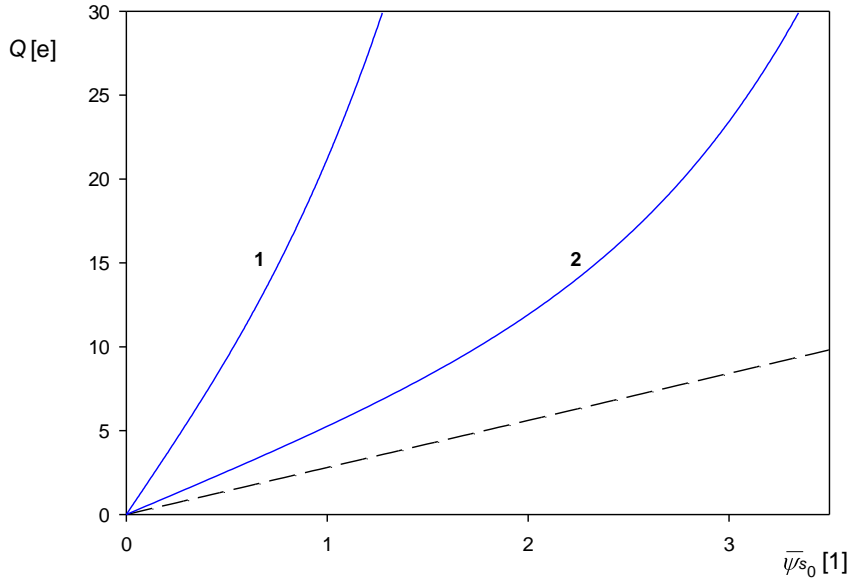
1. the average potential, ionic strength of 0.01 M,  $\kappa a = \kappa_s a = 2.54$
2. the surface potential, ionic strength of 0.01 M,  $\kappa a = \kappa_s a = 2.54$
3. the average potential, ionic strength of 0.15 M,  $\kappa a = \kappa_s a = 0.657$
4. the surface potential, ionic strength of 0.15 M,  $\kappa a = \kappa_s a = 0.657$

The dashed line shows the surface potential acquired in the case of no double-layer.

Subsequently, the non-linear dependencies expressed by Eq.(S54) were numerically inverted in order to obtain the following dependencies of the charge on the average potential  $\langle \psi_s \rangle$ , for the set of the above defined dimensionless parameters, i.e.,

$$Q = f_2^{-1}(\langle \psi_s \rangle, \kappa a, \kappa_s a, a_1/a, R_b/a, \varepsilon/\varepsilon_s) \quad (\text{S55})$$

It is interesting to observe that the derivative of the  $f_2^{-1}$  function in respect to the potential represents the normalized electric capacity of the particle (molecule).



**Fig. S4.** The dependence of fixed charge  $Q$  on the average potential inside obtained for the same set parameters as for Fig. S3.

1. ionic strength of 0.15 M
2. ionic strength of 0.01 M

The dashed line shows the dependence of the nominal charge on the surface potential in the case of no electric double-layer.

As shown in Fig. S4, the slope of the  $Q_0$  vs.  $\langle \bar{\psi}_s \rangle$  dependencies increases with ionic strength and becomes considerable larger than in the case of no electric double-layer (depicted by the dashed line in Fig. S4).

The  $Q$  vs.  $f_2^{-1}(\langle \bar{\psi}_s \rangle)$  functions are useful for developing an efficient scheme to calculate the pH shift within the ion-penetrable region of the molecule. This becomes feasible by observing that the local pH within the molecule is a linear function of the average potential inside, i.e.,

$$\text{pH} = \text{pH}_0 + \frac{1}{\ln(10)} \langle \bar{\psi}_s \rangle \quad (\text{S56})$$

where  $\text{pH}_0$  is the bulk pH.

Thus,

$$\langle \bar{\psi}_s \rangle = \ln(10)(\text{pH} - \text{pH}_0) = \ln(10) \Delta\text{pH} \quad (\text{S57})$$

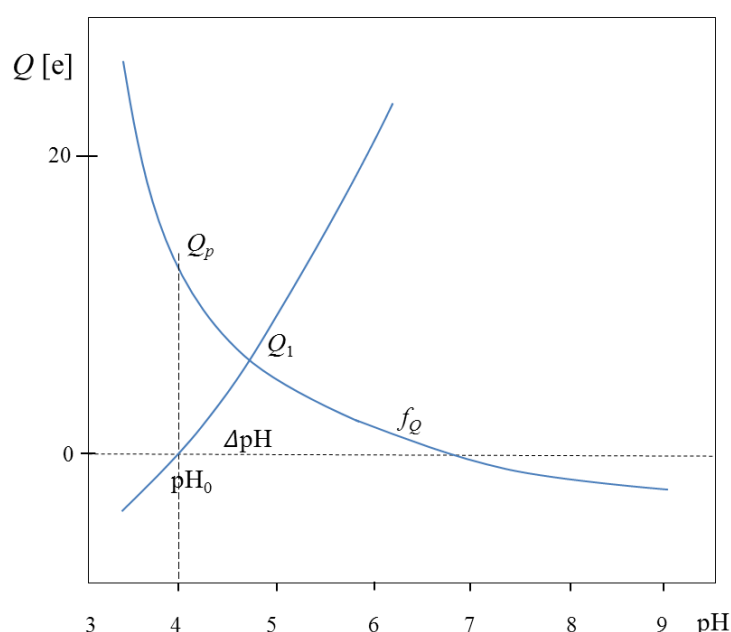
where  $\Delta\text{pH}$  is the pH shift within the molecule.

In consequence Eq.(S55) can be expressed as

$$Q = f_2^{-1}(\Delta \text{pH}) \quad (\text{S58})$$

The results shown In Fig. (S4) suggest that the pH shift should be a non-linear function of the fixed charge.

Comparing the dependence expressed by Eq.(S58) with the nominal charge  $Q_p$  derived using the existing software (for example PROPKA [3] or the H++3.0 [4]) approximated by a convenient interpolating function one can directly determine the pH shift as shown in Fig. S5. This procedure only requires a numerical solution of one nonlinear equation.



**Fig. S5.** A scheme for calculating the pH shift due to electrostatic potential (positive branch) and calculation of the effective (corrected) charge.

A simpler situation appears for the low potential range, where the dependence of the charge on pH becomes a linear function, i.e.,

$$Q = C_Q(x - x_0) \quad (\text{S59})$$

where  $x = \text{pH}$ ,

$x_0 = \text{pH}_0$  is the initial pH (see Fig. S5) and  $C_Q$  is the constant proportional to the electric capacity for the lower potential range.

In this case, one can derive useful analytical solutions describing the pH shift if the nominal charge is interpolated by a convenient polynomial function. For example, using the PROPKA modeling, the nominal charge of the myoglobin molecule can be approximated by

$$f_Q = c_2 x^2 + c_1 x + c_0 \quad (\text{S60})$$

where  $c_2 = 0.94$ ,  $c_1 = -16.2$ ,  $c_0 = 67.8$

Combining Eqs.(S59-60) one can formulate the following expression for calculating the pH shift

$$c_2 x_1^2 + (c_1 - C_Q) x_1 + c_0 + C_Q x_0 = 0 \quad (\text{S61})$$

The real root of this equation, which exists if  $(c_1 - C_Q)^2 / c_2 > 4(c_0 + C_Q x_0)$  is given by

$$x_1 = \frac{C_Q - c_1}{2 c_2} \left\{ 1 - \left[ 1 - \frac{4c_2 (c_0 + C_Q x_0)}{(c_1 - C_Q)^2} \right]^{1/2} \right\} \quad (\text{S62})$$

Therefore, the pH shift and the shifted nominal charge  $Q_1$  can be calculated as follows

$$\begin{aligned} \Delta \text{pH} &= x_1 - x_0 \\ Q_1 &= f_Q(x_1) \end{aligned} \quad (\text{S63})$$

Consequently, the normalized surface potential at  $r = a$  is given by

$$\bar{\psi}_{s0} = f_2(Q_1, \kappa a, \kappa_s a, a_1/a, R_b/a, \varepsilon/\varepsilon_s) \quad (\text{S64})$$

whereas the effective (compensated) charge within the molecule  $Q_c$  can be calculated from the dependence

$$Q_c = 4\pi\varepsilon a \psi_{ch} \bar{\psi}_{s0} \quad (\text{S65})$$

The zeta potential  $\zeta$ , defined as the electric potential in the slip plane [2], is numerically calculated for the given set of the parameters  $Q_1, \kappa a, \kappa_s a, a_1/a, R_b/a, \varepsilon/\varepsilon_s$  solving the non-linear PB equation using the known value of the surface potential  $\bar{\psi}_{s0}$  as the boundary condition at the molecule surface. It is usually assumed that the shear plane is located at the distance from the molecule surface equal to  $\delta_s$ , which is identified with the

hydrated size of the counter-ions. It is assumed that  $\delta_s$  is equal to 0.35 nm that corresponds to the hydrated radius of the Cl<sup>-</sup> ions.

On the other hand, for the linear model, one can calculate the zeta potential using Eq.(S36). For dilute systems, where  $\kappa (R_b - a) \gg 1$ , this equation simplifies to the form

$$\zeta = \psi_{ch} \bar{\psi}_{s0} \frac{a}{a + \delta_s} e^{-\kappa \delta_s} = f_2(Q_1, \kappa a, \kappa_s a, a_1 / a, \epsilon / \epsilon_s) \psi_{ch} \frac{a}{a + \delta_s} e^{-\kappa \delta_s} \quad (\text{S66})$$

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