Supporting Information

Formation of Dielectric layers and Charge Regulation in Protein Adsorption at Biomimetic Interfaces

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S1. Dielectric effect on adsorbed charge

The dielectric properties of a medium have a large impact on the apparent pK_a of acids and bases. This effect has been quantified experimentally by measuring the dissociation of acidic and basic dyes in solutions with different mixtures of water and dioxane.[1] The relation between the medium relative permittivity and change in apparent pK_a from the bulk water value is plotted in Figure S1. Functions are fitted to the plot by weighting the bulk value (at $\varepsilon = 78.36$) to force the plot through this point.

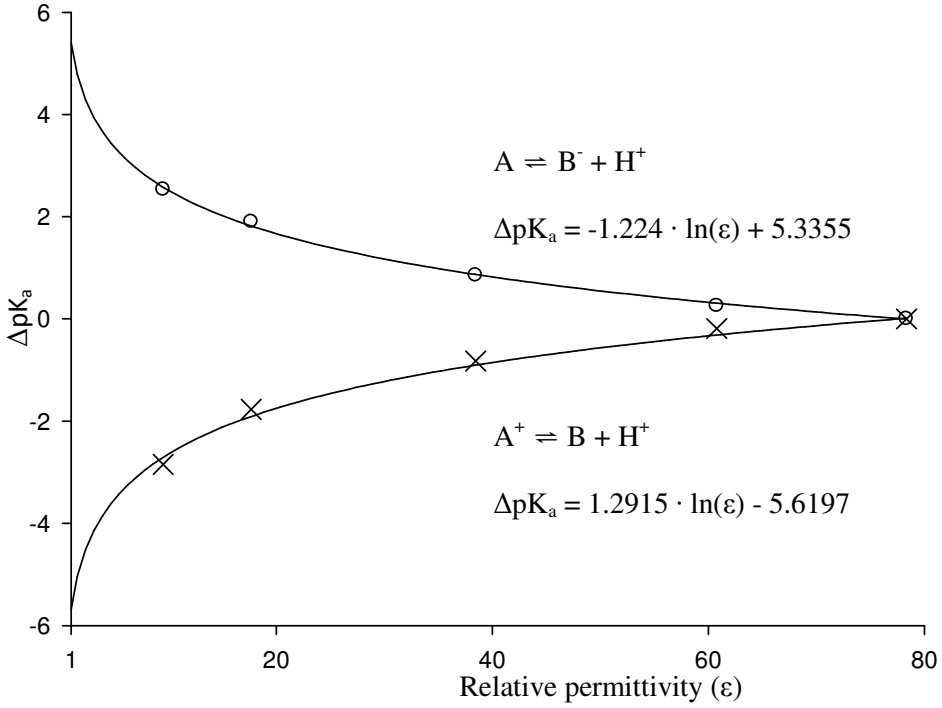

Figure S1. Shift in apparent pK_a (ΔpK_a) from the bulk phase pK_a as a function of local dielectric constant for an acid/base. [Data points from Fernandez and Fromherz][1]

The data in Figure S1 can be used for estimation of the apparent pK_a of amino acids, or other acid/bases, in different dielectric environments, e.g. at a surface or in a dielectric layer. In a protein

the equilibrium $A \rightleftharpoons B^- + H^+$ is relevant for the amino acids glutamate, aspartate and tyrosine, besides the C-terminal (S1) and the function is given:

$$\Delta pK_a = -1.22 \cdot \ln(\varepsilon) + 5.34 \quad \text{(S1)}$$

Further, the equilibrium $A^+ \rightleftharpoons B + H^+$ is relevant for the amino acids arginine, lysine, histidine, and the N-terminal and is described by equation (S2).

$$\Delta pK_a = 1.29 \cdot \ln(\varepsilon) - 5.62 \quad \text{(S2)}$$

These simple functions can then be used to calculate the change in apparent pKa from the local dielectric environment in the form of the relative permittivity (ε).

The next section shows the calculation to correct the apparent pKa for electrostatic effects.

S2. Electric effect on adsorbed charge

At a charged surface the distribution of ions will be different than in bulk phase as is reflected in the difference in pH at the surface and in bulk. E.g. at a negatively charged surface the concentration of H^+ will be higher resulting in a lower concentration based pH as described by the Gouy-Chapmann theory. Thus the effect of surface potential (φ_S) on the charge of an acid or base can be calculated from the potential dependence on the pH:

$$pH_S = pH_{Bulk} + 0.434\frac{F\varphi_S}{RT} \quad \text{(S3)}$$

and the charge is calculated from the apparent pKa for the equilibrium $A^+ \rightleftharpoons B + H^+$:

$$z = \frac{10^{pKa}}{10^{pH(\varphi)} - 10^{pKa}} \quad \text{(S4)}$$

Or the equilibrium $A \rightleftharpoons B^- + H^+$:

$$z = -\frac{10^{pH_S}}{10^{pH_S} - 10^{pKa}} \qquad \text{(S5)}$$

In a protein the equilibrium $A^+ \rightleftharpoons B + H^+$ is relevant for the amino acids arginine, lysine, histidine, and the N-terminal, while $A \rightleftharpoons B^- + H^+$ is relevant for glutamate, aspartate, tyrosine, and the C-terminal. The net-charge of a protein, z_{net}, is the sum of all the amino acids in equations (S4) and (S5). The charge, z, for different values of apparent pK_a is plotted in Figure S2.

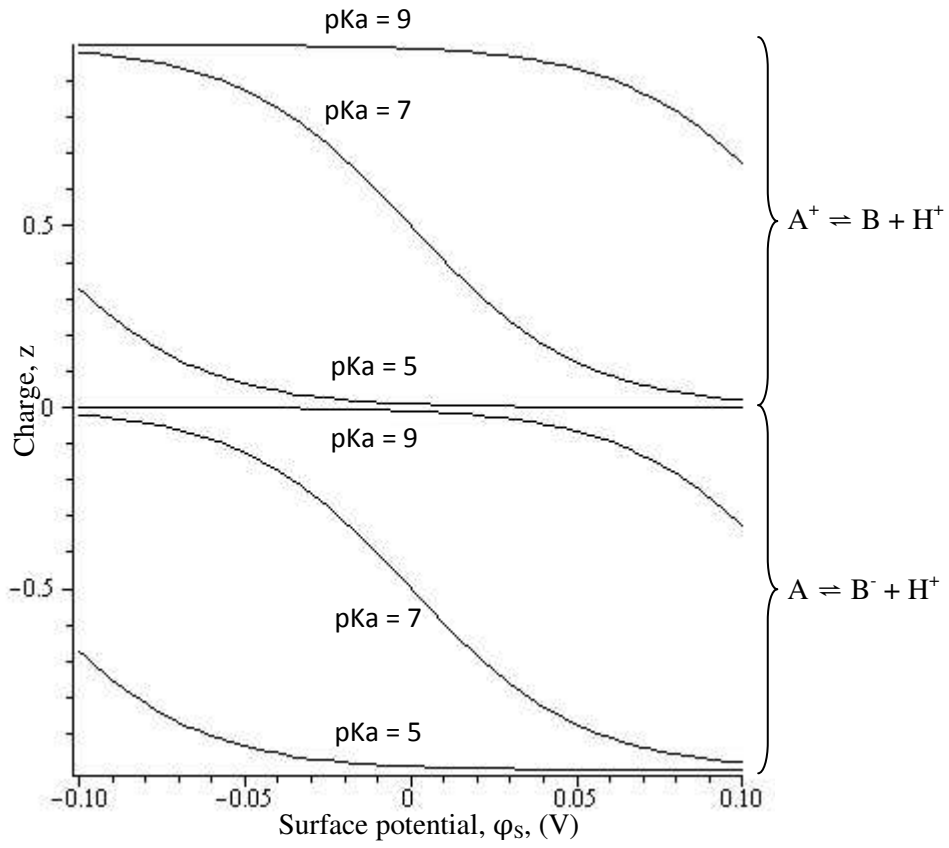

Figure S2. Charge, z, for different values of apparent pKa as a function of potential. $pH_{Bulk} = 7$.

Figure S2 show that an acid or base may shift between being neutral to having a full charge in a relative narrow potential range. An effect which is stronger the closer pH is to the apparent surface

pKa of the acid/base. If the charge is adsorbed and found directly at the surface then the relevant potential is the surface potential φ_s. If the acid/base is found at a distance from the charged surface, e.g. an amino acid on an adsorbed protein, the potential at that distance may be calculated from the Gouy-Chapmann theory. If the potential in bulk solution is taken as zero the potential at distance x from the charged surface can be calculated from:

$$\varphi(x) = \frac{4RT}{F}\tanh^{-1}\left(\exp\left(-F\sqrt{\frac{2\cdot c_{\mathrm{w}}}{RT\varepsilon_0\varepsilon_{\mathrm{w,eff}}}}\cdot x\right)\cdot\tanh\left(\frac{F}{4RT}\cdot\varphi_{\mathrm{s}}\right)\right) \qquad \text{(S6)}$$

In Figure S3 the potential distribution near a charged surface is calculated from equation (S6) to illustrate the distance dependence on the potential. The calculation is done for different values of the surface potential (φ_s) which is the potential at $\varphi(x = 0)$.

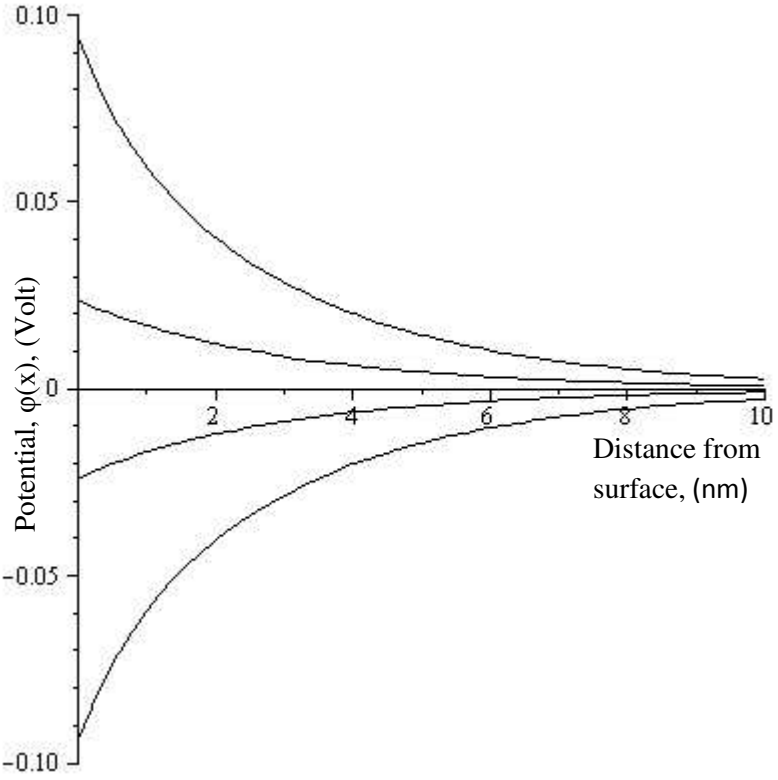

Figure S3. Potential distribution near a charged surface calculated as a function of distance at different surface potentials. The surface potentials are the intersections with the y-axis since φ_s equals $\varphi(x = 0)$.

Figure S3 show that the electrical potential mediated by a charged surface declines rapidly with distance. However at 0.5 – 1 nm which is the estimated average distance of adsorbed amino acids in proteins at hydrophobic interfaces, the potential can still be quite pronounced. In summary the charge of an amino acid positioned on an adsorbed protein is a function of 1) the relative permittivity around the amino acid, which may depend on its position, and 2) the potential around the amino acid which depends on its distance from the charge interface.

REFERENCES

1. Fernandez, M. S.; Fromherz, P. *J. Phys. Chem.* **1977,** *81* 1755-1761.