Electronic Supplementary Information

Dangerous liaisons: anion-induced protonation in phosphate-polyamine interactions and their implications for charge states of biologically relevant surfaces

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1. Size of the microparticles by DLS

The size of the SPs was determined by DLS employing a Zetasizer Nano (Nano ZSizer-ZEN3600, Malvern, U.K.) in water at 25ºC employing a distribution fitting method. The size of the unmodified SPs was determined to be 250±60 nm (**Fig. SI 1**).

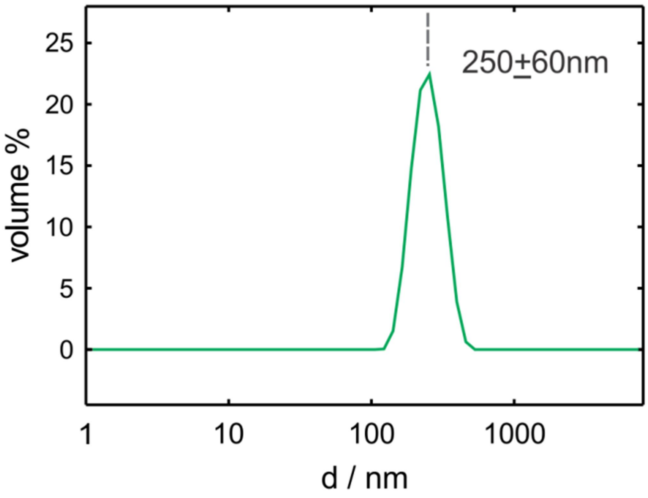

Fig. SI 1. Size distribution of the SiO_2 microparticles determined by DLS in water at 25°C.

2. Experimental results of the zeta-potential

The zeta-potential of the SiO_2@PAH microparticles (full circles) strongly depends on the presence of phosphate anions as shown in **Fig. SI 2** for solutions of different pH. As a control experiment, we have also measured the effect of *Pi* concentration on the zeta-potential of bare microparticles (empty circles). As shown in **Fig. SI 2**, no significant changes are observed in this case. In pH conditions, the results for SiO_2@PAH microparticles can be satisfactorily fitted to **eq. 14** (dash lines in **Fig. SI 2**).

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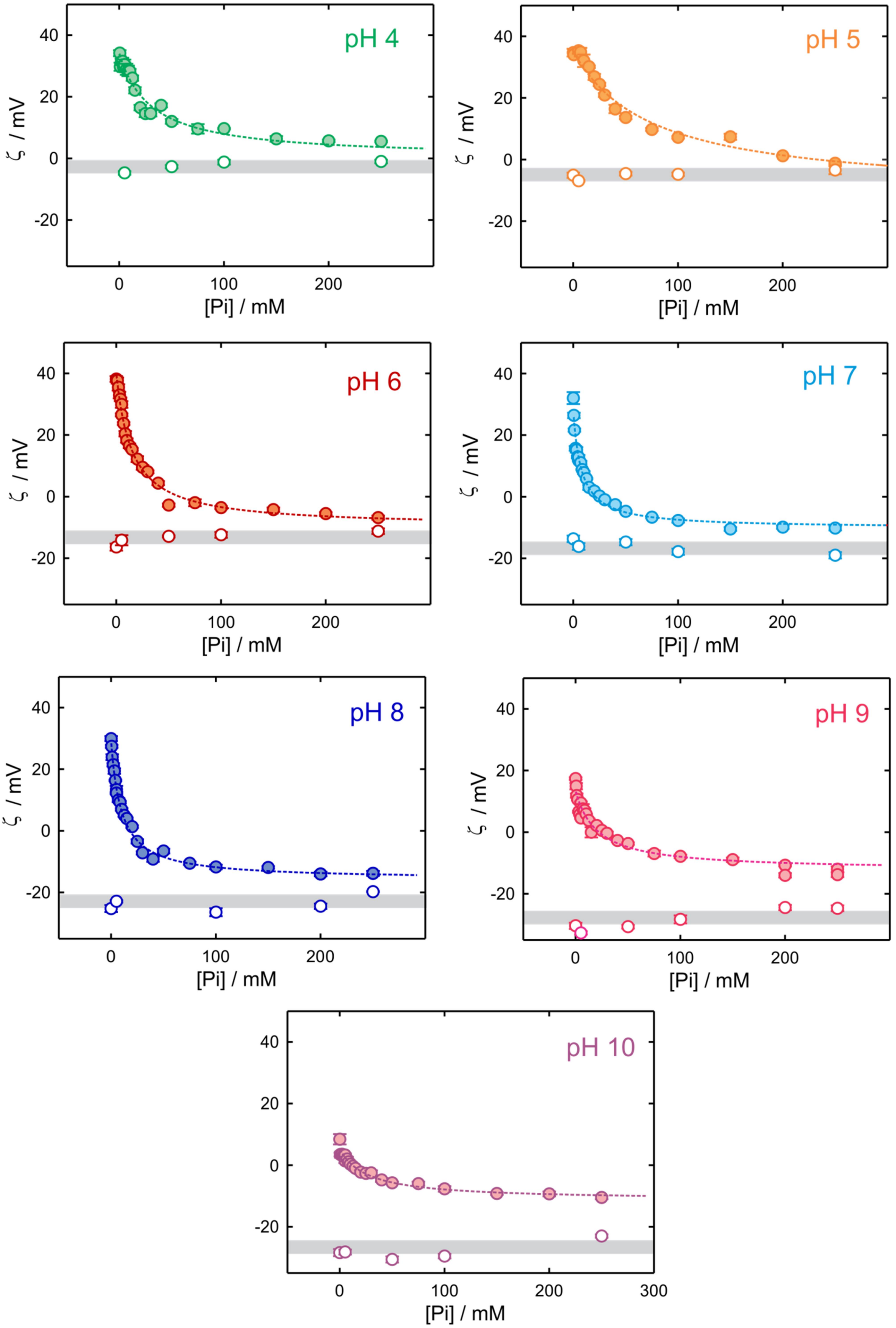

Fig. SI 2. Zeta-potential of the bare SiO$_2$ microparticles (empty circles) and SiO$_2$@PAH microparticles (full circles) as a function of the phosphate concentration in 0.1 KCl solutions of several adjusted pH values. Dash line means the fitting to eq. (14).

3. A model for Phosphate Binding to Amine Units

Let us consider the dissociation equilibriums of phosphates in solution

$$H_3PO_4 \rightleftharpoons H_2PO_4^- + H^+ \quad pK_{a1} = 2.2 \quad K_{a1} = 5.7\ 10^{-3}; \qquad \textbf{(SI 1)}$$

$$H_2PO_4^- \rightleftharpoons HPO_4^{2-} + H^+ \quad pK_{a2} = 7.2 \quad K_{a2} = 6.2\ 10^{-8}; \qquad \textbf{(SI 2)}$$

$$HPO_4^{2-} \rightleftharpoons PO_4^{3-} + H^+ \quad pK_{a3} = 12.7 \quad K_{a3} = 2.2\ 10^{-13}; \qquad \textbf{(SI 3)}$$

Let $[Pi]$ be the total phosphate bulk concentration, that is

$$[Pi] = \left[H_3PO_4\right] + \left[H_2PO_4^-\right] + \left[HPO_4^{2-}\right] + \left[PO_4^{3-}\right] \ ; \qquad \textbf{(SI 4)}$$

The distribution functions for phosphate species in solution can be then calculated as

$$\alpha_0 = \frac{\left[H_3PO_4\right]}{[Pi]};\ \alpha_1 = \frac{\left[H_2PO_4^-\right]}{[Pi]};\ \alpha_2 = \frac{\left[HPO_4^{2-}\right]}{[Pi]};\ \alpha_3 = \frac{\left[PO_4^{3-}\right]}{[Pi]} \qquad \textbf{(SI 5)}$$

Employing the preceding values for the dissociation constants, the distribution functions as a function of pH can be calculated as shown in **Fig. SI 3.**

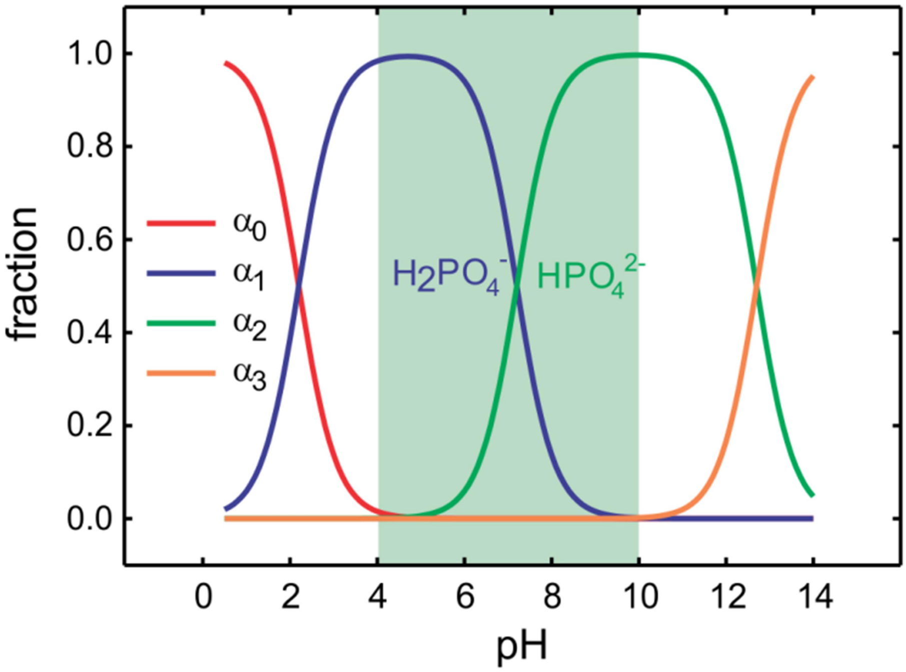

Fig. SI 3. Calculated distribution functions for phosphate species as a function of pH.

According to these plots, the main phosphate species in the range 4-10, are singly and doubly charged anions.

Let us assume that the association equilibriums between phosphate species and amines is mainly electrostatically driven. Taking into account that the only phosphate species whose concentrations are appreciable in this pH range are $H_2PO_4^-$ and HPO_4^{2-}, the whole description of the binding and proton dissociation equilibriums in the range 4-10 can be achieved by considering the following equilibriums

$$(NH_3)^+ + H_2PO_4^- \rightleftharpoons (NH_3Pi) \qquad K_{11} \qquad \textbf{(1)}$$

$$(NH_3)^+ + HPO_4^{2-} \rightleftharpoons (NH_3Pi)^- \qquad K_{12} \qquad \textbf{(2)}$$

Here parentheses are employed to designate surface species. By considering Langmuir-type of binding isotherms, expressions of the former constants can be deduced

$$K_{11} = \frac{\left[(NH_3Pi)\right]}{\left[H_2PO_4^-\right]\left[(NH_3)^+\right]} \qquad \textbf{(SI 6)}$$

$$K_{12} = \frac{\left[(NH_3Pi)^-\right]}{\left[HPO_4^{2-}\right]\left[(NH_3)^+\right]} \qquad \textbf{(SI 7)}$$

Here it is important to note that, although we just use square brackets for concentrations, they mean bulk concentrations for species in solution ($\left[H_2PO_4^-\right]$and $\left[HPO_4^{2-}\right]$) and surface concentrations for the amino or amino-bound surface groups ($\left[(NH_2)\right];\left[(NH_3)^+\right];\left[(NH_3Pi)\right];\left[(NH_3Pi)^-\right]$).

Let us now consider the proton dissociation equilibrium of the surface amino groups

$$(NH_3)^+ \rightleftharpoons (NH_2) + H^+ \qquad K_a \qquad \textbf{(3)}$$

And it will be useful to define the fraction of protonated amine as

$$\theta_+ = \frac{\left[(NH_3)^+\right]}{\left[(NH_3)^+\right] + \left[(NH_2)\right]}, \qquad \textbf{(13)}$$

By employing an *extended Henderson-Hasselbalch equation* (see next section), it is possible to write

$$\theta_+ = \frac{1}{1 + 10^{n_o(pH - pKa)}} \qquad \textbf{(SI 8)}$$

where n_o is an exponent that accounts for the p*Ka* distribution of the surface groups as it is explained in the next section.

Also the proton dissociation of bound phosphate species could be considered

$$(NH_3Pi) \rightleftharpoons (NH_3Pi)^- + H^+ \qquad K_{app} \qquad \textbf{(4)}$$

By employing equilibriums **(1)**, **(2)** and **(SI 2)**, it is easy to prove that $K_{app} = K_{a2}\dfrac{K_{12}}{K_{11}}$**.** It will also be useful to define the fraction of negatively charged associated species

$$\alpha_- = \left[(NH_3Pi)^-\right] / \left\{\left[(NH_3Pi)^-\right] + \left[(NH_3Pi)\right]\right\} \qquad \textbf{(10)}$$

Once again, an *extended Henderson-Hasselbalch* model for equilibrium **(4)** yields

$$\alpha_- = \frac{1}{1+10^{-n_{app}(pH-pKapp)}} \qquad \textbf{(SI 9)}$$

where n_{app} accounts for the p*Ka* distribution of the surface groups.

In the pH range 4-10 it is possible to consider that

$$[Pi] = \left[H_2PO_4^-\right] + \left[HPO_4^{2-}\right] \qquad \textbf{(SI 10)}$$

whereas the total surface concentration of bound phosphates can be written as

$$\left[(NH_3Pi)\right] + \left[(NH_3Pi)^-\right] = \left(K_{11}\left[H_2PO_4^-\right] + K_{12}\left[HPO_4^{2-}\right]\right)\left[(NH_3)^+\right] \qquad \textbf{(SI 11)}$$

where we have employed **eqs SI 6** and **SI 7.**

The global phosphate binding could be described as follows

$$(NH_3)^+ + Pi \rightleftharpoons (NH_3Pi) + (NH_3Pi)^- \qquad K_B \qquad \textbf{(SI 12)}$$

$$K_B = \frac{\left[(NH_3Pi)\right] + \left[(NH_3Pi)^-\right]}{\left[(NH_3)^+\right]\left[Pi\right]} \qquad \textbf{(SI 13)}$$

Using **eq. SI 11**,

$$K_B = \frac{\left(K_{11}\left[H_2PO_4^-\right] + K_{12}\left[HPO_4^{2-}\right]\right)}{\left[Pi\right]} \qquad \textbf{(SI 14)}$$

which can be simplified to

$$K_B = K_{11}\alpha_1 + K_{12}\alpha_2 \qquad \textbf{(7)}$$

4. Distribution of Binding Constants and n_{app}

Both, heterogeneity of adsorption energy of the binding sites and lateral ligand interactions can be modeled by employing an extension of the Langmuir equation that is often referred as Langmuir-Freundlich equation,

$$\theta_i = \frac{(K_i c_i)^m}{1+(K_i c_i)^m} \qquad \textbf{(SI 15)}$$

The parameter m is called *heterogeneity index*. For an ideal adsorption, m=1 and it reduces to the Langmuir model. Typically, when the coefficient is higher than 1, *cooperativity* is said to take place and the equation is also referred as Hill's equation.[1,2] For values lower than 1, *negative cooperativity* is said to occur and it is also assigned to the existence of lateral repulsive interactions between bound species.[3]

Particularly, when equation **eq. SI 15** refers to the protonation equilibrium it is also named as *extended Henderson-Hasselbalch equation*[1] and it is often employed for the mathematical description of the acid-base equilibrium in polyelectrolytes[4,5] and surface confined groups,[6] and it is usually considered as an *apparent number* of protons (n_{app}) that effectively bind to each binding site. Although other advanced models can be employed for the treatment of heterogeneous or interacting binding systems, the use of n_{app} allows the numerical analysis of the experimental data keeping the simplicity of the equations.

Note that for this kind of model, the binding constant (as defined for an ideal binding equilibrium) has not a single value but a distribution of them. Even, the *heterogeneity index* has been theoretically related to the width of the adsorption energy distribution by Sips.[7]

5. The Expression of the zeta-potential

Being zeta-potential a measure of the surface charge, we could consider that it is proportional to the surface concentrations of charged species. By taking into account the charged surface species, it is possible to write

$$\zeta \propto \left[(NH_3)^+\right]-\left[(NH_3Pi)^-\right] \qquad \textbf{(8)}$$

By employing **eq. SI 13**, the former expression can be written as

$$\zeta \propto \left[(NH_3)^+\right](1-\alpha\_K_B\left[Pi\right]) \qquad \textbf{(SI 16)}$$

Let Γ_{NH_2} be the total surface concentration of amino groups,

$$\Gamma_{NH_2} = \left[(NH_2)\right] + \left[(NH_3)^+\right] + \left[(NH_3Pi)\right] + \left[(NH_3Pi)^-\right] \tag{5}$$

Using **eq. SI 11-20**,

$$\Gamma_{NH_2} = \left[(NH_3)^+\right]\left\{1+\frac{\left[(NH_2)\ \right]}{\left[(NH_3)^+\right]} + \left(K_{11}\alpha_1 + K_{12}\alpha_2\right)\left[Pi\right]\right\} \tag{SI 17}$$

so that

$$\left[(NH_3)^+\right] = \Gamma_{NH_2}\left\{1+\frac{\left[(NH_2)\ \right]}{\left[(NH_3)^+\right]} + K_B\left[Pi\right]\right\}^{-1} \tag{SI 18}$$

By replacing this expression into **eq. SI 16**,

$$\zeta \propto \Gamma_{NH_2}(1-\alpha_{-}K_B\left[Pi\right])\left\{1+\frac{\left[(NH_2)\right]}{\left[(NH_3)^+\right]} + K_B\left[Pi\right]\right\}^{-1} \tag{9}$$

In the absence of binding anions, $\left[Pi\right]=0$ and

$$\zeta_0 \propto \Gamma_{NH_2}\left\{1+\frac{\left[(NH_2)\right]}{\left[(NH_3)^+\right]}\right\}^{-1} \tag{11}$$

whose introduction into **eq. (9)** allows writing

$$\zeta / \zeta_0 = \frac{(1-\alpha_{-}K_B\left[Pi\right])}{\left\{1+\theta_{+}K_B\left[Pi\right]\right\}} \tag{12}$$

Taking into account the previous equations, the experimental dependence of the change of the zeta-potential on the phosphate concentration in solution ([*Pi*]) could be written as

$$\zeta = \zeta_0\frac{1-A\left[Pi\right]}{1+B\left[Pi\right]} \tag{14}$$

where we have defined the parameters *A* and *B* as

$$A = (K_{11}\alpha_1 + K_{12}\alpha_2)/(1+10^{-n_{app}(pH-pKapp)}) \tag{16}$$

$$B = (K_{11}\alpha_1 + K_{12}\alpha_2)/(1+10^{n_o(pH-pKa)}) \tag{17}$$

6. Fitting of the values of *A* and *B*

Simplifications could be achieved by considering just the ratio

$$A/B = (1+10^{n_o(pH-pKa)})/(1+10^{-n_{app}(pH-pKapp)}), \qquad \textbf{(SI 19)}$$

However, calculating this quotient from fitted values of *A* and *B* has a relatively high error and values are not secure for further fittings. An iterative procedure was employed to obtain a single set of parameters (K_{12}/K_{11} and n_{app}) to satisfactorily fit the experimental results for both *A* and *B*. The results for *A / B* in the range of pH 4-9 were employed for the initial guess of K_{12}/K_{11} and n_{app} (**Fig. SI 4**). Then, these values were employed for the fittings of *A* and *B* and then recalculated in an iterative way.

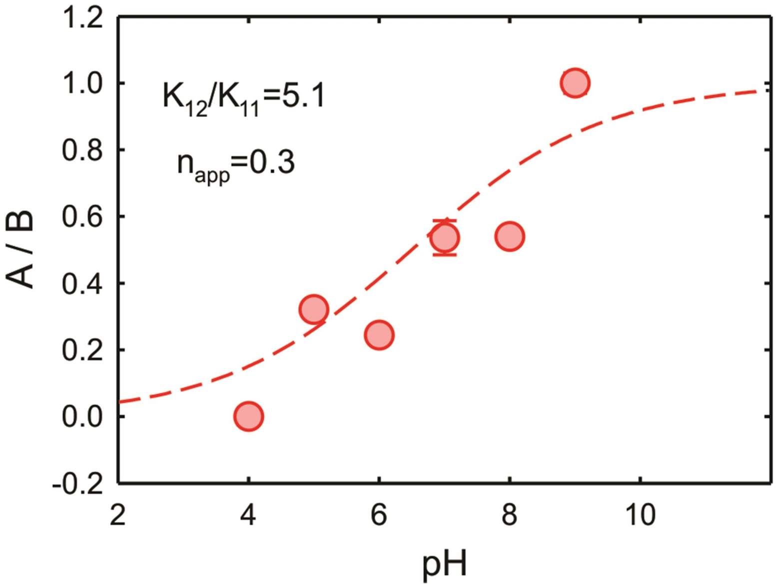

Fig. SI 4. Calculated values of the quotient *A* / *B* from the fitted parameters as a function of pH. Dash line means the calculated values employing the **eq. SI 19**.

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