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Proteins and polyampholytes interacting with polyelectrolyte brushes and microgels: the charge reversal concept revised

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Abstract

Weak polyampholytes and globular proteins among them can be efficiently absorbed from solution by polyelectrolyte brushes or microgels even if the net charge of the polyampholyte is of the same sign as that of the brush/microgel. We use a mean-field approach for calculating free energy of insertion of a probe polyampholyte molecule into a polyelectrolyte brush/microgel. We anticipate that insertion of the polyampholyte into similarly charged brush/microgel may be thermodynamically favorable due to the gain in the cumulative re-ionization free energy of the pH-sensitive acidic and basic residues. Importantly, we demonstrate that the polyampholyte (protein) charge sign inversion upon transfer from the bulk of the solution into the brush/microgel does not provide sufficient condition to assure negative re-ionization free energy balance. Thus (in the absence of other driving or stopping mechanisms) charge sign inversion does not necessarily provoke spontaneous absorption of the polyampholyte into the brush/microgel.

Introduction

Interaction and electrostatically driven complexation of charged macromolecules or (bio)nanocolloids with polyelectrolyte brushes, branched polyelectrolytes and ionic microgels is actively investigated both experimentally and theoretically which is motivated primarily by biomedical applications.¹⁻⁷

Polyelectrolyte brushes are arrays of ionically charged polymer chains end-tethered to planar substrate ("planar polyelectrolyte brushes") or to the surface of colloidal particles ("colloidal polyelectrolyte brushes").⁸⁻¹¹ The latter resemble micellar-like nanostructures formed upon self-assembly of amphiphilic ionic block copolymers in aqueous media.¹² Strong local electrostatic field created by brush-forming polyions allows for entrapping of a major fraction of mobile counterions, which are inevitably present in the solution, in the inner volume of the polyelectrolyte brush. As a result, the brush swells due to excess osmotic pressure exerted by these counterions.^{13,14} Similar effects of localization of counterions and osmotic swelling occur in strongly branched polyelectrolytes¹⁵ (stars, dendrimers, dendrigrafts, etc.) and in cross-linked polyelectrolyte microgels.¹⁶

When the brush/microgel and the "guest" polyelectrolyte are charged oppositely, then Coulomb interactions lead to interpolyelectrolyte complexation with concomitant coacervation and collapse of the brush/microgel that may provoke the loss of colloidal stability.

Charged globular protein can be absorbed from the solution in the intramolecular volume of polyelectrolyte brush-like architectures. A striking feature discovered by M. Ballauff and coworkers is that absorption of the proteins may occur on the "wrong" side of the isoelectric point, that is, when the net charge of the protein globule has the same sign as the charge of the polyelectrolyte brush¹⁷⁻¹⁹ or microgel^{20,21} (see also recent comprehensive reviews^{22,23}).

Although non-electrostatic forces may contribute to the overall protein-polyelectrolyte interaction balance, it was convincingly demonstrated^{17,18} that an increase in the ionic strength in the solution suppresses protein absorption into polyelectrolyte brushes. The latter observation suggest that driving force for the absorption is of electrostatic origin.

A number of hypotheses were proposed to explain this counterintuitive phenomenon. All these hypotheses were based on the fact, that globular proteins comprise amino acid residues with both positive and negative ionic charges. Moreover, these positively or negatively charged residues are weak basic (tertiary amine) or acidic (carboxyl) groups and can be either ionized (through protonation or deprotonation, respectively) or deionized depending on the pH in the surrounding medium. Hence, most of globular proteins can

be classified as weak or pH-sensitive polyampholytes. The overall charge of the polyampholyte vanishes at some particular $\text{pH}=\text{pI}$ corresponding to the isoelectric point (IEP), whereas the polyampholyte acquires net negative or positive charge at pH above or below IEP, respectively.

Importantly, acidic and basic residues on the globule surface are not randomly distributed, but rather form "patches" of pH-dependent negative and positive surface charge. Therefore, even above (below) IEP one finds patches of positive (negative) charge on the globule surface. As it was suggested by M. Ballauff and coworkers,^{19,24} this patchy charge distribution plays the key role in the protein adsorption by similarly charged polyelectrolyte brushes. It was demonstrated theoretically²⁴ using self-consistent field approach that there is an essential asymmetry in the interaction of brush-forming polyelectrolyte chains with patches of charge with different signs on the globular surface: The free energy gain due to polyelectrolyte chains adsorption onto oppositely charged patch exceeds the free energy penalty for polyelectrolyte depletion from similarly charged patch. This asymmetry is related to localization of small mobile counterions near strongly charged patches on the globule surface and their release upon adsorption of polyelectrolyte chains on these patches, which was also confirmed by molecular dynamics simulations.²⁵⁻²⁷ As a result, free energy gain due to polyelectrolyte attraction to the oppositely charged patches may overcompensate the free energy losses due to depletion from similarly charged patches even if the latter occupies larger area on the globule surface and provide the same sign of the net charge of the globule as that of the polyelectrolyte brush.

An alternative hypothesis ("charge reversal") proposed in refs^{28,29} is based on the fact that both degree of dissociation of carboxylic groups and degree of protonation of the amine groups depend on the local pH, that is protein is a weak (pH-sensitive) polyampholyte. Because of the counterion entrapping, local pH in polyelectrolyte brush (or in polyelectrolyte microgel) differs from that in the bulk of the solution. That is, pH in the polycationic brush is higher and pH in the polyanionic brush is lower than that in the bulk of the solution, pH_b . (Here and below subscript "b" refers to the bulk of the solution (the buffer)). Therefore, if $\text{pH} \leq \text{pI} \leq \text{pH}_b$ the protein is charged negatively in the solution, but acquires positive charge inside polyanionic brush. Similarly, if $\text{pH}_b \leq \text{pI} \leq \text{pH}$ the protein is charged positively in the solution but acquires negative charge inside polycationic brush. Hence, the net charge of the protein globule may change the sign upon transfer of the globule from the solution into the similarly charged brush. It is anticipated, that the resulting Coulomb attraction between the brush and the protein drives adsorption of the protein by the brush.

Although the charge reversal hypothesis provides a plausible explanation

of the protein absorption by charged brush/microgel on the "wrong" side of the IEP, as we demonstrate below, the condition $\text{pH} \leq \text{pI} \leq \text{pH}_b$ (or $\text{pH}_b \leq \text{pI} \leq \text{pH}$) is not sufficient for providing thermodynamic driving force for the absorption of negatively (positively) charged protein by polyanionic (polycationic) brush. A proper account of the free energy of re-ionization of acidic and basic residues leads to stronger requirement rather than simple reversal of charge of the protein upon transfer into the brush for assuring a decrease in the free energy of ionic interactions in the system which provides driving force for such absorption and can make it a spontaneous process.

Re-ionization free energy of a polyampholyte

Consider a polyampholyte molecule (e.g., the protein globule) comprising N_+ monomer units capable of acquiring positive (elementary) charge upon protonation, and N_- monomer units capable of acquiring negative (elementary) charge upon dissociation of a proton. When the molecule is immersed into solution with buffered local pH, the fractions of positively and negatively charged ionizable monomer units are, respectively

$$\alpha_+ = (1 + K_+/[H^+])^{-1} \equiv (1 + 10^{pH-pK_+})^{-1} \quad (1)$$

and

$$\alpha_- = (1 + [H^+]/K_-)^{-1} \equiv (1 + 10^{pK_- - pH})^{-1} \quad (2)$$

where $[H^+]$ is local concentration of hydrogen ions, K_+ and K_- are respective acidic ionization constants of basic and acidic monomer groups.

The overall net charge of the polyampholyte (measured in the elementary charge units) equals

$$Q = \alpha_+ N_+ - \alpha_- N_- \quad (3)$$

A polyelectrolyte brush (or polyelectrolyte microgel) which is in equilibrium with the bulk of the solution with fixed pH_b and salt concentration (i.e. with fixed chemical potentials of all mobile ionic species) is characterized by an excess electrostatic potential Ψ (which is also referred to as Donnan potential) with respect to its reference (zero) value in the bulk of the solution. Below we consider negatively charged (polyanionic) brush or microgel, where the excess potential is negative, $\Psi \leq 0$. Therefore, local concentration of hydrogen ions inside the brush (or in the microgel) can be expressed as

$$[H^+] = [H^+]_b \exp(-e\Psi/k_B T) \quad (4)$$

where concentration of hydrogen ions in the bulk of the solution is by definition $[H^+]_b = 10^{-\text{pH}_b}$.

The free energy of the polyampholyte molecule inserted into the brush can be presented as

$$F/k_B T = N_- \left(\alpha_- \ln \alpha_- + (1 - \alpha_-) \ln(1 - \alpha_-) - \alpha_- \ln K_- - \alpha_- \psi + \alpha_- \ln[H^+]_b \right) + N_+ \left(\alpha_+ \ln \alpha_+ + (1 - \alpha_+) \ln(1 - \alpha_+) + \alpha_+ \ln K_+ + \alpha_+ \psi - \alpha_+ \ln[H^+]_b \right) \quad (5)$$

which comprises (i) mixing entropies of ionized and non-ionized monomers (logarithmic terms); (ii) electrostatic free energies of positively and negatively charged monomers (the reduced Coulomb potential inside the brush is defined as $\psi \equiv e\Psi/k_B T$); (iii) ionization free energies through respective ionization constants K_- and $K_+ = K_w/K_{base,+}$ where $K_w = [H^+][OH^-] = 10^{-14}$; and (iv) accounts for equilibrium with the reservoir with fixed chemical potential of hydrogen ions, $k_B T \ln[H^+]_b$.

Minimization of the free energy, eq 5, with respect to α_+ and α_- eq 4 leads to eqs 1 and 2 for equilibrium degrees of ionization of basic and acidic monomer units, respectively. Upon substitution of α_+ and α_- into eq 5 one finds that the free energy contribution due to ionization of positively and negatively charged monomer units of polyampholyte inside the brush can be presented as

$$F_{ion}/k_B T = N_+ \ln(1 - \alpha_+) + N_- \ln(1 - \alpha_-) \quad (6)$$

and the reference state $F_{ion} = 0$ corresponds to absence of charges, $\alpha_+ = \alpha_- = 0$.

Transfer of a polyampholyte molecule from the bulk of the solution into the brush/microgel leads to change in ionization degree of both basic and acidic monomer units. The concomitant change in the free energy can be expressed, with the account of eq 6 as

$$\Delta F_{ion}/k_B T = N_+ \ln\left(\frac{1 - \alpha_+}{1 - \alpha_{b+}}\right) + N_- \ln\left(\frac{1 - \alpha_-}{1 - \alpha_{b-}}\right) \quad (7)$$

where α_{b+} , α_+ , α_{b-} , α_- and the respective degrees of ionization of basic and acidic monomer units in the bulk of the solution and in the brush/microgel.

Since all mobile ions are distributed between the brush and the bulk of the solution according to Boltzmann law, eq 4, lower electrostatic potential inside the brush/microgel implies that local concentration of H^+ ions inside the brush/microgel is larger than in the bulk of the solution. That is, pH in the brush/microgel is smaller than pH in the bulk of the solution which we denote as pH_b . Consequently, as follows from eqs 1 and 2, degree of ionization of acidic monomer units inside polyanionic brush is lower and

degree of ionization of basic monomer units inside the polyanionic brush is higher than in the bulk of the solution.

Because basic monomer units are stronger ionized inside the polyanionic brush than in the bulk of the solution, $\alpha_+ \geq \alpha_{b+}$, the first term in eq 7 is negative. On the contrary, acidic monomer units are weaker ionized inside the polyanionic brush than in the bulk of the solution, $\alpha_- \leq \alpha_{b-}$, and the second term in eq 7 is positive. Hence, the sign of ΔF_{ion} is determined by the balance between re-ionization free energies of basic and acidic monomer units and depends on polyampholyte composition (numbers of acidic and basic monomer units), pH_b in the reservoir, ionization constants of basic and acidic monomers and on the shift of the electrostatic potential between the solution and interior of the brush/microgel.

Polyampholyte absorption threshold vs isoelectric points

At this point it is convenient to introduce a set of dimensionless variables:

$$x = K_-/[H^+]_b \equiv 10^{pH_b - pK_-} \quad (8)$$

controlled by the bulk value of pH, the asymmetry of ionization constants characterized by parameter

$$u = \frac{K_-}{K_+} \equiv 10^{pK_+ - pK_-} \quad (9)$$

and fraction of positively charged ionizable monomer units

$$f_+ = \frac{N_+}{N_- + N_+} \equiv \frac{N_+}{N_\Sigma} \quad (10)$$

were $N_+ + N_- = N_\Sigma$ is total number of ionizable (basic and acidic) monomer units in the polyampholyte.

Finally,

$$\lambda = \exp(e\Psi/k_B T) \quad (11)$$

serves as externally controlled (e.g. by salt concentration) parameter. We remark that $\lambda \leq 1$ since $\Psi \leq 0$ for an anionic brush/microgel. Disregarding gradients in the charge distribution on grafted (brush-forming) or crosslinked polyelectrolyte chains we can present the excess potential (the Donnan potential) as (see e.g. ref¹⁵)

$$\lambda \equiv \exp\left(\frac{e\Psi}{k_B T}\right) = -\frac{\alpha_p c_p}{c_s} + \sqrt{1 + \left(\frac{\alpha_p c_p}{c_s}\right)^2} \leq 1 \quad (12)$$

where α_p is fraction of (negatively) permanently charged monomer units and c_p is polymer concentration inside the brush/microgel and c_s is concentration of all types of monovalent mobile ions in the bulk of the solution. Here we remark that in brushes/microgels formed by weak (pH-sensitive) polyelectrolytes the degree of ionization α_p depends itself on pH and salt concentration in the buffer and increases as a function of c_s that was predicted theoretically^{30,31} and confirmed in experiments^{33,34} and computer simulations.³⁵

Using eqs 1, 2 and 4 the degree of ionization of basic and acidic monomer units in the bulk of the solution and inside the brush/microgel can be expressed as

$$\alpha_{b+} = \frac{u}{x+u}; \alpha_+ = \frac{u}{u+\lambda x} \quad (13)$$

$$\alpha_{b-} = \frac{x}{1+x}; \alpha_- = \frac{\lambda x}{1+\lambda x} \quad (14)$$

The insertion free energy, eq 7, can be then expressed as

$$\frac{\Delta F_{ion}}{N_{\Sigma} k_B T} = \ln \left(\left(\frac{x+1}{\lambda x+1} \right)^{1-f_+} \left(\frac{\lambda(x+u)}{\lambda x+u} \right)^{f_+} \right) \quad (15)$$

The condition $\Delta F_{ion} \leq 0$ is fulfilled when

$$\left(\frac{x+1}{\lambda x+1} \right)^{\frac{1-f_+}{f_+}} \left(\frac{\lambda(x+u)}{\lambda x+u} \right) \leq 1 \quad (16)$$

that is, at $x \leq x_{abs}$ where x_{abs} is found from the equation

$$\left(\frac{x_{abs}+1}{\lambda x_{abs}+1} \right)^{\frac{1-f_+}{f_+}} \left(\frac{\lambda(x_{abs}+u)}{\lambda x_{abs}+u} \right) = 1 \quad (17)$$

The net charge of the polyampholyte molecule in the bulk of the solution and inside the brush (gel) can be expressed as

$$Q_b = \alpha_{b+} N_+ - \alpha_{b-} N_- = N_{\Sigma} \left(f_+ \frac{x^2 + 2ux + u}{(x+1)(x+u)} - \frac{x}{x+1} \right) \quad (18)$$

and

$$Q_{in} = \alpha_+ N_+ - \alpha_- N_- = N_{\Sigma} \left(f_+ \frac{(\lambda x)^2 + 2u\lambda x + u}{(\lambda x+1)(\lambda x+u)} - \frac{\lambda x}{\lambda x+1} \right) \quad (19)$$

respectively. As follows from eqs 18 and 19,

$$Q_b(x=0) = Q_{in}(x=0) = N_{\Sigma} f_+$$

$$Q_b(x = \infty) = Q_{in}(x = \infty) = -N_{\Sigma}(1 - f_+)$$

The net charge of the polyampholyte in the bulk of the solution vanishes, $Q_b = 0$, in the isoelectric point $x = x_{IEP}$ specified as

$$x_{IEP} = \frac{u}{2} \cdot \frac{2f_+ - 1}{1 - f_+} + \sqrt{\frac{u^2}{4} \left(\frac{2f_+ - 1}{1 - f_+} \right)^2 + \frac{uf_+}{1 - f_+}} \quad (20)$$

As follows from eqs 18, 20 the net charge of the polyampholyte in the bulk of the solution $Q_b \leq 0$ at $x \geq x_{IEP}$ and $Q_b \geq 0$ at $x \leq x_{IEP}$.

Inside the brush the net charge of the polyampholyte vanishes at $x = x'_{IEP}$ where

$$x'_{IEP} = \lambda^{-1} x_{IEP} \quad (21)$$

Hence, the net charge of the polyampholyte inside the brush vanished at $x = x'_{IEP} \geq x_{IEP}$, i.e., at $\text{pH}_b \geq \text{pI}$. In the range of $x_{IEP} \leq x \leq \lambda^{-1} x_{IEP}$ the polyampholyte is charged negatively in the bulk of the solution, but positively inside the brush/microgel, i.e., charge sign inversion occurs upon insertion of the polyampholyte into the brush/microgel.

It is easy to check that in the particular case of $u = 1$, eq 20 has a simple form:

$$x_{IEP} = \frac{f_+}{1 - f_+} \quad (22)$$

and solution of eq 17 can be presented as

$$x_{abs} = \frac{1 - \lambda^{f_+}}{\lambda^{f_+} - \lambda} \quad (23)$$

The analysis of eqs 17, 20, 21 proves that the inequality

$$x_{IEP} \leq x_{abs} \leq x'_{IEP} \equiv \lambda^{-1} x_{IEP} \quad (24)$$

holds at arbitrary u as long as $\lambda \leq 1$ (negatively charged brush), which is also illustrated in Figures 1 and 2.

In **Figure 1** the dependences of the polyampholyte molecule net charge outside and inside the brush, Q_b and Q_{in} , respectively, and their difference, $\Delta Q = Q_{in} - Q_b$, are plotted as a function of $x = 10^{\text{pH}_b - \text{pK}^-}$. As one can see from Figure 1, in the limit of $x \rightarrow \infty$ (extreme alkaline pH_b range) the polyampholyte is negatively charged both in the buffer and inside the brush. A decrease in x (in pH_b) leads to a decrease in the magnitude of the polyampholyte negative charge both in the buffer and inside the brush. Inside the brush the polyampholyte charge vanishes at $x = x'_{IEP}$ while the polyampholyte in the buffer remains negatively charged. Further decrease

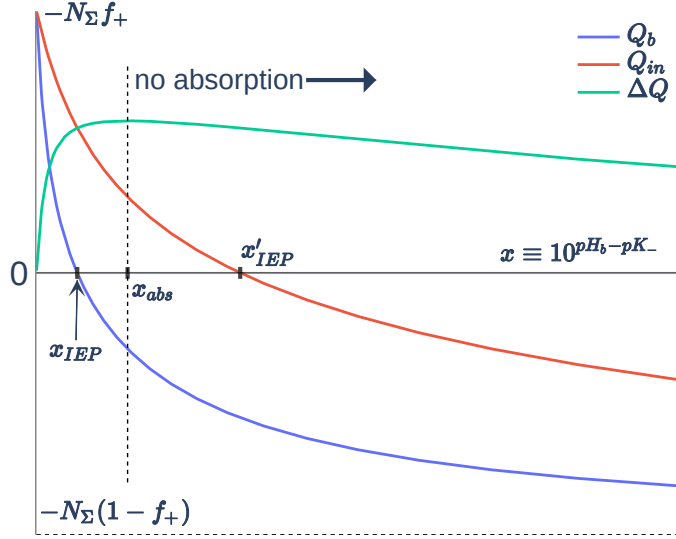


Figure 1: Dependences of the polyampholyte molecule net charge outside and inside the brush, Q_b and Q_{in} , respectively, on $x = 10^{pH_b - pK_-}$ at $f_+ = 0.5$; $u = 1$; $\lambda = 0.2$

in pH_b results in increasing positive charge of the polyampholyte inside the brush (and simultaneous decreasing magnitude of the negative charge outside the brush), but the insertion free energy penalty remains positive, i.e., there is no electrostatic driving force for the polyampholyte uptake in the brush. Only when pH_b drops down to the value corresponding to x_{abs} , the insertion free energy becomes negative.

Hence, ionic interactions provide driving force for spontaneous absorption of polyampholyte by anionic brush/microgel in the range of the pH_b corresponding to the conditions $x \leq x_{abs}$, when the net charge of the polyampholyte is still negative in the bulk of the solution, but positive inside the brush. However, the inversion of the polyampholyte charge sign inside the brush does not necessarily imply negative insertion free energy, i.e., there is no driving force for the absorption in the $x(pH_b)$ range corresponding to $x_{abs} \leq x \leq \lambda^{-1}x_{IEP}$ and the polyampholyte is repelled from the polyelectrolyte brush.

As it follows from eq 20, the value of x_{IEP} corresponding to vanishing net polyampholyte charge in the solution depends only on the polyampholyte composition (fraction of positive charged residues f_+) and ratio of ionization constants $u = K_-/K_+$. The values of x'_{IEP} and x_{abs} depend additionally on λ , that is, on the magnitude of excess electrostatic potential inside the brush/microgel, see eqs 17, 21.

The dependences of the threshold values of x_{IEP} , x'_{IEP} , and x_{abs} on f_+ (at $\lambda, u = const$), on u (at $\lambda, f_+ = const$ and on λ (at $f_+, u = const$) are presented in **Figures 2a,b,c**.

Figure 2a and 2b illustrate that x_{IEP} , x'_{IEP} and x_{abs} increase (corresponding pH_b values grow) when the fraction f_+ of basic monomer units in the polyampholyte or the value of pK_+ increase. As one can see from Figure 2c, x_{abs} and x'_{IEP} grow upon a decrease in λ (increasing magnitude of negative electrostatic potential inside the brush), whereas x_{IEP} (the isoelectric point in the solution) is obviously independent of λ . Remarkably, both x_{abs} and \tilde{x}_{IEP}/x_{abs} , do increase upon a decrease in λ . This is easy to check in the particular case of $u = 1$ when

$$\begin{aligned} x'_{IEP}/x_{IEP} &= \lambda^{-1}; \\ x_{abs}/x_{IEP} &\sim \lambda^{-f_+}; \\ x'_{IEP}/x_{abs} &\sim \lambda^{f_+-1} \end{aligned}$$

Hence, for smaller λ (stronger anionic charge of the brush or lower ionic strength of the solution), both the polyampholyte charge sign inversion and the insertion free energy sign inversion occur at larger x , i.e. at higher pH_b . Therefore, at given $\text{pH}_b \geq \text{pI}$ the uptake of polyampholyte in the brush/microgel can be provoked by a decrease in λ caused, e.g, by a decrease in the ionic strength of the solution which is consistent with experimental observation.¹⁸

In **Figure 3** we present 3D diagram of states for the polyampholyte molecule as a function of composition, f_+ , excess electrostatic potential inside the brush/microgel, $\lambda = \exp(e\Psi/k_B T)$, and pH in the solution, for a typical set of ionization constants $K_- = 5.5$ and $K_+ = 4.5$. The lower (blue) surface corresponds to the pI of the polyampholyte in the solution, above it the polyampholyte in the buffer is negatively charged. The upper (green) surface corresponds to the polyampholyte charge inversion threshold: below this pH value in the buffer the polyampholyte gets positively charged (the charge sign inversion occurs) upon insertion from the buffer into the brush/microgel. The brownish surface sandwiched between the blue and the green ones corresponds to absorption threshold: the re-ionization free energy upon insertion of the polyampholyte into the brush/microgel is positive at higher pH_b and negative at lower pH_b .

Crossing of the boundary $\text{pH}_b(x'_{IEP})$ upon a decrease in λ or in pH_b , as well as upon an increase in f_+ leads to the charge sign inversion for the polyampholyte inserted into the brush, but does not imply appearance of the driving force for the absorption. Further decrease in λ or in pH_b (an increase

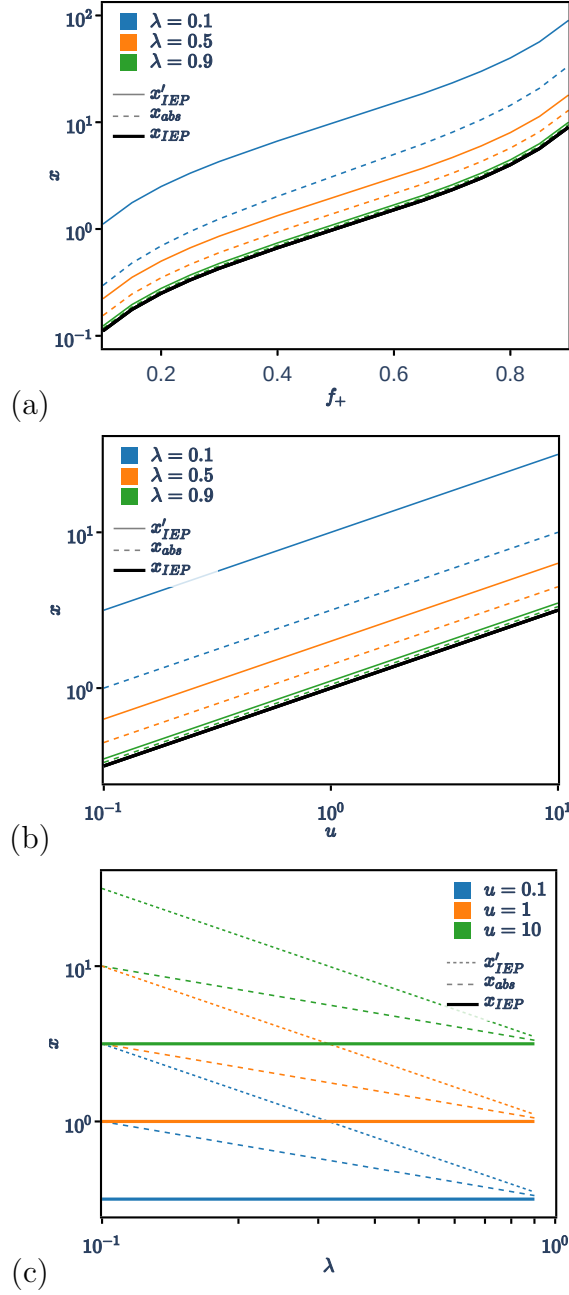


Figure 2: The dependences of x_{IEP} , x'_{IEP} , and x_{ads} on f_+ for $\lambda = 0.1; 0.5; 0.9; u = 1$ (a), on u for $\lambda = 0.1; 0.5; 0.9; f_+ = 0.5$ (b) and on λ for $u = 0.1; 1.0; 10; f_+ = 0.5$ (c).

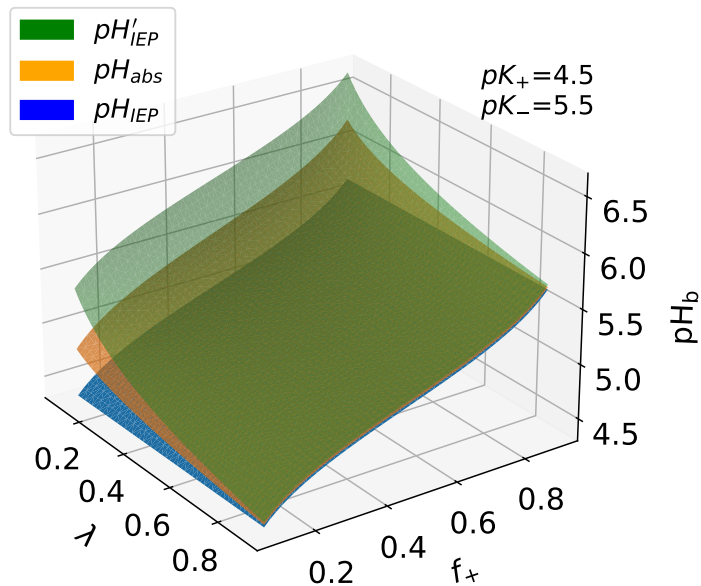


Figure 3: Diagram of states for the polyampholyte molecule in $(f_+, \lambda = \exp(e\Psi/k_B T), \text{pH}_b)$ coordinates for $\text{p}K_- = 5.5$ and $\text{p}K_+ = 4.5$

in f_+) associated with the crossing of the $\text{pH}_b(x_{abs})$ boundary is required to make insertion of the polyampholyte into the brush thermodynamically favorable.

In **Figures 4a** and **b** we show the cross-sections of the 3D diagram presented in Figure 3 corresponding to variation of fraction f_+ of cationic groups at constant λ (Figure 4a) and to variation in the excess electrostatic potential inside the brush/microgel, that is, variation in λ at constant composition of the polyampholyte (constant f_+). Evidently the larger the fraction f_+ of cationic groups, the higher are pH thresholds corresponding to charge sign inversion and to the onset of absorption. However, the difference between these two characteristic pH_b values does not noticeably change upon variation in f_+ . The pH -range at which re-ionization favours absorption widens and pH'_{IEP} and pH_{abs} increase when λ decreases that corresponds to larger difference of the electrostatic potential between the brush/microgel and the buffer. Such decrease in λ can be caused, e.g., by increasing fraction of negatively charged monomer units in the brush or by a decrease in the salt concentration in the buffer (see eq 12). Obviously at $\lambda \rightarrow 1$ all the three surfaces separating different charge inversion/absorption regimes intersect at the line corresponding to IEP of the polyampholyte in the solution, given by

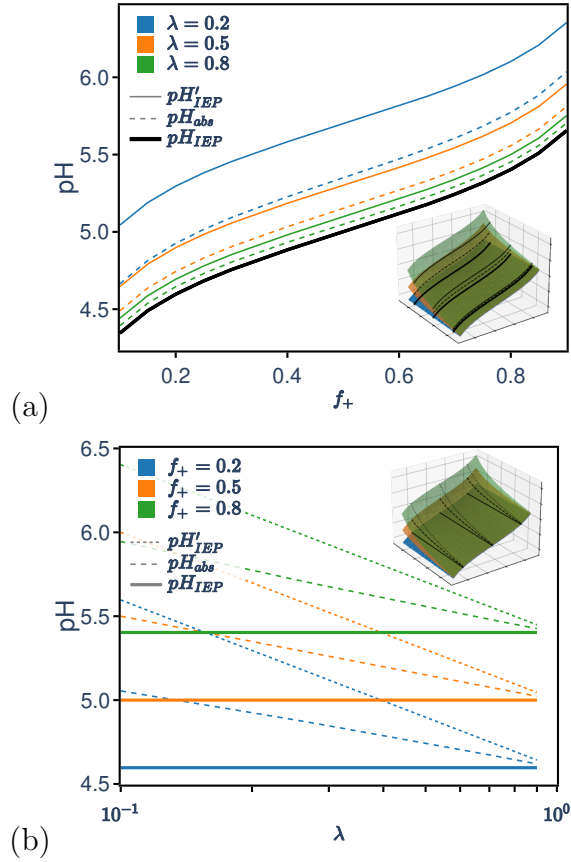


Figure 4: The crosssections of the 3D diagram of states (shown in inserts) at constant values of λ (indicated in the figure) and varied fraction of cationic monomer units f_+ (a) and at constant values of f_+ (indicated in the figure) and varied λ (b).

eq 20.

Comparison of theory to experiment

Our theory describes the electrostatically driven uptake of protein from buffer solution by polyelectrolyte brushes/microgels. We model polyelectrolyte brush/microgel as a reservoir comprising polyelectrolyte chains with the average concentration c_p of monomer units, and mobile monovalent ions distributed via Donnan equilibrium with buffer solution. The gradients in polymer concentration and chain ionization are neglected. The electrostatic potential difference Ψ between the interior of polyelectrolyte brush/microgel

and buffer solution is accounted for via parameter λ (eq 12) in terms of the average concentration c_p of the monomer units, their degree of ionization, α_p , and the concentration c_s of mobile ions in buffer solution. The brush geometry and chemical nature of the tethered polyelectrolytes are accounted via the dependence $c_p(\alpha_p, c_s)$. For strong polyelectrolytes, e.g., poly(styrenesulfonic acid), α_p is constant specified by the degree of polystyrene sulfonation, while for weak polyelectrolytes, e.g., poly(acrylic acid), α_p is pH-sensitive, and is governed by the brush geometry, grafting density of polyelectrolyte chains, pH_b and the ionic strength in buffer solution.¹⁵

To confront the predictions of our model to experiment, we focus on uptake of globular proteins by colloidal polyelectrolyte brushes.^{17,36} Absorption of bovine serum albumin (BSA) in poly(acrylic acid) (PAA) spherical brushes decorating poly(styrene) latex particles was thoroughly investigated in ref.¹⁷ A detailed characterization of the experimental system (L16) was performed in ref.³⁶ It was concluded by the authors that BSA uptake by PAA spherical brushes occurred via two, time separated stages. During the first (quick and reversible) stage, BSA progressively accumulates inside PAA brushes. During the second (later) stage, protein molecules bind to polyelectrolyte chains exhibiting minor changes in the secondary structure. At this stage, an additional, non-electrostatic protein - chain interactions are plausible. Notably, our current model addresses the first stage of the electrostatically driven protein uptake, in which the structure of the polyelectrolyte brush/microgel remains essentially unperturbed by interaction with proteins.

The pH-sensitivity of PAA brush was experimentally confirmed by 3-fold increase in the brush thickness L upon an increase in pH_b from $\text{pH}_b = 2$ up to neutral conditions, $\text{pH}_b = 7$, at low salt, $c_s = 10\text{mM}$ (Figure 2 in ref¹⁷). The increase in L of the spherical brush formed by polyelectrolytes with contour length $L_c = 209\text{ nm}$ and N_p acidic groups per chain (tethered with grafting density σ to the surface of core particle with radius $R_c \approx 50\text{ nm}$), from $L \approx 100\text{ nm}$ ($\text{pH}_b = 5$) to $L \approx 120\text{ nm}$ ($\text{pH}_b = 7$) leads to the decrease in the average concentration of monomer units, $c_p = N_p \sigma L^{-1} / [1 + L/R_c + (L/R_c)^2/3]$, approximately twice. At low salt (i.e., in the osmotic polyelectrolyte brush regime with $\alpha_p c_p / c_s \gg 1$), the degree of ionization α_p of monomer units with dissociation constant K_p varies as $\alpha_p \sim (K_p / c_p)^{1/2}$.¹⁵ As a result, parameter $\lambda \sim c_s / (\alpha_p c_p) \sim c_p^{-1/2}$ slightly increases (by approximately 1.3 times) upon an increase in pH_b from 5 to 7.

The IEP of BSA was experimentally estimated as $\text{pI} = 5.1$, indicating that charging inversion of the protein occurs in the examined range of pH_b . That is, overall protein charge Q_b changes its sign from positive to negative at $\text{pH}_b > 5.1$. By modelling BSA molecule as a compact globule containing

N_Σ available ionizable groups with dissociation constants $K_- = K_+$ (i.e., $u = 1$), the free energy of protein insertion (eq 15) reduces to

$$\frac{\Delta F_{ion}}{N_\Sigma k_B T} = \ln \left(\frac{x+1}{\lambda x+1} \right) - \ln \left(\frac{x_{abs}+1}{\lambda x_{abs}+1} \right) \quad (25)$$

As it follows from Figure 4, the choice of f_+ and λ affects the value of pH_{abs} , corresponding to the protein absorption threshold (shown by dashed lines in Figures 4a and 4b). Recall that $\Delta F_{ion} < 0$ (and the protein absorption is promoted) if $x < x_{abs}$, or, equivalently $\text{pH}_b < \text{pH}_{abs}$. By choosing, e.g., $f_+ = 0.5$ and $\lambda = 0.05$, one finds $\Delta F_{ion} < 0$ for experimentally examined¹⁷ values of $\text{pH}_b = 5.1$ and 6.2 , and $\Delta F_{ion} > 0$ for $\text{pH} = 7.2$. The reduction in absolute value of $\Delta F_{ion} < 0$ is also expected upon addition of salt in buffer solution via the increase in $\lambda \sim c_s$.

In the electrostatically driven protein uptake, the slope of the equilibrium absorption isotherm at low concentrations of BSA is governed by $\exp(-\Delta F_{ion}/k_B T)$. By introducing the difference between x_{abs} and x , $\Delta x = (x_{abs} - x)$ with $0 < \Delta x \ll x_{abs}$, the free energy of protein absorption reduces to

$$\begin{aligned} \frac{\Delta F_{ion}}{N_\Sigma k_B T} &= \ln \left(\frac{x_{abs} + 1 - \Delta x}{\lambda x_{abs} + 1 - \lambda \Delta x} \right) - \ln \left(\frac{x_{abs} + 1}{\lambda x_{abs} + 1} \right) \\ &\approx \Delta x \frac{(1 - \lambda^{-1})}{(1 + x_{abs})(\lambda^{-1} + x_{abs})} \sim -\Delta x \end{aligned} \quad (26)$$

since $\lambda = \exp(e\Psi/k_B T) < 1$. Therefore a larger deviation from the absorption threshold, $\Delta x = (x_{abs} - x) > 0$, should lead to the larger slope of absorption isotherm.

These expectations are in accord with the data in Figures 6 and 8 in ref.¹⁷ A decrease in the initial slope of the absorption isotherm is clearly seen in Figure 6 upon increasing c_s . As for the pH-dependence of the slope in Figure 8, it is difficult to distinguish the difference in slopes for $\text{pH}_b = 5.1$ and $\text{pH}_b = 6.2$ due to extremely sharp increase in the absorbed amount at small concentrations of BSA. However, at $\text{pH}_b = 7.2$, the dramatic decrease in slope is clearly seen, indicating reduction/elimination of the electrostatic driving force. Overall, the conclusion about the electrostatic origin of BSA absorption by PAA colloidal spherical brushes¹⁷ is in reasonable agreement with our theory.

Discussion and conclusions

To summarize, our theory proves that transfer of a polyampholyte molecule (e.g., globular protein) containing pH-sensitive acidic and basic monomeric

groups from the buffered solution into a similarly charged polyelectrolyte brush (or a microgel) can lead to a decrease in the ionic part of the free energy of the system thus making uptake of the polyampholyte molecule thermodynamically favorable. The negative balance in the free energy is assured by overcompensation of the free energy losses due to suppression of ionization of similarly (with respect to the brush) charged monomeric groups by gain in the free energy due to promoted ionization of the oppositely charged ones.

One of the predictions of our theory is separation of the protein absorption threshold by a pH-window from IEP in buffer solution. For both weak and strong polyelectrolyte brushes/microgels, $\lambda = \exp(e\Psi/k_B T) \sim c_s/(\alpha_p c_p)$ decreases upon decreasing c_s in buffer solution and increasing charge concentration ($\alpha_p c_p$) in the polyelectrolyte brush/microgel, widening the window between x_{abs} and x_{IEP} as $x_{abs}/x_{IEP} \sim \lambda^{-f_+}$ (see also Figure 2c).

Hence, in full accordance with experimental observations,^{17,18} our theory correctly predicts that an increase in salt concentration at constant buffer pH_b leading to an increase in $\lambda = \exp(e\Psi/k_B T)$ reduces the driving force and may ultimately suppress uptake of a polyampholyte (protein) by the similarly charged polyelectrolyte brush or microgel at sufficiently high ionic strength of the solution.

When the net re-ionization free energy balance is negative, the polyampholyte net charge changes its sign upon transfer from the bulk of the solution in the brush (charge inversion). However, the opposite is not true, i.e., in spite of earlier claims,²⁸ the charge inversion is not sufficient for making the absorption thermodynamically favorable. In other words, even if the charge inversion of polyampholyte inside the brush/microgel occurs, the re-ionization free energy upon insertion may be still positive. A closer approach to the IEP in terms of pH_b is required to make insertion free energy negative.

Upon calculating the contribution to the free energy of the system related to the presence of ionically charged (ionizable) monomeric groups as a function of the polyampholyte parameters (f_+ , K_- , K_+) and environmental controlled parameters (pH_b, $\lambda = \exp(e\Psi/k_B T)$) we used a number of simplifying approximations.

At fixed pH_b and c_s , uptake of protein molecules from buffer solution by polyelectrolyte brush/microgel is presumed to occur without changing the state of polyelectrolyte brush (i.e., at a fixed value of λ). This assumption is justified for small protein concentrations in buffer solution and no binding of protein molecules to polyelectrolyte chains. Under these conditions, the protein uptake is electrostatically driven, and is accompanied by re-ionization of protein amino acids. For naturally disordered proteins, re-ionization could incorporate all N_Σ ionizable amino acids in the primary sequence. How-

ever, for globular proteins with compact ternary structure, the number N_{Σ} of re-ionizable amino acids could be much less and associated merely with protein/water interface. Notably, the initial slope of the protein absorption isotherm is governed by $\exp(-\Delta F_{ion}/k_B T)$ and is mediated, in particular, by buffer parameters c_s and pH_b .

Another essential assumption used is neglecting (anti)cooperativity of re-ionization process, that is, dependence of the effective ionization constants of the ionogenic groups on their position inside polyampholyte molecule and on the actual charge of the polyampholyte. This approximation implies that ionization of each acidic or basic monomer group is controlled solely by average electrostatic potential inside the brush/microgel and is not affected by charge state of other ionogenic groups in the polyampholyte. Evaluation of the effective ionization constants requires specifying the spacial distribution of ionogenic groups in the polyampholyte molecule (on the surface of the protein globule) that is far beyond the scope of mean-field approach applied here. We also neglected spacial gradients in the charge density and related electrostatic potential distribution inside the brush/microgel.

Furthermore, we assumed pH and $\lambda = \exp(e\Psi/k_B T)$ to be independently controlled parameters that is the case for strong polyelectrolyte brushes/microgels but not for weak (pH-sensitive) ones, where electrostatic potential Ψ is a function of pH in the buffer as well. The dependence of λ on pH_b introduces new feature in the pH-controlled polyampholyte absorption by weak polyelectrolyte brush/microgel and will be studied in our forthcoming publication. The interplay between pH-dependent ionization of weak polyelectrolyte gel and absorbed by the gel proteins was recently investigated by Longo and Szleifer,³⁷ who pointed that re-ionization of the protein upon transfer into the gel significantly enhances absorption.

Finally, we have to stress that we have evaluated only the contributions to the free energy change related to re-ionization of ionogenic monomer groups upon insertion of a polyampholyte (or a protein globule) into polyelectrolyte brush/microgel. There are other contributions to the free energy which have to be accounted for to evaluate the net free energy balance. The insertion of a globule into the brush requires the work to be done against excess osmotic pressure acting in the brush. This leads to a positive contribution ΔF_{vol} to the full insertion free energy which has to be overcompensated by negative ionic contribution ΔF_{ion} evaluated above. The re-ionization may provide a dominant driving force for uptake of weakly charged polyampholytes, intrinsically disordered proteins or, in some cases, of globular proteins with quasi-uniform surface distribution of basic and acidic residues. For most of globular proteins with pronounced patchiness in distribution of positive and negative surface charge the asymmetry of interactions of both with brush-

or gel-forming polyelectrolyte chains and counterion release can be equally or even more relevant in the overall balance of interactions leading to the protein uptake on the "wrong" side of the IEP.

In spite of all these limitations and reservation, we believe that our theory provides a valuable insight into generic physical mechanisms of spontaneous uptake of weak polyampholytes (e.g. globular proteins) by similarly charged polyelectrolyte brushes (or microgels).

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Conflict of interests

The authors declare no conflict of interest.

References

- [1] Kabanov, V.A. In: Dubin, P.; Bock, J.; Davies, R.M.; Schulz, D.N. Thies, C. Editors, *Macromolecular complexes in chemistry and biology* Berlin: Springer , 1994, p. 151-174.
- [2] Kabanov, A.V.; Kabanov, V.A. Interpolyelectrolyte and block ionomer complexes for gene delivery: physico-chemical aspects. *Advanced Drug Delivery Reviews* **1998**, 30 (1-3), 49-60.
- [3] Pergushov, D.V.; Müller, A.H.E.; Schacher, F.H. Micellar Interpolyelectrolyte Complexes. *Chemical Society Reviews* **2012**, 41, 6888-6901.
- [4] Schallon, A.; Synatschke, C.V.; Jerome, V.; Müller, A.H.E.; Freitag, R. Nanoparticulate Nonviral Agent for the Effective Delivery of pDNA and si RNA to Differentiated Cells and Primary Human T Lymphocytes. *Biomacromolecules* **2012**, 13, 3463-3474.
- [5] Malmsten, M; Bysell, H.; Hansson, P. Biomacromolecules in Microgels - Opportunities and Challenges for Drug Delivery. *Current Opinion in Colloid and Interface Science* **2010**, 15,435-444.
- [6] Bysell, H.; Mansson, R.; Hansson, P.; Malmsten, M. Microgels and Microcapsules in Peptide and Protein Drug Delivery. *Adv. Drug Delivery Rev.* **2011**, 63,1172-1185.
- [7] Xu, W.; Rudov, A.A.; Schroeder, R.; Portnov, I.V.; Richtering, W.; Potemkin, I.I.; Pich, A. Distribution of Ionizable Groups in Polyampholyte Microgel Controls Interactions with Captured Proteins: From Blockade and "Levitation" to Accelerated Release. *Biomacromolecules* **2019**, 20, 1578-1591.
- [8] Birshtein, T.M.; Amoskov, V.A. Polymer Brushes. *Polymer Science, Ser. C* **2000**, 42, 172-207.
- [9] Ballauff, M.; Borisov, O. V. Polyelectrolyte brushes. *Current Opinion in Colloid and Interface Science* **2006**, 11, 316-323.
- [10] Minko, S. *Responsive Polymer Materials: Design and Applications* Blackwell Publishing Ltd.: Oxford, 2006
- [11] Rühle, J. ; Ballauff, M.; Biesalski, M.; Dziezok, P.; Gröhn, F.; Johannsmann, D.; Houbenov, N.; Hugenberg, N.; Konradi, R.; Minko S.; Motornov, M.; Netz, R.R.; Schmidt, M.; Seidel, C.; Stamm, M.; Stephan,

- T.; Usov, D.; Zhan, H. Polyelectrolyte Brushes. *Advances in Polymer Science* **2004**, 165, 79-150.
- [12] Borisov, O.V.; Zhulina, E.B.; Leermakers, F.A.M.; Müller, A.H.E. Self-assembled structures of amphiphilic ionic block copolymers: theory, self-consistent field modeling and experiment. *Advances in Polymer Science* **2011**, 241, 57-129.
- [13] Pincus, P.A. Colloid stabilization with grafted polyelectrolytes. *Macromolecules*, **1991**, 24, 2912-2919.
- [14] Borisov, O.V.; Birshtein, T.M.; Zhulina, E.B. Collapse of grafted polyelectrolyte layer. *J.Phys.II*, **1991**, 1, 521-526.
- [15] Borisov, O.V.; Zhulina, E.B.; Leermakers, F.A.M.; Ballauff, M.; Müller, A.H.E. Conformations and solution properties of star-branched Polyelectrolytes. *Advances in Polymer Science* **2011**, 241, 1-55.
- [16] Kramarenko, E.Yu.; Khokhlov, A.R.; Yoshikawa, K. Collapse of Polyelectrolyte Macromolecules Revisited. *Macromolecules* **1997**, 30, 3383-3388.
- [17] Wittmann, A.; Haupt, B.; Ballauff, M. Adsorption of proteins on spherical polyelectrolyte brushes in aqueous solution. *Phys.Chem.Chem.Phys.* **2003**, 5, 1671-1677.
- [18] Wittmann, A.; Ballauff, M. Interaction of proteins with linear polyelectrolytes and spherical polyelectrolyte brushes in aqueous solution. *Phys.Chem.Chem.Phys.* **2006**, 8, 5269-5275.
- [19] Becker, A.L.; Henzler, K.; Welsch, N.; Ballauff, M.; Borisov, O.V. Proteins and polyelectrolytes: A charge relationship. *Current Opimion in Colloid and Interface Science* **2012**, 17, 90-96.
- [20] Welsch, N.; Lu, Y.; Dzubiella, J.; Ballauff, M. Adsorption of proteins to functional polymeric nanoparticles. *Polymer* **2013**, 54, 2835-2849.
- [21] Yigit, C; Welsch, N.; Ballauff, M.; Dzubiella, J. Protein Sorption to Charged Microgels: Characterizing Binding Isotherms and Driving Forces. *Langmuir* **2012**, 28, 14373-14385.
- [22] Achazi, K.; Haag, R.; Ballauff, M.; Dervede, J.; Kizhakkedathu, J.N.; Maysinger, D.; Multhaup, G. Understanding the interactions of polyelectrolyte architectures with proteins and biosystems. *Angewandte Chemie Int. Ed.* **2020**, 59, DOI: 10.1002/anie.202006457

- [23] Czeslik, C.; Wittemann, A. Adsorption mechanism, secondary structure and local distribution of proteins at polyelectrolyte brushes. *Colloid and Polymer Science* **2020**, *298*, 775-789.
- [24] Leermakers F.A.M.; Ballauff, M.; Borisov, O.V. On the mechanisms of interaction of globular proteins with polyelectrolyte brushes. *Langmuir* **2007**, *23*, 237-247.
- [25] Yigit, C.; Heyda, J.; M.; Ballauff, M.; Dzubiella, J. Like-charged protein-polyelectrolyte complexation driven by charge patches. *J.Chem. Phys.* **2015**, *143*, 064905.
- [26] Yigit, C.; Kanduc, M.; Ballauff, M.; Dzubiella, J. Interaction of charged patchy protein models with like charged polyelectrolyte brushes. *Langmuir* **2017**, *33*, 417-427.
- [27] Xu, X.; Angioletti-Uberti, S.; Lu, Y.; Dzubiella, J.; Ballauff, M. Interaction of Proteins with Polyelectrolytes: Comparison of Theory to Experiment. *Langmuir* **2019**, *35*, 5373-5391.
- [28] Biesheuvel, P.M.; Wittemann, A. A modified box model including charged polyelectrolyte brush: self-consistent field theory. *J.Phys.Chem. B* **2005**, *109*, 4209-4214.
- [29] de Vos, W.M.; Leermakers, F.A.M.; de Keizer, A.; Cohen Stuart, M.A.; Klein J.M. Field theoretical analysis of driving forces for the uptake of proteins by like-charge polyelectrolyte brushes: effects of charge regulation and patchiness. *Langmuir* **2010**, *26*, 249-259.
- [30] Zhulina, E.B.; Birshtein, T.M.; Borisov, O.V. Theory of ionizable polymer brushes. *Macromolecules* **1995**, *28*, 1491-1499.
- [31] Klein Wolterink, J.; Leermakers, F.A.M.; Fler, G.J.; Koopal, L.K.; Zhulina, E.B.; Borisov O.V. Screening in Solutions of Star-Branched Polyelectrolytes. *Macromolecules* **1999**, *32*, 2365-2377.
- [32] Currie, E.P.K.; Sieval, A.B.; Fler, G.J.; Cohen Stuart, M.A. Polyacrylic Acid Brushes: Surface Pressure and Salt-Induced Swelling. *Langmuir* **2000**, *16*, 8324-8333.
- [33] Konradi, R.; Rhe, J. Interaction of Poly(methacrylic acid) Brushes with Metal Ions: Swelling Properties. *Macromolecules* **2005**, *38*, 4345-4354.

- [34] del Castillo, G.F.D.; Hailes, R.L.N.; Dahlin, A. Large Changes in Protonation of Weak Polyelectrolyte Brushes with Salt Concentration - Implications for Protein Immobilization. *J. Phys. Chem. Lett.* **2020**, *11* 5212-5218.
- [35] Landsgesell, J.; Hebbeker, P.; Rud, O.; Lunkad, R.; Kosovan, P.; Holm, C. Grand-Reaction Method for Simulations of Ionization Equilibria Coupled to Ion Partitioning. *Macromolecules* **2020**, *53*, 3007-3020.
- [36] Guo, X.; Ballauff, M. Spatial Dimensions of Colloidal Polyelectrolyte Brushes As Determined by Dynamic Light Scattering. *Langmuir* **2000**, *16*, 8719-8726.
- [37] Longo, G.S.; Szleifer, I. Adsorption and protonation of peptides and proteins in pH responsive gels. *J. Phys. D: Appl. Phys.* **2016**, *49* 323001.