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# 1 Polyelectrolyte Brush Interacting with Ampholytic Nanoparticles as 2 Coarse-Grained Models of Globular Proteins: Poisson–Boltzmann 3 Theory

4 Tatiana O. Salamatova, Mikhail Y. Laktionov, Ekaterina B. Zhulina, and Oleg V. Borisov\*



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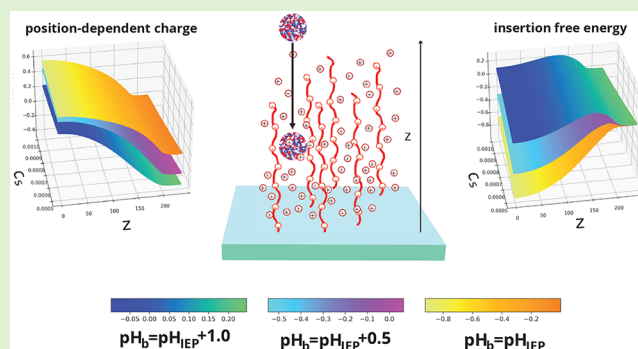


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Supporting Information

5 **ABSTRACT:** The self-consistent field Poisson–Boltzmann frame-  
6 work is applied to analyze equilibrium partitioning of ampholytic  
7 nanoparticles (NPs) between buffer solution and polyelectrolyte  
8 (PE) polyanionic brush. We demonstrate that depending on pH  
9 and salt concentration in the buffer solution, interactions between  
10 ionizable (acidic and basic) groups on the NP surface and  
11 electrostatic field created by PE brush may either lead to the  
12 spontaneous uptake of NPs or create an electrostatic potential  
13 barrier, preventing the penetration of NPs inside PE brush. The  
14 capability of PE brush to absorb or repel NPs is determined by the  
15 shape of the insertion free energy that is calculated as a function of  
16 NP distance from the grafting surface. It is demonstrated that, at a  
17 pH value below or slightly above the isoelectric point (IEP), the  
18 electrostatic free energy of the particle is negative inside the brush and absorption is thermodynamically favorable. In the latter case,  
19 the insertion free energy exhibits a local maximum (potential barrier) at the entrance to the brush. An increase in pH leads to the  
20 shallowing of the free energy minimum inside the brush and a concomitant increase in the free energy maximum, which may result in  
21 kinetic hindering of NP uptake. Upon further increase in pH the insertion free energy becomes positive, making NP absorption  
22 thermodynamically unfavorable. An increase in salt concentration diminishes the depth of the free energy minimum inside the brush  
23 and eventually leads to its disappearance. Hence, in accordance with existing experimental data our theory predicts that an increase  
24 in salt concentration suppresses absorption of NPs (protein globules) by PE brush in the vicinity of IEP. The interplay between  
25 electrostatic driving force for NP absorption and osmotic repelling force (proportional to NP volume) indicates that for large NPs  
26 with relatively small number of ionizable groups osmotic repulsion overcomes electrostatic attraction preventing thereby absorption  
27 of NPs by PE brush.



## 1. INTRODUCTION

28 Electrostatic interactions between charged biomacromolecules  
29 and proteins play an important role in many biological  
30 processes.<sup>1–4</sup> A better understanding of the interactions  
31 between natural polyelectrolytes and proteins could thus  
32 have both fundamental scientific importance and direct  
33 medical implications. Synthetic polyelectrolytes (PEs) and  
34 colloidal polyelectrolyte nanostructures could mimic natural  
35 polyelectrolytes and their assemblies to unravel mechanisms of  
36 molecular processes in living nature, including those of direct  
37 medical relevance (e.g., inflammation or coagulation cascades).  
38 On the other hand, colloidal PE nanostructures, such as  
39 polyelectrolyte brushes, microgels, or block polyelectrolyte  
40 micelles can be used in medical and biotechnological  
41 applications (e.g., drug and gene delivery, inhibition of viral  
42 infection, etc.).<sup>5–14</sup>

43 PE brushes (layers of charged macromolecules terminally  
44 attached to a planar substrate or to the surface of colloidal  
45 particles and immersed in aqueous solution) play a prominent

46 role here, and their interactions with charged species have been  
47 actively explored experimentally and theoretically (see ref 1 for  
48 a comprehensive review). Planar, spherical, and cylindrical PE  
49 brushes are distinguished according to the geometry of the  
50 grafting surface. The latter closely mimic natural glycosami-  
51 noglycans (GAGs) (e.g., aggrecan in articular cartilage,  
52 envisioned as a cylindrical brush with keratan sulfate and  
53 chondroitin sulfate side chains end-tethered to the core  
54 protein<sup>15</sup>), or heparan-sulfate proteoglycans (HSPG) emanat-  
55 ing from the cell surface.<sup>16</sup>

56 In living systems, globular proteins interact with charged  
57 biomacromolecular assemblies that often have complex brush-

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58 like architectures (e.g., layers of bacterial extracellular  
59 polysaccharides, endothelial glycocalyx, complexes of aggre-  
60 canes with hyaluronic acid, etc.) comprising both weak  
61 (carboxyl) and strong (sulfate) anionic groups. Synthetic PE  
62 brushes can be formed by macromolecules comprising either  
63 strong (permanently charged) or weak (pH-sensitive)  
64 ionizable groups. Proteins, on the other hand, are weak  
65 polyampholytes (PAs) that bear pH-sensitive cationic and  
66 anionic groups on their surface. Hence, the net charge of the  
67 protein globule depends on pH, and vanishes in the so-called  
68 isoelectric point,  $pH_{IEP}$ , in which the net charges of cationic  
69 and anionic groups exactly match each other. Hence, the  
70 protein globule possesses a net positive or negative charge at  
71  $pH \leq pH_{IEP}$  or  $pH \geq pH_{IEP}$ , respectively.

72 Under physiological conditions, biomacromolecular brushes  
73 and most of globular proteins are similarly (negatively)  
74 charged, so that their interactions could be mediated by the  
75 Coulomb electrostatic repulsions. On the other hand, seminal  
76 works of M. Ballauff and co-workers convincingly demon-  
77 strated that synthetic polyanionic brushes can efficiently uptake  
78 globular proteins above (on the “wrong side”) the IEP, that is,  
79 when the PE brush and the protein are both negatively  
80 charged.<sup>17–20</sup> It was also demonstrated that absorption of the  
81 proteins is efficient at low and suppressed at high ionic  
82 strength due to the electrostatic nature of attraction between  
83 similarly (negatively) charged PE brushes and proteins.

84 Two possible mechanisms were proposed to explain this  
85 experimental observation: First, it was suggested that, due to  
86 the Donnan equilibrium, pH inside the polyanionic brush may  
87 be lower than  $pH_{IEP}$  even when pH in the buffer is higher than  
88  $pH_{IEP}$  so that the protein which is negatively charged in the  
89 buffer, acquires positive charge inside the negatively charged  
90 brush and, therefore, is spontaneously absorbed by the  
91 brush.<sup>21–23</sup> An alternative hypothesis exploits the fact that  
92 cationic and anionic groups on the protein globule surface are  
93 distributed inhomogeneously and give rise to “patches” of  
94 positive and negative charges. Even when in total the globule is  
95 charged negatively, adsorption of the brush-forming polyanions  
96 on the (minority) patches of positive charge leads to the gain  
97 in the electrostatic free energy which overcompensates the free  
98 energy losses due to depletion from the negatively charged  
99 patches.<sup>24,25</sup> Recent experiments on complexation between  
100 polyelectrolytes and green fluorescent protein mutants with  
101 varied patchiness in the surface charge distribution<sup>26</sup>  
102 convincingly supports the importance of this mechanism of  
103 polyelectrolyte–protein binding. Both hypotheses provide  
104 qualitatively plausible explanation of the protein absorption  
105 by similarly charged PE brushes though both lack quantitative  
106 theoretical justification. Presumably, the reionization may  
107 serve as the dominant driving force for binding of proteins  
108 with fairly uniform (nonpatchy) distribution of cationic and  
109 anionic groups on the globule surface, by strongly charged PE  
110 brushes, including biological ones (e.g., HSPG). This is in a  
111 line with results of numerical self-consistent field calculations  
112 for protein-like ampholytic particles interacting with planar PE  
113 brushes<sup>23</sup> and molecular dynamics simulations of short  
114 ampholytic molecules interacting with a starlike PE.<sup>27</sup> At the  
115 same time, the short-range nonelectrostatic interactions  
116 between protein amino acid residues and the brush monomer  
117 units could also be significant, and in a general case, absorption  
118 of proteins by PE brushes is governed by both types of  
119 interactions.<sup>20</sup>

In our recent paper<sup>28</sup> we have demonstrated that charge  
inversion due to reionization of a weak ampholytic nano-  
particle (NP) upon insertion into a similarly charged PE brush  
or microgel is a necessary but insufficient condition for the  
negative balance in the differential free energy. In other words,  
even if the NP, being negatively charged in the buffer, acquires  
a positive charge inside the negatively charged brush, the net  
ionic interaction balance may disfavor its uptake by the brush.  
This qualitative conclusion was made on the basis of a simple  
Donnan model that disregards the spatial distribution of an  
electrostatic field inside the PE brush/microgel.

Advances in the theory of polyelectrolyte brushes<sup>29,30,32</sup>  
provide a full quantitative analytical description of the  
electrostatic potential and local concentrations of mobile  
ions inside and outside of planar PE brushes within the  
accuracy of the Poisson–Boltzmann approximation. This  
theoretical approach has been recently extended<sup>33</sup> to brushes  
formed by branched polyelectrolytes that mimic natural  
biomacromolecular brushes, and can be applied with  
reasonable accuracy to PE brushes on surfaces of large  
colloidal particles.<sup>34</sup>

In this study, we focus exclusively on the electrostatic driving  
forces with aim to unravel different mechanisms regulating  
uptake of nanoparticles and globular proteins by PE brushes.  
We assimilate globular proteins to weak polyampholytic  
nanoparticles (NPs) with fixed volume and surface area  
covered with both acidic and basic ionizable groups. Although  
such coarse-graining of globular proteins is rather reduction-  
istic, it could still illuminate general trends in the electrostatic  
regulation of the protein–PE brush interactions. The aim of the  
present paper is to implement the Poisson–Boltzmann  
framework to analyze the position-dependent ionization and  
insertion free energy of weak (pH-sensitive) polyampholytic  
NPs, and to examine the effect of environmental conditions  
(pH, salt concentration) and the brush grafting density on the  
shape of insertion free energy curves. This approach provides  
the distribution profiles of NPs inside the brush and may be  
further used to access the diffusion rates of NPs through PE  
brushes.

The rest of the paper is organized as follows: In section 2 we  
introduce our theoretical model and present dominant  
contributions to the NP free energy in the brush. A brief  
summary on the Poisson–Boltzmann theory of PE brushes and  
the review of electrostatic potential distribution is given in  
section 3, while more detailed derivations are delegated to the  
SI. Sections 3.1 and 3.2 are devoted to formulation of the  
electrostatic and osmotic contributions to the insertion free  
energy, respectively. The insertion free energy profiles  
calculated under varied conditions for NPs with one anionic  
and one cationic type of ionizable sites are presented in section  
3.3. Finally, in section 4 we overview the results and outline  
our conclusions.

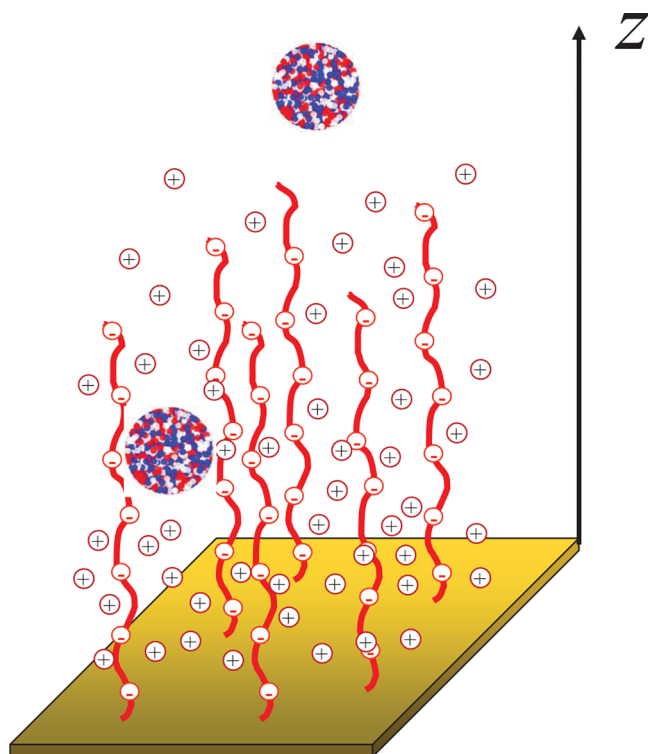
## 2. METHOD

**2.1. Model.** We consider a polyampholytic nanoparticle  
(NP), as a model of a protein in its globular conformation, that  
comprises  $N_+$  ionogenic groups (sites) capable of acquiring  
positive (elementary) charge upon protonation, and  $N_-$   
monomer units capable of acquiring negative (elementary)  
charge upon dissociation of hydrogen ion. The NP has volume  
 $V$  and ionizable groups are assumed to be evenly distributed on  
its surface with area  $\Sigma$ .

180 In the general case, the NP might contain several types of  
181 cationic and anionic ionogenic groups (i.e., amino acid  
182 residues), each characterized by specific acidic ionization  
183 constant  $K_{i\pm}$ . We ascribe the ionization constant  $K_{i-}$  to  $N_{i-}$   
184 anionic groups ( $i_- = 1, 2, \dots$ ) and the ionization constant  $K_{i+}$  to  
185  $N_{i+}$  cationic groups ( $i_+ = 1, 2, \dots$ ). Hence, the total number of  
186 ionizable groups is

$$N_{\Sigma} = N_{+} + N_{-} = \sum_{i+} N_{i+} + \sum_{i-} N_{i-} \quad (1)$$

187  
188 The NP is interacting with a polyelectrolyte brush, Figure 1.  
189 The brush is formed by strong (quenched) polyelectrolytes



**Figure 1.** Schematics of an amphoteric NP insertion into a polyelectrolyte brush. Blue, red, and white areas on the globule surface correspond to cationic, anionic, and neutral residues, respectively.

190 (polyacids) with degree of polymerization  $N \gg 1$  end-  
191 tethered to the surface with density  $\sigma = a^2/s$  ( $a$  is length of  
192 monomer unit and  $s$  is the grafting area per chain). The PE  
193 chains are assumed to be intrinsically flexible (with Kuhn  
194 segment length on the order of monomer unit length,  $a$ ), and  
195 have a fraction  $\alpha$  of (negatively) charged monomer units.

196 The pH in the solution (in the buffer) is fixed to the value of  
197  $\text{pH}_b$ . The solution contains monovalent (positively and  
198 negatively charged) mobile ions of low molecular weight salt  
199 with concentration (number density)

$$c_{b+} = c_{b-} = c_s \quad (2)$$

that controls the Debye screening length

$$\kappa^{-1} = (8\pi l_B c_s)^{-1/2} \quad (3)$$

Here,  $l_B = e^2/\epsilon k_B T$  is the Bjerrum length ( $e$  is the elementary  
charge,  $\epsilon$  is the dielectric permittivity of the solvent,  $k_B$  is the  
Boltzmann constant, and  $T$  is the temperature). In the  
following we assume that  $l_B = a$ , which leads to the  
proportionality factor  $\approx 5$  between molar concentration and  
volume fraction  $C_s = c_s a^3$  of the salt ions.

Because of excess electrostatic potential  $\Psi(z) \leq 0$  created by  
PE brush at distance  $z$  from the grafting surface (with the  
reference value  $\Psi(z = \infty) = 0$ ), and respective gradient in the  
local concentration of hydrogen ions, the replacement of the  
NP from buffer solution into PE brush at distance  $z$  is  
accompanied by reionization of its cationic and anionic groups,  
and leads to the concomitant change in the NP free energy,

$$\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z) \quad (4)$$

Here,  $\Delta F_{\text{ion}}(z)$  and  $\Delta F_{\text{vol}}(z)$  account for reionization free  
energy and for the work against excess osmotic pressure inside  
the brush, respectively.

**2.2. Electrostatic Potential of a Quenched Polyelectrolyte Brush.** The reduced self-consistent electrostatic  
potential,  $\psi(z) \equiv e\Psi(z)/k_B T$ , in a negatively charged (anionic)  
PE brush can be presented as

$$\psi_{\text{in}}(z) = \frac{z^2 - H^2}{H_0^2} + \psi_{\text{in}}(H), \quad 0 \leq z \leq H \quad (5)$$

Here,  $z$  is the distance from the grafting surface,  $H$  is the total  
thickness of the brush (cutoff of the polymer density profile),  
and  $H_0$  is the characteristic length

$$H_0/a = \sqrt{\frac{8}{3\pi^2}} N\alpha^{1/2} \quad (6)$$

The value of constant  $\psi_{\text{in}}(H)$  depends on the potential  
calibration, and ensures continuity of the potential at the brush  
edge,  $\psi_{\text{in}}(H) = \psi_{\text{out}}(H)$ . The potential outside the brush,  
 $\psi_{\text{out}}(z)$ , coincides with that of a uniformly charged plane with  
the surface charge per unit area equal to the residual (negative)  
charge per unit area of the brush

$$\tilde{q} = \int_0^H \rho(z) dz = -\frac{H}{2\pi l_B H_0^2} \quad (7)$$

with the residual charge density  $\rho(z)$  specified by the Poisson  
equation,

$$\frac{d^2 \psi_{\text{in}}(z)}{dz^2} = -4\pi l_B \rho(z) \quad (8)$$

(Here and below, charge is measured in the elementary charge,  
 $e$ , units.) The electrostatic potential outside the brush is thus  
formulated as<sup>30</sup>

$$\psi_{\text{out}}(z) = -2 \ln \left[ \frac{(\kappa \tilde{\Lambda} + \sqrt{(\kappa \tilde{\Lambda})^2 + 1} - 1) + (\kappa \tilde{\Lambda} - \sqrt{(\kappa \tilde{\Lambda})^2 + 1} + 1) e^{-\kappa(z-H)}}{(\kappa \tilde{\Lambda} + \sqrt{(\kappa \tilde{\Lambda})^2 + 1} - 1) - (\kappa \tilde{\Lambda} - \sqrt{(\kappa \tilde{\Lambda})^2 + 1} + 1) e^{-\kappa(z-H)}} \right] \quad (9)$$

with the Gouy–Chapman length



$$\tilde{\Lambda} = \frac{1}{2\pi l_B |\bar{q}|} = \frac{H_0^2}{H} \quad (10)$$

The latter controls distributions of the electrostatic potential and small mobile ions outside the brush, i.e., at  $z \geq H$ . The potential defined by eq 9 vanishes at  $z \rightarrow \infty$ . The cutoff of the polymer density profile,  $z = H$ , is found from the condition of conservation of number of monomer units in grafted chains. The minimal value reached by the electrostatic potential at the grafting surface,  $z = 0$ ,

$$\psi_{in}(0) = -\frac{H^2}{H_0^2} + \psi(H) = -\frac{H^2}{H_0^2} + 2 \ln \left[ \frac{\sqrt{(\kappa \tilde{\Lambda})^2 + 1} - 1}{\kappa \tilde{\Lambda}} \right] \quad (11)$$

is the increasing function of salt concentration  $c_s$  in the buffer but the decreasing function of the grafting density  $\sigma$ . However, the potential difference across the brush,

$$-\psi_{in}(0) + \psi(H) = \frac{H^2}{H_0^2} \quad (12)$$

decreases as a function of salt concentration and increases upon an increase in the grafting density, because the brush thickness  $H$  decreases as a function of salt concentration, but increases as a function of  $\sigma$ .

The polymerization degree  $N$  of the grafted PE chains weakly affects  $\psi(0)$ , while the brush thickness  $H \sim N$ . More detailed derivation and typical shapes of the electrostatic potential profile inside and outside the brush are presented in SI.

Notably, the parabolic shape of electrostatic potential  $\psi_{in}(z)$  in eq 5 holds if the stretched brush-forming chains are still far from full extension (i.e.,  $H \leq L = aN$ ). An approach to full extension of the tethered PE chains ( $H \rightarrow L$ ) modifies electrostatic potential  $\psi_{in}(z)$  as<sup>31</sup>

$$\psi_{in}(z) = \frac{3}{\alpha} \ln \frac{\cos(\pi z/2L)}{\cos(\pi H/2L)}$$

Electrostatic potential  $\psi_{out}(z)$  outside strongly stretched PE brush is given by eq 9 with renormalized Gouy–Chapman length

$$\tilde{\Lambda} = \frac{H_0^2}{H} \frac{(\pi H/2L)}{\tan(\pi H/2L)}$$

In the following we assume that the tethered PEs are moderately charged (with degree of ionization  $\alpha < 1$ ) to ensure the parabolic shape of  $\psi_{in}(z)$ .

### 3. RESULTS AND DISCUSSION

**3.1. Ionic Contribution to the Free Energy.** Transfer of NP from the bulk of solution in PE brush is accompanied by the change in ionization states of both basic and acidic groups on NP surface. The ionic part of the differential free energy can be expressed as<sup>28</sup>

$$\Delta F_{ion}(z)/k_B T = \sum_{i+} N_{i+} \ln \left( \frac{1 - \alpha_{i+}(z)}{1 - \alpha_{bi+}} \right) + \sum_{i-} N_{i-} \ln \left( \frac{1 - \alpha_{i-}(z)}{1 - \alpha_{bi-}} \right) \quad (13)$$

where  $\alpha_{bi+}$ ,  $\alpha_{i+}(z)$ ,  $\alpha_{bi-}$ , and  $\alpha_{i-}(z)$  are the respective degrees of ionization of basic and acidic monomer units on the surface of NP placed at distance  $z$  from the grafting surface and in the bulk of the solution (at  $z = \infty$ ).

Here,

$$\alpha_{i+}(z) = (1 + K_{i+}/[H^+(z)])^{-1} \equiv (1 + 10^{\text{pH}(z) - \text{p}K_{i+}})^{-1} \\ = \left( 1 + \frac{1 - \alpha_{bi+}}{\alpha_{bi+}} \exp(\psi(z)) \right)^{-1} \quad (14)$$

and

$$\alpha_{i-}(z) = (1 + [H^+(z)]/K_{i-})^{-1} \\ \equiv (1 + 10^{\text{p}K_{i-} - \text{pH}(z)})^{-1} \\ = \left( 1 + \frac{1 - \alpha_{bi-}}{\alpha_{bi-}} \exp(-\psi(z)) \right)^{-1} \quad (15)$$

The respective degrees of ionization of ionogenic groups  $i\pm$  in the buffer are

$$\alpha_{bi+} = (1 + K_{i+}/[H^+]_b)^{-1} = (1 + 10^{\text{pH}_b - \text{p}K_{i+}})^{-1} \quad (16)$$

and

$$\alpha_{bi-} = (1 + [H^+]_b/K_{i-})^{-1} = (1 + 10^{\text{p}K_{i-} - \text{pH}_b})^{-1} \quad (17)$$

$[H^+(z)]$  is the local concentration of hydrogen ions (specifying local  $\text{pH}(z) = -\log[H^+(z)]$ ),  $\text{pH}_b \equiv \text{pH}(z = \infty) = -\log[H^+(z = \infty)]$ , and  $K_{i+}$  and  $K_{i-}$  are respective acidic ionization constants of basic and acidic groups.

According to eqs 14 and 15, ionization of acidic and basic ionogenic groups is controlled by local electrostatic potential  $\psi(z)$  in the brush, while the Coulomb interactions between ionized groups on NP surface are disregarded. These interactions could shift the ionization constants with respect to “bare” constants  $K_{i+}$  and  $K_{i-}$  (see refs 35 and 36). Within the applied here approximation,  $\{N_{i\pm}\}$  and  $\{K_{i\pm}\}$  are considered as independent sets of variables. We also neglect the electrostatic potential gradient on the length scale of NP spatial dimensions.

The spacial distribution of hydrogen ions can be expressed as

$$[H^+(z)] = [H^+]_b \exp(-\psi(z)) \quad (18)$$

As long as hydrogen ions are distributed between PE brush and solution according to eq 18, lower electrostatic potential inside the brush,  $\Psi(z) \leq 0$ , indicates that local concentration of  $H^+$  ions inside the brush is larger than in the bulk of the solution. As a result, the degree of ionization of basic residues inside polyanionic brush is higher, while the degree of ionization of acidic residues is lower than in the bulk of the solution (eqs 14 and 15). The sign of  $\Delta F_{ion}(z)$  is determined by the balance between reionization free energies of basic and acidic monomer units (the first and the second terms in eq 13, respectively).

The net charge on NP (measured in the elementary charge units  $e$ ) depends on its position  $z$  with respect to the grafting surface and can be expressed as

$$Q(z) = \sum_{i+} N_{i+} \alpha_{i+}(z) - \sum_{i-} N_{i-} \alpha_{i-}(z) \quad (19)$$

while the NP charge in the buffer is given by

$$Q_b = Q(z = \infty) = \sum_{i+} N_{i+} \alpha_{bi+} - \sum_{i-} N_{i-} \alpha_{bi-} \quad (20)$$

The isoelectric point,  $\text{pH}_{IEP}$ , corresponds to  $\text{pH}_b$  at which the NP charge in the buffer vanishes,  $Q_b = 0$ .

We focus primarily on the case of  $\text{pH}_b \geq \text{pH}_{IEP}$ , that is, when the NP in the bulk of the solution is charged negatively,

336  $Q_b \leq 0$ , i.e., similarly to the brush. Because the electrostatic  
337 potential  $\psi(z)$  created by the brush is a monotonically  
338 increasing function of  $z$  (i.e., monotonically increasing in  
339 absolute value upon approaching the grafting surface), the net  
340 charge of the NP  $Q(z)$  monotonically increases upon  
341 approaching the grafting surface and, depending on the sets  
342 of parameters  $\{N_{\pm}, K_{\pm}\}$ ,  $\{c_s, \text{pH}_b\}$ ,  $\{\alpha, N, \sigma\}$ , may remain  
343 negative or invert its sign to positive at some particular  
344 distance  $z^*$  from the grafting surface, so that

$$Q(z = z^*) = 0$$

345 Remarkably,  $z^*$  may correspond to the NP position either  
346 inside, or outside the brush (close to its edge). The position  $z^*$   
347 can be found from the equation

$$348 \psi(z^*) = -\Delta\text{pH}_b / \log_{10} e \approx -2.3\Delta\text{pH}_b \quad (21)$$

349 with

$$\Delta\text{pH}_b = \text{pH}_b - \text{pH}_{\text{IEP}}$$

350 which implies (with the account of eq 18) that local  
351  $\text{pH}(z = z^*)$  at  $z = z^*$  coincides with  $\text{pH}_{\text{IEP}}$  for NP in solution.  
352 Using eqs 13 and 19, one can demonstrate that

$$353 \left( \frac{\partial \Delta F_{\text{ion}}(z)}{\partial z} \right)_{z^*} = 0 \quad (22)$$

354 that is, the ionization free energy passes through a maximum at  
355  $z = z^*$ , i.e., in the point of the charge inversion. Indeed,

$$\frac{\partial \Delta F_{\text{ion}}(z)}{\partial z} = \frac{\partial \Delta F_{\text{ion}}}{\partial \lambda} \cdot \frac{\partial \lambda(z)}{\partial z}$$

356 where  $\lambda(z) \equiv \exp(\psi(z))$ . By taking the derivative of the r.h.s.  
357 of eq 13 with respect to  $\lambda(z)$  (with the account of eqs 14 and  
358 15), one arrives at

$$359 \frac{\partial \Delta F_{\text{ion}}(z)}{\partial z} = \frac{1}{\lambda} \frac{\partial \lambda(z)}{\partial z} \cdot Q(z) = \frac{\partial \psi(z)}{\partial z} \cdot Q(z) \quad (23)$$

360 where the position-dependent charge  $Q(z)$  of NP is given by  
361 eq 19. As soon as  $\lambda(z)$  is a positive, monotonously increasing  
362 function of  $z$  in the whole range of  $z \in [0, \infty)$ , eq 23 indicates  
363 that  $\Delta F_{\text{ion}}(z)$  exhibits a maximum in the NP charge inversion  
364 point,  $z = z^*$ .

365 When  $\Delta F_{\text{ion}}(z = 0) \leq 0$  and  $z^* \geq 0$ , absorption of NP by the  
366 brush is thermodynamically favorable, and the minimum in  
367  $\Delta F_{\text{ion}}(z)$  (reached at the grafting surface,  $z = 0$ ) is separated  
368 from the exterior solution by the potential barrier with the  
369 height  $\Delta F_{\text{ion}}(z^*)$  located at  $z = z^*$ . As we demonstrate below,  
370 the charge inversion may occur at  $z = z^* \geq 0$  even when  
371  $\Delta F_{\text{ion}}(z = 0) \geq 0$ . In this case, the localization of positively  
372 charged NP inside polyanionic brush corresponds to a  
373 metastable state. Finally, when  $z^*$  decreases down to zero,  
374 the charge inversion inside the brush does not take place and  
375 the insertion free energy  $\Delta F_{\text{ion}}(z)$  becomes a monotonically  
376 decreasing function of  $z$ . As a result, the Coulomb force pushes  
377 NP out of the brush and no absorption of NPs by PE brush  
378 occurs spontaneously.

379 Obviously, if NP is charged positively in the solution,  
380  $Q_b \geq 0$ , then  $\Delta F_{\text{ion}}(z) \leq 0$  at  $\forall z \in [0, \infty)$  and is a  
381 monotonically increasing function,  
382  $\partial \Delta F_{\text{ion}}(z) / \partial z \geq 0 \forall z \in [0, \infty)$ .

383 **3.2. Osmotic Contribution to the Free Energy.** The  
384 second term in eq 4 accounts for the work against excess

osmotic pressure of mobile ions inside the brush and in the 385  
double electrical layer outside. If the concentrations of mobile 386  
monovalent ions of low molecular weight salt in the buffer (at 387  
 $z = \infty$ ) are  $c_{b+} = c_{b-} = c_s$ , then their concentrations at arbitrary 388  
distance  $z$  from the grafting surface are given by the Boltzmann 389  
law, 390

$$c_{\pm}(z) = c_s \exp(\mp\psi(z)) \quad (24) \quad 391$$

and the excess osmotic pressure is 392

$$\Pi(z) / k_B T = c_+(z) + c_-(z) - 2c_s = \quad (25) \quad 393$$

$$c_s (\exp(\psi(z)/2) - \exp(-\psi(z)/2))^2 = 4c_s \sinh^2(\psi(z)/2) \quad (26) \quad 394$$

Hence, the osmotic contribution to the insertion free energy, 395

$$\Delta F_{\text{vol}}(z) / k_B T = 4Vc_s \sinh^2(\psi(z)/2) \quad (27) \quad 396$$

is proportional to NP volume  $V$  and monotonically decreases 397  
with  $z$ . Hence, the derivative of this second term in the free 398  
energy in eq 4 always provides a thermodynamic force 399  
expelling the NP from the brush. 400

We remark that in sufficiently dense or/and weakly charged 401  
brushes the nonelectrostatic (excluded volume) interactions 402  
provide the dominant contribution to the osmotic pres- 403  
sure.<sup>37–39</sup> However, in the present work we focus at sufficiently 404  
strongly charged brushes in which the electrostatic interactions 405  
dominate over the short-range repulsions between monomer 406  
units. 407

**3.3. NP with Two Types of Ionizable Sites.** Below we 408  
focus on a particular case when the NP comprises one type of 409  
cationic groups with ionization constant  $K_+$  and one type of 410  
anionic groups with the ionization constant  $K_-$ . This 411  
simplification allows us to analyze the main trends in NP 412  
interaction with PE brush at variable environmental conditions 413  
( $\text{pH}_b$  and  $c_s$ ) and NP volume  $V$ . 414

The fraction of positively charged ionizable monomer units 415  
is defined as 416

$$f_+ = \frac{N_+}{N_- + N_+} \equiv \frac{N_+}{N_{\Sigma}} \quad (28) \quad 417$$

where  $N_+ + N_- = N_{\Sigma}$  is the total number of ionizable (basic 418  
and acidic) groups, and the ionic contribution  $\Delta F_{\text{ion}}$  in eq 13 419  
with  $i_+ = i_- = 1$  reduces to 420

$$\Delta F_{\text{ion}}(z) / k_B T = N_{\Sigma} \left[ f_+ \ln \left( \frac{1 - \alpha_+(z)}{1 - \alpha_{b+}} \right) + (1 - f_+) \ln \left( \frac{1 - \alpha_-(z)}{1 - \alpha_{b-}} \right) \right] \quad (29) \quad 421$$

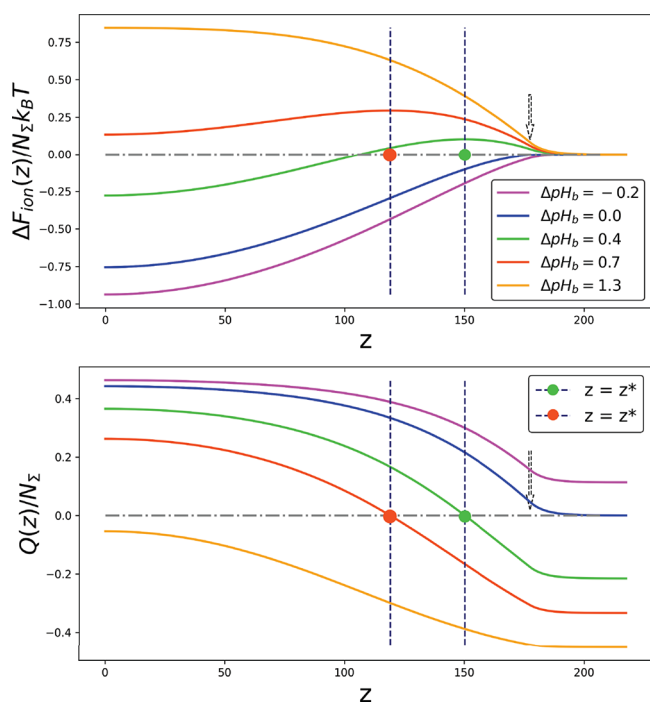
In section 3.3.1, we start with the analysis of  $\Delta F_{\text{ion}}(z)$ , which 422  
is the dominant contribution to the overall free energy, 423  
 $\Delta F(z) \approx \Delta F_{\text{ion}}(z)$ , in the case of small NPs. In the following 424  
section 3.3.2 we consider the interplay between  $\Delta F_{\text{ion}}(z)$  and 425  
 $\Delta F_{\text{vol}}(z)$  which becomes essential for increasingly bulky 426  
charged species and may prevent NP absorption by PE 427  
brush even if the reionization free energy,  $\Delta F_{\text{ion}}$ , provides a 428  
driving force for it. 429

**3.3.1. Reionization Free Energy for NP Interacting with** 430  
**Polyelectrolyte Brush.** Below we present typical ionization free 431  
energy profiles  $\Delta F_{\text{ion}}(z)$  normalized by the total number of 432  
ionizable sites  $N_{\Sigma}$  calculated for the NP with  $f_+ = 0.5$  at varied 433  
 $\text{pH}_b$ , reduced salt concentration  $C_s = c_s a^3$  and grafting density 434  
 $\sigma = a^2/s$ . We use model equal values of ionization constants 435  
 $pK_- = pK_+ = 5$  that assures maximal impact of the reionization 436

437 free energy to the overall insertion free energy balance and  
 438 corresponds at  $f_+ = 0.5$  to the IEP  $\text{pH}_{\text{IEP}} = 5$ . In real experiment  
 439 chosen parameters are sufficiently close to the  $\text{pK}_-$  value for  
 440 carboxylic groups and not far from  $\text{pK}_+$  value for, e.g.,  
 441  $\text{N}(\text{CH}_3)_2$  groups.

442 The degree of polymerization of the brush-forming chains  
 443  $N = 300$ . Notably,  $C_s$  and  $\sigma$  affect the magnitude of the excess  
 444 electrostatic potential created by the brush, whereas  $\text{pH}_b$   
 445 controls degrees of ionization of the cationic and anionic  
 446 residues and the net charge of the globule  $Q_b$  in the buffer.

447 In Figure 2a,b, the position-dependent free energy  $\Delta F_{\text{ion}}(z)$   
 448 and charge  $Q(z)$  are plotted as a function of distance  $z$  from



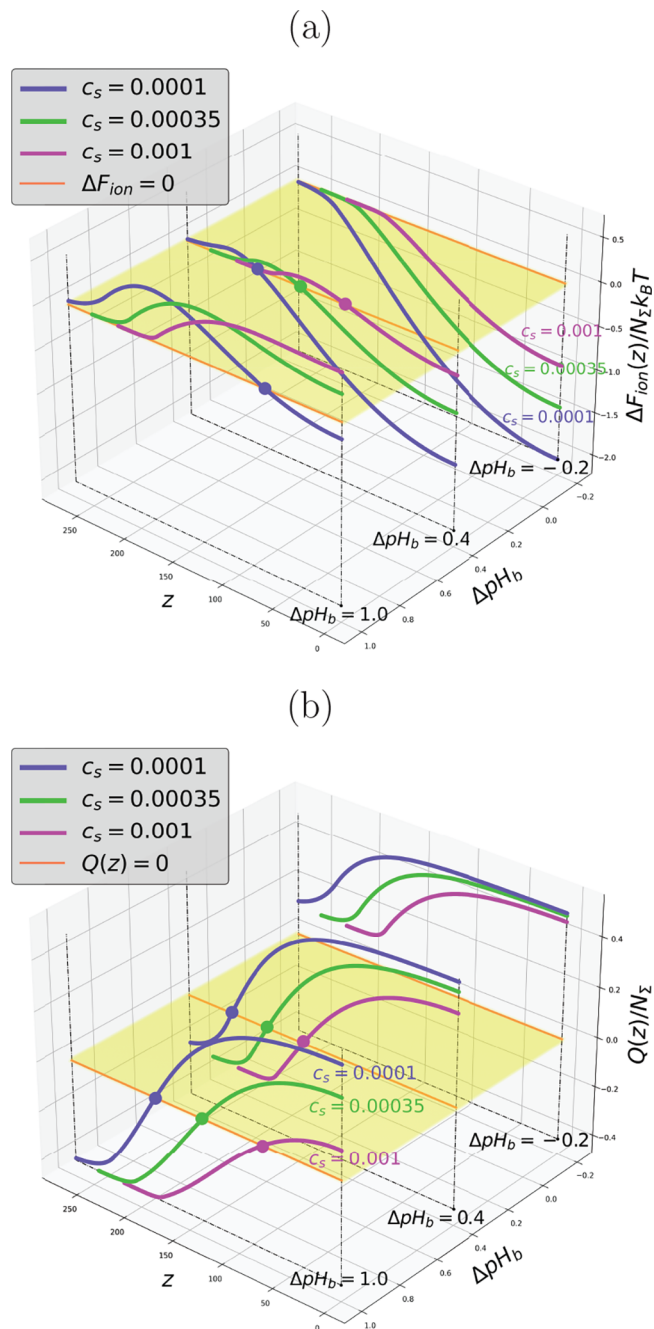
**Figure 2.** Position-dependent insertion free energy  $\Delta F_{\text{ion}}(z)$  (a) and the NP charge  $Q(z)$  (b) both normalized by  $N_\Sigma$ , as a function of the distance  $z$  from the grafting surface for a series of  $\text{pH}_b$  values around the IEP at fixed salt concentration  $C_s = 10^{-3}$ . Arrows indicate the upper boundary of the brush. Dashed vertical lines indicate the charge inversion point,  $z = z^*$ . Dashdot horizontal lines indicate  $\Delta F_{\text{ion}}(z) = 0$  and  $Q(z) = 0$ . Colored circles in panels (a) and (b) correspond to the charge inversion points  $z = z^*$ .

449 the grafting surface for a series of  $\text{pH}_b$  values at constant salt  
 450 concentration  $C_s = 10^{-3}$ .

451 In Figure 3a,b, we show cross sections of 2D profiles of the  
 452 insertion free energy  $\Delta F_{\text{ion}}(z, \Delta\text{pH}_b)$ , and net charge  
 453  $Q(z, \Delta\text{pH}_b)$  of the NP plotted at three selected values of  
 454 salt concentration  $C_s$ .

455 As one can see from Figures 2b and 3b and follows from eq  
 456 19, because of reionization of cationic and anionic sites in the  
 457 electrostatic potential of the polyanionic brush, net charge  
 458  $Q(z)$  of the NP is always a decreasing function of  $z$ .

459 Sufficiently far above the IEP (e.g., at  $\Delta\text{pH}_b = 1.3$  in Figure  
 460 2a,b), the NP is negatively charged in the buffer and remains  
 461 negatively charged,  $Q(z) \leq 0 \forall z \in [0, \infty)$ , though  $|Q(z)|$   
 462 decreases in the absolute value upon insertion of the NP into  
 463 the brush. The insertion free energy in this case is positive,  
 464  $\Delta F_{\text{ion}}(z) > 0 \forall z \in [0, \infty)$ , and monotonously decreasing



**Figure 3.** Cross sections of the 2D profiles of the insertion free energy  $\Delta F_{\text{ion}}(z, \Delta\text{pH}_b)$  (a) and NP charge  $Q(z, \Delta\text{pH}_b)$  (b), both normalized by  $N_\Sigma$ , for a set of values of salt concentration (with the corresponding color code, as indicated at the curves). Colored circles in panel (a) indicate points of vanishing free energy,  $\Delta F_{\text{ion}}(z, \Delta\text{pH}_b) = 0$ ; colored circles in panel (b) correspond to the charge inversion points  $z = z^*$ .

function of  $z$ ,  $\partial\Delta F_{\text{ion}}(z)/\partial z \leq 0$ , that is, the NP is expelled  
 from the brush.

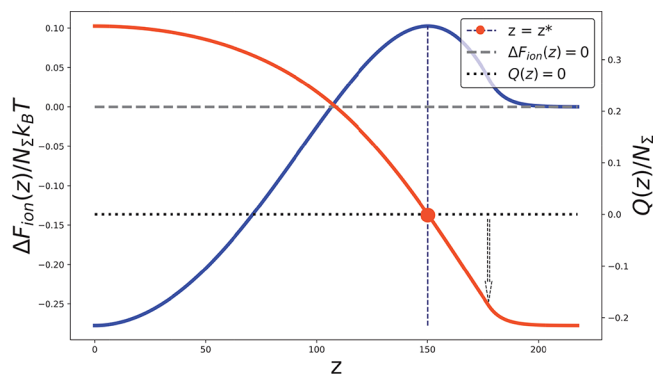
On the contrary, below the IEP, at  $\Delta\text{pH}_b < 0$ , the charge of  
 the NP is positive,  $Q(z) > 0 \forall z \in [0, \infty)$  (Figures 2b and 3b).  
 The free energy  $\Delta F_{\text{ion}}(z) < 0 \forall z \in [0, \infty)$  is negative and  
 monotonously decreases upon approaching the grafting surface  
 (Figures 2a and 3a). In this case, the NP and the brush are  
 oppositely charged, and the NP is driven into the brush by the  
 attractive Coulomb force. The depth of the free energy edge  
 minimum at the grafting surface,  $\Delta F_{\text{ion}}(z = 0)$ , grows upon a



475 decrease in  $\Delta pH_b$ , or upon an increase in the magnitude of the  
 476 negative electrostatic potential at the surface which can be  
 477 induced either by a decrease in salt concentration  $C_s$  (Figure  
 478 S2a) or by an increase in grafting density  $\sigma$  (Figure S2b). The  
 479 same applies also at the IEP,  $pH = pH_{IEP}$ , when the NP charge  
 480 is vanishing far away from the brush, but becomes positive and  
 481 monotonously increases when NP approaches and enters the  
 482 brush.

483 The most peculiar patterns in  $\Delta F_{ion}(z)$  and  $Q(z)$  are  
 484 observed at  $\Delta pH_b \geq 0$ , i.e., slightly above the IEP, as illustrated  
 485 by Figure 2a,b and the cross-section of the  $\Delta F_{ion}(z, \Delta pH_b)$   
 486 surface at  $\Delta pH_b = 0.4$  in Figure 3a,b. In this case NP is charged  
 487 negatively in the buffer,  $Q_b < 0$ , but inverts the sign of its  
 488 charge upon embedding into the brush at  $z = z^*$ . That is, the  
 489 NP charge  $Q(z \geq z^*) \leq 0$  and  $Q(z \leq z^*) \geq 0$ . As  
 490 demonstrated above, the free energy  $\Delta F_{ion}(z)$  passes through a  
 491 maximum in the charge inversion point  $z = z^*$ , i.e.,  $\Delta F_{ion}(z)$  is  
 492 an increasing function of  $z$  at  $z \in [0, z^*]$  and a decreasing  
 493 function of  $z$  at  $z \in [z^*, \infty)$ . Obviously,  $z^* \rightarrow \infty$  at  $pH_b \rightarrow$   
 494  $pH_{IEP}$ .

495 In Figure 4 we present a typical zoomed profile of the free  
 496 energy  $\Delta F_{ion}(z)$  with maximum, corresponding to the  $Q(z)$



**Figure 4.** Characteristic dependence of the  $\Delta F_{ion}(z)$  with a maximum at  $z = z^*$  and corresponding dependence  $Q(z)$  with charge inversion at  $z = z^*$  plotted for  $\Delta pH_b = 0.4$  both normalized by  $N_\Sigma$ . Salt concentration is  $C_s = 10^{-3}$ , grafting area per chain is  $s/a^2 = 100$ . Arrow indicate the upper boundary of the brush ( $z = H$ ), and the charge inversion point  $z = z^*$  is indicated by vertical dashed line and a red circle.

497 sign inversion point, and  $\Delta F_{ion}(z = 0) < 0$ . This free energy  
 498 profile indicates the presence of electrostatic driving force for  
 499 NP absorption by PE brush.

500 Remarkably, similar patterns in the position-dependent net  
 501 charge were observed in Molecular Dynamic simulations<sup>27</sup> of  
 502 ampholytic oligopeptide interacting with strongly charged  
 503 starlike polyelectrolyte: in the vicinity of the IEP the  
 504 oligopeptide charge inversion was observed upon approaching  
 505 the center of the star. However, quantitative comparison of our  
 506 results to those presented in ref<sup>27</sup> is challenging because of  
 507 essentially different spatial distribution of polymer density and  
 508 electrostatic field in a planar PE brush and in a single PE star.

509 As seen in Figure 3a,b, an increase in salt concentration (at a  
 510 given  $\Delta pH_b$ ) leads to the shift in the charge inversion point  $z^*$   
 511 toward the grafting surface, the decrease in  $Q(z)$ , and  
 512 narrowing of the presurface potential well which simulta-  
 513 neously becomes more shallow.

514 Upon a moderate increase in  $pH_b$  above the IEP, i.e., upon a  
 515 decrease in the NP charge  $Q_b < 0$  in the buffer, the charge

inversion point  $z^*$  is shifted toward the grafting surface, the  
 magnitude of the maximum in  $\Delta F_{ion}(z)|_{z=z^*}$  increases, and the  
 depth of the presurface potential well,  $|\Delta F_{ion}(z = 0)|$ , decreases.  
 In this regime, absorption of NPs by the brush remains  
 thermodynamically favorable, but requires overcoming of the  
 potential barrier with maximum at  $z = z^*$ . At sufficiently large  
 number of ionizable groups on NP surface,  $N_\Sigma \sim 10^2$ , the  
 barrier may increase up to  $\sim 10k_B T$  thus completely hindering  
 NP absorption by PE brush kinetically.

When  $\Delta pH_b$  exceeds the threshold value  $\Delta pH_b^{(abs)} \geq 0$  (that  
 is,  $pH_b$  exceeds  $pH_b^{(abs)} > pH_{IEP}$ ), the free energy  $\Delta F_{ion}(z)$   
 becomes positive at  $\forall z \in [0, \infty)$ . The free energy minimum at  
 $z = 0$  remains separated from the bulk of the solution by the  
 potential barrier located at  $z = z^*$ . Hence, the NP location  
 inside the brush at  $0 \leq z \leq z^*$  corresponds to a metastable  
 state although the globule possesses positive net charge,  
 $Q(z \leq z^*) \geq 0$  (see, e.g., the curves corresponding to  
 $\Delta pH_b = 0.7$  in Figure 2a,b). Indeed, the local free energy  
 minimum at  $z = 0$  is separated from the global free energy  
 minimum  $\Delta F_{ion}(z = \infty) = 0$  by a potential barrier at  $z = z^*$ ,  
 which implies that, by definition,<sup>40</sup> the position of the NP in  
 the brush corresponds not to an equilibrium, but to a  
 metastable state.

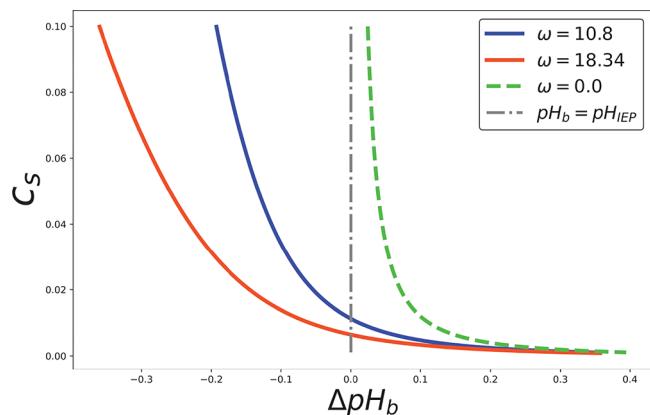
Further increase in  $pH_b$  results in simultaneous increase in  
 the magnitude of the potential barrier at  $z = z^*$  and its  
 displacement toward the grafting surface and concomitant  
 decrease in the depth and width of the potential well.  
 Eventually,  $z^* \rightarrow 0$ , and the free energy  $\Delta F_{ion}(z)$  becomes a  
 positive and monotonously decreasing function of  $z$ , so that  
 the NP–PE brush interaction becomes purely repulsive.

Because the position-dependent net charge  $Q(z)$  and the  
 free energy  $\Delta F_{ion}(z)$  depend on the brush local electrostatic  
 potential  $\psi(z)$ , both properties are strongly affected by salt  
 concentration in the buffer. The magnitude of  $\psi(z)$  decreases,  
 and its range shrinks as the salt concentration in the buffer  
 increases (see Figure S2a in the Supporting Information).  
 Therefore, the equilibrium absorption threshold can be crossed  
 also by an increase in the salt concentration at constant  $pH_b$ , as  
 demonstrated by series of the cross sections of the  
 $\Delta F_{ion}(z, \Delta pH_b)$  surfaces corresponding to  $\Delta pH_b = 1.0$  and  
 different values of  $C_s$  in Figure 3a,b. For given set of parameters  
 $\{f_+, K_+, K_-\}$  the defined above threshold  $pH_b^{(abs)}$  depends on  
 salt concentration  $C_s$ , as demonstrated in Figure 5.

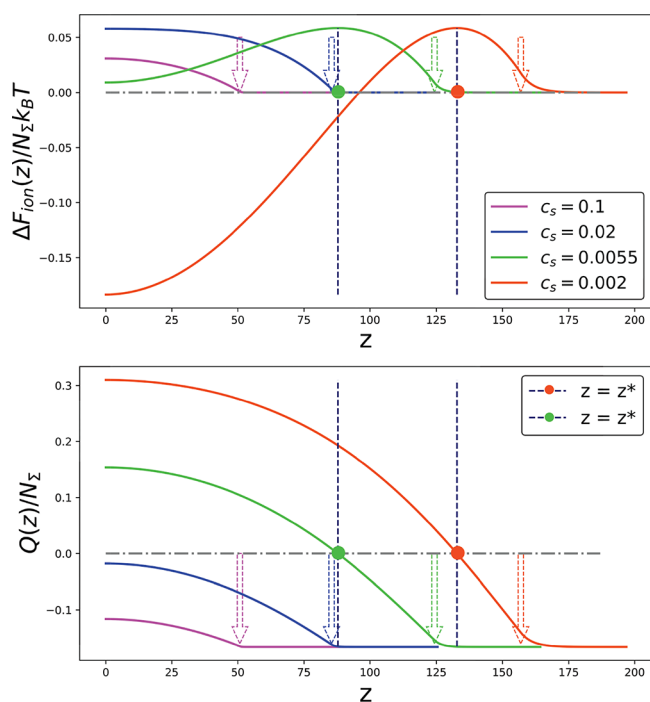
In Figure 6a,b, we present  $\Delta F_{ion}(z)$  and  $Q(z)$  profiles for a  
 number of selected salt concentrations  $C_s$  and constant  
 $\Delta pH_b = 0.3$ . In Figure 7a,b, we show cross sections of 2D  
 profiles of the insertion free energy  $\Delta F_{ion}(z, c_s)$ , and net charge  
 $Q(z, c_s)$  of the NP plotted at three selected values of  $\Delta pH_b$   
 above the IEP. The values of  $pH_b$  are chosen sufficiently close  
 to  $pH_{IEP}$  so that, at low salt concentration,  $C_s = 10^{-3}$ , the  
 $\Delta F_{ion}(z)$  exhibits pronounced maxima at the charge inversion  
 points and the edge minima at  $z = 0$ .

An increase in salt concentration leads to weakening of the  
 electrical field created by PE brush, and the magnitude of the  
 electrostatic potential  $|\psi(z = 0)|$  decreases. Upon an increase in  
 salt concentration, the charge inversion point  $z = z^*$  is shifted  
 to a smaller  $z$ , the free energy at the grafting surface,  
 $\Delta F_{ion}(z = 0)$ , increases, changes its sign from negative to  
 positive, and  $\Delta F_{ion}(z)$  acquires a monotonously decreasing  
 shape corresponding to expulsion of NPs from PE brush. The  
 larger the  $\Delta pH_b$ , the smaller the salt concentration is needed to  
 suppress charge inversion and eliminate the potential well at  
 the grafting surface.

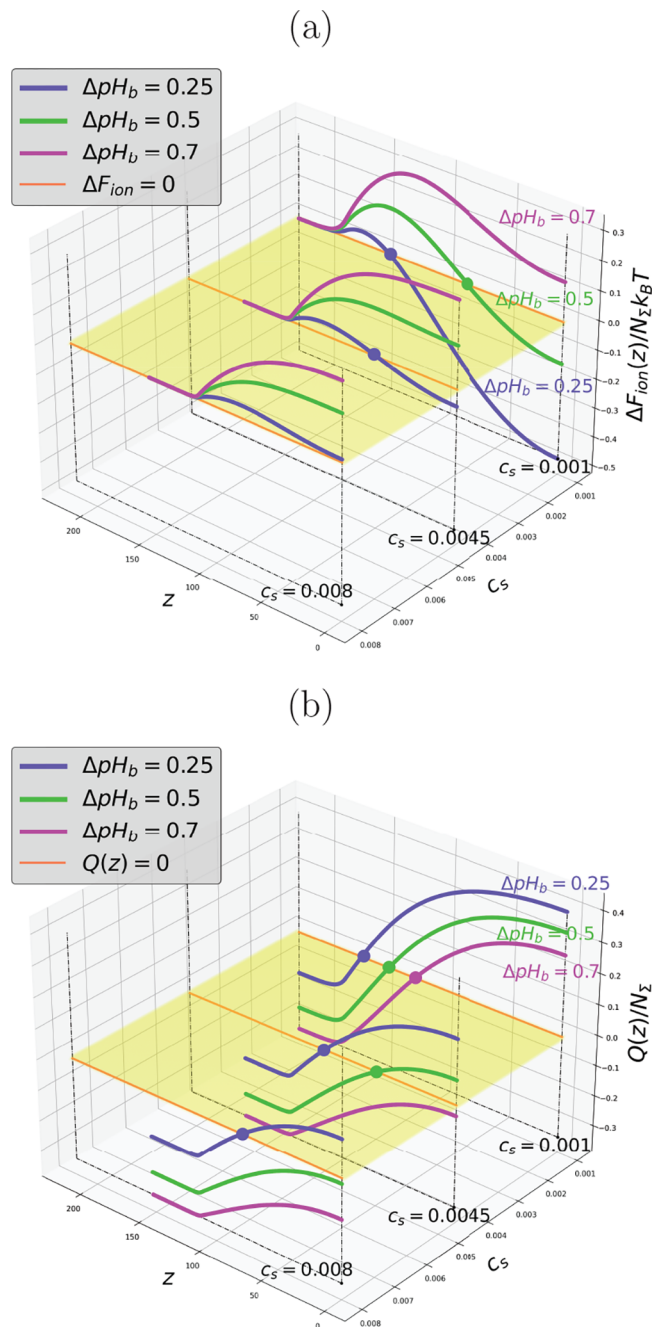




**Figure 5.** Dependences of salt concentration  $C_s^{(\max)}$  corresponding to inversion of sign of  $\Delta F_{\text{ion}}(z=0)$  (dashed line) and  $C_s^{(\max, \omega)}$  corresponding to inversion of sign of  $\Delta F_{\text{ion}}(z=0) + \Delta F_{\text{vol}}(z=0)$  (solid lines) for selected values of  $\omega$  (see color code in the figure) on deviation  $\Delta \text{pH}_b$  from the IEP.



**Figure 6.** Position-dependent insertion free energy  $\Delta F_{\text{ion}}(z)$  (a) and the NP charge  $Q(z)$  (b), both normalized by  $N_\Sigma$ , as a function of the distance  $z$  from the grafting surface for a series of salt concentration  $C_s$  values at  $\Delta \text{pH}_b = 0.3$ . Arrows indicate the salt-dependent position of the upper boundary of the brush. Dashed vertical lines indicate the charge inversion points,  $z = z^*$ . Dashdot horizontal lines indicate  $\Delta F_{\text{ion}}(z) = 0$  and  $Q(z) = 0$ . Colored circles in panels (a) and (b) correspond to the charge inversion points  $z = z^*$ .



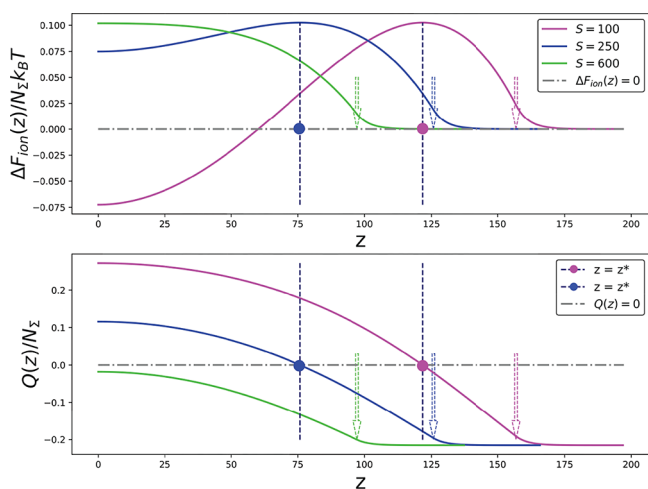
**Figure 7.** Cross sections of the 2D profiles of the insertion free energy  $\Delta F_{\text{ion}}(z, c_s)$  (a) and NP charge  $Q(z, c_s)$  (b), both normalized by  $N_\Sigma$ , for a set of values of  $\text{pH}_b$  (with the corresponding color code, as indicated at the curves). Colored circles in panel (a) indicate points of vanishing free energy,  $\Delta F_{\text{ion}}(z, \Delta \text{pH}_b) = 0$ ; colored circles in panel (b) correspond to the charge inversion points  $z = z^*$ .

579 Remarkably, if  $\Delta \text{pH}_b > 0$  and  $Q|_{z=0} < 0$  or, on the contrary,  
 580  $\Delta \text{pH}_b < 0$  and  $Q|_{z=0} > 0$ , the addition of salt does not lead to  
 581 qualitative changes in the shape of the  $\Delta F_{\text{ion}}(z)$  curves: They  
 582 are monotonously decreasing or monotonically increasing in  
 583 the former and in the latter case, respectively, but their  
 584 magnitudes (the height of the potential barrier or the depth of the potential  
 585 well) as well as the range of the potential  
 586 (proportional to the brush thickness  $H$ ) decrease.

587 The effect of the grafting density  $\sigma = a^2/s$  on the  $\Delta F_{\text{ion}}(z)$   
 588 curves is illustrated by Figure 8: An increase in the grafting

density enhances electrostatic potential, causes additional  
 stretching of the brush-forming PE chains (an increase in the  
 brush thickness  $H$ ) and might drive NP absorption into  
 sufficiently dense PE brush.

3.3.2. *Interplay between NP Charge and Volume.* For an  
 ampholytic NP (protein globule) the ionic contribution to the  
 insertion free energy  $\Delta F_{\text{ion}}(z)$  is complemented by the osmotic  
 term  $\Delta F_{\text{vol}}(z)$ . In this subsection we unravel the competitive  
 effects of the electrostatic (attractive) and osmotic (repulsive)  
 forces on NP absorption by PE brush.



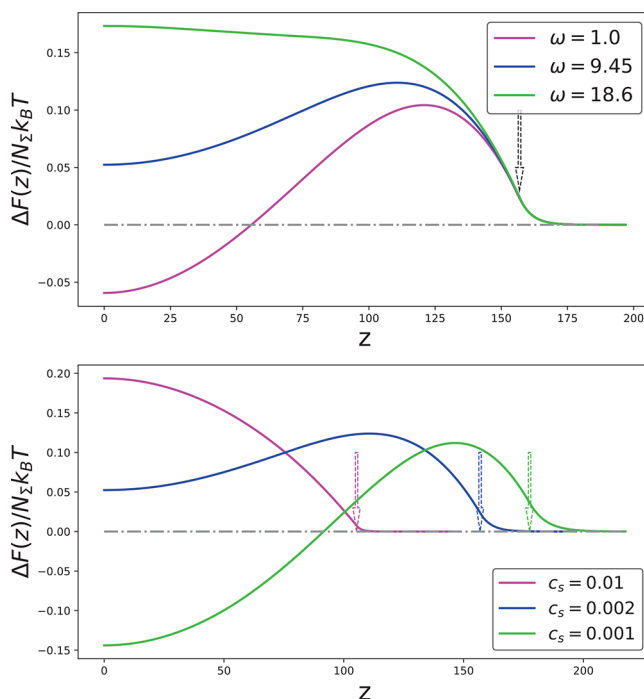
**Figure 8.** Position-dependent insertion free energy  $\Delta F_{\text{ion}}(z)$  (a) and the NP charge  $Q(z)$  (b) both normalized by  $N_{\Sigma}$ , as a function of the distance  $z$  from the grafting surface for a series of  $s = a^2/\sigma$  values at  $C_s = 10^{-3}$ ,  $\Delta\text{pH}_b = 0.4$  (with the corresponding color code, as indicated at the curves). Dashed vertical lines indicate the charge inversion point,  $z = z^*$ . Dashdot horizontal lines indicate  $\Delta F_{\text{ion}}(z) = 0$  and  $Q(z) = 0$ . Colored circles in panels (a) and (b) correspond to the charge inversion points  $z = z^*$ .

**Table 1. Proteins Corresponding to the  $\omega$  Coefficient**

$\omega$	name of the protein
2.0	human serum albumin
3.2	Des-Phe B1 bovine insulin
3.5	phospholipase A2 (PLA2) from <i>Naja naja</i> venom
3.7	$\alpha$ -lactalbumin
4.9	human interleukin-6
5.3	human hemoglobin A2
6.1	human sex hormone-binding globulin
7.5	interleukin-37
8.6	bovine $\beta$ -lactoglobulin
10.3	bromoperoxidase A1
15.7	human interleukin-17A
29.2	pumpkin seed globulin

For given globule volume  $V$ ,  $\Delta F_{\text{vol}}(z)$  in eq 27 (as well as  $628$   
 $|\psi(z)|$  in eqs 5 and 9) is a decreasing function of salt  $629$   
concentration  $c_s$  in the buffer, and an increasing function of the  $630$   
brush grafting density  $\sigma = a^2/s$ .  $631$

Consider first the scenario when  $\Delta\text{pH}_b \geq 0$ , and the  $632$   
 $\Delta F_{\text{ion}}(z)$  curve exhibits a characteristic pattern with the  $633$   
maximum at  $z = z^*$  and the minimum at the grafting surface,  $634$   
 $\Delta F_{\text{ion}}(z = 0) \leq 0$ . In this case, the ionic interactions provide a  $635$   
driving force for the globule absorption above IEP,  $636$   
accompanied by the NP charge reversal. In Figure 9a we  $637$   $\text{9}$   
illustrate the evolution of the insertion free energy profile,  $638$   
 $\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z)$ , upon an increase in the  $639$   
parameter  $\omega$ . As one can see in Figure 9a, at small values of  $\omega$   $640$   
(NPs with relatively small volume  $V$  and large number  $N_{\Sigma}$  of  $641$   
ionizable groups) the  $\Delta F(z)$  curve has the same shape as  $642$   
 $\Delta F_{\text{ion}}(z)$ . That is, exhibits a maximum close to the edge of the  $643$



**Figure 9.** Total free energy curves  $\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z)$  normalized by  $N_{\Sigma}$  corresponding to different globule volume to the number of charge ratio  $\omega$  at  $C_s = 0.002$  (a) and at fixed  $\omega = 9.45$  and varied salt concentration  $C_s$  (b). Other parameters are  $s/a^2 = 100$ . The brush boundary,  $z = H$ , is indicated by the arrows. Dashdot horizontal lines indicate  $\Delta F(z) = 0$ .

599 While the ionic part of the insertion free energy,  $\Delta F_{\text{ion}}(z)$ ,  
600 may exhibit different patterns as a function of  $z$ , the osmotic  
601 contribution  $\Delta F_{\text{vol}}(z)$  given by eq 27 is always a positive and  
602 monotonously decreasing function of  $z$ . As a result, at  
603  $\Delta\text{pH}_b > 0$  and  $\Delta F_{\text{ion}}(z) > 0$  at any  $z \geq 0$ , the net free energy  
604 is positive,  $\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z) > 0$  as well, and NP  
605 absorption by PE brush is unfavorable. Even when the ionic  
606 interactions provide a driving force for globule absorption, (i.e.,  
607  $\Delta F_{\text{ion}}(z = 0) < 0$ ), the net insertion free energy  $\Delta F(z = 0)$  may  
608 be still positive due to the osmotic term,  $\Delta F_{\text{vol}}(z = 0)$ ,  
609 overcompensating the attractive ionic contribution.  
610 Using eqs 4, 27, and 29, the NP insertion free energy can be  
611 presented as

$$\Delta F(z) = N_{\Sigma} \left[ f_+ \ln \left( \frac{1 - \alpha_+(z)}{1 - \alpha_{b+}} \right) + (1 - f_+) \ln \left( \frac{1 - \alpha_-(z)}{1 - \alpha_{b-}} \right) \right] + 4\omega C_s \sinh^2(\psi(z)/2) \quad (30)$$

612 where we have introduced the following parameter

$$\omega = V/a^3 N_{\Sigma} \quad (31)$$

615 which is equal to the average globule volume per ionizable  
616 group. Because  $\Delta F_{\text{ion}}(z) \sim N_{\Sigma}$  (eq 29) and  $\Delta F_{\text{vol}}(z) \sim V$  (eq  
617 27), the relative strength of the osmotic and ionic  
618 contributions,  $\Delta F_{\text{vol}}/\Delta F_{\text{ion}}$ , depends on the parameter  $\omega$ .  
619 Remarkably,  $\omega$  is an increasing function of the globule  
620 dimensions (since  $N_{\Sigma}$  does not grow faster than the globule  
621 surface area  $\Sigma$ ).

622 In Table 1 we list approximate estimates of the parameter  $\omega$   
623 for a number of globular proteins, using values of  $V$  and  $N_{\Sigma}$   
624 from the literature.<sup>41</sup> Notably, the values of  $\omega$  in Table 1  
625 constitute lower boundaries, since all the ionizable residues  
626 (not necessarily located at the globule surface) have been  
627 accounted for in the corresponding protein.

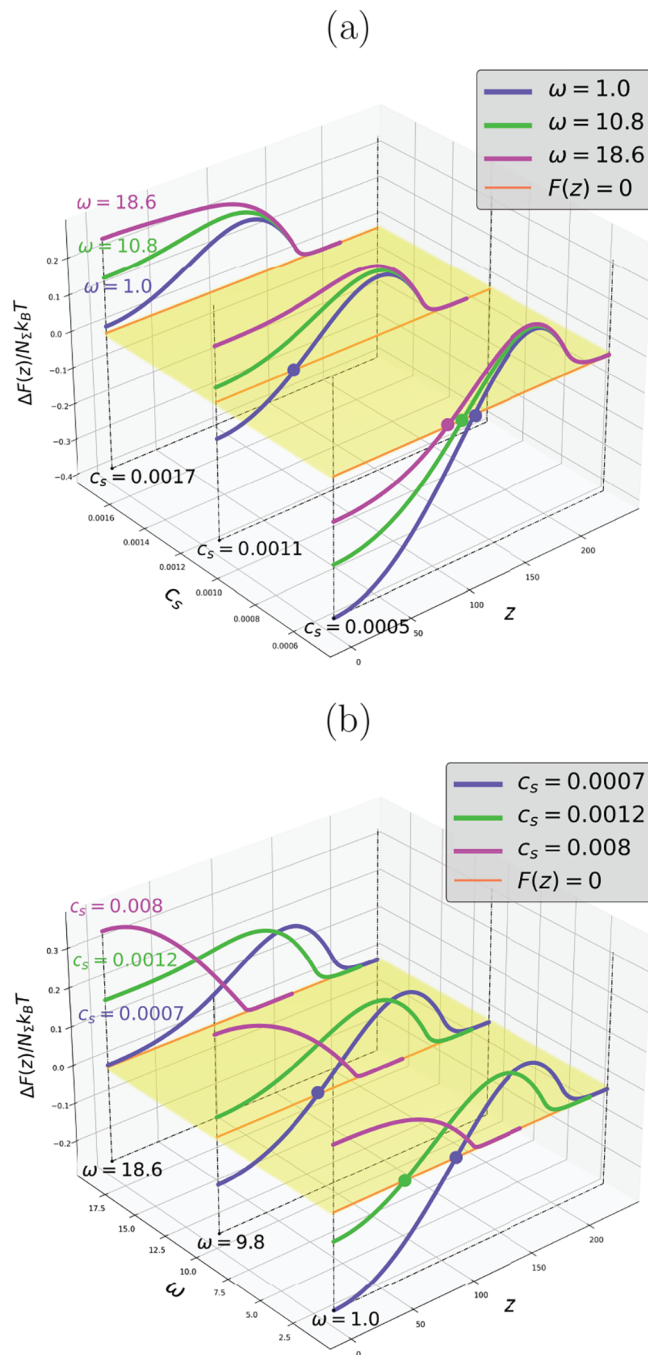
644 brush and reaches a negative value at the grafting surface, but  
 645 with the curve as a whole moving upward. Importantly,  
 646 position of the maximum,  $z = z_{\max}$  is shifted toward the  
 647 grafting surface with respect to  $z^*$  ( $z_{\max} \leq z^*$ ), and does not  
 648 correspond anymore to the globule charge inversion threshold  
 649 (i.e.,  $Q(z = z_{\max}) > 0$ ). Further increase in the globule volume  
 650  $V$  (i.e., an increase in  $\omega$ ) results in further shift of the  
 651 maximum toward the grafting surface, and the increase in both,  
 652  $\Delta F(z_{\max})$  and  $\Delta F(z = 0)$ . The latter becomes eventually  
 653 positive indicating that the osmotic repulsion overcompensates  
 654 the electrostatic attraction making globule absorption  
 655 thermodynamically unfavorable. At large  $\omega$ ,  $\Delta F(z)$  become a  
 656 monotonously decreasing function of  $z$  with  $\Delta F(z) > 0$  at any  
 657  $z \geq 0$ .

658 An increase in salt concentration leads to the simultaneous  
 659 decrease in both, the electrostatic driving force for NP  
 660 absorption and the osmotic repulsion, and eventually  
 661 suppresses the globule absorption by PE brush (as seen in  
 662 Figure 9b). The combined effects of the NP volume-to-charge  
 663 ratio  $\omega$  and salt concentration  $c_s$  are illustrated also by cross  
 664 sections of the 2D profiles of the insertion free energy curves,  
 665  $\Delta F(z, c_s)$  and  $\Delta F(z, \omega)$ , presented in Figure 10a and b,  
 666 respectively. Remarkably, an increase in  $\omega$  or/and in salt  
 667 concentration  $c_s$  leads to the decrease in depth of the minimum  
 668 in  $\Delta F(z)$  (and, eventually, its disappearance), but does not  
 669 affect its position at  $z = 0$ .

670 As it follows from our analysis, only small protein globules  
 671 with large number of ionizable groups are efficiently absorbed  
 672 by PE brushes at  $\Delta pH_b \geq 0$ , while bigger ones are not (unless  
 673 other driving forces are involved).

674 We now examine the interplay between forces of electro-  
 675 static attraction and osmotic repulsion in the case  $\Delta pH_b \leq 0$ ,  
 676 i.e., when  $Q(z) \geq 0$  at any  $z \geq 0$ . The free energy profiles  
 677  $\Delta F(z)$  corresponding to progressively increasing values of  $\omega$   
 678 are presented in Figure 11a and Figure 12a. They demonstrate  
 679 qualitatively new trends compared to the case  $\Delta pH_b \geq 0$ . For  
 680 example, at  $\omega = 15$  the ionic contribution to the free energy  
 681 dominates,  $\Delta F(z) \leq 0$  at any  $z$ , and thermodynamically most  
 682 favorable position of the globule is close to the grafting surface.  
 683 An increase in  $\omega$  leads to the shift of the whole  $\Delta F(z)$  curve  
 684 upward and change in its shape: The repulsive osmotic  
 685 contribution becomes dominant close to the grafting surface,  
 686 so that  $\Delta F(z = 0)$  becomes positive, and a potential well  
 687 develops at the periphery of the brush where the negative ionic  
 688 contribution  $\Delta F_{\text{ion}}(z) < 0$  dominates over the osmotic  
 689 repulsion. The width and the depth of the potential well  
 690 decrease upon an increase in  $\omega$ , and its position is shifted to  
 691 the edge of the brush. Eventually, at large  $\omega$  the peripheral  
 692 potential well disappears, and  $\Delta F(z)$  becomes a monotonously  
 693 decreasing function of  $z$ , indicating expulsion of globules from  
 694 PE brush.

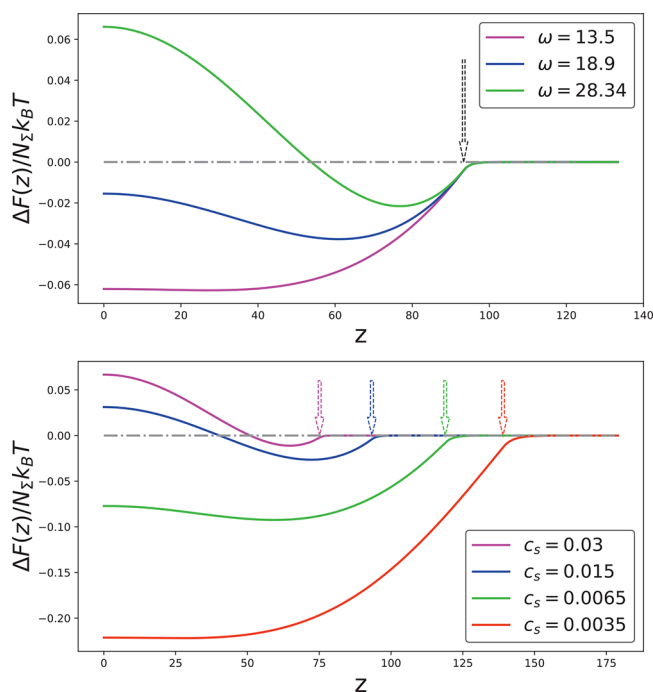
695 Another trend is observed at  $\Delta pH_b \leq 0$  if  $\omega$  is small and  
 696 kept constant, but the salt concentration increases, Figures 11b  
 697 and 12b: Notably the magnitudes of both electrostatic  
 698 (negative) and osmotic (positive) contributions to the free  
 699 energy diminish upon an increase in salt concentration  $C_s$ . At  
 700 very low salt concentration the electrostatic attraction  
 701 dominates over osmotic repulsion at any distance from the  
 702 surface and  $\Delta F(z)$  is a monotonously increasing function of  $z$ .  
 703 At higher salt concentrations, the osmotic (repulsive) part of  
 704 the free energy starts to dominate in the proximal to the  
 705 grafting surface region, while in the distal region ( $z \approx H$ ) the  
 706 electrostatic attraction,  $\Delta F_{\text{ion}}(z)$ , provides the dominant



**Figure 10.** Cross sections of the 2D profiles of the total free energy curves  $\Delta F(z, c_s) = \Delta F_{\text{ion}}(z, c_s) + \Delta F_{\text{vol}}(z, c_s)$  (a) and  $\Delta F(z, \omega) = \Delta F_{\text{ion}}(z, \omega) + \Delta F_{\text{vol}}(z, \omega)$  (b), both normalized by  $N_s$ , for a set values of  $\omega$  corresponding to different globule volume to the number of charges ratio (a) and different salt concentrations (b) (with the corresponding color code, as indicated at the curves).  $s/a^2 = 100$ . Colored circles in panels indicate points of vanishing free energy,  $\Delta F(z, c_s) = 0$  and  $\Delta F(z, \omega) = 0$ .

contribution to  $\Delta F(z)$ . As a result, a minimum in  $\Delta F(z)$  707  
 develops in the distal region. The depth of this minimum 708  
 decreases and its position  $z_{\min}$  is shifted toward the grafting 709  
 surface upon an increase in salt concentration and a 710  
 concomitant decrease in the brush thickness  $H$ . Hence, when 711  
 the osmotic contribution to the free energy becomes 712  
 comparable to the ionic one due to sufficiently large globule 713  
 volume or high salt concentration, the insertion free energy 714





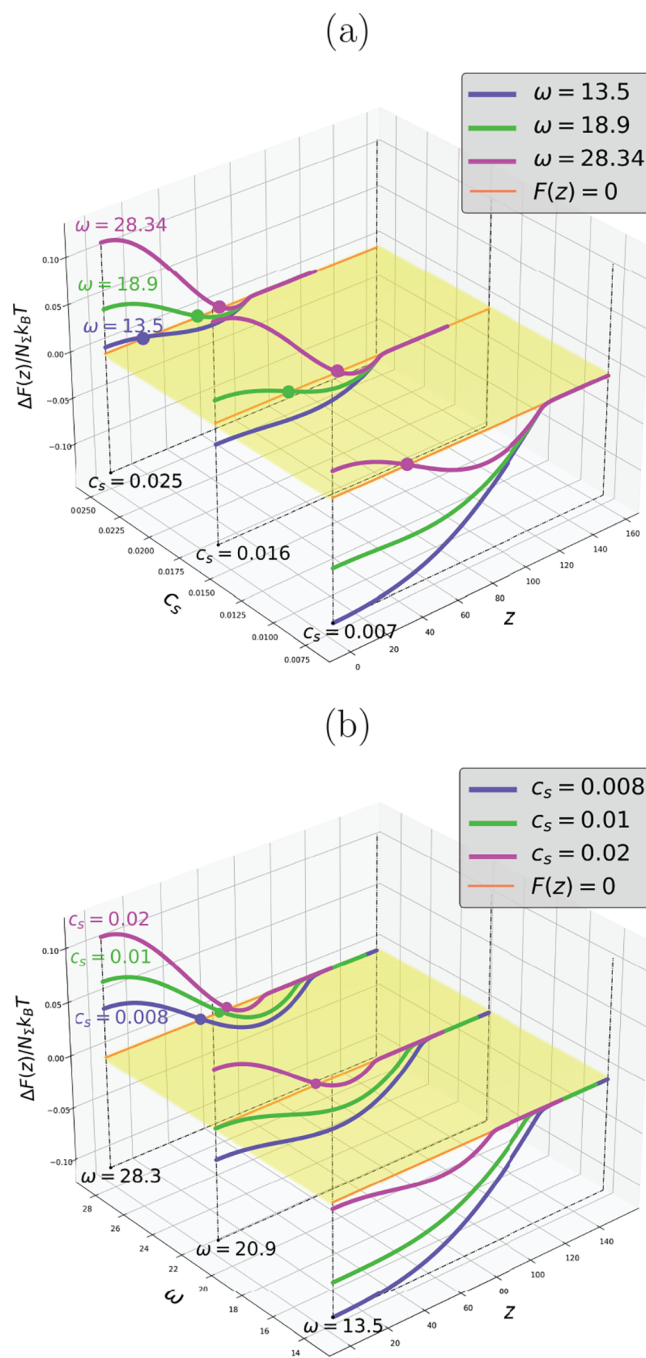
**Figure 11.** Total free energy curves  $\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z)$  normalized by  $N_s$ , at different globule volume to the number of charges ratio  $\omega$ .  $c_s = 0.015$  (a) and at  $\omega = 24.3$  and varied salt concentration (b). The brush boundary,  $z = H$ , is indicated by the arrows. Dashdot horizontal lines indicate  $\Delta F(z) = 0$ .

715 curves decrease from  $\Delta F(z = 0) \geq 0$  in the proximal to the  
 716 grafting surface region and pass through a minimum (with  
 717  $\Delta F(z = z_{\text{min}}) \leq 0$ ) localized in the distal region of the brush.  
 718 Solid lines in the diagram in Figure 5 correspond to the salt  
 719 concentration  $c_s^{(\text{max}, \omega)}$  separating regimes  $\Delta F(0) \leq 0$   
 720 (absorption) and  $\Delta F(0) > 0$  (repulsion). As follows from  
 721 Figure 5, this boundary is systematically displaced toward  
 722 lower salt concentration when  $\omega$  (the globule bulkiness)  
 723 increases.

#### 4. CONCLUSIONS

724 In this paper we have applied the analytical self-consistent field  
 725 Poisson–Boltzmann approach to study thermodynamics of  
 726 interactions between planar PE brushes and globular proteins,  
 727 modeled as nanoparticles (NPs) comprising weak cationic and  
 728 anionic groups on their surfaces. The position-dependent  
 729 insertion free energy  $\Delta F(z)$  and the net charge  $Q(z)$  of the NP  
 730 were calculated as a function of environmental conditions (pH  
 731 and salt concentration  $c_s$  in the buffer), numbers of cationic  
 732 and anionic ionizable groups on the NP surface, the NP  
 733 volume  $V$ , and grafting density  $\sigma$  of the brush-forming  
 734 polyelectrolyte chains.

735 The insertion free energy  $\Delta F(z) = \Delta F_{\text{ion}}(z) + \Delta F_{\text{vol}}(z)$   
 736 accounts for the position-dependent ionization contribution  
 737  $\Delta F_{\text{ion}}(z)$  of NP in the electrostatic field induced by a planar PE  
 738 brush, and for the work  $\Delta F_{\text{vol}}(z)$  performed against excess  
 739 osmotic pressure of mobile ions. As long as the electrostatic  
 740 potential  $\psi(z)$  induced by permanently charged (“quenched”)  
 741 PE brush can be tuned by changing the ionic strength in the  
 742 solution,  $\Delta F_{\text{vol}}(z)$  is controlled solely by the globule volume  $V$   
 743 and the salt concentration  $c_s$  in the buffer. On the contrary,  
 744  $\Delta F_{\text{ion}}(z)$  can be expressed as a function of ionization degrees of  
 745 cationic,  $\alpha_+(z)$  and anionic,  $\alpha_-(z)$  groups that depend on both,



**Figure 12.** Cross sections of the 2D profiles of the total free energy curves  $\Delta F(z, c_s) = \Delta F_{\text{ion}}(z, c_s) + \Delta F_{\text{vol}}(z, c_s)$  (a) and  $\Delta F(z, \omega) = \Delta F_{\text{ion}}(z, \omega) + \Delta F_{\text{vol}}(z, \omega)$  (b), both normalized by  $N_s$  for a set values of  $\omega$  corresponding to different globule volume to the number of charges ratio (a) and different salt concentrations (b), respectively (with the corresponding color code, as indicated at the curves).  $s/a^2 = 100$ . Colored circles in panels indicate points of vanishing free energy,  $\Delta F(z, c_s) = 0$  and  $\Delta F(z, \omega) = 0$ .

the buffer pH<sub>b</sub>, and the ionic strength (via  $\psi(z)$ ). Importantly, 746  
 $\alpha_-(z)$  decreases providing positive contribution to  $\Delta F_{\text{ion}}(z)$ , 747  
 whereas  $\alpha_+(z)$  increases providing negative contribution to 748  
 $\Delta F_{\text{ion}}(z)$  upon insertion of NP in polyanionic brush (with the 749  
 electrostatic potential  $\psi(z) \leq 0$  monotonously increasing as a 750  
 function of  $z$ ). Generalization to the case of polycationic PE 751  
 brush is straightforward by changing sign of  $\psi(z)$  from negative 752  
 to positive. 753



754 In calculating the insertion free energy  $\Delta F(z)$  we  
755 implemented a number of approximations:

- 756 (i) An uptake of NPs (protein globules) from buffer  
757 solution by PE brush is presumed to occur without  
758 changing the conformational state of the globule and  
759 spatial distribution of electrostatic potential  $\psi(z)$   
760 induced by the brush. That is, NPs are considered as  
761 probes. This approximation is valid at small protein  
762 concentrations  $c_b^{(\text{globules})}$  in buffer solution, and at no  
763 strong binding of NPs to the brush-forming PE chains.  
764 (ii) Small values of  $c_b^{(\text{globules})} \ll 1$  allow us to specify the  
765 density profiles  $c_b^{(\text{globules})}(z)$  of absorbed in the brush  
766 globules as

$$767 \quad c_b^{(\text{globules})}(z) = c_b^{(\text{globules})} \exp[-\Delta F(z)] \quad (32)$$

768 and the related partition coefficient,

$$769 \quad \frac{\langle c_b^{(\text{globules})} \rangle}{c_b^{(\text{globules})}} = \frac{1}{H} \int_0^H \exp[-\Delta F(z)] dz \quad (33)$$

770 The latter specifies the ratio of average concentration  
771  $\langle c_b^{(\text{globules})} \rangle$  of globules inside the brush, and concentra-  
772 tion  $c_b^{(\text{globules})}$  in the buffer solution. The limitation  
773  $c_b^{(\text{globules})} \ll 1$  is most important at  $\text{pH}_b \leq \text{pH}_{\text{IEP}}$  when  
774 absorption of large amounts of NPs by colloidal  
775 polyelectrolyte brushes unavoidably leads to collapse of  
776 the brush and loss of colloid stability. This restriction is  
777 less important if PE chains are grafted to planar  
778 substrates, although perturbation of the PE brush  
779 structure upon absorption of large amounts of NPs  
780 also brings the theory to the limit of its validity.

- 781 (iii) Reionization upon insertion is assumed to occur for all  
782  $N_\Sigma$  ionizable groups on NP. However, for globular  
783 proteins with compact ternary structure, only amino acid  
784 residues localized at the globule-water interlace are  
785 involved in (re)ionization process, leading therefore to  
786 concomitant renormalization of  $N_\Sigma$ .  
787 (iv) Our approach does not account for additional attraction  
788 between NP and PE brush due to charge–charge  
789 correlations that provide negative contribution to the  
790 insertion free energy  $\Delta F(z)$ . The charge–charge  
791 correlations are most important for protein globules  
792 with pronounced nonuniform (“patchy”) distribution of  
793 cationic and anionic groups on the globule surface. In  
794 the latter case, the brush-forming PE chains may adsorb  
795 on the oppositely charged patches of charge, but be  
796 depleted from similarly charged patches.<sup>24</sup>  
797 (v) Our model neglects also the short-range nonelectrostatic  
798 interactions between the brush-forming PE chains and  
799 the surface of NPs that could be mediated by the solvent  
800 quality or temperature. In spite of the anticipated  
801 dominant role of electrostatic forces, such interactions  
802 should be necessarily taken into account for full  
803 quantitative prediction of absorption threshold and  
804 diffusion rates for specific proteins.

805 By implementing the above approximations, and using the  
806 self-consistent Poisson–Boltzmann approach, we have dem-  
807 onstrated that the insertion free energy profiles  $\Delta F(z)$  exhibit  
808 complex patterns as a function of  $z$  depending on the  
809 environmental conditions ( $\text{pH}_b$ ,  $c_s$ ) and the globule volume  
810  $V$  normalized by the number of ionizable groups  $N_\Sigma$ . The  
811 depth of the minima and the height of the maxima in  $\Delta F(z)$

profiles are mediated by the number  $N_\Sigma$  of ionizable groups, 812  
and the globule volume  $V$ . Notably, the implemented approach 813  
allows us to specify the lower  $\text{pH}_b$  boundary for uptake of NPs 814  
by PE brush. 815

The analysis of our results indicates that one can distinguish 816  
three characteristic ranges of  $\text{pH}_b$  with respect to the NP 817  
isoelectric point,  $\text{pH}_{\text{IEP}}$ , in the buffer: 818

**$\text{pH}_b > \text{pH}_{\text{IEP}}$ .** The NP is strongly negatively charged in the 819  
buffer,  $Q_b < 0$ . The NP charge  $Q$  remains negative upon 820  
insertion in polyanionic brush, but decreases in magnitude 821  
upon approach to the grafting surface, where the electrostatic 822  
potential of PE brush is the strongest. The  $\Delta F_{\text{ion}}(z)$  and  $\Delta F(z)$  823  
are monotonously decreasing functions of  $z$  indicating 824  
expulsion of NPs from the brush. The magnitude of the 825  
repulsive free energy grows as a function of  $\text{pH}_b$  (due to the 826  
increase in  $|Q_b|$ ), but decreases upon an increase in the ionic 827  
strength in the buffer solution. 828

**$\text{pH}_b \geq \text{pH}_{\text{IEP}}$ .** The NP is charged negatively in the buffer, 829  
 $Q_b \leq 0$ , but acquires positive net charge upon insertion (or 830  
approach to PE brush). The charge inversion,  $Q(z) = 0$ , occurs 831  
at  $z = z^*$  at which  $\Delta F_{\text{ion}}(z)$  passes through a maximum. At 832  
 $z \leq z^*$  the globule charge becomes positive and keeps 833  
increasing whereas the ionic part of the free energy,  $\Delta F_{\text{ion}}(z)$ , 834  
decreases reaching either positive or negative value at the 835  
grafting surface,  $z = 0$ . For small globules,  $\Delta F(0) < 0$  indicates 836  
their accumulation in the brush. At  $\Delta F(0) > 0$ , the local 837  
minimum in  $\Delta F_{\text{ion}}(z)$  at the grafting surface,  $z = 0$ , 838  
corresponds to a metastable state separated by the potential 839  
barrier from the bulk of the solution. Hence, even when the 840  
NP net charge changes its sign from negative to positive, 841  
absorption of NPs by PE brush is not necessarily 842  
thermodynamically favorable though NPs may be kinetically 843  
trapped there. 844

An increase in the ionic strength of the solution weakens the 845  
electrostatic potential  $\psi(z)$  and hinders reionization of anionic 846  
and cationic groups of the inserted in the brush NP. As a result, 847  
the charge inversion point,  $z^*$ , and the corresponding 848  
maximum in  $F_{\text{ion}}(z)$  are displaced toward the grafting surface 849  
(where the magnitude of  $\psi(z)$  is larger), the free energy 850  
 $\Delta F_{\text{ion}}(z = 0)$  grows and turns from negative to positive 851  
indicating passing the absorption threshold. Eventually, at high 852  
salt concentrations the potential  $\psi(z)$  becomes too weak to 853  
induce reionization of the NP inside the brush, and the NPs 854  
are repelled by the brush. 855

A progressive increase in the NP volume  $V$  (an increase in 856  
 $\omega$ ) at constant ionic strength leads to the growth of repulsive 857  
osmotic contribution  $\Delta F_{\text{vol}}(z)$  at constant  $\Delta F_{\text{ion}}(z)$ . As a result, 858  
the  $\Delta F(z)$  curves are shifted upward while the position of 859  
maximum,  $z_{\text{max}}$  is displaced toward the grafting surface. 860  
Importantly, now the position of maximum,  $z_{\text{max}}$  does not 861  
coincide with the charge inversion point,  $z^*$ , and 862  
 $Q(z = z_{\text{max}}) \geq 0$ . Further increase in the NP volume  $V$  leads 863  
to  $\Delta F(z = 0) \geq 0$  and eventual transformation of  $\Delta F(z)$  into 864  
monotonously decreasing curve. That is, expulsion of the bulky 865  
globule from the brush in spite of the charge inversion (which 866  
can be traced in the  $\Delta F_{\text{ion}}(z)$  and  $Q(z)$  curves). 867

**$\text{pH}_b \leq \text{pH}_{\text{IEP}}$ .** The NP is positively charged in the buffer 868  
and, therefore, attracted to the PE brush by the Coulomb 869  
force. The  $\Delta F_{\text{ion}}(z) \leq 0$  at all  $z$ , and the NP positive charge 870  
grows upon insertion into the brush and approach to the 871  
grafting surface. However, absorption of a bulky globule can be 872  
hindered and even fully suppressed due to osmotic (repulsive) 873  
term,  $\Delta F_{\text{vol}}(z)$ . An increase in the globule volume (and in 874

875  $\Delta F_{\text{vol}}(z)$ ) at constant salt concentration  $c_s$  leads to the increase  
876 in  $\Delta F(z)$  primarily in the proximal to the grafting surface  
877 region, flattening of the presurface potential well, and  
878 eventually leading to the appearance of the presurface  
879 maximum with  $\Delta F(z = 0) \geq 0$ . The maximum is separated  
880 from the bulk of the solution by the minimum which  
881 progressively gets more shallow and narrow, and is displaced  
882 to the external boundary of PE brush. A similar evolution of  
883  $\Delta F(z)$  curve is observed for globules with fixed  $V$  and  $N_{\Sigma}$  upon  
884 variations in the salt concentration. The minimum in  $\Delta F(z)$  is  
885 shifted toward the periphery of the brush (and progressively  
886 disappears) upon an increase in  $c_s$ . The theory thereby predicts  
887 that below IEP the interplay between ionic attraction and  
888 osmotic repulsion may result in preferential accumulation of  
889 the absorbed protein globules in the central or even peripheral  
890 regions of the brush, whereas the proximal to the grafting  
891 surface region is depleted from globules. This feature is most  
892 attractive in design of bionanoreactors in which accumulation  
893 of the proteins (enzymes) in the brush is required, but their  
894 direct contacts with underlying substrate (that could lead to  
895 nonspecific adsorption, denaturation, and loss of enzymatic  
896 activity) should be avoided.

897 We remark that amplitudes of the minima and maxima in  
898  $\Delta F(z)$  curves that determine the thermodynamic stability of  
899 the protein absorbed state or the probability of overcoming the  
900 potential barrier separating the absorbed state from the bulk of  
901 the solution or from the grafting surface depend on the  
902 number of charged groups and volume of the globule. With  
903 typical values of  $N_{\Sigma} \sim 10^2$ , the potential well easily reaches the  
904 depth of a few  $k_B T$  under experimentally relevant conditions.  
905 Furthermore, reionization and the NP absorption on the  
906 “wrong side” of the IEP is suppressed by increasing salt  
907 concentration (in full agreement with the experimental data<sup>20</sup>),  
908 but might be operational for sufficiently densely grafted PE  
909 brushes that induce strong local electrostatic field.

910 To summarize, our theory predicts how the electrostatic and  
911 osmotic parts of the insertion free energy change upon  
912 insertion of ampholytic NP containing pH-sensitive acidic and  
913 basic groups in a planar PE brush from the solution in a wide  
914 range of the buffer  $\text{pH}_b$ . On the “wrong side” of the isoelectric  
915 point, the negative balance in the free energy may be assured  
916 by overcompensation of the free energy losses due to  
917 suppression of ionization of similarly (with respect to the  
918 brush) charged monomeric groups by gains due to enhanced  
919 ionization of the oppositely charged ones, and be accompanied  
920 by the change in the sign of the net charge of NP inside the  
921 brush. However, the local minimum in the NP free energy  
922 inside the brush may correspond to a metastable state even if it  
923 is accompanied by the net charge reversal. Importantly,  
924 knowledge of the insertion free energy profile  $\Delta F(z)$  allows  
925 also for the calculation and control of the diffusion rates of  
926 NPs through the brush to be considered in our forthcoming  
927 publication.

## 928 ■ ASSOCIATED CONTENT

### 929 **SI** Supporting Information

930 The Supporting Information is available free of charge at  
931 <https://pubs.acs.org/doi/10.1021/acs.biomac.2c01153>.

932 Detailed derivation of the electrostatic potential  
933 distribution in the planar PE brush (PDF)

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### 958 Notes

The authors declare no competing financial interest.

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## 963 ■ REFERENCES

- (1) Achazi, K.; Haag, R.; Ballauff, M.; Dervedde, J.; Kizhakkedathu, J. N.; Maysinger, D.; Multhaupt, G. Understanding the Interaction of Polyelectrolyte Architectures with Proteins and Biosystems. *Angew. Chem., Int. Ed.* **2021**, *60* (8), 3882–3904.
- (2) Nie, C. A. X.; Pouyan, P.; Lauster, D.; Trimpert, J.; Kerkhoff, Y.; Szekeres, G. P.; Wallert, M.; Block, S.; Sahoo, A. K.; Dervedde, J.; Pagel, K.; Kaufner, B. B.; Netz, R. R.; Ballauff, M.; Haag, R. Polysulfates Block SARS-CoV-2 Uptake through Electrostatic Interactions. *Angew. Chem., Int. Ed.* **2021**, *60* (29), 15870–15878.
- (3) Kapelner, R. A.; Yeong, V.; Obermeyer, A. C. Molecular determinants of protein-based coacervates. *Curr. Opin. Colloid Interface Sci.* **2021**, *52*, 101407.
- (4) Yeong, V.; Werth, E. G.; Brown, L. M.; Obermeyer, A. C. Formation of biomolecular condensates in bacteria by tuning protein electrostatics. *ACS Cent. Sci.* **2020**, *6*, 2301–2310.
- (5) Kabanov, A. V.; Kabanov, V. A. Interpolyelectrolyte and block ionomer complexes for gene delivery: physico-chemical aspects. *Adv. Drug Delivery Rev.* **1998**, *30* (1–3), 49–60.
- (6) 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.
- (7) Miyata, K.; Nishiyama, N.; Kataoka, K. Rational design of smart supramolecular assemblies for gene delivery: chemical challenges in the creation of artificial viruses. *Chem. Soc. Rev.* **2012**, *41*, 2562–2574.
- (8) Yu, T.; Liu, X.; Bolcato-Bellemin, A.-L.; Wang, Y.; Liu, C.; Erbacher, P.; Qu, F.; Rocchi, P.; Behr, J.-P.; Peng, L. An amphiphilic dendrimer for effective delivery of small interfering RNA and gene silencing in vitro and in vivo. *Angew. Chem., Int. Ed.* **2012**, *124*, 8606–8612.

- 994 (9) Liu, X.; Zhou, J.; Yu, T.; Chen, C.; Cheng, Q.; Sengupta, K.;  
995 Huang, Y.; Li, H.; Liu, C.; Wang, Y.; Posocco, P.; Wang, M.; Cui, Q.;  
996 Giorgio, S.; Fermeglia, M.; Qu, F.; Pricl, S.; Shi, Y.; Liang, Z.; Rocchi,  
997 P.; Rossi, J. J.; Peng, L. Adaptive Amphiphilic Dendrimer-Based  
998 Nanoassemblies as Robust and Versatile siRNA Delivery Systems.  
999 *Angew. Chem., Int. Ed.* **2014**, *126*, 12016–12021.
- 1000 (10) Fan, X.; Zhao, Y.; Xu, W.; Li, L. Linear-Dendritic Block  
1001 Copolymer for Drug and Gene Delivery. *Mater. Sci. Eng., C* **2016**, *62*,  
1002 943–959.
- 1003 (11) Malmsten, M.; Bysell, H.; Hansson, P. Biomacromolecules in  
1004 Microgels - Opportunities and Challenges for Drug Delivery. *Curr.*  
1005 *Opin. Colloid Interface Sci.* **2010**, *15*, 435–444.
- 1006 (12) Bysell, H.; Mansson, R.; Hansson, P.; Malmsten, M. Microgels  
1007 and Microcapsules in Peptide and Protein Drug Delivery. *Adv. Drug*  
1008 *Delivery Rev.* **2011**, *63*, 1172–1185.
- 1009 (13) Hachim, D.; Whittaker, T. E.; Kim, H.; Stevens, M. M.  
1010 Glycosaminoglycan-based biomaterials for growth factor and cytokine  
1011 delivery: Making the right choices. *J. Controlled Release* **2019**, *313*,  
1012 131–147.
- 1013 (14) Braatz, D.; Dimde, M.; Ma, G.; Zhong, Y.; Tully, M.;  
1014 Grotzinger, C.; Zhang, Y.; Mavroskoufis, A.; Schirner, M.; Zhong, Z.;  
1015 Ballauff, M.; Haag, R. Toolbox of Biodegradable Dendritic (Poly  
1016 glycerol sulfate)-SS-poly(ester) Micelles for Cancer Treatment:  
1017 Stability, Drug Release, and Tumor Targeting. *Biomacromolecules*  
1018 **2021**, *22* (6), 2625–2640.
- 1019 (15) Kiani, C.; Chen, L.; Wu, Y. J.; Yee, A. J.; Yang, B. B. Structure  
1020 and functions of aggrecan. *Cell Res.* **2002**, *12*, 19–32.
- 1021 (16) Cagno, V.; Tseligka, E. D.; Jones, S. T.; Tapparel, C. Heparan  
1022 sulfate proteoglycans and viral attachment: True receptors or  
1023 adaptation bias? *Viruses* **2019**, *11* (7), 596.
- 1024 (17) Wittemann, A.; Haupt, B.; Ballauff, M. Adsorption of proteins  
1025 on spherical polyelectrolyte brushes in aqueous solution. *Phys. Chem.*  
1026 *Chem. Phys.* **2003**, *5*, 1671–1677.
- 1027 (18) Wittemann, A.; Ballauff, M. Interaction of proteins with linear  
1028 polyelectrolytes and spherical polyelectrolyte brushes in aqueous  
1029 solution. *Phys. Chem. Chem. Phys.* **2006**, *8*, 5269–5275.
- 1030 (19) Becker, A. L.; Henzler, K.; Welsch, N.; Ballauff, M.; Borisov, O.  
1031 V. Proteins and polyelectrolytes: A charge relationship. *Curr. Opin.*  
1032 *Colloid Interface Sci.* **2012**, *17*, 90–96.
- 1033 (20) Walkowiak, J.; Gradzielski, M.; Zauscher, S.; Ballauff, M.  
1034 Interaction of Proteins with a Planar Poly(acrylic acid) Brush:  
1035 Analysis by Quartz Crystal Microbalance with Dissipation Monitoring  
1036 (QCM-D). *Polymers* **2021**, *13*, 122.
- 1037 (21) Yigit, C.; Welsch, N.; Ballauff, M.; Dzubiella, J. Protein  
1038 Sorption to Charged Microgels: Characterizing Binding Isotherms  
1039 and Driving Forces. *Langmuir* **2012**, *28*, 14373–14385.
- 1040 (22) Biesheuvel, P. M.; Wittemann, A. A modified box model  
1041 including charged polyelectrolyte brush: self-consistent field theory.  
1042 *J. Phys. Chem. B* **2005**, *109*, 4209–4214.
- 1043 (23) de Vos, W. M.; Leermakers, F. A. M.; de Keizer, A.; Cohen  
1044 Stuart, M. A.; Kleijn, J. M. Field theoretical analysis of driving forces  
1045 for the uptake of proteins by like-charge polyelectrolyte brushes:  
1046 effects of charge regulation and patchiness. *Langmuir* **2010**, *26*, 249–  
1047 259.
- 1048 (24) Leermakers, F. A. M.; Ballauff, M.; Borisov, O. V. On the  
1049 mechanisms of interaction of globular proteins with polyelectrolyte  
1050 brushes. *Langmuir* **2007**, *23*, 237–247.
- 1051 (25) Xu, X.; Angioletti-Uberti, S.; Lu, Y.; Dzubiella, J.; Ballauff, M.  
1052 Interaction of Proteins with Polyelectrolytes: Comparison of Theory  
1053 to Experiment. *Langmuir* **2019**, *35*, 5373–5391.
- 1054 (26) Kim, S.; Sureka, H. V.; Kayitmazer, A. B.; Wang, G.; Swan, J.  
1055 W.; Olsen, B. D. Effect of protein surface charge distribution on  
1056 protein-polyelectrolyte complexation. *Biomacromolecules* **2020**, *21*,  
1057 3026–3037.
- 1058 (27) Lunkad, R.; Barroso da Silva, F. L.; Kosovan, P. Both Charge-  
1059 Regulation and Charge-Patch Distribution Can Adsorption on the  
1060 Wrong Side of the Isoelectric Point. *J. Am. Chem. Soc.* **2022**, *144*,  
1061 1813–1825.
- (28) Laktionov, M. Y.; Zhulina, E. B.; Borisov, O. V. Proteins and  
1062 polyampholytes interacting with polyelectrolyte brushes and micro-  
1063 gels: the charge reversal concept revised. *Langmuir* **2021**, *37*, 2865–  
1064 2873.
- (29) Zhulina, E. B.; Borisov, O. V. Structure and Interactions of  
1066 Weakly Charged Polyelectrolyte Brushes: Self-Consistent Field  
1067 Theory. *textit J. Chem. Phys.* **1997**, *107*, 5952–5967.
- (30) Zhulina, E. B.; Klein Wolterink, J.; Borisov, O. V. Screening  
1069 Effects in Polyelectrolyte Brush: Self-Consistent Field Theory.  
1070 *Macromolecules* **2000**, *33*, 4945–4953.
- (31) Lebedeva, I. O.; Zhulina, E. B.; Borisov, O. V. Self-consistent  
1072 Field Theory of Polyelectrolyte Brushes with Finite Chain  
1073 Extensibility. *J. Chem. Phys.* **2017**, *146*, 214901–6.
- (32) Zhulina, E. B.; Borisov, O. V. Poisson-Boltzmann Theory of  
1075 pH-Sensitive (Annealing) Polyelectrolyte Brush. *Langmuir* **2011**, *27*,  
1076 10615–10633.
- (33) Zhulina, E. B.; Borisov, O. V. Brushes of Dendritically  
1078 Branched Polyelectrolytes. *Macromolecules* **2015**, *48* (19), 1499–  
1079 1508.
- (34) Zhulina, E. B.; Boulakh, A. B.; Borisov, O. V. Repulsive Forces  
1081 between Spherical Polyelectrolyte Brushes in Salt-Free Solution. *Z.*  
1082 *Phys. Chem. (Berlin, Ger.)* **2012**, *226*, 625–643.
- (35) Lunkad, R.; Murmiliuk, A.; Tosner, Z.; Stepanek, M.; Kosovan,  
1084 P. Role of pK<sub>a</sub> in Charge Regulation and Conformation of Various  
1085 Peptide Sequences. *Polymers* **2021**, *13*, 214.
- (36) Lunkad, R.; Biehl, P.; Murmiliuk, A.; Blanco, P. M.; Mons, P.;  
1087 Stepanek, M.; Schacher, F. H.; Kosovan, P. Simulations and  
1088 Potentiometric Titrations Enable Reliable Determination of Effective  
1089 pK<sub>a</sub> Values of Various Polyzwitterions. *Macromolecules* **2022**, *55*,  
1090 7775–7784.
- (37) Halperin, A.; Kröger, M.; Zhulina, E. B. Colloid-brush  
1092 interactions: The effect of solvent quality. *Macromolecules* **2011**, *44*,  
1093 3622–3638.
- (38) Merlitz, H.; Wu, C.-X.; Sommer, J.-U. Inclusion Free Energy of  
1095 Nanoparticles in Polymer Brushes. *Macromolecules* **2012**, *45*, 8494–  
1096 8501.
- (39) Laktionov, M. Y.; Shavykin, O. V.; Leermakers, F. A. M.;  
1098 Zhulina, E. B.; Borisov, O. V. Colloidal particles interacting with a  
1099 polymer brush: a self-consistent field theory. *Phys. Chem. Chem. Phys.*  
1100 **2022**, *24*, 8463–8476.
- (40) Landau, L. D.; Lifshitz, E. M. *Statistical Physics*; Pergamon  
1102 Press: Oxford, 1986.
- (41) RCSB PDB: Homepage. <https://www.rcsb.org> (accessed  
1104 2022–08–21). 1105