

Supporting information for:

Equilibrium Adsorption of Hexahistidine on pH-Responsive Hydrogel Nanofilms

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1 Theoretical Approach and Molecular Model

Consider the adsorption of a molecular species of interest within a thin hydrogel film that is chemically grafted to a planar surface (see Fig. S1). The hydrogel is formed by a network of crosslinked polyacid chains, which is in contact with a solution that contains the adsorbate (a), water (w), protons (H^+), hydroxyl ions (OH^-), and monovalent anions ($-$) and cations

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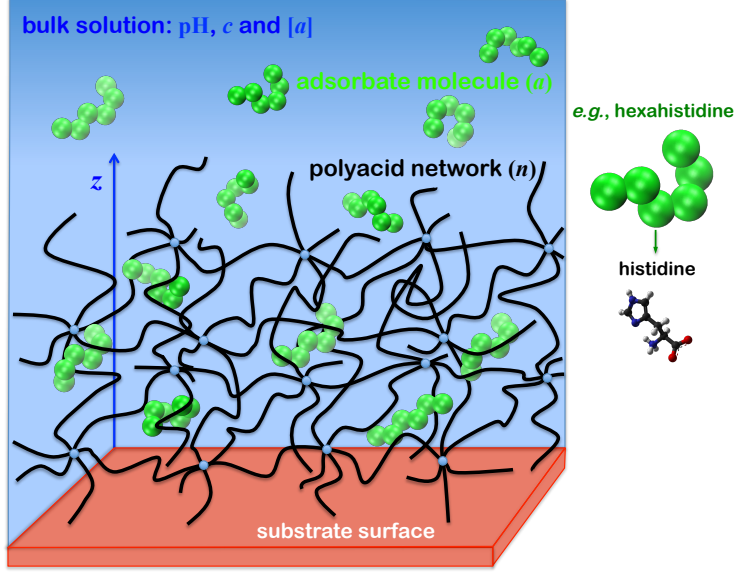


Figure S1: Schematic representation of the system of interest. The picture shows a pH-sensitive polymer network that can adsorb hexahistidine (or other solute molecules) from a solution with controlled pH, as well as salt concentration, c , and adsorbate concentration, $[a]$. This figure has been included in the paper, and it is reproduced here for the sake of clarity.

(+) due to dissociated salt (NaCl, for example). Far away from the surface, the pH of the bulk solution, its salt concentration, c , and the concentration of the adsorbate species, $[a]$, are all externally controlled. Such control over the composition of the solution provides a bath for all of the free species in the system, including the relevant solute, and it fixes their chemical potentials. The polymer network (n) is pH-responsive because each of its segments bears a weak acidic group that can be either protonated (np) or deprotonated (nd). Thus, we consider the adsorption of different molecules in a thin film hydrogel of polyacrylic acid, for example. The adsorbate species of interest are small molecules that can be either permanently charged, or contain acidic/basic groups. Our approach to investigate the equilibrium adsorption within the film consists in defining a detailed molecular-level theory, which is an extension of the one developed for grafted weak polyelectrolyte layers.^{S1,S2} We have recently used this approach to investigate the swelling of the same type of grafted hydrogel films considered in the present study.^{S3} In this section, we describe the general features of the theory, with explicit emphasis on the modifications introduced to model the adsorption inside the film. The first step in this methodology consists in writing the total

Helmholtz free energy of the system, expressed as

$$\begin{aligned}
\beta F = & \sum_{\alpha_n} P_n(\alpha_n) \ln(P_n(\alpha_n)) \\
& + A \int_0^\infty dz \langle \rho_n(z) \rangle f_n(z) (\ln f_n(z) + \beta \mu_{nd}^0) \\
& + A \int_0^\infty dz \langle \rho_n(z) \rangle (1 - f_n(z)) [\ln(1 - f_n(z)) + \beta \mu_{np}^0] \\
& + \sum_{\gamma=w,+, -, H^+, OH^-} A \int_0^\infty dz \rho_\gamma(z) [\ln(\rho_\gamma(z) v_w) - 1 + \beta \mu_\gamma^0] \\
& + A \int_0^\infty dz \sum_{\alpha_a} \rho_a(\alpha_a, z) [\ln(\rho_a(\alpha_a, z) v_w) - 1 + \beta \mu_a^0(\alpha_a)] + \beta F_{\text{chm}}^a \\
& + A \int_0^\infty dz \left[\langle \rho_q(z) \rangle \beta \Psi(z) - \frac{1}{2} \beta \epsilon (\nabla \Psi(z))^2 \right] + \beta U_{\text{vdw}},
\end{aligned} \tag{1}$$

where z is the coordinate that measures the distance from the supporting surface of total area A , and $\beta = \frac{1}{k_B T}$ is the inverse of the temperature, T . The first contribution to F is the conformational entropy of the gel that results from the elasticity of the polymeric structure, where $P_n(\alpha_n)$ gives the probability of finding the network in its conformation α_n . The next two terms together contain the combinatorial free energy of the acid-base equilibrium of network units as well as the self-energies of the protonated and deprotonated monomers. Angle brackets represent an ensemble average over the set of conformations of the network. Then,

$$\langle \rho_n(z) \rangle = \sum_{\alpha_n} P_n(\alpha_n) \rho_n(\alpha_n, z) \tag{2}$$

gives the ensemble average local density of polymer, where $\rho_n(\alpha_n, z)$ is the number density of segments at z when the polymer network is in conformation α_n . Moreover, $f_n(z)$ is the local degree of dissociation of the network, which gives the fraction of the segments located at z that are charged. In other words, the local density of charged network units is $f_n(z) \langle \rho_n(z) \rangle$, while that of uncharged segments is $(1 - f_n(z)) \langle \rho_n(z) \rangle$. The standard chemical potentials of the deprotonated (charged) and protonated (uncharged) network monomers are μ_{nd}^0 and μ_{np}^0 , respectively. The sum over the free species in Eq. (1), excluding the adsorbate, is the

translational (mixing) entropy and self energies of these molecules. The quantities $\rho_\gamma(z)$ and μ_γ^0 are respectively the local number density and standard chemical potential of the mobile species γ , with $\gamma \in \{w, H^+, OH^-, +, -\}$, while v_w is the volume of a water molecule. The following term in Eq. (1) accounts for the translational and configurational degrees of freedom of the adsorbate molecule as well as its self energy. In this contribution to the free energy, $\rho_a(\alpha_a, z)$ gives the z -dependent number density of adsorbate molecules in conformation α_a , and $\mu_a^0(\alpha_a)$ is the corresponding standard chemical potential. Then,

$$\langle \rho_a(z) \rangle = \sum_{\alpha_a} \rho_a(\alpha_a, z) \quad (3)$$

is the total density of adsorbate molecules at z . In this context, angle brackets should also be interpreted as an ensemble average, where the probability for a molecule with center of mass at z to be in conformation α_a is given by

$$P_a(\alpha_a, z) = \frac{\rho_a(\alpha_a, z)}{\langle \rho_a(z) \rangle}. \quad (4)$$

In the form that the last-mentioned contribution to F has been expressed, it is also valid when the species has no conformational degree of freedom, by considering its only configuration. In that case, the expression reduces to the translational entropy and self-energy of the species. The following term, βF_{chm}^a , is the chemical free energy of the adsorbate that accounts for the acid-base equilibrium when the species contains titratable groups. In this work, we study the adsorption of different homooligomers inside a polyacid hydrogel film. Three separate situations are considered depending on whether the units of the oligomers are permanently charged, or bear acidic or basic groups. In these last two cases, F_{chm}^a describes the mixing between the protonated and deprotonated units of the adsorbate molecule as well as their

self energies, which can be written as

$$\begin{aligned} \beta F_{\text{chm}}^a = & A \int_0^\infty dz \langle n_a(z) \rangle g_a(z) [\ln(g_a(z)) + \beta \mu_{ap}^0] \\ & + A \int_0^\infty dz \langle n_a(z) \rangle (1 - g_a(z)) [\ln(1 - g_a(z)) + \beta \mu_{ad}^0], \end{aligned} \quad (5)$$

where $\langle n_a(z) \rangle$ is the local density of ionizable adsorbate segments, with $g_a(z)$ being the fraction of them that are deprotonated. The standard chemical potentials of the protonated and deprotonated monomers of the adsorbate are μ_{ap}^0 and μ_{ad}^0 , respectively. The density of segments is calculated over the set of conformations of the molecule; that is

$$\langle n_a(z) \rangle = A \int_0^\infty dz' \sum_{\alpha_a} \rho_a(\alpha_a, z') m_a(\alpha_a, z', z), \quad (6)$$

where the delocalized quantity $m_a(\alpha_a, z, z')$ represents the number of segments that a single adsorbate chain with center of mass at z contributes to z' . We emphasize that F_{chm}^a is only to be included for adsorbate species having weak electrolyte units, and should not be considered if these molecules have permanent or no charge. The next term in Eq. (1) is the electrostatic energy, where $\Psi(z)$ is the z -dependent electrostatic potential, and ϵ denotes the dielectric permittivity of the medium. In this contribution to the free energy, $\langle \rho_q(z) \rangle$ is the average total electric-charge density at z , given by

$$\langle \rho_q(z) \rangle = f_n(z) \langle \rho_n(z) \rangle q_n + \sum_{\gamma=+,-,H^+,OH^-} \rho_\gamma(z) q_\gamma + f_a(z) \langle n_a(z) \rangle q_a, \quad (7)$$

where q_n is the electric charge of the deprotonated network segment, q_a is that of the ionized unit of the adsorbate molecule, and q_+ , q_- , q_{H^+} and q_{OH^-} denote the electric charge of the different charged free species. To express the total density of charge, we have introduced the local degree of charge of adsorbate units, $f_a(z)$, whose relation to the degree of protonation

depends on whether these segments bear acidic or basic groups; that is,

$$f_a(z) = \begin{cases} 1 - g_a(z) & \text{for acidic groups} \\ g_a(z) & \text{for basic groups.} \end{cases} \quad (8)$$

Equation (7) is also valid when the adsorbate units carry fixed charges, by setting the solute degree of ionization to unity at each position. The last term in Eq. (1) contains the total energy due to the van der Waals attractions between the different species in the system, U_{vdw} .

In addition, two physical constraints must be satisfied by the free energy. First, at each distance from the surface, the available volume must be completely occupied by some of the molecular species. This is the incompressibility constraint that accounts for the inter-molecular repulsions (excluded volume interactions), which can be expressed as

$$\langle \rho_n(z) \rangle v_n + \sum_{\gamma=w,+, -, H^+, OH^-} \rho_\gamma(z) v_\gamma + \langle n_a(z) \rangle v_a = 1, \quad (9)$$

where v_n and v_a are respectively the volumes of monomers of the network and the adsorbate, and v_+ , v_- , v_{H^+} , v_{OH^-} are the volumes of the corresponding free species. In addition, we require the system to be globally electroneutral, which is

$$\int_0^\infty \langle \rho_q(z) \rangle dz = 0. \quad (10)$$

At this point, the quantities to determine in Eq. (1) consist of the probability density function of network conformations, $P_n(\alpha_n)$, the local density distribution of adsorbate molecules, $\rho_a(\alpha_a, z)$, the local densities of all the other free species, $\rho_\gamma(z)$, with $\gamma \in \{w, -, +, H^+, OH^-\}$, the local degree of charge of network segments, $f_n(z)$, and that of the adsorbate units, $f_a(z)$ (or alternatively their degree of protonation, $g_a(z)$), and the electrostatic potential, $\Psi(z)$. Functional optimization of the free energy with respect to each of these quantities, subject

to the two aforementioned constraints, yields expressions for each of these functions. For the mobile species, except for the adsorbate, we obtain:

$$\rho_\gamma(z)v_w = a_\gamma \exp(-\beta\pi(z)v_\gamma - \beta\Psi(z)q_\gamma) \quad (11)$$

with $q_w = 0$, where $\pi(z)$ are the local Lagrange multipliers conjugated with the incompressibility constraint, Eq. (9), at each distance from the surface. The activity of a free species γ , $a_\gamma = e^{\beta\mu_\gamma - \beta\mu_\gamma^0}$, introduces the dependence of $\rho_\gamma(z)$ on the chemical potential of the species, μ_γ , which results from requiring the system to be in equilibrium with the bath solution. In other words, the chemical potential of each of the free species -including the adsorbate- must be identical in both the system and the bath solution. These activities are completely determined by the bath pH, c , and $[a]$. The local density of the different conformations of the adsorbate can be written as

$$\begin{aligned} \rho_a(\alpha_a, z) = & e^{\beta\mu_a - \beta\mu_a^0(\alpha_a)} x_a(\alpha_a, z) \\ & \times \exp\left(-A \int_0^\infty dz' m_a(\alpha_a, z, z') [\beta\Psi(z')q_a + \beta\pi(z')v_a]\right). \end{aligned} \quad (12)$$

The factor, $x_a(\alpha_a, z) \equiv 1$ for an adsorbing molecule with permanently charged units, while for one containing basic or acidic groups:

$$\begin{aligned} x_a(\alpha_a, z) = & \left(\frac{g_a^b}{1 - f_a^b}\right)^{m_a} e^{-m_a\beta\mu_{ad}^0} \\ & \times \exp\left(-A \int_0^\infty dz' m_a(\alpha_a, z, z') \ln(f_a(z'))\right), \end{aligned} \quad (13)$$

where m_a is the degree of oligomerization of the chain, and f_a^b and g_a^b are respectively the degrees of charge and protonation of an adsorbate segment in the bulk solution. The bulk degree of charge and protonation are both completely determined by the intrinsic logarithmic acidity constant of the titratable group, pK_a , and the solution pH. These quantities (f_a^b and g_a^b) are related to each other by the same expression given by Eq. (8). In the case of a

non-oligomeric adsorbing species, Eq. (12) is still valid through setting m_a to 1. To obtain Eq. (12), we have assumed that the molecule of interest is water-soluble, which is modeled by setting the adsorbate-adsorbate van der Waals attractions to zero. Using the same model, gel monomers are also considered to be soluble in water. Moreover, we are primarily interested in the effect that charge regulation by both the adsorbent material and the adsorbate has on the thermodynamics of adsorption. For that reason, adsorbate-gel van der Waals attractions are neglected. Then, the total energy due to these interactions is $U_{\text{vdw}} = 0$. The local fraction of charged segments, in the case of an acidic/basic adsorbate species, can be obtained from

$$\frac{f_a(z)}{1 - f_a(z)} = \frac{f_a^b}{1 - f_a^b} \exp(-\beta\Psi(z)q_a). \quad (14)$$

The probability of a conformation α_n of the polymer network is given by

$$P_n(\alpha_n) = \frac{1}{Q_n} \exp\left(-A \int_0^\infty dz \rho_n(\alpha_n, z) [\ln f_n(z) + \beta\Psi(z)q_n + \beta\pi(z)v_n]\right), \quad (15)$$

where the factor Q_n imposes $\sum_{\alpha_n} P_n(\alpha_n) = 1$. The local degree of dissociation of network segments that minimizes the free energy functional can be obtained from the following expression

$$\frac{f_n(z)}{1 - f_n(z)} = \frac{K_n^0}{a_{H^+}} \exp(-\beta\Psi(z)q_n), \quad (16)$$

where K_n^0 is the unitless equilibrium constant that describes the dissociation of the acidic groups of the network. The variation of the free energy with respect to the electrostatic potential results in the Poisson equation,

$$\epsilon \nabla^2 \Psi(z) = -\langle \rho_q(z) \rangle. \quad (17)$$

At this point, the only quantities that remain to be determined are the local osmotic pressure, $\pi(z)$, and the electrostatic potential, $\Psi(z)$, which are obtained by solving at each position the incompressibility constraint and the Poisson equation, Eqs. (9) and (17), respectively.

Once these interaction fields are known, all the functions that compose the free energy can be calculated. Any thermodynamic quantity of interest can then be obtained in a straightforward manner.

We have described the theory in general terms, which allows for its application to study a variety of systems, including different adsorbates and hydrogels. The specific molecular details of the polymer network and adsorbate species enter the theoretical framework through their sets of conformations, $\{\alpha_n\}$ and $\{\alpha_a\}$, respectively. The input of the theory that incorporates the molecular architecture of the polymer network is the local density-distribution of segments, $\{\rho_n(\alpha_n, z), \forall \alpha_n \in \{\alpha_n\}\}$. The molecular conformations of the network are generated using Molecular Dynamics (MD) simulations. We study the adsorption of homooligomers and single monomers within a thin hydrogel film having some of its weak polyacid chains chemically grafted to a surface. The network is composed of 25 segment-long polyacid chains inter-connected at six-coordinated crosslinking segments. Most of these chains connect two crosslinks, apart from the topmost chains that have their solution-side ends free, and the chains that are grafted by one of their ends to fixed positions on the surface. Grafting points are arranged on the surface forming a square lattice. The logarithmic acidity constant of a network monomer is taken as $\text{pK}_n = 5$ to represent a carboxylic acid such as acrylic acid.^{S4} Thus, the adsorbent material we are modeling can be a polyacrylic acid hydrogel chemically grafted to a solid substrate. In our recent work,^{S3} we have described the molecular model used for the polymer network more extensively as well as provided detailed information on the MD simulations employed to generate conformations of the network.

The molecular information of the adsorbate of interest is incorporated into the formalism via the input, $\{m_a(\alpha_a, z, z'), \forall \alpha_a \in \{\alpha_a\}\}$, which gives the number density of segments of a single molecule, for all of the different conformations of the species and for each position of its center of mass. We are most interested in the adsorption within the polyacrylic acid gel of short n -mers of histidine (his_n). We consider different degrees of oligomerization, from a single histidine molecule up to his_{12} . Each residue of the his_n is represented by a single bead

centered at the position of the C_α with $\text{pK}_a = 6$ of its conjugated acid.^{S4} These oligomers are modeled using a rotational isomeric state model in which each bond can assume one out of three isoenergetic configurations.^{S5} Each of the self-avoiding conformations generated using this method is rotated 36 times by random Euler angles, which yields a total number of 72180 conformations for his_{10} and 614420 for his_{12} , for example. The bond length is taken as $l_a = 0.5$ nm, which gives the effective distance between the centers of two neighboring unified groups. The volume of a histidine residue is $v_a = 0.15$ nm³. We also consider the adsorption of aspartic acid, modeled as having the same diameter as a histidine molecule, volume $v_a = 0.12$ nm³, and $\text{pK}_a = 3.7$. In addition, we investigate the adsorption of different permanently charged molecules, which are modeled as spherical particles with diameter l_a , equal to that of a histidine residue to allow for a straightforward comparison, and volume $v_a = \pi \frac{l_a}{6}$.

2 Additional Results

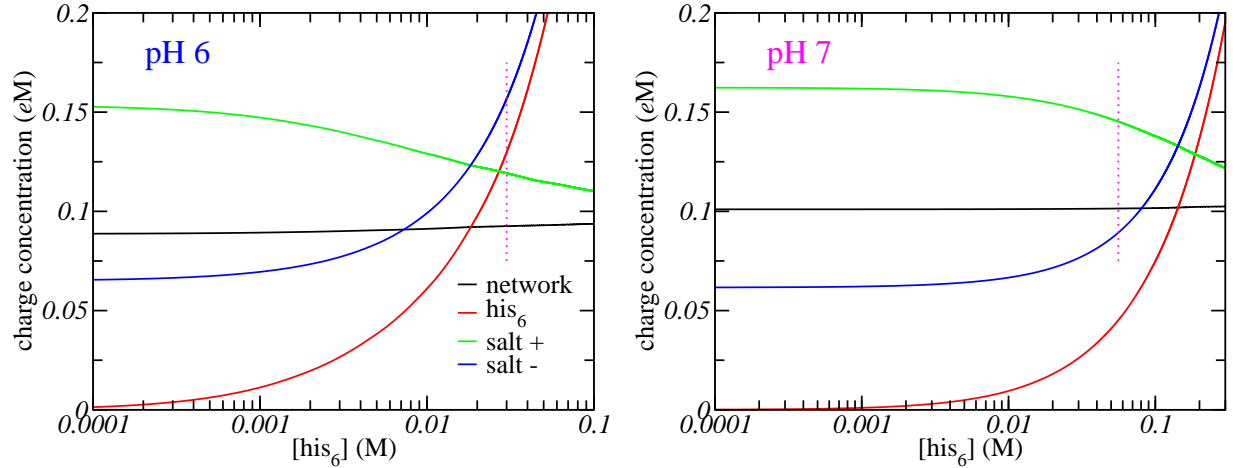


Figure S2: Concentration of electric charge (in absolute value) of the different species inside the film as a function of bulk concentration of hexahistidine. A dotted magenta line, parallel to the vertical axis, gives the his_6 concentration of maximum adsorption at the corresponding bulk pH and c (see article's Figure 4B). The solution pH is 6 and 7 in the left and right panels respectively. In both panels, the grafting density is $\sigma = 0.012$ nm⁻² and the bulk salt concentration is $c = 100$ mM.

Figure S2 shows the concentration of charge (in absolute value) of the different species

inside the film as a function of the bulk concentration of hexahistidine, $[\text{his}_6]$. First, notice that the total concentrations of positive and negative charge are balanced inside the film. The electroneutrality of the film is a condition obtained as a result of minimizing the free energy of the entire system. In our calculations, we only impose that the total charge must be zero when considering the whole system from the supporting surface to the bulk solution (global electroneutrality). Global electroneutrality is a much weaker constraint than imposing charge neutrality of the film.

The two panels of Fig. S2 correspond to conditions such that the adsorption isotherms (Γ vs. $[\text{his}_6]$) display a maximum as a function of the bulk concentration of hexahistidine (see Figure 4B of the article). The his_6 concentrations of such maxima are marked by dashed magenta lines in both panels. Maximum adsorption occurs near the $[\text{his}_6]$ where the adsorbed hexahistidine balances the charge of the network. At higher concentrations, the network cannot adsorb his_6 through electrostatic attractions, and the adsorption hexahistidine drives the additional incorporation of significant amounts of salt anions to neutralize the charge of the film.

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