Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for
Lysozyme Adsorption in pH-responsive Hydrogel Thin-Films: The non-trivial Role of Acid-Base Equilibrium

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S1. Molecular Theory

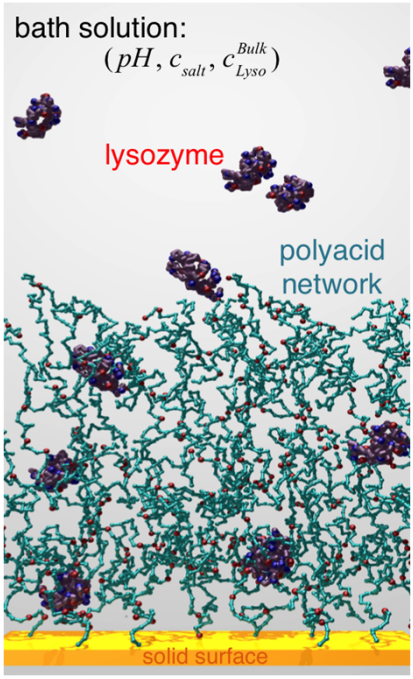

Figure S1. Schematic representation of the system of interest; a grafted polyacid network is in contact with an aqueous salt solution that contains lysozyme. Far above the film the solution pH, salt concentration and protein concentration are all externally controlled.

Consider the adsorption of lysozyme on a hydrogel thin film that is grafted to a planar surface. The hydrogel is formed by a network of crosslinked polyacid chains, in contact with a solution that contains lysozyme ($Lyso$), water (w), protons (H^+), hydroxyl ions (OH^-), and monovalent cations ($+$) and anions ($-$) due to dissociate salt ($NaCl$, for example). Far from the surface, the pH of the bulk solution, its salt concentration, c_{salt}, and the concentration of the lysozyme, c_{Lyso}^{Bulk}, are all externally controlled (see Figure S1).

To study the adsorption of lysozyme, we use a molecular theory that is an extension of the one developed to investigate the adsorption of his-tag within the same kind of thin hydrogel films.[1] The first step in this methodology consists in writing the total Helmholtz free energy of the system:

$$F = -TS_{ConfN} + F_{ChmN} - TS_{Mix} - TS_{TR} + F_{ChmP} + U_{Elect} \quad (S1)$$

where T is the temperature. The first term in Equation S1 contains the conformational entropy of the polymer network that makes the backbone of the hydrogel film; this entropy can be expressed as:

$$S_{ConfN} = -k_B \sum_{\alpha_N} P_N(\alpha_N)\, ln(P_N(\alpha_N)) \quad (S2)$$

where k_B is the Boltzmann constant and $P_N(\alpha_N)$ is the probability of finding the polymer network in its conformation α_N.

The term F_{ChmN} contains the mixing free energy of the acid-base equilibrium of network segments as well as the self-energies of the protonated and deprotonated segments. Assuming that the system is inhomogeneous only in the direction perpendicular to the supporting surface, this contribution can be written as:

$$F_{ChmN} = k_B T A \int_0^\infty dz \langle \rho_{Pol}(z) \rangle \left\{ f_{Gel}(z) \left[\ln f_{Gel}(z) + \beta \mu_{A^-}^0 \right] + (1 - f_{Gel}(z)) \left[\ln (1 - f_{Gel}(z)) + \beta \mu_{AH}^0 \right] \right\} \quad (S3)$$

where the coordinate z measures the distance from the supporting surface of total area A. The angle brackets in the local density of polymer segments, $\langle \rho_{Pol}(z) \rangle$, represent an ensemble average over the set of network conformations; *i.e*,

$$\langle \rho_{Pol}(z) \rangle = \sum_{\alpha_N} P_N(\alpha_N) \rho_{Pol}(\alpha_N, z) \quad (S4)$$

where $\rho_{Pol}(\alpha_N, z)$ is the number density of segments at z when the polymer network is in conformation α_N. In Eq. S3, $f_{Gel}(z)$ is the local degree of dissociation of the network, which gives the fraction of the segments located at z that are deprotonated (charged). Then, the local density of charged network segments is $f_{Gel}(z)\langle \rho_{Pol}(z) \rangle$, while that of uncharged segments is $(1 - f_{Gel}(z))\langle \rho_{Pol}(z) \rangle$. The standard chemical potentials of the deprotonated (charged) and protonated (uncharged) network segment are $\mu_{A^-}^0$ and μ_{AH}^0, respectively.

The third term in the free energy (Eq. S1) is the mixing (translational) entropy of the small mobile species (w, H^+, OH^-, Na^+ and Cl^-), expressed as a sum over the these species:

$$S_{Mix} = -k_B A \sum_{\gamma \in \{w, H^+, OH^-, +, -\}} \int_0^\infty dz \rho_\gamma(z) \left[\ln \left(\rho_\gamma(z) v_w \right) - 1 + \beta \mu_\gamma^0 \right] \quad (S5)$$

The quantities $\rho_\gamma(z)$ and μ_γ^0 are respectively the local density and standard chemical potential of mobile species γ, with $\gamma \in \{w, H^+, OH^-, +, -\}$, while v_w is the volume of a water molecule.

The fourth contribution to the free energy accounts for both the translational and rotational degrees of freedom of the protein; this contribution can be expressed as:

$$S_{TR} = -k_B A \int_0^{\infty} dz \sum_{\theta_{Lyso}} \rho_{Lyso}(\theta_{Lyso},z)\left[\ln\left(\rho_{Lyso}(\theta_{Lyso},z)v_w\right) - 1 + \mu_{Lyso}^{0}\right] \quad (S6)$$

where μ_{Lyso}^{0} is the standard chemical potential of lysozyme. The quantity $\rho_{Lyso}(\theta_{Lyso},z)$ gives the position dependent number density of lysozyme molecules in orientation θ_{Lyso}. Then, the total density of protein with center of mass at z is

$$\langle\rho_{Lyso}(z)\rangle = \sum_{\theta_{Lyso}} \rho_{Lyso}(\theta_{Lyso},z) \quad (S7)$$

The angle brackets in this equation represent an ensemble average over all the possible rotations of the protein, where the probability of finding the protein at z in conformation θ_{Lyso} is given by

$$P_{Lyso}(\theta_{Lyso},z) = \frac{\rho_{Lyso}(\theta_{Lyso},z)}{\langle\rho_{Lyso}(z)\rangle} \quad (S8)$$

The fifth term in Eq. S1 is the chemical free energy of the protein that accounts for the acid-base equilibrium of the different titratable amino acids; this term is written as:

$$F_{ChmP} = k_B T A \int_0^{\infty} dz \sum_{aa \in \{Asp,Glu,Tyr,Arg,His,Lys\}} \langle n_{aa}(z)\rangle\left\{g_{aa}(z)\left[\ln\left(g_{aa}(z)\right) + \beta\mu_{aa,p}^{0}\right] + (1 \quad (S9)$$

where $\langle n_{aa}(z)\rangle$ is the local density amino acid segments type aa (with $aa \in \{Asp,Glu,Tyr,Arg,His,Lys\}$), and $g_{aa}(z)$ gives the fraction of such segments that are protonated. The standard chemical potentials of type aa protonated and deprotonated residues are $\mu_{aa,p}^{0}$ and $\mu_{aa,d}^{0}$, respectively. The local density of type aa residues can be expressed as:

$$\langle n_{aa}(z)\rangle = A\int_0^{\infty} dz' \sum_{\theta_{Lyso}} \rho_{Lyso}(\theta_{Lyso},z')m_{aa}(\theta_{Lyso},z',z) \quad (S10)$$

where the quantity $m_{aa}(\theta_{Lyso},z,z')$ gives the number of segments type aa that a single lysozyme molecule with center of mass at z contributes to z'. Note that Eq. S10 is valid for all kinds of amino acids including the non-titratable (neutral) ones.

The last term in F (Eq. S1) is the electrostatic energy of the system:

$$U_{Elect} = k_B T A\int_0^{\infty} dz\left[\langle\rho_q(z)\rangle\beta\psi(z) - \frac{1}{2}\beta\epsilon(\nabla\psi(z))^2\right] \quad (S11)$$

where $\psi(z)$ is the electrostatic potential, and ϵ denotes the dielectric permittivity of the medium. In the electrostatic energy, $\langle\rho_q(z)\rangle$ represents the average total charge density at z, given by:

$$\langle\rho_q(z)\rangle = q_N f_{Gel}(z)\langle\rho_{Pol}(z)\rangle + \sum_{\gamma=+,-,H^+,OH^-} q_\gamma\rho_\gamma(z) + \sum_{aa\in\{Asp,Glu,Tyr,Arg,His,Lys\}} q_{aa} f_{aa}$$

where q_N is the electric charge of the deprotonated network segment, and q_+, q_-, q_{H^+}, and q_{OH^-} denote the electric charge of the corresponding free species. The local degree of charge of type aa titratable residues, $f_{aa}(z)$, gives the fraction of such units at z that are ionized having electric charge q_{aa}. Depending on whether the residue bear an acidic or basic functional group, the degree of charge is related to that of protonation by the following relation:

$$f_{aa}(z) = \begin{cases} 1 - g_{aa}(z) & for\ acidid\ groups \\ g_{aa}(z) & for\ basic\ groups \end{cases} \quad (S13)$$

In addition, the free energy given by Eq. S1 must satisfy two physical constrains. First, the system must be electroneutral. This global constrain implies that

$$\int_0^\infty \langle \rho_q(z) \rangle dz = 0 \quad (S14)$$

The second physical constraint is the local incompressibility of the system, which accounts for the intermolecular repulsions (excluded volume interactions). This constraint requires that at each position, the available volume must be completely occupied by some of the molecular species:

$$1 = \langle \rho_{Pol}(z) \rangle v_N + \sum_{\gamma = w, +, -, H^+, OH^-} \rho_\gamma(z) v_\gamma + \sum_{aa \in \text{all amino acids}} \langle n_{aa}(z) \rangle v_{aa} \quad (S15)$$

where v_N is the volume of a network segment, v_{aa} is the volume of the amino acid residue type aa, and v_w, v_+, v_-, v_{H^+}, v_{OH^-} are the molecular volumes of the corresponding free species. Note that in the incompressibility constraint, the index aa runs over all residue types (neutral and titratable amino acids).

In addition, the system is in equilibrium with a solution of controlled composition, which fixes the chemical potential of all the free species, including proteins. This implies that the chemical potential of these species must be the same everywhere, inside the hydrogel for adsorbed molecules and in the solution at any distance from the surface.

Equations S2 to S13 give explicit expressions for all the physicochemical contributions to the free energy. We now need to determine the following functions: the probability density function of network conformations, $P_N(\alpha_N)$, the local number density of lysozyme molecules for each rotation, $\rho_{Lyso}(\theta_{Lyso}, z)$, 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, $f_{Gel}(z)$, and that of protein residues $f_{aa}(z)$ (or equivalently their local degree of protonation $g_{aa}(z)$), and the electrostatic potential, $\psi(z)$. Functional optimization of the

proper thermodynamic potential with respect to each of these quantities, subject to the two aforementioned constrains (Equations S14 and S15), yields expressions for each of these functions.

For the mobile species, except for the lysozyme, we obtain:

$$\rho_\gamma(z)v_w = a_\gamma \exp\left(-\beta\pi(z)v_\gamma - \beta\psi(z)q_\gamma\right) \quad (S16)$$

including for water with $q_w = 0$. The local Lagrange multiplier, $\pi(z)$, is introduced to enforce the incompressibility constraint, Eq. S15, at each distance from the surface. This Lagrange multiplier is related to the local osmotic pressure in the system. The activity of free species γ, $a_\gamma = \exp\left(\beta\left(\mu_\gamma - \mu_\gamma^0\right)\right)$, 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. These activities are completely determined by composition of the bath solution (pH, c_{salt}, and c_{Lyso}^{Bulk}), using the incompressibility and charge-neutrality of this solution.

The local density of a given rotation θ_{Lyso} of lysozyme can be written as:

$$\rho_{Lyso}(\theta_{Lyso},z) = a_{Lyso}x_{Lyso}\exp\left(-A\int_0^\infty dz' M_{Lyso}(\theta_{Lyso},z,z')\right) \quad (S17)$$

where a_{Lyso} is the activity of the protein, and the quantity in the integral is

$$M_{Lyso}(\theta_{Lyso},z,z') = \sum_{aa\ \in\ \{Asp,Glu,Tyr,Arg,His,Lys\}} m_{aa}(\theta_{Lyso},z,z')\left(\beta\psi(z')q_{aa} + \ln f_{aa}(z')\right) + \sum_{aa\ \in\ all\ amino} \quad (S18)$$

The factor multiplying the activity in Eq. S17 is

$$x_{Lyso} = \prod_{aa\ \in\{Asp,Glu,Tyr,Arg,His,Lys\}} \left(\frac{g_{aa}^b}{1-f_{aa}^b}\right)^{cn_{aa}} \exp\left(-cn_{aa}\,\beta\mu_{aa,d}^0\right) \quad (S19)$$

where f_{aa}^{b} and g_{aa}^{b} are respectively the degree of charge and protonation of type aa amino acid residues in the bulk solution, which are related to each other by the same expression as Eq. S13. The bulk degree of charge, and thus that of protonation, is completely determined by the bulk pH and the intrinsic pKa of the amino acid. The composition number, cn_{aa}, gives total number of residues type aa that the protein bears (see Table 1 in the article) and satisfies

$$cn_{aa} = A\int_{0}^{\infty} dz' m_{aa}\left(\theta_{Lyso}, z, z'\right) \quad (S20)$$

for any position z and rotation θ_{Lyso}.

The last quantity to express for the protein is the local fraction of charged residues, which can be obtained from

$$\frac{f_{aa}(z)}{1-f_{aa}(z)} = \frac{f_{aa}^{b}}{1-f_{aa}^{b}}\exp\left(-\beta\psi(z)q_{aa}\right) \quad (S21)$$

while for the local degree of charge of network segments we obtain:

$$\frac{f_{Gel}(z)}{1-f_{Gel}(z)} = \frac{K_N^0}{a_{H^+}}\exp\left(-\beta\psi(z)q_N\right) \quad (S22)$$

where K_N^0 is the unit-less equilibrium constant that describes the dissociation of the acidic groups of the network. This equilibrium constant is an intrinsic property of the acid group and one of the inputs of the theory.

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

$$P_N(\alpha_N) = \frac{1}{Q_N}\exp\left(-A\int_{0}^{\infty} dz\rho_{Pol}(\alpha_N, z)\left[\beta\psi(z)q_N + \ln f_{Gel}(z) + \beta\pi(z)v_N\right]\right) \quad (S23)$$

where the factor Q_N is the normalization constant that ensures that $\sum_{\alpha_N} P_N(\alpha_N) = 1$.

Furthermore, the variation of the free energy with respect to the electrostatic potential, assuming that ϵ does not depend on position, results in the Poisson equation,

$$\epsilon\nabla^2\psi(z) = -\langle\rho_q(z)\rangle \quad (S24)$$

At this point, the only remaining unknowns are the local osmotic pressure, $\pi(z)$, and the electrostatic potential, $\psi(z)$, which are obtained by solving at each distance from the surface the incompressibility constraint (Eq. S15) and the Poisson equation (Eq. S24) (17). Once these interaction fields are known, all terms in the free energy can be computed. Thus, any thermodynamic quantity of interest can be obtained in a straightforward manner.

To allow for the application of the molecular theory to different systems, we have described the approach in general terms. In particular, the only modification needed to study other proteins is changing the set of titratable amino acids. Moreover, the approach is also valid for different polyelectrolyte layers/networks.

The specific molecular details of the protein enter the theoretical framework through the number density of type aa residues $m_{aa}(\theta_{Lyso},z,z')$, which must be provided as an input for each type of residue defined in the protein model. We remind the reader that $m_{aa}(\theta_{Lyso},z,z')$ gives the number of segments type aa that a single protein molecule in configuration (rotation) $\theta_{Lyso}$with center of mass at z contributes to z'. The molecular information of the other free species is introduced through quantities such as their volume v_γ and electric charge q_γ.

Moreover, the input that incorporates the molecular information of the polymer network is the number density of polymer segments $\rho_{Pol}(\alpha_N,z)$ that must be given for the whole set of conformations of the network, $\{\alpha_N\}$.

In the next section, we describe the molecular model used to describe the protein and polymer network.

S2. Molecular Model

S2.1. Polymer Network

The input necessary to solve the equation describes includes the set of conformations for the polymer network and the protein. The necessary quantity for the polymer network is the conformation dependent local density of segments, $\{\rho_{Pol}(\alpha_N,z),\forall z\ and\ \alpha_N \in \{\alpha_N\}\}$. The molecular conformations of the network, $\{\alpha_N\}$, are generated using Molecular Dynamics (MD) simulations. The network is composed of 25 segment-long 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 some 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, with grafting density σ. All units of the network are spherical and have the same diameter (segment length), $l_N = 0.5\ nm$, and molecular volume, $v_N = \frac{\pi}{6} l_N^3 = 0.0655\ nm^3$.

The intrinsic logarithmic acidity constant of a network segment is taken as $pK_N = 5$ to represent a carboxylic acid such as acrylic acid. Thus, the hydrogel we are modeling could be a polyacrilic acid network chemically grafted to a solid substrate. In our recent work, 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.[1,2]

S2.2 Lysozyme Coarse Grain Model

We use a coarse-grained model to represent lysozyme, starting from the position of all atoms obtained from the crystallographic structure PDB file (193L). Each protein residue (lysozyme has 134 amino acid residues) is represented by a single bead, which is a solid sphere of diameter $d = 0.6\ nm$, centered at the position of the corresponding α-carbon (see Figure S2). The volume of each bead is $v_{CG} = 0.0655\ nm^3$, which is the same for all types of residues ($v_{aa} = v_{CG}, \forall\ aa$). The protein has full 3D rotational and translational freedom, but the relative position of all beads remains frozen to the initial coarse-graining of the PDB structure.

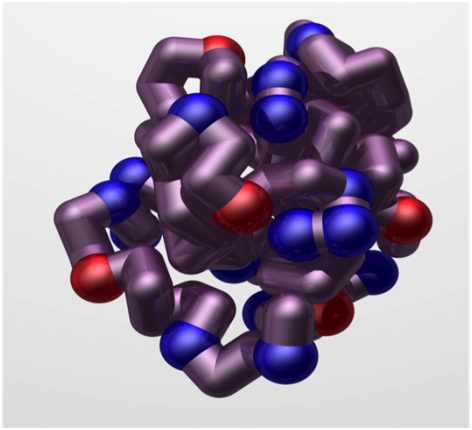

Figure S2. The scheme illustrates the coarse grained model used to describe lysozyme. Basic amino acid residues are shown in blue while acidic ones are colored using red. Charge neutral residues are displayed as purple elbows.

Lysozyme has six different types of titratable amino acids: Aspartic acid (Asp), Glutamic acid (Glu), Tyrosine (Tyr), Arginine (Arg), Histidine (His) and Lysine (Lys). Each of these amino acids is characterized by an intrinsic pKa, pKa_{aa} with $aa \in \{Asp, Glu, Tyr, Arg, His, Lys\}$. All the remaining residues are considered as charge neutral. In the article, Table 1 shows the pKa of each type of titratable amino acid residue as well as the composition number of each residue type, including the neutral type.

The matrix $m_{aa}(\theta_{Lyso},z,z')$ gives the total number of segments type aa at z' contributed by a single protein in rotational state θ_{Lyso} and with center of mass at z. This quantity incorporates the molecular details of the protein into the theory. For each residue type and protein rotation, $m_{aa}(\theta_{Lyso},z,z')$ must be supplied as an input for all positions of the residue and the protein's center of mass.

S3. Maximum Adsorption

In this section, we obtain an approximate value for the maximum lysozyme concentration possible inside the film. Let us consider the close packing of spheres with the diameter of the protein $d_{Lyso} \sim 2.8\,nm$ (calculated as twice the radius of gyration of the crystallographic structure PDB file (193L)). The volume fraction occupied by these spheres is

$$f_{cp} = \frac{\pi}{\sqrt{18}} \approx 0.74$$

The maximum density of lysozyme, ρ_{Lyso}^{max}, can be approximated by that of the closed packed spheres. Then,

$$\rho_{Lyso}^{max} = \frac{f_{cp}}{v_{Lyso}} \approx 0.064\ nm^{-3}$$

where $v_{Lyso} = \frac{\pi}{6} d_{Lyso}^{3}$ is the volume of a lysozyme molecule (approximated by that of solid sphere). This number density is equivalent to a molar concentration

$$c_{Lyso}^{max} \approx 0.1\ M$$

S4. Additional Results

In this section, we present results that complement or support those discussed in the article. Figure S3 shows adsorption profiles, Γ as a function of pH, for different salt concentrations. These curves are discussed in more detail in the article. Briefly, the adsorption, Γ, depends non-monotonically on the solution acidity with a maximum in the intermediate pH range and negligible adsorption at either extreme acid or basic pH.

When $pH = pI$, the isoelectric point, protein molecules in the bulk solution have zero net charge. In our lysozyme model, $pI \approx 10.9$, in agreement with experimental results.[3] If $pH > pI$, solution proteins are negatively charged. Therefore, adsorption under these conditions is not *a priori* expected. In Figure S3, however, we see that at low salt concentration there is adsorption for pH values above the solution isoelectric point of the protein. As c_{salt} increases, the adsorption under these conditions eventually vanishes.

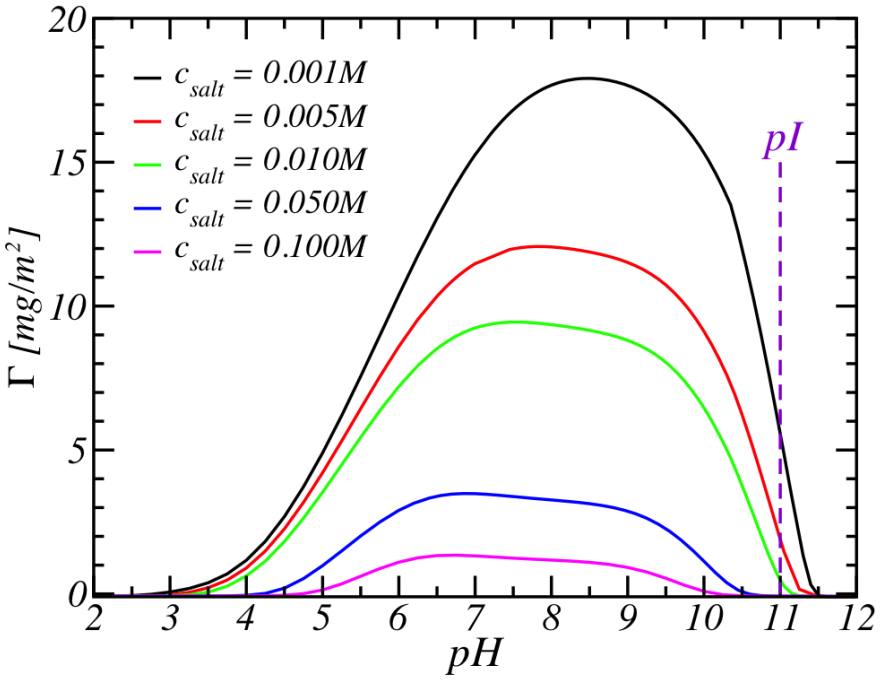

Figure S3. Lysozyme adsorption, Γ, as a function of the solution pH for different salt concentrations. The network grafting density is $\sigma = 0.084\ nm^{-2}$, and the bulk protein concentration is $c_{Lyso}^{Bulk} = 10^{-4} M$.

As a result of optimizing the free energy of the system, we obtain an expression for the local number density of lysozyme $\rho_{Lyso}(z)$ (see Section S1), which is proportional to the molar concentration, $c_{Lyso}(z)$. The effect of the solution pH on the distribution of protein as a function of the distance from the surface is illustrated in Figure S4. The conditions corresponding to neutral and acidic solution pH are shown in panel A, while those corresponding to basic solutions are presented in panel B.

Three different regions can be clearly distinguished in each of the z-dependent profiles shown in Figure S4. Far from the surface (large z), the protein concentration approaches the bulk concentration, c_{Lyso}^{Bulk}. The interfacial region begins near the top the film and extends for a few tens of nanometers ($50\ nm \lesssim z \lesssim 80\ nm$); as z decreases in this interface, lysozyme concentration goes from its bulk value to the value observed inside

the film. Finally, the concentration established within the film can be significantly different from that of the bulk solution.

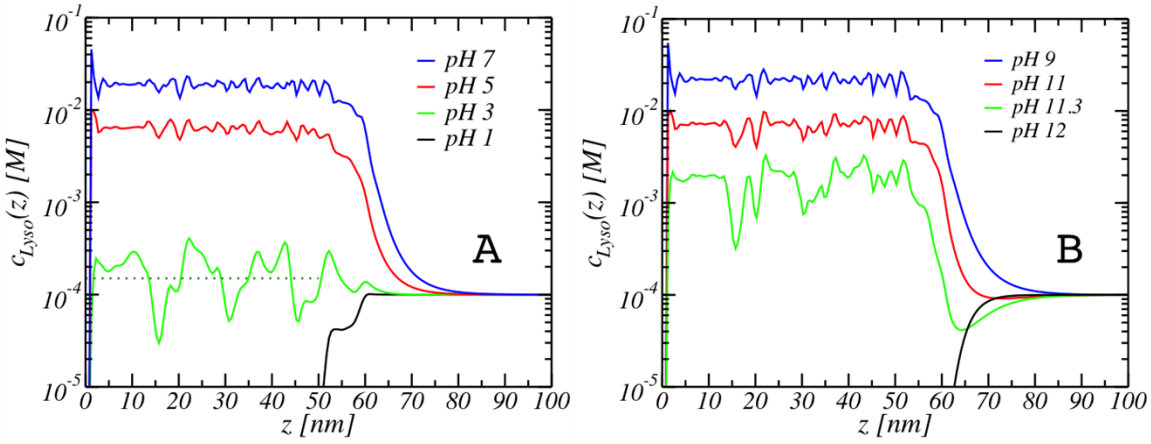

Figure S4. Lysozyme concentration profiles as a function of distance from the substrate, z, at different pH values. Neutral pH and acidic conditions are shown in panel A, while basic solutions are displayed in panel B. The green dotted line in panel A shows the average concentration inside the film, $\langle c_{Lyso} \rangle$, at $pH\ 3$. The protein and salt concentrations in the bulk solution are $c_{Lyso}^{Bulk} = 1 \times 10^{-4}\ M$ and $c_{salt} = 1 \times 10^{-3}\ M$, respectively.

At strong acid conditions ($pH\ 1$), lysozyme concentration drops significantly in the interior of the film (see Figure S4A). The network is nearly charge-neutral, and the electrostatic attractions with the positively charged protein are negligible. Thus, the protein is depleted from the film due to steric and entropic repulsions.

The lysozyme concentration established inside the hydrogel is well characterized by its volume average:

$$\langle c_{Lyso} \rangle = \frac{1}{H_{Gel}} \int_0^{H_{Gel}} c_{Lyso}(z) dz$$

where H_{Gel} is the film thickness defined by

$$H_{Gel} = \frac{\int_0^{\infty} (2z)\langle \phi_{Pol}(z) \rangle dz}{\int_0^{\infty} \langle \phi_{Pol}(z) \rangle dz}$$

In the last equation, $\langle \phi_{Pol}(z) \rangle$ is the (ensemble average) local volume fraction of network polymer. At $pH\,3$, the average concentration of adsorbed protein is similar to the bulk concentration, as observed in Figure S4A (see green dotted line). The regular features observed in the concentration profiles, particularly at $pH\,3$, are associated with the structure of the network. The density of the network and consequently the density of localized negative charge are higher in the regions near the most likely positions of the crosslinks.

The concentration of lysozyme inside the hydrogel increases almost two orders of magnitude at $pH\,5$, as seen in Figure S4A. A similar situation occurs at $pH\,7$, where the adsorbed concentration reaches $\langle c_{Lyso} \rangle \approx 0.02\,M$, which is near the maximum possible, estimated considering the close packing of hard spheres ($c_{Lyso}^{max} \approx 0.09\,M$, see Section S3).

The concentration profiles for basic solutions are shown in panel B of Figure S4. At these conditions, the adsorbed concentration decreases as pH increases until the protein is completely depleted from the film at sufficiently high pH. At $pH\,11.3$, above the protein pI, the results of Figure S4B predict significant adsorption. The local concentration of lysozyme inside the hydrogel is more than an order of magnitude higher than that of the bulk solution. There is a region near the top of the film, however, where depletion is predicted (*i.e.,* $c_{Lyso}(z)$ is locally smaller than in the bulk). In particular, the protein concentration has a local minimum around $60 - 70\,nm$. At this pH, the protein is

negatively charged in the bulk solution (alike the polymer network). To favor adsorption, the protein becomes positively charged inside the film (see article's Figure 4B). The local minimum of the z-dependent concentration of lysozyme at pH 11.3 occurs near the position of charge reversal ($Z_{Lyso}(z) = 0$), as shown in Figure 4B of the article. At this point near the top of the film, the protein changes its net charge from negative (above; up to the bulk solution) to positive (below; down to the supporting surface). Therefore, the protein-network electrostatic interactions change from repulsive (above this position) to attractive (below).

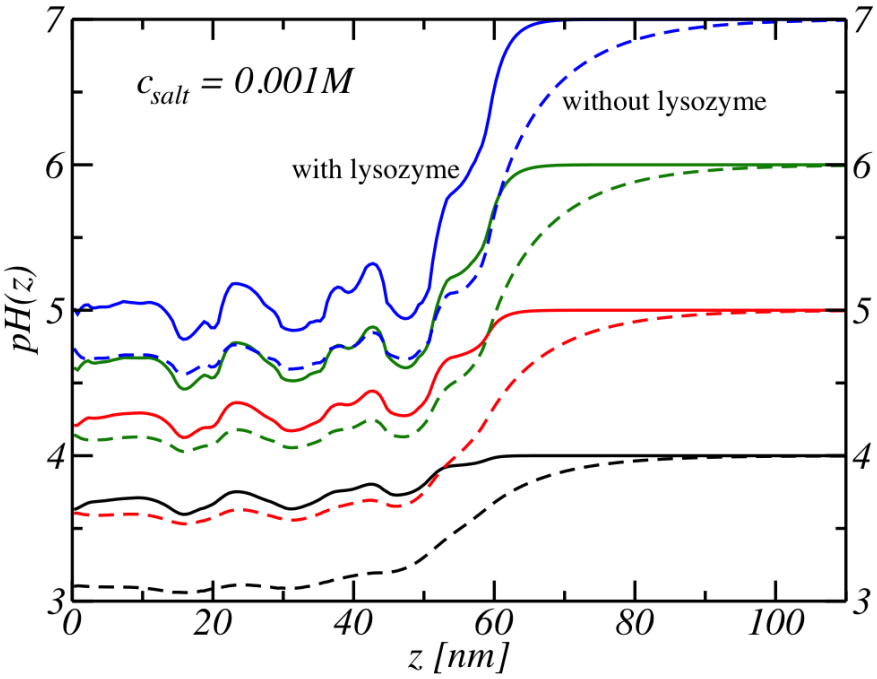

Figure S5. Local pH a function of distance from the substrate, z, for a variety of bulk solution conditions. Solid lines correspond to solutions with lysozyme concentration $c_{Lyso}^{Bulk} = 10^{-4}\,M$, while dashed lines represent solutions containing no protein. The network grafting density is $\sigma = 0.084\,nm^{-2}$, and the salt concentration is $c_{salt} = 1 \times 10^{-3}\,M$.

Next, we investigate further the molecular organization inside and near the hydrogel film by looking at the local pH variations. In the interior of the gel, pH drops significantly respect to the experimentally controlled (bulk) value. This drop in pH

depends sensibly on the bulk solution composition (pH, c_{salt}, and c_{Lyso}^{Bulk}). Moreover, Figure S5 shows that the interfacial region, where the local pH goes from its bulk to its film value, is shorter for lysozyme solutions. That is, the presence of the protein reduces the length of the interface. The reason for this behavior is twofold. First, the pH drops less inside the film for lysozyme solutions, as shown in Figure S5. More importantly, the length of this interfacial region depends mainly on the ionic strength of the bulk solution.[1] As the ionic strength decreases, the film-solution interface becomes longer. In the cases shown in Figure S5, the ionic strength is higher for the lysozyme solutions, since the protein is also electrically charged.

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