Supplementary Material

Figure S1: (a) Tapping-mode AFM height images of a 5μm x 5μm area of CNWs spread onto a mica surface and (b) transverse height profiles determined along lines 1 to 5 in image (a).

The nonlinear regression method has been effectively used in the characterization of titratable functional groups of proteins.\textsuperscript{1, 2} Figure S2 shows the potentiometric titration curve obtained for a collagen sample. The result show good agreement between experimental and simulated data. Simulated values were obtained by considering the presence of five functional groups titratable in the collagen (Table S1). This number of groups was defined statistically using the method described by Masini et al.\textsuperscript{2}
The results obtained using the calculation method for a collagen sample given in Table S1 are the averages of three titrations. Groups with pKa 2.992 and 4.389 resulting from the fitting procedure are assigned the β and γ-carboxylic groups, which correspond to the groups present in the side-chains of aspartic and glutamic acids, respectively. The pKa value of 6.279 corresponds to the imidazole groups of histidine, while the pKa of 7.797 and 9.649 correspond mainly to the sum of the phenolic groups from tyrosine and ε-amine groups from lysine. The sum of the carboxyl groups gives the total of anionic functional groups, while the sum of the others provides the total of the cationic functional groups. Actually, in the determination of the total cationic groups, it was not possible to separate the minor contribution of the phenolic (anionic) groups. This, there is an error in the calculation of this value, which is difficult to avoid using this method to determine the cationic groups. However, we believe that the obtained value of the total cationic groups (765 mmol .Kg⁻¹), together with the value of zeta potential obtained at pH ~4 (+ 15.1 mV), is representative of the cationic nature of this polymer, which can be considered weakly charged (in comparison to traditionally-used polyelectrolytes for multilayered films).
Table S1. Fit results of experimental data of the potentiometric titration of collagen considering the presence of five titratable functional groups.

<table>
<thead>
<tr>
<th>Ionizable functional group</th>
<th>pKa</th>
<th>Sítios / mmol g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carboxilic and γ-carboxilic</td>
<td>2.992 ± 0.004</td>
<td>0.612 ± 0.008</td>
</tr>
<tr>
<td>Imidazole</td>
<td>4.389 ± 0.007</td>
<td>0.513 ± 0.003</td>
</tr>
<tr>
<td>Imidazole</td>
<td>6.279 ± 0.052</td>
<td>0.086 ± 0.001</td>
</tr>
<tr>
<td>ε-amino and phenols</td>
<td>7.797 ± 0.032</td>
<td>0.142 ± 0.0003</td>
</tr>
<tr>
<td>ε-amino and phenols</td>
<td>9.649 ± 0.022</td>
<td>0.537 ± 0.013</td>
</tr>
</tbody>
</table>

To determine the isoelectric point (pI) of collagen, the linear titration method was performed, in which constant volumes of titrant were added. We first carried out the titration of 22 ml of HCl solution (0.022 mol L⁻¹) in the presence of KNO₃ (0.035 mol L⁻¹) with seventy injections of 0.200 mL of titrant (NaOH 0.0571 mol L⁻¹). Later, another titration was performed under the same conditions, with the presence of 0.108 g of collagen. The proton-binding capacity was determined using equation (1)³

\[
Q = \frac{V_0 + V_t}{m}\left([H^+]|_i - [OH^-]|_i - [H^+]|_f + [OH^-]|_f\right)
\]  

(1)

where \(V_0\) and \(V_t\) are the volumes of the background electrolyte and added titrant, and \(m\) is the mass of the collagen. Subscripts ‘\(i\)’ and ‘\(f\)’ refer to the HCl solution and collagen solution. The pI in \(Q\) was determined to be equal to zero.

Figure S3 shows the proton-binding isotherm obtained for the collagen sample. For positive values there is an adsorption of protons, while at negative values, there is a released of protons. The pH at which \(Q\) is zero defines the pI and signifies that there is an equilibrium between the adsorption and release of protons (there is no excess of negative or positive charges). In this way, the value obtained for the pI is 6.95 (Figure S3). This value is in agreement with the results found by other authors.⁴
Figure S3. Isotherms of binding protons obtained for collagen.

Figure S4: Tapping-mode AFM height images of a 1μm x 1μm area for 10-bilayer collagen/CNW thin film grown on a glass substrate. (a) The last layer adsorbed was a CNW layer; (b) the same film with an additional top layer of collagen.
References