An Electrostatic Model to Understand and Adjust the Orientation of Adsorbed Proteins via an External Electric Field

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Abstract

Under the most common experimental conditions, the adsorption of proteins to solid surfaces is a spontaneous process that leads to a rather compact layer of randomly oriented molecules. Due to the importance of this process for the development of catalytic surfaces, a number of existing computational and experimental approaches try to predict and control the orientation of such molecules. However, and despite their own advantages, these tend to be either too expensive computationally, or oversimplified, undermining their ability to predict the most appropriate experimental conditions to maximize the catalytic activity of adsorbed proteins. To address this current need, we present an efficient computational approach to model the behavior of proteins near surfaces in the presence of an external electric field, based on continuum electrostatics. Our model can not only estimate the overall affinity of the protein with the surface, but also their most likely orientation as a function of the potential applied. In this way, a rational selection of the potential can be performed to maximize the accessibility of the protein’s active site to the solvent. The model relies on the Poisson-Boltzmann equation and was implemented in an extension of the code PyGBe that includes an external electric field, and renders the electrostatic component of the solvation free energy. Thus, the presented approach yields useful simulations on computational resources that are readily available in workstations and small clusters. To demonstrate the feasibility of this technique, we investigate the adsorption of trypsin onto a carbon electrode under potentiostatic conditions both numerically and experimentally. We found that even though the adsorption process is largely dominated by hydrophobic effects, the orientation of trypsin can be controlled through an external potential, influencing the position of the active sites, and resulting in an important change in the catalytic activity of the surface.
Introduction

Controlling the orientation of proteins as they adsorb to surfaces is a challenging and critical endeavor. This issue is specially important for the development of biosensors and biocatalysts because the orientation of proteins with respect to the sorbent surface may affect the availability of the active site. Since the adsorption process results from the combination of hydrophobic and electrostatic forces, it can be rationalized considering the physico-chemical properties of the surface, the protein’s 3D structure and amino acid sequence, as well as the experimental conditions. While hydrophobic substrates tend to interact with the hydrophobic core of the protein, driving conformational changes, hydrophilic surfaces tend to interact with the charged and polar functional groups of the protein’s surface, thus influencing their orientation. Along the same lines, recent studies have demonstrated that proteins appear to sense variations in the topography of their nanoscale environments, also potentially resulting in alterations of their orientation. In addition, several groups have developed approaches to influence the adsorption process (amount, orientation, kinetics, etc.) of proteins based on the natural heterogeneous distribution of charges, by performing chemical modifications on either the -NH$_2$ or the -COOH terminal groups, or by directed mutations. Alternatively, and although it can only be applied to conductive materials, the adsorption process can also be influenced by the application of an external electric field, potentially affecting the orientation.

Given the importance of the orientation on the resulting bioactivity, the adsorption process has also been investigated with several computational models, ranging from highly detailed (but slow), molecular dynamics, to more phenomenological (yet faster), coarse-grained approaches. The latter methods are usually based on bead-type descriptions, that agglomerate several atoms into a single effective bead. This comes at the cost of higher levels of parameterization. A possibility to overcome this limitation is to restrict the approximated representation to the solvent only, leaving the protein with an all-atom description. In this regard, implicit-solvent models are an interesting alternative because although they consider
the solvent as a continuum dielectric medium, they provide a complete molecular description of the solute. This sets a framework where continuum electrostatic theory can be easily applied. Under this premise, the ions present in the solvent are considered as point charges that are free to move in response to an electric field, and they arrange at equilibrium according to Boltzmann statistics. In turn, this gives rise to the Poisson-Boltzmann equation to solve for the electrostatic potential. The continuum description makes the calculation of free energies easy, and rather than studying the problem dynamically, a thermodynamic-state analysis becomes natural. Despite these advantages, two important drawbacks of this method remain: the assumption of a rigid solute and the need for well-parameterized dielectric constants. However, the implicit-solvent model remains an efficient approach to study the orientation of proteins at adsorption. While some computational studies have included the effect of external electric fields on the adsorption process, mainly with all-atom molecular dynamics, there is still a critical need to develop faster computational models to accurately describe and potentially predict how proteins would behave in the proximity to a surface charged via an external electric field.

Aiming to address this need and advance the development of bio-catalysts, this report describes an efficient computational model to predict the adsorption of proteins to conductive surfaces, as well as the possibility to affect their orientation using an external electric field. This model represents a new approach to describe adsorption under an external field using the Poisson-Boltzmann equation, enabling researchers to perform useful computations on workstations and small clusters, which are currently available in most universities and research centers around the world. In particular, the model extends the implicit solvent Poisson-Boltzmann model implemented in PyGBe, to include an external electric field. PyGBe is a boundary-element solver, that computes the electrostatic component of the solvation free energy. Here, this free energy is used to calculate the probability of different orientations. Due to its industrial and clinical importance, trypsin was the model protein of choice to validate the outcomes of the model. To this end, the catalytic activity of trypsin
(adsorbed on carbon electrodes at different external fields) serves as an experimental validation for the model.

Materials and Methods

Mathematical model

The Poisson-Boltzmann equation with a boundary element method (BEM)

The implicit solvent model considers an infinite solvent domain that contains salt and has a high permittivity, with a low-dielectric cavity: the solute domain. These two regions are interfaced by the molecular surface, in this case, the solvent-excluded surface. Applying continuum electrostatic theory gives rise to a system of partial differential equations for the potential, where the Poisson-Boltzmann (solvent region) and Poisson (solute region) equations are coupled through continuity conditions on the molecular surface. This model can be extended to consider surfaces with imposed charge or potential, or external electric fields. The mathematical formulation to model the setup from Fig. 1 with a charged surface and an external field, is

\[
\begin{aligned}
\nabla^2 \phi_1(r) &= - \sum_{k=1}^{N_c} \frac{q_k}{\epsilon_1} \delta(r, r_k) \quad r \in \Omega_1 \\
\nabla^2 \phi_2(r) &= \kappa^2 \phi_2(r) \quad r \in \Omega_2 \\
\phi_1 &= \phi_2 + \phi_e \quad r \in \Gamma_1 \\
\frac{\epsilon_1 \partial \phi_1}{\partial n} &= \epsilon_2 \left( \frac{\partial \phi_2}{\partial n} + \frac{\partial \phi_e}{\partial n} \right) \\
-\epsilon_2 \left( \frac{\partial \phi_2}{\partial n} + \frac{\partial \phi_e}{\partial n} \right) &= \sigma_0 \quad r \in \Gamma_2,
\end{aligned}
\]

where \( \mathbf{n} \) is a unit vector normal to the surfaces \( \Gamma_1 \) and \( \Gamma_2 \), pointing into the solvent, \( \sigma_0 \) is the surface charge on \( \Gamma_2 \), and \( \epsilon_1 \) and \( \epsilon_2 \) are the dielectric constants in the corresponding regions. The total electrostatic potential in \( \Omega_1 \) is \( \phi_1 \), whereas in \( \Omega_2 \) this is decomposed into
Figure 1: Sketch of a molecule interacting with a charged surface under an external electric field: $\Omega_1$ is the protein, $\Omega_2$ is the solvent, $\Gamma_1$ is the SES, and $\Gamma_2$ the surface with a prescribed charged density.

$\phi_e = -E_0 z$, due to the external field ($E_0$), and the remaining $\phi_2$.

Using Green’s second identity on Eq. (1), we can write $\phi_1$ and $\phi_2$ in terms of boundary integral equations as

$$
\begin{align*}
\phi_1(r) &= -K_{r,L}^{\Gamma_1} (\phi_1, \Gamma_1) + V_{r,L}^{\Gamma_1} \left( \frac{\partial}{\partial n} \phi_1, \Gamma_1 \right) + \frac{1}{\epsilon_1} \sum_{k=1}^{N_e} \frac{q_k}{4\pi |r-r_k|} \quad r \in \Omega_1 \\
\phi_2(r) &= K_{r,Y}^{\Gamma_2} (\phi_2, \Gamma_2) - V_{r,Y}^{\Gamma_1} \left( \frac{\partial}{\partial n} \phi_2, \Gamma_1 \right) + K_{r,Y}^{\Gamma_2} (\phi_2, \Gamma_2) - V_{r,Y}^{\Gamma_2} \left( \frac{\partial}{\partial n} \phi_2, \Gamma_2 \right) \quad r \in \Omega_2
\end{align*}
$$

where,

$$
\begin{align*}
V_{r,a}^{\Gamma_a} (\varphi) &= \int_{\Gamma_a} G(r, r') \varphi(r') dr' \\
K_{r,a}^{\Gamma_a} (\varphi) &= \int_{\Gamma_a} \frac{\partial G}{\partial n}(r, r') \varphi(r') dr'
\end{align*}
$$

are the single and double layer potentials of a distribution $\varphi(r_a)$ on $\Gamma_a$, evaluated at a point $r$ located anywhere in the domain, except $\Gamma_a$. $G(r, r')$ is the Green’s functions of the Poisson or linearized Poisson-Boltzmann equations. These expressions are also known as the Laplace
and Yukawa potentials, respectively:

\[
G_L(r, r') = \frac{1}{4\pi|r - r'|} \\
G_Y(r, r') = \frac{e^{-\kappa|r - r'|}}{4\pi|r - r'|}
\]  

(4)

with \(\kappa\) the inverse of the Debye length. We can build a system of equations to compute the potential and its normal derivative on the surface by taking the limit as \(r \to \Gamma_1\) and \(r \to \Gamma_2\), which leaves

\[
\frac{\phi_{1, \Gamma_1}}{2} + K_{\Gamma_1, L}^T (\phi_{1, \Gamma_1}) - V_{\Gamma_1, L}^T \left( \frac{\partial}{\partial n} \phi_{1, \Gamma_1} \right) = \frac{1}{\epsilon_1} \sum_{k}^N \frac{q_k}{4\pi|r_{\Gamma_1} - r_k|} \\
\frac{\phi_{2, \Gamma_1}}{2} - K_{\Gamma_1, Y}^T (\phi_{2, \Gamma_1}) + V_{\Gamma_1, Y}^T \left( \frac{\partial}{\partial n} \phi_{2, \Gamma_1} \right) - K_{\Gamma_2, Y}^T (\phi_{2, \Gamma_2}) + V_{\Gamma_2, Y}^T \left( \frac{\partial}{\partial n} \phi_{2, \Gamma_2} \right) = 0 \\
\frac{\phi_{2, \Gamma_2}}{2} - K_{\Gamma_1, Y}^T (\phi_{2, \Gamma_1}) + V_{\Gamma_1, Y}^T \left( \frac{\partial}{\partial n} \phi_{2, \Gamma_1} \right) - K_{\Gamma_2, Y}^T (\phi_{2, \Gamma_2}) + V_{\Gamma_2, Y}^T \left( \frac{\partial}{\partial n} \phi_{2, \Gamma_2} \right) = 0
\]  

(5)

where the subscripts on \(V\) and \(K\) indicate the surface where \(r\) is evaluated. Then, we apply the interface conditions from Eq. (1), to write Eq. (5) in matrix form as

\[
\begin{bmatrix}
\frac{1}{2} + K_{\Gamma_1, L}^T & -V_{\Gamma_1, L}^T & 0 \\
\frac{1}{2} - K_{\Gamma_1, Y}^T & \frac{\epsilon_1}{\epsilon_2} V_{\Gamma_1, Y}^T & -K_{\Gamma_2, Y}^T \\
-K_{\Gamma_1, Y}^T & \frac{\epsilon_1}{\epsilon_2} V_{\Gamma_1, Y}^T & \left( \frac{1}{2} - K_{\Gamma_2, Y}^T \right)
\end{bmatrix}
\begin{bmatrix}
\phi_1(r_{\Gamma_1}) \\
\frac{\partial}{\partial n} \phi_1(r_{\Gamma_1}) \\
\phi_2(r_{\Gamma_2}) \\
\frac{\partial}{\partial n} \phi_2(r_{\Gamma_2})
\end{bmatrix}
= \frac{1}{\epsilon_1} \sum_{k}^N \frac{q_k}{4\pi|r_{\Gamma_1} - r_k|} \\
\left( \frac{1}{2} - K_{\Gamma_1, Y}^T \right) \phi_{e, \Gamma_1} + V_{\Gamma_1, Y}^T \frac{\partial}{\partial n} \phi_{e, \Gamma_1} + V_{\Gamma_2, Y}^T \left( \frac{\sigma_0}{\epsilon_2} + \frac{\partial}{\partial n} \phi_{e, \Gamma_2} \right) \\
-K_{\Gamma_1, Y}^T \phi_{e, \Gamma_1} + V_{\Gamma_1, Y}^T \frac{\partial}{\partial n} \phi_{e, \Gamma_1} + V_{\Gamma_2, Y}^T \left( \frac{\sigma_0}{\epsilon_2} + \frac{\partial}{\partial n} \phi_{e, \Gamma_2} \right)
\]  

(6)

We solve the linear system in Eq. (6) to obtain the electrostatic potential on the surface with the GMRES solver implemented in the boundary element method software PyGBe. This code assumes a piecewise constant distribution of the potential and its
normal derivative on the triangulated molecular surface, and uses centroid collocation. In this work, we extended the model in PyGBe to account for an external electric field. These extensions were performed on a fork\textsuperscript{1} of the official GitHub\textsuperscript{2} repository of the code.

PyGBe solves the integrals on the surface with Gauss quadrature rules depending on the distance between the collocation point and the boundary element. In particular, it uses $K$ points if they are far away, $K_{\text{fine}}$ if they are close by, and a semi-analytical technique\textsuperscript{37} with $N_k$ points on each edge of the triangle if the integral is singular. In this context, a matrix-vector product becomes an N-body problem, where Gauss quadrature nodes serve as sources of mass, and collocation points as evaluation centers. PyGBe accelerates each vector-product in the GMRES solver with a treecode algorithm\textsuperscript{38–40}. The treecode groups the Gauss points in a tree structure, and approximates far-field interactions between a box and a collocation point with a Taylor series expansion of order $P$. The multipole-acceptance criterion $\theta > \frac{R_b}{R}$ defines if a box is far enough, where $R_b$ and $R$ are the box size and the distance between the collocation point and the cluster of Gauss nodes, respectively.

**Energy calculation**

We are interested in computing the interaction free energy of specific conformations of trypsin near the charged surface, to determine the probability of each occurrence. The interaction free energy is the difference in free energy between the setup with trypsin and the charged surface interacting under the external electric fields ($\Delta G_{\text{sys total}}$), and each entity isolated without an external field ($\Delta G_{\text{tryp total}}$ and $\Delta G_{\text{surf total}}$). This is

$$\Delta G_{\text{int}} = G_{\text{sys total}} - G_{\text{tryp total}} - G_{\text{surf total}}.$$

The total free energy $G_{\text{total}}$ has three sources: solvation ($\Delta G_{\text{solv}}$), surface ($G_{\text{surf}}$), and Coulomb ($G_{\text{coul}}$). In the first place, the solvation free energy is the free energy difference of a

\textsuperscript{1}https://github.com/UrzuaSergio/ElectricFieldPyGBe
\textsuperscript{2}https://github.com/pygbe
system in vacuum and dissolved states. For the solute molecule, this can be computed as:

$$\Delta G_{solv} = \frac{1}{2} \int_{\Omega} \rho \phi_{\text{react}} = \frac{1}{2} \sum_{k=0}^{N_q} q_k \phi_{\text{react}}(r_k)$$  \hspace{1cm} (8)$$

where $\phi_{\text{react}}$ is the change in electrostatic potential as the solute is placed inside the solvent.

If we subtract the Coulomb potential out of Eq. (2), $\phi_{\text{react}}$ appears as

$$\phi_{\text{react}}(r) = -K_{r,L}^{\Gamma_1} (\phi_{1,\Gamma_1}) + V_{r,L}^{\Gamma_1,\Gamma_1} \left( \frac{\partial}{\partial n} \phi_{1,\Gamma_1} \right).$$  \hspace{1cm} (9)$$

Secondly, the surface free energy ($G_{\text{surf}}$) is calculated with:

$$G_{\text{surf}} = \frac{1}{2} \int_{\Gamma} (\phi_2 + \phi_e) \sigma_0 d\Gamma = \frac{1}{2} \sum_{j=1}^{N_p} (\phi_2(r_j) + \phi_e(r_j)) \sigma_0 A_j$$  \hspace{1cm} (10)$$

where the sum is over all $N_p$ boundary elements on the charged surface.

Finally, as the Coulomb energy does not change as the molecule is dissolved, it cancels out of the calculation.

**Sampling orientations**

By having the interaction free energy for every orientation from Eq. (7), we can determine its probability of occurrence using Boltzmann statistics. In particular, the probability of finding the system in a state $\lambda$ is

$$P(\lambda) = \frac{\int_{\Lambda} \exp \left( -\frac{\Delta G_{\text{int}}}{k_B T} \right) d\gamma}{\int_{\Lambda} \exp \left( -\frac{\Delta G_{\text{int}}}{k_B T} \right) d\gamma}$$  \hspace{1cm} (11)$$

where $\Lambda$ considers all possible states, $k_B$ is the Boltzmann constant, and $T$ is the temperature.

We sample all possible orientations by aligning the dipole moment vector of trypsin to the normal vector to the surface, and tilting trypsin an angle $\alpha_{\text{tilt}}$ that varies from 0° to 180°.
Then, we rotate about the dipole moment vector an angle $\alpha_{\text{rot}}$ from 0° to 360°, to obtain every possible orientation. This way, we can compute the integrals in Eq. (11) as

$$\int_{\lambda} \exp \left( -\frac{\Delta G_{\text{int}}}{k_B T} \right) d\gamma = \int \int \exp \left( -\frac{\Delta G_{\text{int}}}{k_B T} \right) d\alpha_{\text{rot}} d\alpha_{\text{tilt}}$$

(12)

where the angles $\alpha_{\text{tilt}}$ and $\alpha_{\text{rot}}$ define the state $\lambda$. This process considers a small constant distance between trypsin and the surface of 2Å, as we are studying the orientation at adsorption.

**Experimental details**

**Reagents**

We used sodium bicarbonate, trypsin from porcine pancreas (T4799), and a trypsin activity kit (MAK290) purchased from Sigma-Aldrich (St. Louis, MO, USA). The aqueous solutions were prepared using 18 MΩ·cm water (NANOpure Diamond, Barnstead; Dubuque, IA) and analytical reagent grade chemicals. The phosphate buffer solution was prepared by dissolving anhydrous Na$_2$HPO$_4$ (Fisher Scientific; Fair Lawn, NJ, USA) in ultrapure water. We measured the pH of the solutions using a glass electrode connected to a digital pH meter (Orion 420A+, Thermo; Waltham, MA, USA) and adjusted with 0.1M solutions of HCl.

**Fabrication of carbon electrodes**

Following the procedure described in previous publications from our group, we used electrodes obtained by pyrolysis of paper strips (4.5 cm × 1.5 cm; Whatman 3MM chromatography paper; GE Health Care; Pittsburgh, PA) using a tube furnace (Type F21100, Barnstead–Thermolyne; Dubuque, IA, USA). The quartz tube was first flushed with forming gas (5% H$_2$ / 95% Ar, 1 L/min) for 5 min (to remove the O$_2$ and avoid oxidation reactions) and then allowed to reach a temperature of 1000°C, at a rate of 20°C min/1. After 1 h, we turned off the tube furnace and allowed to cool-down to room temperature while maintaining
the flow of forming gas. Finally, the pyrolyzed samples were removed from the furnace and stored in a Petri dish until use. The pyrolyzed paper layers were fixed to a Plexiglas substrate with double-sided tape and cut using a commercial 30W CO\textsubscript{2} laser engraver (Mini24, Epilog Laser Systems; Golden, CO, USA). The resulting electrodes featured a circular pad (where the reaction takes place, 0.50 cm\textsuperscript{2}) and a stem, similar to those previously reported.\textsuperscript{44} Then, we applied silver paint (SPI Supplies; West Chester, PA, USA) to improve the electrical connection with the alligator clip connected to the potentiostat. To prevent water from wicking up the stem of the electrode (and increasing the electrode area), we applied parafilm to base of the stem, between the circular pad and the contact area.

**Electrochemical Techniques**

We performed cyclic voltammetry (CV) on all electrodes to verify their functionality and electrical connections. In all cases, a standard three-electrode cell comprised of the carbon electrode, a silver/silver chloride (Ag|AgCl|KCl\textsubscript{sat}), and a platinum wire were used as working, reference, and counter electrode, respectively. The CV experiments used a CHI660A Electrochemical Analyzer (CH Instruments, Inc.; Austin, TX). For the adsorption of the trypsin under the application of a selected potential, we used the amperometry mode. For these cases, the electrode was immersed for 15 min in a solution containing 1 mg/mL of trypsin dissolved in phosphate buffer (0.1M, pH=8), with periodic mixing. These electrodes were then removed from the cell, thoroughly rinsed with DI water to remove any unbound protein from the surface, and immediately assayed for activity.

**Trypsin Activity Assays**

The activity of the adsorbed layer of trypsin was assessed colorimetrically following the cleavage of a substrate to generate p-nitroaniline (p-NA). Considering that the commercial kit is designed to accommodate small liquid samples placed on a microplate reader, the procedure was slightly modified. Specifically, 2 \(\mu\)L of the substrate were mixed with 98 \(\mu\)L
of the buffer provided and with 400 µL of DI water in a standard eppendorf tube. The latter was added to ensure that, upon insertion, the electrode remained immersed and in direct contact with the solution containing the substrate. After two hours at room temperature, the electrode was removed from the tube, the solution centrifuged (10,000 rpm for 3 min) and then measured using an spectrophotometer (GENESYS™ 10 Series, Thermo; Madison, WI) at 405 nm.

Results

Catalytic activity of trypsin adsorbed under applied potential.

In order to determine the effect of the potential applied to the electrode on the adsorption process, we measured the activity of trypsin immediately after the adsorption step. It is important to mention that these results involved an adsorption step of only 15 min, selected (along pertinent experimental conditions) to minimize the possibility to induce polarization of the protein and the subsequent formation of multilayers. Thus, we evaluated the catalytic activity of the resulting substrate by following the cleavage of a substrate to generate p-nitroaniline (which absorbs light at 405 nm). The results are summarized in Figure 2 where the resulting enzymatic activity is presented as a function of the potential applied during the adsorption stage. As it can be observed, all substrates showed significant activity of the adsorbed enzyme, where the highest (19 ± 2 U) and lowest (15.2 ± 0.5 U) activity correspond to adsorption at potentials of -1.0V or +1.0V, respectively. As expected, a control experiment, performed by adsorbing the enzyme at open circuit potential, rendered an intermediate activity (17.5 U).

The differences observed in Fig. 2 which we can associate to the effect of the electric field on the orientation of the adsorbed enzyme, served as motivation to develop the computational model.
Figure 2: Enzymatic activity of the carbon electrodes modified by adsorbing trypsin at different potentials. Electrodes were immersed for 15 min in a solution containing 1 mg/mL trypsin dissolved in 0.1 M phosphate buffer, pH = 8

Verification of the numerical model with an analytical solution.

The Appendix shows a derivation of a closed expression for the interaction of a charged sphere with a spherical cavity that contains a centered charge. Here, we use the setup in Fig. 11 to validate our numerical implementation on PyGBe, with $\sigma_0 = -80 \, \text{C/m}^2$, $E_0 = -0.8 \, e^-/\epsilon_0 \text{Å}^2$, $a_1 = a_2 = 4 \, \text{Å}$, and $R = 14 \, \text{Å}$. Table 1 shows the numerical parameters of these runs. In this case, the analytical solution computed with Eq. (20) was $\Delta G_{\text{solv}} = -652.43 \, \text{kcal/mol}$ and $G_{\text{surf}} = -2.88 \cdot 10^8 \, \text{kcal/mol}$. Figure 3 plots the error convergence with mesh density, where we see the expected $1/N$ behavior.

Table 1: PyGBe parameters for verification runs.

| # Gauss points | Treecode | GMRES |
|----------------|----------|-------|
| $K$ $K_{\text{fine}}$ $N_k$ $N_{\text{crit}}$ $P$ $\theta$ tol. |
| 4 | 37 | 9 | 300 | 15 | 0.5 | $10^{-9}$ |
Simulations of trypsin adsorbing on a charged surface under an external field

Numerical parameter selection

The interaction energy in Eq. (7) is a difference between large numbers. Then, we need to be extremely careful about the accuracy of our simulations. We performed simulations of trypsin placed 2 Å away from a 100×100×10 Å surface, charged with ±0.04 C/m², under a field of $E_0 = \pm 0.01$ V/Å and $E_0 = 0$ V/Å, and studied the mesh convergence. In this case, the numerical parameters of PyGBe were specially tight (see Table 2) to ensure that the numerical approximations of the integration and treecode did not contaminate the mesh convergence. We parameterized the charge and radii of trypsin with pdb2pqr and Nanoshaper considering pH=8, and triangulated its surface with Nanoshaper.

We performed simulations with 2, 4, 8, and 16 elements per Å² for $\alpha_{tilt} = 154^\circ$ and $\alpha_{rot} = 90^\circ$, and used Richardson extrapolation to obtain an approximate exact solution. These values are detailed in Table 3. Figure 4 shows the error convergence with respect to the extrapolated value, with the expected $1/N$ behavior.
Table 2: PyGBe parameters for verification runs.

| # Gauss points | Treecode | GMRES |
|----------------|----------|-------|
| K | K_{fine} | N_{k} | N_{crit} | P | θ | tol. |
| 4 | 19 | 9 | 500 | 6 | 0.5 | 10^{-5} |

Table 3: Approximate exact solution for solvation, surface, and interaction energies using Richardson extrapolation for α_{tilt} = 154° and α_{rot} = 90°.

| Surf. charge C/m² | Field V/Å | ΔG_{solv}^{extra} kcal/mol | G_{surf}^{extra} kcal/mol | ΔG_{int}^{extra} kcal/mol |
|-------------------|-----------|---------------------------|--------------------------|--------------------------|
| -0.04             | 0         | -607.1535                 | 18.3495                  | -0.8243                  |
| -0.04             | 0.01      | -609.2285                 | -15.1939                 | -36.4427                 |
| 0.04              | 0         | -606.0682                 | 19.4380                  | 1.3495                   |
| 0.04              | -0.01     | -604.0047                 | -14.1031                 | -30.1280                 |

Figure 4: Error convergence as a function of grid size for cases with $E_0=0$ V/Å and $σ_0=0.04$ C/m² (top left), $E_0=0$ V/Å and $σ_0=-0.04$ C/m² (top right), $E_0=-0.01$ V/Å and $σ_0=0.04$ C/m² (bottom left), and $E_0=0.01$ V/Å and $σ_0=-0.04$ C/m² (bottom right).
Orientation probability calculation

To understand the role of electrostatics in the catalytic activity difference described in Fig. 2, we performed Poisson-Boltzmann simulations of trypsin adsorbed on a charged surface with an external field, and studied its orientation. In these calculations, the external field was set to $\pm 0.01 \text{ V/Å}$ (aiming to approximate an applied potential of $\pm 1 \text{ V}$, selected for the experiments described in Fig. 2). Likewise, permittivity values of $\epsilon_2=80$ (solvent) and $\epsilon = 4$ (solute), and an inverse of Debye length $\kappa=0.175 \text{ Å}^{-1}$, valid for 0.1 M of phosphate buffer, were considered.

In this case, we used a mesh density of 8 elements per Å$^2$ on the molecular surface, and 2 on the electrode. The code parameters for PyGBe are presented by Table 4. With this setting, the interaction energies were at most 2.52% away from the extrapolated values in Table 3.

Table 5 shows the preferred orientation for different combinations of surface charge ($\sigma_0=\pm 0.04 \text{ C/m}^2$) and electric field ($E = \pm 0.01$ and 0 V/Å), with the corresponding interaction energy $\Delta G_{\text{int}}$. We can see that there is an attractive interaction only when the field and charge have opposite signs, whereas the interaction is weak when there is no electric field. The images in Fig. 6 show the probability distribution for the attractive cases, and Fig. 10 is a detailed view of trypsin in its preferred orientation for these cases, with the active sites highlighted.

Table 6 is a study of the effect of salt concentration for the attractive energy cases in Table 5. The reaction of the system to the salt concentration depends on the combination of charge and field: if the charge is negative and the field is positive, the interaction is lowered by salt, however, it is the opposite when the charge is positive and the field is negative.

Table 4: PyGBe parameters for trypsin runs.

| # Gauss points | Treecode | GMRES |
|----------------|----------|-------|
| $K$ $K_{\text{fine}}$ $N_k$ $N_{\text{crit}}$ P $\theta$ tol. | $4$ $19$ $9$ $500$ $6$ $0.5$ $10^{-5}$ |
Table 5: Preferred orientation of trypsin adsorbed on a charged surface under an electric field $\kappa = 0.175$.

| Surf. charge C/m² | Field V/Å | $\Delta G_{\text{int}}$ kcal/mol | $\alpha_{\text{tilt}}$ | $\alpha_{\text{rot}}$ |
|-------------------|-----------|---------------------------------|------------------------|------------------------|
| -0.04             | 0         | -0.9713                         | 148                    | 140                    |
| -0.04             | 0.01      | -56.833                         | 24                     | 240                    |
| -0.04             | -0.01     | 34.742                          | 152                    | 120                    |
| 0.04              | 0         | -0.373                          | 40                     | 0                      |
| 0.04              | 0.01      | 10.071                          | 8                      | 20                     |
| 0.04              | -0.01     | -30.101                         | 148                    | 90                     |

Figure 5: Orientation distribution for $\sigma_0=-0.04$ C/m² (left) and $\sigma_0=0.04$ C/m² (right), without external field.
Figure 6: Orientation distribution for $\sigma_0=-0.04 \, \text{C/m}^2$ (left) and $\sigma_0=0.04 \, \text{C/m}^2$ (right), and an applied external field of $E_0=0.01 \, \text{V/Å}$ (left) and $E_0=-0.01 \, \text{V/Å}$ (right).

Figure 7: Interaction energy $\Delta G_{\text{int}}$ at different orientations for $\sigma_0=-0.04 \, \text{C/m}^2$ (left) and $\sigma_0=0.04 \, \text{C/m}^2$ (right), and no external applied field.

Figure 8: Interaction energy $\Delta G_{\text{int}}$ at different orientations for $\sigma_0=-0.04 \, \text{C/m}^2$ (left) and $\sigma_0=0.04 \, \text{C/m}^2$ (right), and an applied external field of $E_0=0.01 \, \text{V/Å}$ (left) and $E_0=-0.01 \, \text{V/Å}$ (right).
Figure 9: Interaction energies and their respective tilt angles for trypsin adsorbed on a charged surface under an electric field as a function of the ionic strength. Exact values are detailed in Table 6 of the Appendix.

Discussion

Validity of an electrostatic model to explain the experimental observations

Emulating an experimental setup with computer simulations is a challenging task. The real behavior of proteins during experiments is usually far more complex than most models can describe. Specialized models developed to account for complete phenomena are generally too expensive for researchers to obtain useful simulations. While the present case is not an exception, we have identified different mechanisms that explain experimental observations, where simulations can be used to describe specific aspects of the behavior.

To begin with, in the experiments, the external field polarized the electrodes, inducing a surface charge on them. In the numerical model this effect was accounted by the surface charge ($\sigma_0$ in Eq. (1)). Then, the value of $\sigma_0$ to appropriately represent the system should have opposite sign with respect to the external field. In particular, the polarization due to a positive electric field induces a negative apparent surface charge on the electrode, and vice-versa for a negative electric field. These opposite sign combinations of electric field and surface charge yielded the most favorable electrostatic interactions in Table 5, and are studied deeper in Figs. 6 through 10.
Figure 10: Preferred orientation for adsorbed cases. The active site in each case is marked in red. Note that the active site in the top figure is more exposed to the solvent than in the bottom figure.
Also, the Poisson-Boltzmann model only accounts for electrostatic effects, which is just one component of the trypsin-electrode interaction. In fact, for hydrophobic surfaces like the carbon electrodes, this attractive interaction is dominated by the hydrophobic effect, through dewetting of the electrode-protein interface. Moreover, the behavior reported on other carbon substrates, the low density of functional groups of the substrates and the concentration of buffer used (0.1M phosphate, pH=8), also support the general notion that the adsorption process is mostly driven by hydrophobic interactions. The latter is confirmed by the catalytic activity results without external potential from the middle bar of Fig. 2. In that case, the electrode was not polarized by an external field \((\sigma_0 = 0)\), and the activity results indicate good coverage of trypsin on the surface. Then, the difference in catalytic activity for positive and negative external fields (two side bars in Fig. 2) can be attributed to the electrostatic contributions, as it is based on the response of trypsin to the external electric field, and how this affects the orientation at adsorption. This is where Poisson-Boltzmann simulations become particularly useful.

The Poisson-Boltzmann model by itself is not free of approximations. First, it considers a rigid molecular geometry. This may seem like a major limitation, specially considering carbon surfaces may induce conformational changes on trypsin. However, these changes would happen in all conditions described in Fig. 2, making them comparable. Moreover, it is important to consider that such changes are likely to span beyond the timescale selected for the experiments, and that an external field may provide an stabilizing effect on secondary structures. Also, the simulations were performed at a single protonation state, valid for an isolated trypsin at the corresponding pH, however, the surface may induce pKa shifts. Even though this effect, known as charge regulation, has an important impact on electrostatics, its influence on the adsorption orientation can be neglected.
The effect of an external field on the catalytic activity

There are a number of possible causes to explain the differences in catalytic activity described by Fig. 2, which not only refer to the amount of enzymes adsorbed on the surface, but also their conformation. Considering the hydrophobic contributions to the adsorption process, and the enhancement provided by favorable electrostatic interaction free energies in Table and Fig. 8, we do not expect to see major differences in trypsin coverage on the surface of the electrodes prepared under different applied potentials. This is further supported by comparing $\Delta G_{\text{int}}$ with positive and negative electric fields in Fig. 8. In that case, the surface has higher electrostatic affinity with trypsin when the electric field is positive (see colorbars in Fig. 8). This suggests that, if trypsin coverage plays a role in the overall catalytic activity, the latter would be higher with a positive electric field. However, Fig. 2 shows the opposite result: a negative electric field has higher activity.

Other researchers have proposed that local changes of the pH, due to electrolysis of the solvent, could also induce aggregation of proteins and affect the adsorbed amount. However, the selected carbon electrodes showed rather poor electrochemical activity towards the reduction $\text{O}_2$. In addition, under these conditions the application of a negative potential (-1.0V vs Ag|AgCl|KCl$_{\text{SAT}}$) would lead to a local increase in the pH, bringing the protein closer to its isoelectric point (10.5) and thus further decreasing the general influence of (attractive) electrostatic interactions. Moreover, the application of an external electric field can also induce the accumulation of proteins due to a polarization effect, but this is a rather slow process that would not render significant differences in our electrodes.

This motivates us to consider the effect of the external electric field on the orientation as the driving mechanism behind the catalytic activity difference in Fig. 2.

The position of the active sites for the preferred orientations with positive or negative external fields are different (see Fig. 10). With positive surface charge and negative electric field, the active sites are facing out of the electrode, making them accessible for a trypsin-binding antigen. On the other hand, the active sites when the surface charge is negative and
the electric field is positive face towards the electrode, making them less accessible. Then, an enhanced catalytic activity with a negative electric field is expected.

The external field also has an effect on the shape of the orientation distribution. In particular, this distribution is narrower with the electric field is applied (Fig. 6) compared to the case without an external field in Fig. 5. This is the result of an energy landscape with a deeper well when the external field is present, and yields less random orientations at adsorption. The comparison between Fig. 7 and Fig. 8 further emphasises this point. The interaction energies without an external field are close to zero (see colorbar in Fig. 7) indicating a weak electrostatic interaction, despite the fact that the surface is charged in this computational experiment. On the other hand, with an external field, energies are more negative (see colorbar in Fig. 8), and hence, more favorable, regardless of the sign of the external field. Note that the situation modeled in Figs. 5 and 7 are not equivalent to the experiment of the middle bar in Fig. 2 as in that case, the electrode is not polarized by an electric field, making $\sigma_0 = 0$.

The consistently favorable electrostatic interaction in Fig. 8 adds to the already attractive hydrophobic component. Then, the electrode never repels trypsin, however, the molecule tends to rotate and orient to its most likely configuration from Fig. 6. Similar numerical techniques based on the boundary element method could be used to study the hydrodynamics in the trypsin rotation as it approaches the electrode, however, we leave such study for future work.

**Effect of ionic strength**

Figure 9 presents the interaction free energy for the preferred orientation, as a function of ionic strength. The angles with highest probability change only slightly, but the impact on the interaction energy is substantial. With a positive electric field and negative surface charge, the electrostatic contribution to the interaction decreases with salt concentration, which agrees with similar studies. This is a somewhat expected behavior, as the shielding
effect of free ions limits the influence of electrostatics. On the contrary, with a negative
electric field and positive surface charge, electrostatics was enhanced by the ionic strength.
With no salt in the solvent, the positive free energy indicates a repulsive interaction, probably
due to trypsin’s positive net charge. This trend turns around and becomes more attractive
as salt concentration increases, which reveals that the influence of the aggregate, monopole-
type charge is screened, and local interactions between specific regions of the protein and
the surface dominate.

Conclusions

Being able to control the orientation of proteins adsorbed on surfaces is key in a variety
of applications, such as biosensors and biocatalysis, and it can be accomplished using of
electric fields. In this work, we present an efficient computational model, based on the
Poisson-Boltzmann equation, to simulate proteins near surfaces under an external electric
field. With this tool, we can compute protein-surface interaction free energies, and determine
the most likely conformation at adsorption. We chose trypsin interacting with a carbon
electrode to be our model problem, as it exhibited important changes in catalytic activity
depending on the sign of the applied external field. Even though the adsorption process
is dominated by hydrophobic interactions, we were able to identify that electrostatics was
the driving mechanism behind the catalytic activity difference, and more specifically, its
effect on the orientation. Our numerical experiments support this conclusion, as the active
sites for the preferred orientation with negative external field are exposed to the solvent, as
opposed to when the field was positive. This motivates the use of computational studies with
implicit-solvent models and electrostatics in the application of electric fields for fine tuning
of molecular immobilization on surfaces. As future work, we plan to use this approach to
the aid the design of more sensitive surfaces in biotechnological settings.
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Conflicts of Interest

Authors declare no conflict of interest related to the material.

Appendix

A closed expression for spheres with Legendre polynomials

This section describes a derivation towards a closed expression for the electrostatic potential of a spherical molecule with a centered charge interacting with a charged sphere (see Fig. 11), based on Legendre polynomials. The result of this derivation is useful to verify the numerical implementation, leading to the results in Fig 3. Considering azimuthal symmetry,

![Figure 11: Sketch of a spherical molecule interacting with a charged sphere under an external electric field.](image)

the solution of the coupled system in Eq. (11) can be written as...
\[ \phi_1 = \sum_{j=0}^{\infty} C_n r^n P_n(\cos \theta_1) + \frac{q_k}{4\pi \epsilon_1} \sum_{n=0}^{\infty} \frac{r_k^n}{r^{n+1}} P_j(\cos \theta_1) \]

\[ \phi_2 = \sum_{n=0}^{\infty} a_n K_n(\kappa r_1) P_n(\cos \theta_1) + \sum_{n=0}^{\infty} b_n K_n(\kappa r_2) P_n(\cos \theta_2) \]

\[ \phi_e = -E_0 r_1 P_1(\cos \theta_1) \]

\[ \phi_1 = \phi_2 + \phi_e \quad ; \quad \Gamma(r = a_1) \]

\[ \epsilon_1 \frac{\partial \phi_1}{\partial n} = \epsilon_2 \left( \frac{\partial \phi_2}{\partial n} + \frac{\partial \phi_e}{\partial n} \right) \]

\[ -\epsilon_2 \left( \frac{\partial \phi_2}{\partial n} + \frac{\partial \phi_e}{\partial n} \right) = \sigma_0 \quad ; \quad \Gamma_2(r = a_2) \]

(13)

where \( P_n \) and \( K_n \) are the \( n^{th} \) Legendre polynomial and modified spherical Bessel function of second kind, respectively. Using the addition theorem, \( \phi_2 \) and \( \phi_e \) are written as

\[ \phi_2 = \sum_{n=0}^{\infty} a_n K_n(\kappa r_1) P_n(\cos \theta_1) + \sum_{n=0}^{\infty} b_n K_n(\kappa r_2) P_n(\cos \theta_2) \]

\[ \phi_2 = \sum_{n=0}^{\infty} b_n K_n(\kappa r_2) P_n(\cos \theta_2) + \sum_{n=0}^{\infty} a_n K_n(\kappa r_1) P_n(\cos \theta_1) \]

\[ \phi_e = -E_0 r_1 P_1(\cos \theta_1) = E_0 r_2 P_1(\cos \theta_2) - E_0 R \]

(14)

where \( i_m \) corresponds to the modified spherical Bessel function of the first kind. Moreover, \( B_{nm} \) is defined as

\[ B_{nm} = \sum_{\nu=0}^{\infty} A_{nm}^\nu K_{n+m-2\nu}(\kappa R) \]

(15)

where \( R \) is the center-to-center distance, and \( A_{nm}^\nu \) is related to the Gamma function, which is given by the following expression.

\[ A_{nm}^\nu = \frac{\Gamma_{n-\nu+0.5} \Gamma_{m-\nu+0.5} \Gamma_{n-m} + 0.5(n + m - n\nu)! (n + m - 2\nu + 0.5)}{\pi \Gamma_{n+m-n-\nu+1.5} (n - \nu)! (m - \nu)! \nu!} \]

(16)

Evaluating the expressions in Eq. (13) at \( \Gamma_1 \), and applying the interface conditions on
the potential and electric displacement yields

\[ C_j a_j^i + \frac{q_k}{4\pi\epsilon_1} \left( \frac{r_k^j}{a_j^{i+1}} \right) = a_j K_j(\kappa a_1) + \sum_{n=0}^{\infty} b_n (2j + 1) B_{nj i j}(\kappa a_1) - E_0 r_1 \delta_{ij} \]

\[ \frac{1}{\epsilon_1} \left( C_j a_j^{i-1} - \frac{q_k(j + 1)}{4\pi\epsilon_1} \left( \frac{r_k^j}{a_j^{i+1}} \right) \right) = a_j K'_j(\kappa a_1) + \sum_{n=0}^{\infty} b_n (2j + 1) B_{nj i j}(\kappa a_1) - E_0 \delta_{ij}. \]

(17)

Rearranging terms, this leads to

\[ C_j = \frac{1}{a_j^i} \left( a_j K_j(\kappa a_1) + \sum_{n=0}^{\infty} b_n (2j + 1) B_{nj i j}(\kappa a_1) - E_0 \delta_{ij} - \frac{q_k}{4\pi\epsilon_1} \frac{r_k^j}{a_j^{i+1}} \right) \]

\[ \sum_{n=0}^{\infty} \left( a_n \left[ \kappa K'_n(\kappa a_1) - \frac{1}{\epsilon_1} \frac{n}{\epsilon_2} K_n(\kappa a_1) \right] \delta_{nj} + b_n (2j + 1) B_{nj} \left[ \kappa i'_n(\kappa a_1) - \frac{\epsilon_1}{\epsilon_2} \frac{j}{a_1} i_j(\kappa a_1) \right] \right) \]

\[ = - \frac{q_k(2j + 1)}{4\epsilon_2} \left( \frac{r_k^j}{a_j^{j+2}} \right) - E_0 \left( \frac{\epsilon_1}{\epsilon_2} j - 1 \right) \delta_{ij} \]

(18)

Now, applying the conditions on \( \Gamma_2 \) gives

\[ \sum_{n=0}^{\infty} b_n \kappa K'_n(\kappa a_2) \delta_{nj} + a_n (2j + 1) B_{nj} \kappa i'_j(\kappa a_2) = - \frac{\sigma_0}{\epsilon_2} \delta_{0j} - E_0 \delta_{1j} \]

(19)

We can now use Eqs. (13), (17), and (18) to generate a linear system for every index \( j \), as

\[
\begin{bmatrix}
I_{jn} & L_{jn} \\
M_{jn} & I_{jn}
\end{bmatrix}
\begin{bmatrix}
A_n \\
B_n
\end{bmatrix} =
\begin{bmatrix}
-E_0 \left( \frac{\epsilon_1}{\epsilon} j - 1 \right) \delta_{1j} - \frac{q_k(2j + 1)}{4\pi\epsilon_2} \frac{r_k^j}{a_j^{j+2}} \\
-E_0 \delta_{1j} - \frac{\sigma_0}{\epsilon_2} \delta_{0j}
\end{bmatrix}
\]

(20)
where $A_n$, $B_n$, $I_{jn}$, $L_{jn}$ and $M_{jn}$ are:

\[
A_n = a_n \left( \kappa K'_n(\kappa a_1) - \frac{\epsilon_1 n}{\epsilon_2 a_1} K_n(\kappa a_1) \right)
\]

\[
B_n = b_n \kappa K'_n(\kappa a_2)
\]

\[
I_{jn} = \delta_{jn}
\]

\[
M_{jn} = \frac{(2j + 1) B_{nj} i'_j(\kappa a_2)}{\left( \kappa K'_n(\kappa a_1) - \frac{\epsilon_1 n}{\epsilon_2 a_1} K_n(\kappa a_1) \right)}
\]

\[
L_{jn} = (2j + 1) B_{nj} \left( i'_j(\kappa a_1) - \frac{\epsilon_1 j}{\epsilon_2 a_1} K'_n(\kappa a_2) \right)
\]

Having $A_n$ and $B_n$, we can use Eq. (18) to compute $C_j$, and then Eq. (13) to obtain the electrostatic potential anywhere in the domain.

**Details of simulation results for trypsin adsorbed on a charged surface.**

Table 6: Preferred orientation of trypsin adsorbed on a charged surface under an electric field as a function of the salt concentration of the medium. These results are summarized by Figure 9.

| Surf. charge C/m² | Field V/Å | Salt Concent. mM | $\kappa$ Å⁻¹ | $\Delta G_{int}$ kcal/mol | $\alpha_{tilt}$ | $\alpha_{rot}$ |
|------------------|----------|-----------------|--------------|----------------|--------------|--------------|
| 0                | 1e-12    | 0.04            | 2.478        | 48             | 20           |
| 10               | 0.032    | 50              | -13.840      | 112            | 240          |
| 50               | 0.0725   | 100             | -23.589      | 128            | 170          |
| 100              | 0.1026   | 150             | -27.276      | 144            | 100          |
| 150              | 0.125    |                 | -28.676      | 144            | 90           |

| Surf. charge C/m² | Field V/Å | Salt Concent. mM | $\kappa$ Å⁻¹ | $\Delta G_{int}$ kcal/mol | $\alpha_{tilt}$ | $\alpha_{rot}$ |
|------------------|----------|-----------------|--------------|----------------|--------------|--------------|
| 0                | 1e-12    | -0.04           | -77.402      | 148            | 40           |
| 10               | 0.032    | 50              | -61.549      | 36             | 240          |
| 50               | 0.0725   | 100             | -59.097      | 28             | 240          |
| 100              | 0.1026   | 150             | -58.275      | 28             | 240          |
| 150              | 0.125    |                 | -57.742      | 24             | 240          |
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