Probing protein orientation near charged surfaces with an implicit-solvent model and the PyGBe code

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(Dated: 1 April 2015)

Protein-surface interactions are ubiquitous in biological processes and bioengineering, yet are not fully understood. In the field of biosensors, a key factor in biosensor performance is the orientation of biomolecules near charged surfaces. The aim of this work is developing and assessing a computational model to study proteins interacting with charged surfaces and obtain orientation data. After extending the implicit-solvent model used in the open-source code PyGBe and deriving an analytical solution for simple geometry, our careful grid-convergence analysis builds confidence on the correctness and value of our approach for probing protein orientation. Further computational experiments support it: they study preferred orientations for protein GB1 D4′ and immunoglobulin G. Sampling the free energy for protein GB1 at a range of tilt and rotation angles with respect to the charged surface, we calculated the probability of the protein orientation and observed a dipolar behavior. This result is consistent with published molecular-dynamics simulations and experimental studies using this protein. The case of immunoglobulin G is more challenging due to the large size of the molecule, but it is also more relevant to biosensor technology. The probability distribution of orientations for this protein at varying surface charge and salt concentration suggests that it is easier to control the antibody orientation with low salt concentration and high surface charge. The results also show that local interactions dominate over dipole moment for this protein. In view of its capacity to deal with much larger biomolecules than direct simulation, this implicit-solvent model can offer a valuable approach in biosensor studies.

I. INTRODUCTION

Proteins interacting with surfaces and adsorption mechanisms are ubiquitous and play a role in many biological processes. Along its importance in natural activity, like blood coagulation, adsorption affects biotechnologies like tissue engineering, biomedical implants and biosensors. Yet, despite their importance, a full understanding of protein-surface interactions remains elusive.

In the field of biosensors, protein adsorption needs to be engineered to obtain a successful device. Biosensors detect specific molecules using a nanoscale sensing element, like a metallic nanoparticle or nanowire covered with a bioactive coating. The prevalent way to modify a sensor surface is via a self-assembled monolayer (SAM) of a small charged group, with ligand molecules layered on top to achieve the desired function. Antibodies are a common choice for the ligand molecules, although the newest devices use single-domain or single-chain fragment molecules. Sensing occurs when a target biomolecule binds to the ligand molecule, changing some physical parameter on the sensor, such as current in nanowires or plasmon resonance frequency in metallic nanoparticles.

One of the factors affecting biosensor performance is the orientation of ligand molecules. These have specific binding sites, which need to be accessible to the target molecule for the biosensor to function well. Probing protein orientation is thus one key goal of adsorption studies. The aim of this study is to develop and assess a computational model to simulate proteins near surfaces and obtain orientation data.

We use an implicit-solvent approach based on the Poisson-Boltzmann equation and fixed protein structures. A sensor element, functionalized with the SAM, can be represented as a charged surface that interacts electrostatically with a biomolecule. Ignoring conformational changes of the biomolecule is justified in this application, since binding sites should remain nearly unmodified during the fabrication process.

Implicit-solvent models using the Poisson-Boltzmann equation are popular for computing solvation energies in protein systems but few studies have included the effect of surfaces. Lenhoff and co-workers studied surface-protein interactions using continuum models discretized with boundary-elements and finite-difference methods in the context of ion-exchange chromatography. They realized that van der Waals effects can be neglected for realistic molecular geometries, and that the model is adequate as long as conformational changes in the protein are slight.

As far as we know, the continuum framework has not been used or assessed in the context of protein-orientation studies. One such study used a coarse-grained model of the molecule, represented as a set of spheres and others assigned effective charges at the
We have added the capability of modeling a protein near a charged surface to our code PyGBes, an open-source code that uses GPU hardware. Previously, we verified and validated PyGBes in its use to obtain solvation and binding energies, by comparing with analytical solutions of the equations and with results obtained using the well-known APBS software. In the present work, we derived an analytical solution for a spherical molecule interacting with a spherical charged surface, and used it to verify the code in its new application and study numerical convergence. Using the newly extended code, we studied two proteins (GB1 D4’ and immunoglobulin-G) near charged surfaces to obtain their preferred orientation, and compared ours and several other published results. We anticipate this modeling tool to be useful for understanding the behavior of proteins as they adsorb on SAMs, potentially aiding the design of better ligand molecules for biosensors.

II. IMPLICIT-SOLVENT MODEL FOR PROTEINS NEAR CHARGED SURFACES

The implicit-solvent model uses continuum electrostatics to describe the mean-field potential in a molecular system. A typical system consists of a protein in a solvent, defining two regions: inside and outside the protein, with an interface marked by the solvent-excluded surface (SES). The SES, beyond which a water molecule cannot penetrate into the protein, can be generated by rolling a (virtual) spherical probe of the size of a water molecule around the protein (see Figure 1). Inside the protein, the domain has low permittivity (ε = 2 to 4) and there are point charges located at the positions of the atoms. The solvent region, representing water with salt, has a permittivity of ε ≈ 80. A system of partial differential equations models this situation, with a Poisson equation governing inside the protein and a linearized Poisson-Boltzmann equation governing in the solvent region. Appropriate interface conditions on the SES express the continuity of the potential and electric displacement, completing the mathematical formulation.

This model has been widely applied to investigate interactions between molecules, such as in protein-ligand binding. We are interested here in an extension of the model to consider interactions between proteins and surfaces with an imposed potential or charge. This new setup is sketched in Figure 2, and is described mathematically by the following equations:

\begin{align*}
\nabla^2 \phi_1(\mathbf{r}) &= -\sum_k \frac{q_k}{\epsilon_1} \delta(\mathbf{r}, \mathbf{r}_k) \quad \text{in solute } (\Omega_1), \\
\nabla^2 \phi_2(\mathbf{r}) &= \kappa^2 \phi_2(\mathbf{r}) \quad \text{in solvent } (\Omega_2), \\
\phi_1 &= \phi_2 \quad \text{on interface } \Gamma_1, \\
\epsilon_1 \frac{\partial \phi_1}{\partial \mathbf{n}} &= \epsilon_2 \frac{\partial \phi_2}{\partial \mathbf{n}} \quad \text{on interface } \Gamma_1, \\
\phi_2 &= \phi_0 \quad \text{or} \quad -\epsilon_2 \frac{\partial \phi_2}{\partial \mathbf{n}} = \sigma_0 \quad \text{on surface } \Gamma_2, \quad (1)
\end{align*}

Here, \( \phi_i \) is the potential corresponding to the region \( \Omega_i \) with permittivity \( \epsilon_i \), and \( \phi_0 \) and \( \sigma_0 \) are the set potential or charge on the nanosurface. The surface \( \Gamma_2 \) could correspond to a device such as a biosensor.

**Boundary integral formulation** — We express the system of partial-differential equations in (1) by the corresponding integral equations along the interface and the nanosurface, \( \Gamma_1 \) and \( \Gamma_2 \). Many authors have used the boundary-integral representation of the implicit-solvent model to compute solvation energies of proteins, but apart from work led by Lenhoff, we know of no studies that account for interacting nanosurfaces in the system.
Consider the setting in Figure 2 with prescribed potential at $\Gamma_2$. The application of Green’s second identity on the first two equations of [4] yields:

$$\phi_1 + K^{\Omega_1}_{L,Y}(\phi_{1,r_1}) - V^{\Omega_1}_L \left( \frac{\partial}{\partial n} \phi_{1,r_1} \right) = \frac{1}{\epsilon_1} \sum_{k=0}^{N_q} \frac{q_k}{4\pi|r_{\Omega_1} - r_k|} \text{ on } \Omega_1,$$

$$\phi_2 - K^{\Omega_2}_{Y}(\phi_{2,r_1}) + V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{2,r_1} \right) - K^{\Omega_2}_{Y}(\phi_{2,r_2}) + V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{2,r_2} \right) = 0 \text{ on } \Omega_2, \quad (2)$$

where $\phi_{i,G_j} = \phi_i(r_{G_j})$ is the potential in region $\Omega_i$ evaluated at the surface $\Gamma_j$. $K$ and $V$ are defined as

$$K^{\Omega_i}_{L,Y}(\phi_{i,r_1}) = \iint_{\Gamma_j} \frac{\partial}{\partial n} \left[ G^{L/Y}(r_{\Omega_i}, r_{\Gamma_j}) \right] \phi_{i,r_1} d\Gamma,$$

$$V^{\Omega_i}_{L/Y} \left( \frac{\partial}{\partial n} \phi_{i,r_1} \right) = \iint_{\Gamma_j} \phi_{i,r_1} G^{L/Y}(r_{\Omega_i}, r_{\Gamma_j}) d\Gamma, \quad (3)$$

corresponding to the double- and single-layer potentials of $\phi_{i,G_j}$ and $\frac{\partial}{\partial n} \phi_{i,G_j}$ evaluated in the region $\Omega_i$. The functions $G_L$ and $G_Y$ are the free-space Green’s functions of the Poisson (Laplace kernel) and linearized Poisson-Boltzmann (Yukawa kernel) equations, respectively:

$$G_L(r_{\Omega_1}, r_{\Gamma_1}) = \frac{1}{4\pi|r_{\Omega_1} - r_{\Gamma_1}|},$$

$$G_Y(r_{\Omega_2}, r_{\Gamma_2}) = \frac{\exp(-k|r_{\Omega_2} - r_{\Gamma_2}|)}{4\pi|r_{\Omega_2} - r_{\Gamma_2}|}. \quad (4)$$

We then take the limits $r_{\Omega_1} \rightarrow r_1$, $r_{\Omega_2} \rightarrow r_1$, $r_{\Omega_2} \rightarrow r_2$, and apply the boundary conditions: $\phi_{1,G_1} = \phi_{2,G_1}$, $\epsilon_1 \frac{\partial}{\partial n} \phi_{1,G_1} = \epsilon_2 \frac{\partial}{\partial n} \phi_{2,G_1}$, and $\phi_{2,G_2} = \phi_0$ to get the following system of boundary equations:

$$\frac{\phi_{1,G_1}}{2} + K^{\Omega_1}_{L,Y}(\phi_{1,G_1}) - V^{\Omega_1}_L \left( \frac{\partial}{\partial n} \phi_{1,G_1} \right) = \frac{1}{\epsilon_1} \sum_{k=0}^{N_q} \frac{q_k}{4\pi|r_{G_1} - r_k|} \text{ on } \Gamma_1,$$

$$\frac{\phi_{1,G_1}}{2} - K^{\Omega_1}_{Y}(\phi_{1,G_1}) + \epsilon_1 V^{\Omega_1}_Y \left( \frac{\partial}{\partial n} \phi_{1,G_1} \right) - K^{\Omega_1}_{Y}(\phi_0) + V^{\Omega_1}_Y \left( \frac{\partial}{\partial n} \phi_{2,G_2} \right) = 0 \text{ on } \Gamma_1,$$

$$-K^{\Omega_2}_{Y}(\phi_{1,G_1}) + \epsilon_1 V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{1,G_1} \right) + \frac{\phi_0}{2} - K^{\Omega_2}_{Y}(\phi_0) + V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{2,G_2} \right) = 0 \text{ on } \Gamma_2. \quad (5)$$

Rearranging terms, we write Equation (5) in matrix form, as follows:

$$\begin{bmatrix}
\frac{1}{2} + K^{\Omega_1}_{L,Y} & -V^{\Omega_1}_L \\
-\frac{1}{2} - K^{\Omega_1}_{Y} & \epsilon_1 V^{\Omega_1}_Y \\
-\frac{1}{2} - K^{\Omega_2}_{Y} & \epsilon_2 V^{\Omega_2}_Y
\end{bmatrix}
\begin{bmatrix}
\phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{2,G_2}
\end{bmatrix}
= 
\begin{bmatrix}
\frac{1}{2} + K^{\Omega_1}_{L,Y} & 0 \\
-\frac{1}{2} - K^{\Omega_1}_{Y} & \epsilon_1 V^{\Omega_1}_Y \\
-\frac{1}{2} - K^{\Omega_2}_{Y} & \epsilon_2 V^{\Omega_2}_Y
\end{bmatrix}
\begin{bmatrix}
\phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{2,G_2}
\end{bmatrix}$$

$$= 
\begin{bmatrix}
\frac{1}{2} + K^{\Omega_1}_{L,Y} & -V^{\Omega_1}_L \\
-\frac{1}{2} - K^{\Omega_1}_{Y} & \epsilon_1 V^{\Omega_1}_Y \\
-\frac{1}{2} - K^{\Omega_2}_{Y} & \epsilon_2 V^{\Omega_2}_Y
\end{bmatrix}
\begin{bmatrix}
\phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{2,G_2}
\end{bmatrix}$$

$$+ 
\begin{bmatrix}
\sum_{k=0}^{N_q} \frac{q_k}{4\pi|r_{G_1} - r_k|} \\
-\epsilon_1 V^{\Omega_1}_Y \left( \frac{\partial}{\partial n} \phi_{1,G_1} \right) \\
-\epsilon_2 V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{2,G_2} \right)
\end{bmatrix}. \quad (6)$$

If the surface $\Gamma_2$ has prescribed charge, corresponding to a Neumann boundary condition, $-\epsilon_2 \frac{\partial}{\partial n} \phi_{2,G_2} = \sigma_0$, the equivalent derivation yields

$$\begin{bmatrix}
\frac{1}{2} + K^{\Omega_1}_{L,Y} & -V^{\Omega_1}_L \\
-\frac{1}{2} - K^{\Omega_1}_{Y} & \epsilon_1 V^{\Omega_1}_Y \\
-\frac{1}{2} - K^{\Omega_2}_{Y} & \epsilon_2 V^{\Omega_2}_Y
\end{bmatrix}
\begin{bmatrix}
\phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{2,G_2}
\end{bmatrix}
= 
\begin{bmatrix}
\frac{1}{2} + K^{\Omega_1}_{L,Y} & 0 \\
-\frac{1}{2} - K^{\Omega_1}_{Y} & \epsilon_1 V^{\Omega_1}_Y \\
-\frac{1}{2} - K^{\Omega_2}_{Y} & \epsilon_2 V^{\Omega_2}_Y
\end{bmatrix}
\begin{bmatrix}
\phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{1,G_1} \\
\frac{\partial}{\partial n} \phi_{2,G_2}
\end{bmatrix}$$

$$+ 
\begin{bmatrix}
\sum_{k=0}^{N_q} \frac{q_k}{4\pi|r_{G_1} - r_k|} \\
-\epsilon_1 V^{\Omega_1}_Y \left( \frac{\partial}{\partial n} \phi_{1,G_1} \right) \\
-\epsilon_2 V^{\Omega_2}_Y \left( \frac{\partial}{\partial n} \phi_{2,G_2} \right)
\end{bmatrix}. \quad (7)$$

The formulation detailed in this section differs from the work by Lenhoff and co-workers because they consider an infinite charged surface, modeled using a modified Green’s function to account for the half-space domain. Lenhoff’s approach has the advantage that the charged surface does not require a mesh, but presents difficulties if the surface has a complex geometry. More-
over, an infinite surface may not be a good model if its size is comparable to the protein’s, like it happens with nano-structures.

This formulation can be extended to account for Stern layers and solvent-filled cavities, by adding more surfaces or interfaces. In our code, we deal with multiple surfaces in the manner presented by Altman and co-workers, as described in our previous paper.

III. METHODS

A. Discretization

To numerically solve the system in , we discretize the boundaries into flat triangular panels and assume that and are constant within those panels. The discretized form of the integral operators is as follows:

\[ K_{L, \text{disc}}(\phi(r_i)) = \sum_{j=1}^{N_p} \phi(r_{\Gamma_j}) \int_{\Gamma_j} \frac{\partial}{\partial n} [G_L(r_i, r_{\Gamma_j})] \, d\Gamma_j, \]

\[ V_{L, \text{disc}}(\frac{\partial}{\partial n} \phi(r_i)) = \sum_{j=1}^{N_p} \frac{\partial}{\partial n} \phi(r_{\Gamma_j}) \int_{\Gamma_j} G_L(r_i, r_{\Gamma_j}) \, d\Gamma_j, \]

where \( N_p \) is the number of discretization elements on \( \Gamma_i \), and \( \phi(r_{\Gamma_j}) \) and \( \frac{\partial}{\partial n} \phi(r_{\Gamma_j}) \) are the constant values of \( \phi \) and \( \frac{\partial}{\partial n} \phi \) on panel \( \Gamma_j \) (we are somewhat abusing the nomenclature here by reusing the symbol \( \Gamma \), which previously referred to the complete surface). By collocating \( r_i \) on the center of each panel, we get a linear system of equations which looks just like those in Equations (6) and (7), but its elements are sub-matrices of size \( N_p \times N_p \) rather than integral operators. Looking at Figure 3, each element of a sub-matrix is an integral over one panel \( \Gamma_j \).

![Figure 3: Discretization of a molecular surface. \( \Gamma_i \) is the panel where the collocation point resides and \( \Gamma_j \) the panel being integrated.](image)

FIG. 3: Discretization of a molecular surface. \( \Gamma_i \) is the panel where the collocation point resides and \( \Gamma_j \) the panel being integrated.

with \( r_i \) located at the center of the collocation panel \( \Gamma_i \), as follows:

\[ K_{L, ij} = \int_{\Gamma_j} \frac{\partial}{\partial n} [G_L(r_{\Gamma_i}, r_{\Gamma_j})] \, d\Gamma_j, \]

\[ V_{L, ij} = \int_{\Gamma_j} G_L(r_{\Gamma_i}, r_{\Gamma_j}) \, d\Gamma_j. \]

The terms on the right-hand side and the unknown vectors in the discretized form of Equation (6) are sub-vectors of size \( N_p \). In this case, each element is the evaluation on the collocation panel \( \Gamma_i \), written as

\[ \phi_1, \Gamma_i = \phi_1(r_i), \]

\[ \frac{\partial}{\partial n} \phi_1, \Gamma_i = \frac{\partial}{\partial n} \phi_1(r_i), \]

\[ \sum_{k=0}^{N_q} \frac{q}{4\pi|r_{\Gamma_i} - r_k|} = \sum_{k=0}^{N_q} \frac{q}{4\pi|r_i - r_k|}, \]

where \( r_i \) is located at the center of panel \( \Gamma_i \).

In our numerical solution, integrals are calculated in three ways, depending on how close the panel is to the collocation point. When the collocation point is inside the element being integrated, we use a semi-analytical technique with Gauss points placed along the edges of the element. If the integrated element is closer than 2L from the collocation point — where \( L = \sqrt{2 \cdot \text{Area}} \) — we use a fine Gauss quadrature rule, with 19 or more points per element. Beyond a distance of 2L, elements have only 1, 3, or 7 Gauss points, depending on the case.

B. Treecode-accelerated boundary element method

Most modern implementations of the boundary element method (BEM) use Krylov methods to solve the linear system, usually a general minimal residual method (GMRES), which is agnostic to the structure of the matrix. In practice, Krylov solvers for BEM require \( O(n \cdot N_p^2) \) operations to obtain the unknown vector, where \( n \) is the number of iterations to get a desired residual, and is much smaller than \( N_p \). The \( O(N^2) \) scaling is given by a matrix-vector product (with a dense matrix) done in every iteration; this is the most time-consuming part of the algorithm, and makes BEM prohibitive for more than a few thousand discretization elements.

But when we inspect the approximation of the integrals in with Gauss quadrature rules, we see that the matrix-vector product has the form of an N-body problem, similar to gravitational potential calculations in planetary systems. In this case, the Gauss quadrature points act analogously to planets (sources of mass) and the collocation points are analogous to the locations where the gravitational potential is computed (targets points). There are several ways to accelerate this kind of computations, for example fast-multipole methods.
treecodes\textsuperscript{22} and fast-Fourier-transform methods\textsuperscript{33}. In our numerical solution (developed as the open-source code \textsc{PyGBe}), we accelerate the $N$-body calculation with a treecode\textsuperscript{22,32,33} making this part of the algorithm scale as $O(N \log N)$ rather than $O(N^2)$.

The treecode algorithm groups the sources and targets in a tree-structured set of boxes and approximates interactions between far-away boxes using a series expansion—a Taylor series, in our case. This allows for controllable accuracy that depends on the number of terms used in the expansion and the multipole-acceptance criterion that defines the threshold where the distance between source and target is far enough to approximate the interactions with expansions. Details of our implementation of the treecode in \textsc{PyGBe} can be found in our previous work\textsuperscript{22}.

C. Energy calculation

Figure\textsuperscript{2} shows an arrangement with three types of free energy: Coulombic energy from the point charges, surface energy due to $\Gamma_2$ and solvation energy. The Coulombic energy arises simply from the Coulomb interactions of all point charges. This section describes how we compute the other two components of free energy in the boundary-element framework.

**Solvation free energy**— When a protein is in a solvated state, surrounded by water molecules that have become polarized, its free energy differs from its state in vacuo by an amount known as the solvation energy. Its free energy again differs in the presence of other structures in the solvent, e.g., other proteins or charged surfaces. In this work we use the term solvation energy to more broadly mean the change in free energy of the protein from its state in a vacuum, to its state in the solvent with any other components or structures. In single-molecule settings, this definition of solvation energy coincides with the energy required to solvate the molecule.

To calculate the solvation energy, the total minus the Coulomb potential is applied inside the protein, i.e.,

$$ F_{\text{solv}} = \frac{1}{2} \int_{\Omega} \rho \left( \phi_{\text{total}} - \phi_{\text{Coulomb}} \right) \, \text{d}x = \sum_{k=0}^{N_p} q_k \left( \phi_{\text{total}} - \phi_{\text{Coulomb}} \right)(r_k), $$

where $\rho$ is the charge distribution, consisting of point charges (which transforms the integral into a sum). The total minus Coulomb potential includes the reaction potential—representing the response of the solvent by polarization and rearrangement of free ions—and any effects from the immersed surface. We can also interpret it as the potential generated by the boundary $\Gamma$ of the molecular region $\Omega$. Taking the first expression of Equation\textsuperscript{2} and subtracting out the Coulombic effect yields

$$ \phi_{\text{react}}(r_k) = -k_{L}(\phi_{1,\Gamma}^{\text{c}}) + V_{L}(\frac{\partial}{\partial n} \phi_{1,\Gamma}) \quad (13) $$

Equation (11) requires evaluating $\phi_{\text{react}}$ for each point-charge location $r_k$. We obtain this by discretizing Equation (13) and using the solution of the linear system in Equation (6) or Equation (7) as inputs.

**Surface free energy**— Chan and co-workers\textsuperscript{15,16} derived the free energy for a surface with a set charge or potential. They describe the free energy on a surface as

$$ F = \frac{1}{2} \int_{\Gamma} G_c \sigma_0^2 \, d\Gamma \quad \text{for set charge, and} $$

$$ F = -\frac{1}{2} \int_{\Gamma} G_p \phi_0^2 \, d\Gamma \quad \text{for set potential}, $$

where $\phi_0$ and $\sigma_0$ are the prescribed potential and surface charge, respectively. The potential is given by $\phi(\sigma, R, x) = G_c(R, x) \sigma$ for the first expression and the surface charge by $\sigma(\phi, R, x) = G_p (R, x) \phi$ for the second one. This is valid because we are using a linearized Poisson-Boltzmann model.

Using constant values of $\phi$ and $\frac{\partial \phi}{\partial n}$ per panel, the discretized version of Equation (14) takes the form

$$ F = \frac{1}{2} \sum_{j=1}^{N_p} \phi(r_j) \sigma_{0j} A_j, $$

and

$$ F = -\frac{1}{2} \sum_{j=1}^{N_p} \phi_{0j} \sigma(r_j) A_j. $$

where $A_j$ is the area of panel $j$, and $\sigma = \epsilon \frac{\partial \phi}{\partial n}$. To obtain the surface free energy, we can plug in the solution of the system in Equation (6) or (7) to Equation (15).

**Interaction free energy**— When there are two or more bodies in the solvent, they will interact electrostatically. In order to compute the energy of interaction, we need to take the difference between the total energy of the interacting system and the total energy of each isolated component, where the total free energy is given by

$$ F_{\text{total}} = F_{\text{Coulomb}} + F_{\text{surface}} + F_{\text{solv}}. $$

The interaction free energy is

$$ F_{\text{interaction}} = F_{\text{total}} - \sum_{i=1}^{N_c} F_{\text{comp}}^{\text{comp}_i}, $$

where $N_c$ is the number of components in the system and $F_{\text{comp}}^{\text{comp}_i}$ is calculated over the isolated component $i$. 
D. Orientation sampling of a protein near a charged surface

We are interested in studying the orientation of proteins near self-assembled monolayers (SAM), specifically for biosensing applications. In the framework of the implicit-solvent model, we can represent the SAM as a surface charge density, and use Equation (7) to compute the electrostatic potential. According to the Boltzmann distribution, the probability of finding the system in microstate $\lambda$ depends on the total free energy, $F_{\text{total}}$, as

$$P(\lambda) = \frac{\int_{\lambda} \exp\left(-\frac{F_{\text{total}}}{k_B T}\right) d\lambda}{\int_{\Lambda} \exp\left(-\frac{F_{\text{total}}}{k_B T}\right) d\Lambda},$$  

where $\Lambda$ is the ensemble of all micro-states, $k_B$ the Boltzmann constant and $T$ the temperature. To obtain a probability distribution, we used Equation (18) assuming that electrostatic effects were dominant, and sampled $F_{\text{total}}$ for different orientations. We defined the orientation using the angle between the dipole moment and surface normal vectors as a reference (tilt angle), varying from $0^\circ$ to $180^\circ$. Also, for each tilt angle, we rotated the protein about the dipole moment vector in $360^\circ$ to examine all possible orientations. This process is sketched in Figure 4.

In this case, micro-states are defined by the tilt ($\alpha_{\text{tilt}}$) and rotational ($\alpha_{\text{rot}}$) angles, and we rewrite the integral in the numerator of Equation (18) as

$$\int_{\lambda} \exp\left(-\frac{F_{\text{total}}}{k_B T}\right) d\lambda = \int \int \exp\left(-\frac{F_{\text{total}}}{k_B T}\right) d\alpha_{\text{rot}} d\alpha_{\text{tilt}},$$

where micro-state $\lambda$ is a range of angles $\alpha_{\text{rot}}$ and $\alpha_{\text{tilt}}$.

To assess the performance of the implicit solvent model for investigating protein-surface interactions, we studied the orientation of protein G B1 D4' mutant near a charged surface, since there are molecular dynamics simulations and experimental observations available in the literature that we could compare to. Figure 5 shows the structure of Protein G B1 (PDB code 1PGB), to which we applied mutations E19Q, D22N, D46N and D47N to obtain the D4' mutant, using the SwissPdb Viewer software.

We carried out a similar study for the antibody immunoglobulin G (PDB code 1IGT), a widely used protein in biosensors, whose structure is shown in Figure 6. This is a more interesting case from the point of view of our application, yet we do not have the benefit of published simulations or experiments to compare to.

IV. ANALYTICAL SOLUTION

It is possible to derive a closed-form expression for the free energy of interaction between a spherical molecule with a centered charge and a spherical surface with imposed potential or charge, like the one sketched in Figure 7. There are such analytical expressions for interacting charged surfaces and interacting spherical molecules with multiple point charges inside but not for a situation where surfaces and molecules interact. Having such an analytical solution is of great utility in the de-
development of a computational model for protein-surface interaction, because it will allow for proper code verification.

A. Expansion in Legendre polynomials

The system of partial differential equations from Equation (1) models the electrostatic potential field in the setting of Figure 7. Following Carnie and co-workers, the axial symmetry lets us formulate the solution of Equation (1) as an expansion in Legendre polynomials:

\[
\phi_1 = \sum_{n=0}^{\infty} c_n r_1^n P_n(\cos \theta_1) + \frac{q}{4\pi \epsilon_1 r_1} \quad \text{on } \Omega_1,
\]

\[
\phi_2 = \sum_{n=0}^{\infty} a_n k_n(\kappa r_1) P_n(\cos \theta_1)
+ \sum_{n=0}^{\infty} b_n k_n(\kappa r_2) P_n(\cos \theta_2) \quad \text{on } \Omega_2,
\]

being \(P_n\) the \(n\)-th-degree Legendre polynomial and \(k_n\) the modified spherical Bessel function of the second kind.

We make use of the following addition formula,\(^{13}\)

\[
k_n(\kappa r_2) P_n(\cos \theta_2) = \sum_{m=0}^{\infty} (2m+1) B_{nm} i_m(\kappa r_1) P_m(\cos \theta_1),
\]

to reformulate the expression for \(\phi_2\) in Equation (20) as

\[
\phi_2 = \sum_{n=0}^{\infty} a_n k_n(\kappa r_1) P_n(\cos \theta_1)
+ \sum_{n=0}^{\infty} b_n \sum_{m=0}^{\infty} (2m+1) B_{nm} i_m(\kappa r_1) P_m(\cos \theta_1)
\]

\[
= \sum_{n=0}^{\infty} b_n k_n(\kappa r_2) P_n(\cos \theta_2)
+ \sum_{n=0}^{\infty} a_n \sum_{m=0}^{\infty} (2m+1) B_{nm} i_m(\kappa r_2) P_m(\cos \theta_2).
\]

(22)

Here, \(i_m\) is the modified spherical Bessel function of the first kind; \(B_{nm}\) is defined by

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

(23)

where \(R\) is the center-to-center distance; and \(A_{nm}^{\nu}\) is given by the following expression, with \(\Gamma\) (in this context only) representing the gamma function:

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

(24)

Legendre polynomials are orthogonal to each other, and \(\frac{q}{4\pi \epsilon_1 r_1}\) is independent of \(\theta\). Thus, taking the inner product of the expressions in Equations (20) and (22) with \(P_j(\cos \theta_i)\), where \(i = 1\) or \(2\), yields

\[
\phi_1 \delta_{ij} = c_j r_1^j + \frac{q}{4\pi \epsilon_1 r_1} \delta_{ij}
\]

(25)
for Equation (22).

Applying the interface conditions for $\Gamma_1$ on Equation (25) and the first expression of Equation (26), produces

$$\sum_{n=0}^{\infty} a_n \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{n}{d_1} k_n(\kappa d_1) \right) \delta_{nj} + b_n(2j+1)B_{nj} \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{j}{d_1} i_j(\kappa d_1) \right) = -\frac{\epsilon_1}{\epsilon_2} \frac{q}{4\pi\epsilon_1 d_1^2} \delta_{nj}(j+1), \quad (27)$$

where $d_1$ is the radius of surface 1.

**Constant potential $\phi$ on $\Gamma_2$.**

The application of the boundary condition on $\Gamma_2$, $\phi(\Gamma_2) = \phi_0$, where $\phi_0$ is independent on $\theta_2$, gives

$$\sum_{n=0}^{\infty} a_n(2j+1)B_{nj}j_j(\kappa d_2) + b_n k_n(\kappa d_2) \delta_{nj} = \phi_0 \delta_{nj}. \quad (28)$$

Combining Equations (27) and (28) yields the following system of equations for the coefficients $a_n$ and $b_n$

$$IA + LB = -\frac{\epsilon_1}{\epsilon_2} \frac{q}{4\pi\epsilon_1 d_1^2} e$$

$$MA + IB = \phi_0 e \quad (29)$$

where

$$I_j = \delta_j, n$$

$$c_j = \delta_{0j}$$

$$A_n = a_n \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{n}{d_1} k_n(\kappa d_1) \right)$$

$$B_n = b_n k_n(\kappa d_2)$$

$$L_{jn} = (2j+1)B_{nj} \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{j}{d_1} i_j(\kappa d_1) \right)$$

$$M_{jn} = (2j+1)B_{nj}j_j(\kappa d_2) \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{n}{d_1} k_n(\kappa d_1) \right). \quad (30)$$

**Constant surface charge $\sigma$ on $\Gamma_2$.**

In this case, the application of the boundary condition on $\Gamma_2$, $\sigma(\Gamma_2) = -\epsilon_2 \frac{b_0}{2\pi} r_2 = \sigma_0$, where $\sigma_0$ is independent on $\theta_2$, gives

$$\sum_{n=0}^{\infty} a_n(2j+1)B_{nj}k_j'(\kappa d_2) + b_n k_n'(\kappa d_2) \delta_{nj} = -\frac{\sigma_0}{\epsilon_2} \delta_{nj} \quad (31)$$

Combining Equations (27) and (28) produces a system of equations for the coefficients $a_n$ and $b_n$

$$IA + LB = -\frac{\epsilon_1}{\epsilon_2} \frac{q}{4\pi\epsilon_1 d_1^2} e$$

$$MA + IB = -\frac{\sigma_0}{\epsilon_2} e \quad (32)$$

where

$$L_{jn} = \delta_{jn}$$

$$c_j = \delta_{0j}$$

$$A_n = a_n \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{n}{d_1} k_n(\kappa d_1) \right)$$

$$B_n = b_n \kappa k_n'(\kappa d_2)$$

$$L_{jn} = (2j+1)B_{nj} \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{j}{d_1} i_j(\kappa d_1) \right)$$

$$M_{jn} = (2j+1)B_{nj}k_j'(\kappa d_2) \left( \kappa k_n'(\kappa d_1) - \frac{\epsilon_1}{\epsilon_2} \frac{n}{d_1} k_n(\kappa d_1) \right). \quad (33)$$

**B. Energy calculation**

**Solvation free energy of the molecule**— According to Equation (11), the solvation free energy of a molecule with a centered charge is given by

$$F_{solv} = \frac{1}{2} q \phi_{\text{reac}}(r_1 = 0), \quad (34)$$

and using Equation (20), the reaction potential from Equation (13) is:

$$\phi_{\text{reac}} = \phi - \frac{q}{4\pi\epsilon_1 r} = \sum_{n=0}^{\infty} c_n r^n P_n(\cos \theta_1). \quad (35)$$

Applying the boundary conditions at $\Gamma_1$ on Equation (25), we can rewrite $c_j$ in terms of the already computed $a_j$ and $b_j$:

$$c_j = \frac{1}{d_1} \left( a_j k_j(\kappa d_1) + \sum_{m=0}^{\infty} b_m (2j+1)B_{mj}j_j(\kappa d_1) - \frac{q}{4\pi\epsilon_1 d_1} \delta_{0j} \right). \quad (36)$$

Because the charge is located at $r = 0$, only the $n = 0$ terms of Equation (35) will survive, and the potential at this location is:

$$\phi_{\text{reac}}(r_1 = 0) = a_0 k_0(\kappa d_1) + \sum_{m=0}^{\infty} b_m B_{m0} i_0(\kappa d_1) - \frac{q}{4\pi\epsilon_1 d_1} \delta_{0j} \quad (37)$$
The result from Equation (37) in Equation (34) yields the solvation free energy.

For the isolated molecule, \( R \to \infty \) makes \( B_{nm} \to 0 \), which nullifies the sum in Equation (37) and \( a_0 \) for \( R \to \infty \), from the system in Equation (29), is

\[
a_0^\infty = - \frac{q}{d_1^2} \frac{1}{\varepsilon_2} \frac{1}{4 \pi \kappa k_0'(\kappa d_1) \varepsilon_1} \tag{38}
\]

**Surface free energy with set potential \( \phi_0 \)** — We can expand \( G_p \) from Equation (14) in Legendre polynomials as

\[
G_p = - \frac{\varepsilon_2}{2} \left[ b_0 k_0'(\kappa d_2) + \sum_{n=0}^{\infty} a_n n_0' \left( \frac{\kappa d_2}{k_0} \right) \right] \tag{40}
\]

If the surface is isolated, \( R \to \infty \) makes \( B_{nm} \to 0 \), and the free energy in this case is

\[
F = 2 \pi \kappa \phi_0 d_2^2 \left[ b_0 k_0'(\kappa d_2) + \sum_{n=0}^{\infty} a_n n_0' \left( \frac{\kappa d_2}{k_0} \right) \right] \tag{41}
\]

where \( b_0^\infty \) is taken from the system in (29) considering \( B_{nm} \to 0 \), which results in

\[
b_0^\infty = \frac{\phi_0}{k_0' \kappa d_2}. \tag{42}
\]

**Surface free energy with set charge \( \sigma_0 \)** — We can expand \( G_c \) from Equation (14) in Legendre polynomials as

\[
G_c = \frac{1}{\sigma_0} \left[ \sum_{n=0}^{\infty} b_n k_n'(\kappa d_2) P_n(\cos \theta_2) + \sum_{n=0}^{\infty} a_n n_0' \left( \frac{\kappa d_2}{k_0} \right) \right] \tag{43}
\]

Applying Equation (43) into Equation (14) gives

\[
F = 2 \pi \kappa \sigma_0 d_2 \left[ b_0 k_0'(\kappa d_2) + \sum_{n=0}^{\infty} a_n n_0' \left( \frac{\kappa d_2}{k_0} \right) \right] \tag{44}
\]

For the isolated surface, \( R \to \infty \) and \( B_{nm} \to 0 \), and the free energy is

\[
F = 2 \pi \kappa \sigma_0 d_2^2 b_0^\infty k_0'(\kappa d_2) \tag{45}
\]

where \( b_0^\infty \) is calculated from the system in (32) considering \( B_{nm} \to 0 \), which results in

\[
b_0^\infty = - \frac{\sigma_0}{\varepsilon_2 \kappa k_0'(\kappa d_2)}. \tag{46}
\]

V. RESULTS

To obtain the following results, we extended the PyGBe code to consider surfaces with prescribed charge or potential. For most runs, we used a workstation with Intel Xeon X5650 CPUs and one NVIDIA Tesla C2075 GPU card (2011 Fermi). That includes the code verification runs using the analytical solution and the calculations with protein G B1 D4'. The final case considers the antibody immunoglobulin G, which is a much larger molecule than protein G. For these runs, we used Boston University’s BUNGEE cluster, which has 16 nodes with 8 Intel Xeon CPU cores each, and a total of 3 NVIDIA Tesla Kepler K20 and 26 NVIDIA Tesla M2070/2075 GPUs. All runs were serial: single-CPU and single-GPU. We used the free msms software\(^\text{[11]}\) to generate meshes, and pdb2pqr\(^\text{[15]}\) with an Amber forcefield to determine the charges and van der Waals radii. In these tests, we did not consider a Stern layer for either the protein or the charged surface, nor the presence of solvent-filled cavities inside the protein.

A. Verification against analytical solution

Using the analytical solution detailed in Section IV we carried out a grid-convergence study of PyGBe extended to treat interacting surfaces with biomolecules. The setup consists of a spherical molecule with a 5Å radius and a centered charge of \( 1e^- \), interacting with a spherical surface of 4Å radius and an imposed potential of \( \phi = 1 \). The center-to-center distance between the spheres is 12Å, and they are dissolved in water with salt at 145mM, which gives a Debye length of 8 (\( \kappa = 0.125 \)), and permittivity \( \varepsilon_{sol} = 80 \). The permittivity inside the spherical protein is \( \varepsilon_{mol} = 4 \). Figure 8 shows a sketch of this system.

![Figure 8: Sketch of system used in the convergence study of Figure 9](image-url)

Figure 9 presents the results of the grid-convergence analysis, where the error is the relative difference in interaction free energy between the analytical result from...
Section IV and the numerical solution computed with PyGBe. The observed order of convergence of the three finest meshes was 1.007. Table I presents the numerical parameters used in this case. Recall from section III that we calculate the boundary-element integrals differently for close-by and far-away elements, and use a semi-analytical method for the element that contains the collocation point. The fine Gauss quadrature rule is used for elements closer than 2L from the collocation point, where \( L = \sqrt{A / \pi} \). Area. For the treecode, \( N_{\text{crit}} \) is the maximum number of boundary elements per box, \( P \) is the Taylor expansion truncation parameter and \( \theta \) is the multipole-acceptance criterion. The final numerical parameter is the exit tolerance of the GMRES solver.

**TABLE I:** Numerical parameters used in the code-verification runs with the analytical solution.

| # Gauss points: | Treecode: | GMRES: |
|-----------------|-----------|--------|
| in-element close-by far-away | \( N_{\text{crit}} \) | \( P \) | \( \theta \) | tol. |
| 9 per side | 37 | 3 | 300 | 15 | 0.5 | \( 10^{-9} \) |

**FIG. 9:** Grid-convergence study for the interaction free energy between a spherical molecule with a centered charge and a sphere with potential \( \phi = 1 \). Data sets, figure files plus running/plotting scripts are available under CC-BY.

As seen in Figure 9, the error decays with the average area of the boundary elements \( \left( \frac{1}{N} \right) \), which is the expected behavior considering our previous work. This proves that the extension of PyGBe to treat charged surfaces is solving the mathematical model correctly.

B. First case: protein G B1 D4′

We computed the electrostatic field of protein G B1 D4′ interacting with a 100Å \( \times \) 100Å \( \times \) 10Å block with surface charge density \( \pm 0.05 \text{C/m}^2 \), and investigated its preferred orientation. The protein was centered with respect to a 100Å \( \times \) 100Å face, a distance 2Å above it. As seen in Figure 4, \( \alpha_{\text{crit}} \) is the angle between the protein’s dipole moment and the normal vector to the surface, and \( \alpha_{\text{rot}} \) rotates about the dipole moment. The dipole-moment vector placed at the center of mass of the protein generates an axis, and we used the line of shortest distance between the outermost atom and this axis as a reference vector \( \mathbf{V}_{\text{ref}} \). The rotation angle \( \alpha_{\text{rot}} \) is the angle between the normal vector to a 100Å \( \times \) 10Å side face of the block and \( \mathbf{V}_{\text{ref}} \).

In these cases, we considered a solvent with no salt, i.e., \( \kappa = 0 \) (to compare with other published results), and with relative permittivity 80. The region inside the protein had a relative permittivity of 4.

**Grid-convergence study for protein G B1 D4′** — By means of a grid-convergence study, we make sure that the runs are in the asymptotic range of the model, select a triangle density so that the geometry is well-resolved by the surface mesh, and find adequate values of the simulation parameters for sampling the orientations. Using Richardson extrapolation, we find values of the energy that estimate the exact solution and use them as a reference to calculate estimated errors. This error is simply the relative difference between the energy obtained numerically with each mesh density and the estimated exact value. In this way, we choose the parameters for the sampling runs with confidence that they are both accurate and efficient in computing time.

We computed the solvation and surface energy of a system containing a surface with charge density 0.05C/m\(^2\), and a protein at \( \alpha_{\text{crit}} = 10^\circ \) and \( \alpha_{\text{rot}} = 200^\circ \). The numerical parameters are presented in Table II. Using runs with mesh densities of 2, 4, and 8 elements per square Angstrom, we obtained the values in Table III using Richardson extrapolation: these are the reference values for the error plotted in Figure 10. The observed order of convergence was 0.96 for the solvation energy and 0.94 for the surface energy. For details on the Richardson-extrapolation method for performing grid-convergence analysis, see our previous work. Figure 10 shows errors that are decaying as \( 1/N \) in both the solvation and surface energies for the finest three meshes. This indicates that the calculations are in the asymptotic range and the geometry is well resolved in these cases.

**TABLE II:** Numerical parameters used in the convergence runs with protein G B1 D4′.

| # Gauss points: | Treecode: | GMRES: |
|-----------------|-----------|--------|
| in-element close-by far-away | \( N_{\text{crit}} \) | \( P \) | \( \theta \) | tol. |
| 9 per side | 19 | 7 | 500 | 15 | 0.5 | \( 10^{-8} \) |

**TABLE III:** Extrapolated values of energy for protein G B1 D4′.

| Energy [kcal/mol] | Solvation Surface |
|-------------------|-------------------|
| 222.43 | 317.98 |
Probing orientation of Protein $G B1 D4'$ — We sampled the total free energy every $\Delta \alpha_{\text{tilt}} = 2^\circ$ of tilt angle and $\Delta \alpha_{\text{rot}} = 10^\circ$ of rotation angle, resulting in 3,240 independent runs. The surface mesh had 4 triangles per square Angstrom on the protein geometry and 2 triangles per square Angstrom on the charged surface. Numerical parameters are presented in Table IV.

### Table IV: Numerical parameters used in the runs probing orientation of protein $G B1 D4'$.

| # Gauss points: | Treecode: gmres: | in-element close-by far-away | $N_{\text{crit}}$ | $P \theta$ | tol. |
|-----------------|------------------|-------------------------------|--------------------|------------|------|
| 9 per side 19   | gmres:           | in-element close-by far-away  | 300                | 0.5        | $10^{-5}$ |

With total free energy as the input, the integrals of Equation (19) can be computed by means of the trapezoidal rule. Figure 11 presents the probability of the protein orientation in terms of $\cos(\alpha_{\text{tilt}})$, in intervals of $\Delta \cos(\alpha_{\text{tilt}}) = 0.005$ (Fig. 11a) and $\Delta \alpha_{\text{tilt}}=2^\circ$ (Fig. 11b). Table V presents the average orientation $<\cos(\alpha_{\text{tilt}})>$ for the surface having either positive or negative charge density, and Figure 12 shows the electrostatic potential for the preferred orientation in each case.

### Table V: Average orientation.

| $<\cos(\alpha_{\text{tilt}})>$ | Negative | Positive |
|-------------------------------|---------|----------|
|                               | -0.968  | 0.963    |

C. Second case: immunoglobulin G

We computed the electrostatic field of immunoglobulin G—a protein widely used in biosensors—interacting with a $250\times250\times10\text{Å}$ block, varying the conditions of surface charge and salt concentration. The protein was centered with respect to a $250\times250\text{Å}$ face, at a distance $5\text{Å}$ above it. The solvent had relative permittivity of 80 and the protein of 4.

Grid-convergence study for immunoglobulin G — As in the previous section, we carried out a grid-convergence study to make sure the geometry was well resolved and to find adequate values of the simulation parameters for sampling different orientations. The error in Figure 13 is the relative difference between the energy obtained using PyGBe with each mesh density and the estimated exact value computed with Richardson extrapolation.

In this case, we computed the solvation energy and surface energy of a system consisting of a surface with charge density $0.05\text{C/m}^2$ and a protein with $\alpha_{\text{tilt}} = 31^\circ$ and $\alpha_{\text{rot}} = 130^\circ$. Using the results from runs with a mesh density of 2, 4, and 8 elements per square Angstrom, we added the solvation and surface energies, and used Richardson extrapolation to obtain a value of $-2792.22\text{kcal/mol}$, and an observed order of convergence of 0.85. This is our reference to calculate the errors in Figure 13. There is a slight deviation from the expected value of the observed order of convergence (1.0), which we attribute to the non-uniform mesh generated by MSMS. Even though the mesh density is on average doubled for each run, there is no guarantee that the refinement is homogeneous throughout the whole molecular surface. The numerical parameters are presented in Table VI.

### Table VI: Numerical parameters used in the convergence runs with immunoglobulin G.

| # Gauss points: | Treecode: gmres: | in-element close-by far-away | $N_{\text{crit}}$ | $P \theta$ | tol. |
|-----------------|------------------|-------------------------------|--------------------|------------|------|
| 9 per side 19   | gmres:           | in-element close-by far-away  | 1000               | 0.5        | $10^{-5}$ |

Probing orientation of immunoglobulin G — We sampled the total free energy every $\Delta \alpha_{\text{tilt}} = 4^\circ$ of tilt angle and $\Delta \alpha_{\text{rot}} = 20^\circ$ of rotation angle, resulting in a total of 810 runs. The surface meshes had 2 triangles per square Angstrom throughout. Numerical parameters are presented in Table VII.

### Table VII: Numerical parameters used in the runs probing orientation of immunoglobulin G.

| # Gauss points: | Treecode: gmres: | in-element close-by far-away | $N_{\text{crit}}$ | $P \theta$ | tol. |
|-----------------|------------------|-------------------------------|--------------------|------------|------|
| 9 per side 19   | gmres:           | in-element close-by far-away  | 300                | 0.5        | $10^{-4}$ |

With the computed total free energy, we obtained the probability of each orientation using Equation (19) and
FIG. 11: Orientation probability distribution of protein G B1 D4’. Figures 11a and 11b are the probability with respect to the tilt angle and its cosine, respectively. Figures 11c and 11d are the probability with respect to both the tilt and rotation angle. Data sets, figure files and running/plotting scripts available under cc-by.

FIG. 12: Electrostatic potential of protein G B1 D4’ for the preferred orientations according to Figure 11. Black arrow indicates direction of dipole-moment vector.

The trapezoidal rule. We sampled all combinations with surface charges of $\sigma = \pm 0.05\, \text{C/m}^2$ and $\sigma = \pm 0.2\, \text{C/m}^2$ and salt concentrations of 145mM ($\kappa = 0.125\, \text{Å}^{-1}$) and 9mM ($\kappa = 0.03125\, \text{Å}^{-1}$). For each of these cases, Figures 14 and 15 show a color plot of the probability distribution with respect to the tilt and rotation angles, and a 3D plot of the preferred orientation, where the solvent-excluded surface is colored by the electrostatic potential.
This study verified that PyGBe for a boundary element method with constant elements. Unfortunately, PyGBe charged surfaces, we extended its extension. Section IV derives a closed expression for a spherical molecule with a centered charge interacting with a spherical surface, and we used this expression to carry out a grid-convergence study of the interaction energy (Figure 9). The error decays with the area, which is the expected behavior for a boundary element method with constant elements. This study verified that PyGBe solves the mathematical model correctly.

Here, the numerical parameters were all chosen for high accuracy because discretization error is very small for a spherical geometry. We wanted to make sure that the errors due to integration, the treecode approximation and the GMRES solver were even smaller. With more realistic molecular geometries, however, discretization errors are larger and accuracy requirements with PyGBe are relaxed, resulting in lower runtimes.

D. Reproducibility and data management

We have a consistent reproducibility practice that includes releasing code and data associated with a publication. The PyGBe code was released at the time of submitting our previous publication [23] under an MIT open-source license, and we maintain a version-control repository. As with our previous paper, we also release with this work all of the data needed to run the numerical experiments reported here, including running scripts and post-processing code in Python for producing the figures. To support our open-science goals, we prepared such a “reproducibility package” for each of the results presented in Figures 9, 10, 11, and 13 and the probability plots in Figures 14 and 15. The included running scripts invoke the PyGBe code with the correct input data and meshes (also included), and post-process the results to give the final figure, all with just one command. Please see the respective captions for a reference to the reproducibility packages, hosted on the figshare repository.

VI. DISCUSSION

A. Verification with analytical solution

In order to study the interaction of proteins and charged surfaces, we extended PyGBe to account for surfaces with prescribed charge or potential. Unfortunately, there was no analytical solution available in the literature to compare and verify PyGBe’s extension. Section IV derives a closed expression for a spherical molecule with a centered charge interacting with a spherical surface, and we used this expression to carry out a grid-convergence study of the interaction energy (Figure 9). The error decays with the area, which is the expected behavior for a boundary element method with constant elements. This study verified that PyGBe solves the mathematical

FIG. 13: Grid-convergence study of the solvation plus surface energy for immunoglobulin G interacting with a surface with charge density 0.05 C/m². Data sets, figure files and plotting scripts available under cc-by [19].

B. First case: protein G B1 D4′

The orientation of protein G B1 D4′ near charged surfaces was studied using molecular dynamics (MD) simulations by Liu and co-workers [37] and experimentally by Baio and co-workers [35]. The availability of these published results was a motivation to test PyGBe using this protein.

The results presented in Figure 11 show that for the most likely orientations, the dipole-moment vector is aligned with the vector normal to the interacting surface. This indicates that the dipole moment is the dominant effect that determines the protein’s orientation, over local protein-surface interactions. This result is unsurprising, since protein G B1 D4′ is a relatively small biomolecule.

Moreover, Figure 11 reveals that protein G B1 D4′ behaves like a point dipole, as the most likely orientations shift 180° when the sign of the surface charge is flipped. This is also explained by the dipole moment dominating the orientation.

The dipolar behavior described by our calculations with PyGBe agrees with the experiments done by Baio and co-workers [35] in which they observed opposite orientations of protein G B1 D4′ adsorbed on NH₃⁺ and COO⁻ self-assembled monolayers. With positively charged surfaces, most of the proteins oriented with the N-terminal of the protein pointing away from the surface, while for negatively charged surfaces the opposite occurred, with the C-terminal pointing away from the surface. This agrees with our results in Figure 11 since the dipole moment vector of protein G B1 D4′ points from the C-terminal to the N-terminal.

Liu and co-workers [37] used MD simulations to obtain \(< \cos(\alpha_{\text{tilt}}) > = 0.95\) for \(\sigma = 0.05\) C/m², and \(< \cos(\alpha_{\text{tilt}}) > = -0.85 \pm 0.05\) for \(\sigma = -0.05\) C/m², which agrees well with our results in Table V. MD simulations consider van der Waals interactions and conformational changes of the protein, whereas these are not considered in our approach, explaining the slight differences in \(< \cos(\alpha_{\text{tilt}}) > \).

C. Second case: immunoglobulin G

With the extension of PyGBe verified with an analytical solution (Section VI A) and confirmation that the implicit solvent model can be used to study protein-surface interaction with a small protein (Section VI B),
FIG. 14: Orientation probability distribution and surface potential of the preferred orientation for immunoglobulin G near a negative surface charge. The black arrow indicates the direction of the dipole moment. Data sets, figure files and plotting scripts available under CC-BY[19]
FIG. 15: Orientation probability distribution and surface potential of the preferred orientation for Immunoglobulin G near a positive surface charge. The black arrow indicates the direction of the dipole moment. Data sets, figure files and plotting scripts available under cc-by.

(a) Probability for $\sigma = 0.05 \text{C/m}^2$ and $\kappa = 0.125 \text{Å}^{-1}$

(b) Side view for $\alpha_{\text{tilt}} = 64^\circ$ and $\alpha_{\text{rot}} = 280^\circ$

(c) Probability for $\sigma = 0.2 \text{C/m}^2$ and $\kappa = 0.125 \text{Å}^{-1}$

(d) Side view for $\alpha_{\text{tilt}} = 64^\circ$ and $\alpha_{\text{rot}} = 260^\circ$

(e) Probability for $\sigma = 0.05 \text{C/m}^2$ and $\kappa = 0.03125 \text{Å}^{-1}$

(f) Side view for $\alpha_{\text{tilt}} = 44^\circ$ and $\alpha_{\text{rot}} = 120^\circ$

(g) Probability for $\sigma = 0.2 \text{C/m}^2$ and $\kappa = 0.03125 \text{Å}^{-1}$

(h) Side view for $\alpha_{\text{tilt}} = 76^\circ$ and $\alpha_{\text{rot}} = 160^\circ$
we proceeded to explore the effect of surface charge and salt concentration on the orientation of the antibody immunoglobulin G. Antibodies are widely used in biosensors as ligand molecules, due to their affinity and specificity with the target molecule (antigen), and it is vitally important that they are adsorbed on the sensor with the fragment antigen-binding (Fab) pointing away from the sensor, into the incoming flow containing the antigens.

Figures 14 and 15 present the probability distribution of immunoglobulin G for many orientations (given by $\sigma_{\alpha\beta}$ and $\sigma_{\alpha\alpha}$) varying the surface charge ($\sigma$) and salt concentration ($\kappa$). Figures 14a and 15a show that for low surface charge ($\sigma \pm 0.05\text{C/m}^2$) and high salt concentration ($\kappa = 0.125\text{Å}^{-1}$), there is no clear preferred orientation, to the point that the most likely orientation has a probability of around 10%. This means that adsorbing the antibodies under these conditions would result in a wide range of orientations, which is not favorable for biosensor fabrication.

**Effect of surface charge**— With greater surface charge, in this case $\sigma = \pm 0.2\text{C/m}^2$, the orientation probability distribution gets narrower for positive surface charge, and is maintained for negative surface charge. Figure 15c shows a much clearer preferred orientation, with a probability more than 5× higher for positive surface charge at high salt concentration. For low salt concentrations (Figures 14g and 15g), this effect is even larger.

The results presented on Figures 14 and 15 also show that increasing the surface charge has very little effect on the dipole moment orientation. This is evidence that, in contrast to the case of protein G B1 D4', local interactions dominate over the dipole moment. If the dipole moment were the dominant effect, the dipole moment vector would tend to align to the surface normal as the surface charge increases.

**Effect of salt concentration**— We also varied the Debye length ($\kappa^{-1}$) four-fold. In terms of salt concentration, it means a 16× decrease in the amount of salt.

Like increasing the surface charge, lowering the salt concentration narrows the orientation probability distribution. For $\sigma = \pm 0.05\text{C/m}^2$ (Fig. 14e and Fig. 15e), the effect on positive or negative surface charge is very similar: the preferred orientation is about 2× more likely. However, for $\sigma = \pm 0.2\text{C/m}^2$, the increase is larger with negative surface charge (Fig. 14g), than with positive surface charge (Fig. 15g). The narrower probability distribution is explained by the lower shielding effect caused by the reduced salt content, which at the same time increases the electrostatic interaction.

From the results in Figure 14 and Figure 15, we can conclude that it is easier to control the antibody orientation with low salt concentration and high surface charge, because the orientation probability distribution is the narrowest. In our results, Figures 14b and 15b show the orientation of the antibody at the lowest salt concentration and higher surface charge, but only the orientation in Figure 15a, with positive surface charge, is favorable for biosensing applications, since the Fab fragments are pointing up.

That favorable orientations for biosensing applications are best obtained with high positive surface charge and low salt concentration is consistent with experimental observations by Chen and co-workers. These researchers developed a coarse-grained method known as the united residue model, which qualitatively aligns with our results.

**VII. CONCLUSION**

In this work, we successfully used an implicit-solvent model to study protein orientation near charged surfaces. We present for the first time and apply an extension of our open-source PyGBe code to account for the presence of charged surfaces. The new feature of the code was verified against an analytical solution, which we derived for that purpose.

Using PyGBe, we obtained that protein G B1 D4' behaves like a point dipole near a charged surface, with the dipole-moment vector shifting $\sim 180^\circ$ when the sign of the surface charge flips. Our results compare well with experimental observations and simulations using molecular dynamics, supporting the use of our approach for probing protein orientation near charged surfaces. We applied our approach to immunoglobulin G, a biomolecule that is much larger than protein G B1 (about 125×, by volume) and would be challenging to study via molecular dynamics. Through this study, we realized that this protein is best immobilized on a surface with positive charge, for example with a NH$_4^+$ self-assembled monolayer, using high surface charge and low salt concentration.

We conclude that this implicit-solvent model can offer a valuable approach in biosensor studies. In this application, ligand molecules undergo little conformational change as they adsorb on the sensor surface, and thus the assumption of a rigid structure is valid. In our future work, we intend to use this approach to aid the design of better ligand molecules, by looking at the orientation for different ligand molecule mutants.

**ACKNOWLEDGMENTS**

This work was supported by ONR via grant #N00014-11-1-0356 of the Applied Computational Analysis Program. LAB also acknowledges support from NSF CA-REER award OCI-1149784 and from NVIDIA, Inc. via the CUDA Fellows Program. We are grateful for many helpful conversations with members of the Materials and Sensors Branch of the Naval Research Laboratory, especially Dr. Jeff M. Byers and Dr. Marc Raphael.

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