# Orientation of a Y-shaped biomolecule adsorbed on a charged surface

Yu-Jane Sheng,[1] Heng-Kwong Tsao,[2,*] Jian Zhou,[3] and Shaoyi Jiang[3,†]
[1]*Department of Chemical Engineering, National Taiwan University, Taipei, Taiwan 106, Republic of China*
[2]*Department of Chemical and Materials Engineering, National Central University, Chung-li, Taiwan 320, Republic of China*
[3]*Department of Chemical Engineering, University of Washington, Box 351750, Seattle, Washington 98195-1750*
(Received 20 October 2001; revised manuscript received 29 April 2002; published 23 July 2002)

The adsorption and orientation properties of a Y-shaped biomolecule, which models an immunoglobulin (Ig), on a charged surface are analyzed mesoscopically by Monte Carlo simulations. The orientation is a consequence of the interplay between van der Waals interactions and electrostatic interactions. For adsorption dominated by van der Waals attraction, the molecule prefers lying flat on the surface. For weak attraction, we observe a depletion zone in the concentration profile, which can result in a negative surface excess. A secondary peak is found for strong adsorption. For electrostatically dominated adsorption, the orientation is mainly determined by electric dipole and a vertically adsorbed molecule can be attained as it possesses strong electric dipole. Our study provides an explanation for experimental observations of preferential orientation.

DOI: 10.1103/PhysRevE.66.011911 PACS number(s): 87.15.Aa, 68.43.−h, 87.68.+z

Physical adsorption of immunoglobulins (Ig) onto hydrophobic or hydrophilic surfaces is predominant for the oriented immobilization of antibodies on solid supports, which have been extensively used in diagnostic test systems such as immunoassays [1–3]. Experimental work has been directed toward studying how Ig interacts with solid surfaces on a molecular level regarding its orientation. Although some techniques, such as ellipsometry, reflectometry, and atomic force microscopy [1,3,4], have been developed to directly give the information of Ig orientation upon adsorption to surfaces, a clear understanding has yet to emerge.

The development of theoretical analysis of protein adsorption is primarily along the fronts of detailed molecular models [5] and mesoscopic models [6]. Atomistically detailed simulations are the most realistic and informative approach. Nevertheless, they are computational demanding. One must resort to low surface coverage and do not consider the solvents explicitly. The orientation distribution of protein adsorption ($\sim$20 kDa) has been evaluated [5,7] and the simulations result [7] indicates that the electric moment has a second-order but significant effect on the free energy of adsorption. A thorough exploration of physical factors, such as the net charge, electric dipole, and ionic strength, for the orientation of an antibody molecule ($\sim$150 kDa) adsorbed on the surface is prohibitive by adopting an atomistic model because of the most intensive computational requirement.

On the other hand, mesoscopic approaches avoid detailed molecular descriptions and employ continuum models based on principles of colloidal theories; in particular, the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [8]. The DLVO theory consists of screened Coulomb repulsions and van der Waals attractions. The solvent is represented by a continuum medium of uniform dielectric constant. Within the framework of the DLVO theory, the essential features of protein adsorption have been successfully grasped based on the assumption that the protein is treated as a rigid sphere with its net charge uniformly distributed on the surface or placed in the center [6]. However, this simple model is unable to explain some experimental anomalies, such as adsorption of proteins with a net charge of the same sign as the surface. A compromise may be met by employing a continuum model which takes into account structural characteristics of a protein relevant to protein-surface interactions. For instance, immunoglobulin G (IgG) is traditionally regarded as a Y-shape object. The feasibility of this approach can be assessed, at least qualitatively, by examining its ability to predict the anomalous behavior observed in experiments.

In this paper we adopt a mesoscopic model to depict the adsorption of an antibody. It should be noted that the mesoscopic approach has successfully described the hydrodynamic behavior of an IgG Fab domain by the multiple-sphere array model [9] and the kinetics of antibody-antigen associations by an assembled semisphere model [10]. The basic structure of an intact Ig molecule composes of two heavy chains and two light chains. These polypeptide chains are held together via disulfide bonds. The light chains have one variable domain and one constant domain. The heavy chains have one variable domain and three constant domains for IgG. Consequently, an IgG molecule can be divided into 12 regions, which make up the classical ''Y'' shape. The arms and the base are known as the Fab and Fc fragments, respectively. Accordingly, as shown in Fig. 1, we model an IgG with 12 contact spherical domains. The Fab and Fc parts are represented by 8 and 4 spheres respectively. This choice is consistent with the typical molecular weight ratio of Fab to Fc (2:1). The diameter of each sphere is assigned a value of $d=2a=3$ nm and the angle spanned between two arms is assumed 45°. This approximation gives a molecular volume and an aspect ratio reasonably close to those of an IgG molecule.

Although interactions between neighboring adsorbates may influence surface structures at high surface coverage, interactions between proteins and surfaces play the dominant

*Author to whom correspondence should be addressed. Email address: hktsao@cc.ncu.edu.tw
†Email address: sjiang@u.washington.edu

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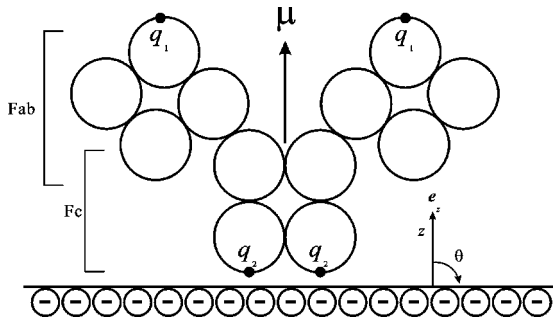


FIG. 1. Schematic of the idealized model system constructed to mimic the adsorption of an antibody onto a charged surface.

role in protein adsorption at low coverage. For models at atomistic levels, protein-surface interaction involves Lennard-Jones interaction and electrostatics. The assumption of pairwise additivity is adopted to calculate the interaction energy. If we treat both antibody and surface as uniform assemblies of similar groups, such as $CH_2$, the interaction can be obtained by integrating the energies of all atoms in one body with all atoms in the other. This process leads to van der Waals attractions (vdW) with a Hamaker constant. Hamaker constants of most condensed phases are found similar even though the media are composed of molecules differing greatly in polarizability and size [11]. In mesoscopic models, the net charge of the mesoscopic object is considered instead of partial charges associated with each atom. In general, the net charge located on the body's surface gives the leading contribution.

Following the underlying, additive assumption, the interaction energy is calculated by summing up vdW ($U_A$) and electrostatic interactions ($U_{el}$) between spheres and surface: $U=U_A+U_{el}+U_{hs}$, where $U_{hs}$ is the excluded volume contribution. vdW is evaluated based on the Hamaker theory [8],

$$U_{A,i}=-\frac{A_i}{6}\left[\frac{a}{r_{z,i}-a}+\frac{a}{r_{z,i}+a}+\ln\left(\frac{r_{z,i}-a}{r_{z,i}+a}\right)\right]. \qquad (1)$$

The typical value of Hamaker constants $A$ for interactions between hydrocarbons is $(1-5)\times10^{-20}$ J. Here $r_{i,z}$ is the vertical distance of the center of the sphere $i$ from the surface. For electrostatic interactions between net charges, the screened Coulombic interaction is used to account for the effects of salt and water solvent. The electrostatic interaction of an assembly of point charges $\{q_ie\}$ with the surface of a uniform density $\sigma e$ can be estimated by [12]

$$U_{el}=\frac{\sigma e^2}{\kappa\varepsilon_r\varepsilon_0}\sum_i q_i\exp(-\kappa r_{i,z}) + \frac{e^2}{8\pi\varepsilon_r\varepsilon_0}\sum_i\sum_j q_iq_j\frac{\exp(-\kappa|\mathbf{r}_i-\mathbf{r}_j^*|)}{|\mathbf{r}_i-\mathbf{r}_j^*|}, \qquad (2)$$

where $\mathbf{r}_j^*$ is the position of the image associated with the charge $q_je$. Equation (2) is obtained by solving the linearized

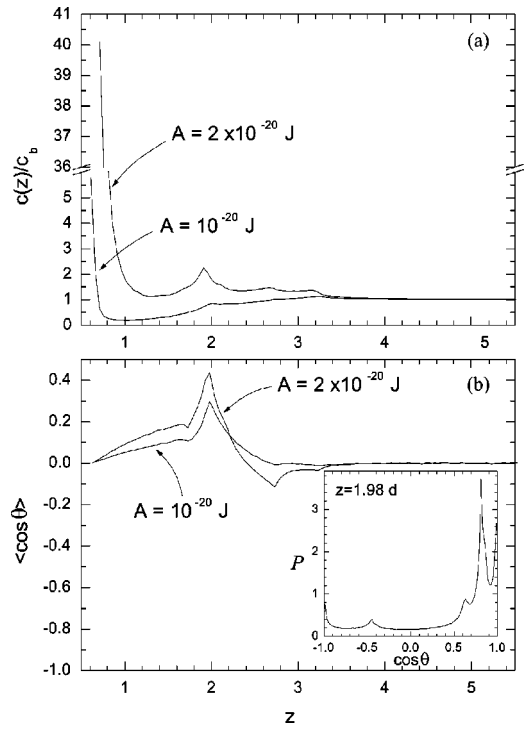


FIG. 2. The effect of van der Waals attractions on distributions of concentration (a) and orientation (b). The probability distribution $P$ of cos $\theta$ at $z=1.98d$ for $A=2\times10^{-20}$ J is shown in the inset.

Poisson-Boltzmann equation. The second term denotes the image contribution and can be neglected in this study. The effects of solvent and ions are reflected through ionic strength (Debye length $\kappa^{-1}$) and dielectric constant ($\varepsilon_r\varepsilon_0$).

The adsorption of an antibody is regarded as a two-body problem (an antibody and a surface) and investigated through Monte Carlo simulations. In the dilute limit, the concentration profile near the surface can be expressed in terms of the effective potential $U_e(z)$, $c(z)/c_b=\exp(-U_e/kT)$, where $c_b$ is the bulk concentration. The effective potential is equivalent to the adsorption free energy for moving an antibody from bulk ($z=\infty$) to a point $z$ [7]. It is related to the ensemble average of the interaction potential at $z$ [13],

$$U_e(z)=-\ln\left\langle\exp\left(-\frac{U(z,\Omega)}{kT}\right)\right\rangle_\Omega. \qquad (3)$$

$\langle\cdot\rangle_\Omega$ denotes ensemble average over all orientations. The antibody at $z$ is rotated randomly at least one million times to compute the average. The random rotation of the antibody is carried out as it does not see the presence of the hard surface. This method is commonly adopted for the second virial coefficient calculation of polymers [13] and can be applied directly to an antibody with realistic molecular structure.

In our simulation the surface charge density is taken to be $\sigma=-0.018\ C/m^2$, which is weaker than the typical value of latex particles. The charge distribution on the antibody is assumed symmetric as shown in Fig. 1. The charges on the top of the arms and on the bottom of the base are $q_1e$ and

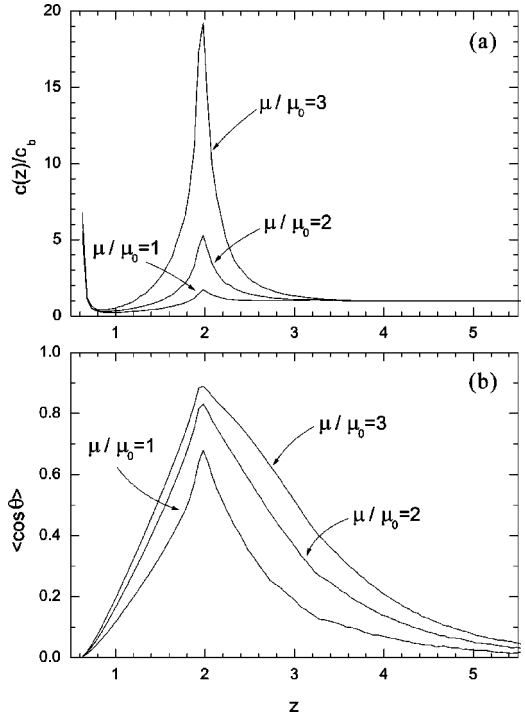


FIG. 3. The effect of electric dipole on distributions of concentration (a) and orientation (b) at $A=10^{-20}$ J. $\mu_0$ denotes electric dipole for $q_1=-q_2=1$.

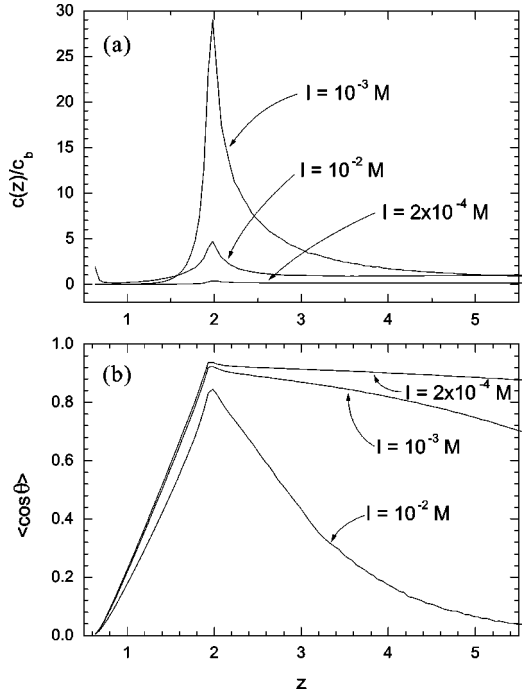


FIG. 4. The influence of net charge on distributions of concentration (a) and orientation (b) at $A=10^{-20}$ J for $q_1=-3$ and $q_2=2$.

$q_2e$, respectively. $|q_i|$ varies from 1 to 3. The ionic strength ranges from 0.001 to 0.1 M. Since the surface is assumed perfectly smooth, protein-surface interactions at close distances cannot be considered. Moreover, to avoid singularity due to physical contact in vdW, a steric exclusion of finite height equivalent to 0.3 nm is included. This assumption is commonly adopted in simulation studies [5,7].

Figure 2 demonstrates the adsorption and orientation properties caused by long-ranged vdW only. The concentration profiles, $c(z)/c_b$, are shown in Fig. 2(a) for $A=1$ and $2\times10^{-20}$ J, respectively. As the distance is within the range of about the height of the antibody, i.e., $\sim 4d$, the attraction becomes significant and $c/c_b$ deviates from unity. The maximum concentration is observed when the center of mass is located at $z\approx0.6d$ for both cases. Note that the center of mass of the antibody is at $z=1.804d$ when it stands on the surface with the Fab fragment pointing toward the bulk. This result implies that the antibody favors to lie flat on the surface when vdW dominate adsorption. In terms of surface excess concentration, $\Gamma=\int_{0.6d}^{\infty}(c-c_b)dz$, one can identify $A=2\times10^{-20}$ J as strong adsorption and $A=10^{-20}$ J as weak adsorption. It is interesting to find that there exists a depletion zone near the surface for weak adsorption. This is because the entropic repulsion due to excluded volume defeats vdW in such a condition. In fact, the surface excess is negative for $A<1.12\times10^{-20}$ J. In other words, such an adsorbent yields an increase in bulk concentrations whereas its surface is occupied with adsorbates. For strong adsorption a second peak is present near $z=1.9d$. This result seems to imply that the vertical ''Y'' orientation may occur. To examine orientation properties, one has to calculate the positional distribution of mean orientation.

The mean orientation at a distance $z$ from the surface is expressed in terms of $\cos\theta$ and evaluated from simulations with a Boltzmann weighting factor. $\theta=0$ corresponds to vertical ''Y'' orientation and $\theta=\pi/2$ represents the antibody lying flat on the surface. Figure 2(b) shows the distribution of mean orientation. At the closest distance, the mean orientation is $\langle\cos\theta\rangle\cong0$. The mean orientation is increased with increasing distance from the surface. It is a consequence of increasing entropy associated with a limiting range of rotation. $\langle\cos\theta\rangle$ reaches a maximum near $z\approx2d$ and then decreases due to unlimited rotation. At the distance where unlimited rotation begins to be possible, vdW may also favor a vertical orientation as shown in Fig. 2(b) inset because of the near contact condition of the antibody base. Far from surfaces, the mean orientation approaches zero due to free rotations in the bulk. The overall mean orientations $\langle\langle\cos\theta\rangle\rangle$ averaged over the range $0.6d\leqslant z\leqslant5.5d$ are 0.024 and 0.002, respectively, for weak and strong adsorption. In accord with Fig. 2(a), one can conclude that for vdW dominated adsorption the antibody prefers lying flat on the surface since this configuration corresponds to the largest attraction. This behavior has been observed on so-called ''hydrophobic'' surface by ellipsometry [4]. Under strong adsorption the probability of observing a vertical orientation is relatively small since its energy barrier $(-U_e)$ is only about 2.5 kT.

When vdW is weak, the adsorption and orientation properties of antibodies are determined by electrostatic interactions, which depend on the charge distribution on the antibody and the ionic strength of the solution $(I)$. The influence

of the charge distribution is manifested through net charge and electric dipole. Consider an antibody without net charge, i.e., $q_1=-q_2$. If we choose the center at the position, $\mathbf{x}_c^q=\Sigma_{i=1}^N \mathbf{x}_i^q/N$, then the electric dipole, defined as $\boldsymbol{\mu}=\Sigma_i q_i(\mathbf{x}_i^q-\mathbf{x}_c^q)$, for $q_1=-1$ is $\mu_0\mathbf{e}_z$. $\mathbf{x}_i^q$ is the charge position. Figure 3(a) shows concentration profiles for various electric dipoles at I=0.01 M with $A=10^{-20}$ J. In addition to the first peak and the depletion zone associated with weak vdW, there exists a second peak at $z\simeq 2d$ due to electrostatic attractions. The probability of staying at this position rises with increasing electric dipole. The effect of electric dipole can be further illustrated from Fig. 3(b), which depicts positional distributions of mean orientation. A nearly vertical orientation is observed at $z\simeq 2d$. The base of the antibody is attracted to the charged surface whereas the arms are repelled from the surface. As a result, the antibody prefers standing vertically.

The effects of net charge and ionic strength are demonstrated in Fig. 4 for $q_1=-3$ and $q_2=2$ with $A=10^{-20}$ J. The experimental anomaly that the adsorption of proteins with a net charge of the same sign as the surface is observed for a range of ionic strength. The antibody is desorbed for lower $I$ but weakly adsorbed for higher $I$. The former is dominated by net charge whereas the latter is controlled by regions of opposite charge. This particular range of $I$ can be assessed by comparing the height of the antibody and the corresponding Debye length, which indicates the effective range of electrostatic interactions. In spite of intricate behavior associated with surface excess, the overall mean orientation increases monotonically with decreasing $I$. As illustrated in Table I, for electrostatically dominated adsorption, the mean orientation is mainly determined by electric dipole though the surface excess is more sensitive to net charge. Note that $\Gamma\propto c_b$ denotes the low coverage limit.

The orientation of adsorbed antibodies was experimentally estimated by antigen binding. An IgG of isoelectric point (pI) 6.9 and its Fab part of pI 8.5 were studied at pH 6–8 [3]. It was observed that the antigen capacity, the mole of bound antigen per mole adsorbed antibody, is absent when the Fab parts are strongly electrostatically attracted by the surface. Evidently, in the experimental range of pH, an IgG possesses an uneven charge distribution, i.e., electric dipole. It is strong enough to yield an orientation with the Fab parts towards the surface, i.e., $\langle\langle\cos\theta\rangle\rangle<0$, which renders the Fab parts inaccessible to antigen binding. With identical Ig coverage, the antigen binding capacity of acidic pretreated IgG was much higher than that of native IgG at pH 7 [2]. Similarly, the immunoreactivity of IgG adsorbed at pH 8 is significantly better than that carried out at pH 4 [14]. In both experiments, pI of antibodies is in the range of pH 6–8 and IgG with higher immunoreactivity displays serious aggregation behavior. The great difference in antigen binding capacity was attributed to different orientation of proteins on the surface. The preferential orientation can be explained by the establishment of stronger electric dipole, which is manifested through the aggregation phenomena and near pI condition.

TABLE I. The effects of charge distributions and ionic strength on surface excess concentration and mean orientation over the region $0.6d\leqslant z\leqslant 5.5d$. The Hamarker constant is $A=1.2\times10^{-20}$ J.

| $q_1$ | $q_2$ | $I$ ($M$) | $\mu/\mu_0$ | $\Gamma$ ($c_b\cdot d$) | $\langle\langle\cos\theta\rangle\rangle$ |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0.769 | 0.019 |
| −1 | 1 | $10^{-2}$ | 1 | 1.017 | 0.147 |
| −2 | 2 | $10^{-2}$ | 2 | 3.252 | 0.310 |
| −3 | 3 | $10^{-2}$ | 3 | 9.988 | 0.527 |
| 0 | 2 | $10^{-2}$ | 1 | 16.977 | 0.108 |
| 0 | 3 | $10^{-2}$ | 1.5 | 54.548 | 0.136 |
| −2 | 0 | $10^{-2}$ | 1 | −1.754 | 0.118 |
| −3 | 0 | $10^{-2}$ | 1.5 | −2.223 | 0.156 |
| −2 | 3 | $10^{-1}$ | 2.5 | 1.006 | 0.046 |
| −2 | 3 | $10^{-2}$ | 2.5 | 14.596 | 0.414 |
| −2 | 3 | $10^{-3}$ | 2.5 | $3.86\times10^{4}$ | 0.875 |
| −3 | 2 | $10^{-2}$ | 2.5 | 1.356 | 0.396 |
| −3 | 2 | $10^{-3}$ | 2.5 | 14.419 | 0.870 |
| −3 | 2 | $10^{-4}$ | 2.5 | −4.940 | 0.913 |
| 2 | −2 | $5\times10^{-3}$ | −2 | 5.951 | −0.389 |
| 3 | −2 | $5\times10^{-3}$ | −2.5 | 37.866 | −0.429 |
| 3 | −3 | $5\times10^{-3}$ | −3 | 21.813 | −0.629 |

In this paper several aspects are not considered. The hydrophobic interaction and hydration force play important roles in biointerface phenomena [11]. They involve water molecules and decay exponentially with distance with a decay length of about 1 nm or less. The electric dipole may exist in both Fab and Fc domains since they can differ in pI. The adsorption in experiments often involve high surface coverage. The present approach can be straightforwardly refined to take into those aforementioned effects in a mesoscopic manner.

H.-K.T. acknowledges support from NSC of Taiwan and S.J. thanks support from DARPA.

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