Biomarker Binding on an Antibody-Functionalized Biosensor Surface: The Influence of Surface Properties, Electric Field, and Coating Density

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ABSTRACT: Antibodies have been used as bioreceptors in biodiagnostic devices for decades, the performances of which are affected by various factors, such as orientation, density, and local environment. While there are extensive works on design and fabrication of various biosensors, little is known about the molecular level interactions between antibodies coated on sensor surfaces and biomarkers suspended in medium. In this work, a coarse-grained model for biomarkers binding on an antibody-functionalized biosensor surface is constructed to study the effects of surface properties and external parameters on antibody orientation and biomarkers binding time. The surface interaction type is found to significantly influence the antibody orientation and biomarker binding time. A proper electric field range is discovered to not only well-orient antibodies but also steer biomarkers toward the surface, consequently reducing the binding time of biomarkers by 2 orders of magnitude. Moreover, a suitable surface coating density of antibodies has been proposed to help antibody orientation as well as biomarker binding. These findings can be used for rational design of biosensors with higher efficiency and more sensitive detections.

INTRODUCTION

Biosensors are effective tools for early diagnosis of diseases and biochemistry analysis.1 After several decades of efforts, current biosensors have become more miniaturized, portable, effective, and sensitive than before. Despite the significant progress achieved in biosensor development, the molecular-level understanding of the biomarker binding process is still limited, which has hindered the further improvements of biosensors.

Various techniques such as electrical, mechanical, magnetic, and optical approaches have been applied to achieve better performance of biosensors.2 The electric field has been widely used as an assistant in biosensor design by improving the selection efficiency.2 The effects of electrostatic interactions on the association process of monoclonal antibodies (Abs) have been studied and compared with experimental results.2,3 The ionic concentration4 and dipole moment of Abs5 which would cause electrostatic forces have been claimed to be able to control the orientation of adsorbed Abs. It has also been verified6 that the affinity and orientation of Abs under the dominance of electrostatic forces are different from those under the dominance of van der Waals interactions. Although the significance of electrostatic effects has been demonstrated7−9 by researchers from different aspects, its effects on the motion of biomolecules are not yet fully understood. To reveal the binding process of biomarkers-Abs10−14 and explore possible contributions of electrokinetics on biosensing, a multiphysics computational model at the molecular level is needed.

The surface density of Abs is another important factor in the biomarker binding process. The monolayer coverage has been experimentally verified to considerably influence the Abs
With a reasonable high surface density, Abs are more feasible to capture biomarkers.\(^{18}\) It is also observed that extremely high surface density may screen it from binding targets. For example, it is found that Abs are more likely to congregate with each other, which is harmful for Abs-antigen binding, at extremely high concentrations.\(^{19,20}\) Therefore, an optimal surface density of Abs should be chosen to achieve the best possible biosensing performance, which calls for a clear understanding of effects of Abs surface coating density on Ab orientation and biomarker binding efficiency. It has also been proven that Ab orientations can be controlled by density on Abs orientation and biomarker binding to achieve the best possible biosensing performance, which calls for an optimal surface density of Abs should be chosen to achieve the best possible biosensing performance, which calls for a clear understanding of effects of Abs surface coating density on Ab orientation and biomarker binding efficiency. It has also been proven that Ab orientations can be controlled by density on Abs orientation and biomarker binding efficiency. It has also been proven that Ab orientations can be controlled by density on Abs orientation and biomarker binding efficiency.

This work aims to develop a multiphysics model to understand the biomarkers binding process on Ab-coated surface at the molecular level. Usually, Molecular dynamics (MD) is used to capture the detailed dynamics of biomolecules interactions. Given the length (micrometers or bigger) and the time scale (microseconds or longer) of our system, pure MD simulations are not feasible because the atomistic molecular dynamics modeling is limited to simulations on the nanometer and nanosecond scale. Thus, a coarse-grained (CG) MD\(^{24}\) method is proposed to model the interaction of Abs-functionalized surface with biomarkers because it not only accesses this time and length scale, but also reduces the computational cost.\(^{25}\)

**METHODS**

**CG Topology.** The CG biosensing system was constructed as a self-assembled monolayer (SAM) coated on Si substrate in a field effect transistor (FET) based biosensor, similar to that in Tian et al.\(^{26}\) While bio-FET was chosen as a typical biosensor in this work, it should be noted that this study of biomarkers binding could be applicable to all kinds of Abs-functionalized sensors.

The substrate with a size of \(50 \times 50 \text{ nm}^2\) was made up of 1824 CG Si\(^{27}\) beads. Each of CG Si beads was mapped from 121 all-atom Si atoms. Alkanethiols were chosen as the SAM molecules, each of which consisted of an alkyl chain as the tail group, a \((-\text{C}=-\text{C}-)_n\) chain as the backbone and a \(-\text{S}-\text{H}\) chain as the headgroup.\(^{28}\) In the coarse-graining process, each alkyl chain was mapped into one bead that was attractive to the substrate; each \((-\text{C}=-\text{C}-)_n\) group was mapped into one bead which was neutral to other molecules; besides, the \(-\text{S}-\text{H}\) groups were mapped into another kind of beads which attached to Abs tightly.\(^{8}\) The SAM length can be adjusted by changing the number of neutral beads.

Immunoglobulin Gs (IgGs) were used as bioreceptors due to their innate high specificity and versatility.\(^{29–36}\) In biosensor design. The IgG contains two light and two heavy chains which are linked by disulfide bonds.\(^{19}\) As shown in Figure 1(a), each heavy chain is constructed by three constant domains denoted as \(\text{C}_{\text{H}1}, \text{C}_{\text{H}2},\) and \(\text{C}_{\text{H}3}\) and a variable domain as \(\text{V}_{\text{H}}\) on the other hand, each light chain consists of one constant domain \(\text{C}_{\text{L}}\) and one variable domain \(\text{V}_{\text{L}}\). Consequently, most of CG models of Abs are formed as a characteristic Y-shaped configuration with 12 parts. The CG model of IgG1 was built up referring to the 12-bead colloidal model of Abs created by Zhou et al.\(^{38}\) and the Elastic Network Normal-Mode analysis constructed by Chaudhri et al.\(^{19,20}\) The all-atom model of the IgG1 molecule was downloaded from the RCSB data bank and was mapped into a 12-bead CG model in which each bead represented one domain. The CG Ab model was treated as symmetric, as shown in Figure 1(b). The total charge of the CG Ab model was neutral, but it had a dipole moment due to its nonuniform internal charge distribution. The dipole moment of Abs was calculated by the Protein Dipole Moment server.\(^{19}\) The mass, charge, and indicated domain of each bead are shown in Table 1. Furthermore, other topological information such as atom types, bonds, angles, and dihedrals are plotted in Figure 1.

![Figure 1. (a) All-atom model of Ab; (b) Detailed topological information on CG Ab model (12 atoms, 4 atom types; 16 bonds, 5 bond types; 16 angles, 5 angle types; 5 dihedrals, and 5 dihedral types). It should be noted that atoms with the same color do not mean they are the same atom type. (c) CG model of Abs.](image-url)

<table>
<thead>
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<th>bead</th>
<th>mass</th>
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<th>indicated domain</th>
</tr>
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<tr>
<td>1,2</td>
<td>12 530</td>
<td>-1</td>
<td>(\text{C}_{\text{H}3})</td>
</tr>
<tr>
<td>3,4</td>
<td>11 936</td>
<td>0</td>
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<td>5,9</td>
<td>11 030</td>
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<td>8,12</td>
<td>10 141</td>
<td>0</td>
<td>(\text{C}_{\text{H}1})</td>
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</tbody>
</table>

Following the same coarse-graining method, the all-atom model of the biomarker\(^{39}\) was mapped into an arrow-shaped CG model using the residue-based CG method, as shown in Figure 2. Four strong binding sites were placed on the head of the biomarker, while two weak binding sites were located on its tail.

After Abs were immobilized on the substrate surface by SAMs, biomarkers were released from a height of 10 nm above Abs. The established CG system for biomarkers binding on Abs-functionalized surface is shown in Figure 3. It should be
noted that the density of SAMs is usually 100 times higher than that of Abs in real situation. However, modeling all free SAMs was not necessary and would dramatically increase the computational cost in our simulations. Thus, only those SAMs connected with Abs were modeled. The motion of each SAM was constrained in a certain zone, which was calculated out according to the surface coating density of SAMs.

CG Potential. The coarse-grained force field is the sum of intermolecular and intramolecular interactions, which can be written as follows:

\[ U_{\text{total}} = U_{\text{inter}} + U_{\text{intra}} \]  

The intermolecular interactions consist of electrostatic and van der Waals interactions. The electrostatic interaction is expressed in Debye form, while the van Der Waals is represented by the Lennard–Jones (LJ) potential:

\[ U_{\text{inter}} = U_{\text{coul}} + U_{\text{lj}} = \frac{q_i q_j}{4\pi\varepsilon_r r} \exp(-kr) + 4\varepsilon_r \left[ \left( \frac{\sigma_{ij}}{r} \right)^{12} - \left( \frac{\sigma_{ij}}{r} \right)^{6} \right] \]  

where \( q_i \) and \( q_j \) represent the net charges on CG sites, \( \varepsilon_r \) indicates the effective dielectric constant, while \( k \) is the Debye screening parameter. \( \sigma_{ij} \) is the well depth for \( ij \)th pair of CG sites, and \( \sigma_{ij} \) is the finite distance at which the interparticle potential for \( ij \)th pair is zero. Determining the value of dielectric constant for protein solutions has been challenging researchers for long. Although a lot of work has been done on evaluating the effective dielectric constant in the presence of explicit/implicit water, no consensus has been reached on how to calculate it within and between two protein molecules. In the current study, the values for proteins and solvents are just fixed at the same with that of Chaudhri et al. The exact value of the effective dielectric constant will be studied along with flexible protein models in the future.

The bond, angle, and dihedral potentials constitute the intramolecular interaction, all of which are defined based on harmonic approximations of interaction strengths:

\[ U_{\text{intra}} = U_{\text{bond}} + U_{\text{angle}} + U_{\text{dihedral}} \]

\[ = \kappa_{\text{bond}}(r - r_0)^2 + \kappa_{\text{angle}}(\theta - \theta_0)^2 + \kappa_{\text{UB}}(r - r_{\text{UB}})^2 + \kappa_{\text{dihedral}}[1 + \cos(\varphi - d)] \]  

where \( \kappa_{\text{bond}}, \kappa_{\text{angle}}, \kappa_{\text{UB}}, \kappa_{\text{dihedral}} \) refer to the spring constants for bond, angle, Urey–Bradley (UB) and dihedral terms, respectively. \( r_0, \theta_0, r_{\text{UB}} \) and \( d \) are equilibrium bond, angle, UB, and dihedral terms, respectively.

All of intramolecule potential data for Abs were extracted from ref 19, while the rest were obtained by applying the Energy Minimization (EM) method. The bond and angle potential parameters were determined following the work of Brandt et al.
Validation of CG Model. To ensure the CG model accurately described the molecular motion, a benchmark case was performed by comparing diffusion coefficients (Ds) of Abs and biomarkers obtained from CG MD results and all-atom results. At 293 K, Abs and biomarkers were released in a fluidic box with periodic boundaries. Their diffusion processes were simulated by both the all-atom MD and CG model. The effects of solvent were represented by the Langevin thermostat.

The Ds of Abs and biomarkers were calculated by the simulation results. Based on Einstein’s theory, the displacement of a Brownian particle is proportional to the square root of the elapsed time.\(^{46}\)

\[
\langle |\Delta r|^2 \rangle = 6D t
\]

where \(\langle \cdot \rangle\) means the time average, \(r\) is the position vector of Abs/biomarkers, \(t\) stands for time, and \(D\) for the diffusion coefficient. Given \(r\) and \(t\), the diffusion coefficient can be calculated.

The Ds of Abs and biomarkers, calculated from both all-atom model and CG model, are listed in Table 2. Referring to the measured value of Abs by Saltzman et al.\(^ {37}\) both all-atom and CG results for the Ab are within the suggested range, indicating good agreement with the experimental results. The Ds of biomarkers from two models were also similar, and agrees with the clinical data.\(^ {38}\) This benchmark case illustrated that our CG system have captured the essential diffusion motion of Abs and biomarkers.

Table 2. Diffusion Coefficients Calculated from Two Models

<table>
<thead>
<tr>
<th>diffusion coefficient</th>
<th>Ab (10(^{-7}) cm(^2)/s)</th>
<th>biomarker (10(^{-7}) cm(^2)/s)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>calculated value</td>
<td>cited value(^ {37})</td>
</tr>
<tr>
<td>all-atom model</td>
<td>4.421</td>
<td>4.4 ± 1.3</td>
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<tr>
<td>coarse-grained model</td>
<td>4.852</td>
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Our coarse-grained model can also be validated by comparing the calculated Gibbs free energy (\(\Delta G\)) differences of antibody-biomarker complexes. Following the work of Novotny et al.,\(^ {49}\) \(\Delta G\) of an antibody–biomarker complex has been calculated based on both full-atomistic and coarse-grained models. \(\Delta G\) of antibody–biomarker complex formation in the coarse-grained model was \(-11.0\) kcal while it was \(-6.8\) kcal in the full-atomistic model. Overall, the \(\Delta G\) difference of 4.2 kcal between the two models was within the acceptable range (difference in the range of \(-9\) ± 3 kcal\(^ {39}\) is reported in Novotny’s work. From these results, our coarse-grained model underestimated the strength of antibody–biomarker complex. This underestimation will be studied in the future through further refinement in the coarse-grained model.

Orientation factor (\(\alpha\)), is derived to describe the orientation of Abs, following a similar definition used by Zhou et al.\(^ {50}\) In our model, the orientation factor is defined as the cosine value of the offsetting angle (\(\theta\)) of Abs from the normal direction of the substrate surface, as shown in Figure 4. A larger \(\alpha\) indicates a better orientation of Abs which represents a higher possibility of binding biomarkers.

The CG MD simulation was run by the LAMMPS\(^ {51}\) package. The time step for the simulation was 0.01 ps. The simulation process was continued until one biomarker bound with Abs. The temperature of the system was 293 K. The dielectric constant for the solution was 20.0, while it was 1.0 for biomolecules.\(^ {19}\) The NVE integration was applied to the system with Langevin dynamics representing the Brownian motion of fluid. Molecules interacted with each other by van der Waals and Coulombic forces. Hydrophobic effects of solvent to rigid CG structures of Abs and biomarkers have been excluded\(^ {19}\) because the solvent is not a necessity in coarse-grained molecular dynamics simulations.\(^ {52}\)

RESULTS AND DISCUSSIONS

The binding processes of biomarkers under different physical conditions were simulated with the developed CG MD model. A typical Ab-biomarker binding process is shown in Figure 5.

In what follows, we will discuss the effects of different parameters on Ab orientation and biomarker binding time. Effect of Surface Properties. In order to understand how surface interaction types between Abs and substrates influence the Abs orientation and biomarker binding, various interaction types ranging from strong attractions to strong repulsions are studied. The attraction types represent the effects of hydrophilic interactions, while the repulsion types represent the effects of hydrophobic interactions.\(^ {53}\) When the surface interaction type is set as attractive, the substrate attracts all SAMs, Abs and biomarkers; whereas, when the surface interaction type is defined as repulsive, the surface is expected to repel those molecules; thereafter, when the surface interaction type is defined as neutral, the surface neither attracts nor repels them. Different interaction types are achieved by adjusting the sigma (\(\sigma\)), epsilon (\(\epsilon\)) and cutoff distance (\(r_c\)) of LJ potentials between the substrate and other molecules. Potential parameters of the substrate under different surface interaction types are listed in Table 3.

The SAM length was set as 0.8 nm while the surface coating density was fixed at 1600/\(\mu\)m\(^2\) in this set of simulations. As shown in Figure 6(a), orientation factors of Abs varies from 0.58 to 0.87 if no electric field is applied. When the electric field is applied, Abs are found to be tightly laid on the surface, referred to the "side-on" as mentioned.\(^ {46}\) When a weak attraction is applied, Abs can easily detach from the substrate after absorption, different from the strong attachment observed under strong attractions. The neutral interaction was applied by setting \(\epsilon\) between the substrate and other molecules as zero along with a reflection wall on the surface of the substrate. Moving molecules would bounce back once they contact the
reflection wall. When the surface interaction type is neutral, Abs oscillate around 30° with vertical axis, resulting in an orientation factor around 0.73. However, when the interaction is repulsive, the orientation factor is above 0.85, implying that the repulsive interaction is more beneficial for the Abs orientations than the neutral or attractive interaction states. With an electric field applied, the orientation factors can be kept above 0.90, under any interaction types.

Figure 5. A typical biomarker binding process, snapshots are taken at 0, 120, 240, 360, 480, 600 ns, respectively. The simulation was run with Ab coating density of 1600/μm², SAM length of 3.2 nm, and electric field strength of 0.1 V/m.

Table 3. Potential Parameters between the Substrate and Other Molecules

<table>
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<tr>
<th>interaction type</th>
<th>σ (Å)</th>
<th>ε (kcal/mol)</th>
<th>r_c (Å)</th>
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<td>1</td>
<td>20.4</td>
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<tr>
<td>weak attraction</td>
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</tr>
<tr>
<td>neutral</td>
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<td>0</td>
<td>6.8</td>
</tr>
<tr>
<td>weak repulsion</td>
<td>6.8</td>
<td>0.5</td>
<td>6.8</td>
</tr>
<tr>
<td>strong repulsion</td>
<td>6.8</td>
<td>1</td>
<td>6.8</td>
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</table>

Figure 6. (a) Orientation factors under different surface interaction types: SA, strong attraction; WA, weak attraction; N, neutral; WR, weak repulsion; and SR, strong repulsion. (b) Biomarker binding time under different surface interaction types.
orientation because the surface interaction type not only affects the behavior of Abs, but also impacts the motion of biomarkers. From Figure 6(b), the weak attraction is demonstrated to be most efficient for biomarkers binding. Without an electric field, the biomarker binding time is 12.67 $\mu$s under the weak attraction, while it is larger than 14.50 $\mu$s under any other interaction types. A strong attraction may trap Abs onto the surface permanently, and decrease the chance of binding Abs which would not happen under weak attractions. The repulsive substrate offers better orientation conditions for Abs, but also repels biomarkers away when they are close to the surface. According to these results, the weak attraction is the most effective interaction type for the binding of biomarkers. The same trend is also observed when an electric field is utilized,

Figure 7. (a) Time history of Abs orientation factors under different conditions. The blue solid line is the time history of Ab orientation factors with an electric field strength of 0.1 V/m and SAM length of 0.8 nm; the black solid line is the time history of Ab orientation factors with an electric field strength of 0.1 V/m and SAM length of 3.2 nm; the red dashed line is the time history of Ab orientation factors without an electric field and SAM length of 0.8 nm; and the green dashed line is time history of Ab orientation factors without an electric field and SAM length of 3.2 nm. (b) A typical snapshot of Abs orientation without an electric field. (c) A typical snapshot of Abs orientation with an electric field.

Figure 8. (a) Abs orientations under different electric field strengths and different SAM lengths. (b) Biomarker binding times under different electric field strengths and different SAM lengths.
where the binding time with weak attractive substrate is the smallest among those five substrate interaction types.

On the basis of the fact that the weak attraction between the substrate and biomolecules helps the binding process of biomarkers most, the following results are all obtained from a weak attractive substrate. In the future, the detailed study of hydrophobic effects will be included in a more precise model with flexible protein structures.

**Effects of Electric Field Strength.** To better understand how an electric field influences Abs orientation and biomarkers binding efficiency, electric field with different strengths were applied to the system. The value of the electric field strength was set as 0, 0.01, 0.05, 0.1, and 0.2 V/m, according to the typical range used in biosensors. A higher value of 0.5 V/m beyond the normal range was also included for comparison purposes. This set of simulations were carried out under the surface coating density of 1600/μm² and the SAM length of 0.8 and 3.2 nm, respectively.

Figure 7 demonstrates how the electric field affects the orientation of Abs. The time histories of Ab orientation factors under various conditions are plotted in Figure 7(a). Abs fluctuate locally with large angles when there is no electric field. With an electric field, the offsetting angles of Abs can be controlled within 20°, which indicates that the orientations of Abs are well constrained. Figure 7, parts (b) and (c), shows schematics of Ab orientations under different electric field conditions. It is shown that Abs may bend and tilt without an electric field, while they are well-orientated and able to bind biomarkers faster under an electric field.

The variation of Ab orientation factors under different electric field strengths and SAM lengths are shown in Figure 8(a). The electric field has little impact on Abs orientations if its strength is smaller than 0.05 V/m; however, when it increases from 0.05 V/m to 0.2 V/m, the Ab orientation factor will increase dramatically; after its strength goes above 0.2 V/m, further rising of the electric field strength will no longer influence the orientation of Abs apparently. It is also shown that shorter SAMs are more beneficial for Abs orientations because a longer SAM will make the Abs more prone to fluctuations. However, the influence of SAM length on Abs orientation is much less dominant compared to that of electric field.

Effects of the electric field on biomarkers binding time are shown in Figure 8(b). The application of an electric field is shown to reduce the biomarker binding time by 2 orders of magnitude, from μs to ns. The biomarker binding time varies from 10 to 100 μs without an electric field, while it is less than one μs with an electric field strength of 0.1 V/m. Moreover, the correlation between electric field strengths and biomarkers binding time is nonlinear. When the field strength is smaller than 0.05 V/m, the electric field is weak and consequently has little impact on the biomarker binding time. Field strengths exceeding 0.05 V/m lead to a dramatic reduction in binding time; however, further increase of the electric field strength above 0.2 V/m will no longer reduce the binding time apparently.

**Effects of Surface Coating Density.** The vital role of surface coating density in biosensor efficiency has been verified by many experimental studies, while our aim is to get a better understanding of the quantitative relationship between the coating density and biomarkers binding time. The coating density is adjusted by changing the number of Abs that are uniformly distributed per square micrometer on the substrate surface. In this set of simulations, the coating densities were selected as 100/μm², 625/μm², 1111/μm², 1600/μm², 3086/μm², and 6400/μm², with an electric field strength fixed at 0.1 V/m and a constant SAM length of 0.8 nm. The effects of coating density on Ab orientation factors and biomarker binding times are illustrated in Figure 9.

The surface coating density affects the orientation of Abs nonlinearly. As shown in Figure 9(a), a higher surface coating density is more helpful for Abs orientation. When the surface coating density of Abs is 6400/μm², under which condition Abs have covered the whole surface without overlapping, the Abs orientation factor is maintained at 0.97 even without an electric field. With smaller surface coating density, Abs have more free space to move, which results in a larger variation in orientations. When the surface coating density is smaller than 2000/μm², the Abs orientation factor decreases almost linearly with the coating density. For extremely small surface coating densities without an electric field, the Ab orientation factor can decrease to 0.72. However, with the assistance of an electric field, the Abs orientation factor can be controlled above 0.94 no matter how small the coating density is.

Figure 9(b) illustrates that the surface coating density can largely decrease the binding time of biomarkers: increasing the surface coating density from 100/μm² to 6400/μm² can reduce the biomarker binding time from ms to μs even without an electric field; a higher surface coating density leads to a shorter binding process of biomarkers. It should be noted that these results are obtained under the situation where Abs are never dense enough to block each other. If the surface coating density...

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*Figure 9.* (a) Effects of surface coating density on Abs orientation factor. (b) Effects of surface coating density on biomarkers binding time.
is so high that Abs are blocked by each other, then the binding capabilities of this surface would be reduced. Under this condition, a higher surface coating density is not beneficial for the binding of biomarkers any more. But this strategy is still widely used in the biosensor application because it can prevent Abs from being washed away by the fluidic flow.

**CONCLUSIONS**

A CG MD model has been developed to study the effects of surface interaction type, electric field strength, and surface coating density on the biomarker binding process. The CG multiphysics computational model has been validated by the fact that CG model results were consistent with full-atomic results when simulating the diffusion process of Abs and biomarkers in the stationary fluid. It should be noted that this is an initial study without testing the convergence of the model, a more refined model will be established in future investigations.

The surface interaction type can affect behaviors of both Abs and biomarkers. With the same physical condition settings, the strong repulsive substrate offers the best orientations for Abs, while the strong attraction makes the orientations of Abs less favorable for biomarker bindings. However, the weak attraction between the substrate and other molecules is the most effective for the binding process when compared with the strong attraction, neutral, and repulsions.

Meanwhile, the electric field is capable of well-orientating Abs and guiding biomarkers toward the substrate, which consequently reduces the binding time and enhances the efficiency. The influence of the electric field is negligible when its strength is smaller than 0.05 V/m. A strength ranging from 0.05 to 0.2 V/m, is found to dramatically reduce the binding time of near-surface biomarkers. Further ascending the electric field strength when it is already above 0.2 V/m will not noticeably cause extra decrease of the biomarker binding time.

Lastly, a higher surface coating density of Abs is favorable for the efficiency of the biosensor system. The binding time changes exponentially with the surface coating density and reduces nearly by 2 orders of magnitude when the coating density increases from 100/μm² to 6400/μm². A denser surface coating can ensure a satisfying orientation of Abs in comparison to sparser ones that do not affect the orientation of Abs at all.

In conclusion, weak substrate—bimolecule attraction, medium electric field strength, and high surface coating density are found to be most effective for fast biomarker binding. The results of this work provide an understanding of the biomarker binding process at a molecular level and guidance to improve biosensor performance.

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**Notes**

The authors declare no competing financial interest.

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