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Effects of external electric fields on lysozyme adsorption by molecular dynamics simulations

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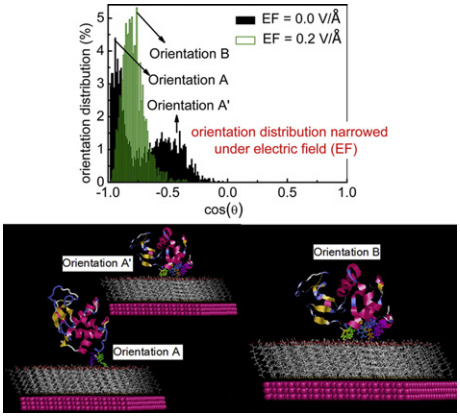
HIGHLIGHTS

- Protein adsorption could generally be promoted by positive electric fields and retarded by negative electric fields.
- Migration of counterions onto surfaces plays a role in lysozyme adsorption under external electric fields.
- An applied electric field may narrow the protein orientation distribution.
- Structural deformation of lysozyme does not increase monotonically with the increasing electric field strength.

GRAPHICAL ABSTRACT



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ABSTRACT

Lysozyme adsorption on carboxyl-terminated self-assembled monolayers under external electric fields has been studied by all-atom molecular dynamics simulations. Lysozyme adsorption on negatively charged surfaces could generally be enhanced by positive electric fields and retarded by negative ones. Under positive electric fields, electrostatic interactions between protein and surface are strengthened; however, the interaction energy descends with field strength increases probably due to the co-adsorption of counterions onto the surface to neutralize surface charge. Comparison of orientation distributions of lysozyme adsorption on the surface in the presence and in the absence of electric fields reveals that an applied electric field could narrow the distribution and therefore helps to immobilize protein on surface with uniform orientation. Orientation angle analysis shows that lysozyme is adsorbed on the surface with “bottom end-on”, “side-on”, “back-on” or “top end-on” orientation under different field strengths, suggesting the possibility of controlling the preferred orientation of lysozyme on surface by applying electric fields. Conformation analysis of protein implies that the structure deformation of adsorbed lysozyme does not increase monotonically with the rising field strength. Under some field strengths, there is no additional structure deformation caused by the electric fields compared with that in the absence of electric fields; while under some other field strengths, there are larger conformational change occurrences. We propose that due to the rearrangement of positions of the local atomic charges of protein to couple its dipole with an external electric field, large position alterations of atoms might

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be avoided and thus conformational changes be restricted. This work may provide guidance for controlling protein adsorption behaviors via external electric fields for applications of protein immobilization and anti-fouling surfaces.

1. Introduction

Protein adsorption is a common process occurring in many biological phenomena [1] and biotechnological applications [2–9]. In some applications such as biosensors, biocatalysis and drug delivery, protein is desired to be attached on surfaces with proper orientation and retained conformation. However, in other applications such as bioseparation, contact lens and anti-fouling coatings, protein deposition may become a major source of contamination. The understanding of protein adsorption amount, orientation and conformation on surfaces will benefit the related biotechnological applications.

In the protein–surface–solution system, protein adsorption behavior is affected by both internal factors [10] (properties of protein, surface and solvent) and external factors [11] (light, thermal, electric field and magnetic field). In the last decades, protein adsorption has been widely studied by theoretical, experimental, and computer simulation approaches [12–27] to understand, predict and ultimately control protein behavior on surfaces. Currently, most studies concentrate on controlling protein behavior by internal factors, such as modifying protein properties [28], designing novel surface materials [17] or modulating solvent microenvironment [29]. However, comparing with internal factors, controlling protein behavior via external factors is superior in convenience and flexibility. An external stimulus could be applied to a given system without modifying properties of components in the system. The direction and/or strength of an external stimulus could be conveniently optimized for different systems in specific applications. Furthermore, an external stimulus applied to surface may be employed to control interfacial properties [30–32], and therefore indirectly control protein behavior at interfaces. Among these external factors, the applied electric field offers a promising means to control the spatial homogeneity, mean orientation and growth rate of protein monolayers [33], since most proteins will align and migrate in response to an electric potential gradient because of its net charge and permanent dipole.

Experimental studies have been carried out to understand the effect of an electric field on protein adsorption behavior and to explore the possibilities to control protein adsorption via an external electric field. Available experimental methods include standard immunoassay [34], measurement of electrical double layer capacitance [35], quartz crystal vibrational analysis (QCV) [36], optical reflectometry [37], optical waveguide lightmode spectroscopy (OWLS) [38] and surface-enhanced resonance Raman spectroscopy (SERRS) [39], etc. Though these methods could provide useful information of protein adsorption under an applied potential, difficulties often encountered in simultaneously measuring adsorption and applying electric fields due to the limitation of measurement techniques [33,37]. Partly because of this, studies on the effects of an applied electric field on protein adsorption behavior are limited and some phenomena are still not well understood. For example, the effect of an external electric field is found to be negligible in some experiments [40] but to be significant in other experiments [33]. Moreover, most experimental studies focus on the protein adsorption kinetics under an applied electric field, while relatively few on the detailed orientation and conformation of adsorbed protein on surfaces. Therefore, people are still far from a full understanding of protein behavior on surfaces under external electric fields, especially at the molecular level.

Molecular simulations are well suited for studying protein adsorption behavior on surfaces and providing molecular-level information [41]. Though molecular simulation method has been used to investigate how an external electric field affects the conformational change of protein in solution [42,43], to the best of our knowledge, it has not been applied to study protein adsorption behavior on surfaces under applied electric fields.

In this work, all-atom molecular dynamics (MD) simulations are used to investigate the effect of external electric fields on protein adsorption behavior on charged surfaces, with the interest in a fundamental understanding of protein adsorption behavior under electric fields at the molecular level and ultimately, a practical possibility of applying external electric fields in controlling protein behavior on surfaces for biotechnological applications.

2. Simulation details

2.1. Simulation models

Lysozyme is often used as a model protein to study adsorption behavior on surfaces since its structure, dynamics, and folding have been extensively studied by a wide range of experimental and theoretical techniques [44]. The X-ray crystal structure of lysozyme (Protein Data Bank entry code: 1HEL) served as the starting configuration of the simulations. Hydrogen atoms were added by CHARMM package [45,46]. Simulations were performed at neutral conditions. The arginine (Arg) and lysine (Lys) residues were taken to be protonated whereas glutamic acid (Glu) and aspartic acid (Asp) residues were taken to be deprotonated; histidine (His) residues were treated as the neutral protonation state (HSD). Four disulfide bonds were added. The N-terminus and the C-terminus were assigned a charge state of $+1e$ and $-1e$, respectively. The protein has a total of 1968 atoms and a net charge of $+8e$.

In our previous work, we found that lysozyme could easily be adsorbed on negatively charged surface with proper orientation [21]. Therefore, self-assembled monolayer with carboxyl group partly deprotonated (COOH-SAM) was adopted in this work. The construction of surface follows Ref. [41]. The $\left(\sqrt{3}\times\sqrt{3}\right)R30^\circ$ structure of $S(CH_2)_9COOH$ self-assembled monolayer (COOH-SAM) surface on Au(111) built using CHARMM package was employed to study protein adsorption. The COOH-SAM is consisted of 168 thiol chains densely packed in a 12×14 array and 1872 gold atoms packed in three layers. Ten thiol chains were deprotonated to represent a surface charge density of $-0.04\ C\ m^{-2}$. The COOH-SAM has the dimension of 59.94 Å × 60.65 Å.

2.2. Simulation methods

We followed a two-step protocol proposed in previous literatures [41,47], i.e., MC simulations were first performed to determine the preferred orientation of protein on surfaces. Then with this orientation as initial configuration, MD simulations were carried out to further study the detailed protein behavior on surfaces. For MC simulations, lysozyme was initially placed at 5 nm above surfaces with a random orientation. The protein was translated and rotated around its center of mass with the acceptance ratio of 0.5 using the Metropolis criteria. During simulations, the protein was modeled as a rigid molecule; the SAMs were fixed in the xy plane; and water was treated as an implicit solvent continuum model. Each simulation was carried out for two million MC steps.

For MD simulations, protein with the preferred configurations obtained from MC simulations was put 1 nm above the surface. Then TIP3 water molecules were added to a simulation box of

59.94 Å × 60.56 Å × 60.65 Å. Any water molecule within 2.8 Å of the protein or SAMs was removed. Counterions, 8 chlorine ions and 10 sodium ions were added to the simulation box to neutralize the simulated system, with the ionic strength corresponding to 0.068 M. To keep water molecules in the simulation box, a weak planar restraint was applied on the top of the box. With the CHARMM36 package, the whole system was initially minimized for 1000 steps by using the conjugate gradient algorithm to remove abnormally close contacts between molecules. Then the system was heated to 310 K for 4000 steps and pre-equilibrated for another 4000 steps. The protein was still constrained at its crystal structure during this stage. Then, the pre-equilibrated configuration of the whole system was loaded into MD simulations. External electric fields were applied in the *z* direction. The fields applied parallel and antiparallel to the z axis are referred to as negative and positive fields, respectively [48]. The initial velocity of each atom was assigned from a Maxwell–Boltzmann distribution at 310 K. The gold atoms and sulfur atoms of SAMs were kept fixed during the simulations. All bonds involving hydrogen atoms were constrained to their equilibrium lengths with SHAKE algorithm [49], allowing for an integration time step of 2 fs to be used. The Berendsen NVT ensemble method [50] was employed to constrain the system temperature at 310 K with a coupling constant of 0.1 ps. The short-range VDW interactions were calculated by a switch potential with a switching function starting at a distance of 10 Å and reaching zero at 12 Å. The long-range electrostatic interactions were treated by PME method. Each MD run was carried out over a period of 20.0 ns. Configurations and trajectories were saved every 4.0 ps for later analysis.

3. Results and discussion

The lysozyme adsorption behavior on negatively charged carboxyl-terminated SAMs under applied electric fields was investigated by a combined MC and MD simulation approaches. Positive field strengths 0.01 (EF_1), 0.05 (EF_2), 0.1 (EF_3) and 0.2 (EF_4) V/Å, as well as negative field strengths −0.01 ($-EF_1$), −0.05 ($-EF_2$), −0.1 ($-EF_3$) and −0.2 ($-EF_4$) V/Å were used. Lysozyme adsorption in the absence of electric field (EF_0) was simulated as a reference. The preliminary configurations of lysozyme on the SAMs were obtained from MC simulations. During the MD simulations, data were collected to analyze the protein–surface distance, orientation distribution, binding residues, root-mean-square deviation (RMSD) of lysozyme. All the statistic data in the paper are based on the latter 10-ns simulations.

3.1. Initial configurations and repeatability of MD simulations

The electrostatic interaction dominated orientation "side-on" (Fig. 1a) obtained from MC simulations was used as the initial configuration for MD simulations. Another possible orientation "end-on" (Fig. 1b) was also employed for initial configuration to check the repeatability of MD simulations. They were put 1 nm above the surface as initial configurations 1 and 2, respectively.

We compared the simulation results of time evolution of total potential energy of lysozyme adsorption in the absence of electric fields using initial configurations 1 and 2. As displayed in Fig. 2, the two energy curves are basically consistent after 10-ns simulation. Energy averaged from 10 to 20 ns for the two simulation cases are −3924 and −3933 kJ/mol, respectively, with 9 kJ/mol (0.2%) difference. The orientation distributions for the simulations with two different initial configurations are also analyzed (Fig. 3). Orientation angle (θ) is employed to quantitatively characterize the orientation of lysozyme on the surface. The orientation angle of the adsorbed lysozyme molecule is defined as the angle between the unit vector normal to the surface and the unit vector along the dipole of lysozyme [29,41]. Fig. 3 shows that the two orientation distribution curves are basically consistent. Considering configuration 1 is preferred where electrostatic interactions dominate the protein adsorption, and electrostatic force may play a major role in protein adsorption under applied electric fields, we choose the "side-on" orientation as the initial configuration for each MD simulation case.

3.2. Protein–surface distance

The minimum protein–surface distance (SSD), defined as the nearest distance between the lysozyme VDW surface and the SAM surface in the *z*-axis, is calculated to trace if lysozyme is adsorbed or not. An increase in the distance implies that protein moves away from surface while a decrease suggests that protein moves to surface. The time evolutions of protein–surface distances under positive and negative electric fields are displayed in Fig. 4. The case in the absence of electric fields is also shown for comparison (black colored in each panel).

When no external electric field is applied, lysozyme is soon adsorbed onto surface at about 0.4 ns. However, at about 10.8 ns, lysozyme desorbs from surface for 0.1 ns before re-adsorbing onto the surface. This phenomenon implies that the lysozyme–surface interaction forces are not strong enough to tightly bind lysozyme on the SAM. With the application of positive electric fields, lysozyme is irreversibly adsorbed on the surface within less time. At EF_1, lysozyme is adsorbed after 1.0 ns. Though there are some small bumps in the SSD curve from 4.0 to 7.0 ns, lysozyme does not desorb from the surface since the SSD value still within 3 Å. Lysozyme just slightly adjusts its configuration to optimize the protein–surface interactions during the period. At EF_2, lysozyme is soon adsorbed on the surface and never desorbs after 1.0 ns. At EF_3 and EF_4, lysozyme is irreversibly

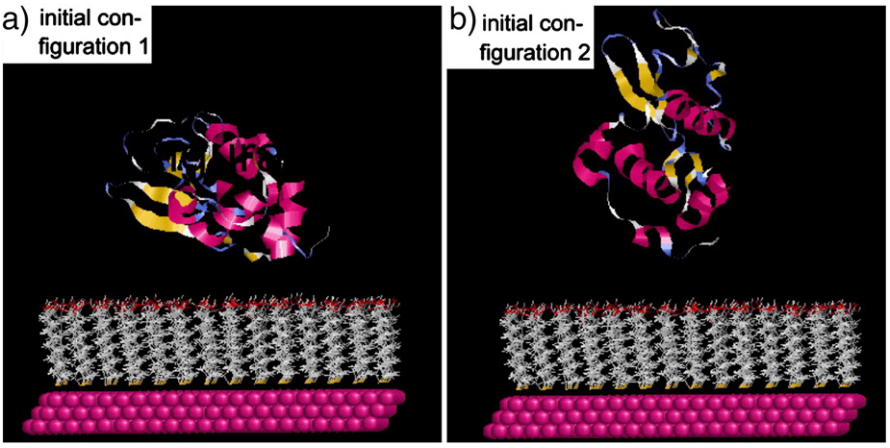

Fig. 1. Initial configurations for MD simulations.

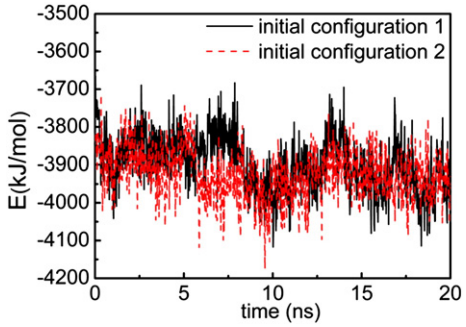

Fig. 2. Time evolution of total potential energy of lysozyme adsorption on SAMs with different initial configurations in the absence of electric fields.

adsorbed on the surface within 2 ns. The comparison of the SSD curves at different electric fields suggests that protein adsorption on the SAMs is enhanced by the applied positive electric fields.

The effects of negative electric fields on protein adsorption process depend on the field strengths. At $-EF_1$, lysozyme is adsorbed on the surface at about 0.4 ns. Though a few small bumps appear in the curve between 11 and 13 ns, it only indicates that the binding force of lysozyme with the SAM is not strong enough but not lysozyme desorbing from surface. Therefore, lysozyme adsorption at $-EF_1$ is not slowed down but slightly facilitated. At $-EF_2$, lysozyme is not irreversibly adsorbed on the SAM until 6.0 ns. At $-EF_3$, lysozyme keeps moving up and down above the surface until it is irreversibly adsorbed on the surface after 10.3 ns. Simulation cases at $-EF_2$ and $-EF_3$ show that protein adsorption is slowed down under negative electric fields. At $-EF_4$, lysozyme is only occasionally adsorbed on the surface during the whole simulation time, indicating that protein adsorption is noticeably inhibited.

The protein–surface distance plots reveal that lysozyme adsorption could be generally promoted by positive electric fields and retarded by negative electric fields, but the extent of promotion or retardation effects on protein adsorption does not change monotonically with the field strengths. To explain this, we calculated the protein–surface interaction energy (U_{PS}) and ions–surface interaction energy (U_{IS}), as listed in Table 1. Subscripts "vdw" and "ele" represent the van der Waals and electrostatic interaction energies, respectively.

It can be seen from Table 1 that under positive electric fields of EF_1, EF_2, EF_3 and EF_4, U_{PS} values are −252.0, −211.3, −192.0 and −192.2 kJ/mol, significantly larger than that at EF_0 (−101.9 kJ/mo), suggesting that protein–surface interactions are strengthened. Since the positive electric fields are applied in the same direction with the inner electric potential produced by the negatively charged SAMs, it will exert a driving force on the positively charged lysozyme towards the surface and therefore strengthen the protein–surface interactions.

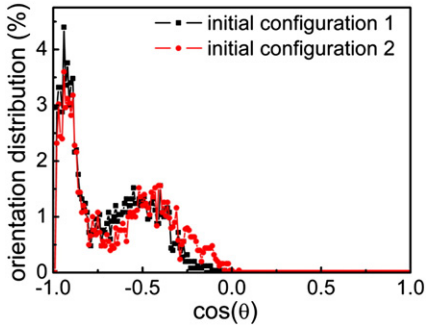

Fig. 3. Orientation distributions of lysozyme adsorption on SAMs with different initial configurations in the absence of electric fields.

However, note that $U_{PS,ele}$ values decline with rising field strengths. This phenomenon is probably attributed to the competitive adsorption of cations in the solution with lysozyme under external electric fields, which will weaken the protein–surface interactions by neutralizing surface charge. With the field strength goes up, there are more cations being adsorbed on the SAMs and the ion–surface interactions are strengthened, as evidenced by the $U_{IS,ele}$ values going up noticeably with the increasing field strengths. At EF_1, $U_{IS,\ ele}$ value is smaller than that at EF_0. Possible reason is that the driving force is still too small to push cations towards the surface at this field strength. In concise, the influence of lysozyme adsorption under positive electric fields is the compromise between two effects, the pulling force on lysozyme to facilitate protein adsorption and the co-adsorption of cations to slow down protein adsorption. Based on the understanding, it is reasonable to infer that under some field strength, lysozyme adsorption may not be significantly affected if the two effects counteract.

With the applied negative electric fields, the situation is also complicated. On one hand, lysozyme is exerted an force to pull it away from surface, which is unfavorable for protein adsorption; on the other hand, cations may migrate away from the SAMs to reduce the competitive adsorption with lysozyme. Therefore, the influence of negative electric fields on lysozyme adsorption depends on the compromise between the above mentioned effects. At $-EF_1$, lysozyme adsorption is not retarded but slightly enhanced probably because lysozyme adsorption is slightly affected while cation adsorption is slowed down, as evidenced by a comparison between U_{PS} values at $-EF_1$ and at EF_0 (Table 1). At $-EF_2$ and $-EF_3$, since the pulling force exerted on lysozyme is still not so high and cation co-adsorption is slowed down, lysozyme could finally adsorb on the surface despite of the retardation. At $-EF_4$, the pulling force dominates and therefore lysozyme adsorption is dramatically inhibited, implying the possibility of using negative electric fields with high strength for anti-fouling applications.

The role of small counterions in protein adsorption is rarely emphasized at molecular level in literatures. Previous experimental [51–54], theoretical [55] and simulation works [29,56] have found that ionic strength has significant effect on protein adsorption. In our work, counterions are correlated to ionic strength. Our findings support the idea demonstrated by Norde [57] that co-adsorption of small ions occurs in protein adsorption. Claesson [58] also observed that small counter-ions co-adsorb with the protein from surface force measurements of lysozyme adsorbed on mica surfaces. Other literature also found that protein adsorption might be sensitive to the type, concentration and charge of ions in solution [59]. Under external electric fields, the counterions from solution may play an even more important role in protein adsorption.

Taking into account the co-adsorption of ions with lysozyme, some seemly contradictory experimental phenomena become understandable. Our findings support the experimental results of the promotion and retardation effects on protein by electric fields. For example, Brusatori et al. [33] found that the adsorption of cytochrome c is considerably enhanced in the presence of an applied potential exceeding 1 V due to the affinity between negatively charged regions of the protein and electrode surface. Moulton et al. [60] observed that the adsorbed amount of human serum albumin and immunoglobulin G increased under positive applied potential and decreased under negative applied potential. Yeh et al. [61] found that the applied electric potential is the major contributor in reducing the adhesive force between protein and surface in an anti-fouling mechanism based on the combined effects of electric field and shear stress. Morrow et al. [62] reported that a negative potential can inhibit protein attachment, or reduce the amount of protein attached. Our simulation work also explains why protein adsorption can be unaffected under some field strengths, which is observed in experimental works [40,63,64]. They found that external EFs exert few influence or only small influence on protein adsorption at solid–liquid interfaces.

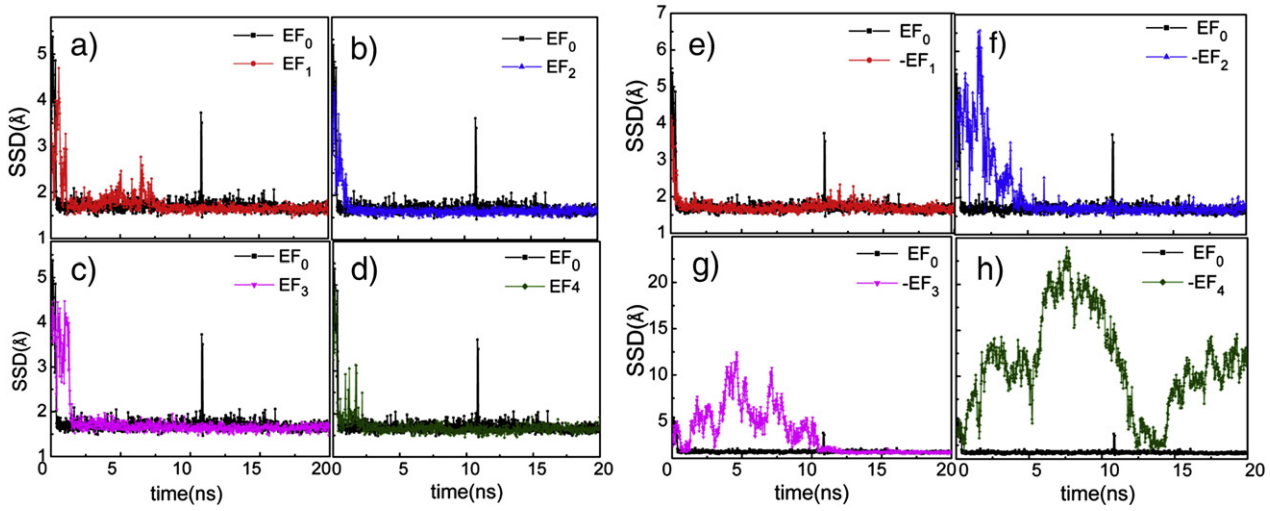

Fig. 4. Time evolution of protein–surface distance under different electric fields.

3.3. Orientation distributions and binding sites

Orientation distributions of lysozyme adsorption on the SAMs under different electric fields are analyzed (Fig. 5). The final configurations of lysozyme on the SAMs are presented in Fig. 6. The binding residues and the minimum distance between the atoms of binding residues and the SAMs (d_{rs}) of each configuration are displayed in Table 2. The case at $-EF_4$ is not shown here because lysozyme is not adsorbed.

In the absence of electric fields (colored black in each panel in Fig. 5), the orientation distribution is wide with two peaks. One represents the possible orientation "bottom end-on" (Fig. 6a) with θ_1 equal to 160.0° and another represents the orientation "side-on" (Fig. 6a) with θ_2 of 113.6°. For the two orientations, the positively-charged Arg128 and Arg125 at the C-terminal of lysozyme are mainly responsible for the binding (Table 2), which is consistent with the simulation results by Kubiak-Ossowska [65]. In the presence of electric fields, the orientation distributions are narrower than that at EF_0 (Fig. 5a–d). This finding indicates that external electric fields may be applied to control the spatial homogeneity of adsorbed proteins, which agrees with the conclusion from Brusatori et al. [65]. At EF_1 and EF_3, lysozyme adopts the "side-on" orientations (Fig. 6b and d) to adsorb on the SAMs, in which the active sites of lysozyme face sideways. At EF_2 and EF_4, "back-on" orientations (Fig. 6c and e) of lysozyme are preferred, in which the active sites face upwards. For either "side-on" or "back-on" orientations, the positively charged residues Lys (Lys1, Lys33) and Arg (Arg5, Arg114, Arg125 and Arg128) play the major role (Table 2), suggesting that electrostatic interactions dominate the protein–surface interactions [20,41]. Besides, for all the preferred orientations, the active sites of lysozyme are accessible, indicating that a positive external electric field may strengthen protein–surface interactions without burying the active sites of lysozyme and thus retain the accessibility for substrates. These orientations detected in our simulations are in agreement with the experimental results [54,66] where attractive electrostatic interactions control the lysozyme adsorption.

In the presence of negative electric fields, the orientation distributions are also narrower than that at EF_0 (Fig. 5e–g), implying that external electric fields may help to tether protein on the surface with uniform orientation. At $-EF_1$ and $-EF_2$, lysozyme is adsorbed on the surface with "back-on" orientations (Fig. 6f and g). However, there are much less residues of lysozyme binding with the SAMs at $-EF_2$ than that at $-EF_1$ (Table 2) because of the weakened protein–surface interactions at $-EF_2$. At $-EF_3$, a totally different lysozyme orientation with θ of 34.9° ("top end-on") is favored (Fig. 6h) where most of the binding residues are neutral. The "top end-on" orientation usually emerges when lysozyme is adsorbed on positively charged surface [21]. However, this orientation is preferred at $-EF_3$, suggesting that protein may adjust greatly the adsorbed configuration to optimize the protein–surface interactions under negative electric fields. The different orientations under different field strengths also imply that it is possible to control protein orientation via the application of electric fields. At $-EF_4$, lysozyme is not adsorbed due to the inhibition effect of strong electric field, as shown in Fig. 6i.

3.4. Protein conformation

To examine the effect of electric fields on the conformational change of lysozyme on the SAMs, the statistical RMSD of lysozyme was calculated.

Table 1
Interaction energy of lysozyme–surface and ions–surface at different electric fields.

EF	Field Strength (V/Å)	interaction energy (kJ/mol)					
		U_{PS}	$U_{PS,ele}$	$U_{PS,vdw}$	U_{IS}	$U_{IS,ele}$	$U_{IS,vdw}$
EF_0	0.00	−101.9	−96.4	−5.5	−436.9	−459.2	22.3
EF_1	0.01	−252.0	−220.2	−31.8	−353.8	−372.1	18.3
EF_2	0.05	−211.3	−185.3	−26.0	−537.3	−566.3	29.0
EF_3	0.10	−192.0	−181.5	−10.5	−706.0	−744.2	38.2
EF_4	0.20	−192.2	−177.9	−14.3	−782.6	−828.2	45.6
$-EF_1$	−0.01	−141.1	−114.2	−26.9	−408.2	−429.6	21.4
$-EF_2$	−0.05	−79.7	−72.2	−7.5	−286.1	−300.7	14.6
$-EF_3$	−0.10	−100.8	−81.6	−19.2	−257.6	−270.6	13.0
$-EF_4$	−0.20	0.0	0.0	0.0	−55.3	−52.7	−2.6

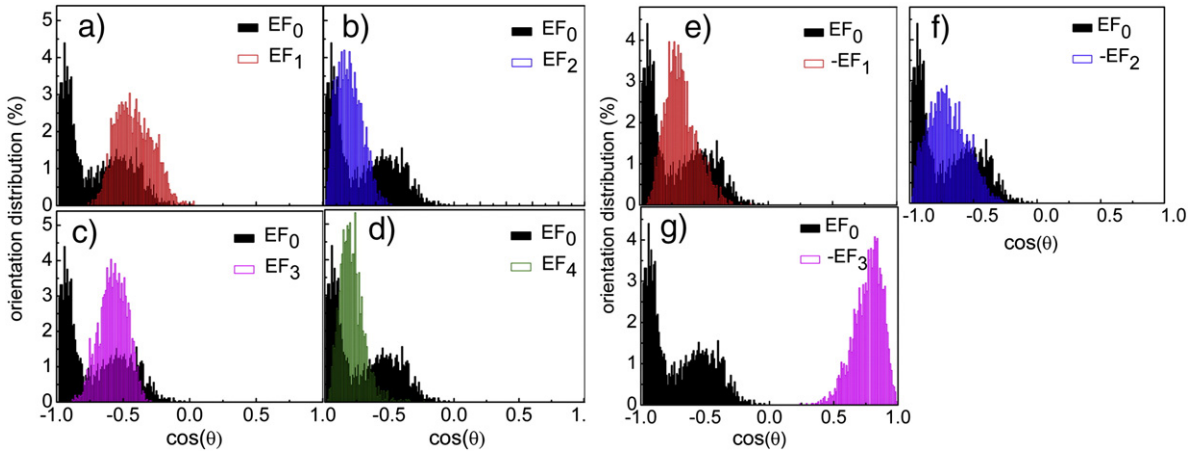

Fig. 5. Orientation distribution of lysozyme adsorption on SAMs under different EFs.

The root mean-square deviations can be used to characterize the conformational changes of proteins [41]. It is defined as

$$\mathrm{RMSD} = \sqrt{\left\langle \sum_{i=1}^{N} m_i \left(r_a - r_{a,ref}\right)^2 \right\rangle / \sum_{i=1}^{N} m_i},$$

where N is the number of protein atoms, m_i is the mass of atom, r_a and $r_{a,ref}$ are the coordinates of one simulated structure and its reference structure, respectively.

In this work, the crystal structure of lysozyme for MD simulations was used as the reference structure. The RMSD value of only the α-carbons of lysozyme (C_α-RMSD) was calculated which could represent the deformation of backbone structure of protein. The results of MD simulations of lysozyme in bulk solution and in the absence of electric fields were also calculated for comparison. The results averaged after 10 ns equilibration are 1.20 (bulk), 1.31 (EF_0), 1.33 (EF_1), 1.44 (EF_2), 1.68 (EF_3), 1.32 (EF_4), 1.30 ($-EF_1$), 1.72 ($-EF_2$), 1.61 ($-EF_3$) and 1.44 ($-EF_4$) Å for different simulation cases.

For lysozyme adsorption with no electric filed applied, the C_α-RMSD value is 1.31, 9.2% larger than that in bulk solution (~1.20 Å), suggesting a surface induced structure alternation in lysozyme. From the superimposed structure of lysozyme adsorbed on surface and the crystal structure of lysozyme (Fig. 7a), It can be seen that the secondary

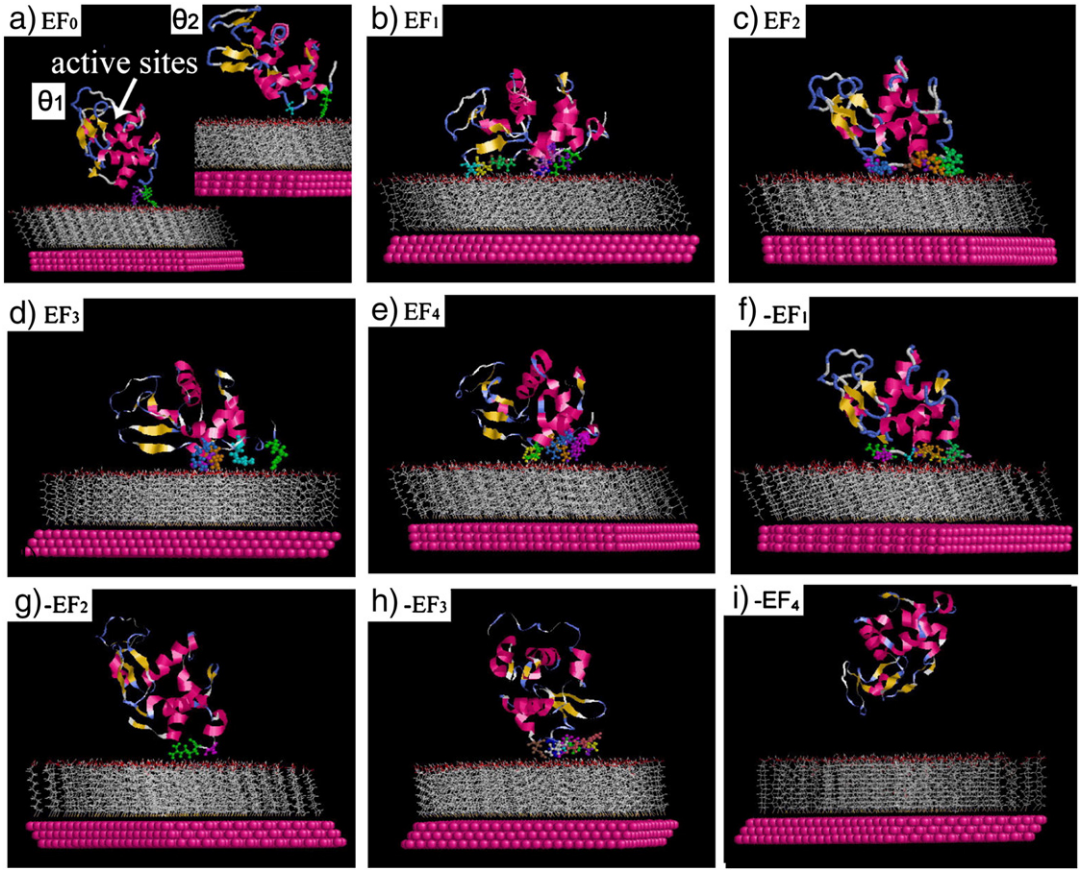

Fig. 6. Final configurations of lysozyme on the COOH-SAM surfaces at different EFs. Water molecules and ions are omitted for clarity. The ball-stick representation is for binding residues.

Table 2
Orientation angles and binding sites of lysozyme adsorbed on the SAM at different EFs.

EF	Field strength (V/Å)	cos(θ) (θ(°))	Binding residues (d_{rs}(Å))
EF_0	0.00	−0.94 (160.0)	Arg125(1.34), Arg128(1.95)
		−0.40 (113.6)	Arg125(1.14), Gly117(2.72)
EF_1	0.01	−0.45 (116.7)	Lys116(1.06), Asn37(1.40), Arg45(1.55), Thr47(1.60), Lys33(1.84),Val2(2.33), Arg114(2.35), Asn44(2.56), Ser36(2.68), Thr43(2.83)
EF_2	0.05	−0.84 (147.1)	Arg128(0.78), Lys1(1.38), Arg5(1.48), Arg125(1.96), Cys6(2,13), Val2(2.15), Gly4(2.22), Glu7(2.66), Ser86(2.98)
EF_3	0.10	−0.59 (126.2)	Arg125(0.95), Arg114(1.11), Val2(1.38), Lys33(2.18), Asn37(2.19), Arg5 (2.74)
EF_4	0.20	−0.76 (139.5)	Asn37(1.68), lys33(2.09), Arg5(2.21), Arg114(2.28), Val2(2.54), Trp123(2.81),
$-EF_1$	−0.01	−0.73 (136.9)	Lys1(1.59), Glu7(1.62), Gly4(1.62), Arg5(1.65), Arg125(1.78), Gly126(1.80), Val2(1.80), Cys6(2.20), Arg128(2.32), Cys127(2.48)
$-EF_2$	−0.05	−0.71 (135.2)	Arg128(1.95), Gly126(2.53)
$-EF_3$	−0.10	0.82 (34.9)	Ser72(1.54), Asn65(1.55), Pro70(1.56), Arg68(1.70), Asn74(1.78), Gly71(1.80), Gly67(2.48), Asn77(2.53), Arg73(2.77)

structure of lysozyme is basically kept except that the α-helix structure near the flexible C-terminal is altered. At EF_1, EF_4 and $-EF_1$, there are little difference in the C_α-RMSD values compared with that at EF_0, indicating no additional structure deformation caused by the applied electric fields. At EF_2, the C_α-RMSD value is slightly larger than that at EF_0, which may be induced by the electric fields. At EF_3, $-EF_2$ and $-EF_3$, the C_α-RMSD values are significantly larger than that at EF_0, suggesting additional conformational changes induced by the external electric fields. Alignment of the final configuration of lysozyme at $-EF_2$ and the crystal structure of lysozyme (Fig. 7b) reveals that parts of the α-helix structure are changed, especially the α-helix structure near C-terminal. However, the secondary structure of the active sites in lysozyme changes little because lysozyme is a hard protein. At $-EF_4$, since lysozyme is not adsorbed onto the surface, the additional conformational change compared with lysozyme in bulk may be induced by the external electric field.

The results of conformational changes of lysozyme under external electric fields are slightly different from expectation since the structure deformation of lysozyme is not simply increase with the field strength. At some field strengths, there is no additional conformational change occurrence compared with that in the absence of electric fields even though the field strength is relatively high. We attribute this phenomenon to a rearrangement of the position of the local atomic charges of protein to couple its dipole with an applied electric field, and this coupling may help to immobilize the inherently flexible regions of protein and hence restrict the occurrence of large conformational changes. Owing to the coupling, a higher strength of applied electric field does not necessarily imply a greater conformational change, as evidenced by the simulation case at EF_4. Similar results were also reported in experimental work [67] and other simulation works [42,43]. Ying et al. found that the adsorbed albumin is found to be stable on the gold surface under the applied potential range of −200–600 mV [67]. Budi et al. observed that moderate strength fields have a stabilizing effect on some regions of the structure of insulin due to the coupling of the helical dipole moment with the applied field [42]. Toschi et al. found that protein aligns its dipole with an applied electric field to lower the energy of the system and the structure deformation does not increase monotonically with the increase of field strength [43].

4. Conclusions

In this work, the effects of external electric fields on adsorption process, orientation and conformation of lysozyme on the negatively charged SAMs were investigated by MD simulations. Protein–surface distance plots show that positive electric fields could promote lysozyme adsorption on the SAMs by strengthening the electrostatic protein–surface interactions, while negative electric fields may inhibit adsorption which has a potential in anti-fouling application. However, the migration of cations onto surface in response to positive electric fields may weaken the electrostatic interactions between protein and surface. By orientation analysis, it was found that the orientation distributions of adsorbed lysozyme under electric fields are narrower than that without electric fields, suggesting that an external electric field may be employed to tether proteins on surface with ordered orientation. In the absence of electric fields, lysozyme is adsorbed with “bottom end-on” or “side-on” orientation; while with the electric fields applied, “side-on”, “back-on” and “top end-on” orientations are preferred, indicating the possibility of immobilizing protein with desired orientation by applying different electric fields since protein needs to translate and rotate to optimize protein–surface interactions. Conformation analysis suggests that the extent of conformational change does not increase simply with the field strengths. Under some field strengths, there is no additional structure deformation induced by the applied electric fields. However, at some other field strengths, lysozyme adjusts greatly the conformation to adapt itself to the electric potential gradient. We propose that protein may rearrange the positions of local atomic charges to couple itself to an applied electric field, and this coupling helps to prevent large

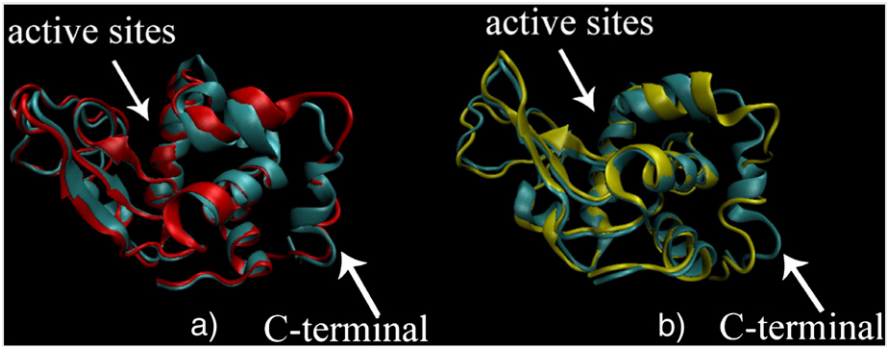

Fig. 7. Superimposed structure of lysozyme at EF_0 (red colored) and $-EF_2$ (yellow colored) with crystal structure (cyan colored).

alteration in the positions of atoms and thus restrict the occurrence of severe structure deformation.

In general, our simulation results are helpful to understand the complicated effects of external electric fields on protein adsorption behavior and provide guidance for protein immobilization and anti-fouling applications.

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