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Molecular dynamics simulation of the enhancement of cobra cardiotoxin and E6 protein binding on mixed self-assembled monolayer molecules

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Abstract
Molecular dynamics simulations are performed on *n*-alkinethiol self-assembled monolayers (SAMs) and their mixture on a gold surface so that the orientations of the binding of cobra cardiotoxin and E6 protein molecules can be selected using the mixing ratio of CH3-terminated SAMs with different chain lengths. The simulations suggest that a SAM surface with different mixing ratios may provide a possible platform for aligning protein molecules with a desired orientation and for enhancing the binding energy of the protein on the designed surface.

1. Introduction

Binding efficiency and selectivity of binding orientation are important factors for improving the detection of antigens/antibodies for protein microarrays. Self-assembled monolayers (SAMs) have been proposed as a platform for enhancing this detection by special designs with blockage of large spaces and randomness of the surface morphology. This study demonstrates the orientational selectivity and binding enhancement for two model proteins (cobra cardiotoxin (CTX) and E6) to nanostructured mixed SAM surfaces with blockage of large spaces by their different chain lengths. The E6 protein is one of the major oncoproteins produced by the human papillomaviruses responsible for cervical cancers [1]. However, the structure of E6 protein has not been fully solved and only a predicted model can be applied. Cobra cardiotoxin [2] is a cytotoxic β-sheet basic polypeptide which is known to cause membrane leakage in many cells including human erythrocytes. The three-dimensional (3D) structures of various CTX homologues in both aqueous and micellar environments [3, 4] are available. Furthermore, the interactions between CTX and lipid membranes have been well studied [2]. CTXs, therefore, are selected as a model verification system for molecular dynamic simulation study of the interaction between protein and SAM molecules.

The specially designed surface is coated with mixtures of *n*-alkinethiol SAMs of different chain lengths (1-decanethiol (C9) and 1-hexanethiol (C5) in the present work), and the surface morphology produced by the different chain lengths provides an additional dimension, giving an additional reaction area on the limited planar area of the underlying gold support. The change in the hydrophobicity arising from the different C9 and C5 compositions will alter the binding affinity between the protein molecules and the SAM surface. The binding enhancement and orientation selectivity have implications for the viability of the detection of E6 and cervical cancer.

The present study performs molecular dynamics (MD) simulations on model systems of a single CTX and a single E6 protein molecule on mixed SAM surfaces with different mixing ratios of C9 and C5. Detailed analysis of the dynamics simulation reveals the physical mechanism of protein binding.

2. Molecular dynamics method

2.1. Initial model systems of CTX and E6 proteins and structure of the SAM molecules

Many cardiotoxin structures have been solved and deposited in protein data banks. We used the NMR structure of CTX A3 (PDB 1I02) as the cardiotoxin model in our simulation because

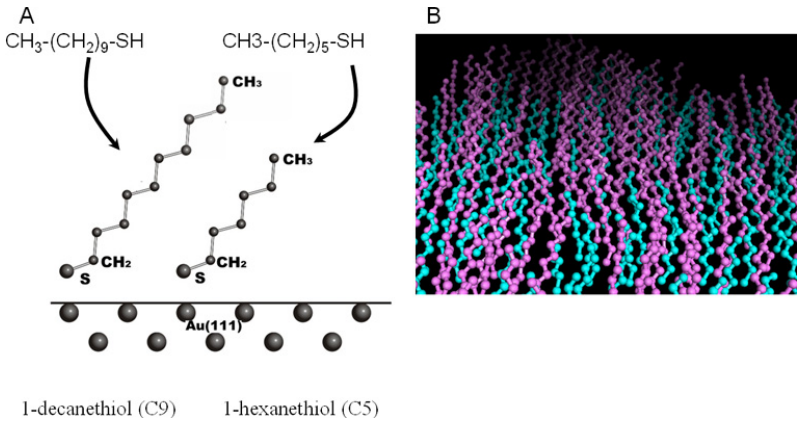

Figure 1. (A) The C5 and C9 molecules are coated on a gold surface. (B) Snapshot of the mixed SAM surface (C5 in cyan and C9 in violet) in simulation.

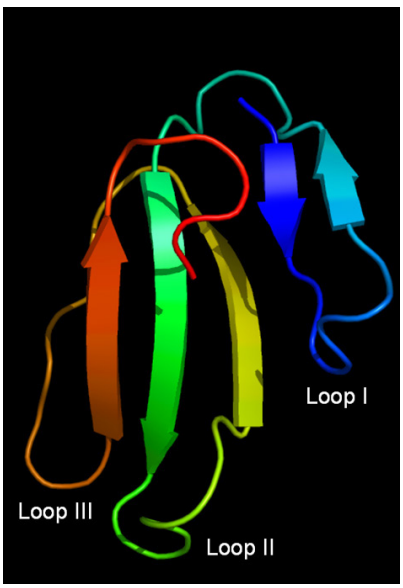

Figure 2. The structure of CTX in a ribbon drawing. The colour is rendered according to the direction of the protein sequences; the N-terminus is in blue and the C-terminus in red. The three major loops (loops I, II and III) are numbered from the N-terminal direction. The molecular image is drawn using PyMol (http://www.pymol.org).

the interactions of CTX A3 [2] with lipid membranes have been well studied.

The E6 protein presents a more challenging problem. Since the E6 protein structure is not available, the structure of E6 protein was predicted by the ROBETTA automatic predication server [5, 6]. ROBETTA returned 10 predicted structures, all of which share the common feature of being an all-α protein. We used the structure with the highest score in our simulation.

2.2. Computational models and details

The molecular dynamics simulation was performed using MOLARIS [7, 8] and the protein–SAM system was represented using the united atom representation [9]. All the parameters of the simulation are taken from the standard library of MOLARIS [8]. The simulation system size is $7.98 \times 6.91 \times 10.00\ \text{nm}^3$ for CTX and $9.98 \times 8.64 \times 15.00\ \text{nm}^3$ for E6. Each system was placed in a rectangular box with periodic boundary conditions and the minimum image convention applied to x and y directions only. For the CTX protein model, the SAM surfaces were constructed by a 16×16 array of the single chain with a sulphur–sulphur spacing of 0.499 nm and a 20×20 array for E6. The modelled SAM surface is shown in figure 1.

The potential functions consist of bond, angle and dihedral terms, as well as non-bonded van der Waals (VDW) and Coulombic interactions. The complete form of the potential function is given by

$$U = \sum_{\text{bonds}} K_b(b - b_0)^2 + \sum_{\text{angles}} K_\theta(\theta - \theta_0)^2 + \sum_{\text{dihedrals}} K_\phi\,[1 - \cos(n\phi - \phi_0)] + \sum_{\text{VDW}} \varepsilon_{ij}\left[\left(\frac{R_{ij}}{r_{ij}}\right)^{12} - 2\left(\frac{R_{ij}}{r_{ij}}\right)^{6}\right] + \sum_{\text{Coulombic}} \frac{332 q_i q_j}{\varepsilon(r_{ij}) r_{ij}}$$

where K_b, K_θ and K_ϕ are the bond, angle, dihedral and improper dihedral force constants, b_0, θ_0 and ϕ_0 are the equilibrium values of bond length, bond angle and dihedral angle; the fourth term on the right-hand side (RHS) is the Lennard-Jones potential energy accounting for the VDW interactions, and ε_{ij} and R_{ij} are the parameters for VDW depth and size, respectively; the last term on the RHS is the electrostatic interactions between charged atoms (q_i is the partial atomic charge). Since the major contributions to the interactions between the protein molecule and the SAM surface are mainly VDW interactions, the simple solvent model of a distance-dependent screening constant $\varepsilon(r_{ij})$ [10] is used to account for solvent effects, though other more sophisticated solvent models such as the surface constrained all-atom solvent model (SCAAS) [11], the local reaction field method [12] or the generalized Born model [13] should be used when electrostatic interactions are important. The mixed-composition SAM surfaces are generated by randomly placing the long and short chains of the SAM molecules on the gold surface while keeping the composition ratio constant.

The Beeman algorithm [14] was used for the integration of the equation of motion with a time step of 1 fs. Initial velocities

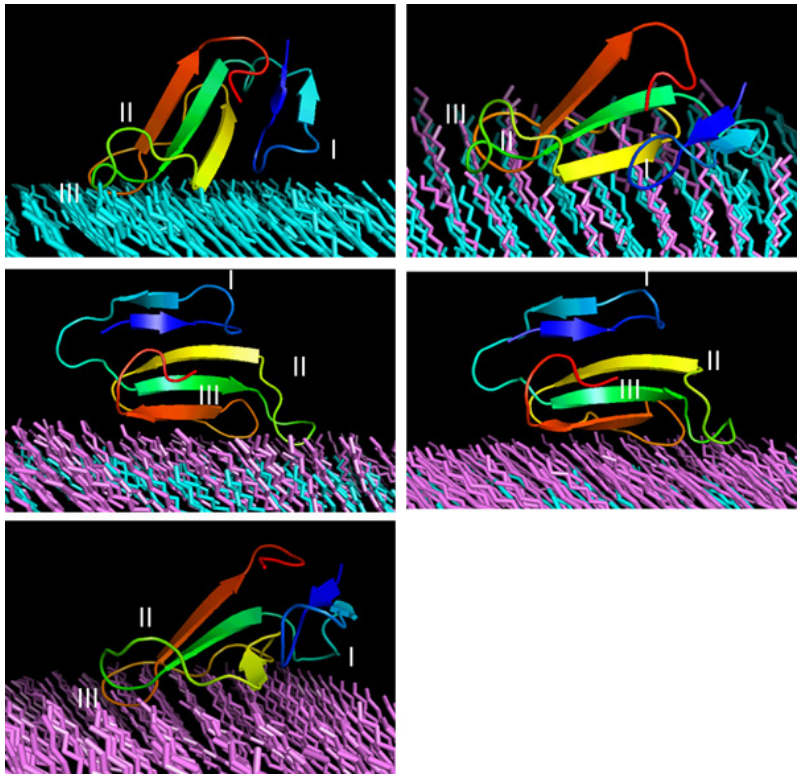

Figure 3. Snapshots of the CTX–SAM simulation. C5 and C9 are coloured in cyan and violet, respectively. From upper-left clockwise are simulations on $C9/(C5 + C9) = 0$, 0.25, 0.5, 0.75 and 1.0.

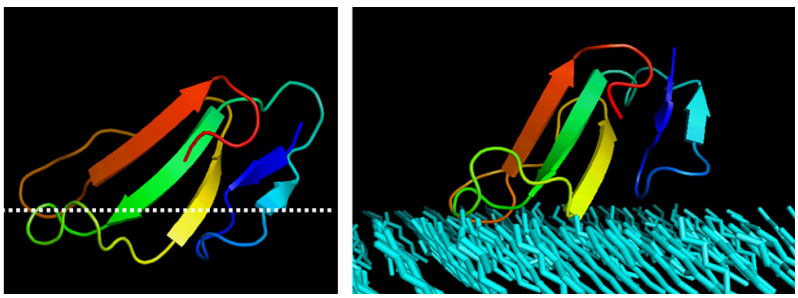

Figure 4. Comparison of the experiment [2] (left) and simulation (left) results of the orientation of CTX on the surface. The simulation was carried out on C5.

were assigned with the Maxwell–Boltzmann distribution at 300 K and the simulation was performed at 300 K. During the initial simulation state, the protein molecule was placed away from the surface at a given distance from the surface such that when the protein is released it will experience the weak attraction between the protein and the surface and will move toward the SAM surface. Since the contributions to the protein–SAM surface interactions come mainly from the hydrophobic interactions, the initial orientation of the protein molecule is selected such that its most hydrophobic surface is directed toward the SAM surface. In this work we computed the hydrophobic surface of the protein using the Eisenberg hydrophobic moment approach [15].

During simulation, we monitor the non-bonded interactions between the protein molecules and the SAM surface as a function of time. For both CTX and E6 protein, the interaction curves become stable after 500 ps, after which we start to collect simulation data. The data collection was 500 ps for E6 protein for each simulation, and 300 ps for CTX.

The binding energy is computed from the non-bonded interactions between the protein molecule and the SAM surface. Since the protein–SAM surface interactions mainly come from the VDW interactions, which arise from the different physical compositions of the long and short hydrocarbon chains, it is reasonable to assume that the relative changes of binding force should be linear to those of binding energy. In this work we are more interested in simulating the relative, instead of the absolute, binding affinities of the protein molecules between the mixed SAM surfaces.

3. Experimental demonstrations

Kidoaki *et al* [16] has reviewed the applications of atomic force microscopic (AFM) probes to the analysis of the

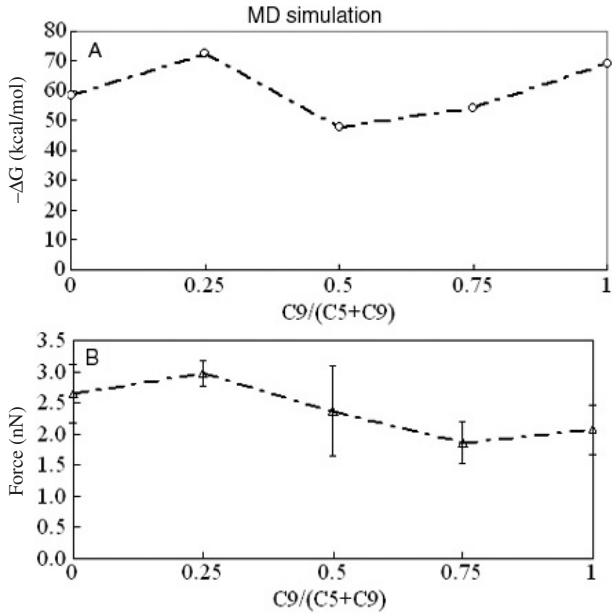

Figure 5. (A) The profile of the computed binding energy of CTX to SAMs of different ratios of C5 and C9. (B) The binding forces obtained from the AFM measurement.

actual interaction forces between protein and material surfaces (e.g. the measurement of single molecular forces between specific protein pairs such as avidin/biotin pairs or antigen/antibody pairs, of the unfolding force of single protein molecules and of non-specific adhesion forces between proteins and surfaces of material such as polystyrene or glass) and the force spectrum is from several fN to nN at the molecular level. Agnihotri *et al* [17] have extended the application to dual-component protein films adsorbed on mica. The force–distance curve can reflect the interaction of the sharp tip of the AFM and a surface at a distance of atomic dimensions. Analysis has showed that the force between them is mainly due to the VDW force of the AFM tip in phosphate-buffered saline (PBS) buffer solution. The present study aims to measure the binding force between the CTX/E6 protein-modified tip and the mixed SAM surface and to identify the orientation of the CTX or E6 protein molecules. These experimental works can be the verification basis for MD simulations.

The detailed experimental process includes the preparation of the AFM tip coated with CTX or E6 protein molecules, the preparation of mixed SAM surfaces and performing interaction force measurements for CTX protein molecules and mixed SAM molecules, which can be found in our previous paper [18]. In the present paper, CTX protein was immobilized with a concentration of 0.015 mg ml^{-1} for the experiments for 2 h and CTX protein was diluted with 6 M guanidine hydrochloride/phosphate buffer at pH = 7.0 to enhance binding of the N-terminal of the CTX protein to the COOH-terminal of the SAM molecules on the Au-coated tip. E6 protein is immobilized with two different concentrations of 0.125 and 0.25 mg ml^{-1} for the experiments for 2 h and is prepared from an expression system in *Escherichia coli* SG13009 cells containing the plasmid pQE30 (Qiagen) harbouring the human papillomavirus HPV-16 in our lab. Finally, the protein molecules are coated on the functional tip of radius less than 30 nm.

4. Simulation results

Cardiotoxin

The cardiotoxin CTX is a well-studied small all-β protein, which shows a characteristic three-finger topology (figure 2). Figure 3 shows typical snapshots of the simulation of CTX on SAM surfaces composed of different ratios of C5 and C9, i.e. C9/(C5 + C9) = 0, 0.25, 0.5, 0.75 and 1. Figure 5(A) shows the computed binding affinity of CTX to the SAM surface. The orientations of CTX on the pure C5 and C9 SAM surfaces are similar. The results are consistent with both experimental and other computational results [2, 19] that the over-all three-finger fold interacts with the membrane by the 'tips' of the fingers or loops. In figure 4, we compare the orientations of CTX from simulation (on C5) and experimental results. Our simulations show that the binding affinities of CTX to C5 and C9 surfaces are similar. However, when the ratio C9/(C5 + C9) = 0.25, the CTX is basically lying flat on the surface, and, due to the larger interaction interface, the binding energy reaches a maximum. When C9/(C5 + C9) = 0.5 and 0.75, the orientations of CTX are again similar—only loops II and III touch the surface, while loop I is on the top of the CTX without interacting with the surface. The interactions are relatively weak on these surfaces. Figures 5(A) and (B) compare the binding affinity profiles from simulation and from AFM measurement. The agreement between simulation and experiment is encouraging. Both profiles shows similar trends and indicate that the binding affinity reaches a maximum when C9/(C5 + C9) = 0.25. From our simulation, loop I appears to play a key role in the orientation of CTX on the SAM surface. The amide proton exchange experiment [10] showed that loop I is situated in the least protected region (for amide proton exchange), while loops II and III are in the most protected regions. Hence, loop I is the most flexible of the three loops and is easily affected by the surface composition.

Figure 6. The modelled structure of E6 from ROBETTA.

E6 protein

The modelled E6 has a four-helix bundle conformation (figure 6). It has an elongated shape with the N- and C-termini at either end. All α helices run in parallel to the longer axis of the protein. We chose two possible orientations of E6 in our simulations due to their similar hydrophobic surfaces toward the SAM surface—one is with the N-terminus (OR1) and the other with the C-terminus pointing (OR2) to the SAM surface, i.e. the directions of the α helices are essentially perpendicular to the surface. Figure 7 shows two typical snapshots of the simulation on $C9/(C5 + C9) = 0.25$ and 0.5. It is obvious that from figure 6 that there is a significant dependence of the orientation of E6 on the SAM composition. For example, when $C9/(C5 + C9) = 0.5$, E6 essentially stands vertical to the SAM surface, but when $C9/(C5 + C9) = 0.25$, the conformation of E6 starts to bend such that its N-terminus touches the surface. Figure 8(A) shows the binding energy profiles obtained from simulation. In general, the binding energy profiles of OR1 and OR2 are quite similar—both peak at certain mixed ratio of SAM. However, the binding energy of OR1 peaks at $C9/(C5 + C9) = 0.25$ and that of OR2 peaks at $C9/(C5 + C9) = 0.5$. Figure 8(B) shows the binding energy profiles obtained from AFM measurements. The agreement between MD simulations and AFM measurements is encouraging. Our results indicate that the mixing ratio of $C9/(C5 + C9)$ dictates the binding energy of the protein molecule to the SAM surface. Thus the peak to average force can be used as an indicator of the protein orientation in the experiments. The general trends of the OR2 simulations agree with the experimental curves (figure 8(B)) better than those of the OR1 simulations. Both experiments and simulations showed similar binding profiles toward the mixed SAM surface. These results suggest that OR2 may be the favoured orientation of the E6 protein when binding toward the SAM surface. However, it should be noted that OR1 at $C9/(C5 + C9) = 0.25$ has the largest binding energy of all. Hence, though our simulation results appear to be consistent with experiment and provide an interesting structural interpretation it is obvious that additional simulations may be necessary to resolve the issue.

5. Conclusion

In this paper, we propose the use of mixed SAM surfaces of the CH_3 tailgroup may affect the binding affinity of a protein molecule and the binding orientation can be selected for detection. We carried out MD simulations of the protein–surface interactions. Since MD provides information at the atomic level about the conformational changes of proteins on different types of surfaces, it provides a molecular interpretation of the force measurements of the protein–surface interactions. In this work we tested two model proteins (CTX and E6) using both simulations and AFM measurements. Our results suggested that a mixed ratio of SAMs might be used to control the orientations of the protein molecule toward the surface. However, more simulations may be needed to obtain more quantitative results. In summary, molecular simulation

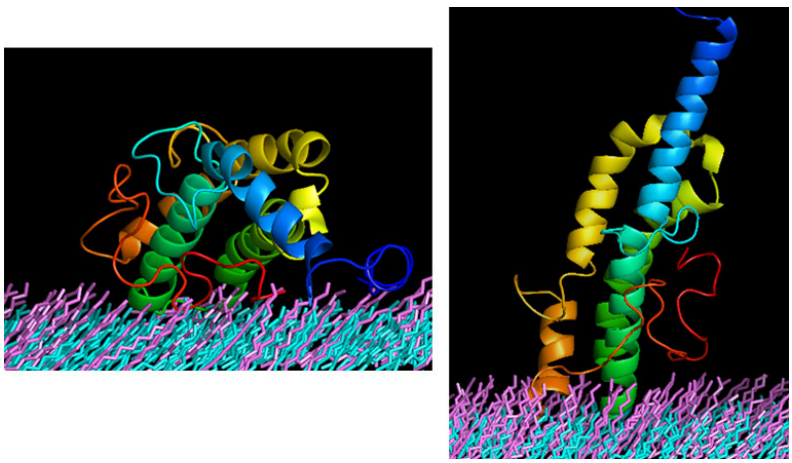

Figure 7. Snapshots of the E6–SAM simulations on $C9/(C5 + C9) = 0.25$ (left) and 0.5 (right).

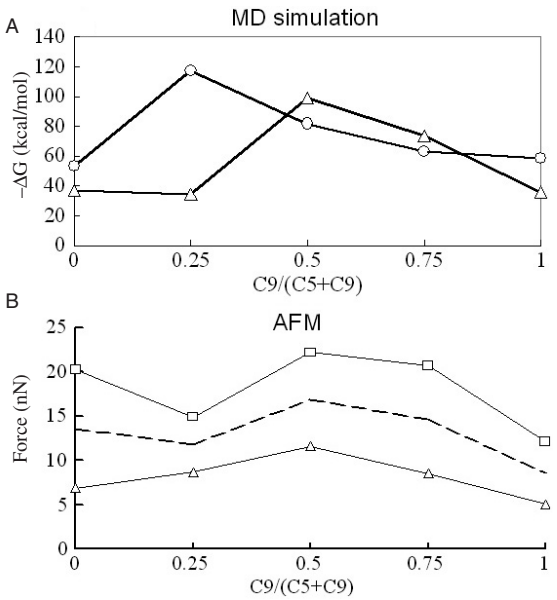

Figure 8. (A) The computed binding energy profiles of E6 to different types of SAM surfaces by simulation using OR1 (circle) and OR2 (triangle). (B) The experimental binding energy profiles of E6 to different types of SAM surfaces by AFM. The squares and triangles represent two sets of AFM measurements (0.250 and 0.125 mg ml^{-1}). The dotted line represents the average values.

is a valuable tool complementary to AFM, providing a link to bridge the structure–energetics relationship at the molecular level.

Acknowledgments

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