Research Article

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Using computational approaches to study dengue virus capsid assembly

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Abstract: Dengue viral capsid plays a significant role in viral life cycle of dengue, especially in viral genome protection and virus-cell fusion. Revealing mechanisms of the viral capsid protein assembly may lead to the discovery of anti-viral drugs that inhibit the assembly of the viral capsid. The E and M-proteins are arranged into heterotetramers, which consists of two copies of E and M-protein. The heterotetramers are assembled into a highly ordered capsid. While many investigations of the interactions between E and M-proteins have been performed, there are very few studies on the interactions between the heterotetramers and their roles in capsid assembly. Utilizing a series of computational approaches, this study focuses on the assembly mechanism of the heterotetramers. Our electrostatic analyses lead to the identification of four binding modes between each two dengue heterotetramers that repeat periodically throughout the virus capsid. Among these four binding modes, heterotetramers in binding modes I, II and IV are attractive. But in the binding mode III the heterotetramers repel each other, making mode III a suitable target for drug design. Furthermore, MD simulations were performed following by salt bridges analysis. This study demonstrates that using computational approaches is a promising direction to study the dengue virus.

Keywords: Dengue Virus, Computational Methods, Electrostatic Energy, Molecular Dynamics, Protein Assembly

MSC: 92C05

1 Introduction

Belonging to the belong to the Flavivirus genus of the family Flaviviridae, dengue virus is one of the most rapidly spreading viruses across tropical and subtropical regions which causes fever and shock. Over 50 million people are infected by the dengue virus in each year. Approximately 2.5 billion people live in the dengue endemic countries [1]. The spread of the dengue virus is recognized as a major urban public health concern by the World Health Organization. Dengue virus is also a potential bioweapon which may threaten the health of human [2].

The atomic resolution structure of dengue type 2 virus was determined in 2013, establishing a detailed structural disposition of the viral capsid. A mature dengue virion has a nucleocapsid core, a lipid bilayer membrane, and a protein capsid shell. While the nucleocapsid core are disordered, the outer protein capsid is very well organized. The capsid is assembled from 180 copies of E proteins and 180 copies of M protein.

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After protein expression and post translational modification, the E and M protein interact to form heterotetramers, which contained 2 copies of E proteins and 2 copies of M proteins. The M protein lies in the groove formed by the transmembrane helices of E protein, where they interact primarily through hydrophobic contact. E proteins and M proteins play significant roles in the life cycle of dengue virus, as they undergo dramatic structural conformational changes in viral maturation and infection[3][4]. Many studies have been focused on the interaction between the E and M proteins, especially on the role of M protein in regulating the conformational changes of the E proteins and resulting spiky and smooth forms of viral capsids. However, there is very few investigations in between their heterotetramers. Using multi-scale computational approaches, here we focused on the interactions among the heterotetramers to characterize the binding features among dengue capsid proteins. By deepening our knowledge on the interactions among the heterotetramers, our studies shed light on the drug design which targets the heterotetramers assembly of the dengue virus.

Various computational and theoretical approaches have been developed to simulate the protein stability [5] and protein-molecule interactions [6-8], therefore can be used to research viral capsid assembly. Even though the scale in length and the complexity of the viral capsids make it extremely challenging for computational simulations to study the assembly mechanism, many computational works have been performed to understand the mechanisms of viral assembly [9]. To reduce the calculation time and extend the capability of the simulation sizes, some works utilized coarse-grained models [10-13] to capture the major information of residues and neglect insignificant atomic details during the simulations. Due to the large size of a viral capsid, very few atomic simulations are performed on an entire viral capsid [14]. Most of the simulation works focus on particular regions of the capsid, such as the scaffold protein-mediated capsomer-capsomer interactions [15]. Electrostatic interactions were identified to be a significant factor for protein-molecule especially protein-protein assembly [16, 17]. Therefore, many works are performed to study the electrostatic interactions in viral capsid assembly [18-20]. In the previous studies, we have successfully calculated the electrostatic potential and electric field lines around a viral capsid [21], which demonstrated that the electrostatic potential distribution surrounding the viral capsid is periodical and the electrostatic interactions play important roles in the viral capsid assembly.

In this study, using several computational tools, we studied the interactions between the dengue heterotetramers at the atomic level. Among all the heterotetramers, we identified four types of binding modes existing in the dengue viral capsid. Our electrostatic potential analysis suggest that these four binding modes vary in strength. Base on the exciting knowledge of heterotetramer orientations, we can conclude that these binding modes are distributed throughout the viral surface. After separating the heterotetramers in particular directions taking into account their centers of masses, the net binding forces between them were calculated. When the heterotetramers are separated in a range of range 6 Å to 30 Å, the binding forces are attractive in three of the binding modes (mode I, II, IV), and repulsive in mode III. These findings are consistent with our electrostatic potential analysis. When the heterotetramers are separated more than 30 Å, the binding forces are then directed to random directions. (Figure 2). This study demonstrates that the computational approach provides a promising direction to study the dengue viral capsid assembly.

2 Methods

The structure of the dengue viral capsid, with a 3.5Å high resolution, was downloaded from the Protein Data Bank (PDB ID 3J27) [22]. Chimera was used [23] to visualize the complete structure of the virus. Four binding modes were identified from the biological assembly of the capsid, which presents all the possible interactions among the heterotetramer in the capsid (Figure 1) and are present all over the capsid in a periodic manner.

Figure 1: Four Binding modes of heterotetramers in dengue viral capsid. (a) Represents all the possible binding modes in the capsid. The involved heterotetramers are colored in blue, orange, purple, red and cyan; (b) binding mode I in which the two heterotetramers are parallel to each other; The interactions between red and purple, or purple and cyan heterotetramers can be considered the same as binding mode I. as they share the same orientation. In binding mode II (c), binding mode III (d), and binding mode IV(e), the two heterotetramers are perpendicular to one another, while they share contacts at different locations of the heterotetramers.

2.1 Delphi calculations

The electrostatic potential maps of the heterotetramers were calculated by Delphi [24, 25]. The electrostatic potential on the surface was visualized with Chimera. Visual Molecular Dynamics (VMD) [26] was utilized to visualize the electric field lines between heterotetramers. In order to illustrate the electric field lines clearly with VMD, the distance between the selected heterotetramers was increased by 20 Å. Field lines passing through the interfaces of each binding mode were mainly studied. The color scale range was set from -3.0 to 3.0 kT/Å.

2.2 DelPhiForce calculations

To gather more information about the electrostatic interactions in dengue capsid, electrostatic binding forces in the four binding modes were calculated using DelPhiForce [27, 28]. Each binding mode was manipulated by fixing a heterotetramer and displacing the other one in a certain range of distance from 6 Å to 40 Å by intervals of 2 Å, generating 12 modified structures. The separation was performed from the center of mass of each heterotetramer following the axis connecting their centers of masses. The electrostatic force from each moved structure was calculated by DelPhiForce calculations, parameters and the boundary conditions were set as polar boundary condition. The dielectric constants were set as 2.0 for proteins and 80 for water environment, respectively. The probe radius for generating molecular surface was 1.4 Å. Salt concentration was set as 0.15M. The boundary condition for the Poisson Boltzmann equation was set as a dipolar boundary condition. The electrostatic binding forces calculated by DelPhiForce were visualized with VMD, representing the forces with arrows (Figure 2).

Binding mode I, II, and IV (Figure 3 (a), (b) and (d)) showed attractive binding forces in a range of 6 to 30 Å; The forces above 30 Å showed to have a random direction. Binding mode III showed a repulsive force from a range of 6 to 30 Å, and consistent with other binding modes, the forces beyond 30 Å become random. In all the four binding forces, the binding mode II has the weakest force.

These calculations only focus on the electrostatic interaction and did not take into account other forces such as Van der Waals and others. This could affect further calculation since we are avoiding other forces.

Figure 2: Electrostatic Forces between the two heterotetramers in all four binding modes. To compare the binding strength, the total forces were calculated the versus the distance. In all four panels, the fixed heterotetramers are presented in electrostatic surface, in which the negatively charged areas are colored in red and positively charged areas are colored in blue. The manipulated heterotetramers are show in transparent grey. The binding forces are represented by yellow arrows. All images are rendered by VMD with a color scale from -3.0 to 3.0 kT/Å°. (a), (b) and (d) Binding modes I, II, and IV present an attractive net force. (c) Binding mode III presents a repulsive net force.

2.3 Molecular Dynamic (MD) simulations

To simulate the interactions between all the four binding modes presented in the viral capsid, MD simulations with an implicit solvent model, Generalized Born model(GB), [29] were carried using the CHARMM36 force field [30]. The dielectric constants for protein and solvent are 2 and 80. The initial temperature for the simulation was set at 300 K with the ion concentration of 0.15 M. The minimization was set at every 2000 steps with 1 fs/step timestep. In each of the four binding modes, a 10 ns simulation is performed, from which four thousand frames were obtained. To study the role of electrostatic interactions, salt bridges that were formed within a distance of 3.2Å by the residues pairs from each binding mode were extracted from the four thousand frames.

3 Results

3.1 Electrostatic surface

The electrostatic surface was obtained via Delphi. The net charge of the heterotetramer is +2e. Visualization of these calculations was performed with VMD. Red colored areas represent a negatively charged surface, white areas represent neutrally charged surface and blue areas represent a positively charged surface. The electrostatic charge surface distribution on an individual heterotetramer shows a particular pattern. Positive residues were predominant in the heterotetramer, negative residues were scattered but mostly in corners (Figure 3).

Figure 3: Electrostatic surface representation for four binding modes. The surface representation of modes I, II, II, IV are shown in panel (a), (b), (c) and (d), respectively. All images are rendered by Chimera with a color scale from -3.0 to 3.0 kT/Å°. Negatively charged areas are colored in red, whereas positively charged areas are colored in blue.

3.2 Electrostatic field lines

The electric field lines and surfaces were displayed in all the binding modes (Figure 4). For the field lines, white lines represent the attractive and repulsive behaviors between heterotetramers. The 20 Å separation of the heterotetramers improved the visualization of the field lines in the area of interest. The results obtained from pdb2pqr [31] showed a net charge for a single heterotetramer was $+2e$ regardless the binding mode and for binding mode I, II, III and IV a net charge of $+4e$. A predominant blue area can be appreciated in all binding modes (Figure 4) and therefore positively charged. The charge of distribution and field lines in each binding mode showed an interesting pattern where mode I as expected has a high attractive interaction, which resulted in denser field lines. Mode II and IV have particularly attractive interactions, but not as strong as binding mode I. On the other hand, Mode III shows predominantly repulsive interactions, hence the poor field lines that were captured. Therefore, we referred these four binding modes as strong electrostatic-attractive binding (mode I), weak electrostatic-attractive binding (mode II and IV), and electrostatic-repulsive binding (mode III).

3.3 Salt bridges

To identify the key interfacial residues, which contribute significantly to the binding interactions, salt bridge formation was analyzed following MD simulations by NAMD. The simulations were done for 10ns for all four binding modes which resulted in to 4000 frames. The salt bridges formed in the 4000 frames was analyzed

Figure 4: Electrostatic field lines of the four binding modes among the heterotetramer of dengue virus. Binding mode I, II, III, IV are shown in panel (a), (b), (c) and (d), respectively, where the interface in each binding mode was zoomed in. All images were rendered by VMD. The color scheme is the same as in Figure 3.

with 3.2 Å bond length cut off. The results show that binding mode I has the greatest number of salt bridges (Figure 5). This is due to the nature of the interaction in mode I where a greater area is involved, and a stronger interaction is taking place. The maximum number of salt bridges reached simultaneously was 8 and the average number of salt bridges per frame was 2.97 (Table 1). Among these salt bridges, the GLU133.C- LYS310.G and Asp192.A-LYS394E are the most stable as they were present close to 50% of the total simulation time. Consistent to the electrostatic field line analyses, these salt bridges are formed in the denser areas of the electric field lines (Figure 4). The maximum numbers of simultaneous salt bridges in binding mode II and IV are 5 and 6, respectively. The corresponding average salt bridge numbers are 1.41 and 1.79, respectively. The amino acids involved in the most stable salt bridge formation for these binding modes were GLU311.G - LYS38.C and GLU172.X - LYS38.E (table S1). It is noteworthy that the salt bridge Glu172.X - Lys38.E in mode IV is highly stable as it is present in 93.05% of the frames from our simulation. Finally, binding mode III presented an interesting salt bridge formation, with a least average per frame of salt bridge formation among all the binding modes (table S1), where only two salt bridges were found. One would expect that binding mode III would share similar outcome as mode II and mode IV due to their similarity of the orientation of the heterotetramer involved. However, our results showed mode III has weakest salt bridge formation, consistent with our electrostatic potential and binding force analysis that repulsive interactions are found mode III. This result suggests a direct relationship between the salt bridge formation and the electrostatic interaction and based on the results we can explain why binding mode III has a weaker electrostatic interaction.

In summary, the average salt bridge number explained the binding forces very well. The binding mode with the highest average salt bridge number has the strongest attractive electrostatic binding interaction. In contrast, the binding mode with the lowest salt bridge number has repulsive electrostatic interactions. The detailed salt bridge pairs in each binding mode are identified, which may serve as the initial target sites for drug design to inhibit the dengue viral capsid assembly.

Figure 5: Salt-bridge formed during the MD simulation of binding mode I (a), II(b), III(c) and IV(d). In each graph, the total number of salt bridges is plotted vs. time from 5ns to 10 ns.

4 Conclusion

In this work we investigated the electrostatic properties and the effects in structure assembly for dengue viral capsid. We found 4 different binding modes which are periodically repeated in the capsid: Binding mode I, which represented two heterotetramers arranged in a parallel orientation and share a greater area of contact. Our results showed that there is a stronger electrostatic attractive interaction at the interface of binding mode I (Figure 4). Further analysis will be done for this mode because its shape is of interest to understand the impact of electrostatic forces in the capsid. The heterotetramers in binding mode II were arranged in a perpendicular orientation and we analyzed the contact area between the heterotetramers. The results showed a predominant attractive electrostatic interaction between the heterotetramers that can be supported by the electric field lines and salt bridge formation. Binding mode III had really interesting results. It was expected to have similar characteristics to mode II and mode IV because of the orientation it is arranged, but interestingly, the results showed very different features. Mode III has the weakest interaction of all modes, as the calculated electrostatic force direction in mode III suggesting repulsive binding force and the salt bridge formation analysis shown that (Table S1) mode III has the lowest average of salt bridge formation compared to

Table 1: Salt bridges formed in all four binding modes during MD simulations, shown with their number of appearances during the simulation and the mean value of the salt bridge.

Binding mode	Salt Bridges	Number	Percentage	Average ^[a]
I	ASP192.A-LYS394.E	630	31.50%	2.81
	GLU195.A-LYS394.C	245	12.45%	
	GLU85.C-LYS88.A	539	26.95%	
	GLU133.A-LYS310.E	691	34.55%	
	GLU133.C-LYS310.G	908	45.50%	
	GLU184.A-LYS388.E	509	25.45%	
	GLU184.C-LYS388.G	1353	67.65%	
II	GLU343.E-ARG286.A	335	16.75%	1.67
	ASP341.G-ARG345.C	368	18.40%	
	GLU311.G-LYS38.C	1395	69.75%	
	GLU338.C-LYS388.G	326	16.30%	
	GLU383.C-LYS388.G	935	46.75%	
III	ASP375.G-ARG345.C	288	14.40%	0.83
	ASP87.E-LYS334.A	1500	75%	
IV	ASP225.E-LYS38.A	117	5.85%	1.82
	ASP290.E-LYS291.X	236	11.80%	
	ASP290.X-LYS38.E	866	43.30%	
	GLU172.X-LYS38.E	1861	93.05%	
	GLU338.X-ARG188X	202	10.1%	
[a] the average salt bridge number for each frame was calculated from 2,000 frames of the last 5 ns simulation				

Mode II and IV. This makes binding mode III an important candidate for deeper study in capsid assembly. Binding mode IV has a slightly stronger attractive interaction than mode II, as the salt bridges in mode IV are formed more frequently during MD simulation.

It is important to note that other interactions in the capsid were not taken into consideration such as Van der Waals force, hydrophobic interactions, etc. In this study, we focused on electrostatic interactions. Our result demonstrated that the electrostatic forces may play significant roles in the capsid assembly. Each binding mode has unique qualities that make it suitable for future analysis such as hydrogen bond analysis, binding energies and the role of salt bridges. The binding mode III has the weakest interaction among all four binding modes, making it a strong target for developing inhibition drugs for viral capsid assembly. For each mode, further calculations can be done in order to explore its precisely characterize their assembly mechanisms, which could lead to the creation of an antibonding mechanism that can combat the virus.

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