Interaction Analysis on the SARS-CoV-2 Spike Protein Receptor Binding Domain Using Visualization of the Interfacial Electrostatic Complementarity

Takeshi Ishikawa,* Hiroki Ozono, Kazuki Akisawa, Ryo Hatada, Koji Okuwaki, and Yuji Mochizuki

C omplex formation between proteins is essential in various biological processes, attracting considerable attention as a promising target for drug discovery. Various computational methods providing a physicochemical insight into protein–protein interactions have been developed. For example, the methods to evaluate the electrostatic complementarity between molecules were proposed to investigate protein–protein or protein–ligand interactions and were mainly used for protein–protein docking. In these studies, electrostatic properties were basically calculated using empirically determined parameters. However, to properly evaluate the effect of the polarization and charge transfer resulting from the complex formation, quantum chemistry-based methods are desired. Recently, Ishikawa et al. proposed a new approach for analyzing the protein–protein interaction based on the ab initio fragment molecular orbital (FMO) method, called visualization of the interfacial electrostatic complementarity (VIINEC). In this method, the electron density (EDN) and electrostatic potential (ESP) calculated in the complex condition are used to visually analyze the electrostatic interaction at the protein–protein interface. It was demonstrated that VIINEC quantitatively evaluates the electronic induced fit by comparison with the ESPs calculated in the isolate condition. Moreover, the degree of the contribution of each amino acid to the electrostatic complementarity between the proteins was quantitatively calculated. A potential application of this method is to provide a physicochemical insight into the change in the molecular interactions caused by the mutations of viral proteins.

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The World Health Organization has declared the outbreak of COVID-19 a global health emergency. To overcome this situation, a great amount of effort has been made in various research fields, including computational studies using the FMO method. For example, Hatada et al. reported an interaction analysis between the SARS-CoV-2 main protease and its inhibitor N3 using the FMO method. Akisawa et al. also performed FMO calculations for SARS-CoV-2 spike proteins (S-protein) complexed with angiotensin-converting enzyme 2 (ACE2) and B38 antibody, in which the fourth-order Möller–Plesset perturbation (MP4) theory was employed. Watanabe et al. performed the FMO calculations associated with the receptor-binding domain (RBD) of the SARS-CoV-2 S-protein, through which the crucial amino acids in the bindings of several neutralizing antibodies were identified. In these FMO studies, so-called interfragment interaction energies (IFIE) were mainly used to analyze the molecular interactions of the target proteins. On the other hand, the electrostatic complementarity at the interface between the proteins, which VIINEC visually represented, was used in this Letter instead of the IFIE-based

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complementarity is visually analyzed. When the pESPs of two proteins at the contact surface, their electrostatic contact grid cells (CGCs). By plotting the pESP values of the located over the contact surface, and they are de considered a contact surface. That is, the density overlap for the molecular contact. In VIINEC, such a ratio is denoted as R. The cuboid region in which the grid data of pEDN were calculated is also given. Visualizations were performed using PyMOL.

In VIINEC, the EDN and ESP of each protein are extracted from the total EDN and ESP of its complex, respectively. They are denoted as partial EDN (pEDN) and partial ESP (pESP), respectively. A separating surface between two proteins of the complex is then defined by the positions where the pEDNs of them have the same value. In other words, a separating surface is made by the zero-value positions of the differential pEDNs between two proteins. Among the separating surfaces, positions, where the pEDN value is greater than a given $\rho_c$, are considered a contact surface. That is, the $\rho_c$ is a kind of criterion of the density overlap for the molecular contact. Detailed information on VIINEC, including the equations of pEDN and pESP, can be found in the previous papers.

In our implementation, the pEDN of each protein is calculated as grid data based on the EDN and ESP of its complex, respectively. They are denoted as partial EDN (pEDN) and partial ESP (pESP), respectively. A separating surface between two proteins of the complex is then defined by the positions where the pEDNs of them have the same value. In other words, a separating surface is made by the zero-value positions of the differential pEDNs between two proteins. Among the separating surfaces, positions, where the pEDN value is greater than a given $\rho_c$, are considered a contact surface. That is, the $\rho_c$ is a kind of criterion of the density overlap for the molecular contact. Detailed information on VIINEC, including the equations of pEDN and pESP, can be found in the previous papers.

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Accordingly, the electrostatic complementarity is quantified by calculating the ratio of the attractive interaction over the contact surface. In VIINEC, such a ratio is denoted as $R^c$. The methodological details have been reported in our previous paper.

In this study, molecular interactions of the RBD of the SARS-CoV-2 S-protein with ACE2 and a neutralizing antibody B38 were analyzed using VIINEC. X-ray structures of the complexes of RBD with ACE2 and B38 were downloaded from PDB (PDB-ID: 6M0J \cite{28} and 7BZ5, \cite{28} respectively). Molecular Operating Environment (MOE) \cite{29} was used to construct the models of the complexes. Hydrogens were added to the complex and optimized with the AMBER10:EHT \cite{30} force field. All waters in the original record were retained, resulting in 80 and 519 waters being included in our RBD/ACE2 and RBD/B38 complex models, respectively. The net charges of RBD and ACE2 in our RBD/ACE2 model were $+2$ and $-24$, respectively. When performing VIINEC, the net charge of each protein should be eliminated because it has an influence to make an overall positive or negative bias on the ESP at the contact surface. Therefore, two chloride ions and 24 sodium ions were added to the RBD and ACE2, respectively, which were placed sufficiently distant from the interface of the proteins. Similarly, the net charges of the RBD and B38 in our RBD/B38 model were $+3$ and $+4$, respectively. Thus, seven chloride ions were added to the model. A detailed discussion on the influence of the net charge in VIINEC can be found in our previous paper. \cite{15} We also prepared isolated protein models with the same atomic coordinates as the corresponding complex models. Graphical information on the models used in this study is also given in Figure S1.

To reiterate, pEDN is calculated as grid data based on the FMO method. Here, a grid with a separation of 0.3 Å covering the interface of the proteins was used, resulting in the total number of grid points being 1,324,575 (87 $\times$ 175 $\times$ 87) for RBD/ACE2 and 2,045,295 (105 $\times$ 151 $\times$ 129) for RBD/B38. FMO calculations were performed at the RI-MP2 level of theory \cite{31,32} where cc-pVDZ basis sets \cite{33} and their auxiliary basis sets \cite{34} were used. Amino acid residues, water molecules, sodium ions, and chloride ions were treated as a single

Figure 1. Separating surfaces constructed using SGCs and the contact surface constructed using CGCs. The SGCs are indicated using gray dots, and the CGCs are indicated using red dots for the cases using 10, 24, and 100 grid points being 1,324,575 (87 $\times$ 175 $\times$ 87) for RBD/ACE2 and 2,045,295 (105 $\times$ 151 $\times$ 129) for RBD/B38. FMO calculations were performed at the RI-MP2 level of theory \cite{31,32} where cc-pVDZ basis sets \cite{33} and their auxiliary basis sets \cite{34} were used. Amino acid residues, water molecules, sodium ions, and chloride ions were treated as a single
Figure 2. pESPs of RBD (a) and ACE2 (b) at the contact surface for the RBD/ACE2 complex. pESP from which the contribution of the E484 of RBD is removed is also given (c). The contact surface with $\rho_c = 10^{-4}$ au is used. pESP values are indicated using colored dots placed at the CGC positions. $R^-$ and $\Delta R^-$ values are also provided. Visualizations were performed using PyMOL.44

Figure 3. pESPs of RBD (a) and B38 (b) at the contact surface of the RBD/B38 complex. The contact surface with $\rho_c = 10^{-4}$ au is used. pESP values are indicated using colored dots placed at the CGC positions. The $R^-$ value is also provided. Visualizations were performed using PyMOL.44
the RBD/ACE2 complex, several positive and negative regions are scattered along the contact surface, resulting in more complicated ESP maps. The intricate patterns of the ESP maps are flipped in the positive and negative and match each other like a dimple key, which is considered to be an essential mechanism of the high affinity between RBD and B38. This result also is discussed in association with the specificity of antibody interaction. That is, the binding of the B38 antibody to the RBD is highly specific because a large electrostatic interaction is created only when the two complicated ESP maps sufficiently match. It is an exciting result that part of the mechanism of the specificity of antibody bindings was addressed using a basic physicochemical quantity such as ESP. Moreover, the ESP maps from which the contribution of several amino acids of B38 is removed are given and discussed together with the IFIE analysis in the Supporting Information (see Figure S3).

In this study, the ESPs of the isolated proteins were also calculated through the CGCs determined using the corresponding complexes. Calculated ESPs are given in Figure 4 panels a1, a2, b1, and b2. Although these ESP maps look similar to those of the corresponding complex (see Figures 2 and 3), calculated \( R^* \) values were significantly smaller than those calculated in the complex condition, i.e., 0.8581 and 0.8387 for RBD/ACE2 and RBD/B38, respectively. These results revealed that the electrostatic complementarity increased by 5.43% and 4.21% for RBD/ACE2 and RBD/B38, respectively, through the complex formation. To further clarify the change in the ESP maps, differential ESPs are given in Figure 4 panels a3, a4, b3, and b4 (the value range of these maps is one-tenth). Notably, the amplitude of positive or negative value is enhanced by forming the complex, thereby increasing the electrostatic complementarity. This is the effect of the electronic induced fit, including the polarization and charge transfer. It is considered one of the vital mechanisms to stabilize the protein–protein complex. The ability to quantitatively evaluate the electronic induced fit is a notable characteristic of VIINEC because common classical force fields do not describe the charge transfer or polarization.

Herein, we reported an application of VIINEC to the complexes of the SARS-CoV-2 S-protein RBD with human ACE2 and a neutralizing antibody. The role of amino acids in complex formation could be analyzed in detail by performing VIINEC in combination with the IFIE analysis. For example, an important mutation in RBD, E484K, was discussed, the specificity of the antibody binding to the target protein was addressed, and the electronic induced fit resulting from the complex formation was quantitatively evaluated to showcase the methodological advantage of VIINEC. Although only the B38 neutralizing antibody was analyzed in this study, many X-ray structures between the RBD and various antibodies have recently been reported. Thus, a systematic application of VIINEC to such complexes may provide a physicochemical insight into the escape mechanism of mutant strains from our immune system. In the calculations of this study, the solvent effect was not taken into account. Many FMO calculations using implicit46–49 and explicit50,51–53 solvent models have been reported. Therefore, we consider that performing VIINEC with a solvent model is an important issue for the future.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpclett.1c02788.

Graphical information on the models used in this study (Figure S1); computational times of the RBD/ACE2 complex (Table S1); computational times of the isolated RBD whose atomic coordinates are the same as RBD in the RBD/ACE2 complex (Table S2); computational times of the isolated ACE2 whose atomic coordinates are the same as ACE2 in the RBD/ACE2 complex (Table S3); computational times of the RBD/B38 complex (Table S4); computational times of the isolated RBD whose atomic coordinates are the same as B38 in the RBD/B38 complex (Table S5); computational times of the isolated B38 whose atomic coordinates are the same as B38 in the RBD/B38 complex (Table S6); IFIE analysis and pESPs of the RBD/ACE2 complex (Figure S3).
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S2); IFIE analysis and pESP of the RBD/B32 complex (Figure S3)  

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Notes
The authors declare no competing financial interest.

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