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# A Computational Study of Fab CHK152 with CHK265 as Anti Chikungunya Virus for Rapid Test Components

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## ABSTRACT

The symptoms of chikungunya disease are generally similar to the symptoms of other viral diseases, except that chikungunya disease can cause joint disorders and even paralysis. Incorrect initial diagnosis will result in inappropriate treatment of the disease Thus it is necessary to study the identified antibodies that can neutralize and protect the body from alphavirus infections, including chikungunya. There are several antibodies that can neutralize chikungunya including CHK152 which neutralizes virus by inhibiting fusion with stabilizing the surface area of the virus, hindering the exposure of the fusion-loop, likely neutralizing infection by blocking fusion. Otherwise CHK265 inhibits the life cycle of the virus in a way neutralize infection by blocking entry at a post-attachment pre-fusion step. The low energy of binding between antigen-antibodies will stabilize the interaction of both. Therefore, this aims to find the best affinity energy for antibodies to be used in the rapid diagnostic component of anti-chikungunya virus (CHIKV). The binding energies were calculated using FireDock an efficient method for the refinement and rescoring of rigid-body docking solutions, through rearrangement of the interface sidechains and adjustment of the relative orientation of the molecules.The results of this study obtained energy scores from CKH152 and CHK265 are -125.78 and -29.94, indicate that CHK265 antibody affinity energy is higher than CHK152. It can be concluded that the best affinity energy for CHIKV diagnostic component is CHK152.

**Keywords:** energy affinity; CHK152; CHK265; E1E2 CHIKV; molecular docking

## INTRODUCTION

Chikungunya virus (CHIKV), a pathogen of the genus alphavirus and the family *Togaviridae* [(1)], but causes excessive complaints of joint pain so that the patient cannot act as usual so that it can materially harm[(2,3)]. Non-compliance with the number of cases reported with the number of sufferers is still common[(4)], this is due to the fact that the examination is still based on the initial symptoms of fever. The fever is similar to fever in other diseases caused by mosquitoes such as dengue[(5-7)], zika[(8)], malaria[(9)]. To establish the right diagnosis in patients with chikungunya is not easy. A practical tool for proper medical treatment is needed to reduce the risk of misdiagnosis[(10)].

One practical alternative chikungunya examination is to use an immunochromatography method which is a specific antigen or antibody detection method in a sample that utilizes the immunological reaction principle, namely the presence of antibody antigen. Imonological reactions occur along the capillary membrane by depending on the migration of micro/ nano particles along the capillary membrane so that they can be applied in the form of strip tests.

Initially the use of this method to detect hCG (human chorionic gonadotropin) in pregnant women, was then developed to detect the presence of specific antigens, and is now also used to detect the presence of specific antibodies[(11)].

Antibodies that can neutralize the chikungunya virus have been widely reported.In this study we focused on studying CHK 152 antibodies which neutralized the process by stabilizing the surface area of the virus and inhibiting the opening of fusion loops[12] and CHK 265 which inhibited the life cycle of viruses through neutralizing infection by blocking entry in post steps attachment and pre-fusion in cells[13]. The structure data of these two antibodies have been stored in the protein data bank (PDB).

Fast Interaction Refinement in molecular docking (Firedock) is a method used to refine and re-evaluate the proteins docking process. Improvement approaches are carried out through van der waals interactions, Atomic Contact Energy (ACE), electrostatic bonds and free energy binding[14,15].

The purpose of this study is to study the structure-affinity relationship of antibody binding to antigens usingdocking methodand to determine the important functional of antibodies that is responsible for good binding. It is expected that a low interaction affinity energy is obtained for the purpose of making the chikungunya rapid test component.

## METHODS

### Preparation of Receptor and Ligands

Refinements structure. A crystal structure of Fab CHK152 (PDB ID: 3J30) and glycoprotein E1E2 CHIKV (PDB ID: 3J2W) was docking. The residu type of cysteine and histidine were adjusted manually,based on their specific chemical environment. A box of TIP3Pwater model was added to the system, where the shortest distancebetween protein and the edge of the box was 10 Å. The system was neutralized by the adding sodium ions.

All the minimization and molecular dynamics (MD) simulations were performed using AMBER16[16] Initial minimization of 5000 steps using the steepest descent algorithm was conducted to water molecules. Then, 5000 steps of conjugate gradient minimization with 500 kcal/mol$Å^2$ of harmonic restraints were applied to the backboneatoms. A final 10000 steps of unrestrained conjugate gradientminimization were performed to remove any sterical clashesamong the atoms.The system was gradually heated to 50°C ≈ 310 K, its optimum temperature, for 60 ps in NVT ensemble using harmonicrestraints of 5 kcal/molA2 on the backbone atoms.Furthermore, 1000 ps of NPT equilibration was performed,where harmonic restraints on the backbone were slowlydecreased by 1 kcal/molA2 until it reaches 0. Then, 10 ns of theproduction run in NPT ensemble was performed with allhydrogen atoms constrained using the SHAKE algorithm. The temperature was controlled with Langevin thermostat with acollision frequency of 1 ps−1, whereas the pressure was controlledusing Berendsen barostat with the coupling constant of1 ps and the target pressure of 1 bar. The time step value duringthe production stage was 2 fs. The nonbonded cutoff value of 9 A was used, and the long-range electrostatics was treatedusing particle mesh Ewald. The MD trajectories were analyzed using the cpptraj module in AmberTools16.

The same thing was done to improve the CHK265 docking (PDB ID: 5ANY)

### In Silico Study of Protein-Protein Interaction

After refinement using MD, which were later refined using FireDock server (http://bioinfo3d.cs.tau.ac.il/FireDock/),an online tool that optimised, refined, reshuffled, and rescored the side chains interface candidate solutions. It also amends the orientation of the relative molecules by confining theflexibility to the side-chains of the interacting surface and allow the movements of small rigid-body[17-18]. Preparing antigens as receptors and antibodies as ligands, choosing antigen antibodies in complex types, choosing full refinement levels.

## RESULTS

The structure of Fab CHK265 and CHK152 crystals obtained at PDB comes from cryo-EM which has a low resolution. Therefore, it is necessary to refine using molecular dynamics to obtain a better structure[19] (table 1). Due to the absence of a complex between the antigen and the CHK152 antibody in the PDB database, it is necessary to combine the E1E2 CHIKV antigen coordinates (PDB ID: 3J2W) with CHK152 antibodies (PDB ID: 3J30). Otherwise CHK265, the crystal structure is already in the form of a complex with E1E2 CHIKV (PDB ID: 5ANY). Then the refinement results are submitted to the Firedock server (http://bioinfo3d.cs.tau.ac.il/FireDock/index.html) to obtain bond energy calculations.

Table 1. Direct calculation of binding energy of cryo-EM structures using FireDock

| Fab | PDB ID | Binding energy (kcal/mol) | Attractive VdW (kcal/mol) | Repulsive VdW (kcal/mol) | H-Bond Energy (kcal/mol) |
|---|---|---|---|---|---|
| CHK265 | 5ANY | -11.82 | -17.42 | 4.46 | -0.52 |
| CHK152 | 3J30 | -18.74 | -26.07 | 40.43 | -6.06 |

Cryo-EM: Cryo-electron microscopy, CHK: Chikungunya virus, PDB: Protein Data Bank

Table 2. Calculation of binding energy of cryo-EM structures using FireDock

| Fab | PDB | Binding Energy (kcal/mol) | Attractive VdW (kcal/mol) | Repulsive VdW (kcal/mol) | H-Bond Energy (kcal/mol) |
|---|---|---|---|---|---|
| CHK265 | 5ANY | -29.96 | -26,11 | 8.07 | -4.76 |
| CHK152 | 3J30 | -125.78 | -69.84 | 20.43 | -12.24 |

Cryo-EM: Cryo-electron microscopy, CHK: Chikungunya virus, PDB: Protein Data Bank

Refinement using dynamic molecular is done in a vacuum to avoid bad contact that can make steric obstruction resulting in repulsion. It is proven that after the complex is cleared the global bond energy, Van der Waals bonds and hydrogen bonds are stronger than the initial conformation (table 2).

Next, visualization and alignment was performed using Biovia Discovery Studio (BDS) with the aim of seeing the homology of the E1E2 CHIKV that will be used when docking CHK265 and CHK152 with PDB ID 5ANY and 3J2W respectively. (Figure. 1)

**A.** **B.**

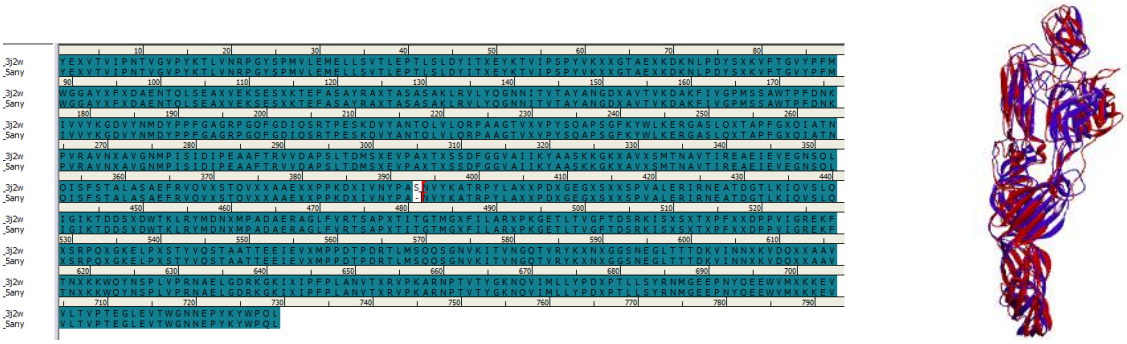
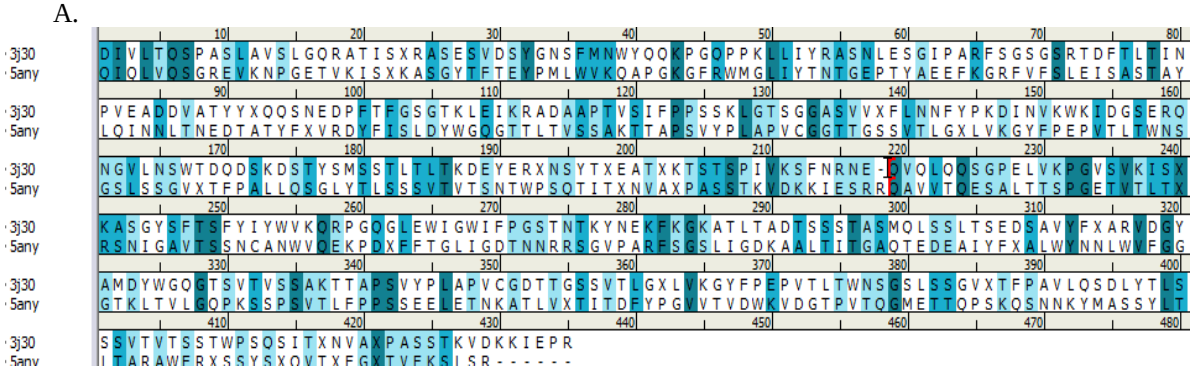


Figure 1. (A) Multiple sequence alignment from cryo-EM dari beberapa glycoprotein E1E2 CHIKV. (B) The superimposition of are glycoprotein E1E2 CHIKV. PDB ID 3J2W and 5ANY, which are visualized in blue and red colors, respectively

The results of the amino acid sequence alignment of the two antigens show that the two antigens have a fairly high similarity, except that the root mean square deviation (RMSD) value is 10.715 Å, indicating that the similarity is quite far.

A.



B. C.

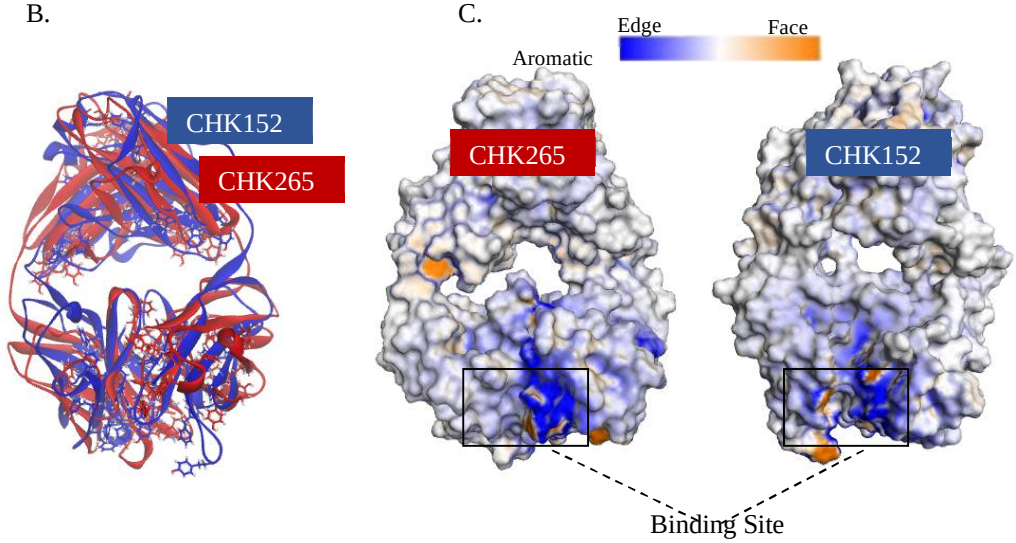



Figure 2. (A) Multiple sequence alignment from CHK265 and CHK152 (PDB ID 5ANY and 3J30). (B) The presence of aromatic residues in CHK265 (red cartoon) and CHK152 (blue cartoon). (C) The aromaticity of CHK265 (left) and CHK152 (right) which are visualized by solvent surface. The edge and face sides of aromatic residues are colored in blue and brown, respectively. Non aromatic residues are colored in white

In contrast to antigens, antibody alignment results show that the two antibodies have different amino acid sequences. (Fig. 2. A) Further analysis of exposure to aromatic compounds (Fig. 2. B), the number of aromatic compounds in both antigens is only 1 compound, CHK265 is more. Using the surface model is increasingly clear in observing the exposure of aromatic compounds. (Figure 2.C) The part that many aromatic compounds are exposed to is usually the active side of the binding to the antigen[20].

**In Silico Study of Protein-Protein Interaction**

We can learn in the picture. 3 and 4 that the Crystal structure superimpose and docking of the CHK265 and CHK152 complexes give RMSD values respectively 1.365 and 0.402 Å (figure 3.A and 4A). Fab's second binding location is on the E2 envelope glycoprotein (Figure 3.B and 4.B), but is on a different domain. For CHK265 binding is on B domain, while CHK152 is A domain[21].

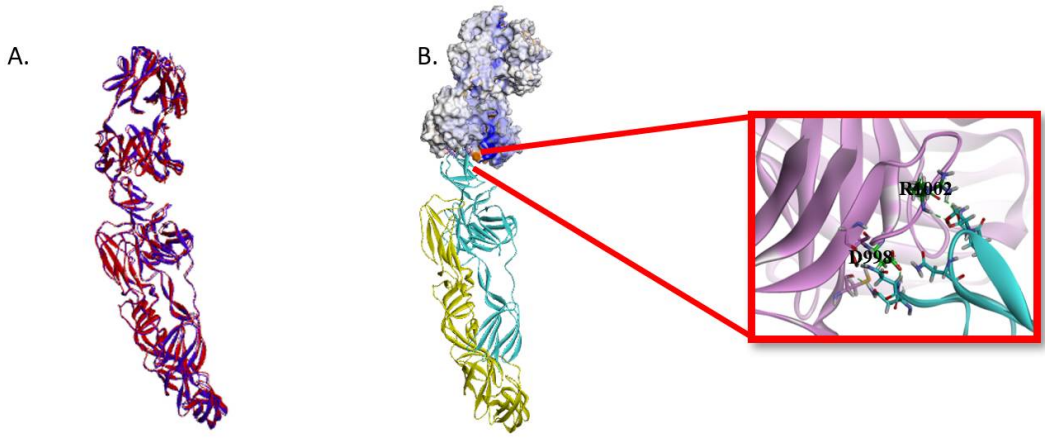



Figure 3. (A) Superimpose CHK265.Crystal structure(blue cartoon and docking (red cartoon). (B) Antigen-antibody interaction from Fab CHK265. Antibody (purple cartoon, E1antigen (yellow cartoon) and E2antigen (blue cartoon) the residual structure of amino acid which makes antigen-antibody bonds strong. Aromatic residues (orange sticks), acids (light green sticks) and bases (dark greensticks).

In the CHK265 complex, the interactions that form hydrogen bonds are very few. Amino acids that play a role in maintaining structural stability through hydrogen bonds are D998 and R1002. (Table. 3) Whereas the CHK152 complex, amino acids has the most role in strengthening interactions including Y761, D1046, R783 and Q947. So that the stability of the bond is maintained and the free energy produced is very good (table 4).

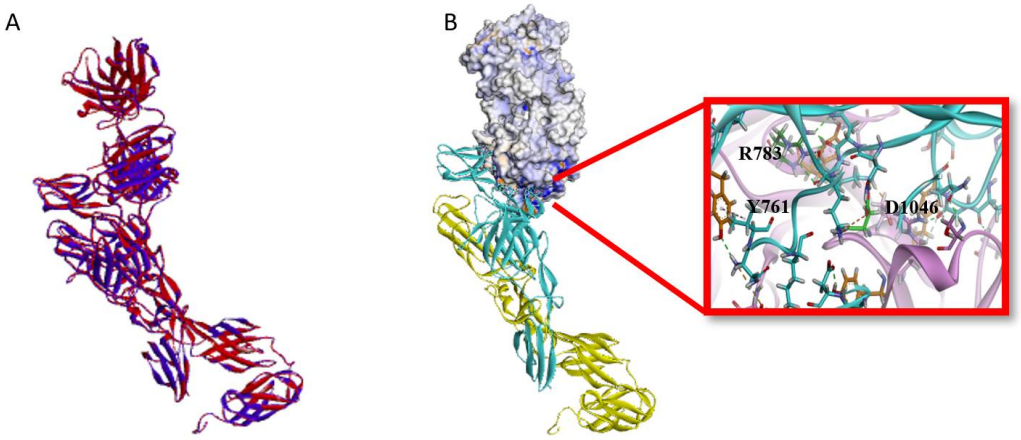



Figure 4. (A) Superimpose CHK152. Crystal structure (blue cartoon) and docking (red cartoon). (B) Antigen-antibody interactions of Fab CHK152. Antibody (purple cartoon), E1antigen (yellow cartoon) and E2 antigen (blue cartoon) the residual structure of amino acid which makes antigen-antibody bonds strong. Aromatic residues (orange sticks), acids (light green sticks) and bases (dark green sticks)

## DISCUSSION

Crystals from cryo-EM have low resolution so that in precision in determining the result structure is less accurate[19]. Briefly minimized (10 ns) can improve the binding energy between antigens and antibodies. The resolution of E1E2 CHIKV (PDB ID: 3J2W) is 5 Å, while the E1E2 CHIKV complex is CHK265 (PDB ID: 5ANY) of 16.9 Å. With a large enough difference in resolution values will result in the introduction of amino

acids will be less appropriate, so the RMSD value obtained is quite high. So it is feared that the interaction between antigens and antibodies is not appropriate. Therefore we feel the need to know the RMSD value between the crystal structure and the docking result structure. The resulting low RMSD value indicates that the structure of the docking is very good.

Tabel 3. Hydrogen bond from CHK265 with E1E2 CHIKV

| No. | Fab CHK265 | | E1E2 CHIKV | | H-Bond Distance |
|---|---|---|---|---|---|
| | Residu | Atom | Residu | Atom | (Å) |
| 1 | C980 | O | N604 | HD21 | 2.03713 |
| 2 | D998 | OD1 | N604 | OD1 | 2.42533 |
| 3 | D998 | OD1 | N605 | H | 1.85698 |
| 4 | T999 | OG1 | N605 | HD22 | 1.87623 |
| 5 | N1000 | HD22 | N573 | OD1 | 1.8499 |
| 6 | R1002 | O | Q570 | HE22 | 1.87037 |
| 7 | R1002 | H | S571 | OG | 2.04422 |
| 8 | R1002 | HH21 | S571 | O | 2.16953 |
| 9 | G1012 | O | N605 | HD21 | 2.19997 |

Tabel 4. Hydrogen bond from CHK152 with E1E2 CHIKV

| No. | Fab CHK152 | | E1E2 CHIKV | | H-Bond Distance |
|---|---|---|---|---|---|
| | Residu | Atom | Residu | Atom | (Å) |
| 1 | Y761 | O | K641 | H | 2.05712 |
| 2 | Y761 | OH | N394 | H1 | 2.39463 |
| 3 | Y761 | O | G640 | HA2 | 2.7764 |
| 4 | Y761 | HA | K641 | O | 2.90053 |
| 5 | G762 | O | R638 | HE | 1.79931 |
| 6 | Y782 | HH | H619 | ND1 | 1.84029 |
| 7 | R783 | HH | H619 | ND1 | 2.6666 |
| 8 | R783 | HH12 | H619 | O | 1.77384 |
| 9 | R783 | HH21 | K620 | O | 2.55362 |
| 10 | R783 | HH22 | H619 | O | 1.92989 |
| 11 | R783 | HH22 | K621 | O | 2.65847 |
| 12 | E788 | OE1 | K620 | HZ3 | 1.82494 |
| 13 | E826 | OE2 | K397 | HZ1 | 1.76857 |
| 14 | E826 | OE2 | N394 | N | 4.95777 |
| 15 | Q947 | OD1 | D601 | OD1 | 4.36854 |
| 16 | Q947 | H1 | N580 | OD1 | 2.14613 |
| 17 | Q947 | HA | N580 | OD1 | 2.6951 |
| 18 | Q947 | HA | D601 | OD1 | 2.24371 |
| 19 | V948 | H | D601 | OD1 | 2.05727 |
| 20 | V948 | HA | N580 | O | 2.53995 |
| 21 | Q949 | H | N580 | O | 2.80991 |
| 22 | S974 | H | Q582 | OE1 | 2.14604 |
| 23 | S974 | HB3 | Q582 | OE1 | 2.6371 |
| 24 | Y979 | HH | D447 | OD2 | 2.01701 |
| 25 | S1001 | HG | D446 | OD2 | 1.60469 |
| 26 | S1001 | HB2 | D446 | OD2 | 2.78428 |
| 27 | N1003 | HD22 | D446 | O | 2.17396 |
| 28 | R1044 | HD2 | G581 | O | 2.73123 |
| 29 | R1044 | HH11 | G581 | O | 1.80464 |
| 30 | D1046 | OD2 | N618 | HA | 2.8237 |
| 31 | D1046 | OD2 | K620 | NZ | 4.84989 |
| 32 | E1046 | OD1 | K621 | NZ | 5.52212 |
| 33 | D1046 | OD1 | N618 | HD21 | 2.47683 |
| 34 | D1046 | OD2 | N618 | HD22 | 1.83387 |
| 35 | D1051 | OD1 | K620 | HZ2 | 2.01695 |
| 36 | Y1052 | HH | T599 | O | 2.13589 |

Hydrogen and electrostatic bonds are non-covalent bonds that are actually weak in strength, but with the increasing number of hydrogen bonds that are formed, it will streng then the binding between antigens and antibodies.

At CHK265 hydrogen bonds that occur in antigens and antibodies are formed from D998 which are negatively charged amino acids with N604 and N605 which are neutral, besides that the bond occurs between R1002 and Q570 and S571 which are neutral too, so the resulting affinity is weak. In addition CHK265 has cross reactive properties, because it is able to interact with other alphaviruses so that they are not sensitive[13]. Unlike CHK152, the binding of antigens and antibodies is surrounded by aromatic amino acids which are neutral, especially Y761 and in the middle there are positive (R783) and negative (D1046) amino acids which mutually stabilize the structure so that the hydrogen bonds are very numerous. So the resulting energy is relatively strong in the binding of antigens and antibodies.

## CONCLUSION

Therefore CHK152 which has a relatively high affinity we choose as lead compound protein which will be used in designing components for the anti chikungunya rapid test. Conversely CHK265 because it has the ability to recognize all alphavirus types should be used for therapy.

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