Probing the interaction of HAV4 peptide (Ac-SHAVAS-NH$_2$) with E-cadherin domain EC1-EC2 by molecular docking

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Abstract. E-cadherin-derived peptides, which are peptide sequences taken from their binding sites, have been shown to be able to modulate cross-cell junction so as to facilitate drug delivery to their targets. One of the E-cadherin-derived peptides, HAV4 (Ac-SHAVAS-NH$_2$) previously has been studied by in vitro and in vivo where the activity of the HAV4 peptide is able to modulate the tight junction between E-cadherin cells. The physicochemical properties of macromolecule such as protein or peptide are very important to understand the function of the macromolecule. Computational chemistry method has good advantage for understanding physicochemical properties in studying the structure, dynamic, and interaction of molecules in support of experimental data. The purpose of this study is to prove the inhibitory activity of interaction between E-cadherin cells and the best conformation of HAV4 peptide by its binding energy and active site parameters. This study uses molecular dynamic simulation and molecular docking. The results of research in 27°C temperature at 20000 ps molecular dynamic simulation which prove the HAV4 peptide has the best conformation at the 2508 ps with conformation energy -47704.4 kJ.mol$^{-1}$. Based on the results of molecular docking simulation, interaction between HAV4 peptide and E-cadherin domain EC1-EC2 show binding energy of -24.393 kJ.mol$^{-1}$ with the Ki inhibition constant of 53.17 µM. This strong interaction occurs in the adhesion arm-acceptor pocket area with residual active sites Trp2, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Asp90, Met92.

1. Introduction
There are three important properties for understanding the function of macromolecules such as proteins [1]. These three properties are structure, dynamic and interaction which play an important role in determining the physicochemical properties of molecules [1–3]. To understand these three properties can be observed in vitro (experimentally with instruments) or in silico (computationally connecting theoretical chemistry with experiments). In experiments, investigating the intermolecular interactions in protein-protein complexes are often difficult, time consuming and expansive. Experimentally the interaction between protein-protein can be determined by calorimetric studies [4] and resonance spectroscopy [5] can provide information about rate constants and bond strength. The analysis of X-ray structure and NMR techniques work well on three-dimensional protein-protein complexes. However, they can only be used limited degree because crystallization of the proteins structure is not easy to do [6]. The application of computational method is a solution for studying molecular structure, dynamics and interactions in supporting the results of experimental data [7].

In its development, computational chemistry divided into four methods, there are ab-initio, semi-empirical, density functional theory (DFT), and molecular mechanics [7,8]. Very large molecules such
as the peptide HAV which sequence of Ac-Ser-His-Ala-Val-Ala-Ser-NH₂ that interact with E-cadherin are viewed by computational methods that can be modeled completely ignoring quantum mechanics. Where does not use a quantum mechanics approach but with molecular mechanics. Molecular mechanics provides a simple algebraic expression for calculating the total energy of a compound of molecules, without having to calculate the wave function or electron density [9].

The molecular docking approach can be used as a modeling of the interactions between small molecules (ligands) with protein or protein with protein, which makes it possible to characterize the behavior of molecules (ligands) at the binding site of the target protein as well as to explain basic biochemical processes [10]. The process of docking is carried out in two basic steps, there are prediction of ligand conformation and its position and orientation within the site and the value of binding affinity where in docking the protein structure is considered a rigid object. The molecular docking approach is an alternative to experimental investigations in drug design because of its ability to predict the position of ligand orientation in complex bonds with protein as receptor at high accuracy [11,12].

The difficulty of healing for brain diseases such as parkinson's and alzheimer's are due to the presence or a protective membrane of the brain, that is the blood-brain barrier (BBB) [13]. Many large molecules such as peptides and protein cannot easily pass through the BBB due to their size, charge and hydrophilicity properties, so drug molecules must have optimal physicochemical properties [14]. In order to penetrate the BBB, the molecules enter through two transport routes, there are the cell membrane or transcellular pathway and through barrier cells (paracellular pathways) [15]. The path that might be passed is paracellular. However, not all peptides can pass through it, this is due to the presence of tight junctions or so-called tight junctions between cells that show minimal porosity so that only small molecules (<11Å) and ions can pass this pathway [16,17].

The formation of tight junctions can be disrupted by modulating interactions between cadherin cells where this modulation can increase junction porosity thus opening up the possibility of increasing drug delivery to the target [18,19]. The inhibition of intercellular junction interactions and increased porosity due to the role of the peptide E-cadherin derivative fragments of groove and bulge sequences [20]. HAV4 peptide is peptide E-cadherin derivative fragment in groove area. Aminof et al (2016) have demonstrated in vitro and in vivo the ability of HAV4 peptide to deliver drug molecules in the BBB and overcome biological barriers such as: metabolic enzyme, tight junction and efflux pumps that prevent drug transport from the systemic circulation to the brain [21]. Siahaan et al (2020) have conducted research in silico on the interaction of ADTC7 cyclic peptide with E-cadherin protein, the result show ADTC7 cyclic peptide with E-cadherin has binding energy of -24.56 kJ.mol⁻¹ and its active site of residues were Val3, Ile24, Pro5, Pro6, Ile7, Ser8, Leu21, Val22, Gln23, Lys25 [22]. Many cadherin peptides have been studied, such as ADT but the molecular interaction mechanism between HAV4 peptides and E-cadherin has not been widely studied.

This study aims to determine the peptide conformation of HAV4 which interacts strongly with E-cadherin (EC1) protein and to determine the active site and binding energy using the molecular docking method. The hypothesis of the molecular interaction between the amino acids of HAV4 will interact with the amino acid E-cadherin to inhibit the cadherin-cadherin interaction.

2. Methods

The amino acid sequence of E-cadherin protein was retrieved from RSCB PDB [23]. E-cadherin domain EC1-EC2 with code pdb (2O72) act as a receptor and the peptide HAV4 act as a ligand. HAV4 peptide was made using PyMol software. The structure of the EC1-EC2 domain and the HAV4 peptide was shown in figure 1.
2.1. Optimization of HAV4 peptide
The optimization of HAV4 peptides was carried out using the GROMACS v.5.1.2 [24]. Optimization with simulating molecular dynamic (MD) to determine the dynamics of the HAV4 peptide so that the conformation and minimum energy were obtained in the water solvent and after the ion was added. MD simulation was carried out for 20 ns to obtain 20000 conformations. In the preparation system, a charmm27 force field was applied and the water solvent tip3p was added to the 1 nm cube space [2]. Next, 4Na\(^+\) and 4Cl\(^-\) ions were added to the system to obtain a physiological concentration of 0.15 M. Energy minimization in the system was carried out to relax excess forces on the system. The equilibrium between the peptide conformation and the solvent was expected at temperature of 300 K and pressure of 1 atm with 100 ps. After the system was equilibrium, a trajectory process by simulating MD for 20 ns (20000 ps). The trajectory results were analyzed, RMSD (root-mean-square-deviation) C\(\alpha\) method for the initial structure and the total energy of the MD simulation for selects 10 conformations that have the lowest energy.

2.2. Molecular docking
Molecular docking was performed using Autodock 4.2 [25]. In molecular docking, there were two stages including Autogrid and Autodock. The search of conformation which E-cadherin as a rigid molecule and HAV4 peptide as a ligand that looks for protein binding site, the search uses the Lamarckian-Genetic algorithm to determine energy binding using a semi-empiric free energy field approach [26]. Set the number of algorithm and the number of evaluation processes to 150 and 10000000 to run the docking process.

3. Results and discussion
3.1. Optimization of HAV4 peptide
Molecular dynamic simulation with GROMACS was carried out with the aim of obtaining the HAV4 peptide which has the best optimization conformation, due to the preparation process of HAV4 peptide from pyMol was not in stable state. The peptide HAV4 undergoes a conformational change every picosecond to 20000 ps (20 ns). The change because the peptide in solution was a dynamic molecule which does not have only one conformation. The dynamic movement of HAV4 peptide could be seen from the simulation results of molecular dynamics on the conformation frame observed at each picosecond time. Molecular dynamic simulation can be divided into three stages, initialization, equilibration and production. The topology of system is cubic as shown in figure 2.
The topology of this system contains HAV4 peptide molecule given 4 Na\(^+\) and 4 Cl\(^-\) ions which are filled with water. The volume of the overall system is 42.9853 mm\(^3\). When the simulation was carried out, the HAV4 peptide in middle box will run randomly. Furthermore, the minimization stage was carried out to relax the system in order to avoid unwanted atomic contaminants. This stage results in a decrease in potential energy to \(-58144\) kJ.mol\(^{-1}\) so as to produce a more stable conformation. Next stage, equilibration was carried out to stabilize the system which is at constant temperature, volume and pressure. So the system reaches a constant state before undergoing molecular dynamic simulation. The final stage in GROMACS is the production process to produce a .trr file which is used for analysis the results of MD simulation, such as total energy and the value of RMSD (Root Mean Square Deviation) shown in figure 3.

![RMSD graph](image)

**Figure 3.** (a) RMSD graph (b) Energy graph.

The first analysis performed was an analysis of RMSD which aims to show the peptide in stable state. The RMSD value was very good if there was not sharp fluctuation and has a small value. RMSD fluctuations that were too sharp indicate the presence of peptide amino acid bonds that were released a certain distance so the peptide is unstable. Based on figure 3 (a), it could be seen that the graph fluctuation are not too sharp. However, there are peak at the several time ps which is marked by a change in the folding-unfolding state which indicates the interaction of amino acid bond was beginning disrupted. The second analysis, total energy was shown in figure 3 (b) where the MD simulation of HAV4 peptide results in changes for each picosecond. Every few picosecond there was conformation change from folding to unfolding and so on. Peptide conformations change because of break and form the hydrogen bond. The unfolding conformation occurs when the peptide was unstable so that it has a higher energy than the folding state. As shown in figure 4, it could be seen there was a slight change in the folding of conformation.

![Conformation with the highest energy and the lowest energy](image)

**Figure 4.** Conformation with the highest energy and the lowest energy.

In figure 4, The peptide conformation at 2508 ps with energy \(-47704.4\) kJ.mol\(^{-1}\) provide information the HAV4 peptide is folding. Furthermore, the peptide conformation at 4186 ps with energy \(-45566.4\) kJ.mol\(^{-1}\) provide information the HAV4 peptide is folding too. It is because the thermal stability of the conformation is ability of the peptide to mountain conformation with the high energy. The stability of
the conformation of a peptide is due to presence of a peptide bond (CO-NH) which has a rigid planar structure, due to the interaction between the double bond electrons of the carbonyl group with the C-N peptide bond. This effect is a resonance that is thought to be sharing of electrons between bonds so the bond was double faint. In general, peptide bonds have a trans conformation, as shown in figure 5.

In figure 5, it is shown that the highest energy and lowest energy conformations give a dihedral angle value that is nearly 180° between the amino acids histidine and serine. This dihedral angle provides information that a stable trans conformation is formed. In the trans, peptide bonds are difficult to undergo rotation. Given the planar nature of the peptide bond which is rigid, rotation is not permitted in the peptide bond. However there is a potential for rotation around the Cα-N and Cα-C bonds. This dihedral angle rotation determine the backbone conformation of the peptide. The angular rotation of Cα-N and Cα-C are referred to as \( \phi \) (phi) and \( \psi \) (psi). The rotation angles of \( \phi \) (phi) and \( \psi \) (psi) in the highest and lowest energy conformations can be seen in table 1.

| \( \phi \) (phi) angle | Conformation at 4186 ps (highest energy) | Conformation at 2508 ps (lowest energy) |
|------------------------|------------------------------------------|----------------------------------------|
| C5-N7-C9-C16            | -99.526                                  | -82.523                                 |
| C16-N18-C20-C34         | -146.505                                 | 76.979                                  |
| C34-N36-C38-C44         | -59.381                                  | -76.405                                 |
| C44-N46-C48-C60         | -58.508                                  | 62.017                                  |
| C60-N62-C64-C70         | -59.783                                  | -156.502                                |
| C70-N72-C74-C81         | -118.435                                 | -73.798                                 |

| \( \psi \) (psi) angle | Conformation at 4186 ps (highest energy) | Conformation at 2508 ps (lowest energy) |
|------------------------|------------------------------------------|----------------------------------------|
| N7-C9-C16-N18          | -0.142                                   | -14.108                                 |
| N18-C20-C34-N36        | 10.054                                   | 50.876                                  |
| N36-C38-C44-N46        | -42.448                                  | -39.653                                 |
| N46-C48-C60-N62        | -48.810                                  | 60.677                                  |
| N62-C64-C70-N72        | -55.802                                  | 158.930                                 |
| N72-C74-C81-N82        | -56.098                                  | -59.512                                 |

From the table 1, each conformation has a different backbone shape which is determined by the rotation angle \( \phi \) (phi) and \( \psi \) (psi). The relationship of dihedral rotation angle is used to obtain information about the peptide folding and the stability of the peptide. As previously explained, the trans conformation is more stable than the cis because the steric effect in trans is smaller and has a minimum energy. Based on table 1 above, the 2508 ps conformation with the lowest energy has a lot more trans...
than the 4186 ps conformation with the highest energy by looking at the angle of rotation of dihedral backbone which have a value close to 180°.

Furthermore, at the lowest energy approach is carried out by looking at the low energy to find the most stable peptide. In this analysis, 10 conformation models were selected from 20000 conformation. The minimum total energy data and RMSD could be seen in Table 2.

Table 2. The total energy resulted from optimization of the HAV4 peptide ligand

| Code | Conformation (ps) | RMSD (nm) | Total Energy Minimum (kJ/mol) |
|------|------------------|----------|-------------------------------|
| K1   | 2508             | 0.141524 | -47704.4                      |
| K2   | 12714            | 0.132685 | -47687.6                     |
| K3   | 11212            | 0.21686  | -47642.9                     |
| K4   | 788              | 0.320556 | -47631.6                     |
| K5   | 9080             | 0.152965 | -47622.6                     |
| K6   | 8948             | 0.169129 | -47619.5                     |
| K7   | 7372             | 0.159283 | -47580.8                     |
| K8   | 19384            | 0.172756 | -47578.2                     |
| K9   | 3561             | 0.126928 | -47559.8                     |
| K10  | 14196            | 0.189527 | -47556.4                     |

Based on the table 2, taking the 10 conformations with the lowest energy are used to find the best conformation to interact with receptor in the molecular docking process.

3.2. Molecular Docking

Molecular docking is used to predict the orientation of ligand bonds with receptor or form stable complex. Docking process is divided into two types, blind docking and oriented docking. All types of docking process consist of two stages, autogrid and autodock. In autogrid stage, the gridbox parameters of the active site of E-cadherin with HAV4 peptide unknown, so a blind docking process was carried out, which set the gridbox size to 60x60x60 Å with spacing 0.636 Å. In autodock stage, the Number of GA Runs parameter is changed to 150 with calculation of 20000000 times resulting in 150 poses for the ligand to obtain the best Gibbs free energy (ΔG). More negative the ΔG value, the better stability level. The result of docking peptide ligand interacting with receptor are obtained in Table 3.

Table 3. The result of docking HAV4 peptide interacting with receptor of E-cadherin.

| Code | Pose | ΔG (kJ/mol) | Ki (mM) |
|------|------|-------------|---------|
| K1   | 54   | -14.60      | 2.74    |
| K2   | 126  | -4.89       | 138.41  |
| K3   | 125  | -5.73       | 99.76   |
| K4   | 73   | -8.66       | 30.17   |
| K5   | 57   | -9.58       | 20.86   |
| K6   | 103  | -8.07       | 38.63   |
| K7   | 69   | -2.67       | 337.31  |
| K8   | 90   | -5.14       | 113.85  |
| K9   | 3    | -8.45       | 33.29   |
| K10  | 71   | -5.56       | 105.08  |

The binding energy taken from 150 poses for each conformation is the most negative with a large population because it has strongest interaction. Table 3 show that K1 conformation has the best stability.
seen from the $\Delta G$ of -14.60 kJ mol$^{-1}$ and has a small $K_i$ value of 2.74 mM. The value of $\Delta G$ contributes to the interactions between protein and ligand, these interaction occur intermolecularly, Van der Waals or hydrogen bonds. The interaction that occurs in the HAV4 peptide with E-cadherin could be seen in Figure 6.

![Figure 6](image)

**Figure 6.** Visualization of (a) complex E-cadherin with HAV4 K1 peptide (b) contact interaction of E-cadherin receptor residues with HAV4 K1 peptide

From figure 6 (a) showed interaction of E-cadherin protein with HAV4 ligand to form a complex and figure (b) the result of molecular docking on K1 show presence of hydrogen bonds and hydrophobic group interactions. The hydrogen bonding is through residues of Val4...Lys25 and His2...Glu23 with the respective hydrogen bond types NH...O and ND1...O at a distance 2.81 Å and 3.07 Å, respectively. In addition to hydrogen interactions other residue contacts occur such as hydrophobic interaction Trp2, Val3, Ile4, Ile24, Ser26, Glu89 and Met 92.

### 3.3. Validation molecular docking

It is necessary to validate the docking process by using the docking method. was carried out by re-tethering the docking ligand pose that has been separated from receptor. Redocking also required to determine the best RMSD value that < 2 Å requirement. Redocking process carried out on the active site ligand which is obtained from docking results. Determination of gridbox focused on active site region to limit space for searching ligand conformation, the gridbox was set to 40x40x40 Å with spacing 0.327 Å. The results of redocking peptide interacting with receptor are obtained in table 4.

| Code | Pose | $\Delta G$ (kJ/mol) | $K_i$ (µM) | RMSD (Å) |
|------|------|---------------------|-----------|----------|
| K1   | 67   | -24.39              | 53.17     | 1.72     |
| K2   | 107  | -12.88              | 5.52      | 1.95     |
| K3   | 102  | -15.73              | 1.76      | 3.82     |
| K4   | 140  | -15.73              | 1.74      | 1.95     |
| K5   | 143  | -14.89              | 2.50      | 2.73     |
| K6   | 31   | -11.75              | 8.73      | 3.82     |
| K7   | 52   | -11.08              | 11.43     | 1.99     |
| K8   | 147  | -10.92              | 12.16     | 1.86     |
| K9   | 101  | -13.13              | 4.97      | 2.84     |
| K10  | 96   | -15.98              | 27.53     | 2.97     |

Based on the redocking results shown in table 4, K1 has smallest $\Delta G$ value compared other conformations, which is -24.39 kJ mol$^{-1}$ so the level of affinity for E-cadherin is higher because the energy needed to bind receptor is lower. By looking at the lower energy produced, the more stable interaction formed between amino acids from protein with peptide ligands, so the HAV4 peptide is able
to inhibit interaction between cadherins. The value of Ki (inhibition constant) is directly proportional to \( \Delta G \), where to produce an inhibitory effect require small concentration, so the value of Ki was getting small. K1 has a Ki value of 53.17 µM which is strong inhibition range. The RMSD value of K1 is 1.72 Å, so it can be said that the molecular docking method was valid because RMSD value is < 2 Å. The interaction formed between HAV4 peptide ligand with E-cadherin from redocking result could be seen in figure 7.

![Figure 7. Visualization of contact interaction E-cadherin receptor residue with the HAV4 K1 peptide ligand from the results of redocking using (a) Ligplus (b) Autodock 4.2 software.](image)

### Table 5. Hydrogen bond and binding site of HAV4 peptide.

| Code | Interaction   | Hydrogen Bond | Amount of Residue |
|------|---------------|---------------|-------------------|
| K1   | Ser1...Lys25  | r (Å)         | Type              |
|      | 2.72          | NH...O        | 10                |
|      | His2...Asp90  | 2.61          | NE2...O           |
|      | Ala3...Trp2   | 3.06          | O...NH            |
| K2   | Ser1...Gln23  | 3.10          | NH...O            |
|      | Val4...Lys25  | 2.63          | NH...O            |
| K3   | His2...Val3   | 2.70          | ND1...O           |
|      | Val4...Lys25  | 2.74          | NH...O            |
|      | Ala5...Lys25  | 2.82          | NH...O            |
| K4   | His2...Gln23  | 2.68          | ND1...O           |
|      | Ala3...Leu21  | 2.85          | NH...O            |
|      | Val4...Leu21  | 2.76          | NH...O            |
|      | Ser6...Ser8   | 2.88          | OG...NH           |
| K5   | Ser1...Leu21  | 2.82          | NH...O            |
|      | His2...Leu21  | 2.96          | NH...O            |
|      | His2...Ser8   | 2.91          | ND1...O           |
| K6   | His2...Lys25  | 3.08          | ND1...O           |
|      | Ala3...Lys25  | 2.07          | NH...O            |
| K7   | Val4...Lys25  | 3.03          | NH...O            |
| K8   | His2...Gln23  | 2.80          | NE2...O           |
|      | Ser6...Leu21  | 2.86          | NH...O            |
| K9   | Ala3...Lys25  | 3.13          | NH...O            |
| K10  | Ser6...Lys25  | 2.50          | NH...O            |

Table 5 provide information on interactions with active site of protein to identify any interactions that occur between ligand and receptor. Based on visualization of redocking results, amino acid residues from K1 have the most interactions. Hydrogen bonding occurs through residues Ser1...Lys25,
His2...Asp90, Ala3...Trp2 with each type of hydrogen bond NH...O, NE2...O, O...NH at successive distances also 2.72, 2.61, 3.06 Å. Other residues that make contact with peptide ligand which have non-bonding interactions were generally electrostatic and van der Waals interactions. Figure 7 show that contact of amino acid residues of E-cadherin with peptides so that it is known active site of E-cadherin and HAV4 are Trp2, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Asp90, Met92. K1 conformation has a dihedral angles \( \psi \) (psi) and \( \phi \) (phi) on good peptide backbone chain could be seen in table 6.

| \( \phi \) (phi) angle | \( \psi \) (psi) angle | K1 |
|-----------------------|-----------------------|----|
| C2-N4-C6'-C7'         | N4-C6'-C7'-N12'       | 29.953 |
| C7'-N12-C14'-C23'     | N12-C14'-C23'-N25     | 97.733 |
| C23'-N25-C27'-C39     | N25-C27'-C29'-N31     | -69.336 |
| C39'-N31-C33'-C34     | N31-C33'-C34'-N39     | -1.041 |
| C34'-N39-C41'-C43     | N39-C41'-C43'-N45     | -154.579 |
| C43'-N45-C47'-C51     | N45-C47'-C51'-N52     | 107.568 |

Table 6. The angles of \( \phi \) (phi) and \( \psi \) (psi) on K1 after simulation molecular docking.

It is known that K1 after docking has more trans conformation in backbone chain than K1 before simulation molecular docking. This indicates that K1 binding with E-cadherin to form a stable conformation.

4. Conclusion
The optimization results of HAV4 peptide with GROMACS showed conformation at 2508 ps was the conformation with the lowest energy of -47704.4 kJ.mol\(^{-1}\). The conformation at 2508 ps with code K1 is a stable conformation for use as a ligand in docking process. The result of docking interaction between HAV4 with E-cadherin where K1 conformation has the lowest binding energy of -24.39 kJ.mol\(^{-1}\). Binding energy showed more stable interaction formed between amino acid from E-cadherin with peptide HAV4 in K1. So that is able to inhibit interaction between cadherins. In addition, the value of K1 was 53.17 µM which is strong inhibition range. The active site of E-cadherin and HAV4 are Trp2, Ile4, Ile24, Lys25, Ser26, Asn27, Ser78, Glu89, Asp90, Met92.

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