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# Assessment of Drug Binding Potential of Pockets in the NS2B/NS3 Dengue Virus Protein

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**Abstract.** Every year an endemic dengue fever estimated to affect over 390 million cases in over 128 countries occurs. However, the antigen types which stimulate the human immune response are variable, as a result, neither effective vaccines nor antiviral treatments have been successfully developed for this disease. The NS2B/NS3 protease of the dengue virus (DENV) responsible for viral replication is a potential drug target. The ligand-enzyme binding site determination is a key role in the success of virtual screening of new inhibitors. The NS2B/NS3 protease of DENV (PDB ID: 2FOM) has two pockets consisting of 37 (Pocket 1) and 27 (Pocket 2) amino acid residues in each pocket. In this research, we characterized the amino acid residues for binding sites in NS3/NS2B based on the hydrophobicity, the percentage of charged residues, volume, depth,  $\Delta G_{\text{binding}}$ , hydrogen bonding and bond length. The hydrophobic percentages of both pockets are high, 59 % (Pocket 1) and 41% (Pocket 2) and the percentage of charged residues in Pocket 1 and 2 are 22% and 48%, and the pocket volume is less than 700 Å<sup>3</sup>. An interaction analysis using molecular docking showed that interaction between the ligand complex and protein in Pocket 1 is more negative than Pocket 2. As a result, Pocket 1 is the better potential target for a ligand to inhibit the action of NS2B/NS3 DENV.

## 1. Introduction

Dengue virus (DENV) infection has become a global concern. There were over 50 million infection cases per year. Not only that, approximately 2.5% people from 500.000 severe dengue cases were reported to die [1]. The developing of an effective DENV treatment is needed to repress the mortality rate caused by DENV infection. In efforts to find a treatment for DENV, characterization of the enzyme binding site is an important step during the virtual screening of new inhibitors. One of the DENV essentials enzymes that essential to be characterized is NS2B/NS3 protease. This enzyme is known to has an essentials role in the maturation of DENV virion and viral replication process [2].

The previous research on NS2BCF-Gy-NS3PRO protease using metapocket analysis and molecular dynamics detected three amino acid residues in NS2B DENV protein and 19 amino acid residues in NS3 DENV protein as binding sites [3]. Other researchers working on NS2B-NS3 2FOM found 12 amino acid residues as binding sites using *CastP* [4]. Altering the structural conformation of DENV NS2B/NS3 has been proposed as a potential means of deactivating the protease, but some uncertainty remains regarding the exact structural arrangement of NS2B/NS3 [5]. However, it is estimated that 60% of



proposed or potential blocking ligands used in drug design never meet initial expectations because the protein targets are found not to be druggable [6]. To address this issue, we determined the residues and druggable binding pocket in NS3/NS2B based on five properties; hydrophobicity, the percentage of charged residues, volume, and depth using computational approach. The Computational approach played an important role to modeled the DENV protein. It can give us the better insight about the protein active site and way to inference the protein activity [7]. We also performed *in silico* molecular docking using 4-hydroxy panduratin A and panduratin A to model the interaction between ligands and protein [8].

## 2. Methods

### 2.1. Preparation of dengue virus NS2B-NS3 protease structure and standard ligand

The complex three-dimensional structure of the DENV NS2B-NS3 protease was obtained from the Protein Data Bank (PDB) database using the specific enzyme ID of 2FOM (<http://www.rcsb.org/pdb/>). The geometry optimization and energy minimization of DENV NS2B-NS3 protease were performed using *MOE2008.10 software*. We chose ligands 4-hydroxy panduratin A and panduratin A as a standard ligand. The structure of the ligands 4-hydroxy panduratin A and panduratin A were drawn using *ACD/Chemsketch* software version 12.01. *MOE2008.10* software was used for optimization of the ligands.

### 2.2 Binding site analysis

The binding sites on the surface protein of the NS3/NS2B of DENV were predicted using *Metapocket 2.0*, *DoGSiteScorer*, and *DEPTH* server. In *Metapocket 2.0* server, the structure of the protein used for prediction was in PDB format, and the amino acid residues will be shown as the result of binding site residues [9–11]. *DoGSiteScorer* was used to determine the 3D structure of the pocket of the protein using the calculation of size, shape and chemical features of the predicted (sub) pockets. PDB and SDF formats were used in this prediction [12,13]. *DEPTH* server calculated cavity volumes and predicted the location of small molecule-binding sites by measuring the closest distance of a residue/atom to the bulk solvent [14–16].

### 2.3 Molecular docking

*Molecular docking* is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. This process was done using the *MOE 2008.10* software through the MOE-dock tools. The method for placement, rescoring and refinement used a matching triangle with 2000000 iterations, London dG, and force field with a population of 1000 repetitions respectively [17].

## 3. Result and Discussion

### 3.1 Binding site prediction

Modeling and characterization of the active site of NS3/NS2B is an important step for screening the potential reactivity of drug candidates [7]. The application *Metapocket 2.0* calculates the geometric and energy interaction between the proteins and target ligands to locate favorable binding sites. The most favorable site is ranked first. NS2B/ NS3 protease of DENV structure contained two pockets. The first site consisted of 37 residues in a chain (7 polar residues, 22 nonpolar residues, and 8 charge residues) and the second binding site consisted of 27 residues (3 polar residues and 11 nonpolar residues and 13 charge residues) in chain A and B (Table 1).

**Table 1.** The amino acid residues in binding pocket 1 and 2 based on residue properties

Pocket	Hydrophobicity		Charge	
	Hydrophilic	Hydrophobic	(+ve)	(-ve)
1	THR_B118;THR_B120	MET_B49; <b>TRP_B69</b> ;VAL_B72	LYS_B117;LYS_B73;LYS_B74;LYS_B84	ASP_B71;ASP_B75;GLU_B86;GLU_B88
	THR_B122; <b>THR_B156</b>	<b>LEU_B76</b> ; <b>TRP_B83</b> ;LEU_B85		
	ASN_B119 <b>ASN_B152</b>	GLY_B87;TRP_B89;GLY_B121		
	ASN_B167	ILE_B123;GLY_B124;VAL_B146		
2	SER_A75;THR_A94	LEU_A53; <b>ALA_A56</b> ;ALA_A57;ILE_A73; <b>LEU_A74</b> ;ILE_A76; <b>LEU_A95</b> ;GLY_A96;TYR_B23;PRO_B106	<b>ARG_B24</b> ;ARG_A55; <b>ARG_A60</b> ;LYS_A87;LYS_B61;HIS_B60	GLU_A52;GLU_A54;ASP_A58;GLU_B19;GLU_A62;GLU_A89;GLU_A90
	ASN_B105			

<sup>a</sup>Black = residues detected by *Metapocket* 2.0; **Black bold**= residues detected by both *Metapocket* 2.0 and *DEPTH*

*DEPTH* server, an application that calculates cavity volumes and predicts the location of small molecule-binding sites, confirmed the *Metapocket* result that there were two cavities suitable for ligand binding. The small molecule predicted can bind to 8 amino acid residues in pocket 1 and 6 amino acid residues in pocket 2 (Table 1). The three amino acid residues with the highest probability of binding were, GLY\_B148, followed by ALA\_B164, and LEU\_B76, are located in pocket 1 as determined by *Metapocket* 2.0.

*DoGSiteScorer* determined the druggable pocket based on physical properties; pocket volume, pocket surface, lipophilic surfaces, and depth [12,13]. Measurement of physical properties has important implications for ligand design. Residues that have a volume  $\geq 500 \text{ \AA}^3$ , depth  $\geq 10.4 \text{ \AA}$ , enclosure  $\geq 0.28$ , percentage of charge residue  $< 26.3$  and hydrophobicity  $\geq -1.12$  generally have the highest probability of forming a druggable pocket [18]. The 2FOM protein has 13 residues which have more than  $10 \text{ \AA}$  of depth in a  $\beta$  chain. The total depth of each pocket is shown in Table 2.

**Table 2.** Drug score of pocket protein by assessment of the physical properties of the pocket using *DoGSiteScorer*

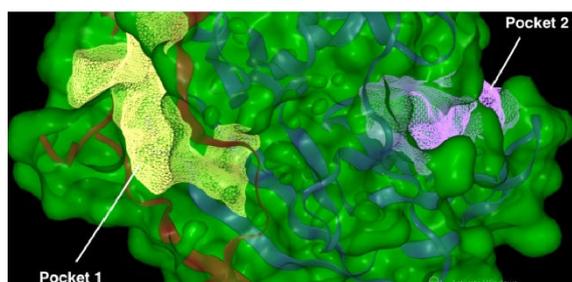
Pocket	Volume [ $\text{\AA}^3$ ]	Surface [ $\text{\AA}^2$ ]	Lipophilic surfaces [ $\text{\AA}^2$ ]	Depth [ $\text{\AA}$ ]	Drug Score
P1	600.00	976.78	576.15	18.92	0.81
P2	573.31	843.74	551.08	16.42	0.78
P3	236.54	457.92	295.86	13.18	0.54
P4	208.45	373.51	202.56	14.27	0.53
P5	154.69	338.81	195.60	9.34	0.32
P6	140.74	336.88	205.71	8.45	0.25
P7	117.70	258.98	184.42	6.34	0.16

legend: undruggable => druggable



The polarity influences the hydrogen bond form because it occurs due to a bond being polar. Hydrogen bonds confer directionality and specificity to the intramolecular interactions in the structures. This interaction is particularly important for proteins, where the hydrogen bond provides the organization for distinct folds and also provides the selectivity in the protein-ligand interactions [19]. So, the number of polar and nonpolar residues will affect the degree of hydrogen bonding in ligand-NS3/NS2B protease complexes. Since the small compounds or ligands will easily find their binding sites at the hydrophobic pocket, hydrophobic pockets are preferable as drug targets. Based on Table 1, both Pocket 1 and 2 have high percentages of hydrophobicity, 59% and 41%, which indicated both

pockets are good for drug target. Furthermore, potential binding sites with a lower frequency of amino acid residues in a charged state are favored as drug targets. The preferable range of charged residues is 10 to  $\leq 26.3\%$  [18]. Since the percentage of charged residues in Pocket 1 and 2 was 21.6% and 48% respectively, only Pocket 1 is indicated as a potentially good drug target. The ligand binding pocket had a wide range of sizes from 100 to 1000 Å<sup>3</sup>. If the pocket volume is lower than 700 Å<sup>3</sup>, the size of potential ligands needs to be considered [20]. Since Pocket 1 and 2 have the volume 600 Å<sup>3</sup> and 573 Å<sup>3</sup> respectively, the small ligand is suitable for this protein. The pocket cavity of NS2B/NS3 Protease is shown in Figure 1.



**Figure 1.** Two drugable pockets of NS3/NS2B protease DENV

### 3.2 Molecular docking

In order to explore the enzyme-substrate interaction and to determine the key residues responsible for interaction, two substrates (4-hydroxypanduratin A and panduratin A) were used as model ligands for the NS3/NS2B protease using the *MOE2008.10* software. Table 3 shows that in all cases there was a negative and low value of free energy of binding ( $\Delta G_{\text{binding}}$ ) interaction between the NS3/NS2B protease pockets and the 2 ligands indicating a potential strong bond in the most favorable conformations. The noncovalent binding will take place as a spontaneous process if it is correlated with a negative binding free energy [21]. Table 3 showed that the interaction between ligand 4-hydroxypanduratin A and panduratin A and NS2B /NS3 protein should occur spontaneously. In Pocket 1 the ligand 4-hydroxypanduratin A formed 2 hydrogen bonds and panduratin A formed one hydrogen bond. In Pocket 2, 4-hydroxypanduratin A is predicted to form 3 hydrogen bonds and panduratin A 4 hydrogen bonds. As mentioned previously hydrophobic pockets are good candidates for druggable pockets even though spectroscopic evidence demonstrates that hydrogen bonds at hydrophobic sites are weaker [19,21].

Even though the  $\Delta G_{\text{binding}}$  for the ligand-protein complex in Pocket 1 is more negative than Pocket 2 (Table 3), the number of hydrogen bonds in Pocket 1 is lower than Pocket 2. There are many factors influencing the stabilization of the ligand-enzyme complex, for example, the distance and angle of hydrogen bonds [22]. In this research, the length of hydrogen bonds ranged from 1.59 to 2.47 (Table 3) which fits the accepted (and most frequently observed) geometry for a hydrogen bond, a length of less than 2.5 Å.

**Table 3.** Result of Molecular docking simulation

Pocket	Ligand	$\Delta G_{\text{binding}}$ (kcal/mol)	Hydrogen bonds	Bond length (Å)
1	4-hydroxypanduratin A	-13.00	Leu_B140 (66%); Lys_B74(45%)	1.79; 1.61
1	Panduratin A	-11.49	Lys_B74(21%)	2.47
2	4-hydroxypanduratin A	-10.77	His_B60(60%); Leu_A53(44%) Lys_B61(48%)	1.75; 1.84; 1.59
2	Panduratin A	-10.80	Glu_B19(90%); Leu_B19(17%); His_B60(63%); Arg_A55(19%)	1.78; 2.3; 1.66 2.08

#### 4. Conclusion

This article described the analysis of amino acid residues for potential binding sites of DENV NS2B/NS3 Protease using *Metapocket*, *Depth*, and *DoGSiteScorer* to perform molecular docking as a part of efforts to discover the potential binding site and new candidates for drug design. Our results clearly identify Pocket 1 as the best candidate as it has a lower overall hydrophobicity and a more spontaneous binding energy ( $\Delta G_{\text{binding}}$ ) than pocket 2.

#### Conflict of Interest

We declare that there is no conflict of interest in this research nor publication

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