

## Plasmepsin

Malaria a dreadful disease is caused by the protozoan parasite, plasmodium. There are several species of plasmodium species, of them four different species are responsible for malaria in human.

Plasmodium falcipuram

Plasmodium vivax

Plasmodium malariae

Plasmodium ovale

One of the crucial drug targets in malaria are plasmepsins.

The aspartic proteases of plasmodium species are known as plasmepsins. Plasmepsins are involved in the hemoglobin degradation inside the food vacuole during the erythrocytic phase of the life cycle. There are ten different isoforms of these proteins and ten genes coding them respectively in plasmodium falciparum (Plm I, II, III, IV, V, VI, VII, IX, X and HAP), it has been suggested that the plasmepsin family is smaller in other human plasmodium species. Expression of Plm I, II, IV, V, IX, X and HAP occurs in erythrocytic cycle, and expression of Plm VI, VII, VIII, occurs in exo-erythrocytic cycle.

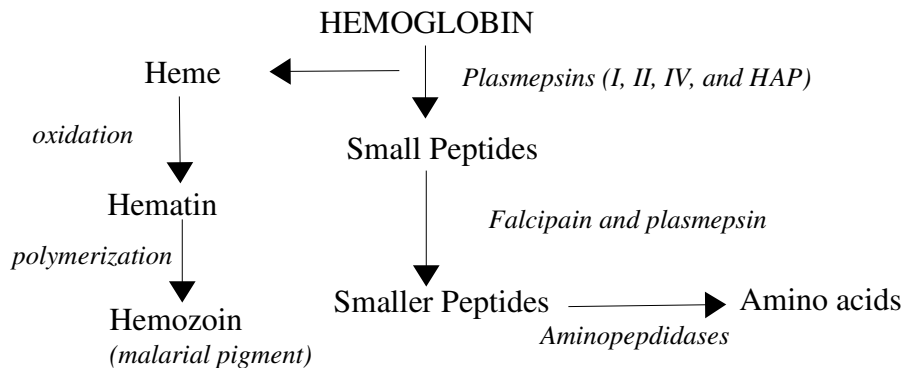


Figure 1. Hemoglobin degradation in plasmodium falcipuram

**Mode of action:** Hemoglobin is degraded by a series of proteases in the digestive food vacuole, the sequential process is represented in figure 1. The two homologous plasmepsin I and II are responsible for the initial attack on the hemoglobin Alpha chain between the residues Phe 33 and Leu 34, in the hinge region, this region is highly conserved and responsible for the stability of the hemoglobin tetramer. The heme is released which is toxic to the parasite and is further oxidized to hematin, also toxic to the parasite. Finally the hematin is polymerized to hemozoin, the malarial pigment. Both plasmepsin I and II are capable of making a initial cleavage in the hemoglobin, the plasmepsins are capable of several other cleavages after the initial attack.

**Homology:** High levels of sequence homology is observed in between different plasmepsin I, II, IV, and HAP, which also lies in the cluster of same gene. Compared to the Plm II binding site, the binding site region of Plm I, IV, and HAP shows 84%, 68%, and 39% identity, respectively.

**Selectivity:** Selectivity between the closely related human aspartic proteases, is one of the important consideration for the development of new drugs for plasmepsins, the closest aspartic protease cathepsin D has 35% sequence similarity to Plm II and even higher identity at the active site.

### Structural information

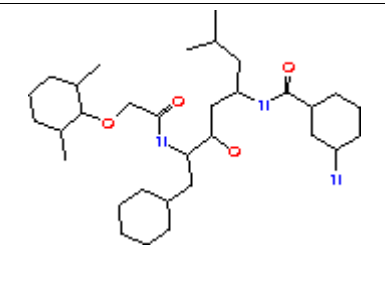
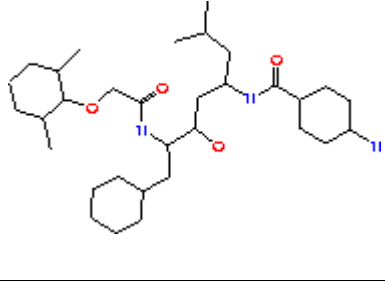
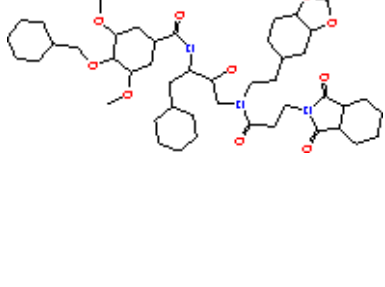
12 different X-ray structures of Plm II are presently available in protein database ([www.pdb.org](http://www.pdb.org)), in the current study we proceeded with four, three of them are monomers (1lee, 1lf2, 1lf3) and one dimer (1ls5), it is a crystallographic artifact and the biologically active form is monomer. All the proteins are crystallized with different inhibitors.

	<i>1lee</i>	<i>1lf2</i>	<i>1lf3</i>	<i>1ls5</i>
<b>Resolution</b>	1.90 Å	1.80 Å	2.7 Å	2.80 Å
<b>Experiment method</b>	X-ray Diffraction	X-ray Diffraction	X-ray Diffraction	X-ray Diffraction
<b>Classification</b>	Hydrolase	Hydrolase	Hydrolase	Hydrolase
<b>EC number</b>	3.4.23.39	3.4.23.39	3.4.23.39	-
<b>Source</b>	P.falciparum	P.falciparum	P.falciparum	P.falciparum
<b>Type of inhibitor</b>	Non-peptidic R36	Non-peptidic R37	Non-peptidic EH5	Peptidic IHN348, 349
<b>No. of monomers</b>	1	1	1	2
<b>Molecular weight</b>	37036	37036	37080	36941
<b>No. of alpha chains</b>	4	4	5	5
<b>No. of beta chains</b>	27	27	28	25
<b>No. of residues</b>	331	331	331	328

	<i>1lee</i>	<i>1lf2</i>	<i>1lf3</i>	<i>1ls5</i>
<i>No. of atoms</i>	2994	2982	2730	5342
<i>No. of water</i>	338	338	52	28
<i>R-factor</i>	0.236	0.2	0.178	0.220
<i>R-free</i>	0.286	0.258	0.224	0.290
<i>Authors</i>	O.A.Asojo,E. Afonina, et al	O.A.Asojo,E. Afonina, et al	O.A.Asojo,S. V.Gulnik et al	O.A.Asojo,S.V .Gulnik et al

Table 1. Structural features of the proteins.

### Different plasmepsins with their crystal ligands

<i>Protein ID</i>	<i>Inhibitor</i>	<i>IUPAC name</i>	<i>Structure of the crystal ligand</i>
1lee	R 36	<i>4-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3- hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide</i>	
1lf2	R 37	<i>3-amino-n-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3- hydroxy-1-isobutyl-5-phenyl-pentyl}-benzamide</i>	
1lf3	EH 5	<i>N-(1-benzyl-3-[[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-propionyl]-[2-(hexahydro-benzo[1,3]dioxol-5-yl)- ethyl]-amino]-2-hydroxy-propyl)-4-benzyloxy-3,5- dimethoxy-benzamide</i>	

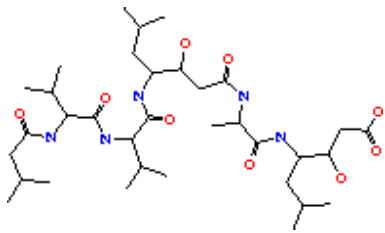
<i>Protein ID</i>	<i>Inhibitor</i>	<i>IUPAC name</i>	<i>Structure of the crystal ligand</i>
1ls5	IHN348 (1ls5_a) IHN348 (1ls5_a) (Pepstatin A)	<i>3-hydroxy-4-isobutyl-4-[aminocarbonyl ethyl(aminocarbonyl-2-hydroxy-5-methylhexyl)tri(aminocarbonyl isobutyl)]butanoic acid</i>	

Table 2. Different plasmepsins with their crystal ligands.

### Experimental Procedure

Different scenarios of proteins have been prepared for docking based on the inclusion of water molecules in the active site. Several tests have been done to know the which water molecules are influencing the docking scores (energy) and rmsd (root mean square deviations). Different names of proteins and their water molecules used during the docking procedure are described in table 3 (below).

<i>protein</i>	<i>Water molecules</i>
1lee	Without water molecule
1lee_2h	Two water molecules (1256, 1270 residues)
1lee_3h	Three water molecules (1065, 1125, 1247 residues)
1lf2	Without water molecule
1lf2_h	One water molecules (585 residue)
1lf3	Without water molecule
1ls5_a	Without water molecule
1ls5_b	Without water molecule

Table 3. Different plasmepsins based on water molecules used during the docking.

## FlexX parameters

The different flexX parameters used during the docking process are

1. Place particle size 1
2. Place particle size 0
3. Maximum overlap volume 2.5
4. Maximum overlap volume 5.0

## Description

**Place particle size:** This is a special feature of flexx where the algorithm places a water molecule by itself. If equals 1, FlexX places spherical objects, called particles, into the active site. Particles can then be used for bridging interactions between the ligand and the protein. The main application of particles is the modeling of discrete water molecules located between the protein and the ligand. If equals to 0, FlexX does not calculate any water molecules by itself. Particle types are defined in the static data file to which the value of amino points.

**Maximum overlap volume:** The overlap test between the protein and ligand consists of two parts. The first condition for overlap is that a protein and a ligand atom exist with an overlap volume greater than maximum overlap volume. Hydrogens are not taken into account in overlap tests. An easy way to switch off the overlap test is to set this parameter to a very high value.

Default value: 2.5 Å

Reasonable range: 0.0-100.0 Å

The combinations of the parameters are used for the docking process.

	<i>Place particle size</i>	<i>Maximum overlap volume</i>
Parameter 1	1	2.5
Parameter 2	1	5
Parameter 3	0	2.5
Parameter 3	0	5

Table 4. FlexX parameter combinations.