STRUCTURAL AND FUNCTIONAL IMPORTANCE OF A NON-CATALYTIC DOMAIN OF FKBP35 FROM *Plasmodium knowlesi*

JOVI SILVESTER

BIOTECHNOLOGY RESEARCH INSTITUTE

UNIVERSITI MALAYSIA SABAH

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JOVI SILVESTER

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IJAZAH: **MASTER OF SCIENCE (BIOTECHNOLOGY)**

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MZ1511009T

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Disahkan Oleh,
NORAZLYNNE MOHD. JOHAN @ JACKLYNE
PUSTAKAWAN
UNIVERSITI MALAYSIA SABAH
(Tanda Tangan Pustakawan)

DR. CALVIN BUDIMAN
PENSYARAH KANAN
INSTITUT PENYELIDIKAN BIOTEKNOLOGI
UNIVERSITI MALAYSIA SABAH
Dr. Calvin Budiman
Penyelia

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, accepts, equations, summaries and references, which have been duly acknowledged.

22 July 2019

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Jovi Silvester

MZ1511009T

CERTIFICATION

NAME	:	**JOVI SILVESTER**
MATRIC NO.	:	**MZ1511009T**
TITLE	:	**STRUCTURAL AND FUNCTIONAL IMPORTANCE OF A NON-CATALYTIC DOMAIN OF FKBP35 FROM *Plasmodium knowlesi*.**
DEGREE	:	**MASTER OF SCIENCE (BIOTECHNOLOGY)**
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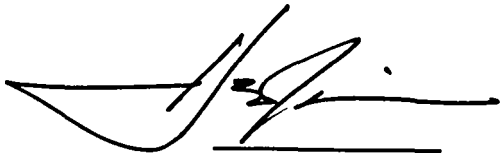
SIGNATURE

1. MAIN SUPERVISOR

DR. CAHYO BUDIMAN



2. CO-SUPERVISOR

ASSOC. PROF. DR. LEE PING CHIN

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ABSTRACT

A 35 kDa FK506-binding protein (FKBP35) from *Plasmodium knowlesi* (PkFKBP35) is considered as a viable target for development of antimalarial drugs without resistant effects. This protein is a member of peptidyl prolyl *cis-trans* isomerase (PPIase) with the ability to catalyze isomerization of *cis*-prolyl bond during protein folding. Sequence alignment of PkFKBP35 with other FKBP35 from *P. falciparum* and *P. vivax* revealed that PkFKBP35 consists of two domains which are the FK506-binding domain (FKBD) and the tetratricopeptide repeat domain (TPRD). FKBD acted as a catalytic domain, while TPRD serves as a non-catalytic domain. Development of new antimalarial drugs is so far focused only on the catalytic domain, while limited studies in the non-catalytic domain. Structurally, non-catalytic domain in other FKBPs was reported to be important for oligomerization of the proteins. There were also some cases that the oligomerization is associated with correct folding of the protein. Nevertheless, whether TPRD, as a non-catalytic domain of PkFKBP35, also structurally play important role for folding and dimerization remain to be investigated. Functionally, as the non-catalytic domain folds into TPR motif, thus this domain was thought to facilitate interaction between FKBP35 and other (partners) proteins. Since TPR motif in other proteins was known to interact to heat shock protein90 (Hsp90), it was also speculated that TPRD of FKBP35 might facilitate interaction between Plasmodium Hsp90, particularly to its C-terminal pentapeptide (MEEVD) and involved in folding machinery of the parasite cells. Interestingly, TPRD of FKBP35 segment contains a calcium-modulated proteins (calmodulin) binding motif (CBM) at its C-terminal. The presence of this motif promotes a speculation that TPRD might also interact with calmodulin and involved in calcium signaling pathway of the parasites. However, no study has been done to confirm these speculations. This study aims to determine the structural and functional roles of the non-catalytic domain (TPRD with its CBM) of PkFKBP35. Structural importance of non-catalytic domain was confirmed through solubility, folding and oligomerization assay. In addition, flexibility analysis revealed and 2D structural analysis of PkFKBP35 using transmission electron microscope revealed that PkFKBP35 was found to be a very dynamic protein with three conformations: circular, hook, elongated. This flexibility is believed regulated by catalytic domain. Further, binding analysis using pull down assay revealed the first evidences of interaction between PkFKBP35 and calmodulin (CaM). The binding was only observed in the presence of calcium ions which suggest that the interaction required an active state of CaM. Further analysis using surface plasmon resonance revealed that full length PkFKBP35 and PkTPRD+ bind to CaM with similar dissociation constant (K_D values). This suggested that TPRD segment with its CBM is really essential for binding to CaM. In addition to the interaction to CBM, PkFKBP35 was also shown to be able to interact to MEEVD of Hsp90. This interaction was also found to be regulated by TPRD. Further, molecular docking analysis revealed that the binding sites of CaM are shared between TPRD and CBM. Altogether, the study demonstrated that non-catalytic domain has important role in protein-protein interaction function of PkFKBP35, mainly in facilitating the interaction to HSP90 or calmodulin. In addition, non-catalytic domain of PkFKBP35 is important for proper folding of this protein, yet, apparently, no involvement in structural flexibility of this protein.

ABSTRAK

(KEPENTINGAN STRUKTUR DAN FUNGSI DOMAIN BUKAN KATALITIK FKBP35 DARIPADA Plasmodium knowlesi)

FK506-pengikat protein bersaiz 35 kDa (FKBP35) dari Plasmodium knowlesi (PkFKBP35) dianggap sebagai sasaran yang berdaya maju untuk pembangunan ubat antimalaria tanpa kesan tentangan. Protein ini adalah ahli peptidyl prolyl cis-trans isomerase (PPIase) dengan keupayaan untuk memangkin isomerization ikatan cis-prolyl semasa lipatan protein. Penjajaran urutan PkFKBP35 dengan FKBP35 yang lain dari P. falciparum dan P. vivax mendedahkan bahawa PkFKBP35 terdiri daripada dua domain yang merupakan domain pengikat-FK506 (FKBD) dan domain pengulangan tetratricopeptide (TPRD). FKBD bertindak sebagai domain pemangkin, sementara TPRD berfungsi sebagai domain bukan pemangkin. Pengembangan ubat antimalarial baru hanya tertumpu pada domain pemangkin, sementara kajian terhad di domain bukan pemangkin. Secara struktural, domain bukan pemangkin dalam FKBP lain dilaporkan penting untuk oligomerisasi protein. Terdapat juga beberapa kes yang oligomerisasi dikaitkan dengan lipatan protein yang betul. Walau bagaimanapun, sama ada TPRD, sebagai domain bukan pemangkin PkFKBP35, juga berperanan penting dalam struktur untuk lipatan dan dimeralisasi untuk disiasat. Secara fungsional, sebagai domain bukan pemangkin dilipat menjadi motif TPR, maka domain ini dianggap memudahkan interaksi antara FKBP35 dan protein (pasangan) lainnya. Oleh kerana motif TPR dalam protein lain diketahui berinteraksi dengan protein kejutan haba 90 (Hsp90), ia juga membuat spekulasi bahawa TPRD FKBP35 mungkin memfasilitasi interaksi antara Plasmodium Hsp90 dan terlibat dalam mesin lipatan sel parasit. Menariknya, segmen TPRD FKBP35 mengandungi protein mengikat kalsium (calmodulin) yang mengikat (CBM) di terminal C-nya. Kehadiran motif ini menggalakkan spekulasi bahawa TPRD mungkin juga berinteraksi dengan calmodulin dan terlibat dalam laluan isyarat kalsium parasit. Walau bagaimanapun, tiada kajian telah dilakukan untuk mengesahkan spekulasi ini. Kajian ini bertujuan untuk menentukan peranan struktur dan fungsi domain bukan pemangkin (TPRD dengan CBM) PkFKBP35. Kepentingan struktur domain bukan pemangkin telah disahkan melalui uji kelarutan, lipatan dan oligomerisasi. Di samping itu, analisis fleksibiliti mendedahkan dan analisis struktur 2D PkFKBP35 menggunakan mikroskop elektron penghantaran mendedahkan bahawa PkFKBP35 didapati sebagai protein yang sangat dinamik dengan tiga bentuk: bulat, cangkuk, memanjang. Tambahan pula, analisis mengikat menggunakan asai tarik ke bawah menunjukkan bukti pertama interaksi antara PkFKBP35 dan calmodulin (CaM). Analisis lanjut menggunakan resonans plasmon permukaan mendedahkan bahawa panjang penuh PkFKBP35 dan PkTPRD+ mengikat CaM dengan pemalar pemisahan yang sama (nilai K_D). Ini menunjukkan bahawa segmen TPRD dengan CBM adalah sangat penting untuk mengikat CaM. Sebagai tambahan kepada interaksi ke CBM, PkFKBP35 juga ditunjukkan dapat berinteraksi dengan MEEVD dari Hsp90. Interaksi ini juga didapati dikawal oleh TPRD. Tambahan lagi, analisis "docking" molekul mendedahkan bahawa tapak interaksi CaM dikongsi di antara TPRD dan CBM. Secara keseluruhannya, kajian menunjukkan bahawa domain bukan pemangkin mempunyai peranan penting dalam fungsi interaksi protein protein PkFKBP35, terutamanya dalam memudahkan interaksi dengan Hsp90 atau calmodulin. Di samping itu, domain bukan katalitik PkFKBP35 adalah penting untuk lipatan protein yang betul, namun, nampaknya, tiada penglibatan dalam fleksibiliti struktur protein ini.

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LIST OF ABBREVIATIONS

bp	Base pair
FKBD	FKBP binding domain
FKBP35	FK506-binding domain 35
P. falciparum	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
cDNA	Complementary Deoxyribonucleic acid
P. vivax	*Plasmodium vivax*
Pf	*Plasmodium falciparum*
Pk	*Plasmodium knowlesi*
PPIase	Peptidyl prolyl isomerase
Pv	*Plasmodium vivax*
rpm	Revolution per minute
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
TEMED	Tetramethylethylenediamine
UV	Ultraviolet ray
EDTA	Ethylenediaminetetraacetic acid
TPRD	Tetratricopeptide repeat domain
DTT	Dithiothreitol
CaM	Calmodulin
CBM	Calmodulin-binding motif
Pvn	Parvulin
Cyp	Cyclophillin
WHO	World health organization
TPR	Tetratricopeptide repeat
FKBP22	FK506-binding domain 22
Hsp90	Heat shock protein 90
FKBP38	FK506-binding domain 38
FKBP12	FK506-binding domain 12
CDC	Center for disease control and prevention
ACT	Artemisinin combination therapy
CsA	Cyaclosporin A
Ca^{2+}	Calcium ions
LB	Luria-Bertani
IPTG	isopropyl β-D-1-thiogalactopyranoside
HCl	hydrochloric acid
FPLC	Fast Protein Liquid Chromatography
SEC	Size exclusion chromatography
CD	Circular Dichroism
NaCl	Sodium chloride
$CaCl_2$	Calcium chloride
w/v	Weigh/volume
APS	Ammonium Persulfate
GMQE	Global Model Quality Estimation

LIST OF SYMBOLS

µg	Micro gram
µm	Micro meter
µM	Micro molar
°C	Degree Celsius
a.u.	Arbitrary unit
g	Gram
kDa	Kilo Dalton
M	Molar
ml	Mili liter
nm	Nano meter
ps	Pico second
%	Percentage
mM	Mili molar
K	Kelvin
Δ	Delta
≈	Approximately
β	Beta
α	Alpha
™	Trademark sign

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Malaria is a global health issue affecting almost half population in the world. This disease is caused by Plasmodium parasites and transmitted to human via the bites of infected female *Anopheles* mosquitoes (WHO, 2016). The number of malaria cases reported in 2016 has increased about 5 million as compared to the number of cases reported in 2015. The numbers of malaria deaths in 2016 as reported by WHO (2017) was 445 000 cases which showed decrease of 1000 cases from 2015 total cases. The malaria cases distribution was categorized into 91% was in the African region, 7% in South-east Asia and 2% from the Eastern Mediterranean (WHO, 2017). The incidence rate of malaria in Malaysia has decreased from 37.0 to 14.7 per 100,000 population in 2006 and 7.1 per 100,000 population in 2012. While in 2016 the rate increase to 7.2 per 100,000 population. The malaria mortality rate was fluctuating throughout the years of 2001-2012 between 0.25 to 0.59, however, in 2016, the mortality rate dropped to just 0.01 per 100,000 population (Ministry of Health Malaysia, 2016; 2012). Malaria Elimination 2011-2020 is a National Strategic Plan that was introduced with the objective to prevent locally-acquired malaria in Malaysia (WHO, 2015). The isolated geographic area with high number of migrant workers causing Sabah and Sarawak to have high number of malaria cases in Malaysia (Ministry of Health Malaysia, 2016). According to WHO Malaria Report 2015, Malaysia with 1.3 million populations at risk of malaria is in the pre-elimination stage of malaria. As of 2010, the risk of contracting malaria for the population in Sabah, Sarawak and West Malaysia were 24.5%, 19.7% and 0.4% respectively.

Malaria is caused by Plasmodium parasites which can be transmitted to human via the bites of infected female Anopheles mosquitoes. There are 4 Plasmodium species that were previously reported to have the ability to infect human such as *P. falciparum, P. malariae, P. vivax,* and *P. ovale*. The *P. falciparum* and *P. vivax* were considered as the most common parasites in Africa and outside the Sub-saharan (World Health Organization, 2016). Recently, *P. knowlesi* was reported as the fifth human malaria parasite. Though, this Plasmodium species was firstly reported to be hosted by macaque monkeys (Wilson *et al.*, 2011). The structure of *P. malariae* and *P. knowlesi* are very similar, thus, it is hard to differentiate both of the Plasmodium species microscopically. Therefore, causing misdiagnosis and classification of both species as a single group for cases notification (William *et al.*, 2013, Cox-Singh *et al.*, 2008).

P. knowlesi natural host are long-tailed and pig-tailed macaques. The macaques are from forested area of Southeast Asia region including Borneo island (Singh & Daneshvar, 2013). Furthermore, the increasing reported cases of *P. knowlesi* in Malaysia since 2008 was a major concern, making Malaysia as the country with the highest percentage of *P. knowlesi* infection in WHO Western Pacific Region. William *et al.* (2014) reported that *P. knowlesi* has increasing number of incidence and has been significantly increased from 2004 to 2013 in Sabah (William *et al.*, 2014). According to Sabah Department of Health malaria report data from 1992-2013, the percentage of malaria cases in Sabah caused by *P. malariae/P. knowlesi* was increasing from 1% in 1992 to 35% in 2011 and to 62% in 2013 (William *et al.*, 2014). Thus, the situation urges serious efforts to eradicate the parasite infection.

Antimalarial drugs are the answer to control and eliminate malaria. The greatest known naturally occurring antimalarial compounds are quinine and artemisinin which extracted cinchona bark and *Artemisia*, respectively (An *et al.*, 2017). The deployment of artemisinin-based combination therapies have contributed greatly in recent decreases in the global malaria problem (Cui *et al.*, 2015). However, there have been recent alarming concerns about the antimalarial drugs resistance in Plasmodium parasites. World Health Organization (WHO, 2015) had reported that out of 5 Plasmodium species that affect human, three of the

plasmodium parasites are known to have antimalarial drugs resistance. The resistance toward antimalarial drugs has led to incomplete elimination of the Plasmodium parasites from patient's blood after treatment using antimalarial drugs which the parasites has developed resistance towards. Furthermore, the problem exaggerated by the ability of the parasites to have cross resistance which caused the treatment of the malaria using antimalarial drugs that has same chemical family or similar modes of action with another antimalarial drugs that the parasites already build resistance toward, become similarly inefficient (World Health Organization, 2016). Thus, resistance to antimalarial medicines is a threat to global strategy to control and eliminate malaria. Therefore, to address this problem, alternative antimalarial drugs must be discover and develop.

Presently, an immunosuppressant drug known as FK506 or also known as tacrolimus was reported to have antimalarial properties with no resistance issue being reported thus far (Monaghan *et al.*, 2017). FK506 was used originally during organ transplantation to momentarily suppress the recipient immune response so that no immune response to reject the transplanted organ. Immunosuppressive effect of this drug is considerably the biggest drawback. Therefore, although FK506 possess antimalarial properties, it is not possible or unfavorable for this drug to be used as an antimalarial drug for long term as it may suppress the patient immune system. Therefore, attempts to find FK506 replacers as antimalarial drugs with no immunosuppressive effect and no resistance effect are necessary (Monaghan *et al.*, 2005).

Monaghan *et al.* (2017) reported that the protein receptor for this drug is a FK506-binding protein (FKBP) which is a member of peptidyl prolyl *cis-trans* isomerase (PPIase). PPIase is a group of enzyme capable of catalyzing slow isomerization of *cis*-prolyl peptide bond which is regarded as a rate-limited step of protein folding (Fanghanel & Fischer, 2004). Three structurally distinct family of PPIase were identified, including cylophilin (Cyp), FKBP and parvulin (Par). These three groups are different in their substrate specificity, inhibitors as well as their cellular roles. FK506 specifically binds to FKBPs group, but not to Cyp or Par. An FK506-FKBP complex inhibits the catalytic activity of the FKBP, protein phosphatase calcineurin and blocking a key step in T-cell activation. This leads to assumption of

the existence of FKBPs member inside Plasmodium parasite cells. The exposure of Plasmodium parasite with FK506 leads to disruption of the vital cellular functions of the FKBPs that cause cell death events.

Based on the genomic sequences of some Plasmodium parasites, it was later confirmed that a single FKBPs member exist inside parasite cells. This protein known as FKBP35, is to date, the only PPIase member exists inside the parasite cells. The study on this protein was done on *P. falciparum* and *P. vivax,* designated as Pf and PvFKBP35, respectively (Alag *et al.*, 2013; Yoon *et al.*, 2007). Previous studies on FKBP35 from *P. falciparum* (PfFKBP35) and *P. vivax* (PvFKBP35) discovered that this protein consists of two domains, N-terminal domain which is highly similar with human FKBP12 (designated as FKBP domain, FKBD), and C-terminal domain with tetratricopeptide repeat motif comprising calmodulin binding motif (designated as tetratricopeptide repeat domain, TPRD) (Kang *et al.*, 2008; Monaghan & Bell, 2005; Yoon *et al.*, 2007). FKBP35 protein has been reported to bind with FK506, exhibit PPIase activity toward tetrapeptide substrate, chaperone function, and inhibit calcineurin's phosphatase activity with or without FK506 (Monaghan & Bell, 2005; Yoon *et al.*, 2007).

It is interesting to note that the genomic DNA of *P. knowlesi* encodes a homolog of FKBP35 (gene ID: PKH_146480), designated as PkFKBP35. It shares a high similarity to PfFKBP35 and PvFKBP35 approximately 80% and 90%, respectively, based on their sequences. Likewise, the primary structure of PkFKBP35 also displays the organization of FKBD followed by tetratricopeptide repeated domain. The presence of FKBD in PkFKBP35 (designated as PkFKBD henceforth) suggests that FK506 might be an effective antimalarial drug for *P. knowlesi* infection. Although many studies have been conducted on PfFKBP35 and PvFKBP35, unfortunately, so far, there is no study on PkFKBP35.

Most of studies were conducted on FKBD, which was identified as a domain responsible for catalytic function of this protein. High similarity of this domain with human FKBP12 revealed that some residues in human FKBP12 that had been reported to be involved in PPIase activity (Y26, F36, D37, R42, F99, W59 and I56) (Fanghanel & Fischer, 2004) are conserved in FKBD from Plasmodium FKBPs. These

residues, interestingly, were also well conserved in other Plasmodium FKBPs, which leads to an acceptable assumption that PkFKBD indeed serves as a catalytic domain. Accordingly, attempts on finding the drug targeting FKBP35 was so far focused on this domain. As an example, Harikishore *et al.* (2013) attempted the screening and discovered the small molecules inhibiting FKBD from *P. falciparum* and discovered a small ligand of displaying inhibitory effect towards this protein. Nevertheless, there are no attempts yet for the screening of inhibitors targeting non-catalytic domain of Plasmodium FKBP35. This is believed due to the less fundamental studies on non-catalytic domain of Plasmodium FKBP35.

TPRD is assumed to be a non-catalytic domain of Plasmodium FKBP35. The sequence homology of TPRD among Plasmodium FKBP35 is considerably high (> 80%) supposing that they share structural and functional properties. It is interesting to note that in multi-domain FKBPs with dimeric structure (e.g., FKBP22, Lp-MIP, FKBP37, and FKBP26), domains that were involved in dimerization were found to have no catalytic activity (non-catalytic domain) (Budiman *et al.*, 2011; Jo *et al.*, 2015; Hackert & Hendrickson, 2011; Tunnicliffe *et al.*, 2001). In addition, structural analysis of TPR motif was also found to facilitate dimerization in some proteins. Therefore, it is reasonable to assume that TPRD might be important for dimerization. Besides, finding on FKBP22 from *Shewanella* sp. SIB1 revealed an interesting relation between dimerization of non-catalytic domain and overall folding of the protein (Budiman *et al.*, 2012). Nevertheless, dimerization of PkTPRD and its association with the folding of PkFKBP35 remain to be experimentally proven.

Structurally, TPR motif consists of multiple repeats of 34 amino acids sharing a degenerate consensus sequence defined by a configuration of small and large hydrophobic amino acids (Zeytuni & Zarivach, 2012). TPR is not a unique domain for Plasmodium FKBP35 since; hitherto, more than 5000 proteins were reported to harbor this motif. Functionally, this motif facilitates involvement of the proteins in many cellular diverse processes, which mainly through mediating the interaction to the other proteins (protein-protein interaction). To note, the formation of protein-protein complexes is essential for many biological functions. The protein-protein interactions are considered as essential for all functional, living

cells (Zeytuni & Zarivach, 2012). Accordingly, TPRD of Plasmodium FKBP35 is considerably a feasible antimalarial drug target as functional inhibition of this domain might lead to disruption of many cellular events of the parasite.

One of protein partner that was reported to interact with TPR motif is heat shock protein 90 (Hsp90). TPR domain (TPRD) of *P. falciparum* (PfTPRD) has been reported to bind to Hsp90. Hsp90 is important molecular chaperone that is involved in the activation or maturation of many keys proteins which play important roles in different types of cellular functions (Pratt et al, 2003; Richter *et al.*, 2003). The interaction is particularly facilitated by pentapeptide of MEEVD located at the C-terminal of Hsp90 (Alag *et al.*, 2009). Docking and molecular dynamic simulation further demonstrated the pentapeptide is accommodated by clamp forming residues and a hydrophobic pocket (Alag *et al.*, 2009). This interaction suggests possible involvement of FKBP35 in protein folding machinery of parasite and play an important role in the pathogenesis of Plasmodium (Yoon *et al.*, 2007). Additionally, this interaction also provides another hotspot for development of antimalarial drug targeting FKBP35 and thus comprehensive understanding on this interaction is unavoidable. Nevertheless, whether interaction between FKBP35 and MEEVD pentapeptide are general feature for the other Plasmodium FKBP35 remain to be addressed. Besides, experimental study on the interaction is so far limited only to TPRD (Alag *et al.*, 2009). Study on the interaction between full length FKBP35 or FKBD towards MEEVD is important to have conclusive proposal on the role of TPRD in the interaction.

The genomic DNA of *P. knowlesi* also contains a gene encoding Hsp90, designated as PkHsp90, with 87% and 90% similarity to PfHspP90 and PvHsp90, respectively, on their amino acid sequences. The presence of PkFKBP35 with PkHsp90 promotes the possibility that these proteins might also associate and involved in wide cellular network and chaperone system of *P. knowlesi*. Monaghan and Bell (2005) finding implied that inhibition of chaperone function might be the promising target for development of antimalarial drug (Monaghan & Bell, 2005). There is no study yet for PkFKBP35, particularly on its interaction to PkHsp90. The study might lead to general understanding on Plasmodium and Hsp90 interaction which is important as a platform for development of novel antimalarial drug. To

note, amino acid sequences alignment revealed that while the clamp forming residues are highly conserved for PfFKBP35 and PkFKBP35, some corresponding amino acid residues forming hydrophobic pocket for MEEVD binding in PfFKBP35 are different to that of PkFKBP35. These differences promote possibility of the uniqueness in binding mechanism between PkFKBP35 and PkHsp90 and possibility to develop specific inhibitor for PkFKBP35. Nevertheless, experimental studies are needed to confirm this assumption.

In addition, the presence of Calmodulin-binding motif (CBM) at the C-terminal tail of TPRD suggested that the FKBP35 also binds to calcium-modulated proteins (Calmodulin / CaM). CaM is a small protein, with approximately 16 kDa in size that capable of binding to calcium ions. Since, FKBP35 has the conserved motif of CBM in their amino acid sequence, it may involve in regulating activities that are done by CaM. CaM is known to regulate several cellular processes that involved calcium ions-dependent signaling pathways (Stull, 2001). In its action, CaM binds to its target protein and regulates function of some target proteins that further affect the downstream cellular pathways. CaM is widely distributed among mammalian and other eukaryotes with high similarity in the amino acid sequences (Hayashi *et al.*, 1998). CaM has 4 calcium ions binding sites which are known as the EF hands, in which upon completion of Ca^{2+} binding, the CaM undergoes structural changes. This conformational change activates the CaM, hence, enables CaM to recognize and bind to its target proteins (Crivici & Ikura, 1995). The interaction between FKBP35 to CaM suggests the involvement of this protein in calcium-mediated signaling pathway of the parasite cells.

Nevertheless, there are no study to date for the interaction between Plasmodium FKBP35 and CaM. Human FKBP38 is so far the only FKBPs member with CBM that been experimentally proved to bind with CaM (Edlich *et al.*, 2007; Edlich *et al.*, 2005). Nevertheless, FKBP38 is structurally different to FKBP35 as this protein stick in the cell membrane. The CBM in FKBP38 is not located at the C-terminal of this protein but close to its transmembrane domain (Kang *et al.*, 2008). Indeed, the binding of CaM to FKBP38 was reported to be observed in catalytic and

non-catalytic domains of this protein, which is questionable. Study on binding between PkTPRD and CaM should confirm two issues:
(1) Whether or not CBM at Plasmodium FKBP35 generate binding affinity to CaM; and
(2) Whether or not binding site of CaM is localized at catalytic and non-catalytic domain.

Altogether, fundamental studies on TPRD of PkFKBP35 are unavoidable to have a comprehensive understanding on the importance of this domain. In this study, first experimental evidences on the role of TPRD for structure and function of PkFKBP35 are provided. Confirmed importance roles of this domain should provide acceptable reasons for targeting this domain, in addition to its catalytic domain, in the development of antimalarial drug with no resistance effect. As this study specifically targets the protein from *P. knowlesi*, this should also provide a platform for development of specific drug targeting this simian malaria parasite.

1.2 Problem Statement

The data from World Health Organization showed that malaria is a major threat on human health worldwide as it risks more than half of the human population. Malaria disease is caused by Plasmodium parasites which are transmitted from the bites of female Anopheles mosquitoes. Currently, there are five major Plasmodium parasites which are known to infect human. The Plasmodium parasites are *P. falciparum, P. malariae, P. vivax, P. ovale,* and *P. knowlesi*. In Malaysia, *P. knowlesi* dominate the reported cases of malaria, this might be due to Southeast Asia is the natural habitat of *P. knowlesi* and the natural hosts are the long-tailed and pig-tailed Macaques.

Medicine for malaria is commercially available and has plays a major part in reducing the fatality rate because of malaria in this last decade. However, the Plasmodium parasites have developed resistance toward the antimalarial drugs that currently being used to combat this disease. Therefore, threatening to hinder the efforts to eradicate malaria.

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