CHARACTERIZATION OF THE FUNCTIONAL DOMAINS OF FKBP35 FROM *Plasmodium knowlesi*

CARLMOND GOH KAH WUN

THESIS IS SUBMITTED IN FULLFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE

BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2019

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: **CHARACTERIZATION OF THE FUNCTIONAL DOMAINS OF FKBP35 FROM *Plasmodium knowlesi***

IJAZAH: **SARJANA SAINS (BIOTEKNOLOGI)**

Saya **CARLMOND GOH KAH WUN**, sesi **2015-2019**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesisi ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepengtingan, Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan Oleh,

CARLMOND GOH KAH WUN
MZ1511001T

Tarikh: 3 SEPTEMBER 2019

(Tanda Tangan Pustakawan)

Dr. Cahyo Budiman
Penyelia

DECLARATION

I, Carlmond Goh Kah Wun, student of Biotechnology Research Institute of University Malaysia Sabah hereby declare that my thesis entitled "Characterization of the Functional Domains of FKBP35 from *Plasmodium knowlesi*" is the result of my work and has not been submitted for any degree or professional qualification, except stated, otherwise by reference or acknowledgement. This study was carried out by me for my Master of Science (MSc.) under the guidance and supervision of Dr. Cahyo Budiman and Mdm Sophia Lau Tiek Ying.

.....

21st AUGUST 2019

CARLMOND GOH KAH WUN
MZ1511001T

CERTIFICATION

NAME	:	**CARLMOND GOH KAH WUN**
MATRIC NO.	:	**MZ1511001T**
TITLE	:	**CHARACTERIZATION OF THE FUNCTIONAL DOMAINS OF FKBP35 FROM *Plasmodium knowlesi***
DEGREE	:	**MASTER OF SCIENCE (BIOTECHNOLOGY)**
VIVA DATE	:	**21st MAY 2019**

CERTIFIED BY,

MAIN SUPERVISOR

Dr. Cahyo Budiman

SIGNATURE

CO-SUPERVISOR

Mdm. Lau Tiek Ying

ACKNOWLEDGEMENT

I would like to thank all those who have contributed to my study. Word is inadequately expressing my deepest gratitude to my supervisor, Dr. Cahyo Budiman for his fruitful comments and invaluable suggestion which have been a very crucial formative influence in my study. Without his expert guidance, support, and encouragement, my research would never come out in the present form.

Completing my master study have been more difficult without the supports and friendship provided by Jovi Silvester, Norzulaiha Abd Karim, Wan Nur Shuhaida Wan Mahadi, Rafida Razali, Herman Umbau Lindang, and Nursyuhada Mohamad Zaini, the members of Protein Research Group at BRI. Not to forgot Fernandes Opook in assistance and guidance me when I started my research and Pressly Matthew whenever I need assistance. I am indebted for all their helps.

Special thanks to Madam Azyyati Padzil from Malaysia Genome Institute (MGI) on her guidance and access to surface plasmon resonance (Biacore) instrument to complete my works.

Next, I wish to thank all the lecturers, staffs, lab assistants, students, and other members of BRI for the direct and indirect guidance and good environment while doing my work in BRI.

I also want to thank the Ministry of Higher Education Malaysia which financially support this research work under FRGS grant (Research grant code: FRGS0395-ST-2/2014). My sincerest gratitude also goes to University Malaysia Sabah for providing and allowing me for providing all the resource in completing this thesis. Lastly, I wish to thank my family for their endless love, financial support and motivation; and thanks to all my friends for their love and encouragement.

Carlmond Goh Kah Wun

21st August 2019

ABSTRACT

High incident of *Plasmodium knowlesi* accompanied by the increase in drug resistance cases in Malaysia Borneo urges us to develop a novel antimalarial drug with no resistance. Despite its advantages as an antimalarial compound without resistance effects, FK506 displayed an immunosuppressive side effect. In attempts to find FK506 replacers, solid fundamental studies on target molecule of FK506 are needed to provide a platform for the development of novel antimalarial drug without the risk of resistance. Previous studies revealed FK506 inhibited function of FKBP35 from *Plasmodium falciparum*. Genome sequence of *P. knowlesi* suggested the presence of its FKBP35 (Pk-FKBP35) in which has high sequence similarity with FKBP35 from *P. falciparum* (Pf-FKBP35) and *P. vivax* (Pv-FKBP35), thus suggesting Pk-FKBP35 is considered a viable target for combating *P. knowlesi*. FKBP35 is a member of peptidyl-prolyl *cis-trans* isomerase (PPIase), consisting FK506-binding domain (FKBD) followed by tetratricopeptide repeated domain (TPRD). There is no study so far on Pk-FKBP35, particularly on the functionality of these domains. In fact, most of the studies on Pf- and Pv-FKBP35 were focused on FKBD region. This is due to the finding that FKBD is the region where FK506 binds and inhibits PPIase catalytic function of this protein. Previous studies on Pf-FKBP35 and Pv-FKBP35 also suggested that this protein might exhibit a dual-function of foldase and chaperone-like activities. However, involvement of the domains of FKBP35 on this dual-function remains poorly understood. High similarity between Pk-FKBP35 and Pf-FKBP35 suggested that Pk-FKBP35 might also exhibit dual-function, yet no experimental evidence was reported. This study aims to understand the regulatory domains for the function of FKBP35 from *Plasmodium knowlesi*. To address, expression system containing gene encoding full-length of Pk-FKBP35 and its derivatives, Pk-FKBD and PK-TPRD were constructed. Each of them was expressed in *Escherichia coli* BL21(DE3). Two steps purifications including Ni-NTA binding affinity followed by gel filtration yielded 109, 162 and 189 mg of proteins from 1 L culture of Pk-FKBP35, Pk-FKBD, and Pk-TPRD respectively. Pk-FKBD showed comparable catalytic PPIase activities with full-length Pk-FKBP35 when tested using synthetic tetrapeptide (Suc-Ala-Leu-Pro-Phe-AMC) with k_{cat}/K_M of 4.7 x 10^5 M^{-1} s^{-1}

while full-length Pk-FKBP35 yielded k_{cat}/K_M of 5.0 x 10^5 M^{-1} s^{-1}. Meanwhile, no PPIase activity was detected when Pk-TPRD was measured. This suggested that catalytic activity of Pk-FKBP35 resides at its FKBD. Further, this PPIase activity was also confirmed to be inhibited by FK506 with IC_{50} values of 310 and 309 for full-length Pk-FKBP35 and Pk-FKBD, respectively. The binary complex of FK506-FKBP35 was also found to be able to extremely increase the inhibition properties toward calcineurin phosphatase activity. This inhibition was also modulated by FKBD region, while TPRD is apparently important to maximize. Dual-function of Pk-FKBP35 was firstly examined on its foldase activity using RNase T1 as a protein substrate. Pk-FKBP35 demonstrated remarkable ability to catalyze slow-folding of RNase T1 with k_{cat}/K_M of 14.0 x 10^5 M^{-1} s^{-1}. Interestingly, Pk-FKBD was also able to catalyse slow-folding of RNase T1, with k_{cat}/K_M of 3.0 x 10^5 M^{-1} s^{-1}, which was lower than Pk-FKBP35. By contrast, no foldase activity was detected on Pk-TPRD. This suggested that Pk-FKBD is essential for foldase activity. However, the presence of Pk-TPRD is still required to maximize the activity. Meanwhile, Pk-FKBP35 was also confirmed to exhibit chaperone-like activity as indicated by its ability to bind to a folding intermediate of α-lactalbumin, with K_D of 24.39 μM, and prevent DTT-induced insulin aggregation in a concentration dependent manner. Further analysis of chaperone activity on Pk-TPRD and Pk-FKBD revealed that Pk-TPRD is a regulatory domain for binding to a folding intermediate of α-lactalbumin with K_D of 15.75 μM and prevention of DTT-induced insulin aggregation. This study confirmed that both domains of Pk-FKBP35 contributed to different function of this protein. Inhibition of any of this domain is believed to cause disruption of Pk-FKBP35 function and therefore promote the death of parasite cells. Altogether, targeting any of this domain in drug development is considered a good strategy to combat malaria diseases.

ABSTRAK

PENCIRI-CIRIKAN DAN FUNGSI DOMAIN FKBP35 DARIPADA Plasmodium knowlesi

Insiden kejadian Plasmodium knowlesi yang tinggi serta peningkatan kes rintangan ubatan di Borneo Malaysia, mendorong kami untuk menghasilkan ubat baharu antimalaria tanpa rintangan. FK506 memberikan kesan imunosupresif walaupun kelebihannya sebagai sebatian antimalaria tanpa kesan rintangan. Dalam percubaan mencari penganti bagi FK506, satu kajian terhadap molekul sasaran pada FK506 diperlukan bagi menyediakan platform untuk membangunakan ubat anti-malarial tanpa rintangan. Kajian terdahulu pada Plasmodium falciparum mendedahkan bahawa FK506 menghalang fungsi FKBP35. Urutan genome mencadangkan kehadiran FKBP35 pada P. Knowlesi (Pk-FKBP35) menunjukkan persamaan yang tinggi dengan FKBP35 dari P. falciparum (Pf-FKBP35) dan P. vivax (Pv-FKBP35), justeru mencadangkan Pk-FKBP35 dianggap sebagai sasaran untuk memerangi jangkitan P. knowlesi di Asia Tenggara. FKBP35 adalah ahli isomerase cis-trans peptidyl-prolyl (PPIase), yang merangkumi domain FK506-pengikat (FKBD) diikuti oleh domain berulang tetratricopeptide (TPRD). Setakat ini, belum ada kajian tentang Pk-FKBP35, terutamanya mengenai fungsi domain-domain ini. Kebanyakan kajian hanya dari P. Falciparum FKBP35 dan P. vivax -FKBP35 dan difokuskan kepada bahagian domain FKBD. Ini disebabkan bahawa penemuan mengenai FKBD adalah kawasan di mana FK506 mengikat dan menghalang fungsi katalitik protein PPIase ini. Kajian terdahulu mengenai Pf-FKBP35 dan Pv-FKBP35 mencadangkan bahawa protein tersebut mungkin menunjukan aktiviti dual-fungsi dalam perlipatan dan aktiviti chaperon. Walau bagaimanapun, penglibatan domain FKBP35 dalam dual-fungsi ini masih kurang difahami. Persamaan yang tinggi antara Pk-FKBP35 dan Pf-FKBP35 menunjukkan bahawa Pk-FKBP35 juga berkemungkinan menunjukkan dual-fungsi, namun tiada bukti eksperimen dilaporkan. Kajian ini bertujuan untuk memahami domain kawalan bagi fungsi FKBP35 dari Plasmodium Knowlesi. Untuk menangani masalah ini, sistem expressi yang mengandungi pengekodan gen penuh Pk-FKBP35 dan derivatifnya, Pk-FKBD dan PK-TPRD telah dibina. Setiap daripada mereka dihasilkan dalam bacteria Escherichia coli BL21

(DE3). Dua langkah penapisan termasuklah afiniti pengikatan Ni-NTA diikuti oleh penapisan gel menghasilkan 109, 162 dan 189 mg protein dari 1 L kultur Pk-FKBP35, Pk-FKBD, dan Pk-TPRD masing-masing. Pk-FKBD menunjukkan aktiviti PPIase katalitik yang sama dengan Pk-FKBP35 apabila diuji menggunakan tetrapeptida sintetik (Suc-Ala-Leu-Pro-Phe-AMC) dengan k_{cat} / K_M 4.7 x 105 M^{-1} s^{-1}, Pk-FKBP35 menghasilkan k_{cat}/K_M of 4.7 x 10^5 M^1 s^{-1}. Sementara itu, tiada aktiviti PPIase pada Pk-TPRD. Ini menunjukkan bahawa aktiviti pemangkin Pk-FKBP35 berada di FKBDnya. Seterusnya, aktiviti PPIase ini juga dihalang oleh FK506 dengan IC_{50} nilai 310 dan 309 nm bagi Pk-FKBP35 dan Pk-FKBD. Kompleks gabungan FK506-FKBP35 juga dapat meningkatkan sifat-sifat penghalangan terhadap aktiviti calcineurin phosphatase. Inhibisi ini dimodulasi oleh bahagian FKBD, pada masa yang sama TPRD penting untuk memaksimumkan inhibisi ini. Aktiviti dual-fungsi ini pertama kali dikaji pada aktiviti penglipatannya menggunakan RNase T1 sebagai protein substrat. Pk-FKBP35 menunjukkan keupayaan yang untuk mempercepatkan perlipatan RNase T1 yang lambat dengan k_{cat}/K_M of 14.0 x 10^5 M^1 s^{-1}. Pk-FKBD juga dapat mempercepatkan perlipatan RNase T1, dengan k_{cat}/K_M of 3.0 x 10^5 M^1 s^{-1}, lebih rendah daripada Pk-FKBP35. Sebaliknya, tiada aktiviti perlipatan dikesan pada Pk-TPRD. Ini mencadangkan bahawa Pk-FKBD penting untuk aktiviti perlipatan. Walau bagaimanapun, kehadiran Pk-TPRD masih diperlukan untuk memaksimumkan aktiviti tersebut. Sementara itu, Pk-FKBP35 disahkan untuk menunjukkan aktiviti chaperon seperti yang ditunjukkan oleh keupayaannya untuk mengikat pada perlipatan sementara α-laktalbumin, dengan nilai K_D 24.39 μM, dan menghalang agregasi insulin yang disebabkan oleh DTT. Analisa lebih lanjut mengenai aktiviti chaperon pada Pk-TPRD dan Pk-FKBD mendedahkan bahawa Pk-TPRD adalah domain kawalan seliaan untuk mengikat kepada pertengahan perlipatan α-laktalbumin dan mencegahan agregasi insulin daripada DTT dengan nilai K_D 15.75 μM. Kajian ini mengesahkan bahawa kedua-dua domain pada Pk-FKBP35 menyumbang kepada dual-fungsi berlainan bagi protein ini. Penghalangan daripada domain-domain ini dipercayai menyebabkan gangguan fungsi Pk-FKBP35 dan menyumbang kepada kematian sel parasit. Kedua-dua domain ini disarankan sebagai penghasilan ubatan yang dianggap sebagai strategi baik untuk memerangi penyakit malaria.

TABLE OF CONTENTS

	Pages
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	v
ABSTRAK	vii
TABLE OF CONTENTS	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xv
LIST OF SYMBOLS	xvi
LIST OF APPENDICES	xvii

CHAPTER 1: INTRODUCTION

1.1 Background Study	1
1.2 Problem Statement	5
1.3 Hypothesis	5
1.4 Aim and Objectives	6

CHAPTER 2: LITERATURE REVIEW

2.1 Malaria	7
2.2 Malaria Life Cycle	11
2.3 Human Malaria and the Emerge of *Plasmodium knowlesi* in Malaysia	12
2.4 Antimalarial drug and resistance	13

2.5 Member of PPIase Family Protein 17

2.6 FKBP35 18

2.7 Structure of FKBP35 18

2.8 Catalytic Activity of PPIase 19

2.9 Structure of FK506 Binding Domain (FKBD) 22

2.10 Structure of Tetratricopeptide Repeat Domain (TPRD) 24

2.11 FK506 Drug 26

2.12 Chaperon Function 28

2.13 Surface Plasmon Resonance (SPR) 31

CHAPTER 3: MATERIALS AND METHODS

3.1 Gene Synthesis and Expression System Construction 34

3.2 Competent Cell Preparation 35

3.3 Transformation of Expression System into *E. coli* BL21 (DE3) 36

3.4 Protein Expression and Purification 36

3.5 SDS PAGE 37

3.6 PPIase Activity 38

3.7 FK506 Inhibition 39

3.8 DTT-induced Aggregation of Insulin Refolding Assay 39

3.9 Surface Plasmon Resonance (SPR) 40

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Codon Optimization 41

4.2 Expression System and Construction of Full-length Pk-FKBP35 49

4.3 Construction of Pk-FKBD and Pk-TPRD 52

4.4 Protein Expression and Purification 53

4.5 PPIase Activity of Protein Expressed and FK506 Inhibition 58

4.6 Calcineurin Activity 67

4.7 Involvement of Pk-FKBP35 in protein folding: Dual-function 69

4.7.1 Foldase Activity 70

4.7.2 Chaperone-like activity 74

4.7.2.1 Binding to a Folding Intermediate of α-lactalbumin 75

4.7.2.2 Insulin Aggregation Assay 80

CHAPTER 5: CONCLUSION

6.1 Conclusion 85

6.2 Future Prospect 88

REFERENCES 89

APPENDICES 103

LIST OF TABLES

		Pages
Table 2.1	Classes of Antimalarial Drug and Example of Respective Class	9
Table 2.2	Currently Used Antimalarial Drugs	15
Table 2.3	Resistance of Antimalarial Drug and their Mechanisms	16
Table 2.4	Important Residues of PPIase Family Protein	18
Table 2.5	Binding Examples of TPR Domain	25
Table 4.1	Comparison of IC_{50} value between Pf-FKBD, Pv-FKBD, and Pk-FKBD	65

LIST OF FIGURES

		Pages
Figure 2.1	Countries with indigenous in 2000 and their status by 2016	10
Figure 2.2	Life cycle of malaria	11
Figure 2.3	Asymmetry unit of Pv-FKBP35 in complex with ligand D44	19
Figure 2.4	PPIase activity at *cis* and *trans* form of prolyl bonds	20
Figure 2.5	NMR solution of Pv-FKBD	23
Figure 2.6	TPR domain of *Plasmodium falciparum*	24
Figure 2.7	Crystal structure of TPRD	25
Figure 2.8	Structure of FK506 and its derivatives	27
Figure 2.9	Mechanism of action of Tacrolimus (FK506)	27
Figure 2.10	Structure of Dnak	30
Figure 2.11	Reconstructed structure of the CIpB Hexamer	31
Figure 2.12	The typical setup of SPR technology	32
Figure 3.1	Schematic representation of the primary structure of full-length PK-FKBP5 and its domain (Pk-FKBD and Pk-TPRD)	35
Figure 4.1	Average GC content and distribution of codon frequency along the length of Pk-FKBP35 gene sequence before and after optimization	44
Figure 4.2	Gene sequence of optimized and unoptimized (original) of Pk-FKBP35	45
Figure 4.3	Amino acid sequence of optimized and unoptimized (original) of Pk-FKBP35	47
Figure 4.4	Plasmid Construction Map of PK-FKBP35	49
Figure 4.5	Agarose gel of pET29b before and after digested	50
Figure 4.6	Colonies of Pk-FKBP35	52

Figure 4.7	Agarose gel bands of Pk-TPRD and Pk-FKBD	53
Figure 4.8	Expression check of full-length Pk-FKBP35, Pk-FKBD and Pk-TPRD	57
Figure 4.9	Comparison of Pk-FKBP35 with other FKBP family protein	60
Figure 4.10	Catalytic activity of full-length Pk-FKBP35 in different concentration	62
Figure 4.11	Catalytic activity of full-length Pk-FKBP35 and its derivatives	64
Figure 4.12	Inhibition of full-length Pk-FKBP35 and Pk-FKBD by FK506 drug	66
Figure 4.13	Effect of Pk-FKBP35 and its derivatives toward calcineurin activity	68
Figure 4.14	Binding of RNase T1 to Pk-FKBP35	71
Figure 4.15	Binding of RNase T1 to Full-length Pk-FKBP35 and its derivatives	73
Figure 4.16 (a)	Sensogram of Biacore X100 showing the binding of full-length Pk-FKBP35 with reduced α-lactalbumin	76
Figure 4.16 (b)	The binding response of Pk-FKBP35	77
Figure 4.17 (a)	Sensogram of Biacore X100 showing the binding of Pk-FKBD and Pk-TPRD with reduced α-lactalbumin	79
Figure 4.17 (b)	The binding response of Pk-TPRD	80
Figure 4.18 (a)	The binding of intermediate insulin with different concentration of full-length Pk-FKBP35.	82
Figure 4.18 (b)	The binding of intermediate insulin with full-length Pk-FKBP35 and its derivatives	83
Figure 6.1	Summary of full-length Pk-FKBP35 and its derivatives on their respective function	87

LIST OF ABBREVIATIONS

bp	Base pair
FKBD	FKBP binding domain
FKBP35	FK506-binding domain
P. falciparum	*Plasmodium falciparum*
P. knowlesi	*Plasmodium knowlesi*
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

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

LIST OF APPENDICES

		Pages
Appendix A	Flow Chart of KOD Plus Mutagenesis for Pk-FKBD and Pk-TPRD	103
Appendix B	iPCR Reaction Cocktail	104
Appendix C	SDS Page Cocktail	104
Appendix D	PPIase Assay Cocktail	105
Appendix E	RNase T1 Cocktail	1105
Appendix F	PPIase Inhibitor Assay Cocktail	106
Appendix G	Insulin Refolding Assay Cocktail	107
Appendix H	pET-29 Map	107

CHAPTER 1

INTRODUCTION

1.1 Background Study

Human malaria is an overwhelming disease that caused more than half of the world's population at risk. Malaria disease is caused by a parasite of Plasmodium genus and it can be transmitted from macaque to human, and between human through bites of female *Anopheles* mosquitoes. In some rare cases, this parasite can be transmitted via infected blood transfusion or through placenta delivery from an infected pregnant mother to the infant in the womb (Malhotra *et al.*, 2006). Based on World Health Organization (2018), there were an estimated 212 million new human malaria cases and up to 445,000 deaths in the year 2016 even though anti-malarial drugs and treatments are available. However, until today, there are no valid licensed for vaccines towards malaria, hence raising the global stress in the finding malarial treatment. Studies found that there are 6 Plasmodium species that caused human malaria, which are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi* and *P. Cynomology*. *P. knowlesi* is the latest species causing malaria in human (Daneshvar *et al.*, 2009).

Among the 6 species of Plasmodium parasite, *P. knowlesi* was found caused most of human malaria in Southeast Asia, with high incidences were reported at the Borneo island, Peninsular Malaysia, Thailand, Singapore, Philippines, Vietnam and Myanmar (Lau & Joveen-Neoh, 2011a, 2011b). However, the most widespread of *P. knowlesi* is in Malaysian Borneo region. In the year of 2004, Singh *et al.* (2004) discovered a large number of *P. knowlesi* malaria were misdiagnosed as *P. malariae*

malaria due to their similarity in microscopic examination by PCR which consequently caused delayed in treatment in Sarawak, Malaysia. Since then, the cases of *P. knowlesi* malaria was reported progressively mostly from Southeast Asia region hence confirming the existence of human malaria that is caused by *P. knowlesi*. Apart from that, large numbers of human malaria were reported by travellers whose visited countries in Southeast Asia, especially Sabah and Sarawak states of Malaysia, were caused by *P. knowlesi* (Bronner *et al.*, 2009; Daneshvar *et al.*, 2009; Figtree *et al.*, 2010; Joveen-Neoh, *et al.*, 2011).

Due to the rapid development of malaria parasite, available antimalarial drugs such as chloroquine and artemisinin begins to exhibit resistant effect (Alag *et al.*, 2010) hence increases the rate of malarial disease infection. William *et al.* (2011) reported that 39% of the malarial patients in Queen Elizabeth Hospital (QEH), Kota Kinabalu suffered from severe *P. knowlesi* malaria, while six of them have died. While Cox-Singh *et al.* (2008) stated that four fatal cases were reported in Sarawak caused by *P. knowlesi* between the years 2004 – 2005. This brought the urge in discovering a new anti-malarial drug for *P. knowlesi* in addition to the most used anti-malarial drug caused resistant to the parasite (Kotaka *et al.*, 2008). One of the ways in drug design is through re-visiting and re-designing the existing anti-malarial drug.

Based on previous studies, malarial activities of *P. falciparum* and *P. vivax* parasite were found to be inhibited by FK506 drugs, this lead to the binding target site of FK506 on Pf- and Pv-FKBP35, a member in FKBP family in both *P. falciparum* and *P. vivax*, which also conserved in *P. knowlesi*, named as Pk-FKBP35 (Alag *et al.*, 2010). Tacrolimus or commonly known as FK506 is an immunosuppressive drug that usually used for prevention of rejection during organ transplant, surprisingly it shows antimalarial activity. FK506 is a 23-membered macrolide lactone that found to binds at peptidyl-prolyl *cis-trans* isomerase (PPIase) domains of various FK506-binding proteins (FKBPs), including FKBPs of Plasmodium species, hence promising malarial drug target site (Alag *et al.*, 2010; Kotaka *et al.*, 2008). However, due to

the immunosuppressive effect of this FK506 drug, novel antimalarial drug replacing FK506 must be developed. To address, comprehensive studies on the function of pk-FKBP35 should contributed to accelerate the development of FK506 replacers.

FKBP35 is a 35 kDa FKBP member protein that can be identified in most *Plasmodium* species, which known to involve in malaria infection (Alag *et al.*, 2009). Pf- and Pv-FKBP35 exist as a multi-domain protein containing N-terminal FK506-binding domain (FKBD) followed by tetratricopeptide domain (TPRD) with a putative calmodulin binding site (Alag *et al.*, 2013; Kumar *et al.*, 2005). FKBD was known to have *cis-trans* peptidyl-prolyl isomerase (PPIase) activities, which function in the conversion of *cis-trans* rotamers of peptidyl-prolyl amide bones (Alag *et al.*, 2010); while TPRD believed to exhibit chaperone function, which both of these functions are related in protein folding (Bianchin *et al.*, 2015). Pf-FKBP35 also found to interact with calcineurin in a dependent and independent manner in the presence of FK506, this highly represents the potential drug target site (Kumar *et al.*, 2005; Monaghan & Bell, 2005). The high sequence similarity of FKBP35 protein in *P. knowlesi* with both *P. falciparum* and *P. vivax* assuming Pk-FKBP35 holding both FKBD and TPRD hence increase the possibility of Pk-FKBP35 exhibit both PPIase and chaperone activities (Kotaka *et al.*, 2008; Yoon, *et al.*, 2007). Due to the multi-domains of FKBP35, it is believed FKBP35 exhibit dual-function which are PPIase activity and chaperone function, that held in each of the domain.

PPIase is a highly conserved protein family that can be found in almost all living organisms. It functions as an enzyme that accelerates the rate-limiting *cis-trans* isomerisation of Xaa-Pro peptide bones. This process occurs during protein folding reactions, especially during refolding of denatured proteins, de novo protein synthesis as well as during the formation of biologically active conformations of polypeptides (Budiman *et al.*, 2012; Schiene-Fischer & Yu, 2001). Based on their inhibitor specificity, PPIase was divided into three families, which are cyclophilins (CyPs), FK506-binding proteins (FKBPs), and parvulins, however all of them having different sequences and structures. The evolving of these proteins was believed to

recognize the specific signature of proteins sequences and supervise *in vivo* protein folding (Schiene-Fischer & Yu, 2001). Studies found that PPIase activity of CyPs can be inhibited by cyclosporine while FKBPs can be suppressed by FK506 and rapamycin. Hence, they usually used as target inhibitor in immunosuppressive drugs development. FKBPs can be found in most Plasmodium species, this indicates that they show PPIase activities. Most of FKBPs family members, including Pf- and Pv-FKBP35 showing PPIase activities (Kumar *et al.*, 2005; Monaghan & Bell, 2005), hence there is a high probability of Pk-FKBP35 having PPIase activity. However, the natural function of FKBPs still poorly understood.

On the other hand, chaperone is a type of protein that functions to assist the proper folding of a protein. Misfolding or aggregation of protein thereby by disrupting the cell function and lead to diseases such as Alzheimer and Parkinson diseases (Caughey & Lansbury Jr, 2003). Hence chaperone plays an important role in preventing as well as refolding of the misfolded protein (Buchner, 1996). Chaperone can be reusable as it will release the properly folded protein. Besides, chaperone tend to control the folding state not only under normal conditions, but as a protection for the protein during extreme/stress conditions such as in different temperature or unsuitable pH that might lead to protein self-destruction hence protein unfolding/aggregation (Hoffmann, *et al.*, 2004). Because of this, most of the chaperones are heat shock protein (HSP) since it can endure in different temperature states. Chaperone usually assists in post-translational mechanisms that can be easily found on endoplasmic reticulum where it provided space for the newly synthesized polypeptide to fold properly. Based on Hoffmann *et al.* (2004), there are two systems of chaperones, foldase and holdase. Foldase support protein folding in ATP-dependent manner while holdase prevent aggregation by binds to protein folding intermediate state, but not support in protein folding.

Some PPIase family member exhibited chaperone function in addition to their PPIase catalytic activity (Alag *et al.*, 2013; Kumar *et al.*, 2005; Yoon *et al.*, 2007). Interestingly, the chaperone function is mostly found in multi-domain PPIase.

In this group of PPIase, catalytic activity and chaperone function are often found to be separately regulated by different domains. FKBP35 from *P. falciparum* was reported to have a dual-function (Kotaka *et al.*, 2008). Given the high similarity between Pf- and Pk-FKBP35, the later protein assumes to exhibit dual-function. Nevertheless, experimental evidence remains to be confirmed. Even more, whether the dual-function of Plasmodium FKBP35 is specifically regulated by different domain is unknown.

In this study, we demonstrated full-length Pk-FKBP35 exhibited both PPIase and chaperone activities comparable to those Pf- and Pv-FKBP35, in addition to identify regulatory domains for the catalytic and chaperone activities of this protein.

1.2 Problem Statement

As a worldwide threat, Malaria needs more attention and efforts to reduce the infection cases. In Southeast Asia, especially at Borneo region, most of the malaria was caused by *P. knowlesi*. There are antimalarial drugs available at the market however most of them were found resistance toward malarial disease and this became a threat to global efforts in controlling and eliminating malaria. Better inhibitor toward FKBP35, a malarial target gene, in combating malarial disease must be developed. However, there are only studies of FKBP35 from other species such as *P. falciparum* and *P. vivax* but no *P. knowlesi*. Moreover, most of the studies only focus on the catalytic part, but less study on dual-function on both PPIase and chaperone activities. Hence comprehensive understanding towards dual-functions of Pk-FKBP35 should be studied.

1.3 Hypothesis

It is hypothesised that Pk-FKBP35 exhibit remarkable PPIase activity which is regulated by PK-FKBD, by accelerating the isomerisation of tetrapeptide; it also hypothesized that Pk-FKBP35 and Pk-FKBD also regulate the function of foldase by accelerating the isomerisation of RNase T1. Lastly, it is hypothesized that Pk-FKBP and Pk-TPRD exhibit chaperone by binding to protein intermediate and prevent protein aggregation.

1.4 Aim and Objectives

This study aims to understand regulatory domains for the function of FKBP35 from *Plasmodium knowlesi*. This understanding may serve as a platform for further development of an antimalarial drug. To address, the objectives of the study are:

(i) To determine the catalytic PPIase activity and inhibition by FK506 of full-length Pk-FKBP35 and its derivatives,

(ii) To determine the foldase activity of full-length Pk-FKBP35 and its derivatives, and

(iii) To determine the chaperone function of full-length Pk-FKBP35 and its derivatives.

REFERENCES

Acharya, K., Stuart, D., Walker, N., Lewis, M., & Phillips, D. (1989). Refined structure of baboon α-lactalbumin at 1.7 Å resolution: Comparison with C-type lysozyme. *Journal of molecular biology, 208*(1), 99-127.

Acharya, K. R., Ren, J., Stuart, D. I., Phillips, D. C., & Fenna, R. E. (1991). Crystal structure of human α-lactalbumin at 1· 7 Å resolution. *Journal of molecular biology, 221*(2), 571-581.

Alag, R., Balakrishna, A. M., Rajan, S., Qureshi, I. A., Shin, J., Lescar, J., . . . Yoon, H. S. (2013). Structural Insights into Substrate Binding by PvFKBP35, a Peptidylprolyl cis-trans Isomerase from the Human Malarial Parasite Plasmodium vivax. *Eukaryotic Cell, 12*(4), 627-634. doi: 10.1128/EC.00016-13

Alag, R., Bharatham, N., Dong, A., Hills, T., Harikishore, A., Widjaja, A. A., . . . Yoon, H. S. (2009). Crystallographic structure of the tetratricopeptide repeat domain of Plasmodium falciparum FKBP35 and its molecular interaction with Hsp90 C-terminal pentapeptide. *Protein Science, 18*(10), 2115-2124.

Alag, R., Qureshi, I. A., Bharatham, N., Shin, J., Lescar, J., & Yoon, H. S. (2010). NMR and crystallographic structures of the FK506 binding domain of human malarial parasite Plasmodium vivax FKBP35. *Protein Science : A Publication of the Protein Society, 19*(8), 1577-1586. doi: 10.1002/pro.438

Alifrangis, M., Nag, S., Schousboe, M. L., Ishengoma, D., Lusingu, J., Pota, H., . . . Lynch, C. (2014). Independent origin of Plasmodium falciparum antifolate super-resistance, Uganda, Tanzania, and Ethiopia. *Emerging infectious diseases, 20*(8), 1280.

Allan, R. K., & Ratajczak, T. (2011). Versatile TPR domains accommodate different modes of target protein recognition and function. *Cell stress and chaperones, 16*(4), 353-367.

Arié, J. P., Sassoon, N., & Betton, J. M. (2001). Chaperone function of FkpA, a heat shock prolyl isomerase, in the periplasm of Escherichia coli. *Molecular microbiology, 39*(1), 199-210.

Austin, C., Davis, J. B., Fliri, H. G., Ford, R. L., & Steadman, V. A. (2015). Prolyl Isomerases as New Therapeutic Targets.

Baughman, G., Wiederrecht, G. J., Chang, F., Martin, M. M., & Bourgeois, S. (1997). Tissue distribution and abundance of human FKBP51, an FK506-binding protein that can mediate calcineurin inhibition. *Biochemical and biophysical research communications, 232*(2), 437-443.

Beigi, L., Karbalaei-Heidari, H. R., & Kharrati-Kopaei, M. (2012). Optimization of an extracellular zinc-metalloprotease (SVP2) expression in Escherichia coli BL21 (DE3) using response surface methodology. *Protein expression and purification, 84*(1), 161-166.

Bennett, J., Cassidy, H., Slattery, C., Ryan, M., & McMorrow, T. (2016). Tacrolimus Modulates TGF-β Signaling to Induce Epithelial-Mesenchymal Transition in Human Renal Proximal Tubule Epithelial Cells. *Journal of clinical medicine, 5*(5), 50.

Bianchin, A., Allemand, F., Bell, A., Chubb, A. J., & Guichou, J.-F. (2015). Two crystal structures of the FK506-binding domain of Plasmodium falciparum FKBP35 in complex with rapamycin at high resolution. *Acta Crystallographica Section D: Biological Crystallography, 71*(6), 1319-1327.

Braakman, I., & Hebert, D. N. (2013). Protein folding in the endoplasmic reticulum. *Cold Spring Harbor perspectives in biology, 5*(5), a013201.

Bronner, U., Divis, P. C., Färnert, A., & Singh, B. (2009). Swedish traveller with Plasmodium knowlesi malaria after visiting Malaysian Borneo. *Malaria Journal, 8*(1), 15.

Buchner, J. (1996). Supervising the fold: functional principles of molecular chaperones. *The FASEB journal, 10*(1), 10-19.

Budiman, C., Angkawidjaja, C., Motoike, H., Koga, Y., Takano, K., & Kanaya, S. (2011). Crystal structure of N-domain of FKBP22 from Shewanella sp. SIB1: Dimer dissociation by disruption of Val-Leu knot. *Protein Science, 20*(10), 1755-1764.

Budiman, C., Bando, K., Angkawidjaja, C., Koga, Y., Takano, K., & Kanaya, S. (2009). Engineering of monomeric FK506-binding protein 22 with peptidyl prolyl cis-trans isomerase. *FEBS Journal, 276*(15), 4091-4101.

Budiman, C., Koga, Y., Takano, K., & Kanaya, S. (2011). FK506-Binding Protein 22 from a Psychrophilic Bacterium, a Cold Shock-Inducible Peptidyl Prolyl Isomerase with the Ability to Assist in Protein Folding. *International Journal of Molecular Sciences, 12*(8), 5261-5284. doi: 10.3390/ijms12085261

Budiman, C., Tadokoro, T., Angkawidjaja, C., Koga, Y., & Kanaya, S. (2012). Role of polar and nonpolar residues at the active site for PPIase activity of FKBP22 from Shewanella sp. SIB1. *FEBS Journal, 279*(6), 976-986.

Capanna, E. (2006). Grassi versus Ross: who solved the riddle of malaria? *International Microbiology, 9*(1), 69-74.

Caughey, B., & Lansbury Jr, P. T. (2003). Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annual review of neuroscience, 26*(1), 267-298.

Chin, W., Contacos, P. G., Coatney, G. R., & Kimball, H. R. (1965). A naturally acquired quotidian-type malaria in man transferable to monkeys. *Science, 149*(3686), 865-865.

Chin, W., Contacos, P. G., Collins, W. E., Jeter, M. H., & Alpert, E. (1968). Experimental mosquito-transmission of Plasmodium knowlesi to man and monkey. *The American journal of tropical medicine and hygiene, 17*(3), 355-358.

Cieplak, A. S. (2017). Protein folding, misfolding and aggregation: The importance of two-electron stabilizing interactions. *PLoS ONE, 12*(9), e0180905. doi: 10.1371/journal.pone.0180905

Cogswell, F. B. (1992). The hypnozoite and relapse in primate malaria. *Clinical microbiology reviews, 5*(1), 26-35.

Cox-Singh, J., Davis, T. M., Lee, K.-S., Shamsul, S. S., Matusop, A., Ratnam, S., . . . Singh, B. (2008). Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clinical infectious diseases, 46*(2), 165-171.

Cox-Singh, J., & Singh, B. (2008). Knowlesi malaria: newly emergent and of public health importance? *Trends in Parasitology, 24*(9), 406-410.

Cox, F. E. (2010). History of the discovery of the malaria parasites and their vectors. *Parasites & vectors, 3*(1), 5.

Cox, F. E. G. (2002). History of Human Parasitology. *Clinical microbiology reviews, 15*(4), 595-612. doi: 10.1128/CMR.15.4.595-612.2002

Cui, L., Mharakurwa, S., Ndiaye, D., Rathod, P. K., & Rosenthal, P. J. (2015). Antimalarial Drug Resistance: Literature Review and Activities and Findings of the ICEMR Network. *The American journal of tropical medicine and hygiene, 93*(3 Suppl), 57-68. doi: 10.4269/ajtmh.15-0007

Daneshvar, C., Davis, T. M., Cox-Singh, J., Rafa'ee, M. Z., Zakaria, S. K., Divis, P. C., & Singh, B. (2009). Clinical and laboratory features of human Plasmodium knowlesi infection. *Clinical infectious diseases, 49*(6), 852-860.

Davis, T. L., Walker, J. R., Campagna-Slater, V., Finerty Jr, P. J., Paramanathan, R., Bernstein, G., . . . Lee, W. H. (2010). Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. *PLoS biology, 8*(7), e1000439.

De León, A., Jiménez-Islas, H., González-Cuevas, M. a., & de la Rosa, A. P. B. (2004). Analysis of the expression of the Trichoderma harzianum ech42 gene in two isogenic clones of Escherichia coli by surface response methodology. *Process Biochemistry, 39*(12), 2173-2178.

de Mello, A. J. (1996). Total Internal Reflection Fluorescence Spectroscopy. *Surface Analytical Techniques for Probing Biomaterial Processes*, 1-41.

De Silva, J. R., Lau, Y.-L., & Fong, M.-Y. (2016). Expression and Evaluation of Recombinant Plasmodium knowlesi Merozoite Surface Protein-3 (MSP-3) for Detection of Human Malaria. *PLoS ONE, 11*(7), e0158998.

DeCenzo, M. T., Park, S. T., Jarrett, B. P., Aldape, R. A., Futer, O., Murcko, M. A., & Livingston, D. J. (1996). FK506-binding protein mutational analysis: defining the active-site residue contributions to catalysis and the stability of ligand complexes. *Protein engineering, 9*(2), 173-180.

Díaz-Villanueva, J., Díaz-Molina, R., & García-González, V. (2015). Protein folding and mechanisms of proteostasis. *International Journal of Molecular Sciences, 16*(8), 17193-17230.

Dobson, C. M. (2003). Protein folding and misfolding. *Nature, 426*(6968), 884-890.

Dobson, M. J. (1994). Malaria in England: a geographical and historical perspective. *Parassitologia, 36*(1-2), 35-60.

Erlejman, A. G., Lagadari, M., & Galigniana, M. D. (2013). Hsp90-binding immunophilins as a potential new platform for drug treatment. *Future medicinal chemistry, 5*(5), 591-607.

Fanghanel, J., & Fischer, G. (2004). Insights into the catalytic mechanism of peptidyl prolyl cis/trans isomerases. *Front Biosci, 9*, 3453-3478.

Farahbakhsh, Z. T., Huang, Q.-L., Ding, L.-L., Altenbach, C., Steinhoff, H.-J., Horwitz, J., & Hubbell, W. L. (1995). Interaction of. alpha.-crystallin with Spin-Labeled Peptides. *Biochemistry, 34*(2), 509-516.

Faust, E. C. (1951). The history of malaria in the United States. *American Scientist, 39*(1), 121-130.

Figtree, M., Lee, R., Bain, L., Kennedy, T., Mackertich, S., Urban, M., . . . Hudson, B. J. (2010). Plasmodium knowlesi in human, Indonesian Borneo. *Emerging infectious diseases, 16*(4), 672.

Fischer, G., Bang, H., & Mech, C. (1984). Determination of enzymatic catalysis for the cis-trans-isomerization of peptide binding in proline-containing peptides. *Biomedica biochimica acta, 43*(10), 1101-1111.

Fivelman, Q. L., Butcher, G. A., Adagu, I. S., Warhurst, D. C., & Pasvol, G. (2002). Malarone treatment failure and in vitro confirmation of resistance of Plasmodium falciparum isolate from Lagos, Nigeria. *Malaria Journal, 1*(1), 1.

Fruman, D. A., Bierer, B. E., Benes, J. E., Burakoff, S. J., Austen, K. F., & Katz, H. R. (1995). The complex of FK506-binding protein 12 and FK506 inhibits

calcineurin phosphatase activity and IgE activation-induced cytokine transcripts, but not exocytosis, in mouse mast cells. *The Journal of Immunology, 154*(4), 1846-1851.

Fulcrand, G., Dages, S., Zhi, X., Chapagain, P., Gerstman, B. S., Dunlap, D., & Leng, F. (2016). DNA supercoiling, a critical signal regulating the basal expression of the lac operon in Escherichia coli. *Scientific reports, 6*, 19243. doi: 10.1038/srep19243

Furutani, M., Ideno, A., Iida, T., & Maruyama, T. (2000). FK506 binding protein from a thermophilic archaeon, Methanococcus thermolithotrophicus, has chaperone-like activity in vitro. *Biochemistry, 39*(2), 453-462.

Göthel, S., & Marahiel, M. (1999). Peptidyl-prolyl cis-trans isomerases, a superfamily of ubiquitous folding catalysts. *Cellular and Molecular Life Sciences CMLS, 55*(3), 423-436.

Guerin, P. J., Olliaro, P., Nosten, F., Druilhe, P., Laxminarayan, R., Binka, F., . . . White, N. J. (2002). Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. *The Lancet infectious diseases, 2*(9), 564-573.

Gustafsson, C., Govindarajan, S., & Minshull, J. (2004). Codon bias and heterologous protein expression. *Trends in Biotechnology, 22*(7), 346-353.

Hamilton, W. L., Claessens, A., Otto, T. D., Kekre, M., Fairhurst, R. M., Rayner, J. C., & Kwiatkowski, D. (2017). Extreme mutation bias and high AT content in Plasmodium falciparum. *Nucleic acids research, 45*(4), 1889-1901. doi: 10.1093/nar/gkw1259

Hanahan, D., Jessee, J., & Bloom, F. R. (1991). [4] Plasmid transformation of Escherichia coli and other bacteria *Methods in enzymology* (Vol. 204, pp. 63-113): Elsevier.

Hanboonkunupakarn, B., & White, N. J. (2016). The threat of antimalarial drug resistance. *Tropical Diseases, Travel Medicine and Vaccines, 2*(1), 10. doi: 10.1186/s40794-016-0027-8

Harikishore, A., Niang, M., Rajan, S., Preiser, P. R., & Yoon, H. S. (2013). Small molecule Plasmodium FKBP35 inhibitor as a potential antimalaria agent. *Scientific reports, 3*.

Hastings, I. M., & Hodel, E. M. (2014). Pharmacological considerations in the design of anti-malarial drug combination therapies–is matching half-lives enough? *Malaria Journal, 13*(1), 62.

Hayer-Hartl, M., Bracher, A., & Hartl, F. U. (2016). The GroEL–GroES chaperonin machine: a nano-cage for protein folding. *Trends in biochemical sciences, 41*(1), 62-76.

Hayer-Hartl, M. K., Ewbank, J., Creighton, T., & Hartl, F. (1994). Conformational specificity of the chaperonin GroEL for the compact folding intermediates of alpha-lactalbumin. *The EMBO journal, 13*(13), 3192-3202.

Hempelmann, E., & Krafts, K. (2013). Bad air, amulets and mosquitoes: 2,000 years of changing perspectives on malaria. *Malaria Journal, 12*(1), 232. doi: 10.1186/1475-2875-12-232

Heppner, D. G. (2013). The malaria vaccine–status quo 2013. *Travel medicine and infectious disease, 11*(1), 2-7.

Hill, A. V. (2011). Vaccines against malaria. *Philosophical Transactions of the Royal Society B: Biological Sciences, 366*(1579), 2806-2814.

Hoffmann, J. H., Linke, K., Graf, P. C. F., Lilie, H., & Jakob, U. (2004). Identification of a redox-regulated chaperone network. *The EMBO journal, 23*(1), 160-168. doi: 10.1038/sj.emboj.7600016

Hu, K., Galius, V., & Pervushin, K. (2006). Structural plasticity of peptidyl– prolyl isomerase sFkpA is a key to its chaperone function as revealed by solution NMR. *Biochemistry, 45*(39), 11983-11991.

Ikura, T., & Ito, N. (2007). Requirements for peptidyl-prolyl isomerization activity: A comprehensive mutational analysis of the substrate-binding cavity of FK506-binding protein 12. *Protein Science, 16*(12), 2618-2625.

Islam, R., Tisi, D., Levy, M., & Lye, G. (2007). Framework for the rapid optimization of soluble protein expression in Escherichia coli combining microscale experiments and statistical experimental design. *Biotechnology progress, 23*(4), 785-793.

Jabs, A., Weiss, M. S., & Hilgenfeld, R. (1999). Non-proline cis peptide bonds in proteins. *Journal of molecular biology, 286*(1), 291-304.

Jaenicke, R., & Seckler, R. (1999). Spontaneous versus assisted protein folding. *Molecular Chaperones and Folding Catalysts Regulation, Cellular Function and Mechanism*, 407-436.

Jiang, X., Oohira, K., Iwasaki, Y., Nakano, H., Ichihara, S., & Yamane, T. (2002). Reduction of protein degradation by use of protease-deficient mutants in cell-free protein synthesis system of Escherichia coli. *Journal of bioscience and bioengineering, 93*(2), 151-156.

Jin, H., Björnsson, A., & Isaksson, L. A. (2002). Cis control of gene expression in E.coli by ribosome queuing at an inefficient translational stop signal. *The EMBO journal, 21*(16), 4357-4367. doi: 10.1093/emboj/cdf424

Jongwutiwes, S., Buppan, P., Kosuvin, R., Seethamchai, S., Pattanawong, U., Sirichaisinthop, J., & Putaporntip, C. (2011). Plasmodium knowlesi malaria in humans and macaques, Thailand. *Emerg Infect Dis, 17*(10), 1799.

Joveen-Neoh, W. F., Chong, K. L., Wong, C. M. V. L., & Lau, T. Y. (2011). Incidence of malaria in the Interior Division of Sabah, Malaysian Borneo, based on nested PCR. *Journal of parasitology research, 2011.*

Kiefhaber, T., Quaas, R., Hahn, U., & Schmid, F. X. (1990). Folding of ribonuclease T1. 1. Existence of multiple unfolded states created by proline isomerization. *Biochemistry, 29*(12), 3053-3061.

Kino, T., Hatanaka, H., Miyata, S., Inamura, N., NISHIYAMA, M., YAJIMA, T., . . . AOKI, H. (1987). FK-506, a novel immunosuppressant isolated from a Streptomyces. *The Journal of antibiotics, 40*(9), 1256-1265.

Kotaka, M., Ye, H., Alag, R., Hu, G., Bozdech, Z., Preiser, P. R., . . . Lescar, J. (2008). Crystal Structure of the FK506 Binding Domain of Plasmodium falciparum FKBP35 in Complex with FK506†‡. *Biochemistry, 47*(22), 5951-5961.

Kumar, R., Adams, B., Musiyenko, A., Shulyayeva, O., & Barik, S. (2005). The FK506-binding protein of the malaria parasite, Plasmodium falciparum, is a FK506-sensitive chaperone with FK506-independent calcineurin-inhibitory activity. *Molecular and biochemical parasitology, 141*(2), 163-173.

Kurland, C., & Gallant, J. (1996). Errors of heterologous protein expression. *Current opinion in biotechnology, 7*(5), 489-493.

Kuwajima, K. (1989). The molten globule state as a clue for understanding the folding and cooperativity of globular-protein structure. *Proteins: Structure, Function, and Bioinformatics, 6*(2), 87-103.

Laemmli, U., Beguin, F., & Gujer-Kellenberger, G. (1970). A factor preventing the major head protein of bacteriophage T4 from random aggregation. *Journal of molecular biology, 47*(1), 69-85.

Lalremruata, A., Ball, M., Bianucci, R., Welte, B., Nerlich, A. G., Kun, J. F., & Pusch, C. M. (2013). Molecular identification of falciparum malaria and human tuberculosis co-infections in mummies from the Fayum depression (Lower Egypt). *PLoS ONE, 8*(4), e60307.

Larentis, A. L., Nicolau, J. F. M. Q., Esteves, G. d. S., Vareschini, D. T., de Almeida, F. V. R., dos Reis, M. G., . . . Medeiros, M. A. (2014). Evaluation of pre-induction temperature, cell growth at induction and IPTG concentration on

the expression of a leptospiral protein in E. coli using shaking flasks and microbioreactor. *BMC Research Notes, 7*, 671. doi: 10.1186/1756-0500-7-671

Lau, T. Y., Joveen-Neoh, W. F., & Chong, K. L. (2011a). High incidence of Plasmodium knowlesi infection in the interior division of Sabah, Malaysian Borneo. *International Journal of Bioscience, Biochemistry and Bioinformatics, 1*(2), 163.

Lau, T. Y., Joveen-Neoh, W. F., & Chong, K. L. (2011b). *Molecular Detection of Plasmodium knowlesi in the Interior Division of Sabah, Malaysian Borneo.* Paper presented at the 2011 International Conference on Food Engineering and Biotechnology IPCBEE, Singapore.

Lee, Y. C. A., Tang, C. S., Ang, L. W., Han, H. K., James, L., & Goh, K. T. (2009). Epidemiological characteristics of imported and locally-acquired malaria in Singapore. *Annals Academy of Medicine Singapore, 38*(10), 840.

Li, T. K., Baksh, S., Cristillo, A. D., & Bierer, B. E. (2002). Calcium-and FK506-independent interaction between the immunophilin FKBP51 and calcineurin. *Journal of cellular biochemistry, 84*(3), 460-471.

Li, Z.-Y., Liu, C.-P., Zhu, L.-Q., Jing, G.-Z., & Zhou, J.-M. (2001). The chaperone activity of trigger factor is distinct from its isomerase activity during co-expression with adenylate kinase in Escherichia coli. *FEBS Letters, 506*(2), 108-112. doi: 10.1016/S0014-5793(01)02896-4

Libertini, G., & Donato, A. D. (1992). Computer-aided gene design. *Protein Engineering, Design and Selection, 5*(8), 821-825.

Lindner, R. A., Kapur, A., Mariani, M., Titmuss, S. J., & Carver, J. A. (1998). Structural alterations of α-crystallin during its chaperone action. *European Journal of Biochemistry, 258*(1), 170-183.

Liu, F., Wang, Y.-Q., Meng, L., Gu, M., & Tan, R.-Y. (2013). FK506-binding protein 12 ligands: a patent review. *Expert opinion on therapeutic patents, 23*(11), 1435-1449.

Liu, W., Li, Y., Learn, G. H., Rudicell, R. S., Robertson, J. D., Keele, B. F., . . . Locatelli, S. (2010). Origin of the human malaria parasite Plasmodium falciparum in gorillas. *Nature, 467*(7314), 420.

Liu, X., Liu, L., Wang, Y., Wang, X., Ma, Y., & Li, Y. (2014). The study on the factors affecting transformation efficiency of E. coli competent cells. *Cell, 5*, x106.

Looareesuwan, S., White, N., Bunnag, D., Chittamas, S., & Harinasuta, T. (1987). High rate of Plasmodium vivax relapse following treatment of falciparum malaria in Thailand. *The Lancet, 330*(8567), 1052-1055.

Lorence, A. (2012). *Recombinant Gene Expression*: Springer.

Luchavez, J., Espino, F., Curameng, P., Espina, R., Bell, D., Chiodini, P., . . . Singh, B. (2008). Human infections with Plasmodium knowlesi, the Philippines. *Emerging infectious diseases, 14*(5), 811.

Maleszka, R., Lupas, A., Hanes, S., & Miklos, G. G. (1997). The dodo gene family encodes a novel protein involved in signal transduction and protein folding. *Gene, 203*(2), 89-93.

Malhotra, I., Mungai, P., Muchiri, E., Kwiek, J. J., Meshnick, S. R., & King, C. L. (2006). Umbilical cord–blood infections with Plasmodium falciparum malaria are acquired antenatally in Kenya. *Journal of Infectious Diseases, 194*(2), 176-183.

Manderson, D., Dempster, R., & Chisti, Y. (2006). A recombinant vaccine against hydatidosis: production of the antigen in Escherichia coli. *Journal of Industrial Microbiology and Biotechnology, 33*(3), 173-182.

März, A. M., Fabian, A.-K., Kozany, C., Bracher, A., & Hausch, F. (2013). Large FK506-binding proteins shape the pharmacology of rapamycin. *Molecular and cellular biology*, MCB. 00678-00612.

Mayxay, M., Pukrittayakamee, S., Newton, P. N., & White, N. J. (2004). Mixed-species malaria infections in humans. *Trends in Parasitology, 20*(5), 233-240.

Meshnick, S. R., & Dobson, M. J. (2001). The history of antimalarial drugs *Antimalarial chemotherapy* (pp. 15-25): Springer.

Michnick, S. W., Rosen, M. K., Wandless, T. J., Karplus, M., & Schreiber, S. L. (1991). Solution structure of FKBP, a rotamase enzyme and receptor for FK506 and rapamycin. *Science, 252*(5007), 836-839.

Mikol, V., Kallen, J., Pflügl, G., & Walkinshaw, M. D. (1993). X-ray Structure of a Monomeric Cyclophilin A-Cyclosporin A Crystal Complex at 2· 1 Å Resolution. *Journal of molecular biology, 234*(4), 1119-1130.

Monaghan, P., & Bell, A. (2005). A Plasmodium falciparum FK506-binding protein (FKBP) with peptidyl–prolyl cis–trans isomerase and chaperone activities. *Molecular and biochemical parasitology, 139*(2), 185-195.

Morgan, R. M., Hernández-Ramírez, L. C., Trivellin, G., Zhou, L., Roe, S. M., Korbonits, M., & Prodromou, C. (2012). Structure of the TPR domain of AIP: lack of client protein interaction with the C-terminal α-7 helix of the TPR domain of AIP is sufficient for pituitary adenoma predisposition. *PLoS ONE, 7*(12), e53339.

Na-Bangchang, K., & Karbwang, J. (2009). Current status of malaria chemotherapy and the role of pharmacology in antimalarial drug research and development. *Fundamental & clinical pharmacology, 23*(4), 387-409.

Nikbakht, H., Xia, X., & Hickey, D. A. (2014). The evolution of genomic GC content undergoes a rapid reversal within the genus Plasmodium. *Genome, 57*(9), 507-511.

Ninan, T., Nalees, K., Newin, M., Sultan, Q., Than, M. M., & Shinde, S. (2012). Plasmodium knowlesi malaria infection in human. *Brunei Int Med J, 8*, 358-361.

Nosten, F., & White, N. J. (2007). Artemisinin-based combination treatment of falciparum malaria. *The American journal of tropical medicine and hygiene, 77*(6_Suppl), 181-192.

Okazaki, A., Ikura, T., Nikaido, K., & Kuwajima, K. (1994). The chaperonin GroEL does not recognize apo-α-lactalbumin in the molten globule state. *Nature Structural and Molecular Biology, 1*(7), 439.

Park, S. T., Aldape, R. A., Futer, O., DeCenzo, M. T., & Livingston, D. J. (1992). PPIase catalysis by human FK506-binding protein proceeds through a conformational twist mechanism. *Journal of Biological Chemistry, 267*(5), 3316-3324.

Parmakelis, A., Russello, M. A., Caccone, A., Marcondes, C. B., Costa, J., Forattini, O. P., . . . Powell, J. R. (2008). Historical analysis of a near disaster: Anopheles gambiae in Brazil. *The American journal of tropical medicine and hygiene, 78*(1), 176-178.

Pattnaik, P. (2005). Surface plasmon resonance. *Applied biochemistry and biotechnology, 126*(2), 79-92.

Peterson, A. W., Halter, M., Tona, A., & Plant, A. L. (2014). High resolution surface plasmon resonance imaging for single cells. *BMC cell biology, 15*(1), 35.

Pirkl, F., & Buchner, J. (2001). Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. *Journal of molecular biology, 308*(4), 795-806.

Poinar, G. (2005). Plasmodium dominicana n. sp.(Plasmodiidae: Haemospororida) from Tertiary Dominican amber. *Systematic parasitology, 61*(1), 47-52.

Pukrittayakamee, S., Imwong, M., Looareesuwan, S., & White, N. J. (2004). Therapeutic responses to antimalarial and antibacterial drugs in vivax malaria. *Acta tropica, 89*(3), 351-356.

Rahfeld, J.-U., Rücknagel, K. P., Schelbert, B., Ludwig, B., Hacker, J., Mann, K., & Fischer, G. (1994). Confirmation of the existence of a third family among

peptidyl-prolyl cis/trans isomerases Amino acid sequence and recombinant production of parvulin. *FEBS Letters, 352*(2), 180-184.

Ramm, K., & Pluckthun, A. (2000). The periplasmic E. coli peptidyl-prolyl-isomerase FkpA (II): isomerase-independent chaperone activity in vitro. *Journal of Biological Chemistry.*

Ramm, K., & PluÈckthun, A. (2001). High enzymatic activity and chaperone function are mechanistically related features of the dimeric E. coli peptidyl-prolyl-isomerase FkpA1. *Journal of molecular biology, 310*(2), 485-498.

Ranganathan, R., Lu, K. P., Hunter, T., & Noel, J. P. (1997). Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell, 89*(6), 875-886.

Richardson, R. T., Alekseev, O. M., Grossman, G., Widgren, E. E., Thresher, R., Wagner, E. J., . . . Michael, G. (2006). Nuclear autoantigenic sperm protein (NASP), a linker histone chaperone that is required for cell proliferation. *Journal of Biological Chemistry, 281*(30), 21526-21534.

Roberts, D. R., Laughlin, L. L., Hsheih, P., & Legters, L. J. (1997). DDT, global strategies, and a malaria control crisis in South America. *Emerging infectious diseases, 3*(3), 295.

Rosano, G. L., & Ceccarelli, E. A. (2014). Recombinant protein expression in Escherichia coli: advances and challenges. *Recombinant protein expression in microbial systems*, 7.

Rostam, M. A., Piva, T. J., Rezaei, H. B., Kamato, D., Little, P. J., Zheng, W., & Osman, N. (2015). Peptidyl-prolyl isomerases: Functionality and potential therapeutic targets in cardiovascular disease. *Clinical and Experimental Pharmacology and Physiology, 42*(2), 117-124.

Ryo, A., Liou, Y.-C., Lu, K. P., & Wulf, G. (2003). Prolyl isomerase Pin1: a catalyst for oncogenesis and a potential therapeutic target in cancer. *Journal of cell science, 116*(5), 773-783.

Sambrook, J., & Russell, D. W. (2006). The Hanahan method for preparation and transformation of competent E. coli: high-efficiency transformation. *Cold Spring Harbor Protocols, 2006*(1), pdb. prot3942.

Saul, F., Arie, J.-P., Vulliez-le Normand, B., Kahn, R., Betton, J.-M., & Bentley, G. (2004). Structural and functional studies of FkpA from Escherichia coli, a cis/trans peptidyl-prolyl isomerase with chaperone activity. *Journal of molecular biology, 335*(2), 595-608.

Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., . . . Moarefi, I. (2000). Structure of TPR Domain–Peptide Complexes. *Cell, 101*(2), 199-210. doi: 10.1016/S0092-8674(00)80830-2

Schiene-Fischer, C., & Yu, C. (2001). Receptor accessory folding helper enzymes: the functional role of peptidyl prolyl cis/trans isomerases. *FEBS Letters, 495*(1-2), 1-6.

Scholz, C., Eckert, B., Hagn, F., Schaarschmidt, P., Balbach, J., & Schmid, F. X. (2006). SlyD proteins from different species exhibit high prolyl isomerase and chaperone activities. *Biochemistry, 45*(1), 20-33.

Scholz, C., Stoller, G., Zarnt, T., Fischer, G., & Schmid, F. X. (1997). Cooperation of enzymatic and chaperone functions of trigger factor in the catalysis of protein folding. *The EMBO journal, 16*(1), 54-58.

Schreiber, S. L., & Crabtree, G. R. (1992). The mechanism of action of cyclosporin A and FK506. *Immunology today, 13*(4), 136-142.

Sharp, P. M., & Li, W.-H. (1987). The codon adaptation index-a measure of directional synonymous codon usage bias, and its potential applications. *Nucleic acids research, 15*(3), 1281-1295.

Shaw, P. E. (2002). Peptidyl-prolyl isomerases: a new twist to transcription. *EMBO Reports, 3*(6), 521-526. doi: 10.1093/embo-reports/kvf118

Shirane, M., & Nakayama, K. I. (2003). Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. *Nature cell biology, 5*(1), 28-37.

Shuman, H. A., & Silhavy, T. J. (2003). Microbial genetics: The art and design of genetic screens: Escherichia coli. *Nature Reviews Genetics, 4*(6), 419.

Singh, B., Sung, L. K., Matusop, A., Radhakrishnan, A., Shamsul, S. S., Cox-Singh, J., . . . Conway, D. J. (2004). A large focus of naturally acquired Plasmodium knowlesi infections in human beings. *The Lancet, 363*(9414), 1017-1024.

Stefani, M. (2004). Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1739*(1), 5-25.

Suzuki, Y., Haruki, M., Takano, K., Morikawa, M., & Kanaya, S. (2004). Possible involvement of an FKBP family member protein from a psychrotrophic bacterium Shewanella sp. SIB1 in cold-adaptation. *European Journal of Biochemistry, 271*(7), 1372-1381. doi: 10.1111/j.1432-1033.2004.04049.x

Suzuki, Y., Win, O. Y., Koga, Y., Takano, K., & Kanaya, S. (2005). Binding analysis of a psychrotrophic FKBP22 to a folding intermediate of protein using surface plasmon resonance. *FEBS Letters, 579*(25), 5781-5784. doi: http://dx.doi.org/10.1016/j.febslet.2005.09.067

Tomala, K., & Korona, R. (2008). Molecular chaperones and selection against mutations. *Biology Direct, 3*(1), 5. doi: 10.1186/1745-6150-3-5

Trager, W., & Jensen, J. B. (1976). Human malaria parasites in continuous culture. *Science, 193*(4254), 673-675.

Uchida, T., Fujimori, F., Tradler, T., Fischer, G., & Rahfeld, J.-U. (1999). Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl cis/trans isomerase. *FEBS Letters, 446*(2-3), 278-282.

Van den Eede, P., Van, H. N., Van Overmeir, C., Vythilingam, I., Duc, T. N., Hung, L. X., . . . Erhart, A. (2009). Human Plasmodium knowlesi infections in young children in central Vietnam. *Malaria Journal, 8*(1), 249.

Van Duyne, G. D., Standaert, R. F., Karplus, P. A., Schreiber, S. L., & Clardy, J. (1991). Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science, 252*(5007), 839-842.

Veith, I. (2015). *The yellow emperor's classic of internal medicine*: Univ of California Press.

Vekshin, N. (2008). How α-crystallin prevents the aggregation of insulin. *Biochemistry (Moscow), 73*(4), 458-462.

von Bayern, A. M. P., Heathcote, R. J. P., Rutz, C., & Kacelnik, A. (2009). The role of experience in problem solving and innovative tool use in crows. *Current Biology, 19*(22), 1965-1968. doi: 10.1016/j.cub.2009.10.037

von Seidlein, L., & Bejon, P. (2013). Malaria vaccines: past, present and future. *Archives of disease in childhood*, archdischild-2013-304173.

Wang, Q., Mei, C., Zhen, H., & Zhu, J. (2012). Codon preference optimization increases prokaryotic cystatin C expression. *BioMed Research International, 2012*.

Weininger, U., Haupt, C., Schweimer, K., Graubner, W., Kovermann, M., Brüser, T., . . . Balbach, J. (2009). NMR Solution Structure of SlyD from Escherichia coli: Spatial Separation of Prolyl Isomerase and Chaperone Function. *Journal of molecular biology, 387*(2), 295-305. doi: https://doi.org/10.1016/j.jmb.2009.01.034

White, N. J. (2004). Antimalarial drug resistance. *The Journal of clinical investigation, 113*(8), 1084-1092.

William, T., & Menon, J. (2014). A review of malaria research in Malaysia. *Med J Malaysia, 69*, 82-87.

William, T., Menon, J., Rajahram, G., Chan, L., Ma, G., Donaldson, S., . . . Yeo, T. W. (2011). Severe Plasmodium knowlesi Malaria in a Tertiary Care Hospital, Sabah, Malaysia. *Emerging infectious diseases, 17*(7), 1248-1255. doi: 10.3201/eid.1707.101017

World Health Organisation (2016). World malaria report 2016 *Geneva: WHO. Embargoed until* (Vol. 13).

World Health Organisation (2017). World malaria report 2017. Retreved from https://www.who.int/malaria/publications/world-malaria-report-2017/en/

Wu, G., Dress, L., & Freeland, S. J. (2007). Optimal encoding rules for synthetic genes: the need for a community effort. *Molecular systems biology, 3*(1), 134.

Wurm, D. J., Veiter, L., Ulonska, S., Eggenreich, B., Herwig, C., & Spadiut, O. (2016). The E. coli pET expression system revisited—mechanistic correlation between glucose and lactose uptake. *Applied microbiology and biotechnology, 100*(20), 8721-8729.

Yoon, H. R., Kang, C. B., Chia, J., Tang, K., & Yoon, H. S. (2007). Expression, purification, and molecular characterization of Plasmodium falciparum FK506-binding protein 35 (PfFKBP35). *Protein expression and purification, 53*(1), 179-185.

Yu, H., Dee, D. R., Liu, X., Brigley, A. M., Sosova, I., & Woodside, M. T. (2015). Protein misfolding occurs by slow diffusion across multiple barriers in a rough energy landscape. *Proceedings of the National Academy of Sciences, 112*(27), 8308-8313.