CHAPTER I
INTRODUCTION

Dengue viruses (DENVs) are positive sense single stranded RNA viruses of the family *Flaviviridae*, genus *Flavivirus* (1). Dengue virus consists of an approximately 11 kb genome (2). The open reading frame of DENV encodes for a single polyprotein which is cleaved by cellular and viral proteases into 3 structural proteins namely Capsid (C), pre-membrane/membrane (prM/M) and Envelope (E) proteins and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) involved in RNA replication, assembly and modulation of the immune response of host cell (3). Dengue virus has 4 closely related serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) depending on their antigenic determinants (1, (4).

DENVs are arthropod borne viruses. They are transmitted among humans by mosquitoes in the genus *Aedes* such as *Aedes agypti* (*Ae. agypti*) and *Ae. albopictus* (1). Dengue symptoms can be classified into undifferentiated fever, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Life threatening dengue is caused by plasma leakage or circulatory failure (5). Recently, DENVs are endemic in more than 100 countries especially in Southeast Asia and the Western Pacific with approximately 100 million reported case each year (6). Dengue fever and DHF are the main cause of hospitalization of children in Southeast Asia (7).

Dengue viruses infect target cells by “receptor mediated endocytosis” in a clathrin-dependent manner. Viral particles enter to host cells in endosomal vesicles. Proton pumps change the pH of the endosome and uncoating of the virus occurs to release the viral RNA, that is translated to a single polyprotein at the rough endoplasmic reticulum (rER). Cellular and viral proteases cleave the single polyprotein into 3 structural proteins (C, prM and E) which form the building block of virus and 7 non-structural proteins. The newly synthesized RNA of viral progeny assembles with the capsid protein forming a capsid-RNA complex (8, 9). Then, these immature particles travel to the trans-Golgi network using the signal sequence for facilitating

translocation, transportation and secretion from rER to Golgi apparatus (9). The 19-aa region at 3' end of capsid sequence and the 15-aa region at 3' end of prM sequence represent the native signal sequence for prM and E, respectively (10). Inside the trans-Golgi network, the immature viral particles become mature after furin-dependent cleavage. The pr cleavage product remains associated with the E protein to prevent premature fusion of the particle during secretion, and pr is liberated only after the particle has been released from the cell to infect other cells (8, 9, (11).

In the viral budding step of yellow fever virus (YFV), the viral non-structural protein 3 (NS3) facilitates viral release by recruitment of a host cellular protein called Alix. Alix can bind with the YPTI motif (a conserved peptide composing of Tyrosine, Proline, Threonine, Isoleucine) on NS3 and recruit other endosomal sorting complex proteins required for transport machinery (ESCRT) associated proteins for viral budding. The YPTI motif is only present in the domain 2 of NS3 helicase of YFV whereas the closely associated motif YIKT and YPKT were found in DENV-2 and DENV-4, respectively. However, the capacity of these DENV motifs in interacting with Alix facilitating viral budding is not known (12, 13).

DENVs are spreading to new areas every year and becoming one of the major emerging diseases. Therefore, an effective dengue vaccine is urgently needed. Nowadays, no commercial vaccine against dengue infection is available. Recently, virus like particle (VLP) which are immunogenic but non-infectious viral particles is one of the vaccine candidates as a substitution for live attenuated vaccine because it is safer (14). Virus like particles (VLPs) are shell-like pseudoviruses, composed of the organization and conformation as native viruses. There is a lipid envelope with 2 type of trans-membrane proteins, (E) and prM/M. These non-infectious particles can trigger strong immune responses because they have the same antigenic determinants as the native virus. Because of the lack of replicative genetic material, VLPs vaccines are more safe than other vaccines and they can be used to explore the mechanism of virus infection (14). Human antibodies raised against the DENV virion are mostly targeted at the E and prM (15). Several dengue VLP vaccines are being developed. Now, we successfully constructed plasmids for the expression of DENV-2 prM/M and E proteins (16) but the limitation of the VLPs production is low yield in cell-based systems. For this reason, signal sequence facilitating protein transportation from rER

to Golgi apparatus is added at the 5' terminus of targeting gene to solve the protein retention in ER leading to improvement of protein expression. In eukaryotes, signal sequences are recognized by signal recognition particle (SRP). They facilitate ribosomal complex and nascent polypeptides to bind with the SRP receptor on the ER and are cleaved off by signal peptidase prior to traveling to Golgi complex (17). One effective signal sequence is the vesicular stomatitis virus glycoprotein (VSV G). This protein has a short hydrophobic N-terminus which shows evidence in ER export facilitation (18). The VSV-G signal sequence is often used in protein research to solve ER retention problems in some proteins such as DENVs E protein (19). This signal can escape from the bulk-flow pathway from ER to Golgi complex (20) and show intracellular accumulation of prM-E proteins in ER.

An alternative strategy to improve VLPs expression is codon optimization. A codon is a series of three nucleotides that encodes for one amino acid. Most amino acids can be coded by more than one triplet of nucleotides. There are 64 different codons, 61 codons encoding for 20 amino acids plus 3 stop codons for terminating the translation process. Each individual organism has a preferred set of codon called codon bias (21). Therefore, codon optimization is a strategy to adapt most of codon into compatible codon usage of their host. This strategy can increase level of protein expression in different cell culture system.

CHAPTER II
OBJECTIVES

Dengue infections cause a significant public health burden in many tropical and sub-tropical countries, and there is still no effective vaccine. Virus-like particles are one possible vaccination strategy, but optimization of yields is still required. To address this issue this project seeks to:

1. Construct mammalian expression vectors containing dengue virus E protein with a variety of potential secretory regulators including native and heterologous signal sequences with or without codon optimization
2. Express the constructs in mammalian cells and optimize expression of dengue E protein in the cell supernatant
3. Determine if exogenous lipid can increase the cellular production of dengue E protein in the transfected cells.