NANYANG TECHNOLOGICAL UNIVERSITY

School of Biological Sciences

Reg. No. 200604393R

Research Theme: **Membrane protein and Cryo-EM**

Research Project Title: **Understanding the molecular mechanism of odorant receptor co-receptor (orco) in *Anopheles gambiae***

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Co-supervisor/ Collaborator(s) (if any): **N/A**

Project Description

a) Background:

Insects play fundamental roles in terrestrial ecosystems. However, several insects such as mosquitoes, flies and ticks act as vectors which harbour and spread infectious diseases such as malaria, dengue, leishmaniasis, chikungunya and yellow fever. Tropical areas like Africa and South-East Asia including Singapore are hotspots for mosquito borne infections. While dengue is the most prevalent vector borne disease in South-East Asia, the fear of malaria might dominate in the near future. Malaria is mainly caused by Plasmodium, a protozoan transmitted by female Anopheles mosquitoes. According to the National Centre for Infectious Diseases, Singapore, malaria caused by *Plasmodium knowlesi* is emerging at the rapid pace in South-East Asia, particularly in eastern Malaysia, causing serious life-threatening complications and death. The World Health Organization (WHO) reports more than 17% of all infectious diseases are vector-borne diseases which cause approximately 700,000 deaths annually. In 2018 about 228 million cases of malaria were reported with more than 405,000 deaths globally. Development of resistance to existing anti-malarial drugs heightened the need of controlling the disease in an innovative way. Most of the repellents and insecticides target mainly nerve and muscle action (85%) of insects. Due to off target effects and non-specificity, the current repellents or insecticides are losing their effectiveness which is becoming a global concern. Therefore, this global burden of malaria, and other mosquito borne diseases reinforces the need for innovative strategies such as targeting the host vector to combat and eliminate these diseases.

The *A. gambiae* mosquito is recognized as a major malaria vector. Like other insects, A. gambiae uses their sense of smell to find food, avoid predators and noxious agents, to find appropriate mating partners, and to find hosts. Hence, olfaction is critical for their survival. The ORs are responsible for the detection and discrimination of smells. Few if any, insecticides directly target the olfactory system thereby precluding a potentially effective strategy in controlling vector-borne insect populations. Therefore, efforts to understand the molecular details underlying insect olfaction are of fundamental interest in guiding novel strategies for safer, specific and effective insecticides and repellents. A clear understanding of *A. gambiae*

olfaction is not only required to develop targeted insecticides and repellents but also the knowledge could be translated to other mosquito species to control various mosquito borne diseases such as dengue, leishmaniasis, chikungunya and yellow fever.

b) Proposed work:

Insect odorant/olfactory receptors (ORs) are heterotetrameric ligand gated non-specific cation channels. It comprises of multiple OR subunits and at least one essential odorant/olfactory receptor co-receptor (Orco) subunit. Insect ORs can exist in three distinct functional states: A resting state, an open state and an inactivated/ desensitized state. Upon agonist binding, ORs switch from a closed to an open conformation. Interestingly, the inactivation/desensitization mechanism is quite distinct from canonical ion channels. Despite the revolutionary works in the past on the functionality of receptors, a clear understanding of the gating mechanism is still elusive. Interestingly, Orco forms functional homotetramer in heterologous expression system which would serve as a surrogate to understand the gating mechanism of Insect ORs. The **aim** of the project is to understand the molecular mechanism of Orco receptor of *Anopheles gambiae.* The focus of this study is to understand the mechanism of activation and to determine how agonist modulates the transitions between the closed, open and inactivated states. Membrane-lipid constituents are known to influence the function of integral membrane proteins. To elucidate the mechanistic basis for olfactory receptor signalling, the structural information of multiple functional states in presence of various lipids is essential. Therefore, in this project we propose to determine high resolution structures of *A. gambiae* Orco receptor in multiple conformational states and conduct protein dynamic measurements in both detergent and lipidic environments using complementary tools such as Cryo electron microscopy (Cryo-EM), Electron paramagnetic Spectroscopy (EPR) and other biophysical techniques. The questions we seek to answer in this proposal mandate a multidisciplinary approach applicable to robust systems that are amenable to both large scale protein production and biophysical/functional characterization. To this end, **our aims will be three-fold**: **first,** we will determine high-resolution Cryo-EM structures of full length AgOrco (*A. gambiae* Orco) in multiple functional states initially in the detergent environment to establish the workflow to stably express and purify the protein. **Second**, we will determine the effect of membrane lipid composition on the gating conformational changes by solving the structure in various synthetic lipid components, such as PC, PG and Cholesterol. **Third**, we will analyse the conformational dynamics underlying channel gating between the different states by EPR spectroscopy. The effect of membrane lipids on the function of olfactory receptors will be investigated by patch-clamp electrophysiology.

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Please apply at the following:

Application portal:

https://venus.wis.ntu.edu.sg/GOAL/OnlineApplicationModule/frmOnlineApplication.ASPX