LKCMedicine PhD Research Project Submission Form

Research Theme (Please indicate as appropriate)

☐ Dermatology & Skin Biology ☐ Family Medicine & Primary Care
☐ Health Systems & Population Health ☒ Infection & Immunity
☐ Metabolic Disorders ☐ Neuroscience & Mental Health
☐ Medical Education ☐ Others (Please specify):

Research Project Title:

The RNA interactome of Chikungunya virus capsid

Project Description:

Chikungunya virus (CHIKV) is transmitted to humans via mosquitoes and is the causative agent of the acute febrile illness called chikungunya fever (CHIKF) that often leads to painful and long lasting arthritic sequelae. Similar to Dengue fever and Zika fever, the disease is prevalent and rapidly spreading in tropical and subtropical countries all around the world. CHIKV also shares the same mosquito vectors as Dengue virus and Zika virus, which has increased the difficulty of prevention, diagnosis and treatment. Better understand the virus life cycle will facilitate therapeutics development against CHIKV and related infectious viruses. The capsid protein is an essential viral structural protein, responsible for virus genome packaging. The protein (aa 1–261 ~ 29 kDa) has two separate domains: the highly variable N-terminal domain (NTD) is highly positively charged and is responsible for binding viral RNA during assembly. The conserved C-terminal domain is a chymotrypsin-like serine protease which is responsible for cleaving capsid from the translating polypeptide. CHIKV CP is a nuclear-cytoplasmic shuttling protein with an active NLS that binds to karyopherin-a for its nuclear translocation. The export receptor CRM1 is responsible for to transport this viral protein out of the nucleus via a nuclear export signal (NES). Both NLS and NES signal sequences have been mapped to the regions of aa 60-99 and 143-155 respectively. More intriguing is that the capsid protein is able to translocate to the host cell nucleolus via the putative nucleolar localization sequence (NoLS, aa 58 - 110). Mutations on these signalling sequences cause various defective phenotypes and have been implicated in vaccine development. Given its important roles in both virus genome packaging and host cell interaction, it is of great interest to study its dynamic interactions with both viral and
host RNAs at different stages of virus replication and different subcellular locations.

**Brief summary of main Methodologies and/or Techniques to be learned during the proposed PhD (experimental or analytical):**

We would like to use complementary approaches to build a dynamic map of capsid-RNA interactome. First, we will prepare the capsid protein, its truncations and mutants, viral genomic and subgenomic RNAs to characterize the capsid-viral RNA interactions in vitro. We use biochemical and biophysical methods combined with high-resolution mass spectrometry to characterize protein-RNA interface. Second, we will label and express the capsid protein in mosquito and human muscle cell lines, isolate the nucleocapsid from the nucleolus, and characterize the RNA sequence identity, map the interface. This will allow us to define the viral – host RNA interactome using cross-linking immunoprecipitation and sequencing (CLIP-seq). Finally, we will then use reverse genetics, virology, and cell biology methods to validate the structure model of capsid-RNA interactome and to further examine the molecular dynamics of the nucleocapsid complexes during viral genome packaging and capsid trafficking to the host nucleus.

**Keywords:**
Chikungunya virus, RNA, Antiviral therapy.
### Supervisor(s)

#### Primary Supervisor

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<thead>
<tr>
<th>Name of Supervisor:</th>
<th>Luo Dahai</th>
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<tbody>
<tr>
<td>Designation:</td>
<td>Associate Professor</td>
</tr>
<tr>
<td>Email:</td>
<td><a href="mailto:luodahai@ntu.edu.sg">luodahai@ntu.edu.sg</a></td>
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#### Co-Supervisor *(need not be determined at this time)*

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### Main Location of Research Work *(Please indicate as appropriate)*

- ☒ LKCMedicine Experimental Medicine Building @ Yunnan Campus
- ☑ LKCMedicine Clinical Sciences Building @ Novena Campus
- Others *(Please specify)*:  
  
### Other Information

1. Does the proposal need IRB’s approval?  
   - ☑ Yes  ☐ No
   - If “Yes”, is the IRB’s approval in place?  
     - ☐ Yes  ☑ No
2. Does the project involve contact with patients?  
   - ☐ Yes  ☑ No
3. Does the project involve contact with animals  
   - ☐ Yes  ☑ No
   - If “Yes”, is the NTU-Institutional Animal Care and Use Committee approval in place?  
     - ☐ Yes  ☐ No
4. Is there a potential for overseas academic exchange as part of this research project?  
   - ☑ Yes  ☐ No
   - If “Yes”, please specify:  
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