SUMMARY

Enveloped viruses have a bilipid envelope on their virion surface and enter host cells through sequential events such as receptor binding and membrane fusion. In many enveloped viruses, membrane fusion is mediated by envelope proteins, including receptor-binding and fusion proteins. The envelope protein E1 of the Chikungunya virus (CHIKV) is a class II fusion protein with a three-domain architecture, predominantly beta-sheet. A hydrophobic fusion loop (FL) at the tip of domain II inserts into the host cell membrane and initiates membrane fusion. In Class I fusion proteins, like HA in the influenza virus, fusion peptides(1-24AA) are present at the N-terminus. They are unordered and buried inside the trimeric interfaces of the fusion protein in the pre-fusion state. Upon insertion into the host cell, these peptides form ordered helices with a boomerang orientation and are inserted only into one leaflet of the membrane. Class I fusion peptides were thoroughly characterized in terms of their sequence conservation of N-terminal glycine residues, positionally conserved polar residues, and aromatic residues. Irrespective of structural conservation, these fusion peptides (in class I) and fusion loops (in class II and class III fusion proteins) perform the same function. We asked questions about what residues make the FL function the same as class I fusion protein, how deep these FL may insert into the membrane, and which orientation is essential for the membrane insertion. To answer these questions, we looked at sequences of class II fusion loops; interestingly, similar sequence conservation like class I fusion peptides is seen in the class II fusion loop of different class II fusion proteins.

We characterized CHIKV fusion loops and other class II fusion lops sequences using MSA and found in alphaviruses fusion loops highly conserved and contain glycine and aromatic residues like class I fusion peptides. In-silico hydropathy analysis predicted that CHIKV FL mostly favored staying at lipid water interfaces near the lipid head group region. Further, to confirm these results, we performed multiple MD simulations using CHIKV E1 monomer, trimer, and CHIKV-FL protein in the presence of the membrane. These results show only a fusion loop region inserting one leaflet of the membrane that mainly interacts with lipid headgroup regions. The bent orientation of the fusion loop is seen, and disulfide bonds between C94 and C60 residues, as well as G83 are required for this orientation. The “WGGAYCFC” region mainly stabilizes

interactions with the membrane. W89 present at the tip of the fusion loop may play an essential role in membrane anchoring when we substitute it with alanine, as the fusion loop is not inserted into the membrane like wildtype protein.

We mutated seven critical residues in the fusion loop region to further validate our *in silico hydropathy analysis and MD simulation* data. Eight mutations were introduced into the wild-type E1 ectodomain and truncated CHIKV-FL by PCR-mediated mutagenesis, and I successfully expressed all eight mutations in E. E.coli and purified them. I characterized all proteins using far-UV CD spectroscopy to analyze their secondary structure content and intrinsic tryptophan fluorescence to confirm their tertiary structures. From liposome-based fusion and membrane association assays, I first identified G83, C60, and C94 as critical residues for virus fusion and entry; mutating these residues to alanine abolishes membrane fusion. All three residues are located at the fusion loop bent region, and the C60 and C94 residues are making disulfide bonds to stabilize the fusion loop. The fluorescence-based membrane insertion data show how this structure inserts into lipid bilayers and that the E1-wildtype inserted up to the C5 acyl region of POPC lipid, whereas G83A, C60A, and C94A did not insert as deeply as E1WT. Both in silico and biochemical data show that the fusion loop residue G83 and the disulfate bonds between C60 and C94 play a crucial role in membrane anchoring and the proper bent orientation of FL within the membrane.

Further, to understand receptor-mediated membrane fusion in CHIKV, we expressed the CHIKV entry protein complex(E1E2i) in insect cells and characterized it biophysically and biochemically. All results showed that the insect-expressed E1E2i protein is structurally similar to previously reported CHIKV entry complex structures expressed in S2 cells and is functionally active.

Overall, this thesis contributed to understanding the CHIKV fusion loop-membrane interactions, which will help understand the mechanism of CHIKV membrane fusion and class II fusion protein. I also contributed several essential resources for studying the structure-function relationship of CHIKV entry proteins.