Structure-based Site Directed Mutagenesis of the NS5 Protein from Dengue Virus

Sherryl Soh[1*]

[1.] Nanyang Technological University (NTU) / Novartis Institute for Tropical Diseases (NITD)

Background/Objective
The flavivirus NS5 is a multi-functional protein harboring an N-terminal methyltransferase (MTase) and a C-terminal RNA-dependent RNA polymerase (RdRp), in which both play essential roles in viral replication in the host cell. Recently solved, the full-length NS5 crystal structure of Dengue virus (DENV) reveals a well-ordered linker region and an inter-domain interface formed by mostly polar residues.

Method
By employing a combination of established biochemical and reverse genetic tools, the functional significance of intra-molecular interactions to viral replication, growth and infectivity was explored.

Result
Several conserved interface residues selected for structure-guided mutagenesis were shown to be crucial for viral replication using infectious clone and transient luciferase-expressing subgenomic replicon system, indicative of the inter-domain cross-talk in NS5 during DENV life cycle. In vitro enzymatic assays demonstrated that the impaired viral replication could in part be attributed to a decrease in their MTase or RdRp activities. Interestingly, replicon experiment showed that some of the mutants displayed comparable viral replicative abilities as wild-type, supported collectively by biochemical assays and steady-state kinetic measurements in which increased RdRp activities correlated to a higher affinity of NS5 towards nucleotide and RNA substrates were detected. However, these mutants produced either lesser or no viable virus as compared to wild-type in the infectious clone, suggesting that they play important but non-enzymatic roles in viral replication and infectivity.

Conclusion
In all, our functional validation of the conserved intra-molecular interactions between MTase and RdRp domains provides a basis to understand how NS5 performs its versatile, multi-functional roles in genome replication and cap formation as well as offers opportunities for the development of effective antiviral drugs targeting NS5.