Understanding molecular interactions between dengue virus capsid protein and lipid droplets

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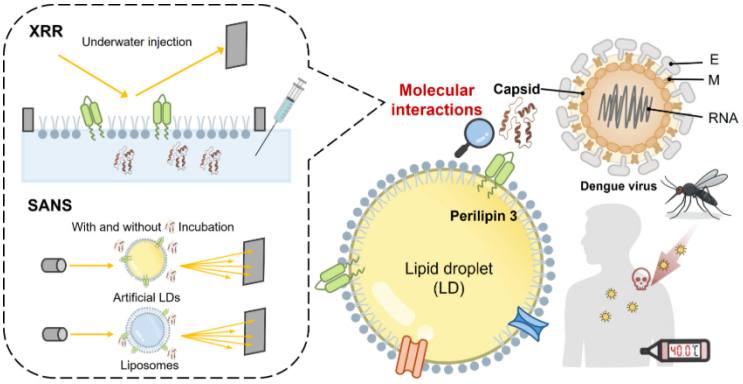
Dengue virus (DENV), a flavivirus transmitted by mosquitoes, infects over 400 million people each year, yet effective antiviral treatments are still lacking. Recent studies show that lipid droplets (LDs), which are intercellular organelles with a neutral lipid core and a phospholipid monolayer, play a key role in the DENV lifecycle. The viral capsid protein (DENV-C) accumulates on LD surfaces and is involved in nucleocapsid formation and viral assembly. However, the molecular details of how DENV-C interacts with LDs remain unclear, particularly the role of its intrinsically disordered N-terminal region in membrane binding. LD surfaces are not made of lipids alone but also contain functional proteins such as Perilipin 3 (PLIN3), which regulate LDs' metabolism. PLIN3 may therefore influence how viral proteins, including DENV-C, are recruited and organized at LDs, but this has not been fully understood.

In this study, biomimetic LD surfaces are created using phospholipid monolayers at the air–water interface in a Langmuir–Blodgett trough. Surface pressure–area (π–A) isotherms showed that the DOPC/DOPE/DAG monolayers collapsed at π ~ 39 mN/m. The PLIN3 underwater injection was conducted at π ~ 25 mN/m, which induced an initial increase in surface pressure followed by a decrease. A two-layer model was used to fit the X-ray reflectivity (XRR) data, yielding a phospholipid monolayer thickness of ~ 24 Å, with no significant changes in either thickness or SLD (scattering length density) observed after the injection of PLIN3, full-length DENV-C protein, or DENV-C pep12-23 (a segment of the protein). However, a change in the SLD of the hydrophilic layer was observed when pep12-23 was injected following PLIN3 incorporation. This result indicates that PLIN3 is essential for DENV-C binding to lipid droplets. Furthermore, complementary quartz crystal microbalance with dissipation (QCM-D) measurements revealed binding kinetics, mass adsorption, and viscoelastic properties as DENV-C and the peptide were added to preformed DOPC/DOPE membranes. Together, these results provide a first mechanistic insight into the role of Plin3 protein in mediating DENV-C binding to the lipid droplet surface, pivotal to future development of antiviral strategies.

Keywords:
Dengue virus, lipid droplets, X-ray reflectivity, monolayer



XRR probes Dengue virus capsid protein adsorption at lipid monolayer, while SANS characterizes structural changes in artificial lipid droplets and liposomes. These model interfaces reveal interactions between Dengue virus Capsid protein and Perilipin-3-functionalized lipid systems.