

Visualising viral membrane fusion by SARS-CoV-2 using cryo-ET

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Host-cell entry of the SARS-CoV-2 virus is dependent on fusion of the viral and host-cell membranes, which enables the release of viral RNA into the host-cell for replication. Membrane fusion is mediated by the spike glycoprotein, which undergoes dramatic conformational transitions from the prefusion to postfusion state to achieve fusion. Given the dynamic, heterogenous nature of membrane fusion and the requirement for both viral and host-cell membrane, static structural biology techniques are not suitable to follow the process. Instead, cryo-electron tomography (cryo-ET) enables membrane fusion intermediates to be captured and visualised in native environments. We have developed a high-throughput cryo-ET workflow to study viral membrane fusion using an *in vitro* reconstitution system. This system allows for visualisation of membrane fusion between virus and host-cell membranes at subnanometre resolution without biosafety hurdles or focused ion-beam (FIB) milling. By combining lentiviral particles pseudotyped with SARS-CoV-2 spike and liposomes coated with the host-cell receptors ACE2, NRP1 and TMPRSS2, we have visualised fusion intermediate structures along the membrane fusion pathway by cryo-ET. Furthermore, in addition to cell-surface entry, receptor-mediated endocytosis is a key mechanism for host-cell entry by the virus. The presence of calcium and low pH have been shown to promote membrane fusion between the virus and endosomal membrane, but the effects of these conditions have not been visualised by structural methods previously. Here, using cryo-ET we reveal that these conditions promote not only virus-host-cell fusion, but also membrane interactions between viral particles to enable viral-viral fusion. Overall, using our *in vitro* reconstitution system and cryo-ET workflow we have progressed our understanding of spike-mediated fusion by SARS-CoV-2, with this developed workflow yielding potential for the study of further diverse viral fusion mechanisms.