Structural Basis of Virus-Cell Fusion and Virus Budding Studied by Solid-State NMR

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Enveloped viruses enter cells by fusing with host cell membranes using viral glycoproteins, and new progeny viruses bud from host cells using viral membrane proteins. Both processes require high membrane curvature and membrane remodeling, and how proteins mediate this curvature generation is a fundamental question that is still poorly understood. I will present our recent structural studies of three membrane proteins that shine light on the mechanism of virus-cell fusion and virus budding using solid-state NMR spectroscopy. 1) We have investigated the structure of the membrane-interacting domains of the fusion proteins of the parainfluenza virus 5 (PIV5) and human immunodeficiency virus (HIV). For the PIV5 fusion protein, the fusion peptide (FP) and the transmembrane domain (TMD) show striking membrane-dependent conformations: the β-sheet conformation correlates with the generation of negative Gaussian curvature and with membrane dehydration, which are required for membrane merger, while the α-helical conformation is found in low-curvature lamellar membranes and forms three-helix bundles. Therefore, the local lipid composition of the membrane regulates the site of virus-cell fusion. Moreover, when the FP and TMD are tethered in a chimera to mimic the post-fusion state, the two domains are found to be not in close contact in the membrane, suggesting that the trimer of hairpin structures of the water-soluble ectodomain do not extend into the membrane. For the HIV fusion protein gp41, we have now measured the oligomeric structure of the membrane-proximal external region (MPER) and the transmembrane domain using solid-state NMR, and show that this segment is trimerized in the lipid membrane. These data give new insights into the structural and functional role of the TMD in class I viral fusion proteins. 2) The influenza virus buds from host cells in a cholesterol-dependent manner using the M2 protein. To understand how cholesterol interacts with M2 to generate membrane curvature, we have determined the cholesterol-binding site of M2 using intermolecular distance measurements and orientation measurements. Our results give unexpected and unique insights represent the first direct determination of the cholesterol-binding structure of a membrane protein in lipid bilayers, and suggest a concrete mechanism for how cholesterol concentration differences between the plasma membrane and the virus envelope drive M2 to achieve membrane scission. Both studies have benefitted from the high gyromagnetic ratio of ^{19}F spins for determining long-range distances that reveal the intermolecular binding and oligomeric assembly of these membrane proteins.