Elucidating membrane protein – cholesterol binding using distance measurements and DNP-enhanced solid-state NMR

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Cholesterol is ubiquitous in mammalian membranes and influences membrane protein structure and function by either direct interactions or indirect effects on membrane physical properties such as rigidity and viscosity. So far most structures of cholesterol-bound membrane proteins have been obtained from monoolein/cholesterol crystals, without clear biological function for the bound cholesterol.

In this work we demonstrate two novel magic-angle-spinning solid-state NMR techniques to elucidate cholesterol-binding sites in membrane proteins bound to lipid bilayers. The first approach employs ^{13}C-^{19}F distance measurements and analysis,[1] while the second approach combines DNP-enhanced ^{13}C-^{13}C correlation experiments with novel biosynthetic labeling of cholesterol.[2] We demonstrate these approaches on the influenza M2 protein, which mediates virus budding and membrane scission in a cholesterol-dependent manner.[3] Using ^{13}C-^{19}F REDOR and distance analysis that accounts for fast methyl rotations, we measured distances between heptafluorinated cholesterol and M2 and found that the cholesterol tail is located near methyl-rich residues in the C-terminal portion of the transmembrane (TM) domain.[1] Moreover, the REDOR dephasing plateaus to ~0.5, indicating that each M2 tetramer binds only two cholesterol. To complement the distance measurement, we used ^{2}H NMR to determine cholesterol orientation. The ^{2}H quadrupolar couplings indicate that bound cholesterol adopts a similar vertical orientation in the lipid bilayer as free cholesterol, indicating that protein binding does not perturb the natural orientation of the sterol.

To further define the structure of the cholesterol-M2 interface, we used a metabolically engineered yeast to express ^{13}C skip-labeled cholesterol.[2] With 1-^{13}C labeled glucose as the precursor, we obtained 15 ^{13}C-enriched sites distributed throughout the molecule. Combining DNP with a ^{13}C-^{13}C double-quantum filter, we obtained high-sensitivity and high-resolution 2D ^{13}C-^{13}C correlation spectra, from which ~15 intermolecular correlations can be extracted.

Combining these ^{13}C-^{19}F and ^{13}C-^{13}C distance constraints and ^{2}H orientation constraints, we obtained an M2-cholesterol docked structural model, which shows extensive interactions between methyl-rich Ile and Leu sidechains of the TM domain and methyl groups of cholesterol. This structural model explains why cholesterol binding does not require a special amino acid sequence motif, but requires the amphipathic helix. The structure and stoichiometry of the M2-cholesterol complex also sheds light on how cholesterol binding causes M2 localization to the neck of the budding virus to cause membrane scission. These distance-based and DNP-enhanced solid-state NMR techniques are generally applicable for elucidating the role of cholesterol in the structures and functions of eukaryotic membrane proteins.

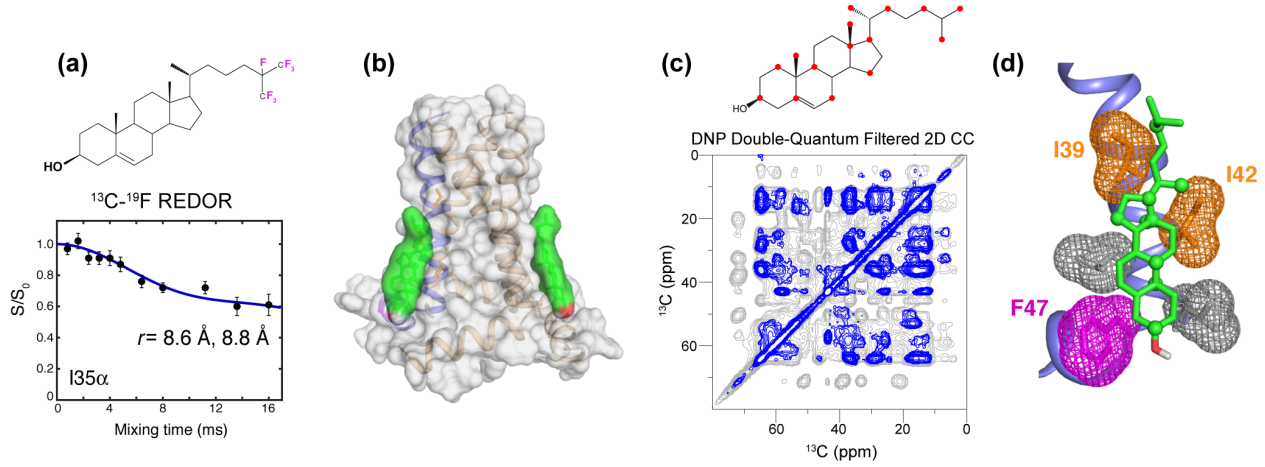

Figure 1. Determining the cholesterol-binding site in influenza M2. (a) Fluorinated cholesterol and ^{13}C-^{19}F REDOR were used to measure protein-cholesterol distances. (b) Surface plot of the M2-cholesterol sub-stoichiometric complex. (c) 1-^{13}C labeled cholesterol from biosynthetic labeling allowed DNP-enhanced 2D ^{13}C-^{13}C correlation spectra to be measured. A ^{13}C-^{13}C double-quantum filter significantly simplified the spectra (blue) and facilitated assignment. (d) The intermolecular ^{13}C-^{13}C correlations indicate which protein sidechains are close to the cholesterol rings.

References:

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