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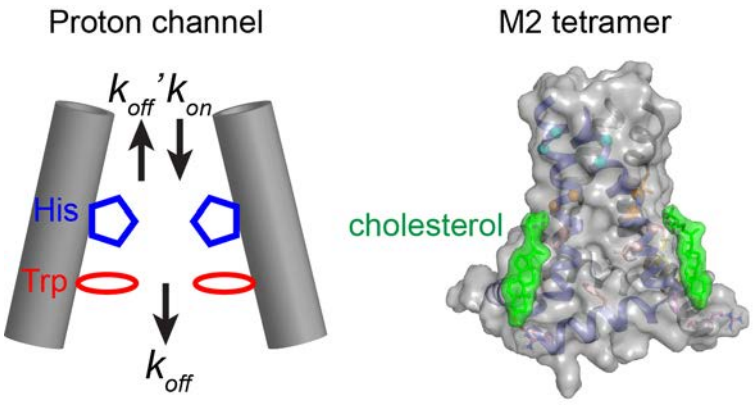
Structure, Assembly and Dynamics of a Viral Membrane Protein Studied by Solid-State NMR

Abstract

The influenza M2 protein forms an acid-activated proton channel that acidifies the virion for virus uncoating, and mediates membrane scission to enable virus budding. In this talk I will present our SSNMR-based comprehensive studies of the structure and dynamics of M2 in lipid bilayers that pertain to both functions.

Using solid-state NMR, we have measured the pH-dependent dynamic structures of the proton-selective histidine and the gating tryptophan in the M2 proton channel. We show that histidine shuttles protons with water molecules, and the protonation equilibria and proton-exchange rates of this shuttling have been obtained. The influence of the protein amino acid sequence on the proton transfer process is examined by comparing influenza A and B viruses' M2 proteins, which have little sequence homology except for the conserved HxxxW motif. By mutating the gating tryptophan residue to a phenylalanine, we found that the asymmetric conductance of protons can be abolished, and histidine can be protonated and activated from protons from the C-terminus. Motionally averaged NMR spectra indicate that both histidine and tryptophan undergo microsecond ring reorientations to transfer protons with water, while relaxation NMR data show that channel water undergoes nanosecond motions that may facilitate Grotthuss hopping of protons to histidine. However, both these motions are much faster than the proton conduction rate of 10-1000 s-1. Using 2D exchange NMR, we have now obtained evidence for tetramer backbone conformational dynamics on the millisecond timescale, in synchrony with proton transport, thus revealing the rate-limiting step of proton transport.



In addition to the proton channel function, M2 mediates membrane scission in a cholesterol-dependent fashion to enable virus budding and release. We have now determined the structure of the cholesterol-binding site of M2 using distance and orientational measurements. The cholesterol-complexed M2 structure gives novel insight into the mechanism of membrane scission.

These results were obtained using a wide range of solid-state NMR techniques, ranging from 13C, 15N and 1H correlation spectroscopy that yields structurally informative chemical shifts, to studies of molecular motions on timescales from nanoseconds to seconds by 2H, 15N, 13C and 1H NMR, and finally to long-range distance measurements using 13C and 19F NMR, together with sensitivity-enhancing dynamic nuclear polarization.