membrane curvature and scission in a cholesterol-dependent mechanism that renders virus budding independent of the cellular ESCRT system.[56] Finally, the C-terminus of the protein interacts with the M1 matrix protein during the formation of the virus particle.[112–115]

Reversal potentials for the M2 TMD in oocyte membranes indicated a 10-fold preference for protons over sodium, potassium or chloride ions.[116] The observation that channel conductance is reversible was consistent with pore-like behaviour,[3,116] yet a slow rate of conductance ($\sim 200\,H^+\,s^{-1}$) in both oocytes and liposomes[117,118] combined with the lack of transport of alkali metal ions were indicative that the water column through the channel complex was interrupted by a selectivity filter. Furthermore, proton conductance only occurs when the external pH is acidic,[116] implicating protonation of ionisable residues as an important step. In this regard, mutagenesis of the highly conserved His37 residue within the TMD resulted in enhanced conductance[106] as well as a loss of selectivity[116] while retaining amantadine sensitivity, indicating that this residue played a fundamental role during M2 gating. Functionally defined models for the M2 TMD channel complex predicted that His37 did indeed line the channel lumen.[119] consistent with Cu^{2+}-mediated inhibition of channel activity.[120] Furthermore, the major gating residue was predicted to comprise the similarly conserved Trp41 residues,[120] resulting in a now well-accepted HxxxW tetrad comprising the gating mechanism for all M2 proteins, although Val27 may also form a secondary gate towards the N-terminal neck of the TMD.[53,72,121] This functional unit has been borne out by extensive structural studies and a range of biophysical techniques employed to study the gating behaviour of M2, which has become a model for prototypic proton channels.

M2 structural studies were expedited by the fact that peptides representing minimal domains were able to recapitulate channel function, allowing the study of tetrameric complexes in membrane-mimetic environments. In all cases, M2 forms a left-handed four-helix bundle[122] with a defined hydrophilic lumen containing both His37 and Trp41 tetrads. Figure 9.1 shows the major M2 structures solved to date in chronological order and separated by technique/conditions.

The first reported structures were solved by solid-state NMR (ssNMR) in Cross's laboratory for the TM domain (22–46), in both the absence (PDB: 1NYJ)[123,124] and the presence of amantadine (PDB: 2H95).[125] This TM structure was subsequently refined by Cady and co-workers in the presence of amantadine (PDB: 2KQT).[79,126] The TM construct was also the subject of crystallographic studies, producing an amantadine-bound structure (PDB: 3BKD/3C9J),[78] which again has been recently refined (PDB: 3LBW).[53] What was apparent from early ssNMR and X-ray studies was that, in the absence of drug or near-neutral pH required to stabilise the closed form of the channel, the C-terminal region comprising the Trp41 tetrad was splayed, such that that the 'gate' could not be formed. However, this was not the case either in refined ssNMR/X-ray structures or for structures comprising the conductance domain where the additional presence of the amphipathic helix resulted in a more compact channel structure where the Trp41 tetrad restricted the C-terminal end