cycle, leading to the proposal that the drug disrupted an M2–HA protein–protein interaction.[5]

The demonstration that M2 formed stable, disulfide-linked tetramers[102] combined with M2-mediated raised Golgi/endosomal pH[7,8,103–105] provided the first clue to M2 channel formation. This was confirmed in seminal studies of M2 expressed in *Xenopus* oocytes, demonstrating amantadine-sensitive monovalent cation conductance that was activated upon reduced external pH.[3] Mutations within M2 known to confer resistance to amantadine also rendered isolated channel activity insensitive to the drug. Amantadine-sensitive channel activity was also shown for M2 peptides corresponding to the minimal TMD (amino acids 21–46) using artificial bilayers.[4]

Thus, M2 became the first example of a virus-encoded ion channel protein where activity of the protein in isolation was directly related to a functional requirement at specific stages in the virus life cycle, which in turn was known to be targeted by small-molecule inhibitors. Several studies showed that M2 displayed selectivity for protons and that gating was both activated by acidic pH and dependent on a highly conserved His37 residue, as demonstrated by rational mutagenesis with subsequent effects on channel gating.[106] The convergent conclusions of multiple studies led to the proposed role of M2 during virus entry as mediating acidification of the virion interior following endosome acidification,[6] which also triggers membrane fusion mediated by HA.[107] Reduced intra-virion pH is thought to uncouple interactions between RNPs and the matrix protein, thereby promoting efficient uncoating following release of the virion contents into the cytoplasm. The role of M2 during particle release applied to influenza strains where the HA0 precursor contained a multi-basic region that enabled intracellular cleavage by furin. This results in HA becoming prematurely primed to undergo fusogenic change in reduced pH environments, such as in the TGN and endosomes where vATPase is active. M2, present in the membrane of such compartments, negates vATPase activity by promoting the flow of protons out of the acidifying secretory compartment, thereby allowing HA to traffic to particle assembly sites on the cell surface in a functional form, able to bind to sialic acid receptors.[7–10,103–105]

9.2.1.2 Structure and Gating of Influenza A Virus M2

M2 is a 97 amino acid (aa) protein with a single TMD which forms disulfide-linked tetramers in membranes.[46,102,108] The 25 N-terminal residues are located on the surface of the plasma/virion membrane and are highly conserved. This has recently been exploited in vaccine development due to the similarity between most influenza A isolates.[109,110] The TMD extends from aa 25 to 46 and is followed by an amphipathic helix (aa 47–62) and the remaining cytosolic domain. Ion channel activity can be recapitulated by a minimal domain including the TMD (aa 22–46),[4] although a longer 'conductance domain' (CD), which includes the amphipathic helices (aa 18–60 or 22–62, depending on the study), displays enhanced channel properties in oocytes.[111] The amphipathic helices are also critical to a recently described function for M2 in mediating