of the channel. This was seen in a structure for a rimantadine-bound channel complex from Chou's laboratory solved by solution NMR (PDB: 2RLF).[80] This was followed by two solution structures for amantadine-resistant variants, S31N (PDB: 2KIH)[127] and V27A (2KWX).[128] Finally, an ssNMR structure of the conductance domain was recently obtained in the presence of lipid bilayers (PDB: 2L0J).[72] In this structure, the position of the amphipathic helices was significantly altered compared with the solution structures, bringing them into closer proximity to the TM region. This is likely due to the influence of various membrane-mimetic environments on protein structure compared with an authentic membrane bilayer. Differences in drug binding are discussed in Section 9.2.1.3; however, these structures combined with related biophysical techniques have provided insight into the mechanism underpinning M2 gating, although this also varies with the construct and experimental system employed.[129] Interestingly, protonation of the His37 tetrad appears to greatly enhance the stability of the assembled tetramer,[47] this being three orders of magnitude higher at pH 6 than pH 9.[111] His37 protonation first occurs at a much higher pH (8.2) compared with His in free solution.[130] One explanation for this comes from molecular mechanics studies around the 2L0J structure, which support a 'dimer of dimers' model for the His37 tetrad, allowing sharing of a single proton between two His residues within each pair.[72] This both results in a strong hydrogen bond between the two residues and also permits one of each pair to undergo cation–π interactions with the indole of the adjacent Trp41. The third protonation event, which occurs at pH $\sim$6, then induces an unstable activated state where indole–imidazole cation–π interactions are disrupted by alterations in the helical bundle, allowing opening of the Tryp41 gate and the flow of protons to occur.[116] Kinetic analyses combined with solution NMR support synchronicity between His37 dimer protonation and Trp41 opening, with conformational changes detected in solution for both residues concomitantly upon reduced pH.[131] A similar mechanism is also supported by molecular dynamics and mathematical models, which also indicate a rate-limiting role for the secondary gate formed by Val27.[132] However, other studies support a 'shuttle' mechanism of proton conductance inconsistent with the formation of His37 dimers, whereby exchange of protons between His37 and water residues are facilitated by imidazole ring reorientations.[133–136] As such, the precise molecular mechanisms of even this 'simple' model for proton channel conductance remain a matter of some debate, potentially due to the different structural orientations, membrane environments and peptides employed affecting the His37 and Trp41 tetrads in distinct structural studies.

[†]Abbreviations: DHPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphatidylcholine; DMPC, 1,2-dimyristyl-*sn*-glycero-3-phosphatidylcholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine; PEG, poly(ethylene glycol); OG, octyl β-D-glucopyranoside; ssNMR, solidstate nuclear magnetic resonance; sNMR, solution nuclear magnetic resonance; X-ray, X-ray crystallography; AM2, influenza A virus M2; BM2, influenza B virus M2.