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from AM2 showed rimantadine binding within the lumen,[145] and the allosteric binding site present on the conductance domain structure solved in bilayers is significantly altered by the positioning of the amphipathic helices.[72]

Hence a seemingly overwhelming body of evidence has accumulated in recent years favouring the lumenal M2–adamantane interaction. However, a number of studies on both conductance domain and TM peptides have identified that these compounds are in fact capable of binding to both sites, although the affinity for the allosteric, membrane-exposed site is lower as judged by surface plasmon resonance (SPR)[146] and allosteric binding is only seen in solid-state studies of TM peptides upon the partitioning of the drug into the lipidic compartment.[79] *In silico* docking to the conductance domain solution NMR structure also exclusively favours binding to the allosteric site,[147] whereas lumenal binding is predicted when performed on the solid-state TM structure.[82] Interestingly, molecular dynamics simulations on the conductance domain again predict binding to both sites, although the nature of binding to each was different.[148] Binding within the lumen resulted in a much more stable interaction, yet had to overcome a significant energy barrier, while binding to the allosteric site was less stable yet binding was significantly kinetically more favourable than binding to the lumen. One unifying possibility could involve a cooperative mechanism of inhibition, whereby rapid kinetic drug binding to the allosteric site serves to stabilise channel complexes such that more stable interactions within the lumen might take place.

In addition, the mechanisms by which the most commonly described adamantane resistance mutants, such as L26F, L28F, V27A, A30T, S31N and G34E, prevent drug binding are not clear. Some, such as V27A, A30T and G34E, affect residues predicted to interact with drug molecules following lumenal binding, making the likelihood of disrupting a stabilising interaction the most obvious mechanism. However, others, such as L26F and L28F, affect residues involved in helical stacking in most of the described structures, making their role less clear. Interestingly, the most significant resistance polymorphism, S31N, found in both pandemic H1N1 and highly pathogenic H5N1 avian strains, has been proposed potentially to affect binding at both sites.[126,127] For lumenal binding, this mutation induces broadening of the channel aperture, which has been proposed to allow drugs to bind only loosely within the lumen, thereby not affecting the flow of protons. However, this mutation also disrupts the peripheral binding site in solution NMR structures (PDB: 2KIH) and induces the formation of less stable tetramers.

Which, then, is the most relevant adamantane binding site in Nature? One confounding issue resulting from the many structural and biophysical studies is the different peptides and conditions used when studying M2–drug interactions. The most obvious difference is that conductance domain peptides contain additional M2 sequence compared with TM peptides and the inclusion of the amphipathic helices appears to stabilise the structure, making it more compact. Although TM peptides contain some key elements of the peripheral binding site such as Asp45, they lack distal residues such as Phe47 and Phe48,