X-ray crystal structures of the influenza A M2 proton channel bound to amantadine, rimantadine, and inhibiting compounds

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Inhibition of the M2 proton channel in the influenza A virus prevents viral replication from occurring. Two of the four FDA-approved drugs for the treatment of influenza infections, amantadine and rimantadine, target the M2 channel. However, because M2 is a membrane protein, structural studies of drug binding to the channel have been limited due to the challenging nature of the target. The only previously published drug-bound crystal structure was limited to 3.5 Å resolution[1]; the presence of amantadine in the channel pore was confirmed by this structure, but the orientation of the drug could not be unambiguously determined and the resolution was too poor for water molecules to be visualized. Here, we have obtained multiple crystal structures of M2 in the presence of drugs and inhibiting compounds using lipidic cubic phase (LCP) crystallization techniques. We present the first crystal structures of rimantadine bound to M2 in both the C_{closed} and C_{open} conformations of the channel (2.0 Å, 2.5 Å), as well as amantadine bound to the C_{closed} conformation (2.0 Å). At this resolution range the orientation of the bound drug is unambiguous, and the ammonium group of the adamantane drugs can be seen interacting with ordered water molecules present within the channel. Additionally, we report the binding of a dual-inhibiting compound to both the wild type channel (2.6 Å) and the drug-resistant V27A mutant (2.5 Å). The position of the bound inhibitor within the channel shifts in the presence of the V27A mutation. These structures further our understanding of drug binding and inhibition within the M2 proton channel and will help guide the design of compounds for the inhibition of drug-resistant mutants of M2.

References:
[1] Stouffer, et al. Nature 2008. 451(7178):596-9.