BST-2/tetherin.[188] However, a hydrophobic region present in the N-terminal region of the protein, reminiscent of M2, led investigators to examine possible viroporin activity.

In 1996, recombinant and oocyte-expressed Vpu was shown to display channel activity in bilayers and cell membranes, respectively, with N-terminal TMD peptides also recapitulating activity *in vitro*.[25,26] Channels showed selectivity for Na^+ and K^+ compared with Cl^-, yet whilst a bacterial cross-feeding assay supported a preference for Na^+, oocyte experiments did not confirm such discrimination and also showed partial permeability to divalent cations. Mutating the TMD sequence abrogated Vpu-mediated enhancement of particle release, leading to viroporin function being implicated during this process.[189,190] This was supported by the finding that the amiloride derivatives 5-(*N*,*N*-hexamethylene)amiloride and 5-(*N*,*N*-dimethyl)amiloride, but not amiloride itself or amantadine, blocked Vpu channel activity in bilayers and also the release of HIV virus-like particles from HeLa cells.[181]

Interestingly, further studies on Vpu indicated that it disrupted, rather than enhanced, the conductance of K^+ ions across oocyte membranes.[187] This may be related to an interaction between the Vpu TMD and mammalian TASK channels, whereby sequence homology between the two proteins led to Vpu abolishing TASK-1 current and, conversely, over-expression of TASK led to a marked impairment of the ability of Vpu to enhance viral particle release.[191] However, multiple studies have demonstrated that the Vpu TMD retained its own channel activity in multiple *in vitro* scenarios[24–26,55,179,181,183,184,192] and scrambling this sequence in simian human immunodeficiency viruses (SHIVs) reduced pathogenicity in macaques.[189] It should be noted, however, that disrupting the TMD may also interfere with Vpu actions on CD4 or tetherin degradation. Interestingly, the same group found that replacing the Vpu TMD with that of M2 generated a pathogenic virus *in vivo* that was sensitive to rimantadine, and this could also be recapitulated by introducing a single histidine residue in place of alanine at position 19.[193,194] This was put forward as proof of principle for the potential of viroporin inhibitors in HIV-1 therapy, yet these mutant/chimeric viruses have not been followed up subsequently. However, in 2010 a novel Vpu inhibitor selected from a bacterial toxicity screen was reported to display an EC_{50} of $\sim 2\,\mu M$ in subtype B HIV-1-infected macrophages.[86] BIT225 is an amiloride derivative and has been reported to block the activity of other viroporins in addition to Vpu, including pestivirus and HCV p7 channels.[87] The targeting of Vpu channel activity by BIT225 is supported by its action on Vpu peptides *in vitro* and also by the compound being ineffective against HIV-2 in culture, which lacks a Vpu protein,[86] although no specific resistance mutations have been described either through rational methods or following long-term virus culture. Nevertheless, Biotron have progressed the compound into clinical Phase 1/2 trials in treatment-naive HIV-1-infected patients to assess safety and tolerability (see www.biotron.com.au). If successful, this will provide a landmark for other viroporin drug development programmes.