non-specific membrane disruption or perturbation, which can manifest as current traces and has led to some proteins being controversially nominated as viroporins.[59,60] In addition, although general ionic preferences can be determined, *e.g.* for cations compared with anions, some viroporins have been shown to conduct ions that are later shown not to be physiologically relevant in cell-based assays – many ion channels will transport ionic species that are present if their favoured ligand is absent, albeit with reduced efficiency/specificity. Finally, it is not generally possible to determine the amount of *functional* peptide/protein present within bilayers at a given time, making it difficult to calculate the kinetics of small-molecule interactions in most cases.

An alternative to artificial bilayers is the use of liposome dye release assays as indirect measurements of channel activity.[1] Although these do not monitor single channel events and are subject to many of the limitations of bilayer systems, they have the advantage of being technically straightforward, reliable and readily expanded into multi-well formats, as illustrated by a recent high-throughput screen conducted by Boehringer Ingelheim for HCV p7.[62] Although indirect with regard to channel activity *per se*, such assays have been used to assess reliably the effects of null mutants and small molecules against a variety of viroporins. The use of liposomes also permits biochemical analysis of proteins as bulk populations, which can often provide additional insight into functional determinants and membrane association/insertion. Dye release assays, like bilayers, illustrate the flexibility of viroporin conductance; p7,[61,62] SH,[18] E5[45] and also M2 and NSP4 (unpublished observations) readily permit the release of anionic carboxyfluorescein dye. However, the usefulness of such assays to drug discovery is supported by the identification of effective small molecules in liposomes that also display efficacy in virus culture.[63,64]

Assessment of viroporin activity in cells has been undertaken in prokaryotes, yeast and mammalian cells using a variety of strategies. Early work identifying membrane permeability for picornavirus 2B, alphavirus 6K, HIV-1 Vpu and others was based on bacterial systems.[24,65–67] These included bacterial lysis assays and also viroporin-induced permeability of bacterial membranes to otherwise impermeant antibiotics, with resultant diminished protein translation. Such techniques have also been used recently to map functional domains in rotavirus NSP4.[23] In addition, Vpu-induced disruption of bacterial membrane Na^+ gradients was demonstrated using a system where this led to leakage of proline from *Escherichia coli* cells, thereby promoting the growth of an adjacent proline-auxotroph indicator strain.[26] These systems are readily amenable to the testing of multiple viroporin variants, making them a convenient means of screening viral proteins for this activity. However, one drawback is the potential for indirect induction of membrane permeability, through either membrane disruption or perturbation of host membrane proteins.

Experiments in non-prokaryotic systems have primarily involved classical electrophysiological set-ups employing *Xenopus laevis* oocyte surface expression, including seminal studies on M2.[3] This powerful, adaptable technique permits the analysis of both single channels and/or populations and