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# Relating structure and function of viral membrane-spanning miniproteins

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Many viruses express small hydrophobic membrane proteins. These proteins are often referred to as viroporins because they exhibit ion channel activity. However, the channel activity has not been definitively associated with a biological function in all cases. More generally, protein–protein and protein–phospholipid interactions have been associated with specific biological activities of these proteins. As research has progressed there is a decreased emphasis on potential roles of the channel activity, and increased research on multiple other biological functions. This being the case, it may be more appropriate to refer to them as 'viral membrane-spanning miniproteins'. Structural studies are illustrated with Vpu from HIV-1 and p7 from HCV.

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## Introduction

Small viral membrane proteins perform multiple biological functions, which are generally associated with much larger proteins. Contributing factors may be the need to take optimal advantage of the limited sequence information encoded in compact viral genomes, and that these proteins are associated with lipid bilayers with different compositions and properties in various organelles, which can influence their structures, interactions, and functions. Here, we utilize two well-characterized examples, virus protein 'u' (Vpu) of the human immunodeficiency virus (HIV-1) [1] and p7 of hepatitis C virus (HCV) [2], to describe the current state of the structural biology of this class of proteins. Neither of these proteins has been crystallized, therefore nearly all of the experimental structural information available is derived from nuclear magnetic resonance (NMR) spectroscopy. We contrast the properties of these proteins with those of the best studied example; the M2 protein of Influenza, which has a well-defined channel function. The structure of its transmembrane channel domain has been extensively characterized by NMR spectroscopy, X-ray crystallography, and other physical methods [3,4].

Despite the differences in their titles, DiMaio's review entitled 'Viral miniproteins' [5••] and that by Nieva *et al.* [6••] entitled 'Viroporins: structure and biological functions' are concerned with the same class of proteins. Viroporins are strongly associated with having channel activity. DiMaio, in a sense, breaks new ground by providing a more general view of these proteins. This is well justified because these proteins have multiple essential activities; even though in many cases they do display channel activity, it is not always clear if and how it is associated with biological functions. Strebel's review [7•] describes functional implications of much of the available structural information on Vpu, including questions about the role of its channel activity. The review by Steinmann and Pietschmann provides essential background on p7 [8].

## NMR of membrane proteins

Solution NMR is applicable to relatively small membrane proteins in model membrane environments, such as mixed organic solvents, detergent micelles, isotropic bicelles, and recently nanodiscs. Despite their limitations, these environments have been used because they enable the proteins to reorient rapidly enough in solution to yield high-resolution NMR spectra. Caution must be exercised in the interpretation of the results as these non-native environments can affect the properties of the proteins. For example, the commonly used solvent 50%TFE/50%water has a propensity to stabilize helices while destabilizing or distorting tertiary and quaternary structure. It may very well be the case that the same sensitivity of the structure to the lipid environment provides a range of functions [9] and makes it difficult to sort out the correct biological structure from the various types of NMR samples that have been studied [10].

Membrane proteins can also be reconstituted into phospholipids. When associated with bilayers, membrane proteins are effectively 'immobilized' on the timescales of the chemical shift and dipole–dipole nuclear spin interactions. Consequently, solid-state NMR methods are required to obtain high-resolution NMR spectra. Under physiological conditions, membrane proteins undergo rotational diffusion about the bilayer normal, which

enables the application of rotationally aligned solid-state NMR [11••]. This is complementary to other solid-state NMR approaches that measure distances and angles in unoriented samples with the application of magic angle spinning (MAS) [12,13] as well as mechanically or magnetically aligned samples for oriented sample solid-state NMR [14].

## Vpu from HIV-1

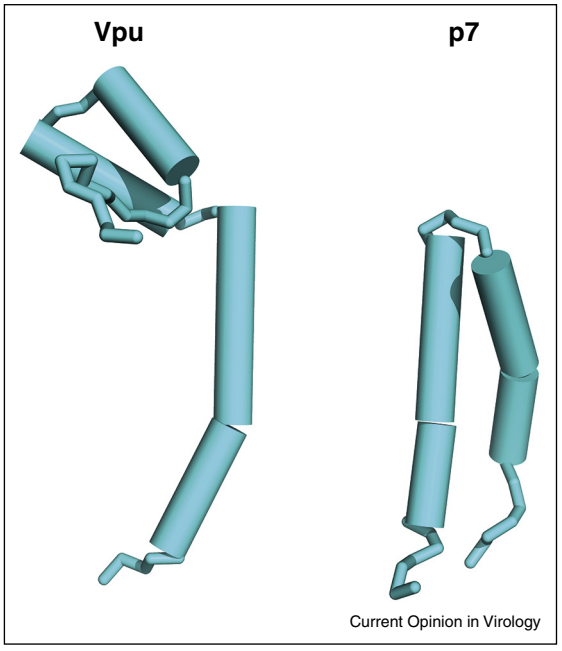
HIV-1 has a single-stranded positive-sense RNA genome of 9.8 kb that is processed to form the structural, regulatory, and envelope proteins characteristic of a retrovirus. In addition it has four accessory proteins, one of which, Vpu, is a small 81-residue membrane protein [1].

The initial structural studies of Vpu were performed by solution NMR in TFE/water on a series of synthetic peptides having between 15 and 50 amino acids [15–19] with sequences corresponded to overlapping sections of the protein. General ideas about the overall architecture of the protein emerged from these studies. More detailed studies required the use of expressed polypeptides because of the opportunities for isotopic labeling [20–24]. From the combination of these studies, a picture of Vpu emerged as having an N-terminal hydrophobic trans-membrane helix, which oligomerizes to form channels, and a C-terminal cytoplasmic domain with a helix–loop–helix arrangement. Significantly, the loop contains two conserved serine residues that are phosphorylated.

The combination of expressed polypeptides and phospholipid bilayer samples provides a near-native environment. Marassi *et al.* [25] studied expressed full-length and truncated constructs of Vpu in phospholipid bilayers by oriented sample solid state NMR, which showed that the protein has two distinct domains. To define the minimum folding units, Ma *et al.* [26] prepared three truncated forms of Vpu and compared their structural and functional properties to those of the full-length protein. Park *et al.* [27] determined the three-dimensional structure of the channel-forming trans-membrane domain (VpuTM). Substitution of alanine at position 18 by a histidine (A18H) has been shown to render HIV-1 infections susceptible to rimantadine [28]. The structure of A18H VpuTM was determined, and compared to that of wild-type Vpu TM [29]. Rotationally aligned studies complement solution NMR studies in yielding a structure for the Vpu monomer (Zhang *et al.*, unpublished results) (Figure 1). This structure is distinguished by having three helical segments, one of which is a hydrophobic membrane-spanning helix that can form a channel; the other two are part of an amphipathic surface domain. Notably, all of the helices can interact with other proteins contributing to the biological functions of Vpu.

In a complementary approach using unoriented samples, Sharpe *et al.* [30] described solid-state magic angle spinning

**Figure 1**




Structural models of Vpu (left) and p7 (right) derived from NMR data (unpublished results).

experiments on the N-terminal half of Vpu. Their data indicated that the transmembrane alpha helix extends beyond the hydrophobic core of the bilayer. Lu *et al.* [31] described measurements that provided new constraints on the oligomerization state of Vpu. Their data indicated that a variety of oligomers coexist in phospholipid bilayers. This is also consistent with at least one of Vpu's activities being associated with its forming channels. Do *et al.* applied magic angle spinning solid-state NMR [32] to expressed full-length Vpu and a construct consisting of residues 372–433 of CD4.

The binding between Vpu and β-TrCP does not require phosphorylation of the serines in the interhelical loop of the cytoplasmic domain of Vpu, however activation of the degradation pathway does. Coadou *et al.* [33–37] used relatively short peptides to elucidate [35] the basis of β-TrCP recognition. The β-TrCP-bound structure of phosphorylated Vpu was found to be similar to the structure of the free peptide in solution and to the structure recognized by its antibody. Gharbi-Benarous *et al.* [36] investigated a 22-amino acid peptide that mimics the phosphorylated Vpu antigen. Phosphorylation of Vpu at sites Ser52 and Ser56 on the DSGXXS motif is required for the interaction of Vpu with the ubiquitin ligase SCF (β-TrCP) that triggers CD4 degradation by the proteasome. The peptide residues forming this bend are recognized by a monoclonal antibody. Evrard-Todeschi *et al.* [37] examined the binding and conformation of phosphopeptides to β-TrCP.