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# Computational analysis of mutations in the transmembrane region of Vpu from HIV-1

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**Abstract**

Vpu is an 81 amino acid integral membrane protein encoded by HIV-1. Its α-helical transmembrane (TM) domain (residues ~6–28) enhances virion release by oligomerizing into bundles and forming ion-conducting channels across the plasma membrane. Its cytoplasmic domain (residues ~29–81) is also α-helical and binds to the transmembrane protein CD4, inducing its degradation. Mutations within the TM domain have been found to abrogate enhanced particle release from the infected cell (Tiganos et al. *Virology* (1998) 251 96–107). A series of computational models of monomeric, pentameric and hexameric $Vpu_{1-31}$ mutants have been constructed, embedded in fully hydrated lipid bilayers and subjected to a 3 ns molecular dynamics (MD) simulation. None of the mutations has any destabilizing effect on the secondary and tertiary structure. One of the mutants, in which the position of a tryptophan residue within the TM domain is altered, is known not to induce CD4 degradation; an extended kinked model of this mutant has been generated ($Vpu_{1-52}$IVW-k) and during subsequent MD simulations, the bend between the TM and a part of the cytoplasmic domain is found to unwind and a complex salt bridge involving Lys-37 is formed.


*Keywords:* Vpu; HIV-1; Mutant; Viral membrane protein; Molecular dynamics simulation; protein structure

## 1. Introduction

Computational methods such as molecular dynamics (MD) simulations can be used to assess whether any mutation in a protein has an effect on the structure and function of the protein before more time consuming experiments have been performed. Recently, MD simulations have been used to perform a computational alanine scanning experiment on the 1:1 human growth hormone–receptor complex [1]. It was shown that the simulation results are in good agreement with the experimental findings of binding free energy differences. In another study, the structural impact of mutations in the fusion domain of the fusion protein gp41 from HIV-1 on its fusogenic activity has been explored [2]. As a result of the simulations, conformational flexibility was suggested as the key factor which might be responsible for the experimental findings [3]. Here, mutants of the viral membrane protein Vpu from HIV-1 are used to evaluate possible implications of these mutations on the structural integrity.

Vpu is a type I integral membrane protein of 81 amino acids with a N terminal transmembrane (TM) domain and a larger cytoplasmic domain ([4–6], reviewed in [7]). The protein acts in two ways: (i) by interaction of its cytoplasmic domain with CD4 which leads to a degradation of CD4 [8–11], (ii) by self-assembly which induces ion channel activity to amplify the release of immature virions from the infected cell [12,13]. The structure of Vpu is reasonably well resolved by NMR [14–17], CD- [18] and FTIR-spectroscopy [19], enabling the generation of working

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a model for Vpu which can be summarized as follows: a TM helix approximately from residue 6 to 29, a second helix (helix-2) from residue 40 to 50 and a third helix (helix-3) from residue 60 to 70. Another short helix or turn is proposed for residues 75 to 78. Using this information, models can be produced for computational analysis.

In this study, experimental investigations on TM domain mutants of Vpu and the consequences of these mutations on its structure are explored using MD simulations. Mutations within the TM domain have been found to modulate the ability of Vpu to enhance particle release [20]. The present exploration serves as a test case to assess the mechanism of this function on an atomic scale. For all of the mutants investigated, membrane integration ability and subcellular translocation was identical to wild type (WT) Vpu [20]. In addition to a modulation of particle release, one mutant also abrogates CD4 degradation. The structural integrity of the TM helix has been found to be essential for both functions of Vpu [20]. Generation of computational models with solely the TM domain (TM helix), either as a single entity or as a pentameric or hexameric assembly embedded in a hydrated lipid bilayer, aims to assess whether these mutations would affect local conformation of the TM domain of the protein. An extended model of Vpu including the first 52 amino acids of one of the mutants is generated because of the dual effect of this mutant: reducing particle release and failing to induce CD4 degradation [20]. The simulation time is held short to enable a large number of models to be produced on a computationally low-cost level.

## 2. Materials and methods

Wild type $Vpu_{1-31}$ (HV1H2) and a series of mutant single helices consisting of the first 31 residues of Vpu (for simplicity referred to as WT-1, FV-1, respectively) and bundles consisting of five (WT-5, FV-5) and six of these helices (WT-6, FV-6) were generated. For one mutant, Vpu-IVW, an extended model with 52 amino acids, referred to as IVW-k, was generated.

The sequences used for the models are:

| | |
|---|---|
| WT | QPIQIAIVA$^{10}$ LVVAIIIAIV$^{20}$ VWSIVIIEYR$^{30}$ K |
| FI mutant | QPIQIA**FI**A$^{10}$ LVVAIIIAIV$^{20}$ VWSIVIIEYR$^{30}$ K |
| KSL mutant | QPIQ**K**A**SL**A$^{10}$ LVVAIIIAIV$^{20}$ VWSIVIIEYR$^{30}$ K |
| FV mutant | QPIQIAIVA$^{10}$ LV**FV**IIIAIV$^{20}$ VWSIVIIEYR$^{30}$ K |
| IVW mutant | QPIQIAIVA$^{10}$ LVVAIIIAIV$^{20}$ **IVSW**VIIEYR$^{30}$ K |
| IVW-k mutant | QPIQIAIVA$^{10}$ LVVAIIIAIV$^{20}$ **IVSW**VIIEYR$^{30}$ KILRQRKIDR$^{40}$ LIDRLIERAE$^{50}$ DS |

The mutations are highlighted in bold and the sequence was adapted from [20]. For model building, a protocol was used which combines a simulated annealing (SA) procedure with short molecular dynamics (MD) simulations based on the program Xplor [21]. This procedure is described in detail elsewhere [22]. The protocol can be briefly summarized as being a two-stage procedure in which in the first stage idealized helices with a rise per residue of 0.15 nm and 3.6 residues per turn were constructed. The tilt angle was set to be 5° for all the helices, either as single entities (see for example [23,24]) or as bundles of five or six helices (see, for example, [23,25]). All atoms of a side chains from a particular residue were superimposed on the Cα-atom. Gradually, the side chain atoms emerge from the position of the Cα-atoms which were restrained in position. During the annealing step (1000 K) weights for bond length, bond angles, planarity and chirality increased. After an initial delay, a repulsive van der Waals term was gradually introduced. Having reached a final scaling factor for the van der Waals term, the structures were cooled to 300 K in steps of 10 K/0.5 ps. To avoid trapping of the side chain atoms in unwanted conformations, the van der Waals radii were reduced to 80% of their full value. This part of the protocol was repeated five times to derive 5 different structures. Each repeat was performed with the different initial velocities needed for a short MD run at 1000 K derived from a Maxwellian random number distribution function. Each of these structures was used in the second stage of the protocol to run 5 short MD simulations at 500 K which differ in their randomly chosen (see above) initial starting velocities. Electrostatic interactions based on the PARAM19 parameter set were introduced at this stage. Harmonic restraints were used to hold the Cα-atoms. These restraints were gradually relaxed while the temperature was reduced from 500 to 300 K. Partial charges on the side chain atoms of the polar side chains were scaled up to a maximum of 0.4 of their full value. At 300 K, a 5 ps MD simulation followed by a 1000-step conjugate gradient energy minimization was performed. The values for the partial charges were also held constant during the 5 ps simulations and minimizations. Distant electrostatic interactions were truncated with a switching function. At the end of the second part of the protocol, 25 structures were generated from which the straightest helix, (in case of the single entities), or the most symmetric model, (for the pseudo five or six fold axes in the bundles), were selected. In general, the bundles were generated by copying a single helix around a central axis holding an inter-helix distance of 0.94 nm.

The extended kinked model, IVW-k was generated from an α-helix as described previously [24] including all the residues, by bending (using SwissPDB viewer software) the helix around residues Glu-28 to Leu-33 so that they adopted the following values at the start of the simulation: $\phi = -59.0°$, $\psi = -25.5°$ for Glu-28; $\phi = -59.2°$, $\psi = -36.1°$ for