
# Disruption of Helix-Capping Residues 671 and 674 Reveals a Role in HIV-1 Entry for a Specialized Hinge Segment of the Membrane Proximal External Region of gp41

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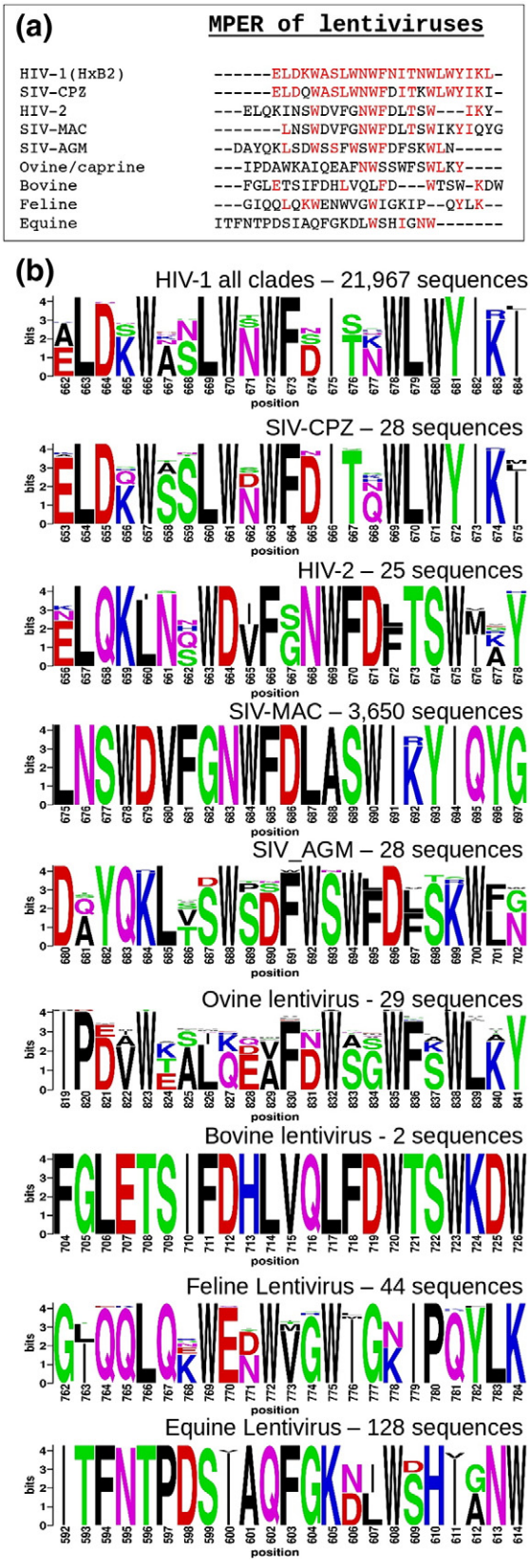
## Abstract

HIV-1 (*h*uman *i*mmunodeficiency *v*irus type *1*) uses its trimeric gp160 envelope (Env) protein consisting of non-covalently associated gp120 and gp41 subunits to mediate entry into human T lymphocytes. A facile virus fusion mechanism compensates for the sparse Env copy number observed on viral particles and includes a 22-amino-acid, lentivirus-specific adaptation at the gp41 base (amino acid residues 662–683), termed the membrane proximal external region (MPER). We show by NMR and EPR that the MPER consists of a structurally conserved pair of viral lipid-immersed helices separated by a hinge with tandem joints that can be locked by capping residues between helices. This design fosters efficient HIV-1 fusion via interconverting structures while, at the same time, affording immune escape. Disruption of both joints by double alanine mutations at Env positions 671 and 674 (AA) results in attenuation of Env-mediated cell–cell fusion and hemifusion, as well as viral infectivity mediated by both CD4-dependent and CD4-independent viruses. The potential mechanism of disruption was revealed by structural analysis of MPER conformational changes induced by AA mutation. A deeper acyl chain-buried MPER middle section and the elimination of cross-hinge rigid-body motion almost certainly impede requisite structural rearrangements during the fusion process, explaining the absence of MPER AA variants among all known naturally occurring HIV-1 viral sequences. Furthermore, those broadly neutralization antibodies directed against the HIV-1 MPER exploit the tandem joint architecture involving helix capping, thereby disrupting hinge function.



## Introduction

Lentiviruses such as HIV-1 (*h*uman *i*mmunodeficiency *v*irus type *1*), the causative agent of acquired immunodeficiency syndrome (AIDS), are encapsulated in a membrane derived from the infected host cell as virus buds (reviewed in Refs. [1] and [2]). A trimeric Env gp160 spike consisting of three pairs of non-covalently associated gp120 and gp41 subunits is the only viral protein on the HIV-1 membrane. The Env gp120 mediates attachment and entry into human $CD4^+$ T lymphocytes upon binding its primary cellular receptor CD4 and CCR5 or CXCR4 co-receptor. Viral infectivity is dependent on membrane fusion between




HIV-1 and the host cell through formation of a gp41 six-helix-bundle complex [3–5]. The efficiency of this mechanism is especially critical, given fewer than 12 copies of Env clustered on each viral particle [6].

The membrane proximal external region (MPER) is a tryptophan-rich segment located at the base of the gp41 subunit and appears to destabilize the viral membrane during the fusion process [7,8]. Deletion of the HIV-1 MPER, or concurrent mutation of three conserved tryptophan residues on its N-terminal helix to alanines, abolishes membrane fusion activity [9,10]. However, the presence of these conserved tryptophans alone is not sufficient to support the viral fusion activity [8]. Other MPER residues, even the exposed, primarily hydrophilic ones, may also be involved despite sequence variability therein. Previously, we solved the solution structure of a clade B HxB2 strain MPER peptide in detergent micelle with an unusual helix–hinge–helix motif [11]. Interestingly, the central hinge region is the target of several broadly neutralizing antibodies (BNAbs), including 4E10, 10E8 and Z13e1 [12–14]. Here, we provide detailed structural and functional results on this specialized hinge region relating to its potentially important role during the intermediate stages of the HIV membrane fusion process.

## Results and Discussion

### MPER sequence conservation and limited variability

Bioinformatics studies show that, while considerably conserved within each lentivirus group, the MPER sequences from HIV-1 and its ancestor SIV-CPZ (chimpanzee) are distinctive from those of HIV-2 and their related SIV-MAC (macaque) and SIV-AGM (African green monkey) sequences and are distant from non-primate lentiviruses (Fig. 1). As shown in Figs. 1b and 2a, the HIV-1 MPER is highly conserved across different clades. Structurally, the clade B HxB2 MPER peptide in dodecyl-phosphocholine (DPC) detergent micelle shows a helix–hinge–helix motif dictated by the segment's unique amphipathic pattern (Fig. 2b), with the membrane-buried residues mostly conserved and solvent-exposed residues relatively

**Fig. 1.** Comparison of MPER segments from nine groups of lentiviruses. The lentiviral segments, 23 amino acid long, N-terminal to their respective annotated transmembrane helices were extracted from SwissProt Database. (a) Sequence alignment with colored residues being identical with the reference sequence (HIV-1 HxB2). (b) Logos showing conservation within each of the nine lentivirus groups. The extremely conserved 3650 SIV sequences from rhesus macaque (SIV-MAC) is a result of infection in research primate centers.