Review

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Future Medicinal Chemistry

Interactions of HIV-1 proteins as targets for developing anti-HIV-1 peptides

Protein–protein interactions (PPI) are essential in every step of the HIV replication cycle. Mapping the interactions between viral and host proteins is a fundamental target for the design and development of new therapeutics. In this review, we focus on rational development of anti-HIV-1 peptides based on mapping viral–host and viral–viral protein interactions all across the HIV-1 replication cycle. We also discuss the mechanism of action, specificity and stability of these peptides, which are designed to inhibit PPI. Some of these peptides are excellent tools to study the mechanisms of PPI in HIV-1 replication cycle and for the development of anti-HIV-1 drug leads that modulate PPI.

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Protein–protein interactions in the HIV-1 replication cycle

Mapping the interactions between proteins derived from host and pathogen origins is essential for understanding the molecular mechanisms of host–pathogen interactions [1–4]. Protein–protein interactions (PPI) play a crucial role in the replication of HIV-1 [5–24]. HIV-1 infection results in an interplay between viral and host proteins or homodimeric/oligomeric viral protein interactions, resulting in a complex interaction network between various proteins [25,26]. The HIV-1-Human Protein Interaction Database (HHPID) identified 1435 human genes encoding 1448 human proteins that interact with HIV-1 proteins, resulting in 2589 unique HIV-1-host protein interactions [27–33]. Thirty two percent of these are direct physical interactions as revealed from binding studies and 68% are indirect interactions such as upregulation through activation of signaling pathways. The database reveals that numerous human proteins interact with more than one HIV-1 protein. Using a quantitative scoring system termed mass spectrometric interaction statistics (MiST), 497 HIV-human PPIs involving 435 individual human proteins and 18 viral proteins have been identified [25,34–40].

HHPID reports 15 essential HIV-1 proteins [25,31,41–44] (Figures 1 & 2). Three fundamental proteins (Gag, Pol, Env) are encoded by the HIV-1 genome and they undergo proteolysis to form the mature proteins. Four structural proteins, matrix (MA), capsid (CA), nucleocapsid (NC) and p6, are products of the proteolysis of Gag. Env proteolysis results in the envelope proteins gp120 and gp41 [45,46]. Pol encodes three enzymes: protease (PR), reverse transcriptase (RT) and integrase (IN). Encapsulated within the virus particle, the three Pol proteins play key functions in the viral replication upon infection. The remaining proteins (Vif, Vpr, Nef, Tat, Rev, Vpu) are accessory proteins [47–50]. The database shows 43 different direct interactions of HIV-1 proteins with human proteins based on activity, binding, inhibition, cleavage, complexation, modulation, deglycosylation and upregulation. Only a part of these interactions are targets for peptide inhibitors and will be discussed here (Figure 2).

Peptides as a tool to study PPI

Understanding PPI requires thorough structural, biophysical and biochemical characterization using recombinant proteins. However, a major hurdle is the expression and purification of the interacting proteins. Some proteins

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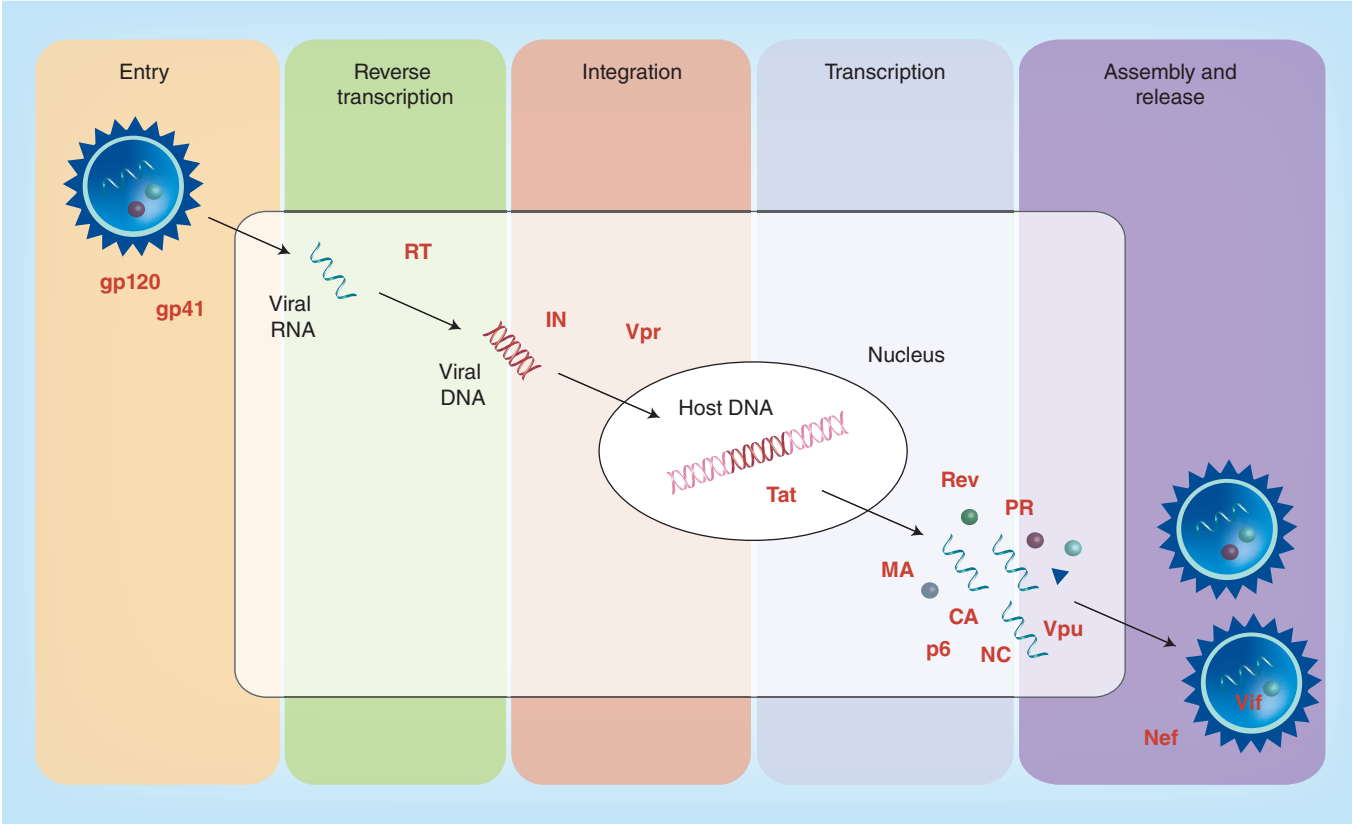

Figure 1. HIV-1 replication cycle with the essential viral proteins highlighted.

are insoluble or toxic to the expressing host, resulting in low yields that hamper structural and quantitative studies. Using peptides for these studies provide many advantages relative to the recombinant proteins. Peptides derived from the interacting proteins enable determination of the specific interaction sites, the affinity and thermodynamic contribution (enthalpy vs entropy) in PPI [51–54]. Chemical synthesis of the peptides makes it possible to overcome the expression and purification related problems of protein production [55–57]. This makes it technically convenient to study the PPI via a full-length protein and a peptide derived from the complementary protein in addition to the interaction between the two full-length proteins. Peptides derived from binding interfaces may bind weaker than the parent protein, partly due to loss of secondary structure. Modifications such as post-translational modifications (e.g., acetylation or phosphorylation) [58,59], labeling (e.g., fluorescein or biotin) or incorporation of non-natural amino acids can be inserted specifically into a protein sequence only using chemical peptide synthesis [60,61]. Peptides are an excellent model for binding studies of protein domains. Upon binding, they can undergo conformational change mimicking the native binding interface [62,63]. This makes peptides a useful tool for discovering drug leads by modulating (either activating or inhibiting) PPI [64–70]. In this review, we present an overview of peptides derived from PPI from different stages of the HIV-1 replication cycle [71] and their implications for anti-HIV-1 drug design.

PART I: interactions between viral & host proteins

Interactions between the viral capsid & host membrane proteins

The initial contact between the virus and the host cell is made between the viral glycoprotein gp120 (originating from the Env polyprotein, PDB: 3DNL, Figure 3A) and the cell surface receptor CD4 [72,73]. CD4 is a host glycoprotein expressed on the surface of T helper cells, regulatory T cells, macrophages, monocytes and dendritic cells. The binding of a highly conserved, nonglycosylated region of gp120 to CD4 results in the viral insertion into the host membrane [74–76]. Upon association with another viral envelope protein, gp41 (PDB: 2ZFC, Figure 3B), which mediates viral entry through membrane fusion, binding to CD4 occurs. This results in a conformational change that allows gp120 to bind to the coreceptors CCR5 or CXCR4 [77,78]; belonging to the family of G protein-coupled receptors and chemokine receptors [79–81]. The viral insertion causes an additional conformational change in the heptad repeat regions (HR1 and HR2) of gp41 [82], resulting in the entry of the viral capsid into the host cell via a fusion

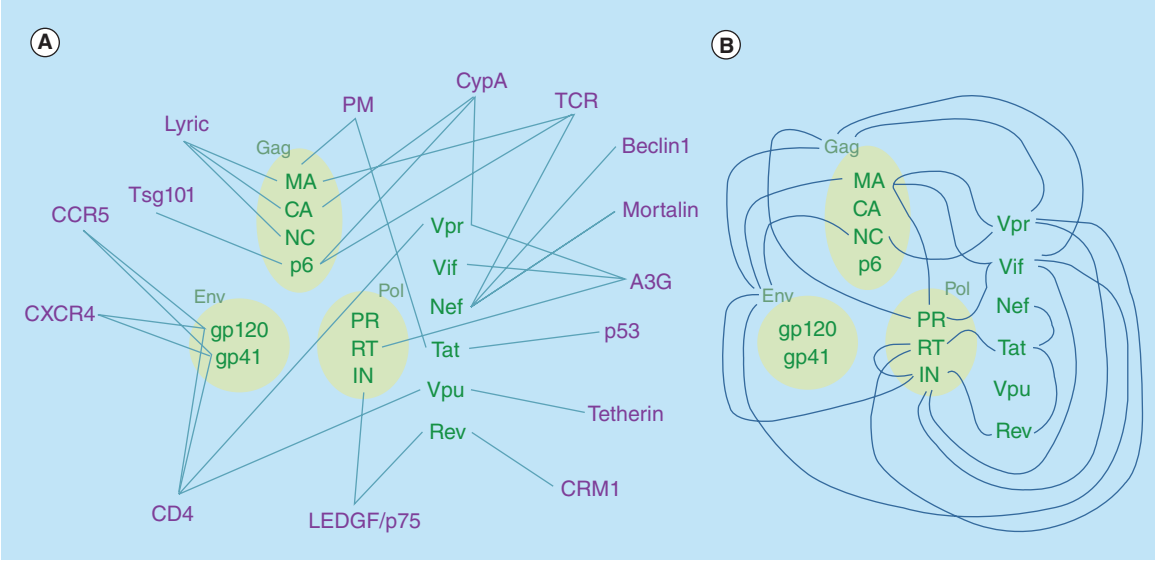

Figure 2. Selected PPI of HIV-1 proteins. (A) An interaction map between direct interactions of HIV-1-proteins (green) with human proteins (purple). **(B)** An interaction map between direct interactions of viral proteins (green). Both the interactions are involved in protein–protein interactions that served as basis for developing inhibitory peptides.
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pore [83]. This leads to the generation of epitopes for neutralizing antibodies that prevent chemokine receptor binding [84,85]. Two inhibitors of fusion and entry are currently used in the clinic. Approved in 2003, the 36-mer peptide inhibitor T20 (Enfuvirtide) blocks a critical conformational change in gp41 responsible for membrane fusion [86]. Maraviroc is a small molecule antiretroviral drug, approved in 2007, which inhibits the interaction between gp120 and CCR5 [86].

Peptides derived from HIV-1 Env & host proteins interactions

The HIV inhibiting peptide database (HIPdb) reveals 110 HIV inhibitory peptides that target the interactions of the viral Env proteins. They aim to prevent the interactions between the virus and cellular cofactors by binding either viral envelope proteins or host proteins [87,88]. Table 1A shows the best HIV-1 inhibitory peptide based on the prediction of antigenicity method for inhibiting Env proteins. The HIV-1 envelope protein gp41 fragment peptide (residues 568–588) is derived from the *N*-heptad region of gp41 Env ectodomain [89]. It specifically binds the phospholipid membrane thereby inhibiting the viral-cell fusion process. Microcalorimetric titrations revealed that a 22-resides tyrosine-sulfated peptide (S22 peptide) derived from the N-terminus of CCR5 showed a strong interaction with the gp120-CD4 complex with K_d = 2.2 μM (Table 1B). The process is both entropically and enthalpically favorable. No binding was observed between the gp120-CD4 complex and an identical peptide lacking the sulfated tyrosine residues [90,91].

HIV-1 gp120 & CXCR4 interactions

One of the functions of gp120 is tethering of the virus to the cellular co-receptor CXCR4. CXCR4 binds the bridging sheet and V3 loop of gp120 [92,93]. The binding between CXCR4 and gp120 involves a conformational rearrangement of gp120. The soluble synthetic peptide, CX4-M1, functionally mimics the HIV-1 co-receptor CXCR4 [85,94]. The interaction interface between gp120 and its cellular co-receptor partner CXCR4 is between the V3 loop of gp120 and the extracellular loops (ECLs) of CXCR4. The CX4-M1 peptides are derived from the ECL region of CXCR4 from different HIV1 strains and binding was determined via direct ELISA [95]. The binding affinities between the peptides and the protein were measured by surface plasmon resonance (SPR) (Table 1C). To confirm specific binding, CX4-M1 was competed with a specific antibody, mAb447–52D, that recognizes the V3 loop of gp120. A peptide binding assay with CX4-M1 and V3 loop peptides

Key terms

gp120: HIV-1 envelope glycoprotein encoded by the HIV *env* gene. The virus entry into cells is anchored by gp120. The process is mediated by the binding of gp120, which is exposed on the surface of the HIV envelope to specific cell surface receptors such as CD4, heparan sulfate proteoglycan. The change in the conformation of gp120 triggers fusion between the viral and host cell membranes.

Integrase: Viral enzyme encoded by HIV-1, which catalyzes the integration of the viral cDNA into the host cell genome. IN performs two enzymatic activities: 3′-end processing in the cytoplasm and strand transfer in the nucleus.

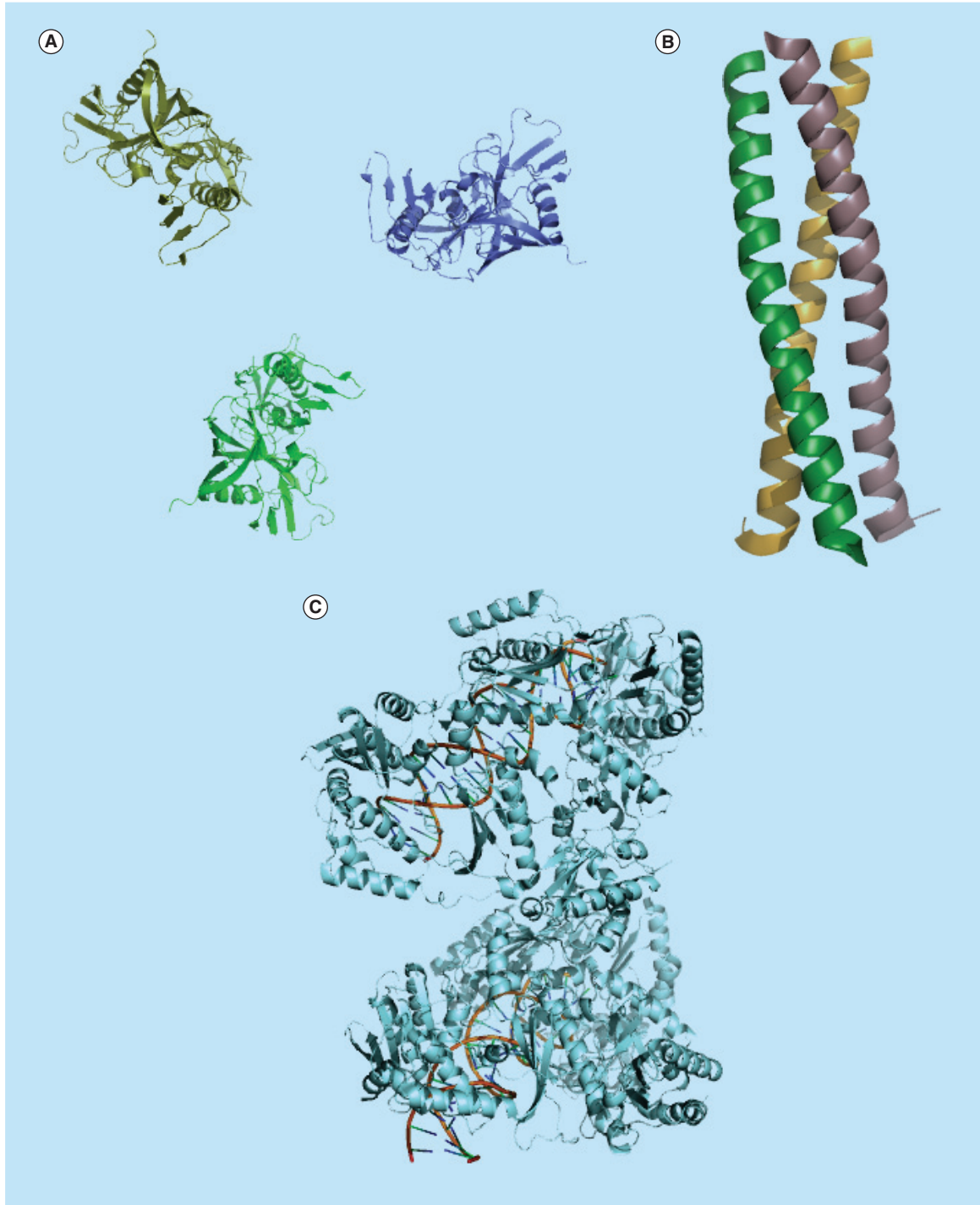

Figure 3. X-ray crystal structures of some HIV-1 proteins. (A) HIV-1 gp120 trimer (PDB: 3DNL) [279]; **(B)** NTD of HIV-1 gp41 trimer (PDB: 2ZFC) [280]; and **(C)** HIV-1 RT with DNA (PDB: 3V4I) [281].

confirmed that the V3 loop is the crucial part of the co-receptor binding site of gp120.

The viral enzyme reverse transcriptase

HIV-1 reverse transcriptase (RT) produces a viral cDNA based on the viral RNA (PDB: 3V4I, Figure 3C). The DNA is later integrated into the host cell genome [96]. RT is a heterodimeric protein with two asymmetric chains termed p51 and p66 [97]. HIV-1 RT has three main activities: RNA-directed DNA polymerization, DNA-directed RNA polymerization and exonuclease via degradation of RNA [98]. RT is the target of numerous small molecule antiretroviral drugs used in the clinic [99]. AZT is a Nucleoside analog RT Inhibitor

Table 1. Peptides that inhibit viral entry.

PPI	Name	Sequence	Ref.
(A) Env and CD4 interaction	HIP962	EINCTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCNIS	[87,88]
	HIP963	CTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHC	[87,88]
	HIP964	ESVKITCARPYQNTRQRTPIGLGQSLYTTRSRSIIGQAHCNIS	[87,88]
	HIP965	EINCTRPNNNTRKSIHIGPGRAFTTGEIIGDIRQAHCNIS	[87,88]
	HIP966	ESVVINCTRPNNNTRRRLSIGPGRAFYARRNIIGDIRQAHCNIS	[87,88]
	HIP953	WMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFRS	[87,88]
	HIP958	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF	[87,88]
	HIP959	YTSLIHSLIEESQNQQEKNEQELLELDKWASLANAA	[87,88]
	HIP1016	WMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFR-SLTVWGIKQLQARILAVERYLK	[87,88]
(B) gp120 and CCR5 interaction	S22	MDYQVSSPIY(SO_3^-)DINY(SO_3^-)YTSEPSQK	[90,91]
(C) gp120 and CXCR4 interaction	(ECL1)CX4-M1	$(ECL1)^{97}$DAVANWYFGNFLCK^{110}	[85,94]
	(ECL2)CX4-M1	$(ECL2)^{182}$DRYICDRFYPNDLWV^{196}	[85,94]
	(ECL3)CX4-M1	(ECL3) ^{262}DSFILLEIIKQGSEFENTVHK^{282}	[85,94]
	V3 loop of HIV-1HxBc2	^{296}CTRPNNNTRKRIRIQRGPGRAFVTIGKIGNMRQAHC^{331}	[92,95]

PPI: Protein–protein interaction.

(NRTI) that acts as a chain terminator of growing DNA strand and was approved as an anti-HIV drug in 1987. In 1996, Nevirapine was approved as the first non-nucleoside RT inhibitor (NNRTI) that inhibits the RT polymerization activity.

The HIV-1 RT & A3G interaction

During reverse transcription, the human cytidine deaminase APOBEC3G (A3G) eliminates HIV-1 infection by inducing deamination of the cytosine residues to uracil in the negative viral DNA strand [100–108]. Using a cell-based co-immunoprecipitation (coIP) assay, the direct interaction of A3G with RT was detected both in transfected cells and in the produced viruses. No other viral components are needed for this interaction. Deletion analysis with a series of T7-tagged RT-deletion mutants (T7-$RT^{1–243}$, T7-$RT^{1–323}$ and T7-$RT^{1–439}$) determined that the RT-binding domain is located at the N-terminal region of $A3G^{65–132}$ [101,109,110]. The polypeptide $A3G^{65–132}$ inhibited the interaction between A3G and the viral RT (PDB: 3VOW, Figure 4A, Table 2A) [101]. The RT-binding polypeptide inhibited the anti-HIV effect of A3G on RT. Competitive coIP in cells co-expressing both RT and A3G using several A3G derived polypeptides showed that $A3G^{65–132}$ significantly disrupted the A3G-RT binding.

Interactions of the HIV-1 integrase

HIV-1 integrase (IN) plays one of the key roles in the viral replication cycle by integrating the reverse transcribed viral cDNA into the host genome (Figure 1) [111–115]. It has three functional domains responsible for integration process: the N-terminal domain (NTD), the catalytic core domain (CCD) and the C-terminal domain (CTD) [116,117]. IN has two enzymatic activities: first, 3′-end processing in the cytoplasm [111,118] in which two IN dimers [119] bind the long terminal repeats (LTR) of the viral DNA and remove a pGT dinucleotide from the 3′-end of each strand. After nuclear transport [120,121], the strand transfer reaction is carried out by an IN tetramer [122–124] resulting in integration of the viral DNA into the host genome. Finally, the single-stranded gaps

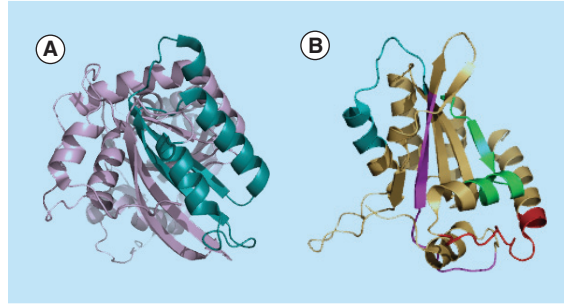

Figure 4. Peptides derived from human APOBEC3G.
(A) Crystal structure of human A3G. The RT-binding A3G 65–132 peptide is shown in cyan (PDB:3VOW) [282], **(B)** Crystal structure of APOBEC3G catalytic core domain (CCD); the Vif-interactions regions are: A3G 211–225 (magenta), A3G 263–278 (cyan), A3G 331–345 (green) and A3G 353–367 (red) (PDB:3IR2) [283].
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Table 2. Peptides that inhibit the viral enzymes reverse transcriptase and integrase.

PPI	Name	Sequence	Ref.
(A) RT & A3G interaction	A3G 65–132	HPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPC-TKCTRDMATFLAEDPKVTLTIFVARLYYFWDPDYQ	[96,101,109,110]
(B) IN dimerization interface	IN 93–107(INH1)	TGQETAYFLLKLAGK	[130–139]
	IN 95–109(α1)	QETAYFLLKLAGRWP	[130–139]
	IN97–102(NL6–5)	TAYFLL	[130–139]
	IN 97–108(NL6)	TAYFLLKLAGRW	[130–139]
	RDNL6	wrgalkllfyat†	[130–139]
	IN 129–139(NL9)	ACWWAGIKQEF	[130–139]
	RDNL9	feqkigawwca†	[130–139]
	IN 129–139W131A (NL9-W3A)	ACAWAGIKQEF	[130–139]
	IN167–187(INH5)	DQAEHLKTAVQMAVFIHNYKA	[130–139]
	IN 171–187(α5)	HLKTAVQMAVFIHNFKR	[130–139]
	IN 196–210(α6)	AGERIVDIIATDIQ	[130–139]
	IN 196–206(α6S)	AGERIVDIIA	[130–139]
	IN 151–175(K156 E G163A D167A)	VESMNEELKKIIAQVRAQAEHLKTAY	[130–139]
(B) Cellular partner proteins	LEDGF/p75 354–378	WIHAEIKNSLKIDNLDVNRCIEALD	[69,153–155]
	LEDGF/p75 355–377	IHAEIKNSLKIDNLDVNRCIEAL	[69,153–155]
	LEDGF/p75 361–370	NSLKIDNLDV	[69,153–156]
	LEDGF/p75 362–369	SLKIDNLD	[69,153–155]
	LEDGF/p75 402–413	KKIRRFVSQVIM	[69,153–156]
(C) Phage display	CP64	c(CVSGHPLWCGGGK)	[158]
	CP65	c(CILGHSDWCGGGK)	[158]

†Inverted sequence with D-amino acids.
PPI: Protein–protein interaction.

between the viral DNA and target DNA are repaired by the host DNA repair machinery [125–127]. The equilibrium between dimeric and tetrameric IN is of extreme importance in the integration process, making it an attractive target for drug design [69]. In 2007, Raltegravir was the first IN inhibitor approved for clinical use. Another IN inhibitor, Elvitegravir, was approved for clinical use in 2012 [128,129]. Both inhibitors block IN by binding directly to the IN-DNA complex formed during the integration of the viral DNA into the host cell genome [128,129].

Peptides derived from the dimerization interface of IN

The dimerization interface of IN is an excellent starting point for peptides that would inhibit dimer formation [130–132]. Several peptides have been designed (PDB: 3L3U, Figure 5A, Table 2B) but due to relatively low binding affinity to IN, they did not succeed in disrupting the dimeric IN and hence were not efficient inhibitors. Some of the peptides (IN $^{95–109}$, IN $^{97–108}$, IN $^{171–187}$ and IN $^{196–210}$) showed very mild IC_{50} for both 3′-processing and strand transfer *in vitro*. IN $^{147–175}$, which is derived from IN, inhibited IN at 600 μM concentration by partly blocking the active site. The peptide inhibited the catalytic activity of IN by binding it through a protein-peptide coiled-coil structure [130,133–135]. Two peptides derived from the α1 and α5 helices of the CCD, (INH1 and INH5) specifically bound to the dimerization interface of the CCD of IN [136]. The IC_{50} for 3′- processing by INH1 was 250 μM and by INH5 was 11 μM. By inhibiting the 3′-endonuclase activity of IN with IC_{50} values in the low micromolar range, three peptides (α1, α5, α6) also inhibited the IN dimerization [137]. The truncated peptide (NL6–5) and retro-inverso-peptides (RDNL6, RDNL9) retained the inhibitory activity by disrupting the IN dimer and tetramer formation [138,139] All the peptides were derived from the CCD of IN, which is the only domain that mediates IN dimerization (Figure 5A) [138,139].

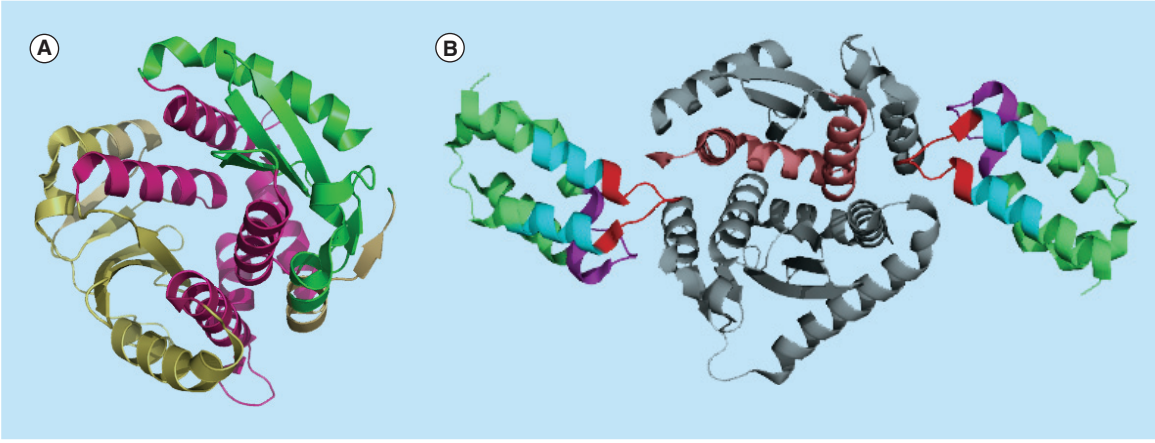

Figure 5. Peptides derived from domains of HIV-1 integrase. (A) Crystal structure of HIV-1 IN catalytic core domain (CCD, beige and green) dimer illustrates the important regions of the IN-dimerization interface from where peptides were derived: IN 93–107 (INH1, α1, NL6) (magenta), IN 171–208 (α5, α6, α6s) (magenta) (PDB:3L3U) [284]. **(B)** Crystal structure of dimeric IN CCD and LEDGF/p75 IBD (gray) showing interacting regions: LEDGF/p75 354–378 (cyan), LEDGF/p75 361–370 (red), LEDGF/p75 402–411 (magenta) [152].
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Peptides derived from cellular proteins that bind IN

Targeting host proteins is risky since it may affect cell viability and produce undesired toxicity. Therefore, the best strategy is to study the interactions between IN and host proteins by finding peptides derived from the IN-binding region of the cellular proteins. These peptides will potentially bind the viral protein and inhibit the interaction with less potential for undesired side effects.

The IN-LEDGF/p75 interaction

In addition to binding the viral DNA [140–142], IN interacts strongly with the cellular transcriptional co-factor LEDGF/p75 [143]. LEDGF/p75 tethers the IN-DNA complex to the host chromatin, where the final integration steps take place [140,144–151]. The IN-LEDGF/p75 is a crucial interaction in the replication cycle, making it as a fundamental target for anti-HIV drug design.

The structure of IN CCD in complex with the LEDGF/p75 IN binding domain (IBD) shows a pseudo two-fold symmetry where an IN CCD dimer binds two LEDGF/p75 IBD at either side (PDB: 2BJ4, Figure 5B) [152]. The IBD interacts with IN via two loops. Our lab rationally designed peptides based on these loops and shorter variants (LEDGF/p75$^{361–370}$, LEDGF/p75$^{402–411}$) [69]. All of these bound IN with micromolar affinities and inhibited the *in vitro* enzymatic activities both in presence and absence of LEDGF/p75 (Table 2B). In addition, these peptides inhibited the integration of viral cDNA and HIV-1 replication in infected cells, by shifting the IN oligomerization equilibrium toward a stable tetramer in the cytosol. Further studies including homology modeling, alanine scan and NMR analysis revealed that all the residues of LEDGF/p75$^{361–370}$ and LEDGF/p75$^{402–413}$ are important for optimal binding and inhibition of IN (Figure 5B) [69,153–156]. A library of cyclic peptides (CPs) derived from LEDGF/p75$^{361–370}$ was screened for *in vitro* IN binding and inhibition [155]. One of these peptides, c(MZ4–1) was a potent and stable inhibitor of IN *in vitro*. NMR and docking studies revealed that c(MZ4–1) possessed a conformation almost identical to the parent IN-binding loop from the IBD of LEDGF/p75. An AlphaScreen assay with these peptides also accounted for IN-LEDGF/p75 interaction [157].

A random peptide phage display strategy was adopted to identify a linear peptide, LEDGF/p75$^{325–530}$, that bound specifically to the IBD of LEDGF/p75. Based on this, small CPs (CP64 and CP65) inhibitors of the IN–LEDGF/p75 interaction (IC_{50} for CP64 is 35.88 μM and IC_{50} for CP65 is 59.89 μM) were developed. These peptides inhibited HIV replication in different cell lines without displaying toxicity (Table 2C) [158]. Saturation transfer difference (STD)

Key terms

Alanine scan: Screening technique for determining of the contribution of specific residues to the function and interactions of a protein. Each residue is sequentially replaced by alanine and the function/interaction of the mutant peptide/protein is compared with the parent peptide. Loss of function/interaction means that the original residue was important for the binding/activity. Alanine is used since it is the simplest chiral residue and thus mimic a loss of a side chain without a conformational change or an introduction of a new function.

Cyclic peptides: Cyclization improves the pharmacological properties of peptides. They are conformationally rigid, resistant to protease degradation and in many cases have improved affinity and specificity as well as cell penetration properties.

NMR confirmed that the residues in CP64 strongly bound to LEDGF/p75 and not to HIV-1 IN.

Stapled peptides that target IN-mediated integration & the IN-LEDGF/p75 interaction

Two-domain crystal structures of IN show that the two monomers of dimeric IN are tethered via strong helix-helix (α1:α5′ and α5:α1′) interactions [159,160]. Using the 'sequence-walking' strategy, two potent IN inhibitors termed NL6 and NL9 [161] were revealed. NL6 has an α-helical structure and is part of the α1 helical domain. A series of hydrocarbon stapled peptides derived from NL6 (NLH2-NLH16, NLX1, NLX2) enhanced interfacial interaction and cell-permeability compared with the parent NL6 peptide through stabilization of the α1 domain [162] as confirmed by CD studies. Increasing the α-helical content also increased the IN inhibitory activity at the 3′-processing step, inhibition of the strand transfer reaction and the IN-LEDGF/p75 interaction, cytoprotective activity (EC_{50}), cell death activity (CC_{50}) and therapeutic index (ratio of CC_{50} to EC_{50}). Combining pairs of α-helical peptides effectively inhibited IN catalytic activities. The most active pair was unstapled NLH5 and stapled NLH6 (IC_{50} values of 9 ±1 μM for 3'-processing and 6 ±1 μM for strand transfer [155]). The pairs were designed with a covalent hydrocarbon staple spanning i and i + 4 residues that did not show inhibition in the alanine scan [163,164]. Most of the stapled peptide pairs inhibit the IN-LEDGF/p75 interaction. Six peptides (NLH2, NLH3, NLH5, NLH6, NLH15, NLH16) inhibited HIV-1 replication in MT-4 cells. Fluorescein-tagged NLH6 (termed NLX-1) penetrated cells and inhibited the target IN. MT-4 cells showed significant cellular uptake of NLX-1, which was localized mainly to the cytoplasm with minimum distribution to the nucleus. The cell-permeability and enhanced potency of the stapled peptides makes them lead IN inhibitors.

Mapping HIV-1 Gag & host cellular proteins interactions

HIV-1 Gag is a viral polyprotein expressed during the late phase of the replication cycle. Cleavage of Gag by the viral PR produces the structural proteins of the mature virion: the matrix (MA), capsid (CA) and nucleocapsid (NC) proteins. MA is the N-terminus of Gag, followed by a CTD termed p6 and two spacer regions that separate CA from NC and NC from p6. The *Gag* products take part in viral self-assembly and release of virions from the infected cells, thus making it critical for viral particle morphogenesis and replication within the living cells [165–174]. The maturation inhibitor Bevirimat is currently in clinical trials. It targets the Gag protein to prevent PR-mediated cleavage at specific Gag sites and also binds CA to prevent core formation [175].

The interaction between Gag p6 & human Tsg101

HIV-1 p6 is a *Gag* cleavage product that plays an important role in regulating capsid processing, facilitating virus budding and incorporation of the viral accessory protein R (Vpr) into virions. These processes require interactions between the human *tumor susceptibility gene 101 (Tsg101)* protein and the CTD of p6 [176]. Tsg101 is a part of the endosomal sorting complex required for transport-I (ESCRT-I), which assists the ubiquitylation of Gag and facilitates viral assembly and budding [177–180]. Successful HIV-1 budding requires an interaction between the tetrapeptide PTAP, derived from residues 3–6 in p6, with the ubiquitin E2 variant (UEV) domain of Tsg101 (PDB: 3OBU, 3OBX, Figure 6). Blocking this interaction inhibits virion formation [181–183].

Peptides containing the PTAP motif are potential inhibitors of the interaction between Gag p6 and Tsg101. A peptide derived from $p6^{5-13}$ bound Tsg101 (Table 3A) [184]. NMR studies showed that the peptide bound to Tsg101 in a groove that interacts with the PTAP residues with K_d = 3 μM [177,183]. The structure showed that binding of E2 ubiquitin-conjugating enzymes to UEV domain of Tsg101 was hampered upon PTAP binding (Figure 6). Structure activity relationship (SAR) studies of this peptide, which included conversion to P^3 polycyclic oxime derivatives in the PTAP domain, improved binding to Tsg101 by 15- to 20-fold [184–186].

To develop effective competitive inhibitors, a technique for genetically selecting CPs that inhibit specifically the p6 Gag-Tsg101 interaction was used [187]. This technique, called SICLOPPS (split intein-mediated circular ligation of peptides and proteins), allowed identification of new CPs that specifically blocked the p6 Gag-Tsg101 interaction and consequently inhibited HIV replication. After several rounds of screening, the selected CPs had no resemblance to the original sequence of the interacting sites in either p6 Gag or Tsg101. Of these, CP11 inhibited the formation of virus-like particles (VLP) in cultured cells with IC_{50} of 7μM and showed better stability compared with the linear $p6^{5-13}$.

The p6 Gag & cyclophilin interaction

Another partner of p6 Gag is the cellular cyclophilin A (CypA) protein, which acts as a prolyl isomerase (PPIases). CypA also acts as a molecular chaperone and assists protein folding, assembly and transportation processes. CypA is incorporated into newly budding particles of HIV-1 and thus can be considered as

a key target in future antiretroviral therapy [188]. p6 contains a relatively high content of proline residues, at positions 5, 7, 10, 11, 24, 30, 37 and 49. Proline cis/trans isomerism was observed for all these proline residues and more than 40% of all p6 Gag proteins show at least one proline in *cis*-orientation. 2D proton NMR of full length p6 Gag or p6 Gag-derived peptides with CypA revealed that it interacts with all proline residues of p6 Gag through a prolyl-peptidyl *cis-trans* isomerase (PPIase).

The modulation of HIV-1 p6 function by CypA was explored by the synthesis of full length p6 and several p6 fragments ($p6^{1-14}$, $p6^{1-21}$, $p6^{23-32}$, $p6^{32-42}$, $p6^{43-52}$ and $p6^{23-52}$) and by using NMR and Surface Plasmon Resonance (SPR) (Table 3A) [188]. Catalytic amount of CypA is sufficient to interact with all the proline residues of $p6^{1-52}$ (molar ratio 1: 283; Table 3A) and hence PPIases activity *in vitro*. However, there was low affinity binding of CypA to p6 fragments compared with binding to full-length p6. Another important inhibitor of CypA is cyclosporine A which was found to suppress both the production and the release of new virions [189,190].

Interactions of HIV-1 Tat

The HIV-1 trans-activator of transcription (Tat) protein is a small viral auxiliary protein that contains 101 or 86 residues, depending on the HIV strain [191,192]. The Tat protein can be divided into six regions: an acidic region (residues 2–11), a cysteine-rich domain (residues 22–37), the hydrophobic core (residues 38–46), a basic region (residues 47–57), the glutamine-rich domain (residues 58–72) and the RGD motif (residues 72–86) [193,194]. The basic region of Tat binds to the negatively charged mRNA in the Tat-activation region (TAR) [195,196]. The binding of Tat to TAR promotes a prolongation of the transcription due to conformational change of the TAR during binding of host cell kinases that phosphorylate the RNA polymerase II complex. The six Arginine residues in Tat^{47-57} are crucial for Tat-TAR recognition [197–200].

The peptide Tat^{47-57} specifically disrupted the TAR-RNA recognition by blocking the production of viral transcript and also interrupted the formation of two cellular cofactors, cyclin T1 and its cognate kinase CDK9, responsible for transcriptional elongation from the viral long terminal repeat (LTR) [197,198,201–205]. Increasing the number of Arginine residues on the hairpin scaffold of Tat-derived peptides dramatically decreased the specificity for binding the TAR-RNA. In contrast, fewer Arginine residues in a Tat-derived peptide of the same length increased the TAR-RNA binding specificity. Arginine-rich Tat peptides have a higher resistance to degradation by proteases (Table 3B) [197,198,201–205].

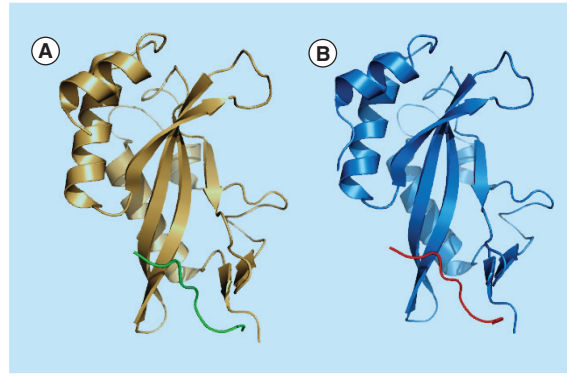

Figure 6. Peptides derived from Tsg101 UEV. (A) Crystal structure of the Tsg101 UEV domain (light brown) in complex with a HIV-1 Gag P7A mutant p6 5–13 PSAP peptide (green) (PDB:30BX) [285]. **(B)** Crystal structure of the Tsg101 UEV domain (blue) in complex with a HIV-1 PTAP (5–13) peptide (red) (PDB:30BU) [285]. For color images please see online www.future-science.com/doi/full/10.4155/FMC.15.46

The Tat-p53 interaction

The cellular tumor suppressor p53 is a homotetrameric transcription factor that induces cell cycle arrest or apoptosis upon oncogenic stress [206]. NMR and x-ray crystallography revealed that the p53 tetramerization domain (p53 Tet; residues 326–355) has a dimer of dimers structure [207,208]. Depending on its concentration, p53 Tet exists in equilibrium among different oligomeric forms [209,210]. p53 inhibits Tat-mediated LTR transcription [211]. The viral Tat binds p53 Tet as was shown by yeast two-hybrid system [209,212]. The CTD of p53 (residues 341–355) interacts specifically with the Tat residues 49–57 in the arginine-rich motif (ARM) [212]. Tat 73–86 can bind p53 with the assistance of cellular proteins such as NF-κB and CBP/p300, as observed by *in vivo* experiments [191,213,214].

To quantitatively understand the molecular basis of Tat-p53 interaction during HIV-1 replication cycle, our laboratory synthesized Tat-derived peptides (Tat^{1-35} and Tat^{47-57}) and studied their binding to the p53 tetramerization domain (Table 3C) [214]. The binding between p53 Tet and Tat^{47-57} is purely cooperative and is temperature-dependent. NMR studies revealed that E343 and E349 from p53 Tet are the major Tat^{47-57} binding residues. The binding mechanism involves electrostatic interactions [214].

The interaction of HIV-Vif with the host cellular protein APOBEC3G

The HIV-1 virion infectivity factor (Vif) is required for the virus replication [215,216]. Vif counteracts A3G

by targeting it for proteosomal degradation and by direct inhibition of its enzymatic activity (PDB: 3IR2, Figure 4B) [217]. Both activities involve a direct interaction between Vif and A3G and thus their inhibition may rescue the antiviral activity of A3G and inhibit HIV-1 propagation [218–220]. Vif binding to

Table 3. Peptides derived from interactions between viral (Gag, Tat, Vif and Vpr) and host proteins.

PPI	Name	Sequence	Ref.
(A) Interaction between MA and TCR			
Gag p6 and Tsg101 interaction	p6 5–13 PSAP peptides	PEPTAPPEE	[177,183–186]
p6 Gag and CypA interaction	p6 1–52	LQSRP5EPTAPPEESFRFGEETTTPSQKQEPIDKELY-PLASLRSLFGSDPSSQ	[188–190]
p6-UEV interaction	Pep#6	TNWYGSG-W	[184–186]
	Pep#8	VLRVHSG-W	[184–186]
	Pep#11	IYWNVSG-W	[184–186]
	Pep#16	TLLVYSG-W	[184–186]
	Pep#112	DGPRGPSTSG-W	[184–186]
	Pep#119	GCPFPPSYSG-W	[184–186]
	Pep#120	PGPVTPGFSG-W	[184–186]
	Pep#122	ARPNRPCRSG-W	[184–186]
	Pep#126	LVPWMPRPSG-W	[184–186]
	Pep#127	PGPCSPVGSG-W	[184–186]
p6 binding	p6 1–52	LQSRP5EPTAPPEESFRFGEETTTPSQKQEPIDKELY-PLASLRSLFGSDPSSQ	[188–190]
	p6 1–21	LQSRPEPTAPPEESFRFGEET	[188–190]
	p6 1–14	LQSRPEPTAPP11EES	[188–190]
	p6 23–52	TPSQKQEPIDKELYPLASLRSLFGSDPSSQ	[188–190]
	p6 23–32	TPSQKQEPID	[188–190]
	p6 32–42	DKELYPLASLR	[188–190]
(B) Interactions of Tat with host proteins			
Tat derived peptides	Tat 47–57	YGRKKRRQRRR	[197,198,200–205]
	AghTat†	YG-Agh-KK-Agh-Agh-Agh-Q-Agh-Agh-Agh	
	AgbTat†	YG-Agb-KK-Agb-Agb-Agb-Q-Agb-Agb-Agb	
(C) The Tat-p53 interaction			
Tat derived peptides	Tat 1–35	MEPVDPNLEPWKHPGSQPTTACSNCYCKVCCWHCQ	[212–214]
	Tat 47–57	YGRKKRRQRRR	[212–214]
	Tat 30–49	CCWHCQLCFLKKGLGISYGK	[212–214]
	Tat 56–76	RGPPQGSKDHQTLIPKQPLPQW	[212–214]
	Tat 65–80	HQVSLSKQPTSQSRGD	[212–214]
	Tat 73–86	PTSQSRGDPTGPKE	[212–214]
P53 derived peptides	p53 326–355	EYFTLQIRGRERFEMFRELNEALELKDAQA	[211–214]
	p53 326–355 R342A	EYFTLQIRGRERFEMFAELNEALELKDAQA	[211–214]
	p53 326–355 L344P	EYFTLQIRGRERFEMFREPNEALELKDAQA	[211–214]
	p53 326–355 L344A	EYFTLQIRGRERFEMFREANEALELKDAQA	[211–214]
	p53 326–355 E346A	EYFTLQIRGRERFEMFRELNAALELKDAQA	[211–214]
	p53 340–351	MFRELNEALELK	[211–214]

†AghTat is *(S)*-2-Amino-6-guanidinohexanoic acid and AgbTat is *(S)*-2-Amino-4-guanidinobutyric acid. These are non natural amino acids.
PPI: Protein–protein interaction.

Table 3. Peptides derived from interactions between viral (Gag, Tat, Vif and Vpr) and host proteins (cont.).

PPI	Name	Sequence	Ref.
(D) Interactions between Vif and host proteins			
Vif and A3G interaction	Vif 14–17	DRMR	[215–231]
	Vif 40–44	YRHHY	[215–231]
	A3G 31–45	NTVWLCYEVKTKGPS	[215–231]
	A3G 98–112	TFLAEDPKVTLTIFV	[215–231]
	A3G 143–157	DGPRATMKIMNYDEF	[215–231]
	A3G 166–180	YSQRELFEPWNNLPK	[215–231]
	A3G 211–225	WVRGRHETYLCYEVE	[215–231]
	A3G 263–277	LDVIPFWKLDLDQDY	[215–231]
	A3G 331–345	AGAKISIMTYSEFKH	[215–231]
	A3G 353–367	HQGCPFQPWDGLDEH	[215–231]
Vif and Cullin5 interaction	$Hx_5Cx_{17-18}Cx_{3-5}H$ (108–139)	LAEDPKVTLTIFVARLYYFWDPDYQEALRSLC	[215–222]
(E) Interactions of Vpr			
Vpr and CypA interaction	Vpr 69–78	FIHFRIGCRH	[236,240,241]
	Vpr 75–84	GCRHSRIGVT	[236,240,241]
	Vpr 75–90	GCRHSRIGVTRQRRAR	[236,240,241]
	Vpr 75–90 (R80A)	GCRHSAIGVTRQRRAR	[236,240,241]
	Vpr 75–90 (R76Q V83I T84I)	GCQHSRIGIIRQRRAR	[236,240,241]
	Vpr 75–90 (R76Q V83I R80A T84I)	GCQHSAIGIIRQRRAR	[236,240,241]
	Vpr 81–90	IGVTRQRRAR	[236,240,241]
	Vpr 87–96	RRARNGASRS	[236,240,241]
	C45D18	DTWPGVEALIRILQQLLFIHFRIGCQHC	[240–242]
	Vpr H1	TLELLEELKNEAVRHFPR	[242]
	Vpx H1	EAFDWLDRTVEAINREAVNH	[242]

†AghTat is *(S)*-2-Amino-6-guanidinohexanoic acid and AgbTat is *(S)*-2-Amino-4-guanidinobutyric acid. These are non natural amino acids.
PPI: Protein–protein interaction.

A3G results in polyubquitination and degradation of A3G by forming a E3 ubiquitin ligase complex consisting of ElonginB and C, Cullin5 and RING finger protein 1 [221,222]. The mutation K128D in A3G abrogated the interaction with Vif [223,224].

The peptides Vif^{14-17}, Vif^{22-26}, Vif^{40-44} and Vif^{69-72} inhibited the A3G-Vif interactions (Table 3D) [225]. Deletion mutagenesis of A3G also showed that Vif^{54-124} and $Vif^{105-156}$ peptides are critical for the interaction [226]. An *in vitro* Vif-A3G binding assay between GST-tagged Vif and His-tagged A3G and a Fluorescence Resonance Energy Transfer (FRET) assay between GST-Vif and biotinylated $A3G^{110-148}$ confirmed their interactions (Table 3D) [227].

Mapping the Vif-A3G interaction by using peptide arrays resulted in defining the precise binding interface (Table 3D) [226–229]. A3G bound nine Vif-derived peptides from three distinct regions in Vif: residues 8–45 from the NTD, residues 154–192 from the CTD containing the conserved motif ^{161}PPLP^{164} and a central region between residues 83–99 [230,231]. The A3G-derived peptides $A3G^{143-157}$, $A3G^{211-235}$ and $A3G^{263-277}$ bound full-length Vif and Vif-CTD. The peptide array experiment revealed that peptides $A3G^{31-52}$, $A3G^{166-180}$, $A3G^{211-225}$, $A3G^{263-277}$ and $A3G^{331-367}$ also bound to Vif.

The interactions of Vpr with host cellular proteins

The viral protein R (Vpr) is the only virion associated regulatory protein and is not a component of the virus polyprotein precursors. It assists the nuclear import of the preintegration complex (PIC) in nondividing host cells [232]. Vpr is crucial for effective HIV-1 infection of target $CD4^+$ T cells and macrophages [233–235]. Vpr interacts

with numerous cellular proteins in order to perform its nuclear import and G_2 cell cycle arrest functions.

Interaction between Vpr & CypA

One of the key Vpr interacting protein is cyclophilin A (CypA) [236]. *Cis-trans* prolyl isomerization of the highly conserved proline residues in Vpr, such as Pro5, Pro10, Pro14 and Pro35, is catalyzed by CypA. SPR experiments showed that the heptapeptide CypA 32–38(^{32}RHFPRIW^{38}) mediates the binding between CypA and the N-terminal region of Vpr [237]. P35A mutation disrupted the Vpr-CypA interaction. In the mutant peptide Vpr$^{75–90}$ (R80A), the replacement in the C-terminal region of Vpr hampered the co-IP of Vpr with CypA [238,239].

The above observations together with the significant amount of CypA in the virion [240] led to the design of Vpr-based peptides to study the Vpr - CypA interaction (Table 3E) [241,242]. SPR and ITC studies revealed the strong binding affinities of C-terminal Vpr$^{75–90}$ (K_d = 0.28 μM) and N-terminal Vpr$^{30–40}$ (K_d = 1 μM) peptides. Other C-terminal Vpr peptides such as Vpr$^{69–78}$, Vpr$^{75–84}$, Vpr$^{81–90}$ and Vpr$^{87–96}$ interacted weakly with CypA. The weakest binding response was observed for mutant peptides such as Vpr$^{75–90}$ R80A (K_d = 7.5 μM) and Vpr$^{75–90}$ R76Q, V83I, R80A, T841 (K_d = 4.7 μM) as compared with the wild type peptide. NMR studies revealed that the mutations did not influence the secondary structure of the C-terminal binding domain of Vpr.

The interaction between Vpr & the WXXF motif of host cell proteins

The conserved WXXF motif of uracil-DNA-glycosylase mediates the intracellular binding of Vpr with uracil DNA glycosylase. Many WXXF-including peptides have domain-specific interactions with Vpr. The fusion of the WXXF dimer to the *chloramphenicol acetyl transferase (CAT)* gene demonstrated that the WXXF dimer-CAT construct induced CAT activity inside the virions through Vpr-dependent docking [243].

Phage display peptide screening predicted that more than 90% peptides having consensus motif WXXF efficiently binds Vpr protein [243,244]. Similarly, Vpr binding peptides from GST-Vpr panning also revealed a WXXF consensus motif [245]. Nine peptides were found to bind Vpr (Table 3E) [243].

The Vpr interaction with cell-surface $\alpha_V\beta_3$ in endothelial cells

Vpr targets mitochondrial membranes to trigger apoptosis and cell death. The internalization of cyclic RGD in endothelial cells for cellular apoptosis is mediated by the cell surface receptors $\alpha_V\beta_3$ integrins. The Vpr induced apoptotic cell death involves the interactions of Vpr with the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocator (ANT) [246,247]. The VDAC and ANT interaction is based on permeability transition pore (PTP) as a result of dynamic multiprotein complex formation at inner and outer mitochondrial membrane contact sites.

A TEAM-VP (Targeted to Endothelial Apoptogenic Mitochondrio-active Vpr-derived Peptide) peptide was designed based on $\alpha_V\beta_3$ binding and endothelial apoptogenic sequences derived from the mitochondria active portion of Vpr. TEAM-VP peptide is combined with a tumor blood vessel RGD-like 'homing' motif and a mitochondrial membranes permealization (MMP)-inducing sequence. It is composed of the cysteine mediated CP sequence GGCRGDMFGC and a Vpr$^{67–82}$ sequence derivative (Table 3E). The cyclic core 'GGCRGDMFGC' of TEAM-VP specifically bound to VDAC and ANT and internalized into $\alpha_V\beta_3$-expessing cells through its cyclic-RGD motif. [248].

PART II: interactions between viral proteins

The Env–MA interaction

The matrix protein p17 (MA) originates from the Gag precursor protein, p55^{gag} [249]. It is N-terminally myristylated and binds to the viral inner membrane or the inner leaflet of the plasma membrane (PM) of the infected cells [250]. MA is involved in nuclear import of the viral DNA [251]. A specific interaction between p17 and Env was revealed by the co-expression of Env proteins that influenced the assembly of Gag particles. The membrane-proximal amino terminus of p17 in the Gag precursor closely associates with the membrane in the mature particle indicating that p17 participates in the specific Env incorporation into the viral particles [252].

Several p17 peptides (p17$^{1-12}$, p17$^{12–29}$, p17$^{30–52}$, p17$^{53–87}$, p17$^{87–115}$ and p17$^{115–132}$), derived from all the six parts of p17, were synthesized (Table 4A) [253,254]. The antigenic epitopes was examined for anti-HIV-1 p17 antibody (p17 Ab) in the serum of an HIV1 carrier. p17$^{1-12}$, p17$^{12–29}$, p17$^{30–52}$ were highly recognized in the serum and led to inhibition of virus multiplication as tested using ELISA. The purified antibodies obtained from the patient using the p17-derivated peptide immunoaffinity columns confirmed that the reactivity of p17$^{30–52}$Ab to p17 was the highest among the antibodies.

The Gag–PR interaction

PR cleaves the Gag and Gag-Pol precursors into active viral proteins such as p1^{gag}, p2^{gag}, p6^{gag}, p7^{gag}, p17^{gag} and p24^{gag} [45,255–257]. The cleavage of the Gag precursors is necessary for maturation and HIV-1 infectivity. p2^{gag} is an inherent suicidal inhibitor of PR due to its strong *in vitro* inhibition of the proteolytic cleavage of the recombinant Gag precursor into functional structural units (p17^{gag} and p24^{gag}) [258]. After the viral maturation, p2^{gag}

inhibits PR activity in released viral particles and thus blocks the autolysis of HIV-1 virions.

PR is one of the most common anti-HIV drug targets and many FDA approved anti-HIV drugs are PR inhibitors [86,97]. The nonapeptide (AEAMSQVTN) derived from the N-terminus of $p2^{gag}$ inhibited HIV-1 PR activity *in vitro* to prevent autolysis of the virion after sequential processing and reorganization of the virion core (Table 4B) [258]. Further SAR studies with $p2^{gag}$ revealed that alanine replacements (M4A and T8A) and deletion of Asn9 from the nonamer (AEAMSQVTN) decreased the PR inhibitory properties. However, the other mutated peptides did not have inhibitory activity.

The Vif–PR interaction

Vif blocks the cleavage activity by directly interacting with PR. Vif stably blocks the premature activation of PR in cytoplasm, which is circumvented during particle assembly [259]. The NTD of Vif (residues 1–96) inhibits the PR cleavage *in vitro* and in bacteria. Both Vif and PR are present in the mature virions [260]. Vif regulates PR in the virion at the early stage of infection [260]. Several Vif-derived peptides inhibited PR-mediated cleavage of Gag *in vitro* and during viral protein expression in peripheral blood lymphocytes [261]. $Vif^{1–38}$ and Vif $^{1–65}$ and $Vif^{10–96}$ peptides were highly stable toward proteolysis. $Vif^{21–65}$ is essential for PR binding and blocking proteolysis (Table 4B). $Vif^{21–65}$ inhibited PR five times better than full-length Vif. Vif-derived peptides such as $Vif^{30–65}$ and $Vif^{78–98}$ specifically inhibited the Vif-PR interaction *in vitro* and blocked the production of viruses in HIV-1-infected cells [262,263]. $Vif^{88–98}$ inhibited PR dimerization. Two PR-derived peptides $PR^{1–9}$ and $PR^{94–99}$ abrogated Vif function as an A3G neutralizer and inhibited Vif-PR binding in a dose-dependent manner [264]. This means that $PR^{1–9}$ competed with PR for the Vif binding site.

Vpr interactions with RT & IN

RT, IN and Vpr are in close spatial proximity within the PIC, allowing them to interact with each other [265]. The interaction between RT and IN involves the single-stranded viral RNA copied into integration-competent double-stranded DNA by RT, DNA polymerase and ribonuclease H (RNaseH). Then the PIC is imported to nucleus by IN and Vpr for integration [266]. RT and IN physically interact with each other and the full-length Vpr and its isolated CTD can interfere with the IN-mediated integration activity *in vitro* [267].

A library of Vpr-derived peptides was screened for their ability to bind directly to RT and IN *in vitro* and to inhibit their enzymatic activities (Table 4B) [265–267]. Dot-blot binding assay showed that the C-terminal Vpr

Table 4. Peptides derived from interactions between viral proteins.

PPI	Name	Sequence	Ref.
(A) Interaction between Env and MA			
Env and MA	p17 l-12	MGARASVLSGGE	[253,254]
	p17 12–29	ELDKWEKIRLRPGGKKQY	[253,254]
	p17 30–52	KLKHIVWASRELERFAVNPGLLE	[253,254]
	p17 53–87	TSEGCRQILGQLQPSLQTGSEELRSLYNTIAVYC	[253,254]
	p17 87–115	CVHQRIDVKDTKEALDKIEEEQNKSKKKA	[253,254]
	p17 115–132	AAADTGNNSQVSQNY	[253,254]
	Env V3	NCTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHC	[253,254]
(B) Interactions of Protease			
Protease and Gag interaction	p2 gag pep#	AEAMSQVTNTATIM	[257,258]
	Nona p2 gag pep#	AEAMSQVTN	[257,258]
	p2 gag pep# mutant1	AEAMSQ	[257,258]
	p2 gag pep# mutant2	AEAMSQV	[257,258]
	p2 gag pep# mutant3	AEAMSQVT	[257,258]
	p2 gag pep# mutant4	VTN	[257,258]
	p2 gag pep# mutant5	VTNTATIM	[257,258]
Protease-Vif interaction	Vif 21–26	WKSLVK	[259–264]
	Vif 41–65	RHHYESPHPRISSEVHIPLGDAR	[259–264]

PPI: Protein–protein interaction.

peptides ($Vpr^{57–71}$ and $Vpr^{61–75}$) efficiently bound RT and IN. Molecular docking of $Vpr^{57–71}$ into the 3D structure of RT and of the two peptides $Vpr^{33–47}$ and $Vpr^{61–75}$ into the IN CCD were carried out to understand the biochemical effects such as steric hindrance and conformational changes of the active sites. DNA polymerase as well as RNase H activities of RT were significantly inhibited by $Vpr^{57–71}$, $Vpr^{65–79}$ and $Vpr^{69–83}$ with IC_{50} values in the range of 0.22–2 μM. DNA primer extension by RT was also inhibited by $Vpr^{53–67}$, $Vpr^{57–71}$, $Vpr^{61–75}$, $Vpr^{65–79}$ and $Vpr^{69–83}$. $Vpr^{33–47}$, $Vpr^{57–71}$, $Vpr^{61–75}$ and $Vpr^{65–79}$ were able to abrogate IN strand transfer activity. The three peptides $Vpr^{57–71}$, $Vpr^{61–75}$ and $Vpr^{65–79}$ inhibited the 3'-end processing activity of IN whereas the disintegration was blocked by $Vpr^{33–47}$, $Vpr^{69–83}$, $Vpr^{57–71}$, $Vpr^{61–75}$ and $Vpr^{65–79}$.

Conclusion & future perspective

In this review, we described the PPI in the HIV-1 replication cycle that are targets for inhibition by peptides and from which inhibitory peptides were derived. These PPI include both viral–cellular and viral–viral protein interactions. Most of the peptides reported are derived from viral–host PPI and not from viral–viral PPI, indicating that the host–viral interactions are more promising drug targets. Current research is focused on developing peptides libraries based on *in vitro* and *in vivo* experiments that will be later modified into small molecule inhibitor. The peptides are discovered using different approaches, and different assays were performed to analyze their quantitative or qualitative binding to viral proteins and their effect on HIV-1 infectivity.

Peptides do not serve only as tools for studying PPI, but have clinical use against HIV. The peptide, Fuzeon® (Enfuvirtide) was approved for clinical use against HIV [84–86,268,269]. Current research in anti-HIV drug design is focused on stabilizing lead peptides using different strategies such as cyclization, peptoids and more [268–273].

Peptides serve as excellent starting points for the design of peptidomimetics and the development of new small molecule drug leads based on their sequences and conformations. Currently, many of the FDA-approved anti-HIV drugs in the clinic, such as Indinavir, Ritonavir, Saquinavir and Lopinavir are the result of gradual conversion from a peptide to a small molecule [269,274–278]. These small molecules are mostly peptidomimetic hydroxyethylene or hydroxymethylamine HIV-1 protease inhibitors. Other types of small molecules such as ADS-J1, ADS-J2, XTT formazan, NB-2, NB-64, AOP-RANTES, PSC-RANTES, Vicriviroc, Maraviroc and Aplaviroc are also the outcome of peptidomimetic approaches. They act by targeting the HIV-1 entry through gp120, gp41, CCR5 and CXCR4. This approach may be used in the future for other PPI as described above.

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Executive summary

- Protein–protein interactions (PPI) are essential in every step of the human immunodeficiency virus (HIV) replication cycle.
- Mapping the interactions between viral and host proteins, as well as between the viral proteins themselves, is a fundamental target for the design and development of new therapeutics.
- Peptides are excellent tools to study the mechanisms of PPI in HIV-1 replication cycle and for the development of anti-HIV-1 drug leads that modulate PPI.
- These peptides can be later developed into small molecules, which can be used as drugs.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Jones S, Thornton JM. Principles of protein-protein interactions. *Proc. Natl Acad. Sci. USA* 93(1), 13–20 (1996).

2 Larsen TA, Olson AJ, Goodsell DS. Morphology of protein–protein interfaces. *Structure* 6(4), 421–427 (2014).

3 Trakselis MA, Alley SC, Ishmael FT. Identification and mapping of protein–protein interactions by a combination of cross-linking, cleavage, and proteomics. *Bioconjug. Chem.* 16(4), 741–750 (2005).

4 Chisholm ST, Coaker G, Day B, Staskawicz BJ. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124(4), 803–814 (2006).

5 König R, Zhou Y, Elleder D *et al.* Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. *Cell* 135(1), 49–60 (2014).

6 Dixon RA, Lamb CJ. Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 41(1), 339–367 (1990).

7 Shapira SD, Gat-Viks I, Shum BO V *et al.* A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell* 139(7), 1255–1267 (2009).

8 Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP. Signaling in plant-microbe interactions. *Science* 276(5313), 726–733 (1997).

9 Gu W, Schneider JW, Condorelli G, Kaushal S, Mahdavi V, Nadal-Ginard B. Interaction of myogenic factors and the retinoblastoma protein mediates muscle cell commitment and differentiation. *Cell* 72(3), 309–324 (1993).

10 Zervos AS, Gyuris J, Brent R. Mxi1, a protein that specifically interacts with Max to bind Myc-Max recognition sites. *Cell* 72(2), 223–232 (1993).

11 Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem. J.* 351(2), 289–305 (2000).

12 Pawson T, Nash P. Protein–protein interactions define specificity in signal transduction. *Genes Dev.* 14(9), 1027–1047 (2000).

13 Moran MF, Koch CA, Anderson D *et al.* Src homology region 2 domains direct protein-protein interactions in signal transduction. *Proc. Natl Acad. Sci. USA* 87(21), 8622–8626 (1990).

14 Imagawa M, Chiu R, Karin M. Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. *Cell* 51(2), 251–260 (1987).

15 Fields S, Song O. A novel genetic system to detect protein–protein interactions. *Nature* 340(6230), 245–246 (1989).

16 Wenger RH. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. *FASEB J.* 16(10), 1151–1162 (2002).

17 Wu JY, Maniatis T. Specific interactions between proteins implicated in splice site selection and regulated alternative splicing. *Cell* 75(6), 1061–1070 (1993).

18 Campbell S, Vogt VM. Self-assembly *in vitro* of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1. *J. Virol.* 69(10), 6487–6497 (1995).

19 Sandalon Z, Oppenheim A. Self-assembly and protein–protein interactions between the SV40 capsid proteins produced in insect cells. *Virology* 237(2), 414–421 (1997).

20 Ceres P, Zlotnick A. Weak protein–protein interactions are sufficient to drive assembly of hepatitis B virus capsids†. *Biochemistry* 41(39), 11525–11531 (2002).

21 Nielsen AL, Oulad-Abdelghani M, Ortiz JA, Remboutsika E, Chambon P, Losson R. Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell* 7(4), 729–739 (2001).

22 Murzina N, Verreault A, Laue E, Stillman B. Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol. Cell* 4(4), 529–540 (1999).

23 Blobel G, Dobberstein B. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67(3), 835–851 (1975).

24 Passow H. Molecular aspects of band 3 protein-mediated anion transport across the red blood cell membrane. In: *Reviews of Physiology, Biochemistry and Pharmacology, Volume 103 SE - 2*. Springer, Berlin Heidelberg, 61–203 (1986).

25 Jager S, Cimermancic P, Gulbahce N *et al.* Global landscape of HIV-human protein complexes. *Nature* 481(7381), 365–370 (2012).

•• **Information database for human and HIV-1 protein interactions.**

26 Brass AL, Dykxhoorn DM, Benita Y *et al.* Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 319(5865), 921–926 (2008).

27 Evans P, Dampier W, Ungar L, Tozeren A. Prediction of HIV-1 virus-host protein interactions using virus and host sequence motifs. *BMC Med. Genomics* 2(1), 27 (2009).

28 Pinney JW, Dickerson JE, Fu W, Sanders-Beer BE, Ptak RG, Robertson DL. HIV–host interactions: a map of viral perturbation of the host system. *AIDS* 23(5), 549–554 (2009).

•• **Comprehensive protein–protein interaction database of HIV.**

29 Von Mering C, Krause R, Snel B *et al.* Comparative assessment of large-scale data sets of protein-protein interactions. *Nature* 417(6887), 399–403 (2002).

30 Maulik U, Bhattacharyya M, Mukhopadhyay A, Bandyopadhyay S. Identifying the immunodeficiency gateway proteins in humans and their involvement in microRNA regulation. *Mol. Biosyst.* 7(6), 1842–1851 (2011).

31 Fu W, Sanders-Beer BE, Katz KS, Maglott DR, Pruitt KD, Ptak RG. Human immunodeficiency virus type 1, human protein interaction database at NCBI. *Nucleic Acids Res.* 37(Suppl. 1), D417–D422 (2009).

32 Moustafa N, Eldin A S, Kassim KS. Predicting HIV-1 human protein interactions using data mining without information loss. *I JMEIT.* 1(1), 25–41 (2013).

33 Dyer MD, Murali TM, Sobral BW. The landscape of human proteins interacting with viruses and other pathogens. *PLoS Pathog.* 4(2), e32 (2008).

34 Gavin A-C, Aloy P, Grandi P *et al.* Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440(7084), 631–636 (2006).

35 Hakes L, Pinney JW, Robertson DL, Lovell SC. Protein-protein interaction networks and biology -what's the connection? *Nat. Biotech.* 26(1), 69–72 (2008).

36 Fahey M, Bennett M, Mahon C *et al.* GPS-Prot: a web-based visualization platform for integrating host-pathogen interaction data. *BMC Bioinformatics* 12(1), 298 (2011).

37 Budayeva H, Cristea I. A mass spectrometry view of stable and transient protein interactions [Internet]. In: *Advancements of Mass Spectrometry in Biomedical Research SE - 11.* Woods AG, Darie CC (Eds.). Springer International Publishing, 263–282 (2014).

38 Li S. Proteomics Defines Protein Interaction Network of Signaling Pathways. In: *Bioinformatics of Human Proteomics SE - 2.* Wang X (Ed.). Springer Netherlands, 17–38 (2013).

39 Morris JH, Knudsen GM, Verschueren E *et al.* Affinity purification–mass spectrometry and network analysis to understand protein-protein interactions. *Nat. Protoc.* 9(11), 2539–2554 (2014).

40 Emig-Agius D, Olivieri K, Pache L *et al.* An integrated map of HIV-human protein complexes that facilitate viral infection. *PLoS ONE* 9(5), e96687 (2014).

41 Ptak RG, Fu W, Sanders-Beer BE *et al.* Cataloguing the HIV type 1 human protein interaction network. *AIDS Res. Hum. Retroviruses* 24(12), 1497–1502 (2008).

42 Wheeler DL, Barrett T, Benson DA *et al.* Database resources of the national center for biotechnology information. *Nucleic Acids Res.* 35(Suppl. 1), D5–D12 (2007).

43 Van Dijk D, Ertaylan G, Boucher C, Sloot P. Identifying potential survival strategies of HIV-1 through virus-host protein interaction networks. *BMC Syst. Biol.* 4(1), 96 (2010).

44 MacPherson JI, Dickerson JE, Pinney JW, Robertson DL. Patterns of HIV-1 protein interaction identify perturbed host-cellular subsystems. *PLoS Comput. Biol.* 6(7), e1000863 (2010).

45 Frankel AD, Young JAT. HIV-1: fifteen proteins and an RNA. *Annu. Rev. Biochem.* 67(1), 1–25 (1998).

46 Tavassoli A. Targeting the protein-protein interactions of the HIV lifecycle. *Chem. Soc. Rev.* 40(3), 1337–1346 (2011).

47 Malim MH, Emerman M. HIV-1 accessory proteins–ensuring viral survival in a hostile environment. *Cell Host Microbe* 3(6), 388–398 (2008).

48 Sakai K, Dimas J, Lenardo MJ. The Vif and Vpr accessory proteins independently cause HIV-1-induced T cell cytopathicity and cell cycle arrest. *Proc. Natl Acad. Sci. USA* 103(9), 3369–3374 (2006).

49 Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM. The challenge of HIV-1 subtype diversity. *N. Engl. J. Med.* 358(15), 1590–1602 (2008).

50 Rappaport M, Kogan J. HIV-1 accessory protein Vpr: relevance in the pathogenesis of HIV and potential for therapeutic intervention. *Retrovirology* 8, 25 (2011).

51 Tong AHY, Drees B, Nardelli G *et al.* A combined experimental and computational strategy to define protein interaction networks for peptide recognition modules. *Science* 295(5553), 321–324 (2002).

52 Benyamini H, Friedler A. Using peptides to study protein–protein interactions. *Future Med. Chem.* 2(6), 989–1003 (2010).

•• **Review about the use of peptides to study protein–protein interactions.**

53 Tolsma SS, Volpert O V, Good DJ, Frazier WA, Polverini PJ, Bouck N. Peptides derived from two separate domains of the matrix protein thrombospondin-1 have anti-angiogenic activity. *J. Cell Biol.* 122(2), 497–511 (1993).

54 Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu. Rev. Immunol.* 20(1), 395–425 (2002).

55 Katzen F, Chang G, Kudlicki W. The past, present and future of cell-free protein synthesis. *Trends Biotechnol.* 23(3), 150–156 (2005).

56 Zhu H, Snyder M. Protein chip technology. *Curr. Opin. Chem. Biol.* 7(1), 55–63 (2003).

57 Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. *Adv. Drug Deliv. Rev.* 54(4), 459–476 (2002).

58 Engelhard VH, Altrich-Vanlith M, Ostankovitch M, Zarling AL. Post-translational modifications of naturally processed MHC-binding epitopes. *Curr. Opin. Immunol.* 18(1), 92–97 (2006).

59 Chandra K, Roy TK, Naoum JN, Gilon C, Gerber RB, Friedler A. A highly efficient *in situ N*-acetylation approach for solid phase synthesis. *Org. Biomol. Chem.* 12(12), 1879–1884 (2014).

60 Kerppola TK. Visualization of molecular interactions by fluorescence complementation. *Nat. Rev. Mol. Cell Biol.* 7(6), 449–456 (2006).

61 Dieterich DC, Lee JJ, Link AJ, Graumann J, Tirrell DA, Schuman EM. Labeling, detection and identification of newly synthesized proteomes with bioorthogonal non-canonical amino-acid tagging. *Nat. Protoc.* 2(3), 532–540 (2007).

62 Lee LC, Hunter JJ, Mujeeb A, Turck C, Parslow TG. Evidence for α-helical conformation of an essential n-terminal region in the human Bcl2 protein. *J. Biol. Chem.* 271(38), 23284–23288 (1996).

63 Sattler M, Liang H, Nettesheim D *et al.* Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. *Science* 275(5302), 983–986 (1997).

64 Eichler J. Peptides as protein binding site mimetics. *Curr. Opin. Chem. Biol.* 12(6), 707–713 (2008).

65 Walensky LD, Kung AL, Escher I *et al.* Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. *Science* 305(5689), 1466–1470 (2004).

66 Murray JK, Gellman SH. Targeting protein–protein interactions: lessons from p53/MDM2. *Pept. Sci.* 88(5), 657–686 (2007).

67 Phan J, Li Z, Kasprzak A *et al.* Structure-based design of high affinity peptides inhibiting the interaction of p53 with MDM2 and MDMX. *J. Biol. Chem.* 285(3), 2174–2183 (2010).

68 Pazgier M, Liu M, Zou G *et al.* Structural basis for high-affinity peptide inhibition of p53 interactions with MDM2 and MDMX. *Proc. Natl Acad. Sci. USA* 106(12), 4665–4670 (2009).

69 Hayouka Z, Rosenbluh J, Levin A *et al.* Inhibiting HIV-1 integrase by shifting its oligomerization equilibrium. *Proc. Natl Acad. Sci. USA* 104(20), 8316–8321 (2007).

70 Lawrence SH, Ramirez UD, Tang L *et al.* Shape shifting leads to small-molecule allosteric drug discovery. *Chem. Biol.* 15(6), 586–596 (2008).

71 Freed E. HIV-1 Replication. *Somat. Cell Mol. Genet.* 26(1–6), 13–33 (2001).

72 Freed EO. HIV-1 and the host cell: an intimate association. *Trends Microbiol.* 12(4), 170–177 (2004).

73 Freed EO, Martin MA. The role of human immunodeficiency virus type 1 envelope glycoproteins in virus infection. *J. Biol. Chem.* 270(41), 23883–23886 (1995).

74 Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 393(6686), 648–659 (1998).

75 Moore JP, Binley J. HIV: envelope's letters boxed into shape. *Nature* 393(6686), 630–631 (1998).

76 Wyatt R, Kwong PD, Desjardins E *et al.* The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* 393(6686), 705–711 (1998).

77 Pancera M, Majeed S, Ban Y-EA *et al.* Structure of HIV-1 gp120 with gp41-interactive region reveals layered envelope architecture and basis of conformational mobility. *Proc. Natl Acad. Sci. USA* 107(3), 1166–1171 (2010).

78 Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. *Ann. Rev. Biochem.* 70(1), 777–810 (2001).

79 Deng H, Liu R, Ellmeier W *et al.* Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 381(6584), 661–666 (1996).

80 Dragic T, Litwin V, Allaway GP *et al.* HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* 381(6584), 667–673 (1996).

81 He J, Chen Y, Farzan M *et al.* CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* 385(6617), 645–649 (1997).

82 Chan DC, Fass D, Berger JM, Kim PS. Core structure of gp41 from the HIV Envelope Glycoprotein. *Cell* 89(2), 263–273 (2014).

83 Marsh M, Helenius A. Virus entry: open sesame. *Cell* 124(4), 729–740 (2006).

84 Doranz BJ, Rucker J, Yi Y *et al.* A dual-tropic primary HIV-1 isolate that uses fusin and the β-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 85(7), 1149–1158 (1996).

85 Gross A, Möbius K, Haußner C, Donhauser N, Schmidt B, Eichler J. Mimicking protein-protein interactions through peptide-peptide interactions: HIV-1 gp120 and CXCR4. *Front. Immunol.* 4(257), 1–11 (2013).

86 Esté JA, Telenti A. HIV entry inhibitors. *Lancet* 370(9581), 81–88 (2015).

87 Rao BS, Gupta KK, Kumari S, Gupta A, Pujitha K. Conserved HIV wide spectrum antipeptides-a hope for HIV treatment. *Adv. Tech. Biol. Med.* 1(1), 1–9 (2013).

88 Teissier E, Penin F, Pécheur E-I. Targeting cell entry of enveloped viruses as an antiviral strategy. *Molecules* 16(1), 221–250 (2010).

89 Pascual R, Contreras M, Fedorov A, Prieto M, Villalaín J. Interaction of a peptide derived from the N-heptad repeat region of gp41 Env ectodomain with model membranes. modulation of phospholipid phase behavior. *Biochemistry* 44(43), 14275–14288 (2005).

90 Huang C, Lam SN, Acharya P *et al.* Structures of the CCR5 N terminus and of a tyrosine-sulfated antibody with HIV-1 gp120 and CD4. *Science* 317(5846), 1930–1934 (2007).

91 Brower ET, Schön A, Klein JC, Freire E. Binding thermodynamics of the N-terminal peptide of the CCR5 coreceptor to HIV-1 envelope glycoprotein gp120†. *Biochemistry* 48(4), 779–785 (2009).

92 Davis CB, Dikic I, Unutmaz D *et al.* Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. *J. Exp. Med.* 186(10), 1793–1798 (1997).

93 Rizzuto CD, Wyatt R, Hernández-Ramos N *et al.* A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science* 280(5371), 1949–1953 (1998).

94 Möbius K, Dürr R, Haußner C, Dietrich U, Eichler J. A functionally selective synthetic mimic of the HIV-1 co-receptor CXCR4. *Chem. Eur. J.* 18(27), 8292–8295 (2012).

95 Gross A, Möbius K, Haußner C, Donhauser N, Schmidt B, Eichler J. Exploring converse molecular mechanisms of anti-HIV-1 antibodies using a synthetic CXCR4 mimic. *Bioorg. Med. Chem. Lett.* 22(19), 6099–6102 (2012).

96 Zennou V, Petit C, Guetard D, Nerhbass U, Montagnier L, Charneau P. HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* 101(2), 173–185 (2000).

97 Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 256(5065), 1783–1790 (1992).

98 Wang J, Smerdon SJ, Jäger J *et al.* Structural basis of asymmetry in the human immunodeficiency virus type 1 reverse transcriptase heterodimer. *Proc. Natl Acad. Sci. USA* 91(15), 7242–7246 (1994).

99 Jochmans D. Novel HIV-1 reverse transcriptase inhibitors. *Vir. Res.* 134(1–2), 171–185 (2008).

•• **Overview of reverse trascriptase inhibitors with the strategy for their development.**

100 Iwatani Y, Chan DSB, Wang F *et al.* Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G. *Nucleic Acids Res.* 35(21), 7096–7108 (2007).

101 Wang X, Ao Z, Chen L, Kobinger G, Peng J, Yao X. The cellular antiviral protein APOBEC3G interacts with HIV-1 reverse transcriptase and inhibits its function during viral replication. *J. Virol.* 86(7), 3777–3786 (2012).

102 Stainforth DA, Aina T, Christensen C *et al.* Uncertainty in predictions of the climate response to rising levels of greenhouse gases. *Nature* 433(7024), 403–406 (2005).

103 Yu X, Yu Y, Liu B *et al.* Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* 302(5647), 1056–1060 (2003).

104 Mangeat B, Turelli P, Caron G, Friedli M, Perrin L, Trono D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424(6944), 99–103 (2003).

105 Miyagi E, Opi S, Takeuchi H *et al.* Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. *J. Virol.* 81(24), 13346–13353 (2007).

106 Li X-Y, Guo F, Zhang L, Kleiman L, Cen S. APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *J. Biol. Chem.* 282(44), 32065–32074 (2007).

107 Guo F, Cen S, Niu M, Saadatmand J, Kleiman L. Inhibition of -primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication. *J. Virol.* 80(23), 11710–11722 (2006).

108 Guo F, Saadatmand J, Niu M, Kleiman L. Roles of Gag and NCp7 in facilitating annealing to viral RNA in human immunodeficiency virus type 1. *J. Virol.* 83(16), 8099–8107 (2009).

109 Druillennec S, Dong CZ, Escaich S *et al.* A mimic of HIV-1 nucleocapsid protein impairs reverse transcription and displays antiviral activity. *Proc. Natl Acad. Sci. USA* 96(9), 4886–4891 (1999).

110 De Rocquigny H, Gabus C, Vincent A, Fournié-Zaluski MC, Roques B, Darlix JL. Viral RNA annealing activities of human immunodeficiency virus type 1 nucleocapsid protein require only peptide domains outside the zinc fingers. *Proc. Natl Acad. Sci. USA* 89(14), 6472–6476 (1992).

111 Craigie R. HIV Integrase, a Brief Overview from Chemistry to Therapeutics. *J. Biol. Chem.* 276(26), 23213–23216 (2001).

112 Delelis O, Carayon K, Saib A, Deprez E, Mouscadet J-F. Integrase and integration: biochemical activities of HIV-1 integrase. *Retrovirology* 5(1), 114 (2008).

113 Schröder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* 110(4), 521–529 (2014).

114 Kalpana G V, Marmon S, Wang W, Crabtree GR, Goff SP. Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5. *Science* 266(5193), 2002–2006 (1994).

115 Stevenson M, Stanwick T L, Dempsey M P, Lamonica CA. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J.* 9(5), 1551–1560 (1990).

116 Chen JC, Krucinski J, Miercke LJ *et al.* Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains: a model for viral DNA binding. *Proc. Natl Acad. Sci. USA* 97(15), 8233–8238 (2000).

117 Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies DR. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* 266(5193), 1981–1986 (1994).

118 Marchand C, Johnson AA, Semenova E, Pommier Y. Mechanisms and inhibition of HIV integration. *Drug Discov. Today Dis. Mech.* 3(2), 253–260 (2006).

• **HIV-1 integration and its role in replication.**

119 Guiot E, Carayon K, Delelis O *et al.* Relationship between the oligomeric status of HIV-1 integrase on DNA and enzymatic activity. *J. Biol. Chem.* 281(32), 22707–22719 (2006).

120 Van Maele B, Busschots K, Vandekerckhove L, Christ F, Debyser Z. Cellular co-factors of HIV-1 integration. *Trends Biochem. Sci.* 31(2), 98–105 (2014).

121 Levin A, Armon-Omer A, Rosenbluh J *et al.* Inhibition of HIV-1 integrase nuclear import and replication by a peptide bearing integrase putative nuclear localization signal. *Retrovirology* 6(112), 1–16 (2009).

122 Leh H, Brodin P, Bischerour J *et al.* Determinants of Mg2+-dependent activities of recombinant human immunodeficiency virus type 1 integrase†. *Biochemistry* 39(31), 9285–9294 (2000).

123 Li M, Craigie R. Processing of viral DNA ends channels the HIV-1 Integration Reaction to Concerted Integration. *J. Biol. Chem.* 280(32), 29334–29339 (2005).

124 Faure A, Calmels C, Desjobert C *et al.* HIV-1 integrase crosslinked oligomers are active *in vitro*. *Nucleic Acids Res.* 33(3), 977–986 (2005).

125 Chaurushiya MS, Weitzman MD. Viral manipulation of DNA repair and cell cycle checkpoints. *DNA Repair* 8(9), 1166–1176 (2009).

126 Lewinski MK, Bushman FD. Retroviral DNA integration–mechanism and consequences. *Adv. Genet.* 55, 147–181 (2005).

127 Parissi V, Caumont A, de Soultrait VR *et al.* The lethal phenotype observed after HIV-1 integrase expression in yeast cells is related to DNA repair and recombination events. *Gene* 322(0), 157–168 (2003).

128 Oyebisi Jegede, John Babu, Roberto di Santo, Damian J, McColl JW, MEQ-M. HIV type 1 integrase inhibitors: from basic research to clinical implications. *AIDS Rev.* 10(3), 172–189 (2008).

129 Freed CSA and EO. Anti-HIV-1 therapeutics: from FDA-approved drugs to hypothetical future targets. *Mol. Interv. Apr.* 9(2), 70–74 (2009).

130 Maes M, Loyter A, Friedler A. Peptides that inhibit HIV-1 integrase by blocking its protein-protein interactions. *FEBS J.* 279(16), 2795–2809 (2012).

• **Review about peptides that target the protein–protein interactions of HIV-1 integrase.**

131 Camarasa M-J, Velázquez S, San-Félix A, Pérez-Pérez M-J, Gago F. Dimerization inhibitors of HIV-1 reverse transcriptase, protease and integrase: A single mode of inhibition for the three HIV enzymes? *Antiviral. Res.* 71(2–3), 260–267 (2006).

132 Soultrait VR d, Desjobert C, Tarrago-Litvak L. Peptides as new inhibitors of HIV-1 reverse transcriptase and integrase. *Curr. Med. Chem.* 10(18), 1765–1778 (2003).

133 Goldgur Y, Dyda F, Hickman AB, Jenkins TM, Craigie R, Davies DR. Three new structures of the core domain of HIV-1 integrase: an active site that binds magnesium. *Proc. Natl Acad. Sci. USA* 95(16), 9150–9154 (1998).

134 Neamati N, Sunder S, Pommier Y. Design and discovery of HIV-1 integrase inhibitors. *Drug Discov. Today* 2(11), 487–498 (1997).

135 Sourgen F, Maroun RG, Frère V *et al.* A synthetic peptide from the human immunodeficiency virus Type-1 integrase exhibits coiled-coil properties and interferes with the *in vitro* integration activity of the enzyme. *Euro J. Biochem.* 240(3), 765–773 (1996).

136 Maroun RG, Gayet S, Benleulmi MS *et al.* Peptide inhibitors of HIV-1 integrase dissociate the enzyme oligomers†. *Biochemistry* 40(46), 13840–13848 (2001).

137 Zhao L, O'Reilly MK, Shultz MD, Chmielewski J. Interfacial peptide inhibitors of HIV-1 integrase activity and

dimerization. *Bioorg. Med. Chem. Lett.* 13(6), 1175–1177 (2003).

138 Van Aerschot A. Oligonucleotides as antivirals: dream or realistic perspective? *Antiviral Res.* 71(2–3), 307–316 (2006).

139 Archakov AI, Govorun VM, Dubanov A V *et al.* Protein-protein interactions as a target for drugs in proteomics. *Proteomics* 3(4), 380–391 (2003).

140 Emiliani S, Mousnier A, Busschots K *et al.* Integrase mutants defective for interaction with LEDGF/p75 are impaired in chromosome tethering and HIV-1 replication. *J. Biol. Chem.* 280(27), 25517–25523 (2005).

141 Turlure F, Devroe E, Silver P A, Engelman A. Human cell proteins and human immunodeficiency virus DNA integration. *Front. Biosci.* 9, 3187–3208 (2004).

142 Al-Mawsawi LQ, Neamati N. Blocking interactions between HIV-1 integrase and cellular cofactors: an emerging anti-retroviral strategy. *Trends Pharmacol. Sci.* 28, 526–535 (2007).

143 Busschots K, Vercammen J, Emiliani S *et al.* The interaction of LEDGF/p75 with integrase is lentivirus-specific and promotes DNA binding. *J. Biol. Chem.* 280(18), 17841–17847 (2005).

144 Llano M, Vanegas M, Fregoso O *et al.* LEDGF/p75 determines cellular trafficking of diverse lentiviral but not murine oncoretroviral integrase proteins and is a component of functional lentiviral preintegration complexes. *J. Virol.* 78(17), 9524–9537 (2004).

145 Cherepanov P, Maertens G, Proost P *et al.* HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J. Biol. Chem.* 278(1), 372–381 (2003).

146 Vandegraaff N, Devroe E, Turlure F, Silver PA, Engelman A. Biochemical and genetic analyses of integrase-interacting proteins lens epithelium-derived growth factor (LEDGF)/p75 and hepatoma-derived growth factor related protein 2 (HRP2) in preintegration complex function and HIV-1 replication. *Virology* 346(2), 415–426 (2006).

147 Ciuffi A, Llano M, Poeschla E *et al.* A role for LEDGF/p75 in targeting HIV DNA integration. *Nat. Med.* 11(12), 1287–1289 (2005).

148 Llano M, Delgado S, Vanegas M, Poeschla EM. Lens epithelium-derived growth factor/p75 prevents proteasomal degradation of HIV-1 integrase. *J. Biol. Chem.* 279(53), 55570–55577 (2004).

149 Cherepanov P, Sun Z-YJ, Rahman S, Maertens G, Wagner G, Engelman A. Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75. *Nat. Struct. Mol. Biol.* 12(6), 526–532 (2005).

150 Llano M, Saenz DT, Meehan A *et al.* An essential role for LEDGF/p75 in HIV integration. *Science* 314(5798), 461–464 (2006).

151 Cherepanov P, Devroe E, Silver PA, Engelman A. Identification of an evolutionarily conserved domain in human lens epithelium-derived growth factor/transcriptional co-activator p75 (LEDGF/p75) that binds HIV-1 integrase. *J. Biol. Chem.* 279(47), 48883–48892 (2004).

152 Cherepanov P, Ambrosio ALB, Rahman S, Ellenberger T, Engelman A. Structural basis for the recognition between HIV-1 integrase and transcriptional coactivator p75. *Proc. Natl Acad. Sci. USA* 102(48), 17308–17313 (2005).

153 Levin A, Benyamini H, Hayouka Z, Friedler A, Loyter A. Peptides that bind the HIV-1 integrase and modulate its enzymatic activity – kinetic studies and mode of action. *FEBS J.* 278(2), 316–330 (2011).

154 Hayouka Z, Levin A, Maes M *et al.* Mechanism of action of the HIV-1 integrase inhibitory peptide LEDGF 361–370. *Biochem. Biophys. Res. Commun.* 394(2), 260–265 (2010).

155 Hayouka Z, Hurevich M, Levin A *et al.* Cyclic peptide inhibitors of HIV-1 integrase derived from the LEDGF/p75 protein. *Bioorg. Med. Chem.* 18(23), 8388–8395 (2010).

156 Maes M, Levin A, Hayouka Z, Shalev DE, Loyter A, Friedler A. Peptide inhibitors of HIV-1 integrase: From mechanistic studies to improved lead compounds. *Bioorg. Med. Chem.* 17(22), 7635–7642 (2009).

157 Al-Mawsawi LQ, Christ F, Dayam R, Debyser Z, Neamati N. Inhibitory profile of a LEDGF/p75 peptide against HIV-1 integrase: Insight into integrase–DNA complex formation and catalysis. *FEBS Lett.* 582(10), 1425–1430 (2008).

158 Desimmie BA, Humbert M, Lescrinier E *et al.* Phage display-directed discovery of LEDGF/p75 binding cyclic peptide inhibitors of HIV replication. *Mol. Ther.* 20(11), 2064–2075 (2012).

159 Wang J-Y, Ling H, Yang W, Craigie R. Structure of a two-domain fragment of HIV-1 integrase: implications for domain organization in the intact protein. *EMBO J.* 20(24), 7333–7343 (2001).

160 Dyda F, Hickman AB, Jenkins TM, Engelman A, Craigie R, Davies D. Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* 266, 1981–1986 (2004).

161 Chen H, Engelman A. Characterization of a replication-defective human immunodeficiency virus type 1 att site mutant that is blocked after the 3′ processing step of retroviral integration. *J. Virol.* 74(17), 8188–8193 (2000).

162 Long Y-Q, Huang S-X, Zawahir Z *et al.* Design of cell-permeable stapled peptides as HIV-1 integrase inhibitors. *J. Med. Chem.* 56(13), 5601–5612 (2013).

163 Desjobert C, de Soultrait VR, Faure A *et al.* Identification by phage display selection of a short peptide able to inhibit only the strand transfer reaction catalyzed by human immunodeficiency virus type 1 integrase†. *Biochemistry* 43(41), 13097–13105 (2004).

164 Li H-Y, Zawahir Z, Song L-D, Long Y-Q, Neamati N. Sequence-based design and discovery of peptide inhibitors of HIV-1 integrase: insight into the binding mode of the enzyme. *J. Med. Chem.* 49(15), 4477–4486 (2006).

165 Freed EO. HIV-1 Gag proteins: diverse functions in the virus life cycle. *Virology* 251(1), 1–15 (1998).

166 Saad JS, Miller J, Tai J, Kim A, Ghanam RH, Summers MF. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. *Proc. Natl Acad. Sci. USA* 103(30), 11364–11369 (2006).

167 Bennett RP, Nelle TD, Wills JW. Functional chimeras of the Rous sarcoma virus and human immunodeficiency virus gag proteins. *J. Virol.* 67(11), 6487–6498 (1993).

168 Craven RC, Leure-duPree AE, Weldon RA, Wills JW. Genetic analysis of the major homology region of the Rous sarcoma virus Gag protein. *J. Virol.* 69(7), 4213–4227 (1995).

169 Dawson L, Yu X-F. The role of nucleocapsid of HIV-1 in virus assembly. *Virology* 251(1), 141–157 (1998).

170 Kenney SP, Lochmann TL, Schmid CL, Parent LJ. Intermolecular Interactions between retroviral Gag proteins in the nucleus. *J. Virol.* 82(2), 683–691 (2008).

171 Wills JW, Craven RC. Form, function, and use of retroviral Gag proteins. *AIDS* 5(6) (1991).

172 Martinez-Hackert E, Anikeeva N, Kalams SA, Walker BD, Hendrickson WA, Sykulev Y. Structural basis for degenerate recognition of natural HIV peptide variants by cytotoxic lymphocytes. *J. Biol. Chem.* 281(29), 20205–20212 (2006).

173 Brander C, Hartman KE, Trocha AK *et al.* Lack of strong immune selection pressure by the immunodominant, HLA-A*0201-restricted cytotoxic T lymphocyte response in chronic human immunodeficiency virus-1 infection. *J. Clin. Investig.* 101(11), 2559–2566 (1998).

174 Goulder PJR, Sewell AK, Lalloo DG *et al.* Patterns of immunodominance in HIV-1–specific cytotoxic T lymphocyte responses in two human histocompatibility leukocyte antigens (HLA)-identical siblings with HLA-A*0201 are influenced by epitope mutation. *J. Exp. Med.* 185(8), 1423–1433 (1997).

175 Li F, Goila-Gaur R, Salzwedel K *et al.* PA-457: a potent HIV inhibitor that disrupts core condensation by targeting a late step in Gag processing. *Proc. Natl Acad. Sci. USA* 100(23), 13555–13560 (2003).

176 Huang M, Orenstein JM, Martin MA, Freed EO. p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. *J. Virol.* 69(11), 6810–6818 (1995).

177 Pornillos O, Alam SL, Davis DR, Sundquist WI. Structure of the Tsg101 UEV domain in complex with the PTAP motif of the HIV-1 p6 protein. *Nat. Struct. Mol. Biol.* 9(11), 812–817 (2002).

178 Garrus JE, von Schwedler UK, Pornillos OW *et al.* Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107(1), 55–65 (2014).

179 VerPlank L, Bouamr F, LaGrassa TJ *et al.* Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55Gag. *Proc. Natl Acad. Sci. USA* 98(14), 7724–7729 (2001).

180 Martin-Serrano J, Zang T, Bieniasz PD. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* 7(12), 1313–1319 (2001).

181 Zhan P, Li W, Chen H, Liu X. Targeting protein-protein interactions: a promising avenue of anti-HIV drug discovery. *Curr. Med. Chem.* 17(29), 3393–3409 (2010).

182 Freed EO. The HIV–TSG101 interface: recent advances in a budding field. *Trends Microbiol.* 11(2), 56–59 (2003).

183 Demirov DG, Ono A, Orenstein JM, Freed EO. Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. *Proc. Natl Acad. Sci. USA* 99(2), 955–960 (2002).

184 Kim S-E, Liu F, Im YJ *et al.* Elucidation of new binding interactions with the human Tsg101 protein using modified HIV-1 Gag-p6 derived peptide ligands. *ACS Med. Chem. Lett.* 2(5), 337–341 (2011).

185 Liu F, Stephen AG, Waheed AA *et al.* SAR by oxime-containing peptide libraries: application to Tsg101 ligand optimization. *ChemBioChem* 9(12), 2000–2004 (2008).

186 Liu F, Stephen AG, Fisher RJ, Burke Jr. TR. Protected aminooxyprolines for expedited library synthesis: application to Tsg101-directed proline–oxime containing peptides. *Bioorg. Med. Chem. Lett.* 18(3), 1096–1101 (2008).

187 Tavassoli A, Lu Q, Gam J, Pan H, Benkovic SJ, Cohen SN. Inhibition of HIV budding by a genetically selected cyclic peptide targeting the Gag–TSG101 interaction. *ACS Chem. Biol.* 3(12), 757–764 (2008).

• **Development of cyclic pepdtide that target HIV-1 Gag–TSG101 interaction.**

188 Solbak SMØ, Reksten TR, Röder R *et al.* HIV-1 p6–Another viral interaction partner to the host cellular protein cyclophilin A. *Biochim. Biophys. Acta* 1824(4), 667–678 (2012).

189 Hatziioannou T, Perez-Caballero D, Cowan S, Bieniasz PD. Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J. Virol.* 79(1), 176–183 (2005).

190 Franke EK, Luban J. Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag–cyclophilin a interaction. *Virology* 222(1), 279–282 (1996).

191 Rana TM, Jeang K-T. Biochemical and functional interactions between HIV-1 Tat protein and TAR RNA. *Arch. Biochem. Biophys.* 365(2), 175–185 (1999).

192 Sodroski J, Patarca R, Rosen C, Wong-Staal F, Haseltine W. Location of the trans-activating region on the genome of human T-cell lymphotropic virus type III. *Science* 229(4708), 74–77 (1985).

193 Bayer P, Kraft M, Ejchart A, Westendorp M, Frank R, Rösch P. Structural studies of HIV-1 Tat protein. *J. Mol. Biol.* 247(4), 529–535 (1995).

194 Churcher MJ, Lamont C, Hamy F *et al.* High affinity binding of TAR RNA by the human immunodeficiency virus type-1 Tat protein requires base-pairs in the rna stem and amino acid residues flanking the basic region. *J. Mol. Biol.* 230(1), 90–110 (1993).

195 Long KS, Crothers DM. Interaction of Human Immunodeficiency Virus Type 1 Tat-derived peptides with TAR RNA. *Biochemistry* 34(27), 8885–8895 (1995).

196 Luo Y, Madore SJ, Parslow TG, Cullen BR, Peterlin BM. Functional analysis of interactions between Tat and the trans-activation response element of human immunodeficiency virus type 1 in cells. *J. Virol.* 67(9), 5617–5622 (1993).

197 Cullen BR. Regulation of HIV-1 gene expression. *FASEB J.* 5(10), 2361–2368 (1991).

198 Calnan BJ, Biancalana S, Hudson D, Frankel AD. Analysis of Arginine-rich peptides from the HIV Tat protein reveals unusual features of RNA-protein recognition. *Genes Dev.* 5(2), 201–210 (1991).

199 Ensoli B, Buonaguro L, Barillari G *et al.* Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J. Virol.* 67(1), 277–287 (1993).

200 Stevens M, De Clercq E, Balzarini J. The regulation of HIV-1 transcription: molecular targets for chemotherapeutic intervention. *Med. Res. Rev.* 26(5), 595–625 (2006).

201 Weeks KM, Ampe C, Schultz SC, Steitz TA, Crothers DM. Fragments of the HIV-1 Tat protein specifically bind TAR RNA. *Science* 249(4974), 1281–1285 (1990).

202 Cordingley MG, LaFemina RL, Callahan PL *et al.* Sequence-specific interaction of Tat protein and Tat peptides with the transactivation-responsive sequence element of human immunodeficiency virus type 1 *in vitro*. *Proc. Natl Acad. Sci. USA* 87(22), 8985–8989 (1990).

203 Gelman MA, Richter S, Cao H, Umezawa N, Gellman SH, Rana TM. Selective Binding of TAR RNA by a Tat-Derived β-Peptide. *Org. Lett.* 5(20), 3563–3565 (2003).

204 Wender PA, Mitchell DJ, Pattabiraman K, Pelkey ET, Steinman L, Rothbard JB. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proc. Natl Acad. Sci. USA* 97(24), 13003–13008 (2000).

205 Wu C-H, Chen Y-P, Mou C-Y, Cheng R. Altering the Tat-derived peptide bioactivity landscape by changing the Arginine side chain length. *Amino Acids* 44(2), 473–480 (2013).

206 Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 408(6810), 307–310 (2000).

207 Jeffrey PD, Gorina S, Pavletich NP. Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science* 267(5203), 1498–1502 (1995).

208 Clore GM, Omichinski JG, Sakaguchi K *et al.* High-resolution structure of the oligomerization domain of p53 by multidimensional NMR. *Science* 265(5170), 386–391 (1994).

209 Longo F, Marchetti MA, Castagnoli L, Battaglia PA, Gigliani F. A novel approach to protein-protein interaction: complex formation between the P53 tumor suppressor and the HIV Tat proteins. *Biochem. Biophys. Res. Commun.* 206(1), 326–334 (1995).

210 Ariumi Y, Kaida A, Hatanaka M, Shimotohno K. Functional cross-talk of HIV-1 Tat with p53 through Its C-terminal domain. *Biochem. Biophys. Res. Commun.* 287(2), 556–561 (2001).

211 Duan L, Ozaki I, Oakes JW, Taylor JP, Khalili K, Pomerantz RJ. The tumor suppressor protein p53 strongly alters human immunodeficiency virus type 1 replication. *J. Virol.* 68(7), 4302–4313 (1994).

212 Li CJ, Wang C, Friedman DJ, Pardee AB. Reciprocal modulations between p53 and Tat of human immunodeficiency virus type 1. *Proc. Natl Acad. Sci. USA* 92(12), 5461–5464 (1995).

213 Cullen R. Mechanism of action of regulatory proteins encoded by complex retroviruses. *Microbiol. Rev.* 56(3), 375–394 (1992).

214 Gabizon R, Mor M, Rosenberg MM *et al.* Using peptides to study the interaction between the p53 tetramerization domain and HIV-1 tat. *Peptide Sci.* 90(2), 105–116 (2008).

215 Fisher AG, Ensoli B, Ivanoff L *et al.* The sor gene of HIV-1 is required for efficient virus transmission *in vitro*. *Science* 237(4817), 888–893 (1987).

216 Gabuzda DH, Lawrence K, Langhoff E *et al.* Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *J. Virol.* 66(11), 6489–6495 (1992).

217 Huthoff H, Malim MH. Cytidine deamination and resistance to retroviral infection: towards a structural understanding of the APOBEC proteins. *Virology* 334(2), 147–153 (2005).

218 Harris RS, Bishop KN, Sheehy AM *et al.* DNA deamination mediates innate immunity to retroviral infection. *Cell* 113(6), 803–809 (2003).

219 Mariani R, Chen D, Schröfelbauer B *et al.* Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* 114(1), 21–31 (2003).

220 Zhang H, Yang B, Pomerantz RJ, Zhang C, Arunachalam SC, Gao L. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424(6944), 94–98 (2003).

221 Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 9(11), 1404–1407 (2003).

222 Stopak K, de Noronha C, Yonemoto W, Greene WC. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. *Mol. Cell.* 12(3), 591–601 (2003).

223 Bogerd HP, Doehle BP, Wiegand HL, Cullen BR. A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl Acad. Sci. USA* 101(11), 3770–3774 (2004).

224 Mangeat B, Turelli P, Liao S, Trono D. A Single Amino Acid Determinant Governs the Species-specific Sensitivity of APOBEC3G to Vif Action. *J. Biol. Chem.* 279(15), 14481–14483 (2004).

225 He Z, Zhang W, Chen G, Xu R, Yu X-F. Characterization of Conserved Motifs in HIV-1 Vif Required for APOBEC3G and APOBEC3F Interaction. *J. Mol. Biol.* 381(4), 1000–1011 (2008).

226 Zhang L, Saadatmand J, Li X *et al.* Function analysis of sequences in human APOBEC3G involved in Vif-mediated degradation. *Virology* 370(1), 113–121 (2008).

- **Vif-A3G interaction as a basis for developing peptide-based inhibitors.**

227 Mehle A, Wilson H, Zhang C *et al.* Identification of an APOBEC3G binding site in human immunodeficiency virus type 1 Vif and inhibitors of Vif-APOBEC3G binding. *J. Virol.* 81(23), 13235–13241 (2007).

228 Reingewertz TH, Britan-Rosich E, Rotem-Bamberger S *et al.* Mapping the Vif–A3G interaction using peptide arrays: a basis for anti-HIV lead peptides. *Bioorg. Med. Chem.* 21(12), 3523–3532 (2013).

229 Schröfelbauer B, Chen D, Landau NR. A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl Acad. Sci. USA* 101(11), 3927–3932 (2004).

230 Donahue JP, Vetter ML, Mukhtar NA, D'Aquila RT. The HIV-1 Vif PPLP motif is necessary for human APOBEC3G binding and degradation. *Virology* 377(1), 49–53 (2008).

231 Dang Y, Wang X, York IA, Zheng Y-H. Identification of a critical T(Q/D/E)x5ADx2(I/L) motif from primate lentivirus Vif proteins that regulate APOBEC3G and APOBEC3F neutralizing activity. *J. Virol.* 84(17), 8561–8570 (2010).

232 De Noronha CM, Sherman MP, Lin HW *et al.* Dynamic disruptions in nuclear envelope architecture and integrity induced by HIV-1 Vpr. *Science* 294(5544), 1105–1108 (2001).

233 Levy DN, Fernandes LS, Williams W V, Weiner DB. Induction of cell differentiation by human immunodeficiency virus 1 Vpr. *Cell* 72(4), 541–550 (1993).

234 Kogan M, Rappaport J. HIV-1 accessory protein Vpr: relevance in the pathogenesis of HIV and potential for therapeutic intervention. *Retrovirology* 8(1), 25 (2011).

235 Emerman M. HIV-1, Vpr and the cell cycle. *Curr. Biol.* 6(9), 1096–1103 (1996).

236 Ardon O, Zimmerman ES, Andersen JL, DeHart JL, Blackett J, Planelles V. Induction of G2 arrest and binding to cyclophilin A are independent phenotypes of human immunodeficiency virus type 1 Vpr. *J. Virol.* 80(8), 3694–3700 (2006).

237 Solbak S, Reksten T, Wray V *et al.* The intriguing cyclophilin A-HIV-1 Vpr interaction: prolyl cis/trans isomerisation catalysis and specific binding. *BMC Struct. Biol.* 10(1), 31 (2010).

238 Di Marzio P, Choe S, Ebright M, Knoblauch R, Landau NR. Mutational analysis of cell cycle arrest, nuclear localization and virion packaging of human immunodeficiency virus type 1 Vpr. *J. Virol.* 69(12), 7909–7916 (1995).

239 Gaynor EM, Chen ISY. Analysis of apoptosis induced by HIV-1 Vpr and examination of the possible role of the hHR23A protein. *Expt. Cell Res.* 267(2), 243–257 (2001).

240 Franke EK, Yuan HEH, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 372(6504), 359–362 (1994).

241 Solbak SMØ, Wray V, Horvli O *et al.* The host-pathogen interaction of human cyclophilin A and HIV-1 Vpr requires specific N-terminal and novel C-terminal domains. *BMC Struct. Biol.* 11, 49 (2011).

242 Luo Z, Butcher DJ, Murali R, Srinivasan A, Huang Z. Structural studies of synthetic peptide fragments derived from the HIV-1 Vpr protein. *Biochem. Biophys. Res. Commun.* 244(3), 732–736 (1998).

243 BouHamdan M, Xue Y, Baudat Y *et al.* Diversity of HIV-1 Vpr interactions involves usage of the WXXF Motif of host cell proteins. *J. Biol. Chem.* 273(14), 8009–8016 (1998).

244 Debouck C. The HIV-1 protease as a therapeutic target for AIDS. *AIDs Res. Hum. Retrovirus* 8(2), 153–164 (1992).

• **Strategies for anti-HIV-1 Protease drug development.**

245 Smith DB, Johnson KS. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. *Gene* 67(1), 31–40 (1988).

246 Sabbah EN, Druillennec S, Morellet N, Bouaziz S, Kroemer G, Roques BP. Interaction between the HIV-1 protein Vpr and the adenine nucleotide translocator. *Chem. Biol. Drug. Des.* 67(2), 145–154 (2006).

247 Jacotot E, Ferri KF, El Hamel C *et al.* Control of Mitochondrial membrane permeabilization by adenine nucleotide translocator interacting with HIV-1 viral protein R and Bcl-2. *J. Expt. Med.* 193(4), 509–520 (2001).

248 Borgne-Sanchez A, Dupont S, Langonne A *et al.* Targeted Vpr-derived peptides reach mitochondria to induce apoptosis of [alpha]V[beta]3-expressing endothelial cells. *Cell Death Differ.* 14(3), 422–435 (2006).

249 Kattenbeck B, Rohrhofer A, Niedrig M, Wolf H, Modrow S. Defined amino acids in the gag proteins of human immunodeficiency virus type 1 are functionally active during virus assembly. *Intervirology* 39(1–2), 32–39 (1996).

250 Gallina A, Mantoan G, Rindi G, Milanesi G. Influence of MA Internal sequences, but not of the myristylated N-terminus sequence, on the budding site of HIV-1 Gag protein. *Biochem. Biophys. Res. Commun.* 204(3), 1031–1038 (1994).

251 Bukrinsky MI, Haggerty S, Dempsey MP *et al.* A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. *Nature* 365(6447), 666–669 (1993).

252 Dorfman T, Mammano F, Haseltine WA, Göttlinger HG. Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. *J. Virol.* 68(3), 1689–1696 (1994).

253 Ota A, Tanaka-Taya K, Ueda S. Cross-reactivity of anti-HIV-1-p17-derivative peptide (P30–52) antibody to Env V3 peptide. *Hybridoma* 18(2), 149–157 (1999).

254 Ota A, Liu X, Fujio H, Sakato Nobuo, Ueda S. Random expression of human immunodeficiency virus-1 (HIV-1) pl7 (epitopes) on the surface of the HIV-1-infected cell. *Hybridoma* 17(1), 73–75 (1998).

255 Kohl NE, Emini EA, Schleif WA *et al.* Active human immunodeficiency virus protease is required for viral infectivity. *Proc. Natl Acad. Sci. USA* 85(13), 4686–4690 (1988).

256 Liu B, Dai R, Tian C-J, Dawson L, Gorelick R, Yu X-F. Interaction of the human immunodeficiency virus type 1 nucleocapsid with actin. *J. Virol.* 73(4), 2901–2908 (1999).

257 Luciw PA. Human immunodeficiency viruses and their replication. In: *Field Virology (3rd Edition)*. Fields BN, Knipe DM, Howely PM, (Eds.), Philadelphia, PA, USA, Lippincott-Raven Publishers; 18 (1996).

258 Misumi S, Kudo A, Azuma R, Tomonaga M, Furuishi K, Shoji S. The p2gagPeptide, AEAMSQVTNTATIM, processed from HIV-1 Pr55 Gag was found to be a suicide inhibitor of HIV-1 protease. *Biochem. Biophys. Res. Commun.* 241(2), 275–280 (1997).

259 Kotler M, Simm M, Zhao YS *et al.* Human immunodeficiency virus type 1 (HIV-1) protein Vif inhibits the activity of HIV-1 protease in bacteria and *in vitro*. *J. Virol.* 71(8), 5774–5781 (1997).

260 Karageorgos L, Li P, Burrell C. Characterization of HIV replication complexes early after cell-to-cell infection. *AIDS Res. Hum. Retroviruses* 9(9), 817–823 (1993).

261 Potash MJ, Bentsman G, Muir T, Krachmarov C, Sova P, Volsky DJ. Peptide inhibitors of HIV-1 protease and viral infection of peripheral blood lymphocytes based on HIV-1 Vif. *Proc. Natl Acad. Sci. USA* 95(23), 13865–13868 (1998).

262 Baraz L, Hutoran M, Blumenzweig I *et al.* Human immunodeficiency virus type 1 Vif binds the viral protease by interaction with its N-terminal region. *J. Gen. Virol.* 83(9), 2225–2230 (2002).

263 Friedler A, Blumenzweig I, Baraz L, Steinitz M, Kotler M, Gilon C. Peptides derived from HIV-1 vif: a non-substrate based novel type of HIV-1 protease inhibitors. *J. Mol. Biol.* 287(1), 93–101 (1999).

264 Hutoran M, Britan E, Baraz L, Blumenzweig I, Steinitz M, Kotler M. Abrogation of Vif function by peptide derived from the N-terminal region of the human immunodeficiency virus type 1 (HIV-1) protease. *Virology* 330(1), 261–270 (2004).

265 Gallay P, Swingler S, Song J, Bushman F, Trono D. HIV nuclear import is governed by the phosphotyrosine-mediated binding of matrix to the core domain of integrase. *Cell* 83(4), 569–576 (1995).

266 Oz Gleenberg I, Avidan O, Goldgur Y, Herschhorn A, Hizi A. Peptides Derived from the Reverse transcriptase of human immunodeficiency virus type 1 as novel inhibitors of the viral integrase. *J. Biol. Chem.* 280(23), 21987–21996 (2005).

267 Bischerour J, Tauc P, Leh H, Rocquigny H de, Roques B, Mouscadet J. The (52–96) C-terminal domain of Vpr stimulates HIV-1 IN-mediated homologous strand transfer of mini-viral DNA. *Nucleic Acids Res.* 31(10), 2694–2702 (2003).

268 Catherine S. Adamson EOF. Anti-HIV-1 therapeutics: from FDA-approved Drugs to Hypothetical Future Targets. *Mol. Interv.* 9(2), 70–74 (2009).

269 Pang W, Tam S-C, Zheng Y-T. Current Peptide HIV Type-1 Fusion Inhibitors. *Antivir. Chem. Chemother.* 20(1), 1–18 (2009).

270 Park M, Wetzler M, Jardetzky TS, Barron AE. A readily applicable strategy to convert peptides to peptoid-based therapeutics. *PLoS ONE* 8(3), e58874 (2013).

271 Henchey LK, Jochim AL, Arora PS. Contemporary strategies for the stabilization of peptides in the α-helical conformation. *Curr. Opin. Chem. Biol.* 12(6), 692–697 (2008).

272 Kilby JM, Hopkins S, Venetta TM *et al.* Potent suppression of HIV-1 replication in humans by T-20, a peptide inhibitor of gp41-mediated virus entry. *Nat. Med.* 4(11), 1302–1307 (1998).

273 White CJ & Yudin AK. Contemporary strategies for peptide macrocyclization. *Nat. Chem.* 3, 509–524 (2011).

274 Kazmierski WM, Kenakin TP, Gudmundsson KS. Peptide, peptidomimetic and small-molecule drug discovery targeting HIV-1 host-cell attachment and entry through gp120, gp41, CCR5 and CXCR4†. *Chem. Biol. Drug. Des.* 67(1), 13–26 (2006).

275 Cai L, Jiang S. Development of peptide and small-molecule HIV-1 fusion inhibitors that target gp41. *ChemMedChem* 5(11), 1813–1824 (2010).

276 Brunton LL, Lazo JS, Parker K. *Goodman and Gilmans's The Pharmacological Basis of Therapeutics (11th Edition)*. McGraw-Hill, USA (2006).

277 Flexner C. HIV drug development: the next 25 years. *Nat. Rev. Drug Discov.* 6(12), 959–966 (2007).

278 Wlodawer A. Rational approach to AIDS drug design through structural biology. *Annu. Rev. Med.* 53(1), 595–614 (2002).

279 Liu J, Bartesaghi A, Borgnia MJ, Sapiro G, Subramaniam S. Molecular architecture of native HIV-1 gp120 trimers. *Nature* 455(7209), 109–113 (2008).

280 Dwyer JJ, Wilson KL, Martin K *et al.* Design of an engineered N-terminal HIV-1 gp41 trimer with enhanced stability and potency. *Protein Sci.* 17(4), 633–643 (2008).

281 Das K, Martinez SE, Bauman JD, Arnold E. HIV-1 reverse transcriptase complex with DNA and nevirapine reveals non-nucleoside inhibition mechanism. *Nat. Struct. Mol. Biol.* 19(2), 253–259 (2012).

282 Kitamura S, Ode H, Nakashima M *et al.* The APOBEC3C crystal structure and the interface for HIV-1 Vif binding. *Nat. Struct. Mol. Biol.* 19(10), 1005–1010 (2012).

283 Shandilya SMD, Nalam MNL, Nalivaika EA *et al.* Crystal Structure of the APOBEC3G Catalytic Domain Reveals Potential Oligomerization Interfaces. *Structure* 18(1), 28–38 (2015).

284 Wielens J, Headey SJ, Jeevarajah D *et al.* Crystal structure of the HIV-1 integrase core domain in complex with sucrose reveals details of an allosteric inhibitory binding site. *FEBS Lett.* 584(8), 1455–1462 (2015).

285 Im YJ, Kuo L, Ren X *et al.* Crystallographic and functional analysis of the ESCRT-I /HIV-1 Gag PTAP interaction. *Structure* 18(11), 1536–1547 (2015).