Drug Discovery Today: Technologies
Vol. 6, No. 1–4 2009
Editors-in-Chief
Kelvin Lam – Pfizer, Inc., USA
Henk Timmerman – Vrije Universiteit, The Netherlands
Non Protein Therapeutics

HIV-derived peptide mimics

Kalle Moebius, Jutta Eichler*

Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich Alexander University, Schuhstraße 19, 91052 Erlangen, Germany

Peptides capable of mimicking functionally important regions of HIV proteins are excellent tools to explore structure and function of HIV proteins. Furthermore, such peptides can serve as starting points for novel therapeutic and preventive anti-HIV strategies, including the development of virus entry inhibitors, as well as immunogens to elicit neutralizing antibodies. Recent advances in the design and generation of HIV mimetic peptides are summarized in this article.

Section Editor:
Peter Timmerman – Pepscan Therapeutics B.V., P.O. Box 2098, 8203 AB Lelystad, The Netherlands

Introduction

According to the UNAIDS 2008 Report on the global AIDS epidemic (http://www.unaids.org/en/KnowledgeCentre/HIVData/GlobalReport/2008/2008_Global_report.asp), more than 33 million people worldwide are infected with the human immune deficiency virus (HIV), the causative agent of the acquired immune deficiency syndrome (AIDS), in 2007, and an estimated 2 million people died from AIDS. The inherent ability of HIV to escape the host immune response, as well its high genetic variability, which is caused by the unusually high error rate of the HIV reverse transcriptase (RT) [1], has greatly hampered the sustained development of therapeutic and preventive drugs. At present, no cure is available for the disease, nor is an effective protective HIV vaccine.

Entry of HIV into its host cell, as well as its replication, is mediated by a range of specific interactions between viral and host cell proteins. Peptides mimicking the binding sites of the proteins involved in these interactions are not only valuable tools to explore the respective interactions at the molecular level, but also candidates for therapeutic intervention through specific inhibition of these interactions. The structural (Gag, Pol and Env), regulatory (Tat and Rev) and accessory (Nef, Vif, Vpu and Vpr) proteins encoded by the RNA of HIV (Fig. 1) have been the target for the design of peptides that mimic their binding and functional properties [2].

In general, synthetic peptides can be considered excellent tools for the mimicry of specific protein sites, because they can be generated as exact copies of protein fragments, as well as in diverse chemical modifications, which includes the incorporation of a large range of nonproteinogenic amino acids, as well as the modification of the peptide backbone. Apart from extending the chemical and structural diversity presented by peptides, such modifications also increase the proteolytic stability of the molecules, enhancing their potential as drug candidates.

Strategy 1: peptides mimicking Env proteins

The ability of HIV to enter its host cell is located in its envelope protein (Env) on the surface of the virus. Env forms trimeric spikes and consists of two glycoproteins (gp120 and gp41).

The initial event in the HIV entry process is the interaction of gp120 with the N-terminal extracellular domain of the host receptor CD4 (Fig. 2). The CD4 binding site (CD4bs) of gp120 represents a conserved region in this otherwise highly variable protein, as it is required to maintain virus infectivity. Peptides mimicking the CD4bs are therefore promising candidates as virus entry inhibitors. Furthermore, the epitopes of neutralizing anti-HIV antibodies, in particular mAb b12 [3] have been shown to overlap the CD4bs, establishing this part of gp120 as a target for immunogen design. On the basis of the gp120–CD4 complex structure [4], a synthetic CD4bs

*Corresponding author: J. Eichler (jutta.eichler@medchem.uni-erlangen.de)
URL: http://www.medchem.uni-erlangen.de/, http://www.medchem.uni-erlangen.de/

 DOI: 10.1016/j.ddtec.2009.09.001

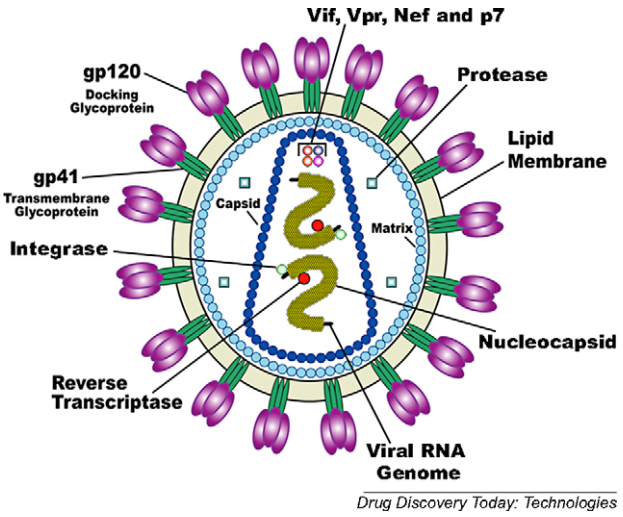

Figure 1. Schematic representation of the human immunodeficiency virus particle depicting the proteins from which mimetic peptides have been derived. The lipid membrane, the RNA molecule and the proteins encoded by HIV are marked. This image is a work of the National Institutes of Health, part of the United States Department of Health and Human Services. As a work of the U.S. federal government, the image is in the public domain.

mimetic peptide was designed and generated, which presents three gp120 fragments that contribute most to its interaction with CD4 [5]. This peptide was shown to compete with gp120 for binding to CD4, as well as to mAb b12. Furthermore, antibodies against this peptide specifically recognized gp120 and competed with mAb b12 for binding to gp120, establishing the molecule as a template for rational vaccine design (Fig. 3).

A peptide that bears no sequential resemblance to the CD4bs, but is recognized by mAb b12, was identified through the screening of phage display libraries [6]. Antibodies against this peptide, however, failed to recognize gp120 [7], indicating that the peptide does not efficiently mimic the epitope of mAb b12.

With the aim of identifying novel epitopes for neutralizing antibodies which could be potentially used as immunogens, phage-displayed peptide libraries were screened for binders to the sera of HIV-infected individuals who did not develop AIDS symptoms over a long period of time (long-term non-progressors, LNTPs) [8]. A range of the identified peptide sequences were homologous to fragments of Env conformational epitopes. Furthermore, immunization of mice with phages presenting such peptides induced antibodies having neutralizing potency against several HIV-1 subtypes. In another study [9], an octapeptide derived from the second conserved region of gp120 was found to be recognized by antisera obtained from LTNPs.

A peptide derived from the V1 loop of gp120 was recognized by antibodies obtained through immunizing guinea pigs with recombinant monomeric and trimeric gp120, respectively [10]. Trimeric soluble gp120, termed gp140, could be obtained by expressing gp120 extended by the short gp41 ectodomain, which contains the Env trimerization information [11].

A peptide derived from the second conserved region (C2) of gp120 was found to cross-react with antibodies raised against human natural anti-vasoactive intestinal peptide (VIP). Although they lack apparent sequence similarities, the reactivity profiles of these two peptides, when tested against a panel of AIDS patient sera, were very similar, indicating a sequence-independent structural mimicry. Using bioinformatic methods, this phenomenon has been attributed to similar physicochemical properties of the peptides [12].

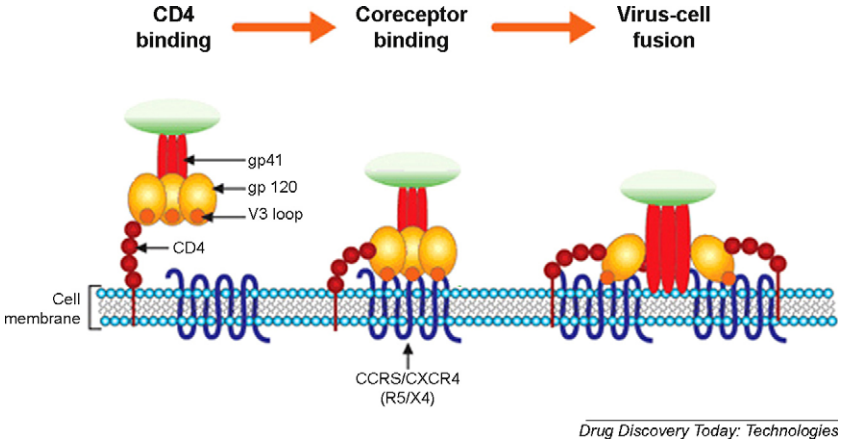

Figure 2. Sequence of HIV interactions during entry into a human host cell. Proteins involved are marked. First, the viral glycoprotein gp120 makes contact with CD4, followed by conformational changes within gp120 that unmask the coreceptor binding site, which then interacts with G-protein-coupled receptors, primarily CCR5 and CXCR4. Further rearrangements lead to the formation of coiled coil structures of gp41 which facilitate fusion of virus and host cell membranes. The figure is derived from [51] by the permission of the author.

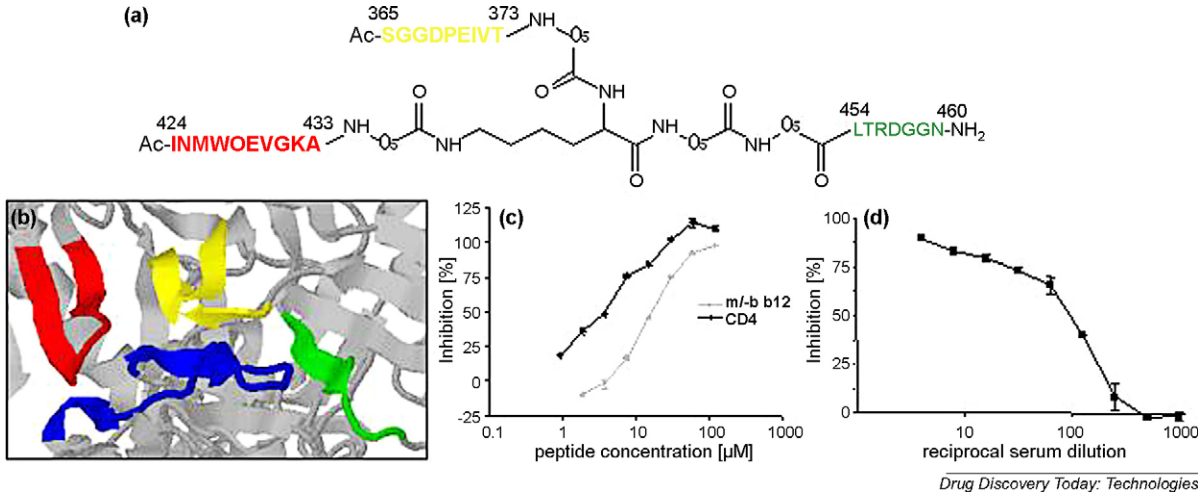

Figure 3. Synthetic mimicry of the CD4bs of HIV-1 gp120 [5]. **(a)** Synthetic peptide presenting the three gp120 CD4bs fragments. **(b)** CD4bs fragments in the crystal structure of a gp120–CD4 complex (pdb code 1rzj). **(c)** Competition of the mimetic peptide with gp120 for binding to CD4 and mAb b12, respectively. **(d)** Competition of anti-peptide antiserum with mAb b12 for binding to gp120.

The structure of a 23 residue peptide corresponding to the fifth conserved region (C5) of gp120, which is thought to directly interact with gp41, has been solved by NMR spectroscopy, providing insight into the interaction of gp120 with gp41 [13].

The V3 loop has been regarded as the principal neutralizing determinant (PND) of gp120, and significant effort has been made to obtain structural and functional information on this domain. Furthermore, the V3 loop is involved in the interaction of gp120 with the second type of cellular receptors, predominantly chemokine receptors CXCR4 and CCR5. Therefore, peptides mimicking the V3 loop are thought to be candidates for entry inhibitors. Because the V3 loop is surface-exposed and therefore well accessible for antibodies, evolutionary pressure has resulted in a large number of V3 variants, which have largely eluded from crystallization so far. Only two crystal structures of gp120 could be solved in which the flexible immunodominant V3 loop was present [14,15].

Alternatively, the structures of peptides presenting the V3 loop of a range of HIV isolates have been obtained in solution by NMR spectroscopy [16]. It should be noted, however, that structures of peptides derived from proteins do not necessarily present their structures within the context of the parent protein [17]. Furthermore, wildtype env, including the V3 loop, is glycosylated, which may result in *in vivo* conformations that are significantly different from structures of recombinant env proteins, or peptides derived from them. To better mimic the V3 loop structure, linear peptides have been conformationally constrained by incorporating a disulfide bridge between cysteine residues flanking an 18 amino acid residue peptide derived from the V3 loop, yielding a β-hairpin peptide [18]. Alternatively, antibodies that recognize the V3 loop in Env can be added to peptides mimicking the V3 loop, thus stabilizing peptide conformations that resemble the V3 loop conformation within the structural context of viral gp120 [19]. Interestingly, these conformations were very similar to those of disulfide-constrained V3 loop peptides. In both cases, the peptides adopted conformations similar to the β-hairpin structure of chemokines, the natural ligands of the receptors (CXCR4 and CCR5) used by HIV as coreceptors [20,21]. Additionally, the acquired results allowed for conclusions about the coreceptor usage switch observed in about half of infected individuals [22]. SPC3, a synthetic multibranched peptide presenting the V3 loop consensus motif GPGRAF was shown to bind specifically to CXCR4 [23], and to be a potent inhibitor of HIV infection of human $CD4^+$ lymphocytes.

To better understand the interaction of the V3 loop with the coreceptors, Galanakis *et al.* solved the NMR structures of various V3 loop peptides in the presence of a peptide corresponding to the N-terminus of CCR5 [24]. On the basis of these structures, electrostatic attractions were proposed as the main driving force for the interaction between these molecules. The V3 loop peptides used in this study, however, were rather short (13–15 amino acids) and presented only the region around the β-turn tip of the V3 loop, not its stem. Furthermore, the CCR5 peptide lacked important sulfate moieties on tyrosine residues, which, in the receptor, strongly influence the net charge of the N-terminus. Therefore, the effects seen in this model system might not mimic the actual gp120–CCR5 interactions. More structural insight into the interaction of the CCR5 N-terminus with gp120 was gained by docking the NMR structure of a peptide presenting the CCR5 N-terminus to the crystal structure of a gp120–antibody complex, which placed the CCR5 peptide at the bridging sheet–V3 loop interface [15]. It should

be noted, however, that the interaction of the coreceptors with gp120 is not solely located in the receptor N-terminus and the V3 loop, respectively. Other regions, in particular the bridging sheet of gp120, as well as the second extracellular loop of the receptors, have also been shown to be involved in that interaction.

Glycoprotein gp41, the membrane-proximal subunit of Env, is responsible for the fusion of viral and host membranes, which is the final step of HIV entry into the host cell.

A 36-mer peptide derived from gp41, termed Enfuvirtide or T20, is the first and thus far only HIV fusion inhibitor approved for clinical use [25]. Binding of this peptide to gp41 prevents viral fusion with the cell membrane. This peptide, however, has become an example for the appearance of drug resistance of HIV, facilitated by mutations during genome replication, enabling the virus to bypass this inhibitor. Indeed, HIV strains that are resistant to Enfuvirtide have already been identified. Such resistance, however, is often accompanied by a reduction of viral fitness, possibly prolonging nonsymptomatic phases after infection [26]. The crystal structure of peptides derived from gp41 has shed some light on the structural basis of the resistance to Enfuvirtide [27].

A second-generation peptide fusion inhibitor, a 39-mer peptide named T1249, is derived from gp41 as well, and binds to gp41 in C-terminal proximity to the Enfuvirtide binding site. Although it is more potent than Enfuvirtide, initial trials with T1249 have been halted because of formulation difficulties [28]. Yet another peptide that binds to the C-terminal region of gp41 has been sequentially modified, resulting in a helical molecule that, as an oligomer, is 3600-fold more effective than Enfuvirtide in preventing viral fusion [29].

Using mirror-image phage display in conjunction with structure-assisted design, a D-amino acid peptide was identified, which, as a trimer, turned out to be a subnanomolar inhibitor of HIV entry [30]. D-Amino acid peptides have the advantage of being resistant to proteolytic degradation. In another study, a peptide containing D-amino acid substitutions, which mimics the gp41 HR2 region, was shown to retain the α-helical conformation of the wildtype sequence [31].

It could be shown that human cells expressing membrane-anchored peptides derived from gp41 were no longer susceptible to HIV infection. Furthermore, a membrane-bound peptide presenting the membrane anchor of gp41 was able to capture N-terminal gp41 peptides, thus functionally mimicking C-terminal gp41 peptides [32].

Using gp41-derived peptides, analysis of the prefusogenic gp120/gp41 interface, which is disrupted upon coreceptor binding, revealed regions of gp41 that are important for its interaction with the gp120 C5 domain [33].

As mentioned above, HIV proteins are able to suppress the host immune response. This effect could be mimicked by peptides derived from Env, which modulate the inflammatory and immunological response of host immune cells. In particular, a peptide derived from gp41 was found to activate the formyl peptide receptor, decreasing interleukin-12 p70, which is associated with immunosuppression [34]. By contrast, peptides derived from the gag protein did not have the same effect on dendritic cells as live virus, implicating that other factors play a role in subsequent immune suppression [35].

Strategy 2: peptides mimicking Pol proteins

The polyprotein encoded by the *pol* gene (Entrez GeneID: 155348) is post-translationally cleaved into four enzymes RT, protease, RNAse and integrase. Inhibitors of these enzymes are components of drug cocktails used in standard anti-retroviral therapy. Most of these enzyme inhibitors are not peptides, but smaller molecules that mimic the substrates of the respective enzymes, thus blocking their active sites.

Nevertheless, peptide mimics of Pol proteins have also been reported, including peptides mimicking the protease dimerization interface, which were found to bind to the monomer, inhibiting the formation of functional homodimeric protease [36,37]. A similar approach was taken by Hayouka *et al.* [38] who could show that peptides derived from Rev interact with integrase and inhibit its activity *in vitro* and in cells by shifting the oligomerization equilibrium of integrase toward the inactive tetrameric state.

Many peptides presenting fragments of HIV proteins have been structurally analyzed in the 1990s [39]. A recent study compared the structure of integrase, which catalyzes the integration of viral cDNA into the host genome, with the structures of peptides presenting integrase fragments that cover its catalytic site. The peptides were shown to adopt different conformations in a concentration-dependent fashion [40].

Strategy 3: peptides mimicking Gag proteins

The protein encoded by the *gag* gene (GeneID: 155348) is proteolytically processed to yield the matrix, nucleocapsid and capsid proteins, as well as p6. The correct assembly of these proteins is crucial for the formation of mature capsids. Consequently, inhibition of Gag proteins is likely to slow down virus replication.

A range of peptides involved in the inhibition of viral replication have been identified [2], many of them derived from Gag proteins. A peptide presenting residues 169–191 of the capsid protein was found to be an inhibitor of capsid assembly [41].

Azizi *et al.* developed a multivalent vaccine composed of 176 linear peptides mimicking predicted epitopes of Env and Gag [42]. Despite the inherent flexibility of linear peptides, immunization of macaques with this cocktail elicited antibodies that were able to neutralize many T cell line adapted (TCLA) and primary HIV isolates. This result demonstrates how the use of multivalent immunogens presenting different

Table 1. Peptides mimicking HIV proteins (selection)

HIV genes	Regions mimicked by peptide(s)	Biological activity/molecular tool for the analysis of	Modifications/notes	Reference
***env* GeneID: 155971**	Structural mimicry *gp41*	Immunogen	Phage display	[8]
	Env (638–673)	Fusion inhibitor	T20	[25]
	Env (620–657)	Fusion inhibitor	Coiled coil	[29]
		Fusion inhibitor	D-Peptide	[30]
		Structure analysis	Disulfide bonds	[33]
	Env (628–661)	Fusion inhibitor	D-Amino acids	[31]
	Env (628–673) *gp120*	Fusion inhibitor/gene therapy	CD34 fusion	[32]
	Env (280–287)	Immunogen	–	[9]
	V1, 2 and 3	Immune response	–	[10]
	133–155	Immunogen	–	[12]
	CD4 binding site	Immunogen	Scaffold	[5]
	Structural mimicry	Immunogen/structure	Phage display	[7]
	V3	Fusion inhibitor	Multibranched	[23]
	V3	Structure analysis	–	[21]
	V3	Structure analysis	–	[20]
	V3	Structure analysis	Disulfide bonds	[18]
***gag* GeneID: 155348**	p17, p24	Immunogen	Allosteric biosensor	[43]
	(362–377 + 461–489)	Immunogen	Multivalent	[42]
***pol* GeneID: 155348**	Whole	Inhibition of dimerization	Review	[37]
	Protease (83–93)	Inhibition of dimerization	–	[36]
	Integrase (IN) (147–175)	Structure analysis	–	[40]
	IN (63–87)	Inhibition of dimerization	–	[52]
Accessory genes				
***vpr* GeneID: 155807**	Vpr (57–71 + 61–75)	Inhibition	–	[46]
***nef* GeneID:156110**	Nef	Immunogen	–	[45]
***tat* GeneID: 155871**	(48–60)	Nuclear targeting	–	[49]
	(49–61)	Inhibition of nuclear transport	Cyclic	[48]
		Inhibition of RNA binding	β-Hairpin	[53]
***rev* GeneID: 155908**		Inhibition of RNA binding	β-Hairpin	[54]
		Inhibition of RNA binding	α-Helical	[55]
		Inhibition of integrase	Inhibits integrase	[38]

antigens can improve the protective capacity of vaccines against infection with diverse HIV strains.

An interesting approach to test HIV peptides for their antigenicity is to incorporate Env and Gag protein sequences into β-galactosidase, which is then activated allosterically upon binding of sera of HIV-infected individuals [43]. Only peptides derived from the Env proteins gp120 and gp41 activated the biomarker significantly. Also, a sequence derived from the CD4 binding site of gp120 induced allosteric activation when incubated with soluble CD4, indicating that the peptide, when inserted into the sequence of β-galactosidase, adopts conformations capable of binding to CD4.

Strategy 4: peptides mimicking accessory proteins

In addition to the structural and regulatory proteins discussed above, the HIV genome also encodes four accessory proteins (Nef, Vif, Vpu, Vpr). During viral replication, these proteins alter cellular pathways via multiple interactions with host cell proteins. Inhibiting these interactions is a promising strategy to inhibit HIV replication.

Peptides derived from Env and Nef, respectively, have been used to enhance mucosal and systemic immune responses against HIV [44].

Two peptides derived from the Vpr protein were found to bind and inhibit the RT, which converts viral RNA into double stranded DNA. The function of integrase, responsible for the integration of this DNA into the human genome, was impaired as well, indicating that interactions between Vpr, RT and integrase are essential for the correct function of these proteins [45].

Strategies for the inhibition of accessory HIV proteins, including the use of peptides, were recently reviewed [46].

Strategy 5: peptides mimicking Tat proteins

The gene *tat* (GeneID: 155871) encodes for a transactivator protein which binds to a region (TAR) on newly synthesized

viral RNA. On the basis of the solution structure of Tat, cyclic peptides were synthesized which functionally mimic the Tat arginine-rich motif. They were tested for their ability to facilitate nuclear localization of peptide conjugates with bovine serum albumin (BSA). It was shown that these peptides hindered Tat-NLS-BSA to enter the nucleus *in vitro*. Additionally, the peptides were found to have inhibitory effects on the interaction of the Rev protein with its corresponding RNA, thus possessing dual functions [47].

Some of the proteins encoded by the HIV genome have properties that are useful for research fields and therapeutic applications unrelated to HIV and AIDS. A prominent example for such applications are arginine-rich peptides derived from Tat [48], which are able to penetrate cellular and nuclear membranes by itself, as well as in conjugates with other, even much larger molecules.

Although the exact mechanism of internalization, in particular the question whether or not it is based on endocytosis [49], has been a subject of controversial discussion, these peptides were the starting point for the development of a whole class of cell-penetrating peptides (CPPs) used for the translocation of molecules, including proteins, into cells, as well as into the nucleus [50]. This process is essential in the development of drugs based on bioactive molecules having intracellular or intranuclear targets.

Conclusion

Over the past decades, peptides that structurally and/or functionally mimic HIV proteins have significantly contributed to the molecular and structural understanding of HIV live cycle and infection pathways (Table 1). Moreover, such peptides are promising candidates for therapeutic and preventive anti-HIV strategies, because they can be used as inhibitors of virus–host interactions that are essential for virus entry and replication. Furthermore, peptides that mimic the binding sites of HIV proteins for their host cell receptors are immunogen candidates for new vaccine strategies. This aspect is of particular importance in light of the persisting neutralizing antibody problem in the field of HIV vaccine development, whose solution will clearly require innovative strategies in the immunogen design. It should be noted, although, that the utility of peptides as bioactive protein mimetics is often limited by the high conformational flexibility of linear peptides, which hamper the mimicry of conformationally defined, less flexible protein sites. This apparent drawback, however, can often be avoided by conformationally constraining peptides through cyclization, or by incorporating chemical moieties into the peptide sequence which either induce or stabilize secondary structures. It can be expected that such complex, conformationally defined peptides will be increasingly useful as mimetics of HIV proteins.

References

1 Bebenek, K. *et al.* (1993) Error-prone polymerization by HIV-1 reverse transcriptase. Contribution of template-primer misalignment, miscoding, and termination probability to mutational hot spots. *J. Biol. Chem.* 268, 10324–10334

2 Kaushik-Basu, N. *et al.* (2008) Peptide inhibition of HIV-1: current status and future potential. *BioDrugs* 22, 161–175

3 Burton, D.R. *et al.* (1994) Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* 266, 1024–1027

4 Kwong, P.D. *et al.* (1998) Structure of an HIVgp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 1998, 648–659

5 Franke, R. *et al.* (2007) Synthetic mimetics of the CD4 binding site of HIV-1 gp120 for the design of immunogens. *Angew. Chem. Int. Ed. Engl.* 46, 1253–1255

6 Zwick, M.B. *et al.* (2001) Identification and characterization of a peptide that specifically binds the human, broadly neutralizing anti-human immunodeficiency virus type 1 antibody b12. *J. Virol.* 75, 6692–6699

7 Saphire, E.O. *et al.* (2007) Structure of a high-affinity 'mimotope' peptide bound to HIV-1-neutralizing antibody b12 explains its inability to elicit gp120 cross-reactive antibodies. *J. Mol. Biol.* 369, 696–709

8 Humbert, M. *et al.* (2007) Mimotopes selected with antibodies from HIV-1-neutralizing long-term non-progressor plasma. *Eur. J. Immunol.* 37, 501–515

9 Djordjevic, A. *et al.* (2007) The presence of antibodies recognizing a peptide derived from the second conserved region of HIV-1 gp120 correlates with non-progressive HIV infection. *Curr. HIV Res.* 5, 443–448

10 Li, Y. *et al.* (2006) Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimeric and monomeric envelope glycoproteins in selected adjuvants. *J. Virol.* 80, 1414–1426

11 Zhang, C.W-H. *et al.* (2001) Expression, purification, and characterization of recombinant HIV gp140. *J. Biol. Chem.* 276, 39577–39585

12 Veljkovic, N. *et al.* (2003) Design of peptide mimetics of HIV-1 gp120 for prevention and therapy of HIV disease. *J. Pept. Res.* 62, 158–166

13 Guilhaudis, L. *et al.* (2002) Solution structure of the HIV gp120 C5 domain. *Eur. J. Biochem.* 269, 4860–4867

14 Huang, C. *et al.* (2005) Structure of a V3-containing HIV-1 gp120 core. *Science* 310, 1025–1028

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

16 Hartley, O. *et al.* (2005) V3: HIV's switch-hitter. *AIDS Res. Hum. Retroviruses* 21, 171–189

17 Zvi, A. *et al.* (1992) Solution conformation of a peptide corresponding to the principal neutralizing determinant of HIV-1IIIB: a two-dimensional NMR study. *Biochemistry* 31, 6972–6979

18 Mor, A. *et al.* (2009) Mimicking the structure of the V3 epitope bound to HIV-1 neutralizing antibodies. *Biochemistry* 48, 3288–3303

19 Dhillon, A.K. *et al.* (2008) Structure determination of an anti-HIV-1 Fab 447-52D–peptide complex from an epitaxially twinned data set. *Acta Crystallogr. D: Biol. Crystallogr.* 64, 792–802

20 Rosen, O. *et al.* (2005) Induced fit in HIV-neutralizing antibody complexes: evidence for alternative conformations of the gp120 V3 loop and the molecular basis for broad neutralization. *Biochemistry* 44, 7250–7258

21 Sharon, M. *et al.* (2003) Alternative conformations of HIV-1 V3 loops mimic beta hairpins in chemokines, suggesting a mechanism for coreceptor selectivity. *Structure* 11, 225–236

22 Ho, S.H. *et al.* (2007) Coreceptor switch in R5-tropic simian/human immunodeficiency virus-infected macaques. *J. Virol.* 81, 8621–8633

23 Barbouche, R. *et al.* (2000) Relationships between the anti-HIV V(3)-derived peptide SPC(3) and lymphocyte membrane properties involved in virus entry: SPC(3) interferes with CXCR(4). *FEMS Microbiol. Lett.* 183, 235–240

24 Galanakis, P.A. *et al.* (2009) NMR evidence of charge-dependent interaction between various PND V3 and CCR5 N-terminal peptides. *Biopolymers* 92, 94–109

25 Champagne, K. *et al.* (2009) Interactions of HIV-1 inhibitory peptide T20 with the gp41 N-HR coiled coil. *J. Biol. Chem.* 284, 3619–3627

26 Lu, J. *et al.* (2004) Relative replicative fitness of human immunodeficiency virus type 1 mutants resistant to enfuvirtide (T-20). *J. Virol.* 78, 4628–4637

27 Bai, X. *et al.* (2008) Impact of the enfuvirtide resistance mutation N43D and the associated baseline polymorphism E137K on peptide sensitivity and six-helix bundle structure. *Biochemistry* 47, 6662–6670

28 Martin-Carbonero, L. (2004) Discontinuation of the clinical development of fusion inhibitor T-1249. *AIDS Rev.* 6, 61

29 Dwyer, J.J. *et al.* (2007) Design of helical, oligomeric HIV-1 fusion inhibitor peptides with potent activity against enfuvirtide-resistant virus. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12772–12777

30 Welch, B.D. *et al.* (2007) Potent D-peptide inhibitors of HIV-1 entry. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16828–16833

31 Gaston, F. *et al.* (2009) Development and characterization of peptidic fusion inhibitors derived from HIV-1 gp41 with partial D-amino acid substitutions. *ChemMedChem* 4, 570–581

32 Egelhofer, M. *et al.* (2004) Inhibition of human immunodeficiency virus type 1 entry in cells expressing gp41-derived peptides. *J. Virol.* 78, 568–575

33 Kim, S. *et al.* (2008) Peptide mimic of the HIV envelope gp120–gp41 interface. *J. Mol. Biol.* 376, 786–797

34 Braun, M.C. *et al.* (2001) Activation of the formyl peptide receptor by the HIV-derived peptide T-20 suppresses interleukin-12 p70 production by human monocytes. *Blood* 97, 3531–3536

35 Granelli-Piperno, A. *et al.* (2004) HIV-1-infected monocyte-derived dendritic cells do not undergo maturation but can elicit IL-10 production and T cell regulation. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7669–7674

36 Bonomi, M. *et al.* (2007) Insight into the folding inhibition of the HIV-1 protease by a small peptide. *Biophys. J.* 93, 2813–2821

37 Camarasa, M.J. *et al.* (2006) Dimerization inhibitors of HIV-1 reverse transcriptase, protease and integrase: a single mode of inhibition for the three HIV enzymes? *Antiviral Res.* 71, 260–267

38 Hayouka, Z. *et al.* (2008) Peptides derived from HIV-1 Rev inhibit HIV-1 integrase in a shiftide mechanism. *Biopolymers* 90, 481–487

39 Frankel, A.D. and Young, J.A. (1998) HIV-1: fifteen proteins and an RNA. *Annu. Rev. Biochem.* 67, 1–25

40 Porumb, H. *et al.* (2004) Circular dichroism and fluorescence of a tyrosine side-chain residue monitors the concentration-dependent equilibrium between U-shaped and coiled-coil conformations of a peptide derived from the catalytic core of HIV-1 integrase. *Biochim. Biophys. Acta* 1699, 77–86

41 Sticht, J. *et al.* (2005) A peptide inhibitor of HIV-1 assembly in vitro. *Nat. Struct. Mol. Biol.* 12, 671–677

42 Azizi, A. *et al.* (2008) Induction of broad cross-subtype-specific HIV-1 immune responses by a novel multivalent HIV-1 peptide vaccine in cynomolgus macaques. *J. Immunol.* 180, 2174–2186

43 Ferraz, R.M. *et al.* (2009) Screening HIV-1 antigenic peptides as receptors for antibodies and CD4 in allosteric nanosensors. *J. Mol. Recognit.* 22, 255–260

44 Pun, P.B. *et al.* (2009) Intranasal administration of peptide antigens of HIV with mucosal adjuvant CpG ODN coentrapped in microparticles enhances the mucosal and systemic immune responses. *Int. Immunopharmacol.* 9, 468–477

45 Gleenberg, I.O. *et al.* (2007) Inhibition of the activities of reverse transcriptase and integrase of human immunodeficiency virus type-1 by peptides derived from the homologous viral protein R (Vpr). *J. Mol. Biol.* 369, 1230–1243

46 Richter, S.N. *et al.* (2009) Strategies for inhibiting function of HIV-1 accessory proteins: a necessary route to AIDS therapy? *Curr. Med. Chem.* 16, 267–286

47 Friedler, A. *et al.* (2000) Development of a functional backbone cyclic mimetic of the HIV-1 Tat arginine-rich motif. *J. Biol. Chem.* 275, 23783–23789

48 Sandgren, S. *et al.* (2002) Nuclear targeting of macromolecular polyanions by an HIV-Tat derived peptide. Role for cell-surface proteoglycans. *J. Biol. Chem.* 277, 38877–38883

49 Richard, J.P. *et al.* (2003) Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake. *J. Biol. Chem.* 278, 585–590

50 Fonsecaa, S.B. *et al.* (2009) Recent advances in the use of cell-penetrating peptides for medical and biological applications. *Adv. Drug Deliv. Rev.* 10.1016/j.addr.2009.06.001

51 Ray, N. and Doms, R.W. (2006) HIV-1 coreceptors and their inhibitors. *Curr. Top. Microbiol. Immunol.* 303, 97–120

52 Fermandjian, S. *et al.* (2001) Self-association of an amphipathic helix peptide inhibitor of HIV-1 integrase assessed by electro spray ionization mass spectrometry in trifluoroethanol/water mixtures. *Rapid Commun. Mass Spectrom.* 15, 320–324

53 Athanassiou, Z. *et al.* (2004) Structural mimicry of retroviral tat proteins by constrained beta-hairpin peptidomimetics: ligands with high affinity and selectivity for viral TAR RNA regulatory elements. *J. Am. Chem. Soc.* 126, 6906–6913

54 Moehle, K. *et al.* (2007) Design of beta-hairpin peptidomimetics that inhibit binding of alpha-helical HIV-1 Rev protein to the rev response element RNA. *Angew. Chem. Int. Ed. Engl.* 46, 9101–9104

55 Mills, N.L. *et al.* (2006) An alpha-helical peptidomimetic inhibitor of the HIV-1 Rev–RRE interaction. *J. Am. Chem. Soc.* 128, 3496–3497