Blocking HIV entry

Oleksandr V. Buzko and Kevan M. Shokat

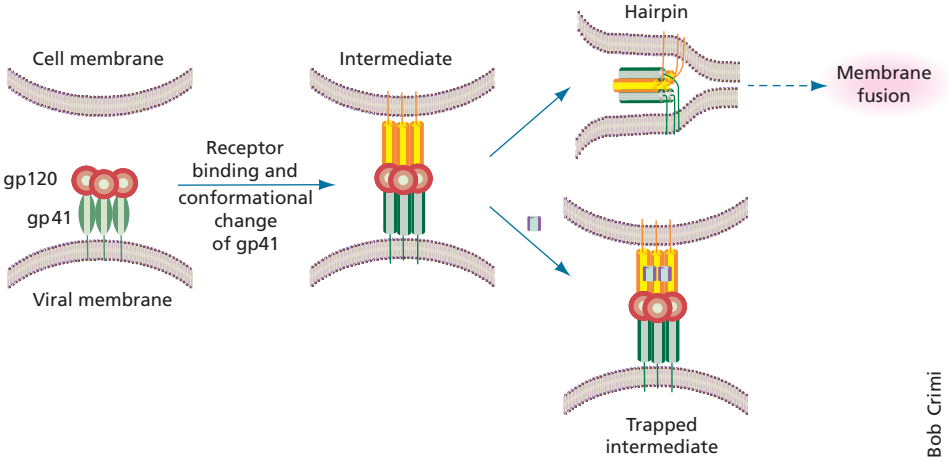
Targeting the fusogenic machinery of HIV with unnatural ligand libraries.

The intense study of the life cycle of HIV has created new and promising targets for drug discovery efforts. The first agents discovered to have therapeutic efficacy in the treatment of AIDS were targeted to viral enzymes (HIV protease[1–3] and reverse transcriptase[4]). The rapid development of drug resistant HIV strains has fueled continued efforts to find new therapeutic anti-HIV agents. In particular, protein components of HIV's fusion machinery have been intensely studied by X-ray crystallography. Such proteins are atypical drug targets because they are not enzymatically active, thus making catalytic activity based screens impossible. Furthermore, such proteins are involved in forming protein–protein complexes, which can be difficult to disrupt using typical small drug-like (<750 Da) molecules. On page 953 of this issue of *Nature Structural Biology*, Ferrer *et al.*[5] describe their efforts to develop non-natural ligands capable of targeting the fusogenic machinery of HIV.

Enveloped viruses enter target cells in a two-step process that involves recognition of the host cell and binding to cell surface receptors followed by fusion of cellular and viral membranes. Glycoproteins incorporated in the viral membrane have been shown to play a central role in both of these processes. In HIV-1, these functions are performed by glycoproteins gp41 and gp120 generated by proteolytic cleavage of the polyprotein precursor gp160 by host cell proteases (Fig. 1). This leads to the formation of a surface subunit (gp120) responsible for recognition and binding to cell surface CD4 receptors[6] as well as CCR5 and CXCR4 chemokine receptors[7]. The gp41 transmembrane component contains a highly helical ectodomain (the extraviral portion of gp41) that provides an efficient mechanism for membrane fusion initiation. The importance of gp41 in the process of viral entry suggests that it could be a promising therapeutic target[8].

The structure of the ectodomain of gp41 has been studied by X-ray diffraction[9,10]. The core structure is a six-helix bundle, in which the C-terminal helices (C34, green in Fig. 1) pack around the N-terminal helices (N36, yellow in Fig. 1). During the binding to target cell surface receptors, the gp41 ectodomain undergoes a conformational change[11,12] that 'activates' it by exposing the hydrophobic N-terminal core of the trimeric structure[13], which initiates membrane fusion. It has been found that the hydrophobic gp41 core has a highly conserved amino acid sequence, which is thought to be responsible for trimerization of gp41[14]. The sequence conservation in this region strongly suggests that viruses are unlikely to develop resistance to agents that target HIV's fusogenic machinery.

The first agents shown to disrupt HIV's fusogenic machinery were peptide fragments of gp41 itself. These peptide inhibitors mimic the sequence of the outer core (C-terminal) of the gp41 ectodomain and bind to the hydrophobic inner core (N-terminal), thus preventing it from contacting the target cell membrane[15–19]. One such peptide, DP-178, has been shown to significantly reduce plasma concentrations of HIV-RNA in clinical trials[20]. This is a surprising result given that the target of the DP-178 peptide is a transient intermediate along the pathway (the so called 'pre-hairpin intermediate). The fact that DP-178 can act in a dominant negative fashion by binding to a required intermediate along the pathway to viral fusion demonstrates that the gp41 ectodomain is a viable drug target). The fact that this target is a transient intermediate during the HIV life cycle makes it an exciting challenge for drug design efforts. The difficulties are compounded by the fact that peptides are often not useful as drugs because of their sensitivity to protease degradation and poor oral bioavailability.



Bob Crimi

Fig. 1 Membrane fusion events associated with HIV entry into a target cell. Upon receptor binding a conformational change in gp41 induces exposure of the highly hydrophobic N-terminal domain of gp41 (yellow), which penetrates the membrane of the target cell. This intermediate appears to be the target of anti-HIV peptides such as DP-178 (purple). In the absence of such inhibitors, the intermediate resolves into another structure, which is depicted in the upper pathway leading to completion of membrane fusion. This figure is based on Fig. 2 in ref. 24.

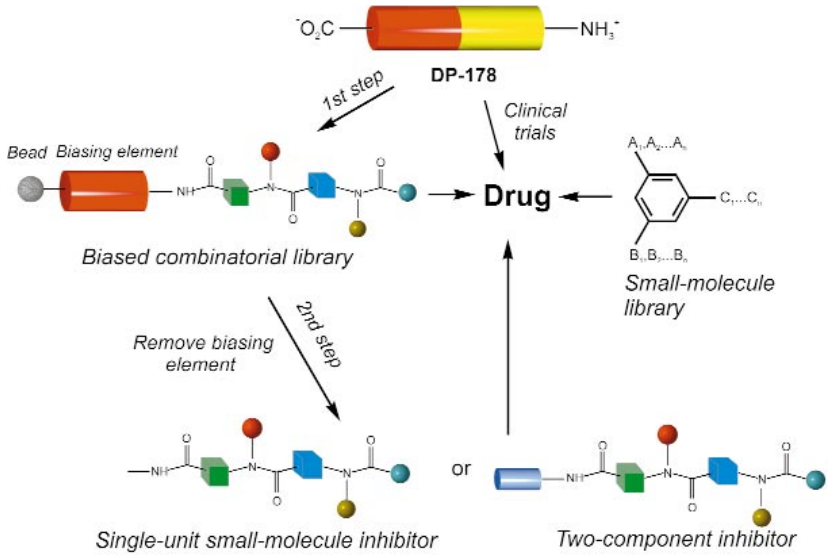


Fig. 2 Stepwise approach to conversion of anti-HIV peptide DP-178 into a small molecule anti-HIV drug. The first step involves biasing of a combinatorial library of small molecules (cubes and spheres represent variable elements) attached to components of the DP-178. In the second step, the biasing element (red cylinder) is removed or replaced with a non-natural positioning element (if complete removal of the biasing element greatly reduces affinity). An alternative approach is to screen a small-molecule library for anti-HIV agents without peptide components as biasing elements.

The challenge addressed by Ferrer *et al.*[5] is the conversion of the anti-HIV agent, DP-178, into a small molecular weight (<750 Da) species capable of binding to the gp41 ectodomain. They began by asking two important questions — which region of DP-178 is required for inhibition and which region of the binding site for DP-178 is most amenable to small-molecule targeting? Several previous reports have suggested that DP-178 is difficult to reduce in size without abolishing its activity[1]. Kim and coworkers highlighted the presence of a deep cavity in the inner core of the gp41 ectodomain that is occupied by Trp 117, Trp 120 and Ile 124 of the outer core α-helices and suggested that this hydrophobic pocket might be amenable to drug targeting[9]. Such a deep hydrophobic cavity is an attractive site for binding of small hydrophobic organic molecules.

What is the best way to identify non-natural molecules that bind to a protein surface when no active site is available for more standard drug discovery approaches? It is worth noting that the disruption of a protein–protein interface requires a significantly higher affinity for the target than is necessary to bind to an enzyme active site and prevent substrate binding. Thus, if one screens large libraries of compounds that are not biased toward binding the target site, chances are that few, if any, hits will be found. Is there a way to use the structure of a known peptide inhibitor to bias a combinatorial library of non-natural binding elements? Such a model of the drug discovery process can be schematically presented as a stepwise transition from the original peptide inhibitor to a small-molecule inhibitor (Fig. 2). The final inhibitor could also be a two-component system including a positioning element, should a single unit be insufficient for specific binding to a target. This general approach has been pioneered by Schreiber and coworkers to discover SH3 ligands[21], and is applied here to the problem of identifying small molecules that target the gp41 ectodomain.

Ferrer *et al.*[5] constructed a combinatorial peptide library of 61,275 members each of which contain a fragment of the gp41 α-helix (residues 125–142, Fig. 2, red helix). Residues 117–124 (WMEWDREI, Fig. 2, yellow helix) of the original gp41 sequence were substituted with non-natural amino acids linked by amide bonds. These residues were chosen for substitution based on the suggestion by Kim and coworkers[9] that they contact the binding site primarily through hydrophobic interactions.

After on-bead screening of the library for binding to the gp41 target using a colorimetric assay, one particularly potent ligand was chosen for further study. Interestingly, the selected ligand contained two carboxyl groups and a cyclopentyl ring. This is somewhat surprising since the randomized library was significantly biased toward hydrophobic moieties designed to be good binders to the hydrophobic inner core. The selected ligand showed potent inhibition of cell fusion and is only ~50-fold less potent in this assay than the natural peptide, DP-178.

The results of the present study indicate that a constrained combinatorial library of binding elements can be successfully used to address the problem of finding novel peptide inhibitors. The procedure could be further improved by collecting more information about structural elements of the inner core of gp41 involved in the complex. As noted by the authors, a crystallographic study of the complex of the gp41 core and the inhibitor would be extremely valuable. The colorimetric assay used for selection of candidate peptides also appears to be a valuable technique for rapid library screening, as it can be coupled with automated imaging systems to allow for faster detection of positive hits[22,23].

Finally, the gp41 ectodomain could also be used as a target for the treatment of AIDS by eliciting neutralizing antibodies. HIV vaccines that have been developed in the past have not had the benefit of having a distinct molecular target as attractive as the gp41 ectodomain. Its transient nature during viral entry may preclude it from being an effective immunogen in HIV infected individuals. However, the GCN4-linked version of gp41 may serve as a good mimic of the natural gp41 ectodomain and, thus, a good immunogen.

To date, vaccine candidates have been shown to elicit neutralizing antibodies to laboratory-adapted isolates of HIV but not primary isolates (reviewed in ref. 24). In contrast, antibodies from actively infected persons can partially neutralize the infectivity of primary isolate viruses. Thus, there is a real need to develop envelope protein preparations from primary isolates that are in active conformations. The idea that fusion-competent immunogens can elicit a strong antibody response was recently tested and validated in a mouse model using a formaldehyde-fixed whole cell vaccine[25]. Together, these advances suggest that drugs targeted against intermediates (or mimics) along the fusion pathway and antibodies raised against the same intermediates hold some promise for the future development of both drugs and vaccines against HIV.

Oleksandr Buzko and Kevan M. Shokat are in the Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94143-0450, USA. Correspondence should be addressed to K.M.S. email: shokat@cmp.ucsf.edu

1. Collier, A.C. *et al. J. Med* **334**, 1011–1017 (1996).
2. Cameron, D.W. *et al. AIDS* 1999. **13**, 212–224 (1999).
3. Humphrey, R.W. *et al. Antiviral Res.* **41**, 21–33 (1999).
4. Henry, K. *et al. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* **19**, 339–349 (1998).
5. Ferrer, M. *et al. Nature Struct. Biol.* **6**, 953–960 (1999).
6. Freed, E.O. & Martin, M.A. *J. Biol. Chem.* **270**, 23883–23886 (1995).
7. Doranz B. J. *et al. J. Exp. Med.* **186**, 1395–1400 (1997).
8. Kazmierski, W.M., Hazen, R.J., Aulabaugh, A. & StClair, M.H. *J. Med. Chem.* **39**, 2681–2689 (1996).
9. Chan, D.C., Fass, D., Berger, J.M. & Kim, P.S. *Cell* **89**, 263–273 (1997).
10. Weissenhorn, W., Dessen, A., Harrison, S. C., Skehel, J. J. & Wiley, D. C. *Nature* **387**, 426–430 (1997).
11. Furuta, R.A., Wild, C.T., Weng, Y. & Weiss, C.D. *Nature Struct. Biol.* **5**, 276–279 (1998).
12. Weng, Y. & Weiss, C. D. *J. Virol.* **72**, 9676–9682 (1998).
13. Sattentau, Q.J., Moore, J.P. *J. Exp. Med.* **174**, 407–415 (1991).
14. Shu, W. & Lu, M. *Biochemistry* **38**, 5378–5385 (1999).
15. Wild, C., Greenwell, T. & Matthews, T. *AIDS Res. Hum. Retroviruses* **9**, 1051–1053 (1993).
16. Callebaut, C. *et al. Virology* **218**, 181–192 (1996).
17. Salzwedel, K., West, J.T. & Hunter, E. *J. Virol.* **73**, 2469–2480 (1999).
18. Lamb, R.A., Joshi, S.B. & Dutch, R.E. *Mol. Membr. Biol.* **16**, 11–19 (1999).
19. Judice, J.K *et al. Proc. Natl. Acad. Sci. USA* **94**, 13426–13430 (1997).
20. Kilby, J. M. *et al. Nature Med.* **4**, 1302–1307 (1998).
21. Feng, S., Kapoor, T.M., Shirai, F., Combs, A.P. & Schreiber, S.L. *Chem. Biol.* **3**, 661–670 (1996).
22. Uber, D.C., Jaklevic, J.M., Theil, E.H., Lishanskaya, A. & McNeely, M.R. *Biotechniques* **11**, 642–647 (1991).
23. Cai, W. *et al. Proc. Natl. Acad. Sci. USA.* **95**, 3390–3395 (1998).
24. Chan, D.C. & Kim, P.S. *Cell* **93**, 681–684 (1998).
25. LaCasse, R.A. *et al. Science* **283**, 357–362 (1999).

history

Holliday model of recombination

The structure of an all-DNA four-way junction is presented on page 913 of this issue of *Nature Structural Biology* and is discussed in the News and Views report on page 897. The historical interest of such structures stems directly from the challenge of uncovering the mechanisms of genetic recombination and, in particular, of explaining a complex genetic phenomenon — the aberrant segregation of alleles.

Aberrant segregation was first clearly noted in the 1950s in genetic studies of certain fungi that allow direct examination of their four haploid products of meiosis. Normally in these fungi, a heterozygous locus yields a 2:2 ratio of products (for example, 2 wild type and two mutant alleles). However in some cases, an aberrant segregation ratio, such as 3:1 or 4:0, can occur — a phenomenon known as gene conversion.

There are two types of meiotic recombination: reciprocal (crossing over) and non-reciprocal (gene conversion). Crossing over results in the exchange of genetic markers that flank the cross over site, whereas gene conversion results in the unidirectional substitution of a small region of one chromosome for the corresponding sequence of the homologous chromosome. Gene conversion is often associated with a nearby cross over, which suggests that these two events are the products of the same recombination pathway.

In the early 1960s, the most common explanation for gene conversion was the copy choice model — that is, replication of the genetic material occurs during or after chromosome pairing and the replication machinery can switch from using one chromosome as a template to using its sister chromosome as a template. However, this model made predictions that were not fully supported by genetic findings in many organisms. Moreover, it could not explain the frequent association of non-reciprocal recombination with reciprocal recombination.

In 1964, Robin Holliday proposed a different model to explain gene conversion, one that portrays a more accurate representation of the molecular mechanisms of cell division and recombination (as we now understand them) and one that illuminates the relationship between gene conversion and crossing over:

"It is suggested that along with the general genetic pairing of homologous genomes at meiosis, effective pairing over short regions of the genetic material occurs at the molecular level by the separation of the strands of the DNA double helices, followed by the annealing of strands from two homologous chromatids. If the annealed region happens to span a heterozygous site,

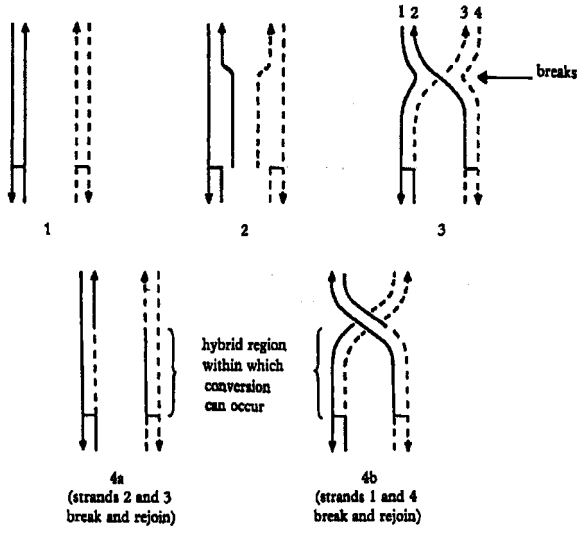


Fig. 1 Holliday's proposal for the intermediate in genetic recombination. Solid lines represent the DNA strands of one chromatid and dashed lines represent those of the other chromatid. The 3' end of a strand is indicated by an arrow. The short horizontal lines mark the positions of the nicks that initiate strand exchange. The numbers below each chromatid pair indicate the order of events, with 4a and 4b representing the possible outcomes of resolution of the four-way junction by breakage and rejoining of different strands. Adapted from ref. 1.