Different from the HIV Fusion Inhibitor C34, the Anti-HIV Drug Fuzeon (T-20) Inhibits HIV-1 Entry by Targeting Multiple Sites in gp41 and gp120*

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Fuzeon (also known as T-20 or enfuvirtide), one of the C-peptides derived from the HIV-1 envelope glycoprotein transmembrane subunit gp41 C-terminal heptad repeat (CHR) region, is the first member of a new class of anti-HIV drugs known as HIV fusion inhibitors. It has been widely believed that T-20 shares the same mechanism of action with C34, another C-peptide. The C34 is known to compete with the CHR of gp41 to form a stable 6-helix bundle (6-HB) with the gp41 N-terminal heptad repeat (NHR) and prevent the formation of the fusogenic gp41 core between viral gp41 NHR and CHR, thereby inhibiting fusion between viral and target cell membranes. Here we present data to demonstrate that, contrary to this belief, T-20 cannot form stable 6-HB with N-peptides derived from the NHR region, nor can it inhibit the 6-HB formation of the fusogenic core. Instead, it may interact with N-peptides to form unstable or insoluble complexes. Our data suggest that T-20 has a different mechanism of action from C34. The interaction of T-20 with viral NHR region alone may not prevent the formation of the fusion active gp41 core. We also demonstrate that the T-20-mediated anti-HIV activity can be significantly abrogated by peptides derived from the membrane-spanning domain in gp41 and coreceptor binding site in gp120. These new findings imply that T-20 inhibits HIV-1 entry by targeting multiple sites in gp41 and gp120. Further elucidation of the mechanism of action of T-20 will provide new target(s) for development of novel HIV entry inhibitors.

Human immunodeficiency virus type 1 (HIV-1)1 envelope glycoprotein (Env), a type I transmembrane protein, plays an important role in the early stage of HIV entry. Its surface subunit gp120 is responsible for virus binding to receptor and coreceptors (1–4), and the transmembrane subunit gp41 mediates fusion of the virus with the target cell (5, 6). Like other type I transmembrane proteins, gp41 consists of cytoplasm (CP), transmembrane (TM), and extracellular domains. The extracellular domain (ectodomain) contains four major functional regions: fusion peptide (FP), N-terminal heptad repeat (NHR or HR1), C-terminal heptad repeat (CHR or HR2), and a tryptophan-rich (TR) region.

Starting form the early 1990s, several peptides derived from the NHR and CHR regions of gp41, designated N- and C-peptides, respectively (Fig. 1A), were discovered to have the viral fusion inhibition activity. The first N-peptide fusion inhibitor, T-21, was identified by Wild et al. (7), which blocked HIV-1 fusion at micromolar concentrations. The first C-peptide fusion inhibitor, SJ-2176, was discovered by our group, which inhibited HIV-1 replication at nanomolar levels (8, 9). Later, another C-peptide fusion inhibitor, T-20, was identified (10, 11) which overlaps SJ-2176 sequence and also has potent anti-HIV activity. Using proteolytic dissection strategies, Lu et al. (12, 13) isolated several pairs of protease-resistant N- and C-peptides from gp41 including N36 and C34, which was later found to form the stable fusogenic core. The peptide C34 is more potent in inhibiting HIV-1 fusion than both SJ-2176 and T-20. Interestingly, C34 overlaps the entire sequence of SJ-2176 with only two additional residues at the N and C termini, respectively.

Extensive structural studies on the peptide pair N36 and C34 have revealed the structure of the fusogenic core gp41. The N- and C-peptides, while stay unstructured on their own, have the tendency to form a stable six-stranded α-helical bundle (6-HB) when mixed with a 1:1 molar ratio. The crystal structures of the 6-HB, based on the work of three independent groups, show that three helices from the N-peptides (the N-helices) associate to form the central trimeric coiled-coil and three helices from C-peptide region (the C-helices) pack obliquely in an anti-parallel configuration into the highly conserved hydrophobic grooves on the surface of the central coiled-coil (14–16). In each of the grooves, there is a highly conserved hydrophobic deep cavity, formed by the cavity-forming sequence (residues 565–581) in the NHR region (Fig. 1), which is critical for viral fusion and stability of the 6-HB (17–19). The 6-HB is extremely thermostable (12, 13) and can be recognized by a conformation-specific monoclonal antibody (mAb), NC-1 (20, 21). In the 6-HB the residues at the “α” and “β” positions in the α-helical wheels of the N-helices can interact with those at the “α” and “β” positions in the C-helices during the process of 6-HB formation (5) (Fig. 1, B and C).

Based on the structure of the 6-HB, a model was proposed to elucidate the mechanism of gp41-mediated membrane fusion.
and the anti-HIV-1 activity of the C-peptides (6). After gp120 binds to CD4 and a coreceptor (e.g. CCR5 or CXCR4), gp41 changes its conformation to a prefusion (intermediate) state by inserting its fusion peptide into the target cell membrane. Then the gp41 N- and C-helices associate to form the 6-HB bringing the viral and target cell membranes into close proximity and

![Diagram of HIV-1 gp41 molecule and its critical sequences in forming 6-HB fusion core.](http://www.jbc.org/)

**A**. Schematic diagram of the HIV-1 gp41 molecule. The residue numbers of each region correspond to their positions in gp160 of HIV-1

**B**. Model of 6-HB formed by the N- and C-helices. The internal trimer is formed through the interaction of the residues located at the a and d positions (in blue) in three N-helices and 6-HB is formed through the interaction of the residues located at the e and g positions (in red) in the N-helices and the a and d positions (in green) in the C-helices.

**C**. Interaction between the gp41 N- and C-helices and the critical sequences in the NHR and CHR regions. The lines between the NHR and CHR regions indicate the interaction between the residues located at e and g positions in NHR and a and d positions in CHR, respectively. Interaction between the sequences spanning residues 530–540 and residues 666–673 is unknown since no crystal structure containing these sequences is available. The critical sequences in NHR and CHR include: (i) GIV (residues 547–549, red) is a determinant of resistance to T-20 in NHR region (36), which is presented in the peptides N36-F10, N36, and N46, but not in the peptide T-21; (ii) LLQLTVWGIKQLQARIL (residues 565–581, green) is the cavity-forming sequence in the NHR region (14, 31); (iii) WMEWDREI (residues 628–635, orange) is the cavity binding sequence in the CHR region (14, 17); and (iv) WASLWNWF (residues 666–673, pink) is a partial tryptophan-rich sequence (11). The N-peptide N36-F10, N36, and T-21 span the N-terminal, middle, and C-terminal portions of the NHR region, respectively. N46 covers both the N-terminal and middle portions of the NHR region. N46, N36, and T-21, but not N36-F10, contain the entire cavity-forming sequence. The C-peptide C34 covers the major portion of the CHR region, including the cavity binding sequence. T-20 overlaps the C-terminal portion of the CHR region and part of the tryptophan-rich sequence, but not the cavity binding sequence.
result in the fusion of HIV-1 with the target cell. It was then, proposed that C-peptide (e.g. C34) may form a heterogeneous 6-HB with the NHR region of gp41, thus, block the formation of fusion-active core of gp41 and inhibit the fusion between the viral and target cell membranes. The understanding of the action of the fusion inhibitor has opened a new avenue to identify antiviral peptides against other viruses with type I transmembrane envelope glycoprotein, such as simian immunodeficiency viruses (SIV) (22), Sendai virus (23), feline immunodeficiency virus (FIV) (24), respiratory syncytial virus (RSV), measles virus (25), Ebola virus (26), Nipah and Hendra viruses (27), and most recently, the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) (28–30).

T-20 (brand name: Fuzeon), developed by Trimeris, Inc. and Hoffmann-La Roche, Ltd., as the first member of a new class of anti-HIV drugs, the HIV fusion inhibitors, was recently licensed by the United States Food and Drug Administration (FDA)\(^2\) for treatment of HIV-infected individuals, including those who failed to respond to prior antiretroviral therapy (31–33). Because the major sequence of T-20 is derived from the CHR region and partially overlaps C34 sequence (Fig. 1, A and C), it has been proposed that T-20 acts in the same way as C34 to inhibit viral fusion by blocking the formation of the fusogenic core of gp41 (6, 33–35).

However, new evidence on the fusion inhibitor T-20 raised doubts about the generality of the above model. Although partially overlapping with the C34 peptide, T-20 does not contain the cavity binding sequence (residues 628–635) (Fig. 1C), which is important for gp41-mediated membrane fusion and the stability of the gp41 core conformation (17–19). There has been no report on the direct interaction of T-20 with the NHR region of gp41 sequence different from that of C34 and other C-peptides. Further elucidating the mechanism of action of T-20 may be different from that of C34.

Here we present data to show that T-20, unlike C34, does not form the stable 6-HB with the N-peptides containing the entire cavity-forming sequence. Instead, it may associate with N-peptides to form unstable or insoluble complexes. In addition, T-20-mediated anti-HIV activity could be abrogated by peptides derived from the membrane-spanning domain in gp41 and the coreceptor binding site in gp120. HIV-1 isolated resistant to T-20 are sensitive to C34-containing peptides, such as T-649 (36). In addition, the viral sensitivity to T-20 is modulated by coreceptor specificity defined by the V3 loop of gp120 (37). These findings suggest the mechanism of action of T-20 may be different from that of C34.

EXPERIMENTAL PROCEDURES

**Peptides**—Peptides T-20, C34, T-21, N36, N46, N36-F10, N36-SIM, N36-HA (a hemagglutinin epitope GGYPFPVPDYAGPG was tagged at the C terminus of N36), T-20-ANAA, T-20-ANAA-octyl, C34-biotin (biotin was conjugated to the N terminus of C34), N36-F, C34-F, and T-20-F (fluorescein isothiocyanate (FITC) was added to the N termini of N36, C34 and T-20, respectively) were synthesized by a standard solid-phase Fmoc method at the Microchemistry Laboratory, the New York Blood Center. The peptides were purified to homogeneity (95% purity) by high-performance liquid chromatography (HPLC) and identified by laser desorption mass spectrometry (PerSeptive Biosystems, Framingham, MA). 5-Helix and 5-Helix (D4) were kindly provided by Dr. Michael Root (Thomas Jefferson University, Philadelphia, PA). HIV-1 Env (H9/HIV-1NG device) was obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, contributed by Drs. Robert Gallo and Douglas Richman, and DAIDS, NIAID, respectively.

**Native Polyacrylamide Gel Electrophoresis (N-PAGE)**—N-PAGE was used to determine the 6-HB formation between the N- and C-peptides as described previously (38). Briefly, an N-peptide (N36, T-21, or N36-F10) was incubated with a C-peptide (C34, T-20 or T-20 derivatives) (the final concentration of each peptide was 40 μM) at 37 °C for 30 min. The sample was mixed with Tris-glycine native sample buffer (Invitrogen, Carlsbad, CA) at a ratio of 1:1 and was then loaded onto a 10 × 1.0 cm precast Tris-glycine gel (18%, Invitrogen, Carlsbad, CA) at 25 μl per well. Gel electrophoresis was carried out with 125 V constant voltage at room temperature for 2 h. The gel was then stained with Coomassie Blue and imaged with a FluorChem 8800 Imaging System.

\(^2\) U. S. FDA, March 13, 2003. FDA approves first drug in new class of HIV treatments for HIV-infected adults and children with advanced disease. FDA News.

![Fig. 2. Analysis of the secondary structures of N36, C34, T-20, and the mixtures of N36+C34 and N36-T-20 by CD spectroscopy. A, CD spectra for N36, C34, and their complex. B, temperature scanning at 222 nm for the N36-C34 complex. Inserted is the first derivative of the curve against temperature, which was used to determine the T_m value. C, CD spectra for N36, T-20, and their complex. Final concentration of each peptide is 10 μM.](http://www.jbc.org/content/jbc/179/16/11261/F2.large.jpg)
Fluorescence Native Polyacrylamide Gel Electrophoresis (FN-PAGE)—FN-PAGE was performed under the same conditions using the same reagents as those for N-PAGE described above except that one of the two peptides was replaced by FITC-conjugated peptide (N36-F, C34-F, or T-20-F) (38). Immediately after electrophoresis, fluorescence bands in the gel were imaged by the FluorChem 8800 Imaging System using a transillumination UV light source with excitation wavelength at 302 nm and a fluorescence filter with emission wavelength at 520 nm. The gel was then stained with Coomassie Blue and imaged again with a FluorChem 8800 Imaging System.

Fluorescence-linked Immunosorbent Assay (FLISA)—C34-F was used in FLISA for detection of the 6-HB (39). Briefly, the mixture of an N-peptide (N36 or N46) and C34-F at final concentration of 1 μM in Dulbecco’s PBS was added to the wells of 96-well polystyrene plates precoated with mAb NC-1 (0.08 μg/ml) in the presence or absence of a peptide competitor. After incubation at room temperature for 30 min, the plate was washed extensively, followed by addition of 150 μl of PBS. The fluorescence intensity was measured by the Ultra 384 reader (Tecan U.S., Inc., Durham, NC) using fluorescence filters with excitation and emission wavelengths at 485 and 535 nm, respectively. The fluorescence polarization values, expressed in millipolarization units (mP), were calculated using the equation: $mP = \frac{1000 \times (I_{S} - I_{sh}) - (I_{P} - I_{sh})}{(I_{S} - I_{sh}) + (I_{P} - I_{sh})}$, where $I_{S}$ is the parallel emission intensity measurement and $I_{P}$ is the perpendicular emission intensity.

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sample measurement, whereas \( I_{SB} \) and \( I_{FB} \) are the corresponding measurements of the background signals in buffer.

For competition experiment, equimolar C34-F and 5-Helix (25 nM) were incubated in the presence of competitive peptides at the graded concentration. The mP values were measured and the percent inhibition of the 6-HB formation by a competitor was calculated using the following equation: 

\[
\% \text{ Inhibition} = \frac{1 - (E - N)(P - N)}{P} \times 100.
\]

\( E \) represents mP value in the presence of a competitive peptide while \( P \) represents mP value in the absence of a competitor. \( N \) corresponds to the wells where only C34-F, but neither 5-Helix nor competitive peptide, was added.

Enzyme-linked Immunosorbent Assay (ELISA)—An ELISA was per-
formed to compare the relative binding affinities of N36 and N36-SIM with C34 to form 6-HB. Briefly, wells of polystyrene plates were coated with 10 μg/ml of mouse anti-HA mAb (Sigma) in 0.1 M Tris-HCl buffer (pH 8.8) and kept overnight at 4 °C, then blocked with 1% nonfat milk. Fifty microliters of N36 or N36-SIM were mixed with 25 μl of C34-biotin (4 μM) and incubated at 37 °C for 30 min, followed by addition of 25 μl of N36-HA (1 μM). After 30 min of incubation at 37 °C, the mixture was transferred to the coated wells and incubated at 37 °C for 1 h. Then streptavidin-labeled horseradish peroxidase (SA-HRP) (Zymed Laboratories Inc., S. San Francisco, CA), and the substrate, 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma) were added sequentially. Absorbance at 450 nm (A450) was determined spectrophotometrically by an ELISA reader (Ultra 384 model, Tecan US, Research Triangle Park, NC). The percent inhibition of N36-HA/C34-biotin 6-HB formation by N36 and N36-SIM, respectively, was calculated as described previously (20), and IC50 values were calculated using the computer program Calcusyn (21).

Circular Dichroism (CD) Spectroscopy—An N-peptide (N36, N46, T-21, or N36-F10) was incubated with a C-peptide (C34 or T-20) at 37 °C for 30 min (the final concentrations of N-peptides and C-peptides were 10 μM in 50 mM sodium phosphate and 150 mM NaCl, pH 7.2). The isolated N- and C-peptides were also tested. CD spectra of these peptides and peptide mixtures were acquired on Jasco spectropolarimeter (Model J-715, Jasco Inc., Japan) at room temperature using a 5.0-nm bandwidth, 0.1-nm resolution, 0.1-εm path length, 4.0-s response time, and a 50-nm/min scanning speed. The spectra were corrected by the
subtraction of a blank corresponding to the solvent. Thermal denaturation was monitored at 222 nm by applying a thermal gradient of 5 °C/min. The melting curve was smoothed, and the midpoint of the thermal unfolding transition (T_m) values was calculated using Jasco software utilities as described previously (40).

HIV-1-mediated Cell-Cell Fusion—A dye transfer assay was used for detection of HIV-1-mediated cell fusion as described previously (8, 41). H9/HIV-1_MN cells were labeled with a fluorescent reagent, Calcein AM (Molecular Probes, Inc., Eugene, Oregon) and then incubated with MT-2 cells (ratio = 1:1.0) in 96-well plates at 37 °C for 2 h in the presence or absence of peptides tested. The fused and unfused Calcein-labeled HIV-1-infected cells were counted under an inverted fluorescence microscope (Zeiss, Germany) with an eyepiece micrometer disc. Four fields per well were counted. The percentage of inhibition of cell fusion by peptides was calculated as described previously (41), and IC_{50} values were calculated using the computer program Calcusyn.

RESULTS

Unlike C34, T-20 Does Not Form Stable 6-HB with N36—To investigate the interaction of T-20 and C34 with N36, respectively, the CD spectrum of equimolar mixtures of C34 + N36 and of T-20 + N36 were recorded. In agreement with the results reported by Lu et al. (13), C34 and N36 separately did not adapt to any stable conformation, characterized by the CD spectra distinctive of random coils, while the equimolar mixture of the two peptides indicated the formation of a helical complex, characterized by the saddle-shaped negative peak in the far UV region of the CD spectrum and the significant increase of molar ellipticity (θ) at 222 nm (Fig. 2A). This helical bundle is relatively stable in phosphate buffer with a T_m about 66 °C (Fig. 2B). The CD spectrum of T-20 was rather similar to that of C34, indicating mainly random coil conformation. However, the spectrum of the T-20 + N36 mixture (equimolar) was distinctively different from that of C34 + N36. The saddle-shaped negative peak in the far UV region was clearly lacking. The θ_{222} was just the sum of the spectra of N36 and T-20, indicating no increase in helical content (Fig. 2C). The CD data confirmed that the addition of T-20 failed to induce any helical structure of N36.

The lack of interaction between T-20 and N36 is also consistent with the results of the FN-PAGE experiments (Fig. 3). When the fluorescence-labeled C34 (C34-F) and N36 were mixed with equal molar ratio, two fluorescence bands were revealed (Fig. 3A, lane 4). The lower band with low fluorescence intensity was the free C34-F, and the upper band with high fluorescence intensity corresponded to the 6-HB, confirmed by Western blot using the 6-HB-specific mAb NC-1 and FLISA with the materials eluted from the upper band as described previously (38). While in the mixture of fluorescence-labeled T-20 (T-20-F) and N36, the only band observable was that of free T-20-F (Fig. 3A, lane 5) with the same intensity as the band of free T-20-F at the same concentration (Fig. 3A, lane 3).

The N-peptide N36 was not observable because it did not have fluorescent label and also because it carries net positive charges. The same experiments were also carried out using fluorescence-labeled N36 (N36-F) and unconjugated C34 or T-20. Again, the 6-HB formed between C34 and N36-F was easily identifiable (Fig. 3C, lane 4), while no band was observed for the mixture of N36-F and T-20 (Fig. 3C, lane 5). The free N36-F (Fig. 3C, lane 1) did not enter the gel because of its net positive charges, which causes it to migrate upward toward the cathode (the negative end), and only appeared as a faint band stacked on the top of the gel. When the same gel was stained by Coomassie Blue (Fig. 3D), the bands of C34, T-20, and the N36-F-C34 complex were observable but not the band of N36-F. These results suggest that T-20, unlike C34, does not interact with N36 to form 6-HB.

In the next experiment, the effects of T-20 on the 6-HB formation between N36 and C34-F were investigated using a FLISA established previously (39). Only the N36-C34-F complex, but not the isolated C34-F or N36, could be captured by the mAb NC-1, which specifically recognized the conformation of the 6-HB (Fig. 4A) (20). As expected, addition of the unconjugated C34 reduced the fluorescent intensity of the C34-F-N36 6-HB in a dose-dependent manner (Fig. 4B) because of the competitive replacement of C34-F by the unconjugated C34. In the contrast, the addition of T-20 did not affect the formation of C34-F-N36 6-HB and the T-20 appeared to be unable to compete with C34-F to form the 6-HB with N36 (Fig. 4B).

The inhibitory activity of T-20 and C34 on formation of the 6-HB was further studied by FN-PAGE. As shown in Fig. 4C, the mixture of N36-C34-F (lane 2) showed a lower band of C34-F and an upper band corresponding to the 6-HB, while the sample containing only C34-F (lane 1) showed only a single fluorescence band located at the lower portion of the gel. When unconjugated C34 was added to the N36-C34-F mixture...
The fluorescence intensity of the C34-F band increased while that of band of the complex decreased. The position of the 6-HB complex band moved up, suggesting that the 6-HB is formed by N36 and a mixture of C34 and C34-F. No effects were observed when T-20 was added to the N36/H18528C34-F mixture. The intensity and position of both the bands of N36/H18528C34-F and free C34-F remained unaffected (lane 4).

The Coomassie Blue staining of the same gel confirmed the same findings (Fig. 4D). When C34 was added to the N36-C34-F mixture (lane 3), the band corresponding to the 6-HB formed between N36 and the mixture of C34-C34-F was smeared and located at a little higher position than that of N36-C34-F 6-HB (lane 2), consistent with the lower mobility of the free, unconjugated C34 compared to the isolated FITC-conjugated C34 (the free, unconjugated C34 was located just above that of the free C34-F in lane 3). As the C34-F in the original N36-C34-F complex was replaced by unconjugated C34, the intensity of the C34-F band was also increased compared to that in lane 2. When free T-20 was added to the N36-C34-F mixture (lane 4), similar as in FN-PAGE, the density and position of the bands corresponding to N36-C34-F 6-HB and the free C34-F remained the same. The band corresponding to the free T-20 was revealed clearly by the isolated third band in the middle between the upper band of the 6-HB and the lower band of C34-F (Fig. 4D).

The gel electrophoresis experiments also showed that the unconjugated C34 inhibited the fluorescent N36-C34-F 6-HB formation in a dose-dependent manner. With the increasing concentrations of C34 added to the N36-C34-F mixture, the fluorescence intensity of the lower band corresponding to C34-F increased and that of the upper band decreased. The position of the upper band moved up gradually, suggesting the secondary structure of N36-F10, C34, T-20, and the mixtures of N36-F10/C34 and N36-F10/T-20 by CD spectroscopy and temperature scan. A, CD spectra for N36-F10, C34, and their complex. B, temperature scanning at 222 nm for N36-F10/C34 complex. C, CD spectra for N36-F10, T-20, and their complex. D, temperature scanning at 222 nm for N36-F10/T-20 complex. Final concentration of each peptide is 10 nM.

![Fig. 8](http://www.jbc.org/)

### Table I

| Peptide       | Residue no. | Sequence                                                                 | IC50 \(_{nm}^b\) |
|---------------|-------------|---------------------------------------------------------------------------|-----------------|
| N36           | 546–581     | SGIVQQQNNLRAIEAQQHLLQLTWGKQGRAIL                                           | 584 ± 46        |
| N36-SIM       | 546–581     | SSIMQQQNNLRAIEAQQHLLQLTWGKQGRAIL                                           | 538 ± 30        |
| N46           | 536–581     | TLTIVQARQLSGIVQQQNLLRAIEAQQHLLQLTWGKQGRAIL                                 | 313 ± 7         |
| N36-F10       | 536–569     | TLTIVQARQLSGIVQQQNLLRAIEAQQHLLQLTWGKQGRAIL                                 | 2200 ± 90       |
| T-21          | 553–590     | NNLRAIEAQQHLLQLTWGKQGRAILH LonelyLKDQ                                      | 247 ± 14        |
| C34           | 628–661     | WMEWDRREINNTSILHSLIESQNRQEQKNEQELL                                         | 1.0 ± 0.2       |
| T-20          | 638–673     | YTLSIHLSIESQNRQEQKNEQELLHDKWNLNWF                                          | 3.0 ± 0.4       |
| T-20-ANAA     |             | YTLSIHLSIESQNRQEQKNEQELLHDKWASLANAA                                        | 1330 ± 57       |
| T-20-ANAA-octyl|           | YTLSIHLSIESQNRQEQKNEQELLHDKWASLANAA-octyl                                  | 21.7 ± 2.7      |

\(^a\) The residue numbers of each region correspond to their positions in gp160 of HIV-1_HXB2.

\(^b\) IC50 means the concentration of a peptide causing 50% inhibition of HIV-1-mediated cell-cell fusion.
6-HBs contain more C34 and less C34-F when higher concentrations of C34 were added (Fig. 4E). When the gel was then stained with Coomassie Blue, the density of the band corresponding to the isolated C34 (just above the band of C34-F) increased with the increasing concentration of C34 added, while the upper 6-HB bands moved up gradually (Fig. 4F). The bands located between the 6-HBs formed by N36-C34-F (lane 1) and N36-C34 (lane 7) were the 6-HBs containing different ratios of C34:C34-F. When the increasing concentrations of T-20 were added to the N36-C34-F mixture, the intensity and position of the bands corresponding to the 6-HB and the isolated C34-F did not change (Fig. 4G), while the intensity of the middle band corresponding to the isolated T-20 increased gradually (Fig. 4H). These confirm that T-20, even at high concentrations, cannot compete with C34 for binding to N36 to form the 6-HB.

Unlike C34, T-20 Does Not Form Stable 6-HB with T-21, Another N-peptide Containing Full-length Cavity-forming Sequence, but Not the T-20-resistant Determinant—Early evidence that shows that T-20 may target the gp41 NHR region is from a CD analysis of the interaction between T-20 and T-21, an N-peptide that is derived from the C-terminal portion of the NHR region and contains the entire cavity-forming sequence (Fig. 1), because addition of T-20 to T-21 results in the decrease of the α-helicity of T-21 (7, 11, 43). But unlike N36, T-21 does not contain the GIV motif, the determinant of T-20 resistance (36, 44). We used similar approaches to compare the secondary structures formed by T-21-C34 and T-21-T-20. T-21, like N36, interacted with C34 to form an α-helical oligomer (Fig. 5A). However, T-21 could not form any α-helical complex with T-20. In contrast, T-20 distorted the α-helical structure (Fig. 5B), consistent with the observation by Wild et al. (11, 43). These results suggest that T-20 may interact with T-21 to change its α-helicity, but this interaction did not result in the formation of α-helical oligomeric structure.

The interaction of T-20 and C34 with T-21 was also examined using N-PAGE and FN-PAGE. As shown in Fig. 6C, T-20 and C34 each gave a band located at the lower portion of the gel. The T-21-C34 mixture gave a lower band corresponding to C34 and an upper band corresponding to the 6-HB. However, the T-21-T-20 mixture exhibited only the lower T-20 band, but no upper 6-HB band (Fig. 5C). With the increasing concentration of T-20, the intensity and the position of either the upper band corresponding to the 6-HB formed by T-21-C34-F or the lower one corresponding to the isolated C34-F did not change, while the intensity of the middle band corresponding to the isolated T-20 increased gradually (Fig. 5, D and E). These results suggest that T-20, unlike C34, did not interact with T-21 to form stable 6-HB, nor inhibit interaction between C34-F and T-21.

Unlike C34, T-20 May Weakly Bind to 5-Helix, but Does Not Block C34 Interacting with 5-Helix to Form 6-HB—Using protein design strategies, Root et al. (45) identified a polypeptide, denoted 5-Helix, which is composed of three N-peptides (N-40, residues 543–582) and two C-peptides (C-38, residues 625–662) connected by -GGSGG- linkers. Since 5-Helix contains five of the six α-helical coils and has one of the three grooves exposed because of the missed C-helix, it can attract a CHR domain of the viral gp41 or a C-peptide, such as C34, to form a stable six-helix bundle. Binding of C34-F to 5-Helix to form 6-HB was investigated using the fluorescence polarization assay. As shown in Fig. 6A, the polarization values (mP) increased with increasing concentration of the 5-Helix until it reached a plateau when the concentration ratio of 5-Helix:C34-F became about 1:1. Under the same conditions, C34-F could not bind to 5-Helix (D4), a 5-Helix mutant with Asp replacement of the four highly conserved residues at the “e” position (Val549, Leu556, Gln563, and Val570) in the third N40 segment, which is responsible for C-peptide binding. This confirms that C34-F can specifically interact with 5-Helix to form 6-HB. T-20-F did not have significant interaction with 5-Helix at 25 nM. When the concentration of 5-Helix was increased to 200 μM (the ratio of 5-Helix:T-20-F is about 8:1), T-20-F showed certain binding activity at a level about 60% of the maximum interaction between 5-Helix and C34 at 1:1 ratio. This suggests that T-20 may bind to 5-Helix, but has much lower binding affinity than C34. In a competition experiment, the unconjugated free C34 could compete with C34-F to bind 5-Helix, resulting in decrease of the polarization values. In contrast, the free, unconjugated T-20, which has similar potent anti-HIV-1 activity to C34, could not block C34-F binding to 5-Helix (Fig. 6B). This further confirmed that T-20 cannot interact with any
polypeptides or constructs with a full-length cavity-forming sequence to form stable 6-HB.

**The Determinant of T-20 Resistance in the NHR Region Is Not the Critical Site Involved in the Interaction Between the NHR and CHR Regions to Form 6-HB**—One major evidence supporting the hypothesis that T-20 targets the NHR region is that the determinant of T-20 resistance in the HIV-1 variants is located in the gp41 NHR region (residues 547–549: GIV) (36, 44). However, clinical data showed that the subtype difference in T-20 susceptibility is unrelated to NHR genetic variation (46). Changing the sequences other than the gp41 NHR region, such as gp120 V3 loop, can also modulate T-20 sensitivity (47, 48). Sensitivity of T-20 also correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics (49). Furthermore, T-20-resistant HIV-1 variants remain fully sensitive to the C-peptide T-649 (residues 628–663), which covers the entire sequence of C34 (residues 628–661) (36). To investigate whether the GIV motif in the gp41 NHR region is important for the interaction between the NHR and CHR to form the 6-HB, we synthesized an N36 analogous peptide, designated N36-SIM, in which the wild-type GIV motif was replaced by T-20-resistant motif SIM (36) and compared the ability of N36 and N36-SIM to form the 6-HB with C34. Both wild-type N36 and N36-SIM had similar activity to interact with C34 to form the 6-HB as determined by FN-PAGE (Fig. 7A) and FLISA (Fig. 7B), and to block the 6-HB formation between N36-HA and C34-biotin (IC50 values of 2.67 ± 0.26 and 2.74 ± 0.19 μM, respectively) as assessed by ELISA (Fig. 7C). In addition, both N36 and N-36-SIM had similar inhibitory activity on HIV-1 Env-mediated cell fusion with IC50 values of 0.584 and 0.538 μM, respectively (Table I). This is in a good agreement with the result obtained from the experiment on T-21, which does not contain the GIV motif, but can interact with C34 to form stable 6-HB (Fig. 5). These results suggest that the GIV motif may be important for T-20 binding and for T-20-mediated anti-HIV-1 activity, but may not be critical for the gp41 NHR and CHR association to form the 6-HB and for C34-mediated HIV-1 fusion inhibitory activity.

**T-20 Interacts with N36-F10, an N-peptide Containing the GIV Motif and Partial Cavity-forming Sequence**—To further investigate the molecular interaction of T-20, a peptide, N36-F10, was synthesized which covers the region of residues 536–569, about 10 amino acids preceding the sequence of N36 (residues 546–581) and contains the GIV motif, the determinant of T-20 resistance. Unlike N36, it does not contain the full-length sequence required for forming the hydrophobic cavity (Fig. 1C). Peptide C34 appeared to be able to interact with N36-F10 but form only an unstable complex with the helical content much lower than that of N36-C34 6-HB (Fig. 8A). The Tm of this N36-F10-C34 complex was only around 36 °C (Fig. 8B) while the Tm of N36-C34 is 66 °C (Fig. 2B) (50). Peptide T-20 could also interact with N36-F10 to form a complex with low helix content (Fig. 8C) and low thermal stability (Fig. 8D, Tm = 48 °C).

The interaction was confirmed by FN-PAGE. The N36-F10-C34-F complex band was not visible because it may be damaged during electrophoresis because of its unstable property (e.g. Tm = 36 °C). Mixing N36-F10 and T-20-F induced a band of the complex (Fig. 9A), indicating that there is an interaction between T-20-F and N36-F10.
Is the interaction of T-20 to N36-F10 enough to explain the inhibitory activity of T-20? T-20 contains a consecutive tryptophan-rich (TR) region at the C terminus (Fig. 1), and the 3 tryptophan residues (Trp<sup>666</sup>, Trp<sup>670</sup>, and Trp<sup>672</sup>) are critical to its inhibitory activity. T-20-ANAA, a mutant T-20 in which the last four WNWF residues were changed to ANAA, shows much less anti-HIV activity (51). Using HIV-1-mediated cell-cell fusion assay, we confirmed that the inhibitory activity of T-20-ANAA is about 450-fold less than that of wild-type T-20 (Table I). In N-PAGE, T-20-ANAA could interact with N36-F10 to form a complex, which means that the WNWF is not critical for the binding of T-20 to N36-F10 (Fig. 9C). These results indicate that although T-20 can bind to gp41 NHR, this binding may not account for its potent anti-HIV activity.

T-20 May Interact with N46, Which Overlaps N36 and N36-F10; but Unlike C34, T-20 Cannot Form Stable 6-HB with N46—Although we have compared the ability of C34 and T-20 to interact with N36 and N36-F10 to form the 6-HB, one may argue that neither of these N-peptides contains the full binding region for C34 and T-20. Therefore, we synthesized a longer N-peptide, N46 (residues 536–581), which covers the sequences of both N36 and N36-F10, and compared the ability of C34 and T-20 to form 6-HB with N46 and to block 6-HB formation between N46 and C34-F. CD analysis indicated that C34 could interact with N46 to form the α-helical bundle, similar to that formed by C34 and N36 (Fig. 10A). However, the mixture of T-20 and N46 did not result in the increase of the α-helicity of N46, but rather caused a decrease in it (Fig. 10B), in agreement with the results obtained from a mixture of T-20 and T-21 (Fig. 5B). These confirm that T-20, unlike C34, cannot form the α-helical oligomer with the peptides derived from the gp41 NHR region, but may interact with the N-peptides to form unstable or insoluble complexes. Noticeably, the N46-C34 complex had higher T<sub>m</sub> value (88 °C, Fig. 10C) than that of the N36-C34 complex (66 °C, Fig. 2B), suggesting that the α-helical oligomer formed by C34 with a longer N-peptide is more stable than that with a shorter N-peptide.

In cell-cell fusion assay, N46 was more effective than N36 and N36-F10 in inhibiting HIV-1 Env-mediated membrane fusion with an IC<sub>50</sub> value of 313 nM (Table I). At the concent-

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**Fig. 11. Interaction of C34 and T-20 with N46 determined by FN-PAGE and FLISA.** A, interaction of N46 (50, 25, 12.5, 0 μM) with C34-F (50 μM) or T-20-F (50 μM), respectively, as determined by FN-PAGE. B, inhibitory activity of C34 and T-20 on the N46-C34-F 6-HB formation as measured by FLISA.
T-20 Targets Multiple Sites in gp120/gp41

...gomers or aggregates that could not enter into the gel.

In FLISA, the N46 could interact with C34-F to form a complex, which could be captured by the conformation-specific mAb NC-1, suggesting that the complex is 6-HB. Formation of the 6-HB by N46 and C34-F could be significantly blocked by C34, but not by T-20 (Fig. 11B).

All the above results suggest that C34 can interact with N46 to form a stable and soluble 6-HB, while T-20 may bind to N46 to form non-α-helical, unstable, or insoluble complexes or aggregates, and T-20 cannot block the 6-HB formation between N46 and C34.

**T-20-mediated Inhibitory Activity Is Abrogated by Peptides Derived from the Coreceptor Binding Region in gp120 and the Membrane-spanning Domain in gp41**—In addition to a target site within the NHR region, T-20 may also bind to a non-specified region at the C terminus of the gp41 ectodomain close to the viral membrane, the membrane-proximal domain (52, 53). Other groups have shown that the sensitivity of HIV-1 to T-20 relates with envelope/coreceptor affinity, receptor density, and fusion kinetics (47, 49). These suggest that T-20 may target multiple sites in both gp41 and gp120. We thus used a complete set of HIV-1, Env (15-mer) peptides to investigate the possible binding regions of T-20, assuming that the peptides derived from the T-20 binding sites in gp41/gp120 can interact with T-20 and block its anti-HIV-1 activity. The peptide C34 and PBS were used as controls. The complete set (212 peptides) covers the whole gp120 and gp41 sequence at a 4 amino acid shift for each peptide. Using HIV-1-mediated cell-cell fusion assay, the Env peptides themselves at final concentration of 12.5 μg/ml in PBS (about 7 μM) have no inhibitory activity (Fig. 12A). T-20 and C34 at 100 nM final concentration can completely inhibit the cell-cell fusion. We mixed the Env peptides (12.5 μg/ml) with T-20 or C34 (100 nM) at 37 °C for 30 min, then detected their inhibitory activity. None of the peptides could block the anti-HIV-1 activity of C34 (Fig. 12B), but the peptides 6312, 6313, 6314 derived from gp120 and 6378, 6379, 6380 derived from gp41 completely abrogated T-20-mediated inhibition of the HIV-1-induced cell-cell fusion (Fig. 12C). The sequence and the blocking activity of these peptides expressed as EC50 (effective concentration of a peptide causing 50% abrogation of the anti-HIV-1 activity mediated by T-20) are shown in Table II.

The overlapping peptides 6312, 6313, 6314 share a sequence of amino acid 417–423, QCKIKQI, which overlaps the sequences of the β-19 to β-20 strands in the gp120 bridging sheet (residues 412–427). This region is centrally located within the coreceptor binding site (2), suggesting that T-20 may interact with this region and block HIV-1 binding to the coreceptor. The overlapping peptides 6378, 6379, 6380 share a common sequence of amino acid 684–691, IFIMIVG, a part of the gp41 membrane-spanning domain, which is thought to be the possible second site in gp41 for T-20 binding. The blocking activity of the peptides 6378, 6379, and 6380 cannot be ascribed to non-specific hydrophobic interaction since other peptides derived from the transmembrane region of HIV-1 gp41 (e.g. peptides 6381 and 6382) and those derived from the FP regions (e.g. 6337 and 6338) did not block T-20-mediated inhibition of cell-cell fusion (Fig. 12C). Furthermore, the scrambled version of the peptide 6380 did not inhibit the anti-HIV-1 activity of T-20 (Table II). These suggest that the peptides 6378, 6379, and 6380 specifically interact with T-20 to block its inhibitory activity on HIV-1 infection.

**The gp120 Coreceptor Binding Region and gp41 Membrane-spanning Region Interact at Different Sites in T-20**—Shai and co-workers reported that C-terminal octylation of SIV gp41-derived T-20-ANAA mutant can rescue this inactive mutant to...
an inhibitory potency similar to that of the wild-type T-20 (62). In the present study, we found that C-terminal octylation can also rescue a low inhibitory activity of HIV-1H9262 gp41-derived T-20-ANAA mutant (Fig. 13A and Table I). Using N-PAGE, T-20-ANAA-octyl has the similar binding ability with N36-F10 as wild-type T-20 and T-20-ANAA (data not shown). Using the blocking assay, gp120 coreceptor binding region-derived peptide 6312 at 1 μM can abrogate both T-20 and T-20-ANAA-octyl (100 nM)-mediated inhibitory activity on cell-cell fusion, while gp41 membrane-spanning domain-derived peptide 6380 at 5 μM can only block the wild-type T-20 anti-HIV-1 activity (Fig. 13B). This indicates that the membrane-spanning domain-derived peptides may interact with the tryptophan-rich sequence at the C-terminal region of T-20, while the interaction site of the gp120 coreceptor binding region-derived peptide is beyond this tryptophan-rich region. It seems that the binding to the gp120 coreceptor binding region contributes more to the inhibitory activity of T-20 because: 1) N36-F10 cannot block T-20 activity completely at 0.8 μM and only has marginal binding activity on T-20-ANAA-octyl anti-HIV-1 activity (Fig. 13B); and 2) the peptides derived from the gp120 coreceptor binding region are much more potent than those from the gp41 membrane-spanning domain in blocking the anti-HIV-1 activity of T-20 (Table II).

**DISCUSSION**

T-20 has been widely used as a proof-of-concept for anti-HIV drugs, albeit the concept of inhibitor reaction was deduced from the work using another C-peptide C34 (6, 12, 14, 33–35). Our studies clearly demonstrated that, lacking the N-terminal cavity binding sequence, T-20 cannot interact with the cavity-forming sequence in the gp41 NHR region to form a stable 6-HB. Thus it is difficult to envision that T-20 would act in the same way as C34 by interrupting the formation of the gp41 core. Instead, our data suggest that T-20 may interact with the N-terminal portion of NHR region containing the highly conserved GIV motif, the membrane-spanning domain in gp41, and the coreceptor binding site on gp120. These interactions, acting together or alone, cause inhibition of viral fusion.

The assumption of the same mechanism of action of T-20 and C34 mainly come from the study that a contiguous 3-amino acid sequence (residues 547–549: GIV) within the NHR region was identified to be critical for T-20-mediated anti-HIV activity. Substitutions at two positions within the GIV sequence, Gly to Ser or Asp and Val to Met, resulted in virus resistance to T-20 (36, 54, 55). However, we found that the GIV motif may not be involved in the interaction between the NHR and CHR to form 6-HB since: 1) N36-SIM, the N36 analog with mutations in the GIV motif, has similar activity as the wild-type N36 to interact with C34 to form the 6-HB and to block the 6-HB formation between N36-HA and C34-biotin (Fig. 7), and 2) T-21 which does not contain the GIV motif, the membrane-spanning domain in gp41, 66 °C). C34 can also interact with the NHR of N36-FA (0.8 μM, N36-F10 (0.8 μM), 6312 (1 μM), and 6380 (5 μM) on T-20 (20 nM) and octylated T-20 mutant (100 nM)-mediated anti-HIV-1 activity. PBS was used as control.**

**FIG. 13.** HIV-1 fusion inhibitory activity mediated by peptides T-20, T-20-ANAA, and T-20-ANAAoctyl in the presence or absence of the peptides N36, N36-F, 6312, and 6380. A, inhibitory activity of T-20, T-20 mutant (T-20-ANAA), and its octyl-derivative against HIV-1-mediated membrane fusion; B, blocking activity of the peptides N36 (0.2 μM), N36-F10 (0.8 μM), 6312 (1 μM), and 6380 (5 μM) on T-20 (20 nM) and octylated T-20 mutant (100 nM)-mediated anti-HIV-1 activity. PBS was used as control.
to the NHR of gp41. FP alone can insert into and fuse biological membranes (56). Extension of FP to include partial sequence of NHR region results in significant enhancement of FP-mediated membrane fusion, suggesting that NHR has a synergistic role in FP-mediated fusogenic activity (57). Mobley et al. (58) demonstrated that T-20 can block hemolysis and cell aggregation induced by FP and the N-peptide fusion inhibitor T-21, implying the interaction of T-20 with the FP. It is plausible that T-20 inhibits the viral fusion by affecting the membrane perturbations associated with the interactions of FP and NHR, which underlie the merging of the viral envelope with the target cell membrane.

In addition to the interaction with the NHR of gp41, T-20 may also target the membrane-spanning domain in gp41 and block the late step of membrane fusion. Peptides derived from the membrane-spanning domain could effectively abrogate T-20-mediated inhibitory activity on cell-cell fusion. However, one may raise doubts about the approach because none of the peptides derived from the gp41 NHR region abrogates C34-mediated anti-HIV-1 activity. This is because the peptides used in this study for Pepscan experiment are too short (15-mer) to block the late step of membrane fusion. Peptides derived from the gp41 NHR and CHR regions to interact with their counterparts for blocking the activity of the corresponding N- and C-peptides. The binding site of T-20 in the gp41 membrane-spanning domain consists of a short sequence with seven residues, IFIMIVG, most of which are hydrophobic. T-20 may bind to this fragment through its hydrophobic residues located in the tryptophan-rich domain. It has been proven that the hydrophobic residues in the tryptophan-rich sequence are critical for the anti-HIV-1 activity of T-20 (11). Removal or replacement of 4 residues, WNWF, at the C terminus of T-20 almost completely eliminated its anti-HIV-1 activity while deletion of 3 residues from the N terminus of T-20 has no effect on its fusion inhibitory activity (11, 51). The tryptophan-rich domain in gp41 is also important for Env-mediated fusion (57) because it may bind to the membrane surface (60) and participate in oligomerization of gp41 to form fusion pore in the membrane (53). It has been reported that the T-20 analog with mutations in the tryptophan-rich domain, T-20-ANAA, has no HIV-1 fusion inhibitory activity, but the membrane-anchoring T-20-ANAA has similar anti-HIV-1 activity as the wild-type T-20 (61). C-terminal ocytlation rescue the inactive ANAA mutant of T-20-like peptide derived from the SIV gp41 (62). These suggest that interaction of T-20 and the target membrane is necessary for T-20-mediated membrane fusion inhibitory activity. The data from our study confirm these observations and suggest that T-20 may also bind to viral membrane by interacting with the membrane-spanning domain at the late step of membrane fusion (post-lipid mixing) to prevent fusion pore formation. Since T-20 can bind to the N-terminal fragment of the NHR region and other sites in gp41, it is understandable that T-20 can capture gp41 in immunoprecipitation experiments (63).

T-20 may interact with the coreceptor binding site on gp120 and block interaction of gp120-CD4 complex with the coreceptor. In the present study, we found that the peptide 6311–6313, which share the sequence of residues 417–423 in the HIV-1 gp120 were able to abrogate T-20-mediated anti-HIV-1 activity. Interestingly, this sequence overlaps the β19 and β20 fragments (residues 412–427) in the bridging sheet, a coreceptor binding site in gp120 (2). It suggests that the coreceptor binding site in gp120 may be another target site for T-20 and interaction of T-20 with this site may account for part of the T-20-mediated HIV-1 fusion inhibitory activity. Our finding is consistent with the mounting new evidence suggesting that T-20 may bind to gp120. Reeves et al. (49, 64) showed that the T-20 sensitivity correlated with gp120/coreceptor affinity and was affected by mutations in the coreceptor binding site on gp120. Most recently, Yuan et al. (65) reported that T-20 bound to gp120 of the X4 but not R5 virus in a CD4-induced, V3 loop-dependent manner and binding of T-20 to gp120 blocked the interaction between the gp120-CD4 complex and the CXCR4 coreceptor, indicating that T-20 preferably interact with CXCR4 binding site on gp120. Alam et al. (66) using surface plasmon resonance (SPR) binding assays found that T-20 can bind to CD4-induced gp120, and the binding region is centrally located within the HIV-1 coreceptor binding site.

The interaction with the gp120 coreceptor binding region and gp41 membrane-spanning domain may be mediated by different regions in T-20. Peptides derived from the gp41 membrane-spanning domain can significantly block inhibition of cell-cell fusion mediated by T-20, but not by T-20-ANAA-ocytl. This suggests that the gp41 membrane-spanning domain may interact with the C-terminal tryptophan-rich region of T-20. In contrast, the peptides derived from the HIV-1 gp120 coreceptor binding region can effectively abrogate the anti-HIV-1 activity mediated by both T-20 and T-20-ANAA-ocytl, indicating that the interaction site of the gp120 coreceptor binding region-derived peptides is located outside the T-20 tryptophan-rich region. Because the peptide T20-ANAA can bind to N36-F10 as wild-type T-20, the binding site for the gp41 NHR region in gp41 may be also located beyond the C-terminal tryptophan-rich domain. Furthermore, since peptides derived from gp120 coreceptor binding region are much more potent in blocking T-20 activity than those from the gp41 membrane spanning domain and the peptide N36-F10, binding of T-20 to the gp120 coreceptor binding region seems to contribute more for the inhibitory activity of T-20.

The present findings indicate that T-20 targets multiple sites in gp41 and gp120 and its anti-HIV activity may be a combinatory effect mediated by different mechanisms of action. Further clarification of these mechanisms may provide new targets for development of novel anti-HIV drugs and vaccines. Since C34 has more potent anti-HIV activity than T-20 and a different mechanism of action from T-20, C34 or its analogs should also be developed for clinical application and used for treatment of patients infected by HIV-1 strains resistant to T-20.

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REFERENCES
1. Sattentau, Q. J., Dalgleish, A. G., Weiss, R. A., and Beverley, P. C. (1986) Science 234, 1120–1123
2. Kwong, P. D., Wyatt, R., Robinson, J., Sweet, R. W., Sodroski, J., and Hendrickson, W. A. (1998) Nature 393, 648–659
3. Bizore, C. D., Wyatt, R., Hernandez-Ramos, N., Sun, Y., Kwong, P. D., Hendrickson, W. A., and Sodroski, J. (1998) Science 280, 1949–1953
4. Peng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996) Science 272, 872–877
5. Moore, J. P., Jameson, B. A., Weiss, R. A., and Sattentau, Q. J. (1993) in Viral Fusion Mechanisms (Benitez, J., ed) pp. 233–289, CRC Press, Boca Raton, FL
6. Chan, D. C., and Kim, P. S. (1998) Cell 93, 681–694
7. Wild, C., Ous, T., McDaniel, C., Bolognesi, D., and Matthews, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10537–10541
8. Jiang, S., Lin, K., Strick, N., and Neurath, A. R. (1993) Nature 365, 113
9. Jiang, S., Lin, K., Strick, N., and Neurath, A. R. (1998) Biochem. Biophys. Res. Commun. 195, 533–538
10. Wild, C., Greenwell, T., and Matthews, T. (1993) AIDS Res. Hum. Retroviruses 9, 1051–1053
11. Wild, C., Shugars, D. C., Greenwell, T. K., McDaniel, C. B., and Matthews, T. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9770–9774
12. Lu, M., Blacklow, S. C., and Kim, P. S. (1995) Nat. Struct. Biol. 2, 1075–1082
13. Lu, M., and Kim, P. S. (1997) J. Biomol. Struct. Dyn. 15, 465–471
Different from the HIV Fusion Inhibitor C34, the Anti-HIV Drug Fuzeon (T-20) Inhibits HIV-1 Entry by Targeting Multiple Sites in gp41 and gp120
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