Supporting Online Materials

Materials and Methods

Molecular biology for generation of *Spodoptera frugiperda* (Sf9) and mammalian expressed CXCR4-WT and CXCR4-T4L-ΔC constructs. The CXCR4-WT DNA was synthesized by DNA2.0 with flanking restrictions sites AscI at the 5' end and FseI at the 3' end. The expression vector designated pFastBac1-830220 is a modified pFastBac1 vector (Invitrogen) containing an expression cassette with an HA signal sequence followed by a FLAG tag at the N-terminus and a PreScission protease site followed by a 10xHis tag at the C-terminus. The components of the expression cassette were introduced using standard PCR based site-directed mutagenesis. The expression cassette also contains restriction sites for AscI and FseI allowing for the standard restriction digest and subsequent ligation of the synthesized CXCR4-WT DNA.

The CXCR4-1 gene, based on wild type CXCR4 and T4L sequences, included the following five additional features: (a) Asn2-Tyr161 of T4L were inserted between Ser229 and Lys230 within the CXCR4 ICL3 region. (b) A two amino acid Gly-Ser linker was inserted at both termini of T4L (T4L1). (c) C-terminal residues 326-352 of CXCR4 were truncated (dC326). (d) Two exogenous restriction sites were added, AscI at the 5' termini and FseI at the 3' termini. And (e) the gene encoding Lys225-Leu226 was modified to AAG CTT to introduce an endogenous restriction site, HindIII. The CXCR4-1 gene was further modified by introducing a thermostabilizing mutation L125^{3,41}W, using standard QuickChange PCR, then sub-cloned into the pFastBac1-830220 vector using the aforementioned restriction sites. Construction of CXCR4-2 was completed by modifying the CXCR4-1 gene. The C terminus was further truncated to residue 320 eliminating a total of 33 amino acids (dC320), using PCR with primer pairs 5'-AAG

ACC TCC GCA CAA CAC GCT TTG ACC AGT GGC CGG CCT CTG GAA GTT CTG TTC CAG GGG-3' and 5'-CCC CTG GAA CAG AAC TTC CAG AGG CCG GCC ACT GGT CAA AGC GTG TTG TGC GGA GGT CTT-3'. In the CXCR4-3 construct, the T4L was inserted between His228 and Gly231. Two residues (Ser229 and Lys230) were truncated and a Ser-Gly-Ser linker was added at the C terminus of T4L (T4L2). This CXCR4-3 construct was amplified using PCR primers encoding exogenous restriction sites (*Hind*III at the 5' end, GCG AAG CTT TCA CAC AAC ATC TTC GAG, and *Afl*II at the 3' end, GCG CTT AAG AGC TTT ACG CTT TTG GTG TCC TGA ACC TGA GTA AGC GTC CCA), and subsequently ligated into the corresponding restriction sites between Lys225 and Lys239 in the CXCR4-2 construct.

The CXCR4-WT DNA expression cassette was subcloned into the mammalian expression vector pACMV-TetO (1). CXCR4-T4L- Δ C constructs were subcloned into the mammalian expression vector pcDNA3.1(-) using the XhoI and EcoRI restriction sites. For the CXCR4- Δ C constructs, the T4L fusion and L125^{3.41}W mutation were subsequently removed using standard QuickChange PCR.

Expression and purification of Sf9-expressed CXCR4 constructs for crystallization. Hightiter recombinant baculovirus (>10⁸ viral particles per ml) was obtained using the Bac-to-Bac Baculovirus Expression System (Invitrogen). Briefly, recombinant baculoviruses were generated by transfecting 5 μ g of recombinant bacmid containing the target gene sequence into Sf9 cells using 3 μ l of FuGENE HD Transfection Reagent (Roche) and Transfection Medium (Expression Systems). Cell suspensions were incubated for 4 days while shaking at 27 °C. P0 viral stocks were isolated after 4 days and used to produce high-titer baculovirus stocks. Viral titers were performed by flow cytometric methods by staining cells with gp64-PE (Expression Systems) (2). Sf9 cells at cell density of $2-3 \times 10^6$ cells/ml were infected with P2 virus at MOI of 5. Cells were harvested by centrifugation at 48 hours post infection and stored at -80 °C until use.

Insect cell membranes were disrupted by thawing frozen cell pellets in a hypotonic buffer containing 10mM HEPES, pH 7.5, 10 mM MgCl₂, 20 mM KCl and protease inhibitor cocktail (Roche). Extensive washing of the raw membranes was performed by repeated centrifugation in the same hypotonic buffer (two - three times), and then in a high osmotic buffer containing 1.0 M NaCl, 10 mM HEPES, pH 7.5, 10 mM MgCl₂, 20 mM KCl, and protease inhibitor cocktail (three - four times), followed by Dounce homogenization to resuspend the membranes in fresh wash buffer thereby separating soluble and membrane associated proteins from integral transmembrane proteins. Highly purified membranes were resuspended in 10 mM HEPES, pH 7.5, 10 mM MgCl₂, 20 mM KCl, 30% (v/v) glycerol, and protease inhibitor cocktail, then flash-frozen with liquid nitrogen and stored at -80 °C until further use.

Purified membranes were thawed on ice in the presence of 200 μ M CXCR4 compound (IT1t or CVX15) and EDTA-free protease inhibitor cocktail (Roche), and incubated at 4 °C for 1 hour. The membranes were then solubilized in 50 mM HEPES, pH 7.5, 500 mM NaCl, 0.5% (w/v) n-dodecyl- β -D-maltopyranoside (DDM, Anatrace), 0.1% (w/v) cholesteryl hemisuccinate (CHS) (Sigma), and 100 μ M CXCR4 compound (IT1t or CVX15) for three hours at 4 °C. The supernatant was isolated by centrifugation at 160,000 × g for 40 minutes, and incubated in 5 mM buffered imidazole, 800 mM NaCl, with TALON IMAC resin (Clontech) overnight at 4 °C. Typically, 2 ml of resin per 1 L of original culture volume was used. After binding, the resin was washed with ten column volumes of 50 mM HEPES, pH 7.5, 800 mM NaCl, 10% (v/v) glycerol, 0.1% (w/v) DDM, 0.02% (w/v) CHS, 20 mM imidazole and 100 μ M CXCR4 compound (IT1t or CVX15), followed by ten column volumes of 25 mM HEPES, pH 7.5, 500 mM NaCl, 10% (v/v)

glycerol, 0.05% (w/v) DDM, 0.01% (w/v) CHS, 10 mM MgCl₂, 5 mM ATP (Sigma) and 100 µM CXCR4 compound (IT1t or CVX15), and five column volumes of 25 mM HEPES, pH 7.5, 500 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) DDM, 0.01% (w/v) CHS and 100 µM CXCR4 compound (IT1t or CVX15). The protein was then eluted with 4 column volumes of 25 mM HEPES, pH 7.5, 500 mM NaCl, 10% (v/v) glycerol, 0.05% (w/v) DDM, 0.01% (w/v) CHS, 300 mM imidazole and 500 µM CXCR4 compound (IT1t or CVX15). PD MiniTrap G-25 column (GE healthcare) was used to remove imidazole and increase the compound concentration to 1 mM. The protein was then treated overnight with His-tagged PreScission protease (home-made) and His-tagged PNGase F (home-made) to remove the C-terminal His-tag and deglycosylate the receptor. The compound concentration was increased to 2 mM in this step. PreScission protease and PNGase F were removed by Ni-NTA superflow resin (Qiagen), which was incubated at 4 °C for 1 hour. The His-tag cleaved receptor was collected in the Ni-NTA column flow through. The receptor was then concentrated to 60-70 mg/ml with a 100 kDa molecular weight cut-off Vivaspin concentrator (Vivascience). Protein purity and monodispersity was tested by SDS-PAGE and analytical size-exclusion chromatography (aSEC). Typically, the protein purity exceeded 95%, and the aSEC profile showed a single peak, indicative of receptor monodispersity.

Lipidic cubic phase crystallization of CXCR4 constructs. Lipidic cubic phase (LCP) crystallization trials were performed using an *in meso* crystallization robot as previously described (*3*). 96-well glass sandwich plates were filled with 40-50 nl protein-laden LCP boluses overlaid by 0.8 µl of precipitant solution in each well and sealed with a glass coverslip. The protein-LCP mixture contained 40% (w/w) receptor solution, 54% (w/w) monoolein, and 6% (w/w) cholesterol. Crystallization set-ups were performed at room temperature (~20 °C). Plates were incubated and imaged at 20 °C using an automated incubator/imager (RockImager 1000, Formulatrix). Initial crystallization conditions (0.1 M HEPES, pH 7.0, 30% (v/v) PEG400, 0.4 M

sodium malonate or sodium citrate) were found by the LCP-FRAP assay (4). After extensive optimization, five distinct crystal forms have been obtained (Table S4). Crystals were harvested directly from LCP matrix using MiTeGen micromounts and flash frozen in liquid nitrogen.

Data collection and structure solution. X-ray data were collected on the 23ID-B/D beamline (GM/CA CAT) at the Advanced Photon Source, Argonne, IL using a 10 μm minibeam at an incidence wavelength of 1.0330 Å and a MarMosaic 300 CCD detector. Crystals were invisible after flash-freezing into liquid nitrogen, and a similar alignment and data-collection strategy was followed as previously described (5-7) for several hundred crystal samples. Most of the crystals diffracted to 2.3 – 3.5 Å resolution when exposed to 1 s of unattenuated beam using 1° oscillation, however, data collection was limited to 10 – 30 frames per crystal, due to the fast onset of radiation damage in the microcrystals. Data were integrated, scaled and merged using XDS and HKL2000 (8-9). A complete data set of CXCR4-2/IT1t (space group *P2*₁) was obtained by merging data collected from 2 crystals. Initial phase information was obtained by molecular replacement using a polyalanine model of the 7 TM α-helices of β₂AR (PDB ID: 2RH1) and a T4L model derived from the A_{2A}AR-T4L structure (PDB ID: 3EML). Data for CXCR4-3/CVX15 were processed at 2.9 Å resolution. All refinements were performed with the Refmac5 software suite (*10*) followed by manual examination and rebuilding of the refined coordinates in program X-fit (*11*) using both $|2F_0-F_c|$ sigma-A weighted and $|F_0-F_c|$ maps, as well as omit maps.

Data collection and refinement statistics for all five crystal forms are shown in Table S1. Electron density omit maps for IT1t and CVX-15 are shown in Fig. S3 and crystal packing diagrams for the different crystal forms are shown in Fig. S4.

Ligand binding assay and functional assays. *Ligand binding assay with Sf9 expressed receptor:* For the competition binding assay, the radio-labeled ligand [³H]BIMA (51.2 Ci/mmol) was

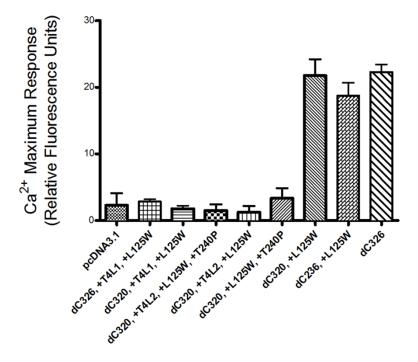
prepared through the tritio-debromination of a potent CXCR4 antagonist, bis(imidazolylmethyl) amine analog (BIMA). For binding assays using the filter plate method, membrane preparations from Sf9 cells expressing CXCR4 were incubated for 120 minutes at room temperature with different concentrations of antagonists (BIMA, ITlt and CVX15) and 6nM [³H]BIMA in 20 mM HEPES, pH 7.5, 1 mM CaCl₂, 5 mM MgCl₂. For CVX15 and BIMA competition assay, the assay buffer also contained 150 mM NaCl and 1% BSA. Unbound radioligand was removed by rapid filtration through a 96-well GF/C filter plate presoaked in 0.3% polyethylenimide (MultiScreen Harvest Plate, Millipore Corp.), and rinsed five times with 500 µL of ice-cold PBS. After drying the harvest plates, 30 µl of BetaScint scintillation liquid (Perkin-Elmer Life Sciences) were added per well. The bound radioactivity was measured using a Packard TopCount NXT. Nonspecific binding was determined in parallel reactions in the presence of an excess of 30 µM unlabelled BIMA. Scintillation proximity assays (SPA) were performed in isoplate-96 white frame clear well microplates (Perkin Elmer). Membranes containing expressed CXCR4 constructs were incubated at room temperature for 60 minutes with different concentrations of antagonists, 6 nM [³H]BIMA, 250 µg of wheat germ agglutinin SPA beads (Perkin Elmer) in assay buffer (50 mM HEPES, pH 7.5, 1 mM CaCl₂ and 10 mM MgCl₂ final concentration). The signal from receptor bound [³H]BIMA was measured using a Packard TopCount NXT and nonspecific binding determined in the presence of 50 µM unlabeled BIMA.

All data were analyzed by nonlinear regression analysis using GraphPad Prism. Equilibrium dissociation constants (K_d) for [³H]BIMA were calculated using homologous competition binding with cold BIMA and apparent affinity (K_i) values for other ligands were calculated using the Cheng-Prusoff equation as $K_i = IC_{50}/(1 + [ligand] / K_d)$. All measurements were performed in triplicate and repeated at least three times. The mean values \pm standard deviation for K_d of [³H]BIMA and K_i of other ligands are shown in Table S3. *Mammalian Expression for ligand binding assays:* HEK293 cells containing pcDNA6/TR were transformed and a stable cell line was selected for inducible expression of CXCR4, as previously described (*12*).

CHO expression and Calcium Flux Assays: Prior to transfection, CHO-K1 cells (ATCC) were grown in 10 cm plates at 37 °C, 5% CO₂, in DMEM/F12 (Gibco #10565) supplemented with 10% FBS. At 90% confluency, the pcDNA3.1(-) expression vectors were transiently transfected using Lipofectamine 2000 according to manufacturer instructions. After six hours, medium was replaced with fresh medium supplemented with 5 mM sodium butyrate.

Calcium flux assays were performed 24 hours after transfection using the FLIPR Calcium 4 assay kit (Molecular Devices) and 2.0×10^5 cells per well in a 96-well plate format. The provided assay buffer was supplemented with 0.1% BSA and 2 mM probenecid. Briefly, cells were detached using PBS + 1 mM EDTA, washed twice in PBS + 0.5% BSA and resuspended in assay buffer. After counting on a Vi-CELL automated cell analyzer (Beckman Coulter), cells were normalized to 2.0×10^6 cells/ml and plated at 100 µl per well plus 100 µl dye. Plates were centrifuged for 3 minutes at 200 × g then incubated for 1 hour at 37°C. CXCL12-dependent increases in cytosolic Ca²⁺ were measured at 37 °C using a FlexStation III microplate reader (Molecular Devices). CXCL12 was prepared as previously described (*13*).

Supplementary Figures:



Supplementary Figure S1. Calcium flux assay in CHO cells transiently transfected with CXCR4 receptor constructs or empty vector pcDNA3.1, showing the effects of T4L, L125W, T240P and various C-terminal truncations on signaling. T4L1 refers to the T4L junction in constructs CXCR4-1 (dC326, +T4L1, +L125W) and CXCR4-2 (dC320, +T4L1, +L125W), and T4L2 is in CXCR4-3 (dC320, +T4L2, +L125W, +T240P). Details are shown in supplementary molecular biology section. C-terminal truncations, dC326 and dC320, refer to truncating C-terminal residues 326-352 and 320-352, respectively. Real-time changes in relative fluorescence units were measured for 150 seconds with addition of 200 nM CXCL12 at 20 seconds. Bars represent the maximum fluorescence change observed +/- standard deviation. Experiments were repeated at least two times in triplicate.

id=71 nSeq=20		
CX4_P21_25_a	1	PCFREENANFNKIFLETTYSIIFLTGIVGNGLVILVMGYQKK LESNT KYRLHLSVABLLFVITLFFMAVDAVAN
CXCR4_HUMAN	1	MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLETIYSIIFLTGIVGNGLVILVMGYQKK-LRSMTSKYRLHLSVADLLFVITLEFWAVDAVAN-
-CXCR5_HUMAN	1	MNYPLTLEMDLENLEDLFWELDRLDNYNDTSLVENHLCPATEGPLMASFKAVFYPVAYSLIFLLGVIGNVLVLVILERHNO-TRSSTETFLFHLAVADLLLVFILPFAVAEGSVG-
-CXCR3 HUMAN	1	MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLNFDRAFLPALYSLLFLLGLLGNGAVAAVLLSRT-ALSSTTFLLHLAVADTLLVLTLEUWAVDAAVQ-
CXCR2_HUMAN	1	MEDENMESDSFEDFWKGEDLSNYSYSSTLPPFLLDAAPCEPES-LEINKYFVVIIYALVFLLSLLGNSLVMLVILYSKV-GRSVTDVYLLNLALADLLFALTLEIWAASKVNG-
CXCR1_HUMAN	1	MSNITDPQMWDFDDLNFTGMPPADEDYSPCMLET-ETLNKYVVIIAYALVFLLSLLGNSLVMLVILYSKV-GKSVTDVYLLNLALADLLFALTLEIWAASKVNG-
CCR6_HUMAN	1	MSGESMNFSDVFDSSEDYFVSVNTSYYSVDSEMLLCSLQEVROFSRLFVEIAYSLICVFGLLGNILVVITFAFYKK-ARSMTVYLLNMAIADILFVUTLEFWAVSHA-IG
CCR9_HUMAN	1	MIPTDFTSPIPNMADDYGSESTSSMEDYVNFNFTDFYCEKNNVROFASHFLPPLYWLVFIVGALGNSLVILVYWYCTR-VKTMTDMFLLNLAIADLFLVTLPFWAIAAA-D
CCR7_HUMAN	1	MDLGKPMKSVLVVALLVIFQVCLCQDEVTDDYIGDNTTVDYTLFESLCSKKDVRNFKAWFLPIMYSIICFVGLLGNGLVVLTYIYFKR-LKTMTTYLLNLAVADILFLLTLFFWAYSAAK
CCRL1_HUMAN	1	
CXCR6_HUMAN	1	
CCR5_HUMAN	î	
CCR2 HUMAN	ĩ	
CCR3_HUMAN	ĩ	
CCR1_HUMAN	1	
-CCR8_HUMAN	î	
CCR4_HUMAN	î	
CCRL2_HUMAN	1	
CXCR7_HUMAN	1	MDLHLFDYSEPGNFSDISWPCNSSDCIVVDTVMCPNMPNKSVLLYTLSFIYIFIFVIGMIANSVVWVNIQAKT-TGYDTHCYILNLAIADLWVVLTIPVWVVSLVQHN
CCR10_HUMAN	1	
CX4_P21_25_a	-	
CA4_F21_25_a		*
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CXCR4_HUMAN	101	<mark>WYF</mark> GNFLC <mark>K</mark> AVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSO <mark>RP</mark> KLLAEKVVYVGVWIPALLLTI <mark>P</mark> DFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLIL <mark>P</mark> G
CXCR4_HUMAN CXCR5_HUMAN	101 114	L - <mark>NYF</mark> GNFLC <mark>K</mark> AVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSO <mark>RP</mark> RKLLAEKVVYVGVWIPALLLTI <mark>P</mark> DFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLIL <mark>P</mark> G L - <mark>NVLGTFLCKTVIALHKVNFYCSSLLLACIAVDRYLAIVHA</mark> VHAY <mark>RHR</mark> RLLSIHITCGTIWLVGFLLAL <mark>PEILF</mark> AKVSQGHHNNSLPRCTFSQENQAETHAWF <mark>TS</mark> RFLYHVAGFLL <mark>P</mark> M
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN	101 114 116	L - <mark>NYF</mark> GNFLC <mark>K</mark> AVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSORPRKLLAEKVVYVGVWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG L -VVLGTFLCKTVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYRHRRLLSIHITCGTIWLVGFLLALPEILFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPM 5 -WVFGSGLCKVAGALFNINFYAGALLLACISFDRYLN <mark>IVHA</mark> TQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVGRTALRVLQLVAGFLLPL
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CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR7_HUMAN	101 114 116 111 102 110 111 121	<pre>NYFGNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSORPEKLLAEKVVYVGVMIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG -WVGTFLCKTVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYHHERLLSIHITCGTINLVGFLLALPEIFAKVSQGHHNNSDRCTFSOENQAETHAWFTSRFLYHVAGFLLPM 6-WVFGSGLCKVAGALFNINFYAGALLLACISFDRYLNIVHAVHLYHRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVQRTALRVLQLVAGFLLPM 6-WVFGSGLCKVAGALFNINFYSGILLLACISFDRYLNIVHATQLYHRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVQRTALRVLQLVAGFLLPM 6-WVFGSGLCKVAGALFNINFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFICLSIWGLSLLALPVLLFRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSFGFIVPL 7-WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFICLSIWGLSLLAFVLLFPKTVYSSNVSPACYEDMGNNTANWRMLLRILPHTFGFIVPL 7-WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFICLGCWGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 7-WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFICLGCWGLSWISSSTFVFNQKYNTQGSDVCEFKYQTVSEFIRKLKMLGLLFGFFIFL 7-WFGTFLCKVVSLLKEVNFYSGILLLCISVDRYLAIVHATRTLTQKRHL-VKFICLGCWGLSWISSSTFVFNQKYNTQGSDVCEFKYQTVSEFIRKKLKMLGLLFGFFIFL 7-WFGTFLCKVVSLLKEVNFYSGILLLCISVDRYLAIVHATRTLTQKRHL-VKFICLGCWGLSVISSSTFVFNQKYNTQGSDVCEFKYQTVSEFIRKKLKLGLLFGFFIFL 7-WFGTFLCKVVSLKEVNFYSGILLLCISVDRYLAIVAATKSFLKSKILCVVWGCFIWVLAAALCIFEILYSQIKEESGIAICTMVYPSDESTKLKSAVLTKVILGFFLFF 7-WFGTFLCKVVSLKEVNFYSGILLTGSVDRYLAIVAAXAVATKAVSAHTKREKKLLYSKMVCFTIWVLAAALCIFEILYSDLQRSS-SEQAMRCSUITEHVEAFTIQVAQMVIGFLVFF 7-WFGCVFFCKLFAIYKMSFFSGULLLCISIDRYVAIVQAXSAHRHARVLISKSVGGWHILATVISIFELLYSDLQRSS-SEQAMRCSUITEHVEAFTIQVAQMVIGFLVF 7-WFGVFFCKLFAIYKMSFFSGULLTCISTDRYVAIVQAXSAHRHARVLISKSCGGWHILATVISIFELLYSDLQRSS-SEQAMRCSUITEHVEAFTIQVAQMVIGFLVF 7-WFGVFFCKLFAIYKMSFFSGULLTCISTDRYVAIVQAXSAHRHARVLUSKSCGGWHILATVISIFELLYSDLQRSS-SEQAMRCSUI</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR1_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR1_HUMAN	101 114 116 111 102 110 111 121 104	<pre></pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR6_HUMAN CCR7_HUMAN CCR1_HUMAN CXCR6_HUMAN	101 114 116 111 102 110 111 121 104 94	<pre>- WYFGNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSORPKLLAEKVVYVGVWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG - WVFGSGLCKVVALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYHHFRLLSIHITCGTIWLUGFLLALPEIFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPM 5 - WVFGSGLCKVAGALFNINFYAGALLLACISFDRYLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVQRTALRVLQLVAGFLLPL 1 - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFICLSIWGLSLLLALPULFRTVYSSNVSPACYEDMGNNTANWRMLRLRILPQSFGFIVPL 2 - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFICLSIWGLSLLLALPULFRTVYSSNVSPACYEDMGNNTANWRMVLRILPHTFGFIVPL 2 - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCWGLSWNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCWGLSWNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 - WIFGTFLCKVVSLLKEVNFYSGULLLCISMDRYIAIVQATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNQKYNTQGSDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPL 3 AVVFSNATCKLLKGIYANNFYSGULLLCISMDRYIAIVQATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNQKYNTQGSDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPL 4 QWK QTFMCKVVNSMYKMNFYSCVLLIMCISVDRYIAIAQAMRAHTWREKRLLYSKMVCFTIWVLAAALCIPETLYSQLKEESGIAICTMVYPSDESTKLKSAVLTLKVILGFFLPF 5 SWVFGVHFCKLIFAIYKMSFFSGMLLLLCISIDRYVAYVQVSQARHRRAVLLISKLSCVGIWILATVLSIPELLYSDLQRS-SEQAMRCSLIFITEHVAFITIQVAQMVIGFIVPL 6 GWUGKMCXITSALYTLNFVSGMQFLACISIDRYVAYTKVPSOSGVGKWIIGCVINMAAILLSIPQLVFYVNDNARCIFIDKAIGYVT 6 GWVGKMCXITSALYTLNFVSMLLLCISIDRYVAYKVYGYOAYKAPGSGVGKWIIGCVINMAAILLSIPQLVFYVNDNARCIFIDEAISTVVLATQMLGFFLPF</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR6_HUMAN CCR7_HUMAN CCR1_HUMAN CCR1_HUMAN CXCR6_HUMAN	101 114 116 111 102 110 111 121 104 94 93	<pre>- WYF GNFLC KAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSORP KLLAEKVVYVGVWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG - WVLGTFLC KTVIALHKVNFYCSSLLLACIAVDRYLAIVHATNAYRHFRLLSIHITCGTIWLUGFLLALPEILFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPM 5 - WVF GSGLC KVAGALFNINFYAGALLLACISVDRYLAIVHATQLYRRGPPARVTLTCLAVWGLCLFALPDFIFLSAHHDE-RLNATHCQYNFPQVGRTALRVLQLVAGFLLPL - WIF GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATQLYRRGPPARVTLTCLAVWGLCLFALPDFIFLSAHHDE-RLNATHCQYNFPQVGRTALRVLQLVAGFLLPL - WIF GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFICLSIWGLSLLLALPVLLFRRTVYSSNVSPACYEDMGNNTANKRMLRILPHTFGFIVPL 2 -WIF GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFVCLGC WGLSMLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 -WIF GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFVCLGC WGLSMLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 -WIF GTFLC KVVSLLKEVNFYSGILLLCISIDRY AIV QATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNQKYNTQGSDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPL 2 -WIF GTFLC KVVSLKKWNFYSGULLTCISIDRYAIN QATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNQKYNTQGSDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPL 3 AVVF SNATCKLLKGIYAINFNCGMLLLCISIDRYAINQAXRAHHWREKRLLYSKNVCFTIWLAAALCIPEILYSQIKEESGIAICTMVYPSDESTKLKSAVLTLKVILGFFLPF 4 - WYF GVHFC KLIFAIYKMSFFSGMLLLCISIDRYAIVQAXSARHHREKRLLYSKNVCFTIWLAAALCIPEILYSQIKEESGIAICTMVYPSDESTKLKSAVLTLKVILGFFLPF 5</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR1_HUMAN CCR1_HUMAN CCR6_HUMAN CCR5_HUMAN CCR2_HUMAN	101 114 116 111 102 110 111 121 104 94 93 105	- WYFGNFLCKAVHVIYTVNLYSSVLIAFISLDRYLAIVHATNSORPEKLLAEKVVYVGVMIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG - WVGTFLCKVVILHKVNFYCSSLLLACIAVDRYLAIVHAVHAYHHERLLJIHITCGTINLVGFLLALPEILFAKVSQGHHNNSLPRCTFSOENQAETHAWFTSRFLYHVAGFLLPM - WVFGSGLCKVAGALFNINFYAGALLLACISFDRYLNIVHATQLYHRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVQRTALRVLQLVAGFLLPM - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCNGLSMNLSLPFIFLRRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSFGFIVPL - WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCNGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 -WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCNGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 -WIFGTFLCKVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGCNGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 -WIFGTFLCKVVSLLKEVNFYSGILLLCISVDRYLAIVHATRTLTQKRHL-VKFVCLGCNGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 3 AWFSNATCKLLKGIYINFYSGULLLCISVDRYLAIVAATKSFRLSS TLEFRSKIICLVVNGLSVIISSSTFVFNQKYNTQGSDVCEFKYQTVSEFIRWKLMLGLELLFGFFIFL 4 GVHECKLFAIYKMSFFSGMLLLCISVDRYLAIVQAYSAHRHRAFVLISKKMVCFTINVLAAALCIPEILYSDLQRSS-SEQAMRCSIITEHVEAFITIQVAQMVIGFLVFI 5 SWYGVHCCKLLGKITSALYTNFYSGMLLLCISIDRYVAVTKVPSQSGVGRFCWIICFCVMMAILLSIPQLVFYTNDNARCIFIFPRYLGTSMKALIQMLEICIGFVVFF 5 GWVEGVMCCKSLGIYTINFYTSMLLICITVDRFIVVAVTKVPSQSGVGRFCWIICFCVMMAILLSIPQLVFYTNDNARCIFIFPRYLGTSMKALIQMLEICIGFVVFF 5 GWVEGNMCCLSUFGIFFIILLTIDRYLAIVHAVF-ALKARTVTFGVTSVITWVAVFASVFGIIFTKCQKEDKVTVCGPYFPRGWNNFHTIMRNILGLVLFL 5 EWVFGNAMCCKLFGLYHIGYFGGIFFIILLTIDRYLAIVHAVF-ALKARTVTFGVTSVITWVAVFASVFGIIFTKCQKEDSYVCGPYFPRGWNNFHTIMRNILGLVLFL
CXCR4_HUMAN CXCR5_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR1_HUMAN CCR1_HUMAN CCR5_HUMAN CCR5_HUMAN CCR3_HUMAN	101 114 116 111 102 110 111 121 104 94 93 105 98	<pre></pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR6_HUMAN CCR1_HUMAN CCR1_HUMAN CXCR6_HUMAN CCCR5_HUMAN CCC8_HUMAN CCC8_HUMAN	101 114 116 111 102 110 111 121 104 93 105 98 98	<pre>- WYFGNFLCKAVHVIYTVNLYSSVLIAFISLDRYLAIVHATNSORPKLLAEKVVYVGVNIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG - WVGTFLCKVVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYKHFRLLSIHITCGTIWLUGFLLALPEIFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPM - WVGSGLCKVAGALFNINFYAGALLLACISFDRYLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVGRTALRWFTSRFLYHVAGFLLPL - WIFGTFLCKVVSLLKEVNFYSGILLLACISFDRYLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYNFPQVGRTALRWFTSRFLYHVAGFLLPL - WIFGTFLCKVVSLLKEVNFYSGILLLACISFDRYLNIVHATQLYRRGPPARVTLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCQYN</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR6_HUMAN CCR1_HUMAN CCR1_HUMAN CXCR6_HUMAN CCR5_HUMAN CCR3_HUMAN CCR3_HUMAN CCR8_HUMAN	101 114 116 111 102 110 111 121 104 93 105 98 98 98	<pre>- WY GNFLC KAVHVIYTVNLYSSVLIAFISLDRYLAIVHATNSORP KLLAEKVVYVGVNIPALLLTIPDFIF ANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPG - WV GTFLC KVVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYHH FRLLSIHITCGTIWLUGFLLALPELFAKVSQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPM 5 - WV GSGLC KVAGALFNINFYAGALLACISVDRYLAIVHATQLYRRGPPARVTLTCLAVWGLCLFALPDFIF LSAHHDE-RLNATHCQYNFPQVGRTALRVLQLVAGFLLPL 5 - WV GSGLC KVAGALFNINFYAGALLACISVDRYLAIVHATQLYRRGPPARVTLTCLAVWGLCLFALPDFIF LSAHHDE-RLNATHCQYNFPQVGRTALRVLQLVAGFLLPL 5 - WI GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFICLSIWGLSLLLAPVLLFRTVYSSNVSPACYEDMGNNTANWRMVLRILPHTFGFIVPL 2 - WI GTFLC KVVSLLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRYL-VKFVCLGC WGLSNNLSLPFFLFRQAYHPN-NSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 - WI GTFLC KVVSLKEVNFYSGILLLACISVDRYLAIVHATRTLTQKRHL-VKFVCLGC WGLSNNLSLPFFLFRQAYHPN-NSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL 2 - WI GTFLC KVVSLKEVNFYSGILLLACISVDRYLAIVQATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNQKYNQGSDVCEPKYQTVSEPIRWKLLMLGLELFGFFIPL 3 AVV SNATCKLLKGIYAINFNCGMLLLCISDRYYIAIVQAMKATKREKRLLYSKMVCFTIWVLAAALCIBETLYSQIKEESGIAICTMVYPSDESTKLKSAVLTLKVILGFFLPF 5 SVV GVHFC KLIFAIYKMSFYSGMLLLCISDRYVAIVQAVSAHRHRAKKLLXSKMVCFTIWVLAAALCIBETLYSQIKEESGIAICTMVYPSDESTKLKSAVLTLKVILGFFLPF 5 SVV GVHC KLIFAIYKMSFYSGMLLLCISDRYVAIVQAVSAHRHRAKVLLISKLSCVGIWILATVLSIPELLYSQLVQSS-SEQAMRCSLITEHVAFITIQVAQMVIGFIVPL 5 SVV GVHC KLIFAIYTNFYSMLLLCITDRYLAVYNTKVPSOSGVGKPCWIIGTCVMMAAILSIPQLVFYTVNDNACIPIDKLICGYHDEAISTVVLATQMTLGFFLPL 6 WV GQVMC KSLLGIYTINFYTSMLLILCITVDRFIVVKATKAYNQQAKRMTWGKVTSLLIWVISLLVSLPQIIYGNVFNLDKLICGYHDEAISTVVLATQMTLGFFLPL 9 WV GNAMCKLFTGLYFIGFFSGIFFIILLTIDRYLAVYHAVFALKARTVTFGVTSVITWVAVFASVGGIIFTKCQKEGLHYTCSSHFPYSQYQFWKNFQTLKIVLGLVLPL 9 WV GNAMCKLFTGLYFIGFFSGIFFIILLTIDRYLAVHAVFALKARTVTFGVTSVITWVAVFASVGGIIFTKCQKEDSYVCGFYFPHSWRHFHTLRMTIGLVPL 9 WV GNAMCKLFTGLYSEFFIILLTIDRYLAVHAVFALKARTVTFGVITSVITWVAVFASVGGIFTKCQKEFYPHCSLALPFHSWRHFHTLRMTIGLVPL 9 WV GNAMCKLFTGLYSEFFIILLTIDRYLAVHAVFALKARTVTFGVITSVITW</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR3_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR7_HUMAN CCR1_HUMAN CCR5_HUMAN CCR2_HUMAN CCR3_HUMAN CCR1_HUMAN CCR4_HUMAN	101 114 116 111 102 110 111 121 104 93 105 98 98 98 98 98	<pre></pre>
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CXCR4_HUMAN CXCR5_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR6_HUMAN CCR1_HUMAN CCR1_HUMAN CCR5_HUMAN CCR3_HUMAN CCR1_HUMAN CCR1_HUMAN CCR4_HUMAN CCR4_HUMAN CCR1_HUMAN	101 114 116 111 102 110 111 121 104 93 105 98 98 98 98 98 102 98 102 98	<pre>- WY GNFLC KAVHVIYTVNLYSSVLIAFISLDRYLAIVHATNSORP KLLAEKVVYVGVNIPALLLTIPDFIF ANVSEADDRYICDRFYPNDLWVVVFOFOHINVGLILPG - WV GTFLC KVVIALHKVNFYCSSLLLACIAVDRYLAIVHAVHAYKH FRL5IHITCGTINUUGFLALPEIFAKVSOGHHNNSLPRCTFSOENOAETHAWFTSRFLYHVAGFLLPM - WV GTGSLC KVAGALFNINFYAGALLLACISFDRYLNIVHATOLYR GPPARVLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCOYNFPOVGRTALRWFTSRFLYHVAGFLLPL - WI GTFLC KVVSLLKEVNFYSGILLLACISFDRYLNIVHATOLYR GPPARVLTCLAVWGLCLLFALPDFIFLSAHHDE-RLNATHCOYNFPOVGRTALRWFTSRFLYHVAGFLLPL - WI GTFLC KVVSLLKEVNFYSGILLLACISFDRYLNIVHATOLYR GPPARVLTVFKUSUGLSULSVISS-NVSPACYEDWONTANWRMLRILPLOSFGFIVPL - WI GTFLC KVVSLLKEVNFYSGILLLACISFDRYLAIVHATRTLTOK RKL-VKFVCLGCMGLSMNLSLPFFLF ROAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL - WI GTFLC KVVSLLKEVNFYSGILLLACISFDRYLAIVHATRTLTOK RKL-VKFVCLGCMGLSMNLSLPFFLF ROAYHPNNSSPVCYEVLGNDTAKWRMVLRILPHTFGFIVPL - WI GTFLC KVVSLLKEVNFYSGILLLCISFDRYVAIVOATKSFRLASTLPRSKIICLVVWGLSVIISSSTFVFNOKYNTQGSDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPL 0 kW 0 GTFMC KVVNSMYKNNFYSCVLLIMCISFDRYAINOOAKRHTWRKRLLYSKMVCFTINVLAAALCIPEILYSQIKEESGIAICTMYYPSDESTKLKSAVLTLKVILGFFLPF 5 WV GVHFCKLIFAIYKMSFFSGMLLLCISIDRYVAIVOAVSAKHHRARVLLISKLSCVGINILATVLSIPELLYSDLQRSS-SEQAMRCSIITEHVEATMKALIQMLEICIGFVVF 6 WV GVMCKSLGIYTINFYSMLLCCITVDRFIVVWKATKAYNQOARRHTWGKVTSLLINVISLLVSLPQIIYGNVFNLDKLICGYHDEAISTVVLATOMTLGFFLPF 5 WV GQVMCKSLGIYTINFYSMLLTCITVDRFIVVWKATKAYNQOARRHTWGKVTSLLINVISLLVSLPQIIYGNVFNLDKLICGYHDEAISTVVLATOMTLGFFLPF 6 WV GNAMCKLFGLYGGIFFIILLTIDRYLAVHAVFALKARTVTFGVTSVITNUVAVFASLPGIIFTRSOKEGLHYTCSSHFPYSQVOFWKNFOTKIVILGLVLL 6 WV GNAMCKLFGLYGLYSEIFFIILLTIDRYLAIVHAVFALKARTVTFGVTSVITNULAVFASLPGIFTRSOKEGHYTCSLAFPHSSNKMNFTKRNILGLVPL 7 WW GNAMCKLFGLYGLYSEIFFIILLTIDRYLAIVHAVFALKARTVTFGVTSVITNULAVFASLPGIFTKCOKEDSVYCGPYFPRGNNFHTIMNILGLVPL 7 WW GNACKLFGLYGLYSEIFFIILLTIDRYLAIVHAVFALKARTVTFGVTSVITNULAVFASLPGIFTKCOKEDTHTCSLHFPHSSLRWKLFQALKLNFGVLYL 7 WW GTVMCXVVSGYYIGFYSSMFFITLSVDRYLAIVHAVFALKARTVTFGVTSVITNULAVFASLANGGLYFSKTQWEFTHHTCSL</pre>
CXCR4_HUMAN CXCR5_HUMAN CXCR2_HUMAN CXCR1_HUMAN CCR6_HUMAN CCR9_HUMAN CCR1_HUMAN CCR1_HUMAN CCR5_HUMAN CCR5_HUMAN CCR3_HUMAN CCR1_HUMAN CCR1_HUMAN CCR4_HUMAN CCR4_HUMAN	101 114 116 111 102 110 111 121 104 94 93 105 98 98 98 98 98 98	<pre></pre>

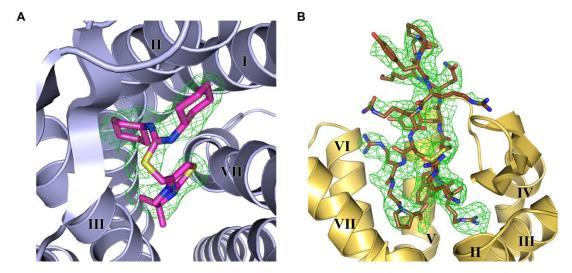
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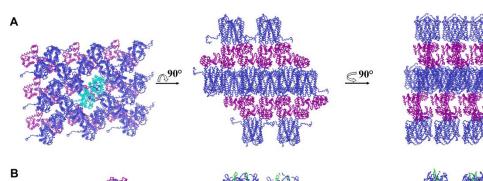
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CX4_P21_25_a	187	IVILSCYCIIISKLSHSGSGSKGHORKALKTTVILILAFFACHLWYIGISIDSFILLEIIKGGEFENTVHKWISIT ALAFFHCCLNULLGAKIKTSAGHALSGRPLEVLFQ
CXCR4_HUMAN	213	IVILSCYCIIISKLSHSKGHQKRKALKTTVILILAFFACNLFYYIGISIDSFILLEIIKQGCEFENTVHKWISIT ALAFFHCCLNFILYAFLGAKFKTSAQHALTSVSRGSSLKILSK
CXCR5_HUMAN	232	LVMGWCYVGVVHRLROAQRRPOROKAVRVAILVTSIFFLCWSPYHIVIFLDTLARLKAVDNTCKLNGSLPVAITMCEFLGLAHCCLNPMLYTRAGVKFRSDLSRLLTKLGCTGPASLCQL
CXCR3_HUMAN	229	LVMAYCY-AHILAVLLVSRORALRAMALVVVVVAFALCNTPYHLVVLVDILMDLGALARNCGRESRVDVAKSVTSGLGYMHCCLNPLLYAFVGVKFRERMMLLLRLGCPNORGLORO
CXCR2_HUMAN	225	LIMLFCYGFTLETLFKAHMGONHRAMRVIFAVVLINLCNLPYNLVLLADTLMRTOVIOETCERRNHIDRALDAT LGILHSCLNPLIYAFIGON RHGLLKILAIHGLISKDSLPKD
CXCR1_HUMAN	216	FVMLFCYGFTLRTLFKAHMGONHRAMRVIFAVVLIFLLCNLPYNLVLLADTLMRTOVIOESCERRNNIGRALDAT ILGFLHSCLNPIIYAFIGON RHGFLKILAMHGLVSKEFLARH
CCR6_HUMAN	228	MFMIFCYTFIVMTLVOAQNSK <mark>AHKAIRVIIAVVLVFLACQIPHNMVLLVTAANLGKM-NRSC</mark> QSEKLIGYTKTVT <mark>UVLAFLHCCLNPVLYAFIGORFR</mark> NYFLKILKDLWCVRRKYKSSG
CCR9_HUMAN	228	VVMACCYTIIIITTLIOAKKSS <mark>HHKALKVTITVLTVE</mark> VLSOF <mark>P</mark> YNCILLVOTIDAYAMFISNCAVSTNIDICFOVTOTIAFFHSCLN <mark>P</mark> VLYVTVGERIRRDLVKTLKNLGCISOAOWVSF
CCR7_HUMAN	237	LAMSFCYLVIIRILOARNFE <mark>RNKAINVIIAVVVVF</mark> IVFOL <mark>P</mark> YNGVVLAQTVANFNITSST <mark>CE</mark> LSKOLNIAYDVTYSLACVRCCVN <mark>P</mark> FLYAFIGVKIRNDLFKLFKDLGCLSQEQLRQW
CCRL1_HUMAN	214	LIMGVCYFITARTLMKMPNIKIS <mark>R</mark> PLKVLLTVVIVFIVTQLPYNIVKFCRAIDIIYSLITSCNMSKRMDIAIQVTSIALFHSCLNPILYY MGAS KNYMKVAKKYGSNRRQROSVE
CXCR6_HUMAN	205	LTMIVCYSVIIKTLLHAGGFOMHRSLMIIFLVMAVFLLTOMPINLMKFIRSTHWEYYAMTSFHYTIMVTATAYLRACLNEVLYATVSLKTRKNFWKLVKDIGCLPYLGVSHO
CCR5_HUMAN	208	LVMVICYSGILKILIRCRNEKK <mark>HHRAVH</mark> LIFIIMIVYFLF <mark>MAP</mark> YNIVLLLNTFQEFFG-LNN <mark>C</mark> SSSNRLDQAMQVI <mark>T</mark> ILGMIHCCIN <mark>P</mark> IIYAFVGEKIRNYLLVFFQKHIAKRFCKCCSI
CCR2_HUMAN	216	LIMVICYSGILKILIRCRNEKK <mark>HHRAVRVIFIIMIVYFLFWIP</mark> YNIVILLNIFQEFFG-LSN <mark>CE</mark> SISQLDQATQVI <mark>T</mark> ILGMIHCCIN <mark>E</mark> IIYAFVGEKIRSLFHIALGCRIAPLQKPVCGG
CCR3_HUMAN	213	LVMAICYTGIIKTLLRCPSKKKYKAIRLIFVIMAVFFIFNT <mark>P</mark> YNVAILLSSYQSILF-GND <mark>CE</mark> RSKHLDLVMLVT <mark>VIAYSHCCMN</mark> PVIYAFVGERIRKYLRHFFHRHLLMHLGRYIPF
CCR1_HUMAN	213	LVMIICYTGIIKILLRRPNEKKSKAVHLIFVIMIIFFLFWT <mark>P</mark> YNLTILISVFQDFLF-THE <mark>CE</mark> QSRHLDLAVQVT <mark>T</mark> VIAYTHCCVN <mark>E</mark> VIAK VGER KKYLRQLFHRRVAVHLVKWLPF
CCR8_HUMAN	212	TIFMFCYIKILHOLKRCQNHN <mark>KTKAIRLVLIVVIASLLFNVPFNVVLF</mark> LTSLHSMHI-LDGCSISQQLTYATHVT <mark>H</mark> IISFTHCCVN <mark>H</mark> VIYALVGEKIKKHLSEIFQKSCSQIFNYLGRQ
CCR4_HUMAN	216	GINLFCYSMIINTLOHCKNEKKNKAVKMIFAVVVLFLGFNTPYNIVLFLETLVELEV-LODCTFERYLDYAIQATTILAFVHCCLNPIIYFLGEKIKKYILOLF-KTCRGLFVLCQYC
CCRL2_HUMAN	214	FIFTFLYVOMRKTLRFREORYSLFKLVFAIMVVFLLMAAPYNIAFFLSTFKEHFS-LSDCKSSYNLDKSVHITKLIATTHCCINELLYAFLDGTKSKULCRCFHLRSNTPLOPRGOS
CXCR7_HUMAN	226	SITAVFYFLLARATSASSDOEKHSSRKIIFSYVVVFLVCHLPYHVAVLLDIFSILHYIPFTCRLEHALFTALHVTQCLSLVHCCVN <mark>P</mark> VLYS <mark>KINRNYR</mark> YELMKAFIFKYSAKTGLTKLI
CCR10_HUMAN	221	GVMVACYALLG <mark>RTI</mark> LAARGPE <mark>HRRALR</mark> VVVALVAA <mark>F</mark> VVLOL <mark>E</mark> YSLALLLDTADLLAARERS <mark>C</mark> PASKRKDVALLVTSGLALARCGIN <mark>E</mark> VLYAFLGLRI MODLRRLLRGGSCPSGPOPRRG
CX4_P21_25_a		
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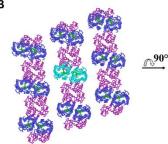
CX4_P21_25_a	306	
CXCR4_HUMAN	332	GKRGGHSSVSTESESSSFHSS
CXCR5_HUMAN	352	FPSWRRSSLSESENATSLTTF
CXCR3_HUMAN	348	PSSSRRDSSWSETSEASYSGL
CXCR2_HUMAN	344	SRPSFVGSSSGHTSTTL
CXCR1_HUMAN	335	RVTSYTSSSVNVSSNL
CCR6_HUMAN	346	FSCAGRYSENISROTSETADNDNASSFTM
CCR9_HUMAN	347	TRREGSLKLSSMLLETTSGALSL
CCR7_HUMAN	356	SSCRHIRRSSMSVEAETTTTFSP
CCRL1_HUMAN	333	EFPFDSEGPTEPTSTFSI
CXCR6_HUMAN	318	WKSSEDNSKTFSASHNVEATSMFQL
CCR5_HUMAN	327	FQQEAPERASSVYTRSTGEQEISVGL
CCR2_HUMAN	335	PGVRPGKNVKVTTQGLLDGRGKGKSIGRAPEASLQDKEGA-
CCR3_HUMAN	331	LPSEKLERTSSVSPSTAEPELSIVF
CCR1_HUMAN	331	LSVDRLERVSSTSPSTGEHELSAGF
CCR8_HUMAN	330	MPRESCEKSSSCQQHSSRSSSVDYIL
CCR4_HUMAN	333	GLLQIYSADTPSSSYTQSTMDHDLHDAL
CCRL2_HUMAN	330	AQGTSREEPDHSTEV
CXCR7_HUMAN	345	DASRVSETEYSALEQSTK
CCR10_HUMAN	340	CPRRPRLSSCSAPTETHSLSWDN
CX4_P21_25_a		

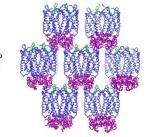
Supplementary Figure S2. Sequence alignment between human chemokine receptors. Conserved cysteine residues are highlighted in yellow, and the four cysteines forming disulfide bonds in CXCR4 (Cys28-Cys274 and Cys109-Cys186) are marked with stars. Other colors represent properties of the conserved residues: blue, positively charged; red, negatively charged; cyan, polar; magenta, aromatic; green, hydrophobic; orange, proline. Intensity of the color is function of residue conservation. First row of the alignment shows CXCR4-2 sequence.

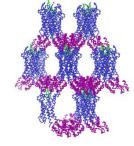


Supplementary Figure S3. Electron density of (**A**) IT1t in CXCR4-2/IT1t, and (**B**) CVX15 in CXCR4-3/CVX15. IT1t and CVX15 are shown in stick representation. Electron density is contoured at 2.5 σ for IT1t and 2.0 σ for CVX15 from an F_{obs} - F_{calc} omit map calculated without the contribution of ligands.



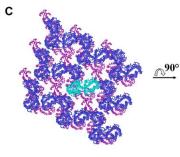


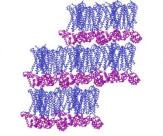


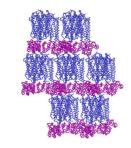


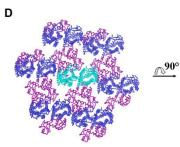
S 90°

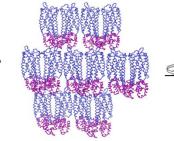
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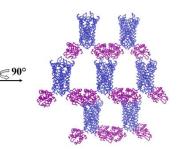


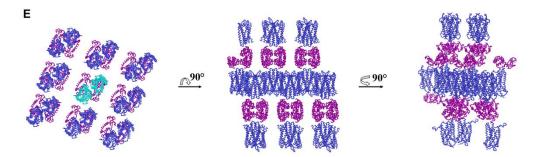












Supplementary Figure S4. Crystal-packing in five crystal forms, showing conserved CXCR4 dimers. CXCR4 is colored blue, and T4 lysozyme is shown in purple. One of the dimers is colored cyan. (A) CXCR4-2/IT1t (space group *P2*₁). (B) CXCR4-3/CVX15 (space group *C2*). (C) CXCR4-2/IT1t (space group *P1*). (D) CXCR4-3/IT1t (space group *P1*). (E) CXCR4-1/IT1t (space group *I222*).

Supplementary Tables:

Supplementary Table S1. Data collection (APS GM/CA 23ID-B/D, *10-µm* beam) and refinement statistics. Highest resolution shell is shown in parentheses.

Structure	CXCR4-2 /IT1t ₁ (A)	CXCR4-3 /CVX15 (B)	CXCR4-2 /IT1t ₂ (C)	CXCR4-3 /IT1t (D)	CXCR4-1 /IT1t (E)
Construct definition	+T4L1†, dC320‡, +L125W	+T4L2†, dC320‡, +L125W, +T240P	+T4L1†, dC320‡, +L125W	+T4L2†, dC320‡, +L125W, +T240P	+T4L1†, dC326‡, +L125W
PDB ID	30DU	3OE0	30E8	30E9	30E6
		Data collection			
Number of crystals	2	14	11	9	3
Space group	$P2_1$	<i>C2</i>	<i>P1</i>	P1	<i>I222</i>
Cell dimensions	-				
a, b, c (Å)	64.5, 83.7, 120.0	82.1, 144.9, 74.0	69.4, 76.6, 91.7	72.8, 72.9, 84.5	71.1, 78.7, 240.6
α, β, γ (°)	90.0, 102.2, 90.0	90.0, 104.5, 90.0	96.0, 97.8, 97.4	64.6, 73.8, 61.3	90.0, 90.0, 90.0
Number of reflections measured	94,558	106,704	93,481	76,109	36,485
Number of unique reflections	41,569	17,656	28,801	24,127	10,233
Resolution (Å)	50.0-2.50	50.0-2.90	50.0-3.10	50.0-3.10	50.0-3.30
	(2.59-2.50)	(3.00-2.90)	(3.15-3.10)	(3.27-3.10)	(3.42-3.30)
R_{sym}^{*}	0.12 (0.49)	0.12 (0.63)	0.15 (0.51)	0.12 (0.51)	0.14 (0.67)
Mean $I/\sigma(I)$	7.2 (1.8)	9.5 (1.5)	12.7 (1.8)	11.2 (1.5)	10.2 (1.5)
Completeness (%)	95.8 (89.0)	94.9 (73.6)	87.3 (59.3)	96.9 (87.7)	94.4 (78.2)
Redundancy	2.3 (1.9)	6.0 (3.9)	3.2 (1.9)	3.2 (2.5)	3.6 (2.2)
		Refinement			
Resolution (Å)	20.0-2.50	20.0-2.90	20.0-3.10	20.0-3.10	6.0-3.20
Number of reflections	41,455	16,707	28,647	24,209	8,310
(test set)	(2,079)	(898)	(1,441)	(1,238)	(406)

Rwork / Rfree	0.239 / 0.286	0.209 / 0.267	0.247 / 0.308	0.251 / 0.283	0.230 / 0.308
Number of atoms	7,803	3,644	10,397	6,804	3,444
Protein	7,425	3,488	10,316	6,750	3,368
Lipids, ligand, and other	243	150	81	54	76
Water	135	6	0	0	0
Overall <i>B</i> values (Å ²)	43	68	98	101	68
CXCR4	38	66	91	99	55
T4 lysozyme	54	72	108	104	93
Ligand	42	67	96	106	69
Lipid	50	—	—	—	59
RMSD					
Bond lengths (Å)	0.012	0.011	0.009	0.009	0.012
Bond angles (°)	1.25	1.28	1.07	1.12	1.43
Ramachandran plot statistics					
(%) (excluding Gly, Pro)					
Most favored regions	90.3	89.6	89.3	87.0	89.6
Additionally allowed regions	9.1	10.0	9.5	12.2	9.8
Generously allowed regions	0.6	0.7	0.7	0.5	0.5
Disallowed regions	0.0	0.0	0.4	0.3	0.0

* $R_{sym} = \sum_{hkl} |I(hkl) - \langle I(hkl) \rangle | \sum_{hkl} \langle I(hkl) \rangle$, where $\langle I(hkl) \rangle$ is the mean of the symmetry-equivalent reflections of I(hkl).

†T4L1 & T4L2: different T4L junction sites. Details are shown in supplementary molecular biology section.

‡dC320 and dC326: C-terminal truncations. C-terminal residues 320-352 and 326-352 were truncated in different constructs.

Antagonist	Structure or sequence	Molecular weight, Da	CXCR4 binding, IC ₅₀ , nM	T _m ∗, °C
IT1t	$N = \bigvee_{N = 1}^{N} \bigvee_{N = 1}^$	404	8.0†	57
CVX15	¹ Arg- ² Arg- ³ Nal- ⁴ Cys- ⁵ Tyr- ⁶ Gln- ⁷ Lys- ⁸ dPro- ⁹ Pro- ¹⁰ Tyr- ¹¹ Arg- ¹² Cit- ¹³ Cys- ¹⁴ Arg- ¹⁵ Gly- ¹⁶ dPro (⁴ Cys— ¹³ Cys disulfide bond)	2115	0.6‡	57

Supplementary Table S2. CXCR4 antagonists in ligand optimization.

* Melting temperature measured using CXCR4-2 construct.
†Radioligand competition assay using [¹²⁵I]CXCL12 and CEM cell membranes (14).
‡Radioligand competition assay using [¹²⁵I]CXCL12 and Jurkat cells (whole cell RLBA).

Supplementary Table S3. The equilibrium constant (K_d) values for BIMA and apparent affinity
(K_i) values determined for various CXCR4 constructs using the filter plate and SPA methods. All
measurements were performed in triplicate and repeated at least three times. The mean values \pm
standard deviation for K_d of [³ H]BIMA and K_i of other ligands are shown.

C	BIMA (K_d , nM)		IT1t (K_i , nM)		CVX15 (K_i , nM)	
Constructs	Filter plate	SPA	Filter plate	SPA	Filter plate	SPA
CXCR4-WT (Sf9)	27.5 ± 1.2	3.5 ± 1.5	11.2 ± 2.7	8.3 ± 1.0	46.7 ± 8.7	1.1 ± 1.0
CXCR4-1 (Sf9)	25.6 ± 1.5	7.2 ± 1.2	14.0 ± 1.7	8.2 ± 1.0	268 ± 53.0	162.1 ± 1.1
CXCR4-2 (Sf9)	36.9 ± 1.2	7.4 ± 1.3	25.6 ± 4.8	7.3 ± 1.4	201 ± 36.1	189.2 ± 1.4
CXCR4-3 (Sf9)	24.4 ± 1.2	5.2 ± 1.2	19.8 ± 5.0	6.6 ± 1.4	55.3 ± 7.0	2.5 ± 1.0
CXCR4-WT (HEK293T)		3.7 ± 1.4		22 ± 1.5		2.3 ± 0.9

Note: K_d and K_i values for CXCR4 constructs were determined using both filter plate and SPA methods for crossvalidation of the data. Due to nonspecific interaction between CVX15, Sf9 membranes, and the filter plate, extensive optimization of assay buffer and membrane treatment was required to obtain reproducible K_d and K_i values in the radioligand binding assay using the filter plate. We then used another assay method (SPA) to ensure that the relative binding affinities between the different CXCR4 constructs are not skewed by the assay methods and conditions.

Crystal form	Construct	Compound	Precipitant solution	Average Crystal size	Average Crystal growth time	Space group	Molecule(s) per AU*	Estimated solvent content
A	CXCR4-2 (+T4L1†,dC320‡, +L125W)	IT1t	100 mM sodium citrate, pH 5.5, 20% (v/v) PEG400, 300 mM sodium malonate, 5 mM Taurine and 2 mM IT1t.	~ 90 µm × 40 µm × <5 µm	10 days	P2 ₁	2	57%
В	CXCR4-3 (+T4L2†, dC320‡, +L125W,+T240P)	CVX15	100 mM Tris, pH 7.0, 25% (v/v) PEG400, 300 mM potassium sodium tartrate and 1 mM CVX15.	~ 40 μm × 30 μm × 10 μm	10 days	<i>C</i> 2	1	68%
С	CXCR4-2 (+T4L1†,dC320‡, +L125W)	IT1t	100 mM MES, pH 6.0, 26% (v/v) PEG400, 300 mM Sodium malonate, 5 mM strontium chloride and 2 mM IT1t.	~ 20 μm × 10 μm × 10 μm	3 days	Р1	3	57%
D	CXCR4-3 (+T4L2†, dC320‡, +L125W,+T240P)	IT1t	100 mM MES, pH 6.0, 27-35% (v/v) PEG400, 270-330 mM sodium malonate, 5 mM hexamine cobalt chloride and 2 mM IT1t.	~ 60 μm × 40 μm × 15 μm	10 days	P1	2	61%
E	CXCR4-1 (+T4L1†,dC326 * , +L125W)	IT1t	100 mM sodium citrate, pH 5.0- 5.5, 20-26% (v/v) PEG400, 280-320 mM sodium malonate, 5 mM nickel chloride and 2 mM IT1t.	~ 60 μm × 10 μm × <5 μm	7 days	I222	1	59%

Supplementary Table S4. Five crystal forms obtained by LCP crystallization.

*AU: asymmetric unit.

†T4L1 & T4L2: different T4L junction sites. Details are shown in supplementary molecular biology section. ‡dC320 and dC326: C-terminal truncations. C-terminal residues 320-352 and 326-352 were truncated in different constructs.

CXCR4	IT1t	Distance (Å)
Asp97 ^{2.63} (OD1)	N4	2.7
Glu288 ^{7.39} (OE1)	N1	2.8
Hydrophobic int	eractions between CXC	R4 and IT1t
CXCR4	IT1t	Distance (Å)
Trp94 ^{2.60} (CZ2)	C18	3.6
Trp94 ^{2.60} (CZ3)	S1	3.8
Trp102 (CZ3)	C20	3.9
Val112 ^{3.28} (CG2)	C19	3.8
Tyr116 ^{3.32} (CE2)	C3	3.6
Arg183 (CZ)	C12	3.8
lle185 (CD1)	C12	3.7
Ile185 (CD1)	C11	3.5
Cys186 (CB)	C21	3.7
Cys186 (O)	S2	3.0
Asp187 (CG)	S2	3.7
Hydrogen bond and salt	bridge contacts between	CXCR4 and CVX1
CXCR4	CVX15	Distance (Å)
Asp187 (OD1)	Arg1 (NH1)	3.1
Asp187 (OD2)	Arg1 (N)	3.2
Arg188 (N)	Arg1 (O)	3.1
His113 ^{3.29} (ND1)	Arg2 (NH1)	2.9
Thr117 ^{3.33} (OG1)	Arg2 (NH2)	2.9
Asp171 ^{4.60} (OD2)	Arg2 (NH2)	3.0
Arg188 (NH2)	Arg2 (O)	3.2
Arg188 (NE)	Arg2 (O)	2.9

Supplementary Table S5. Direct contacts between CXCR4 and ligands.

Arg188 (O)	Cys4 (N)	3.0
Tyr190 (N)	Cys4 (O)	2.9
Asp193 (OD2)	Lys7 (NZ)	2.9
Asp262 ^{6.58} (OD2)	Arg14 (NH1)	3.2
Asp262 ^{6.58} (OD1)	Arg14 (O)	2.9
Intramolec	ular hydrogen bonds of (CVX15
CVX1		Distance (Å)
Arg2 (N)	dPro16 (OXT)	2.6
Nal3 (O)	Arg14 (N)	2.9
Tyr5 (OH)	Arg14 (NE)	3.5
Tyr5 (N)	Cit12 (O)	2.7
Tyr5 (O)	Cit12 (N)	2.8
Lys7 (N)	Tyr10 (O)	2.7
Lys7 (O)	Tyr10 (N)	2.6

. 0	en bonds in CXCR4-2/IT1t	unner
Molecule A atom	Molecule B atom	Distance (Å)
Asn192 (ND2)	Leu266 ^{6.62} (O)	3.0
Asn192 (ND2)	Glu268 (OE2)	2.6
Trp195 ^{5.34} (NE1)	Leu267 ^{6.63} (O)	2.8
Leu266 ^{6.62} (O)	Asn192 (ND2)	3.0
Leu267 ^{6.63} (O)	Trp195 ^{5.34} (NE1)	2.8
Glu268 (OE2)	Asn192 (ND2)	2.6
Hydrophobic	interactions in CXCR4-2/I	T1t dimer
Molecule A atom	Molecule B atom	Distance (Å)
Leu194 ^{5.33} (CD2)	Leu194 ^{5.33} (CD2)	3.5
Leu194 ^{5.33} (CD2)	Val197 ^{5.36} (CG2)	3.6
Val197 ^{5.36} (CG2)	Leu194 ^{5.33} (CD2)	3.3
Val197 ^{5.36} (CG1)	Val198 ^{5.37} (CG2)	3.6
Val198 ^{5.37} (CG2)	Val197 ^{5.36} (CG1)	3.6
Val198 ^{5.37} (CG1)	Phe201 ^{5.40} (CE1)	4.0
Phe201 ^{5.40} (CE1)	Val198 ^{5.37} (CG1)	3.7
Phe201 ^{5.40} (CD1)	Phe201 ^{5.40} (CD1)	4.0
Met205 ^{5.44} (CG)	Met205 ^{5.44} (CE)	4.3
Leu210 ^{5.49} (CD1)	Leu210 ^{5.49} (CD2)	3.5
Leu210 ^{5.49} (CD2)	Leu210 ^{5.49} (CD1)	3.5
Trp195 ^{5.34} (CE2)	Leu267 ^{6.63} (CB)	3.6
Leu267 ^{6.63} (CB)	Trp195 ^{5.34} (CE2)	3.6
Additional hydroph	obic interactions in CXCR4	4-3/CVX15 dimer
Molecule A atom	Molecule B (crystal symmetry related molecule) atom	Distance (Å)
Leu136 ^{3.52} (CD2)	His140 (CE1)	3.6

Supplementary Table S6. CXCR4 homodimer interactions.

His140 (CE1)	Leu136 ^{3.52} (CD2)	3.6
His140 (CE1)	His140 (CG)	3.6

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