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New G-protein-coupled receptor structures provide insights into the recognition of CXCL12 and HIV-1 gp120 by CXCR4

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The G protein-coupled receptor (GPCR) superfamily consists of thousands of integral membrane proteins that exert a wide variety of physiological functions and account for a large portion of the drug targets identified so far. However, structural knowledge of GPCRs is scarce, with crystal structures determined for only a few members including β1 and β2 adrenergic receptors, adenosine receptor, rhodopsin, and dopamine D3 receptor [1]. Recently, Wu *et al*. published in *Science* the structure of CXCR4, which is the first structure of the 19 known chemokine receptors of the GPCR superfamily [2].

The widely expressed chemokine receptor CXCR4 is exclusively activated by CXCL12 (also known as SDF-1). The CXCL12/CXCR4 signaling plays important functional roles in various biological processes such as cell trafficking, cell survival, and proliferation, and is essential for the development (reviewed in [3,4]). The CXCL12/CXCR4 axis has also been indicated to be critical for the homing of CXCR4-expressing tumor cells to tissues secreting CXCL12 such as the bone marrow, lung, liver, and lymph nodes, and their survival and proliferation in the new environment [3,4]. Therefore, the CXCL12/CXCR4 pathway is an important drug target for various types of cancers [5], and one small-molecule inhibitor of the pathway, namely Plerixafor has been approved by the US Food and Drug Administration for the treatment of non-Hodgkin lymphoma and multiple myeloma together with granulocyte-colony stimulating factor. In addition, CXCR4 also functions as a co-receptor for the entry of X4 HIV-1 strains and is a validated pharmaceutical target for modulating the viral entry of HIV-1 into T cells [6].

Precise structural information is valuable for design and development of new regimens against diseases. Although CXCR4 is an important drug target, by the time Wu *et al*. published their results, only one structure of CXCL12 in complex with a peptide derived from the sequence of the N-terminal 38 residues of CXCR4 (p38) was reported [7]. Although the CXCL12-p38 structure elucidated the structural basis for the interaction of CXCL12 with the N-terminus of CXCR4 [7], the structure of CXCR4 is still largely unknown, and the other regions of CXCR4 that potentially interact with CXCL12 has not been identified. Moreover, the dimeric CXCL12-p38 structure might only represent one of the dynamic oligomeric states of the complex. For the fusion of HIV-1, CCR5 is a co-receptor for CD4 for R5 HIV-1 strains, which is also a chemokine receptor of the GPCR superfamily. The structure of the CCR5 N-terminus (residues 2–15) in complex with HIV-1 gp120 and CD4 had been determined recently [8]; however, a similar structure of CXCR4 was not available yet.

In the recent paper by Wu *et al*., five crystal structures of human CXCR4 variants in complexes with a small-molecule isothiourea derivative, namely 1T15, and with a cyclic peptide inhibitor, namely CVX15 at resolution of 2.5–3.2 Å are reported [2]. Comparison of the CXCR4 structures with the previously reported GPCR structures reveals that in CXCR4 the overall architecture of the canonical bundle of seven transmembrane helices is divergent from that in the other GPCR structures with multiple profound differences, underscoring the necessity of obtaining precise structural information with experimentally determined structures rather than constructed homology models. On the extracellular side of CXCR4, the conserved disulfide bonds between Cys28 of the N-terminal segment and Cys274 at the tip of helix VII, and between Cys109$^{3.25}$ and Cys186 of extracellular loop 2 (ECL2) are found to stabilize the N-terminal segment and ECL2 to form the entrance to the ligand-binding pocket, which explains their critical roles in the ligand binding. On the intracellular side, the intracellular part of helix VII is one turn shorter compared with the other GPCR structures, and helix VIII is missing with the C-terminus taking an extended conformation. The CXCR4 mutant (CXCR4-3) carrying a T240$^{6.36}$P mutation

is shown to uncouple the ligand binding and the signaling processes. In the mutant, a region of helix VI (residues Gly231$^{6.27}$ to Pro240$^{6.36}$) is structurally disrupted, which might impair the binding and activation of downstream G proteins.

Then Wu *et al*. compared the binding modes of the small-molecule inhibitor 1T15 and the cyclic peptide CVX15. The two inhibitors have substantial overlap at the binding site. 1T15 occupies part of the ligand-binding pocket that involves helices I, II, II, and VII only, while CVX15 fills almost the entire pocket. On the other hand, the binding of the two different inhibitors results in significant conformational variations in the N-terminus and the extracellular tips of some of the helices forming the pocket, indicating the structural flexibility of the ligand-binding site in CXCR4. In addition, in the CXCR4–CVX15 complexes, the binding of CVX15 induces minor conformational change of the extracellular part of helix V, which leads to closer contacts of the intracellular parts of the receptors, providing another example of the ligand-induced allosteric modulation and possible binding cooperativity of CXCR4.

The oligomeric state and structure of the chemokine–chemokine receptor complexes might be dynamic, which has been indicated in various studies and might have pharmacological effects [7,9,10]. In all the five structures obtained by Wu *et al*., CXCR4 forms a symmetric dimer with consistent contacts between the monomers, suggesting that the homodimer interface identified in these structures is likely biologically relevant. The potential interacting interface between the chemokine and the chemokine receptor was also discussed. For the binding of CXCL12 (SDF-1) which is the natural ligand of CXCR4, a ‘two-site’ model has been proposed [11]. In the model, the chemokine core domain (site 1) may serve as the initial docking site to interact with the N-terminal region of CXCR4, and the‘ N-terminus region (site 2) of CXCL12 may bind to the groove formed by the helices of CXCR4 to trigger the signaling [11]. The newly reported CXCR4 structures reveal new insights into the binding of ‘site 2’ of CXCL12 at the ligand-binding pocket [2]. Furthermore, the constructed model of the CXCR4–CXCL12 complex identifies the potential binding interface and suggests the structural basis for the critical role of Lys1 of CXCL12 [2].

Based on the structural information of CXCR4 [2] and CCR5 [8], a model for early stages of the HIV-1 entry process was proposed by Wu *et al*. [2], in which the binding of gp120 of HIV-1 to CXCR4 also employs a ‘two-site’ mechanism analogous to the ‘two-site’ model of the CXCL12 binding to CXCR4. For the viral entry, the binding of gp120 to the receptor CD4 induces conformational changes of gp120, which leads to recruitment of CXCR4 or CCR5 as a co-receptor. The sulfotyrosine-containing N-terminus of the co-receptor serves as ‘site 1’ for the initial interaction with the V3 loop of gp120, which results in conformational change of the V3 loop, the subsequent interactions of the rearranged V3 loop with the extracellular loops and the ligand-binding pocket (‘site 2’) of the co-receptor. The structural details of CXCR4 revealed in the report by Wu *et al*. and the implications for the binding of CXCL12 and gp120 of HIV-1 at ‘site 2’ of CXCR4 are of great value for understanding the signal transduction via the receptor and for the design of new strategies targeting the viral fusion of HIV-1.

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