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# Dimerization of chemokine receptors in living cells: key to receptor function and novel targets for therapy

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**Chemokine receptors control and mediate a diverse array of physiological and pathogenic processes. Many seven transmembrane (TM) G-protein-coupled receptors (GPCRs), including chemokine receptors, exist as homo- or heterodimers. Growing evidence indicates that the dimeric form is the basic functional structure of these receptors. Hetero-dimerization may allow for enhanced or specific functions of receptors and may be essential for receptor activity. Thus, dimers may provide new targets for chemokine receptor-based therapies. Synthetic peptides of TM regions of chemokine receptors may interfere with homologous interactions and inhibit functional activity of the receptors. Therefore, TM peptides and possibly compounds that target dimers and/or signaling of chemokine receptors may have therapeutic applications.**

The GPCR superfamily is composed of more than 800 distinct proteins. GPCRs mediate a variety of diverse physiological and pathogenic processes. Chemokine receptors are a subset of the GPCR superfamily and, in humans, this group includes at least 22 members [1] that regulate the trafficking and homing of leukocytes, migration of cerebellar granule cells, cancer cells, hematopoietic stem cells, and that mediate inflammation, allograft rejection, wound healing, atherosclerosis, angiogenesis, HIV-1 infection, embryogenesis and hematopoisis [2–4]. Chemokine receptors are therefore attractive targets for the treatment of many diseases including cancer, AIDS, atherosclerosis and inflammation [5,6]. The concept that GPCRs, including chemokine receptors, exist as dimers or oligomers has advanced rapidly in recent years based on structural and functional studies. The evidence for chemokine receptor dimerization and its role in chemotaxis and metastasis is discussed in this review. Methods to abrogate chemokine receptor dimerization are also explored as a way to modulate chemokine signaling that may be detrimental to the host.

## Techniques applied in the study of cellular protein–protein dimerization

Several techniques are commonly used for the study of receptor dimer formation [7]. Recent advances in energy transfer methods enable the study of protein–protein dimerization in living cells. Energy transfer in conjunction with molecular biology techniques allows us to examine dimer interface, or domains/residues important for chemokine receptor dimerization/conformation in living cells. Confocal microscopy/photobleaching in conjunction with proteins fused to cyan fluorescence protein (CFP) or yellow fluorescence protein (YFP) can be used to measure fluorescence resonance energy transfer (FRET) between interacting proteins [8] (Fig. 1) and can provide valuable information about the subcellular localization of dimers. Expression of these receptors should be controlled at physiological or pathological levels. Similarly, flow cytometry equipped with appropriate lasers can be used for FRET analysis of large number of cells over a short time frame. This assay could be used for high-throughput screening of compounds that interfere with protein–protein interactions in living cells. Trimerization can also be analyzed between three proteins fused with CFP, YFP or mRFP [9]. Homogeneous time resolved FRET (HTRF) reveals cell surface dimerization by conjugating anti-receptor antibodies to long-lived fluorophores such as europium and allophycocyanin. Bioluminescence resonance energy transfer (BRET) measures energy transfer in a given cell population by using fusion proteins genetically linked to GFP or Renilla luciferase (Rluc). Atomic force electron microscopy can visualize protein monomers, dimers or oligomers [10].

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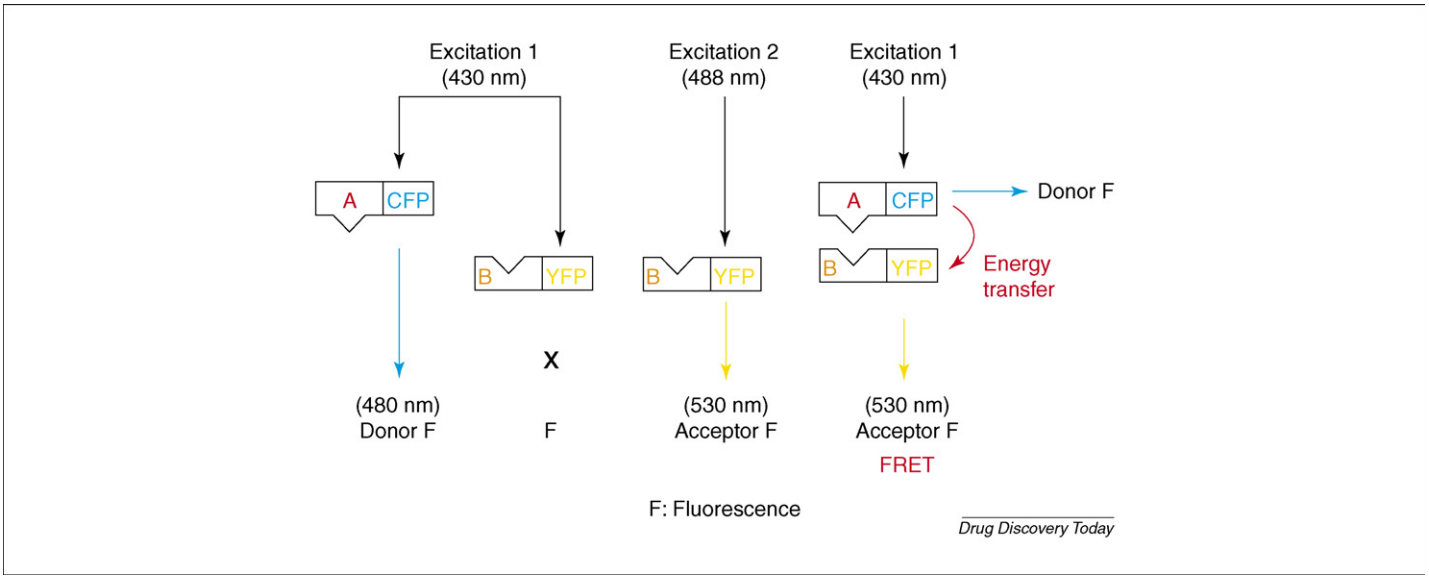


**FIGURE 1**

Schematic of FRET. This represents a simplified FRET measurement using a flow cytometry. FRET will be detected when energy is transferred from a higher energy fluorophore donor (CFP) to a receptor (YFP) non-radioactively. The transfer rate is inversely correlated with the $6^{th}$ power of the distance (within 1–10 nm) between the donor and acceptor that have an overlap of donor emission and acceptor absorption spectra. A: protein A; B: protein B.

## Chemokine receptor dimers and function

GPCRs share many structural features, including a bundle of seven TM helices connected by six loops of varying lengths. Dimer is the minimal functional structure of a GPCR. Crystal structures have been defined for rhodopsins [11], and recently for the human β2-adrenergic receptor [12–14], members of subfamily A that constitutes ~90% of all GPCRs. Crystallized GPCRs can exist as homo- or heterodimers, as part of larger oligomeric complexes, or as monomers [15,16]. Paracrystalline arrays of dimers of native rhodopsin were revealed by infrared-laser atomic-force microscopy in disc membranes of mouse retinae [10].

### *Homodimerization of chemokine receptors*

Homodimerization has been reported for several chemokine receptors. Several early studies suggested that homodimerization was induced by ligand binding. For example, CCL2/MCP-1-induced CCR2 dimerization while CXCL12 induced CXCR4 dimerization [17,18]. CCR5 homodimers or oligomers were increased in the presence of ligands [19] and were noted to interfere with HIV-1 infection [20], suggesting that monomeric CCR5 is the native form mediating infection. Recently, constitutive dimerization has been reported for CCR2 [21], CCR5 [19,22,23], CXCR1, CXCR2 [24,25] and CXCR4 [8,21,26], by biochemical methods, BRET assay and FRET assay, indicating that chemokine receptors associate with each other in the absence of ligand binding. It is probable that dimers might be stabilized by ligands in early studies that showed ligand-induced receptor dimerization using IP-WB methods. Ligand binding may induce a fractional increase in energy transfer in BRET assays that is above the signal detected before ligand binding. This increase may be because of ligand-induced conformational changes that occur without any change in the number of dimers per cell [21].

### *Heterodimerization of chemokine receptors*

Receptor oligomerization is a pivotal aspect of the structure and function of GPCRs that has been shown to have implications for receptor trafficking and signaling. Heterodimerization of GPCRs was found between γ-aminobutyric $acid_B$ ($GABA_B$) receptors R1 and R2 (type C GPCR), and between opioid receptors κ and δ. Hetero-oligomers with enhanced functional activity are also formed between receptors for dopamine (D2R) and somatostatin (SSTR)5. The dimerization of D2R with SSTR5 creates a novel high affinity receptor for dopamine and SST agonists. Additionally, dimerization of SSTR1 with SSTR5 was induced by their respective agonists and was required for receptor activation. Angiotensin receptor, AT1, in association with bradykinin B2 receptor increased the action of G alpha(q) and G alpha(i) receptor activation and modulated endocytic trafficking [27]. Co-activation of both dopamine D1 and D2 receptors that exist as a heterodimer was required for the induction of calcium flux whereas this calcium signal was not induced by the selective agonist for either receptors [28].

Like other GPCRs, chemokine receptors may also form heterodimers. CXCR4 and CCR5 are the primary co-receptors for X4 and R5 HIV-1 isolates. Heterodimer of CCR2V64I and CXCR4 or CCR5 was induced by the mixture of their respective ligands. Heterodimerization between CCR5 and CCR2V64I was suggested to be the mechanism by which individuals with the CCR2V64I allele have delayed AIDS progression [29,30], as heterodimers were less efficient in allowing HIV infection of cells than those expressing CXCR4 or CCR5 in the absence of CCR2V64I. Likewise, heterodimerization of CCR5 delta32 with CXCR4 or with normal CCR5 was associated with resistance to X4 and R5 human immunodeficiency virus type 1 in primary CD4+ cells, respectively [31], possibly by retaining co-receptors intracellularly. However, it is not clear that native CCR5 can directly bind CXCR4 and one study reported only weak binding of CCR5 and CXCR4 by BRET assay [26]. Other examples of constitutive heterodimers include studies showing CXCR1 and CXCR2 interact as well as forming respective homodimers. No interactions were noted for either of them with another GPCR, alpha(1A)-adrenoreceptor [25], indicating

the specificity of CXCR1 and CXCR2 interaction. CCR2 and CXCR4 were shown to heterodimerize by BRET analysis [21].

The role of chemokine dimers in facilitating chemokine receptor dimerization has yet to be determined. It has been suggested by several studies that heterodimers of chemokines can be detected *in vitro*. First, CCR2 homodimers were induced by its ligand CCL2/MCP-1 [17], which forms homodimers. Secondly, the heterodimerization of CCR2bV64I with CXCR4 was induced by the mixture of the respective ligands CCL2 and CXCL12/SDF-1α [29]. Third, CCR2bV64I dimerized with CCR5 when exposed to a mixture of CCL2 and CCL5/RANTES [30]. The mixture of CCL2 and CCL5 at a dose less than the effective dose of each in inducing adhesion showed strong effects (synergy) in an adhesion assay, indicating a gain-of-function for the mixture [30]. Fourth, the formation of heterodimers has been reported between CCL3 and CCL4 [32], between CXC chemokines platelet factor-4 and CXCL8/IL-8 [33], and between CC chemokines CCL2 and CCL8 [34], though effects on receptor dimerization have not been studied. However, it has been reported that chemokine CCL4 dimers do not bind to CCR5 [35], and no direct evidence indicates that chemokine dimers can induce chemokine receptor dimerization.

Of note, negative cooperation between chemokines was reported recently. CXCL12 and its antagonist AMD3100 can inhibit CCL2 binding only in cells co-expressing CCR2 and CXCR4, but not CCR2 alone, suggesting that binding of CXCL12 to CXCR4 in the heterodimer trans-inhibited the binding of CCL2 to its receptor CCR2 in the receptor heterodimer. Since CCL2 binding to cells only expressing CCR2 was not affected, the trans-inhibition is unlikely because of the formation of a CXCL12-CCL2 dimer that failed to bind receptors. Conversely, this allosteric trans-inhibition is also demonstrated for CCL2 and its inverse agonist TAK799 in cells co-expressing CXCR4 and CCR2 [36].

Chemokine receptors may also associate with cell surface molecules other than chemokine receptors. For example, CCR5 formed dimers with a GPCR mu opioid receptor. In addition, co-immunoprecipitation of CXCR4 or CCR5 with CD4 in the presence of HIV-1 X4-gp120 or R5-gp120, respectively, indicates the formation of a complex that comprises a chemokine receptor-CD4-gp120 hetero-oligomeric structure [37,38]. CCR5 and CD4 hetero-association induced by gp120 plays important roles in HIV-1 infection, but current data are not conclusive regarding whether the two molecules are directly associated in the absence of virus envelope glycoprotein. Three groups reported a direct association [39–41], but others failed to observe hetero-interaction of CD4 with CCR5 [42,43]. Constitutive as well as gp120-induced association of CXCR4 with CD4 have been reported [38,42] and a direct association was found between rat CXCR4 and human CD4 [44].

Another interesting association has been described for chemokine receptor CXCR4 and CD26, the dipeptidyl peptidase IV involved in chemokine processing [45]. Endogenous CD26 expression on transplanted cells negatively regulates hematopoietic stem cell homing and engraftment by proteolytically processing CXCL12, which is secreted by bone marrow stromal cells and attracts CXCR4 expressing cells to the bone marrow [46]. CXCR4 expression is co-modulated with CD26 expression by CXCL12 on lymphocytes [46].

Chemokine receptors share many structural features including a bundle of seven TM helices. The TM domains may not only determine the protomer structure of a receptor but also play an important role in mediating intramolecular interactions. It has been demonstrated recently that TM1 and TM4 are important for homo-dimerization of CCR5 based on mutagenesis analysis and peptide-blocking studies [19]. Likewise, TM4 was involved in the homo-dimerization of D2 dopamine receptor and C5a [47,48]. In addition, two models developed from atomic force microscopy analysis of native mouse rhodopsin [49,50] and from cryo-electron microscopy analysis of squid rhodopsin [51] suggest that TM4 plays a central role in rhodopsin dimerization, though the relative contributions of TM5 or TM3 differ in the two models. Further study with cross-linking of substituted cysteines indicates that residues across the entire TM4 region are included in the dimer interface of dopamine D2 receptor [52]. Of note, early studies have found that TM-VI was important for the β-adrenergic receptor dimer [53] and the leukotriene B4 receptor BLT1 dimer [54]. A recent report indicates that TM I, TM II, and H8 of rhodopsin are important for dimerization [55]. The proposed interfaces from three models of rhodopsin are quite different. It is an open question as to which model is more close to the native situation or if the receptor can exist in different conformational forms. The structural results raise the interesting possibility that interactions of GPCR dimers may not be fixed, but could have flexibility in mechanisms of conformational pairing.

Since chemokine receptors are members of subfamily A of 7TM-GPCRs they could, in theory, have rhodopsin-like structure as well. Therefore, homotypic interactions between CXC receptors, between CC receptors, or between CXC receptor and CC receptor are not unreasonable. Thus, monomers within receptor dimers may interact with each other in a fashion similar to that of other 7TM-GPCRs.

### *Intracellular dimerization of chemokine receptors*

Homo- and heterodimers of GPCRs can form intracelluarly and may initiate this interaction during biosynthesis. Immature forms of GPCRs, including the receptors for oxytocin, vasopressin and serotonin 5-$HT_{2c}$, were present as dimers during transit through the endoplasmic reticulum [56,57]. Energy transfer between CCR5 molecules was detected in endoplasmic reticulum subfractions as well as the plasma membrane when interactions were evaluated using BRET assay [22]. Confocal analysis of CXCR4-eCFP-transfected cells showed that CXCR4 was located in vesicle-like structures in the cytoplasm. Intracellular CXCR4 molecules were also detected in human primary T cells, HeLa cervical cancer cells and NCI 2126 non-small cell lung carcinoma cells, indicating that intracellular CXCR4 is a natural form of the native protein [8]. Intracellular CXCR4 was also found in CD34+ stem cells [58]. Dimerization of intracellular CXCR4 observed by confocal photobleaching FRET [8] supported an assertion that CXCR4 proteins may traffic to the cell surface as dimers and remain dimeric in the plasma membrane. Intracellular CXCR4 probably includes mature and immature species and one study demonstrated immature dimeric forms of CXCR4 by immunoprecipitation [26]. Assembly of CXCR4 dimers intracellulary prior to cell surface expression may explain the fact that CXCL12 does not increase CXCR4 dimers in several studies using energy transfer methods [8,21,26]. All therapies targeting CXCR4 molecules should consider the impact of an intracellular pool of CXCR4. It will be

interesting to see whether small molecules can be developed which dissociate both cell surface and intracellular CXCR4 dimers and prevent intracellular CXCR4 trafficking to the cell surface. Small molecules may achieve best therapeutic potential in dimers with fewer contacting sites.

### *Receptor dimers and chemotaxis*

Many eukaryotic cells are able to sense external gradients of chemoattractants and respond with asymmetric changes in cell morphology and motility. In response to local chemokine gradients, pseudopodia form predominantly at the leading edge during chemotaxis or directed cell migration. Chemokine receptors coupled to the G protein transduce signals and induce concurrent changes in actin, myosin, and other molecules that enable the cell to move toward the chemoattractant. For example, the increase of phosphatidylinositol (3,4,5) trisphosphate and signaling of PI3Ks and phospholipase A(2) may lead to directional sensing [59,60]. The small GTPase Rac is thought to regulate cell movement by influencing actin cytoskeletal organization and membrane ruffling. CXCL12-induced migration of Jurkat T lymphocytes involves G(i)- and Gβγ-induced Rac activation as well as G(i)-independent Rho activation that induces phosphorylation of myosin light chain. B lymphocyte migration induced by CXCL12 involves BTK and PLC-γ2 [61]. Cell migration also depends on the activation of mitogen-activated protein kinase (MAPK), which can regulate myosin motor function, an event critical for cell contraction. Cell migration plays a key role in development, immunity, tissue homeostasis, wound healing, metastasis and atherosclerosis. The gradients of chemokines, the type and levels of chemokine receptors and intracellular signal cascades are key factors influencing chemotaxis.

Several studies suggested that homodimers of CXCR2 or CCR2 are essential for chemotaxis induced by their specific ligands [17,24] as demonstrated by the loss of activity in cells cotransfected with mutated and wild-type receptors. A TM4 peptide that reduces CXCR4 homologous interactions as detected by FRET, blocked CXCL12-induced chemotaxis [8]. The role of chemokine receptor dimers in chemotaxis mediated by other chemokine receptors has yet to be determined.

Chemokine receptors are widely expressed on immune cells and mediate chemotaxis, and control the site, intensity and outcome of immune responses. They should be the receptors mediating anti-tumor activities by directing immune cells to the site of tumor growth. Paradoxically, however, cancer cells employ chemokine receptors to migrate or metastasize from primary site to other tissues or organs.

### *Chemokine receptors in cancer metastasis*

Cancer metastasis is associated with poor prognosis. Preventing migration of cancer cells from the primary tumor to distal organs/tissues and bone marrow may significantly prolong overall survival. Many chemokine receptors are involved in normal cell migration and metastasis (Tables 1 and 2). The differential expression of chemokine receptors determines the traffic and homing of leukocytes. Likewise, the profile of chemokine secretion by tissues and organs leads to the preferential metastasis of certain tumors that have differential expression of chemokine receptors. Among the 22 currently identified chemokine receptors, 13 were reported to be involved in cancer metastasis. CXCR4 appears to play an essential role in the metastasis of breast cancer [62]. The newly identified CXCR7 shares the same ligand, CXCL12, with CXCR4 which is also known to be important for tumor growth, but does

**TABLE 1**

**CXC chemokine receptors and their functions**

| | | Receptors with indicated functions |
|---|---|---|
| Migration | | CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7 |
| Effects of C-tail deletion on | Ca++ | No effects observed for CXCR1, CXCR2, CXCR4 |
| | Endocytosis | CXCR1, CXCR2, CXCR4 were affected |
| | Migration | CXCR1, CXCR2, CXCR3, CXCR4 were affected |
| Homodimer | | CXCR1 dimer was detected<br>CXCR2 dimerization is important for migration and signaling<br>CXCR4 dimerization is important for migration and signaling |
| Heterodimer | | CXCR1 with CXCR2, CXCR4 with CCR2B, CCR5Δ32, CD4, or CD26 |
| Receptor activation involving | Clathrin | CXCR1, CXCR2, CXCR4 |
| | Actin Polymerization | CXCR1, CXCR2, CXCR3, CXCR4 |
| | Beta-arrestins | CXCR1, CXCR2, CXCR3, CXCR4 |
| | Tyrosine phosphorylation | CXCR1:FAK, Pyk2, Cbl CXCR2: FAK, Cbl CXCR3: Src<br>CXCR4: FAK, Pyk2, Stat, Src, Cbl, Lck, SLP-76 p52Shc |
| Expressed on cancer cells | | CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CXCR7 |
| Involved in metastasis | | CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR7 |

**TABLE 2**

**CC and CX3C chemokine receptors with reported function**

| | *Receptors with indicated function* |
|---|---|
| **Migration** | CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CX3CR1 |
| **Metastasis** | CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9, CCR10, CX3CR1 |
| **Homodimer** | CCR2 dimerization is important for migration and Ca++<br>CCR5 dimerization is important for migration, signaling and Ca++ |
| **Heterodimer** | CCR2 with CCR5 and CXCR4, CCR5 with CCR2 and C5aR |
| Receptor activation involving | |
| **Clathrin** | CCR2, CCR4, CCR5, CCR7 |
| **Actin polymerization** | CCR1, CCR2, CCR3, CCR5, CCR7, CCR9, CCR10 |
| **Arrestins/dynamin** | CCR5, CCR7 |
| **Tyrosine kinase** | CCR5: Stat1,3, Lck<br>CCR7: migration blocked by Jaks inhibitor |
| Expressed on tumor cells | CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9, CCR10, CX3CR1 |

not directly mediate cancer cell metastasis and calcium mobilization [63]. CXCR4 is also expressed in 27 other cancers, including melanoma, colorectal cancer, prostate cancer, ovarian cancer, lung cancer, hepatocellular carcinoma, nasopharyngeal cancer, esophageal cancer, leukemia and lymphoma. High-level expression of CXCR4 on primary tumors is associated with poor prognosis [64].

There are different strategies of targeting CXCR4 or other chemokine receptors. Targeting CXCL12/CXCR4 with antibodies reduced metastasis in animals. Small molecule antagonists of CXCR4 [65] and peptides mimicking CXCL12/SDF-1 [66] also inhibited cancer cell growth and migration. Targeting CXCR4 with siRNA was effective in controlling metastasis of breast cancer [67].

Interestingly, preformed CXCR4 dimers [21,26] are important in ligand-induced CXCR4 functions [8]. Constitutive expression of dimeric CXCR4 was found in malignant living cells by FRET [8]. Although CCR5 dimerization may be important for cell surface targeting, the relationship between CCR5 dimerization and receptor function has yet to be determined [68]. We have shown that

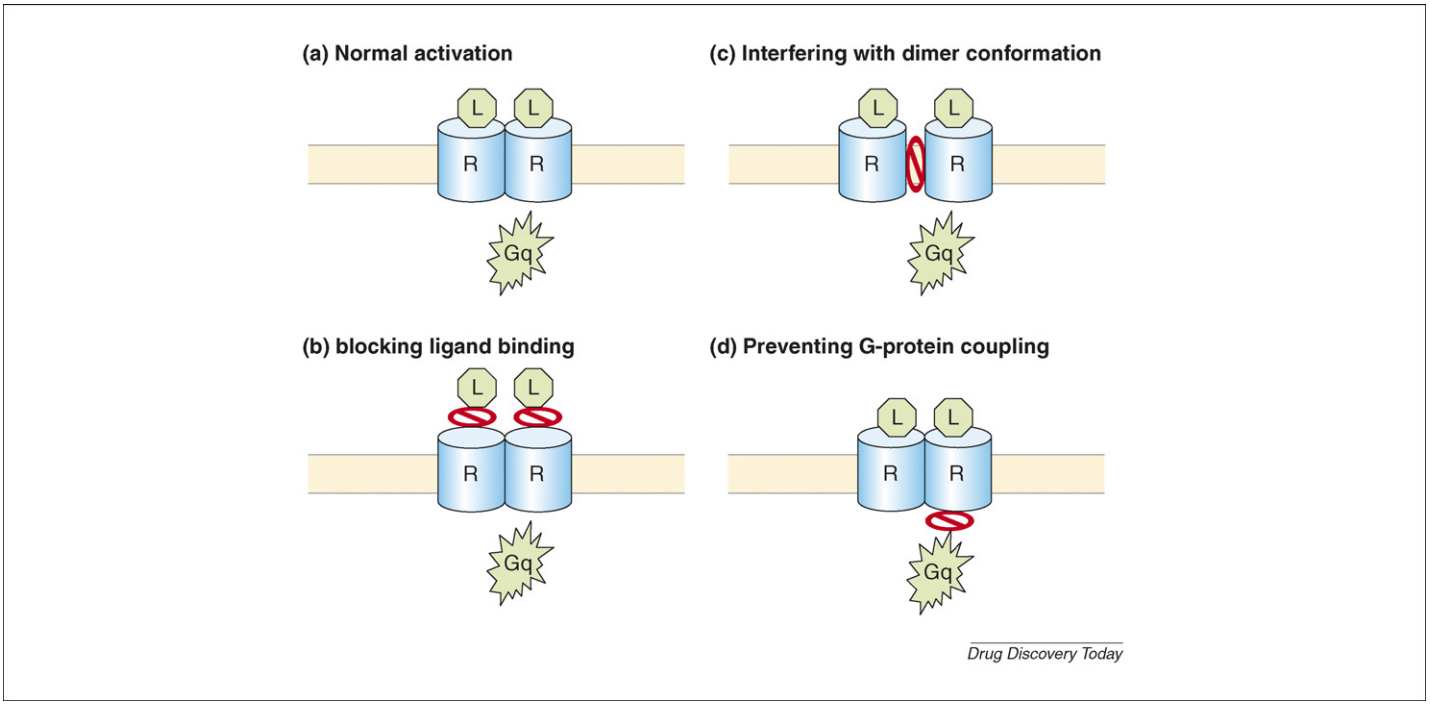


**FIGURE 2**

Mechanisms of action for potential GPCR-targeting drug candidates. **(a)** Proposed model for GPCR activation showing two ligands, two receptors, and one G-protein. **(b)** Targeting ligand binding with antibodies, peptides, or compounds, which is likely to affect the ligand-induced conformational changes. **(c)** Interfering with protomer interactions by targeting dimers (possibly at the interface) with peptides and compounds. The outcomes of interference could be (1) by reducing ligand-induced conformational changes, (2) increasing the distance between protomers in a dimer, (3) or in some cases the dimer may be disrupted. All these outcomes could affect receptor signaling. **(d)** Targeting G-protein coupling by peptides and compounds and controlling receptor signaling. L: ligand; R: receptor; : drug candidates.

CXCR4-TM4 peptide, a synthetic peptide of the transmembrane region 4 of CXCR4, decreases energy transfer between protomers of CXCR4 homodimers and inhibits CXCL12-induced migration and actin polymerization in malignant cells [8]. Thus, it may be possible to use peptides to specifically control CXCL12-directed migration of cancer cells and to reduce the number of CXCL12 attracted tumor-associated macrophages, which promote tumor growth and metastasis. Our study of CXCR4 TM4 peptide suggests that chemokine receptor dimers/signaling are potential targets for effective cancer therapy.

Other synthetic peptides of CXCR4 TMIV (X442), TMII (X422), TM6 and TM7 were modified by adding a few residues at terminals and have been shown to inhibit calcium mobilization, X4 HIV-1 infection [69], CXCL12 inhibition of cAMP [21] and ligand-induced conformational changes in CXCR4 dimers, but failed to inhibit the constitutive BRET of CXCR4 [21]. The inhibition of signaling by X442 or X422 was stated to be CXCR4 specific. The role of these two peptides in migration has yet to be determined. In addition to interfering at a dimer interface, TM peptides may intercalate into the monomeric subunit to inactivate the GPCR [69]. Data of mutants with amino residue substitution of DRY motif on intracellular loop 2 and residue N119 of CXCR4 suggest that mutations could affect receptor conformation and activity. A good correlation between BRET and Gαi activation was found for R134A and N119K, but not for N119S that had reduced BRET, but with constitutive activation [70]. Of note, although only ligand-induced conformational change was inhibited by AMD3100 and X442, an inverse agonist (that inhibits the constitutive action of the receptor), T14012, inhibited both ligand-induced conformational change and basal BRET between monomers of CXCR4 dimer. Thus, it is possible to target both ligand-induced conformational changes and constitutive homologous interactions with specific agents. An inverse ligand turns constitutive active receptors into inactive ones, such as IP-10 for KSHV-GPCR [71], IP-10 and CXCL12 for HHV-8 ORF74 [72], and two compounds (UK-396,794, UK-438,235) for CCR5 [73]. Data with T14012 suggest that one possible mechanism of action of inverse agonists may modify homologous interactions of the two protomers in a dimer, for example to increase the distance between the two monomers of a dimer. Results with CCR5 TM4 peptide blocking experiments [19], CXCR4 TM peptides and T14012 together with data showing dissociation of constitutive dimer by TM-VI of the β-adrenergic receptor [53] and leukotriene B4 receptor BLT1 [54] support the assertion that in addition to inhibiting ligand-induced conformational changes, the conformation of the constitutive dimer (Fig. 2), rather than the number of dimers, may be altered by therapeutics.

Therapeutic peptides are in preclinical and clinical studies. How to increase the half-life of therapeutic peptides and proteins in circulation and tissues is challenging. Proper modification, such as pegylation at specific sites/orientation, could significantly increase the half-life of peptides while maintaining their therapeutic activity [74–76]. Pegylated peptides could be injected weekly, instead of daily. Several pegylated therapeutic proteins, such as pegylated interferon alpha 2a and pegylated interferon alpha 2b, are on the market.

## Conclusions

In summary, homodimers or heterodimers of chemokine receptors can be detected on the cell surface and in the cytoplasm of normal and malignant cells. Growing evidence supports the concept that receptor dimers or oligomers are probably the basic functional unit of chemokine receptors. Chemokine receptor dimers are found in the absence of ligands. Chemokines induce conformational changes of receptor dimers. Synthetic peptides of the transmembrane regions of chemokine receptors may be an effective tool to study the role of receptor dimerization in receptor activities, such as signal transduction, Ca++ mobilization, chemotaxis and endocytosis. TM4 peptides and their modified forms (such as pegylated), and possibly other TM peptides and compounds that interfere with chemokine receptor dimerization, conformation and/or signaling, thereby controlling migration of receptor expressing cells, may be useful for the treatment of cancer, cardiovascular disease, graft rejection/GVHD, infectious disease and inflammatory conditions. It is possible that similar strategies may be used to target dimer/signaling of other members of subfamily A GPCRs under pathologic conditions. Interfering with HIV-1 protease dimerization and potently inhibiting of HIV-1 replication by non-peptidyl small molecule inhibitors [77] suggests that compounds (including peptides) based on mechanism of dimer interference may have broad therapeutic potential.

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