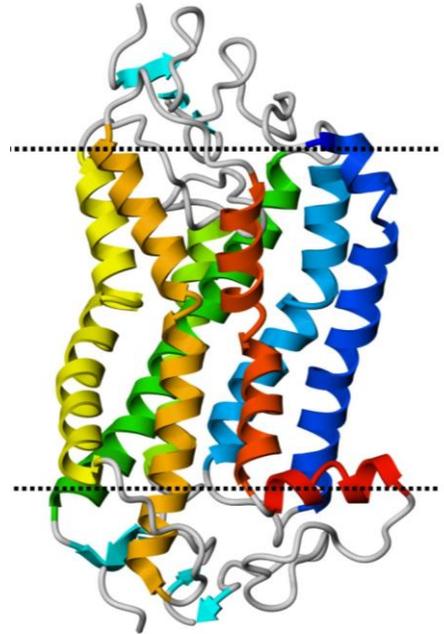


## Investigation of molecular switches at G-protein-coupled receptors: chemokine receptor CXCR4 and formyl peptide receptors FPR1-3

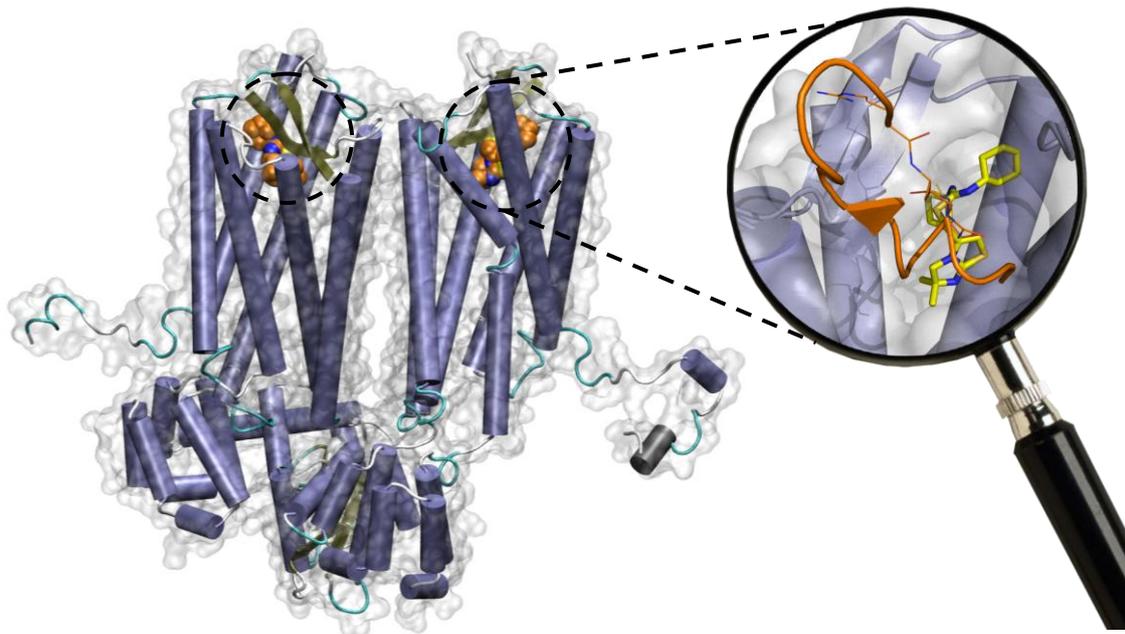
G protein-coupled receptors (GPCRs) are a large protein family of seven-transmembrane-domain receptors which are fundamental in controlling of cellular responses (Fig. 1). They convey signals through changes of their conformation. GPCRs are prototypic allosteric proteins with the ability to adopt numerous conformations, many of which interact with cellular partners, not only G proteins, to initiate cellular biochemical processes. GPCRs are involved in many diseases, and because of that they are also targets of around half of all modern medicinal drugs. Nonetheless, it is still not known how GPCRs respond to extracellular ligands and how their mutations lead to disease.



**Fig. 1.** The general structure of GPCR

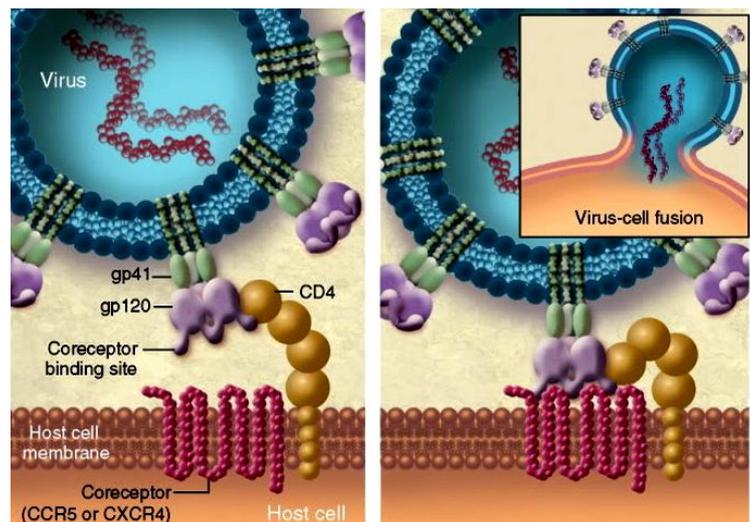
Chemokine receptors are critical regulators of cell migration in the context of immune surveillance, inflammation, and development. The G protein-coupled chemokine receptor CXCR4 is specifically implicated in cancer metastasis and HIV-1 infection. Recently (November 2010), the structure of this receptor was published [1]. In the five independent crystal structures of CXCR4, the receptor is bound to an antagonist small molecule IT1t (Fig. 2) and a cyclic peptide CVX15 at 2.5 to 3.2 Å resolution. These structures can be very helpful in understanding of the interactions between CXCR4 with natural ligands as well as the HIV-1 glycoprotein gp120 of viral envelope (Fig. 3).

The chemokine receptor CXCR4 is one of the coreceptors for HIV entry and more than 10 members of GPCRs have been shown to work as coreceptors for human immunodeficiency virus type 1 (HIV-1), HIV type 2 (HIV-2), and simian immunodeficiency viruses (SIVs). Additionally, the interaction between CXCR4 and its ligand SDF-1 is involved in various disease conditions, such as cancer cell metastasis, leukemia cell proliferation, rheumatoid arthritis and pulmonary fibrosis. Therefore, CXCR4 inhibitors have potential as novel therapeutics for the treatment of these diseases as well as HIV infection [2]. Numerous efforts have been made to develop a new class of anti-HIV agents [3] that target these coreceptors as an additional or alternative therapy to standard HAART. Current therapeutic intervention in HIV infection relies upon 20 different drugs. Despite the impressive efficacy shown by these drugs, there are unexpected adverse effects, such as mitochondrial toxicity, lipodystrophy, and drug resistance even to entire drug classes. Thus, there is now a great need for new antiretroviral drugs.



**Fig. 2.** The crystal structure of CXCR4 dimer with the ligand bound (PDB code: 3ODU)

Formyl peptide receptors (FPRs) plays important roles in protection of monocytes against HIV-1 infection. However, they are also efficiently used as a coreceptor by HIV/SIC strains similarly to other chemokine receptors CCR5 or CXCR4 [4]. Furthermore, the peptides derived from HIV-1, HIV-2, Ebola virus, SARS coronavirus and coronavirus 229E exhibit high affinity binding to the FPRs [5]. FPRs have also been found to interact with many structurally diverse pro- and anti-inflammatory ligands associated with different diseases including amyloidosis, Alzheimer's disease and prion disease [6]. How these receptors recognize such diverse ligands and how they contribute to disease pathogenesis and host defence are questions currently under investigation that could lead to new therapeutic targets. Agonists of FPRs could define a novel way for anti-inflammatory drug discovery by mimicking the way our body disposes of inflammation. This could be a viable approach to regulate aberrant inflammatory responses in the case of several chronic rheumatic and cardiovascular pathologies [7]. The understanding of action of the FPR ligands, particularly in the brain, could lead to novel anti-inflammatory therapeutics for the treatment of a variety of clinical conditions, including stroke [8].



**Fig. 3.** HIV infects the host cell

Our current understanding of function of GPCRs was changed from simple On-Off Switches to microprocessor-like action of GPCRs [9]. Especially the phenomenon of functional selectivity, whereby certain ligands initiate only portions of the signalling mechanisms mediated by a given receptor, opened new horizons for drug discovery. Currently, there is a need to discover new receptor-ligand behaviours and quantify the effect of a drug on these complex systems. Specifically, some agonists selectively activate cellular pathways associated with cell type and some antagonists actively induce receptor internalization without activation. In addition, the effects of allosteric modulators can be linked to the nature of the co-binding ligands. Agonists are now known to have multiple efficacies that are associated with selected signalling pathways coupled to the receptor. The so called functional selectivity (biased agonism and biased antagonism) is especially interesting in terms of mechanism of action and potential therapeutic application.

Although GPCRs interact with very diverse sets of ligands the membranous part of GPCRs share extensive similarities having seven transmembrane helices linked by relatively short loops. Each receptor undergoes a series of conformational rearrangements controlled by molecular switches leading to partial or full activation and the dynamic character of GPCRs is thought to be essential for their diverse physiological functions. Transition between these intermediate states involves the disruption of intramolecular interactions that stabilize the basal state of a receptor. Such profound changes are evoked by the action of molecular switches. The major switches proposed so far for different GPCRs include the “rotamer toggle switch” involving the CWxPx(F/H) sequence on transmembrane helix TM6, the switch based on the NPxxY(x)<sub>(5,6)</sub>F sequence linking TM7 and H8, the “3-7 lock” interaction connecting TM3 and TM7 (involving Schiff base-counterion interaction in rhodopsin), and the “ionic lock” linking transmembrane helices TM3 and TM6 and employing the (D/E)RY motif on TM3.

The action of molecular switches concurrent to binding of ligands (analogs of morphine) was investigated by our group in  $\mu$ OR,  $\delta$ OR and  $\kappa$ OR opioid receptor models [10, 11]. On the basis of conducted molecular dynamics simulations we proposed that both agonists and antagonists bound in the same binding site to Y3.33 but only agonists were able to move deeper into the receptor binding site and to reach H6.52. We observed that the movement from Y3.33 to H6.52 induced a break of the TM3-TM7 connection (“3-7 lock”). A concerted motion of W6.48 and H6.52, so called “extended rotamer toggle switch”, was also observed which may imply a temporal but also spatial (agonists were bound to H6.52) dependence between them. Recently, we investigated the structurally similar guanidino-naltrindole (GNTI) compounds, where 5'-GNTI is an antagonist while 6'-GNTI is an agonist of the  $\kappa$ OR opioid receptor, to explore how a subtle alteration of the ligand structure influences the receptor activity [12].

In the current project the similar investigations of the influence of ligand binding on early activation steps in CXCR4 and FPRs chemokine receptors are proposed. The structure of CXCR4 is already known while the structures of FPR1-3 receptors will be derived by

homology modeling procedure employing the CXCR4 template. Only the membranous part of the receptor will be built on the receptor template while the extra-membranous loops will be constructed using the *ab initio* CABS method. A range of docking methods including the simulated annealing procedure for ligand docking will be used followed by all-atom molecular dynamics simulations to determine the immediate changes in the structure of the ligand-receptor complexes. The natural ligands of chemokine receptors as well as currently used drugs will be studied including peptide ones – therefore a combination of theoretical methods with experimental constraints will be applied. To understand the detailed function of chemokine receptors and to design potential drugs, we need the information on how the receptor conformation changes upon ligand binding, which is not available from the crystal structure itself.

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