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# MODELING THE ALLOSTERIC MODULATION OF CCR5 FUNCTION BY MARAVIROC

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## **Abstract**

Maraviroc is a non-peptidic, low molecular weight CC chemokine receptor 5 (CCR5) ligand that has recently been marketed for the treatment of HIV infected individuals. This review discusses recent molecular modeling studies of CCR5 by homology to CXC chemokine receptor 4, their contribution to the understanding of the allosteric mode of action of the inhibitor and their potential for the development of future drugs with improved efficiency and preservation of CCR5 biological functions.

## A. Introduction

CC Chemokine Receptor 5 (CCR5) belongs to the wide family of G-protein coupled receptors (GPCRs) composed of seven membrane spanning helices that are connected by extracellular and intracellular loops (ECL and ICL), an extracellular N-terminal domain and a cytosolic C-terminal tail. CCR5 is a receptor for small chemotactic cytokines called CC chemokines including CCL-3, -4, -5 and -8 that participates in innate immunity and in the initiation of adaptive immune responses. [1] CCR5 also serves as a CD4 coreceptor for the entry of R5-tropic strains of HIV into activated CD4<sup>+</sup> T-lymphocytes and macrophages. The receptor works by binding the CD4-bound form of the viral envelope glycoprotein gp120, allowing the target cell and viral membranes to come closer and to fuse. Its crucial role in HIV infection is exemplified by individuals homozygous for the *CCR5*Δ32 allele who do not express functional CCR5 and are highly protected against HIV. [2] This has raised the hypothesis that blocking of the receptor could represent a feasible approach to fight HIV. Over the last years however, it has become increasingly evident from studies in mouse models that the lack of CCR5 results in impaired host defenses against infection by a variety of pathogens. [3] Epidemiologic studies also associated homozygosity for the *CCR5*-Δ32 allele with increased severity of clinical outcomes in infections with flaviviruses (West Nile virus and Tickborne encephalitis virus), indicating that some of the functions of CCR5 may not be dispensable and raising concerns about the safety of long-term inhibition of the receptor in HIV infection. [4] This review sheds light on emerging low molecular weight, allosteric regulators of CCR5 that have the potential to inhibit HIV entry while preserving other receptor functions and presents advances on molecular modeling approaches that help explain how these molecules act and may sustain further development of inhibitors. Our recent work on Maraviroc (MVC) binding

to a CCR5 model built by homology to the crystal structure of human CXC chemokine receptor 4 (CXCR4) is presented as a case study. [5]

## **B. Allosteric regulation of CCR5 by non peptidic, low molecular weight compounds as promising long term anti-HIV therapy**

Approaches to treat AIDS using CCR5 as a target include so far gene therapy strategies that aim at interfering with the expression of the receptor in patient's cells [6] and blockade of the receptor by ligands such as monoclonal antibodies [7] or modified chemokine derivatives. [8] These ligands are orthosteric inhibitors of gp120 binding to CCR5 because they attach to extracellular domains of the coreceptor, which the viral glycoprotein also binds to. Chemokines with agonist activity in addition remove CCR5 from the cell surface by promoting internalization of the receptor and in some cases by inhibition of its recycling. [9,10] A third class of CCR5 ligands acting as HIV entry inhibitors comprises structurally diverse non-peptidic, low molecular weight compounds (Fig. 1): TAK-779, the first inhibitor discovered by Takeda Pharmaceuticals, [11] its derivative TAK-652, [12] Aplaviroc (APL) (AK602 or GW873140) licensed by GlaxoSmithKline and whose development was discontinued because of hepatic toxicity in clinical trials, [13] Schering-Plough's Vicriviroc (SCH-D or SCH-417690) that continues to be evaluated in clinical trials [14] and Pfizer's Maraviroc (MVC) that is used for the treatment of patients who are infected with R5-HIV only. [15] These compounds prevent gp120 from binding to CCR5 but the mechanism involved differs from that of orthosteric ligands. Indeed, in recent radioligand dissociation kinetic experiments we demonstrated that MVC and TAK779 accelerate the dissociation rate of radiolabeled CCL3 or gp120-soluble CD4 complexes from CCR5, clearly indicating that the inhibitors could interact with the receptor occupied by either of both radioligands. These

experiments thus suggested that TAK779 and MVC bind to allosteric sites of CCR5 (that is, domains of CCR5 that are separate from the orthosteric binding site, which chemokines and gp120 bind to) and, while doing so, modify the receptor conformation in such a way that the receptor is no longer accessible to orthosteric ligands or the virus itself. [16] However, although MVC binds CCR5 with comparable affinity and dissociates CCR5 radioligands less efficiently, as compared to TAK-779, we also found that it was 100-fold more potent for inhibiting HIV infection. This suggests that MVC binding to CCR5 not only acts by blocking the viral envelope glycoprotein binding to target cells but also alters other stages of HIV-1 entry and infection. Other examples of differential effects of allosteric inhibitors on different CCR5 functions have been reported in the literature. For example, while TAK-779 more potently inhibits CCL3L1-induced internalization of CCR5 than HIV infection, the reverse is observed for TAK-652. [17] Similarly, some viruses are resistant to some inhibitors while retaining susceptibility to others. [18-20] These data are intimately related to the so-called “probe dependence” feature of allosteric inhibitors, which, in contrast to orthosteric antagonists, allows them to modulate different receptor functions or the binding of different orthosteric ligands to different extents ranging from inhibition to enhancement. [21,22] As another example, APL inhibits HIV infection at concentrations that permit CCL5 binding to the receptor and CCL5-mediated chemotaxis and internalization, [13] but prevent the binding of CCL3. [23] Thus, CCR5 allosteric inhibitors represent promising therapeutic tools because they can silence some functions of the receptor while preserving others. In this sense, the functional design of small molecule allosteric regulators of CCR5 could offer the unique possibility to inhibit HIV infection while preserving the immune functions of the receptor. The experimental structure of CCR5 is not yet available. Modeling techniques nevertheless could predict it and propose ligand binding modes for a molecular interpretation of allostery and the prospective design of new compounds.

### **C. Modeling the three-dimensional structure of CCR5**

Since 2000, many models of CCR5 have been proposed by different research groups. They were built by homology to template GPCRs, so that progresses made in modeling reflect the breakthroughs achieved in solving GPCR structures by X-ray crystallography. [24] For eleven years, CCR5 models were based on distant GPCR homologs, mainly bovine rhodopsin, but also human  $\beta$ 2 adrenergic receptor or human adenosine A2A receptor. The requisites for reliable modeling of CCR5 from rhodopsin, from sequence alignment guided by hydrophathy-profile and the presence of rhodopsin-like GPCR motifs to the incorporation of angle and distance restraints during the refinement of coordinates by molecular mechanics are well described in reference [25]. Rhodopsin-based CCR5 models represent low-accuracy models whose reliability depends on the level of knowledge-based constraints introduced upon modeling. In any cases, the seven transmembrane domains (7TM) are the most consistent part of the model, since their fold is common to all GPCR structures. [24,26]

In 2011 was released the first structure of a peptide GPCR, namely human CXC chemokine receptor 4 (CXCR4). [27] CXCR4 is a close homolog of human CCR5. There is 29% of sequence identity between the two receptors, with up to 53.3% of identical residues in individual transmembrane domain (TM). The structure of CXCR4 revealed structural distinctive characteristics in the length and the straightness of helices. The extracellular end of helix 2 (TM2) especially undergoes a  $\sim 120^\circ$  rotation in CXCR4 as compared to rhodopsin. This distortion is due to the TxP motif conserved across chemokine receptors. [28] The intra- and extra-cellular domains of CXCR4 are well defined in the crystal structure. They differ from those observed in other GPCRs although they include common secondary structure elements. [29] For example, the second extracellular loop (ECL2) adopts a  $\beta$ -hairpin

conformation in both CXCR4 and rhodopsin, but is oriented outward in CXCR4 whereas it positions deeper into the 7TM bundle in rhodopsin. Last, a parallel and symmetric dimeric assembly of the receptors was observed in all five CXCR4 crystal structures presented in reference [27], thereby reinforcing the general belief that the chemokine receptors exist as dimeric entities.

Only few models of CCR5 obtained by homology to CXCR4 are reported in the literature. [5,30,31] The model we proposed in 2011 [5] has afforded the precise definition of the three-dimensional structure for most of the receptor, especially the 7TM bundle, the extracellular loops and a portion of the N-terminal domain. It has provided details on the organization of the receptor, revealing networks of aromatic residues that bridge the transmembrane helices and connect the 7TM bundle to the extracellular loops. The model also well paired the hydrogen-bonding groups of the few polar residues present in the hydrophobic 7TM bundle. The model delineates a wide, deep and open pocket in the 7TM bundle for the binding of TAK-779, APL, MVC and other small inhibitors (reviewed in [32]). Using docking, we demonstrated that on the whole the pocket better accommodates true CCR5-binders than their decoys (i.e. similar compounds with no affinity for the receptor). For the sake of comparison, although the rhodopsin-based models are sufficient to describe some of the structural determinants for ligand binding (e.g., the carboxylate group of Glu283 - Glu7.39 according to Ballesteros-Weinstein numbering scheme- which is critical for the binding to CCR5 of all inhibitors but TAK-779, is available to establish an ionic bond with the central positively charged nitrogen of inhibitors), they fail to discriminate true binders from decoys upon structure-based virtual screening as well as CXCR4-based model, unless the transmembrane cavity is refined to capture the structural features important for ligand binding. [33] For example, Trp86 (Trp2.60) in TM2, which is conserved across CC chemokine receptors, contributes to the binding of chemokines, gp120 and MVC. [5,34] The side chain of Trp86 is

directed towards the cavity in CXCR4-based models, whereas it faces the lipid bilayer in rhodopsin-based models unless a kink is enforced in helix 2 during the modeling procedure.

The modeling approaches to CCR5 structure as well as the predictive power of the different models are summarized in Table 1. If only the CXCR4-based model provides solid structural clues for the molecular understanding of allostery, the rhodopsin-based models have proven their particular applicability in drug discovery, especially with identification of non peptidic agonists [35] and the optimisation of allosteric CCR5 modulators. [31,36] Similarly, it was recently demonstrated that models of the  $\beta$ 2 adrenergic receptor can perform as well as the crystal structure in structure-based virtual screening. [37]

#### **D. Modeling ligand binding to CCR5 helps understanding allostery**

The next step towards the understanding of allostery is the mapping on the receptor of the binding sites for orthosteric and allosteric ligands. Site-directed-mutagenesis (SDM) constitutes an invaluable tool to achieve this goal. The interpretation of SDM data is however not a trivial task. First of all some mutations can indirectly influence ligand binding. Similarly, robust controls are necessary to attest that changes in ligand binding do not result from misfolding. For example, the mutation of Trp248 (Trp6.48) in TM6 was shown to deteriorate the binding of ligands and also the receptor expression. [5] An additional level of complexity in assessing the effects of mutations on ligand binding depends on the choice of the receptor functional assay that is used as readout. Regarding the studies on the binding of allosteric compounds to CCR5, the different functional assays that have been developed so far include (i) direct binding experiments of tritiated forms of these compounds, (ii) inhibition of antibody or radiolabeled chemokine binding to CCR5, (iii) inhibition of cell-cell fusion or (iv) inhibition of HIV entry. Overall, while these assays produced reproducible and

consistent results, some differences have been reported depending on which receptor function was investigated. For instance, while we observed that the Y251A (Tyr6.51) mutation moderately affects MVC in its ability to displace  $^{125}\text{I}$ -CCL3 binding to CCR5, [5] more dramatic effects were reported using fusion inhibition assays. [32] Similarly, the replacement of Thr195 (Thr5.39) was found to increase by 12-fold the  $\text{IC}_{50}$  values for inhibition of  $^{125}\text{I}$ -CCL5 binding to CCR5 by APL [38] but did not change the effect of the inhibitor on fusion. [32] The discrepancies can be explained by the fact that the different functional assays can have different sensitivities. [32] Alternatively, some mutations could have differential effects on allosteric inhibition of different functions of CCR5, in agreement with the “probe-dependent” nature of allosteric inhibitors here above discussed. For those reasons, comparing SDM data issued from different functional assays reported in the literature is an obligatory step to achieve accurate pictures of ligand binding sites into receptors.

The analysis of the SDM results is greatly facilitated by accounting for structural data. The three-dimensional structure of CCR5 built by homology to CXCR4 especially discriminated between residues that are involved in the receptor folding (the tightly-packed ones) and those that directly interact with orthosteric or allosteric ligands (the surface-exposed ones). The above mentioned Trp248 (Trp6.48) is a typical example of a key structural residue. It constitutes a hub of the network of aromatic residues in the 7TM bundle, so that its replacement by any other amino acid has important structural and functional consequences. Similarly, a possible role of Ile198 (Ile5.42) in the dynamics of the receptor was described in reference [5]. This residue is located in TM5 one helix turn upstream of a hinge region defined by a GxxxP motif, which was locked by a thermostabilizing mutation in the CXCR4 variant used for crystallisation. [27]

Altogether, SDM and three-dimensional data provided an accurate and credible mapping of ligand binding sites: the CCR5 chemokine CCL3 and the viral glycoprotein gp120 bind to extracellular domains of the receptor, especially ECL2, while MVC inserts in the receptor 7TM bundle. Using docking experiments, we could further define the binding sites and tested the simultaneous binding of orthosteric and allosteric ligands. [5] We proposed a model of interaction between CCR5 and CCL3 (or gp120) by performing manually the rigid-body docking of the orthosteric ligand into the receptor in order to replicate the interaction mode that was observed in the crystallographic structure of the complex between CXCR4 and the peptide antagonist CVX15,[27] *i.e.* hydrogen bonds were established between the ECL2 of CCR5 and a  $\beta$ -sheet in CCL3 (or the V3 loop in gp120) to form a single  $\beta$ -sheet from the  $\beta$ -strands of the two molecular partners. Experiments of automatic docking of MVC into CCR5 yielded multiple poses for the inhibitor that roughly delimit three different sites, yet overlapping, in the transmembrane pocket: one deeply buried (site 3 in reference [5]), an upper one between helices 1, 2, 3 and 7 (site 1) and an upper one between helices 3, 5, 6 and 7 (site 2) (Fig. 2). The area common to sites 1 and 3 corresponds to the binding site of small molecule antagonist It1 in CXCR4. [27] Interestingly, the modeled complex between CCR5 and either gp120 or CCL3 leaves room for MVC to access the 7TM bundle of the receptor between helices 1, 2, 3 and 7 (Fig. 3), thereby adding to the understanding of our experimental data that MVC could promote dissociation of either  $^{125}\text{I}$ -CCL3 or  $^{35}\text{S}$ -gp120 prebound to CCR5. [16] Interestingly, the dose-dependent experiments of MVC-induced dissociation of radioligands from CCR5 suggested that ligand-occupied CCR5 has a lower affinity for the inhibitor than the free receptor, consistent with the fact that allosteric interactions are reciprocal (*i.e.* the radioligands are expected to modulate the binding of MVC similarly as MVC modulates that of the radioligands). [16] In this context, MVC could move from the low affinity site to another site of higher affinity that is accessible only when

the receptor is free (for example, MVC could theoretically pass from the superficial site 1 to the deepest site 3 by a simple translation without necessity of conformational changes for the ligand adaptation to the two sites). Alternatively, the upper site could shift from a MVC-low affinity state to a high-affinity state once the radioligand is dissociated. Once bound with high affinity to CCR5, MVC may prevent efficient binding of orthosteric ligands to the receptor probably by perturbing TM-ECL2 interactions. [5]

### **E. Perspective: one step closer towards a complete picture of the system**

Many studies were undertaken during the last twelve years to understand the mode of action of non-peptidic, small molecules inhibiting CCR5. SDM and homology modeling from the crystal structure of CXCR4 added to the comprehension of the molecular determinants in CCR5 which participate in allosteric inhibition of the receptor by MVC. Such a knowledge should prove useful in designing future allosteric compounds preserving important CCR5 functions in immunity while inhibiting HIV and devoid of adverse effects on health. [31]

Noteworthy, a major complexity in studying structure/function relationships of CCR5 arises from the equilibrium between different conformational and homo- and hetero-oligomeric states of the receptor. [39,40] Its influence on the binding and the effects of allosteric inhibitors remains to date difficult to assess by molecular modeling approaches. For example, although our SDM and docking experiments have proposed distinct binding sites for MVC and orthosteric ligands, thus agreeing well with an allosteric mode of action for the inhibitor, the possibility cannot be ruled out that small molecule CCR5 inhibitors also transmit allosteric effects from one receptor to another in a CCR5 dimer, as recently suggested. [41] Similarly, evidences indicated that CCR5 undergoes regulations that are of allosteric nature once it is engaged in receptor homo- or hetero-dimers, [42] but to what extent this influences the binding of small allosteric ligands to the receptor and their efficiency as HIV entry inhibitors

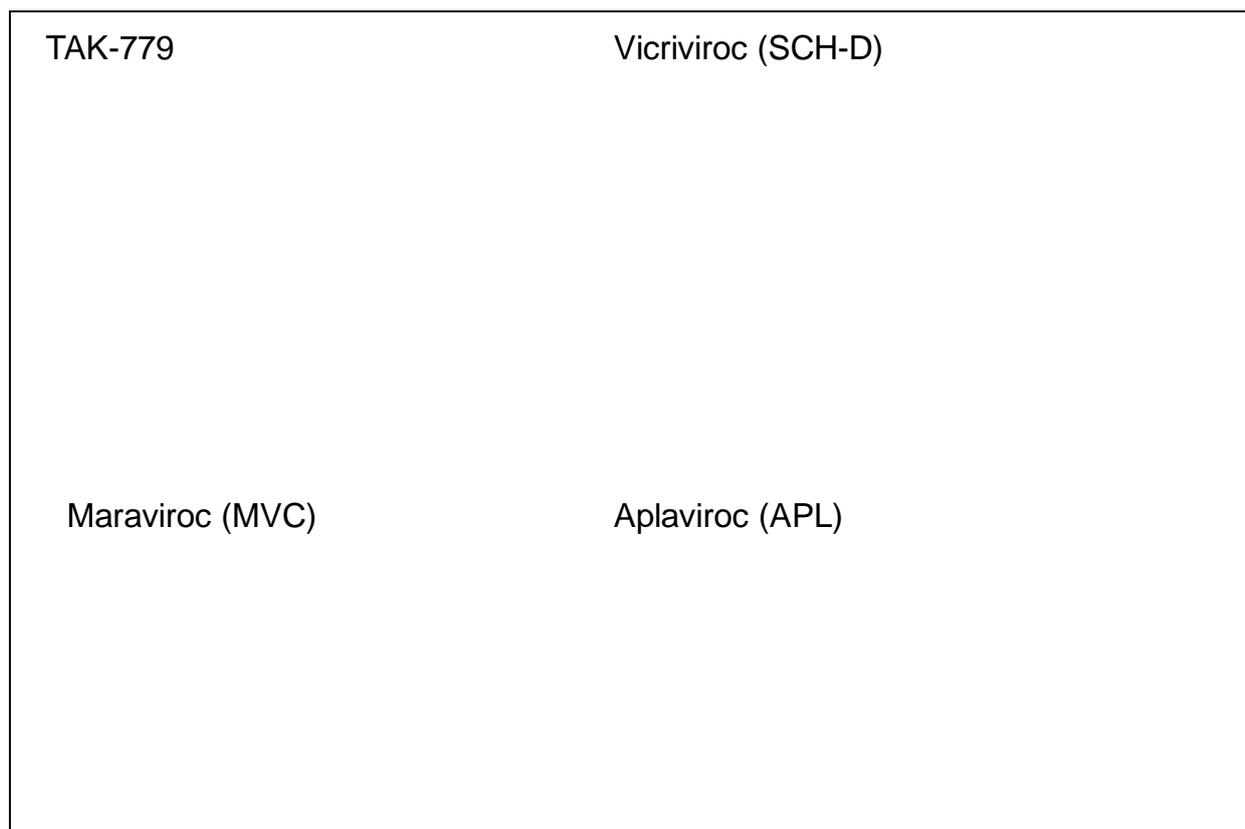
has remained poorly studied. Yet, in support of such a possibility, a role for CCR5 conformational state in the binding of small molecule CCR5 inhibitors was actually suggested by us and others. [16,43] Finally, it is not known yet if disruption of dimer formation may take part in the inhibitory process of small molecule CCR5 ligands. Indeed, we recently described residues in the CCR5 putative dimer interface whose mutation abrogated gp120 binding (and presumably HIV entry). [5] Designing molecules that would target this interface could help elucidate these critical issues regarding the contribution of receptor dimers in HIV entry and infection and their sensitivity to allosteric inhibitors.

<b>Models</b>	<b>Unbias, low accuracy</b> <sup>[5], [44]</sup>	<b>Biased, fair accuracy</b> <sup>[5], [31], [32]</sup>	<b>Unbiased, good accuracy</b> <sup>[5], [30], [31]</sup>
<b>Modeling approaches</b>			
Template for TMs	rhodopsin*	rhodopsin	CXCR4
Template for loops	PDB-derived library of loops or no loops	PDB-derived library of loops or no loops	CXCR4
<b>Refinement of model</b>			
The TxP motif	/	kink in helix 2	/
Inter-residue constraints derived from experimental data	/	e.g. vicinity of the side chains of Phe2.59 and Leu3.28 [28]	/
<b>Customization of the 7TM</b>			
Selection of rotamers	/	e.g. carboxylate of Glu7.39	/
Shaping the pocket according to the presumed active conformation of known binders	/	available for inter-molecular ionic bond	/
<b>Applications</b>			
Identification of residues in the orthosteric site	poor	poor	fair
Identification of residues in the allosteric site	fair	good	good
Identification of networks of intra-molecular interactions	fair	partial	good
Performance in virtual screening by docking [5]			
- Sensitivity: retrieval of CCR5-binders	fair	good	good
- Specificity: discard of decoys	fair	fair	good
Docking of ligands**	poor	fair	good

**Table 1: Approaches to CCR5 modeling and predictive power of models.**

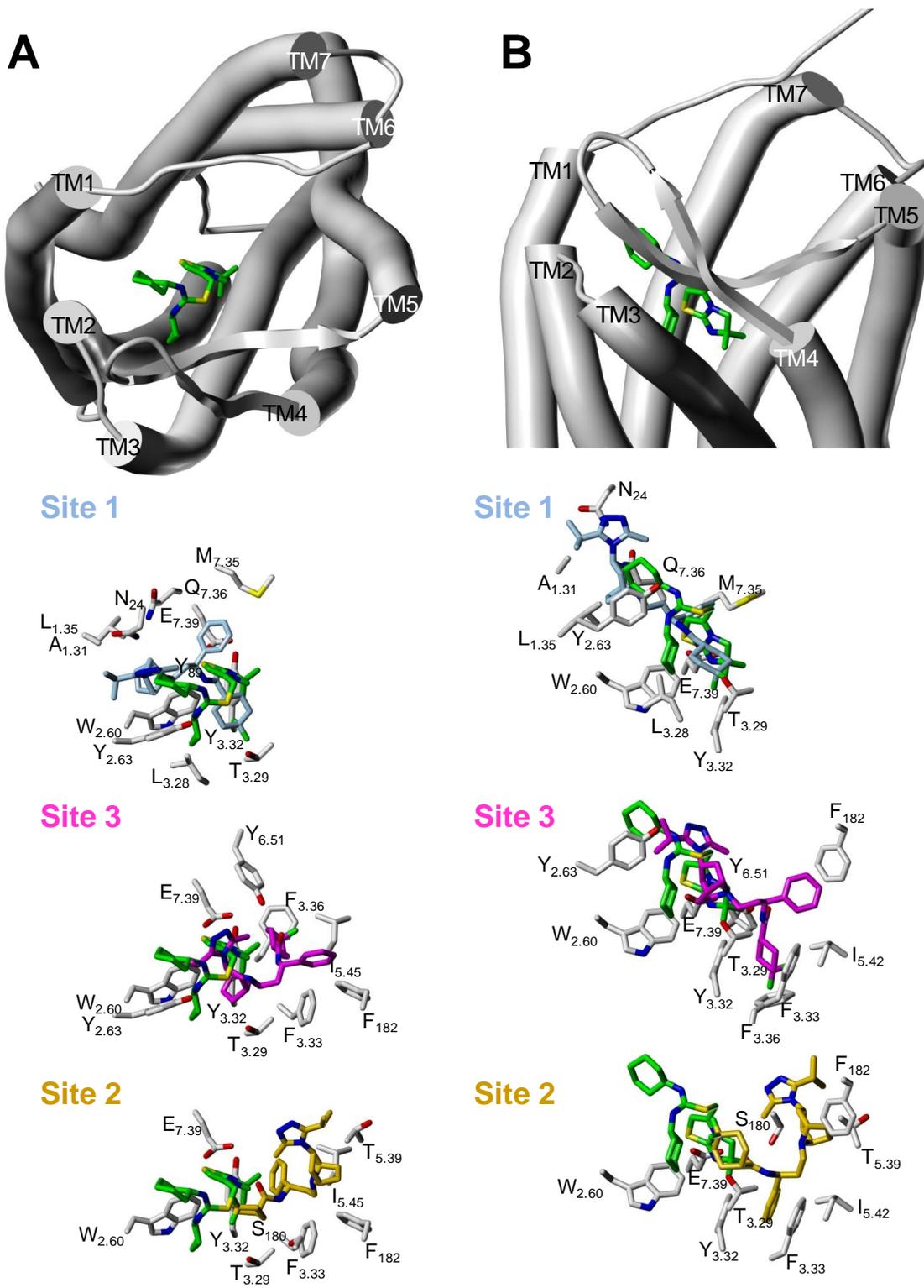
\* Models of CCR5 were also built from the coordinates of  $\beta$ 2 adrenergic and adenosine  $\alpha$ 2A receptors, but they have a lower predictive power than the ones based on bovine rhodopsin.

\*\*Ability to generate reasonable poses, which are not necessarily correctly ranked by scoring functions



**Figure 1: Chemical structure of CCR5 inhibitors.**

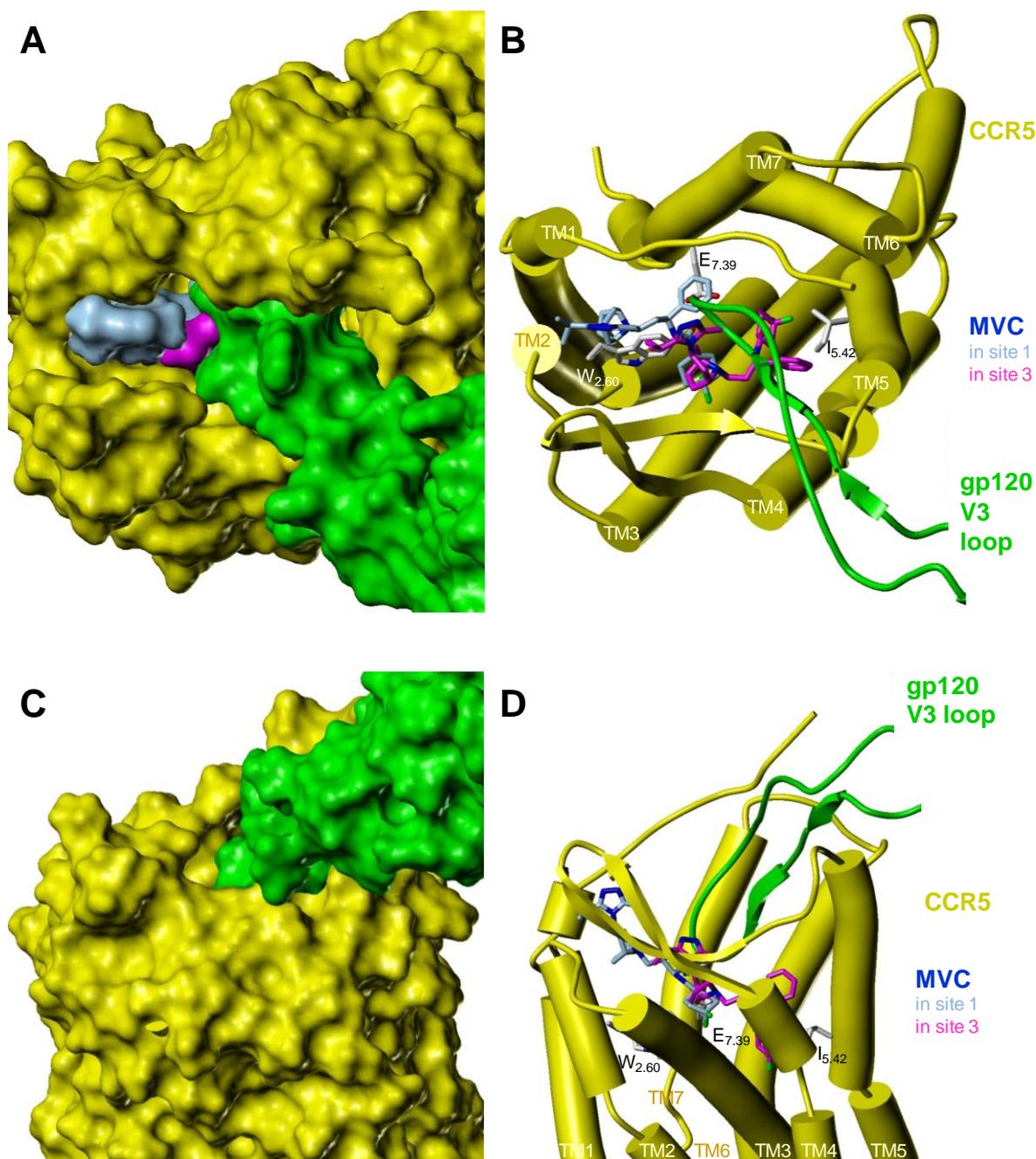
At physiological pH, the four compounds are positively charged. They however contain different chemical scaffolds and do not define a simple unique pharmacophore or a consensual three-dimensional shape. [45]



**Figure 2: Possible binding modes for MVC in the 7TM bundle of CCR5**

From top to bottom are displayed the crystal structure of CXCR4 (grey ribbon) in complex with the antagonist It1 (carbon atoms colored in green), and representative docked poses of MVC into the CXCR4-based model of CCR5 (capped sticks) in site 1 (MVC carbon atoms colored in light blue), in site 3 (MVC carbon atoms colored in magenta) and in site 2 (MVC

carbon atoms colored in orange). The side chains in CCR5 binding sites are labeled according to the Ballesteros-Weinstein numbering scheme (except for Asn24 in the N-terminal domain, and for Ser180 and Phe182 in ECL2). For the sake of clarity, It1 position is shown in all views. The panel B reflects an orientation orthogonal to the view in panel A. All the images are at the same scale



**Figure 3: Complex between CCR5, Gp120 and MVC.**

Proteins are either represented by their solvent-excluded molecular surface (A,C) or by their secondary structures displayed as ribbons (B,D). The side chains of residues discussed in the text are displayed as capped sticks. MVC is either represented by its solvent-excluded surface (A,C) and by capped sticks (B,D, heavy atoms only). Two different poses of MVC are shown. The panels A and B reflect the same orientation. The panels C and D reflect an orientation orthogonal to the view in panels A and B. All the panels are at the same scale.

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