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Type	Oral communication
Session	Molecular Dynamics Simulations
Title	CXCL10-CXCR3 complex formation, different isoforms result in different binding modes
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Motivation

CXCR3 is a G-protein coupled receptor expressed principally on leukocytes, monocytes and epithelial cells; it is involved in leukocyte traffic, integrin activation, cytoskeletal changes and chemotactic migration, by binding to its classical ligands, CXCL-9/10/11 (1).
Three splicing variants of CXCR3 are known: CXCR3a, the most common isoform, consisting of 368 amino acid residues; CXCR3b resulting from an alternative splicing of the CXCR3 mRNA with a 52 aa extended N-terminal domain when compared to the isoform a, instead CXCR3-alt is a significantly truncated variant not involved in classical ligand binding (1). CXCL10, the interferon-γ-inducible protein (IP-10) belongs to the CXC family of chemokines and acts as an immunoinflammatory mediator, inhibits angiogenesis and displays antitumor properties (2).
Several studies indicated that the CXCR3 N-terminal domain plays a key role in determining binding affinity, receptor selectivity, and also in regulating allosteric signaling through the receptor (3). Moreover, sulfation of tyrosine in chemokine receptors is emerging as a post-translational modification that substantially contributes to ligand binding (2). Tyr 27 and 29, two of the N-terminal tyrosine can be sulfonated. Additionally, CXCR3b displays two other potential sulfonable tyrosines in the extended N-term domain, at position 6 and 40; these differences may affect the binding mechanism and the receptor activation of CXCR3b rather than CXCR3a (4).
Finally, Kleist and co-workers hypothesize a “two-step” model, where receptor binding and activation can be dissociated (5). In the first step, the chemokine binds to the N-term domain of the receptor. In the second, residues on IP10 N-terminal bind to the binding cavity on the transmembrane domain of the receptor and induce the allosteric communication to the cytosol.
In this work, we analyse by molecular dynamics (MD) simulation the binding mechanism of CXCL10 on CXCR3, evaluating the effect of tyrosine sulfonation and taking into account different sulfotyrosine combinations, on binding mode and affinity. In parallel, we analyse variations between CXCR3 isoforms a and b to highlight the different allosteric communication toward the cytoplasmic C-term domain, responsible of G-protein binding.

Methods

We modelled CXCR3a and b isoforms, both were included in a double layer membrane with CHARMM-GUI server (http://www.charmm-gui.org/) and through the MD we optimized the structure conformation. The binding between CXCL10 and CXCR3 representative conformation was simulated employing a supervised MD approach (6).

Results

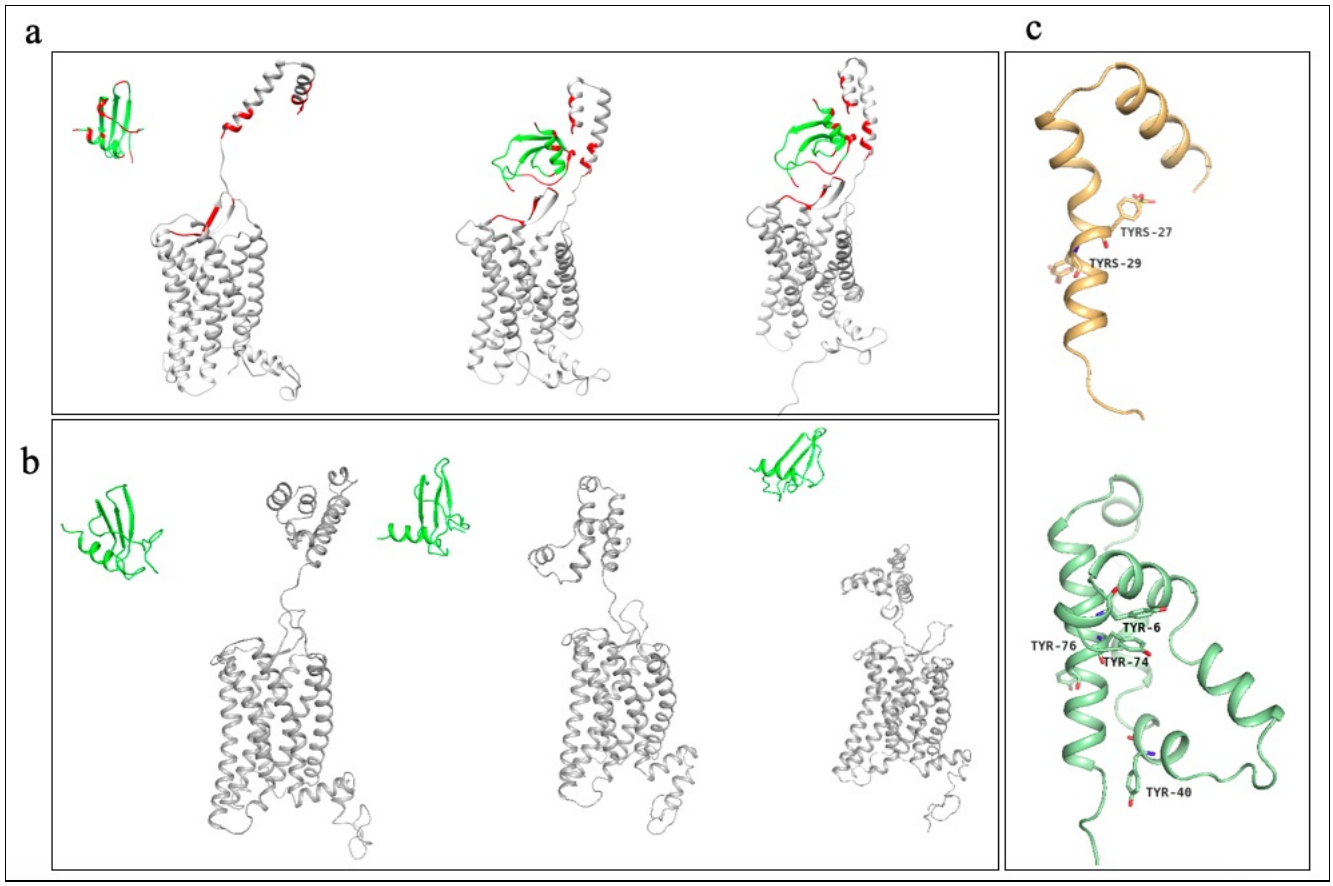
Several simulations were collected for each complex, in order to obtain a consistent result. In particular, the supervised MD was performed in 3 runs, using the same conditions, starting from the same equilibrated system. A list of selected residues from the N-term of CXCR3 and all the extra-cellular loop were adopted for the computation of the binding site instead the entire chemokine was considered as ligand in order to perform the supervision on the distance between the receptor (CXCR3) and the ligand (CXCL10). All simulations return the CXCL10 correctly positioned in CXCR3a binding site within the first 15ns (fig 1a). While, CXCR3b simulations doesn’t reach the complex formation due to the closing up of the N-term domain, and interrupt at 9 Å of ligand-receptor distance (fig 1b).
CXCR3a complex simulations resulted consistent in both ligand-receptor decreasing energies, related to minor R-L distances and similarities in pattern of contacts, involving both residues from the CXCR3 N-terminal and the extra-cellular β-sheet, which has been described as an important player for the receptor activation.
A second round of supervised MD will be performed to evaluate the second step of the “two-step” model. The next step will be the evaluation of the effect of tyrosine sulfation (Y27, Y29 for both receptors and Y6, Y40 only for CXCR3b) (fig 1c) on binding mode and affinity.

Info

(1) Lacotte, S.; Brun, S.; Muller, S.; Ann. NY Acad. Sci. 2009, 1173:310-7.
(2) Gao, J.M.; Xiang, R.L.; Jiang, L.; et al. Acta Pharmacol Sin 2009, 30:193–201.
(3) Raucci, R.; Colonna, G.; Giovane, A.; et al. Biochim Biophys Acta. 2014, 1844:1868-80.
(4) Boyé, K.; Billottet, C.; Pujol, N.; et al. Scientifoc Reports. 2017, 7: 10703.
(5) Kleist, A.B.; Getschman, A.E.; Ziarek, J.J.; Biochem Pharmacol. 2016, 15:114:53-68.
(6) Salmaso, V.; Sturlese, M.; Cuzzolin, A.; Moro, S.; Structure. 2017, 25:655-662.

Fig 1. CXCR3a is represented in the first line, and CXCR3b in the second. (a) screenshots of CXCR3a-CXCL10 simulation, residues involved in R-L contacts are in red. (b) screenshots of CXCR3b-CXCL10 simulation. (c) possible sulfonated N-term Tyr.

Figure



Availability	-

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