GPR17 molecular modelling: interactions with non-conventional pro-inflammatory ligands

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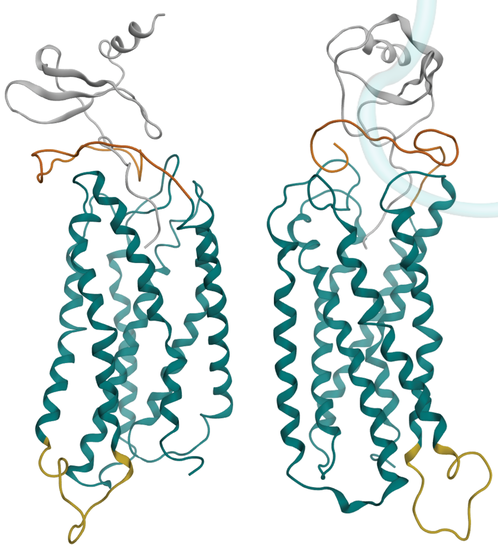
ABSTRACT [1]

GPR17 is a class A-GPCRs operated by different classes of ligands, such as uracil nucleotides, cysteinyl-leukotrienes and oxysterols. Similar to other receptors of the same class, GPR17 can associate into homo- and hetero-dimers. Recent findings suggest its promiscuous behaviour namely the possibility to be operated by ligands able to transversely interact with more than one GPCRs. In fact, both GPR17 and CXCR2 are operated by oxysterols, and both GPR17 and CXCRn ligands have demonstrated roles in orchestrating inflammatory responses and oligodendrocyte precursor cell (OPC) differentiation to myelinating cells in acute and chronic diseases of the CNS. Here we demonstrate that GPR17 can be activated by the chemokine stromal-derived factor-1 (SDF-1), a ligand of CXCR4 and CXCR7, and investigate the underlying molecular recognition mechanism, by combining *in silico* modelling data with *in vitro* validation in (i) a classical reference pharmacological assay for GPCR activity and (ii) a model of maturation of primary OPCs. We also demonstrate that cangrelor, a GPR17 orthosteric antagonist, can block the SDF-1-mediated activation of GPR17 in a concentration-dependent manner. The ability of GPR17 to respond to different classes of GPCR ligands suggests that this receptor modifies its function depending on changes occurring in the extracellular mileu changes occurring under specific pathophysiological conditions and advocates it as a strategic target for neurodegenerative diseases with an inflammatory/immune component.

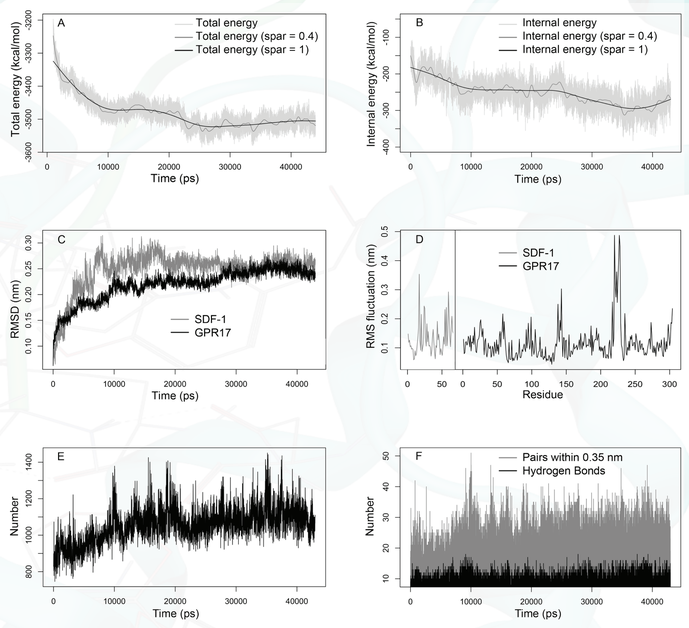
MODELING AND MOLECULAR DYNAMICS SIMULATION



We modeled the GPR17::SDF-1 complex using functional and structural information on SDF1 and on CXCR4 as partial homologous template for GPR17:

- Crystal structures are available for both CXCR4 and SDF-1.
- Functional evidence suggests interaction between SDF1 and CXCR4.
- A model of CXCR4::SDF-1 has been published by Tamamis et al. [2].
- P2Y12 and Rho are the most appropriate partial homologous templates for the TM bundle of GPR17.

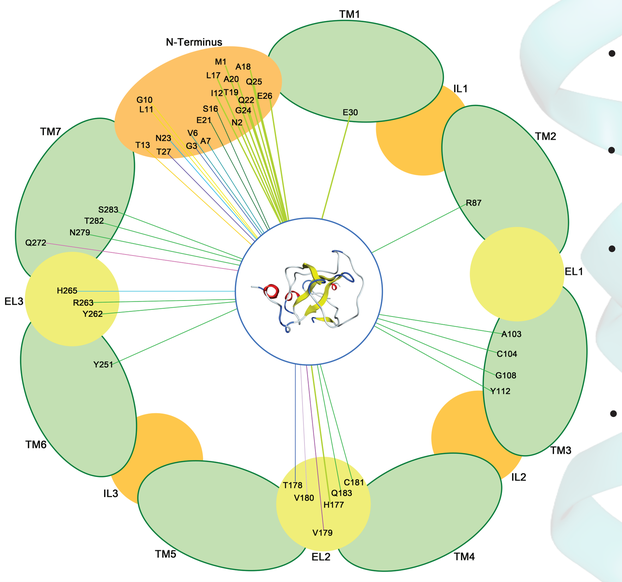
Comparative model of the GPR17::SDF-1 complex.
Regions built using $P2Y_{12}$ as template are represented as green ribbons; residues transferred from other templates are highlighted in yellow (IL3, from rhodopsin) and orange (N-terminus, from the CXCR4::SDF-1 in Tamamis at al. [2]). SDF-1 (from the CXCR4::SDF-1 in Tamamis at al. [2]) is in grey.



In order to investigate the molecular behaviour of the GPR17::SDF-1 complex, we carried out a 43 ns MD simulation with CHARMM using a Generalized Born method with a simple switching (GBSW) model of implicit membrane/solvent. The evaluation of the stability of the GPR17::SDF-1 complex during the production phase was based both on energetic and geometric parameters. The general stability of the GPR17::SDF-1 complex is confirmed by the tendency of the MD simulations to reach convergence after 20 ns.

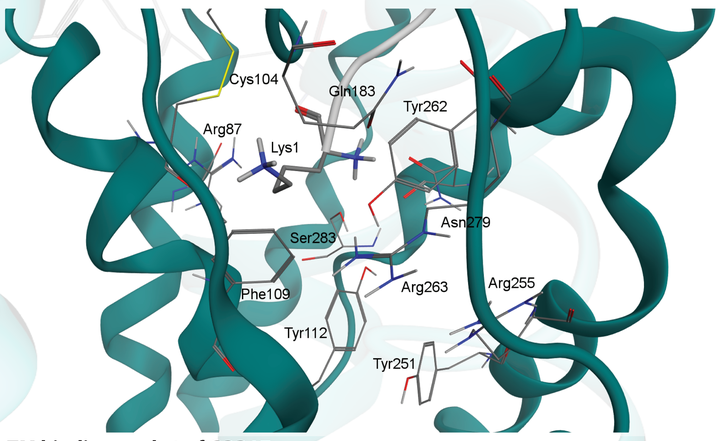
Stability of GPR17::SDF-1 during MD simulation.
Total energy and interaction energy (light grey) are plotted vs time in panels (A) and (B), respectively. Panel (C) shows the RMSD profiles vs time for SDF-1 (grey) and GPR17 (black), whereas panel (D) shows the RMS fluctuation, expressed in nm, computed for the α-carbons of SDF-1 (light grey, residues 1–68) and GPR17 (dark grey, residues 1–304). Panel (E) reports as a function of time the total number of contacts between all pairs of residues, panel (F) the total number of hydrogen bonds occurring between SDF-1 and GPR17 (in black); panel (F) also shows the number of atom pairs < 0.35 nm apart (in grey).

INTERACTION BETWEEN GPR17 AND SDF-1



- During the whole MD simulation we found eighty-six pairs of residues involved in the GPR17::SDF-1 interaction.
- Twenty-one pairs of these residues form interactions conserved in the CXCR4::SDF-1 complex [2].
- Among the conserved interactions, ten are stable throughout the MD simulation: Lys1-Tyr112; Lys1–Tyr251; Lys1–Asn279; Ser–Gln25; Asn33–Asn2; Gln48–Ala18; Gln48–Ala18; Gln48–Thr19; Gln48–Ala20; Glu21–Lys27.
- Our *in silico* data suggest that the binding surface of SDF-1 on GPR17 is diffused and that both the N-terminus and the ELs are important for molecular recognition, as already described for many peptide ligands of GPCRs.

GPR17::SDF-1 interaction spatial map.
Intermolecular interactions between pairs of residues in GPR17::SDF-1 are represented by lines; multiple interactions are represented with light green lines.



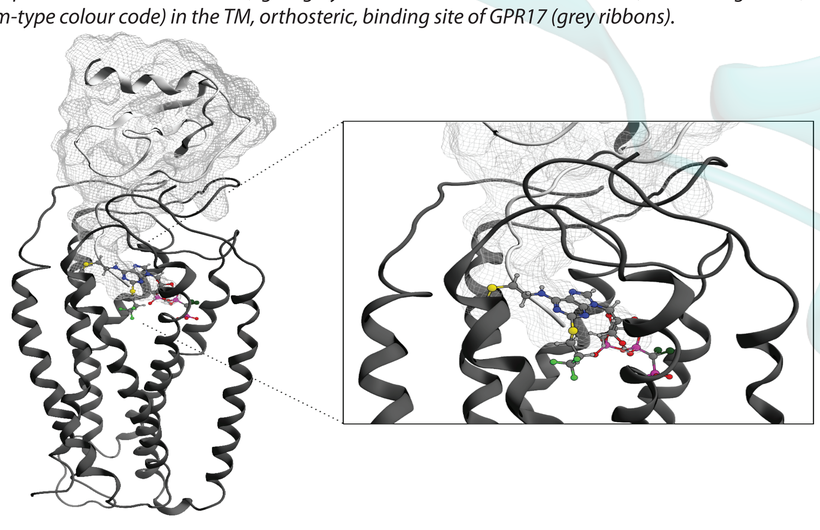
TM binding pocket of GPR17.
Representative picture of the residue Lys1 (labelled sticks), at the extremity of SDF-1 N-terminus (white ribbons) entering the TM binding pocket of GPR17 (green ribbons).

TM are also involved:
Lys1 of SDF-1, essential for the chemotactic activity toward CXCR4, exhibits specific H-bond interactions with the –OH groups of Tyr112 (TM3), Tyr251 (TM6), and Tyr262 (EL3), and with polar groups of residues Asn279 (TM7) and Gln183 (EL2). While our previous studies on GPR17 reported that all these residues are likely involved in the recognition of uracil nucleotides, in SDF-1 neither His255 nor Arg255 are directly involved in the recognition mechanism.

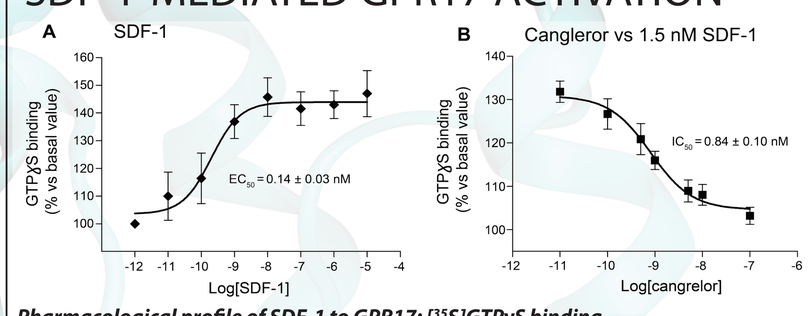
GPR17::SDF-1 artwork, powered by Studio Bozzetto.

The nucleotide-derivative antagonist cangrelor [4] binds the same TM binding pocket, establishing specific interactions with some residues involved in the SDF-1 recognition; this features suggest that cangrelor may hamper the entrance of SDF-1 N-terminus in the TM binding site.

Uracil nucleotide binding site of GPR17.
Superposition between SDF-1 (light grey molecular surface and ribbons) and cangrelor (sticks, atom-type colour code) in the TM, orthosteric, binding site of GPR17 (grey ribbons).



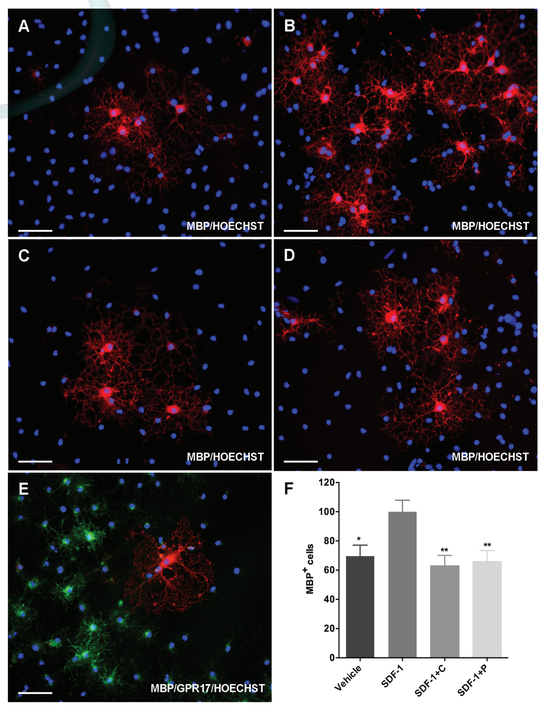
SDF-1-MEDIATED GPR17 ACTIVATION



Pharmacological profile of SDF-1 to GPR17: [^{35}S]GTPγS binding.
((A) Membrane aliquots obtained from 1321N1 cells transfected with hGPR17 were incubated with different SDF-1 concentrations, and [35S]GTPγS binding assay was performed. (B) Effect of the GPR17 receptor antagonist cangrelor on ligand-stimulated [35S]GTPγS binding. Membranes from human GPR17-transfected cells were pre-incubated for 10 min with cangrelor (0.1 nM–100 nM), then stimulated with SDF-1 at the constant concentration of 1.5 nM (10 fold over the EC50 value).

[^{35}S]GTPγS binding experiments performed on 1321N1 cells stably transfected with GPR17 demonstrate that SDF-1 is able to increase the GTPγS binding to cell membranes, with affinity constant values of 0.14 ± 0.03 nM. This effect is completely antagonized by the well-known GPR17 orthosteric antagonist cangrelor, further confirming that SDF-1 specifically bound GPR17 and behaved as a receptor agonist.

SDF-1-mediated activation of GPR17 in OPCs.
MBP-expressing cells (in red) treated with vehicle (A) and SDF-1 alone (B), or in combination with cangrelor (C) and plerixafor, an CXCR4 antagonist (D). (E) Control cell cultures expressing GPR17 (in green) and MBP (in red). Cell nuclei are labelled with Hoechst 33258 (in blue). (F) Percentage of MBP+ cells after treatment with vehicle and with SDF-1 alone, or in combination with cangrelor and plerixafor.



CONCLUSIONS

Besides activating its cognate receptors CXCR4 and CXCR7, SDF-1 can also activate GPR17 with comparable potency, as predicted by our *in silico* simulation. In our *in vitro* models, the activation of GPR17 by SDF-1 efficiently promotes the maturation of OPCs, a well-known key event of the myelination pathway. This emphasizes the pathophysiological relevance of the cross-talk between these receptors in the regulation of OPC maturation and myelination.

In primary OPC cultures, treatment with SDF-1 increases the number of MBP+ cells with respect to control. This increase is not observed when SDF-1 is added to cells either in combination with cangrelor or plerixafor. In both antagonist groups, the number of MBP+ cells is comparable to, or slightly lower than, the one measured in the vehicle-treated cells.

References

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Acknowledgements

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