Elucidating the bioactive conformation of CCR5 Chemokine Receptor inhibitors

G. Tedesco[†]

[†] Cresset, New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, SG8 0SS, UK

Abstract

In such cases where the crystallographic information about a target is scarce or unavailable (for example GPCRs, ion channels and novel targets), field pharmacophore modeling as implemented in FieldTemplater[1] can help understanding how active compounds interact with their protein target and which parts of those active molecules are involved in binding. In this case study, working from just a few 2D structures of known active CCR5 Chemokine Receptor inhibitors, we show how FieldTemplater was able to predict the bioactive conformation for these ligands as evinced from the X-ray data of the CCR5 inhibitor Maraviroc.

Method

FieldTemplater is a tool for comparing molecules using their electrostatic and hydrophobic fields in order to find common patterns. When applied to several structurally-distinct molecules with a common activity, FieldTemplater can determine the bioactive conformation and relative alignments of these molecules without requiring any protein information.

The CCR5 Chemokine Receptor is a G-Protein Coupled Receptor (GPCR) involved in the viral entry pathway for HIV. CCR5 inhibitors have accordingly potential therapeutic applications as AIDS treatments.

Five highly potent CCR5 inhibitors (shown in Figure 1) were extracted from the literature.[2] The high activity at CCR5 and the presence of a basic Nitrogen in all the chosen ligands supports the hypothesis that all compounds potentially share a common binding mode.

As these ligands are large (MW between 515 and 580) and flexible (number of rotatable bonds between 8 and 11), they can potentially be aligned in a number of different ways and the identification of their bioactive conformation, in the absence of X-ray data, is non-trivial.

FieldTemplater generates a series of conformations that the ligands might adopt at physiological conditions. It analyzes these conformations to find sets that show a high molecular field similarity (and hence have similar shape/binding properties). Where all the ligands with a common activity align well, it is very likely that this is the bioactive conformation.

FieldTemplater conditions appropriate to the analysis of large molecules with complex ring systems were chosen to generate the binding hypothesis. In particular, the maximum number of conformations to be generated was set to 200, within an energy window of 3kcal/mol and

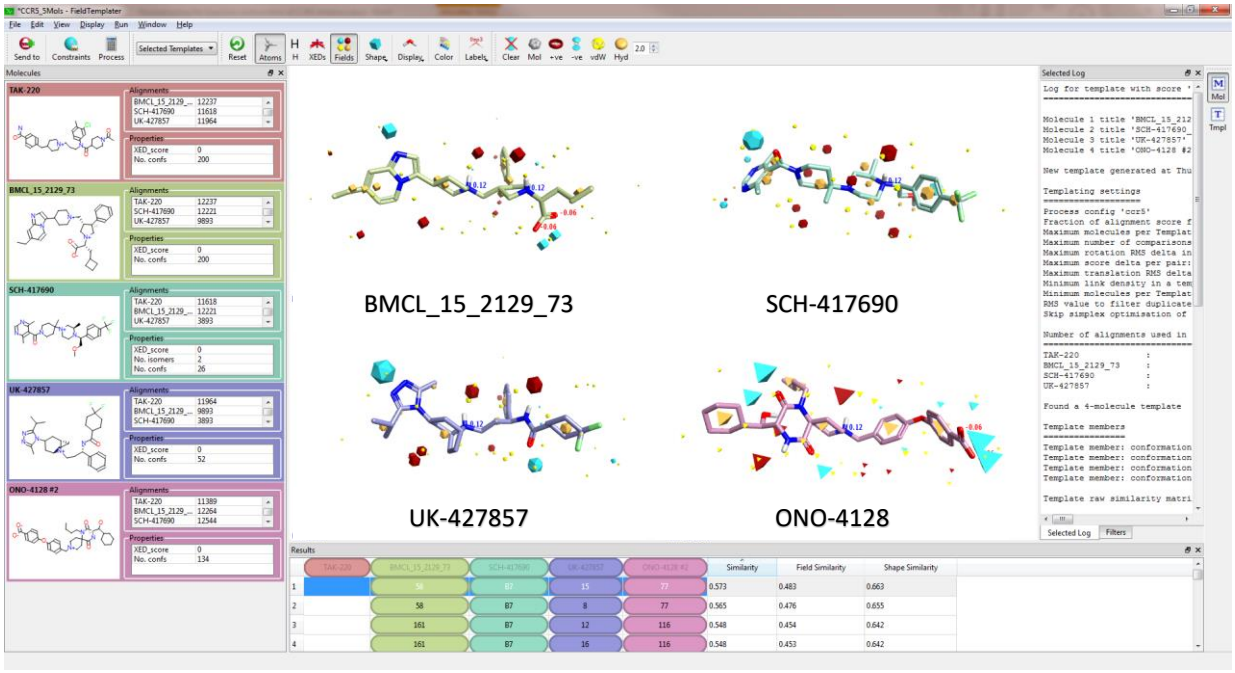

Figure 1. FieldTemplater binding mode hypotheses (templates) for the highly potent CCR5 receptor inhibitors shown. Field points are colored as follows: Blue: negative field points; Red: positive field points; Yellow: van der Waals surface field points; Gold/Orange: hydrophobic field points.

applying an energy minimization cut-off of 0.05 kcal/mol/A; the conformational analysis of the complex ring systems was carried out separately, and the chosen conformation for the ring was then kept fixed; a ratio of field and shape similarity of 0.5 (corresponding to 50% field and 50% shape) was used for the alignments; and the minimum number of molecules required to form a binding hypothesis was set to 4 (out of 5 total molecules).

Results

Approximately 30 possible alignments (templates) were identified by FieldTemplater. However, no consistent alignment was found for all 5 ligands, while templates including 4 out of 5 total molecules were identified.

The top scoring template, in terms of overall shape and field similarity, is shown in Figure 1. Main features in this template are:

a) a positive field point (red polyhedra in Fig. 1) mapped by the protonated basic Nitrogens of UK-427857 (Maraviroc), BMCL_15_2129_73, ONO-4128;
b) a second positive field point mapped by the amide NH of UK-427857, by the second protonated basic N of BMCL_15_2129_73 and by the protonated basic N of SCH-417690;
c) a negative field point (blue) on the left hand side of the compounds, mapped by the triazole ring of UK-427857, the imidazo-pyridine ring of BMCL_15_2129_73, the carbonyl Oxygen of the amide group of SCH-417690, and by one of the carbonyl Oxygens on the spiro ring of ONO-4128;

d) a third positive field point on the left hand side, underneath all ligands;
e) hydrophobic field points (gold/orange) mapped by the aliphatic and aromatic rings on the right hand side and in the middle of the ligands.

The alignment of ONO-4128 in this template is somehow unexpected, with the carboxylate group lying on the right hand side (rather than mapping the negative field point on the left hand side). Other template solutions found by FieldTemplater indeed capture this alternative alignment. In the absence of X-ray data for ONO-4128, its actual binding mode to CCR5 remains to be further elucidated.

The accuracy of the FieldTemplater solution was investigated by comparing the conformation of UK-427857 to that present in the published CCR5 crystal structure (PDB 4MBS). This was achieved by transfering the top scoring template (Fig. 1) into the main Forge interface. The X-ray conformation of this ligand bound to the CCR5 receptor, as derived from the 4MBS PDB was downloaded directly into the Forge interface and split into protein and ligand. The two conformations were then aligned in a rigid manner based on their field points and shape.

As can be seen in Figure 2, the FieldTemplater result aligns extremely well with the experimental conformation with an overall field and shape similarity of 0.87. The right end side of the molecules is almost perfectly overlaid while the only relevant difference can be observed on the left hand side, where the triazole ring is flipped of 180°. This happens as an almost symmetric pattern of hydrophobic field points surrounds the triazole group.

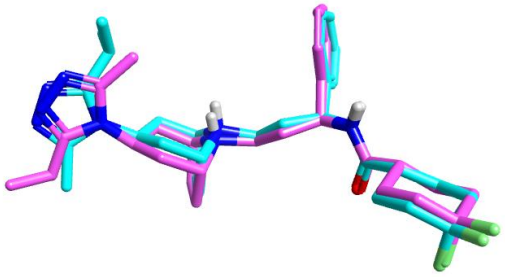

Figure 2. Conformation of Maraviroc identified by FieldTemplater (magenta) overlaid to the X-ray conformation from the 4MBS PDB (blue).

Conclusion

FieldTemplater was able of correctly reproduce the bioactive conformation of Maraviroc, a CCR5 receptor inhibitor, without making use of the X-ray information about the binding mode of this ligand. Additionally FieldTemplater indicates the relative alignments and likely bioactive conformations of 3 further CCR5 inhibitors.

In such cases where the X-ray structure of the target is scarce or unavailable, field pharmacophore modeling as implemented in FieldTemplater can help understanding how active compounds interact with their protein target and which parts of those active molecules are involved in binding, in the absence of any protein information.

References and Links

1. http://www.cresset-group.com/products/forge/fieldtemplater/
2. The inhibitors were TAK-220 from J Med Chem. 2006 **49,** p2784-93; compound 73 taken from *Bioorganic and Medicinal Chemistry Letters* **15**, p2129 (BMCL_15_2129_73); Vicriviroc (SCH-417690); Maraviroc (UK-417690); Aplaviroc (ONO-4128).