A General Method for Defining the 3D Active Site Requirements of GPCRs: CCK2 Ligands Example

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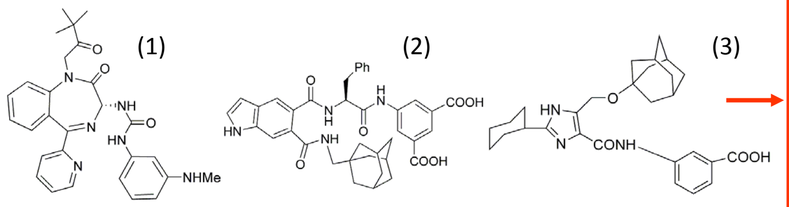
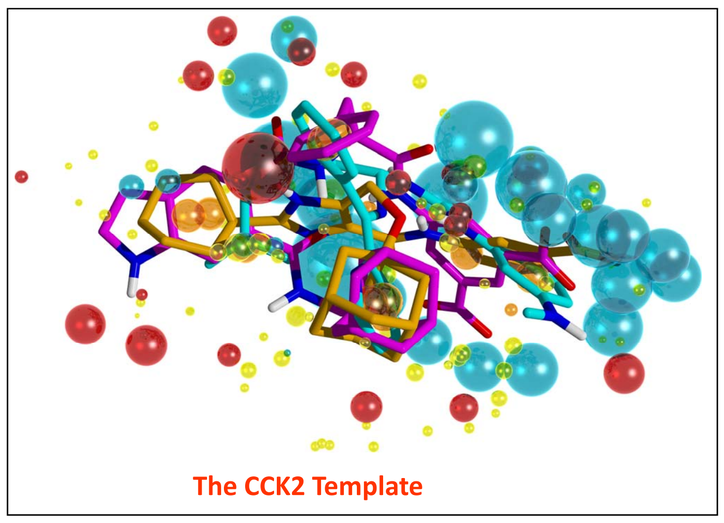
Introduction

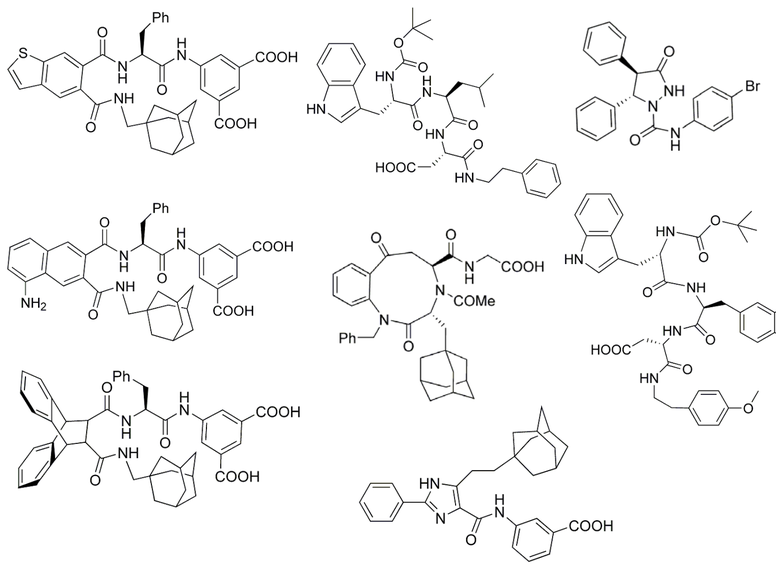
Structure-based drug design is widely accepted as a valuable tool to aid lead optimisation for targets with an x-ray structure. However, designing ligands for GPCR receptors, such as CCK2, presents a greater challenge due to the lack of good 3D structural data on the targets. Certain groups have found homology models based on rhodopsin useful, but there can be large errors in these models. We have therefore approached this problem from the ligands' viewpoint.

Cresset's molecular fields [1] model the binding characteristics of a ligand. They have been extensively validated for virtual screening by us and some of the major pharma companies. We have now applied fields to create a model to predict bioactive conformations of ligands in the absence of x-ray data. Our reasoning is that if a set of diverse ligands can adopt a conformation in which they all display the same field pattern, then this alignment must be an hypothesis for their bioactive conformation.





The CCK2 Template



Work Flow

Take 3 structurally-diverse ligands highly active in the CCK2 in vitro rat stomach functional assay [2]

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Use FieldTemplater to derive a bioactive conformation model: **The CCK2 Template**

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Use FieldAlign to align diverse molecules with a range of CCK2 activities to the template

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Plot the field similarity value of each molecule to the template against the biological activity

Fields and Field Points

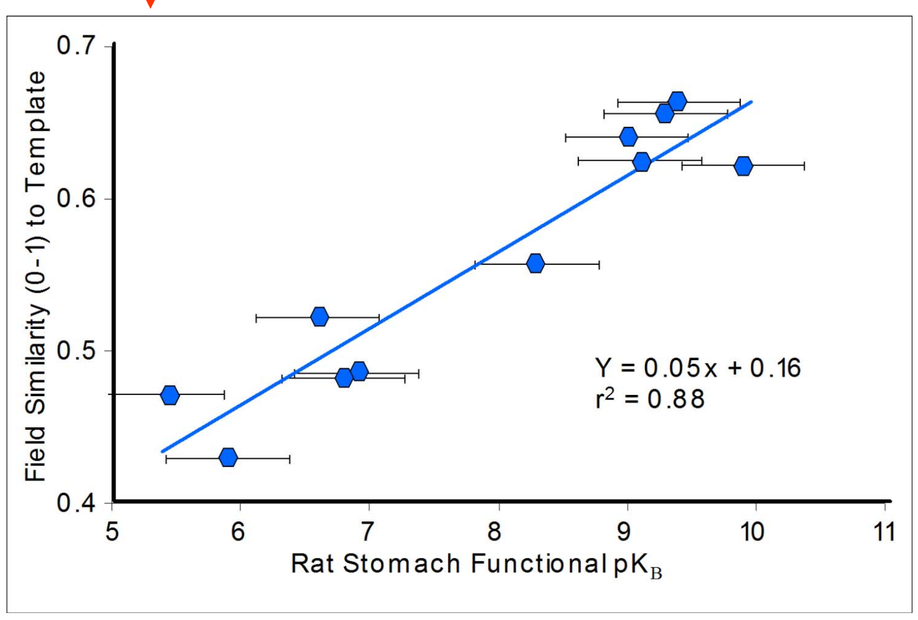
Cresset describes molecules by 4 molecular fields: electrostatic (+ve and –ve), hydrophobic and steric. The fields model the binding properties of compounds. We place fieldpoints (coloured spheres) at the sites where the fields are strongest. Molecules with similar field point patterns are expected to show similar binding characteristics to an active site, irrespective

FieldTemplater

FieldTemplater takes 3 or more structurally-diverse 2D molecules and finds those conformations that can display a common field pattern. This pattern is an hypothesis for the binding features required for activity and the molecules' conformations are an hypothesis for their bioac-

FieldAlign

FieldAlign takes 2D molecular structures and aligns them to a 3D reference molecule (here, the CCK2 template) using molecular fields. A similarity value is generated for each molecule's field to the template field. The more similar the molecular fields, the higher the similarity value (1 = identical).



Note: It is advantageous to use highly active compounds to create the template, since they have the optimal field for activity. Use of poorly active compounds will result in a poorly predictive model.

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Additional Validation of the CCK2 Template Model

We used the homology model of Anders et al [3] of CCK2 with its docked peptide agonist ligand, CCK8 (Asp-Tyr(SO3)-Met-Gly-Trp-Met-Asp-Phe(amide)),to validate our CCK2 template model. It has been shown that only the last 4 residues of CCK8 are essential for activity.

Validation Work Flow

Extract CCK8 from the homology model of the protein-ligand complex. This is our reference molecule for FieldAlign

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Use FieldAlign to align molecules 1 to 3 (used to create the CCK2 template) onto the CCK8 molecular field, starting from their 2D structure.

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Analyse the conformation and location on CCK8 of these 3 molecules

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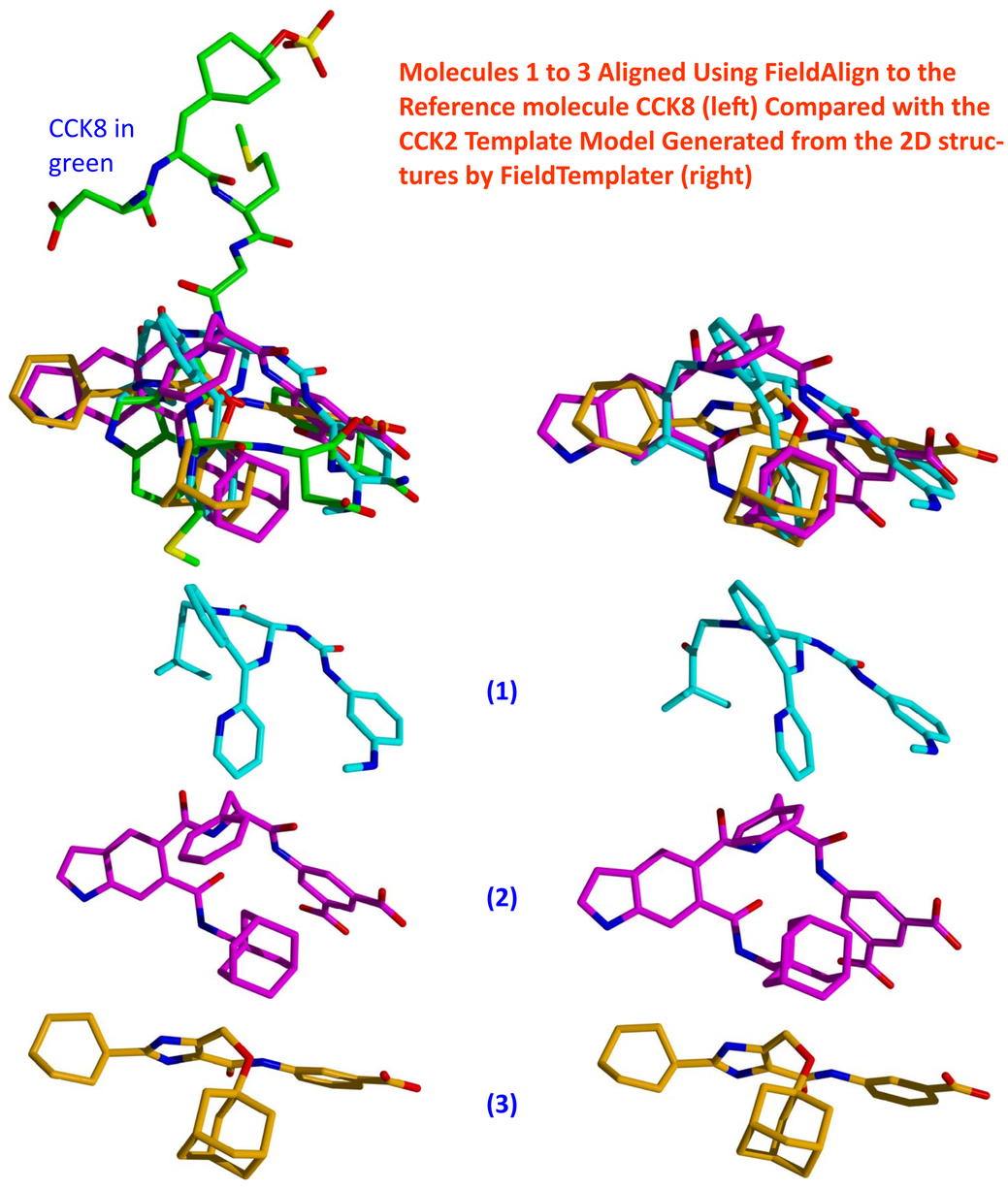
If our template model agrees with the homology model, then the 3 molecules should all (a) adopt highly similar conformations to those found by FieldTemplater and (b) align with the last 4 residues of CCK8.



Molecules 1 to 3 Aligned Using FieldAlign to the Reference molecule CCK8 (left) Compared with the CCK2 Template Model Generated from the 2D structures by FieldTemplater (right)

Conclusions

1. A CCK2 template model for activity has been constructed from 3 active compounds, starting from their 2D structure, by looking for a set of conformations in which they all display a common field pattern.
2. The template correctly predicts the activity of a set of test compounds using a field similarity measure.
3. The conformation of molecules 1 to 3 in the template is highly similar to the conformation they adopt on alignment with the CCK8 ligand from the CCK2 receptor homology model. This adds credence to the validity of our template model.
4. Molecules 1 to 3 all align with the last four residues of CCK8 which corresponds to the region of the peptide essential for activity, providing further evidence that the template model may indeed represent their bioactive conformation.

References

1. T. Cheeseright et al, J. Chem. Inf. Model.; **46**, 665-676 (2006)
2. I.M. Buck et al, J. Med. Chem.; **48**, 6803-6812 (2005)
3. J. Anders et al, Biochemistry, **38**, 6043- 6055 (1999)