Fragment Fields in Drug Discovery

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Introduction

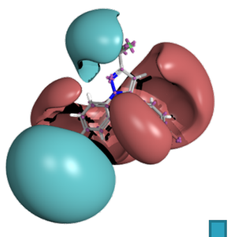
Cresset's field point descriptors are widely used for molecular alignment and similarity scoring[1,2]. The similarity scoring uses a field sampling technique, in which the field extrema from one molecule are used as sampling points into the analytically-calculated field of the other molecule.

One of the many benefits of this approach is that it provides a very compact information rich representation of molecules. More importantly, in moving from drug sized molecules to fragments this information content does not degrade as quickly as other 3D or 2D methods.

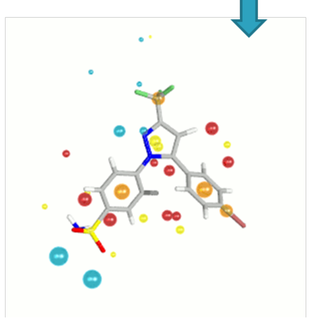
In this study we illustrate the benefit of using fields in the context of fragment based drug discovery.

Fields

The field points of a molecule are generated by locating the points in space where the electrostatic and steric fields of a molecule are locally maximal. Each such point is labelled with the strength of the field at that point. We use four fields: positive and negative electrostatic, "shape" (van der Waals), and "hydrophobic" (a density function correlated with steric bulk and hydrophobicity).



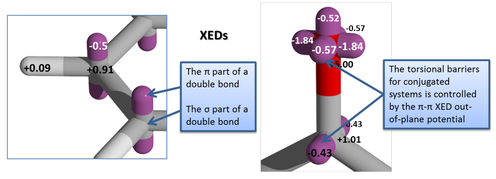
3D Molecular Electrostatic Potential (MEP)



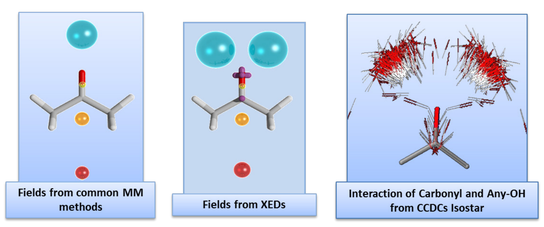
Field Points

The XED Force Field

To generate good field patterns, it is necessary to use a force field with a more complex electrostatic model. The XED force field, first developed by J. G. Vinter[3] in 1994, puts additional point electrostatic charges around atoms with pi orbitals and lone pairs.

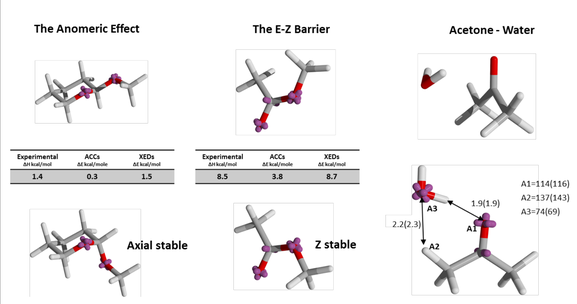


This 'multipole' approach offers advantages over atom centred charge (ACC) monopole based molecular mechanics - providing a better match to observed data.

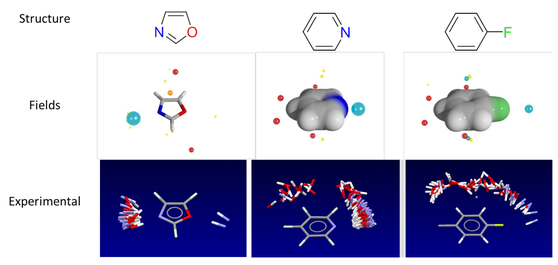


XED's on Fragment's

Application of the XED force field provides greater accuracy[4,5] compared with conventional molecular mechanics:



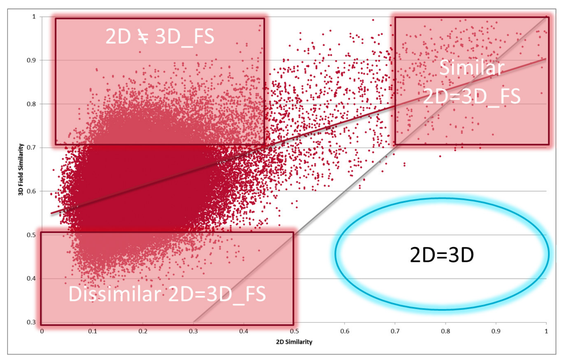
Irrespective of size, for instance when choosing to model very large (protein), medium (drug) or small (fragment) systems:



Fragment Library Analysis

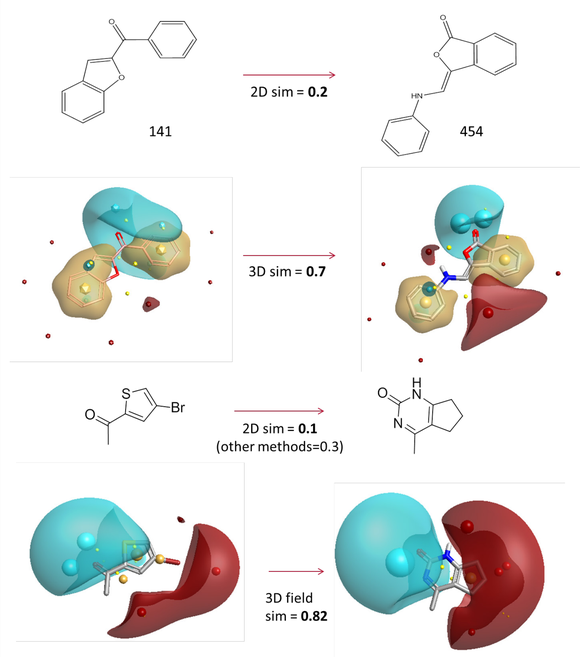
The analysis of fragment libraries is challenging since conventional metrics for determining diversity/similarity generally use summed features eg. feature encoded bit strings as a means of comparing molecules.

A set of 7,907 diverse RO3 compliant fragment library from Bionet was analysed and an all-by-all similarity matrix was determined. The plot 2D v 3D (Field) similarity is shown below.



On the whole, the library is indeed diverse using both 2D and 3D field metrics - yet a significant proportion of the library has very low 2D similarity and high 3D field similarity (3D_FS).

Two example fragments below illustrate this interesting and potentially very useful result.

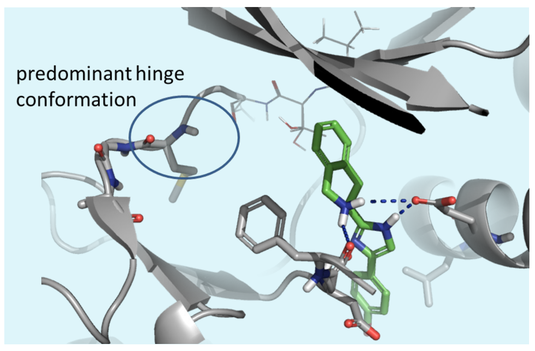


Exploitation of such examples in either increasing fragment library diversity via their removal, or enriching fragment hit IP via their specific employment - should add value.

Protein Kinase P38

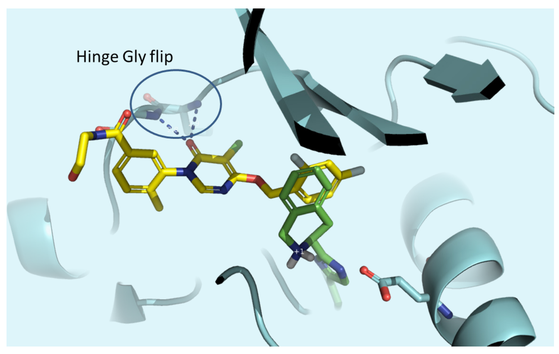
Map Kinase P38 is a protein kinase with a key role in the MAP kinase signal transduction pathway and is implicated in a number of inflammatory diseases including IBS, Crohns disease and rheumatoid arthritis. P38 has thus received much attention as a target with potential for the treatment of these conditions.

Several X-ray structures of bound inhibitors are known for p38 and together they reveal a particularly plastic ATP binding site. Below is an example of a fragment bound into the DFG-out conformation of P38 (PDB:3K3I).



A great deal of interest has been focused on accessing inactive states of kinase enzymes such as the DFG-out forms. However, selectivity in the kinase area remains a constant challenge.

A further illustration of the plasticity of P38 is shown below in an example inhibitor complex exploiting a Gly flip at the backbone hinge region close to the Thr gatekeeper (PDB:3ROC and 3HUB*).

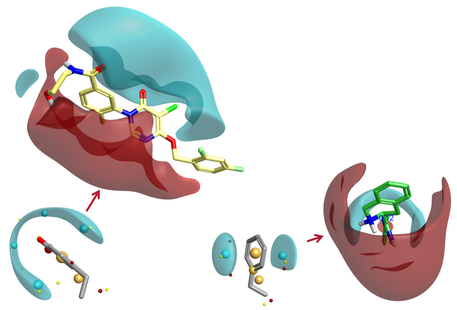


It is thought that an enhancement in selectivity towards P38 is achieved by this relatively rare residue pairing which in turn allows this specific conformation to be adopted.

Superimposed in this picture is the DFG out ligand fragment from the previous example (in green). A potentially useful activity/selectivity profile might be obtained by combining both DFG-out and Gly flipped modes of inhibitor binding. To this end, we embarked on a virtual exercise using our automated fragment replacement tool Fieldstere.

P38 Inhibitor Field Analysis

To address the feasibility of connecting these two binding modes in a single entity a field analysis was conducted on these two and related inhibitors*.



Gly rich loop Tyr from PDB:3HUB*

DFG-out Phe from PDB:3K3I

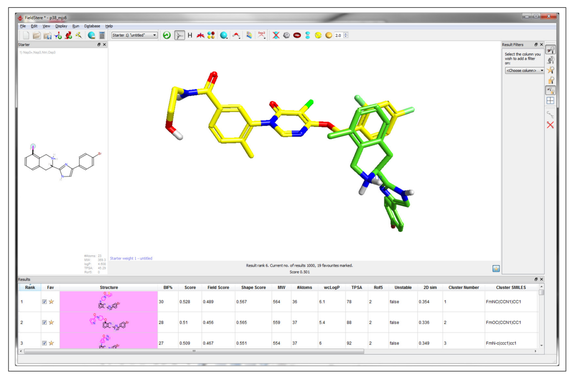
The positive patches (red surface) in both are consistent with the adoption of DFG-out Phe and Glycine rich loop Tyr residue positions found in these alternative loop configurations; both of which provide complementarity electron density to the Pi face (blue surface) from these two key residues.

*a closely related p38 inhibitor complex with alternative Gly loop

Fragment Expansion with FieldStere

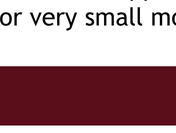
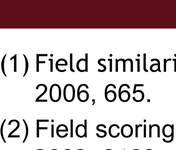
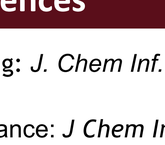
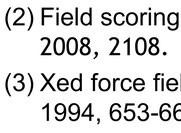
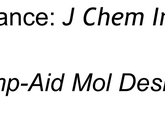
Having established that the two fragments were compatible, both could be used as reference molecules in a FieldStere project:

- The DFG-out ligand from PDB: 3K3I as 'seed'
- The flipped hinge ligand PDB: 3ROC as the reference to be scored against
- Scoring weighting 80:20 Ref:Seed.



P38: FieldStere results

Example output molecules from this fully automated method, are shown in the table below:

Structure	Rank	Sim	Structure	Rank	Sim
	1	0.53		11	0.49
	4	0.51		13	0.49
	6	0.50		53	0.48

All the examples would be predicted to be both 'DFG-out' and 'hinge flipped' P38 inhibitors and thus likely to be highly selective and inactive state targeted.

The electrostatic fields of the compound ranked '1' is shown against the two combined fields of the 'seed' and 'reference' below:

Conclusions

Cresset's Field based software is an extremely useful enabling technology for drug discovery; with wide applicability for use with either large or very small molecules

References

(1) Field similarity scoring: *J. Chem Inf. Mod.,* 2006, 665.

(2) Field scoring performance: *J Chem Inf Model*, 2008, 2108.

(3) Xed force field: J *Comp-Aid Mol Design*, **8**, 1994, 653-668.

(4) Anomeric effect: *J Comp Chem* **17,** 1996, 429-449.

(5) Acetone water: *Science* **257** 942, 1992.

Acknowledgements

Thanks to Andy, Mark, Tim and Rob for amusing discussions, support, pictures and access to excellent software.

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