Using Molecular Fields to Determine the Bound Conformation of Ligands in the Absence of X-ray Crystallographic Data

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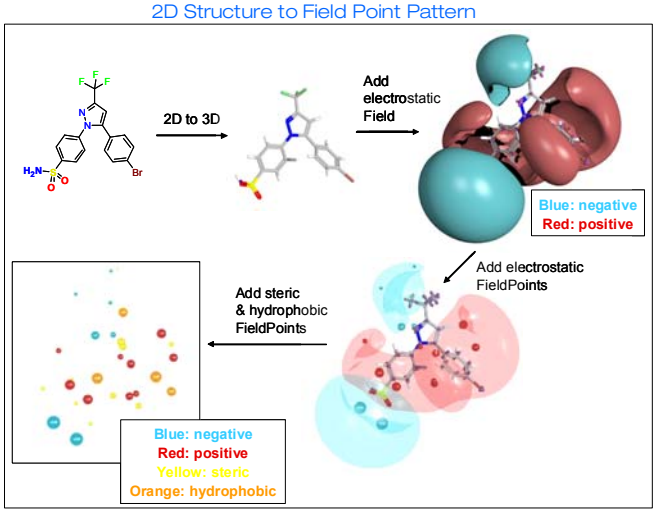
Background to Molecular Field Technology

Chemists are used to visualising compounds by considering their 2D or 3D atom and bond structure. However, this may not be the most appropriate way to compare compounds and interpret protein binding and SAR data. Proteins interact with electrons, not atoms, so we have developed technology to visualise compounds in terms of their electrostatic Fields [1]. Steric and hydrophobic fields are also calculated to give a more complete physicochemical description of molecules. Fields model the binding properties of a compound and explain why compounds with different structures can bind to the same biological target. Fields have been used for virtual screening [2] to identify novel hits with the same Field pattern as a known active. No protein structure is required, so targets can be tackled which are not appropriate for virtual screening using docking studies, such as GPCRs and ion channels.

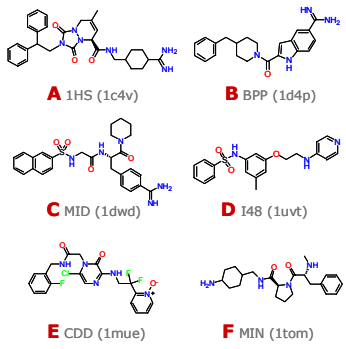
2D Structure to Field Point Pattern



References
1. Cheeseright, T.; Mackey, M.; Vinter, A., *DDT BioSilico*, **2004**, *2*, 57-60.
2. Cheeseright, T.; Mackey, M.; Rose, S. and Vinter, A., *J. Chem. Inf. Model.* (accepted for publication November 2005)

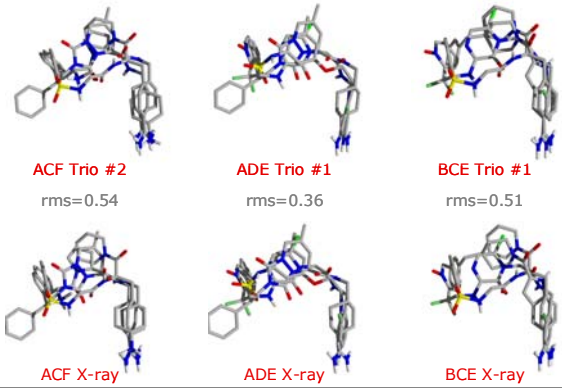
Thrombin Inhibitors Results

6 compounds with x-ray data on the protein-ligand pair were used.
Trio templates were found for 19 of the 20 possible trios.
The best trio (with respect to closest field fit; rank = 1) was also the closest fit to the x-ray data in 6 cases.
The best fit to the x-ray data was consistently found in the top few ranked trios where a good RMS fit was found (<1.3A).



Trio	Rank	RMS
A-D-E	1	0.36
A-C-D	1	0.50
B-C-E	1	0.51
A-C-F	2	0.54
A-E-F	5	0.57
A-B-C	2	0.59
C-E-F	3	0.65
A-B-F	3	0.76
C-D-F	3	0.72
A-B-E	1	0.73
B-E-F	1	0.85
B-C-F	1	0.96
B-C-D	8	1.76
A-D-F	23	2.45
A-B-D	72	2.52
D-E-F	63	2.66
C-D-E	13	2.76
B-D-F	7	2.82
B-D-E	2	4.50

Thrombin examples of 3 trio overlays compared to the x-ray structures



Generation of a Bound Conformation Model

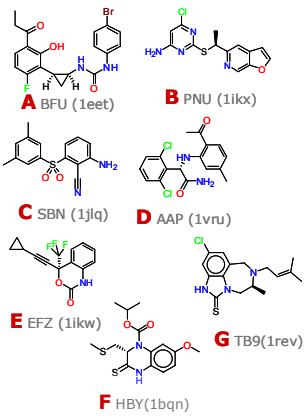
Field technology has been extended to generate bound conformation hypotheses for small sets of diverse ligands binding at the same site. Fields model the binding interactions of a ligand, so compounds that bind at the same site must be able to adopt conformations in which they all display the same field pattern to the target. Therefore we need to identify those conformations and find the common pattern. Accurate bound conformation models can increase understanding of SAR and reduce lead optimisation times. This is obviously valuable for targets which lack x-ray crystallographic data. Bound conformation hypotheses ('Trio Templating') have been generated for thrombin and NNRTI HIV inhibitors and validated by comparison with x-ray data from protein-ligand pairs.

Trio Templating: Validation Protocol

- Take 3 molecules (A, B C) and generate up to 100 conformations of each (A1 to A100, B1 to B100, C1 to C100)
- Combine all pairwise alignments (Ai on Bj, Bi on Cj, Ci on Aj for i,j=1-100) and find 'templates' i.e. field patterns common to all 3 molecules e.g A20,B5,C89
  A template is generated if A, B and C overlay within defined constraints
- Optimise, then score and rank each template by field pattern similarity
  Most similar overlays (templates) have highest score (rank = 1)
- Compare each molecule in each template to the 'true' answer found from aligning PDB x-ray structures
  Average heavy atom RMS < 1.3A = Correct
  Average heavy atom RMS > 2.0A = Wrong

NNRTI HIV Inhibitors Results

7 compounds with x-ray data on the protein-ligand pair were used.
Trio templates were found for 30 of the 35 possible trios.
The best trio (with respect to closest field fit; rank = 1) was also the closest fit to the x-ray data in 14 cases.
The best fit to the x-ray data was consistently found in the top few trios.



Trio	Rank	RMS
A-B-E	4	0.57
A-B-C	4	0.71
C-D-E	1	0.73
B-C-E	1	0.74
A-C-E	1	0.76
E-F-G	2	0.90
B-E-G	5	1.04
B-C-D	7	1.06
B-D-E	1	1.14
A-E-F	6	1.19
B-D-G	1	1.21
A-E-G	4	1.22
B-E-F	1	1.26
A-C-D	2	1.26

Trio	Rank	RMS
C-E-G	1	1.31
D-E-G	1	1.32
C-D-G	1	1.42
D-E-F	3	1.57
B-F-G	1	1.61
C-E-F	1	1.67
A-B-F	6	1.88
A-B-D	9	1.93
C-D-E	3	2.34
B-C-F	6	2.36
A-D-E	1	2.60
B-C-G	5	3.00
D-F-G	2	3.14
B-D-F	3	3.29
A-F-G	1	3.76
A-D-F	1	4.00

HIV examples of 3 trio overlays compared to the x-ray structures