June 2011 Chicago

Ligand fitting with Coot

..and more?

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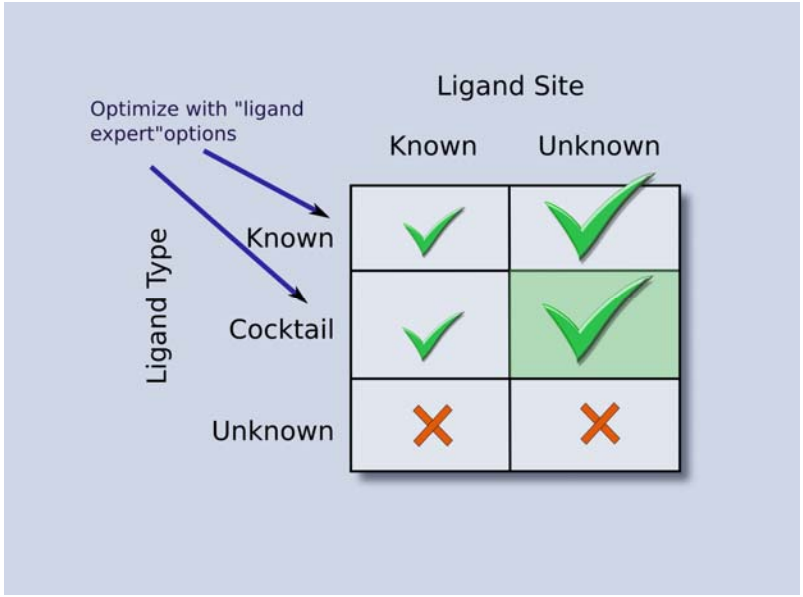
Fitting Ligands



Ligand Fitting

- *c.f.* Oldfield (2001) *Acta Cryst. D* X-LIGAND
- Somewhat different torsion search algorithm
- Build in crystal-space

REFMAC Monomer Library
chem_comp_bond

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loop_
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_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
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_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_esd
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 ALA      N    CA      single     1.458    0.019
 ALA      CA   HA      single     0.980    0.020
 ALA      CA   CB      single     1.521    0.033
 ALA      CB   HB1     single     0.960    0.020
 ALA      CB   HB2     single     0.960    0.020
 ALA      CB   HB3     single     0.960    0.020
 ALA      CA   C       single     1.525    0.021
 ALA      C    O       double     1.231    0.020
```

REFMAC Monomer Library
chem_comp_tor

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_chem_comp_tor.atom_id_3
_chem_comp_tor.atom_id_4
_chem_comp_tor.value_angle
_chem_comp_tor.value_angle_esd
_chem_comp_tor.period
 TRP   chi1  N   CA  CB  CG  180.000   15.000   3
 TRP   chi2  CA  CB  CG  CD1  90.000   20.000   2
```
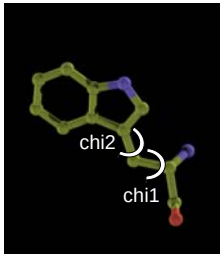
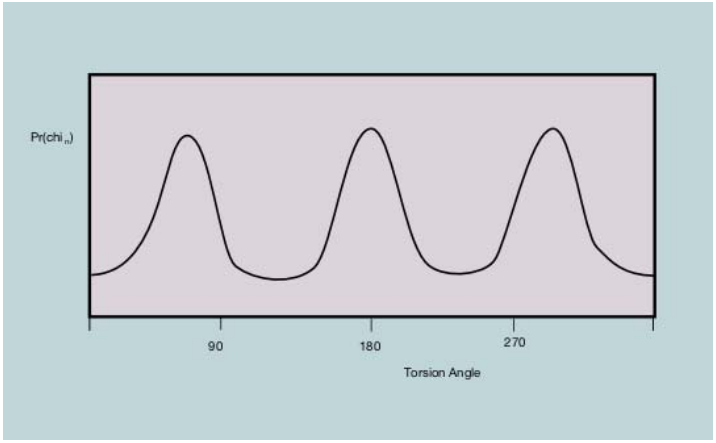
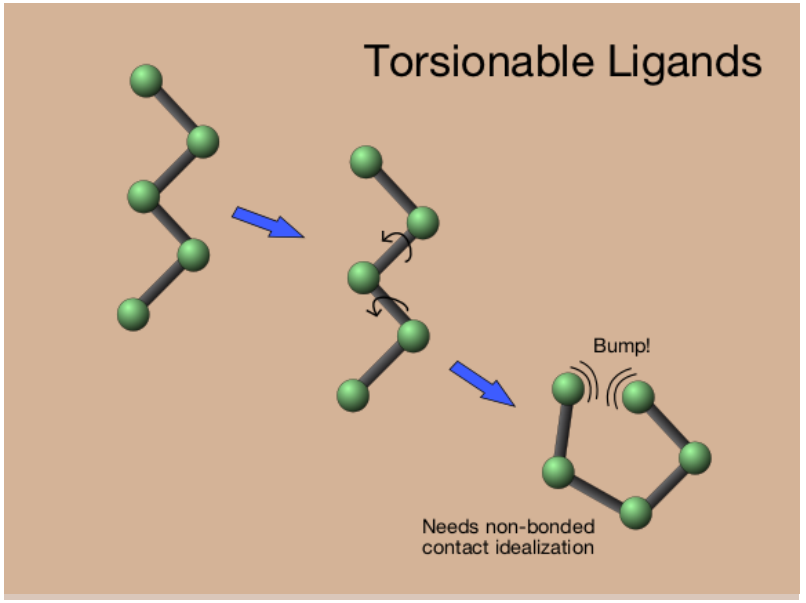



Ligand Torsionable Angle Probability from CIF file



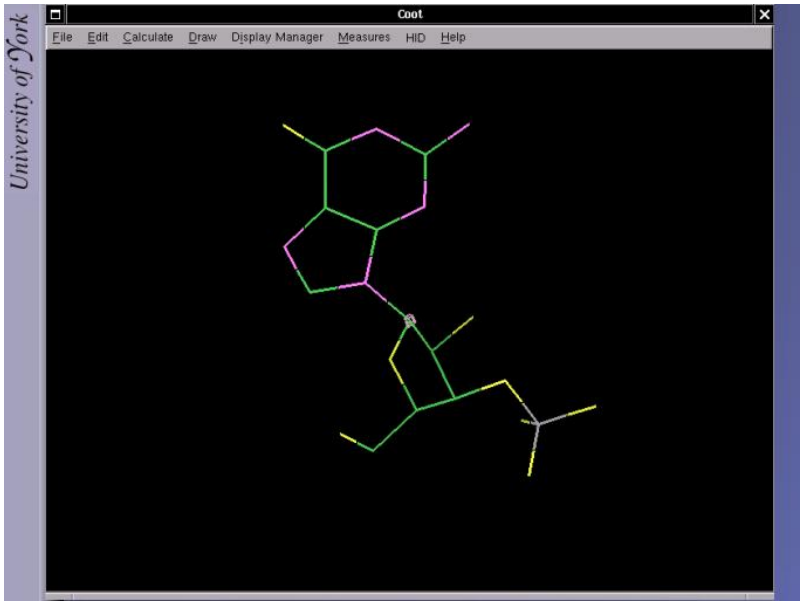


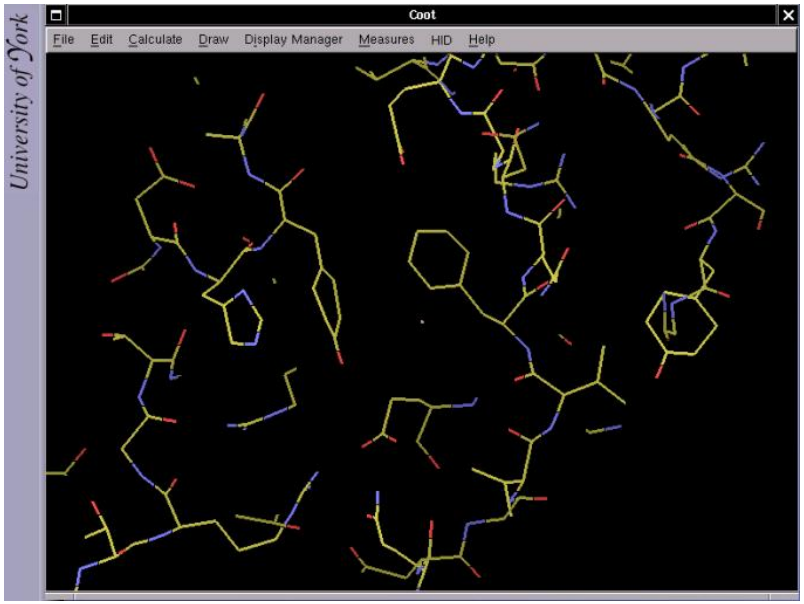
Crystal Space

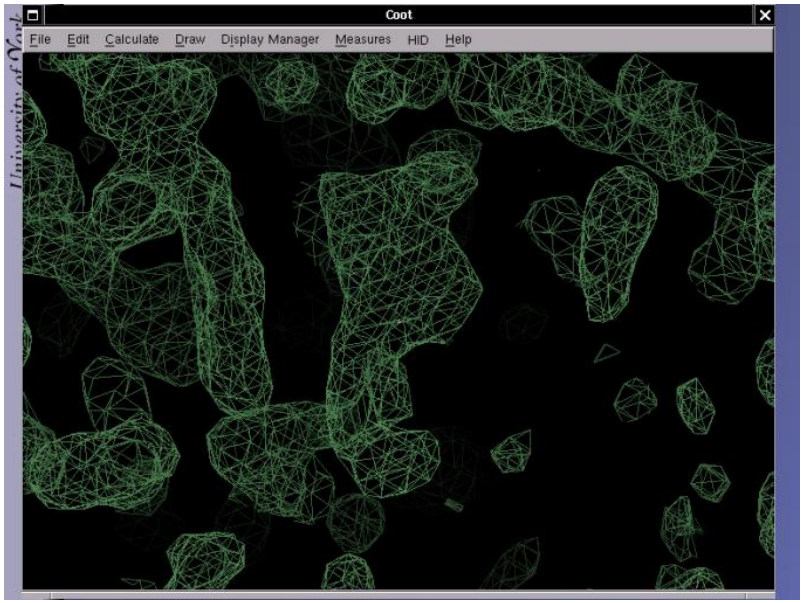
- Build in “crystal space”
  - Like real-space, but wrapped by crystal symmetry
    - Like “Asteroids”
  - Assures only one real-space representation of map features
    - Build everything only once
    - No symmetry clashing
  - However, more difficult to calculate real space geometries
    - …such as bonds, torsions

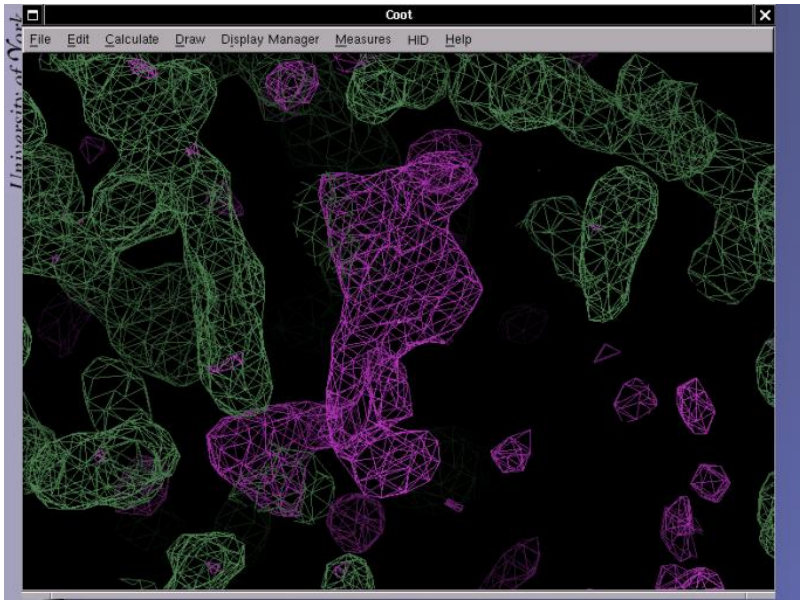
Crystal space

- Building in crystal space is good:
  - We don’t need to define where the protein is and create an extended map that surrounds it
  - We don’t have to worry about the relative position of the ligand and the protein
    - Unknown “BORDER” parameter
  - We find (and fit) each site exactly once
    - No symmetry problems



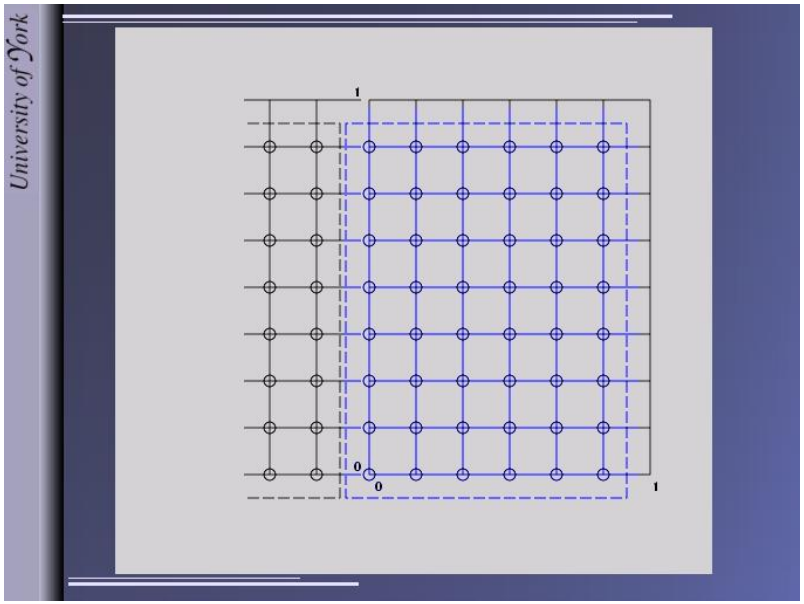


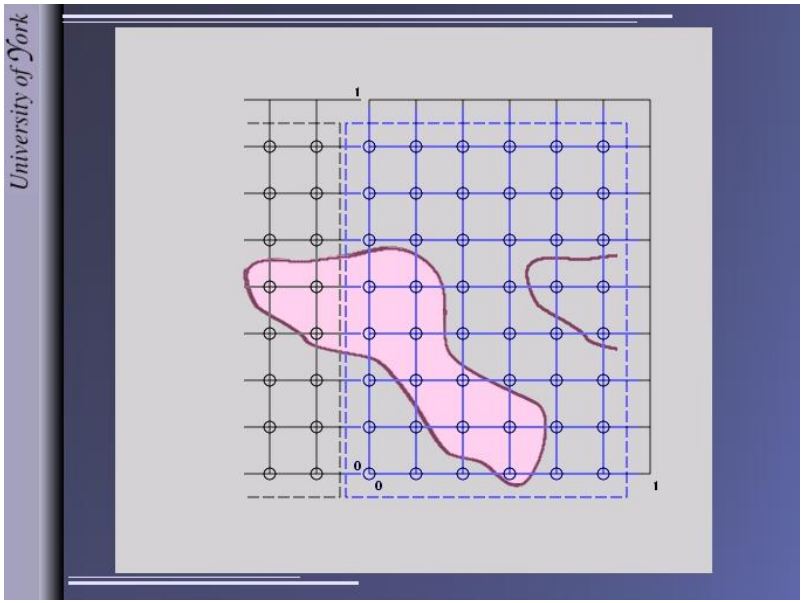




Clipper Map Mapping

- Clipper maps
  - Appear to be “infinite”
  - Density value can be queried anywhere in space

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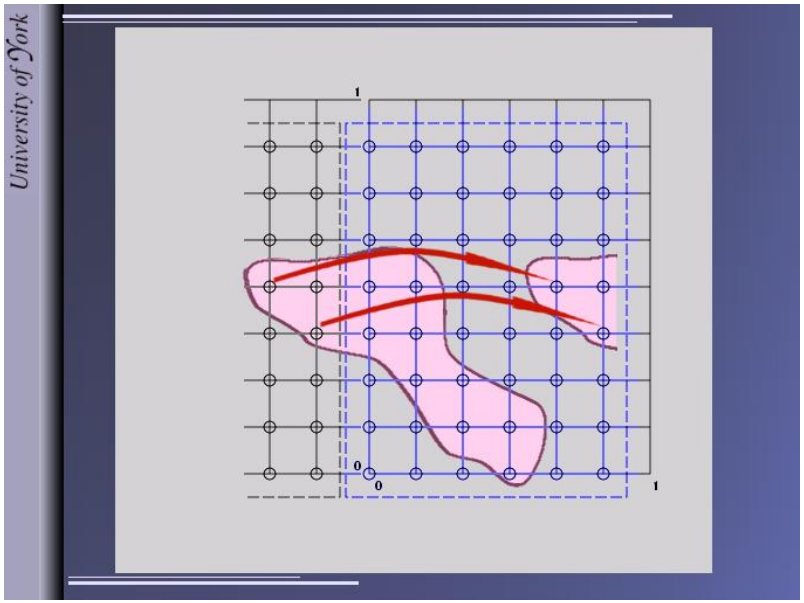
0
1


University of York
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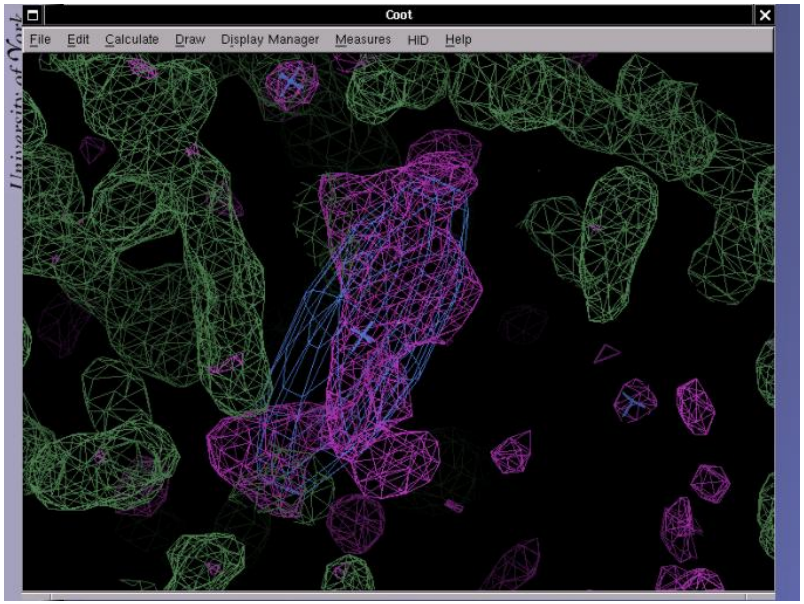
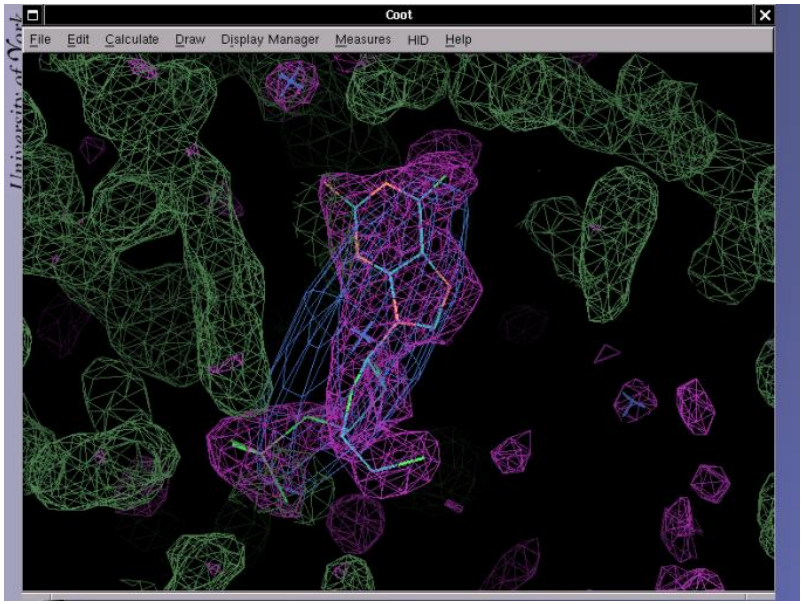
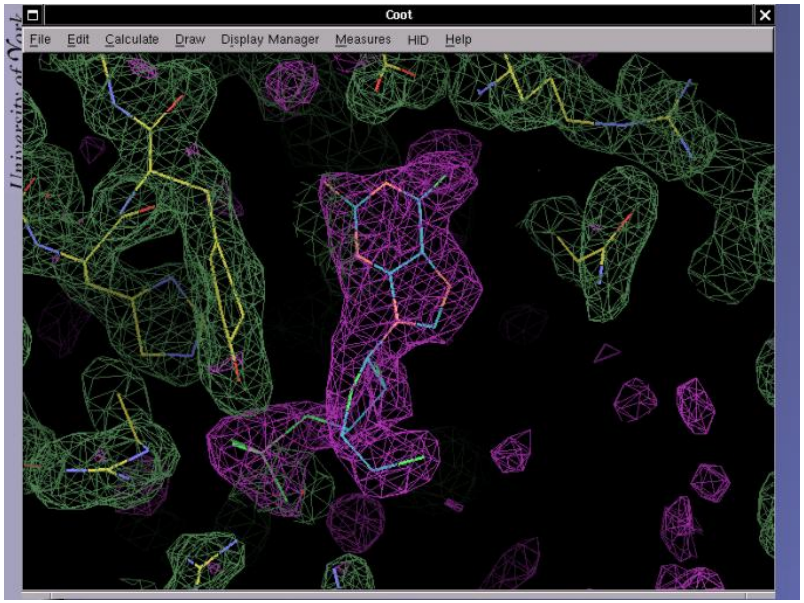

Coot
File Edit Calculate Draw Display Manager Measures HID Help


Coot
File Edit Calculate Draw Display Manager Measures HID Help



Conformation Idealization

- Each conformer is passed through the “Regularization” function of Coot
  - Non-bonded terms included
- Better to have hydrogen atoms on the model
- Slows things down a good deal…
  - May not be the best method to explore conformational variability for many rotatable bonds

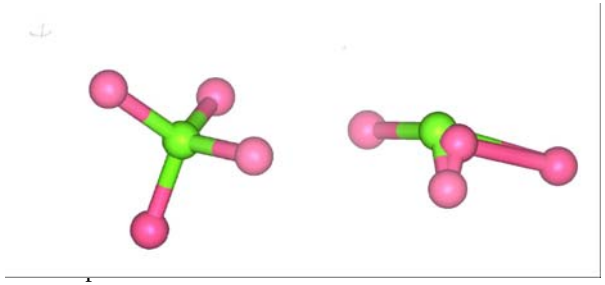
Ligand Overlay

- Algorithm and Code by Eugene Krissinel
- Tries to overlay different ligands/monomers by graph matching
- Useful for “database” ligands where atom names are not selected by hand
- Has been used as the basis of the function which “mutates” residues to alternative monomer types
  - e.g. phosphorylation

Ligand validation

Ligand validation
Why validate?

- 70.000 structures in PDB
- 11.000 'ligands' in 50.000 structures



- 1.65 A resolution
- R/Rfree 0.15/0.19

Ligand validation

- How to validate ligand geometry?
  - Compare observed structure to restraints
    Coot: Validate->Geometry analysis

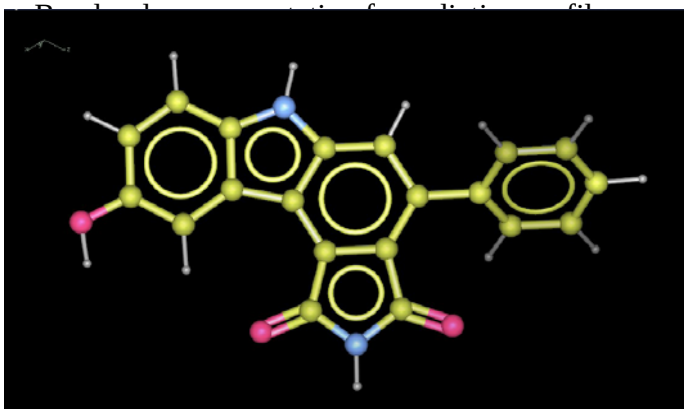
  - But what if parametrization (dictionary) is wrong?

»How to validate ligand geometry?

- QM
  - CPU hungry
  - In vacuo
  - Low energy ≠ (?) bound ligand
- PDB (e.g. ValLigURL)
  - Good cofactor structures
  - Less useful for novel ligands
  - (occ.) questionable quality
- CSD
  - Using small molecule geometries
  - e.g. Mogul

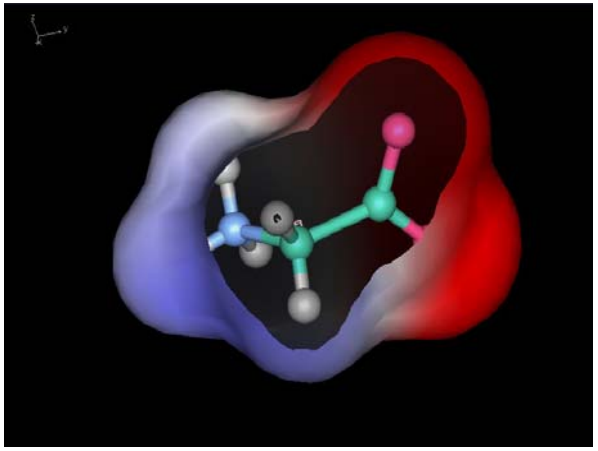
Ligand representation

Ligand representation



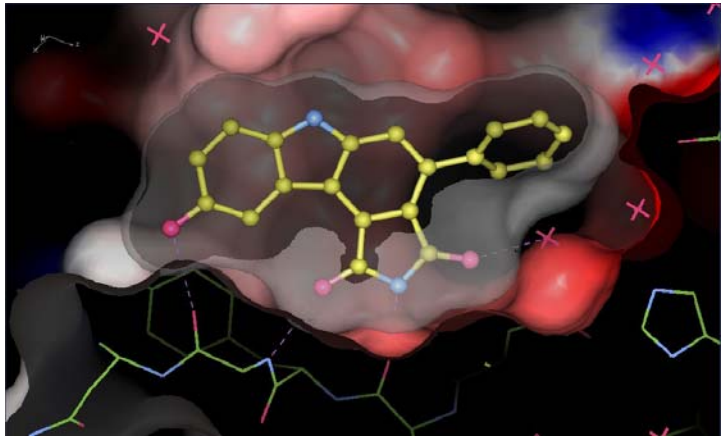
Surfaces

- Partial charges



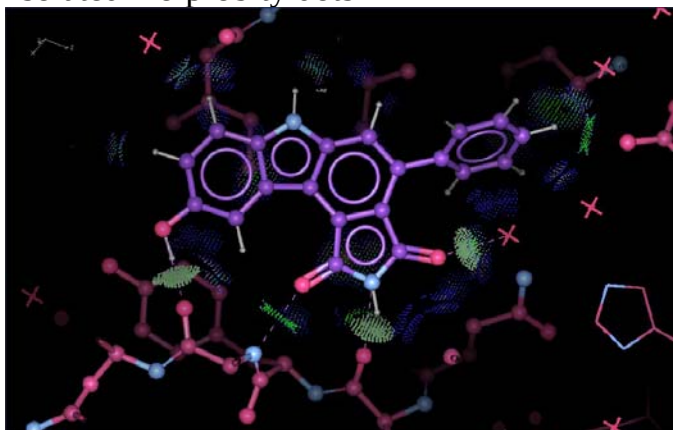
Surfaces

- Transparent surfaces – surface complementarity



Binding mode analysis

- Binding site highlighting
- Isolated molprobity dots



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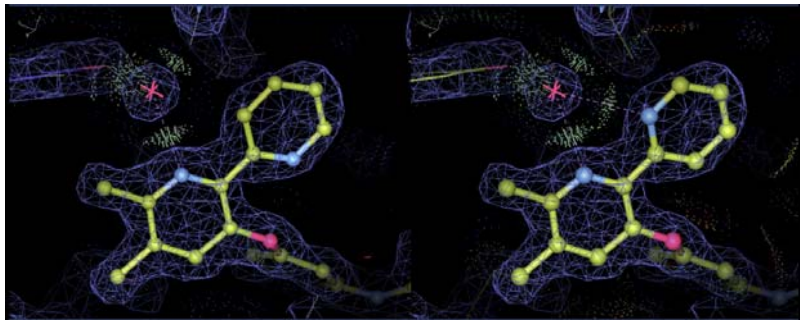
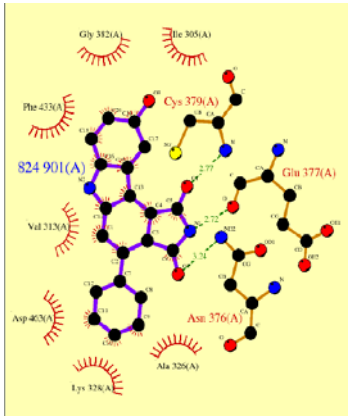
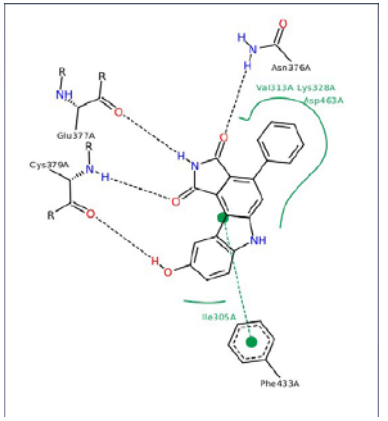


Ligand environment

- 2D ligand pocket layout (ligplot, poseview)





Ligand environment layout

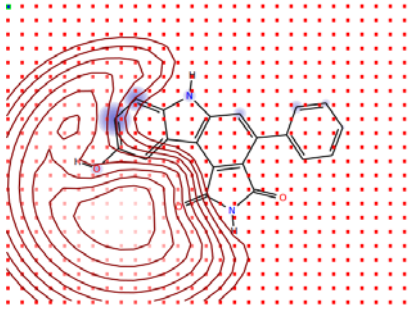
- Binding pocket residues
- Interactions
- Substitution contour
- Solvent accessible halos

Ligand environment layout

- Considerations
  - 2D placement and distances should reflect 3D metrics
    - As much as possible
  - Residues should not overlap the ligand
  - Residues should not overlap each other
  - H-bonded residues should be close to atoms to which they are bonded
  - Etc.
  - c.f. Clark & Labute (2007); poseview

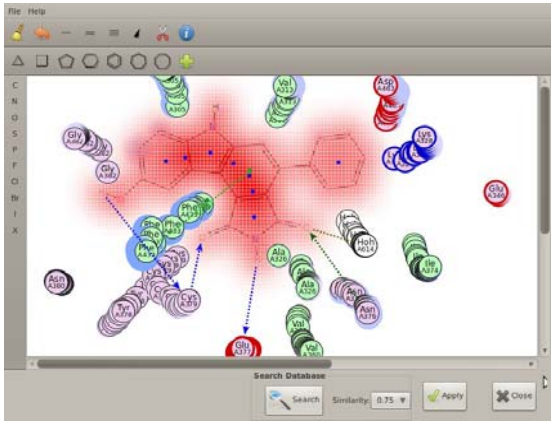
Ligand environment layout

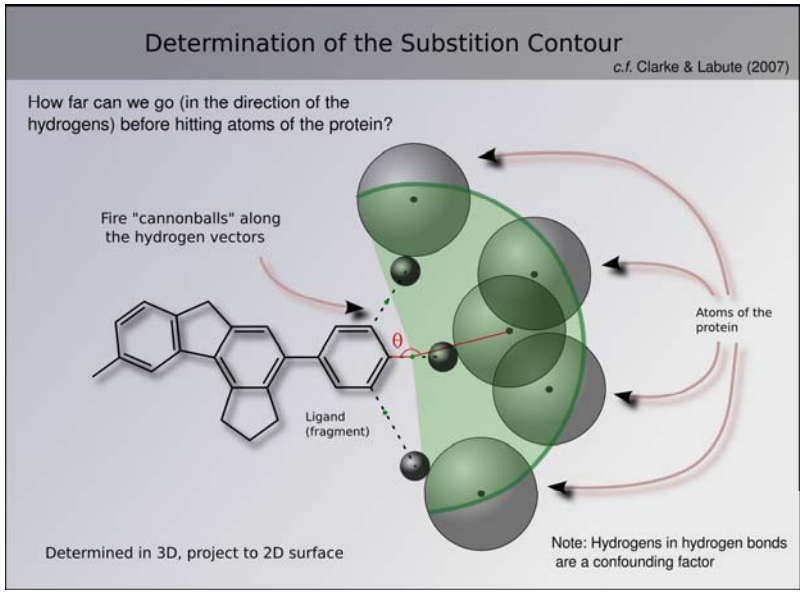
- Initial residue placement



Ligand environment layout

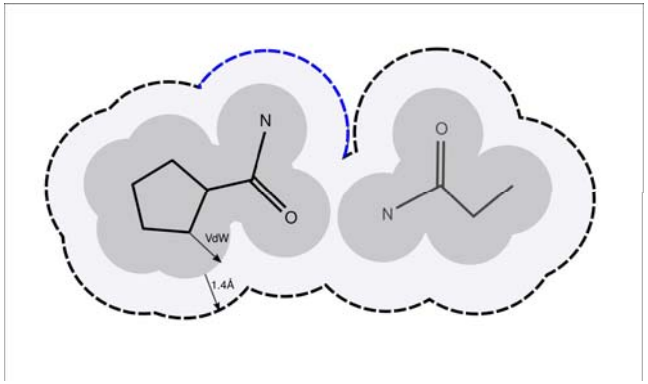
- Residue position minimization

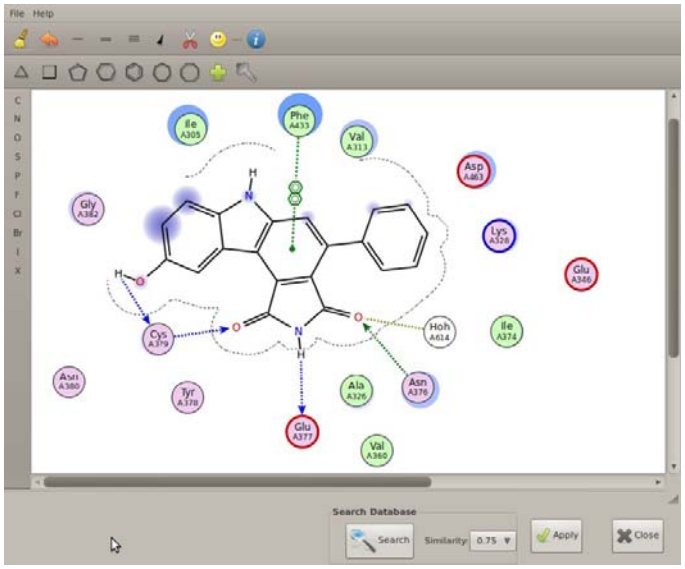




Solvent exposure calculation

- Identification of solvent accessible atoms
- Different from substitution contour





What is Non-Crystallographic Symmetry?

- 2 or more copies of a molecule in the unit cell not related by crystallographic symmetry
- Crystallographic copies of molecules are (of course) treated as if they were exactly the same across the unit cell – and indeed across the whole crystal
- Non-crystallographically related molecules provide different representations of the same molecule
  - This can be useful for model-building
  - But difficult to use in practice

Handling NCS...

Handling NCS

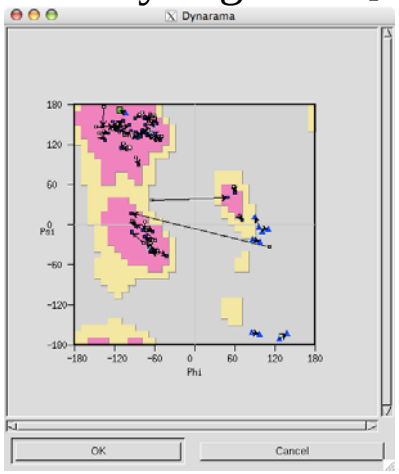
- What are the Problems?
- Strict NCS:
  - NCS should appear like crystallographic symmetry does [exact copies]
- Non-Strict NCS:
  - Molecules are different
  - How to cope with differences, but minimize unnecessary rebuilding?

Handling NCS

Typical Scenario:

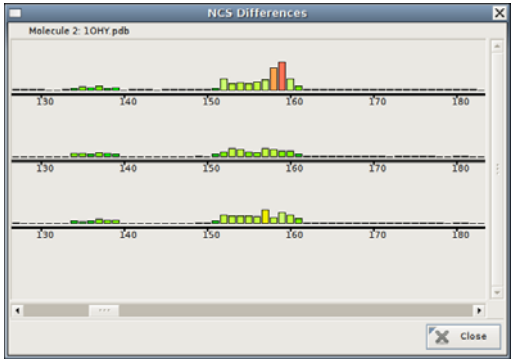
- I have done an LSQ overlap of my NCS-related molecules and from the graph, have seen significant deviations in the positions of some side-chains.
- Why are they different?

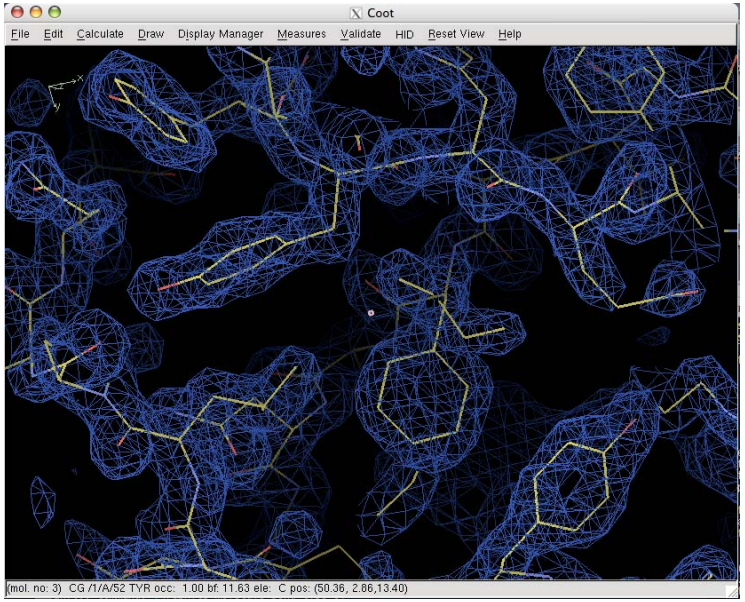
…or Kleywegt Plots[*]

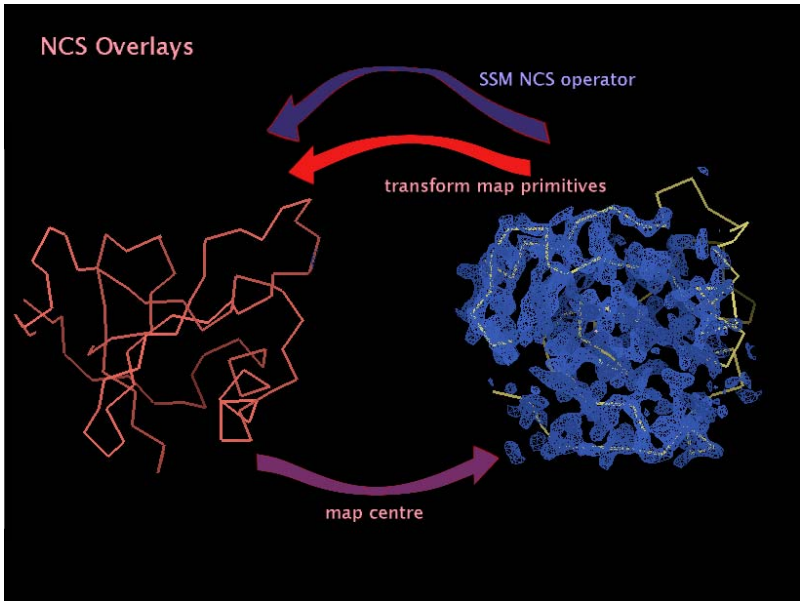


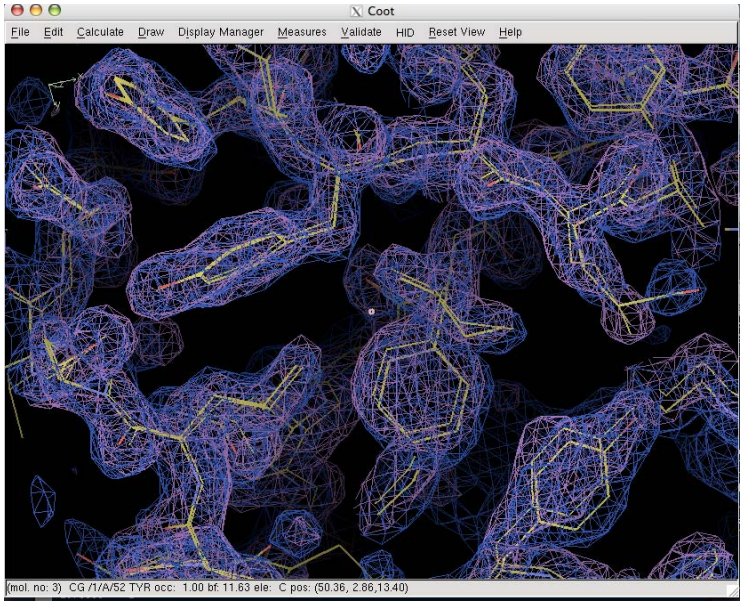
[*] Named by George Sheldrick

...or new NCS Differences graph







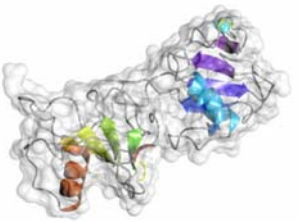


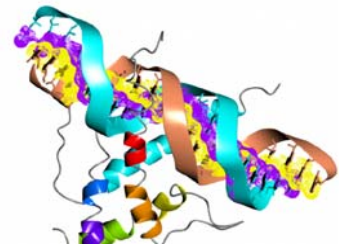
NCS Model-modification Tools

- Automatic detection of NCS
  - And their operators
- Copy Master NCS molecule to others
  - Applies NCS transformation
- Copy NCS Master residue-range
- Change NCS Master chain
- NCS Skipping

CCP4MG

- New CCP4MG (v. 2.5)
- Easy to use
- Picture wizard
- Annotations
- PISA interface
- Movies
- Online updates





http://www.ysbl.york.ac.uk/~ccp4mg/

Acknowledgements

- Paul Emsley
- Kevin Cowtan
- Eleanor Dodson
- Keith Wilson

http://www.biop.ox.ac.uk/coot/

or

Google: Coot

or for WinCoot

http://www.ysbl.ac.uk/~lohkamp/coot

- Libraries, dictionaries
  - Alexei Vagin, Eugene Krissinel, Stuart McNicholas
  - Dunbrack, Richardsons
- Coot Builders and Testers
  - William Scott, Ezra Peisach
  - York YSBL, Dundee, Glasgow (early adopters)
  - Coot Mailing List subscribers