Model-Building with Coot
An Introduction

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Coot

- Molecular Graphics application
  - Protein Crystallographic model-building tools
    (Crystallographic Object-Oriented Toolkit)
  - Aim: “Slick and powerful” interface to CCP4

- Interface to other programs: SHELXL, Refmac, Libcheck, Probe&Reduce (Molprobity), EBI, EDS, Povray, Raster3D, PHENIX

- Several model-building and validation tools
Feature Integration

Refinement

- External
  - e.g. REFMAC

- Internal

Validation

- Internal

- External
  - e.g. MolProbity
Real Space Refinement


- **Major feature of Coot**
  - Gradient minimizer (BFGS derivative)
  - Based on mmCIF standard dictionary
  - Minimizing bonds, angles, planes, non-bonded contacts, torsions, [chiral volumes]

- Provides “interactive refinement”
- Different minimizer to Refmac…
  - …means “nice & tight” geometry
    - Chi squareds

Faster & Animated
Refinement “Traffic Lights”

“Traffic Lights” represent the chi-squared values for each of the refined geometry types.
Refinement Techniques

- Auto-zone
- Single-Atom Drag
- Over-dragging
- Ramachandran Refinement
- Sphere refinement
- Coming Soon..?
  - Dials, PowerMate, spaceballs
  - Wii Refinement
Generic Objects

- A generic object can be any set of coloured lines or points
- Mechanism to display and close
  - (not “clickable”)
- Generic Objects from .vu files
- MAPMAN “Bones”
- MolProbity Dots
Some more Coot Tools...

- Alternate Conformations
- Ligand fitting
- Rigid-body Fitting
  - Steepest Descent
  - Simplex (slower but better)
- “Move Molecule Here”
- Water Search
Symmetry generation re-written
Electron Density: “Continuous Crystal”
Rotamer Database Molprobity Option

Select Rotamer

1: 47.08% Chi_1 = -1
2: 31.71% Chi_1 = -1
3: 11.06% Chi_1 = -1
4:  8.17% Chi_1 = -1

Accept  Cancel
Other Tools

- Reverse chain direction
- 180° side-chain flip
- Planar peptide restraints
- “Chi” angles for ligands
- Dots, ball&stick
- Fill-partial-residues (de-chainsaw)
Chainsawing

Model-trimming

VAL -> LEU by sequence alignment

Schwartzzenbacker et al. (2004) Acta Cryst D60 1229
Least Squares Fitting

- Least Squares Superposition:

![Least Squares Superposition](image)
Low Resolution Tools
Extra Restraints....
Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane

Extended Plane in Coot

(add-planar-peptide-restraints)
Ramachandran Restraints

- **Scenario:**
  - I have a loop, with poor density, I know the atoms are there somewhere and I want to provide a “reasonable” model.

- **Controversial Feature?**
  - Ramachandran Plots have been used for “validation” - but here we are deliberately optimizing them.

- Ramachandran Plots can be added to the geometry target function.
Tweaking a Ramachandran Outlier
Tweaking Phi and Psi
Controversial?

“... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model...”

Gerard Kleywegt & Alwyn Jones (1996)

But to quote Jane Richardson:

Do you want a better structure – or a better idea of the quality of your structure?
Adding Torsion Angle Restraints

- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”

- Add Pseudo-bonds
Restrain the Hydrogen-bonding atom distances
Helix-Building
**Alpha Helix Placement**

- **Scenario:** Looking at a new map, not built with automatic tools:
  - “I can see that there’s a helix here - build it for me!”

- **From a given point:**
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D Rotation search to find best fit
  - Score based on density at CB positions
  - Trim ‘n Grow
Centering the Rotation point
Cylinder Search

Pick the orientation that encapsulates the most electron density

2 orientation axes
2 x 1-D Helix orientation searches
All search models
(for the “up” orientation)
Fitting Strands
Placing Strands

- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures
Strand fitting algorithm

- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution
Strand Translation Search (along the tube axis)
Strand Rotation Search
(around the tube axis)
Not all is rosy...
Fitting Strands caveat

- In the case of strand-fitting, the initial translation search centring the cylinder is not performed (the search cylinder is too thin)

- The user is responsible for centring the search point “in the middle of the tube”

- Not at a C-alpha position
Handling NCS...
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

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
Coot Futures...

- **Aim:**
  - Slick, easy to use
  - Powerful
  - Smooth interface to external applications

- **Under Development**
  - Interesting things move quickly
  - There may be bugs