Model-Building of Proteins Using X-ray Data With Coot

Paul Emsley
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Which coot?

- Coot-0.7.1-pre revision 4600 (or so)
- Linux:
  - [http://www.ccp4.ac.uk/coot/nightlies/pre-release/](http://www.ccp4.ac.uk/coot/nightlies/pre-release/)
- Mac OS X
  - Bill Scott's Nightlies
- Windows
  - Bernhard Lohkamp's WinCoot
- Tutorial Data:
  - lmb.bioch.ox.ac.uk/coot/tutorial
    - get *ligand* files
Modelling Proteins with Coot

- Overview
  - Low resolution side-chains
  - Tools for EM
  - Tools for NCS
Acknowledgments, Collaborators

Kevin Cowtan  
Eugene Krissinel  
Stuart McNicholas  
Martin Noble  
Alexei Vagin  

Bernhard Lohkamp
Feature Integration

Refinement

External

Internal
e.g. REFMAC

Validation

External
e.g. MolProbity

Validation, Model Building and Refinement should be used together
Real Space Refinement

- Major Feature of Coot
  - Gradient-based minimiser (BFGS derivative)
  - Geometry library is the standard CIF-based Refmac dictionary
    - Minimise deviations in bond length, angles, torsions, planes, chiral volume, non-bonded contacts
    - Including links and modifications
- Provides “interactive” refinement
- Subject to substantial extension

Representation of Results:

Student Reaction:

“Oh, I don't look at that window...”
(I maximise the window immediately)
Representation of Results:

Second attempt...

Student Reaction:

“Oh, box of meaningless numbers.

Go away”
Representation of Results: “Traffic Lights”

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

- Single-Atom Drag
  - Over-dragging
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept
Low Resolution Model-Building

- “Backrub” rotamers
Current Low Resolution Rotamer Search

Previous

Rotamer Search

+ Rigid Body Refinement
New Low Resolution Rotamer Search

After Fitting Tools in KING/Molprobity
Networking...

- PDBe interface...
- Drag and drop
  - Also with drugbank
PDBe Recent Structures

JSON parser, network threaded code
Using the API
RCrane: Semi-Automated Building of RNA
Handling EM maps
Partitioning Maps: Watershed Algorithm

1D-analog

New region created

Different segments
Finding Holes

- An implementation of
  - Smart, Goodfellow & Wallace (1993) Biophysics Journal 65, 2455
  - Atomic radii from AMBER
  - I used
    - radii from CCP4 monomer library
    - sans simulated annealing
**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

Using 2 rotation axes
2 x 1-D Helix orientation searches
Handling NCS...
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
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
- NCS Ligands
Interfaces and Assemblies: Interface to PISA
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Projection from the surface of a doughnut: $2 \times 360^\circ$

(linear scaling)
Peptide Backbone Geometry
Typical 2D Projection of Ramachandran Plot