June 2011 Chicago

Model-Building with Coot

An Introduction, low resolution tools

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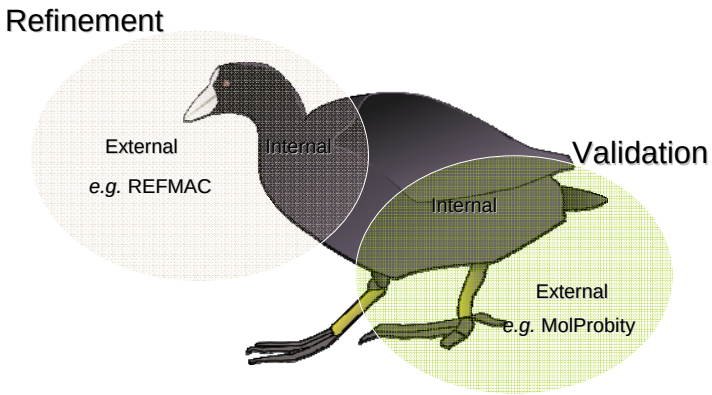
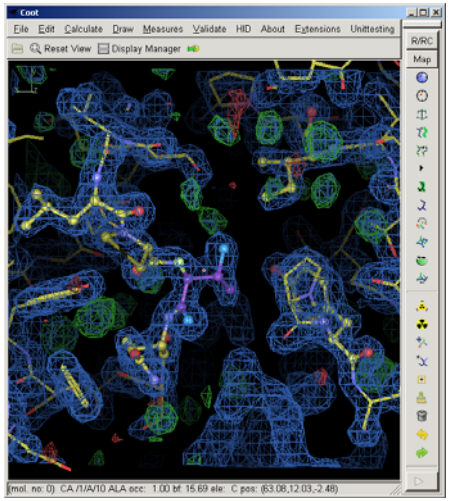
Coot

- Molecular Graphics application
  - Protein crystallographic model-building tools (Crystallographic Object-Oriented Toolkit)
  - Aims:
    - Model building, completion, validation
    - "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

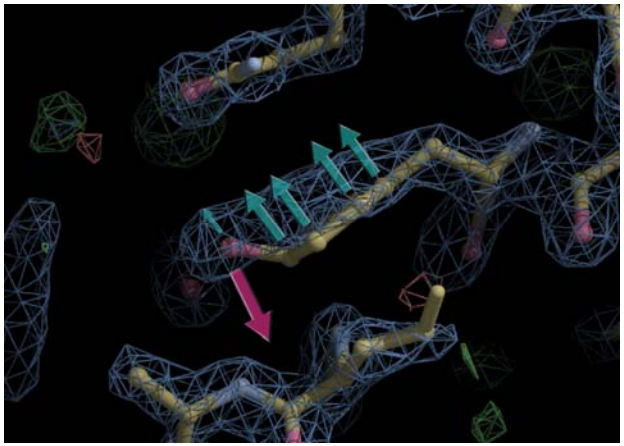
Feature Integration

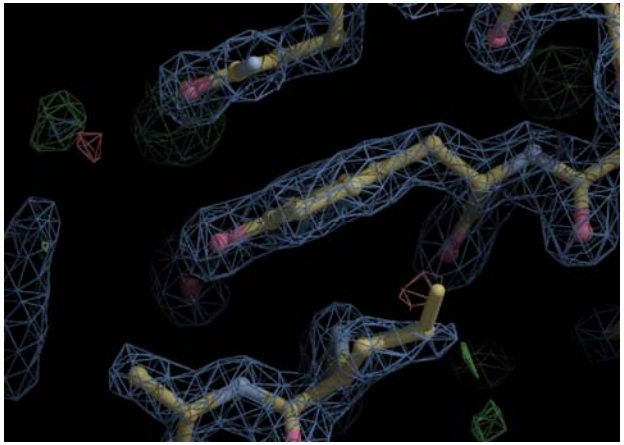




What is “Refinement”?

- The adjustment of model parameters (co-ordinates) so that the calculated structure factors match the observations as nearly as possible
  - In “one-shot” real-space refinement, such as in Coot, this translates to:
    - move the atoms into as high density as possible while minimizing geometrical distortions





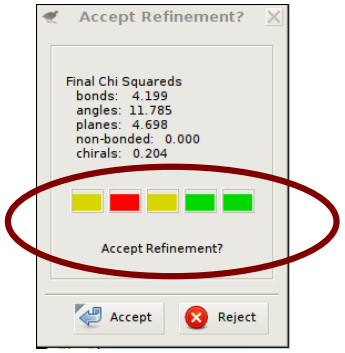
Real Space Refinement

Diamond, R. (1971). *Acta Cryst*. A 27, 436-452.

- Major feature of Coot
  - Gradient minimizer (BFGS derivative)
  - Based on mmCIF standard dictionary
  - Minimizing bonds, angles, planes, non-bonded contacts, torsions, [chiral volumes, Ramachandran]
- Provides “interactive refinement”
- Different minimizer to Refmac…
  - …means “nice & tight” geometry
    - Chi squareds

Faster & Animated

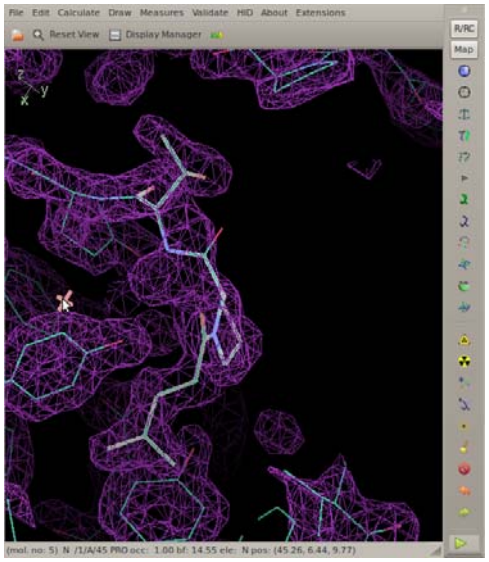
Refinement “Traffic Lights”



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

Refinement Techniques

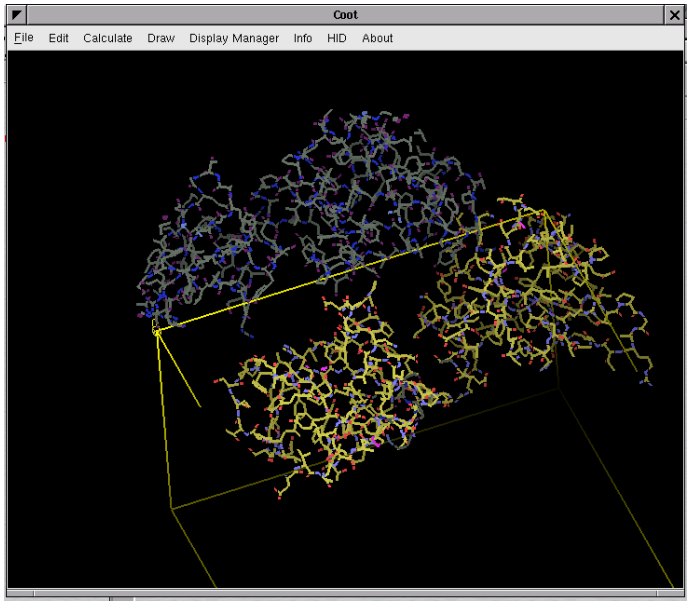
- Auto-zone
- Single-Atom Drag
  - Over-dragging
- Ramachandran Refinement
- Sphere refinement
- Coming Soon..?
  - Dials, PowerMate, spaceballs
  - Wii Refinement

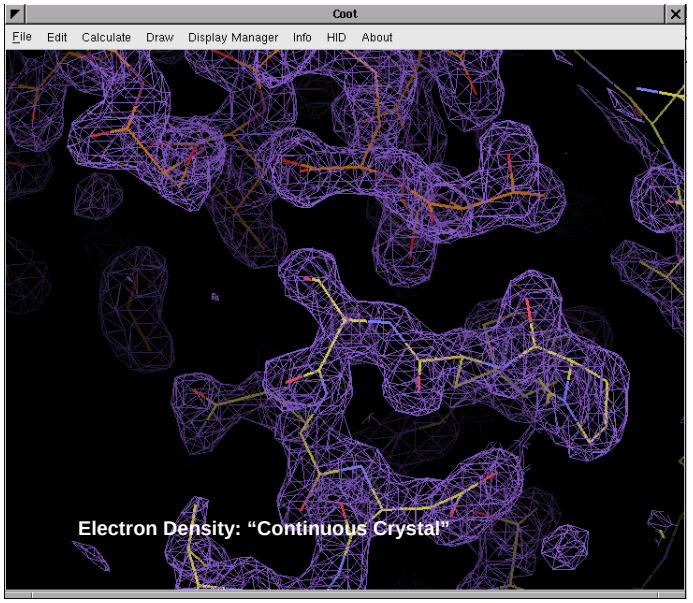


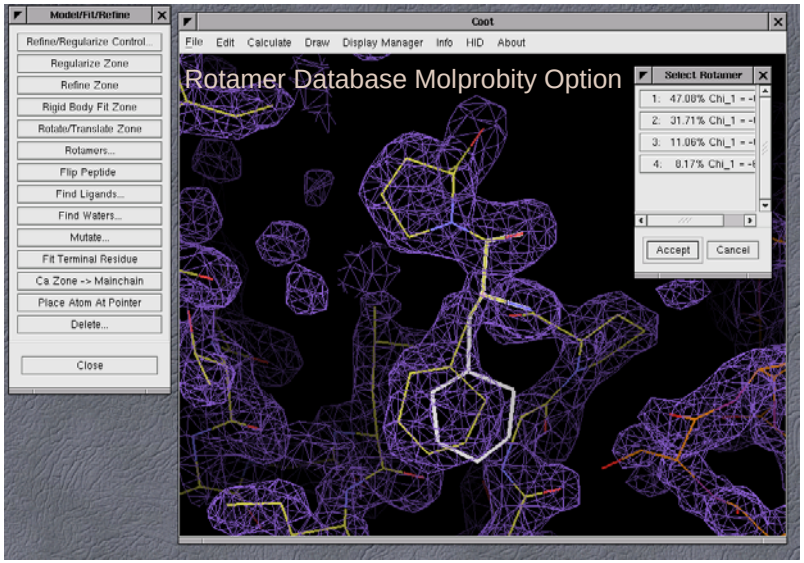
Over-dragging

“Sphere” Refinement

- Given an “Active” Residues
  - Fine a sphere of residues around it and use them all for refinement
    - NOT just a linear selection
  - Residues from different chains (or different parts of the same chain) interact
  - Make CYS-CYS or glycosylation links as you find them
    - Use the group and link_list chem_link in the dictionary

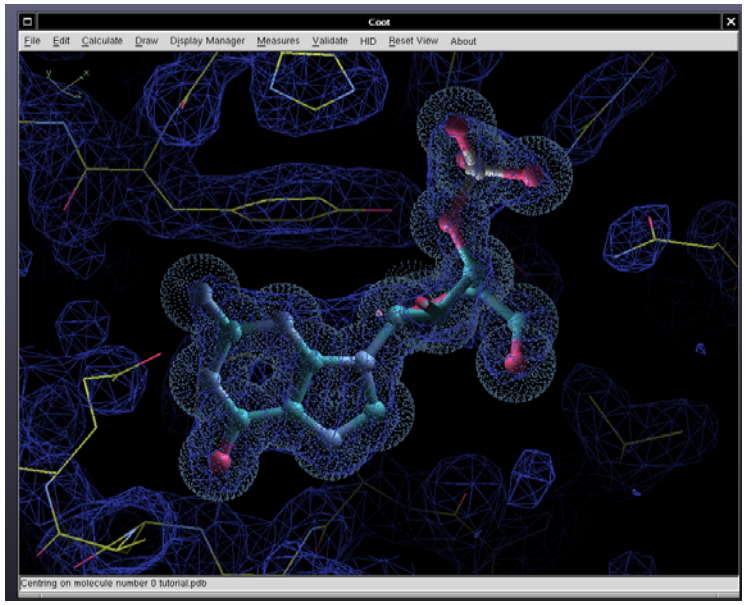






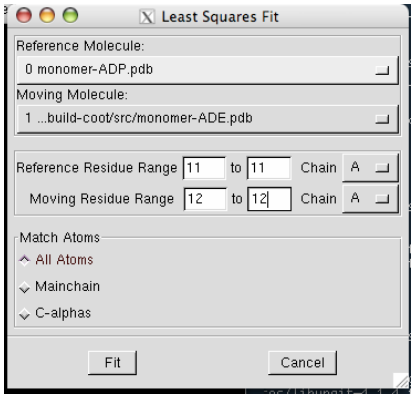
Some more Coot Tools…

- Alternate Conformations
- Ligand fitting/search
- Rigid-body Fitting
  - Steepest Descent
  - Simplex (slower but better)
- “Move Molecule Here”
- Water Search
- Fill-partial-residues (after MR)
- Dots, ball&stick representation



Superpositions

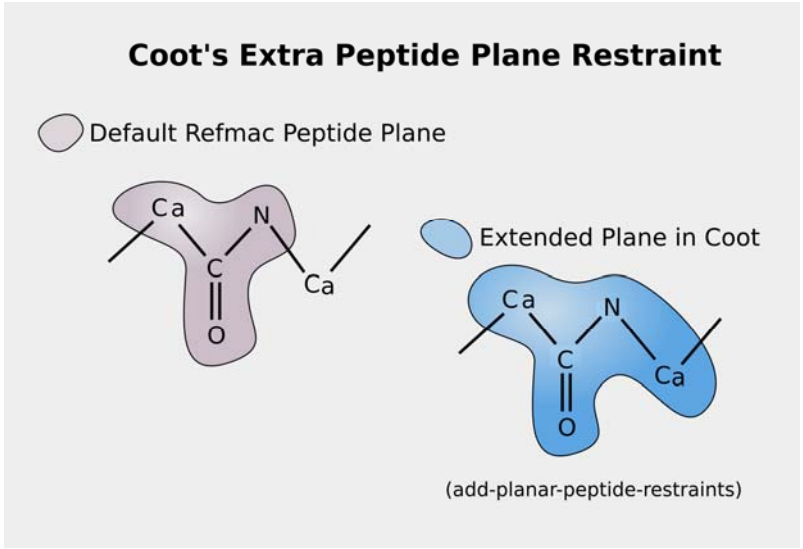
- S(econdray) S(tructure) M(atching)
- Least Squares Superposition:



Low Resolution Tools

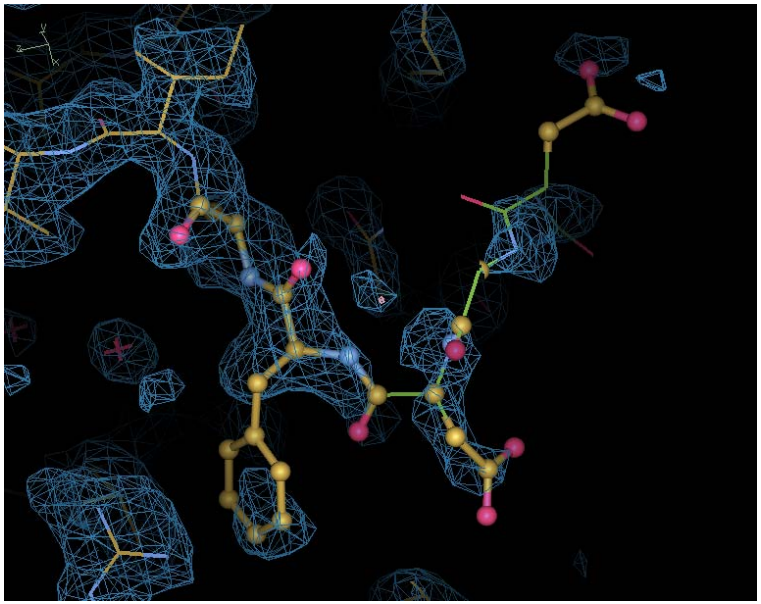
Extra Restraints....

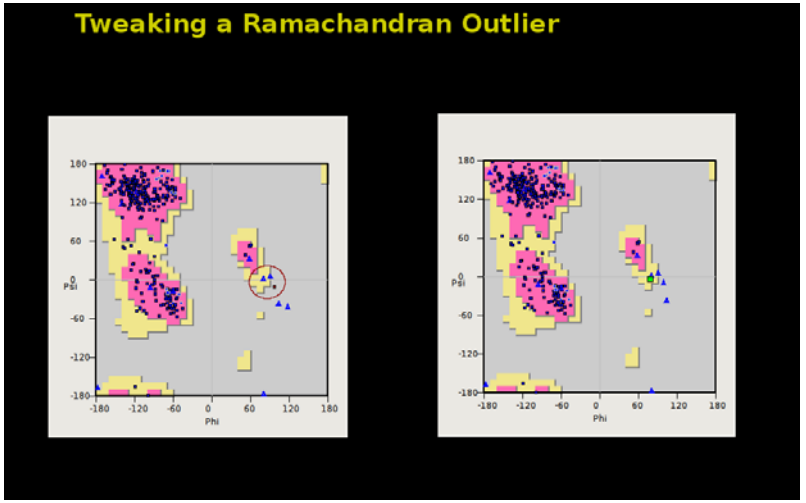
- Peptide plane
- Ramachandran restraints
- Secondary Structure restraints
- Remove degree of freedom
  - Torsion angle restraints
  - Backrub rotamers

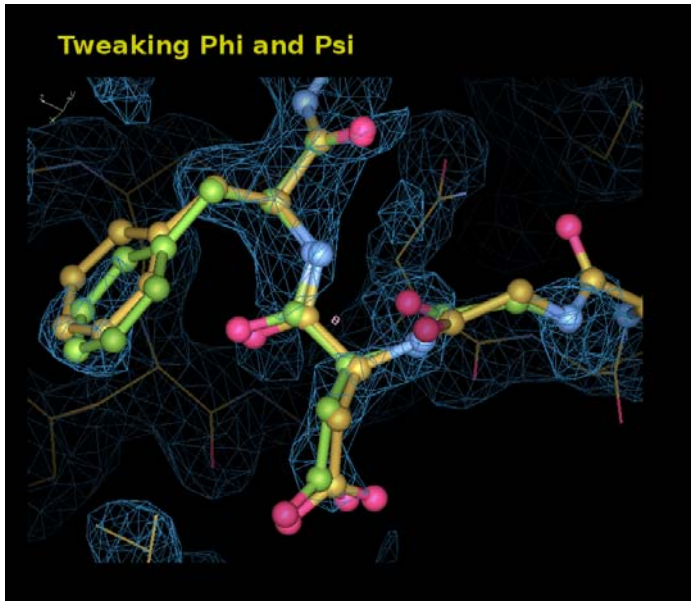


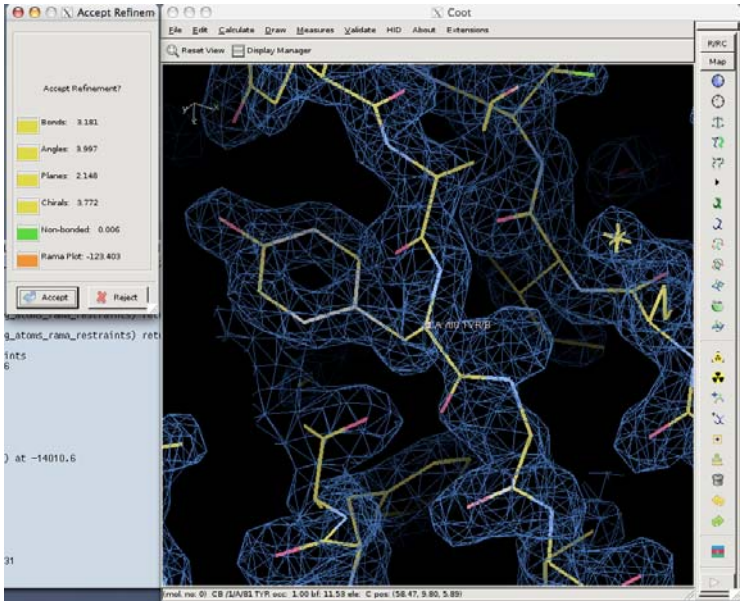
Ramachandran Restraints

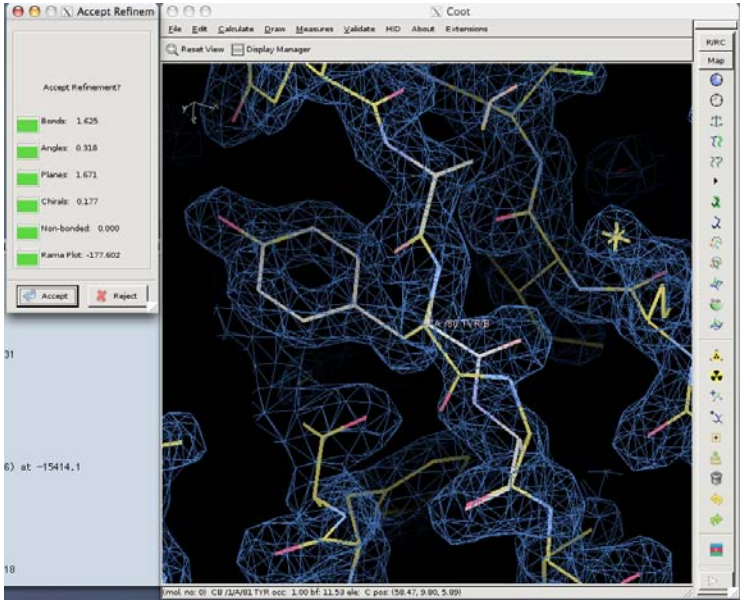
- Scenario:
  - I have a loop, with poor density, I know the atoms are there somewhere and I want to provide a "reasonable" model
- Controversial Feature?
  - Ramachandran Plots have been used for "validation" - but here we are deliberately optimizing them
- Ramachandran Plots can be added to the geometry target function











Ramachandran Restraints

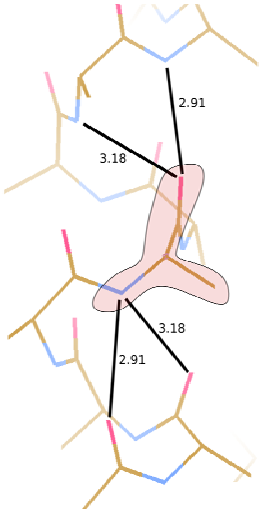
- Controversial?
  - "... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model..."
    - Gerard Kleywegt & Alwyn Jones (1996)
- But to quote Jane Richardson:
  - Do you want a better structure – or a better idea of the quality of your structure?

Adding Torsion Angle Restraints

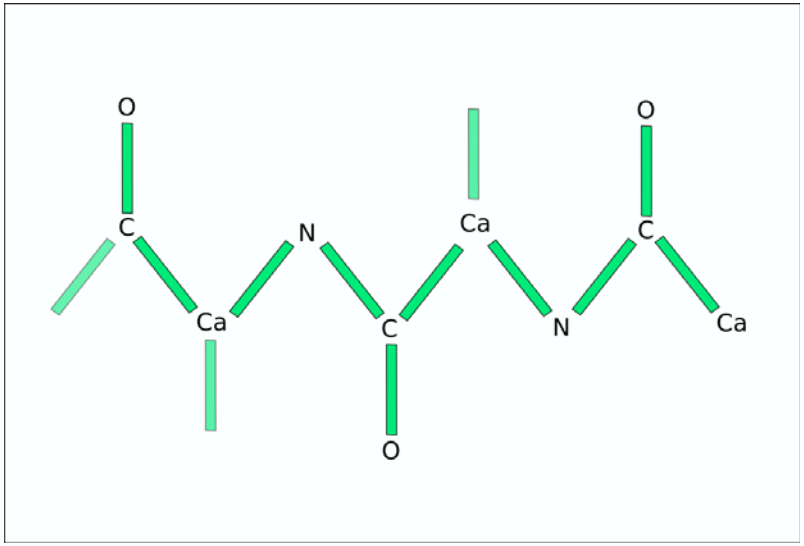
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”
    - i.e. trapped in local minimum

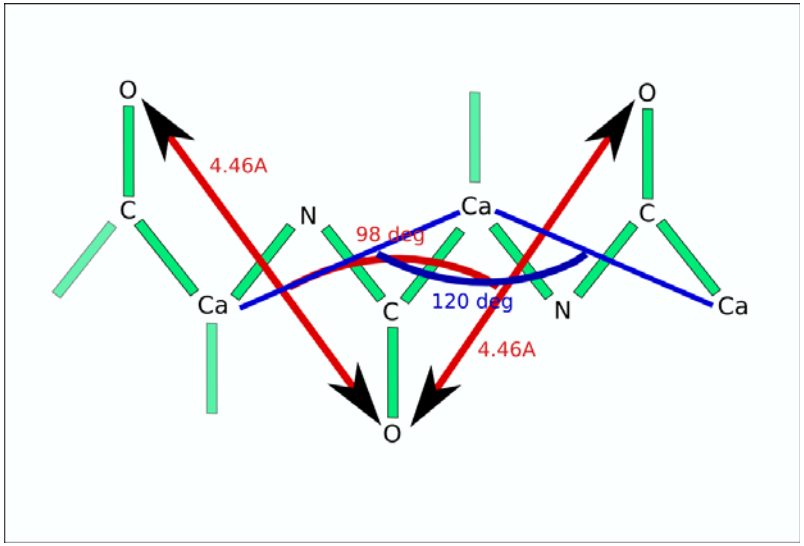
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints

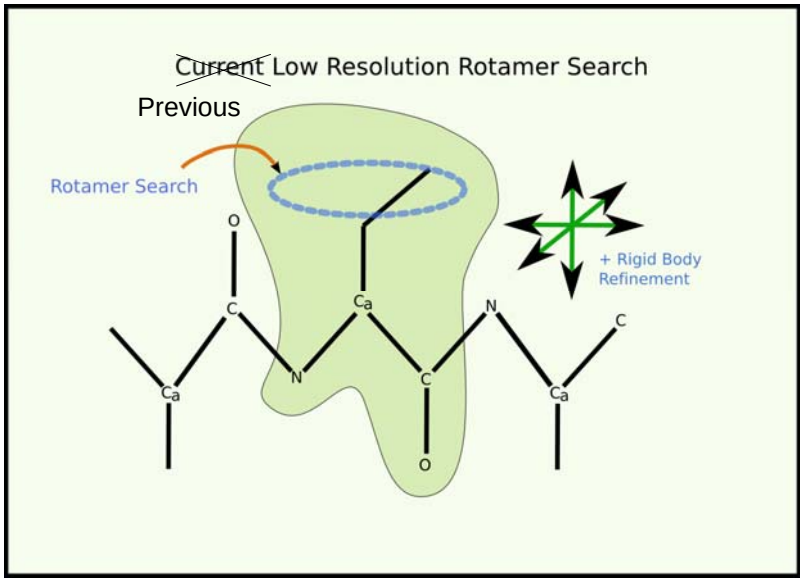


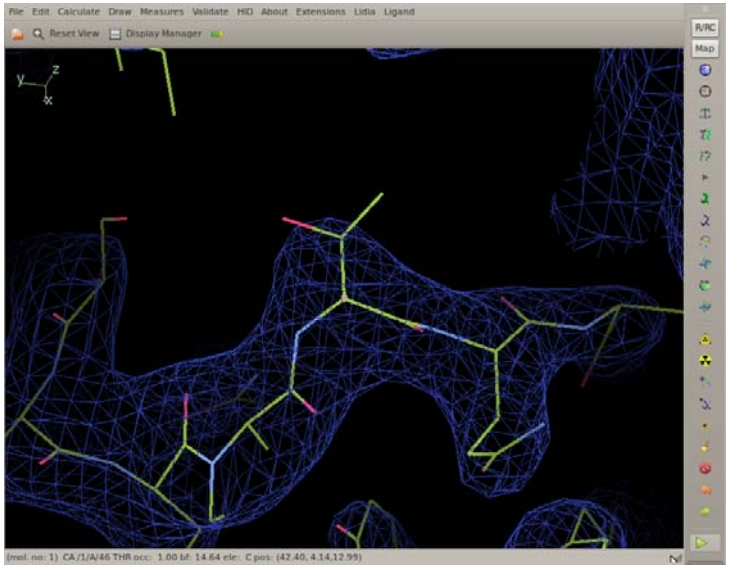
Restrain the Hydrogen-bonding atom distances

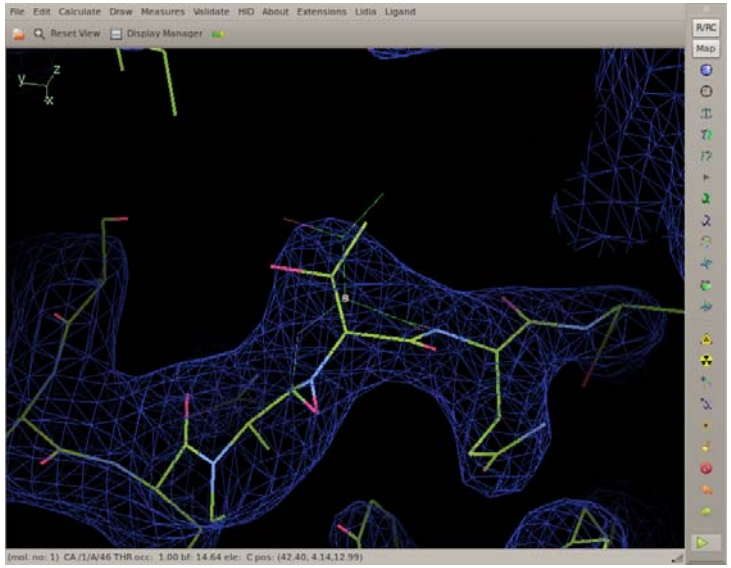


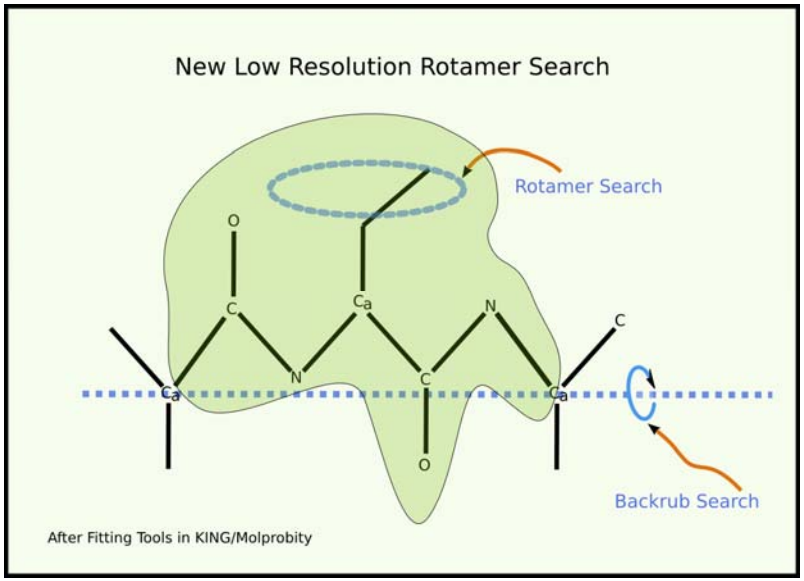


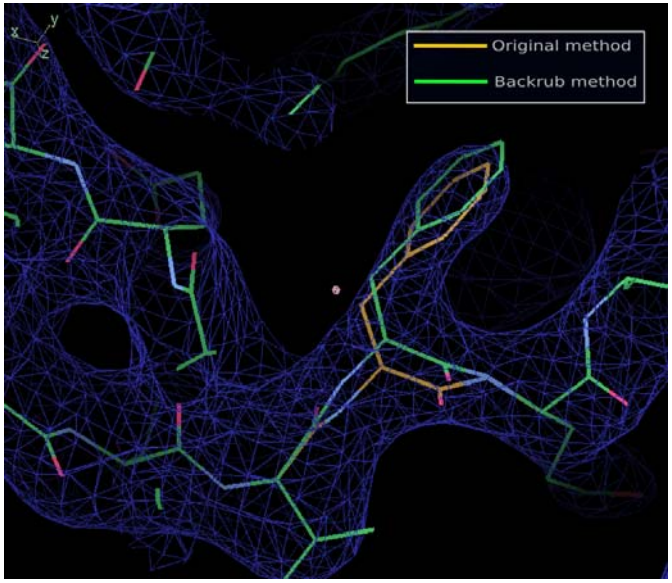
“Backrub” Rotamers







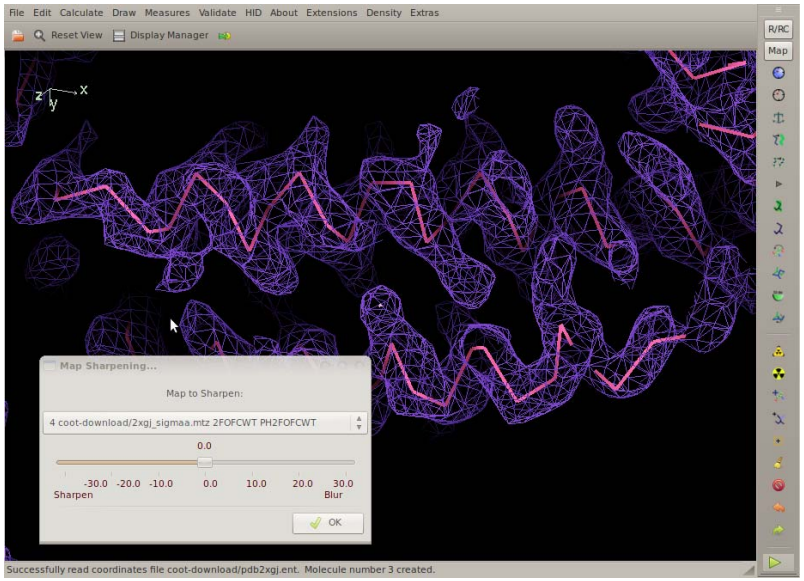


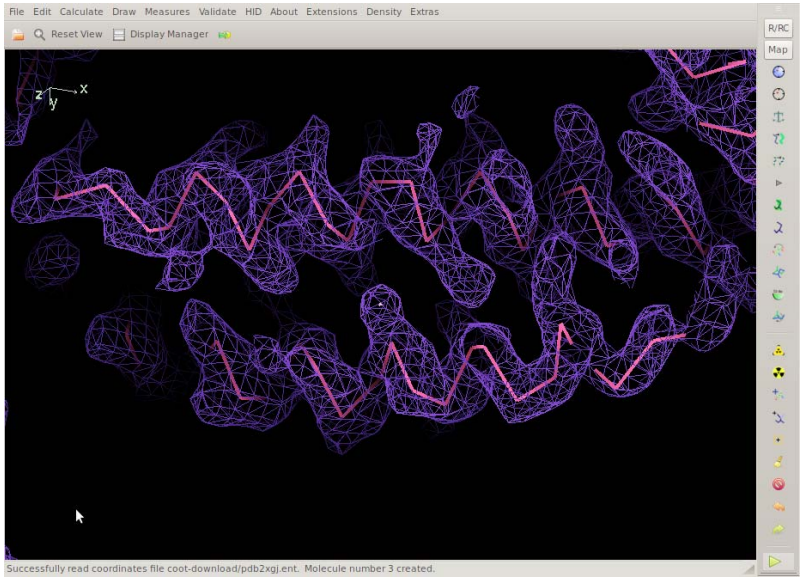


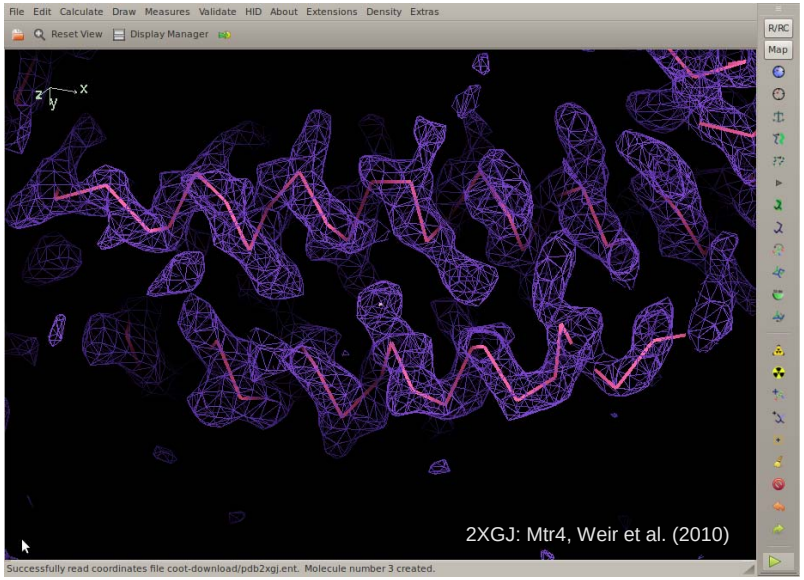
Map Sharpening

Which B-factor shall I use to get the most interpretable map?

Interactively adjust the structure factor amplitudes and re-generate the map with FFT and recontouring...





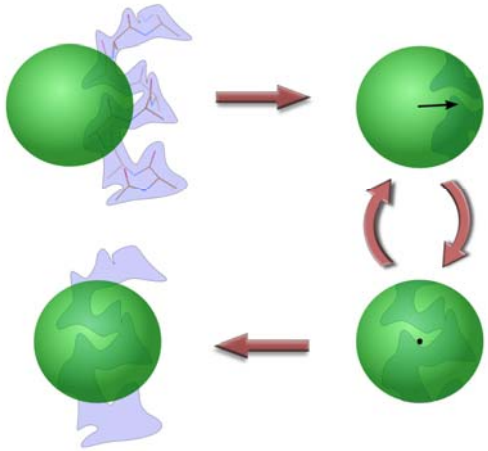


Helix-Building

Alpha Helix Placement

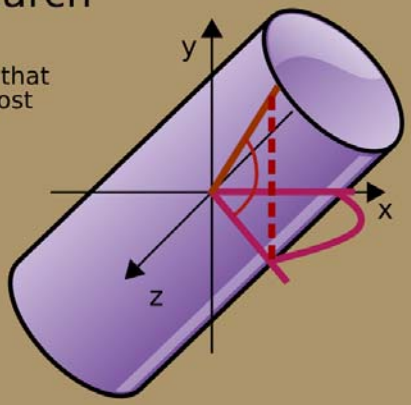
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D Rotation search to find best fit
  - Score based on density at CB positions
  - Trim 'n Grow

Centering the Rotation point

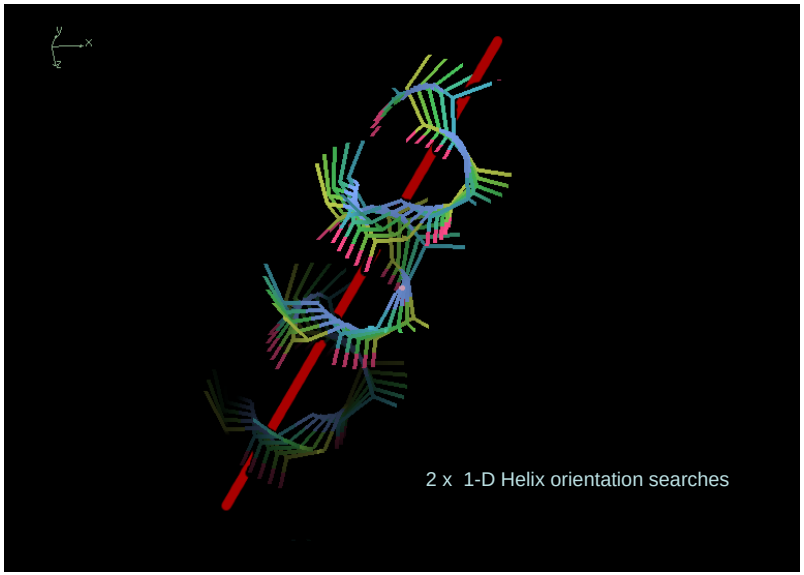


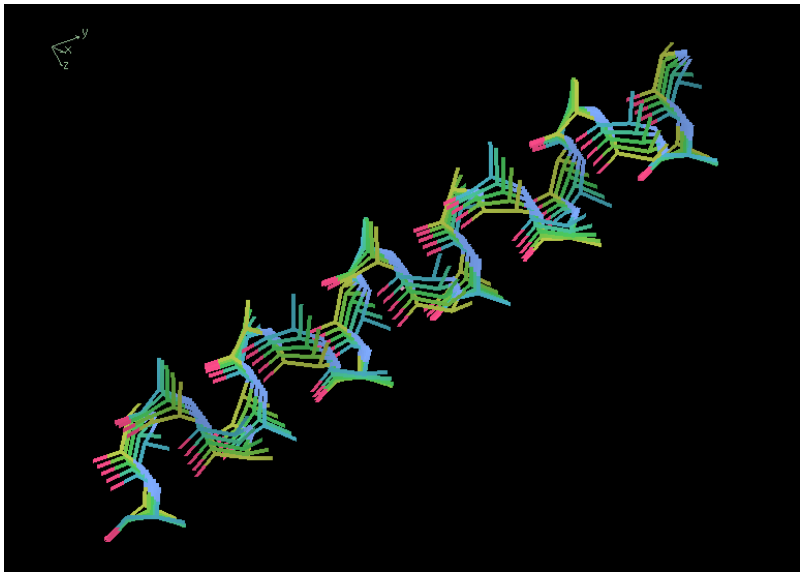
Cylinder Search

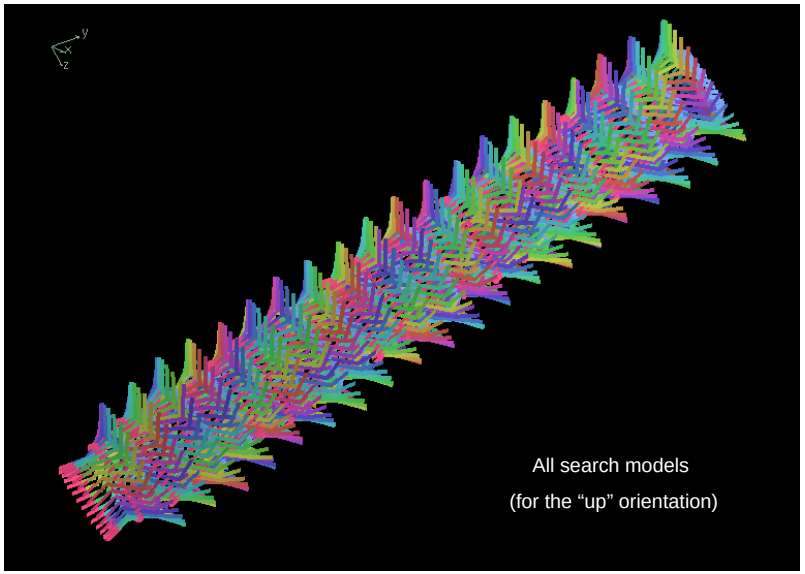
Pick the orientation that encapsulates the most electron density

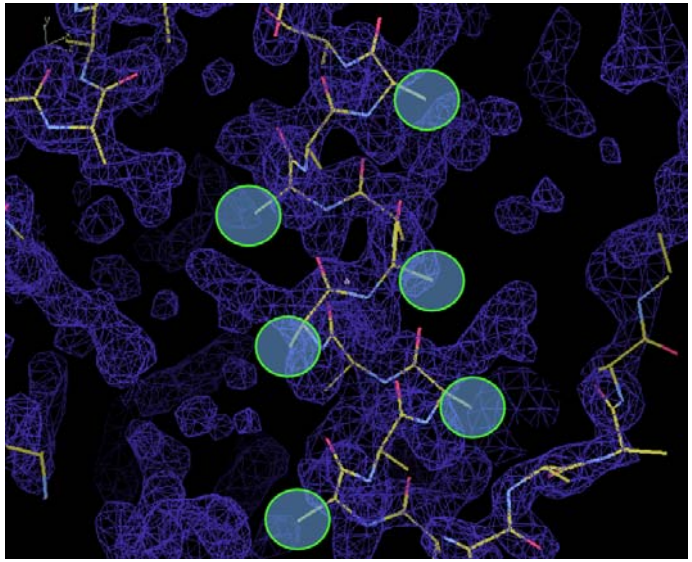


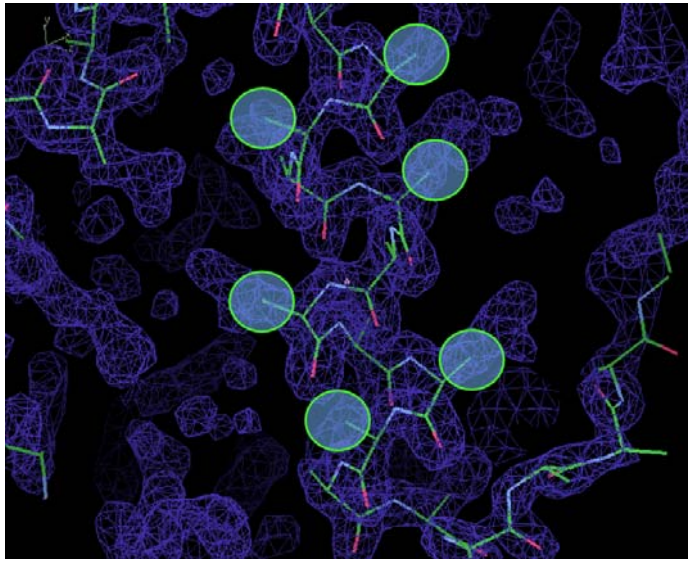
2 orientation axes











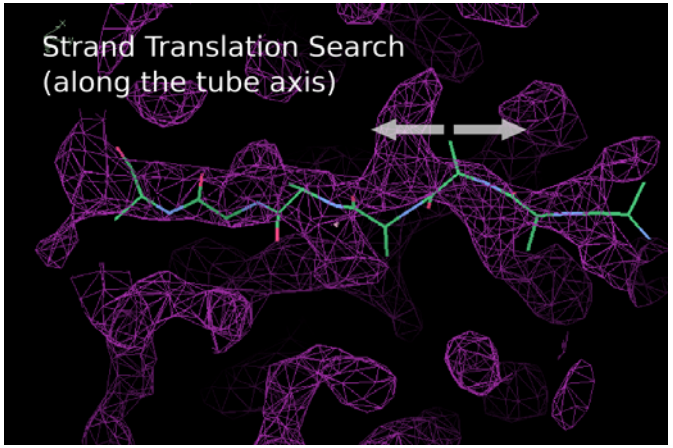
Fitting Strands

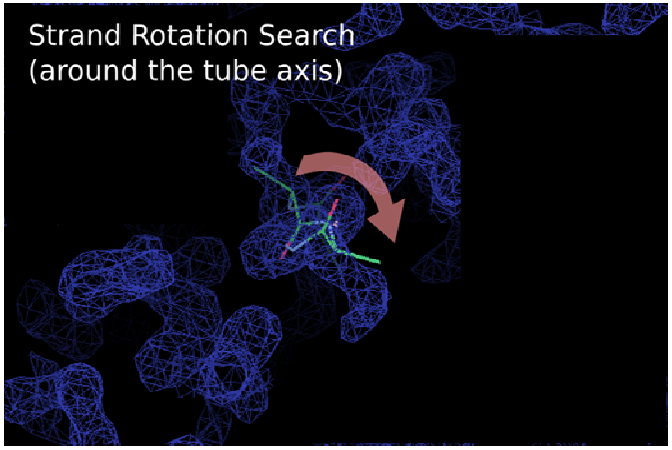
Placing Strands

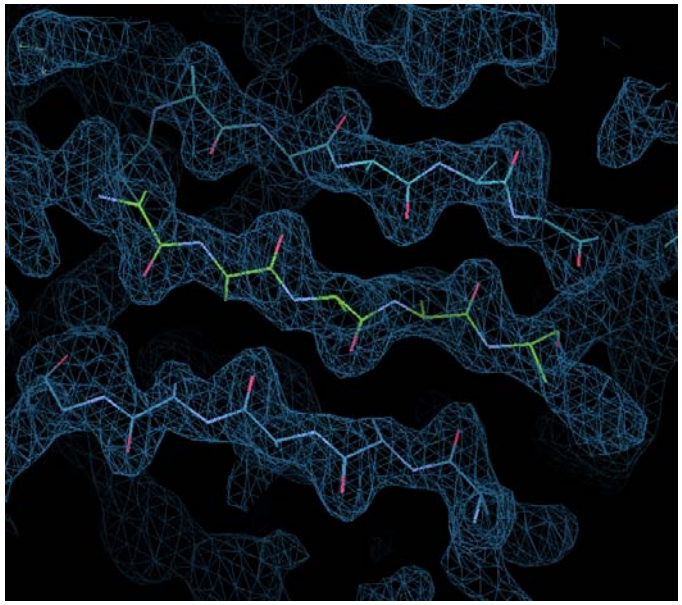
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

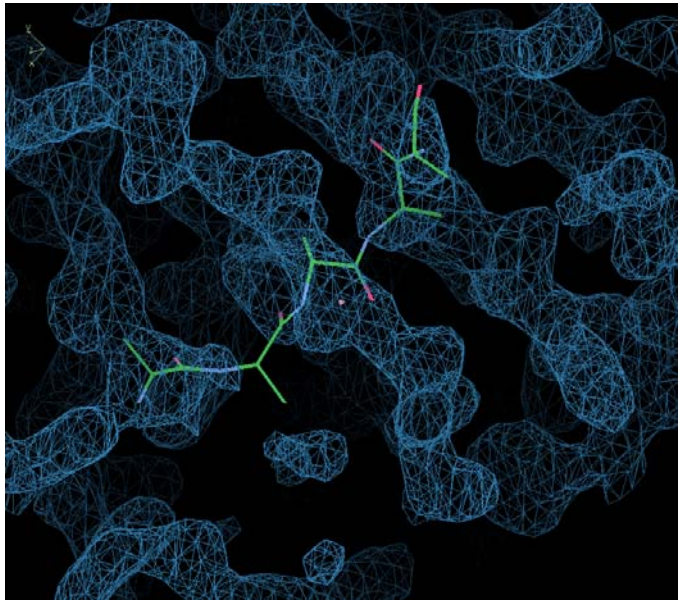
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution







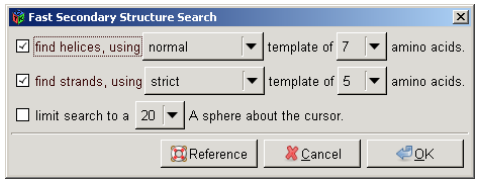
Not all is rosy...



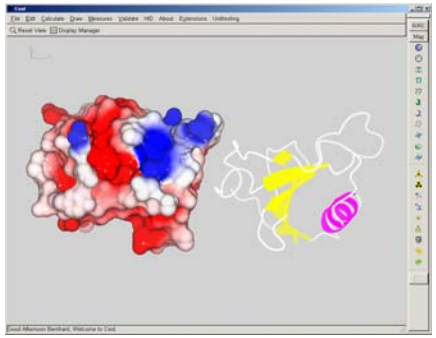
Fitting Strands caveat

- 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

Automated Fast Secondary Structure Search



Coot Futures…



- Aim:
  - Slick, easy to use
  - Powerful
  - Smooth interface to external applications
- Under Development
  - Interesting things move quickly
  - There may be bugs

Further information

- Coot WIKI
  - http://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/Coot
- Coot BB (mainling list)
  - http://www.biop.ox.ac.uk/coot/mailing-list.html
- Coot documentation
  - http://www.biop.ox.ac.uk/coot/docs.html

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