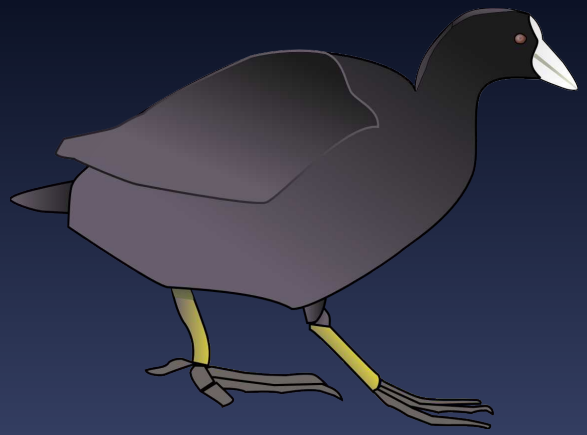
December 2011 Okinawa



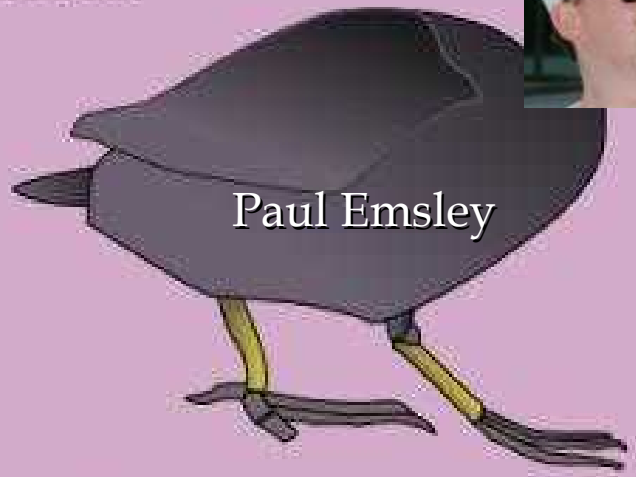
Model-Building with Coot

An Introduction, low resolution tools

Bernhard Lohkamp

Karolinska Institutet

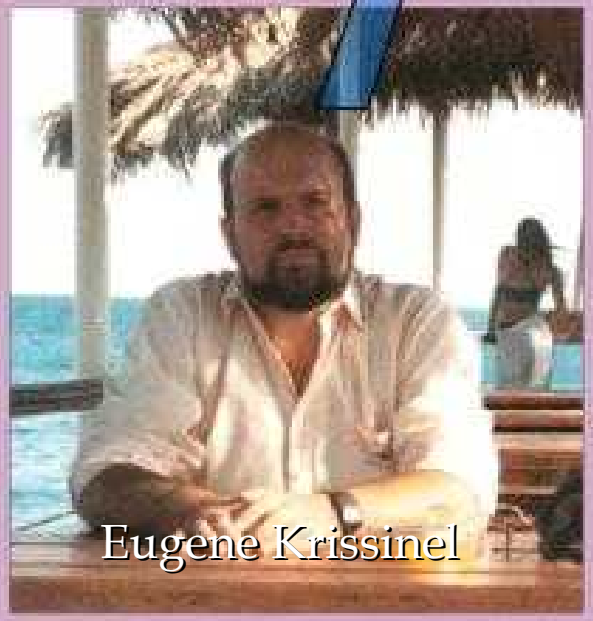
Coot Contributors:



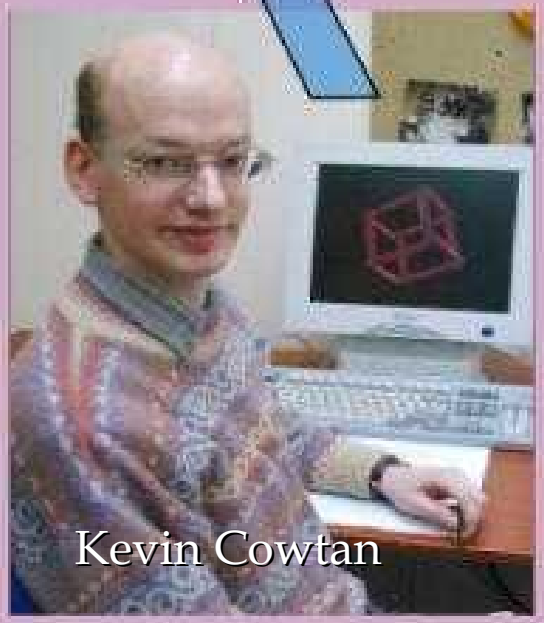
Paul Emsley

Stuart McNicholas

Alexei Vagin



Eugene Krissinel



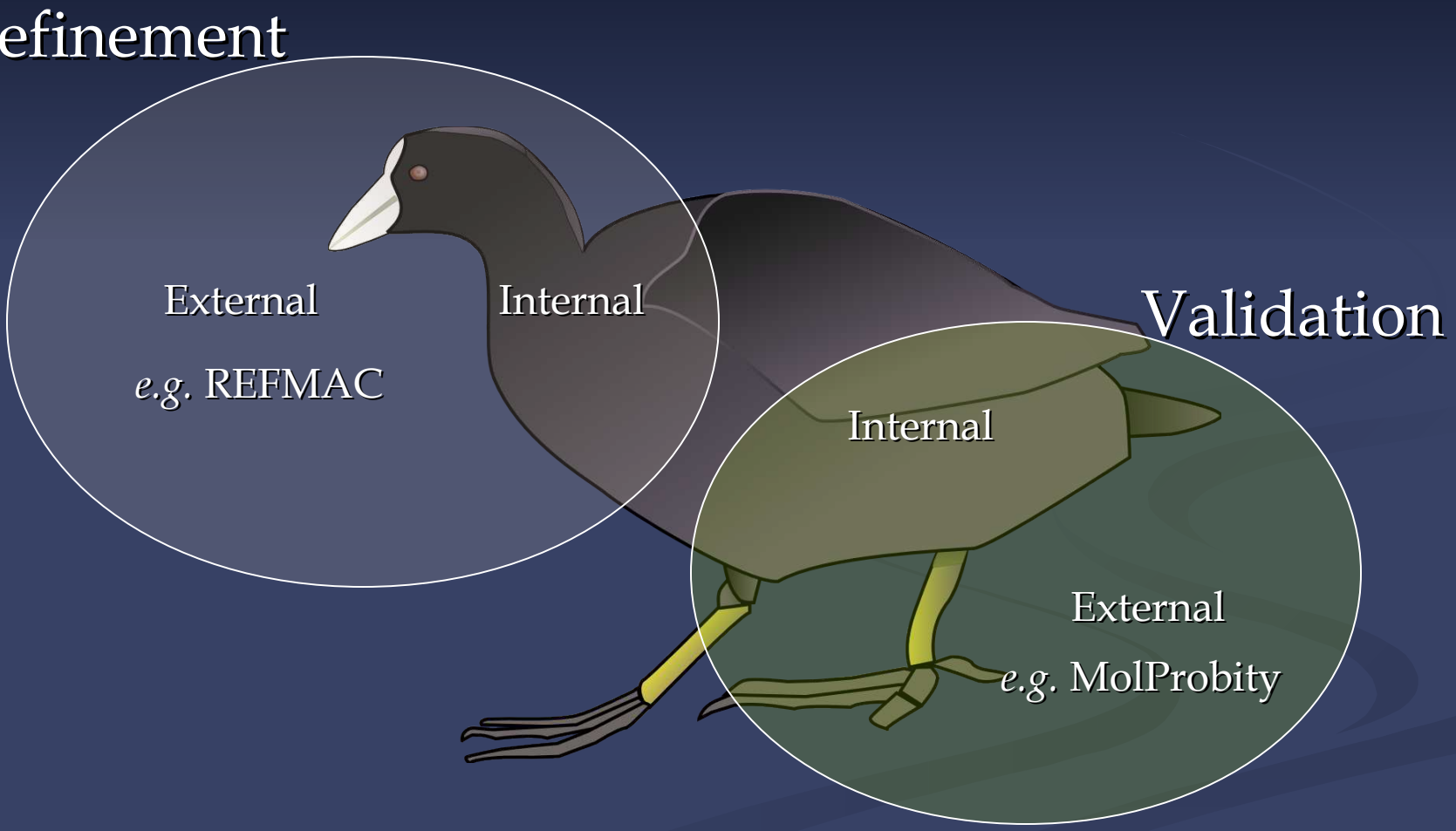
Kevin Cowtan

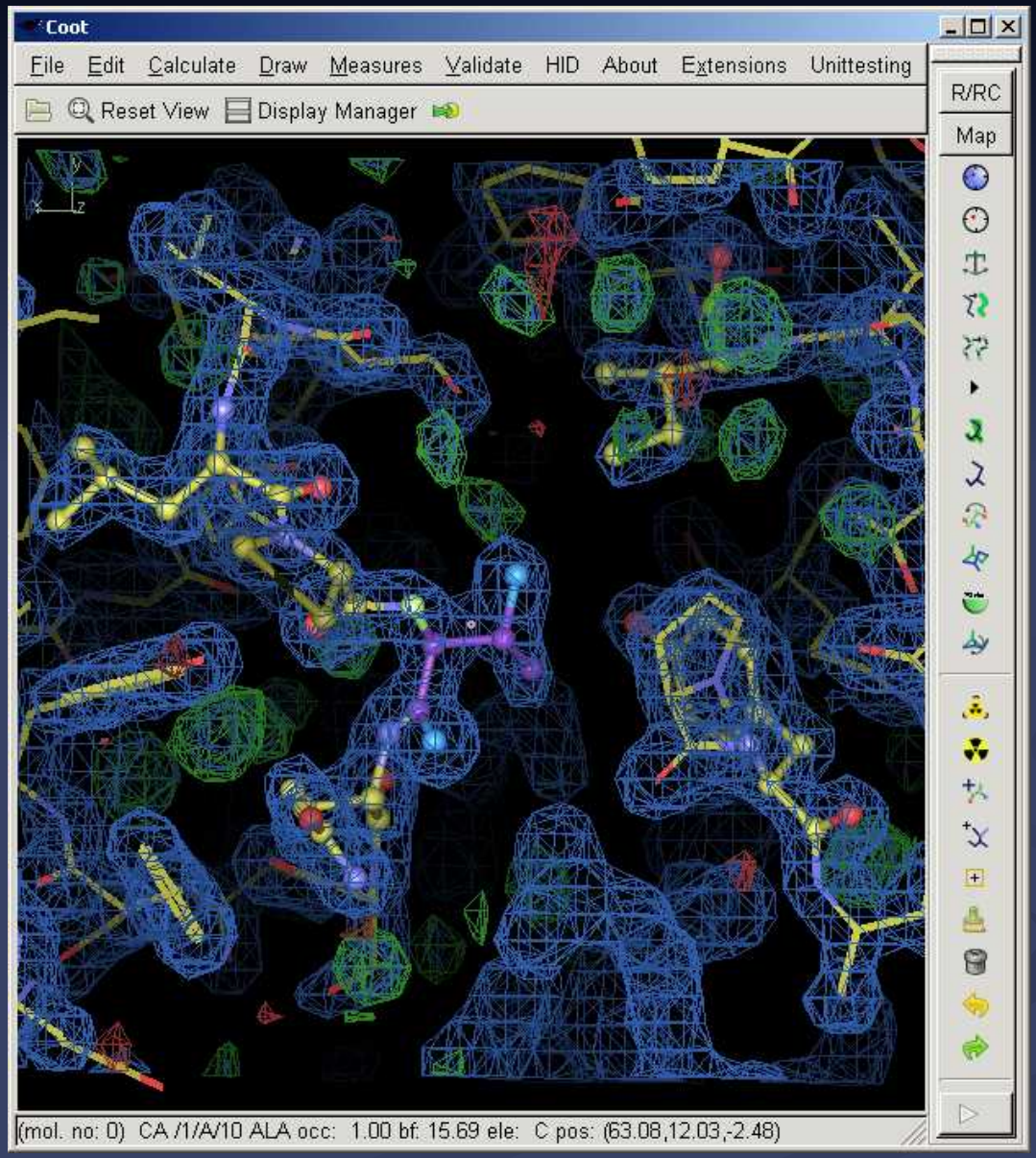
Bernhard Lohkamp

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




Coot
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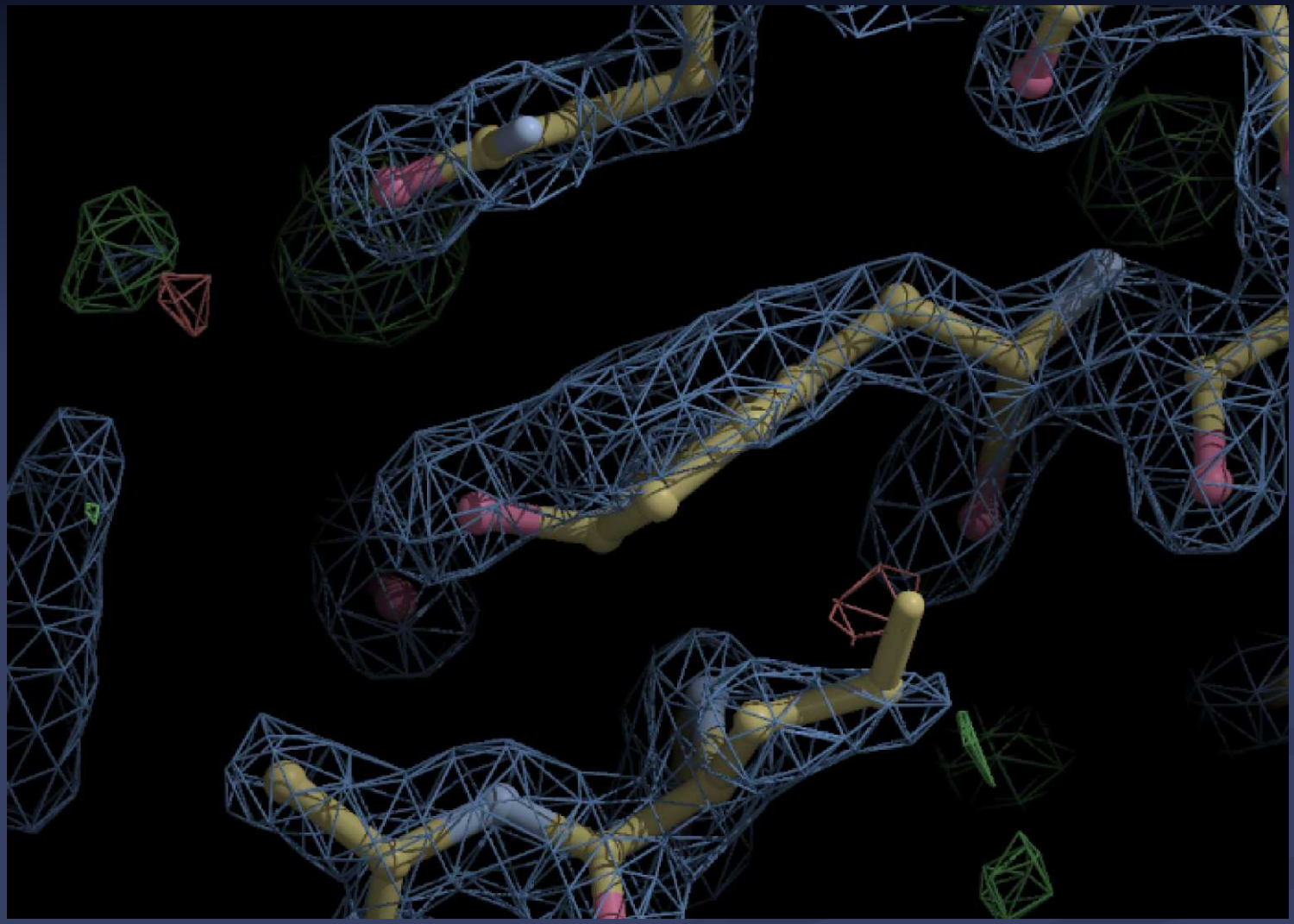
Map
(mol. no: 0) CA /1/A/10 ALA occ: 1.00 bf: 15.69 ele: C pos: (63.08,12.03,-2.48)

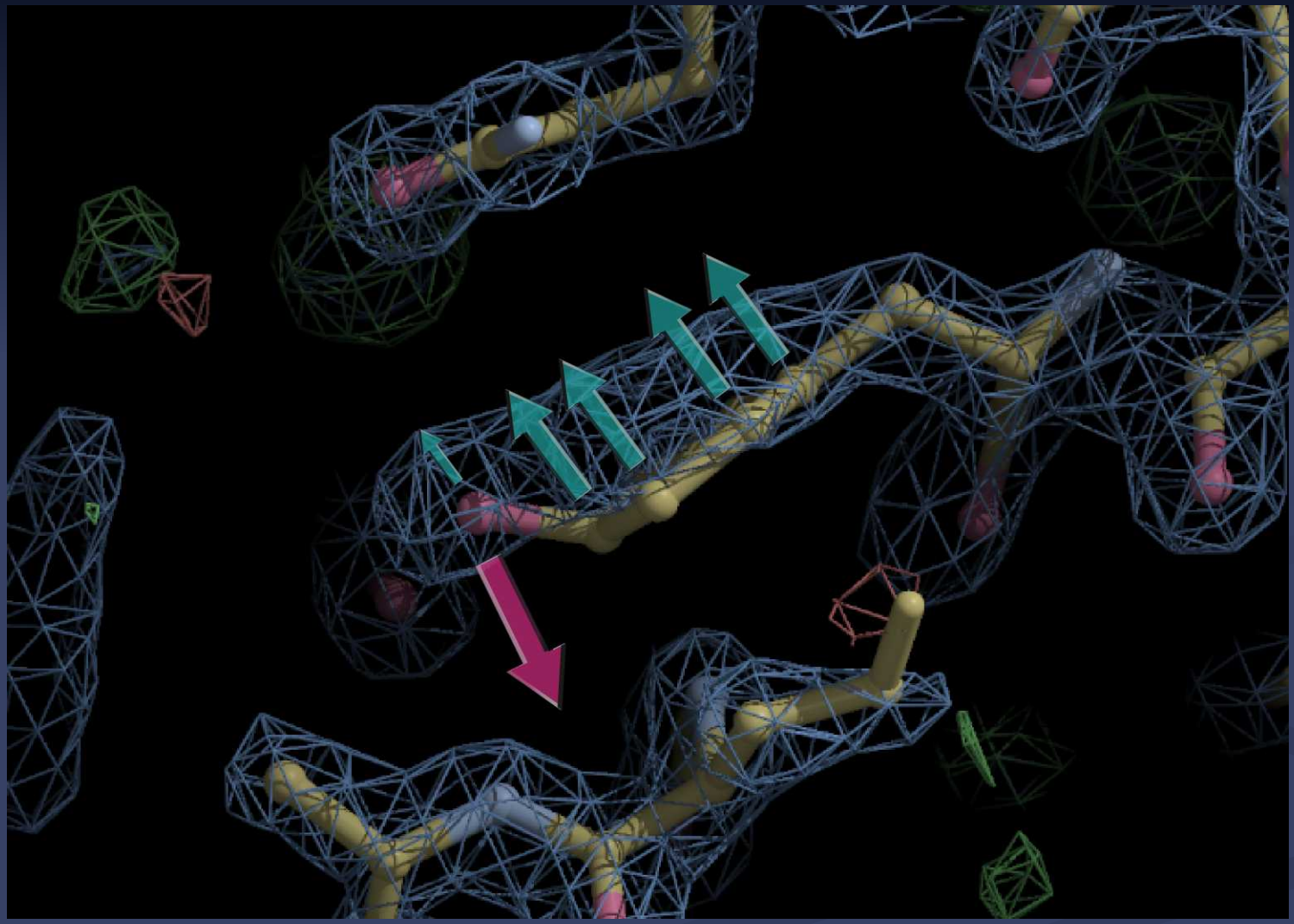
What is "Refinement"?

- 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

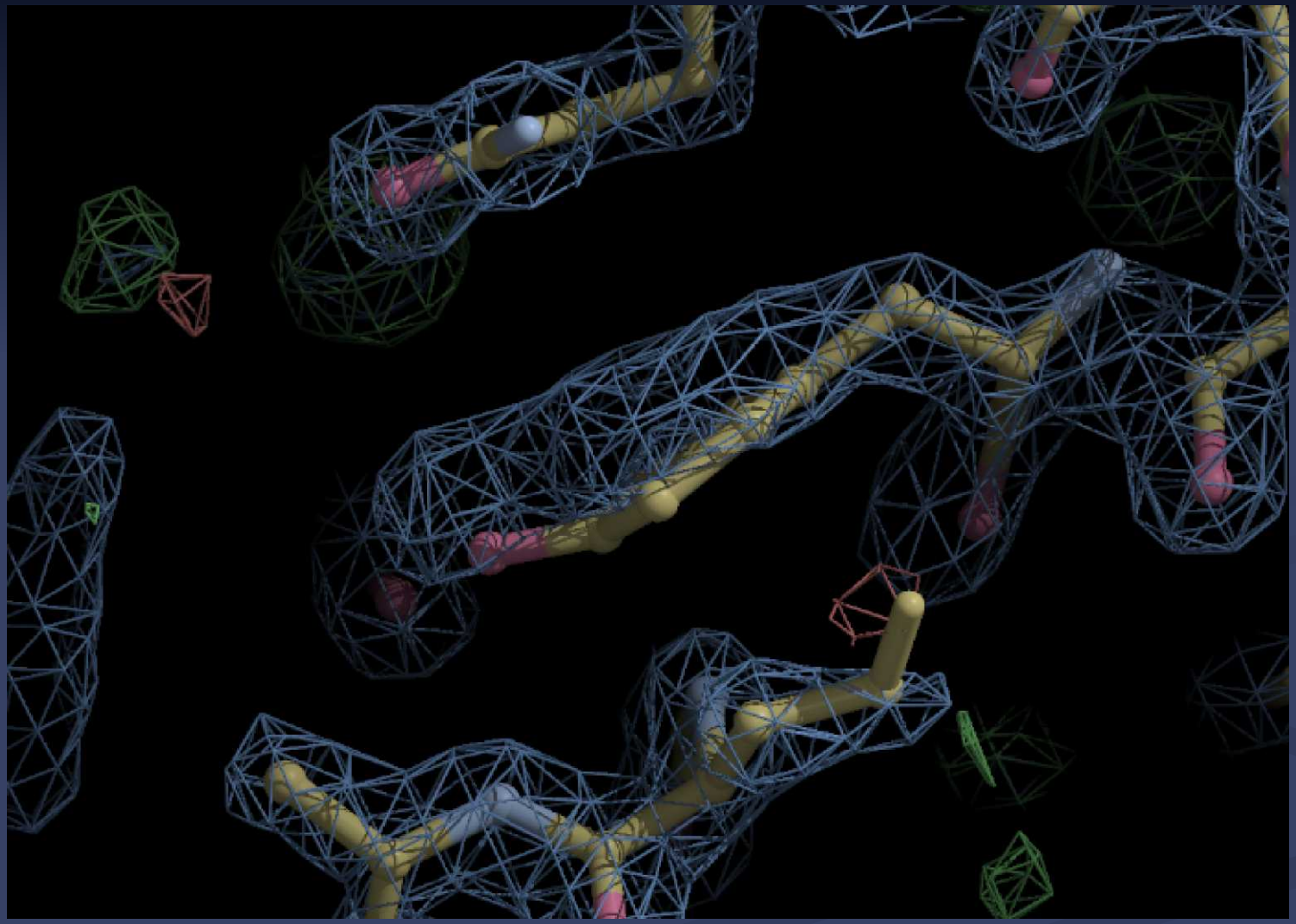
Distorted Geometry Pre-Refinement



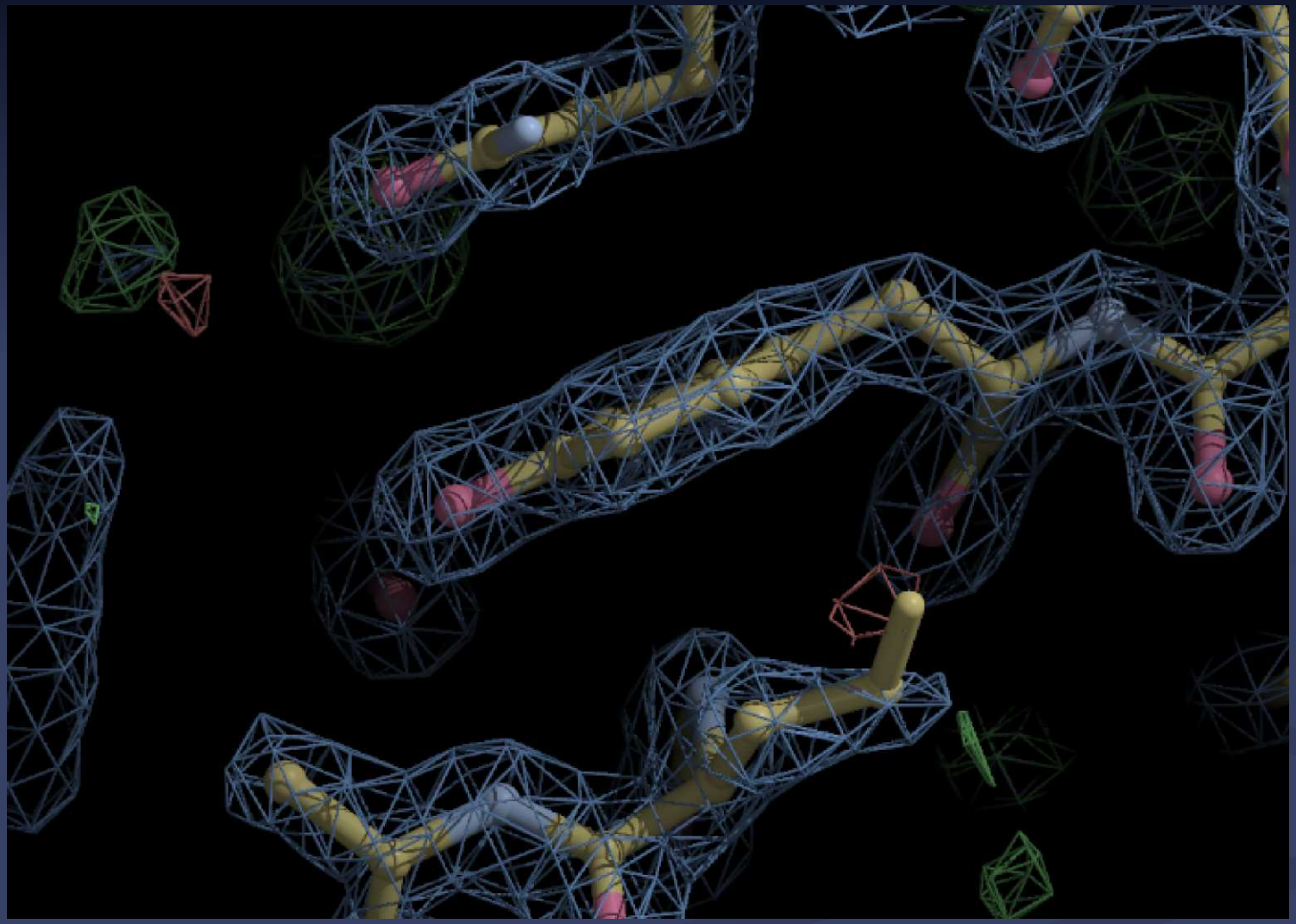
Refinement Gradients



Refinement: Cycle 3



Refinement Cycle 200: Minimized



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

Representation of Results:

File Edit View Terminal Help

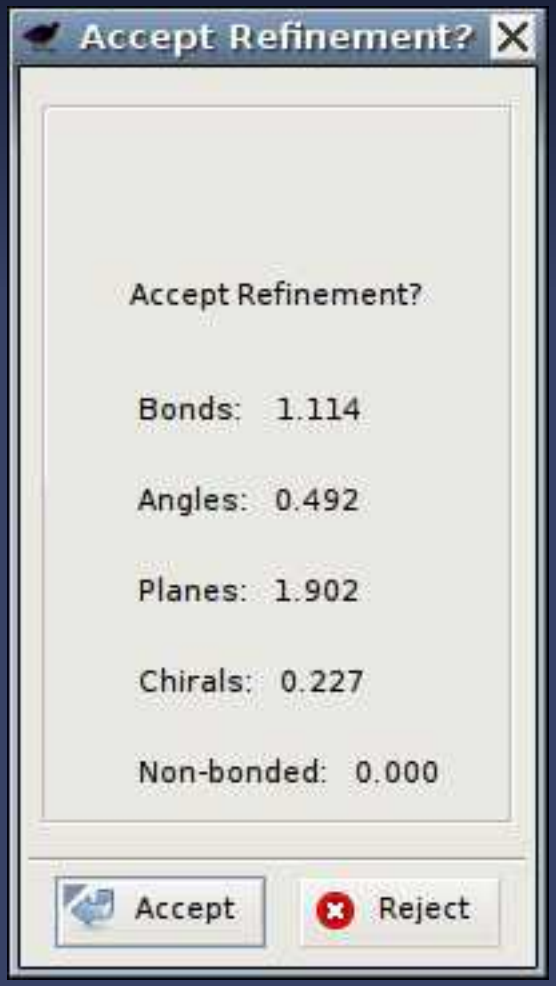
```
created 32 bond       restraints
created 38 angle      restraints
created 1 plane      restraints
created 5 chiral vol restraints
created 76 restraints

   INFO:: [spec: "A" 45 ""] [spec: "A" 46 ""] link_type :TRANS:
   INFO:: [spec: "A" 45 ""] [spec: "A" 44 ""] link_type :TRANS:
Link restraints:
   2 bond    links
   6 angle   links
   4 plane   links
Flanking residue restraints:
   4 bond    links
   12 angle   links
   8 plane   links
INFO:: made 668 non-bonded restraints
initial distortion_score: -16033.2
    Initial Chi Squareds
bonds:      1.15701
angles:     0.847832
torsions:   N/A
planes:     1.6176
non-bonded: 0
chiral vol: 0.705728
rama plot:  N/A
Minimum found (iteration number 67) at -16275.9
    Final Estimated RMS Z Scores:
bonds:      1.19412
angles:     0.713337
torsions:   N/A
planes:     1.05134
non-bonded: 0
chiral vol: 0.522415
rama plot:  N/A
 SUCCESS
 TIME:: (dragged refinement): 332.657
```

The first attempt

Student Reaction:

“Oh, I don't look at that window...”

Representation of Results:



Second attempt...

Student Reaction:

“Oh, box of meaningless numbers.

Go away”

Representation of Results: "Traffic Lights"

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

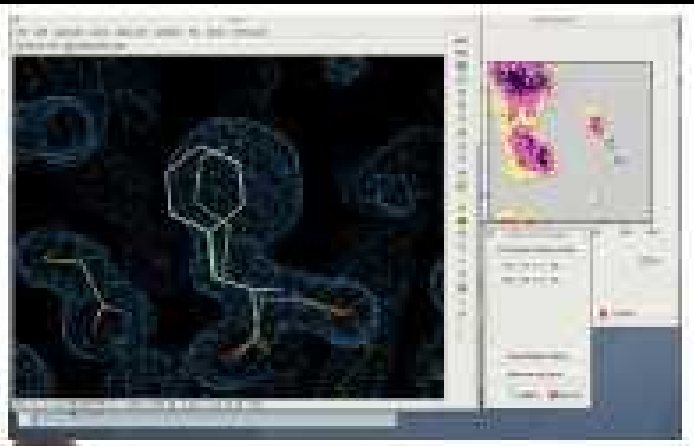
Good refinement

Bad refinement

Refinement Techniques

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

Wii Refinement?

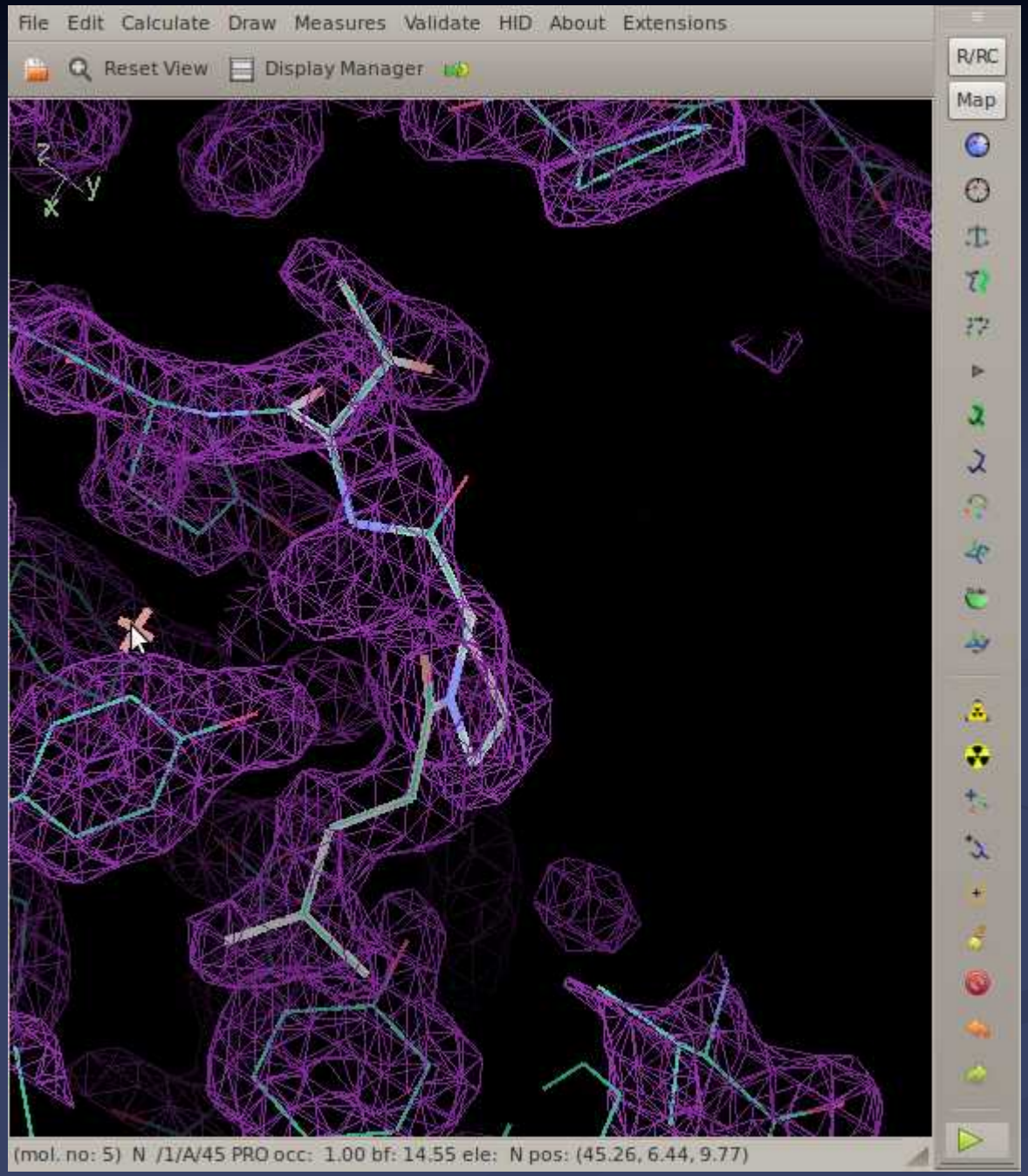


Maybe in 2012 (a P. Emsley* estimation...)

* although me to do...

Wii Refinement?

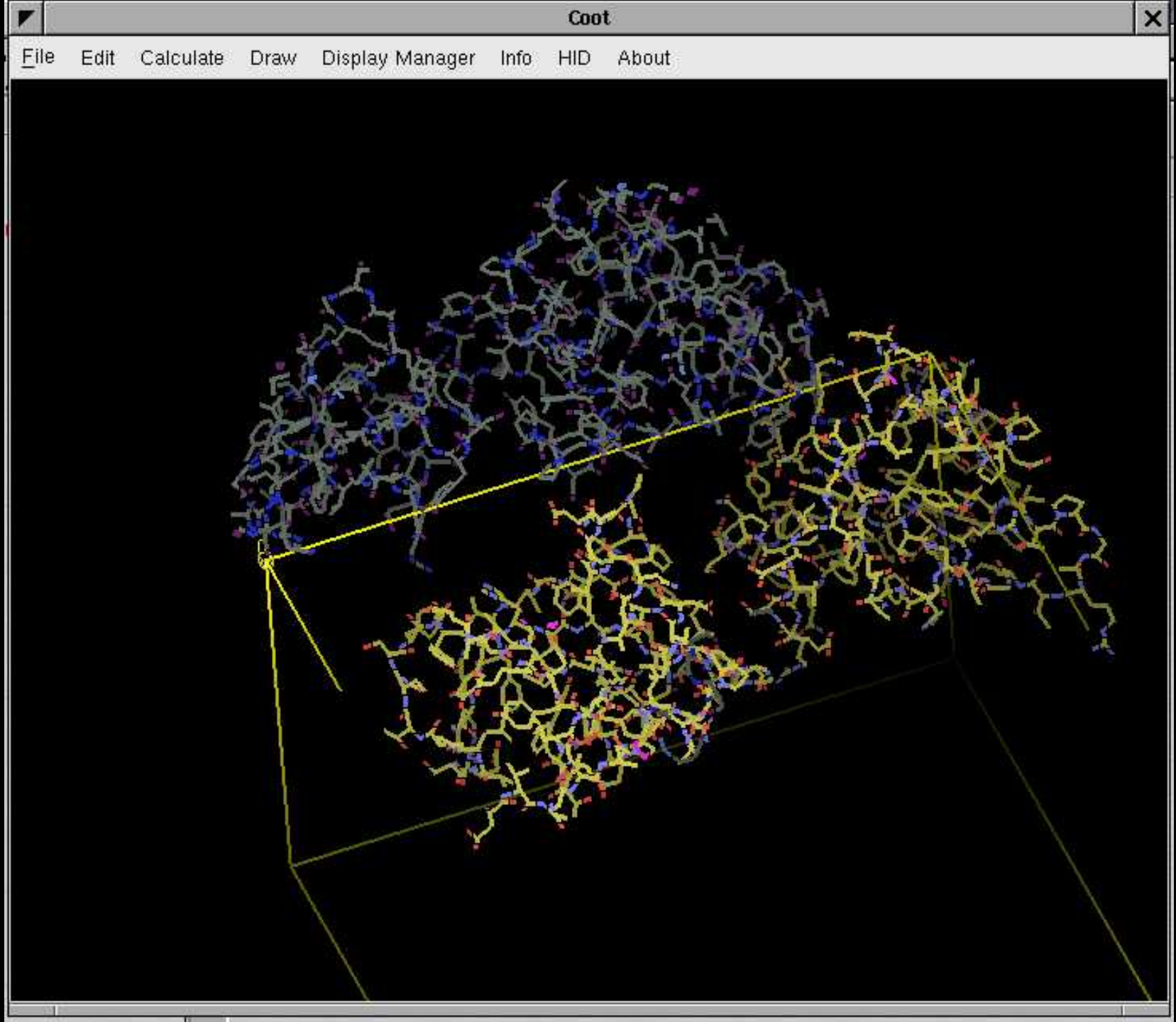
= maybe in 2??? in reality...



Over-dragging

"Sphere" Refinement

- Given an "Active" Residues
  - Define 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


Coot
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Edit
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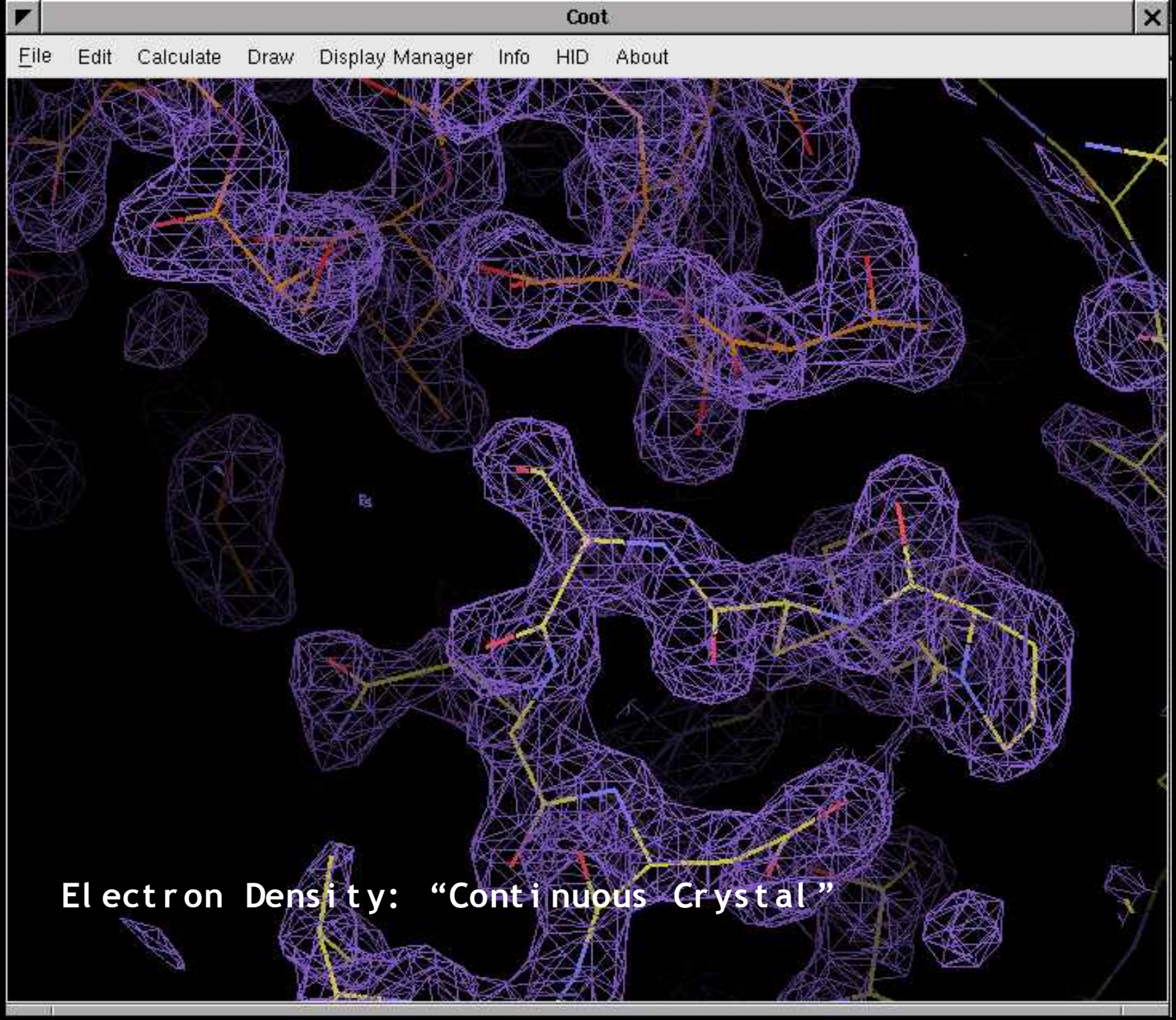
Draw
Display Manager
Info
HID
About


Coot
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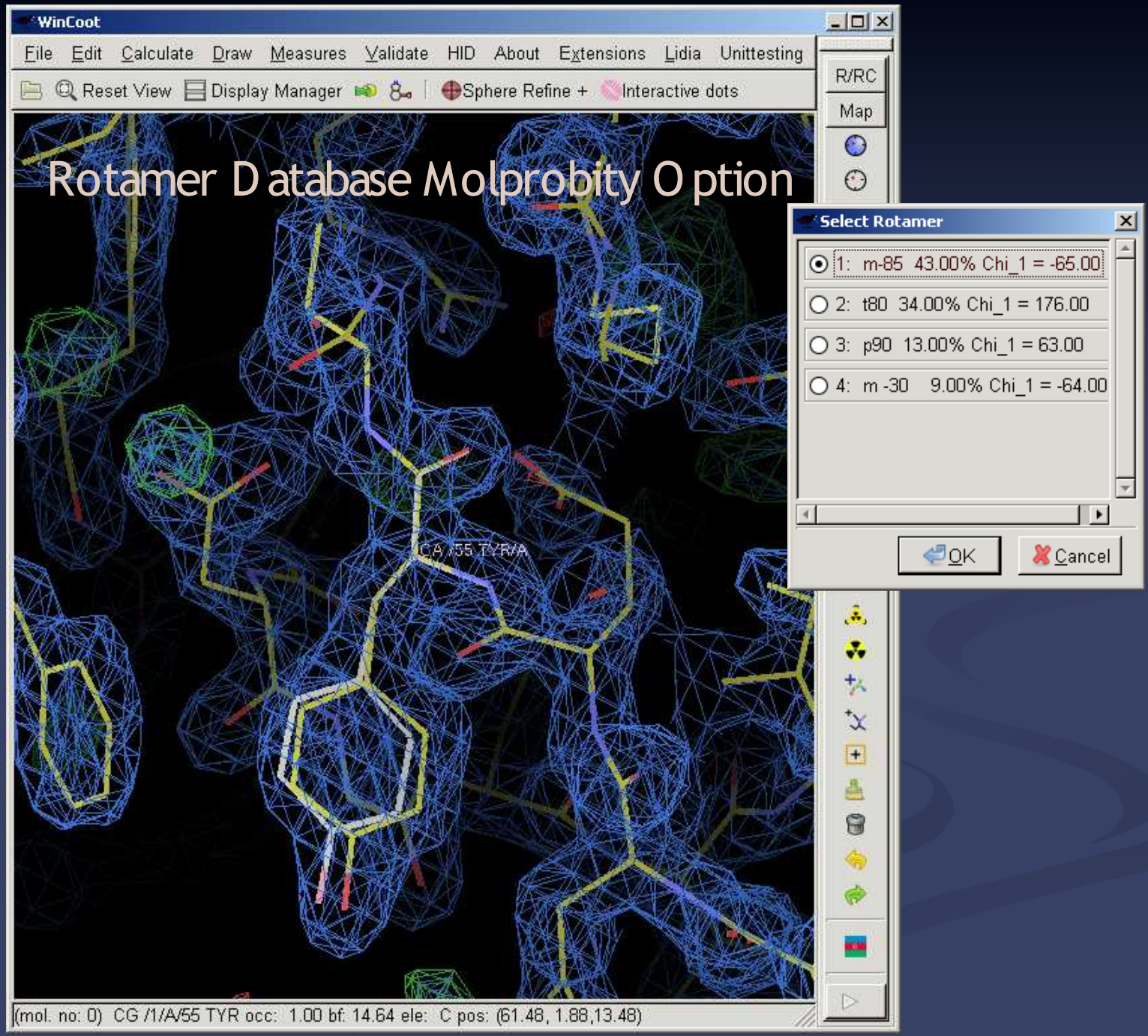
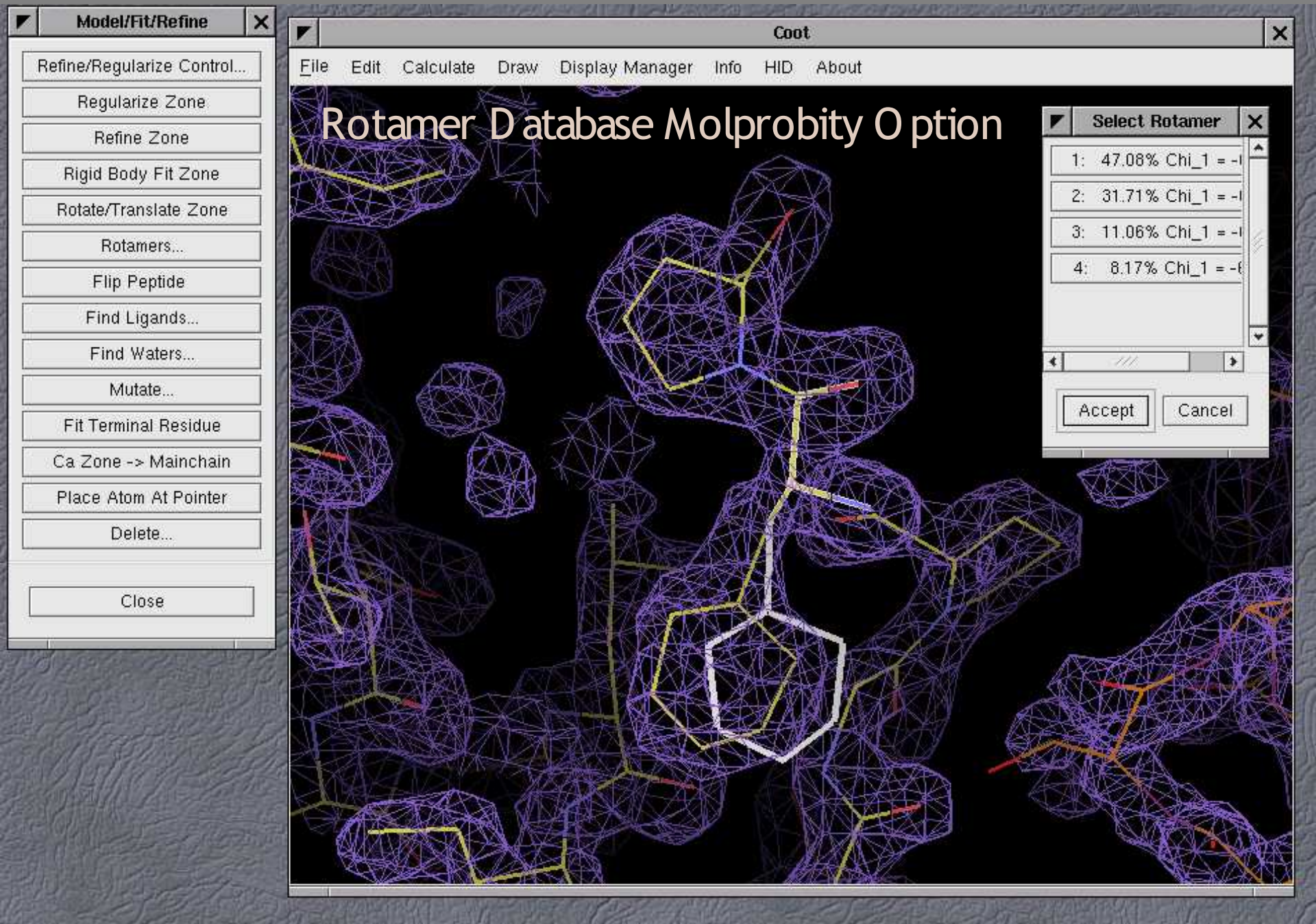
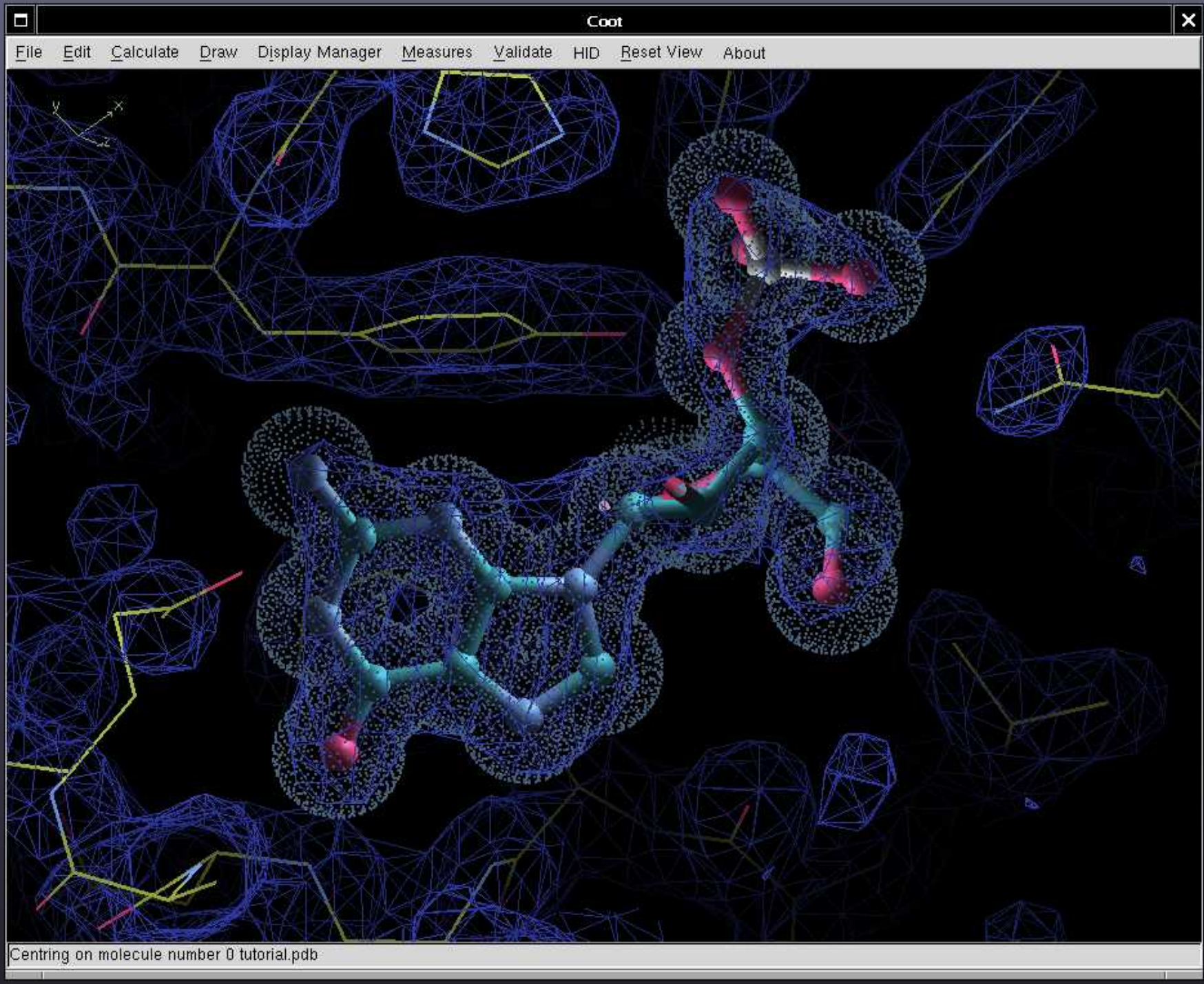
Calculate
Draw
Display Manager
Info
HID
About
Electron Density: “Continuous Crystal”


WinCoot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia Unittesting
Reset View
Display Manager
Sphere Refine +
Interactive dots
R/RC
Map
Rotamer Database Molprobity Option
Select Rotamer
1: m-85 43.00% Chi_1 = -65.00
2: t80 34.00% Chi_1 = 176.00
3: p90 13.00% Chi_1 = 63.00
4: m -30 9.00% Chi_1 = -64.00
OK
Cancel
CA /55 TYR/A
(mol. no: 0) CG /1/A/55 TYR occ: 1.00 bf: 14.64 ele: C pos: (61.48, 1.88,13.48)


Model/Fit/Refine
Refine/Regularize Control...
Regularize Zone
Refine Zone
Rigid Body Fit Zone
Rotate/Translate Zone
Rotamers...
Flip Peptide
Find Ligands...
Find Waters...
Mutate...
Fit Terminal Residue
Ca Zone -> Mainchain
Place Atom At Pointer
Delete...
Close
Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About
Rotamer Database Molprobity Option
Select Rotamer
1: 47.08% Chi_1 = -
2: 31.71% Chi_1 = -
3: 11.06% Chi_1 = -
4: 8.17% Chi_1 = -
Accept
Cancel

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


Coot
File
Edit
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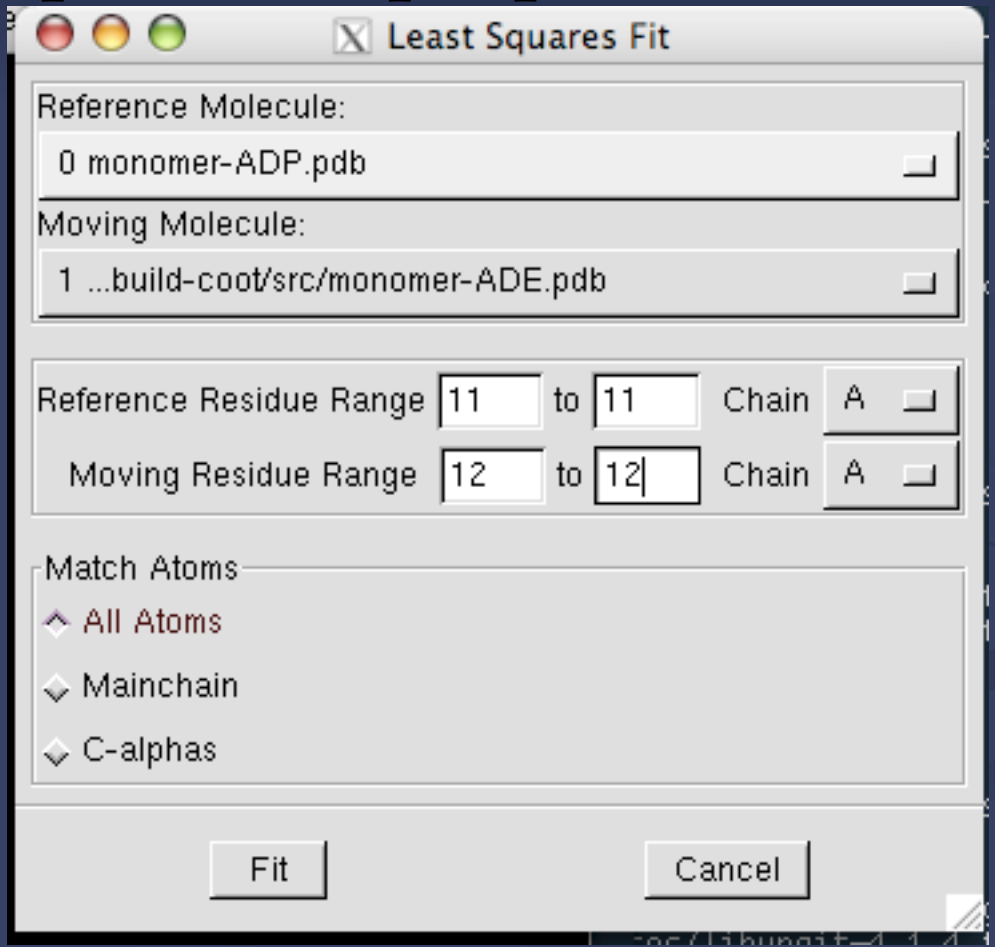
Draw
Display Manager
Measures
Validate
HID
Reset View
About
Centring on molecule number 0 tutorial.pdb

Superpositions

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



Low Resolution Tools

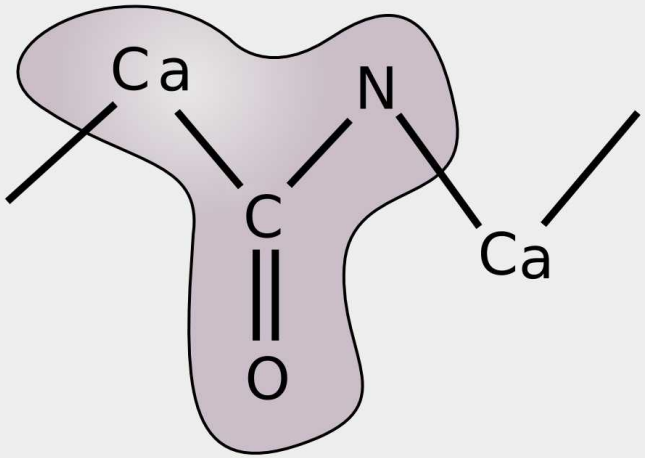
Extra Restraints....

Extra Restraints....

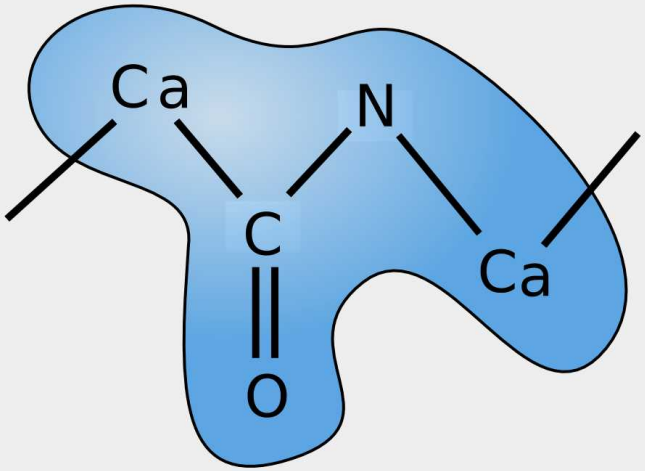
- Peptide plane
- Ramachandran restraints
- Secondary structure restraints
- Remove degree of freedom
  - Torsion angle restraints
  - Backrub rotamers
- Manually add restraints

Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane



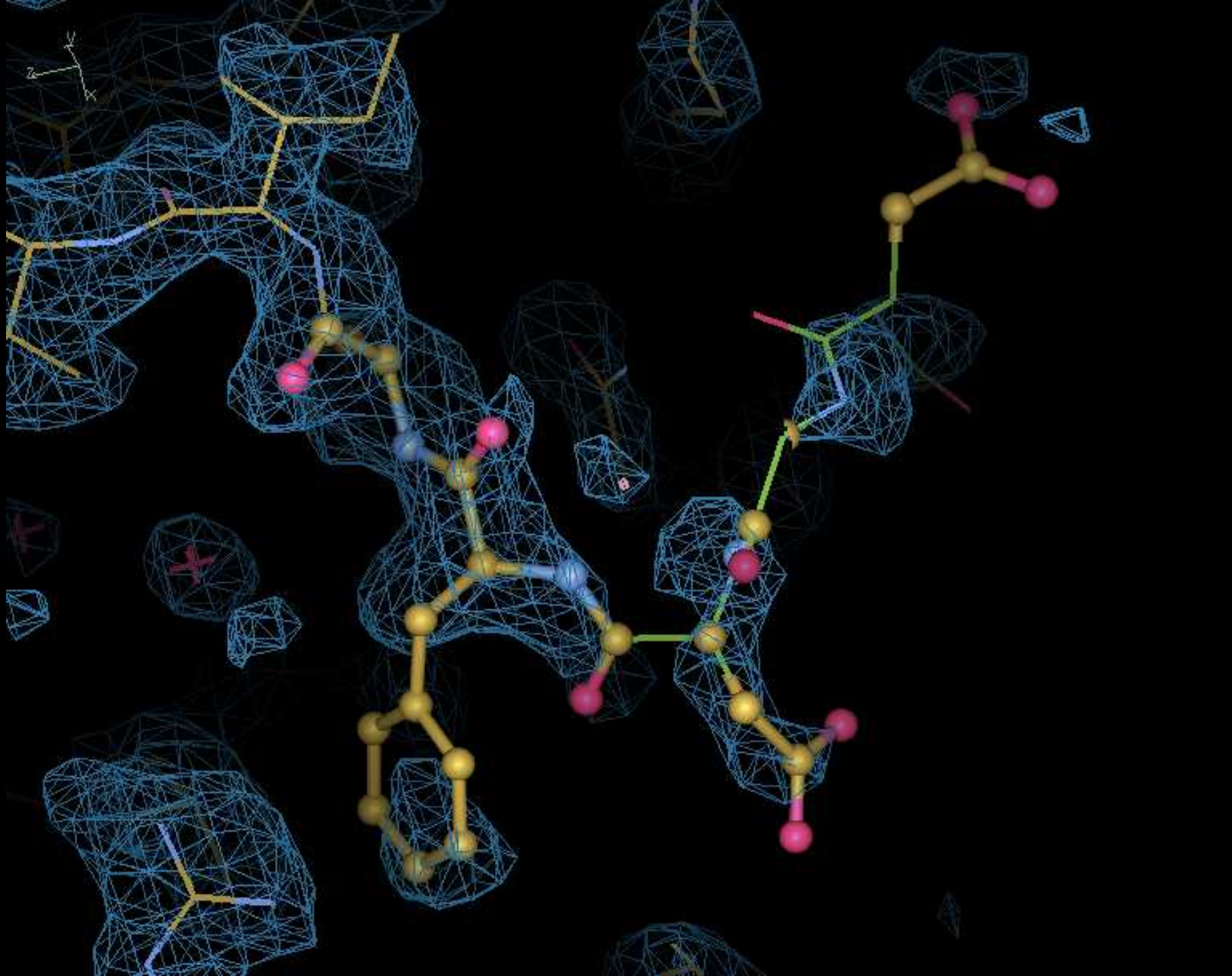
Extended Plane in Coot



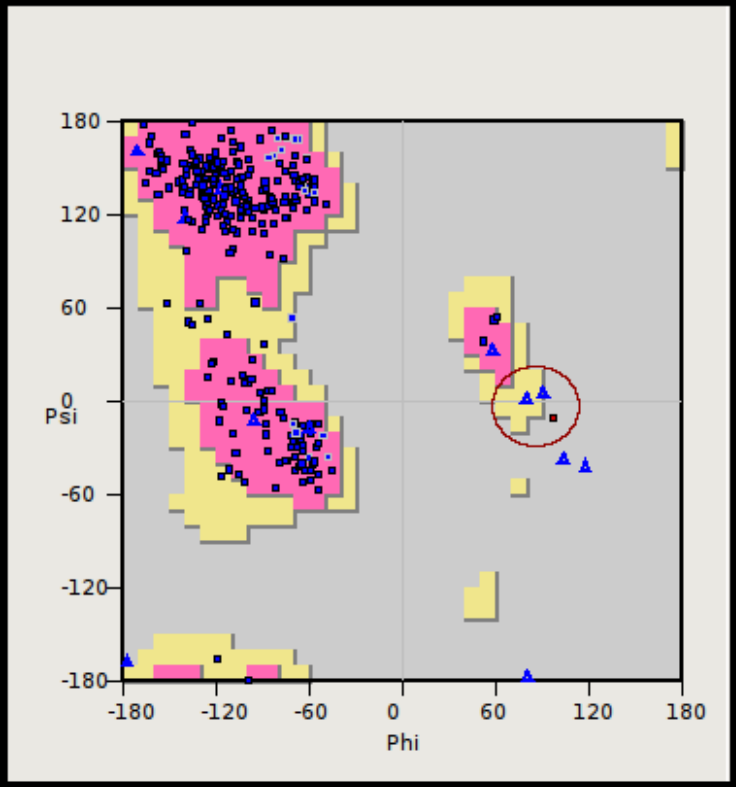
(add-planar-peptide-restraints)

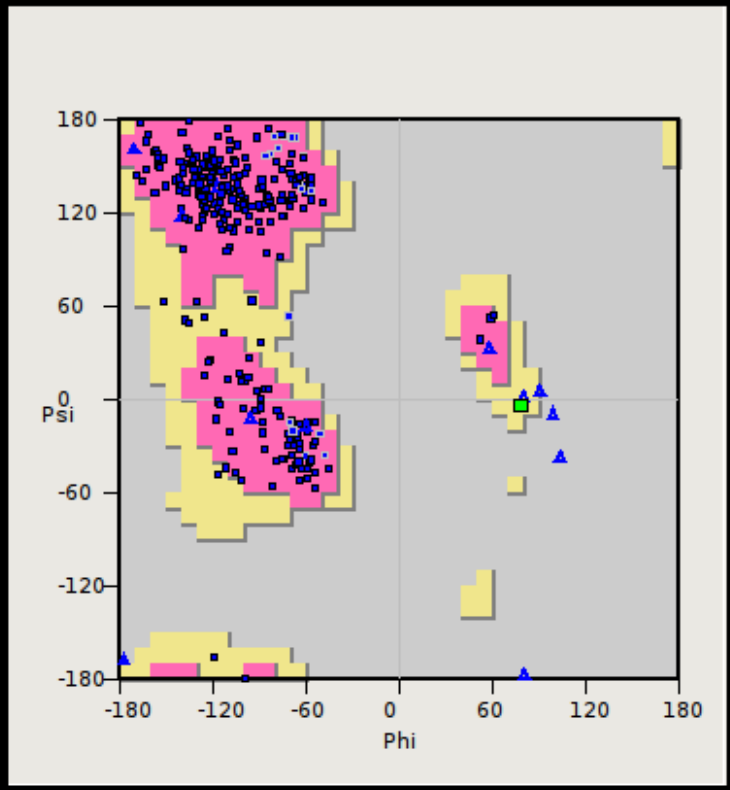
Ramachandran Restraints

- 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

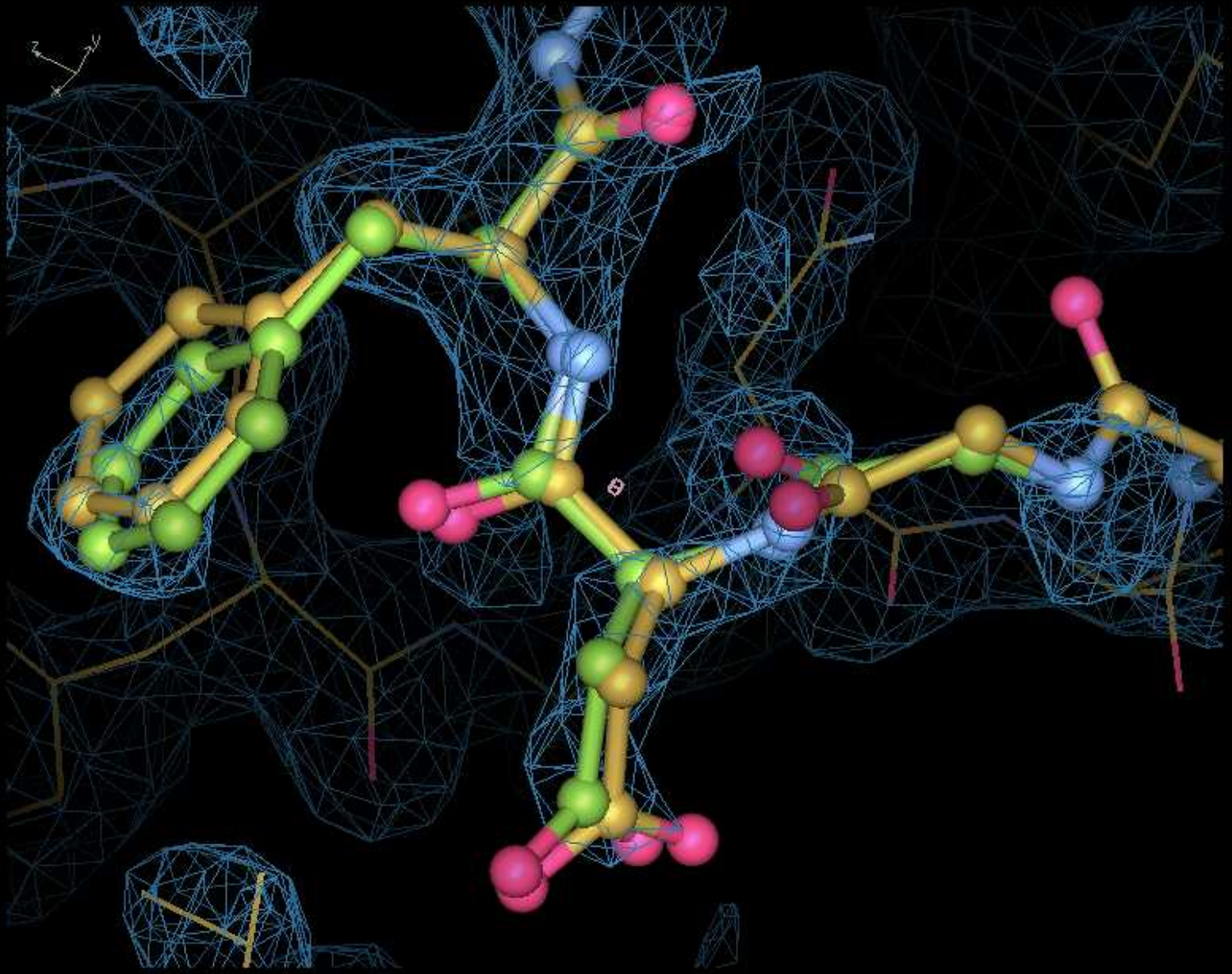


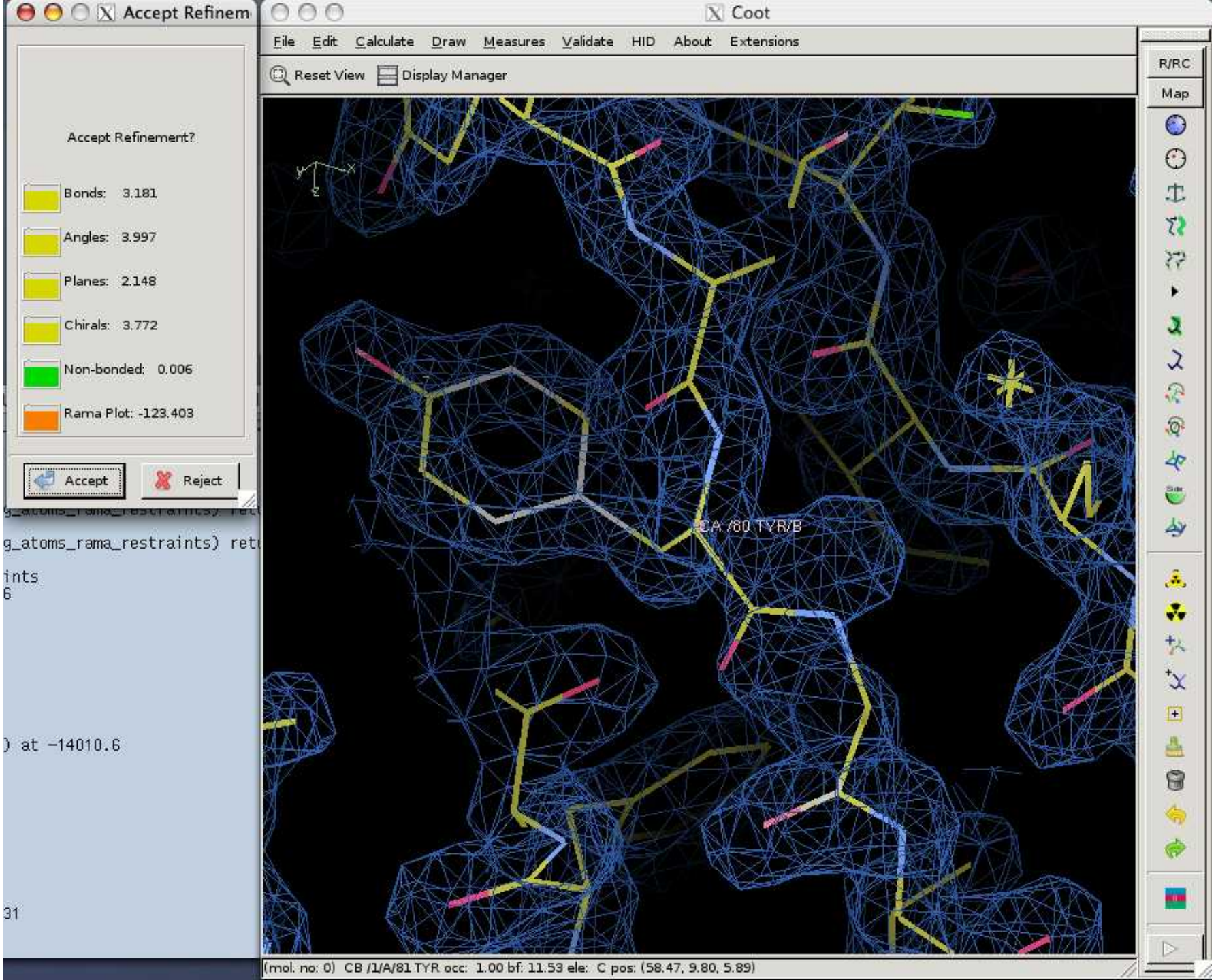
Tweaking a Ramachandran Outlier

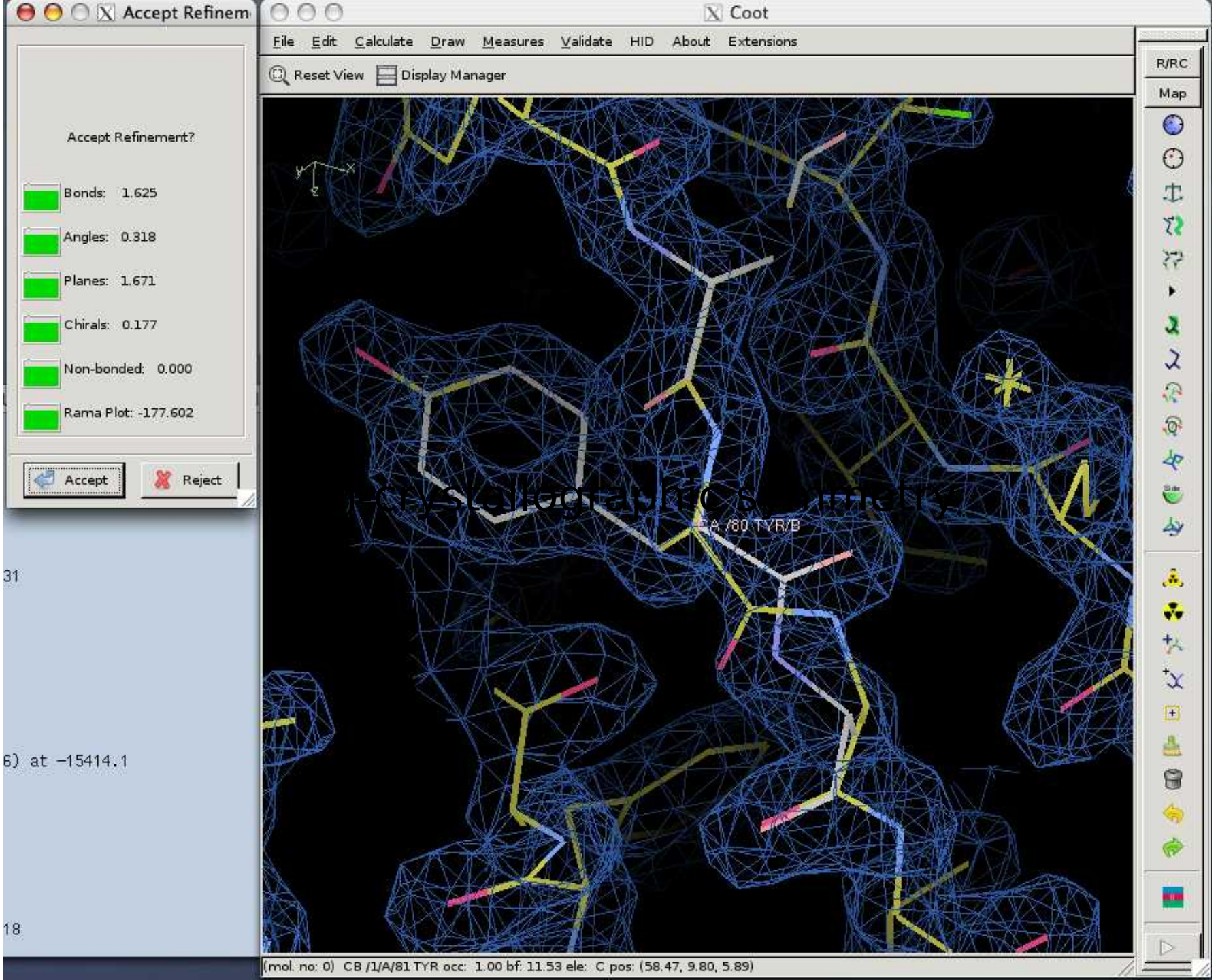




Tweaking Phi and Psi




Accept Refinem
Accept Refinement?
Bonds: 3.181
Angles: 3.997
Planes: 2.148
Chirals: 3.772
Non-bonded: 0.006
Rama Plot: -123.403
Accept
Reject
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
CA /80 TYR/B
(mol. no: 0) CB /1/A/81 TYR occ: 1.00 bf: 11.53 ele: C pos: (58.47, 9.80, 5.89)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Accept Refinement?
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Angles: 0.318
Planes: 1.671
Chirals: 0.177
Non-bonded: 0.000
Rama Plot: -177.602
Accept
Reject
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
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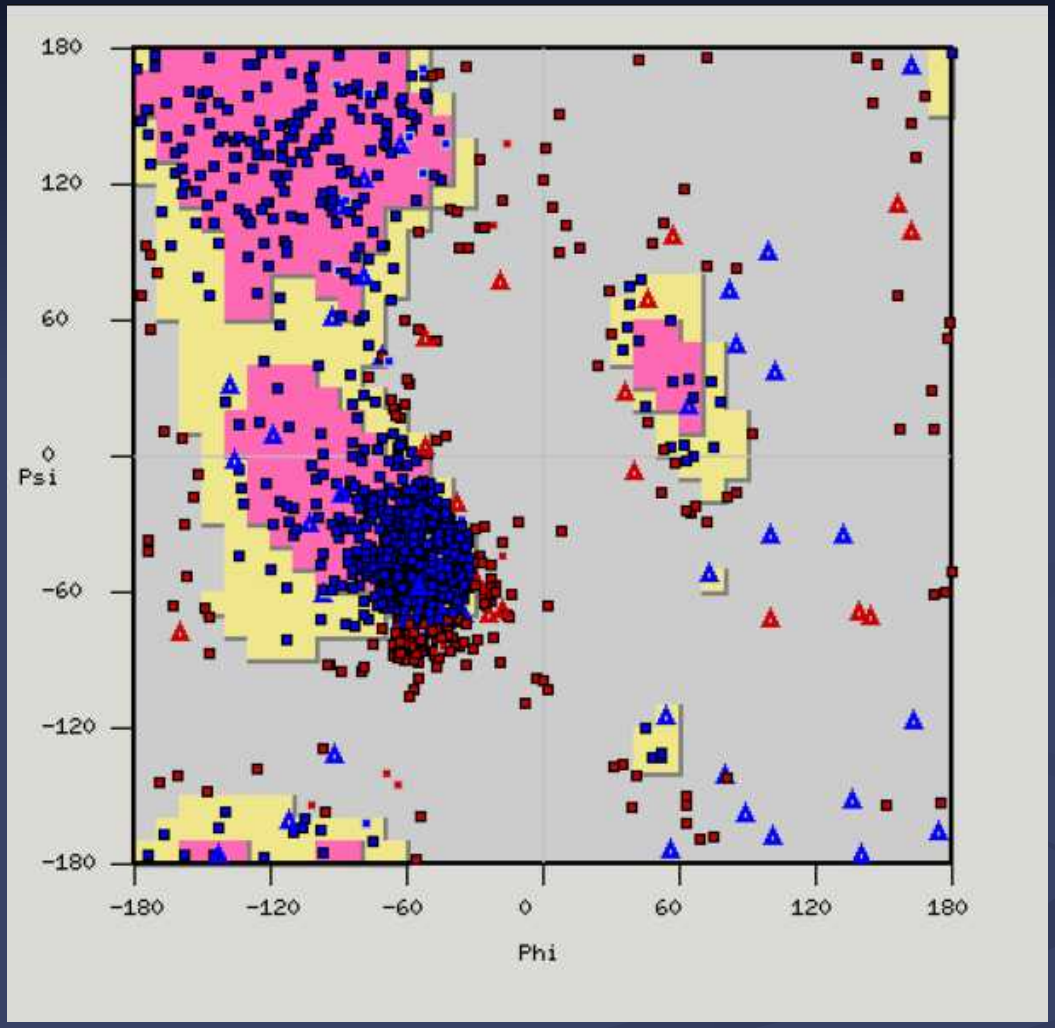
/80 TYR/B
(mol. no: 0) CB /1/A/81 TYR occ: 1.00 bf: 11.53 ele: C pos: (58.47, 9.80, 5.89)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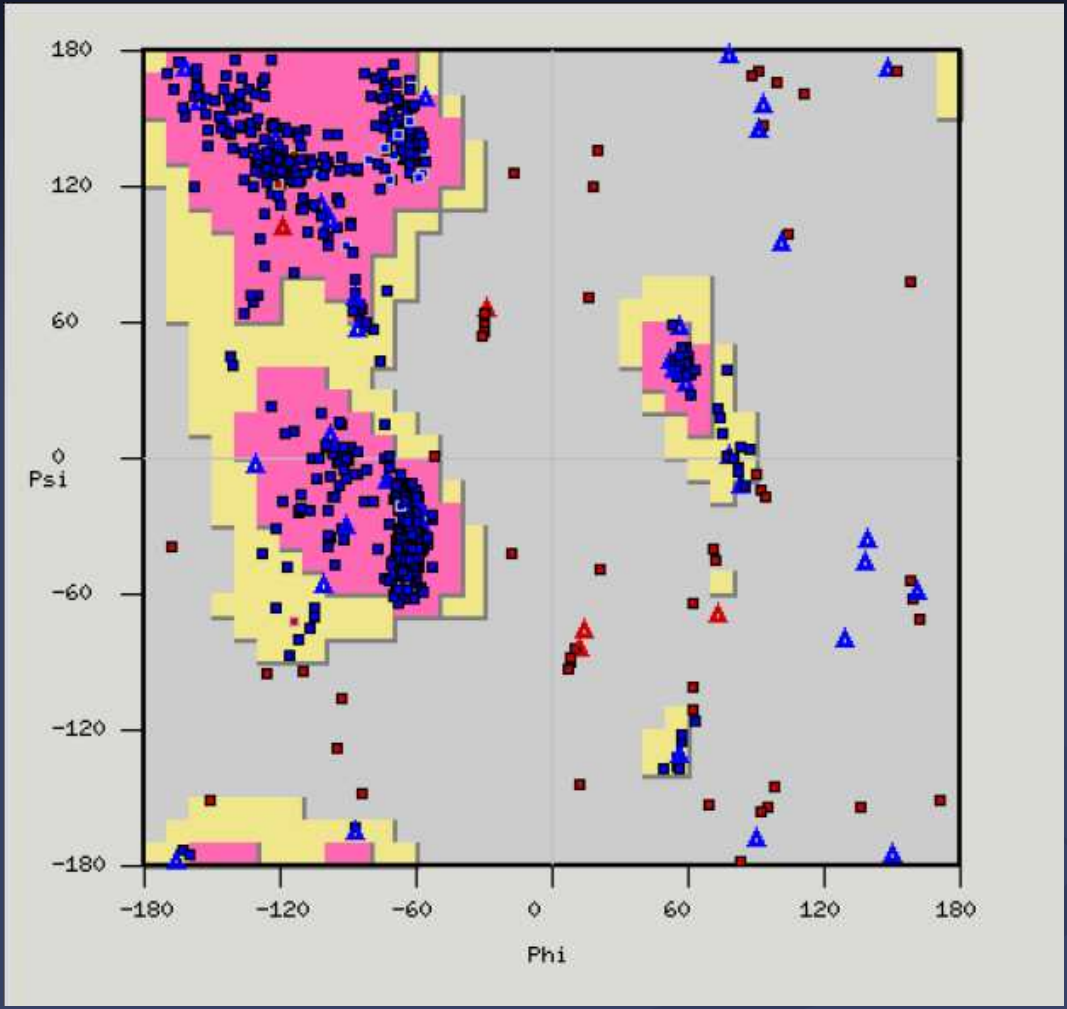
120
60
0
Psi
-60
-120
-180
-180
-120
-60
0
60
120
180
Phi


180
120
60
0
Psi
-60
-120
-180
-180
-120
-60
0
60
120
180
Phi

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?

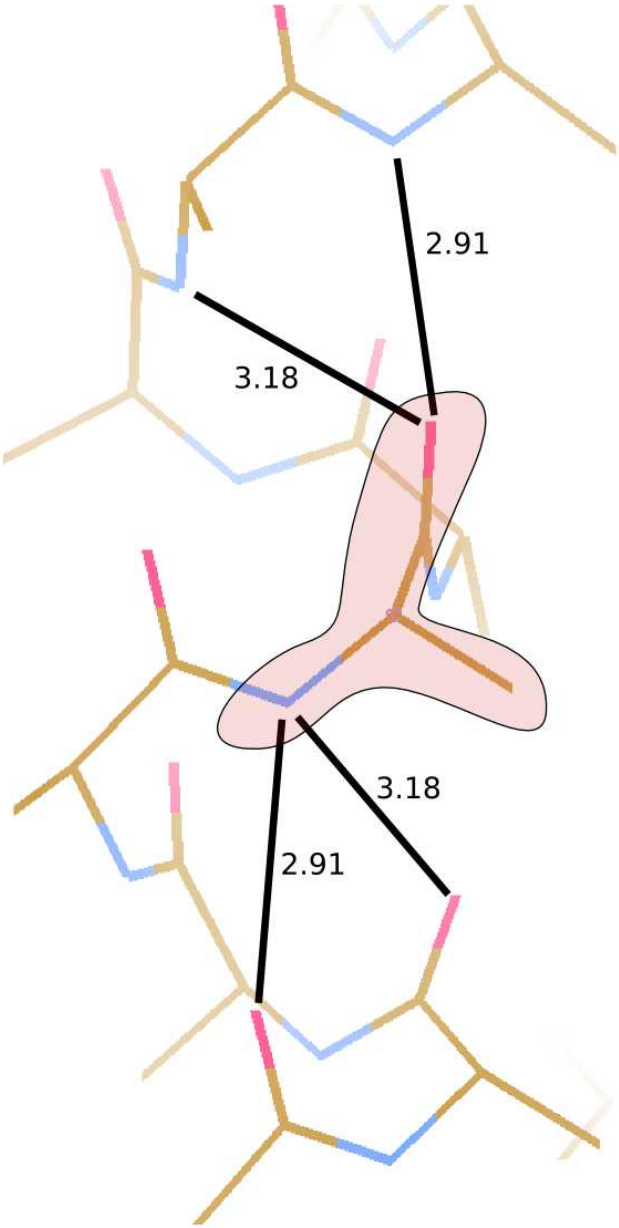
Adding Torsion Angle Restraints

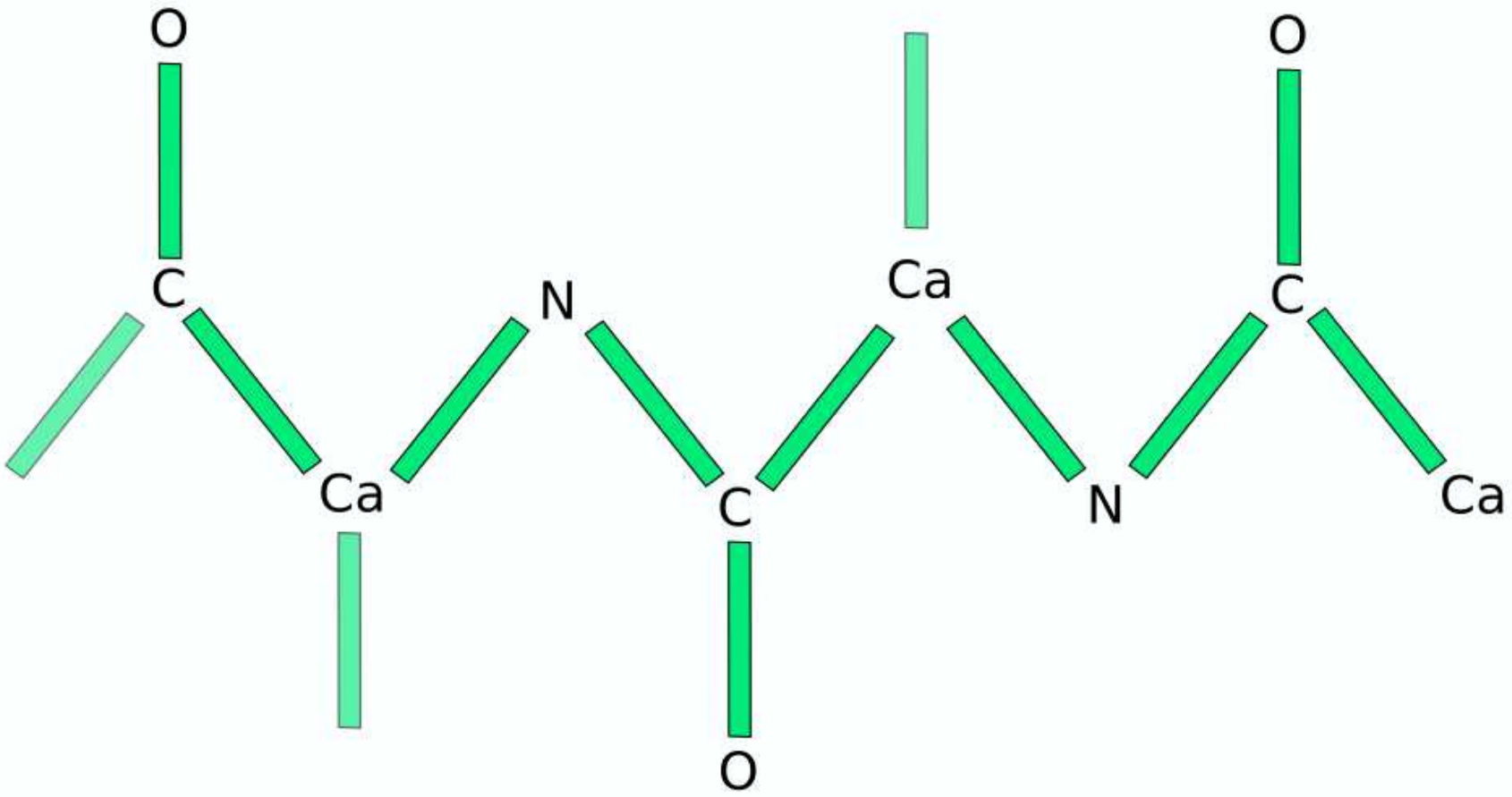
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”
    - i.e. trapped in local minimum

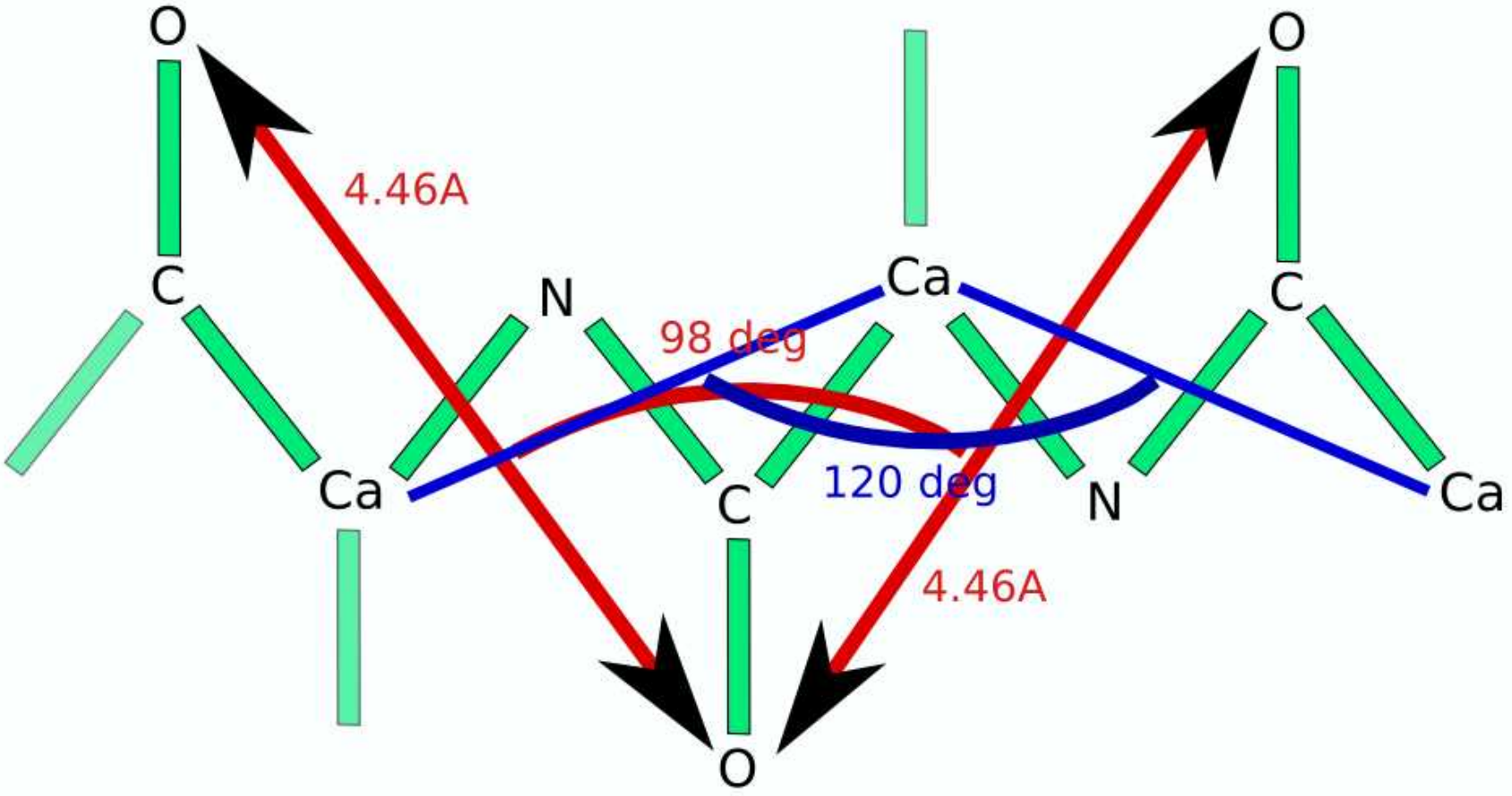
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints

Restrain the Hydrogen-bonding atom distances



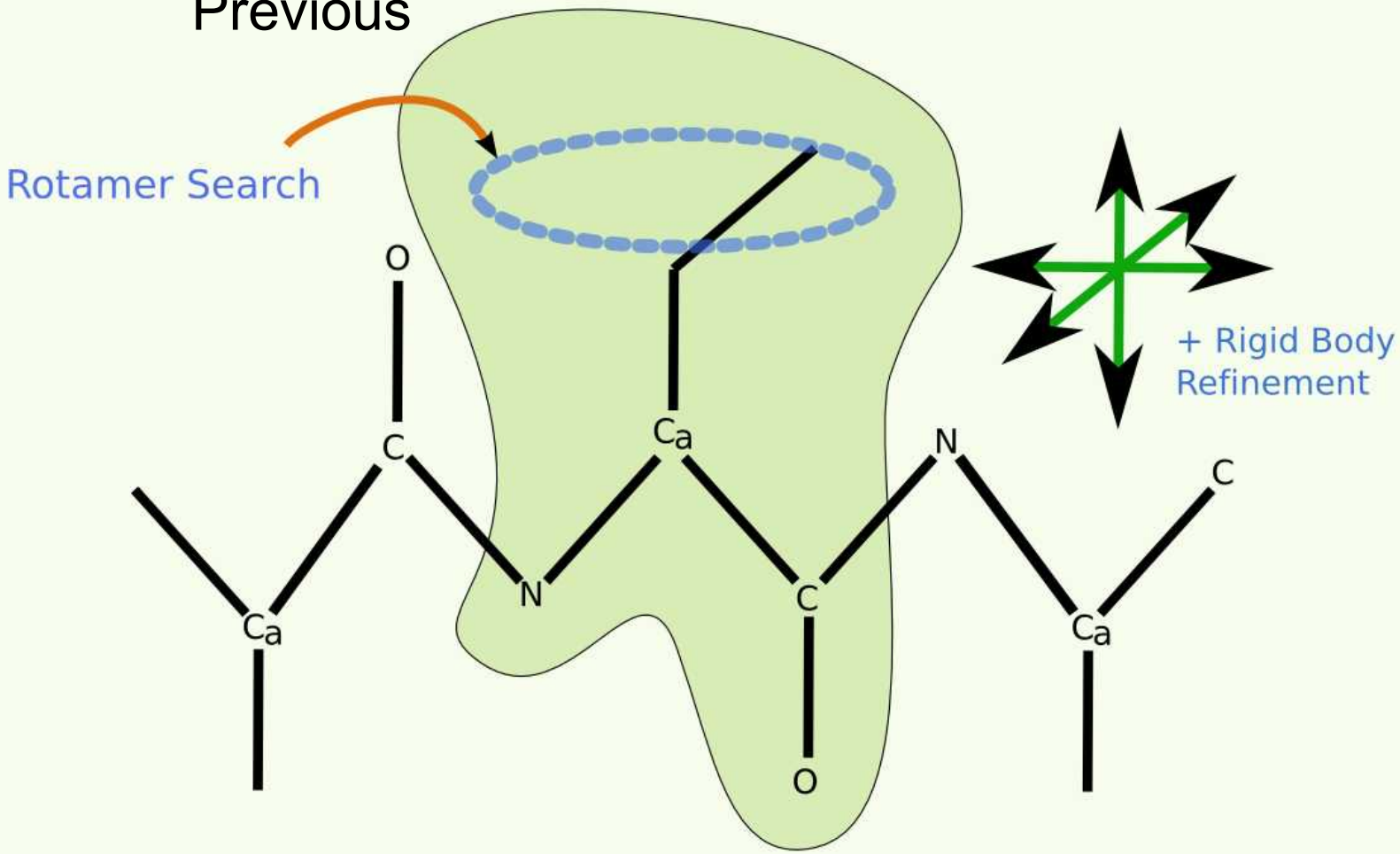

O
C
Ca
N
Ca
C
O
O
C
N
Ca

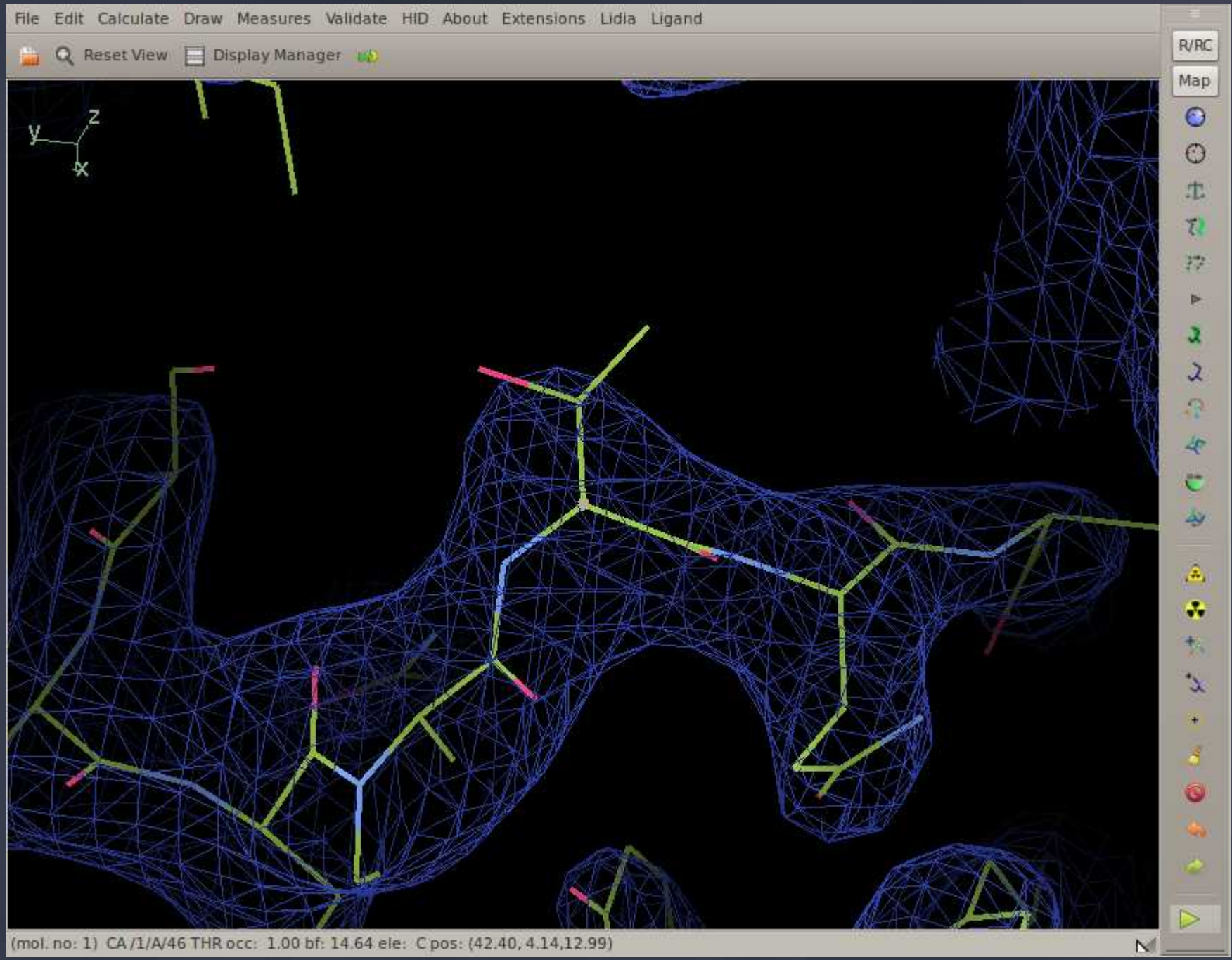

O
4.46A
C
N
Ca
98 deg
Ca
O
C
120 deg
N
Ca
4.46A
O

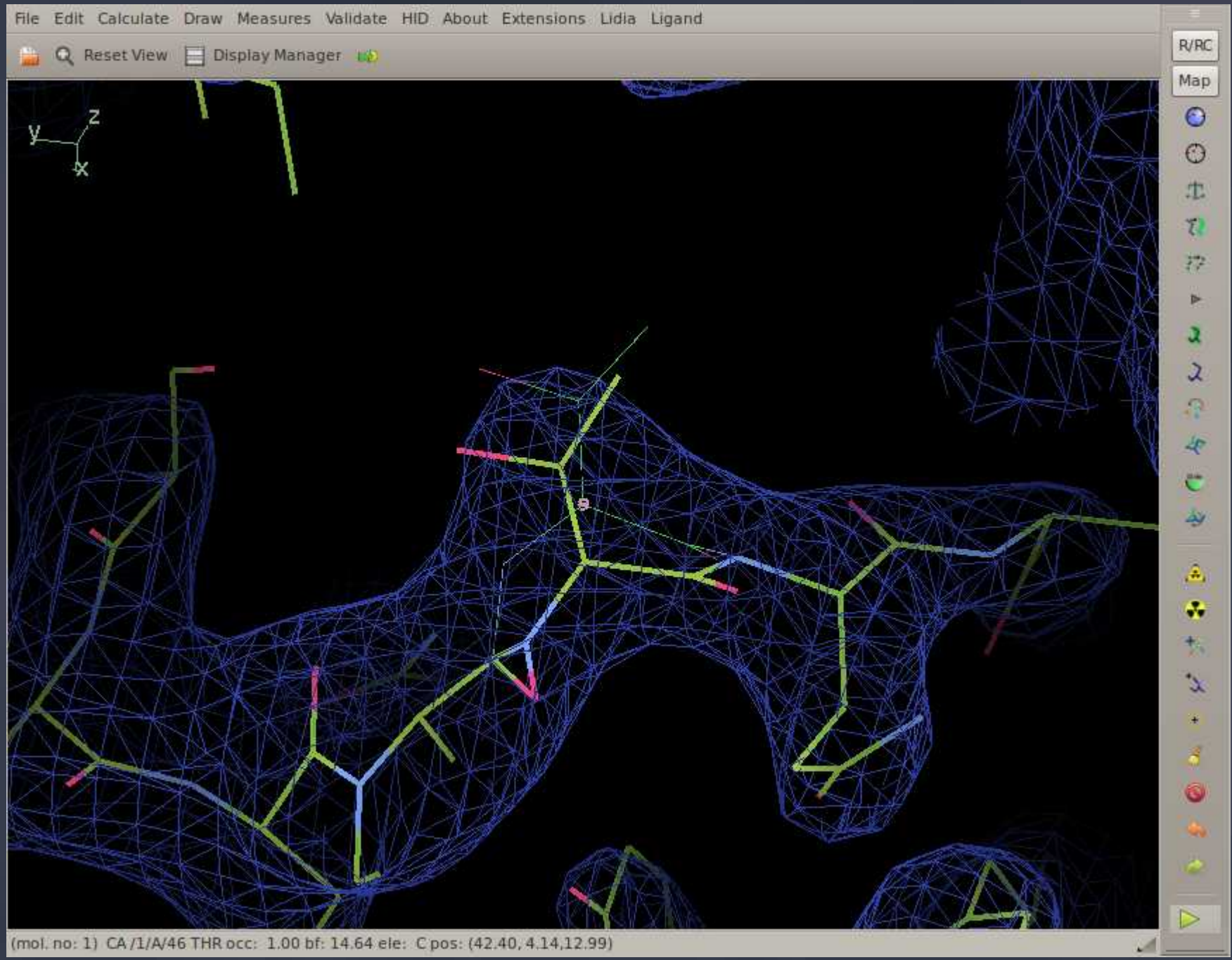
“Backrub” Rotamers

~~Current~~ Low Resolution Rotamer Search

Previous




File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
Display Manager
R/RC
Map
z
y
x
(mol. no: 1) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)


File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
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Map
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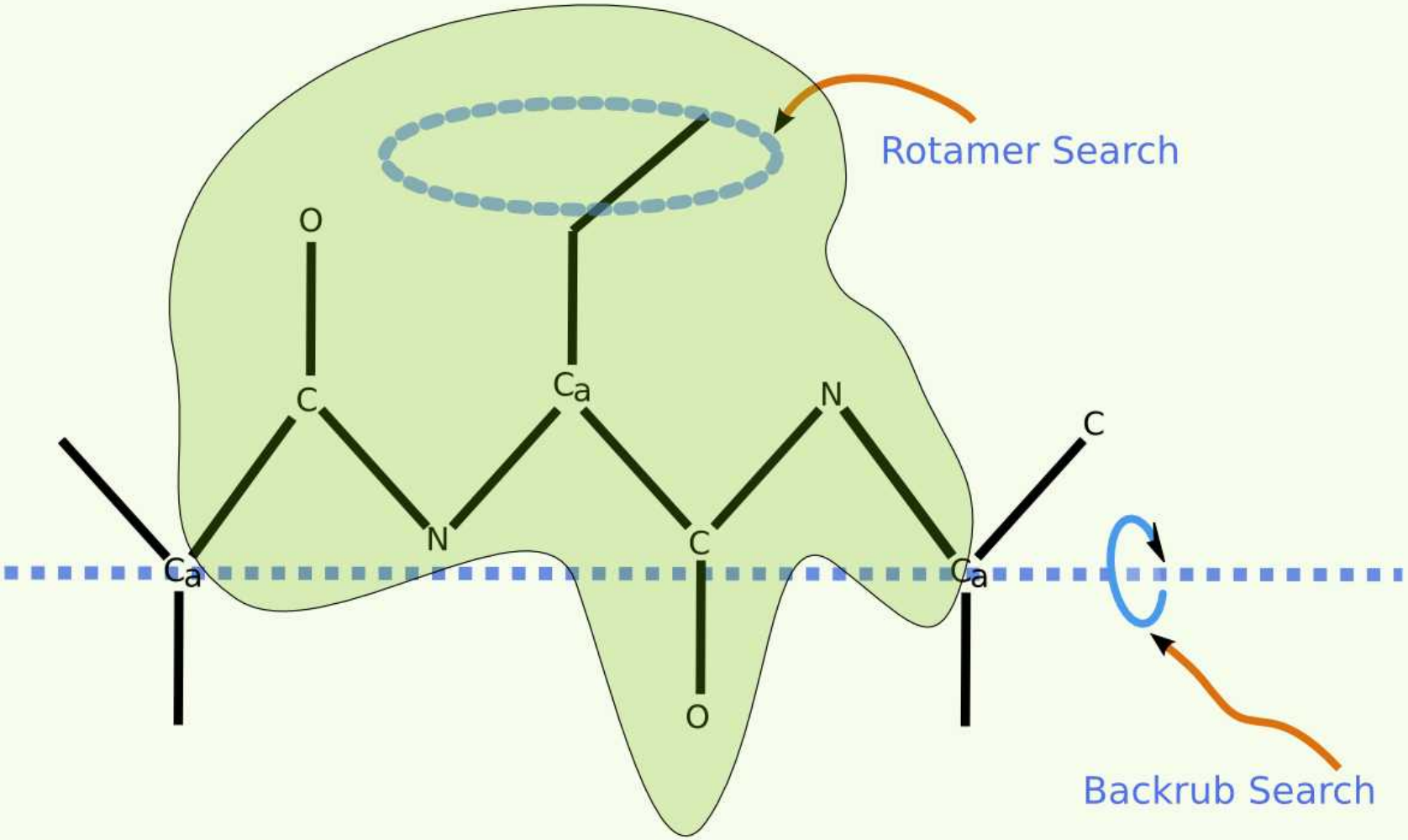
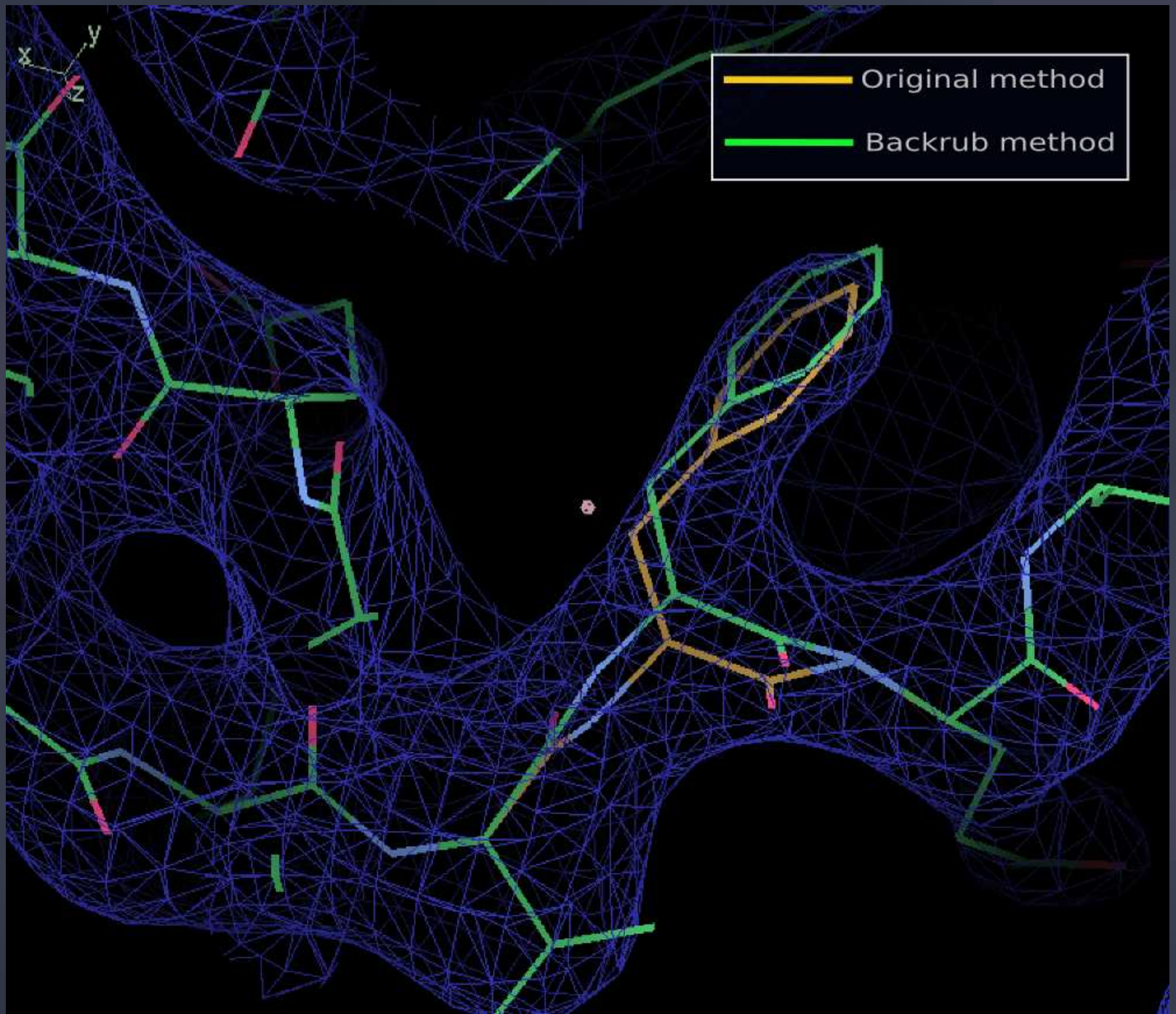
(mol. no: 1) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)

New Low Resolution Rotamer Search



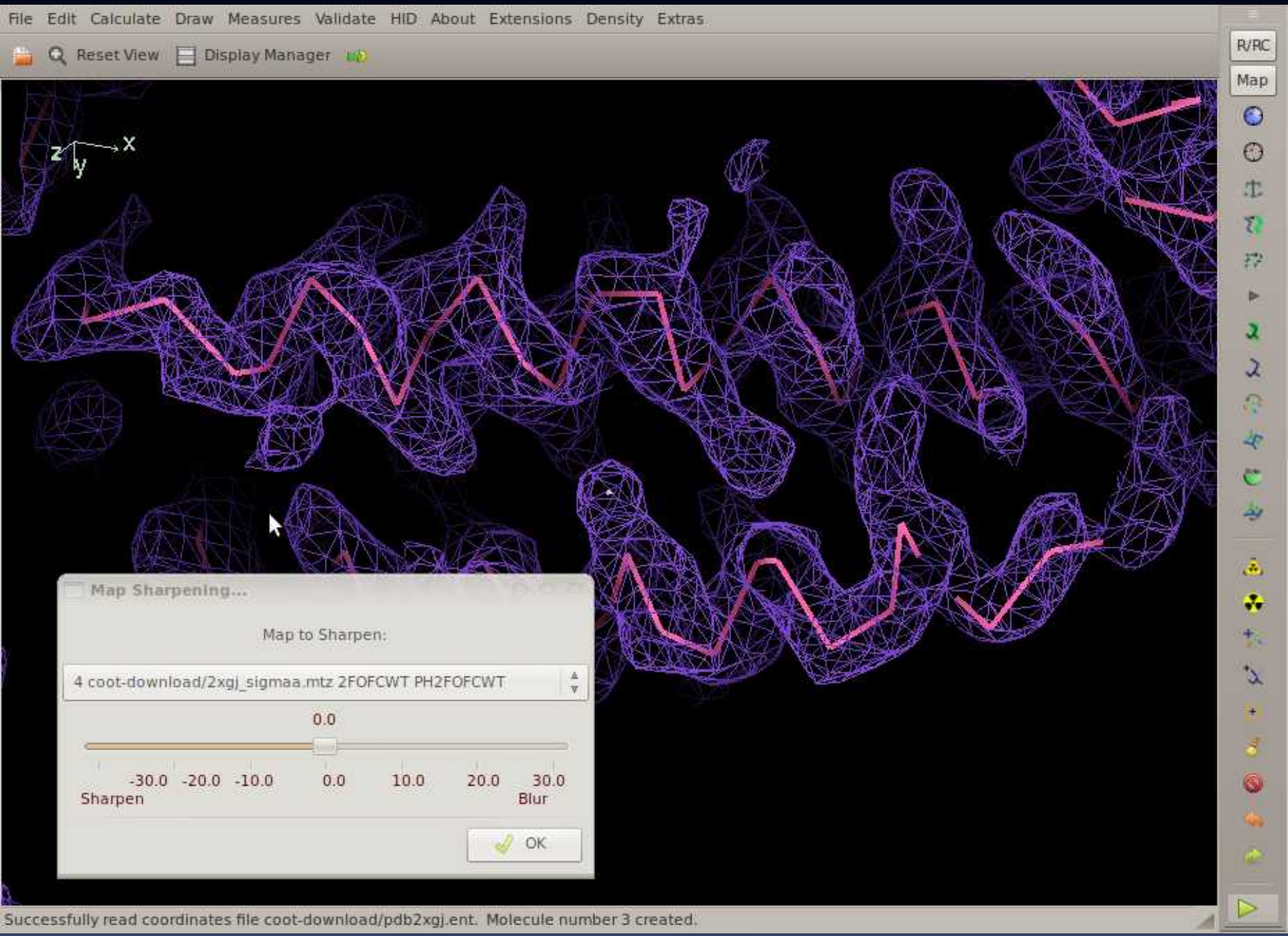
After Fitting Tools in KING/Molprobity

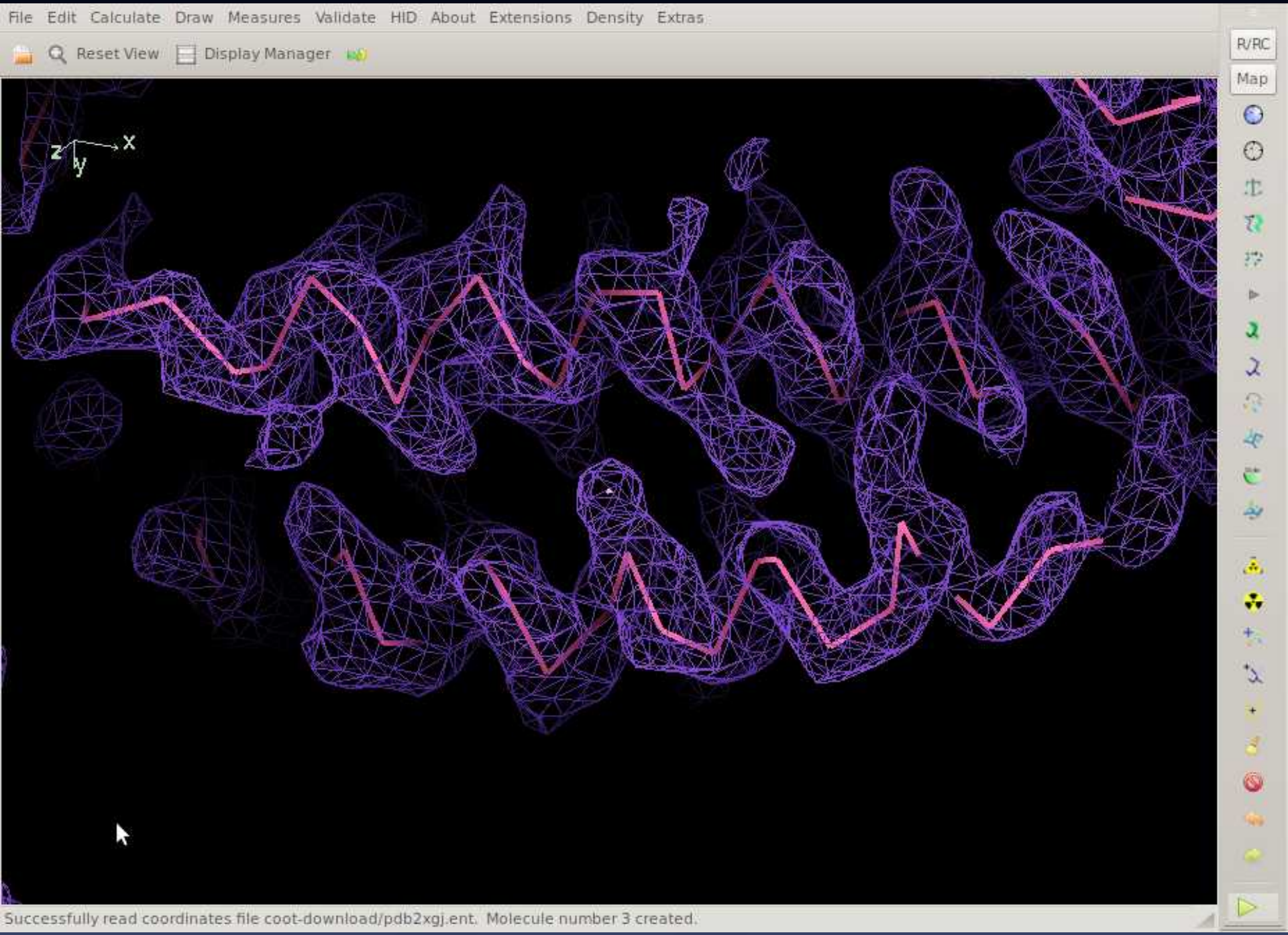

x
y
z
Original method
Backrub method

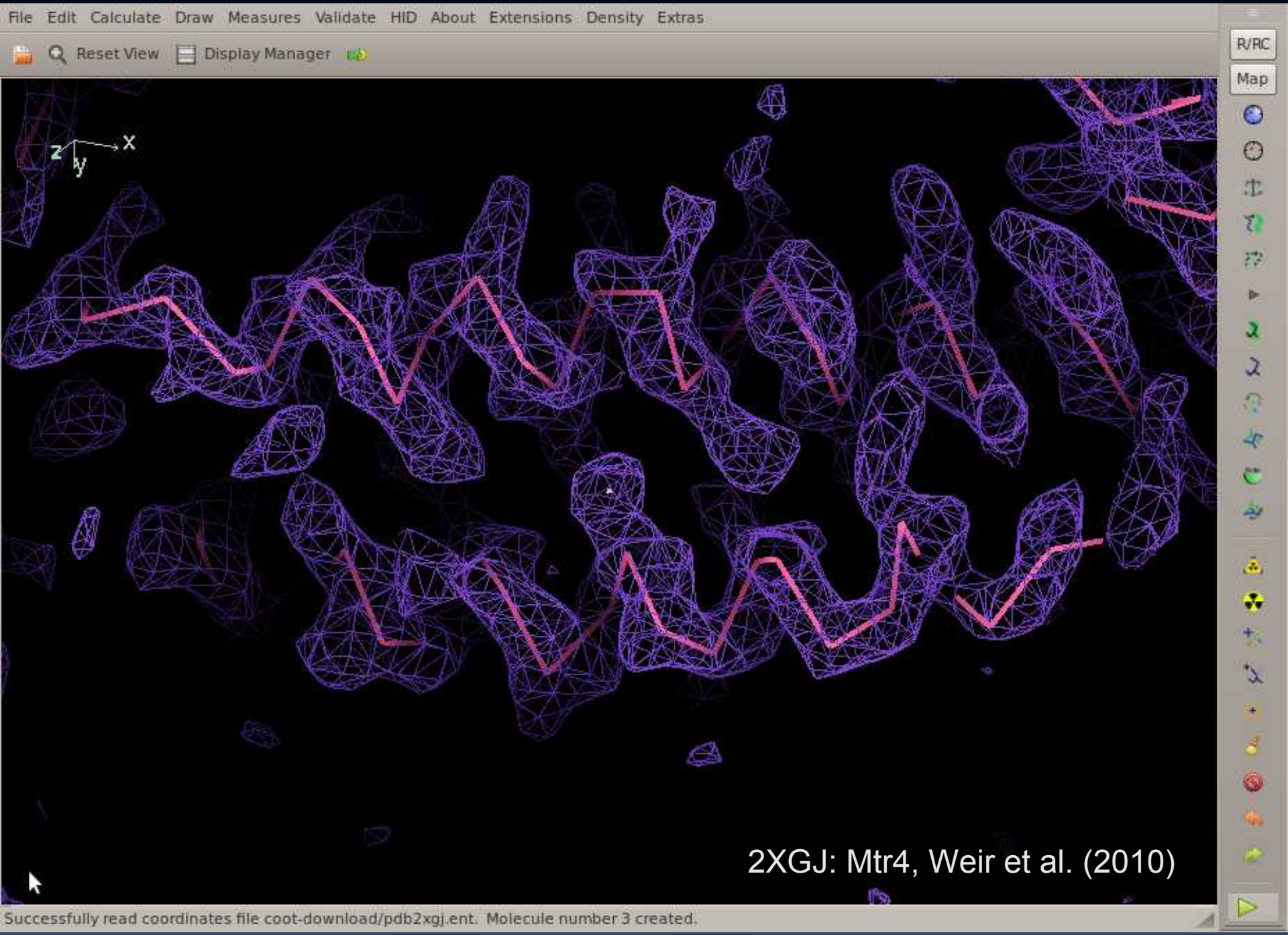
Map Sharpening

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...


File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
Map Sharpening...
Map to Sharpen:
4 coot-download/2xgj_sigmaa.mtz 2FOFCWT PH2FOFCWT
0.0
-30.0 -20.0 -10.0 0.0 10.0 20.0 30.0
Sharpen
Blur
OK
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.


File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.


File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
2XGJ: Mtr4, Weir et al. (2010)
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.

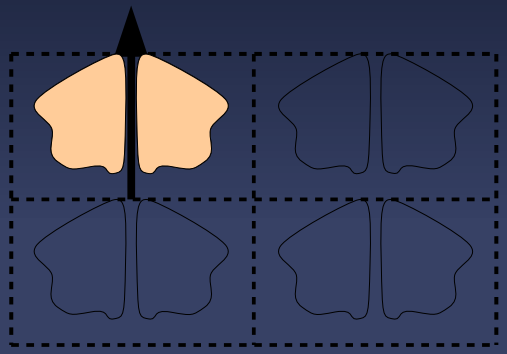
Handling NCS...

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

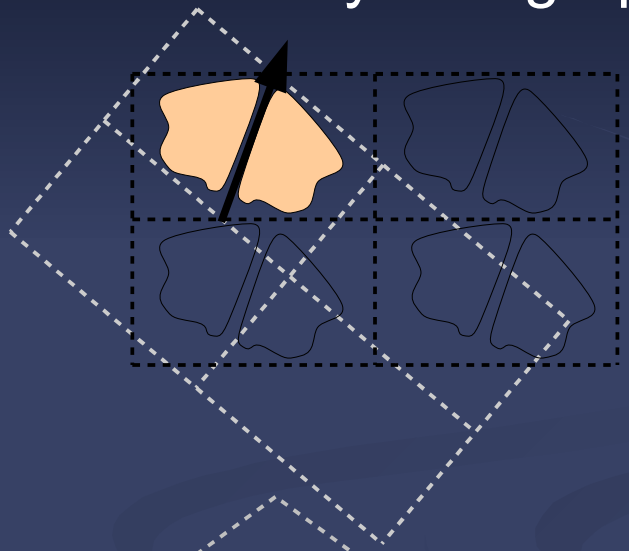
Non-crystallographic symmetry

Crystallographic

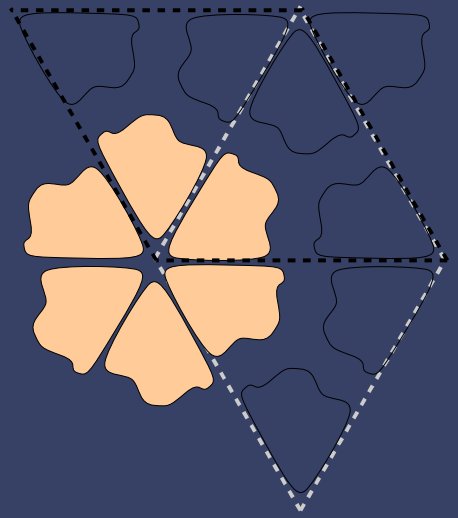


Aligned
2-fold

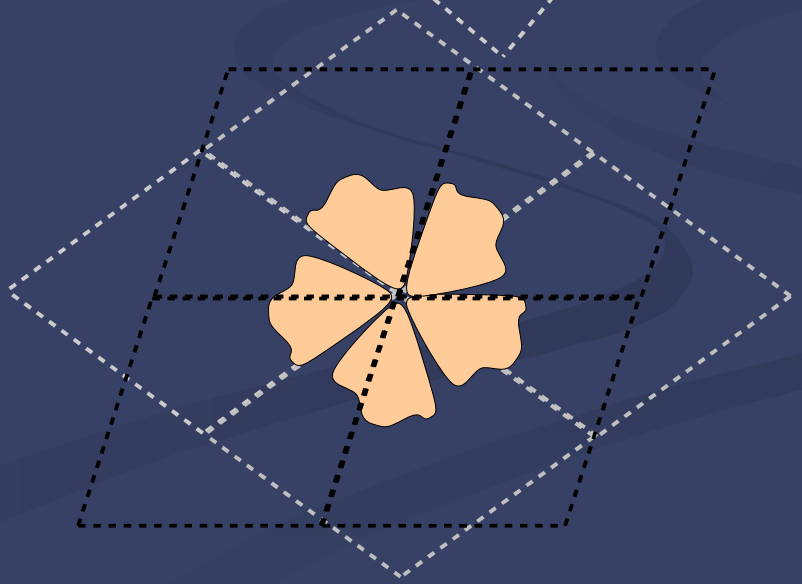
Non-crystallograpic



Unaligned
2-fold



Aligned
6-fold



Aligned
5-fold

Handling NCS

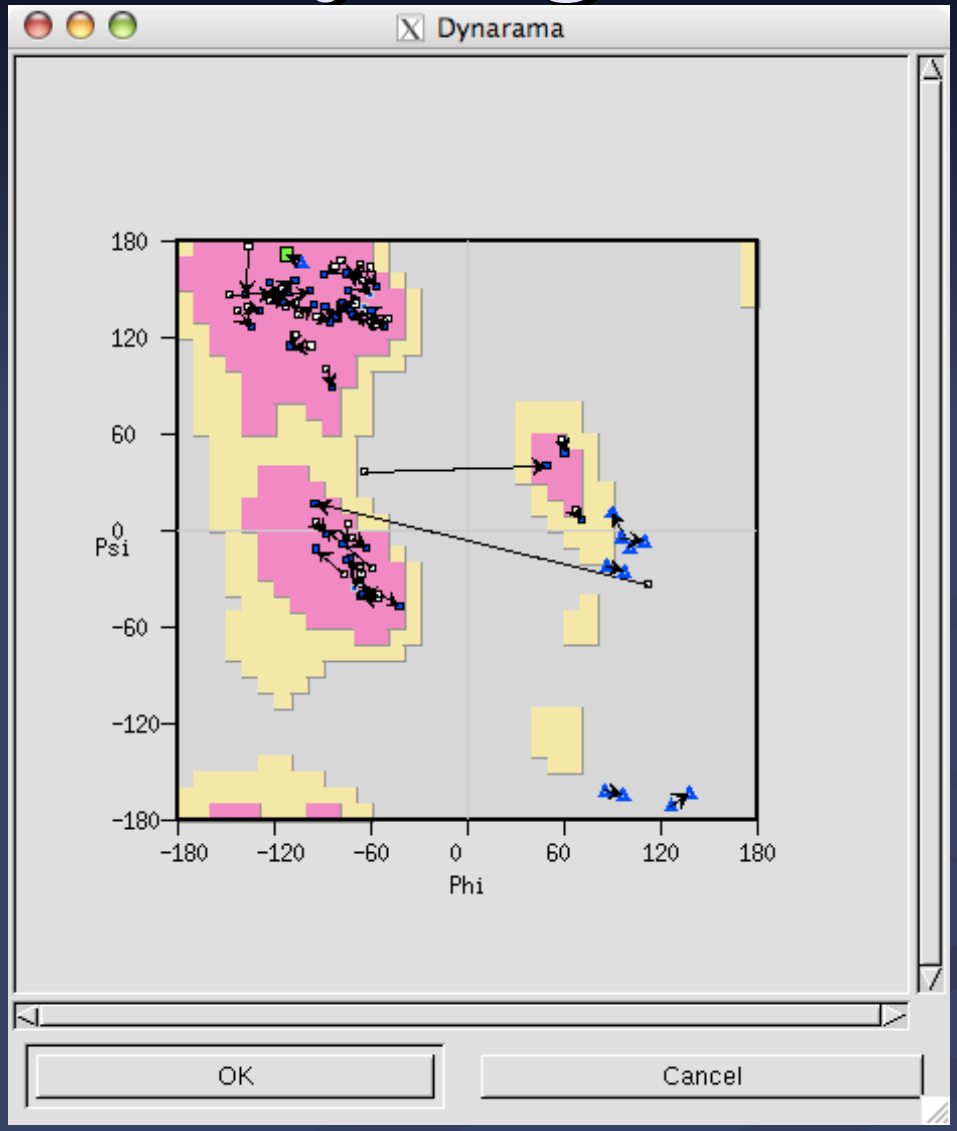
- What are the Problems?
- Strict NCS:
  - NCS should appear like crystallographic symmetry does [exact copies, e.g. virus]
- Non-Strict NCS:
  - Molecules are different
  - How to cope with differences, but minimize unnecessary rebuilding?

Handling NCS

Typical Scenario:

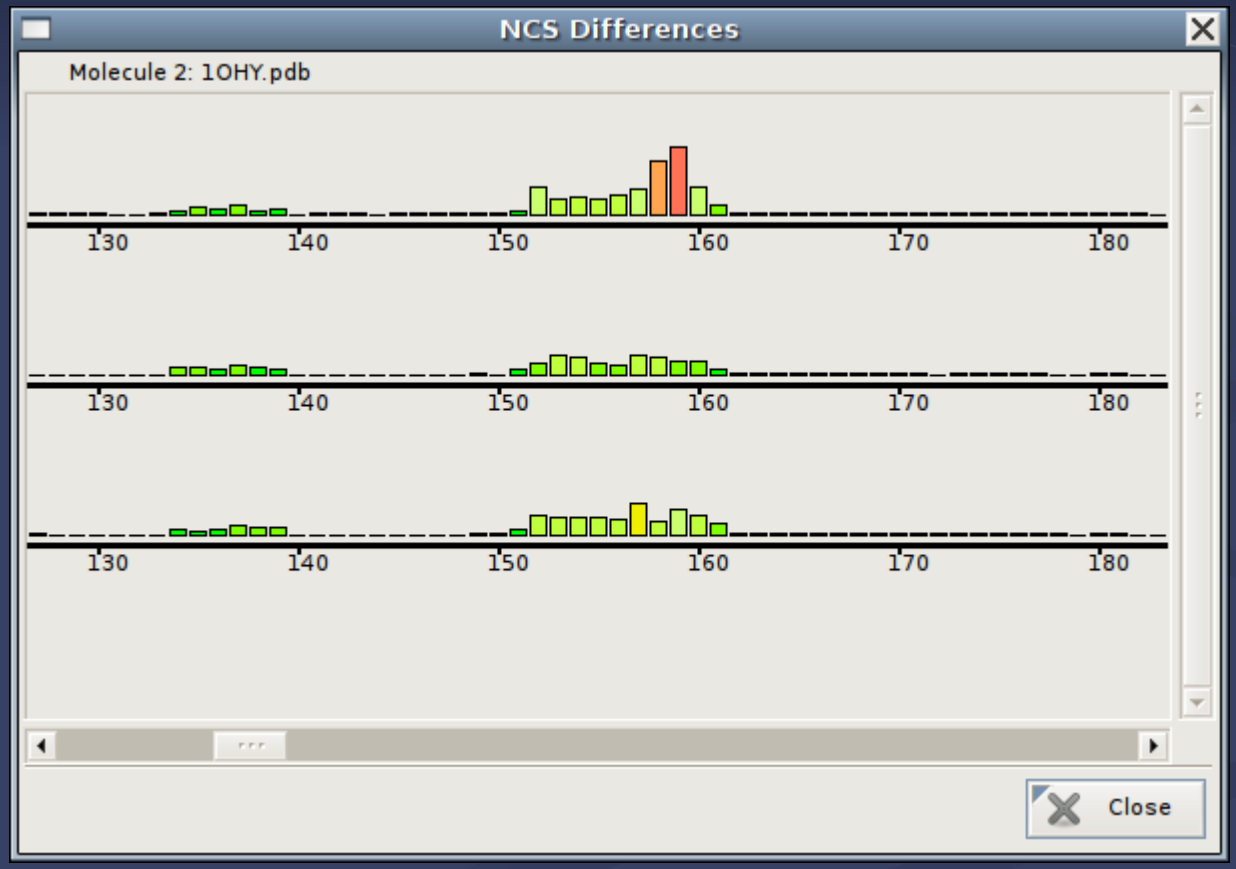
- I have done an LSQ overlap of my NCS-related molecules and from the graph, have seen significant deviations in the positions of some side-chains.
- Why are they different?

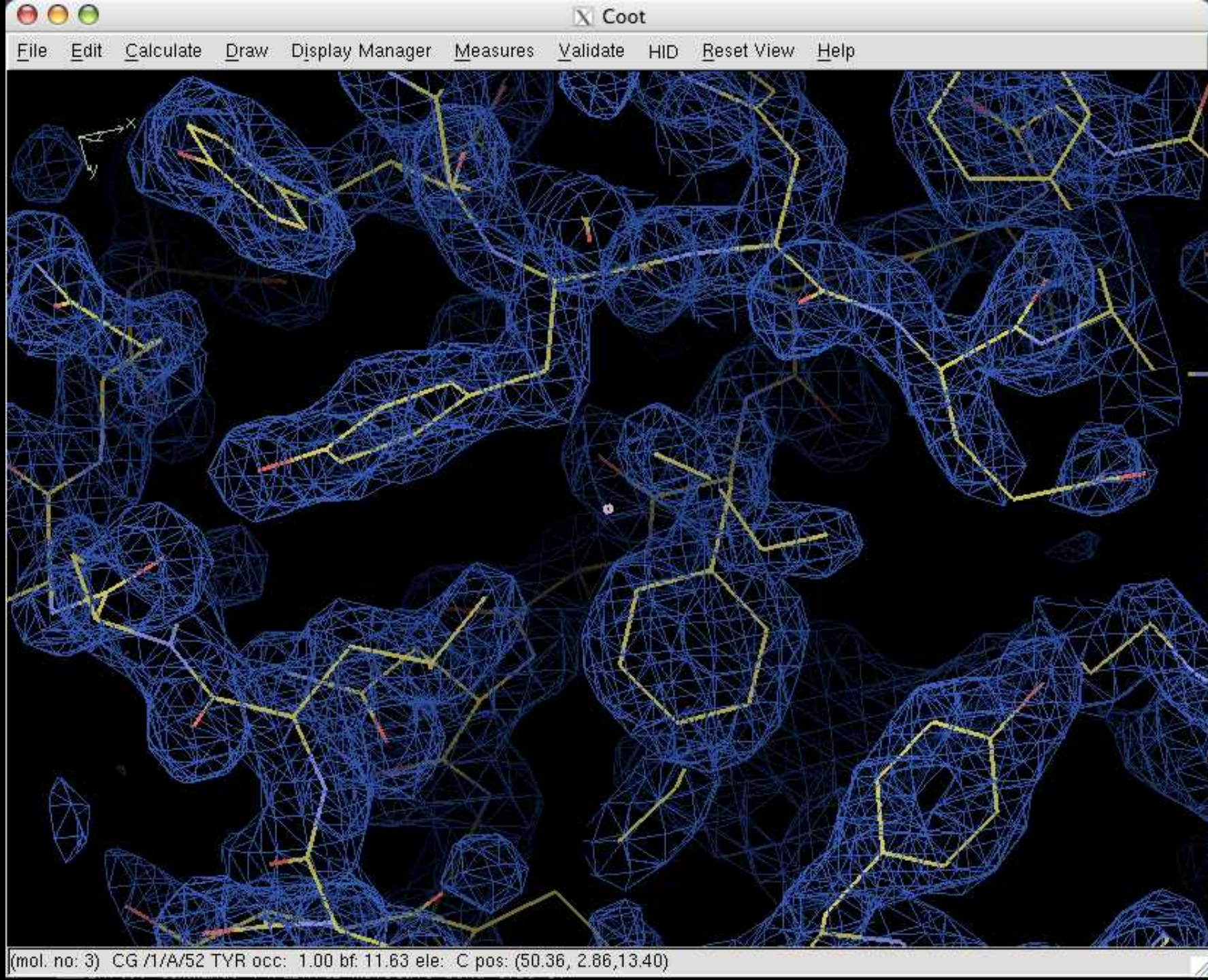
…or Kleywegt Plots[*]



[*] Named by George Sheldrick

...or NCS Differences graph

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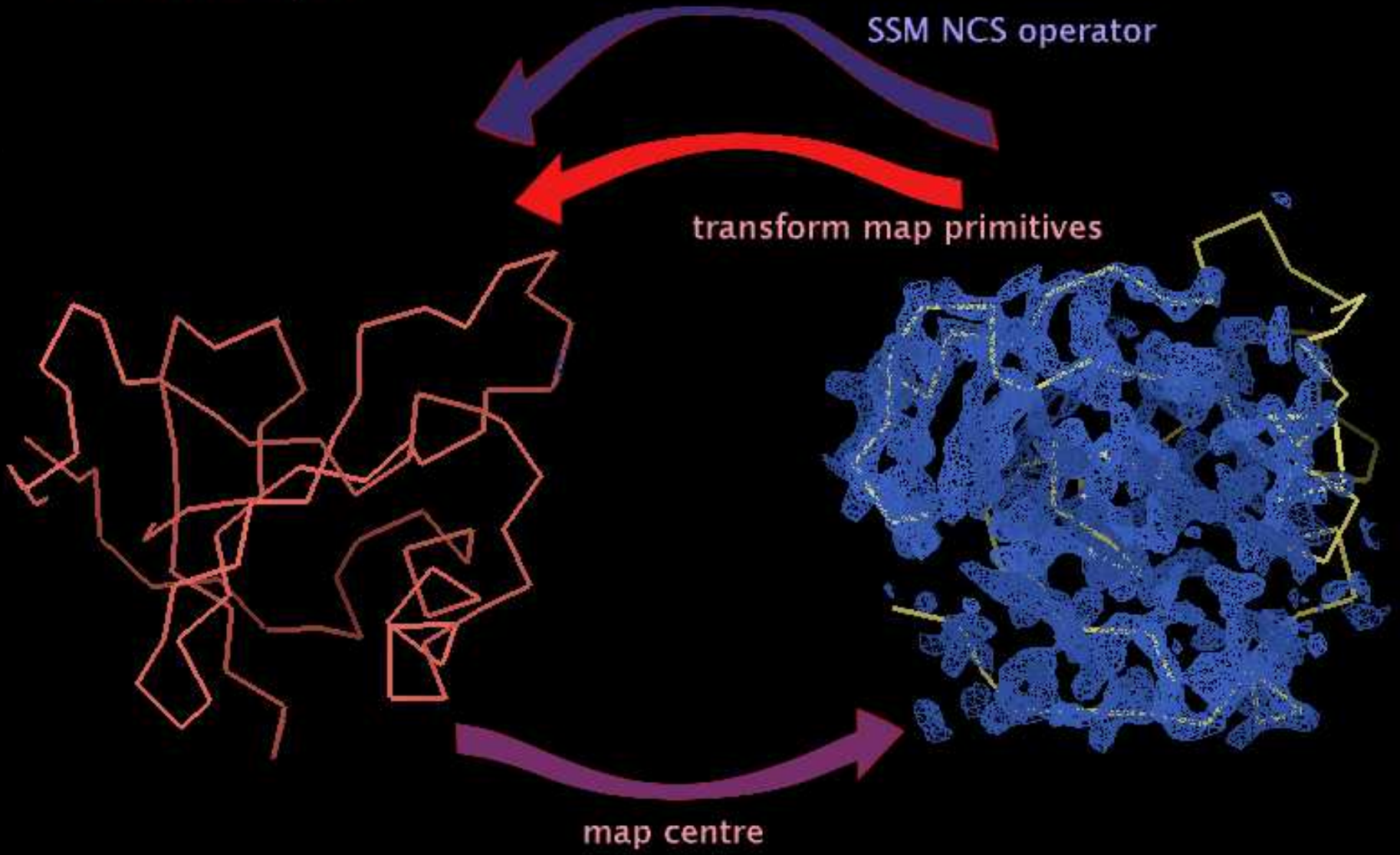
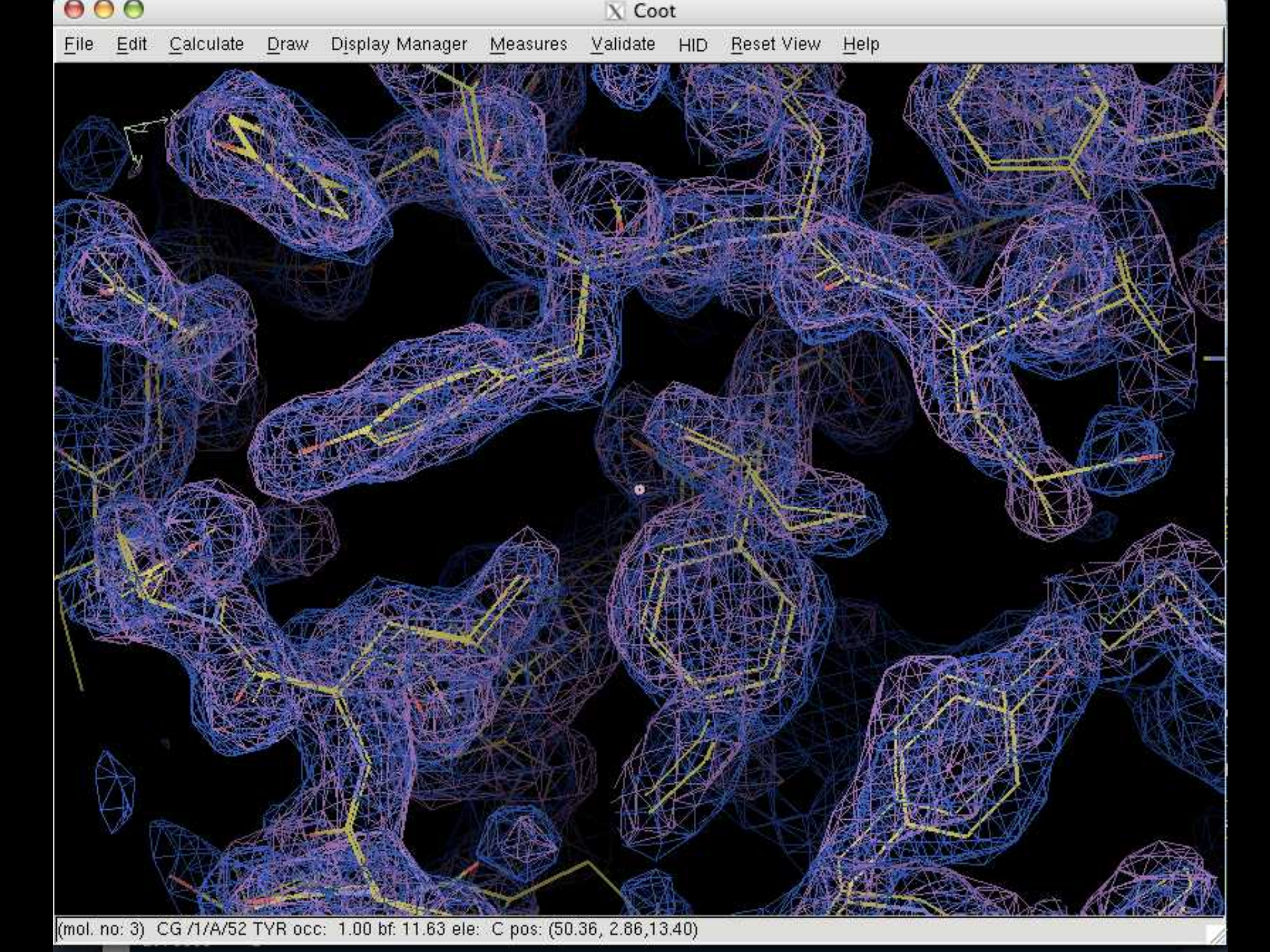
(mol. no: 3) CG /1/A/52 TYR occ: 1.00 bf: 11.63 ele: C pos: (50.36, 2.86,13.40)

NCS Overlays




Coot
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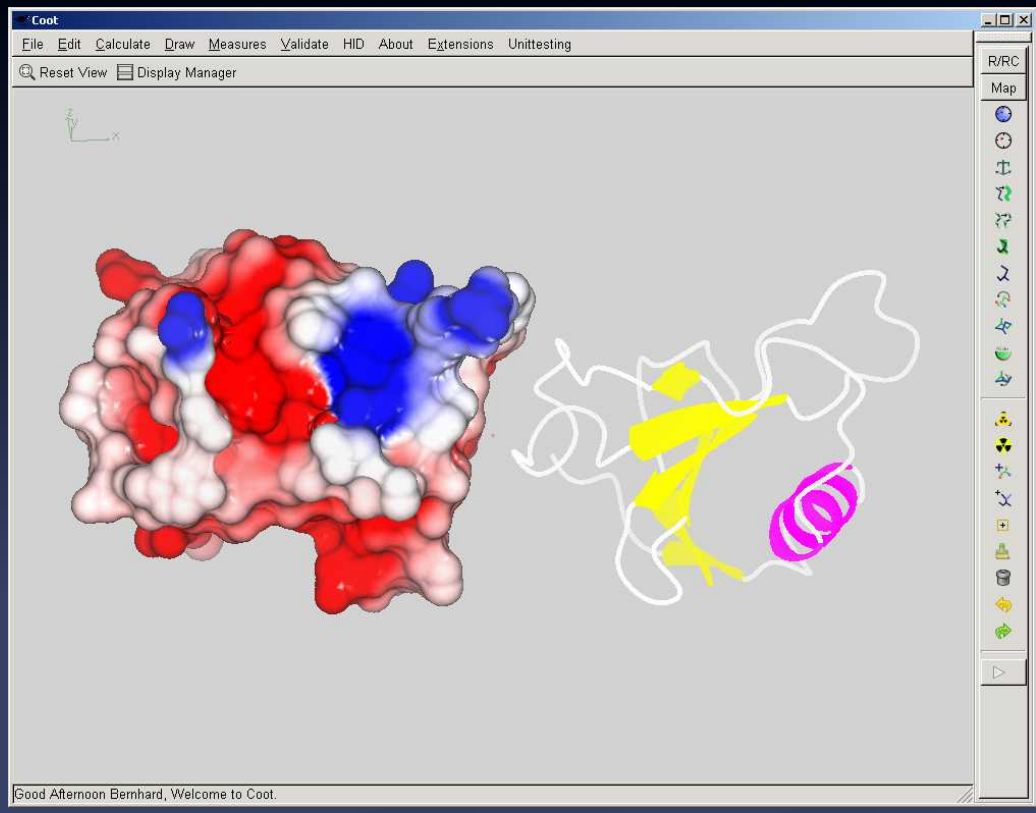
Measures
Validate
HID
Reset View
Help
(mol. no: 3) CG /1/A/52 TYR occ: 1.00 bf: 11.63 ele: C pos: (50.36, 2.86,13.40)

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

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