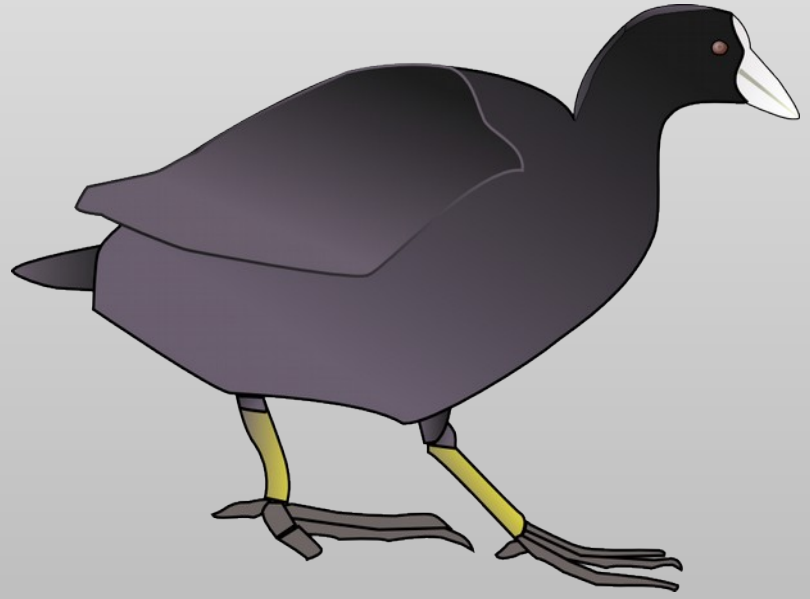
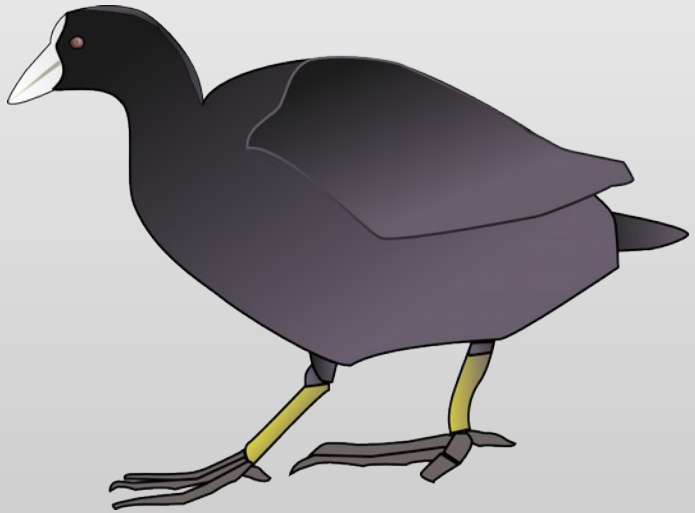
Model-Building of Proteins Using X-ray and Cryo-EM Data With *Coot*



Paul Emsley

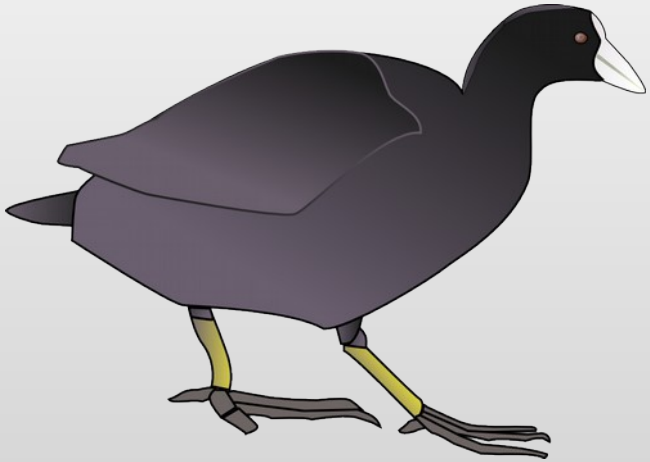
May 2017

Modelling Proteins with *Coot*



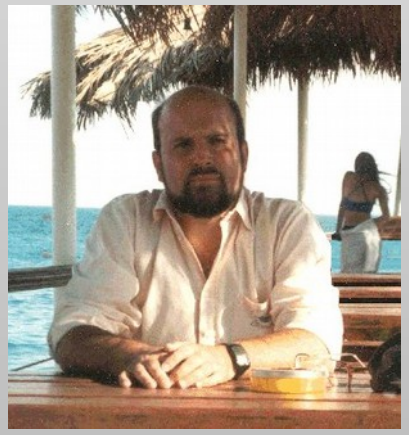
- Overview
  - Low resolution side-chains
  - Tools for EM
  - Tools for NCS

Acknowldegments, Collaborators

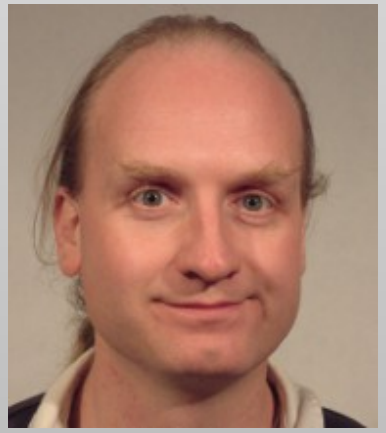


Bernhard Lohkamp

Kevin Cowtan



Eugene Krissinel

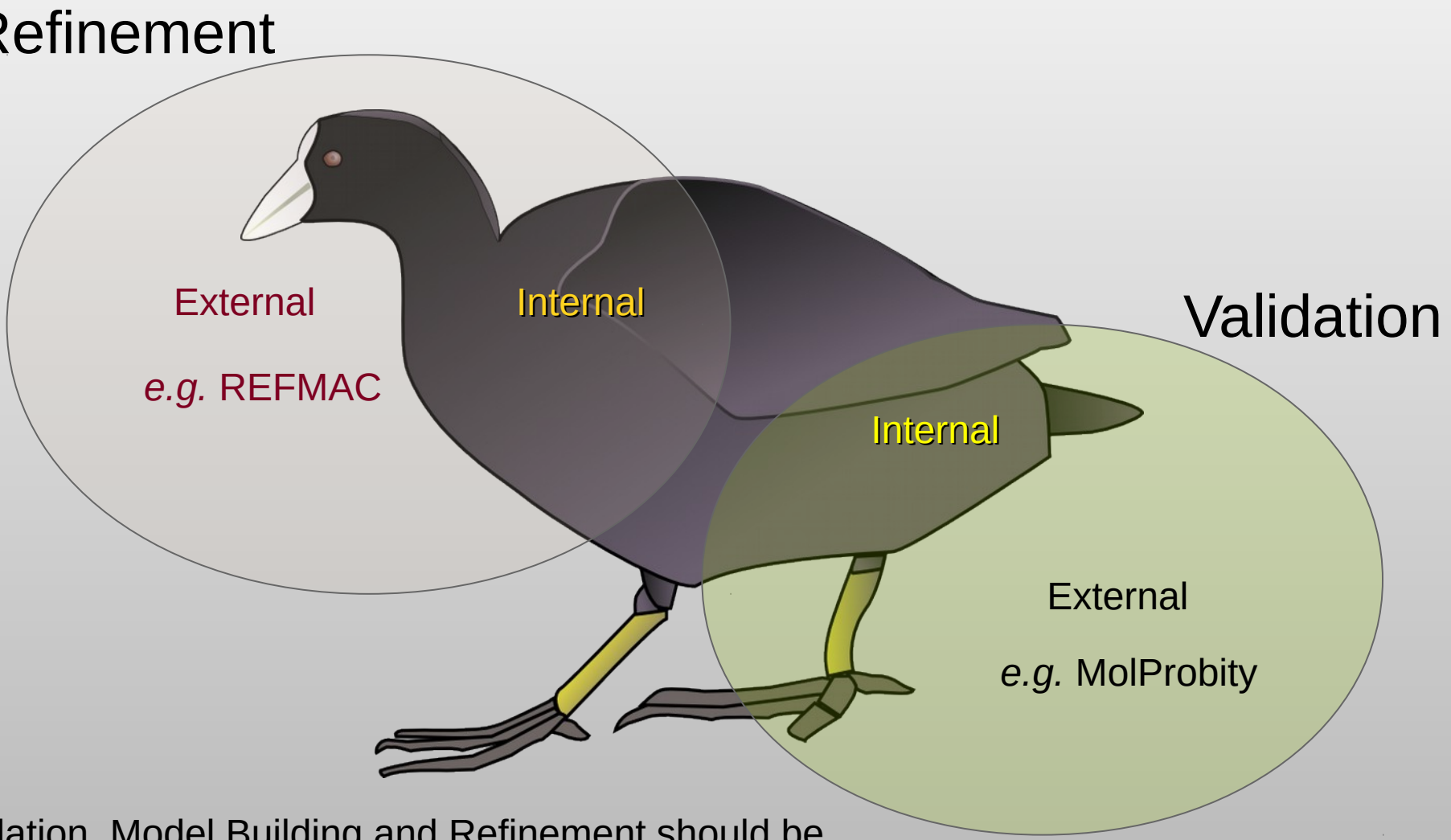


Stuart McNicholas

Martin Noble

Alexei Vagin

Feature Integration



Validation, Model Building and Refinement should be used together

Real Space Refinement

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

- 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:

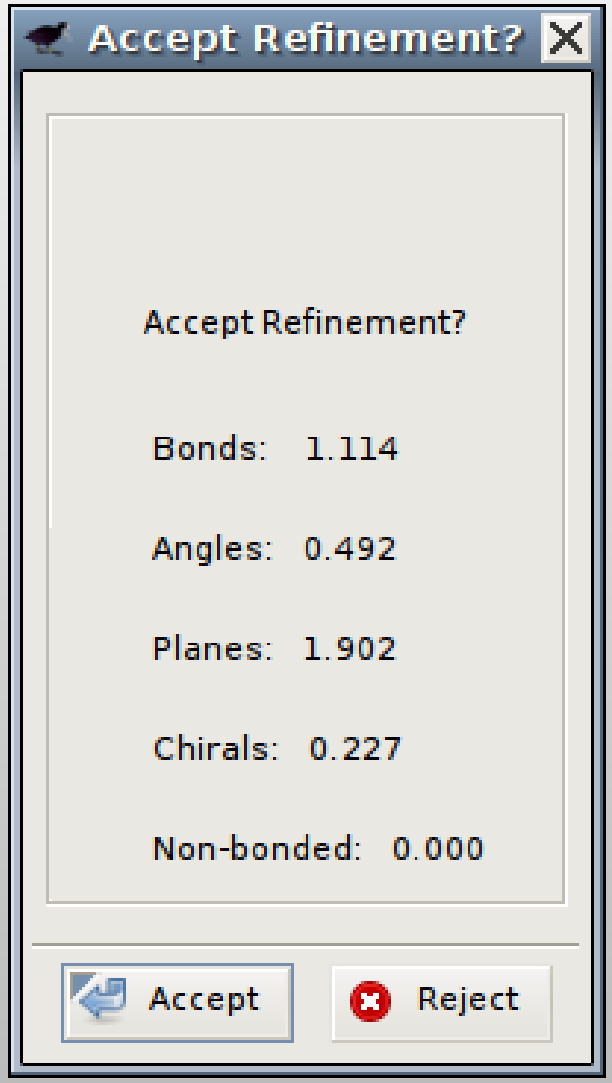
```
File  Edit  View  Terminal  Help
created 32 bond       restraints
created 38 angle      restraints
created 1 plane      restraints
created 5 chiral vol restraints
created 76 restraints

   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...”
(I maximise the window immediately)

Representation of Results:



Second attempt...

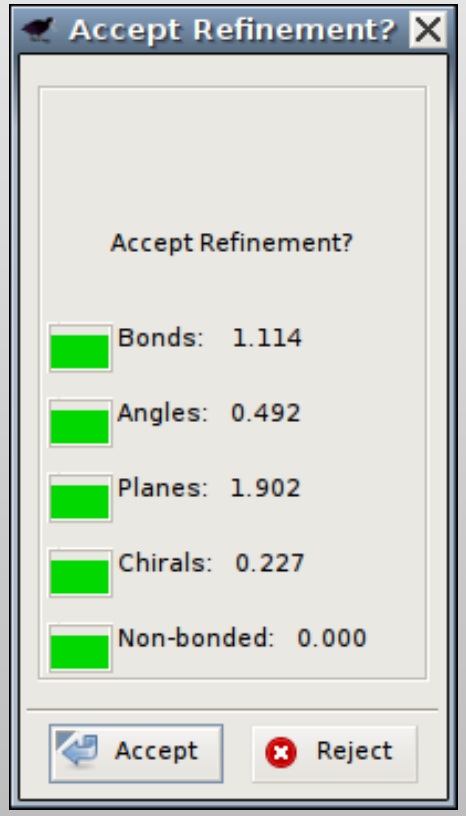
Student Reaction:

“Oh, box of meaningless numbers.

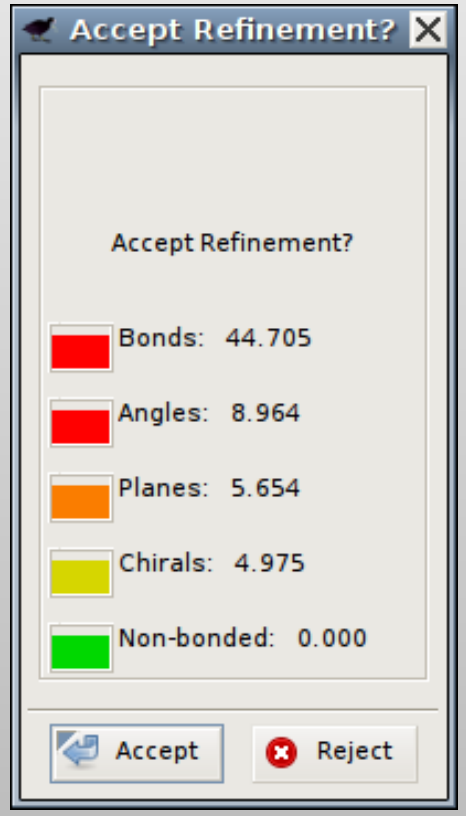
Go away”

Representation of Results: “Traffic Lights”

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



Good refinement

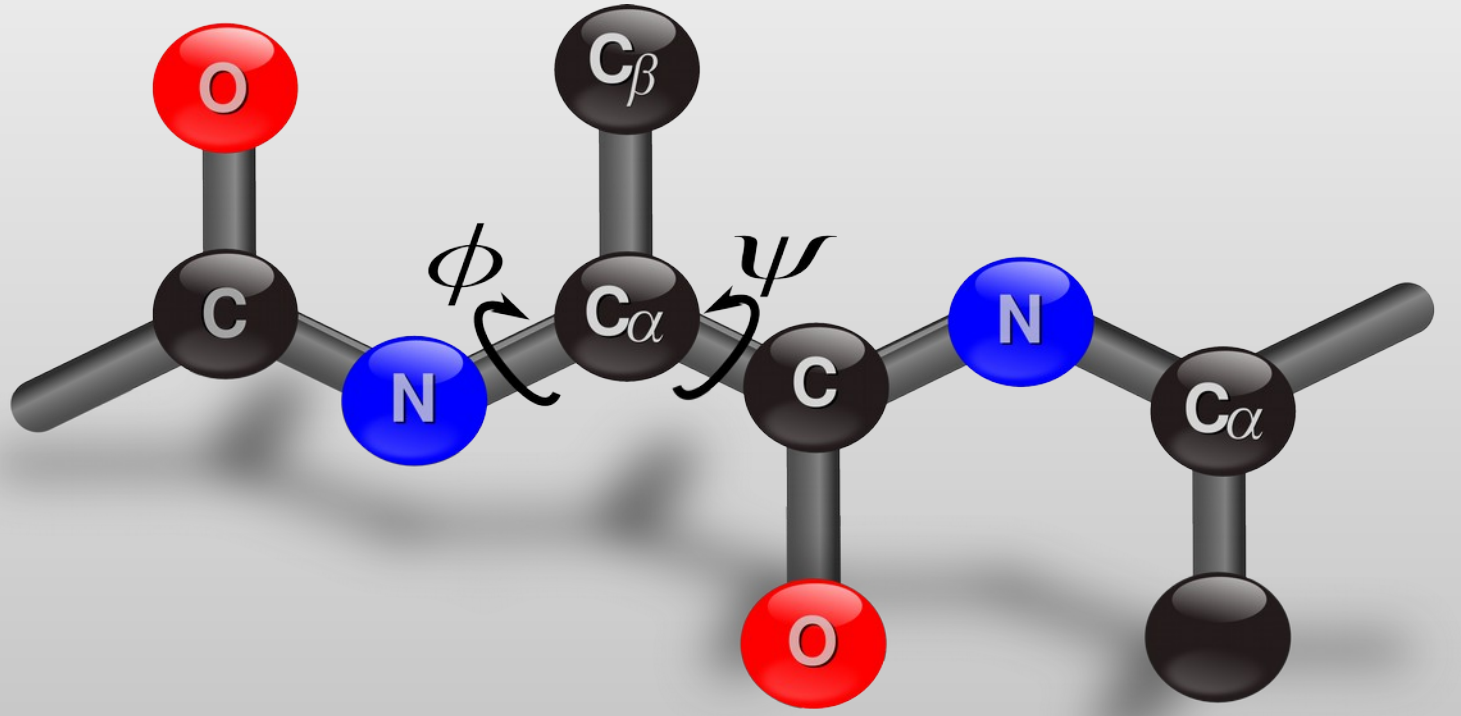


Bad refinement

Refinement Techniques

- Single-Atom Drag
  - Over-dragging
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept

Peptide Backbone Geometry

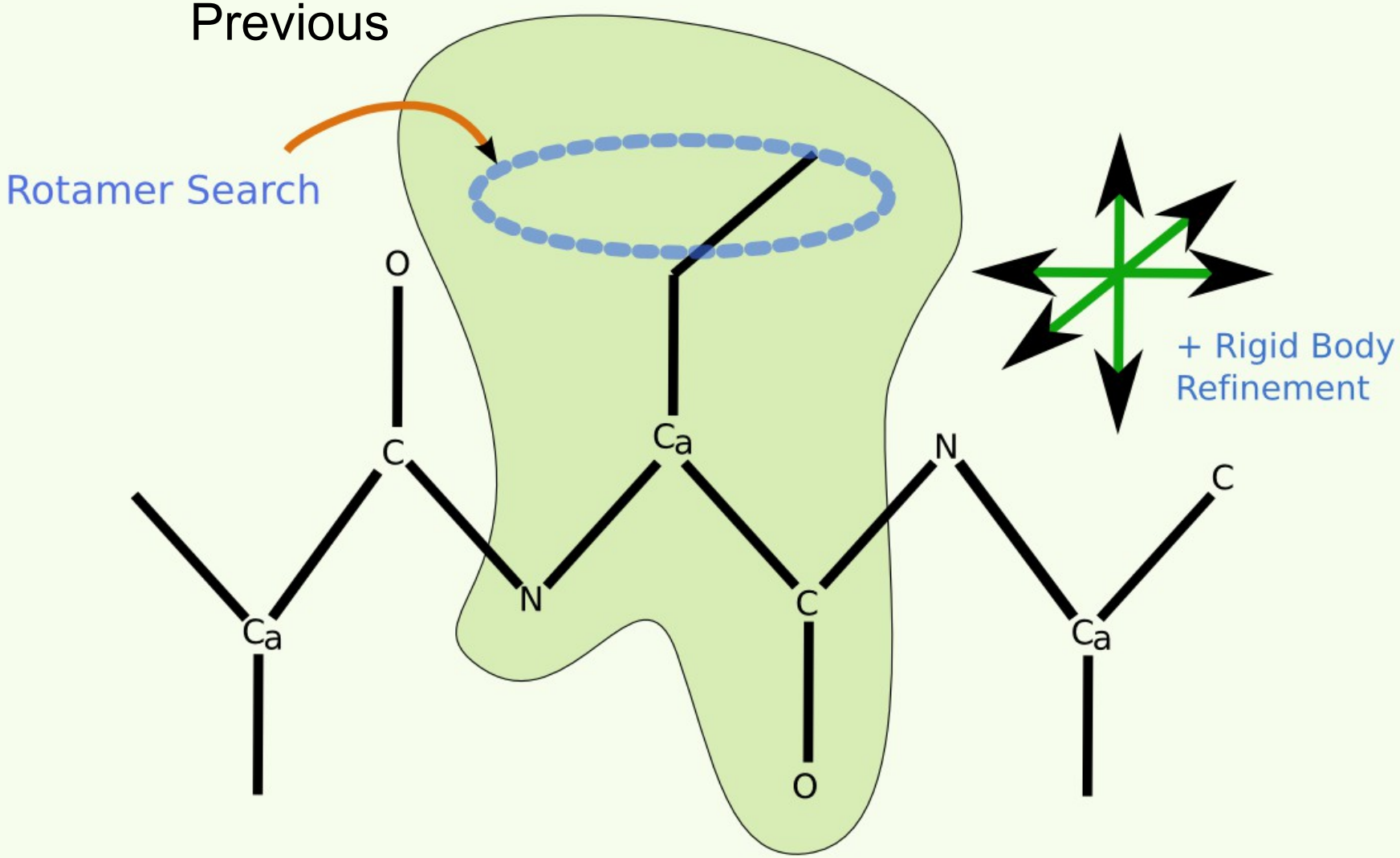


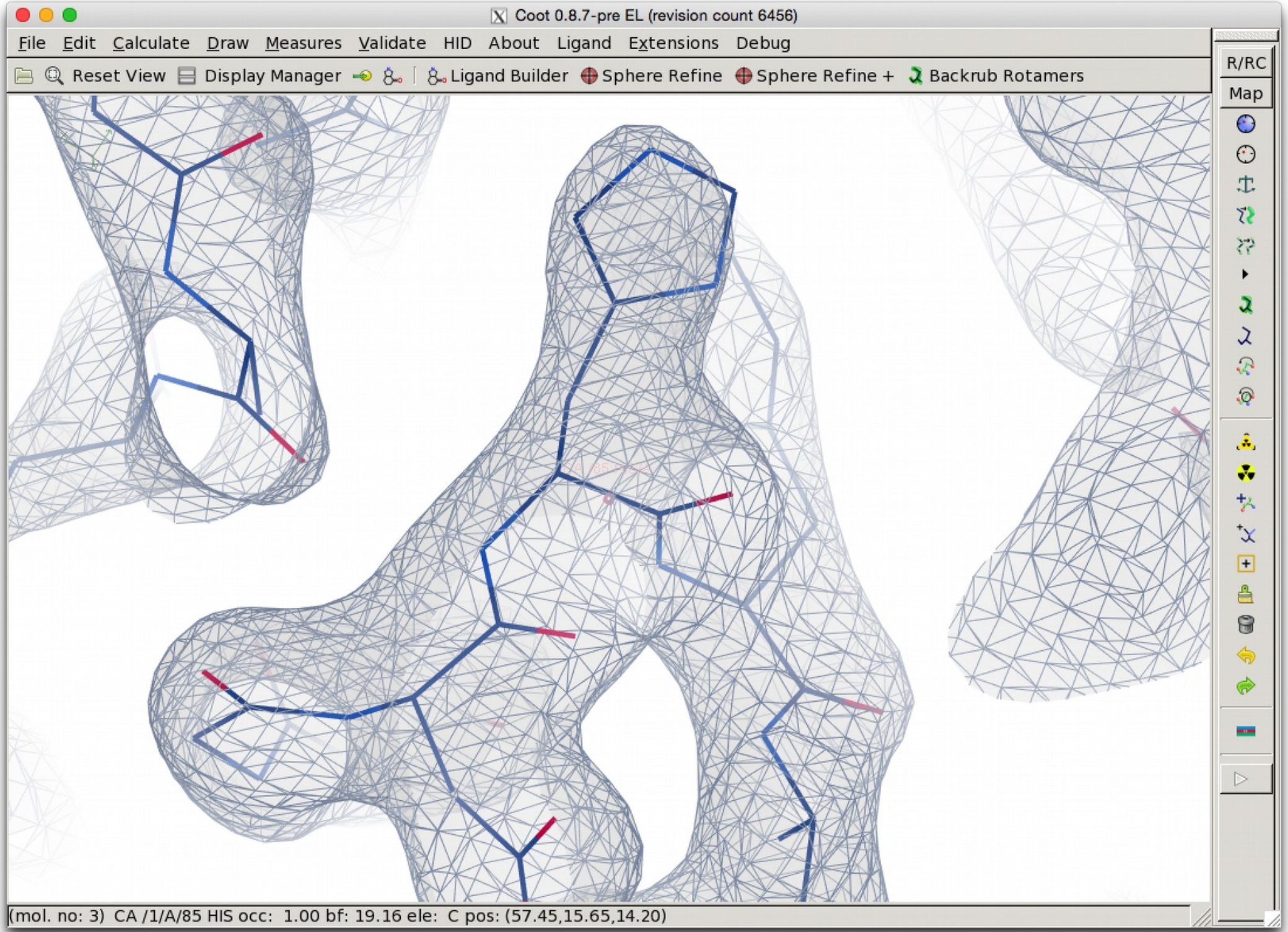
Low Resolution Model-Building

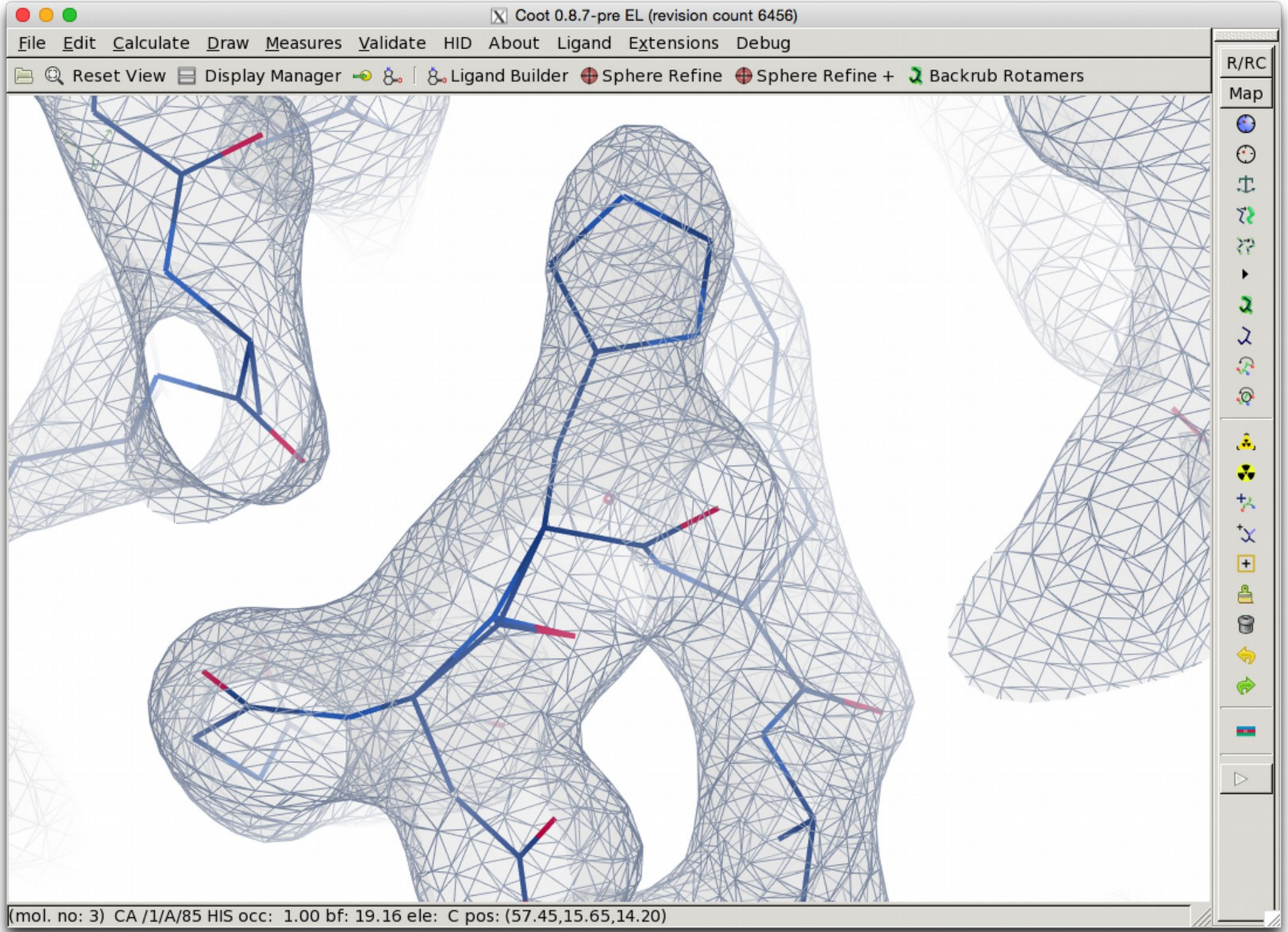
- “Backrub” rotamers

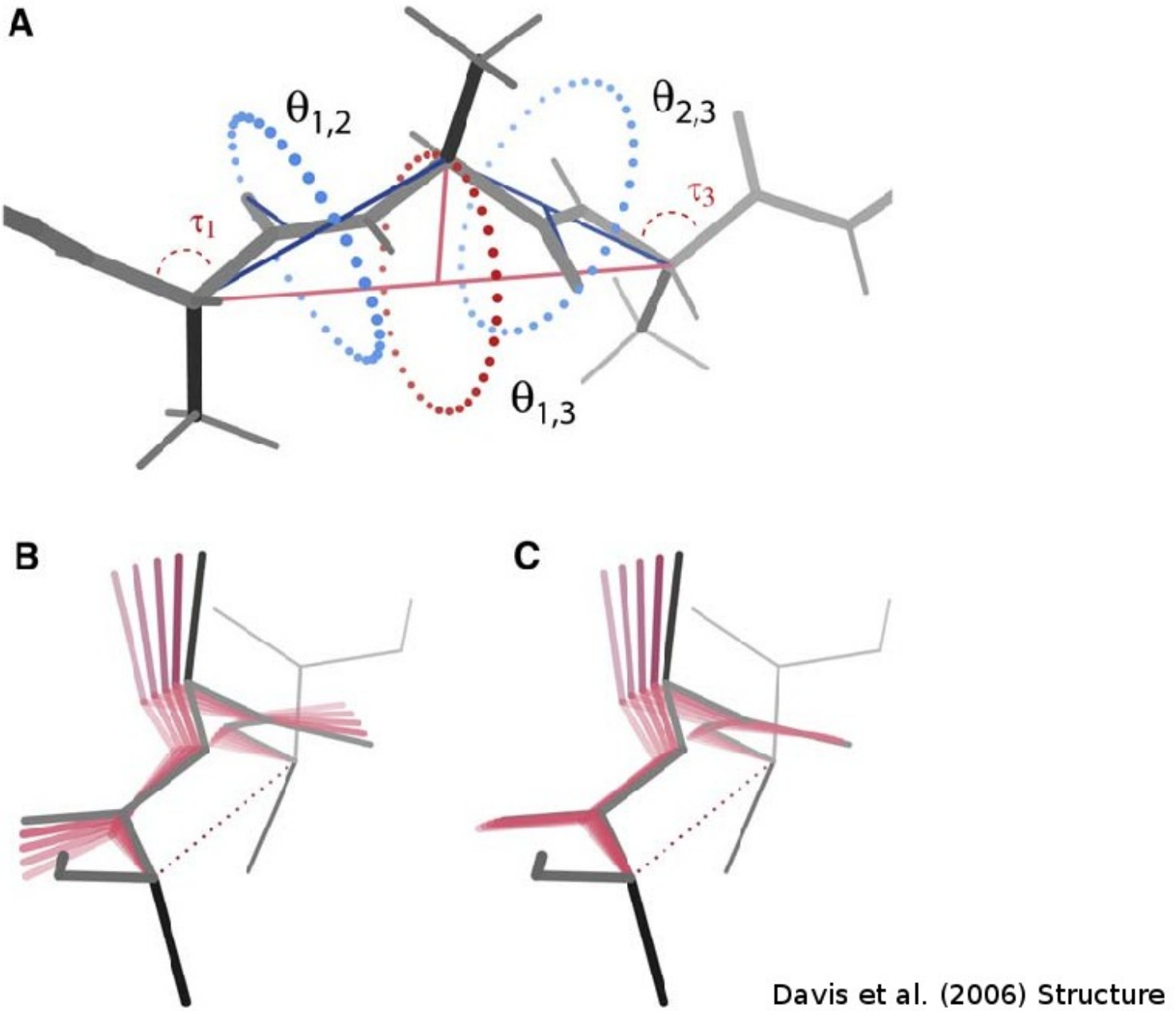
~~Current~~ Low Resolution Rotamer Search

Previous



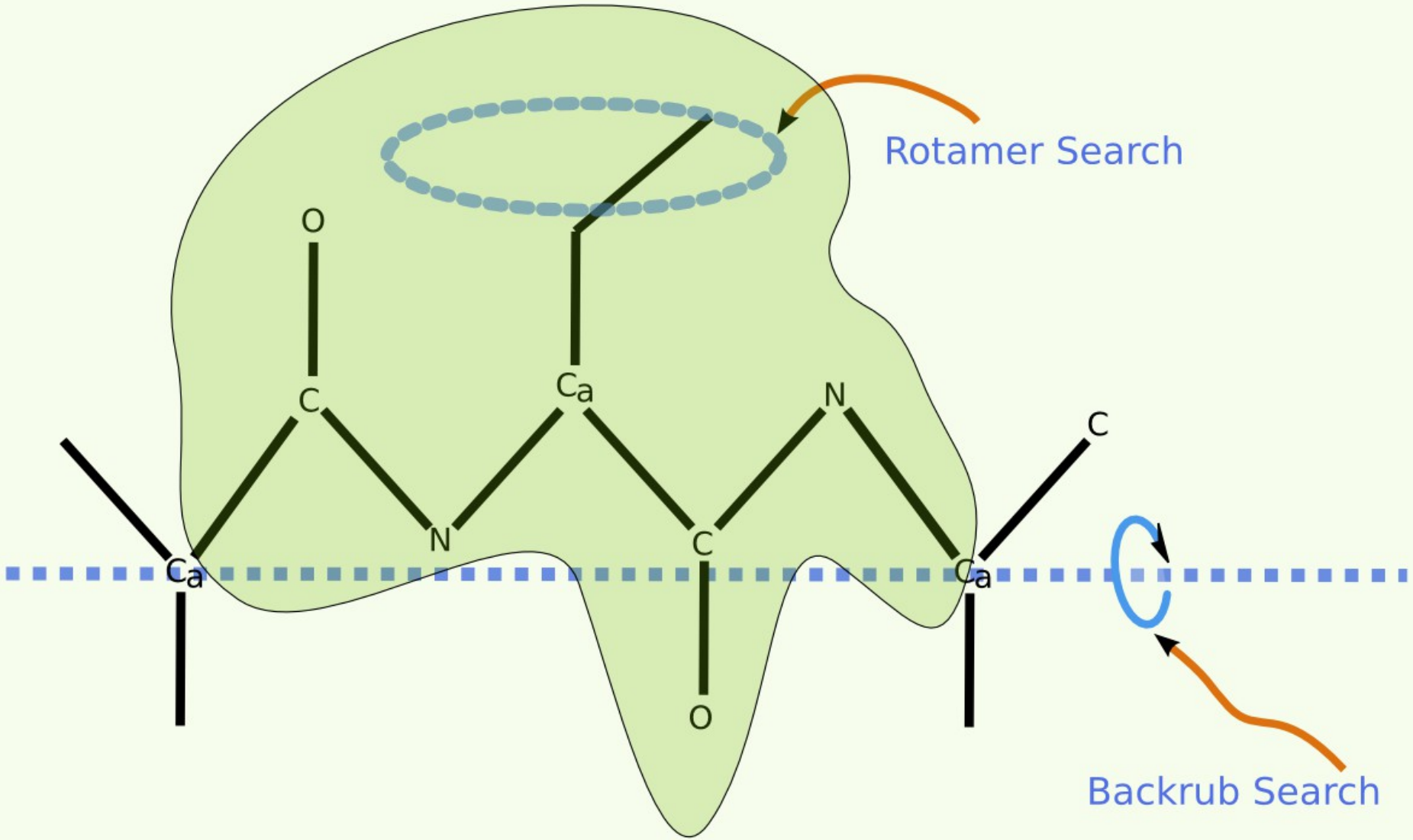

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)

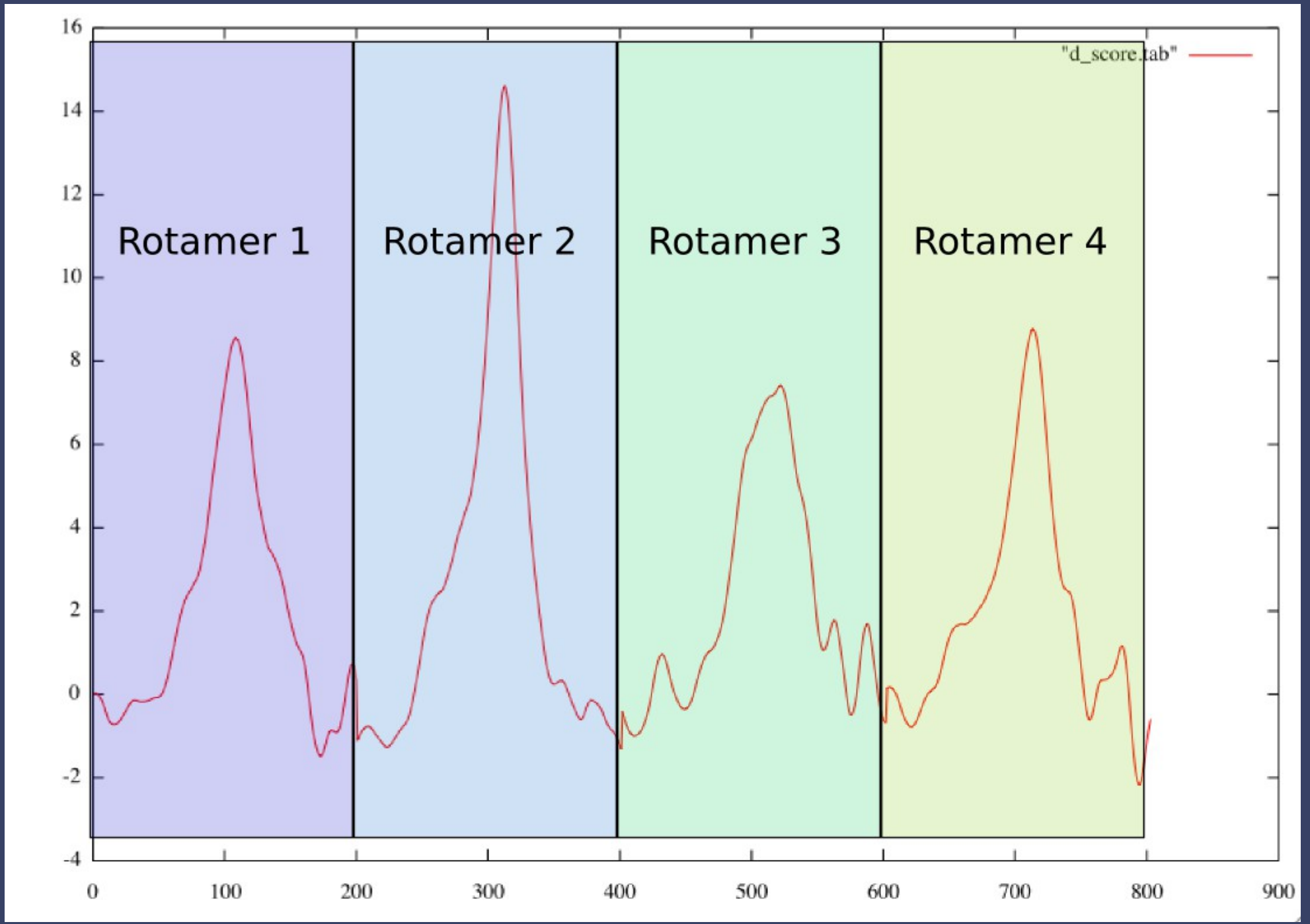


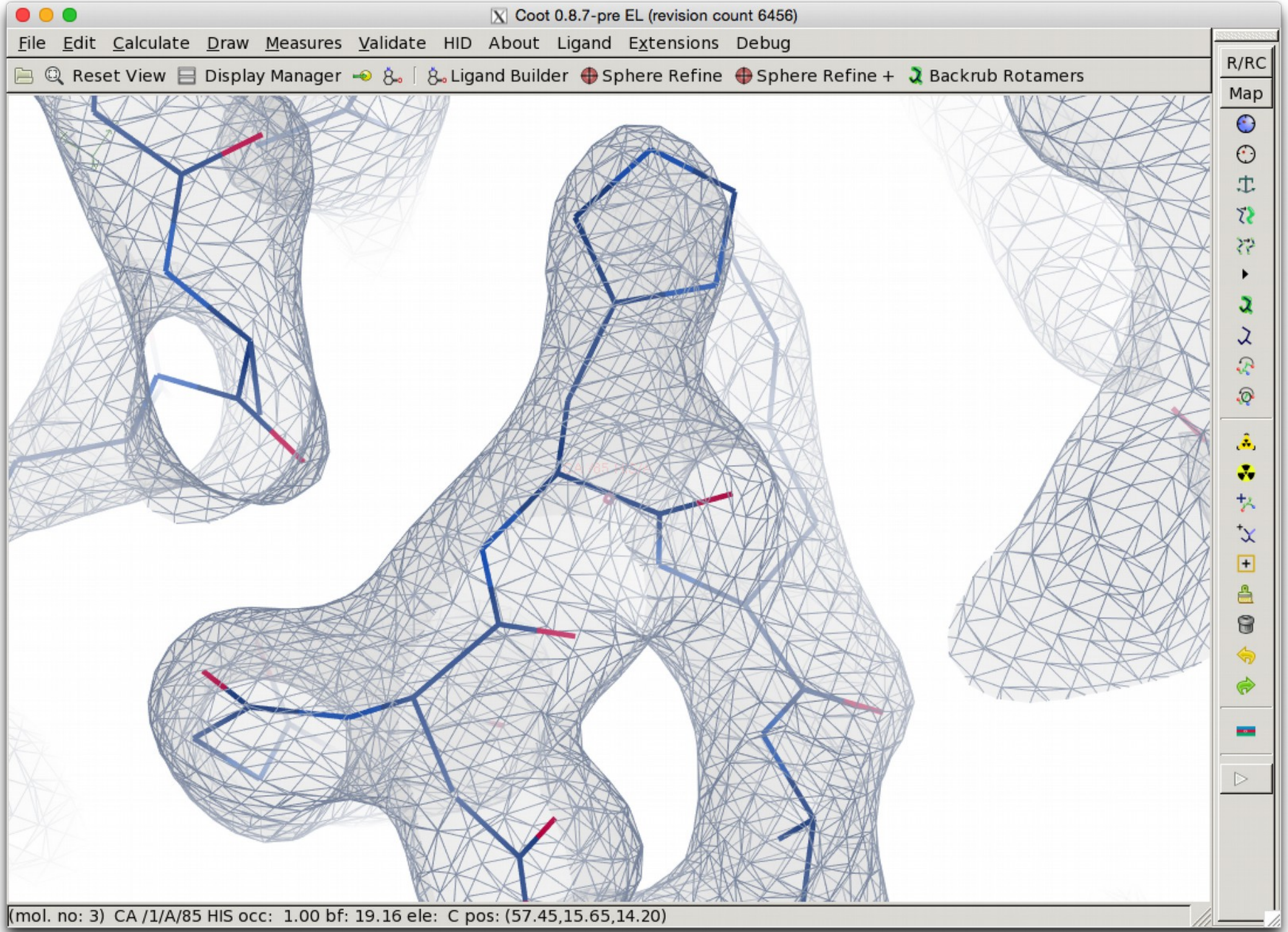
Davis et al. (2006) Structure

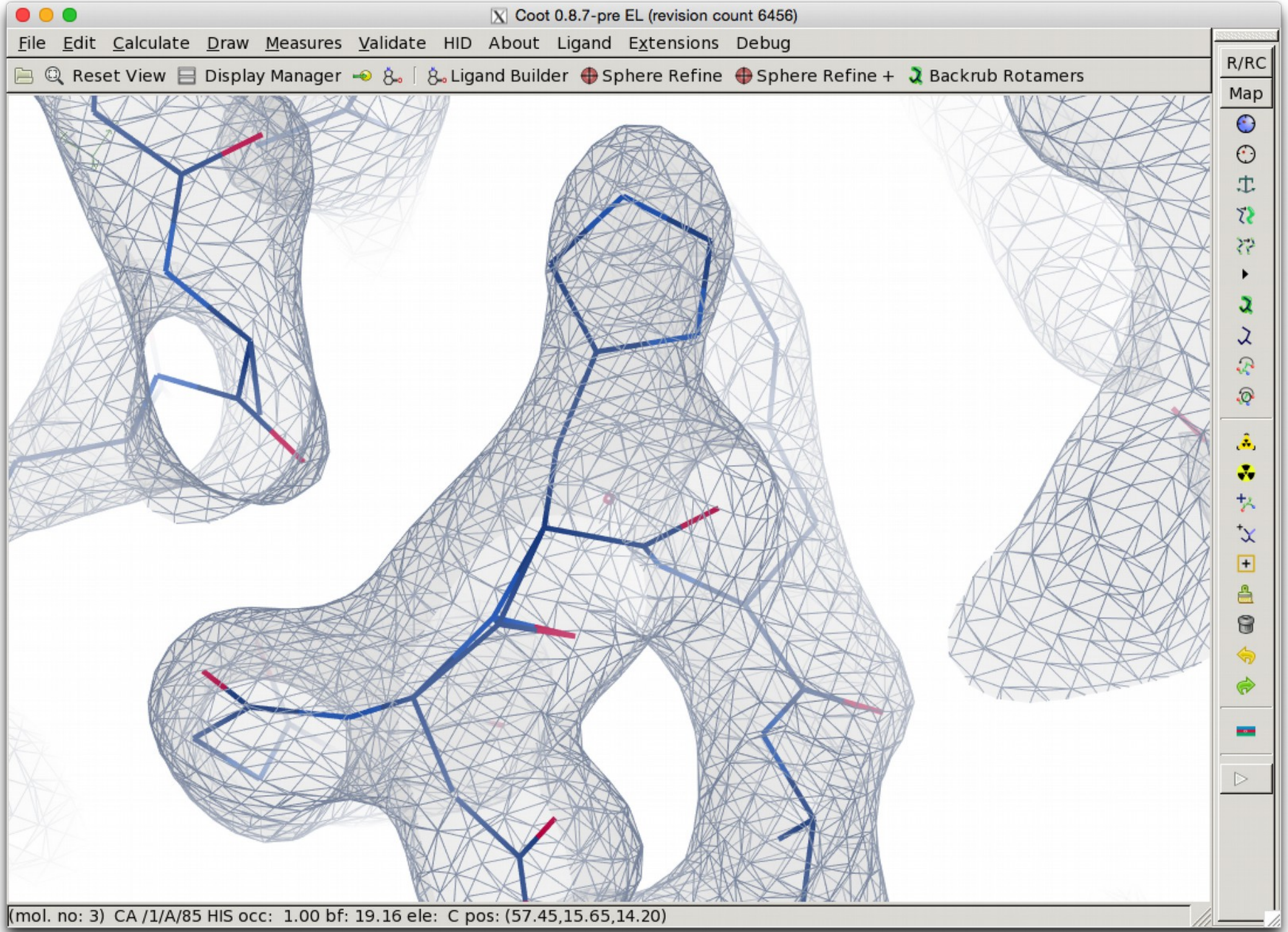
New Low Resolution Rotamer Search

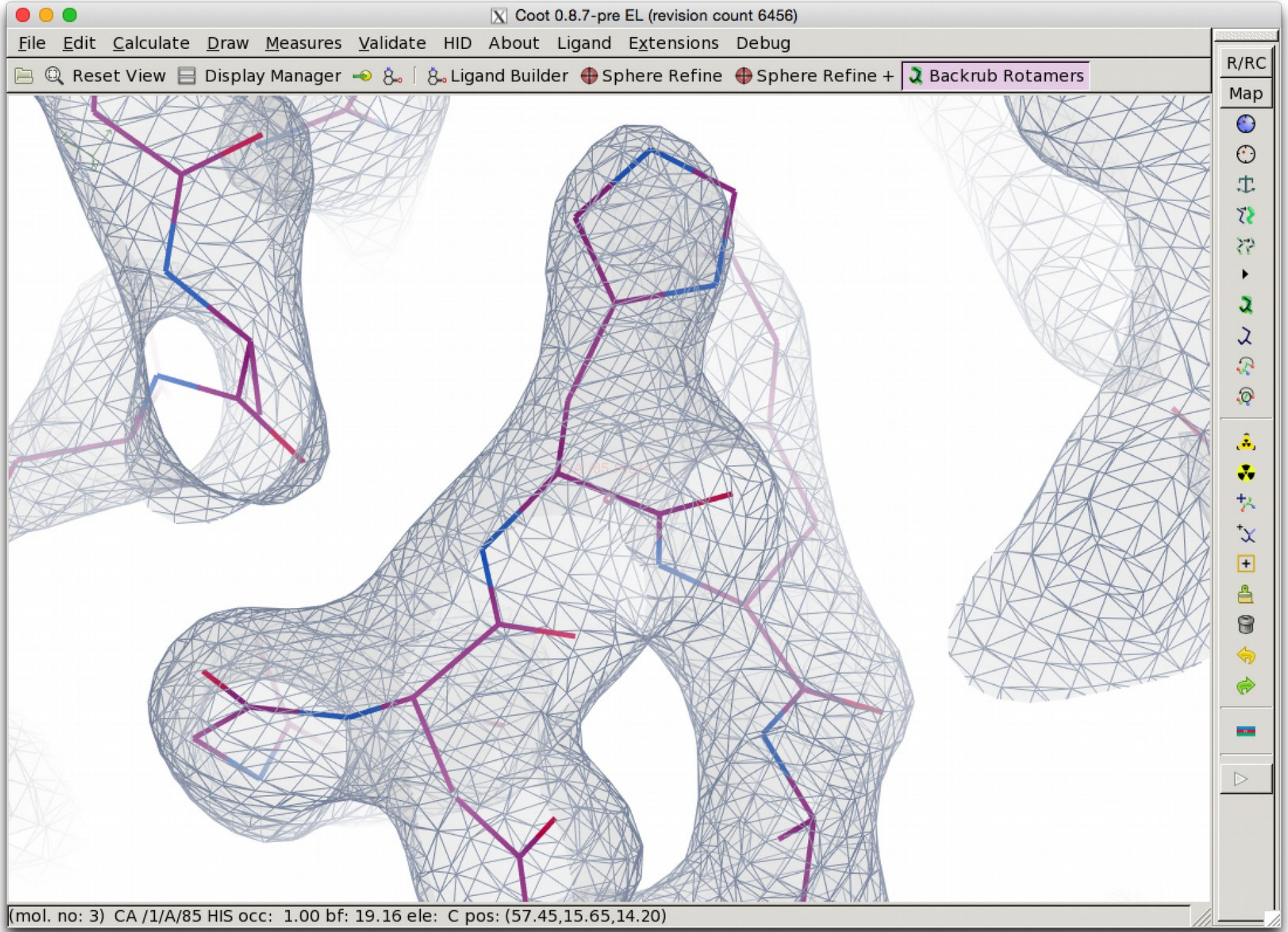


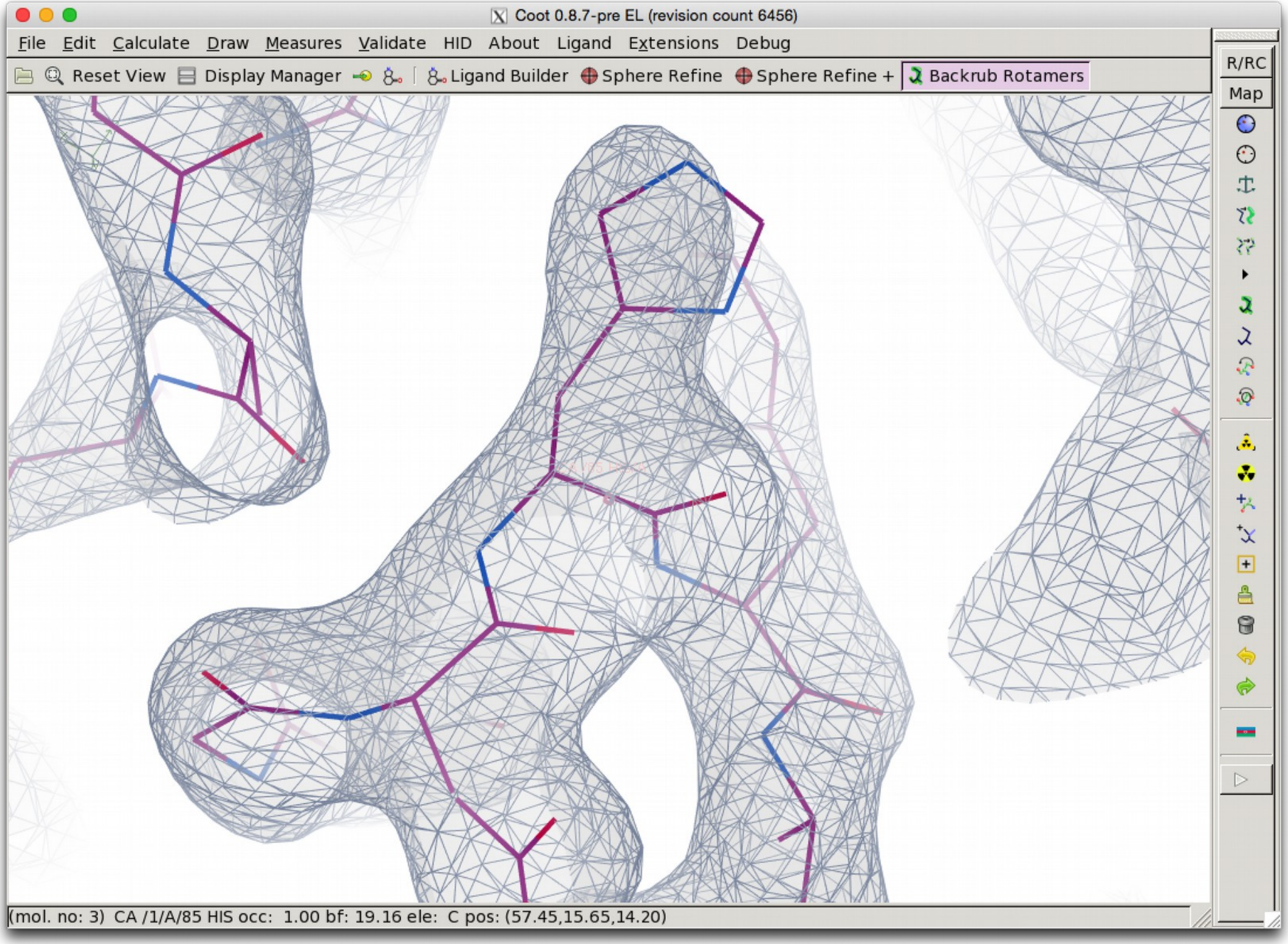
After Fitting Tools in KING/Molprobity

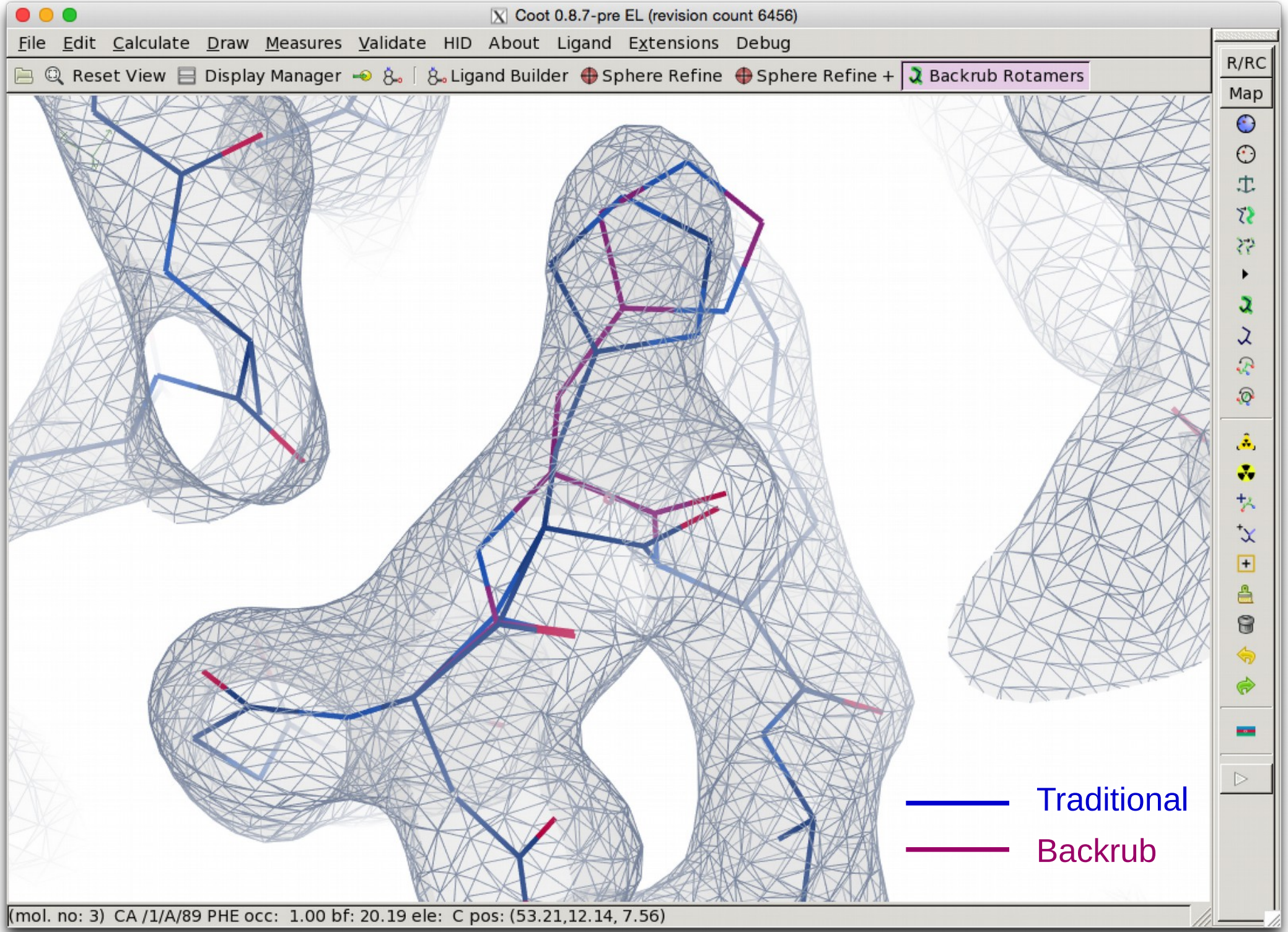

"d_score.tab"
Rotamer 1
Rotamer 2
Rotamer 3
Rotamer 4
16
14
12
10
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
800
900

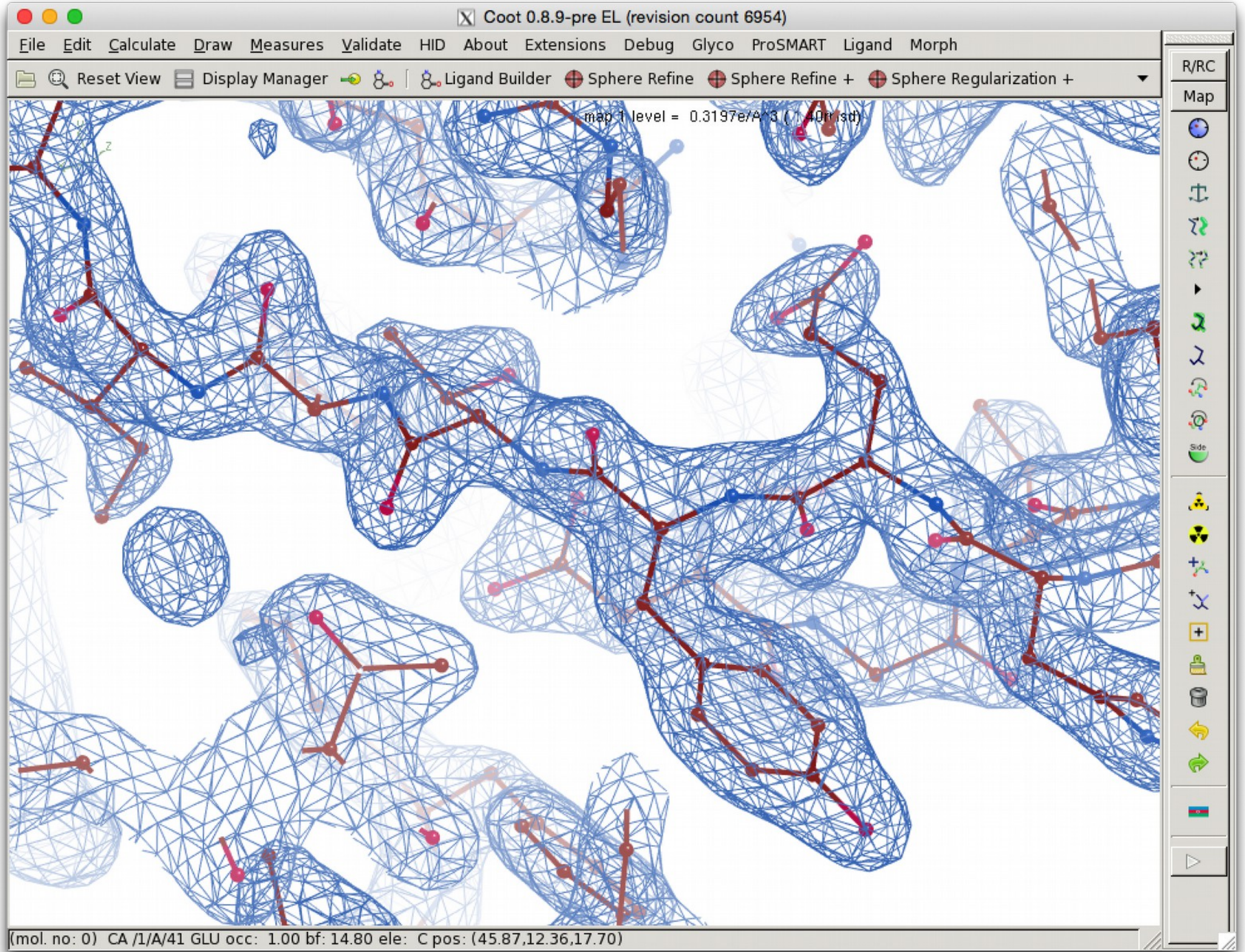

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)

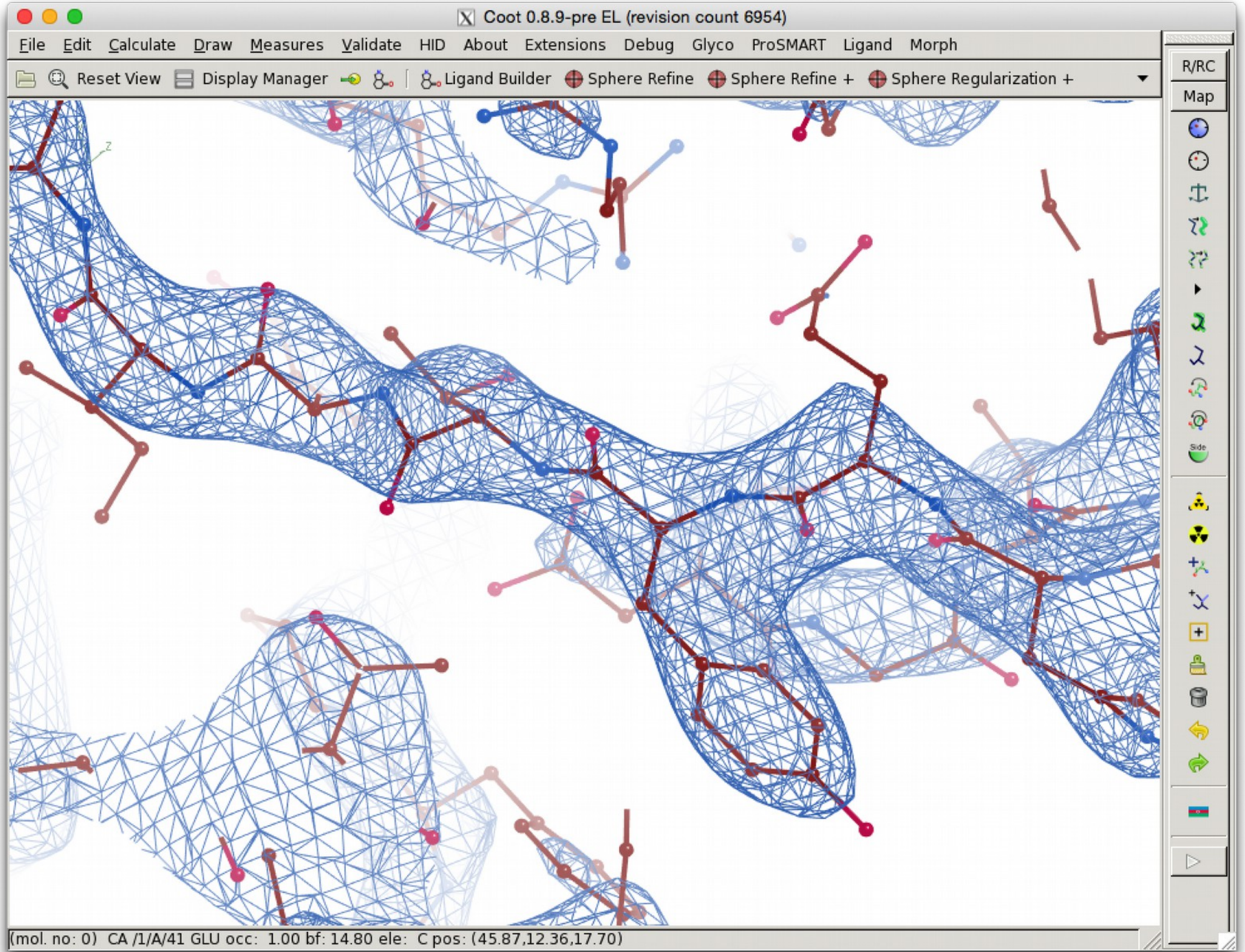

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)

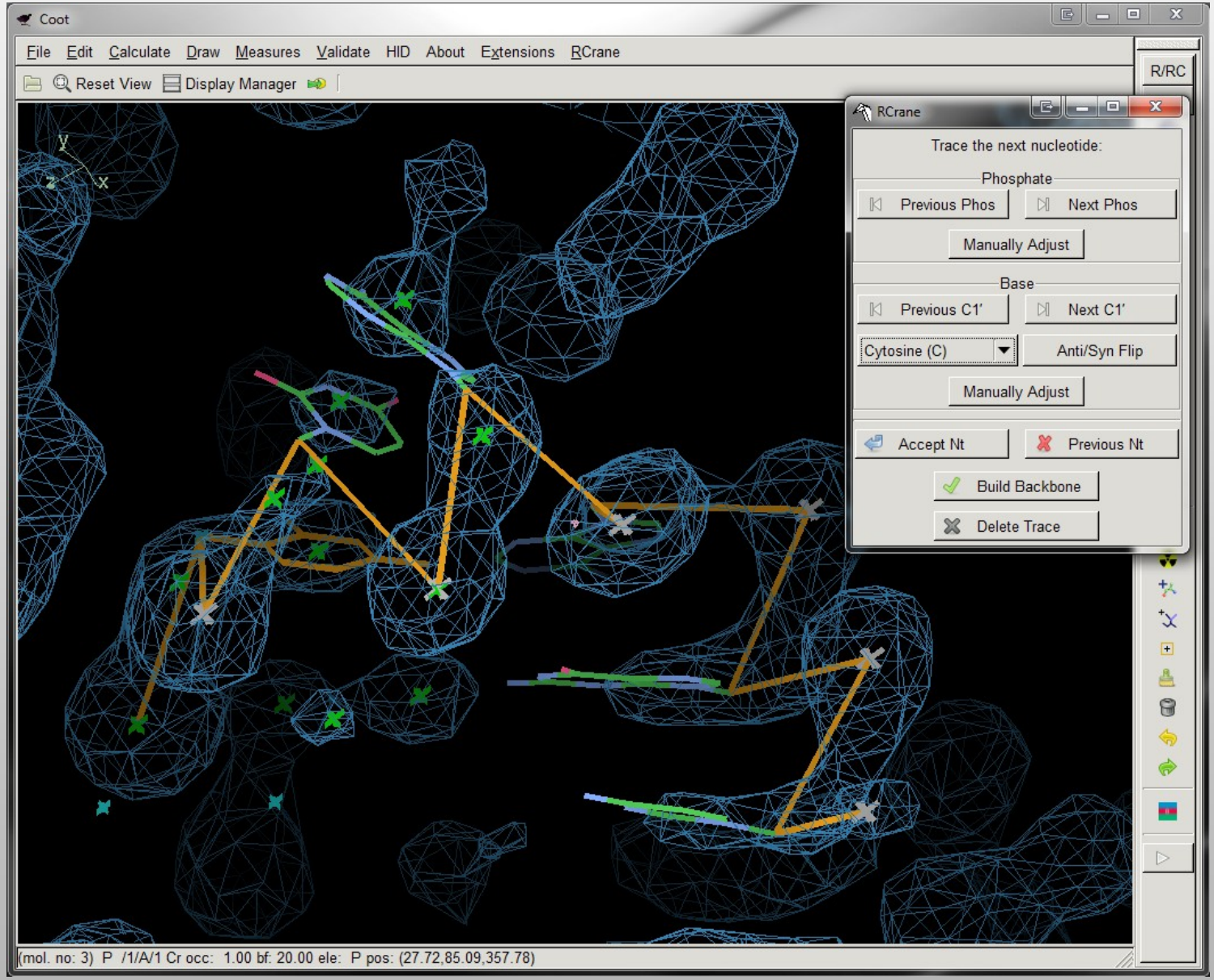

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
Traditional
Backrub
(mol. no: 3) CA /1/A/89 PHE occ: 1.00 bf: 20.19 ele: C pos: (53.21,12.14, 7.56)

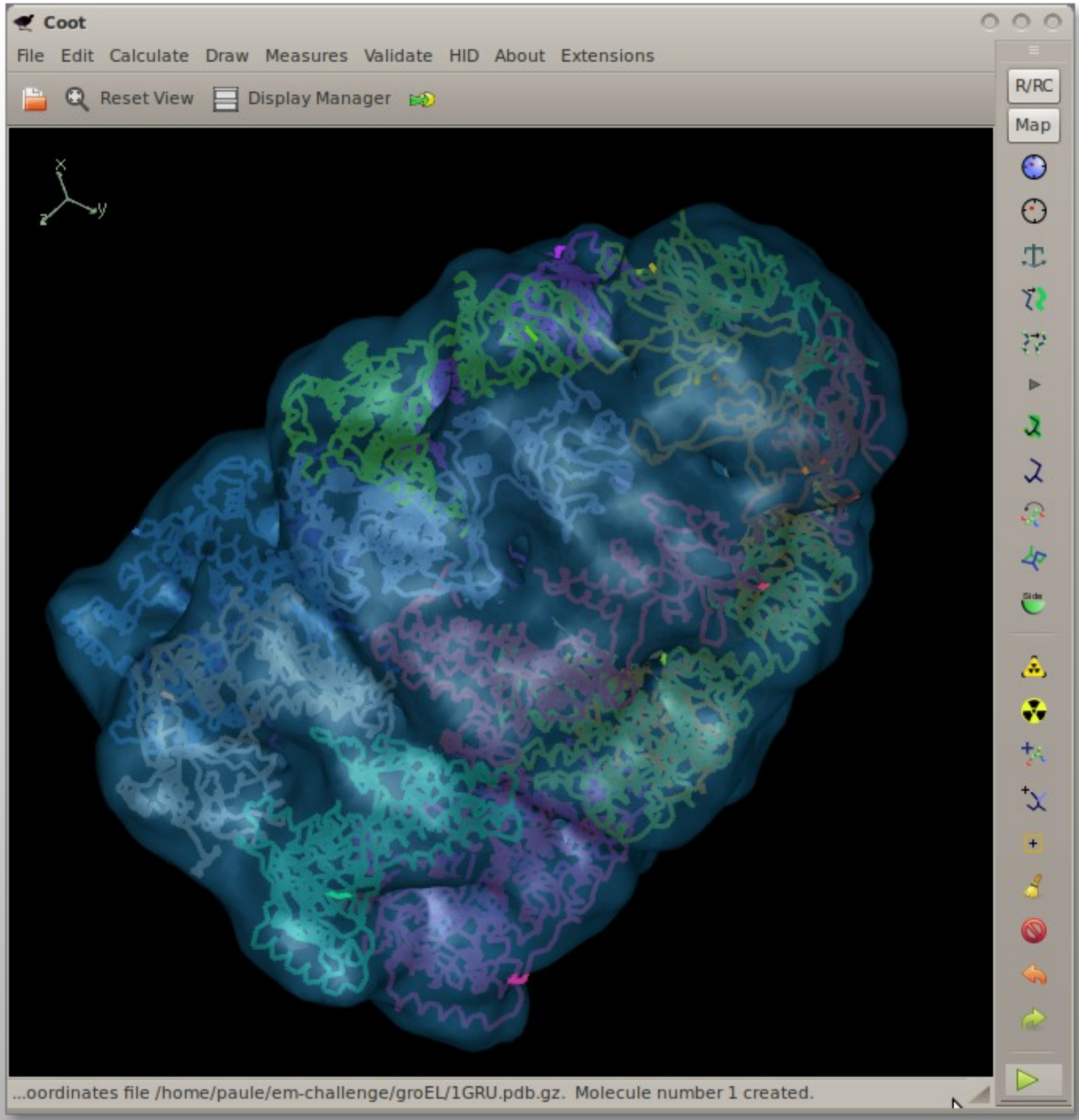

Coot 0.8.9-pre EL (revision count 6954)
File Edit Calculate Draw Measures Validate HID About Extensions Debug Glyco ProSMART Ligand Morph
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Sphere Regularization +
R/RC
Map
map 1 level = 0.3197e/A^3 (1.40rms)
(mol. no: 0) CA /1/A/41 GLU occ: 1.00 bf: 14.80 ele: C pos: (45.87,12.36,17.70)

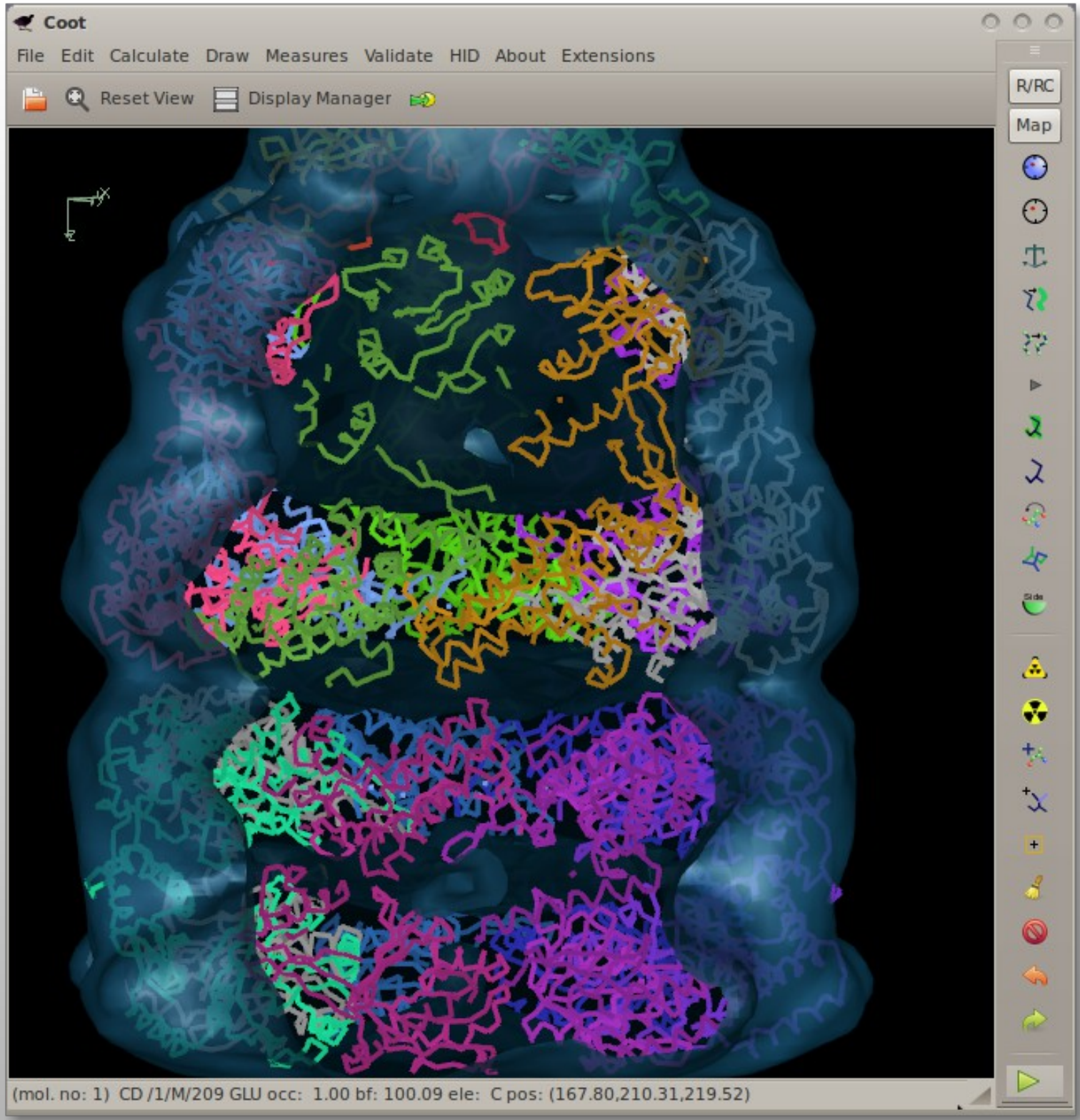

Coot 0.8.9-pre EL (revision count 6954)
File Edit Calculate Draw Measures Validate HID About Extensions Debug Glyco ProSMART Ligand Morph
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Sphere Regularization +
R/RC
Map
Side
(mol. no: 0) CA /1/A/41 GLU occ: 1.00 bf: 14.80 ele: C pos: (45.87,12.36,17.70)

RCrane: Semi-Automated Building of RNA



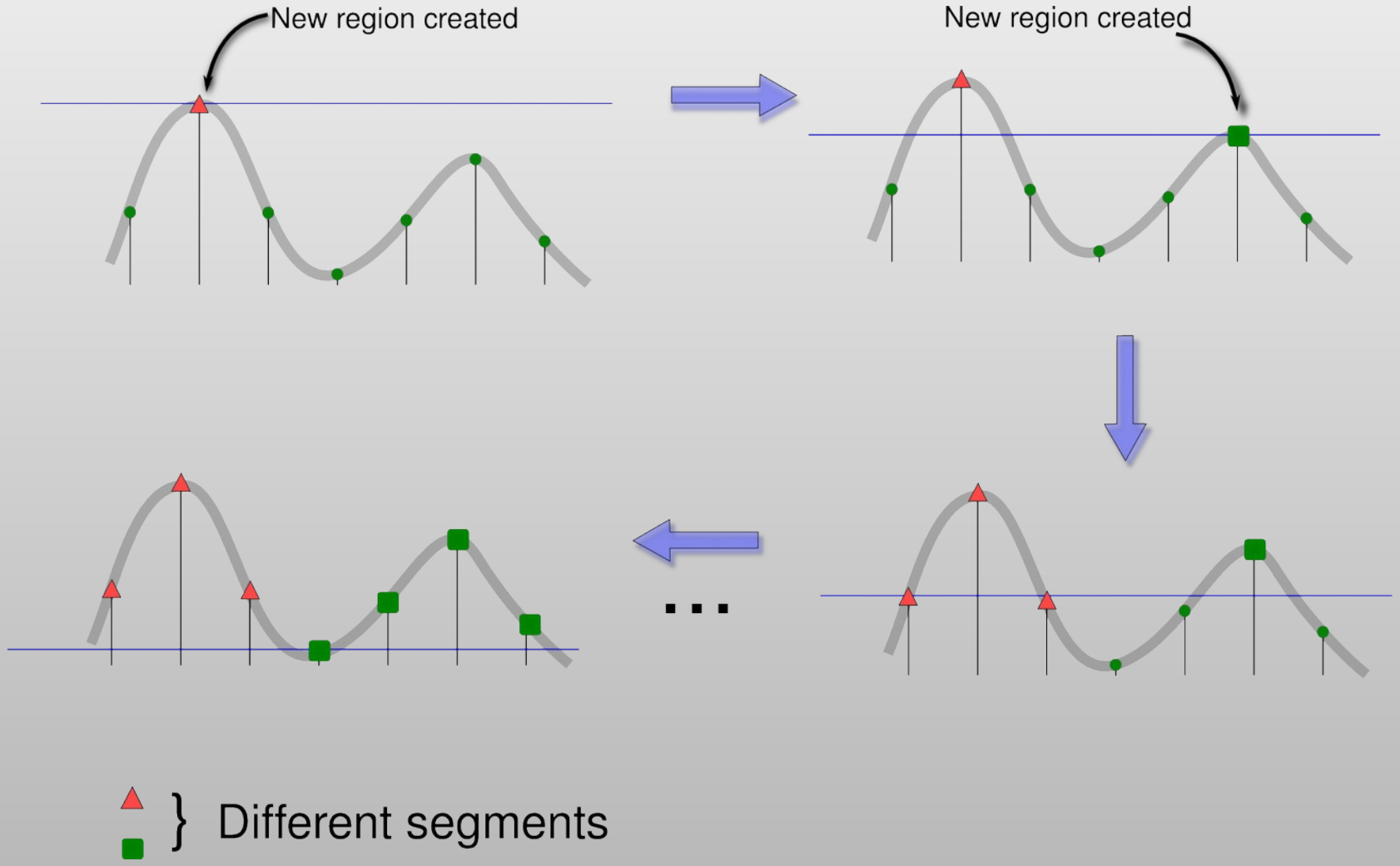
Handling EM maps

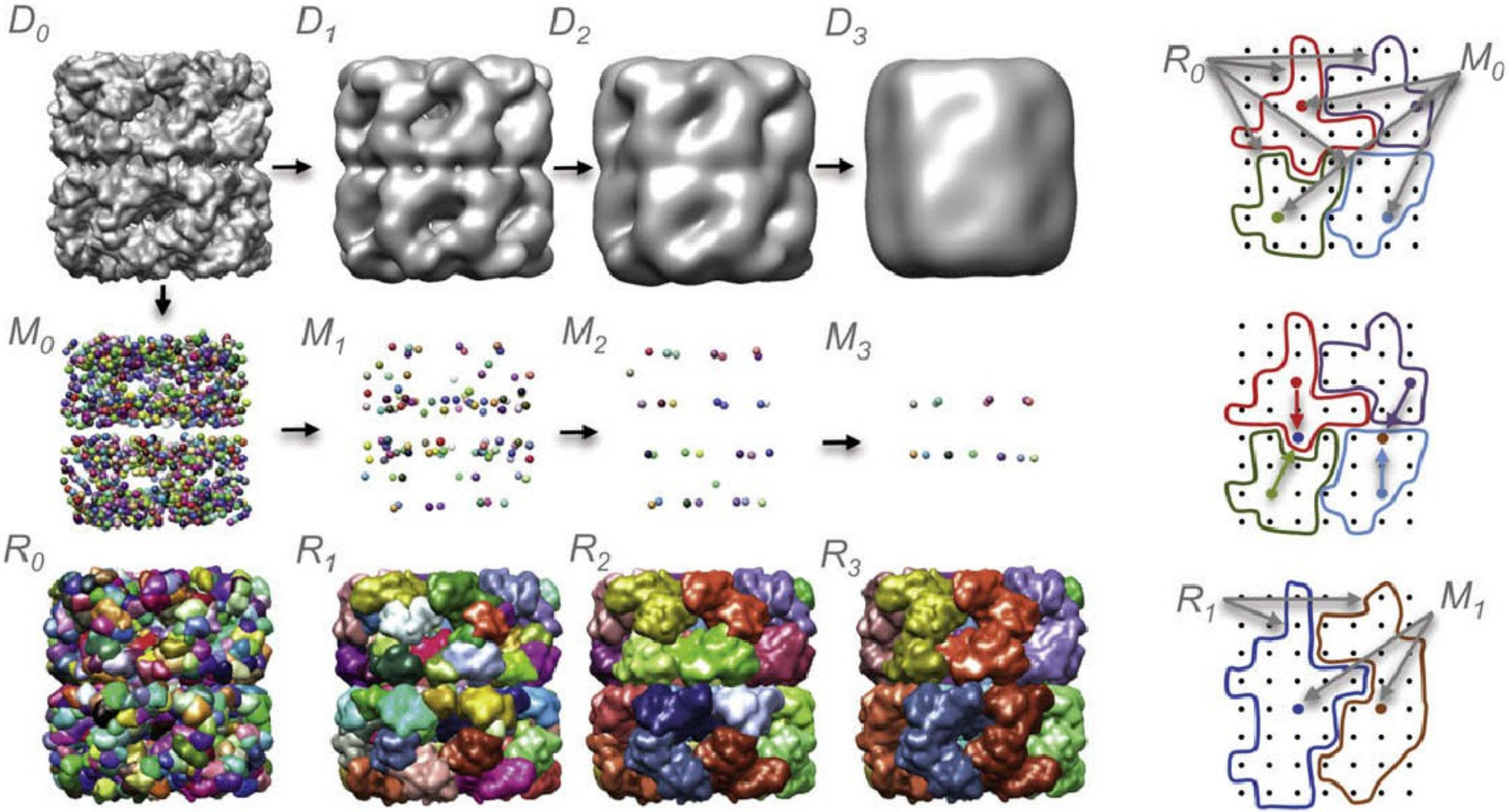

Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
...oordinates file /home/paule/em-challenge/groEL/1GRU.pdb.gz. Molecule number 1 created.


Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
(mol. no: 1) CD /1/M/209 GLU occ: 1.00 bf: 100.09 ele: C pos: (167.80,210.31,219.52)

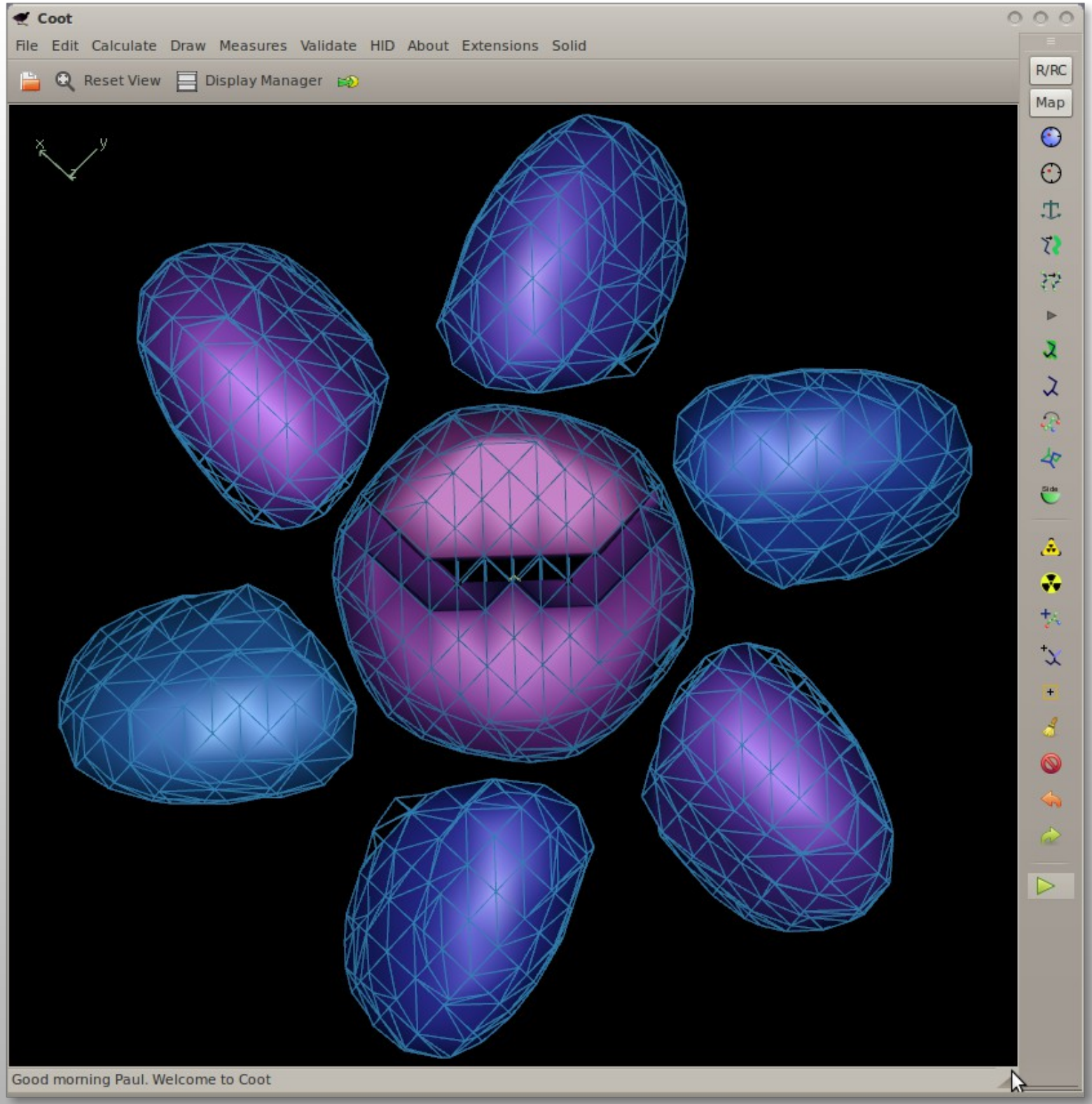
Partioning Maps: Watershed Algoritm

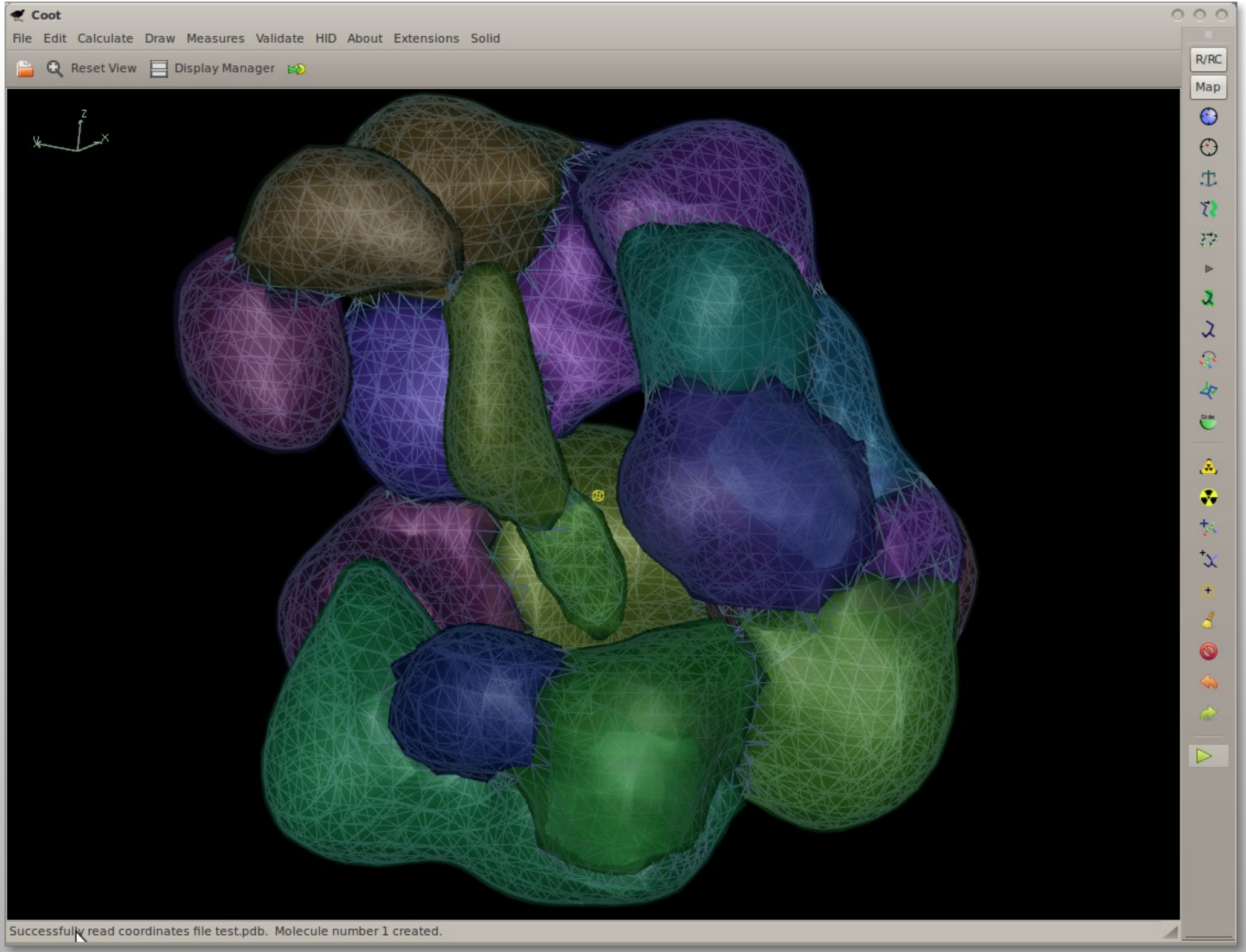
1D-analog

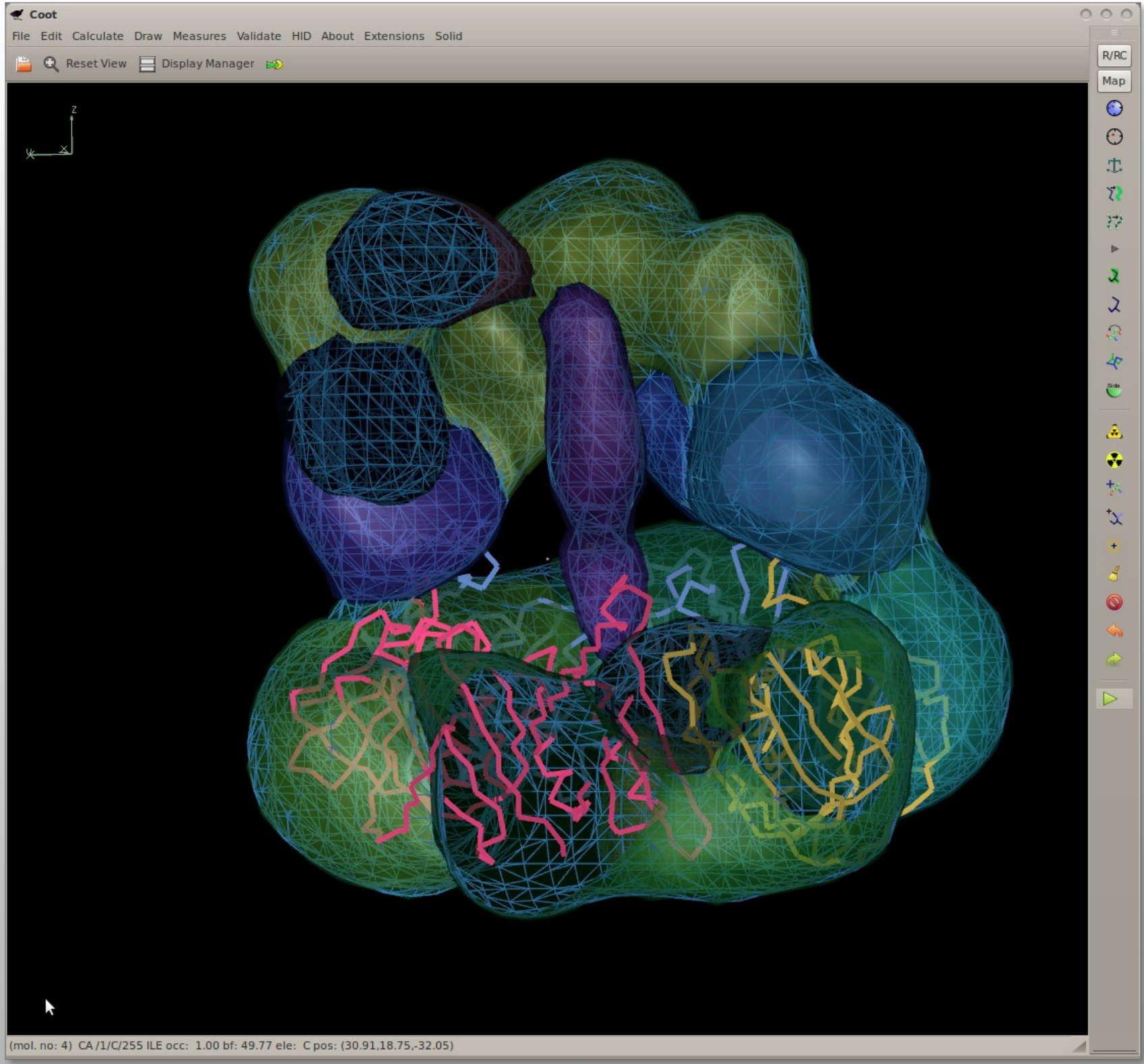




Pintilie *et al.* (2010) J.Struct.Biol.


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Solid
Reset View
Display Manager
R/RC
Map
Good morning Paul. Welcome to Coot


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Solid
Reset View
Display Manager
R/RC
Map
Z
Y
X
Successfully read coordinates file test.pdb. Molecule number 1 created.

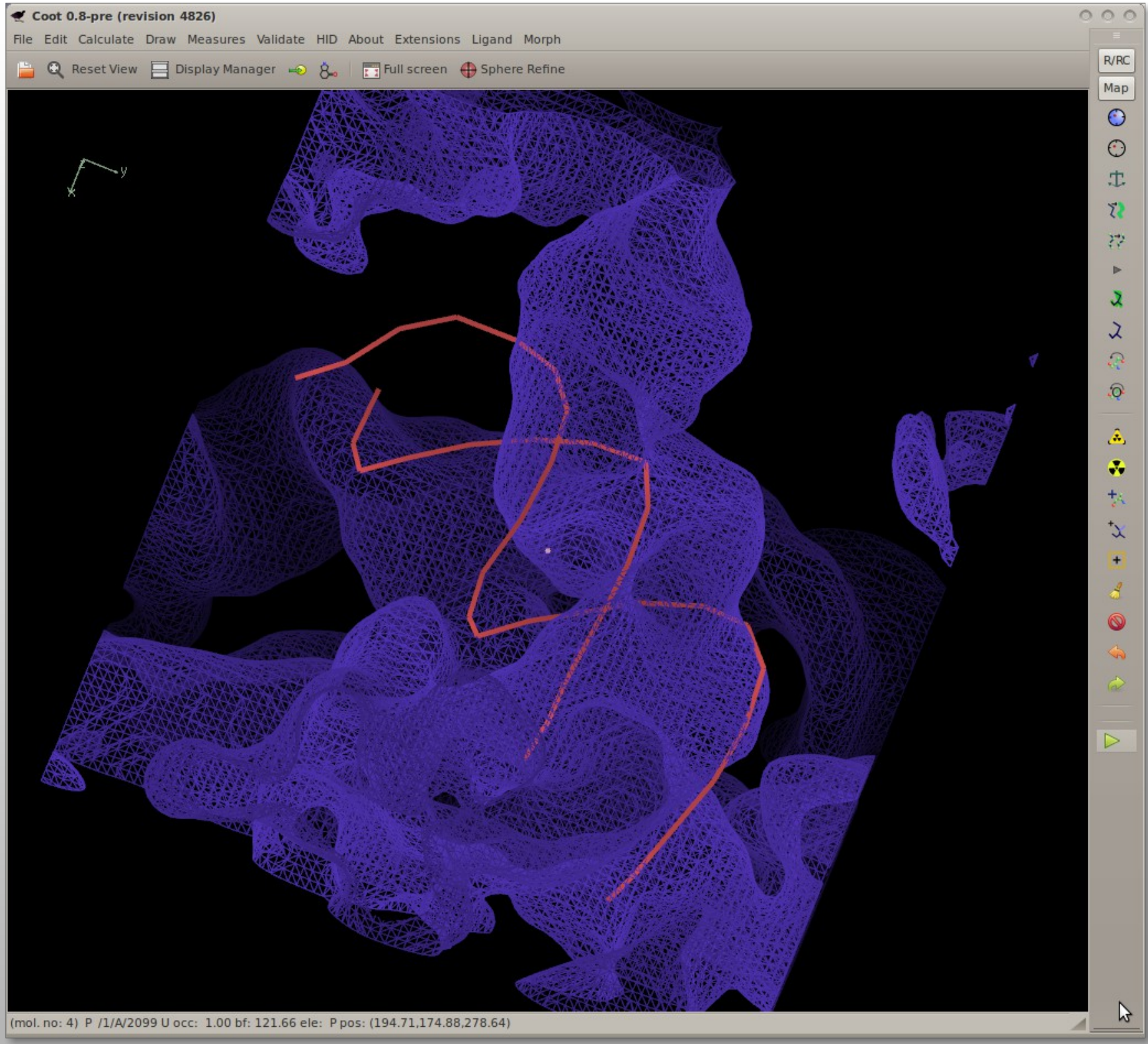

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Solid
Reset View
Display Manager
R/RC
Map
(mol. no: 4) CA /1/C/255 ILE occ: 1.00 bf: 49.77 ele: C pos: (30.91,18.75,-32.05)

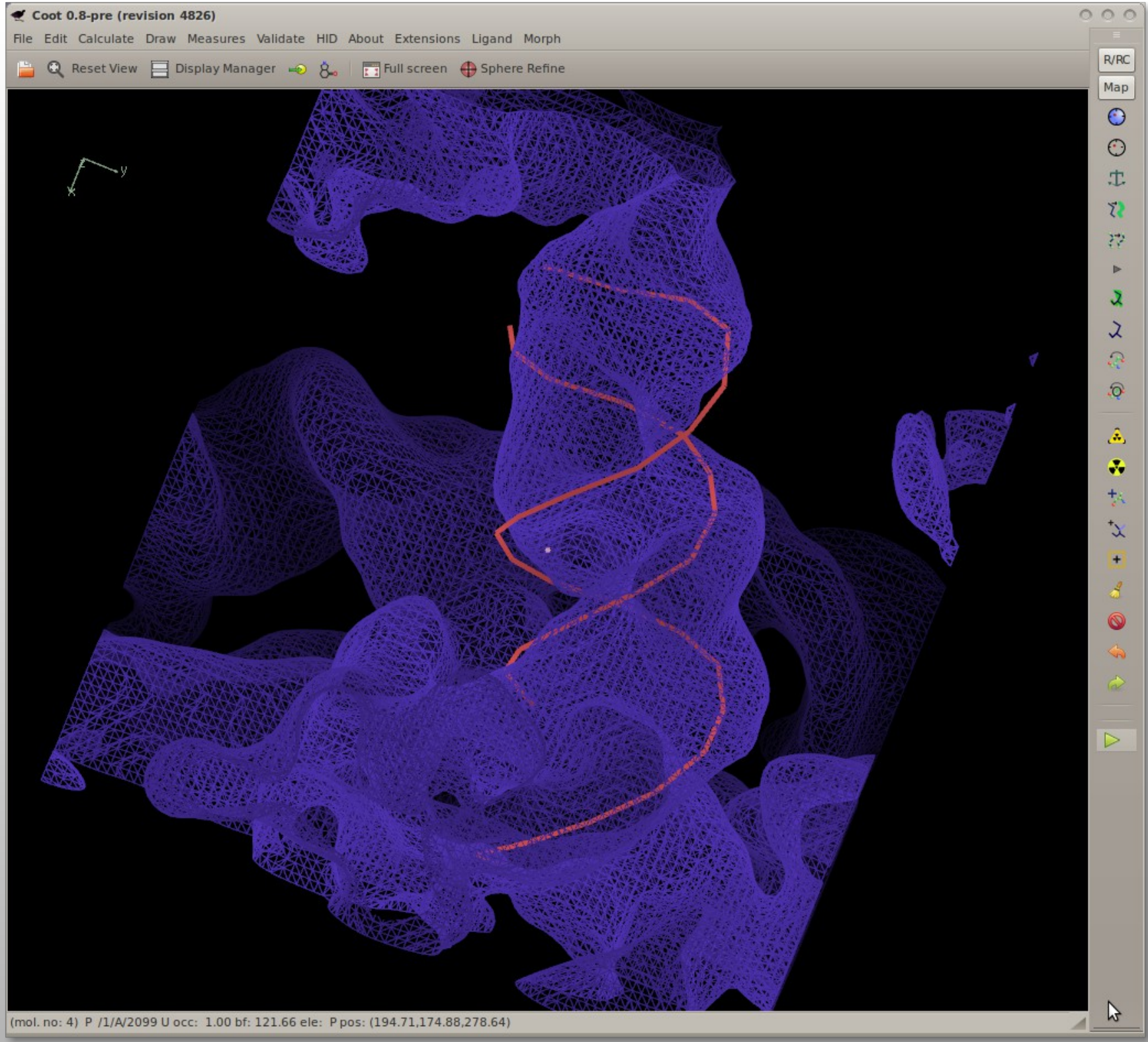
Jiggle Fit

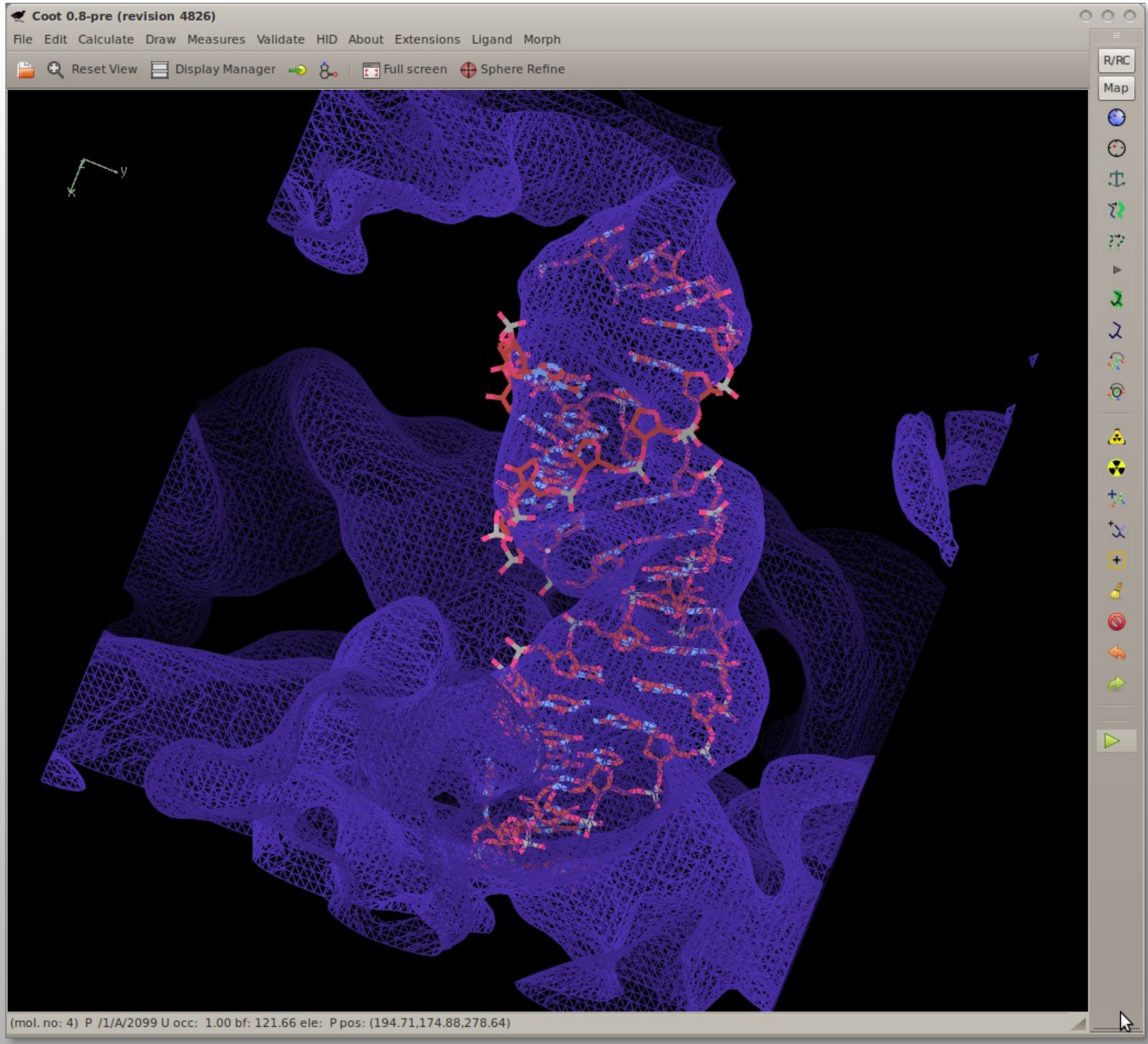
- How do I rotate and translate these atoms to fit the density?
  - 6-dimensional problem
- Originally used to fit simple ligands/solvent molecules to blobs of density
- Now extended to fit arbitrary atom selections
  - *e.g.* by Chain

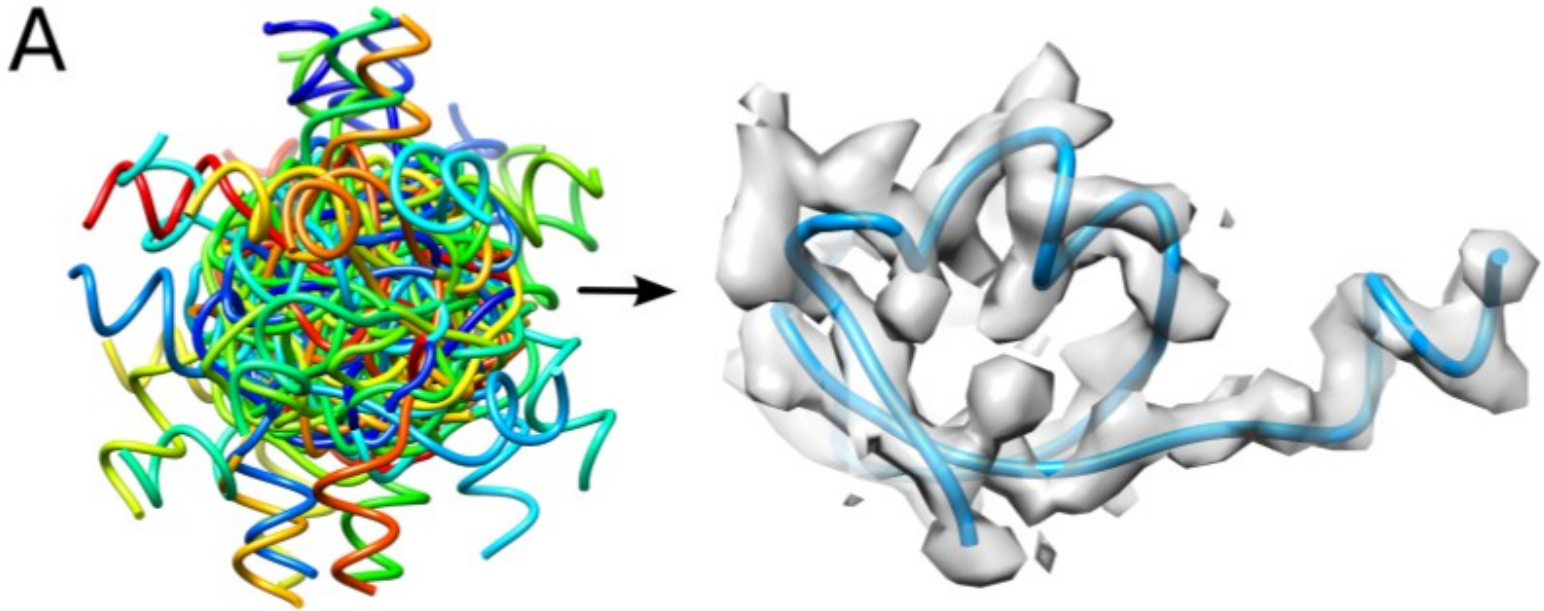
Jiggle Fit: How it Works

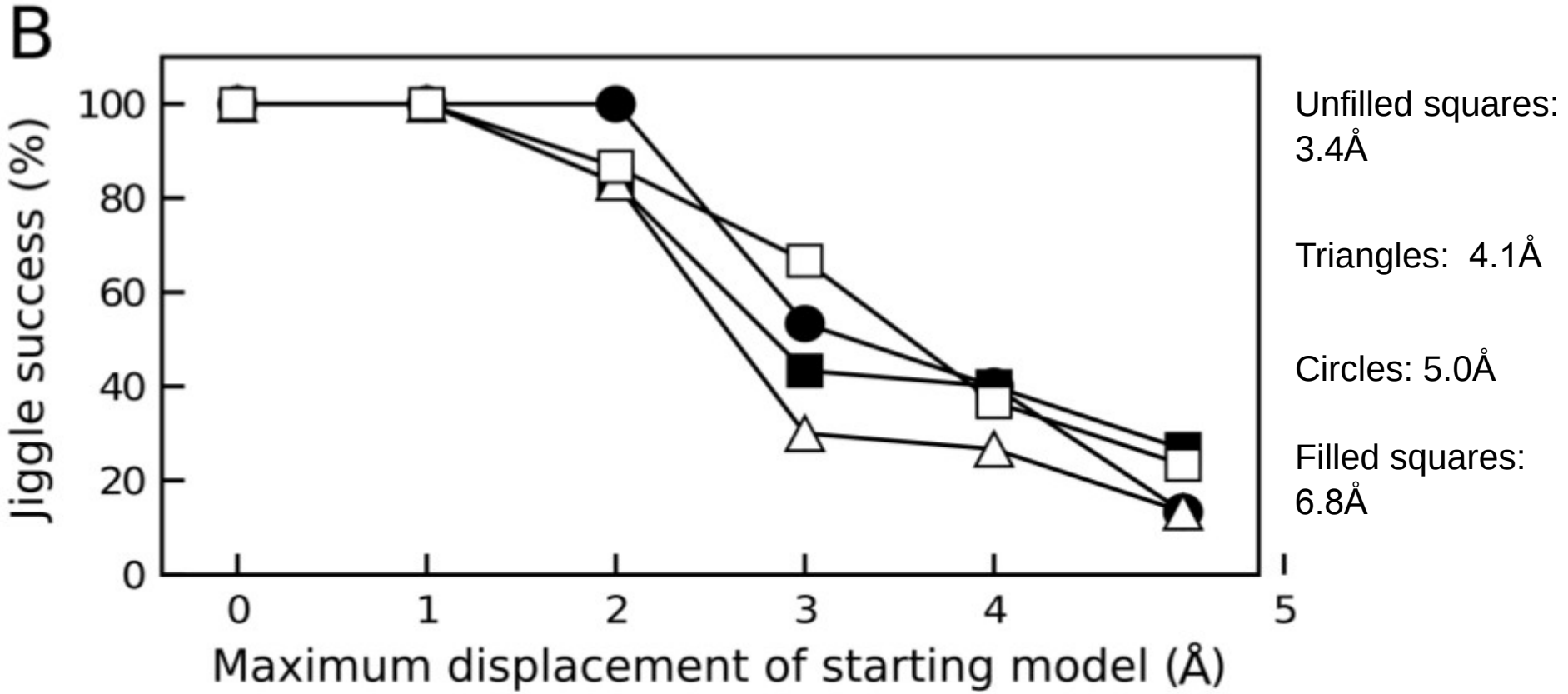
- Loop *n* (say 1000) times:
  - Generate random angles and translations
  - Transform atom selection by these rotations and translation
  - Score and store the fit to density
- Rank density fit scores,
  - Pick top 20 solution, for each of them
    - Rigid body fit and score solutions
    - Pick the highest scoring solution if it's better than the starting model)
- Radius of Convergence is larger when using a low-pass map


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)


A


B
Jiggle success (%)
100
80
60
40
20
0
0
1
2
3
4
5
Maximum displacement of starting model (Å)
Unfilled squares: 3.4Å
Triangles: 4.1Å
Circles: 5.0Å
Filled squares: 6.8Å

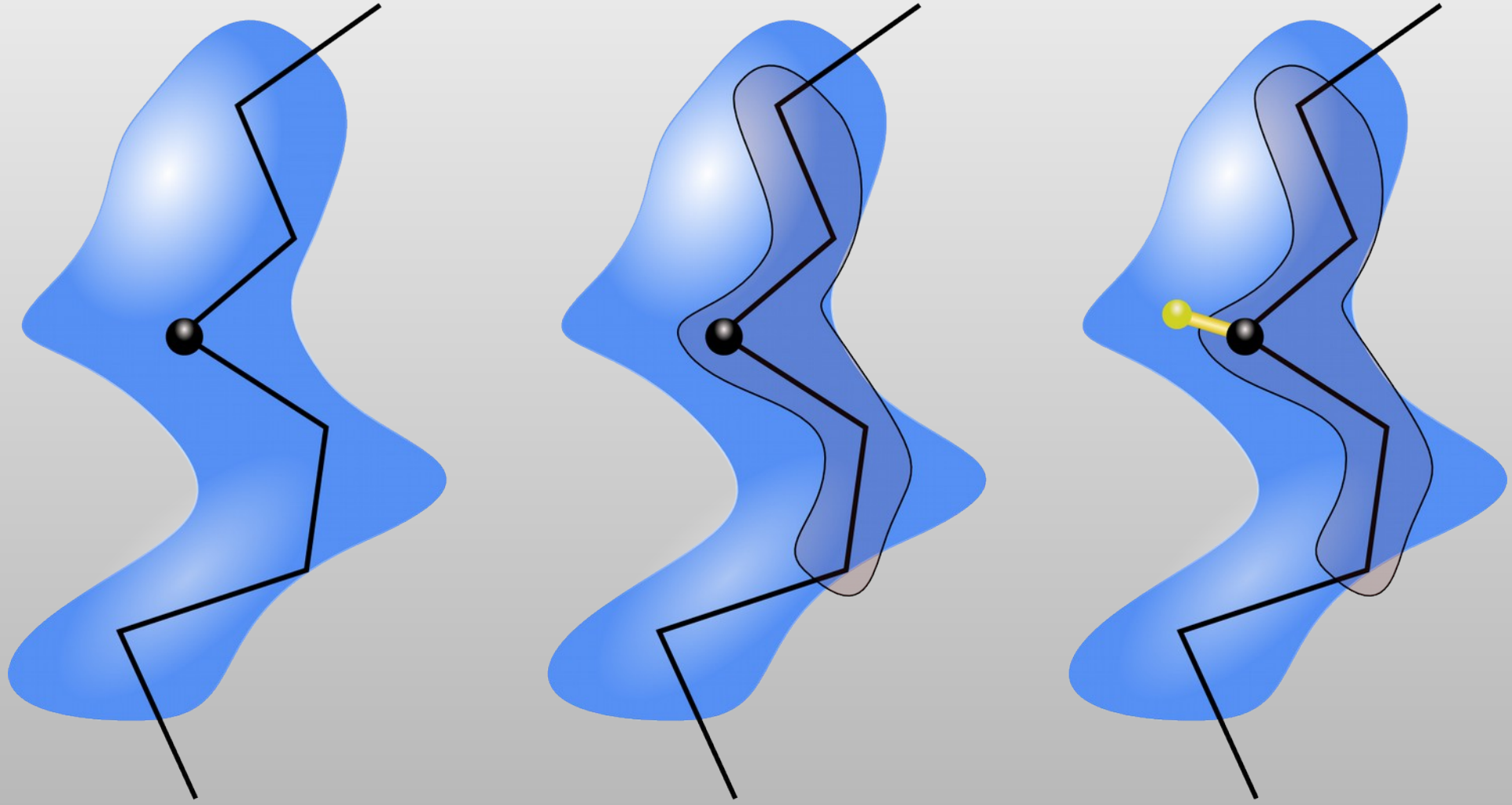
So we have our ideal RNA or homologous protein sitting roughly in the density

(not a great fit)

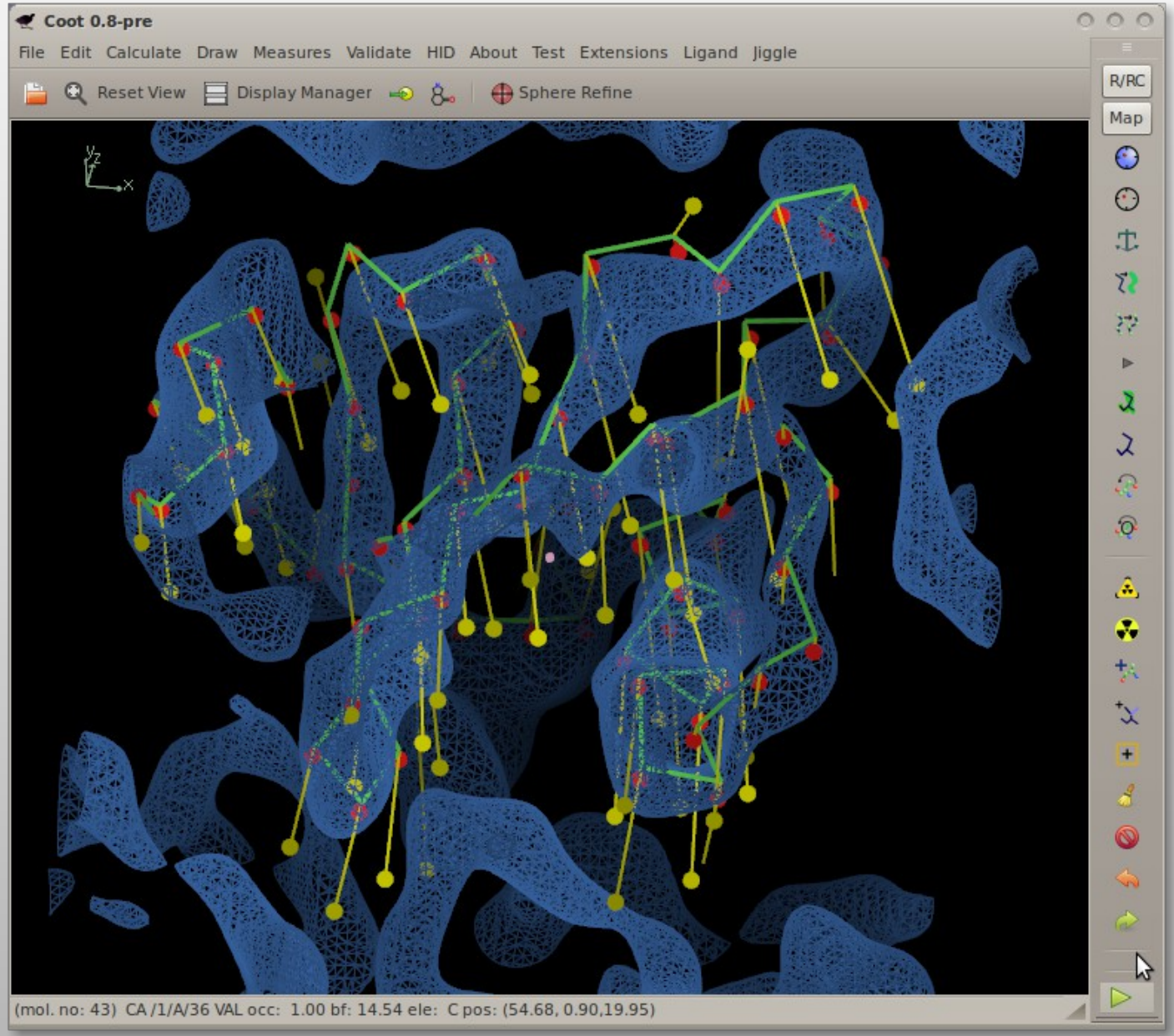
Model Morphing: How it Works

- For each residue in a chain, we ask:
  - where does a small fragment centred on this residue want to go?
  - (Robust) average the transformations and apply them on a per-residue basis
- Repeat

Model Morphing: Generating the Raw RTs

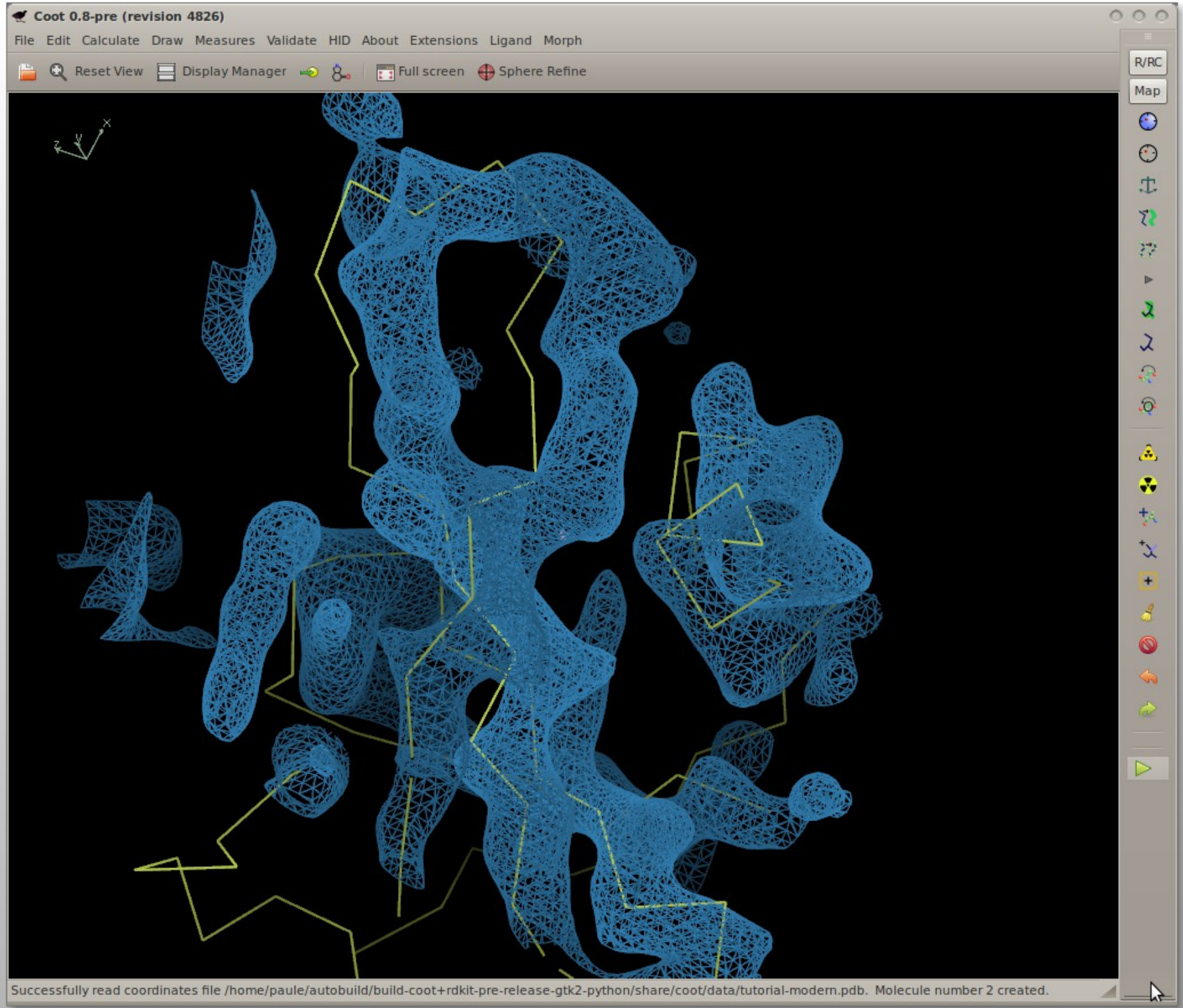


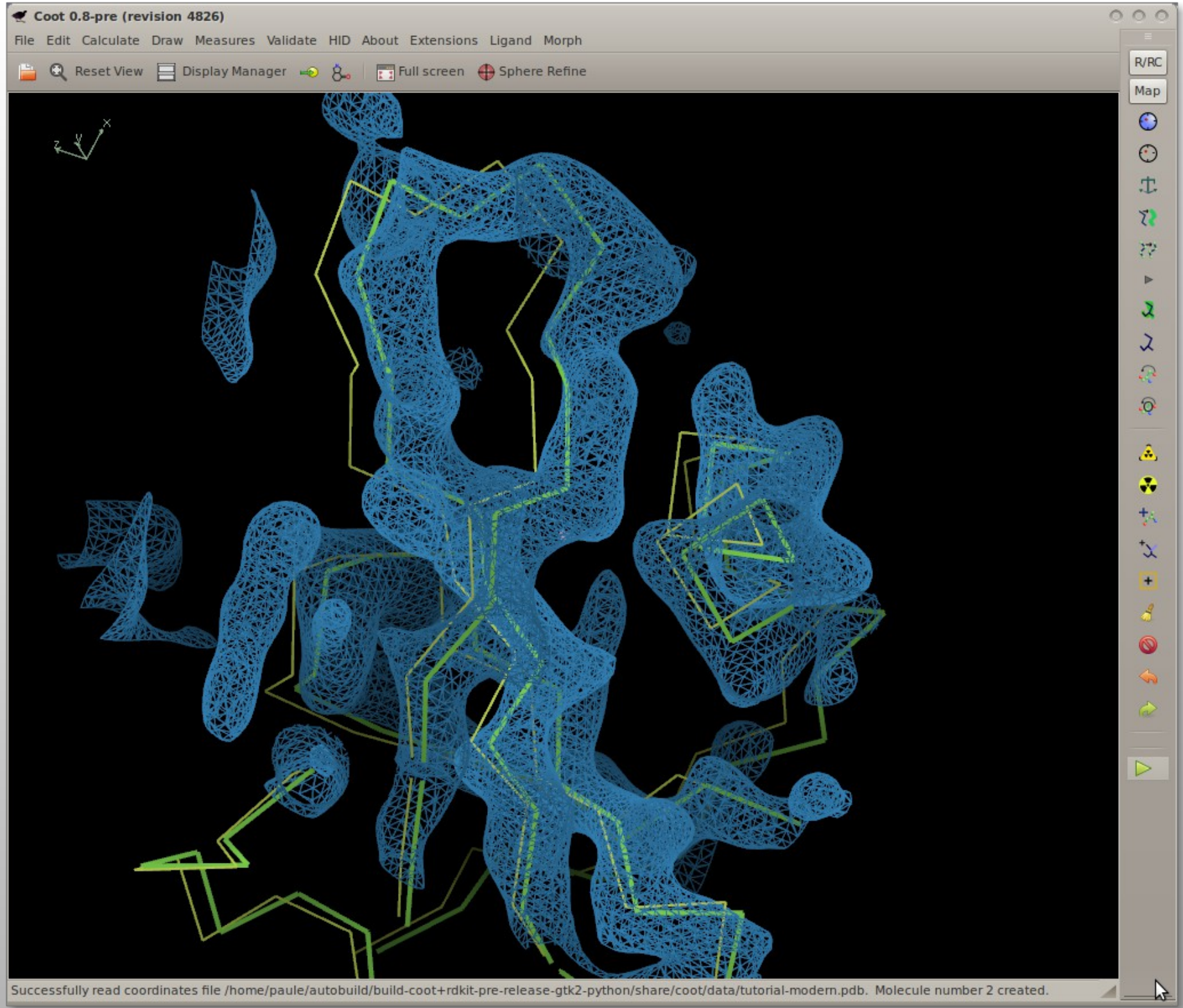
Model Morphing: Example

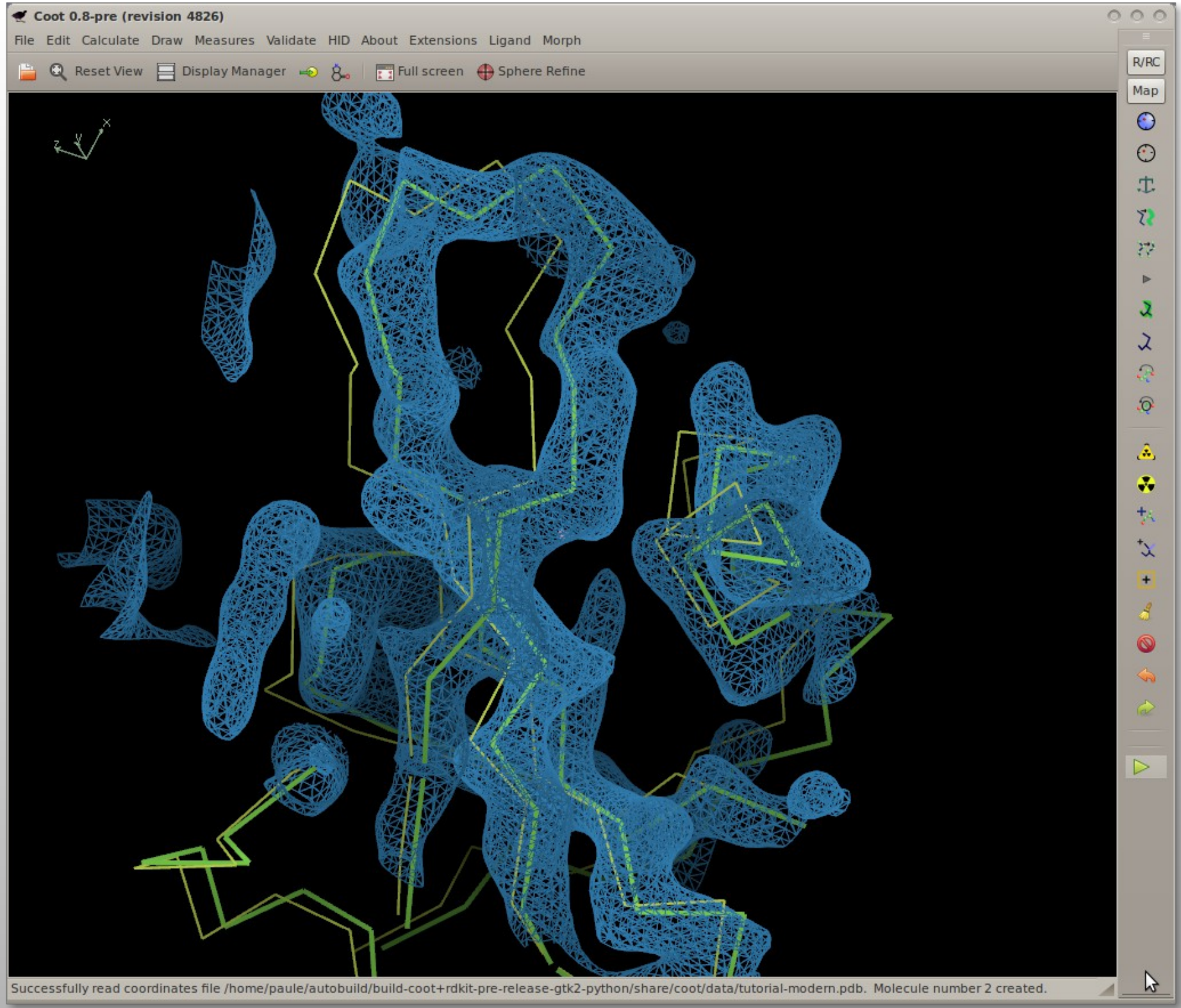


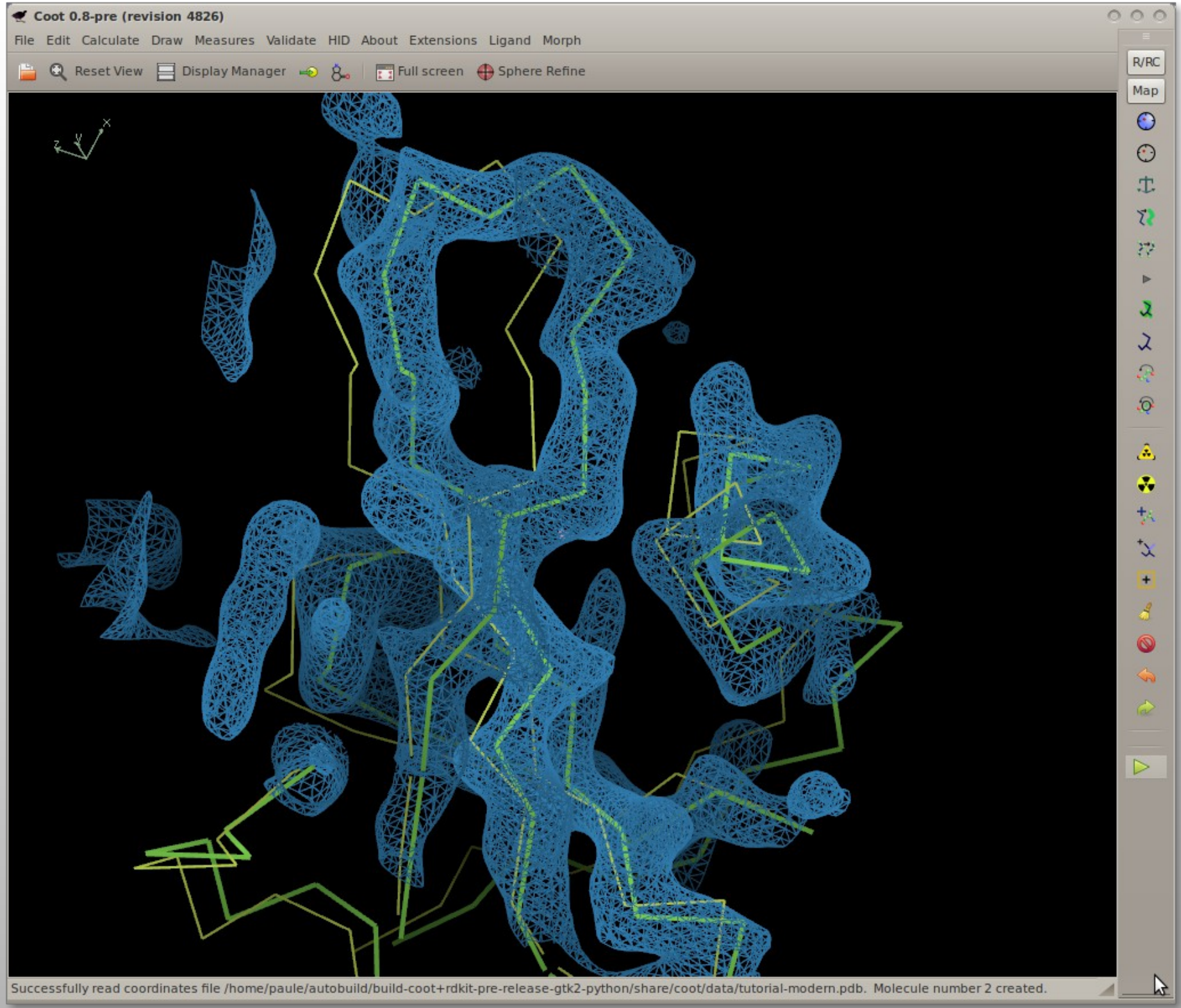
Model Morphing: Robust Averaging

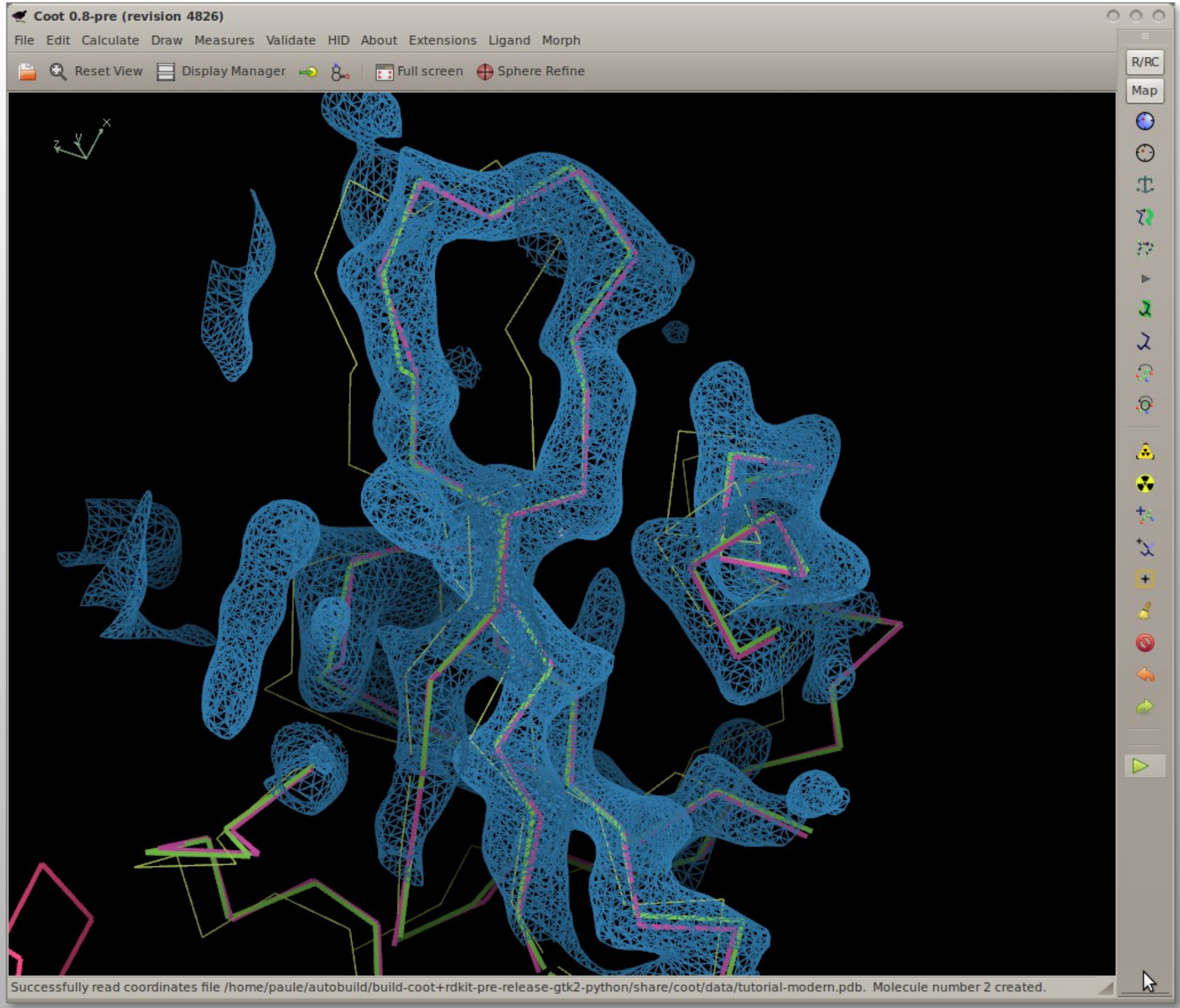
- What are the residues in the environment of a residue?
  - What are their RTs?
  - Create a metric 'distance', sort on that
  - Discard the top and bottom 25%
  - Use remaining RTs to generate average
  - ...which is then applied to central residue
- Repeat for all residues
- Larger environment radii make the shifts smaller/more conservative
  - More cycles needed


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

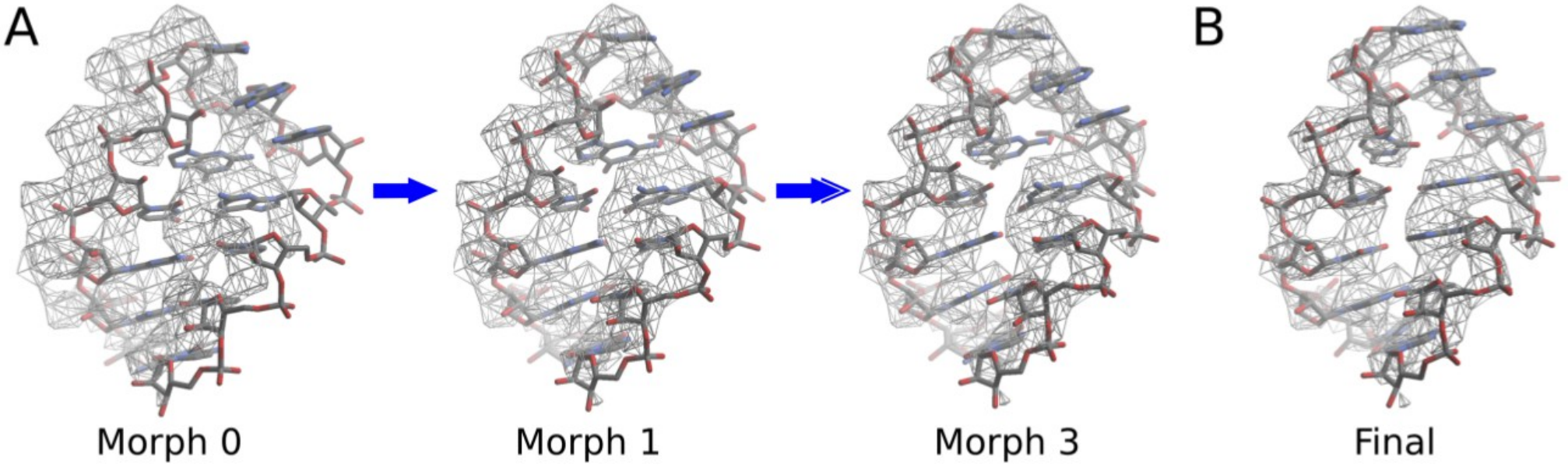

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.


Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Model Morphing



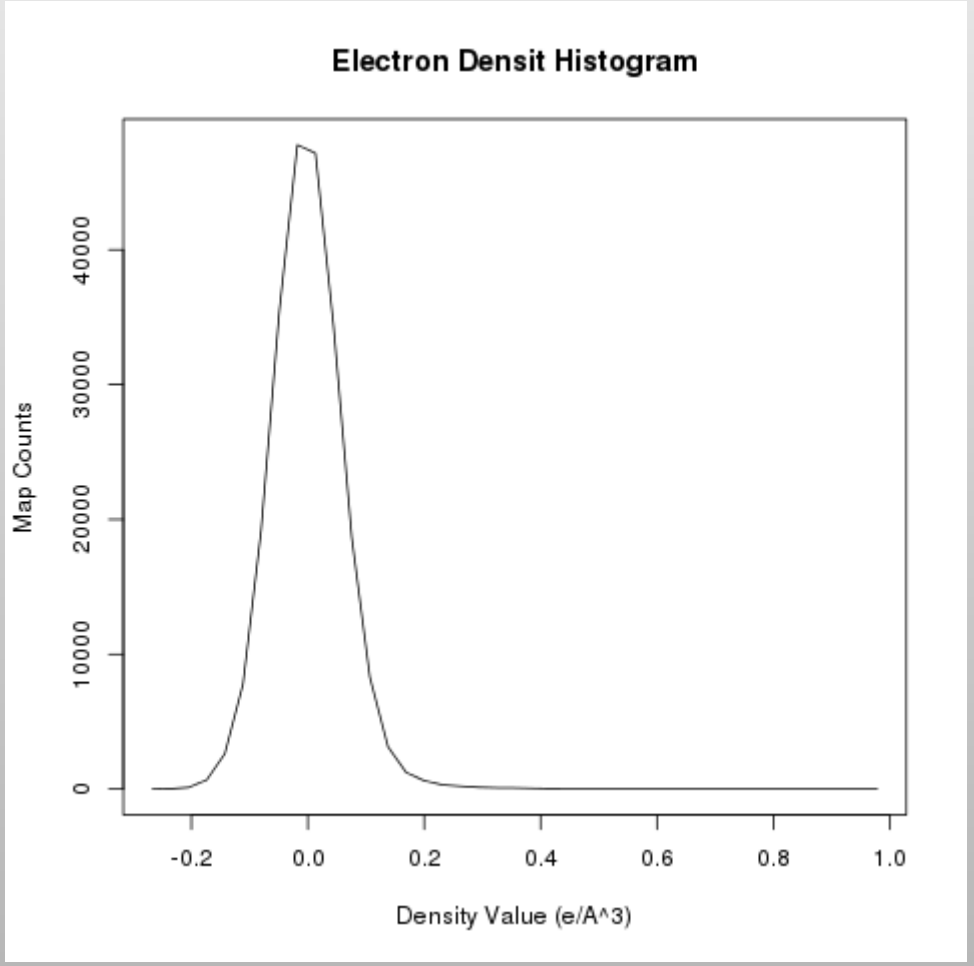
Helix Fitting

- The distribution of electron density is quite unlike that of x-ray maps
  - e.g. You don't see main-chain atoms at 4 rmsd in x-ray maps
    - regions of dense electron density contribute negatively to helix score

Helix Fitting

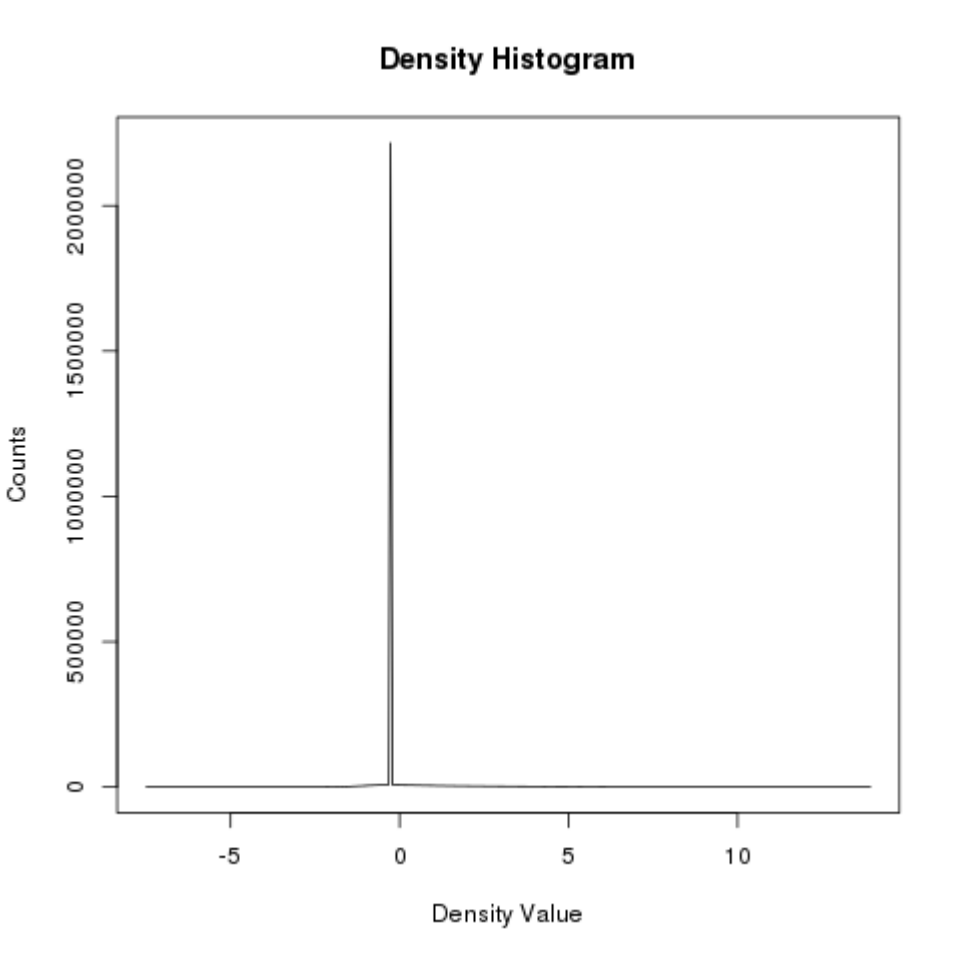
- The distribution of electron density is quite unlike that of x-ray maps

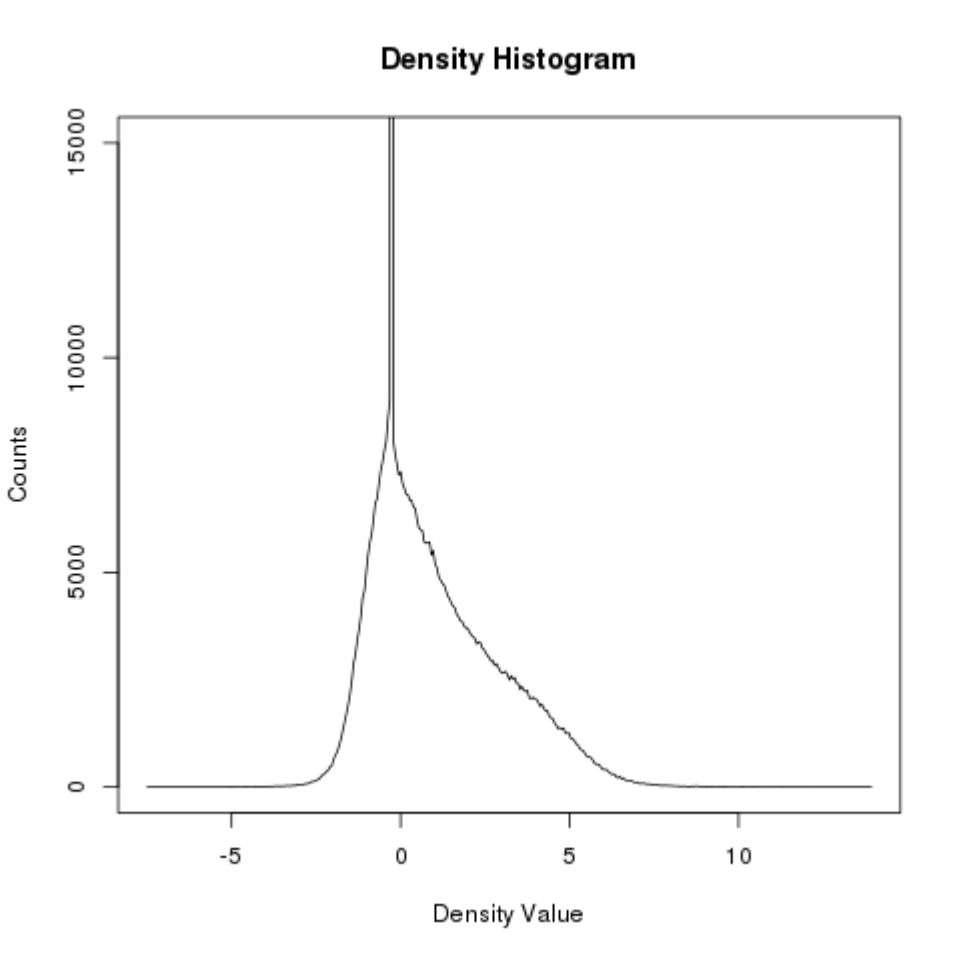
Typical Density Histogram from an X-ray map



Helix Fitting

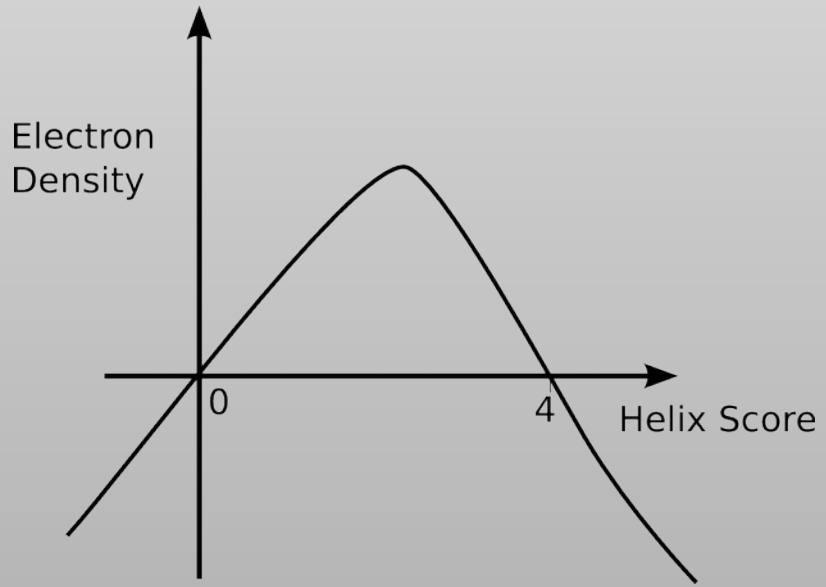
- The distribution of electron density is quite unlike that of x-ray maps

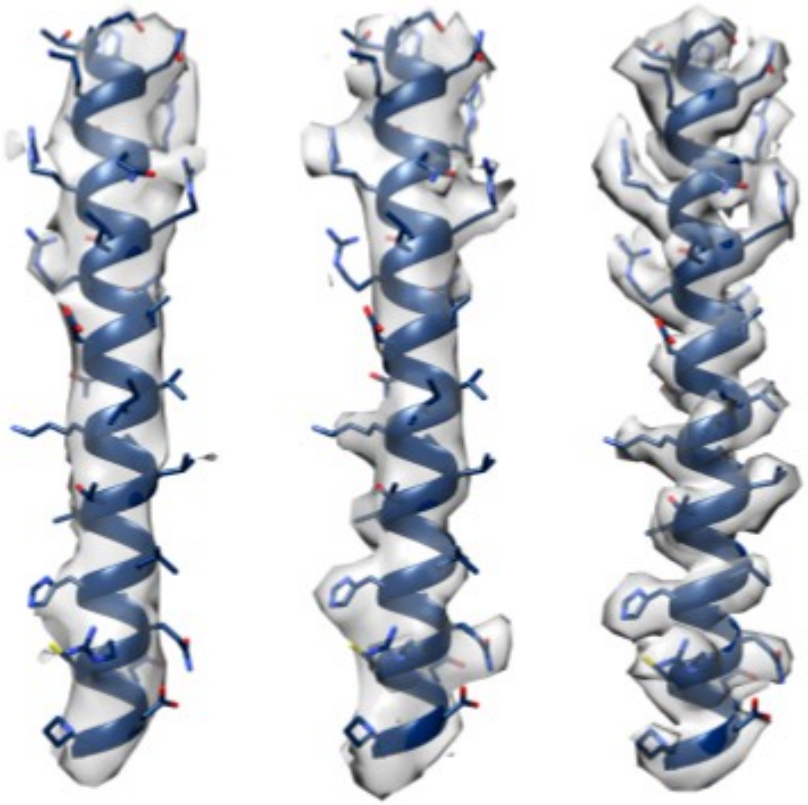




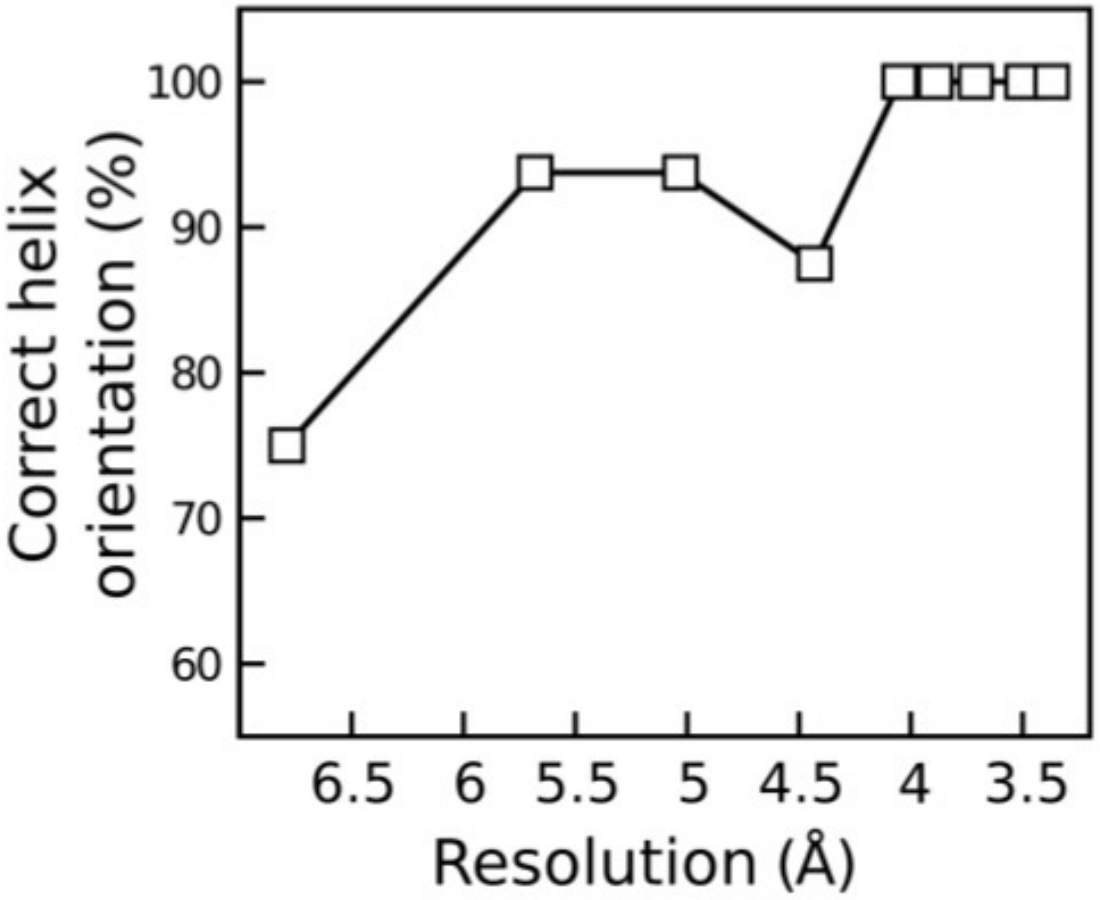
Helix Fitting

- The distribution of electron density is quite unlike that of x-ray maps
  - e.g. You don't see main-chain atoms at 4 rmsd in x-ray maps
    - regions of dense electron density contribute negatively to helix score
  - These EM maps were sharpened and in a big box of mostly nothing
  - Lots to see at 4 rmsd





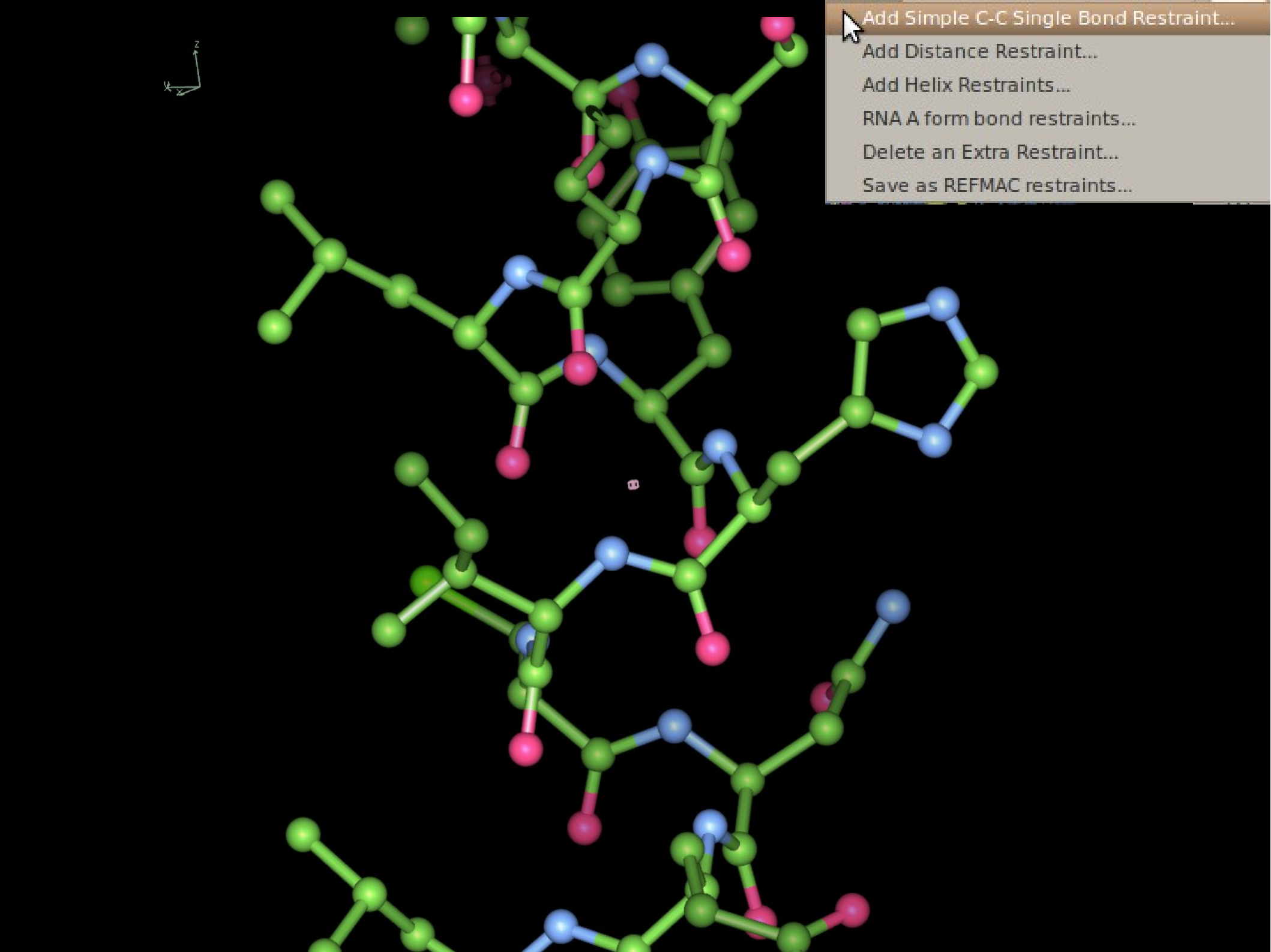
6.8Å 5.0Å 3.2Å

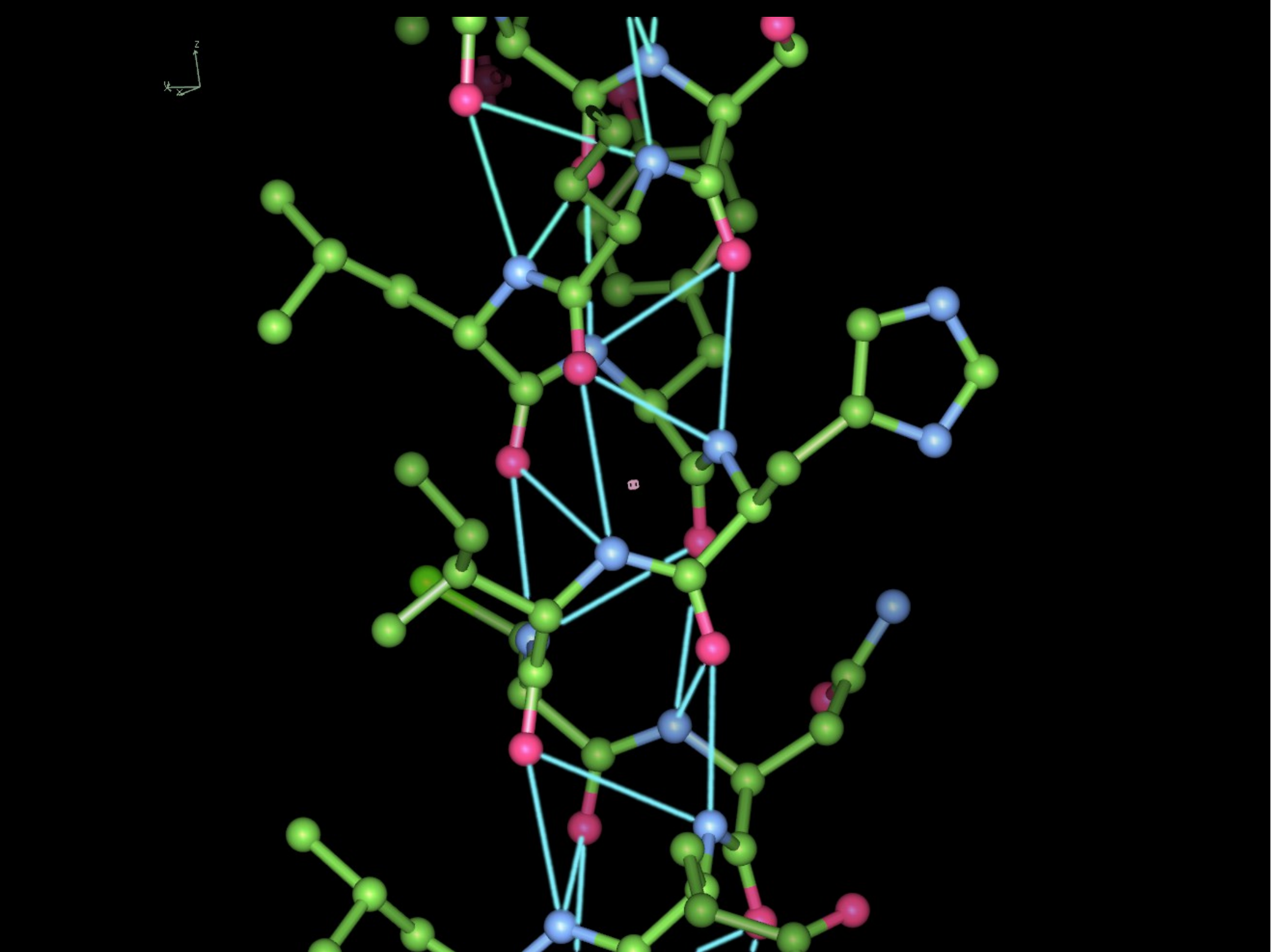

Correct helix orientation (%)
100
90
80
70
60
6.5
6
5.5
5
4.5
4
3.5
Resolution (Å)

Additional Restraints

Restraints Editing in *Coot*

- Distance Restraints:
  - Alpha helices, A-form RNA, B-form DNA
- Add and delete individual restraints
  - User-selectable sigma
- Select 2 residues for range
- User-defined torsion restraints
- Input from ProSMART
- Output to Refmac

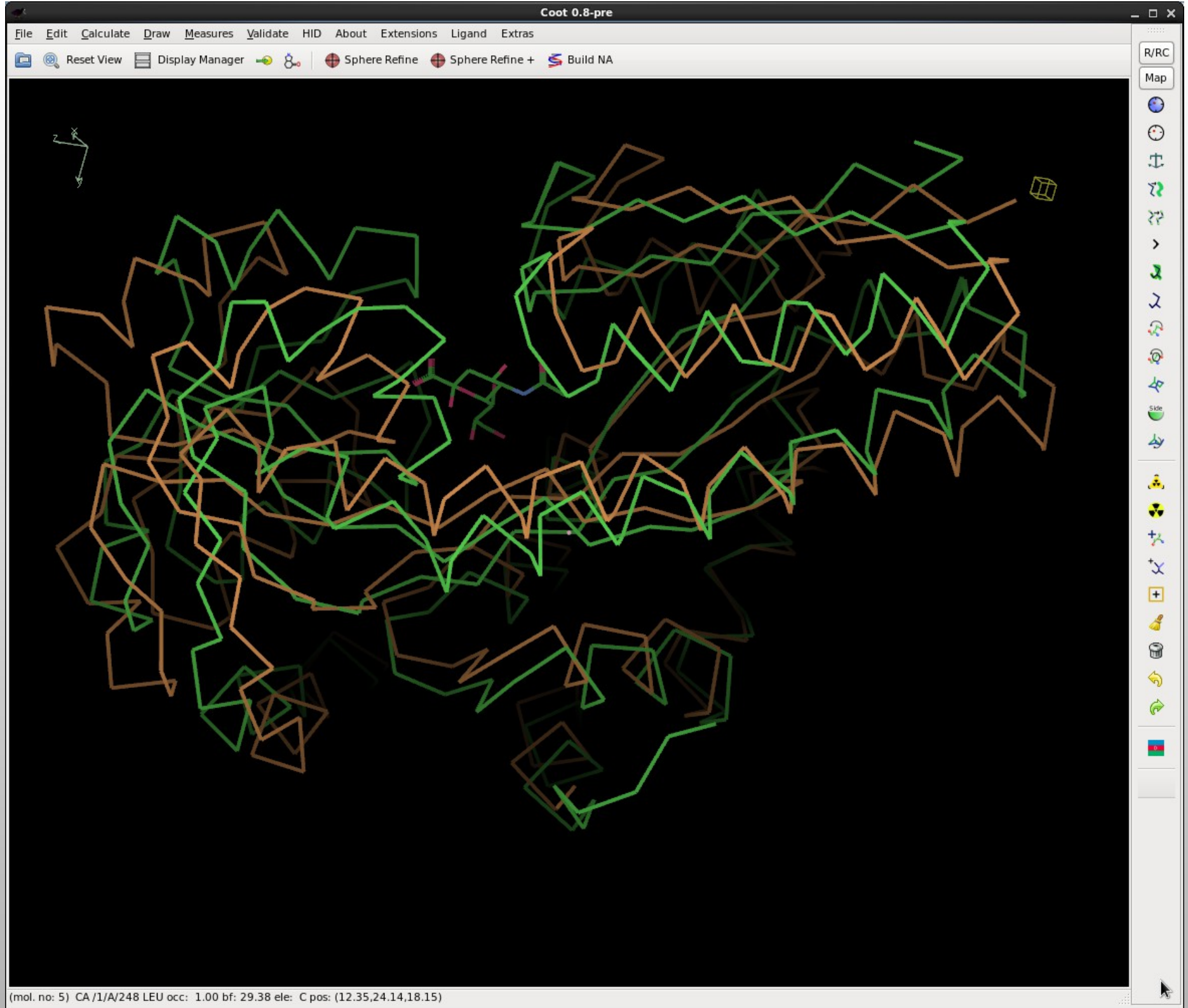

Add Simple C-C Single Bond Restraint...
Add Distance Restraint...
Add Helix Restraints...
RNA A form bond restraints...
Delete an Extra Restraint...
Save as REFMAC restraints...

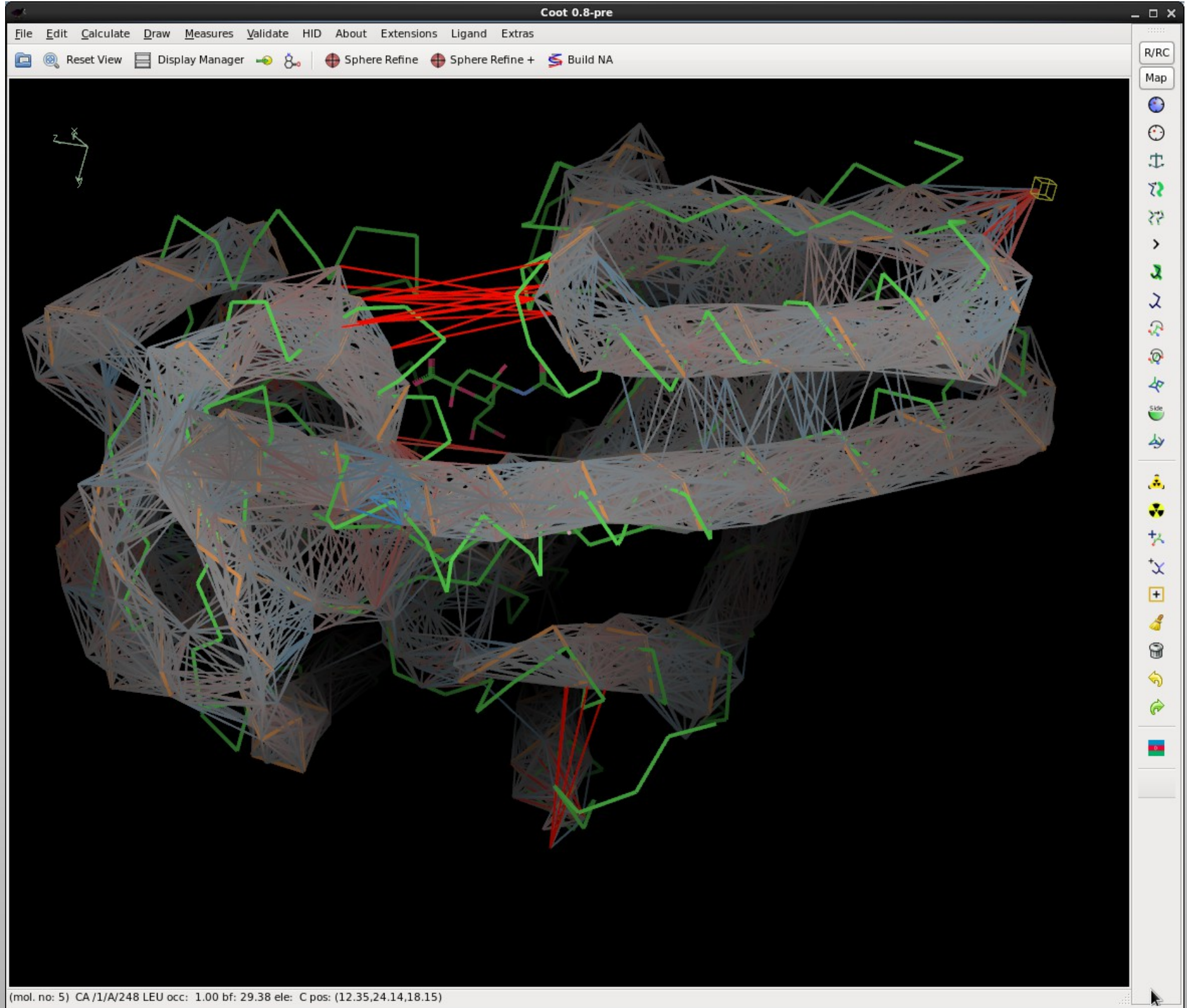


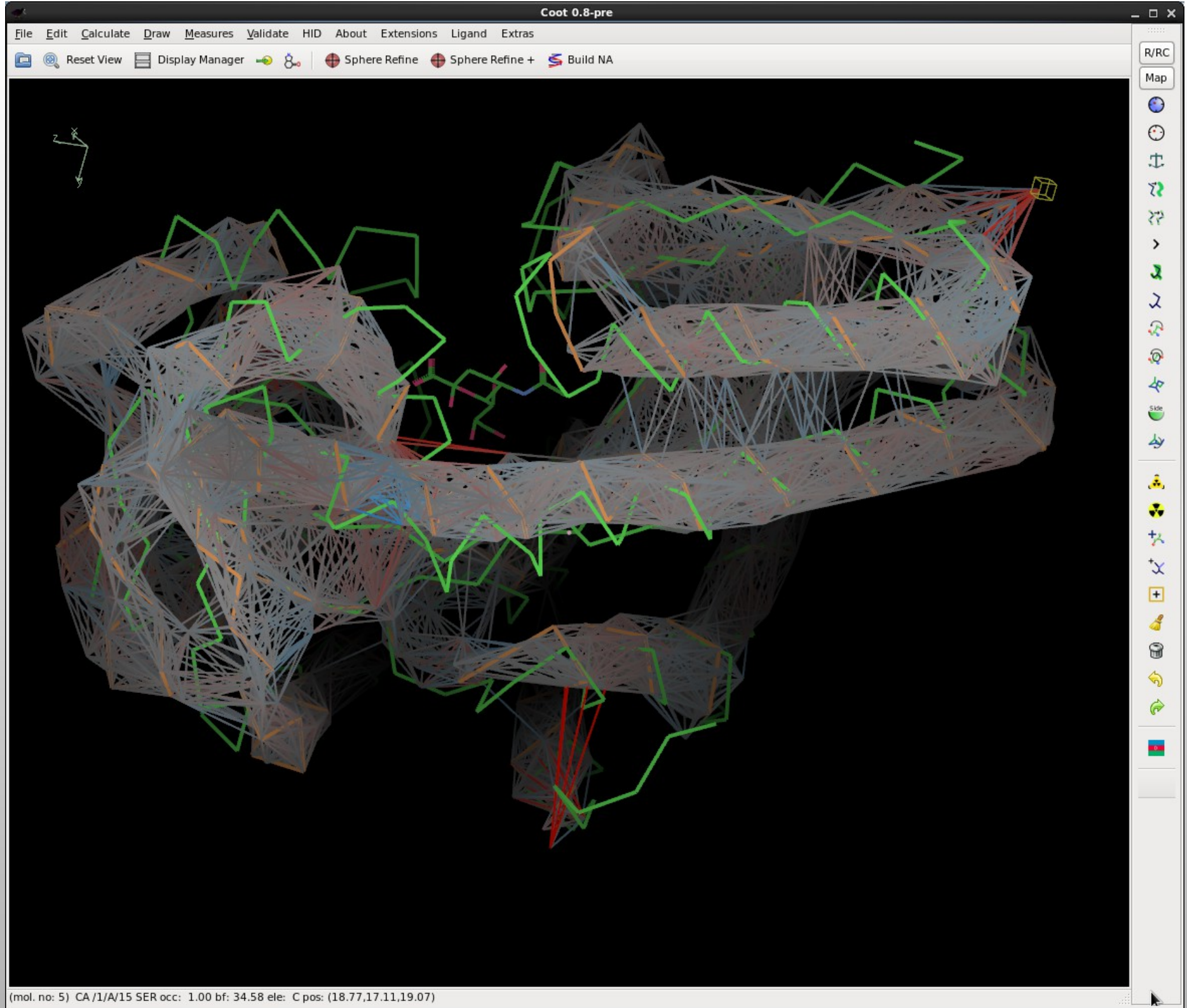
ProSMART Interface

- Use previous-solved “template” structures to inform the refinement of the (low resolution) target protein
- Conformation-independent structural comparison/superposition
- and restraint generation


Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)


Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)

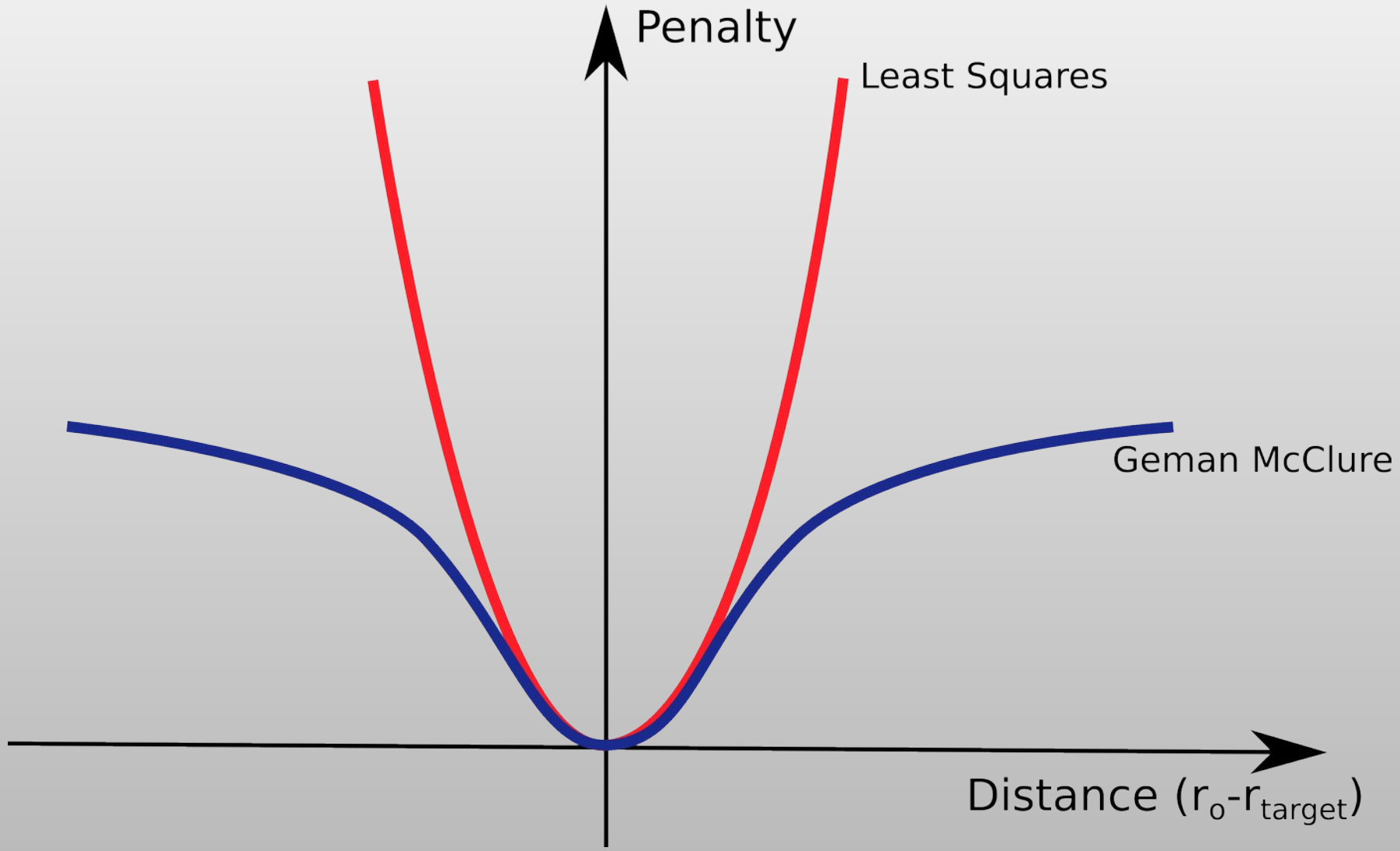

Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)


Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/15 SER occ: 1.00 bf: 34.58 ele: C pos: (18.77,17.11,19.07)

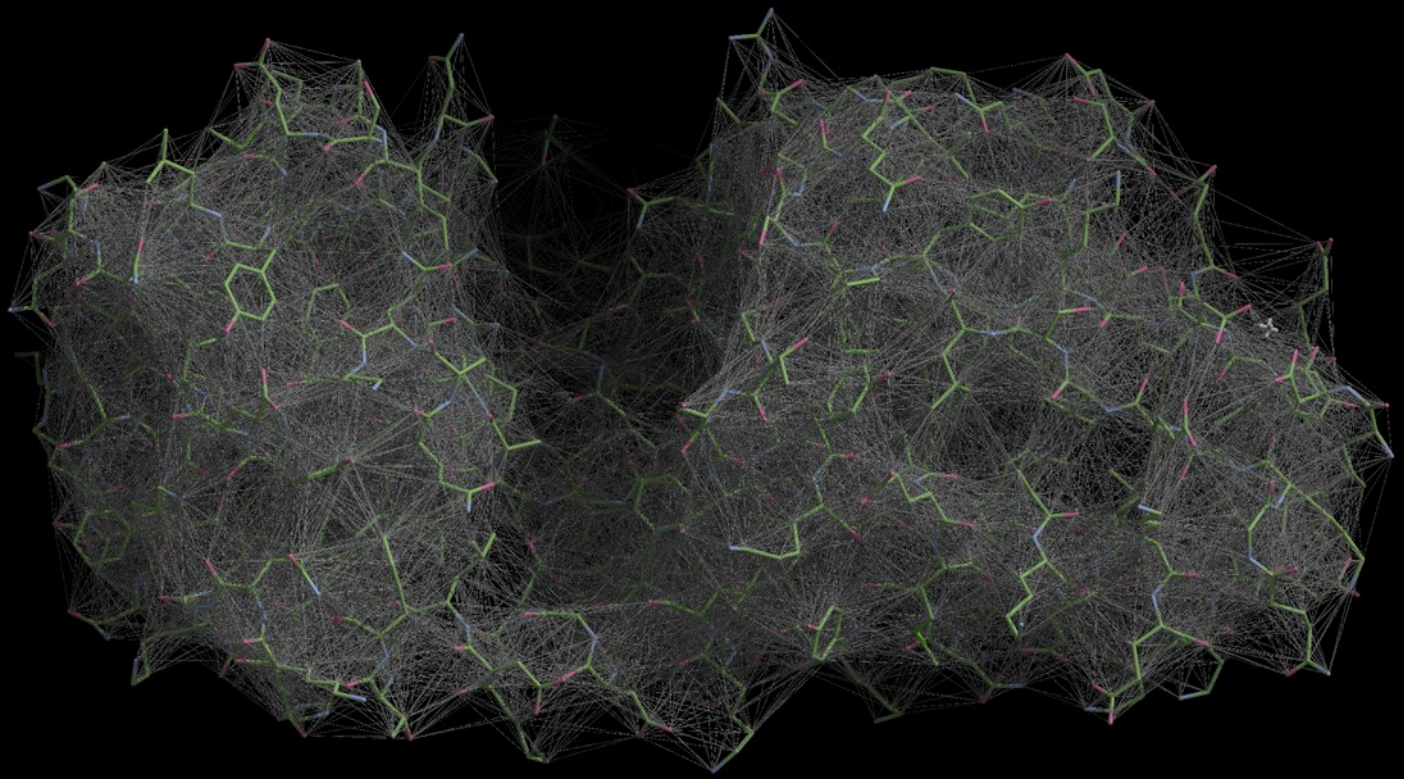
ProSMART integration

- ProSMART generates distance restraints from homologous structures
  - to be applied to current model for refinement
  - now available in *Coot*

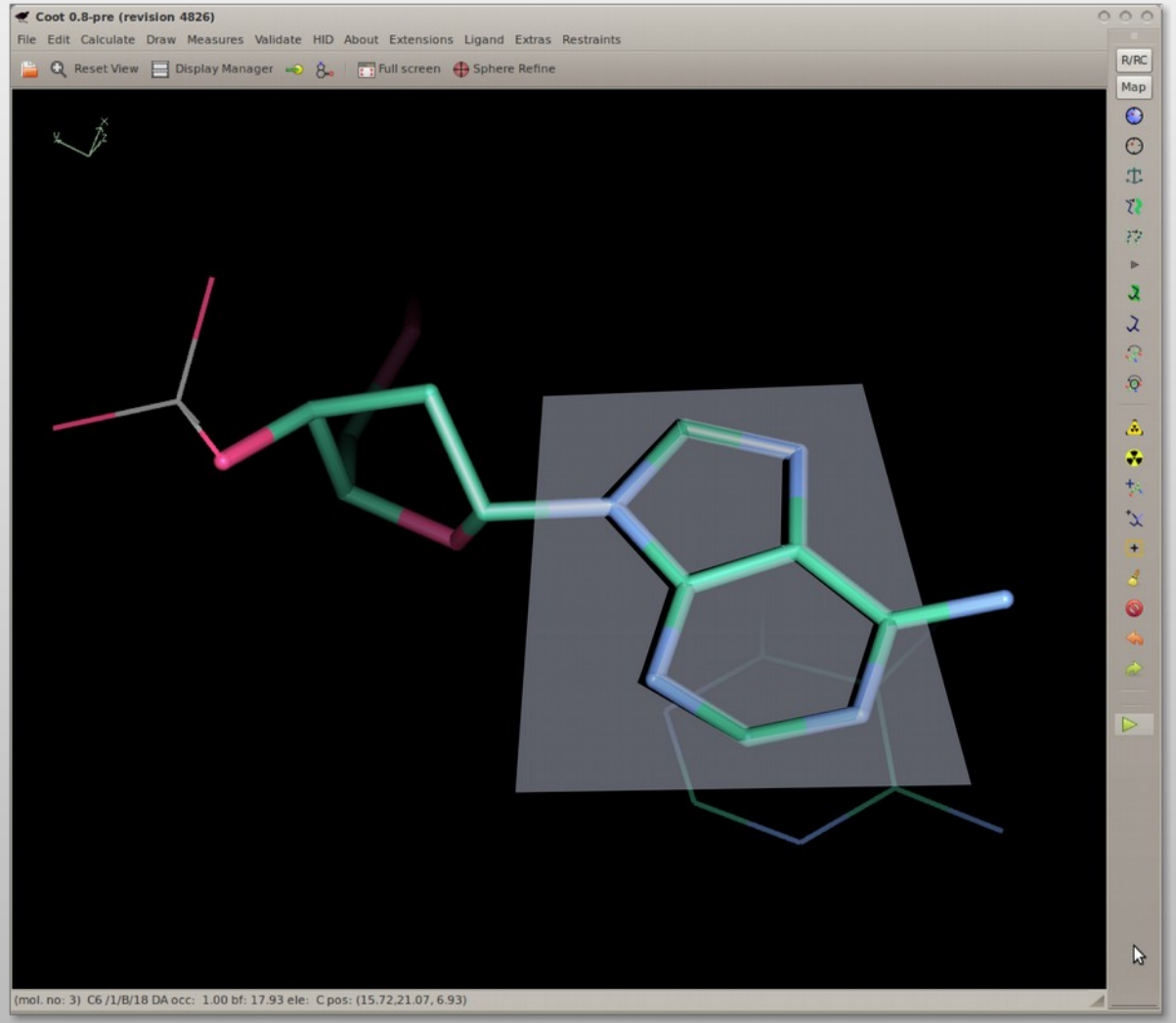
Modified Target Function



ProSMART Restraints

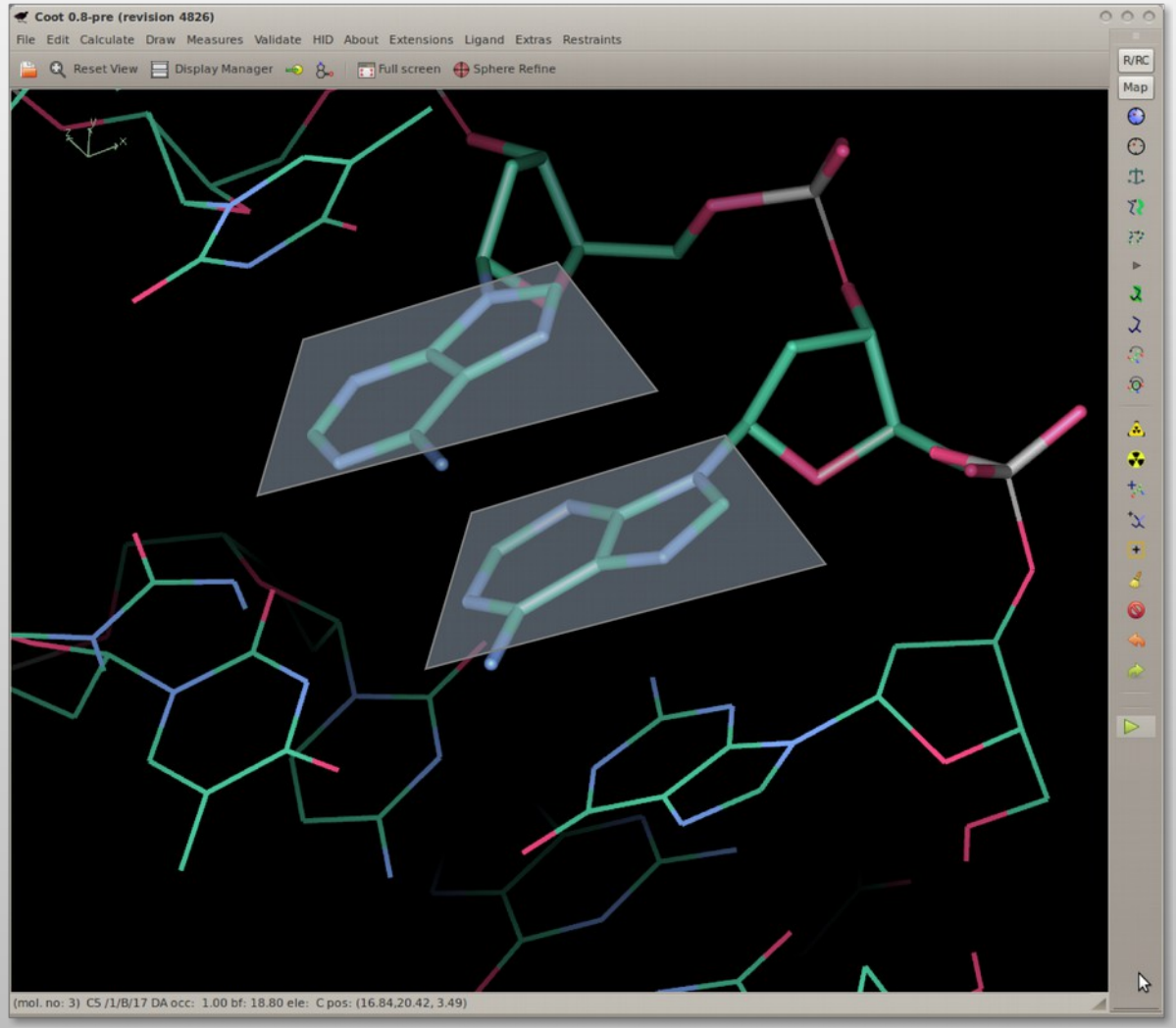


Plane Restraints



Derivativaties are an eigenvector scaled by out-of-plane distance

Parallel Planes Restraints



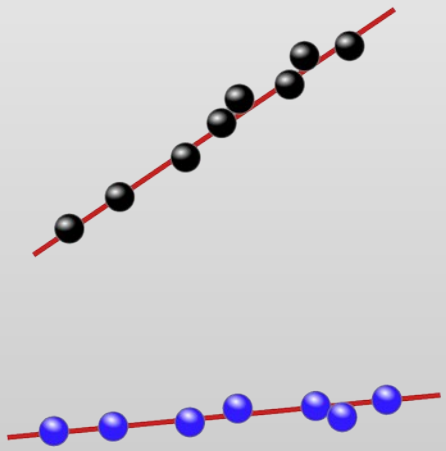
$$S = (a_1 - a_2)^2 + (b_1 - b_2)^2 + (c_1 - c_2)^2$$

Not easy to use in Coot

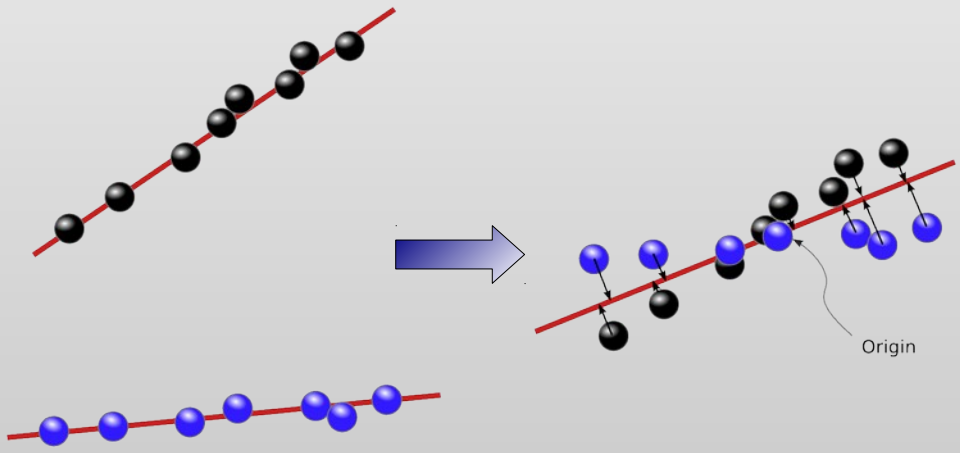
Parallel Planes Restraints

- Also, we have considered parallel-planes distance restraints
  - More tricky still to implement
  - Not implemented yet (not in *Coot*, anyway)

Parallel Planes Restraints

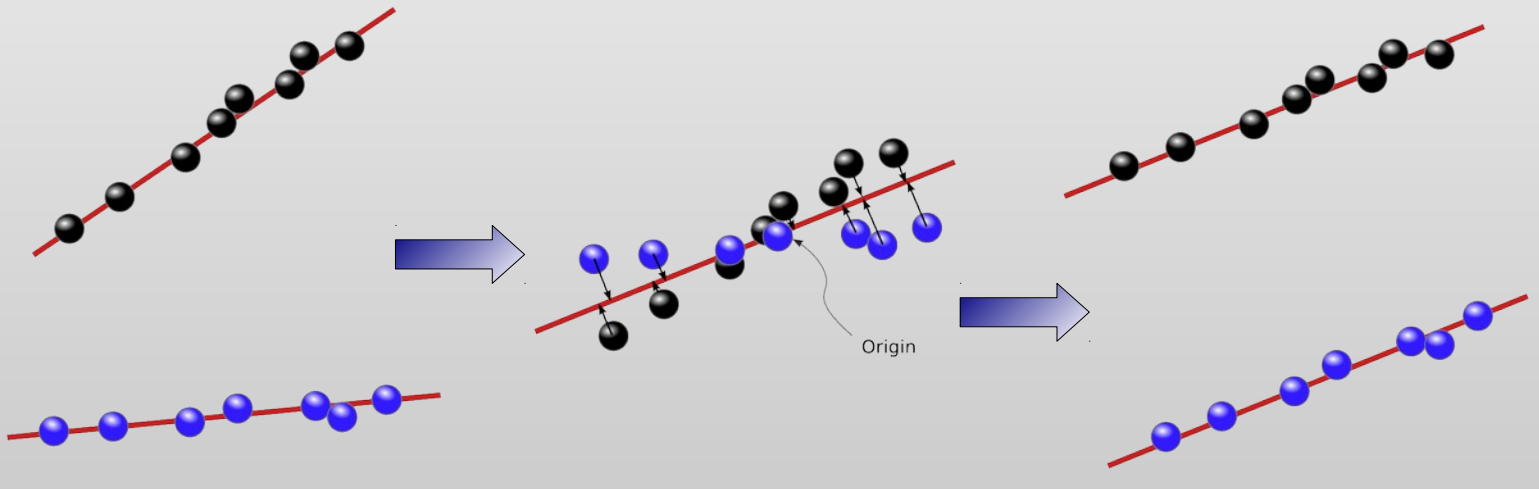


Parallel Plane Restraints



Shift to Origin

Parallel Planes Restraints

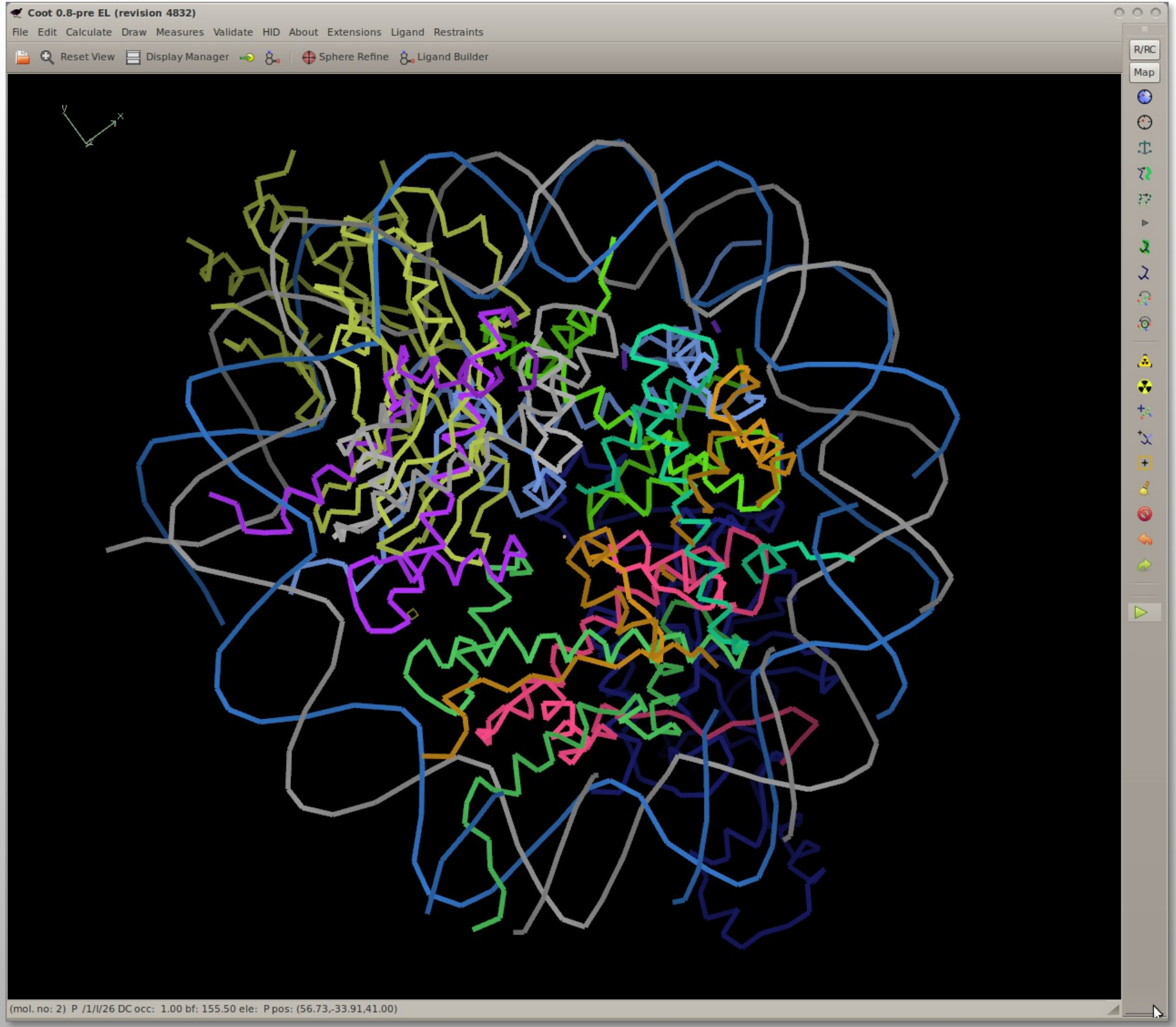


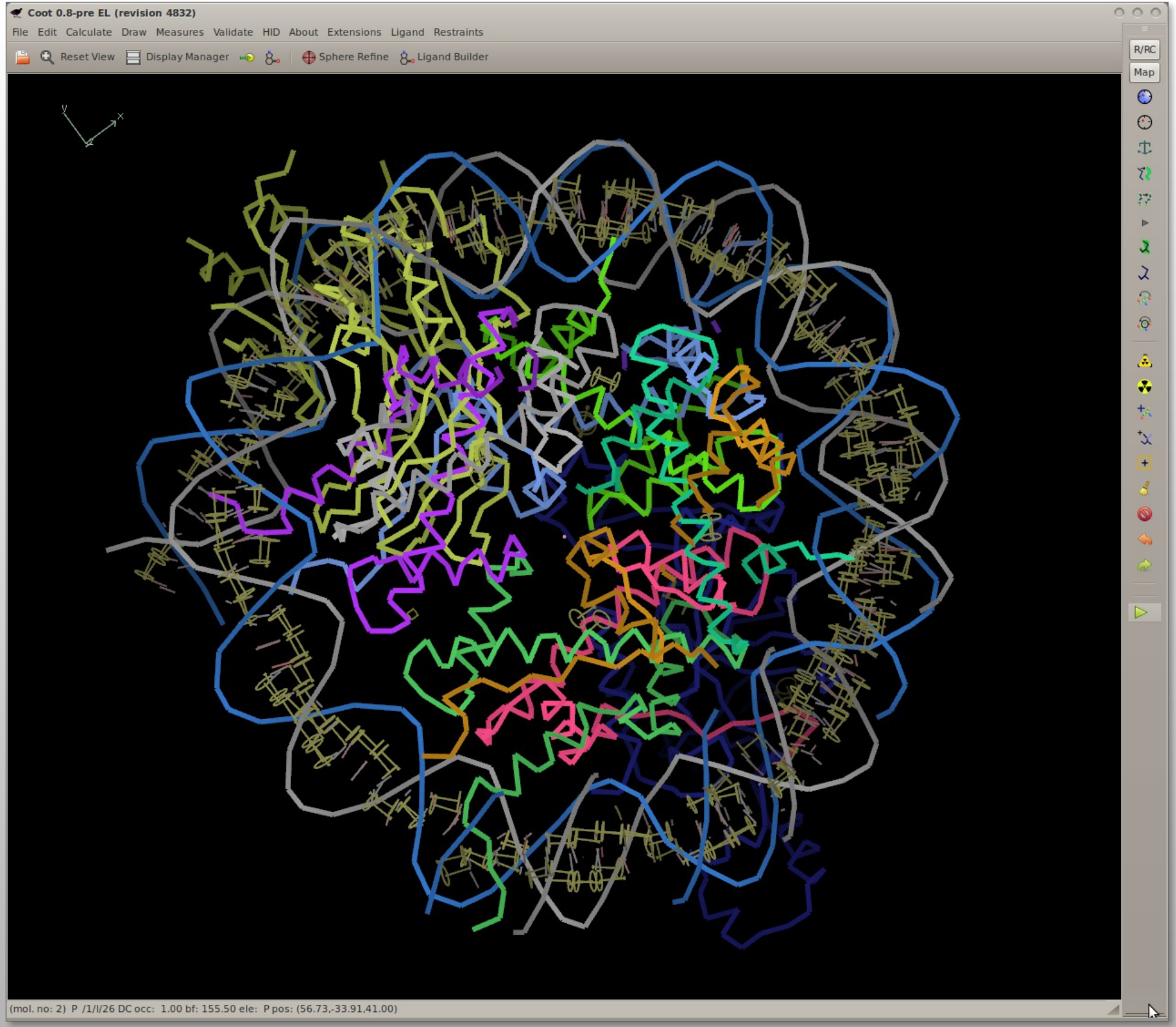
Shift to Origin

Move Back to Molecule

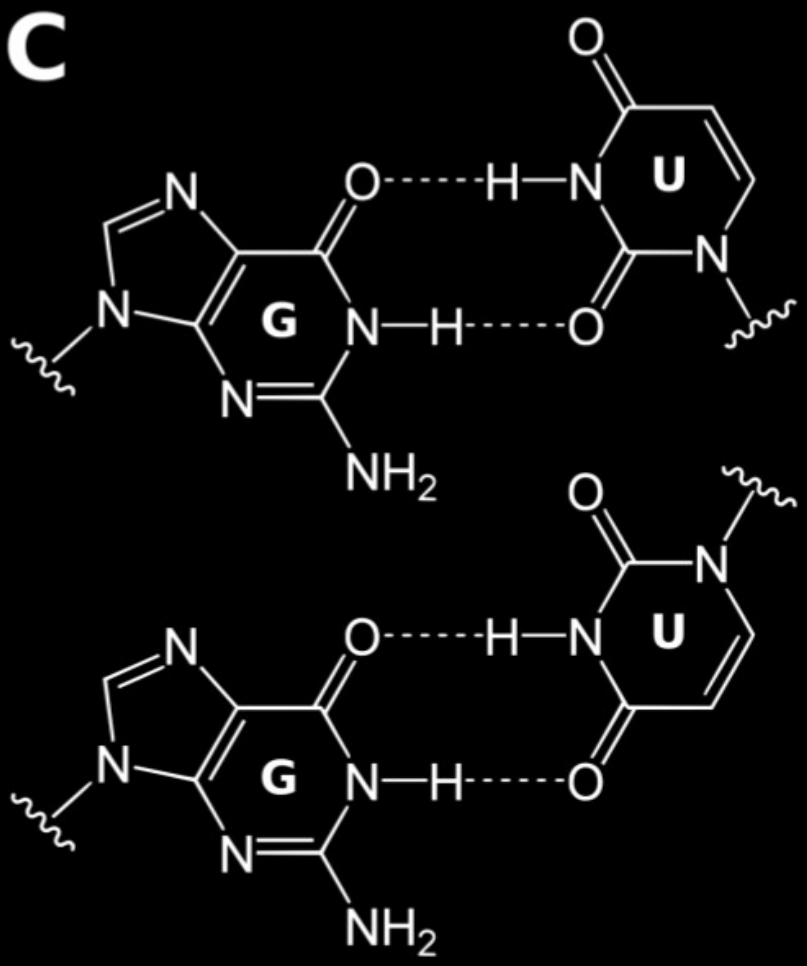
Automatic Generation of Base Pairing and Stacking Restraints

- Fei Long's `libg_d`
  - Provide it with a model and it writes out Refmac restraint descriptions
  - ... which *Coot* can also read
  - *Coot* can also create user-define base-paring and stacking restraints

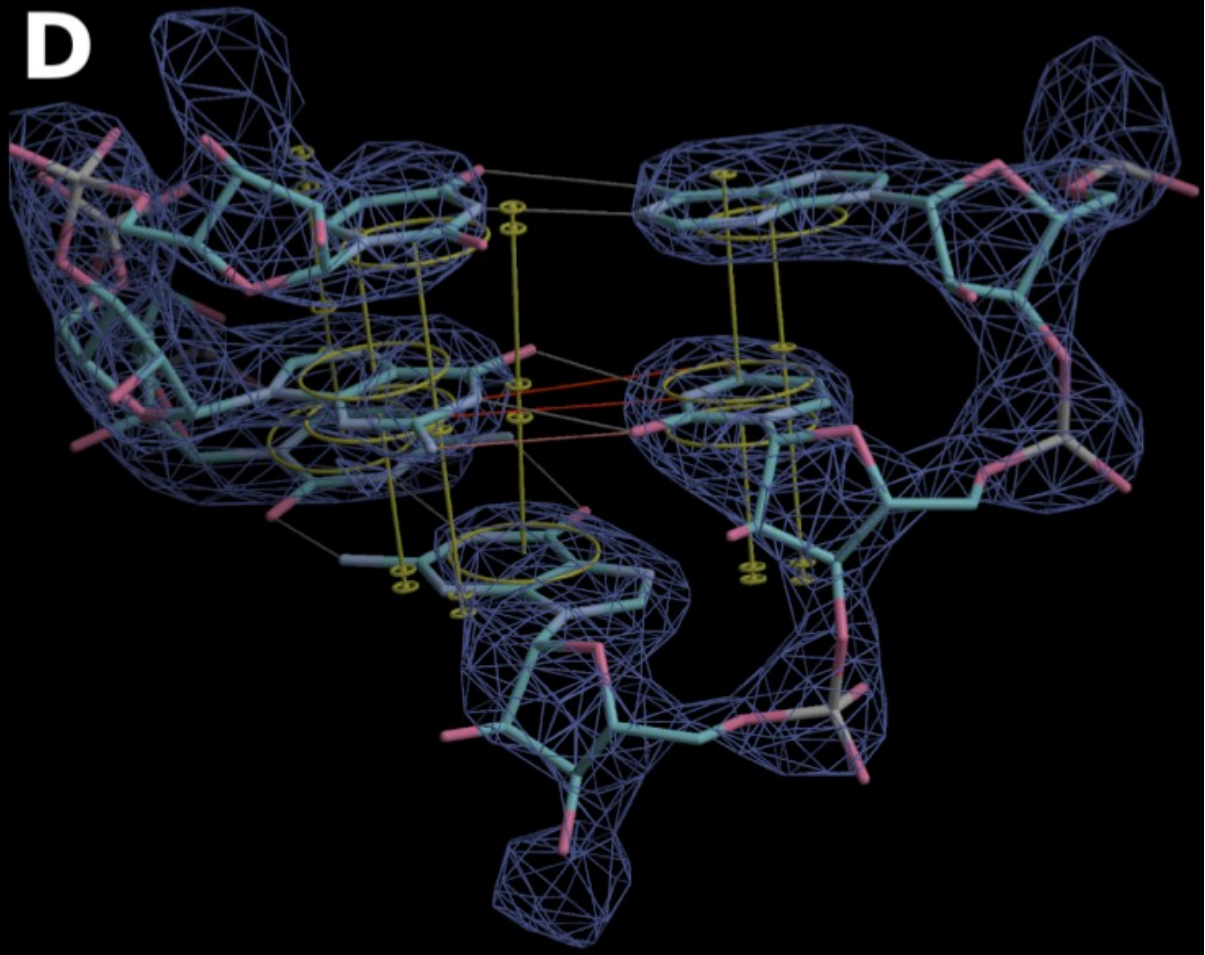

Coot 0.8-pre EL (revision 4832)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints
Reset View
Display Manager
Sphere Refine
Ligand Builder
R/RC
Map
(mol. no: 2) P /1/I/26 DC occ: 1.00 bf: 155.50 ele: P pos: (56.73,-33.91,41.00)


Coot 0.8-pre EL (revision 4832)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints
Reset View
Display Manager
Sphere Refine
Ligand Builder
R/RC
Map
(mol. no: 2) P /1/I/26 DC occ: 1.00 bf: 155.50 ele: P pos: (56.73,-33.91,41.00)

Libg restraints



(Watson Crick and)
Wobble, Reverse Wobble



Representation in Coot

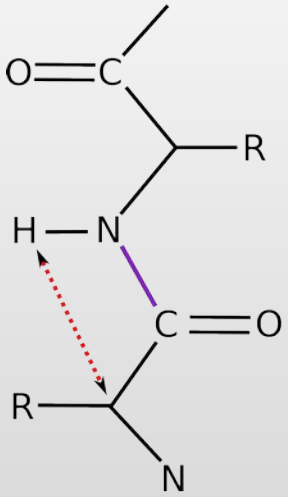
cis-Peptides

- What is a cis-peptide?
- Peptide restraints in Coot 2004-2015

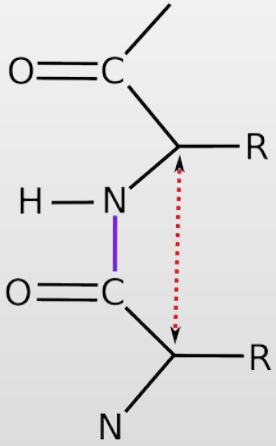
cis-Peptides

- A number of paper have been published recently highlighting the unusually large number of cis-peptides in some structures:
  - Croll: The rate of cis-trans conformation errors is increasing in low-resolution crystal structures *Acta Cryst.* (2015). **D**71, 706-709
  - Touw *et al*.: Detection of trans–cis flips and peptide-plane flips in protein structures *Acta Cryst.* (2015). **D**71, 1604-71614

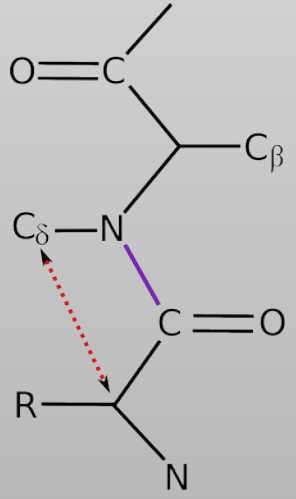
cis-Peptides



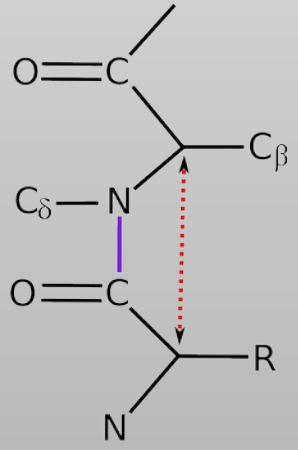
trans-peptide



cis-peptide

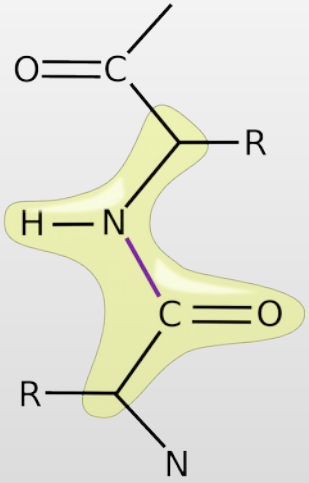


PRO *trans-peptide*

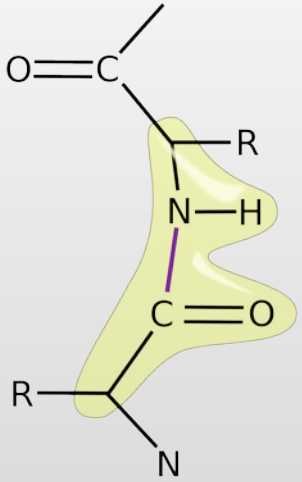


PRO *cis-peptide*

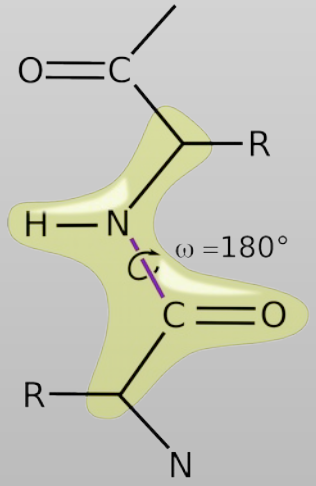
cis-Peptides



trans-peptide
with plane restraints

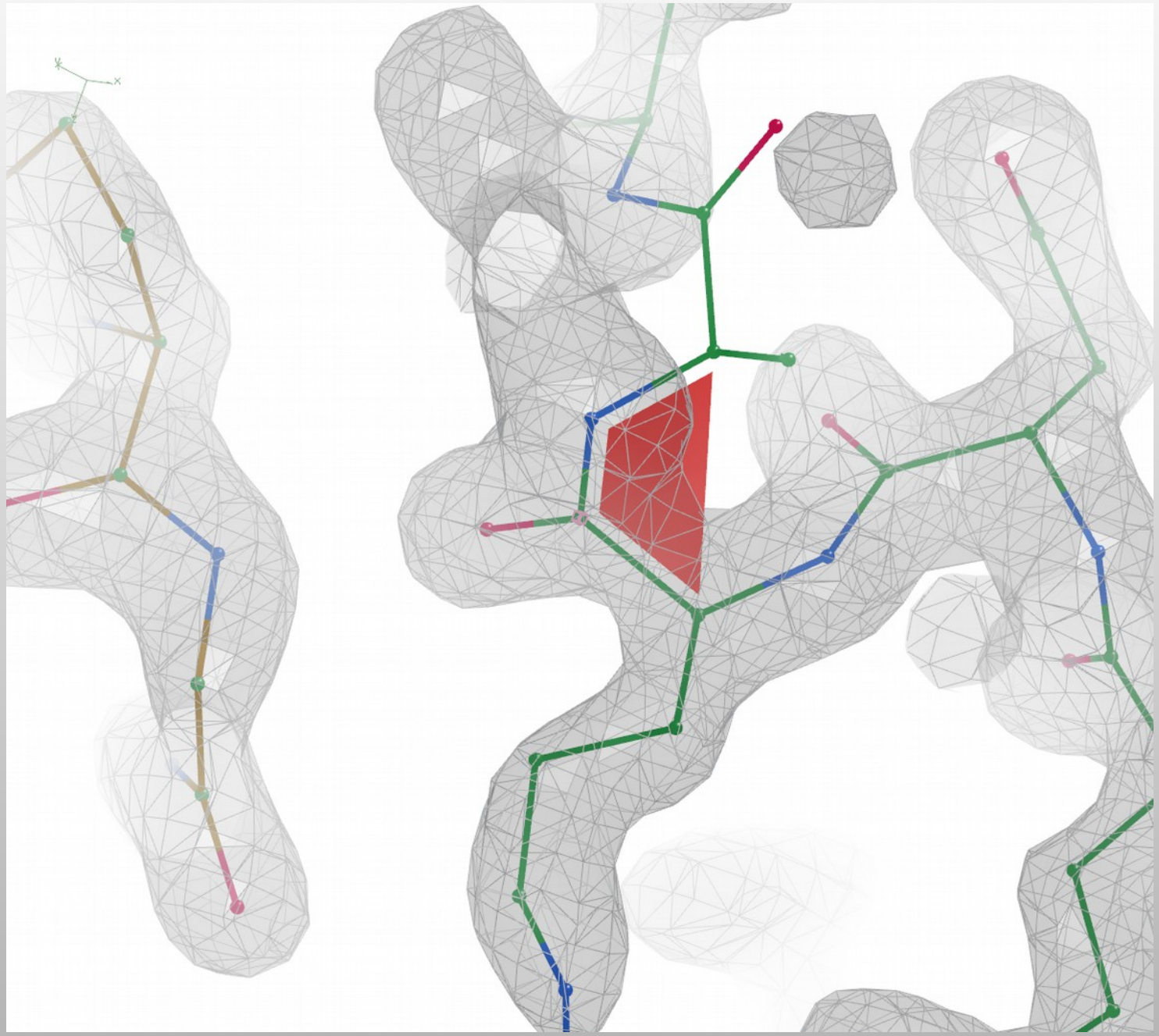


cis-peptide
with plane restraints

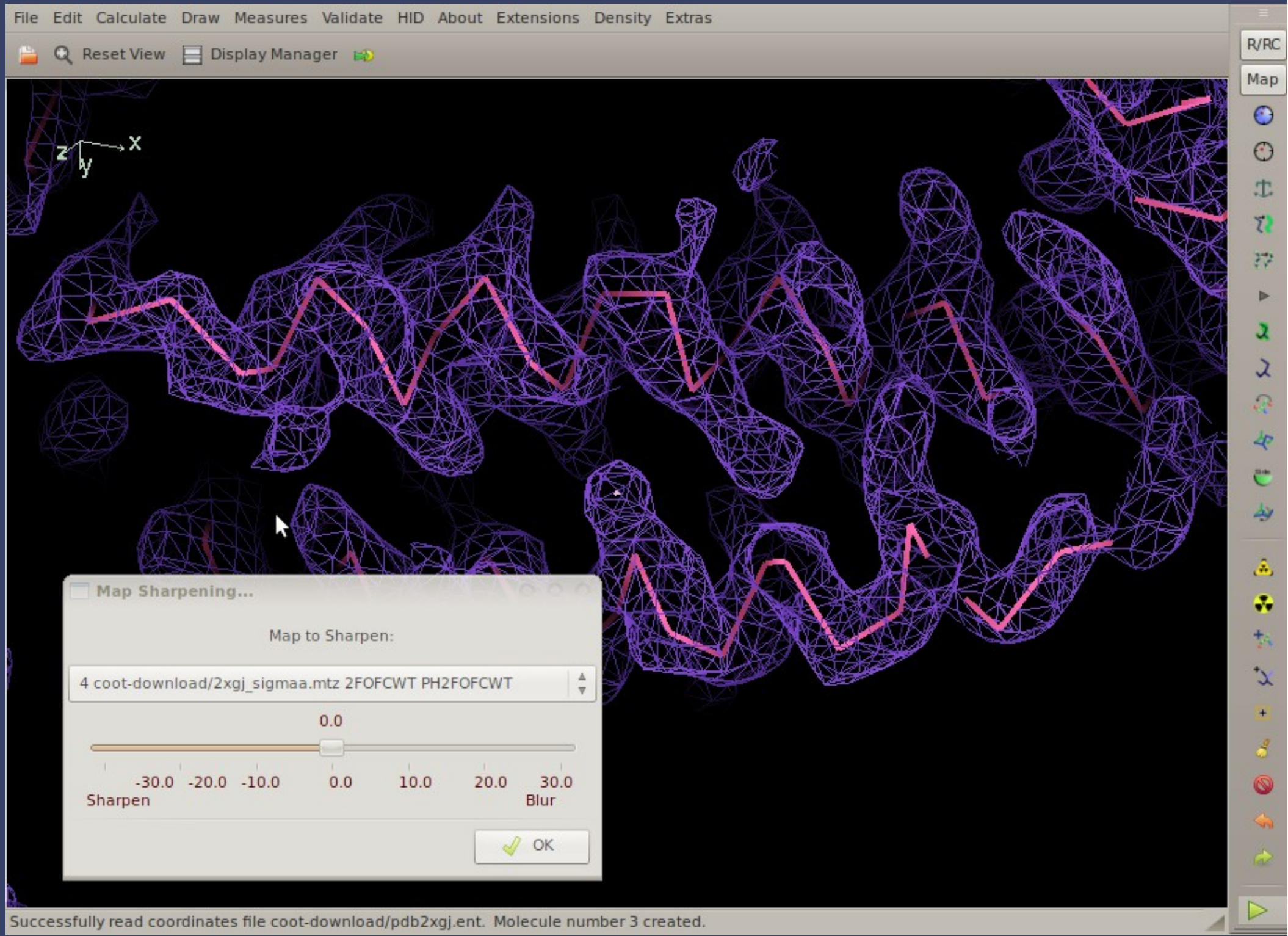


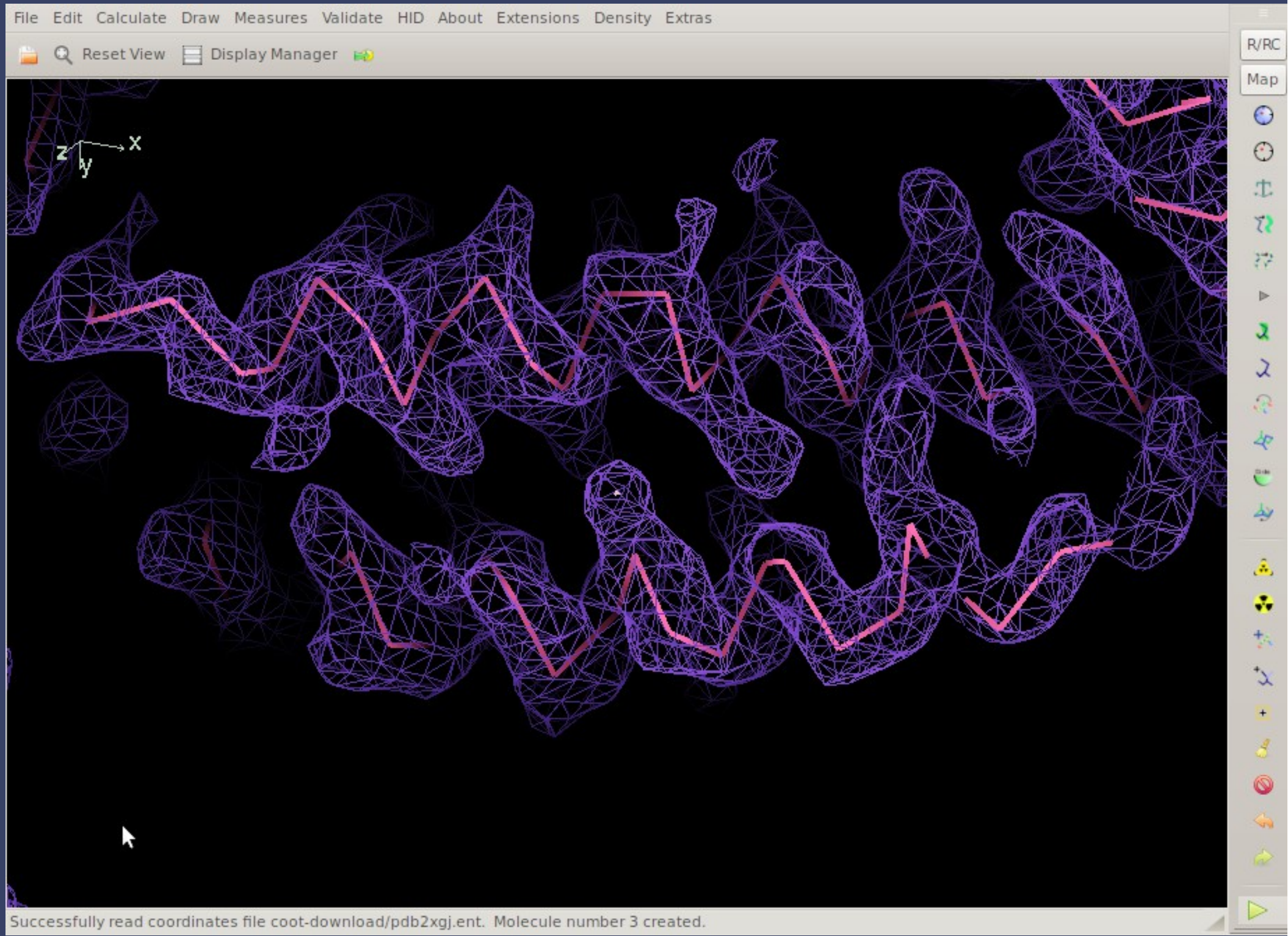
trans-peptide
with plane and trans restraints

cis-peptide Representation



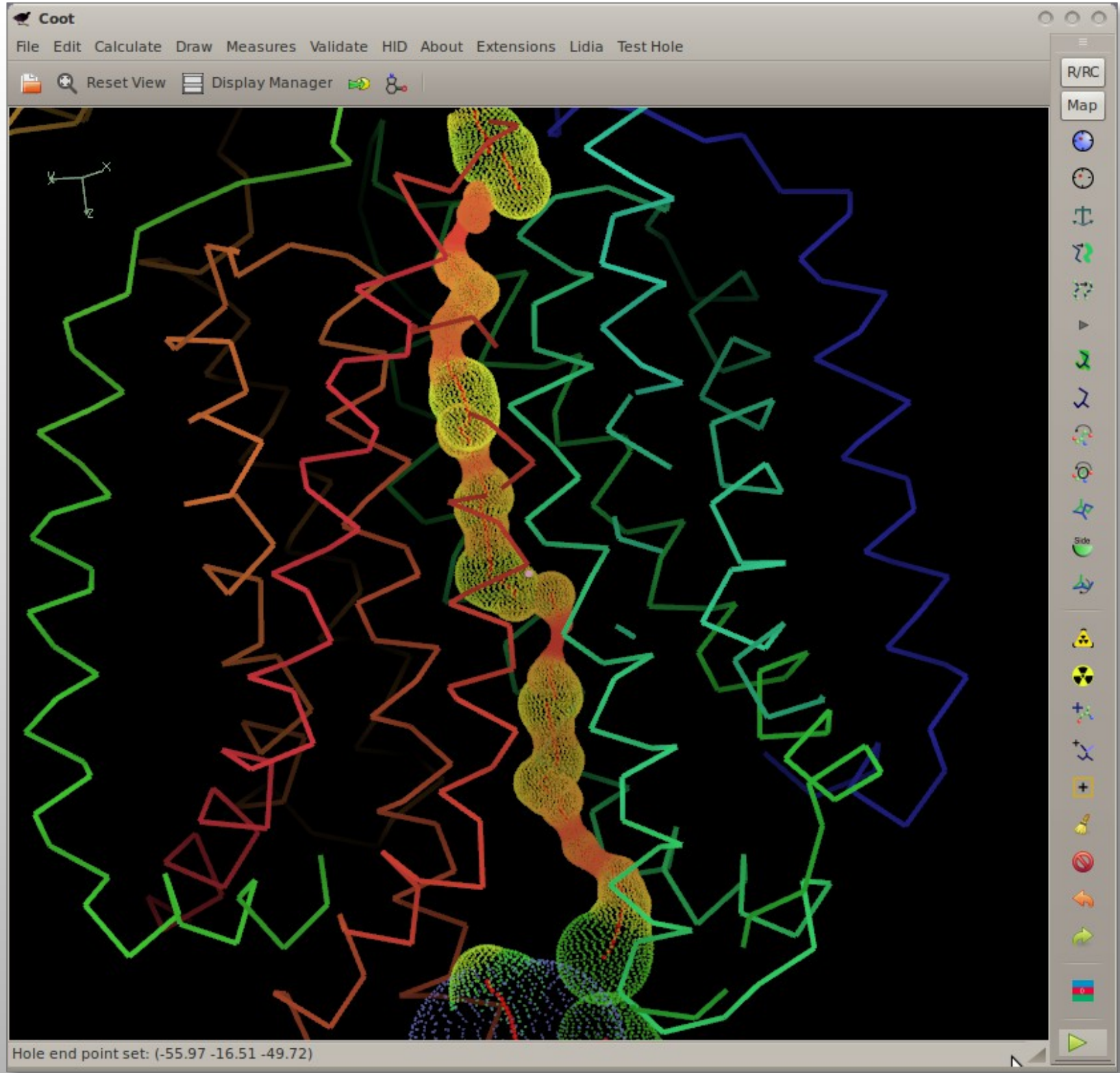
Interactive Map Sharpening


File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
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.

Finding Holes

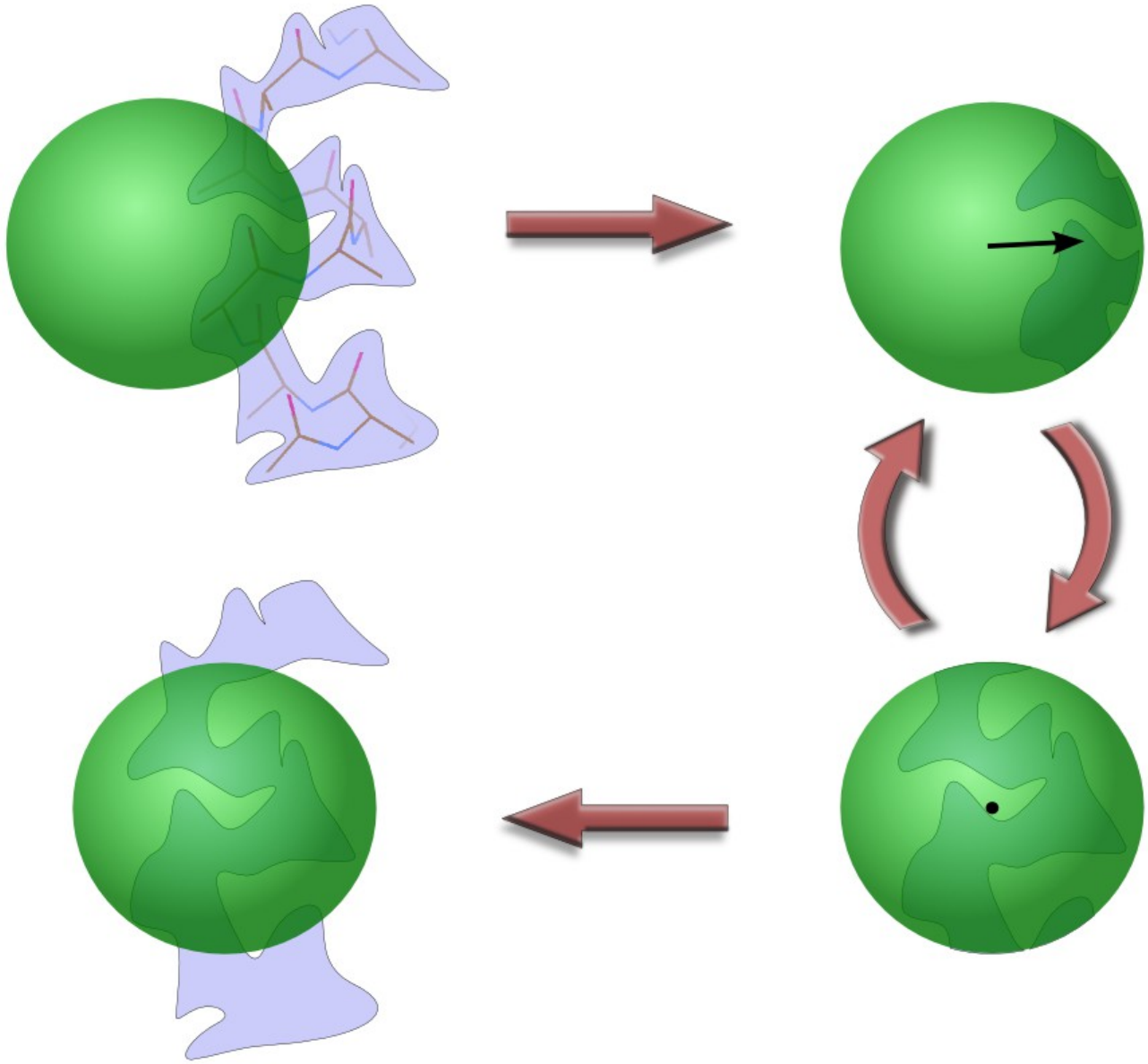
- An implementation of
  - Smart, Goodfellow & Wallace (1993) Biophysics Journal **65**, 2455
  - Atomic radii from AMBER
  - I used
    - radii from CCP4 monomer library
    - sans simulated annealing


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia Test Hole
Reset View
Display Manager
R/RC
Map
Side
Hole end point set: (-55.97 -16.51 -49.72)

Alpha Helix Placement

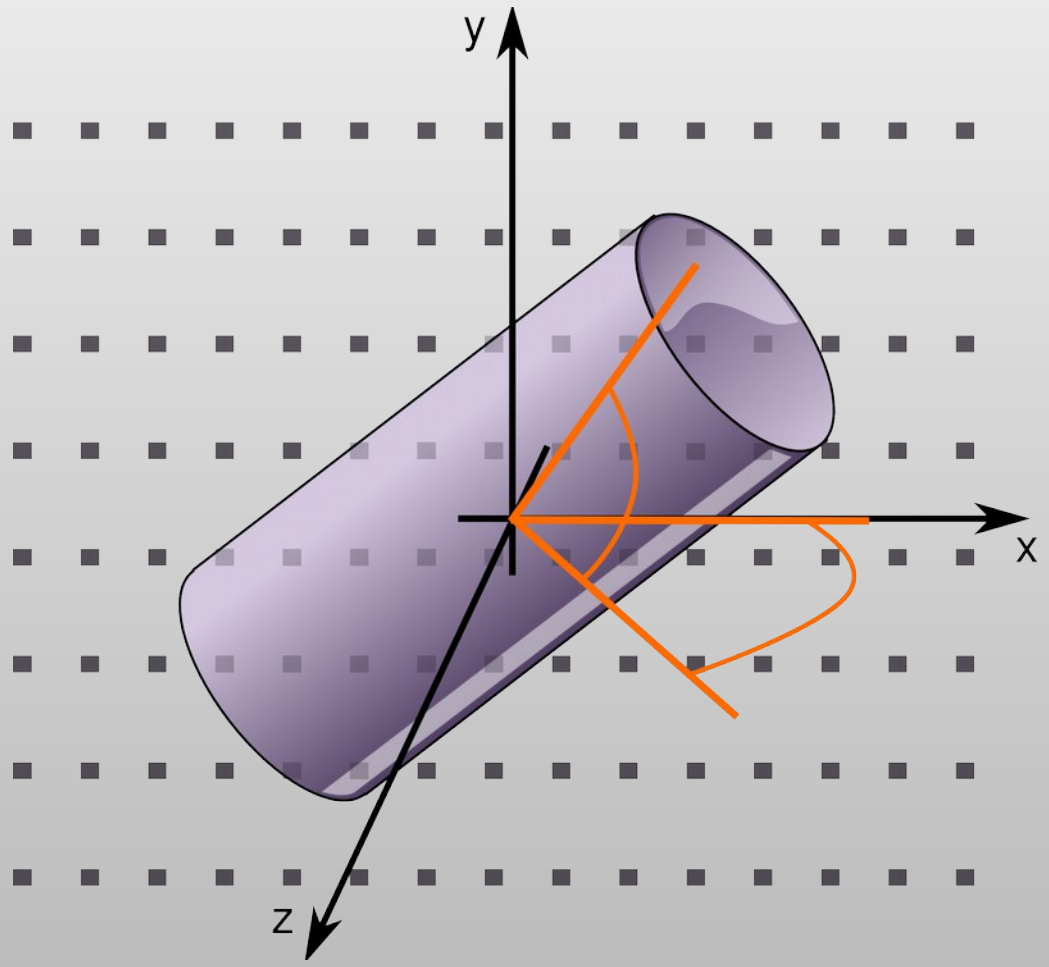
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

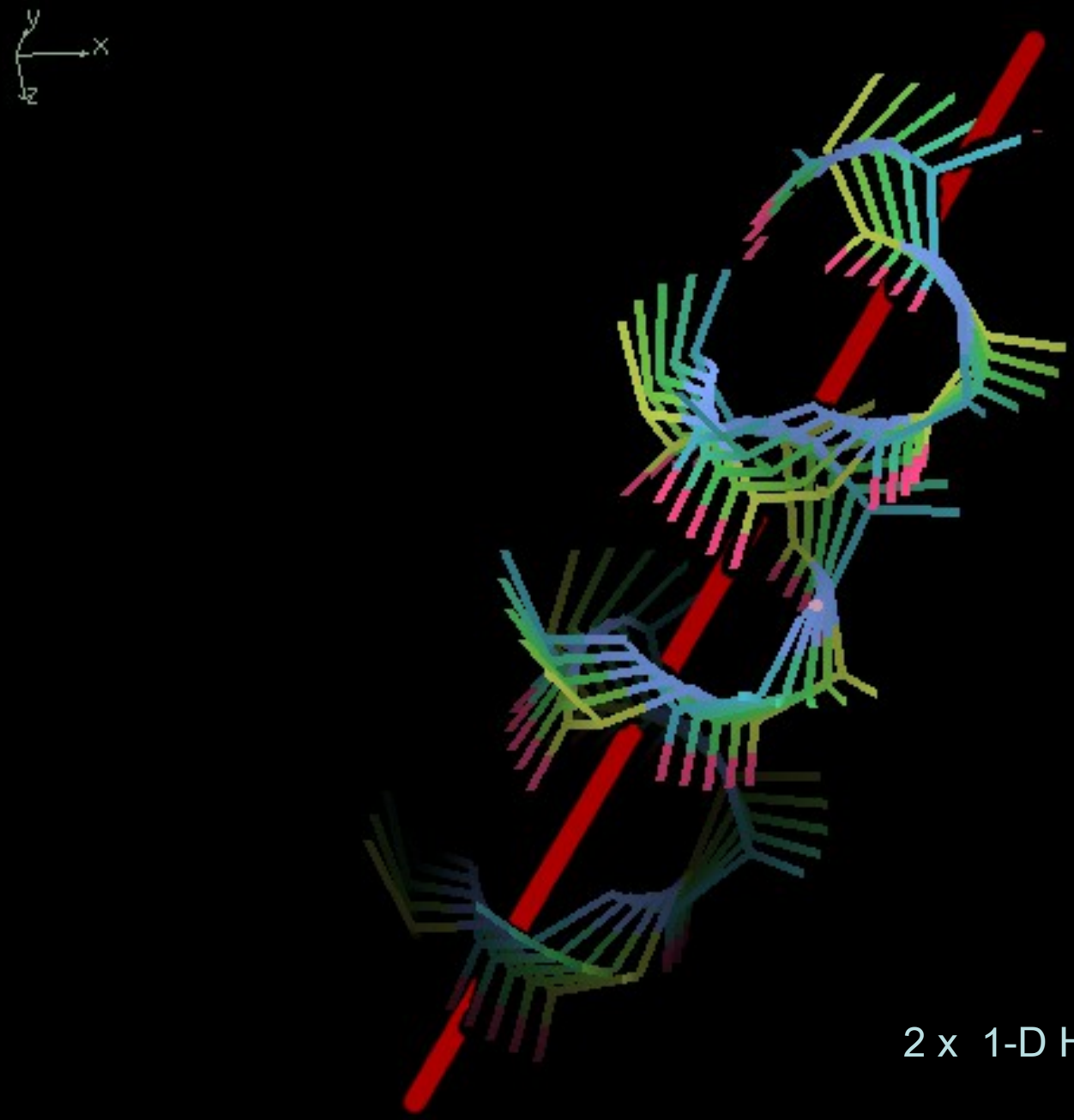


Cylinder Search

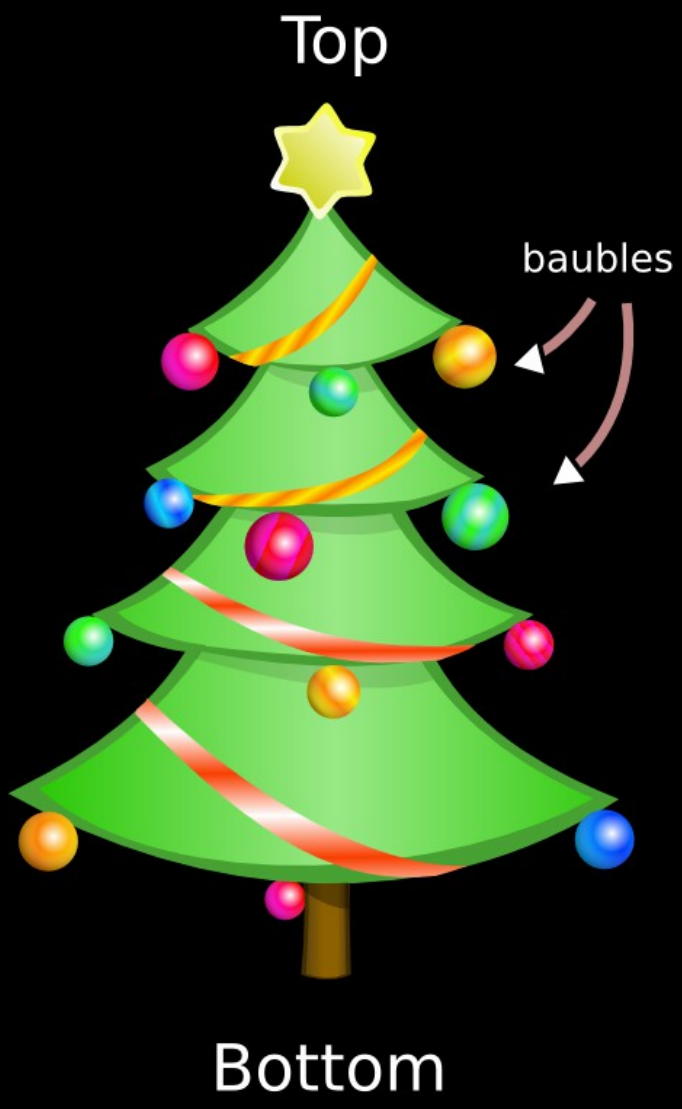
- Pick the orientation that encapsulates the most electron density

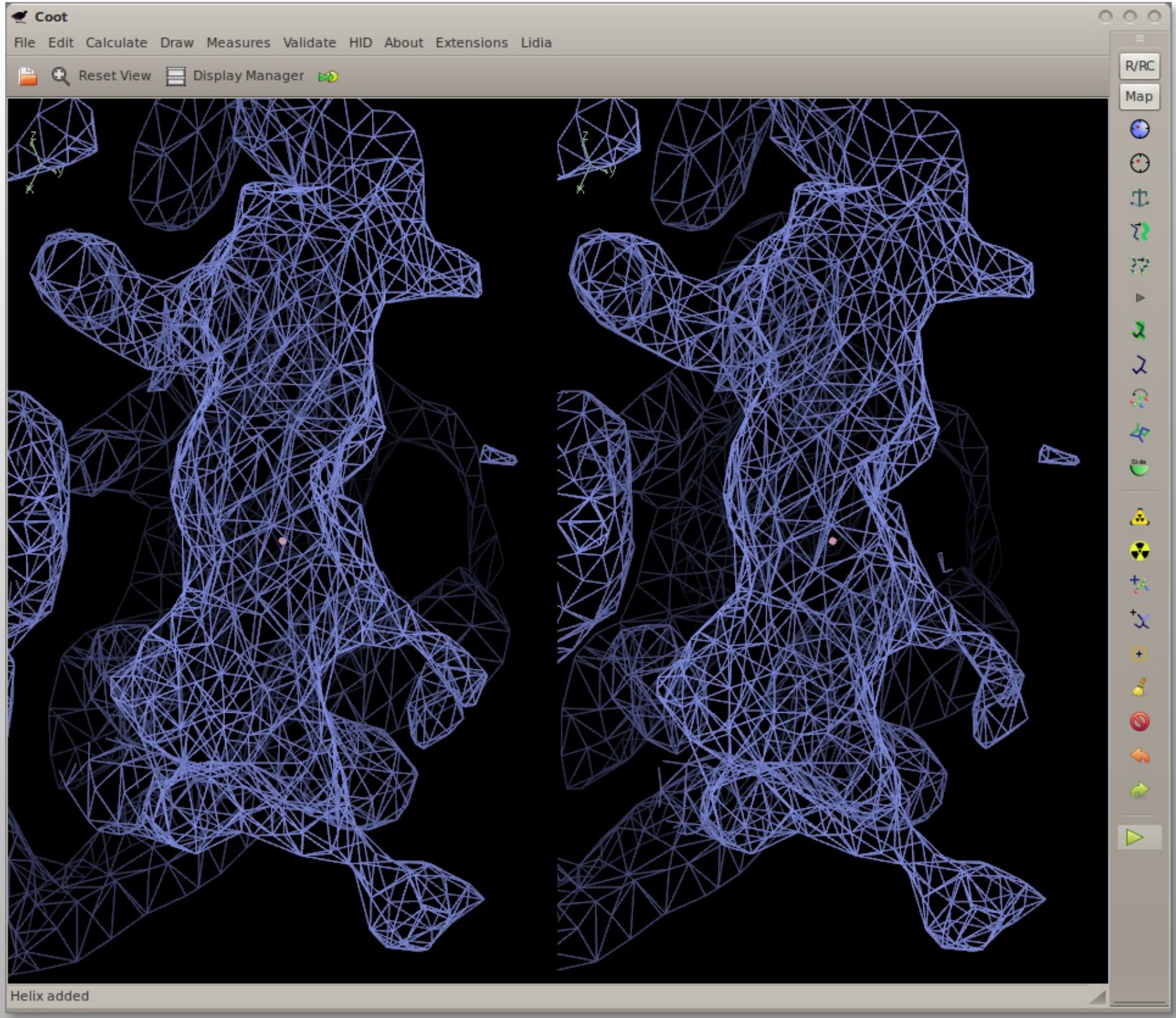


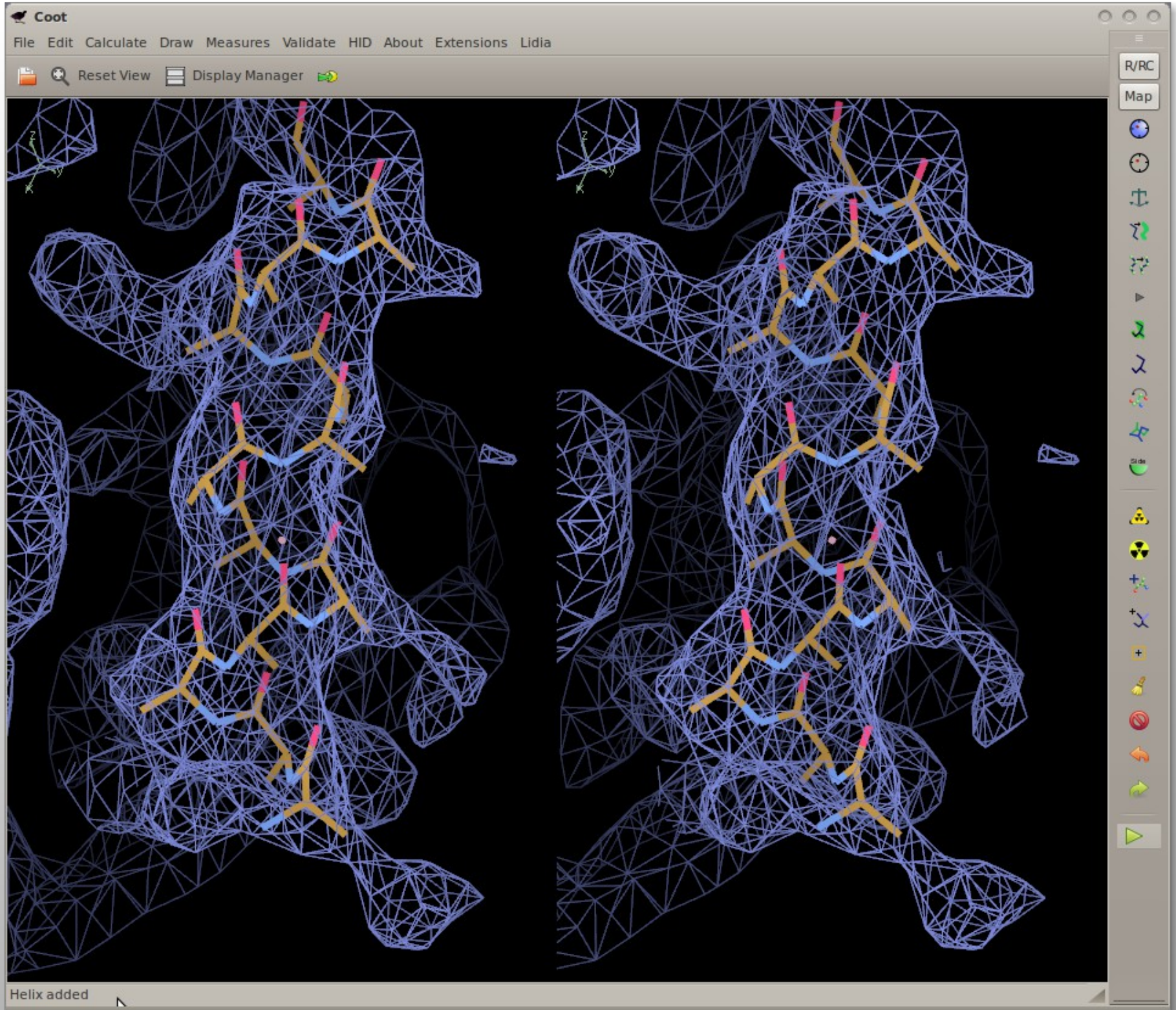
Using 2 rotation axes

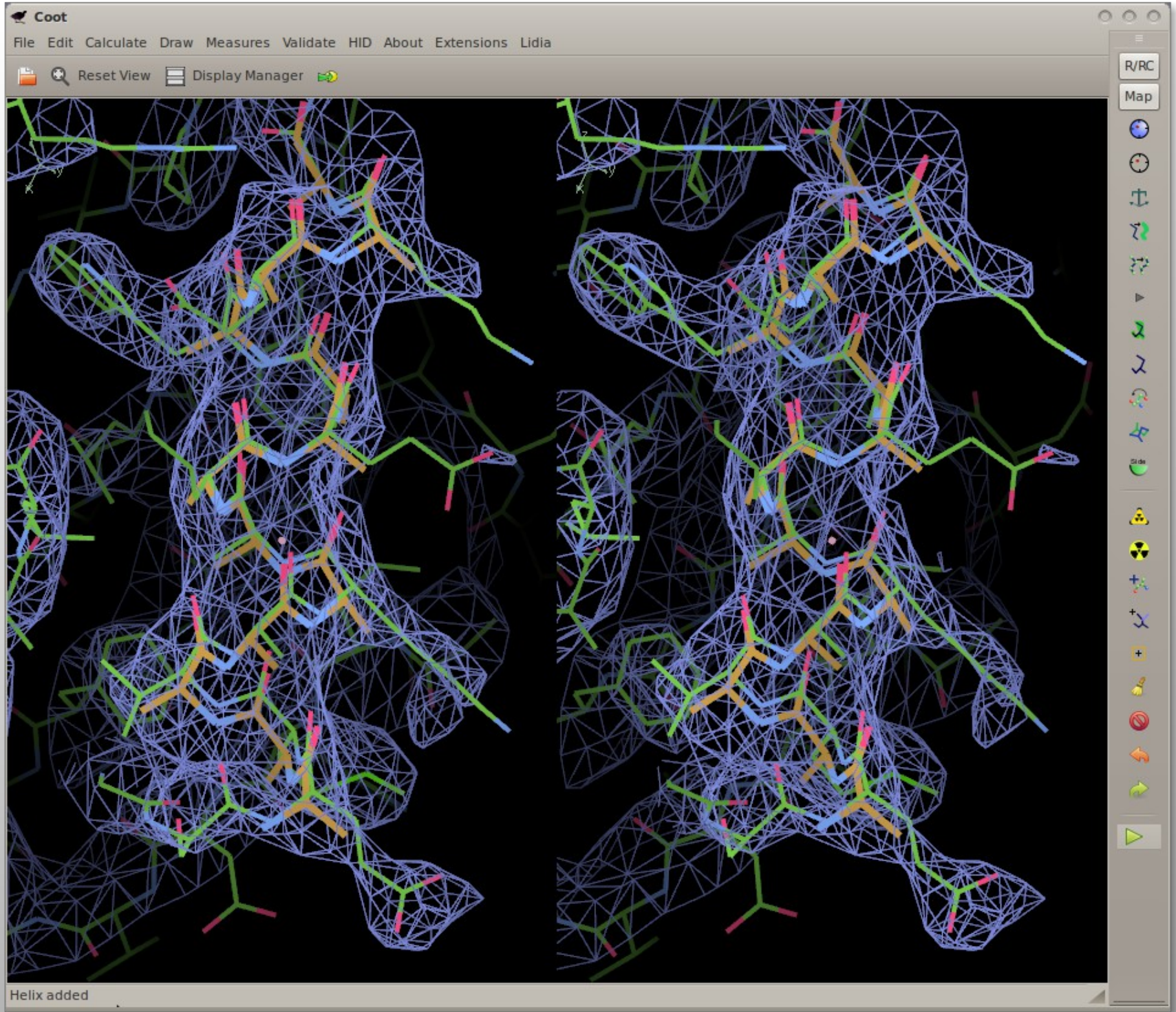


2 x 1-D Helix orientation searches


Top
baubles
Bottom


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added

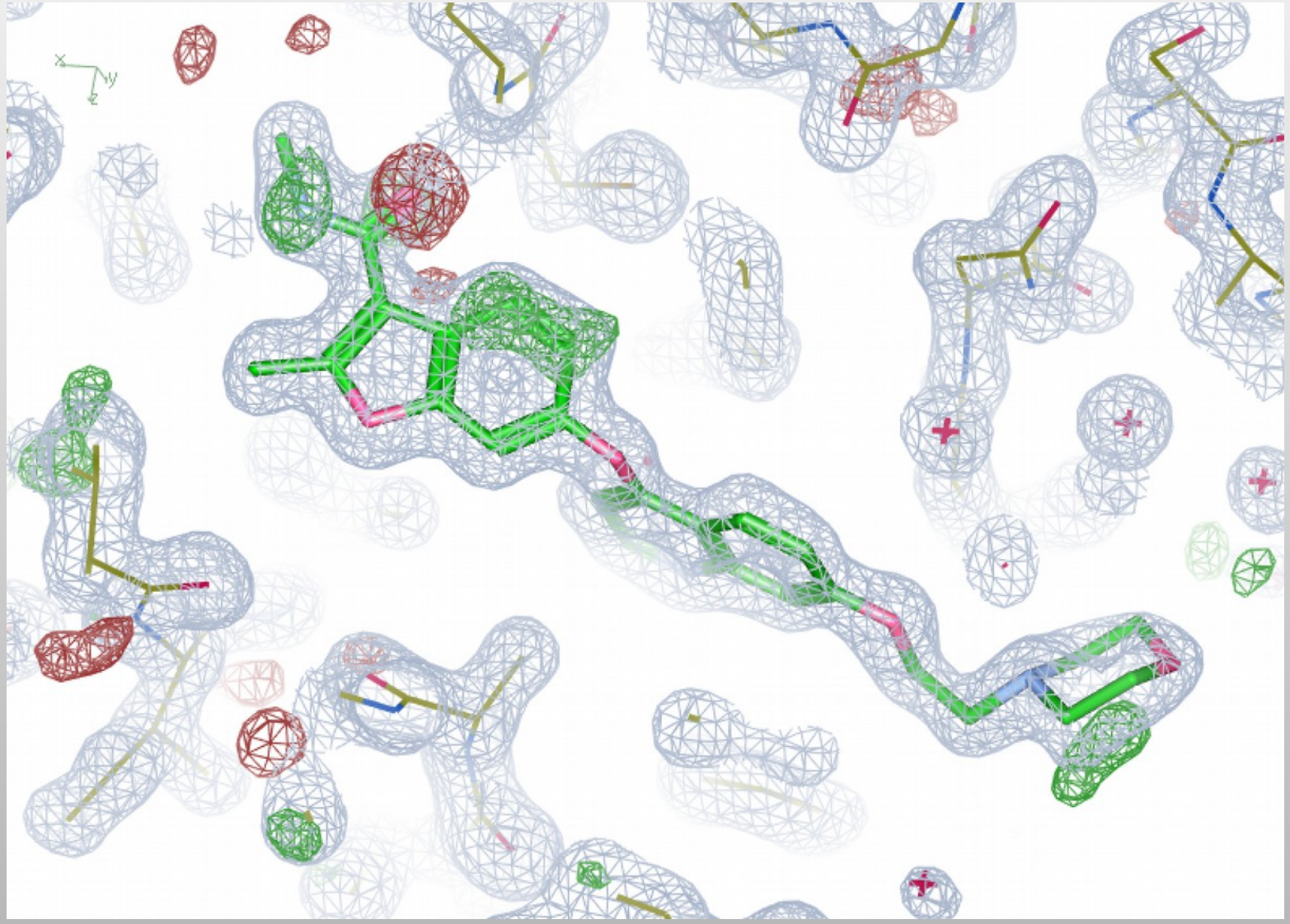

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added

Making Density Slides with *Coot*

- White background
- “High” Oversampling (2.3x)
- Pale gray (or very pastel) density colour
- Enable Cut-glass mode 5-10%
- Anti-aliased Coot
  - `$ setenv __GL_FSAA_MODE 5`
  - 0.8.3 will do a better job of anti-aliasing out the box
  - (transfer to CCP4-built binaries)

Example Density Slide



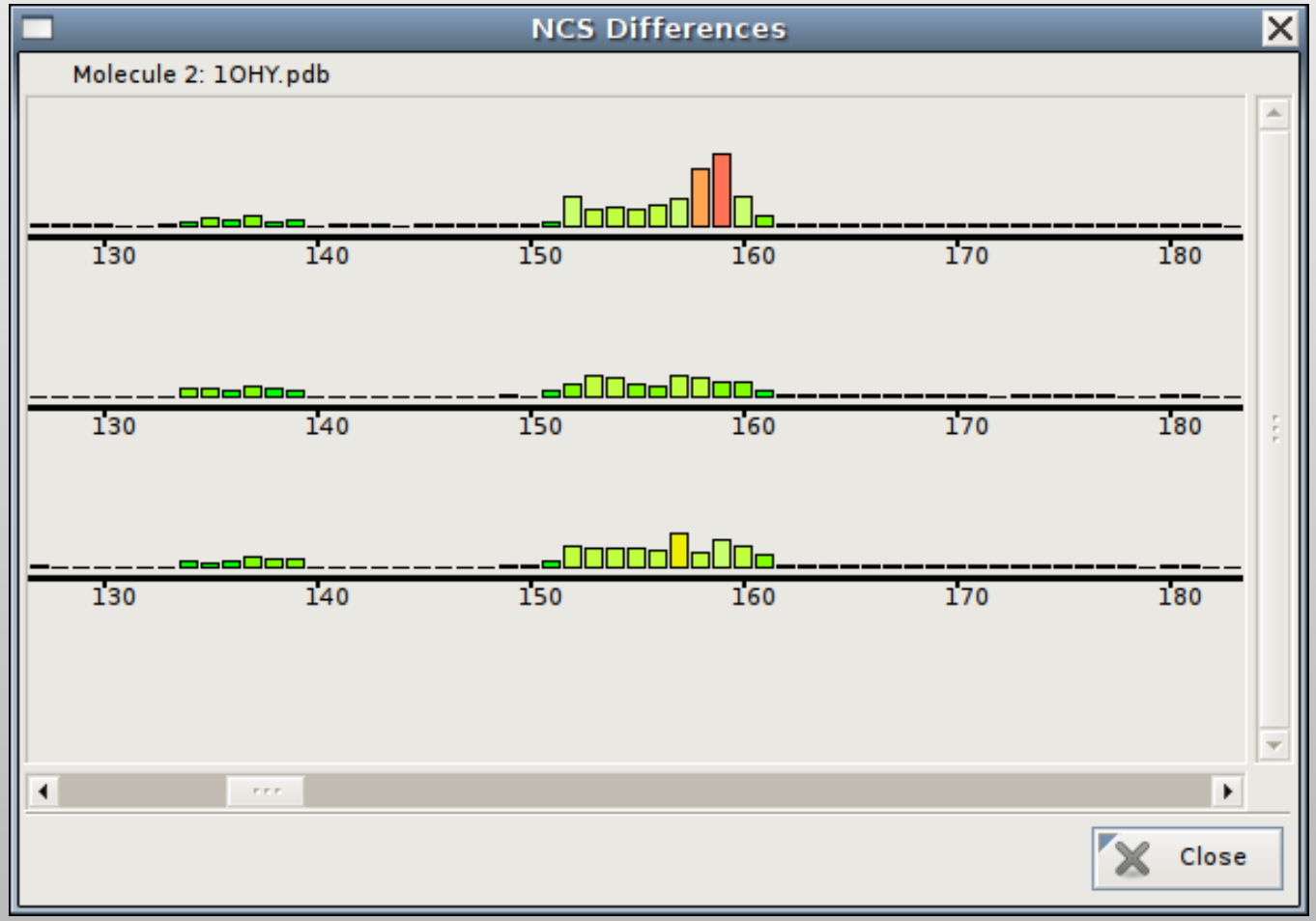
Handling NCS...

Handling NCS

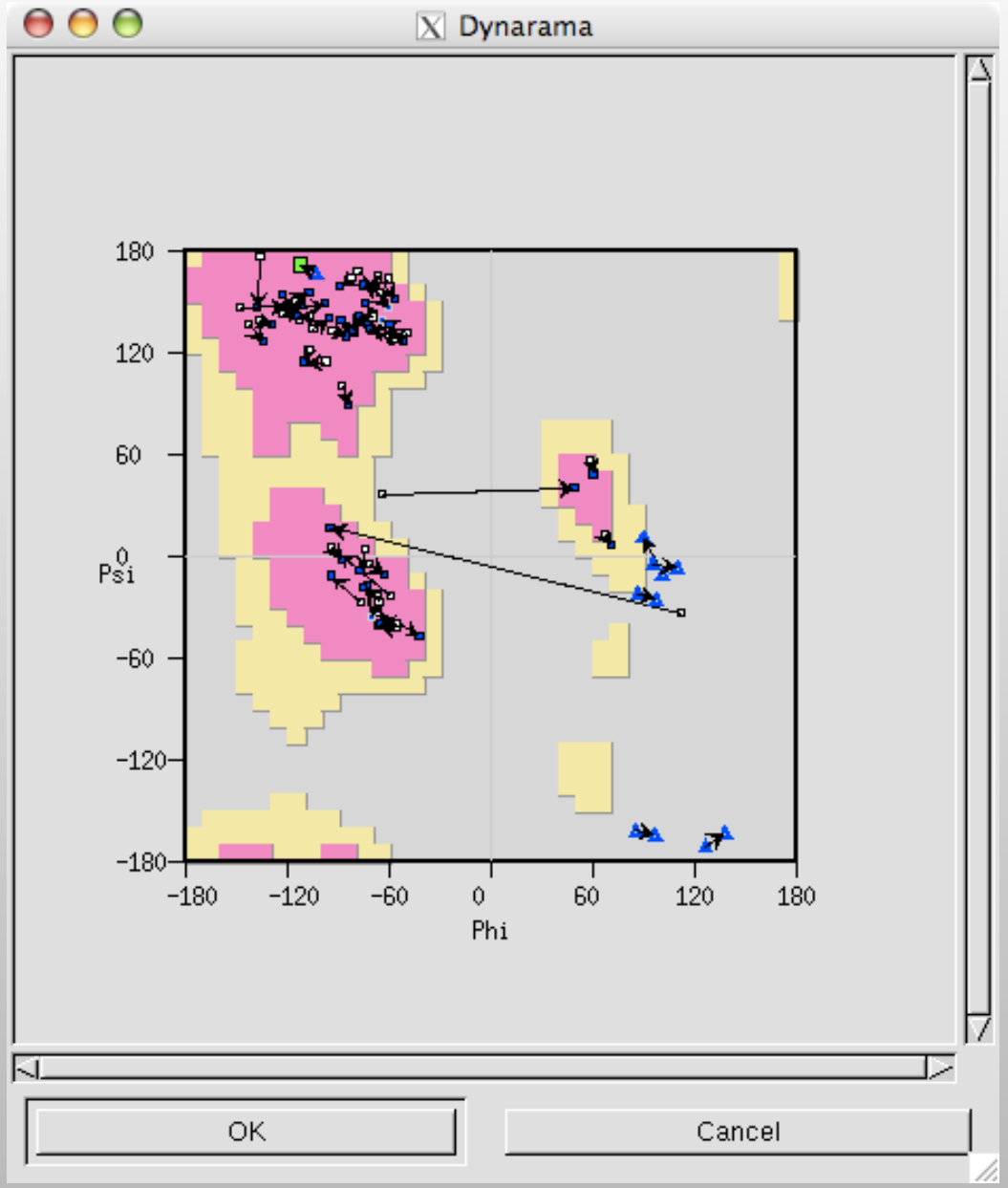
Typical Scenario:

- 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?

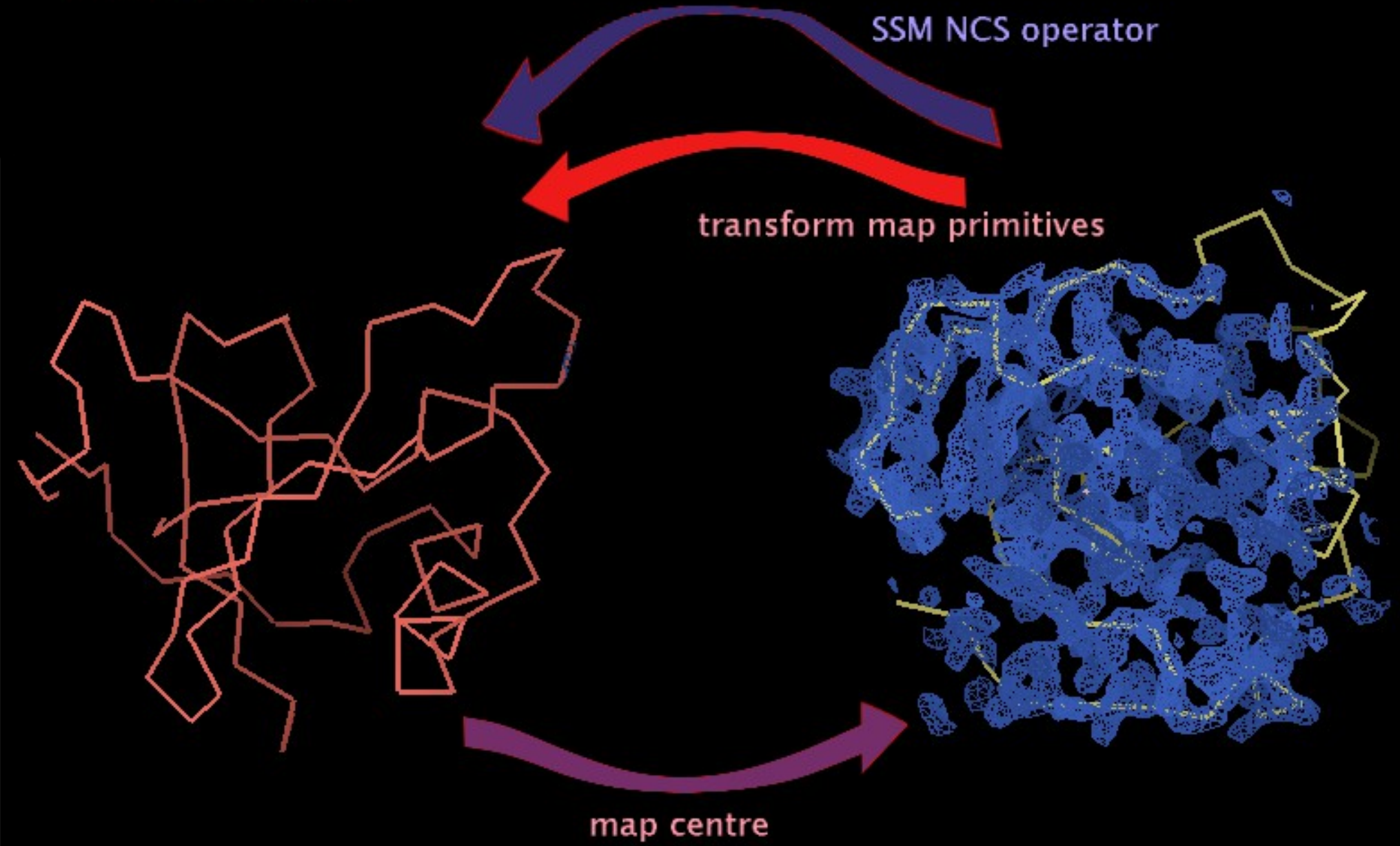
… NCS Differences graph

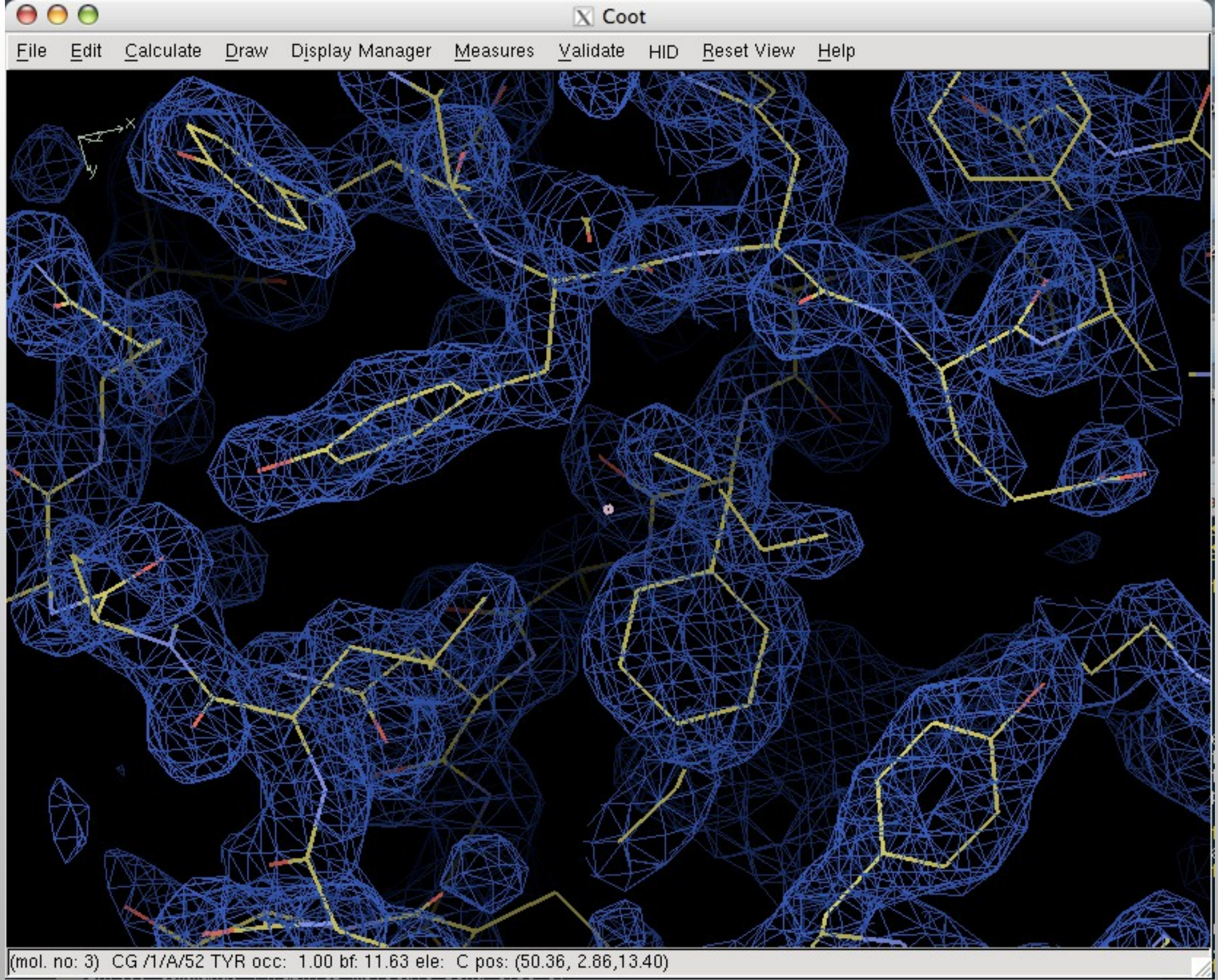


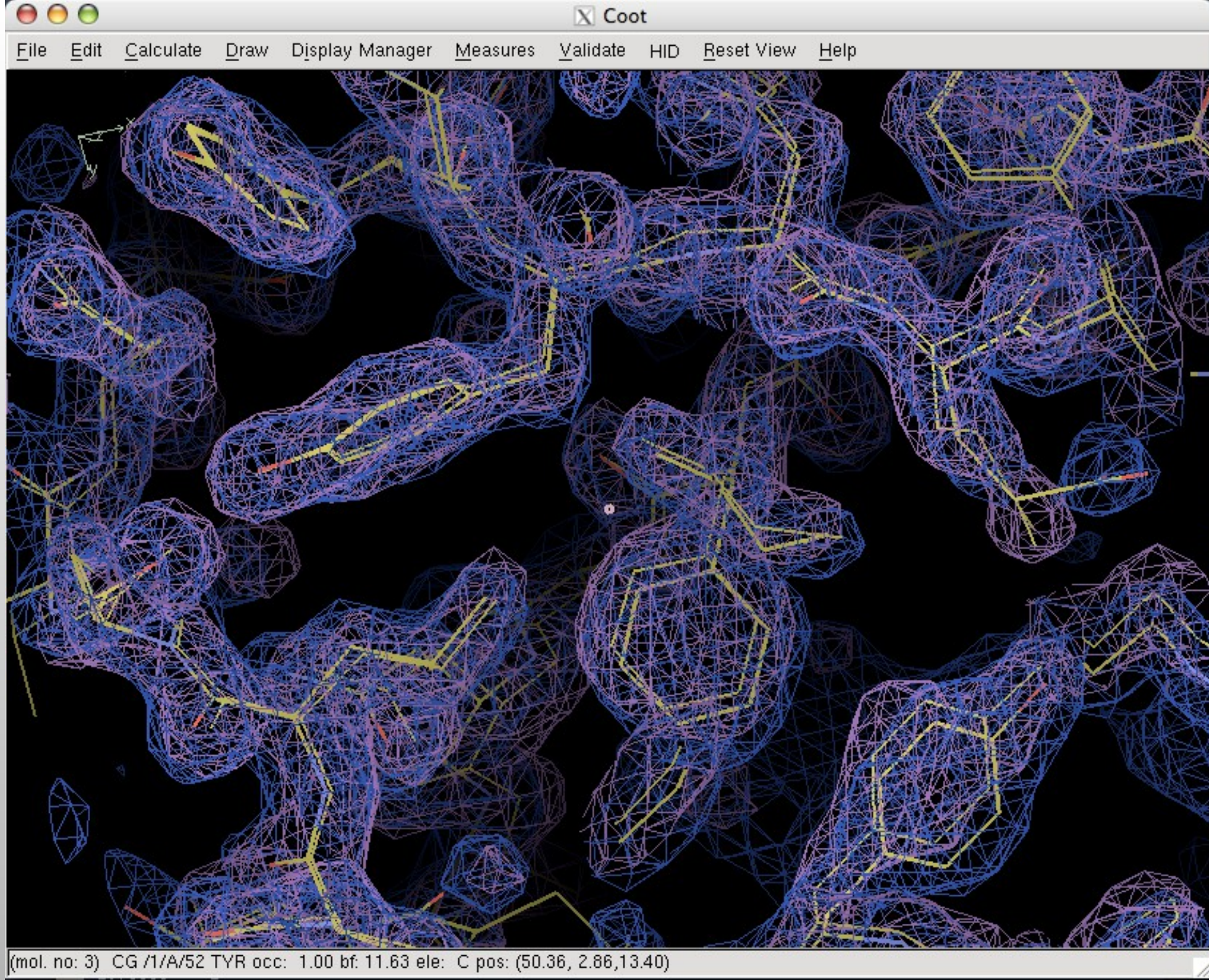
…or ”Kleywegt” Plots



NCS Overlays




Coot
File
Edit
Calculate
Draw
Display Manager
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)


Coot
File Edit Calculate Draw Display Manager 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

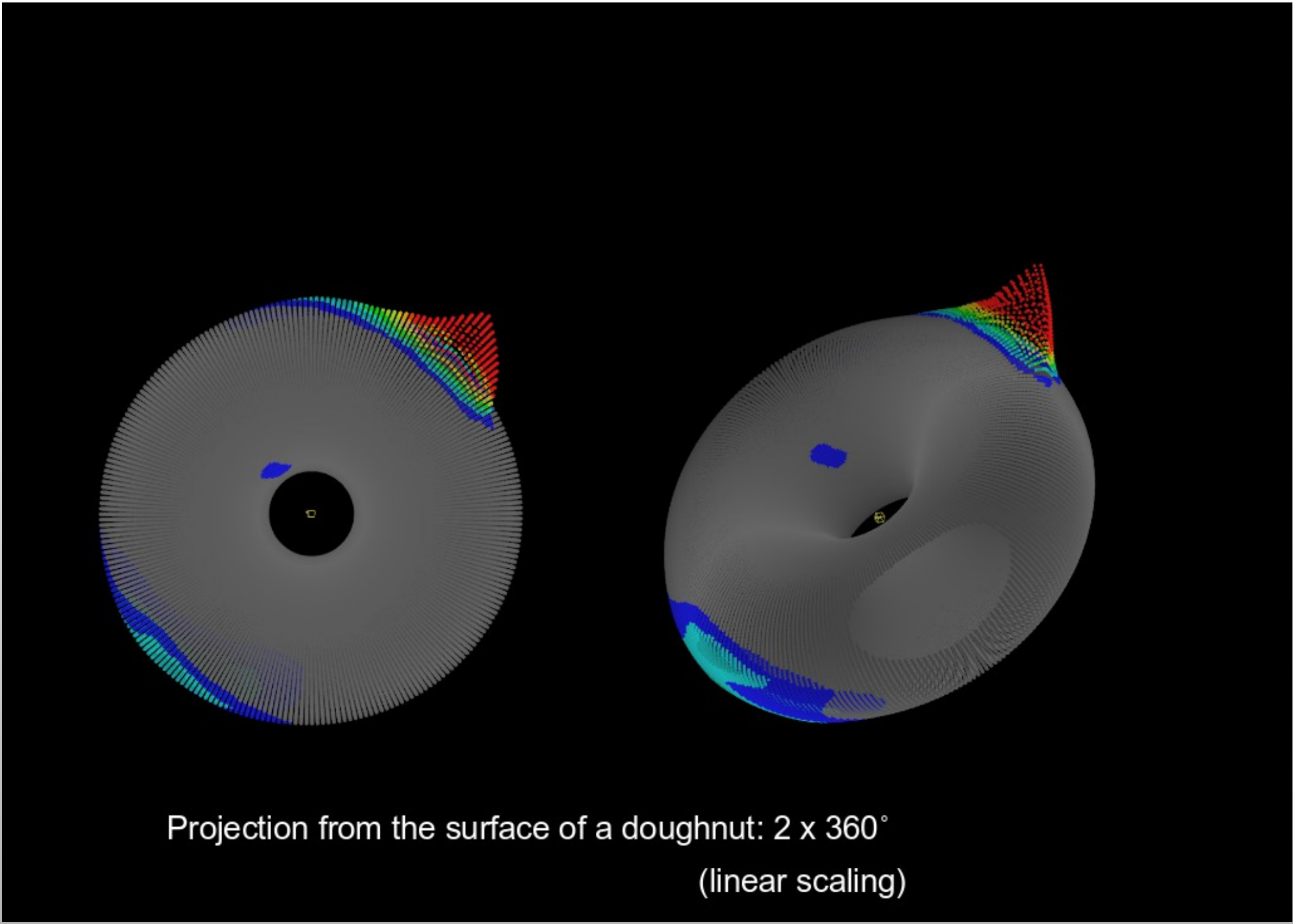
- Automatic detection of NCS
  - And their operators
- Copy Master NCS molecule to others
  - Applies NCS transformation
- Copy NCS Master residue-range
- Change NCS Master chain
- NCS Skipping
- NCS Ligands

Acknowledgements

- Keith Wilson
- Eleanor Dodson
- Garib Murshudov

- Libraries, dictionaries
  - Alexei Vagin, Eugene Krissinel
  - Richardsons (Duke)

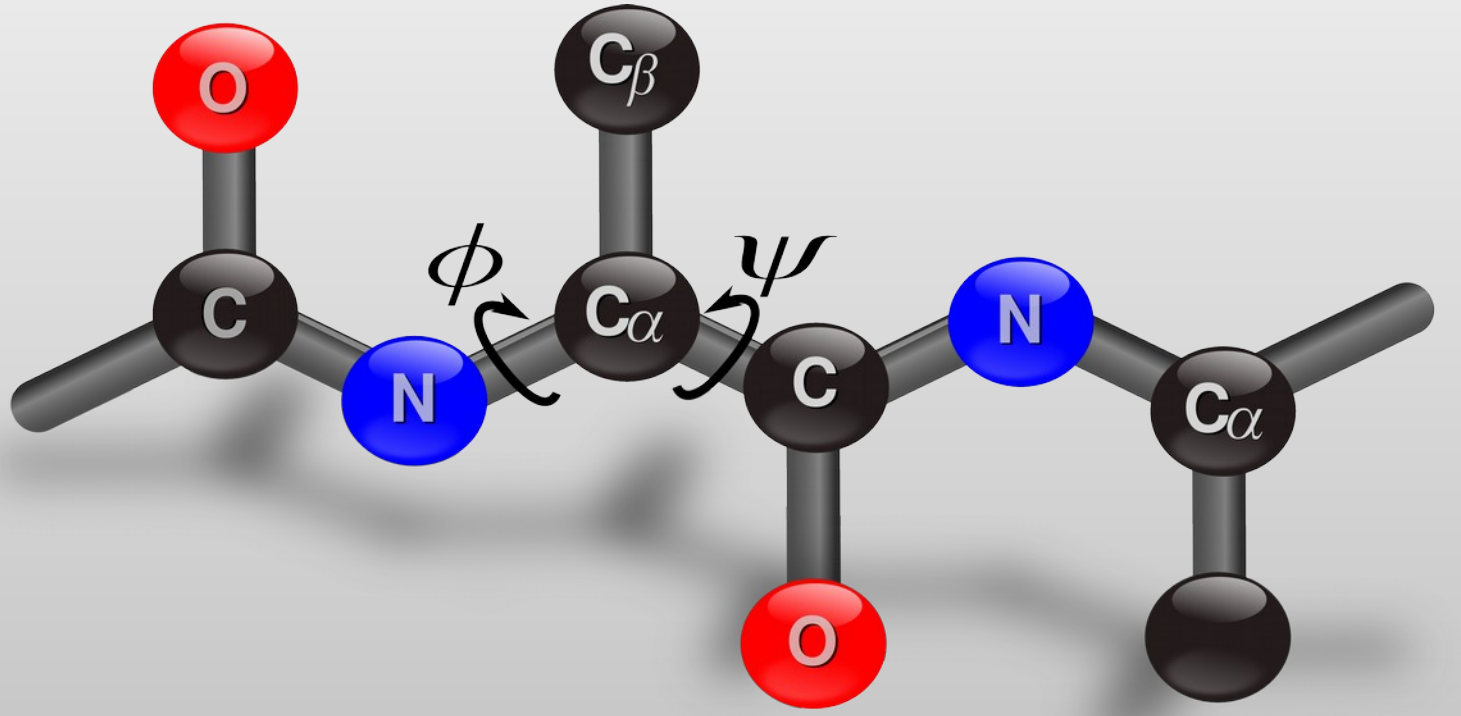
- Funding
  - BBSRC, CCP4 & MRC



Projection from the surface of a doughnut: 2 x 360°

(linear scaling)

Peptide Backbone Geometry



Typical 2D Projection of Ramachandran Plot