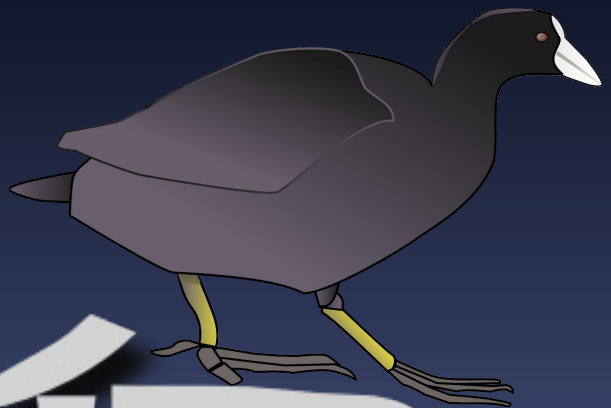

Common Coot
(*Fulica atra*)



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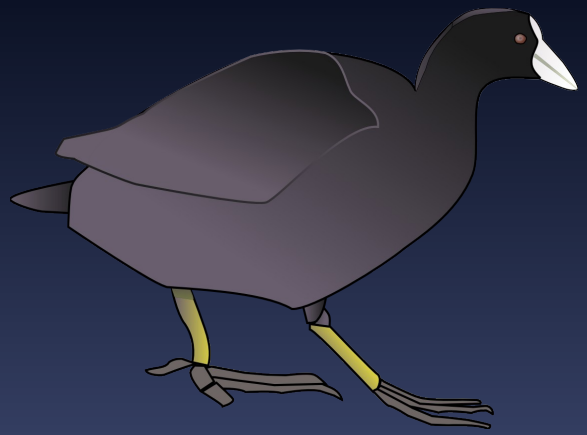
Introduction to Coot

Mar 2011 Shanghai



Model-Building with Coot

An Introduction, low resolution tools(, NCS)

(Paul Emsley)

(University of Oxford)

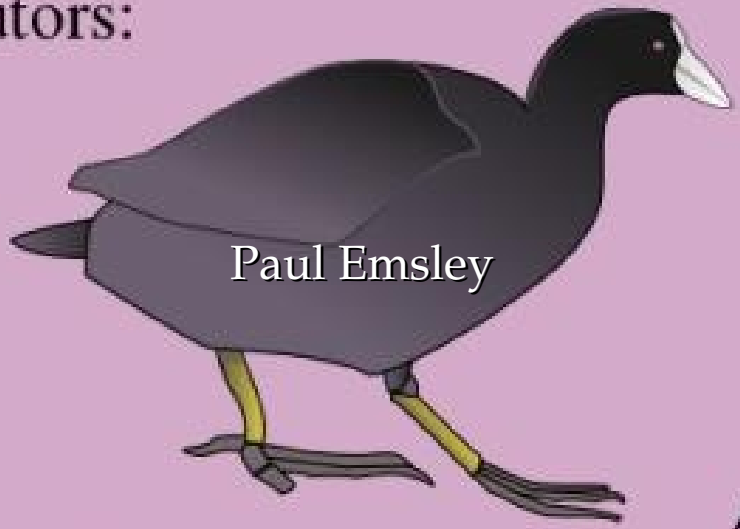
Bernhard Lohkamp

Karolinska Institutet

Coot

- Molecular Graphics application
  - Protein crystallographic model-building tools (Crystallographic Object-Oriented Toolkit)
  - Aims:
    - Model building, completion, validation
    - Fill the gap where automated methods fail
    - “Slick and powerful” interface to CCP4
- Interface to other programs: SHELXL, Refmac, Libcheck, Probe&Reduce (Molprobity), EBI, EDS, Povray, Raster3D, PHENIX, ...

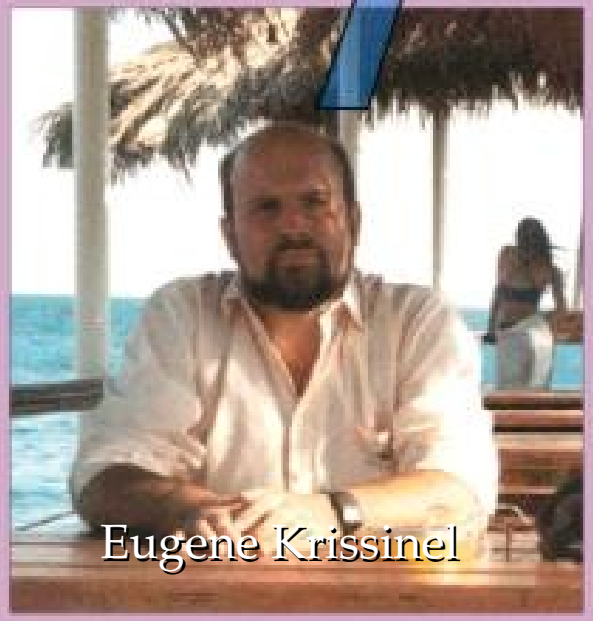
Coot Contributors:



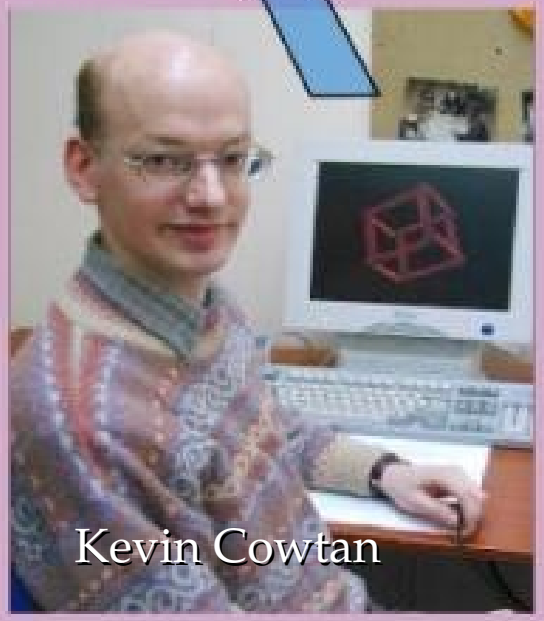
Paul Emsley

Stuart McNicholas

Alexei Vagin



Eugene Krissinel

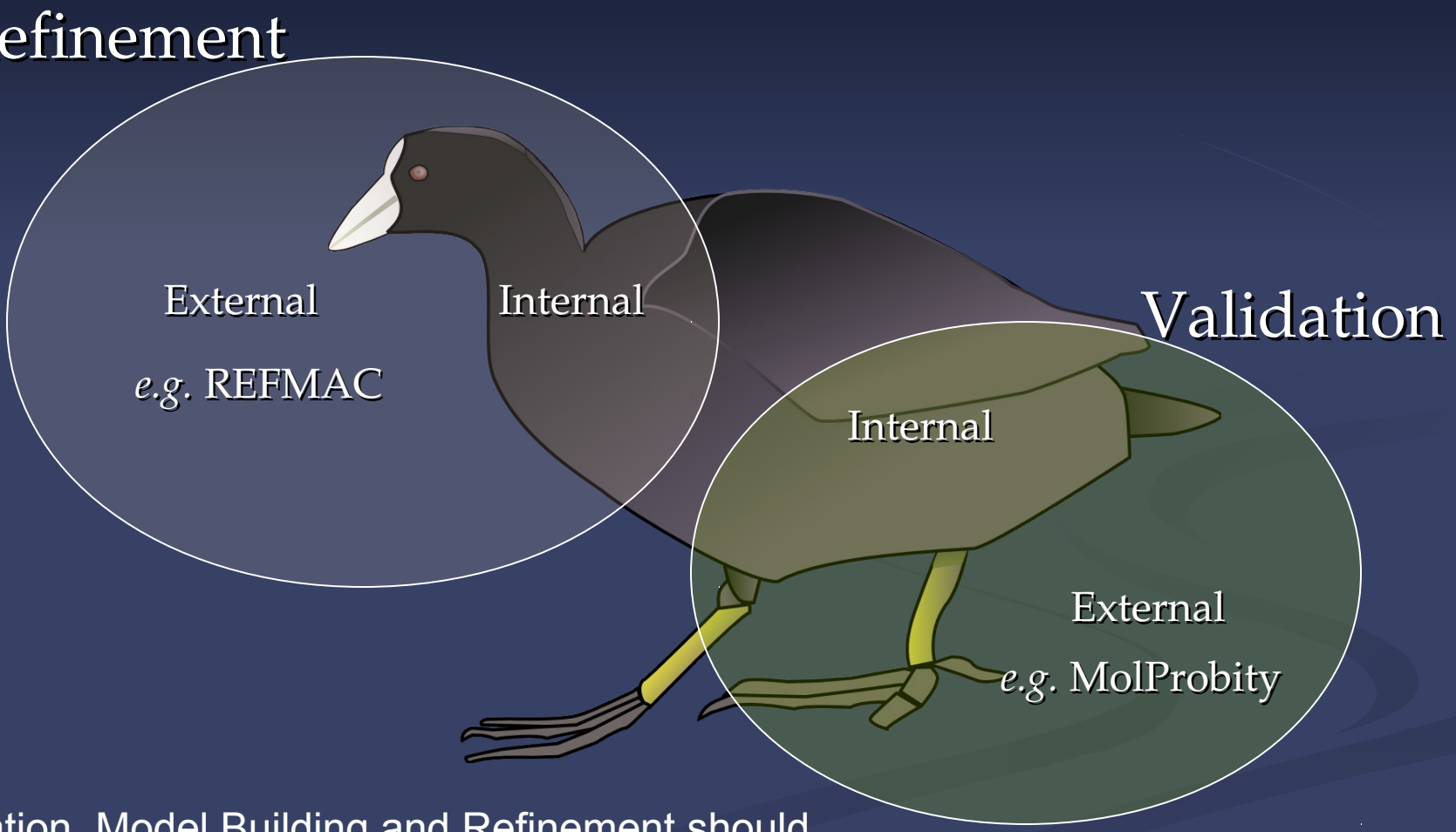


Kevin Cowtan

+

Bernhard Lohkamp

Feature Integration



Validation, Model Building and Refinement should be used together

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

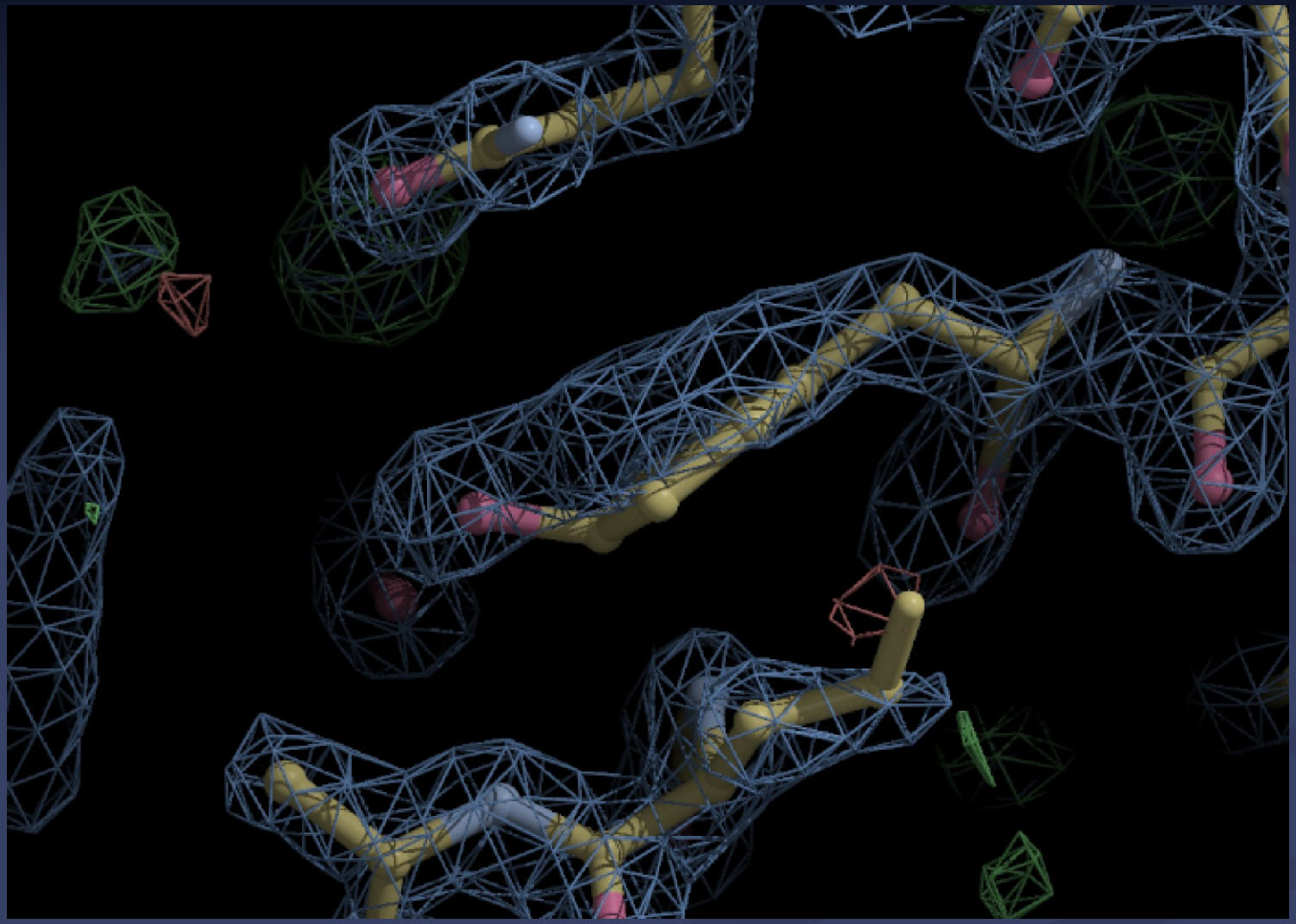
What prior geometric information do we have?

- We know chemistry....
  - We know bond lengths and uncertainties
  - We know bond angles and uncertainties
  - We know the chiral centres
  - We know which atoms should lie in a plane
  - We know (more or less) about torsions
- We combine the gradients from the data with those from molecular mechanics in the minimisation

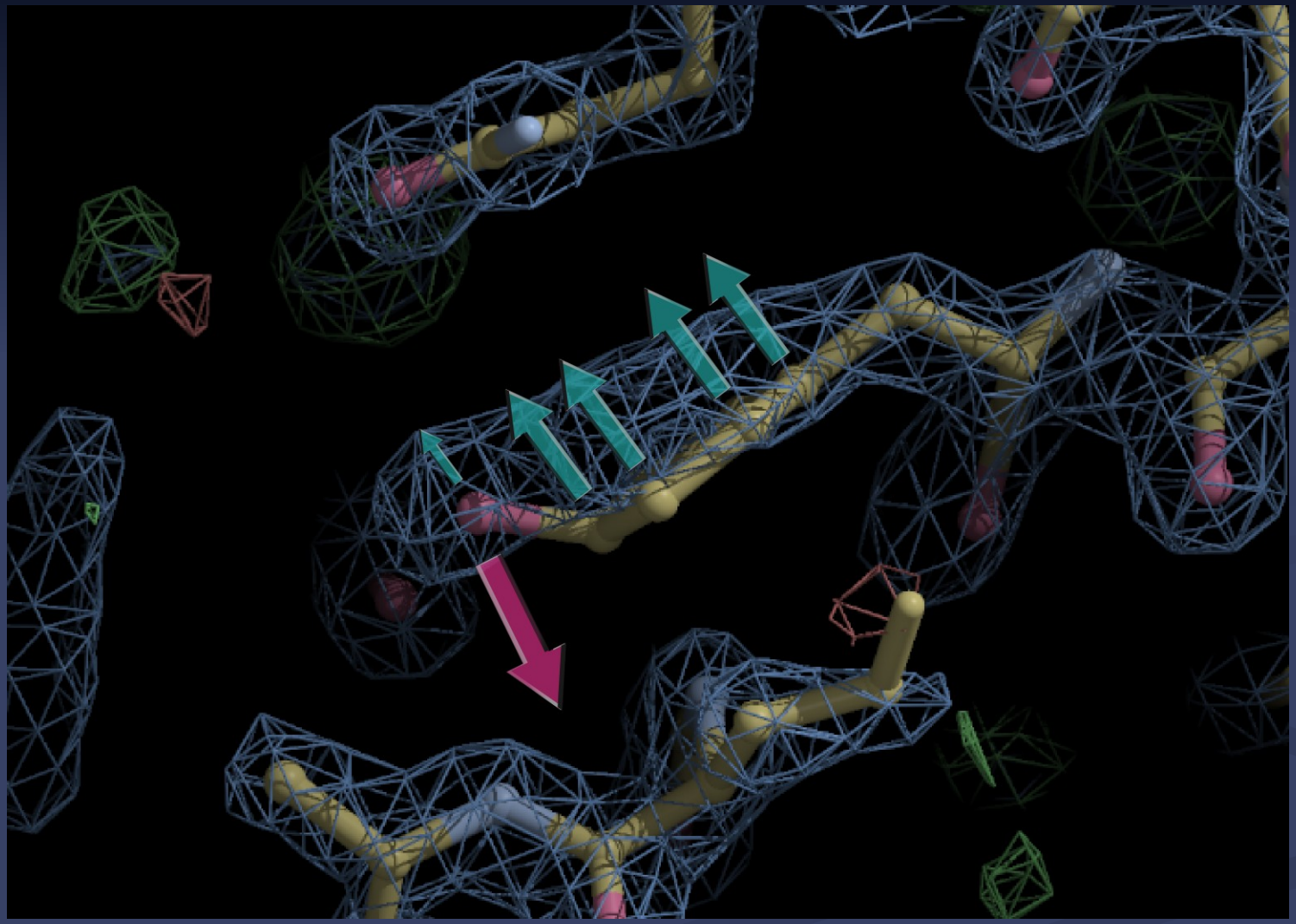
REFMAC Monomer Library `chem_comp_bond`

```
loop_
_chem_comp_bond.comp_id
_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
_chem_comp_bond.type
_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_esd
 ALA      N    H       single     0.860    0.020
 ALA      N    CA      single     1.458    0.019
 ALA      CA   HA      single     0.980    0.020
 ALA      CA   CB      single     1.521    0.033
 ALA      CA   C       single     1.525    0.021
 ALA      C    O       double     1.231    0.020
```

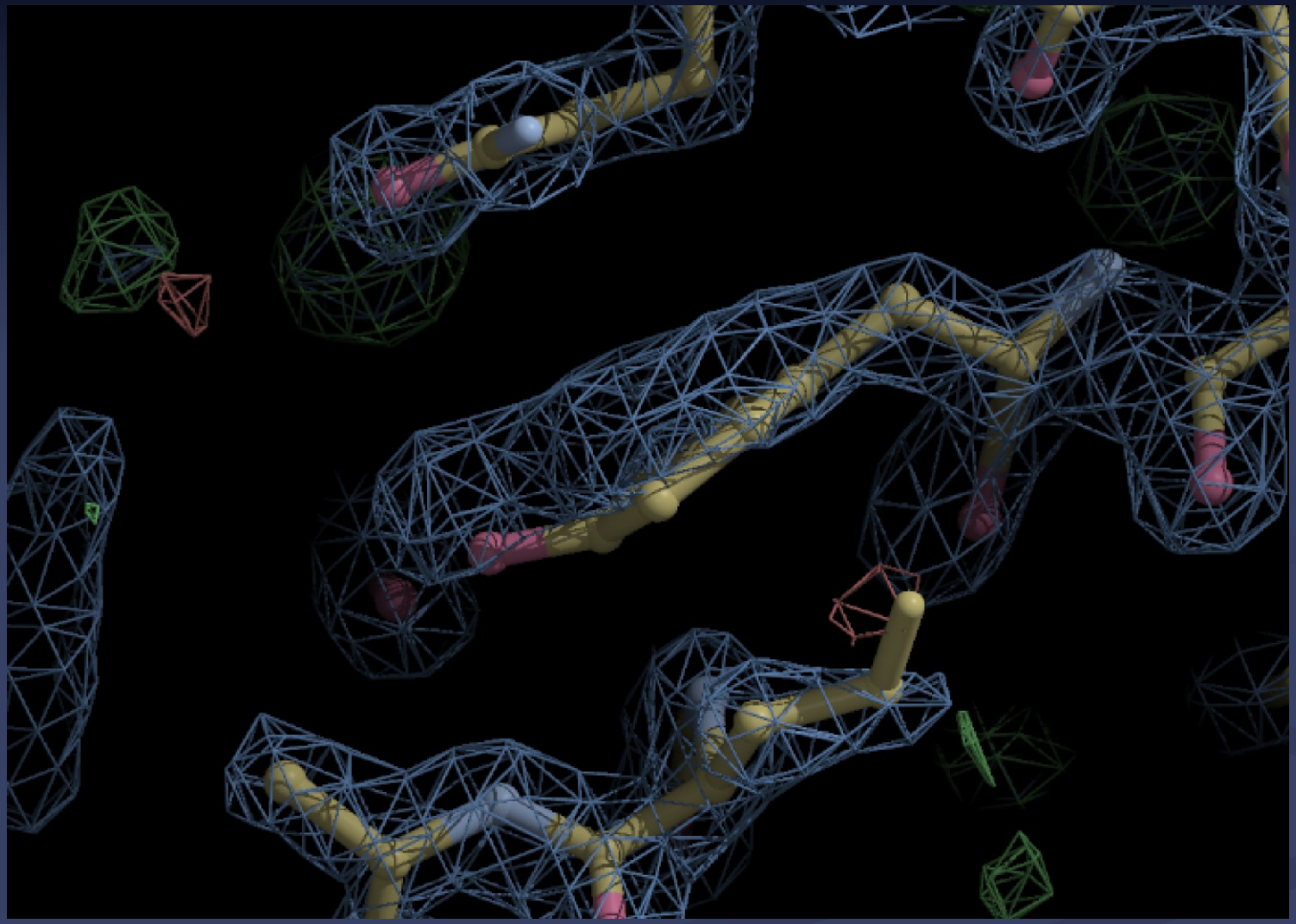
Distorted Geometry Pre-Refinement



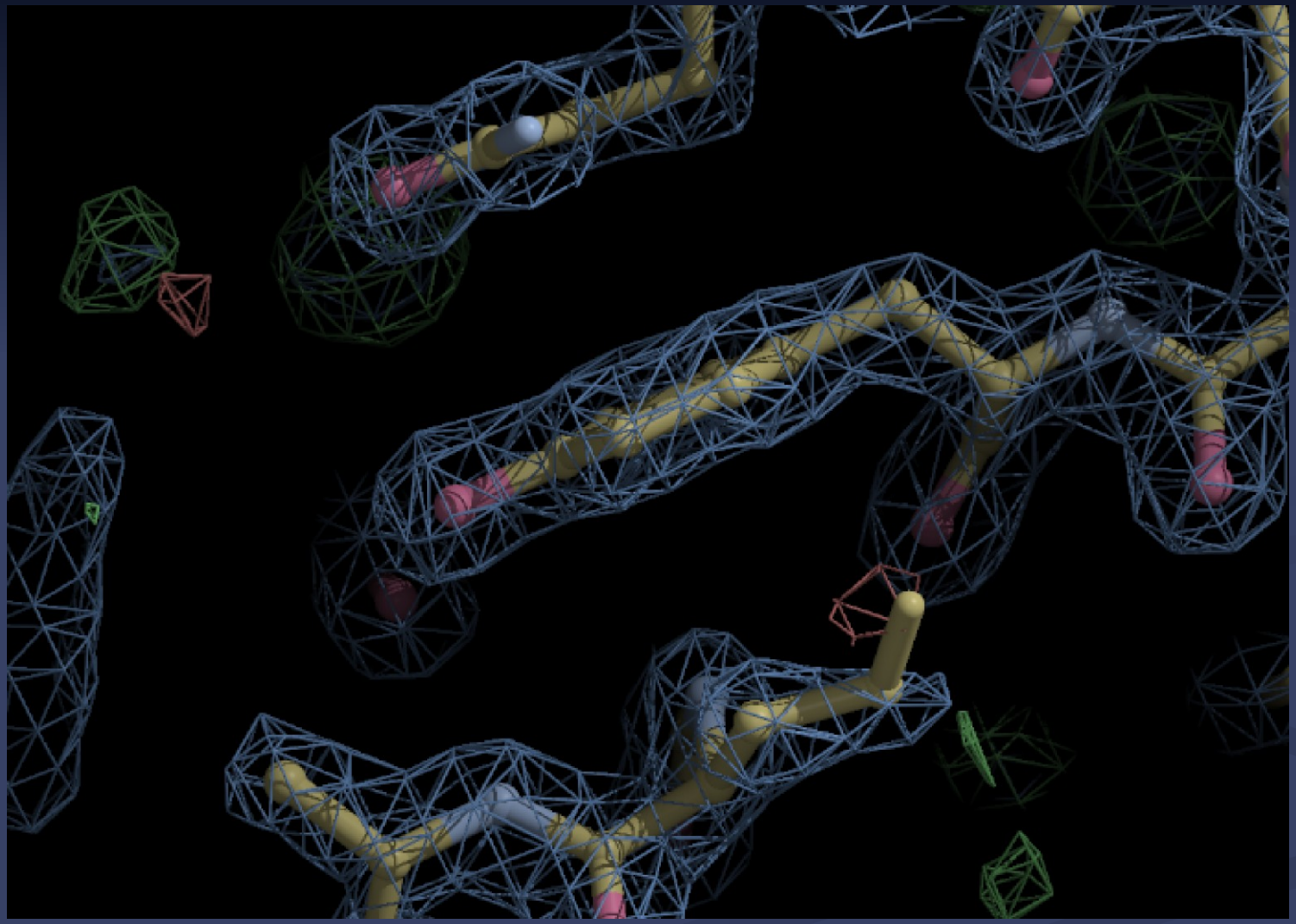
Refinement Gradients



Refinement: Cycle 3



Refinement Cycle 200: Minimized



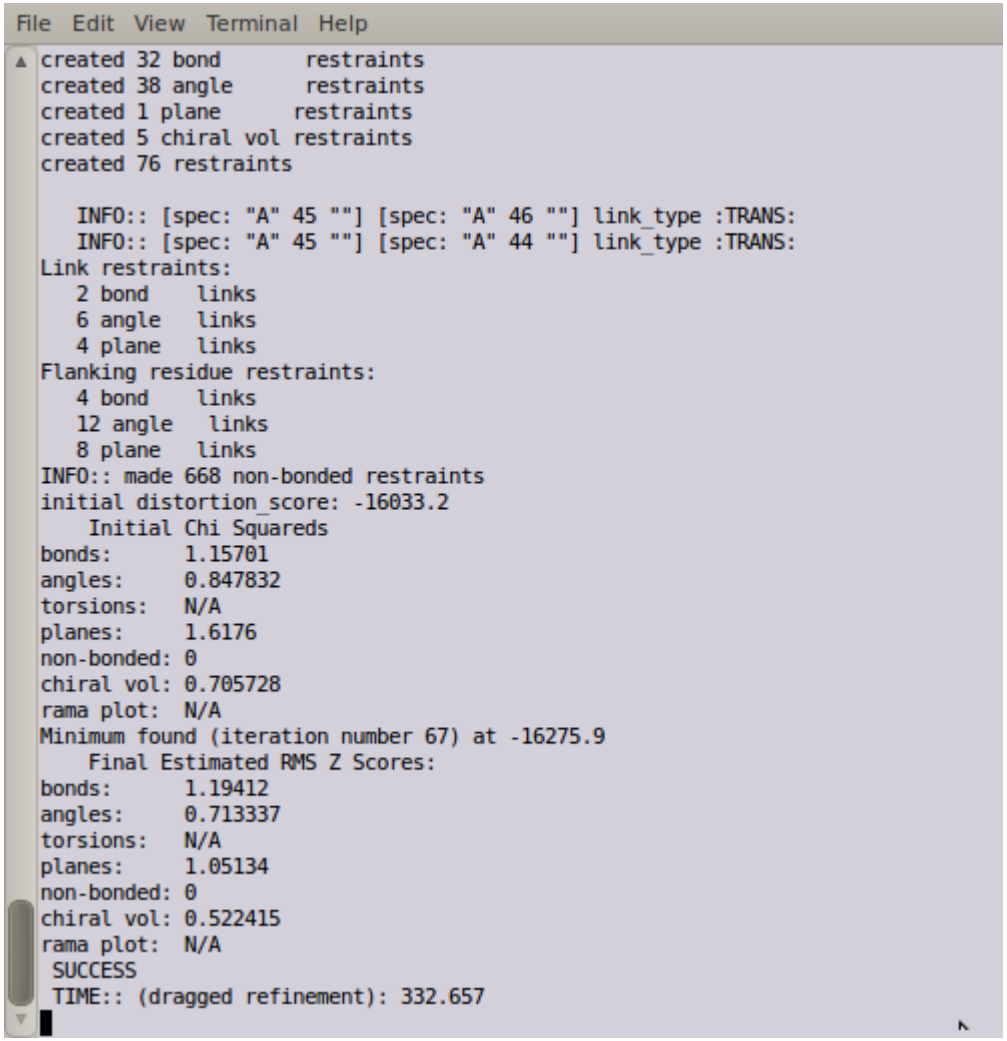
Real Space Refinement

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:

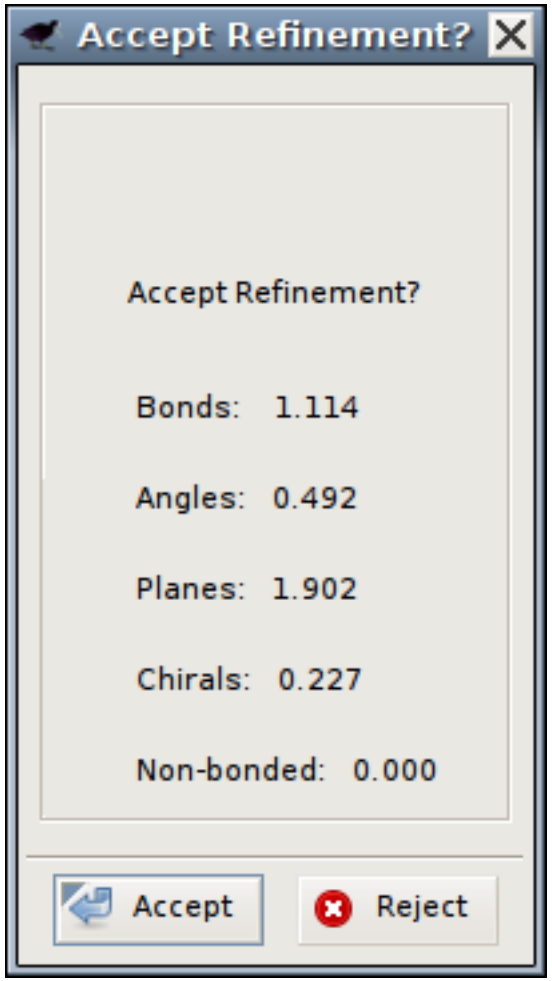


The first attempt

Student Reaction:

“Oh, I don't look at that window...”

Representation of Results:



Second attempt...

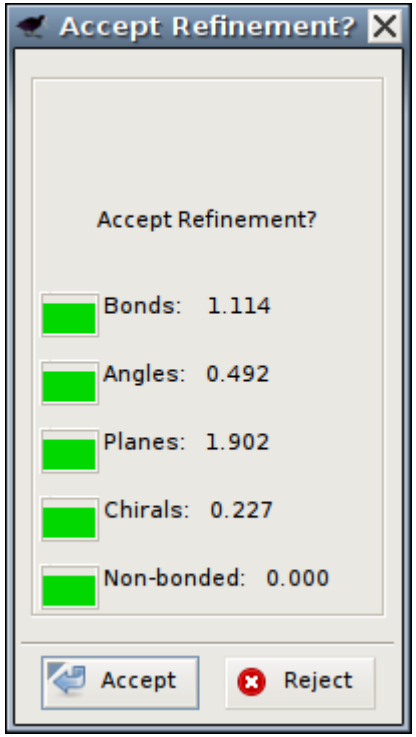
Student Reaction:

“Oh, box of meaningless numbers.

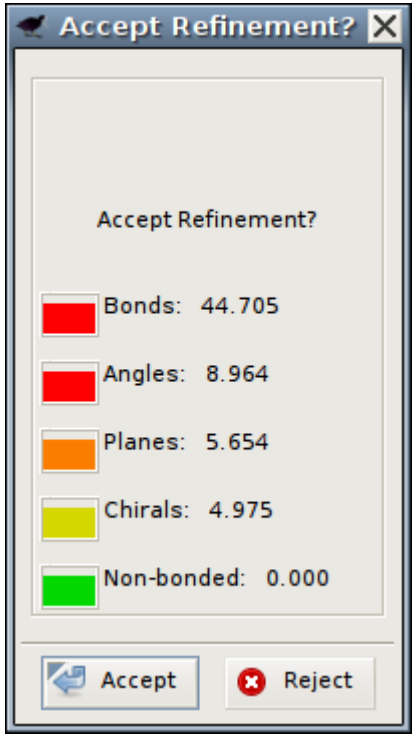
Go away”

Representation of Results: “Traffic Lights”

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



Good refinement

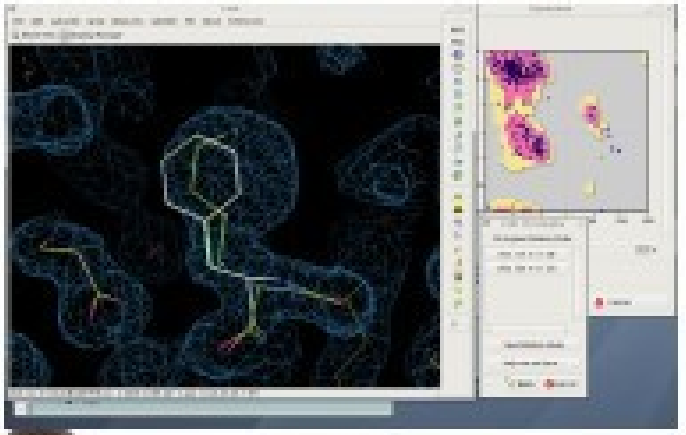


Bad refinement

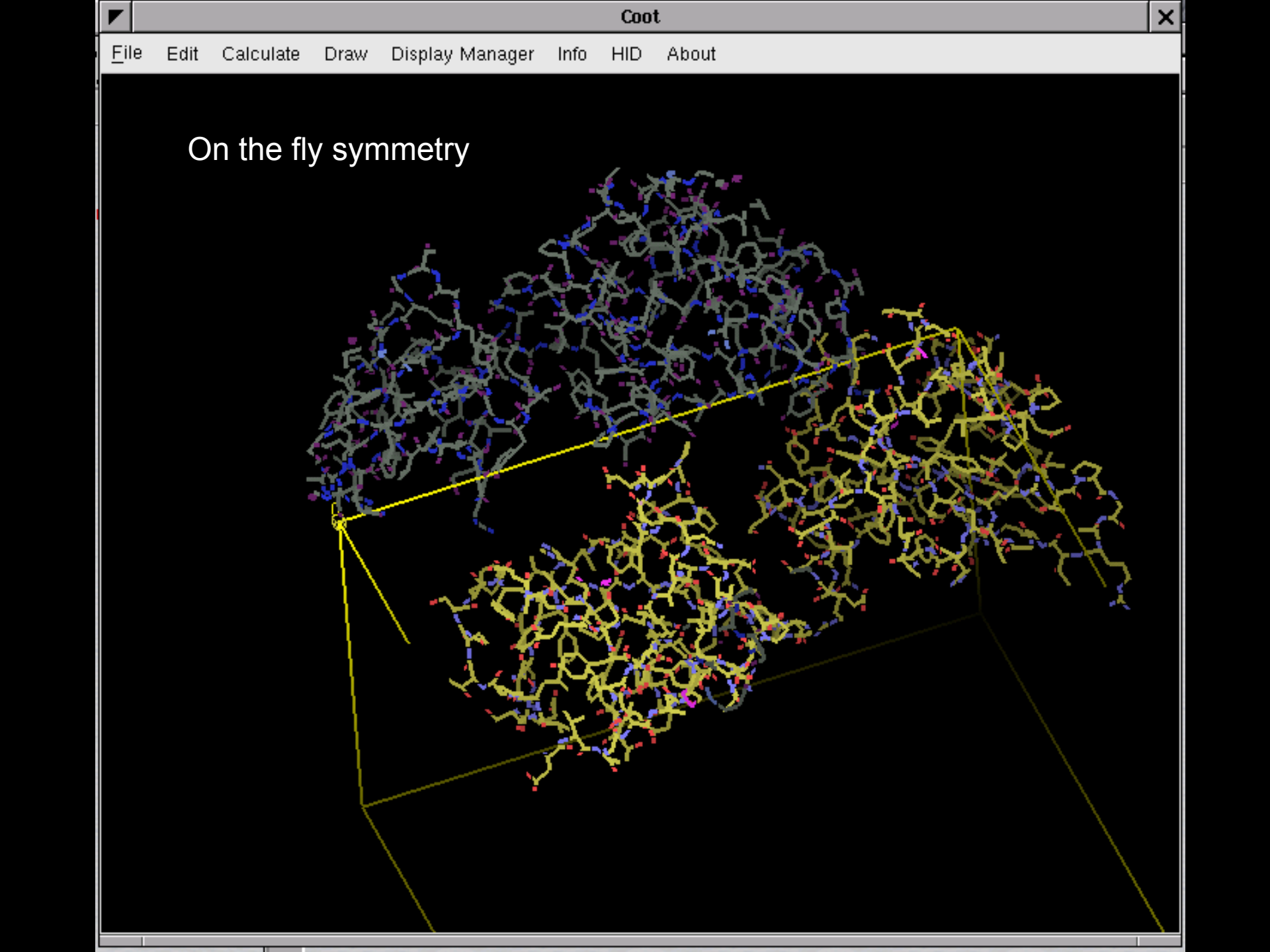
Refinement Techniques

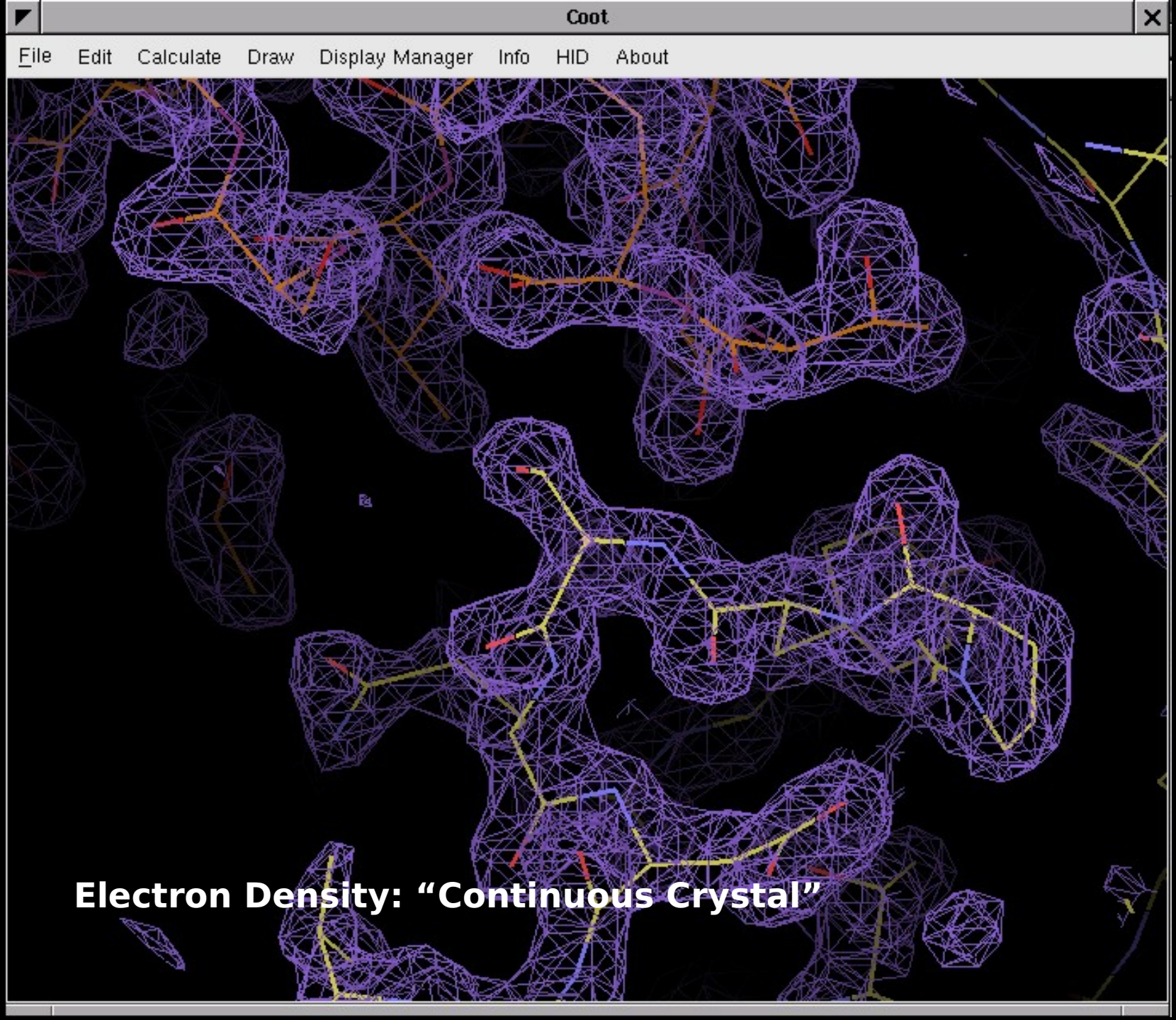
- Single-Atom Drag
  - Over-dragging
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept
- Ramachandran Refinement
- Coming Soon..?
  - Wii Refinement

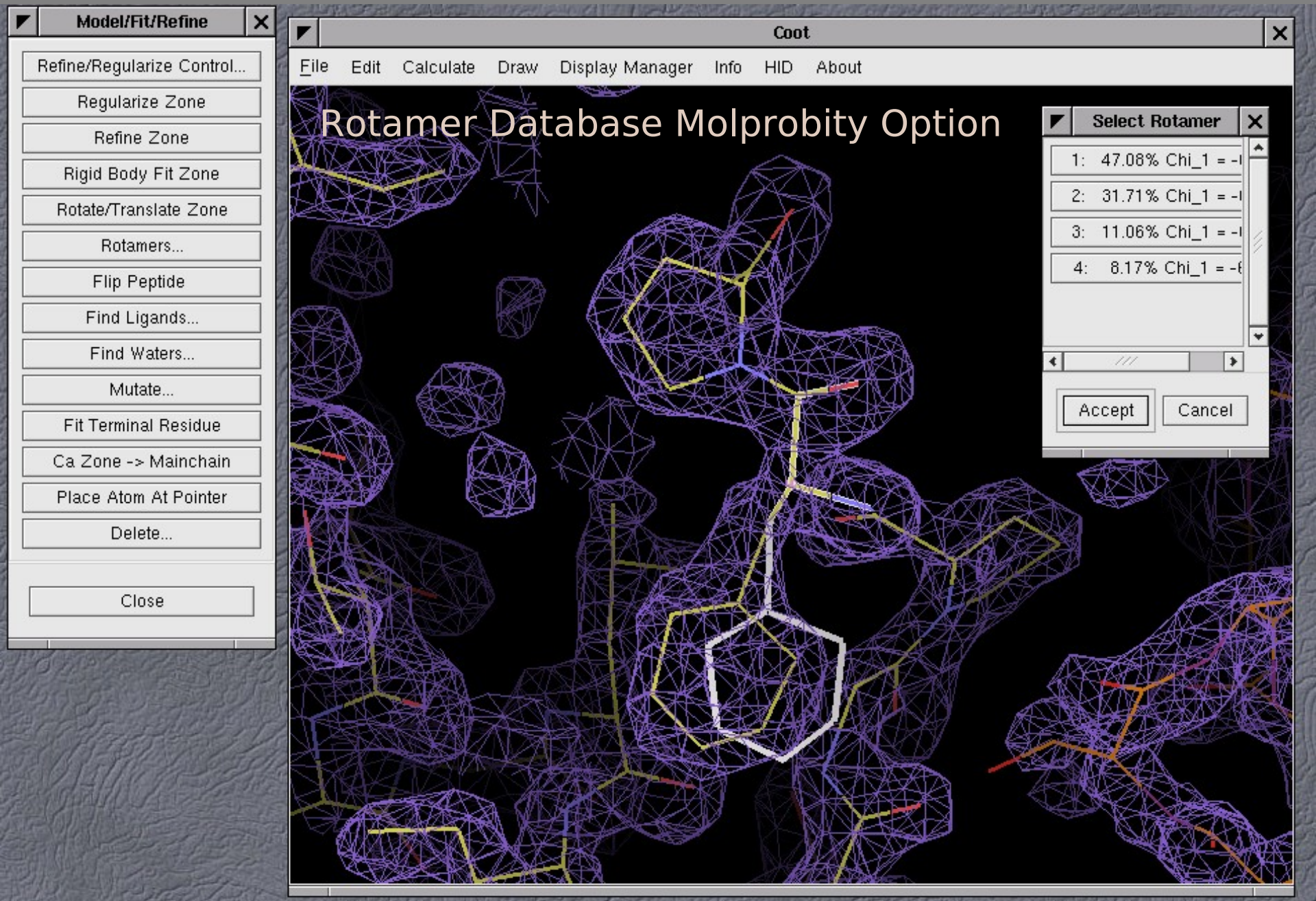
Wii Refinement?



Wii Refinement?

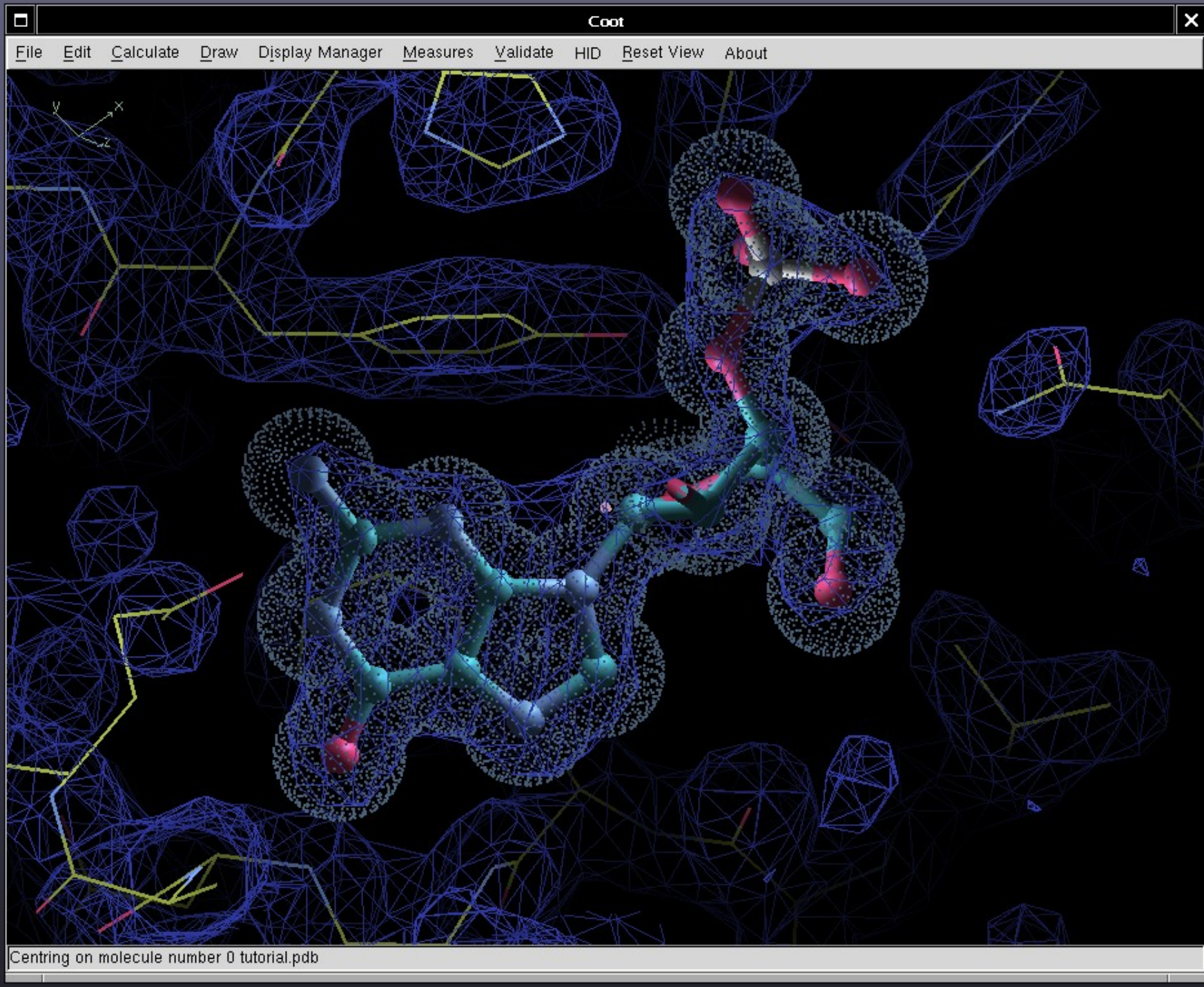

Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About
On the fly symmetry


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


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

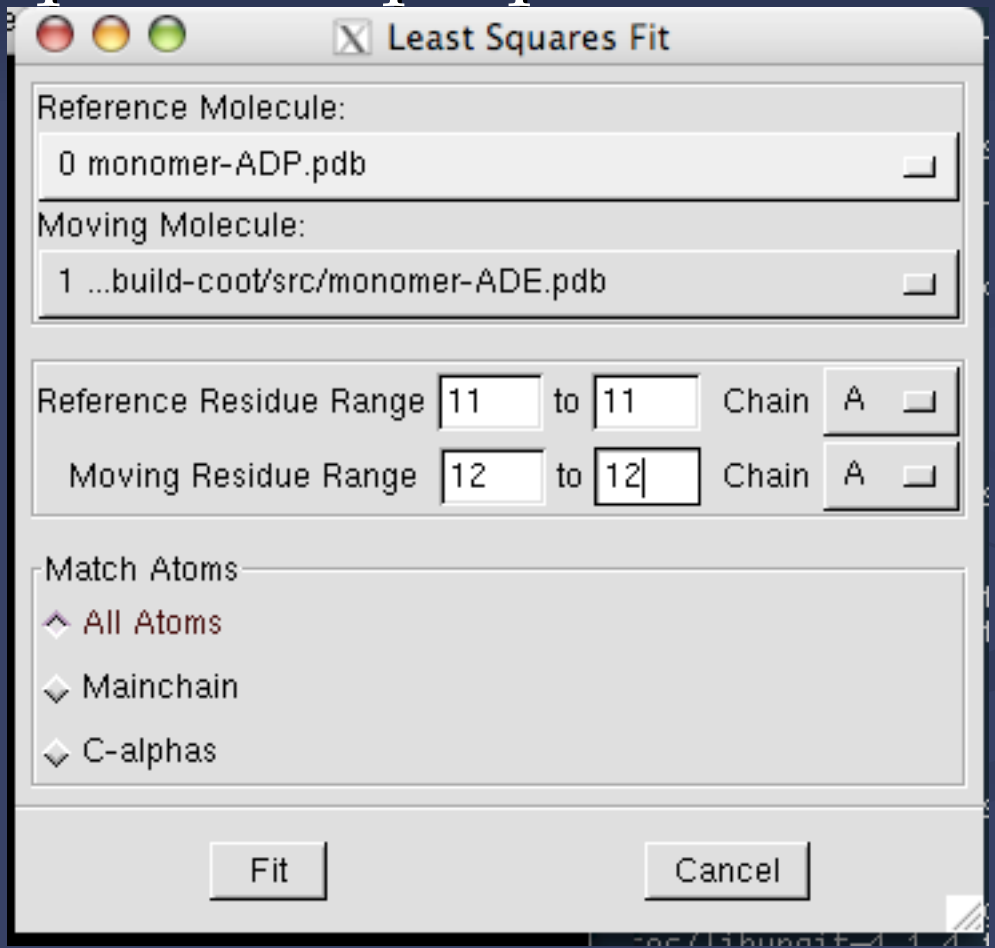
Some more Coot Tools…

- 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
Calculate
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

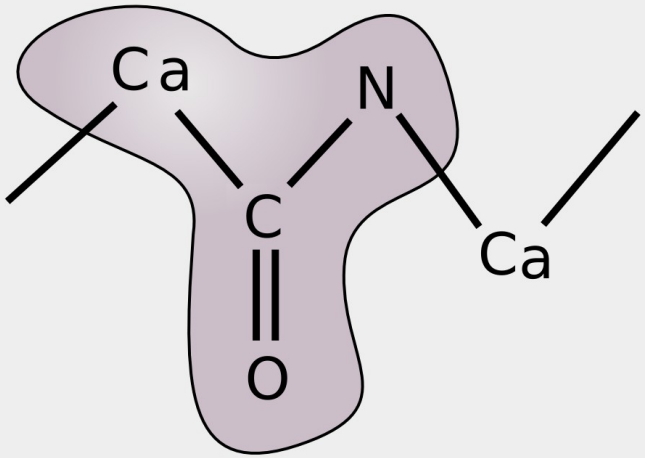
Extra Restraints....

Extra Restraints....

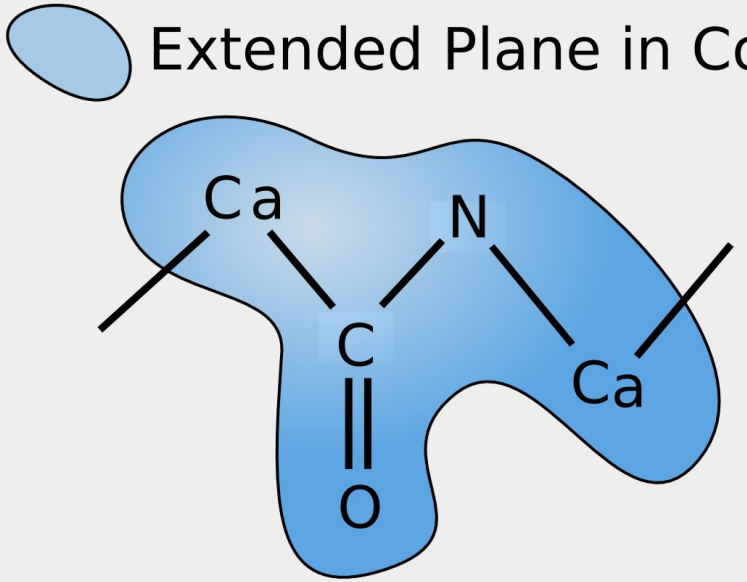
- Peptide plane
- Ramachandran restraints
- Secondary Structure restraints
- Remove degree of freedom
  - Torsion angle restraints
  - Backrub rotamers

Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane



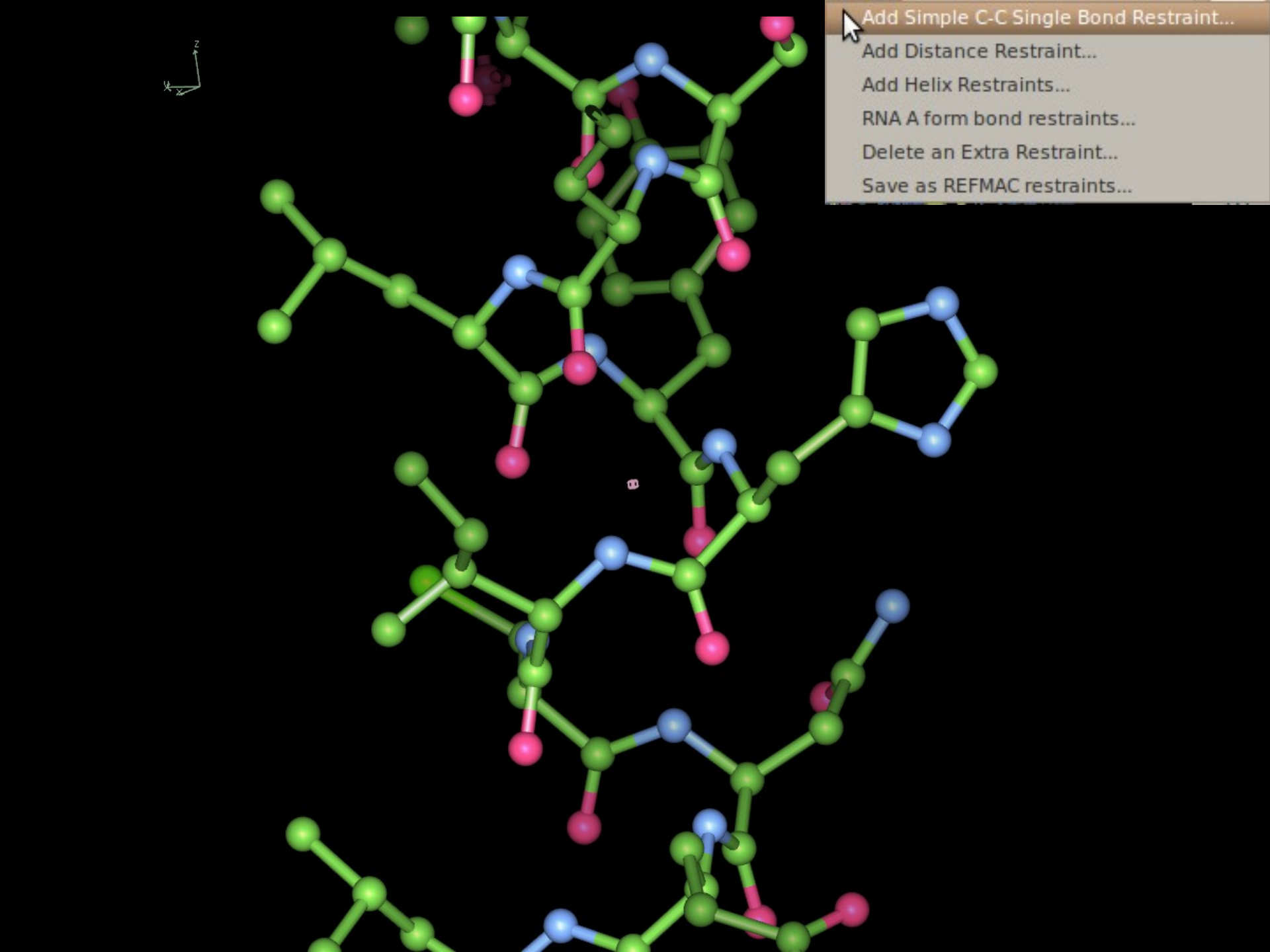
Extended Plane in Coot

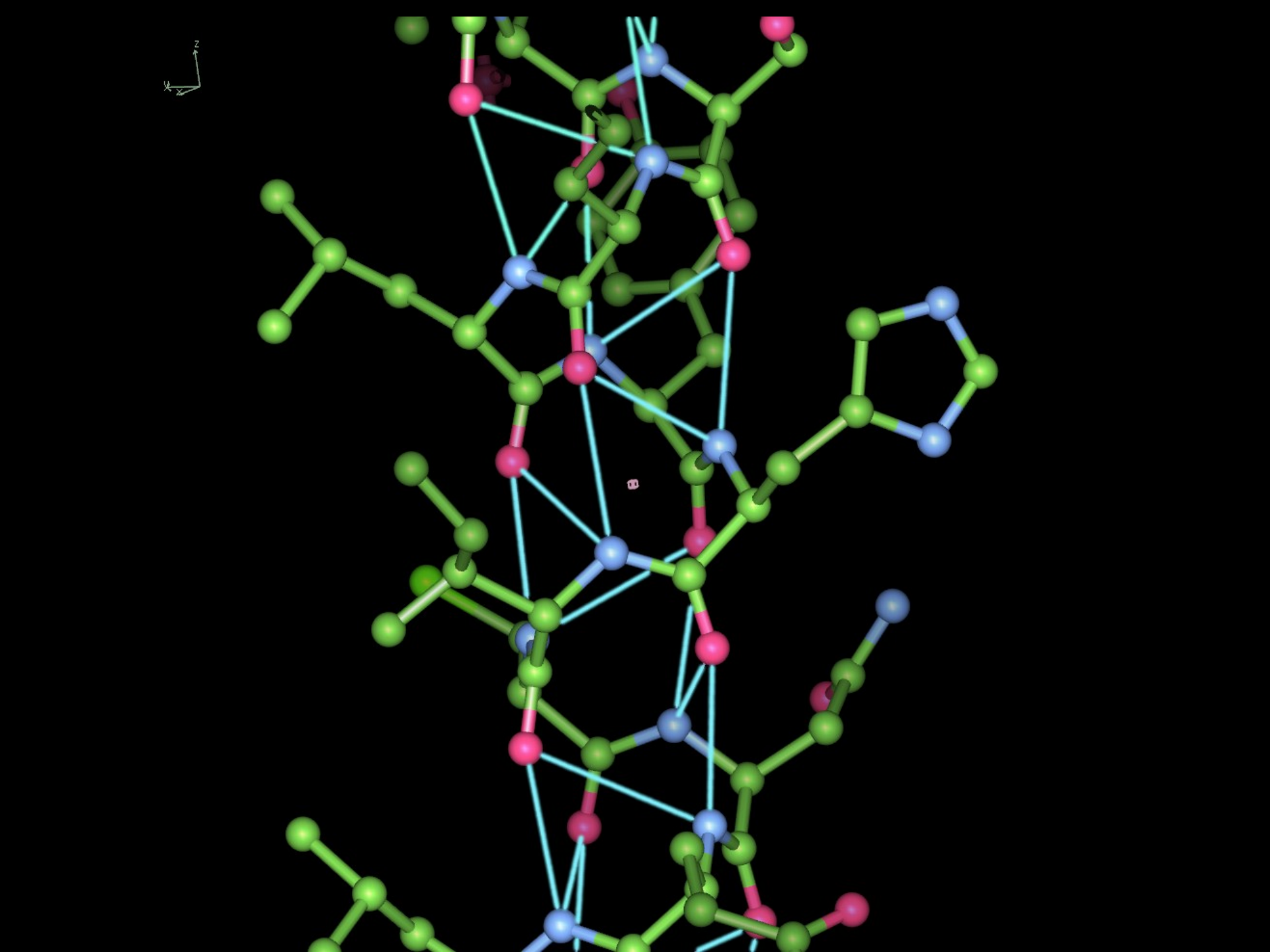


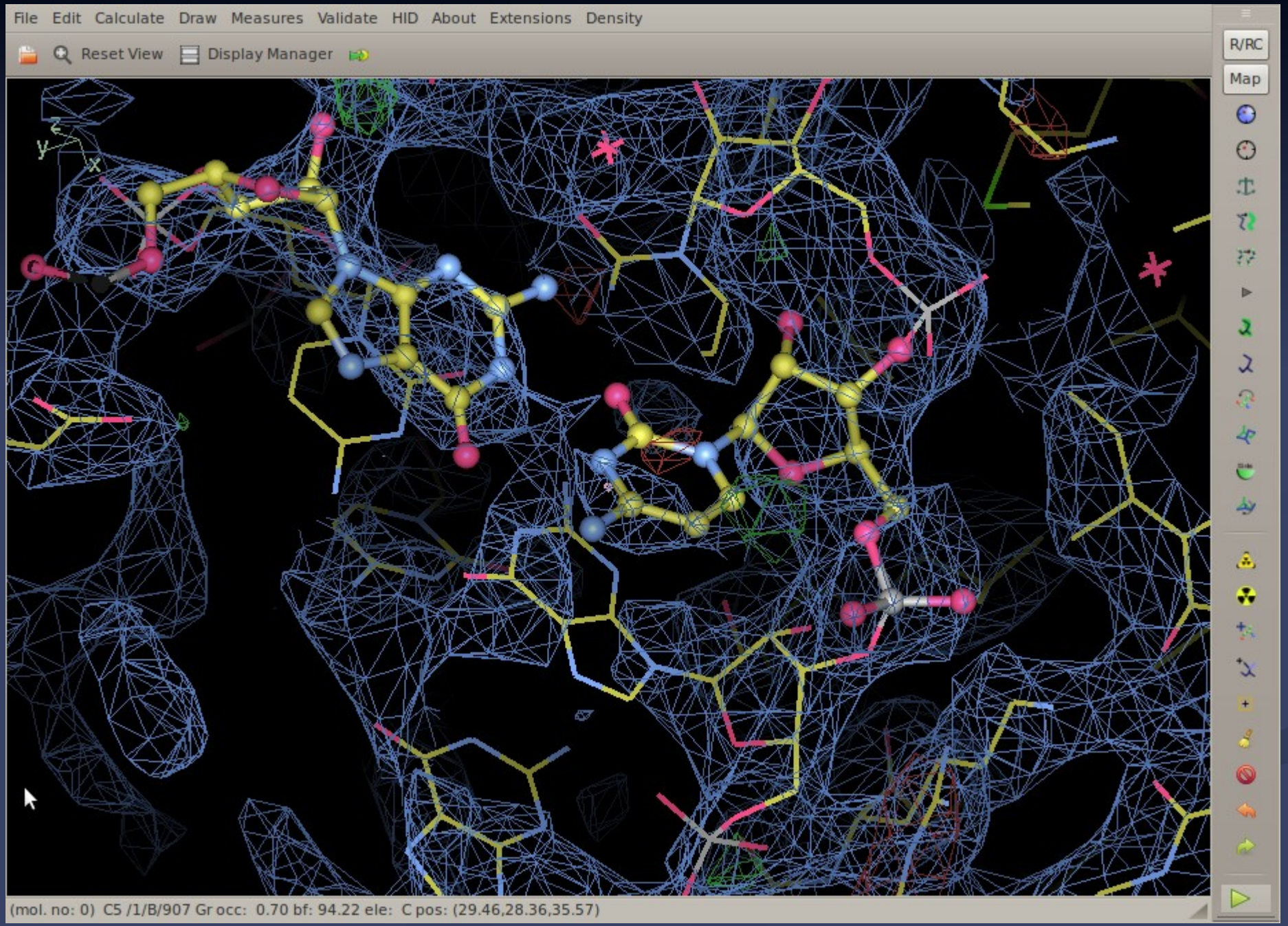
(add-planar-peptide-restraints)

Restraints Editing in Coot

- Distance Restraints:
  - Alpha helices, A-form RNA
- Add and delete individual restraints
  - User-selectable sigma
- Select 2 residues for range
- Future: All molecule
  - Beta strands
- User-defined Torsion restraints


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...


Z
X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R/RC
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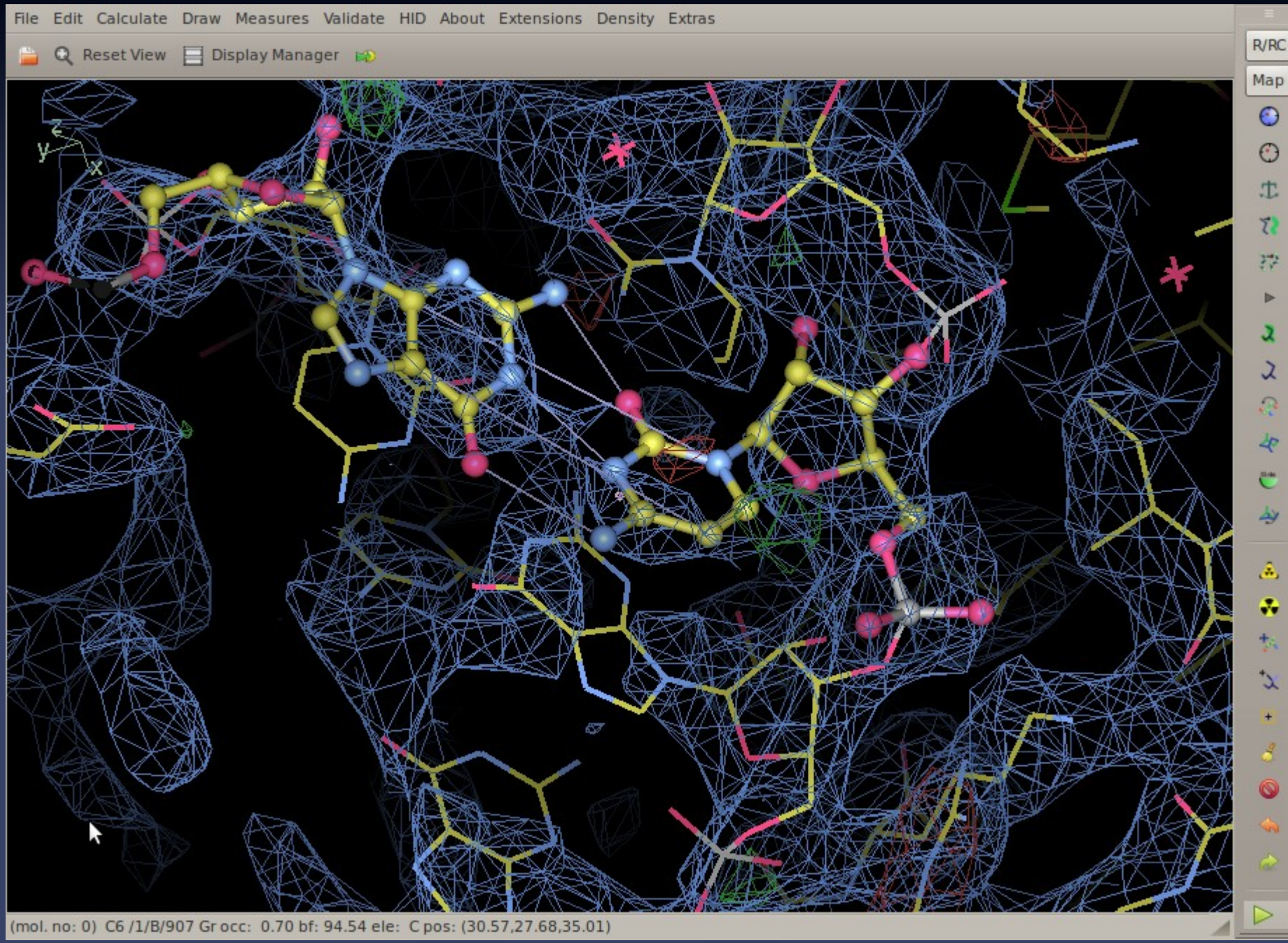
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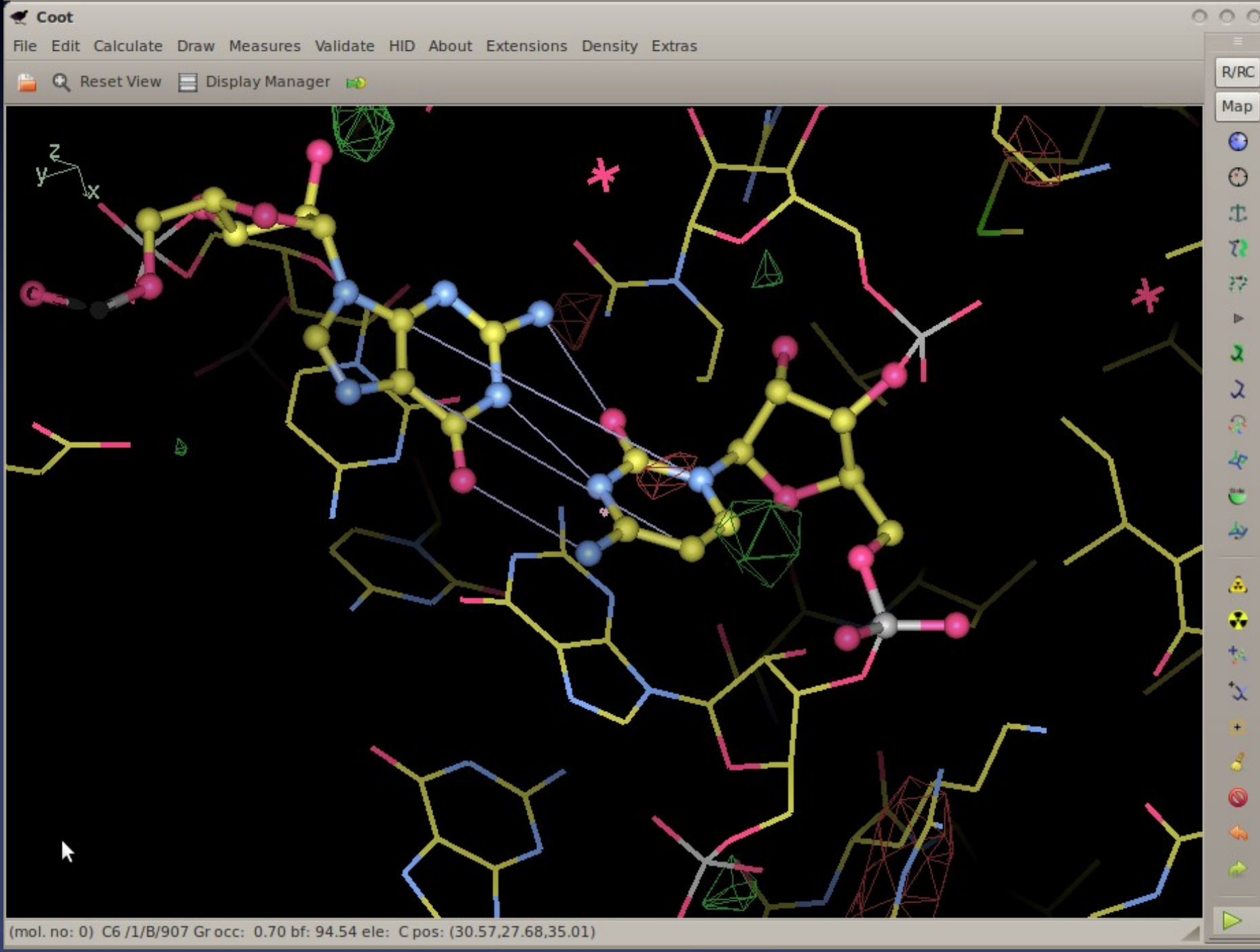
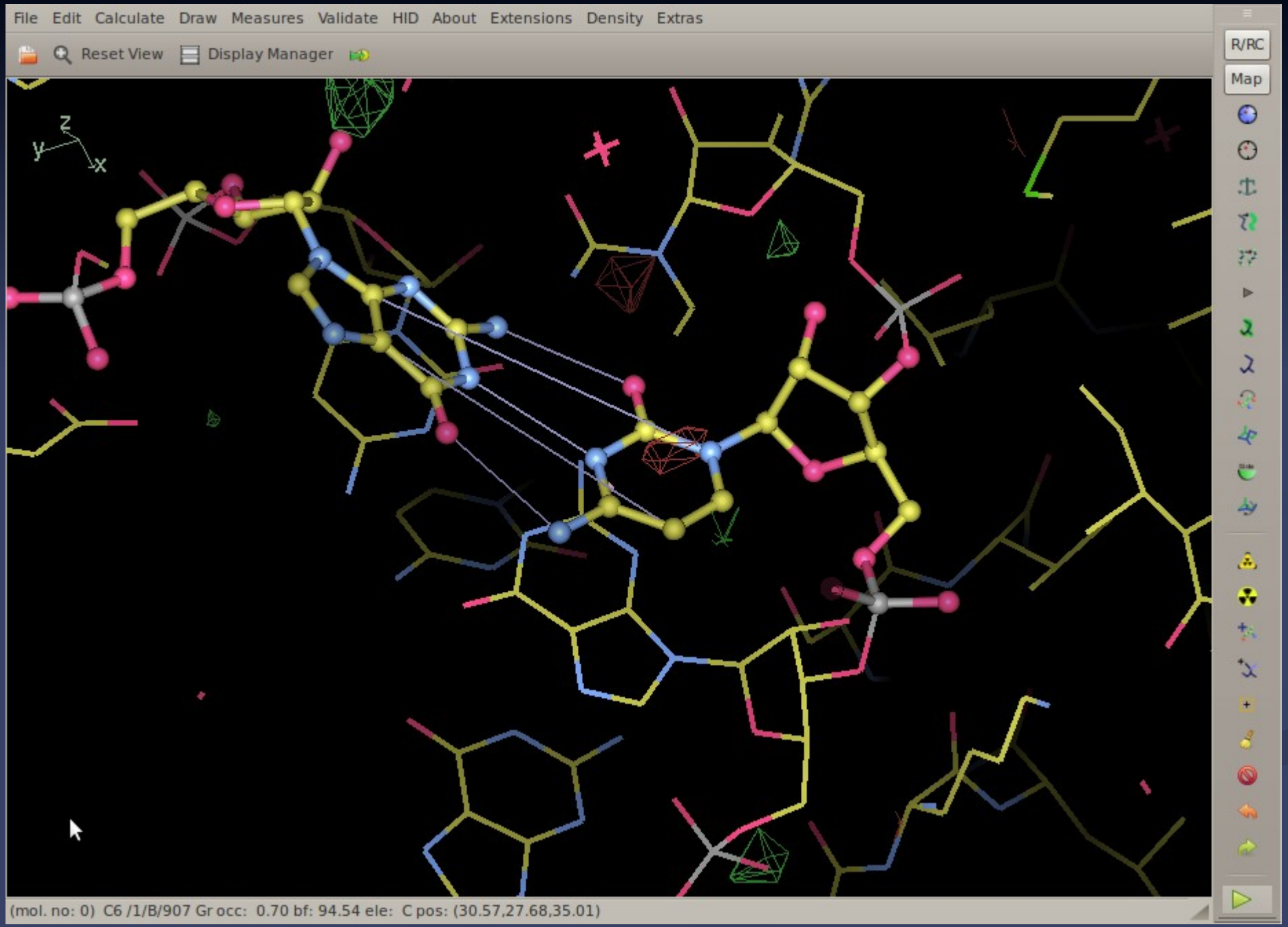
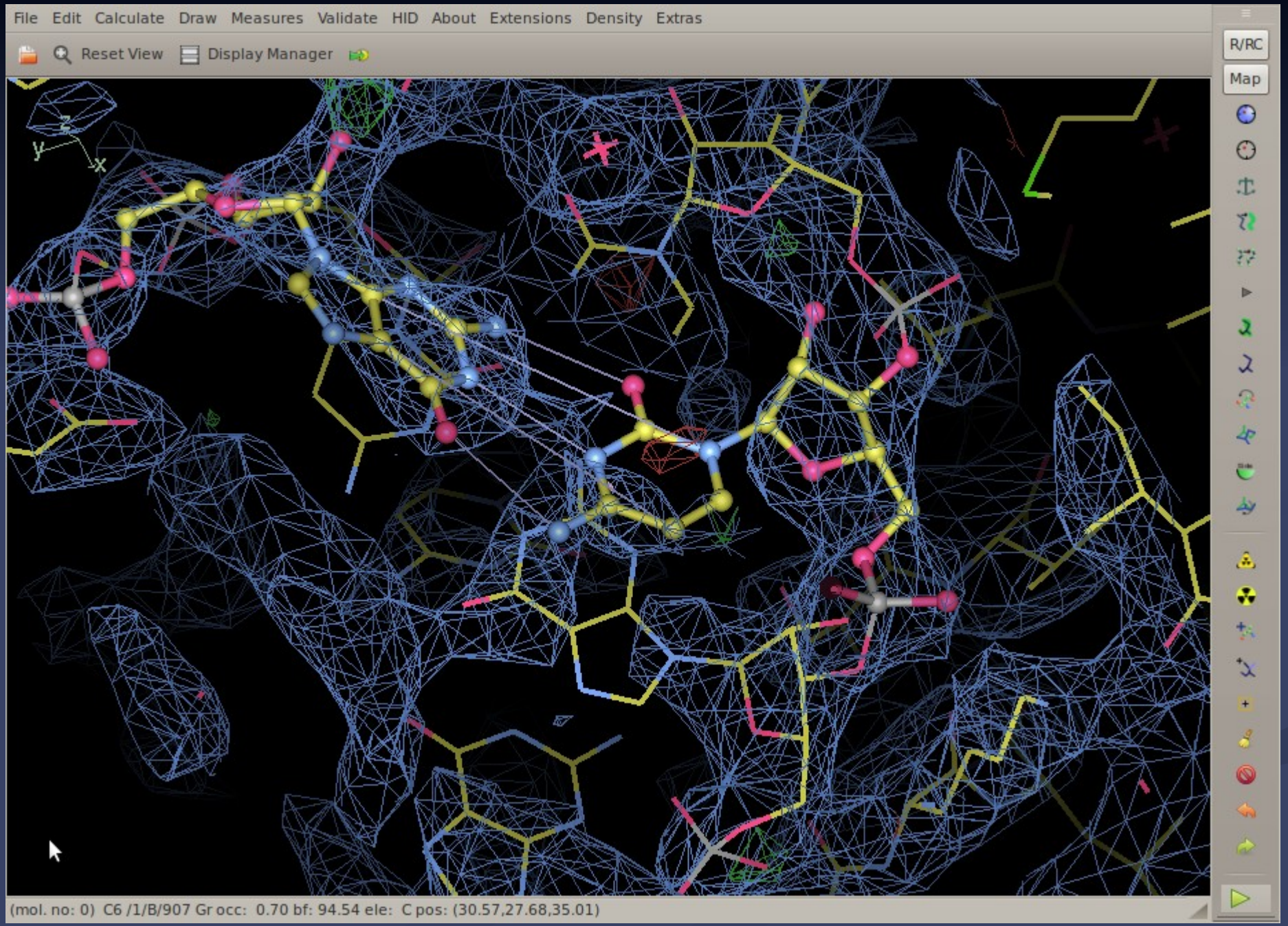

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
(mol. no: 0) C6 /1/B/907 Gr occ: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)


File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
Z
Y
X
(mol. no: 0) C6 /1/B/907 Gr occ: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)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Reset View
Display Manager
R/RC
Map
(mol. no: 0) C6 /1/B/907 Gr occ: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)

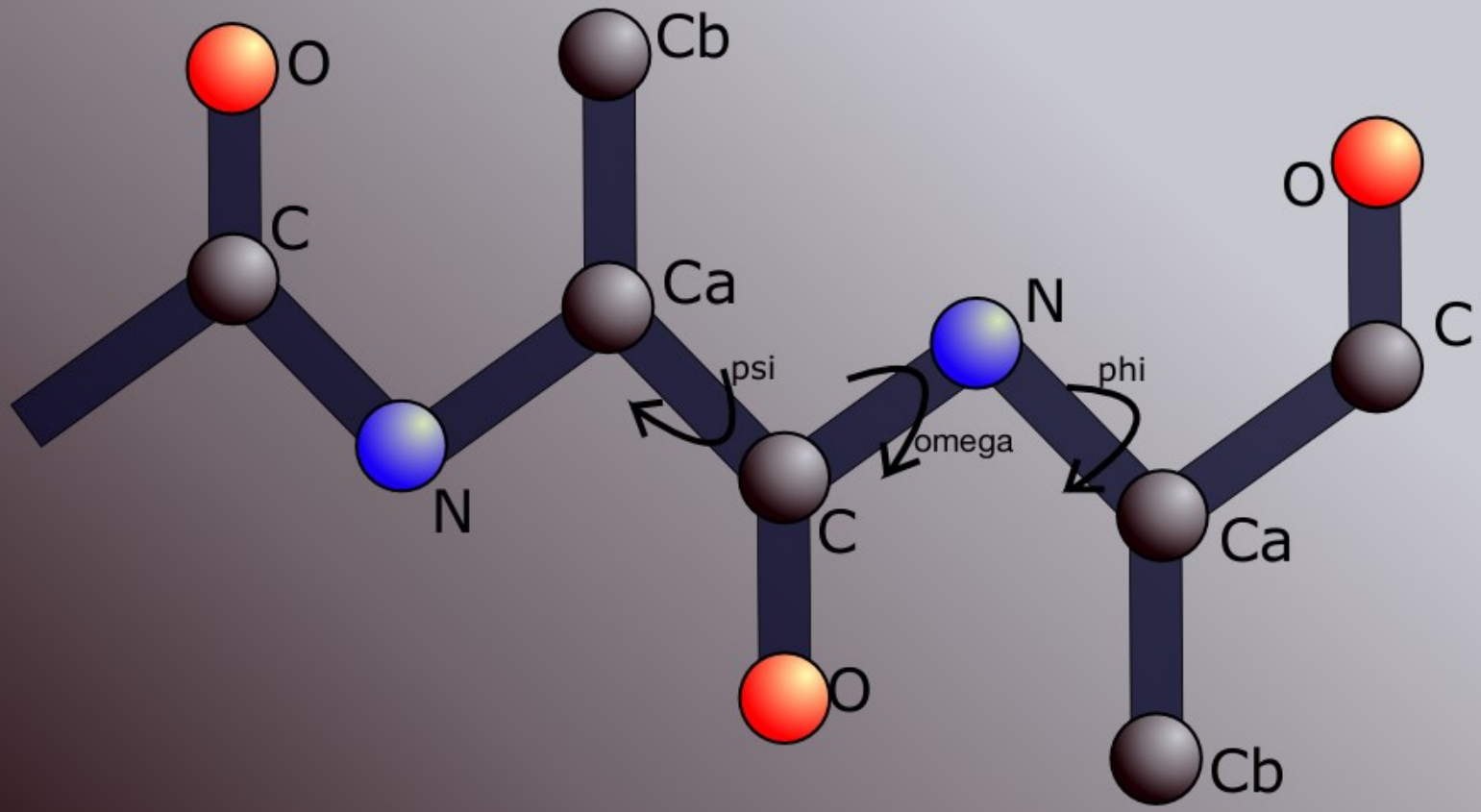
Export as Refmac Restraints:

- EXT DIST FIRST CHAIN A RESI 55 INS . ATOM CA SECOND CHAIN A RESI 55 INS . ATOM C   VALUE 1.54 SIGMA 0.05

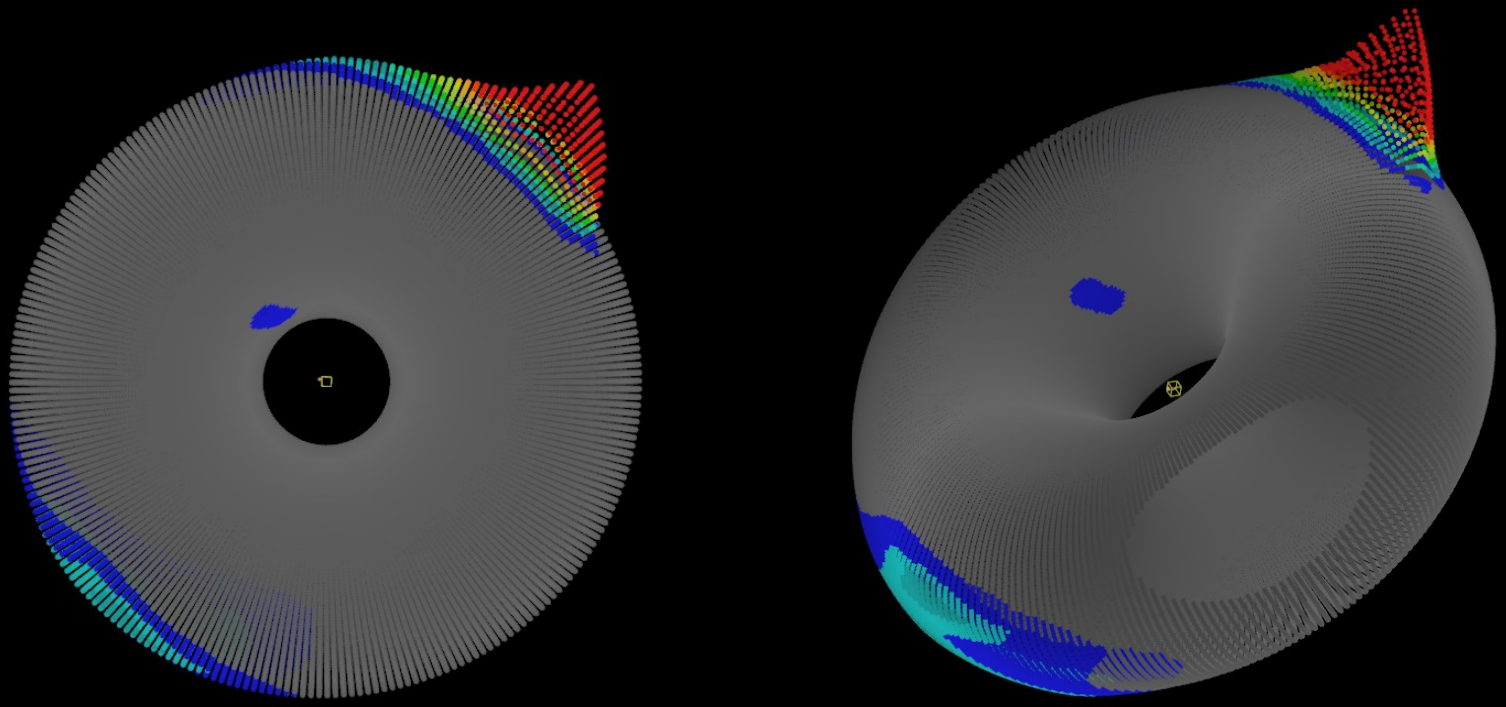
Ramachandran Restraints

- 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

Peptide Torsion Angles



Ramachandran Plot Representation



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

(linear scaling)

1 Torsion Angle Derivatives

If we have a torsion angle, θ, say, then the derivates needed are

$$\frac{\partial \theta}{\partial x_1} \tag{1}$$

for the atom positional parameters y_1, z_1, x_2 and so on. These Coot already has in analytical form.

2 Ramachandran Derivatives

Kevin Cowtan provides a "Log Ramachandran" plot, $R(\phi, \psi)$, where the value of the plot should be added to the target function and minimized.

$$R(\phi, \psi), \quad \frac{\partial R(\phi, \psi)}{\partial \phi} \quad \text{and} \quad \frac{\partial R(\phi, \psi)}{\partial \psi} \tag{2}$$

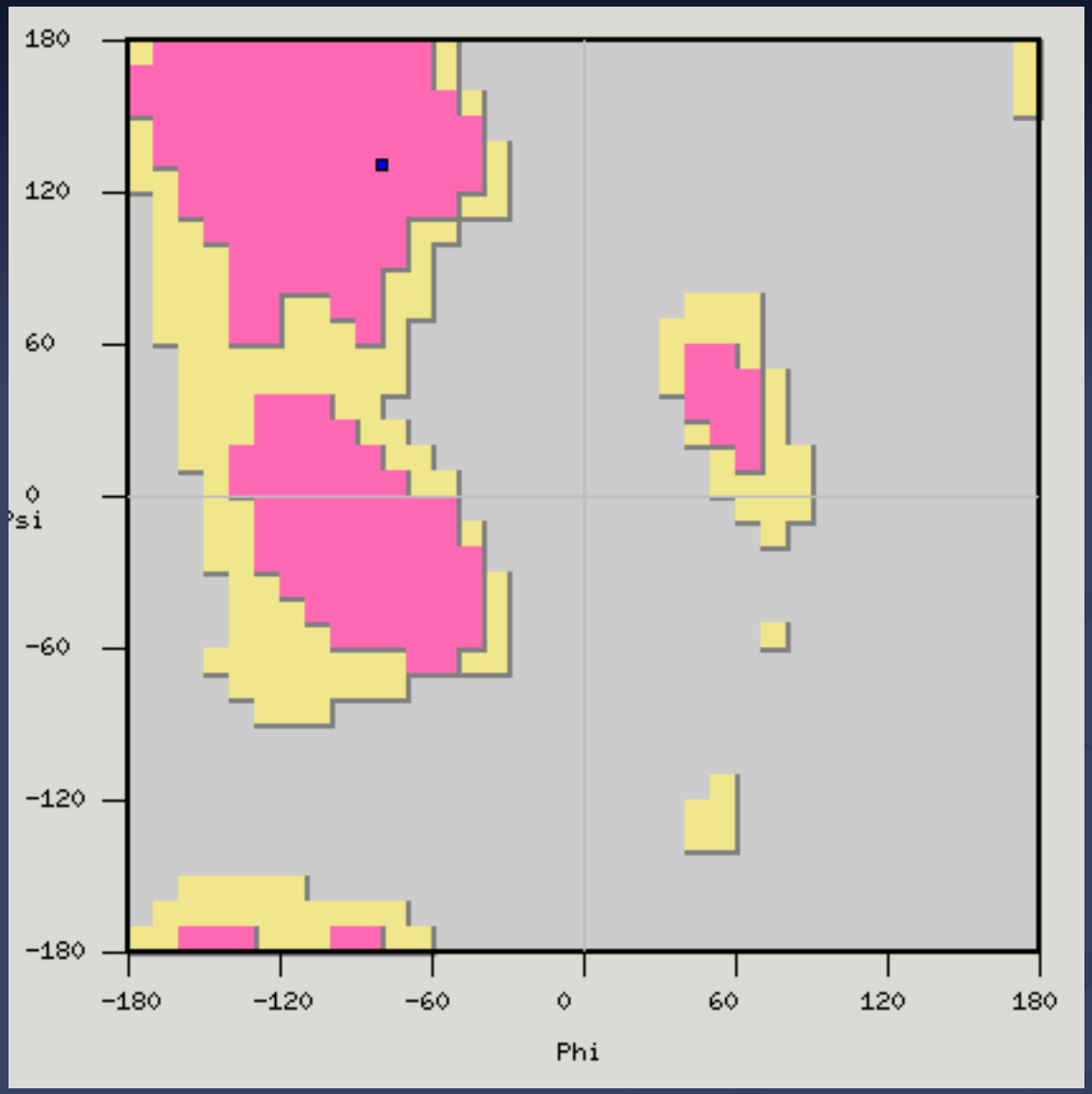
To use this in refinement, I need:

$$\frac{\partial R(\phi, \psi)}{\partial x_1} \quad \text{(and so on)} \tag{3}$$

And this can be calculated from:

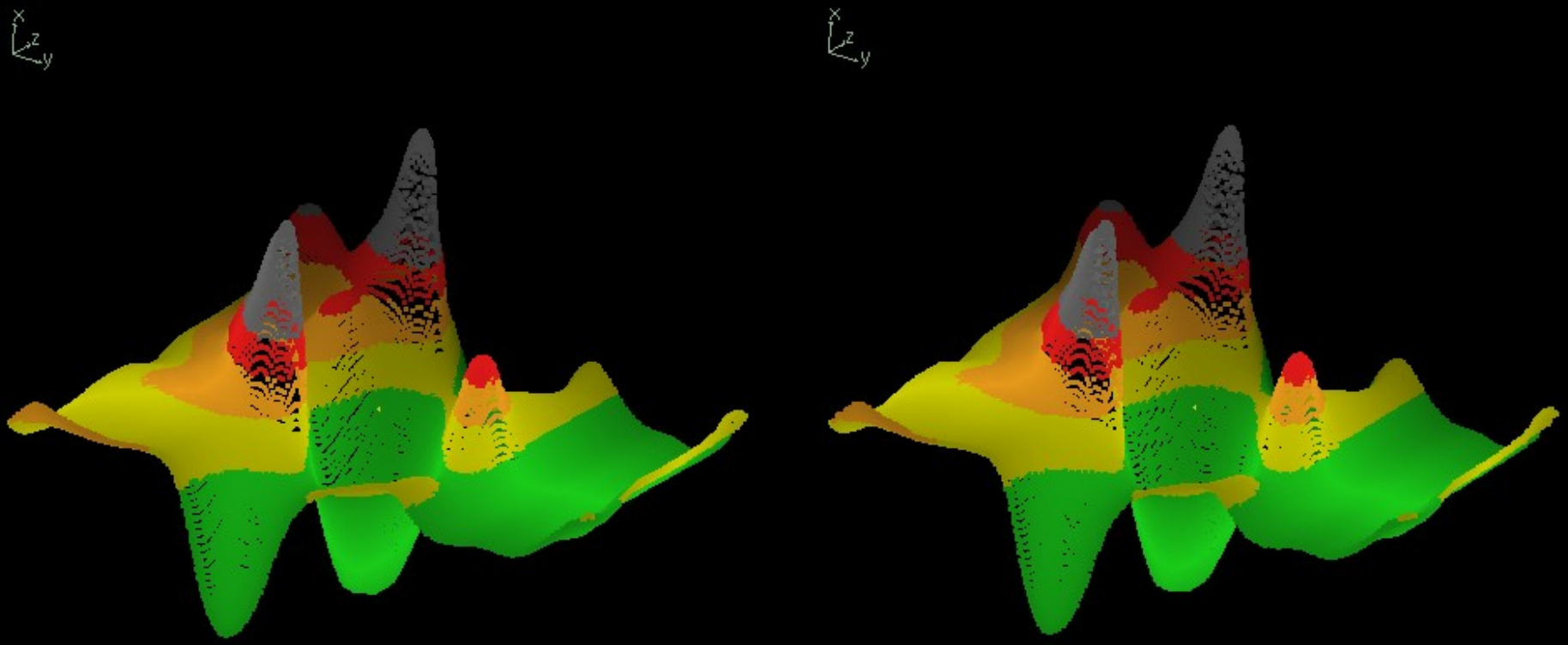
$$\frac{\partial R(\phi, \psi)}{\partial x_1} = \frac{\partial R(\phi, \psi)}{\partial \phi}\frac{\partial \phi}{\partial x_1} + \frac{\partial R(\phi, \psi)}{\partial \psi}\frac{\partial \psi}{\partial x_1} \tag{4}$$

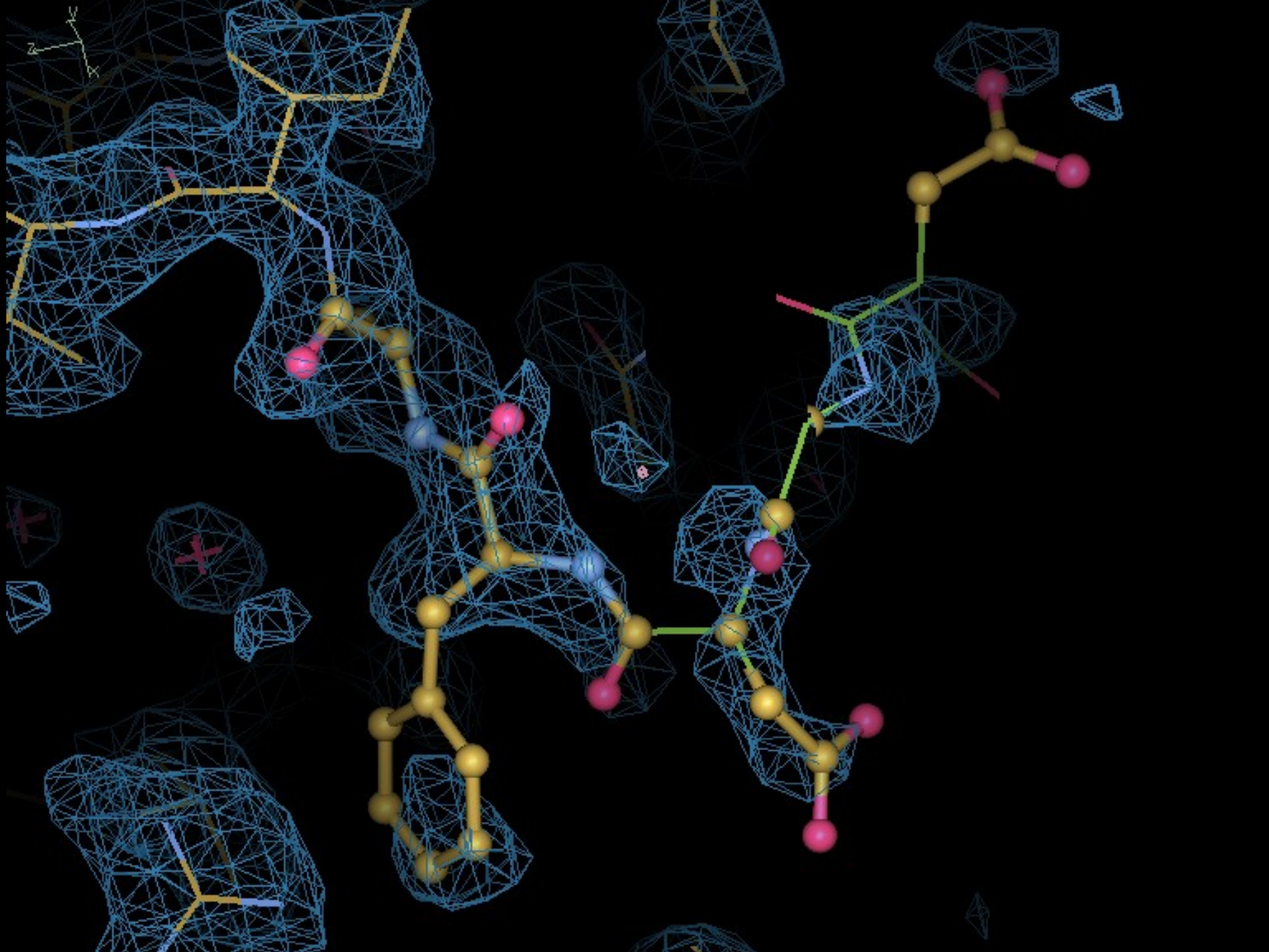
Conventional Ramachandran Plot As Rendered by Coot



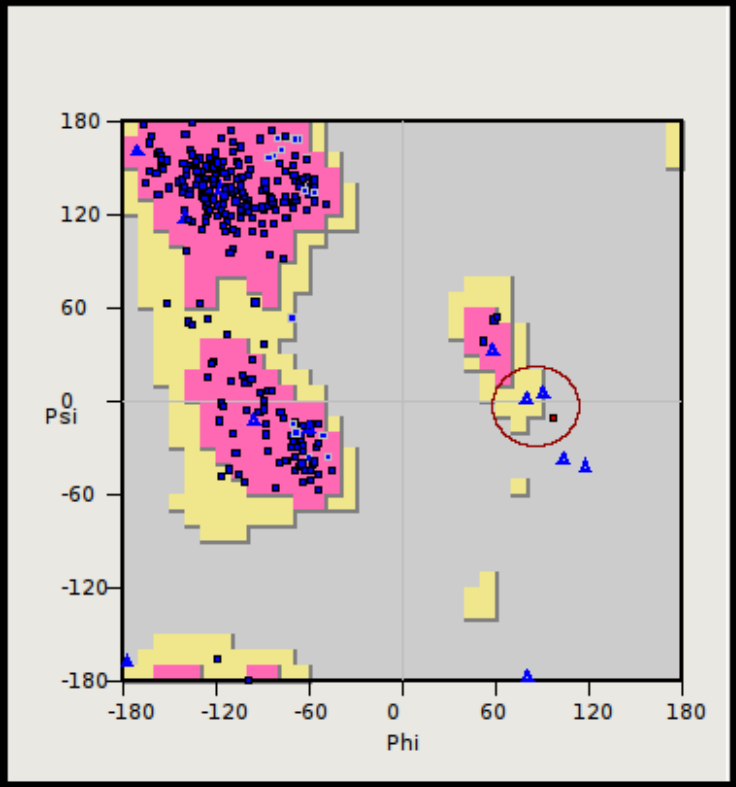
Based on Richardsons' "Top500"

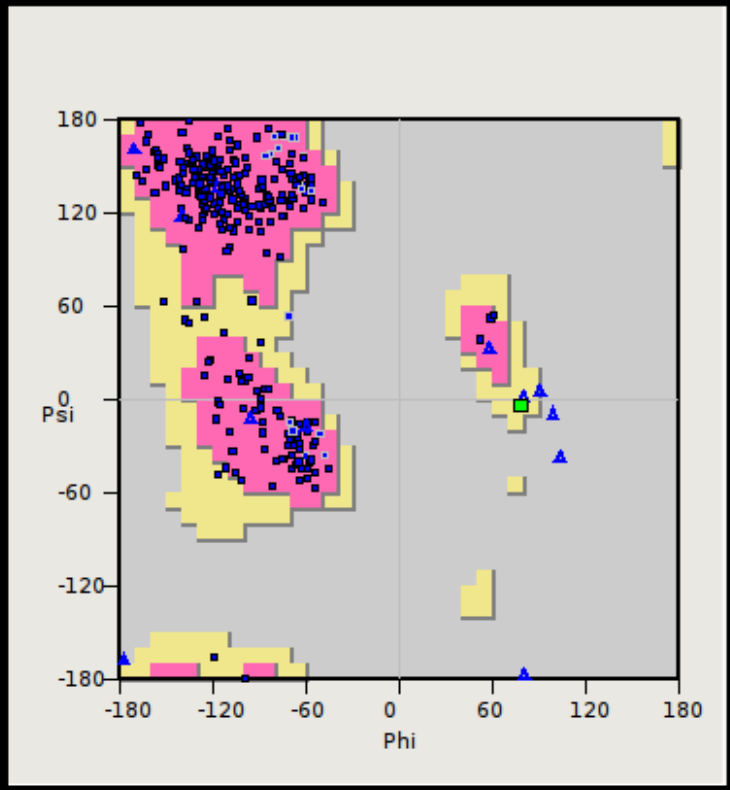
Negatively enhanced disallowed regions



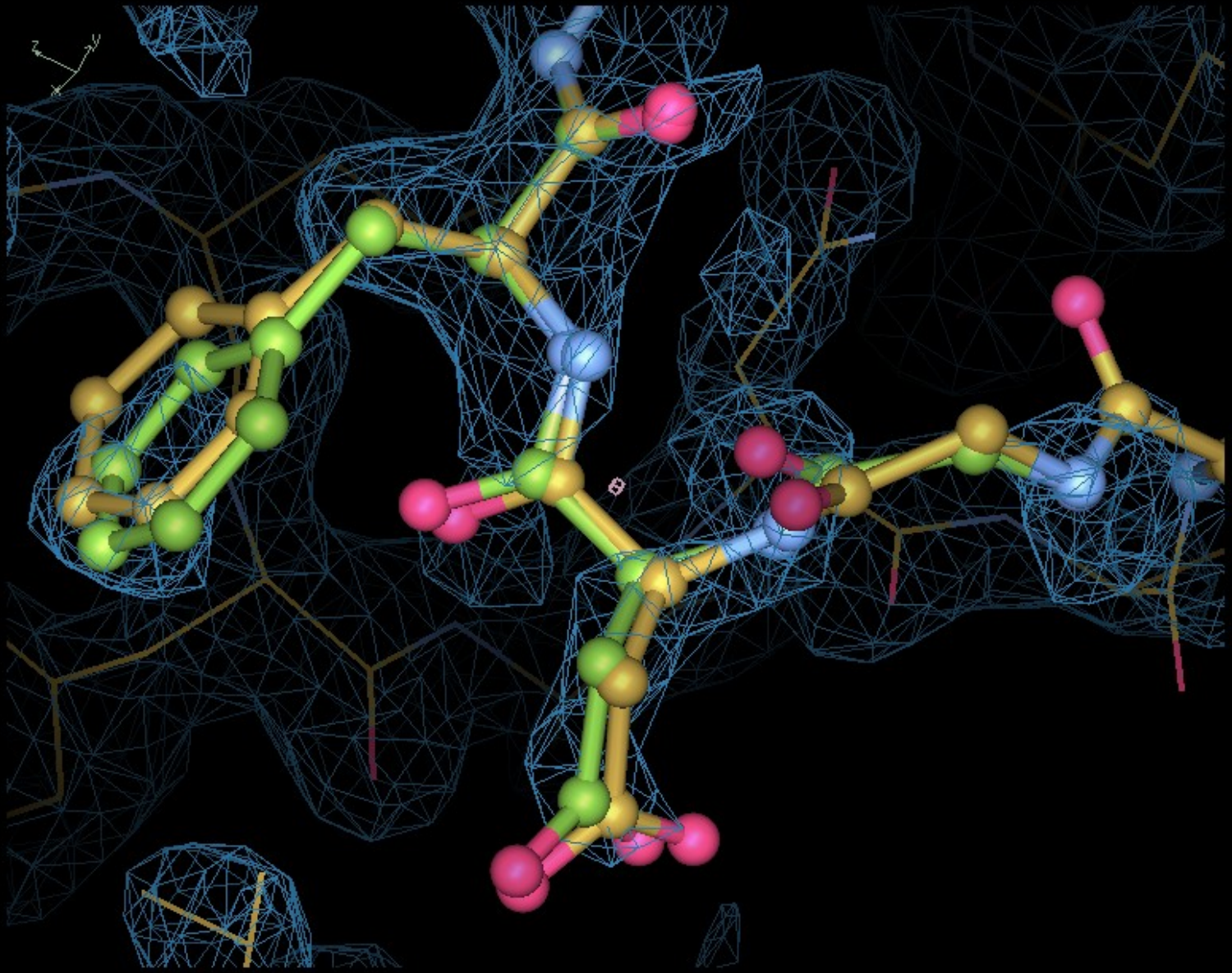

Y
Z
X

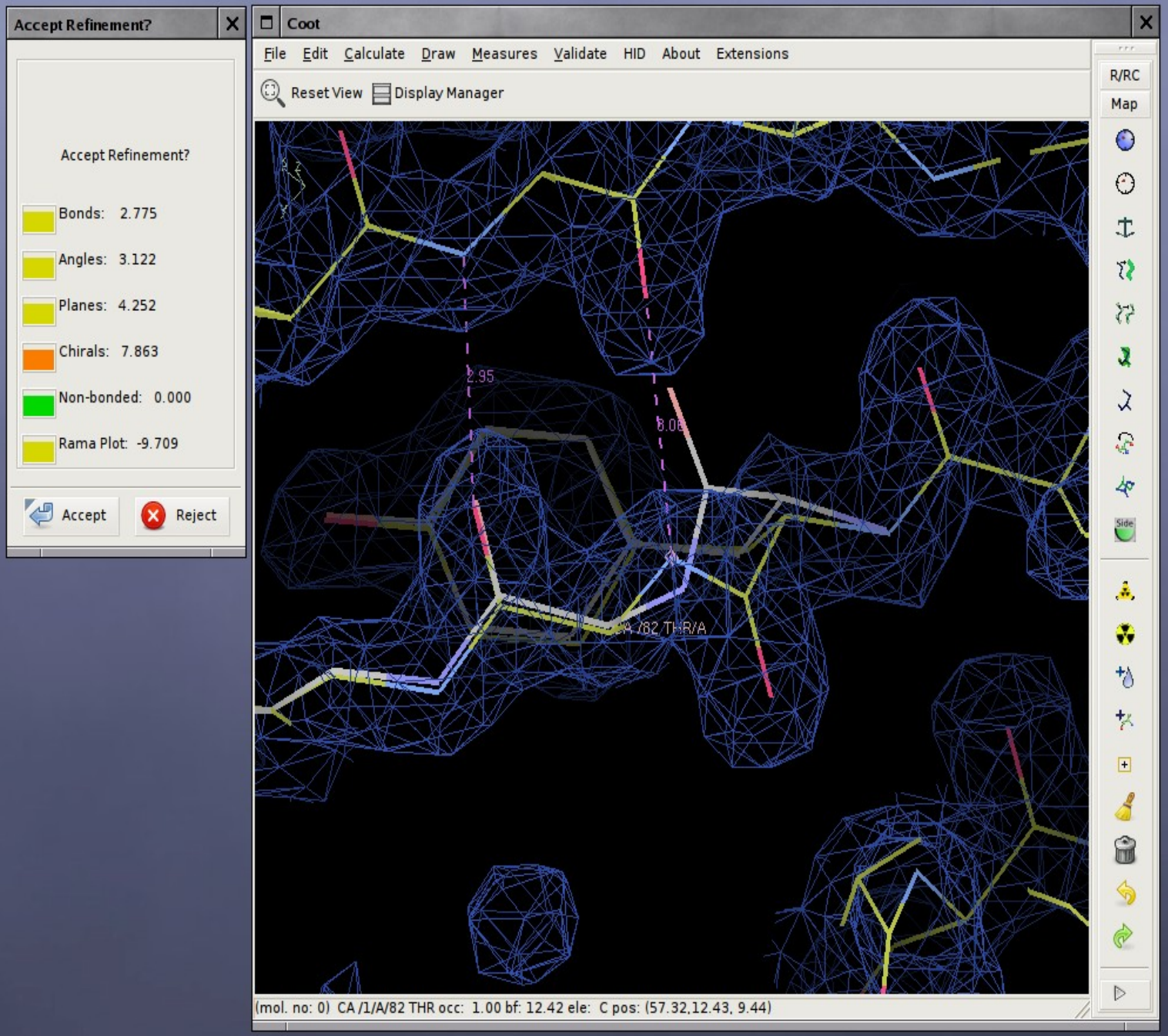
Tweaking a Ramachandran Outlier

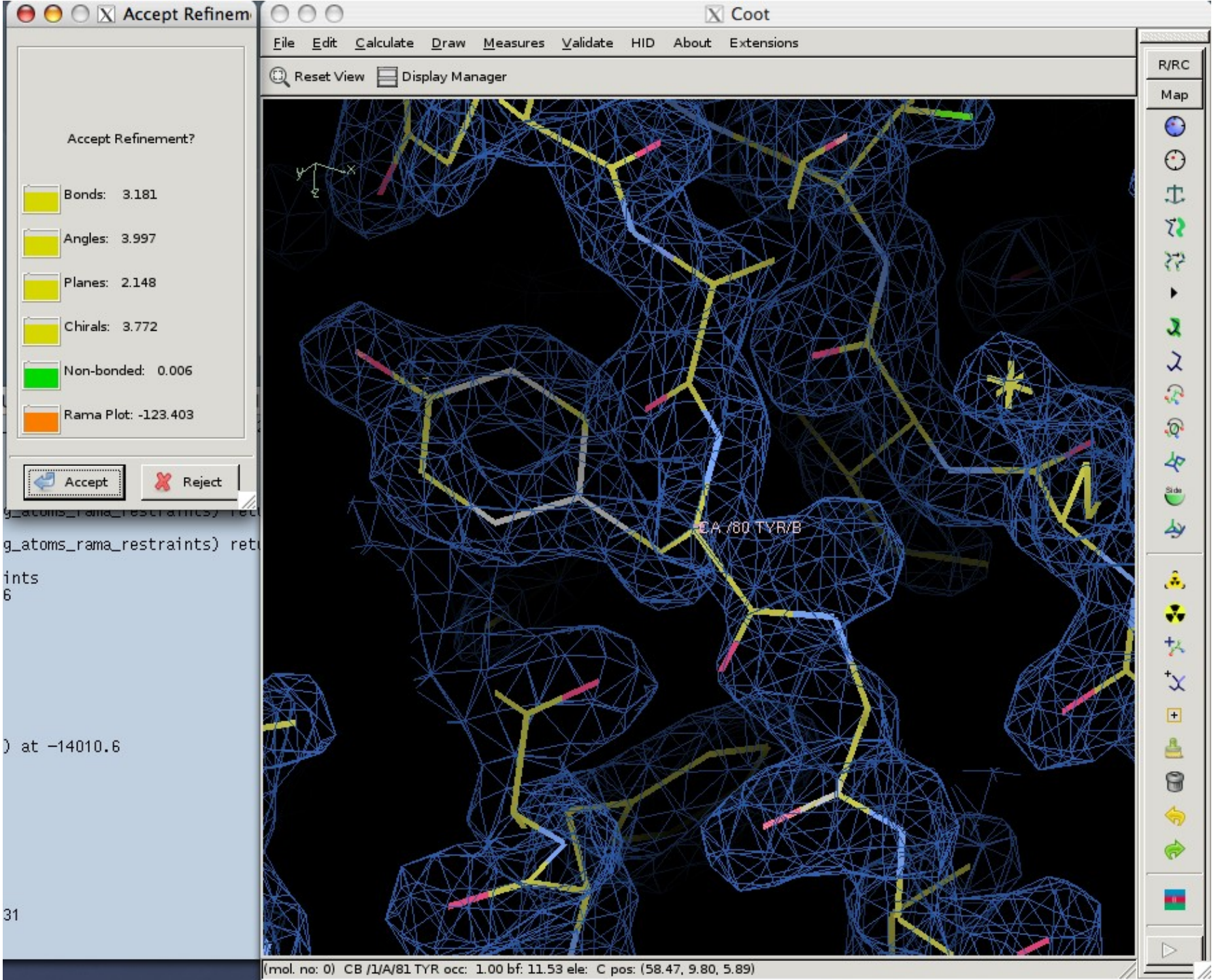


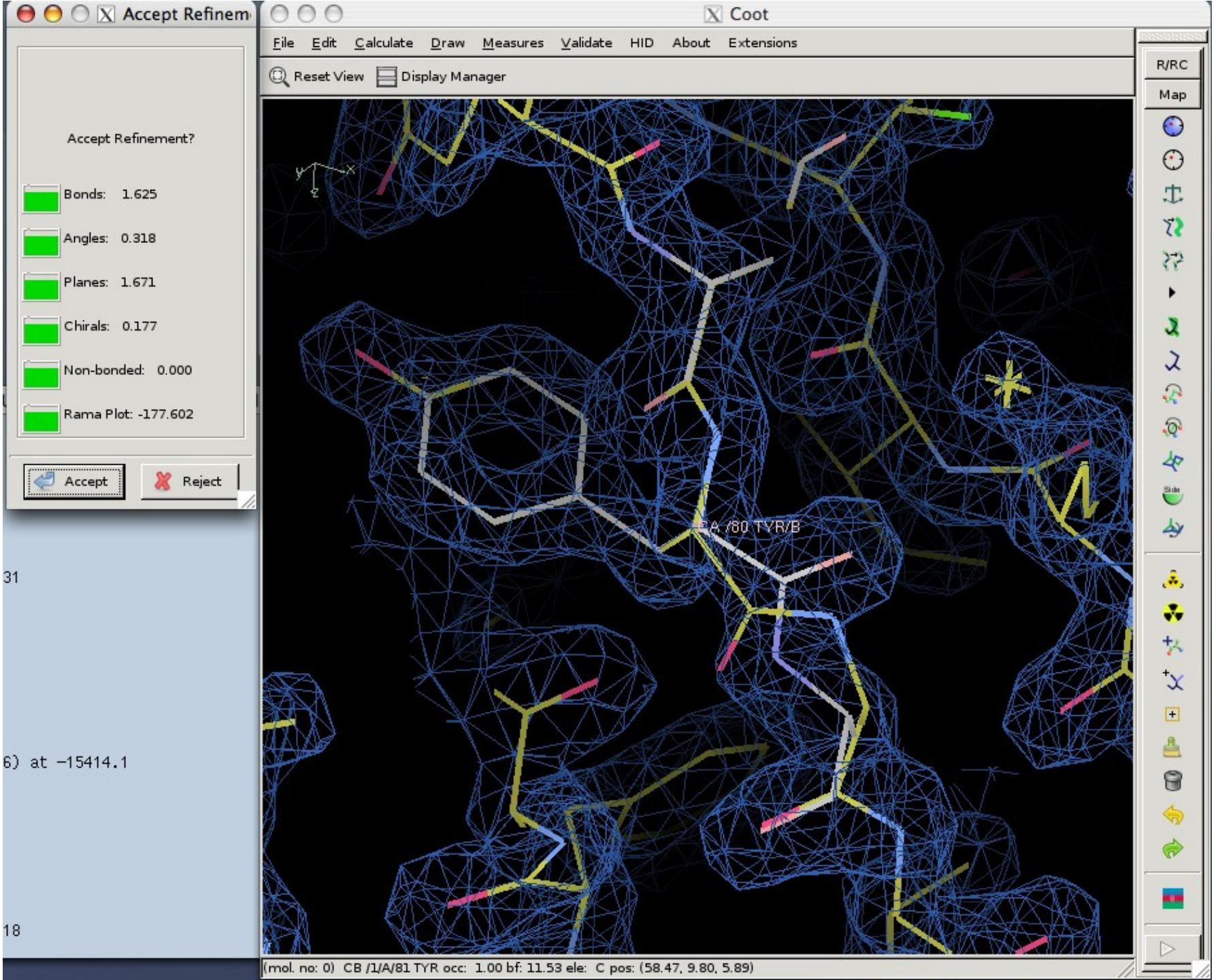


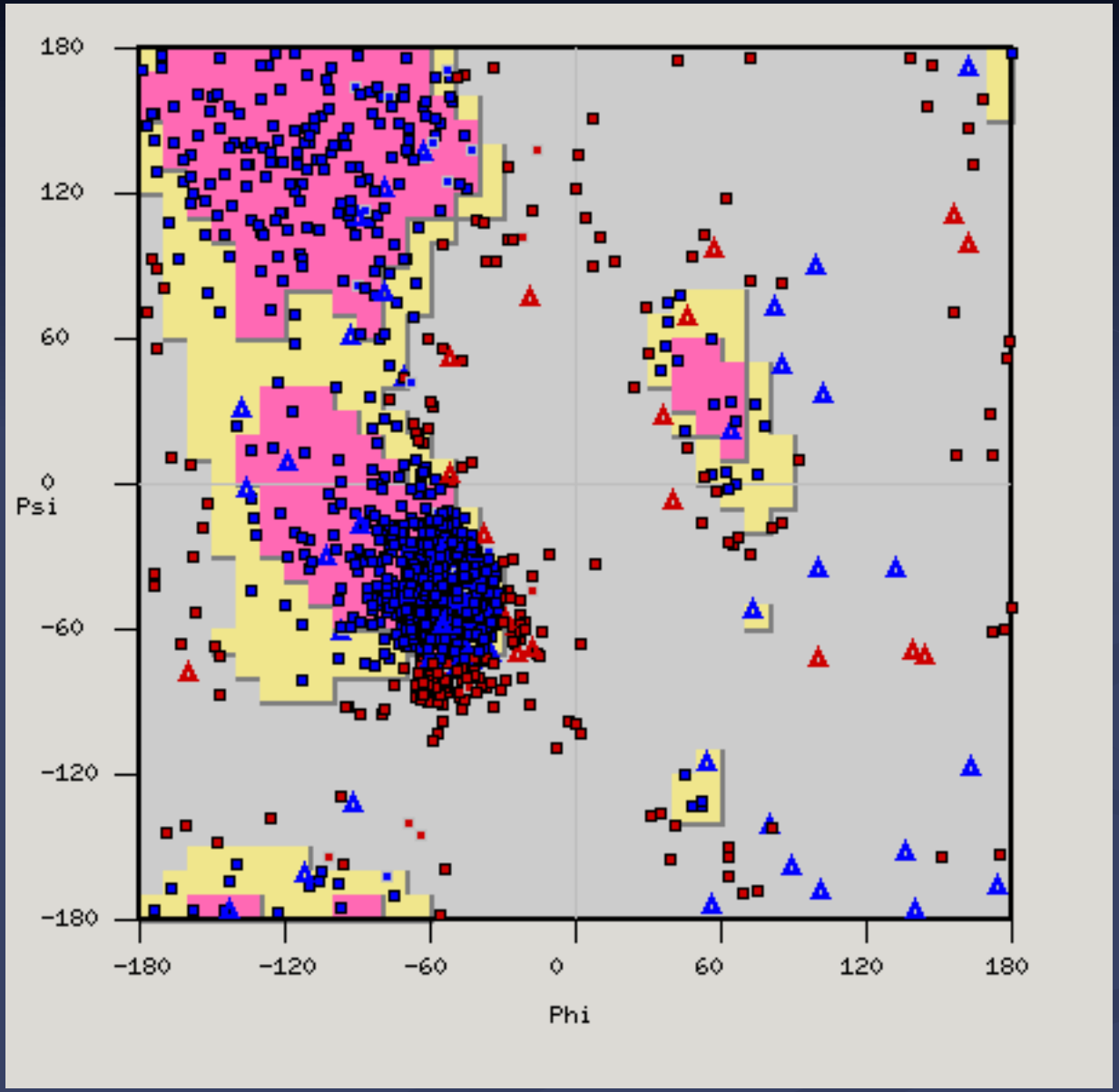
Tweaking Phi and Psi

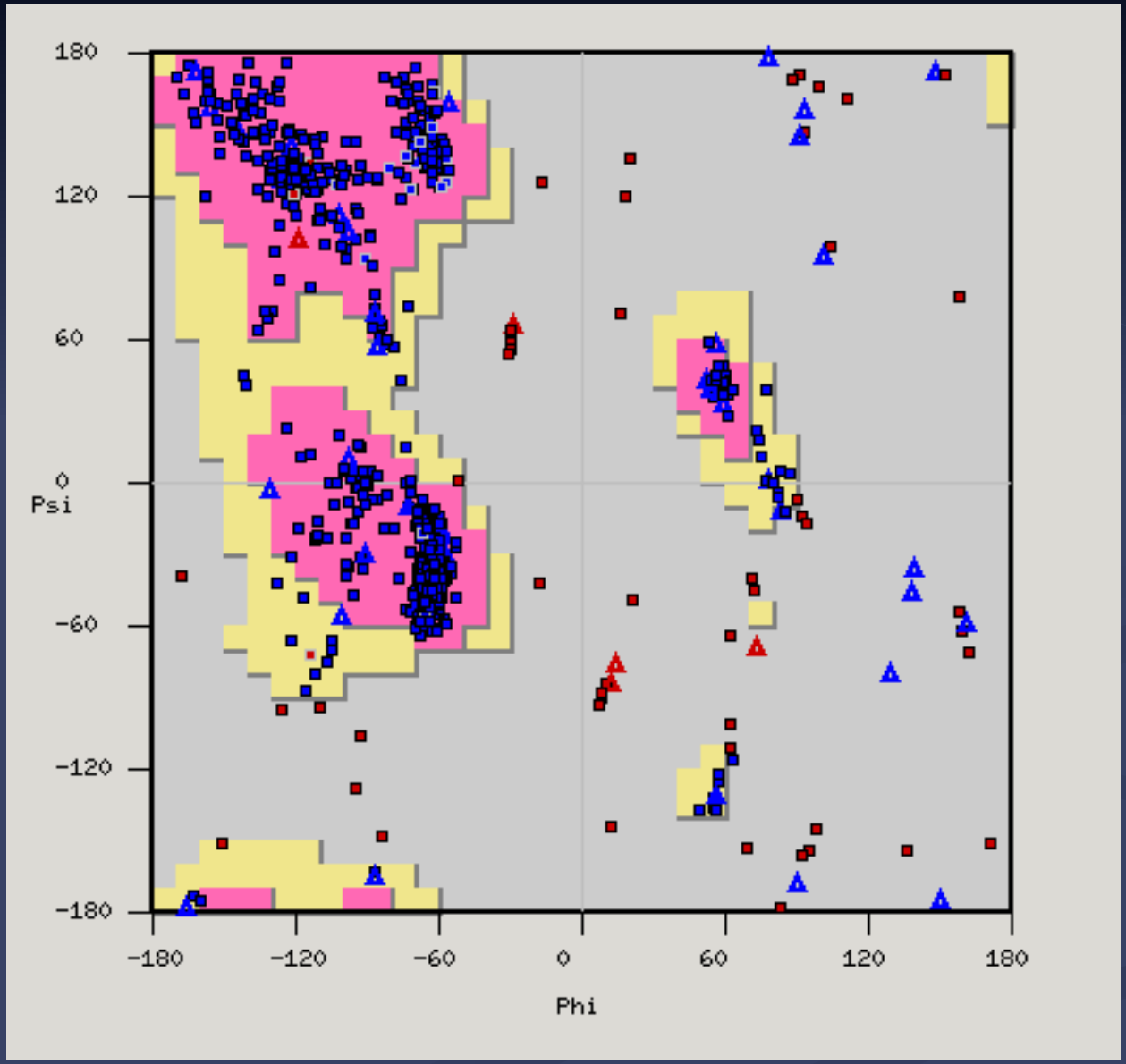



Accept Refinement?
Accept Refinement?
Bonds: 2.775
Angles: 3.122
Planes: 4.252
Chirals: 7.863
Non-bonded: 0.000
Rama Plot: -9.709
Accept
Reject
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
Side
2.95
6.08
A /82 THR/A
(mol. no: 0) CA /1/A/82 THR occ: 1.00 bf: 12.42 ele: C pos: (57.32,12.43, 9.44)


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
A /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)


Accept Refinem
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
Accept Refinement?
Bonds: 1.625
Angles: 0.318
Planes: 1.671
Chirals: 0.177
Non-bonded: 0.000
Rama Plot: -177.602
Accept
Reject
A /80 TYR/B
31
6) at −15414.1
18
(mol. no: 0) CB /1/A/81 TYR occ: 1.00 bf: 11.53 ele: C pos: (58.47, 9.80, 5.89)


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

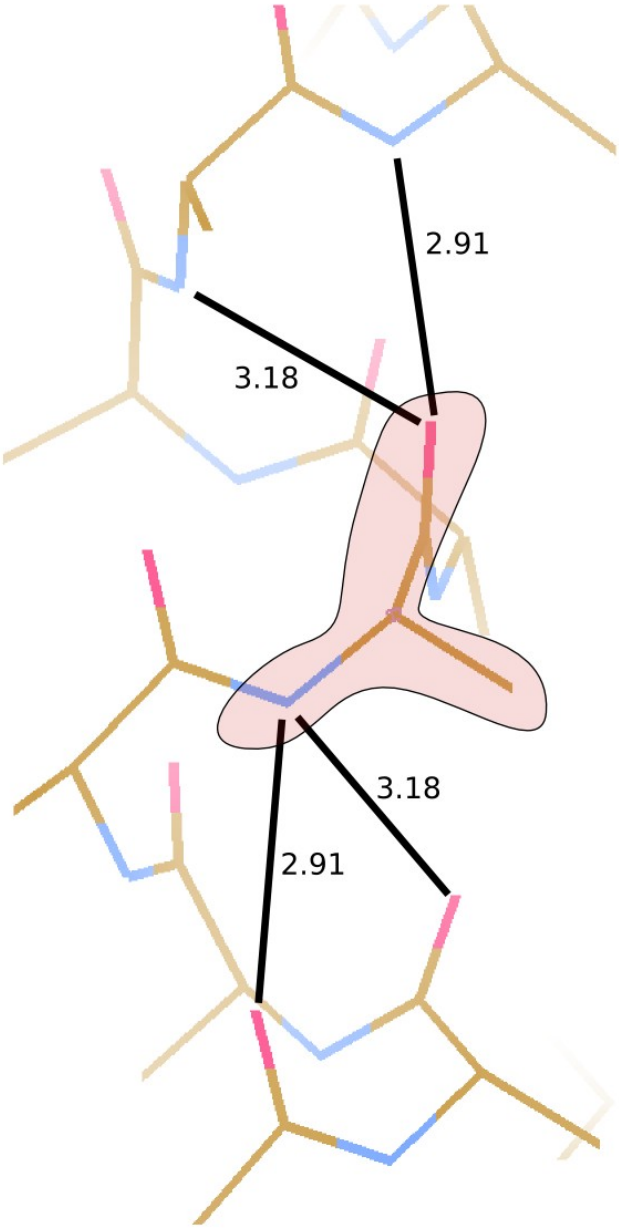
- Controversial?
  - "... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model..."
    - Gerard Kleywegt & Alwyn Jones (1996)
- But to quote Jane Richardson:
  - Do you want a better structure – or a better idea of the quality of your structure?

Adding Torsion Angle Restraints

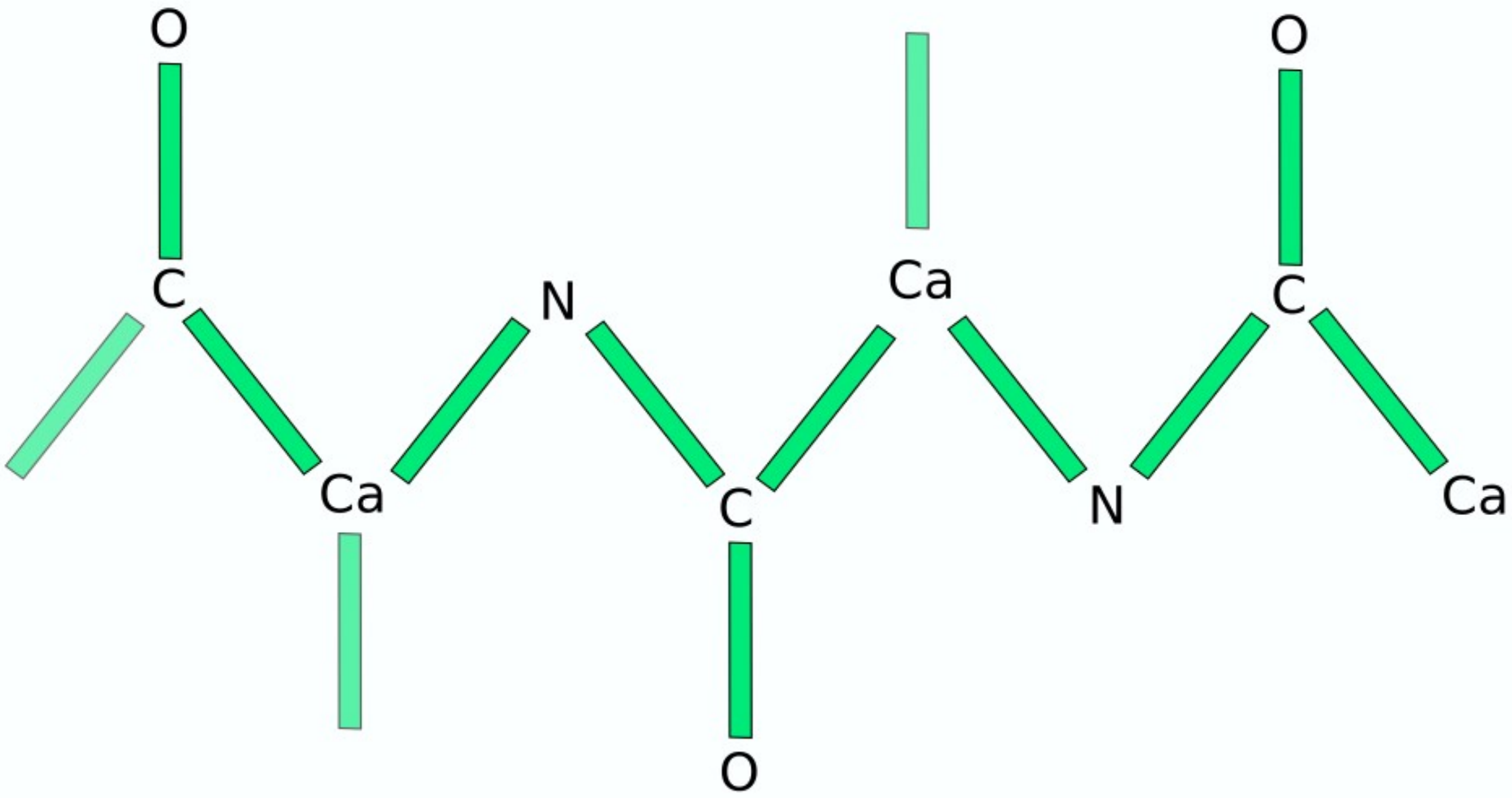
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region "stuck and unsatisfied"
    - i.e. trapped in Local Minimum

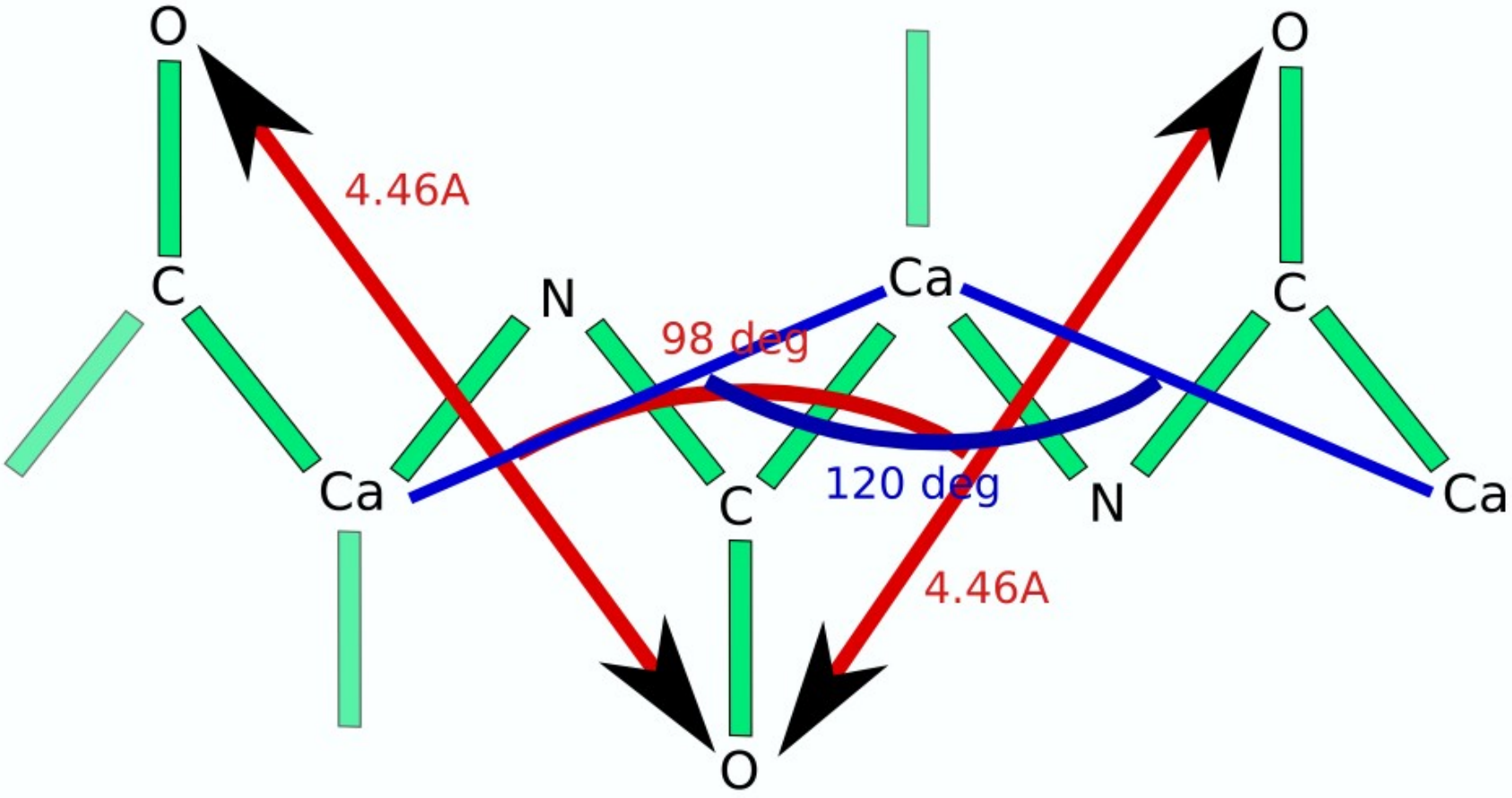
- Add Pseudo-bonds (c.f. Brigcogne & Buster)

Alpha Helix pseudo-bond restraints



Restrain the Hydrogen-bonding atom distances

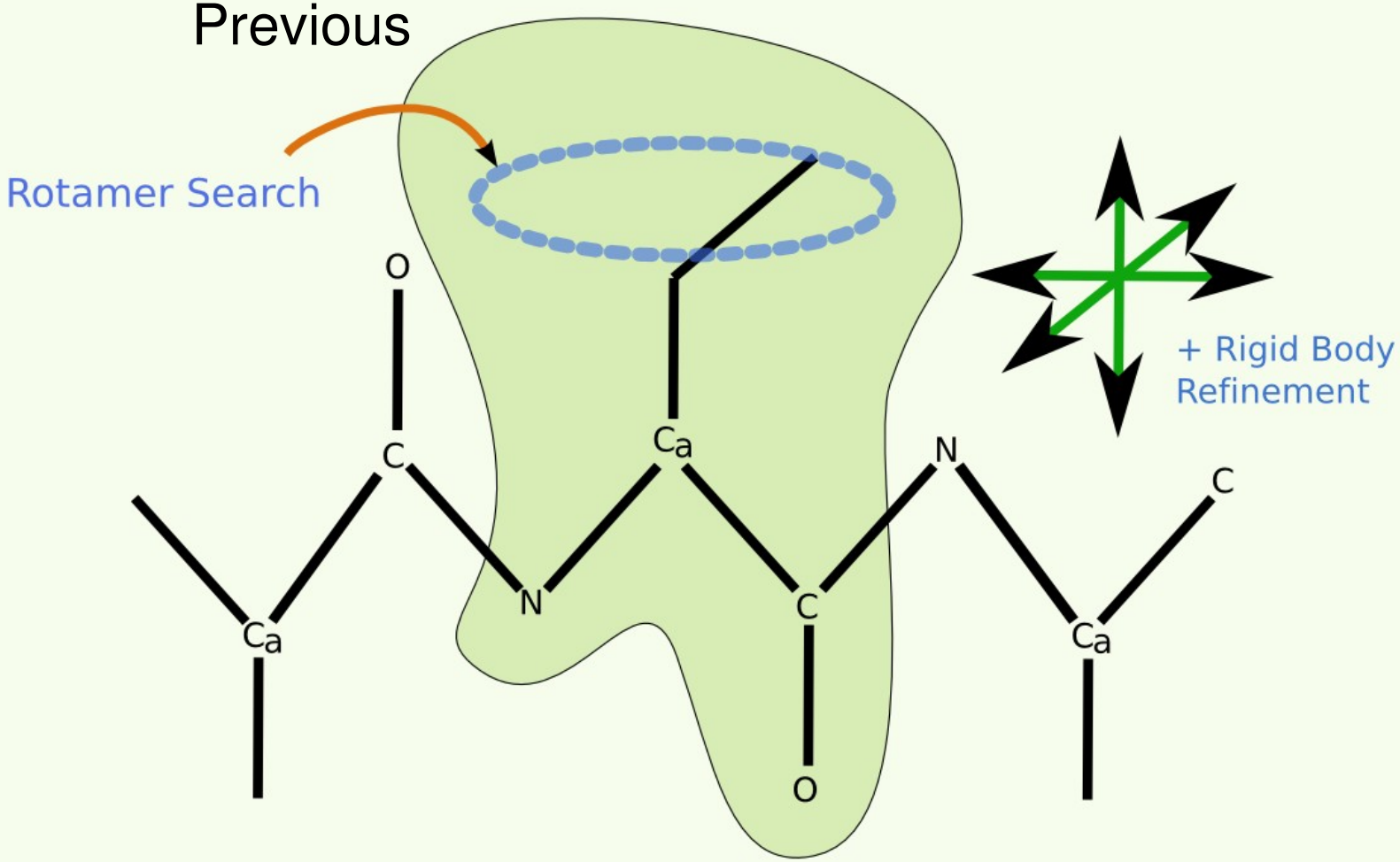

O
C
Ca
N
Ca
C
O
O
C
Ca
N
Ca

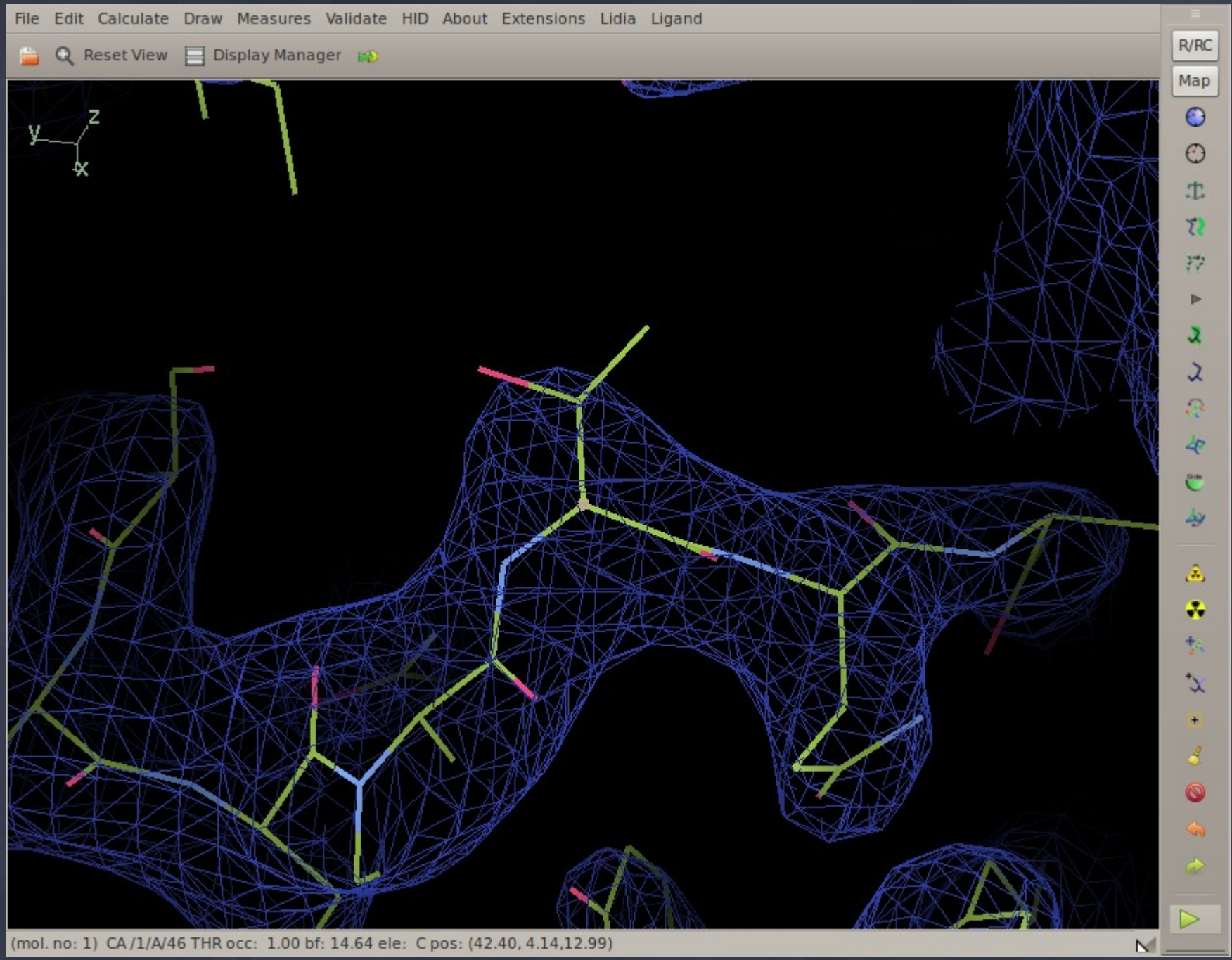

O
4.46A
C
N
Ca
O
C
98 deg
Ca
C
120 deg
N
Ca
4.46A
O

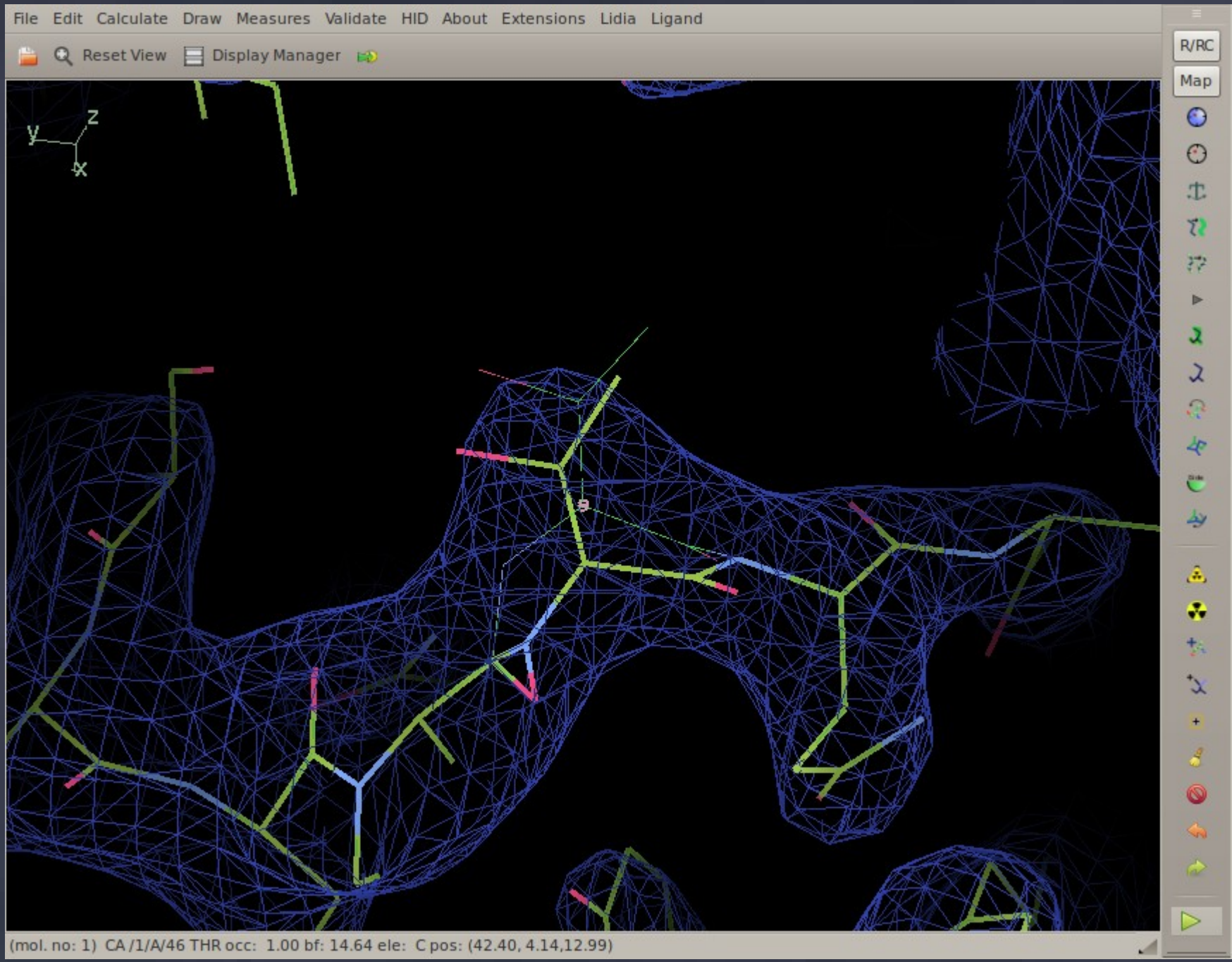
“Backrub” Rotamers

~~Current~~ Low Resolution Rotamer Search

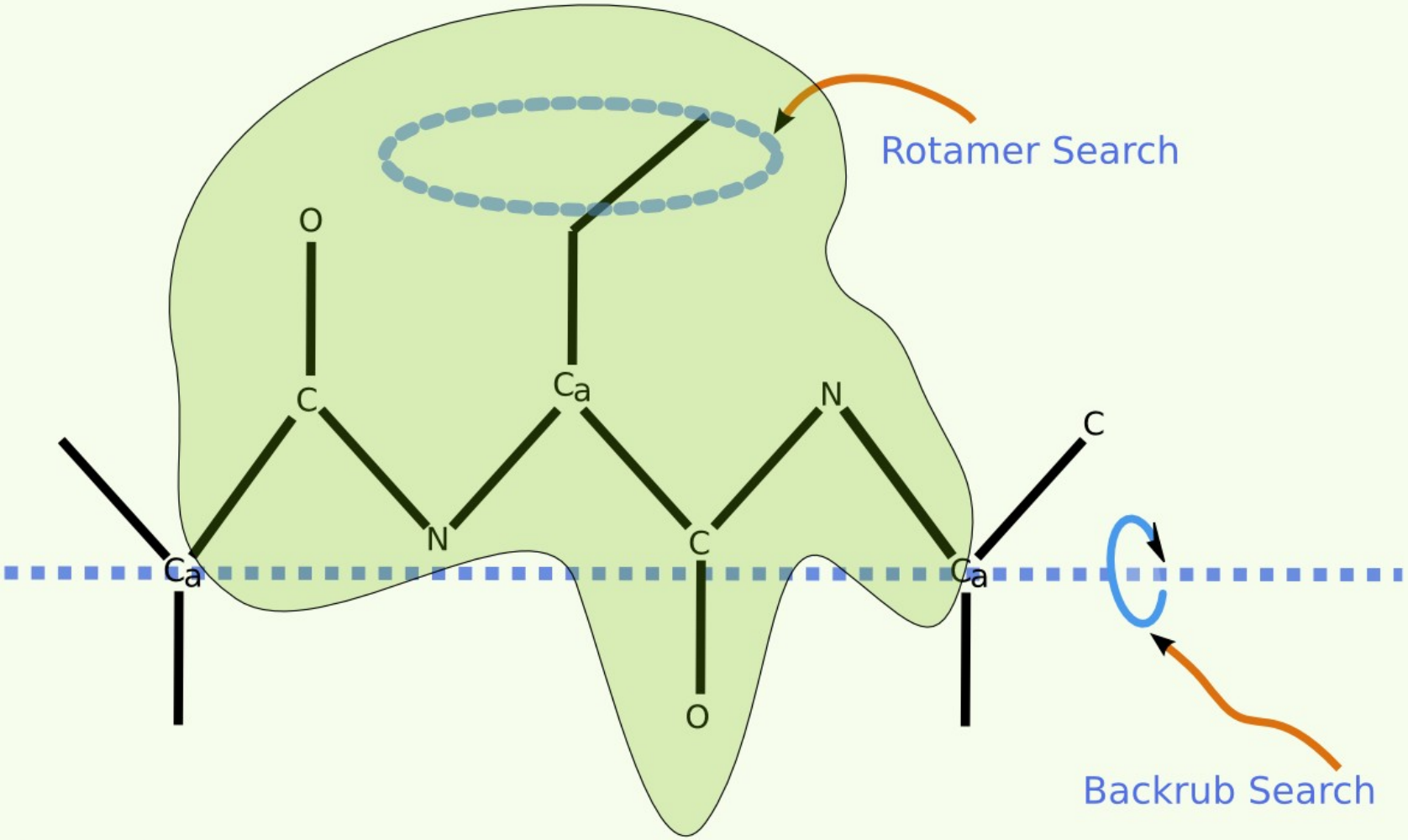
Previous



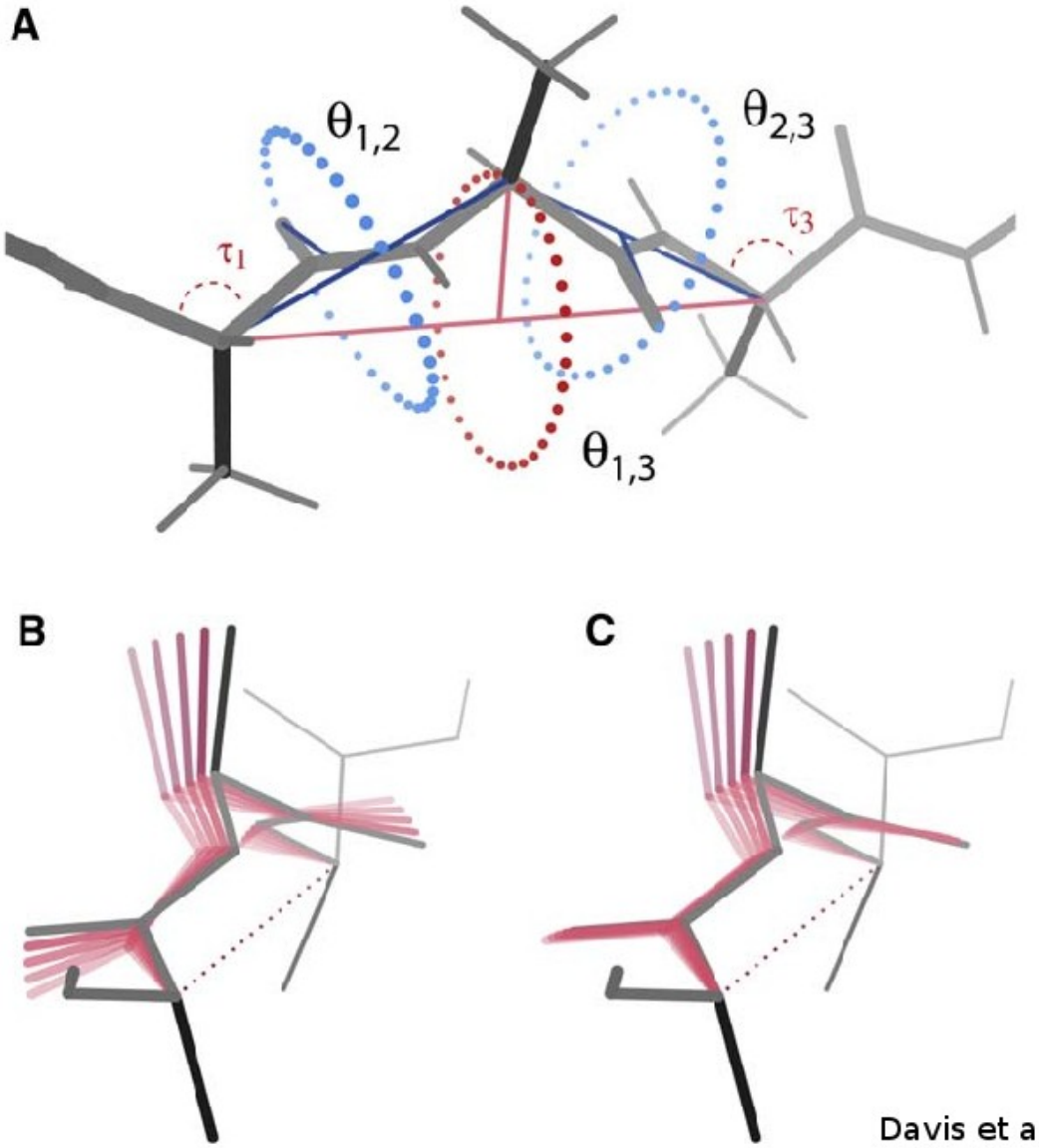

File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
Display Manager
R/RC
Map
Z
Y
X
(mol. no: 1) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)


File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
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)

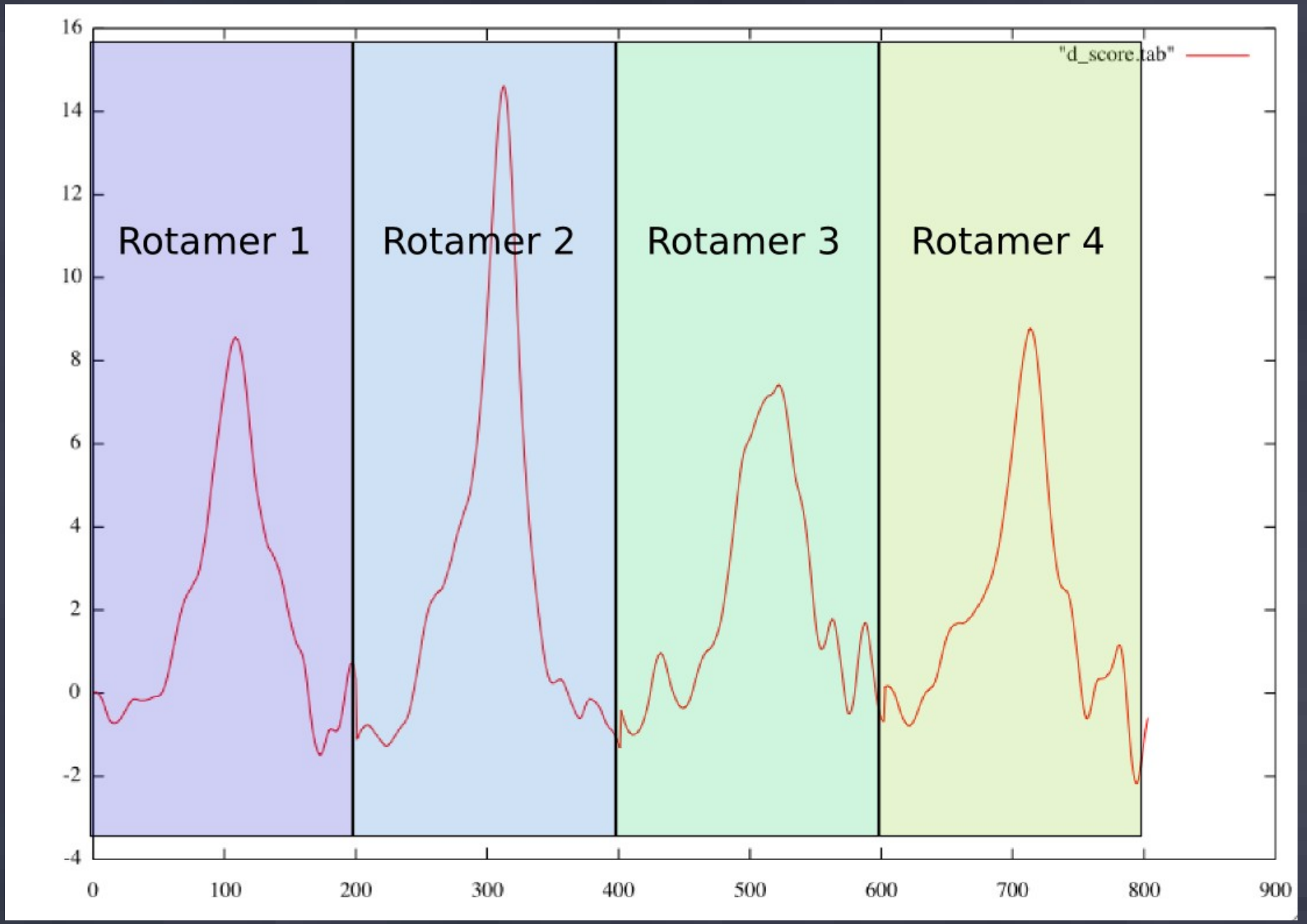
New Low Resolution Rotamer Search

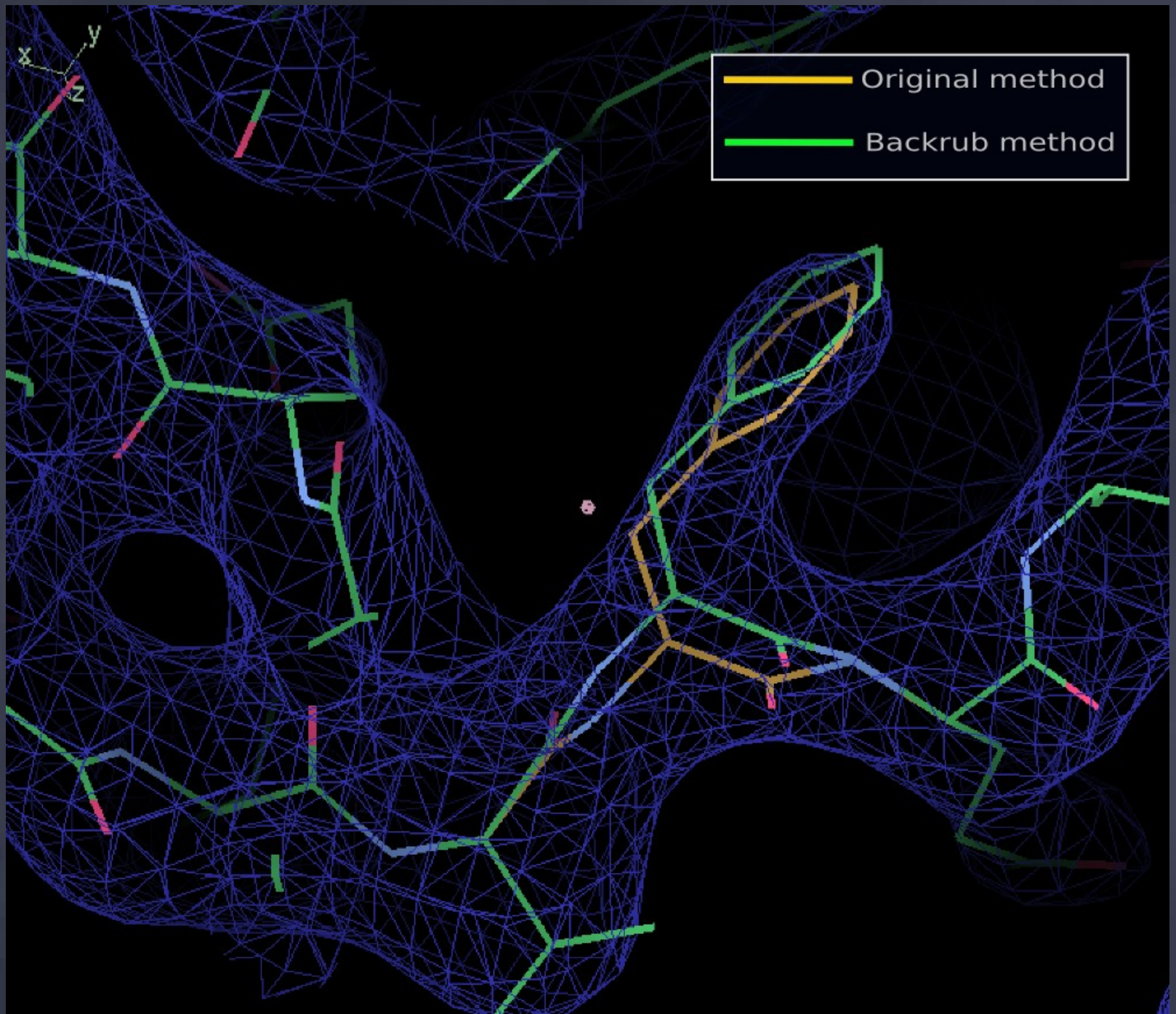


After Fitting Tools in KING/Molprobity



Davis et al. (2006) Structure

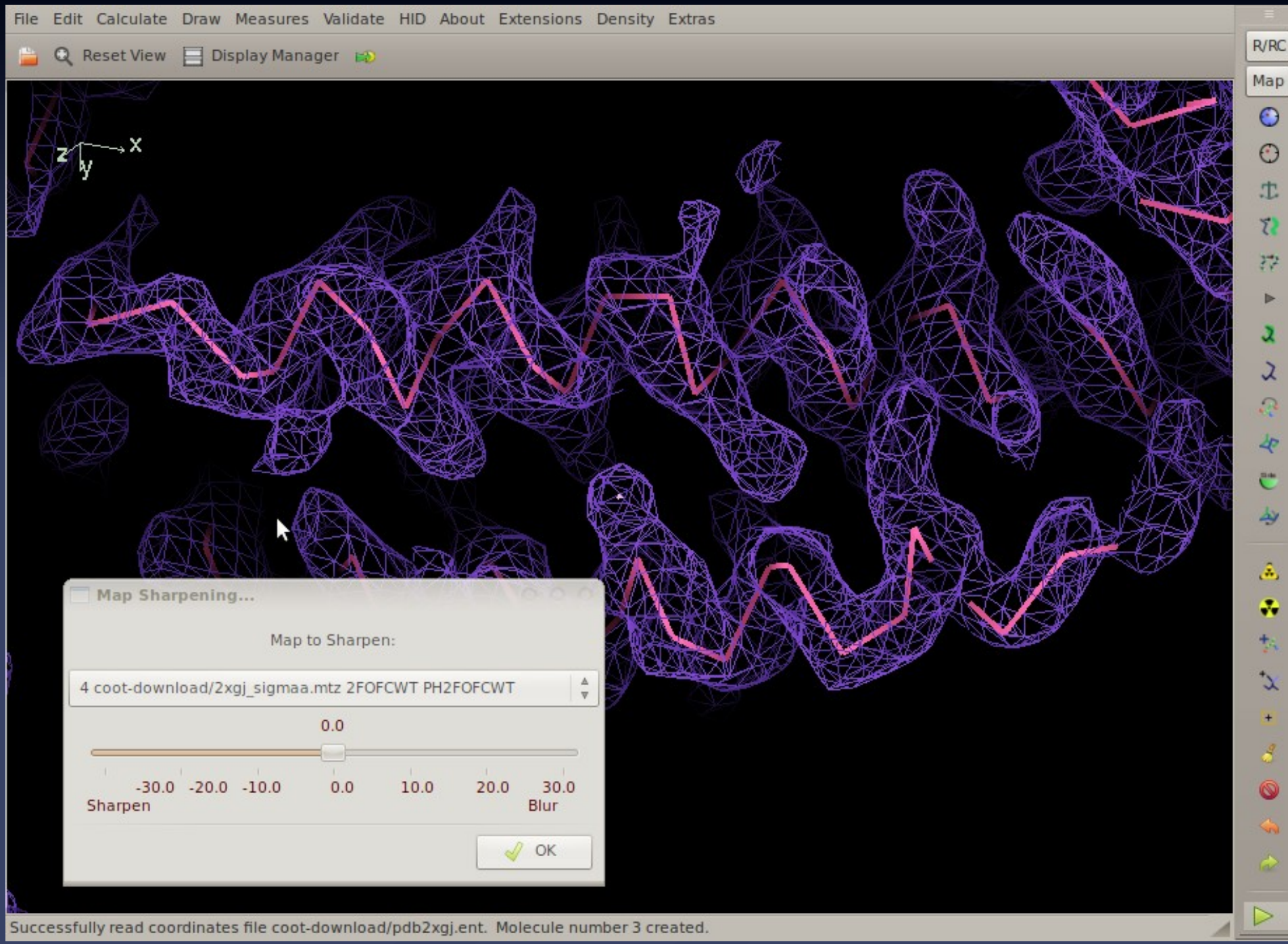

"d_score.tab"
Rotamer 1
Rotamer 2
Rotamer 3
Rotamer 4
16
14
12
10
8
6
4
2
0
-2
-4
0
100
200
300
400
500
600
700
800
900

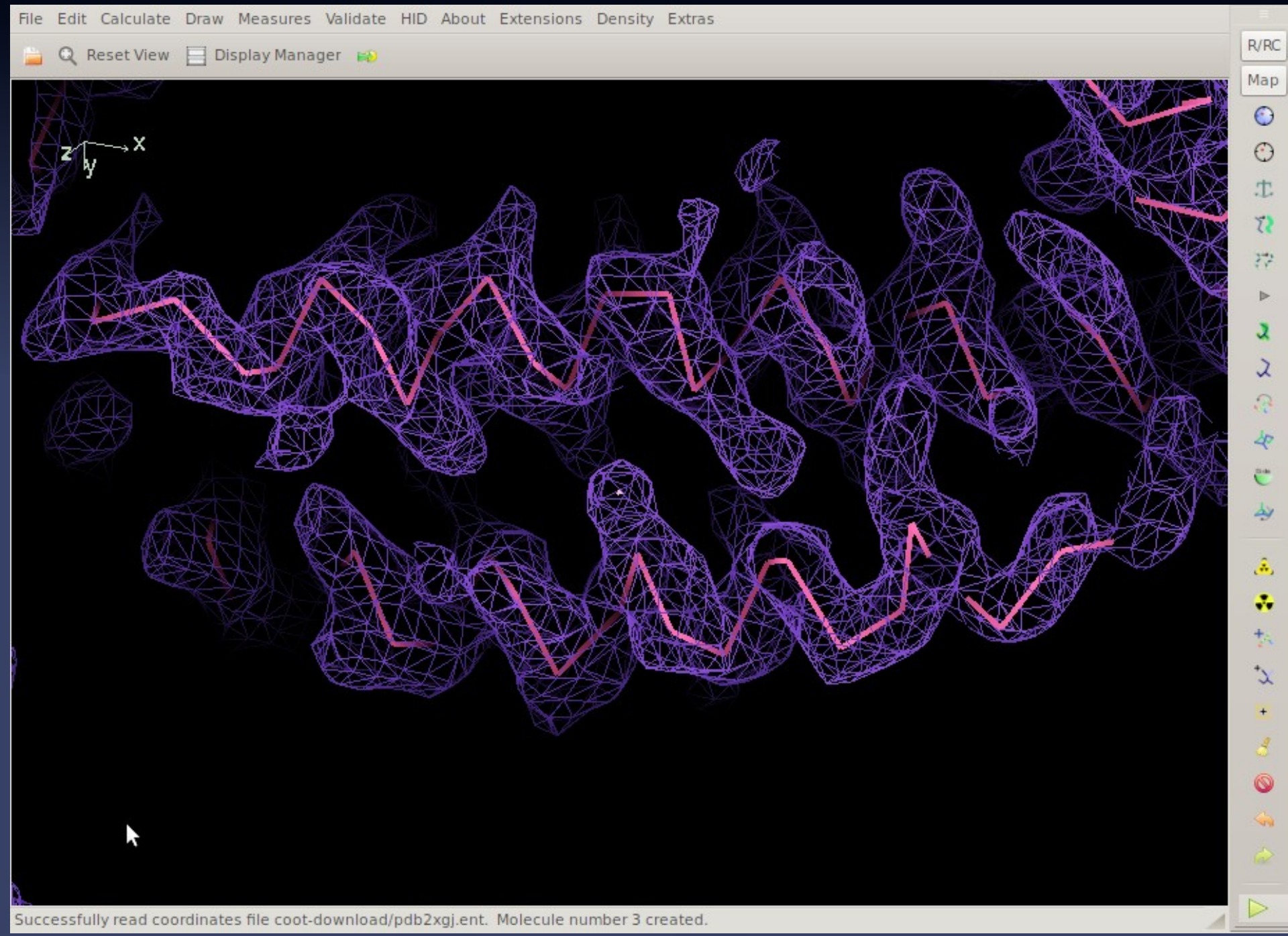

y
x
z
Original method
Backrub method

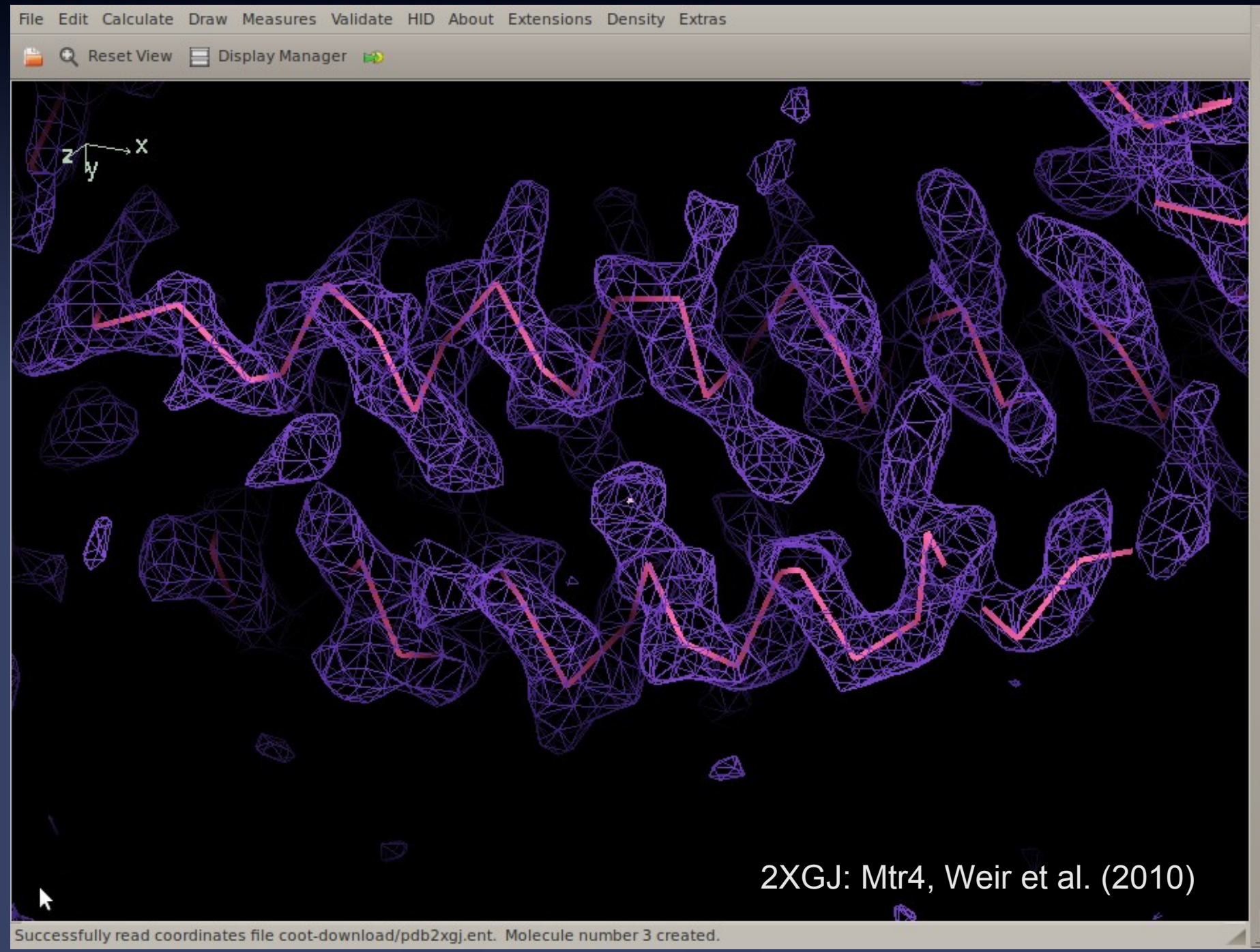
Sharpening

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

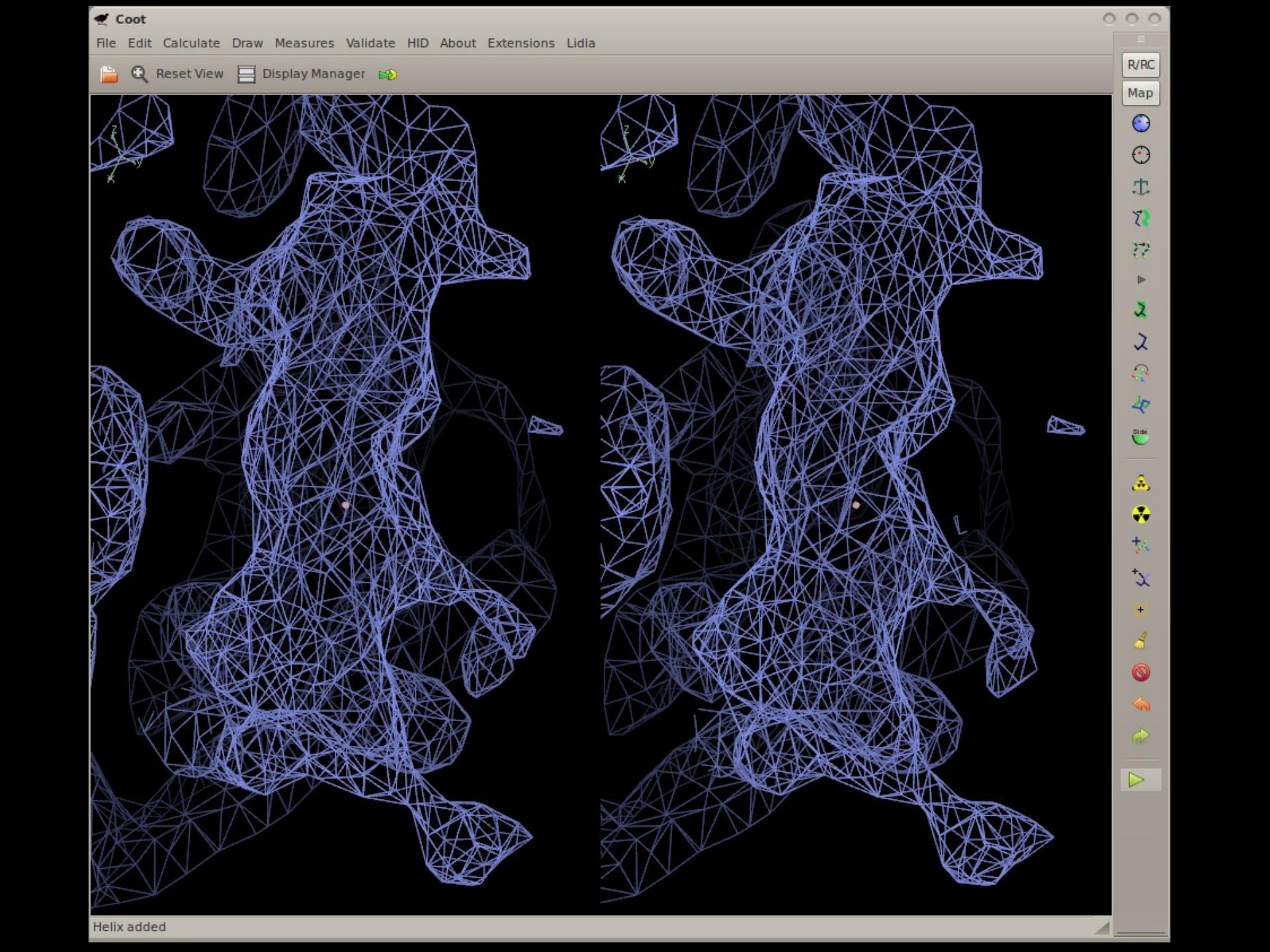
Interactively adjust the structure factor amplitudes and re-generate the map with FFT and recontouring...


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

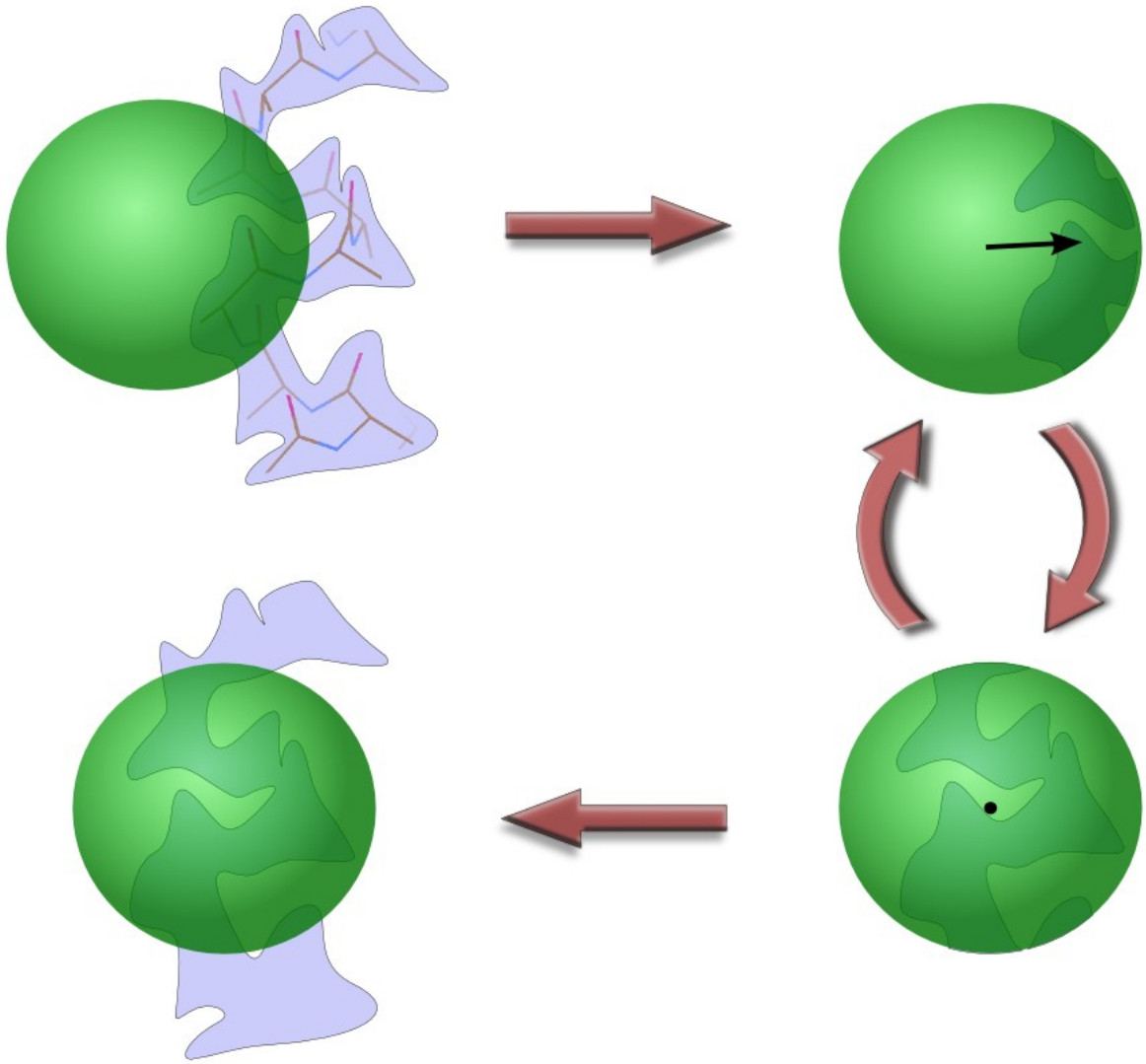
Helix-Building


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

Alpha Helix Placement

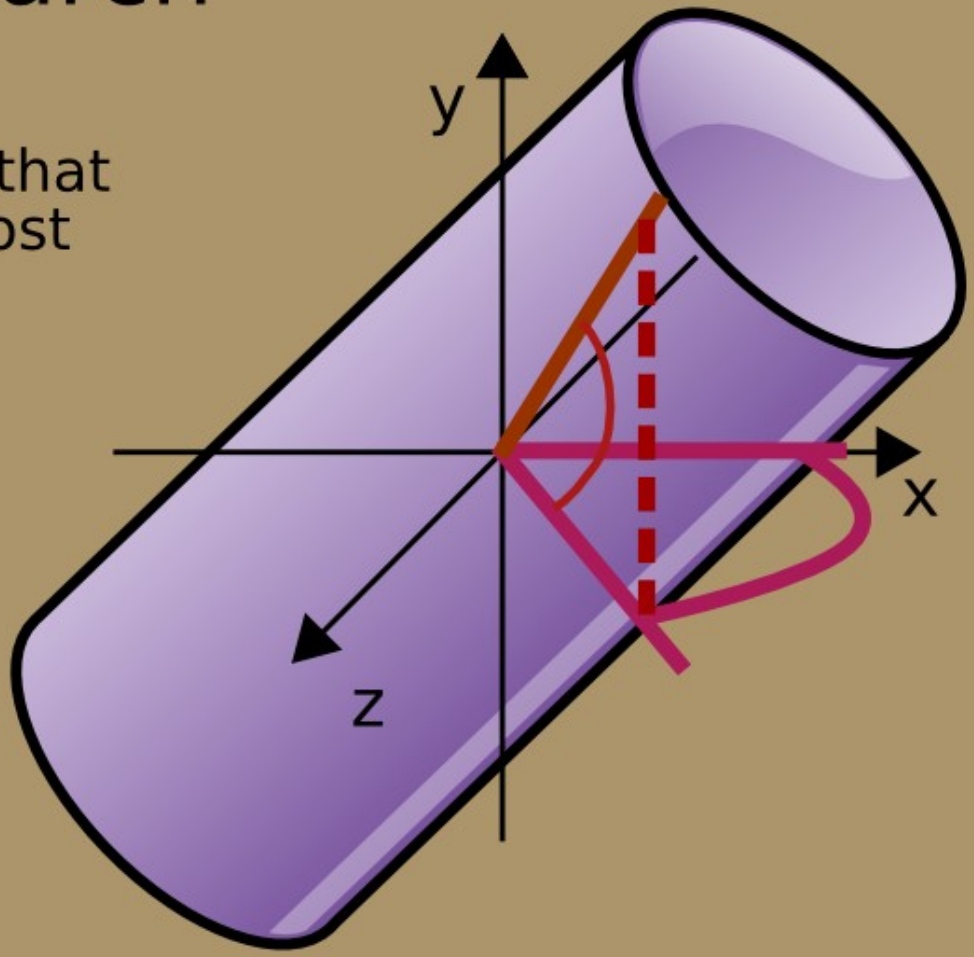
- Scenario: Looking at a new map, not built with automatic tools:
  - “I can see that there’s a helix here - build it for me!”
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

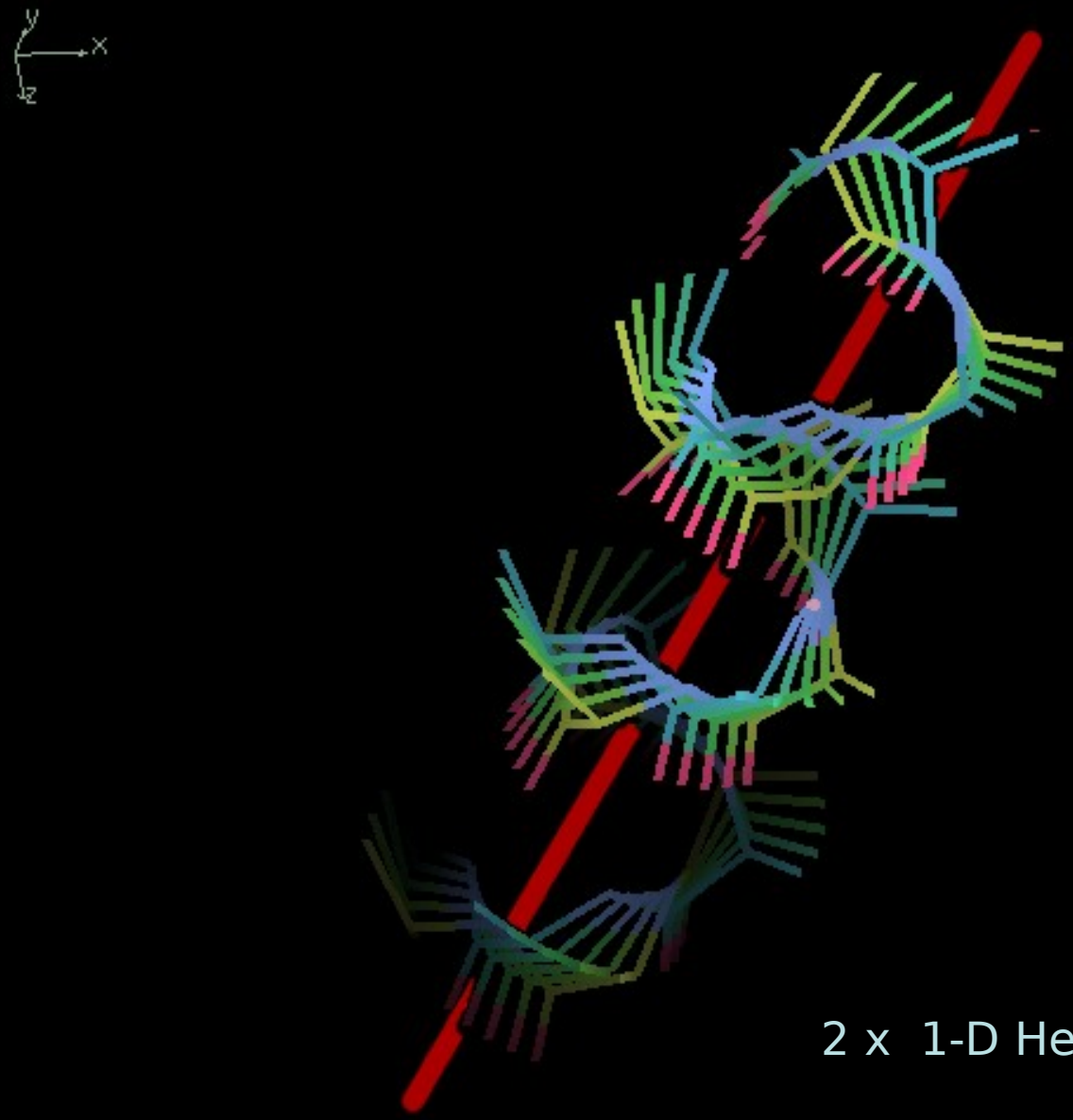


Cylinder Search

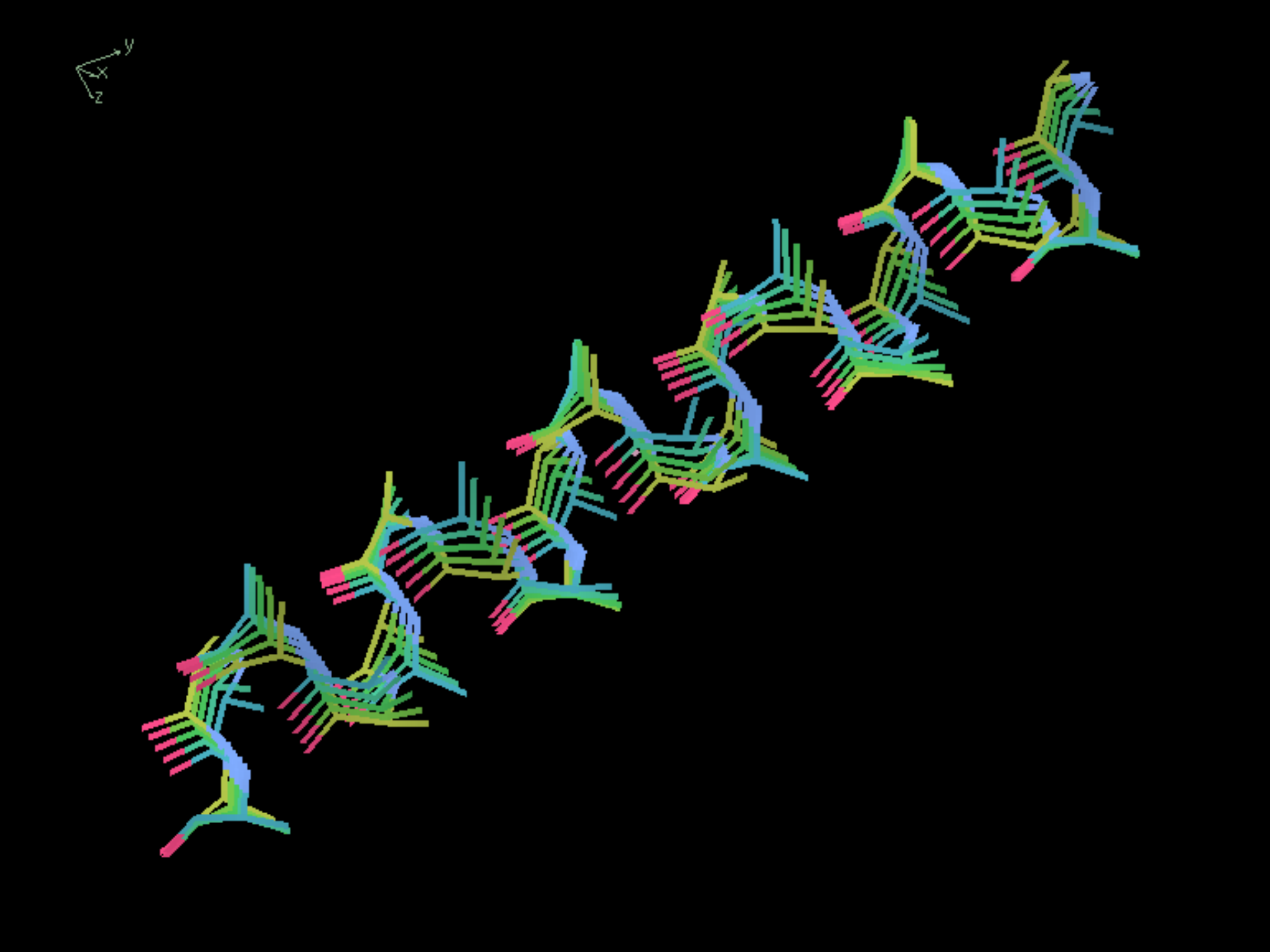
Pick the orientation that encapsulates the most electron density

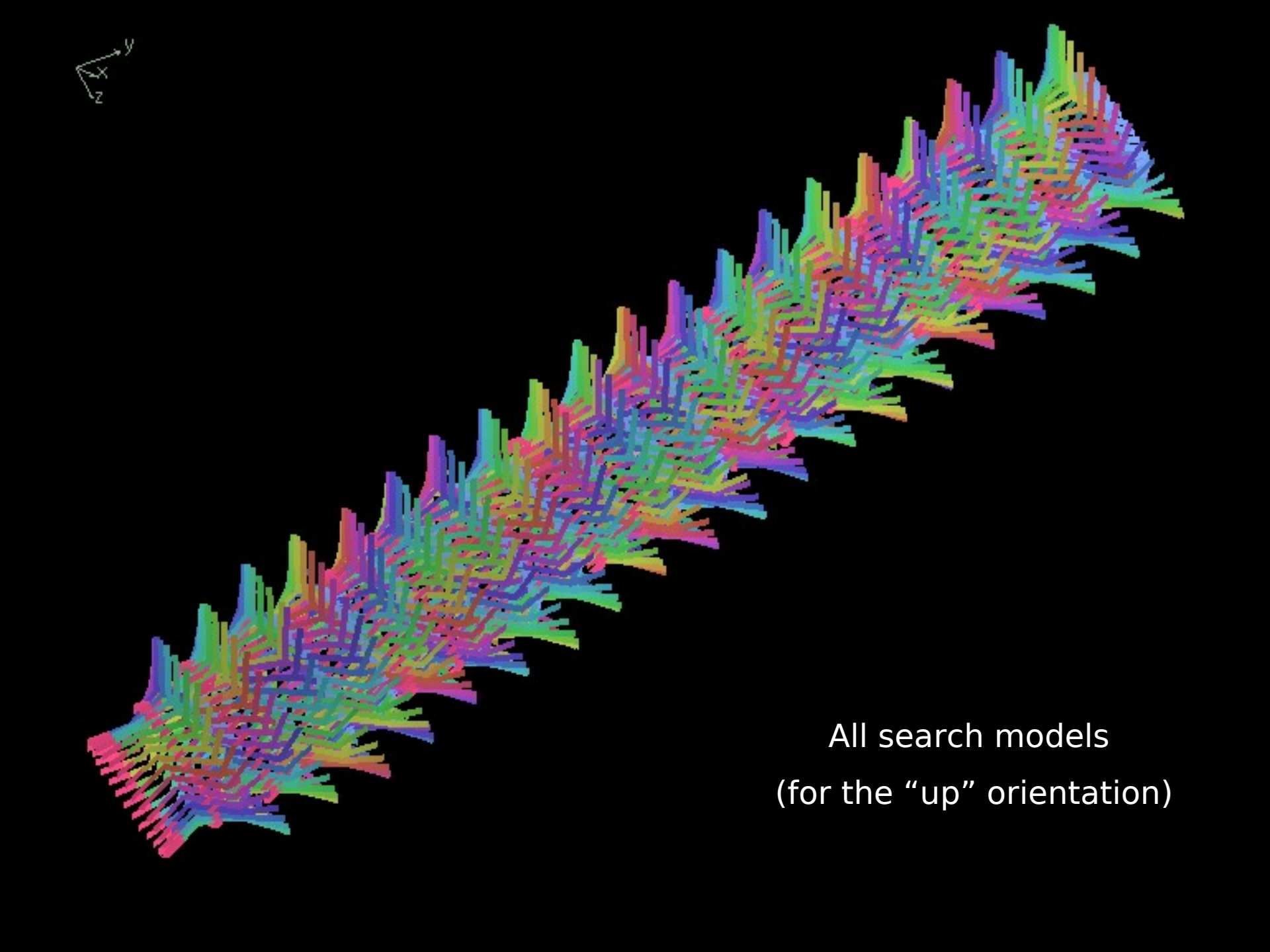


2 orientation axes



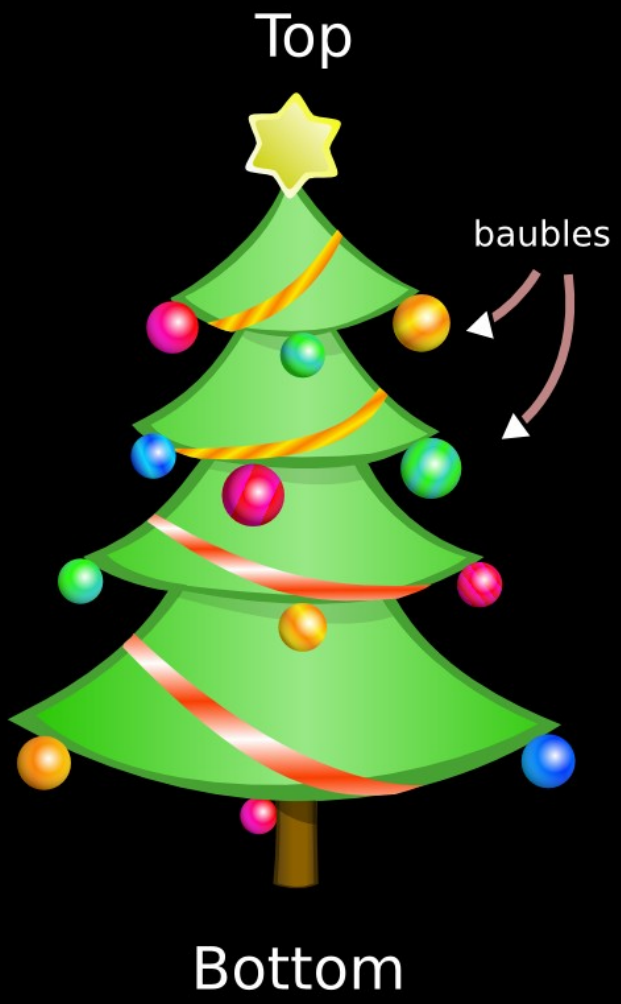
2 x 1-D Helix orientation searches

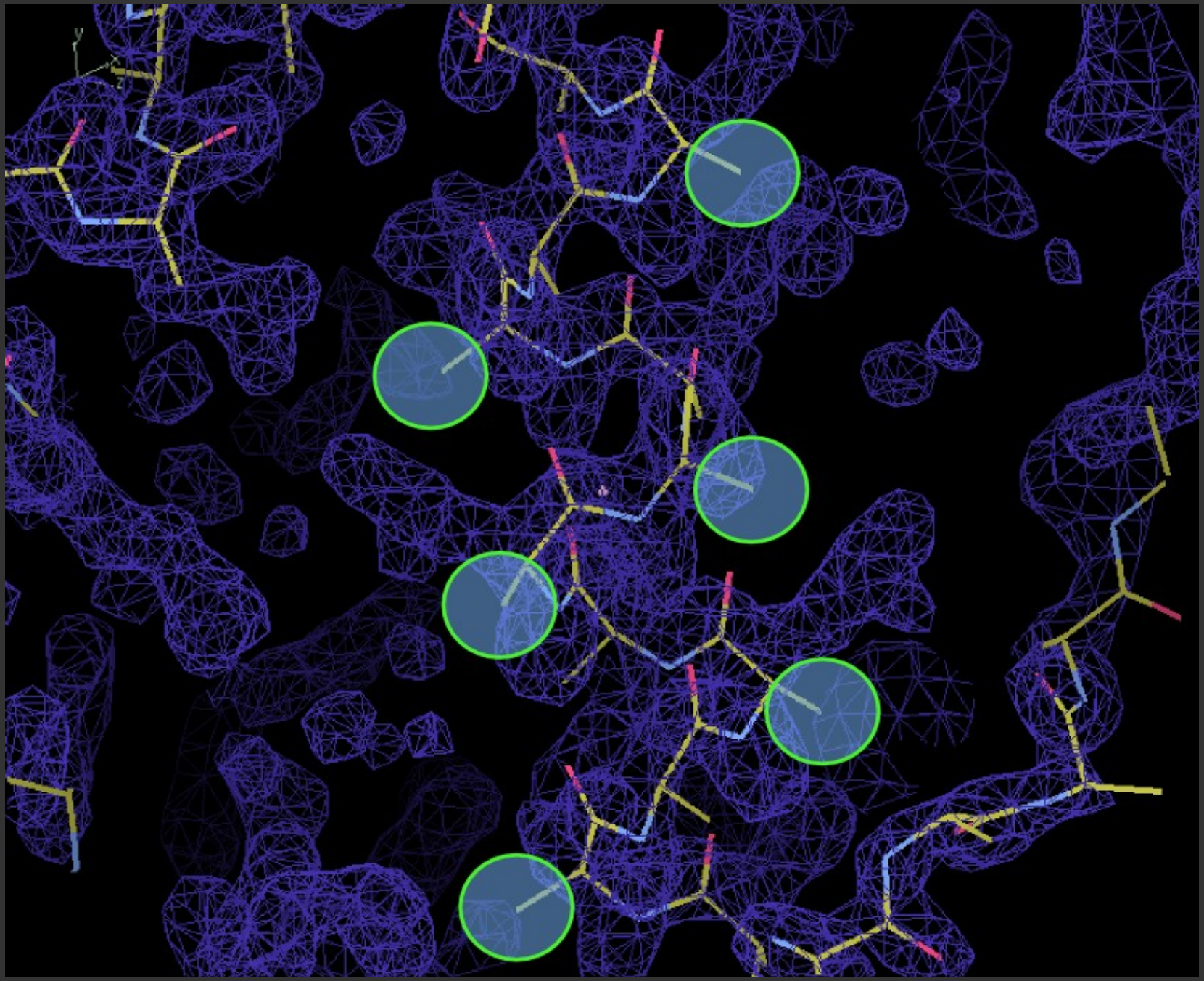

y
x
z

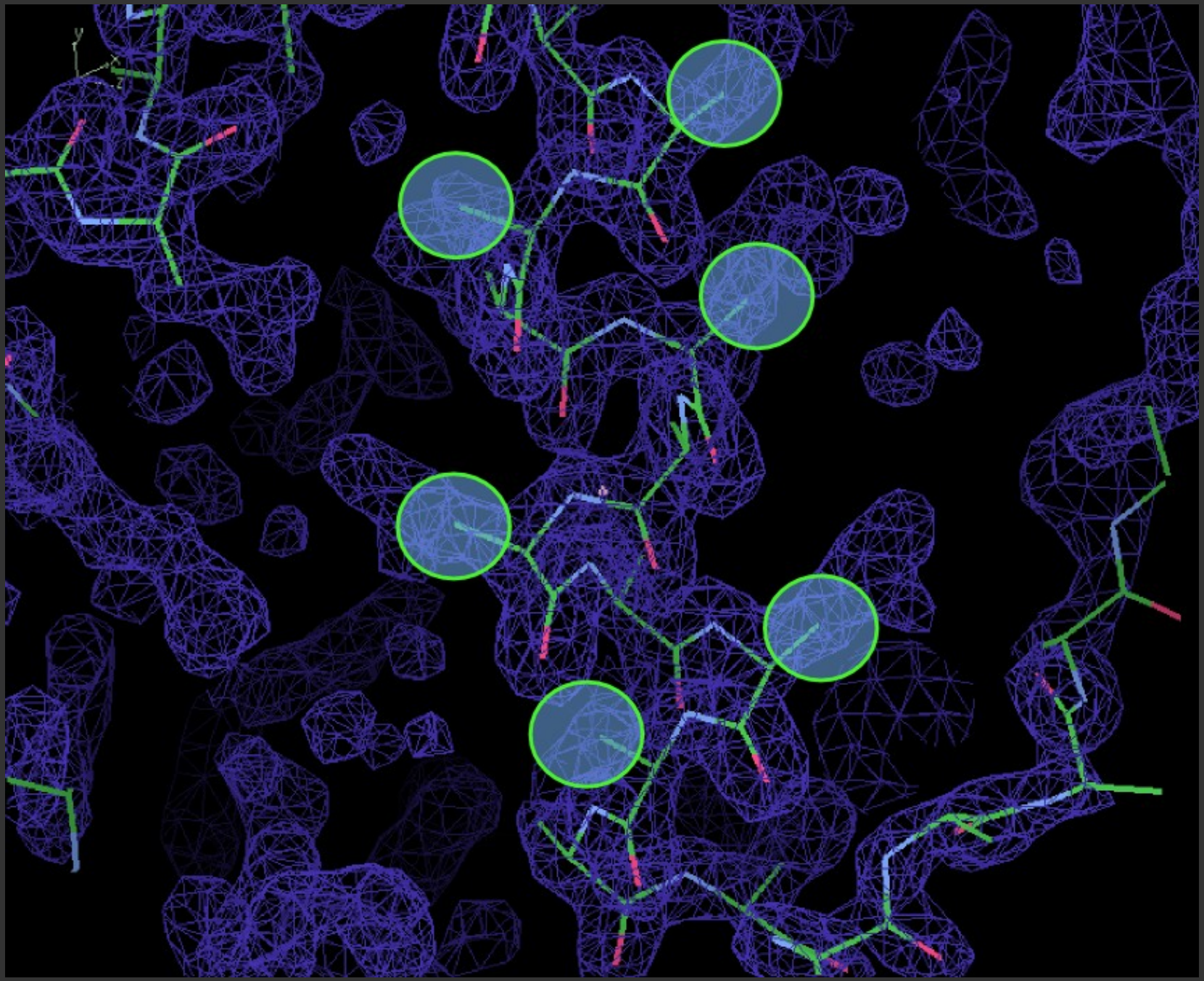


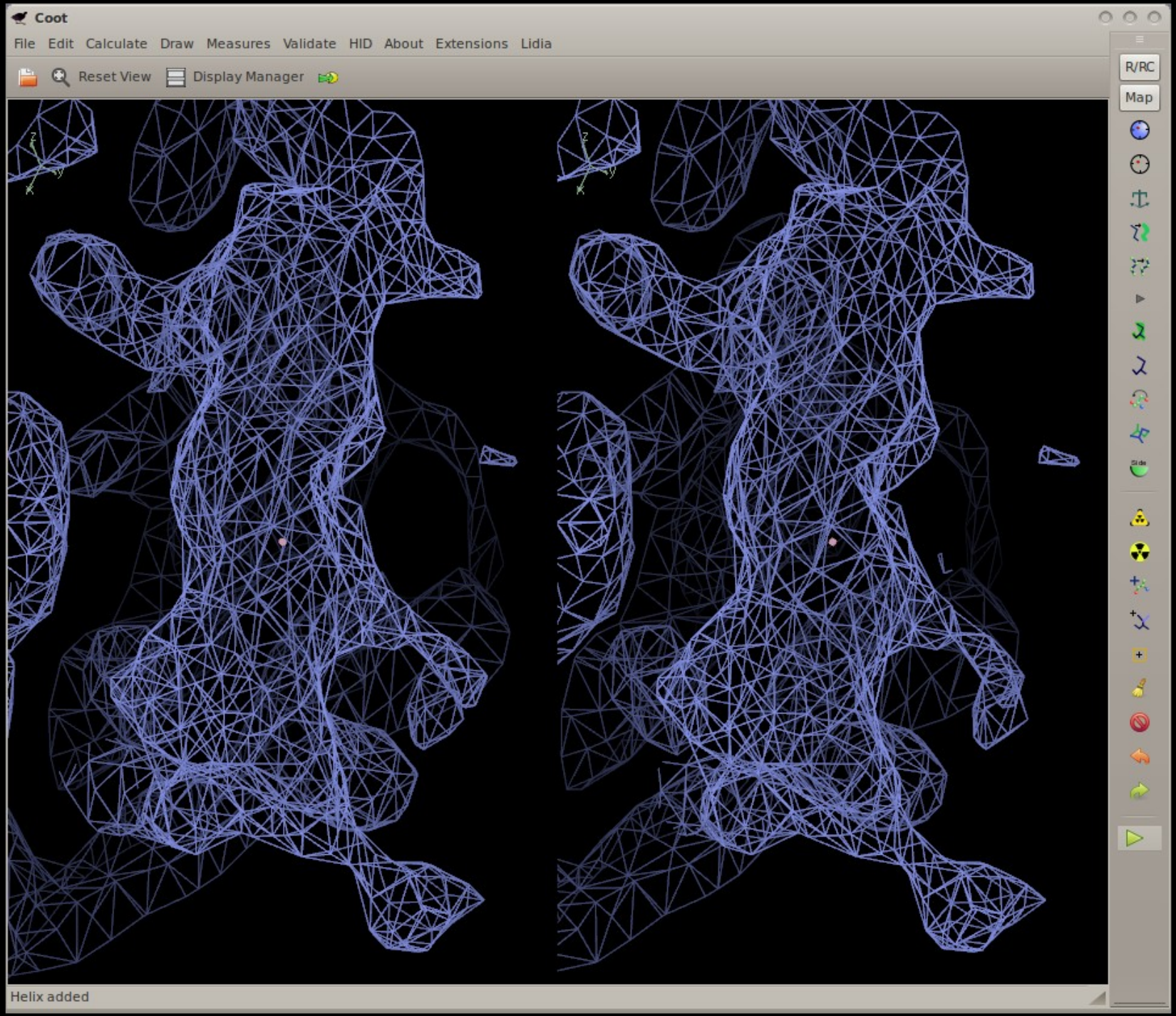
All search models

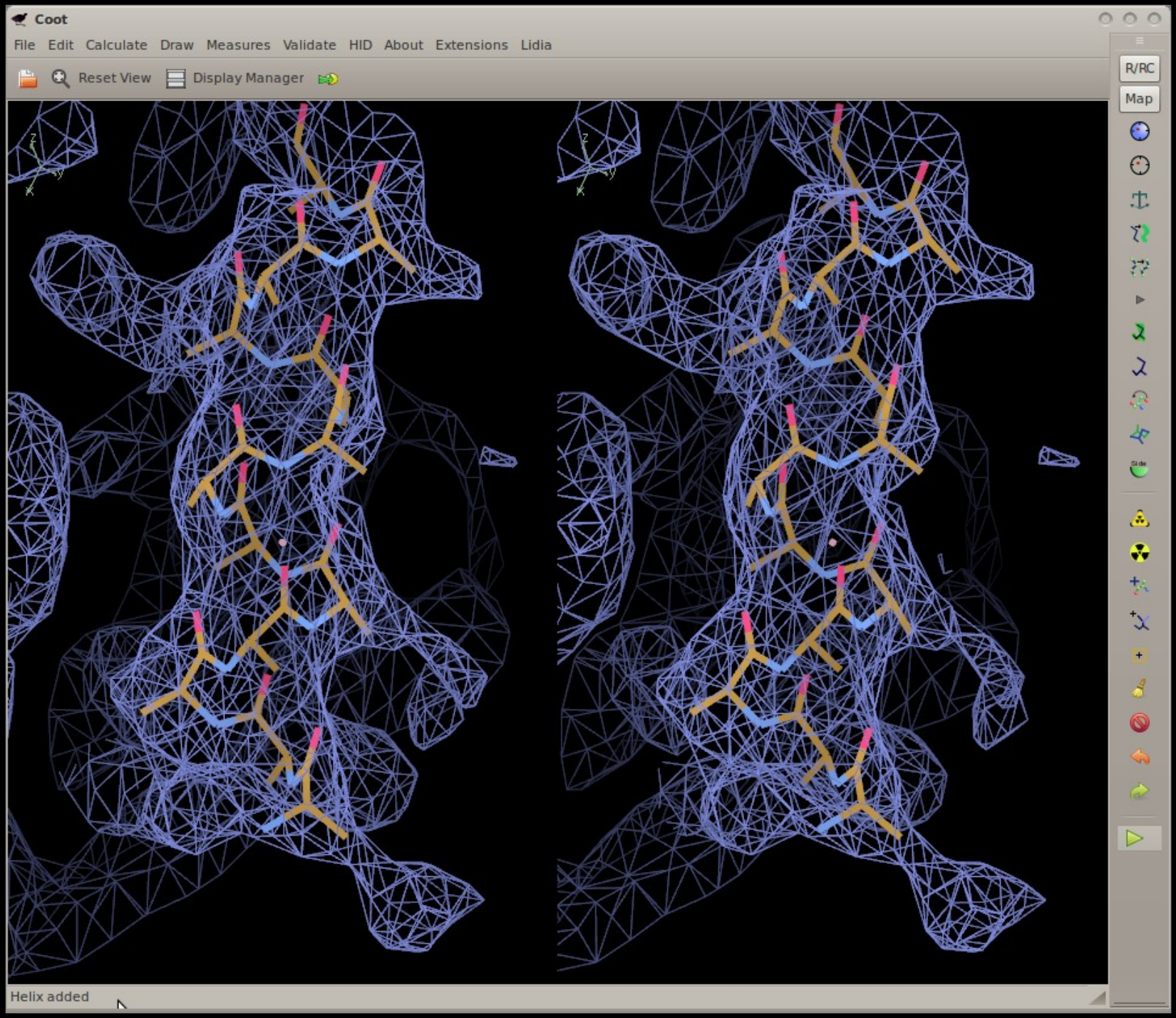
(for the “up” orientation)

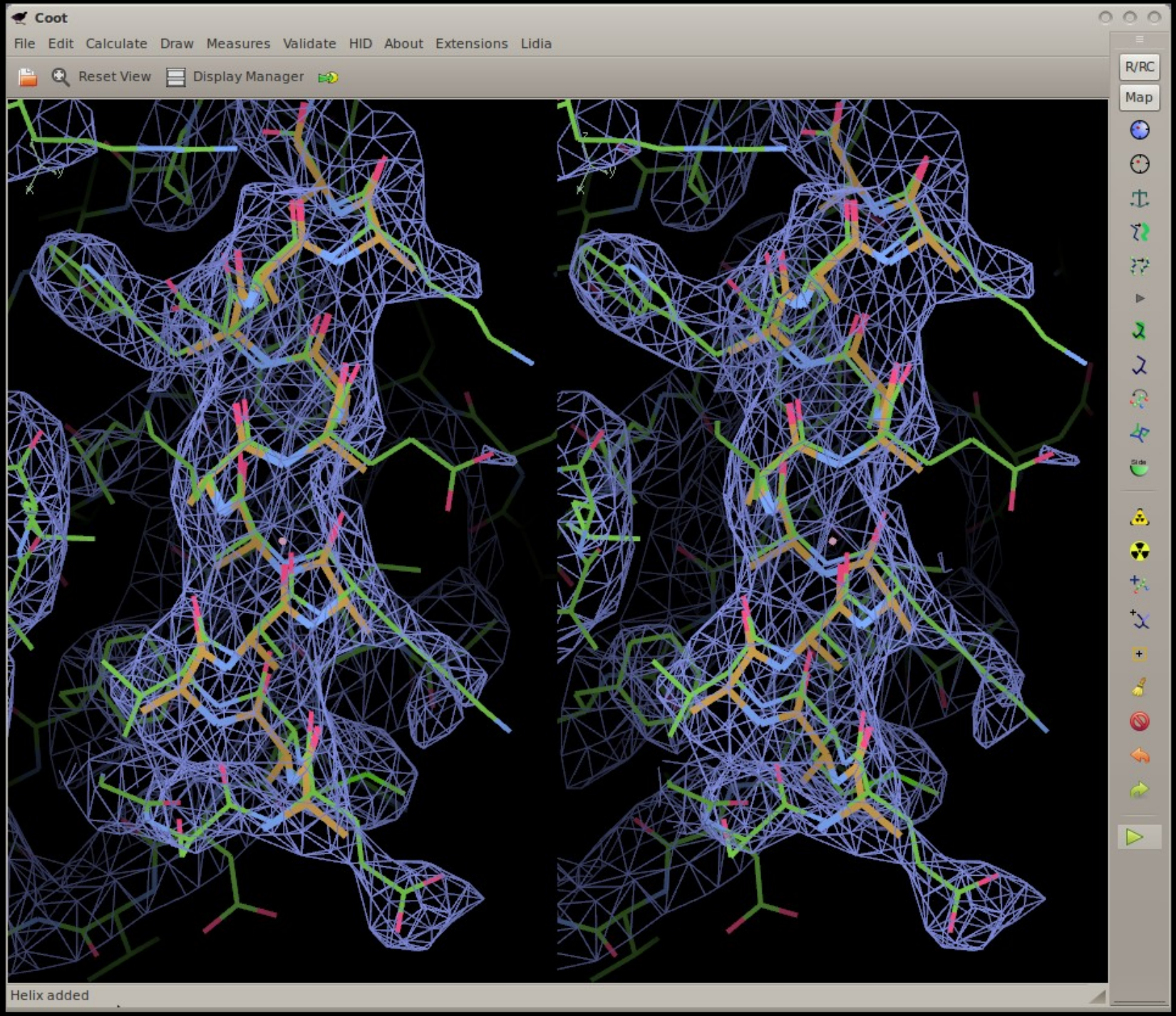

Top
baubles
Bottom






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

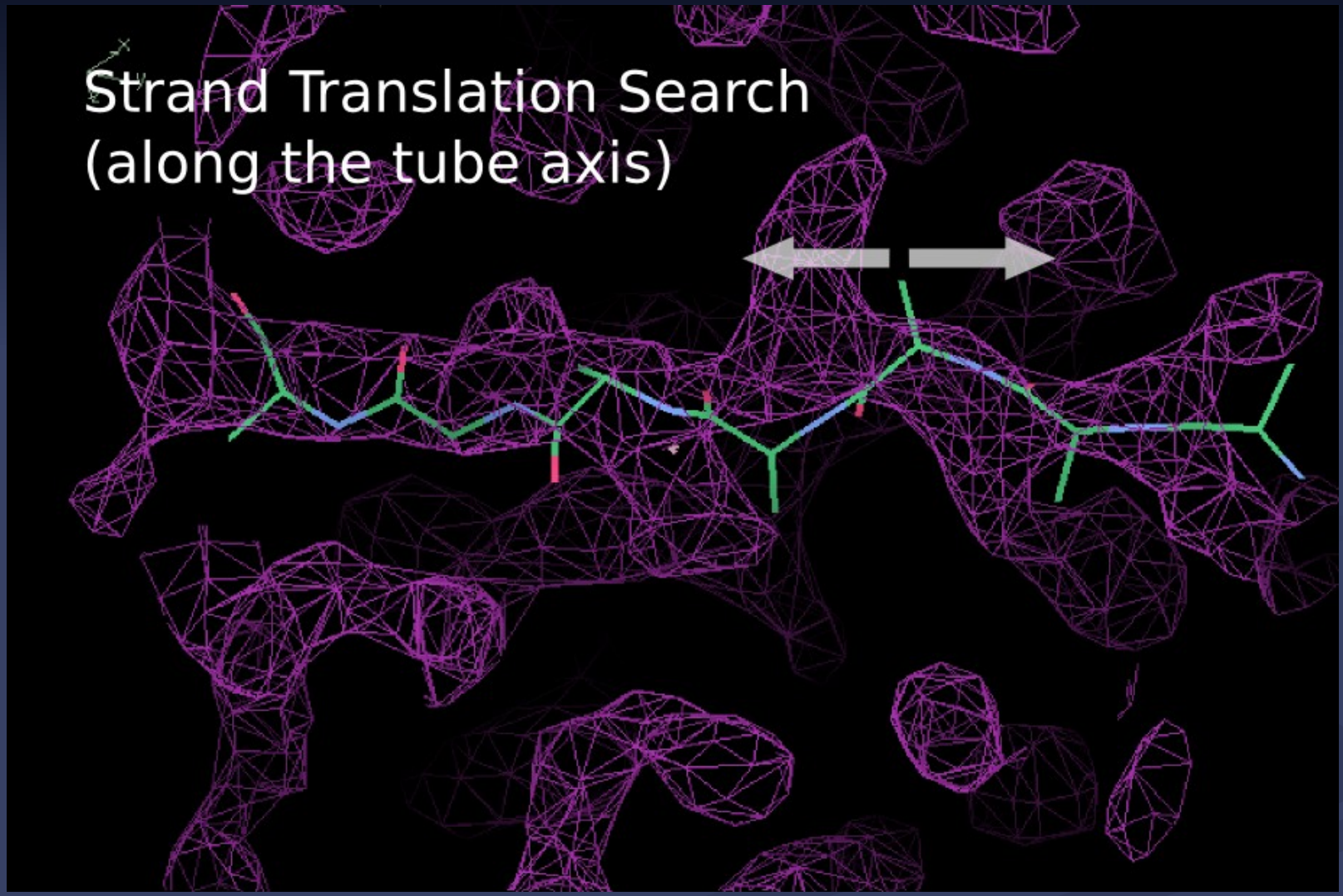
Fitting Strands

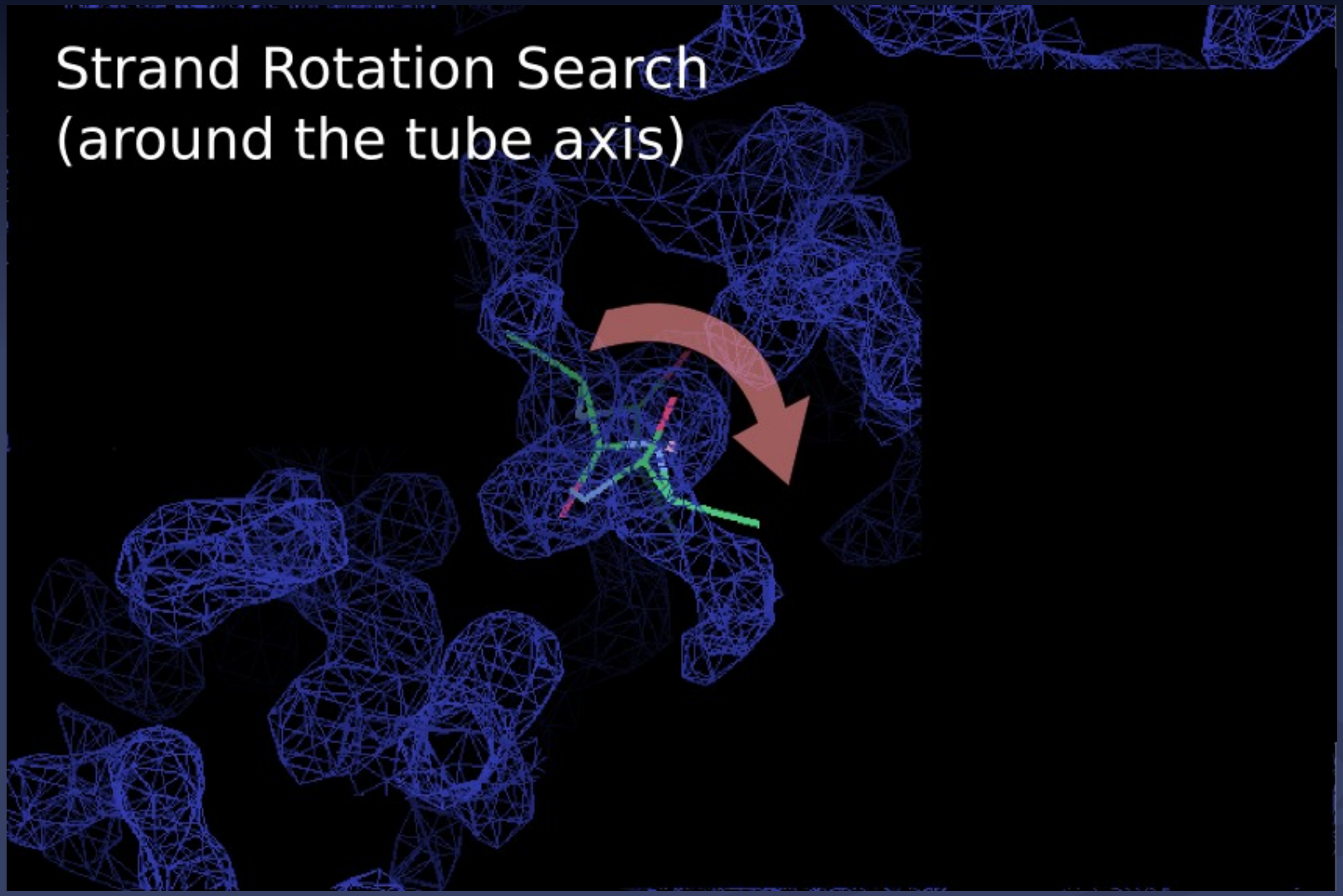
Placing Strands

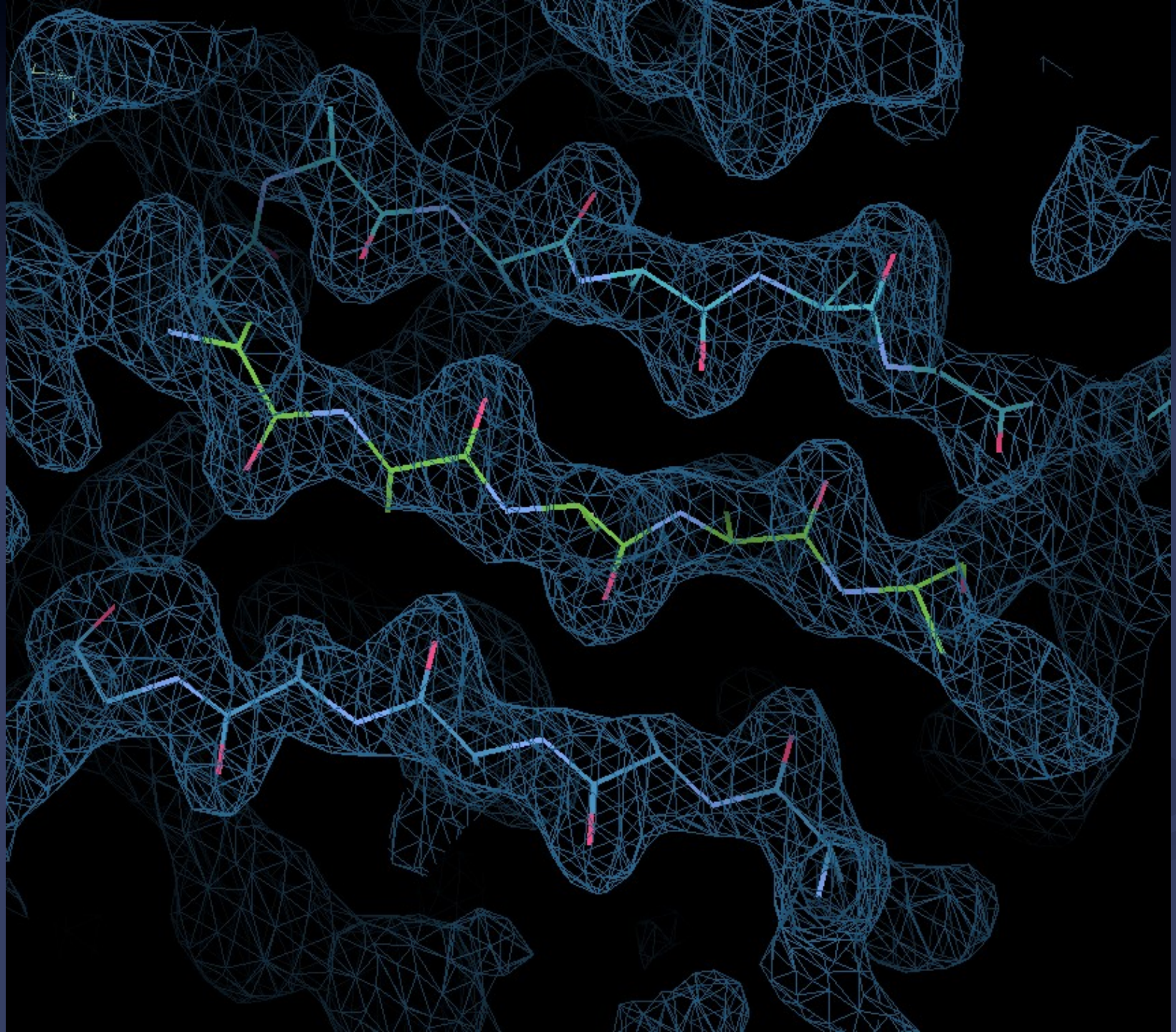
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

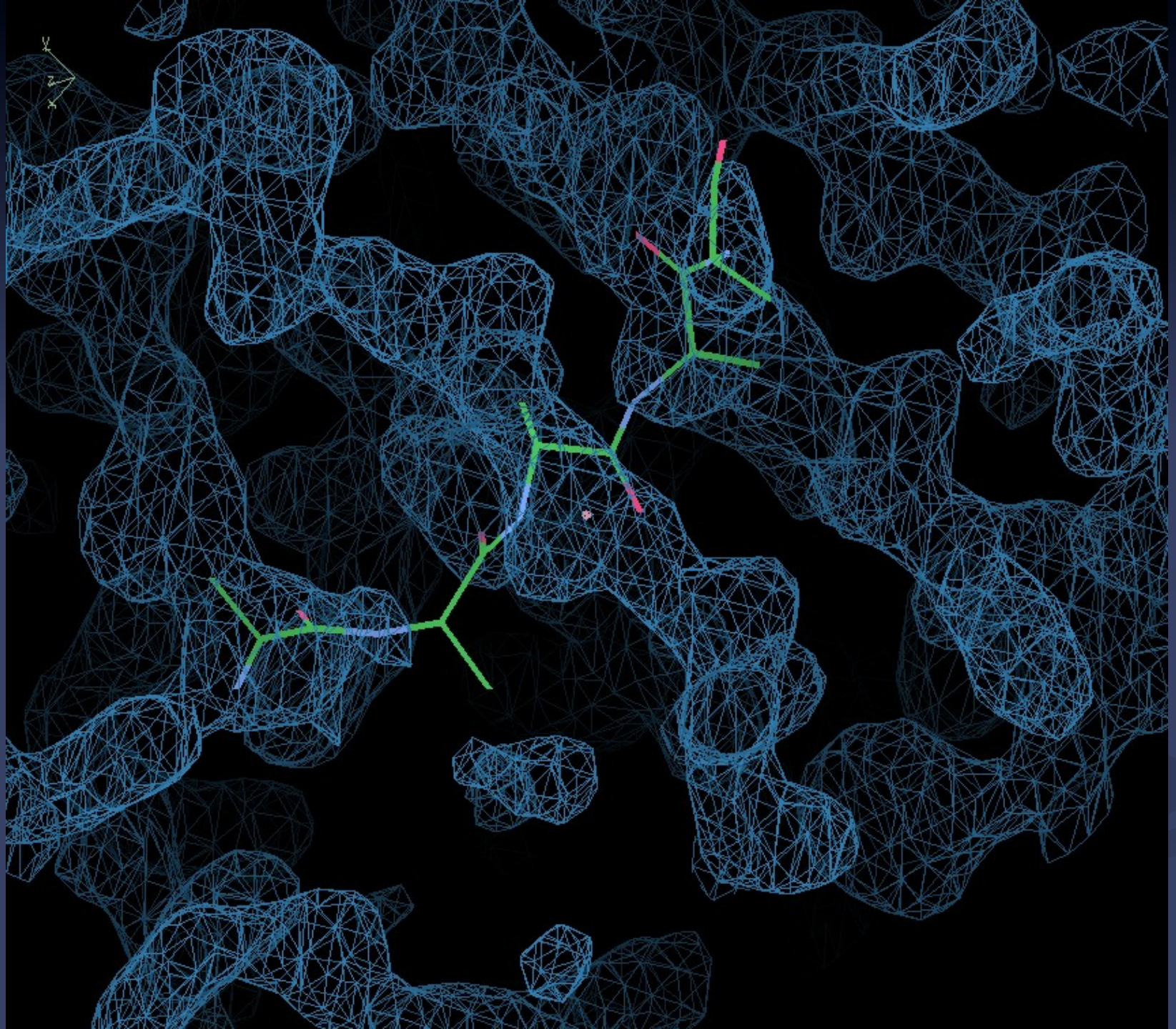
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution


Strand Translation Search
(along the tube axis)


Strand Rotation Search
(around the tube axis)



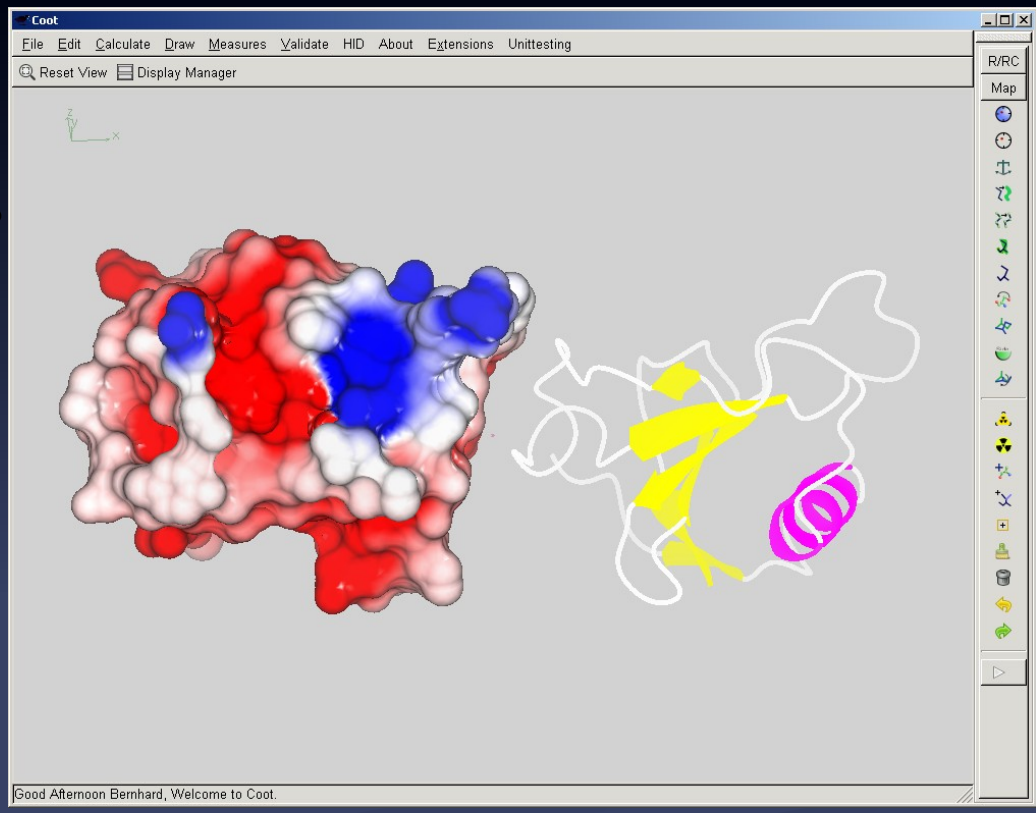
Not all is rosy...



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

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:/lmb.bioch.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

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

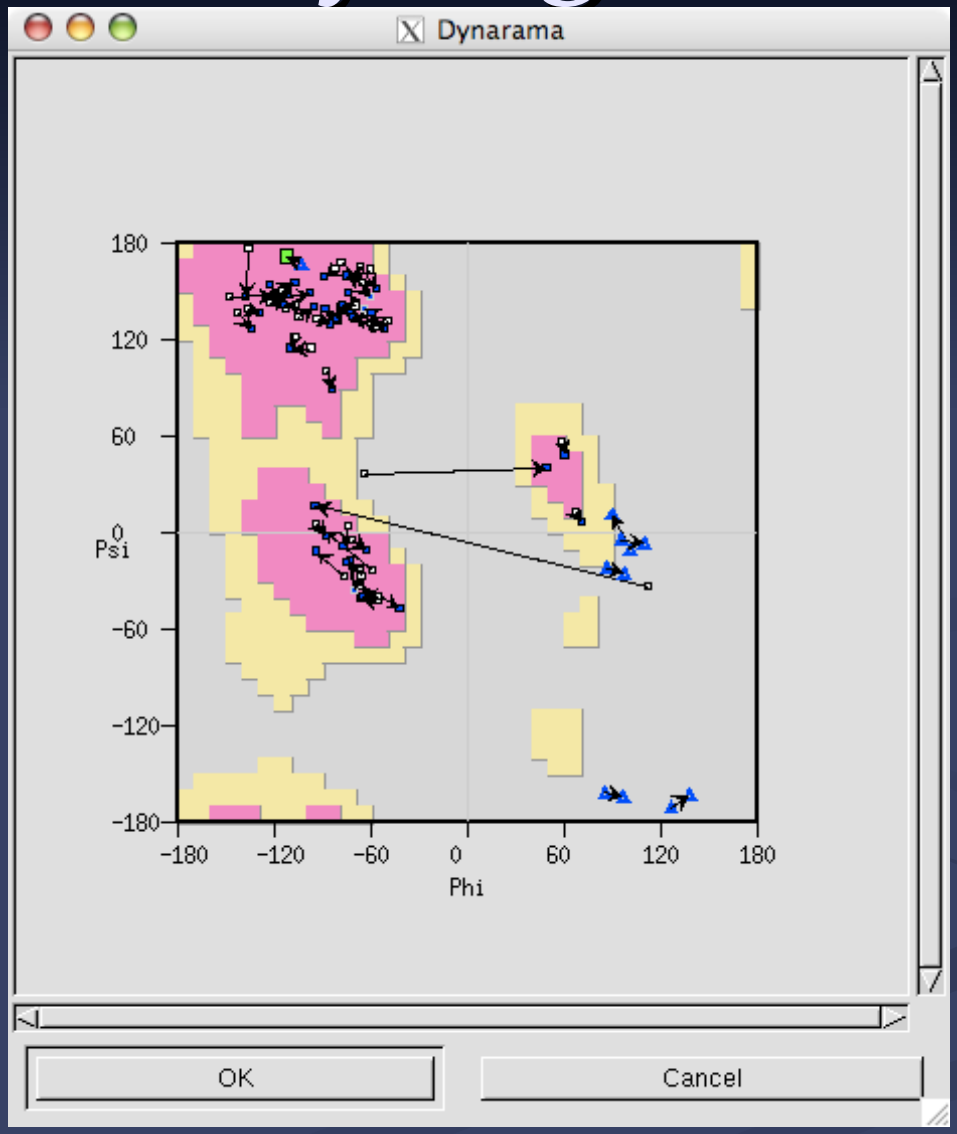
- What are the Problems?
- Strict NCS:
  - NCS should appear like crystallographic symmetry does [exact copies]
- Non-Strict NCS:
  - Molecules are different
  - How to cope with differences, but minimize unnecessary rebuilding?

Handling NCS

Typical Scenario:

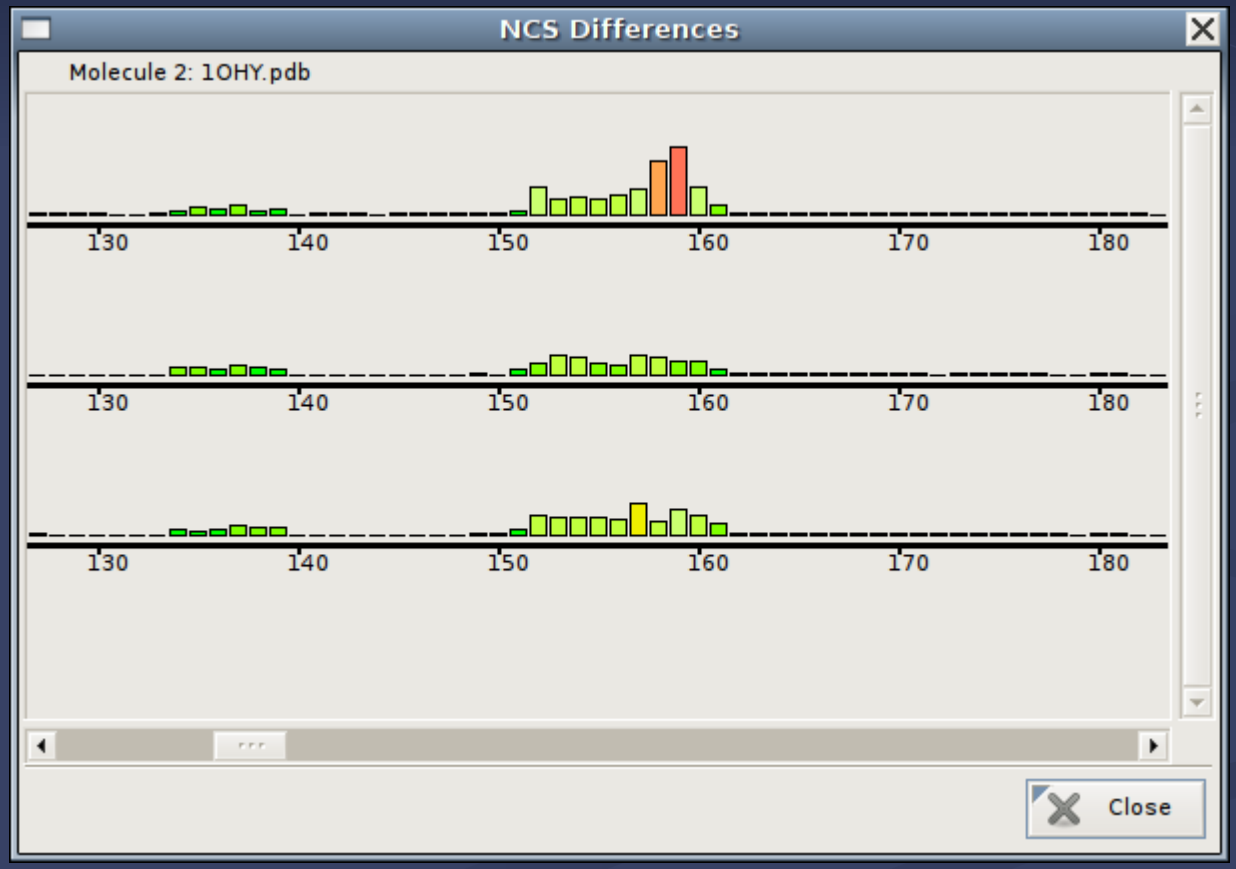
- I have done an LSQ overlap of my NCS-related molecules and from the graph, have seen significant deviations in the positions of some side-chains.
- Why are they different?

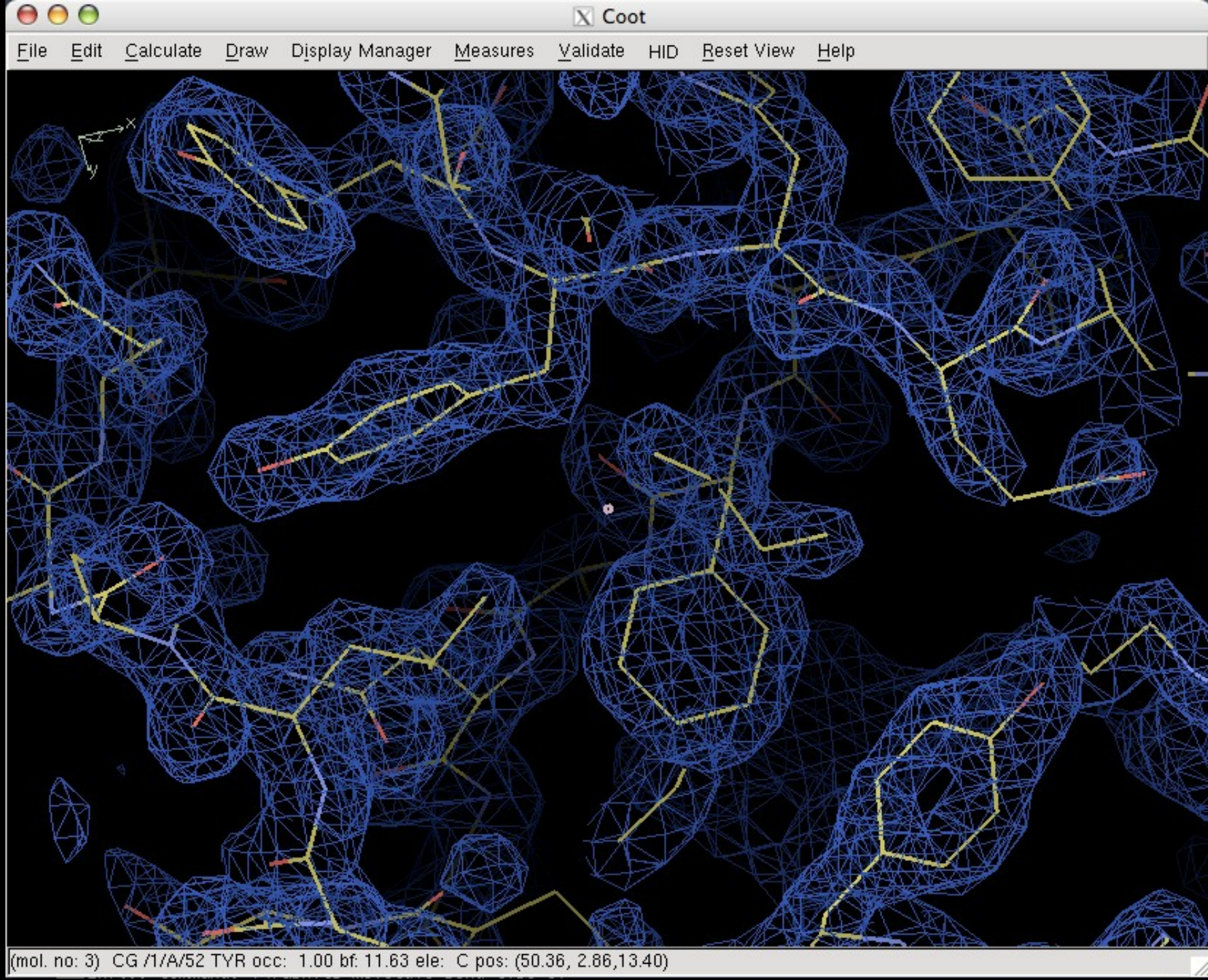
…or Kleywegt Plots[*]



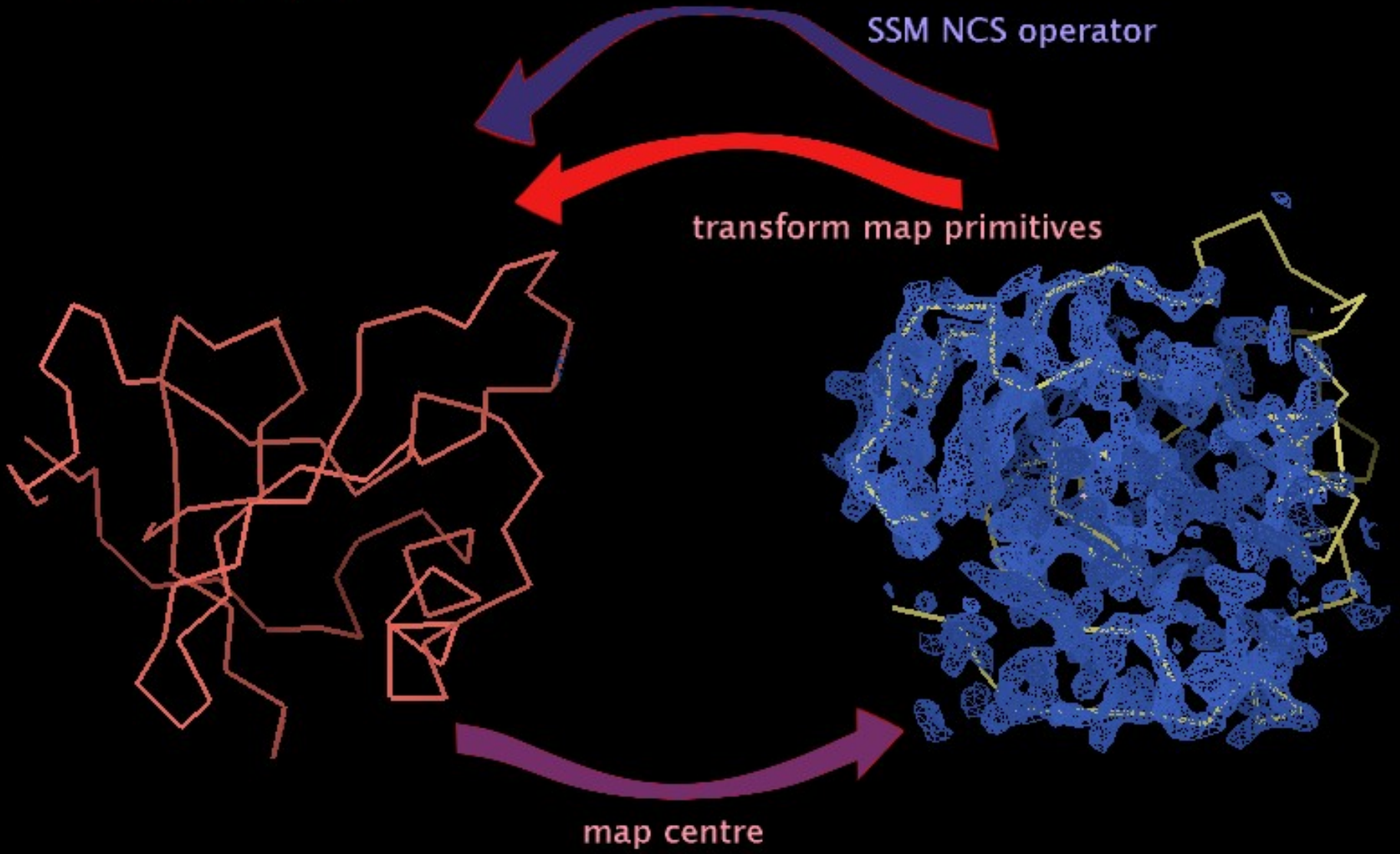
[*] Named by George Sheldrick

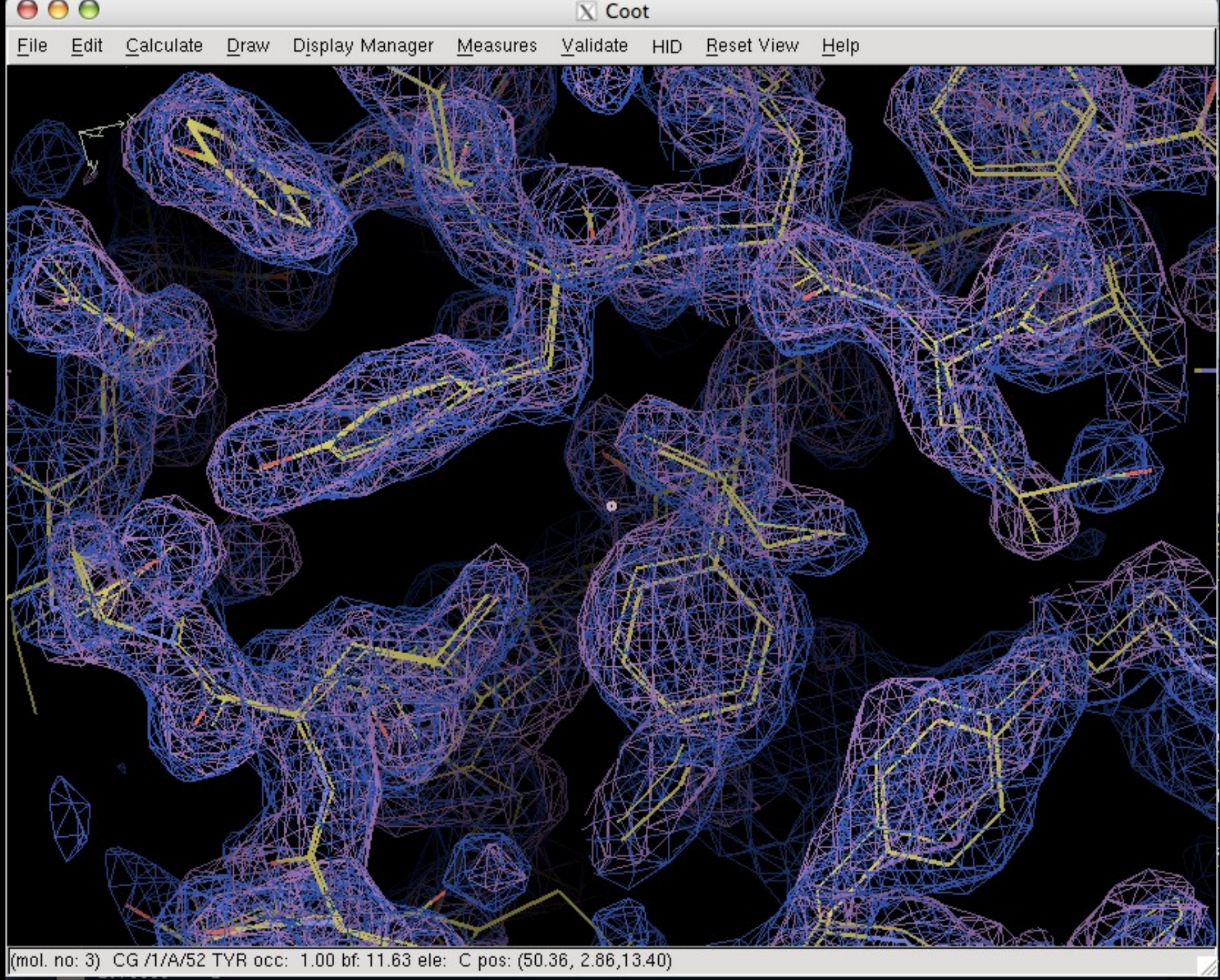
...or NCS Differences graph




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 Overlays




Coot
File
Edit
Calculate
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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