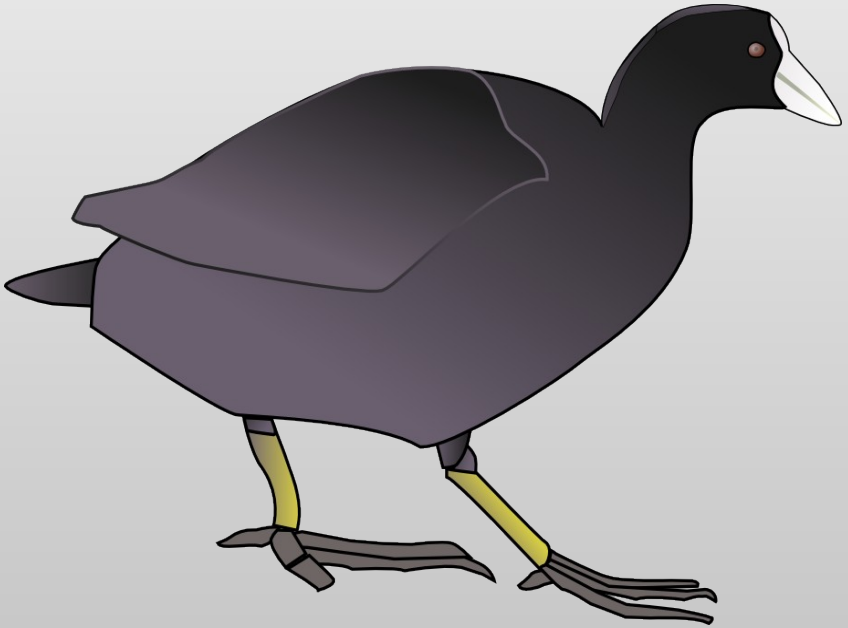
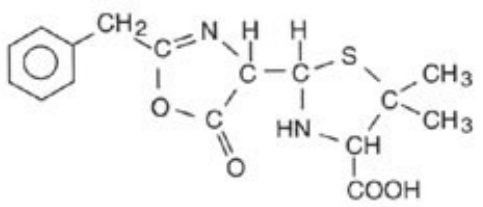
Coot



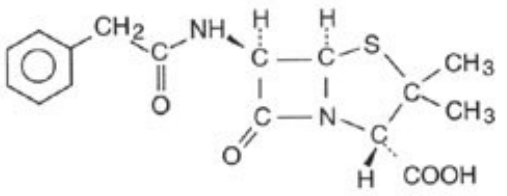
Judit Debreczeni

December 2014

(a)



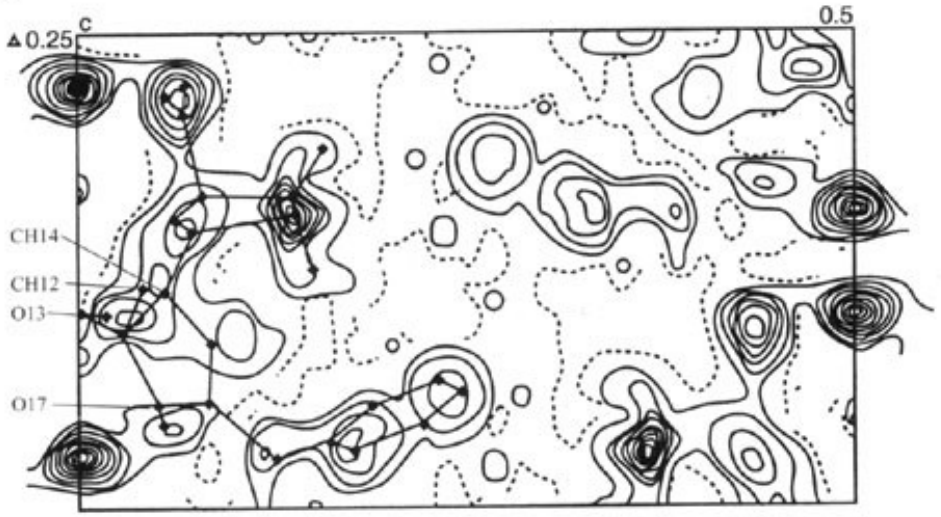
Oxazolone



β-Lactam

Is there a 4-membered ring?

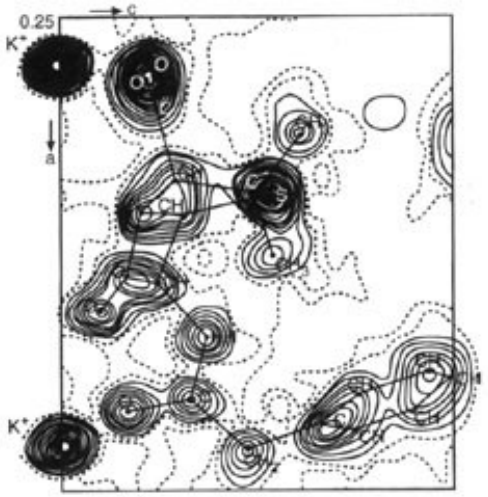
(b)



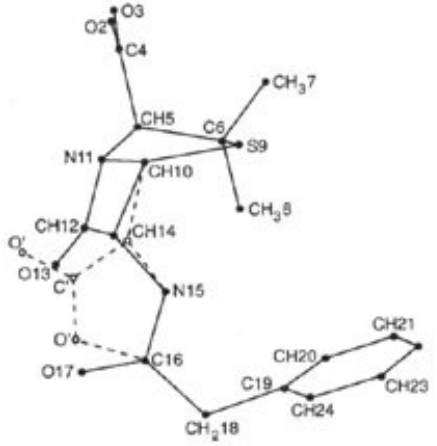
First Projection Fourier map
Phased by Rb

Dotted bonds indicate the bonding for the oxazalone structure.

(c)



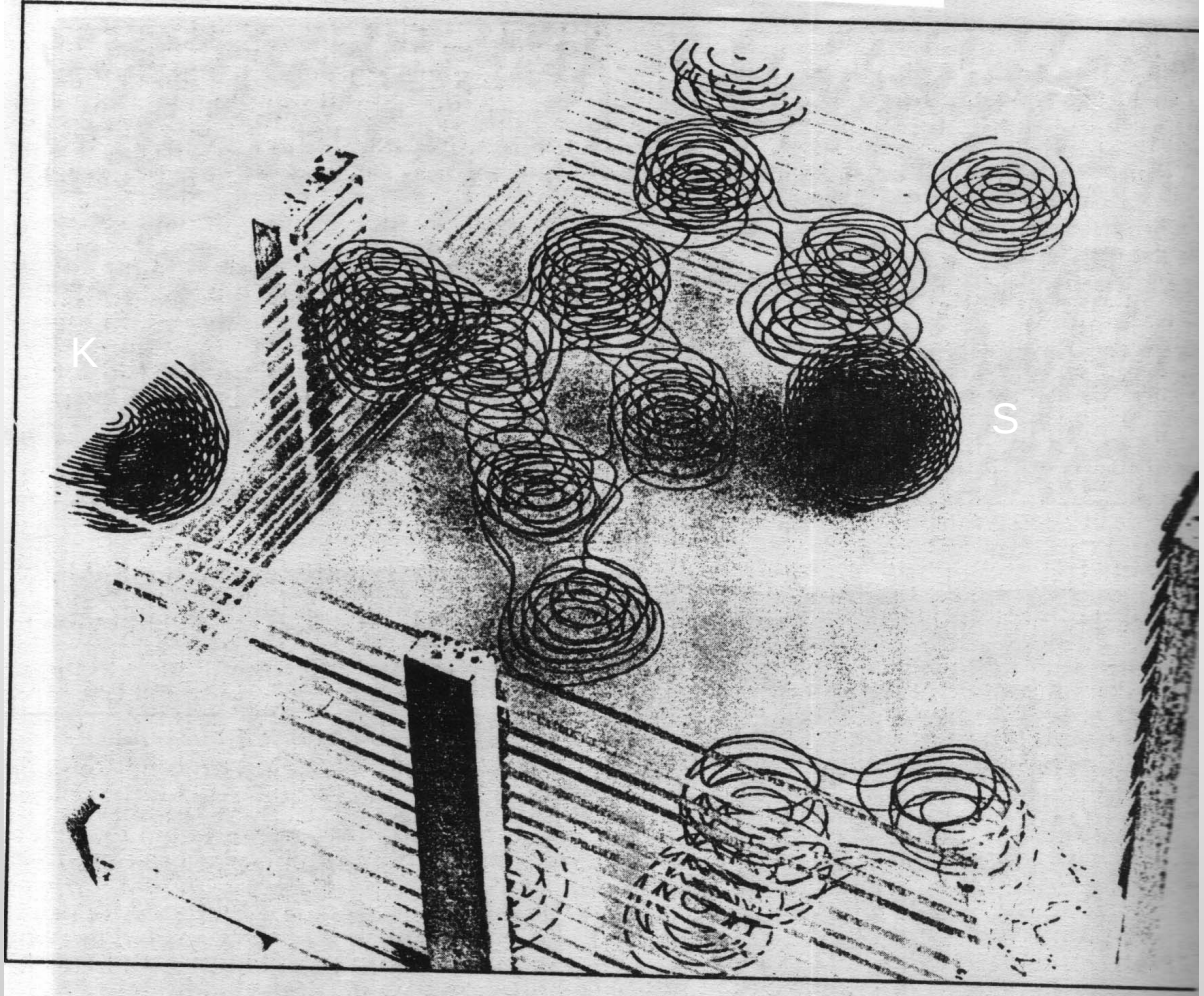
(d)

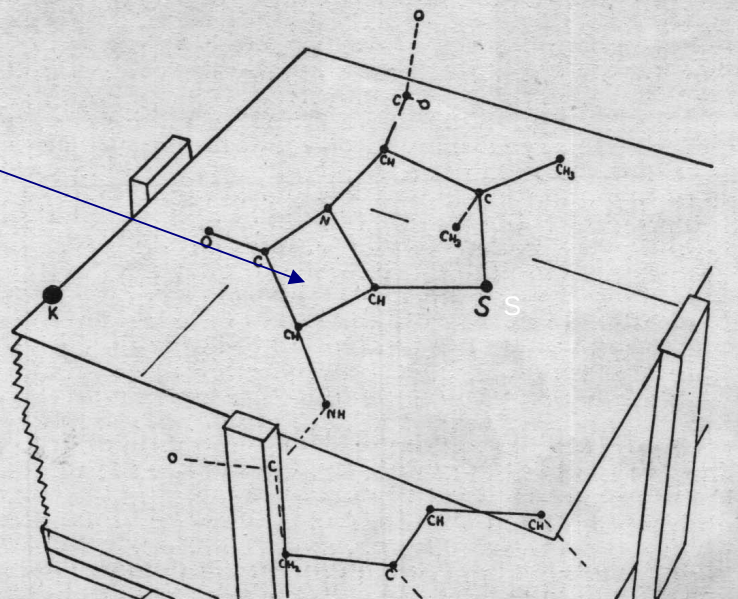


Final map Yes!!

The 3-dimensional penicillin G map calculated in 1944. This unequivocally determined its chemical structure.
(The hand however is wrong.)

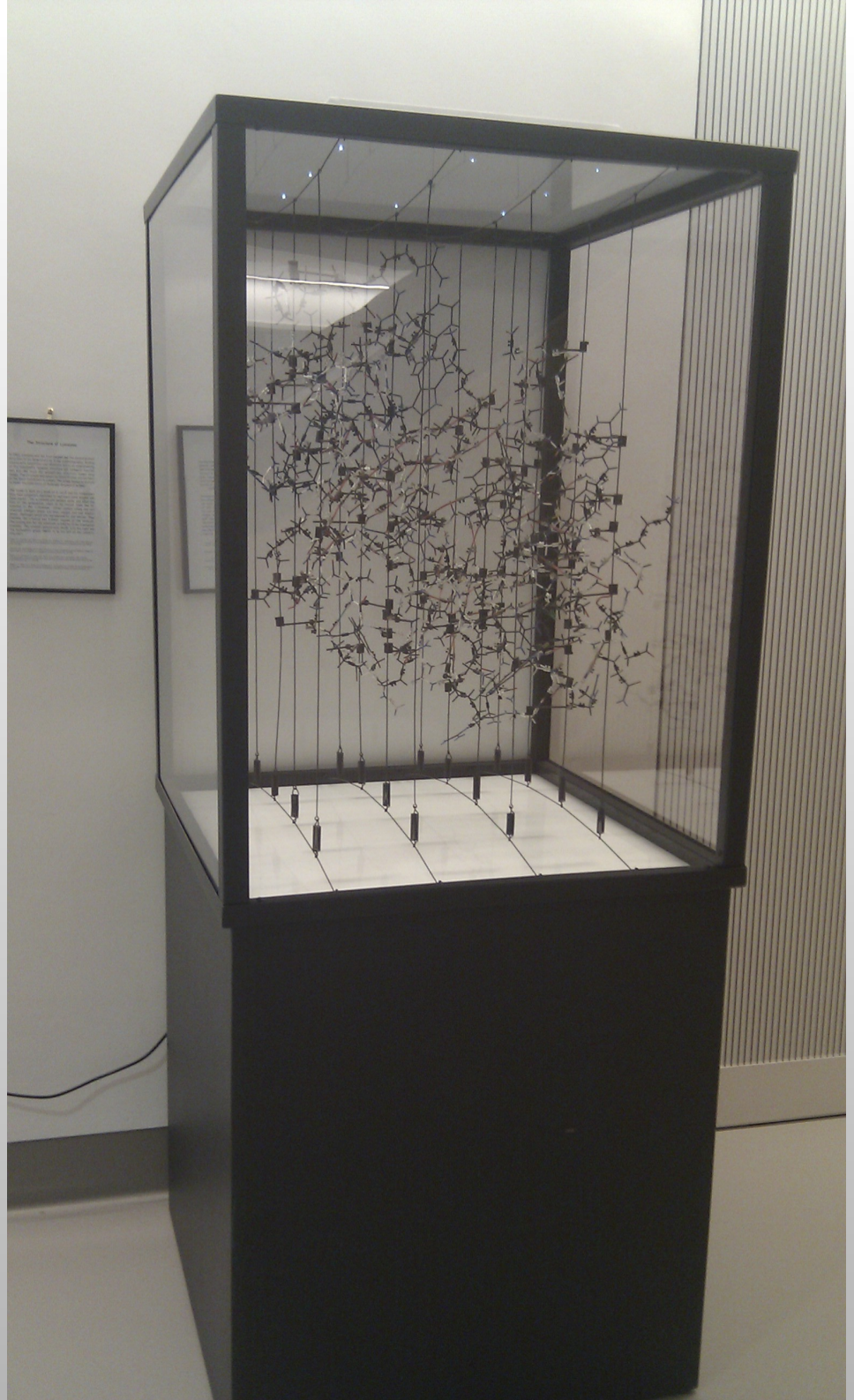
The chemical interpretation of the electron density map. The four membered beta-lactam ring, the centre of the controversy.



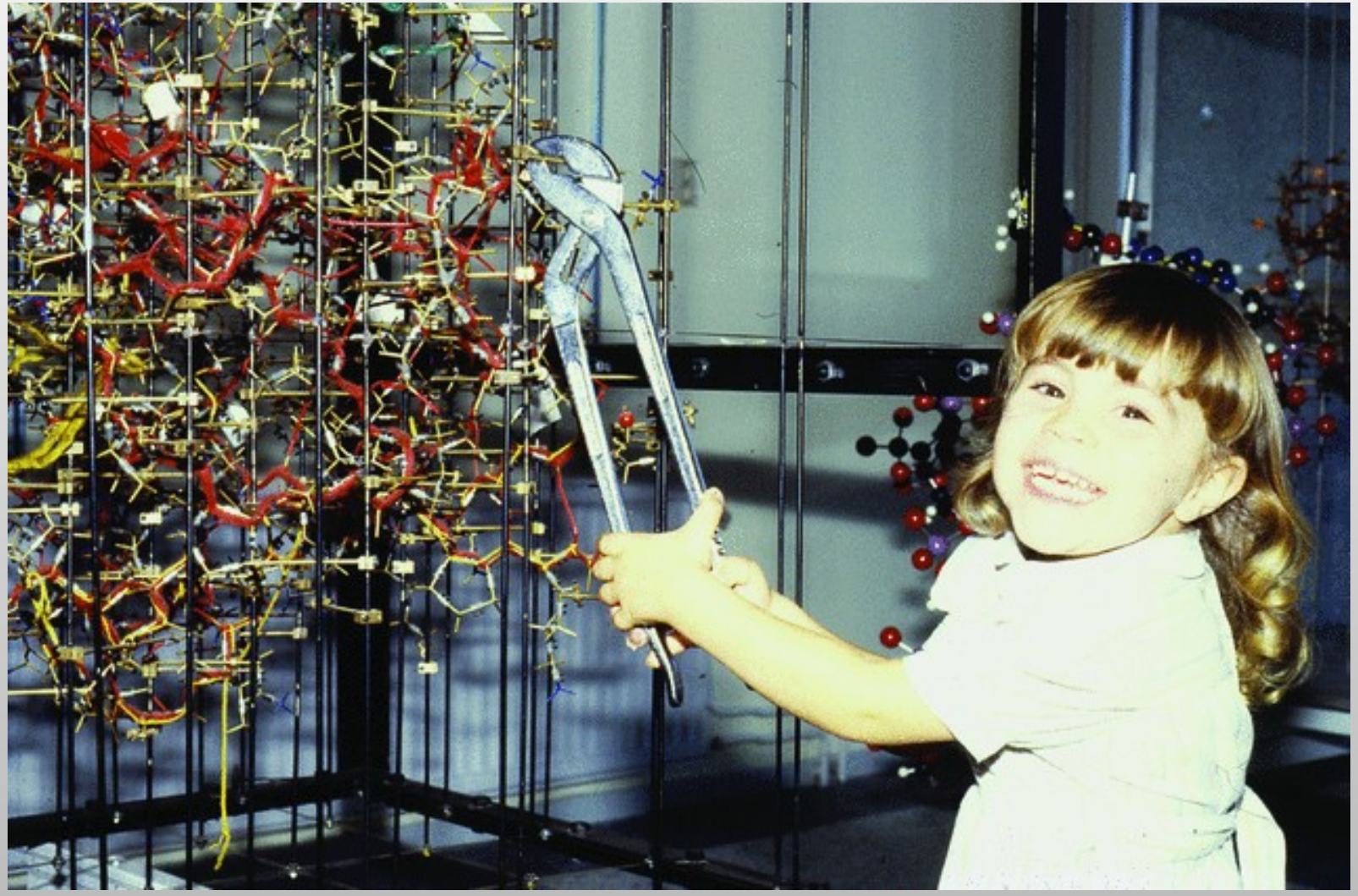


DCH building first insulin model

Lysozyme 1965



Kendrew wire model of alcohol dehydrogenase that is about to undergo a round of rebuilding by Maelle Cambillau



T. Alwyn Jones (2004)

- Moorhen

- Coot

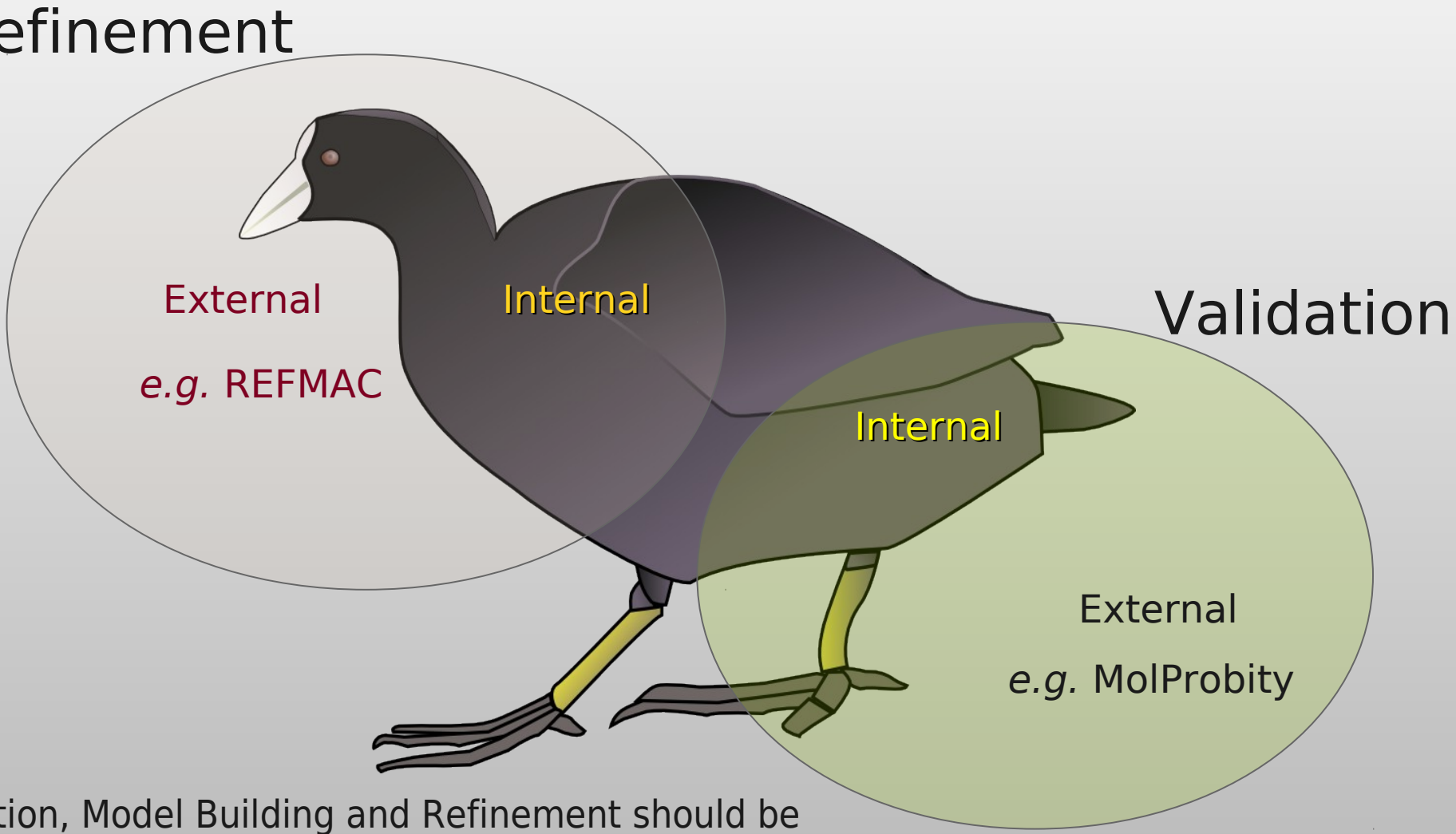
Outline

- Model building and refinement
  - Real space refinement
  - Other useful tools
  - Helix and strand placement
  - Automated fast sec structure search
  - Loop fitting
- Low res
  - Additional restraints:
    - Torsion angle restraints
    - Ramachamdran restraints
    - Planar peptide restraints
    - Prosmart interface
    - Additional restraints
  - Backrub rotamers
  - Map sharpening
  - Jiggle fit

Coot

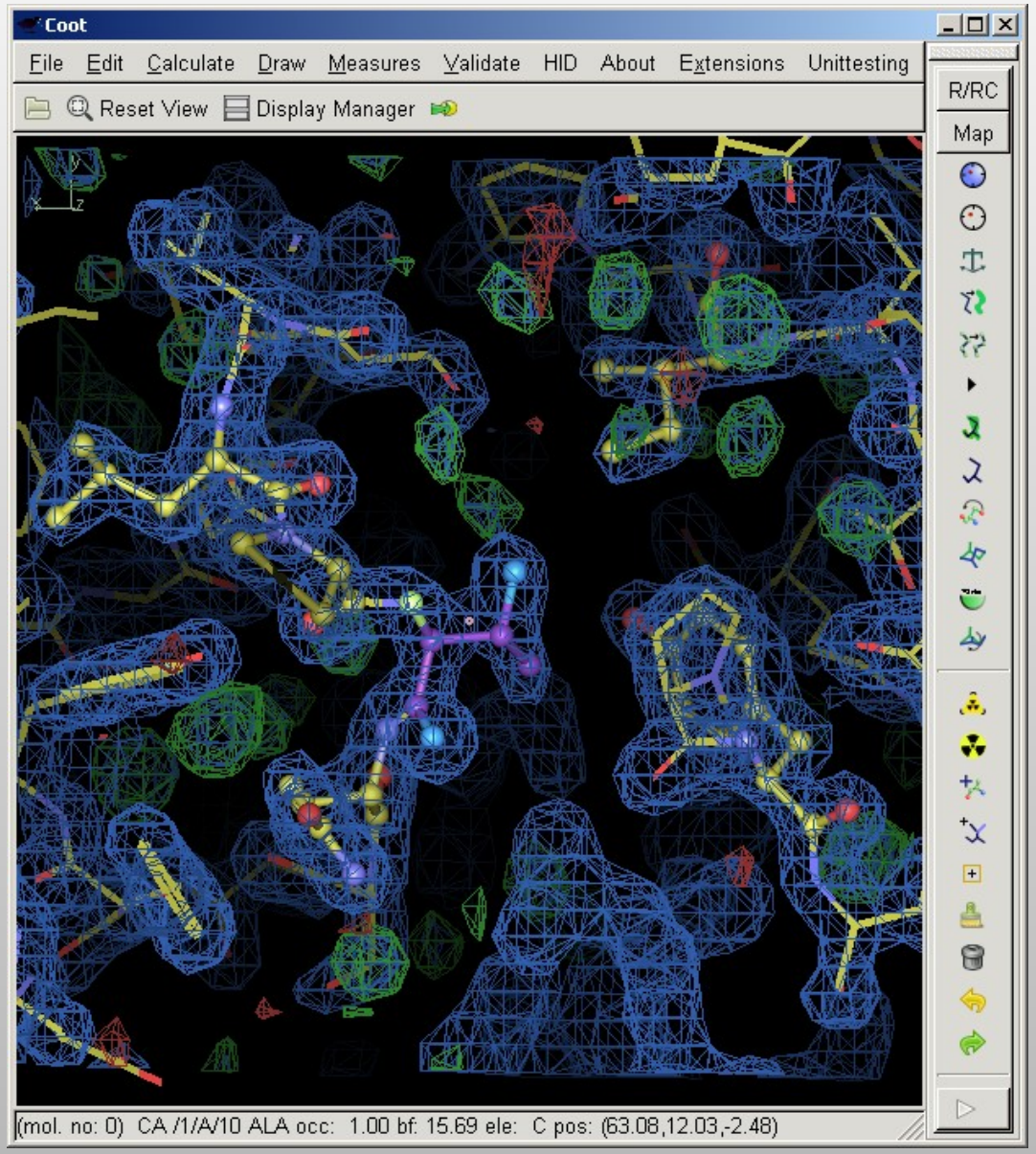
- Crystallographic Object-Oriented Tool-kit
- Primarily a tool for the interpretation of electron density generated from X-ray data
  - with tools for modelling:
    - rotate/translate, rotamers,
    - refinement & regularization
    - add, delete
    - ligand fitting
  - Interface to other programs: SHELXL, Refmac, Libcheck, Probe&Reduce (Molprobity), EBI, EDS, Povray, Raster3D, PHENIX, ...
- A “workhorse”, not a show-pony

Feature Integration

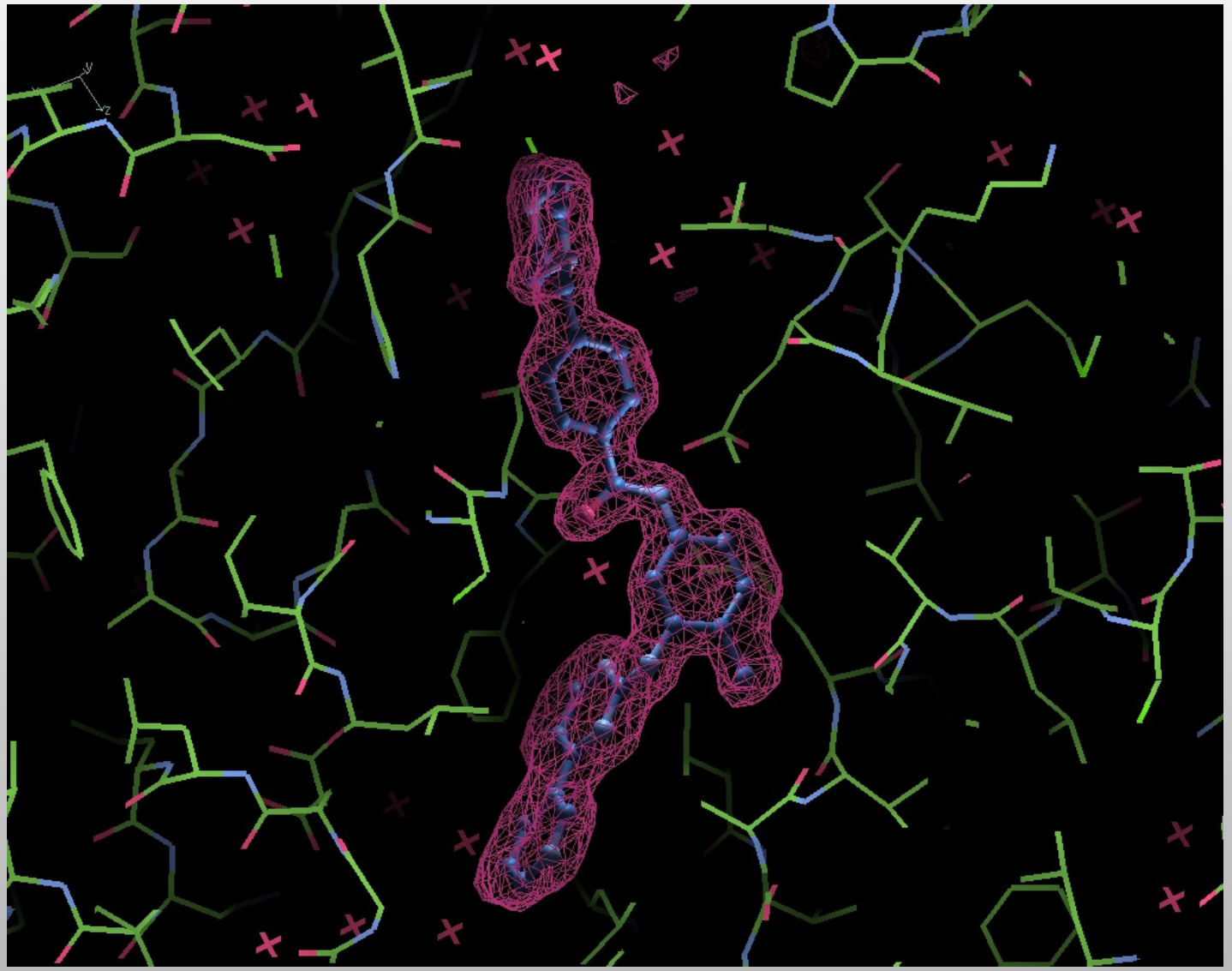


Validation, Model Building and Refinement should be used together

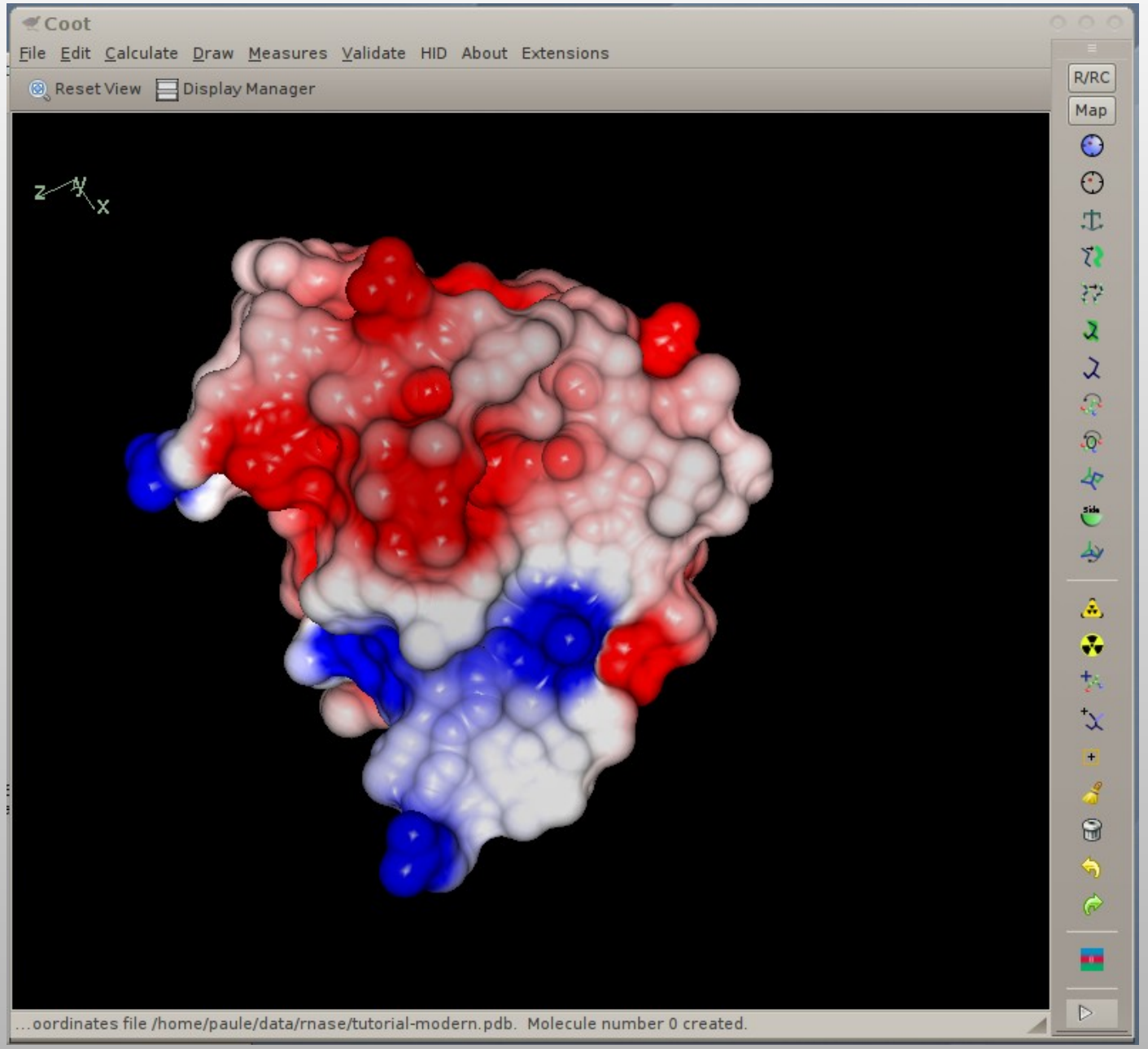
What does it look like?



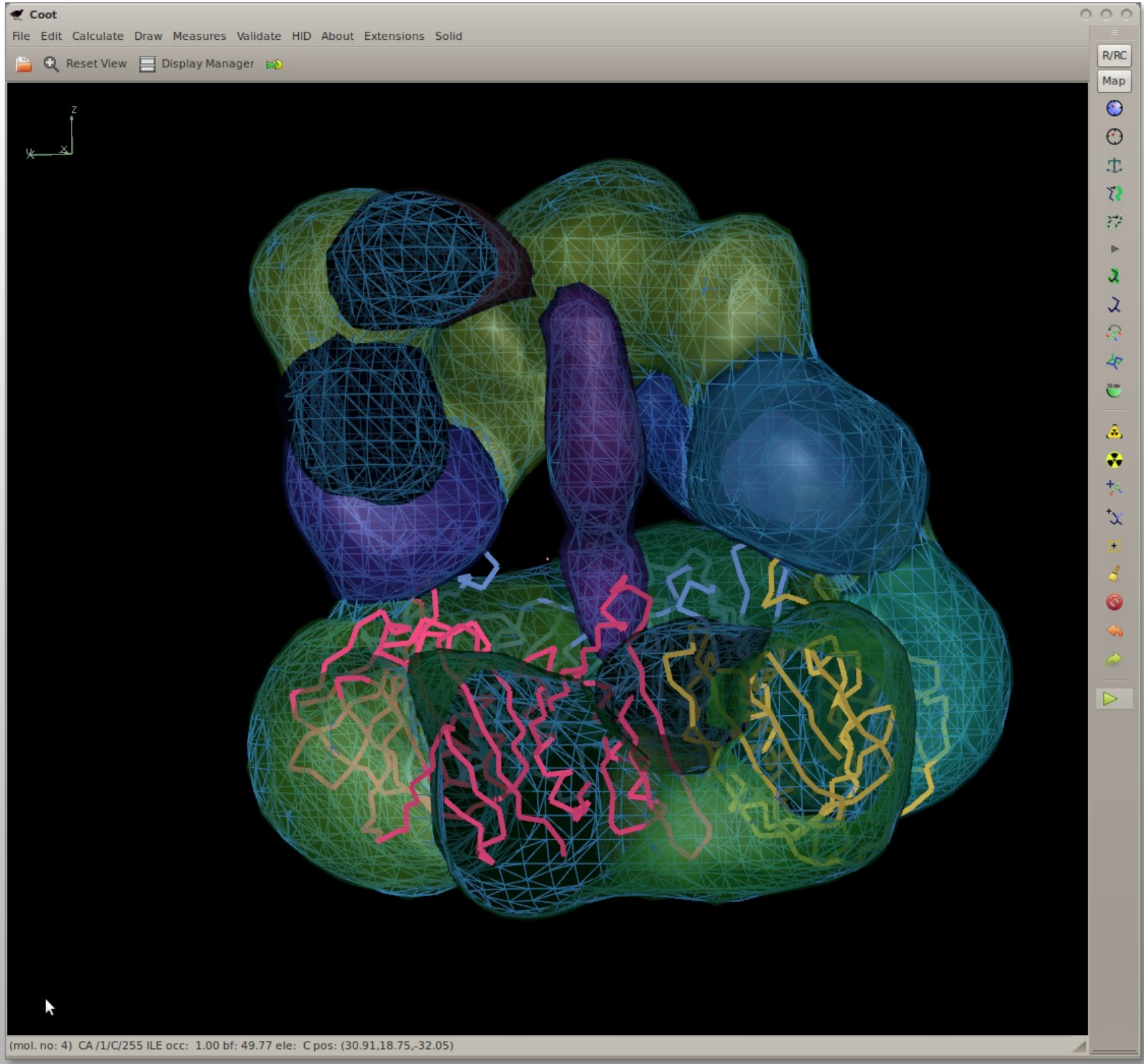
Some Representation Tools



Some Representation Tools



Gruber & Noble (2007)


Coot
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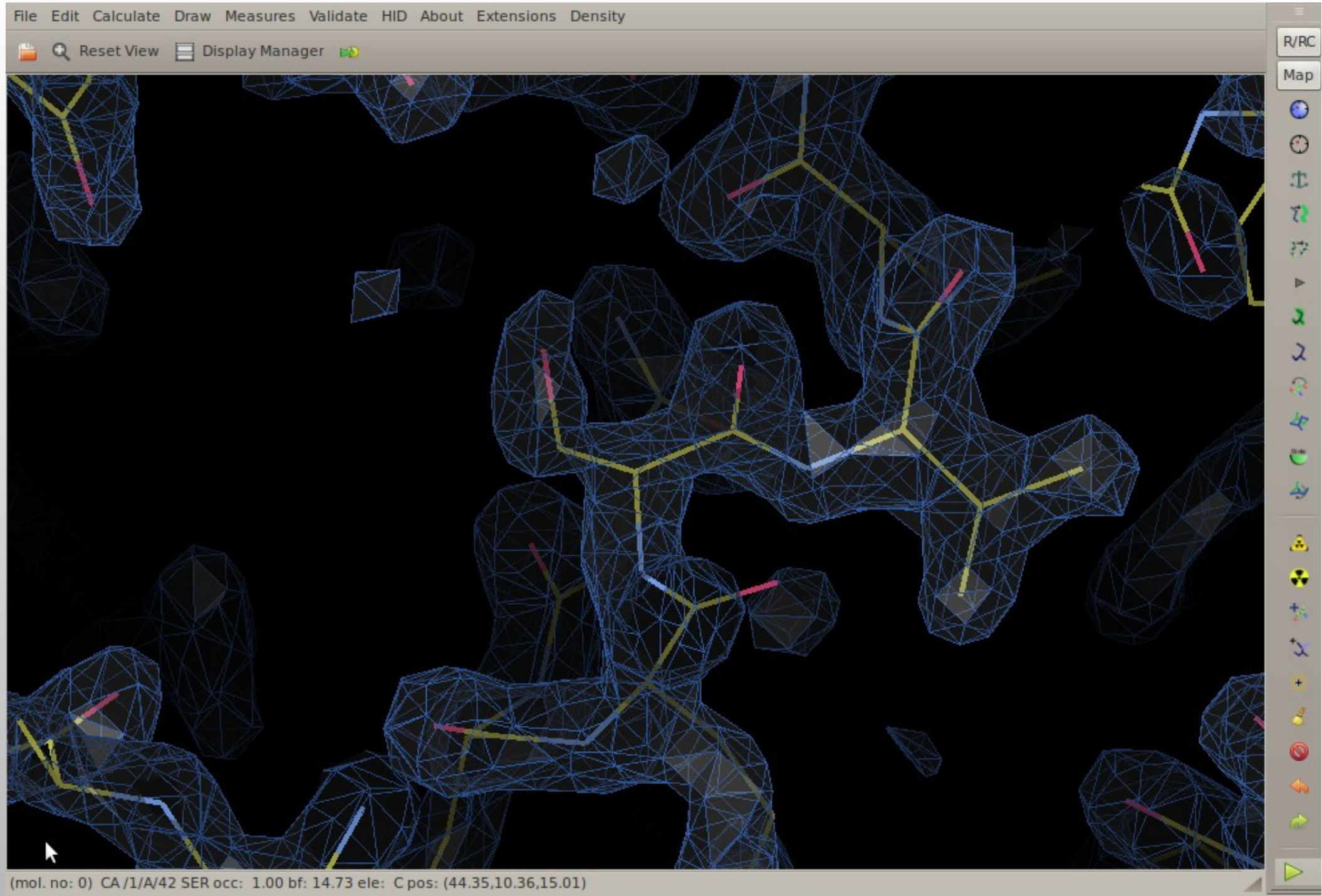
Reset View
Display Manager
R/RC
Map
(mol. no: 4) CA /1/C/255 ILE occ: 1.00 bf: 49.77 ele: C pos: (30.91,18.75,-32.05)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Reset View
Display Manager
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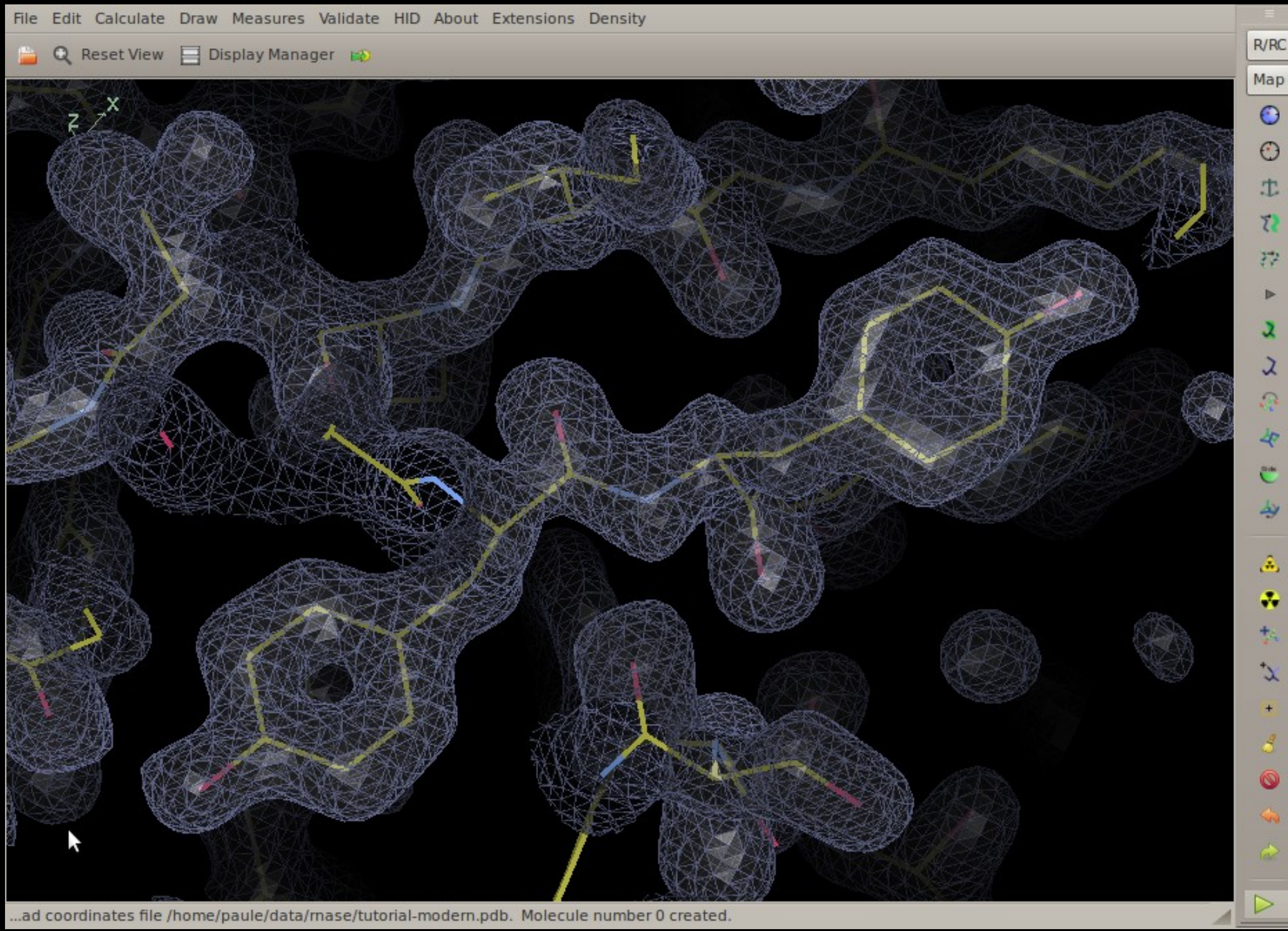
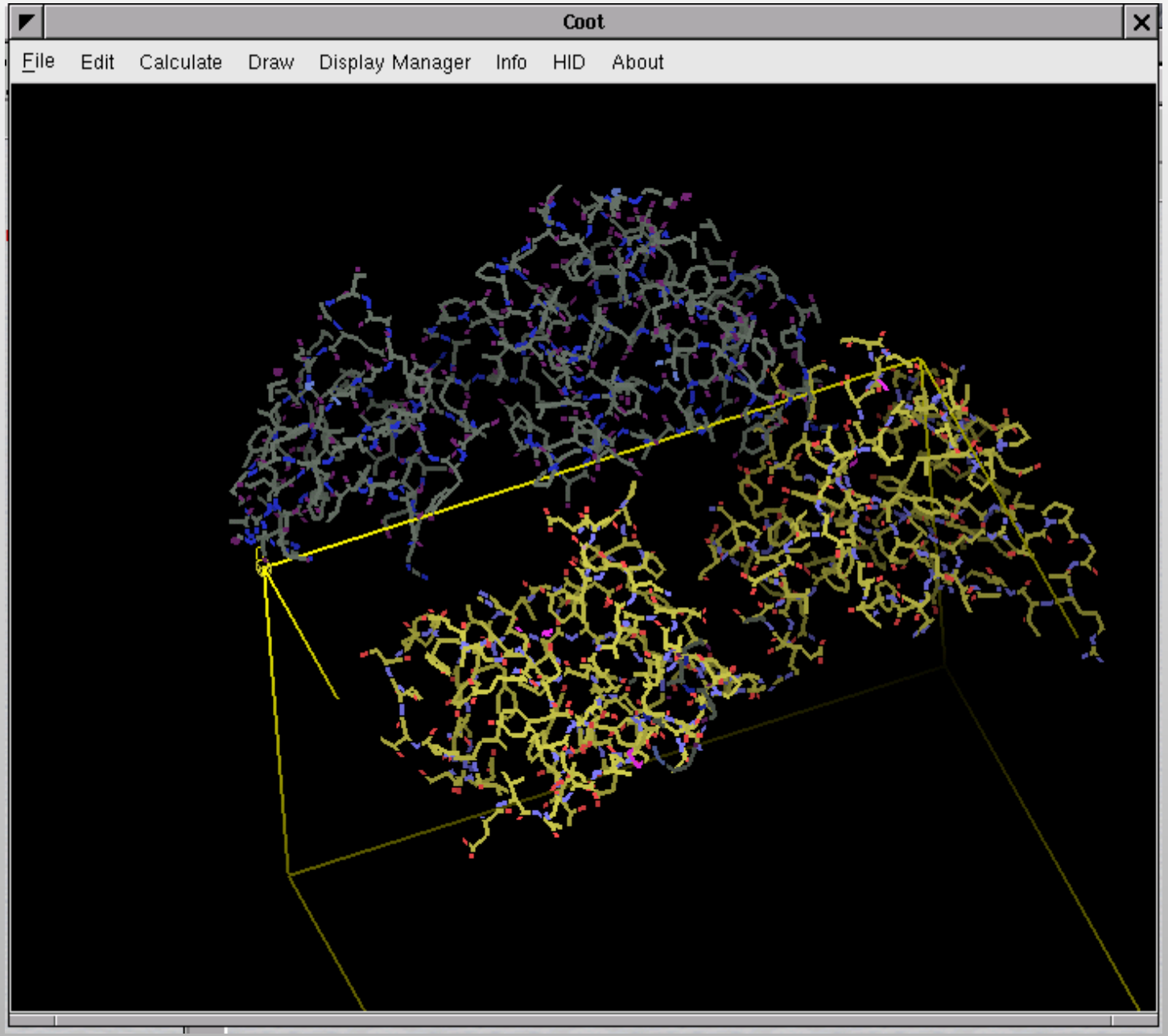
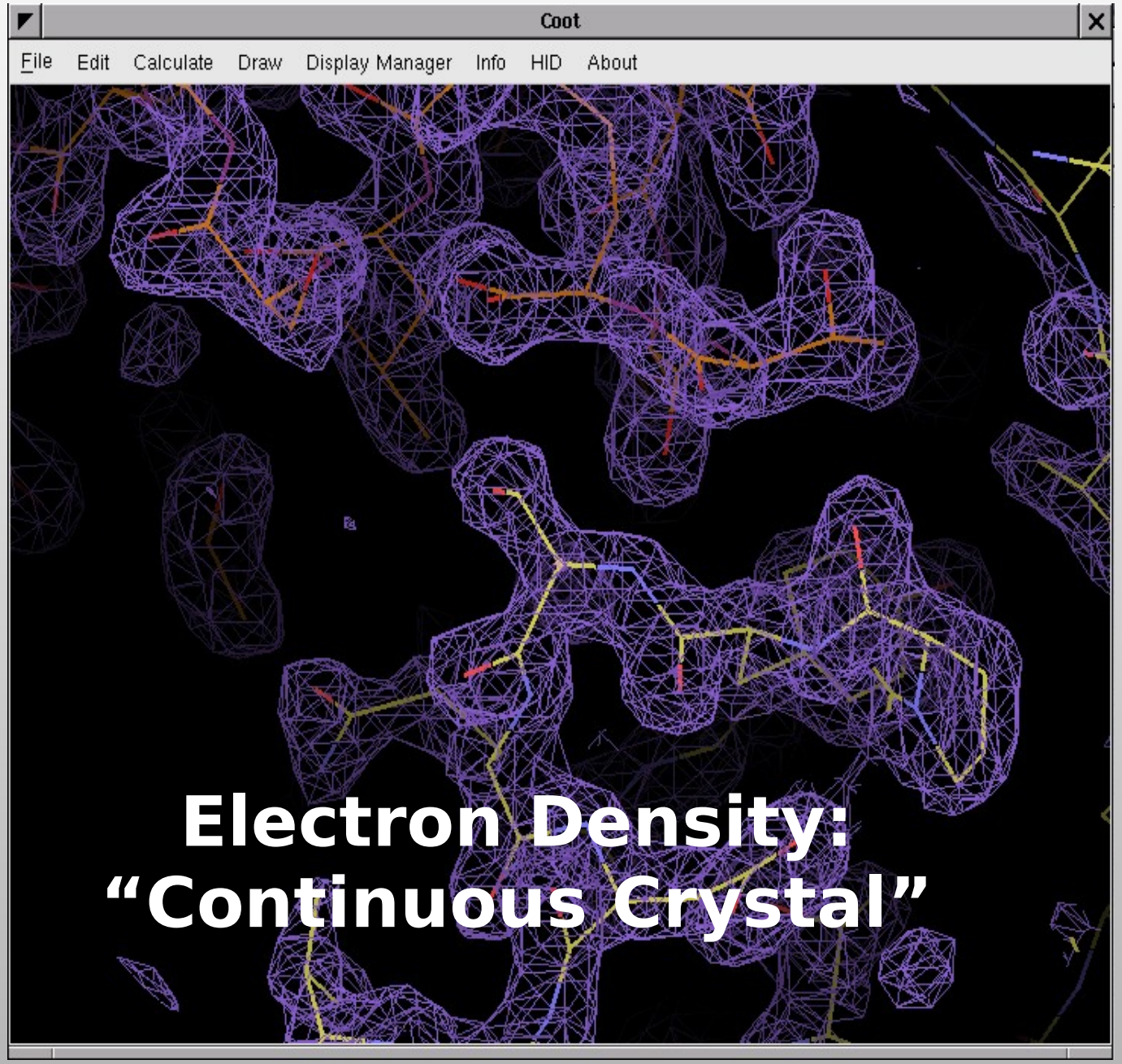
Map
(mol. no: 0) CA /1/A/42 SER occ: 1.00 bf: 14.73 ele: C pos: (44.35,10.36,15.01)


File Edit Calculate Draw Measures Validate HID About Extensions Density
Reset View
Display Manager
R/RC
Map
Z
X
...ad coordinates file /home/paule/data/rnase/tutorial-modern.pdb. Molecule number 0 created.

What does it look like?



What does it look like?



Real Space Refinement

Real Space Refinement

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

  move the atoms into as high density as possible while minimizing geometrical distortions

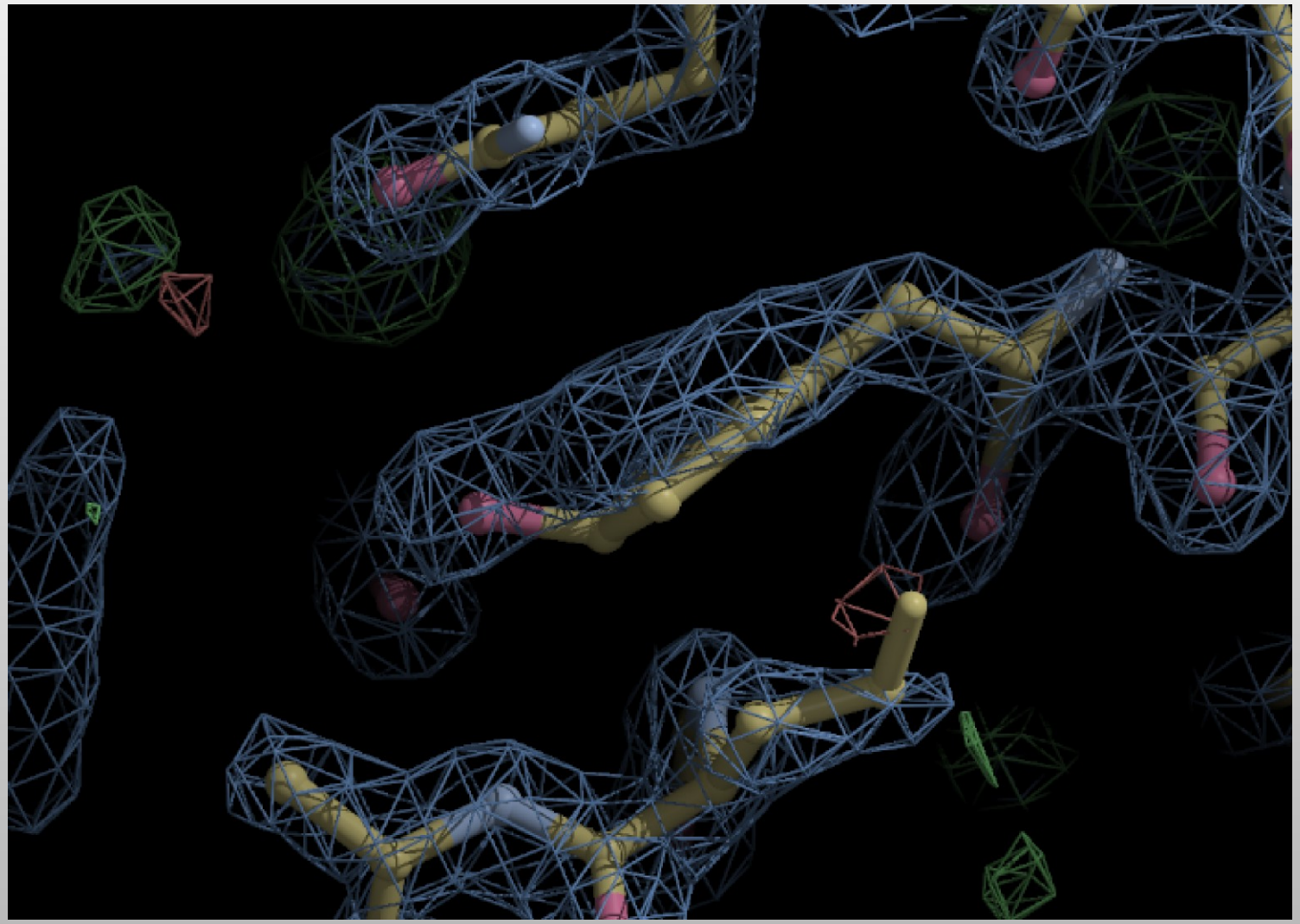
Real Space Refinement

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

- Major Feature of Coot
  - Gradient-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, Ramachandran
    - Including links and modifications
- Provides “interactive” refinement – fast and animated
- Nice and tight geometry
  (can set X-ray/geometry weight)
- Subject to substantial extension

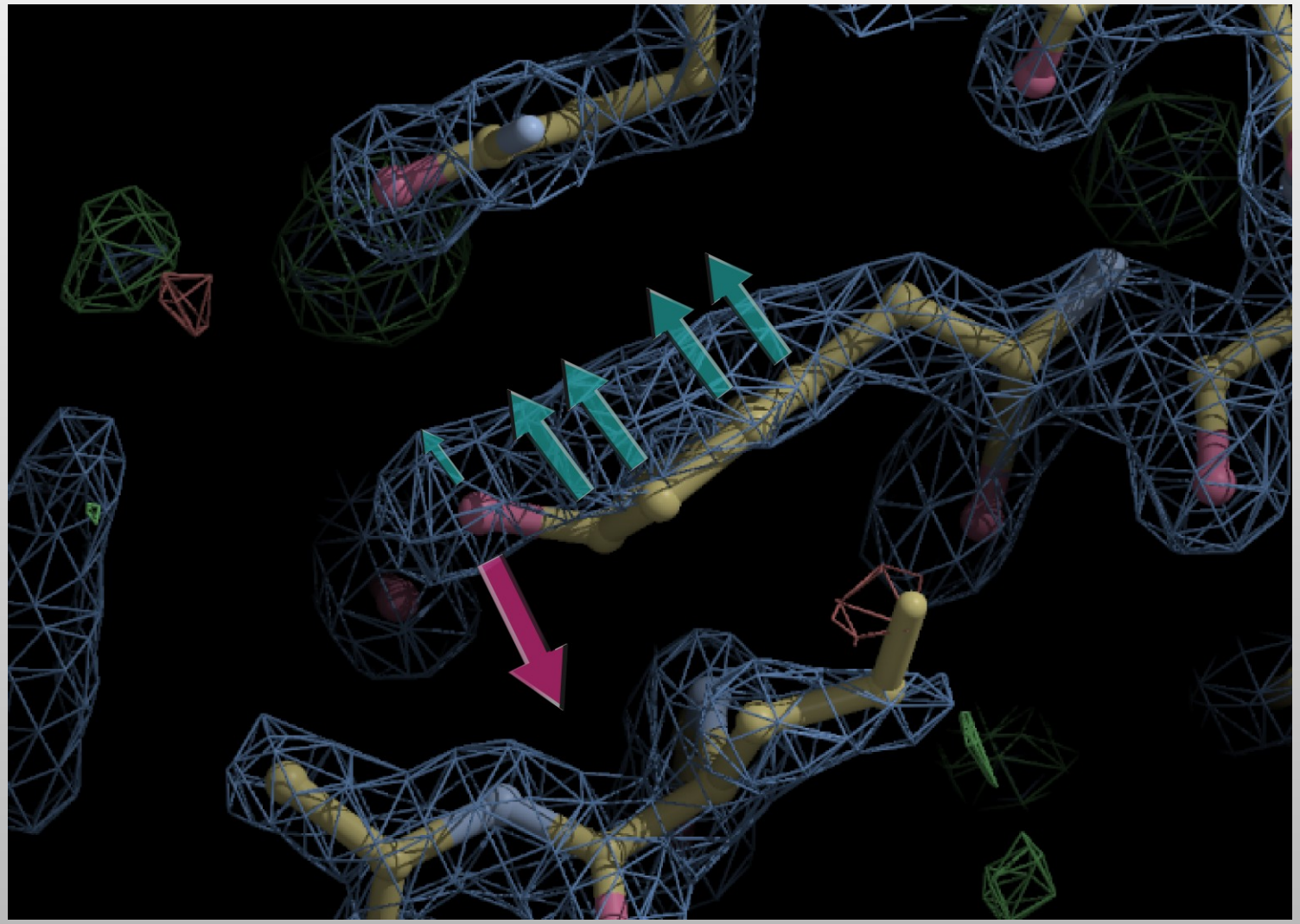
Real Space Refinement

- Distorted geometry pre-refinement



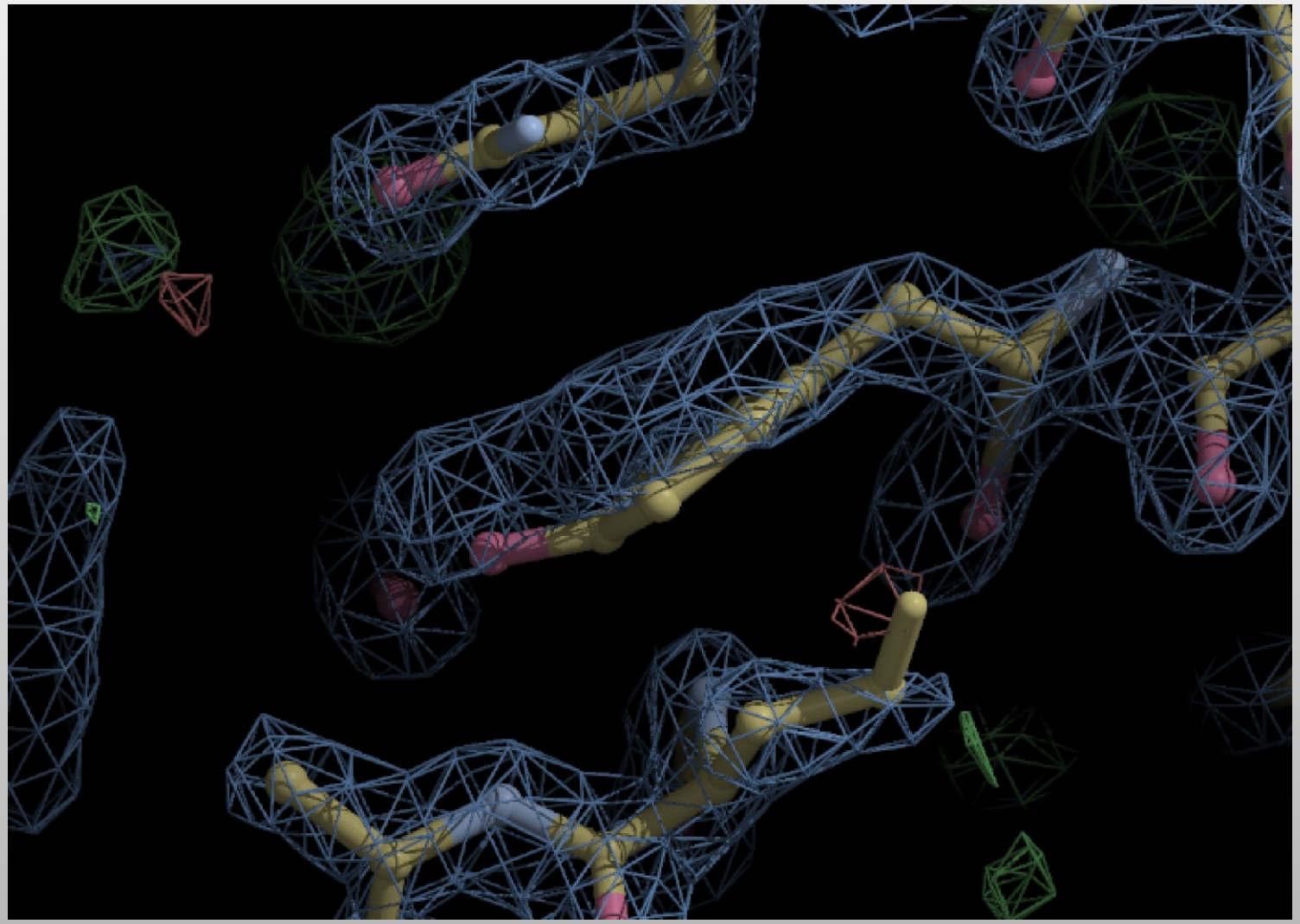
Real Space Refinement

- Refinement gradients



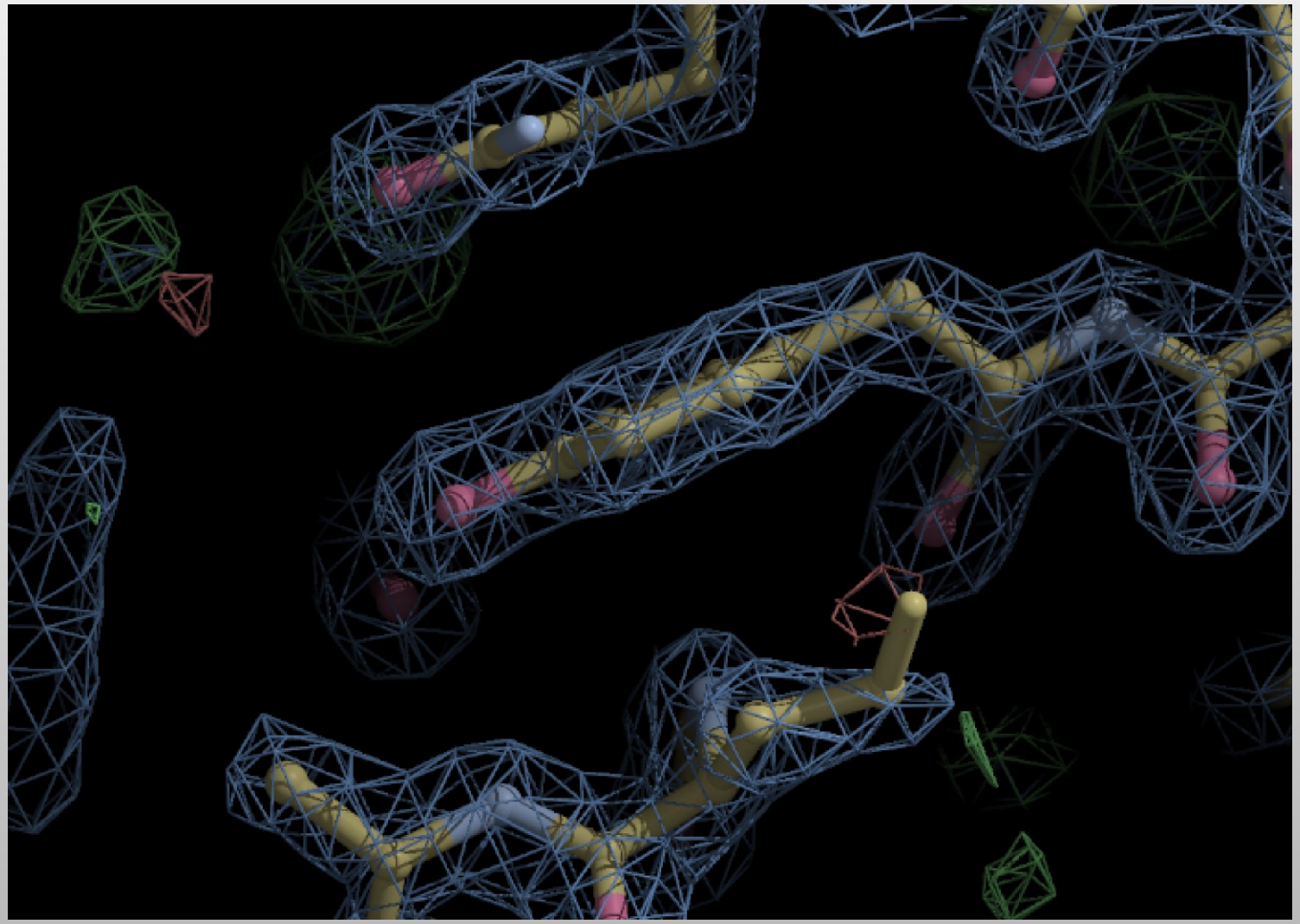
Real Space Refinement

- Refinement cycle 3



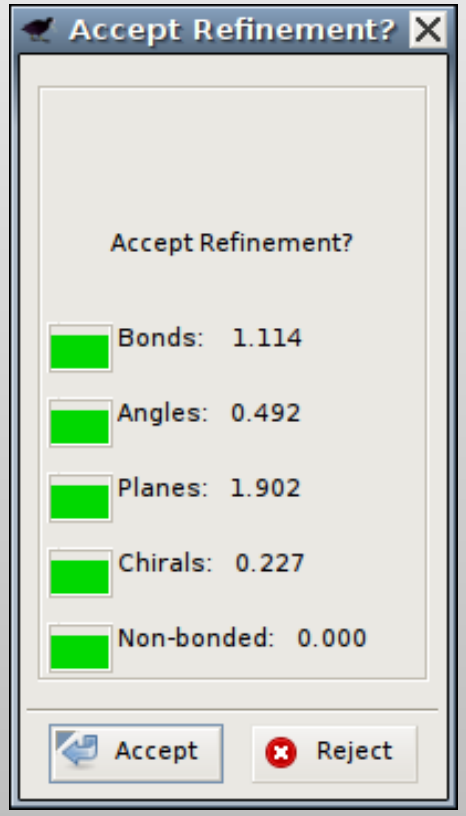
Real Space Refinement

- Refinement cycle 200: minimised

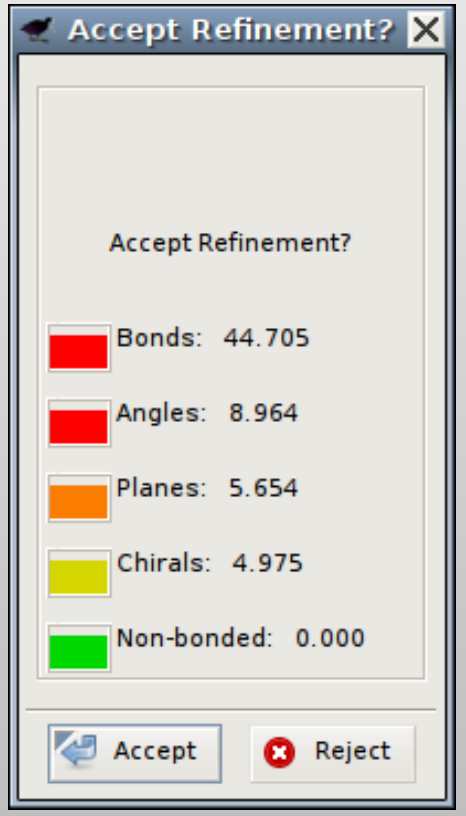


Representation of Results: “Traffic Lights”

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



Good refinement



Bad refinement

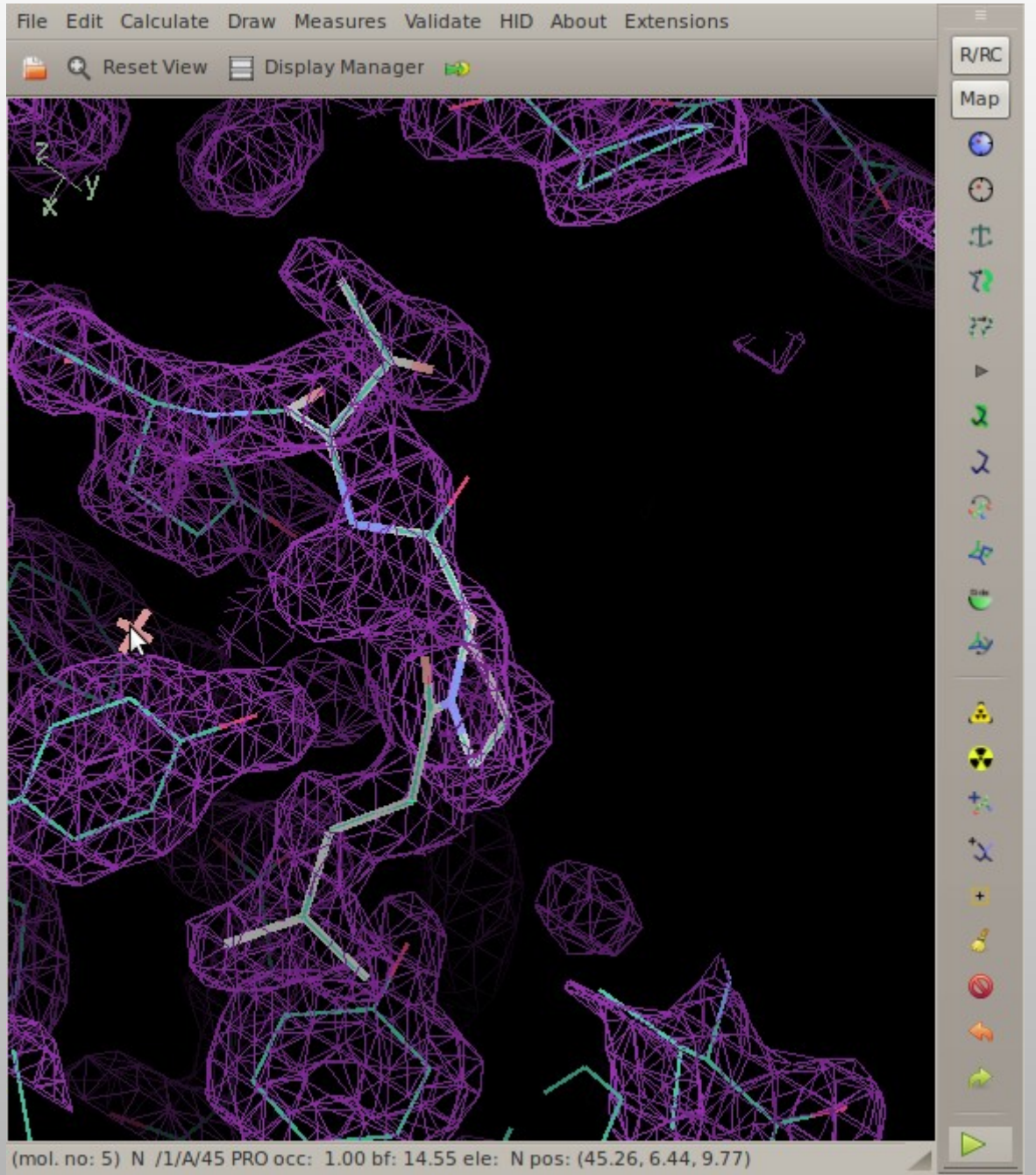
Refinement Techniques

- Auto-zone
- Single-Atom Drag
  - Over-dragging
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept
- Ramachandran Refinement
- Sphere refinement

Overdragging

- Overdragging

  Crtl left-mouse



Sphere refinement

- Given an “Active” Residue
- 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
- Most powerful keybinding!

Some more Coot Tools…

- Rotamer search
- Chi angle editing
- Alternate Conformations
- Add terminal residue
- Ligand fitting/search
- Rigid-body Fitting
  - Steepest Descent
  - Simplex (slower but better)
- “Move Molecule Here”
- Water Search
- Fill-partial-residues (post-MR)
- “All model” tools (post-MR) eg stepped refine
- Additional representations:
  dots, ball&stick, CA trace etc representation

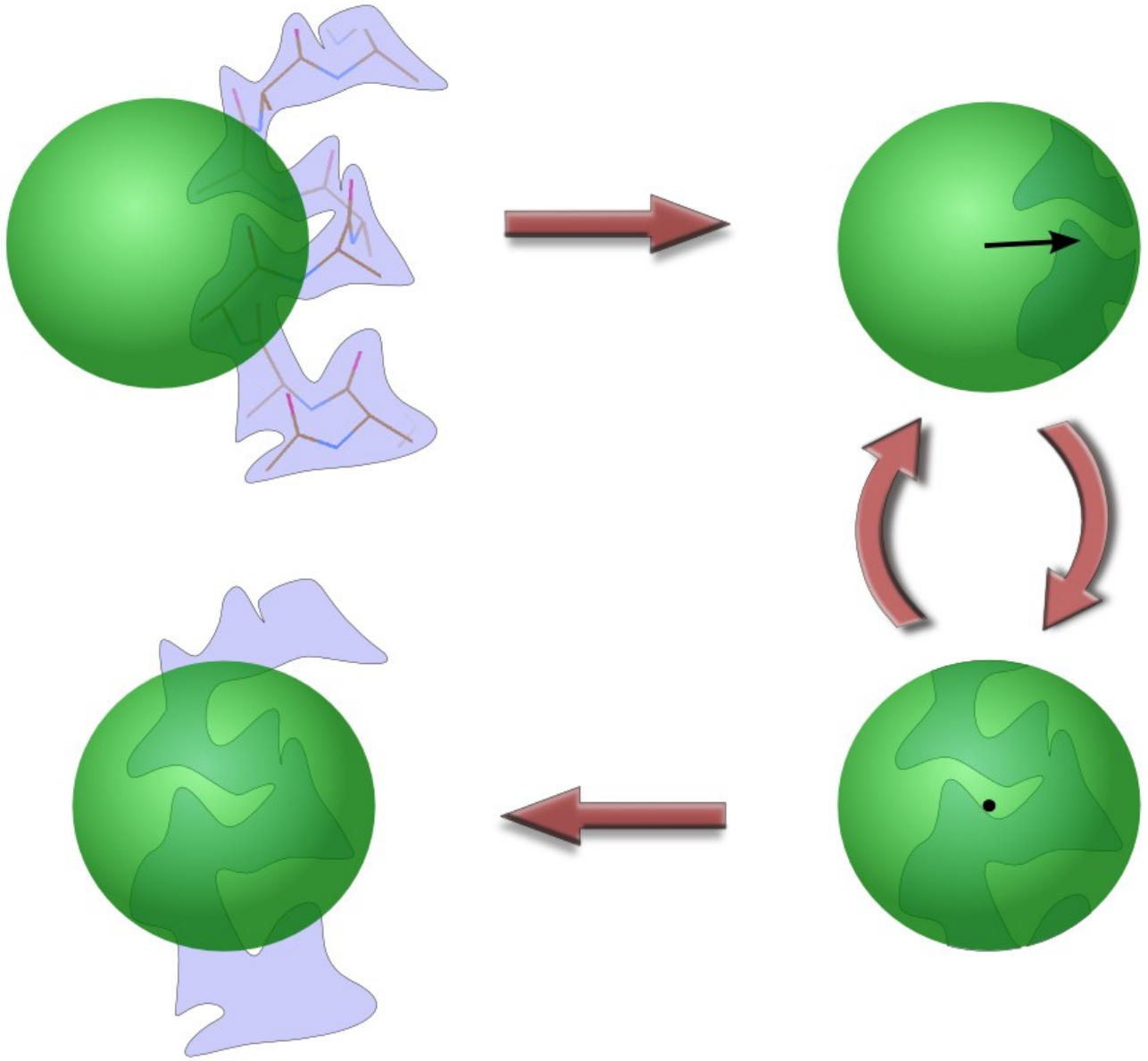
... and many more

Building from scratch

Alpha Helix Placement

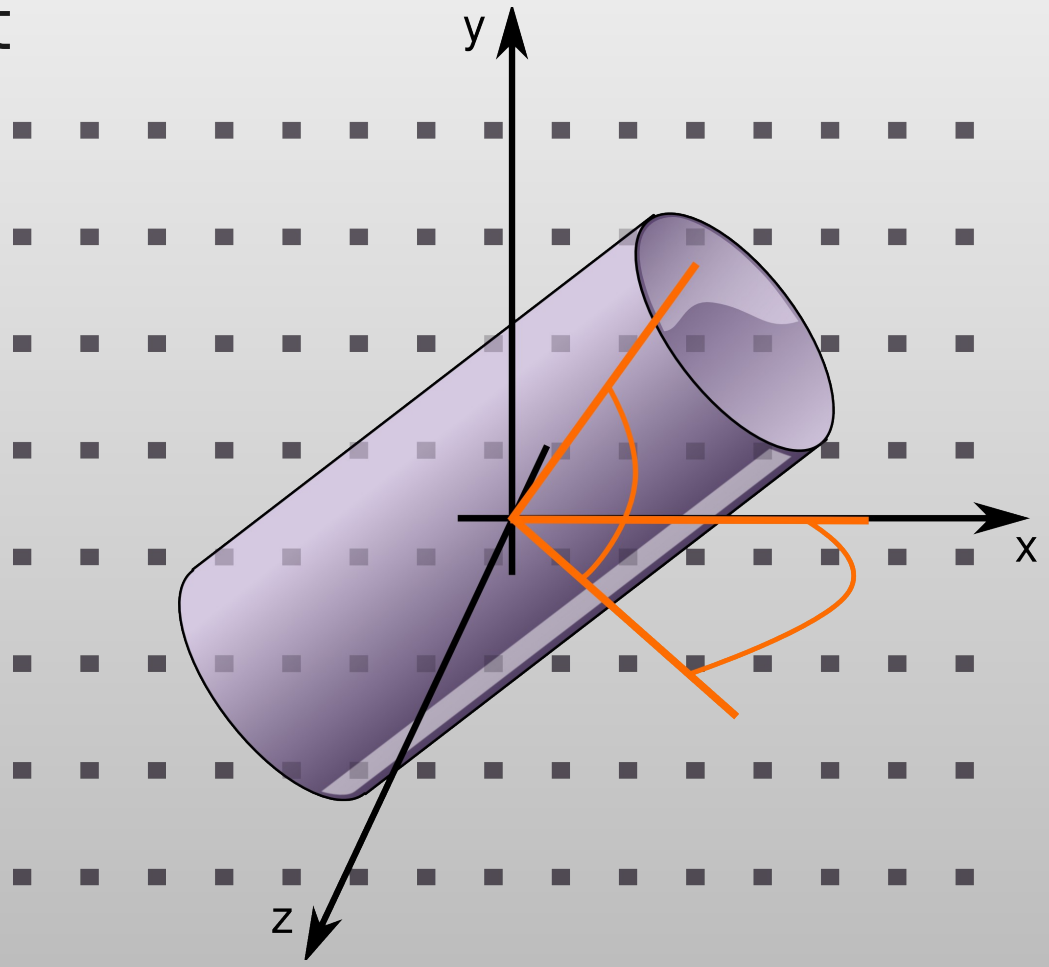
- Scenario: Looking at a new map, not built with automatic tools:
  - “I can see that there’s a helix here - build it for me!”
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

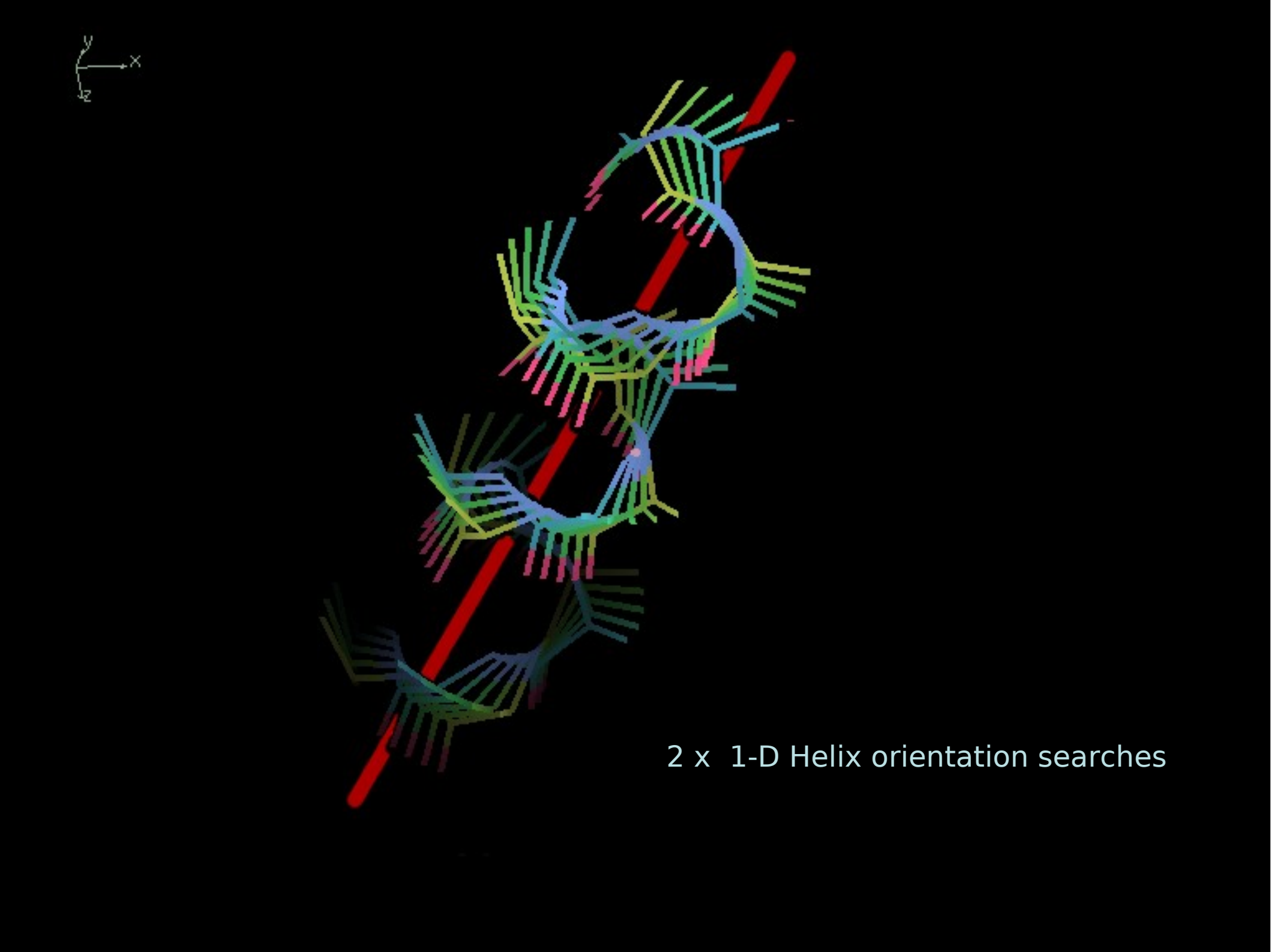


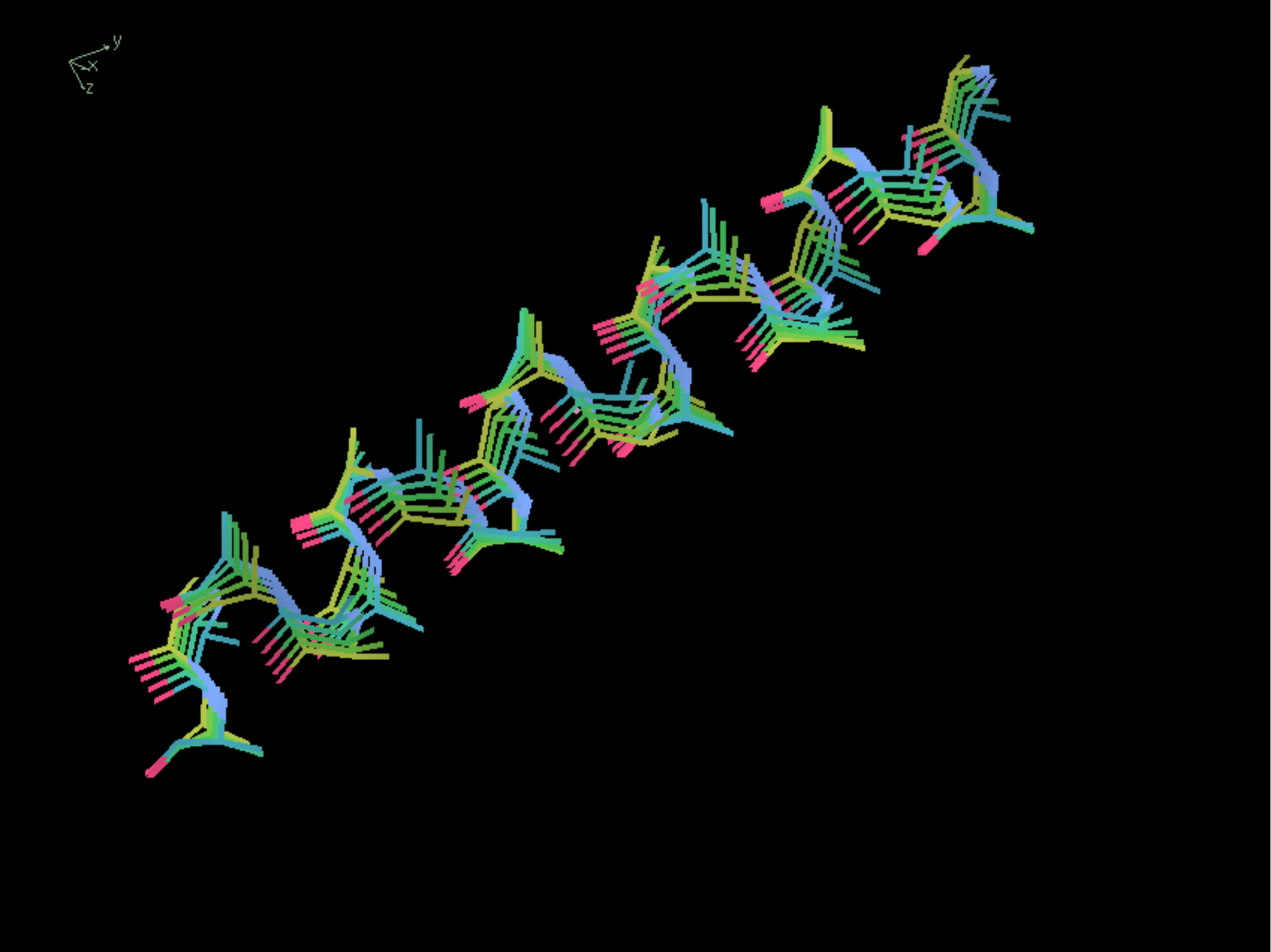
Cylinder Search

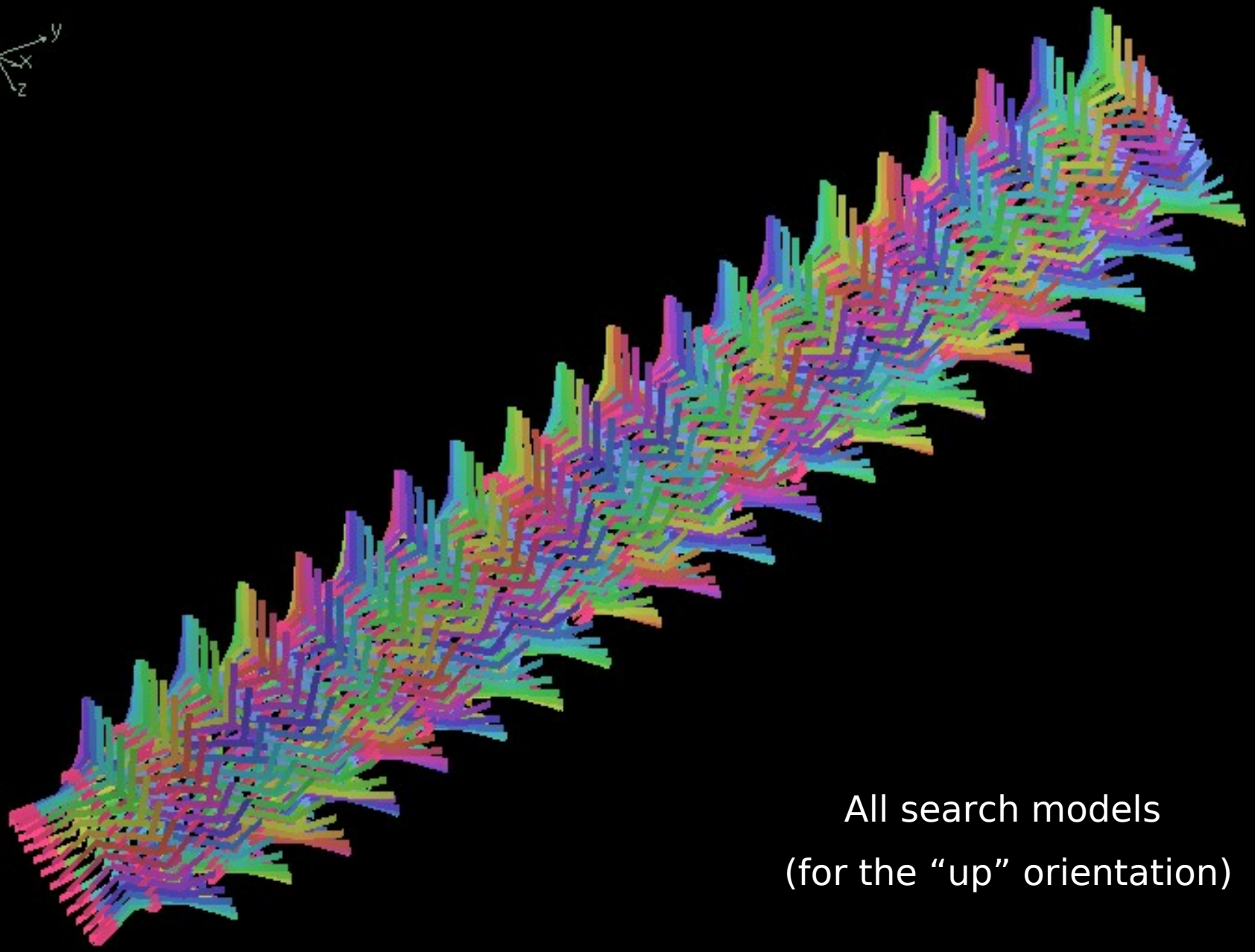
- Pick the orientation that encapsulates the most electron density



Using 2 rotation axes

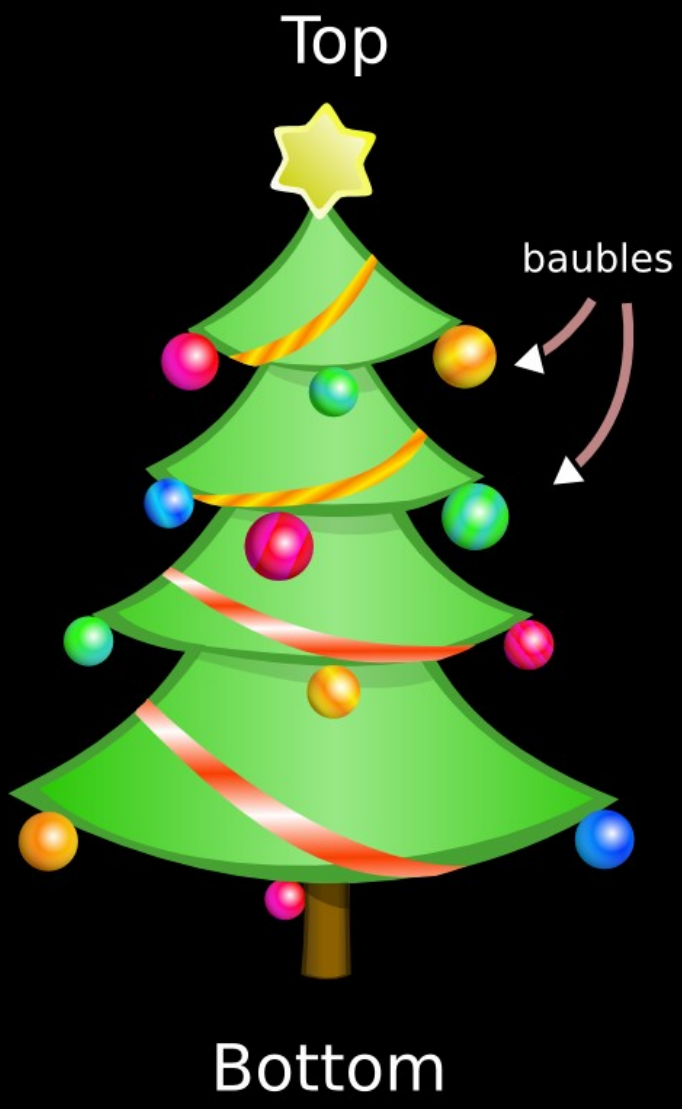

y
x
z
2 x 1-D Helix orientation searches

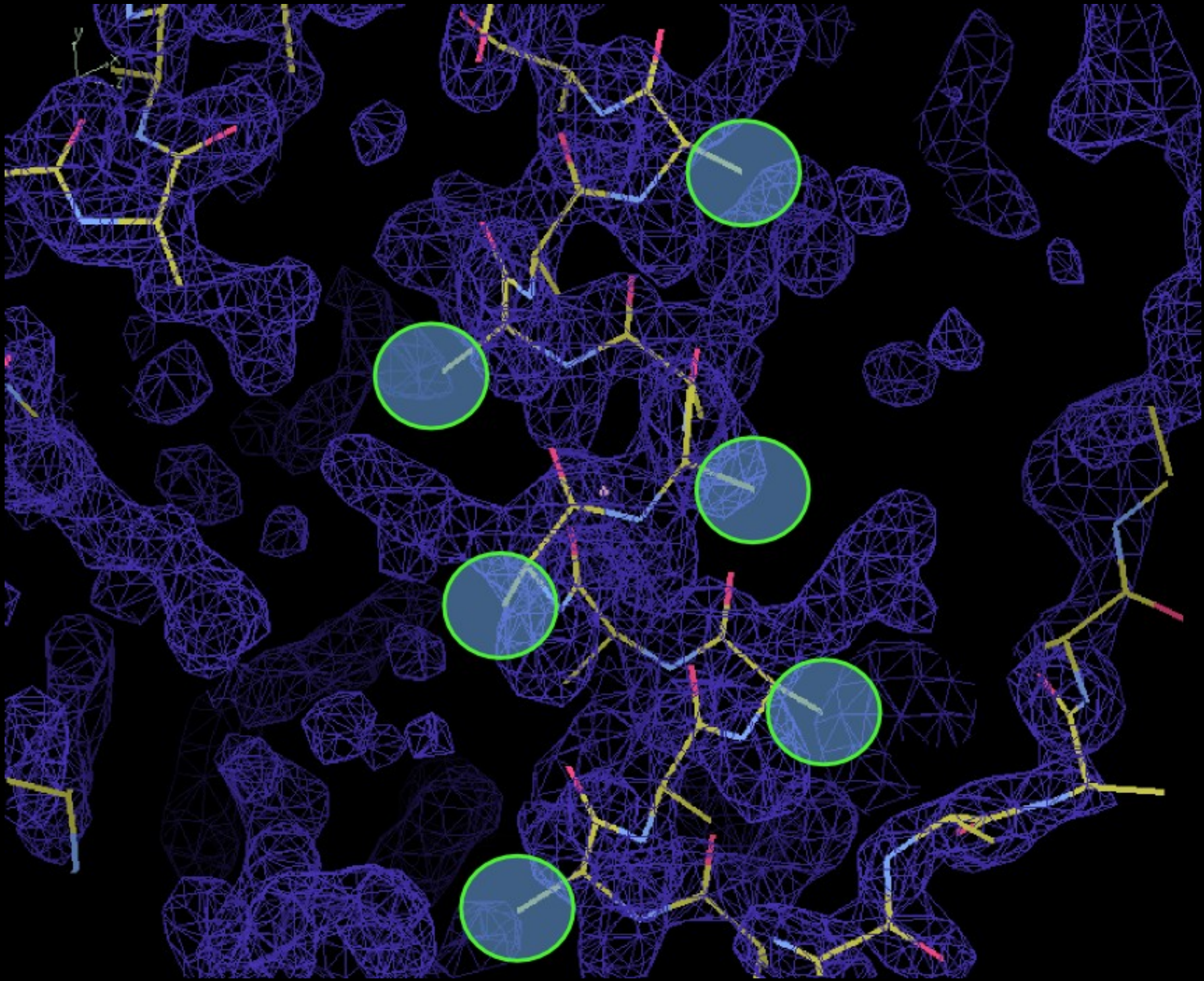

y
x
z

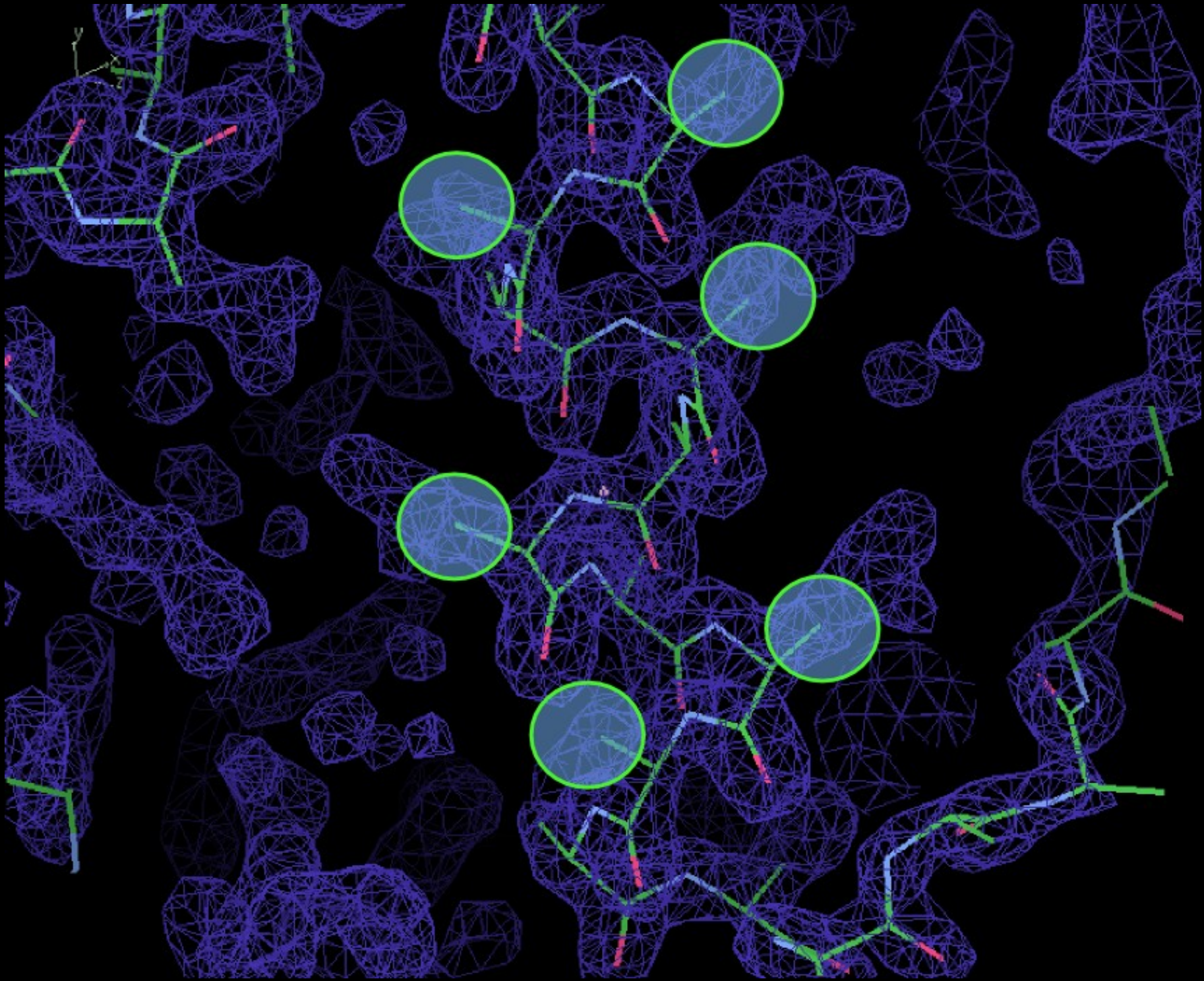


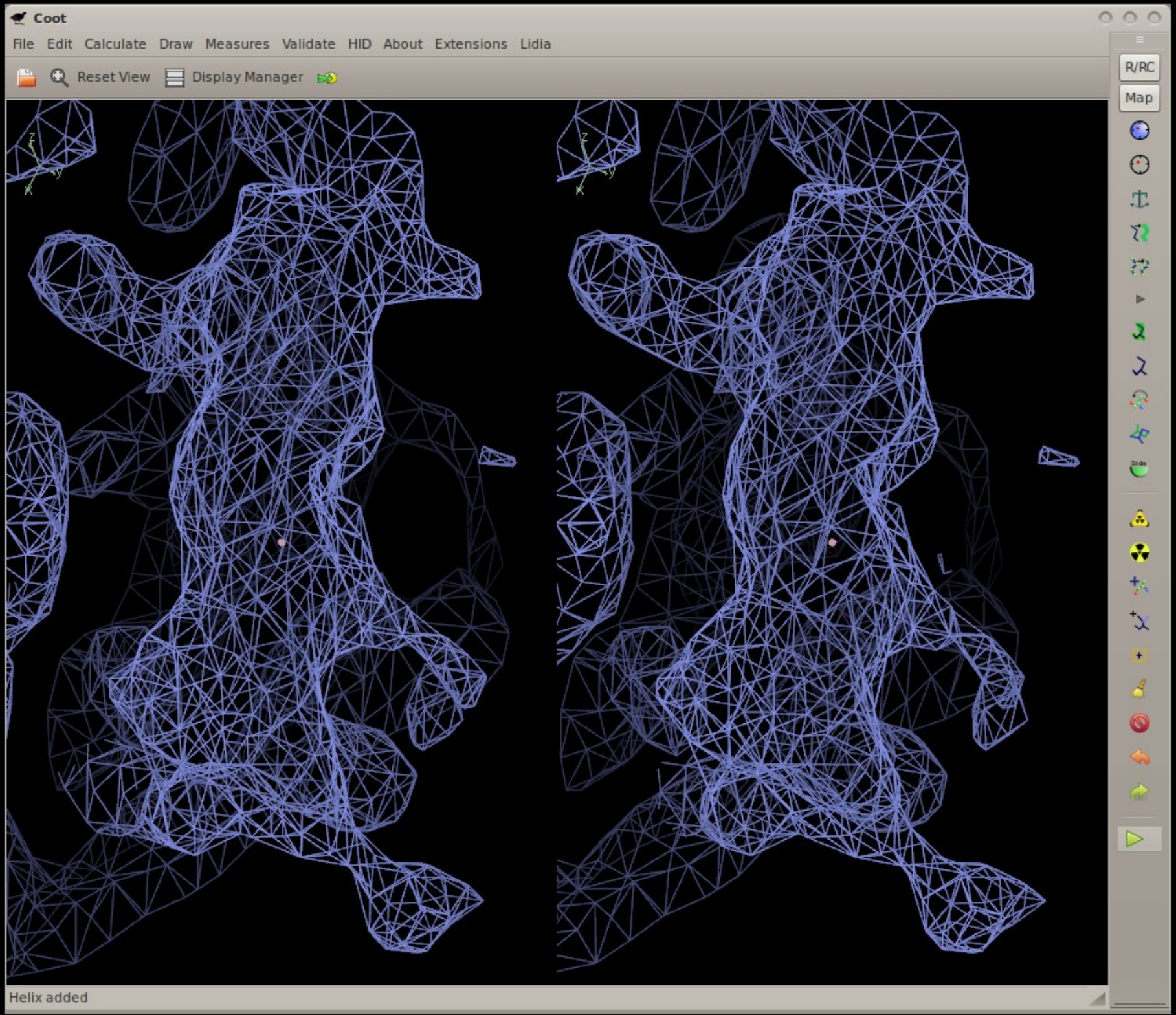
All search models

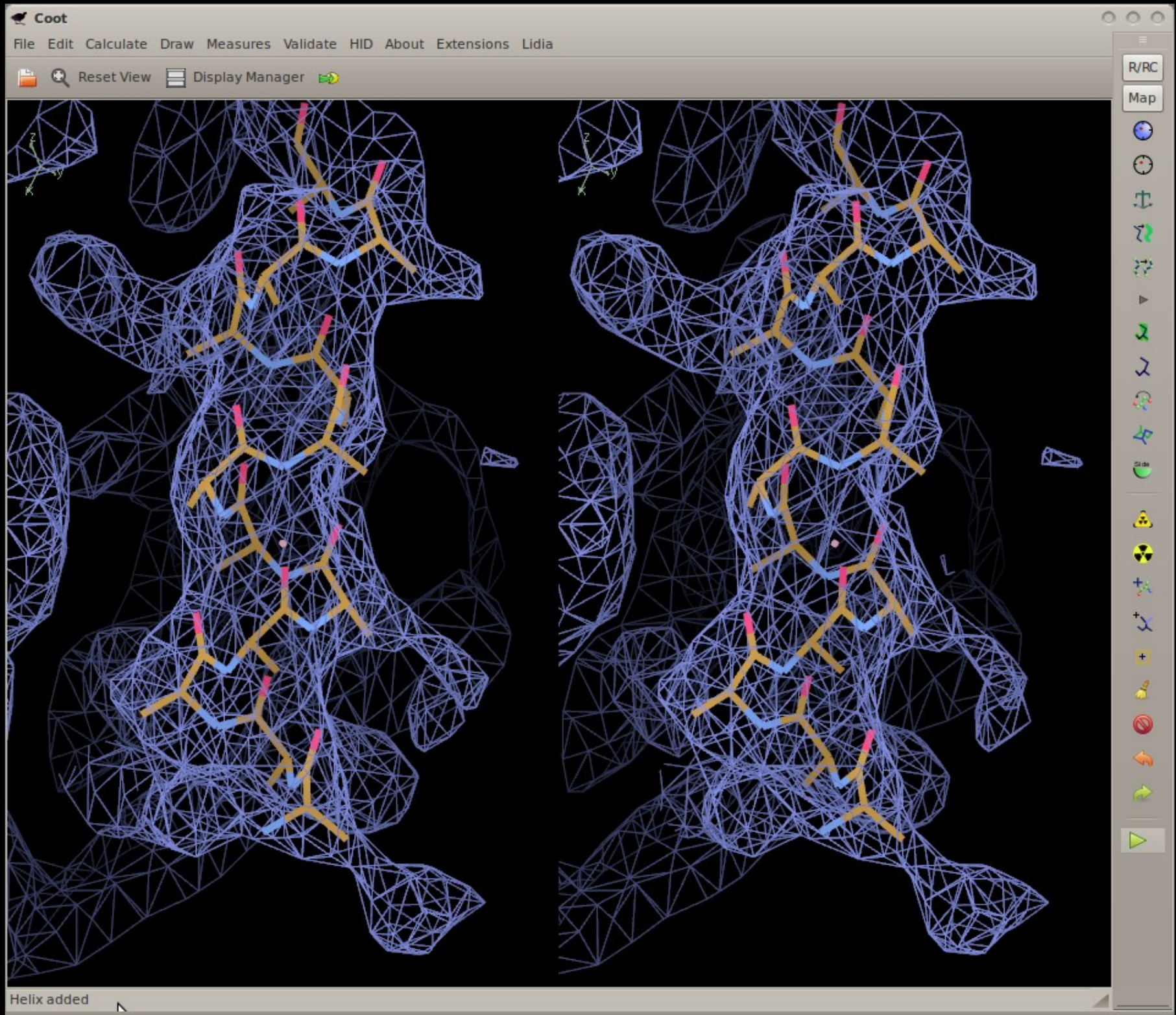
(for the “up” orientation)


Top
baubles
Bottom






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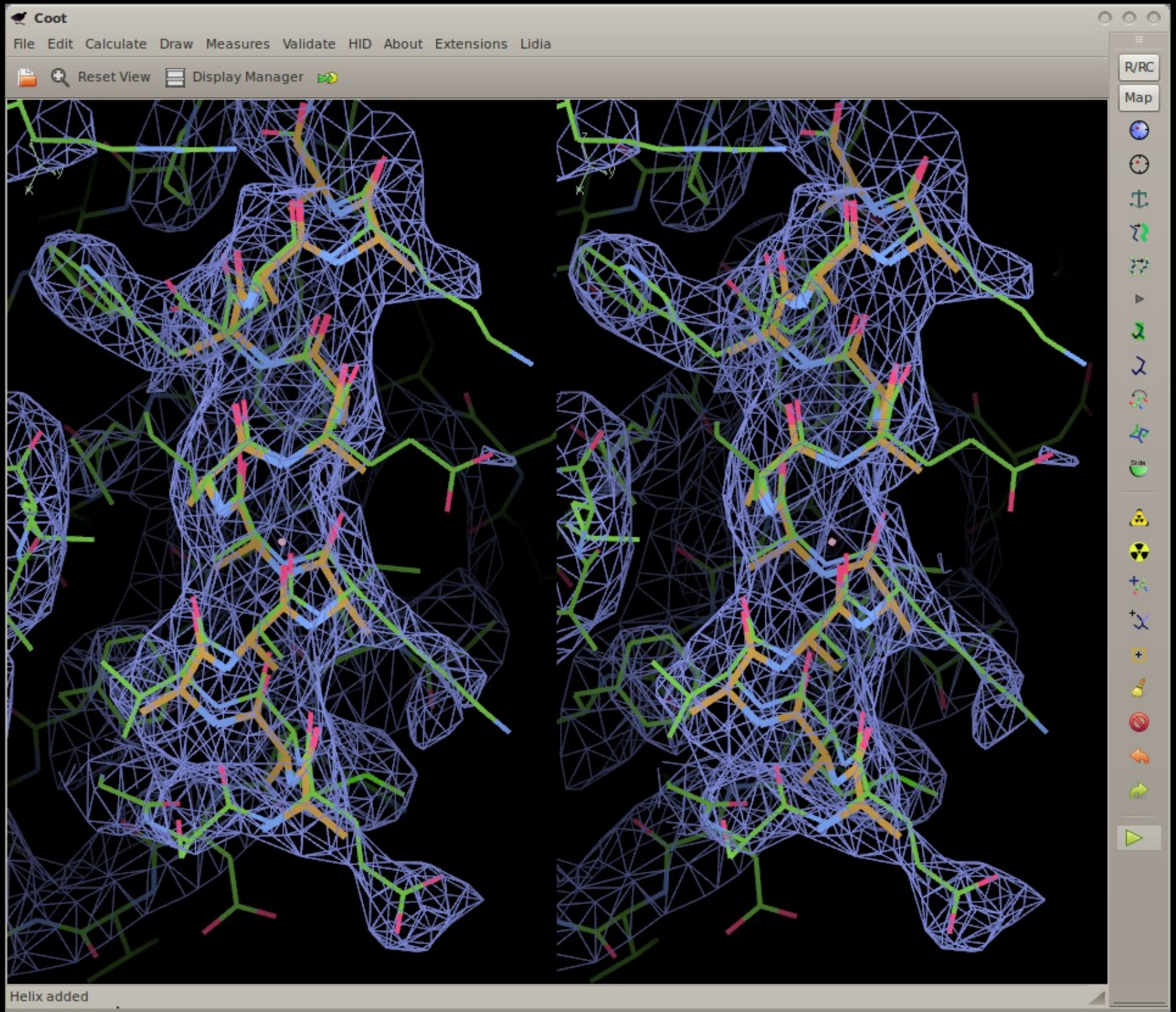
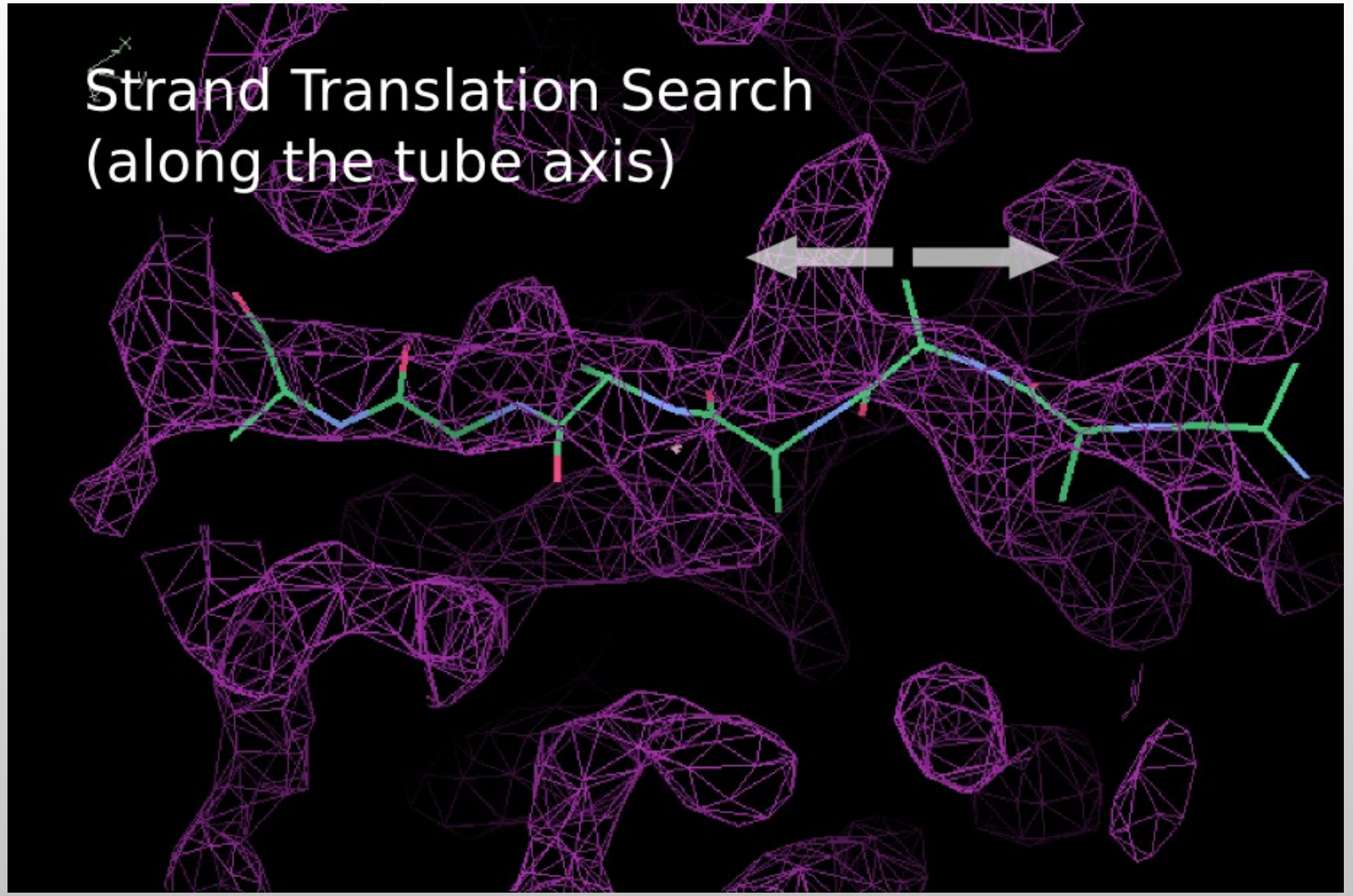

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added

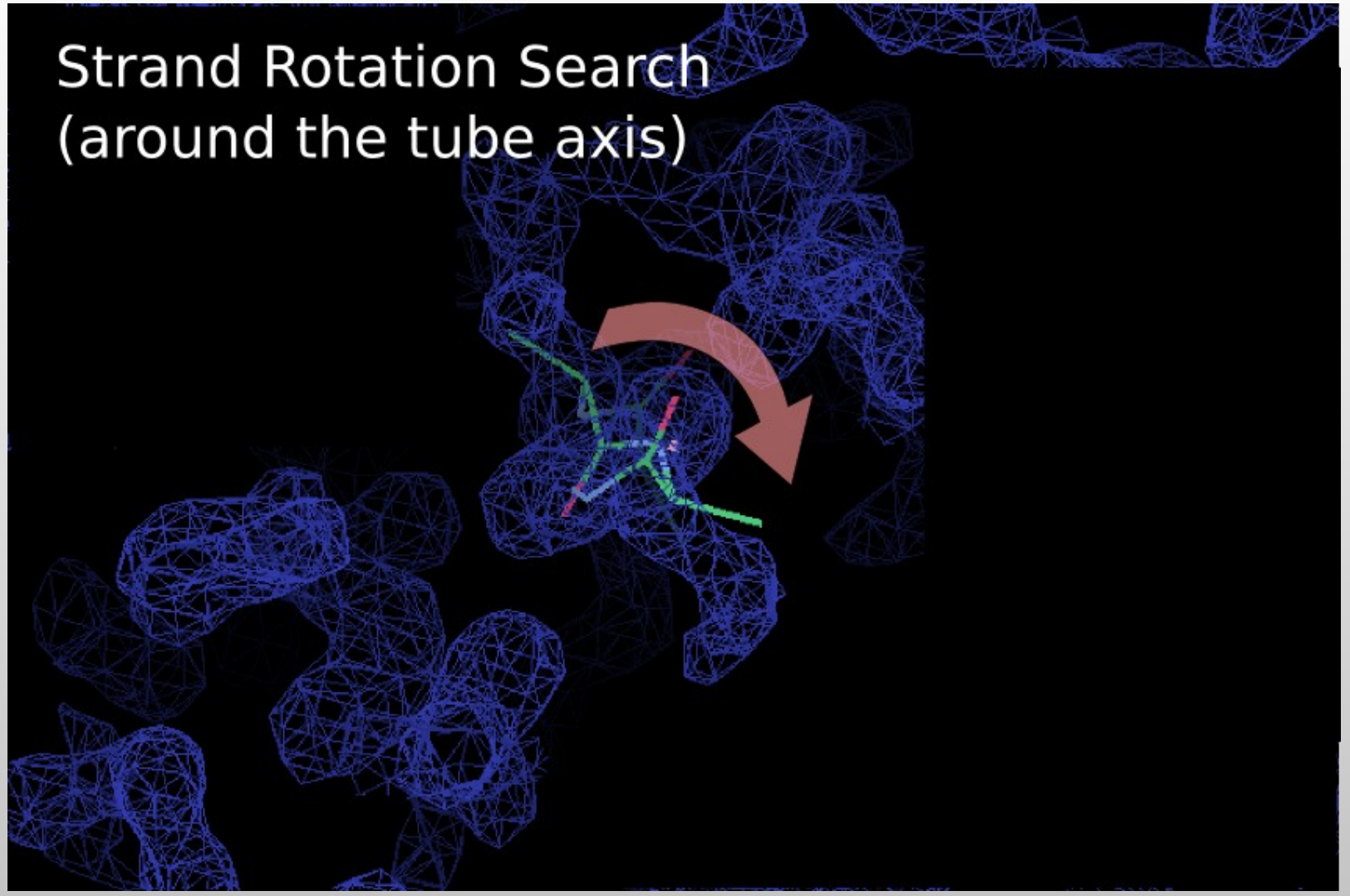
Placing Strands

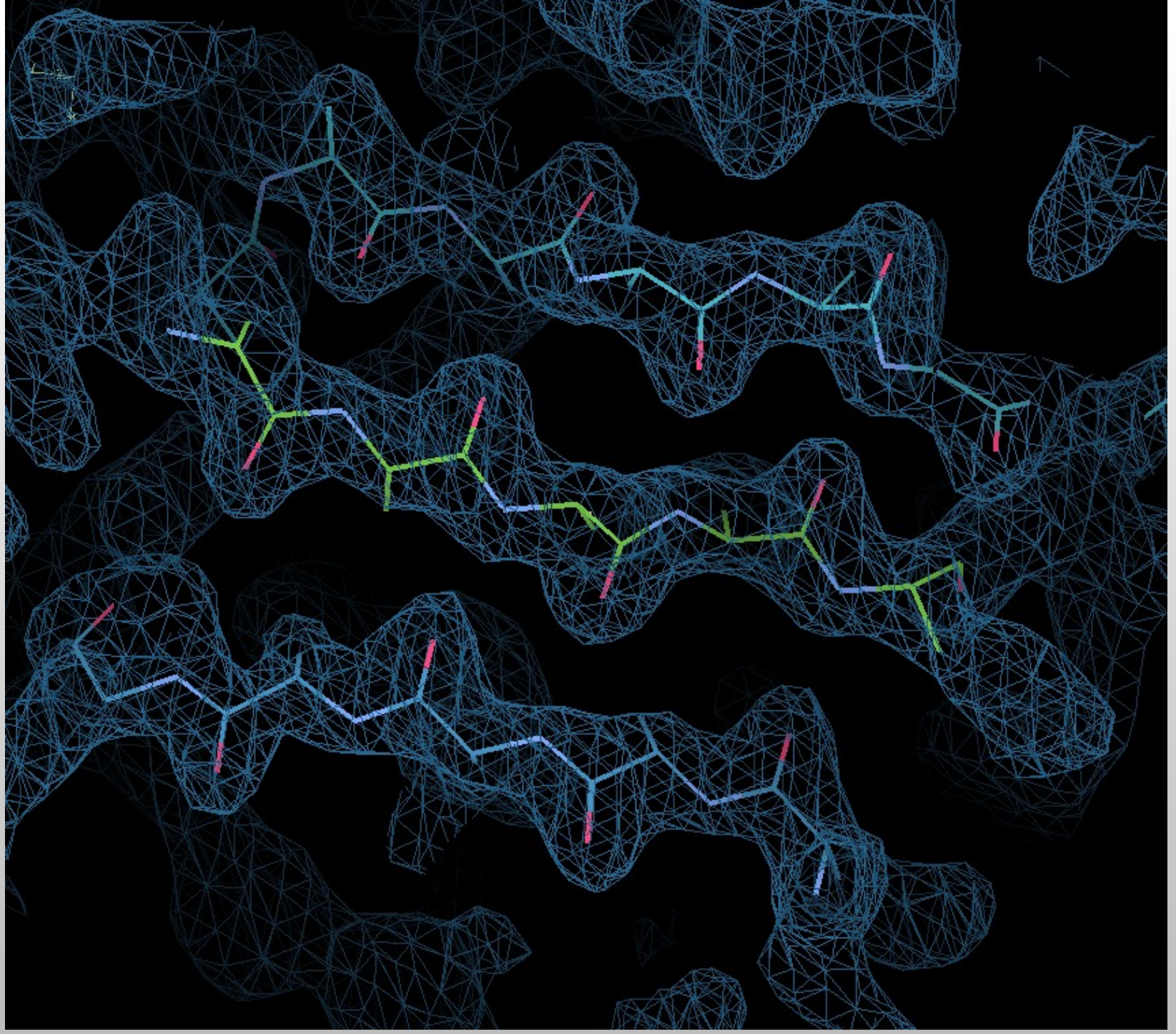
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

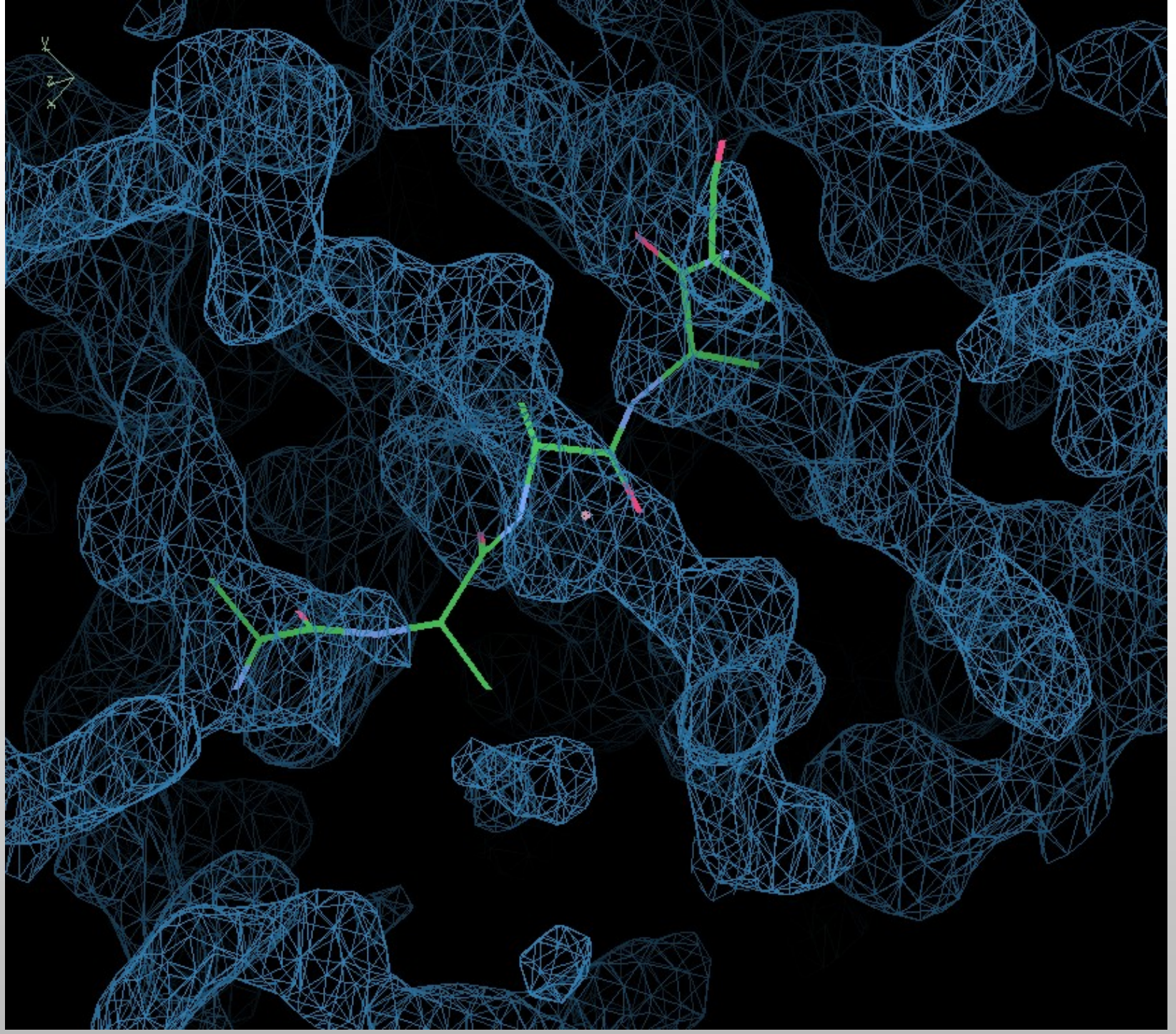
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution


Strand Translation Search
(along the tube axis)


Strand Rotation Search
(around the tube axis)



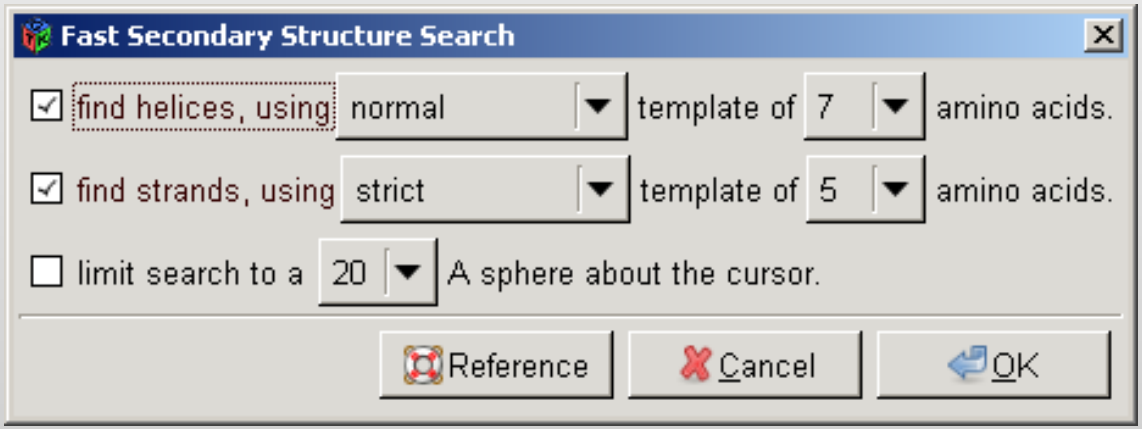
Not all is rosy...



Fitting Strands caveat

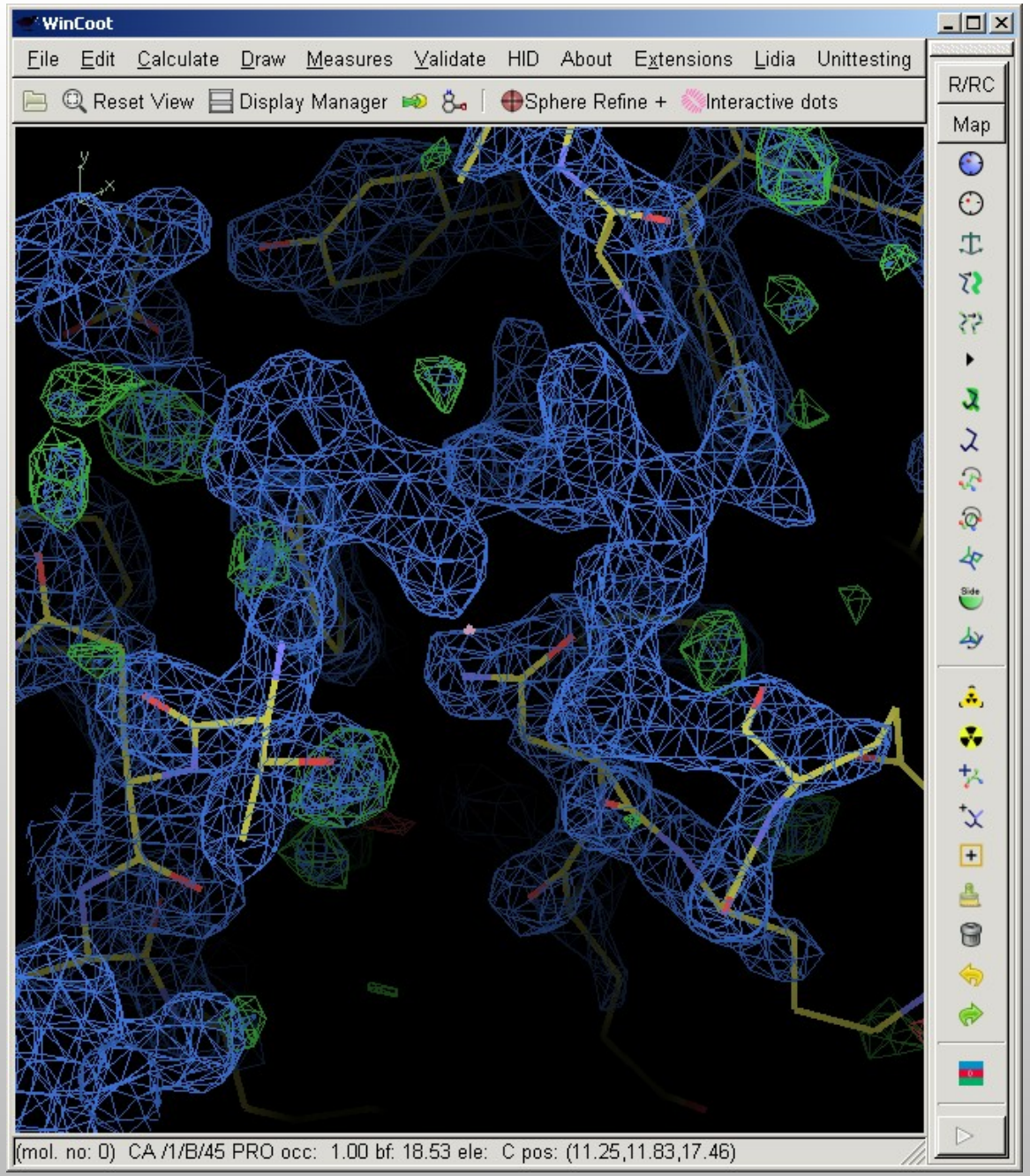
- In the case of strand-fitting, the initial translation search centring the cylinder is not performed (the search cylinder is too thin)
- The user is responsible for centring the search point “in the middle of the tube”
- Not at a C-alpha position

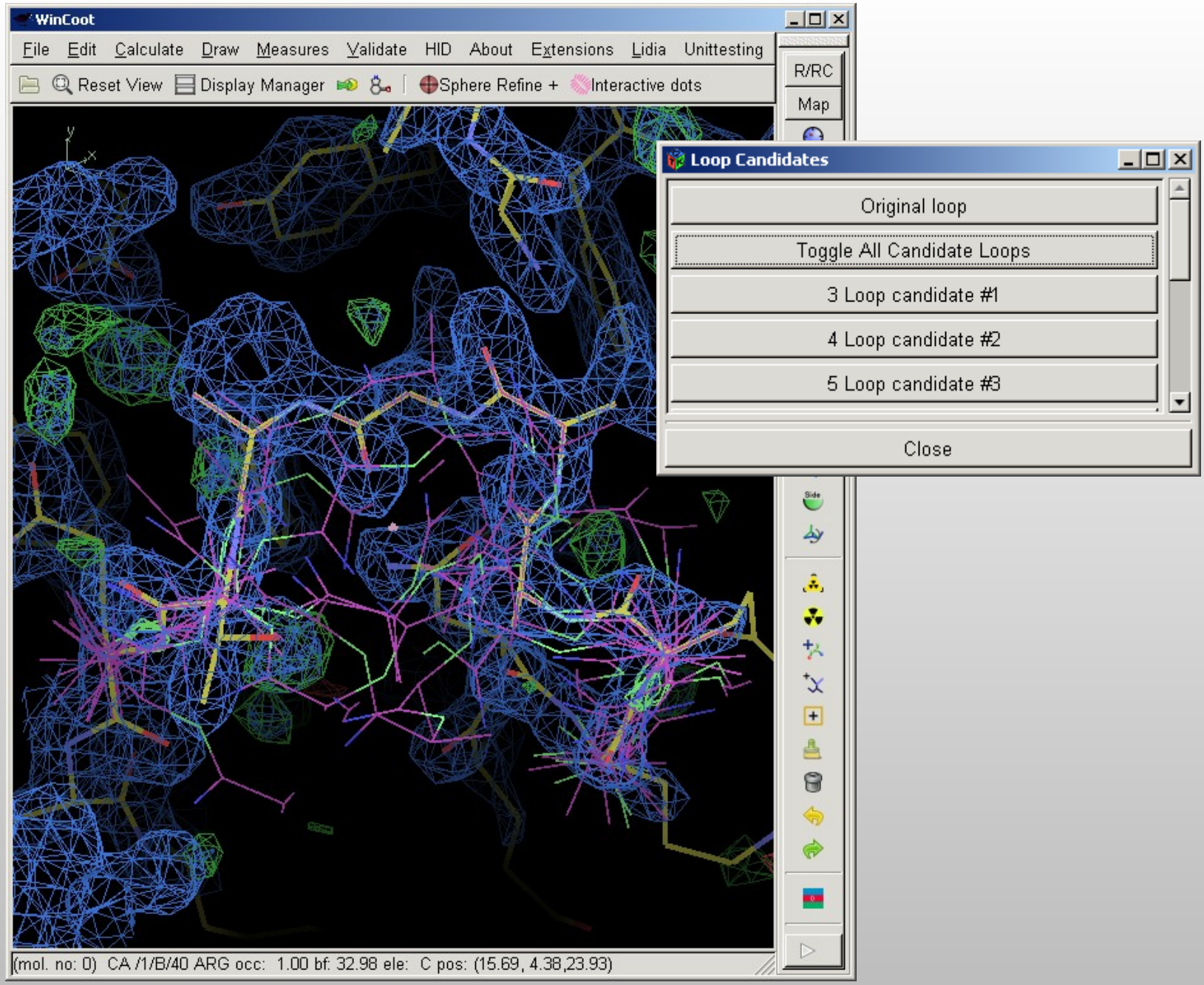
Automated Fast Secondary Structure Search



Loop fitting

- Simple loop fitting
  - Add residue by residue (from both termini)
- DB loop
  - Fitting fragments from database


WinCoot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia Unittesting
Reset View
Display Manager
Sphere Refine +
Interactive dots
R/RC
Map
(mol. no: 0) CA /1/B/45 PRO occ: 1.00 bf: 18.53 ele: C pos: (11.25,11.83,17.46)


WinCoot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia Unittesting
Reset View
Display Manager
Sphere Refine +
Interactive dots
R/RC
Map
Loop Candidates
Original loop
Toggle All Candidate Loops
3 Loop candidate #1
4 Loop candidate #2
5 Loop candidate #3
Close
(mol. no: 0) CA /1/B/40 ARG occ: 1.00 bf: 32.98 ele: C pos: (15.69, 4.38,23.93)

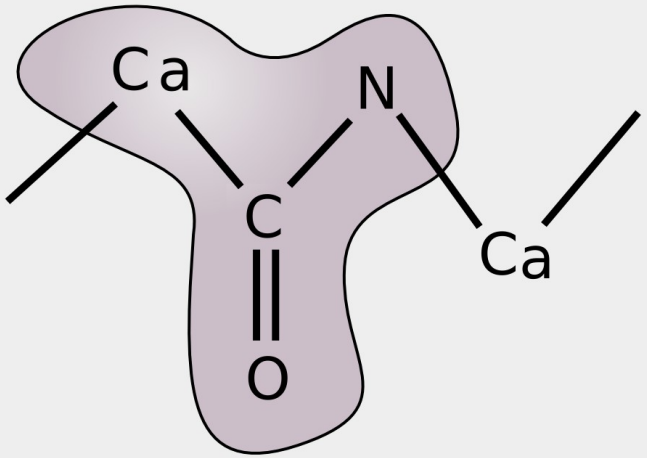
Tools for low resolution

Extra Restraints....

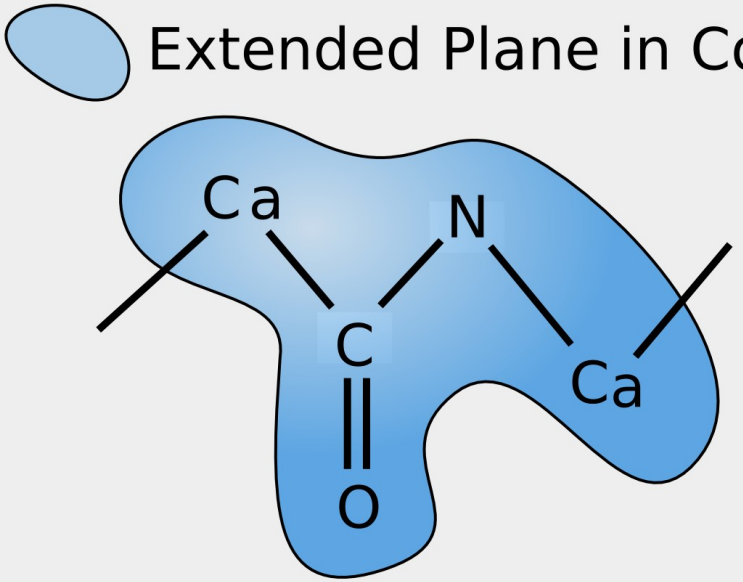
- Peptide plane
- Ramachandran restraints
- Secondary structure restraints
- Remove degree of freedom
  - Torsion angle restraints
  - Backrub rotamers
- Manually add restraints

Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane



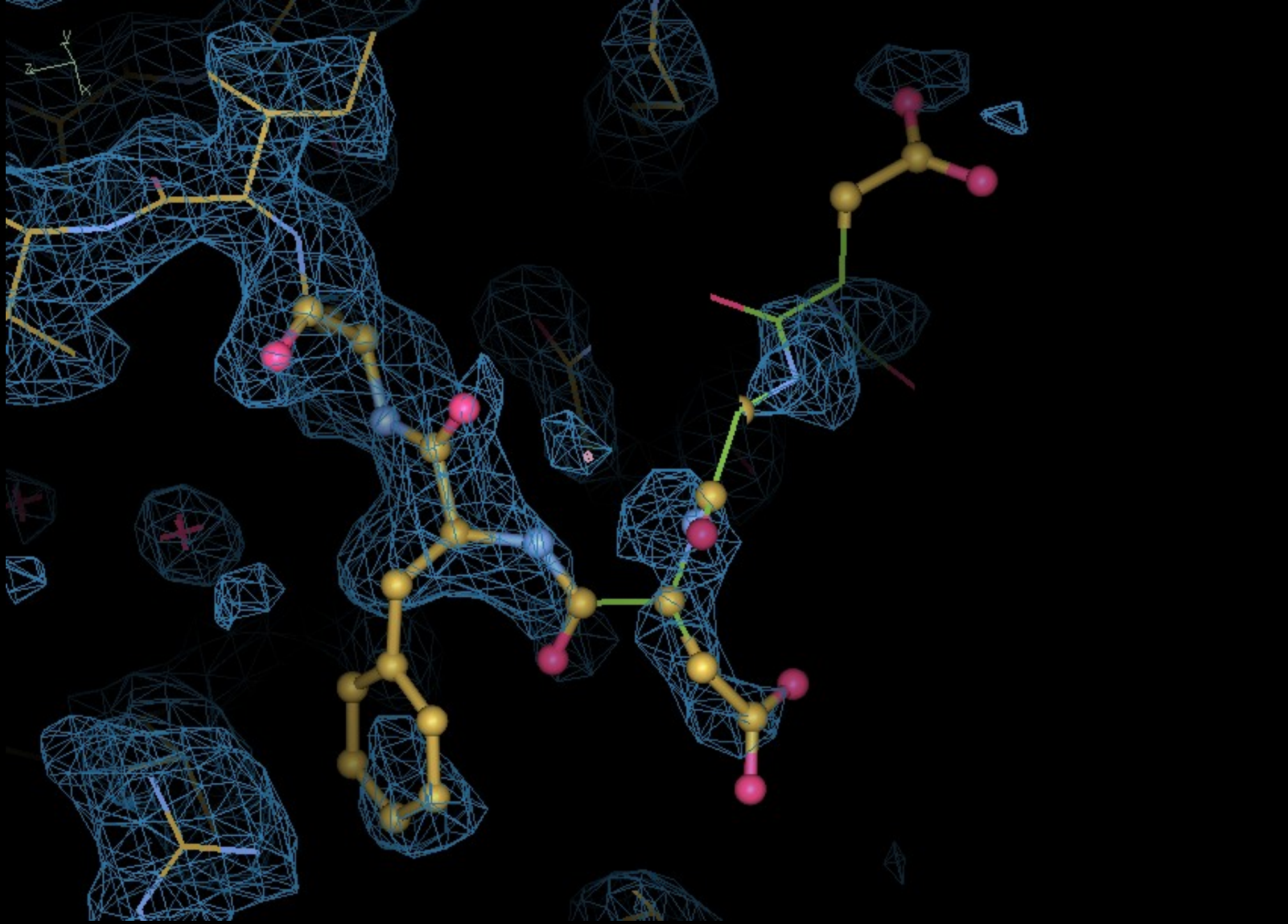
Extended Plane in Coot



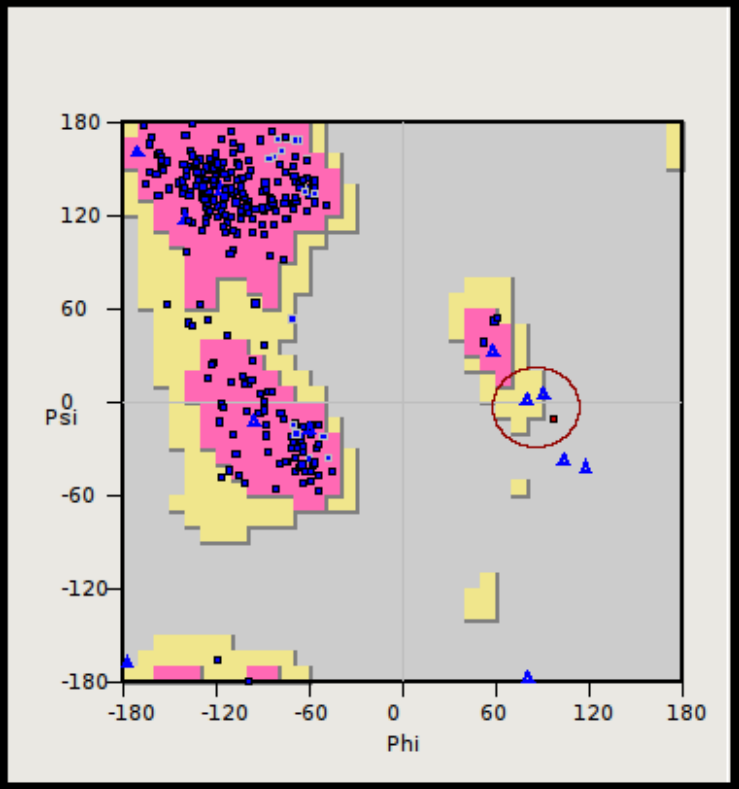
(add-planar-peptide-restraints)

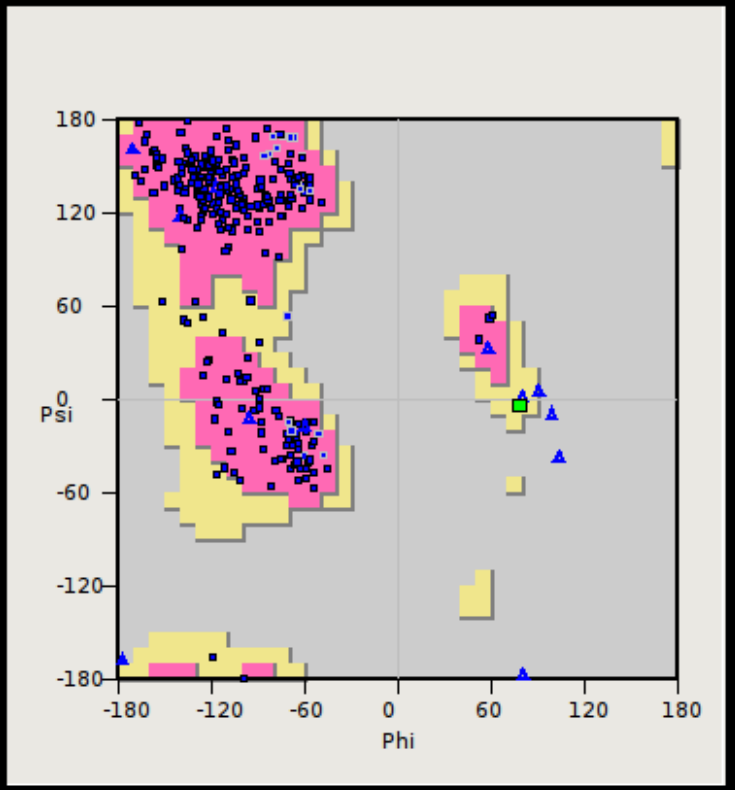
Ramachandran Restraints

- 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

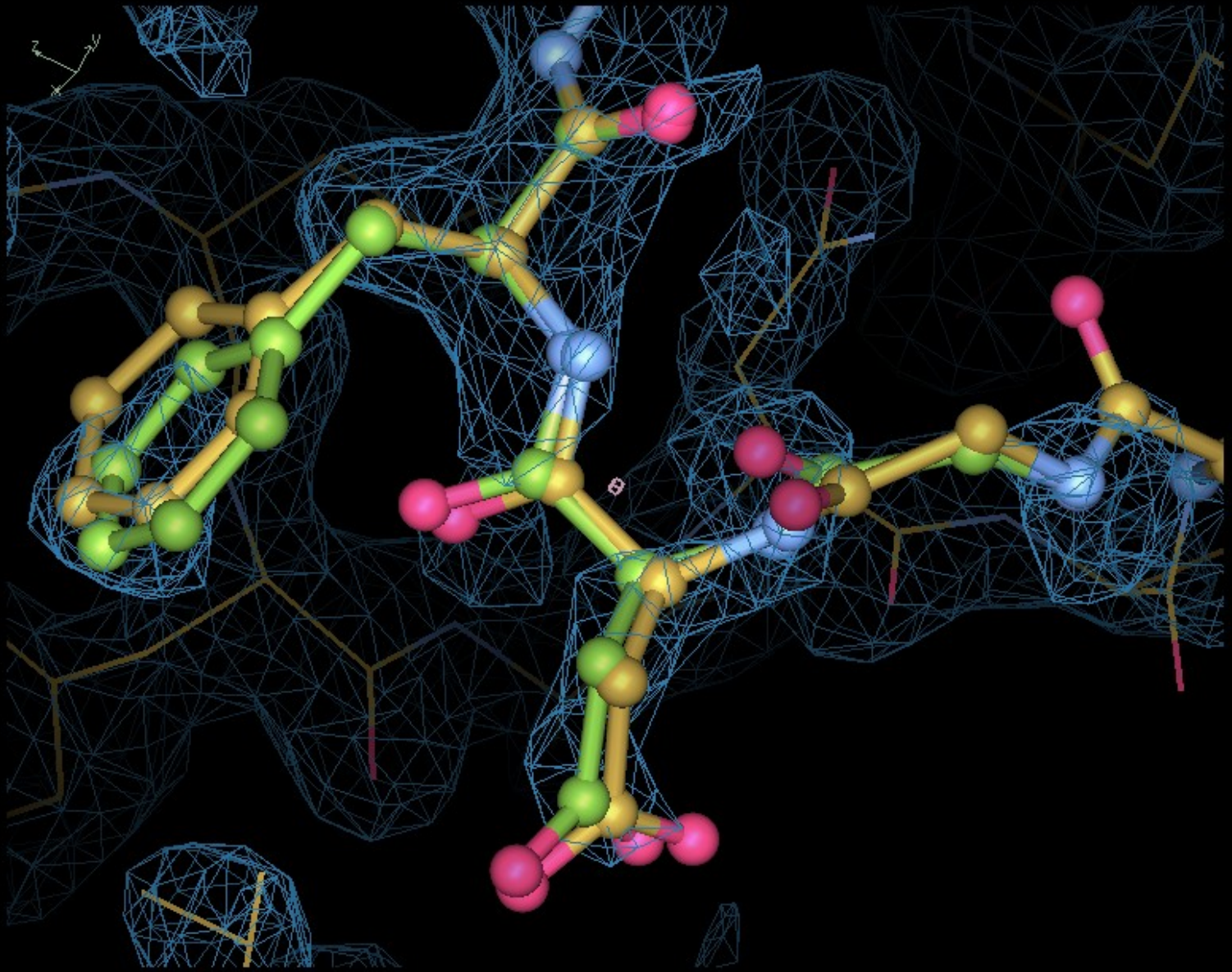

Y
Z
X

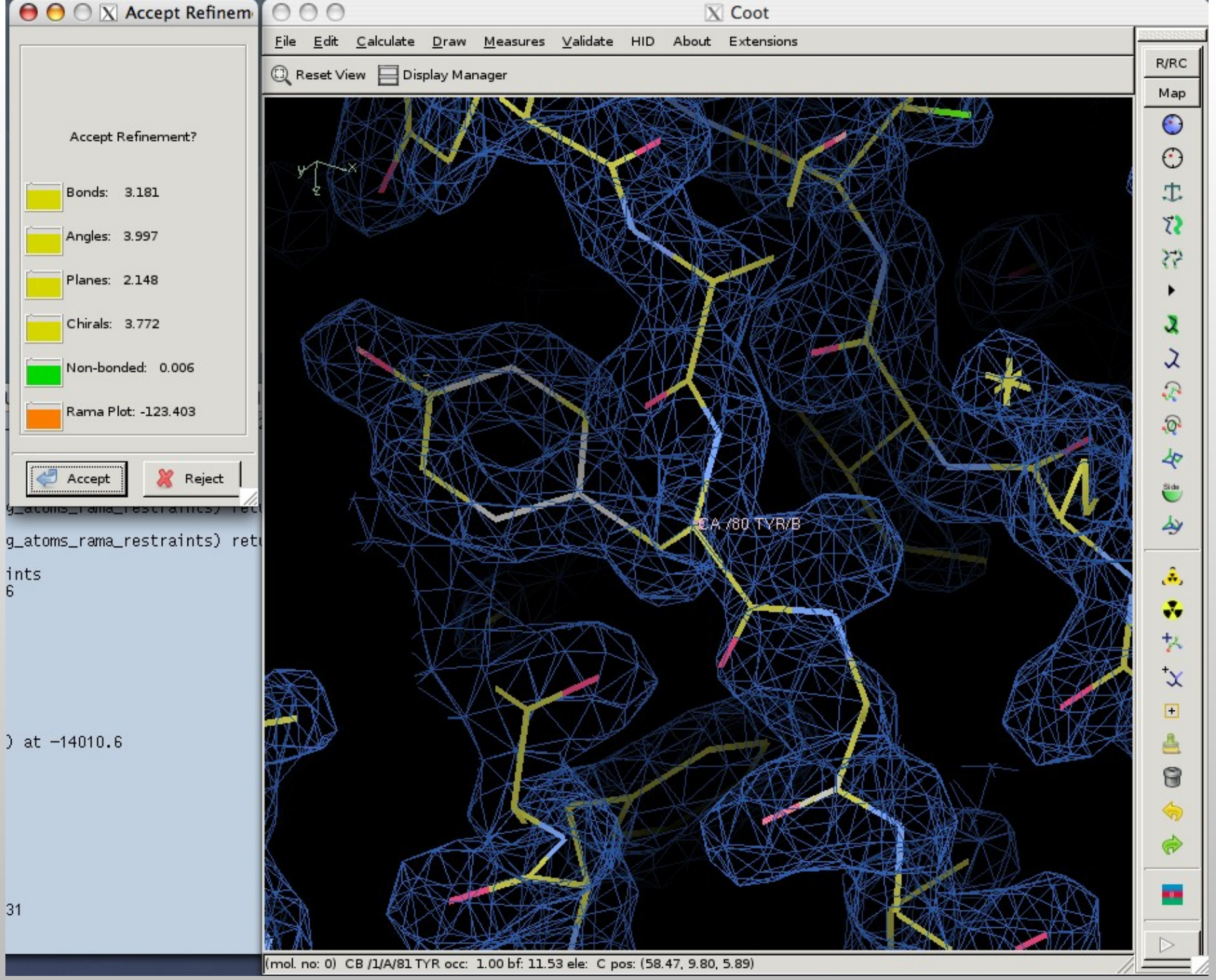
Tweaking a Ramachandran Outlier

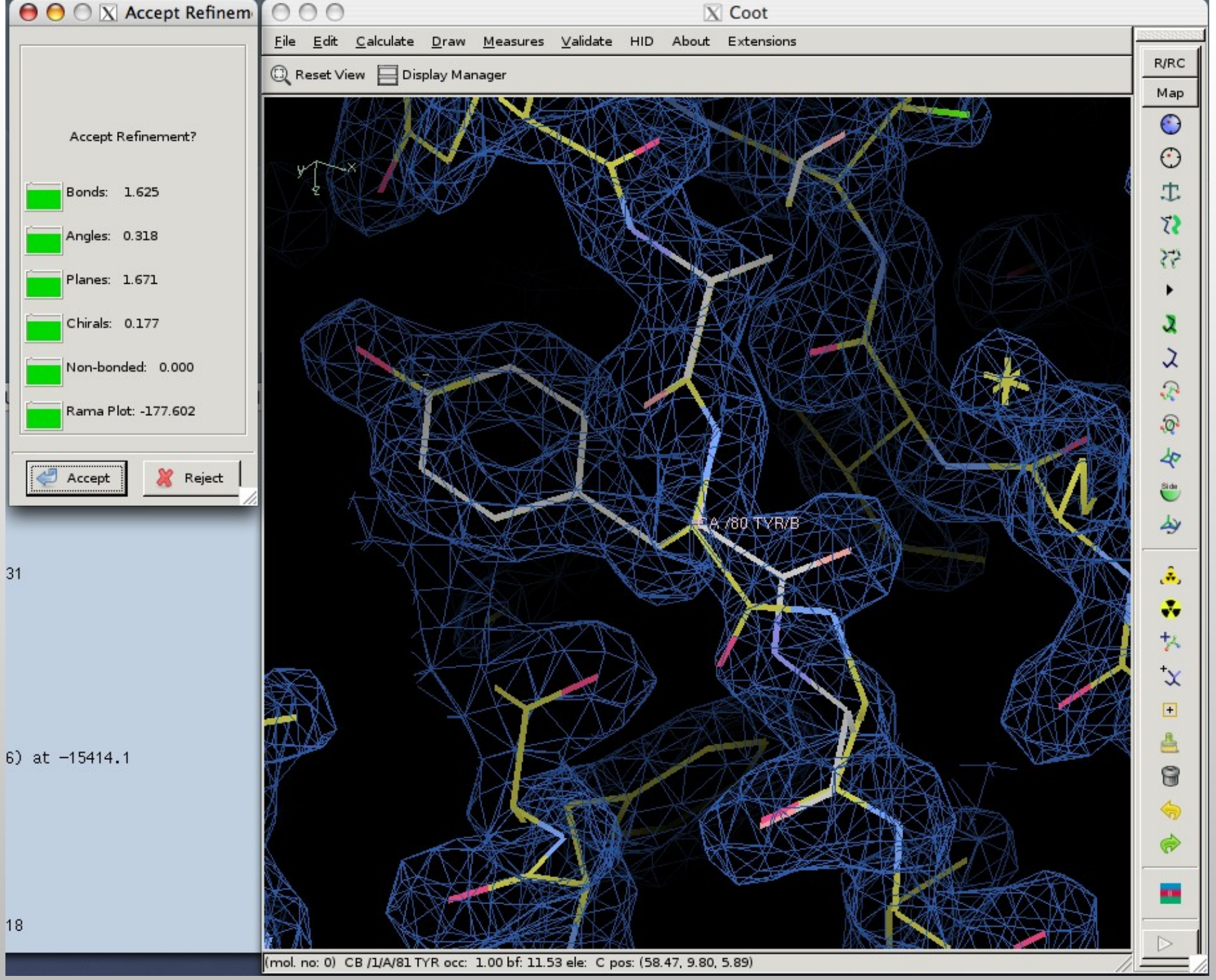




Tweaking Phi and Psi




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
g_atoms_rama_restraints) retu
ints
6
) at -14010.6
31
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)


Accept Refinem
Accept Refinement?
Bonds: 1.625
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
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)
31
6) at −15414.1
18

Ramachandran Restraints

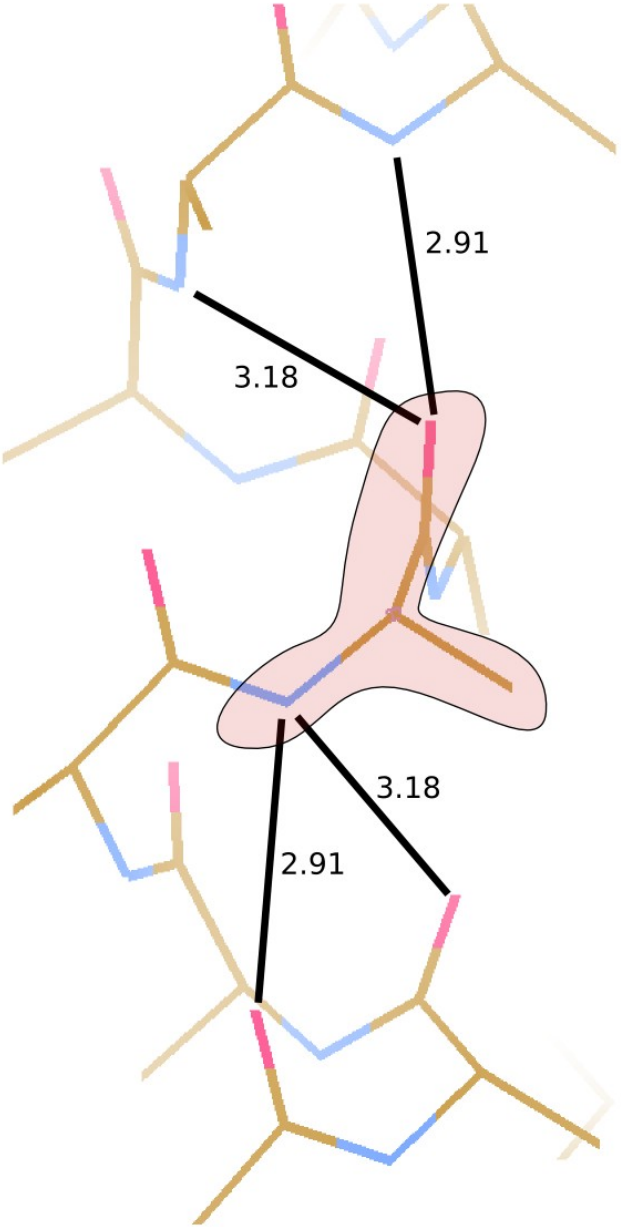
- Controversial?
  - “... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model...”
    - Gerard Kleywegt & Alwyn Jones (1996)
- But to quote Jane Richardson:
  - Do you want a better structure – or a better idea of the quality of your structure?

Adding Torsion Angle Restraints

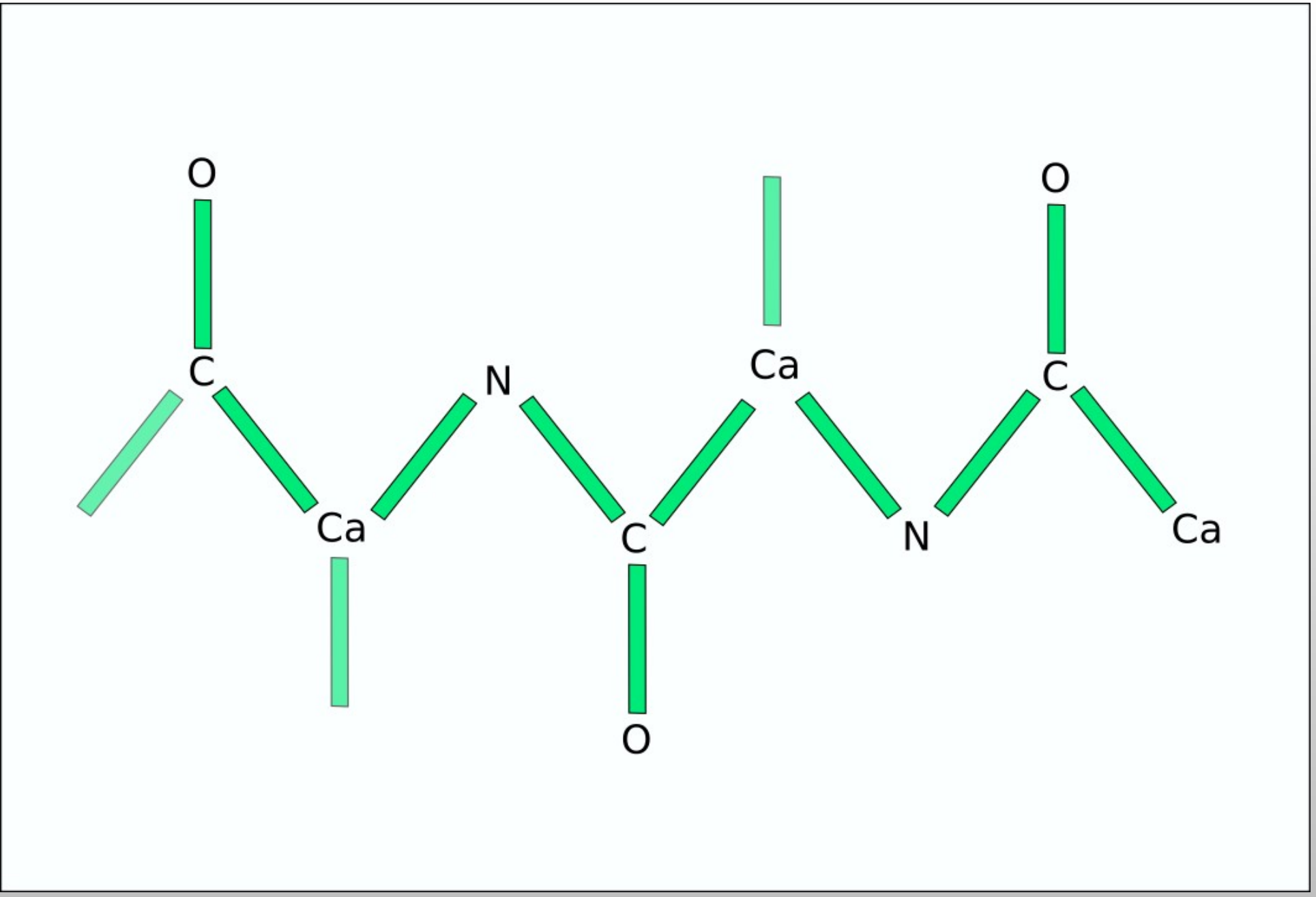
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”
    - i.e. trapped in local minimum

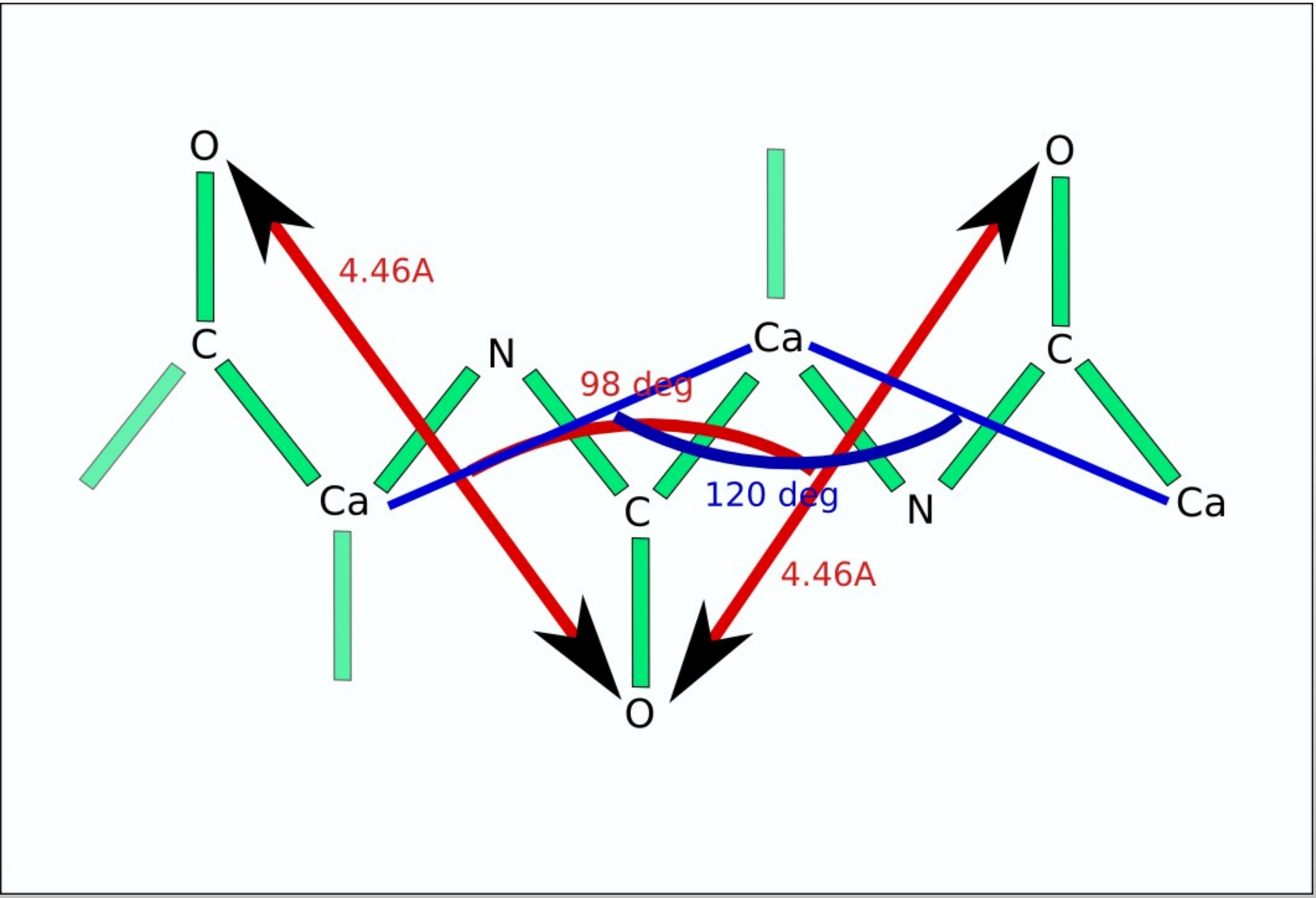
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints



Restrain the Hydrogen-bonding atom distances

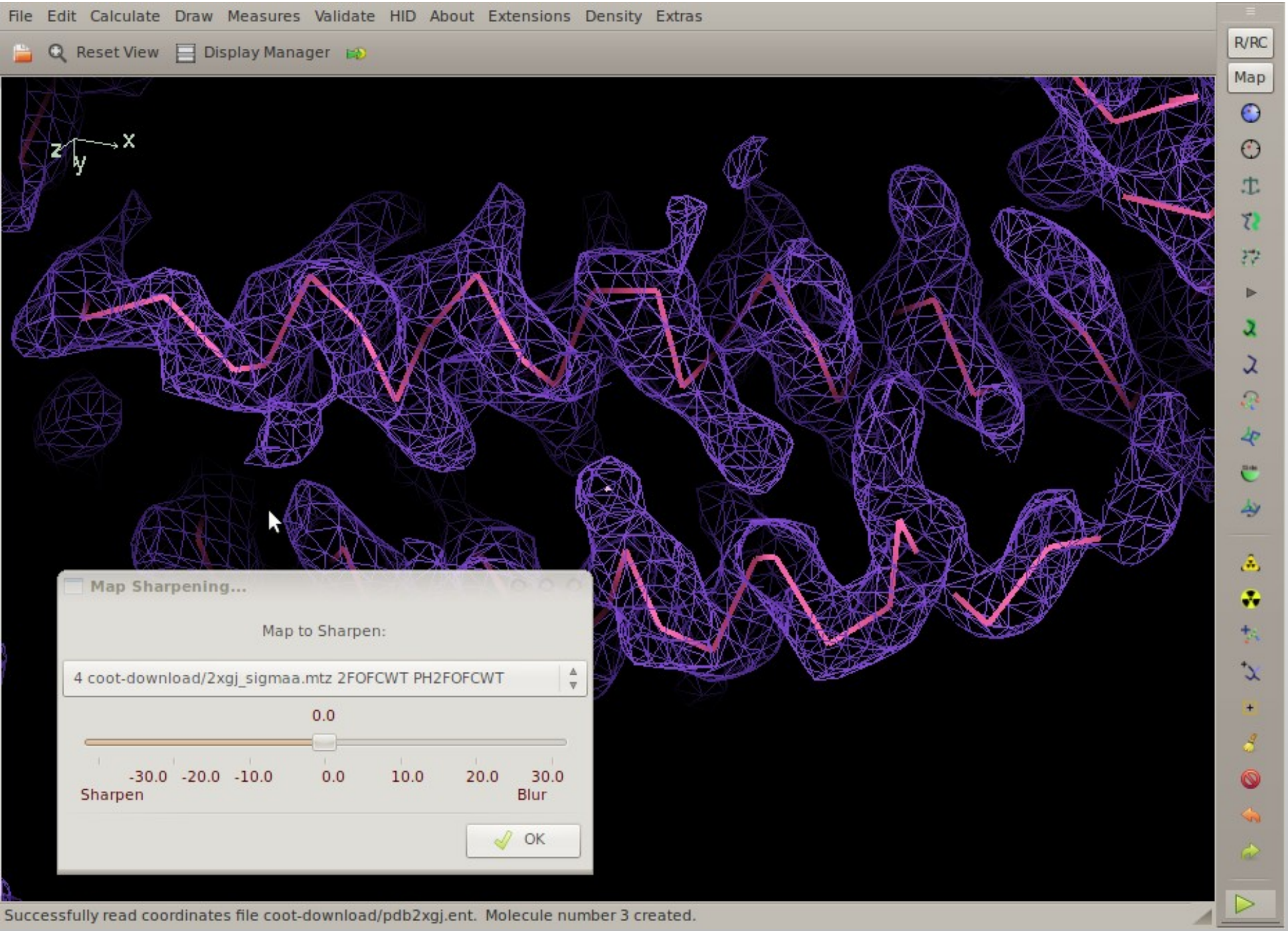

O
C
Ca
N
C
O
Ca
N
Ca
C
O
Ca

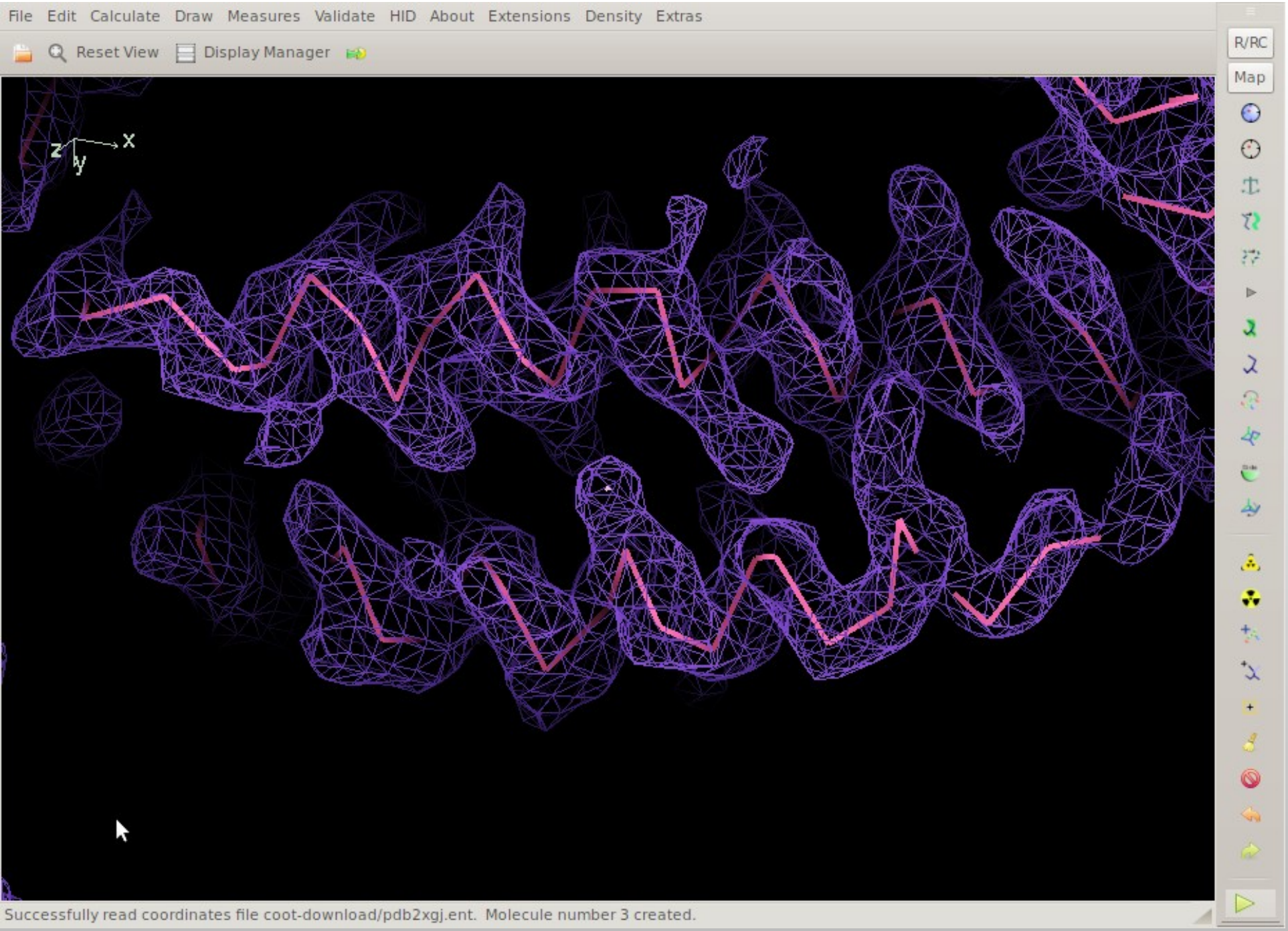

O
4.46A
C
N
Ca
98 deg
Ca
O
C
Ca
C
120 deg
N
Ca
4.46A
O

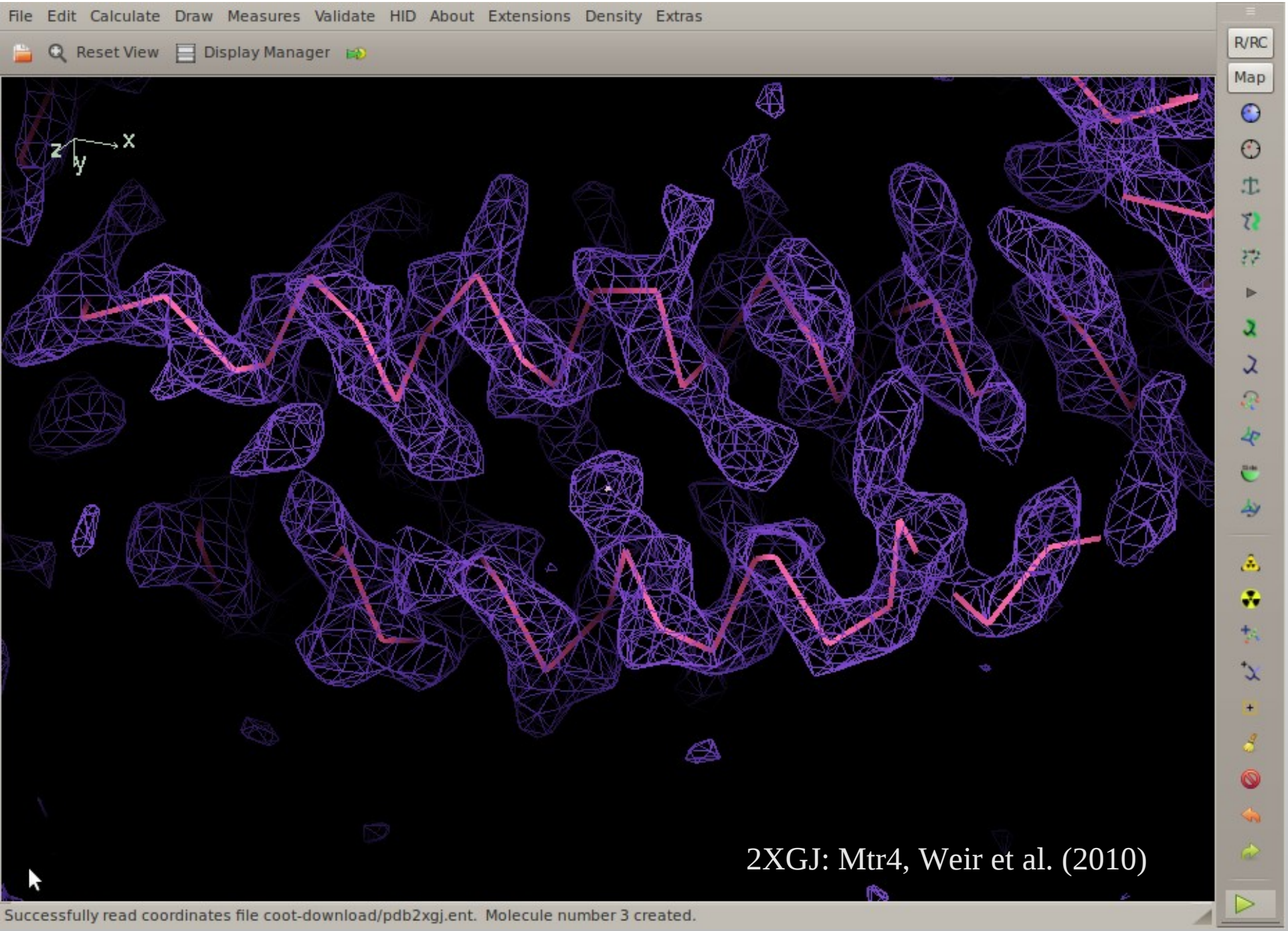
Map Sharpening

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.

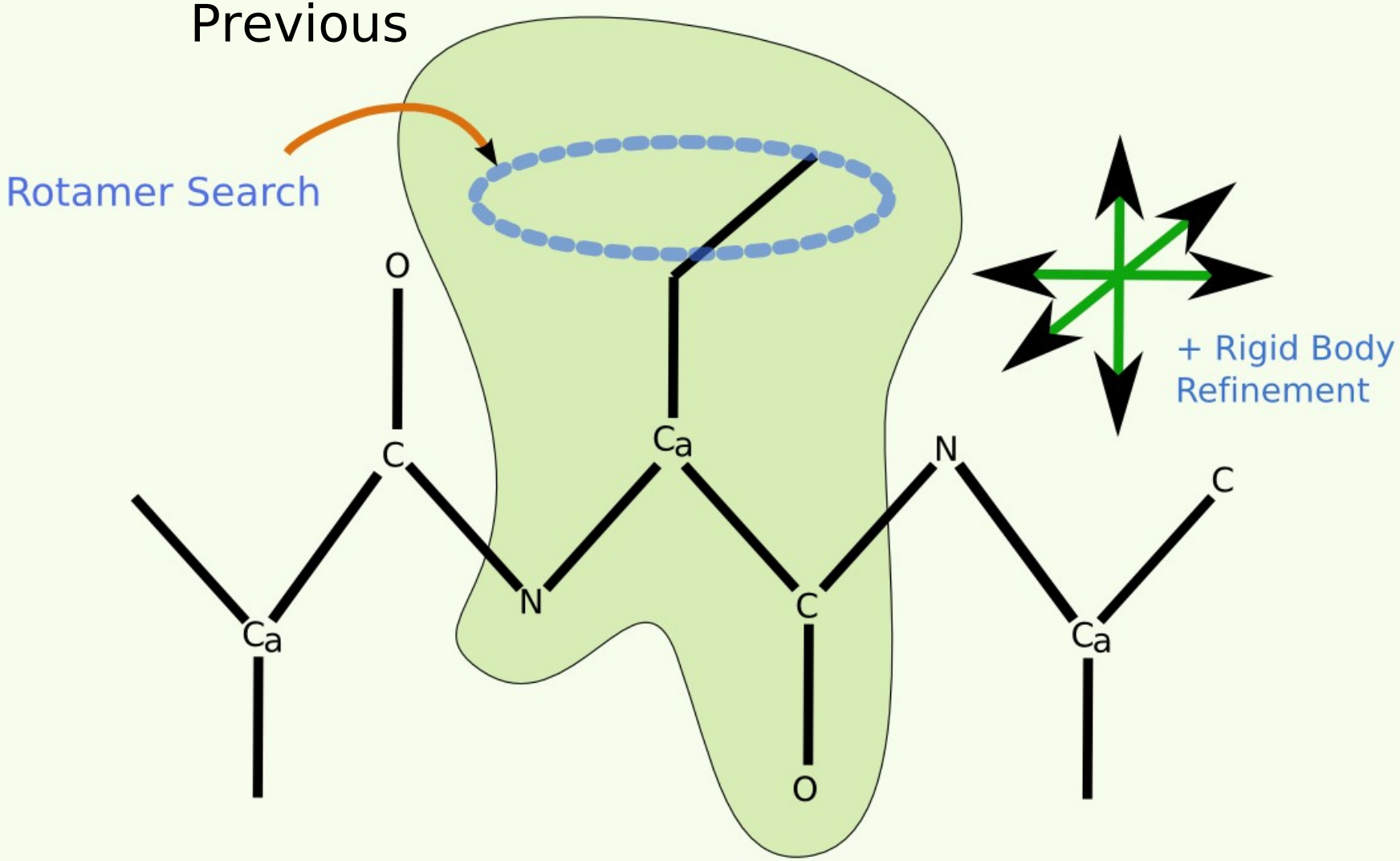

File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
2XGJ: Mtr4, Weir et al. (2010)
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.

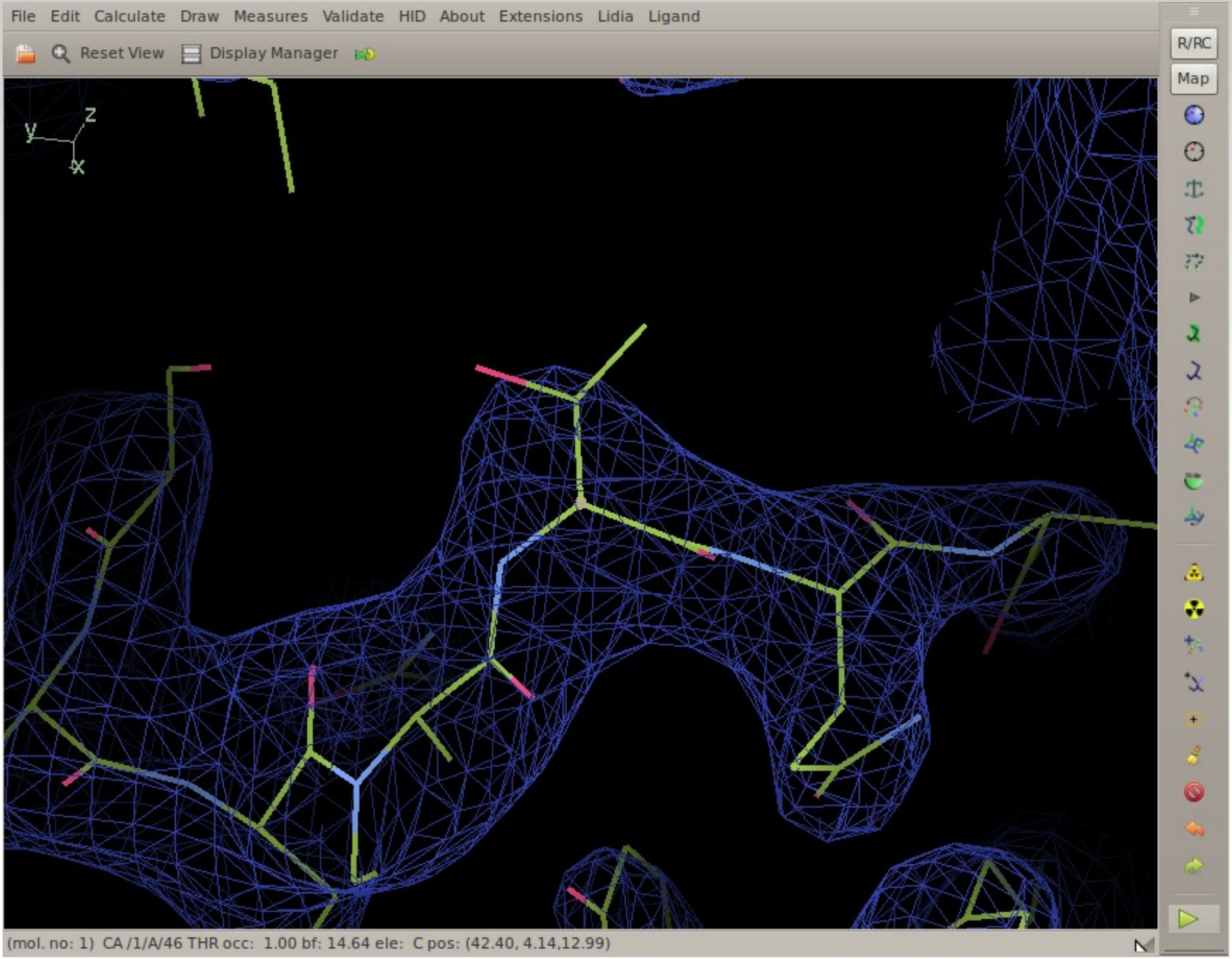
”Backrub Rotamers”

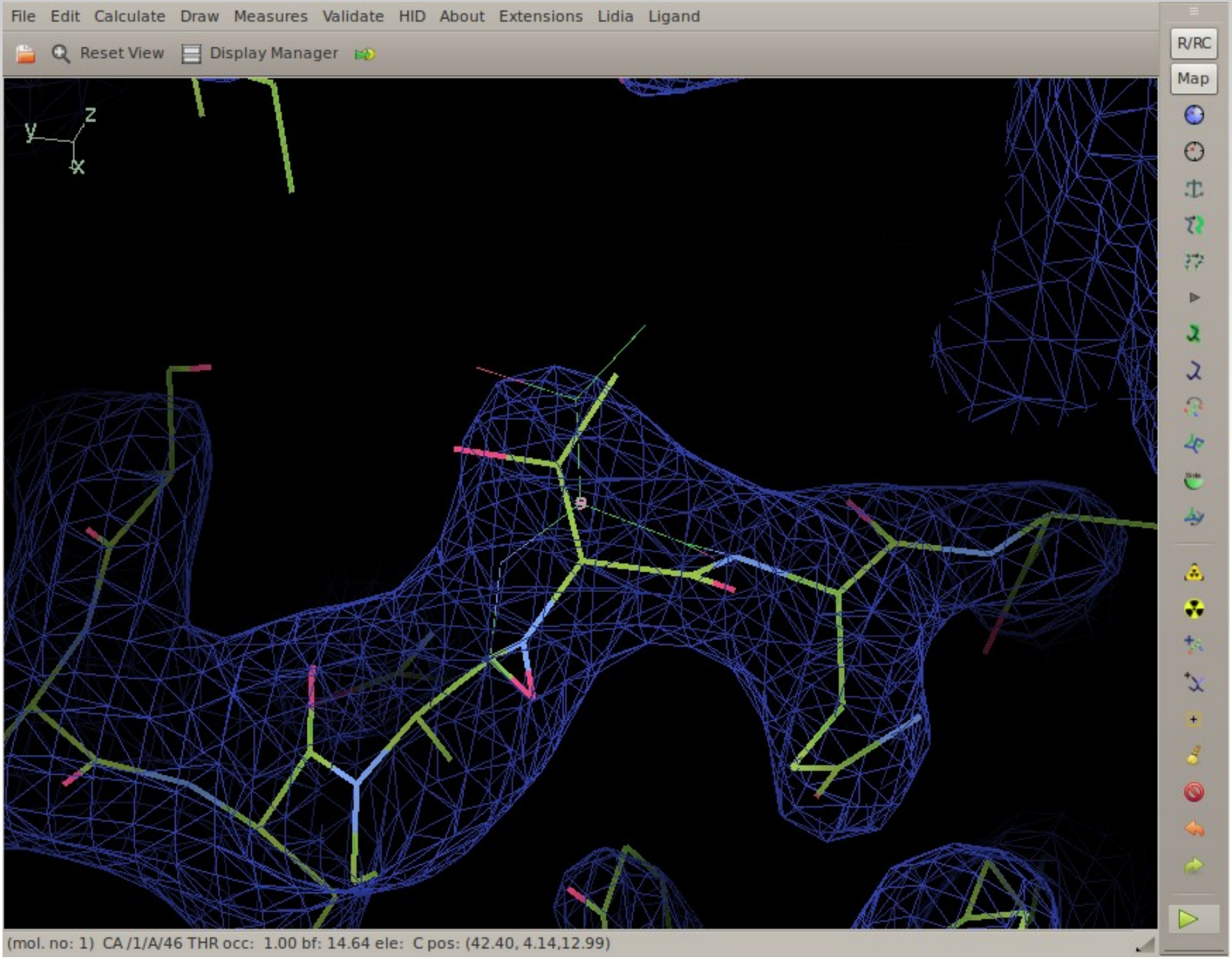
- High probability models with low resolution data

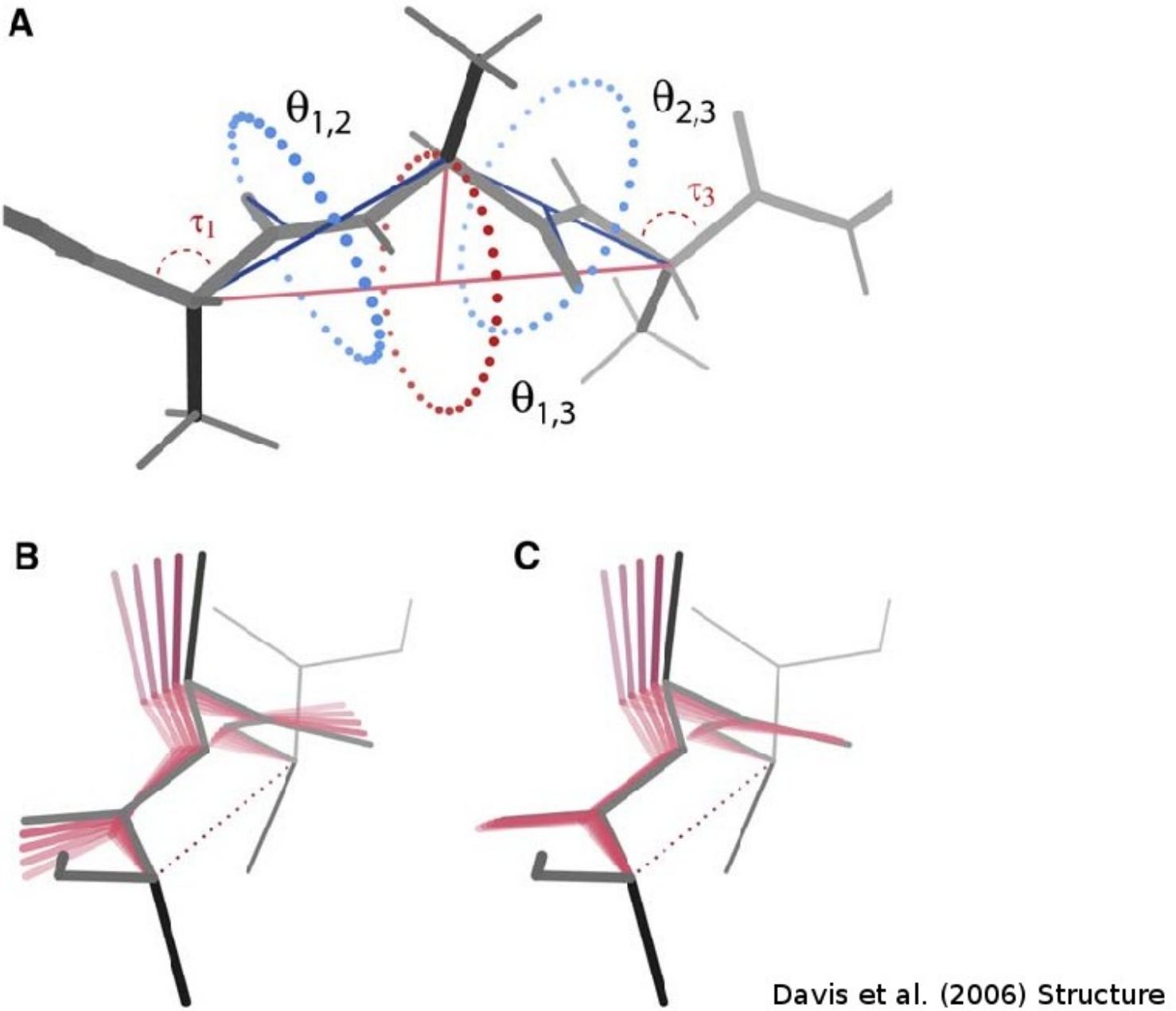
~~Current~~ Low Resolution Rotamer Search

Previous

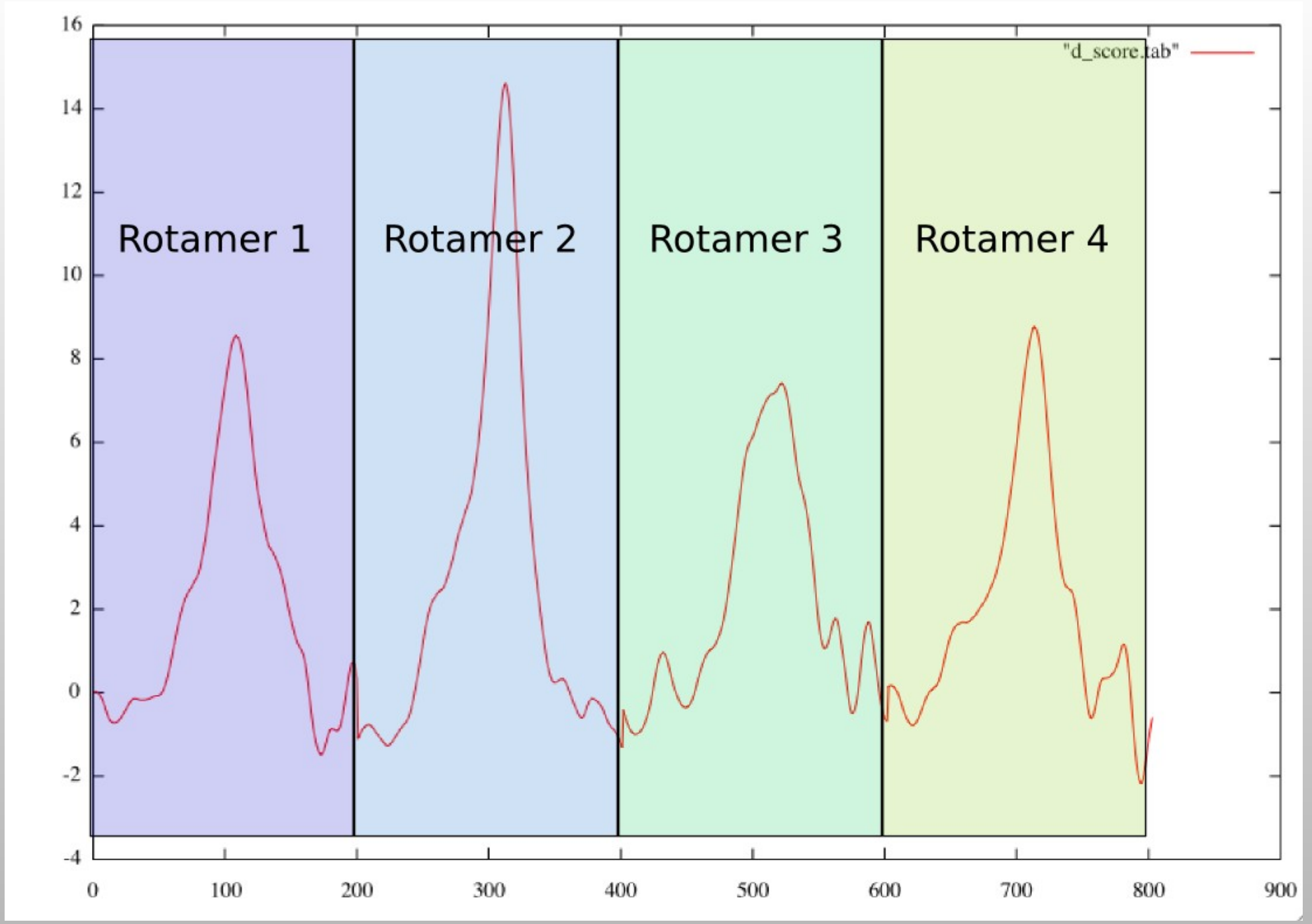



File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
Display Manager
R/RC
Map
Z
Y
X
(mol. no: 1) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)


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)



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
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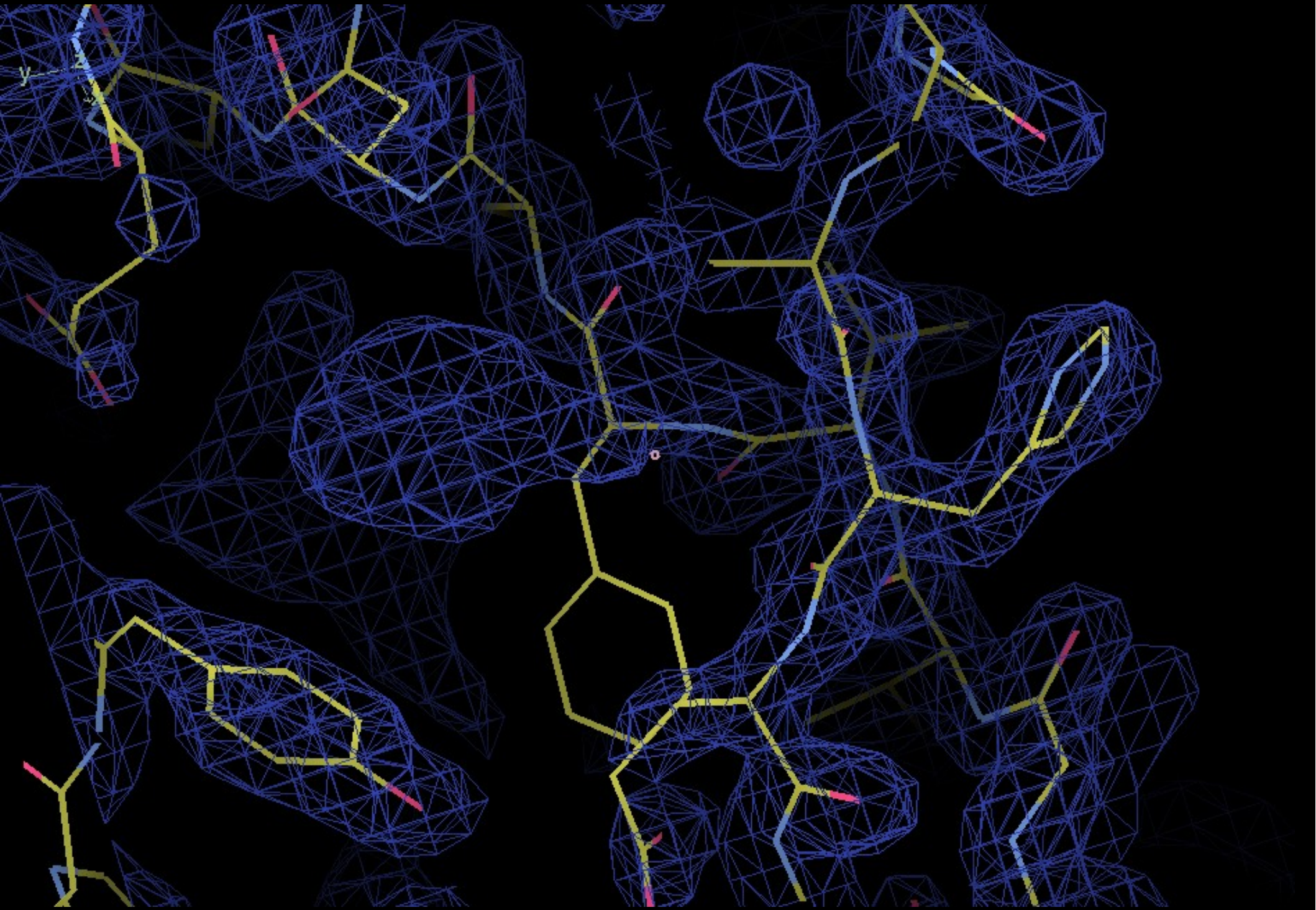
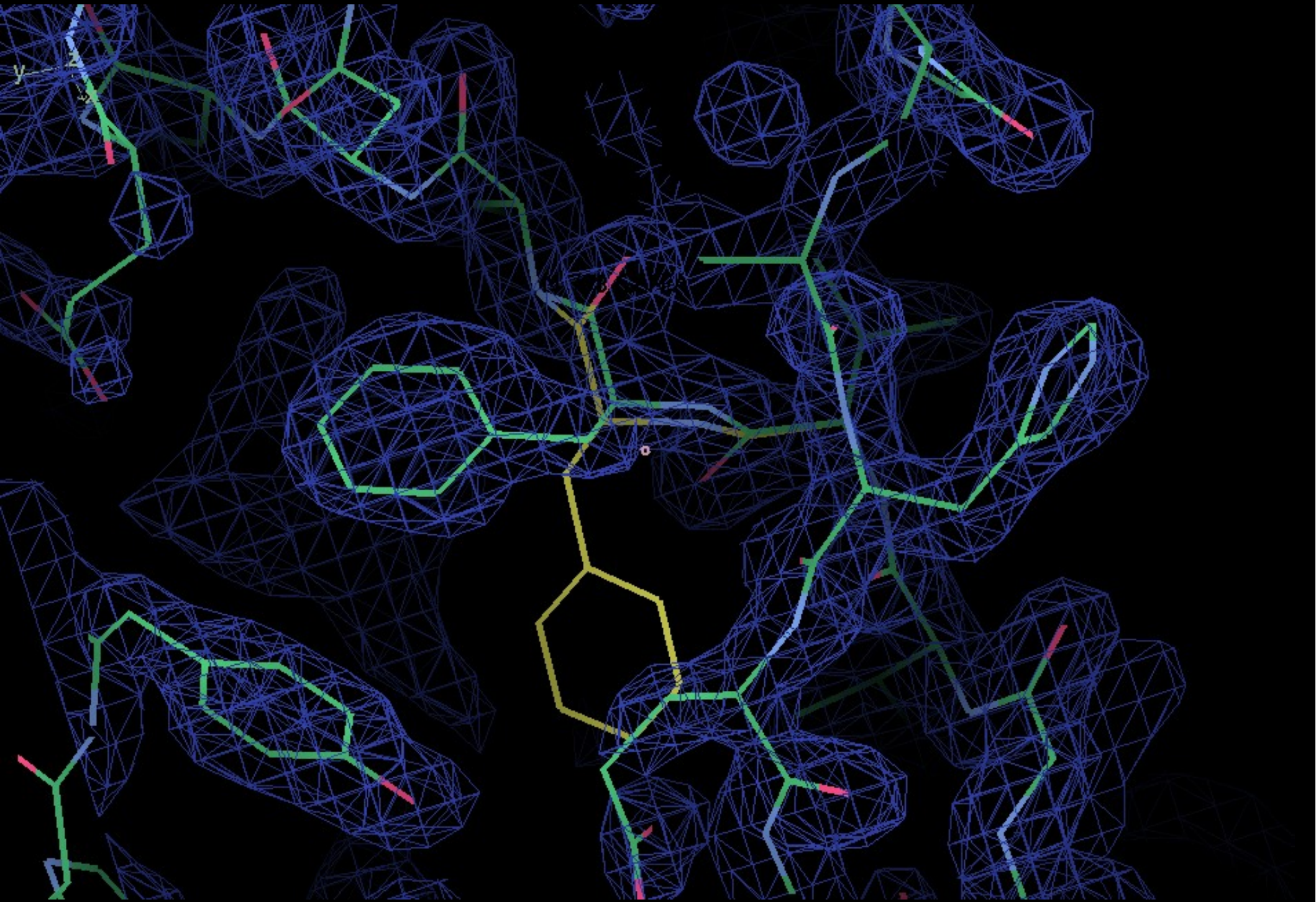
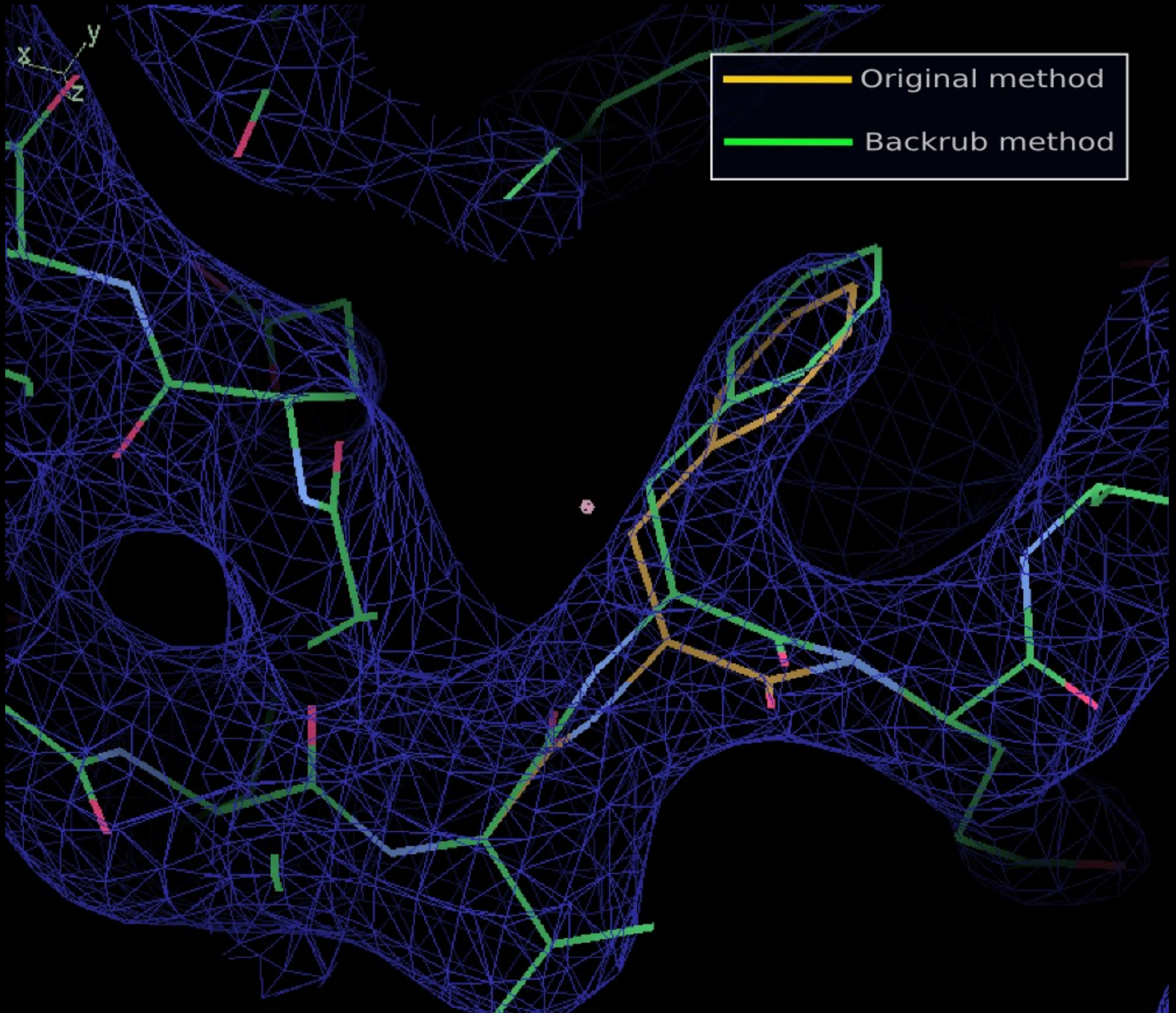
-4
0
100
200
300
400
500
600
700
800
900






y
x
z
Original method
Backrub method

To turn it on...

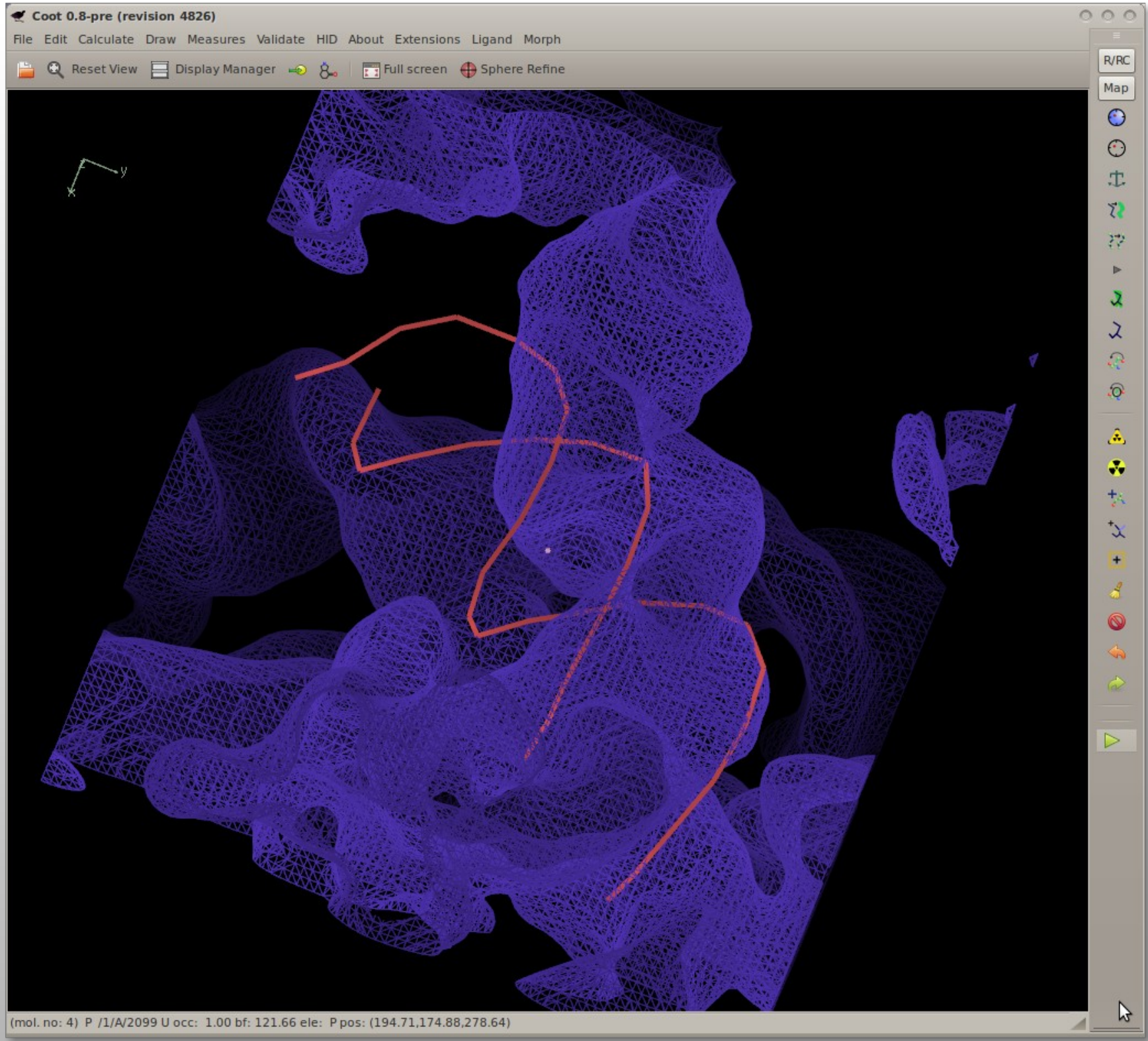
- (ROTAMERSEARCHLOWRES)
- Default for resolutions worse than 2.6A
- Extensions → Modeling → Use Backrub rotamers

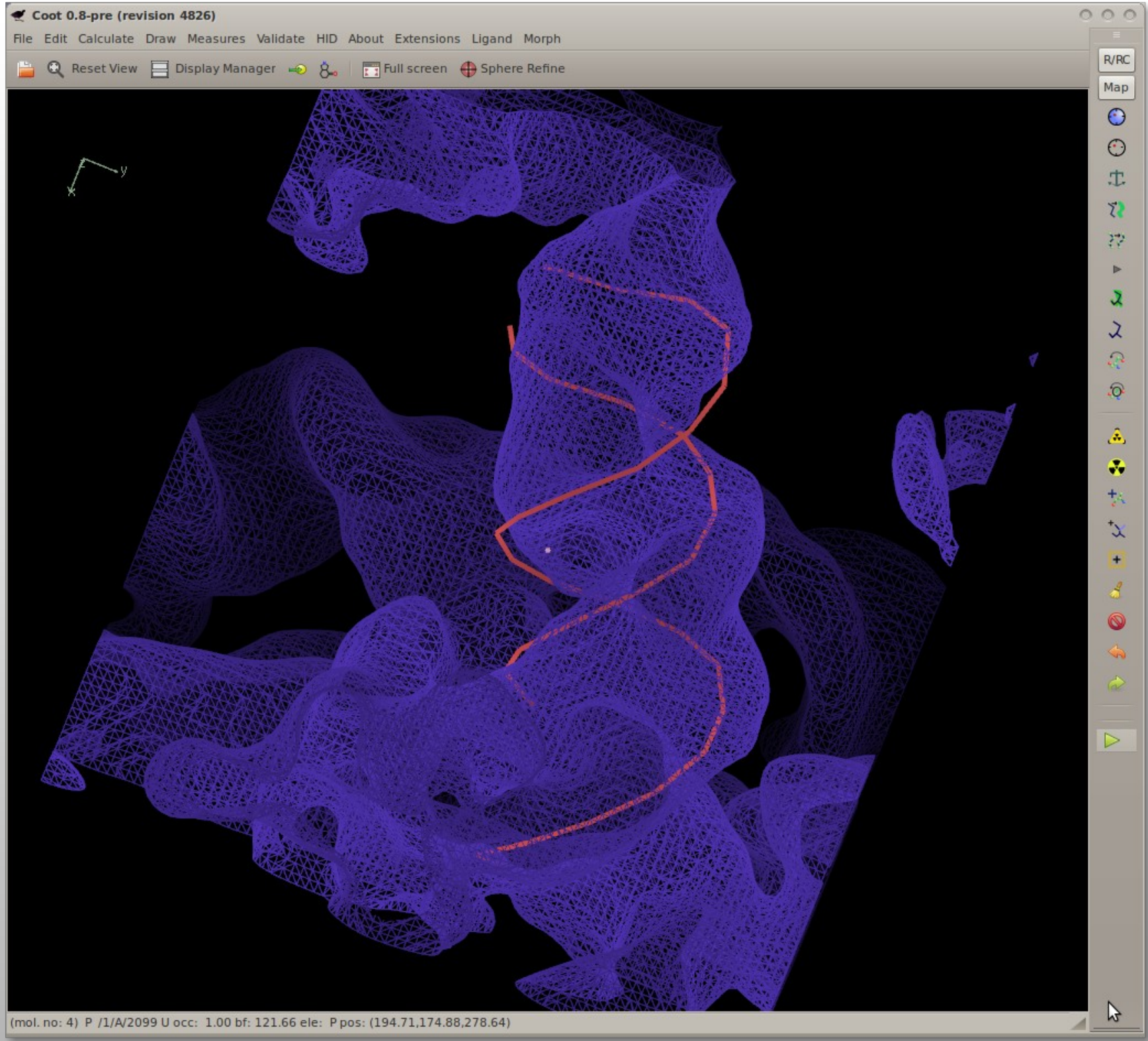
Jiggle Fit

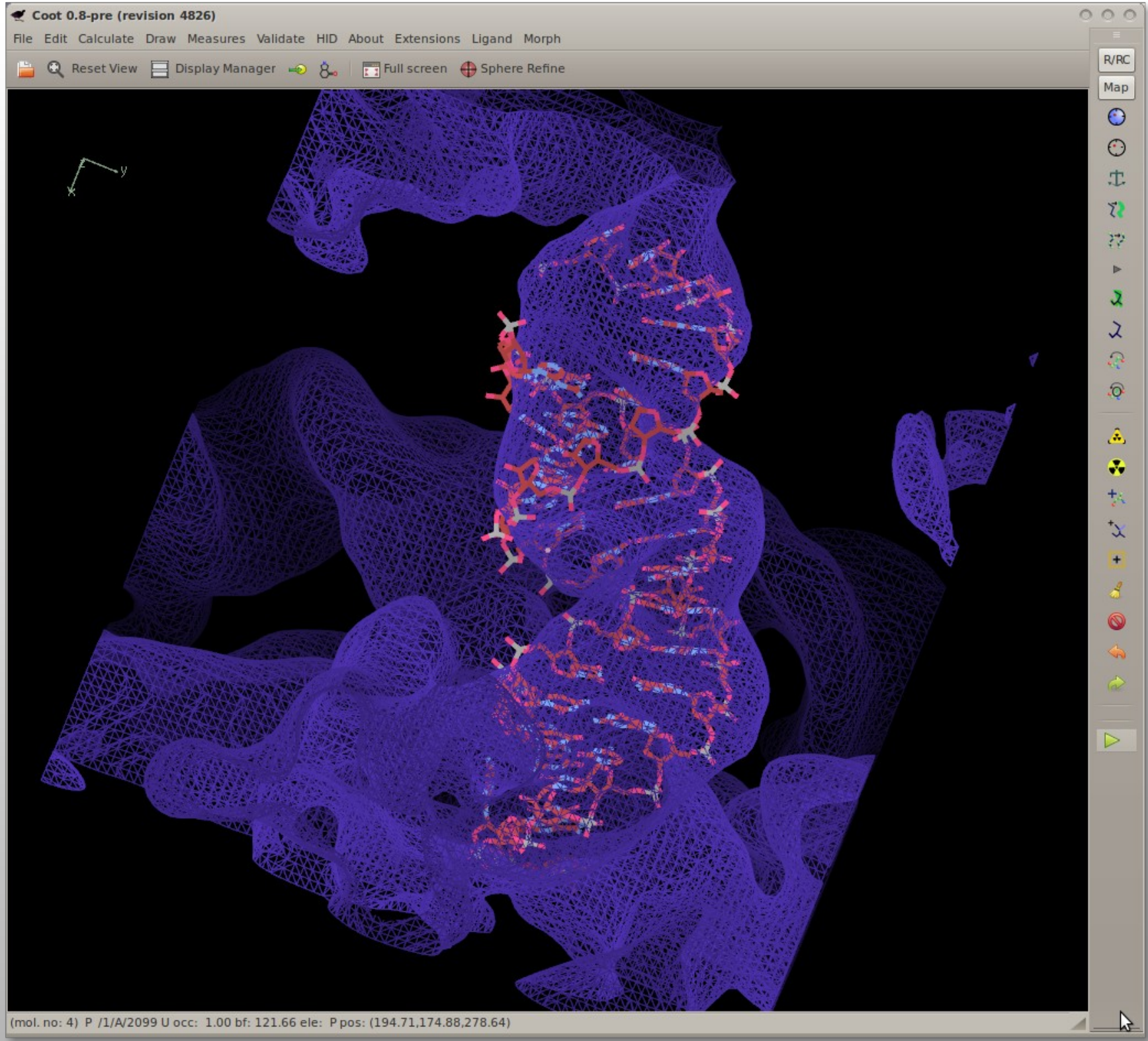
- How do I rotate and translate these atoms to fit the density?
  - 6-dimensional problem
- Originally used to fit simple ligands/solvent molecules to blobs of density
- Now extended to fit arbitrary atom selections
  - *e.g.* by Chain

Jiggle Fit: How it Works

- Loop 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

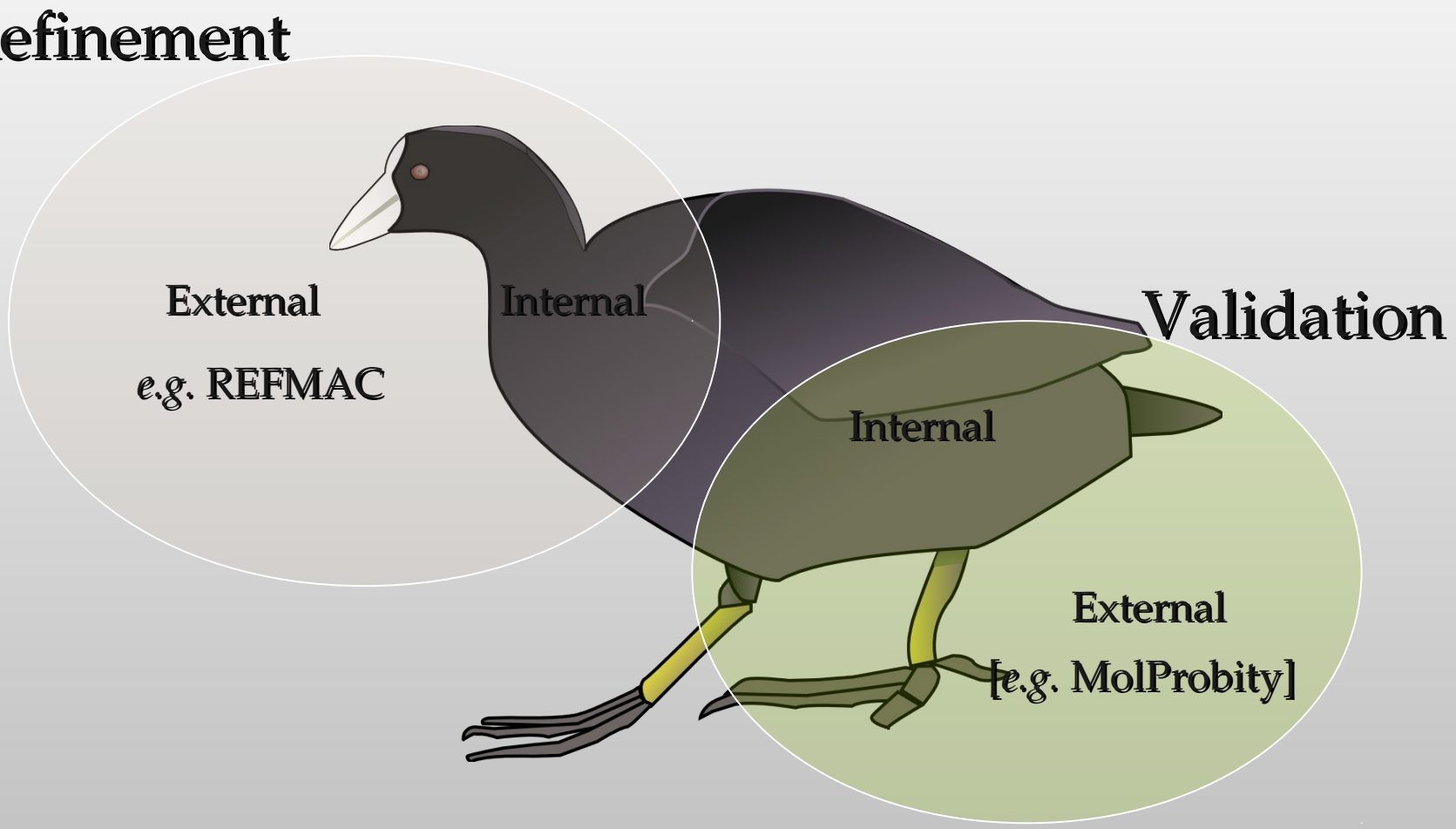

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

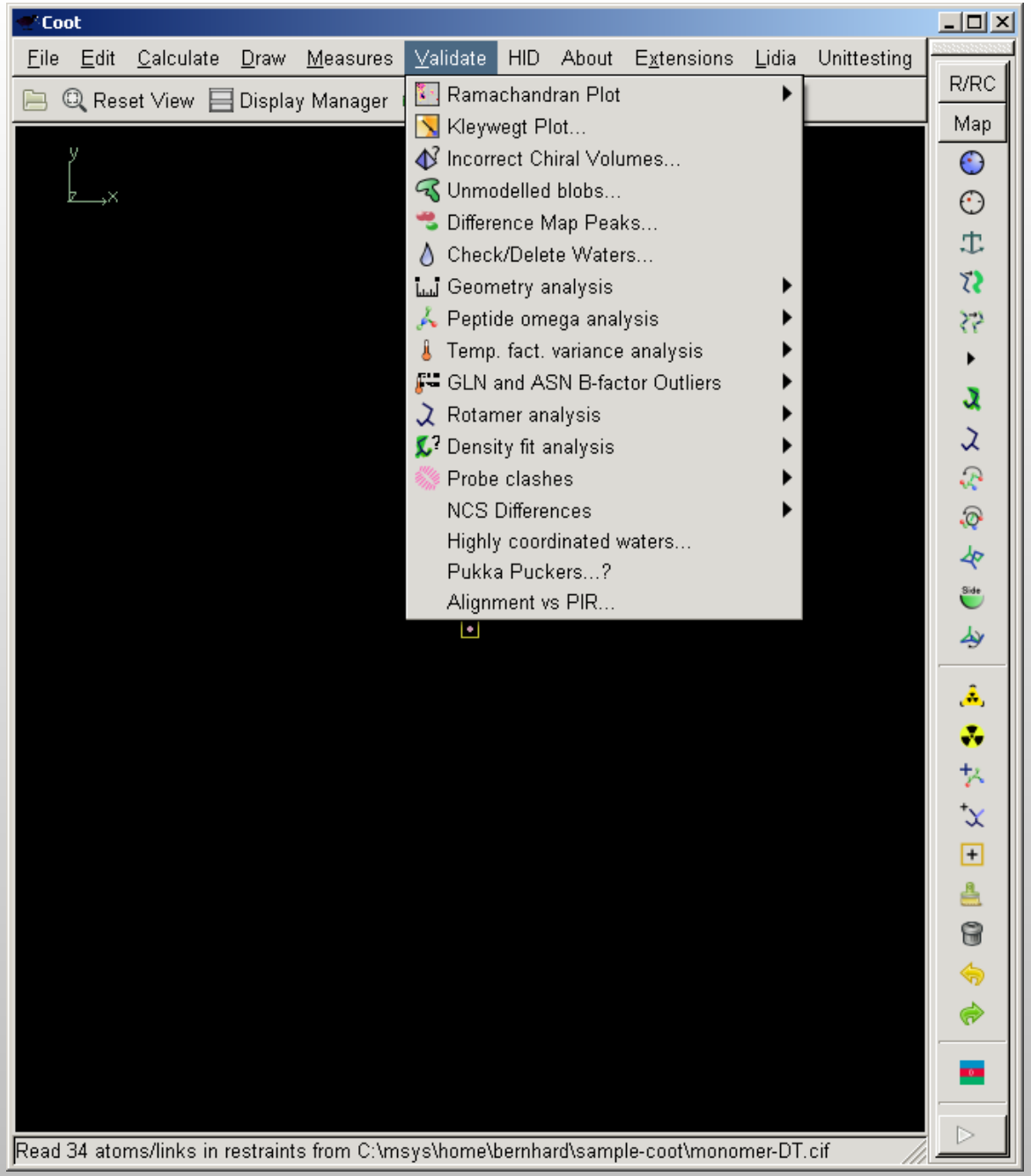

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

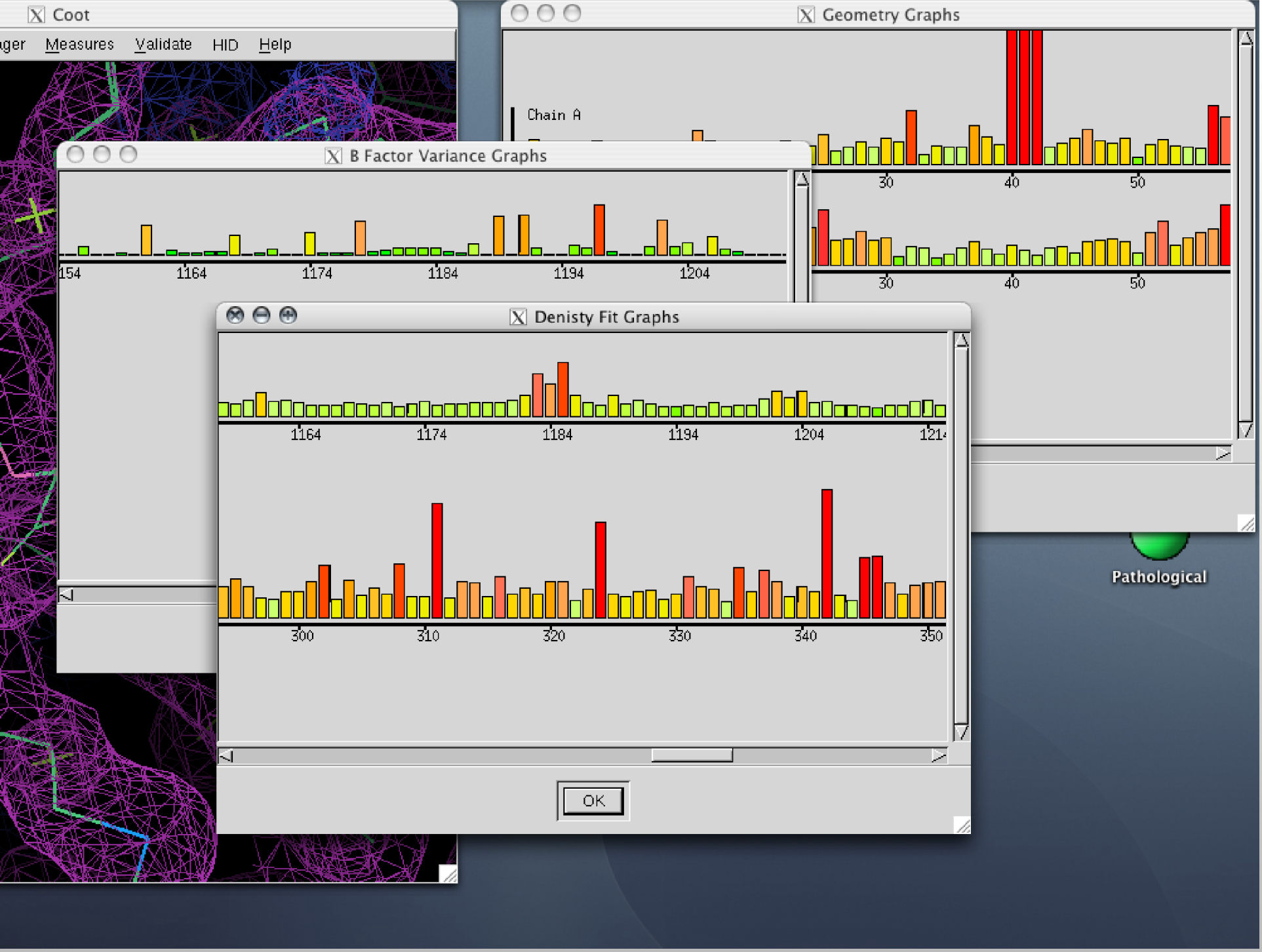

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
y
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

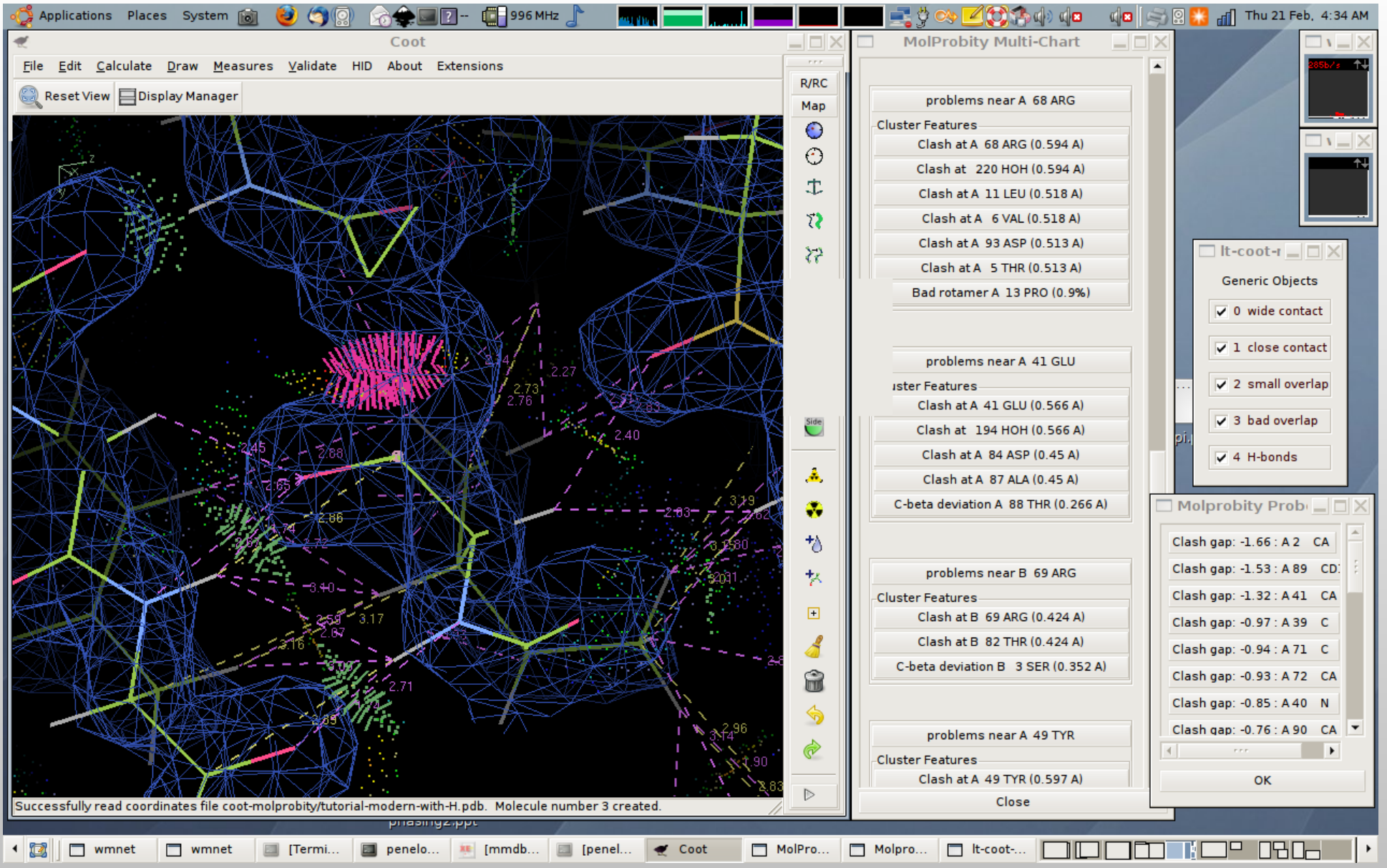
Validation...

Feature Integration




Coot
File
Edit
Calculate
Draw
Measures
Validate
HID
About
Extensions
Lidia
Unittesting
Reset View
Display Manager
Ramachandran Plot
Kleywegt Plot...
Incorrect Chiral Volumes...
Unmodelled blobs...
Difference Map Peaks...
Check/Delete Waters...
Geometry analysis
Peptide omega analysis
Temp. fact. variance analysis
GLN and ASN B-factor Outliers
Rotamer analysis
Density fit analysis
Probe clashes
NCS Differences
Highly coordinated waters...
Pukka Puckers...?
Alignment vs PIR...
R/RC
Map
Read 34 atoms/links in restraints from C:\msys\home\bernhard\sample-coot\monomer-DT.cif


Coot
ger
Measures
Validate
HID
Help
Geometry Graphs
Chain A
30
40
50
B Factor Variance Graphs
154
1164
1174
1184
1194
1204
Denisty Fit Graphs
1164
1174
1184
1194
1204
300
310
320
330
340
350
OK
Pathological


Applications Places System
996 MHz
Thu 21 Feb, 4:34 AM
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
Side
Successfully read coordinates file coot-molprobity/tutorial-modern-with-H.pdb. Molecule number 3 created.
MolProbity Multi-Chart
problems near A 68 ARG
Cluster Features
Clash at A 68 ARG (0.594 A)
Clash at 220 HOH (0.594 A)
Clash at A 11 LEU (0.518 A)
Clash at A 6 VAL (0.518 A)
Clash at A 93 ASP (0.513 A)
Clash at A 5 THR (0.513 A)
Bad rotamer A 13 PRO (0.9%)
problems near A 41 GLU
ıster Features
Clash at A 41 GLU (0.566 A)
Clash at 194 HOH (0.566 A)
Clash at A 84 ASP (0.45 A)
Clash at A 87 ALA (0.45 A)
C-beta deviation A 88 THR (0.266 A)
problems near B 69 ARG
Cluster Features
Clash at B 69 ARG (0.424 A)
Clash at B 82 THR (0.424 A)
C-beta deviation B 3 SER (0.352 A)
problems near A 49 TYR
Cluster Features
Clash at A 49 TYR (0.597 A)
Close
lt-coot-r
Generic Objects
0 wide contact
1 close contact
2 small overlap
3 bad overlap
4 H-bonds
Molprobity Prob
Clash gap: -1.66 : A 2 CA
Clash gap: -1.53 : A 89 CD
Clash gap: -1.32 : A 41 CA
Clash gap: -0.97 : A 39 C
Clash gap: -0.94 : A 71 C
Clash gap: -0.93 : A 72 CA
Clash gap: -0.85 : A 40 N
Clash gap: -0.76 : A 90 CA
OK
wmnet
wmnet
[Termi...
penelo...
[mmdb...
[penel...
Coot
MolPro...
Molpro...
lt-coot-...

Other Programs

- Molprobity Suite
  - molprobity.biochem.duke.edu
- WHATCHECK
- VERIFY-3D