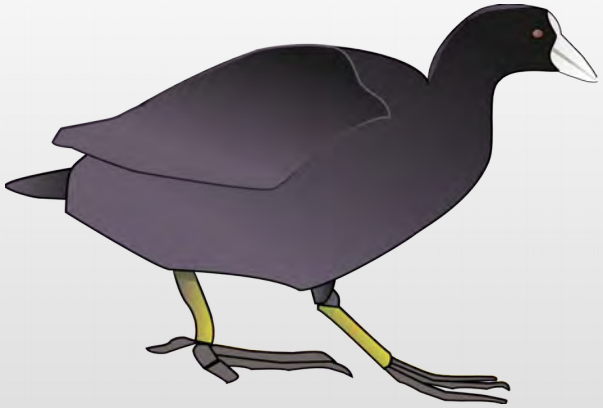


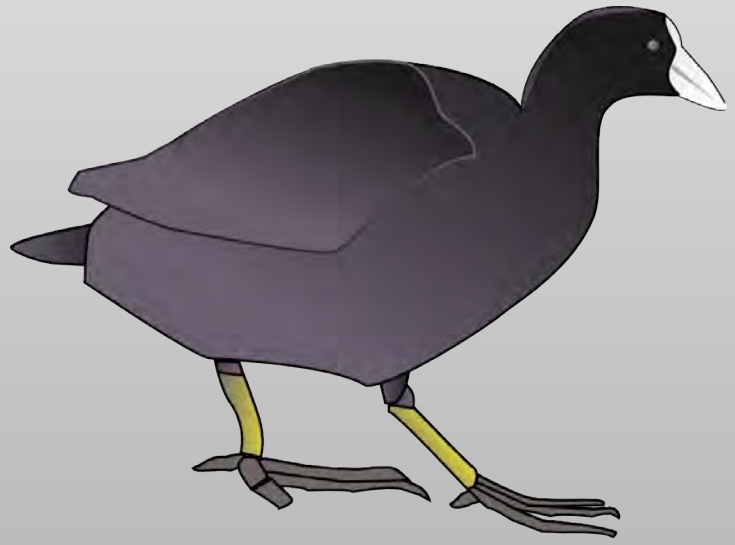
大鷭
大鳥類
クート

オオバン

Introduction to Coot

Model-Building with *Coot:*

An Introduction
and low resolution tools



Bernhard Lohkamp

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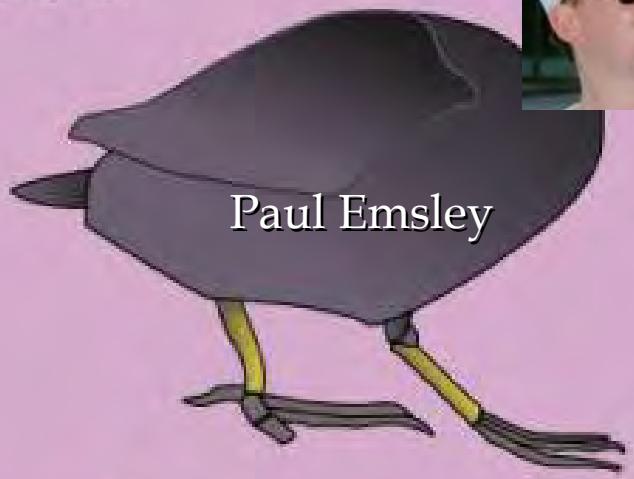
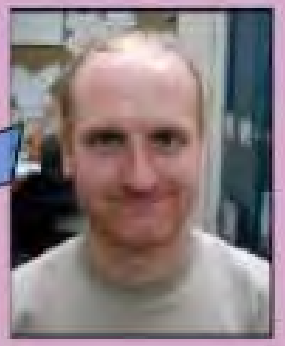
Bernhard.Lohkamp@ki.se

Tsukuba PF November 2014

Coot Contributors:



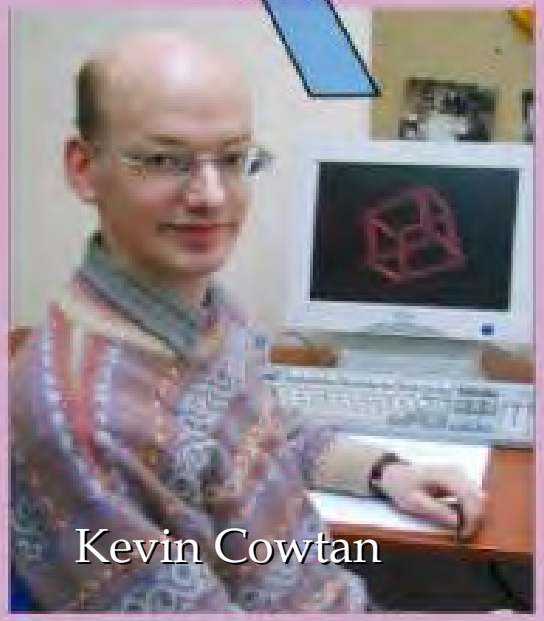
Paul Emsley



Stuart McNicholas

Alexei Vagin

Eugene Krissinel



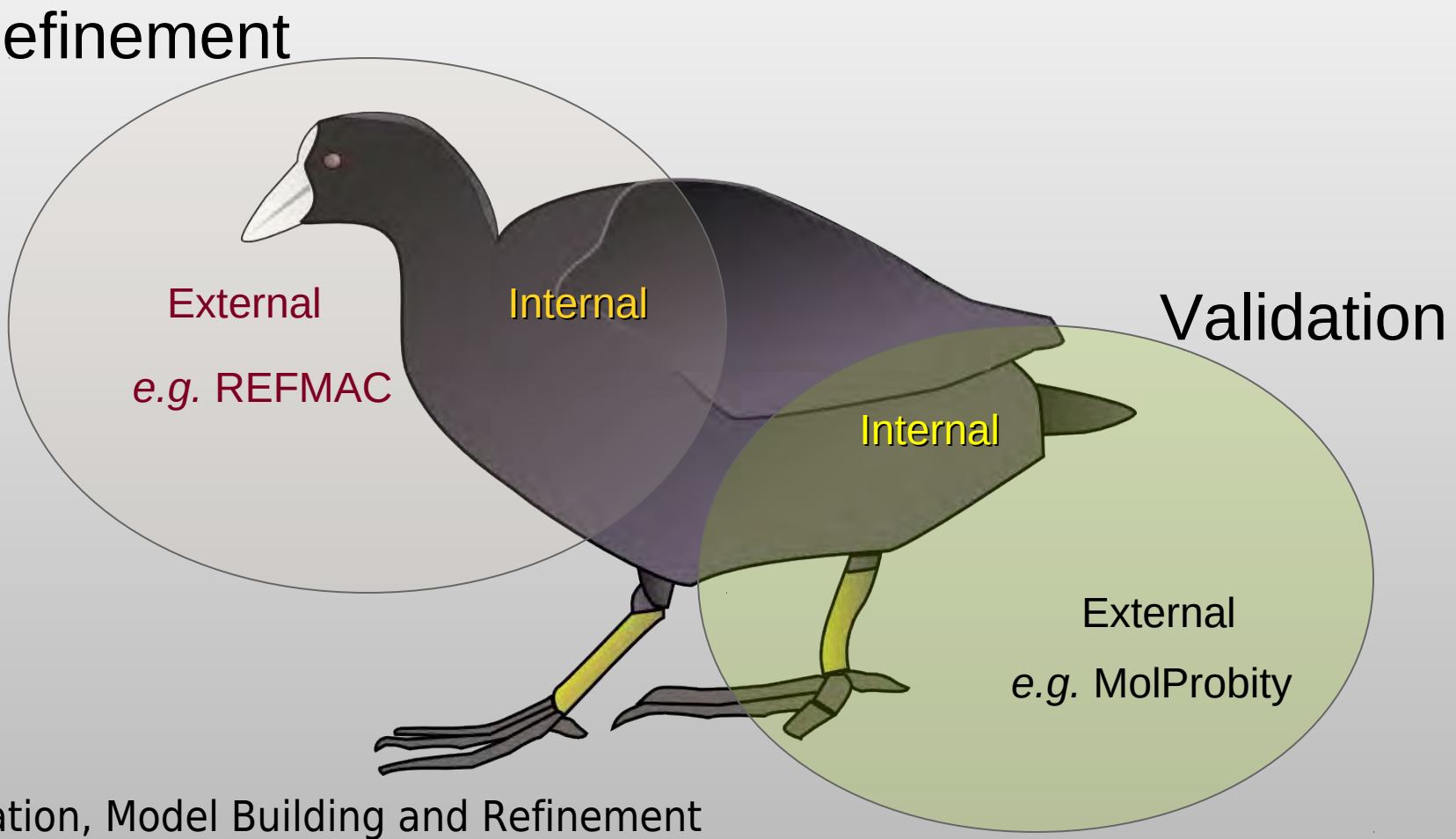
Kevin Cowtan

Bernhard Lohkamp

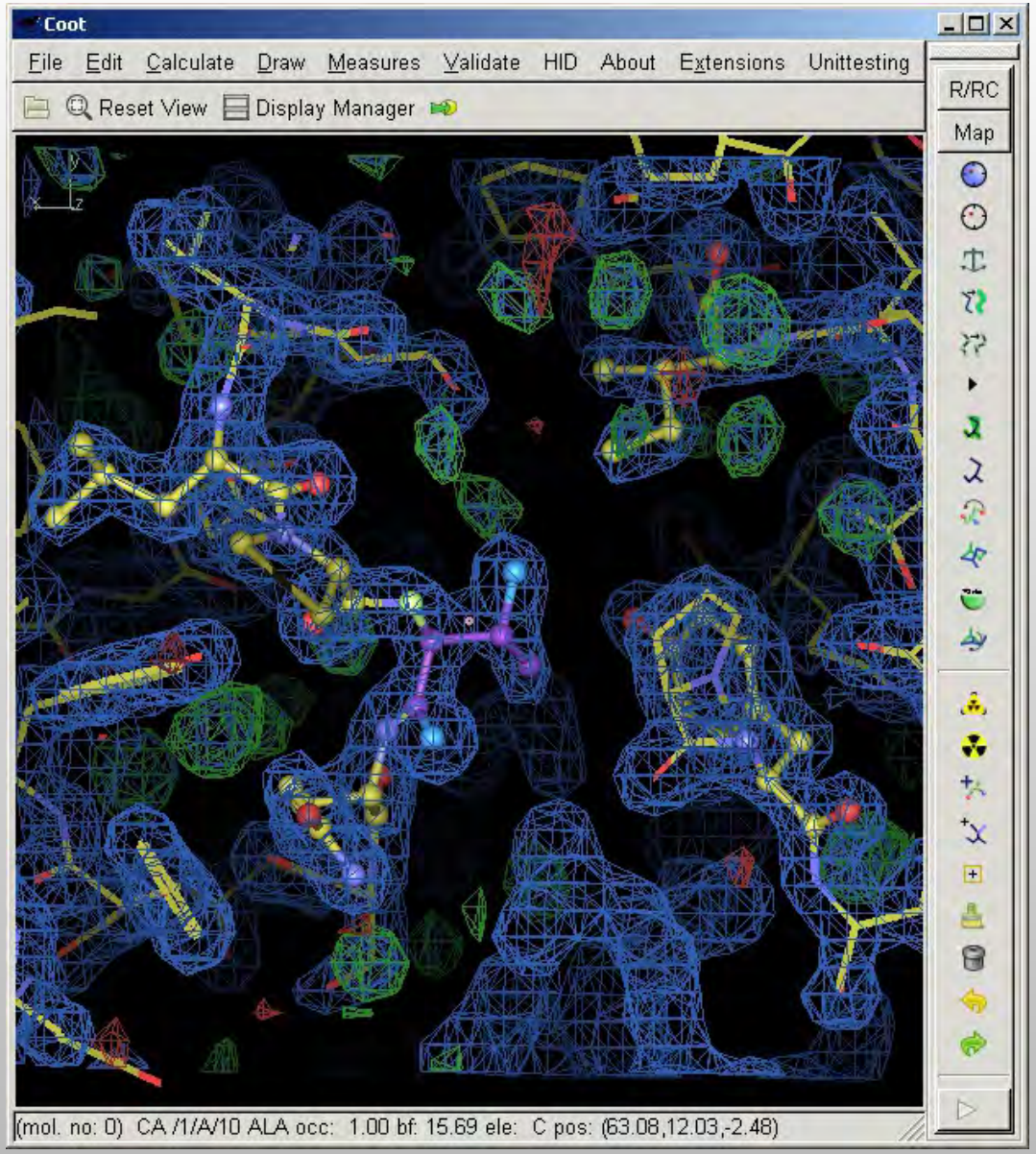
Coot

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

Feature Integration



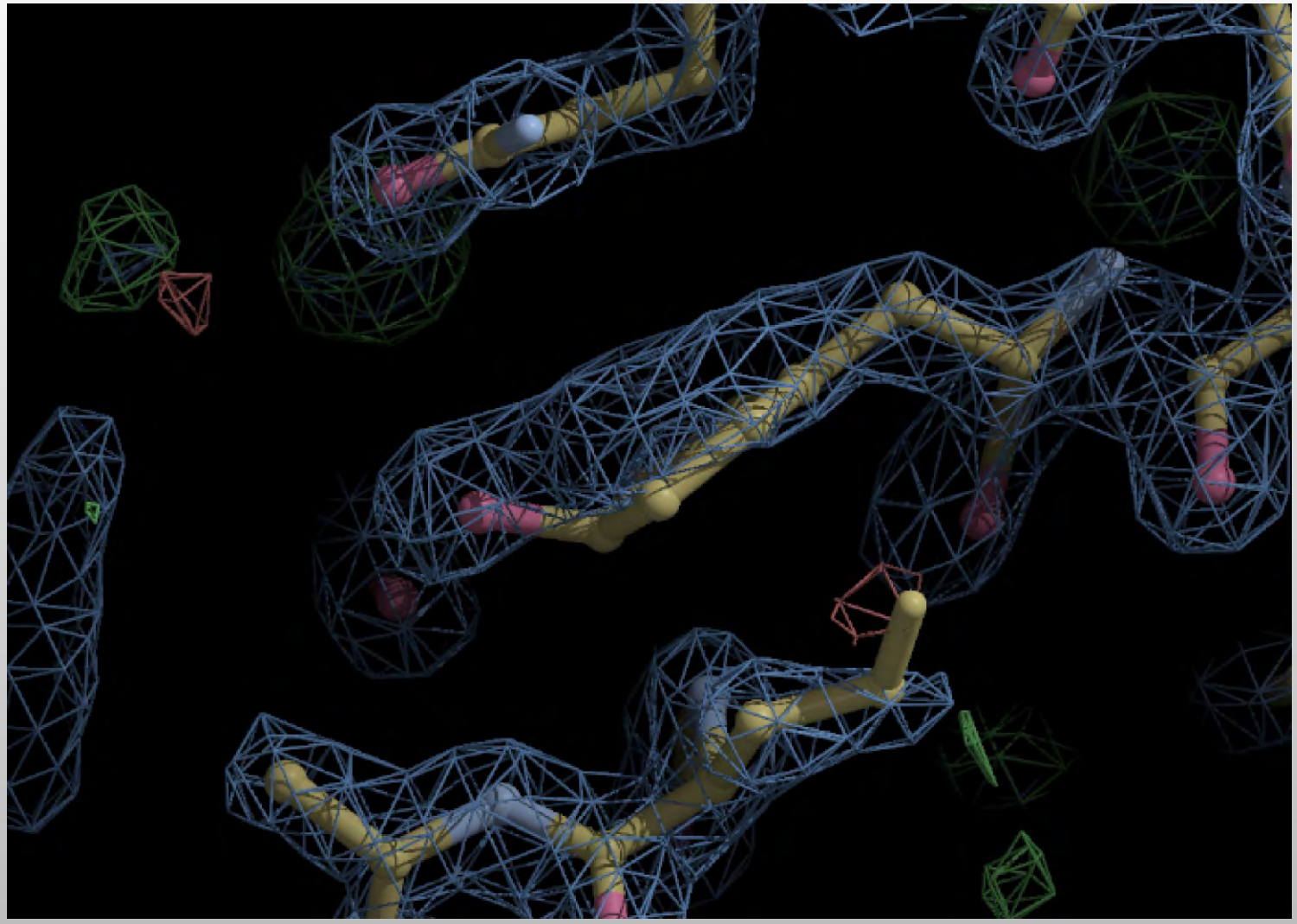
Validation, Model Building and Refinement should be used together


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Unittesting
Reset View
Display Manager
R/RC
Map
(mol. no: 0) CA /1/A/10 ALA occ: 1.00 bf: 15.69 ele: C pos: (63.08,12.03,-2.48)

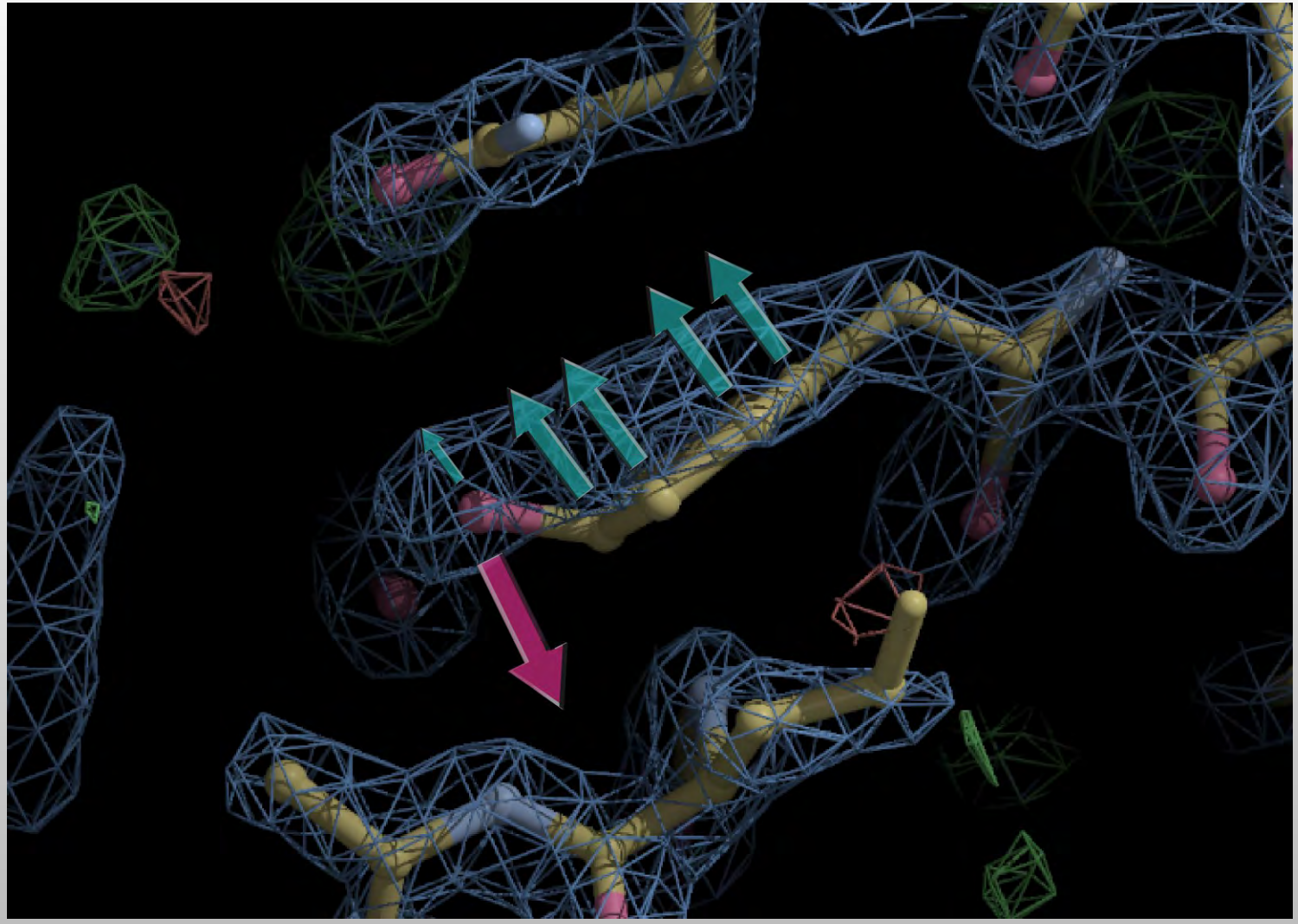
What is “Refinement”?

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

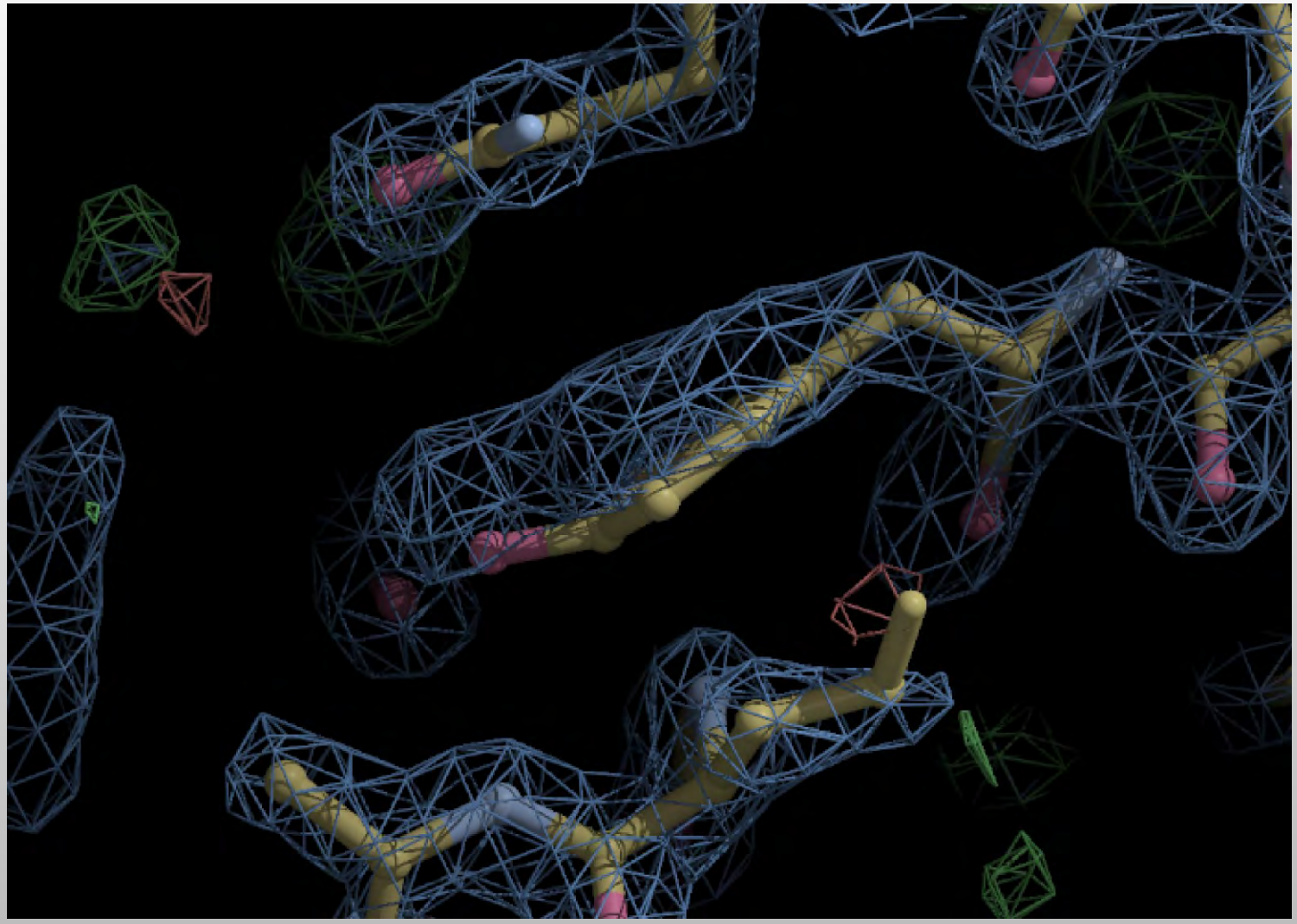
Distorted Geometry Pre-Refinement



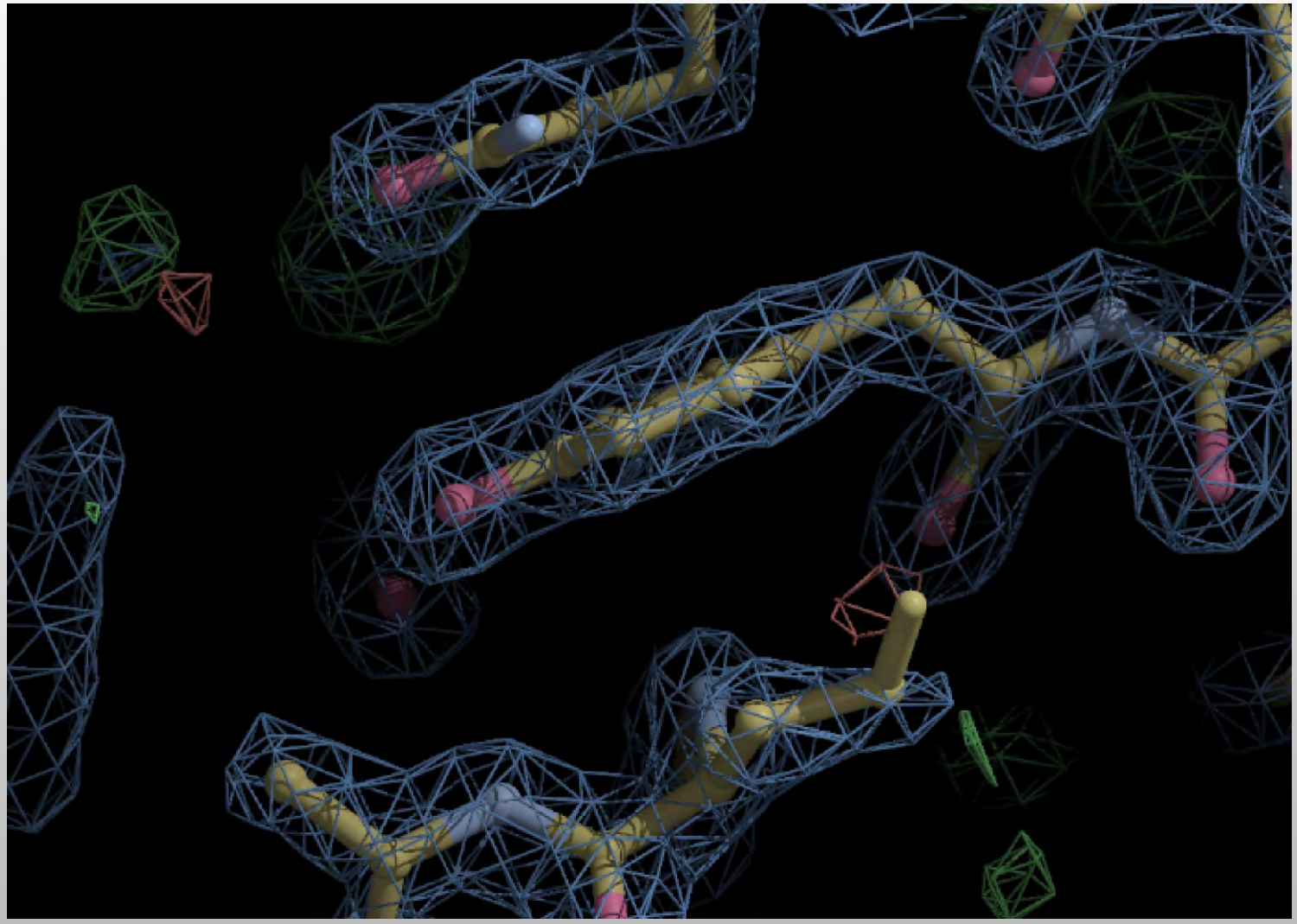
Refinement Gradients



Refinement: Cycle 3



Refinement Cycle 200: Minimized



Real Space Refinement

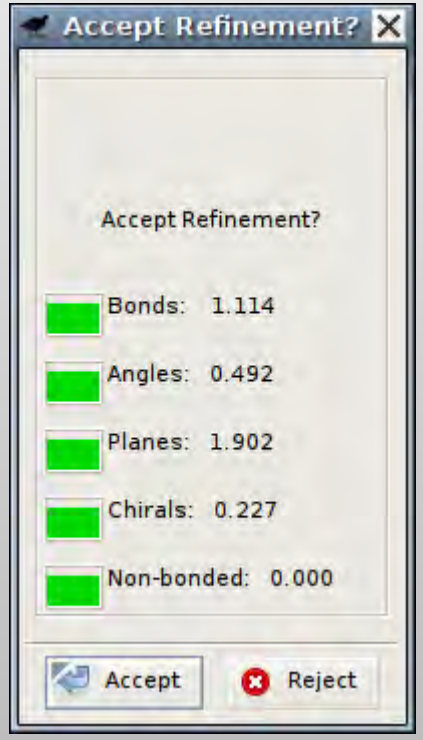
Diamond, R. (1971). *Acta Cryst*. A 27, 436-452.

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

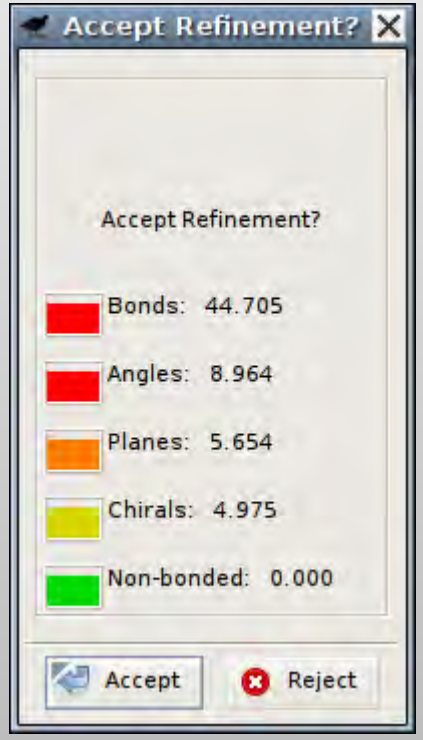
Faster & Animated

Representation of Results: “Traffic Lights”

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



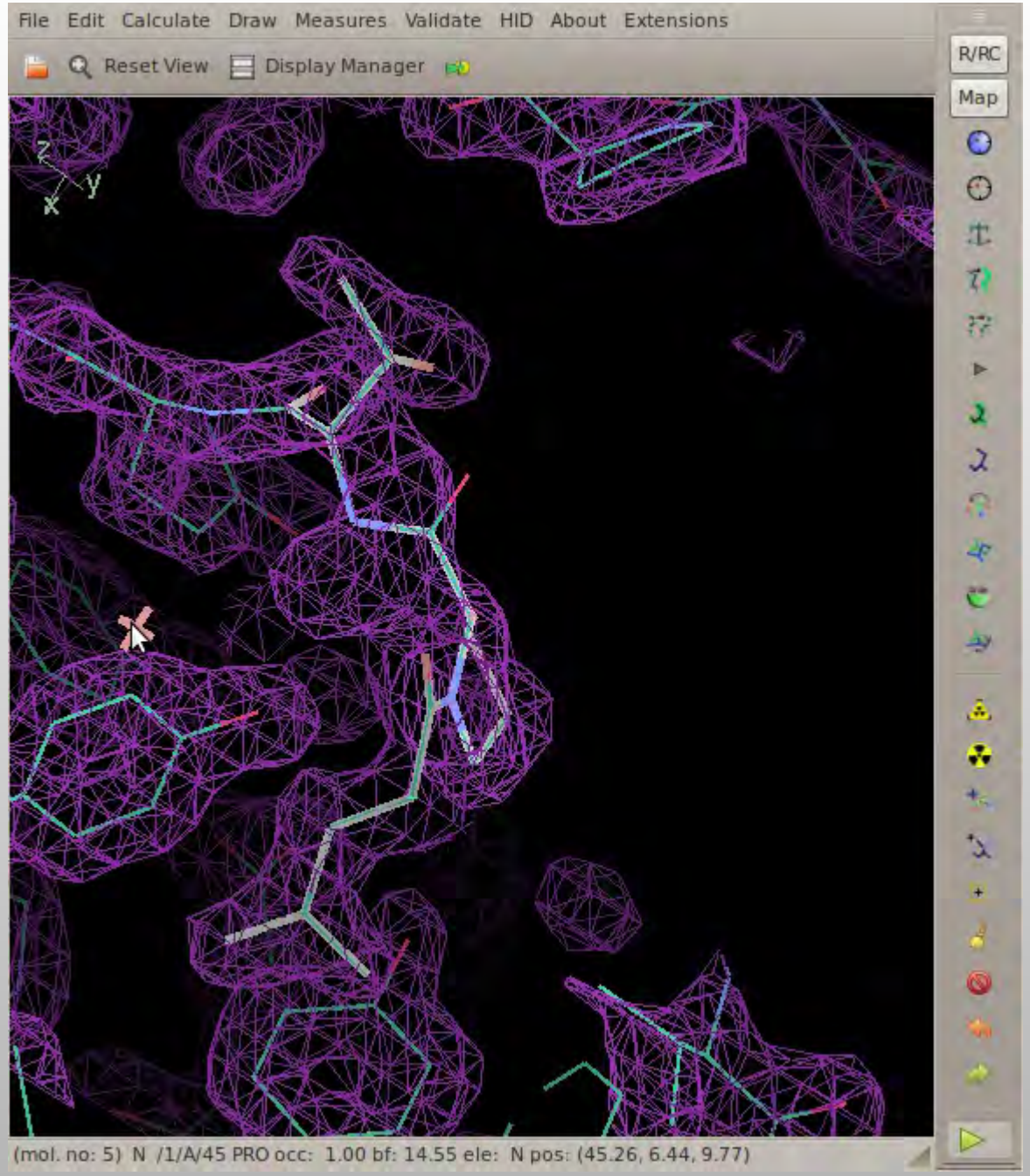
Good refinement



Bad refinement

Refinement Techniques

- Auto-zone (A-key → keybindings)
- Single-Atom Drag
  - Over-dragging
- Ramachandran Refinement
- Sphere refinement
- Coming Soon..?
  - Dials, PowerMate, spaceballs
  - Wii Refinement

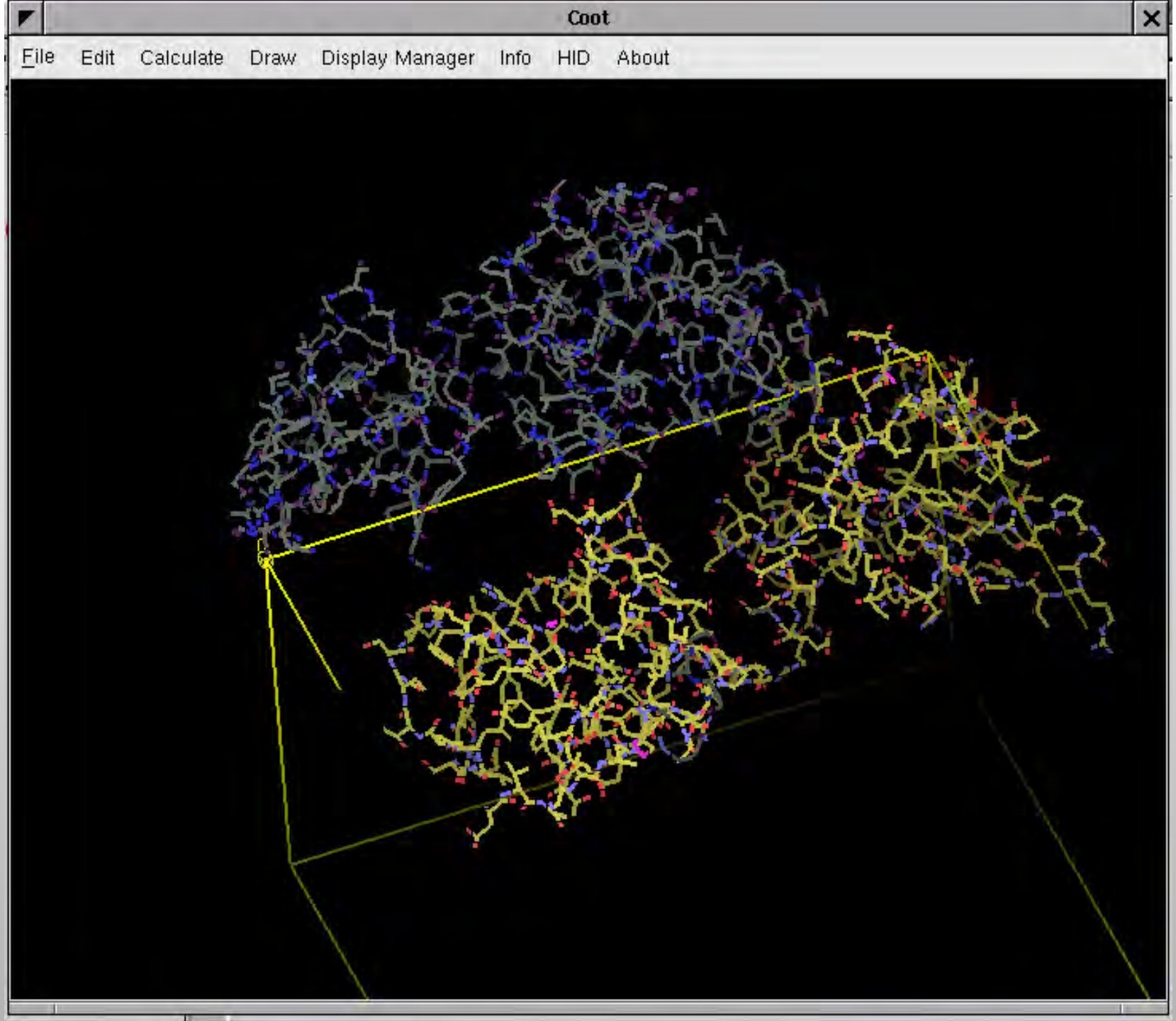


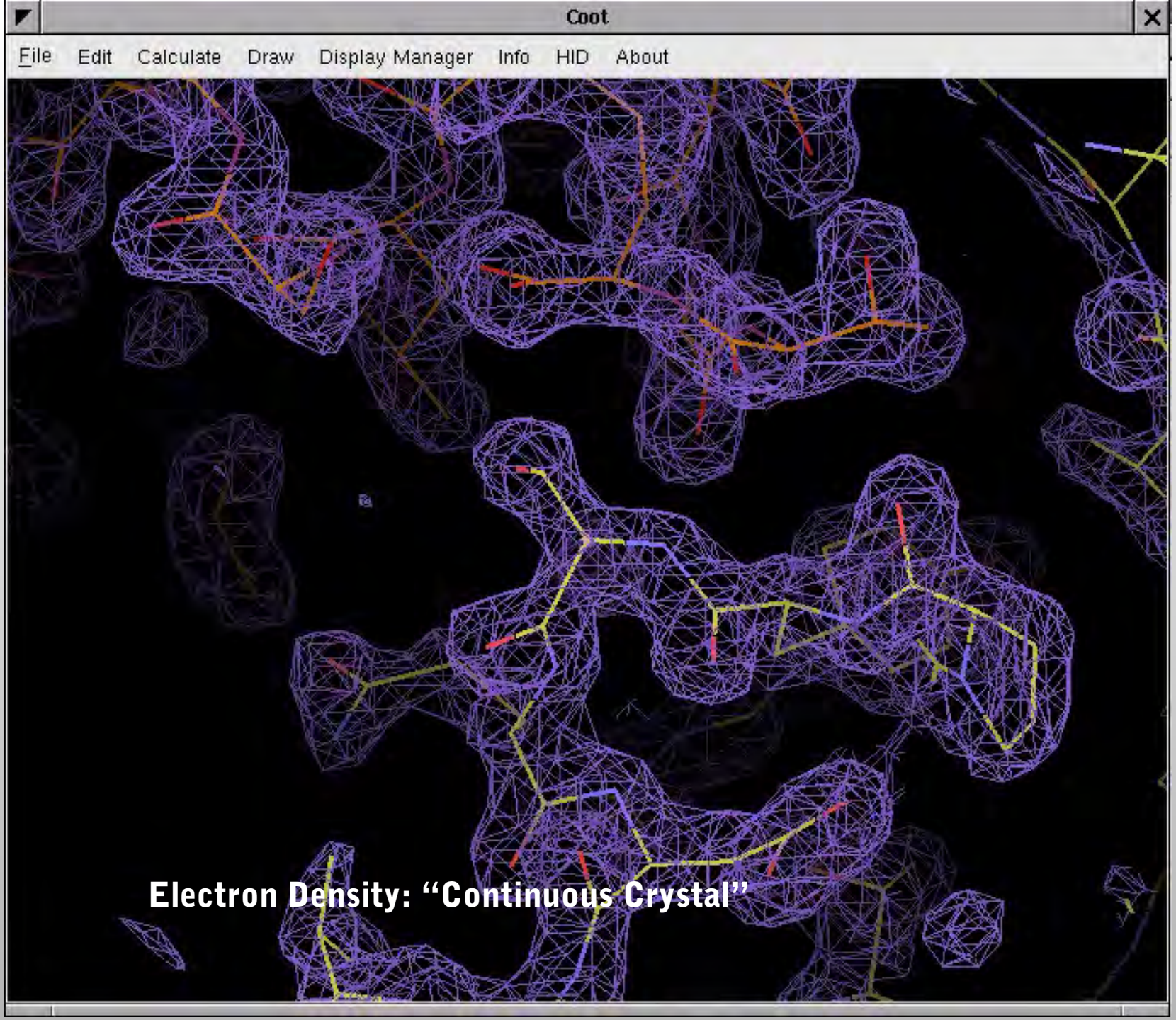
Over-dragging

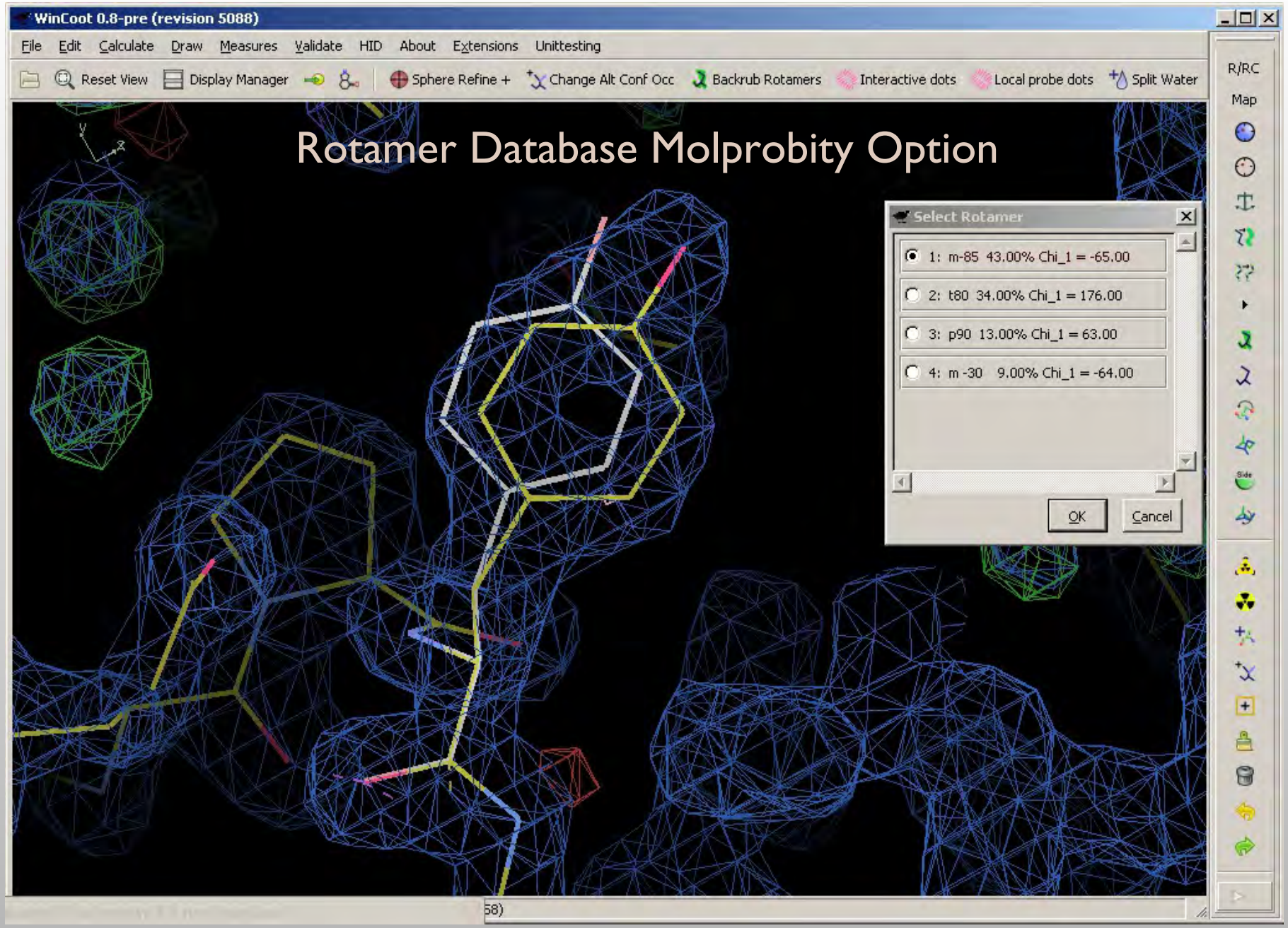
(Ctrl-left mouse)

“Sphere” Refinement

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

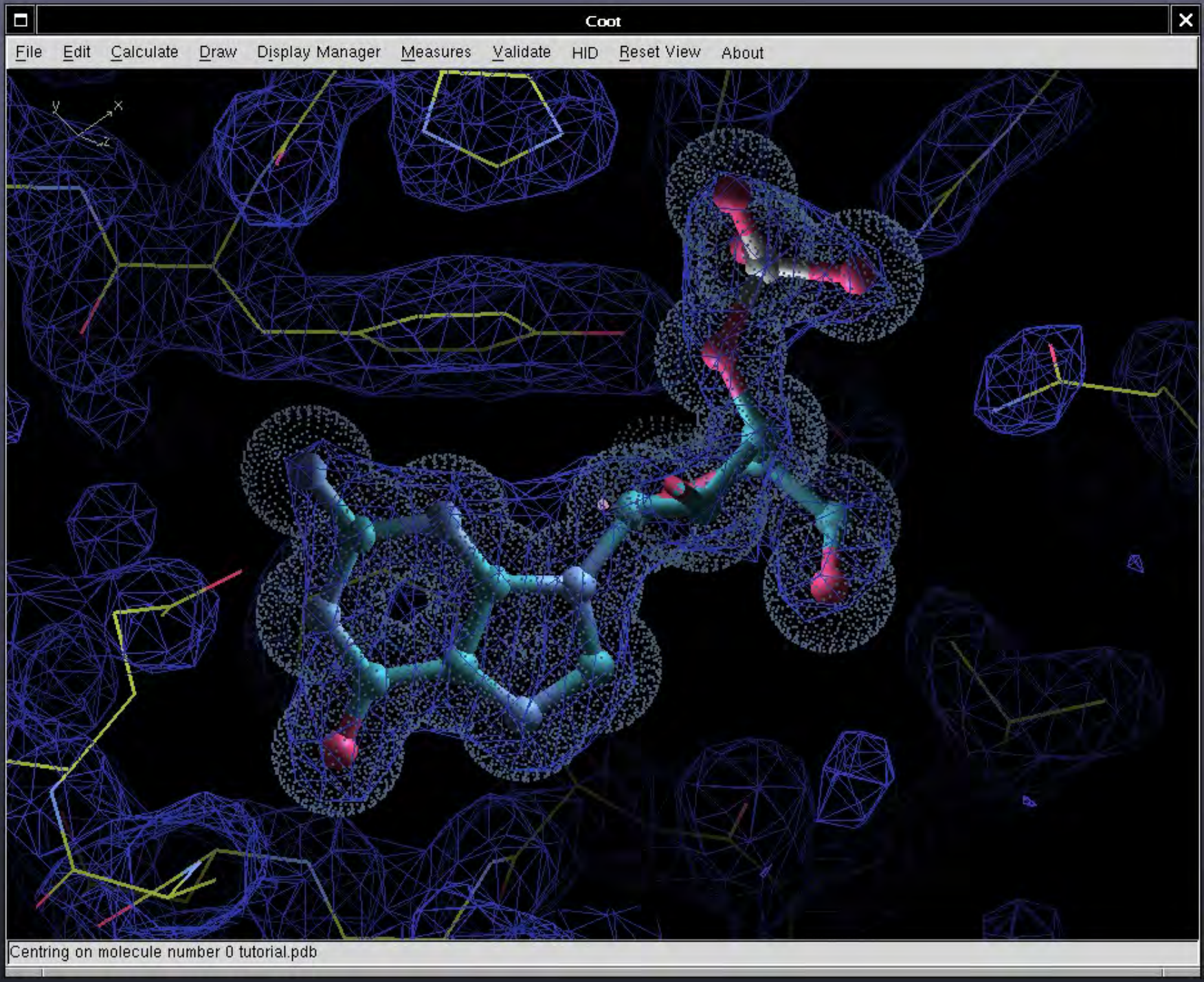

Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About


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


WinCoot 0.8-pre (revision 5088)
File Edit Calculate Draw Measures Validate HID About Extensions Unittesting
Reset View
Display Manager
Sphere Refine +
Change Alt Conf Occ
Backrub Rotamers
Interactive dots
Local probe dots
Split Water
R/RC
Map
Rotamer Database Molprobity Option
Select Rotamer
1: m-85 43.00% Chi_1 = -65.00
2: t80 34.00% Chi_1 = 176.00
3: p90 13.00% Chi_1 = 63.00
4: m -30 9.00% Chi_1 = -64.00
OK
Cancel

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

Low Resolution Tools

Extra Restraints....

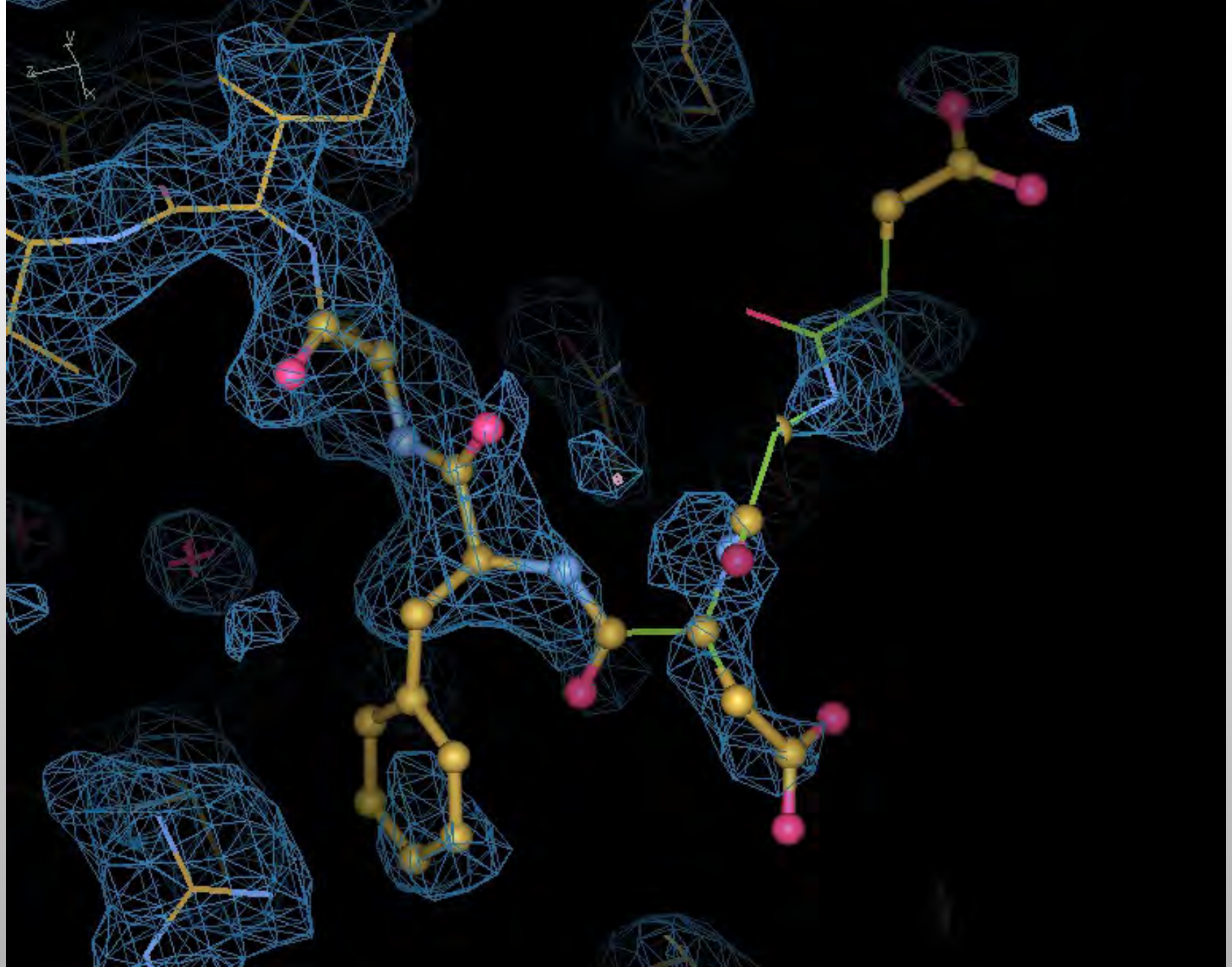
Extra Restraints....

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

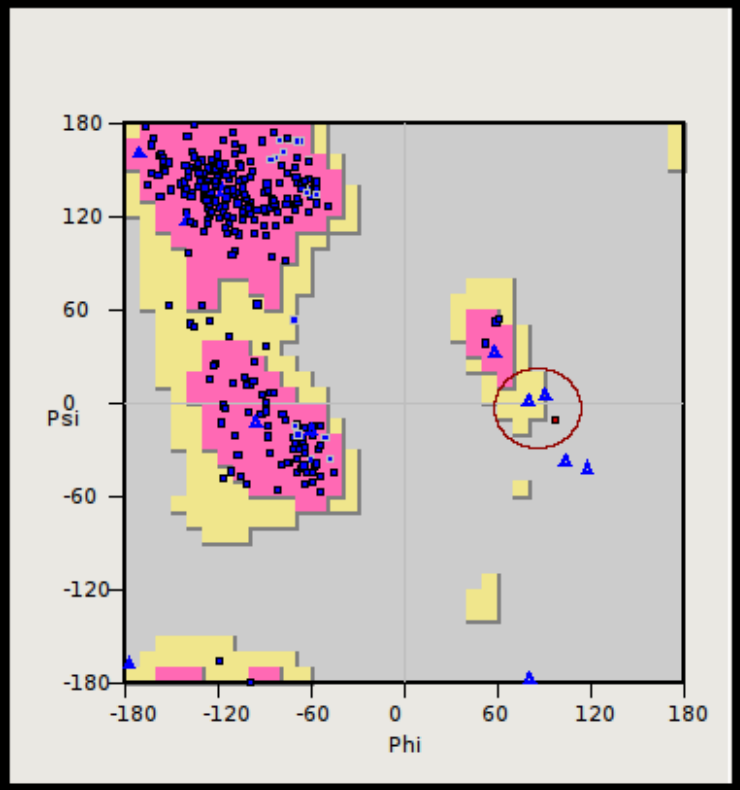
- Map sharpening
- [Jiggle fit]
- [Morphing]

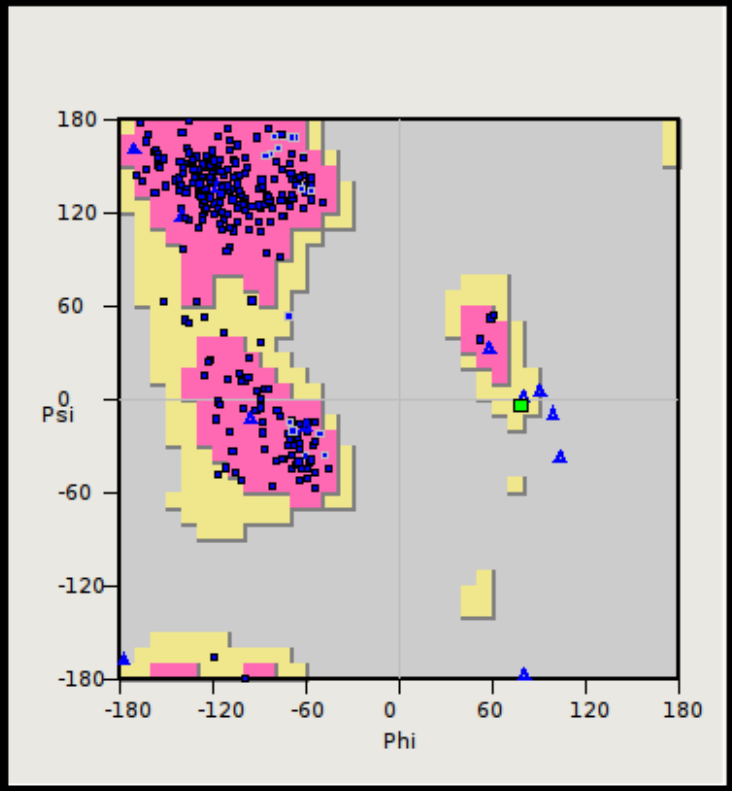
Ramachandran Restraints

- 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

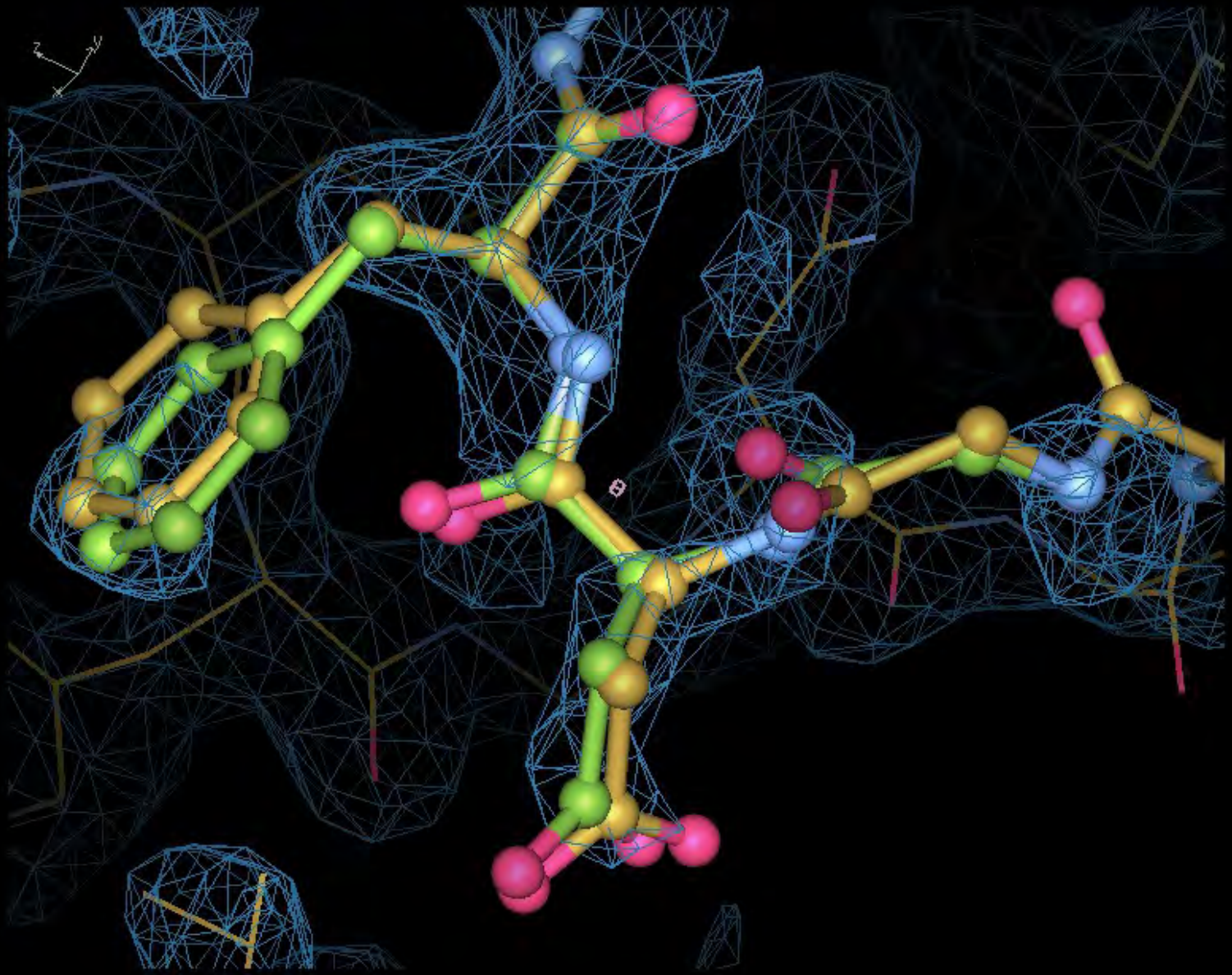

Y
Z
X

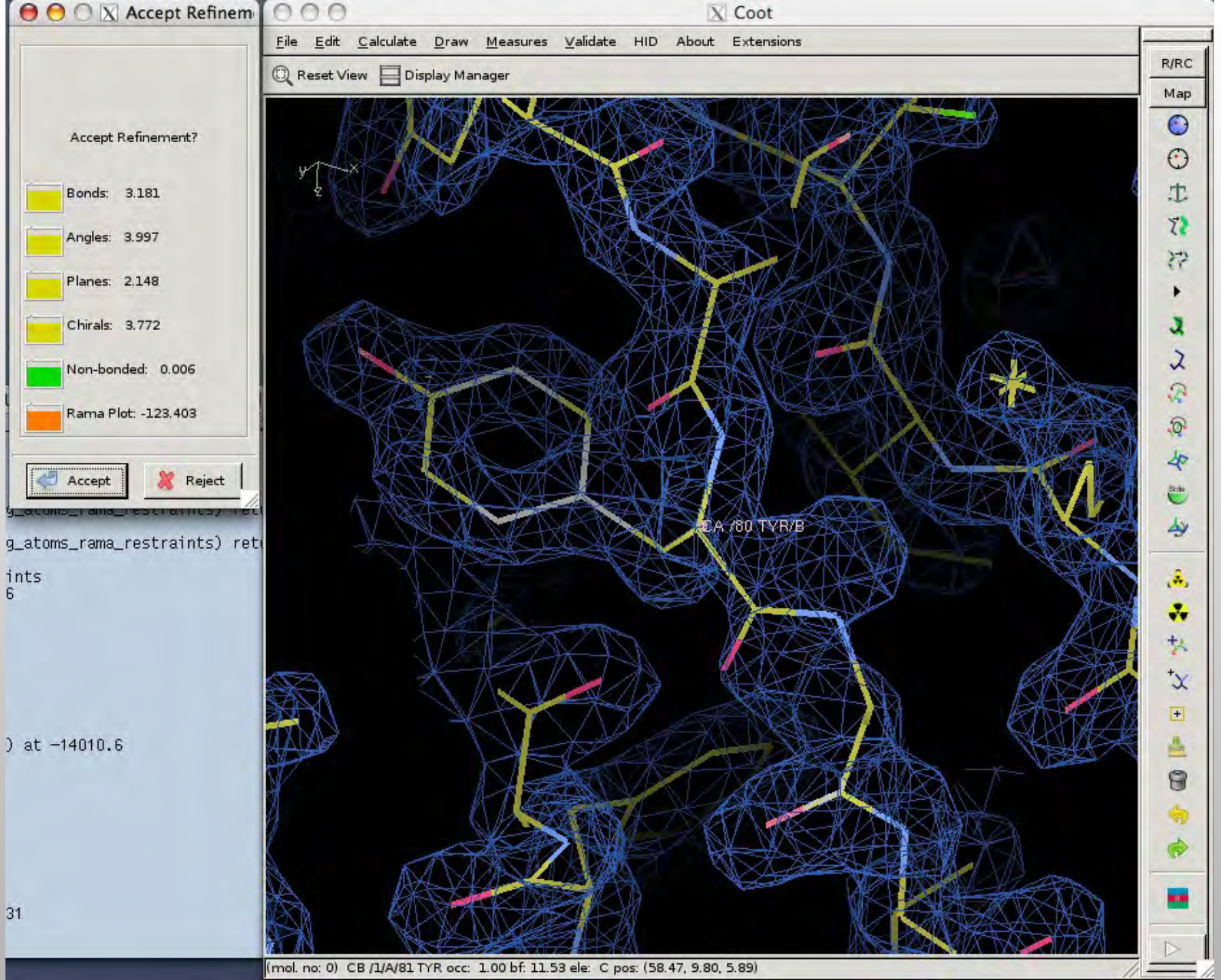
Tweaking a Ramachandran Outlier

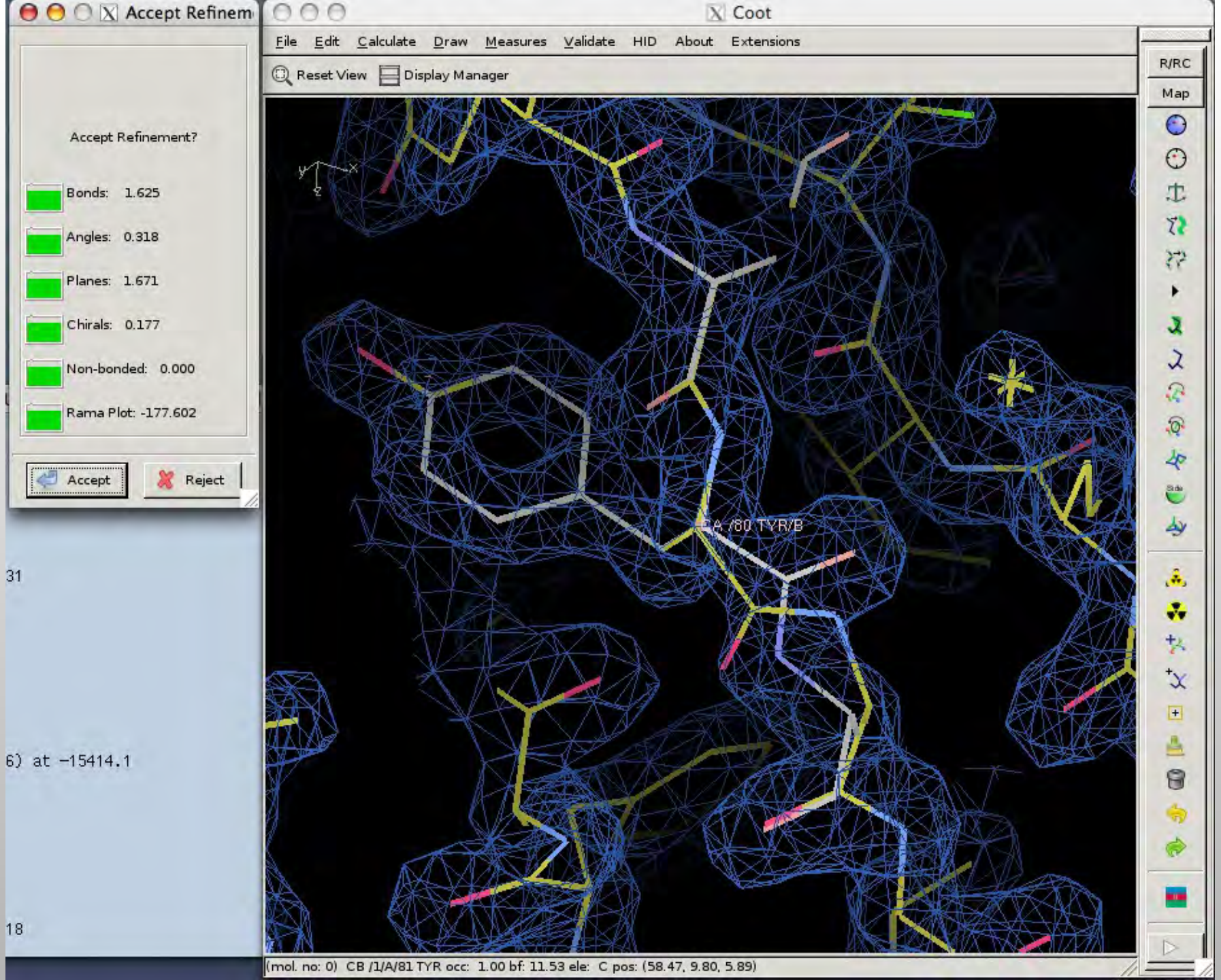




Tweaking Phi and Psi




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
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?

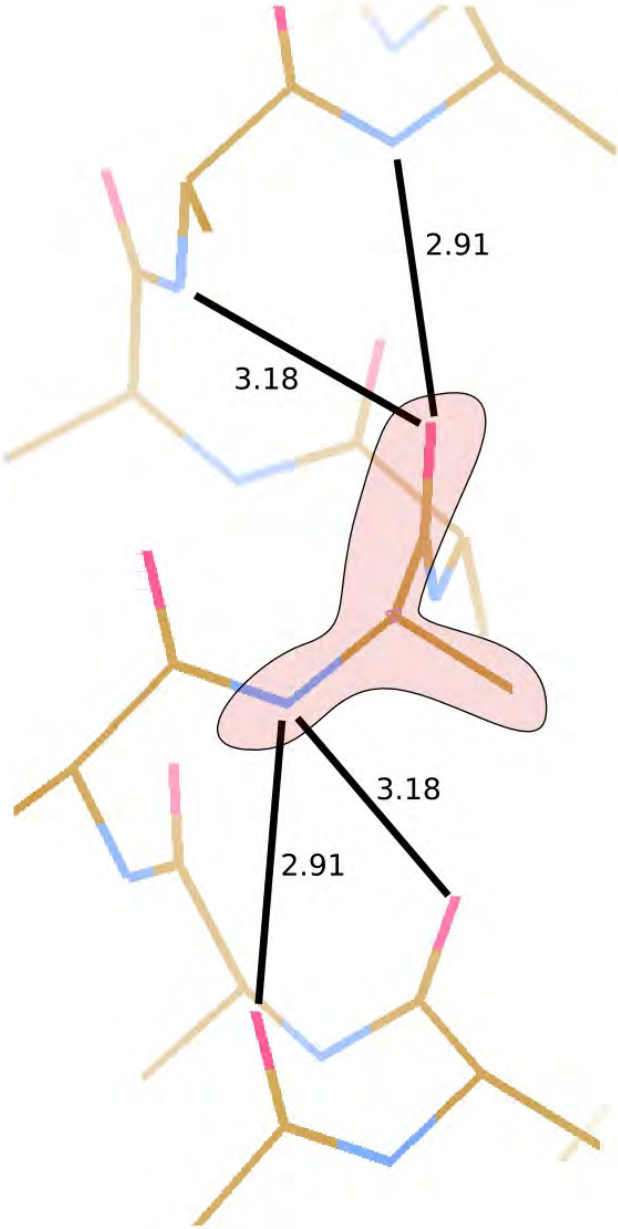
Adding Torsion Angle Restraints

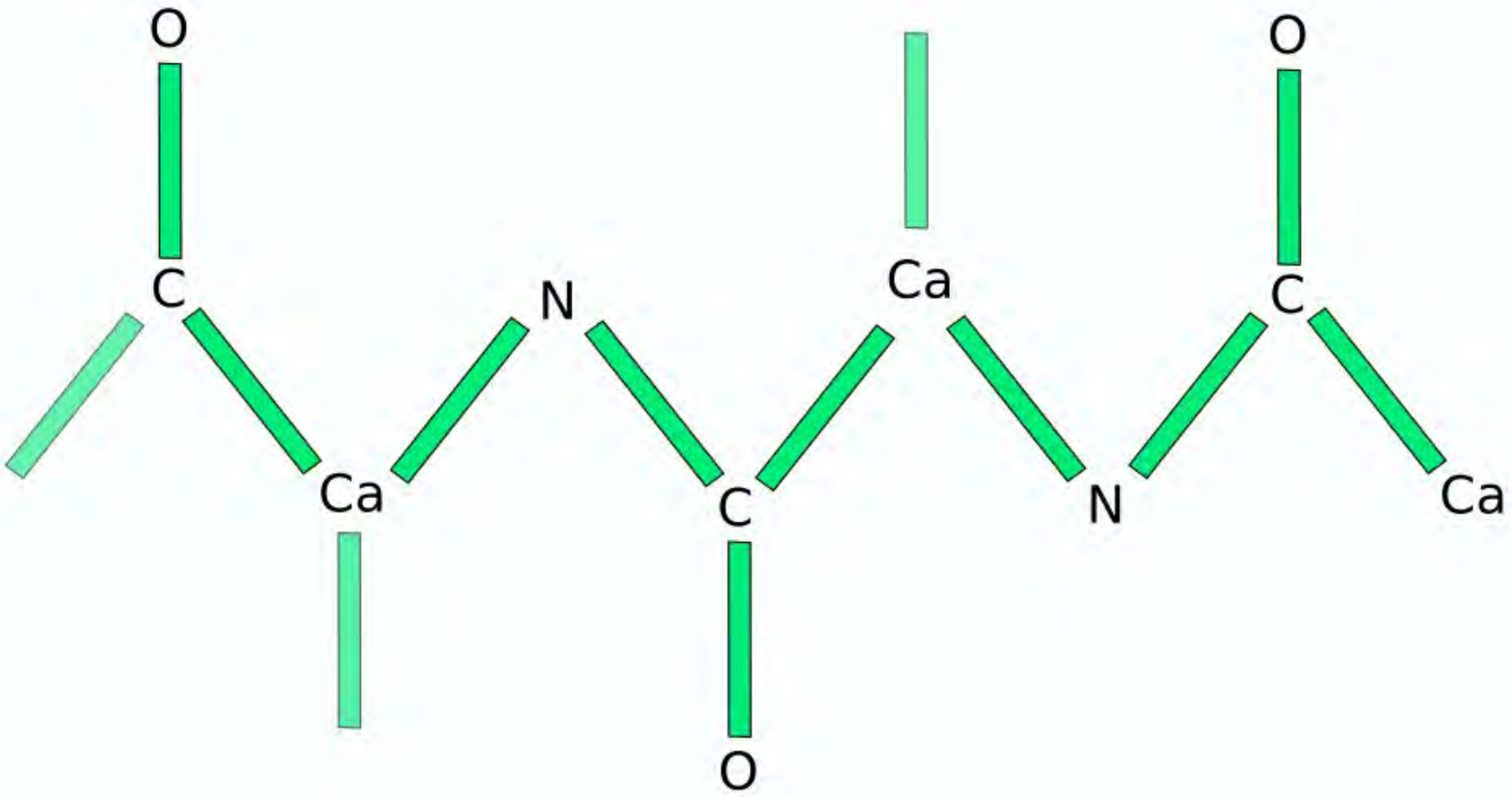
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”
    - i.e. trapped in local minimum

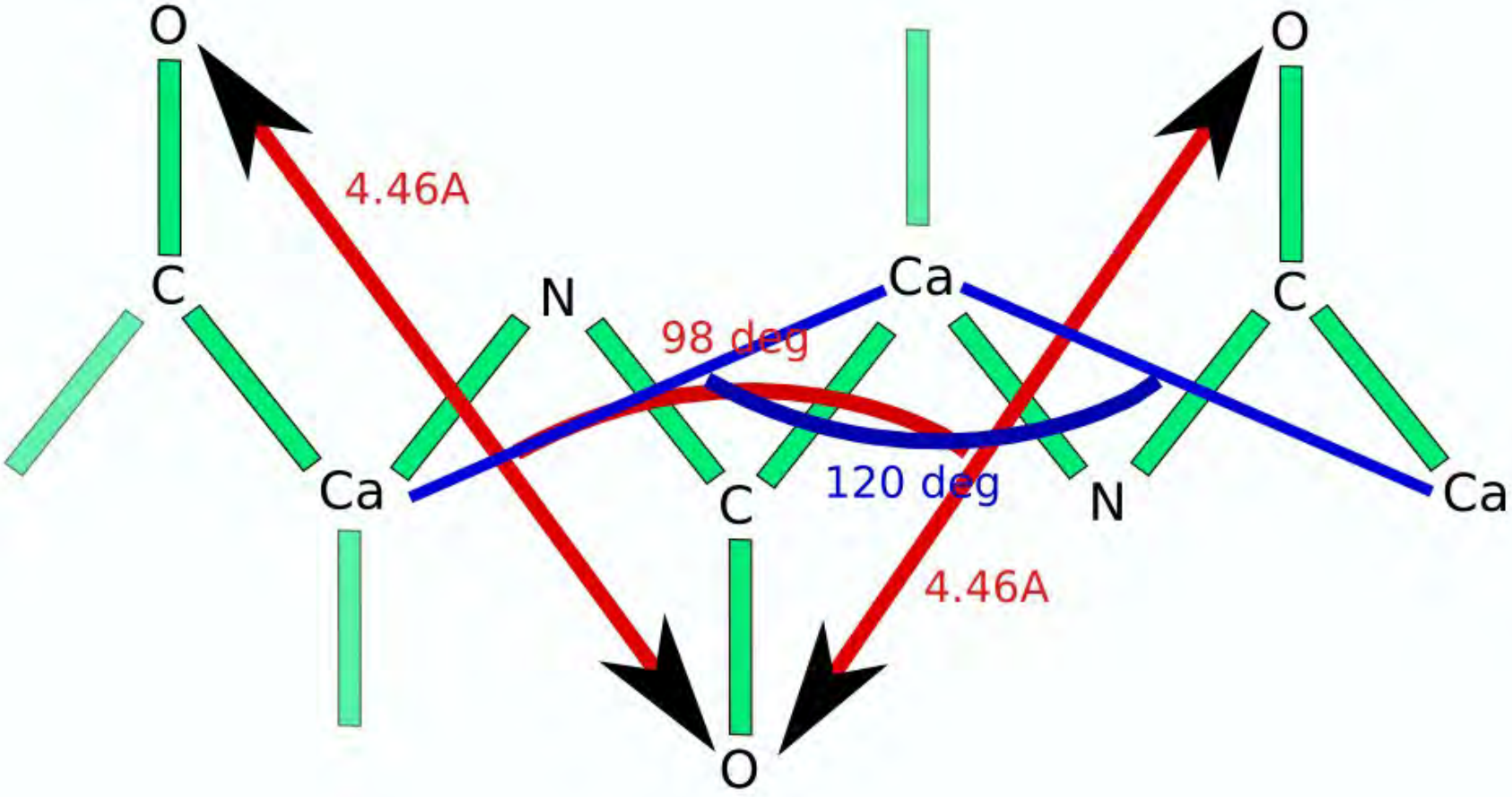
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints

Restrain the Hydrogen-bonding atom distances




O
C
Ca
N
Ca
C
O
O
C
N
Ca

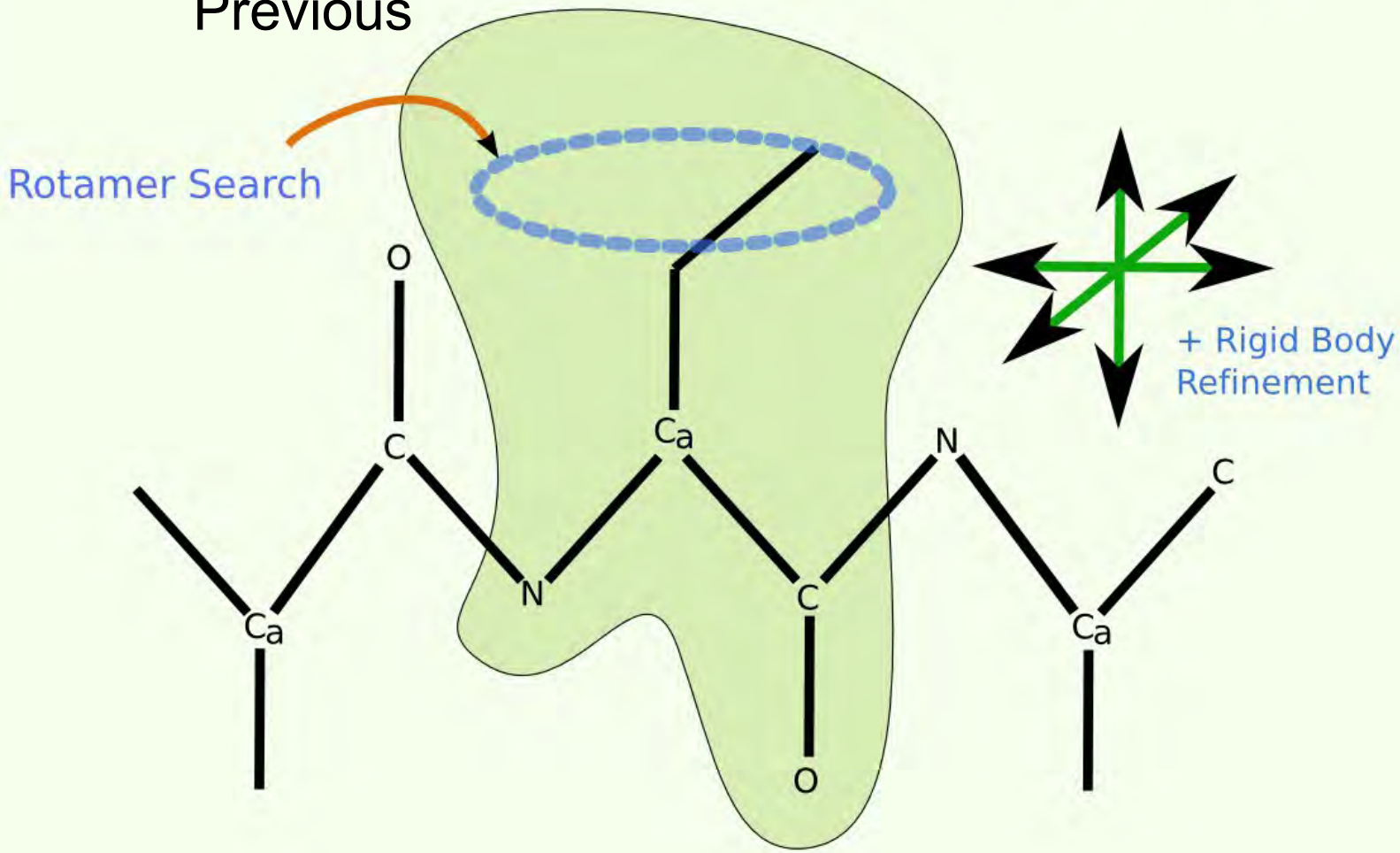

O
4.46A
C
N
Ca
O
C
Ca
98 deg
120 deg
Ca
C
N
4.46A
O

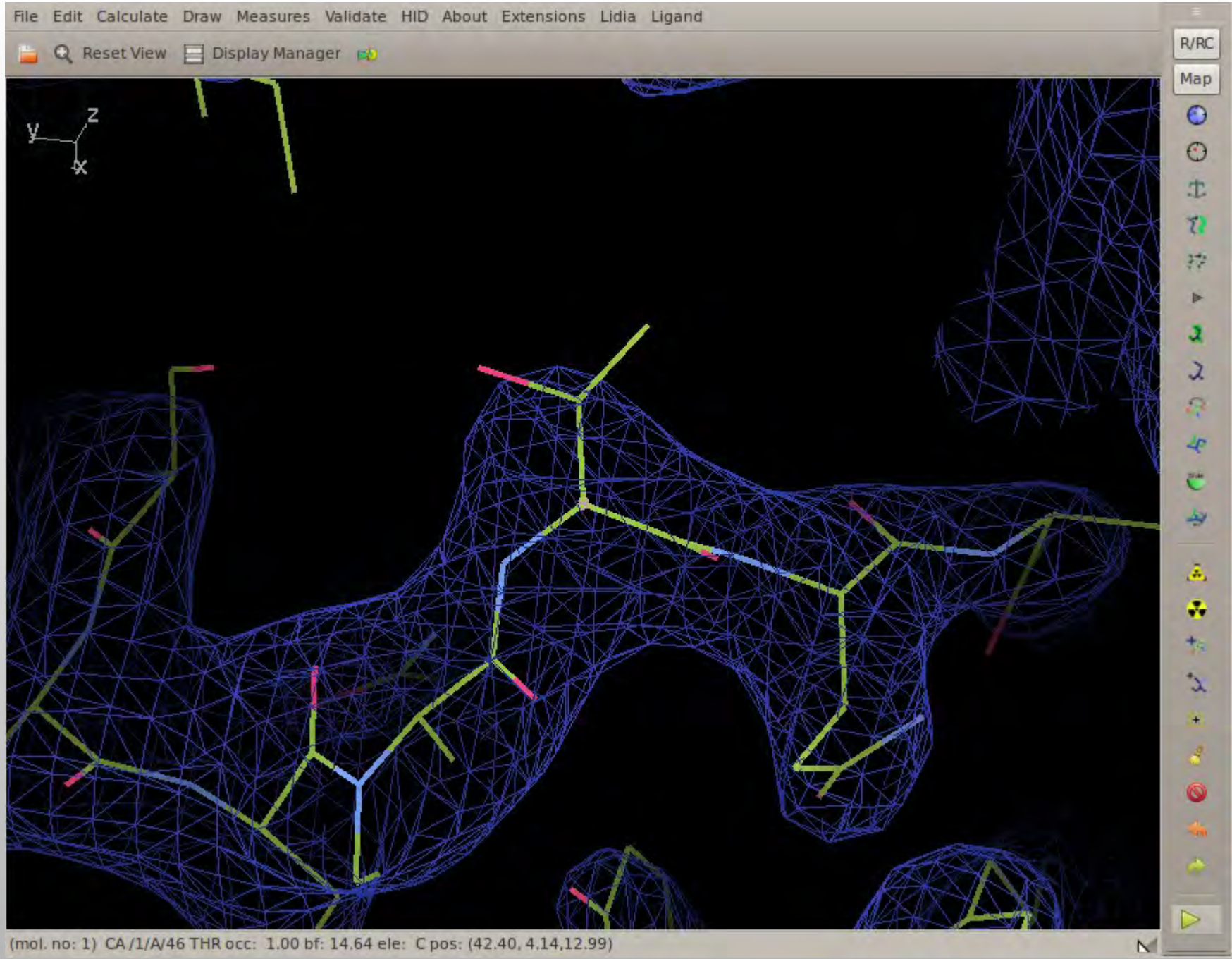
”Backrub Rotamers”

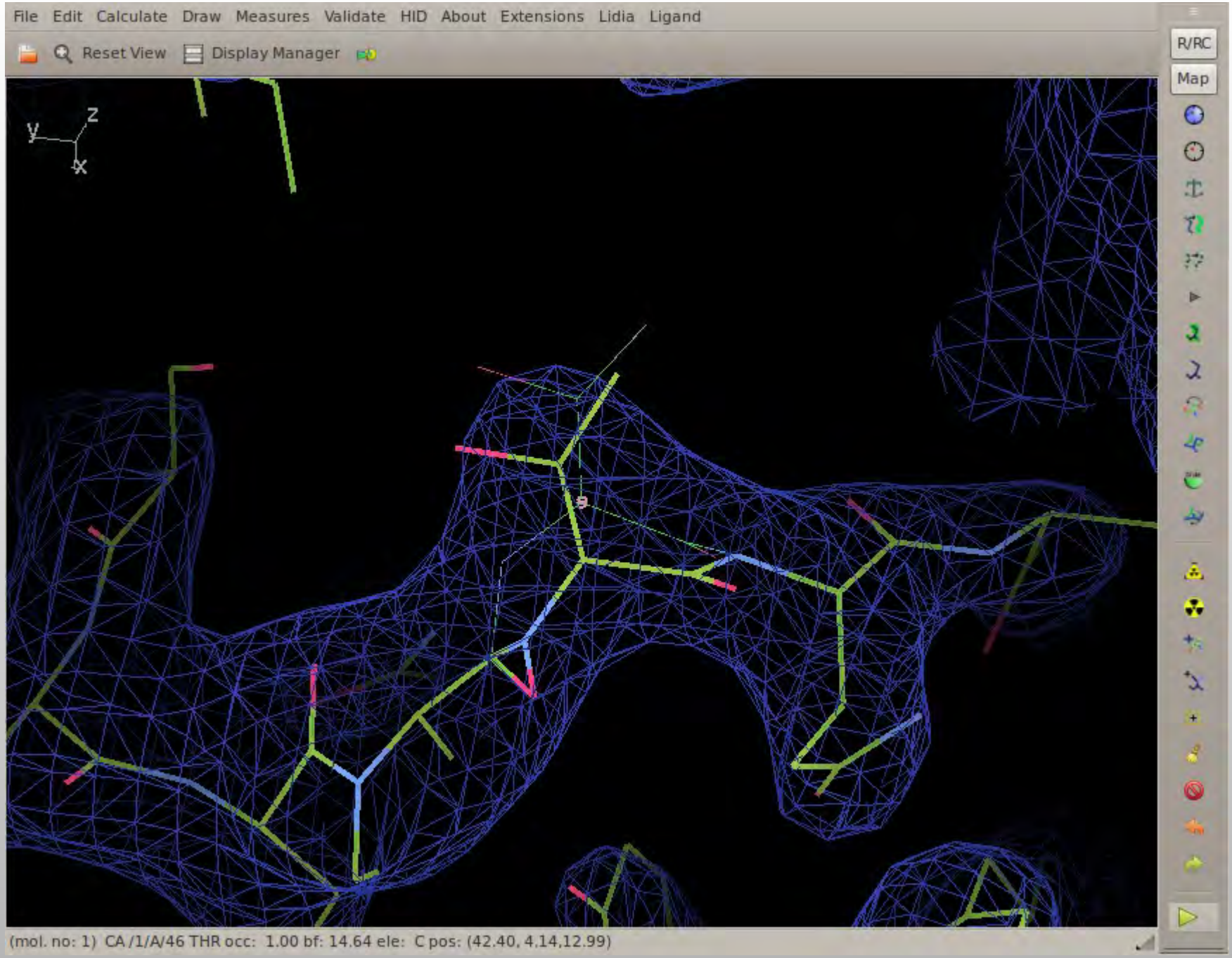
- High probability models with low resolution data

~~Current~~ Low Resolution Rotamer Search

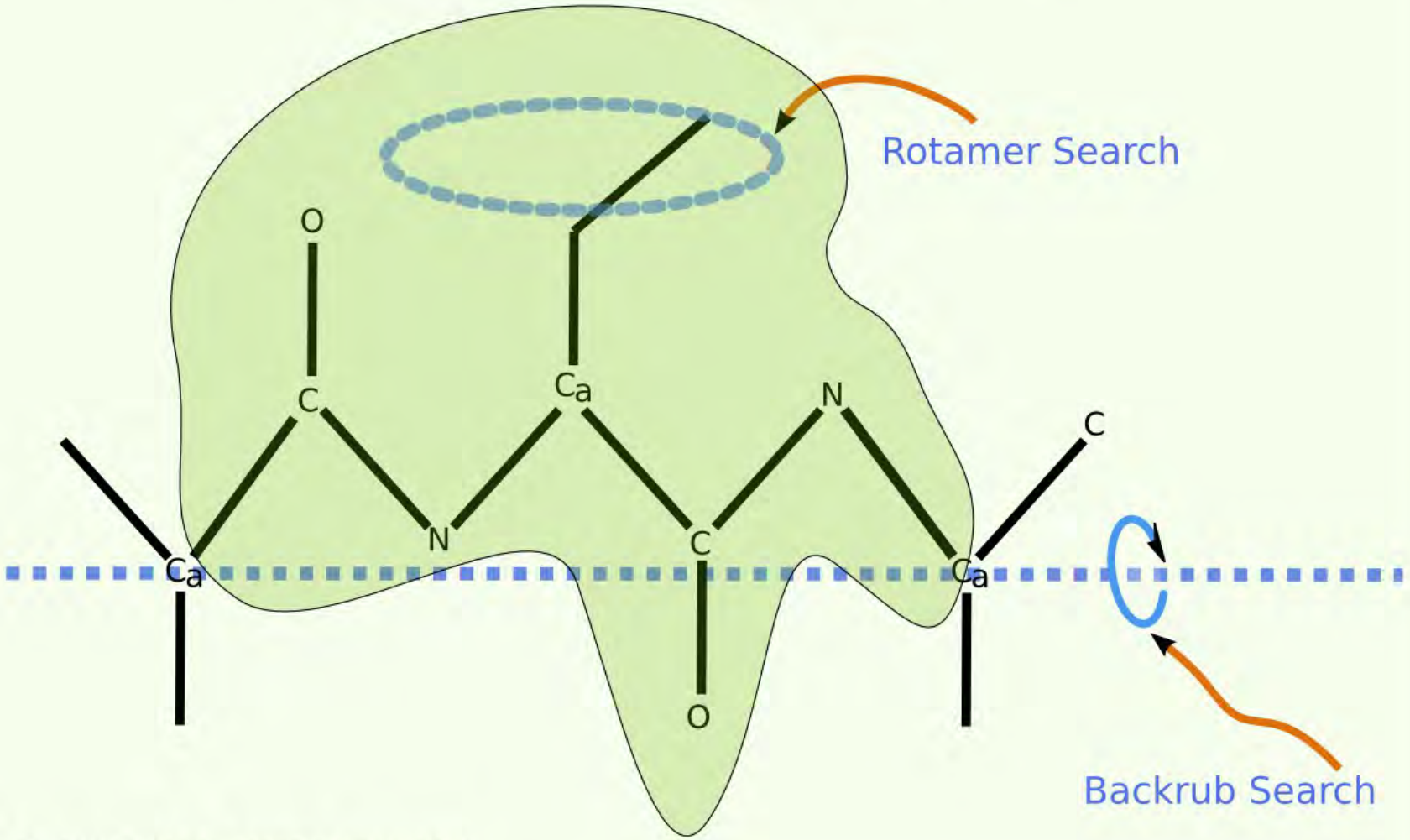
Previous



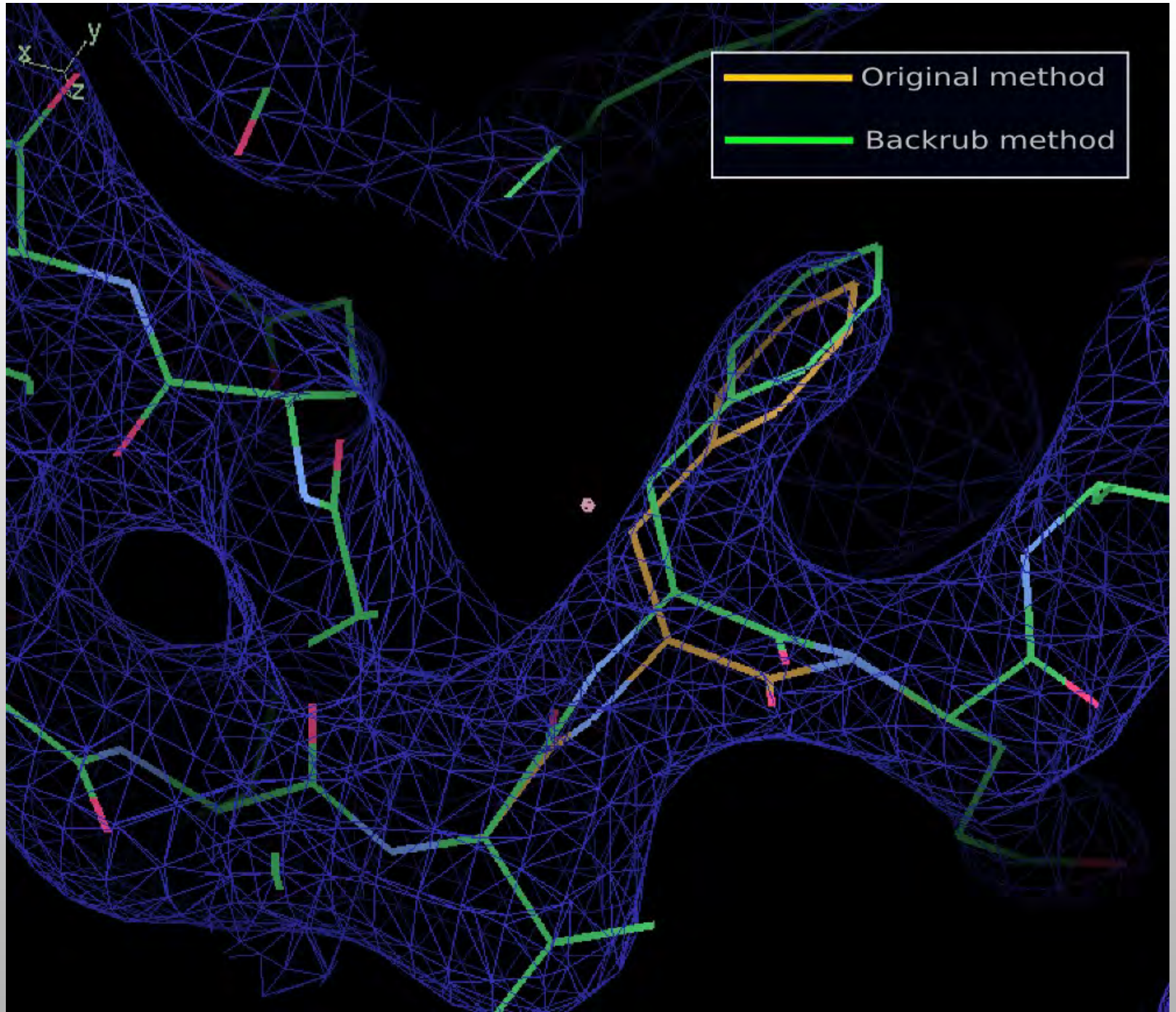

File Edit Calculate Draw Measures Validate HID About Extensions Lidia Ligand
Reset View
Display Manager
R/RC
Map
Z
Y
X
(mol. no: 1) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)


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)

New Low Resolution Rotamer Search



After Fitting Tools in KING/Molprobity


y
x
z
Original method
Backrub method

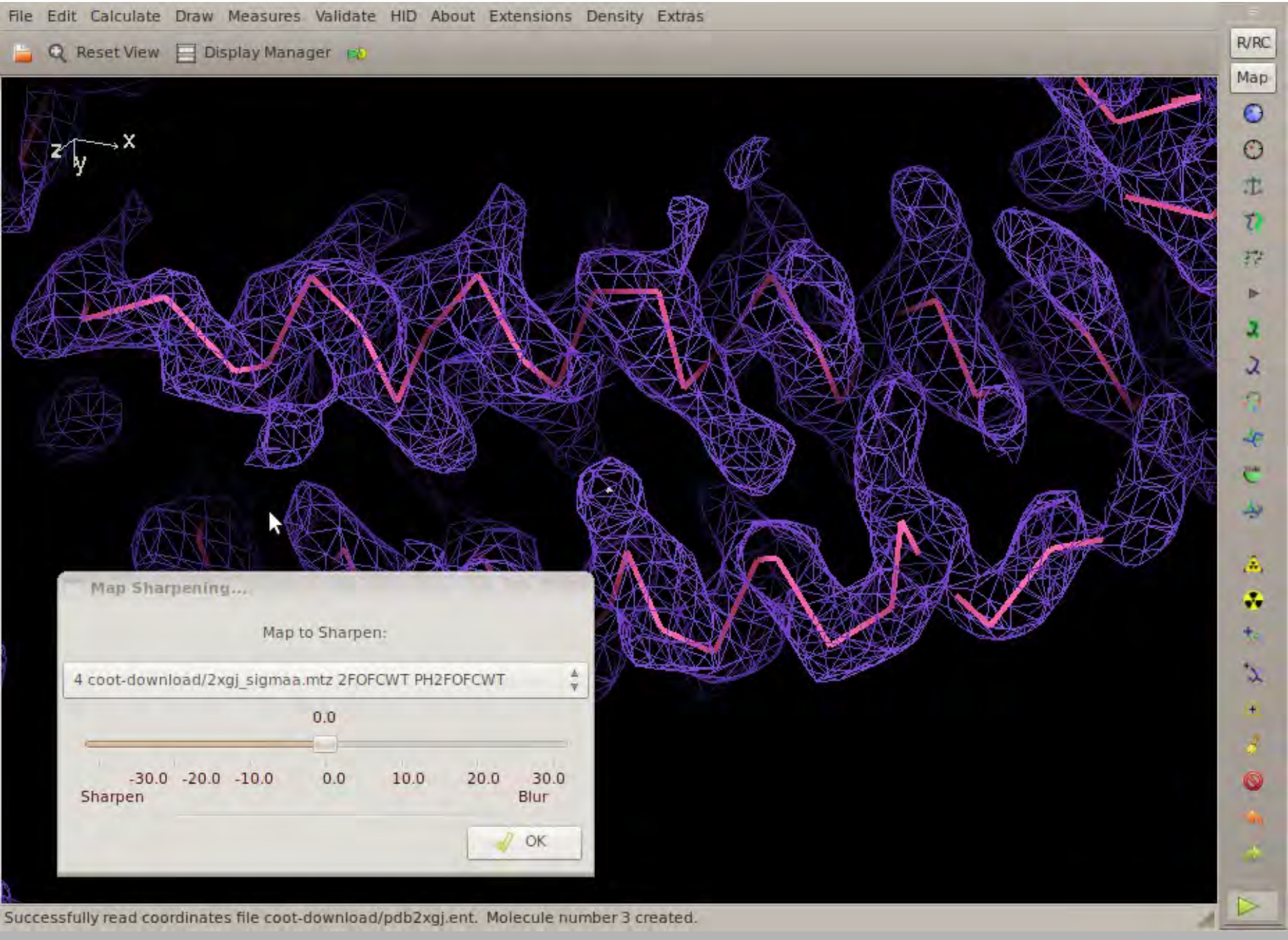
To turn it on...

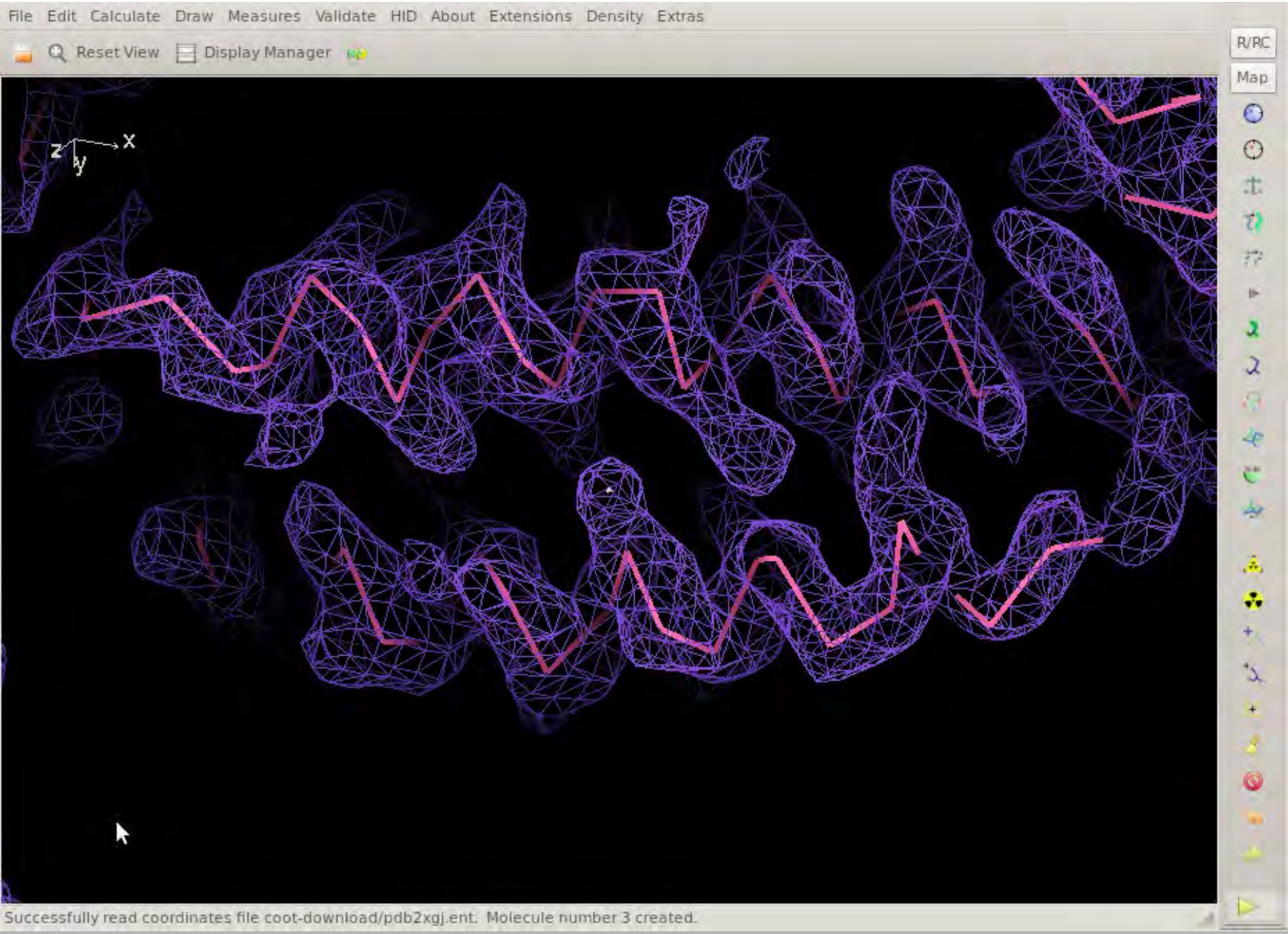
- automatically for resolution > 2.9Å
- via
  - Extensions → Modelling → Rotamer Search
  - scripting:
    - guile: `(ROTAMERSEARCHLOWRES)`
    - python: `set_rotamer_search_mode(ROTAMERSEARCHLOWRES)`
  - toolbutton

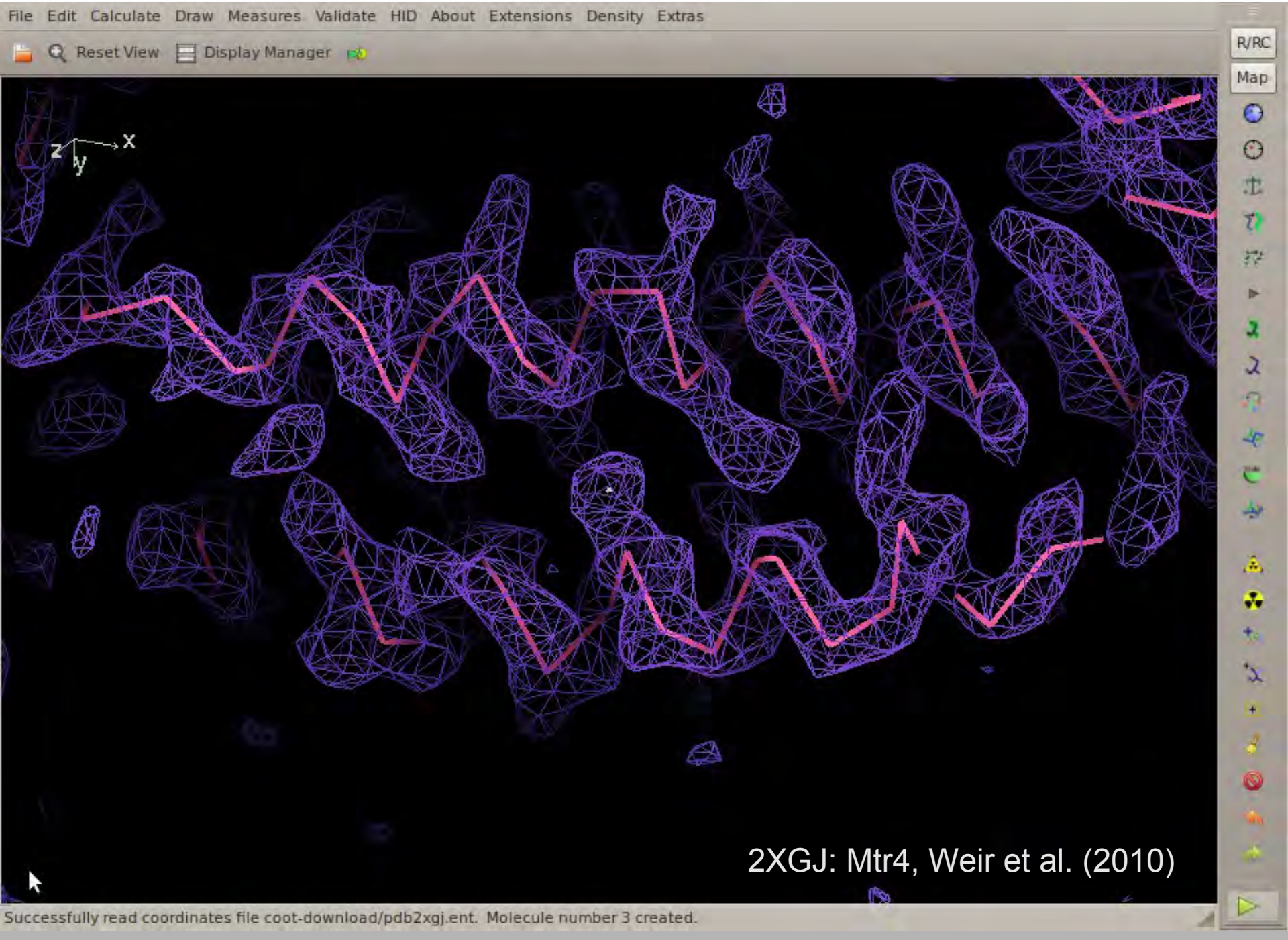
Map Sharpening

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...

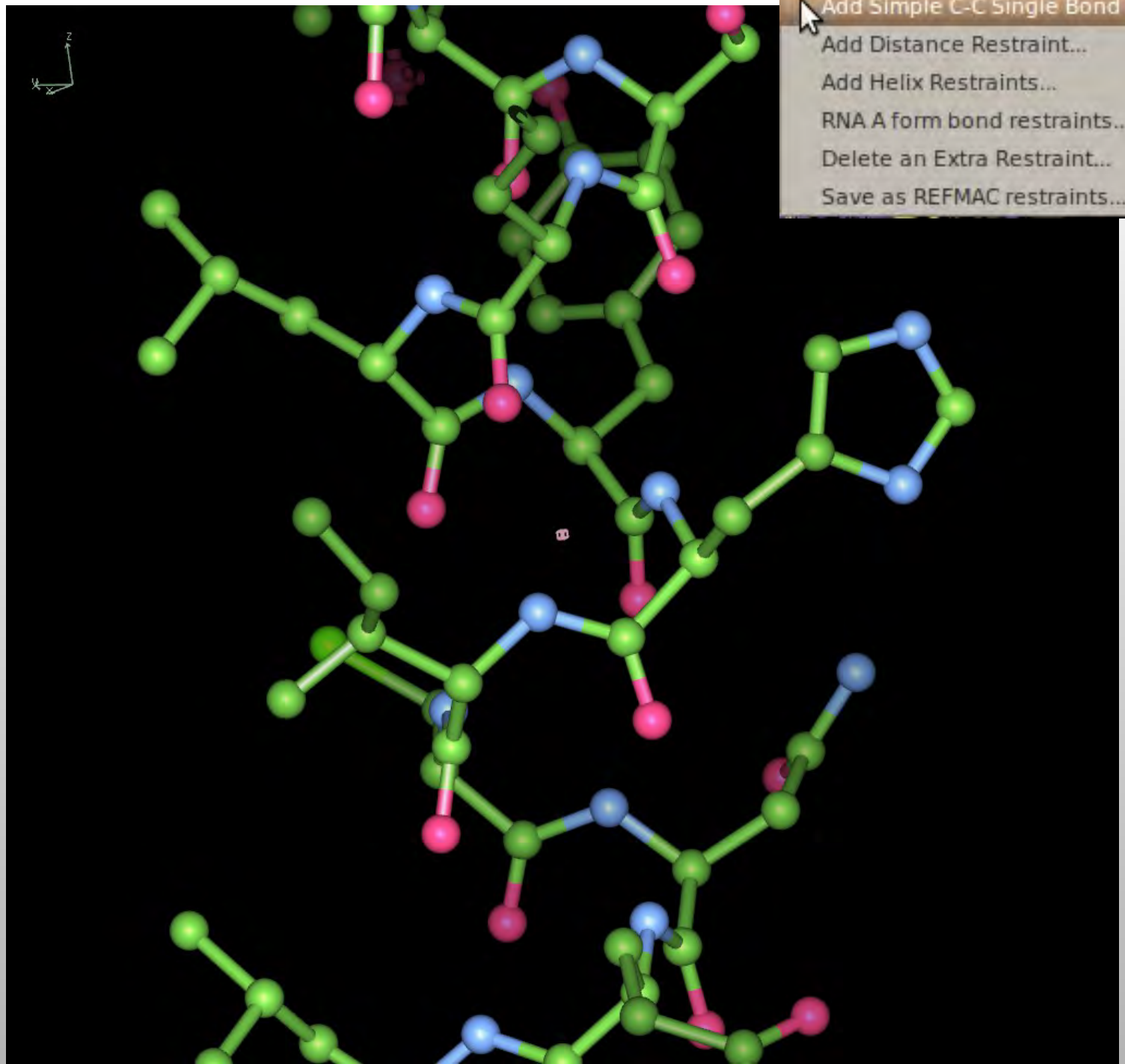

File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
Map Sharpening...
Map to Sharpen:
4 coot-download/2xgj_sigmaa.mtz 2FOFCWT PH2FOFCWT
0.0
-30.0 -20.0 -10.0 0.0 10.0 20.0 30.0
Sharpen
Blur
OK
Successfully read coordinates file coot-download/pdb2xgj.ent.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

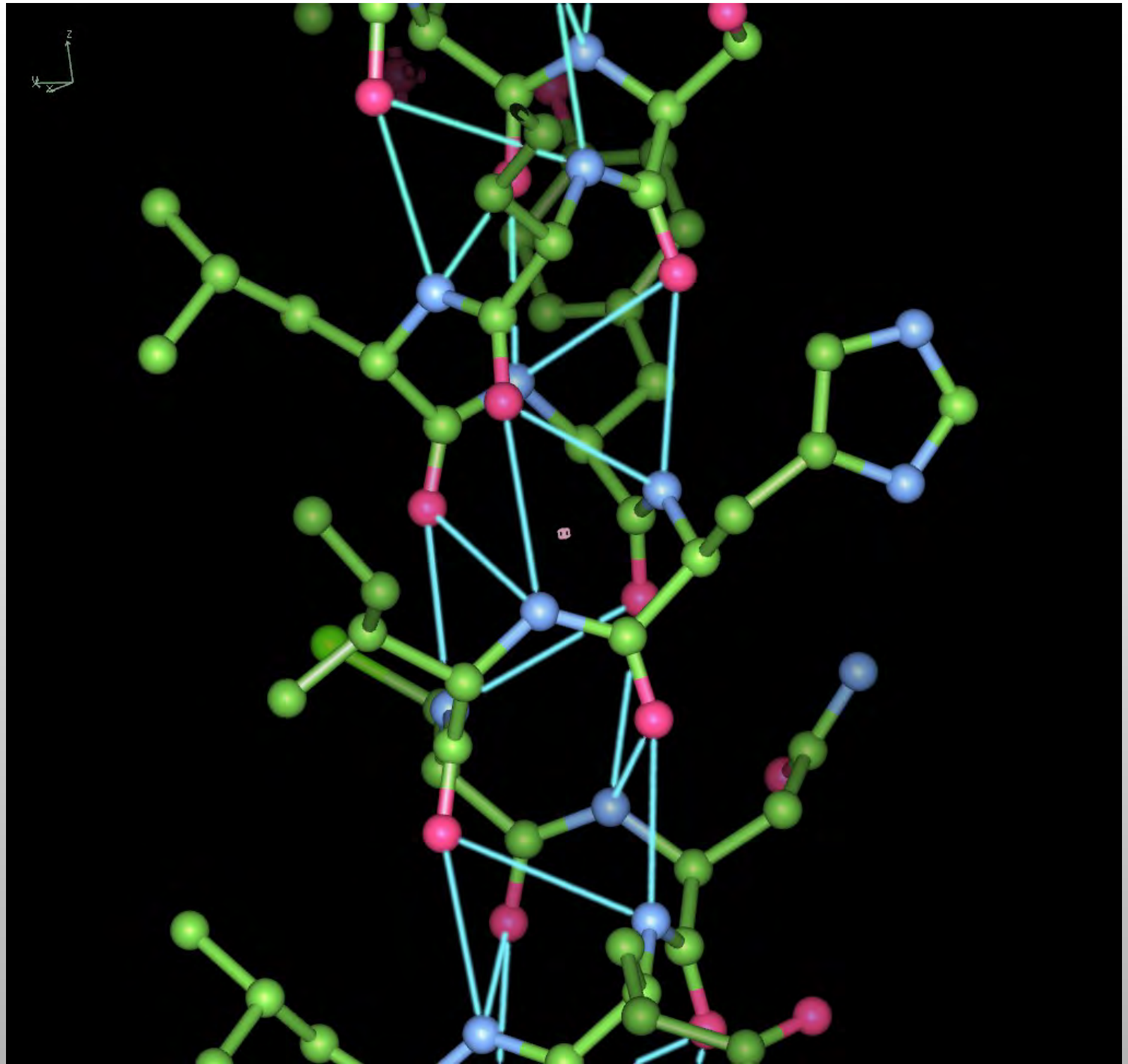

File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.

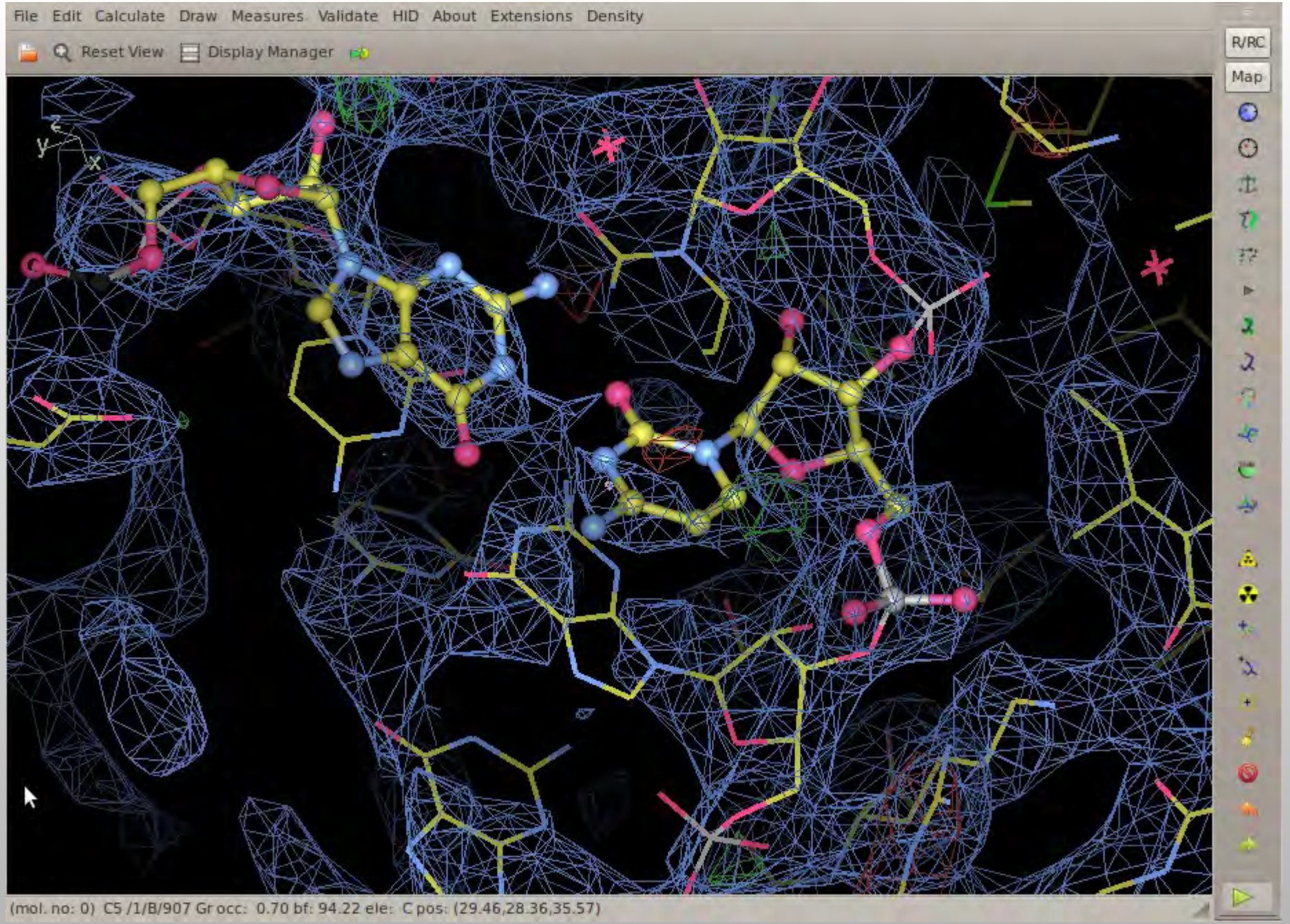

File Edit Calculate Draw Measures Validate HID About Extensions Density Extras
Reset View
Display Manager
R/RC
Map
z
x
y
2XGJ: Mtr4, Weir et al. (2010)
Successfully read coordinates file coot-download/pdb2xgj.ent. Molecule number 3 created.

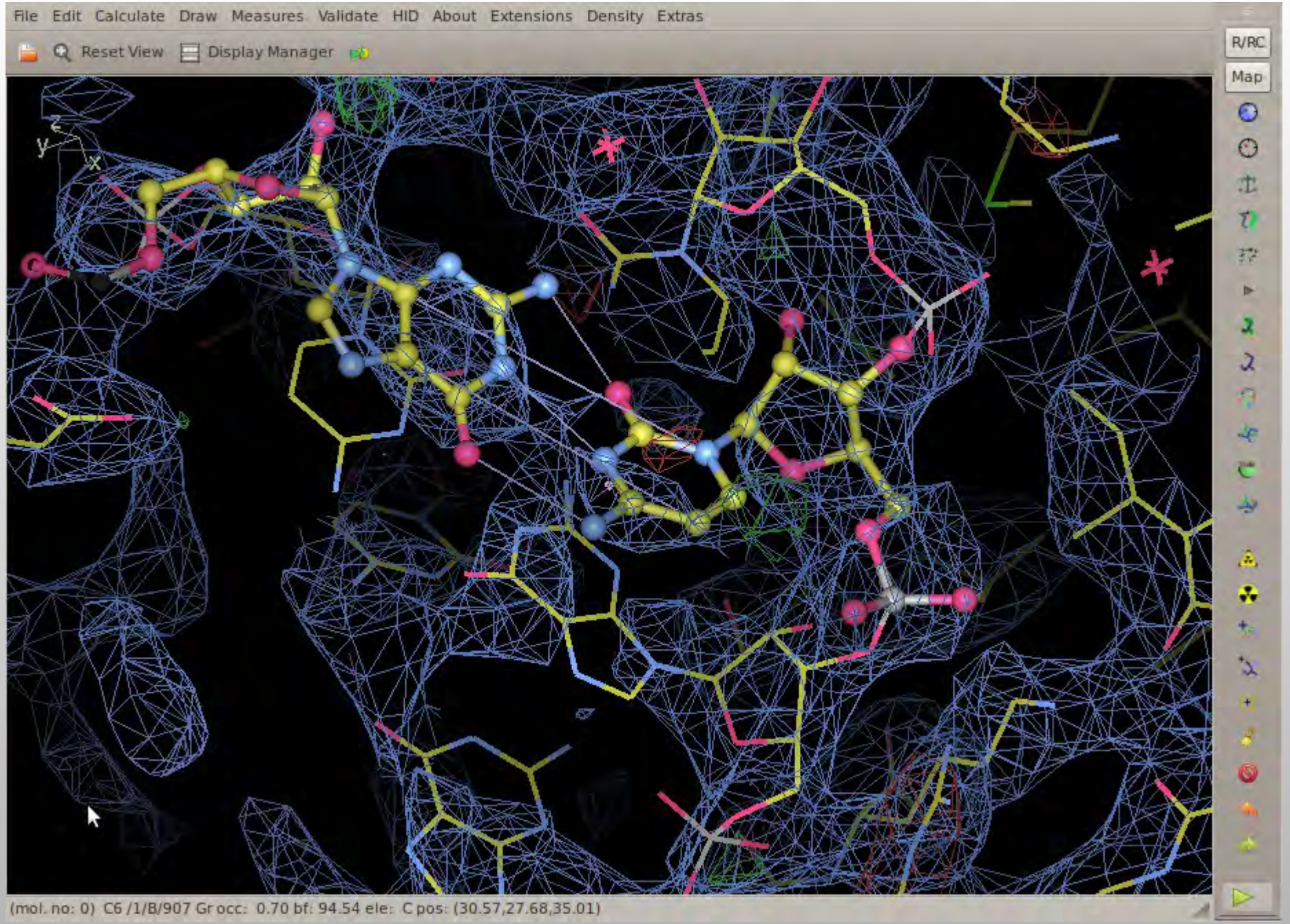
Restraints Editing in *Coot*

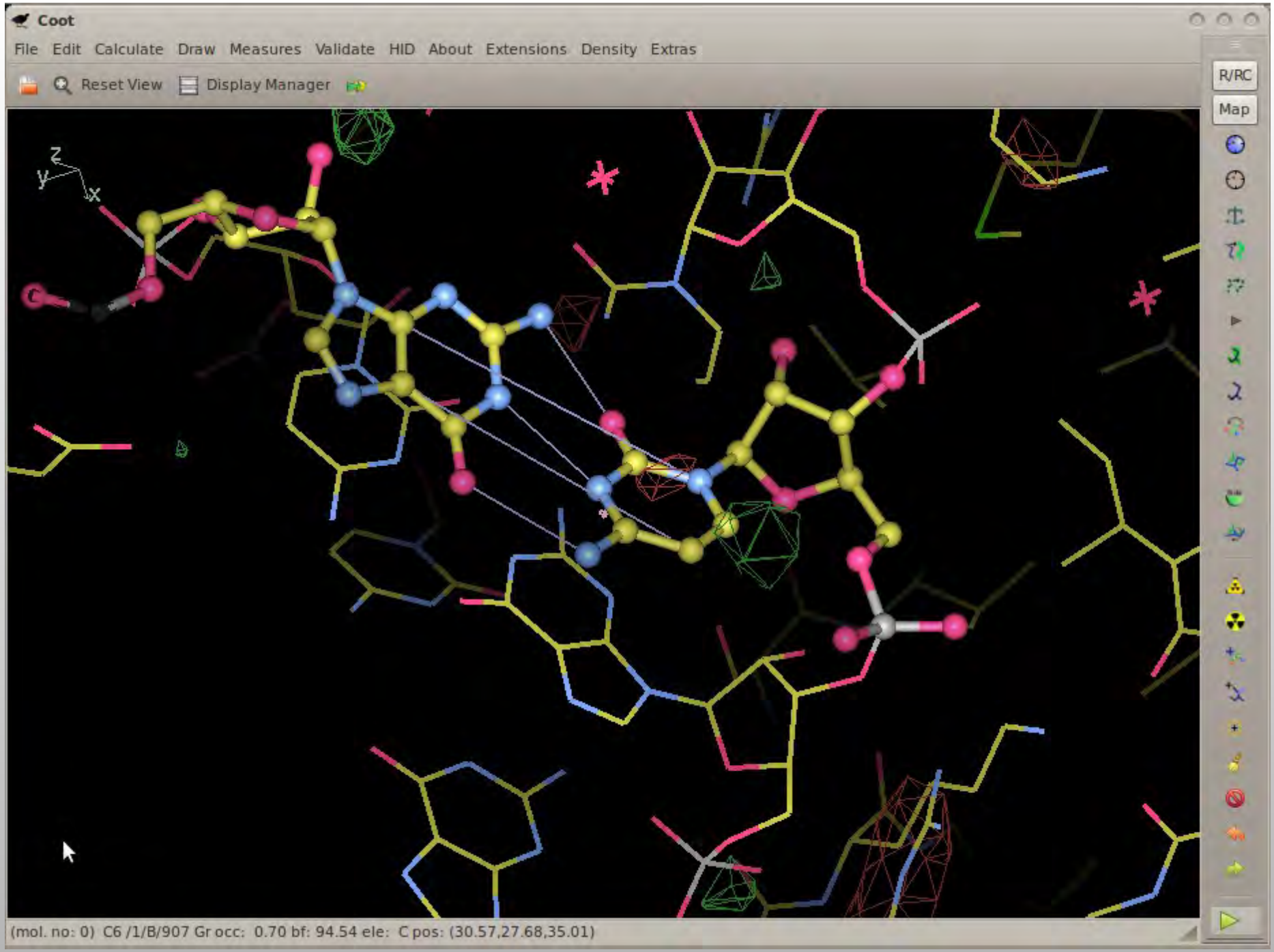
- Distance Restraints:
  - Alpha helices, A-form RNA, B-form DNA
- Add and delete individual restraints
  - User-selectable sigma
- Select 2 residues for range
- User-defined torsion restraints
- Input from ProSMART
- Output to Refmac
- [planar restraints]

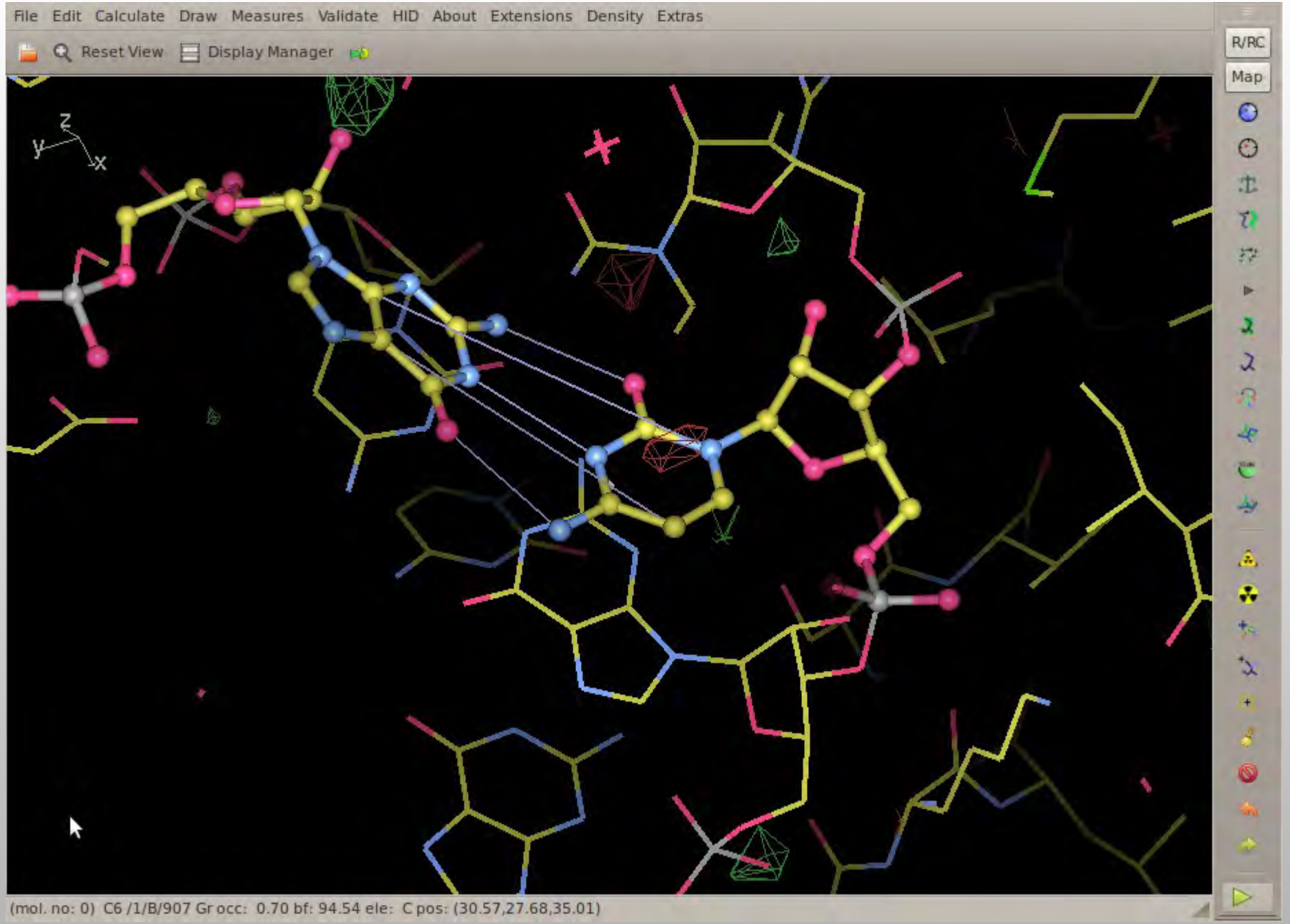

Add Simple C-C Single Bond Restraint...
Add Distance Restraint...
Add Helix Restraints...
RNA A form bond restraints...
Delete an Extra Restraint...
Save as REFMAC restraints...




File Edit Calculate Draw Measures Validate HID About Extensions Density
Reset View
Display Manager
R/RC
Map
(mol. no: 0) C5 /1/B/907 Gr occ: 0.70 bf: 94.22 ele: C pos: (29.46,28.36,35.57)


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)


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)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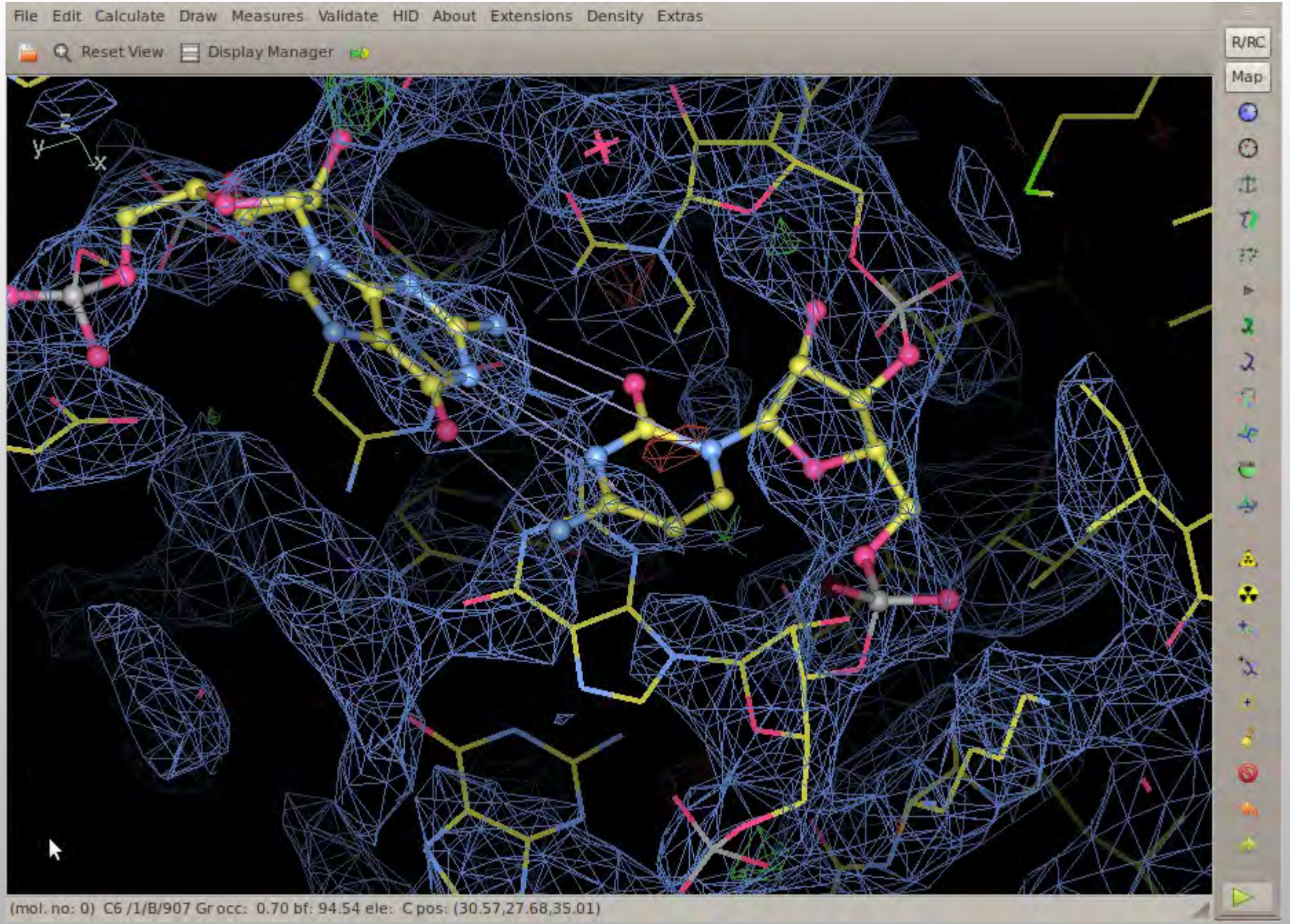
Reset View
Display Manager
R/RC
Map
Z
Y
X
(mol. no: 0) C6 /1/B/907 Gr occ: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)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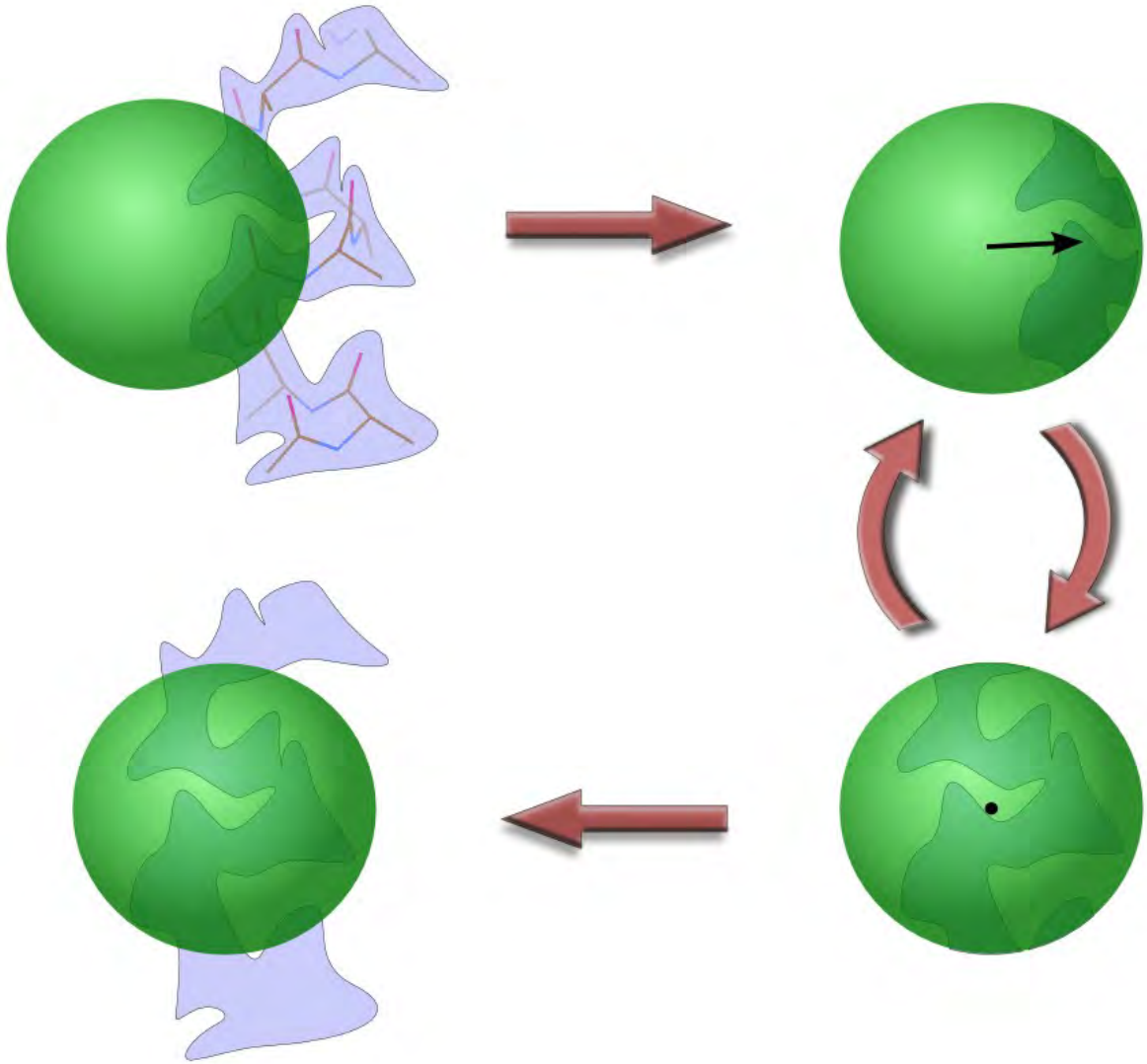
Reset View
Display Manager
R/RC
Map
(mol. no: 0) C6 /1/B/907 Gr occ: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)

Helix-Building

Alpha Helix Placement

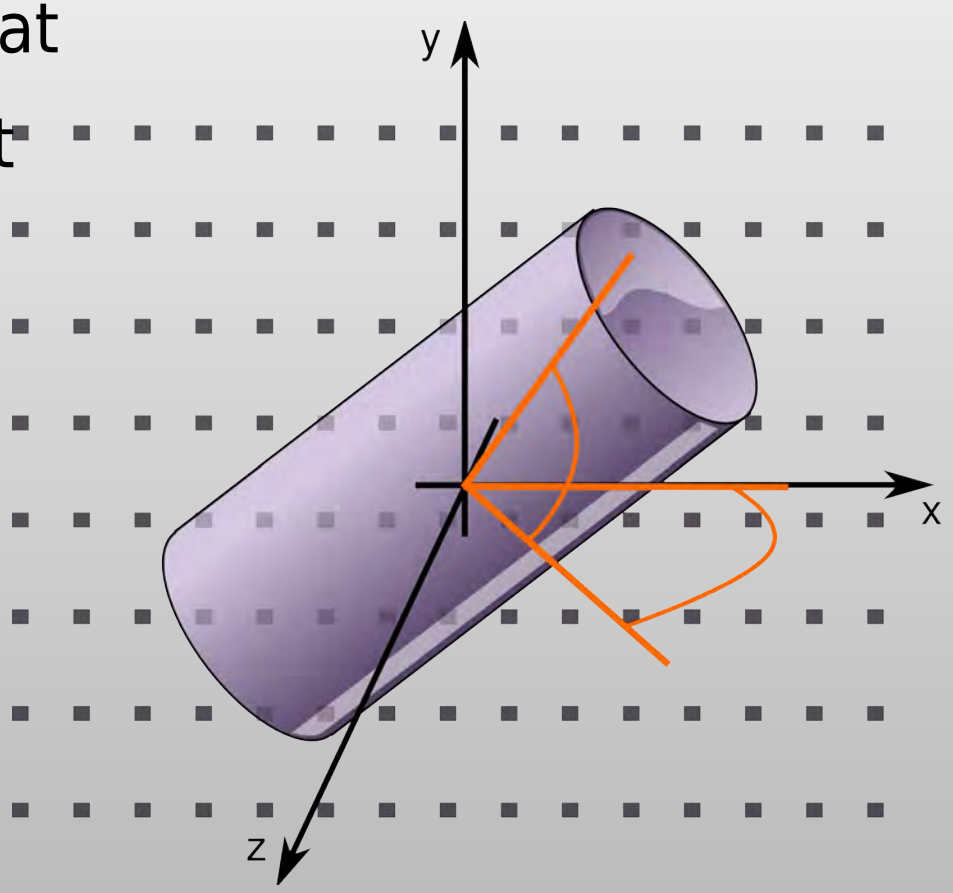
- Scenario: Looking at a new map, not built with automatic tools:
  - “I can see that there’s a helix here - build it for me!”
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

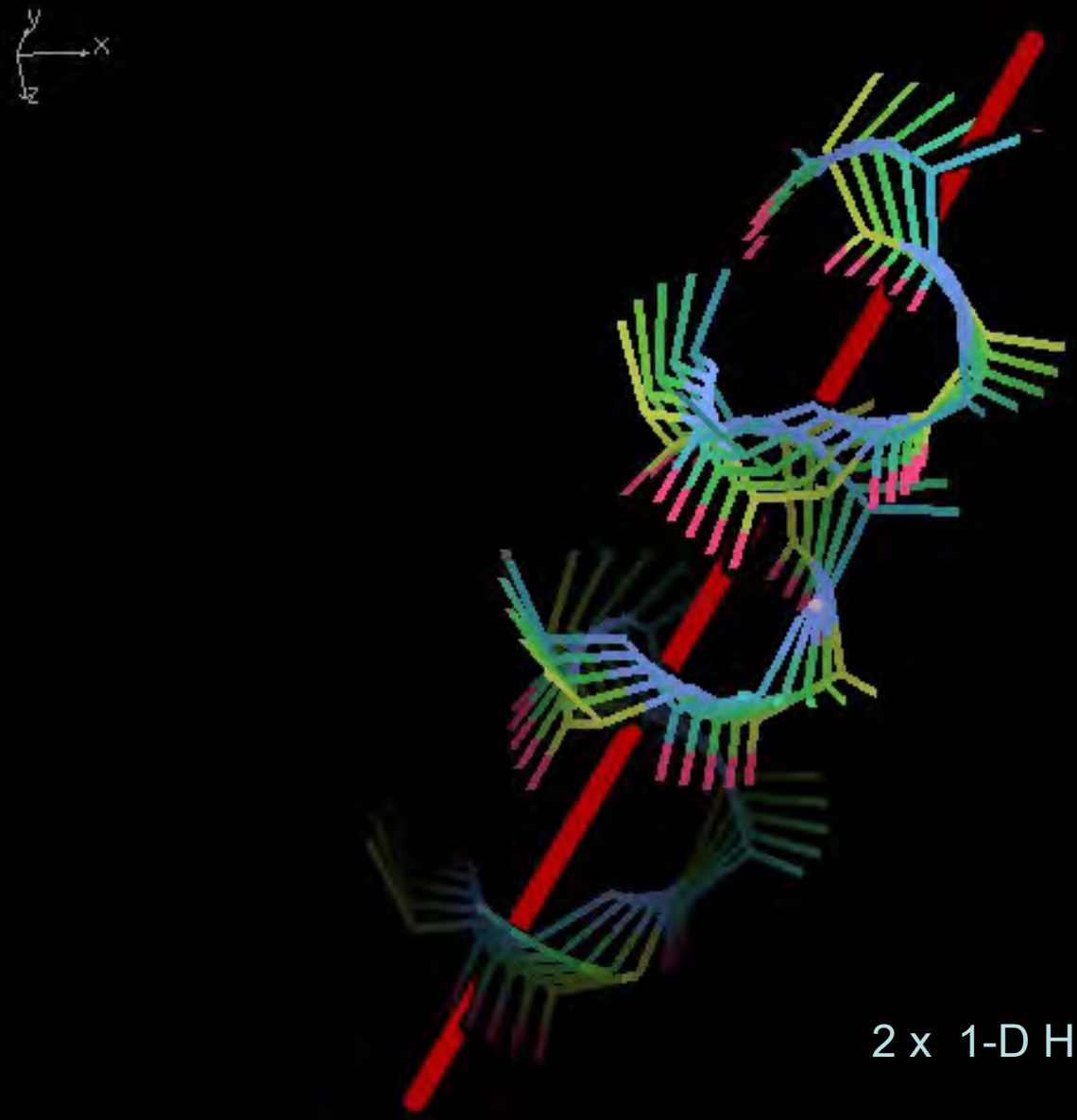


Helix Fitting: Cylinder Search

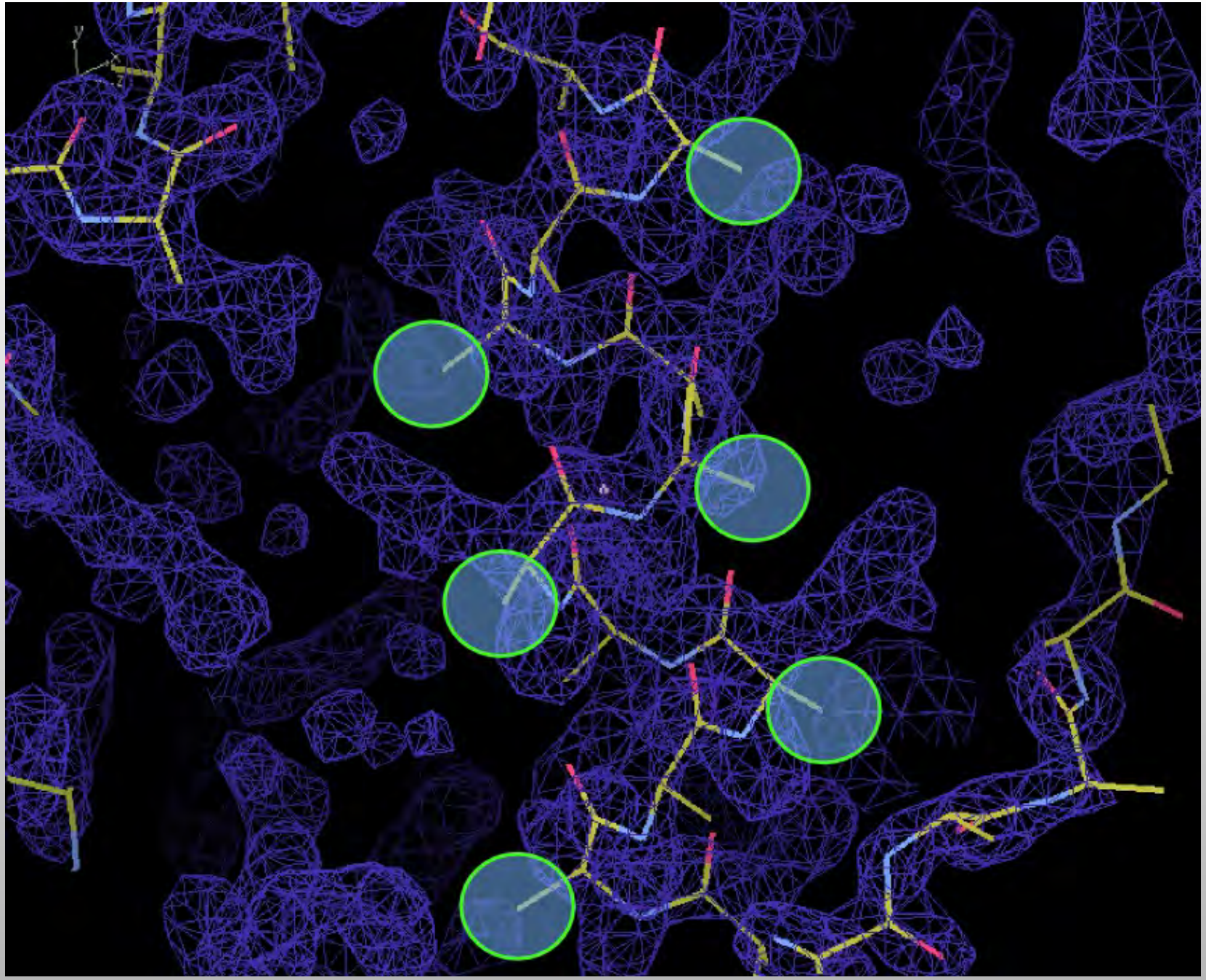
- Pick the orientation that encapsulates the most electron density

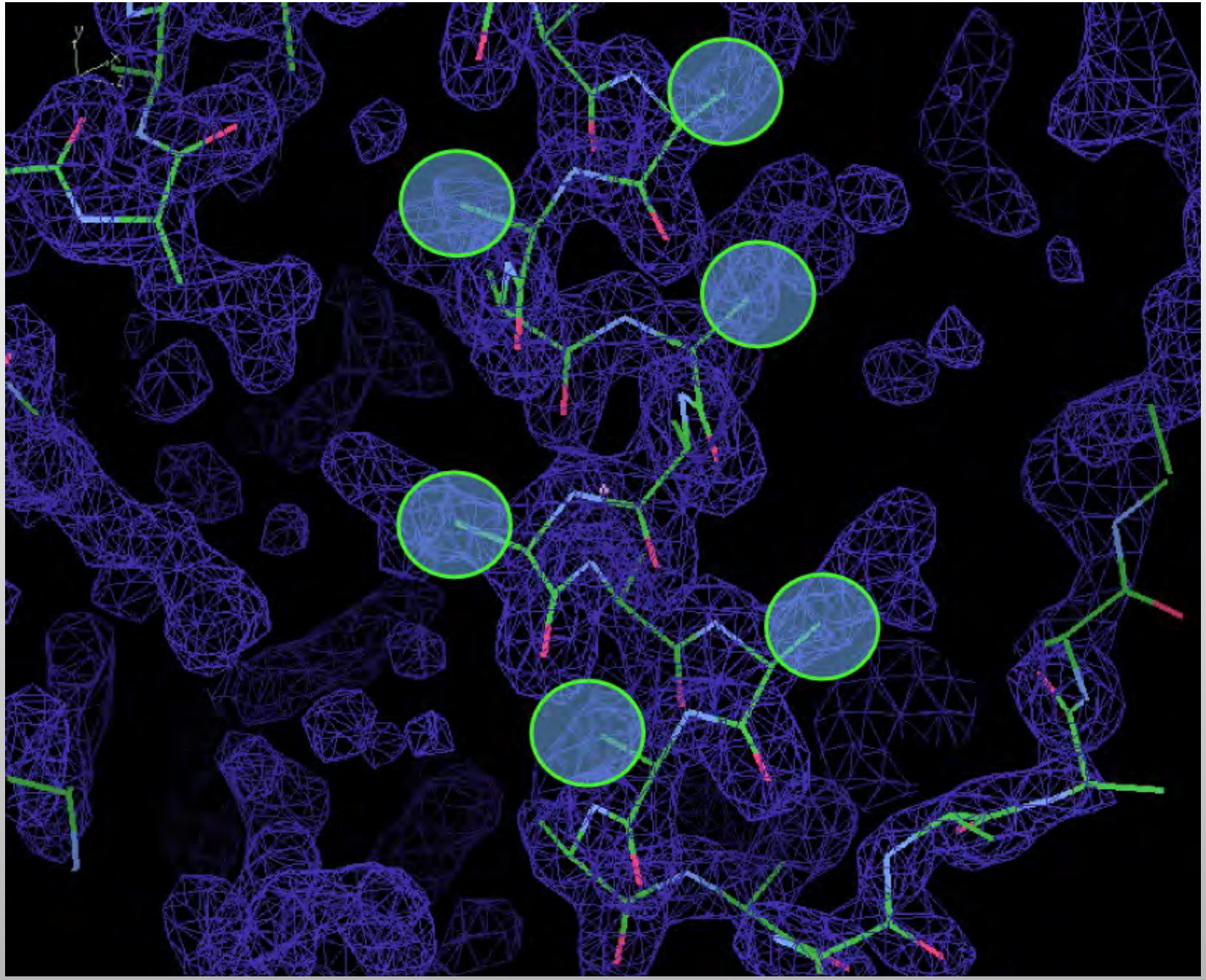


Using 2 rotation axes



2 x 1-D Helix orientation searches





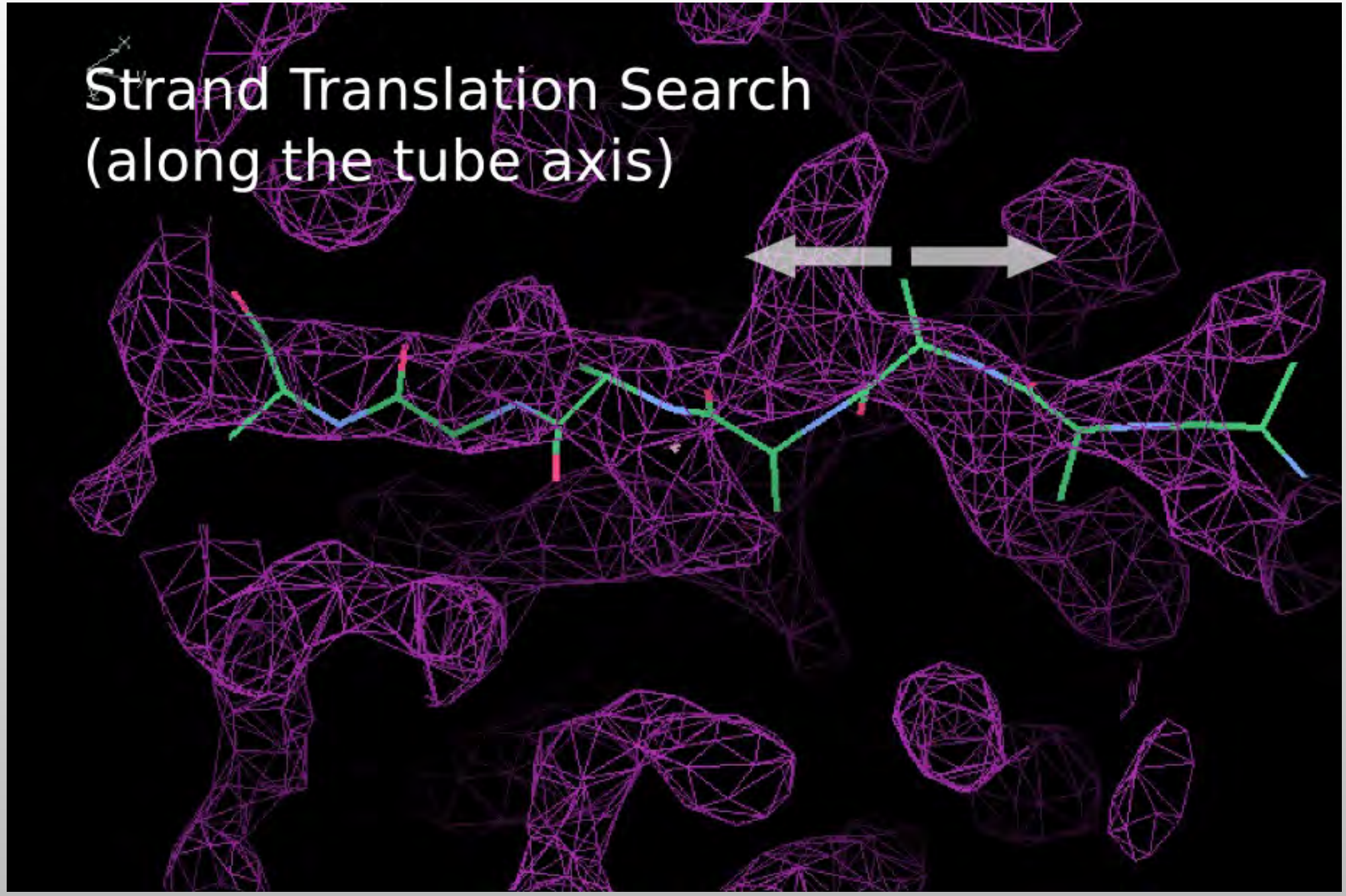
Fitting Strands

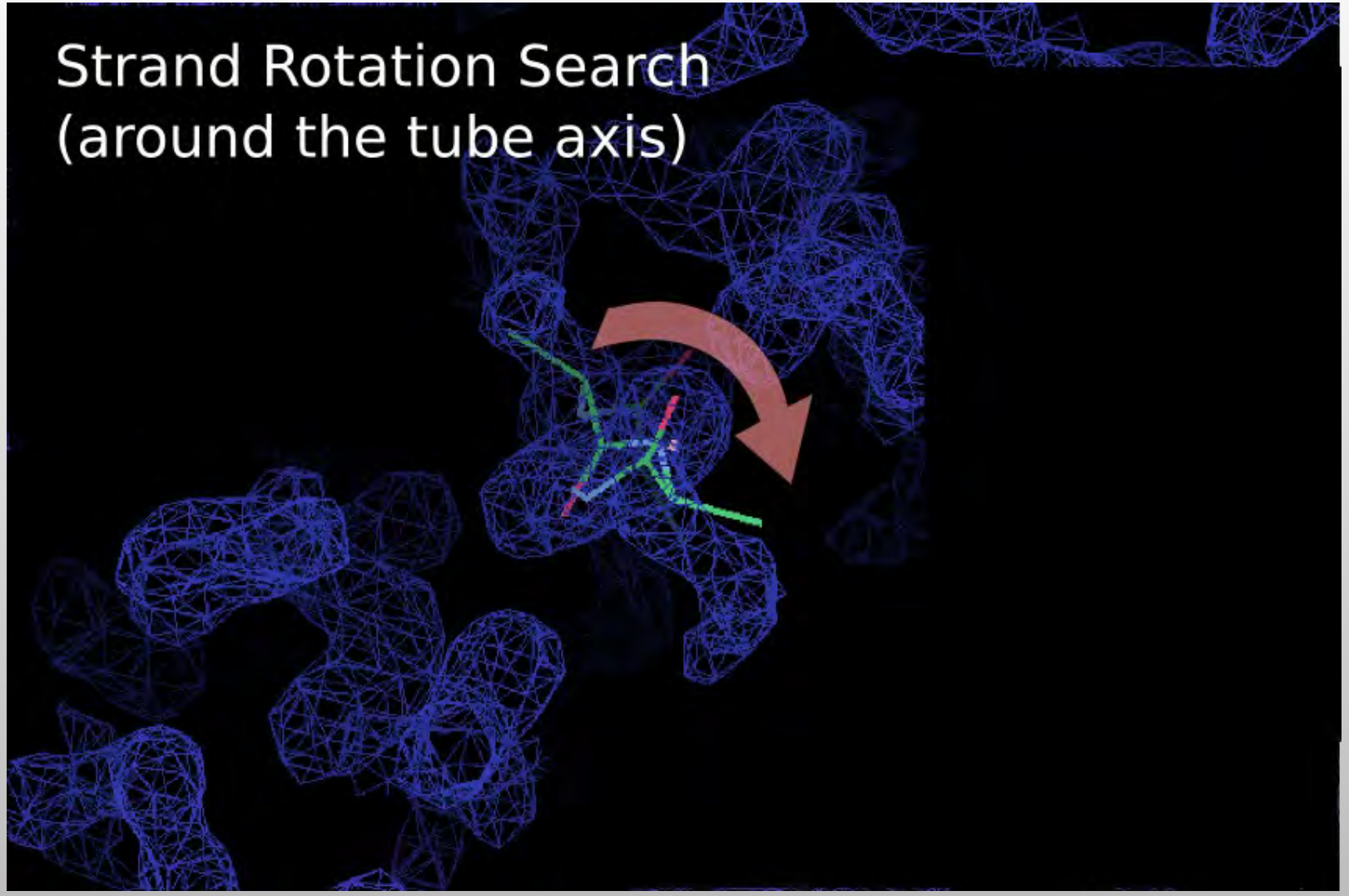
Placing Strands

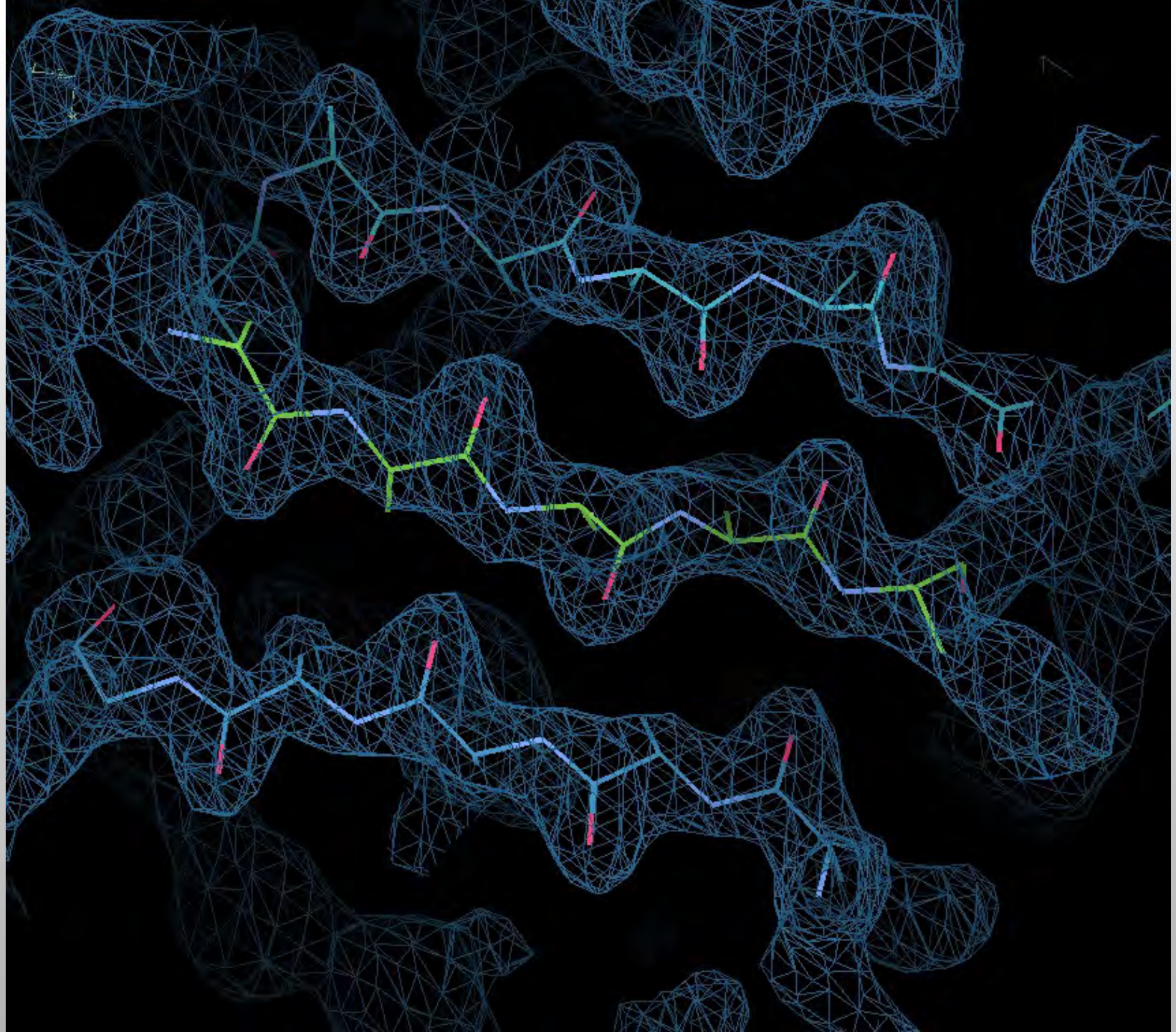
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

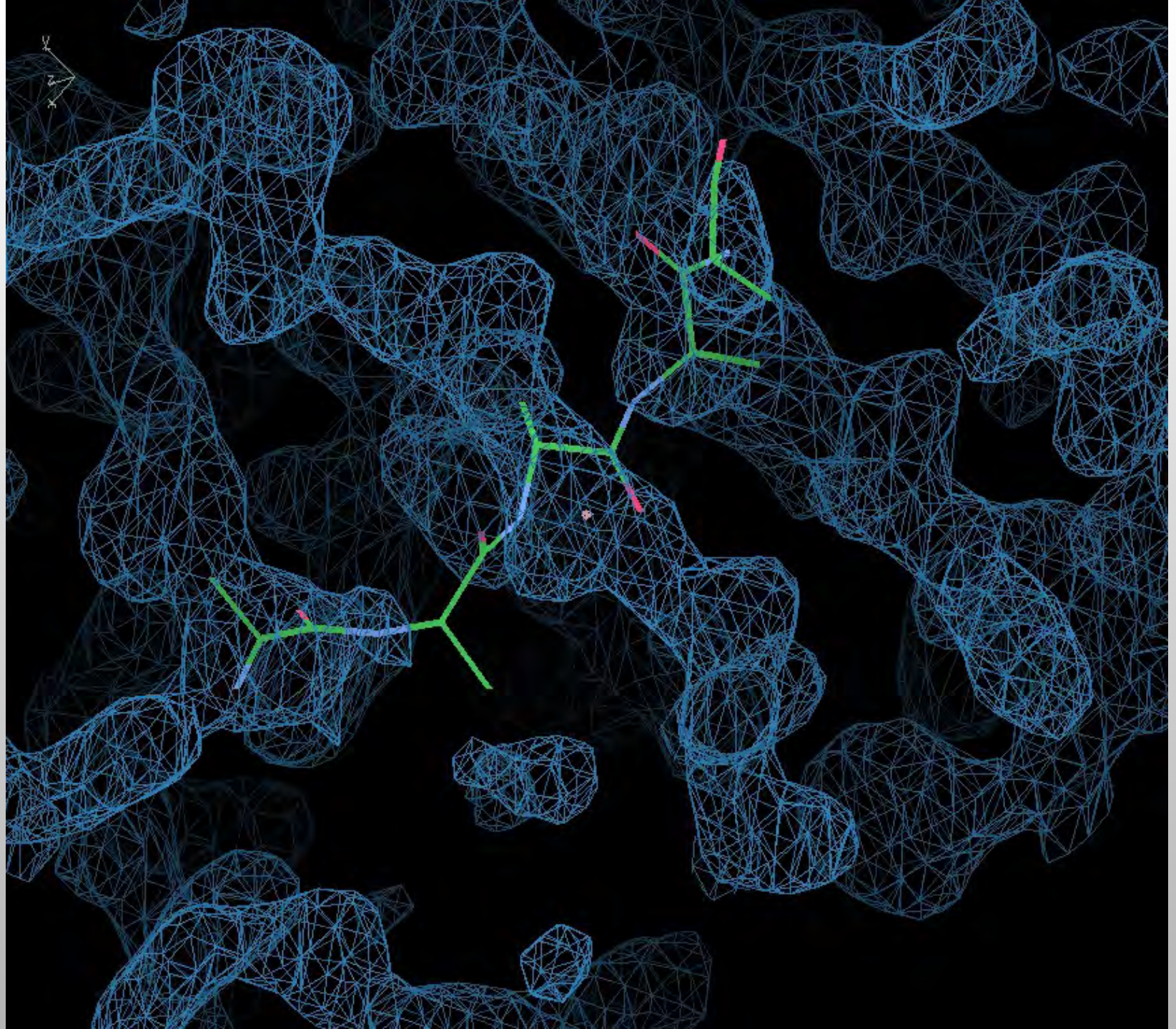
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution


Strand Translation Search
(along the tube axis)


Strand Rotation Search
(around the tube axis)



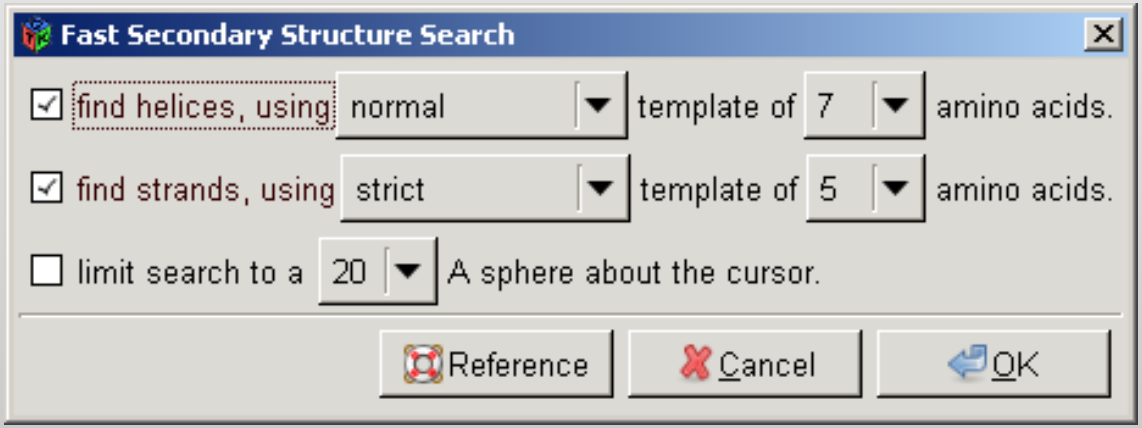
Not all is rosy…



Fitting Strands caveat

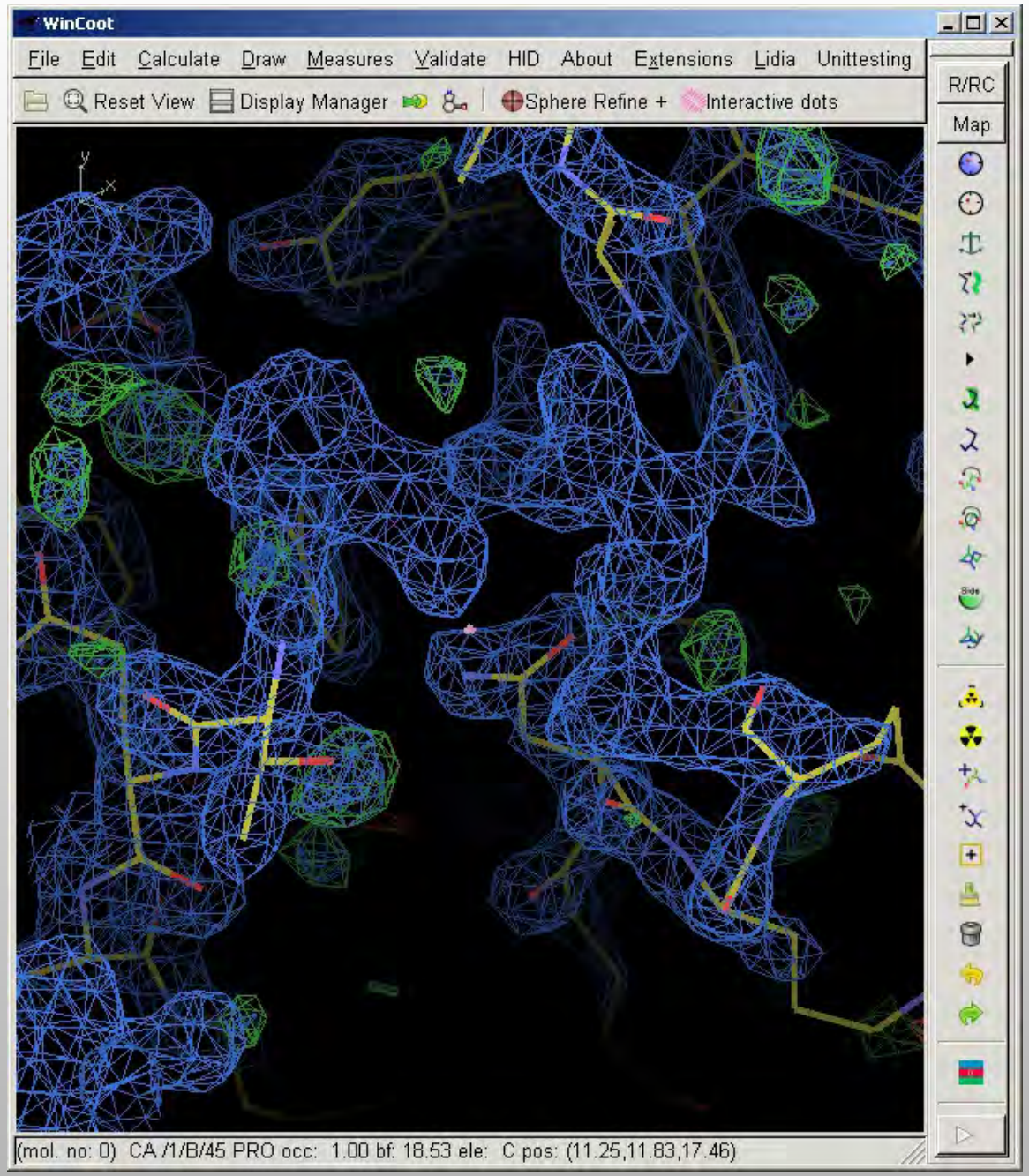
- In the case of strand-fitting, the initial translation search centring the cylinder is not performed (the search cylinder is too thin)
- The user is responsible for centring the search point “in the middle of the tube”
- Not at a C-alpha position

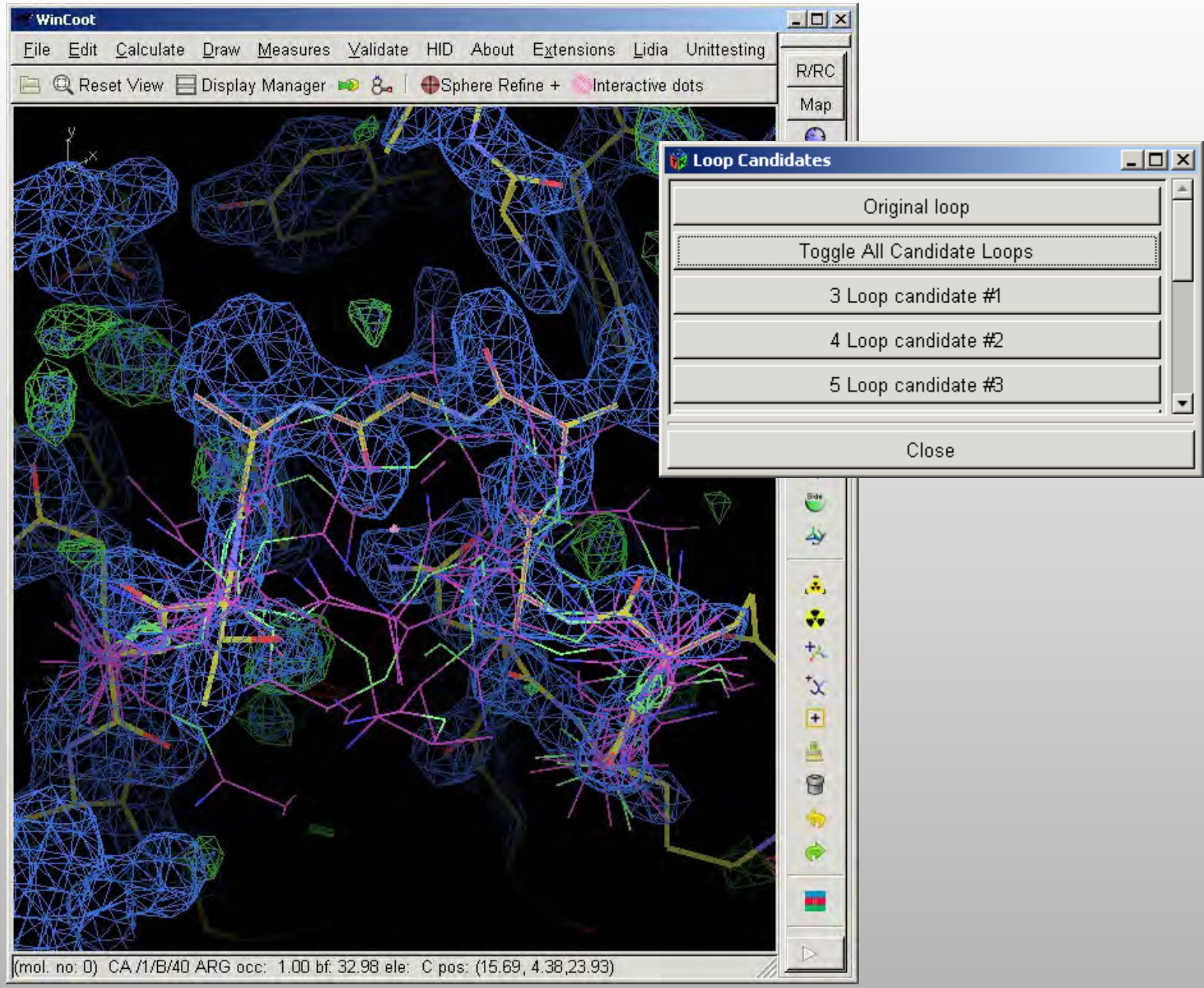
Automated Fast Secondary Structure Search



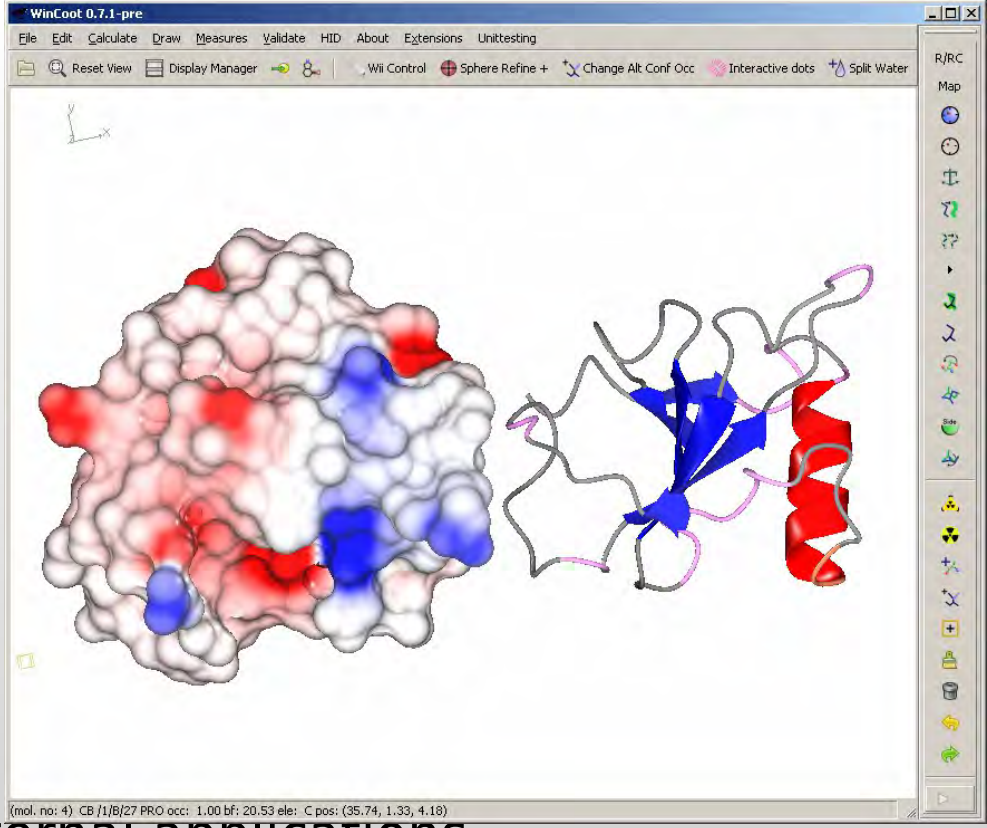
Loop fitting

- 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)

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 (mailing list)
  - http://www.jiscmail.ac.uk/lists/coot.html
- Coot documentation
  - http://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/docs/

Acknowledgements

- Paul Emsley
- Kevin Cowtan
- Eleanor Dodson
- Keith Wilson

http://www2.mrc-lmb.cam.ac.uk/personal/pemsley/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