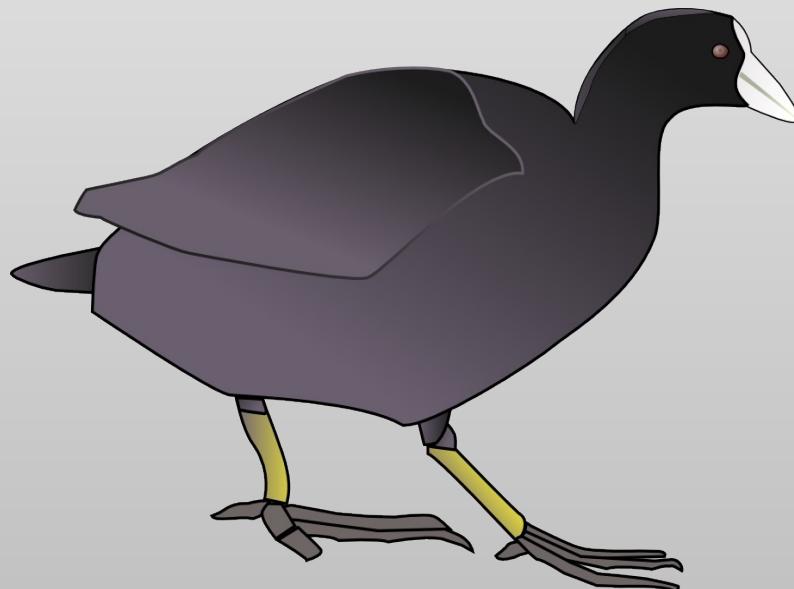


A photograph showing two Common Coots swimming on a body of water with small ripples. The birds have dark grey bodies, white patches on their wings, and distinctive white patches around their eyes. They are facing towards the right side of the frame.

Common Coot
(*Fulica atra*)

Model Building

An Introduction to *Coot*



Bernhard Lohkamp

Karolinska Institutet, Sweden

Lund June 20221

Model-Building

- Place protein atoms in (best) electron density

Automated model building



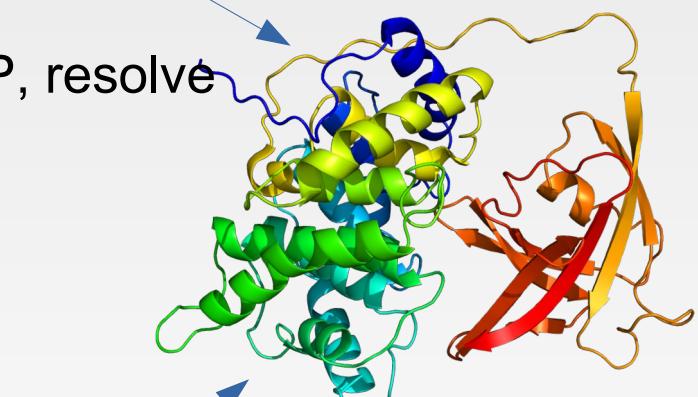
e.g. buccaneer, ARP/wARP, resolve

“Manual”(*) model building



e.g. Coot

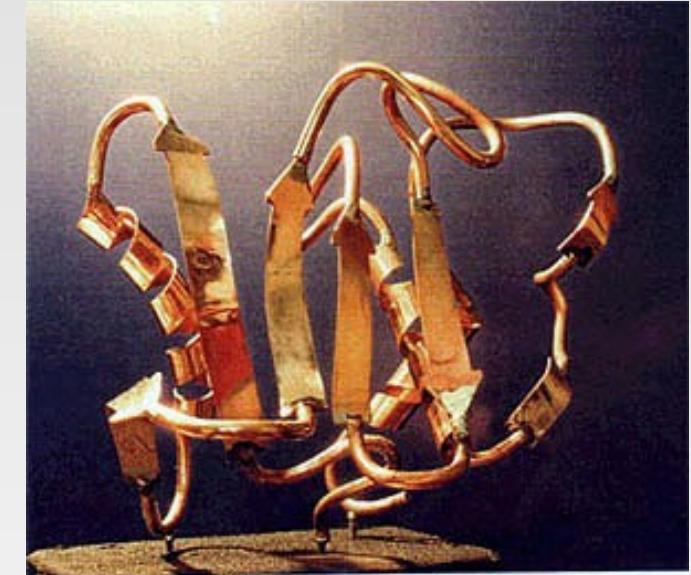
(*) rather interactive real-space refinement



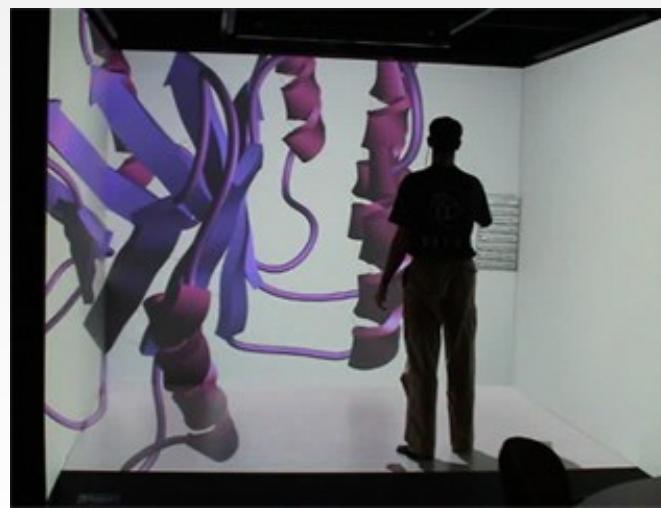
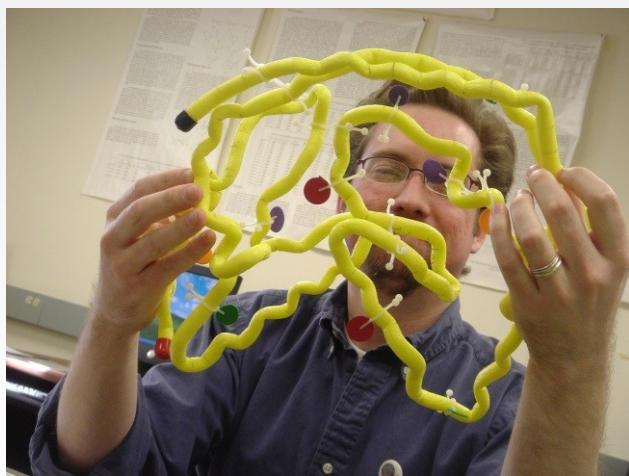
Model-Building



Kendrew (1957)



Rubin

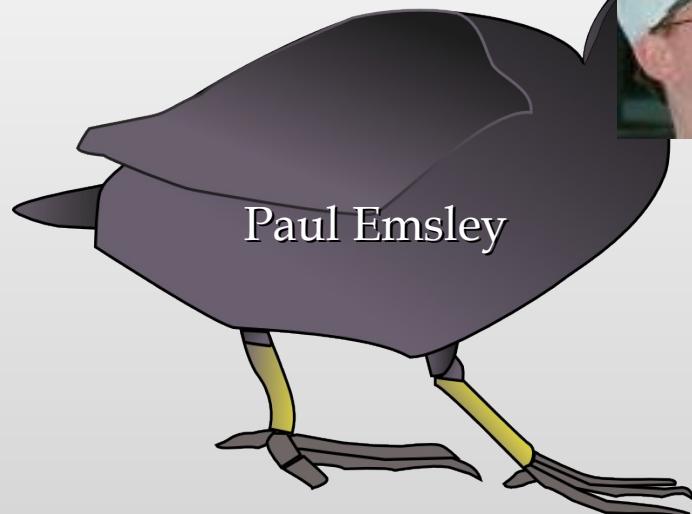


... but why bother?

- In the days of **Automation**
 - why build something **Interactive**?
- Automated model-building for complete models is still impossible
 - It takes a brain to validate
- Concerted correction/improvement of a model is difficult on the larger scale
- Coot is built with Novice users in mind
 - (but not exclusively)

It's fun!!!

Coot Collaborators

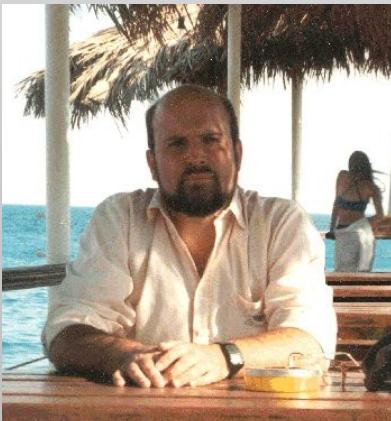


Paul Emsley

Bernhard
Lohkamp



Kevin
Cowtan



Eugene
Krissinel



Stuart
McNicholas



Martin
Noble



Alexei
Vagin

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, AceDrg, Probe&Reduce (MolProbity), EBI, EDS, Povray, Raster3D, PHENIX, ...
- Several model-building and validation tools

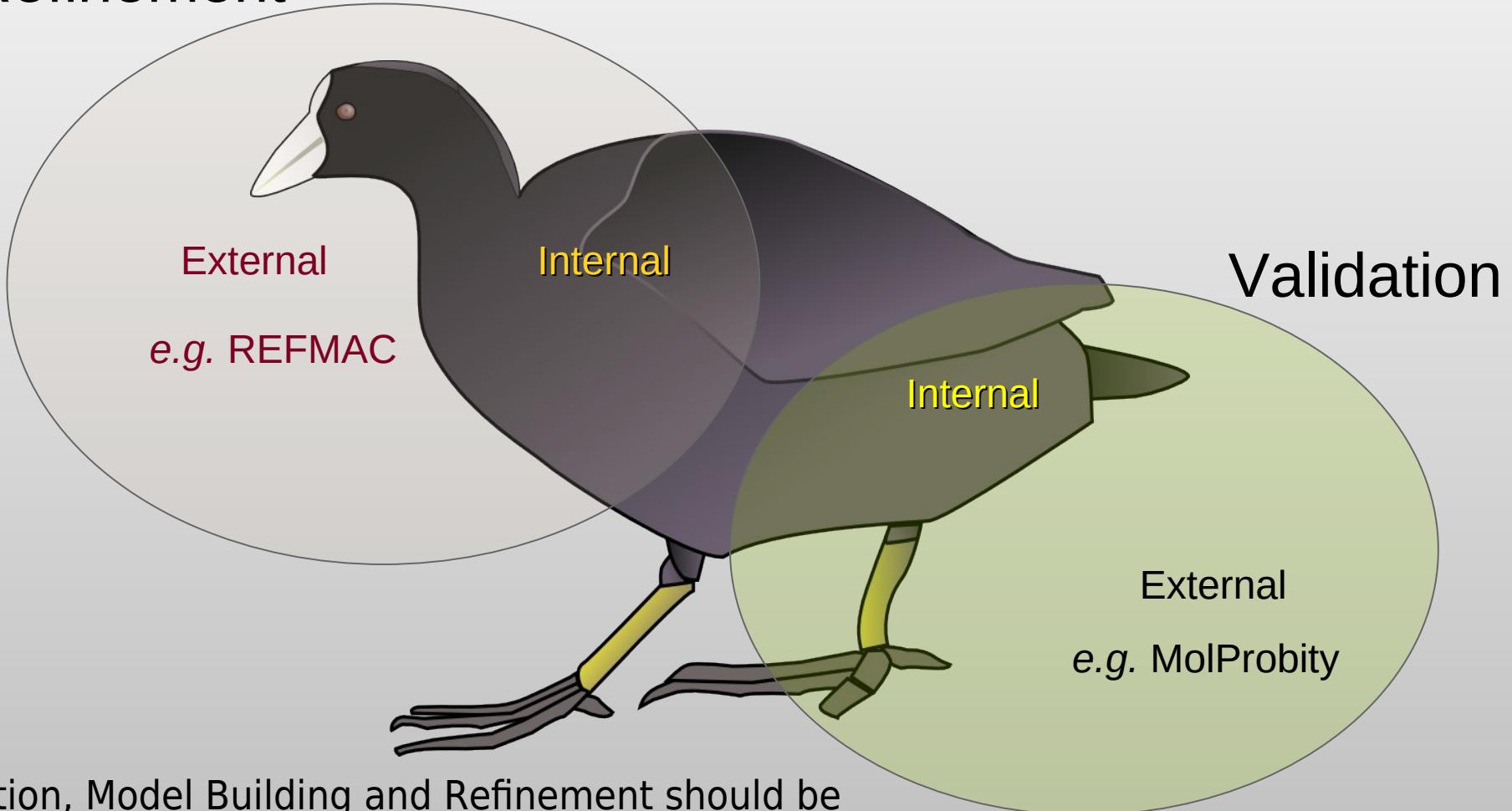
(*) EM tools now too

(some) Coot features

- displays maps and atomic macromolecular models
- allows model manipulation, e.g.
 - structure idealization & real space refinement
 - manual rotation/translation, rigid body fitting & rotamer selection
 - ligand search, solvation, mutations
 - Ramachandran plots, non-crystallographic symmetry (NCS)...
- interfaced to other programs
 - Refinement
 - Phasing
 - Model validation and analysis

Feature Integration

Refinement

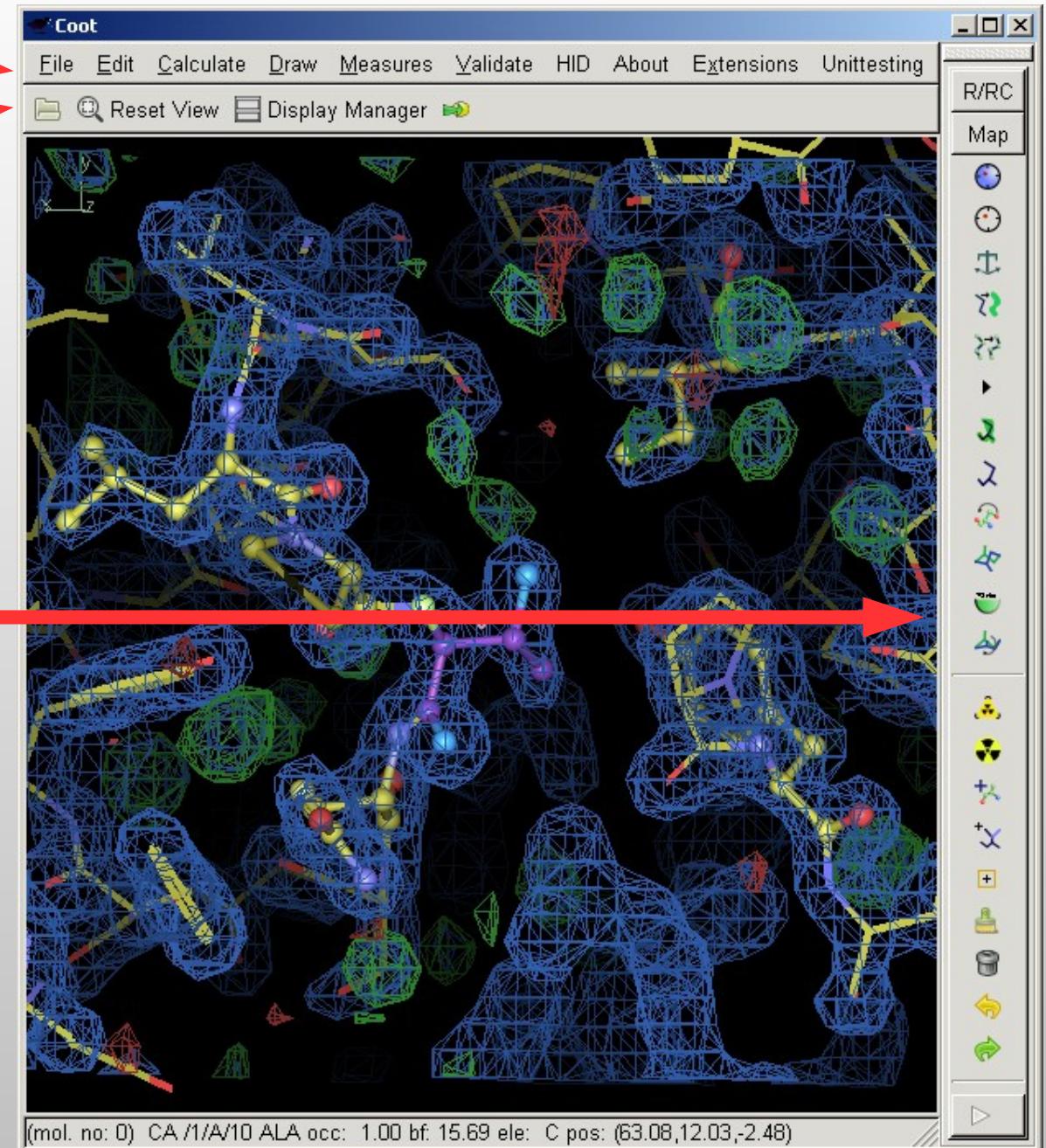


Validation, Model Building and Refinement should be used together

Menus

**Main toolbar
(customizable)**

**Model/fit/refine
Toolbar
(access to main
functions)**



What is “Refinement”?

- The adjustment of model parameters (coordinates) so that the calculated structure factors match the observations (map) 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

Refinement

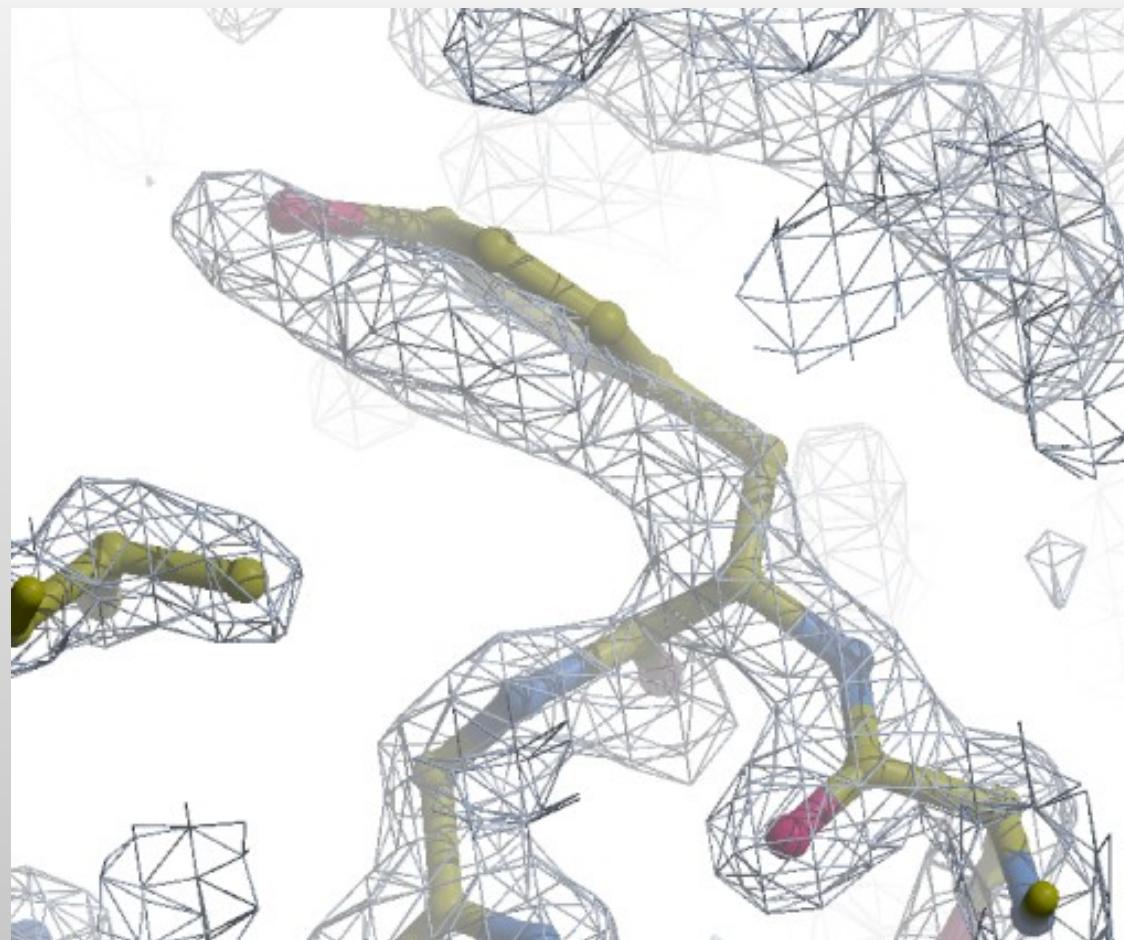
real vs reciprocal space

- Real Space
 - Refinement against electron density (map; E_{map})
- Reciprocal Space
 - Refinement against structure factors (E_{xray})

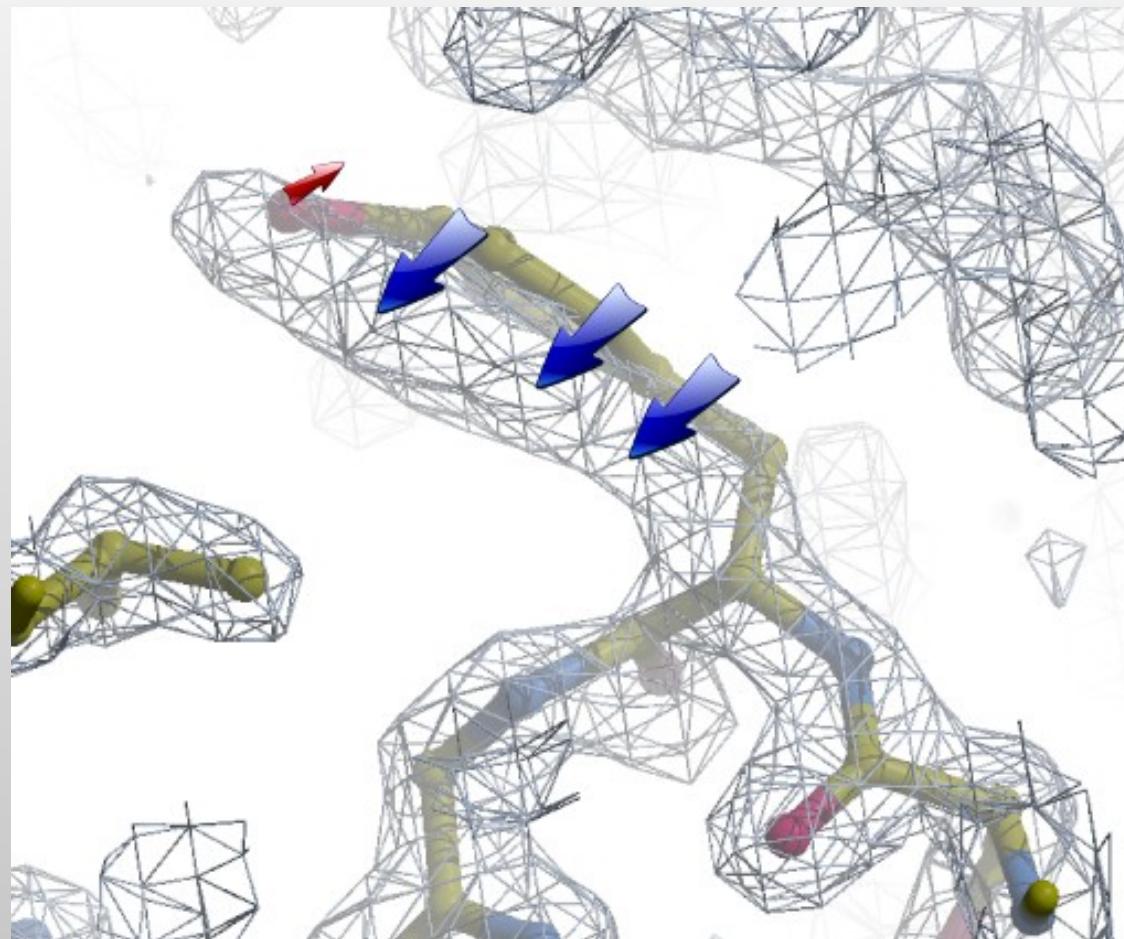
Minimize:

$$E_{\text{total}} = E_{\text{map/xray}} + w \cdot E_{\text{geometry}}$$

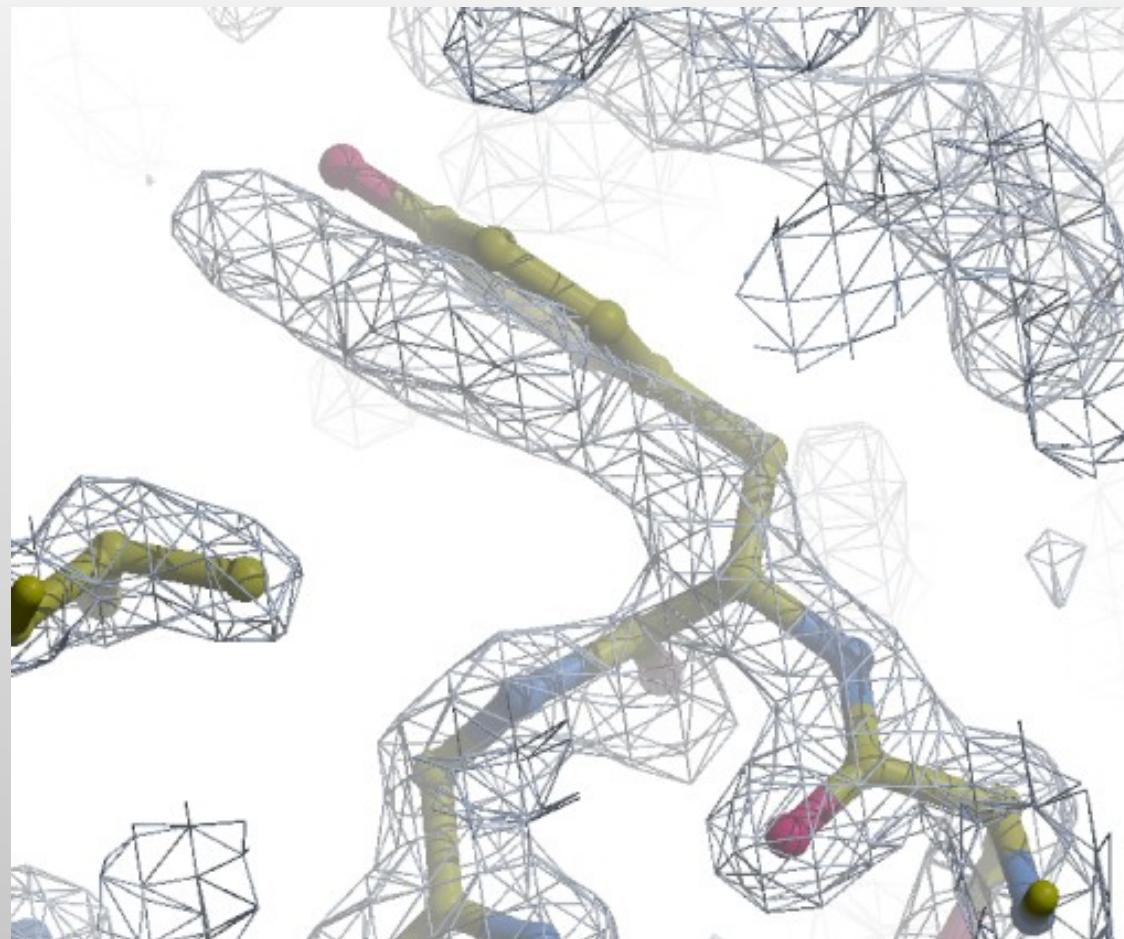
Distorted Geometry Pre-Refinement



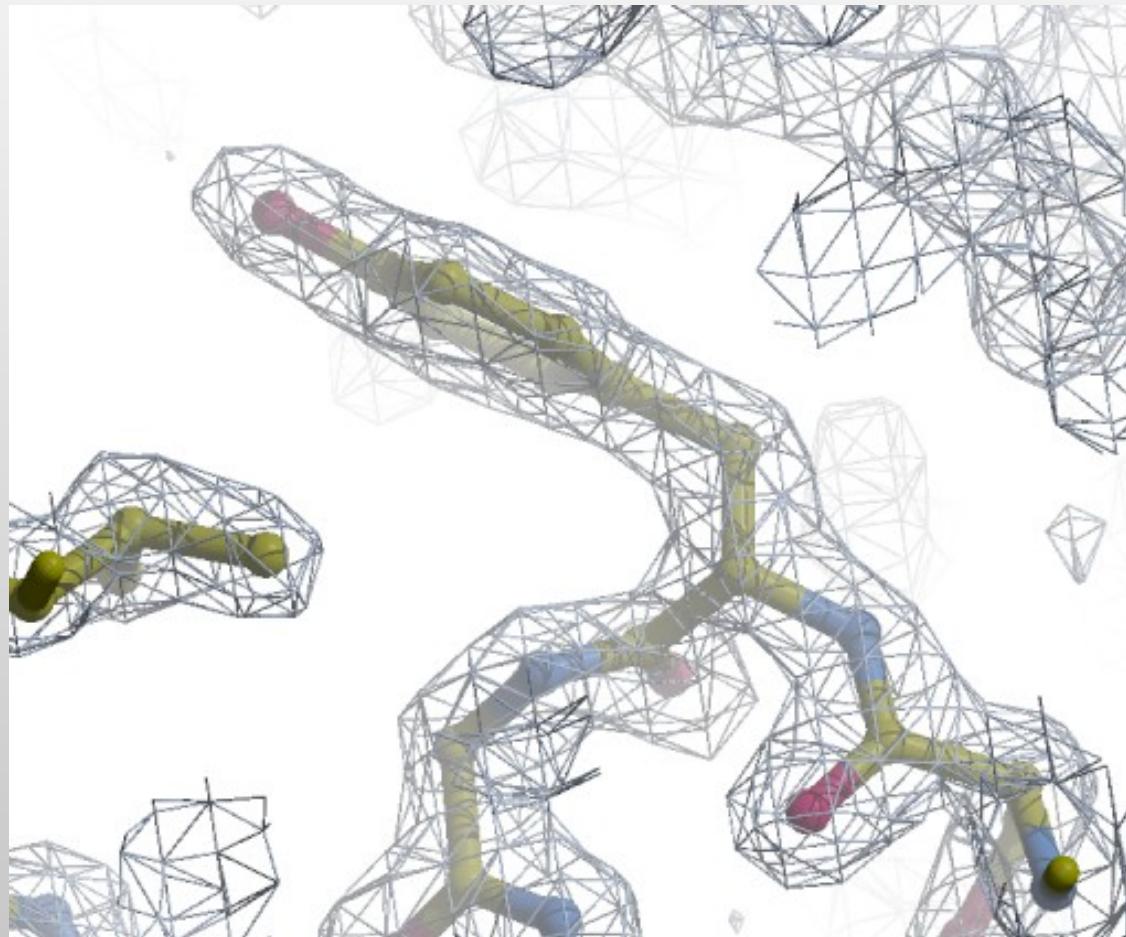
Refinement Gradients



Refinement: Cycle 3



Refinement Cycle 200: Minimized



Real Space Refinement

- Major feature of Coot

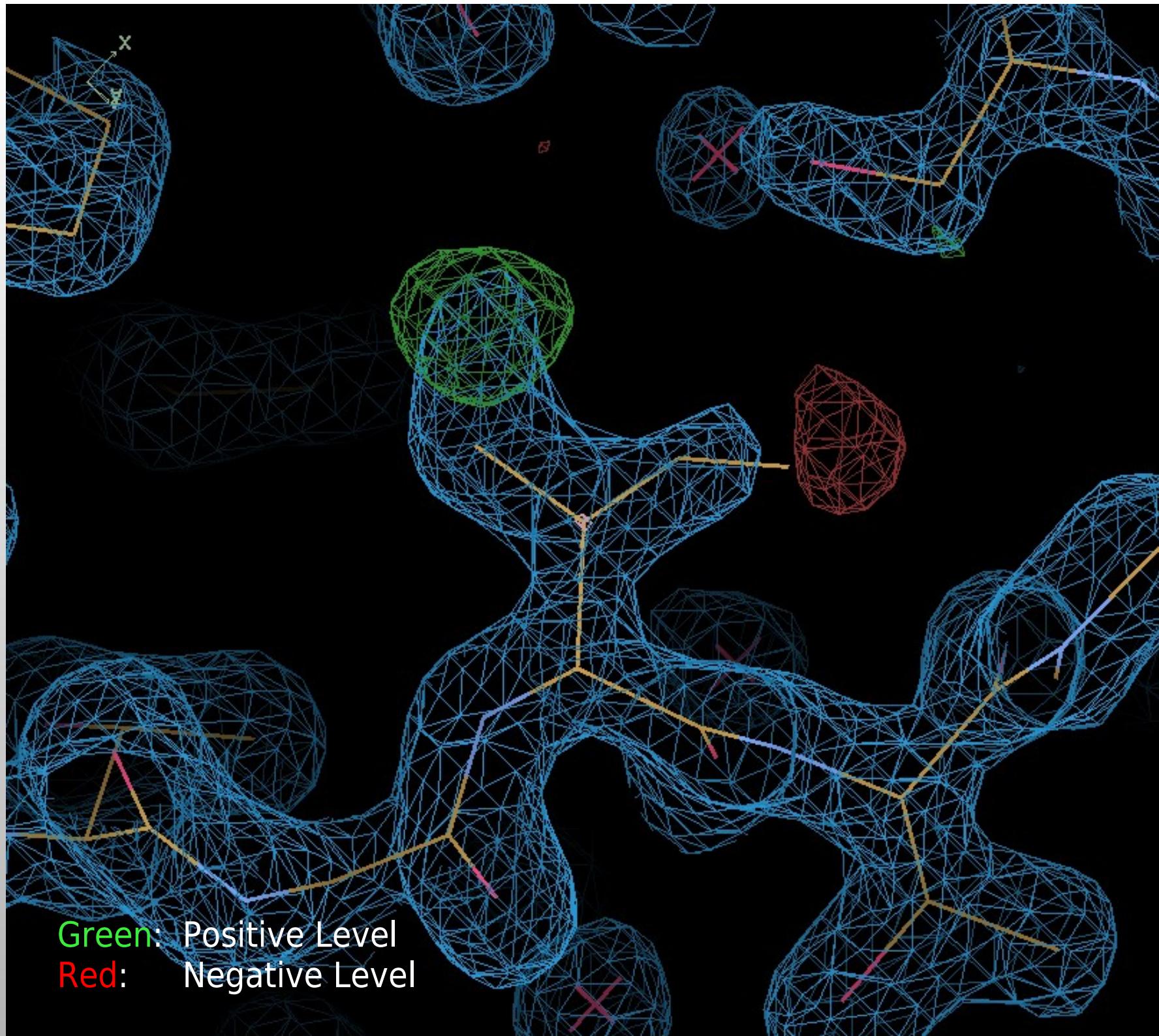
Diamond, R. (1971). *Acta Cryst. A*
27, 436-452.

- Gradient minimizer (BFGS derivative)
- Based on mmCIF standard dictionary
- Minimizing bonds, angles, planes, non-bonded contacts, torsions, [chiral volumes, Ramachandran]
- Provides “interactive refinement”
 - Atom positions can be moved after refinement (white)
- Chi squares (easy evaluation of result)
- Threaded (0.9 onwards):
 - Target function and derivative evaluation, model and map all happen simultaneously now
 - => more atoms, smoother updates and/or closer to the minimum

Fast & Animated

Which map to use?

- Direct maps
 - Calculated from experimental amplitudes and phases inferred from diffraction or model
 - $2F_o - F_c$, density modified map (experimentally phased, MR or refinement), usually blue
 - Covers the model (if $F_o \approx F_c \Rightarrow F_o$)
- Difference map
 - Highlight errors in model
 - $F_o - F_c$, usually red (negative, i.e. too much model, $F_c > F_o$) and green (positive, i.e. not enough model, $F_o > F_c$)
- Composite map
 - Combination of above
 - e.g. $3F_o - 2F_c \approx 2F_o - F_c + F_o - F_c$



Green: Positive Level

Red: Negative Level

Representation of Refinement Results:

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

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

The first attempt

Student Reaction:

“Oh, I don't look at that window...”

Representation of Refinement Results:



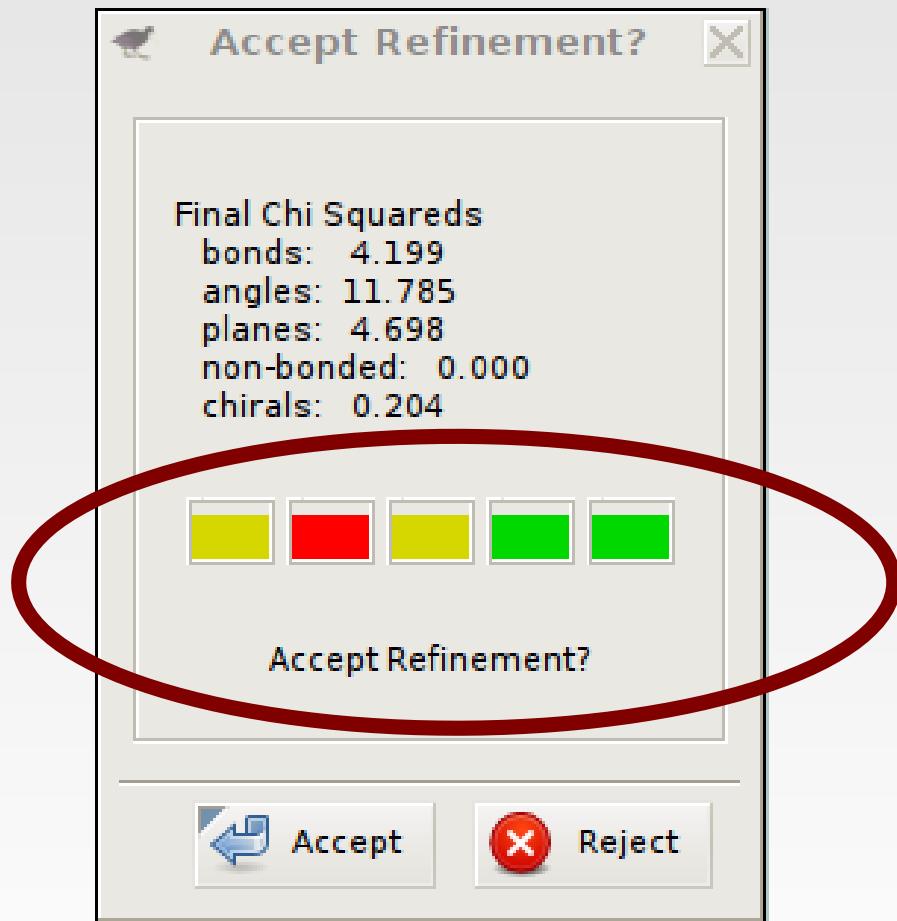
Second attempt...

Student Reaction:

“Oh, box of meaningless numbers.

Go away”

Refinement “Traffic Lights”



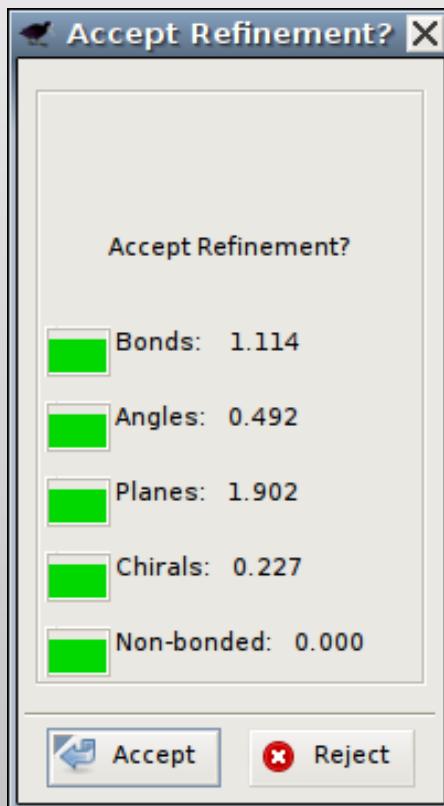
“Traffic Lights” represent the chi-squared values for each of the refined geometry types

e.g. bonds length (b):

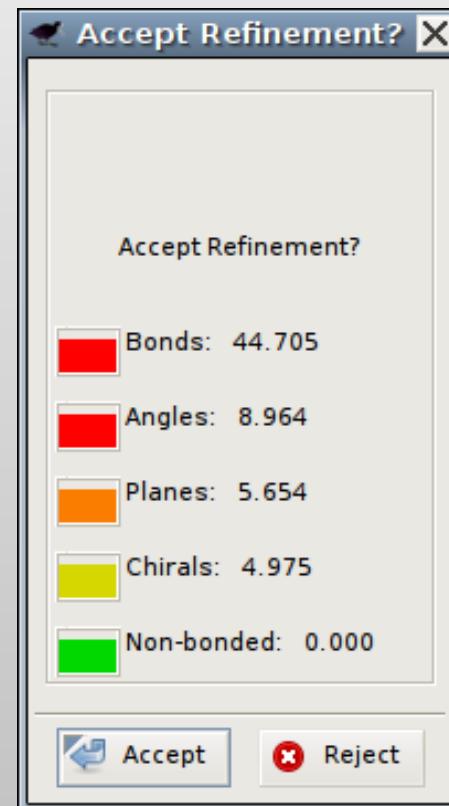
$$\chi^2_{bonds} = \frac{\sum (b_i - b_0)^2}{\sum \sigma_i^2}$$

Representation of Results: “Traffic Lights”

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



Good refinement

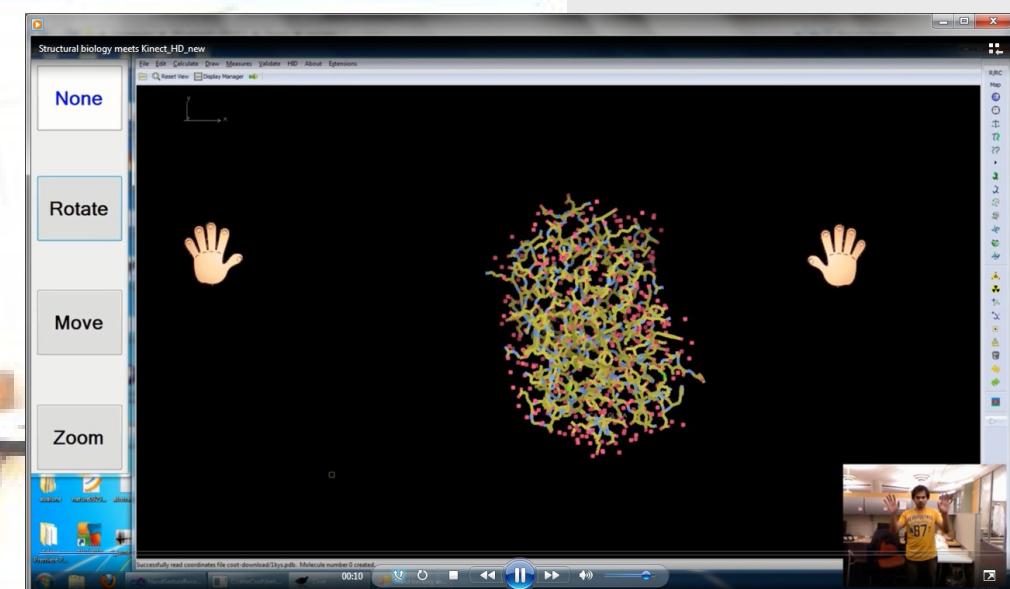
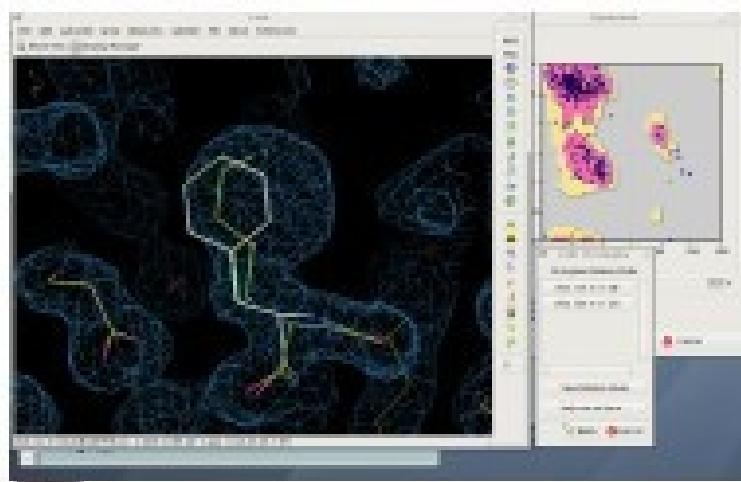


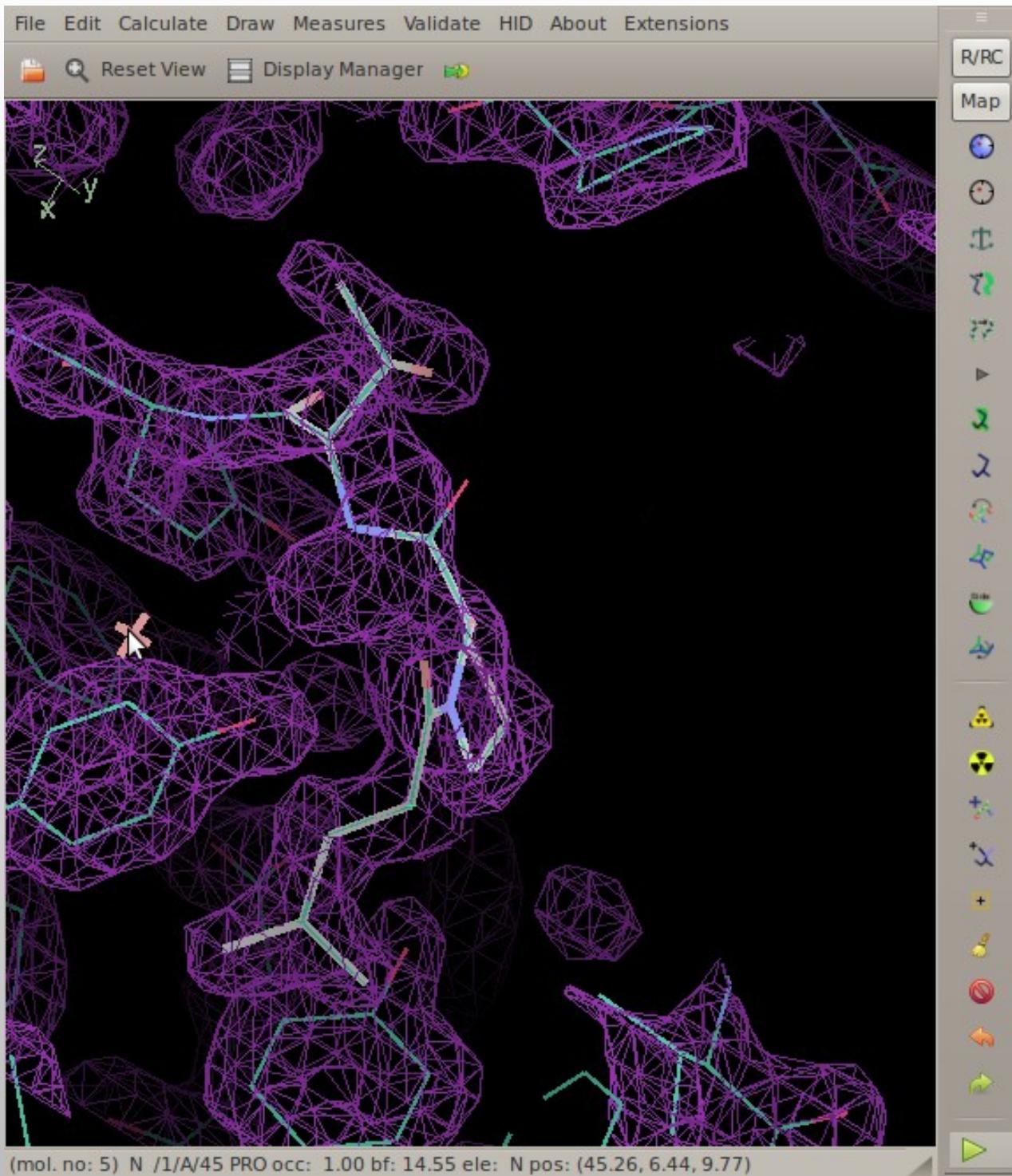
Bad refinement

Refinement Techniques

- Single-Atom Drag
- Key-bindings:
 - Triple Refine
 - Single Residue Refine with Auto-accept
- Ramachandran Refinement
- Sphere refinement
- Crankshaft Peptide Optimisation
- Coming at some point..?
 - VR (Hamish Todd)
 - AR

Wii/VR Refinement?





Single-drag

(Ctrl-left mouse)

(over dragging –
out of density: not
required any more
usually)

Coot Key-binding Crib-Sheet

Coot Crib Sheet

October 14, 2016

1 Keyboard

1.1 Dialog Shortcuts

F6 Post Go To Atom window
F7 Post Display Control Window

1.2 Previous/Next Residue

"Space" Next Residue
"Shift" "Space" Previous Residue

1.3 Closest Residue

"p" go to an atom of the closest residue (the "CA" atom if the residue has one)

1.4 Go To Residue

Ctrl+<Residue-number><Enter>
Jump to the give residue (you can provide a chain-id too!)

1.5 Next NCS Chain

"o" - other NCS chain.

1.6 "Undo" Move

"u" to undo the move recent screen recentering (e.g. move back after recentering after reading a new PDB file)

¹The chain-id goes directly before the residue number, i.e. Ctrl+g <Chain-id><Residue-number><Enter>

1.7 Previous/Next Rotamer

When in "Rotamer" mode, these keyboard shortcuts are available:
" " Next Rotamer
" " Previous Rotamer

1.8 Keyboard Chi Angles

Instead of pressing the buttons in the Chi Angles button box, you can use keyboard "1" for Chι1, "2" for Chι2 etc.

1.9 Keyboard Contouring

Use "+" or "-" to change the contour level

1.10 Keyboard Labelling

"l" to label closest atom

1.11 Quick Save As

Ctrl+s to save the state and any unsaved molecules (to default file names).

1.12 Keyboard Residue Info

Ctrl+i then click on residue to open Residue Info dialog

²note: focus must be in the graphics window, not the Rotamer dialog

1.13 Keyboard Translation

Keypad 3 Push View (+Z translation)
Keypad . Pull View (-Z translation)

1.14 Keyboard Undo/Redo

Ctrl-z Undo last modification
Ctrl-y Redo last modification
u Undo last move/navigation

1.15 Editing

Ctrl-c Copy active molecule
Ctrl-x Delete active residue

1.16 Keyboard Zoom and Clip

n Zoom out
m Zoom in
d Slim clip
f Fatten clip

1.17 Crosshairs

c cross-hairs

1.18 Skeleton

s: Generate skeleton around current point³

1.19 Continuous Rotate

i: Toggle continuous spin

1.20 Baton Mode

b: toggle into baton rotate mode⁴

³if a skeleton is being displayed
⁴rather than view rotate mode

2 Mouse

Mouse actions are occasionally augmented with keyboard modifiers:

Left-mouse Drag	Rotate view
Ctrl Left-Mouse Drag	Translate view
Shift Left-Mouse Click	Label Atom
Right-Mouse Drag	Zoom in and out
Shift Right-Mouse Drag	Change clipping and Transparency
Screen Z	
The movement is along or across axes:	
up/right/down/left shift	up/right/down/left shift
up/left/down/right chan	up/left/down/right chan slab
Ctrl Shift Right-Mouse Drag	Rotate View about Screen Centre on atom
Middle-mouse Click	Increase map contour level
Scroll-wheel Forward	Decrease map contour level

Intermediate (white) atoms can be dragged around by clicking on them

Left-mouse Drag	Move all intermediate atoms by linear shear
Left-mouse Drag with "A" key:	as above with non-linear shear
Left-mouse Drag with "Ctrl":	Move a single atom

3 Refinement Extras

Use "A" to define a residue range⁵ with a single-click. Useful in Refinement and Regularization.

- Click "Real Space Refine Zone"
- Click on an atom
- Press the "A" key

⁵+/ - n residues from the current residue

4 Template Key-bindings

E	Flip Ligand
G	Go To Blob (under cursor)
H	Neighbors
J	Jiggle Fit This Residue
K	Fit Partial Side-chain
R	Refine Active Residue
T	Triple Residue Refine
X	Refine Active Residue and Auto-accept
W	Add Water
Y	Add Terminal Residue
Shift-Q	Rotamer Dialog for Residue
Shift-R	Sphere Refine
Shift-A	Accept Baton Position
Shift-B	Sphere Regularize
Shift-P	Delete Residue Hydrogens
Shift-V	Undo Symmetry View
Shift-X	Edit Chi Angles
Shift-W	Add Water to Blob
Shift-4	Ball and Stick for Ligand

1

2

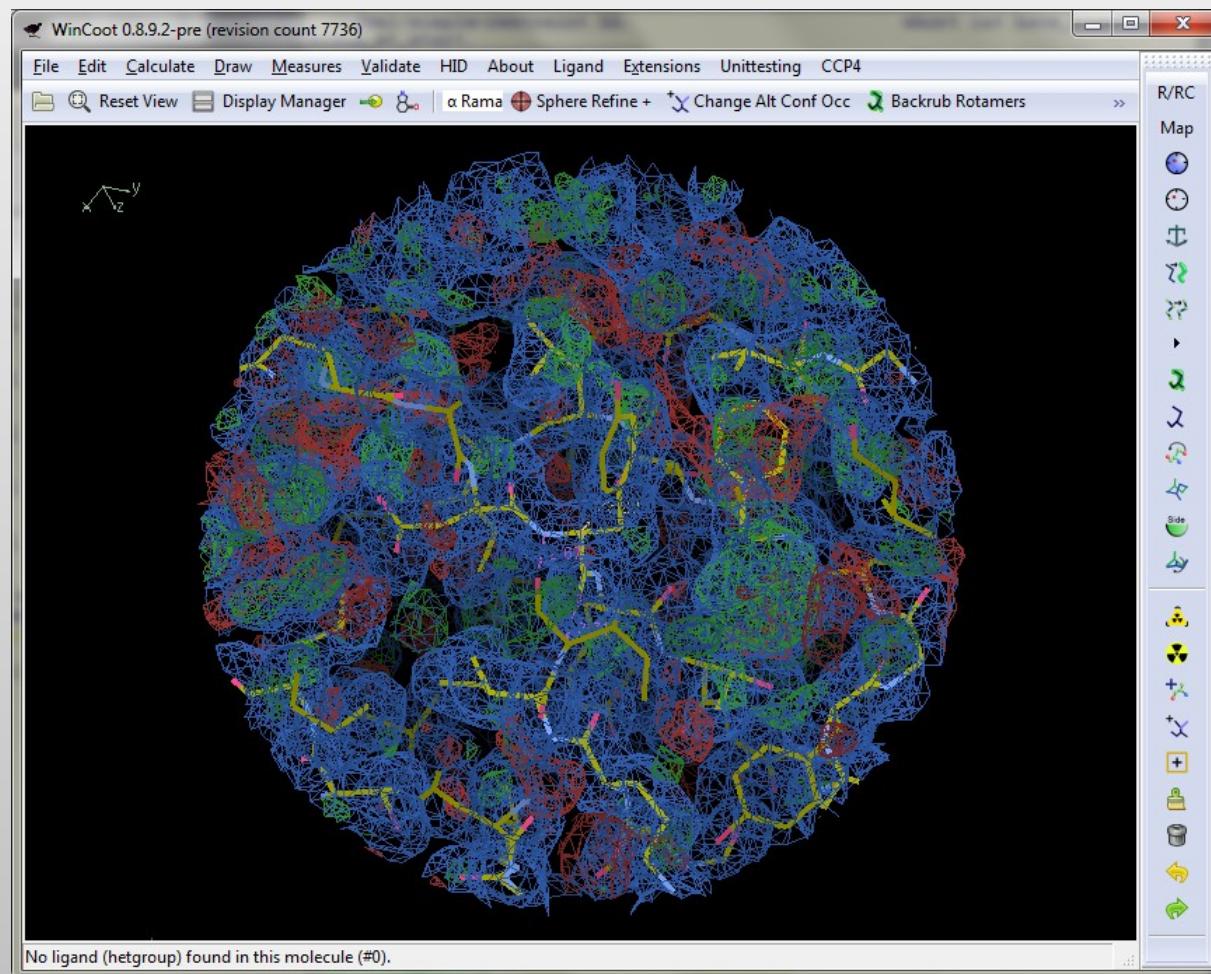
3

“Sphere” Refinement

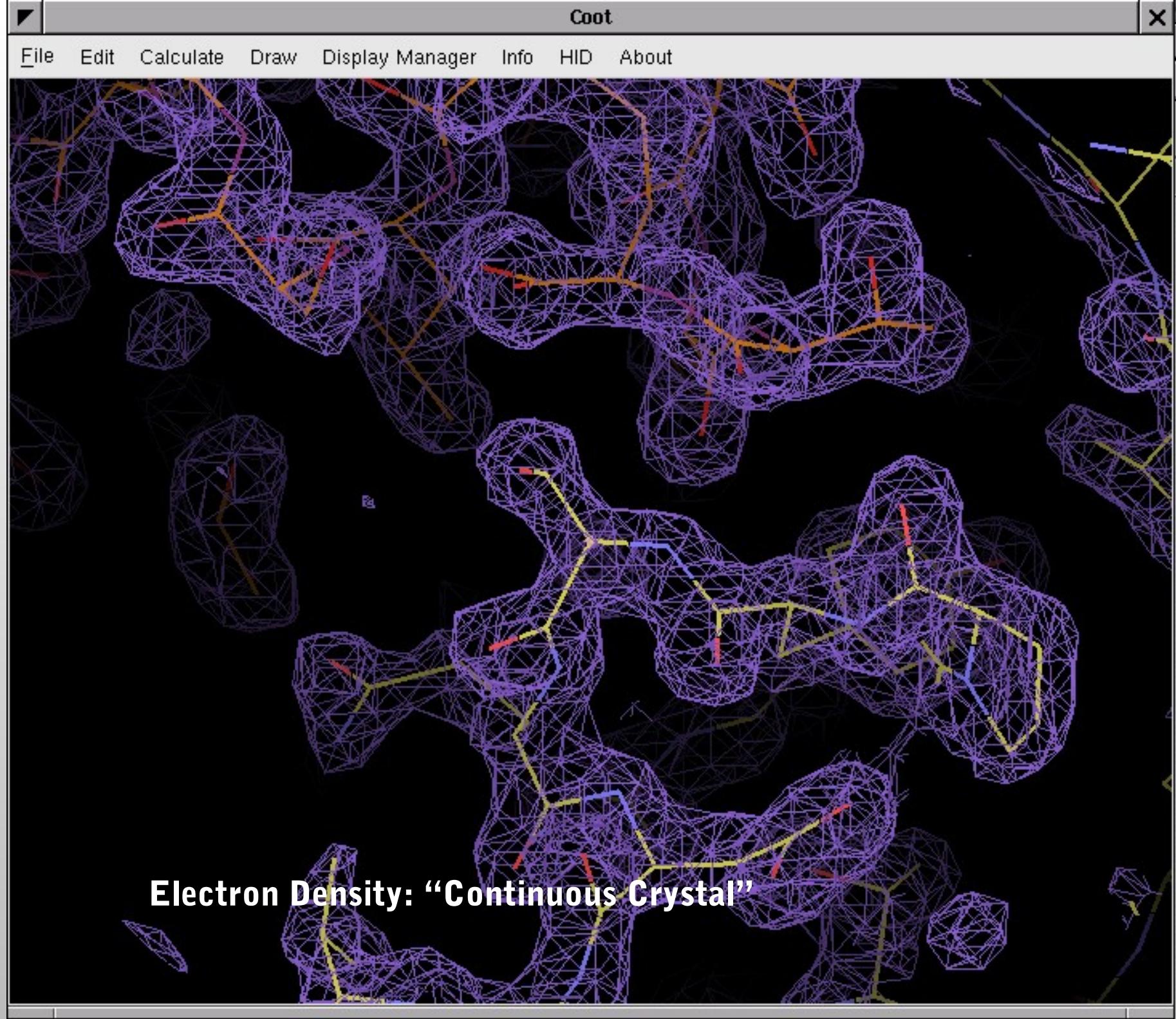
- Given an “Active”* Residues
 - Define a sphere of residues around it and use them all for refinement
 - NOT just a linear selection
 - Residues from different chains (or different parts of the same chain) interact
 - Make CYS-CYS or glycosylation links as you find them
 - Use the group and link_list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

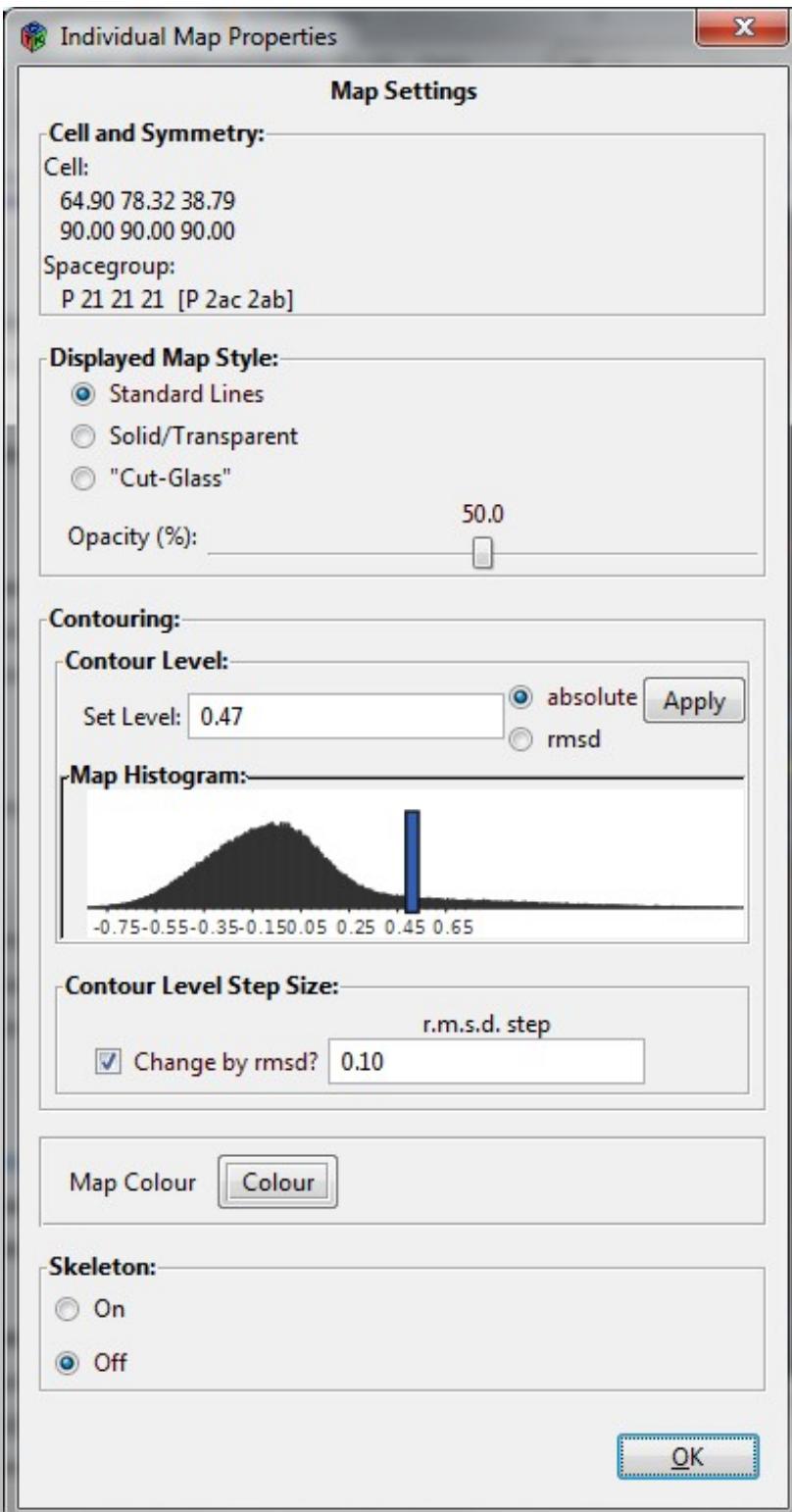
* Active residue = residue closest to the screen centre

Limit model and map display



- Map
 - Sphere
 - Use Preferences to set radius
- Coordinates
 - All
 - Within radius
 - Symmetry...

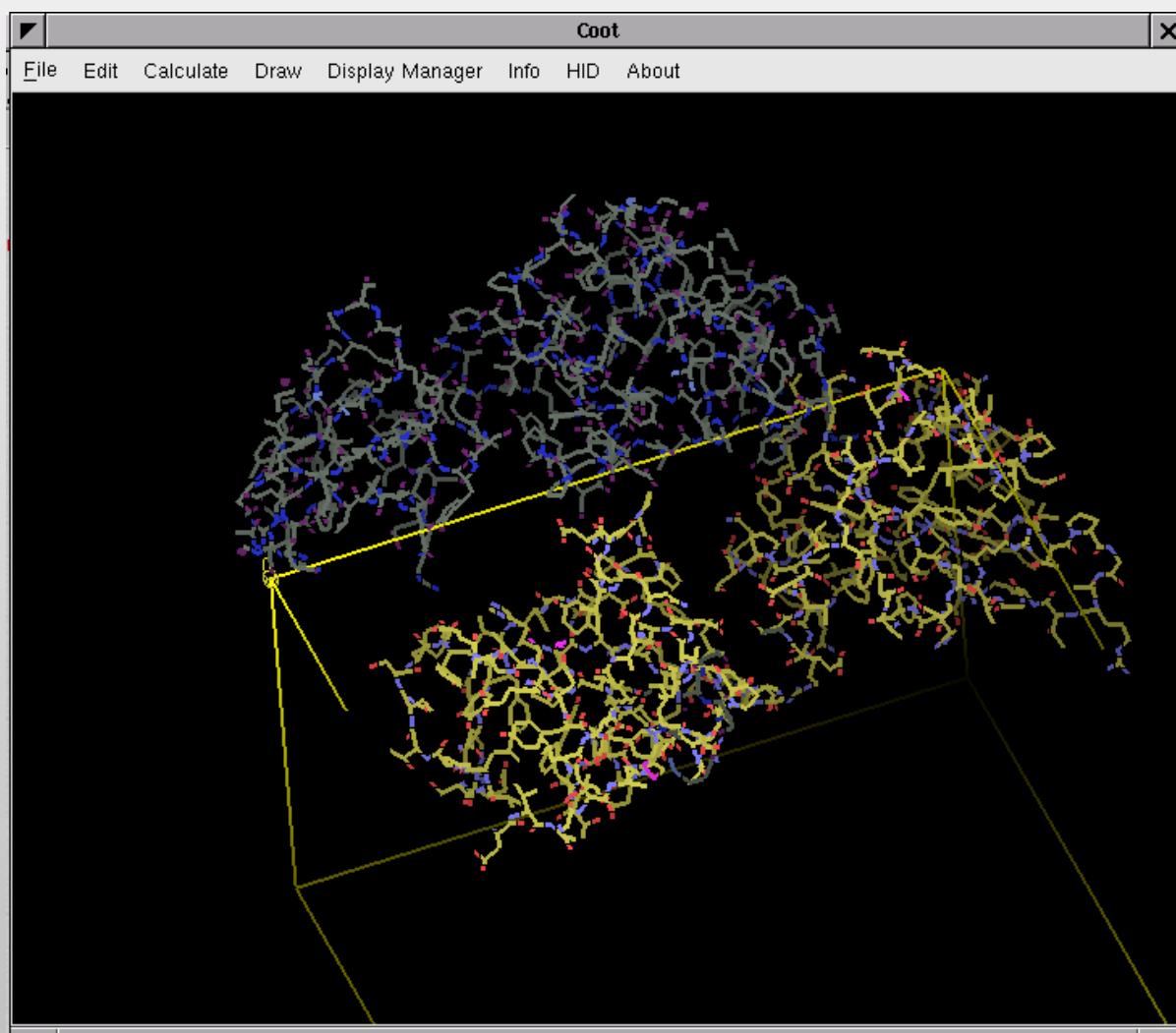




Map properties

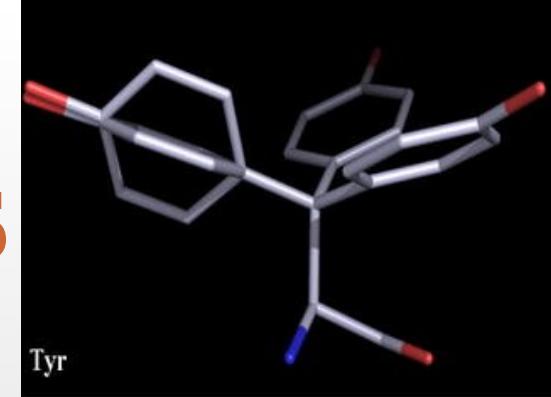
- Different display styles
 - Standard
 - Solid
 - Cut-glass
- Histogram (interactive)

Symmetry

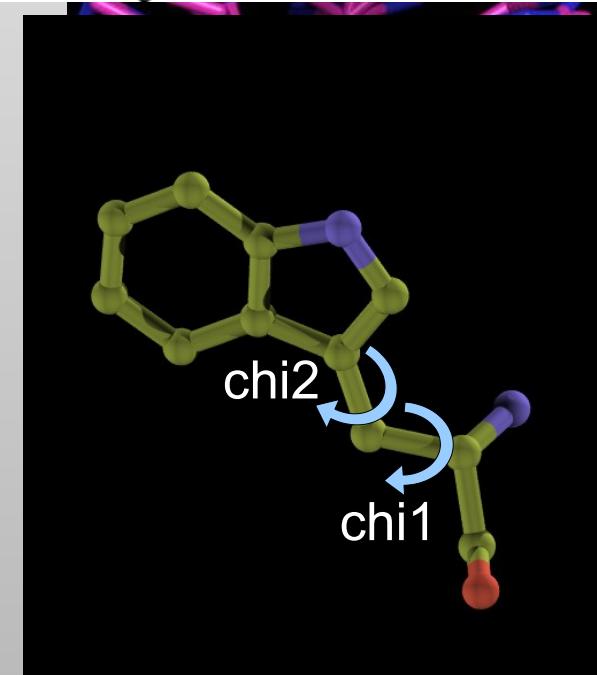
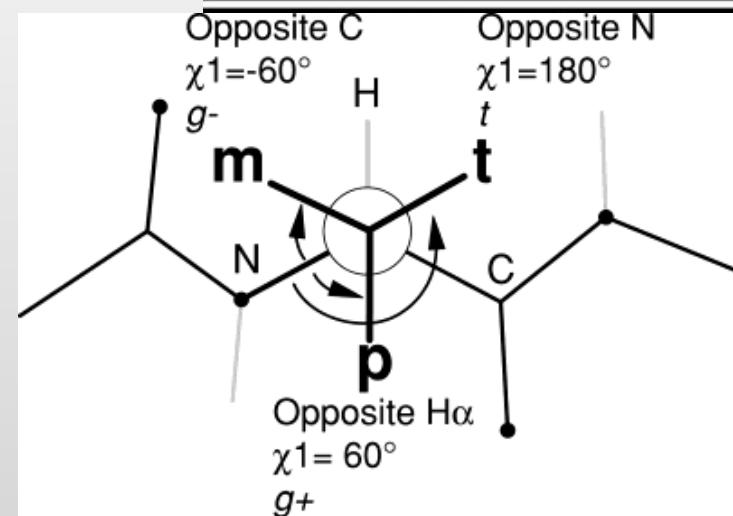


- Display
 - crystallographic symmetry copies
 - Unit cell
- Investigate crystal packing (voids?)
- Helps tracing/model building

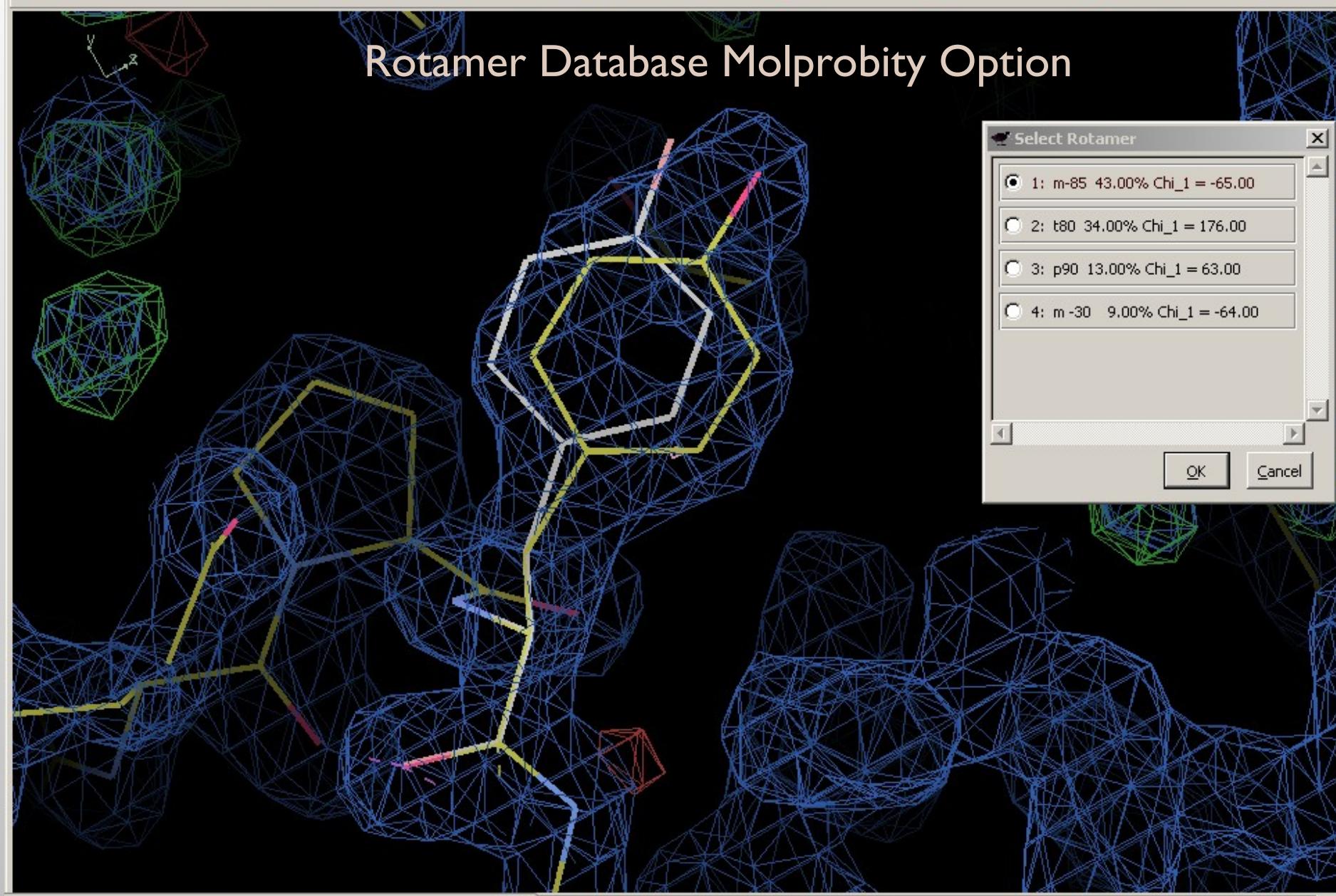
Side-chain Rotamers



- Rotamers are preferred configurations of a side-chains rotatable bonds
- “preferred” means these configurations occur more frequently in a set of reference protein structures
- “preferred” because they are low-energy conformations (staggered rather than eclipsed)
- Several Rotamer “databases” exist



Rotamer Database Molprobity Option

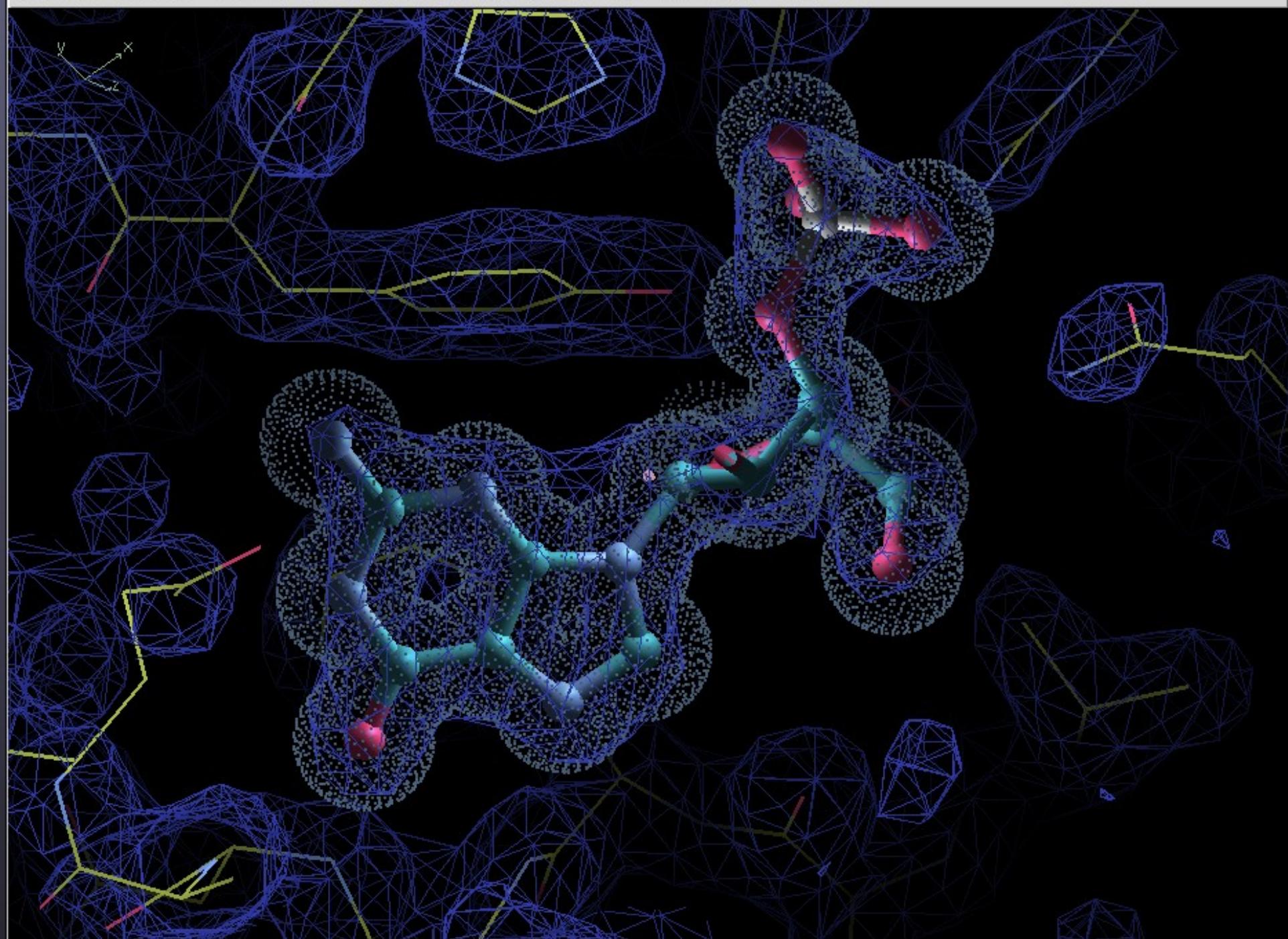


Some more Coot Tools...

- Add Terminal Residue
 - ϕ,ψ hypothesis scoring
- 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



Low Resolution/Extra Tools

Extra Restraints....

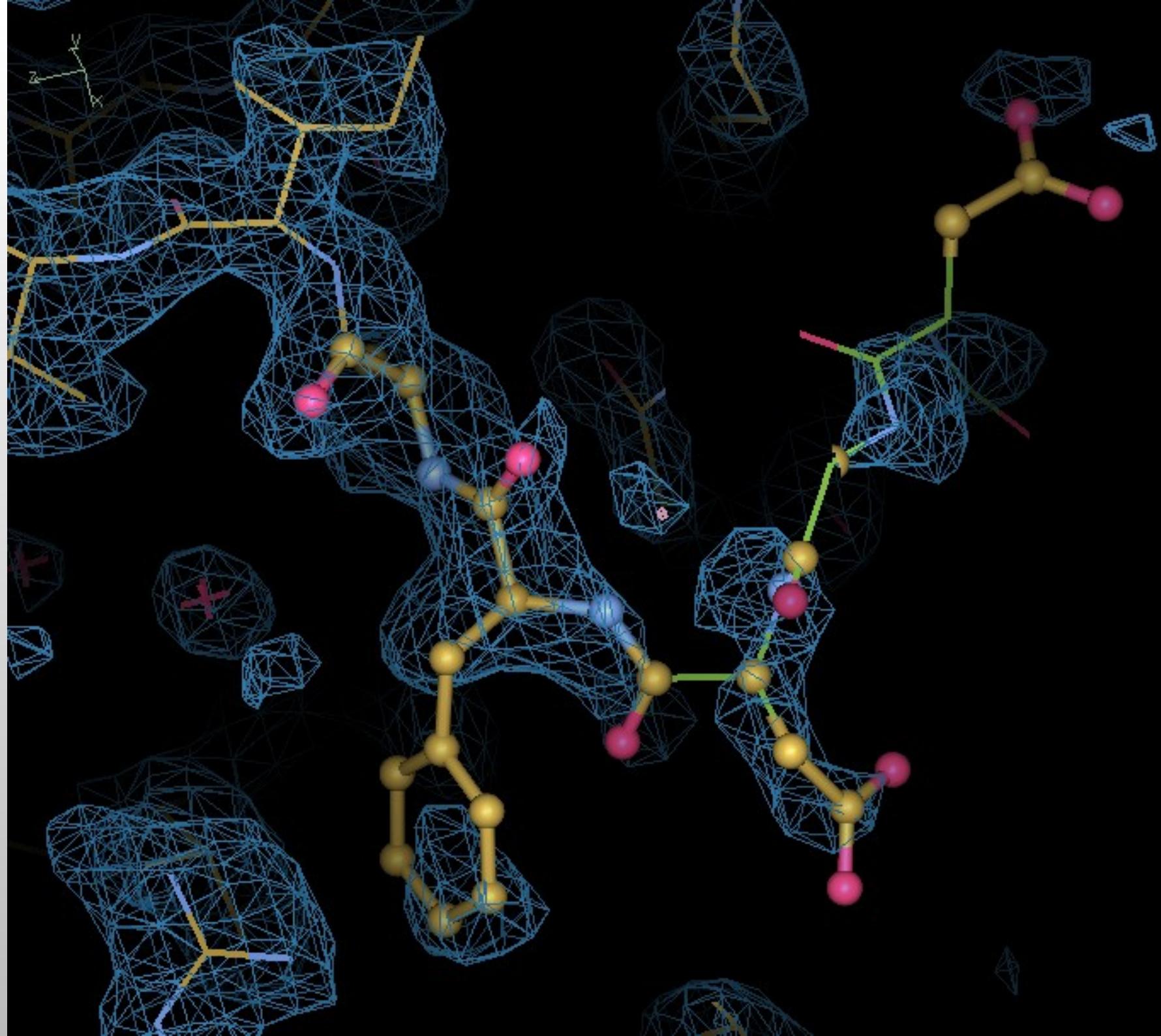
Extra Restraints....

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

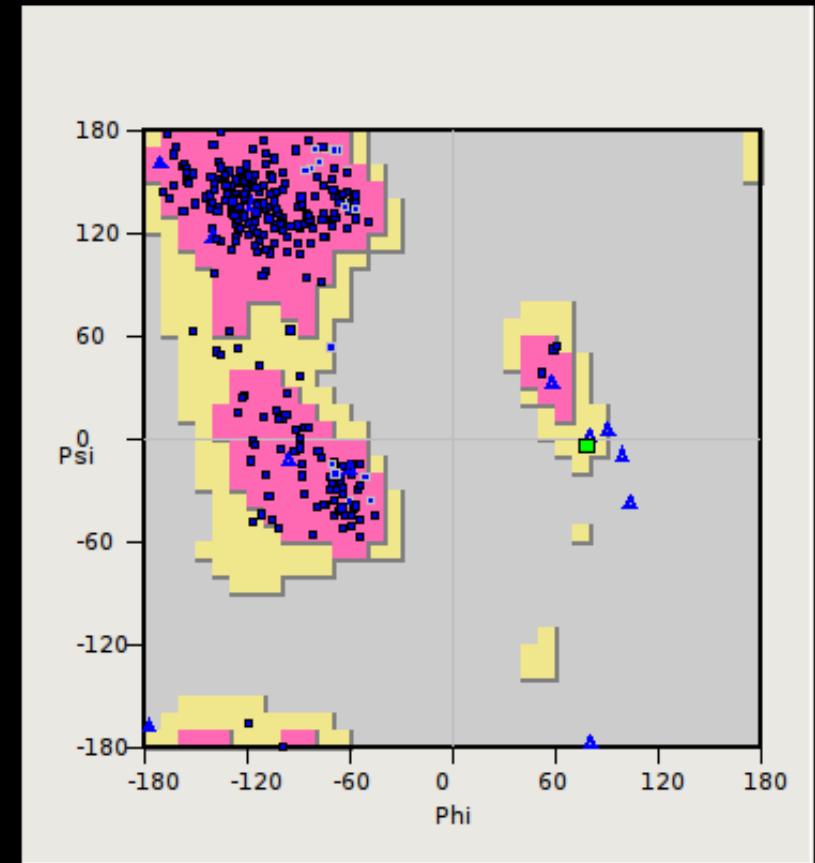
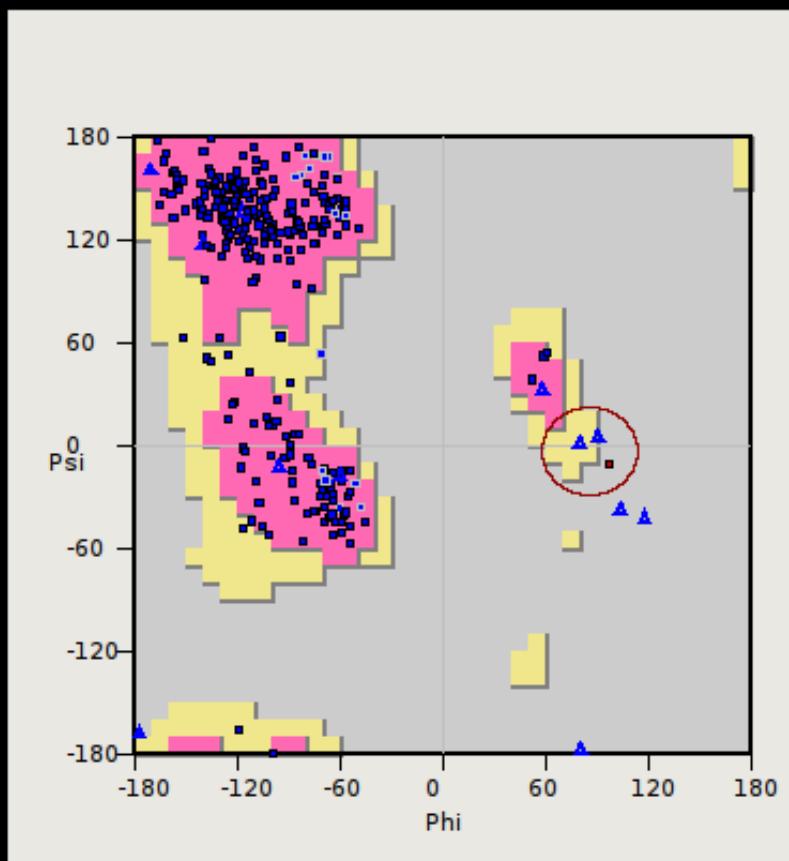
- Map sharpening
- [Jiggle fit]
- [Morphing]

Ramachandran Restraints

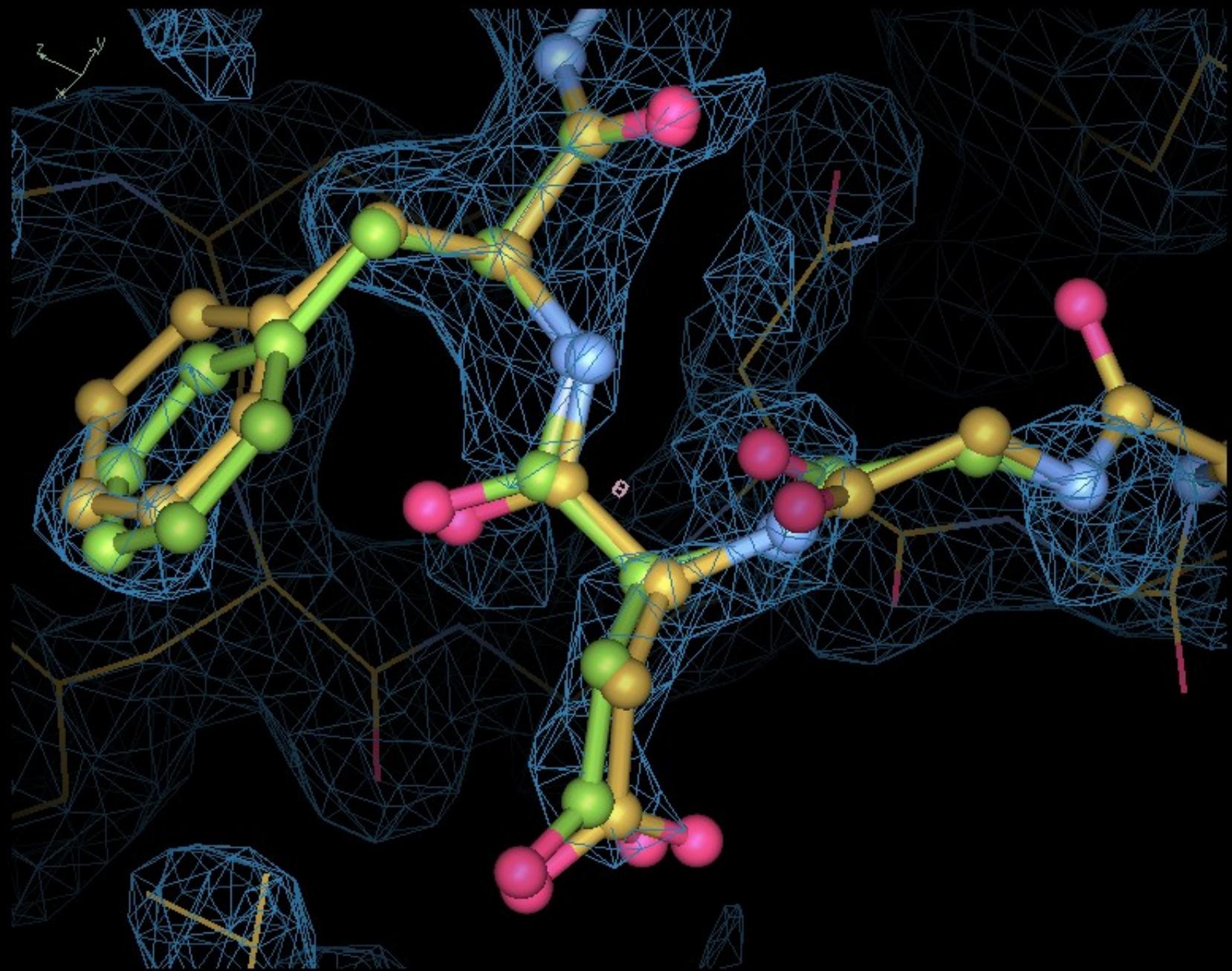
- Scenario:
 - I have a loop, with poor density, I know the atoms are there somewhere and I want to provide a “reasonable” model
- Controversial Feature?
 - Ramachandran Plots have been used for “validation” - but here we are deliberately optimizing them
- Ramachandran Plots can be added to the geometry target function



Tweaking a Ramachandran Outlier



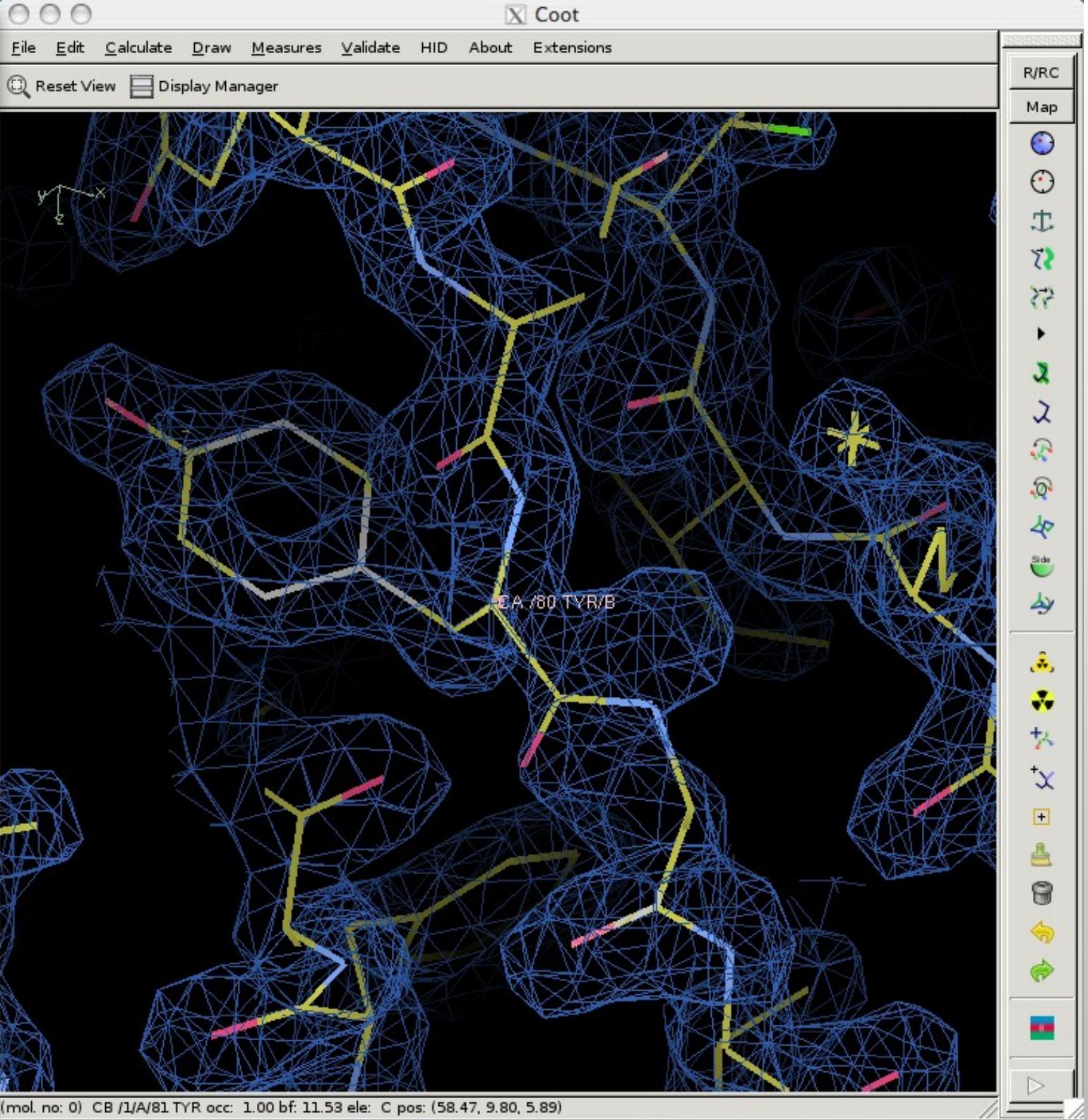
Tweaking Phi and Psi



Accept Refinement



```
g_atoms_rama_restraints) reti  
ints  
6  
  
) at -14010.6
```



X Accept Refinement

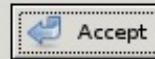
X Coot

File Edit Calculate Draw Measures Validate HID About Extensions

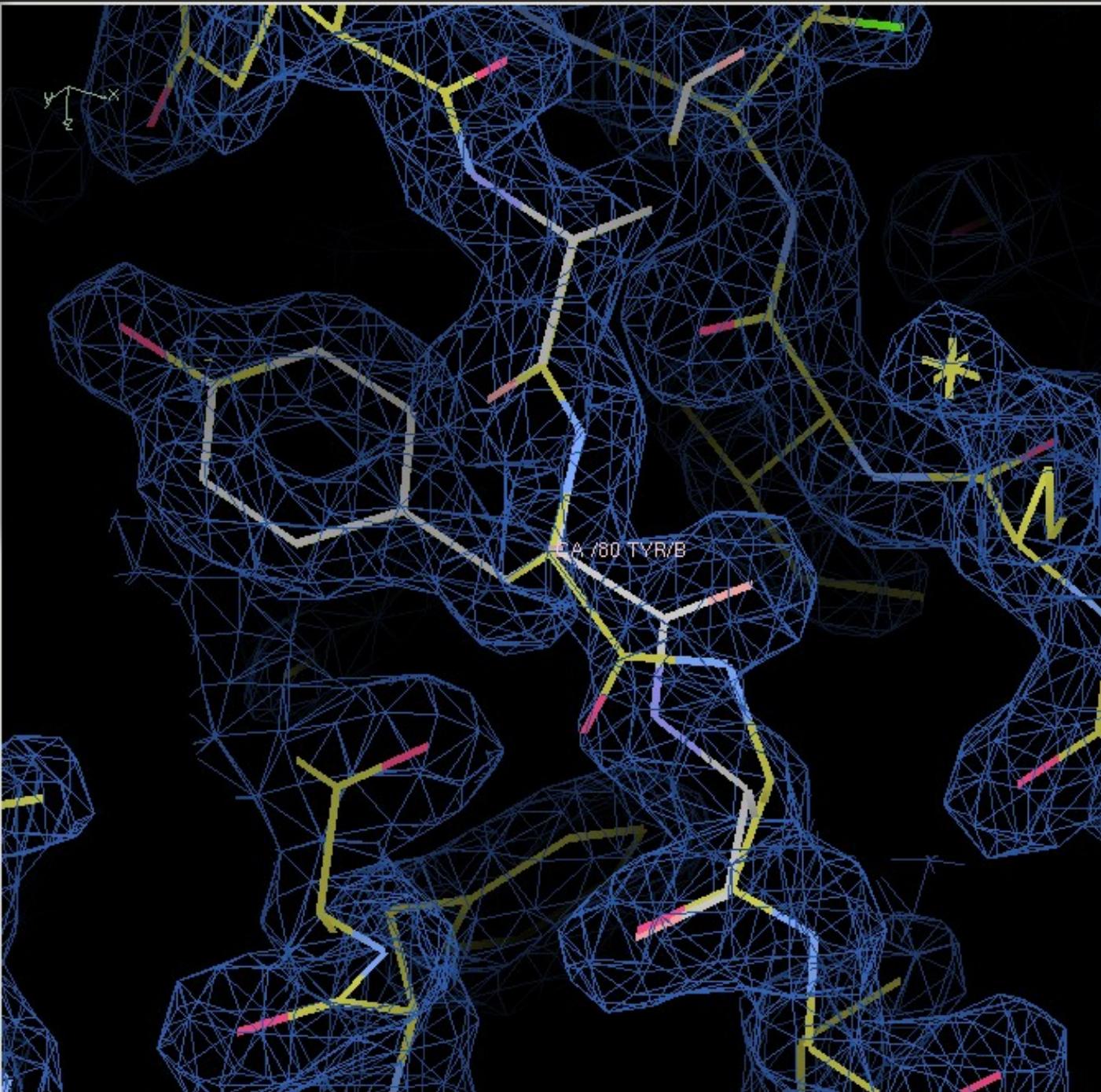
Reset View Display Manager

Accept Refinement?

- Bonds: 1.625
- Angles: 0.318
- Planes: 1.671
- Chirals: 0.177
- Non-bonded: 0.000
- Rama Plot: -177.602

 Accept

 Reject



R/RC

Map



Ramachandran Restraints

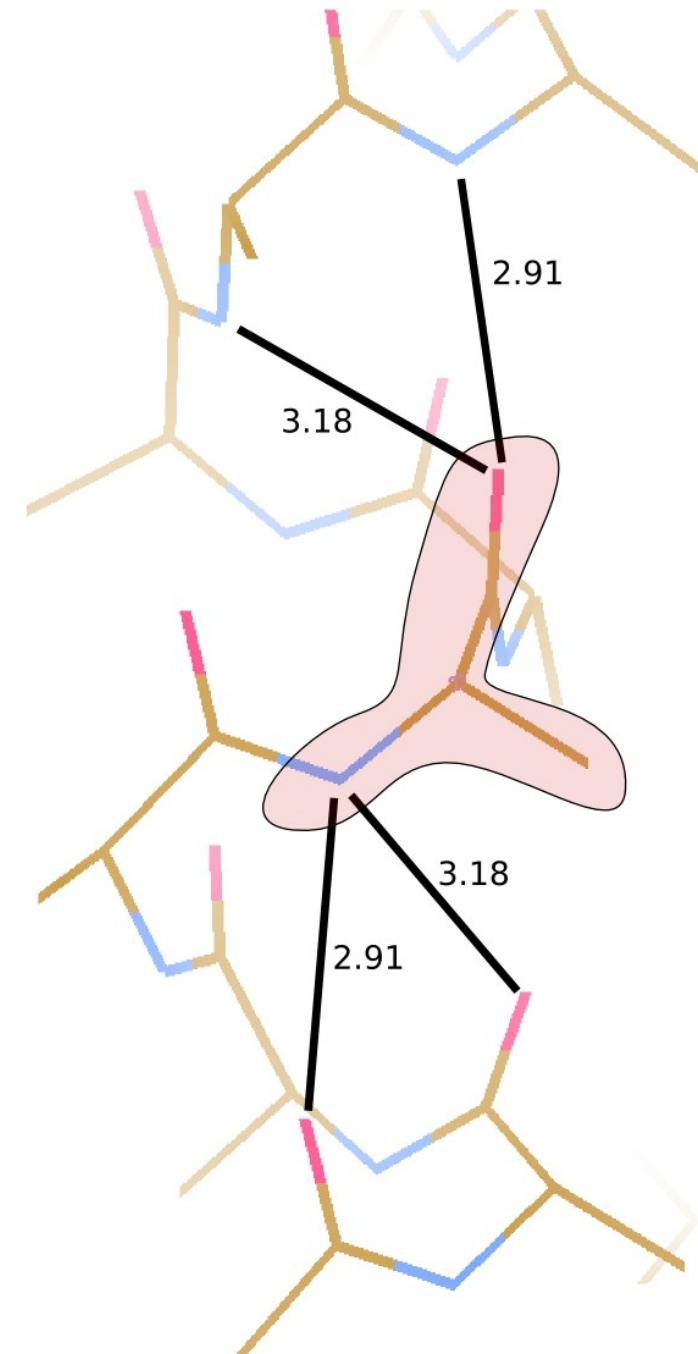
- Controversial?
 - “... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model...”
 - Gerard Kleywegt & Alwyn Jones (1996)
- But to quote Jane Richardson:
 - Do you want a better structure - or a better idea of the quality of your structure?

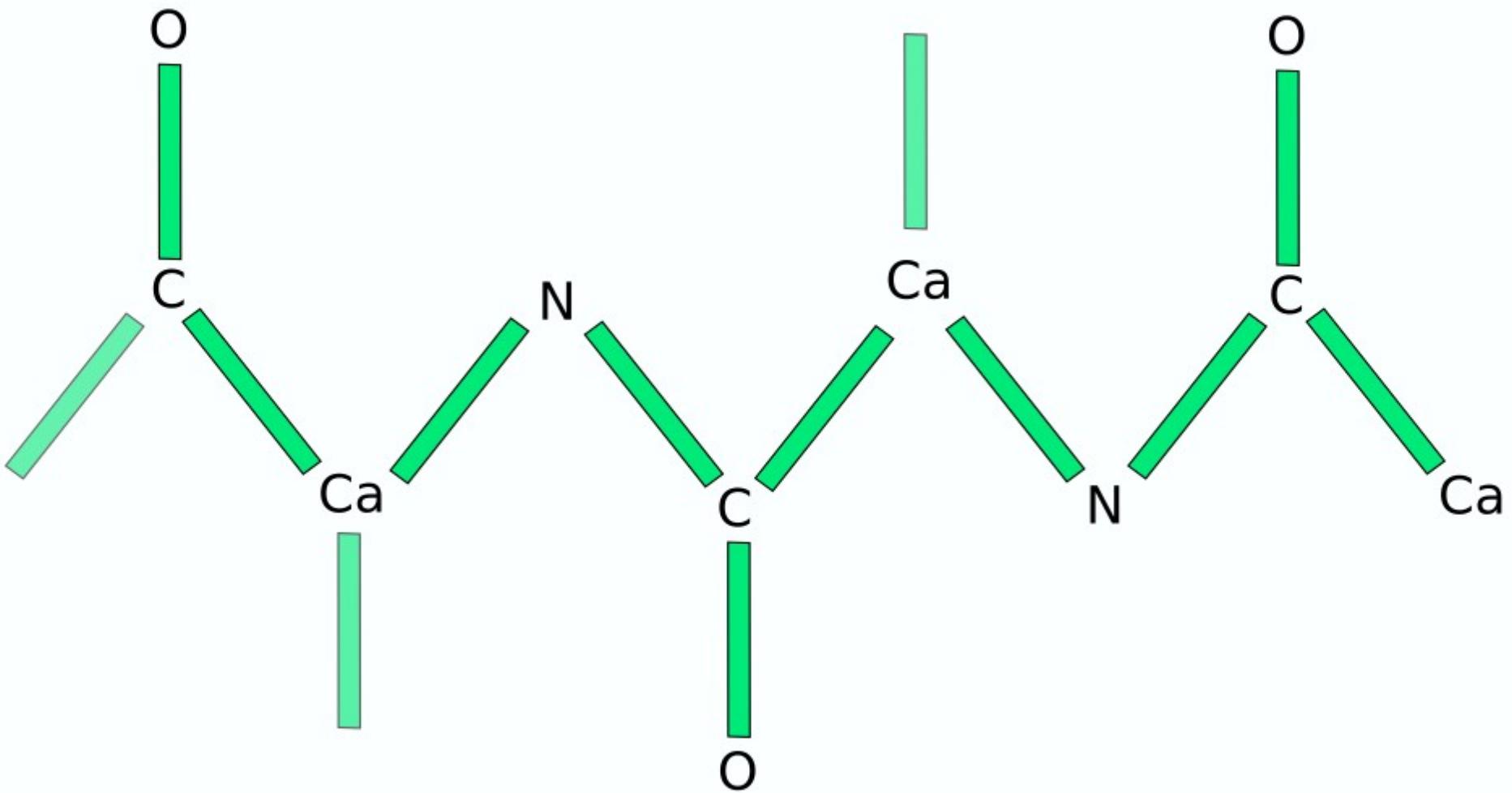
Adding Torsion Angle Restraints

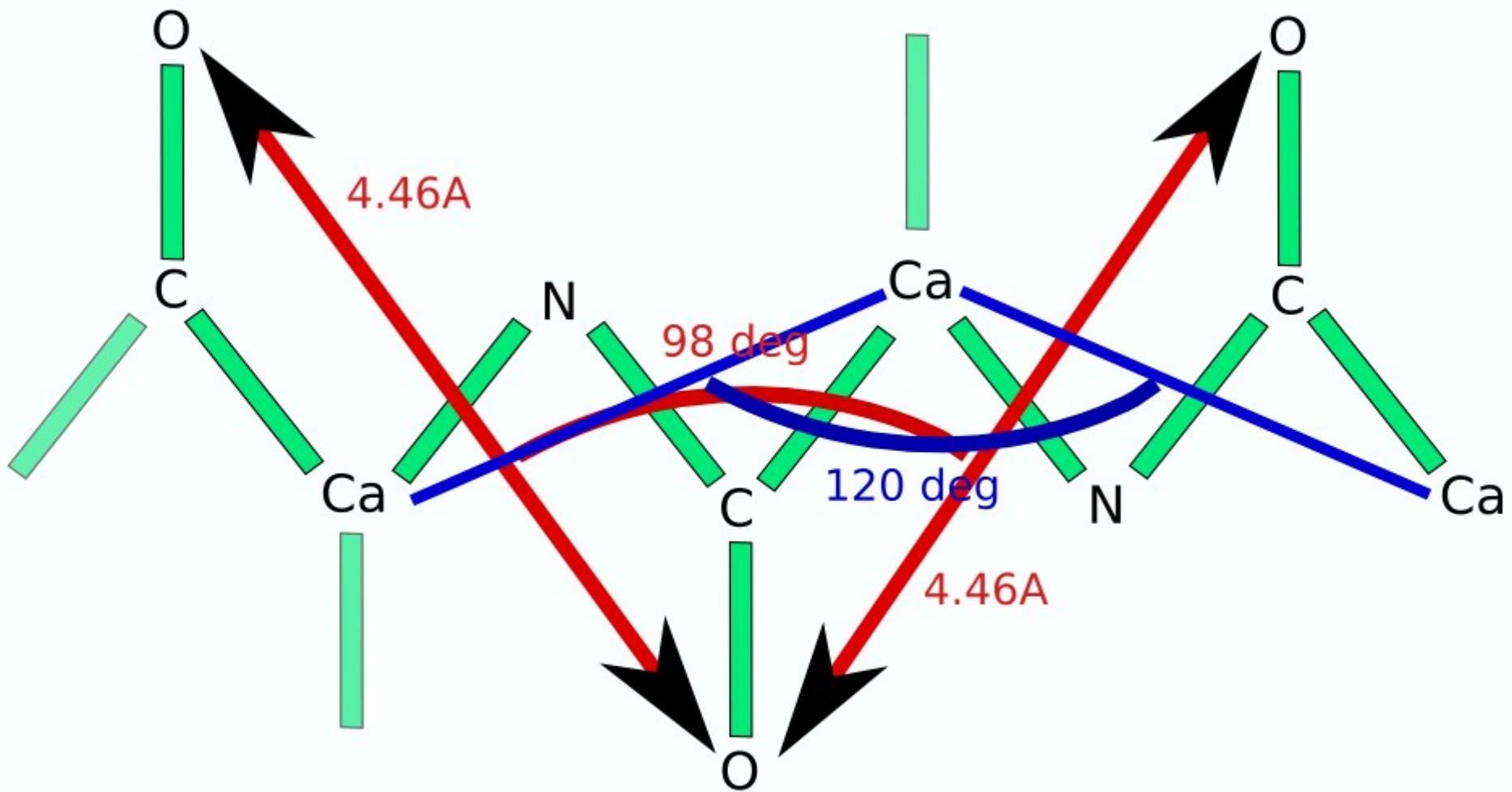
- Torsion angle refinement is slow (relatively)
 - Simple addition of these restraints to the geometry target function
 - often makes the region “stuck and unsatisfied”
 - i.e. trapped in local minimum
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints

Restrain the Hydrogen-bonding atom distances







Activate restraints

start here

Refinement and Regularization Parameters

(revision count 7736)

Draw Measures Validate HID R/RC Ligand Extensions Unittesting CCP4

Display Manager α Rama Sphere Refine + Change Alt Conf Occ Backrub Rotamers >>

R/RC

Map

Refinement and Regularization Parameters

For Regularization and Refinement:

- Use Torsion Restraints
- Use Planar Peptide Restraints
- Use Trans Peptide Restraints
- Ramachandran Restraints

Mainchain Restraints

- No Secondary Structure Restraints
- Alpha Helix Restraints
- Beta Strand Restraints

Weight Matrix

Refinement Weight Estimate

OK

Refinement weight, the smaller the tighter geometry

No ligand (hetgroup) found in this molecule (#0).

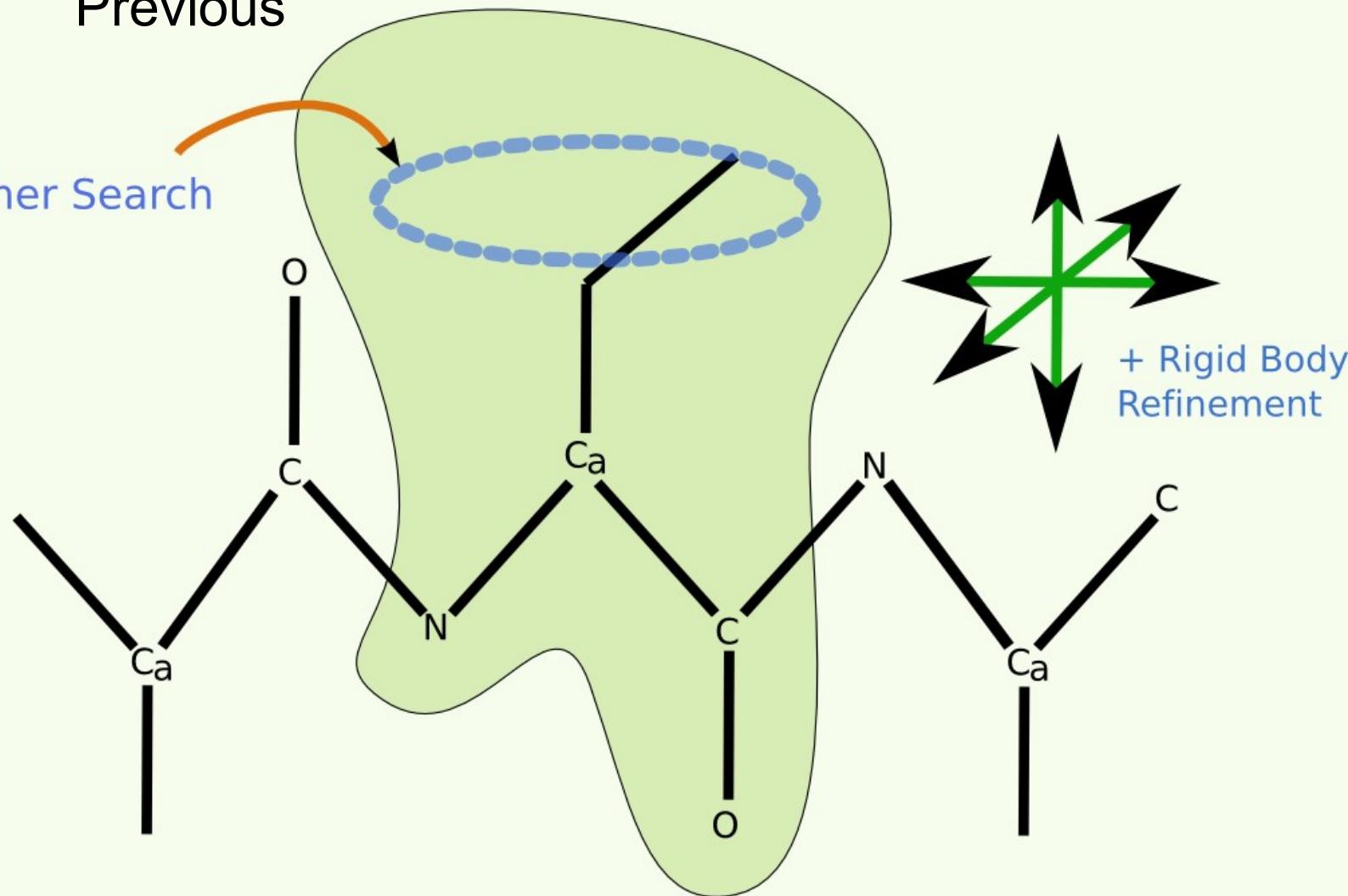
"Backrub Rotamers"

- High probability models with low resolution data

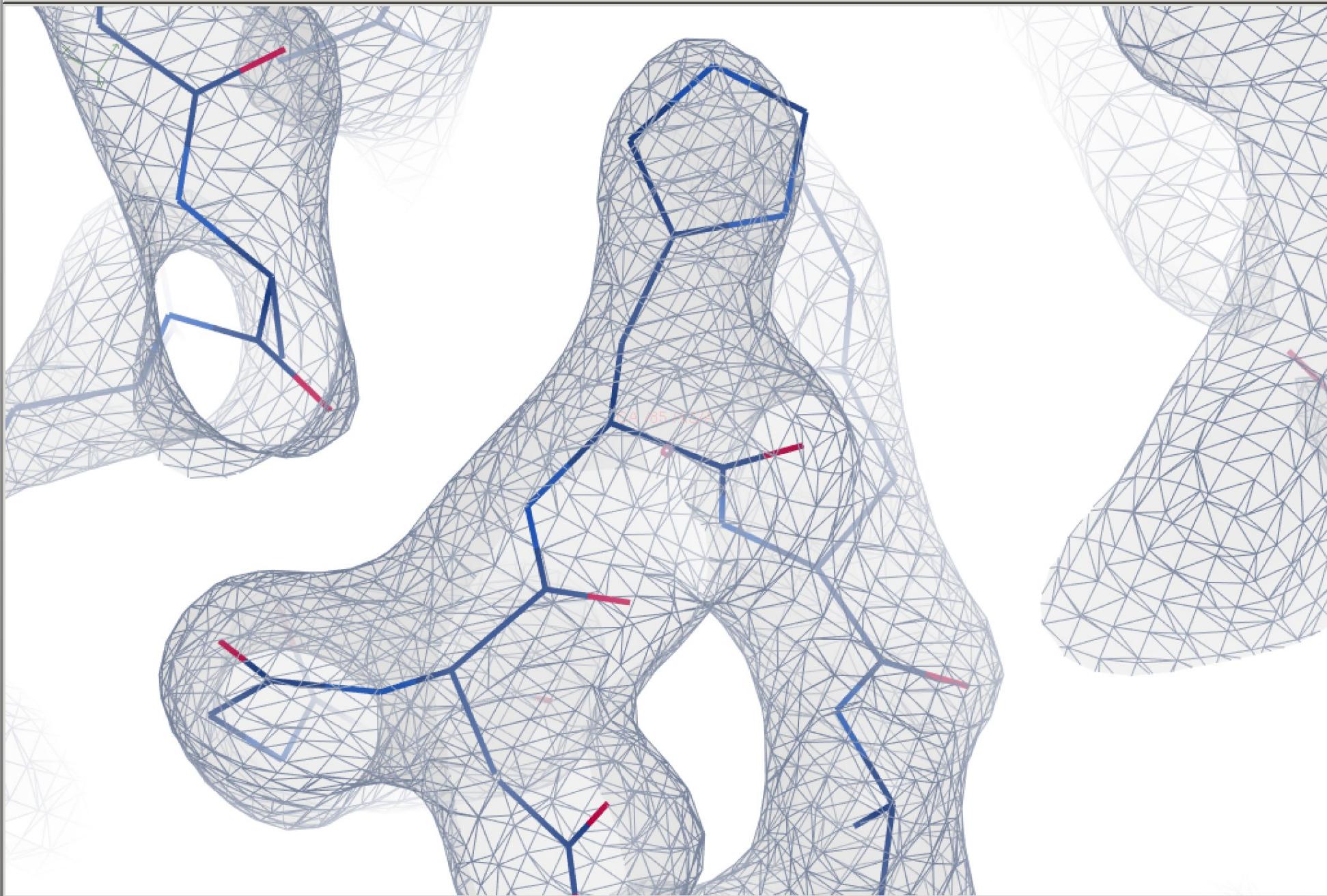
~~Current~~ Low Resolution Rotamer Search

Previous

Rotamer Search



+ Rigid Body
Refinement

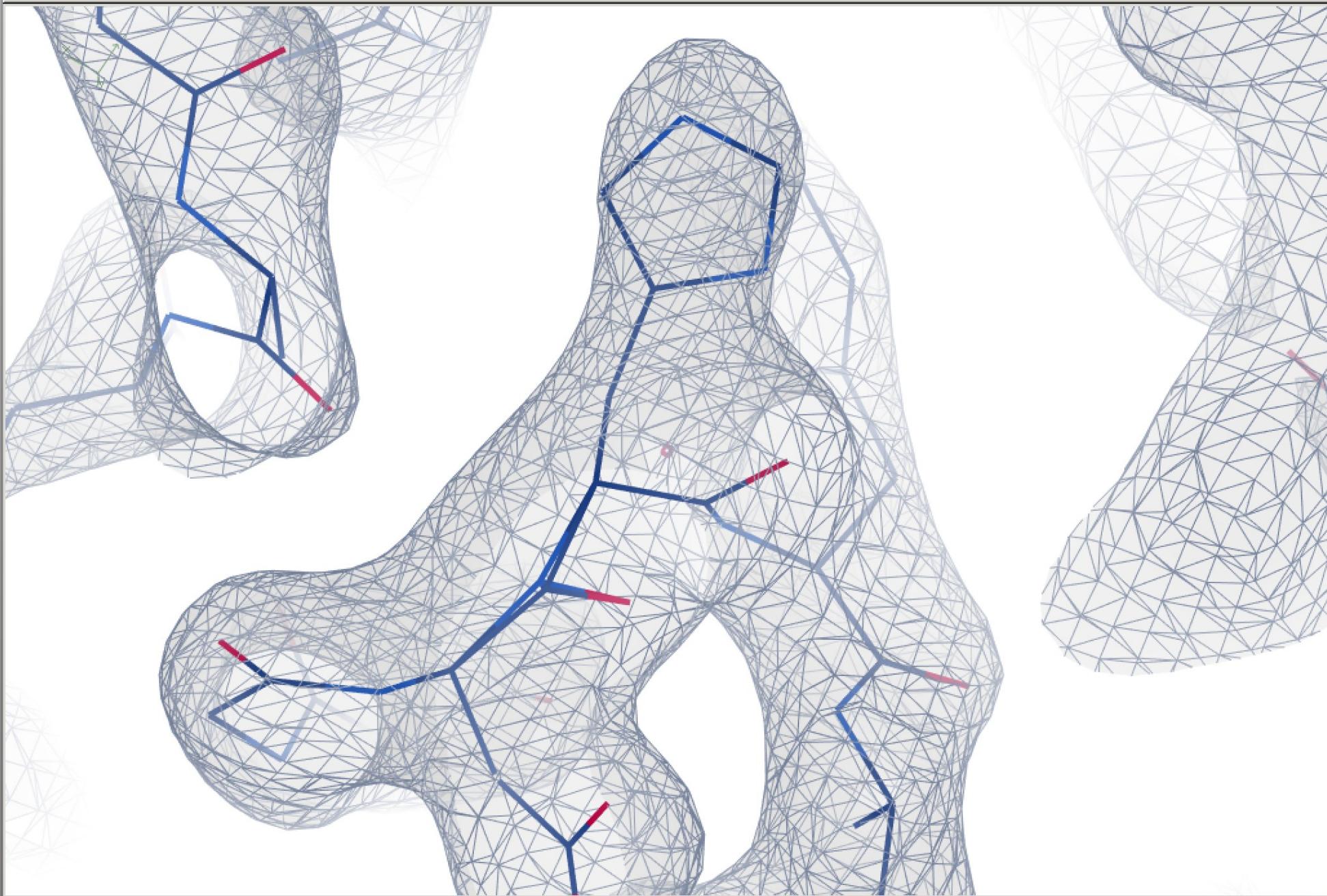


(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)

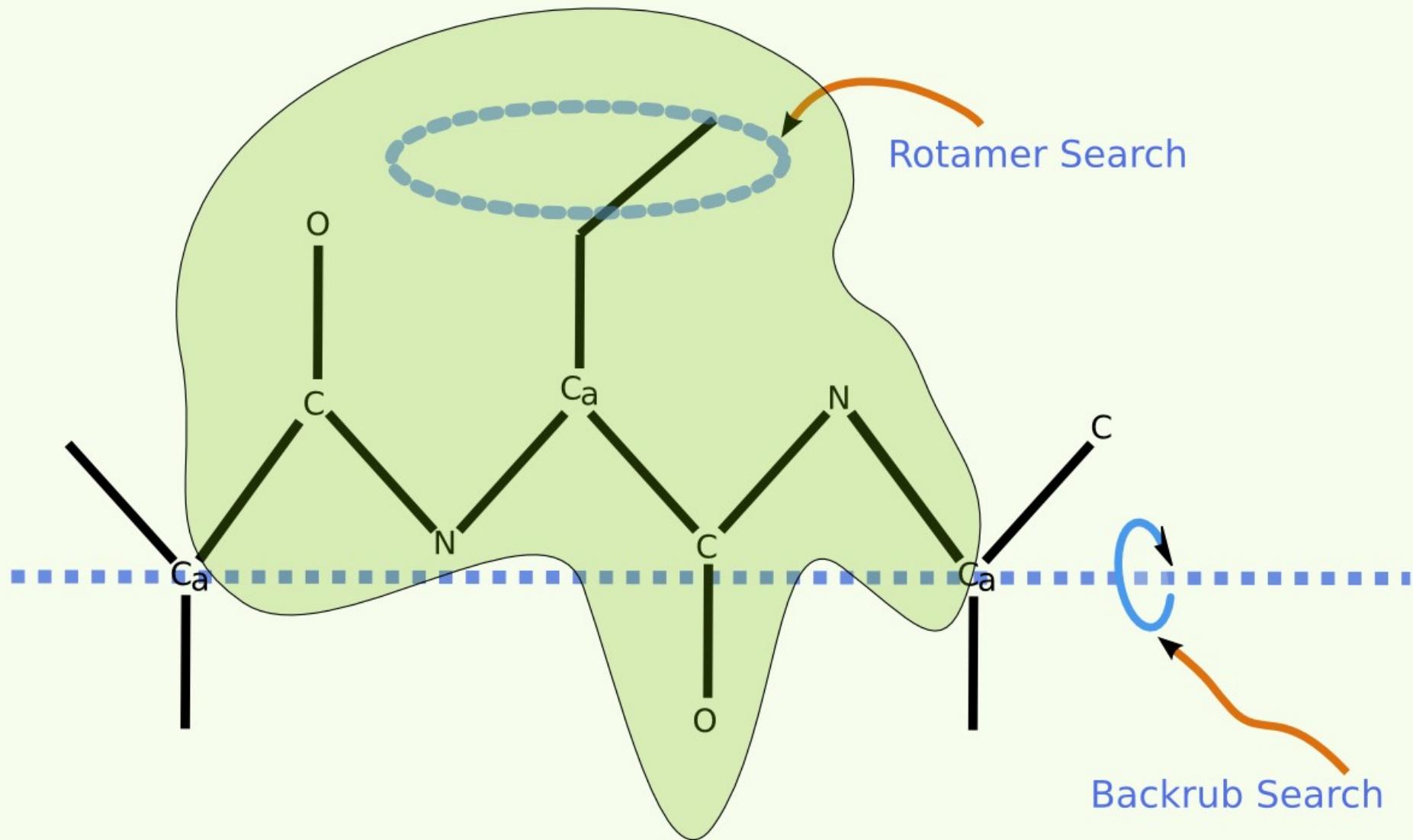
R/RC

Map

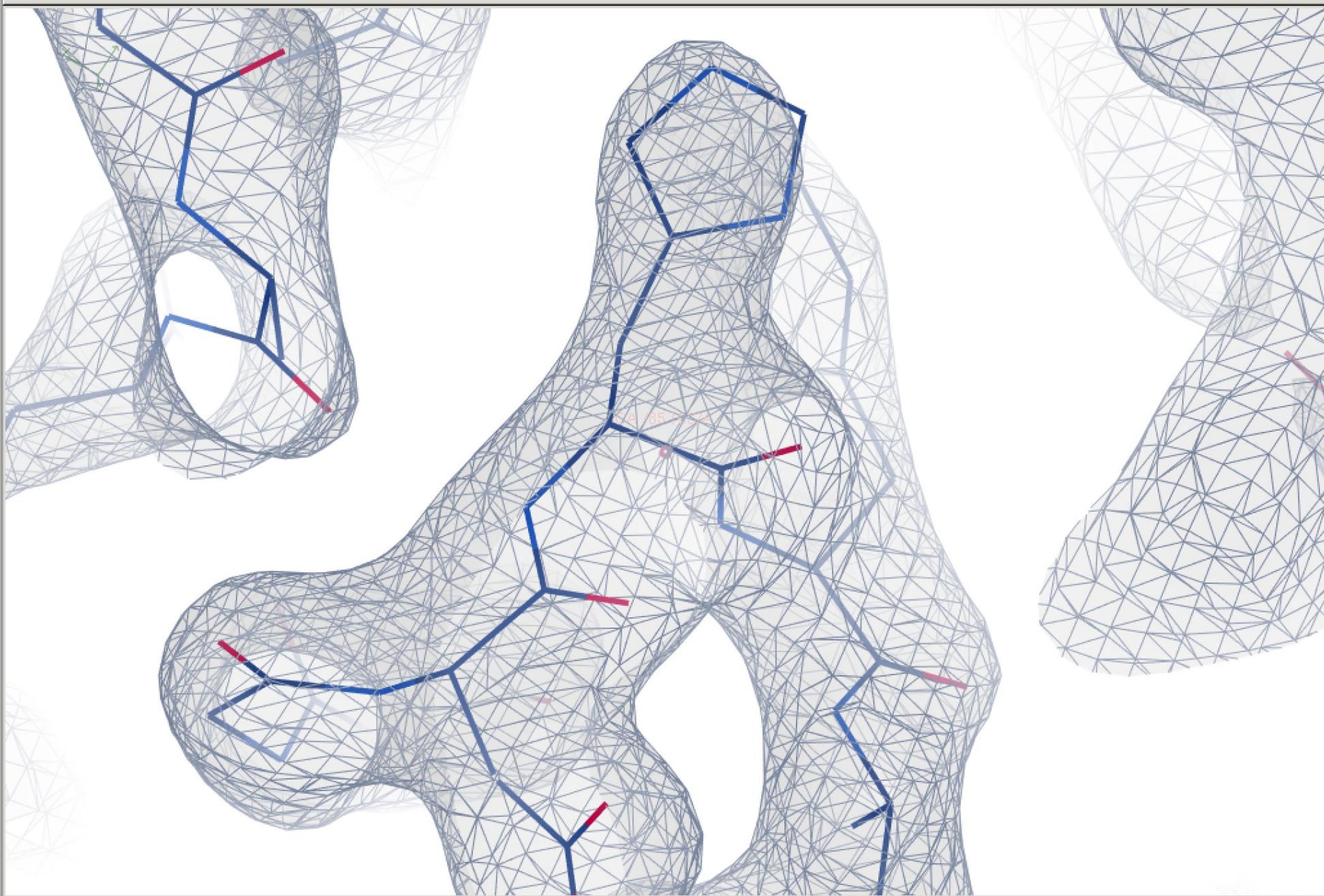


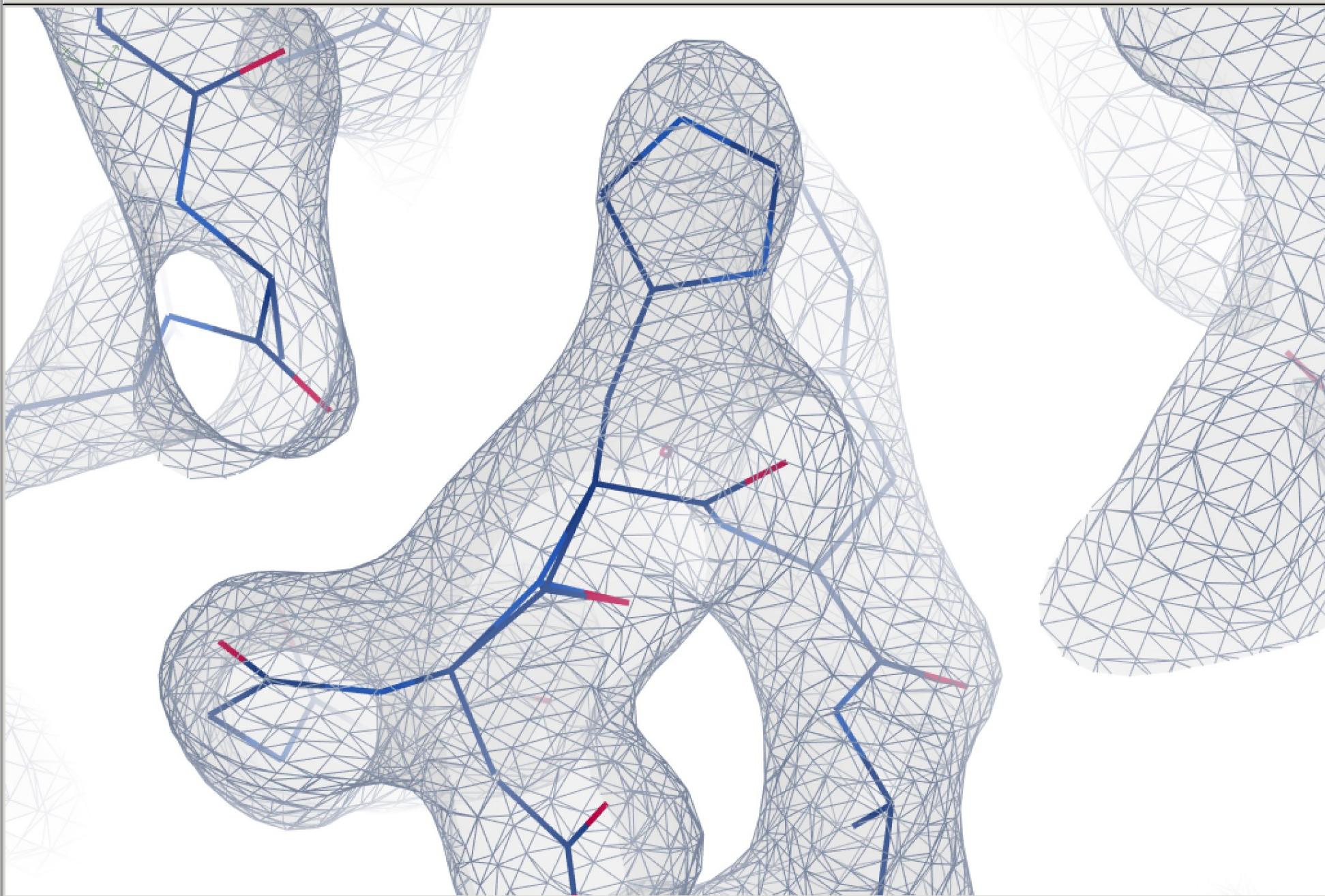


New Low Resolution Rotamer Search



After Fitting Tools in KING/Molprobity



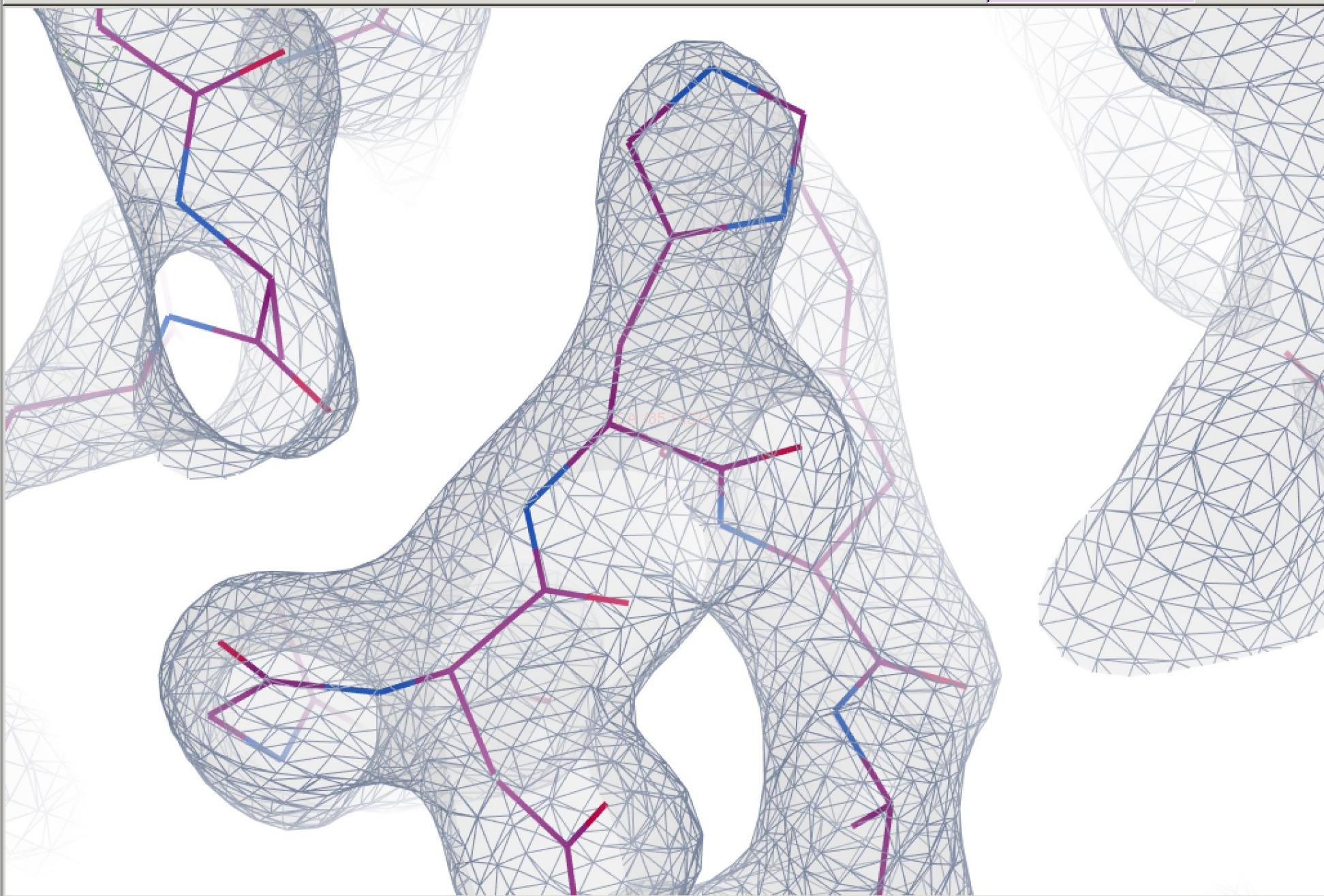


R/RC

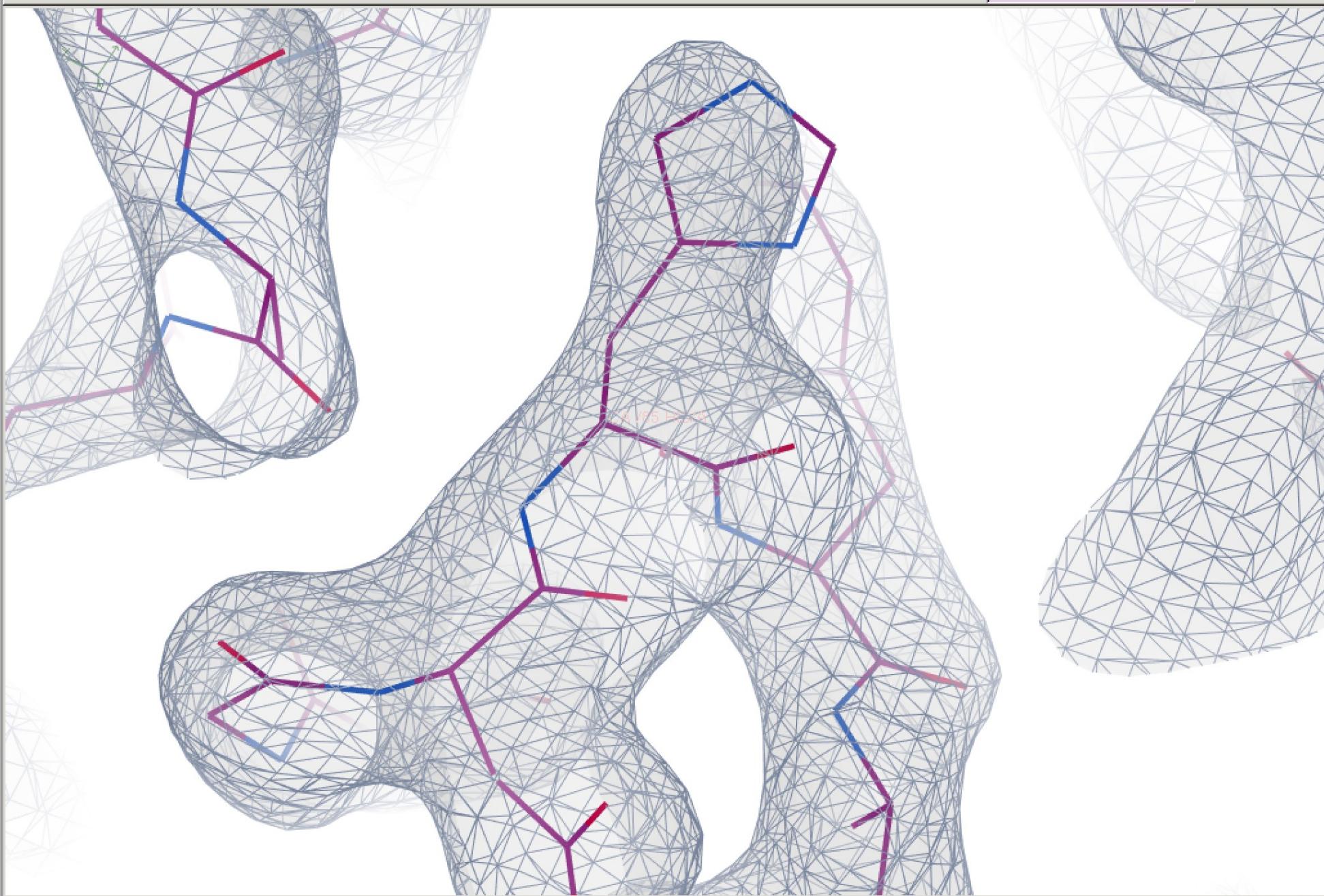
Map



▷

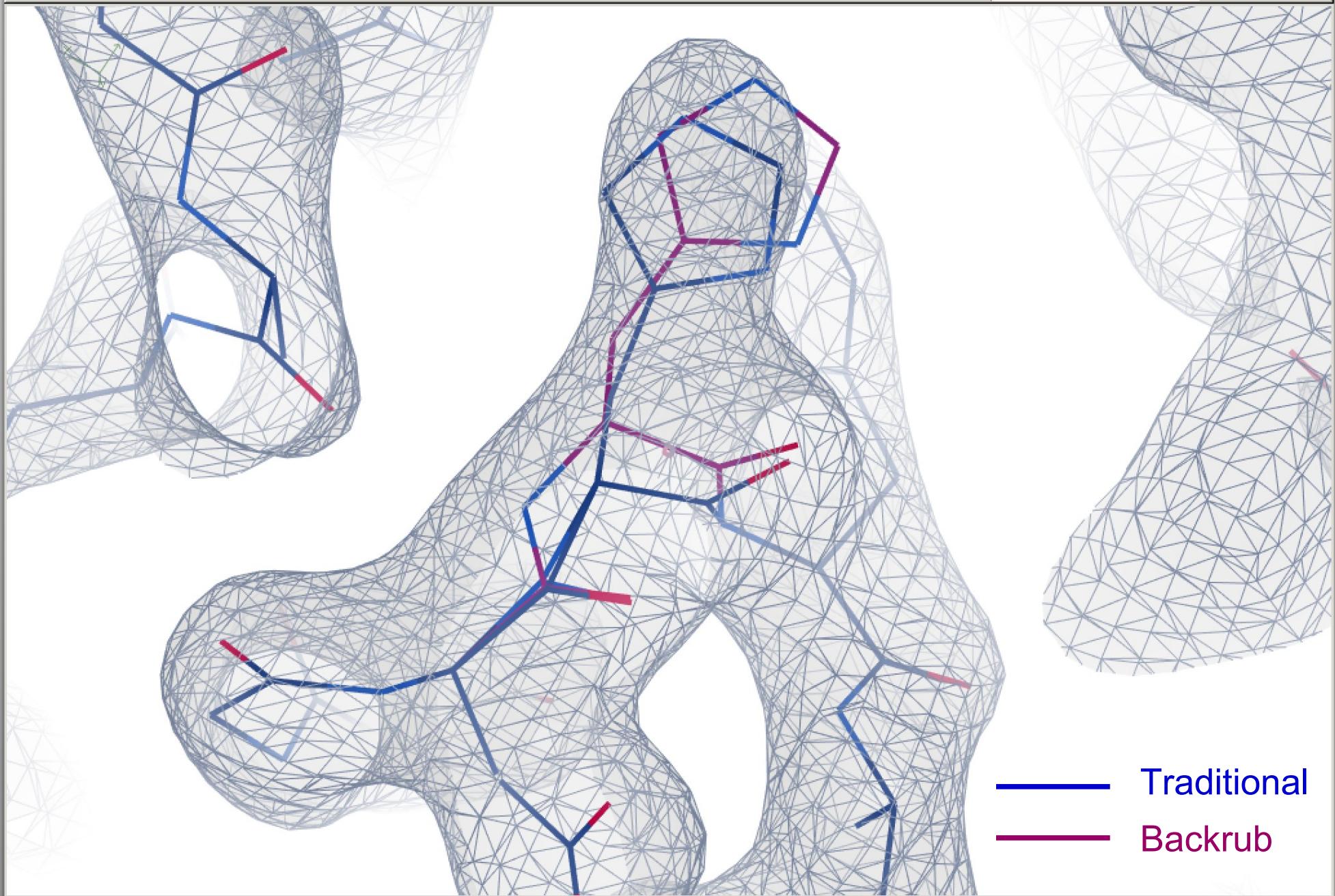


(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)



(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)





To turn it on...

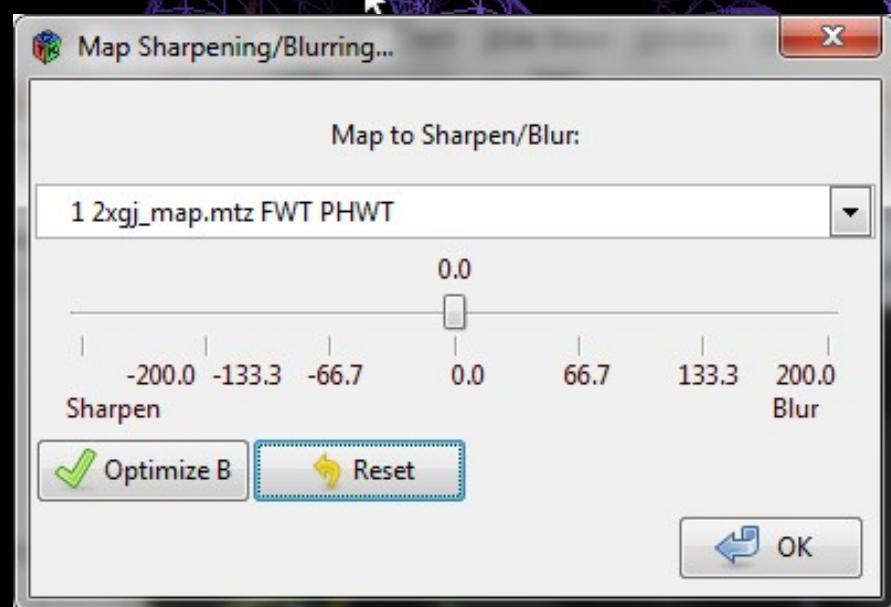
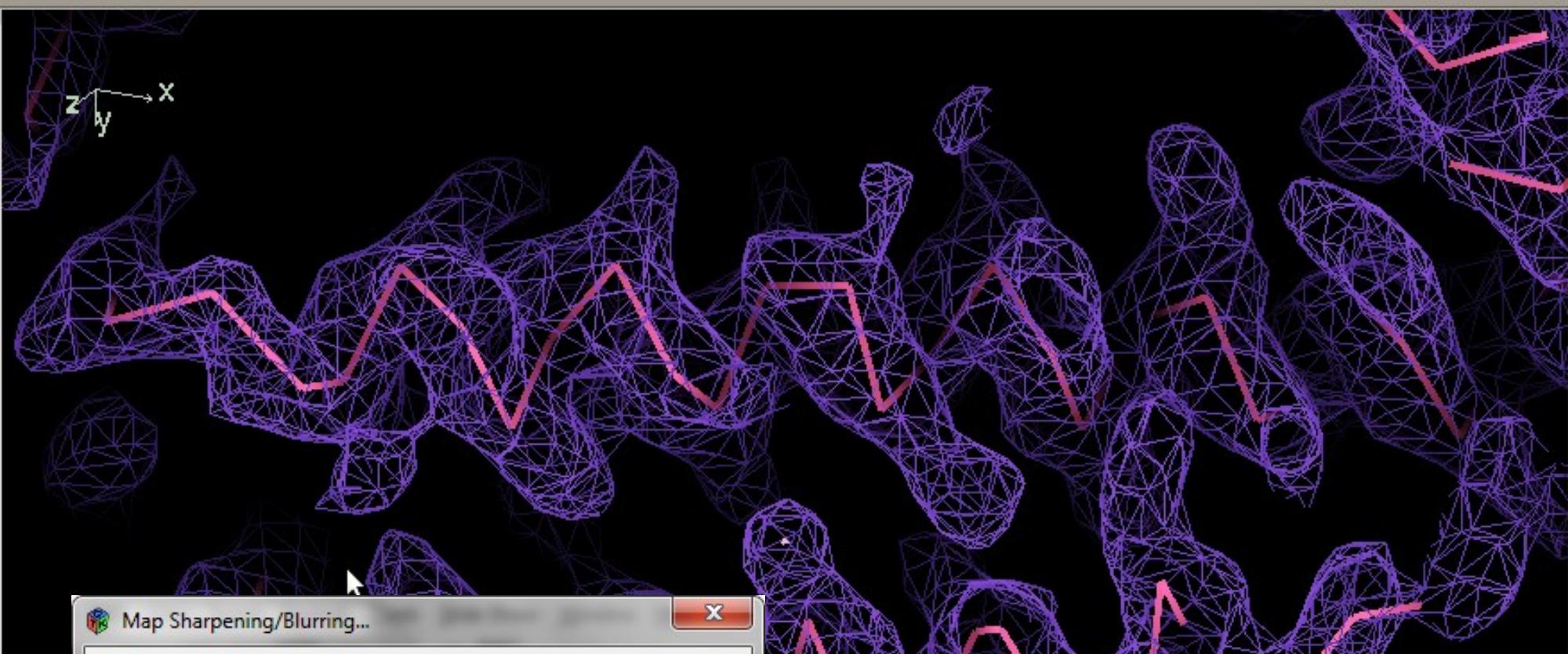
- automatically for resolution > 2.9Å
- via
 - Extensions → Modelling → Rotamer Search
 - scripting:
 - **guile**: (ROTAMERSEARCHLOWRES)
 - **python**: set_rotamer_search_mode(ROTAMERSEARCHLOWRES)
 - toolbutton

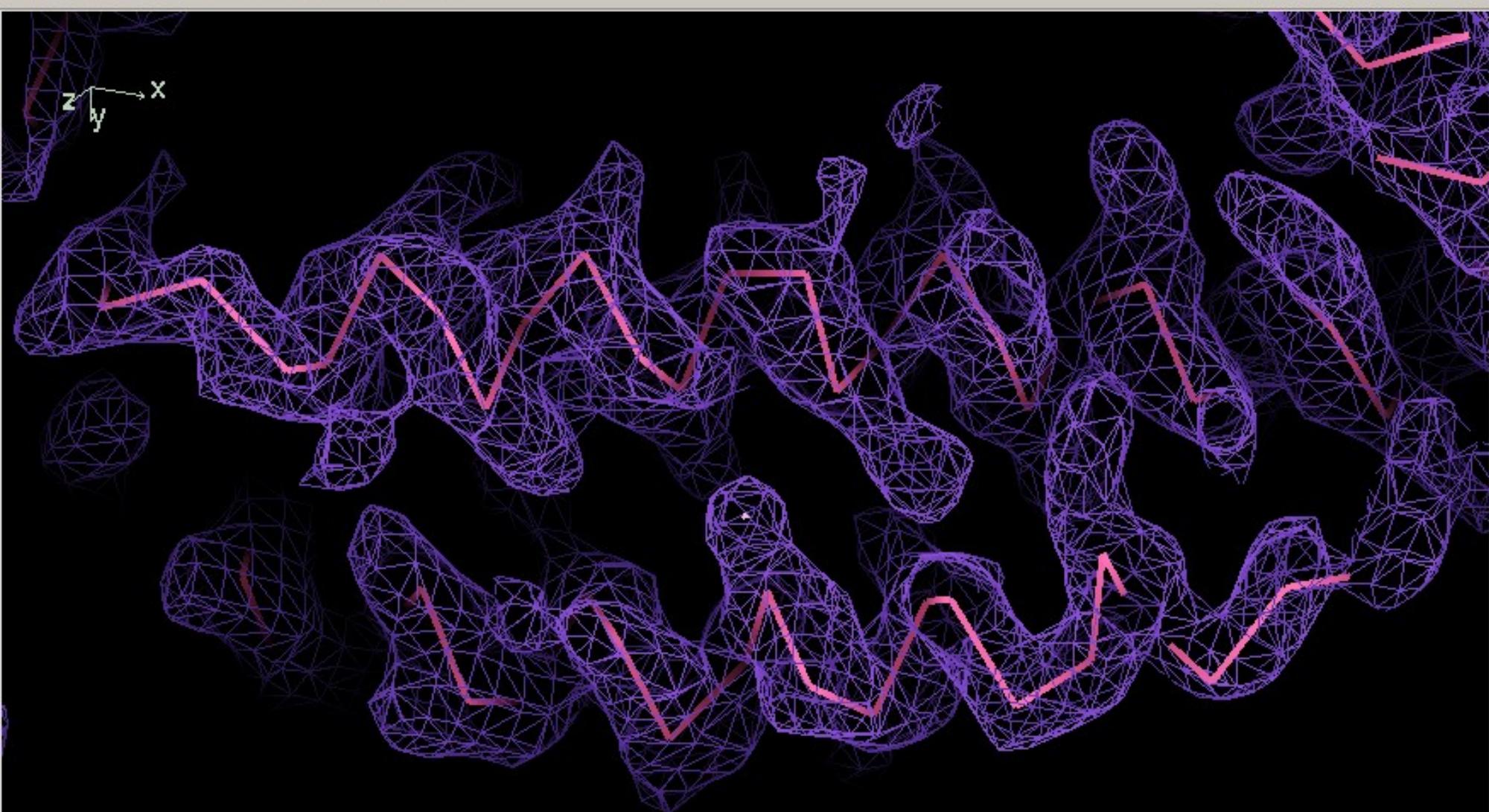
Map Sharpening

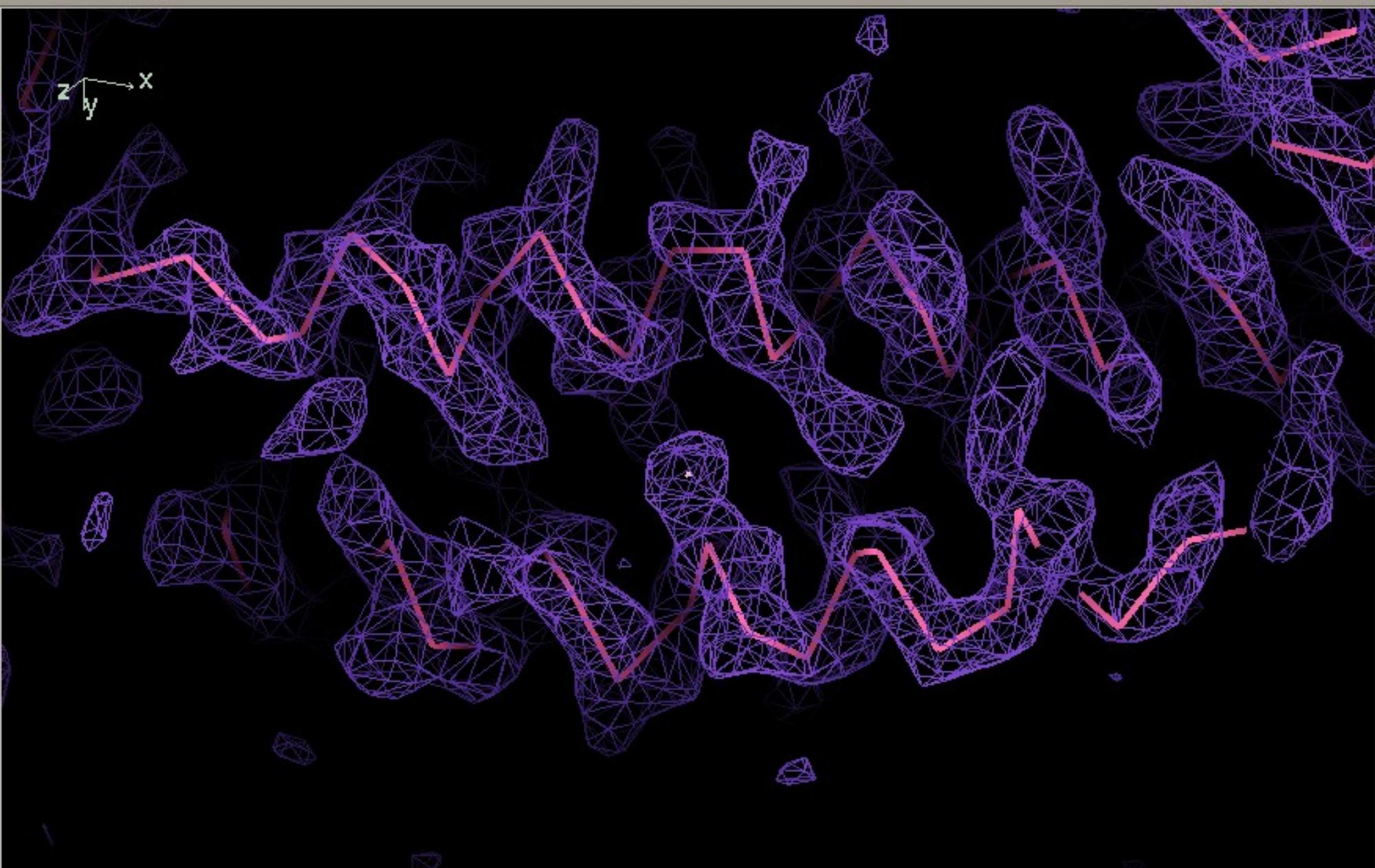
Which B-factor shall I use to get the most interpretable map?

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

Try to optimise using map kurtosis





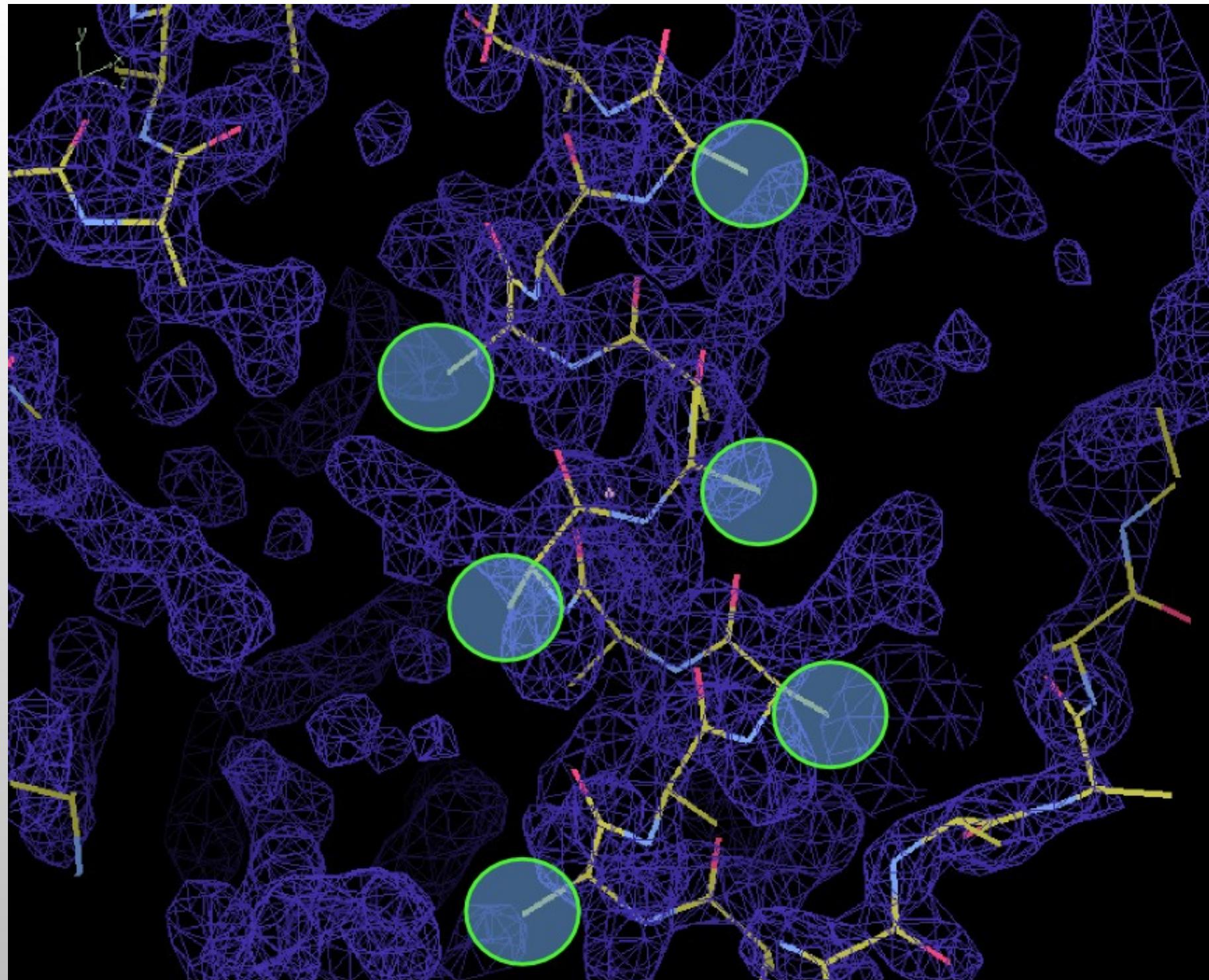


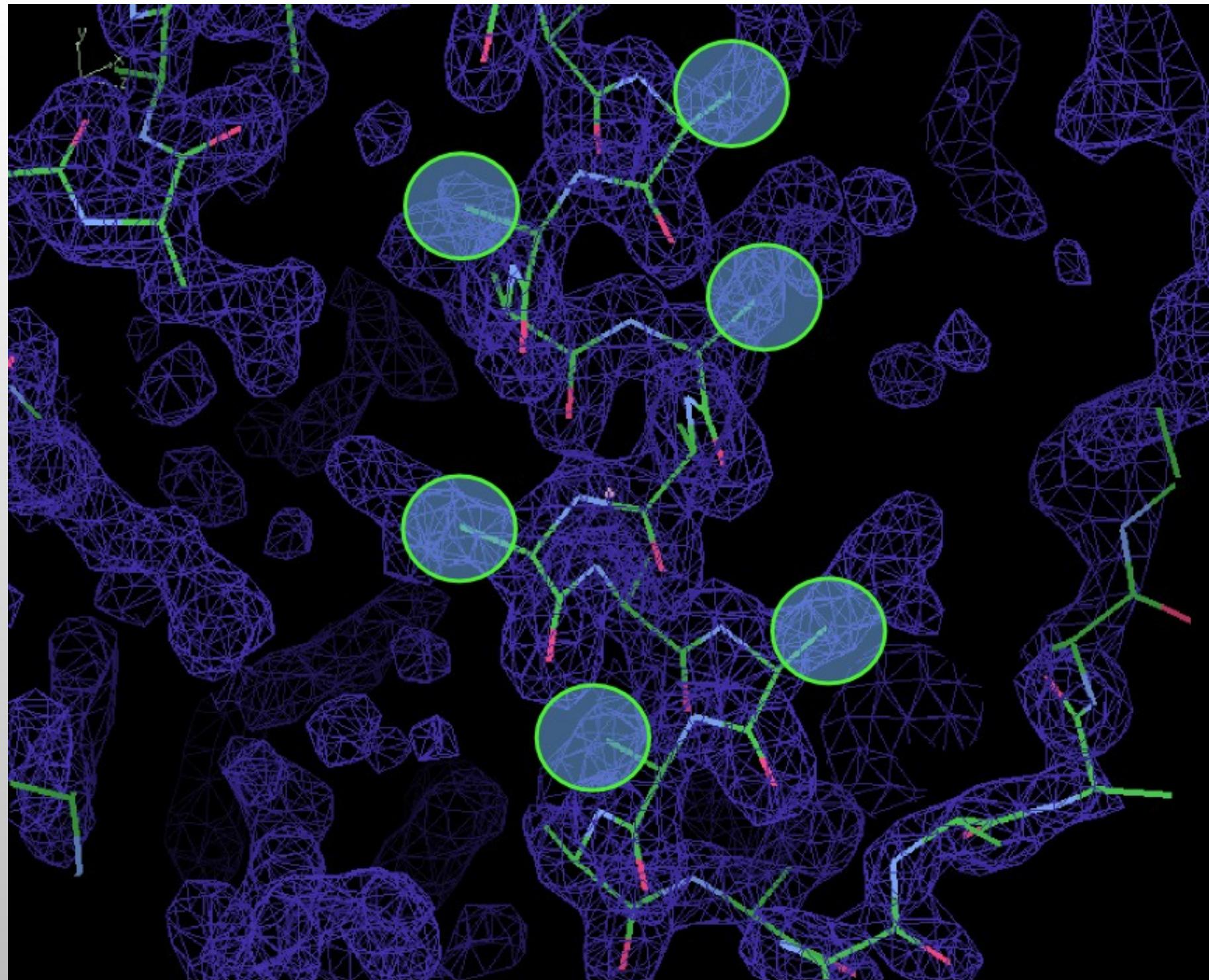
2XGJ: Mtr4, Weir et al. (2010)

Secondary structure building

Alpha Helix Placement

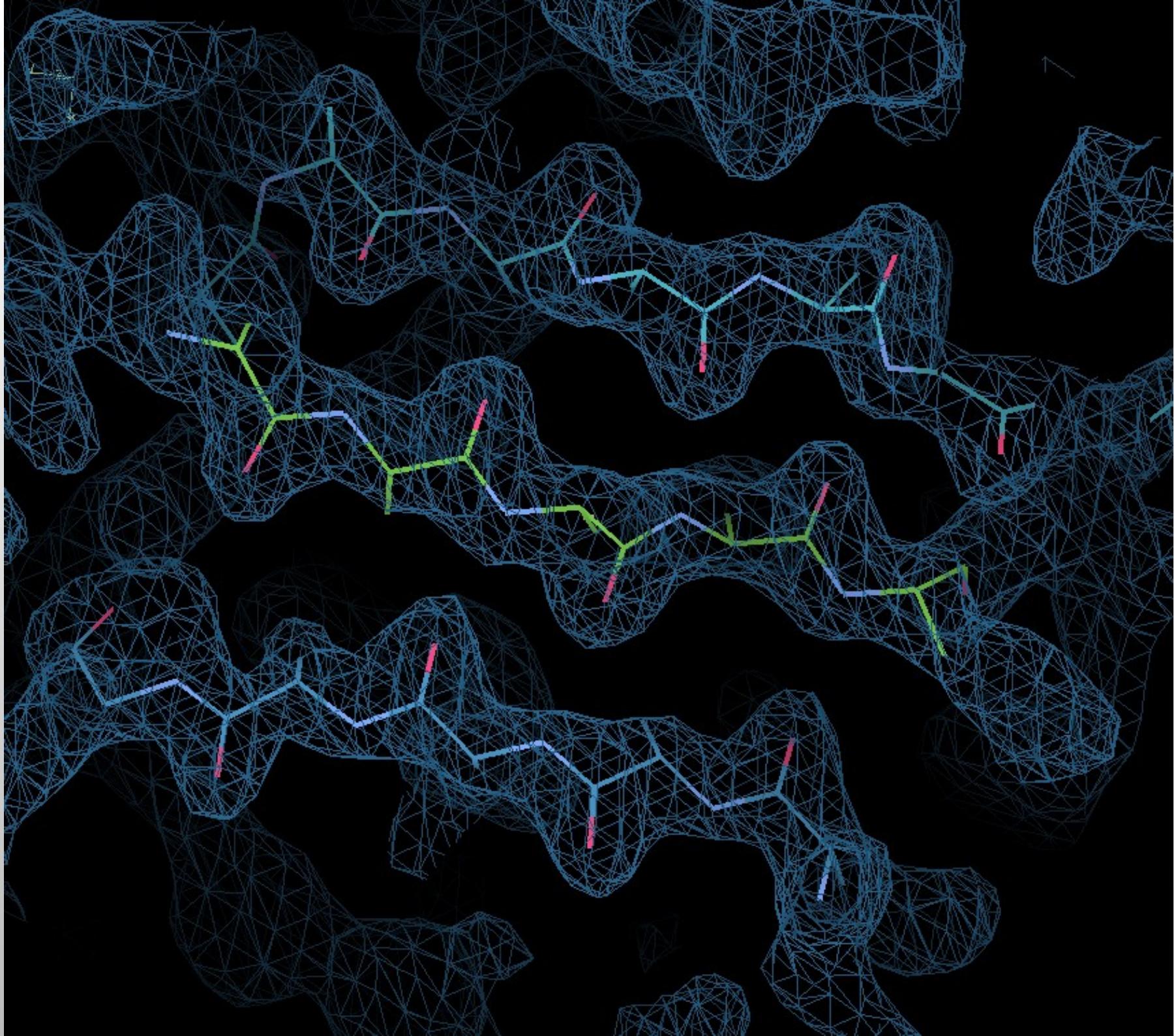
- Scenario: Looking at a new map, not built with automatic tools:
 - “I can see that there’s a helix here - build it for me!”
- From a given point:
 - Move to local averaged maximum
 - Do a 2D MR-style orientation search on a cylinder of electron density
 - Build a helix (both directions)
 - 1D Rotation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



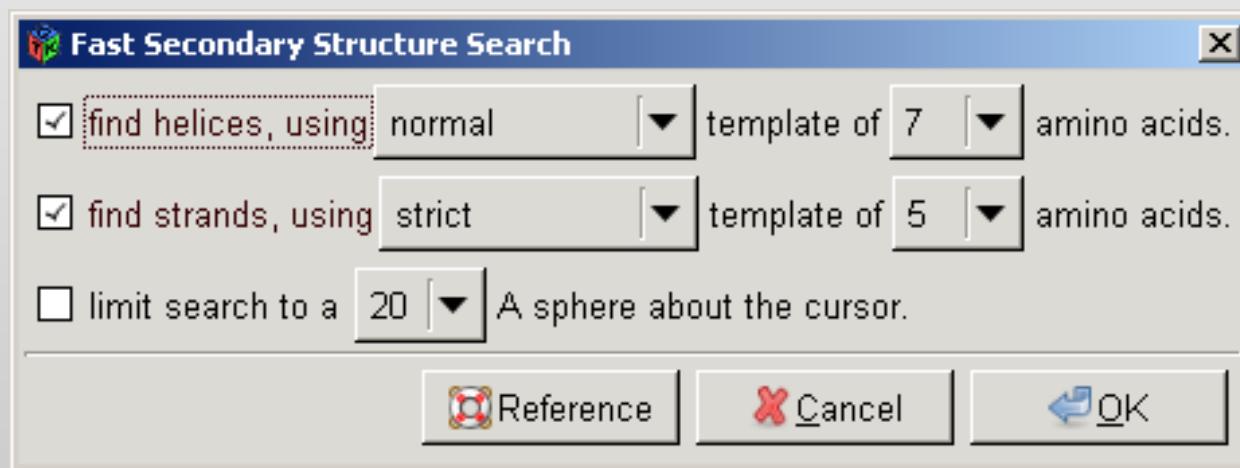


Strand Placement

- Similar but 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

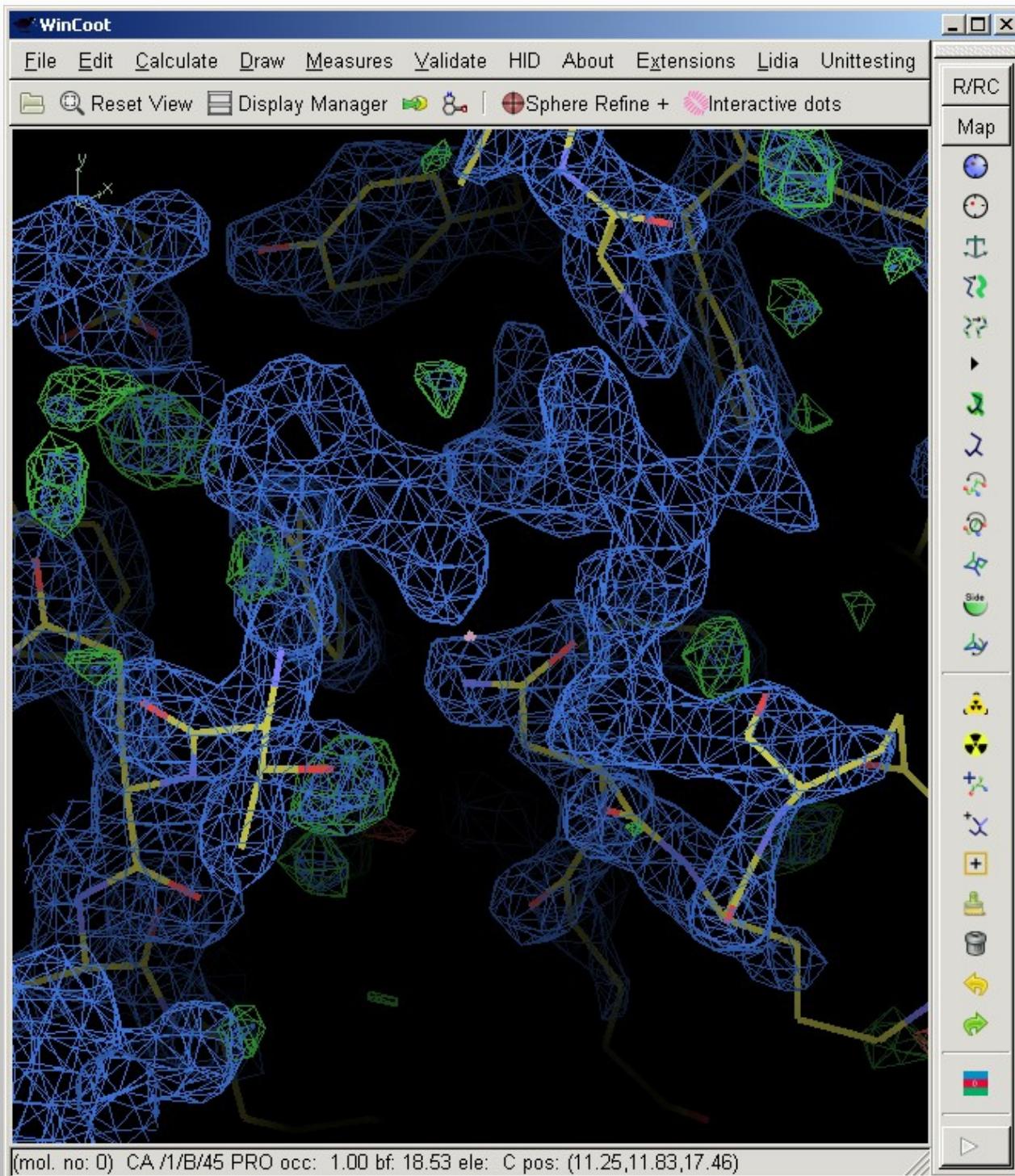


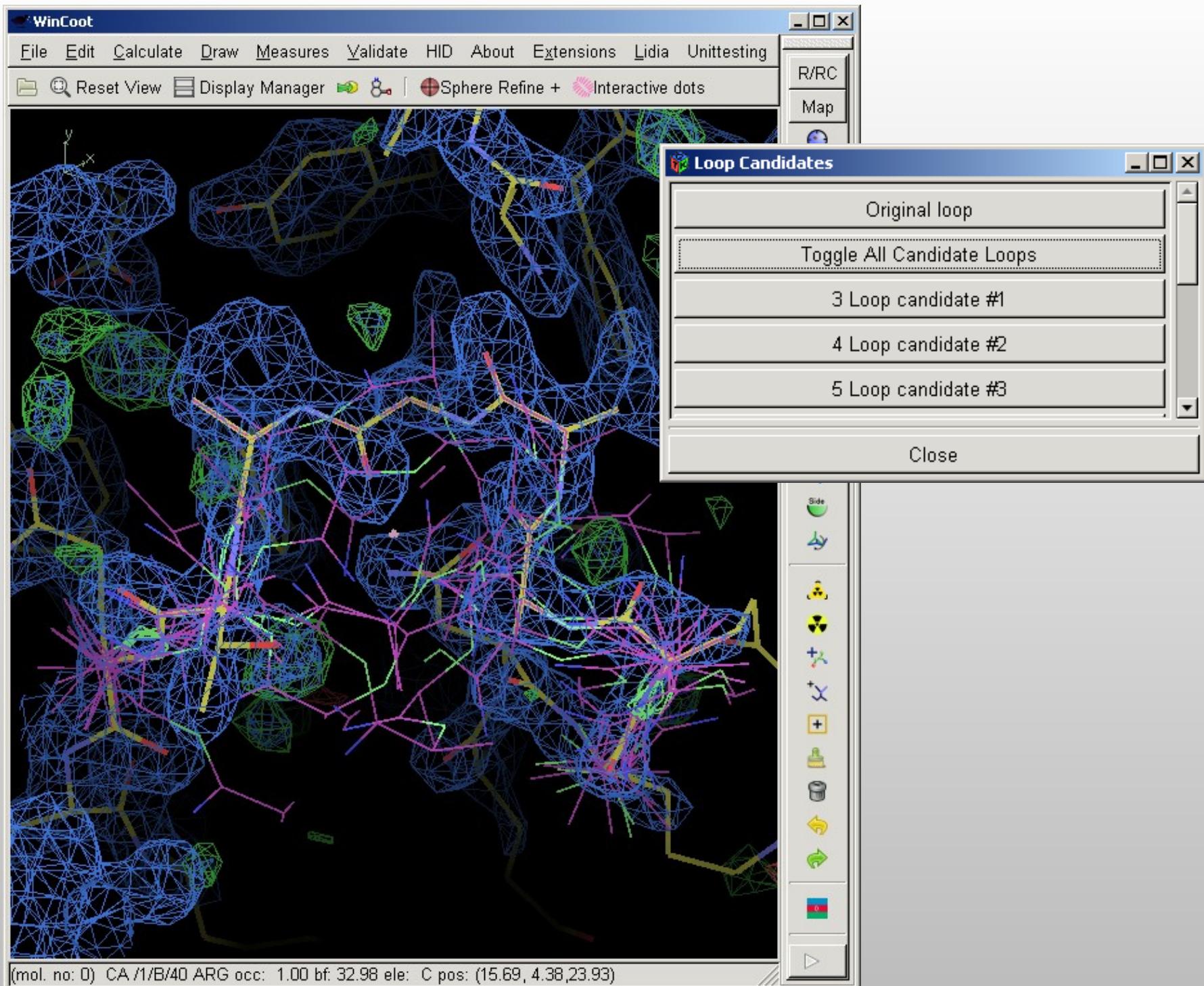
Automated Fast Secondary Structure Search



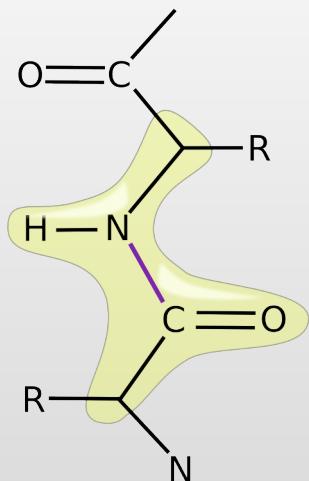
Loop fitting

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

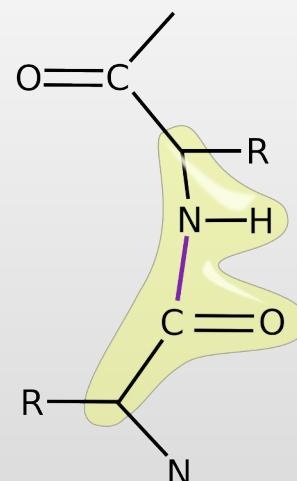




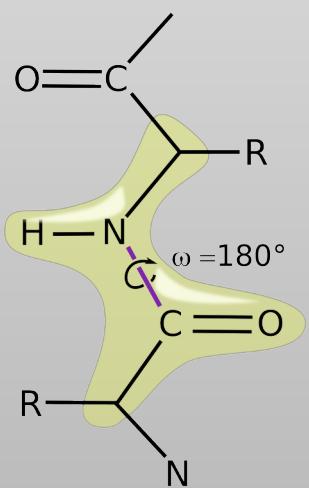
cis-Peptides



trans-peptide
with plane restraints



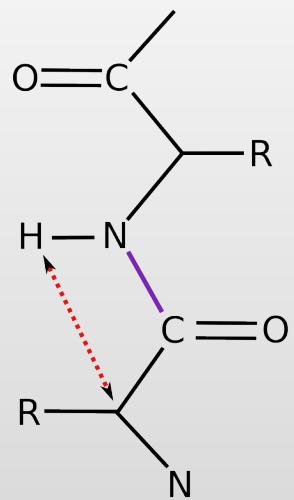
cis-peptide
with plane restraints



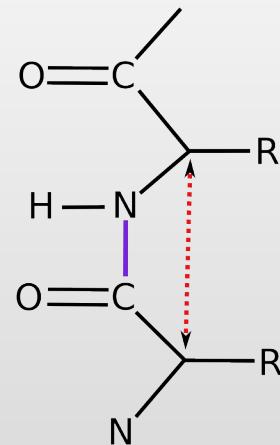
trans-peptide
with plane and trans restraints

Now replaced by trans-peptide
pseudo-bond centre distance
restraints

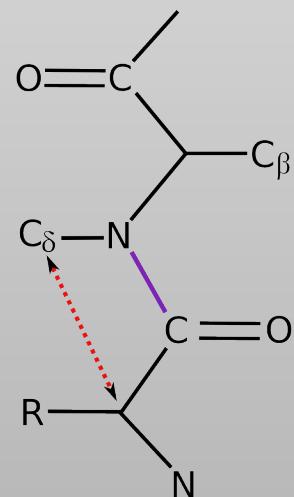
cis-Peptides



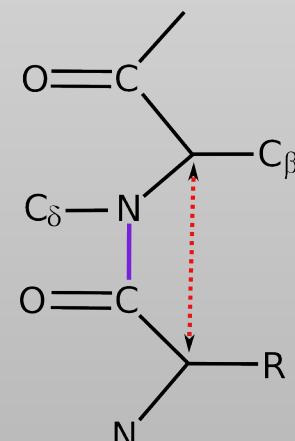
trans-peptide



cis-peptide

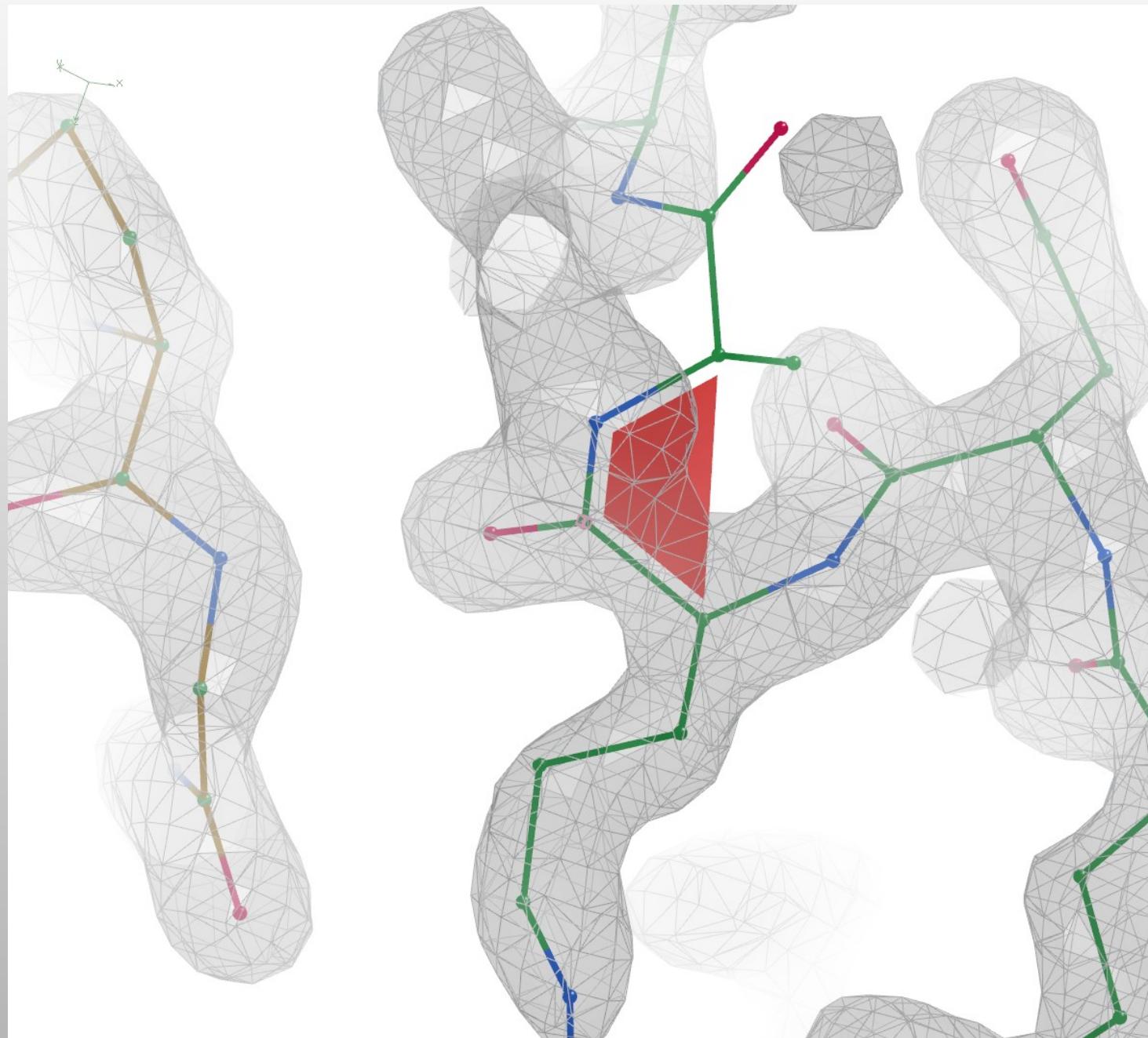


PRO *trans-peptide*



PRO *cis-peptide*

cis-peptide Representation



Pre-PRO



Twisted-trans



Non-pre-PRO



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

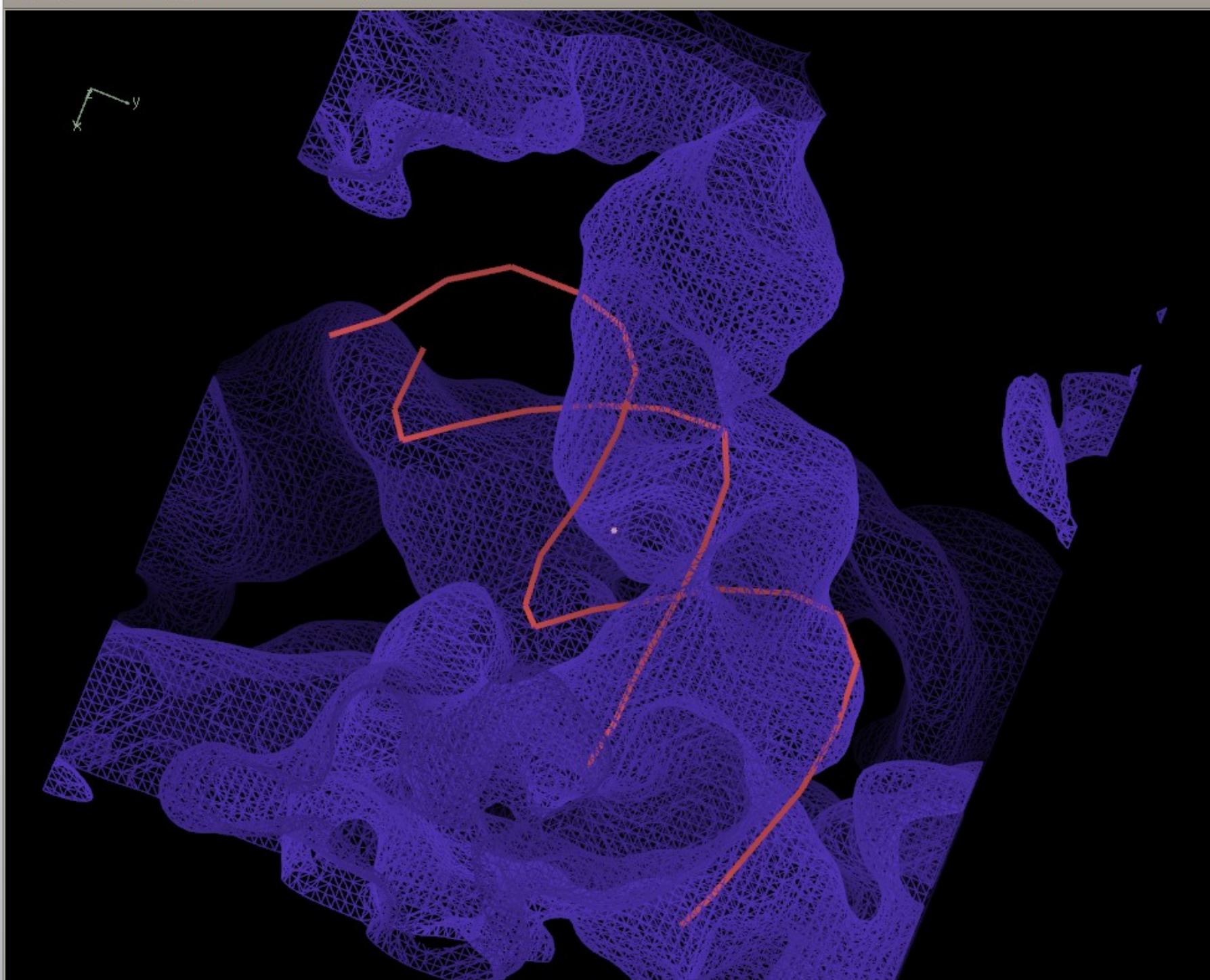
Jiggle Fit: How it Works

- 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

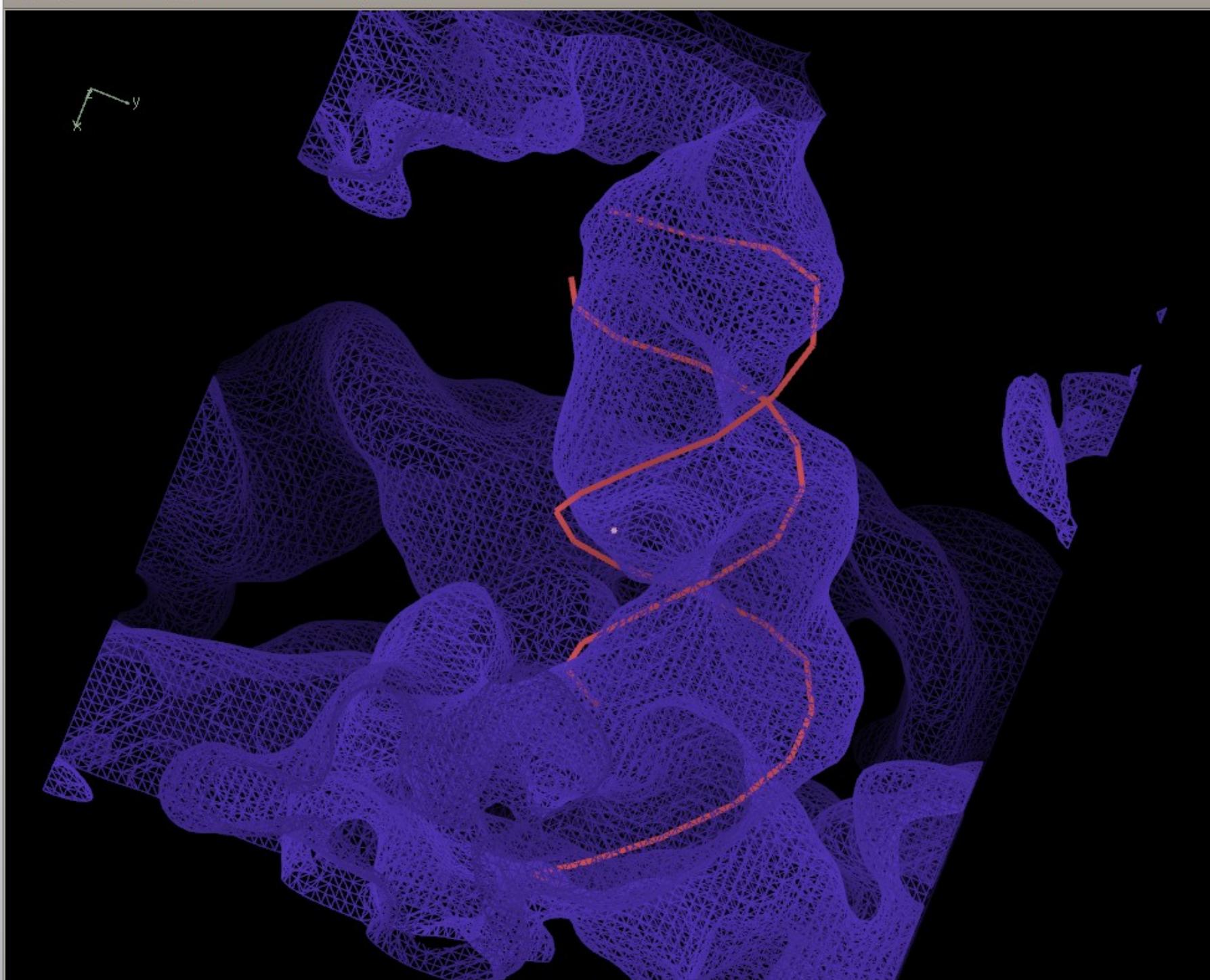
Reset View Display Manager Full screen Sphere Refine



Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

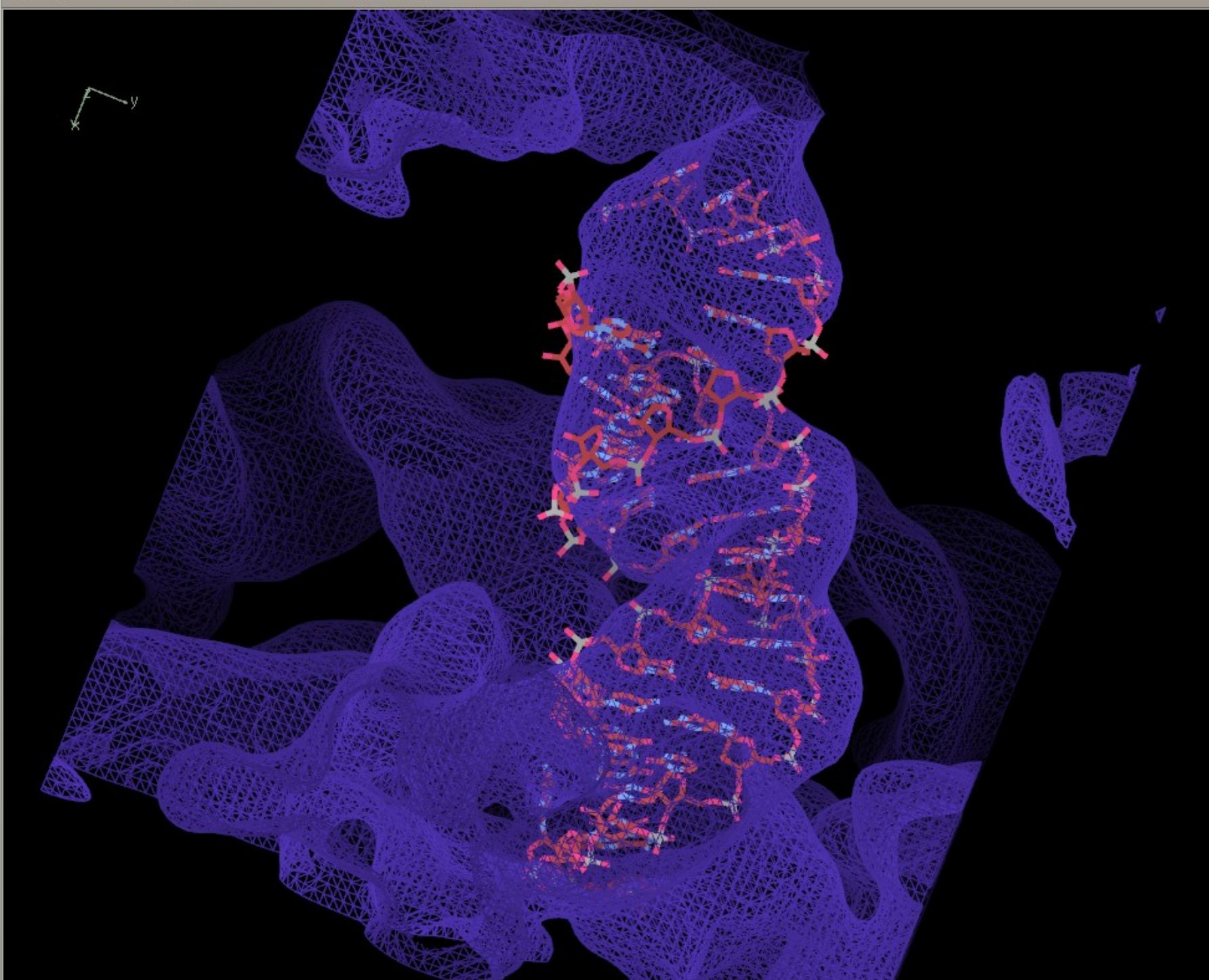
Reset View Display Manager Full screen Sphere Refine



Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

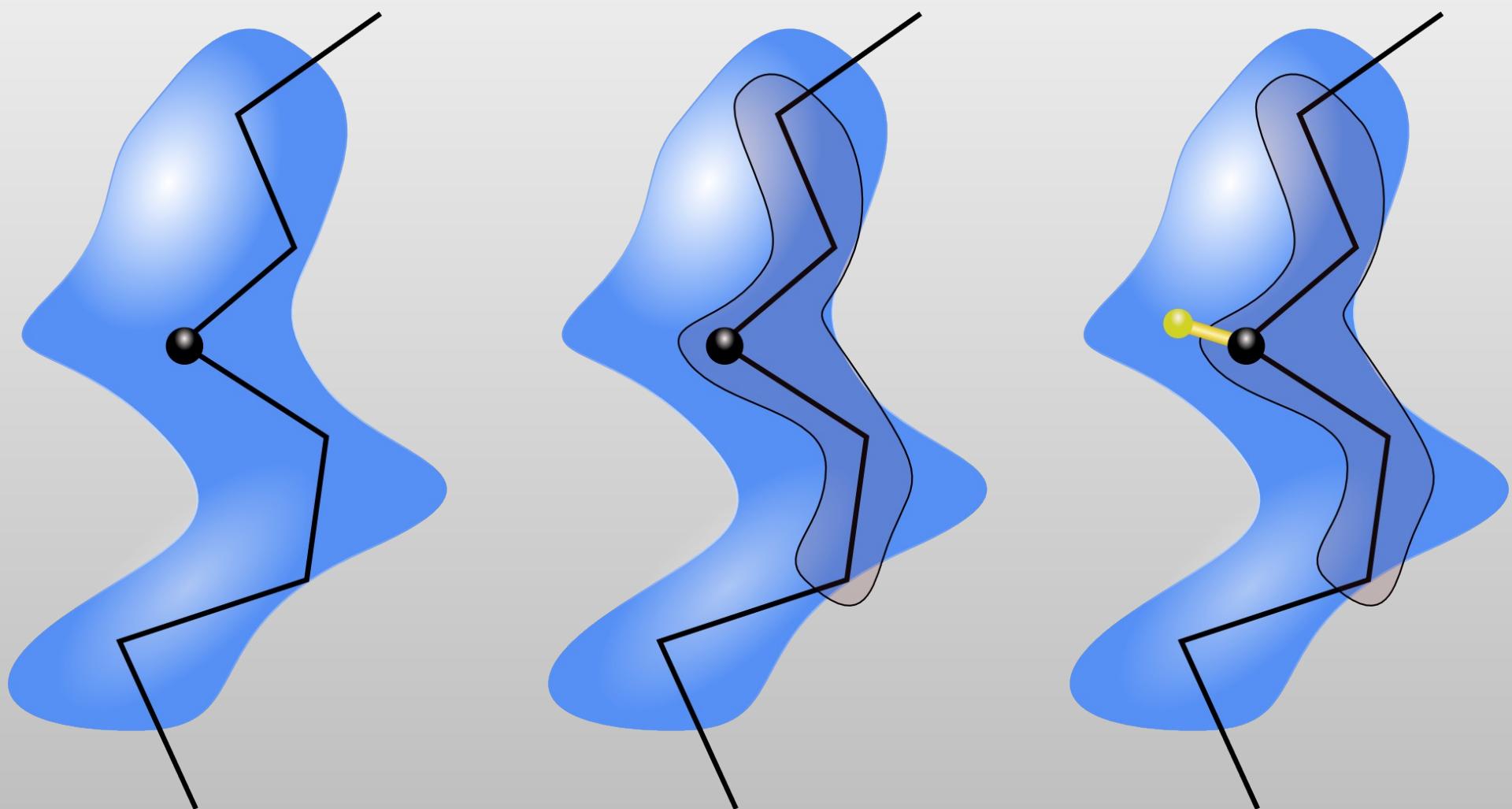
Reset View Display Manager Full screen Sphere Refine



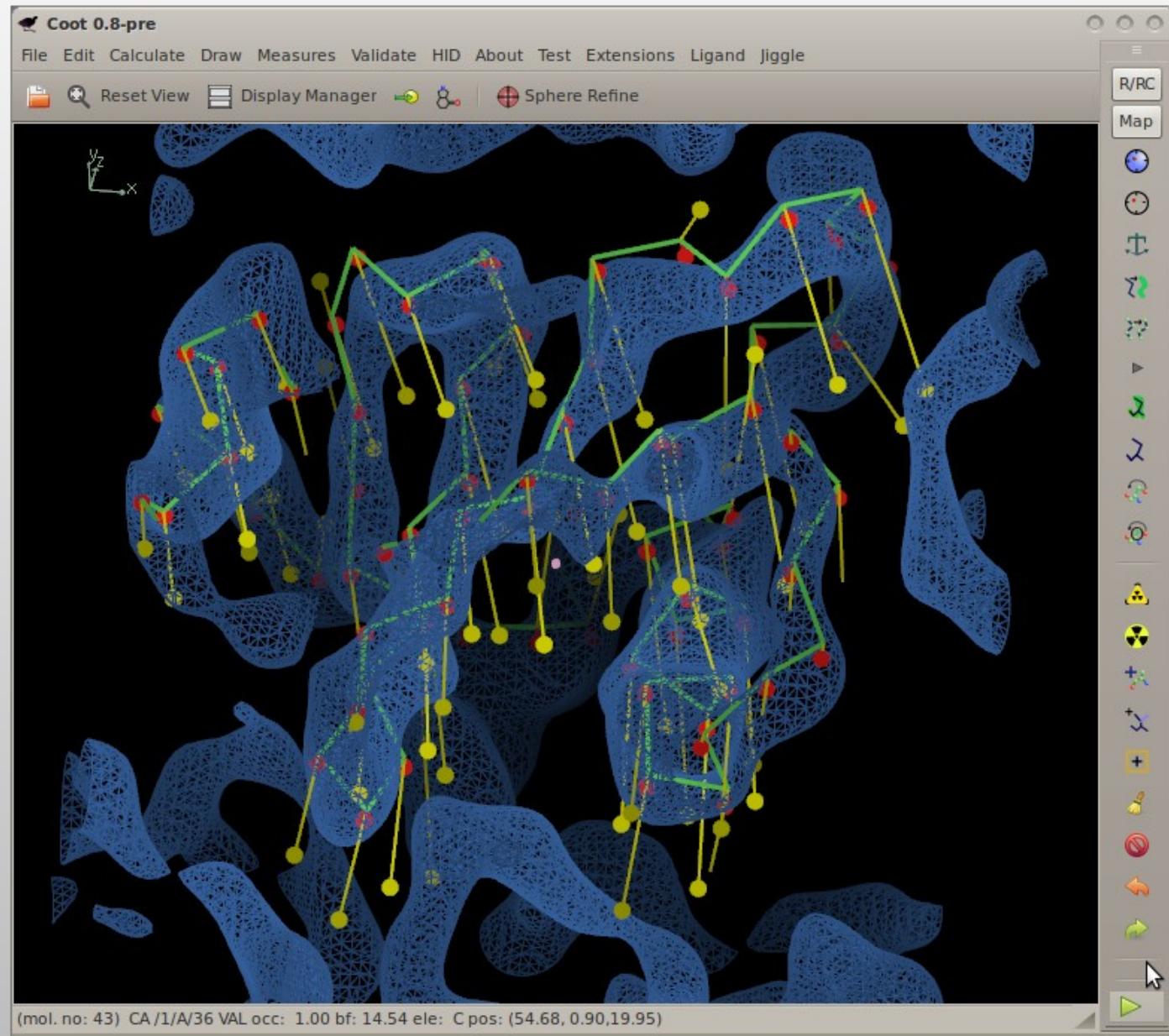
Model Morphing: How it Works

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

Model Morphing: Generating the Raw RTs



Model Morphing: Example



Model Morphing: Robust Averaging

- What are the residues in the environment of a residue?
 - What are their RTs?
 - Create a metric 'distance', sort on that
 - Discard the top and bottom 20%
 - Use remaining RTs to generate average
 - ...which is then applied to central residue
- Repeat for all residues
- Larger environment radii make the shifts smaller/more conservative
 - More cycles needed

Coot 0.8-pre (revision 4826)

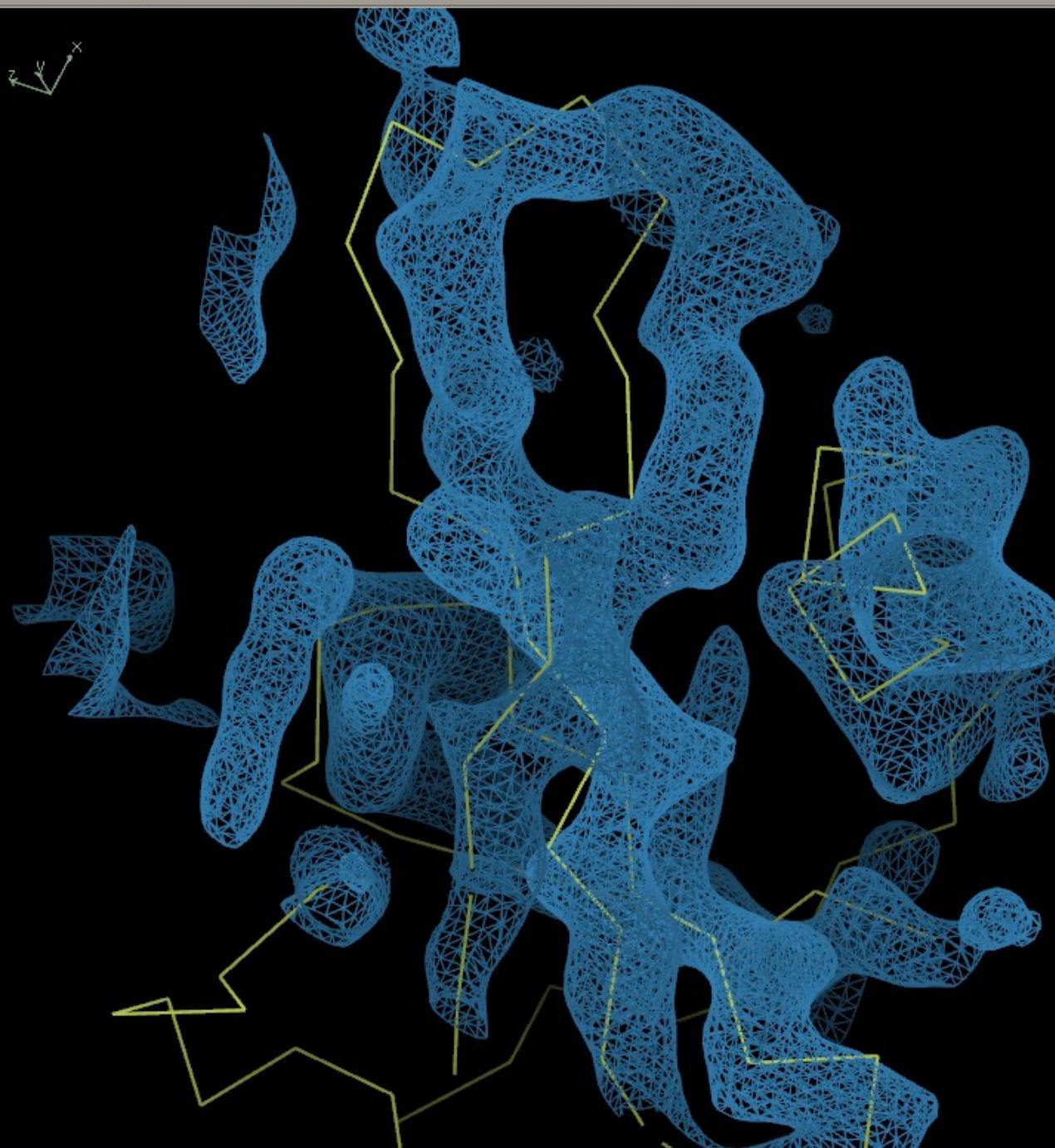
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

Reset View Display Manager Full screen Sphere Refine



R/RC

Map



Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

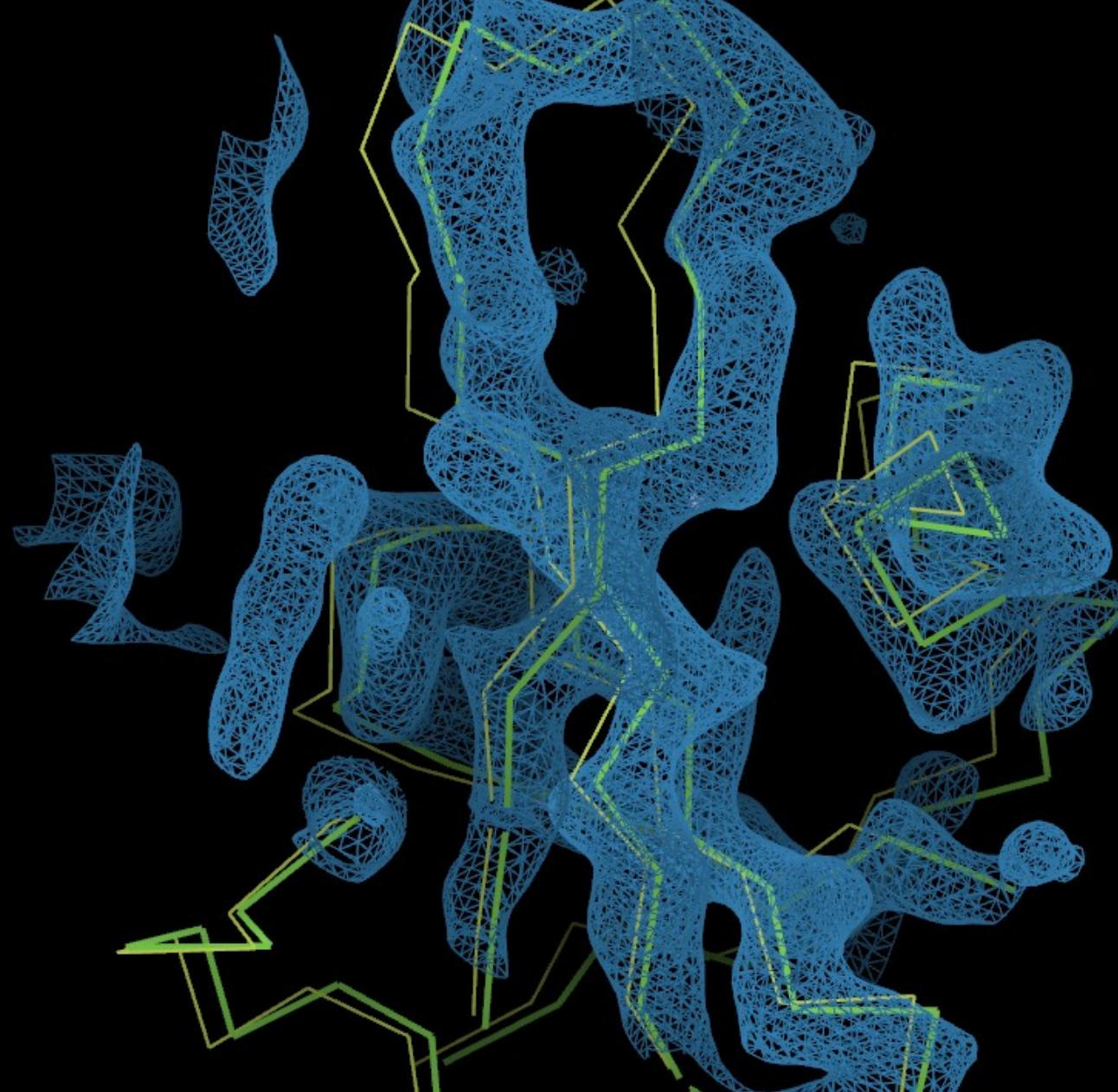
Reset View Display Manager

Full screen

Sphere Refine

R/RC

Map

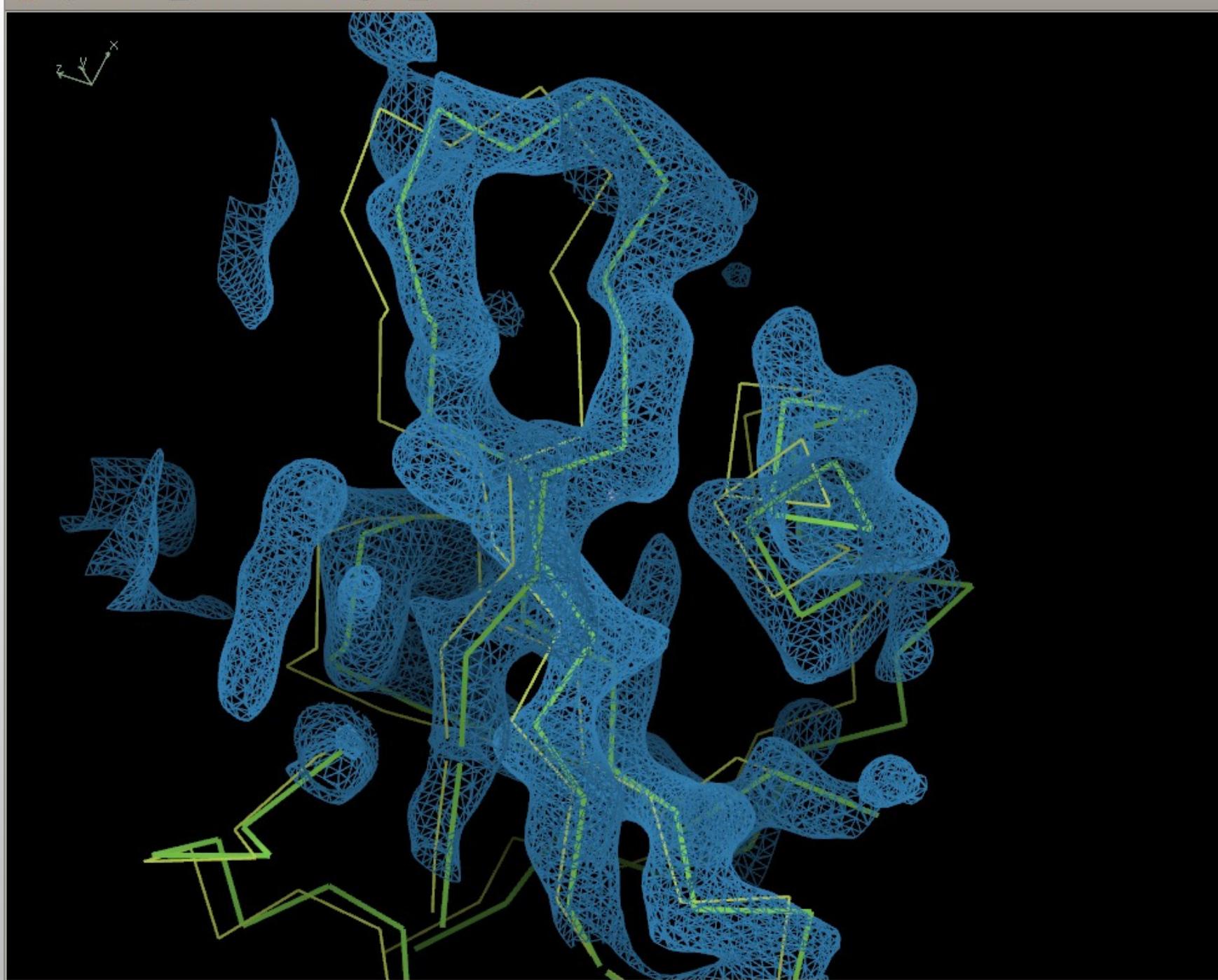


Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

Reset View Display Manager Full screen Sphere Refine

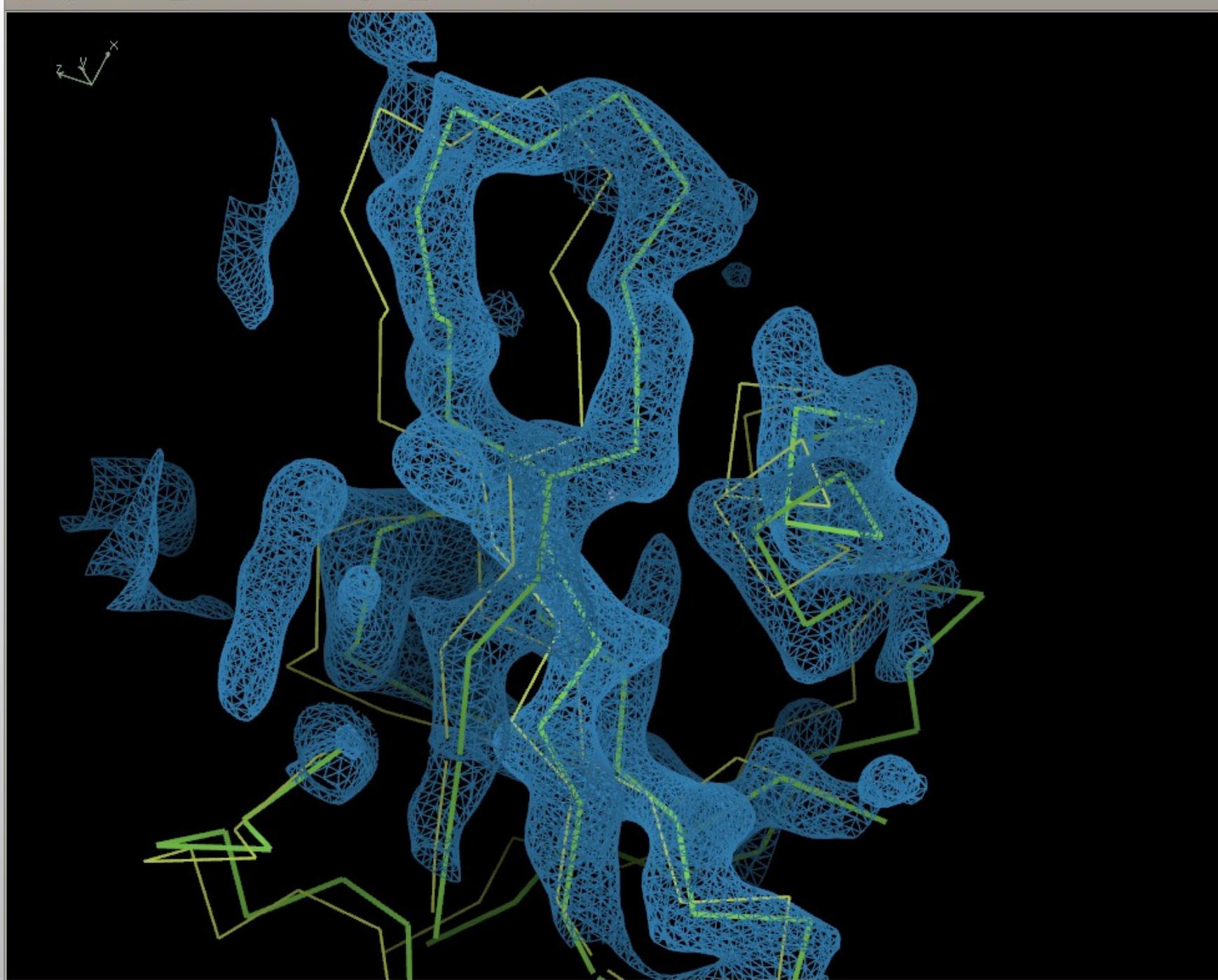


Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

Reset View Display Manager Full screen Sphere Refine



Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Coot 0.8-pre (revision 4826)

File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph

Reset View Display Manager



Full screen

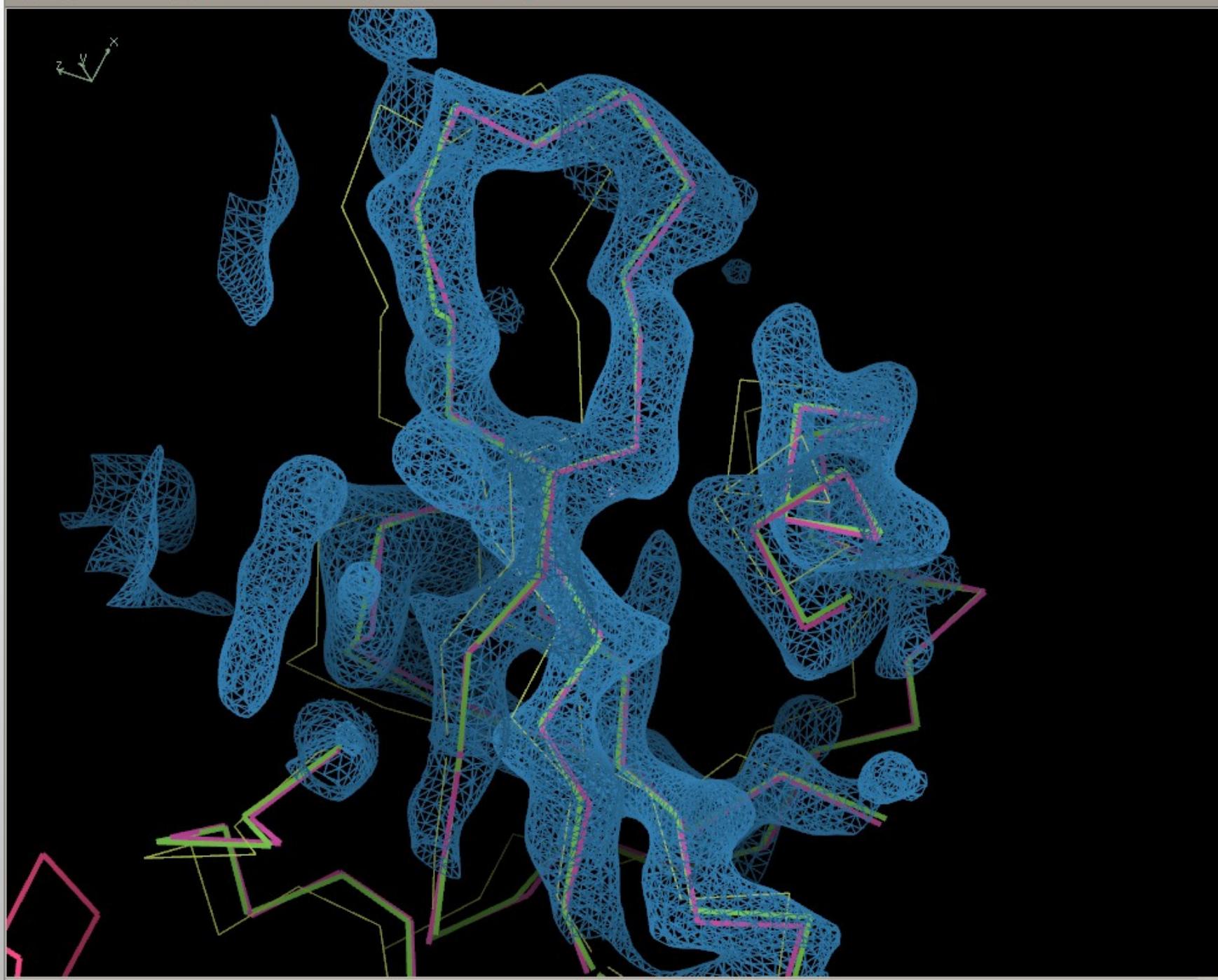


Sphere Refine



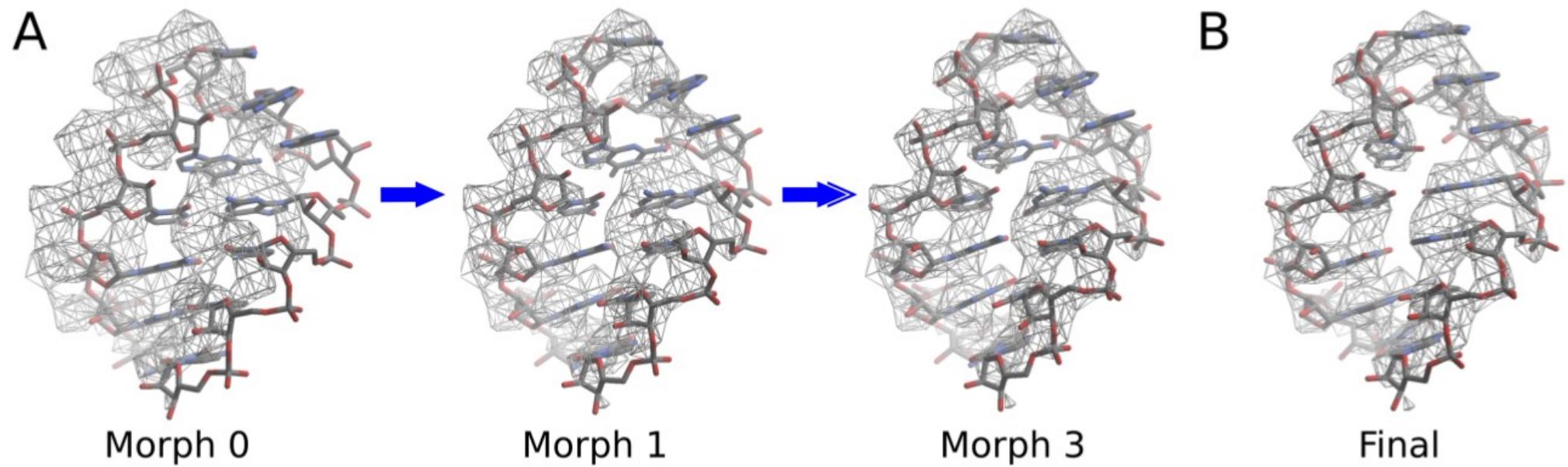
R/RC

Map



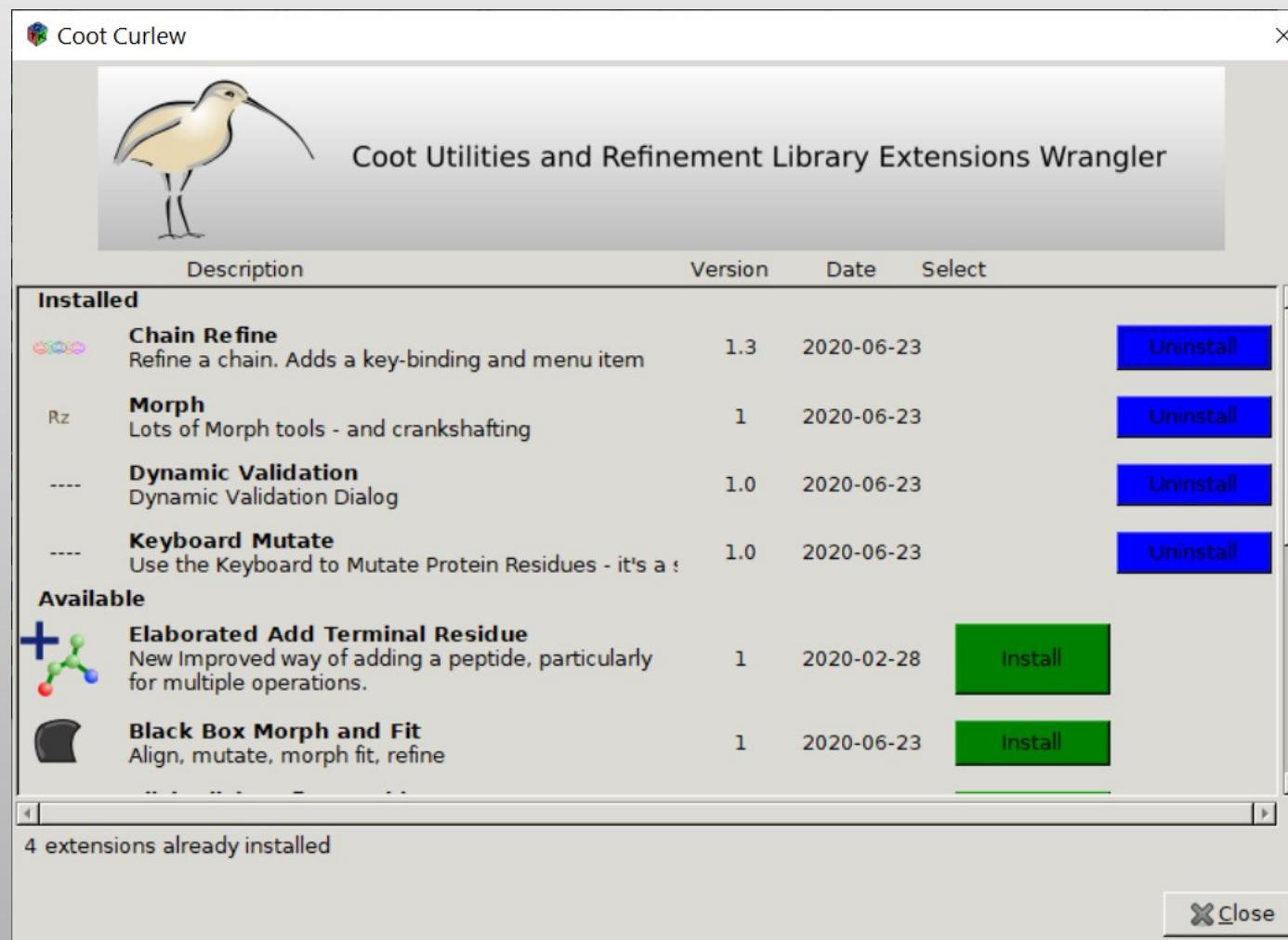
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. Molecule number 2 created.

Model Morphing



CURLEW: Coot Utilities and Refinement Library Extention Wranger

- Easy access to "interesting" Coot scripts



A Few Tools More...

- More restraints
 - ProSMART
 - User defined
 - Planes, DNA (libg) etc.
- Carbohydrate-fitting
 - N-linked glycosylation
- Use of NCS
 - Copy chains
- Scripting

Ligands in Coot

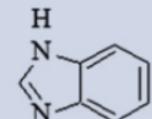
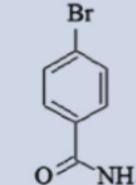
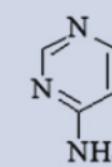
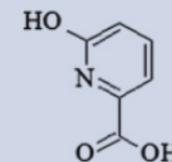
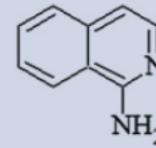
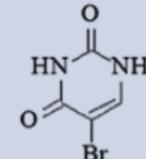
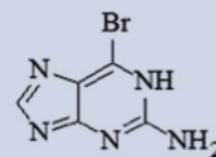
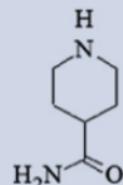
- Ligand fitting
- Importing and building ligand from scratch
 - AceDRG, PRODRG, pyrogen, LIBCHECK
- Validation
- Representation
 - Surfaces
- Analysis
 - Molprobity, LIDIA

Ligand building

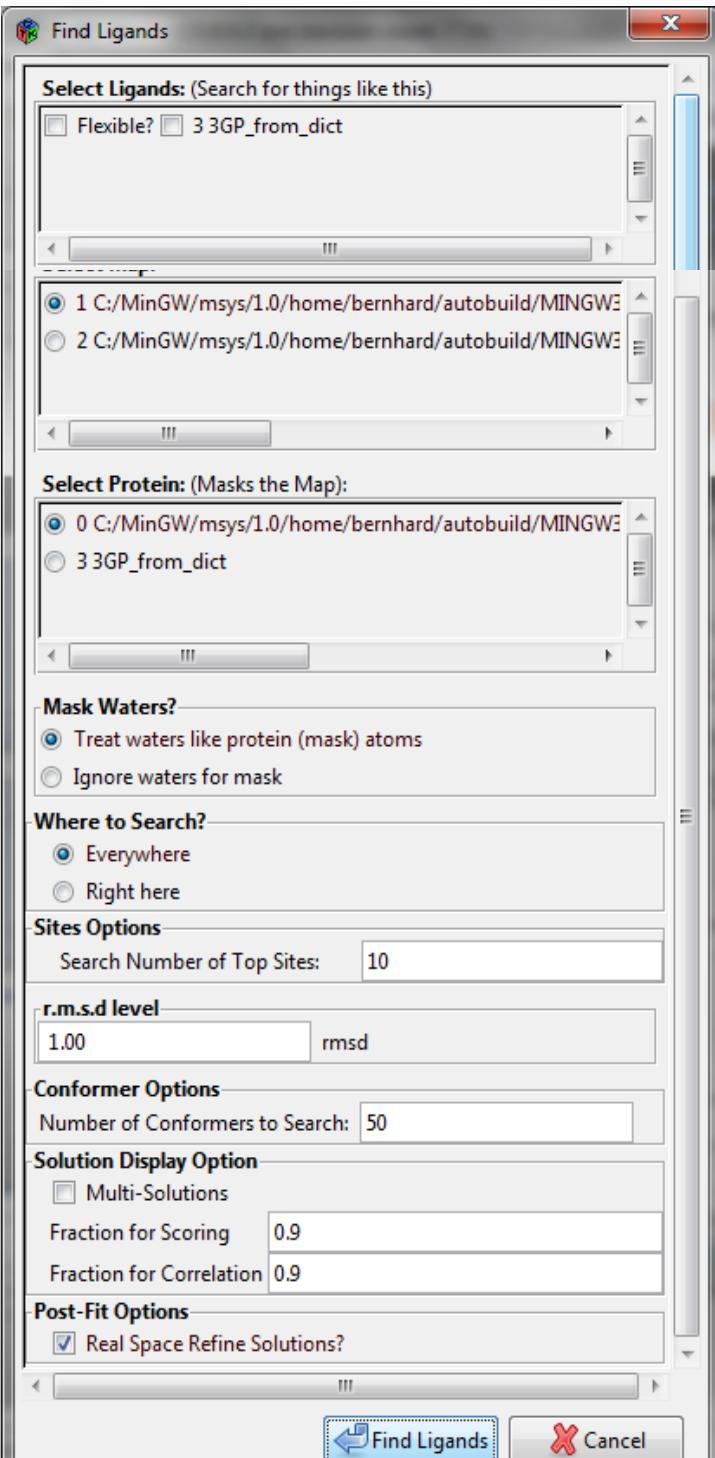
Ligand Site

		Known	Unknown
Ligand Type	Known		
	Cocktail		
	Unknown		

Cocktail Examples



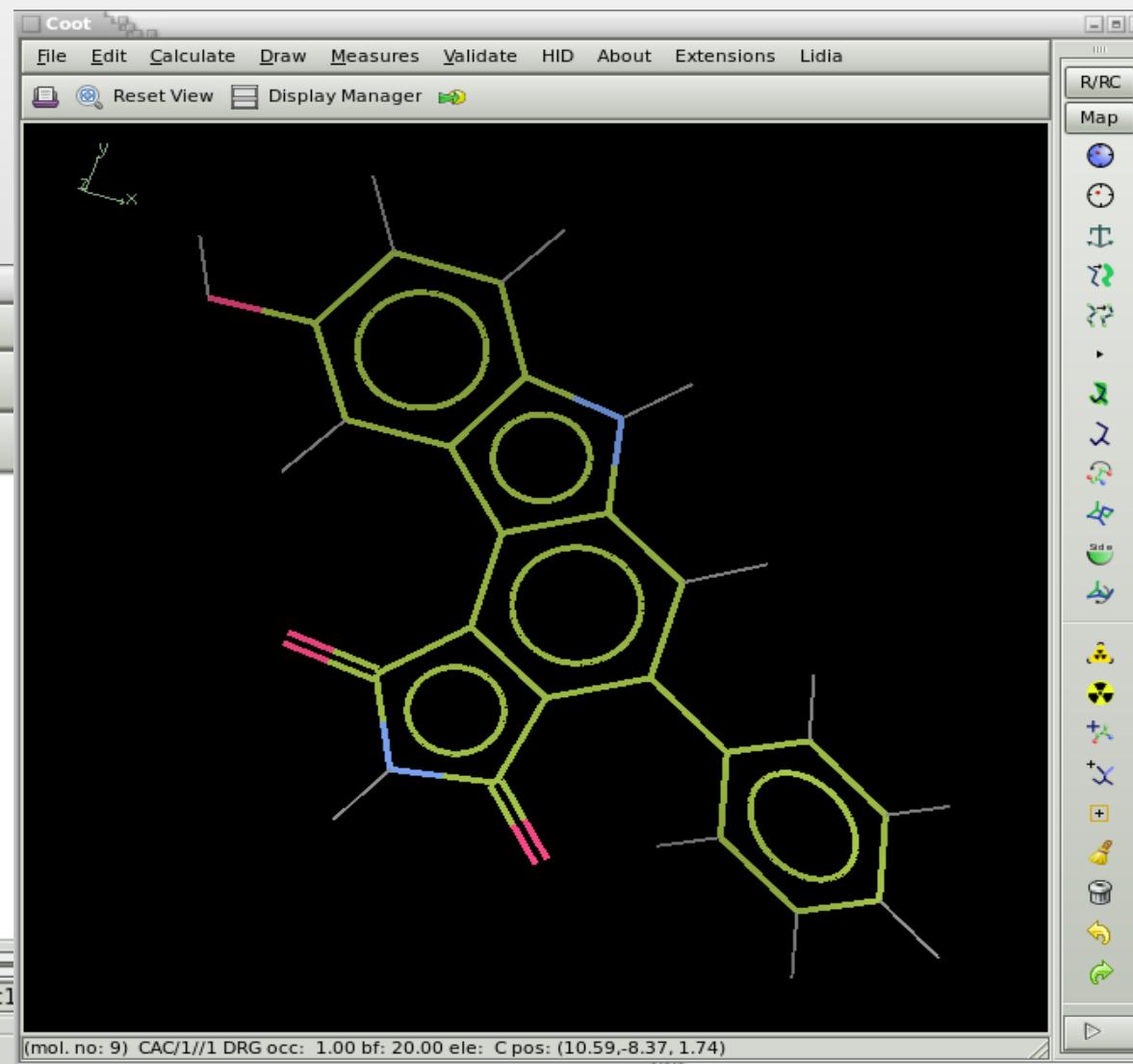
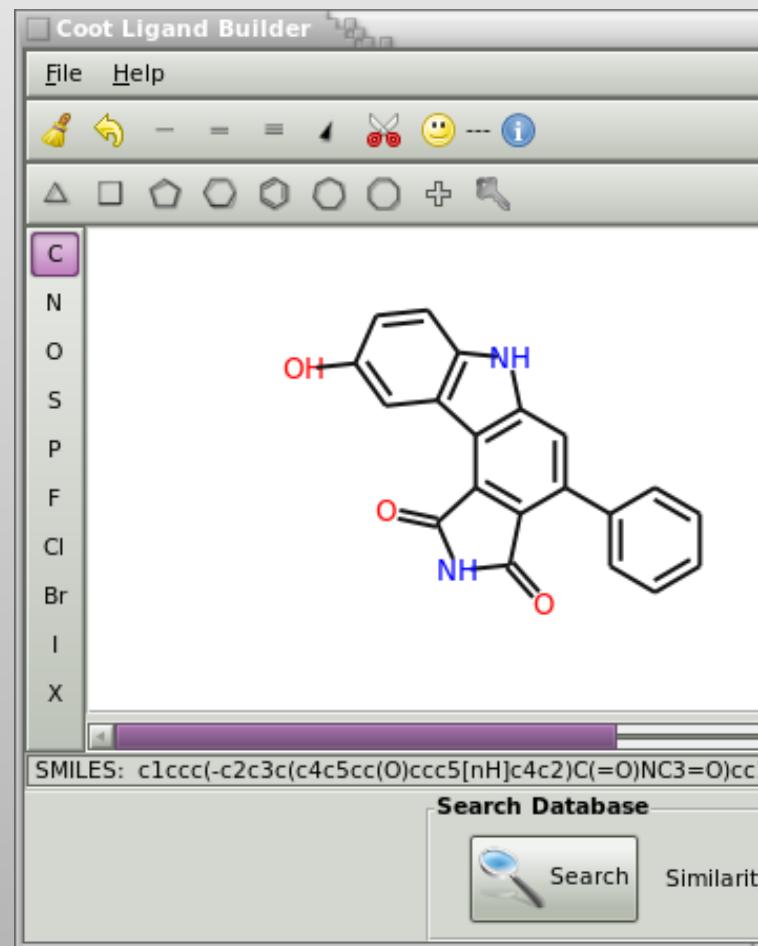
Ligand search in Coot



- Which ligand (flexibility?)
- Which map
- What to do with the protein (mask)
- Where to search
- How many sites to find
 - Acceptance levels
- Map level
- No of conformers
- Real space refine

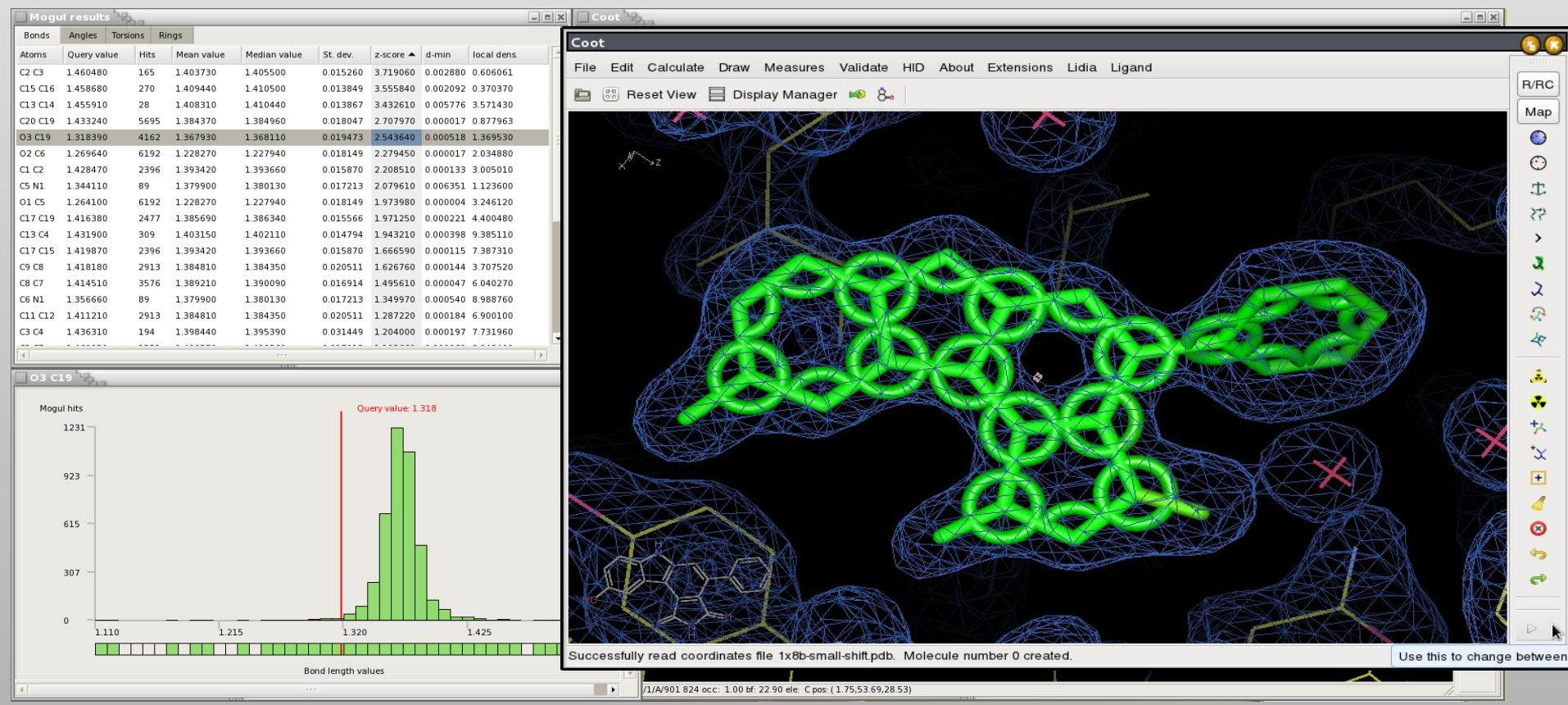
2D Ligand Builder

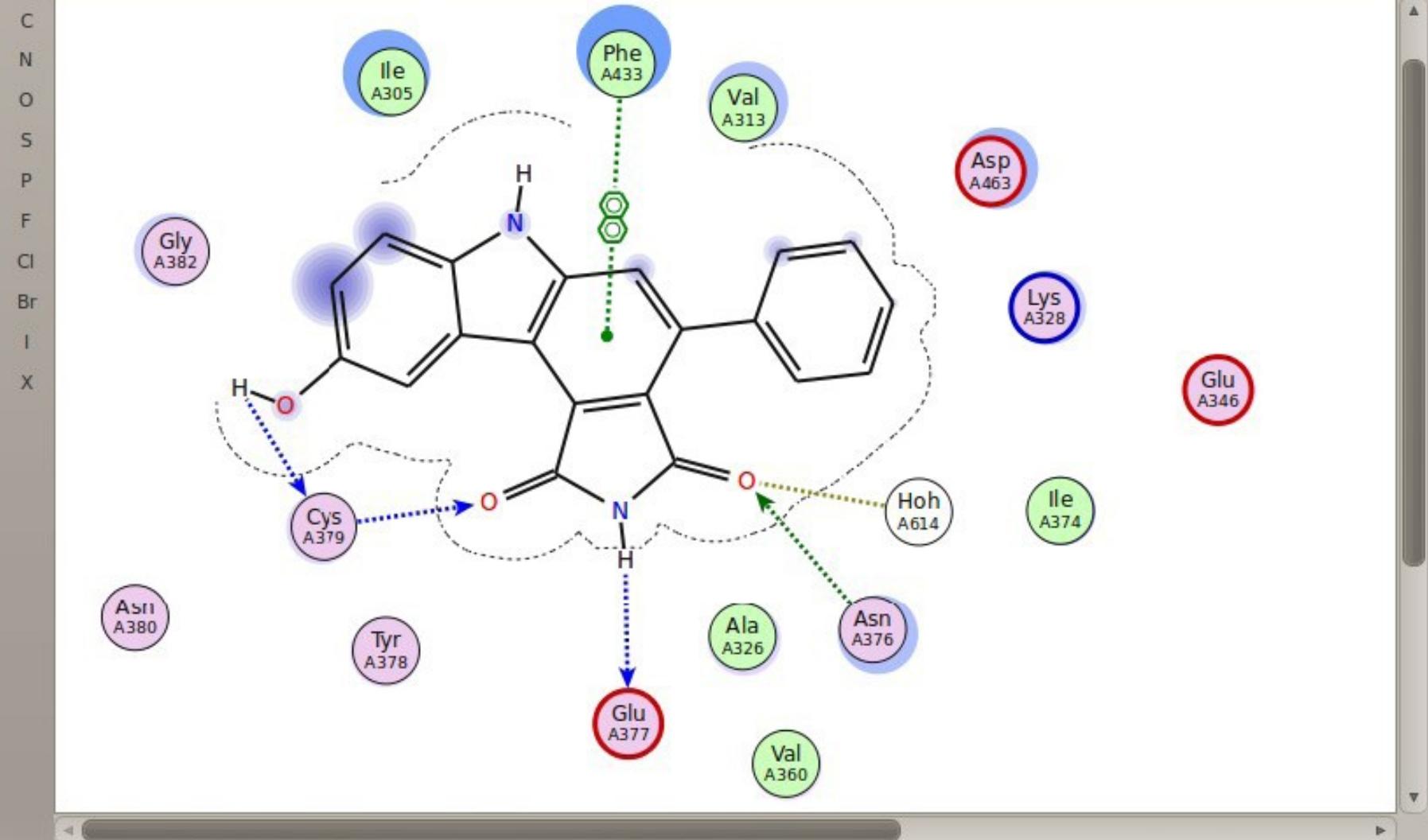
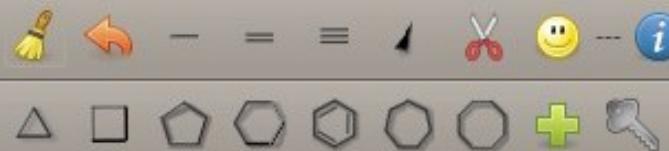
- Free sketch
- AceDRG (PRODRG)



Ligand Validation

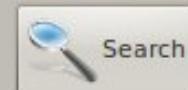
- Mogul plugin in Coot (distortions can be shown without)
 - Run mogul, graphical display of results
 - Update restraints (target and esds for bonds and angles)
 - Ligand distortion can be analysed without Mogul





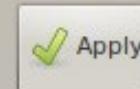
Interactive:
Mouse over interacting residues =>
Highlighted in structure

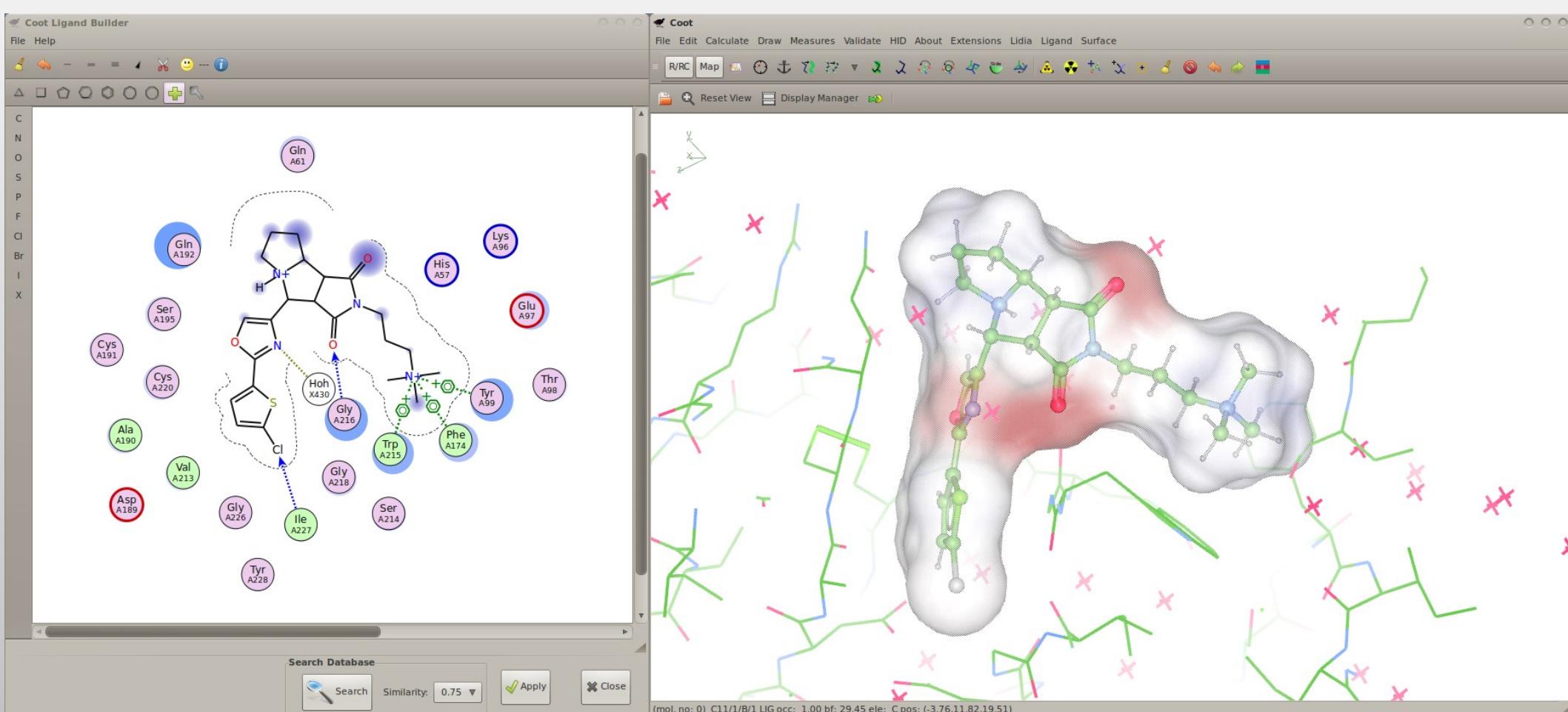
Search Database



Search

Similarity: 0.75 ▾





A bit of validation and blobology

Validation of model only

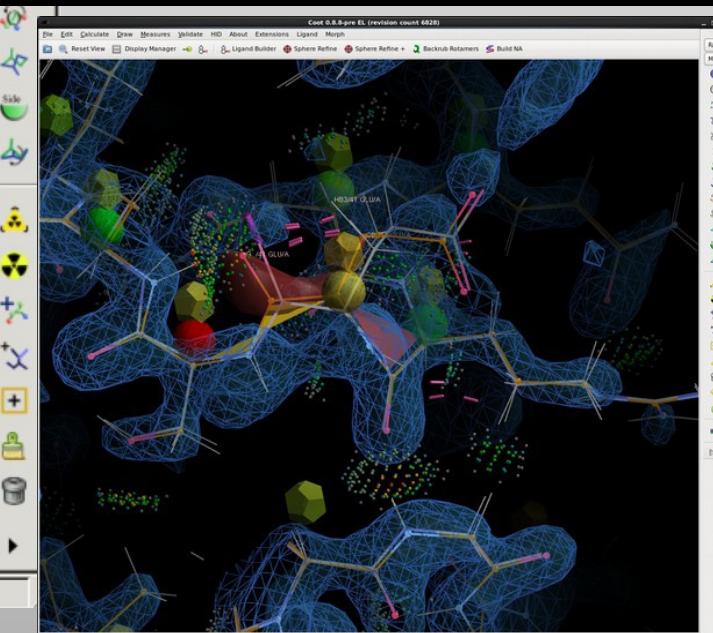
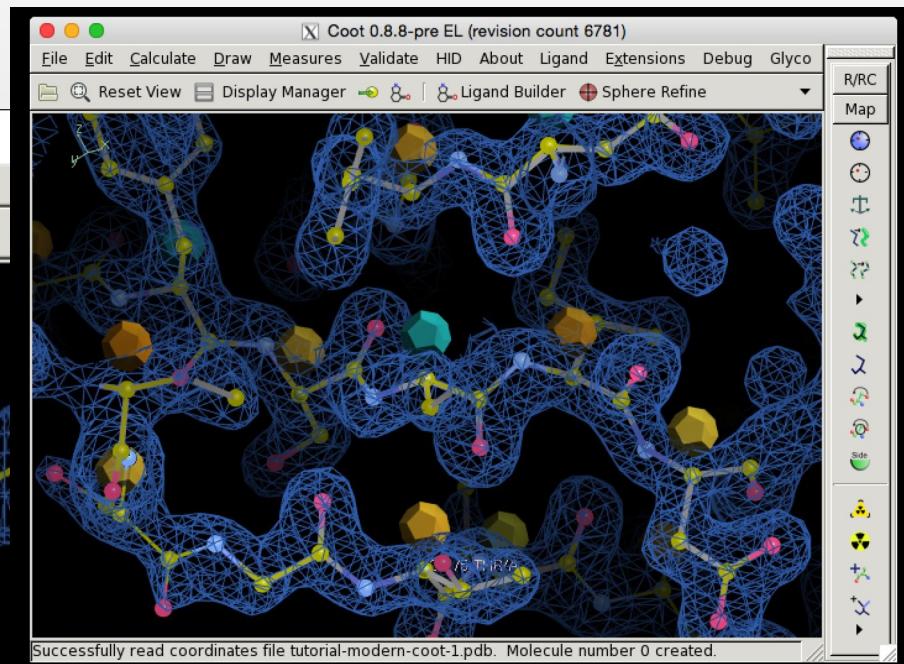
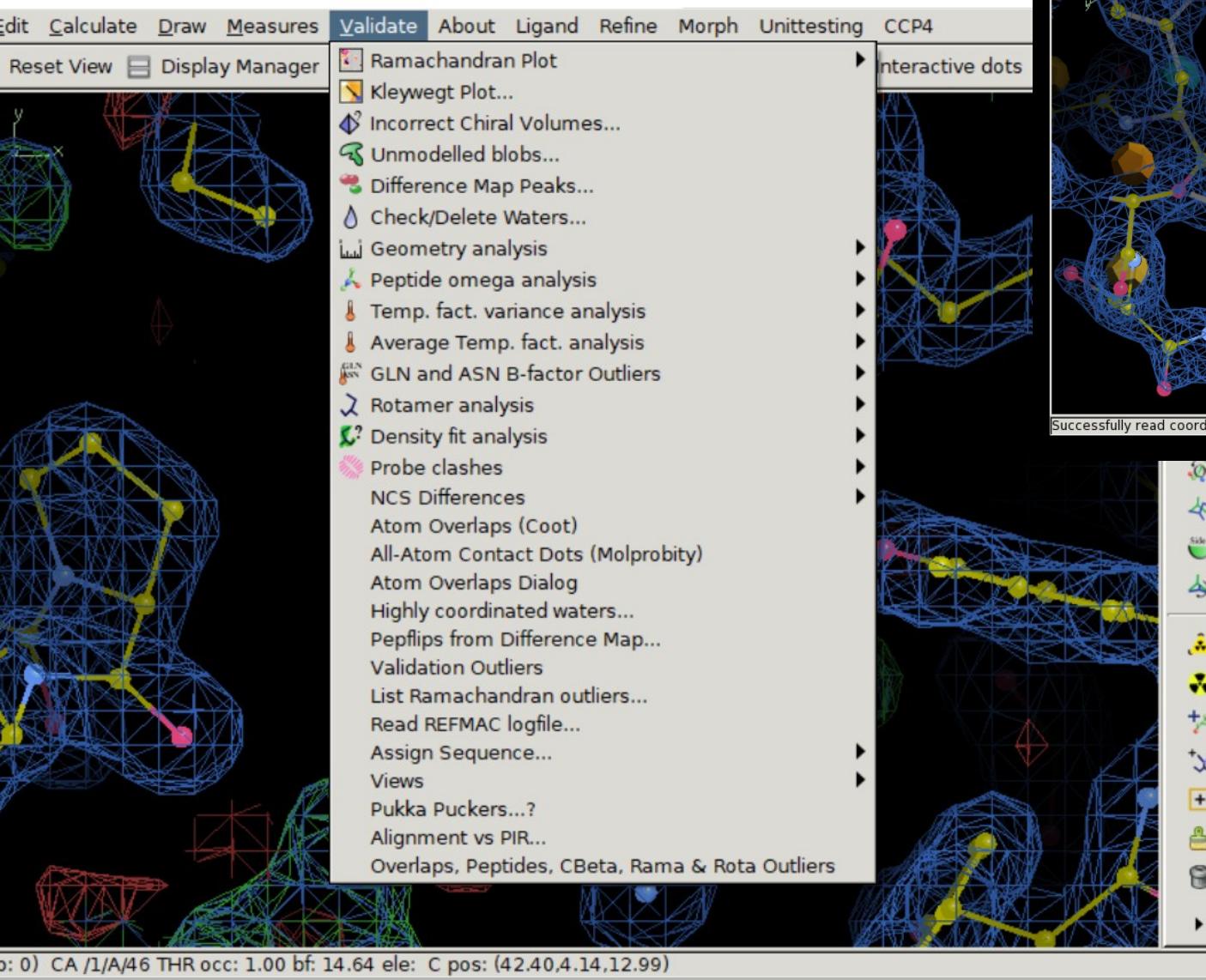
- Ramachandran Plot
 - Kleywegt Plot (NCS differences)
- Geometry Analysis
- Peptide ω Analysis
- Temperature Factor Analysis
- Rotamer Analysis
- Clashes

Validation of model fit to density

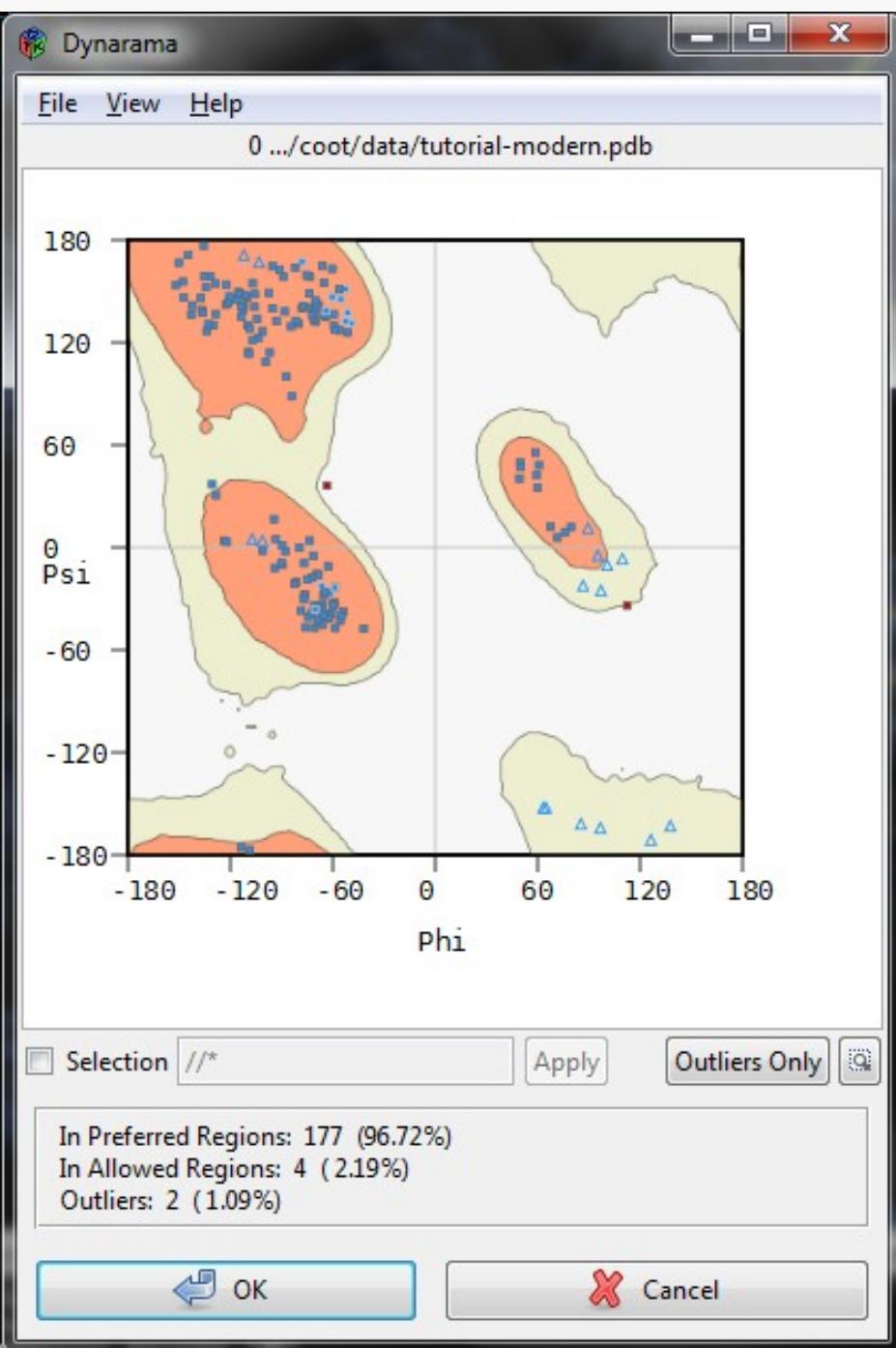
- Density Fit Analysis
- Difference Map Peaks
 - Variance analysis at water positions
- Unmodelled blobs

Validation tools in Coot

Coot 0.9.4.1 EL



New Ramachandran plot

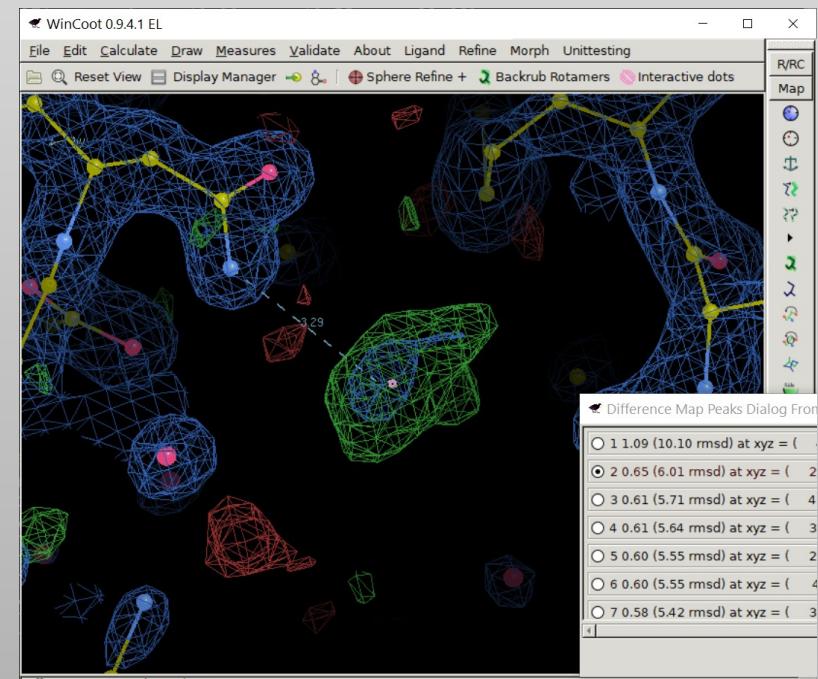
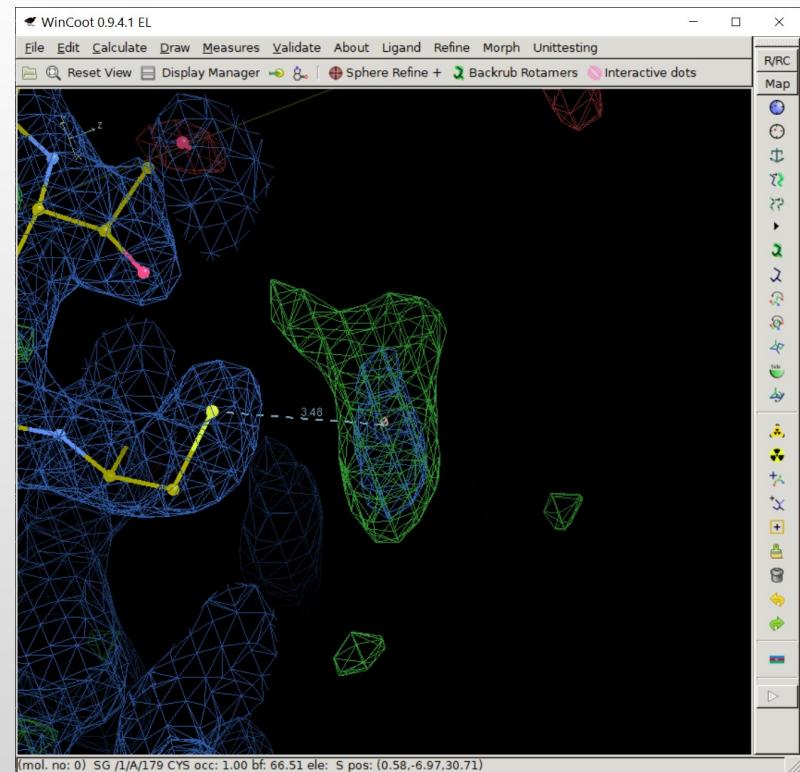


- Smooth outline
- Outliers only
- Selection only
- Save pdf
- Stand-alone
- Quick change to Kleywegt plot (incl. chains)



Blobology

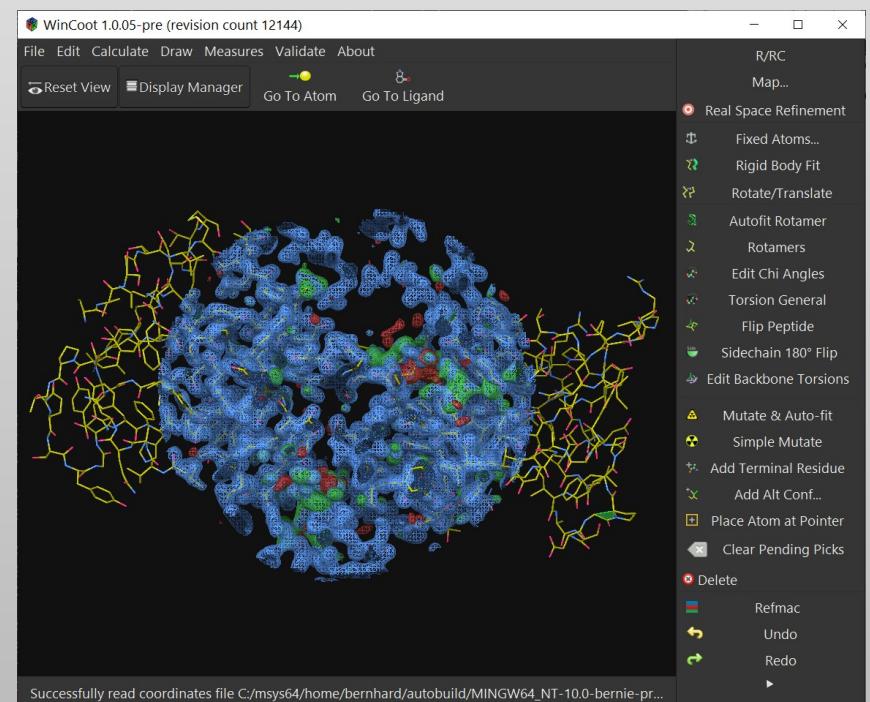
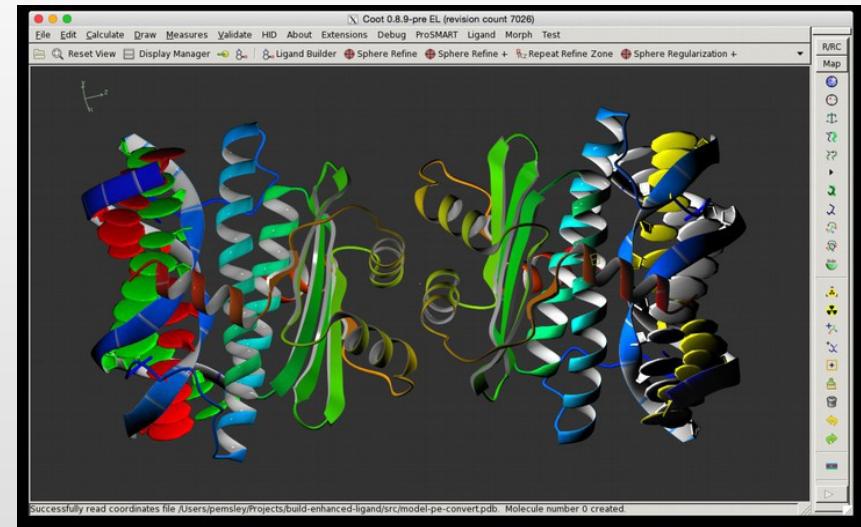
- What can be there?
 - Crystallisation solution
 - Protein buffer
 - Purification buffers
 - Medium (and metabolites)
- Can it be there? i.e. do we have sensible interactions
 - Distance? (use pointer or environment distance)
 - Correct chemistry?
 - Check symmetry
- Accept that not everything can be modelled but provide the best possible model



Coot Present, and Futures...

- Aim:
 - Slick, easy to use
 - Powerful
 - Smooth interface to external applications
- Under Development
 - Interesting things move quickly
 - There may be bugs

Python 3, GTK3 – Coot 1.0



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/>
- YouTube
 - Various tutorial
 - <https://www.youtube.com/c/PaulEmsley>

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

<https://github.com/bernhardcl/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
- Funding
 - BBSRC, CCP4, MRC, RÅC

Which map to use? Which contour level?

- Coot defaults:
 - Direct maps: 1.5 rmsd
 - Difference maps: 3 rmsd
- Adjust contour level with mouse wheel so that there is no noise!
 - Use solvent region for decision making
 - Often 1-1.2 rmsd is more appropriate
 - May be locally different

How do we get the atoms into the density?

- Which atoms (groups) are placed?
 - Single atoms
 - Amino acids/nucleic acid
 - Secondary structure elements
 - Backbone (baton)
- How to place?
 - Manual move
 - Computational fit (refinement)
 - Both, interactive
 - All atoms are “blue”, no distinction between the “heavy” atoms (unless atomic resolution)

Side chain density what to do if there isn't any?

- Lower the contour level, maybe something shows up

- Possible “solutions”

- 1) Mutate to Ala:

- Doesn't reflect the truth

- PDB will complain that there is a sequence mismatch

- 2) Stub the residue (i.e. remove all atoms beyond C β)

- Again, not reality

- PDB will complain about missing atoms

- 3) Set occupancies to 0 (or low value)

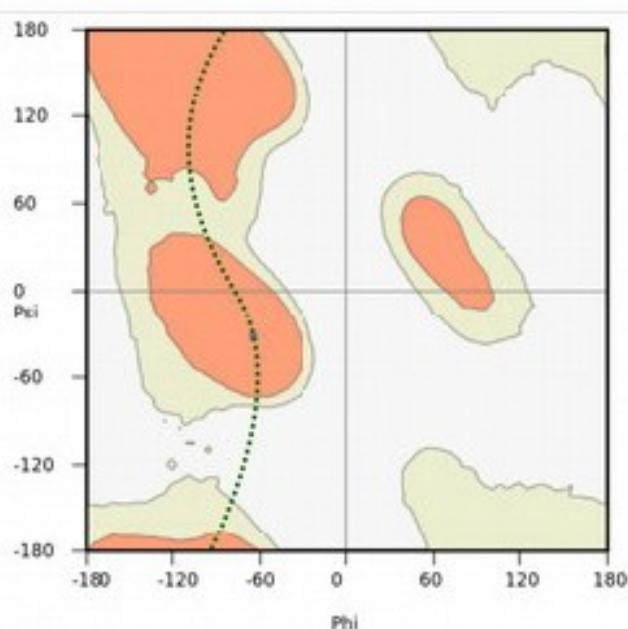
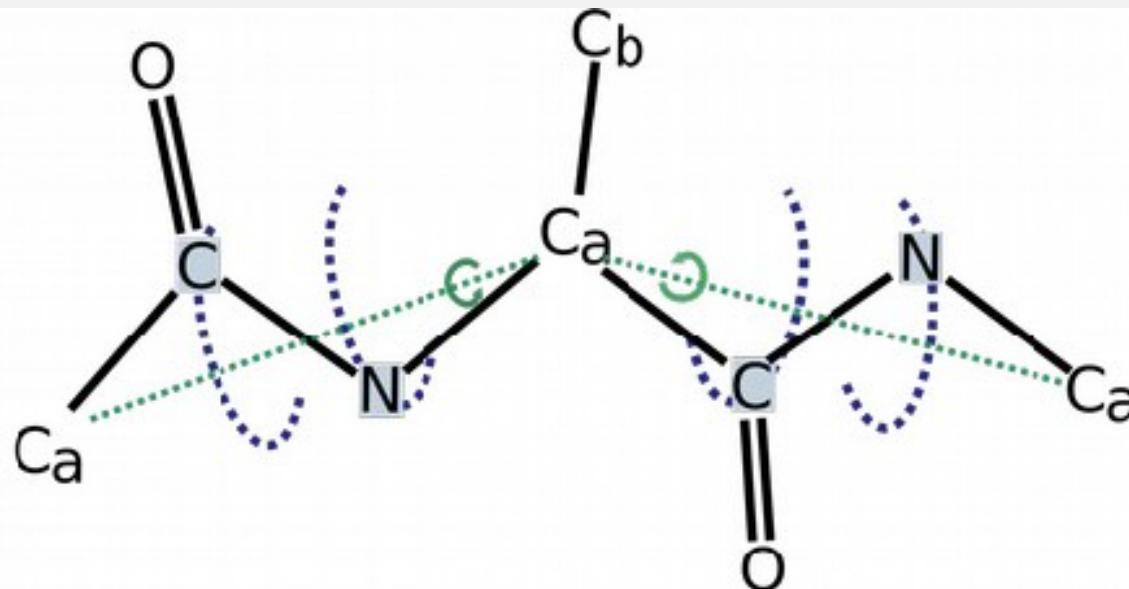
- May be deceiving (0 occ not always clear)

- Possible “distorted” side chains

Side chain density what to do if there isn't any?

- 4) Keep all atoms (and let refinement inflate B-factors)
 - Use a “fitting” (not clashing) high probable rotamer
 - Suggests a fixed position (unless you check B-factors)
- 5) Multiple conformations
 - Data may not justify this (resolution)
 - Confusing (?), which rotamers and how many

Crankshaft Peptide Optimisation



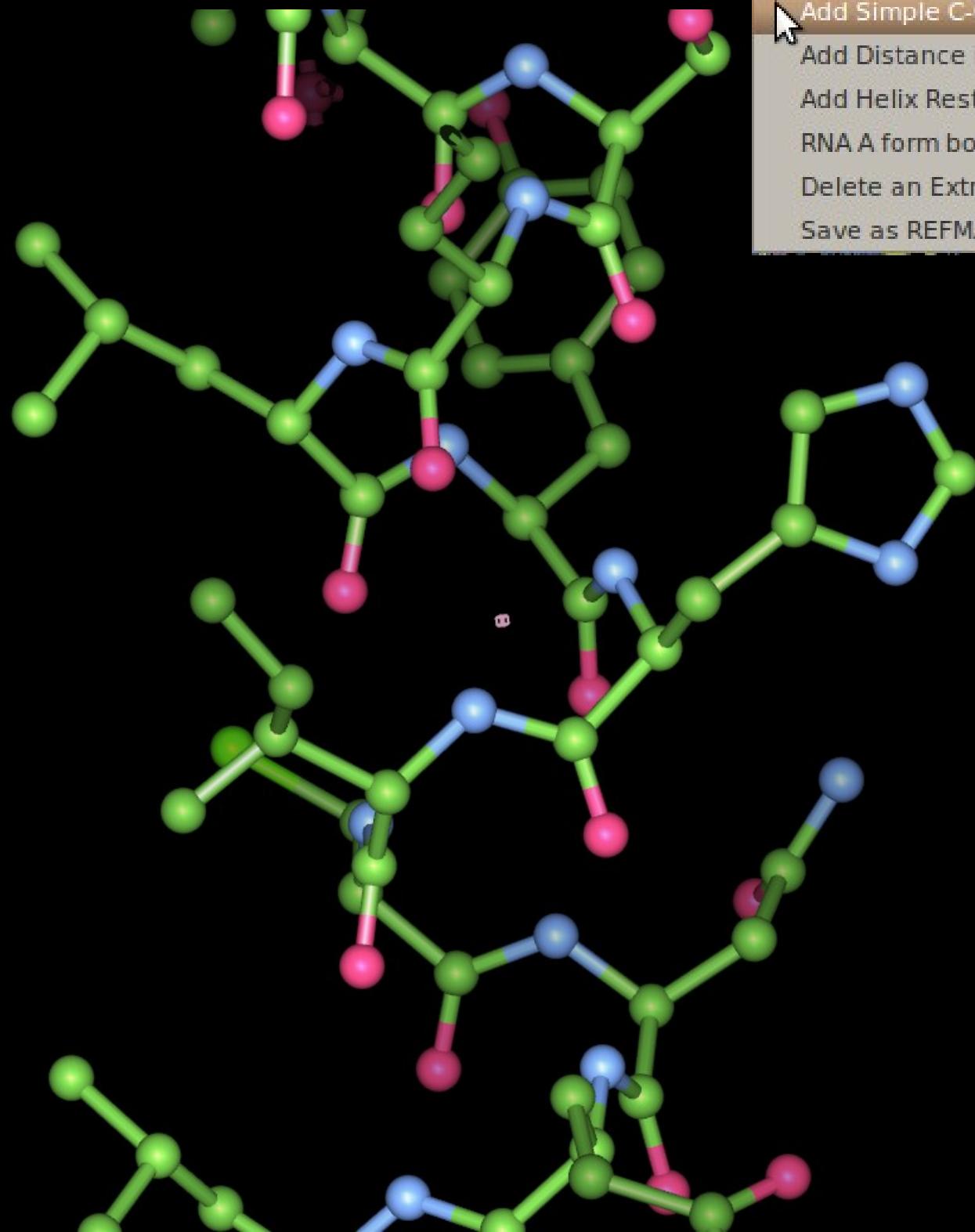
- Rotation around C α -C α vectors creates new positions for C and N atoms, leading to new $\{\phi_i, \psi_i\}_{i=1-3}$ angles and positions in the Ramachandran Plot
 - 1 neighbour each side \rightarrow 3 residue
 - 2 neighbours each side \rightarrow 5 residue
- Pertsemlidis et al. (2007) Statistical Applications in Genetics and Molecular Biology, 4(1), 35
- Useful discussions: Z. Otwinowski

Crankshaft Peptide Optimisation

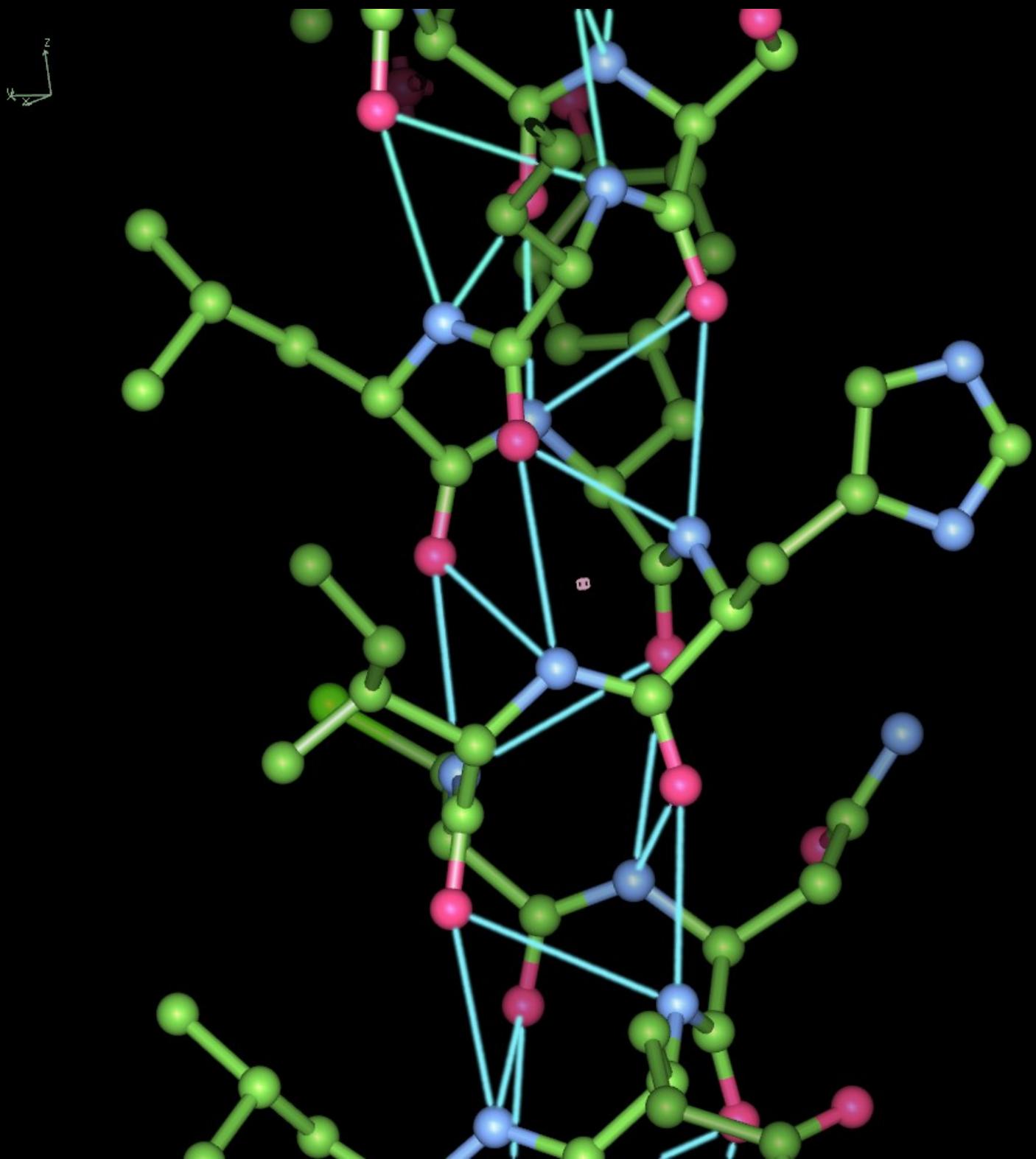
- By rotation of the peptide atoms around a C α -C α vector for a number of residue pairs, choose solutions for which ϕ, ψ most probable
 - cis \rightarrow trans conversion (if needed) is the first step
 - a number of local-minima solutions are generated
 - each of which are (simultaneously) evaluated by real-space refinement
 - and assessed by posterior model distortion (model probability)
 - fit to map (likelihood) is used but has little discriminatory power for cryo-EM maps

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 (ProSMART interface)
- 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...](#)



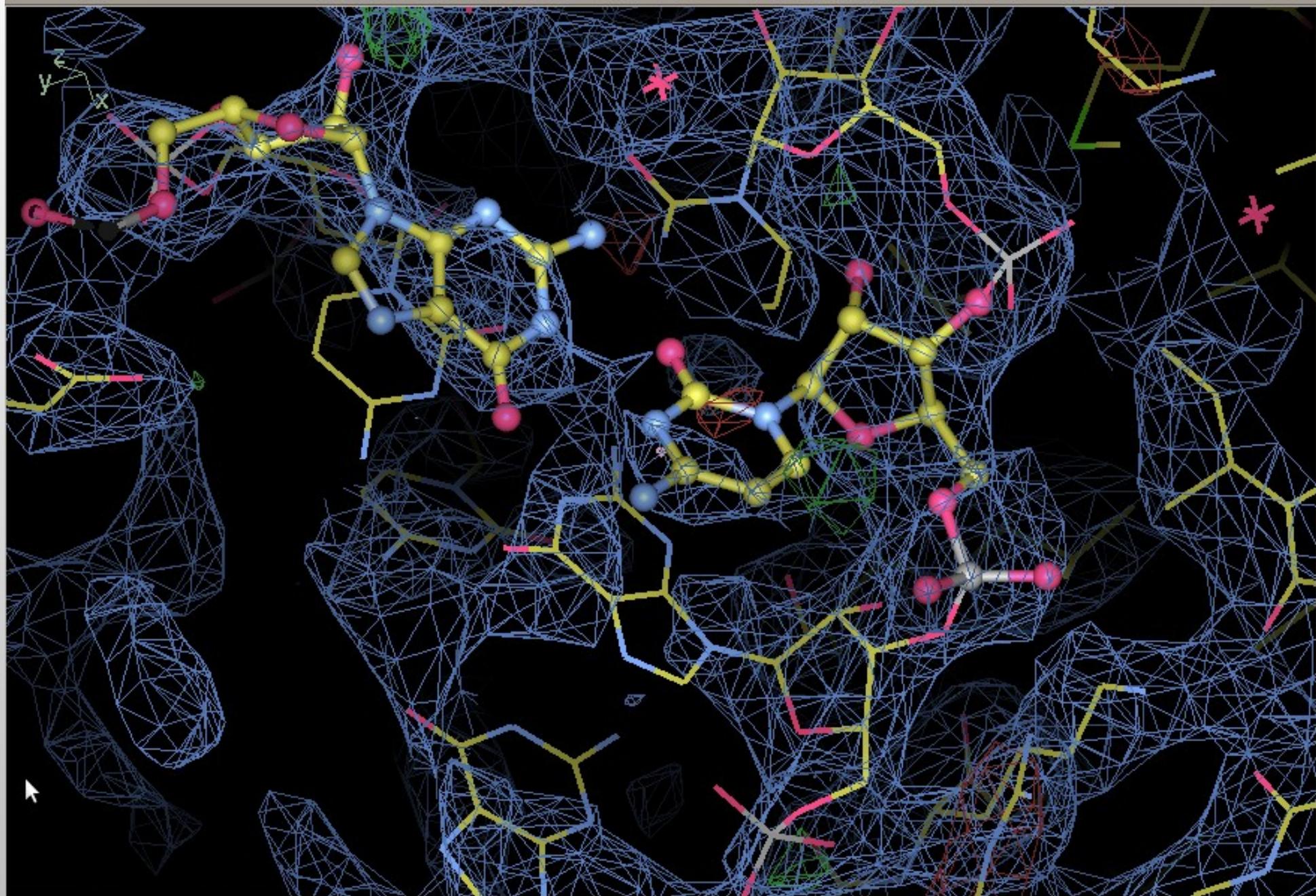
File Edit Calculate Draw Measures Validate HID About Extensions Density

Reset View

Display Manager

R/RC

Map



(mol. no: 0) C5 /1/B/907 Grocc: 0.70 bf: 94.22 ele: C pos: (29.46,28.36,35.57)

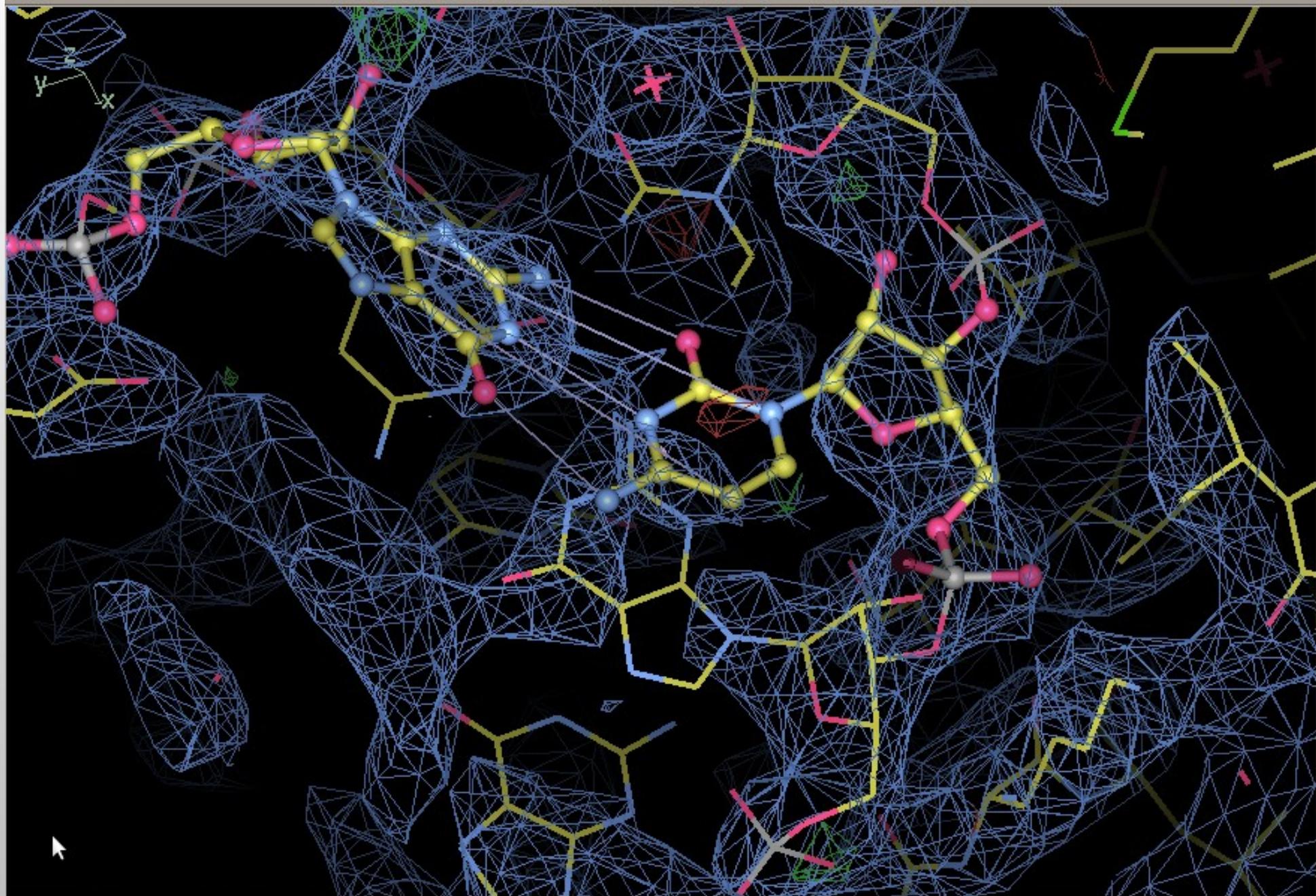
File Edit Calculate Draw Measures Validate HID About Extensions Density Extras

Reset View

Display Manager

R/RC

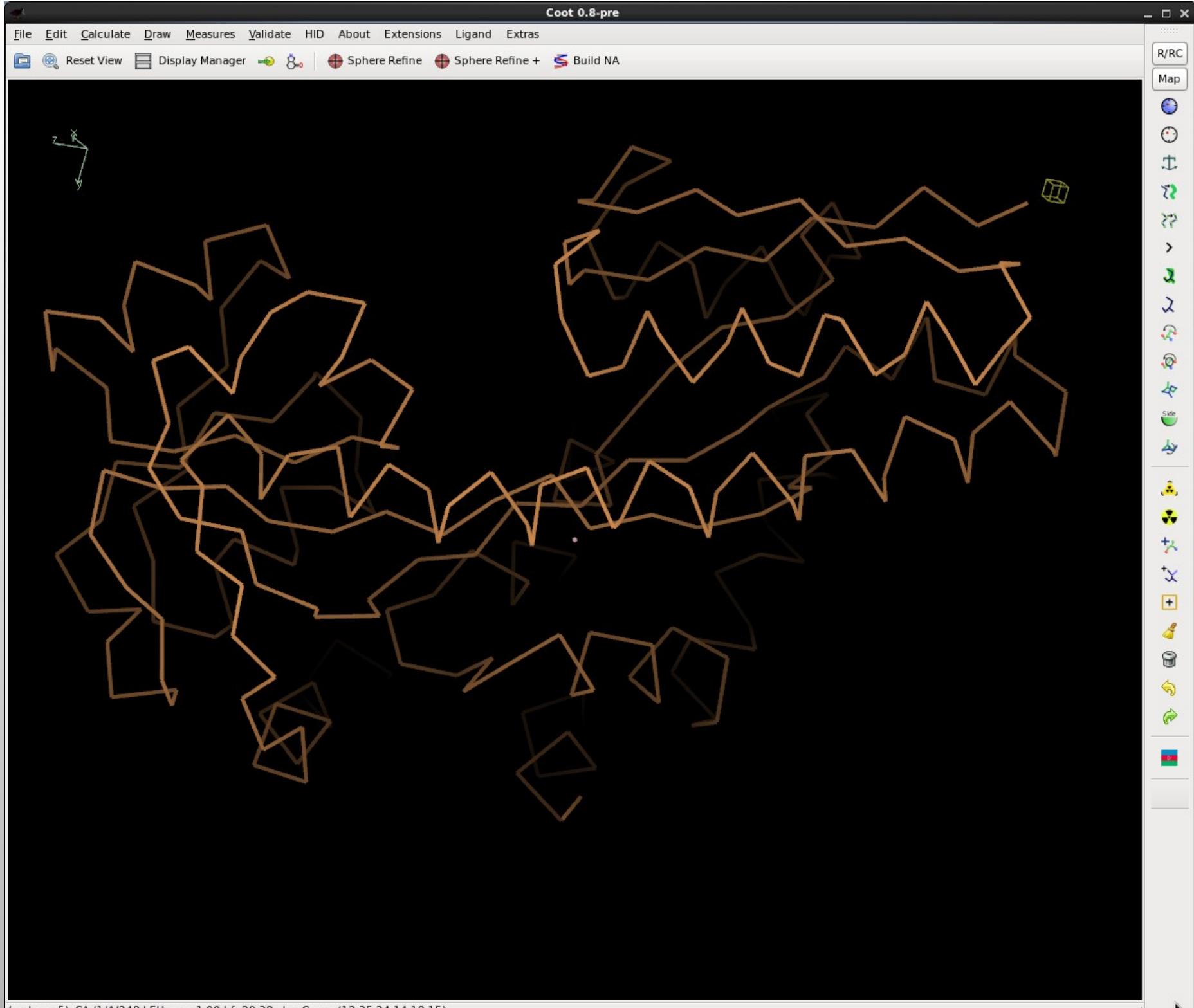
Map

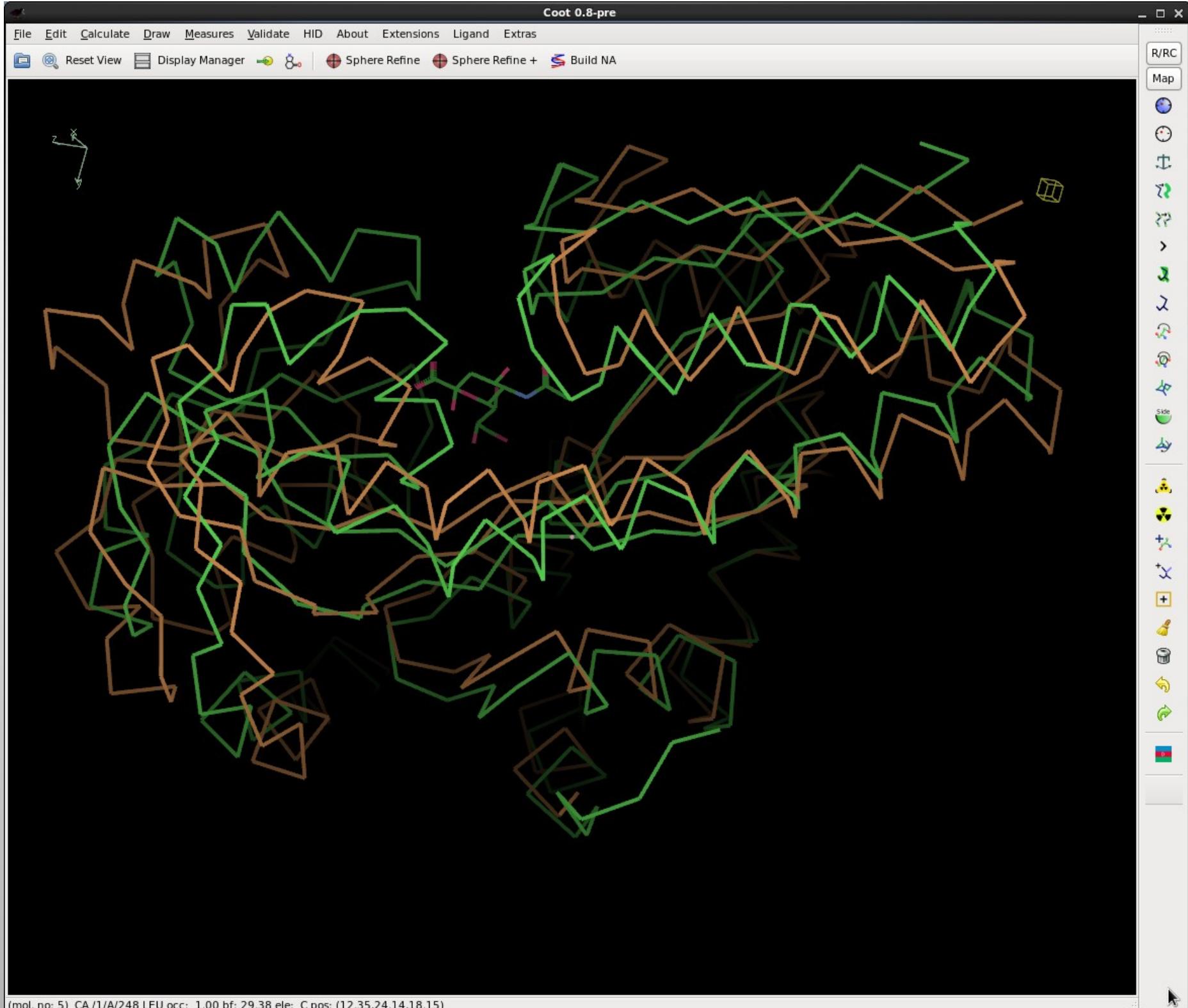


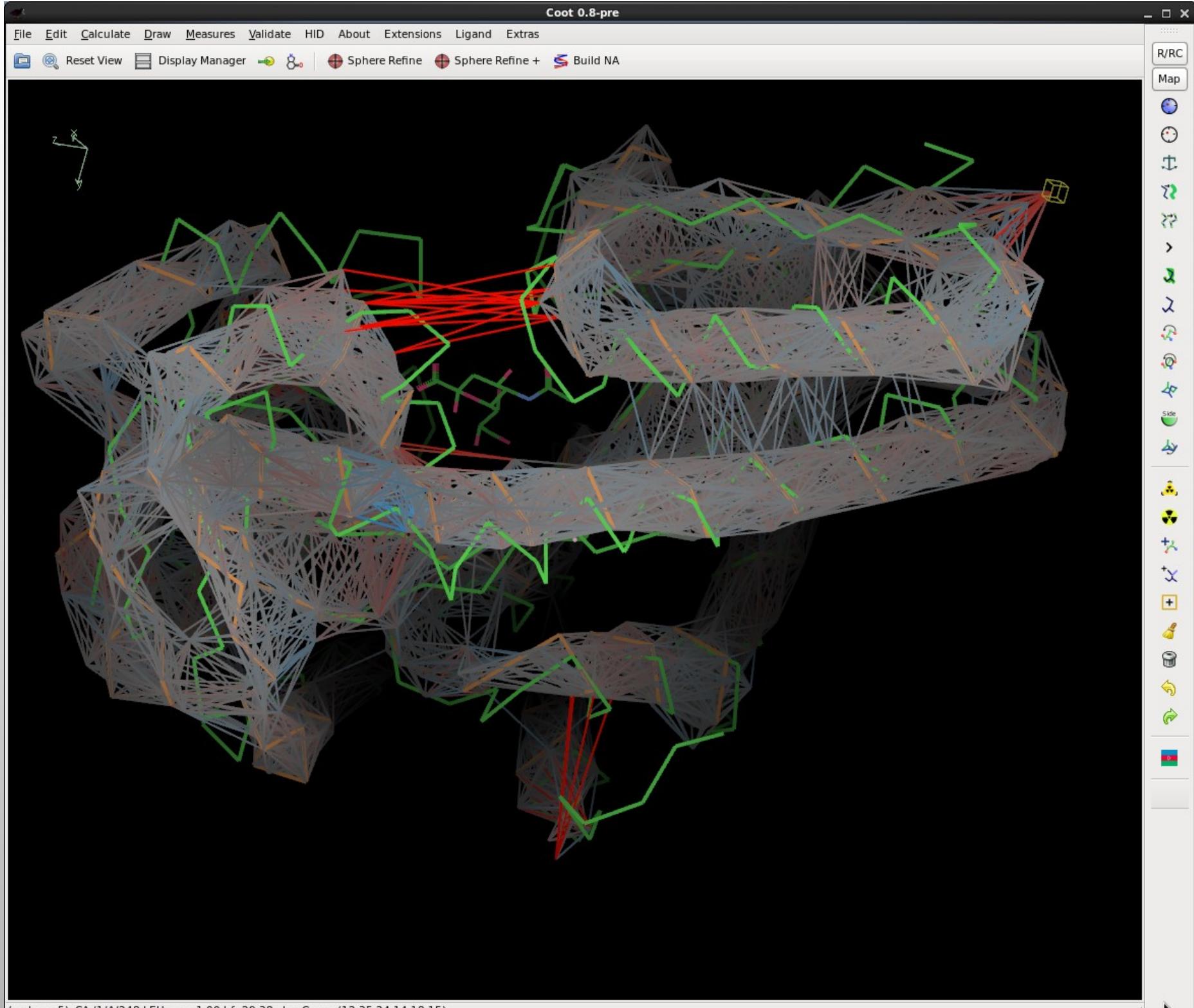
(mol. no: 0) C6 /1/B/907 Grocc: 0.70 bf: 94.54 ele: C pos: (30.57,27.68,35.01)

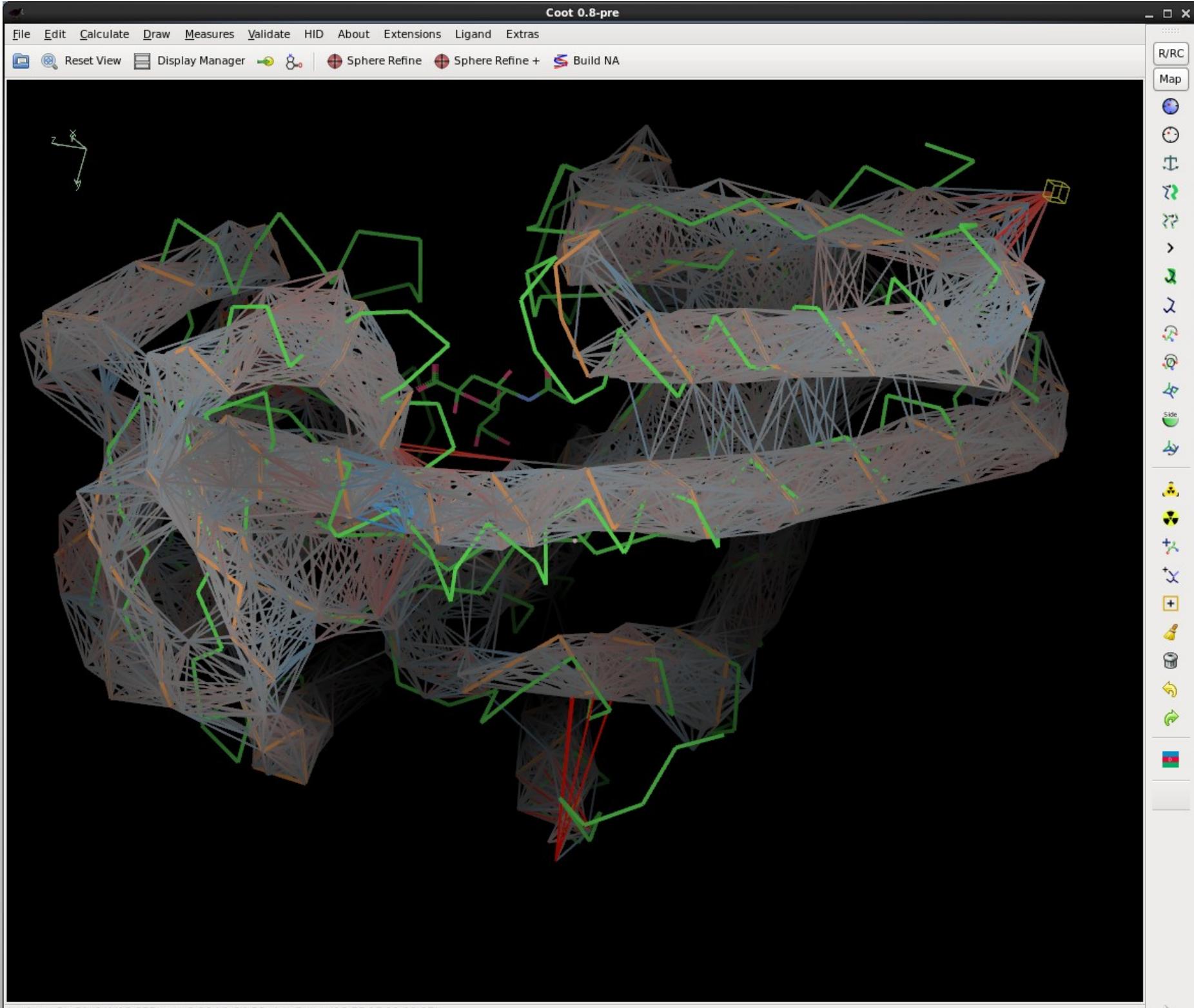
ProSMART Interface

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









Scripting

- Python or scheme
- 100s of functions are scriptable
- Accessed via:
 - the command line: **--script**
 - the GUI: Calculate -> Run Script...
 - Interactive: Calculate -> Scripting
- Use **--no-graphics** for “batch mode”

SSM Overlay by Scripting

- (superpose-with-atom-selection *imol1 mol2 atom-selection-string-1 atom-selection-string-2 move-copy-flag*)
- e.g. in scheme (superpose-with-atom-selection 0 1 “//A/20-120” “//B/30-130” 0)
- e.g. in Python superpose_with_atom_selection(0, 1, “//A/20-120”, “//B/30-130”, 0)
- General command:
 - Scheme: (**scheme-command arg1 arg2 ...**)
 - Python: **python_command(arg1, arg2, ...)**

More on Scripting

- If something is boring, stop it
 - Write a script
 - Or get someone to do it for you
 - me?
- Scripting available in Python or Scheme (lisp)
- Scripting example available on the mailing list
 - and the Coot Wiki

Some key bindings

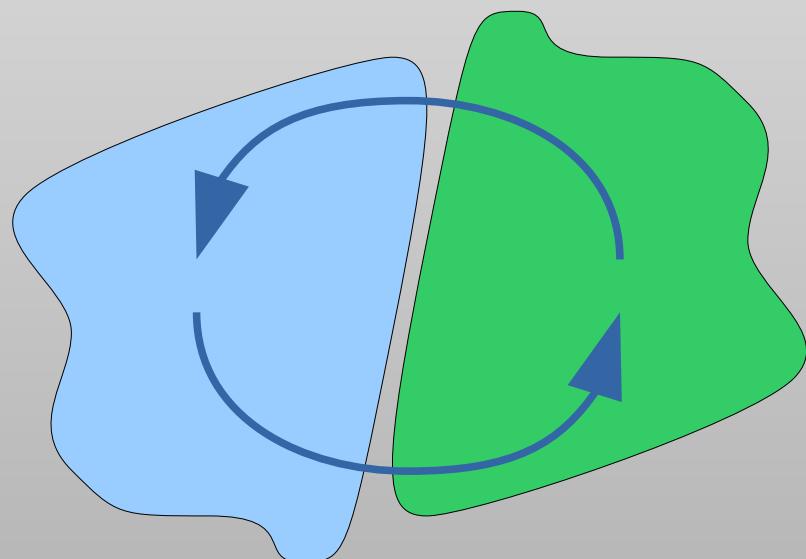
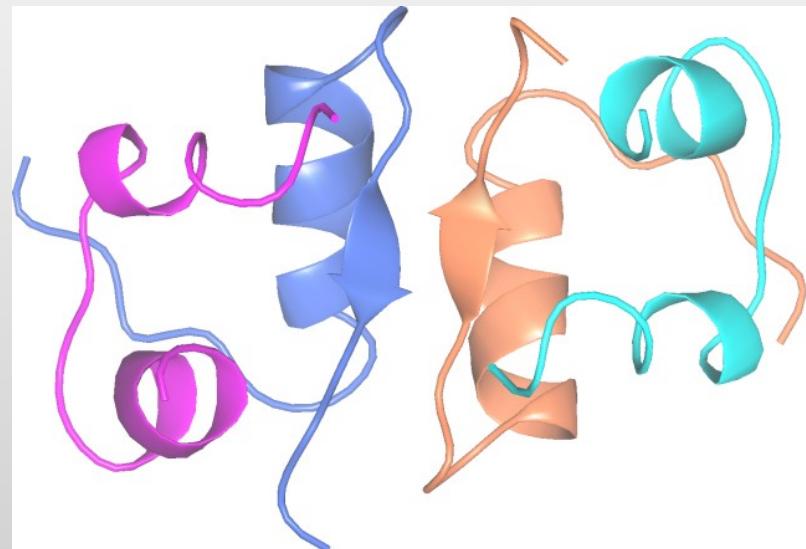
- Any function can be bound to a key
 - Allows for personalization/customization
- Here's how you do it:

```
(add-key-binding "x" (lambda () (refine-active-residue)))
```
- Makes Coot easy to use
 - (but harder to learn)
- <http://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/docs/coot-keys-and-buttons.pdf>

Handling NCS...

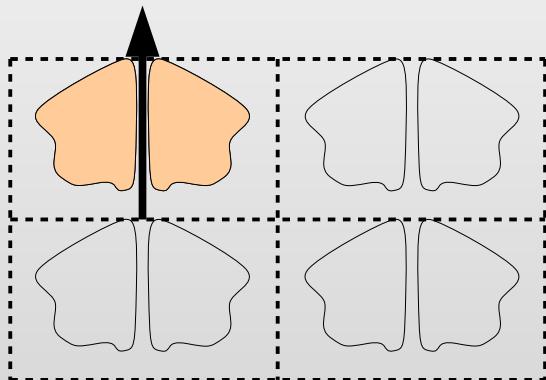
What is Non-Crystallographic Symmetry (NCS)?

- 2 or more copies of a molecule in the unit cell not related by crystallographic symmetry
- NCS related molecules provide different representations of the same molecule



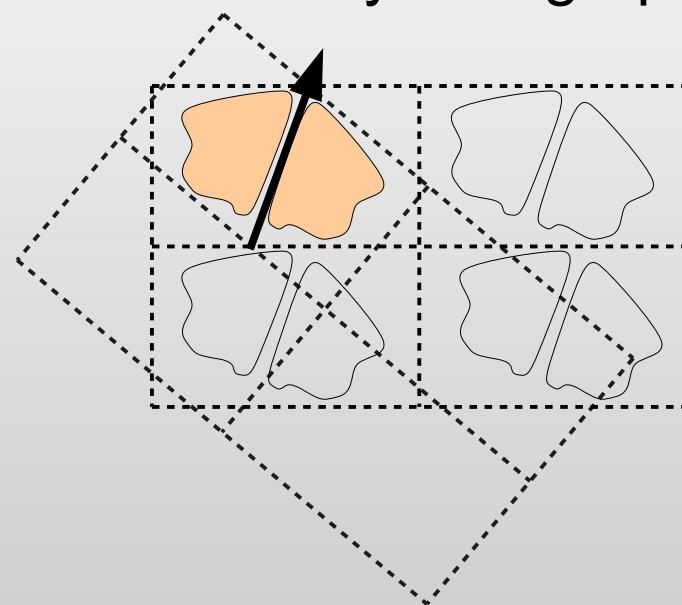
Non-crystallographic symmetry

Crystallographic



Aligned
2-fold

Non-crystallographic



Unaligned
2-fold

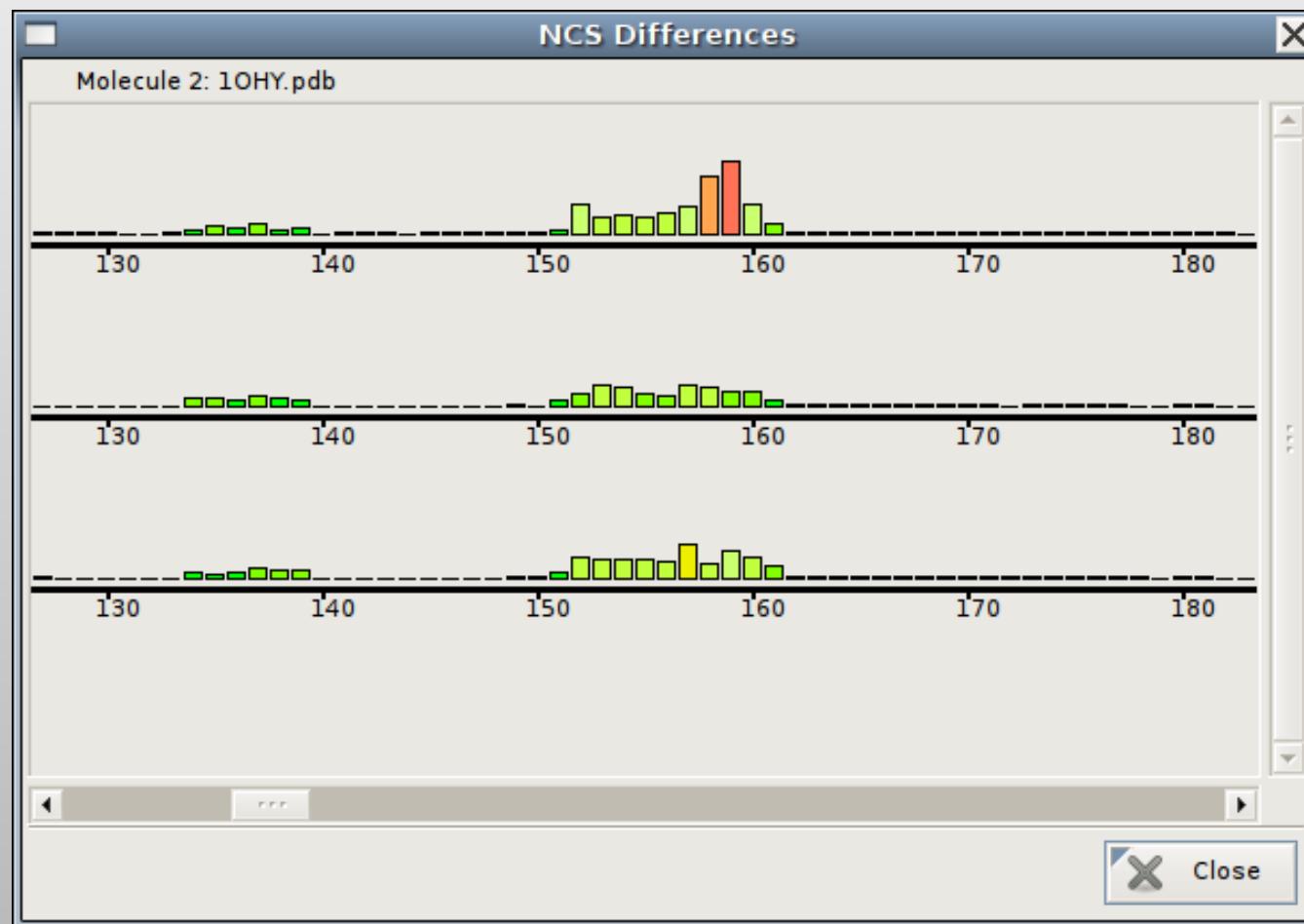
- What are the problems?
 - Molecules are different
 - How to allow for differences, but minimize unnecessary rebuilding?

Handling NCS

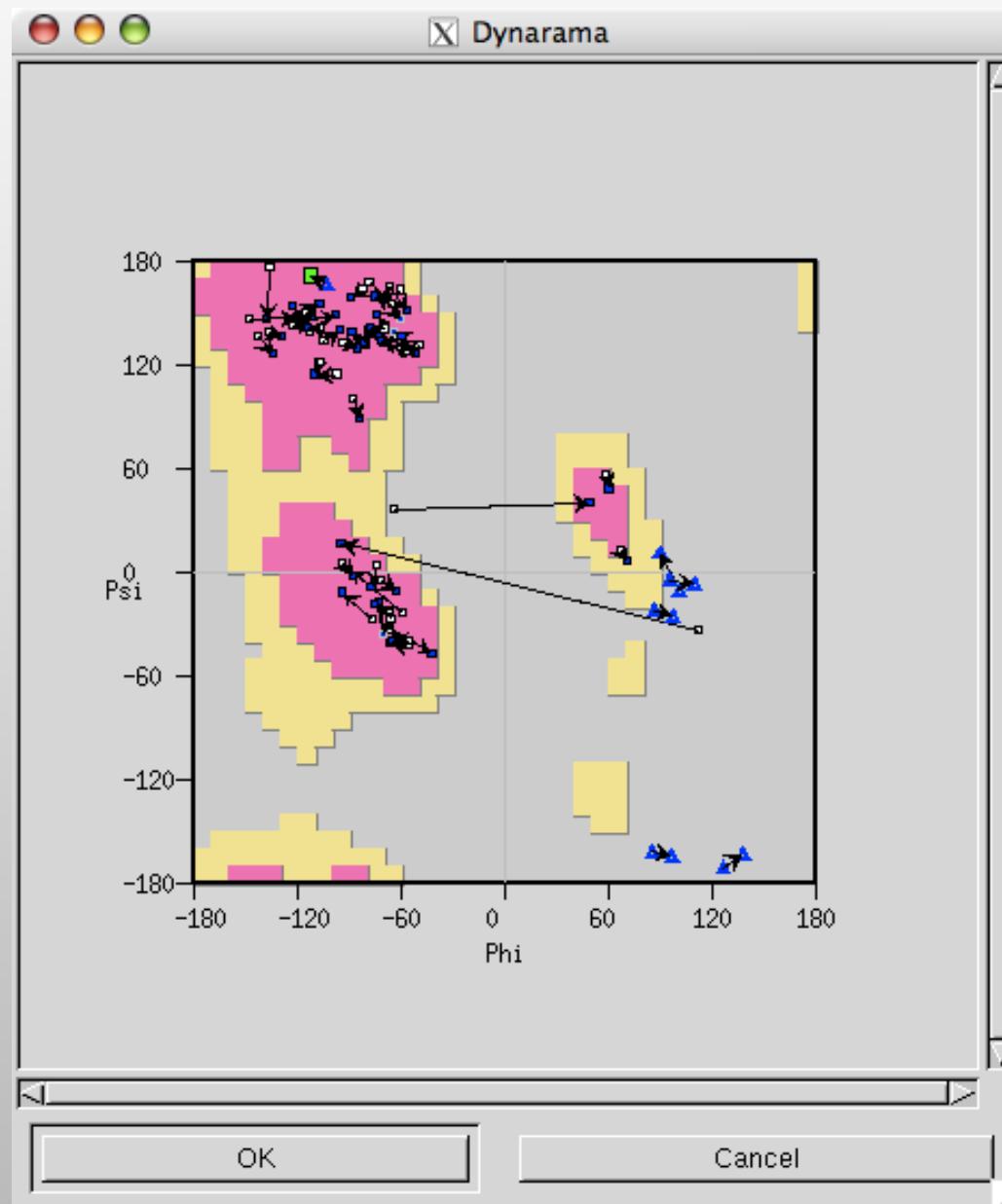
Typical Scenario:

- 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 NCS Differences graph



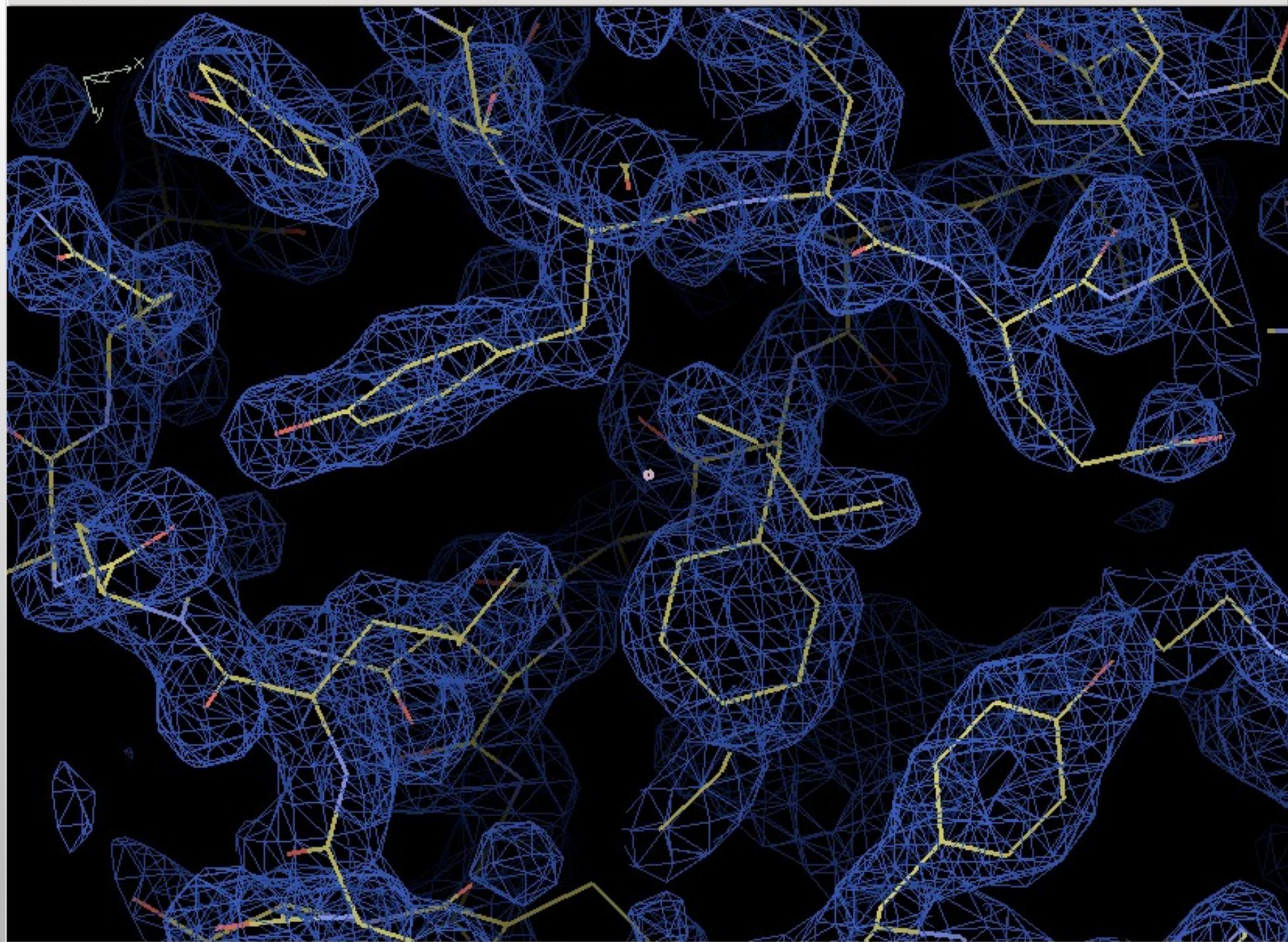
...or Keywelt Plots[*]



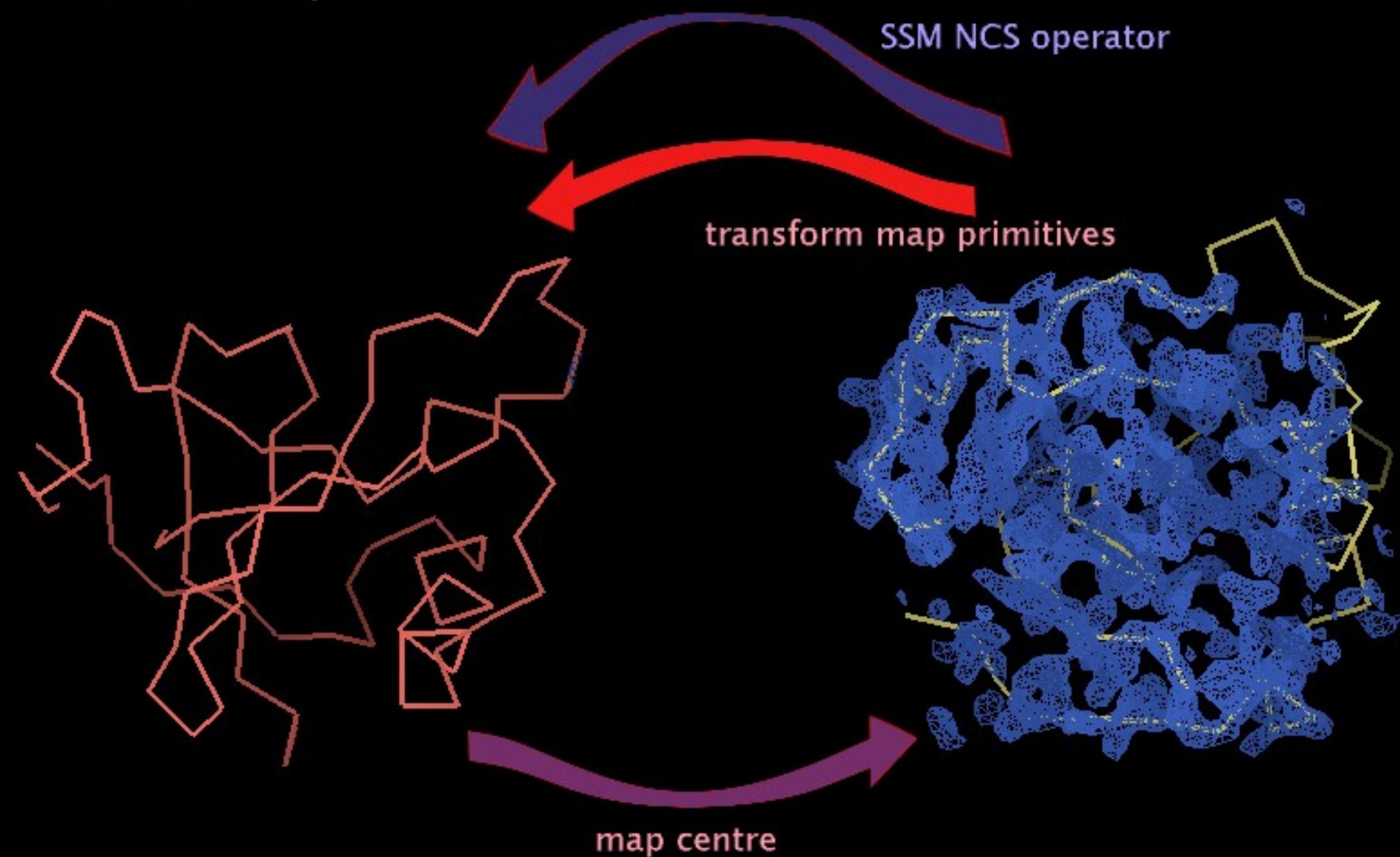
[*] Named by George Sheldrick

Coot

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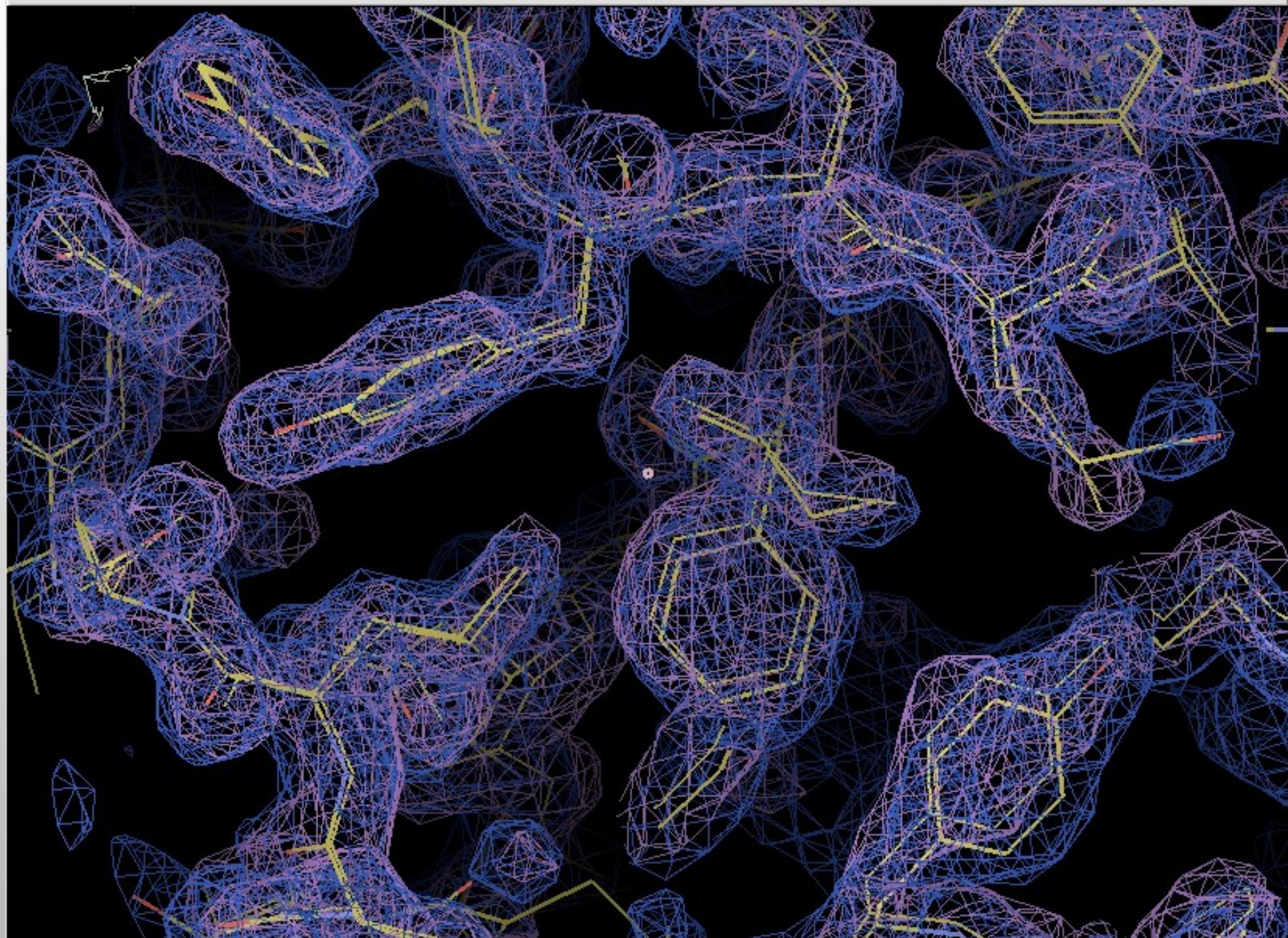


NCS Overlays



Coot

File Edit Calculate Draw Display Manager Measures Validate HID Reset View Help



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

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 ('o' key)