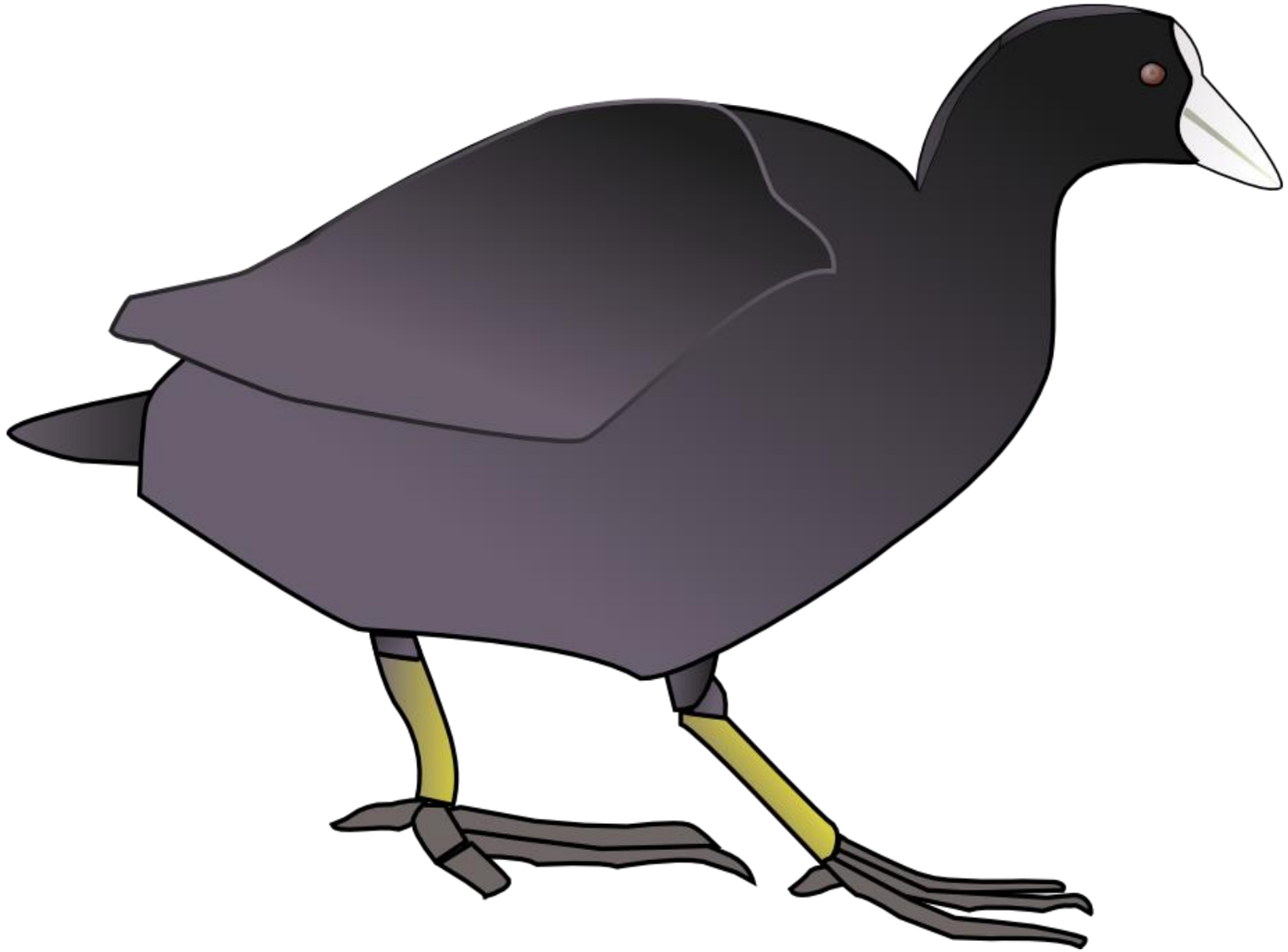
Coot



Judit Debreczeni (AZ, UK)

2020 December, DLS

Acknowledgements

Credit where it's due:

- Developers:
  - Paul Emsley
  - Bernhard Lohkamp
  - Kevin Cowtan
- Libraries:
  - Alexei Vagin
  - Garib Murshudov
  - Eugene Krissinel
  - Greg Landrum

Useful links

Downloads:

- Coot: https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/binaries/
- wincoot: https://bernhardcl.github.io/coot/
- CCP4: http://www.ccp4.ac.uk/download
- CCPEM: https://www.ccpem.ac.uk/download.php

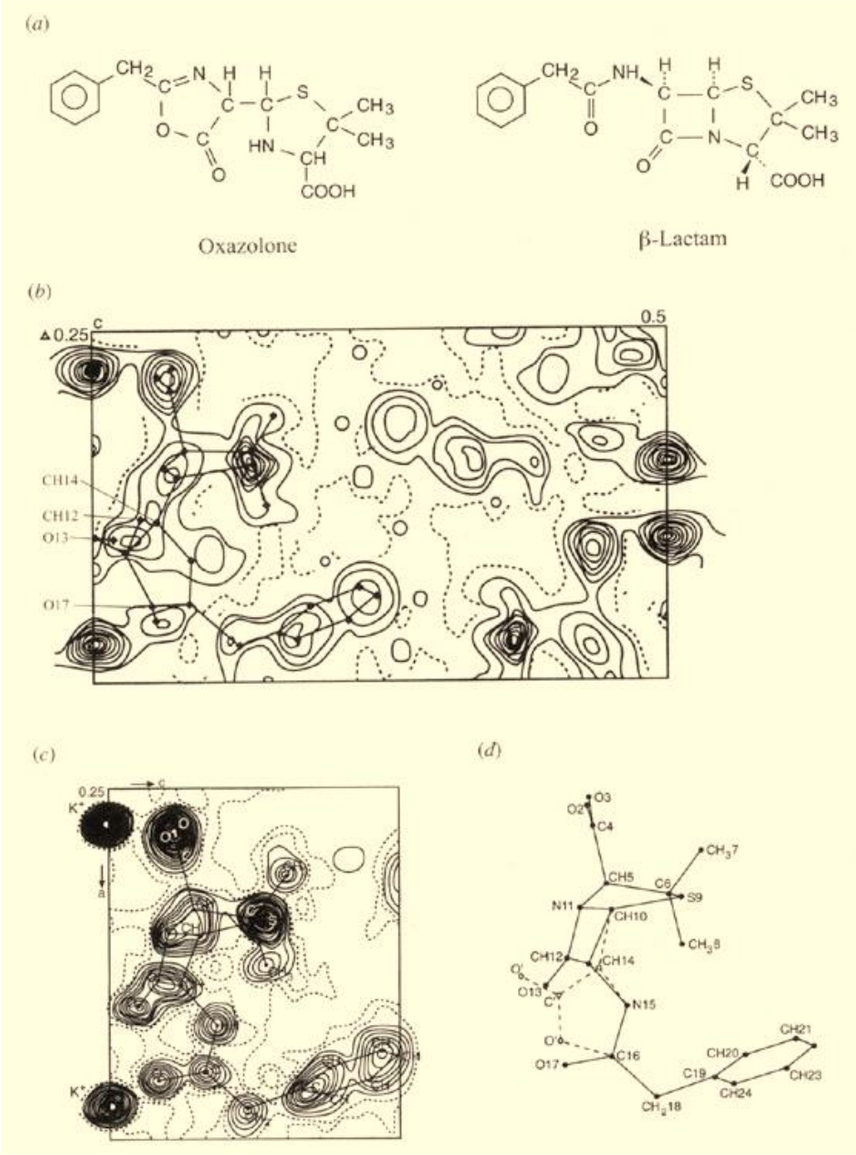
Manual: https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/docs/

Blog: https://pemsley.github.io/coot/

Moorhen

Coot

Once upon a time…



Is there a 4-membered ring?

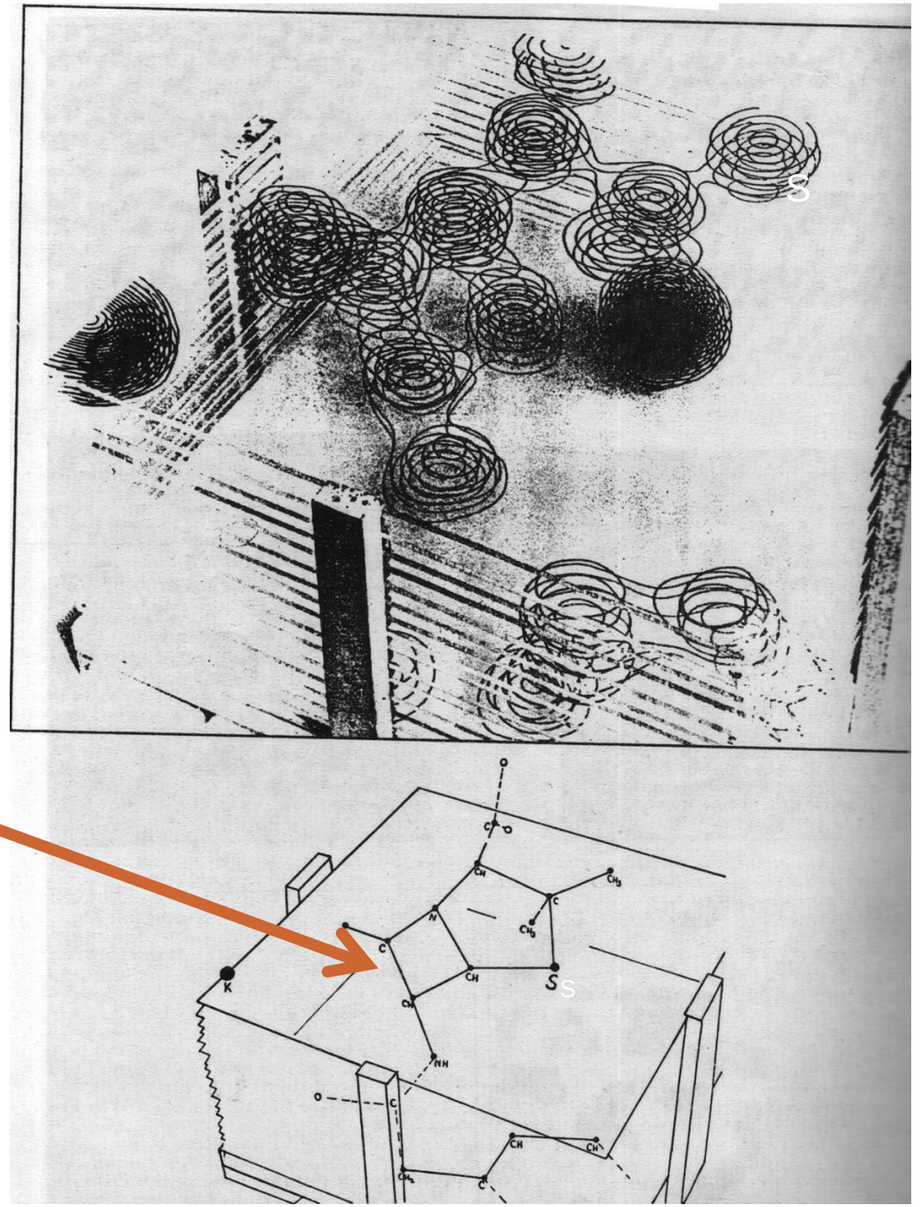
- First projection of Fourier map
- Final map
  (yes, there is a 4-membered ring)

Once upon a time…

The 3-dimensional penicillin G map calculated in 1944. This unequivocally determined its chemical structure.

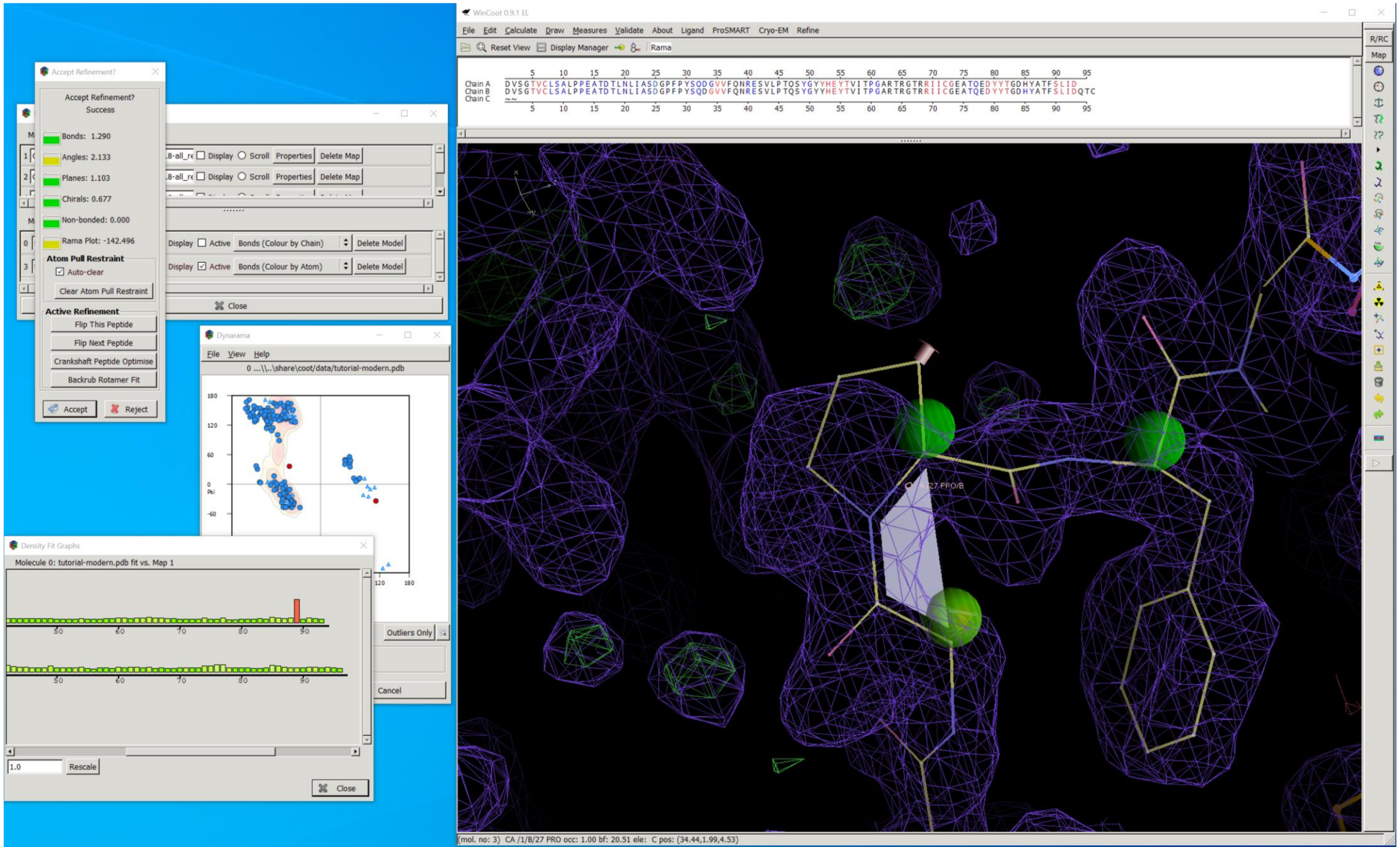
(The hand however is wrong.)

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



First model of insulin…

Right now…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Accept Refinement?
Success
Bonds: 1.290
Angles: 2.133
Planes: 1.103
Chirals: 0.677
Non-bonded: 0.000
Rama Plot: -142.496
Atom Pull Restraint
Auto-clear
Clear Atom Pull Restraint
Active Refinement
Flip This Peptide
Flip Next Peptide
Crankshaft Peptide Optimise
Backrub Rotamer Fit
Accept
Reject
Dynarama
File View Help
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Density Fit Graphs
Molecule 0: tutorial-modern.pdb fit vs. Map 1
Outliers Only
Cancel
1.0
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File Edit Calculate Draw Measures Validate About Ligand ProSMART Cryo-EM Refine
Reset View
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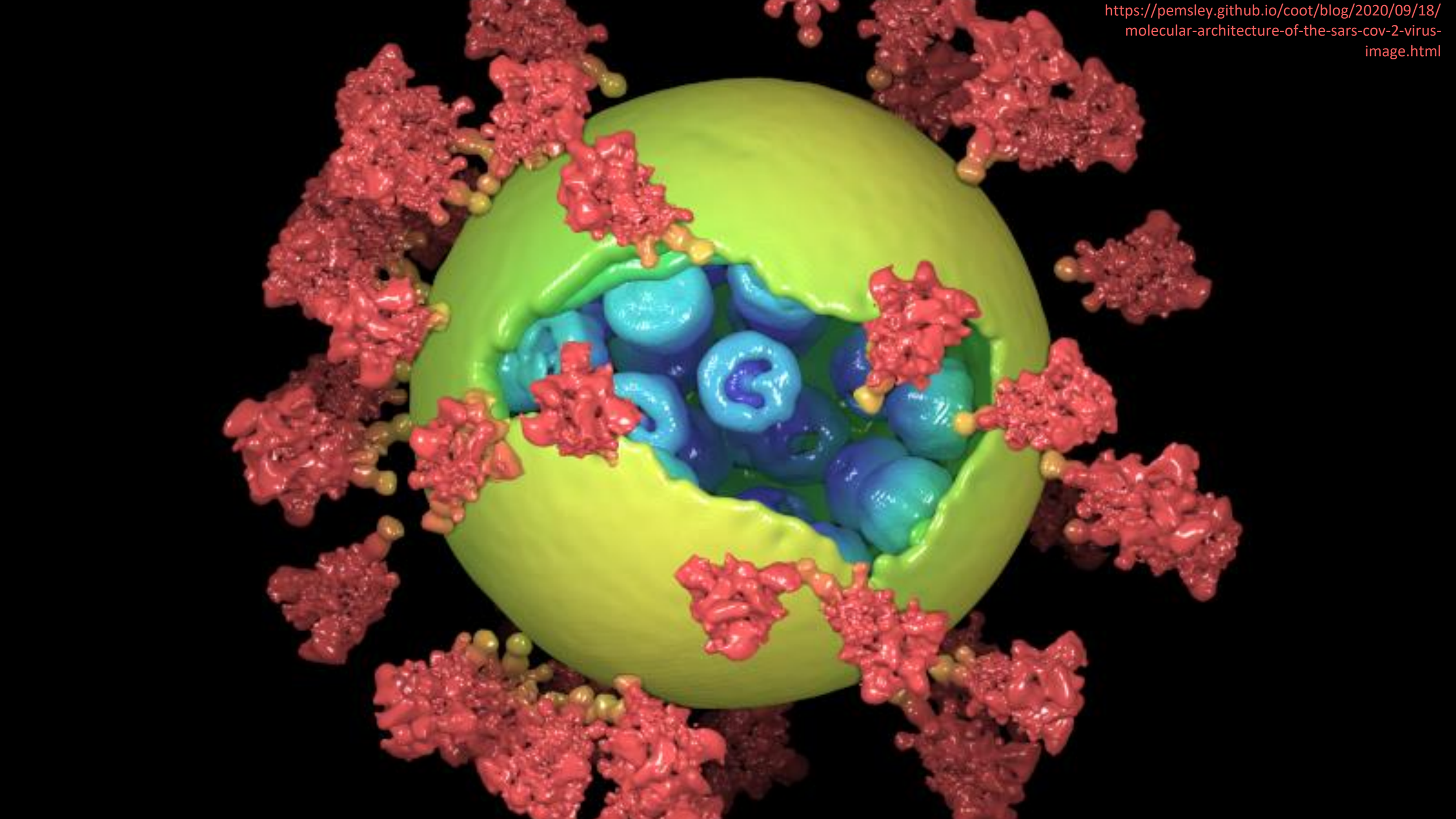
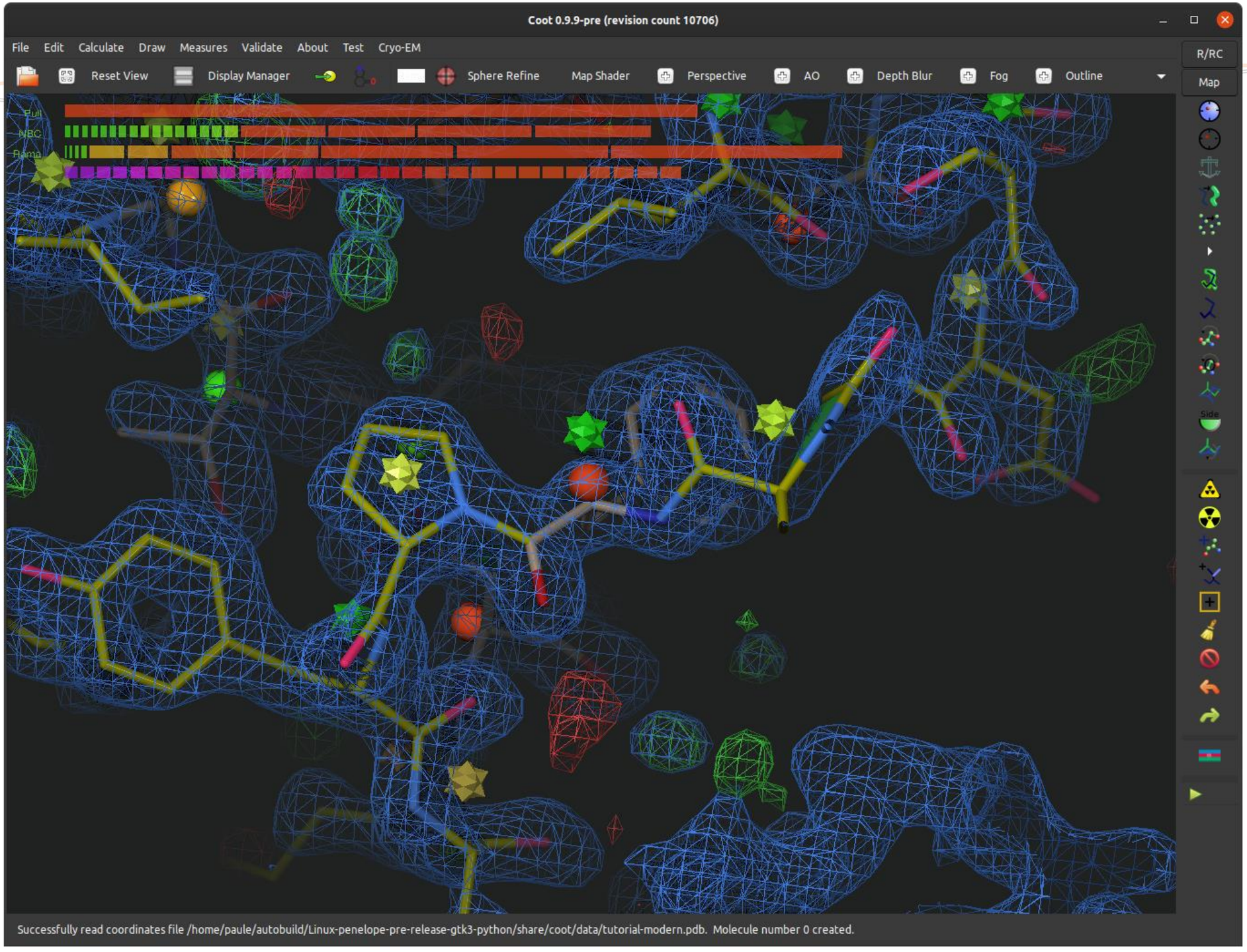
Chain A
Chain B
Chain C
(mol. no: 3) CA /1/B/27 PRO occ: 1.00 bf: 20.51 ele: C pos: (34.44,1.99,4.53)



https://pemsley.github.io/coot/blog/2020/09/18/molecular-architecture-of-the-sars-cov-2-virus-image.html

The future…?

- instead of
  - multiple windows
  - flat view and little depth
  - obsolete components (GTK1/2, Python2.x, OpenGL v1)
- the future is:
  - single full screen view
  - HUD representation of metrics
  - more depth using shading, occlusion, shadows, blur, fog depth
  - modern technology (Python3, Gtk+3, Gtk4, Vulcan)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Fog
Outline
R/RC
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Rama
Side
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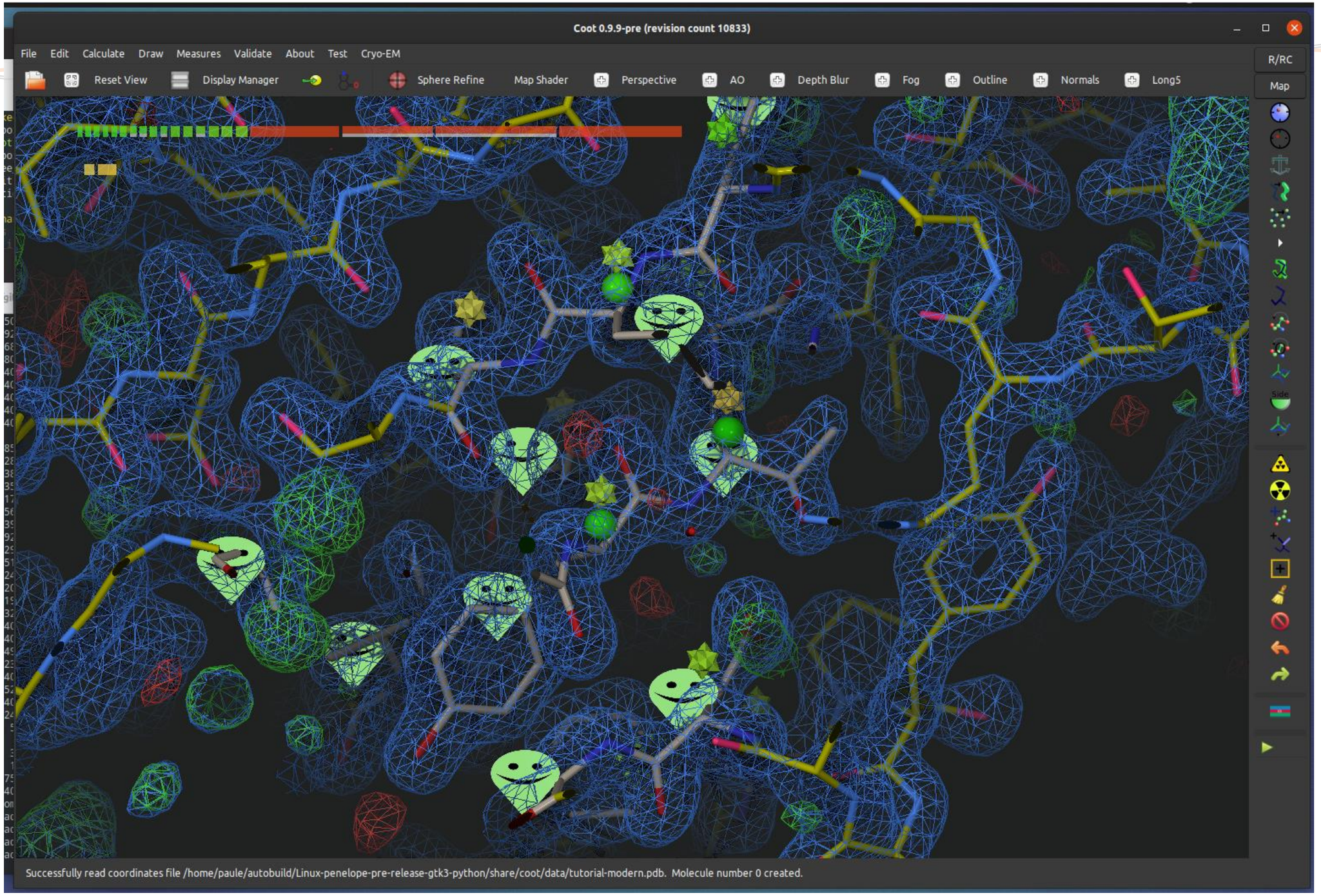
Holmes Street
Parker Street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Map
Side
Successfully read coordinates file /home/paule/autobuild/Linux-penelope-pre-release-gtk3-python/share/coot/data/tutorial-modern.pdb. Molecule number 0 created.

Outline

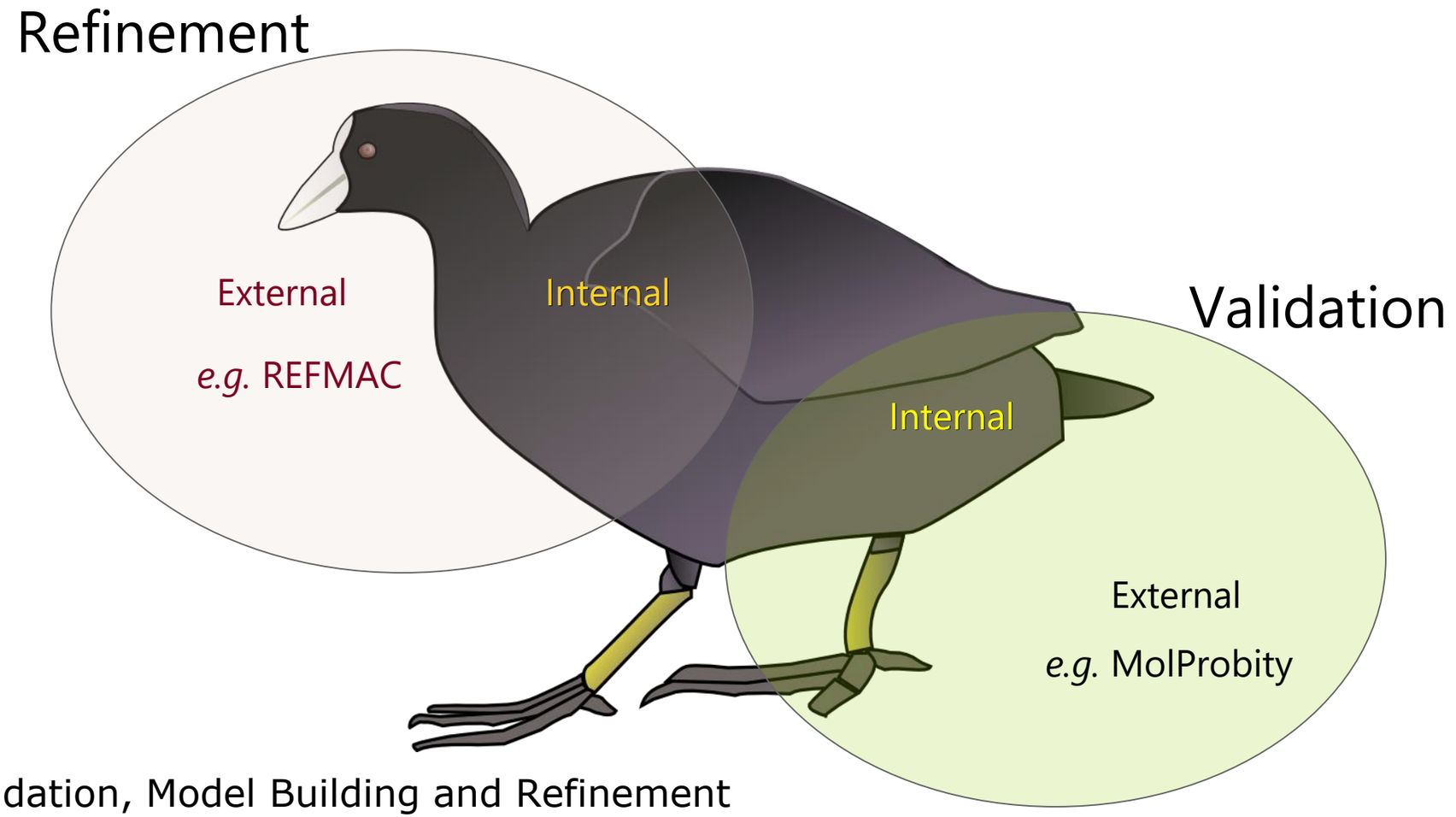
- Look and feel
- Speed: preferences and key bindings
- Extra resources: Curlew
- Real space refinement – 0.8.x vs 0.9.x
- Low resolution tools
- Validation
- Handling NCS

Coot

- Crystallographic Object-Oriented Tool-kit
- Primarily a tool for the interpretation of electron density generated from X-ray data (now CryoEM as well)
- 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

Coot

- Interactive model-building, refinement and validation
- (Fast) automation over interaction
- Functionality over speed
- Speed over beauty
- Beauty over ugly (as long as it doesn't cost too much time)

A workhorse not a show pony
– Optimised for the world of chicken-wire

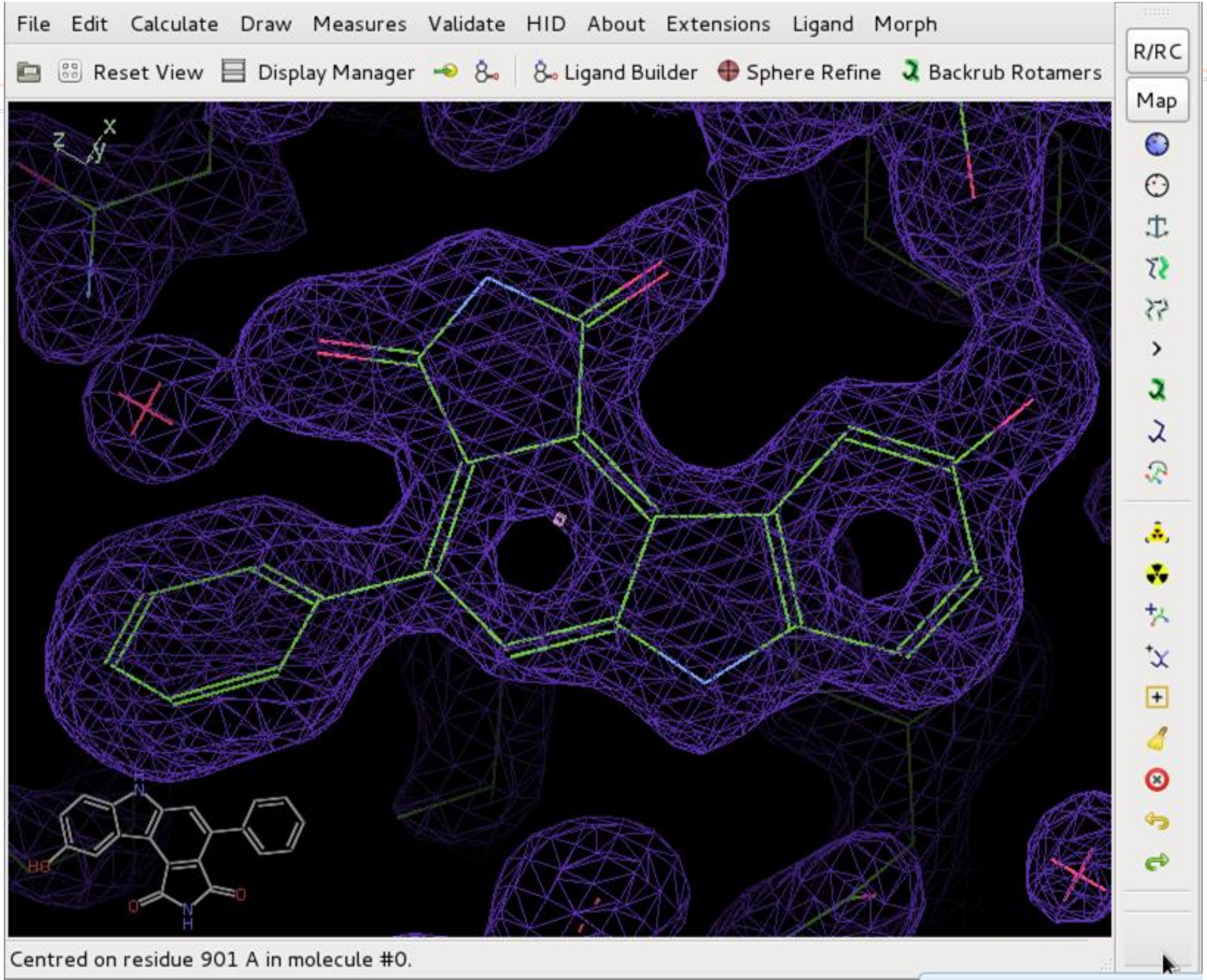
Presentation quality screenshots with *Coot*

Standard Screenshot

- Density is too dark for high illumination seminar rooms

Better option:

- Edit → Background Colour → White
- Draw → Additional Representations → Ball & Stick → Add Representation
- Resample the map (1.8 → 2.2)
- Map Colour → “Light Blue/Grey”


File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
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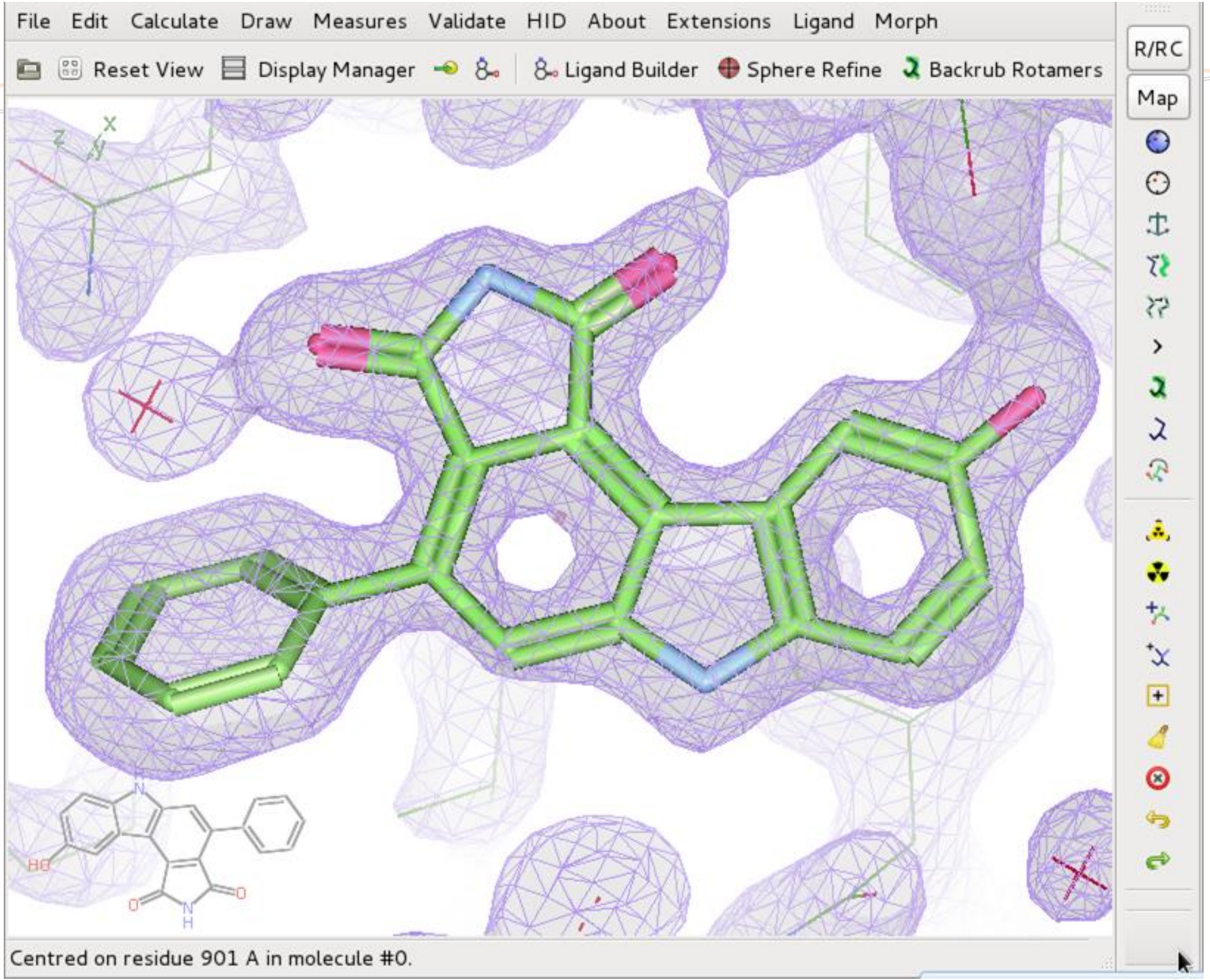
Map
Centred on residue 901 A in molecule #0.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Reset View
Display Manager
Ligand Builder
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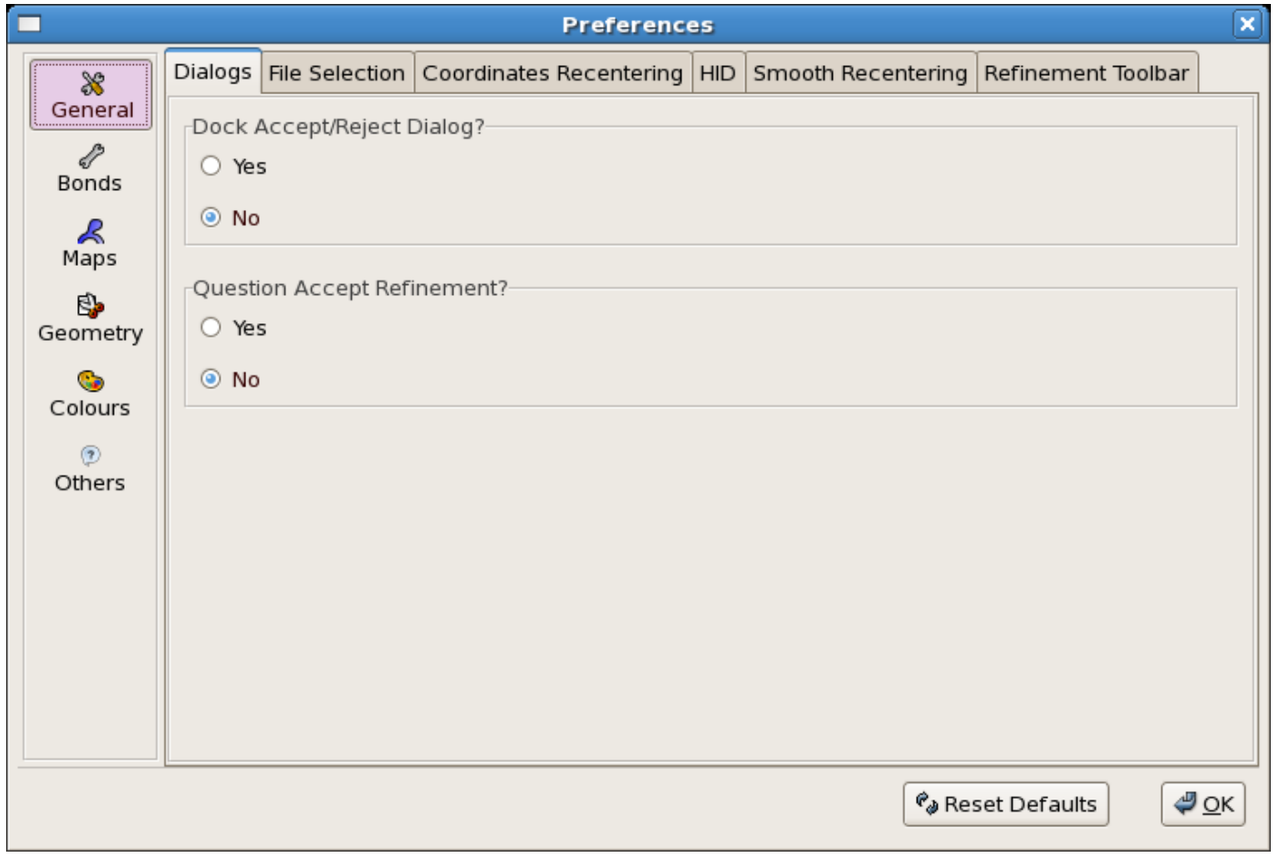
Backrub Rotamers
R/RC
Map
Centred on residue 901 A in molecule #0.

Speed: user preferences

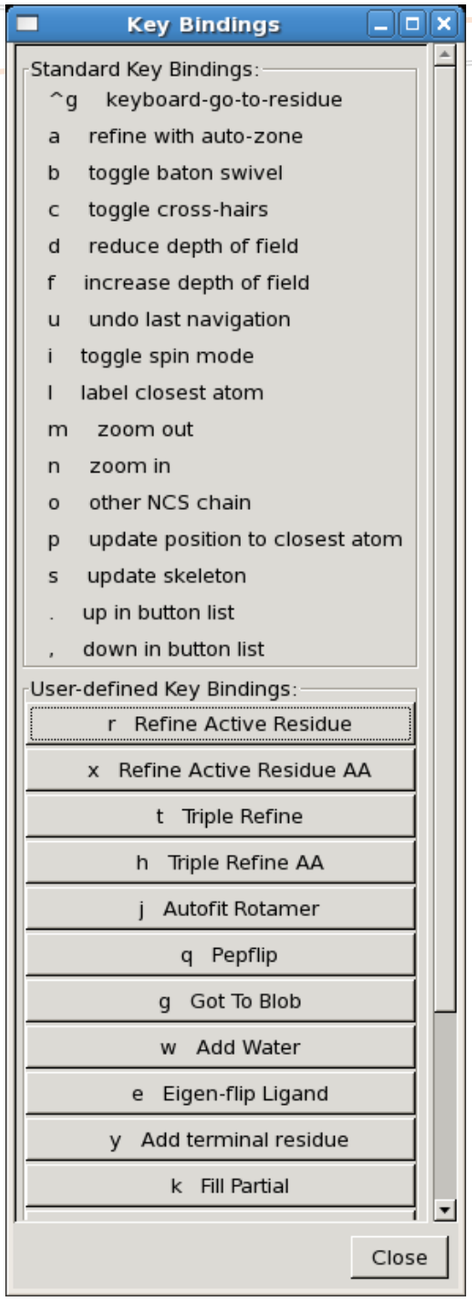
Preferences tabs

Preferences groups



Speed: key bindings

- Short-cuts to speed up common tasks
- Some build-in
- User defined ones
  examples on Coot WIKI pages:
  https://strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/Coot



Speed: key bindings

- example: active residue refinement → key “x”

in scheme:

```
(add-key-binding "Refine Active Residue" "x"
 (lambda () (refine-active-residue)))
```

in python

```
add_key_binding("Refine Active Residue", "x",
 lambda: refine_active_residue())
```

- Makes Coot easy to use (but harder to learn)
- Crib sheet available here:

https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/docs/crib-sheet.pdf

demo?

Curlew

- Coot Utility Refinement Library Extension Wrangler mechanism to install extensions, tweaks etc
- new in 0.9.x
- File→Curlew

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

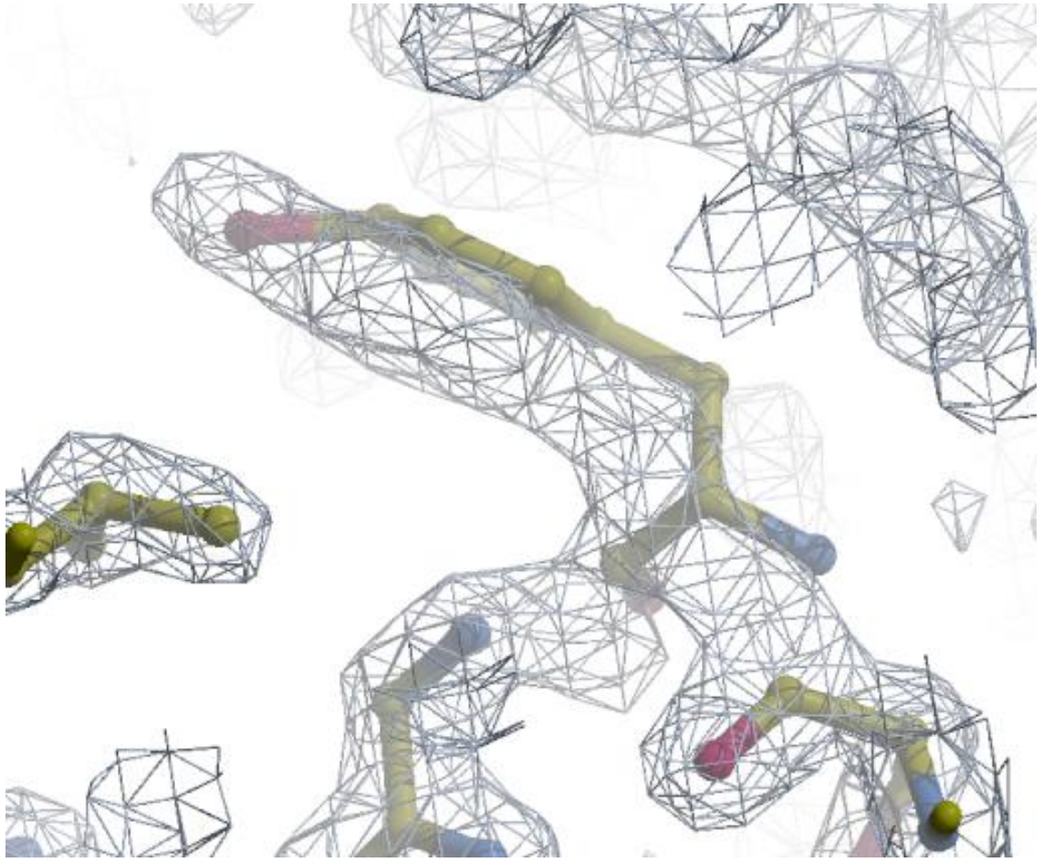
Real Space Refinement

- 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

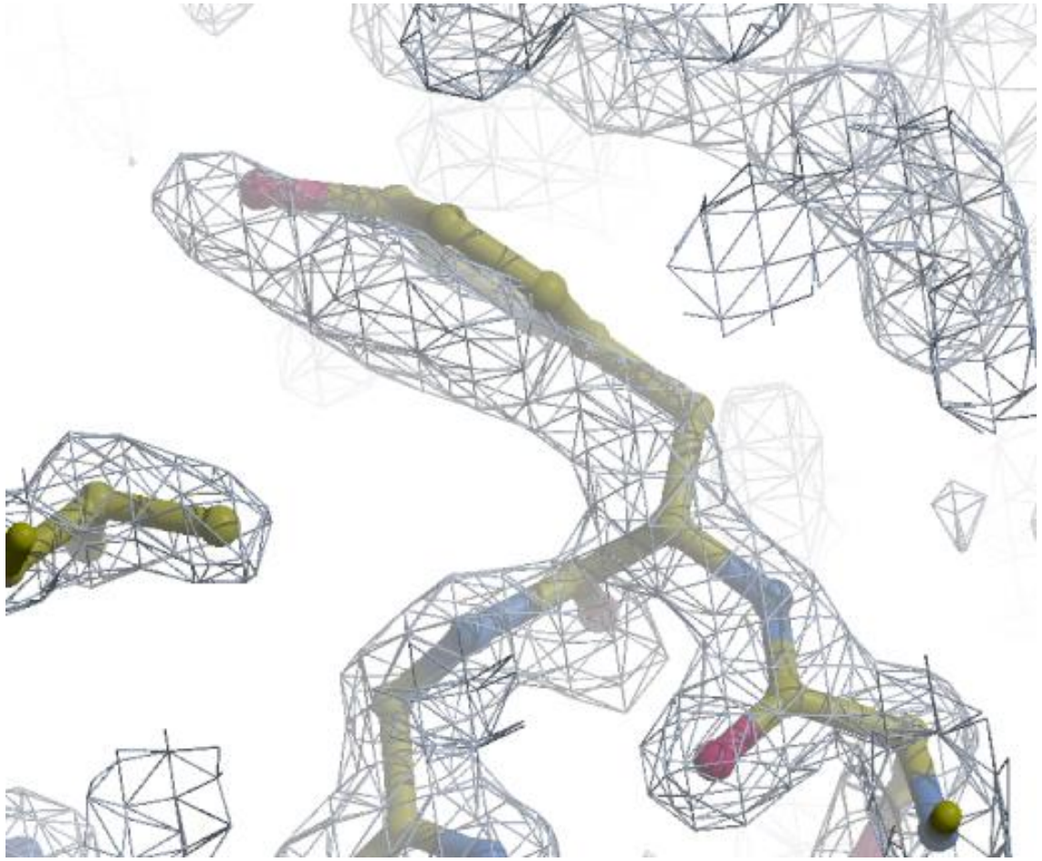
Real Space Refinement

- refmac monomer library (e.g.: bonds in Ala)

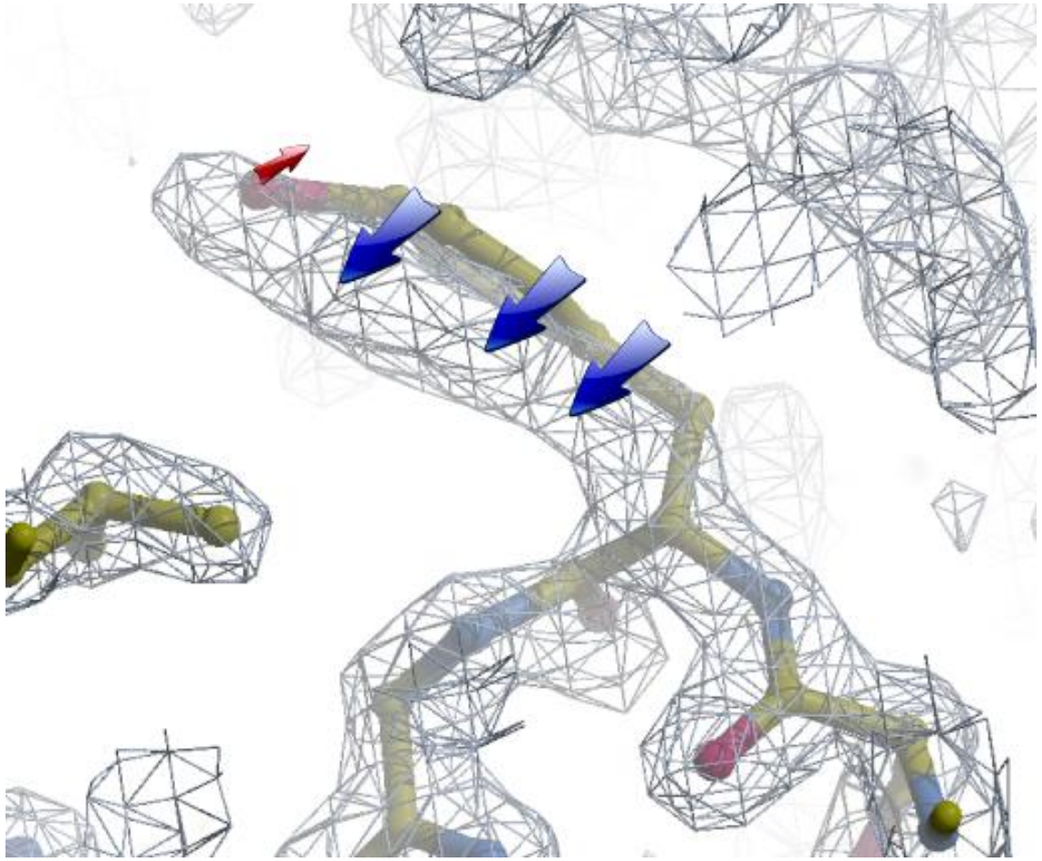
```
loop_
_chem_comp_bond.comp_id
_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
_chem_comp_bond.type
_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_esd
 ALA      N    H       single     0.860    0.020
 ALA      N    CA      single     1.458    0.019
 ALA      CA   HA      single     0.980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



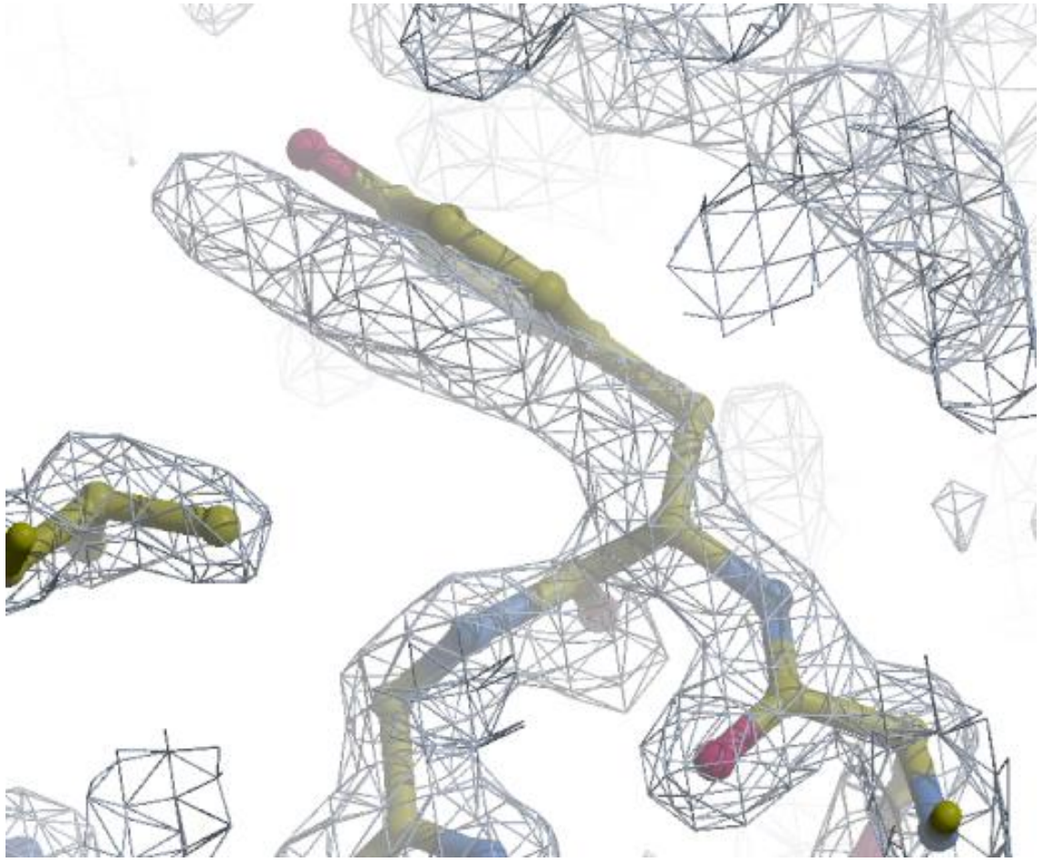
distorted geometry



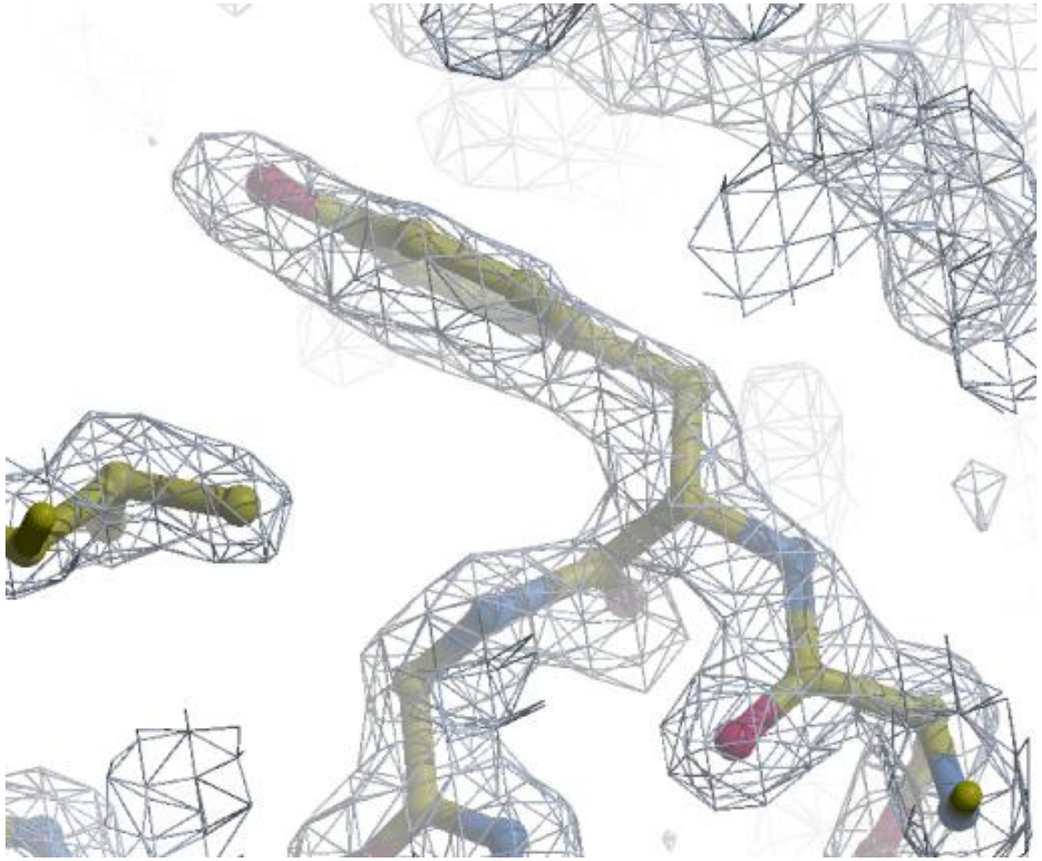
mainchain atoms connect



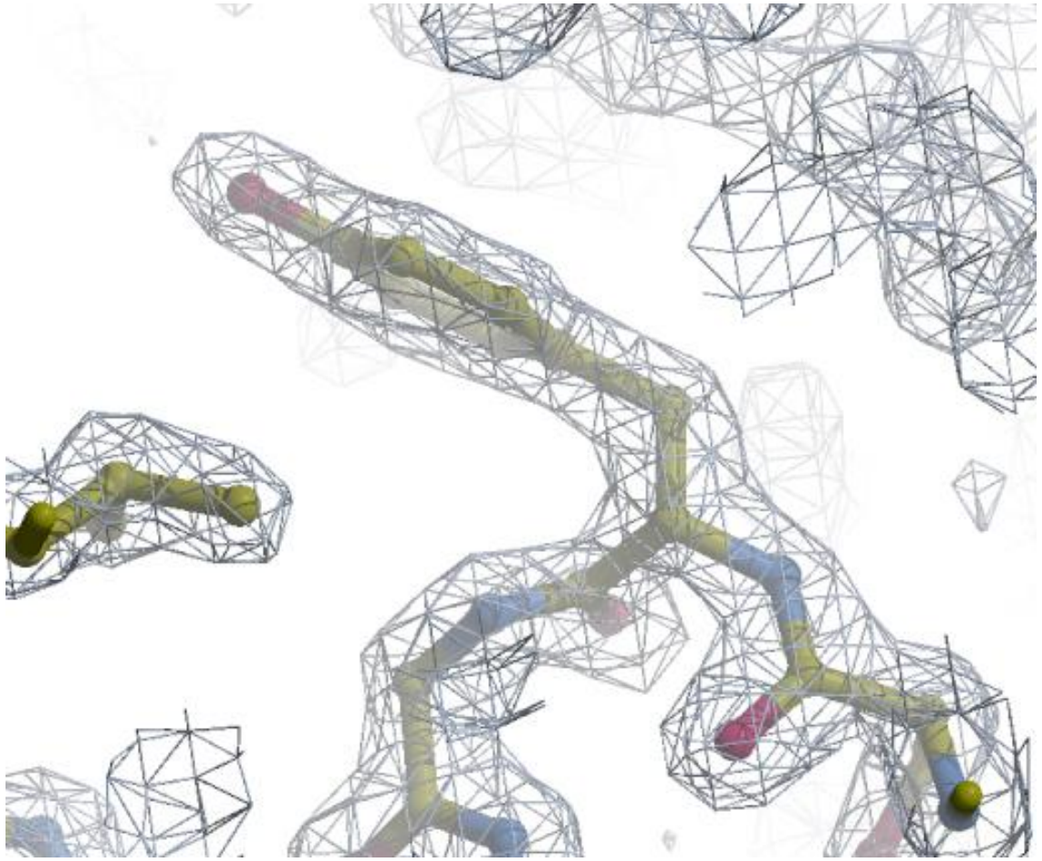
refinement
gradients



after a few cycles of refinement



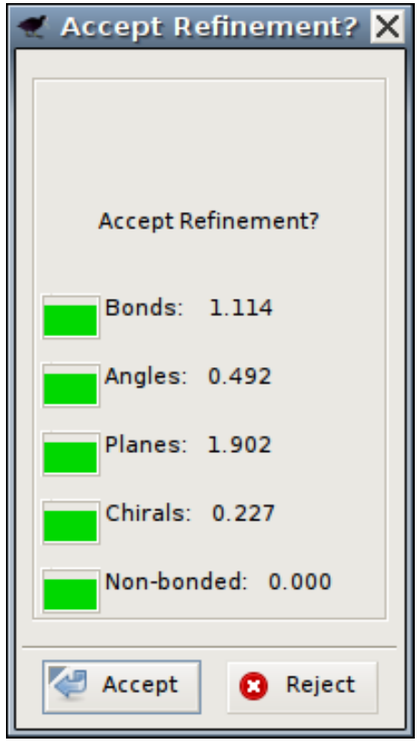
more cycles

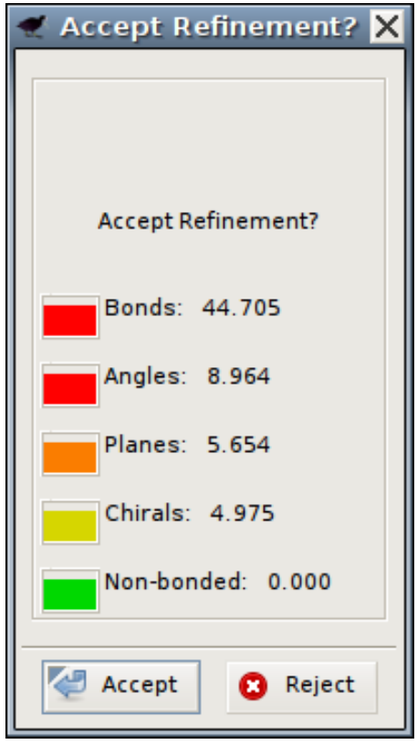


convergence

Results: traffic lights

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





Refinement techniques

- Auto-zone
- Single-Atom Drag
- ~~Over-dragging~~ Obsolete! Instead: pink stick, green peas
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept
  - Sphere refinement

- Ramachandran Refinement

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!

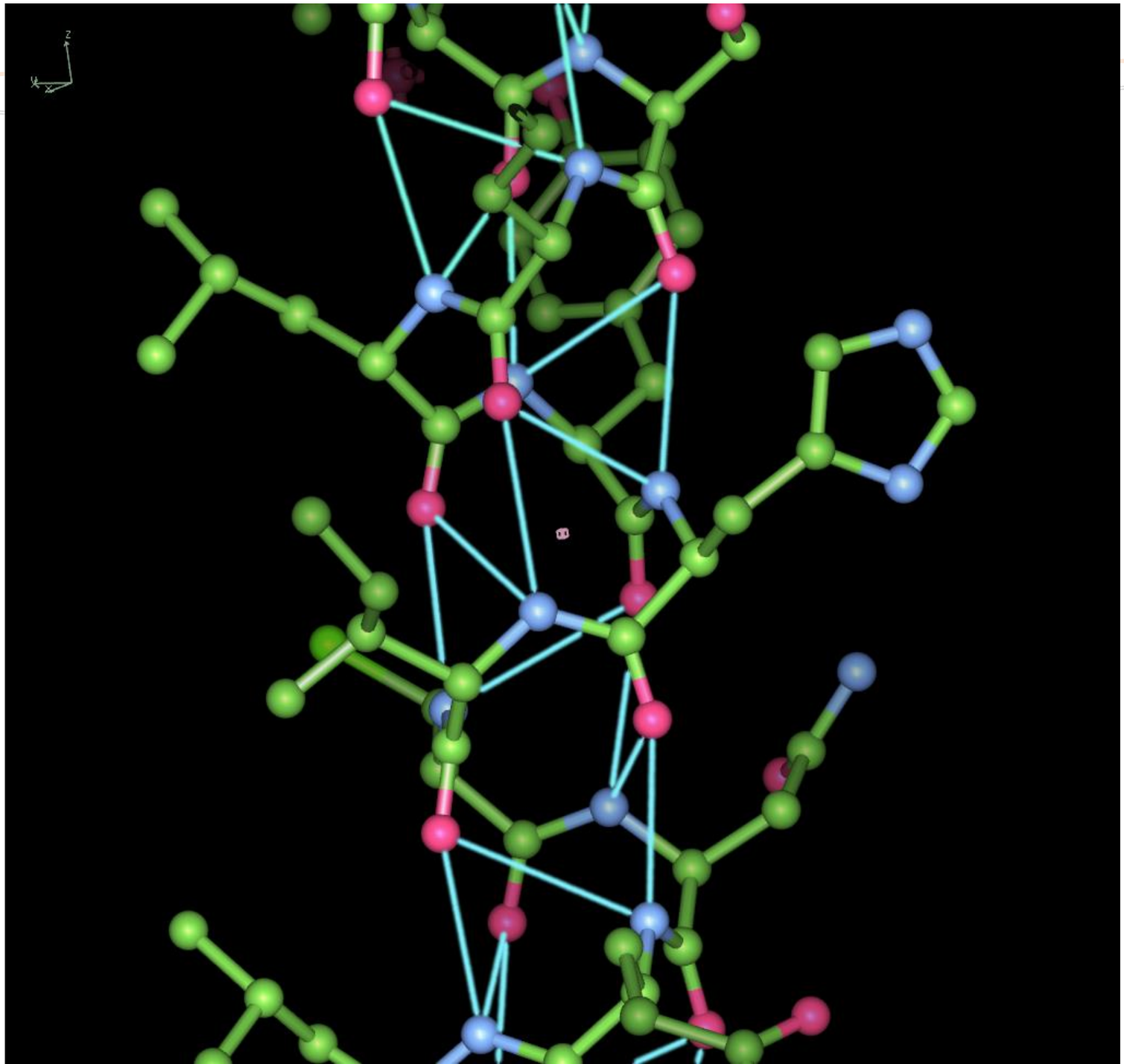
Pink Stick, Green Peas

- new real space refinement mode
- new feel: instead of rubber sheet movement, atoms are refined dynamically
- gentle tugs instead of yanking of atoms
- Pink Stick:
  - shows the difference between where an atom is and where you want it to go.
  - should be short and often difficult to see. If you see several long pink sticks, then that's usually a sign that something has gone wrong.
- Green Peas: markers for Ramachandran probabilities

demo?

ProSMART interface

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




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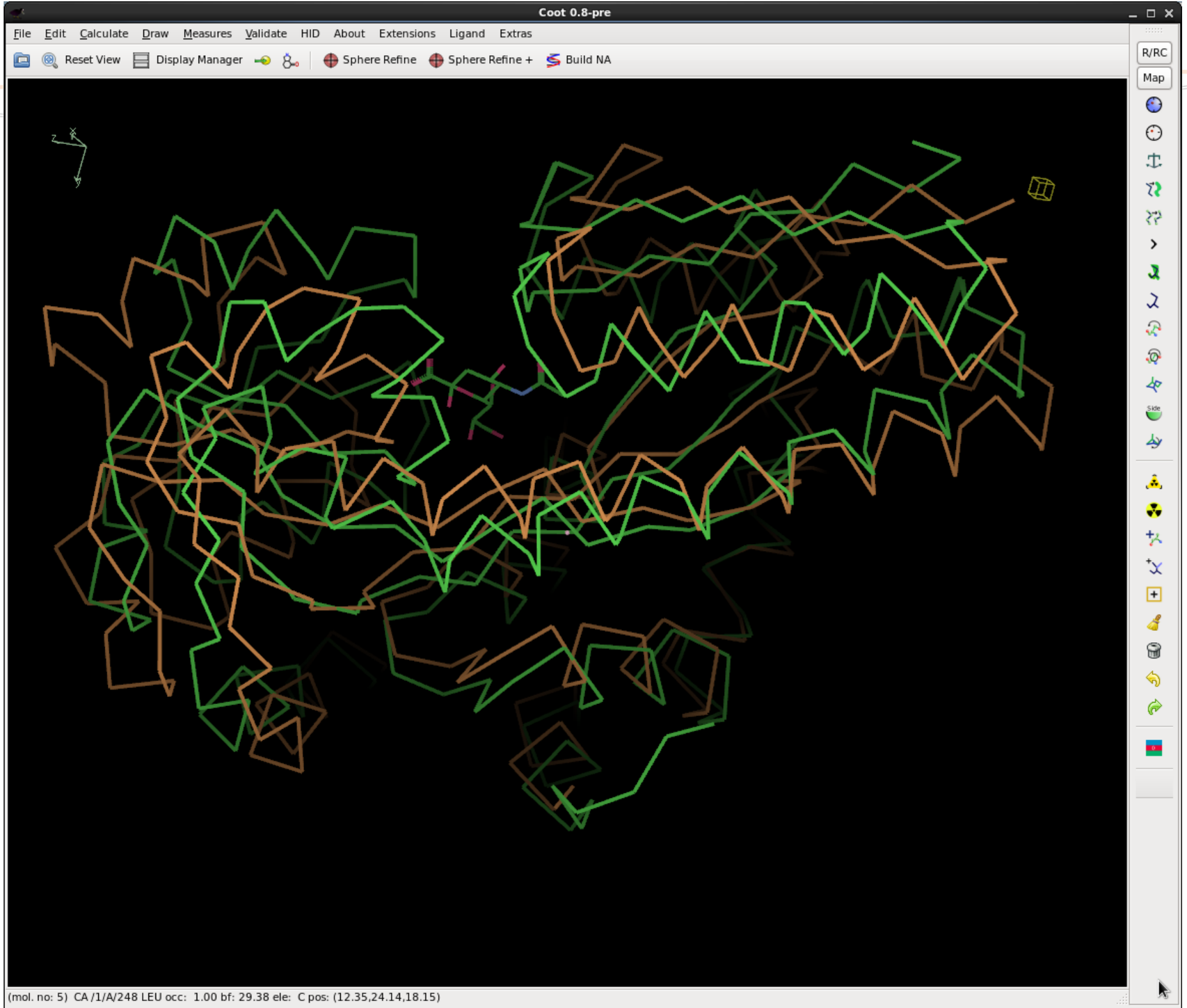
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)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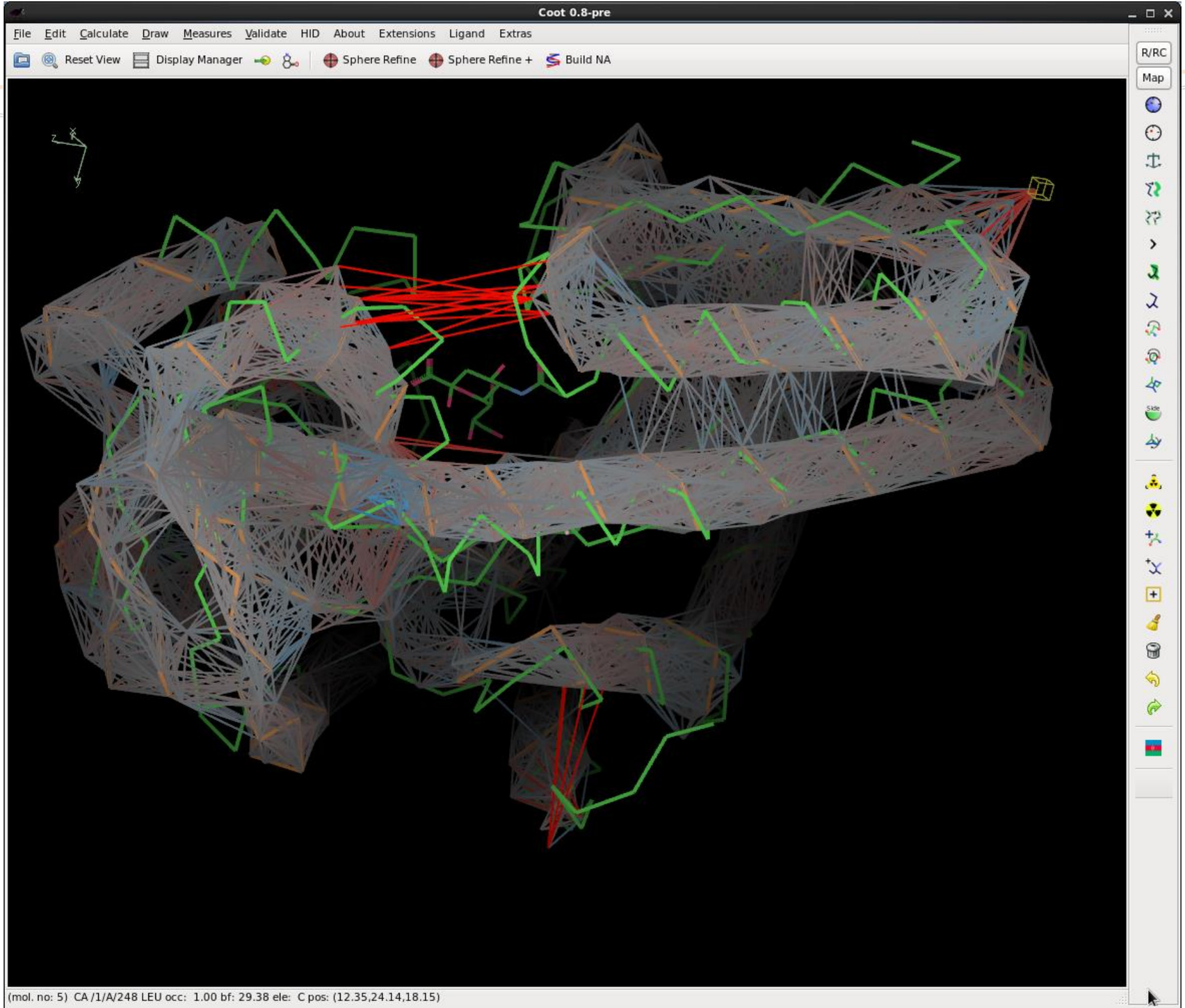
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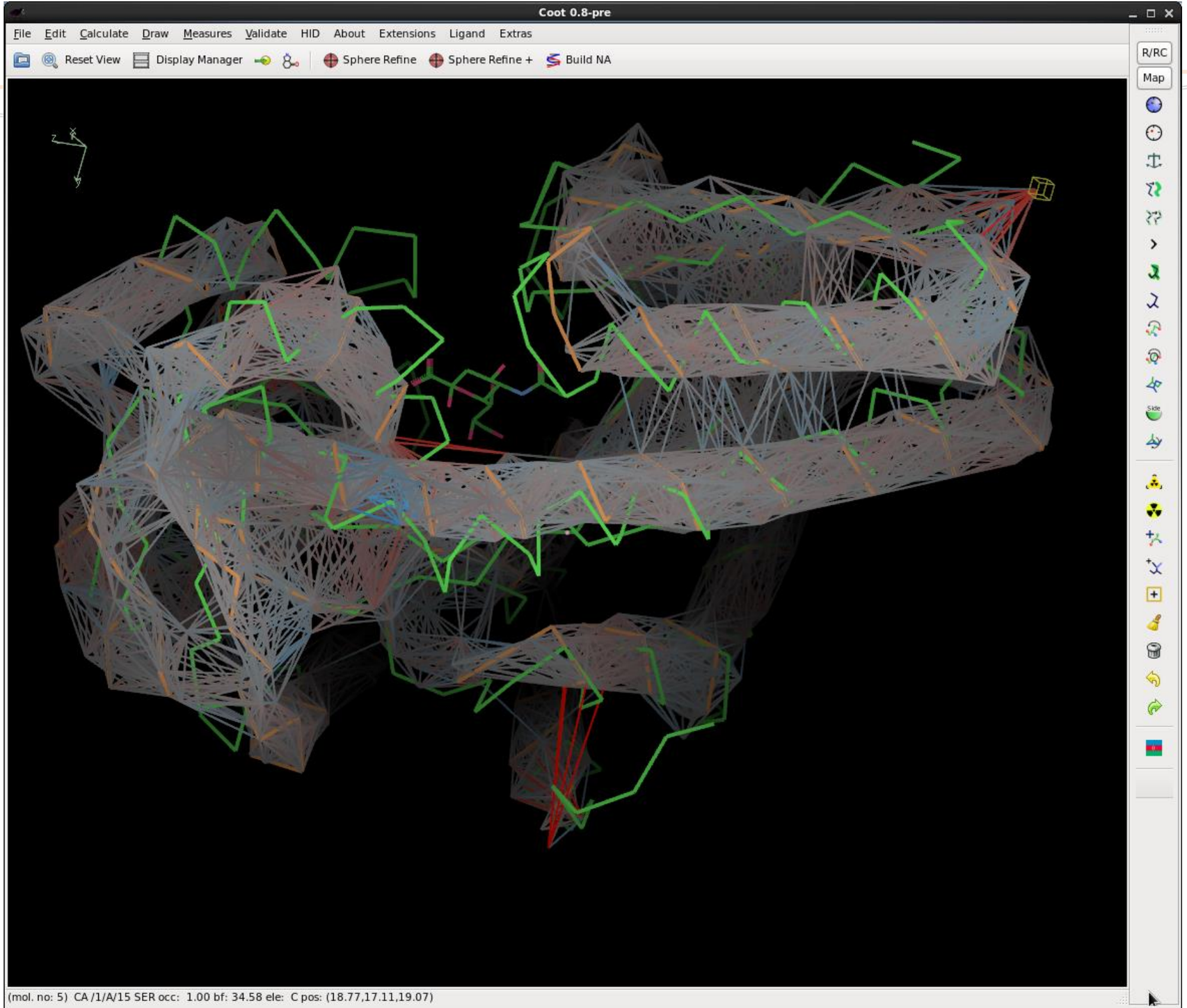
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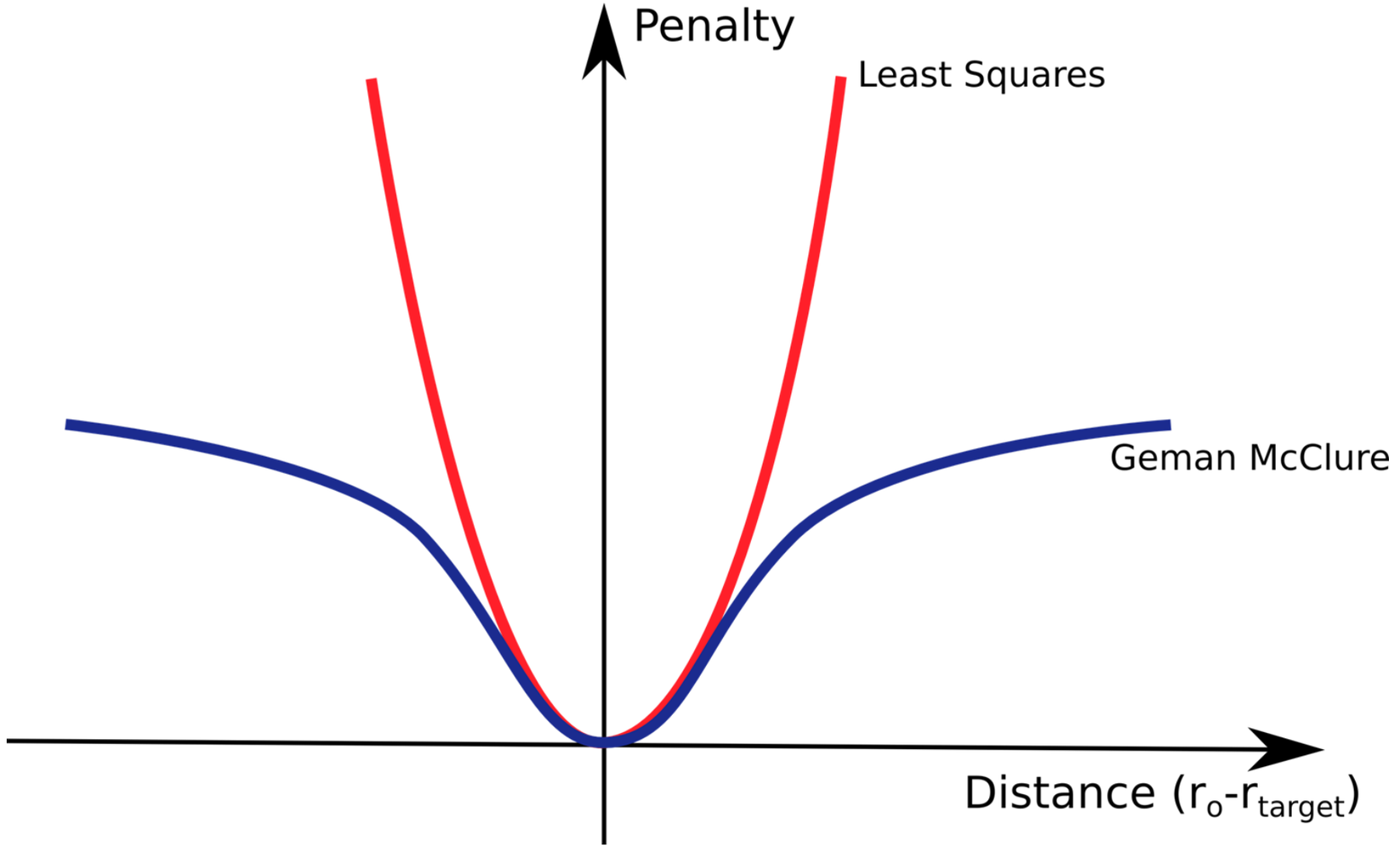
Modified Target Function



Chain Refinement

- a powerful low resolution fitting tool
- refines all atoms of a chain
- ProSMART restraints are recommended (self or external)
- particularly useful with the new **proportional editing** function (Ctrl+scroll for changing the radius)
- Ctrl+scroll to adjust the radius

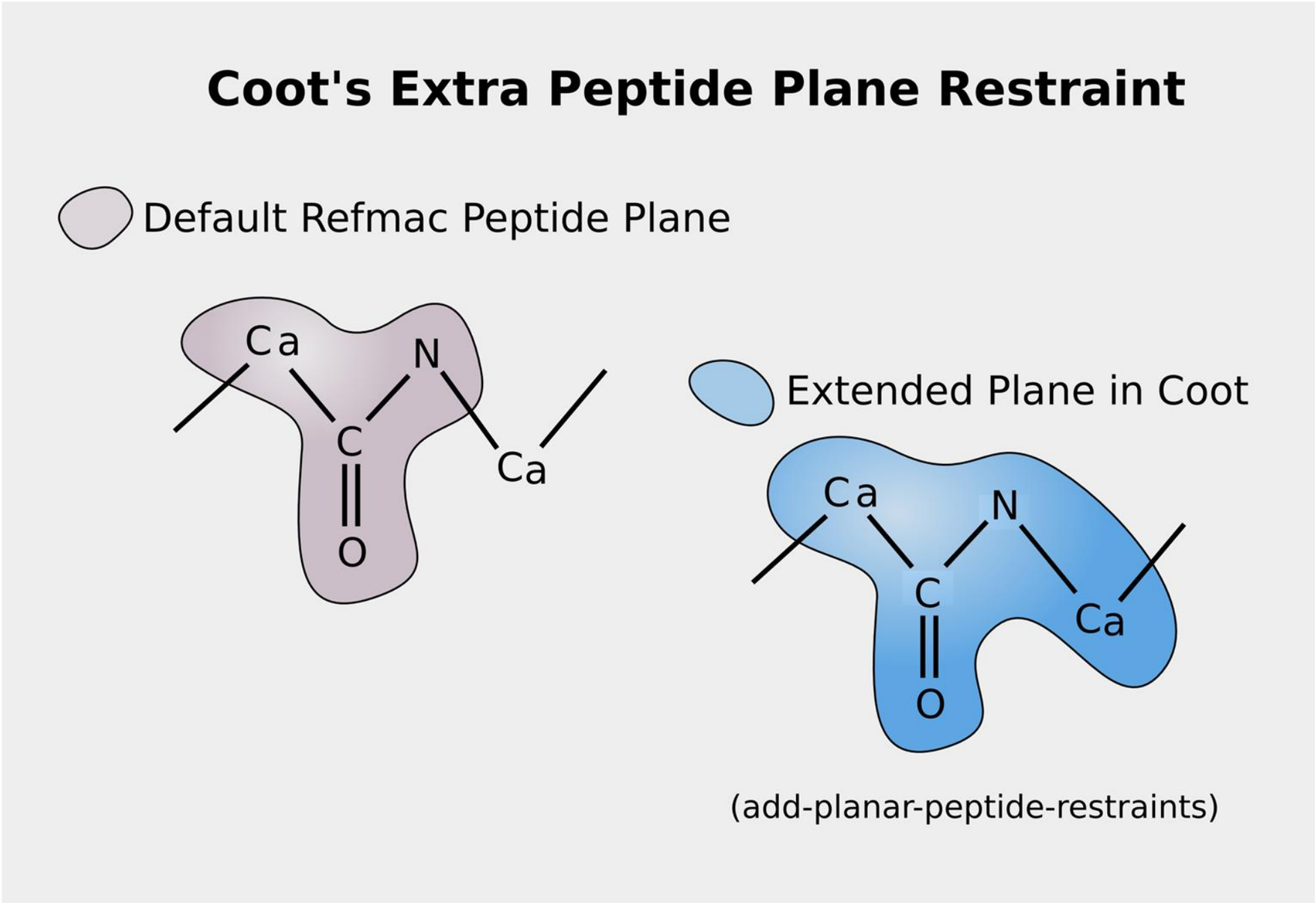
demo?

Tools for low resolution

Tools for low resolution

- Additional restraints
  - Peptide plane restraints
  - Ramachandran restraints
  - Secondary structure restraints
  - ProSmart interface
- Backrub rotamers
- Jiggle Fit
- Map sharpening

Additional 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

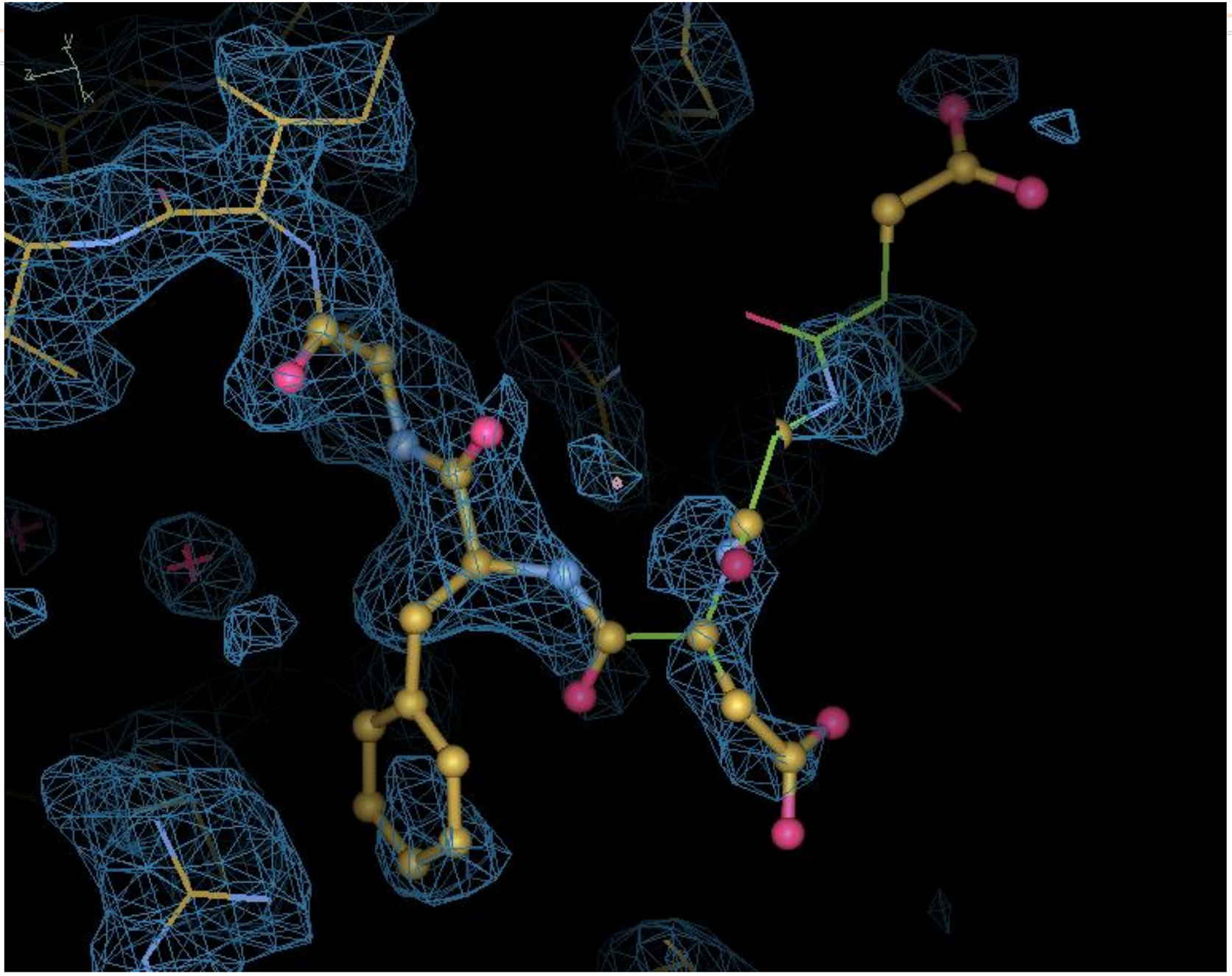
- Controversial Feature?

  Ramachandran Plots have been used for “validation” - but here we are deliberately optimizing them

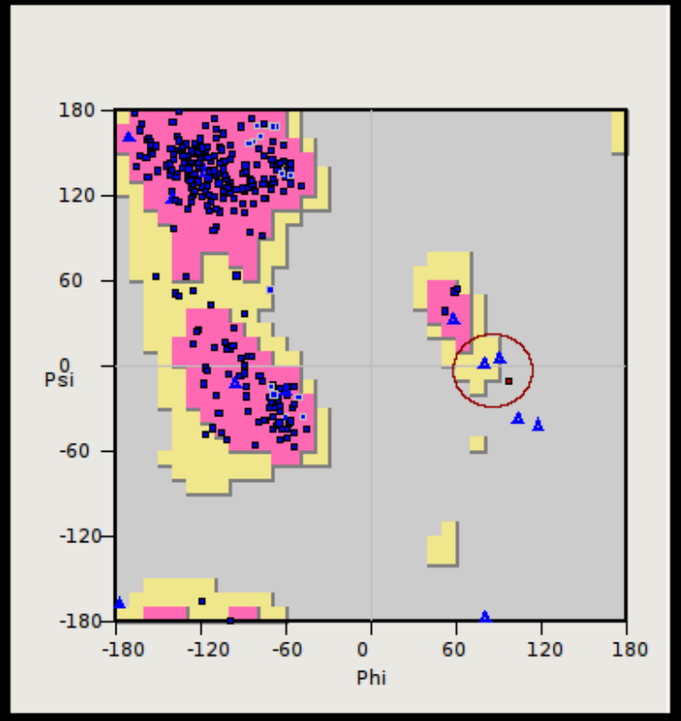
- But to quote Jane Richardson:

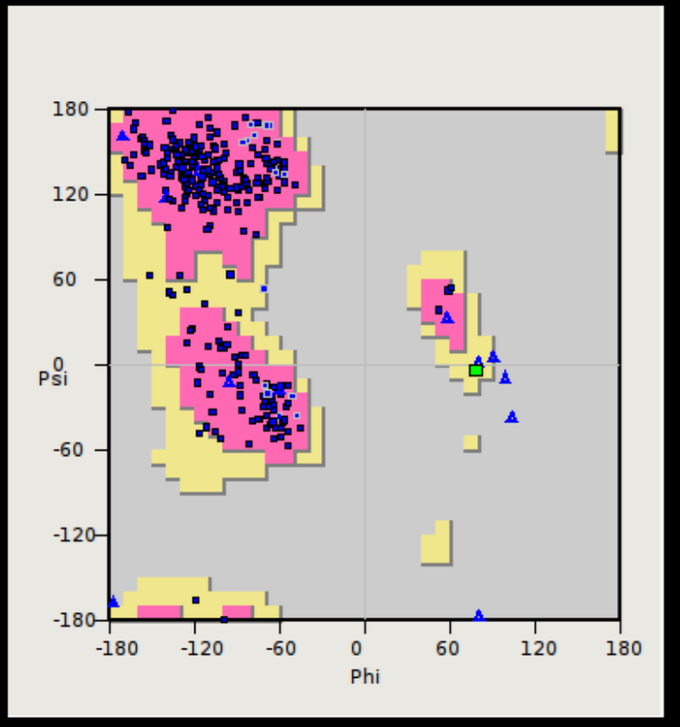
  Do you want a better structure – or a better idea of the quality of your structure?

- Ramachandran Plots can be added to the geometry target function

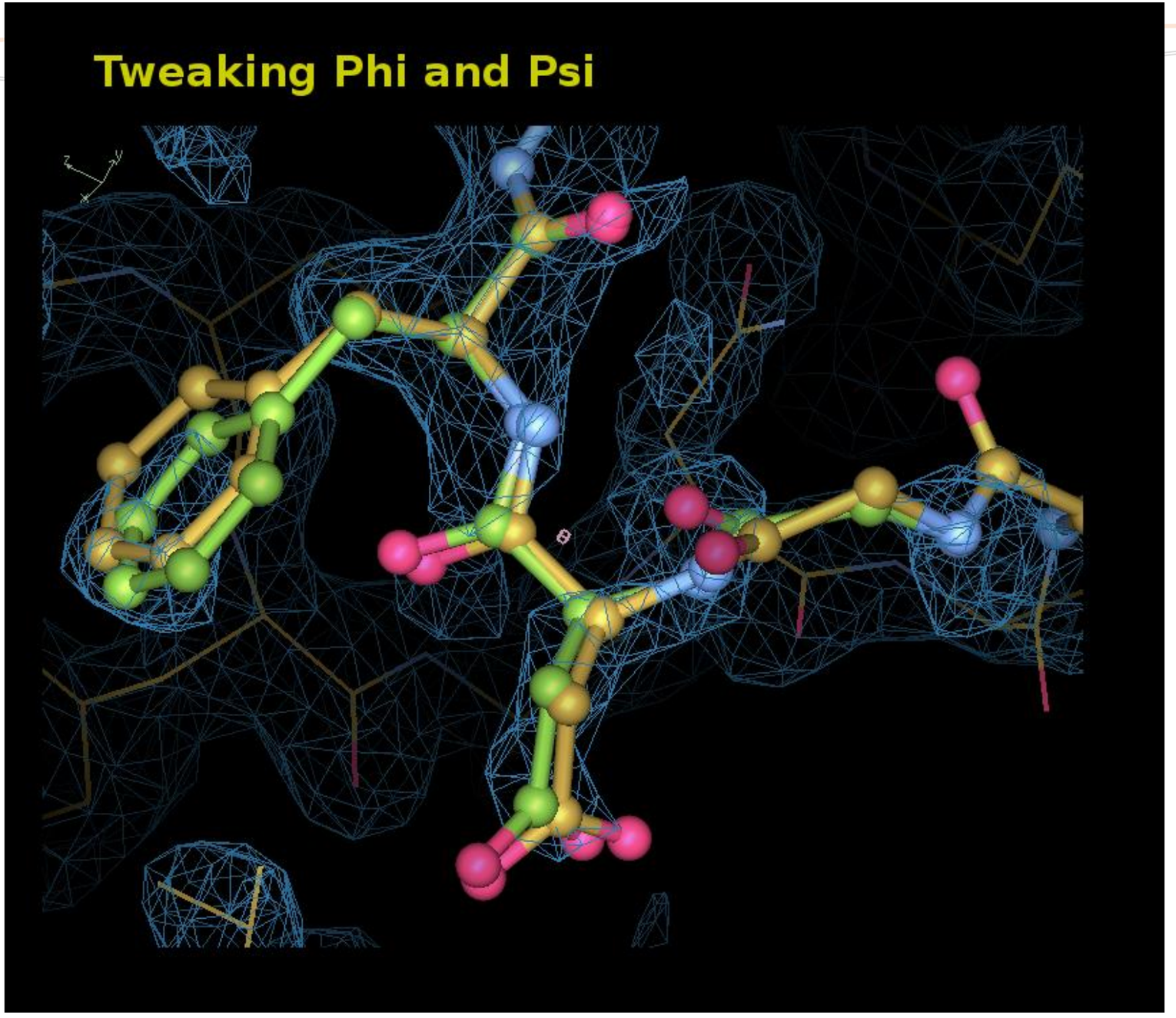


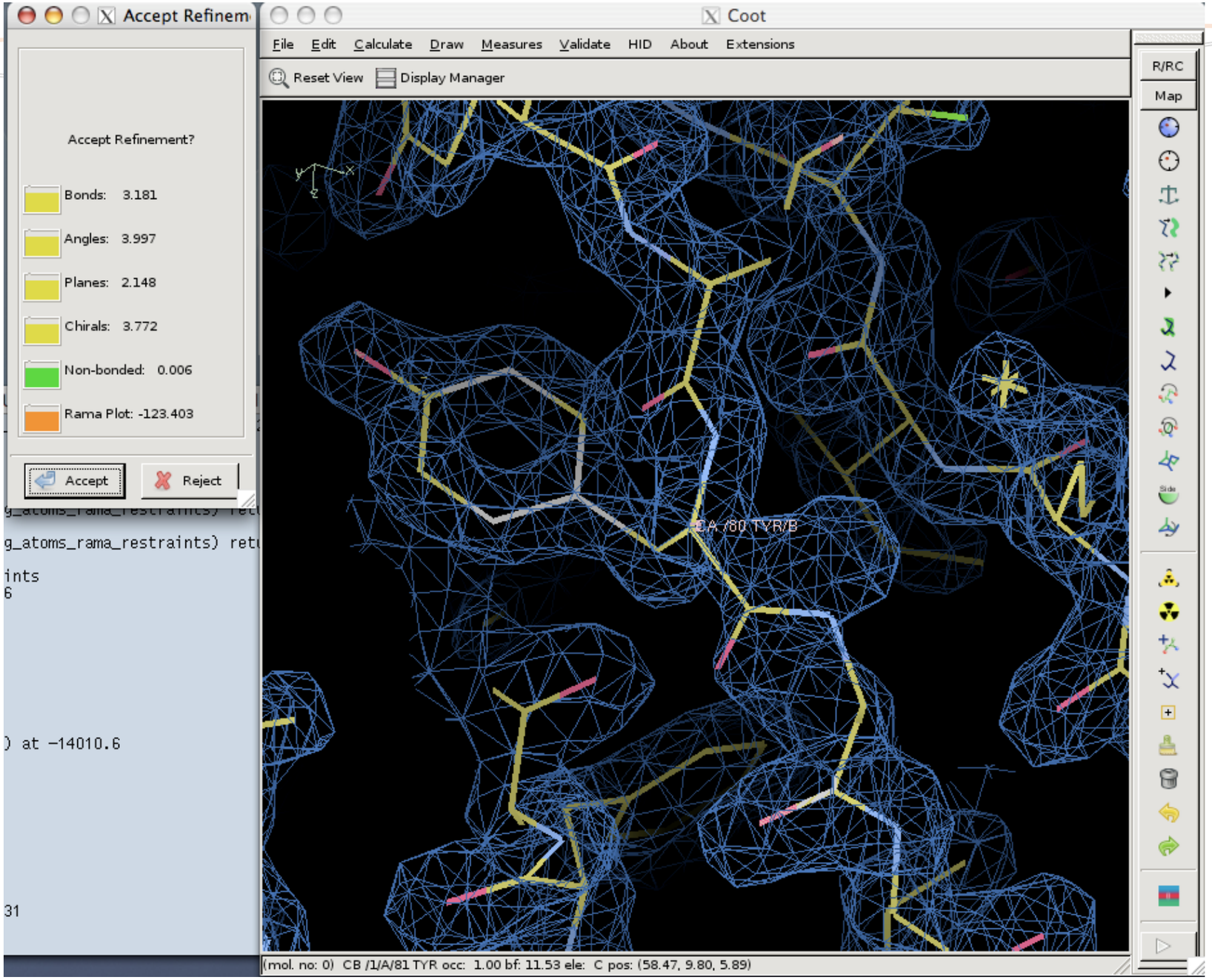
Tweaking a Ramachandran Outlier





Tweaking Phi and Psi

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Planes: 2.148
Chirals: 3.772
Non-bonded: 0.006
Rama Plot: -123.403
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Reject
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) at -14010.6
31
Coot
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R/RC
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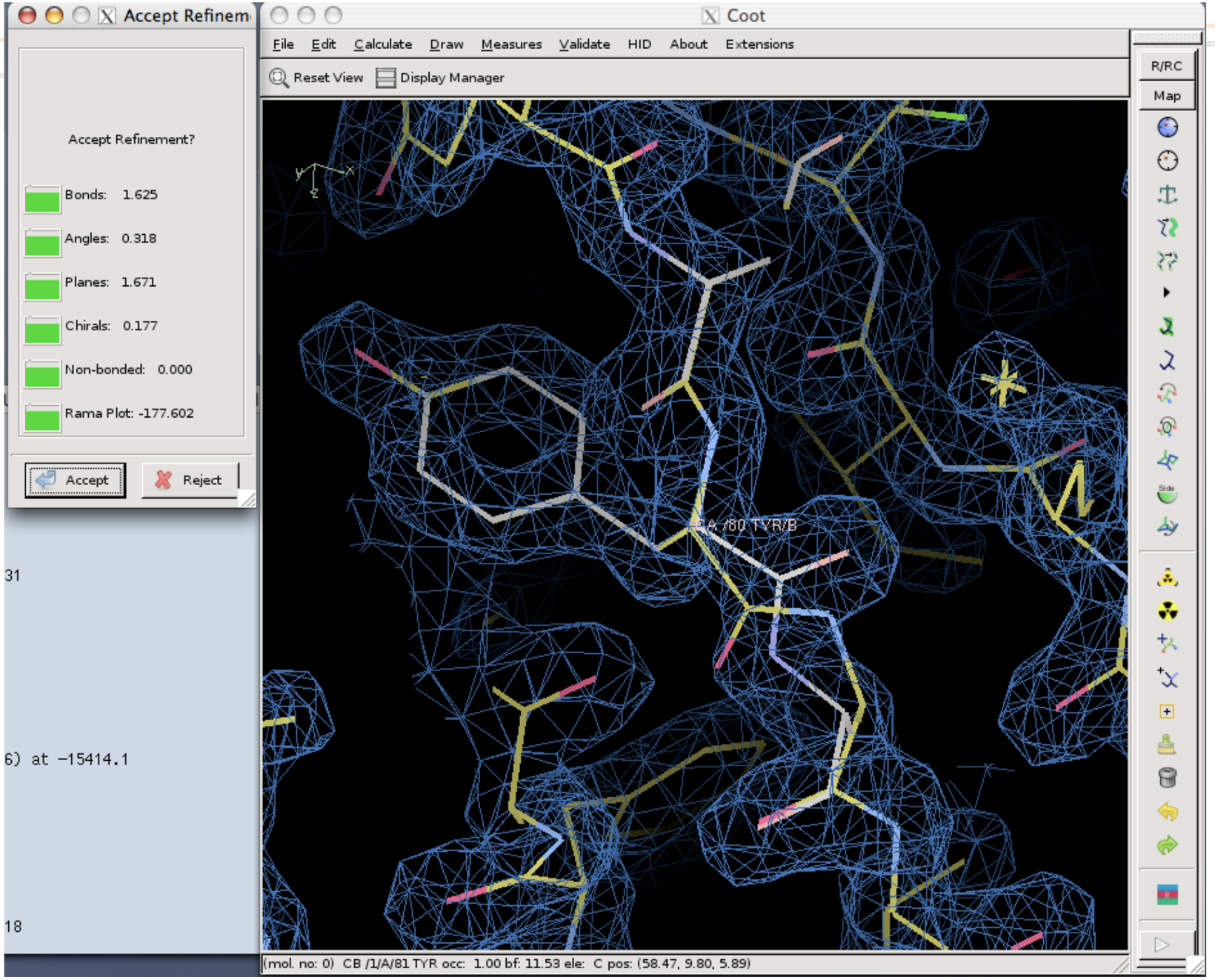
(mol. no: 0) CB /1/A/81 TYR occ: 1.00 bf: 11.53 ele: C pos: (58.47, 9.80, 5.89)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Accept Refinement?
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Angles: 0.318
Planes: 1.671
Chirals: 0.177
Non-bonded: 0.000
Rama Plot: -177.602
Accept
Reject
Coot
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

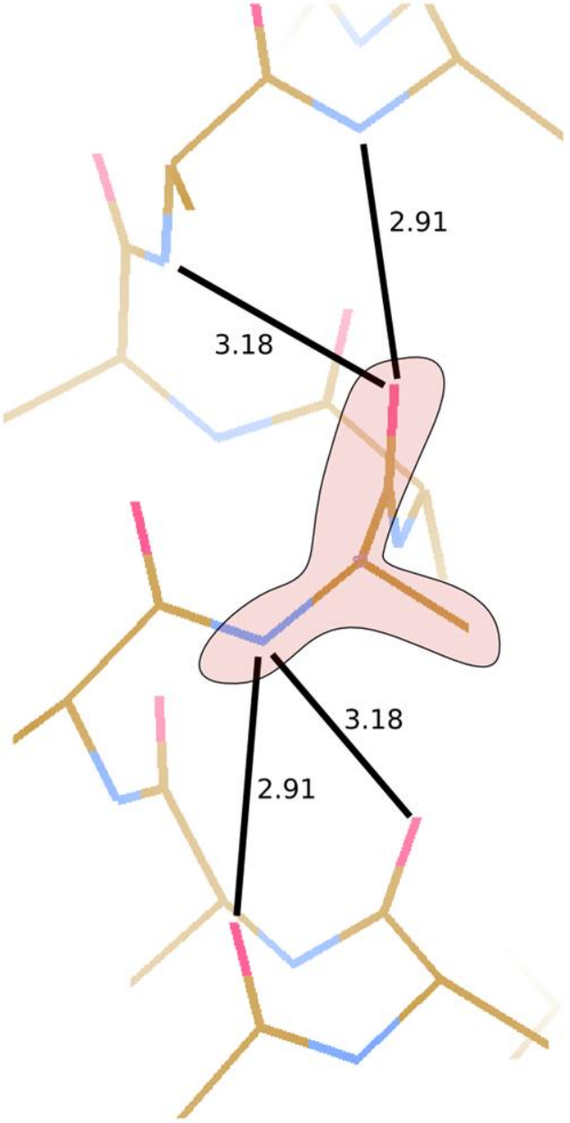
Torsion angle restraints

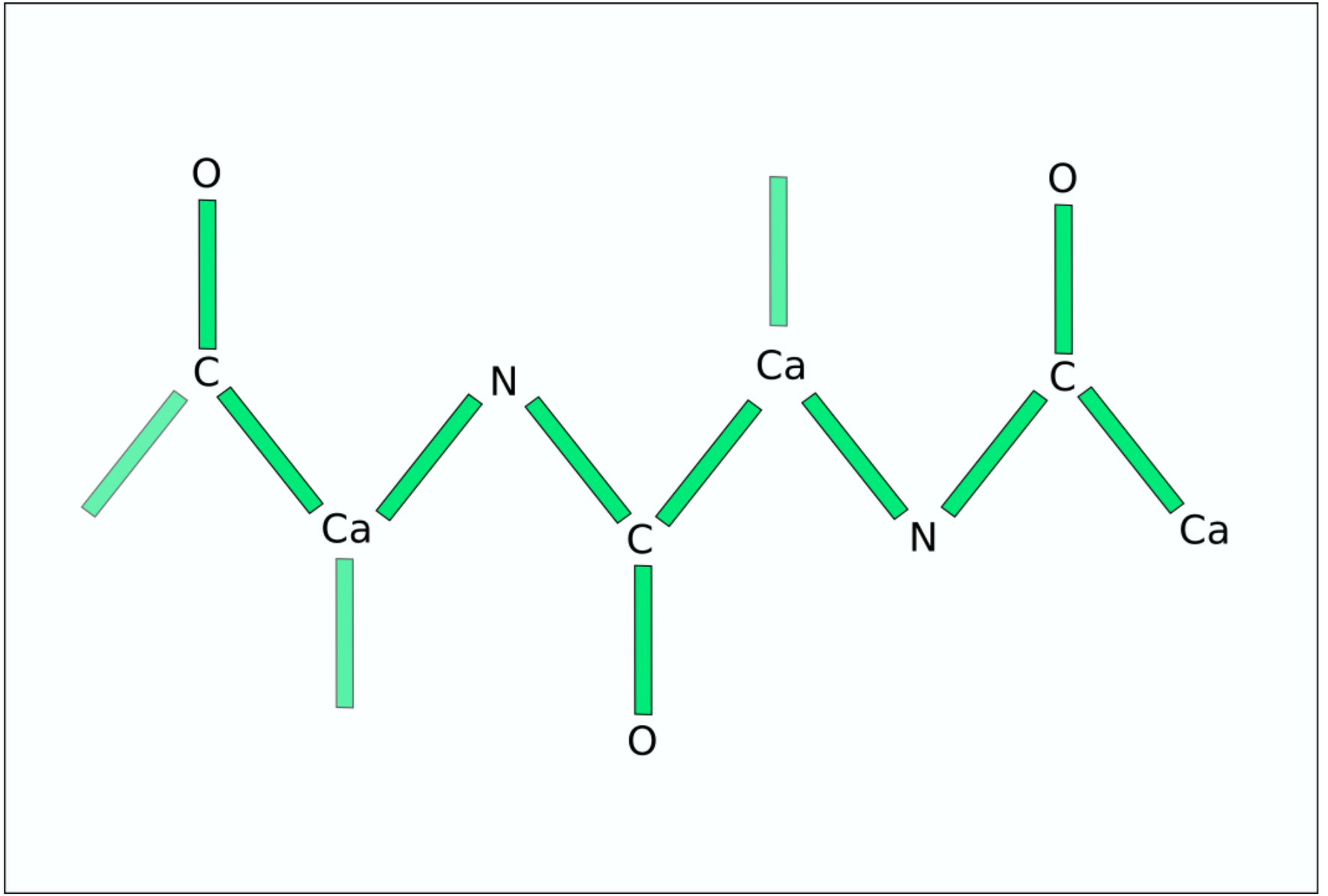
- Torsion angle refinement is slow (relatively)
- Simple addition of these restraints to the geometry target function often makes the region “stuck and unsatisfied”

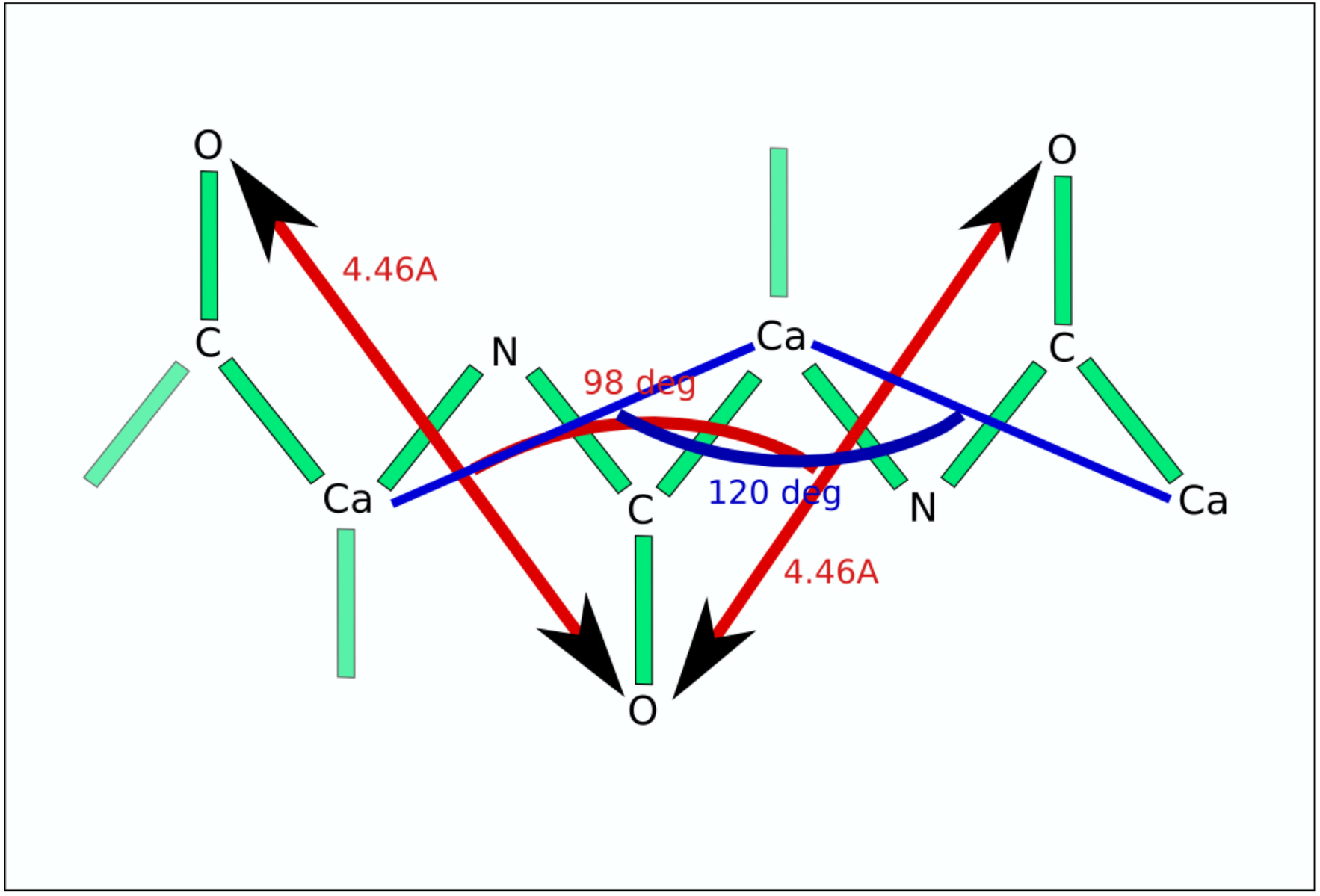
  i.e. trapped in local minimum
  → apply pseudo-bond restraints insead

Alpha Helix pseudo-bond restraints

Restrain the Hydrogen-bonding atom distances




O
C
Ca
N
C
O
Ca
N
C
O
Ca


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

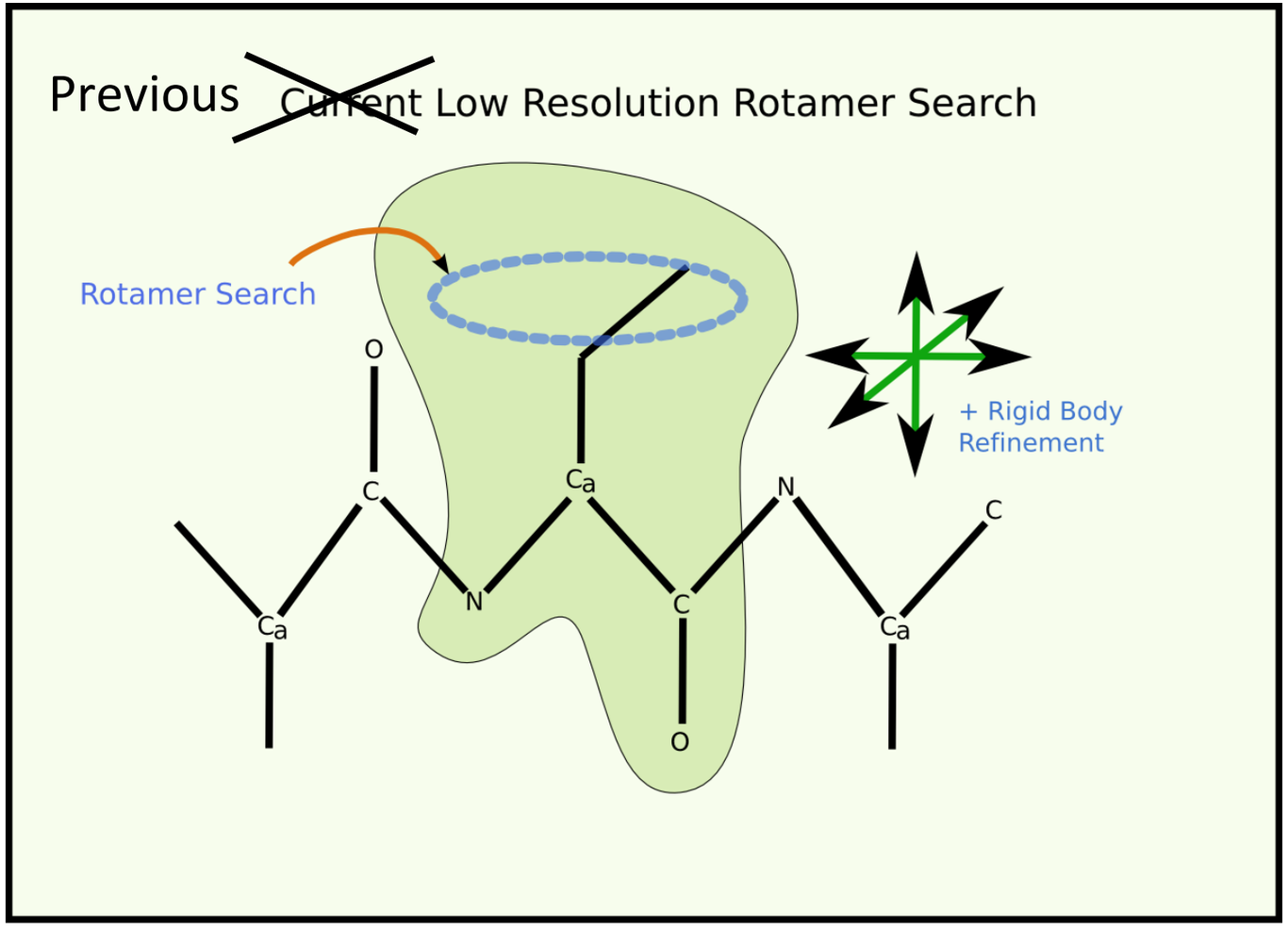
Backrub Rotamers

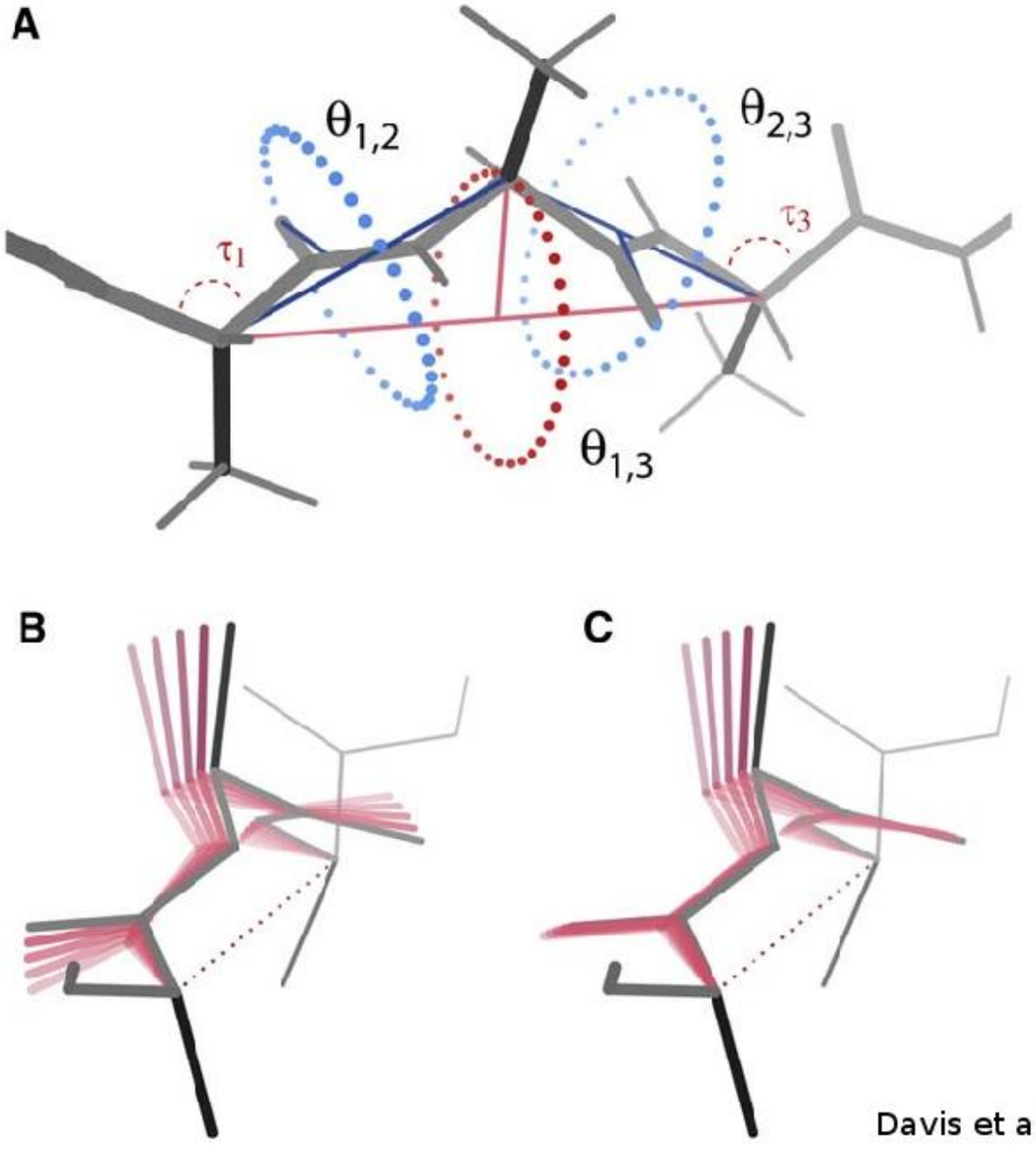
- High probability models with low resolution data
- Rotamers are preferred configurations of a side-chains rotatable bonds, where “preferred” means these configurations occur more frequently in a set of reference protein structures
- “preferred” because they are low-energy conformations
- Several Rotamer “databases” exist

  best: (Son of) Penultimate Rotamer Library

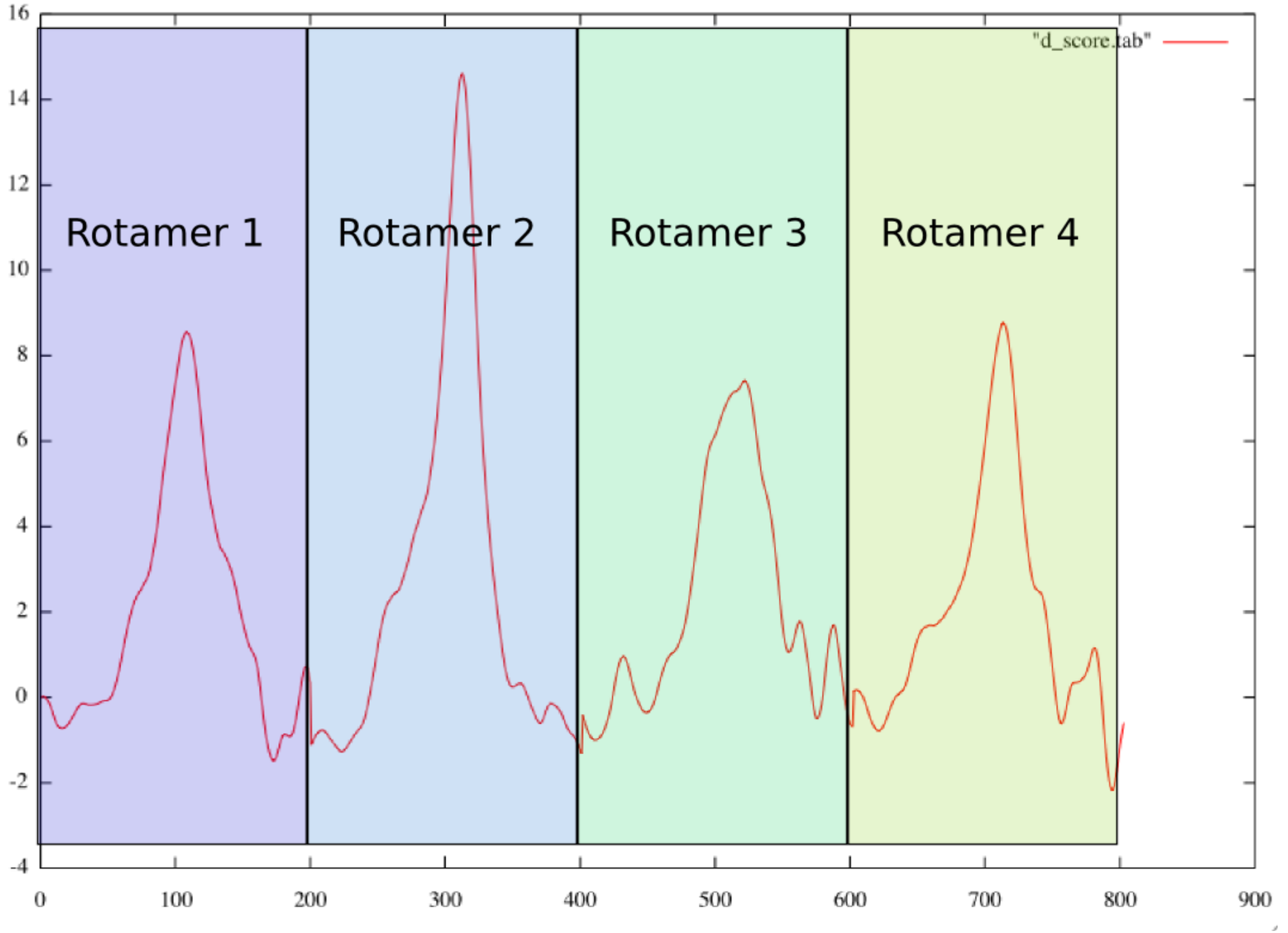
- To activate it:
  - (ROTAMERSEARCHLOWRES)
  - Default for resolutions worse than 2.6A
  - Add a toolbar button (right click on bar…)

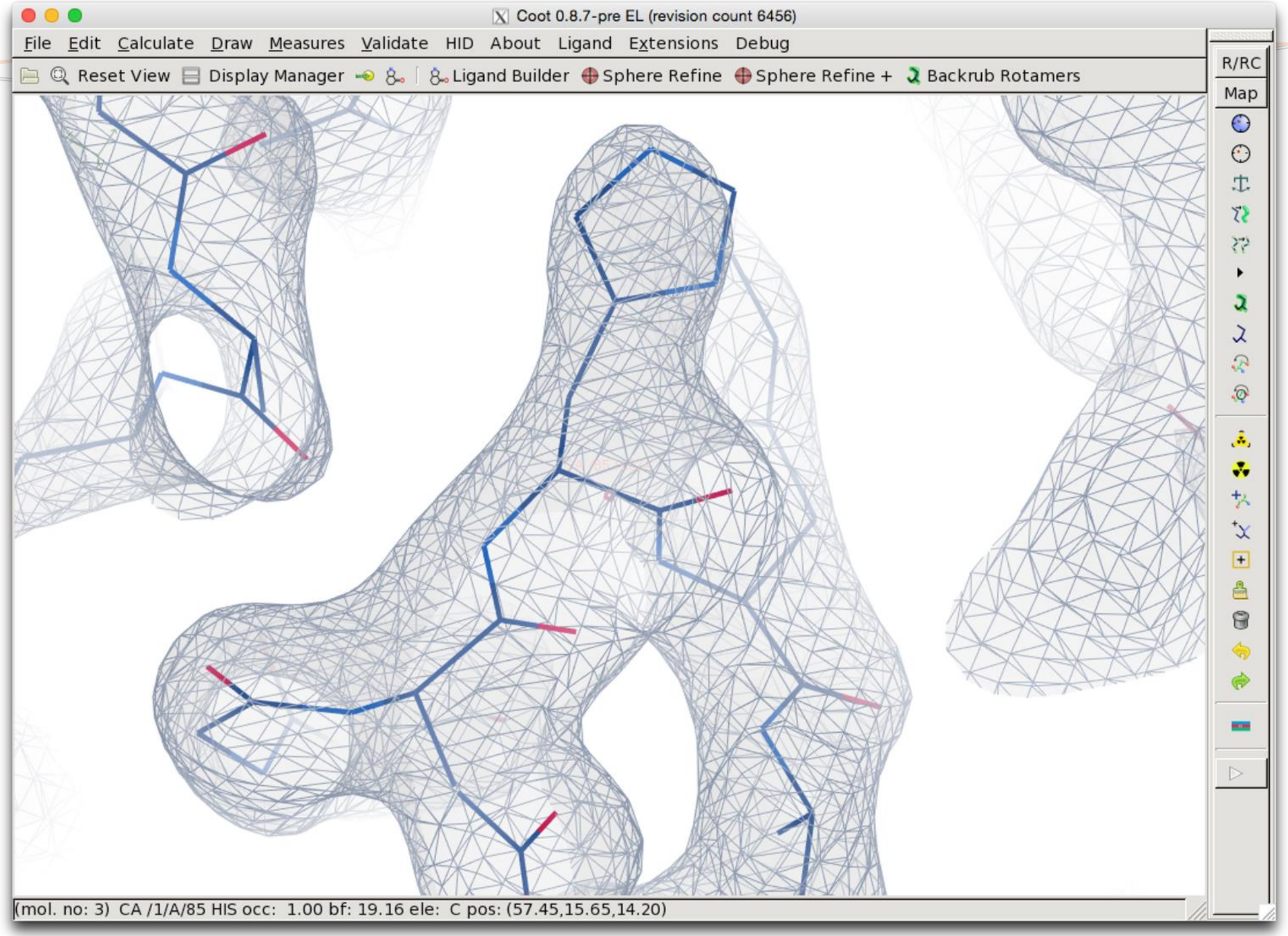
Backrub Rotamers

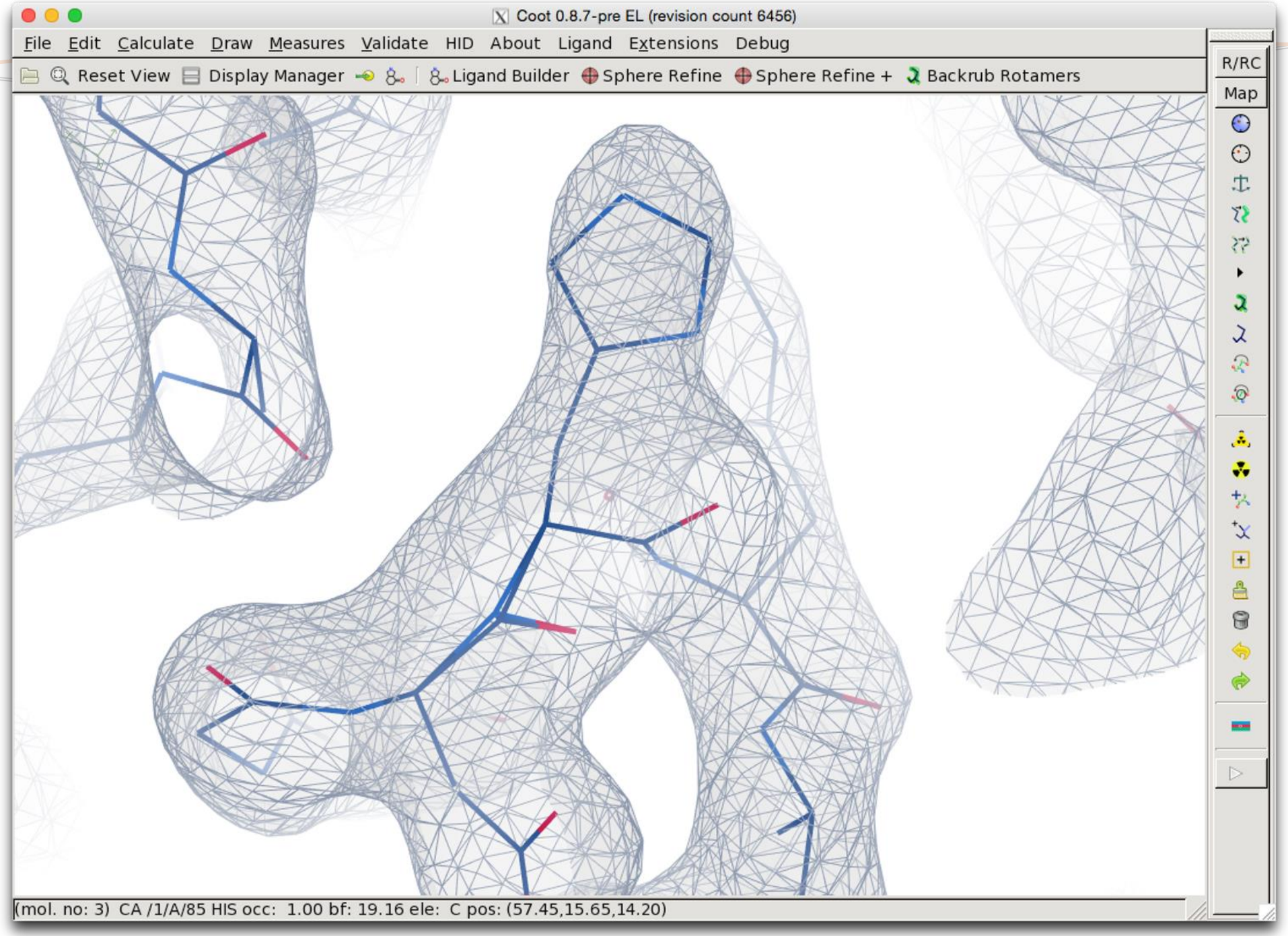




Davis et al. (2006) Structure


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


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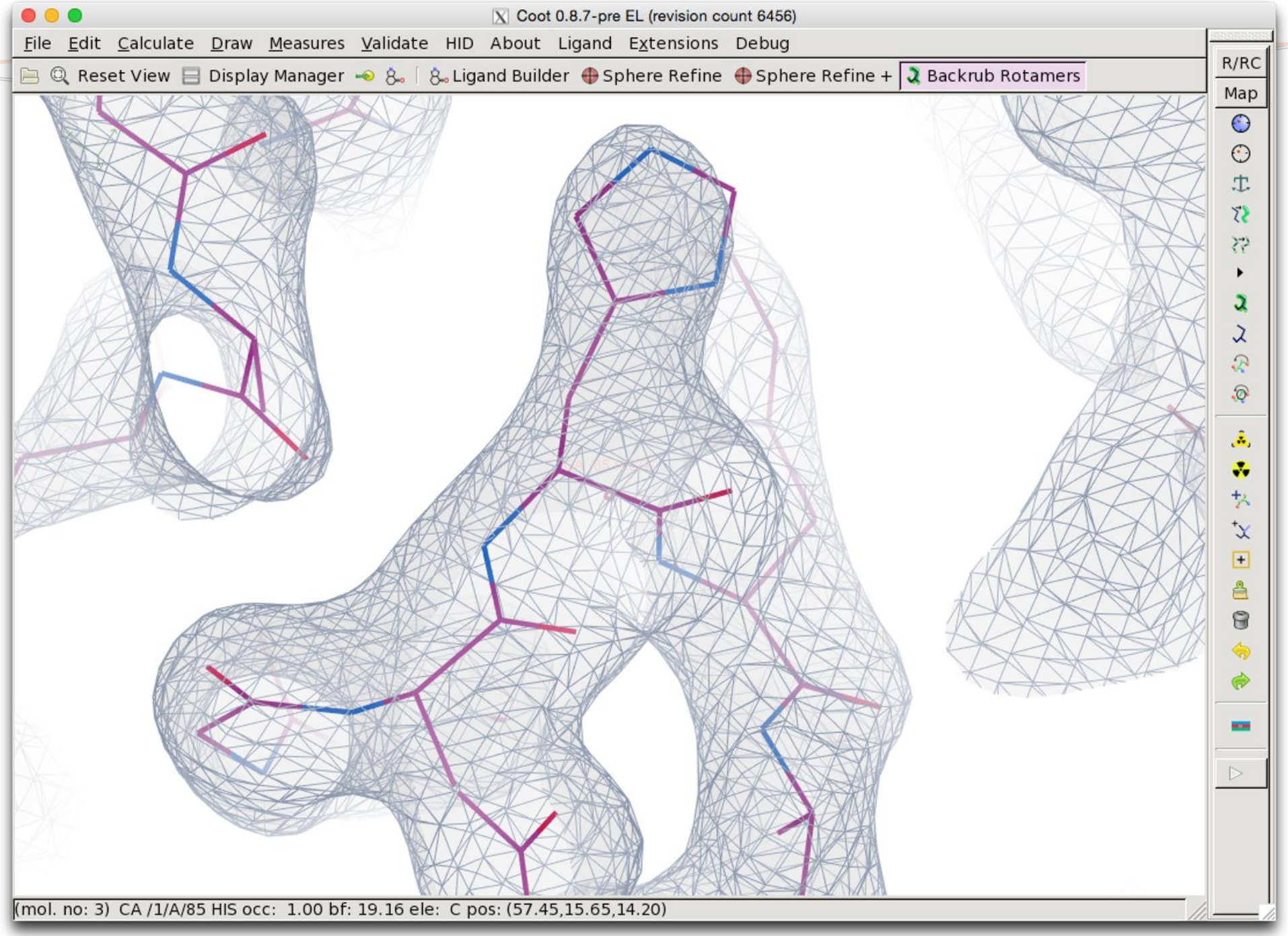
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)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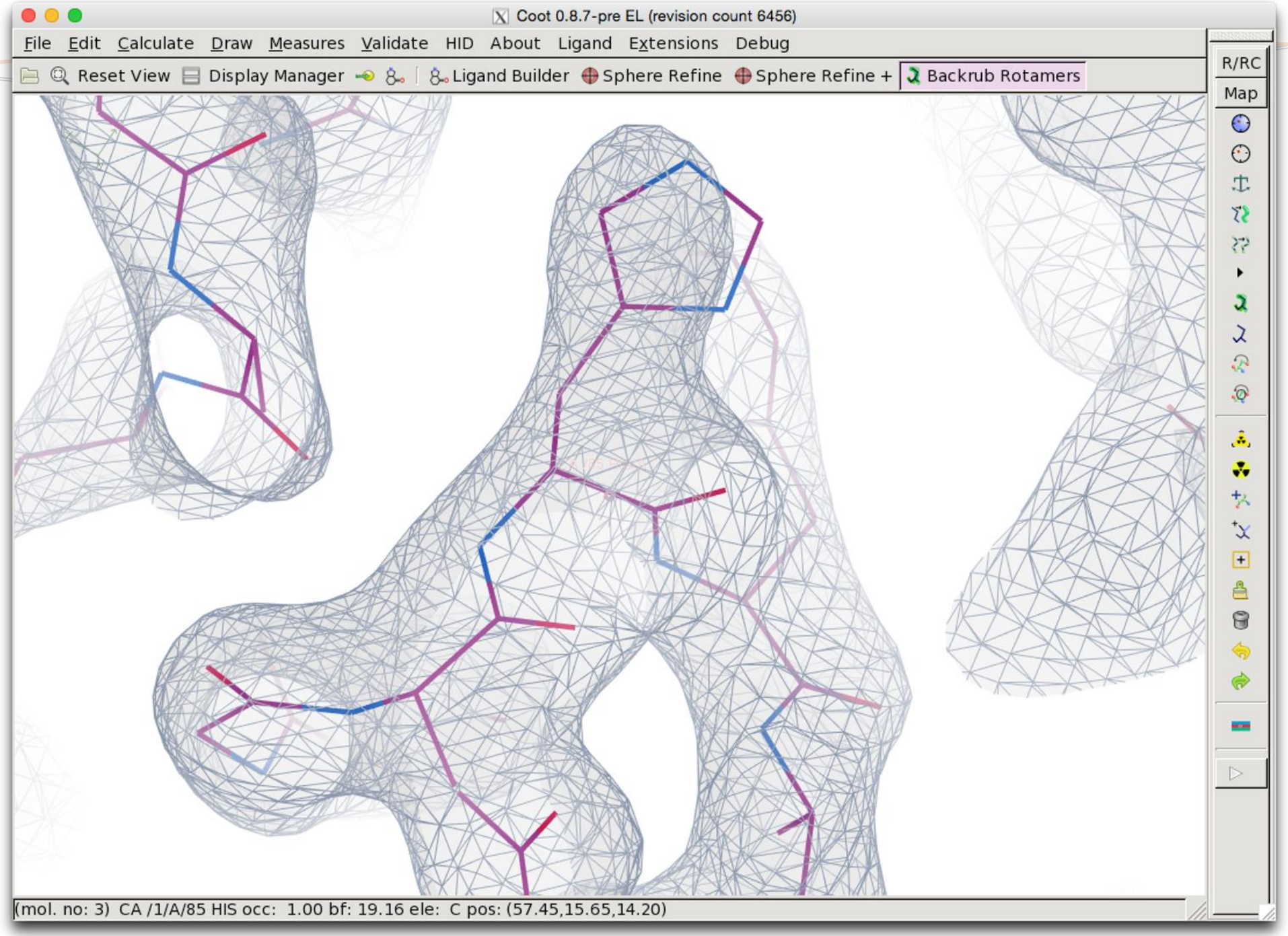
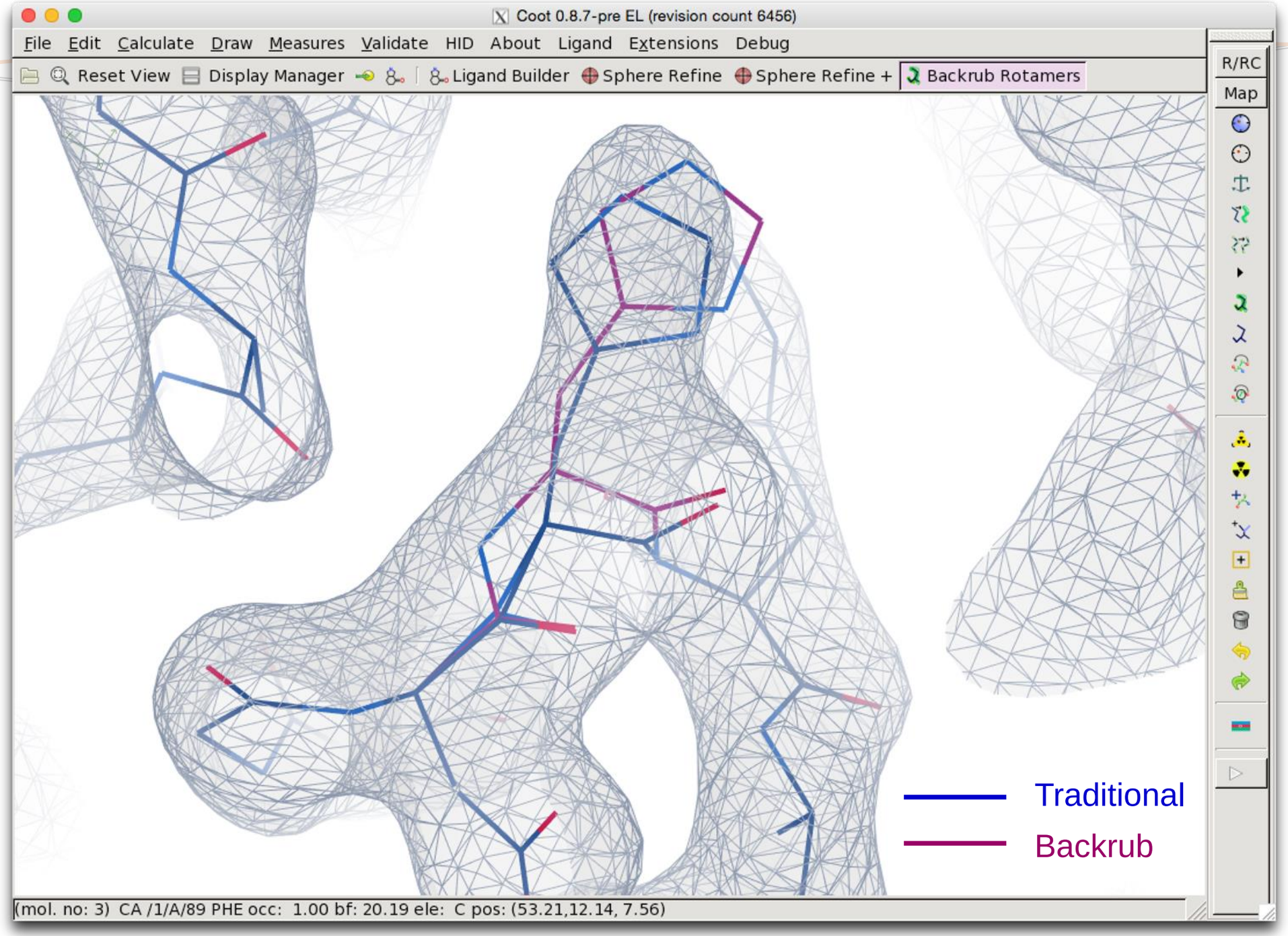
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


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


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

Jiggle Fit

- How do I rotate and translate these atoms to fit the density?

  6-dimensional problem

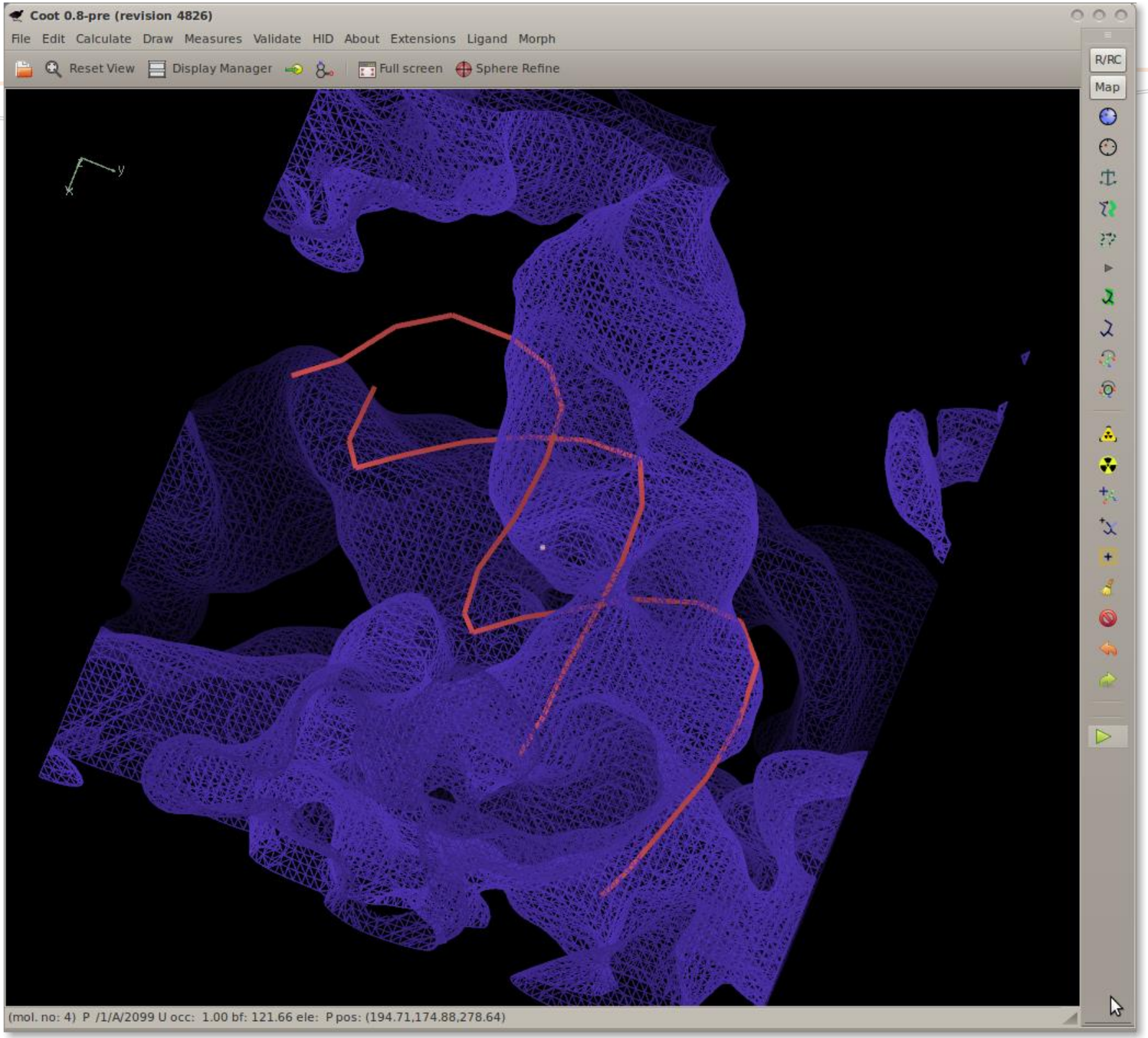
- Originally used to fit simple ligands/solvent molecules to blobs of density (with more or less success)

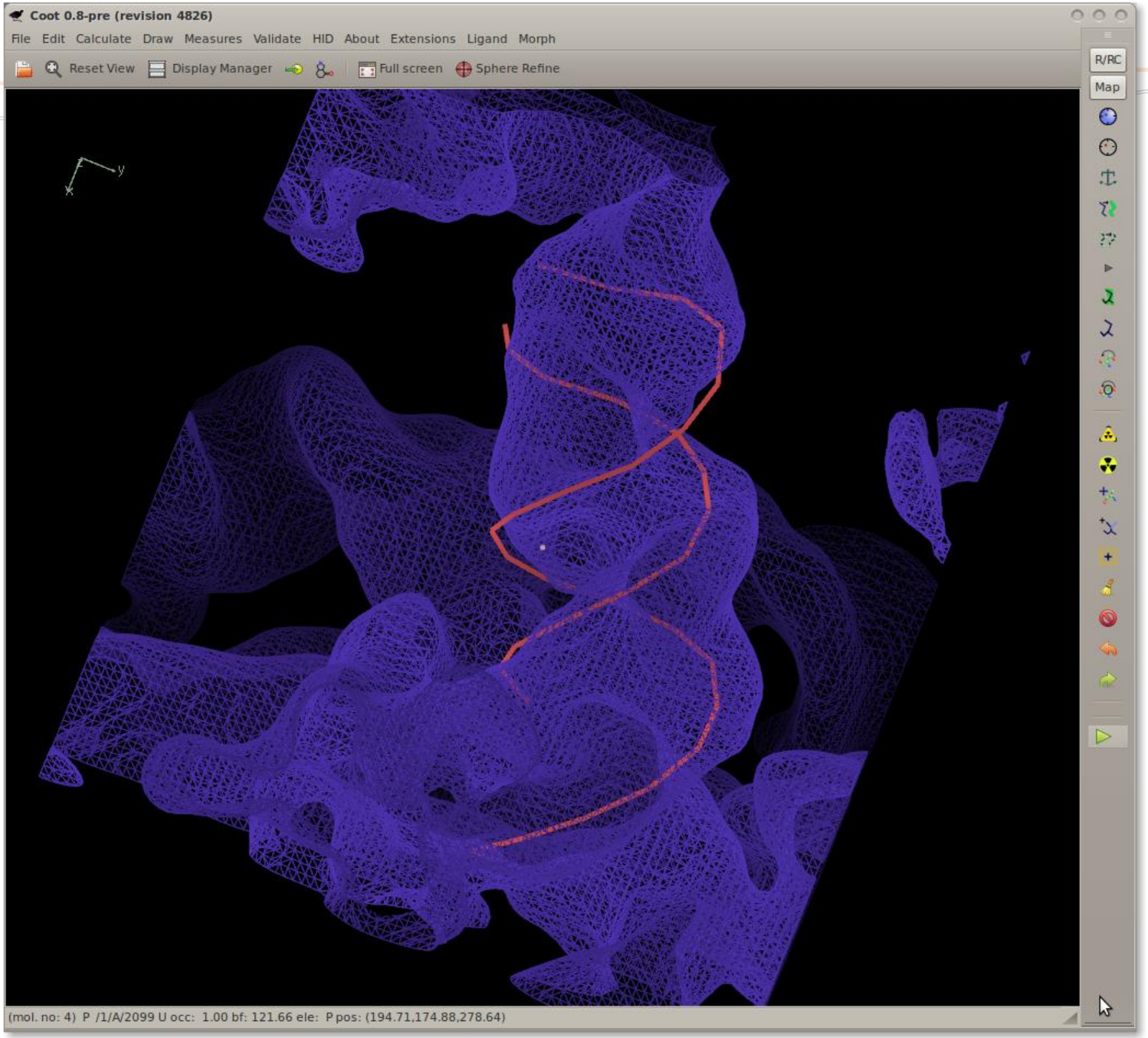
- Now extended to fit arbitrary atom selections

  e.g. by Chain

Jiggle Fit

- 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
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)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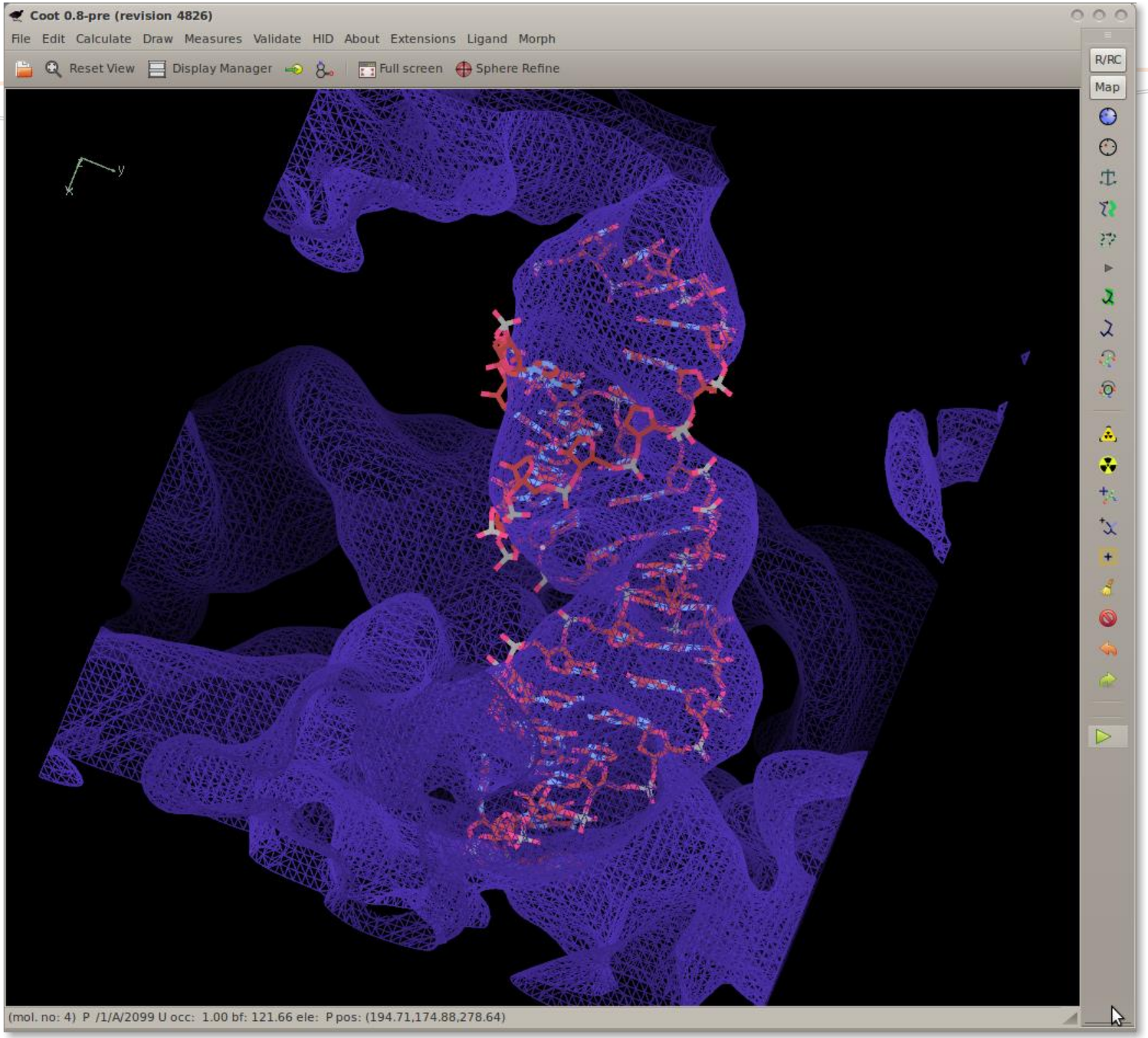
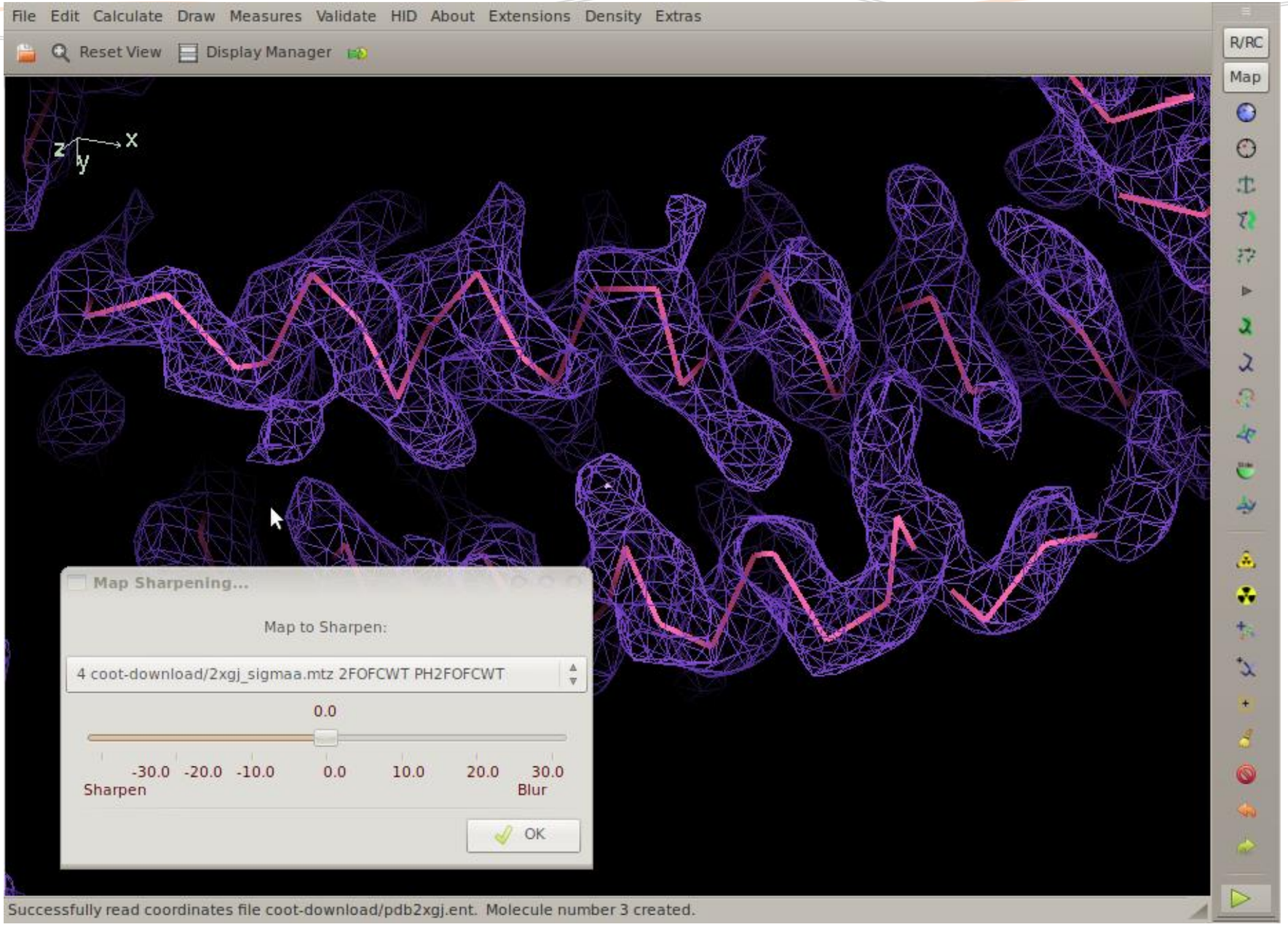
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

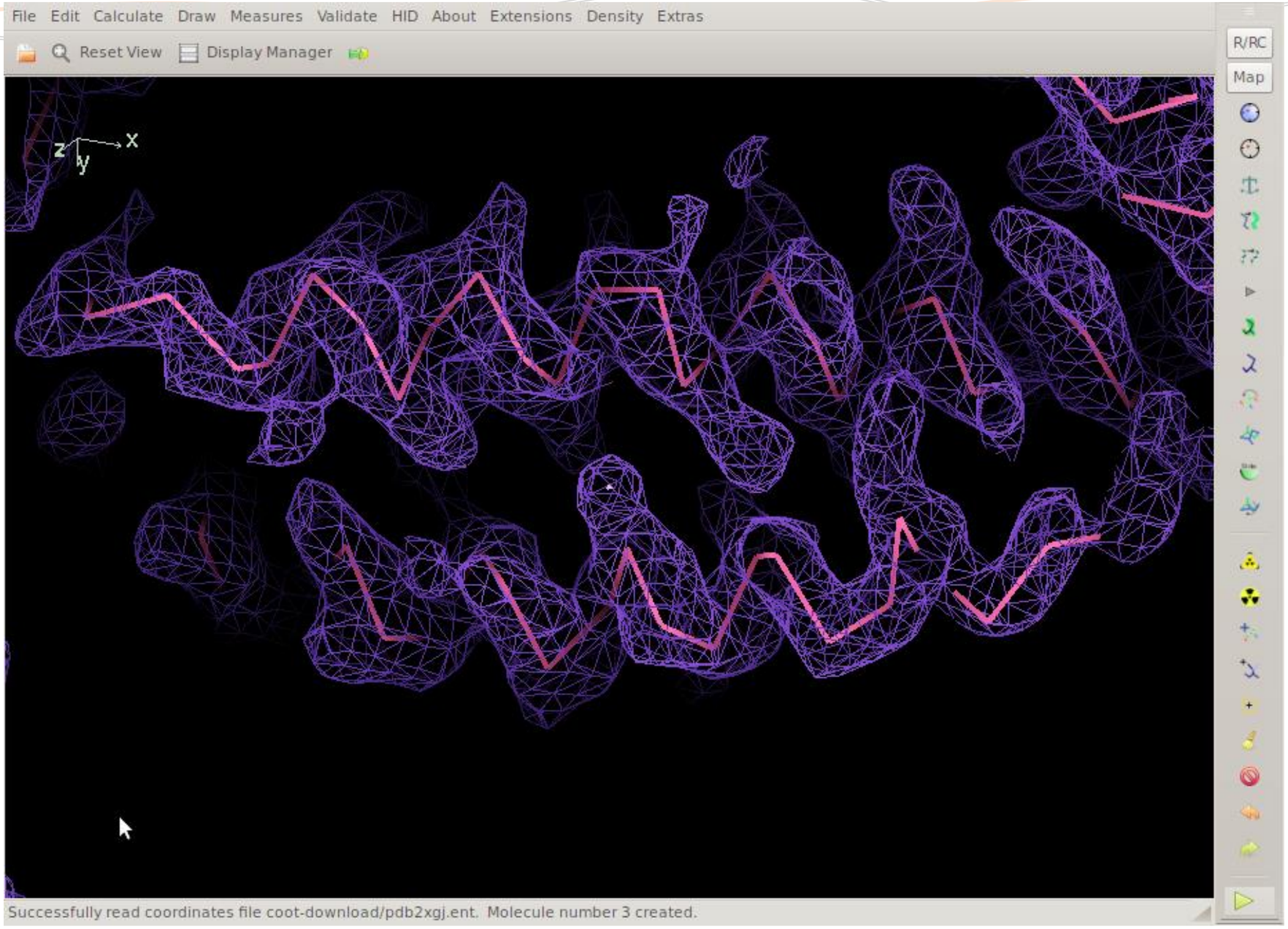

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

Map Sharpening

- Fine tuning the B-factor: which B-factor shall I use to get the most interpretable map?

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


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


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


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

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

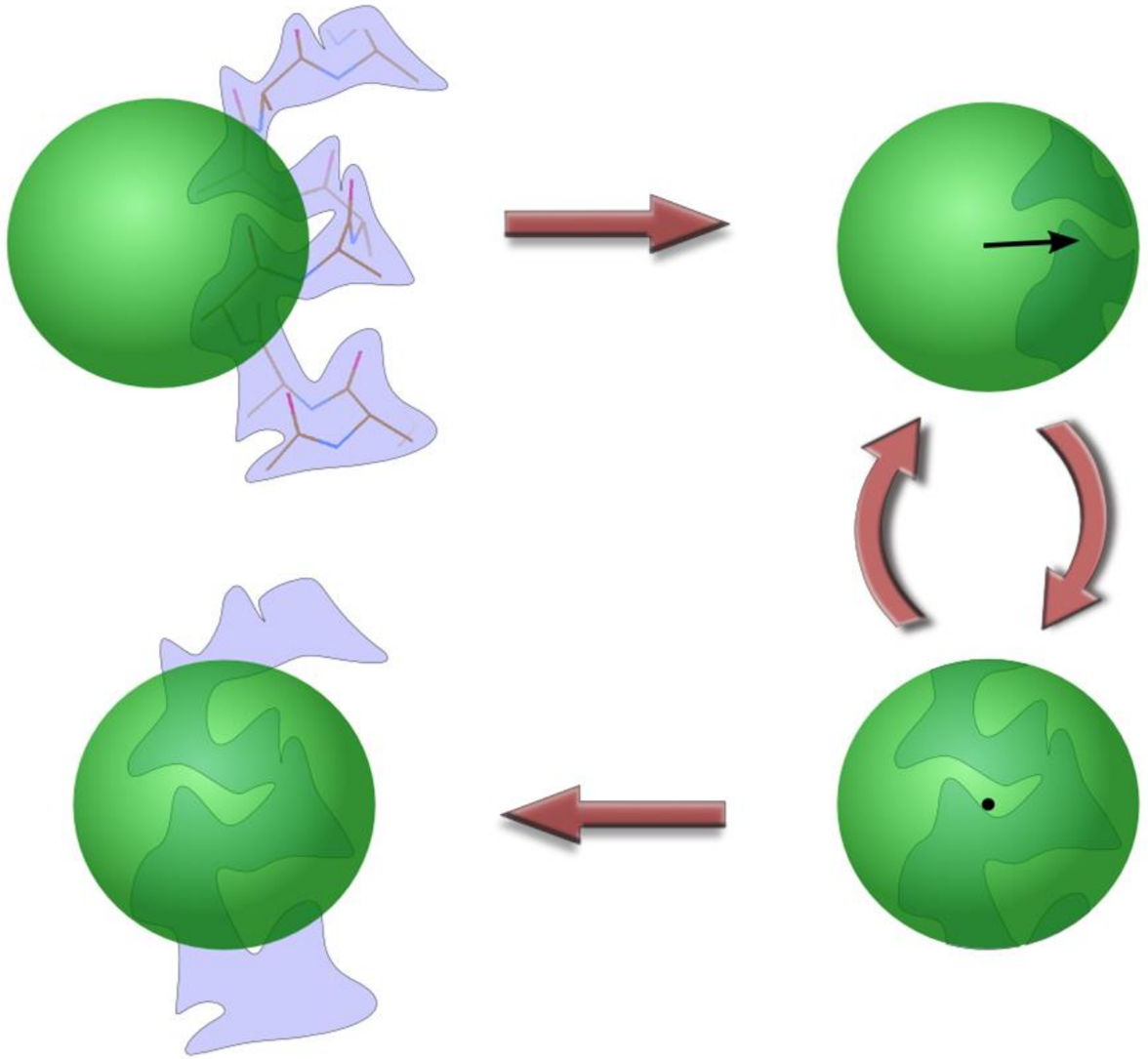
Building from scratch

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

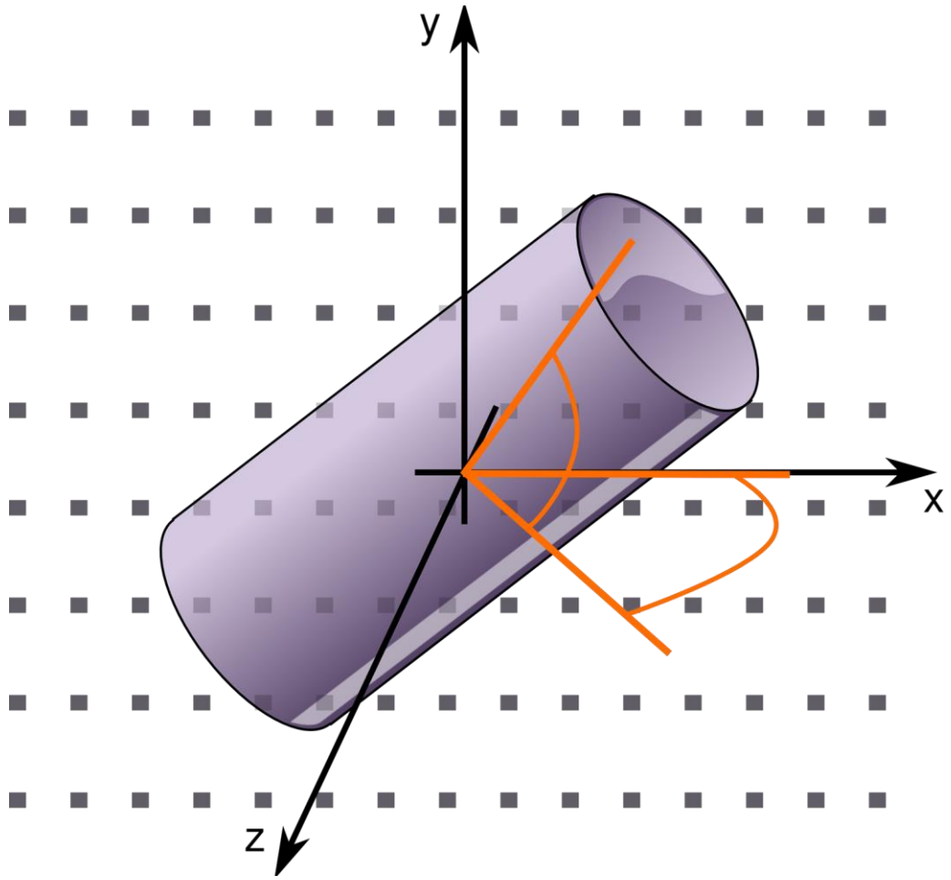
Centering the point of rotation

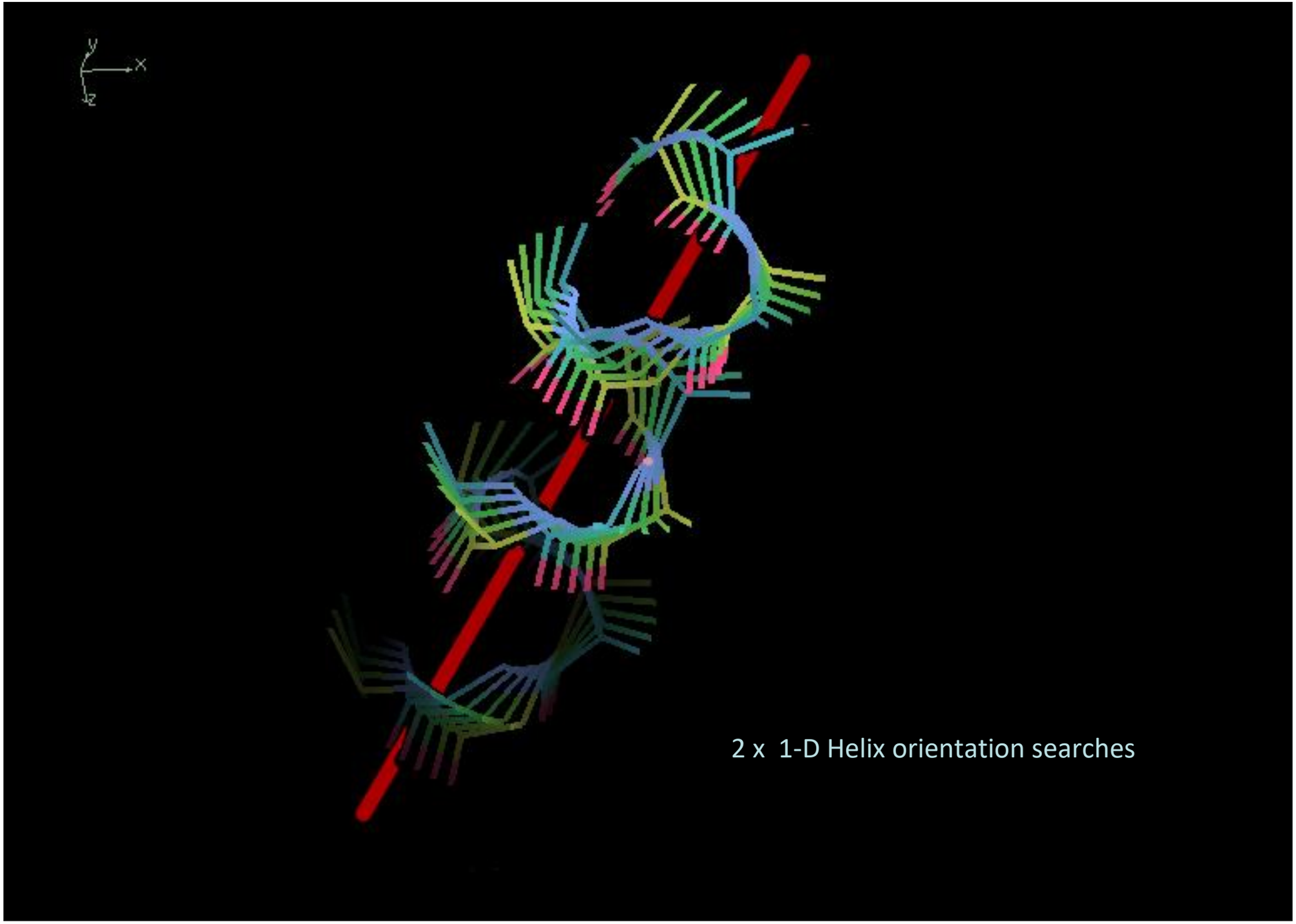


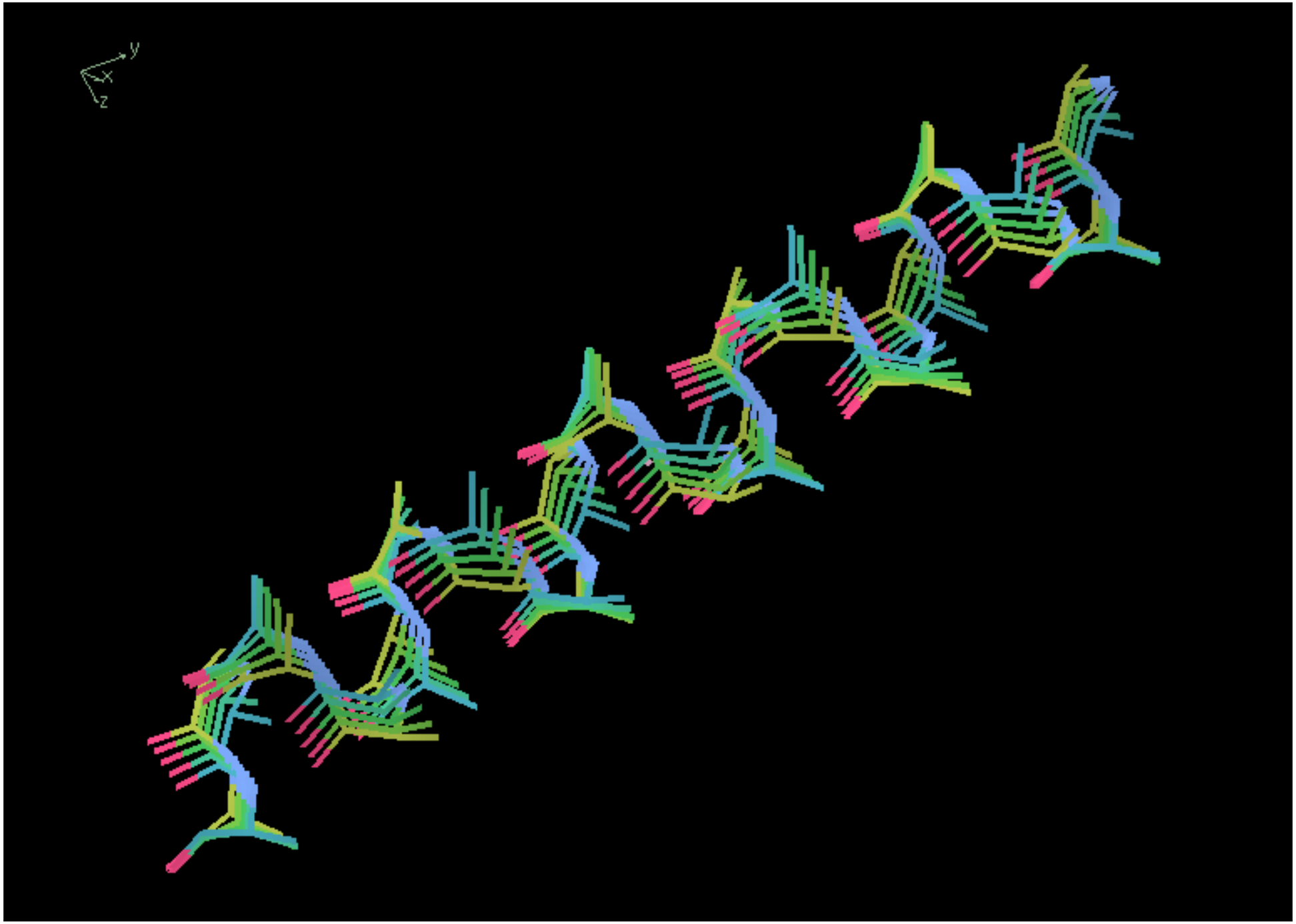
Cylinder search

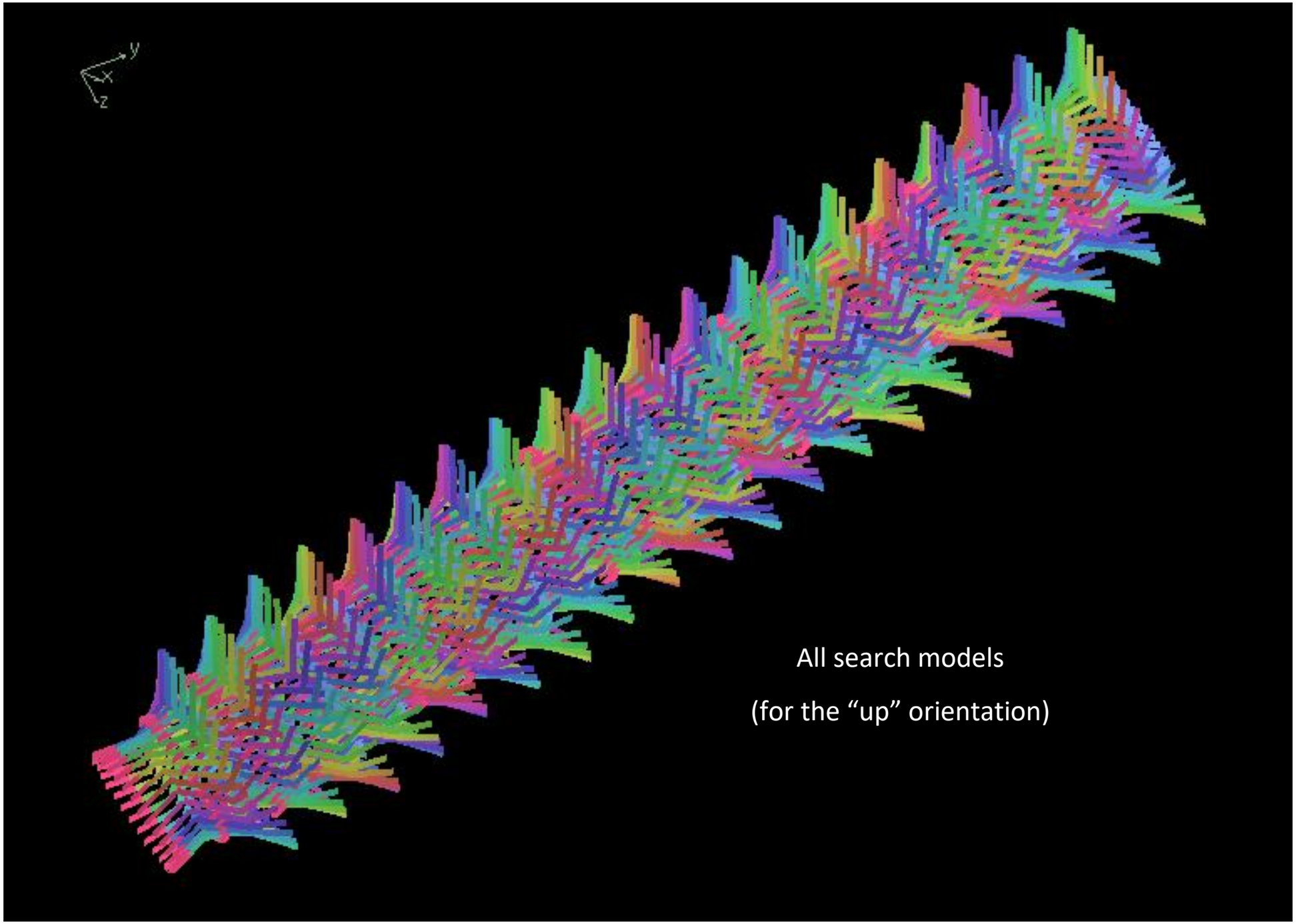
Pick the orientation that encapsulates the most electron density

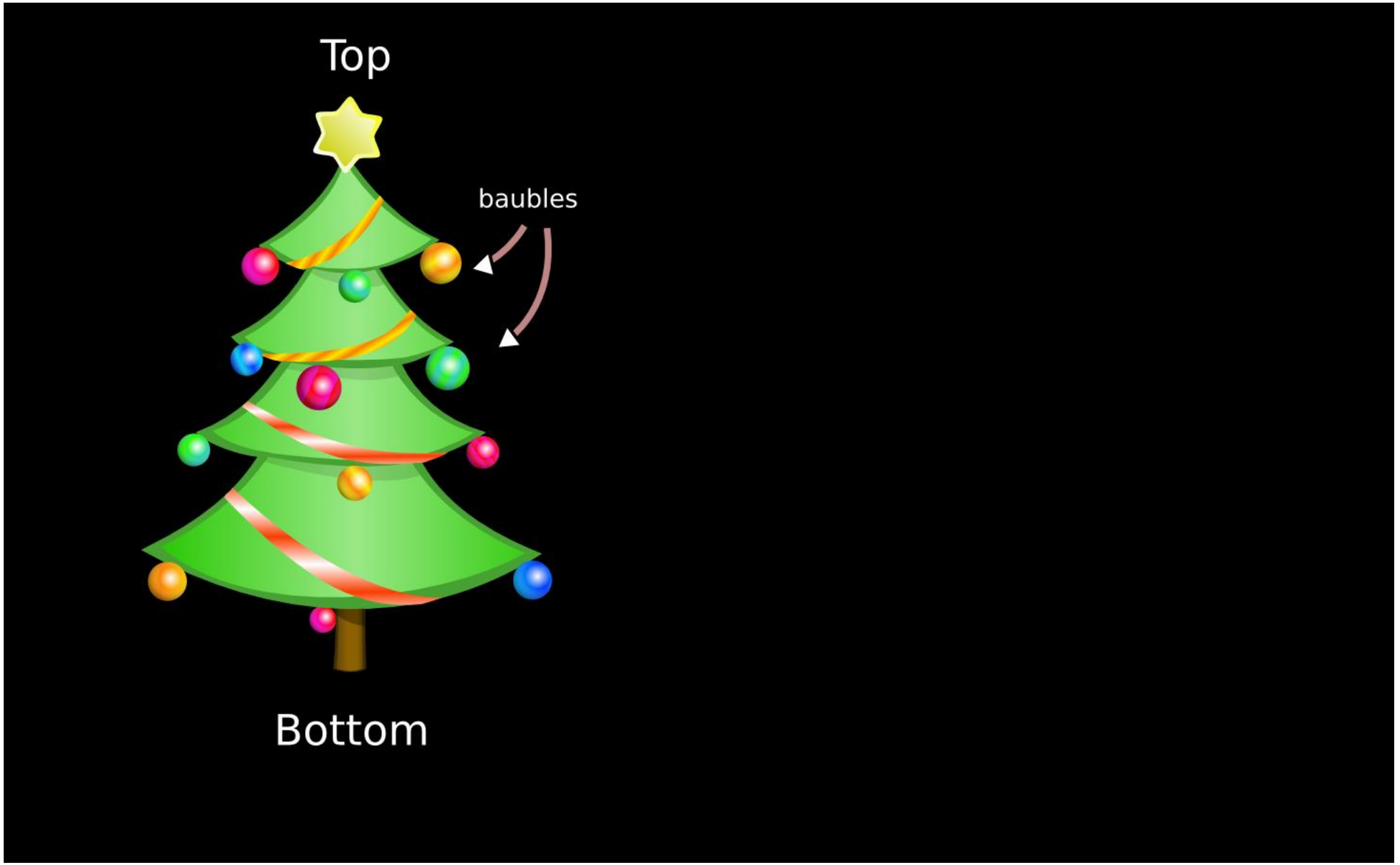
Using 2 rotation axes



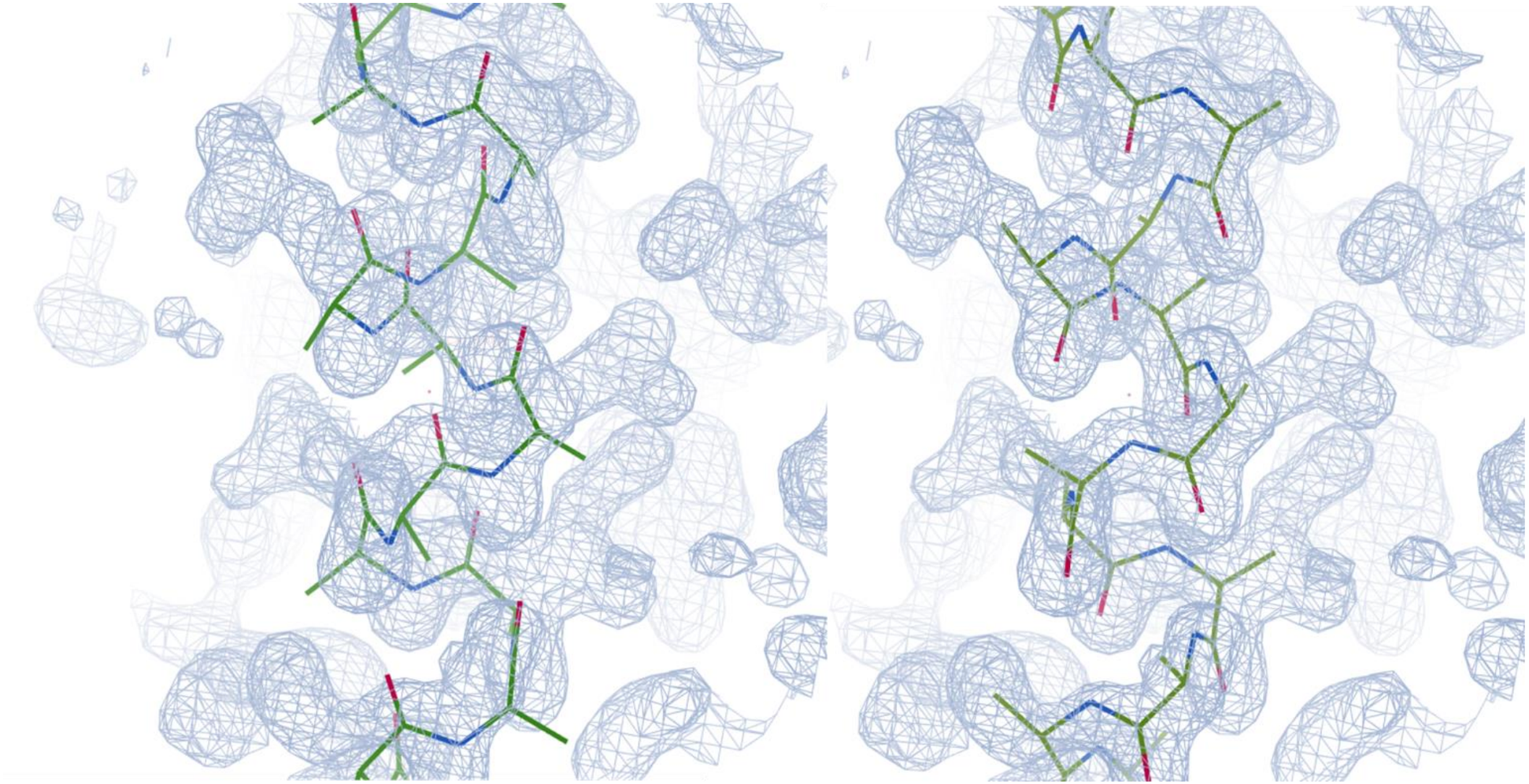

Y
X
Z
2 x 1-D Helix orientation searches


y
x
z

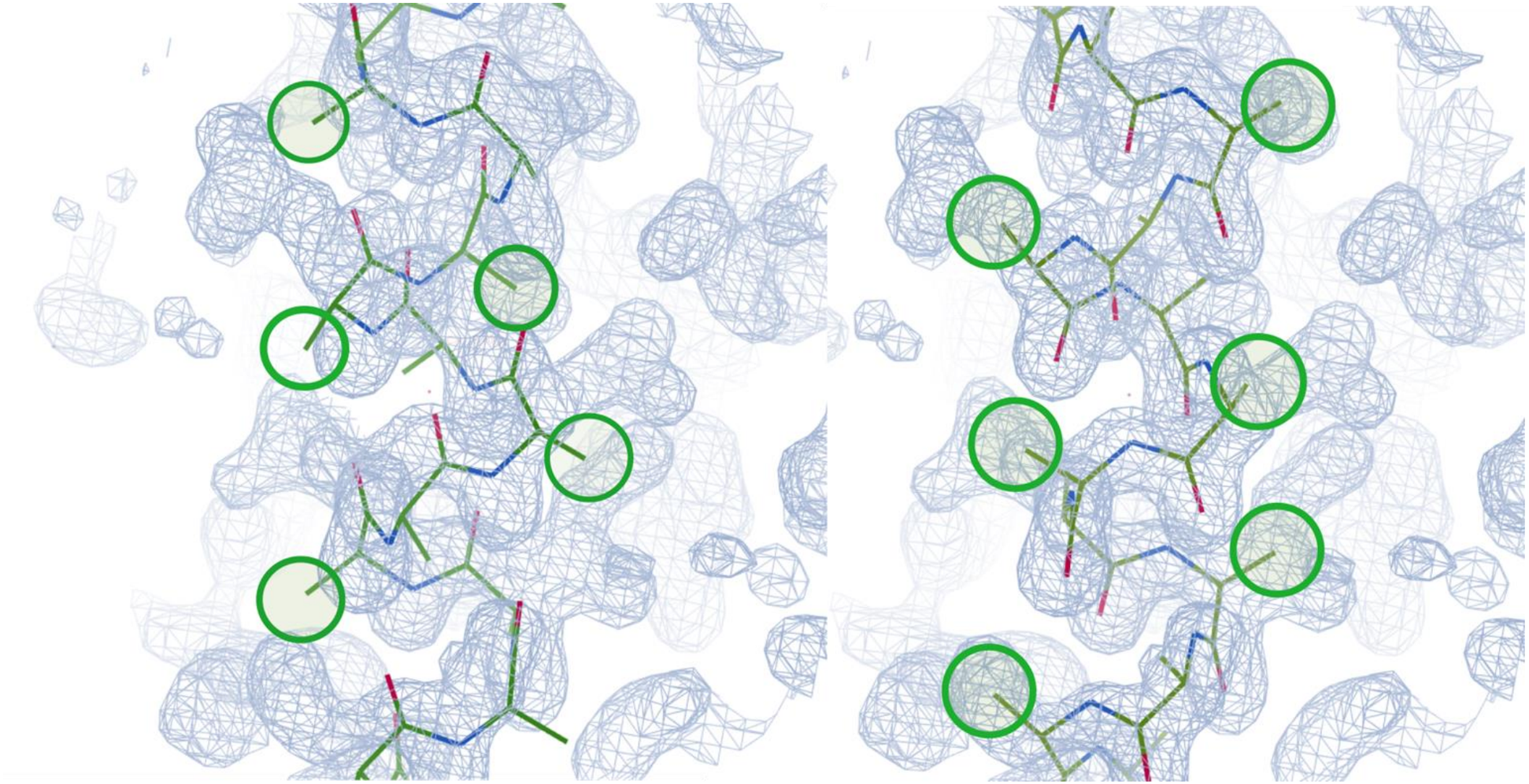

y
x
z
All search models
(for the “up” orientation)


Top
baubles
Bottom

Helix fitting in two orientations

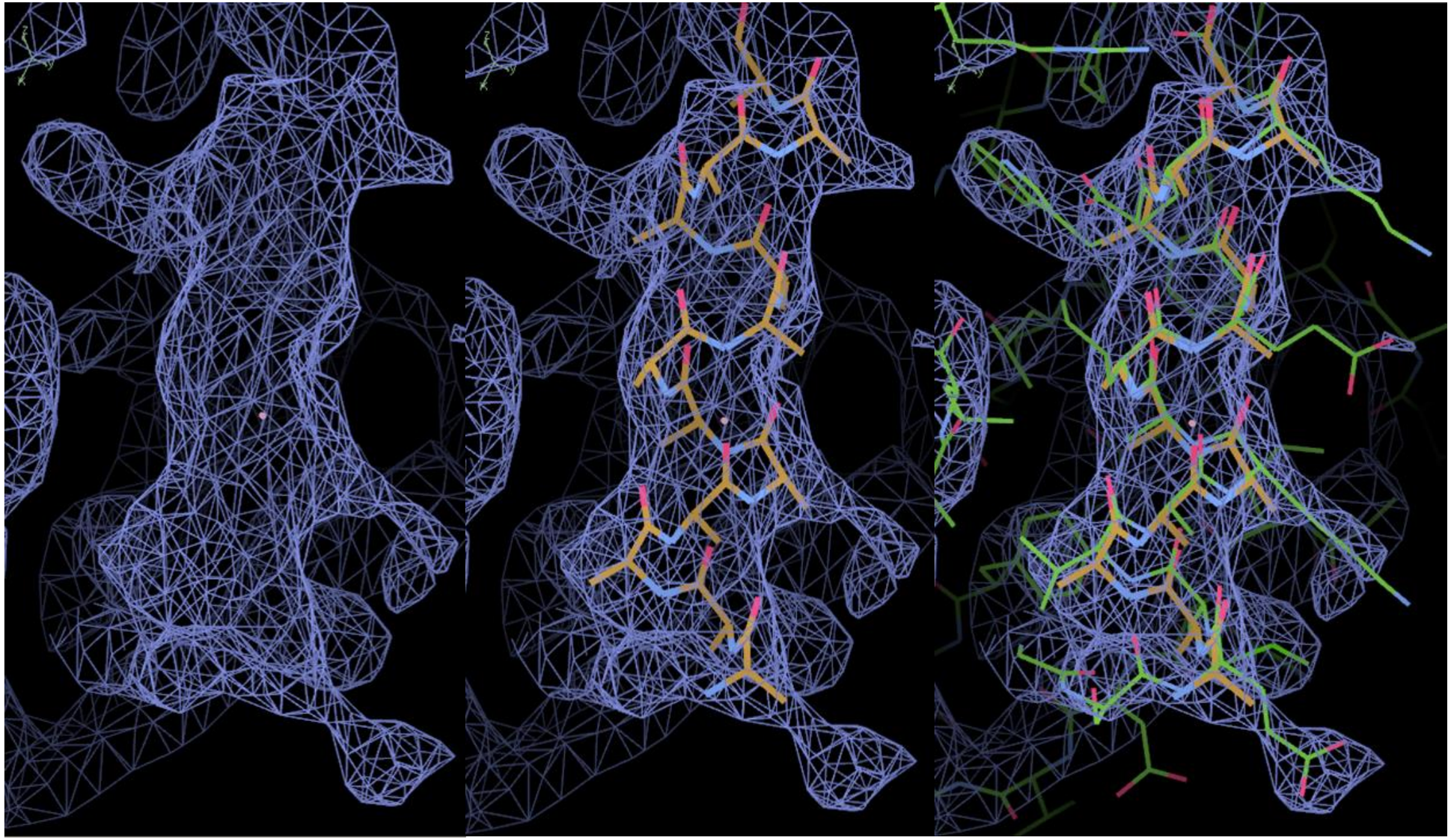


Scoring



c-betas are not fitted and are used for scoring

Helix fitting in action

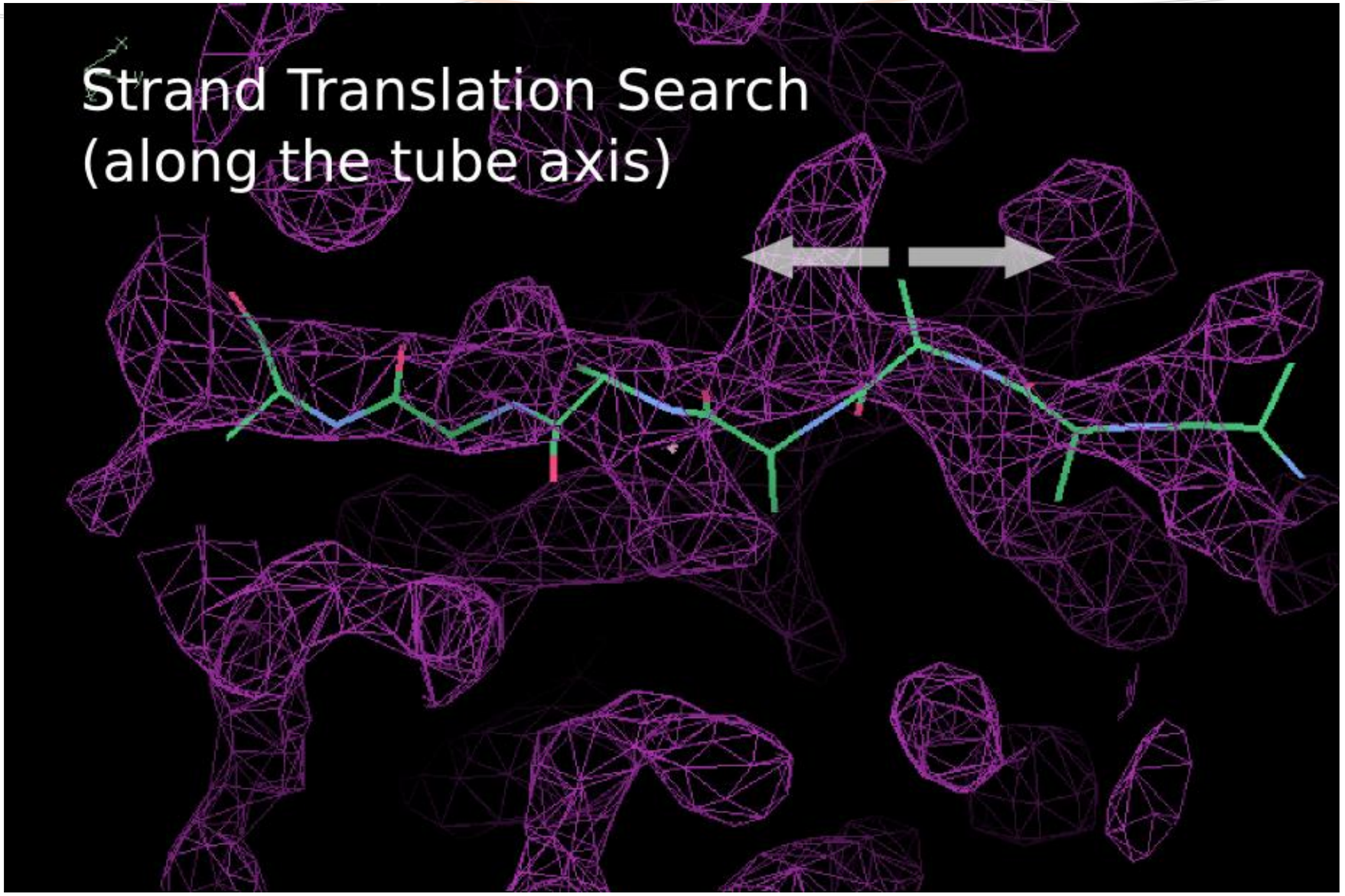


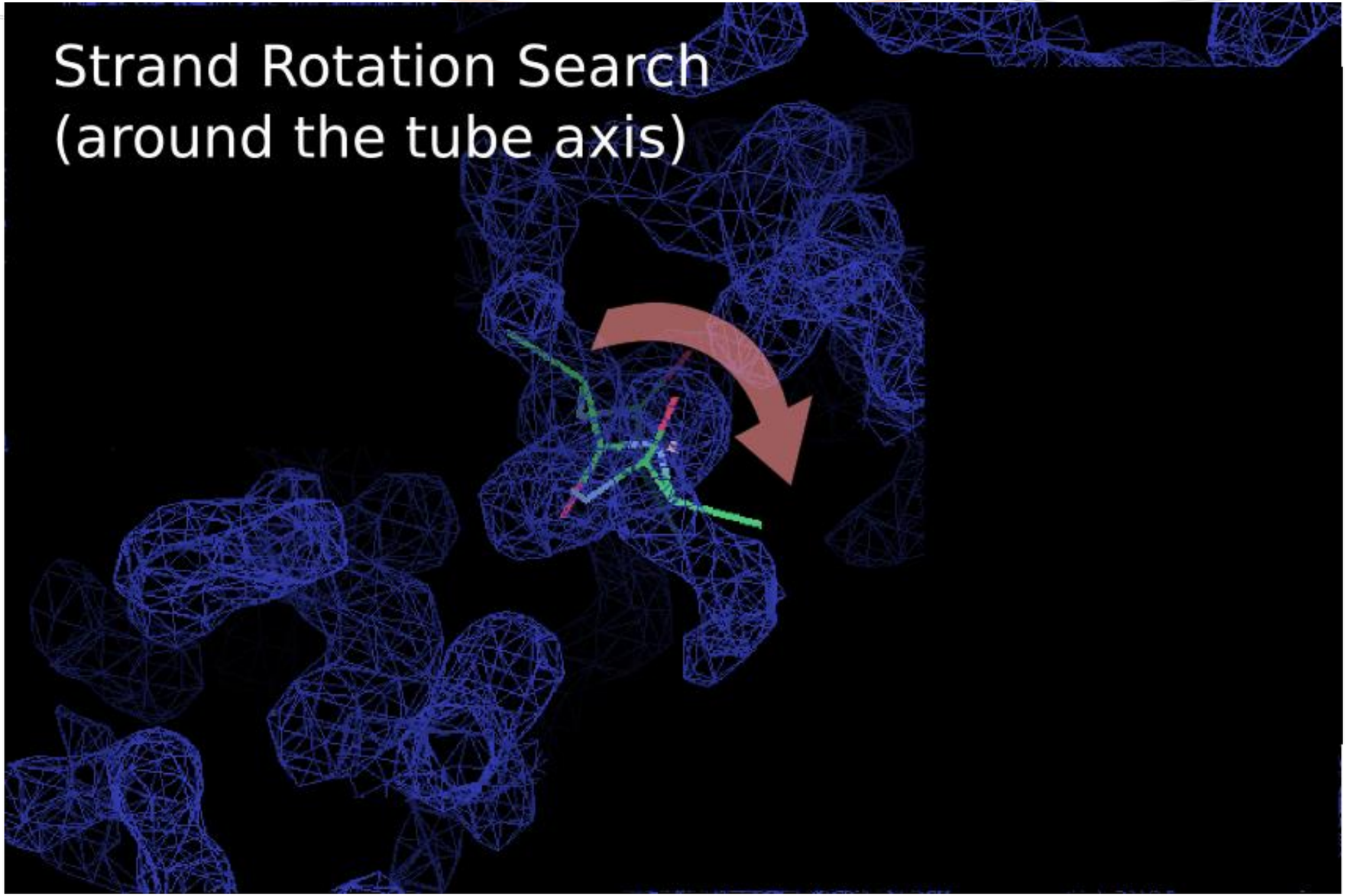
Placing strands

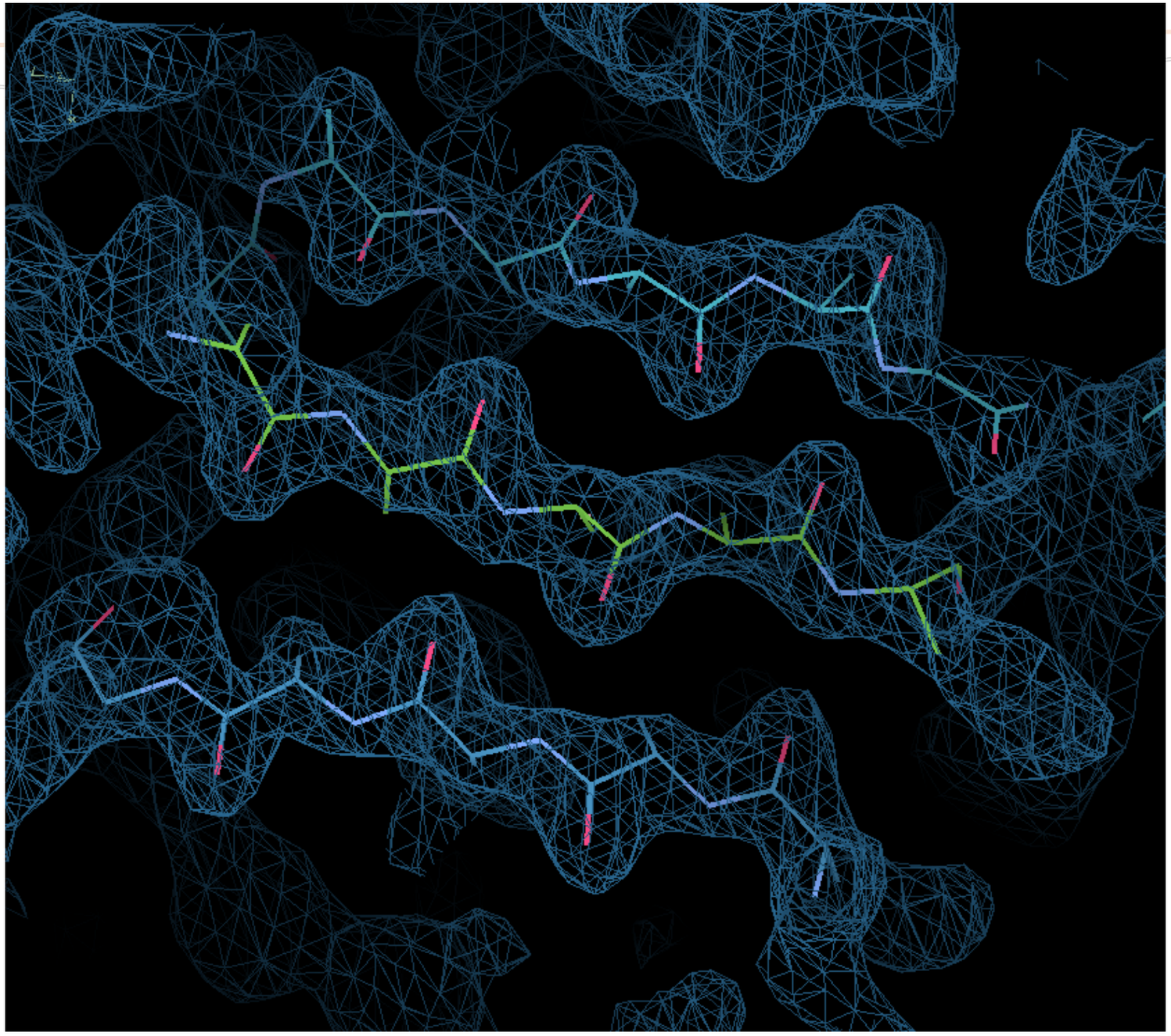
- Unlike Helices, Strands have to be treated as non-idealized
- Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
- Use selections from a “database” of good structures

Strand fitting algorithm

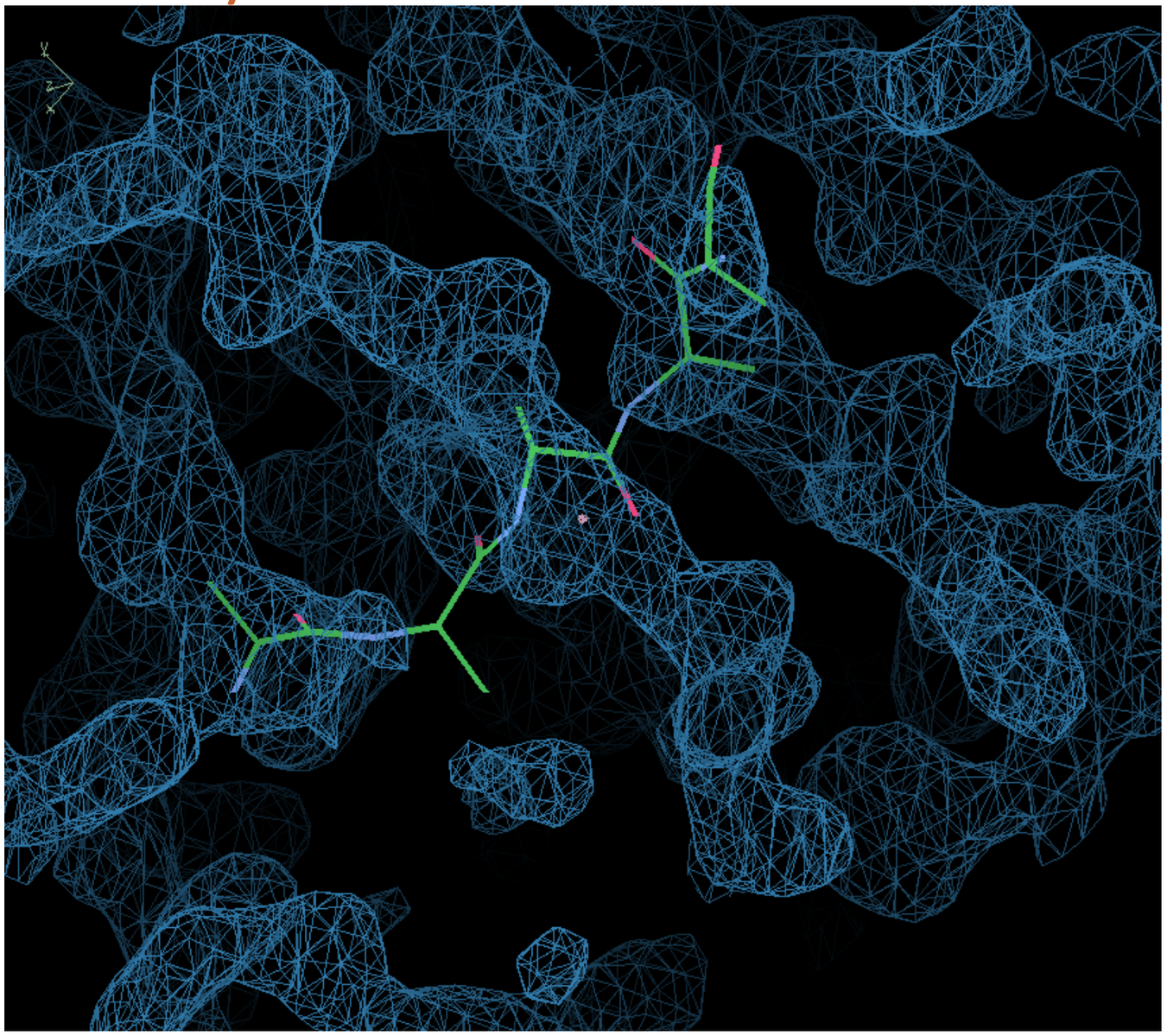
1. Cylinder search
2. Get N fragments of length l from database
3. 1-D Translation search along the tube
4. 1-D Rotation search around the tube
5. Direction flip search
6. Rigid body refine best solutions
7. Real-space refine best solution


Strand Translation Search
(along the tube axis)


Strand Rotation Search
(around the tube axis)



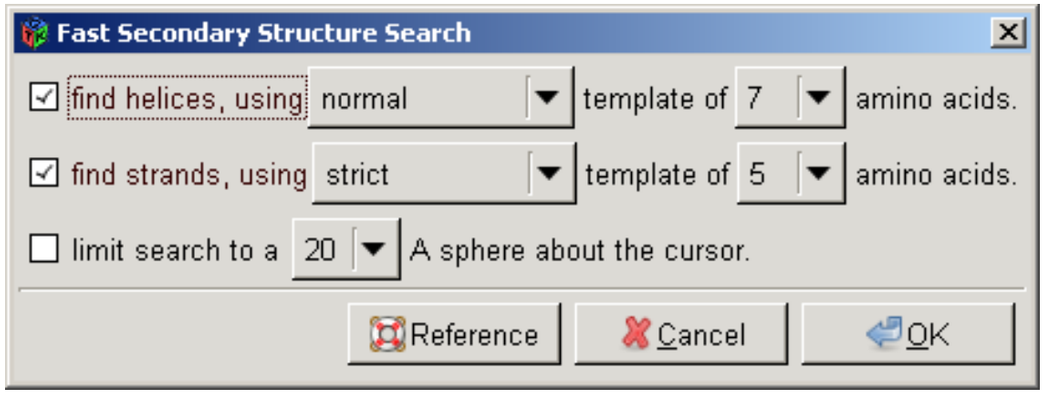
Not all is rosey…



Strand fitting 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 secondary structure search



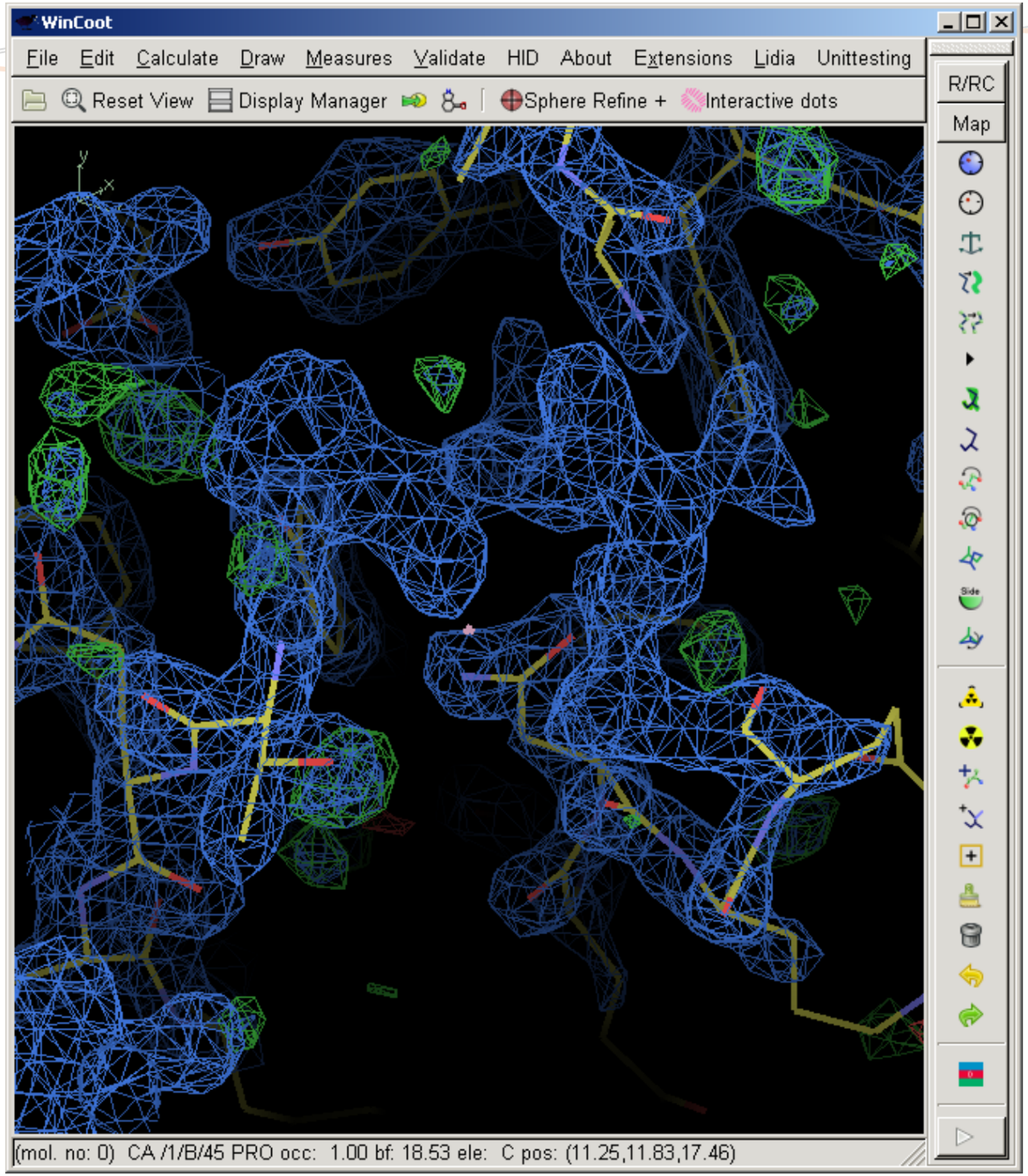
Loop fitting

- Simple loop fitting

  Add residue by residue (from both termini)

- DB loop

  Fitting fragments from database



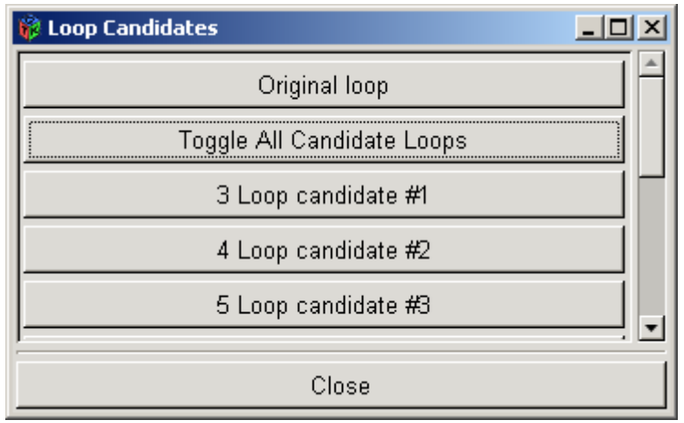
Loop fitting

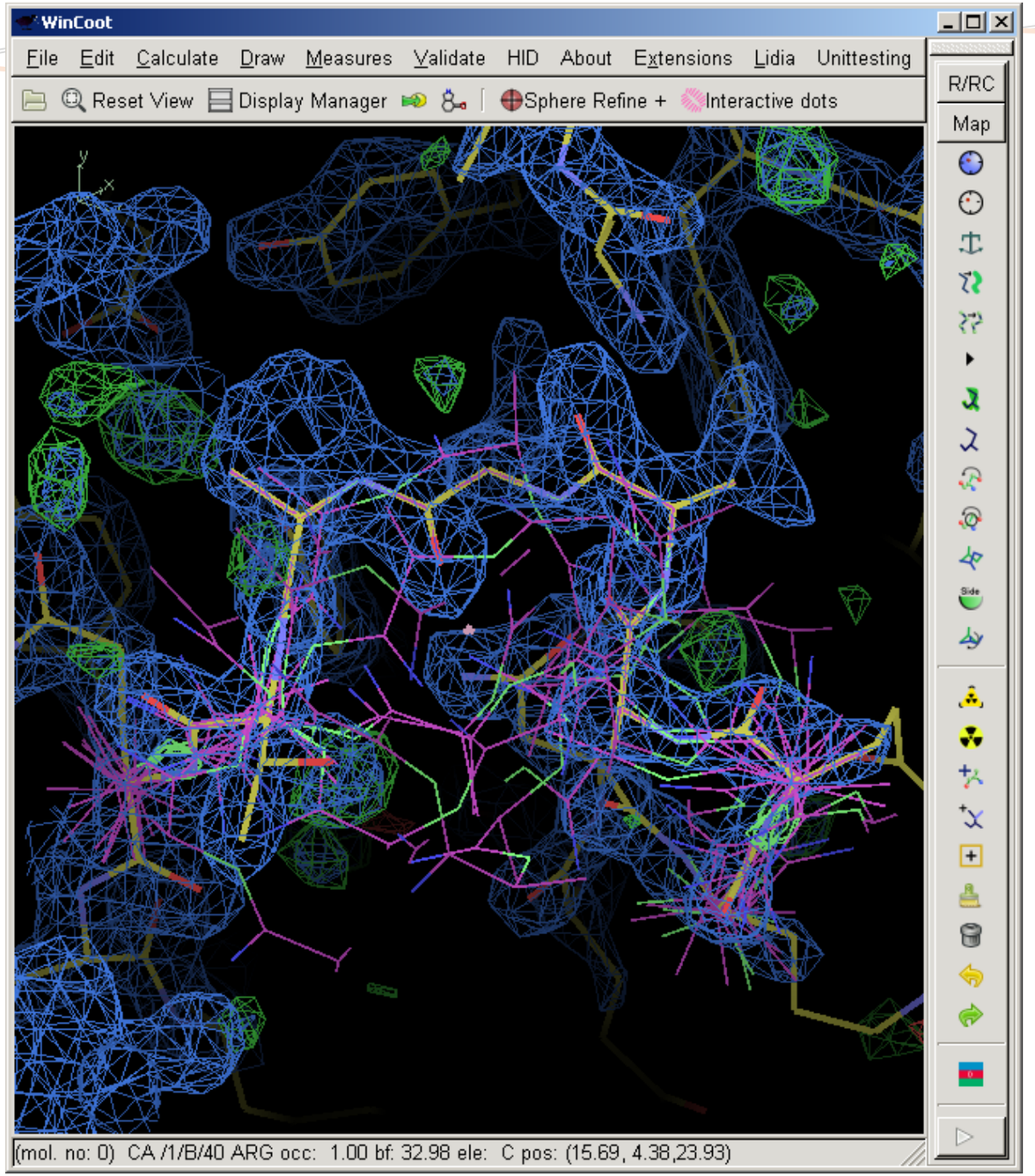
- Simple loop fitting

  Add residue by residue (from both termini)

- DB loop

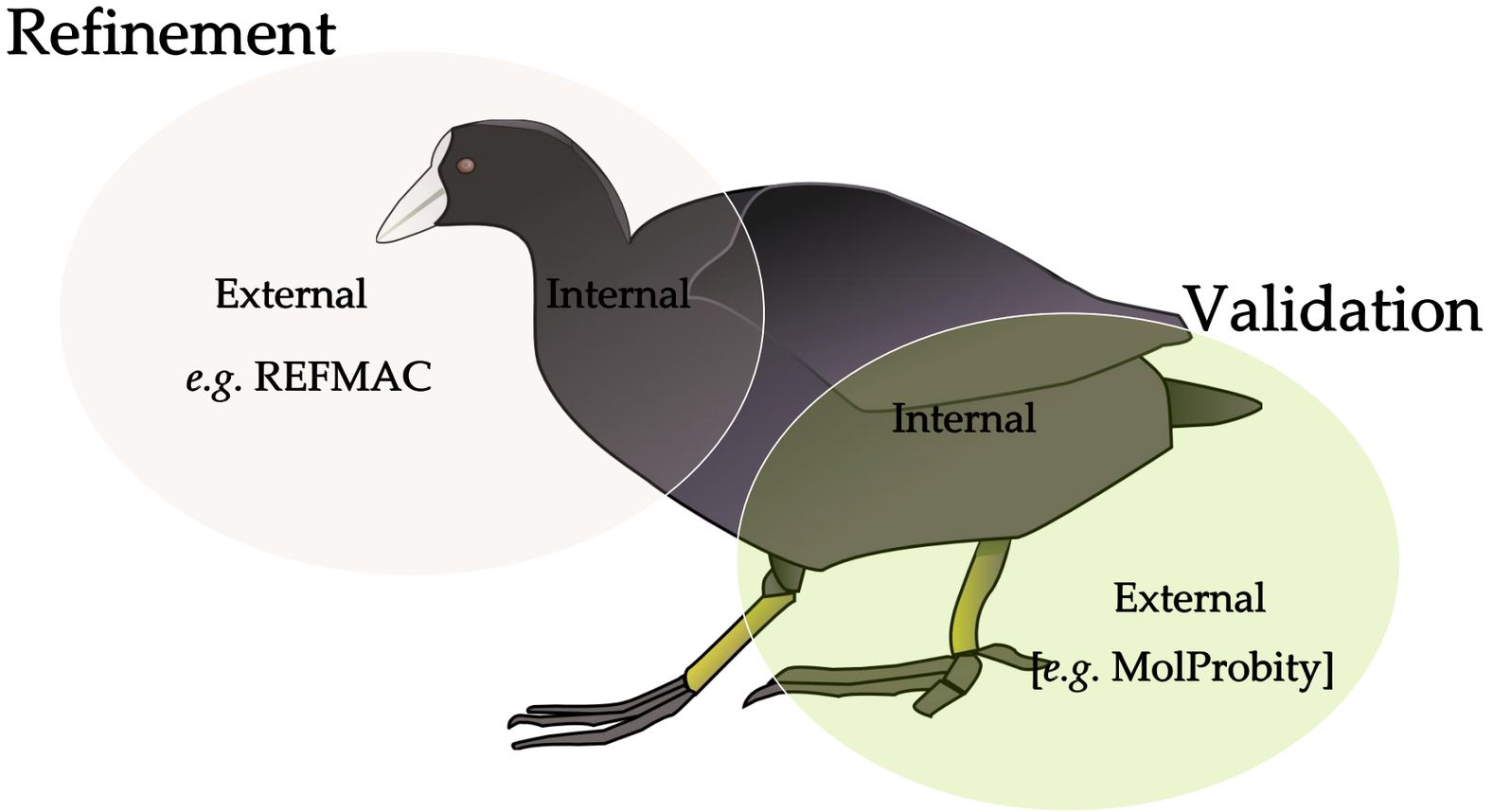
  Fitting fragments from database

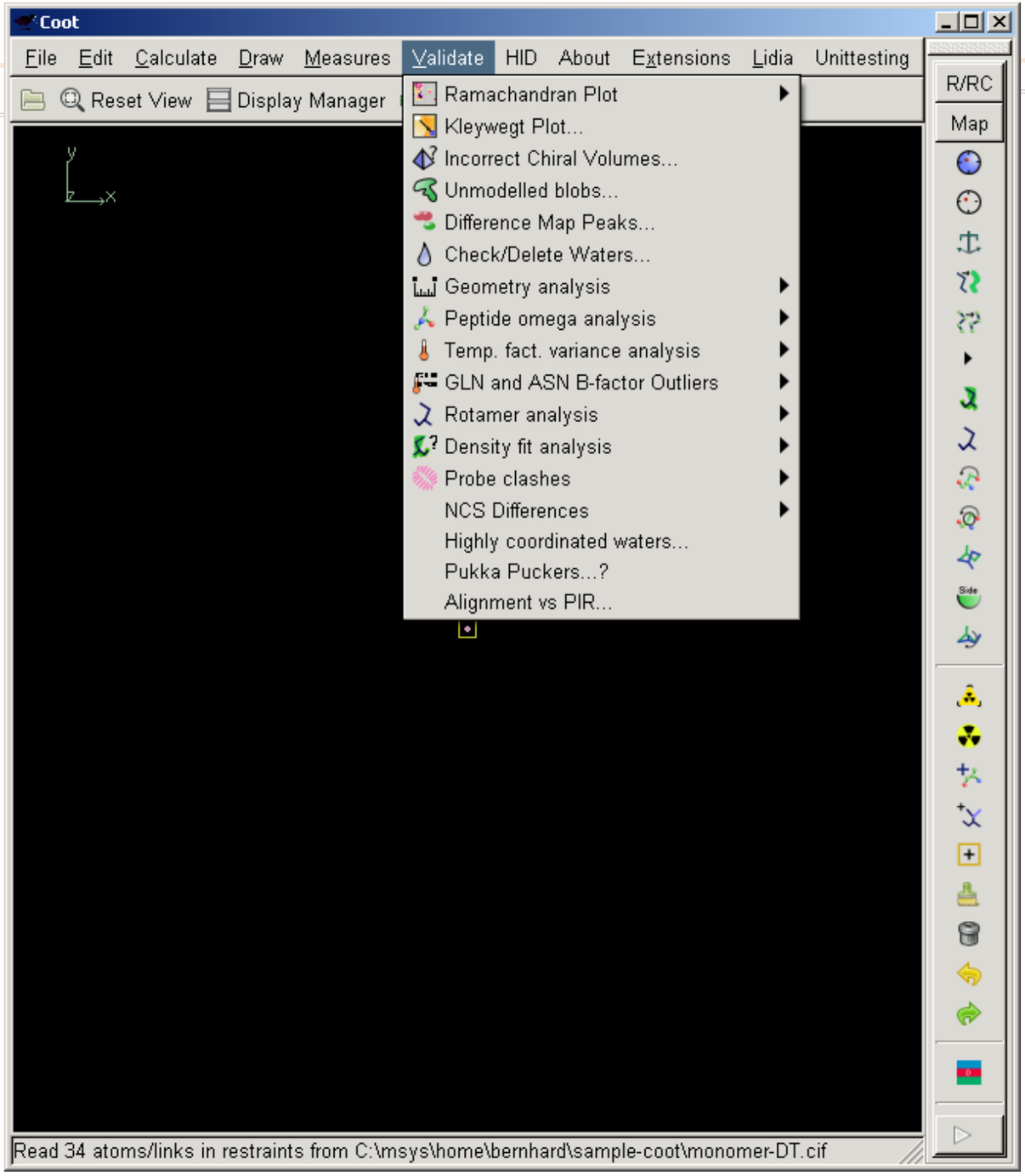




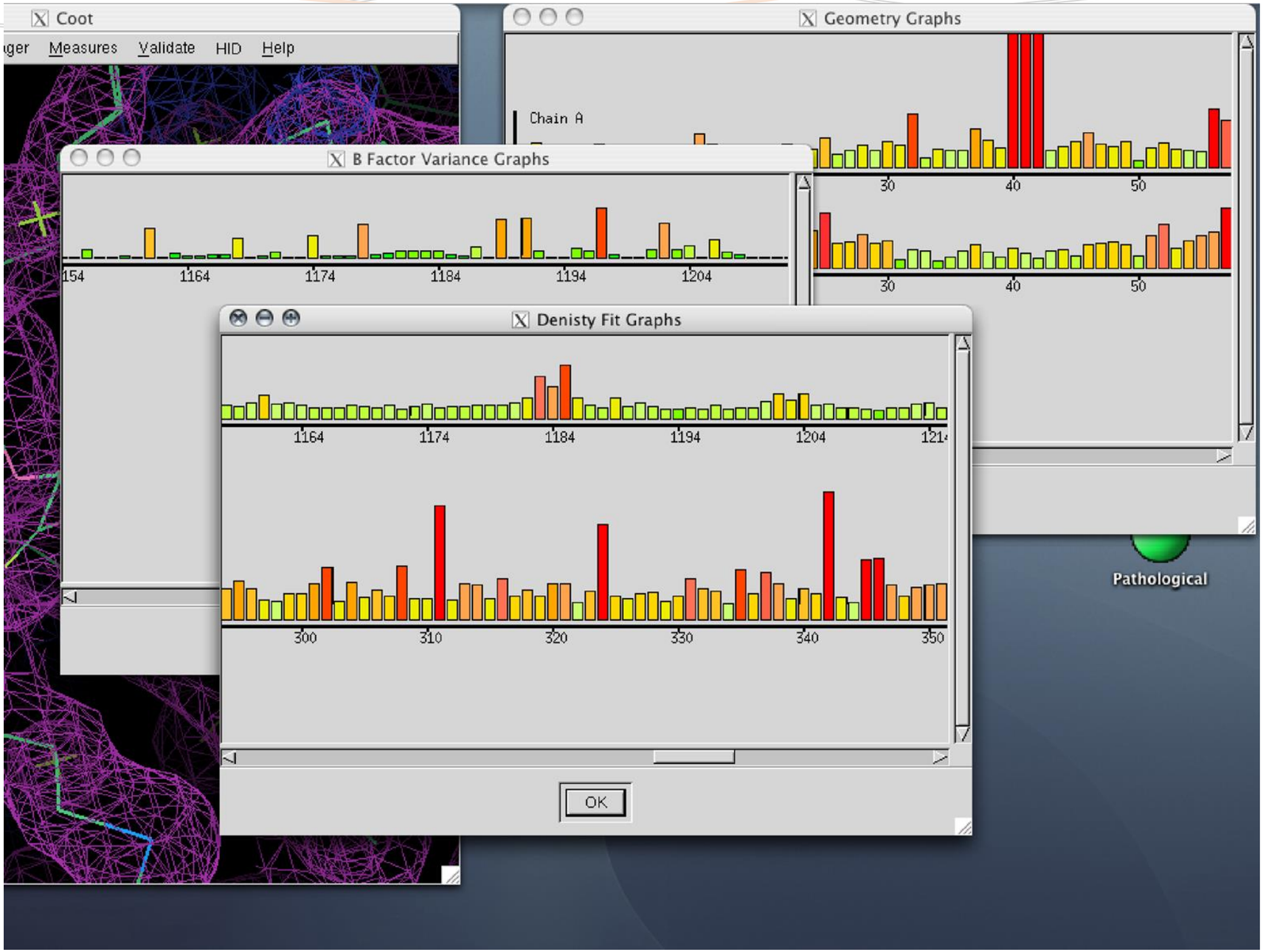
Validation

Feature integration

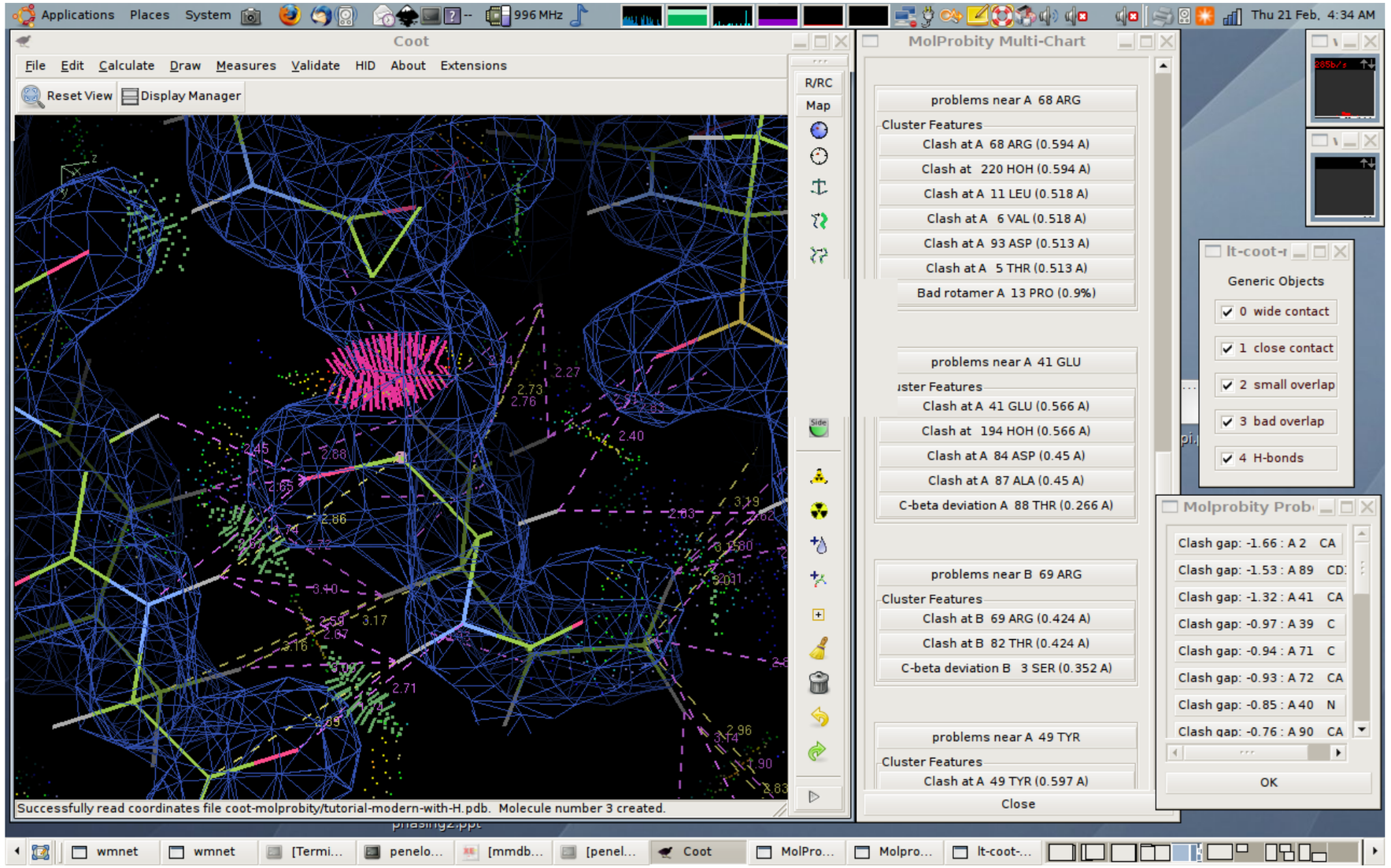



Coot
File
Edit
Calculate
Draw
Measures
Validate
HID
About
Extensions
Lidia
Unittesting
Reset View
Display Manager
Ramachandran Plot
Kleywegt Plot...
Incorrect Chiral Volumes...
Unmodelled blobs...
Difference Map Peaks...
Check/Delete Waters...
Geometry analysis
Peptide omega analysis
Temp. fact. variance 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

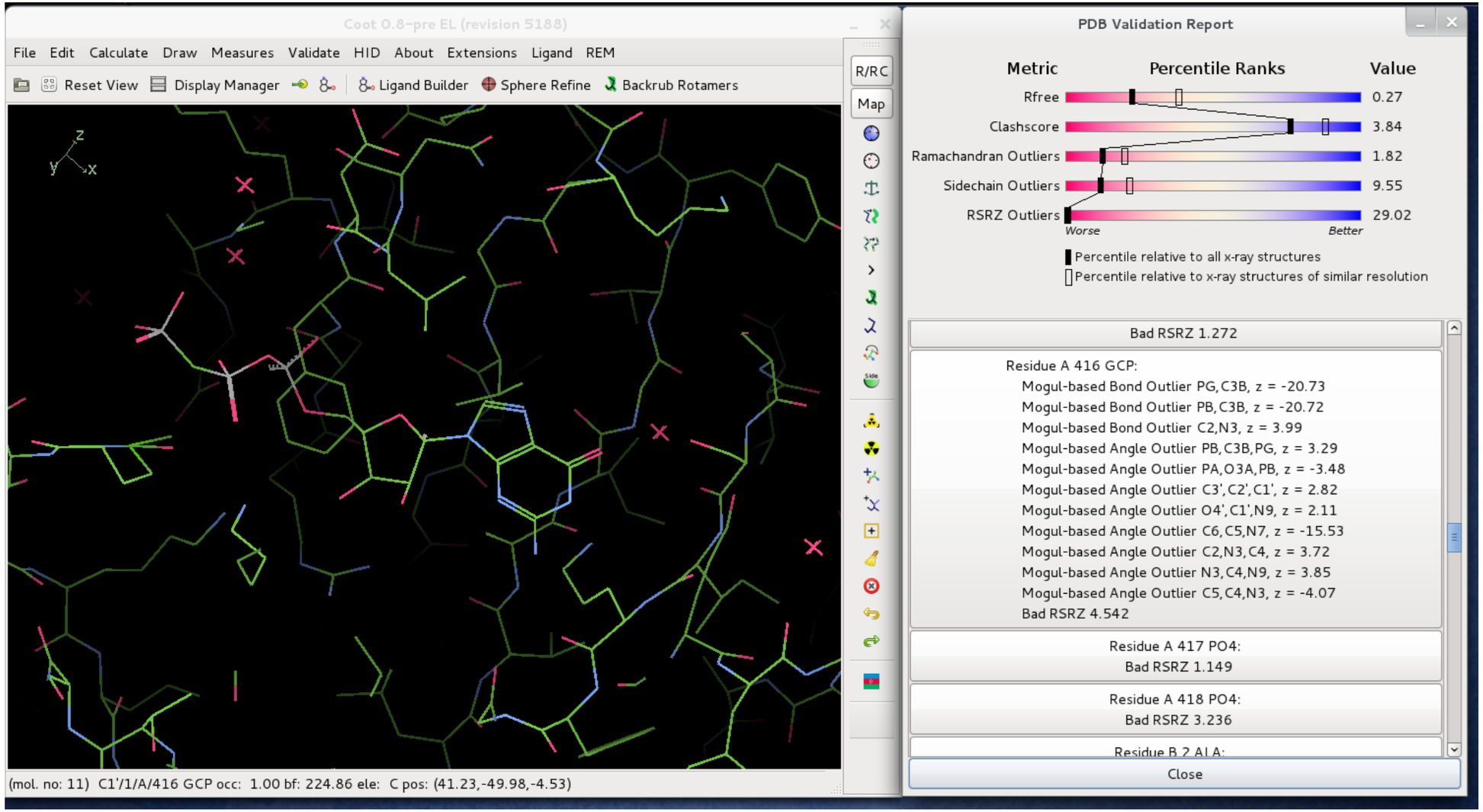
Charts


Coot
ger Measures Validate HID Help
Geometry Graphs
Chain A
30 40 50
30 40 50
B Factor Variance Graphs
154 1164 1174 1184 1194 1204
Denisty Fit Graphs
1164 1174 1184 1194 1204 121
300 310 320 330 340 350
OK
Pathological

Molprobity integration



PDB validation report parsing



NCS: non-crystallographic symmetry

NCS: non-crystallographic symmetry

- 2 or more copies of a molecule in the unit cell not related by crystallographic symmetry
- Crystallographic copies of molecules are (of course) treated as if they were exactly the same across the unit cell – and indeed across the whole crystal
- Non-crystallographically related molecules provide different representations of the same molecule
  - This can be useful for model-building
  - But difficult to use in practice

NCS: non-crystallographic symmetry

Problems?

- Strict NCS:

  NCS should appear like crystallographic symmetry does [exact copies]

- Non-Strict NCS:

  Molecules are different

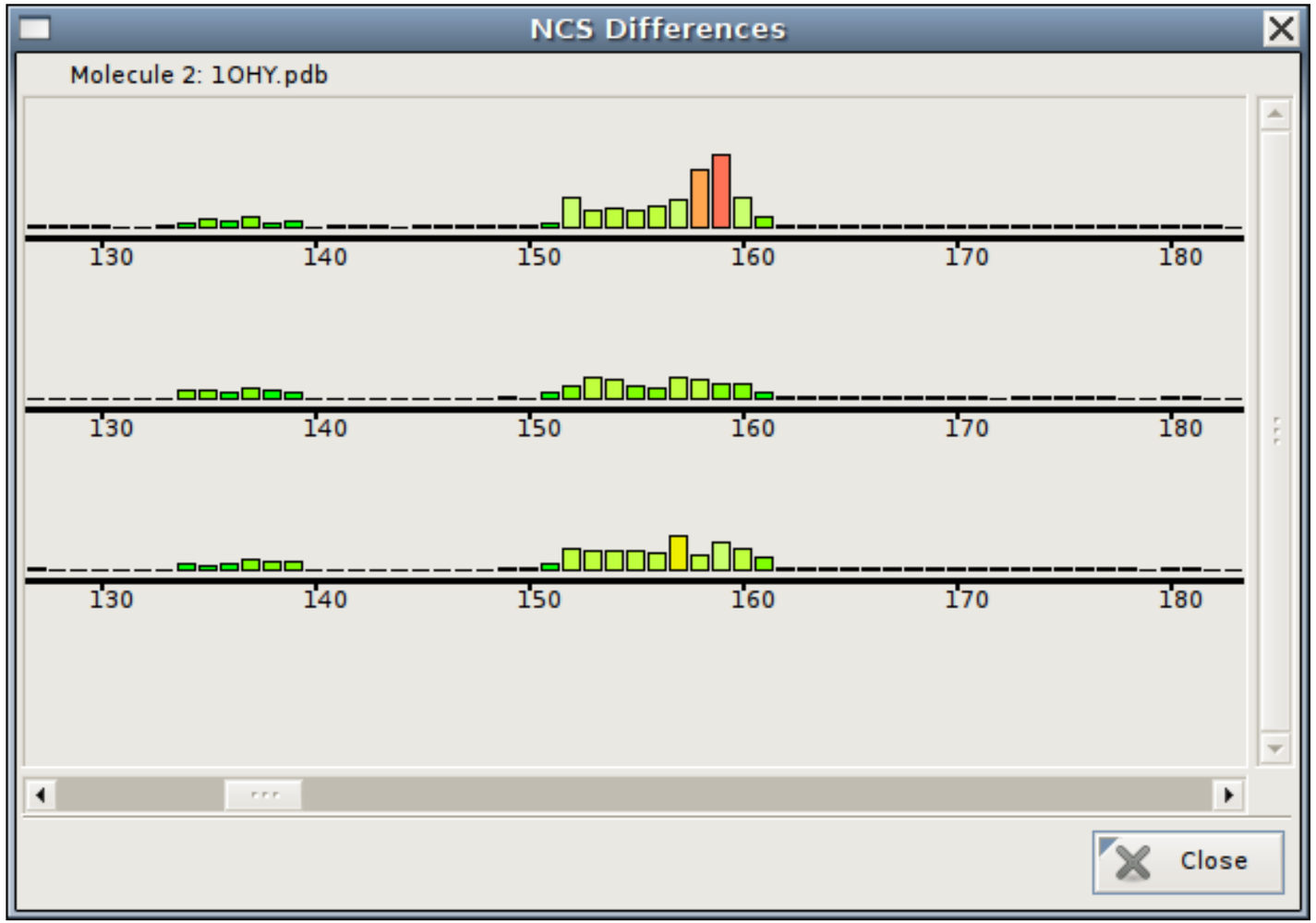
  How to cope with differences, but minimize unnecessary rebuilding?

NCS: non-crystallographic symmetry

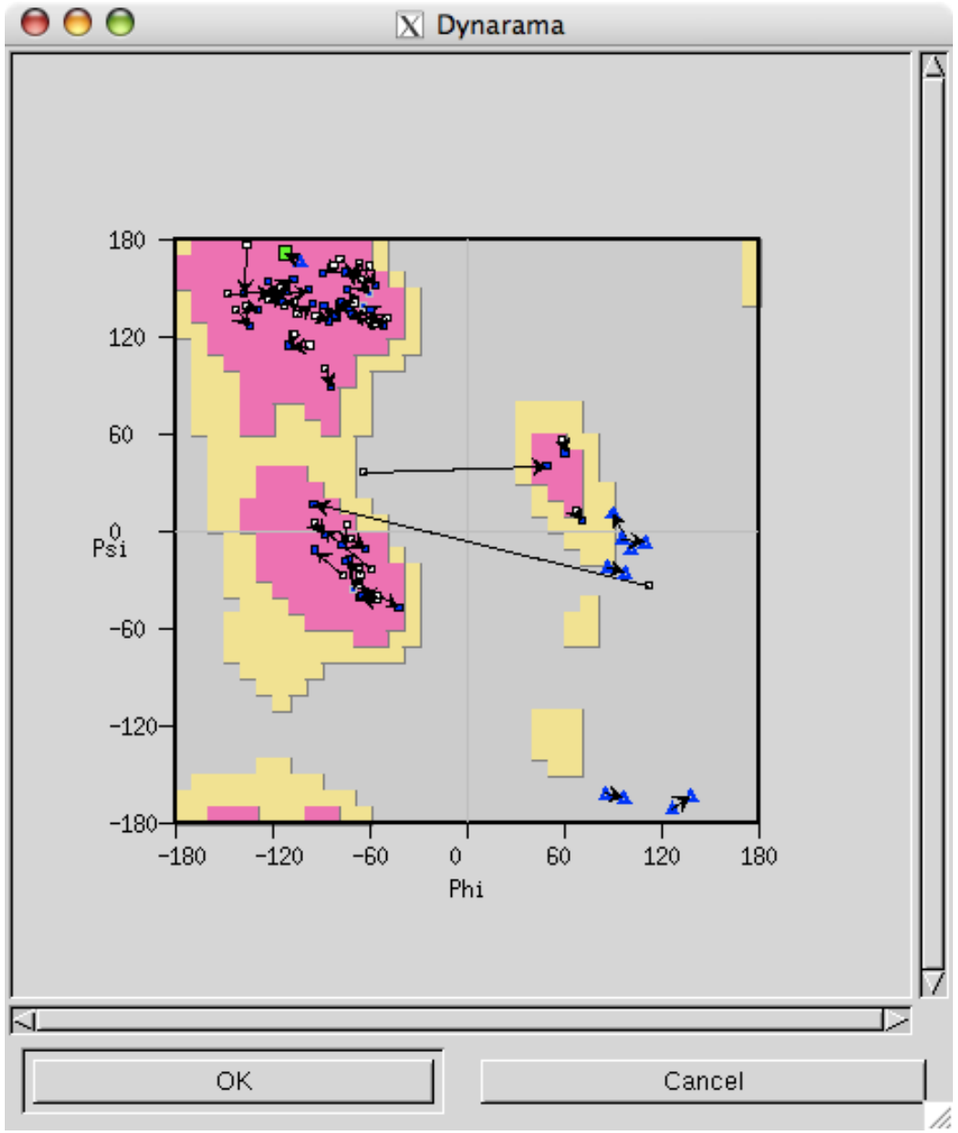
Typical Scenario:

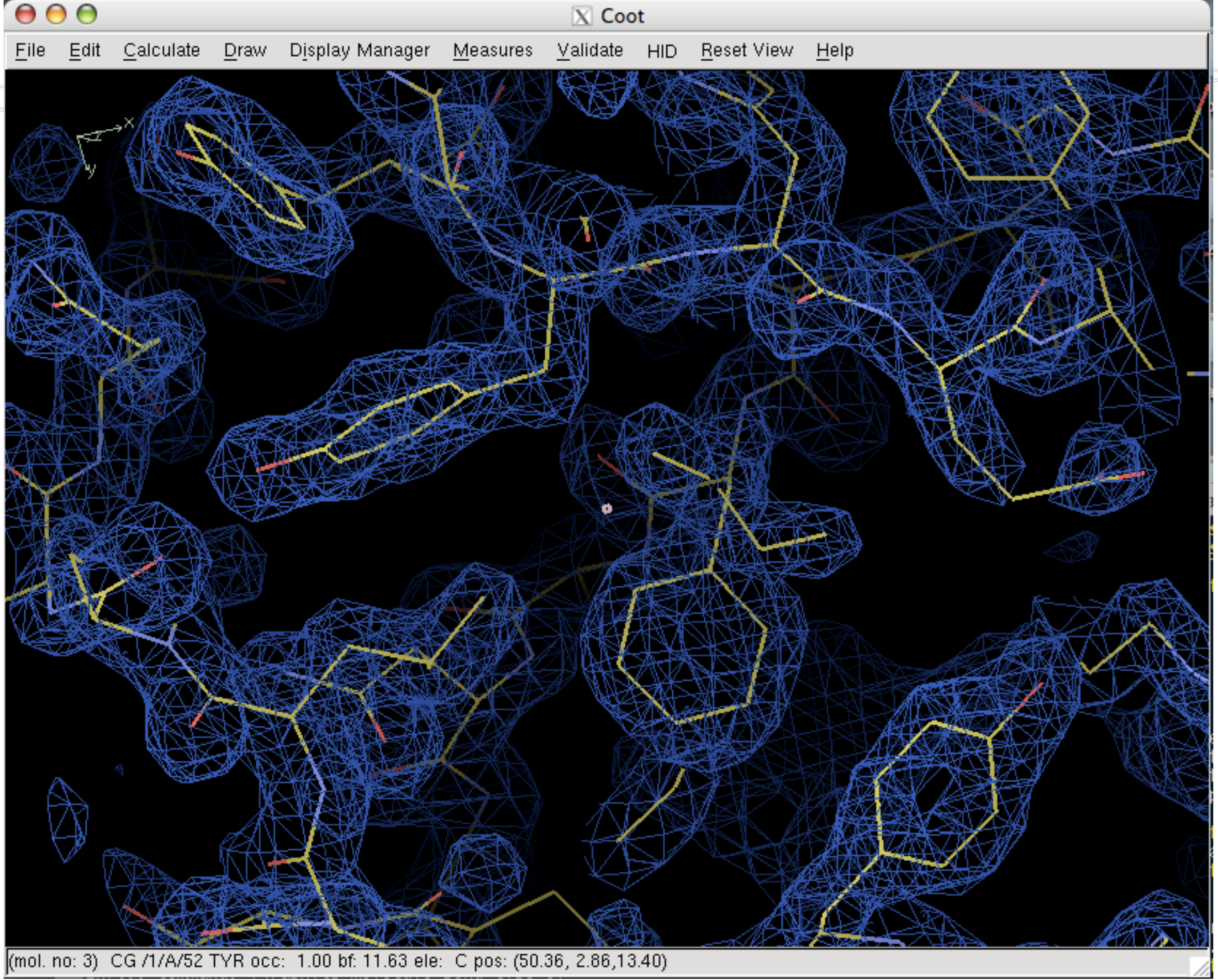
- I have done an LSQ overlap of my NCS-related molecules and from the graph, have seen significant deviations in the positions of some side-chains.
- Why are they different?

NCS differences graph



Kleywegt plot

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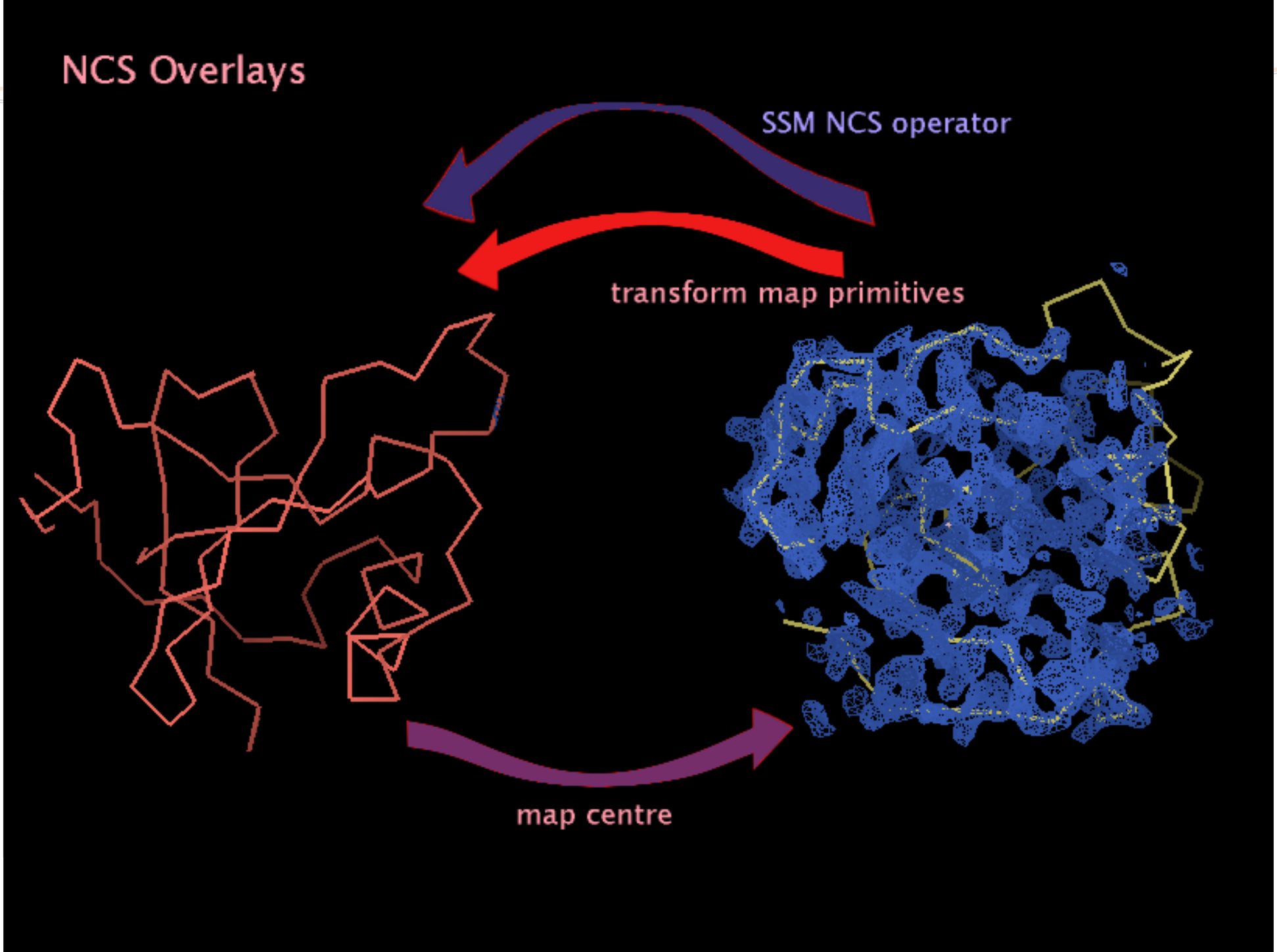
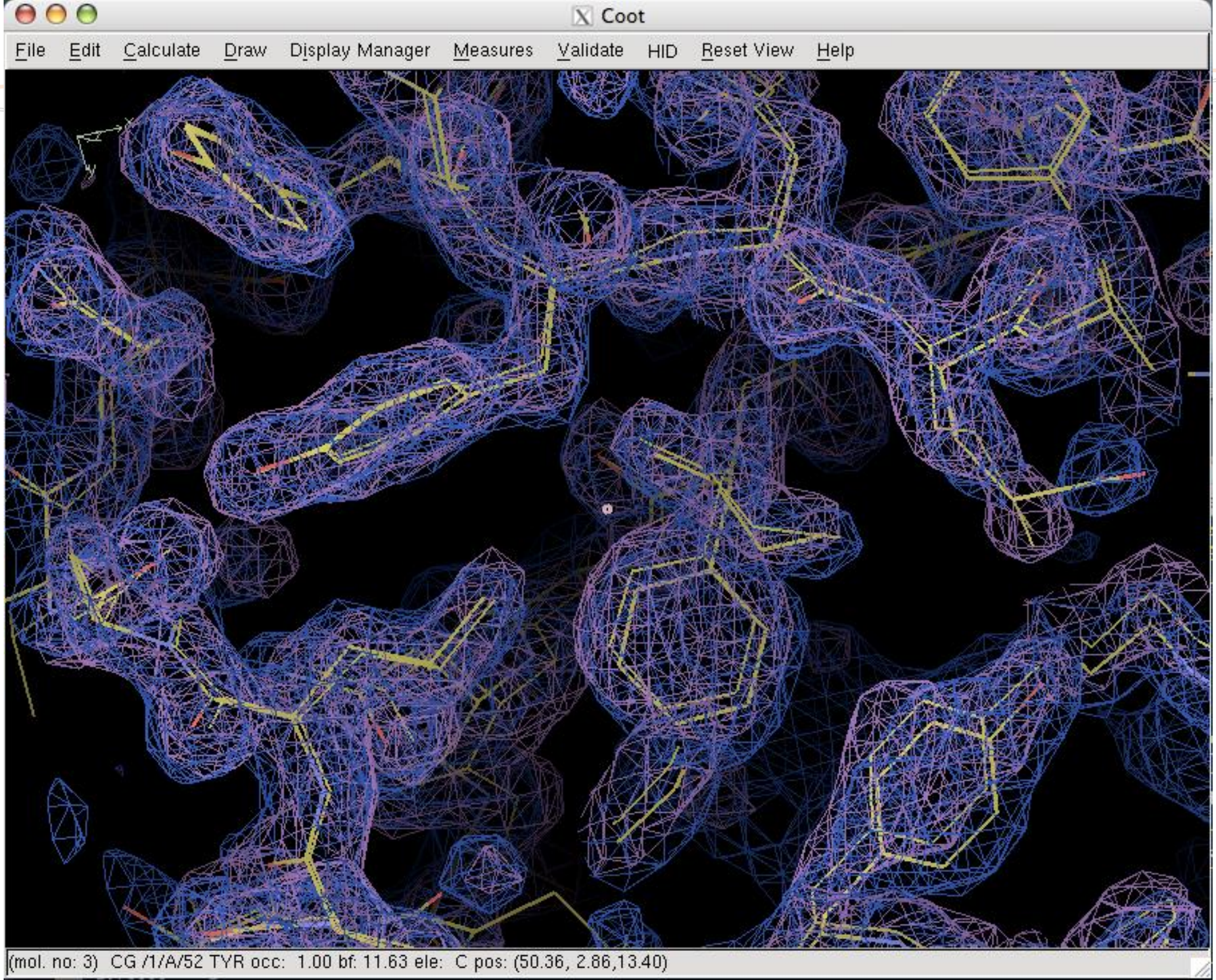
(mol. no: 3) CG /1/A/52 TYR occ: 1.00 bf: 11.63 ele: C pos: (50.36, 2.86,13.40)


NCS Overlays
SSM NCS operator
transform map primitives
map centre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(mol. no: 3) CG /1/A/52 TYR occ: 1.00 bf: 11.63 ele: C pos: (50.36, 2.86,13.40)

NCS 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)
- NCS ligands


Kokay
2012