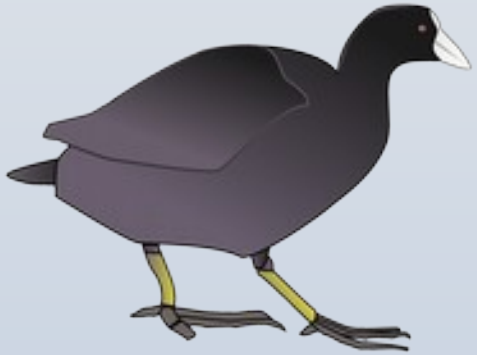
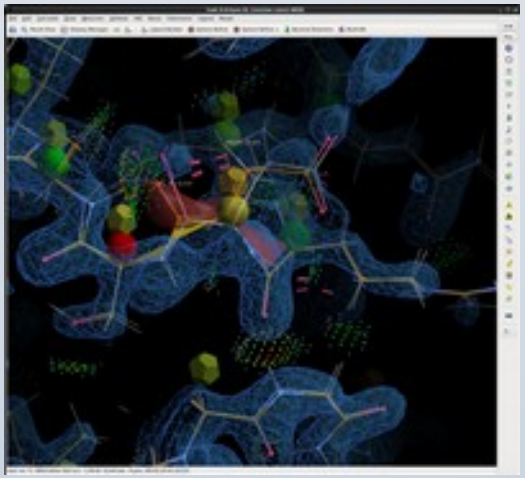
Model-Building with Modern *Coot*

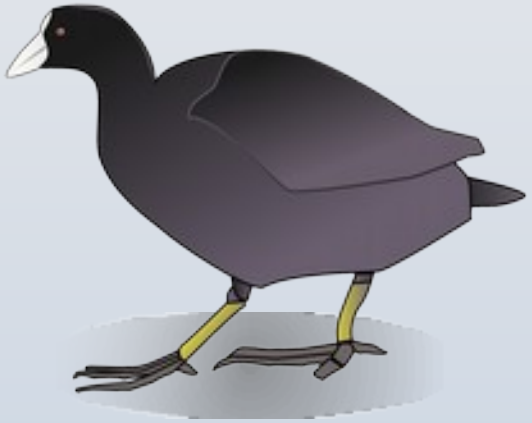




Paul Emsley

Scripps, Feb 2019
EOS Version

Model-building with Modern *Coot*



- Overview:
  - Jiggle Fit
  - Morphing
  - Refinement & Restraints
    - Local distance restraints (ProSMART)
    - cis-peptides
    - “Pink stick, green pea”
  - Recent updates:
    - CURLEW
    - GLSL, Ribbons, VR, AR.

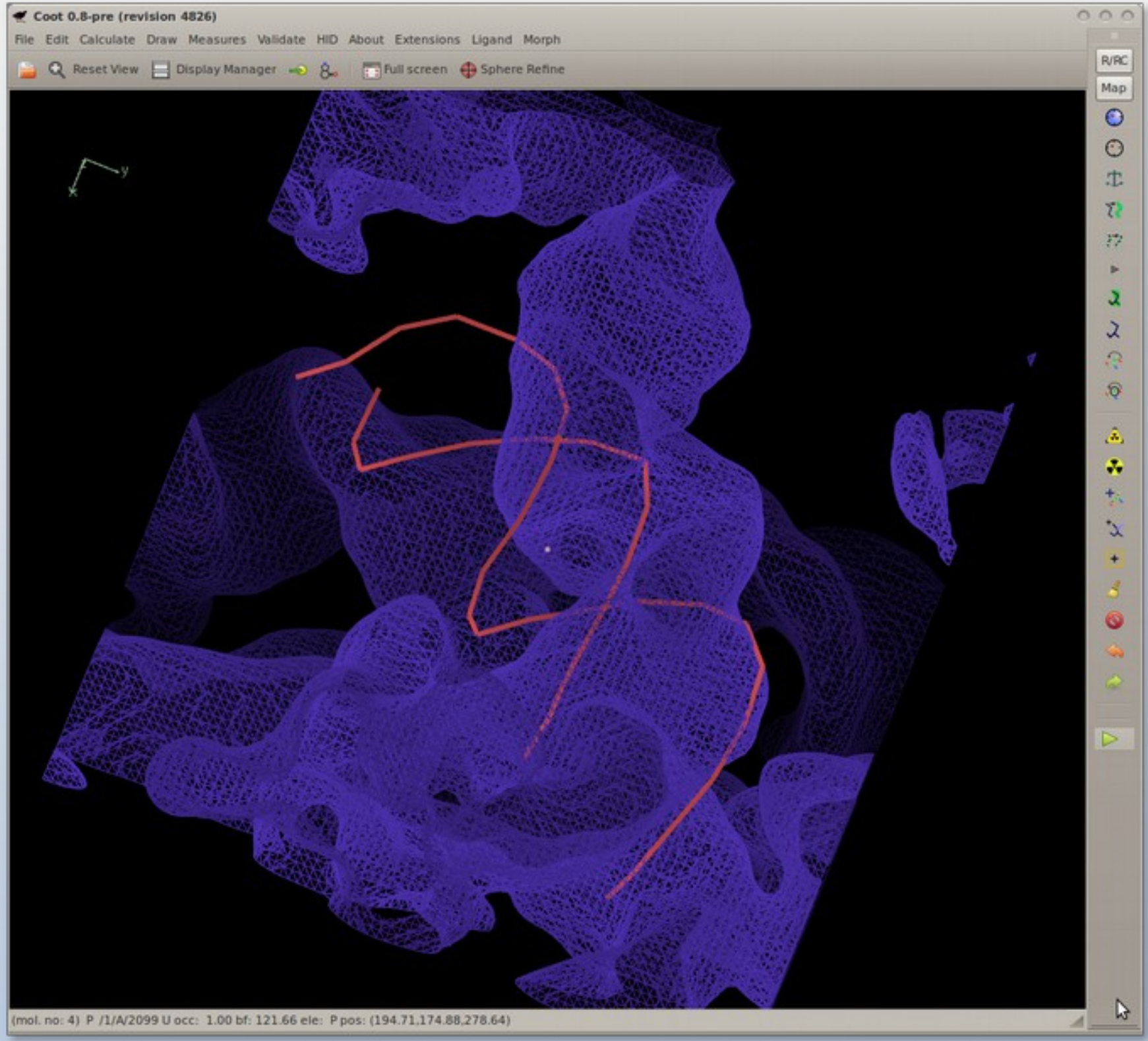
A New Branch

- from which a new binary `coot-experimental` is built
- ~700 revisions ahead of the master branch
- This new branch is actively developed
- Less stable
- More interesting
- Not always clear how to use the new functions
- (or that the new functions are there)

A New Branch

- New multi-threaded sections:
- Refinement
  - Target function and derivative evaluation, model and map all happen simultaneously now
  - Which means: more atoms, smoother updates and/or closer to the minimum
- All-atom contact dots
- Ramachandran Score
- Rotamer Score
- Add Terminal Residue
  - ϕ,ψ hypothesis scoring
- Crankshaft Peptide Optimisation
  - 3 or 5 residue
  - simultaneous evaluation of $\kappa 1$, $\kappa 2$, ... solutions

} "intermediate"/immediate

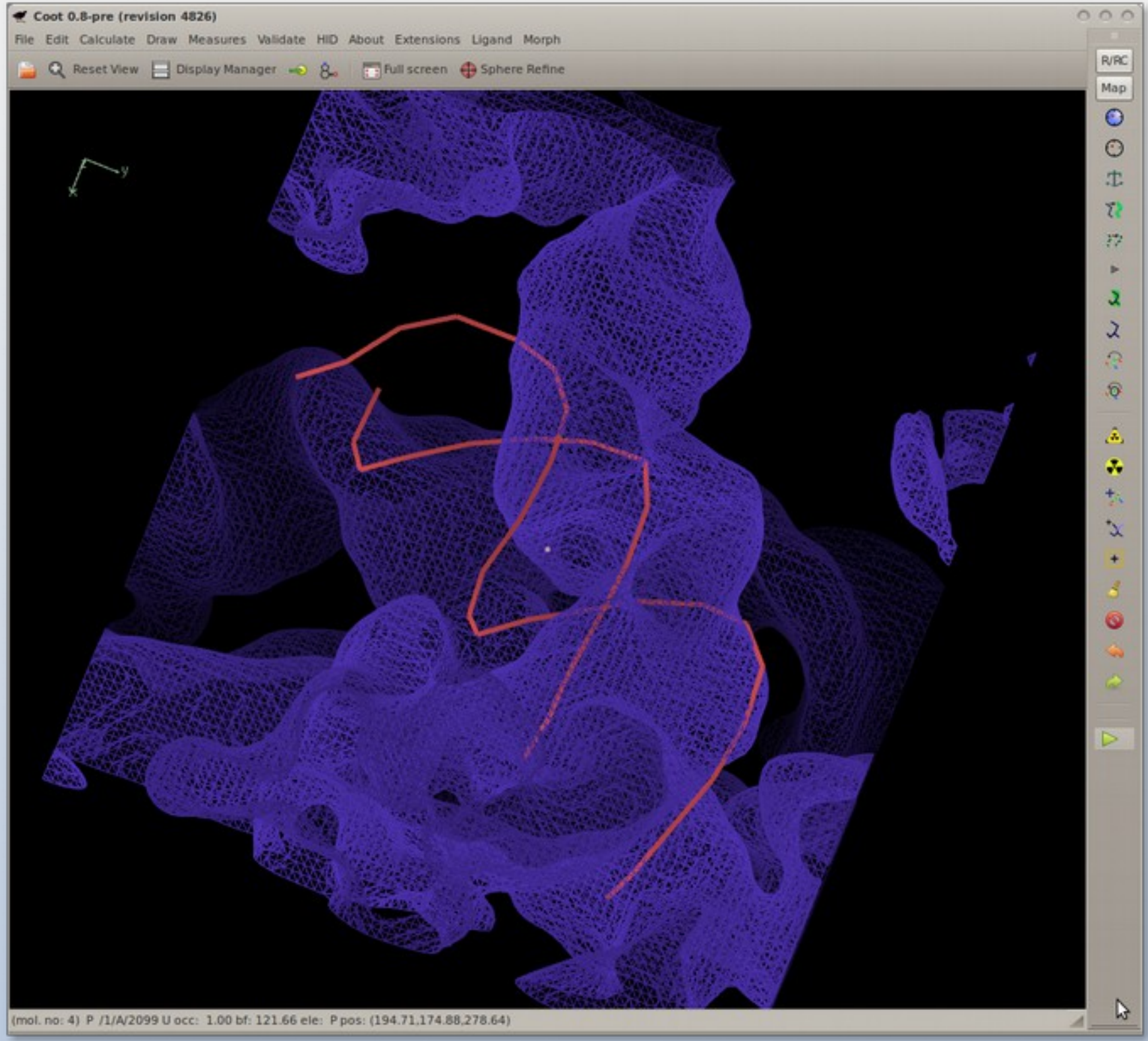

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

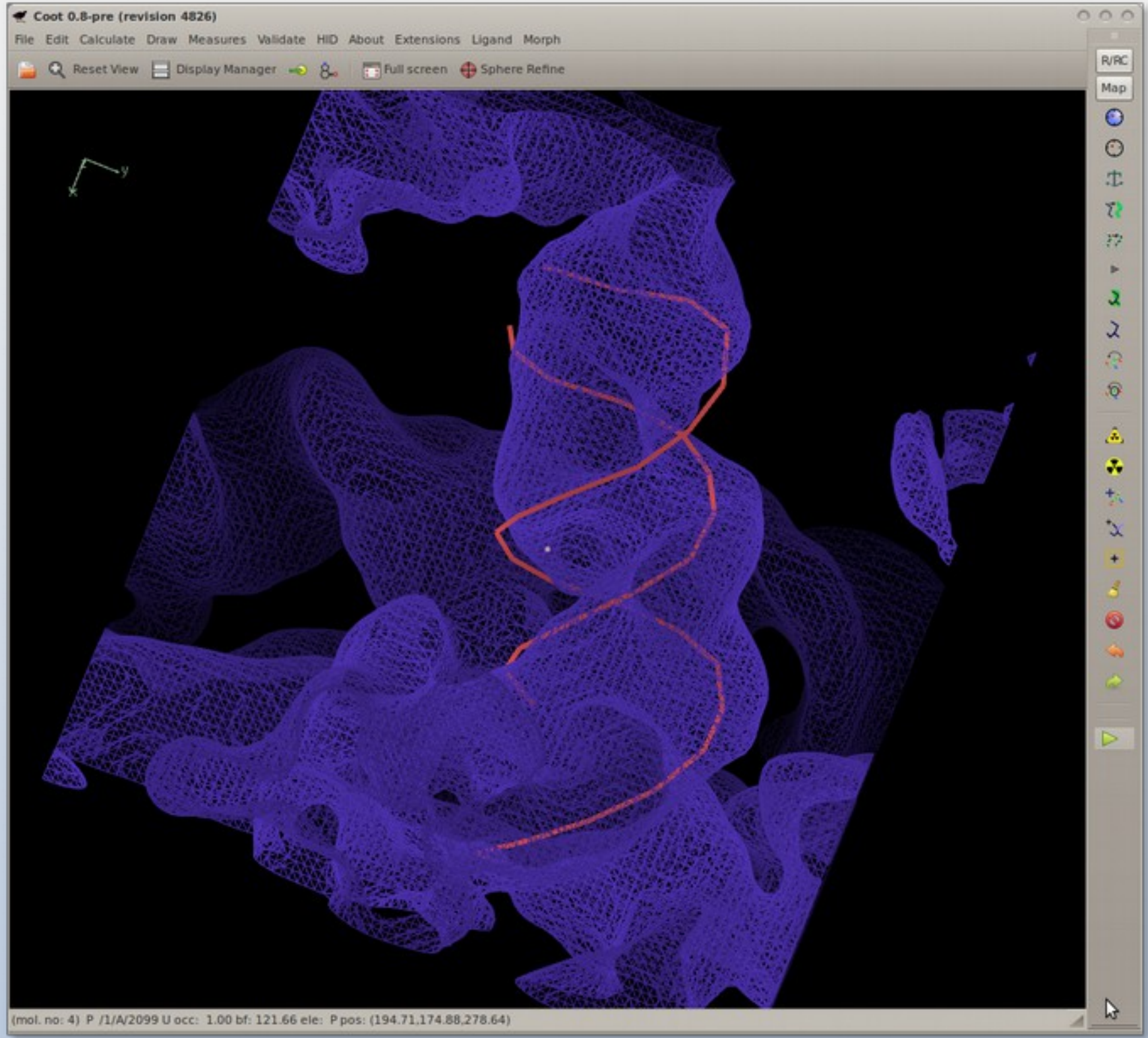
Jiggle Fit

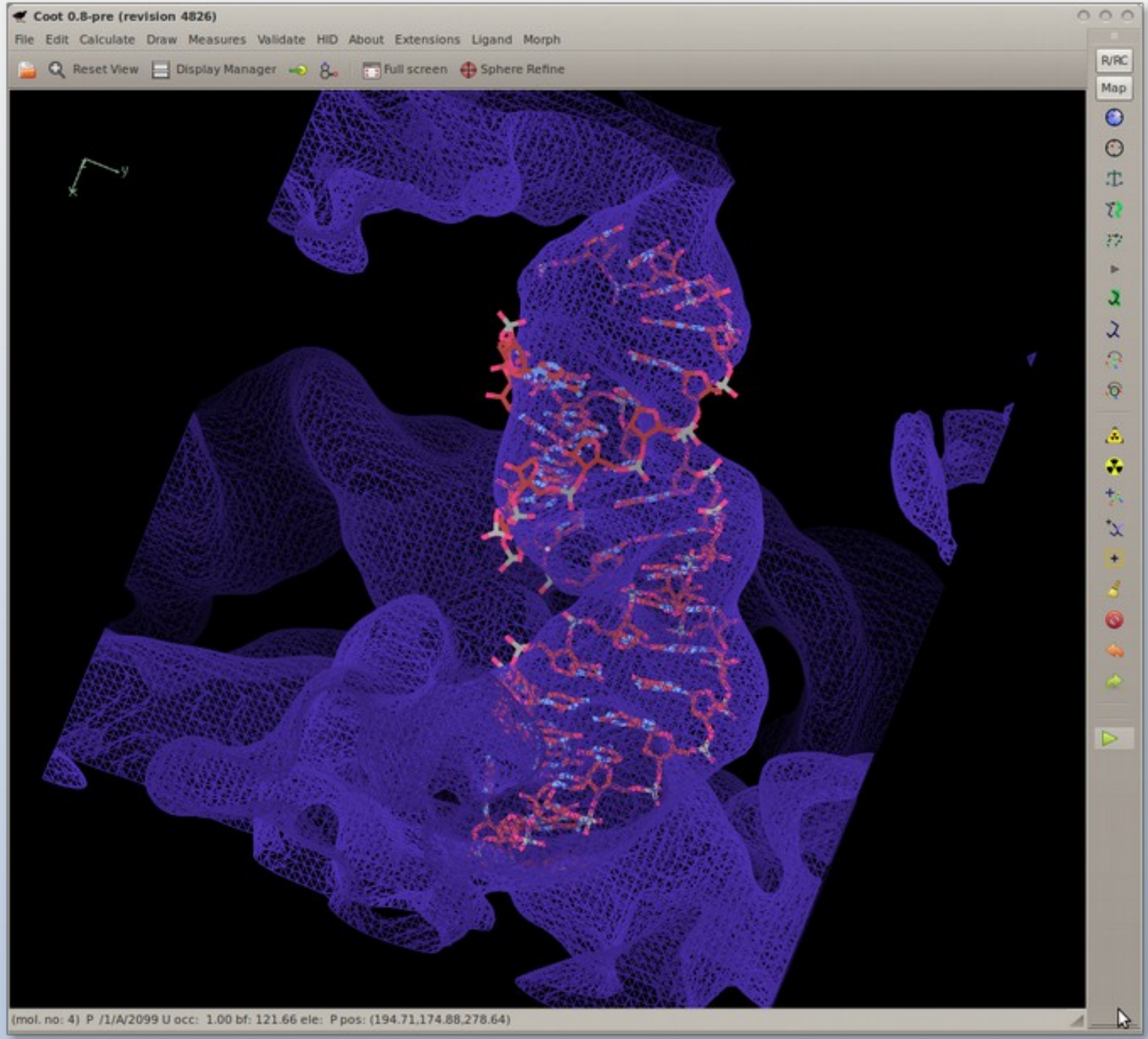
- How do I rotate and translate these atoms to fit the density?
  - 6-dimensional problem
- Originally used to fit simple ligands/solvent molecules to blobs of density
- Now extended to fit arbitrary atom selections
  - *e.g.* by Chain

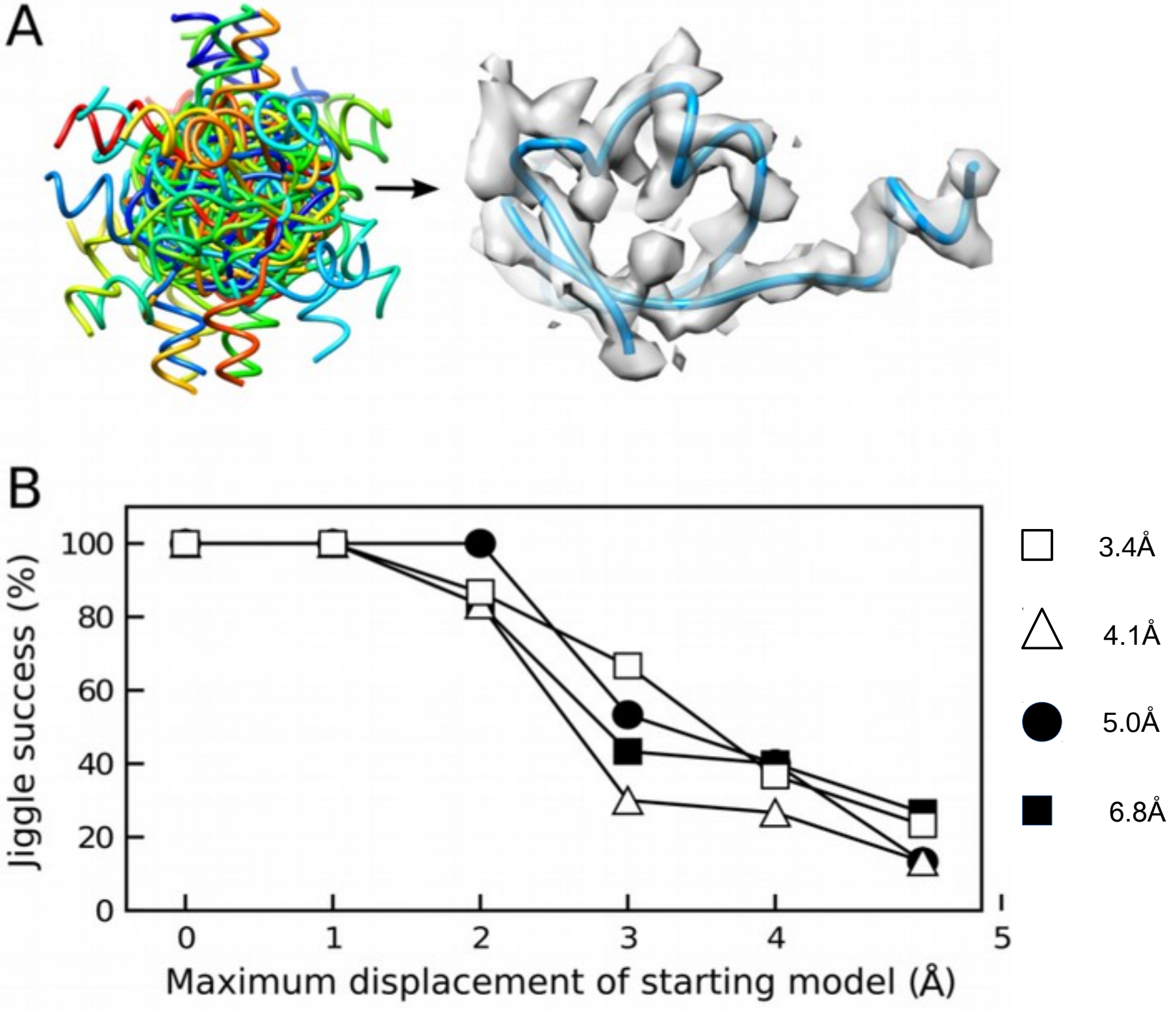
Jiggle Fit: How it Works

- Loop *n* (say 1000) times:
  - Generate sample angles and translations
  - Transform atom selection by these rotations and translation
  - Score and store the fit to density
- Rank density fit scores,
  - Pick top 10 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)


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)


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)


A
B
Jiggle success (%)
100
80
60
40
20
0
0
1
2
3
4
5
Maximum displacement of starting model (Å)
3.4Å
4.1Å
5.0Å
6.8Å

- Note: There are other tools now, such as ProSHADE
  - for symmetry detection and model placement
  - New/Still undergoing testing

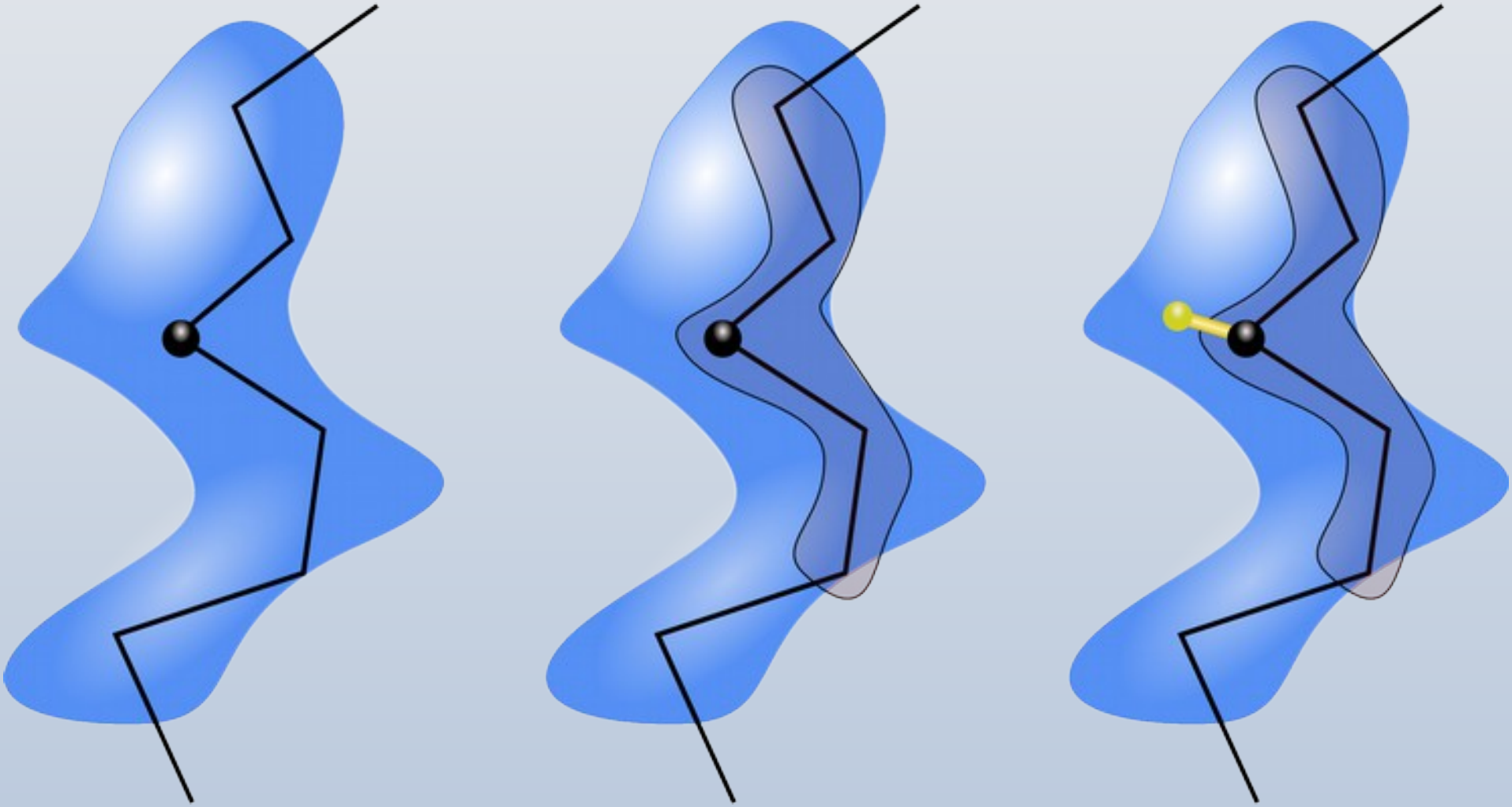
- So we have our ideal RNA or homologous protein sitting roughly in the density

  (not a great fit)

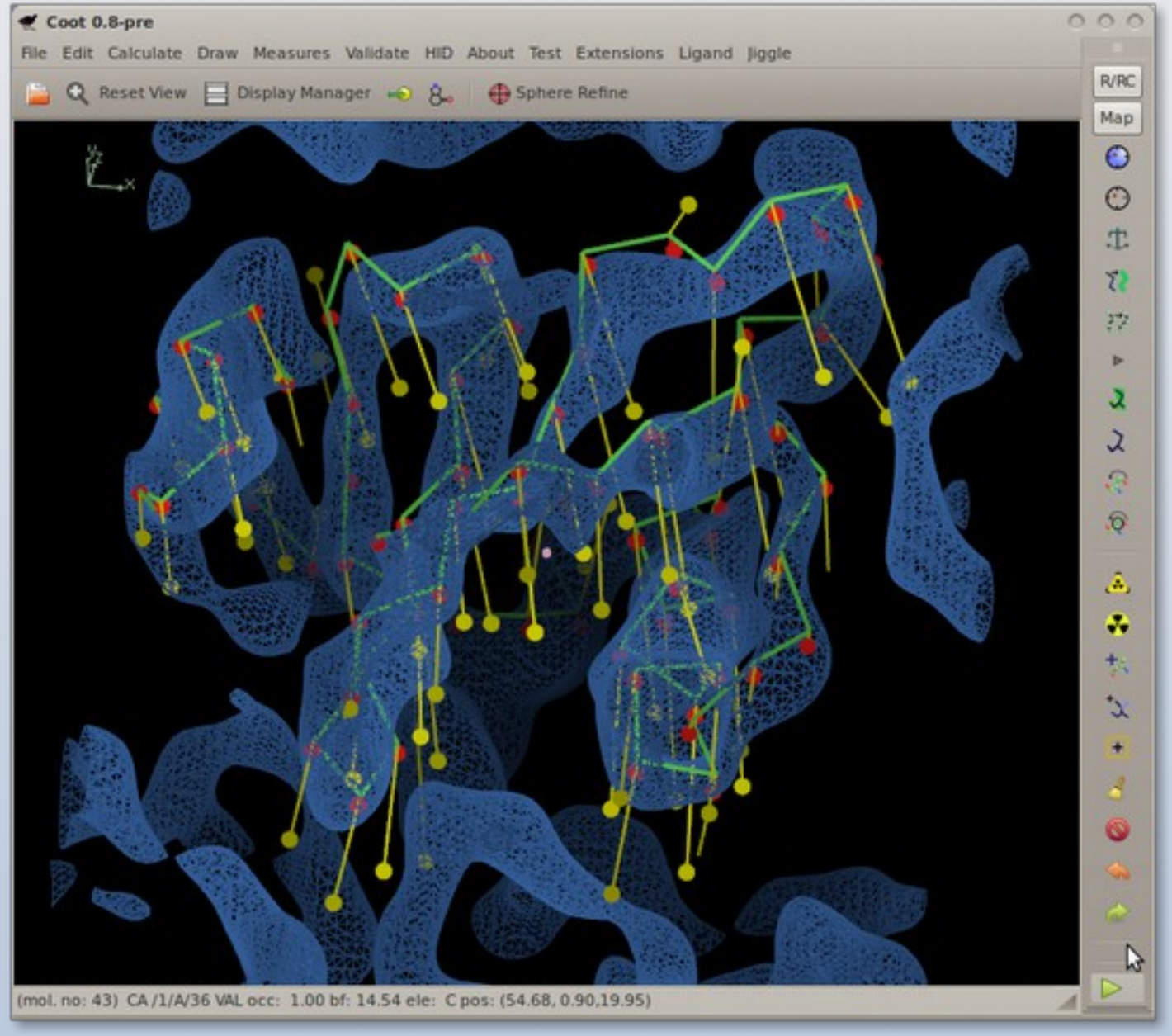
Model Morphing: How it Works

- 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

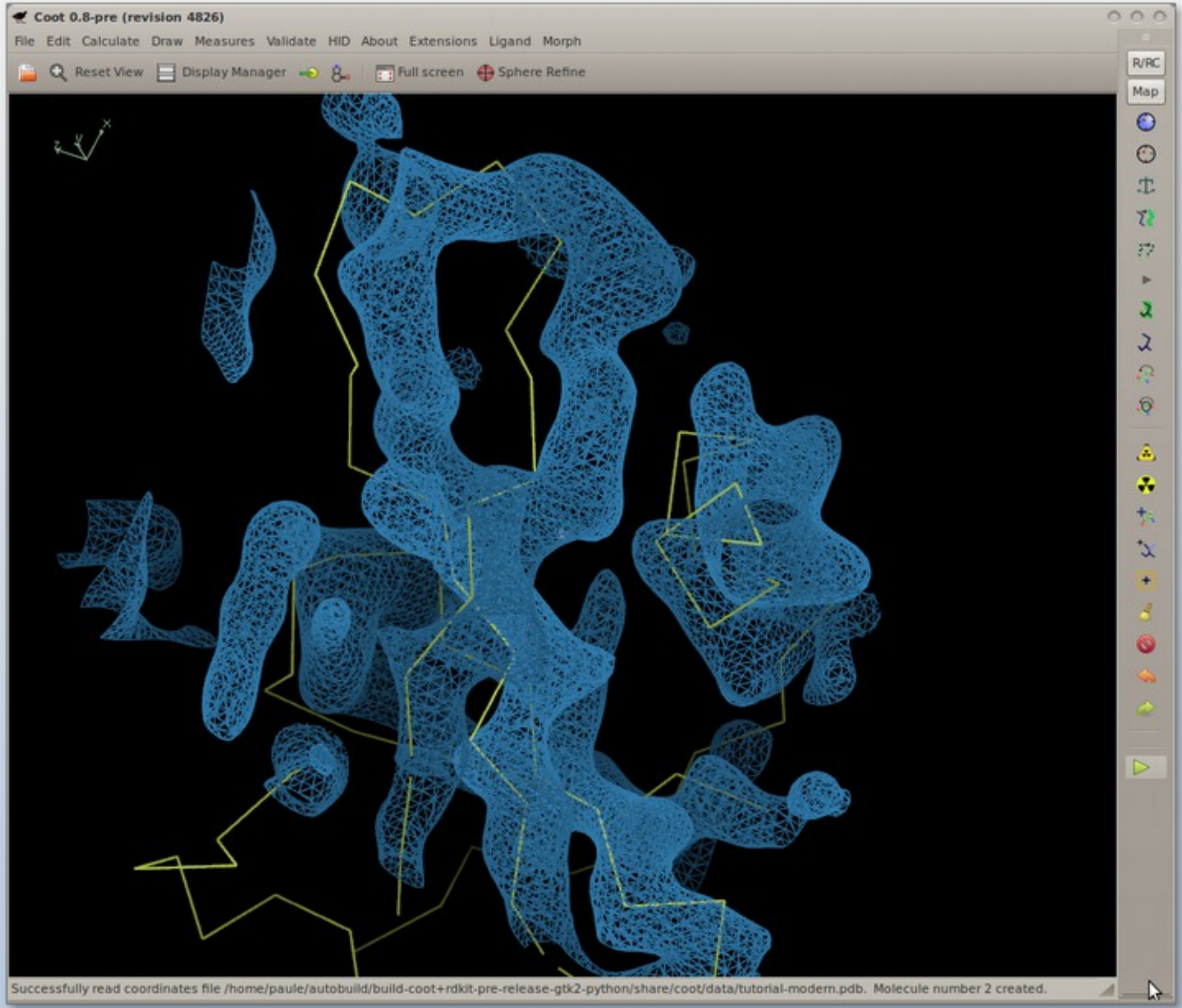


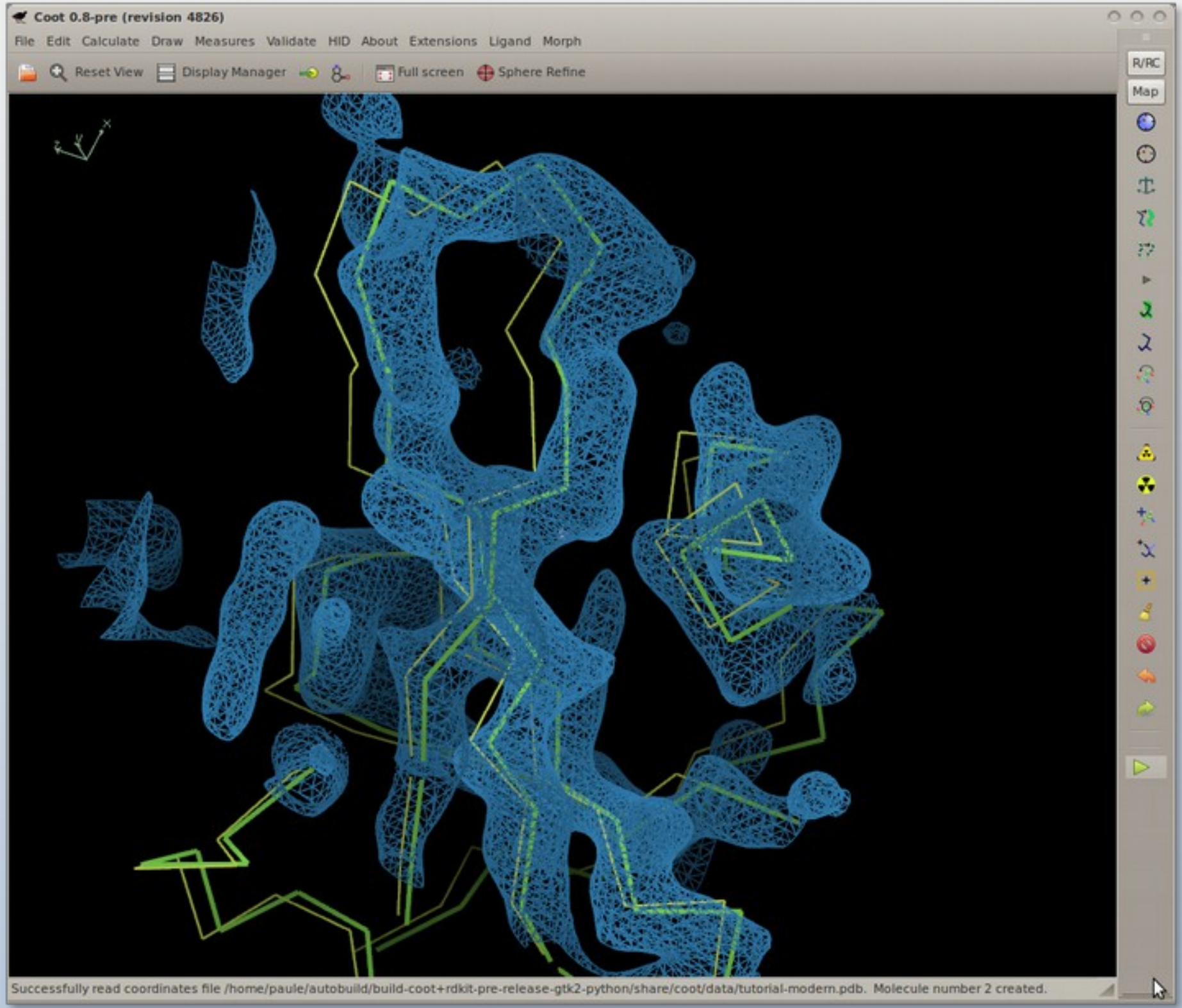
Model Morphing: Example

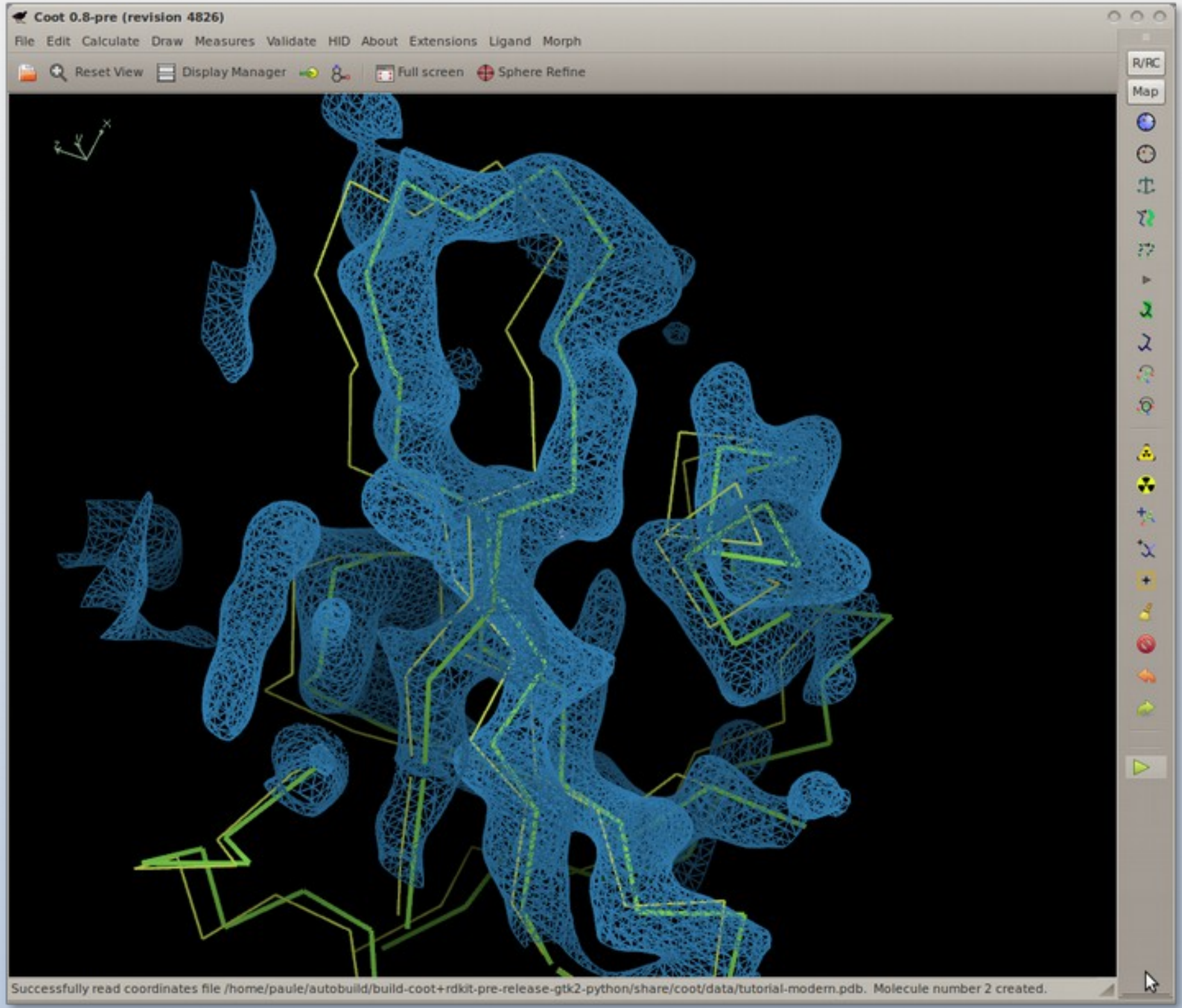


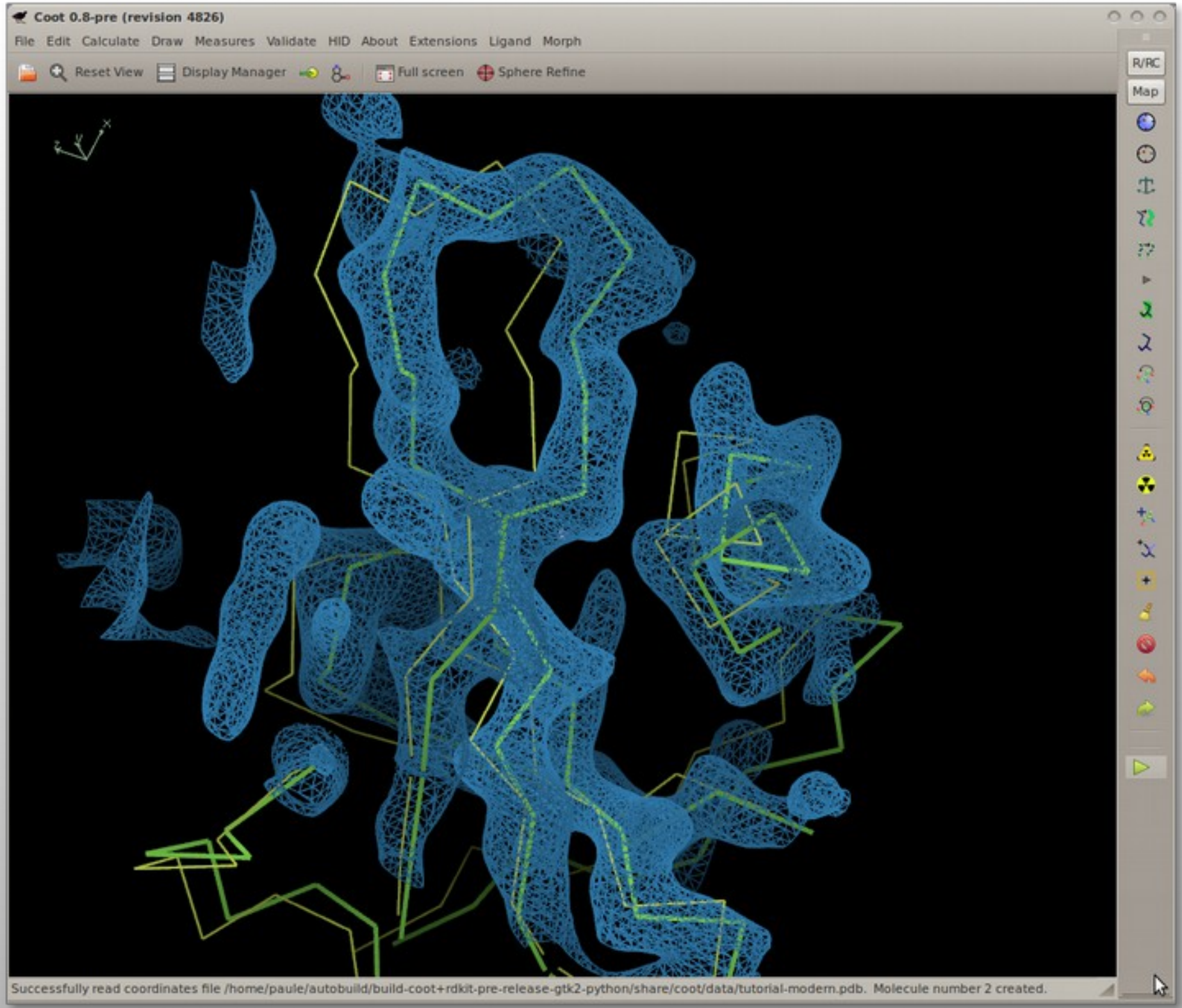
Model Morphing: Robust Averaging

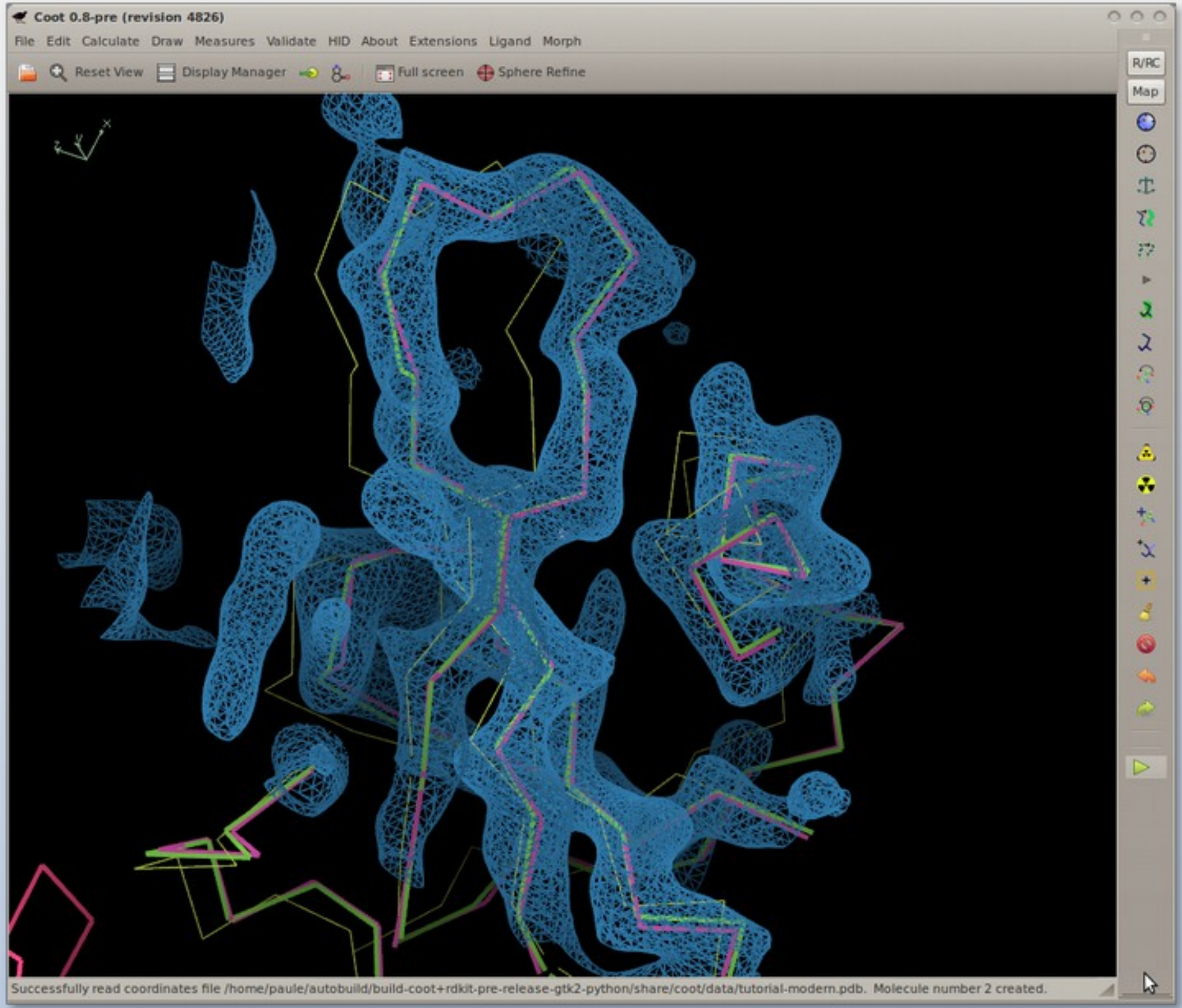
- What are the residues in the environment of a residue?
  - What are their RTs?
  - Create a metric 'distance', sort on that
  - Discard the top and bottom 25%
  - Use remaining RTs to generate average
  - ...which is then applied to central residue
- Repeat for all residues
- Larger environment radii 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.

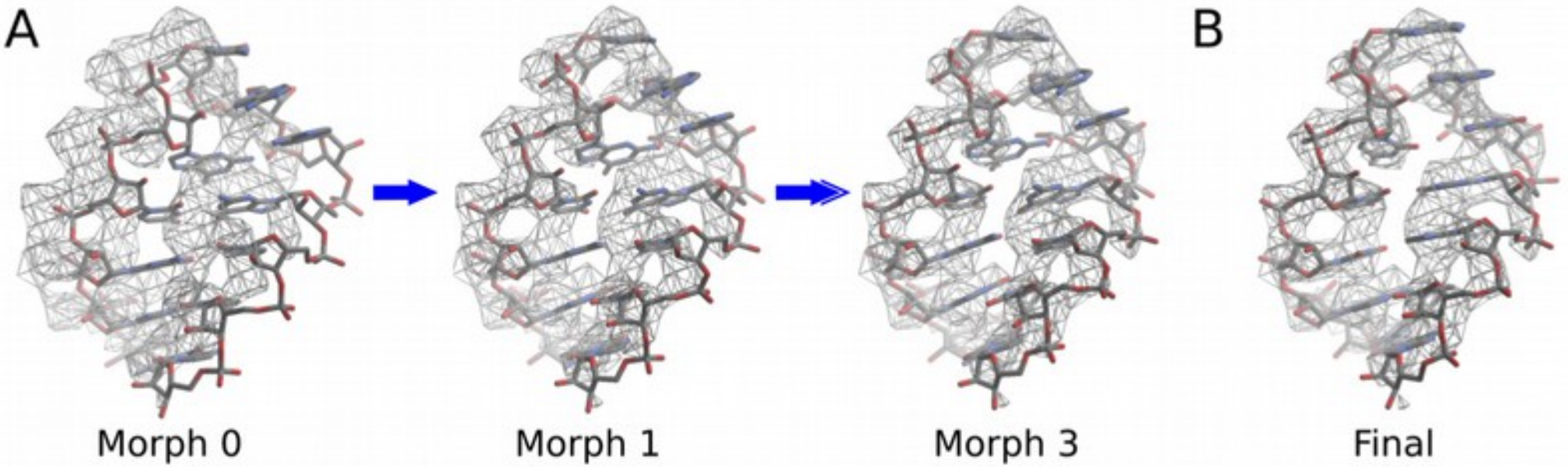

Coot 0.8-pre (revision 4826)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Morph
Reset View
Display Manager
Full screen
Sphere Refine
R/RC
Map
Successfully read coordinates file /home/paule/autobuild/build-coot+rdkit-pre-release-gtk2-python/share/coot/data/tutorial-modern.pdb. 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
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
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



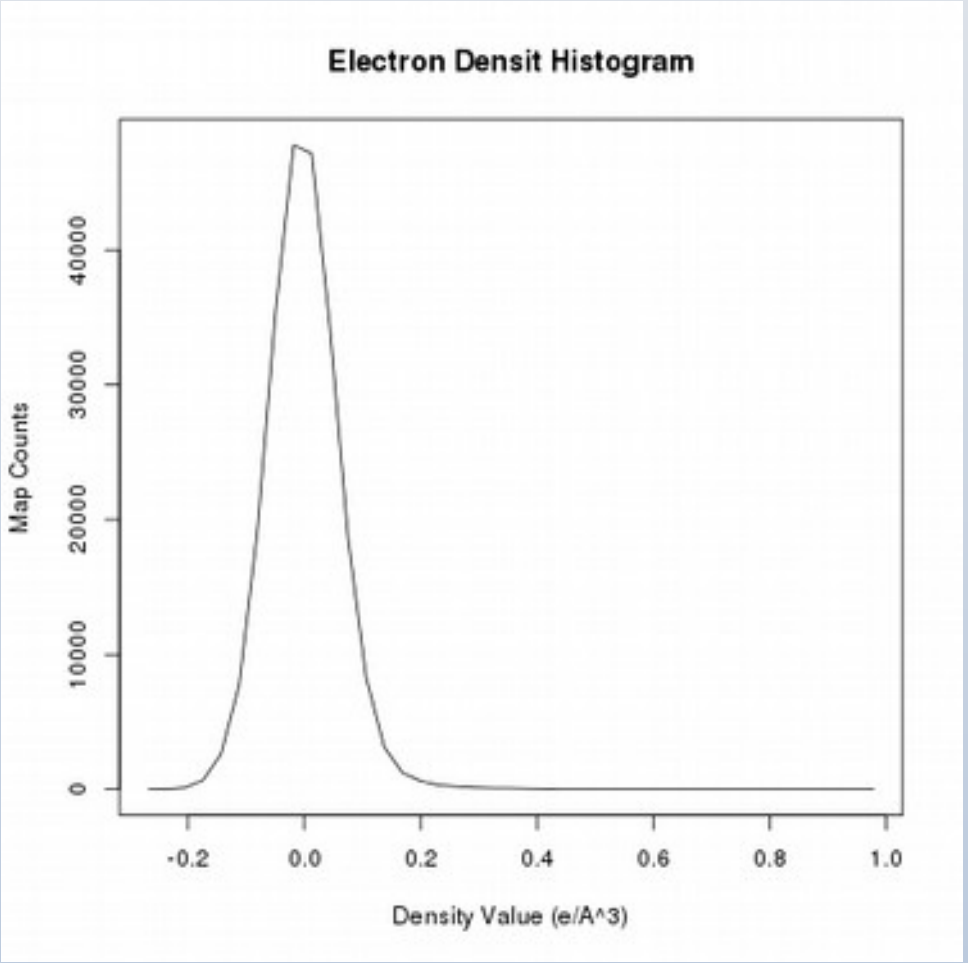
Helix Fitting

- The distribution of electron density is quite unlike that of x-ray maps
  - e.g. You don't see main-chain atoms at 4 rmsd in x-ray maps
    - regions of dense electron density contribute negatively to helix score in x-ray maps

Helix Fitting

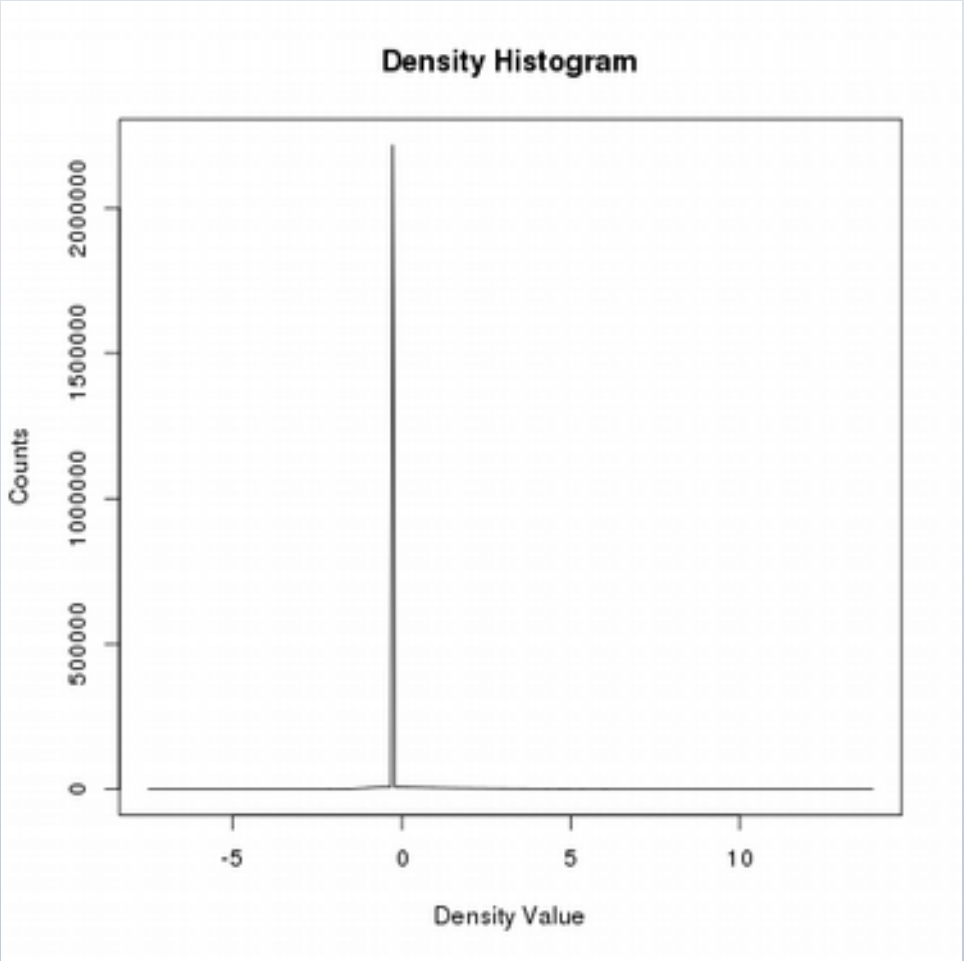
- The distribution of electron density is quite unlike that of x-ray maps

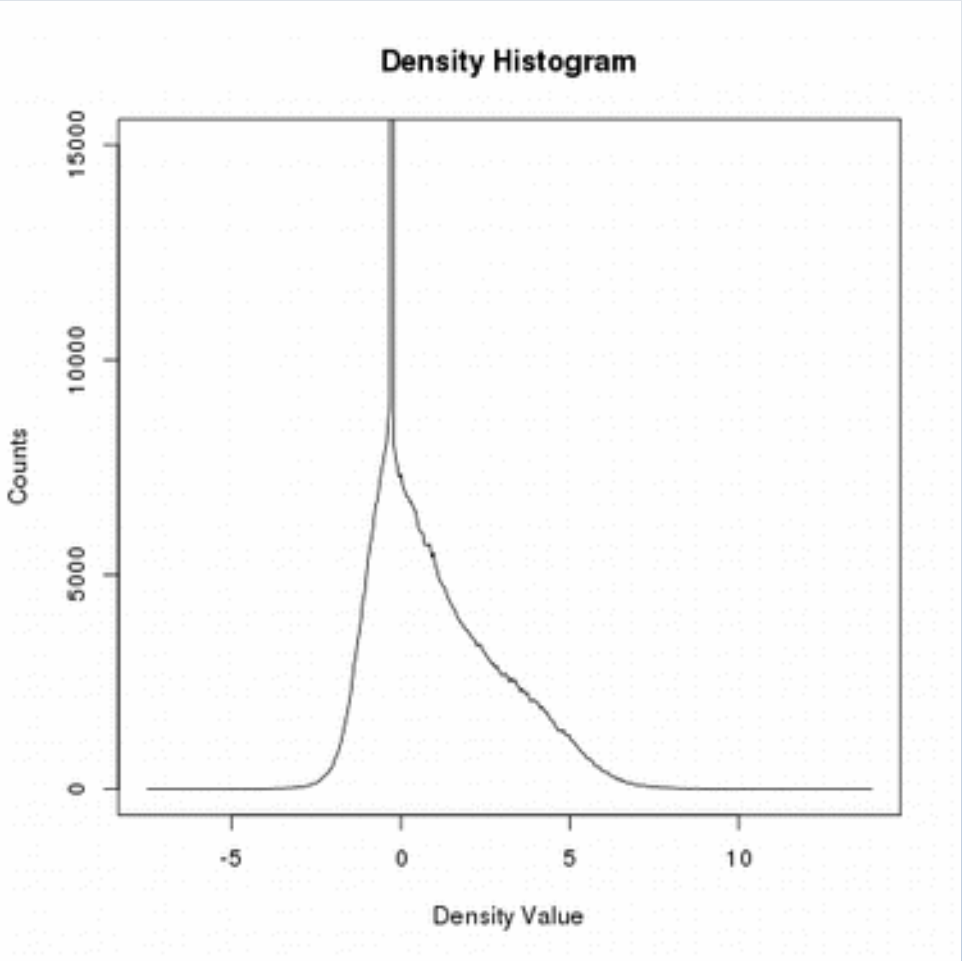
Typical Density Histogram from an X-ray map



Helix Fitting

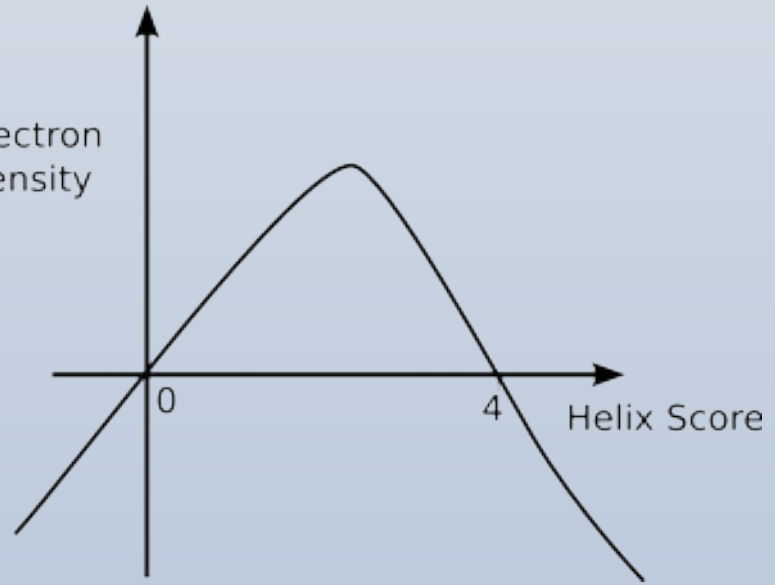
- The distribution of electron density is quite unlike that of x-ray maps





Helix Fitting

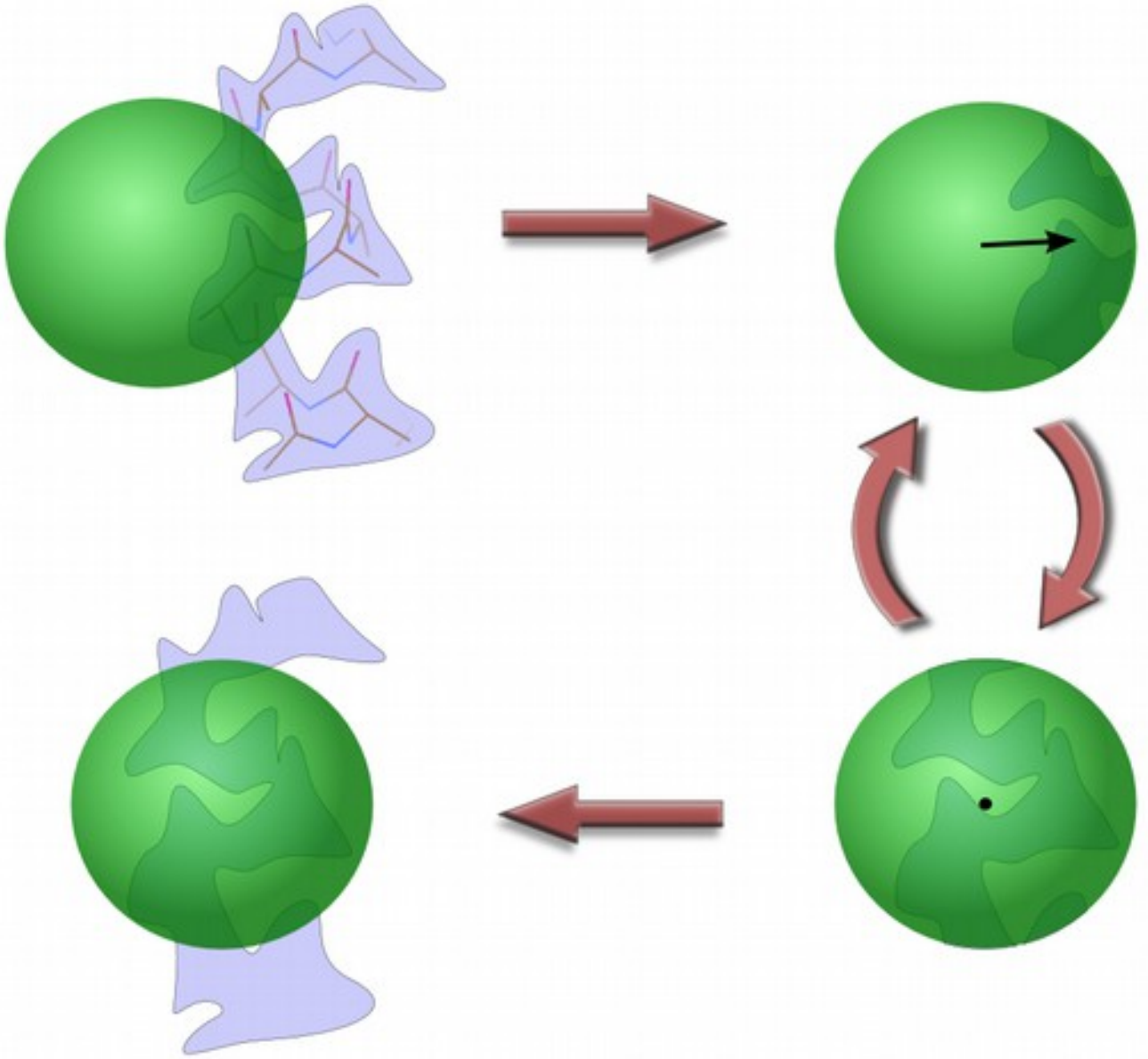
- The distribution of electron density is quite unlike that of x-ray maps
  - e.g. You don't see main-chain atoms at 4 rmsd in x-ray maps
    - regions of dense electron density contribute negatively to helix score
  - These EM maps were sharpened and in a big box of mostly nothing
  - Lots to see at 4 rmsd



Alpha Helix Placement

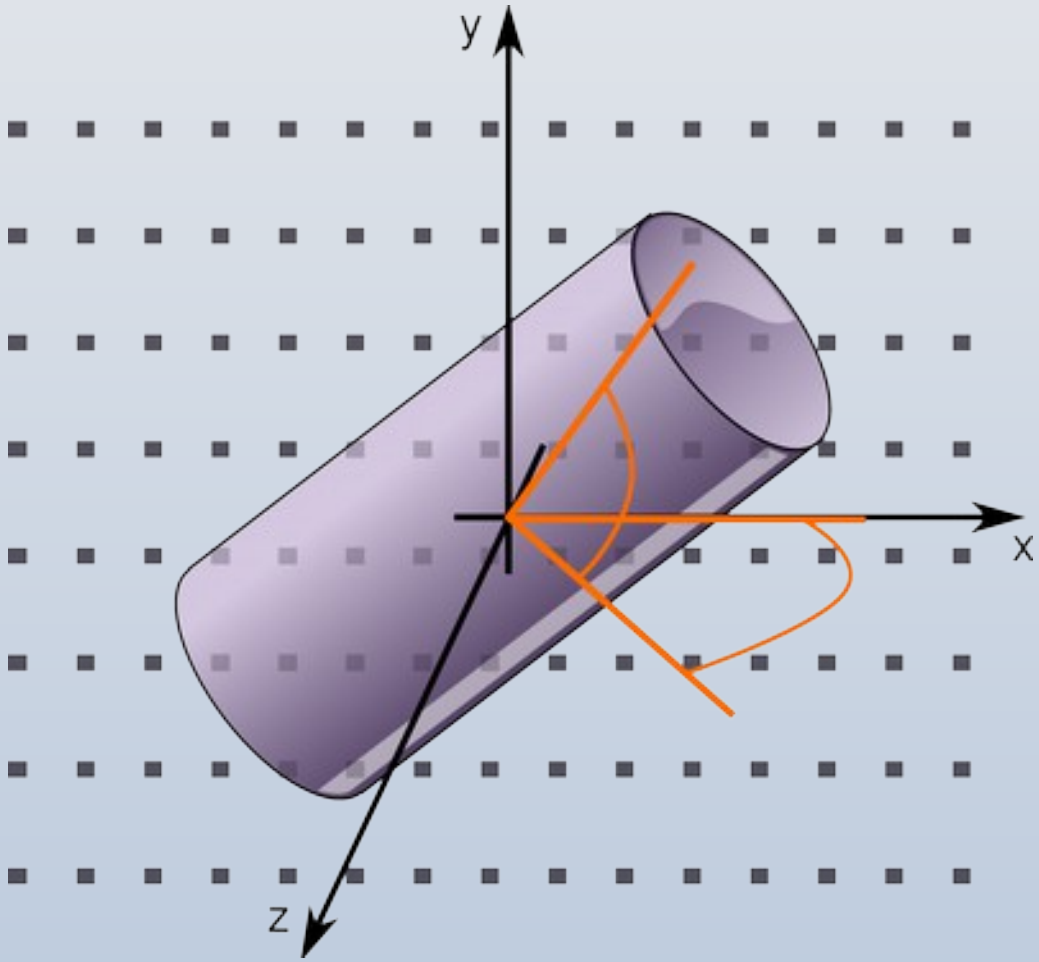
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point



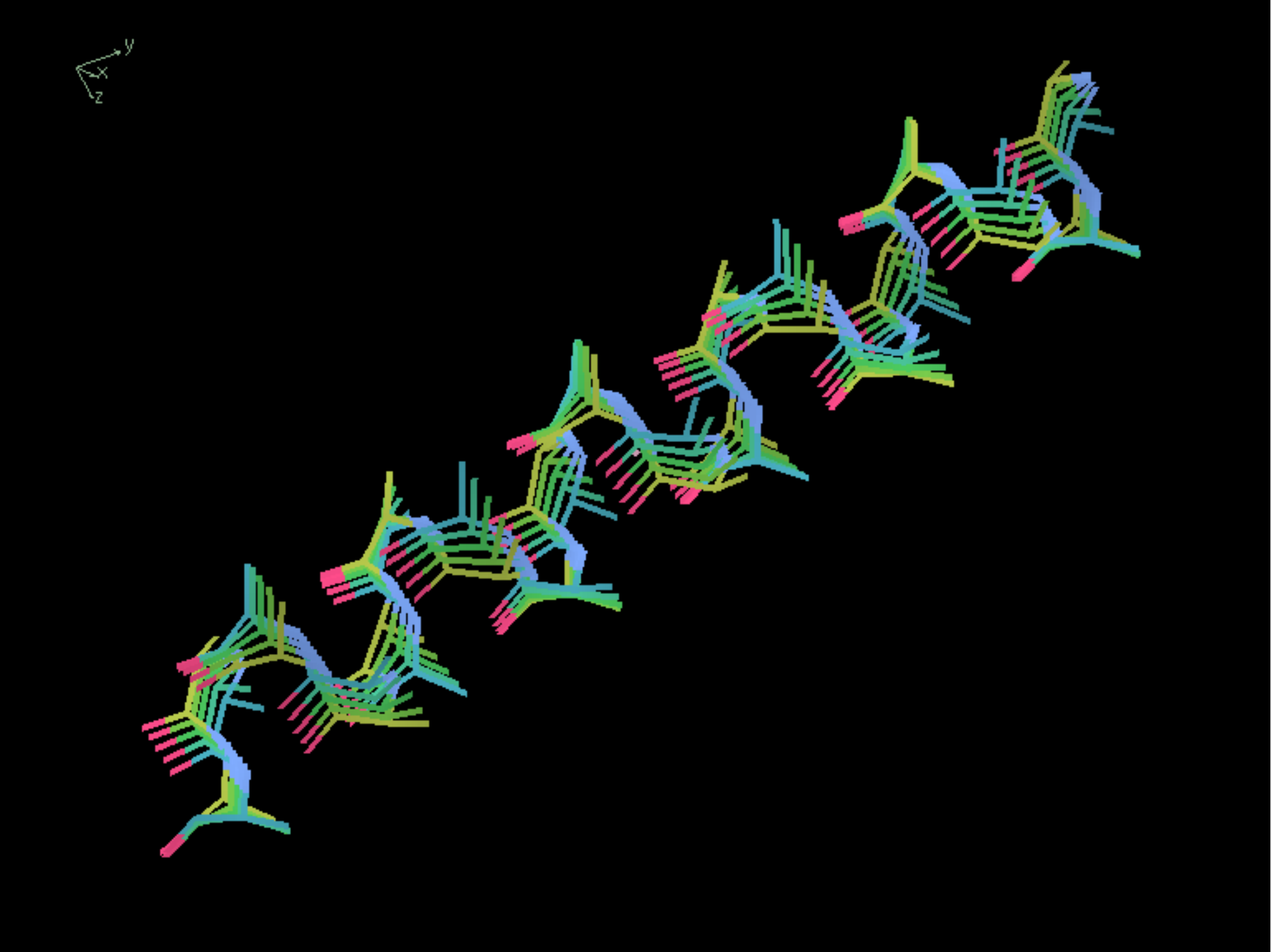
Helix Fitting: Cylinder Search

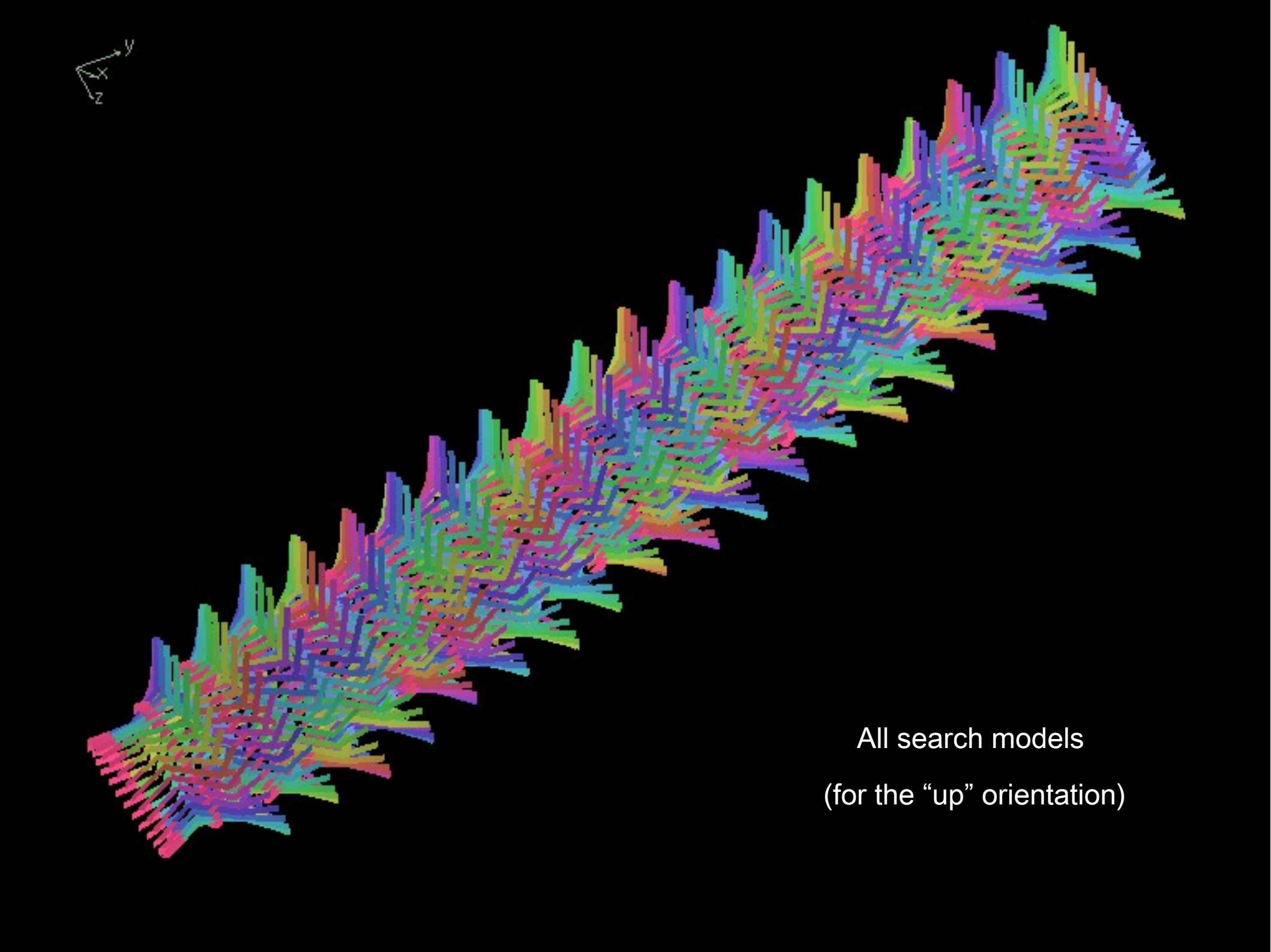
- Pick the orientation that encapsulates the most electron density

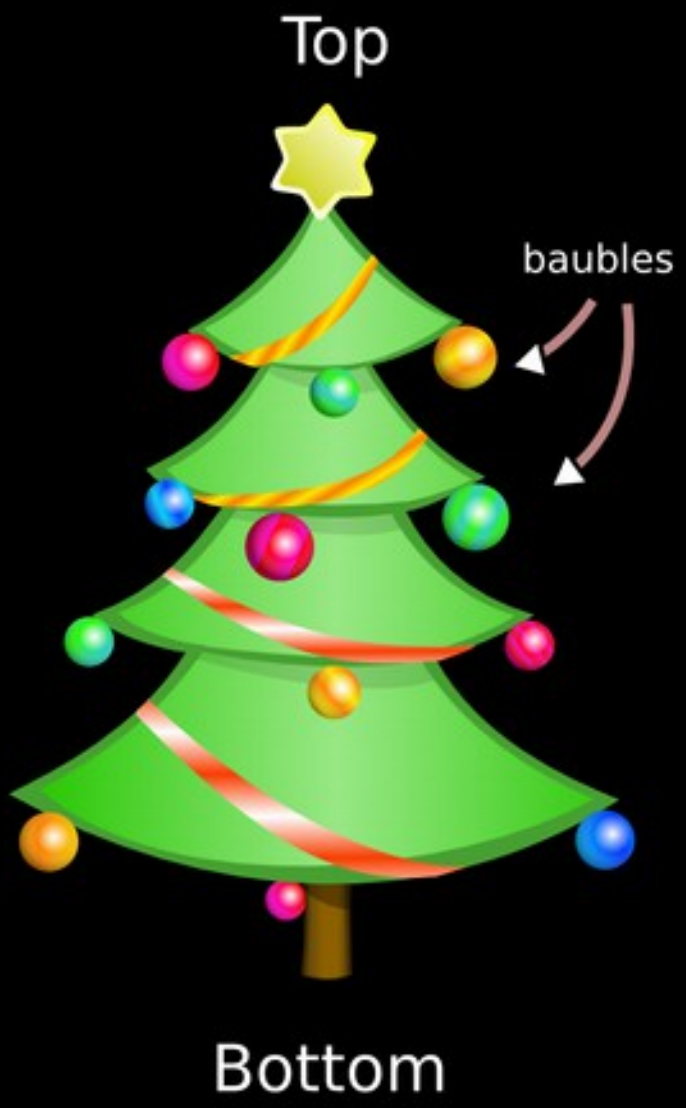


Using 2 rotation axes

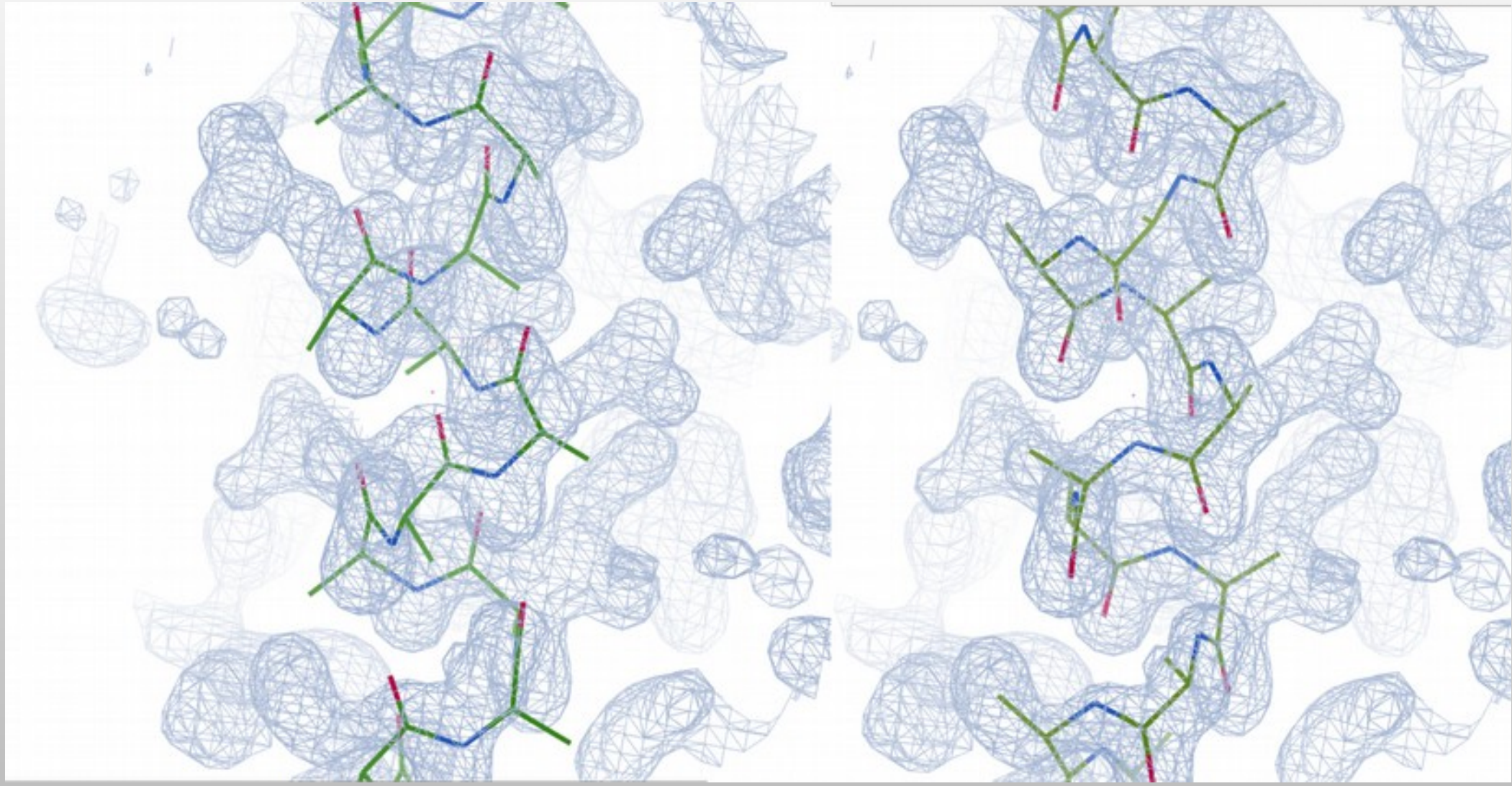
2 x 1-D Helix orientation searches


y
x
z

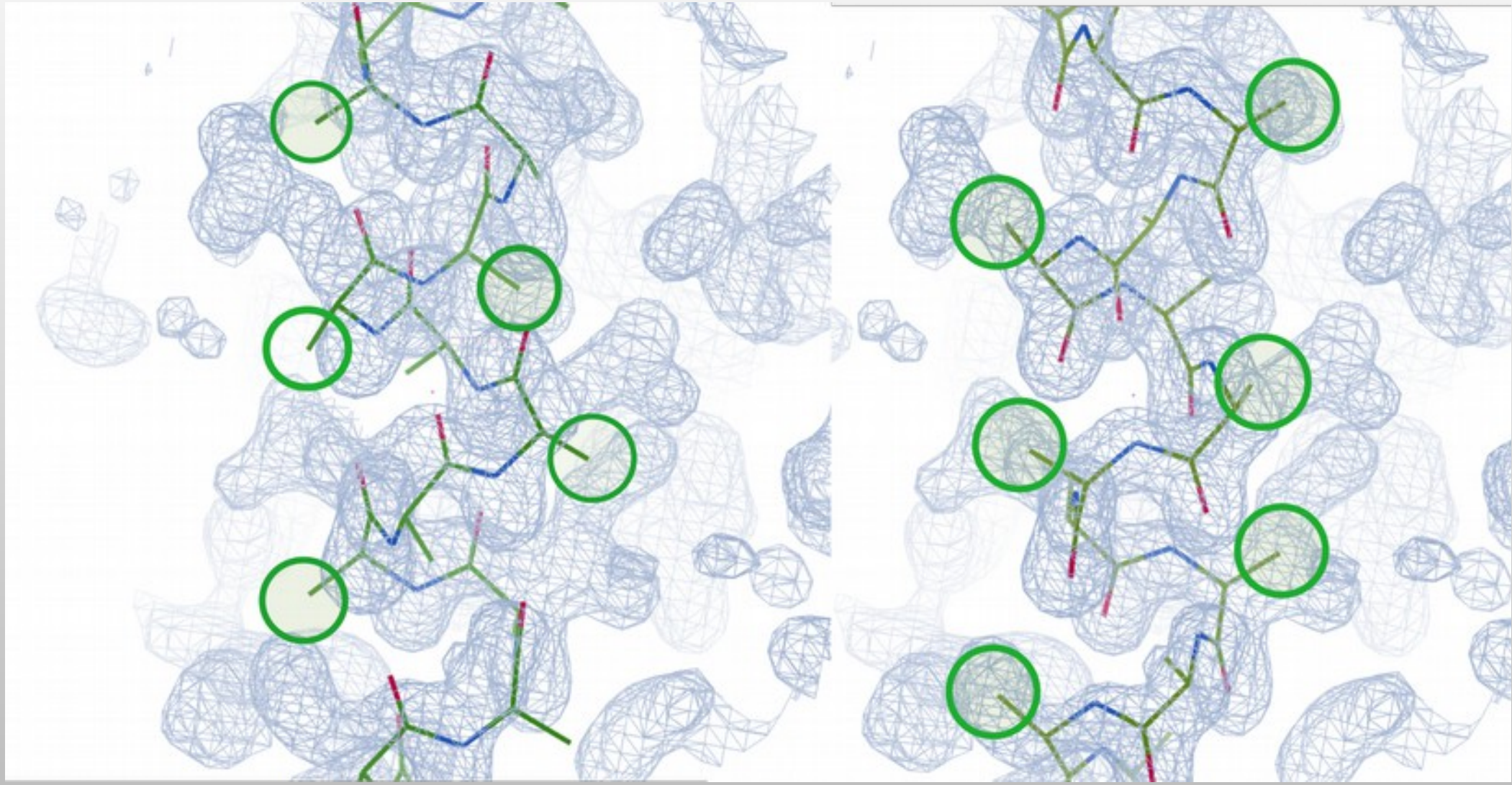

y
x
z
All search models
(for the “up” orientation)


Top
baubles
Bottom

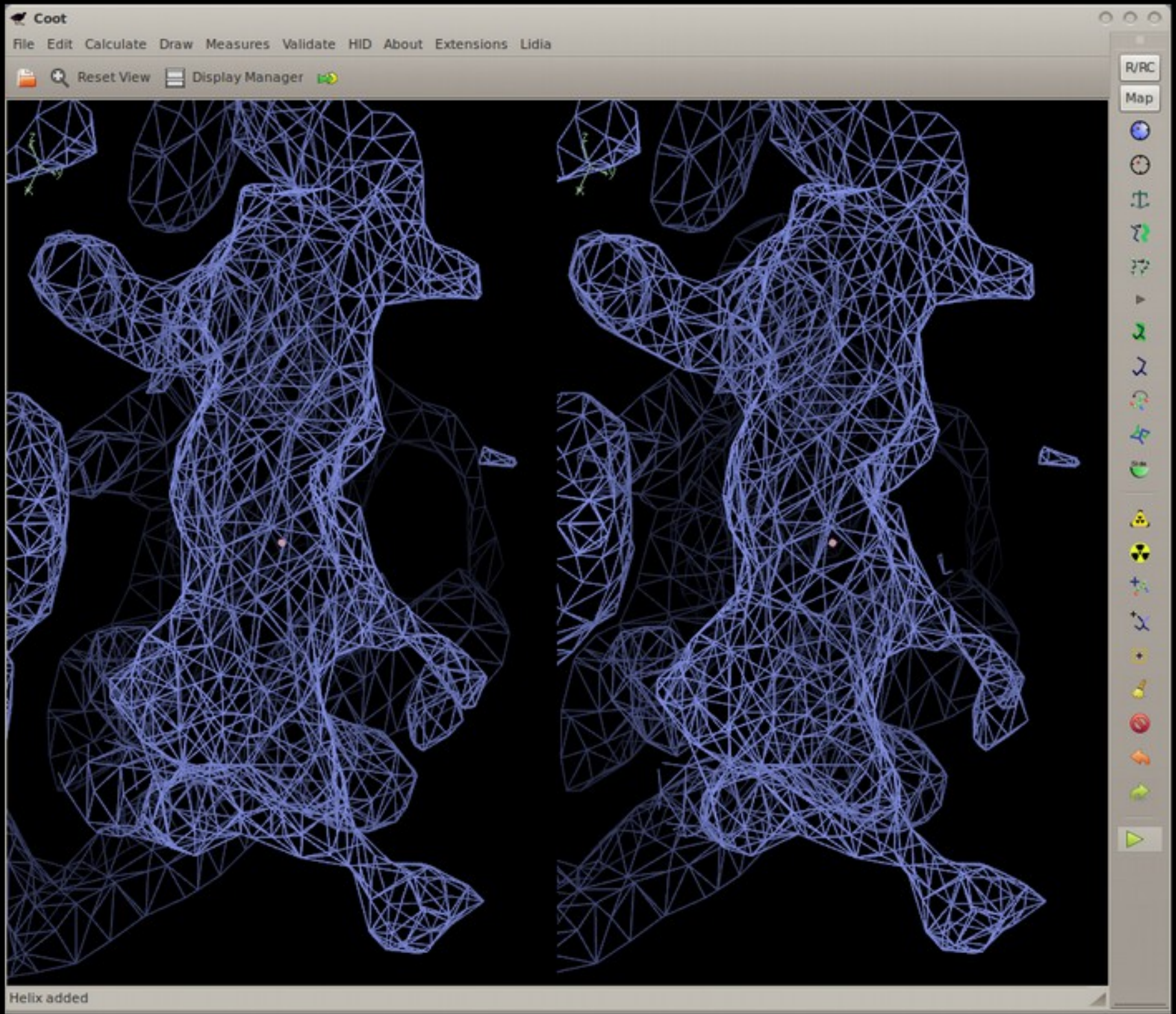
Helix Fitting
Comparing orientation hypotheses

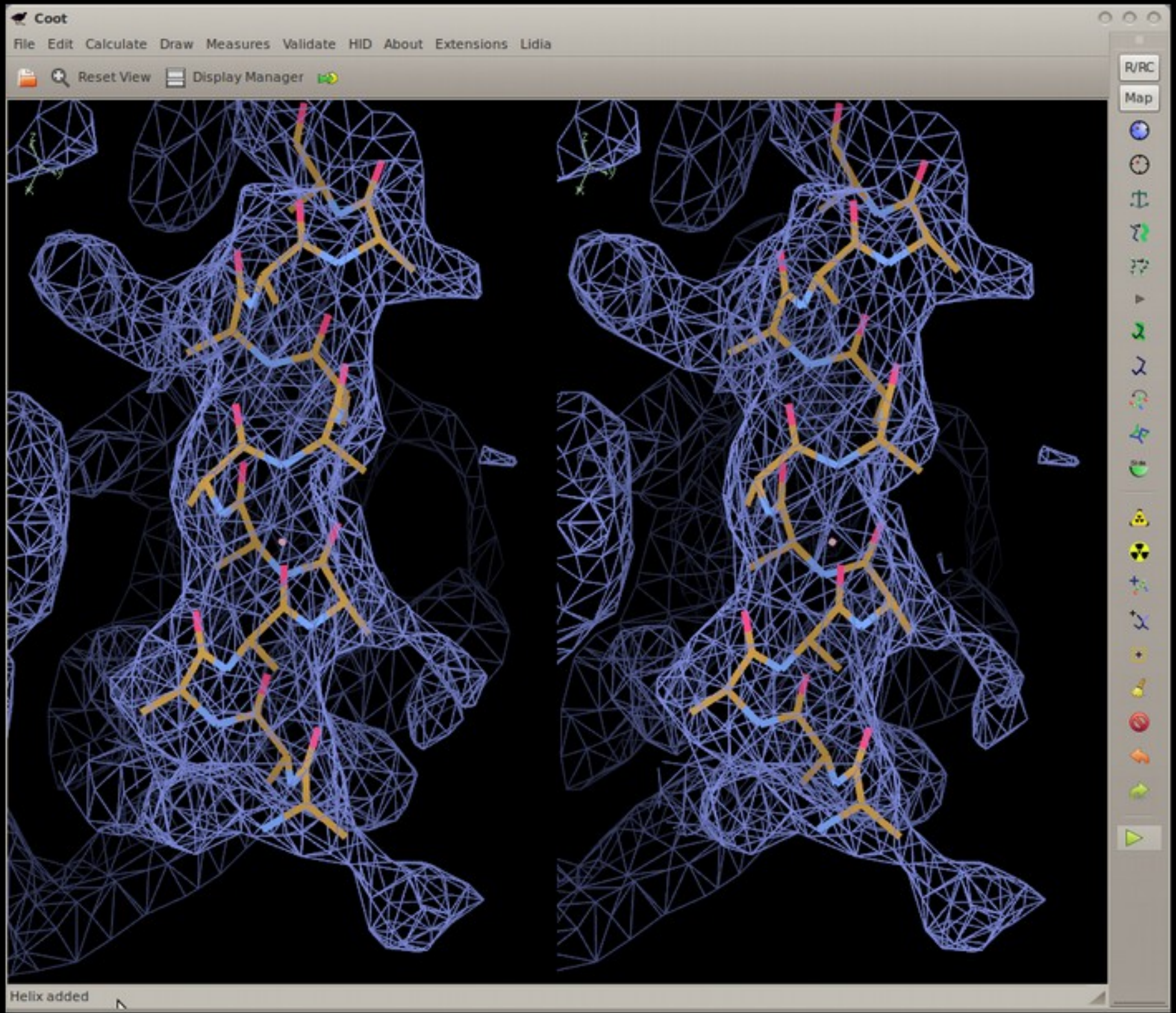


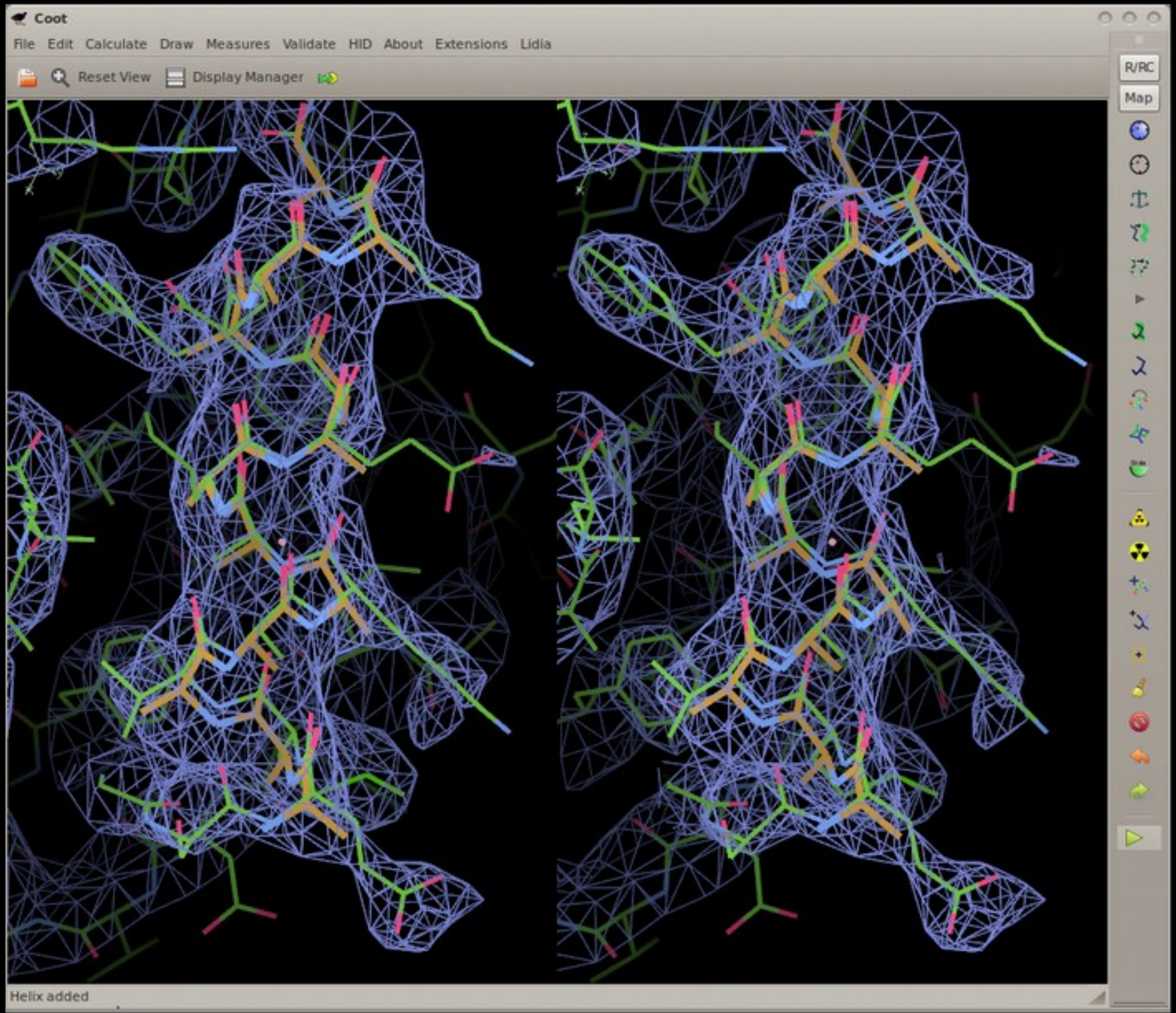
Helix Fitting
Comparing orientation hypotheses



c-betas are not fitted and are used for scoring


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

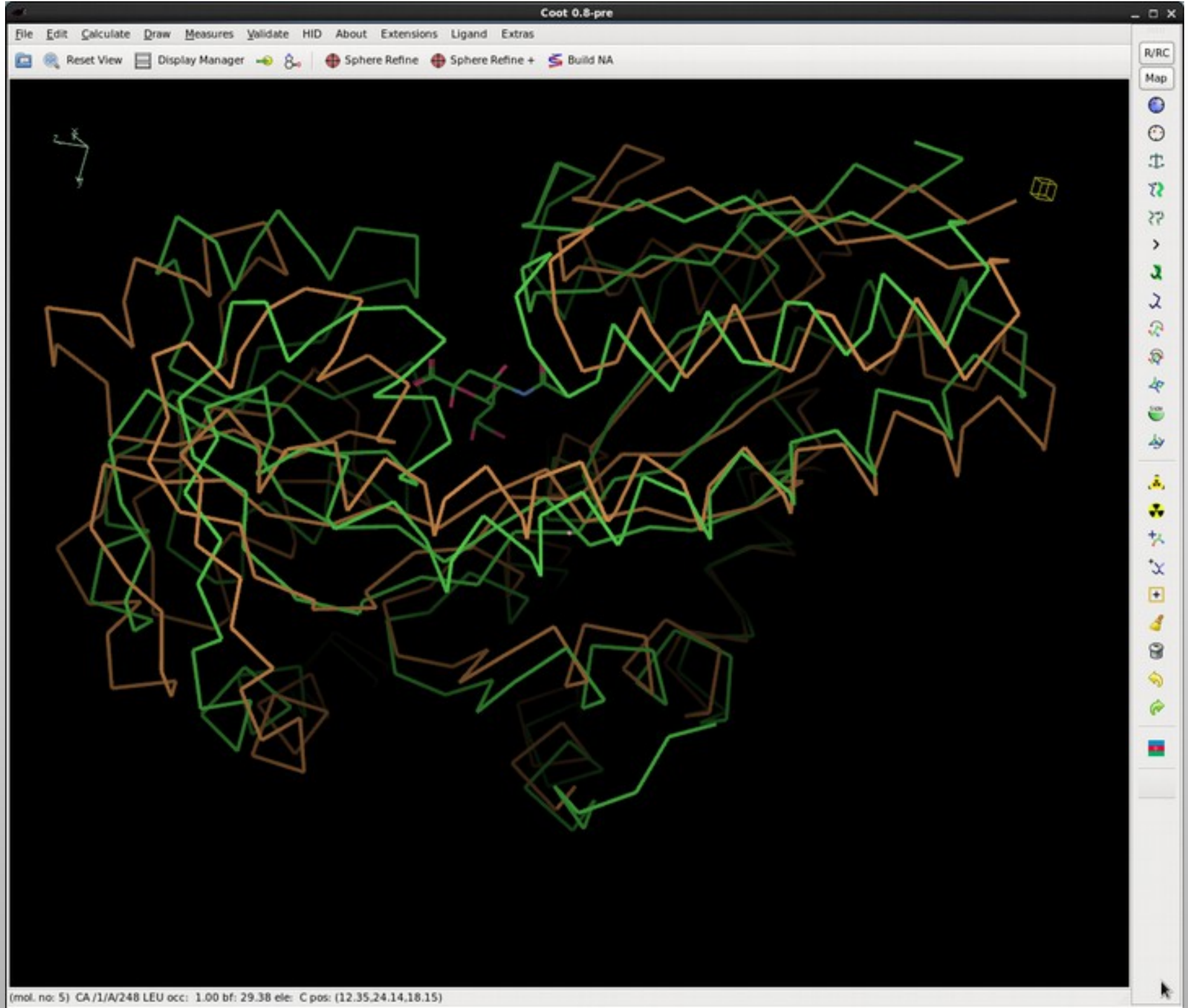

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added

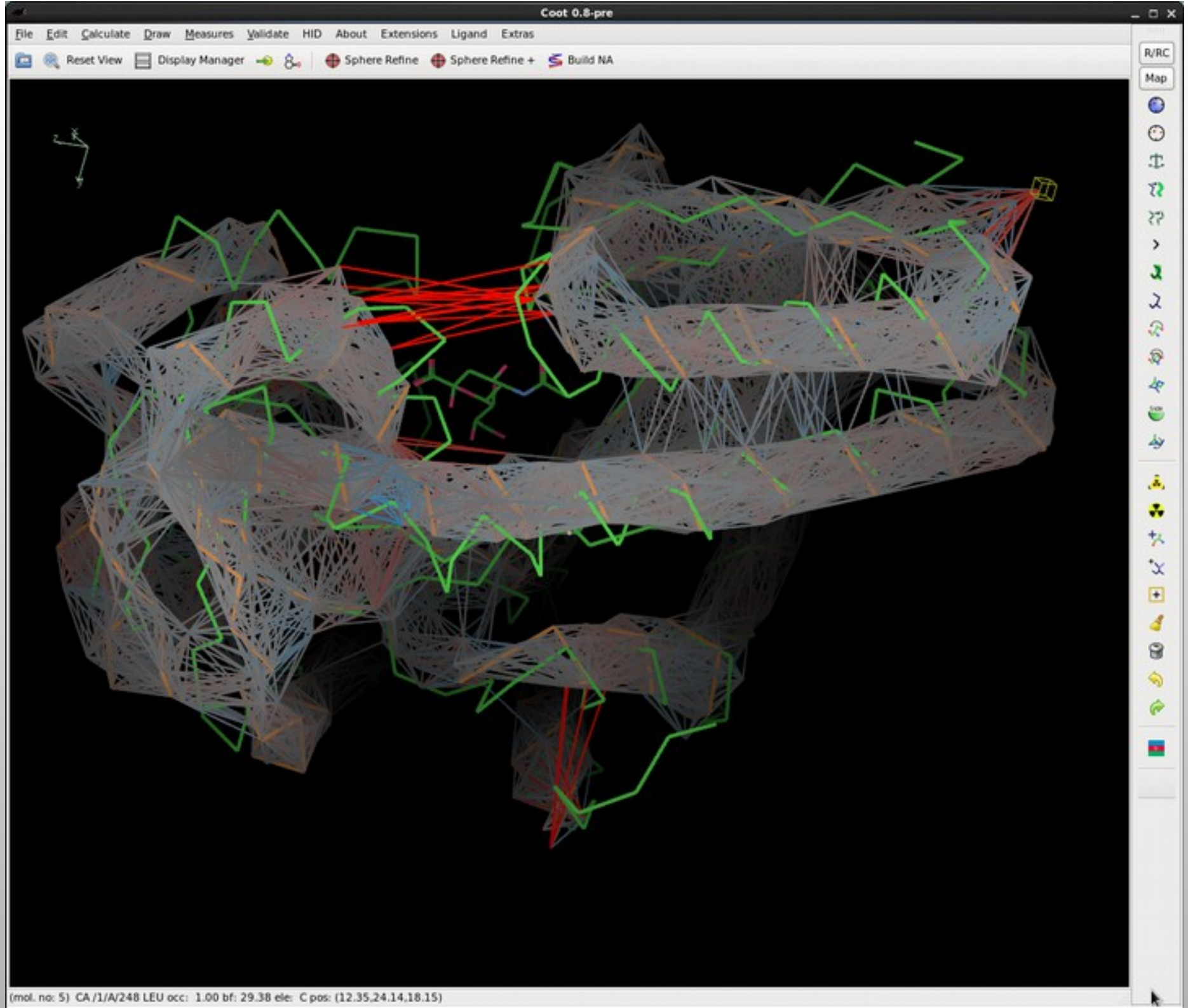

Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia
Reset View
Display Manager
R/RC
Map
Helix added

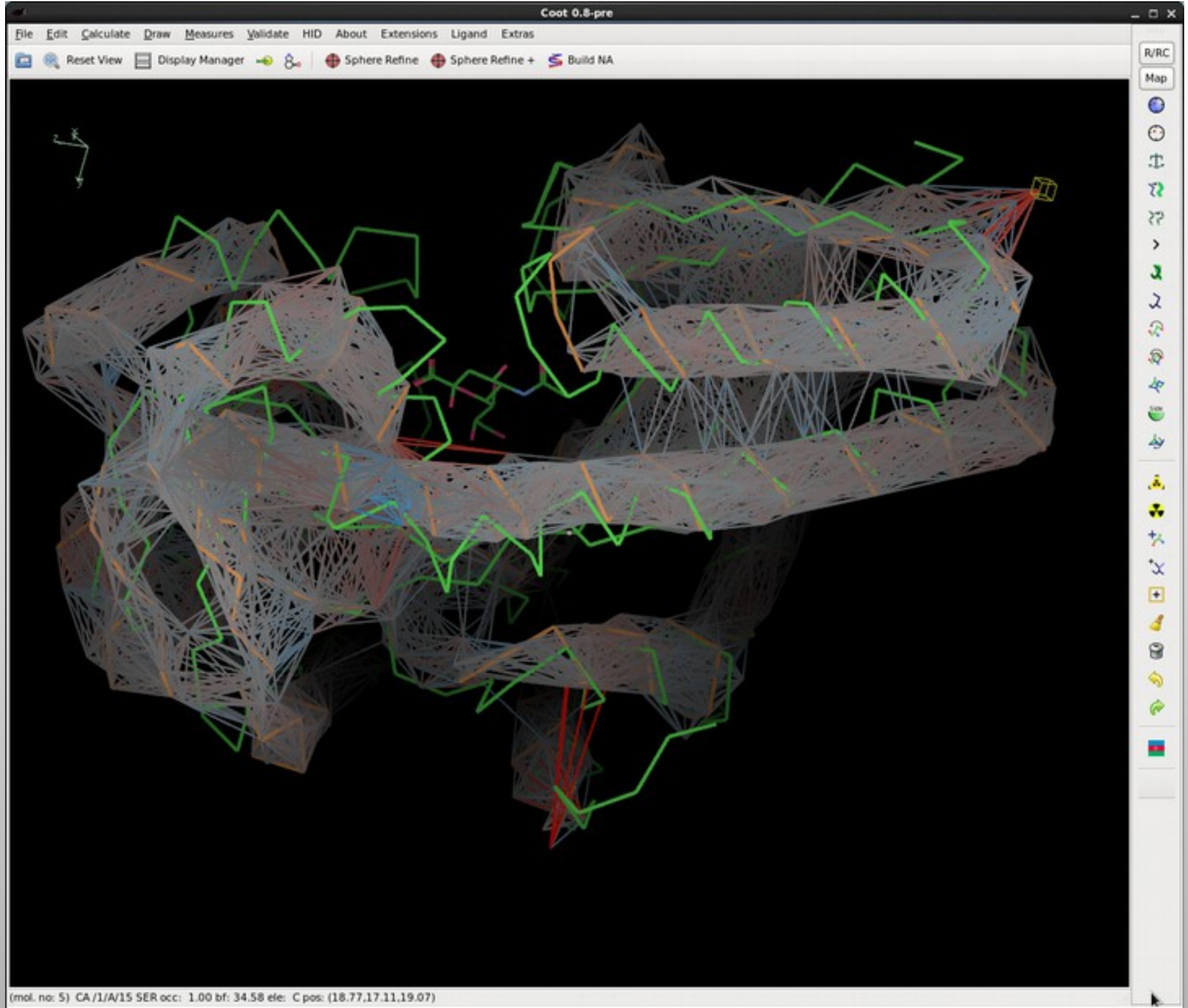
ProSMART

- Use previous-solved “template” structures to inform the refinement of the (low resolution) target protein
- by local-distance restraint generation

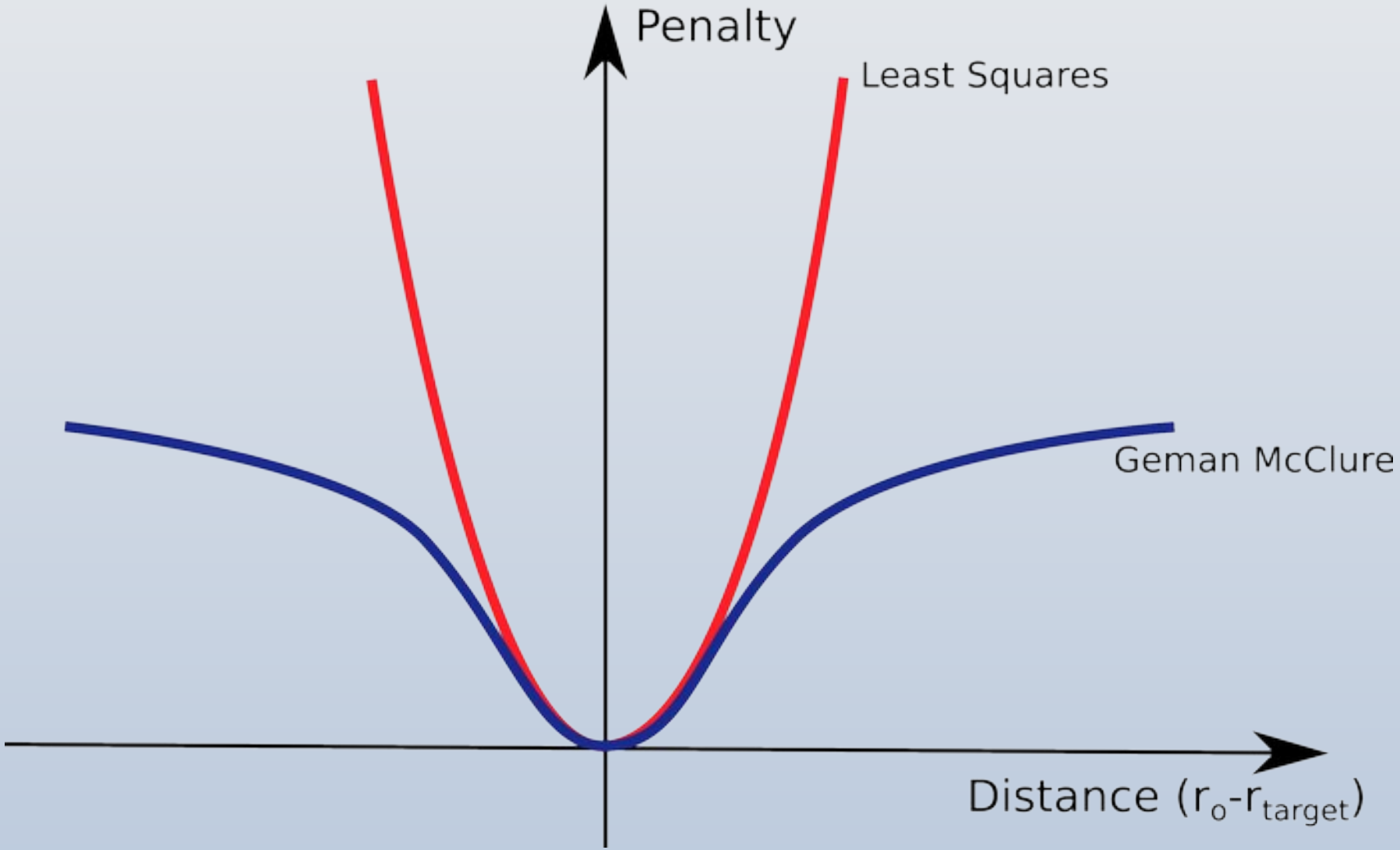

Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)


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

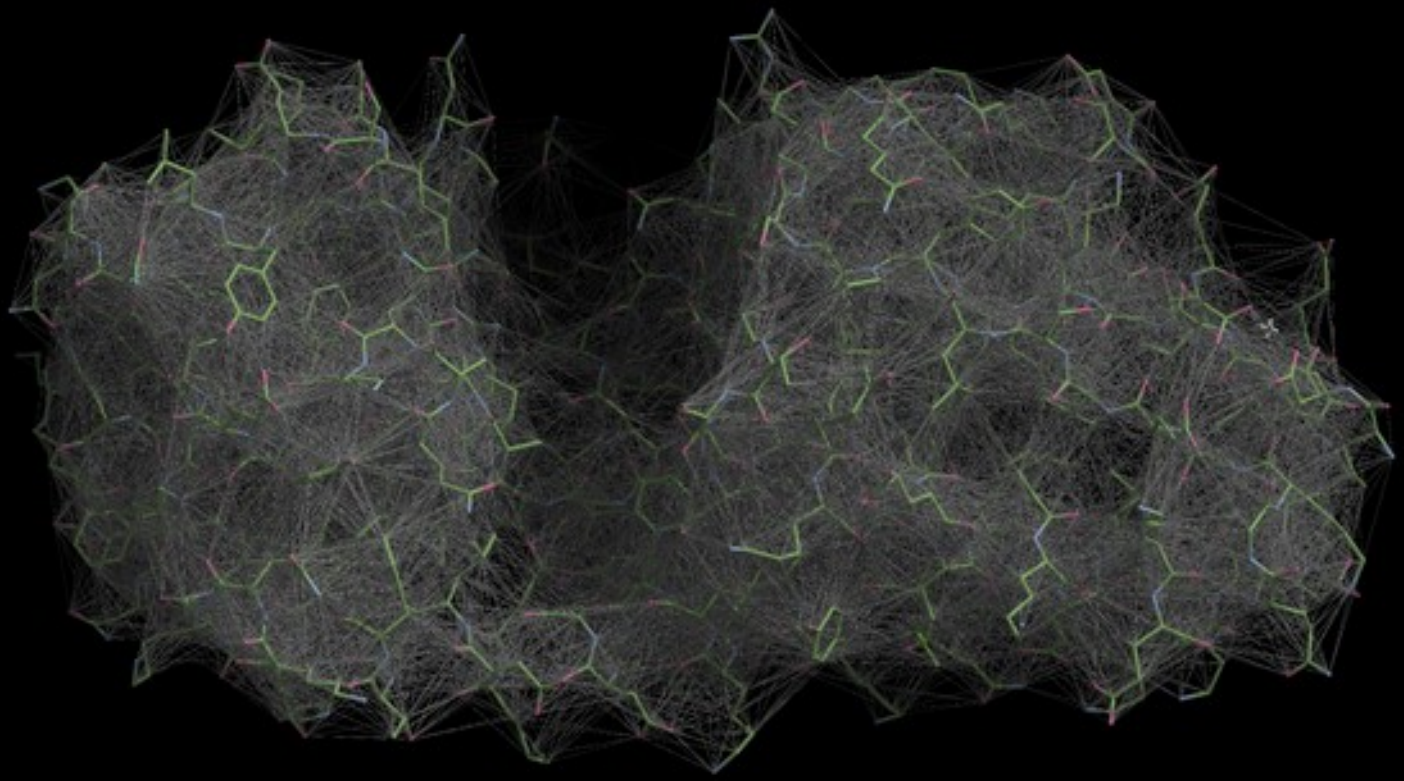

Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/248 LEU occ: 1.00 bf: 29.38 ele: C pos: (12.35,24.14,18.15)


Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/15 SER occ: 1.00 bf: 34.58 ele: C pos: (18.77,17.11,19.07)

Modified Target Function



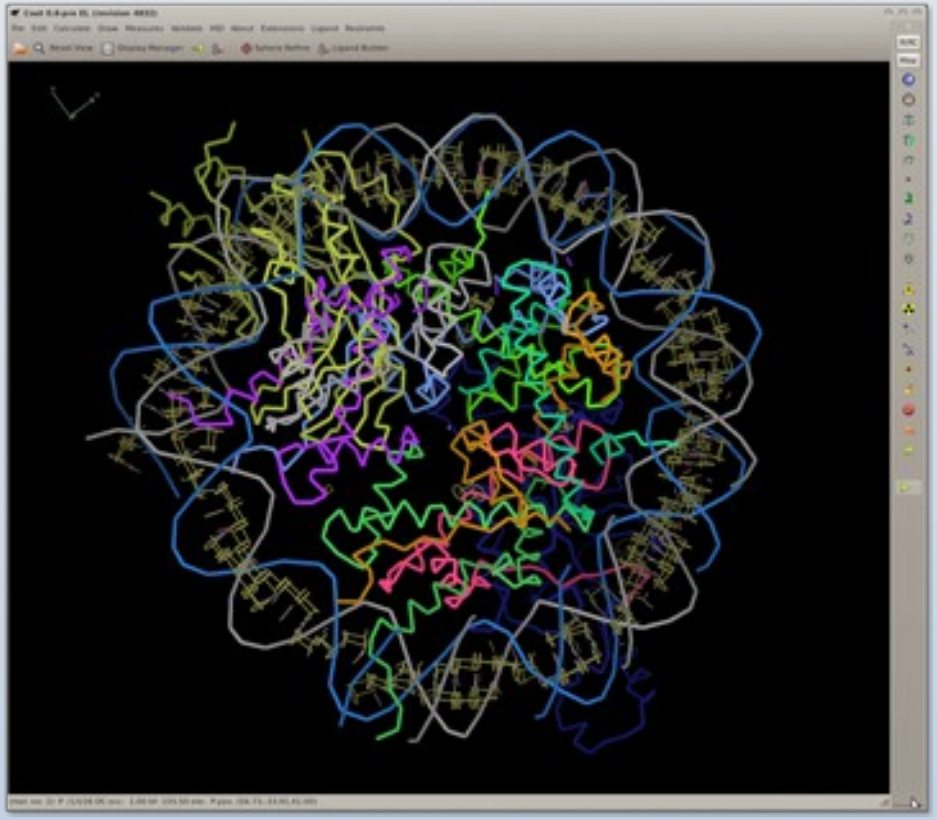
ProSMART Restraints



“ProSMART-like” Restraints

- Instead of using a reference model, more often I use “Self” restraints
  - which can be calculated internally
  - the starting model is the “reference” from which the ideal distances are calculated
  - message to the refinement:
  - “keep the local environments similar to how they were when you started”

Automatic Generation of Base Pairing and Stacking Restraints

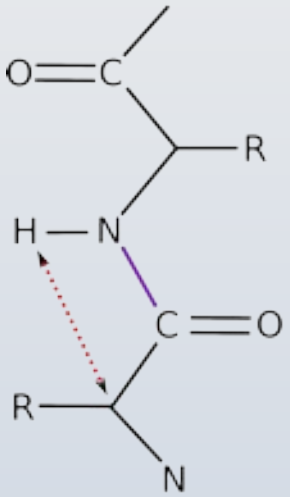


- Fei Long's `libg`
  - Provide it with a model and it writes out Refmac restraint descriptions
  - ... which *Coot* can also read
  - *Coot* can also create user-define base-paring and stacking restraints

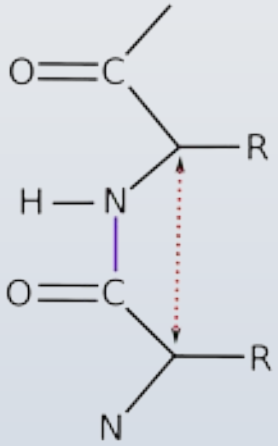
cis-Peptides

- A number of paper have been published recently highlighting the unusually large number of cis-peptides in some structures:
  - Croll: The rate of cis-trans conformation errors is increasing in low-resolution crystal structures *Acta Cryst.* (2015). **D**71, 706-709
  - Touw *et al.*: Detection of trans–cis flips and peptide-plane flips in protein structures *Acta Cryst.* (2015). **D**71, 1604-71614

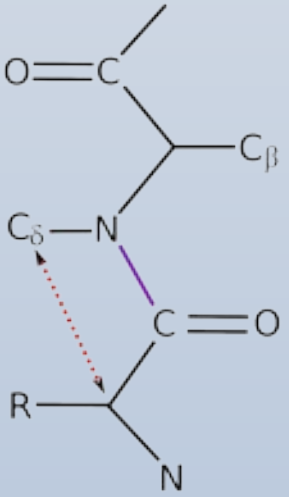
cis-Peptides



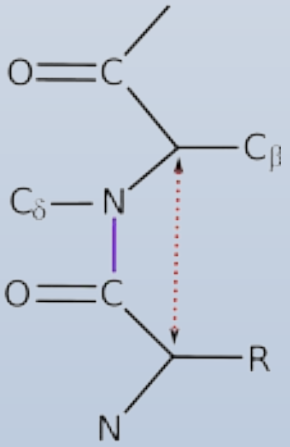
trans-peptide



cis-peptide

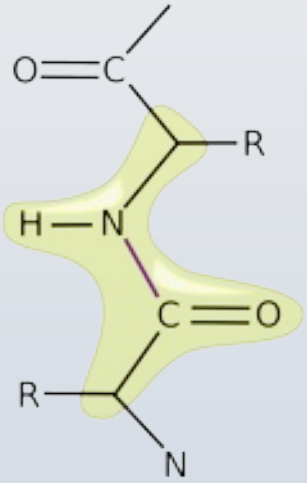


PRO *trans-peptide*

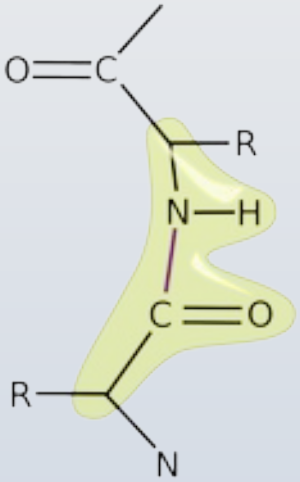


PRO *cis-peptide*

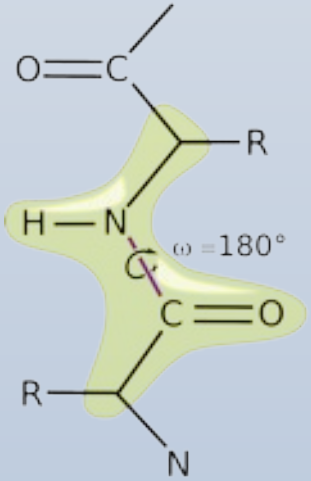
cis-Peptides



trans-peptide
with plane restraints



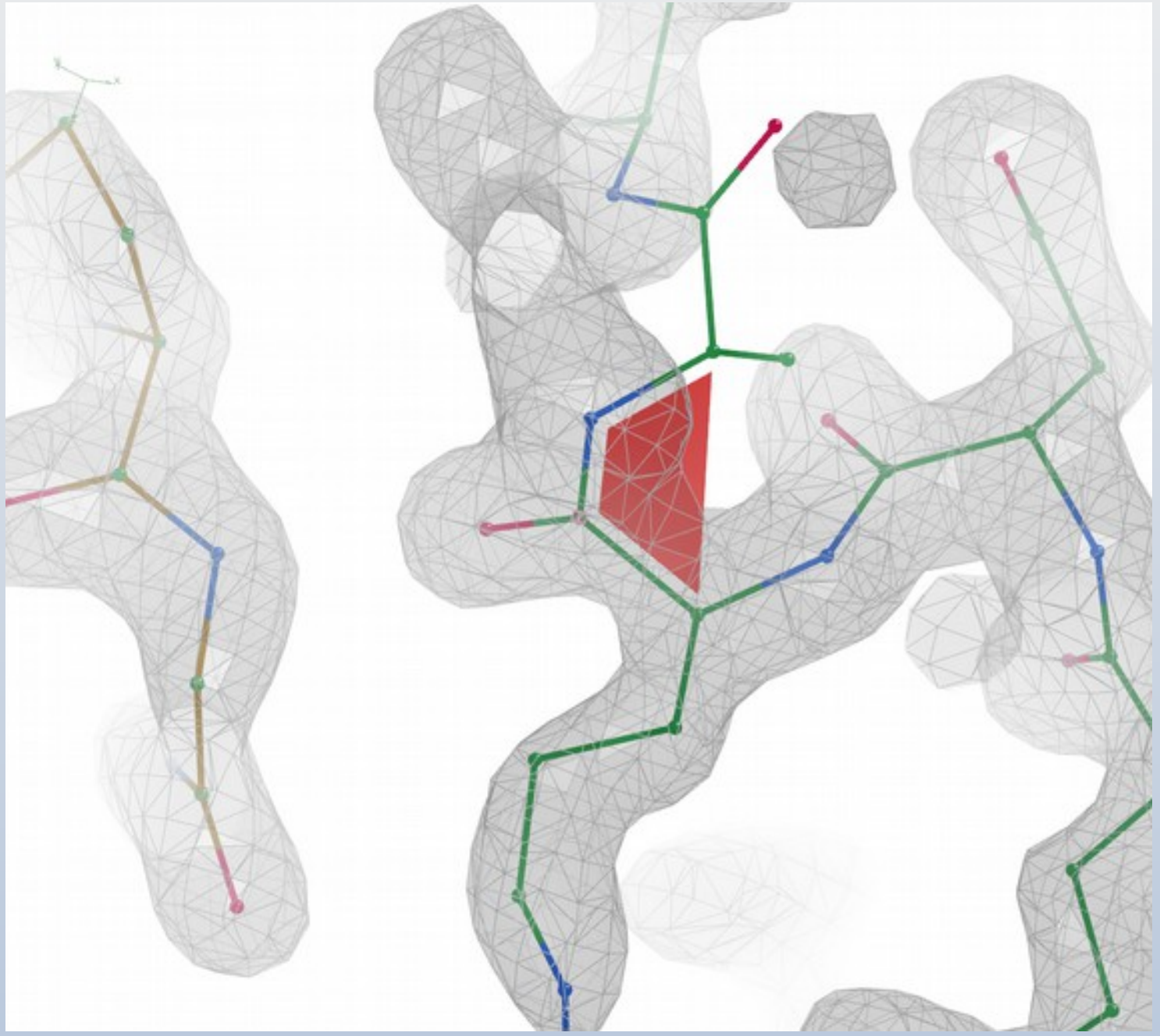
cis-peptide
with plane restraints



trans-peptide
with plane and trans restraints

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

cis-peptide Representation



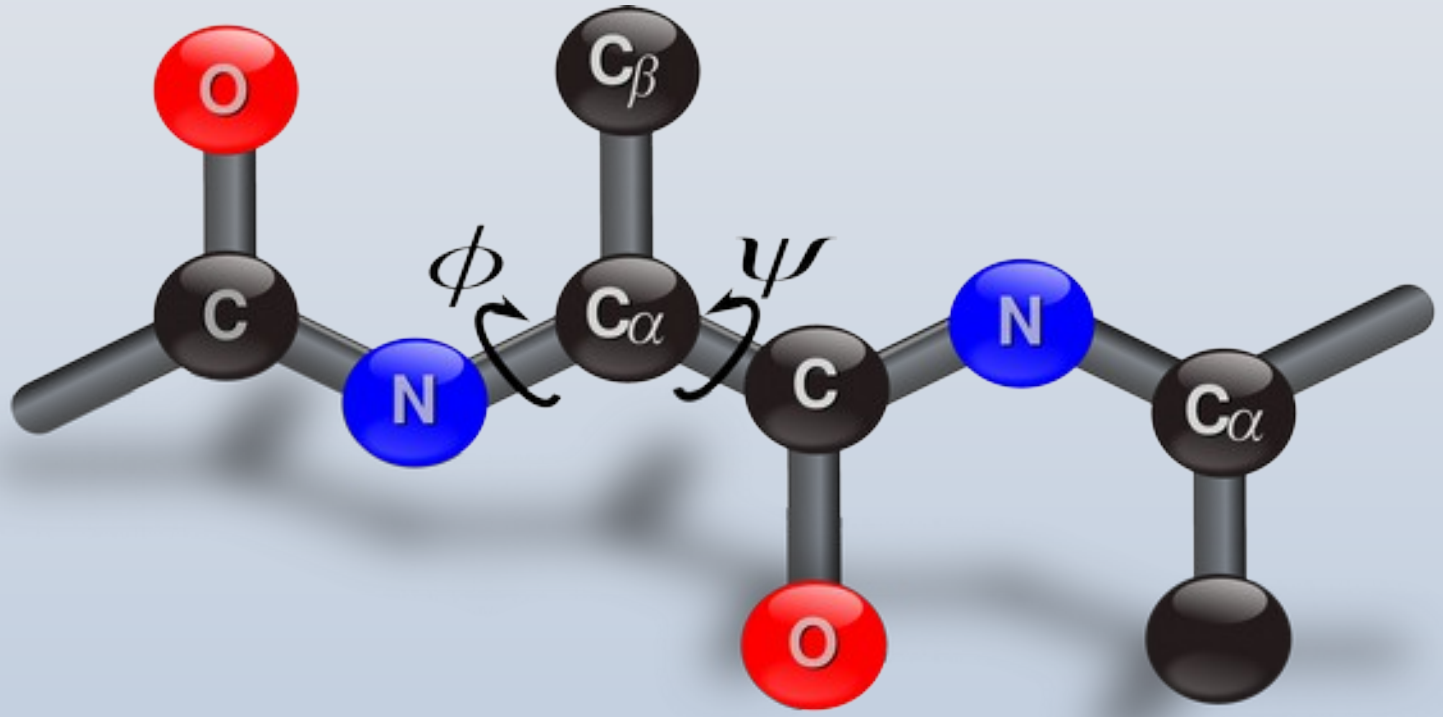
Pre-PRO

Twisted-trans

Non-pre-PRO

Rotamers:

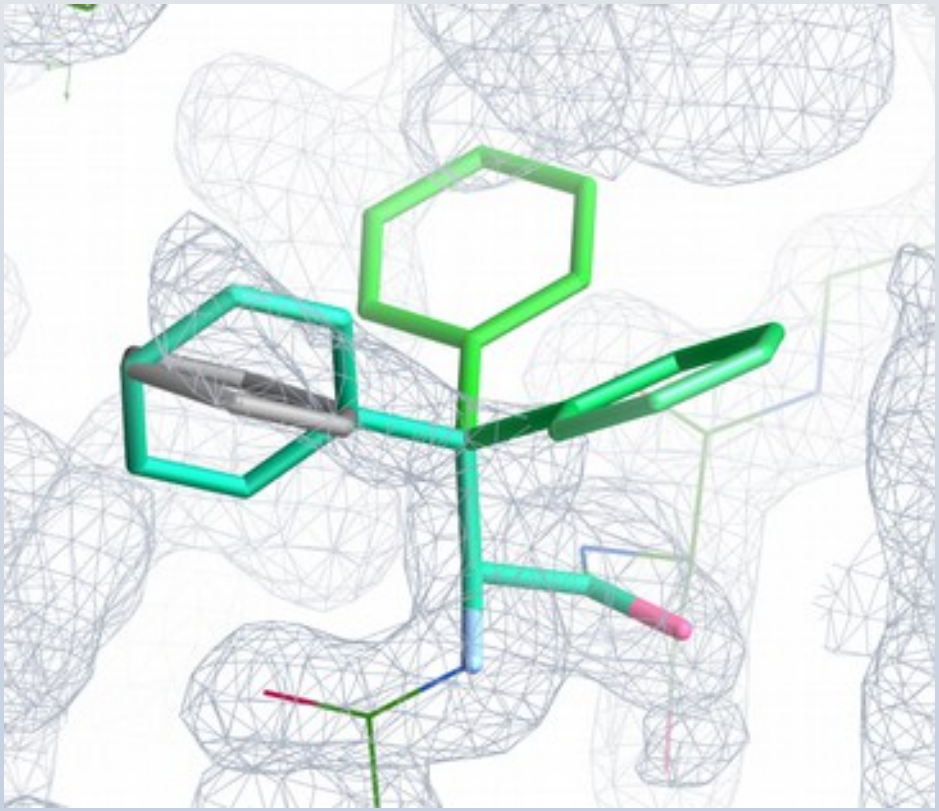
Peptide Backbone Geometry

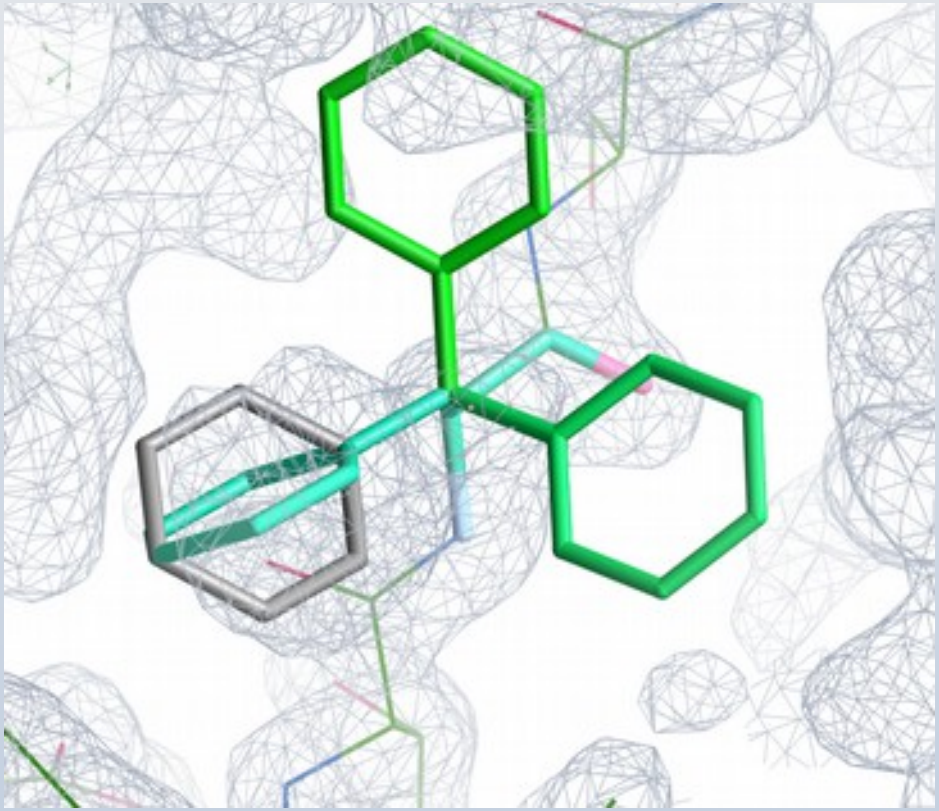


Rotamers

- 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
  - (Son of) Penultimate Rotamer Library

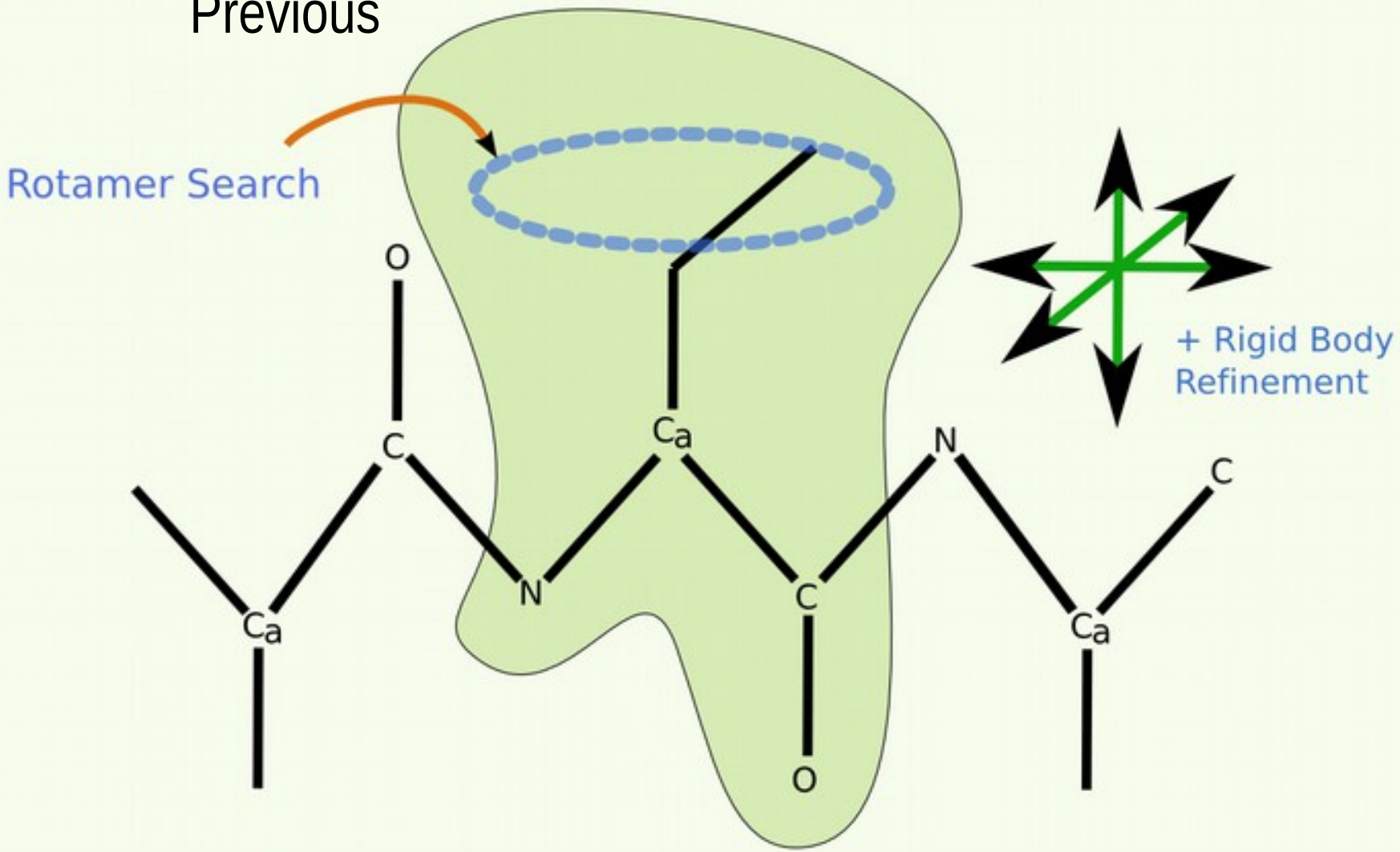
4 PHE Rotamers

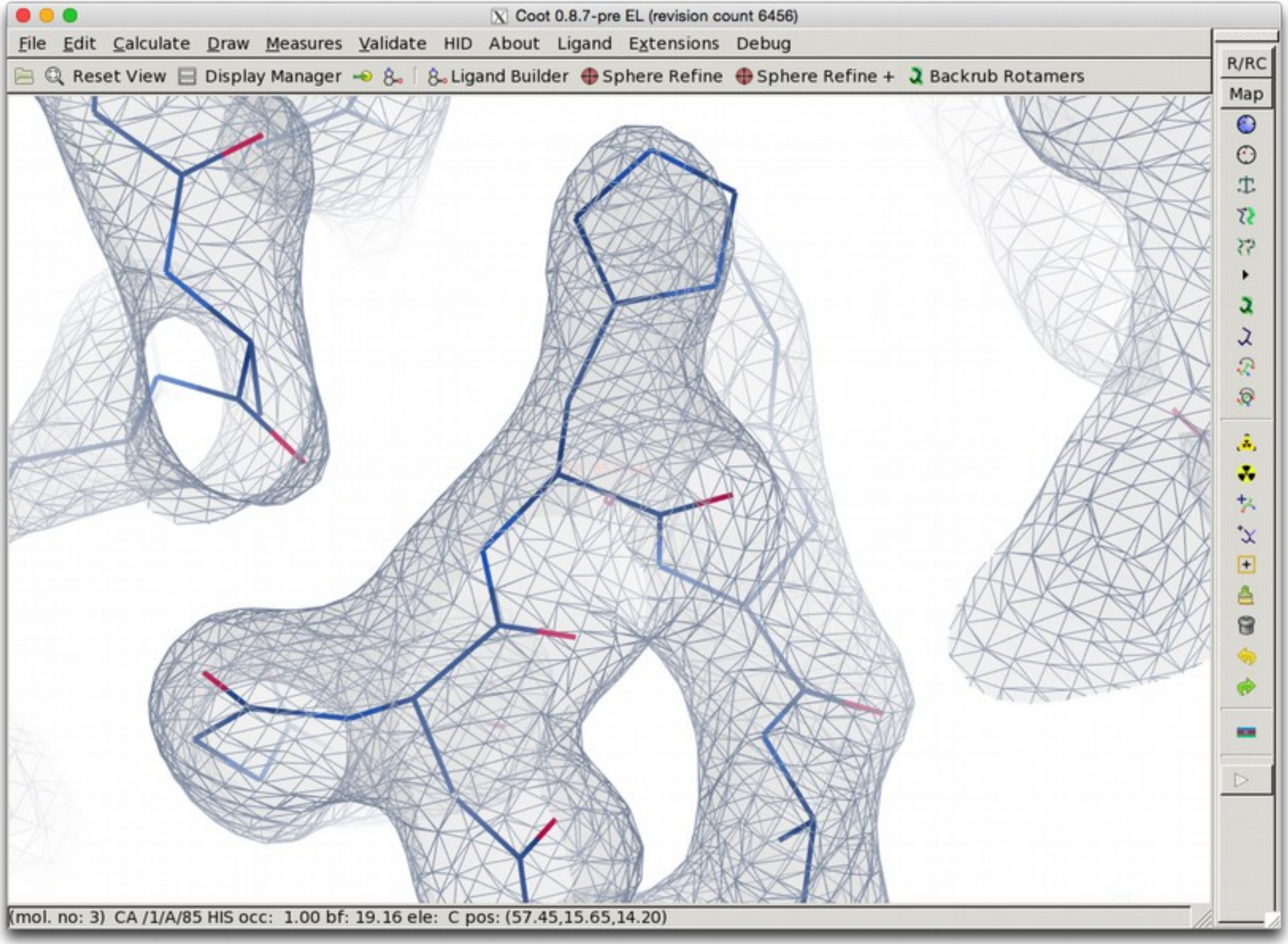


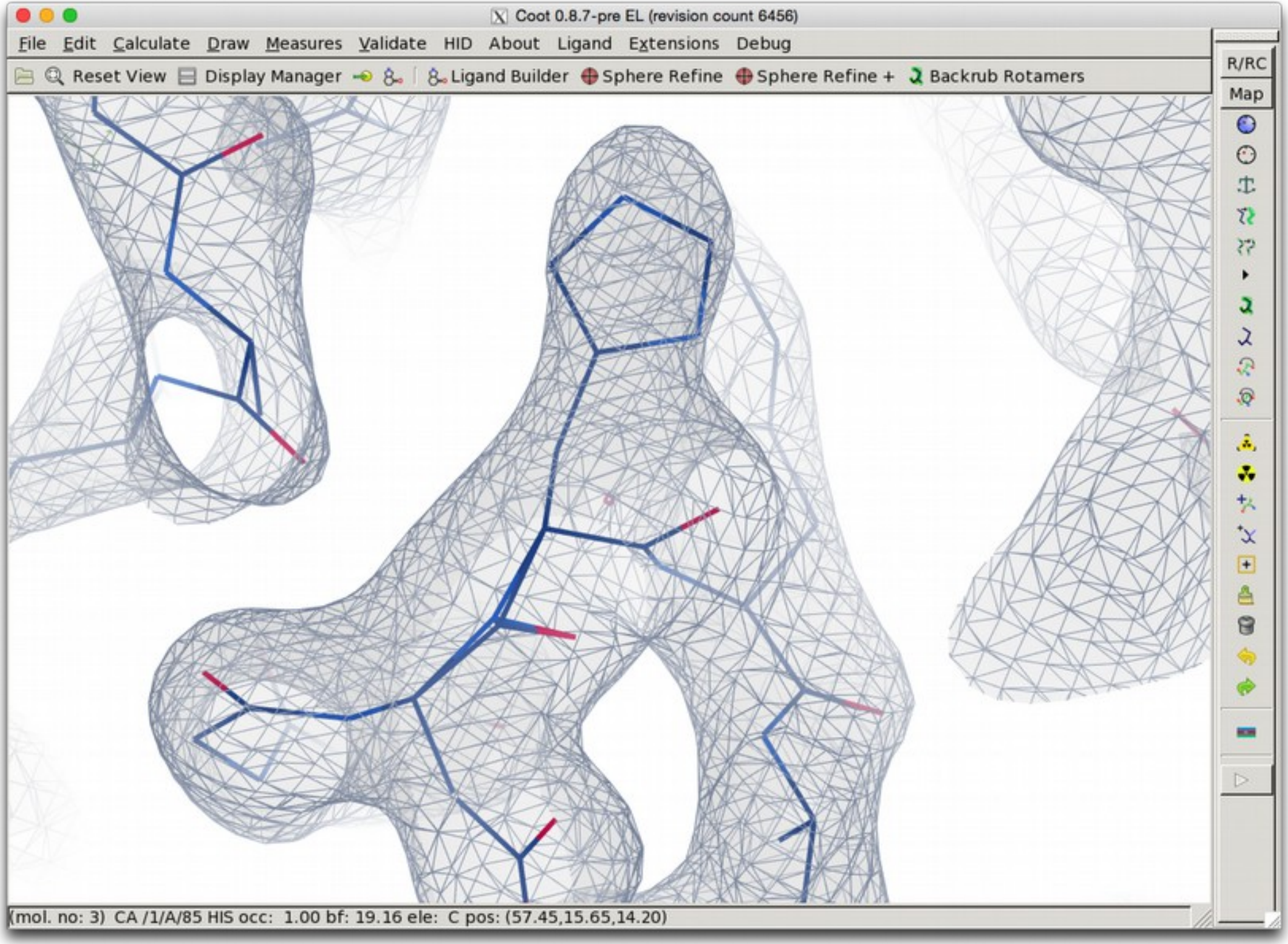


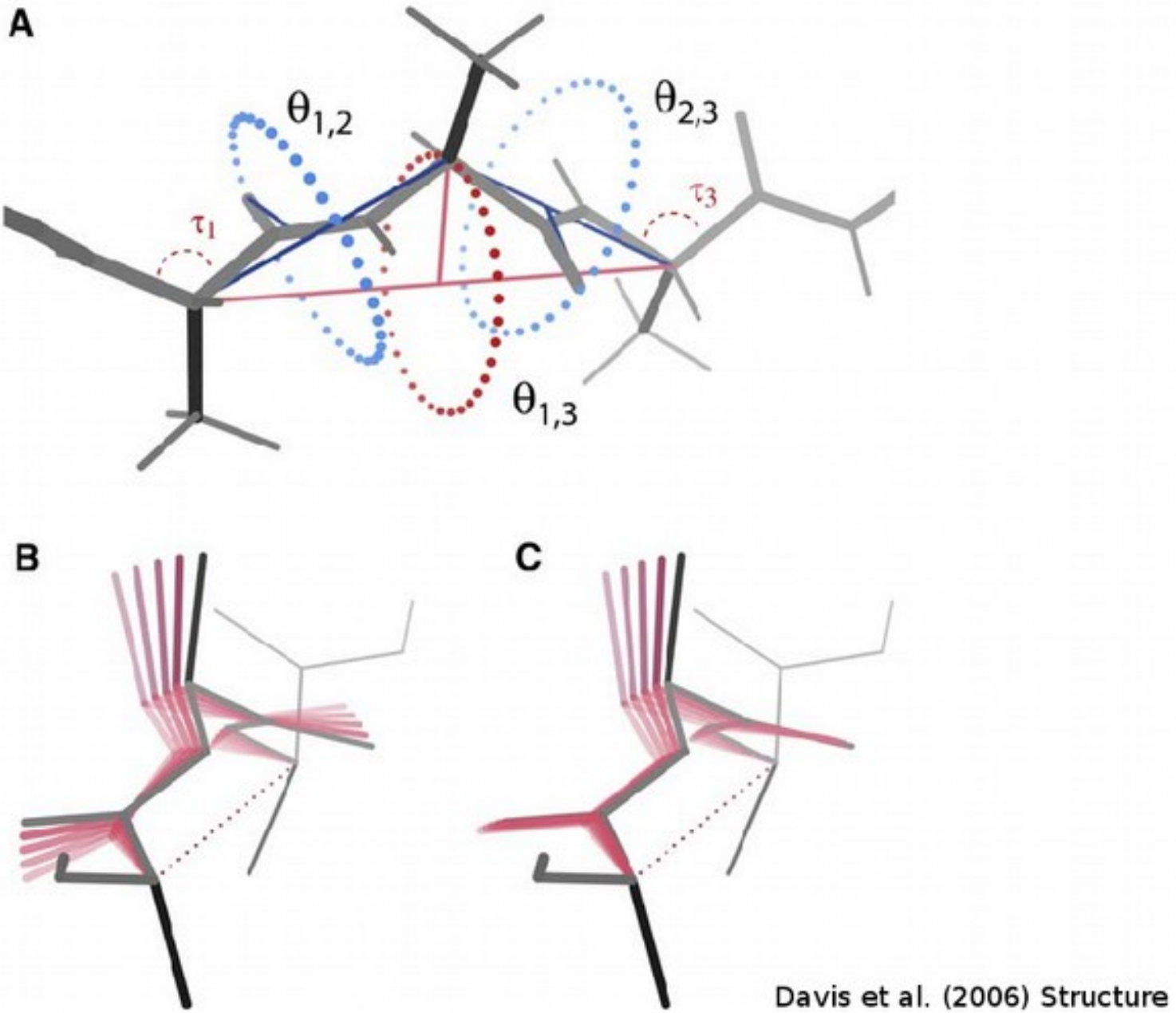
~~Current~~ Low Resolution Rotamer Search

Previous



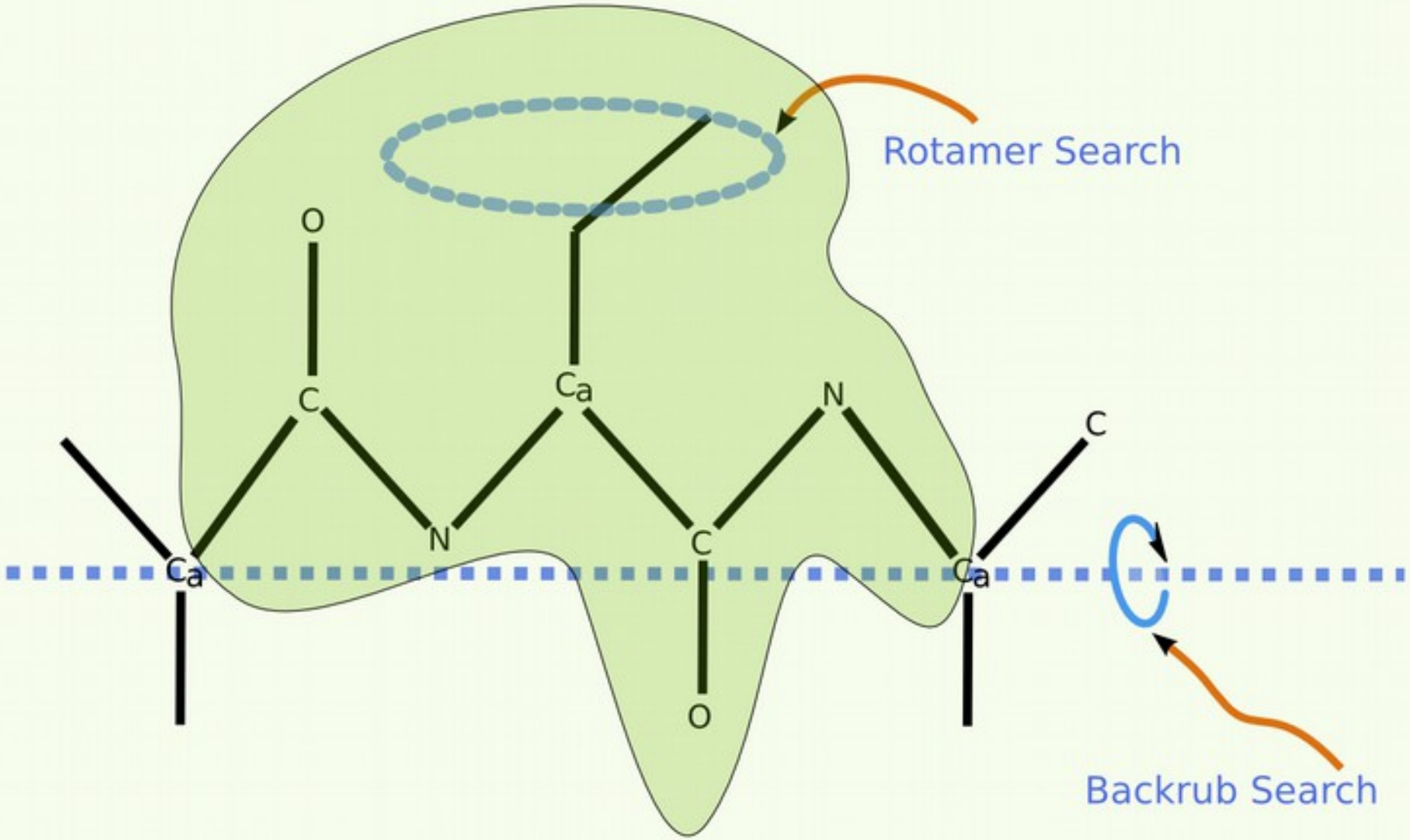

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


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

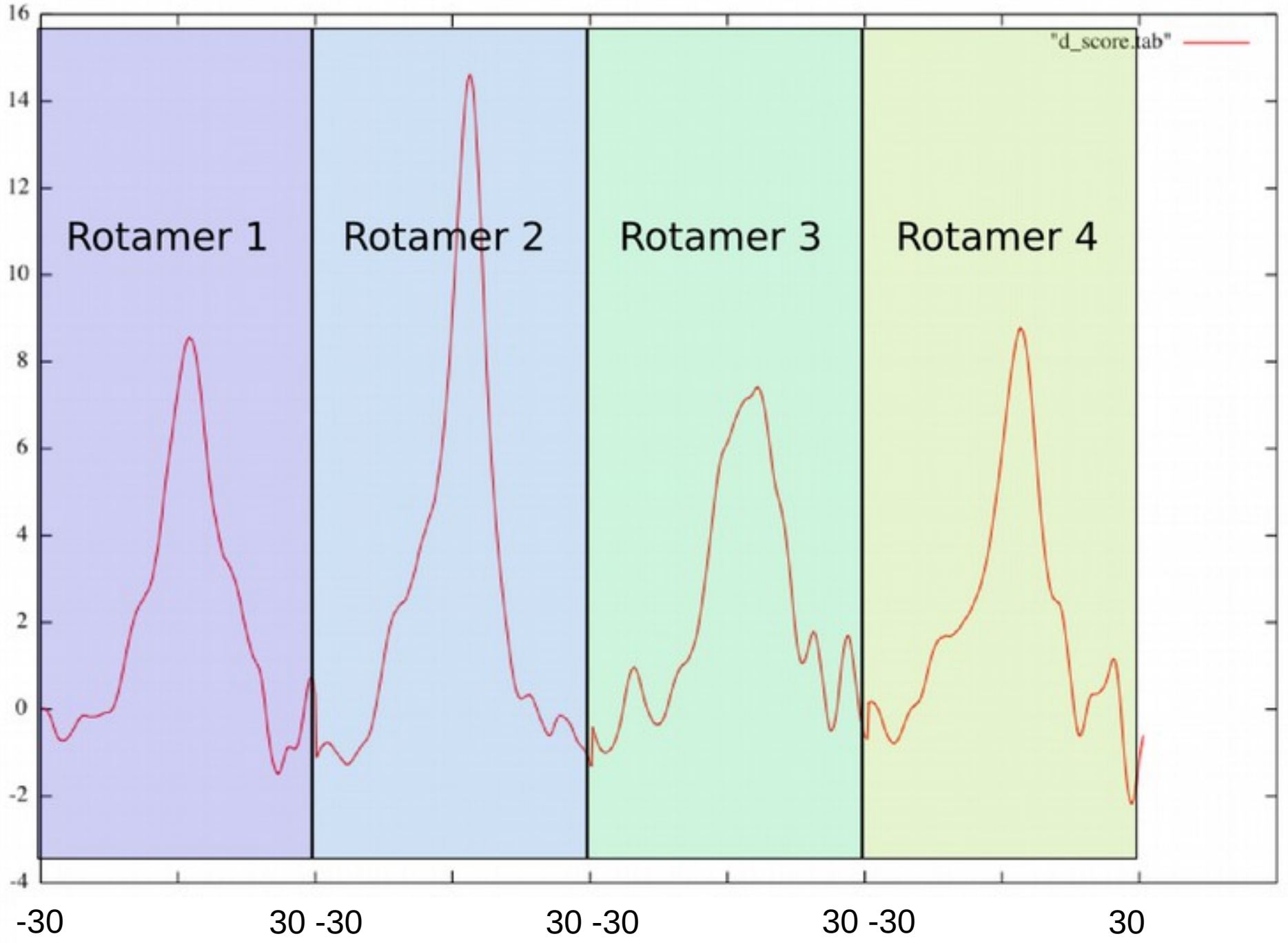


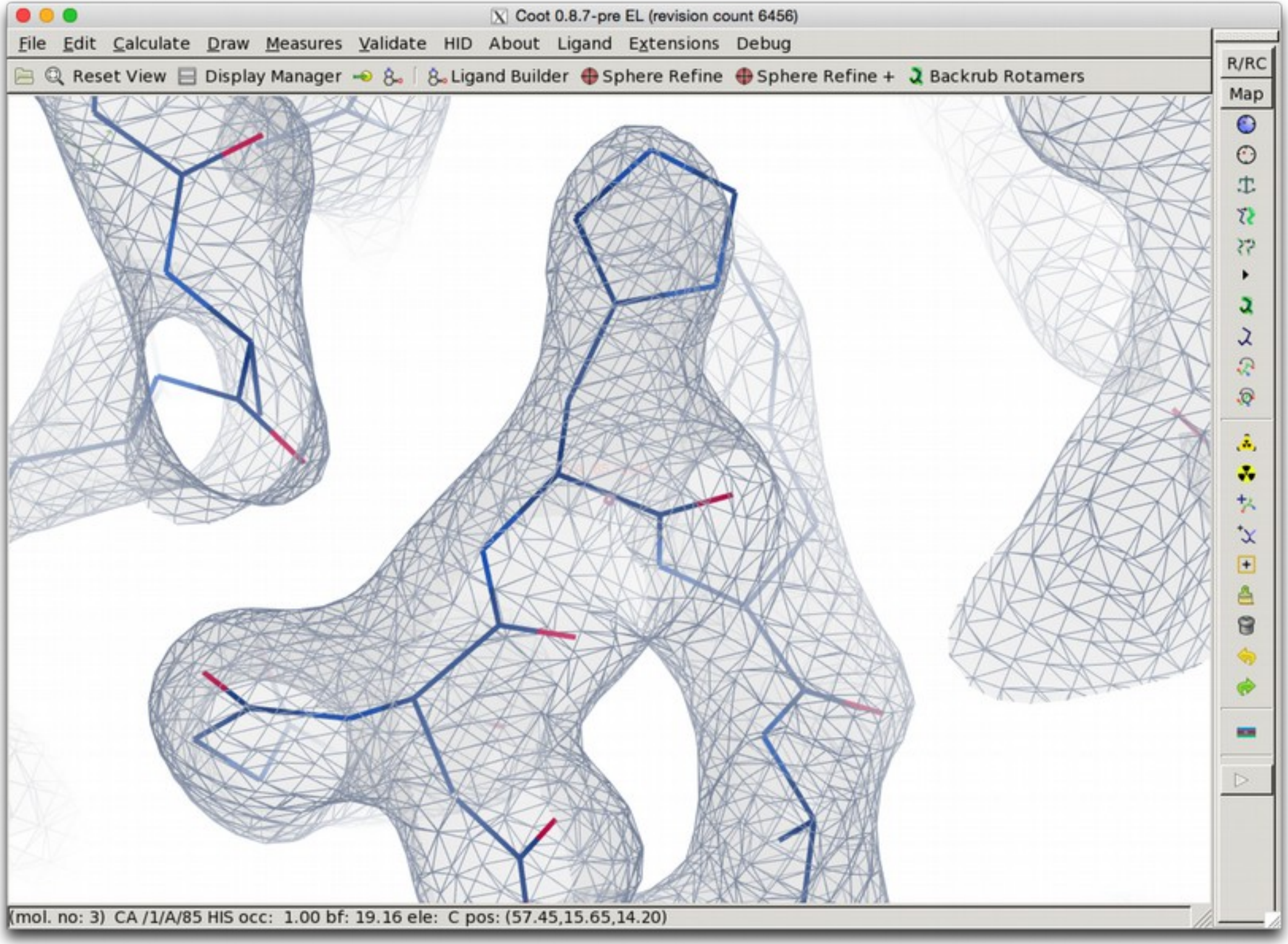
Davis et al. (2006) Structure

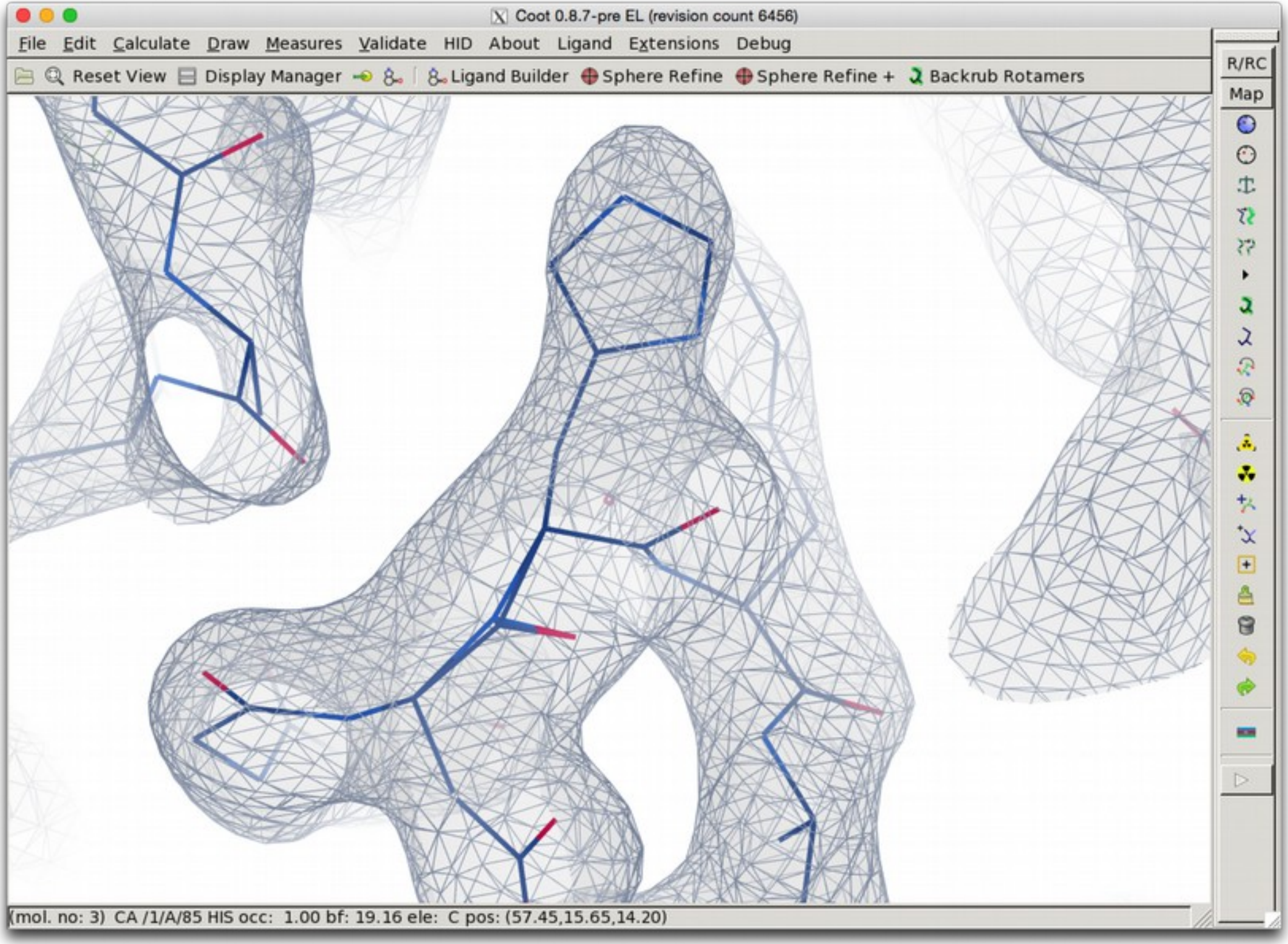
New Low Resolution Rotamer Search

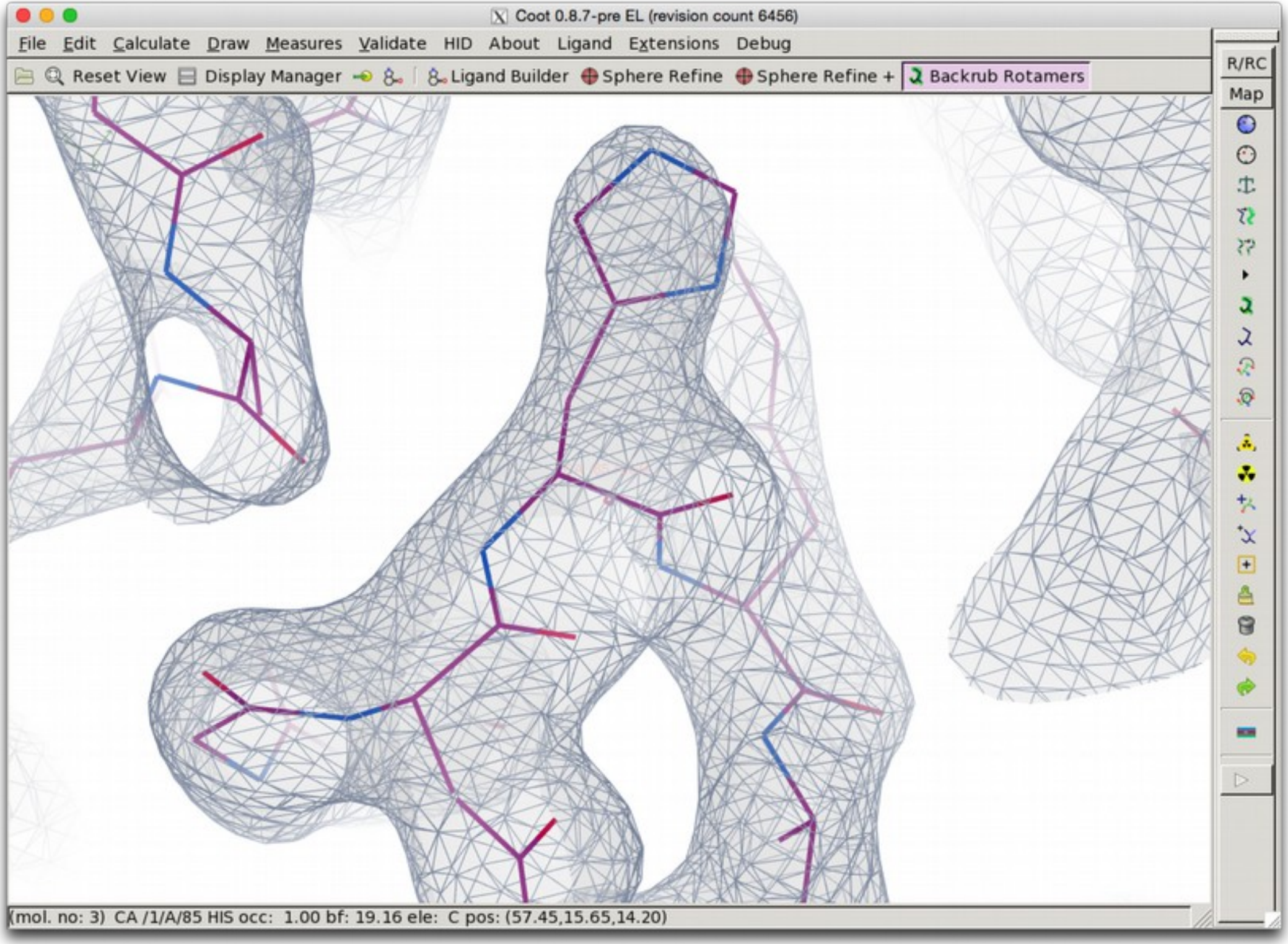


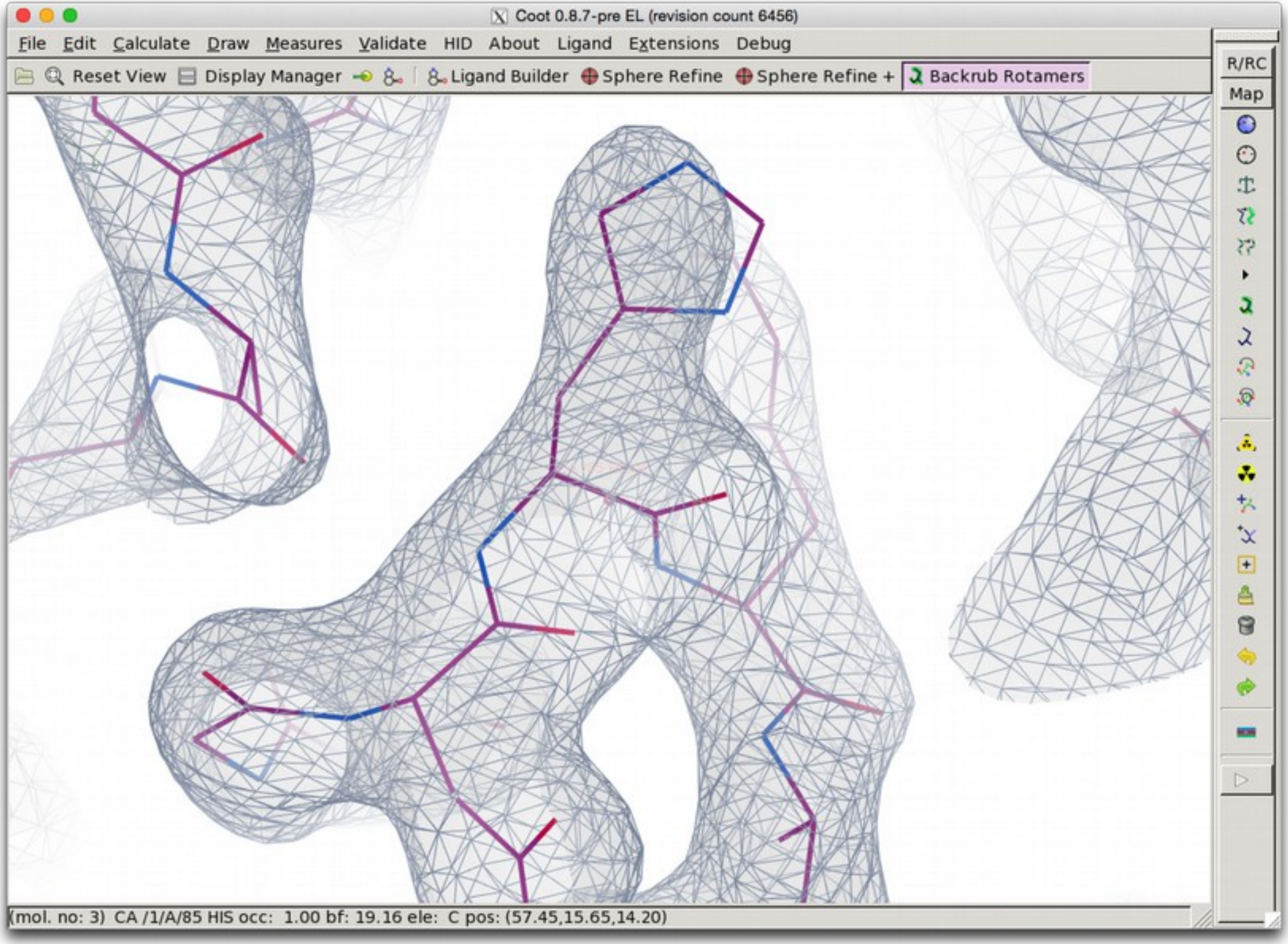
After Fitting Tools in KING/Molprobity

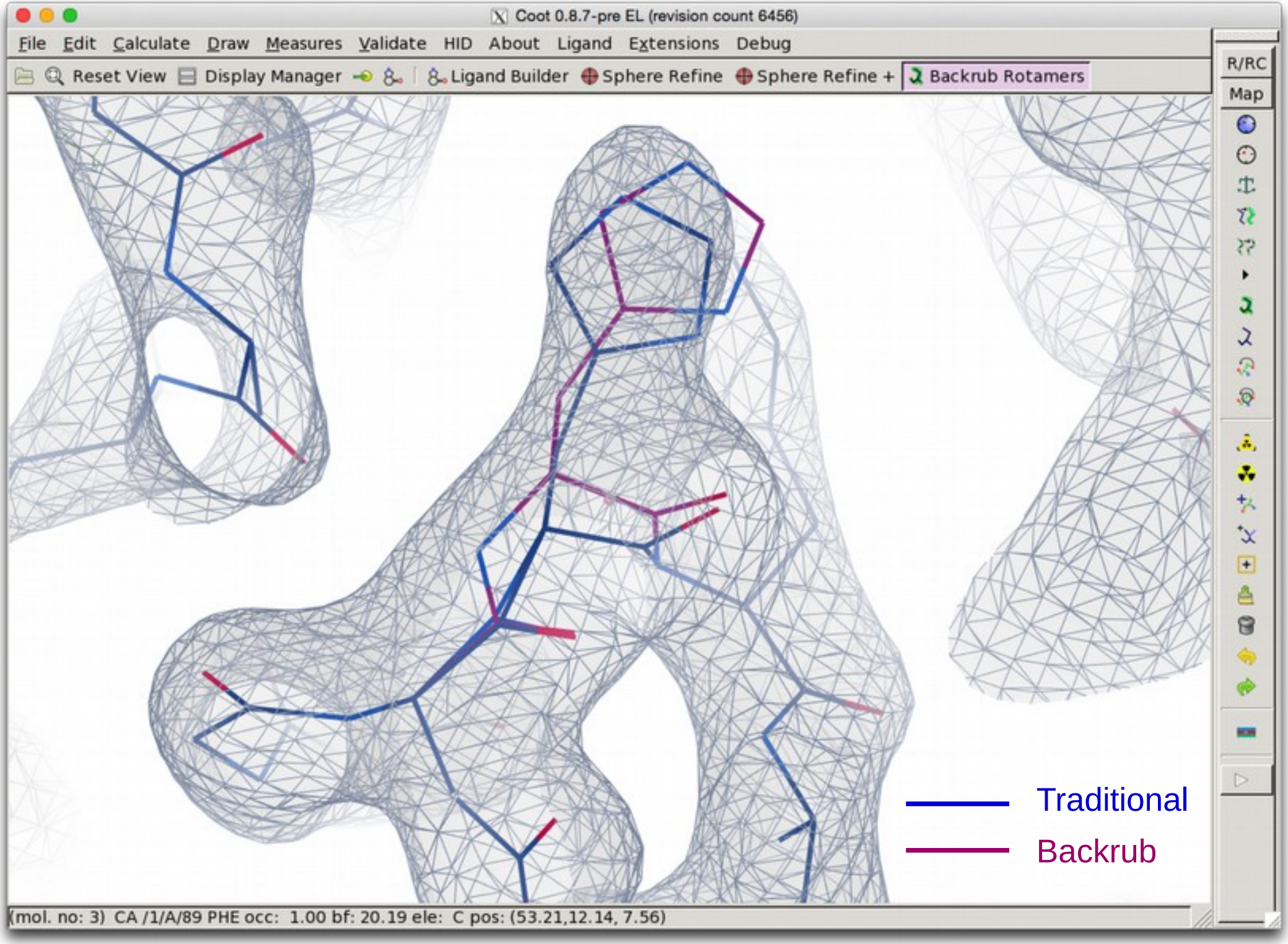

"d_score.tab"
Rotamer 1
Rotamer 2
Rotamer 3
Rotamer 4
16
14
12
10
8
6
4
2
0
-2
-4
-30
30 -30
30 -30
30 -30
30

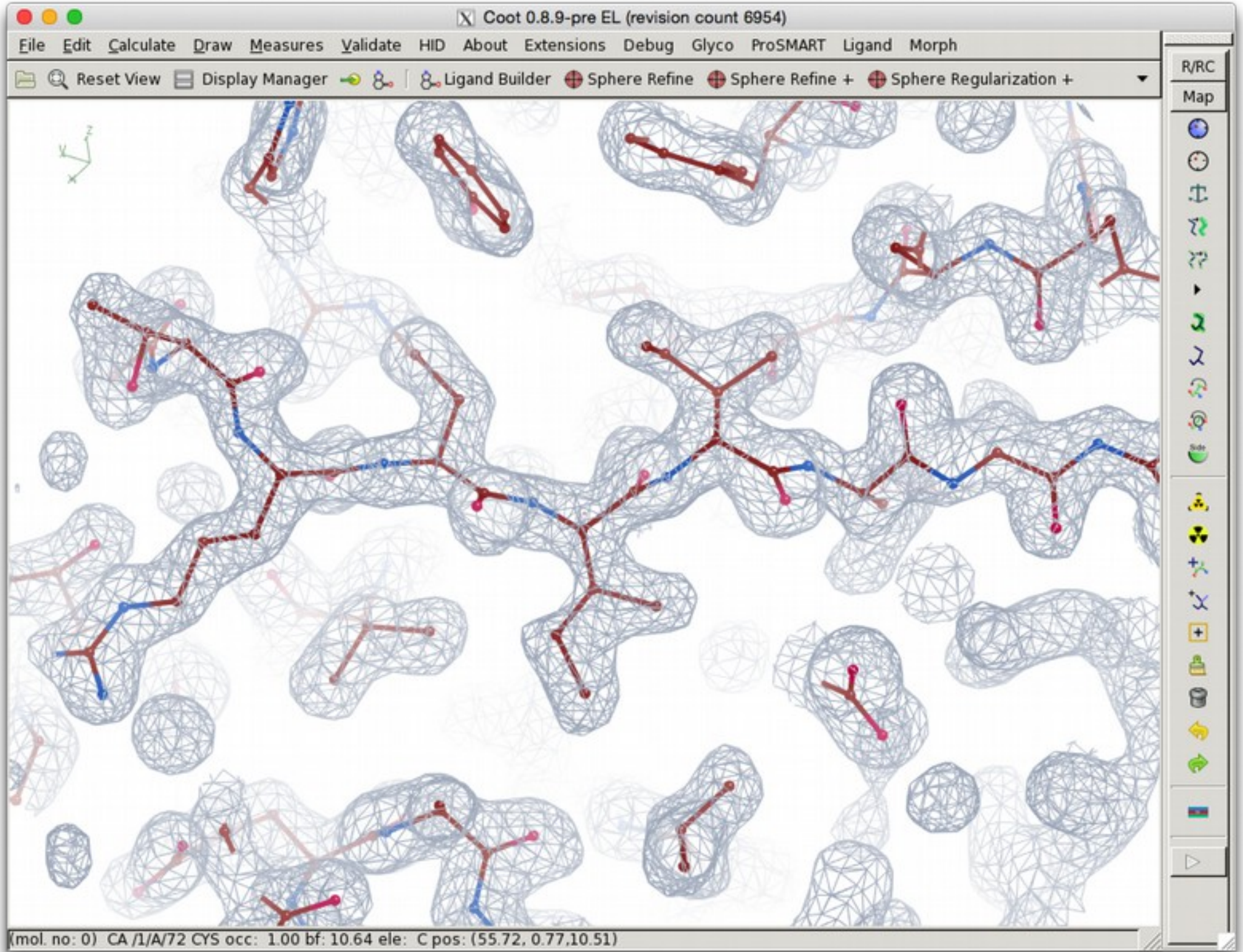

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)

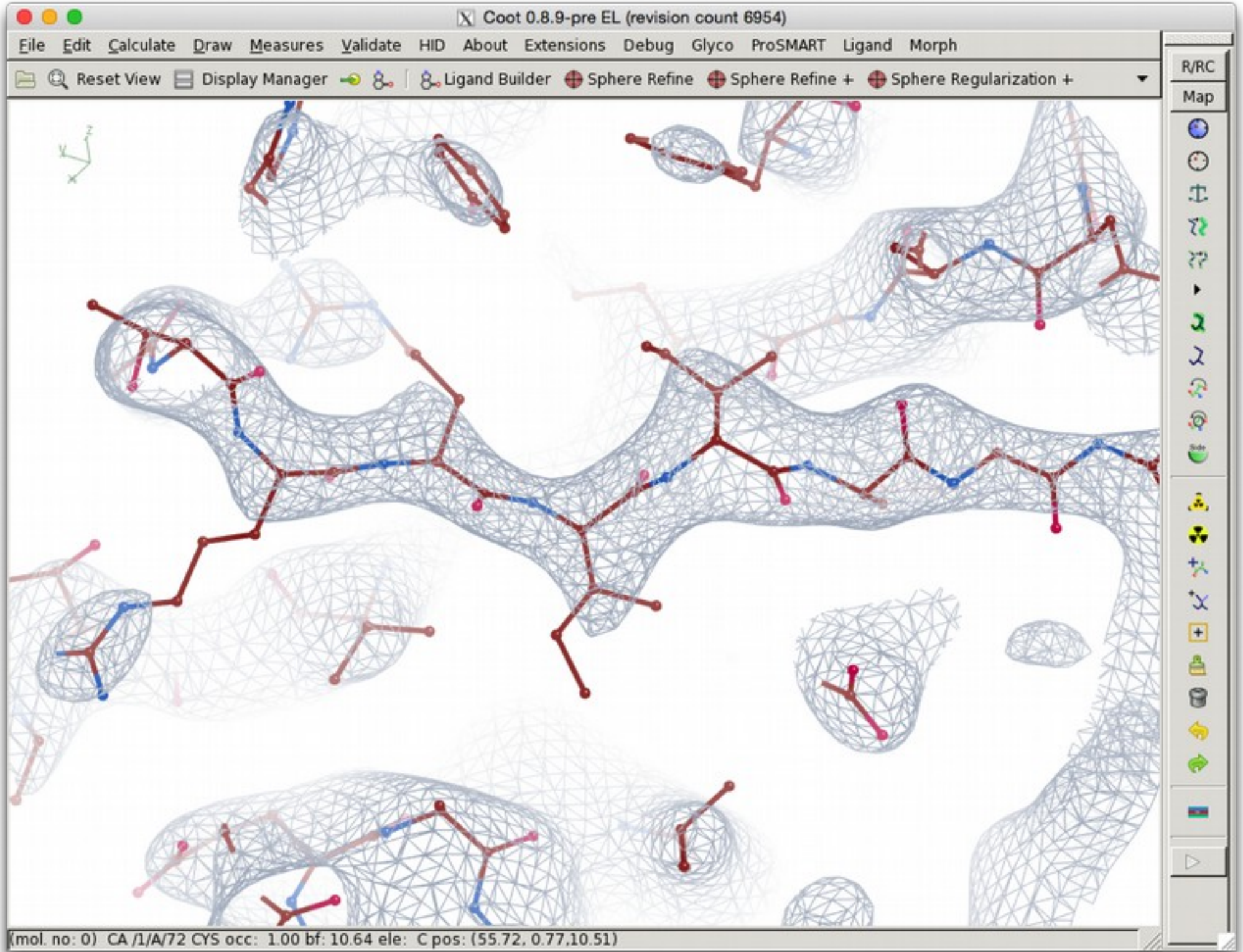

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


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)

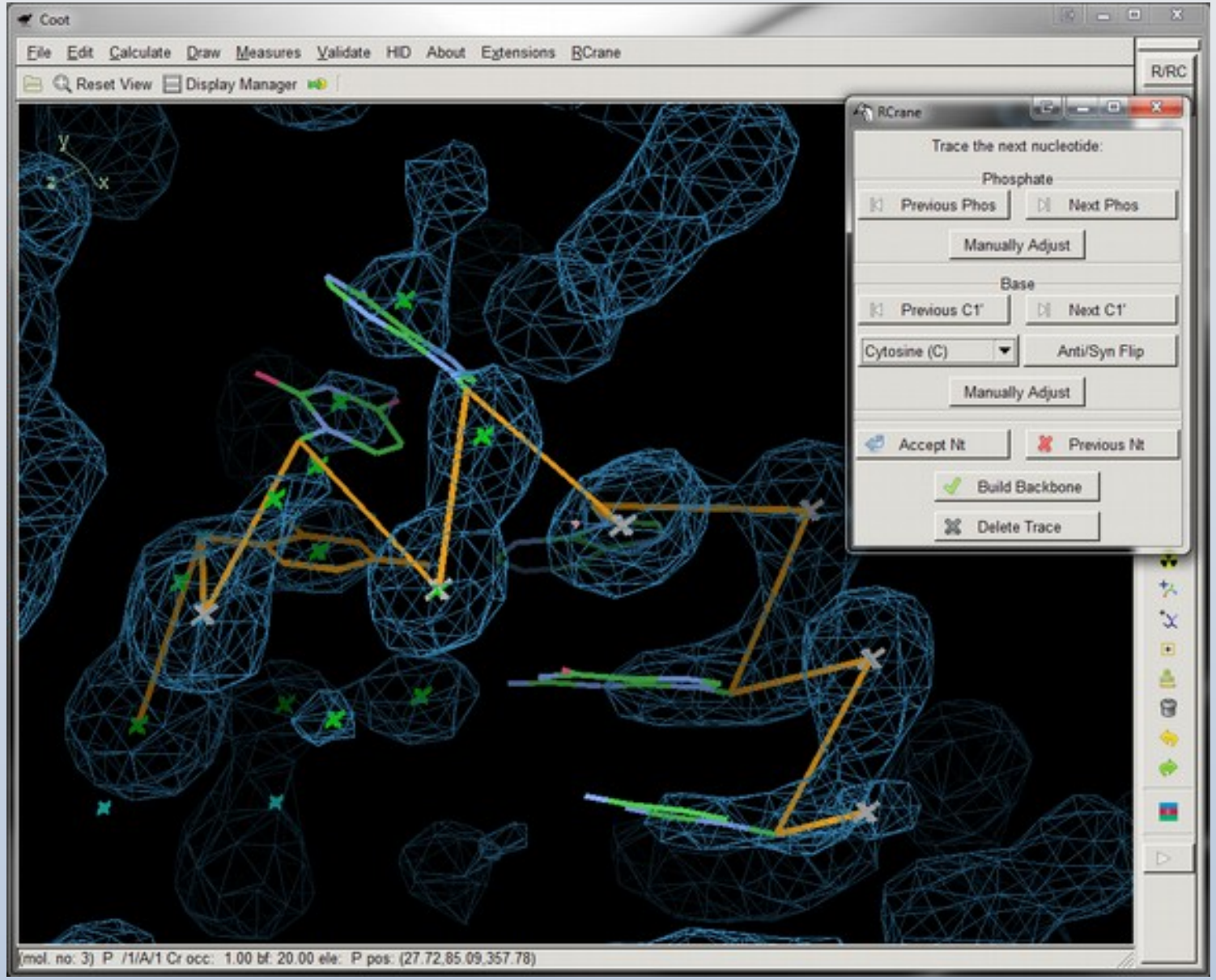

Coot 0.8.7-pre EL (revision count 6456)
File Edit Calculate Draw Measures Validate HID About Ligand Extensions Debug
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


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)

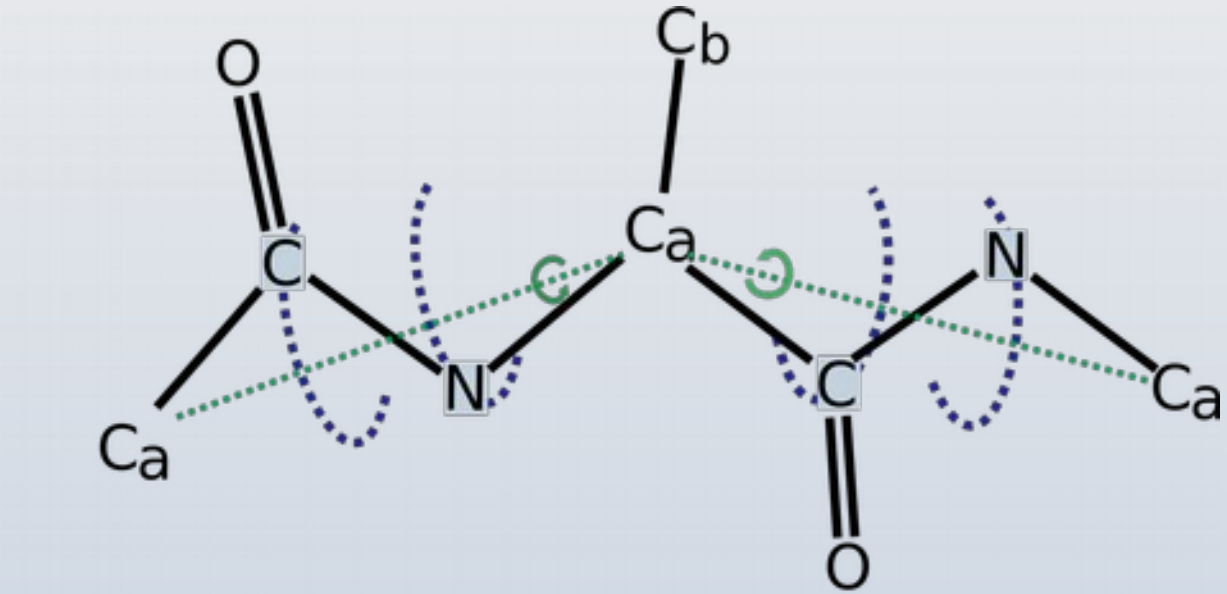

Coot 0.8.9-pre EL (revision count 6954)
File Edit Calculate Draw Measures Validate HID About Extensions Debug Glyco ProSMART Ligand Morph
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Sphere Regularization +
R/RC
Map
(mol. no: 0) CA /1/A/72 CYS occ: 1.00 bf: 10.64 ele: C pos: (55.72, 0.77,10.51)

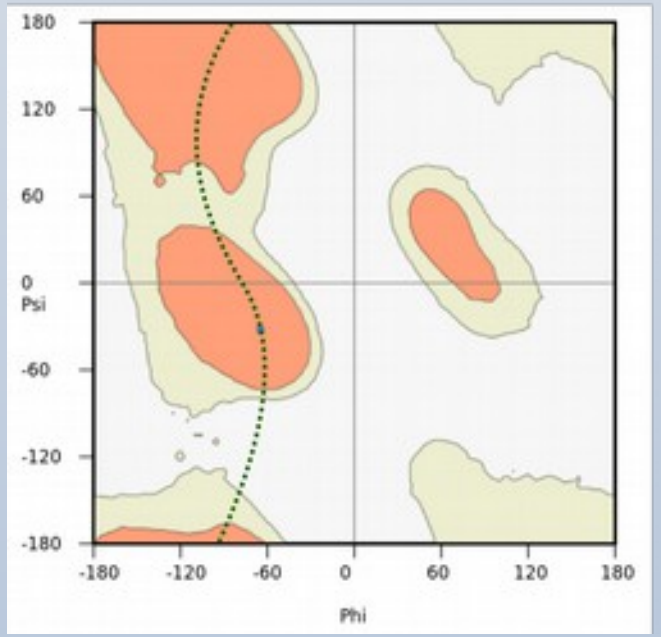

Coot 0.8.9-pre EL (revision count 6954)
File Edit Calculate Draw Measures Validate HID About Extensions Debug Glyco ProSMART Ligand Morph
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Sphere Regularization +
R/RC
Map
(mol. no: 0) CA /1/A/72 CYS occ: 1.00 bf: 10.64 ele: C pos: (55.72, 0.77,10.51)

RCrane: Semi-Automated Building of RNA



Crankshaft Peptide Optimisation





Rotation around Cα-Cα vectors creates new positions for C and N atoms, leading to new $\{\varphi_i,\psi_i\}_{i=1\text{-}3}$ angles and positions in the Ramachandran Plot

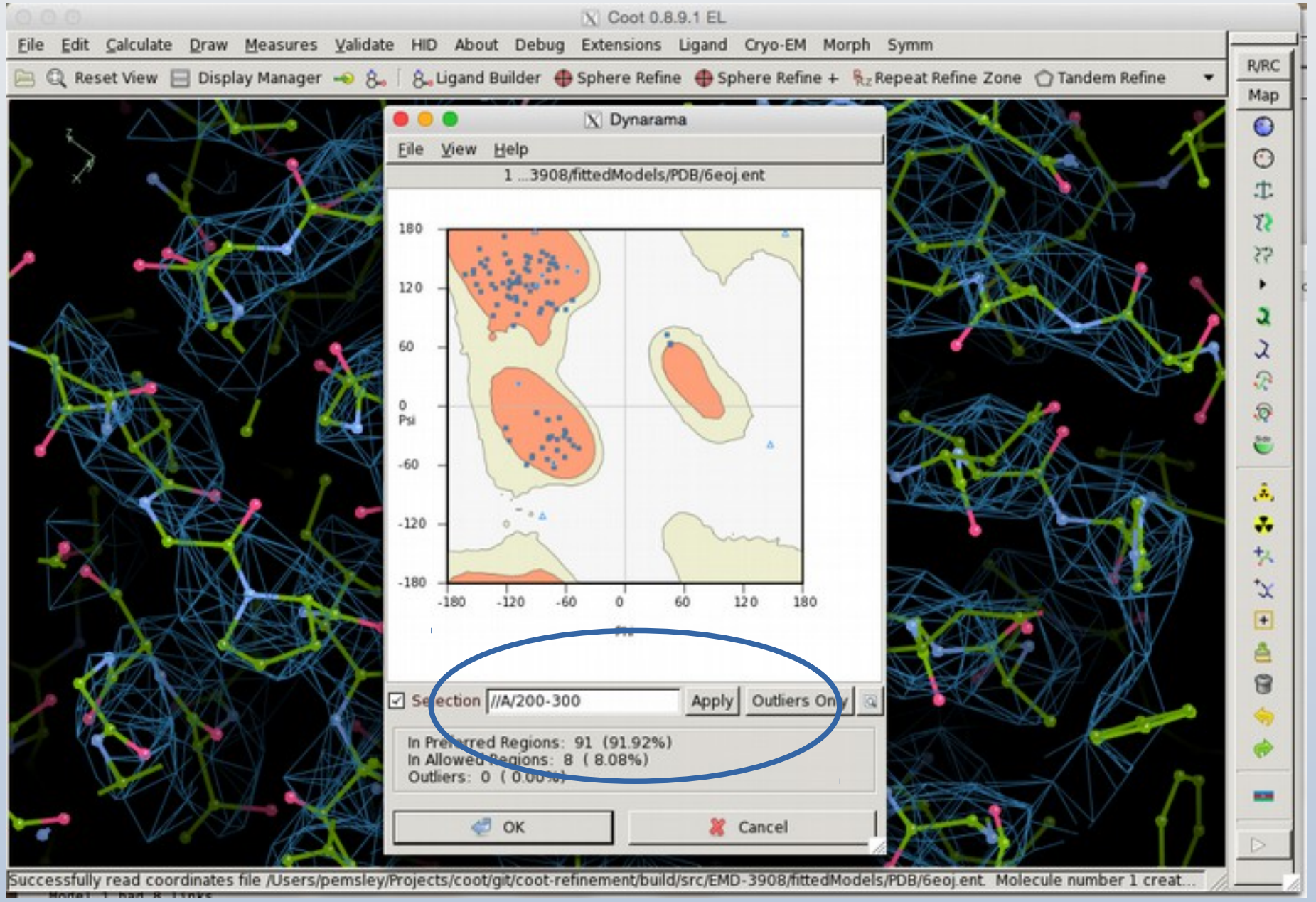
1 neighbour each side ⟶ 3 residue
2 neighbours each side ⟶ 5 residue

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 Cα-Cα vector for a number of residue pairs, choose solutions for which ϕ,ψ most probable
  - *cis*⟶*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

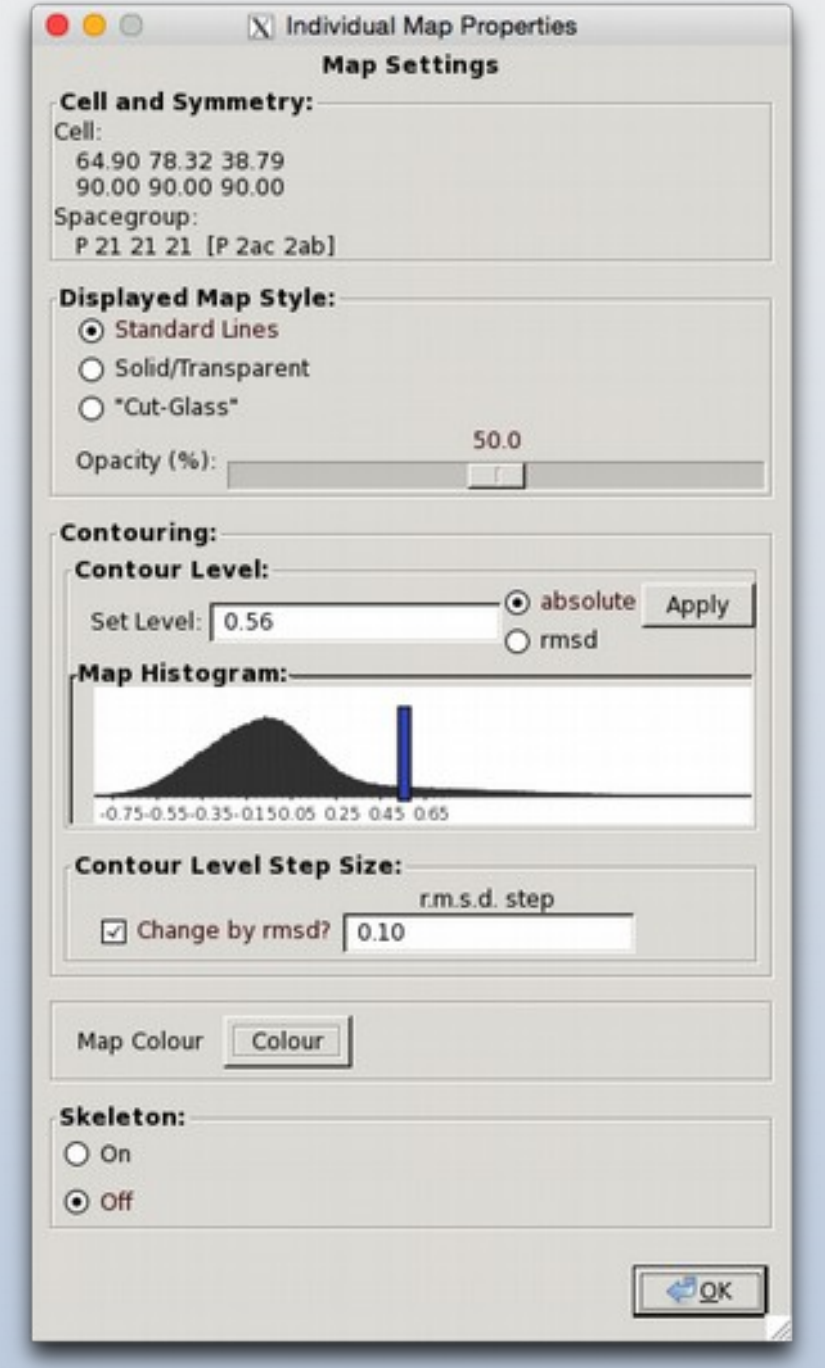
The New Ramachandran Plot



CURLEW:
Coot Utilites and Refinement Library Extention Wranger

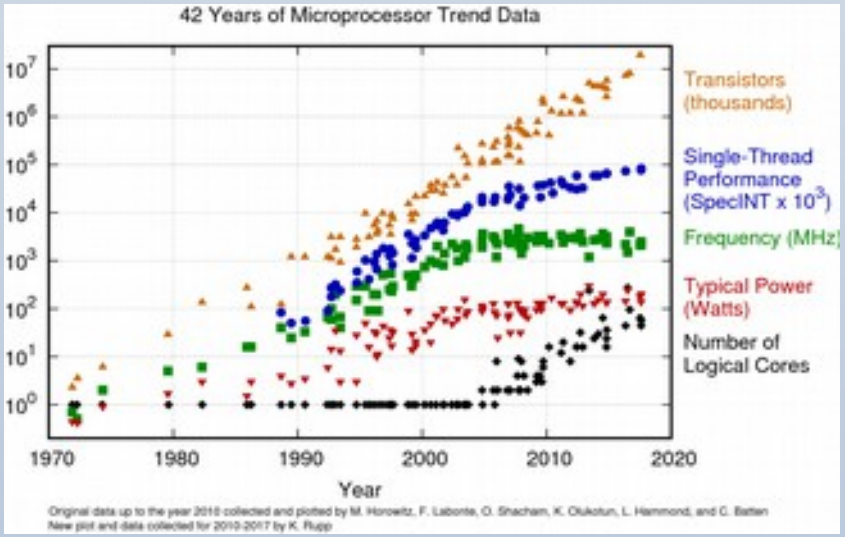
- Easy access to ”interesting” *Coot* scripts

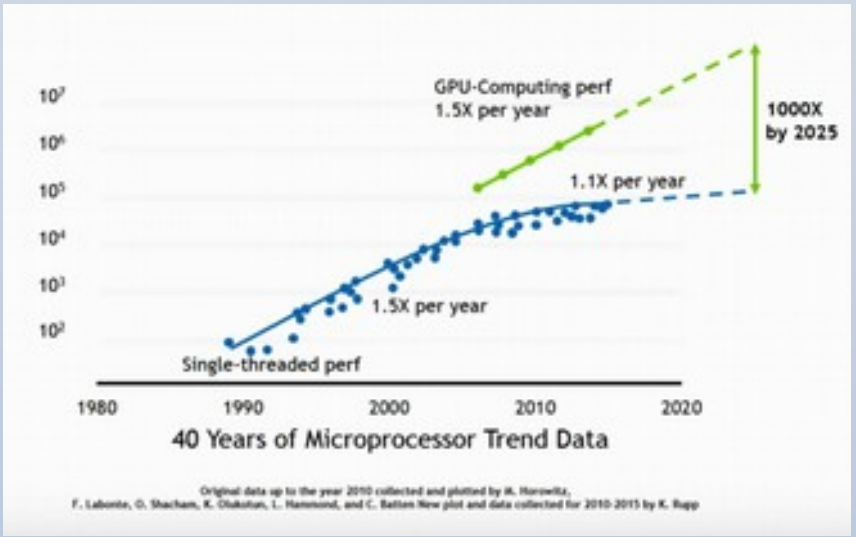
Map Properties



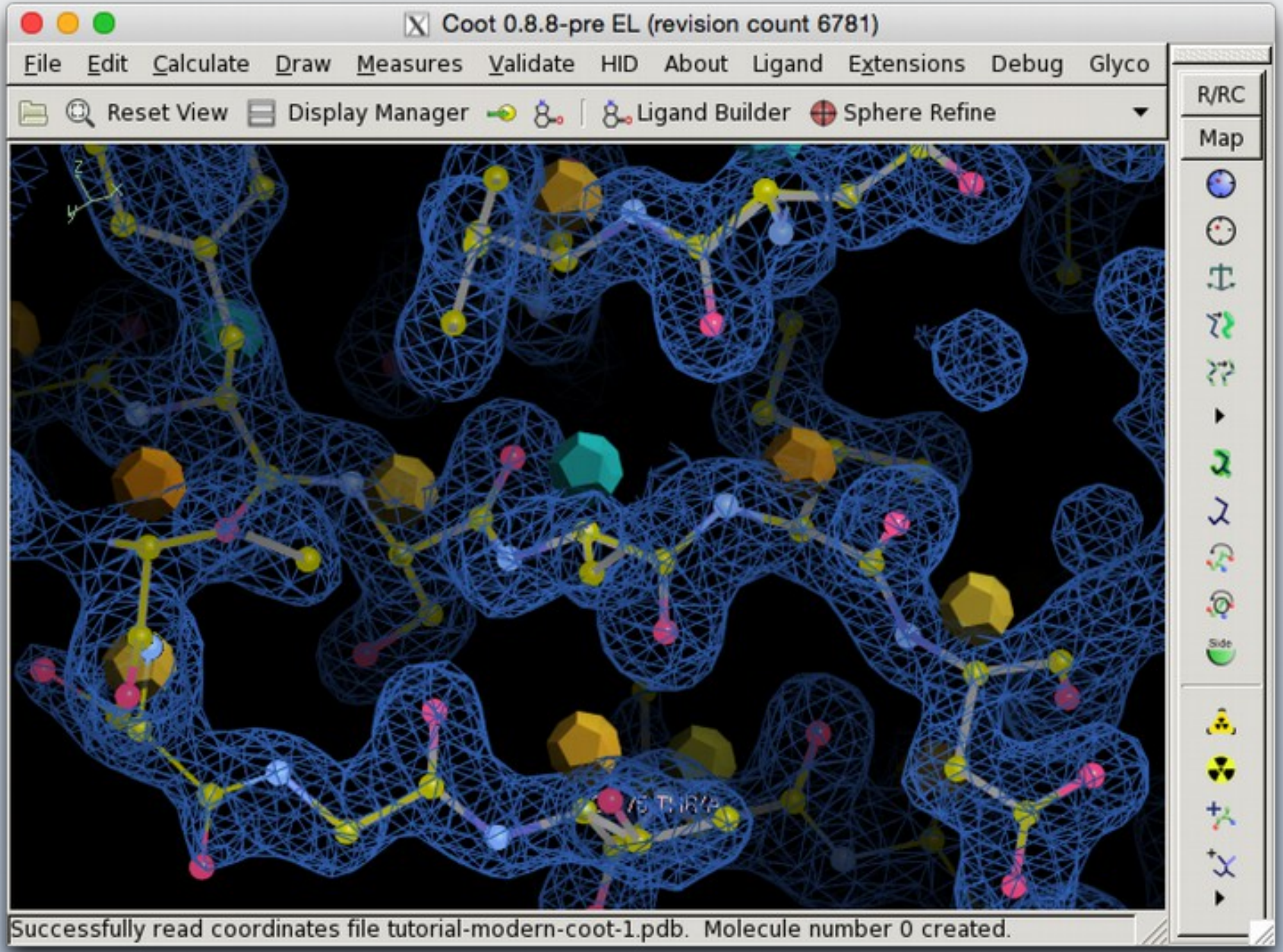
Coot is (or has historically been) Ugly

- The menus needs some re-working
- But most of all the graphics are tired-looking and slow
  - recent transparent objects and animation are an improvement
  - ignoring the developments in computation

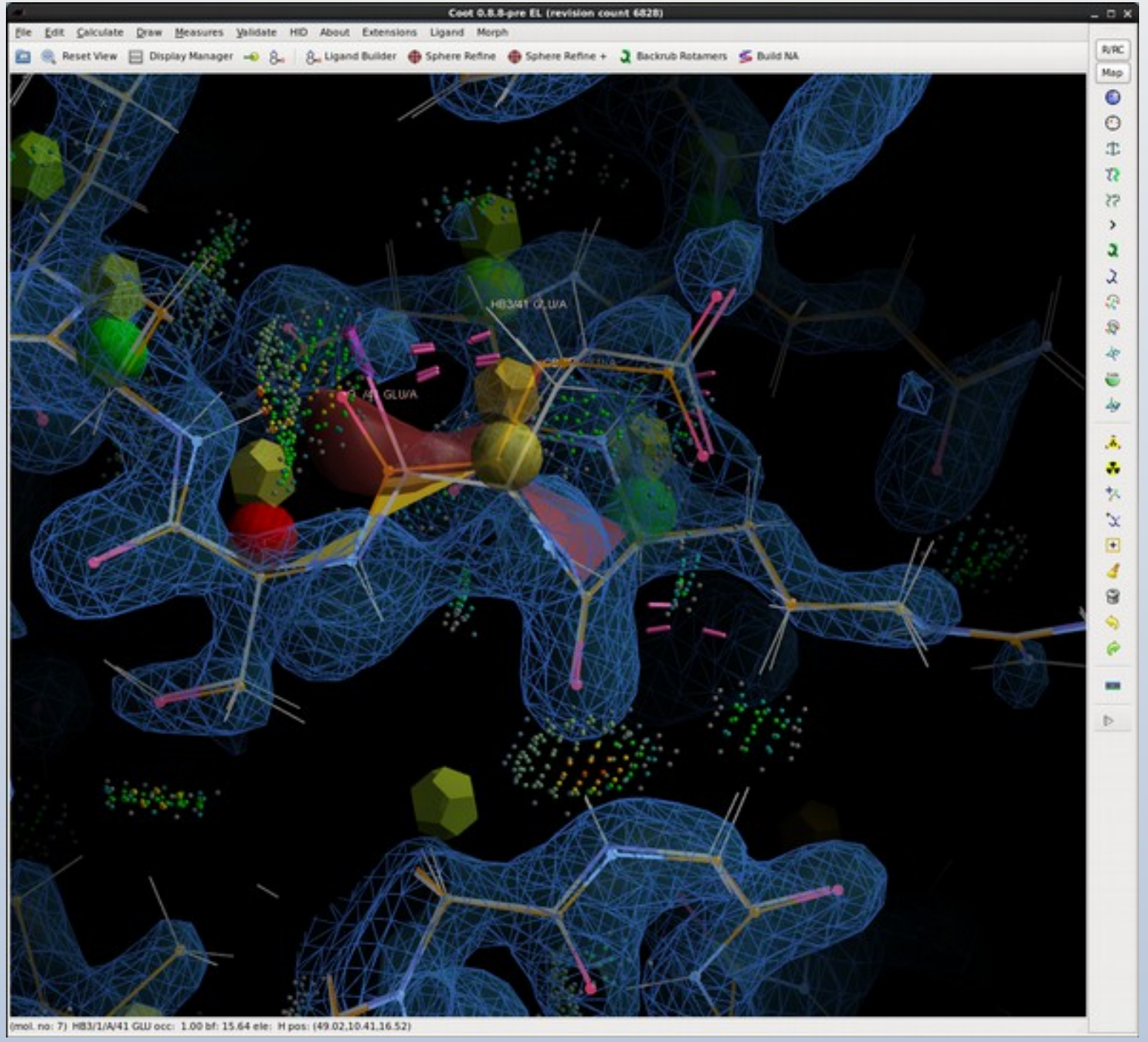




Interactive Rotamer Goodness



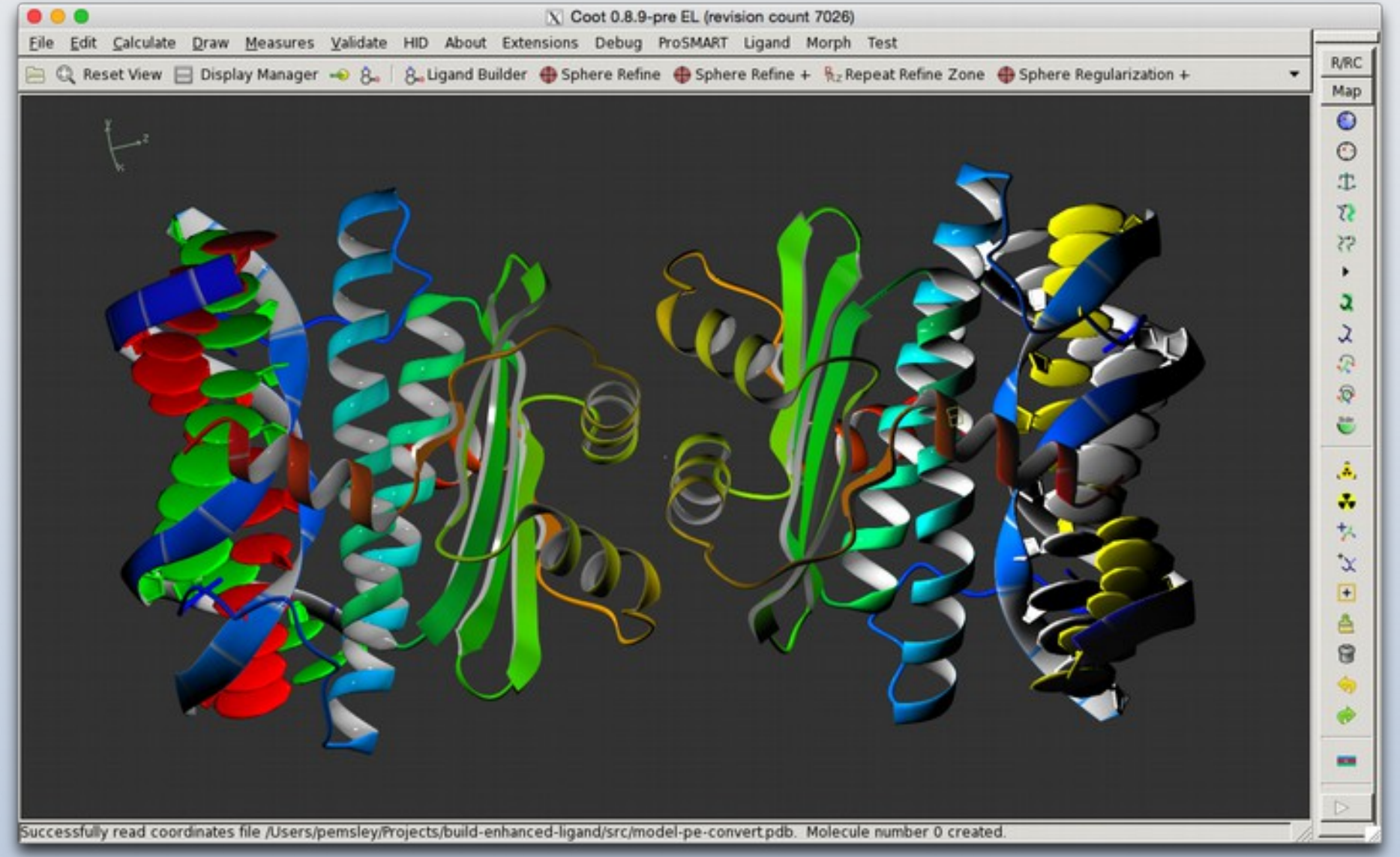
Multi-Criteria Markup



Rotamer and Ramachandran Markup

- `set_show_intermediate_atoms_rota_markup(1)`
- `set_show_intermediate_atoms_rama_markup(1)`

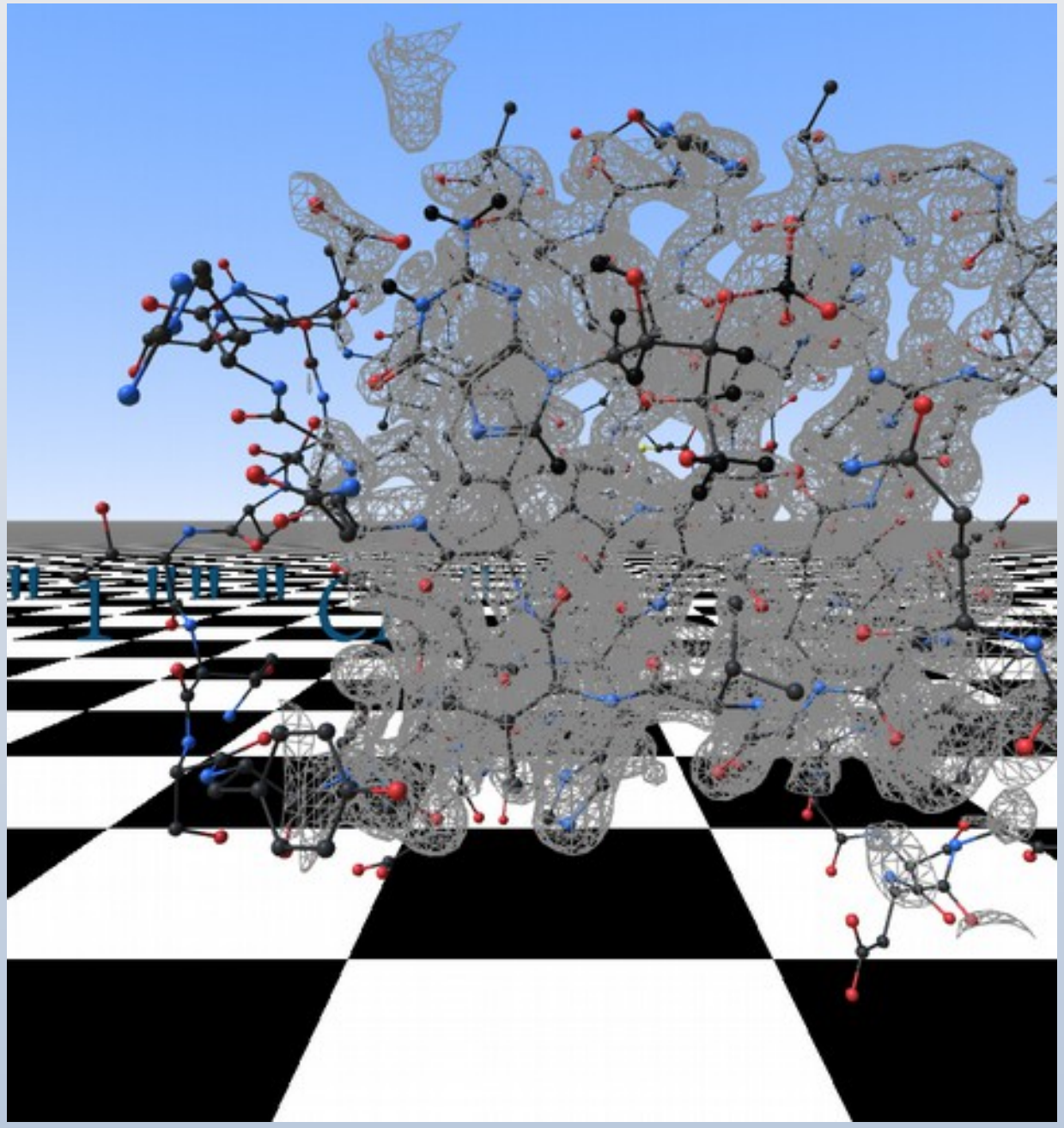
Coot Futures: GPU Ribbons



with Martin Noble

Coot Futures: Virtual Reality

Hamish Todd



- **An Intuitive Interface:**
- Stereoscopic Representation
- Greater Field of View
- 2 Hands with Articulation
- However:
  - current tools are not immediately transferable
  - because: nausea

CootVR

- Demonstrated at CCP-EM Meeting in Keele in April

CootVR Example Video

```
<switch>
```

- Augmented-Reality *Coot*?

with Martin Noble

Acknowledgements

- LMB:
  - Garib Murshudov, Rob Nicholls,
  - Alexey Amunts, Alan Brown

- Kevin Cowan, Bernhard Lohkamp

- Libraries & Dictionaries:
  - Jane & Dave Richardson
  - Alexei Vagin
  - Eugene Krissinel