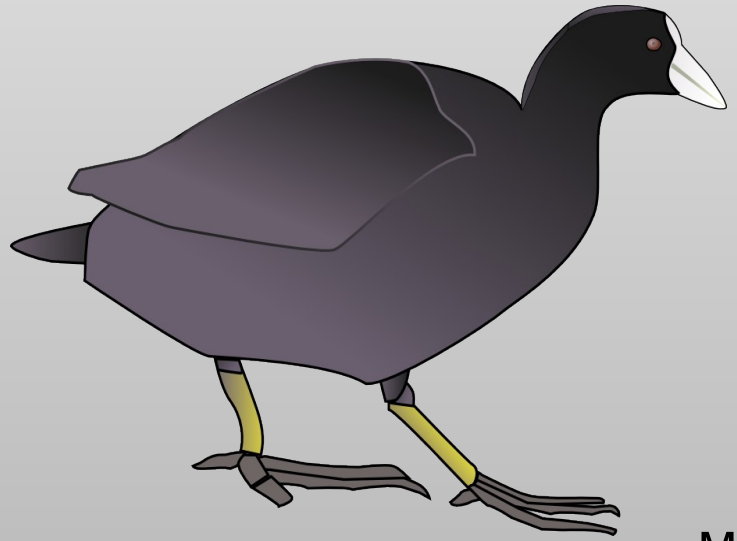
Tools for Cryo-EM Map Fitting



Paul Emsley
MRC Laboratory of Molecular Biology

March 2016

Coot (Paul Emsley)

Coot

- Crystallographic Object-Oriented Tool-kit
- Primarily a tool for the interpretation of electron density generated from X-ray data
  - with tools for modelling:
    - rotate/translate, rotamers,
    - refinement & regularization
    - add, delete
    - ligand fitting
  - A “workhorse”, not a show-pony

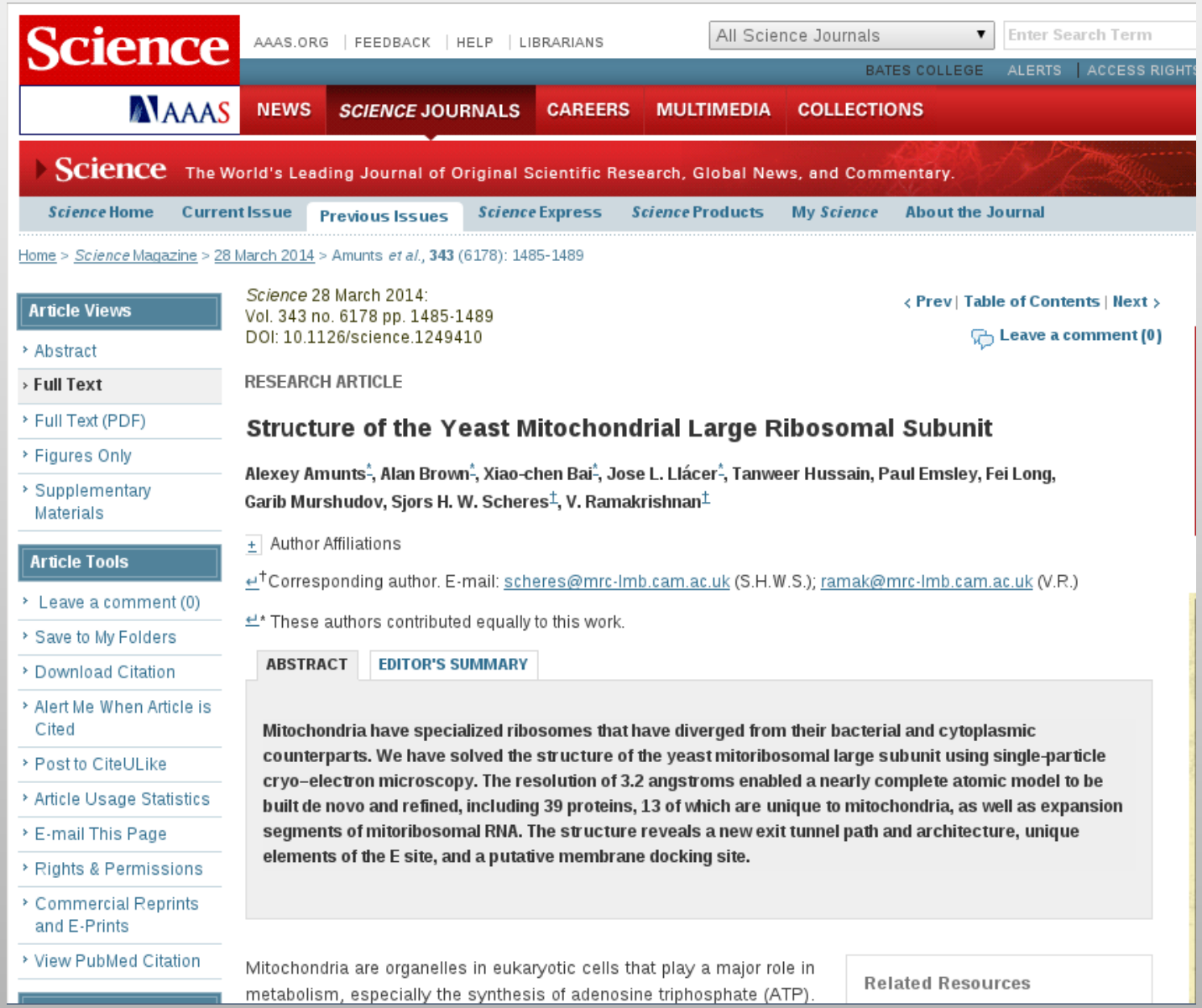
For What is Coot Useful?

What resolution ranges for cryo-EM?

- “Resolution Revolution” maps
- 2-3.8A is the strength
- seeing side-chains and purines vs pyrimidines

Local good fit of model to density

Yeast Mitochondrial Large Ribosomal Subunit



Science 28 March 2014:
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RESEARCH ARTICLE

Structure of the Yeast Mitochondrial Large Ribosomal Subunit

Alexey Amunts[*], Alan Brown[*], Xiao-chen Bai[*], Jose L. Llácer[*], Tanweer Hussain, Paul Emsley, Fei Long, Garib Murshudov, Sjors H. W. Scheres[†], V. Ramakrishnan[†]

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[*] These authors contributed equally to this work.

ABSTRACT | EDITOR'S SUMMARY

Mitochondria have specialized ribosomes that have diverged from their bacterial and cytoplasmic counterparts. We have solved the structure of the yeast mitoribosomal large subunit using single-particle cryo–electron microscopy. The resolution of 3.2 angstroms enabled a nearly complete atomic model to be built de novo and refined, including 39 proteins, 13 of which are unique to mitochondria, as well as expansion segments of mitoribosomal RNA. The structure reveals a new exit tunnel path and architecture, unique elements of the E site, and a putative membrane docking site.

Mitochondria are organelles in eukaryotic cells that play a major role in metabolism, especially the synthesis of adenosine triphosphate (ATP).

Related Resources

Acta Crystallographica Section D
Biological Crystallography
ISSN 1399-0047

Tools for macromolecular model building and refinement into electron cryo-microscopy reconstructions

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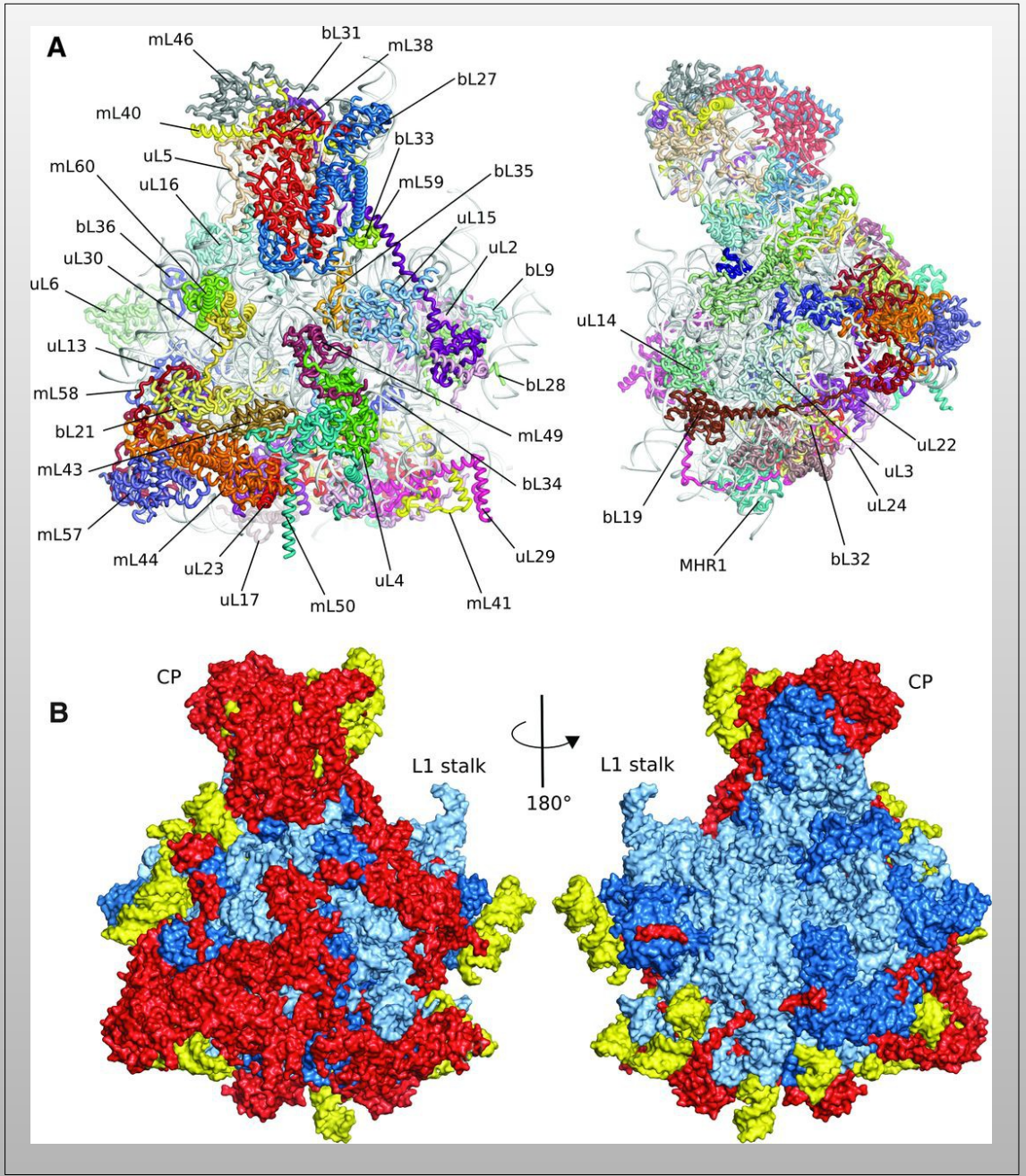
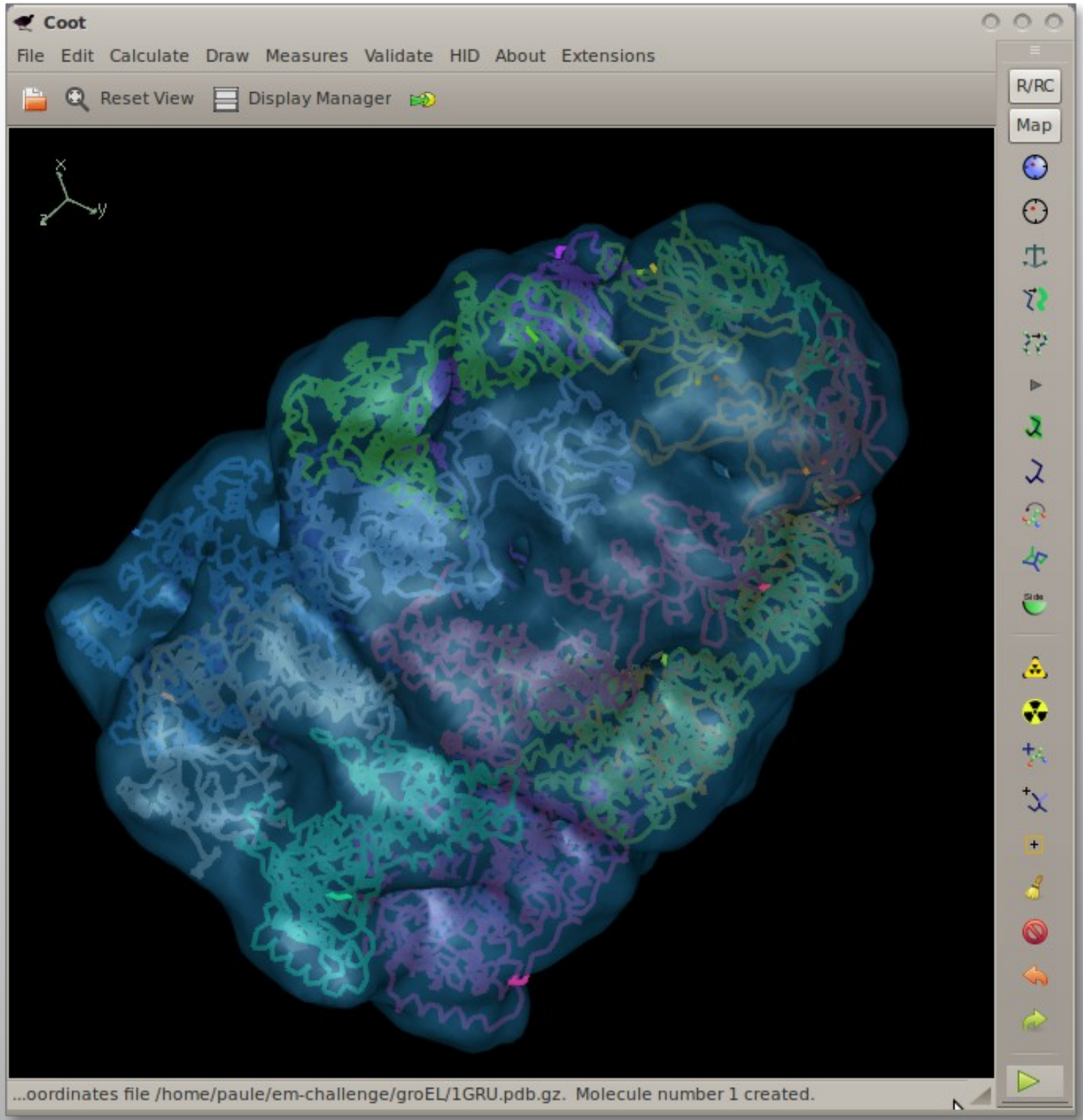
The recent rapid development of single-particle electron cryo-microscopy (cryo-EM) now allows structures to be solved by this method at resolutions close to 3 Å. Here, a number of tools to facilitate the interpretation of EM reconstructions with stereochemically reasonable all-atom models are described. The *BALBES* database has been repurposed as a tool for identifying protein folds from density maps. Modifications to *Coot*, including new Jiggle Fit and morphing tools and improved handling of nucleic acids, enhance its functionality for interpreting EM maps. *REFMAC* has been modified for optimal fitting of atomic models into EM maps. As external structural information can enhance the reliability of the derived atomic models, stabilize refinement and reduce overfitting, *ProSMART* has been extended to generate interatomic distance restraints from nucleic acid reference structures, and a new tool, *LIBG*, has been developed to generate nucleic acid base-pair and parallel-plane restraints. Furthermore, restraint generation has been integrated with visualization and editing in *Coot*, and these restraints have been applied to both real-space refinement in *Coot* and reciprocal-space refinement in *REFMAC*.

New Components



blue: conserved
red: found in both
yellow: yeast only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Display Manager
R/RC
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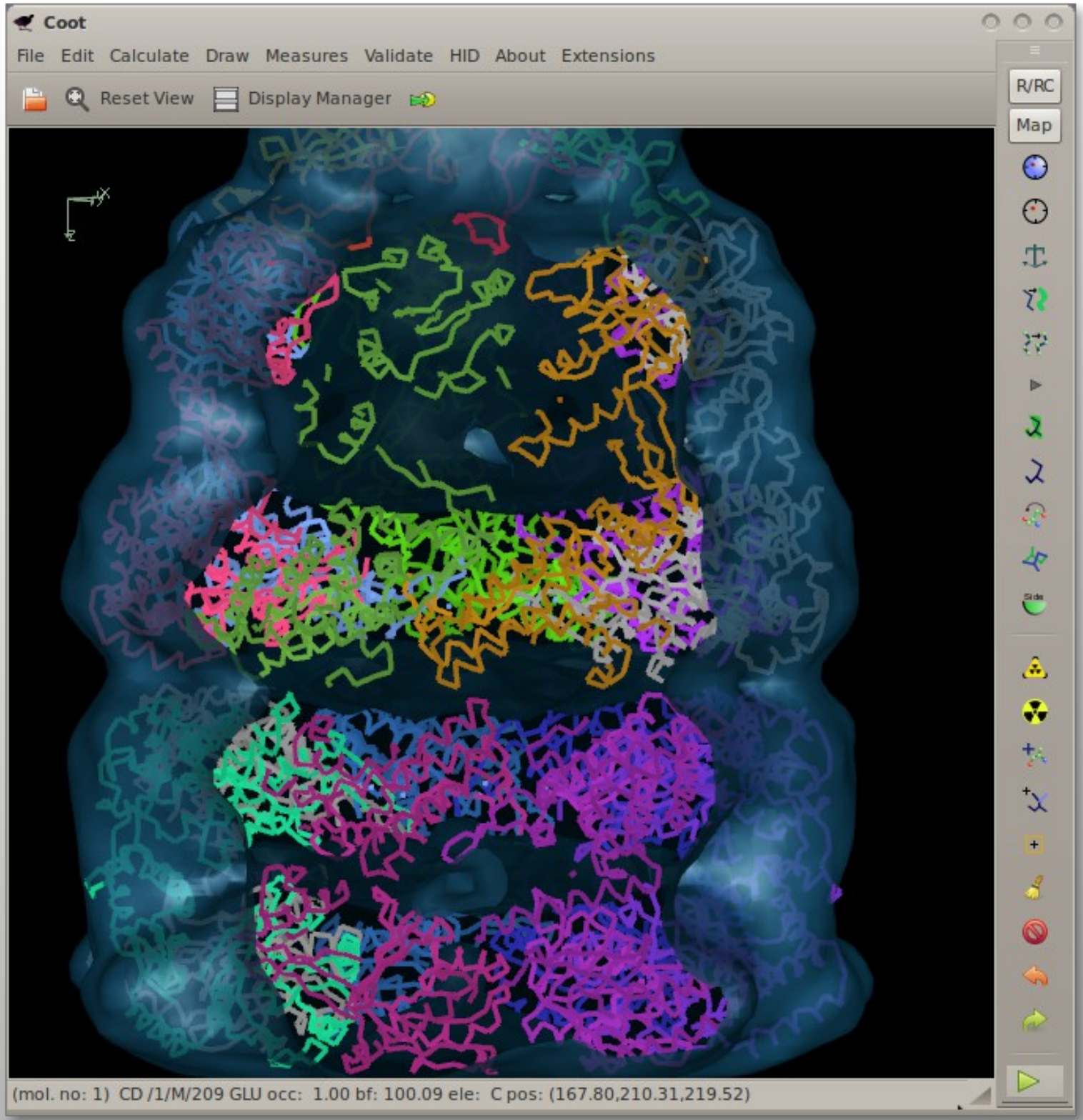
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R/RC
Map
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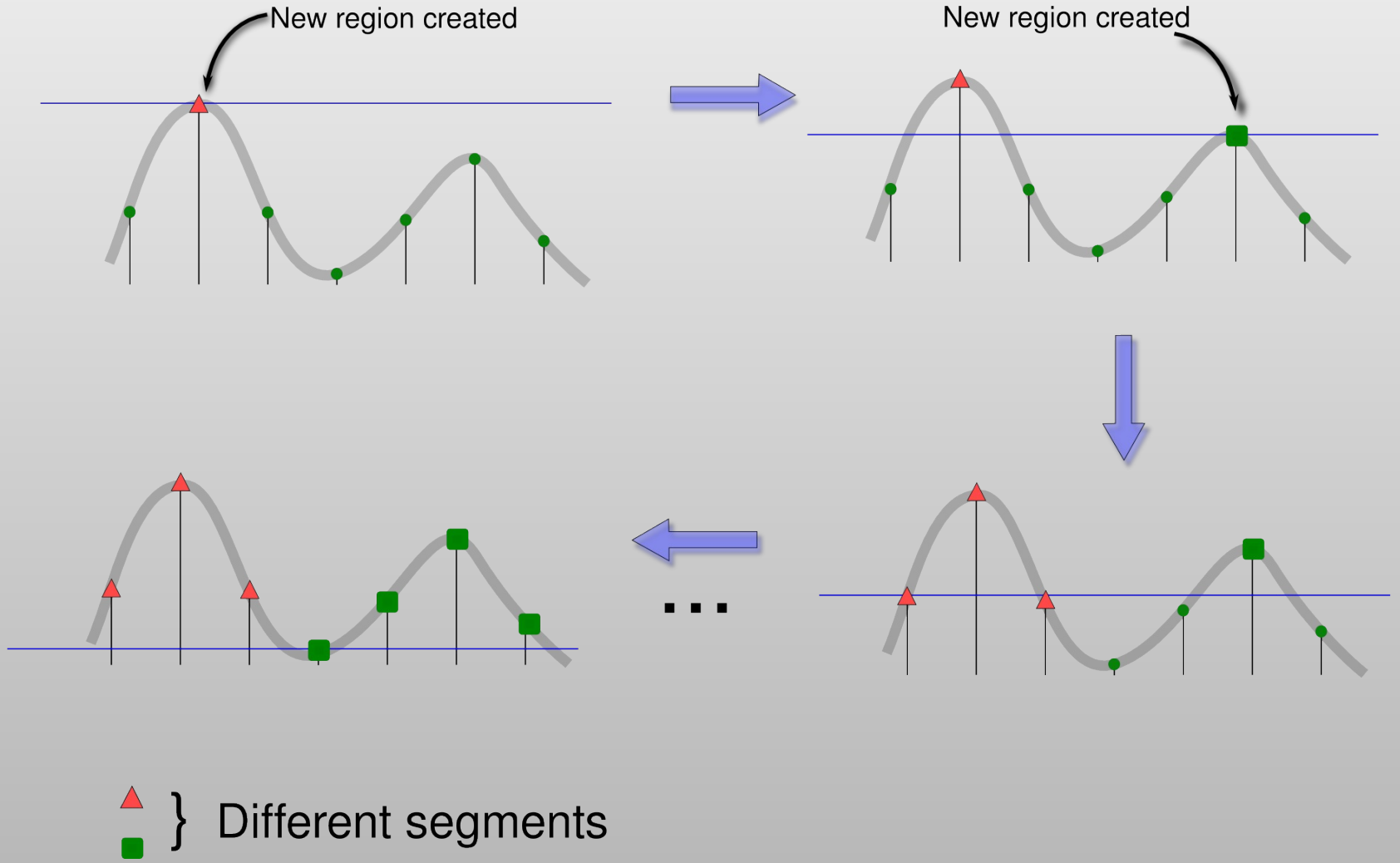
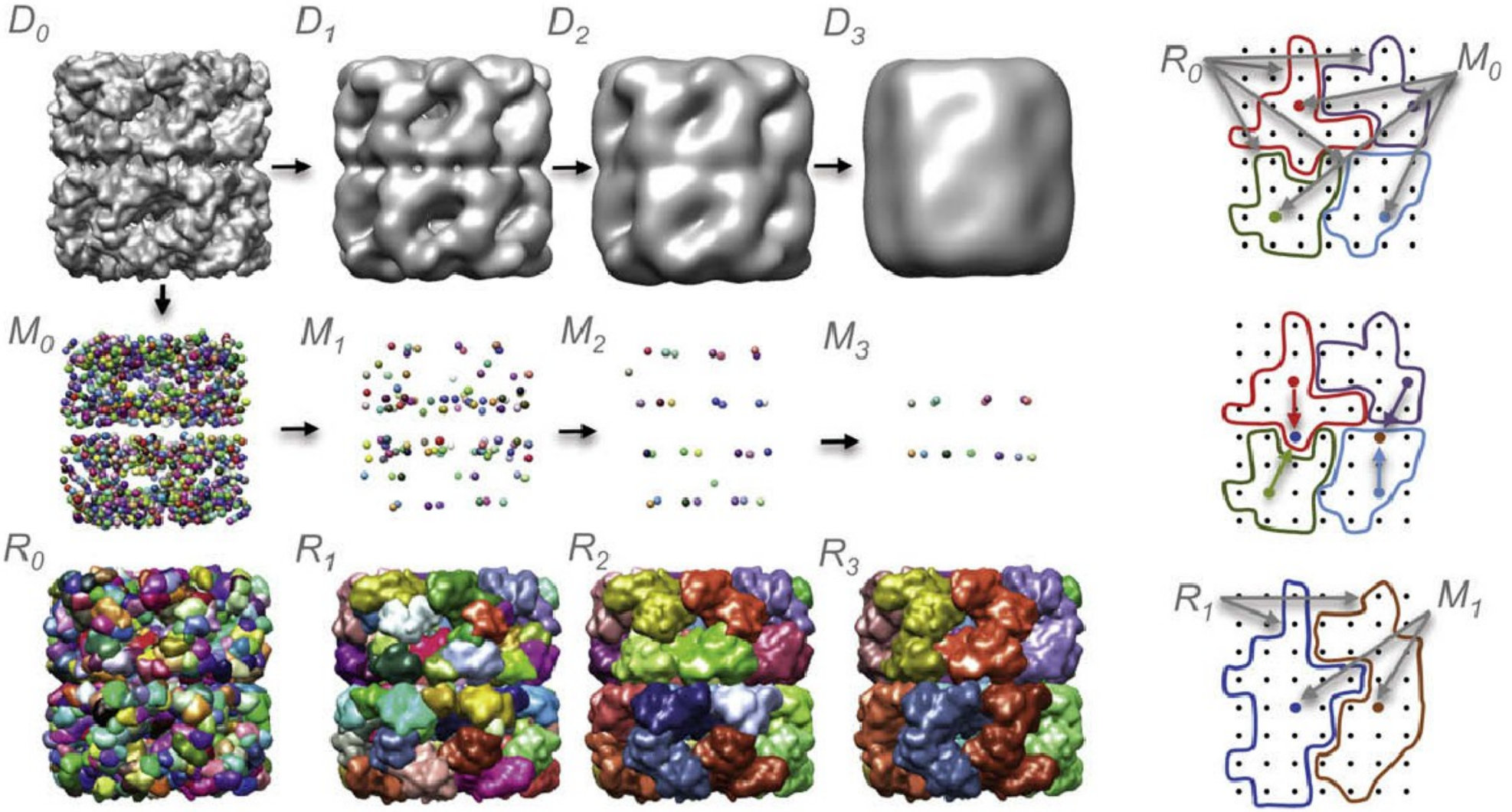
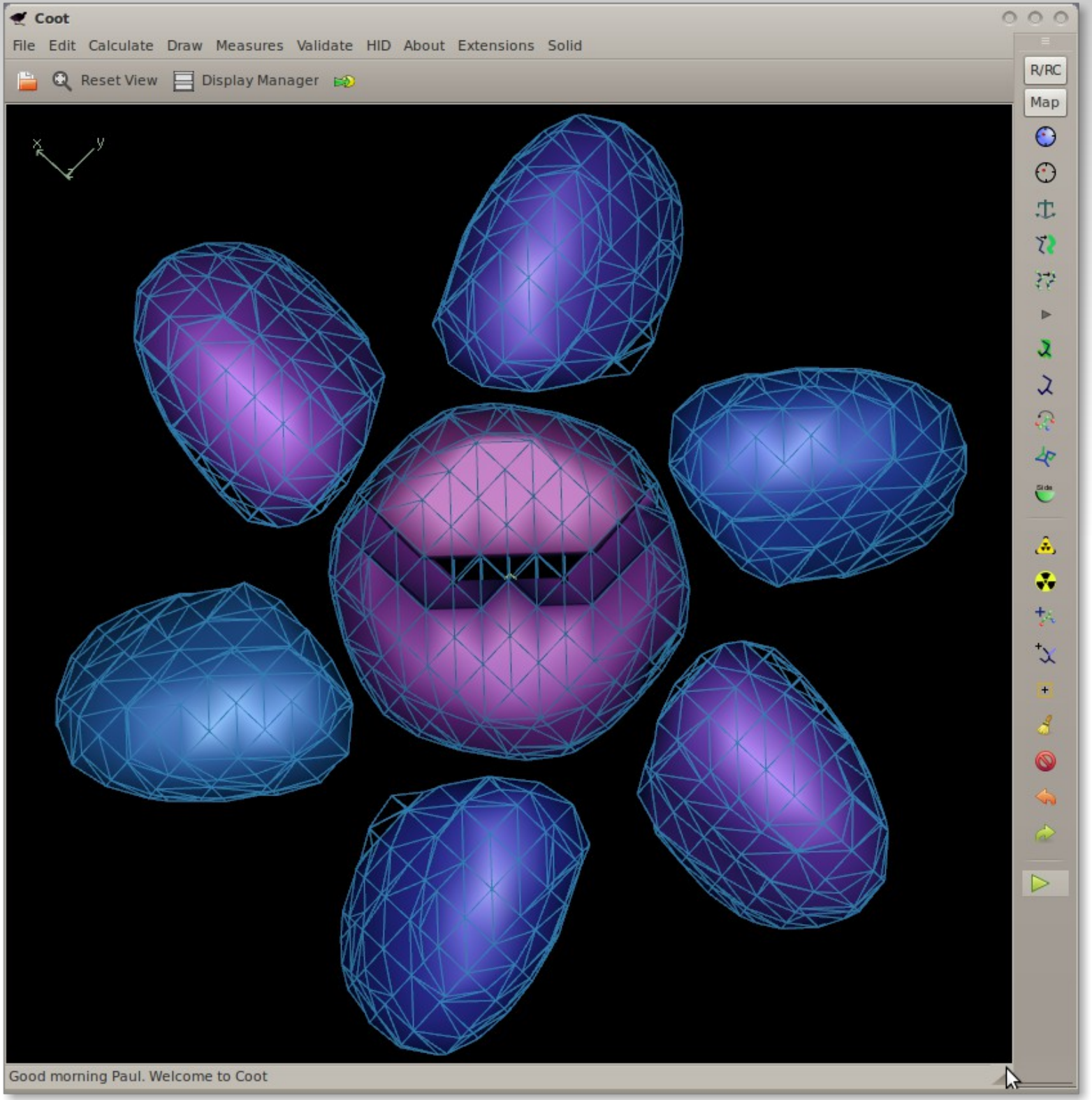
Partioning Maps: Watershed Algorithm

1D-analog





Pintilie *et al.* (2010) J.Struct.Biol.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File Edit Calculate Draw Measures Validate HID About Extensions Solid
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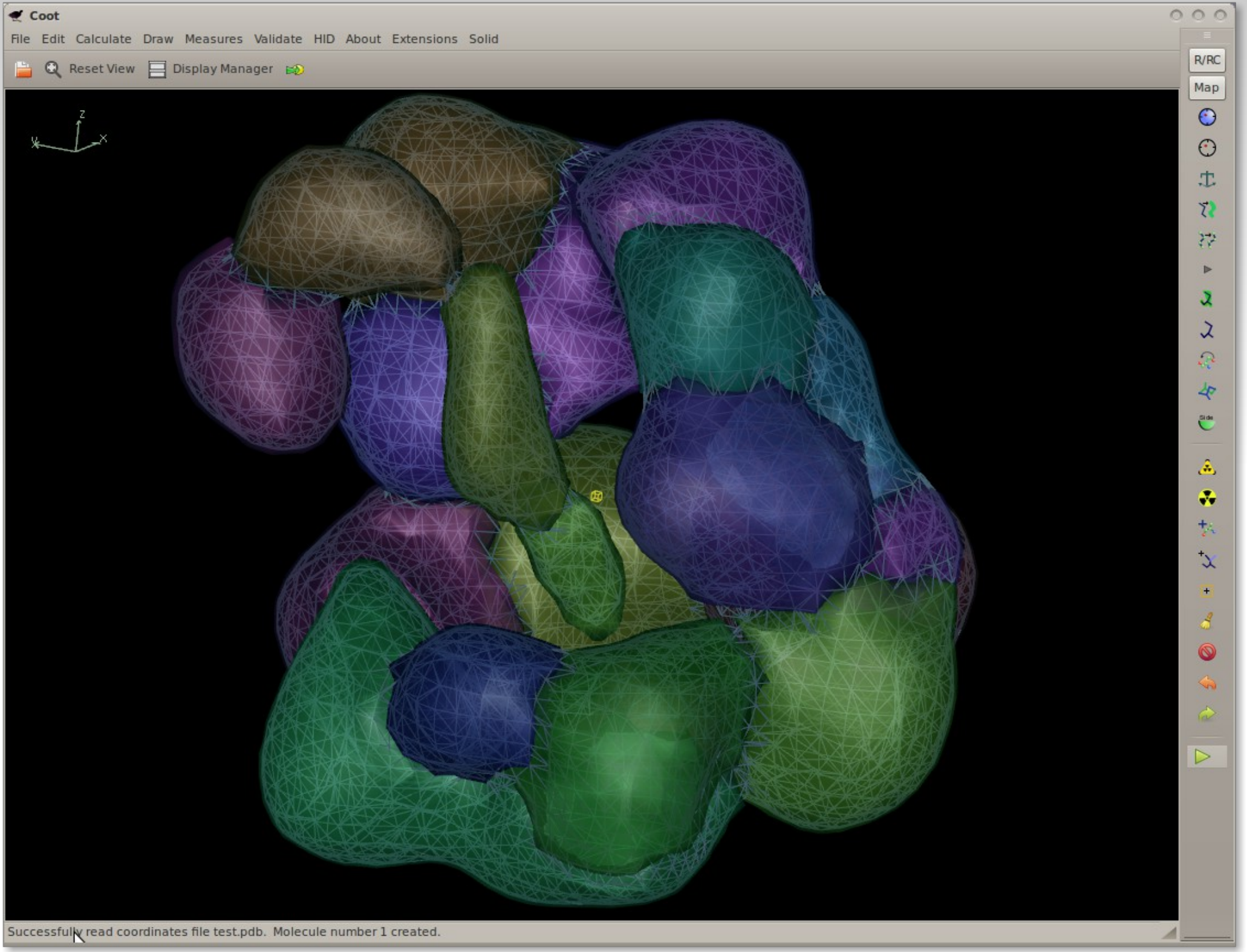
Display Manager
R/RC
Map
Good morning Paul. Welcome to Coot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Y
X
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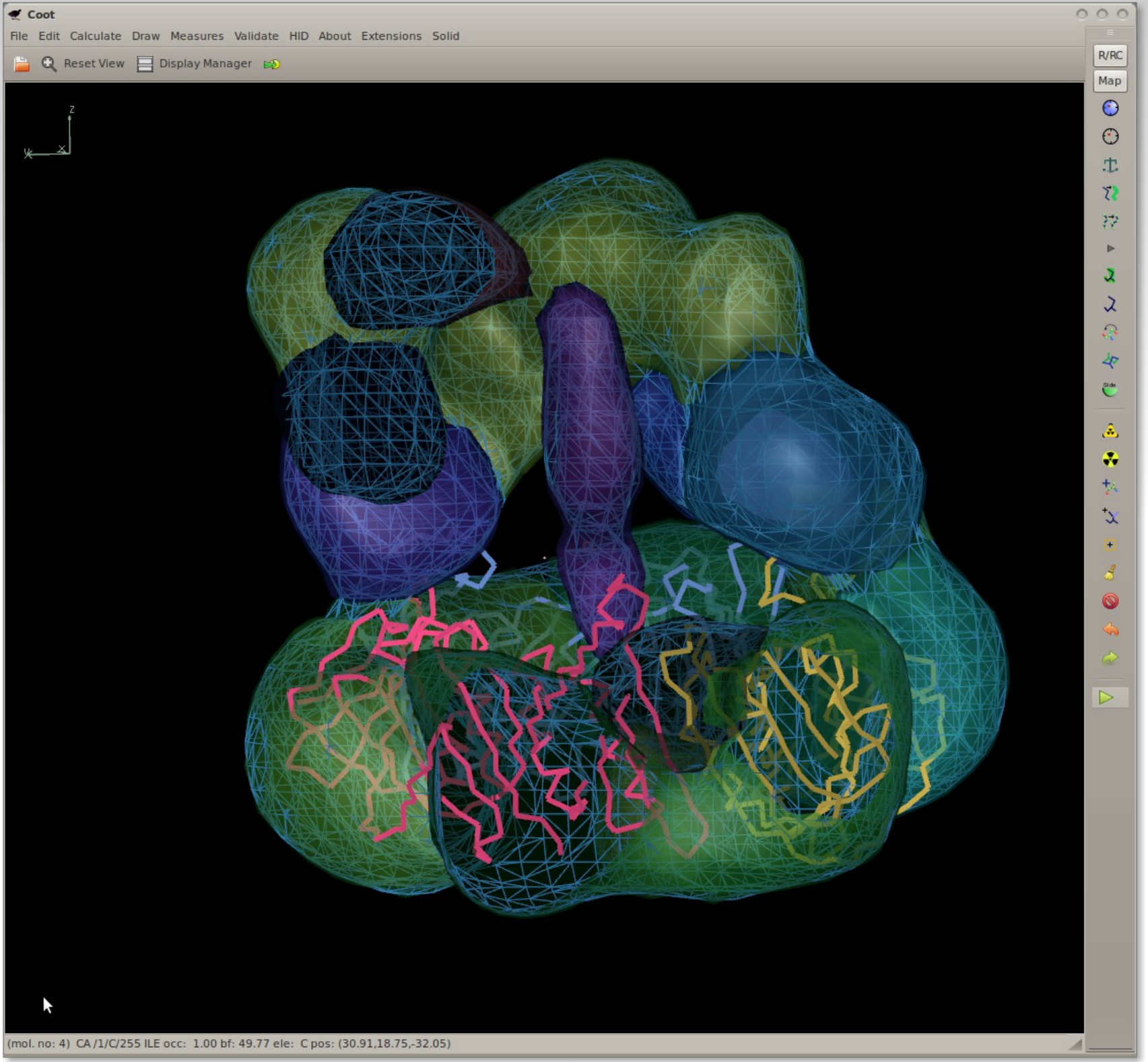
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Display Manager
R/RC
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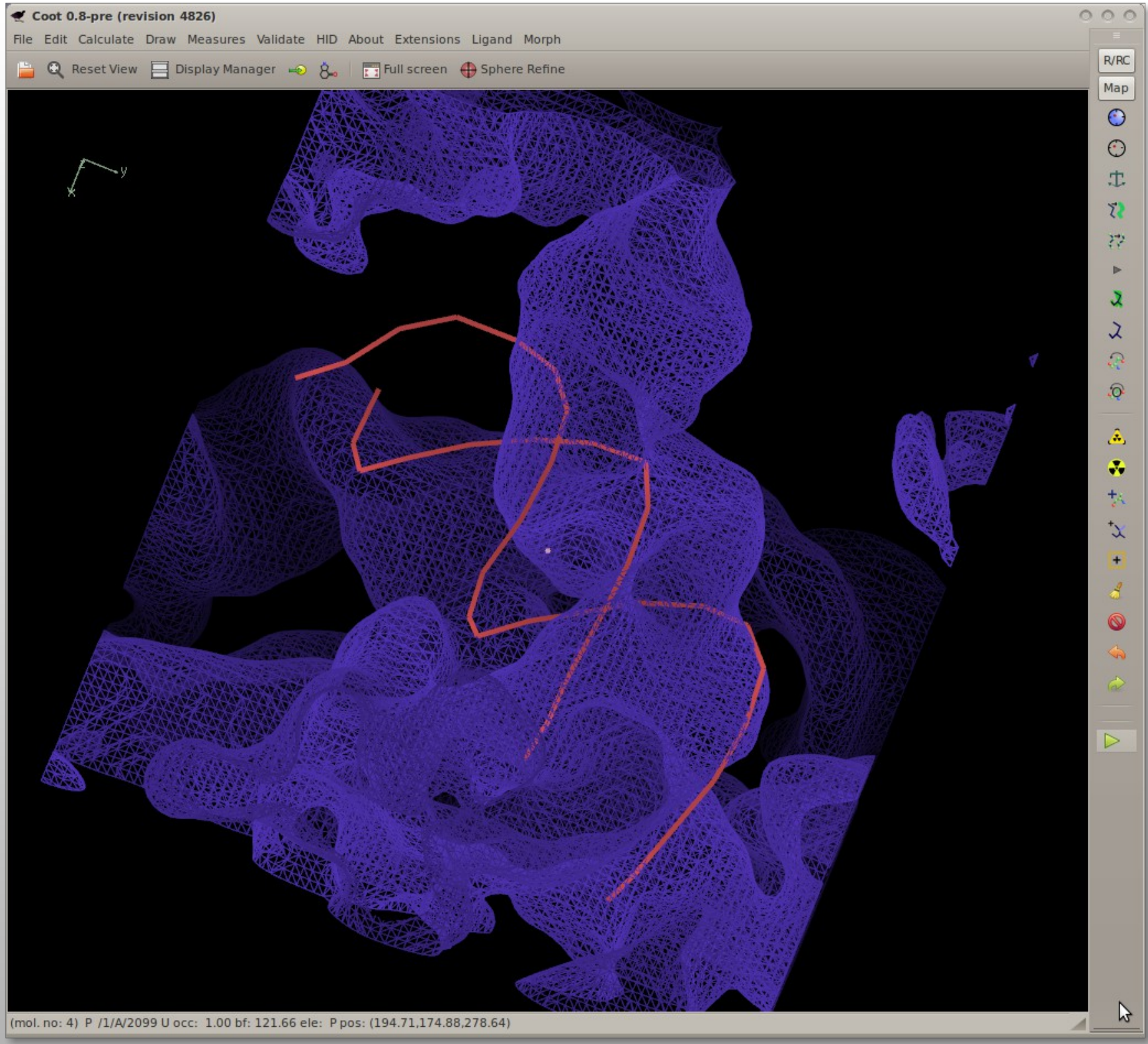
Jiggle Fit

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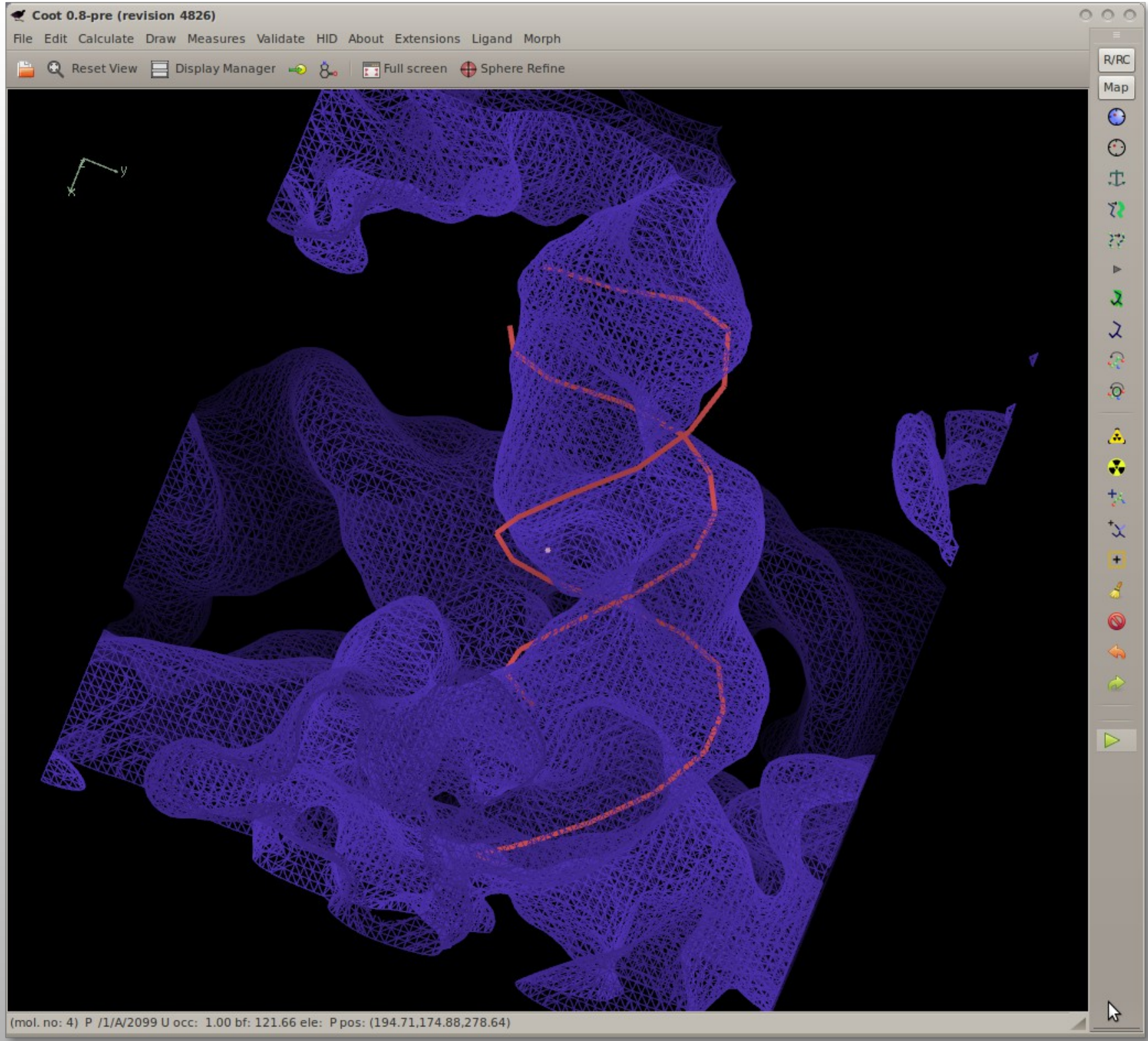
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)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Sphere Refine
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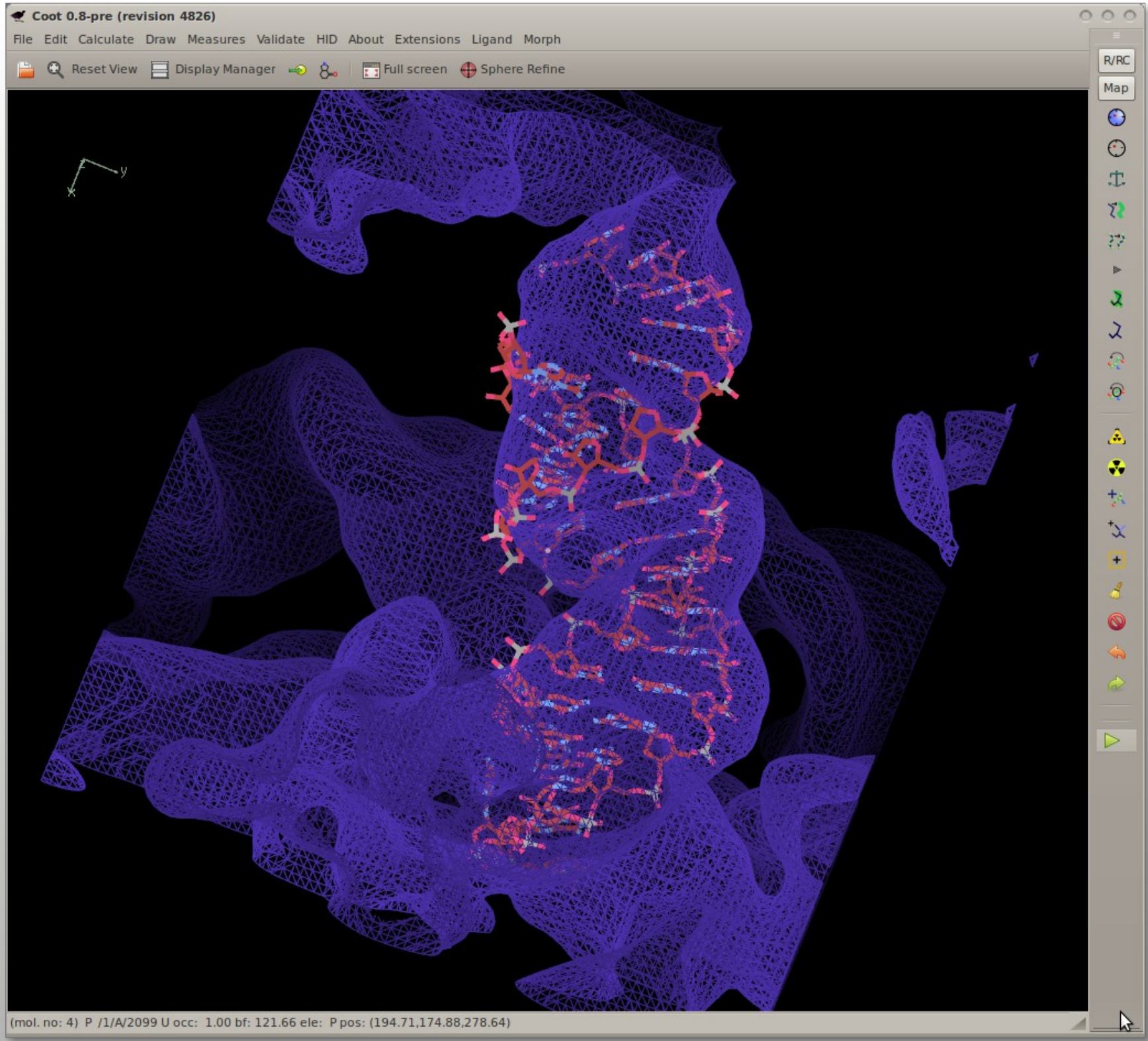
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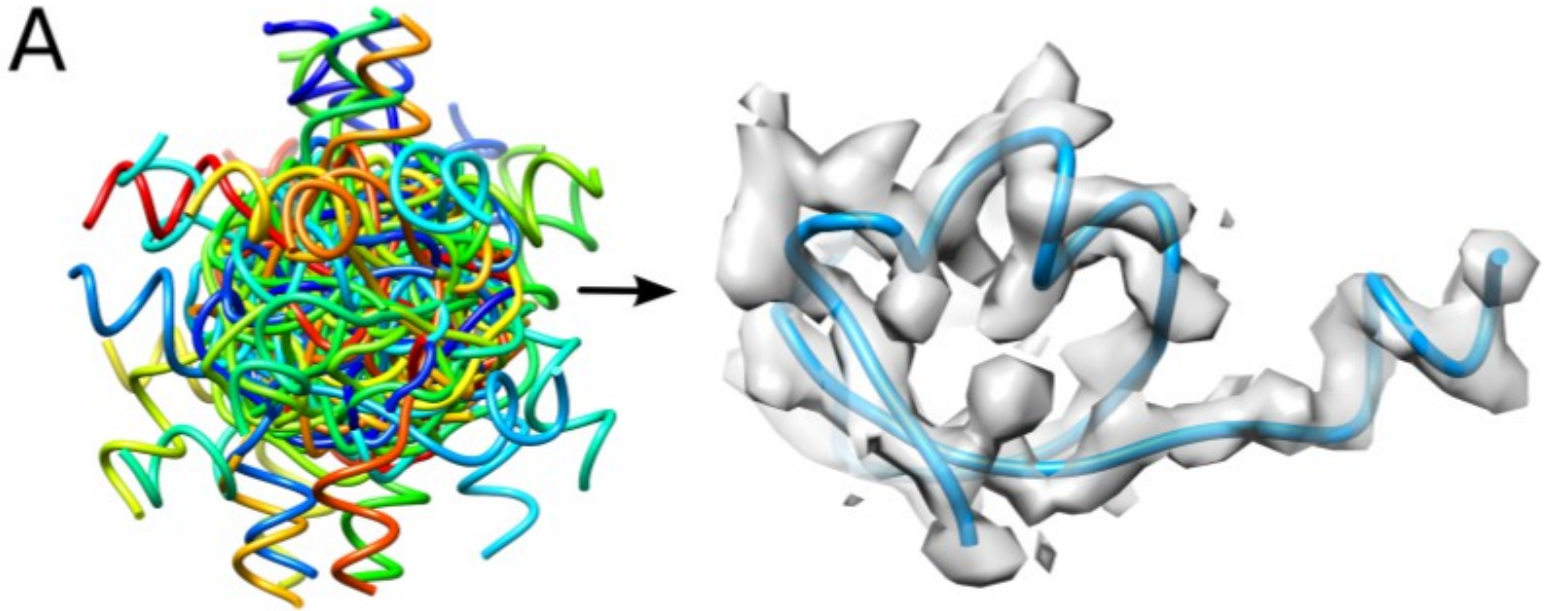
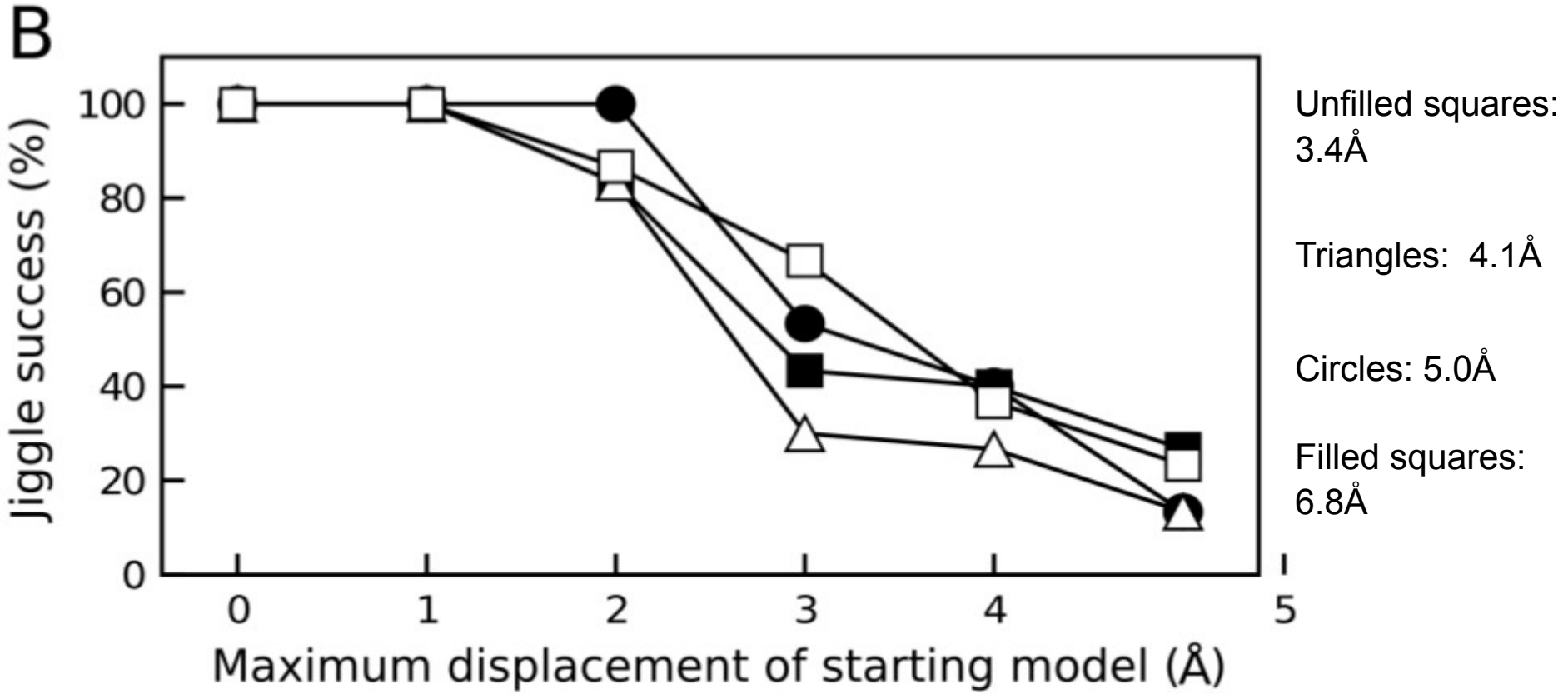

A


B
Jiggle success (%)
100
80
60
40
20
0
0
1
2
3
4
5
Maximum displacement of starting model (Å)
Unfilled squares: 3.4Å
Triangles: 4.1Å
Circles: 5.0Å
Filled squares: 6.8Å

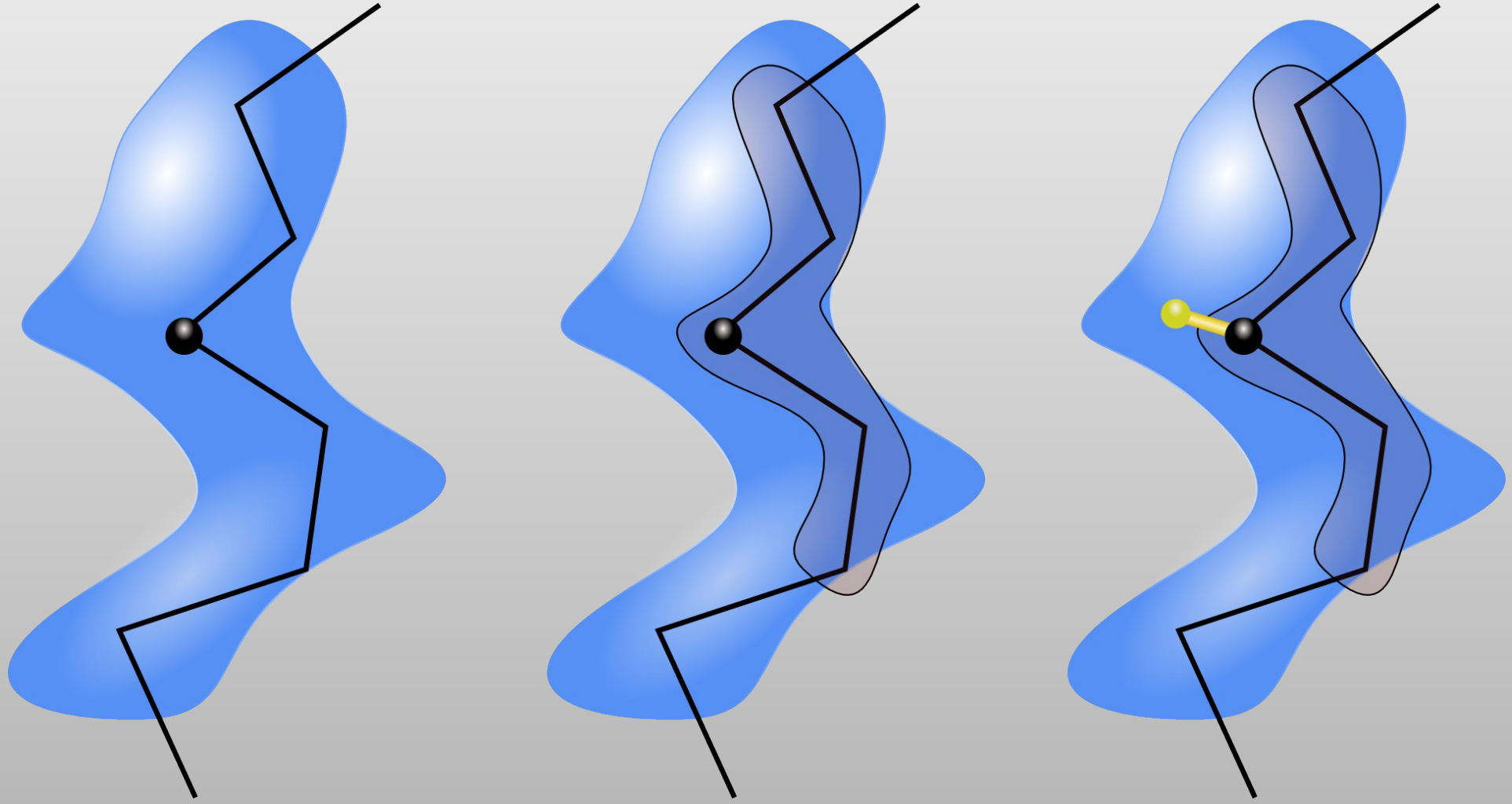
So we have our ideal RNA or homologous protein sitting roughly in the density

(not a great fit)

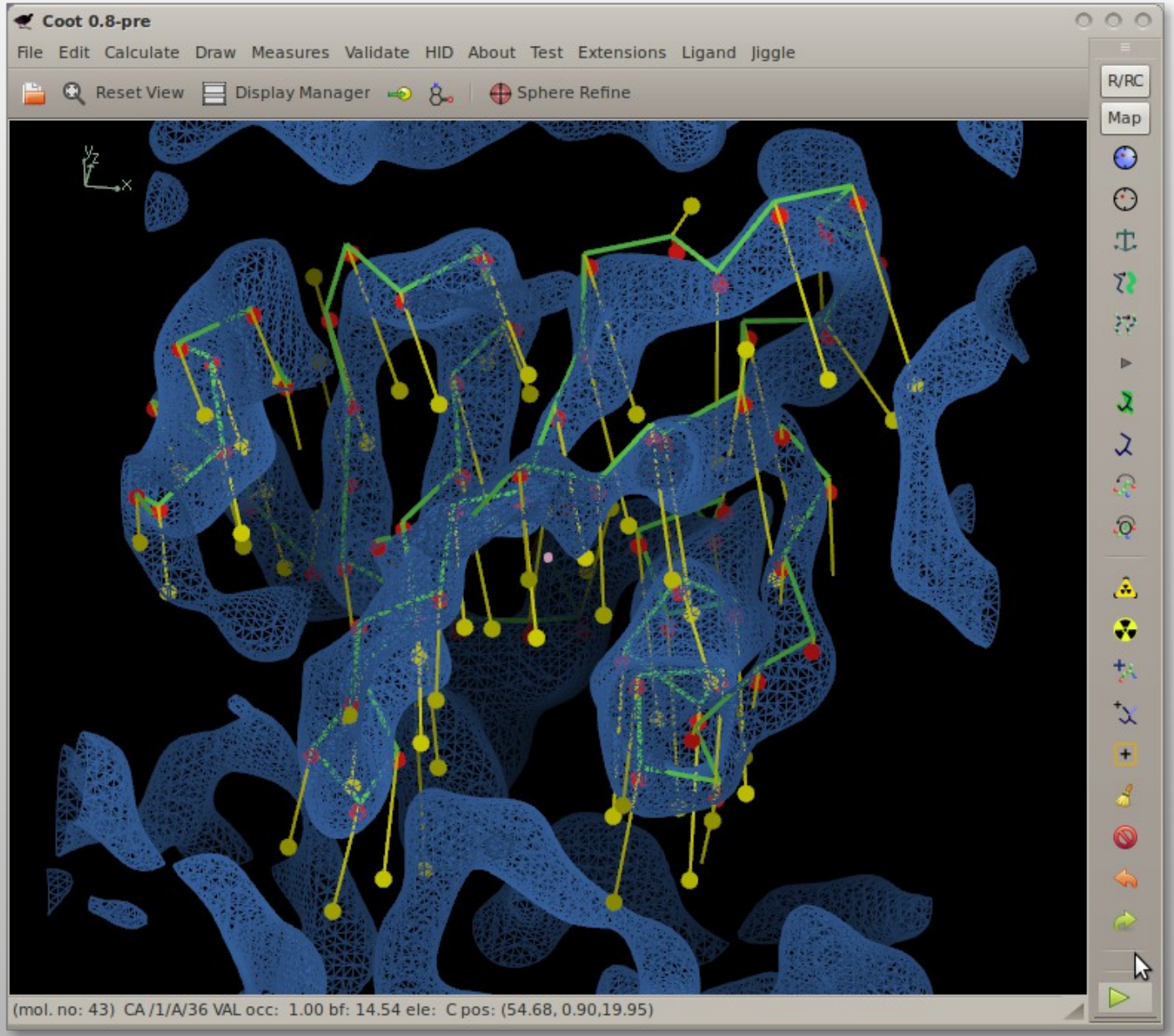
Model Morphing: How it Works

- 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

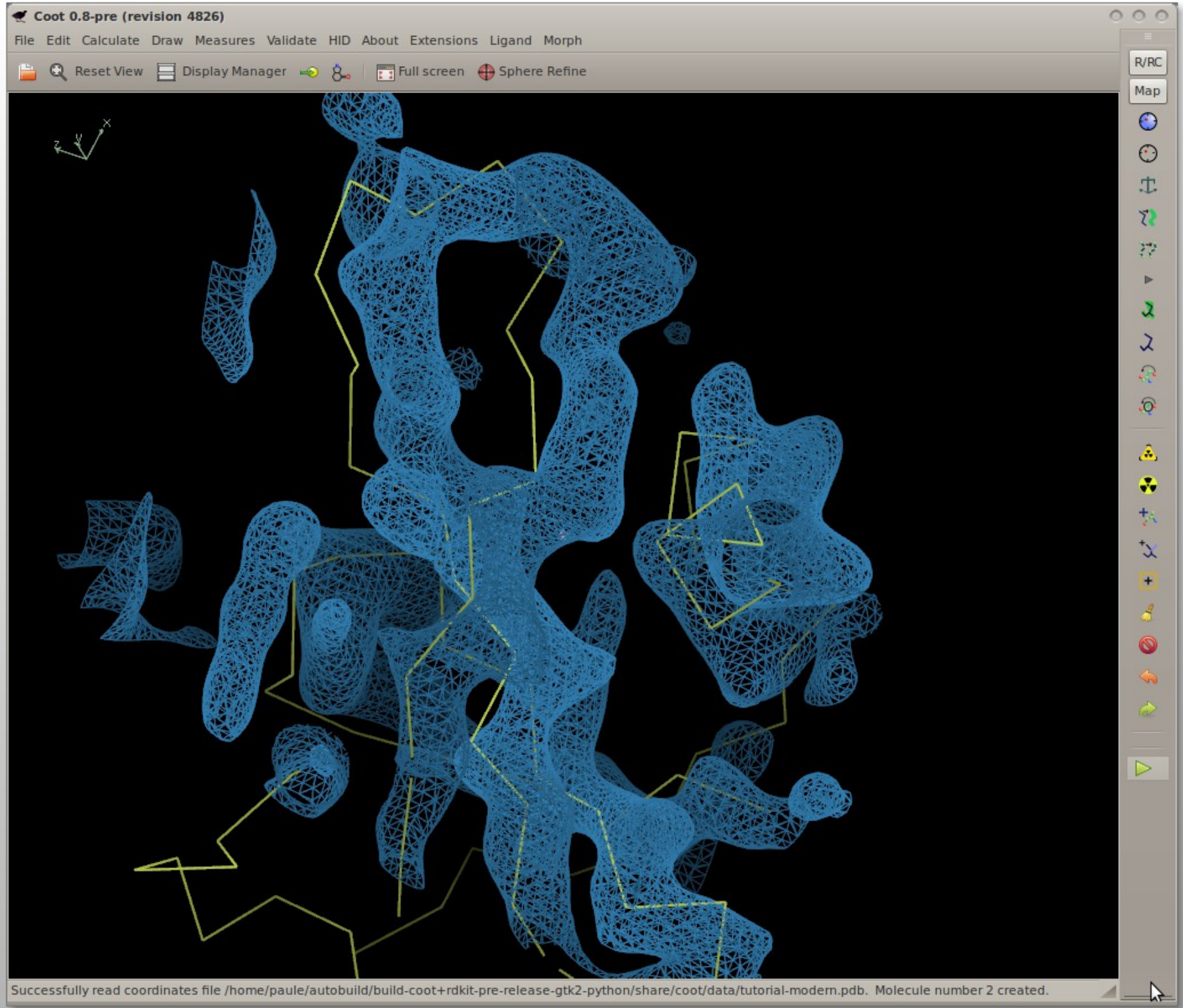


Model Morphing: Example



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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Full screen
Sphere Refine
R/RC
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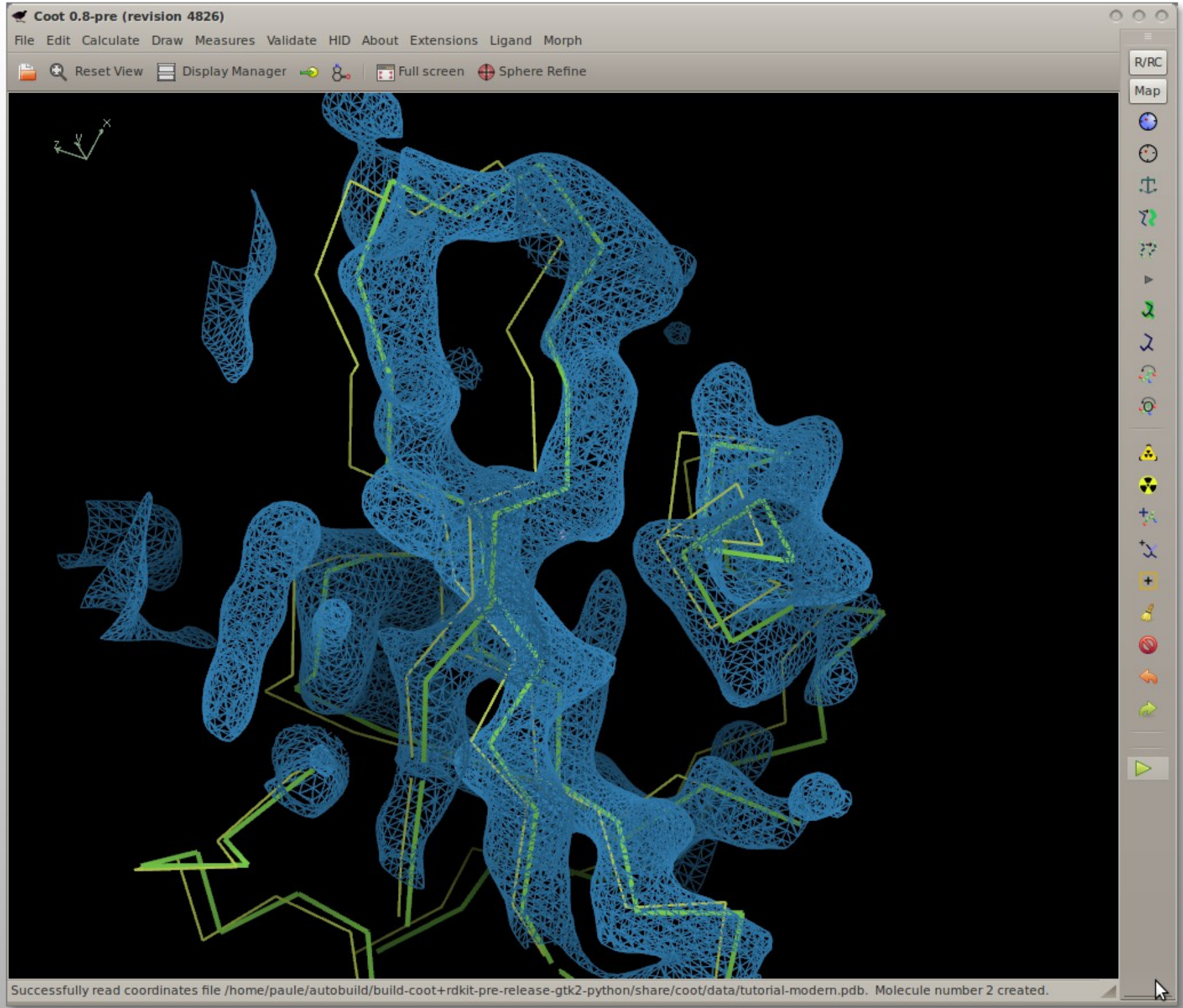
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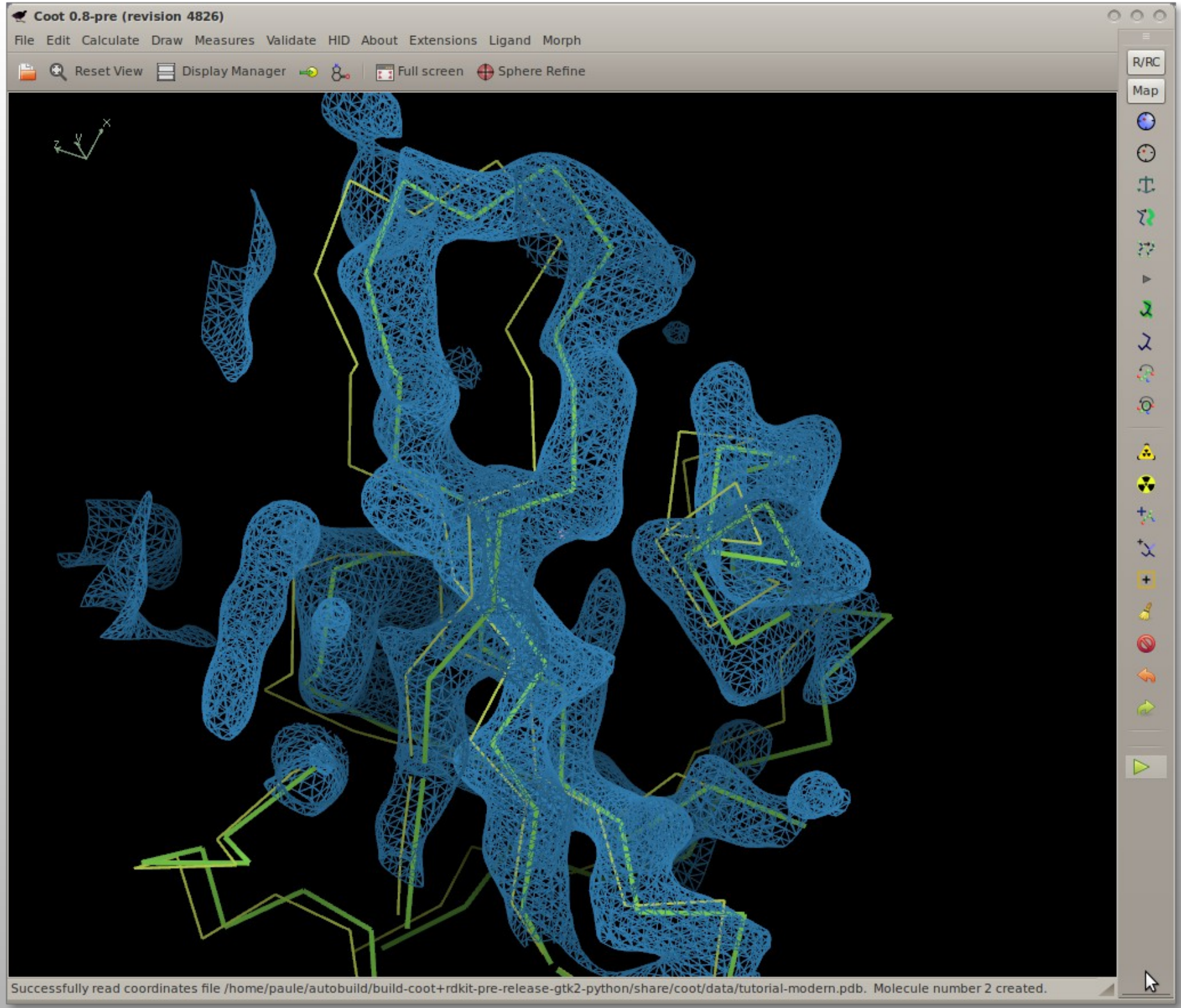
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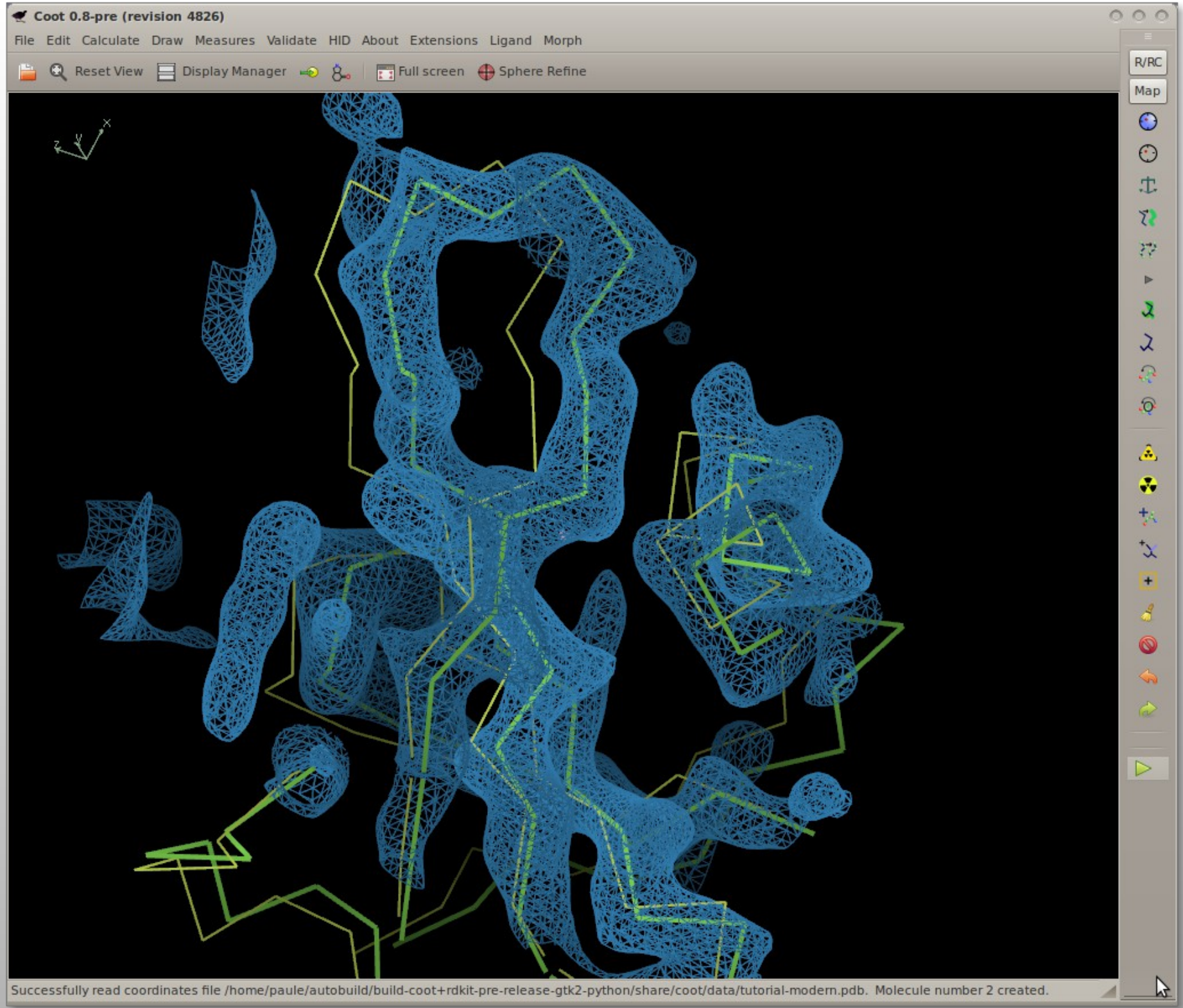
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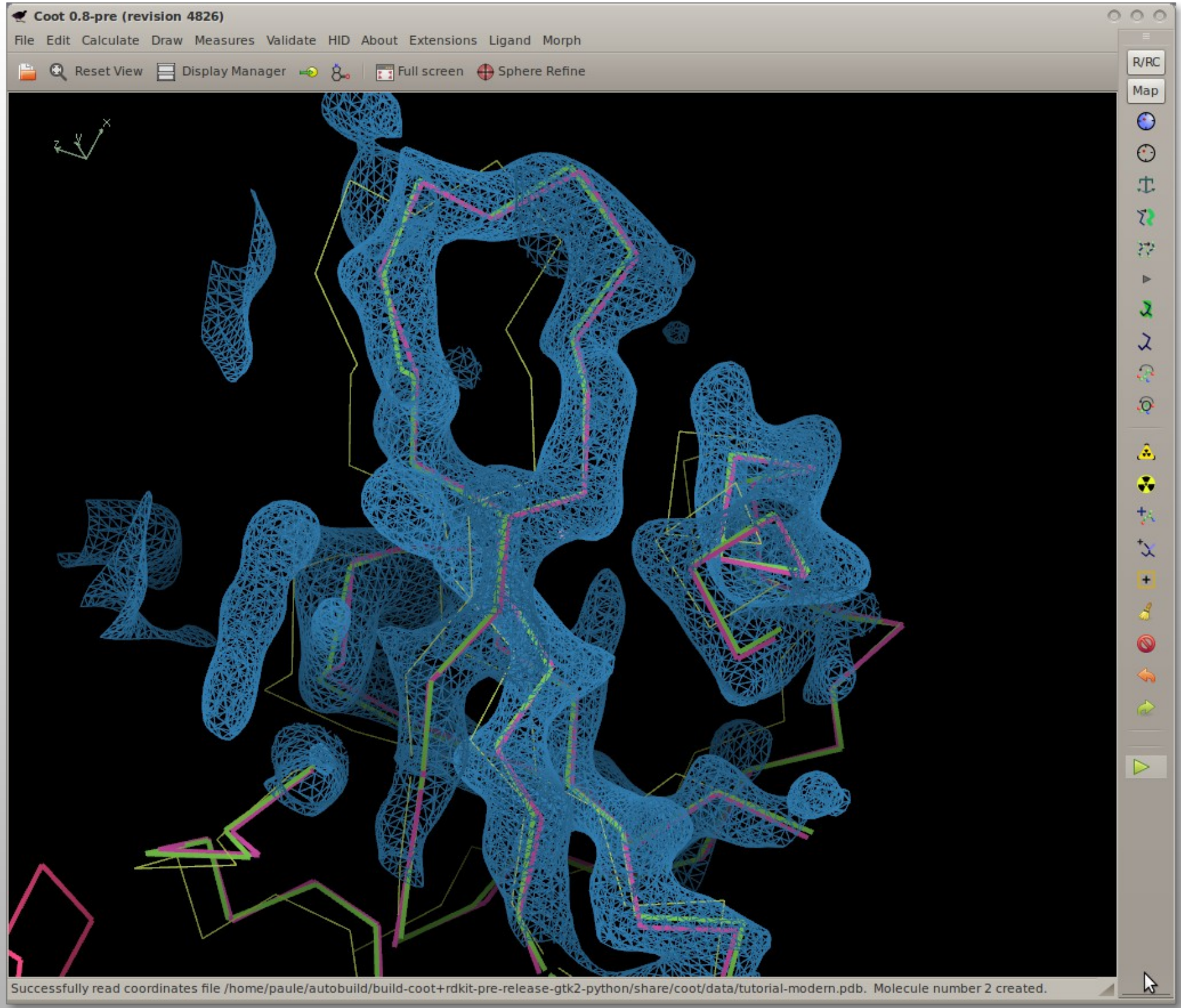
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Map
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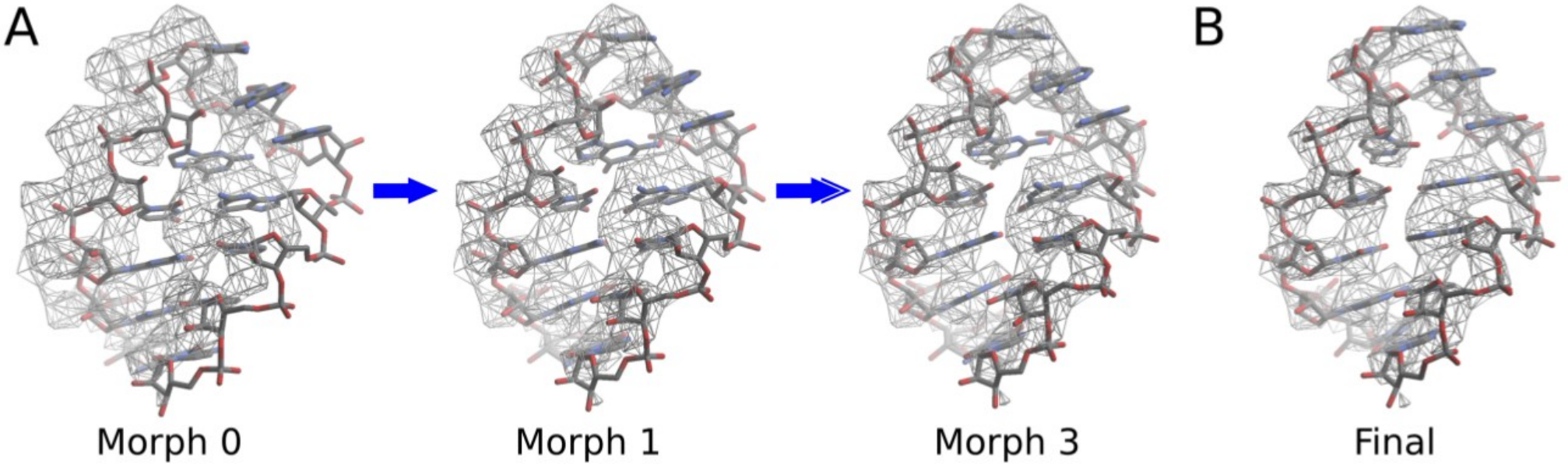
Model Morphing



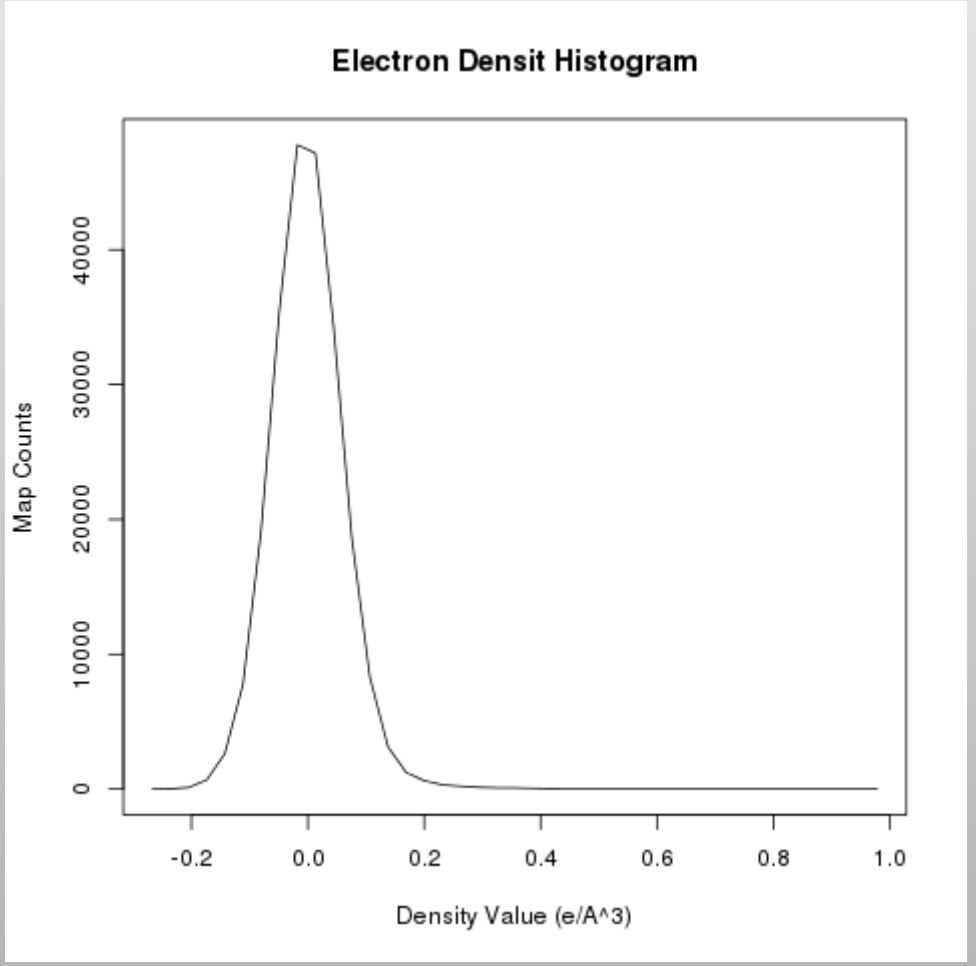
Helix Fitting

- 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

Helix Fitting

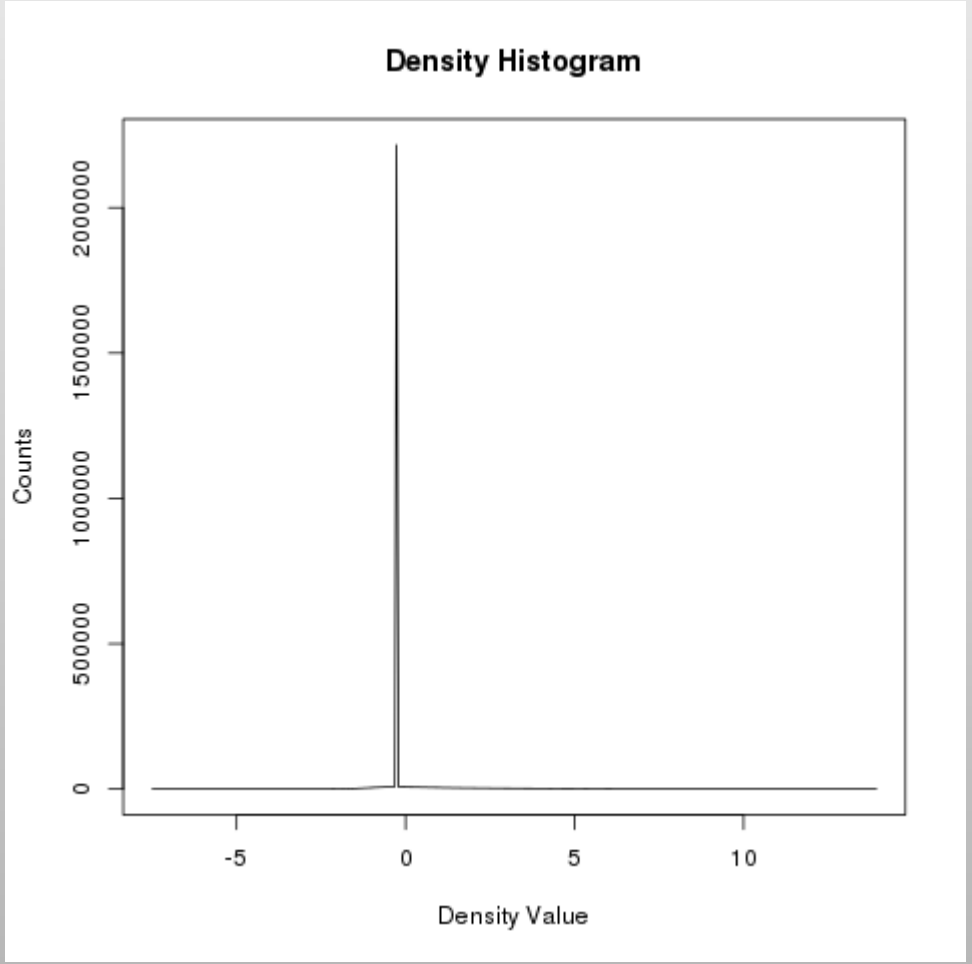
- The distribution of electron density is quite unlike that of x-ray maps

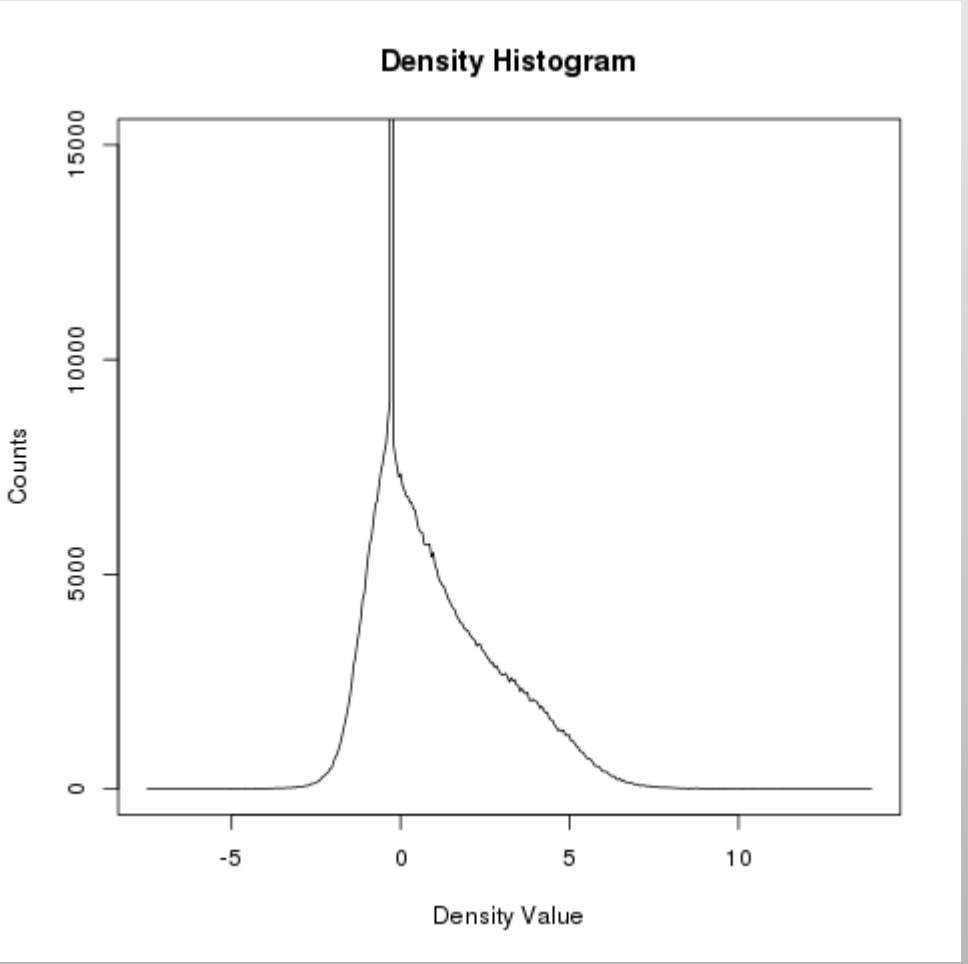
Typical Density Histogram from an X-ray map



Helix Fitting

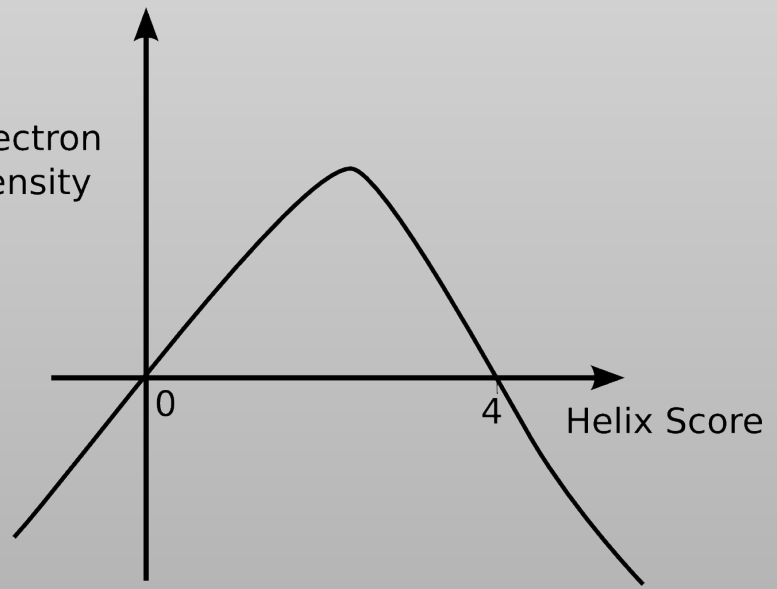
- The distribution of electron density is quite unlike that of x-ray maps

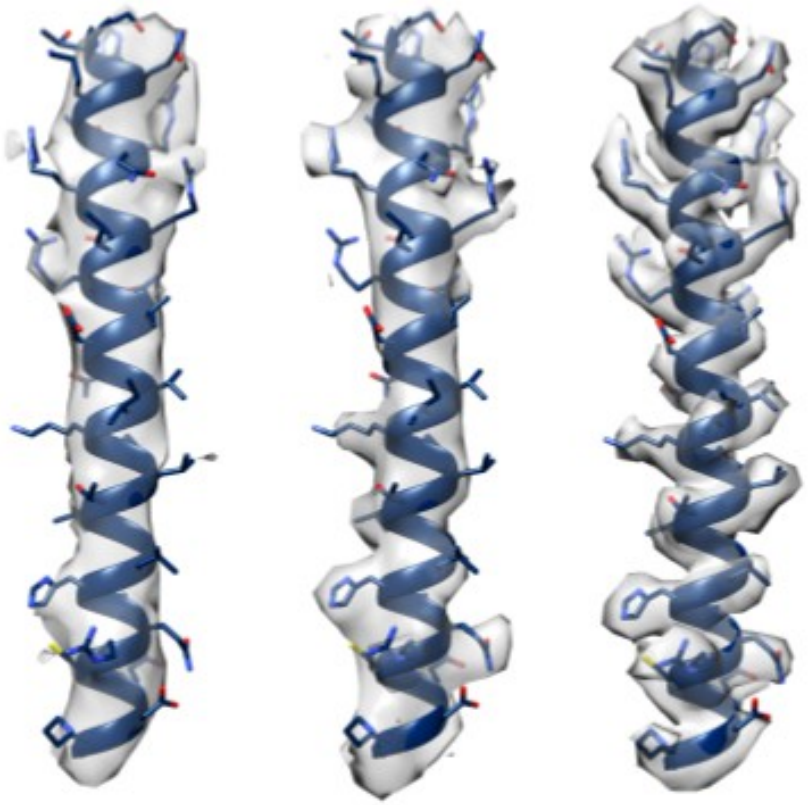




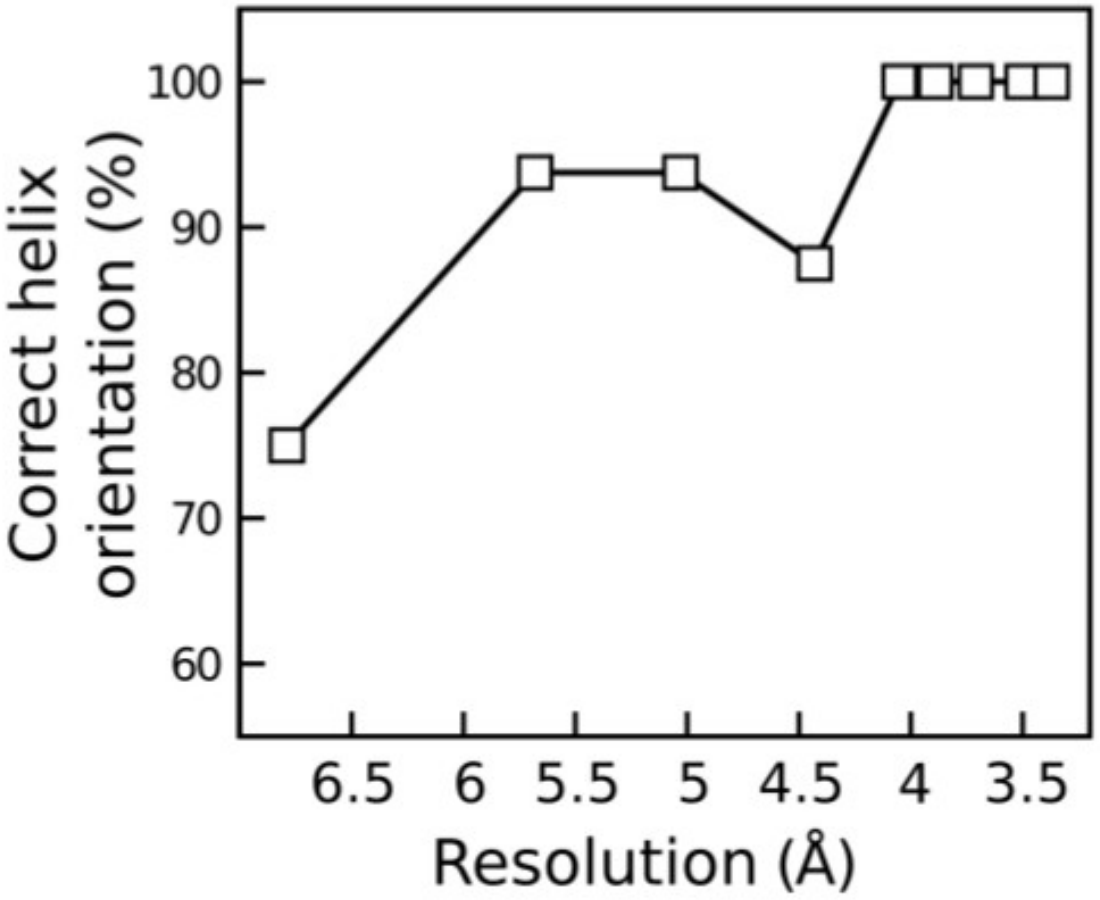
Helix Fitting

- 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





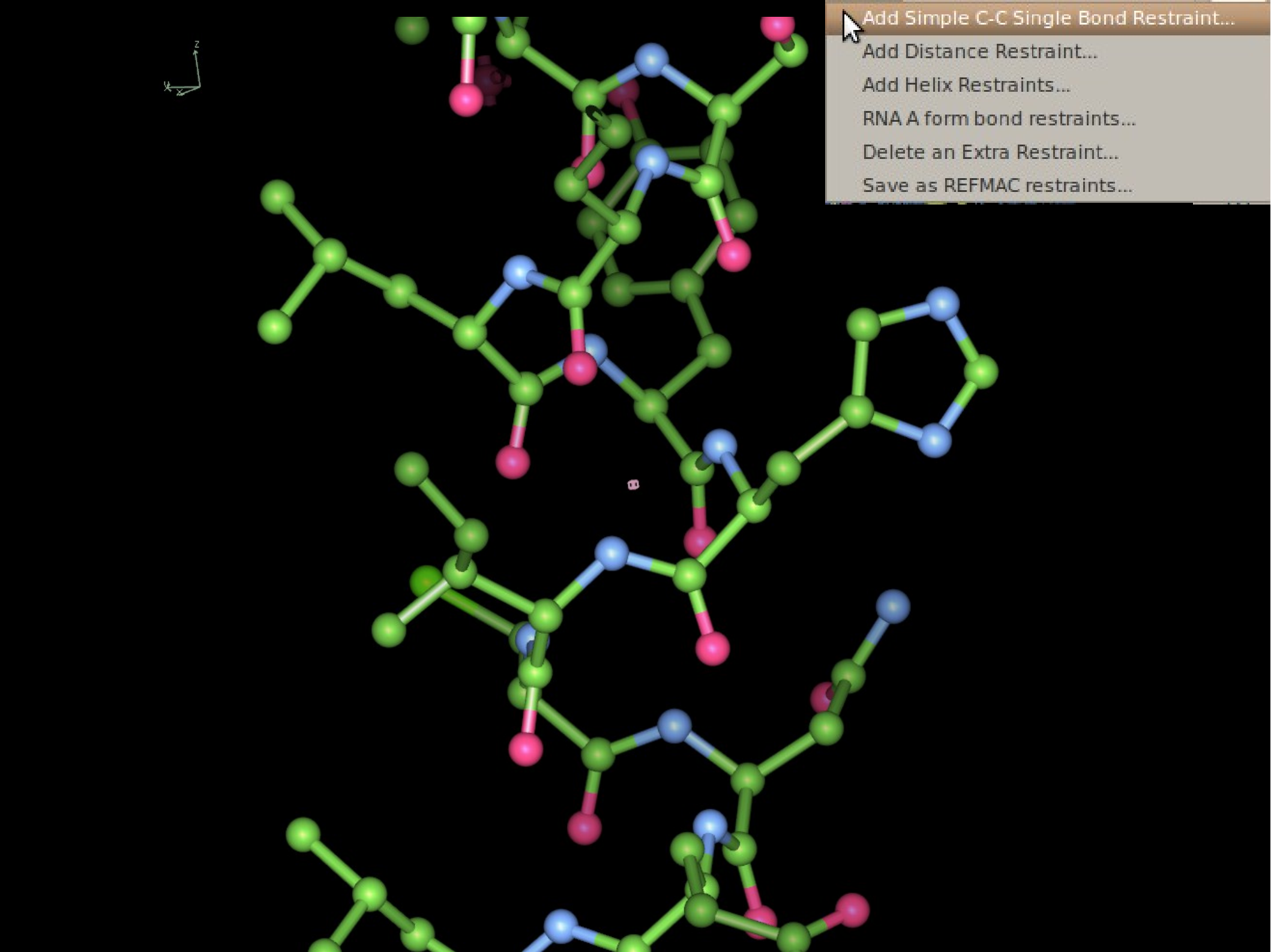
6.8Å 5.0Å 3.2Å

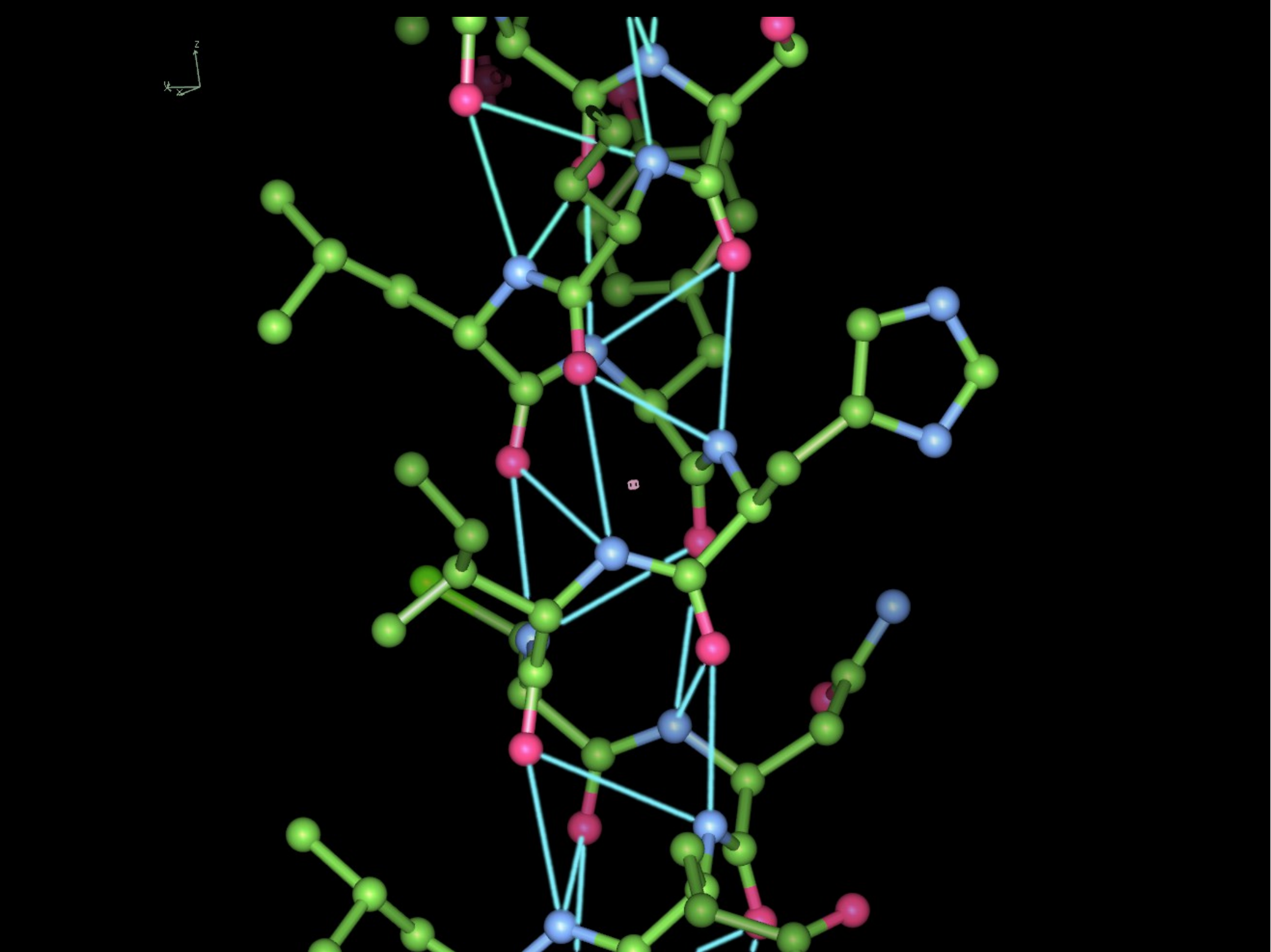

Correct helix orientation (%)
100
90
80
70
60
6.5
6
5.5
5
4.5
4
3.5
Resolution (Å)

Additional Restraints

Restraints Editing in *Coot*

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


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...



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
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
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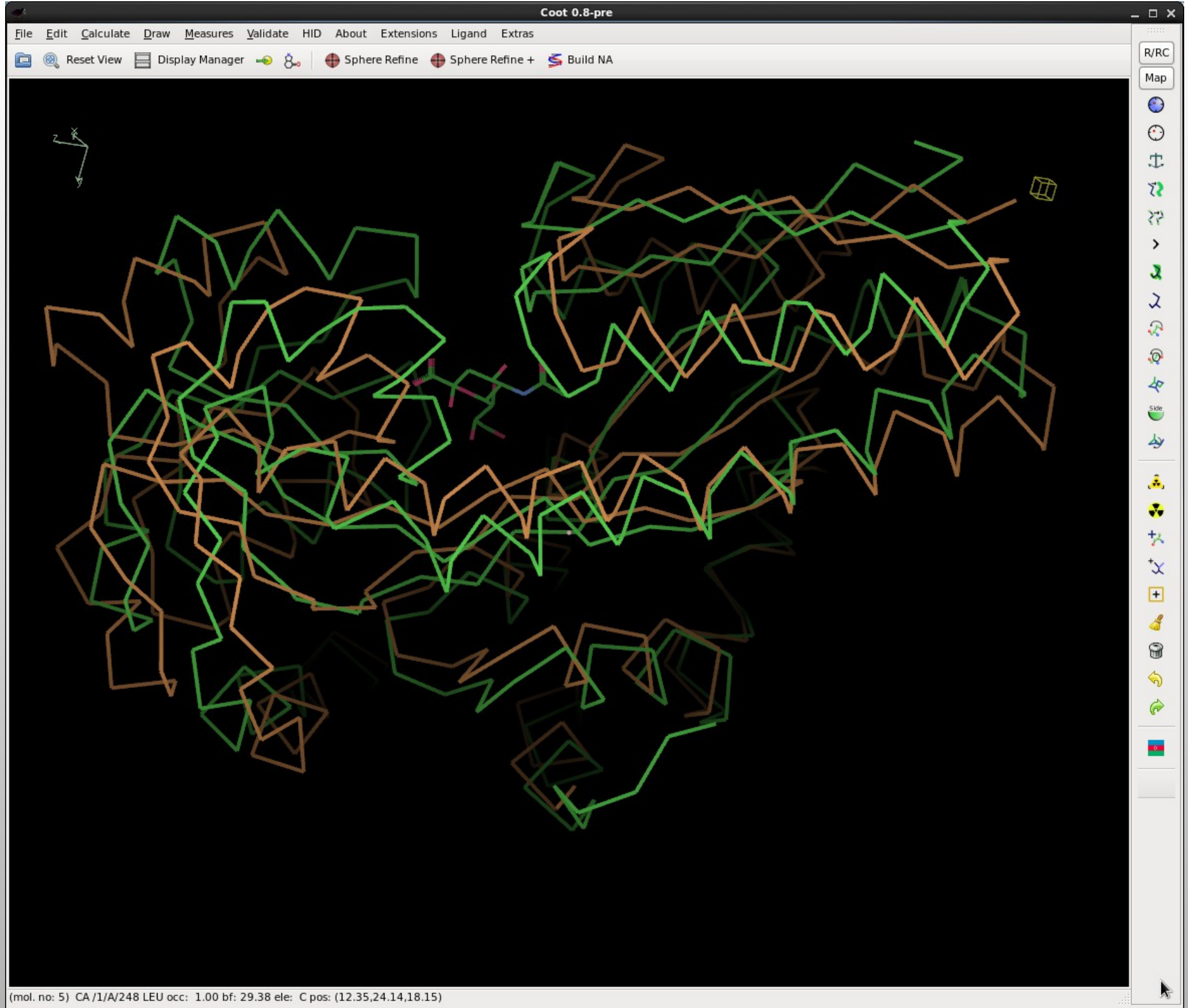
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R/RC
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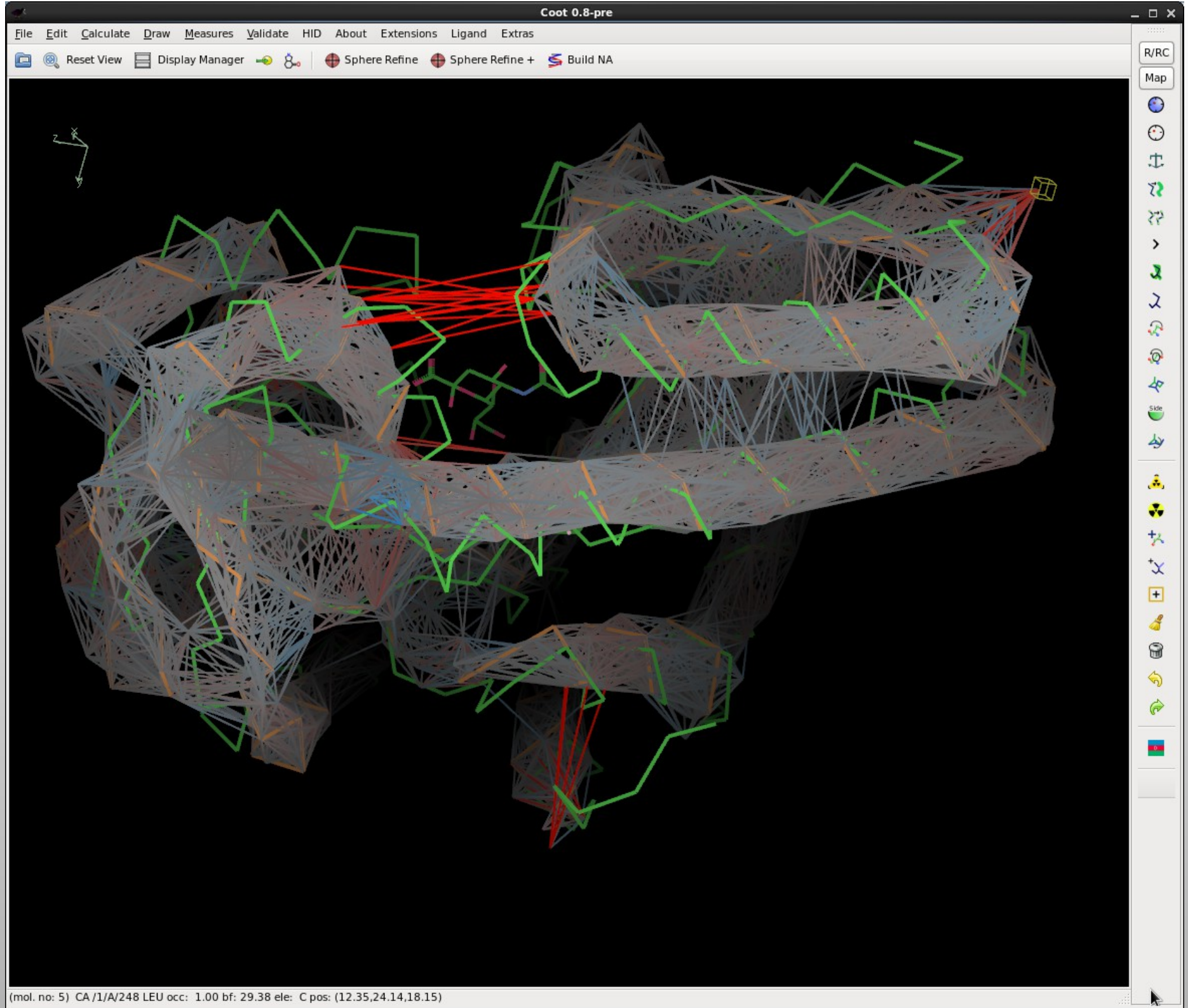
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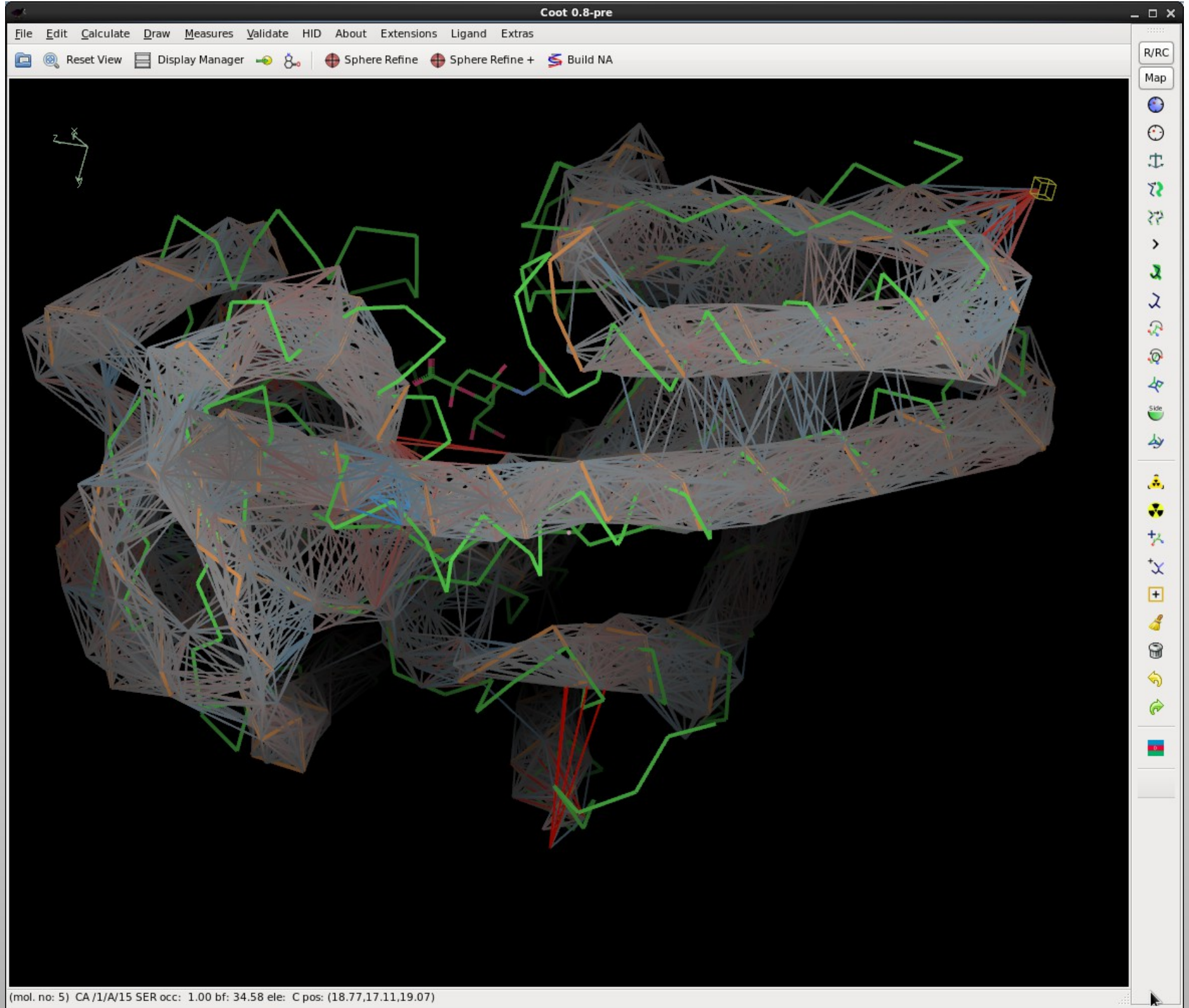
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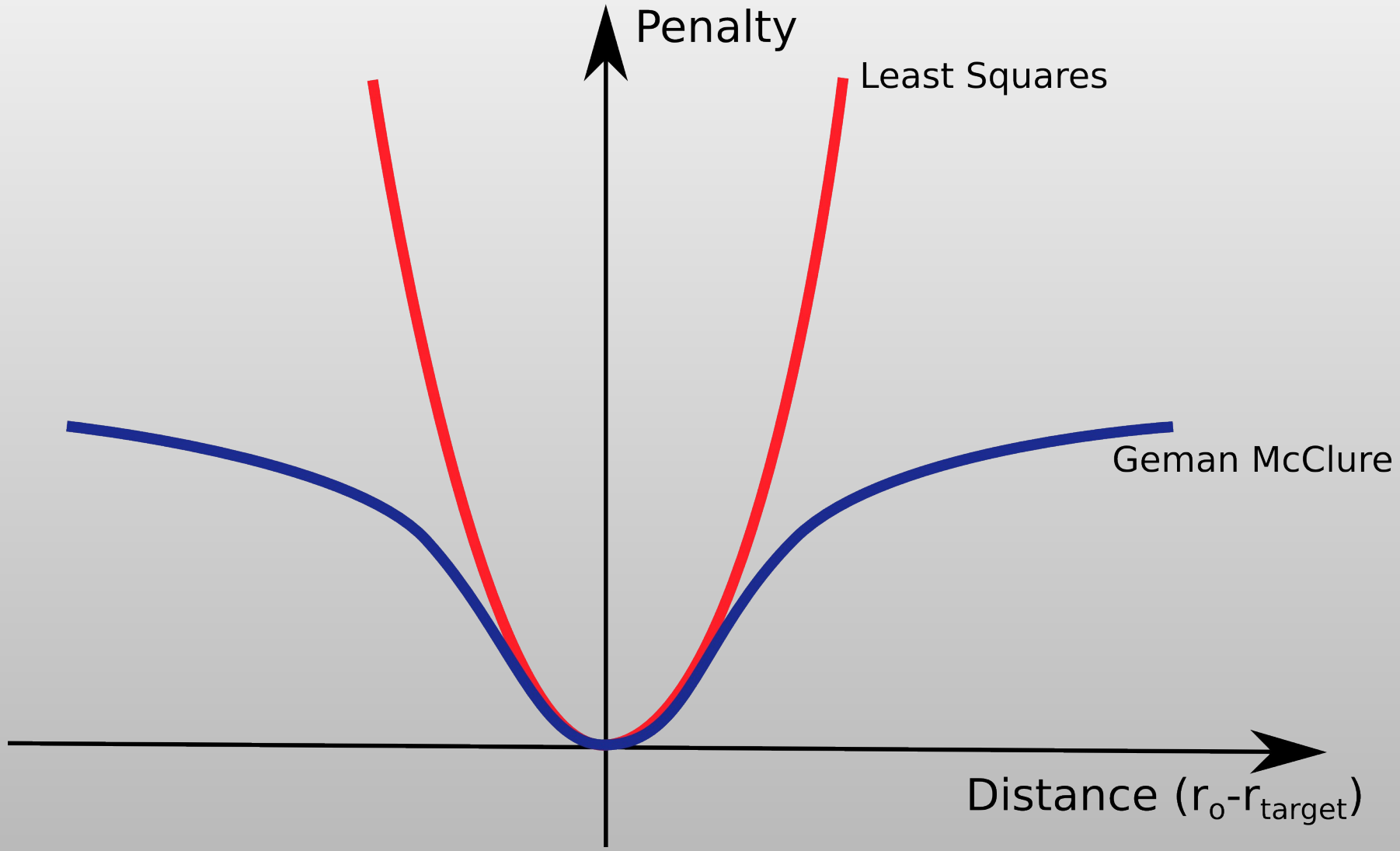
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
(mol. no: 5) CA /1/A/15 SER occ: 1.00 bf: 34.58 ele: C pos: (18.77,17.11,19.07)

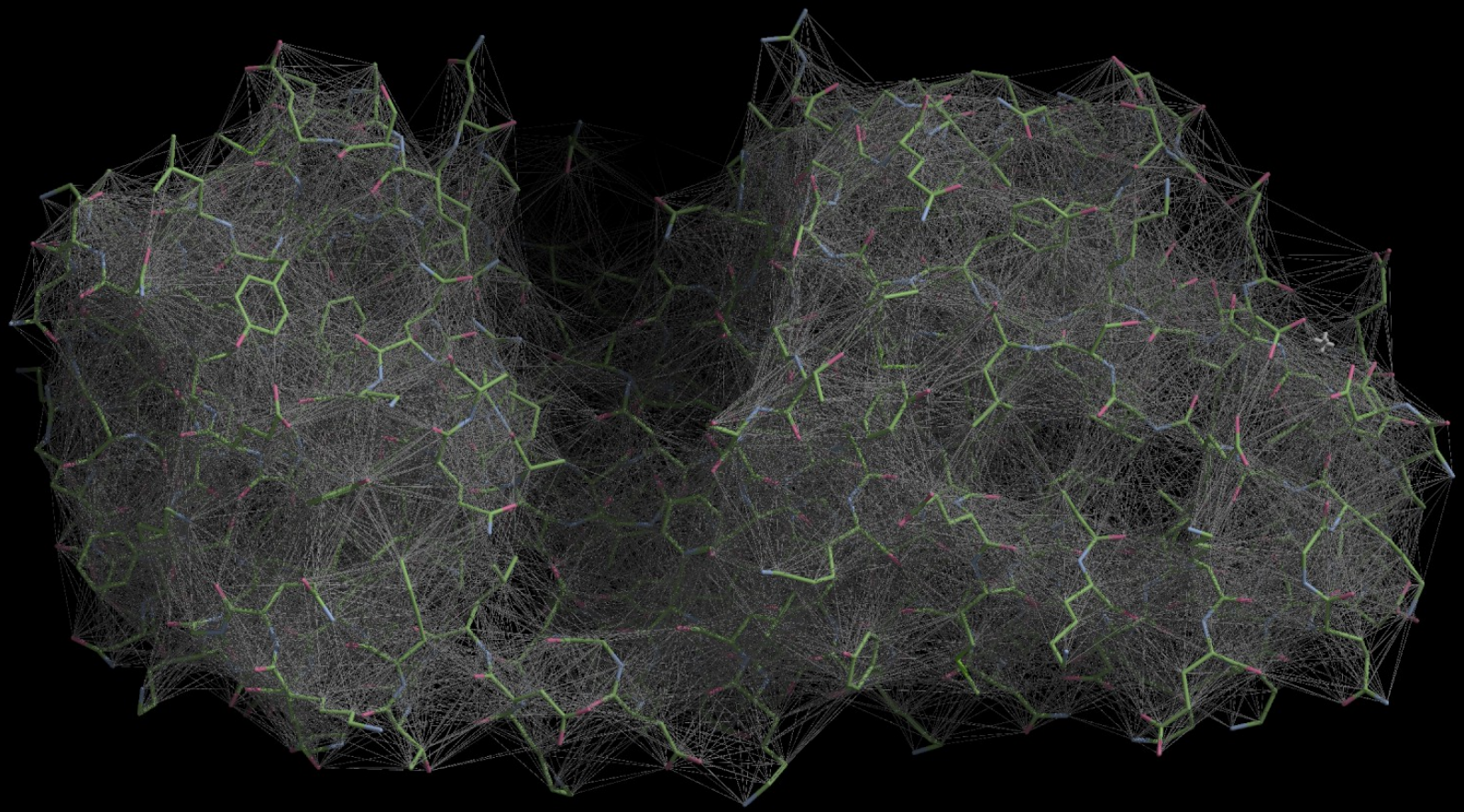
ProSMART integration

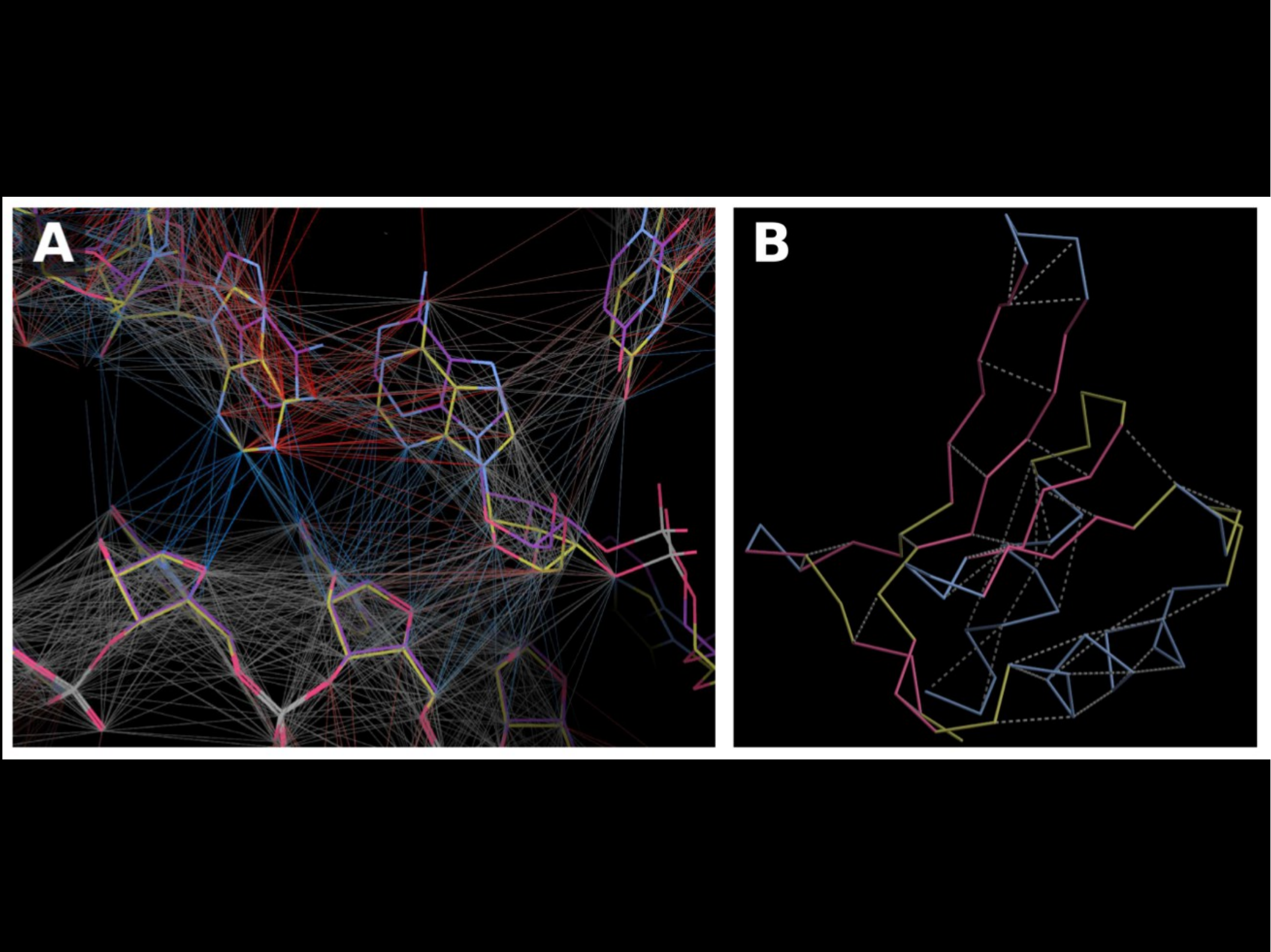
- ProSMART generates distance restraints from homologous structures
  - to be applied to current model for refinement
  - now available in *Coot*

Modified Target Function

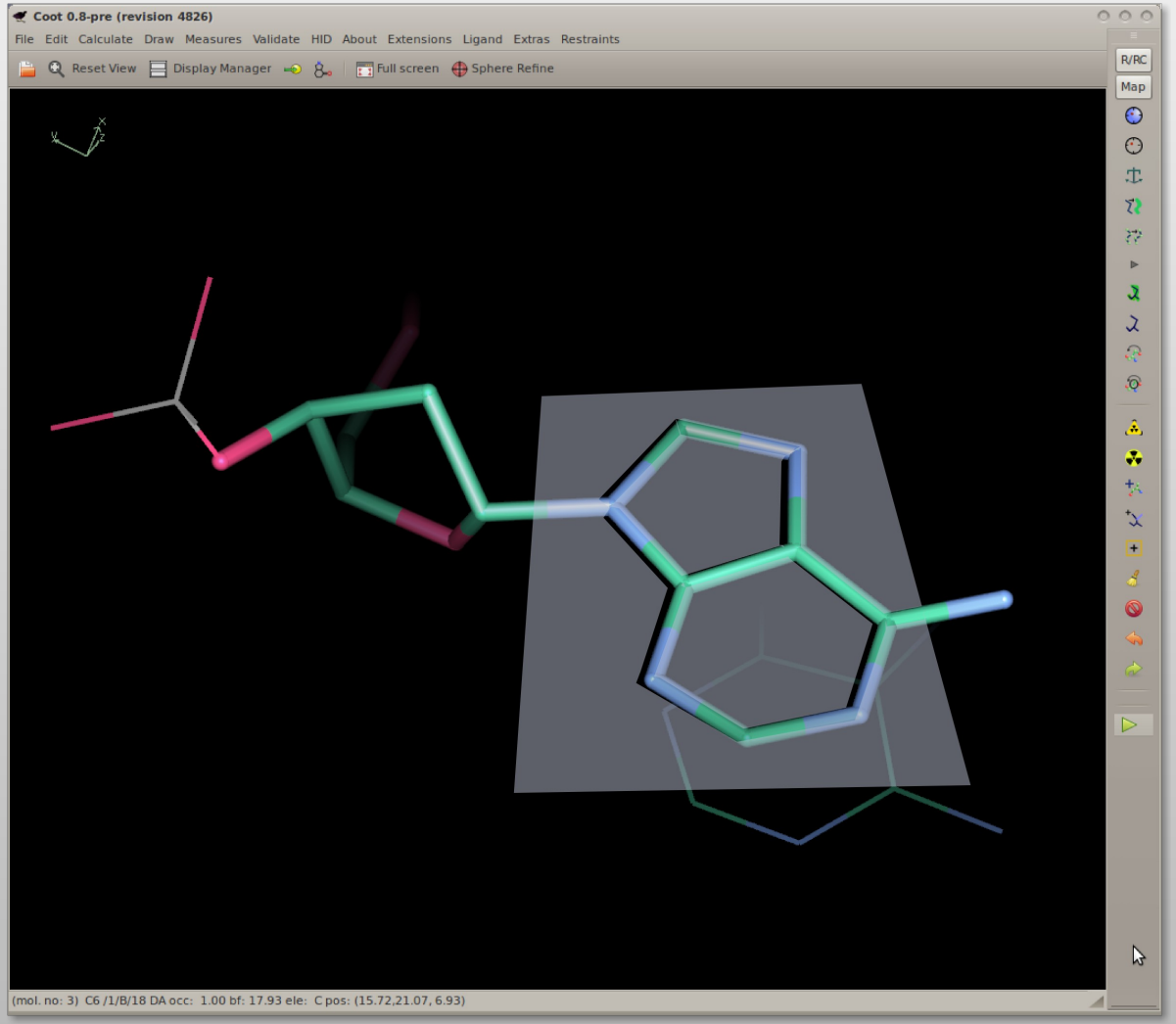


ProSMART Restraints



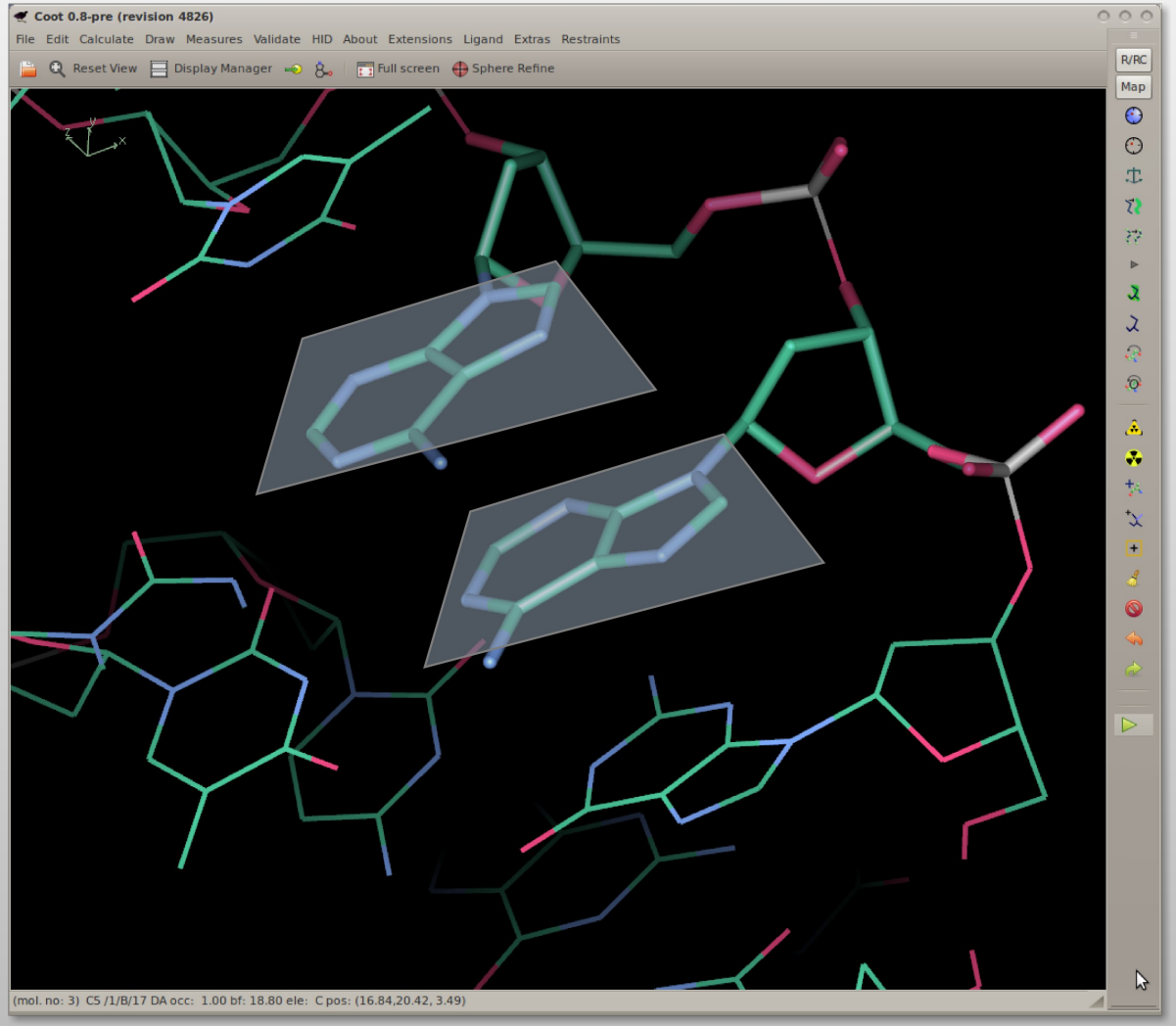

A
B

Plane Restraints



Derivativaties are an eigenvector scaled by out-of-plane distance

Parallel Planes Restraints



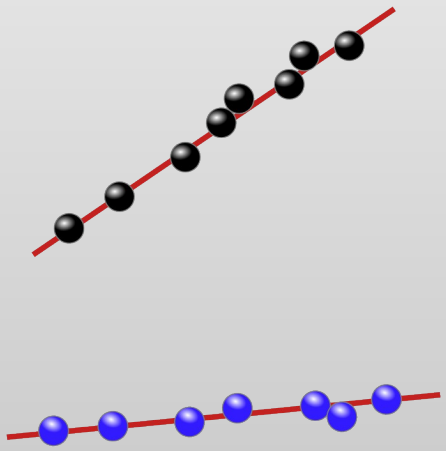
$$S = (a_1 - a_2)^2 + (b_1 - b_2)^2 + (c_1 - c_2)^2$$

Not easy to use in Coot

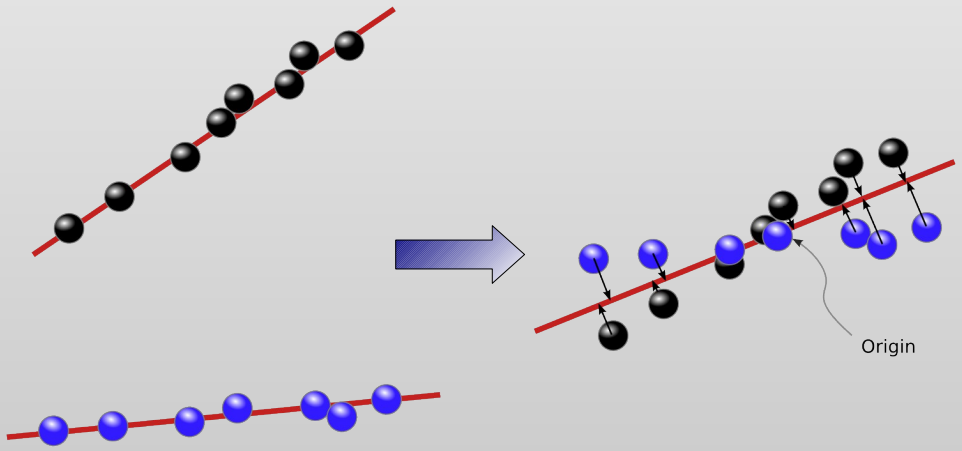
Parallel Planes Restraints

- Also, we have considered parallel-planes distance restraints
  - More tricky still to implement
  - Not implemented yet (not in *Coot*, anyway)

Parallel Planes Restraints

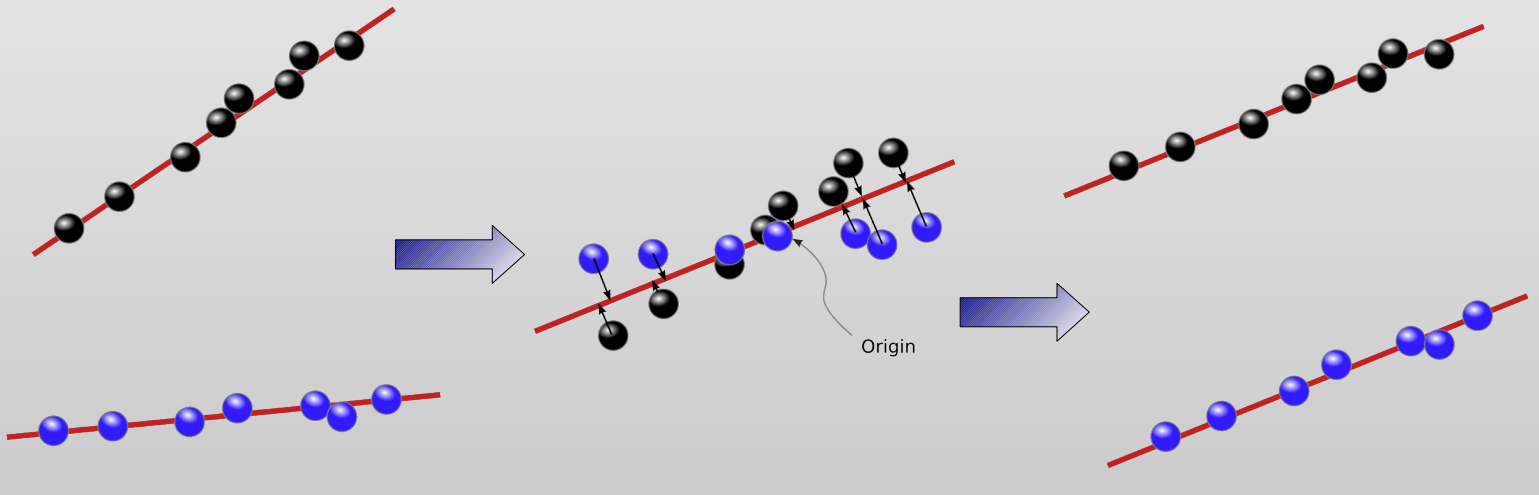


Parallel Plane Restraints



Shift to Origin

Parallel Planes Restraints

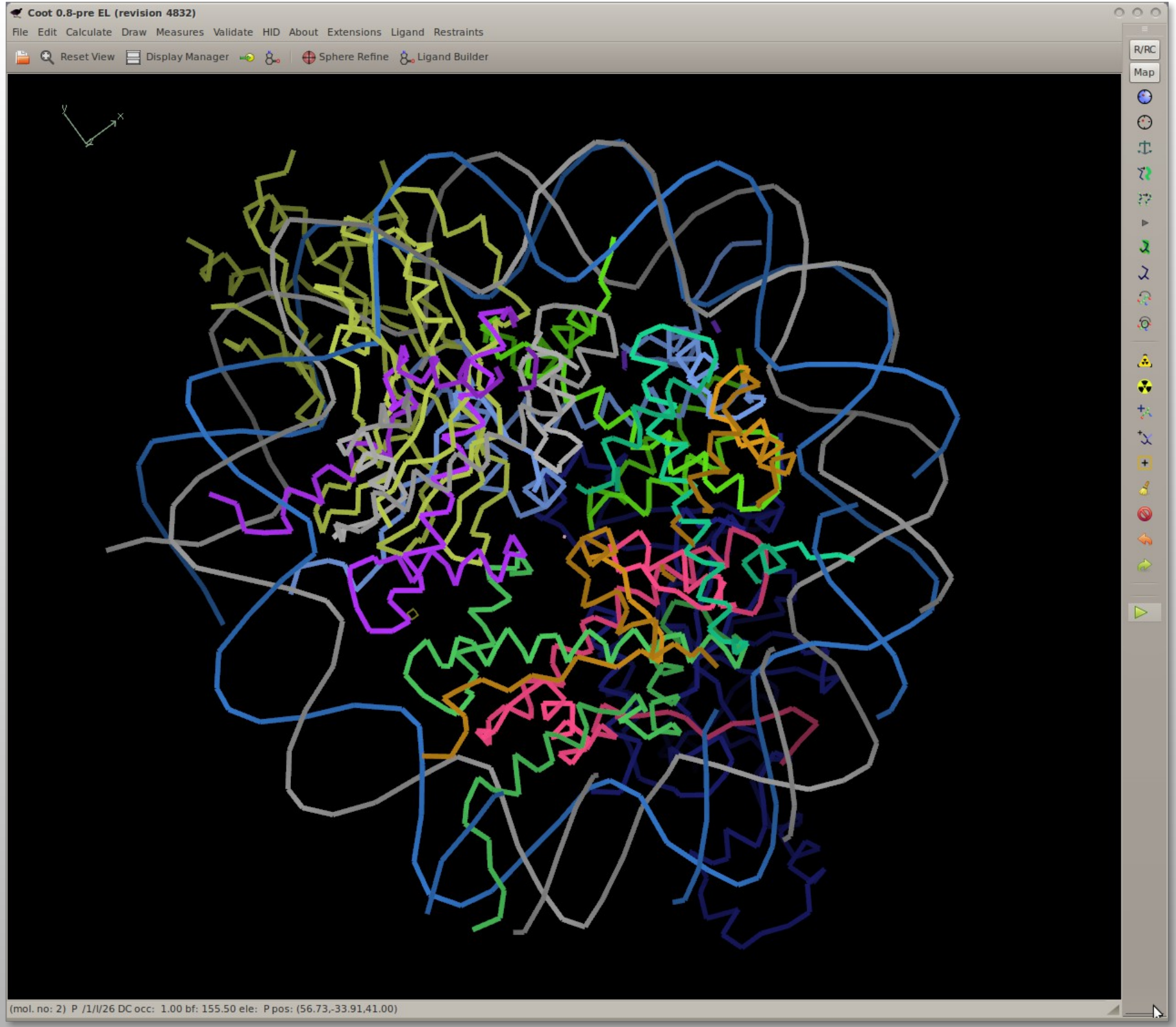


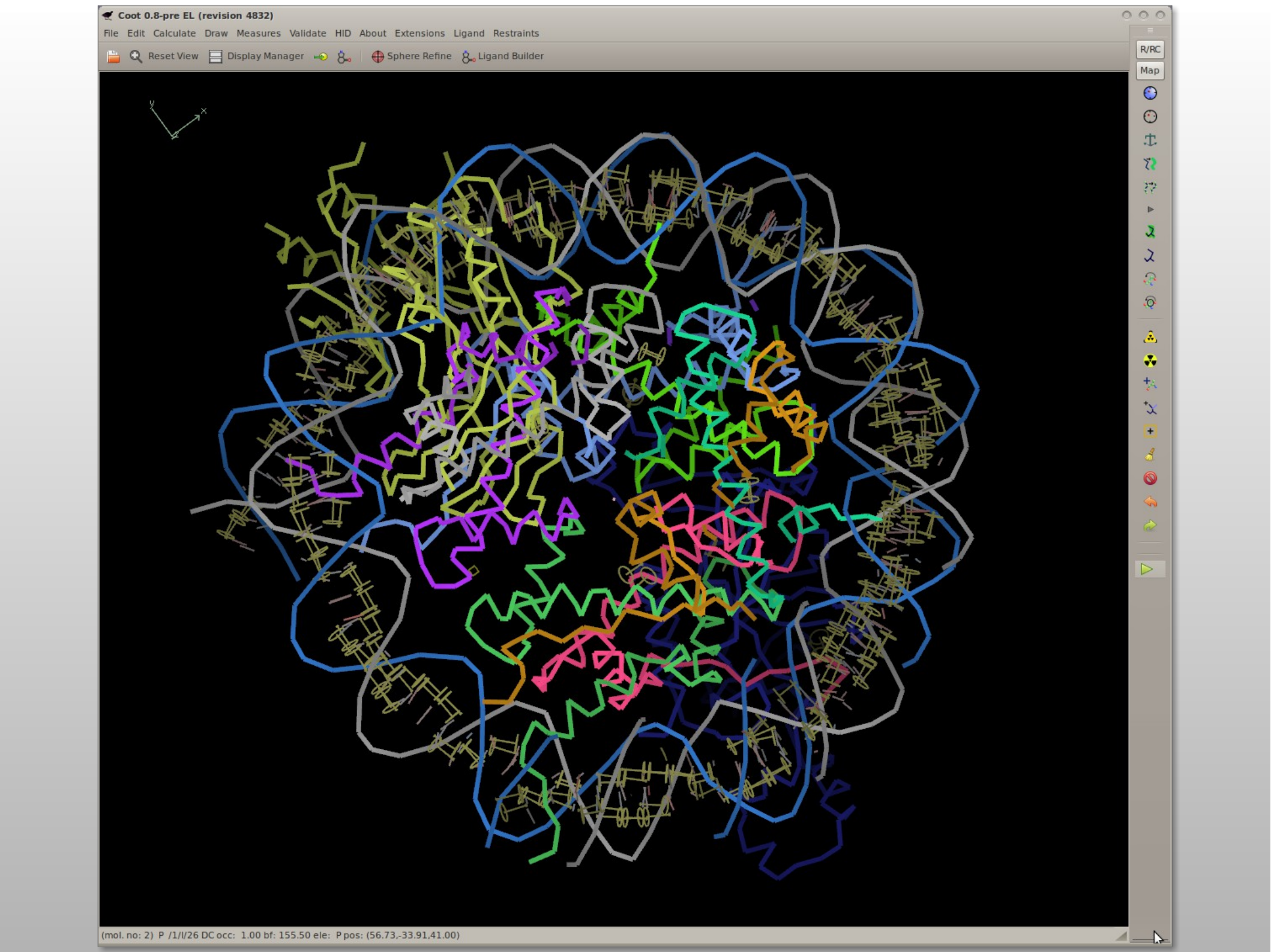
Shift to Origin

Move Back to Molecule

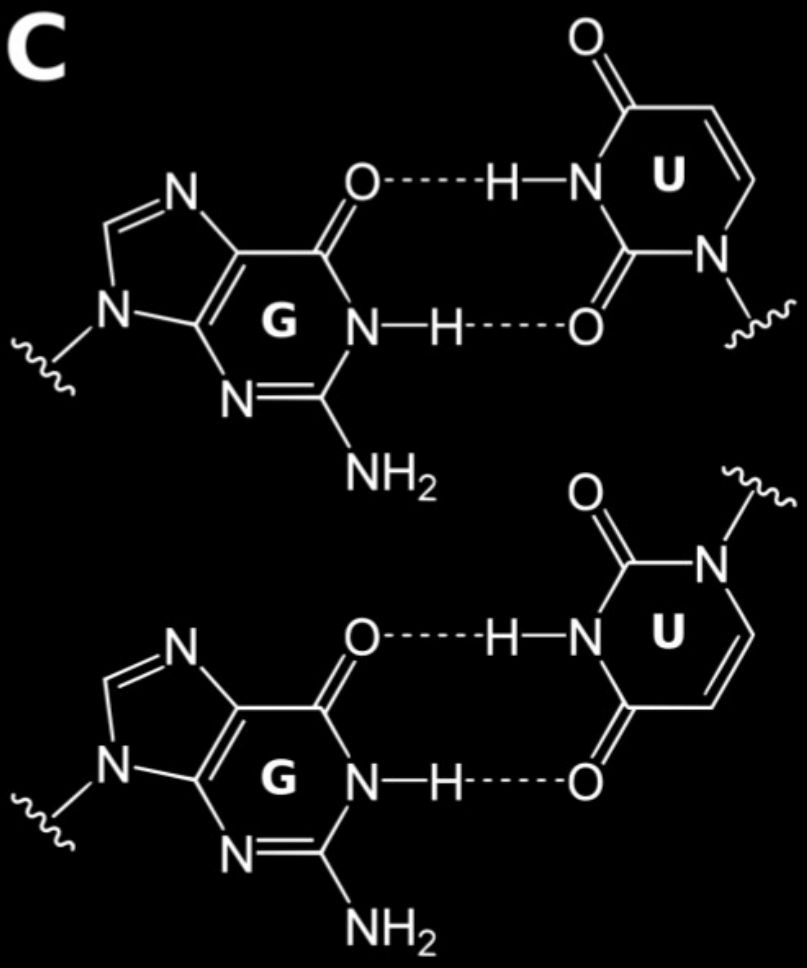
Automatic Generation of Base Pairing and Stacking Restraints

- Fei Long's `libg_d`
  - 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

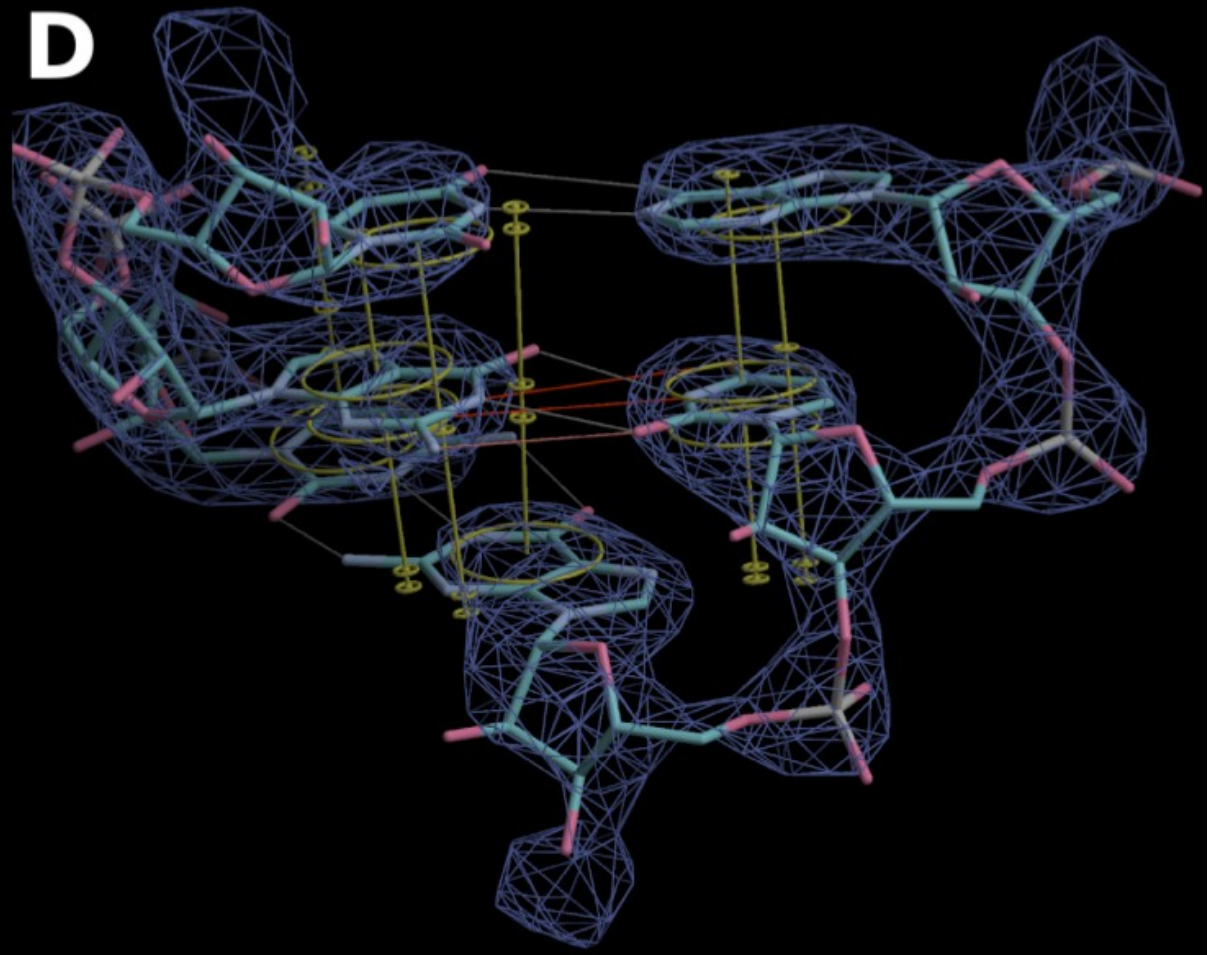

Coot 0.8-pre EL (revision 4832)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints
Reset View
Display Manager
Sphere Refine
Ligand Builder
R/RC
Map
(mol. no: 2) P /1/I/26 DC occ: 1.00 bf: 155.50 ele: P pos: (56.73,-33.91,41.00)


Coot 0.8-pre EL (revision 4832)
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Restraints
Reset View
Display Manager
Sphere Refine
Ligand Builder
R/RC
Map
(mol. no: 2) P /1/I/26 DC occ: 1.00 bf: 155.50 ele: P pos: (56.73,-33.91,41.00)

Libg restraints



(Watson Crick and)
Wobble, Reverse Wobble



Representation in Coot

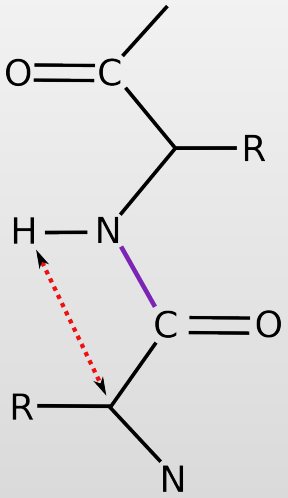
cis-Peptides

- What is a cis-peptide?
- Peptide restraints in Coot 2004-2015

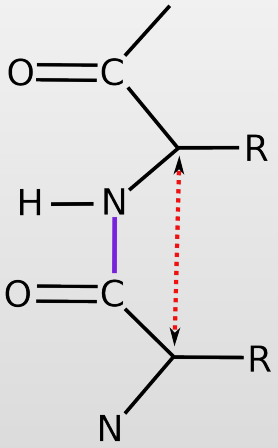
cis-Peptides

- 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

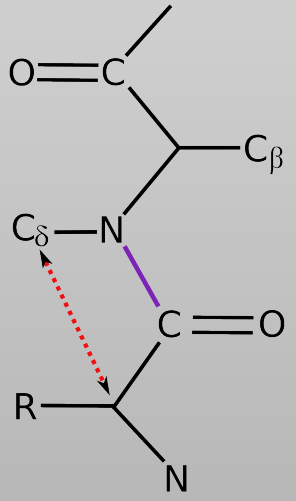
cis-Peptides



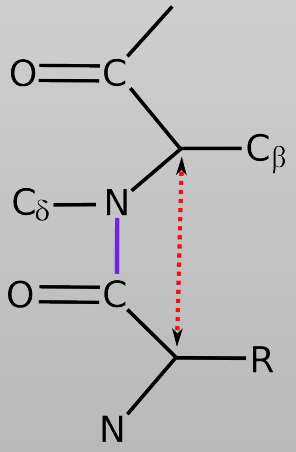
trans-peptide



cis-peptide

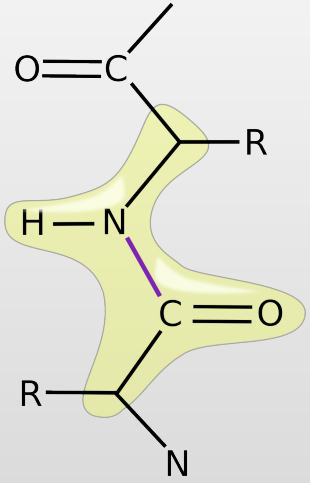


PRO *trans-peptide*

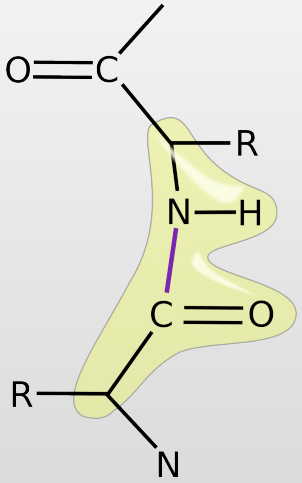


PRO *cis-peptide*

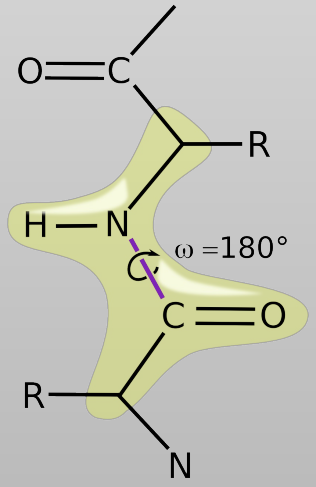
cis-Peptides



trans-peptide
with plane restraints

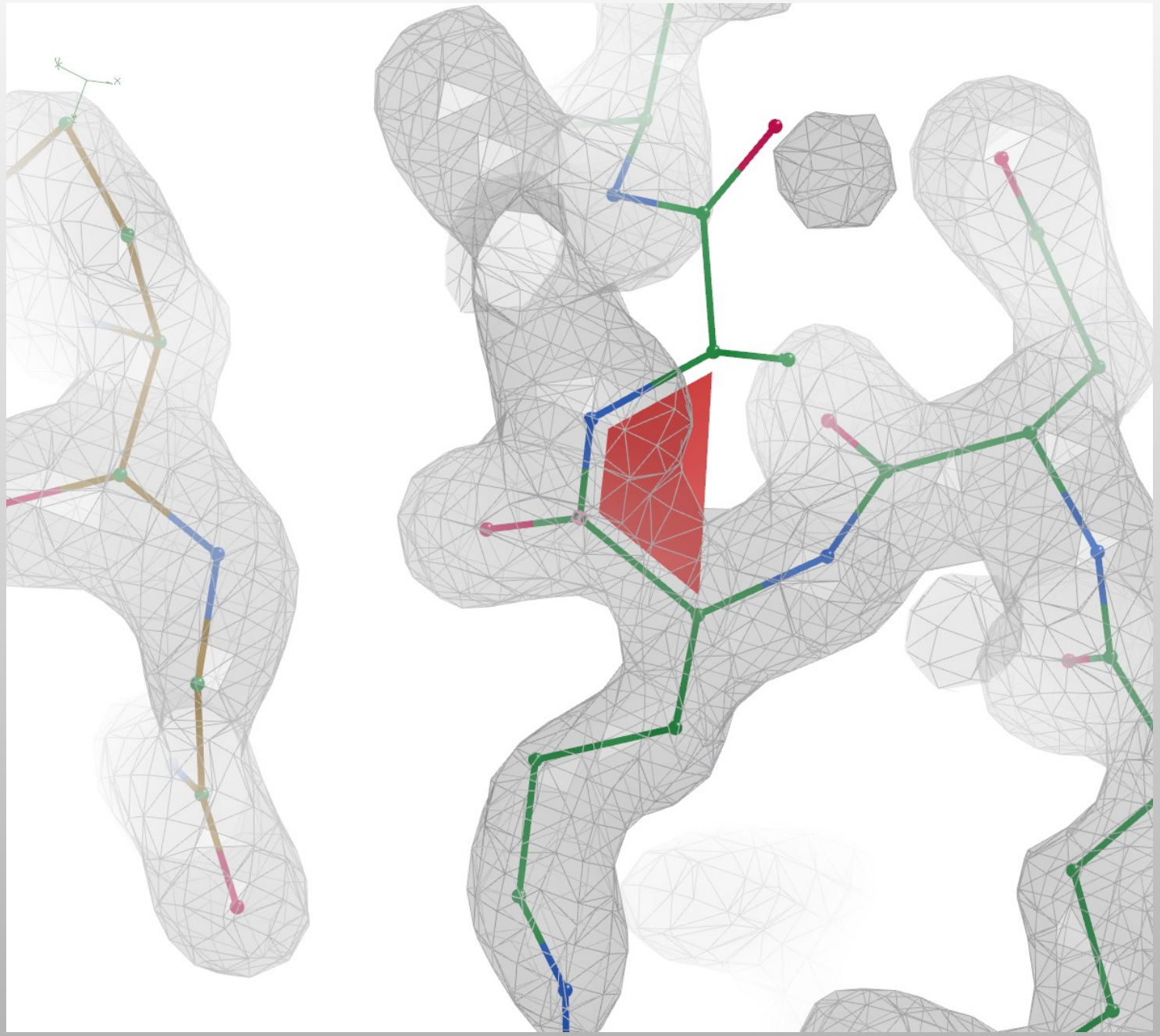


cis-peptide
with plane restraints

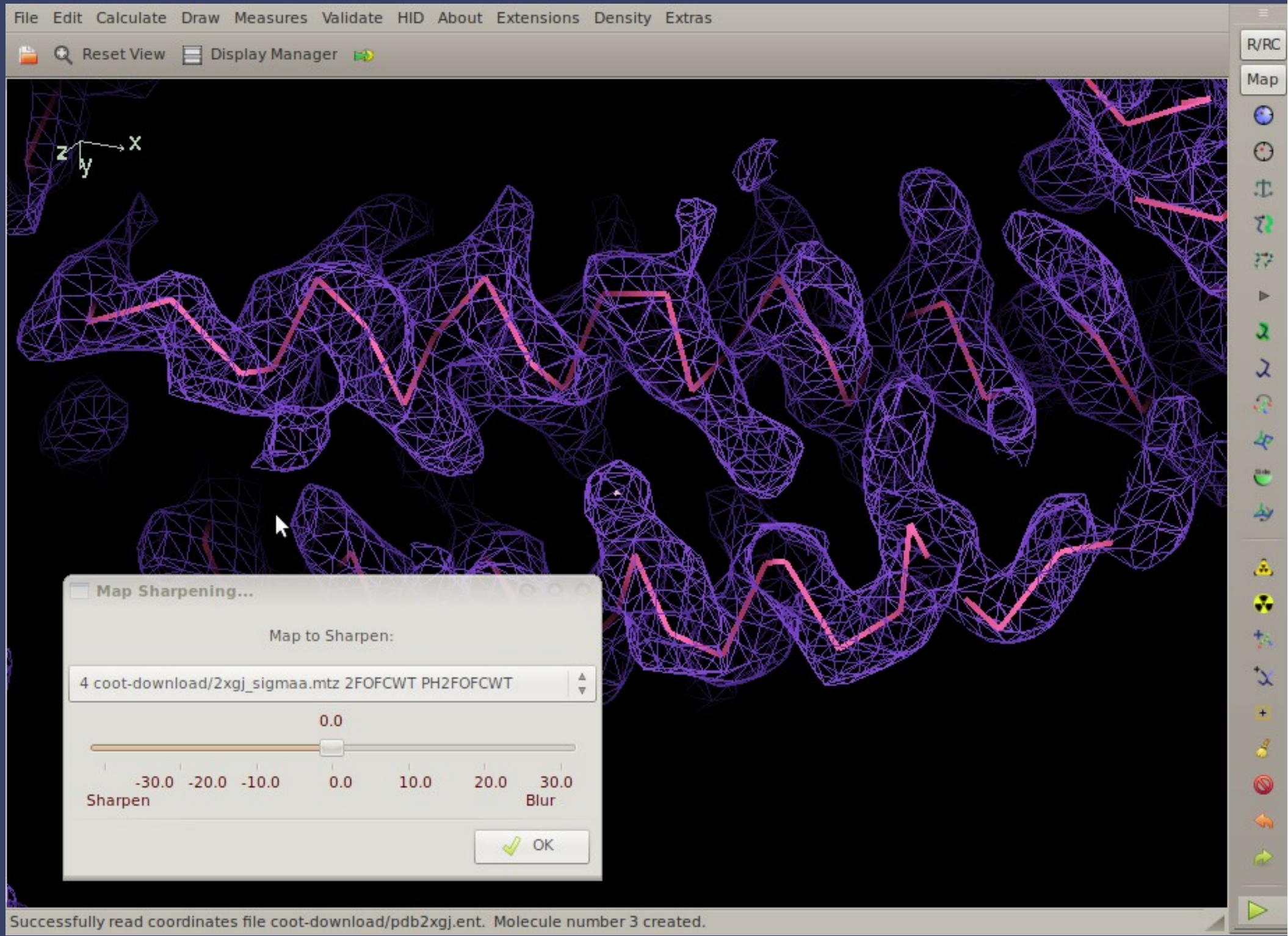


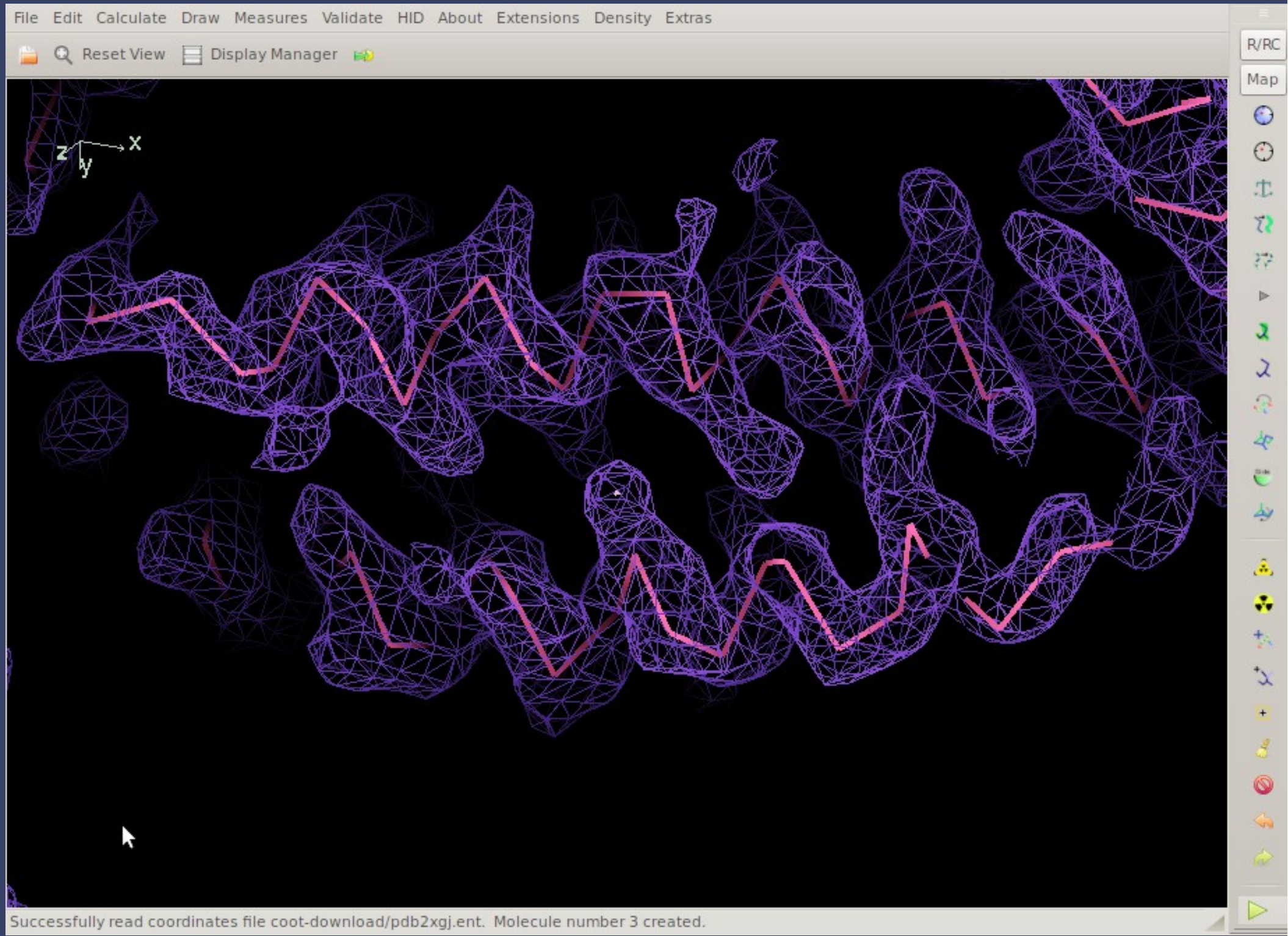
trans-peptide
with plane and trans restraints

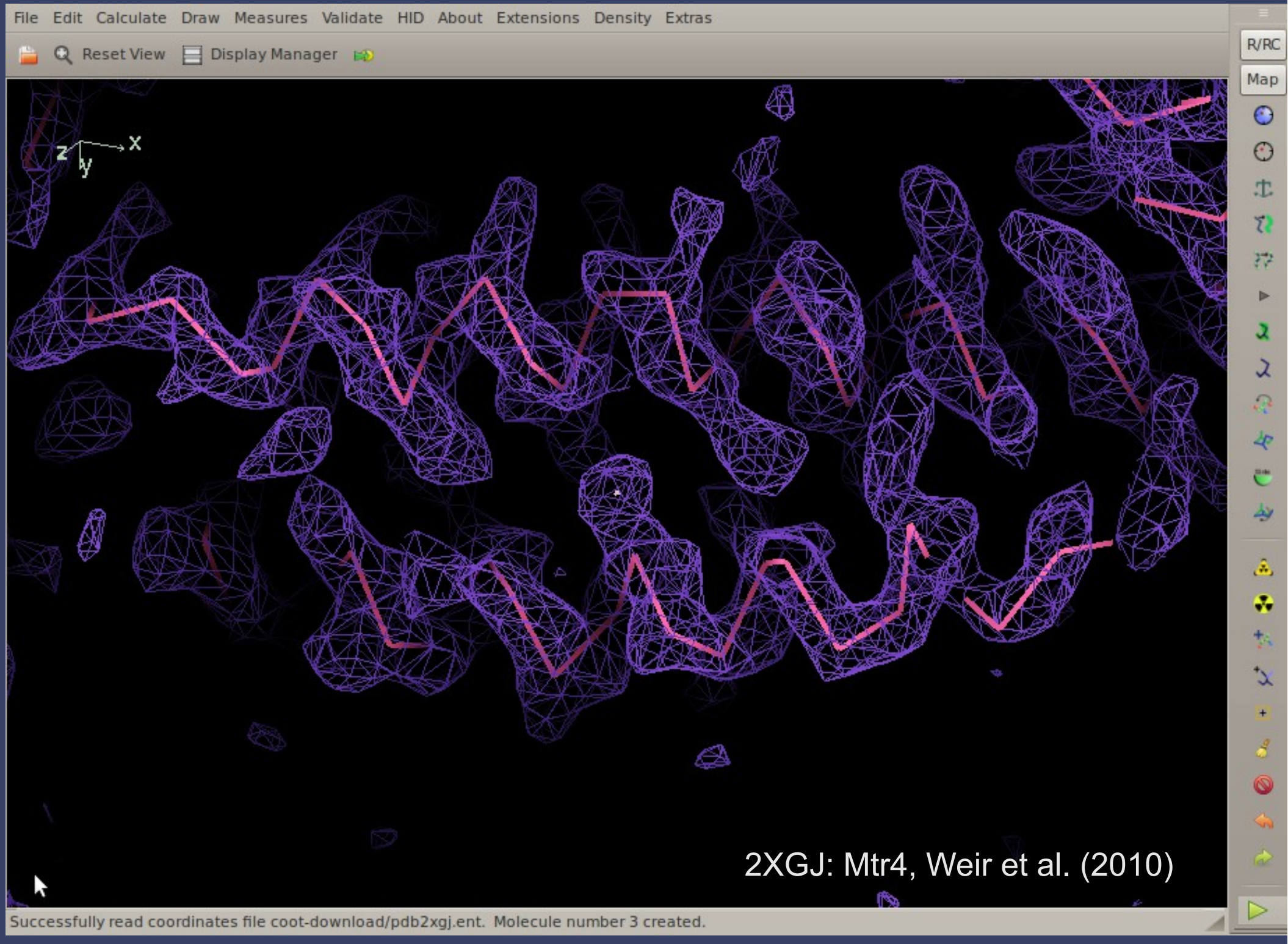
cis-peptide Representation



Interactive Map Sharpening


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 Resources

- Coot Mailing list on JiscMail
- Oliver Clarke's Dropbox

Acknowledgements

- LMB:
  - Garib Murshudov, Rob Nicholls, Fei Long,
  - Alexey Amunts, Alan Brown
- Kevin Cowan, Bernhard Lohkamp
- Libraries & Dictionaries:
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  - Alexei Vagin
  - Eugene Krissinel