Tools for Cryo-EM Map Fitting

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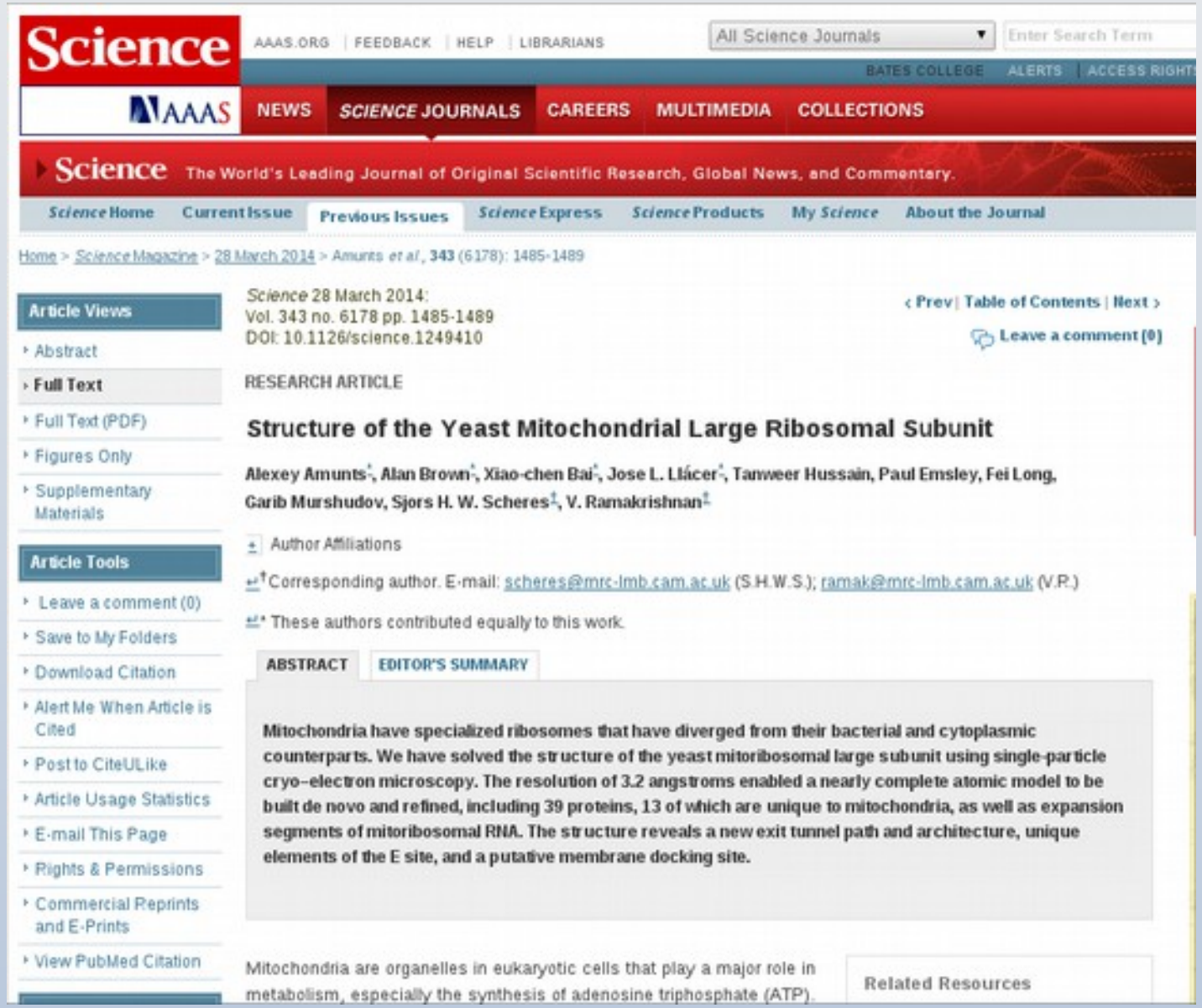
Nov 2018

Cryo-EM Model-building

- Typically, we need to move more atoms that one does for crystallography
- the maps are lower resolution and the starting model is further from where you want them to be – usually systematically so
- addressing these needs has been the focus of my work extending/changing *Coot* for cryo-EM

Yeast Mitochondrial Large Ribosomal Subunit



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RESEARCH ARTICLE

Structure of the Yeast Mitochondrial Large Ribosomal Subunit

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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
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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*.

Science

REPORTS

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Architecture of eukaryotic mRNA 3′-end processing machinery

Ana Casañal,[1*] Ananthanarayanan Kumar,[1*] Chris H. Hill,[1] Ashley D. Easter,[1] Paul Emsley,[1] Gianluca Degliesposti,[1] Yuliya Gordiyenko,[1,2] Balaji Santhanam,[1] Jana Wolf,[1] Katrin Wiederhold,[1] Gillian L. Dornan,[1] Mark Skehel,[1] Carol V. Robinson,[2] Lori A. Passmore[1†]

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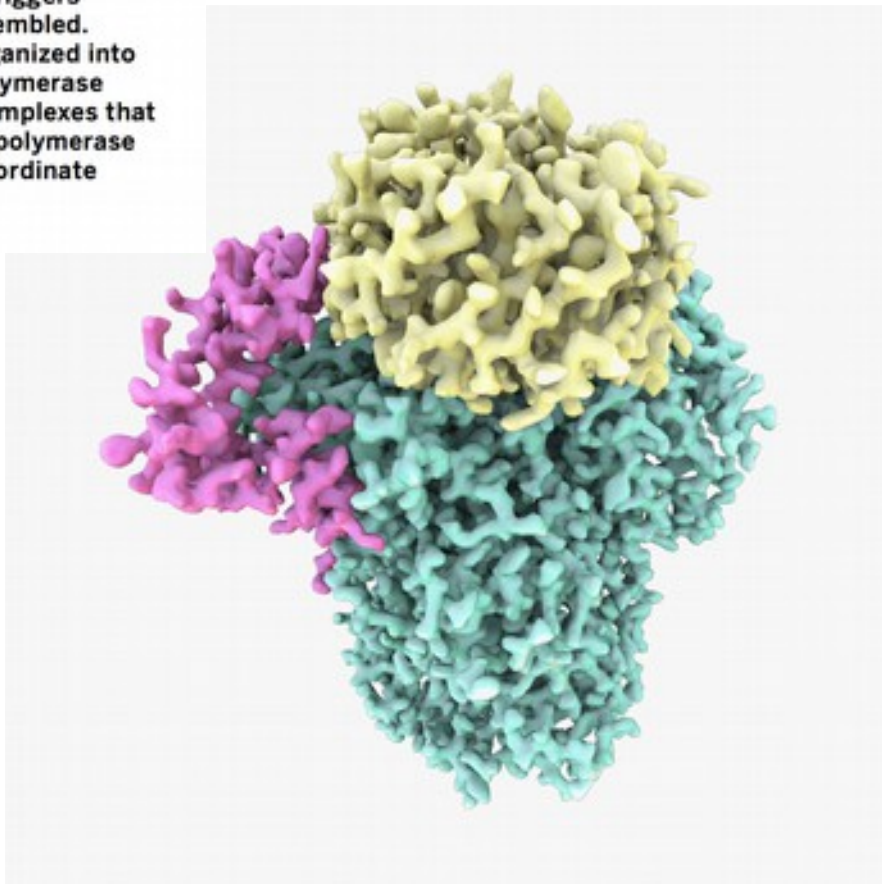
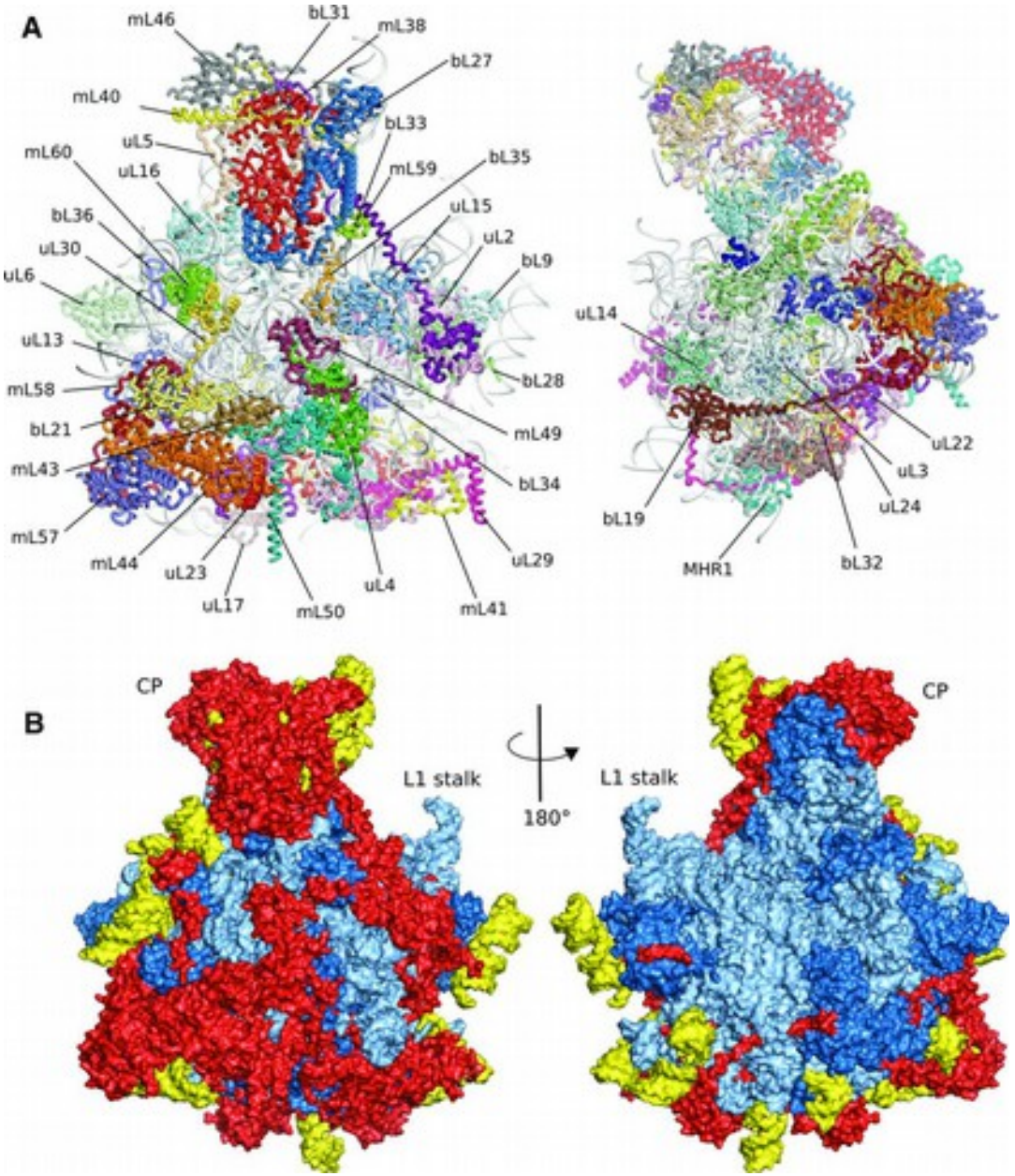
Newly transcribed eukaryotic pre-mRNAs are processed at their 3′-ends by the ~1 MDa multiprotein cleavage and polyadenylation factor (CPF). CPF cleaves pre-mRNAs, adds a poly(A) tail and triggers transcription termination but it is unclear how its different enzymes are coordinated and assembled. Here, we show that the nuclease, polymerase and phosphatase activities of yeast CPF are organized into three modules. Using cryo-EM, we determine a 3.5 Å resolution structure of the ~200 kDa polymerase module. This reveals four beta propellers in an assembly strikingly similar to other protein complexes that bind nucleic acid. Combined with in vitro reconstitution experiments, our data show that the polymerase module brings together factors required for specific and efficient polyadenylation, to help coordinate mRNA 3′-end processing.



Cryo-EM Model-building

- Autozone multi-residue
- Sphere Refine, Sphere Refine+
  - sphere regularize, sphere regularize +
- Geman-McClure distance restraints
- Multi-threading/parallel processing
- Backrub rotamers

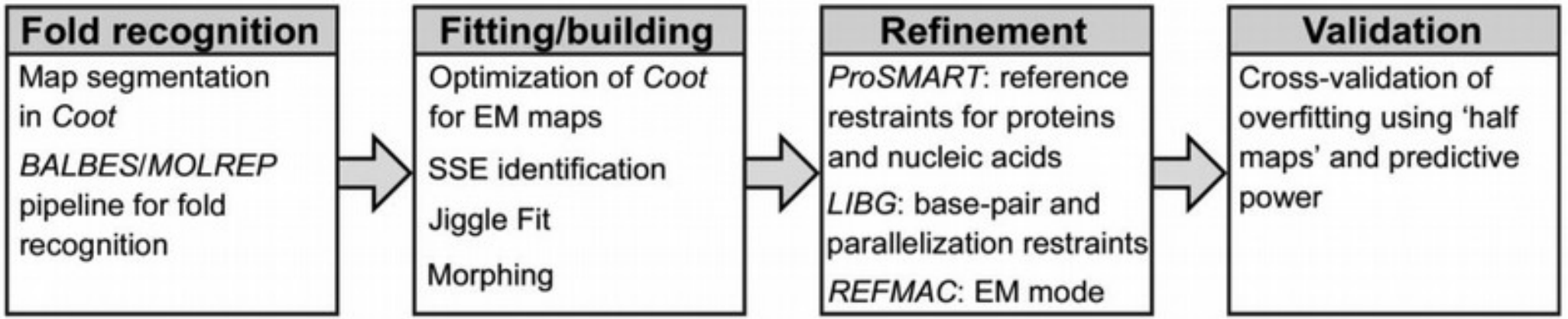
New Ribosome Components



blue: conserved
red: found in both
yellow: yeast only

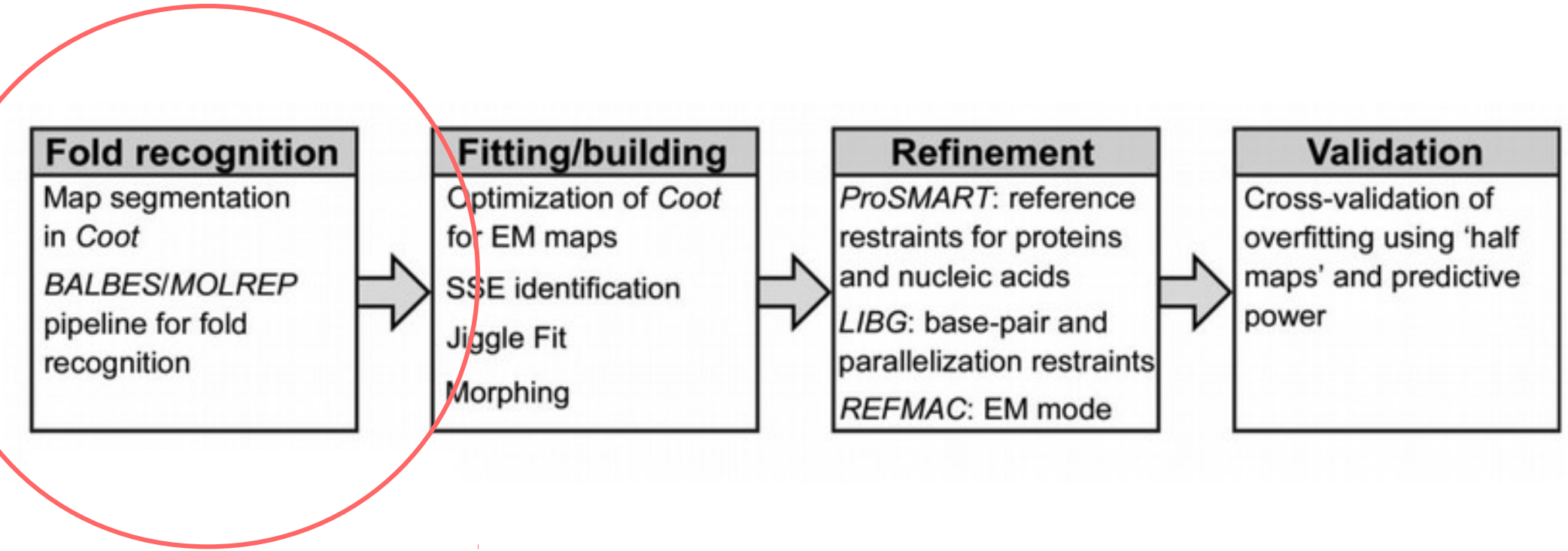
Model-Building Tools

Recent Developments



Model-Building Tools

Recent Developments

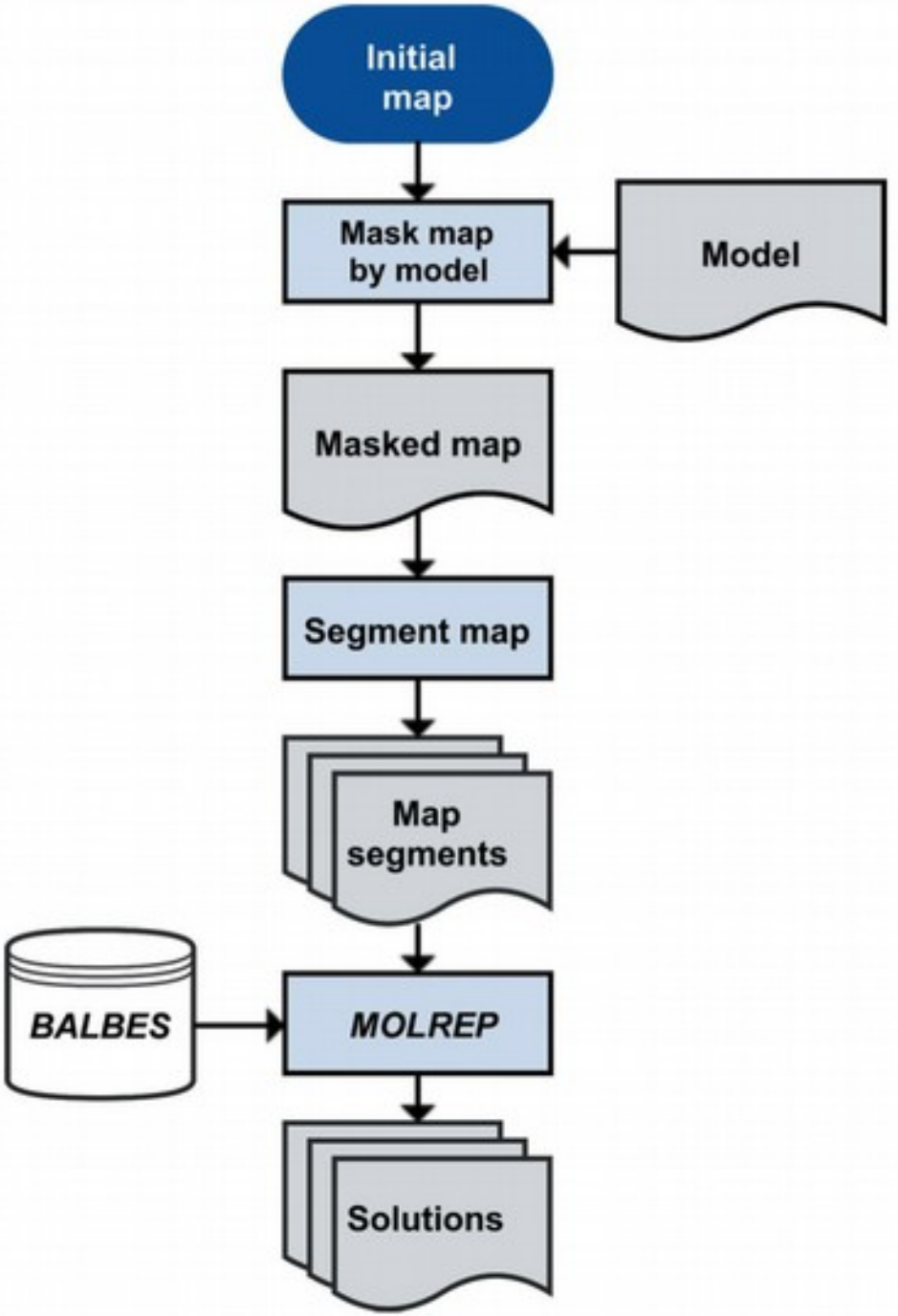


Cryo-EM data

- Ability to collect data from native sources
  - in such cases, the composition of the complex may not be known
  - Mere "docking" of high resolution structures/fragments cannot work
  - At 4Å, it may be possible to trace the backbone
  - At better than 4Å it may be possible to assign the amino-acid sequence
    - thus search a sequence database for possible matches

Cryo-EM data

- Alternatively, use fold recognition
- Using the BALBES database
  - (originally design for molecular replacement)
  - screen domains against unknown density


Initial map
Mask map by model
Model
Masked map
Segment map
Map segments
BALBES
MOLREP
Solutions

Fold Recognition

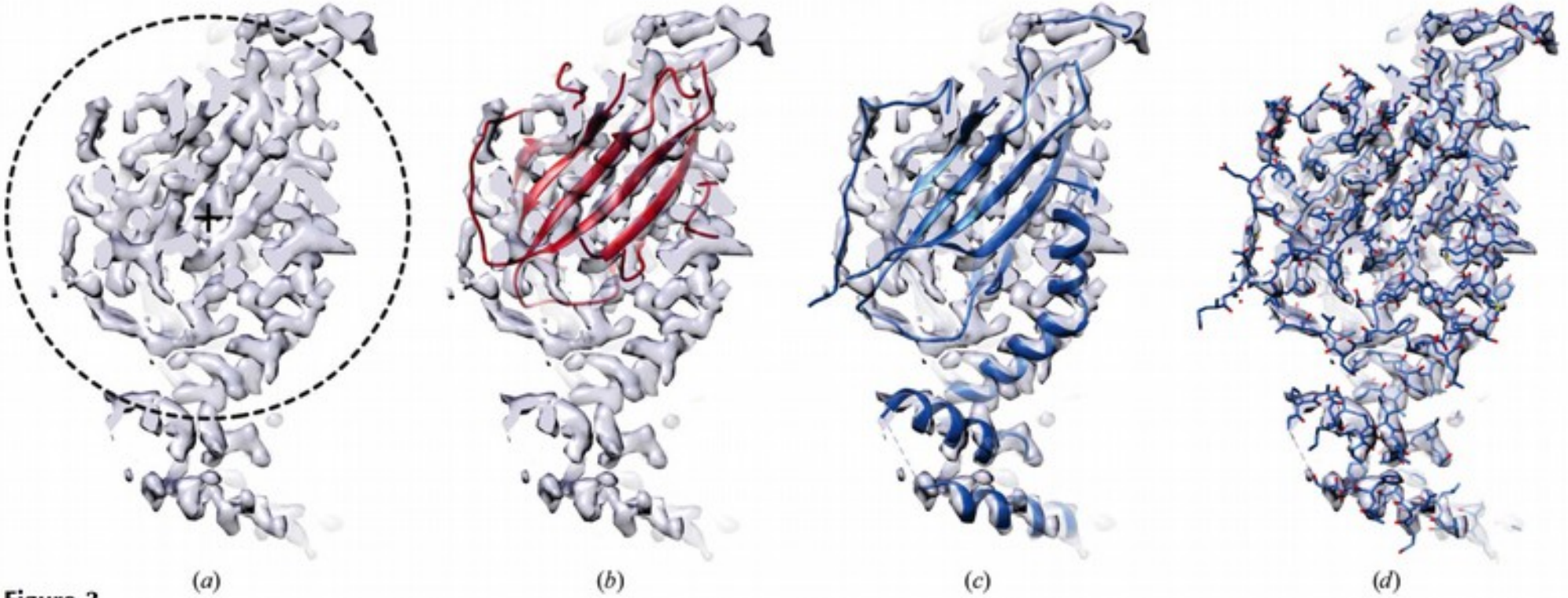
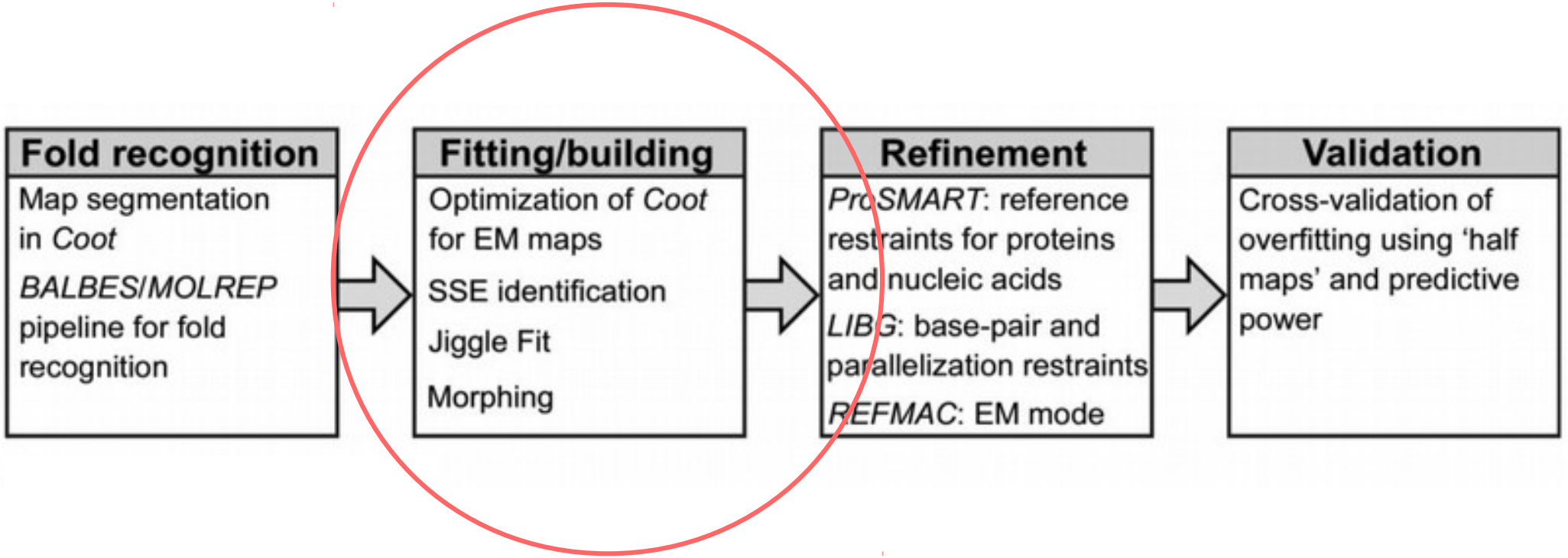


Figure 3
Fold recognition can identify template molecules for model building. (*a*) Density map corresponding to the final model of the mitoribosomal protein mL38 with the segmented search map indicated. (*b*) Top solution from the *BALBES–MOLREP* pipeline. (*c*, *d*) Final refined model of mL38 in (*c*) cartoon and (*d*) full-atom representation.

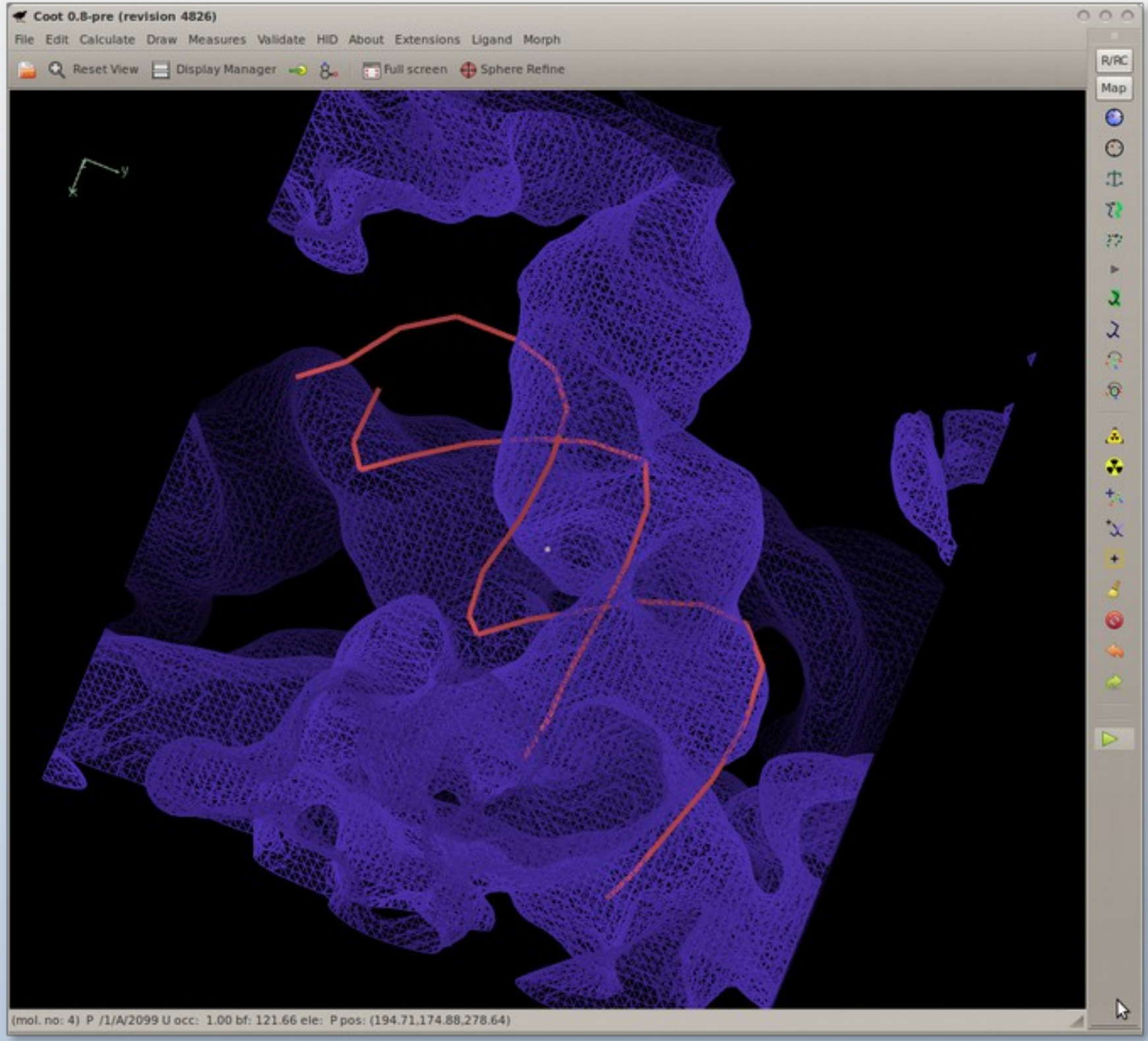
Model-Building Tools

Recent Developments



For What is Coot Useful?

- What resolution ranges for cryo-EM?
  - “Resolution Revolution” maps
  - 2-3.8Å is the strength
  - seeing side-chains and purines vs. pyrimidines
- Local good fit of model to density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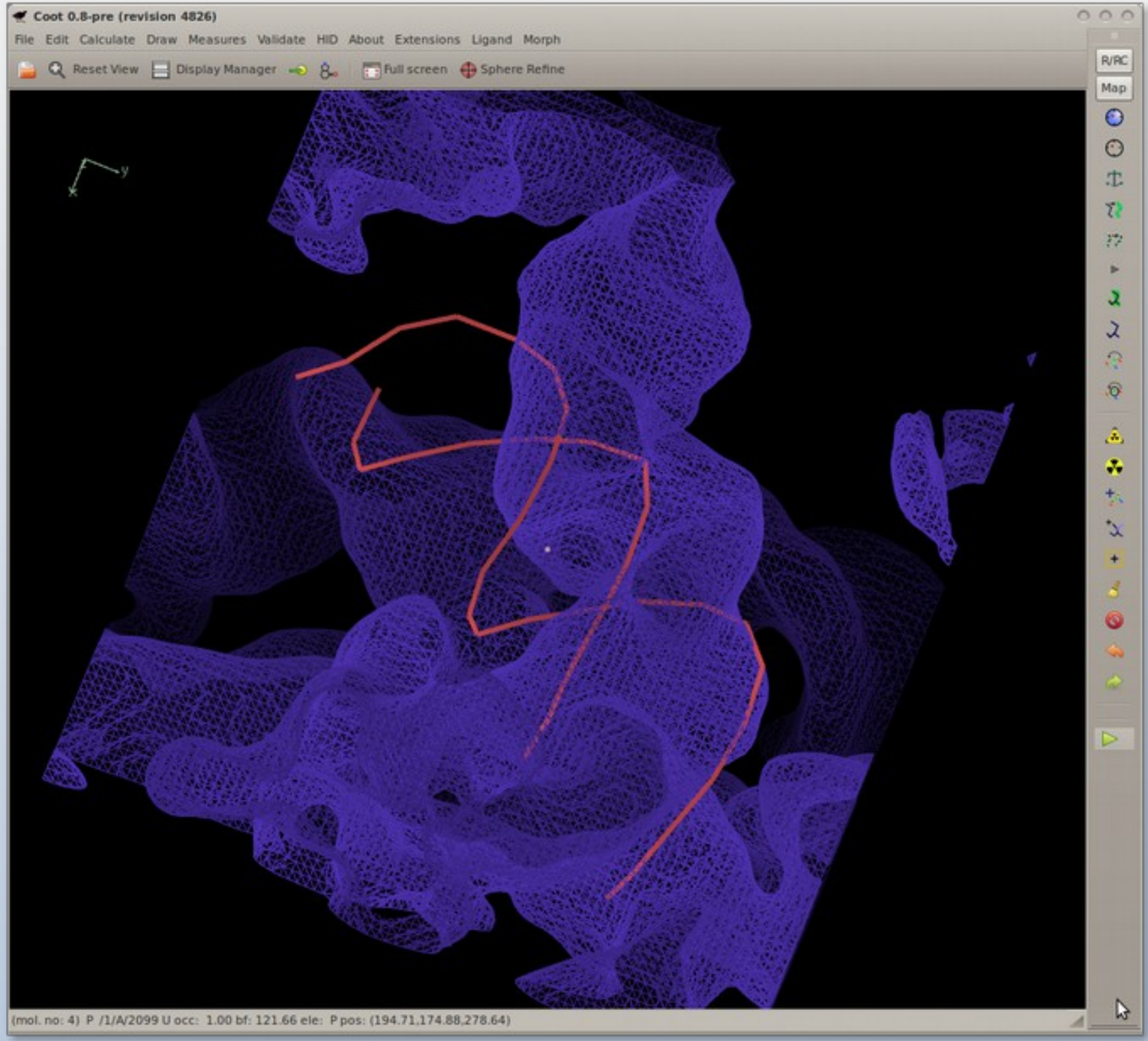
(mol. no: 4) P /1/A/2099 U occ: 1.00 bf: 121.66 ele: P pos: (194.71,174.88,278.64)

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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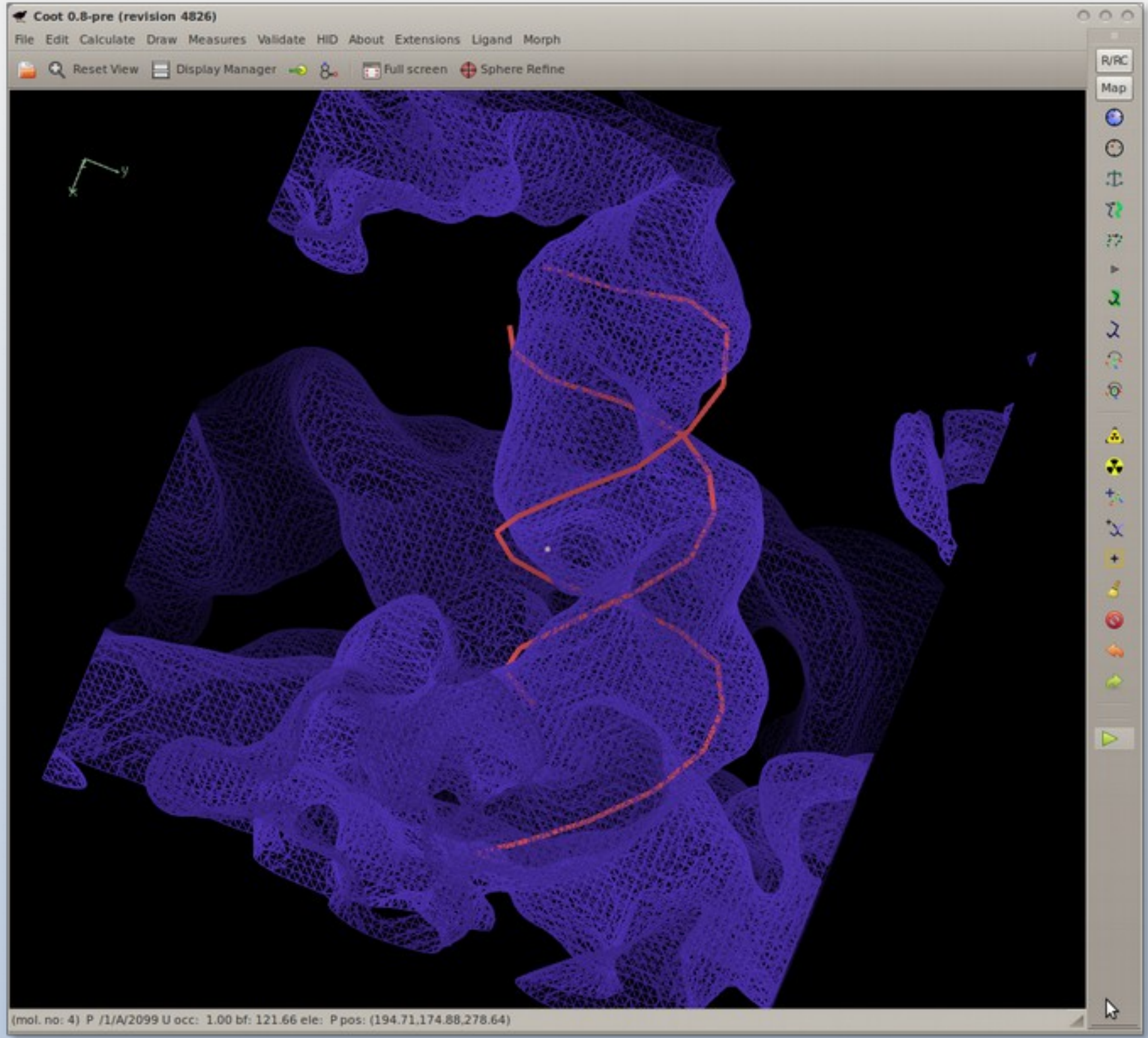
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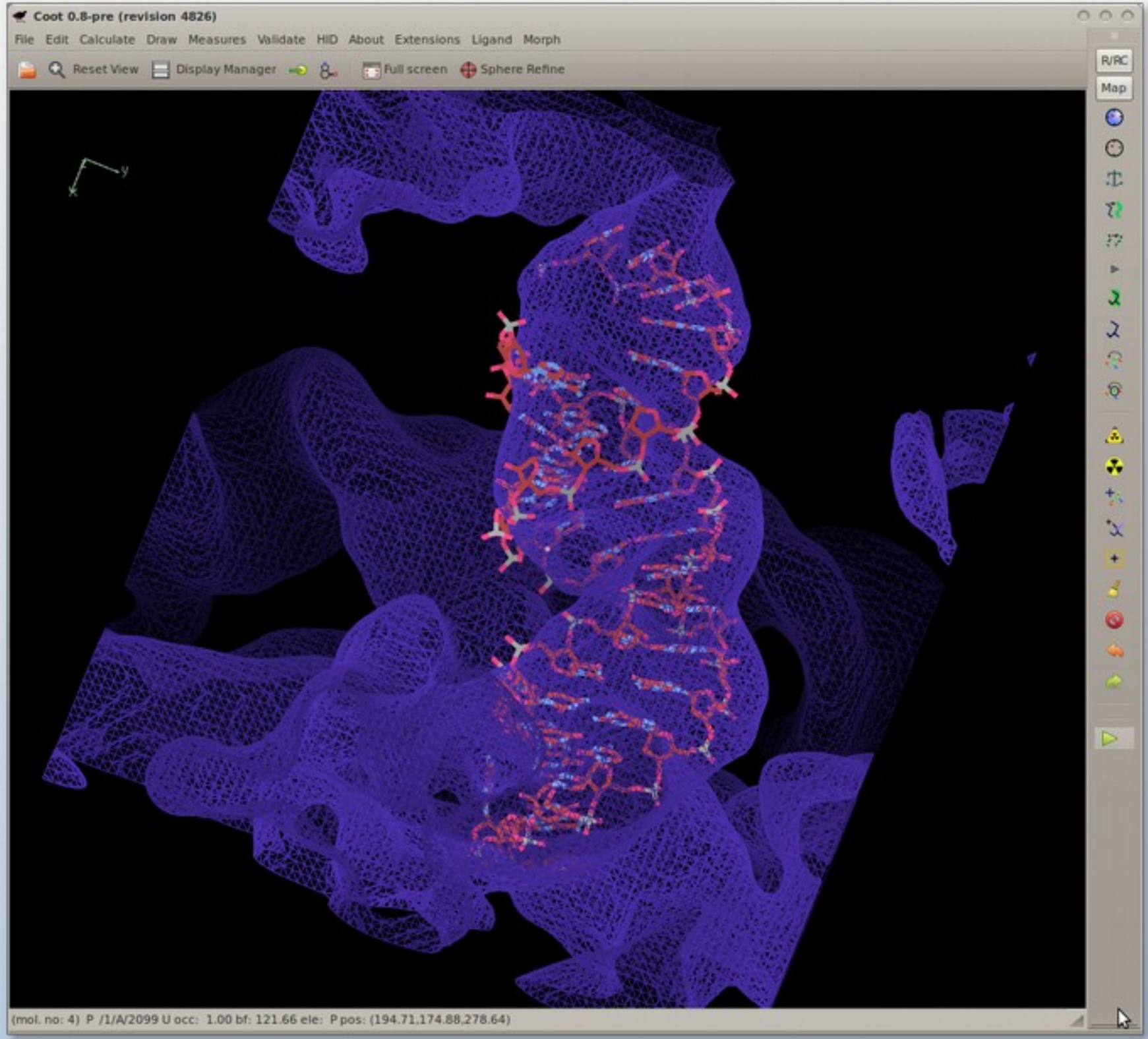
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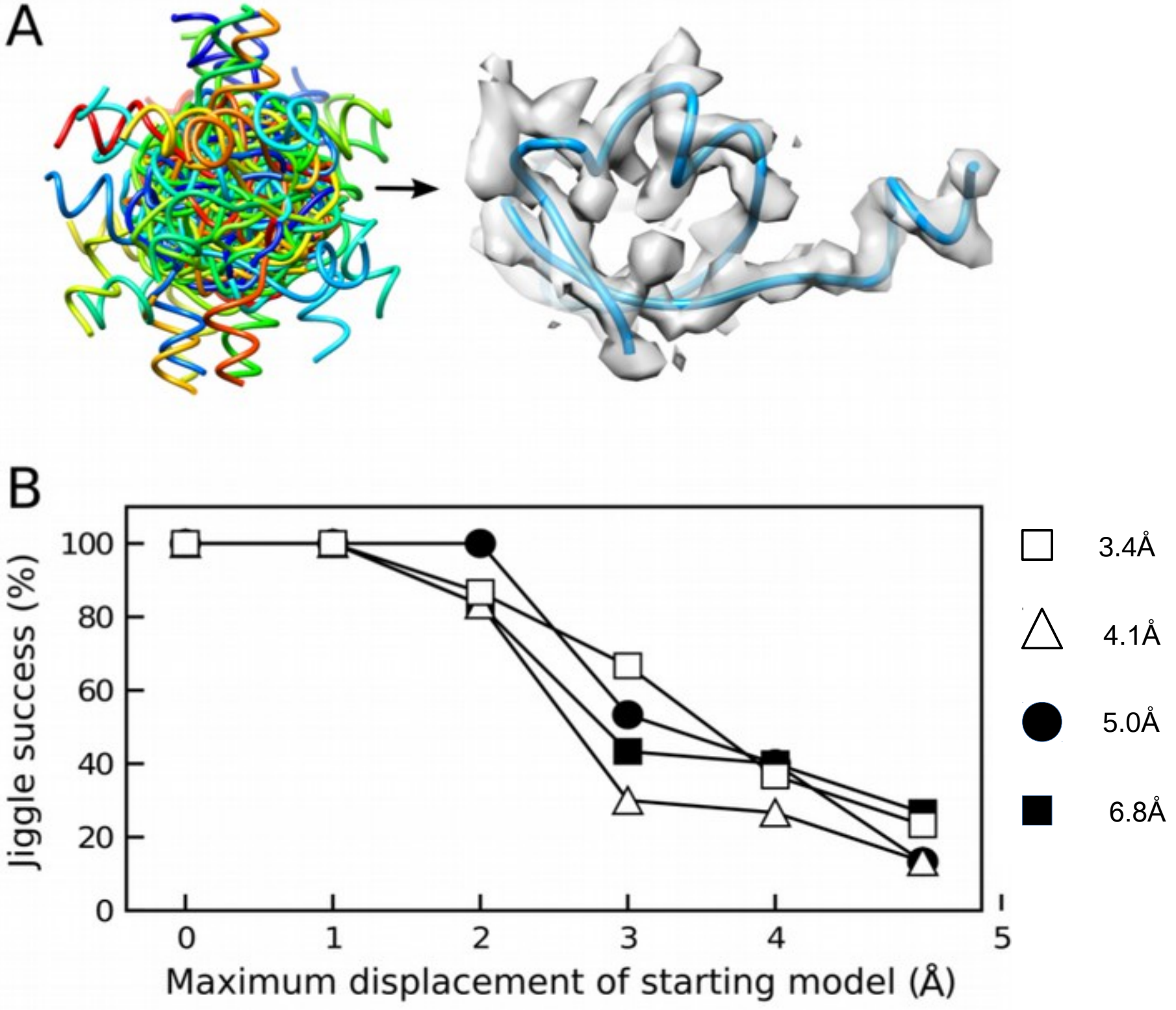

A
B
Jiggle success (%)
100
80
60
40
20
0
0
1
2
3
4
5
Maximum displacement of starting model (Å)
3.4Å
4.1Å
5.0Å
6.8Å

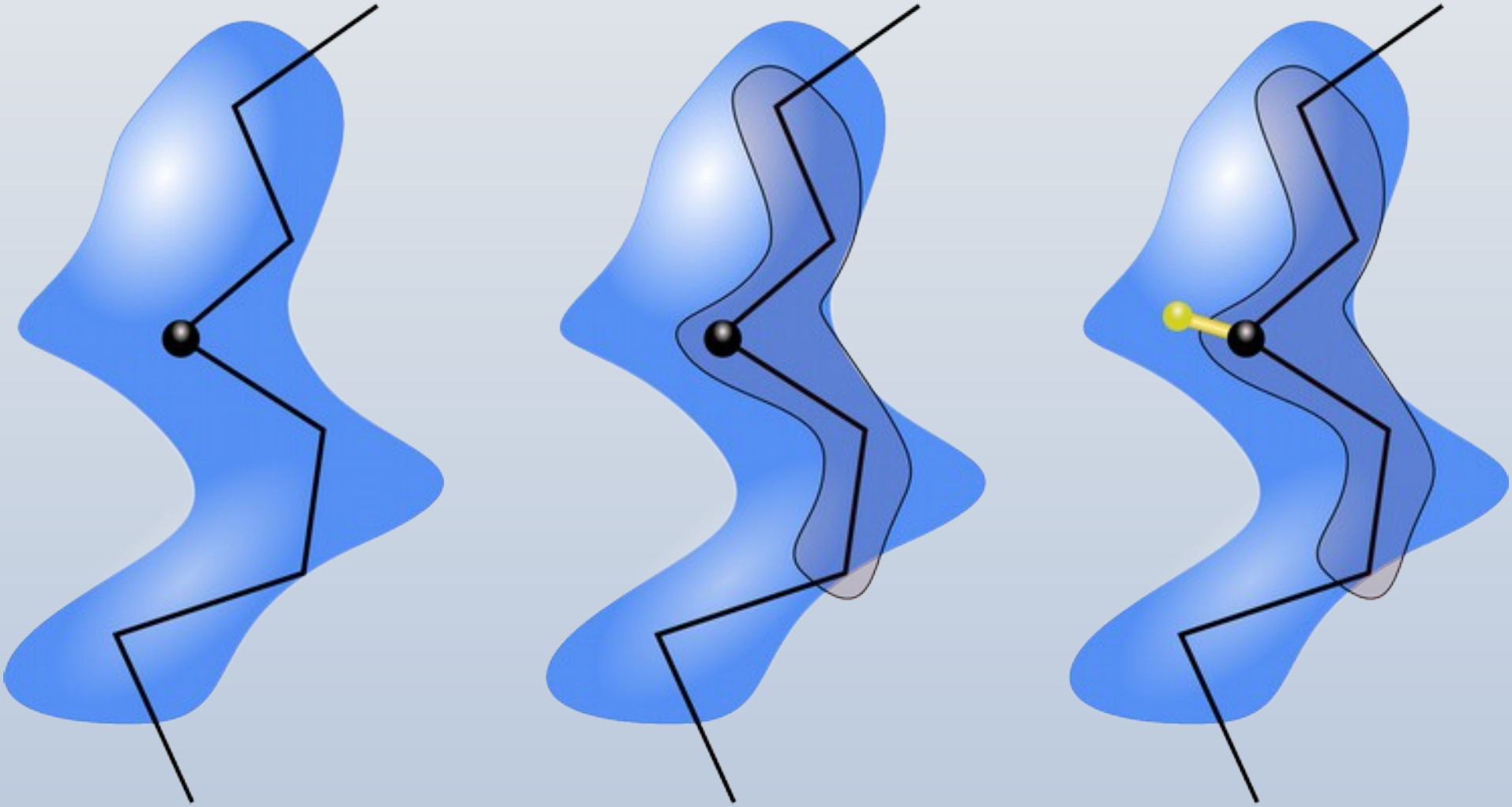
So we have our ideal RNA or homologous protein sitting roughly in the density

(not a great fit)

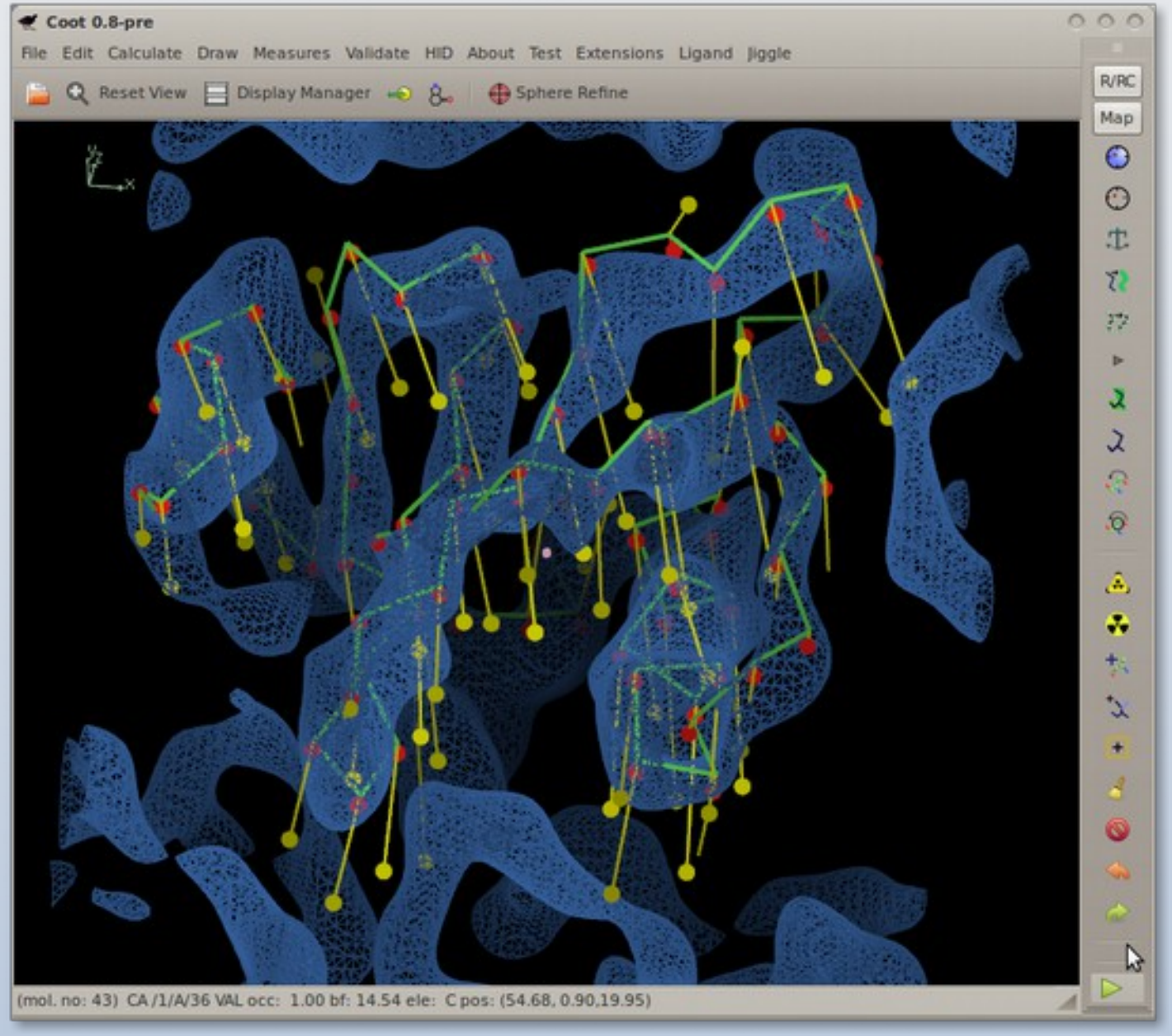
Model Morphing: How it Works

- 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

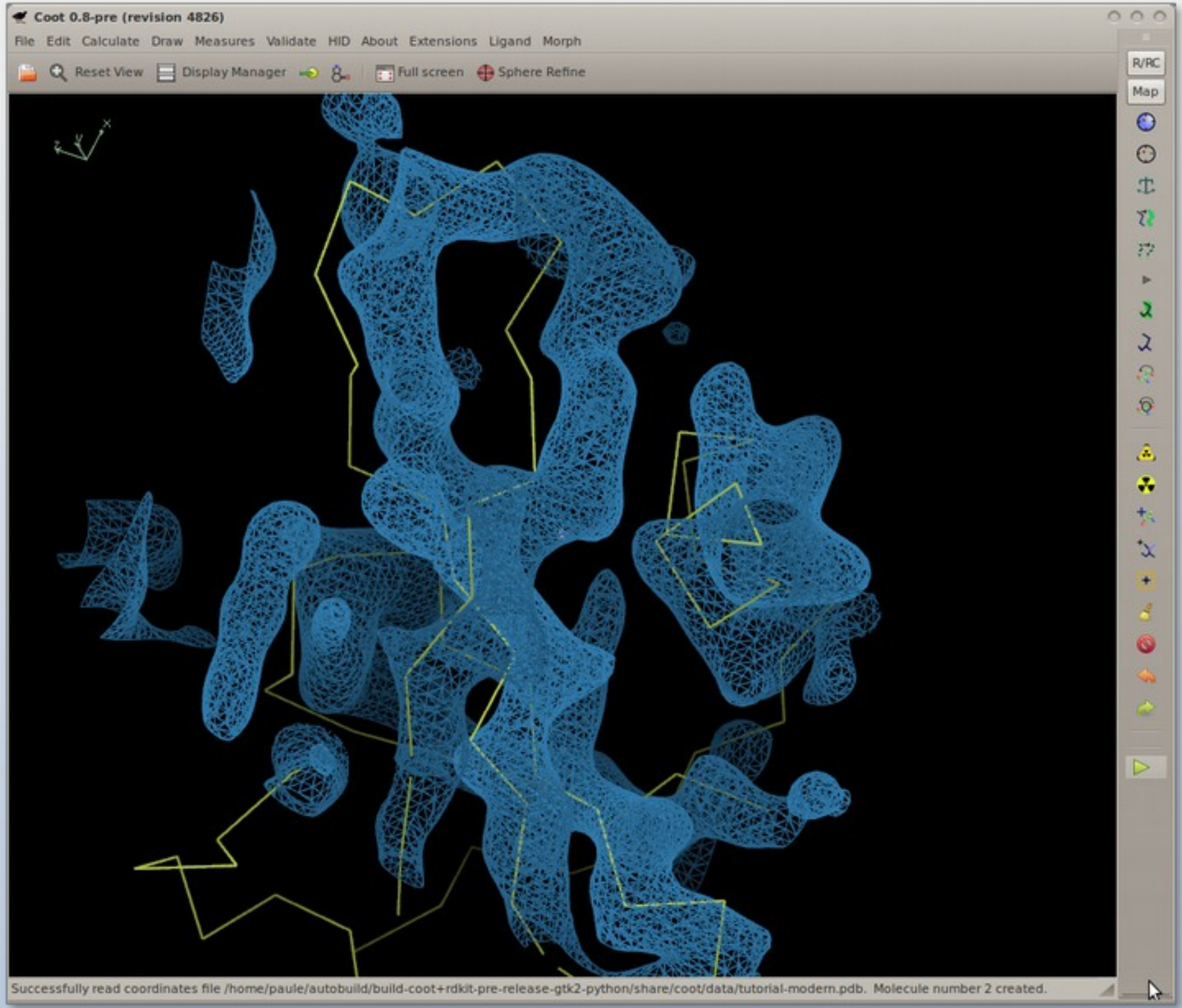


Model Morphing: Example



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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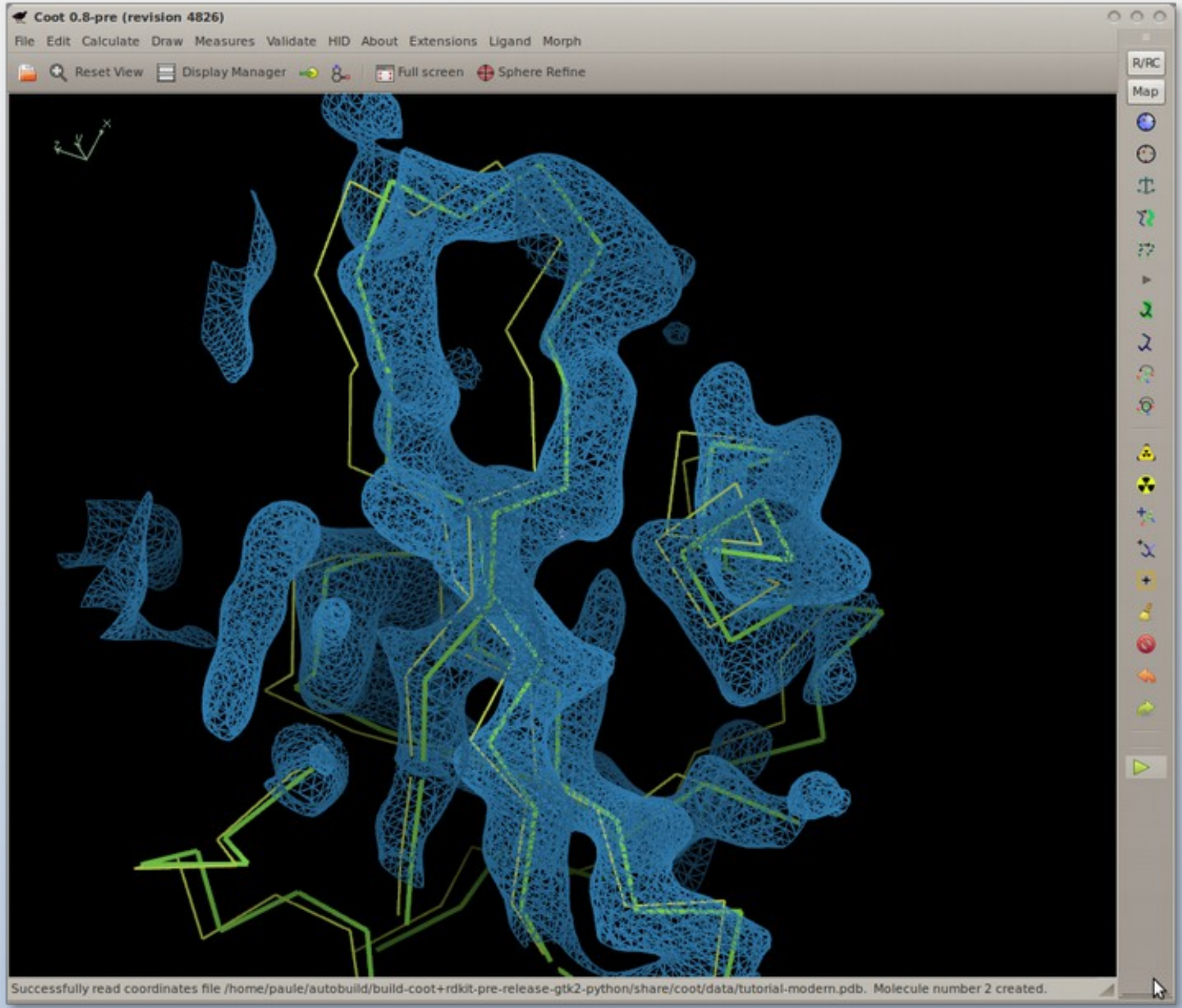
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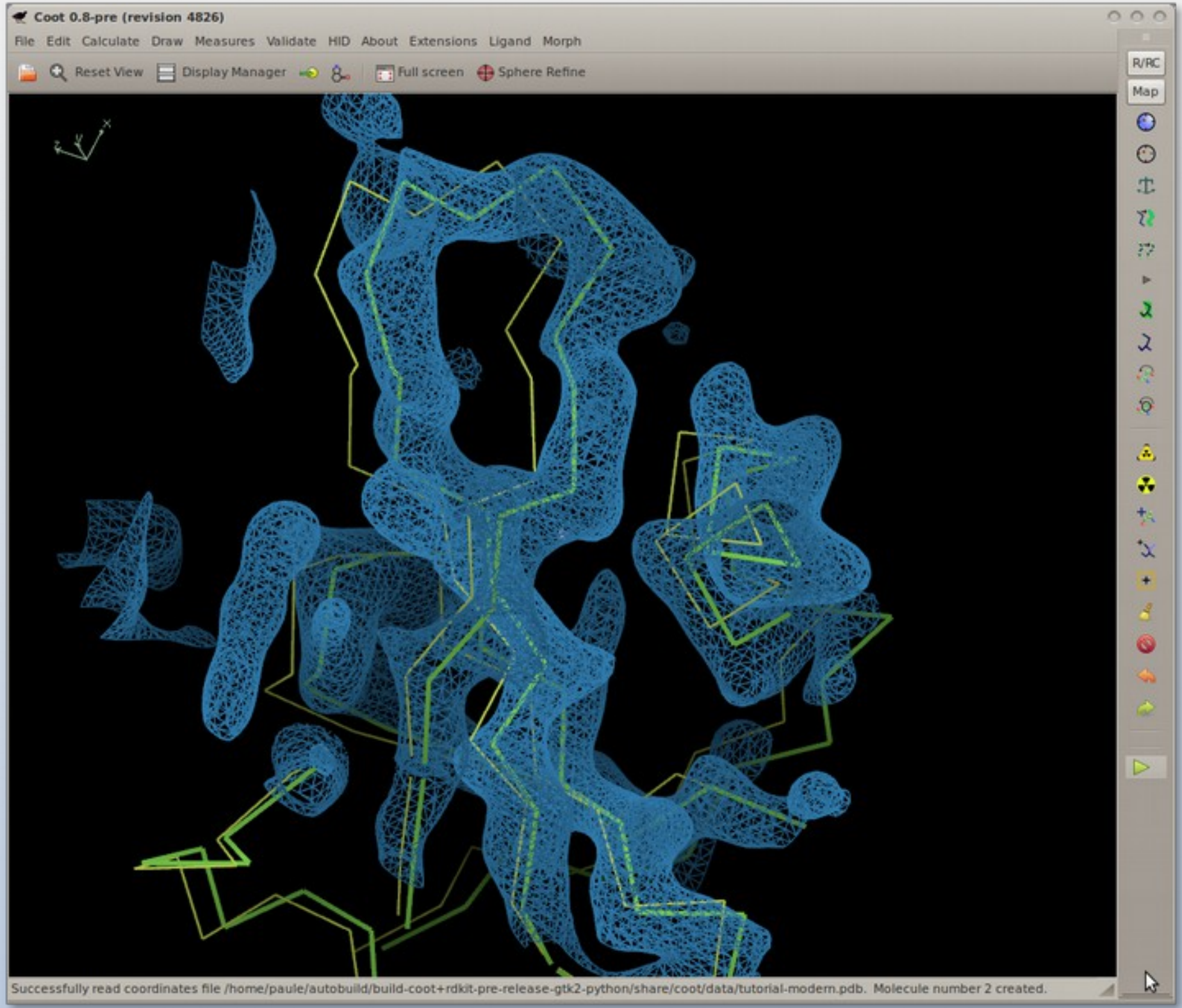
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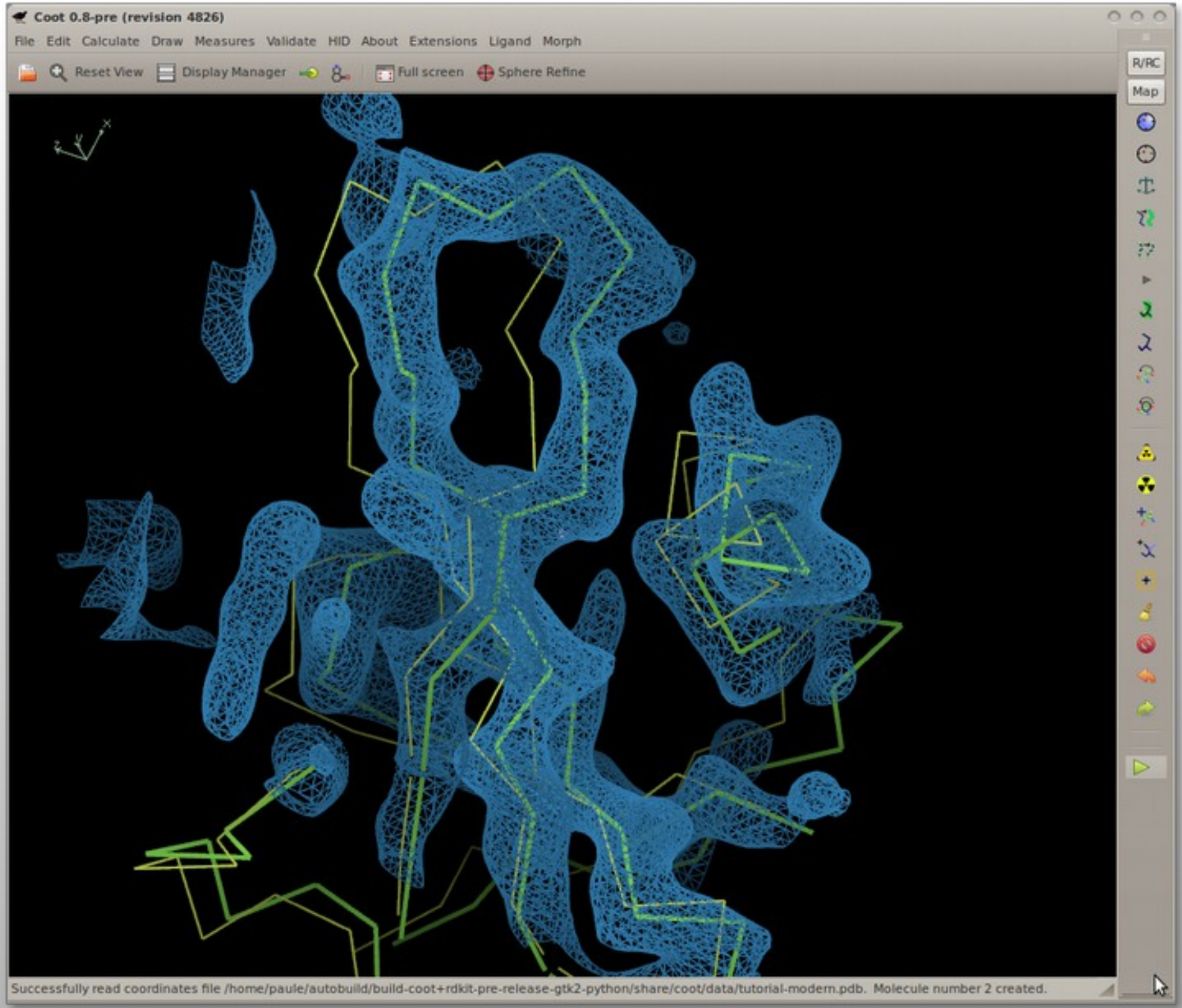
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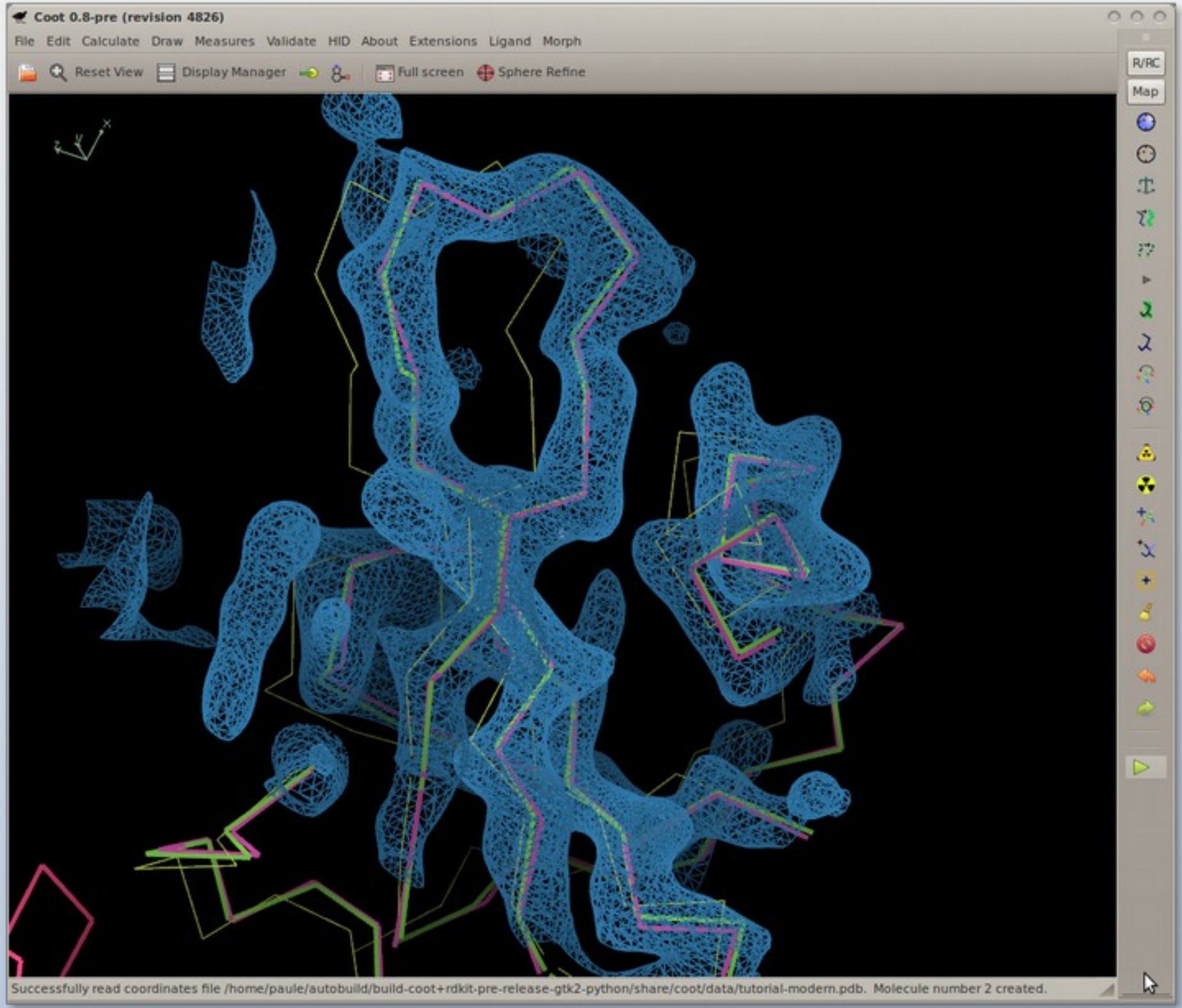
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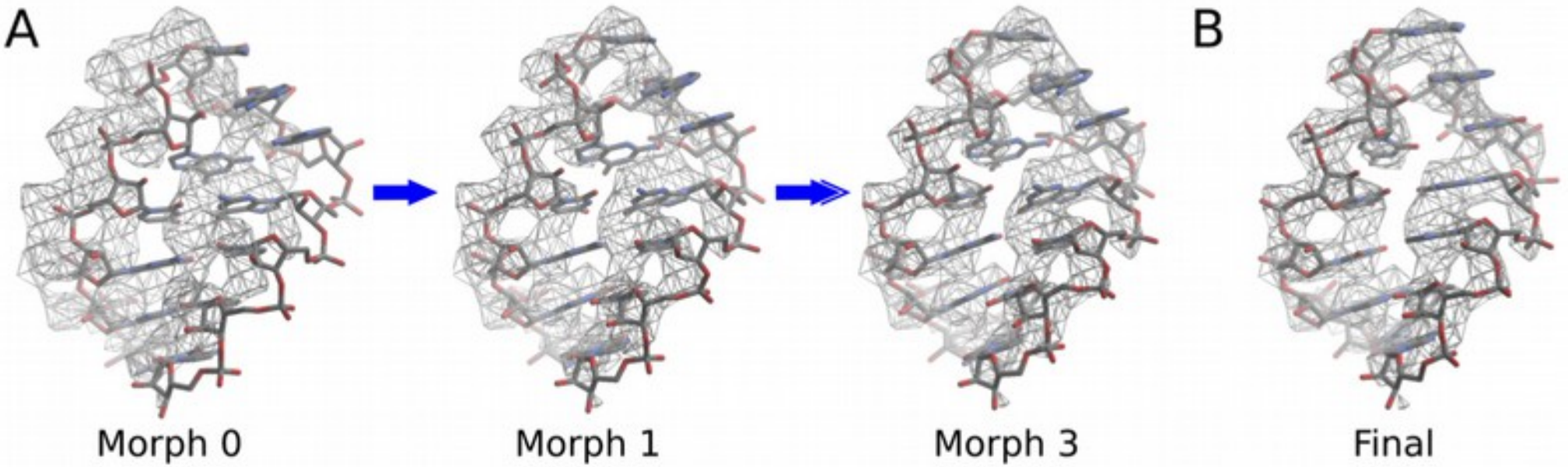
Model Morphing



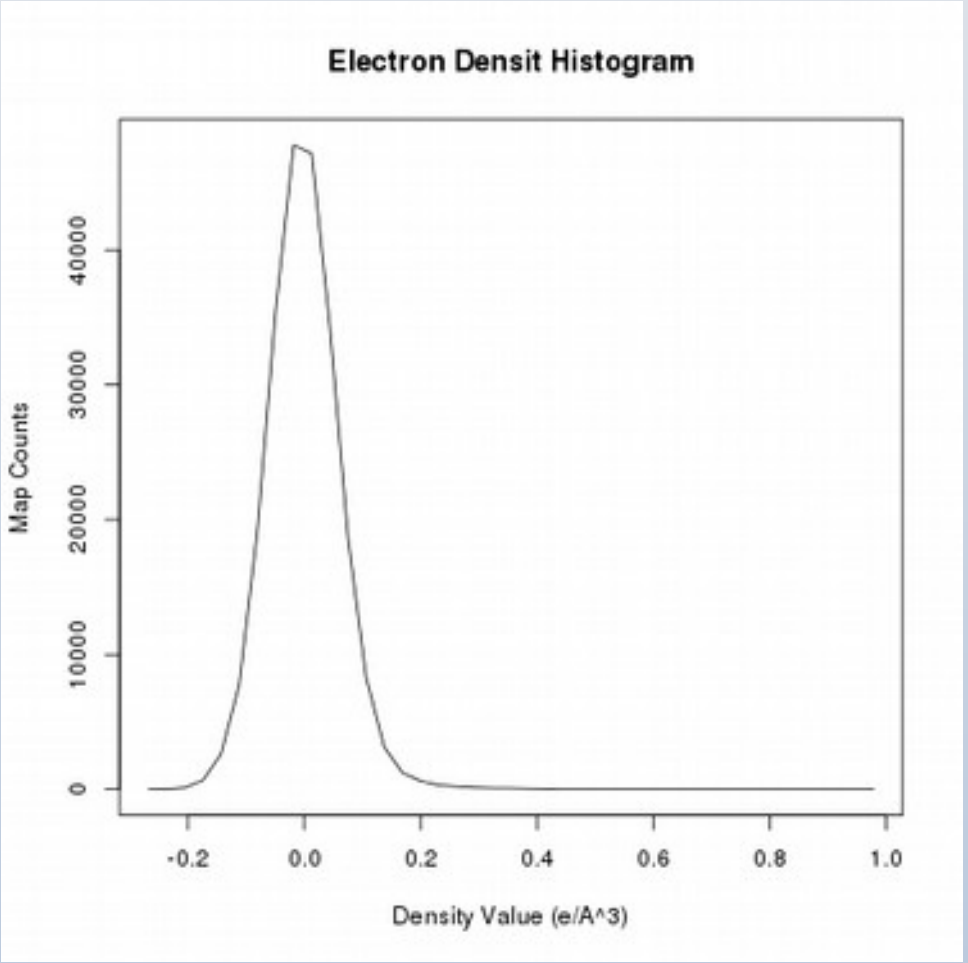
Helix Fitting

- 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 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

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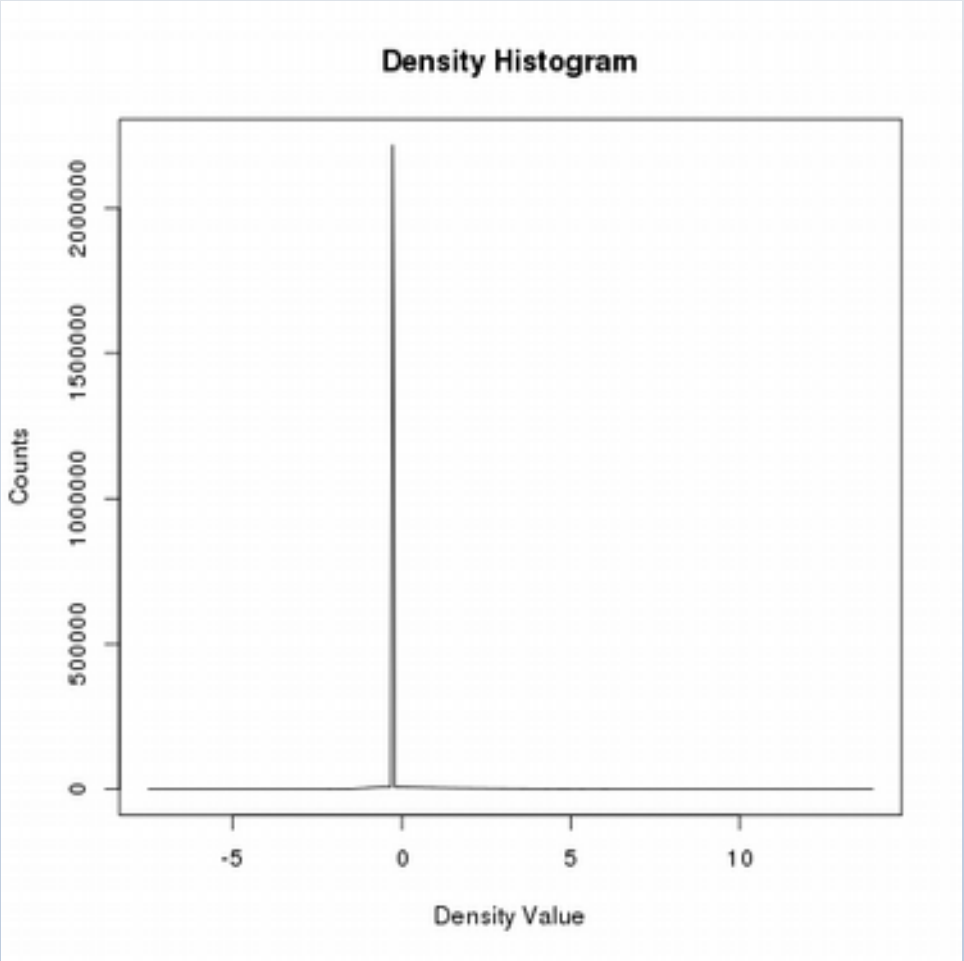
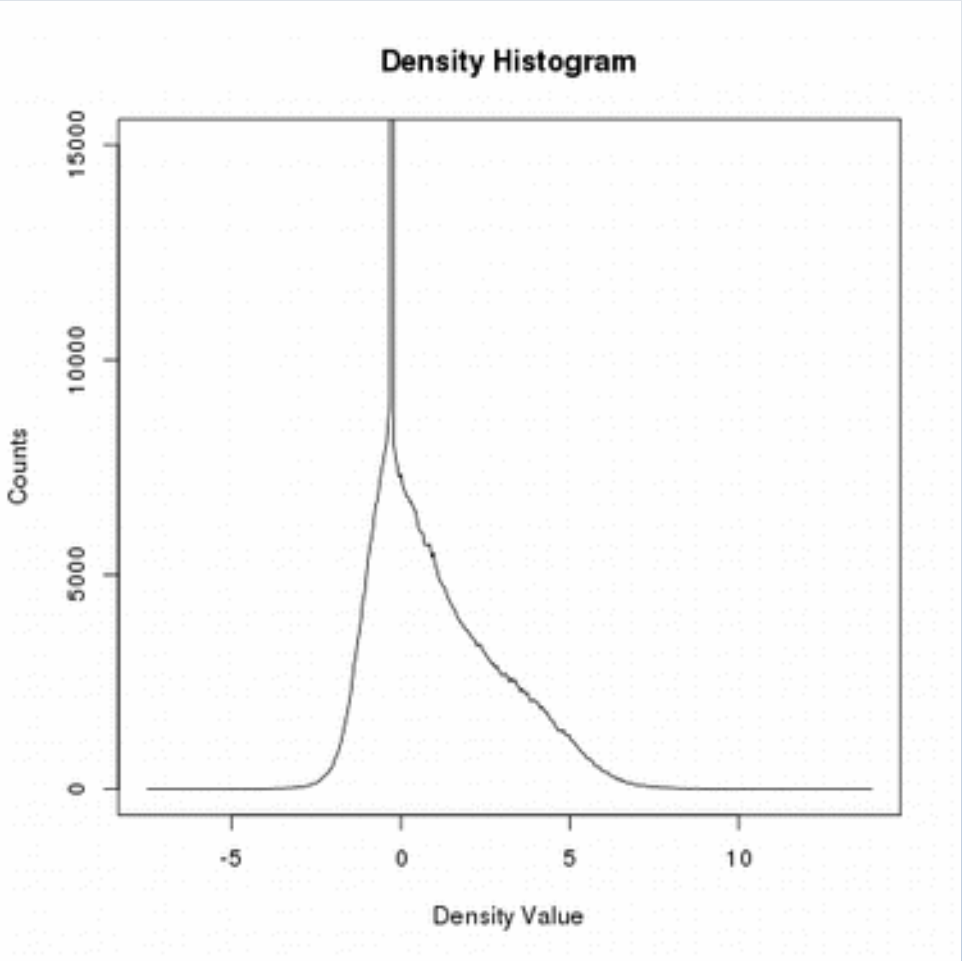
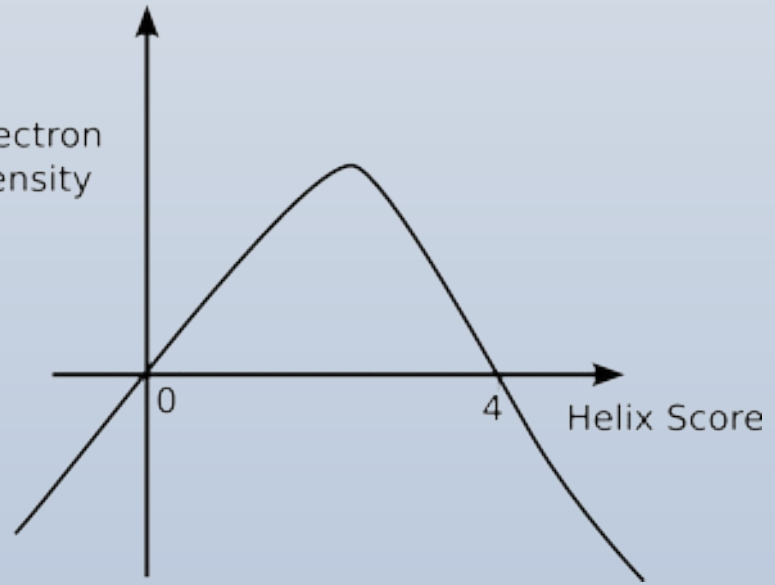




Helix Fitting

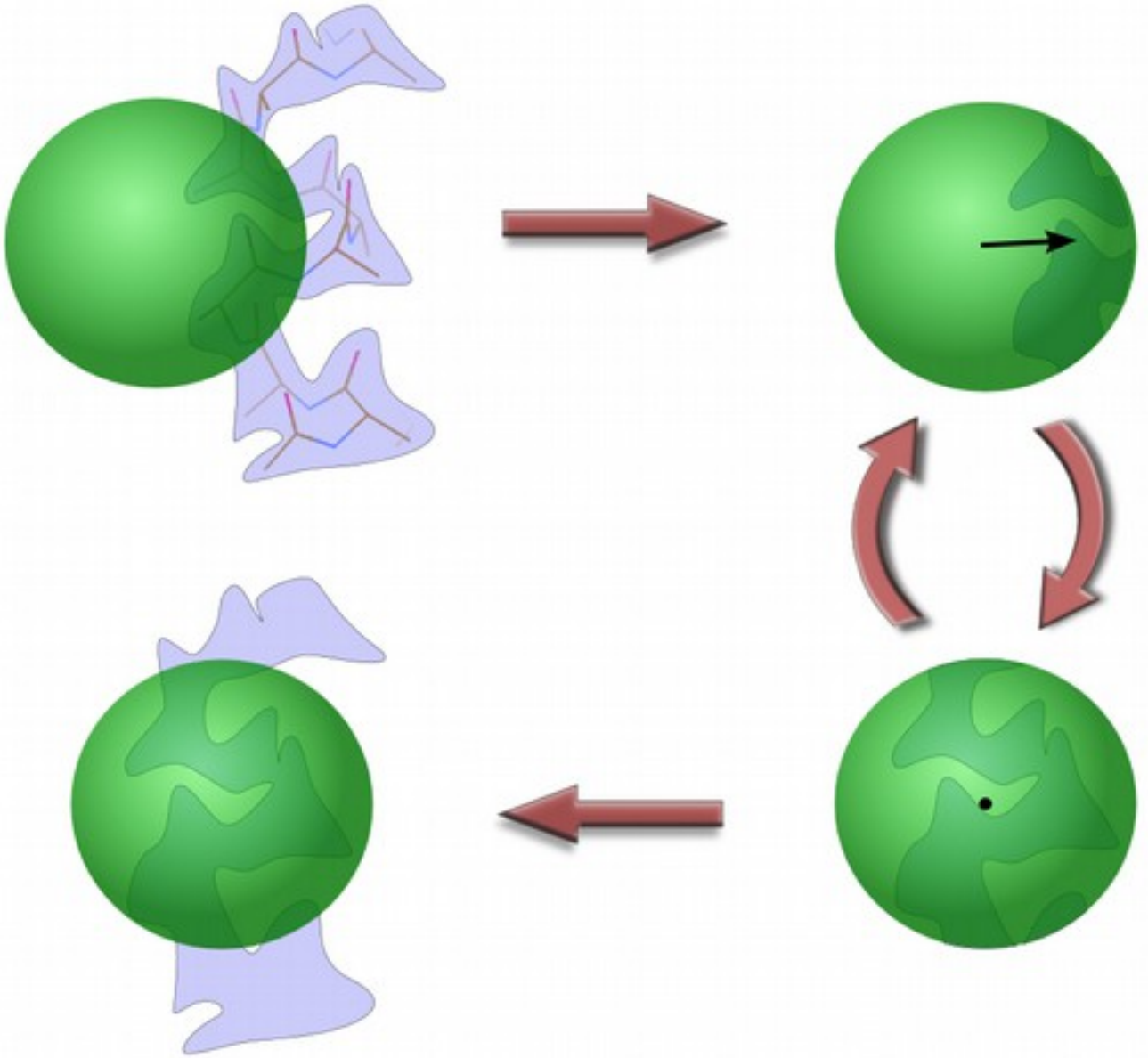
- The distribution of electron density is quite unlike that of x-ray maps
  - e.g. You don't see main-chain atoms at 4 rmsd in x-ray maps
    - regions of dense electron density contribute negatively to helix score
  - These EM maps were sharpened and in a big box of mostly nothing
  - Lots to see at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



Alpha Helix Placement

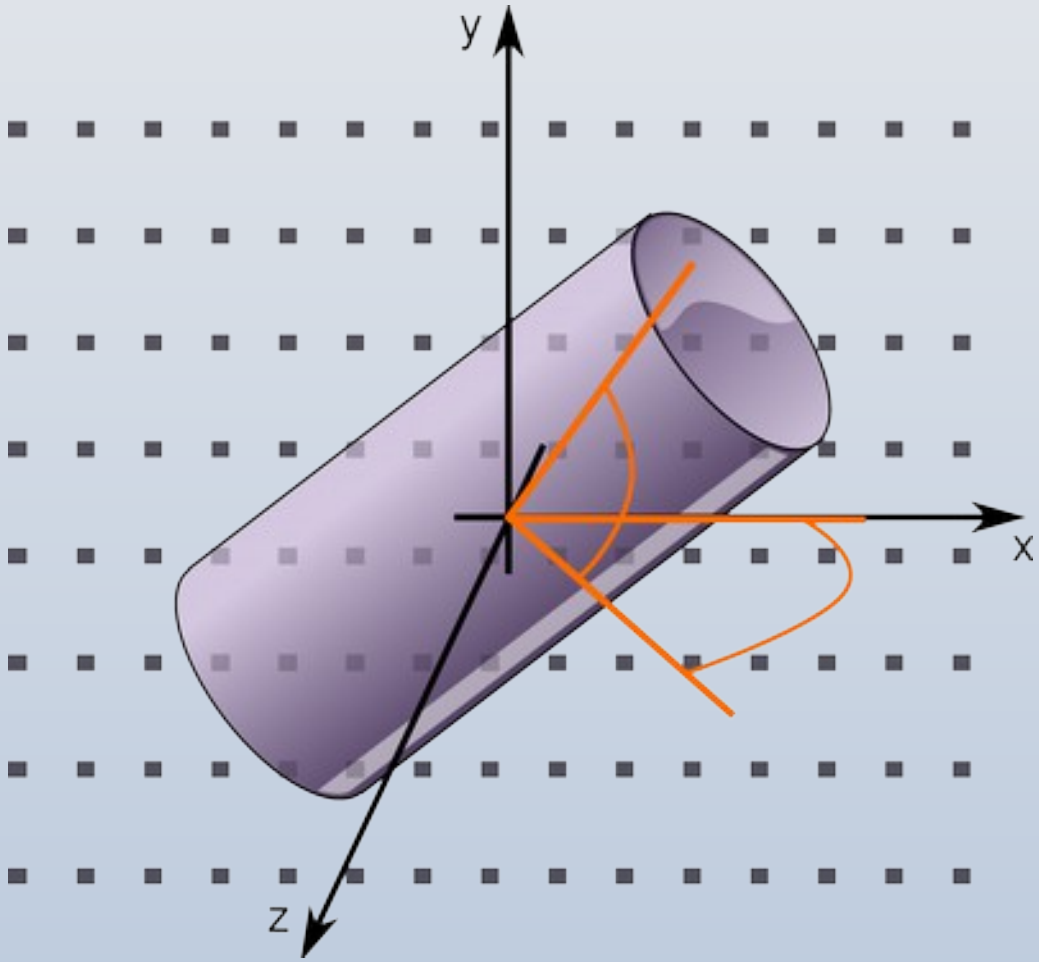
- **Scenario: Looking at a new map, not built with automatic tools:**
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  -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

Centering the Rotation point



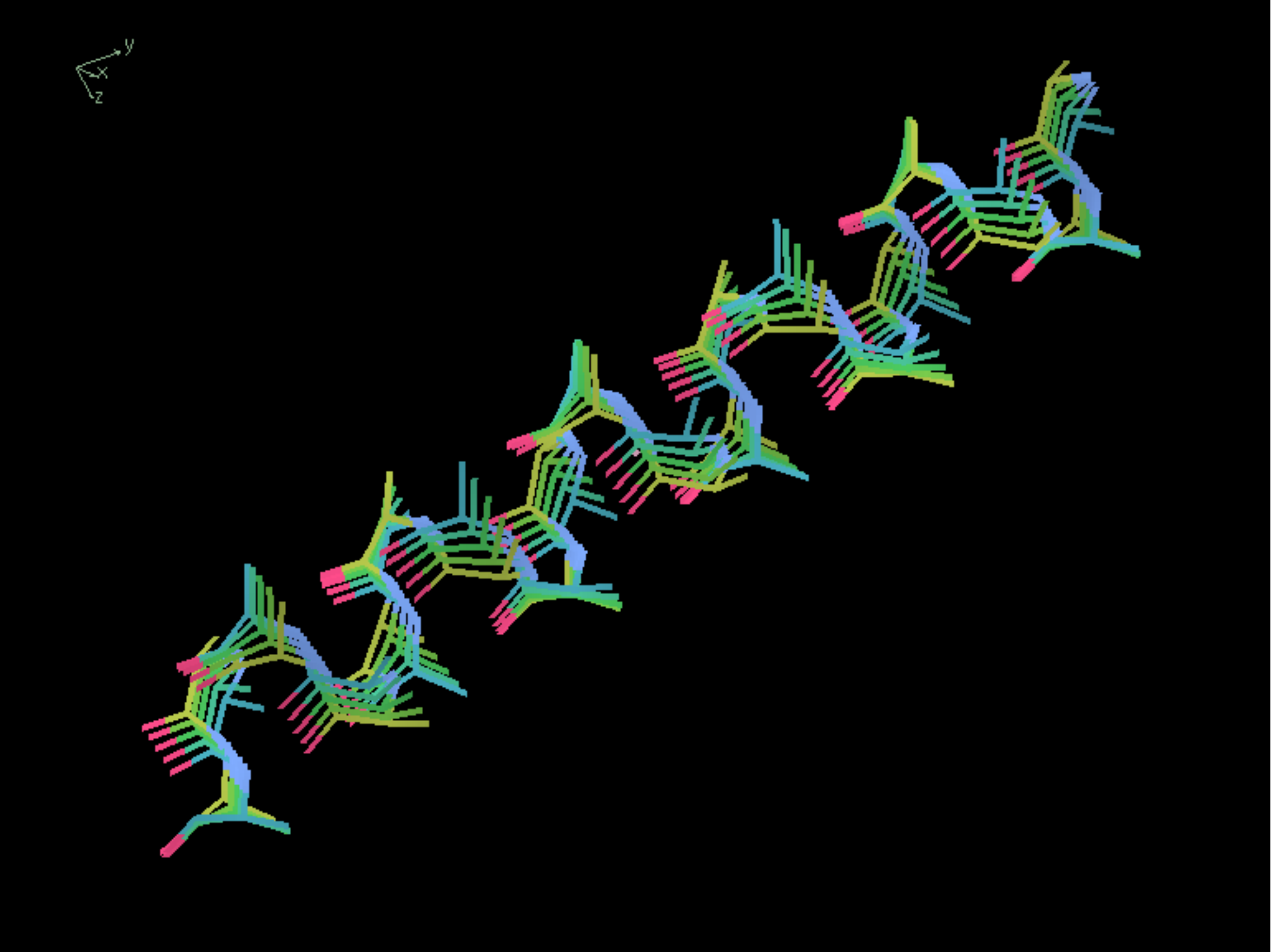
Helix Fitting: Cylinder Search

- Pick the orientation that encapsulates the most electron density



Using 2 rotation axes

2 x 1-D Helix orientation searches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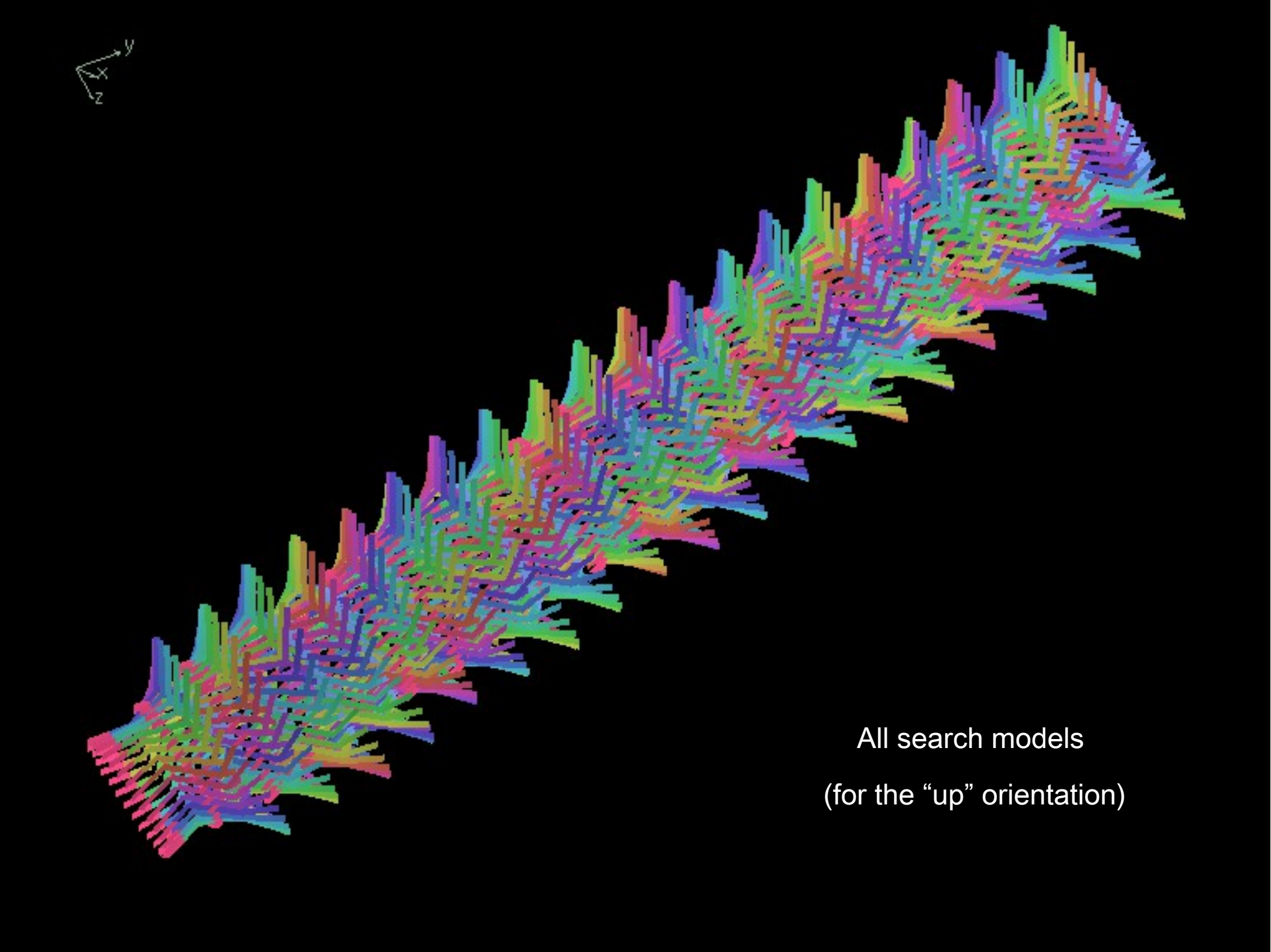
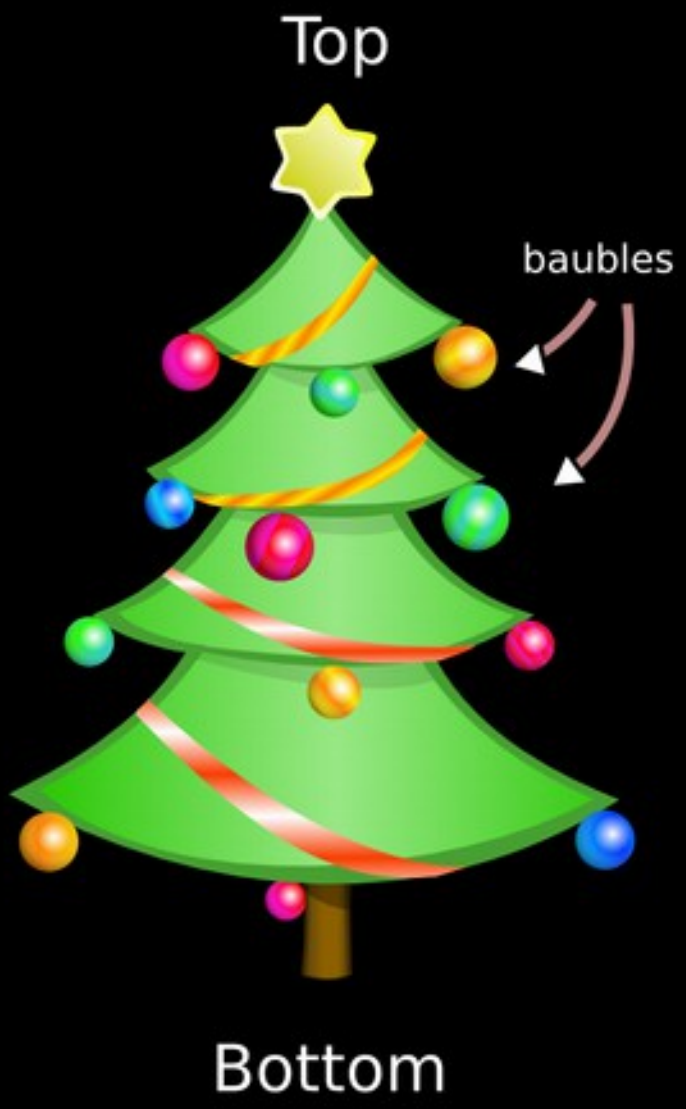
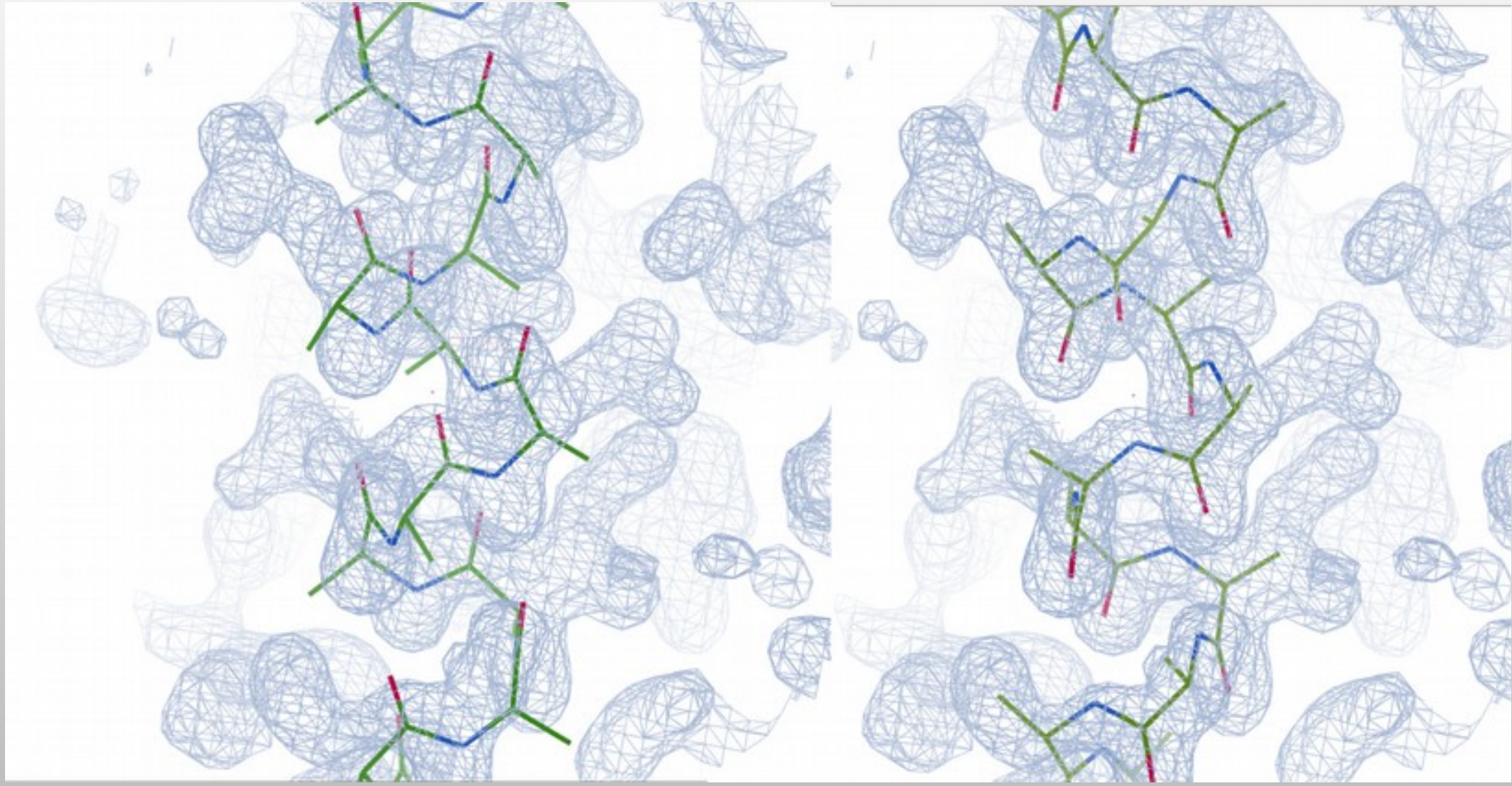
x
z

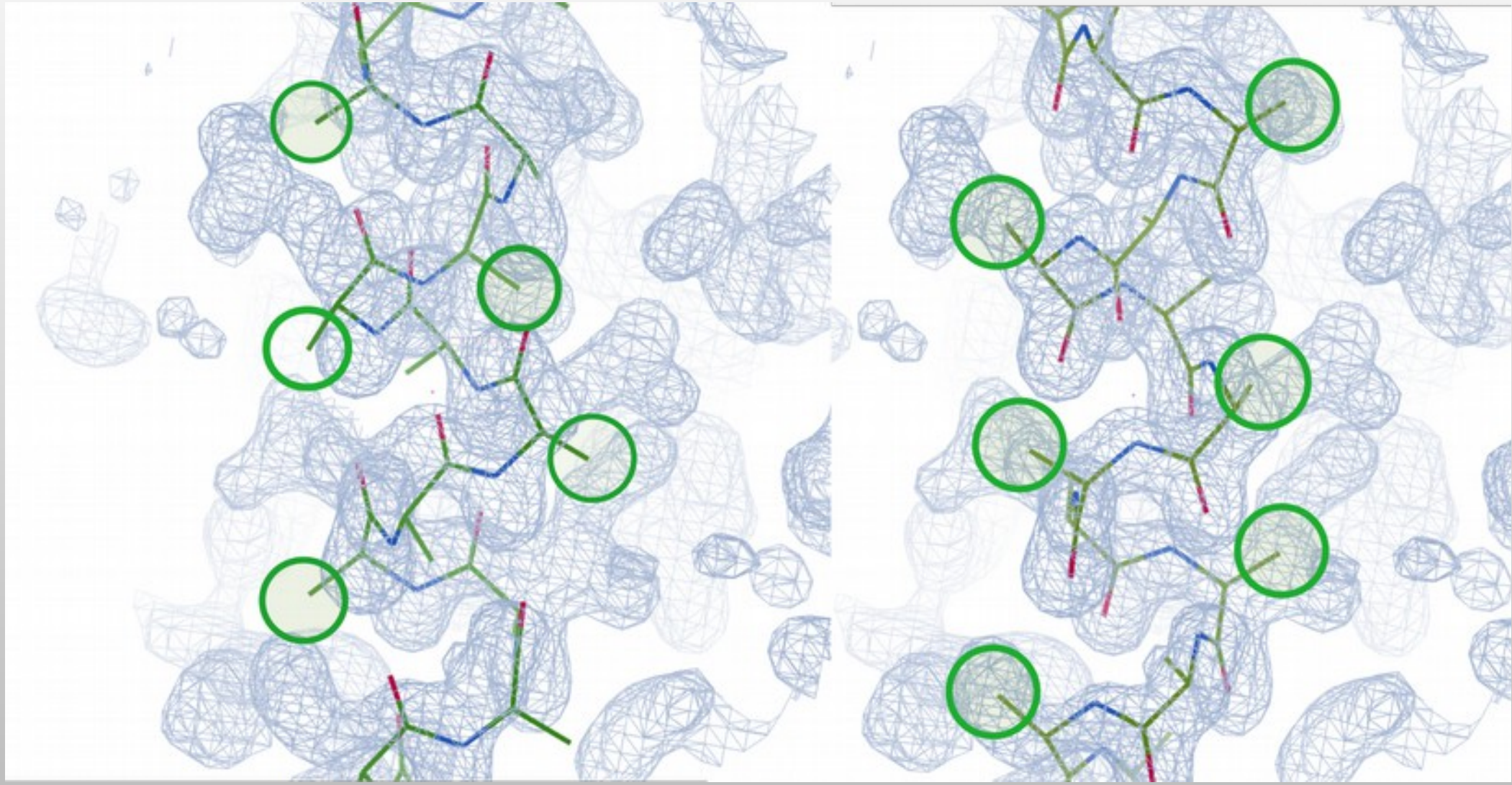

y
x
z
All search models
(for the “up” orientation)


Top
baubles
Bottom

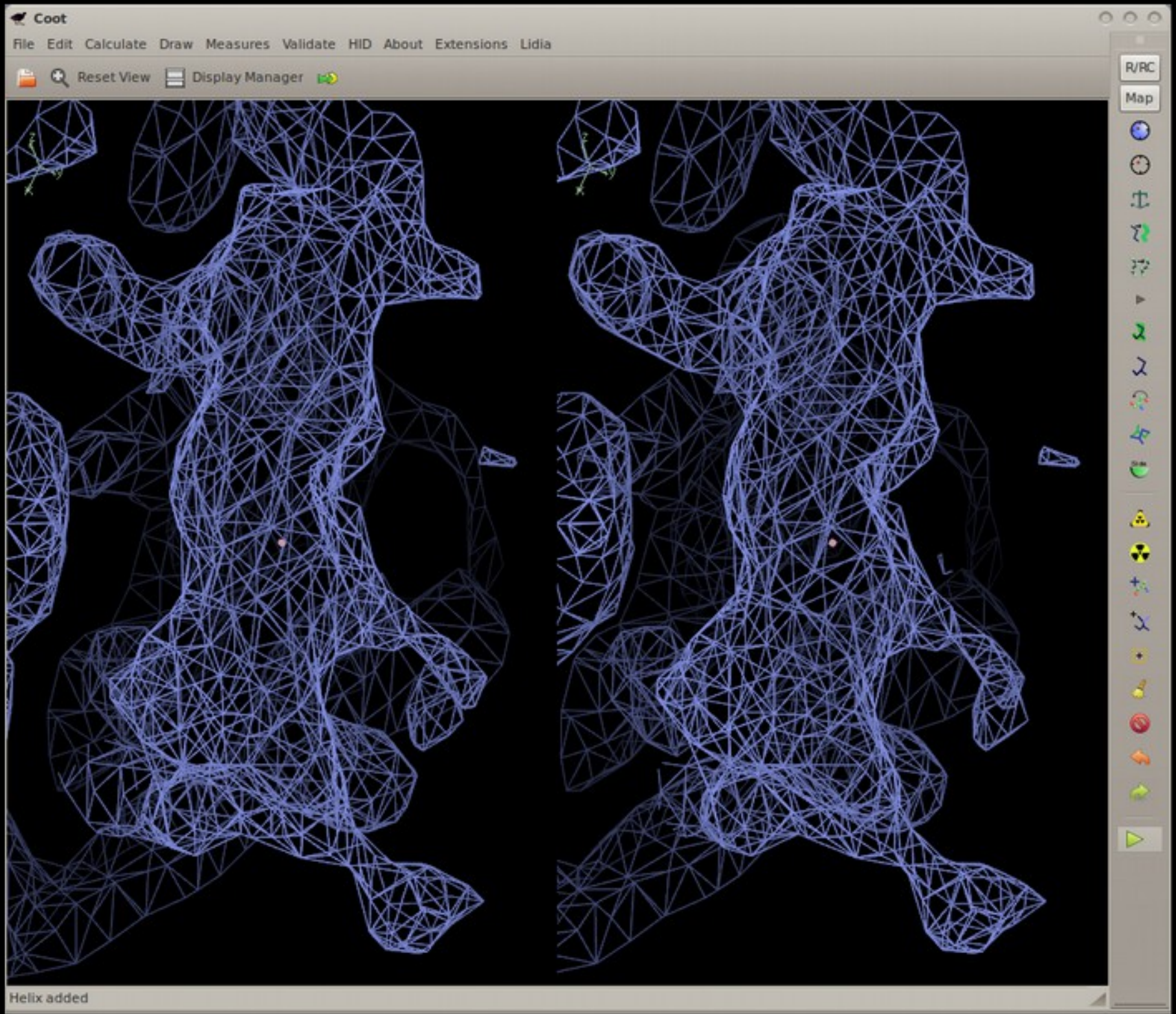
Helix Fitting
Comparing orientation hypotheses

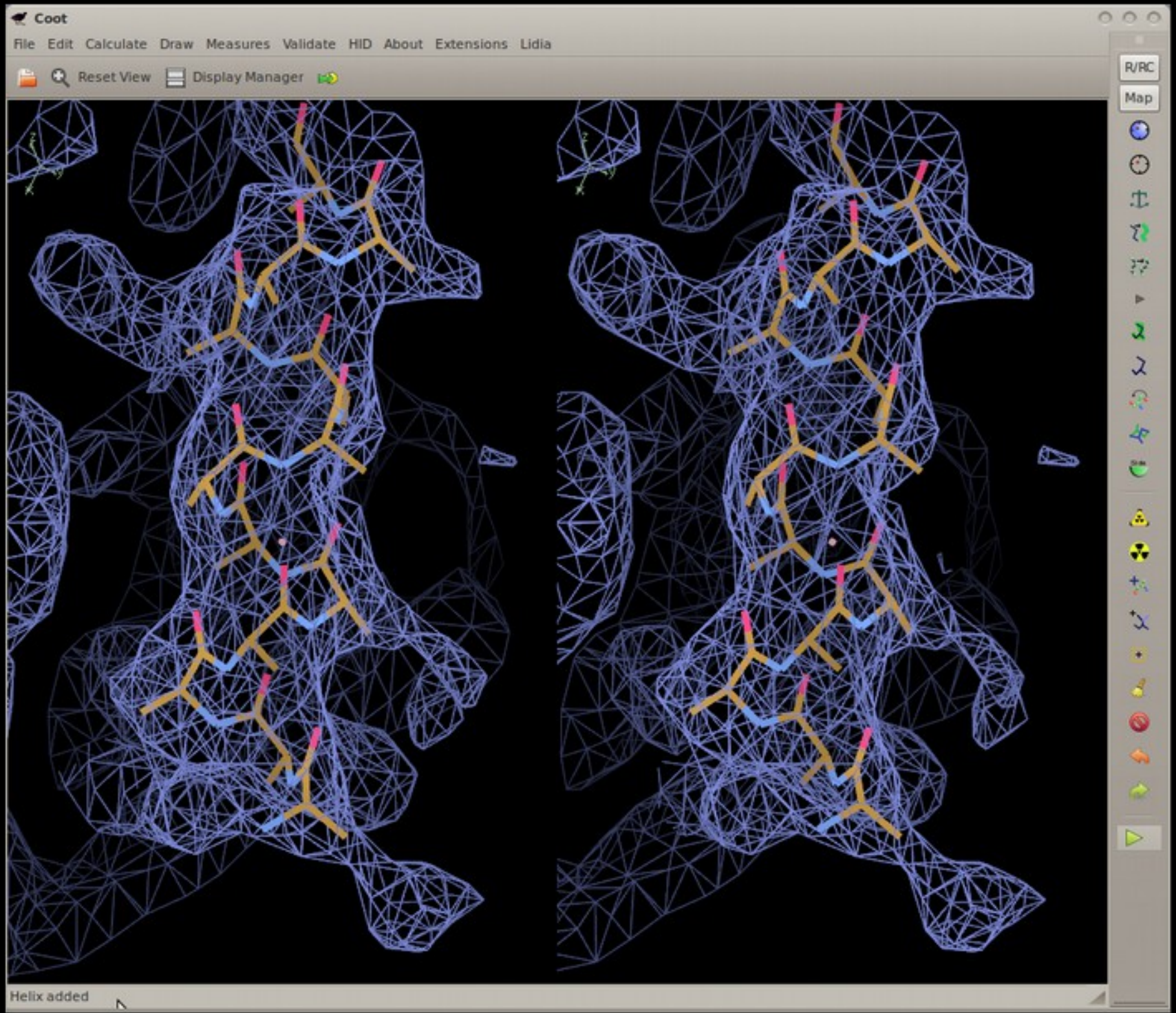


Helix Fitting
Comparing orientation hypotheses



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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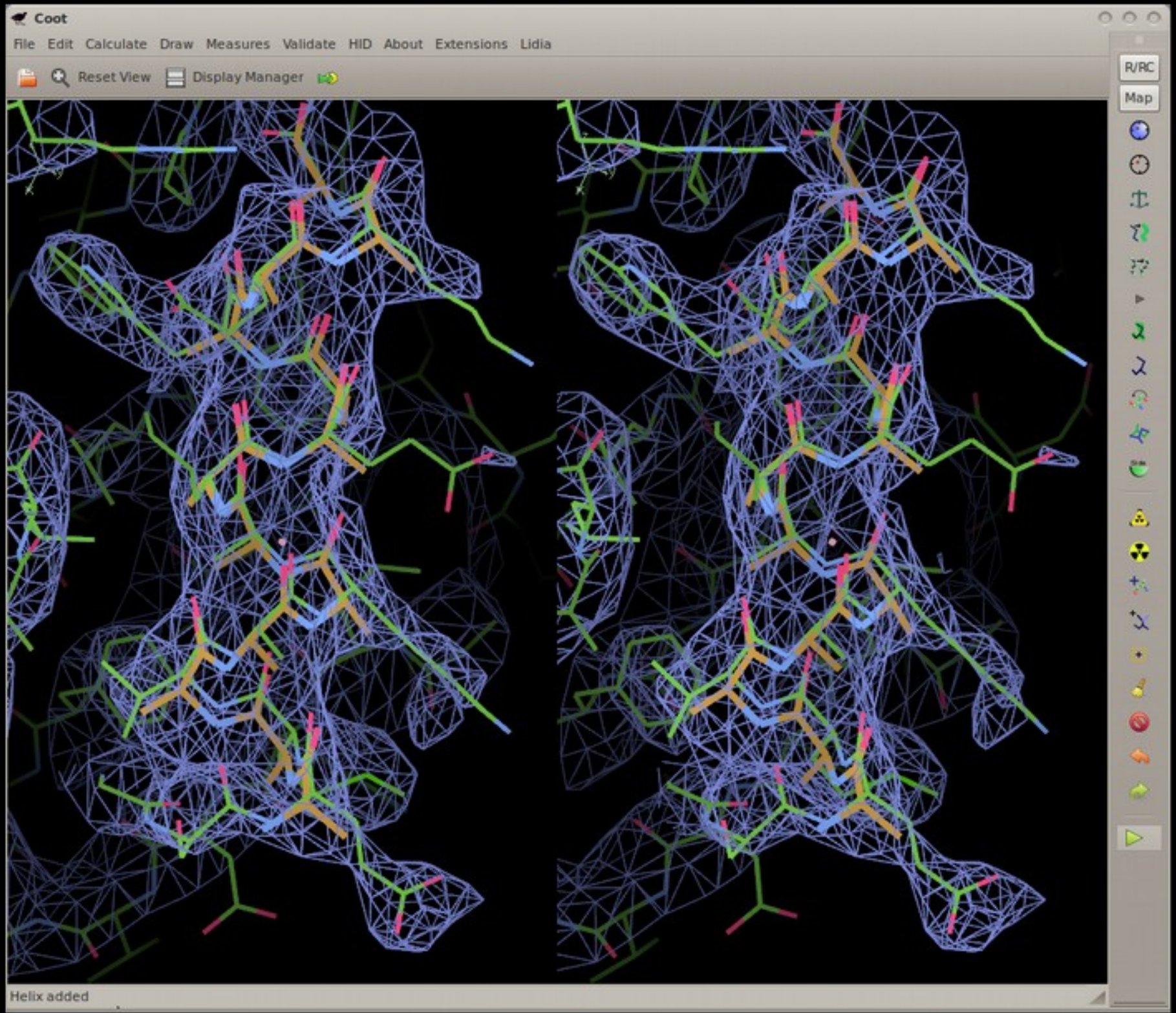
Map
Helix added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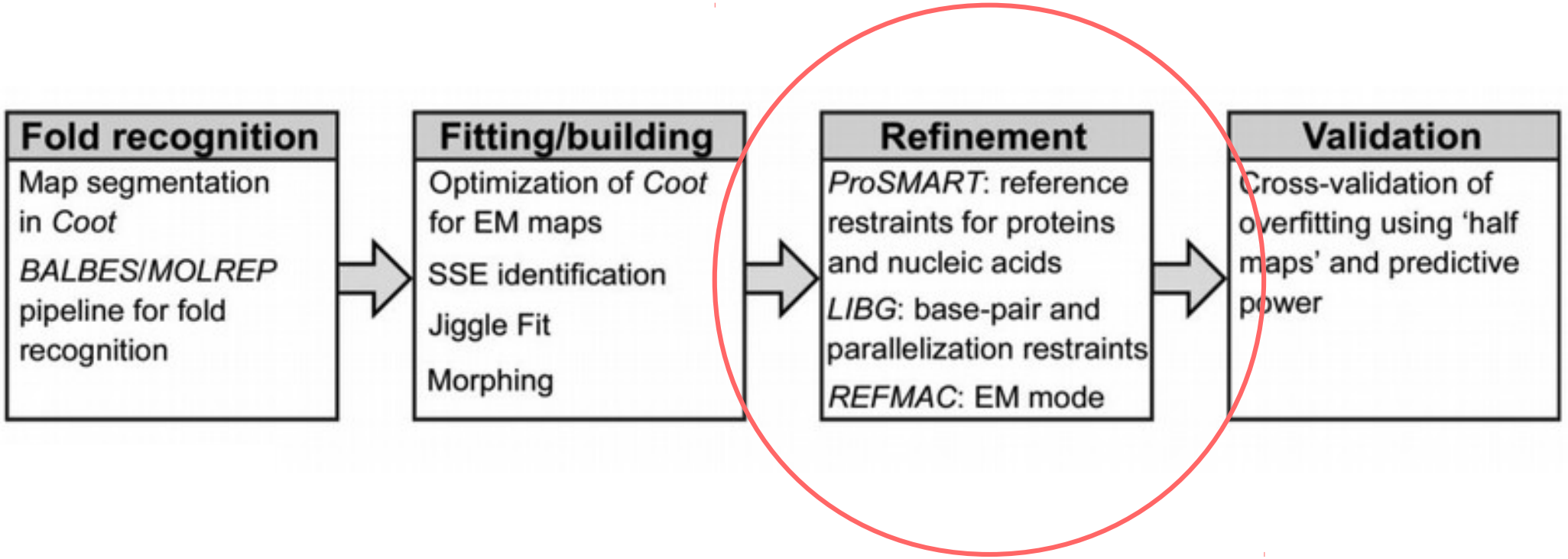
Reset View
Display Manager
R/RC
Map
Helix added

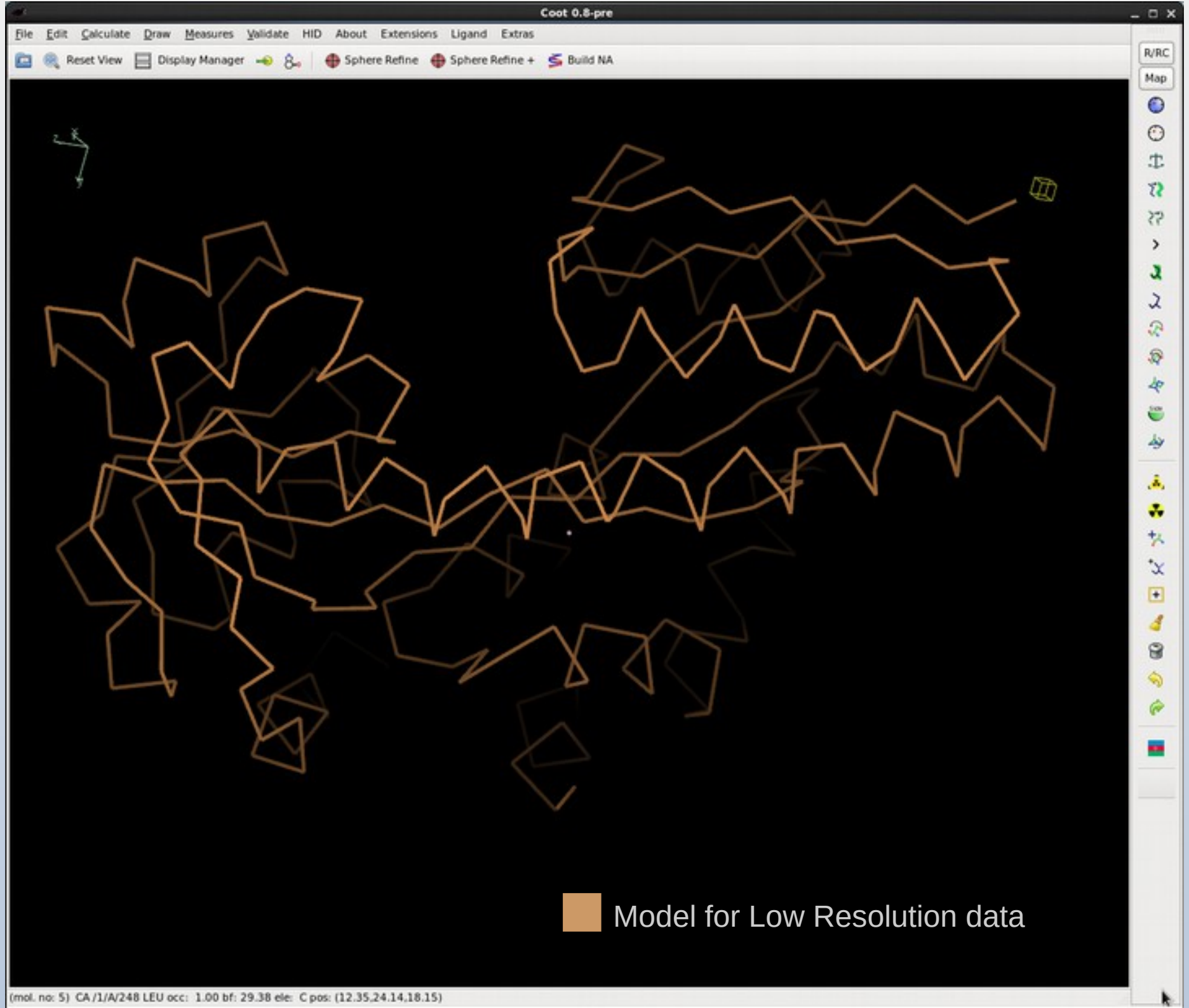
Model-Building Tools

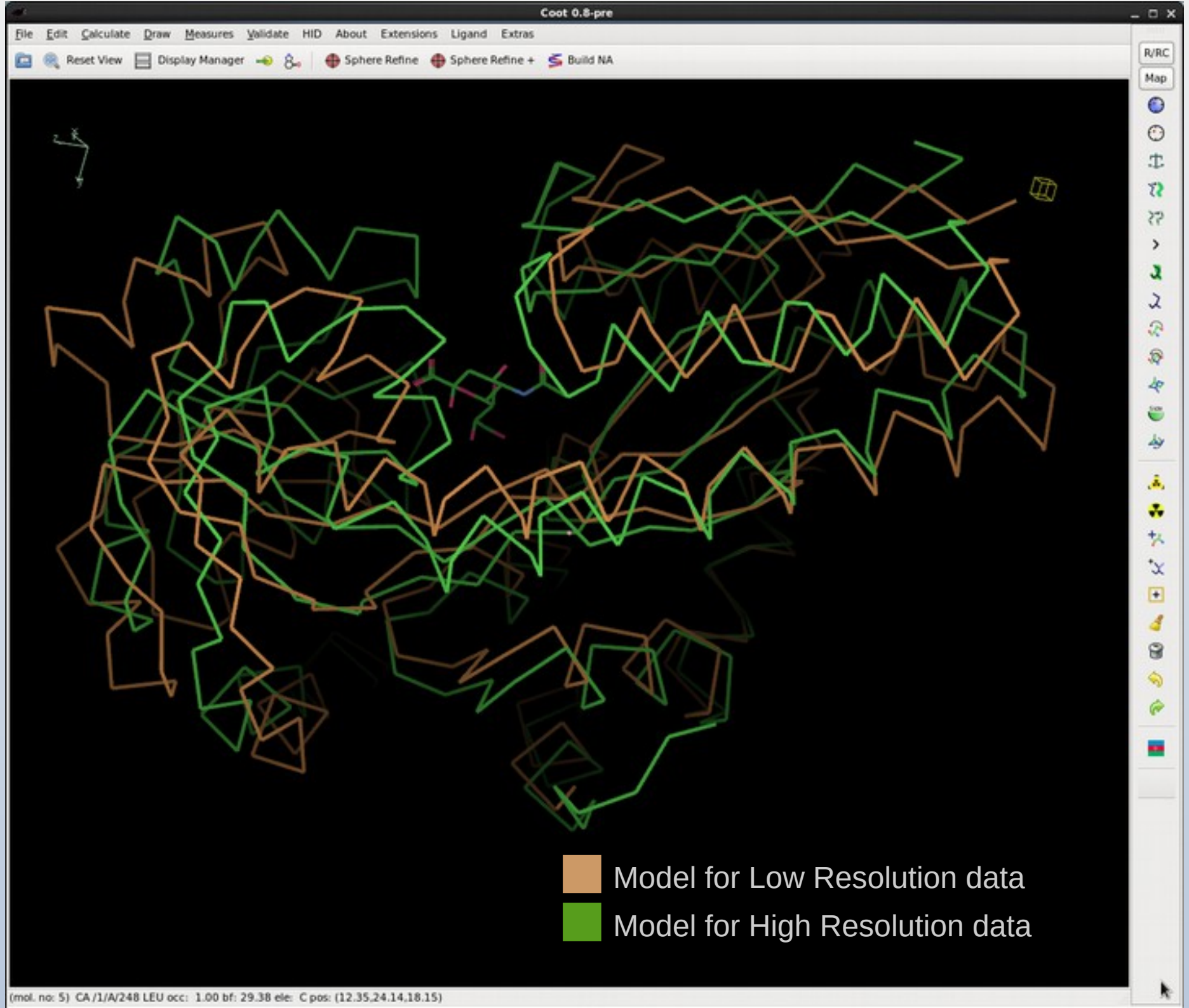
Recent Developments



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
Model for Low Resolution data
(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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Map
Model for Low Resolution data
Model for High Resolution data
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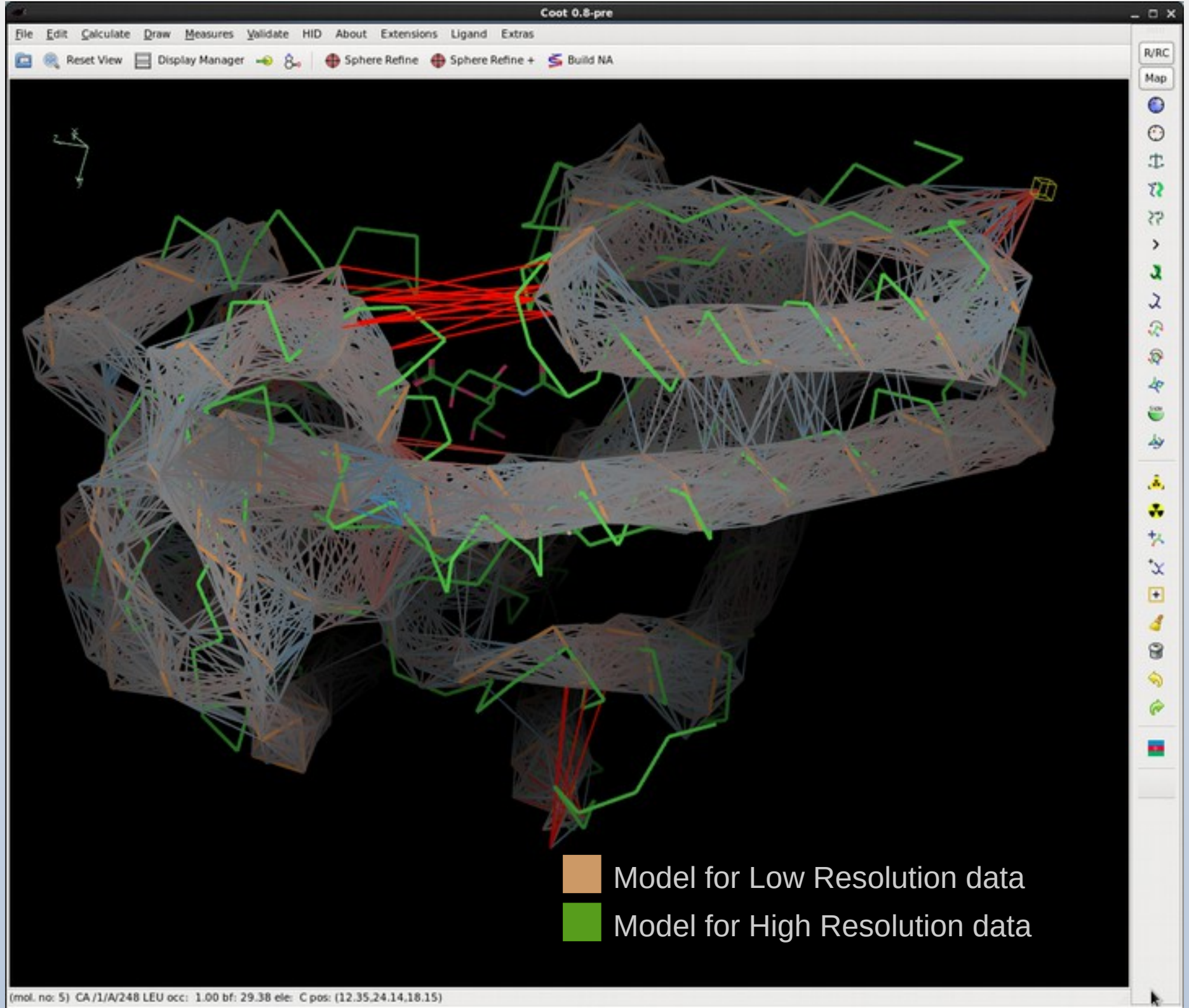
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R/RC
Map
Model for Low Resolution data
Model for High Resolution data
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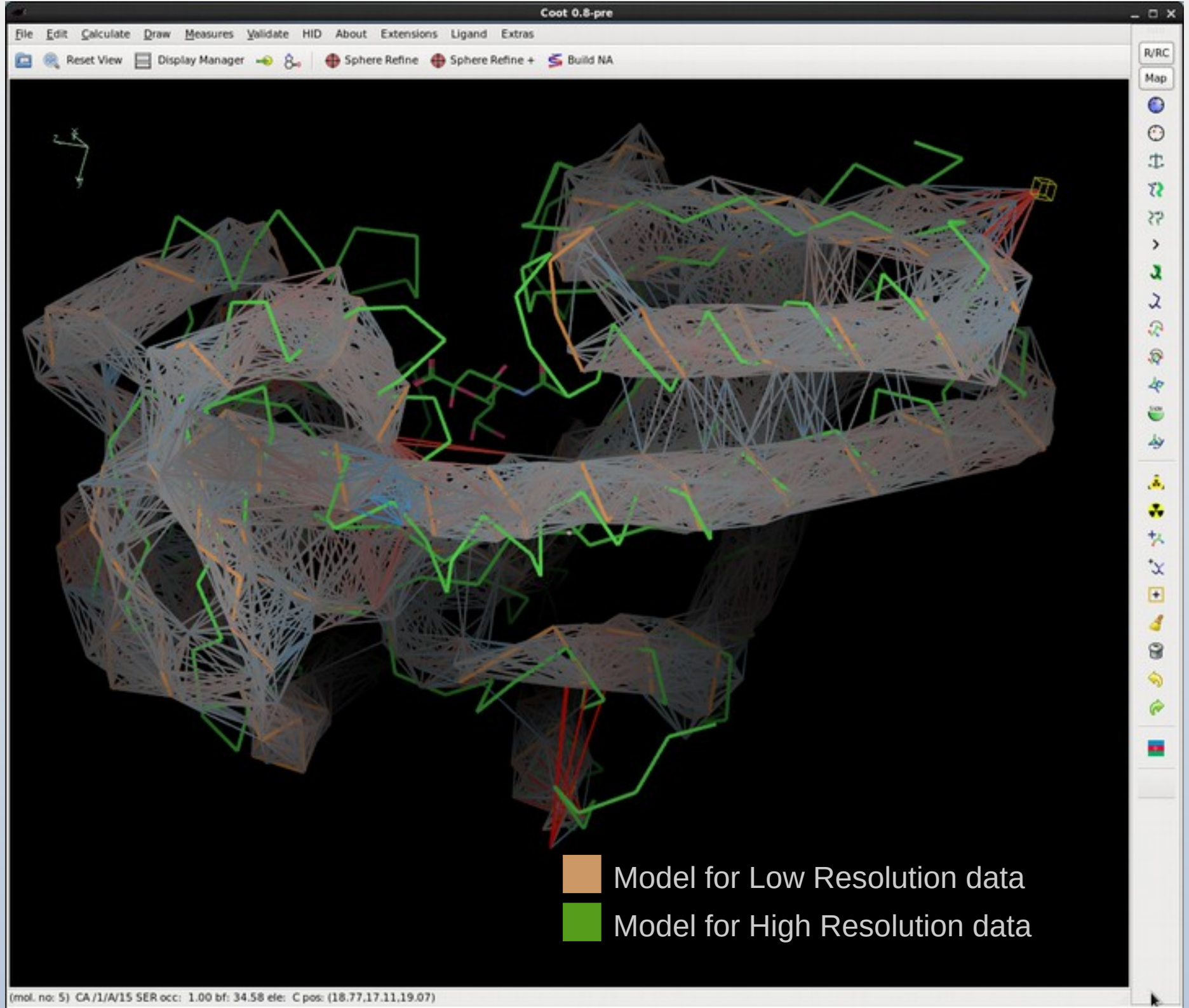
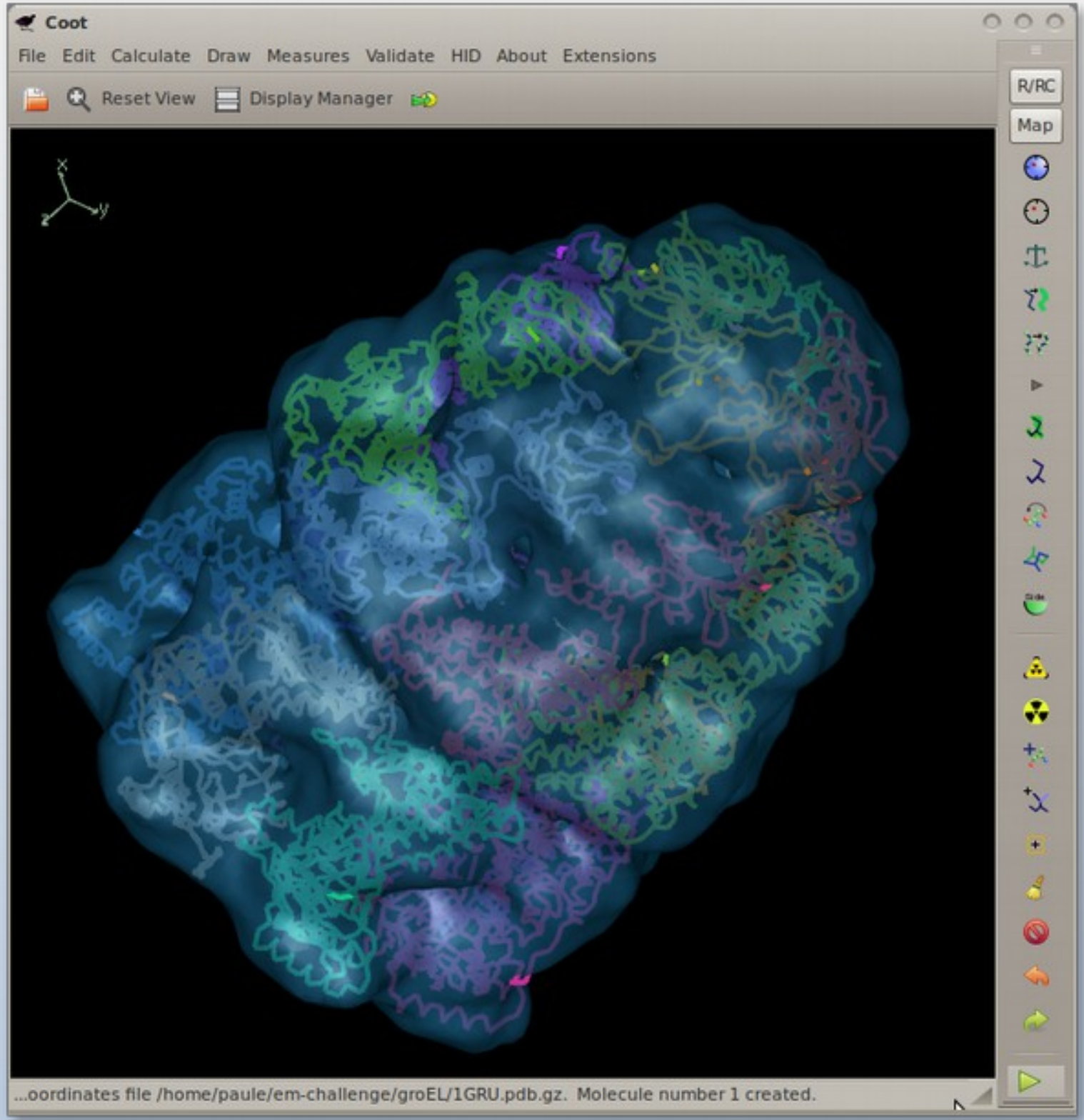

Coot 0.8-pre
File Edit Calculate Draw Measures Validate HID About Extensions Ligand Extras
Reset View
Display Manager
Sphere Refine
Sphere Refine +
Build NA
R/RC
Map
Model for Low Resolution data
Model for High Resolution data
(mol. no: 5) CA /1/A/15 SER occ: 1.00 bf: 34.58 ele: C pos: (18.77,17.11,19.07)

When Fragment Fitting Fails...

- When MOLREP, Jiggle-fit, Buccaneer fail (or is inappropriate)
- But there's still map left to interpret...
- What to do?

- Baton building


Coot
File Edit Calculate Draw Measures Validate HID About Extensions
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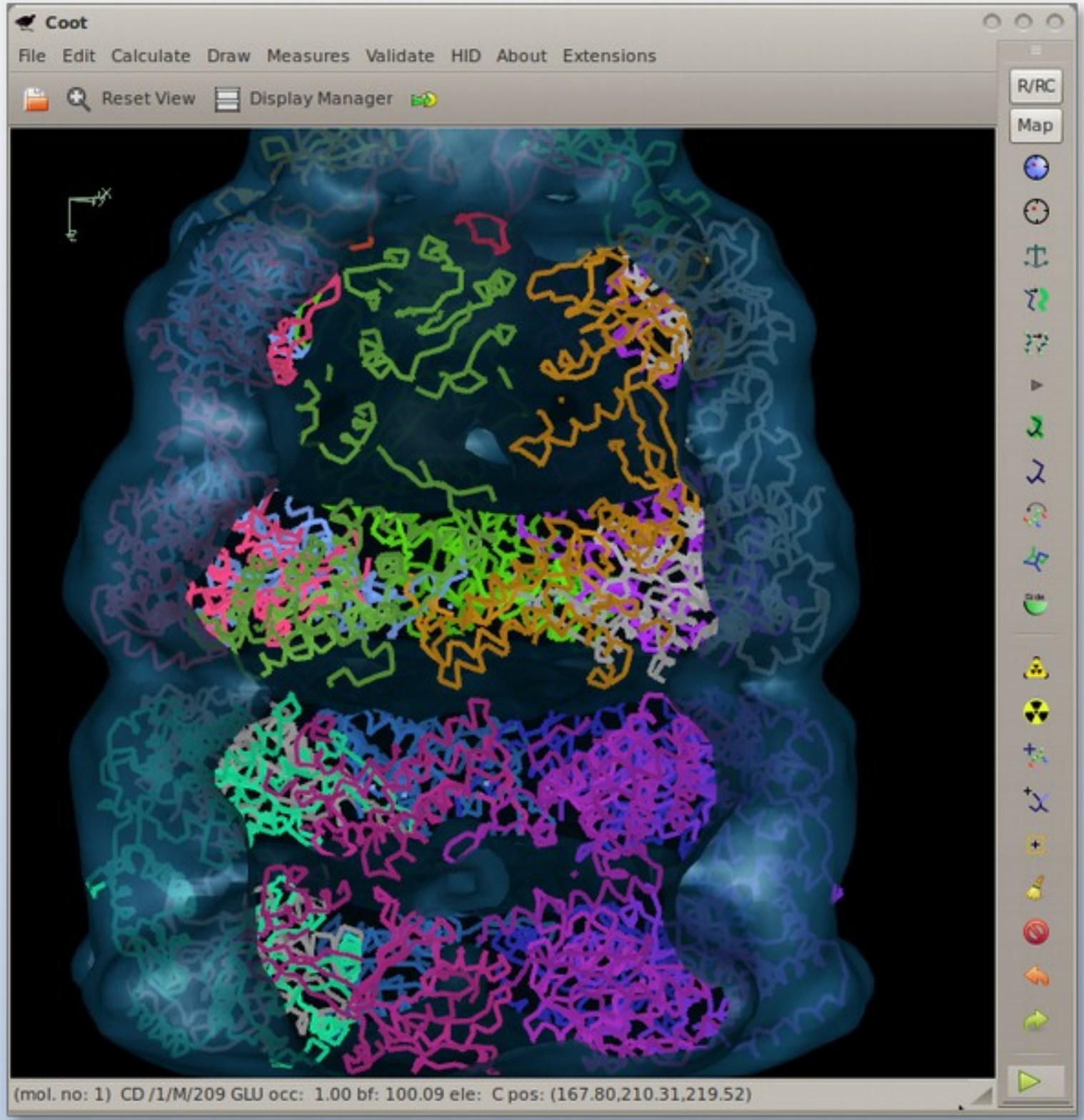
Display Manager
R/RC
Map
...oordinates file /home/paule/em-challenge/groEL/1GRU.pdb.gz. Molecule number 1 created.


Coot
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Reset View
Display Manager
R/RC
Map
(mol. no: 1) CD /1/M/209 GLU occ: 1.00 bf: 100.09 ele: C pos: (167.80,210.31,219.52)

Acknowledgements

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

- Kevin Cowan, Bernhard Lohkamp

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