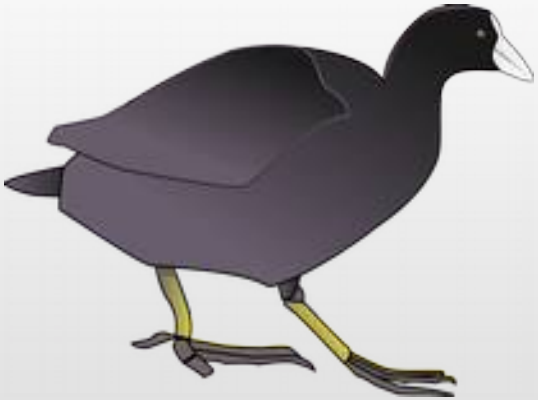
Oct 2019 CSHL



Model-Building using x-ray data

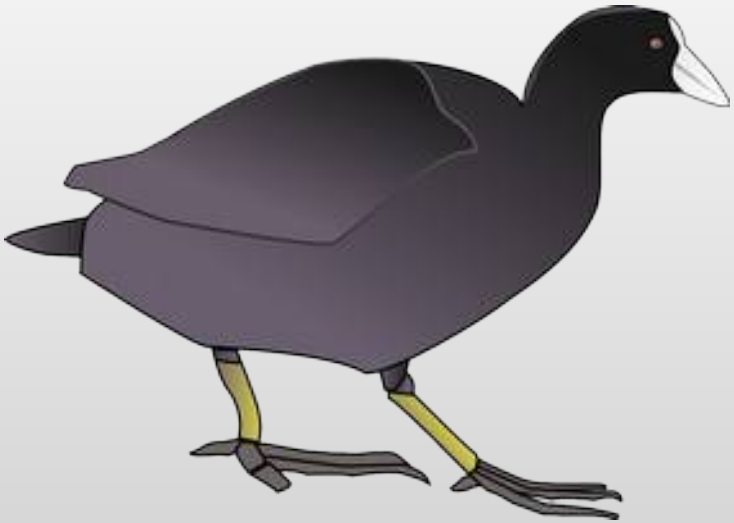
with *Coot*

Paul Emsley,
MRC Laboratory of Molecular Biology
Cambridge, UK

Note to self

- Expand rotamers, (trans/eclipsed/gauche torsions)
- Expand phi, psi
- Discuss Rama restraints

Coot Collaborators



Bernhard Lohkamp

Kevin Cowtan

Eugene Krissinel

Stuart McNicholas

Martin Noble

Alexei Vagin

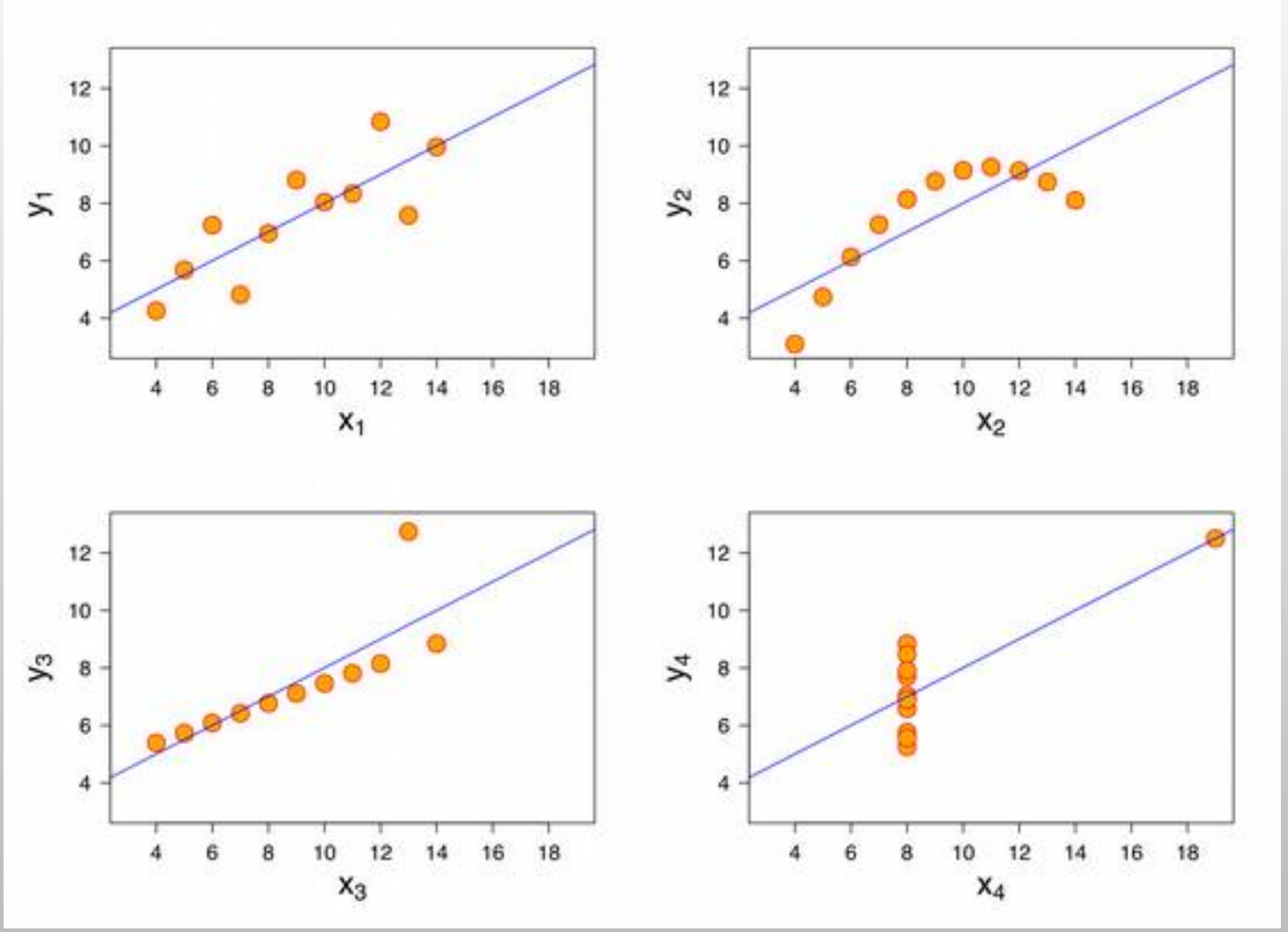
A bit of context

- Why use 3D graphics?

Summary Statistics

- Are useful, but don't tell the whole story
- Let's say we have 10 data points
  - X mean 9
  - Y mean 7.5
  - correlation 0.816
  - regression $y = 3 + \frac{1}{2} x$

View Your Data and Model

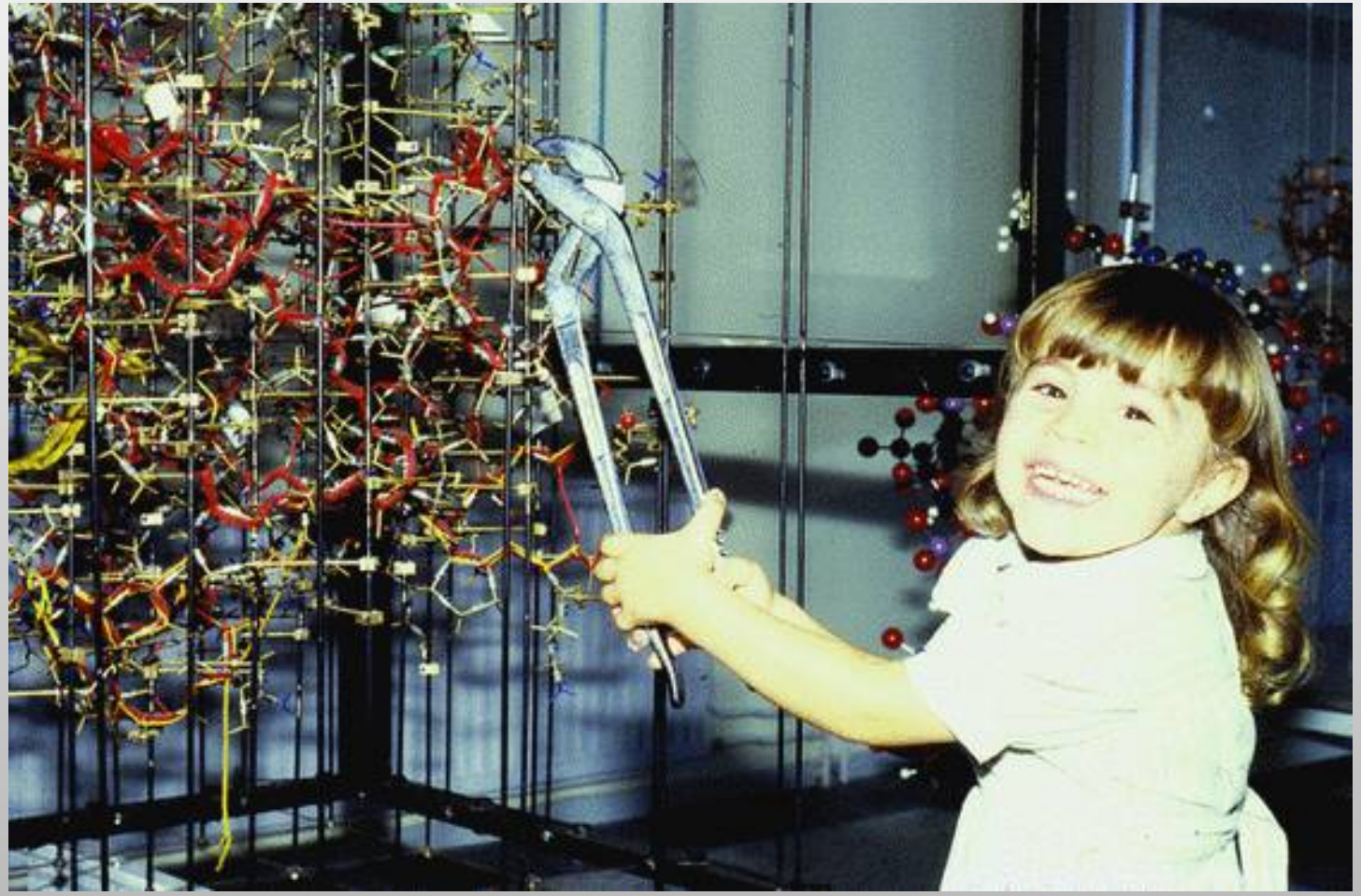


Anscombe's Quartet

“Manual Model Building”

DCH building first insulin model

Kendrew wire model of alcohol dehydrogenase that is about to undergo a round of rebuilding by Maelle Cambillau



T. Alwyn Jones (2004)

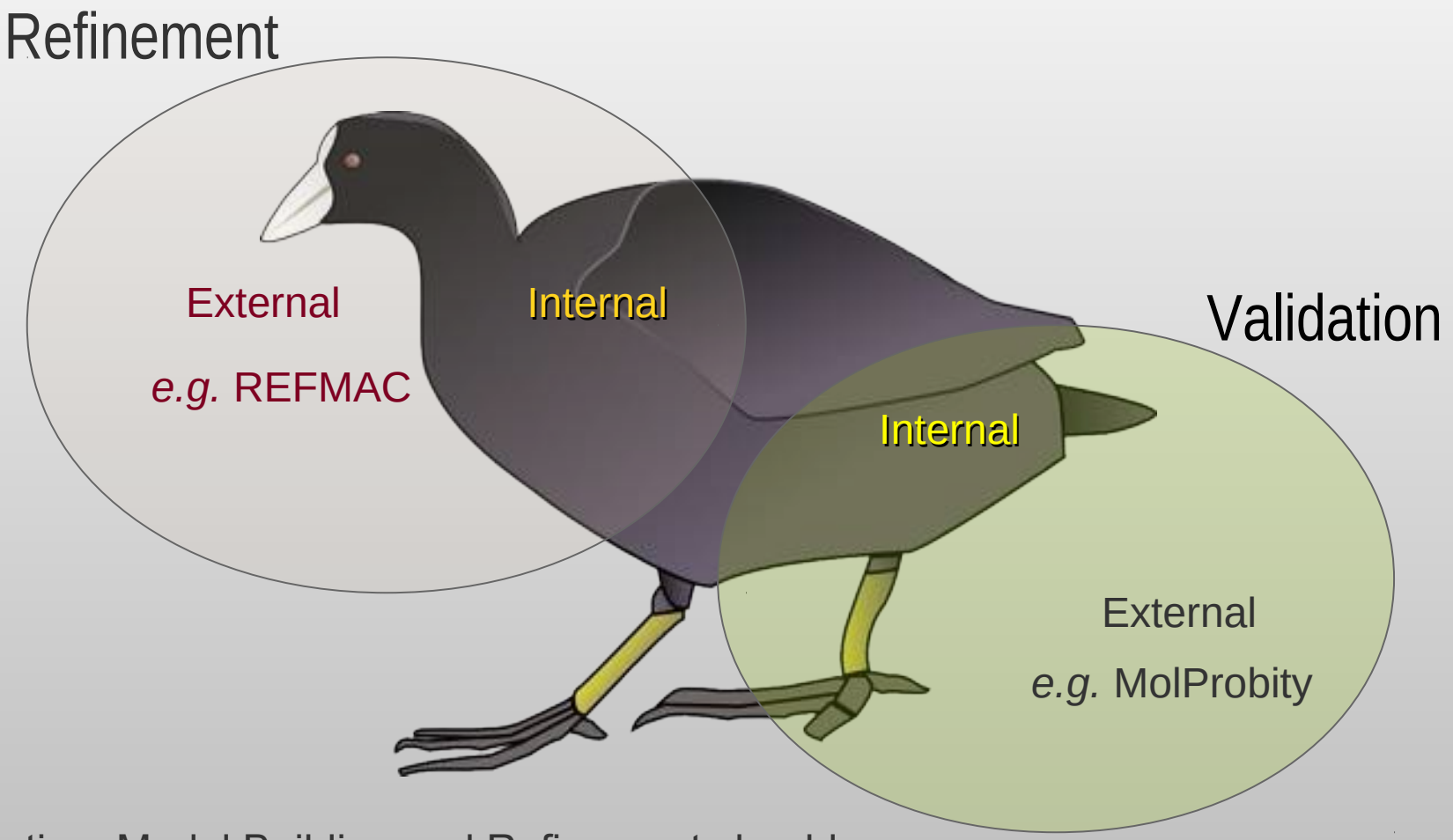
Coot

- Molecular Graphics application
  - Protein Crystallographic model-building tools
  - Designed to “fill the gap” where automatic methods fail
    - (generally, we don't use molecular graphics programs to do what automatic methods can do)
- Interface to other programs: SHELXL, Refmac, Libcheck, Probe&Reduce (Molprobity), EBI, EDS, Povray… and others

But Why Bother?

- Automated model-building for complete models is still impossible
  - It takes a brain to validate
- Concerted correction/improvement of a model is difficult on the larger scale

Feature Integration



Validation, Model Building and Refinement should be used together

What is “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 minimizer (BFGS derivative)
  - Based on mmCIF standard dictionary
  - Minimizing bonds, angles, planes, non-bonded contacts, torsions, chiral volumes
  - Additional user-defined restraints,
    - •secondary structure restraints
    - •homologous protein local environment restraints
- Provides “interactive refinement”

Refinement in *Coot* has been extended in several ways...

What prior geometric information do we have?

- We know chemistry....
  - We know bond lengths and uncertainties
  - We know bond angles and uncertainties
  - We know the chiral centres
  - We know which atoms should lie in a plane
  - We know (more or less) about torsions
- We combine the gradients from the data with those from molecular mechanics in the minimisation

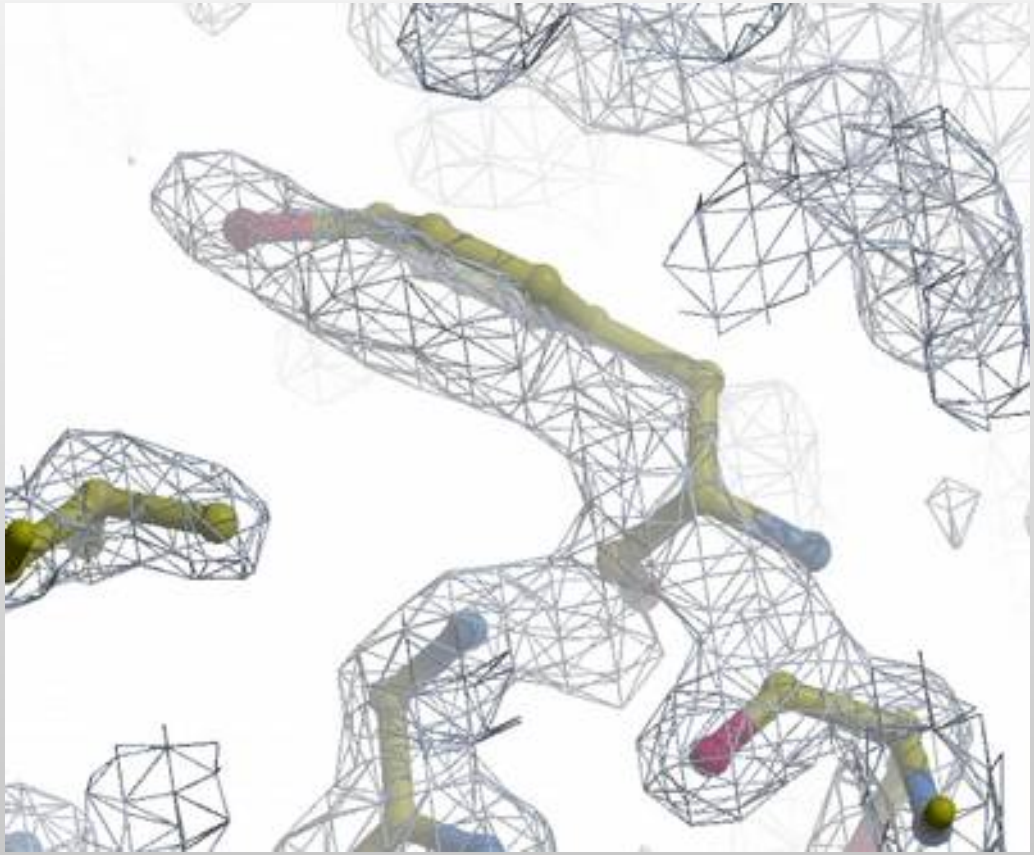
REFMAC Monomer Library
chem_comp_bond

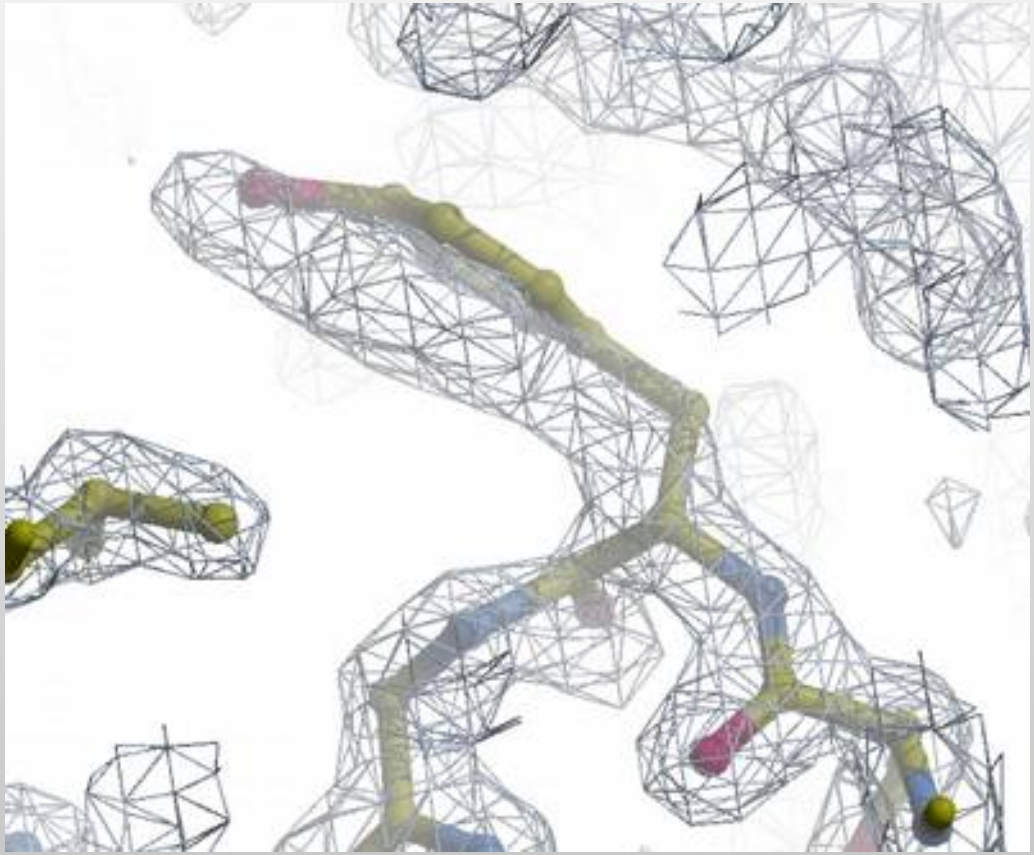
```
loop_
_chem_comp_bond.comp_id
_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
_chem_comp_bond.type
_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_esd
 ALA      N    H       single     0.860    0.020
 ALA      N    CA      single     1.458    0.019
 ALA      CA   HA      single     0.980    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
```

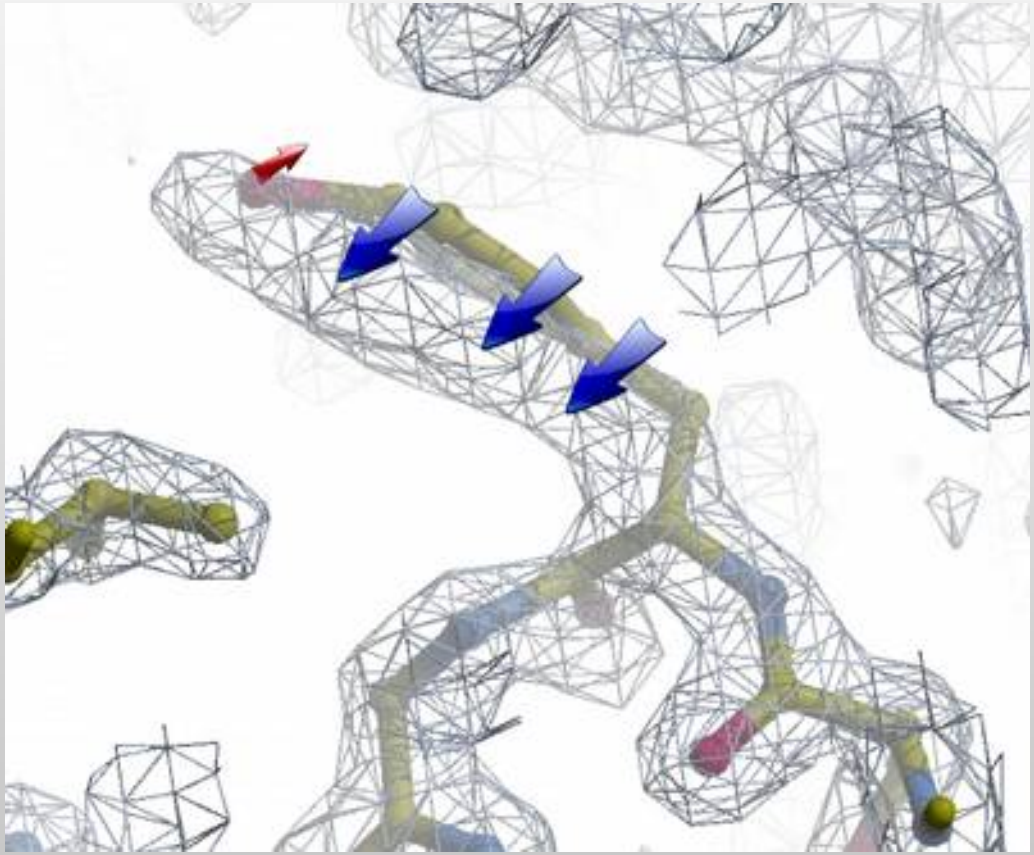
APPENDIX *A*
Regularization and refinement derivatives

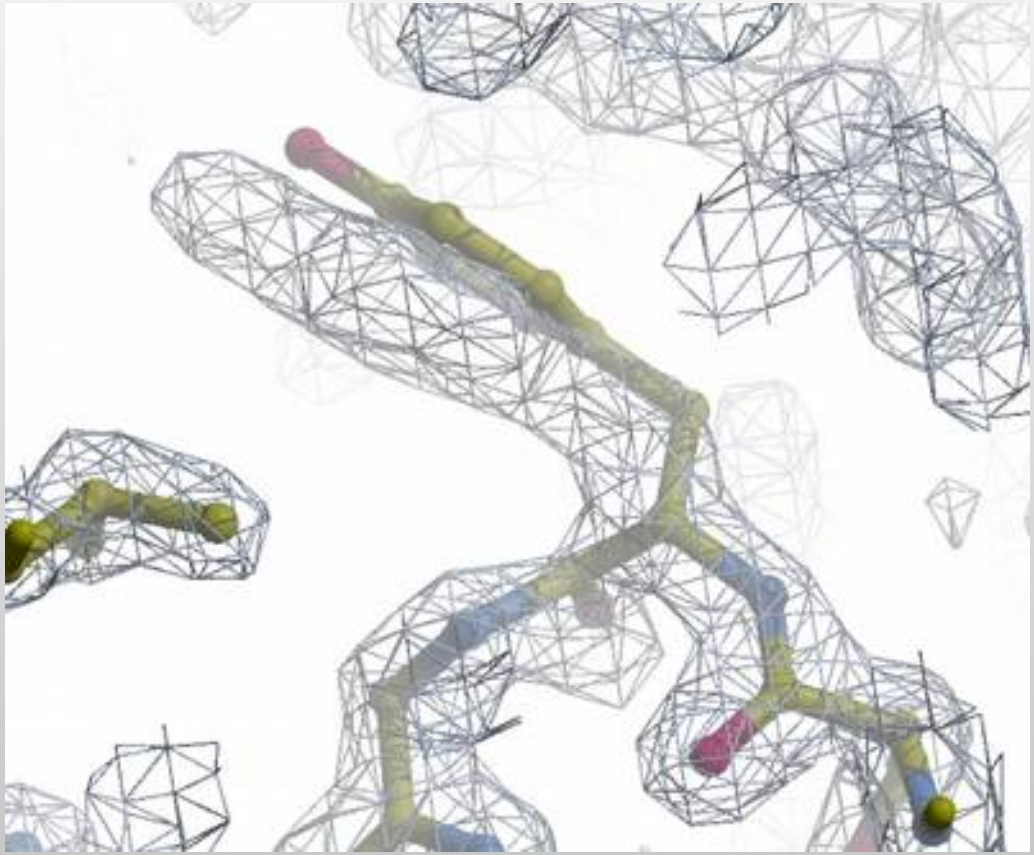
The function that we are trying to minimize is S, where

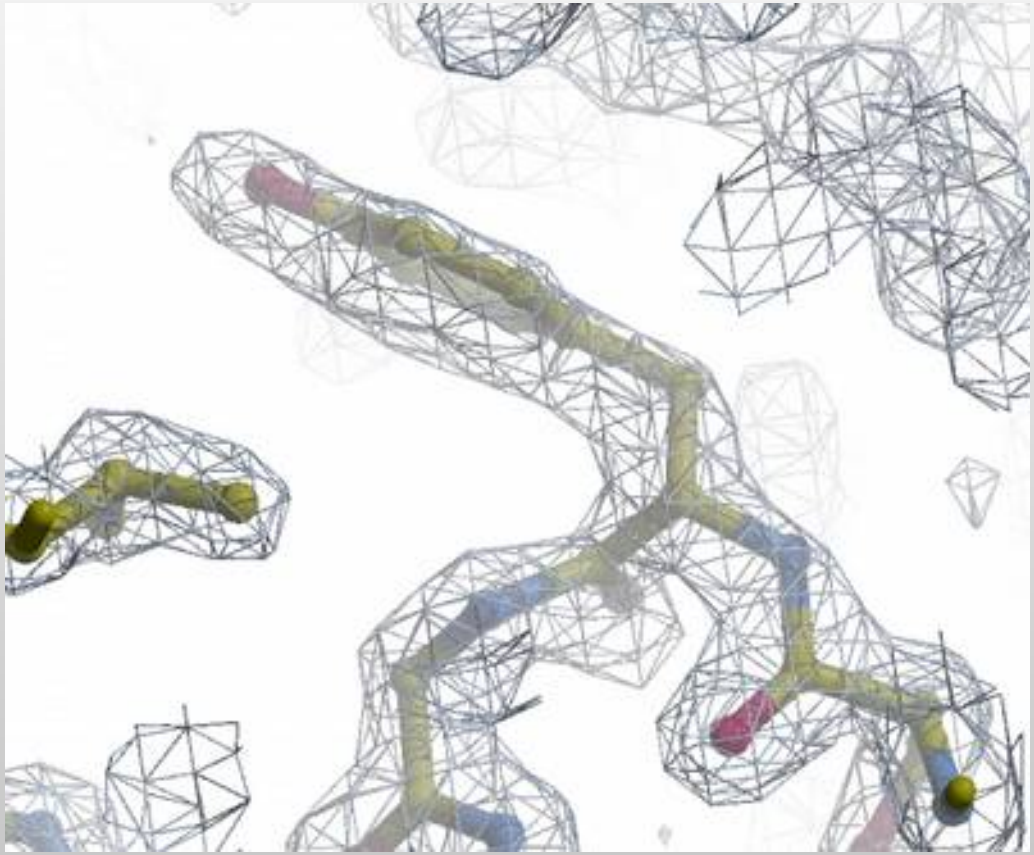
$$S = S_{\text{bond}} + S_{\text{angle}} + S_{\text{torsion}} + S_{\text{plane}} + S_{\text{nbc}} + S_{\text{chiral}}$$

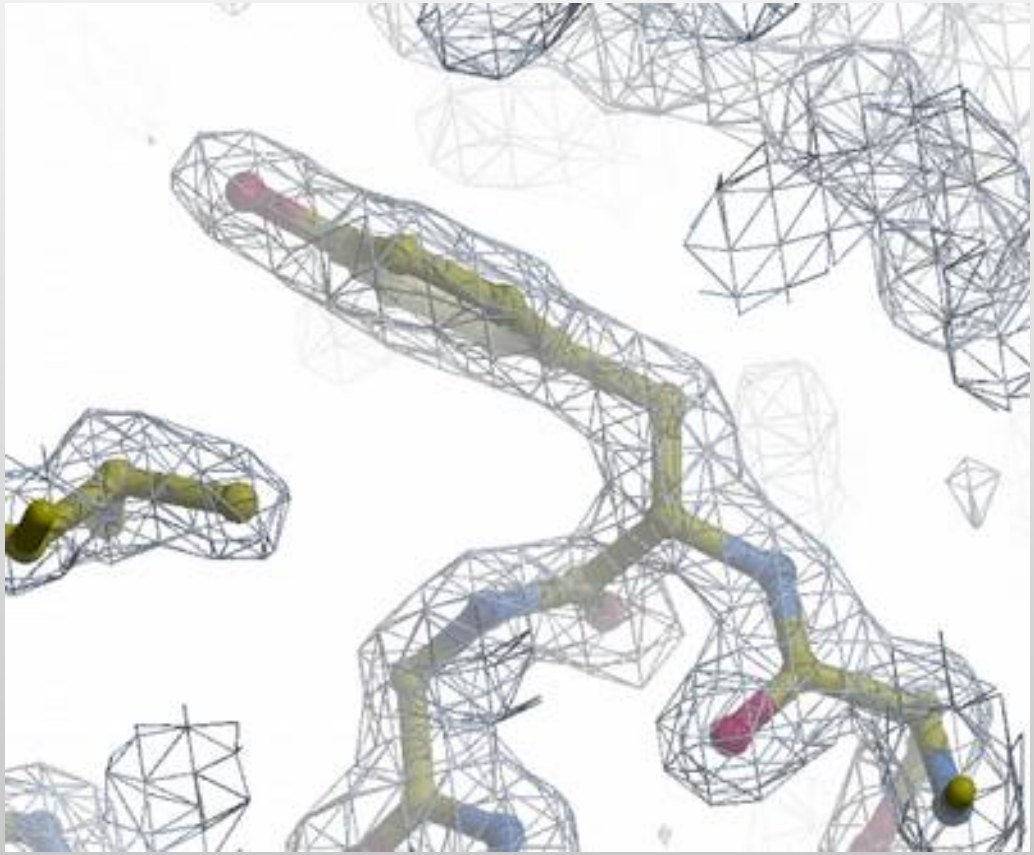








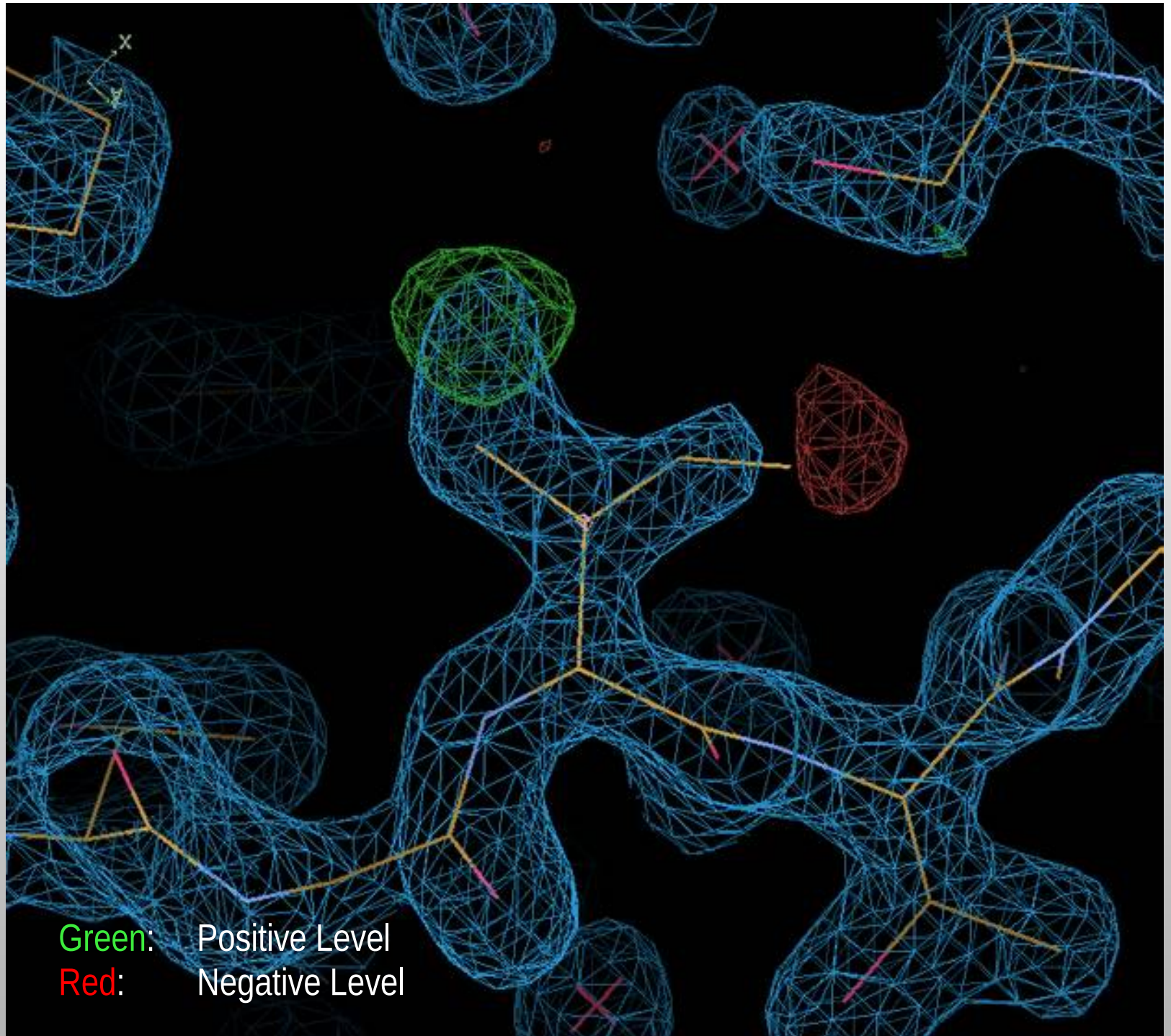




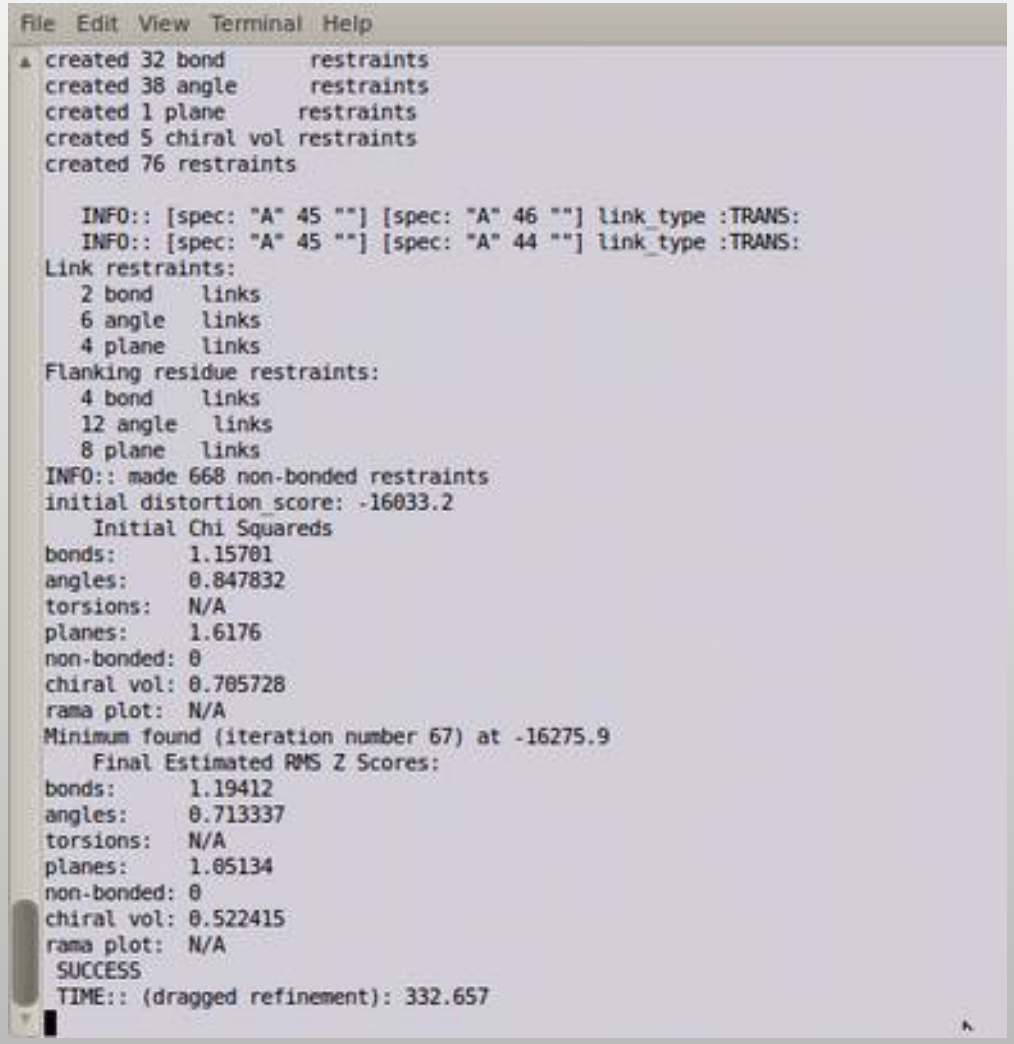
Different types of electron density maps

- "Experimental" maps
  - maps that result directly from the crystallographic data analysis: MIR, MAD, SAD
- Direct Maps:
  - where the atoms are
- Coefficients F_o-F_c ("difference map")
  - Identifies errors in the model. Locations in space where there should be atoms show positive peaks, while locations where the model contains atoms that should not be there show negative peaks.

Morten Kjeldgaard


Green: Positive Level
Red: Negative Level

Representation of Results:

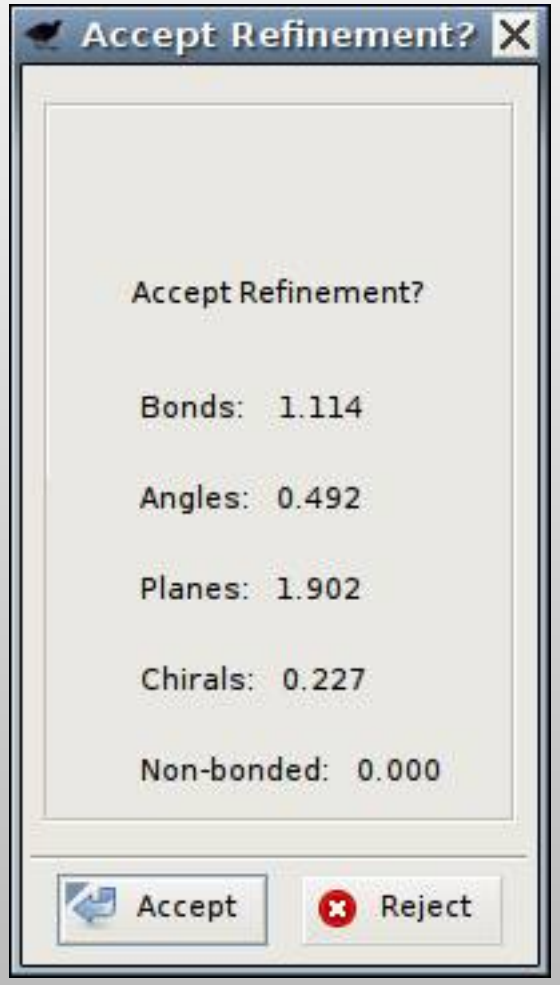


The first attempt

Student Reaction:

“Oh, I don't look at that window...”

Representation of Results:



Second attempt...

Student Reaction:

“Oh, box of meaningless numbers.

Go away”

Representation of Results: “Traffic Lights”

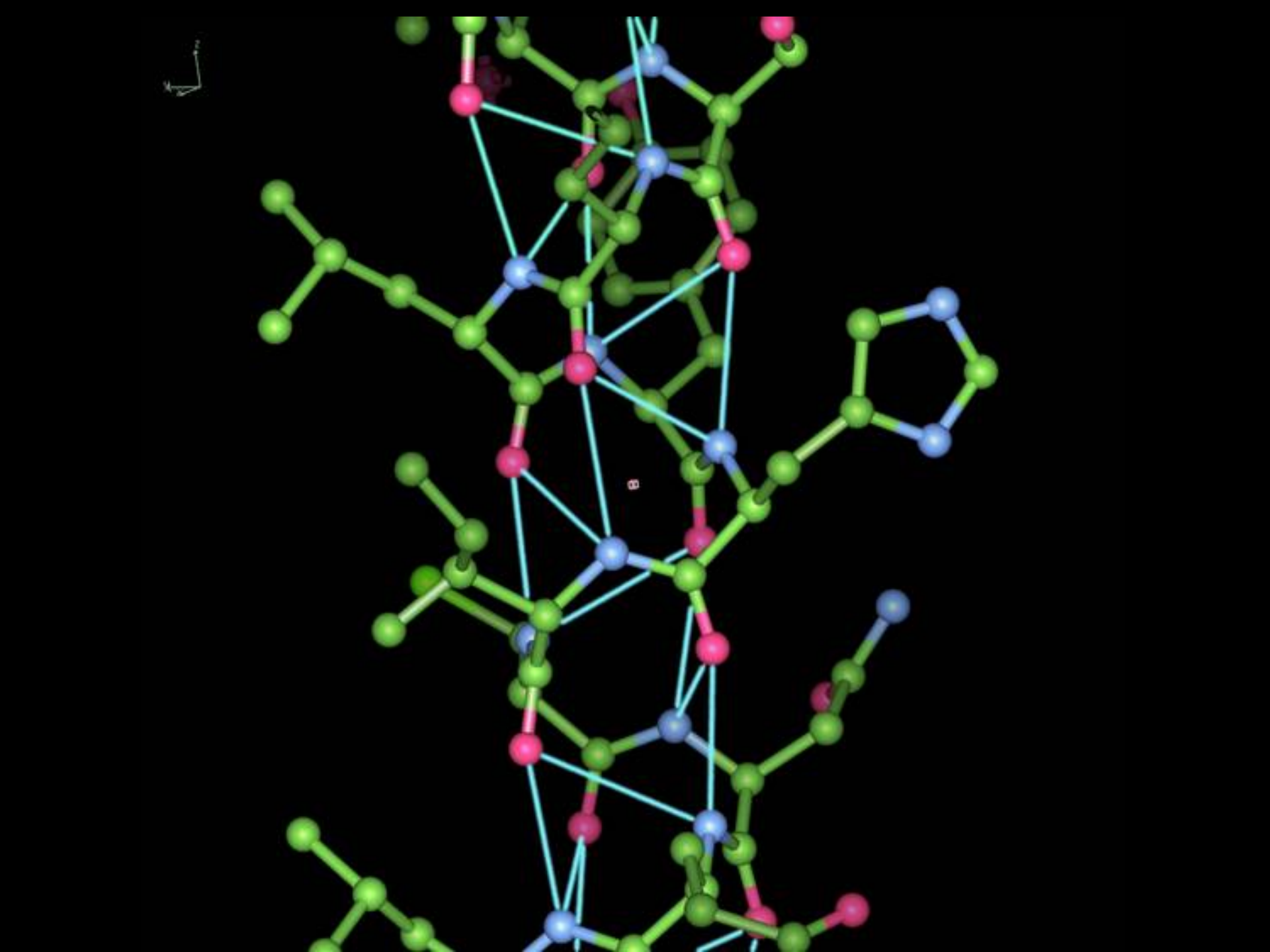
“Traffic Lights” represent the RMSd values for each of the refined geometry types

Good refinement

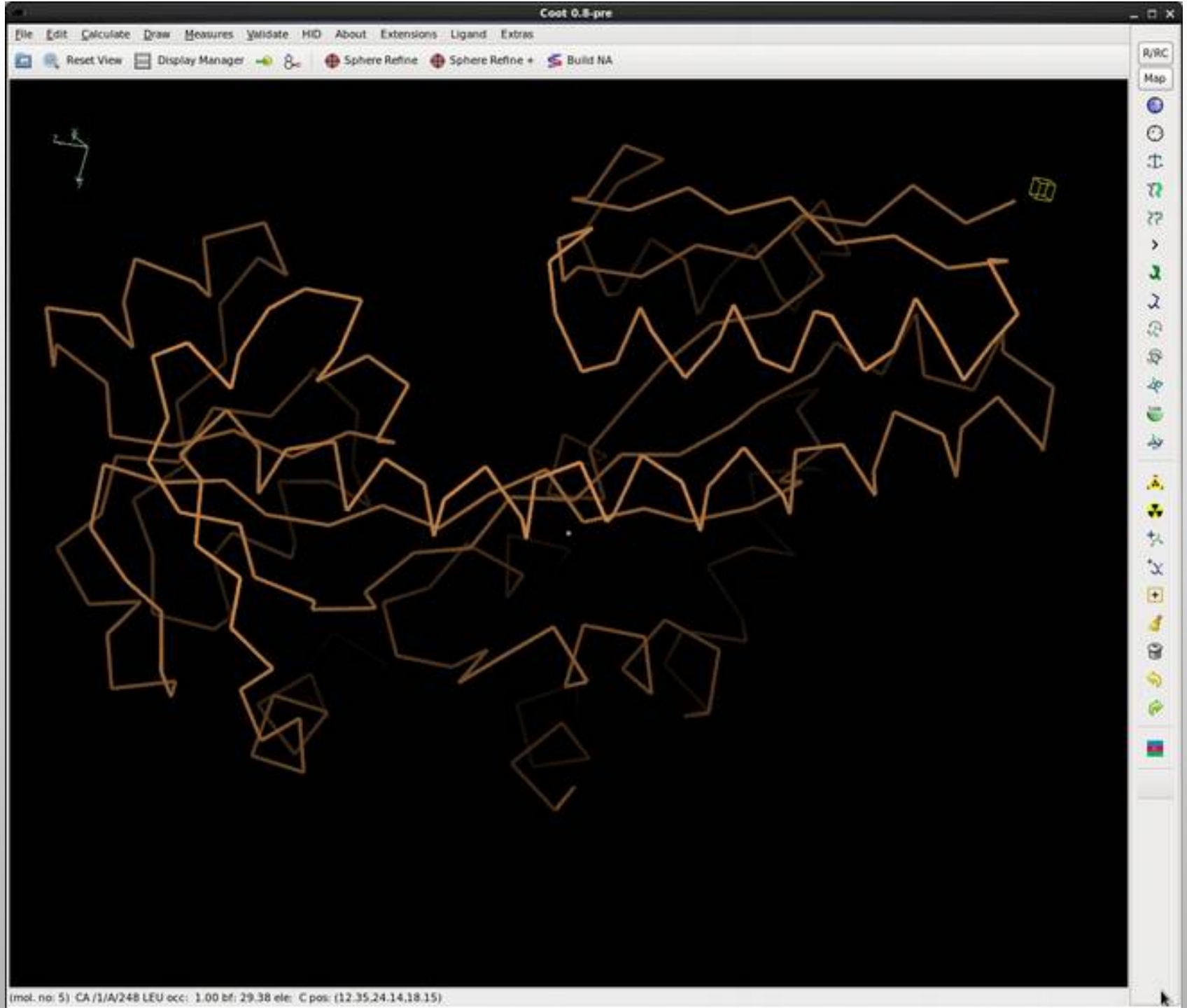
Bad refinement

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



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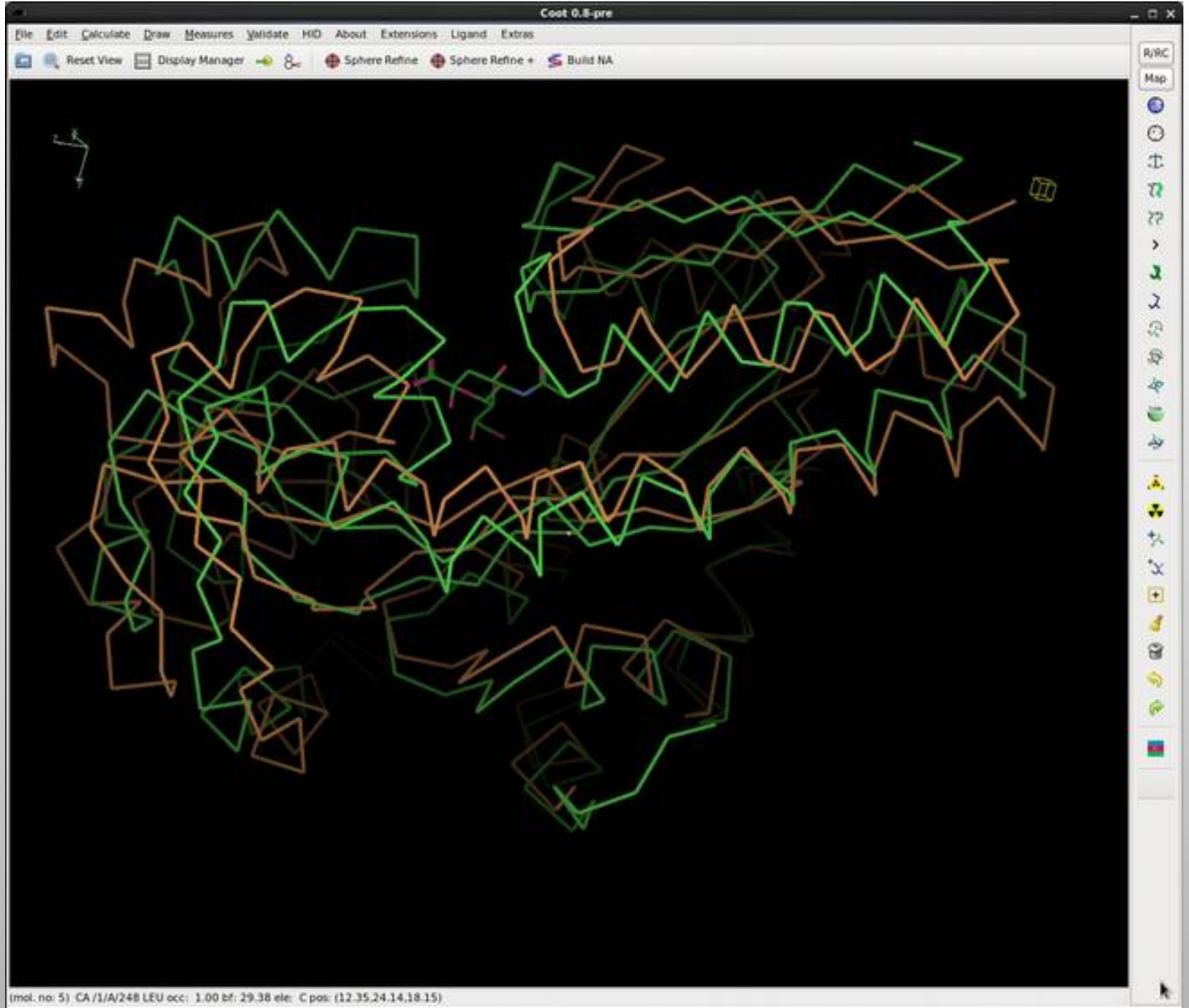
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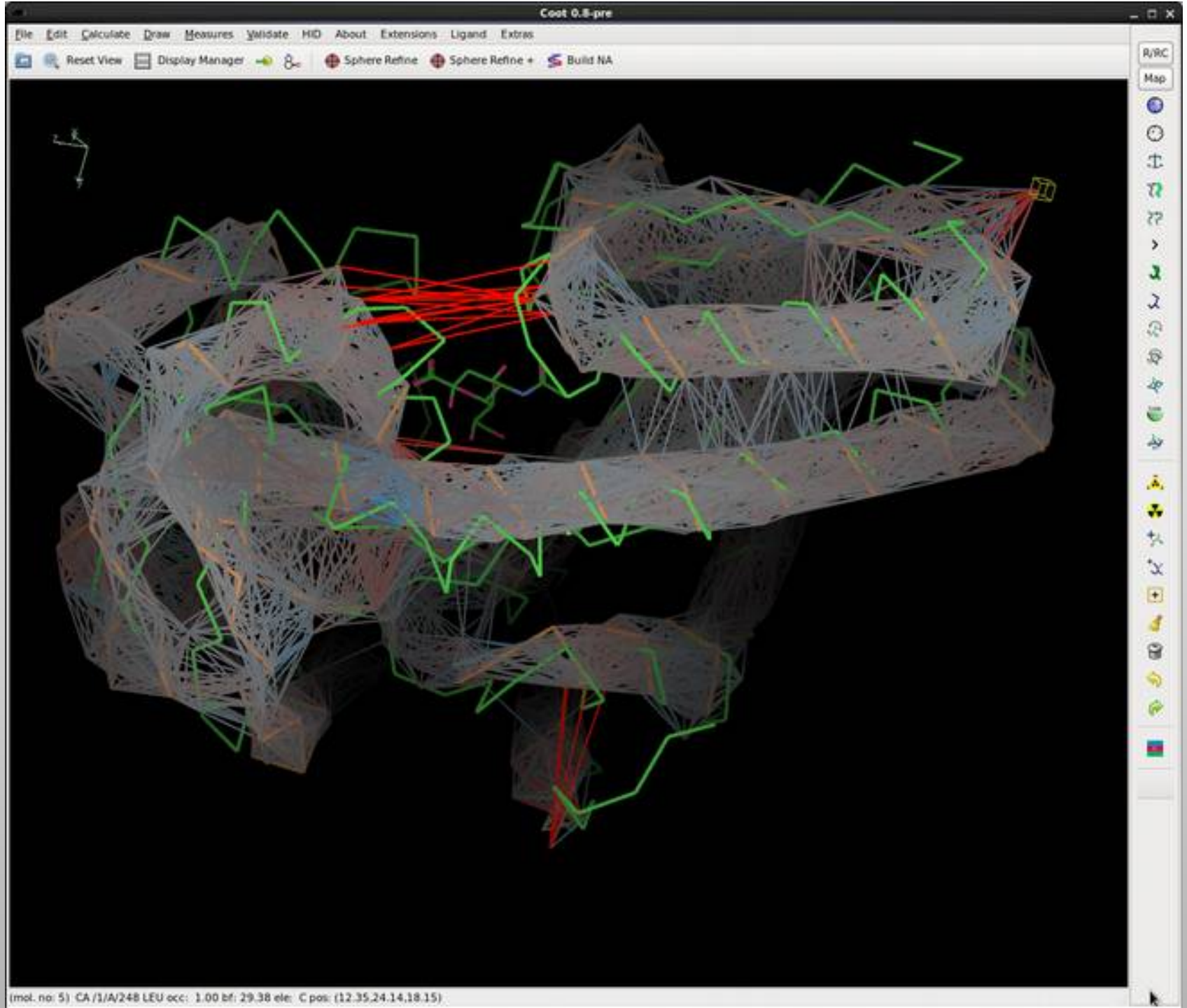
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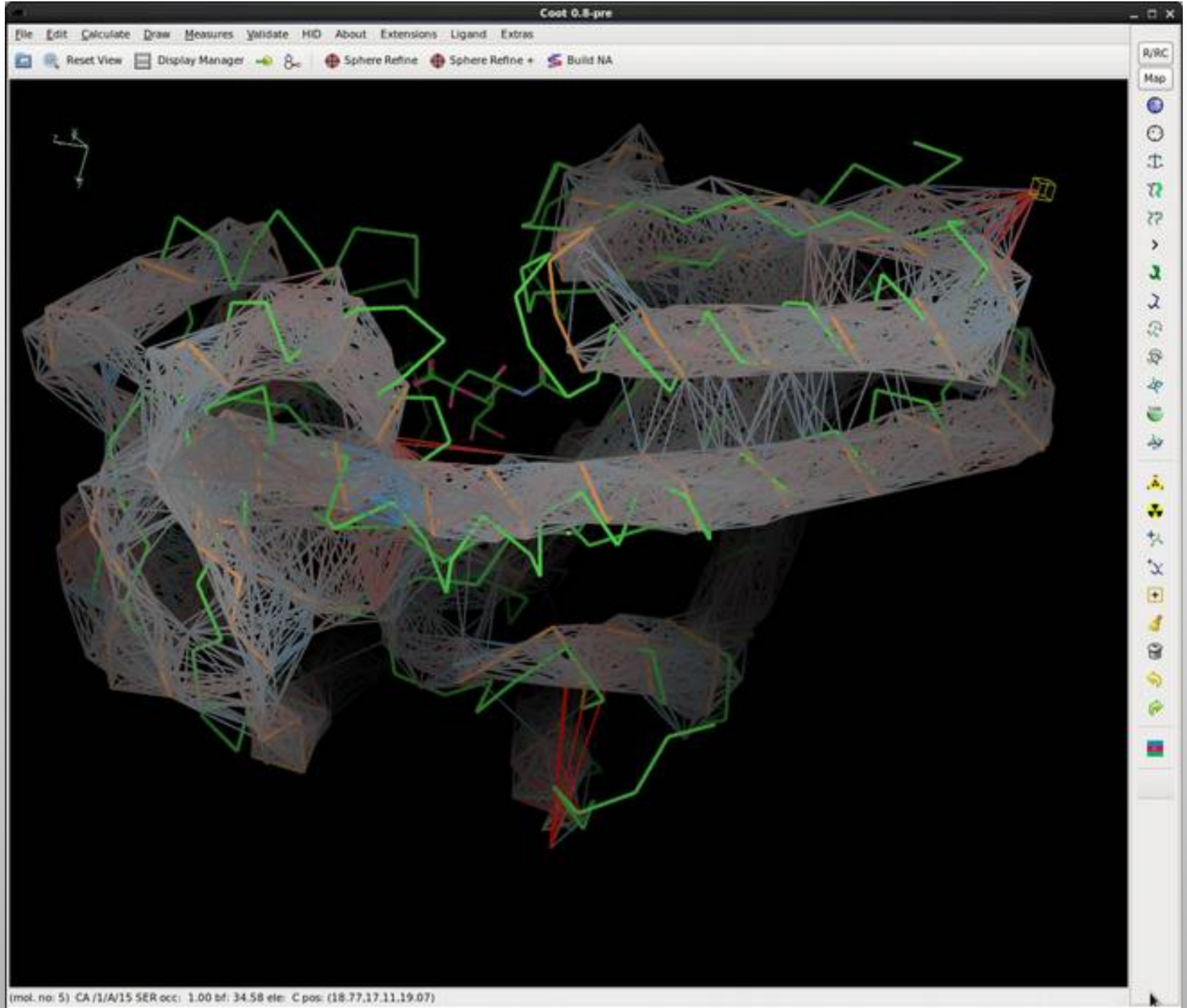
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Build NA
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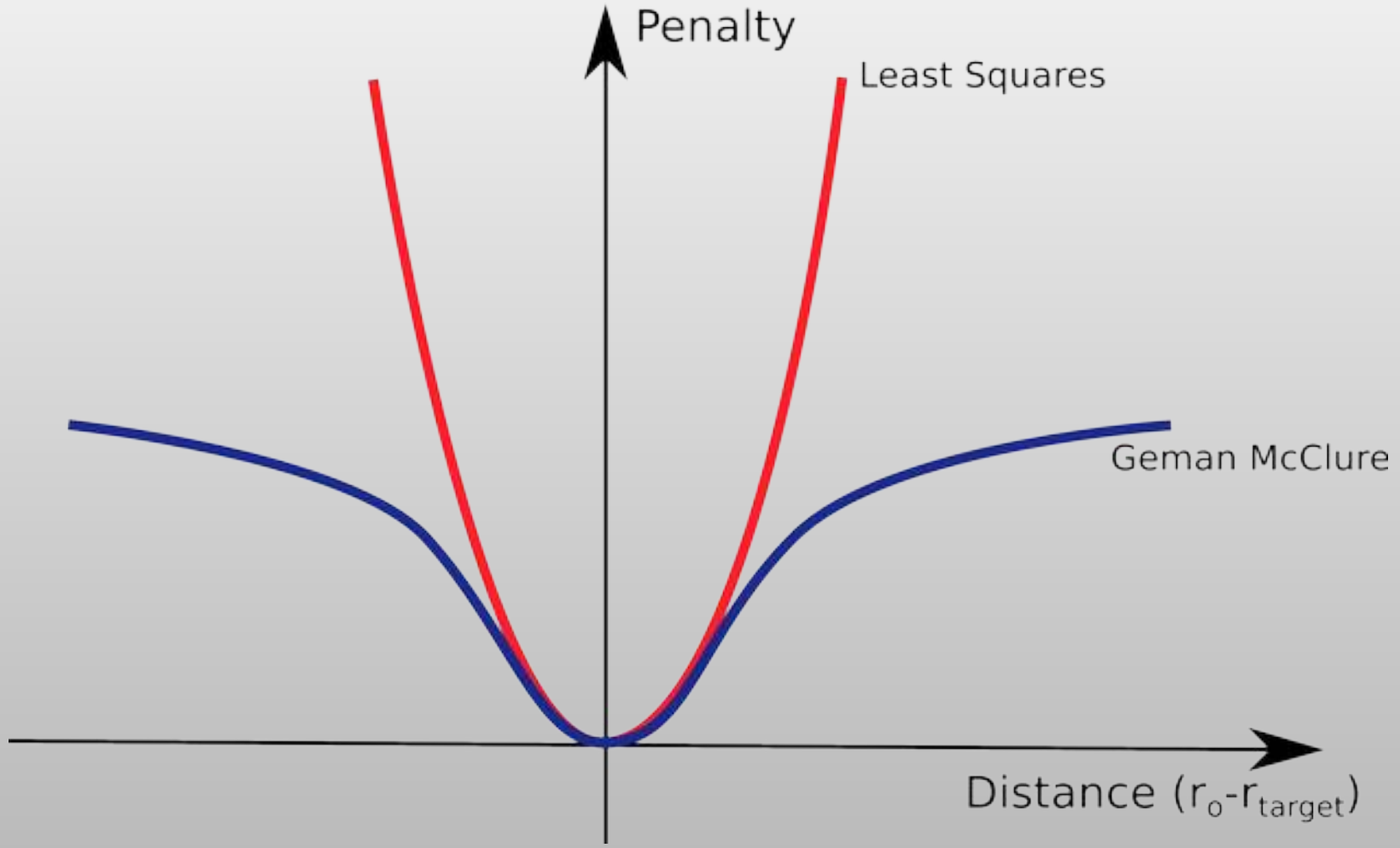
Map
(mol. no: 5) CA /1/A/15 SER occ: 1.00 bf: 34.58 ele: C pos: (18.77,17.11,19.07)

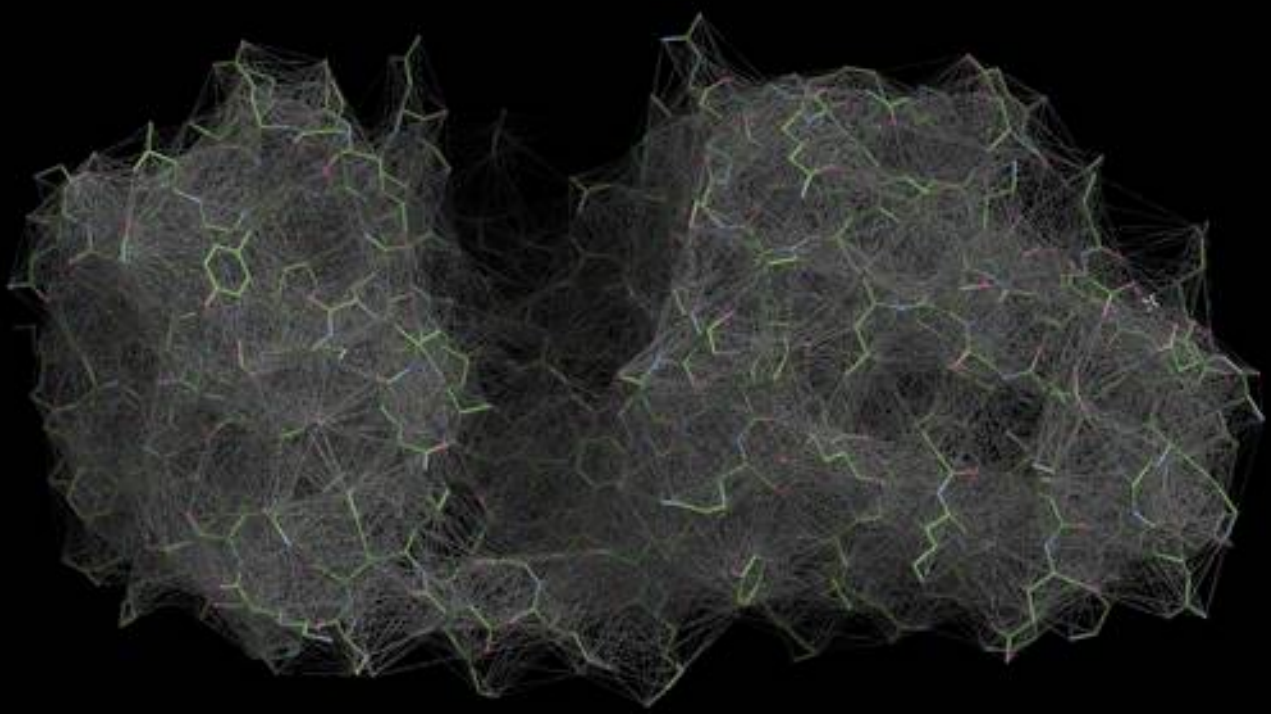
ProSMART integration

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

Modified Target Function



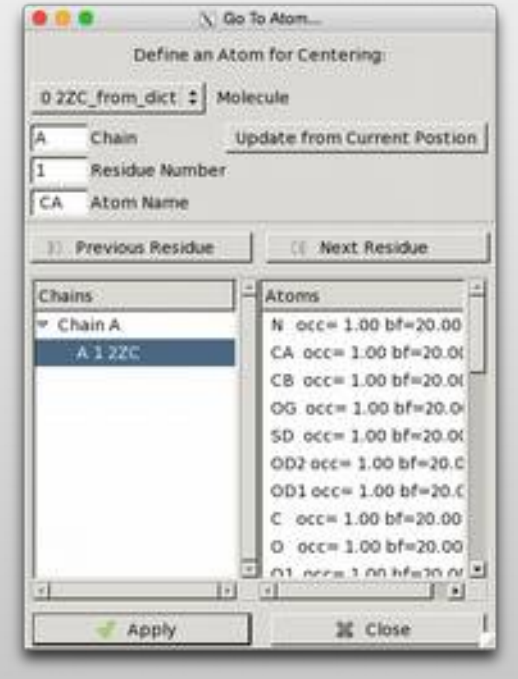
ProSMART Restraints



A note on *Coot*'s GUI

- It used to be clean
- Now lots of features have been added without much thought
- “Somewhat difficult to navigate”
- “Hidden” hot-keys

IISTDTIDIW

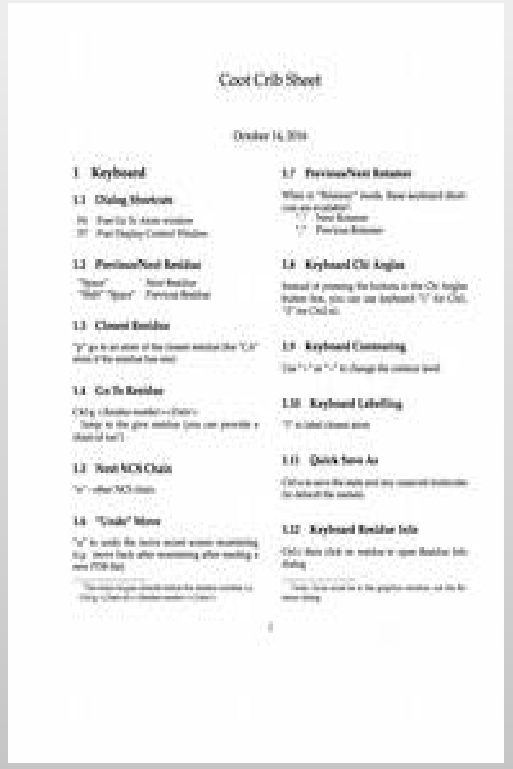


- If I See This Dialog Then I'm Doing It Wrong

Refinement Techniques

- Single-Atom Drag
  - Over-dragging
- Key-bindings:
  - Triple Refine “T”, with auto-accept: “H”
  - Single Residue Refine: “R” with Auto-accept: “X”
  - Add Residue: “Y”
  - Autofit rotamer” “J”
    - Residue Flip: E, Shift: Opt-Alt- → Rotate: Ctl Shft - →
  - Hybridization-aware residue fragment rotation: “Shift F”

Coot Key-binding Crib-Sheet



Coot Crib Sheet

October 14, 2016

1 Keyboard

1.1 Dialog Shortcuts

1.2 Previous/Next Residue

1.3 Closest Residue

1.4 Go To Residue

1.5 Next NCS Chain

1.6 "Undo" Move

1.7 Previous/Next Rotamer

1.8 Keyboard Ctrl Angles

1.9 Keyboard Contouring

1.10 Keyboard Labelling

1.11 Quick Save As

1.12 Keyboard Residue Info

1.13 Keyboard Translation

1.14 Keyboard Undo/Redo

1.15 Editing

1.16 Keyboard Zoom and Clip

1.17 Coordinate

1.18 Skeleton

1.19 Continuous Rotate

1.20 Rotate Mode

2 Mouse

3 Refinement Extras

4 Template Key-bindings

Common Moves not Typically Used

- Quickly fix gross errors:
- (say side-chain built into main-chain density)
- Eigen flip residue
- Rotate/translate residue
- “x” refine

Biggest issue learning coot...

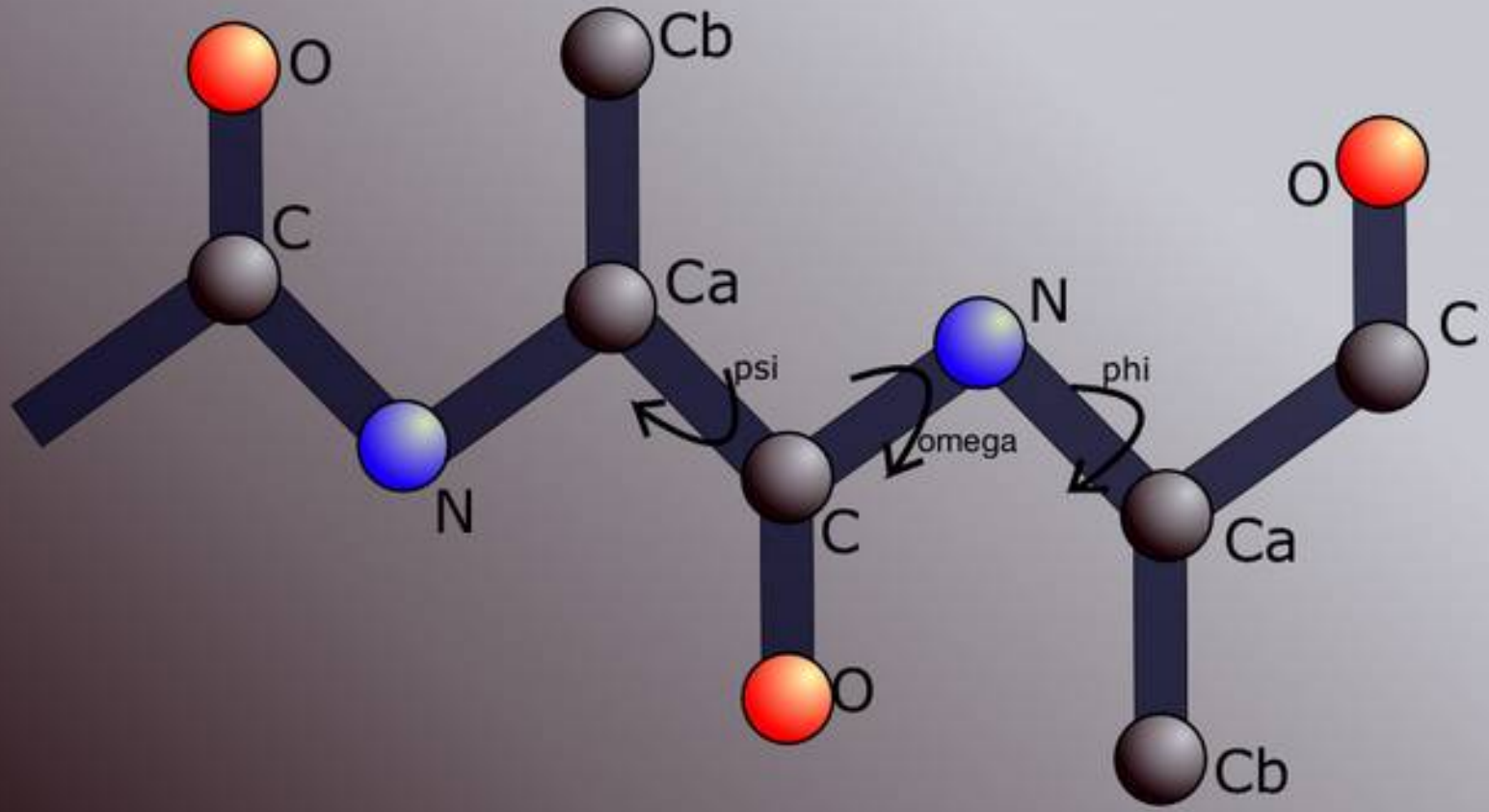
- to recognise which tool is suitable for the current problem
- Here are some examples:
- 'H' Refinement
- 'J' Rotamer
- 'E' eigen-flip

clicking on atoms is slow, try not to do that

Rotamer Searching

- Two methods
  - Traditional
  - Backrub

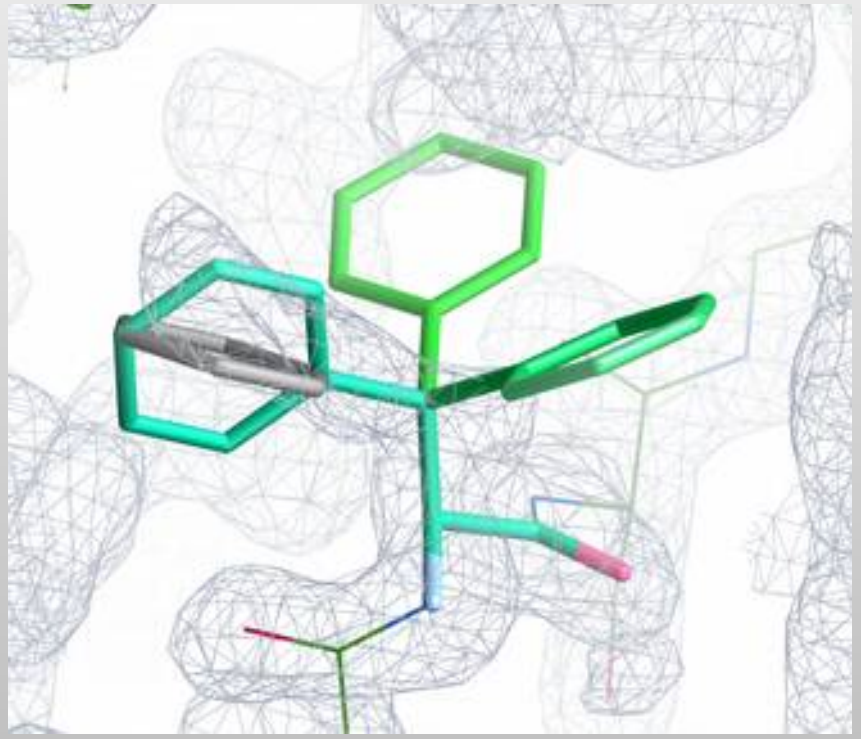
Peptide Torsion Angles

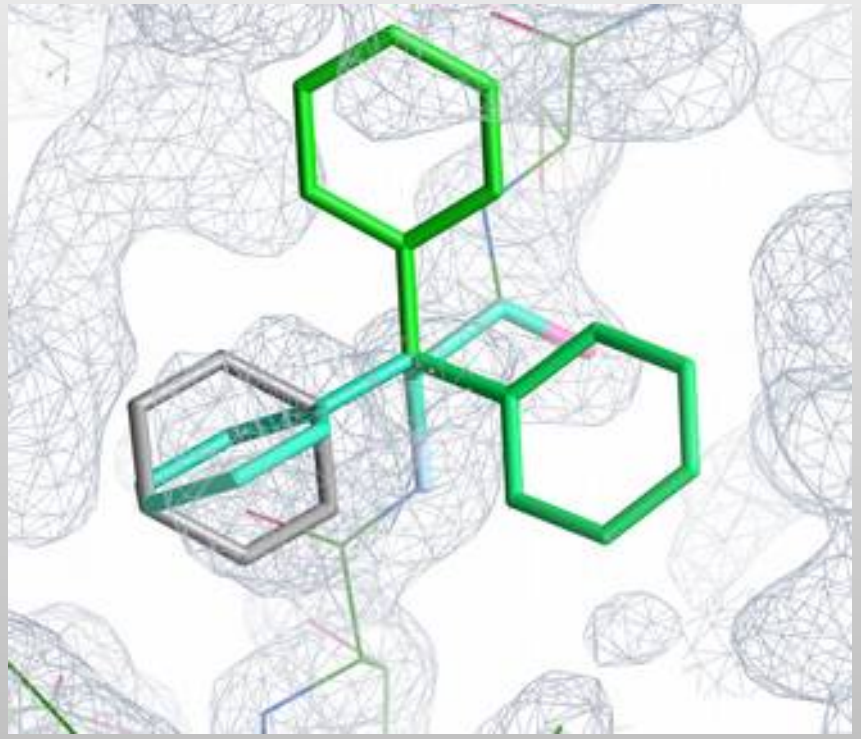


Rotamers

- 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
  - best: (Son of) Penultimate Rotamer Library

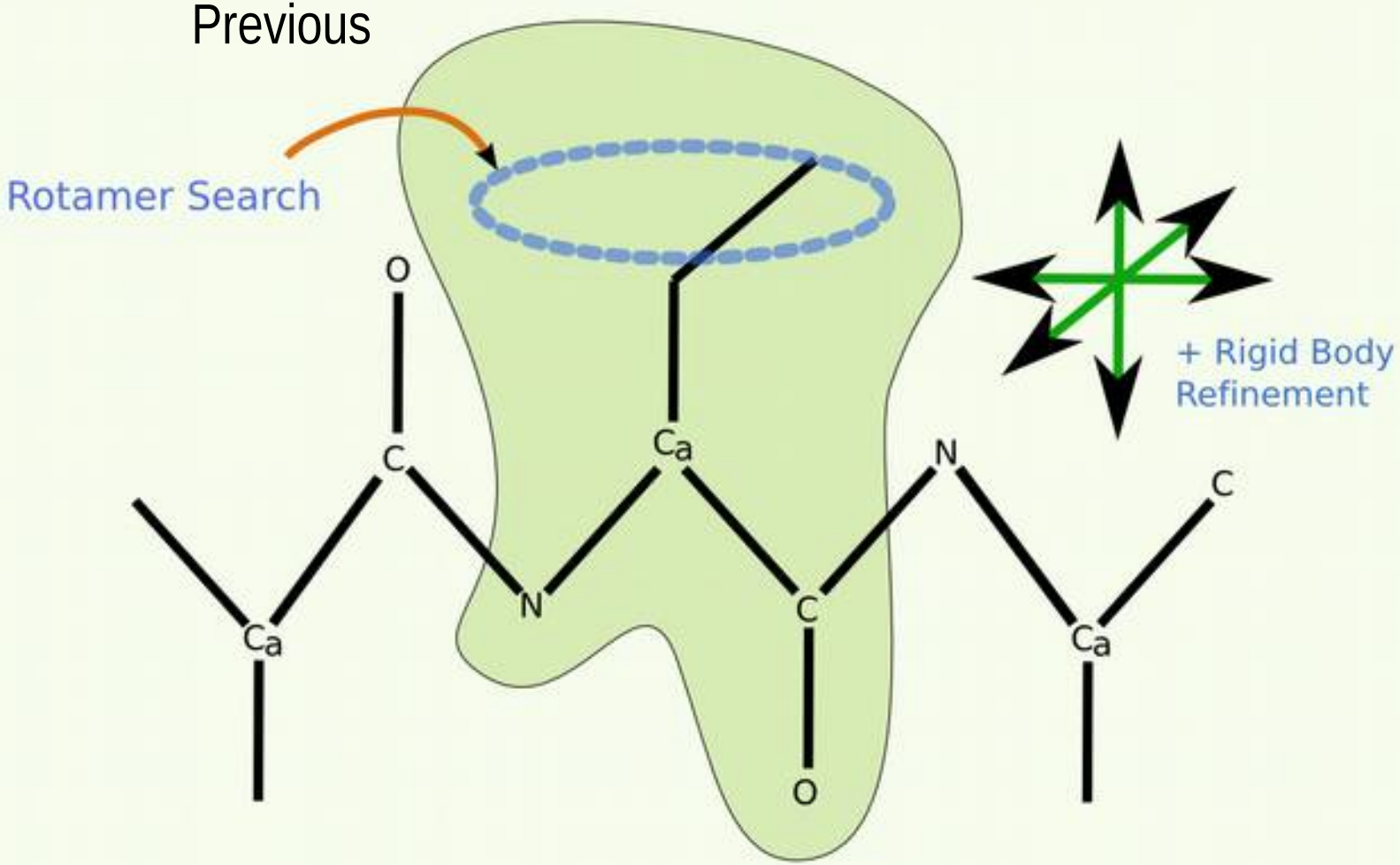
4 PHE Rotamers

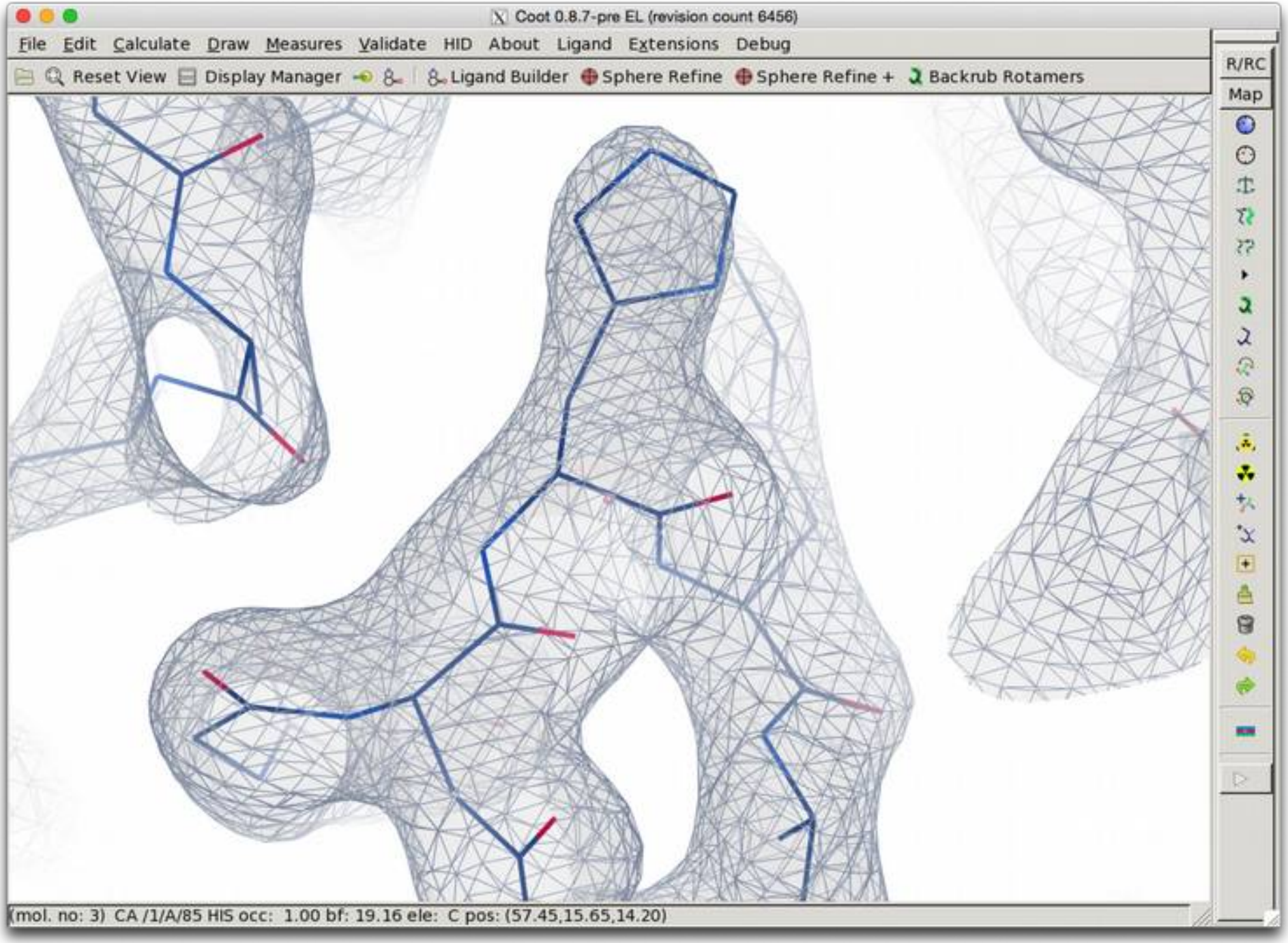




~~Current~~ Low Resolution Rotamer Search

Previous

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Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
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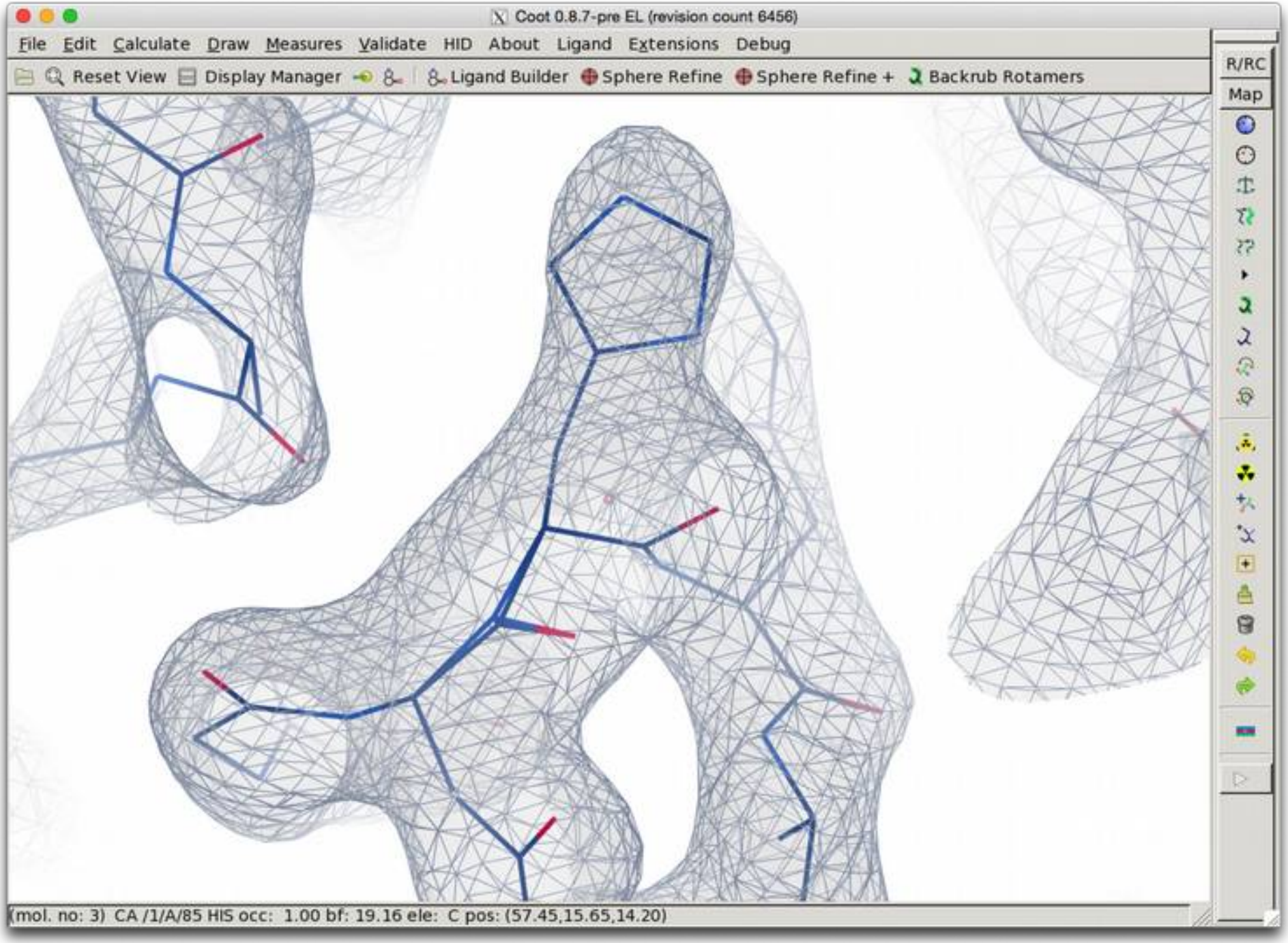
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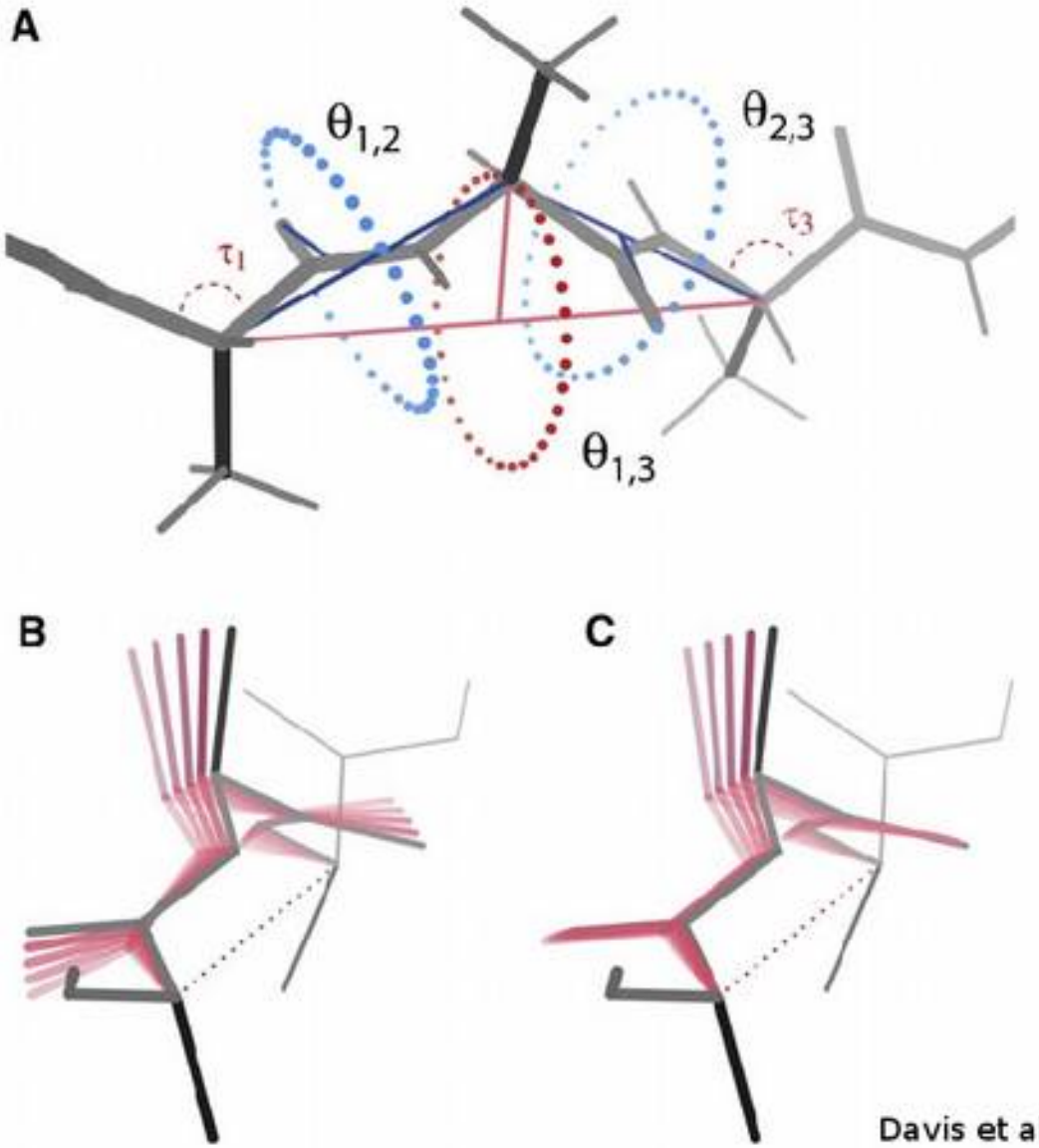
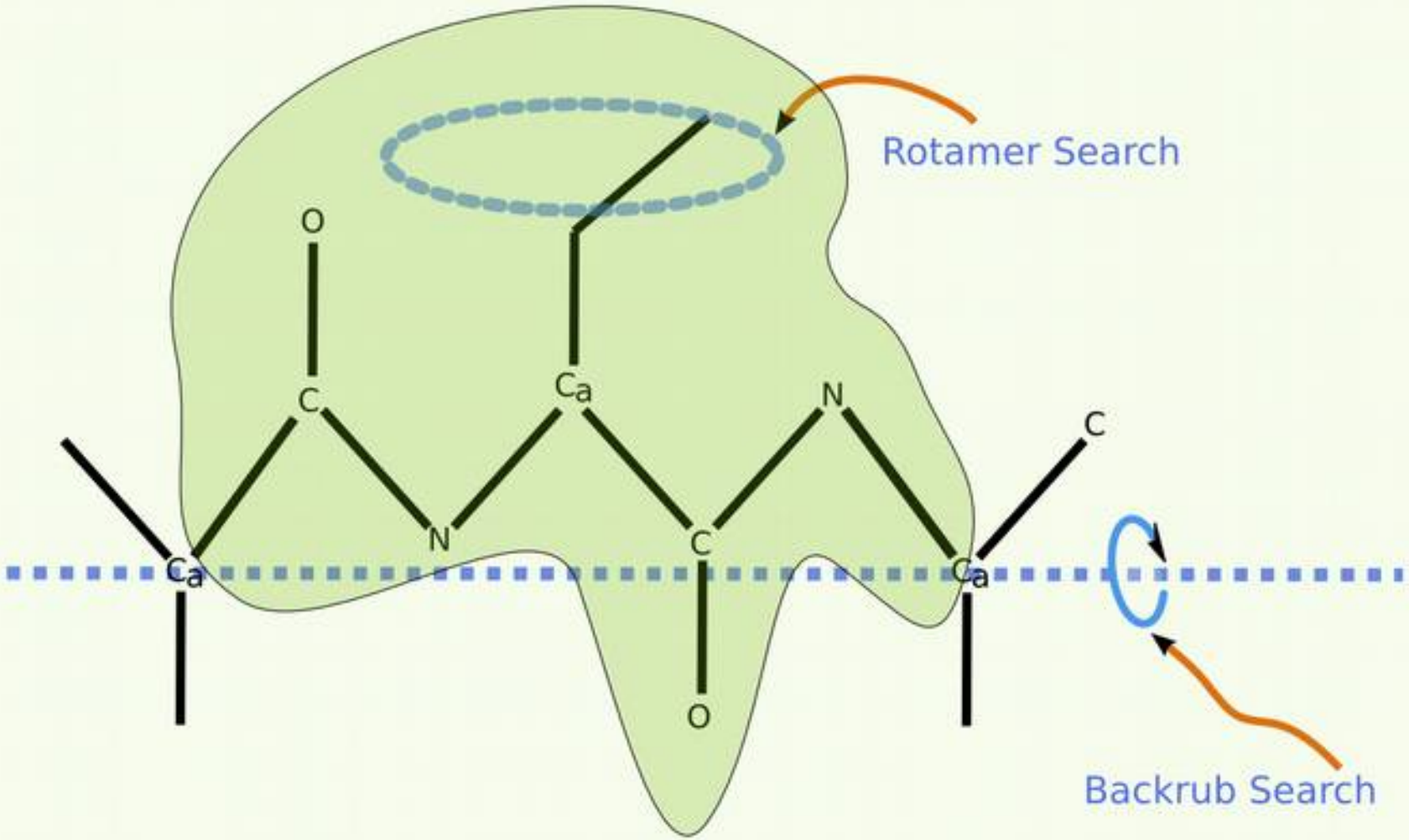
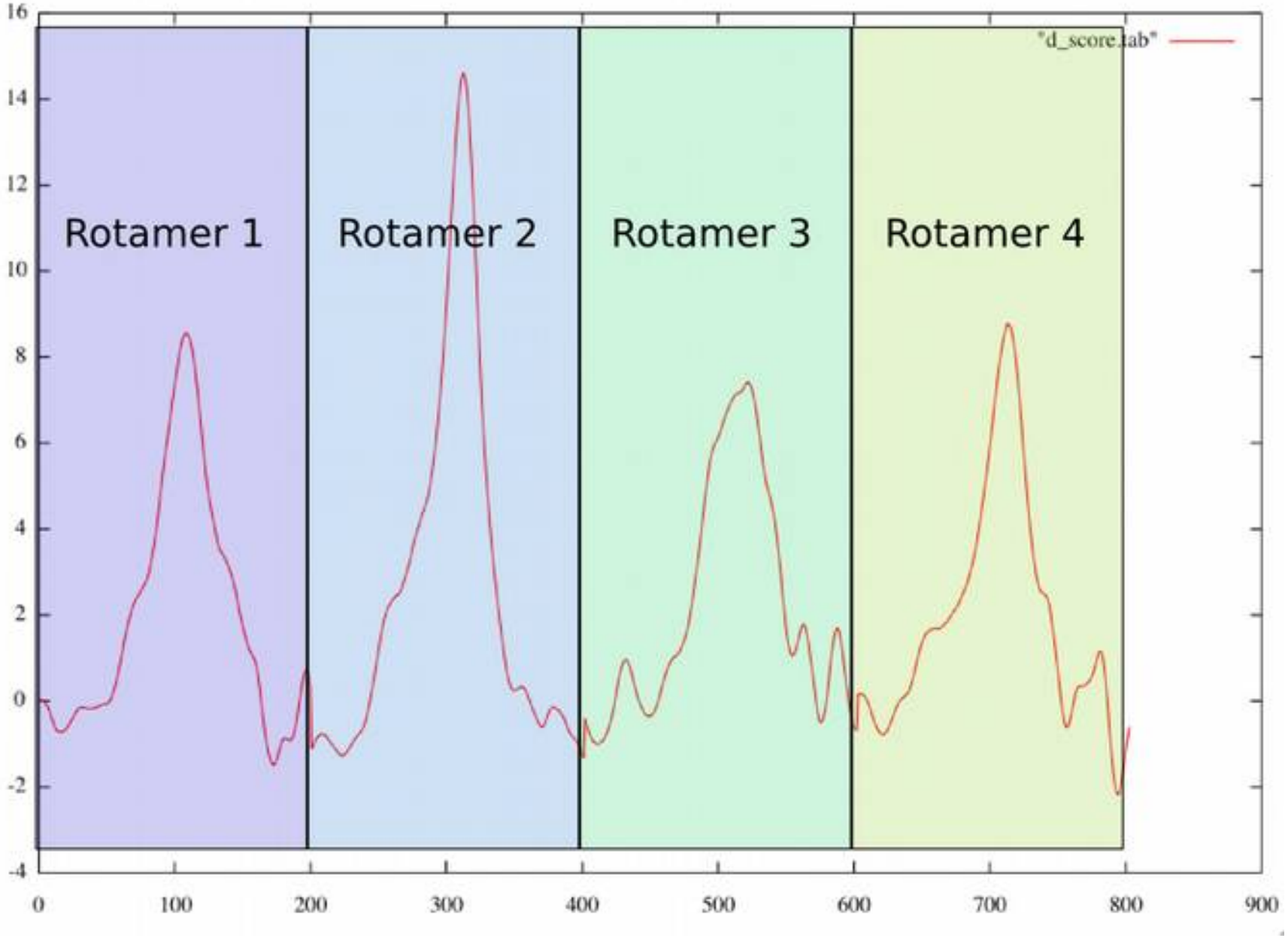


Davis et al. (2006) Structure

New Low Resolution Rotamer Search



After Fitting Tools in KING/Molprobity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Rotamer 1
Rotamer 2
Rotamer 3
Rotamer 4
16
14
12
10
8
6
4
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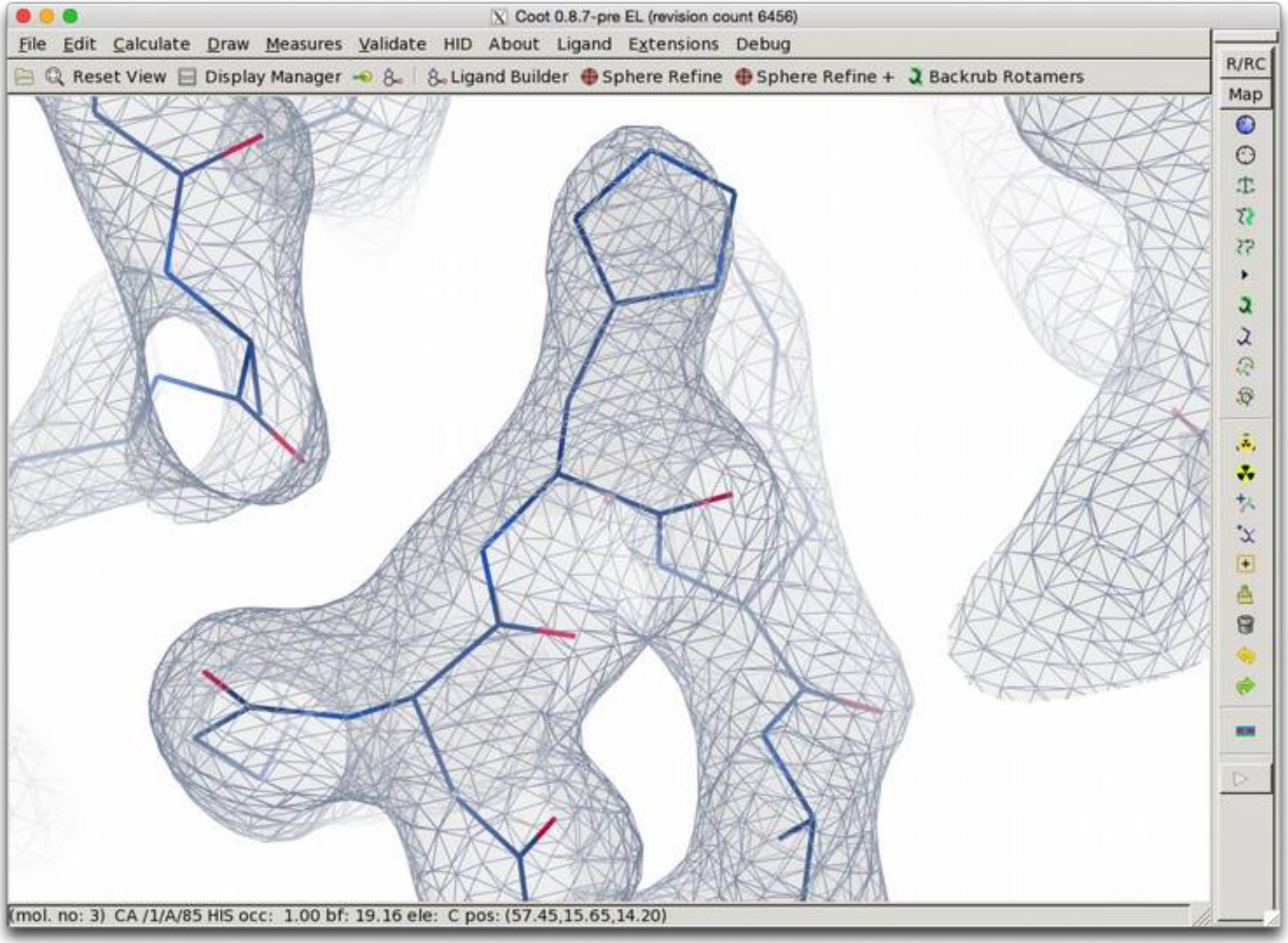
200
300
400
500
600
700
800
900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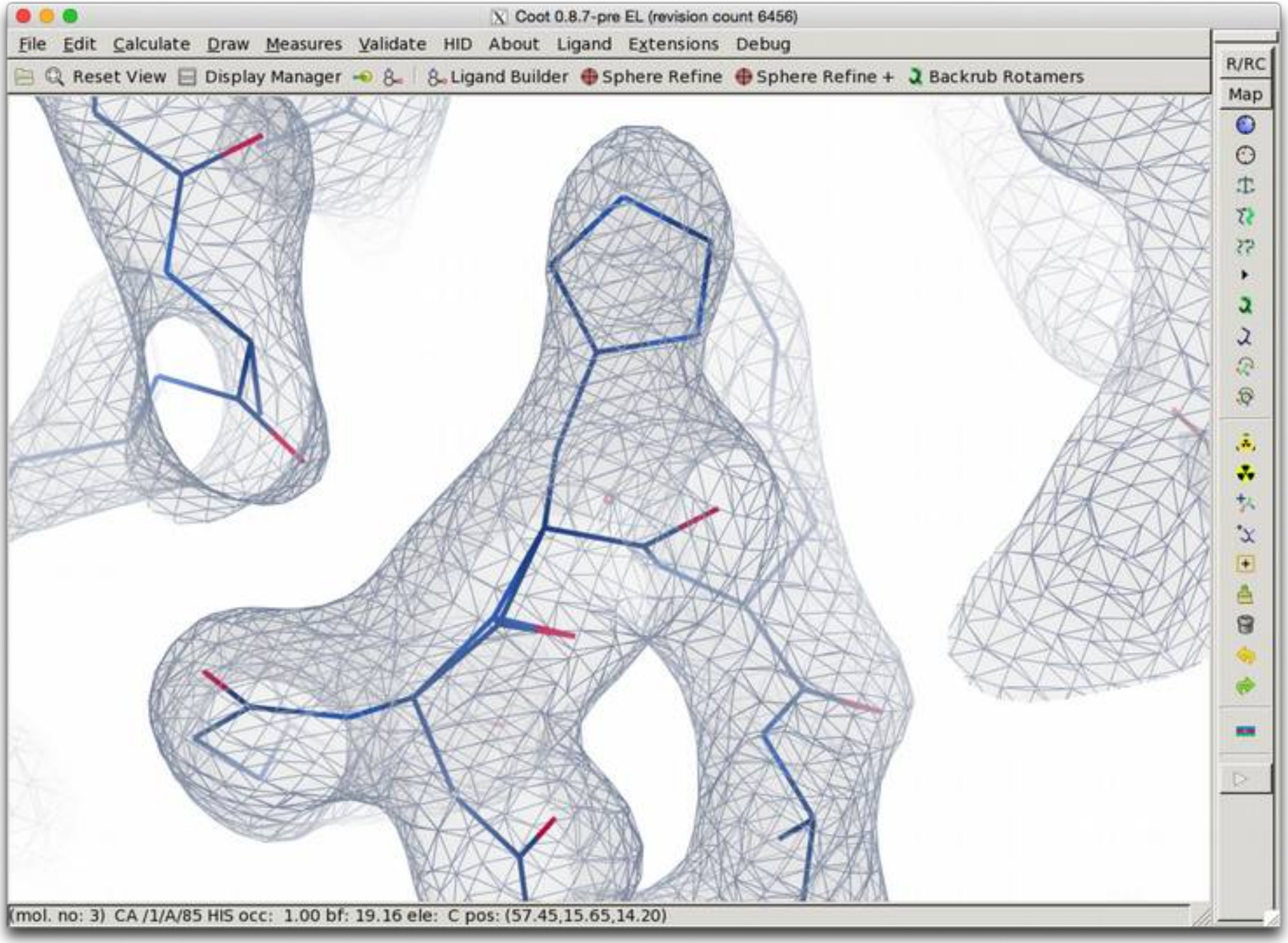
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)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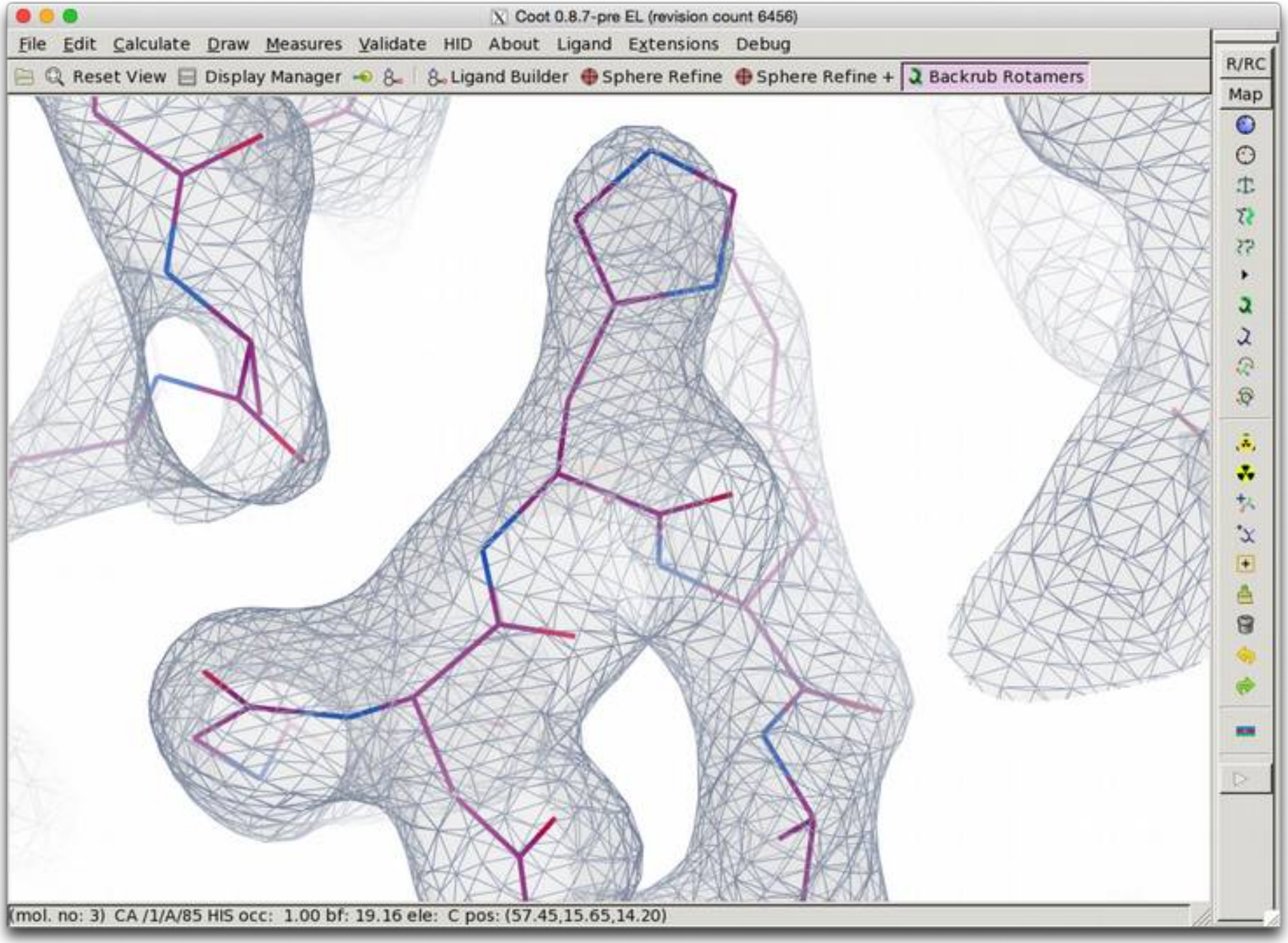
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)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Reset View
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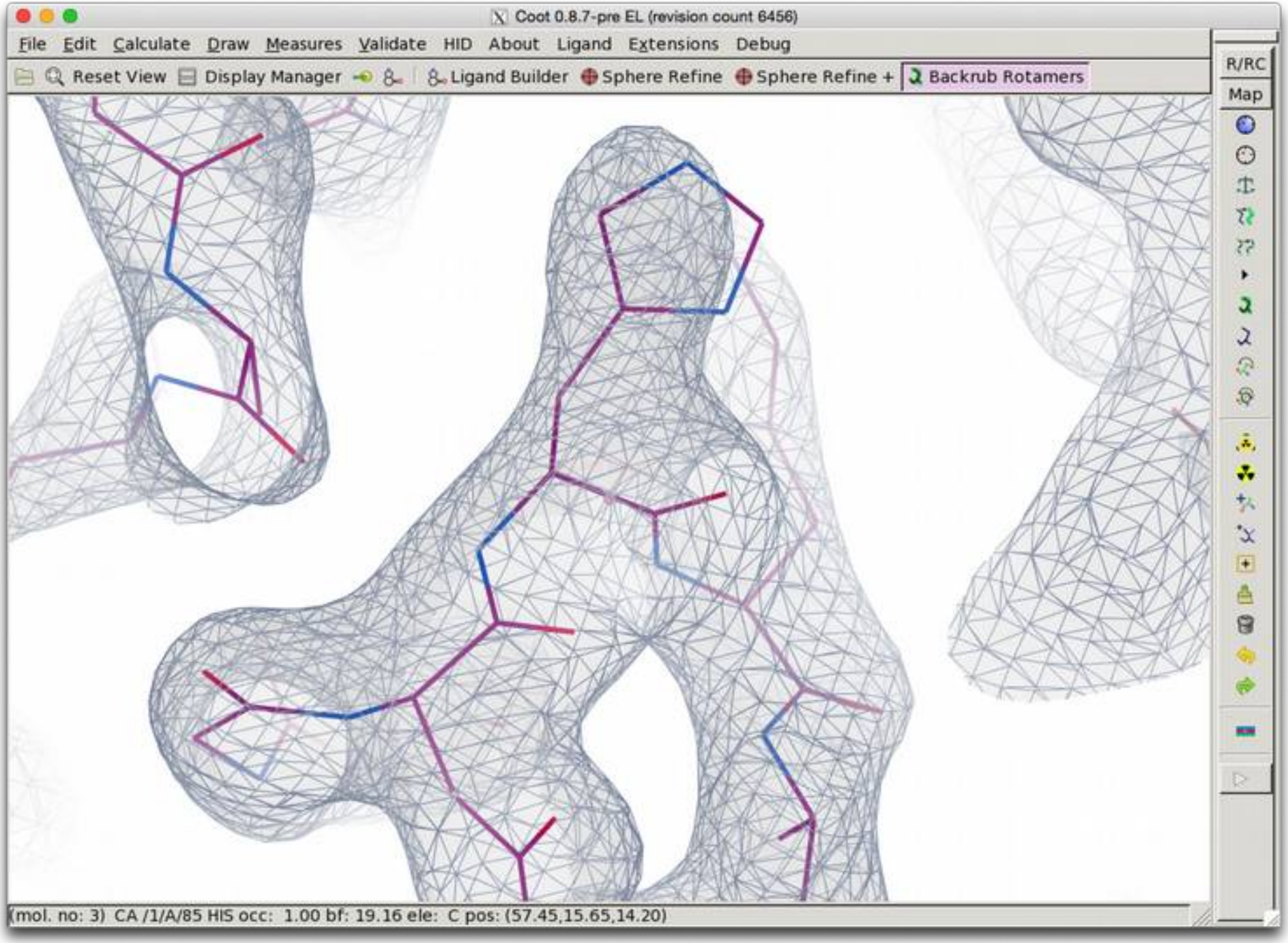
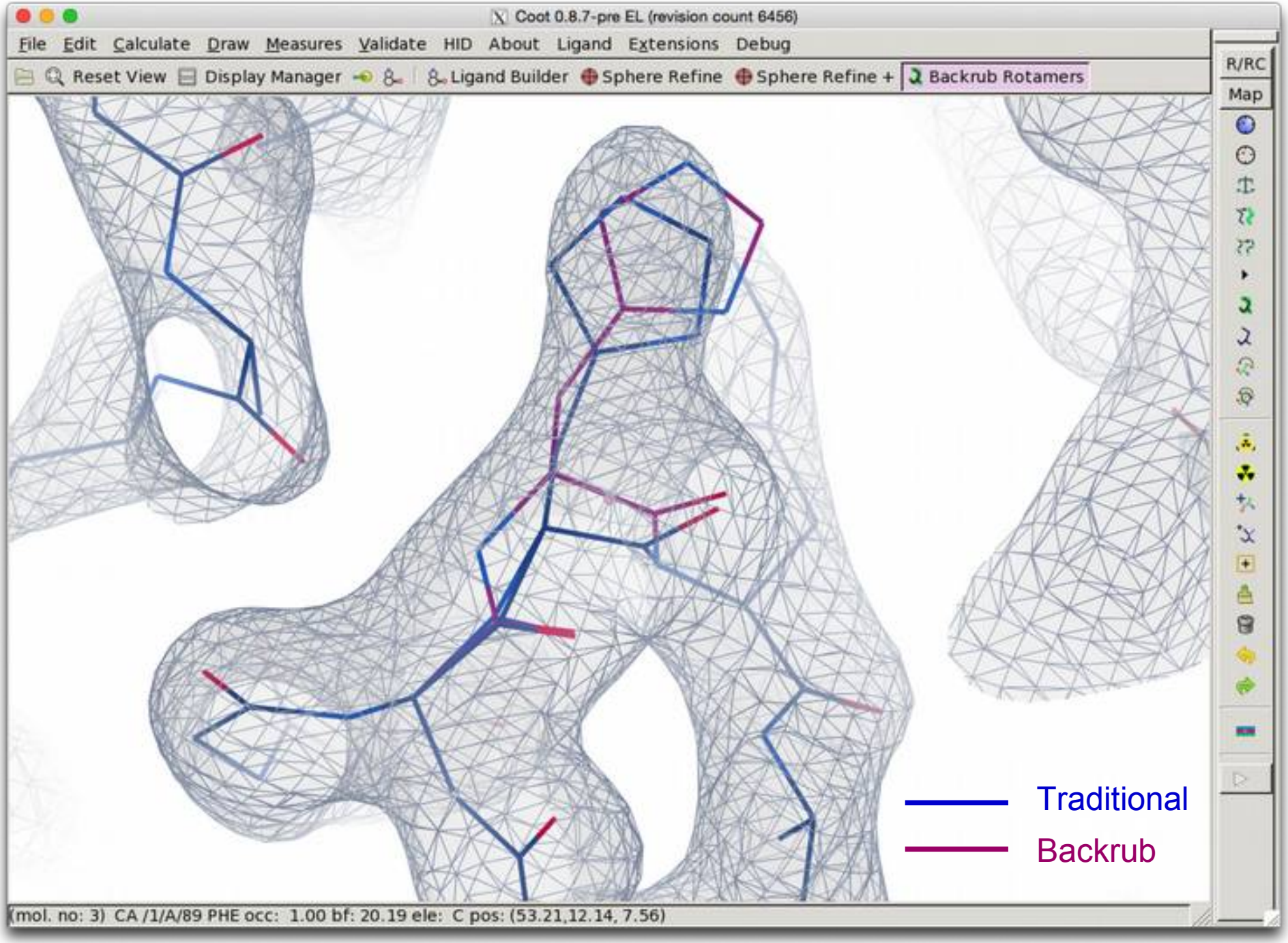
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
(mol. no: 3) CA /1/A/85 HIS occ: 1.00 bf: 19.16 ele: C pos: (57.45,15.65,14.20)


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)
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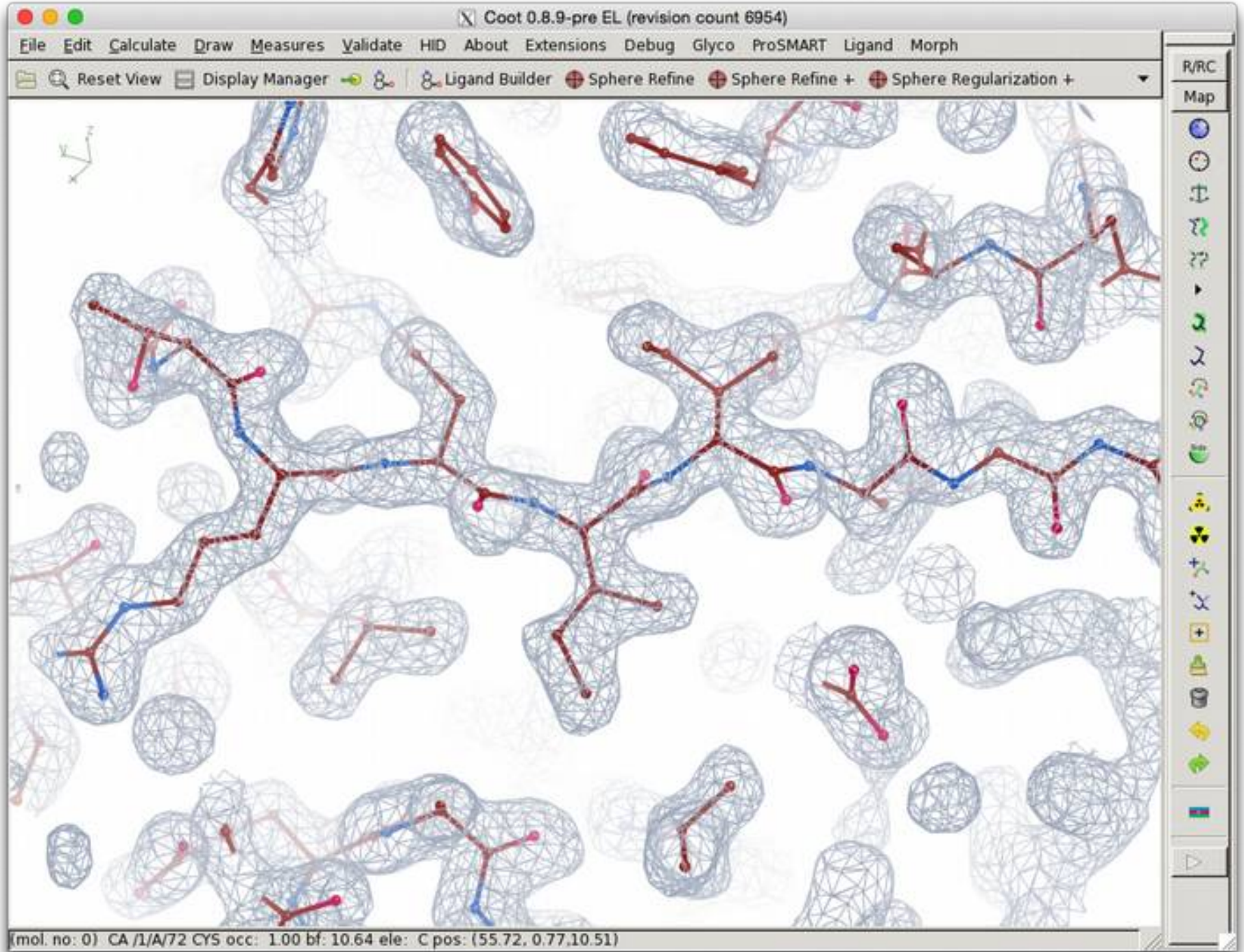
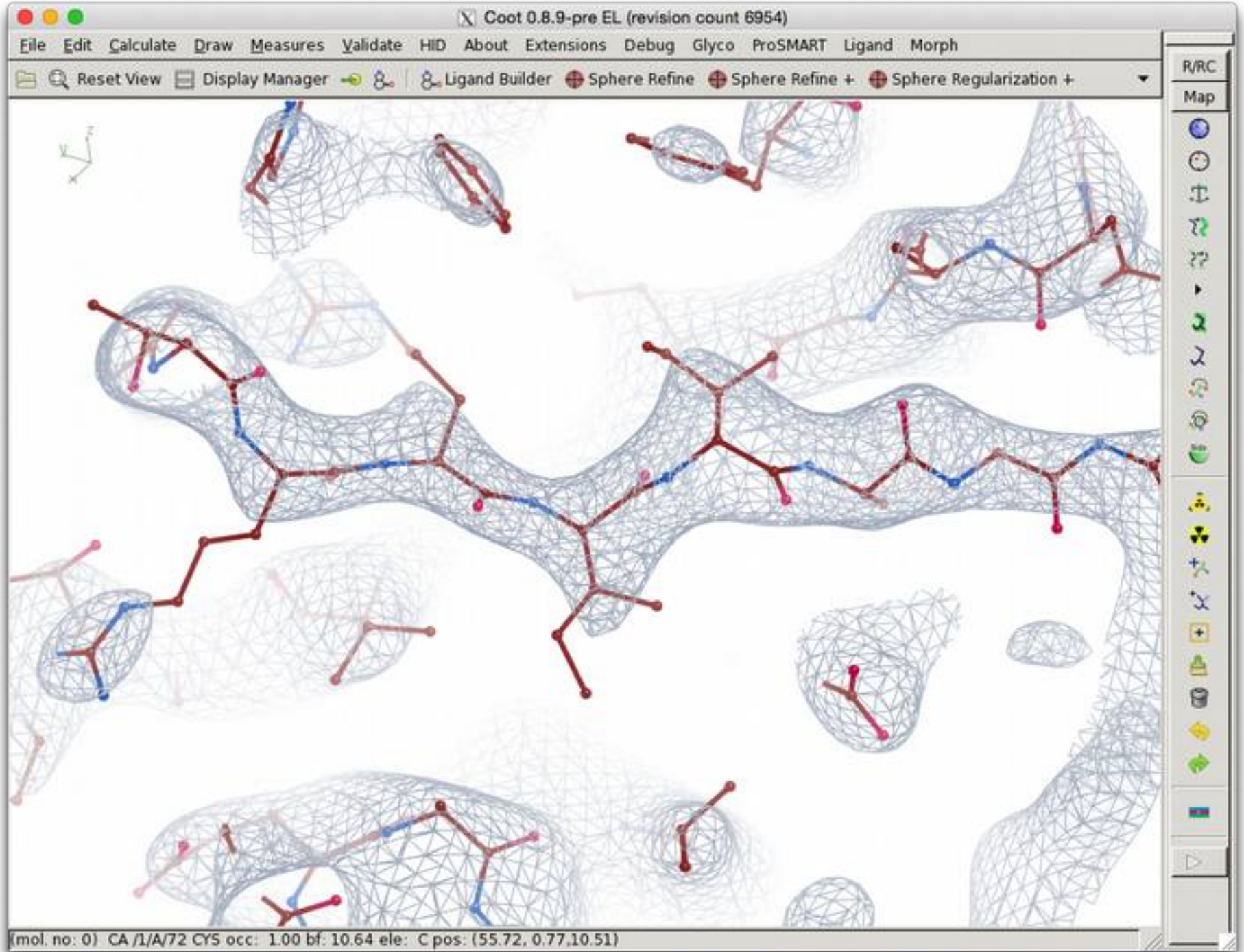
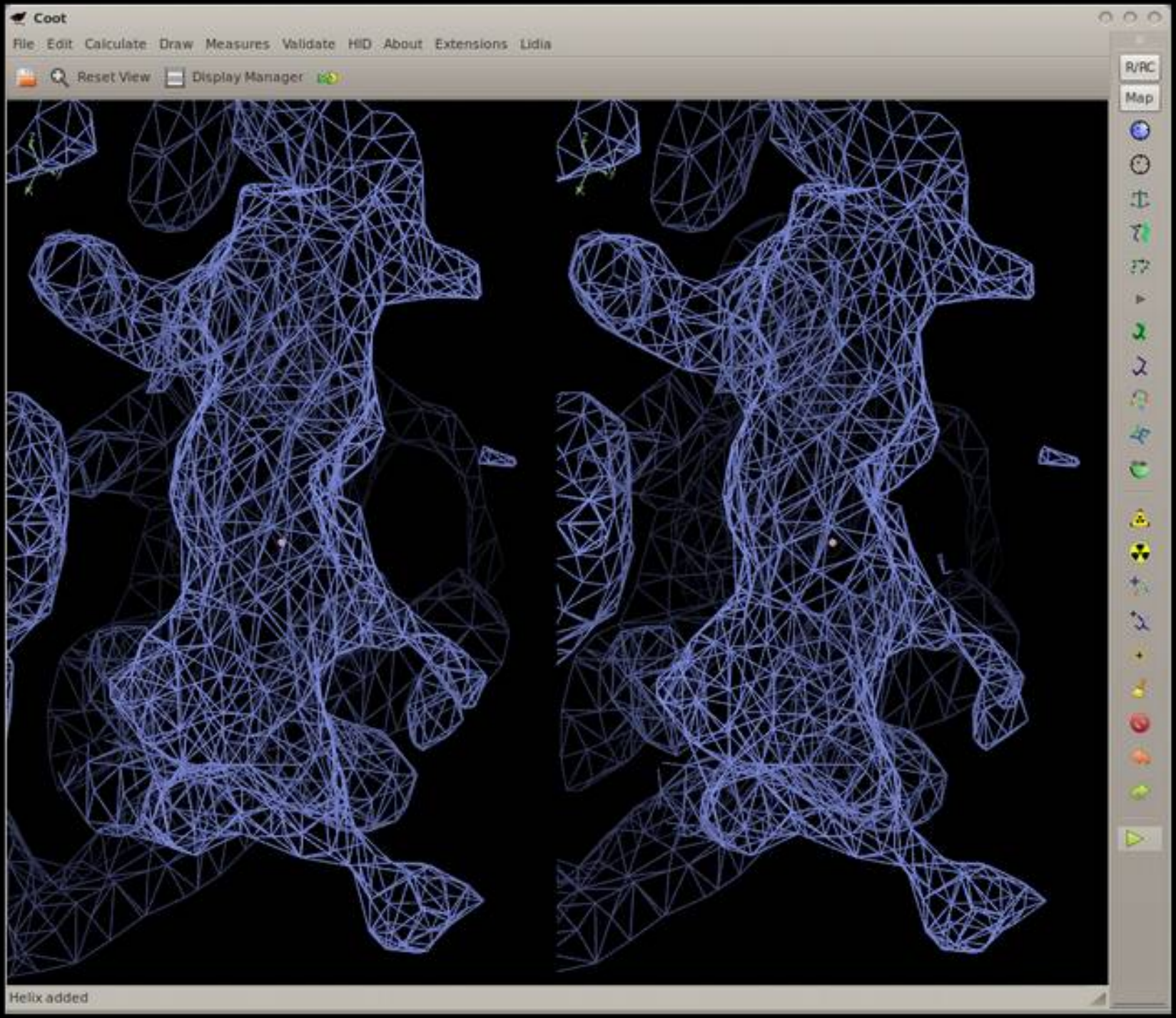
Reset View
Display Manager
Ligand Builder
Sphere Refine
Sphere Refine +
Backrub Rotamers
R/RC
Map
Traditional
Backrub
(mol. no: 3) CA /1/A/89 PHE occ: 1.00 bf: 20.19 ele: C pos: (53.21,12.14, 7.56)


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)

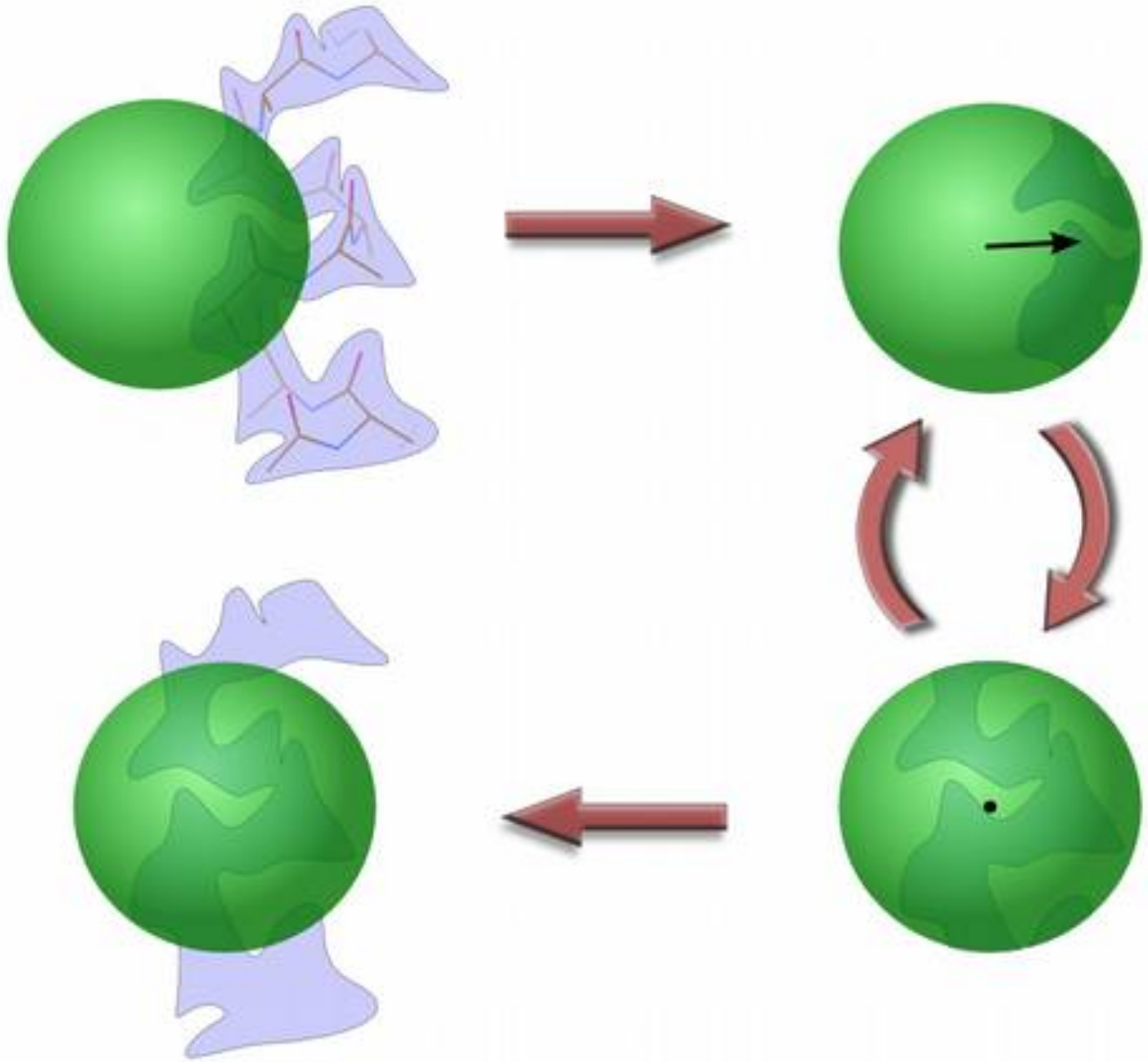
Helix-Building


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

Alpha Helix Placement

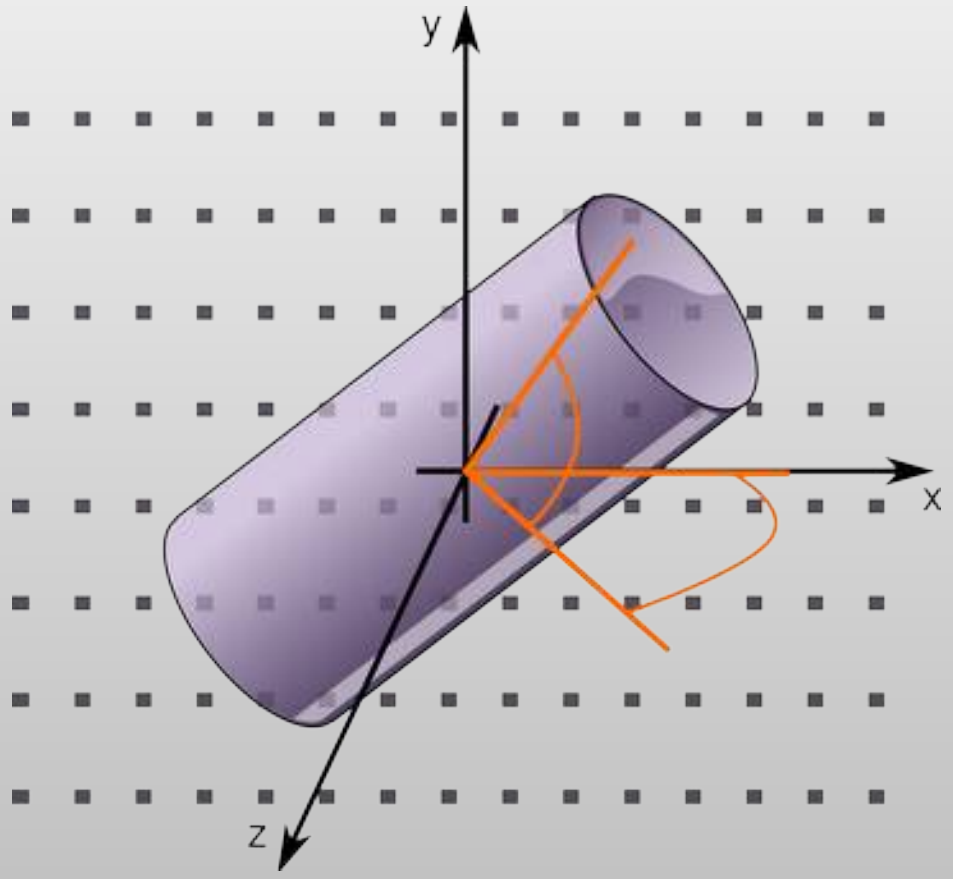
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

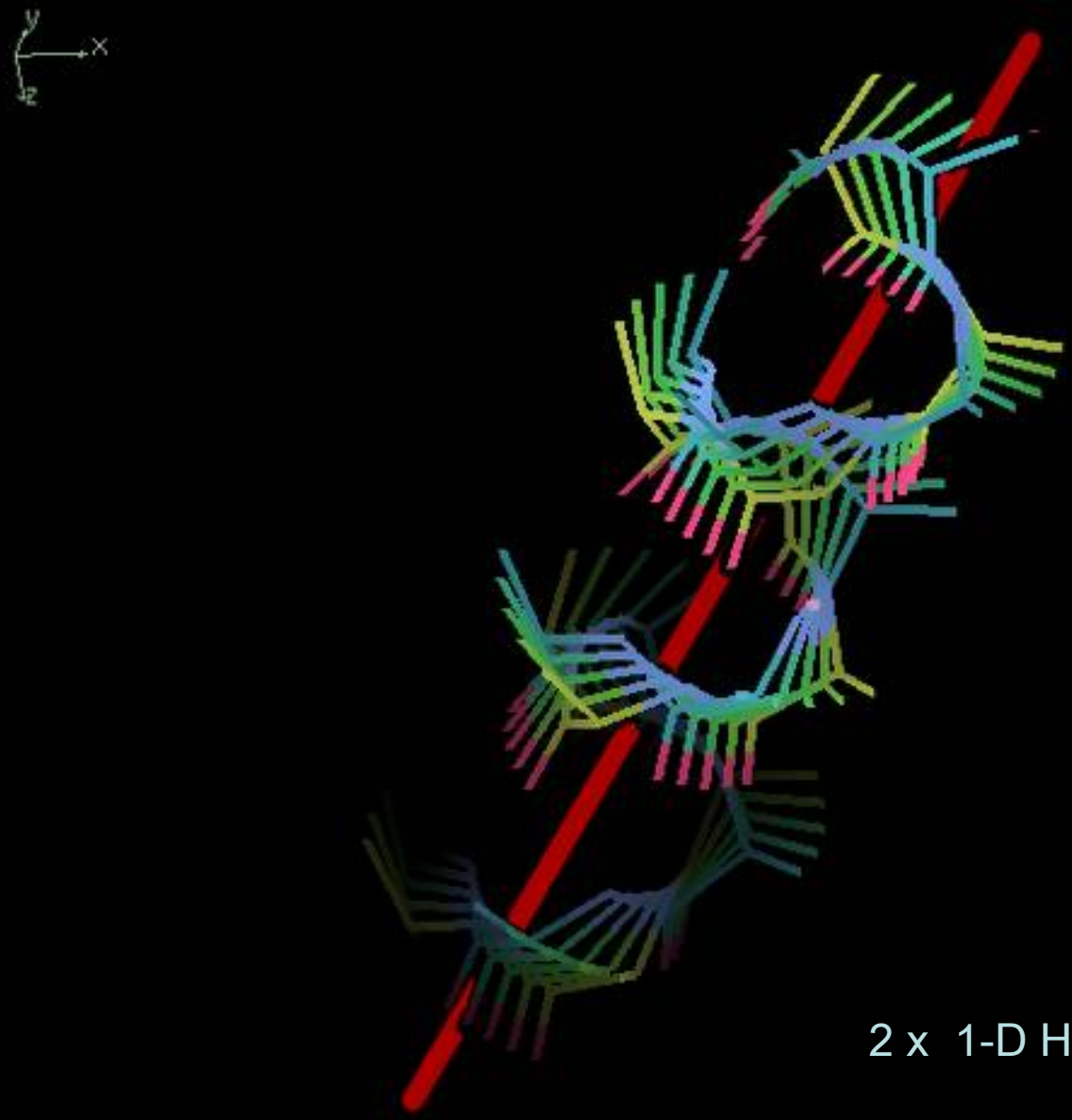


Helix Fitting: Cylinder Search

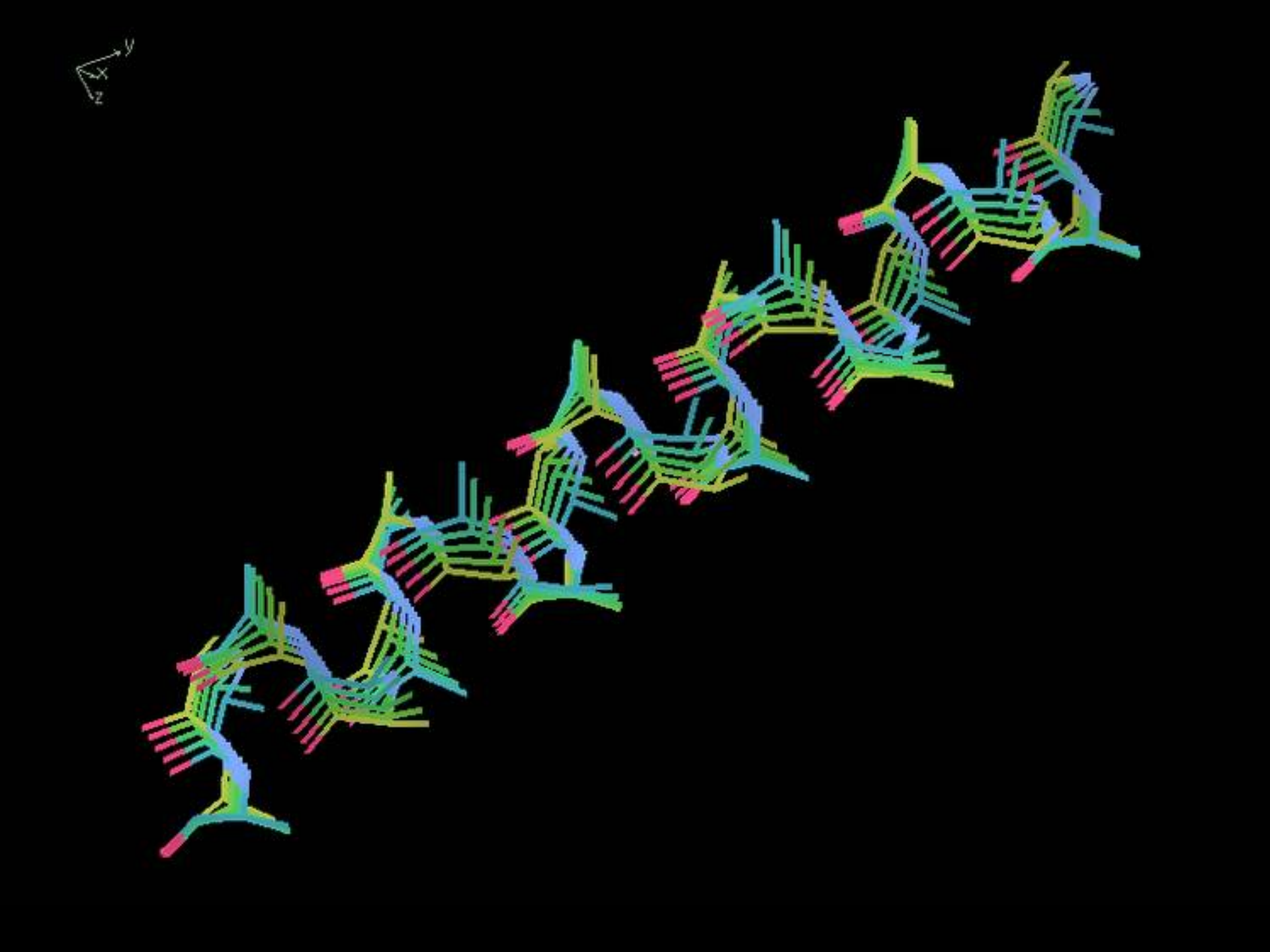
- Pick the orientation that encapsulates the most electron density

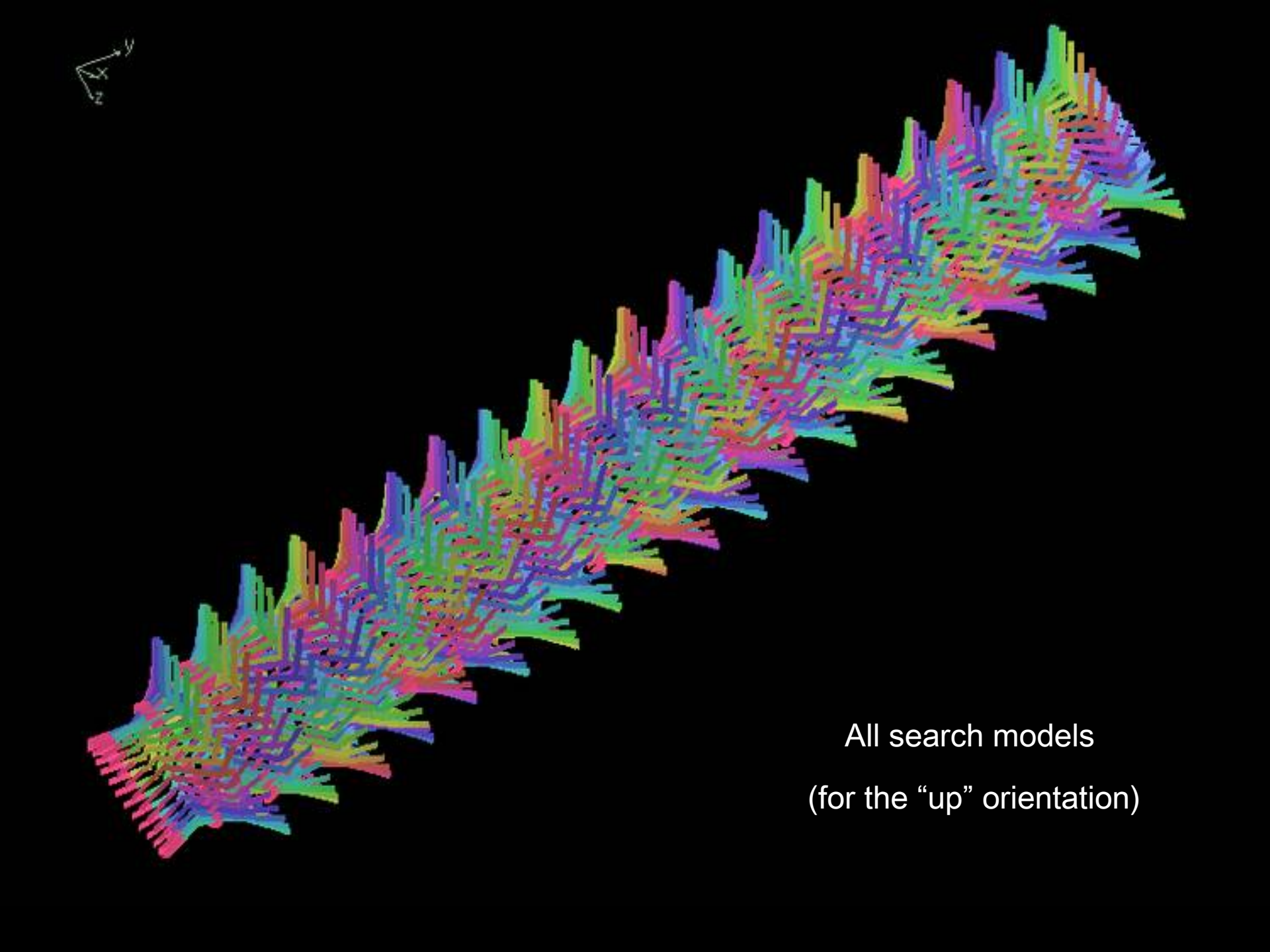


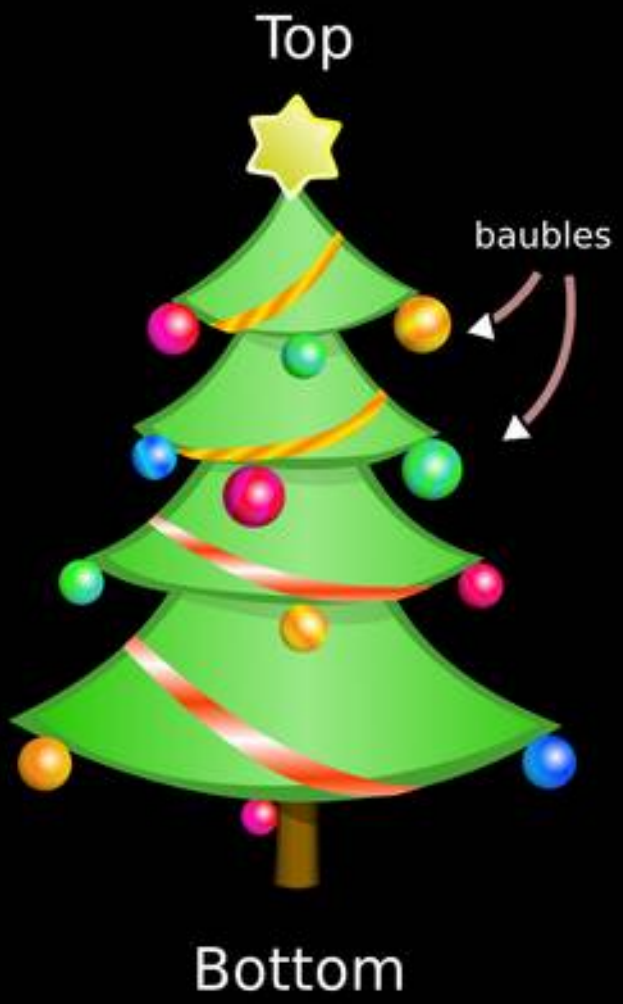
Using 2 rotation axes



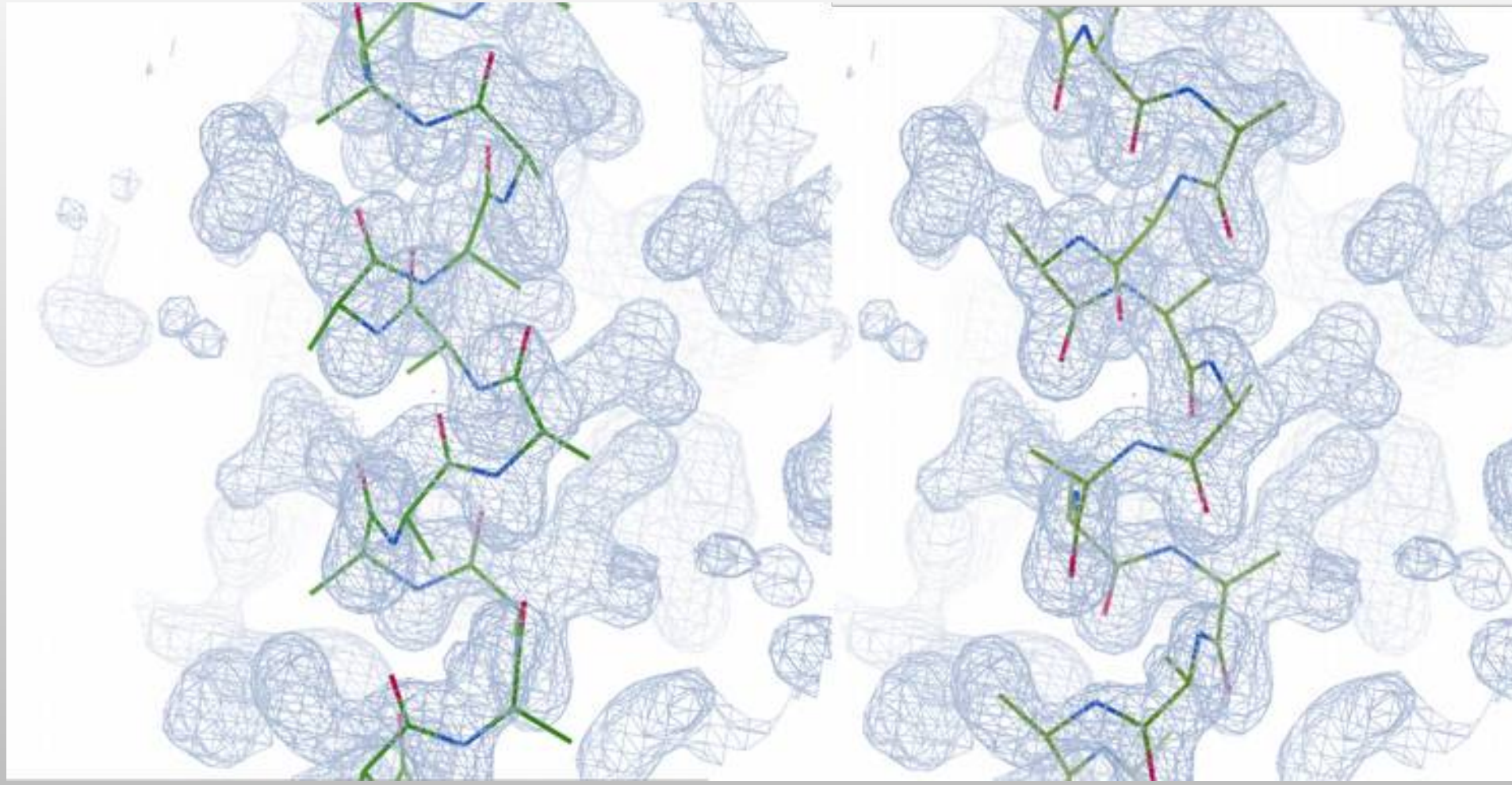
2 x 1-D Helix orientation searches


y
x
z

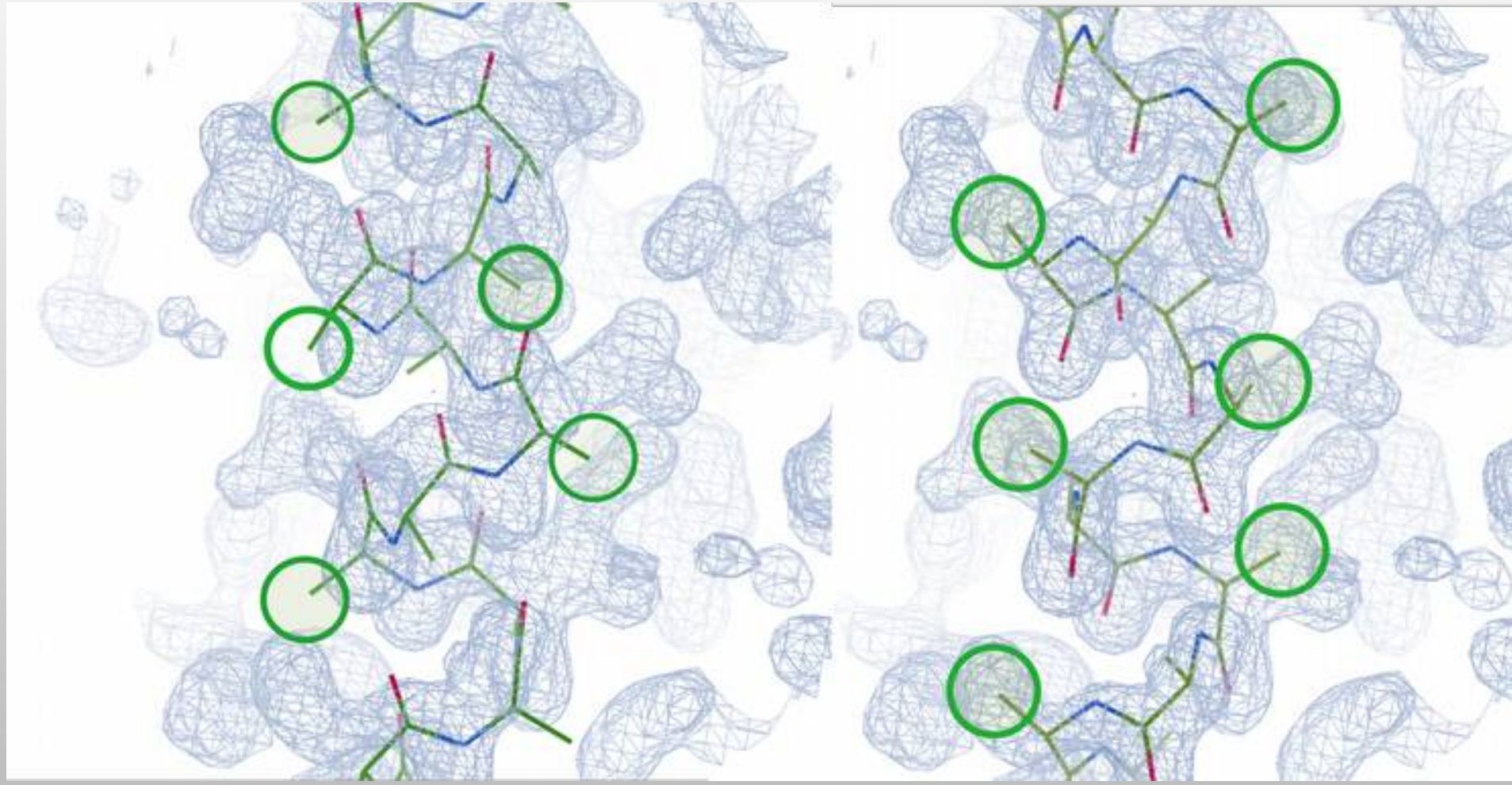

All search models
(for the “up” orientation)


Top
baubles
Bottom

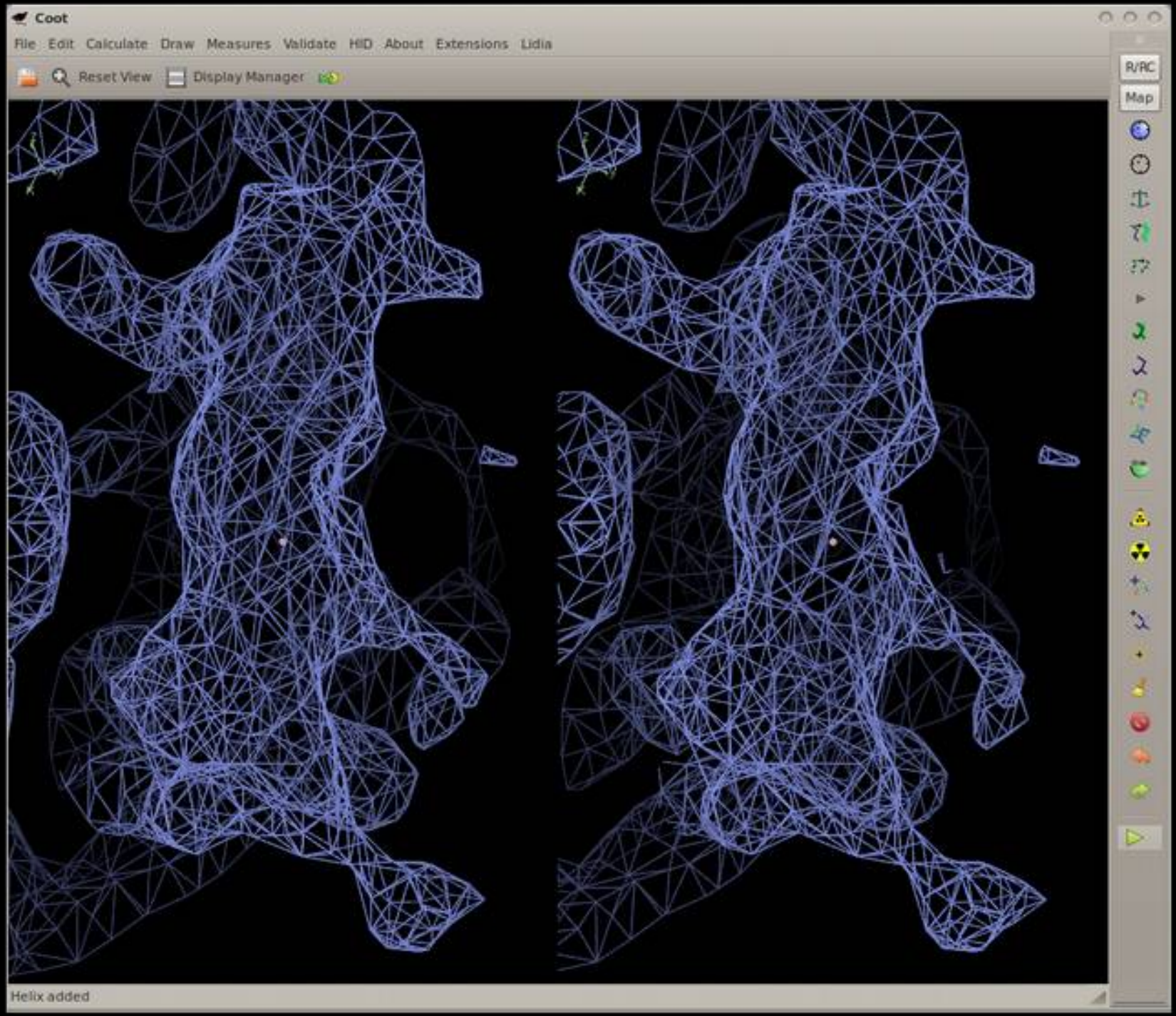
Helix Fitting
Comparing orientation hypotheses

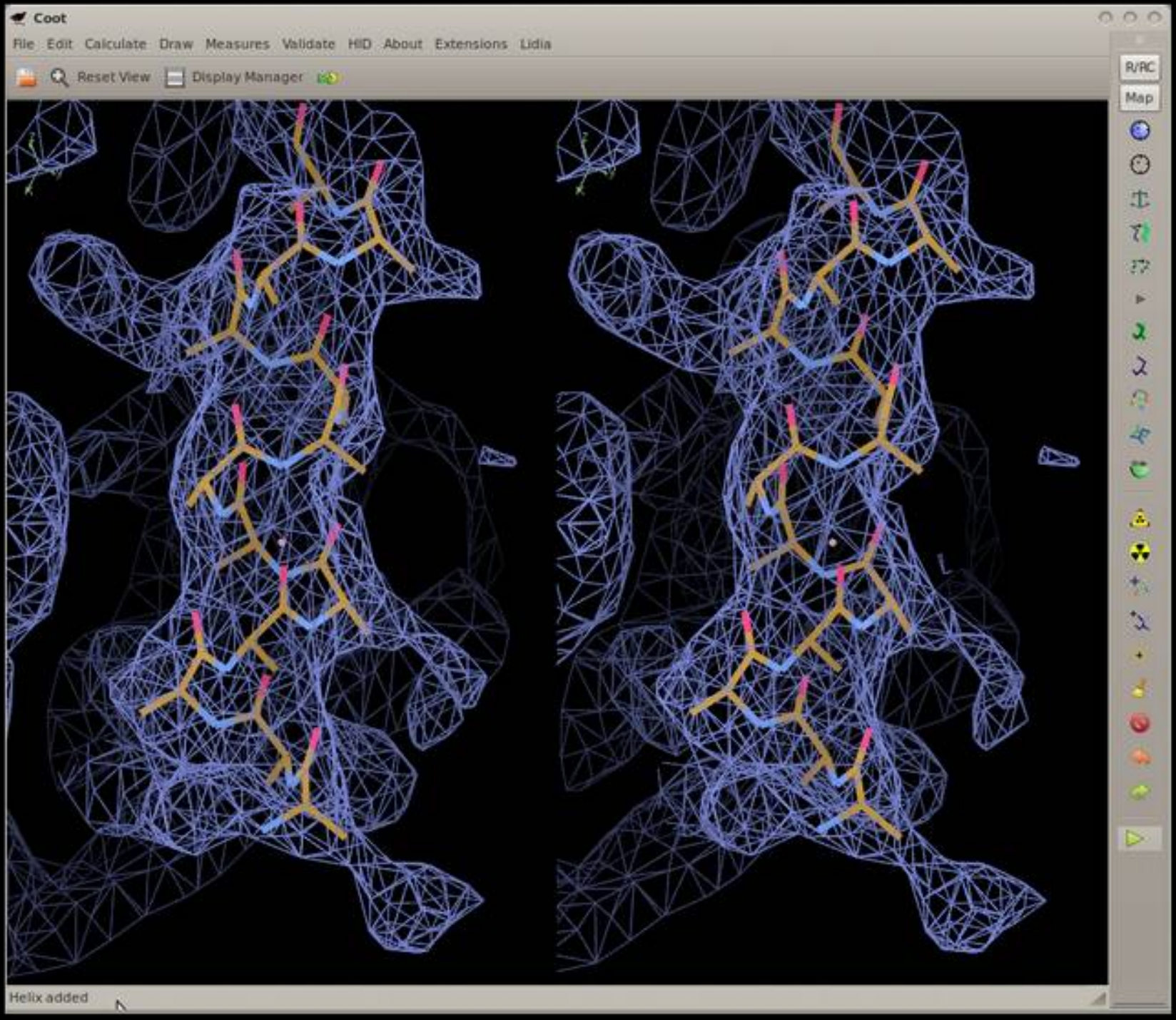


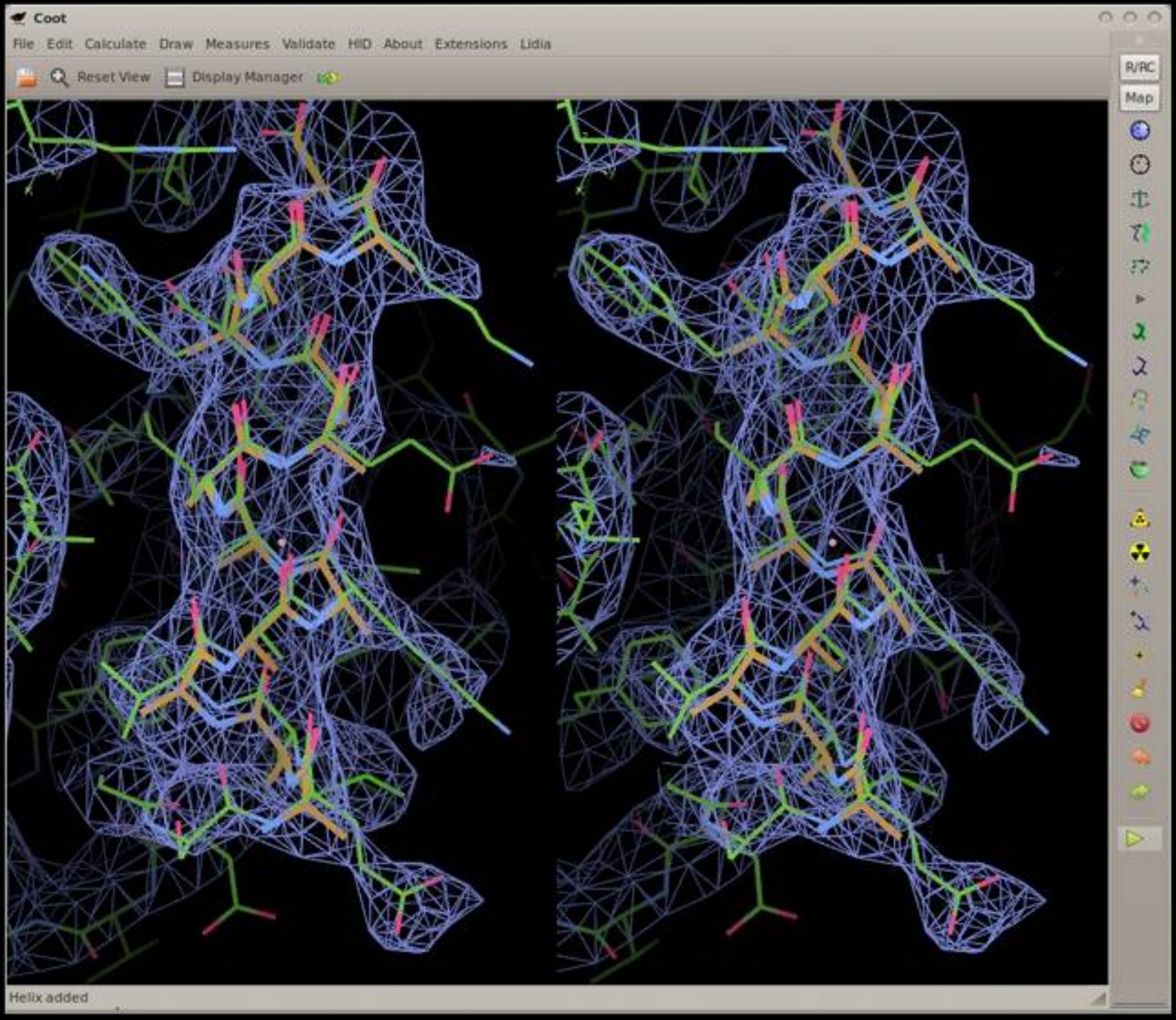
Helix Fitting
Comparing orientation hypotheses



c-betas not fitting 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

cis-Peptides

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

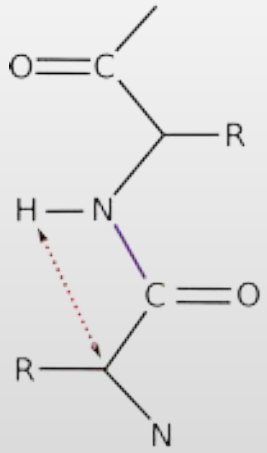
Merging

- Merging Fragments is much easier than it used to be
- (for overlapping fragments)

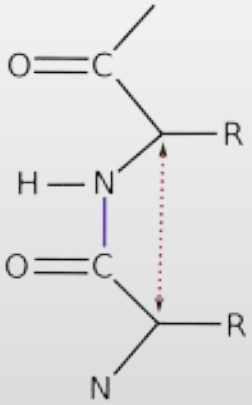
cis-Peptides

- 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

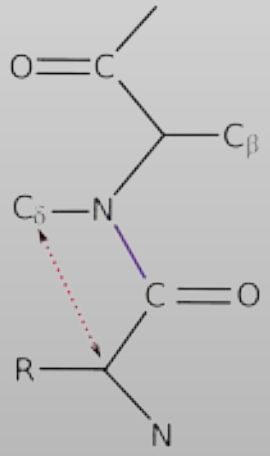
cis-Peptides



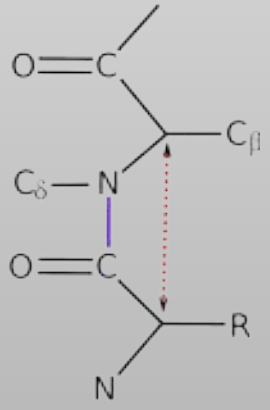
trans-peptide



cis-peptide

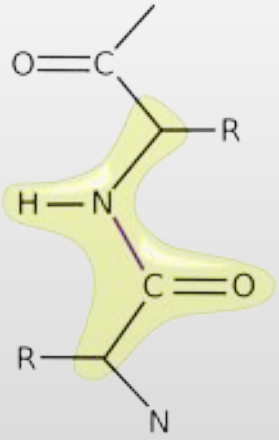


PRO *trans-peptide*

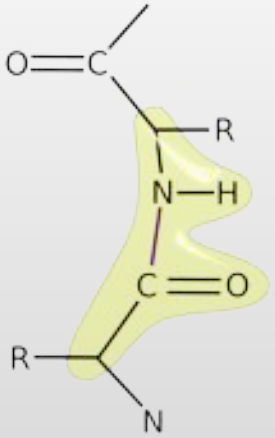


PRO *cis-peptide*

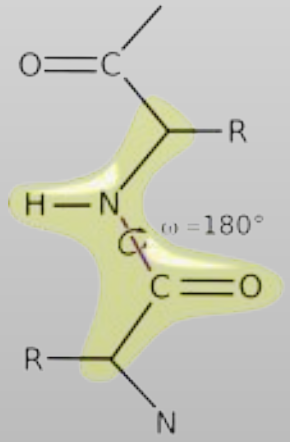
cis-Peptides



trans-peptide
with plane restraints

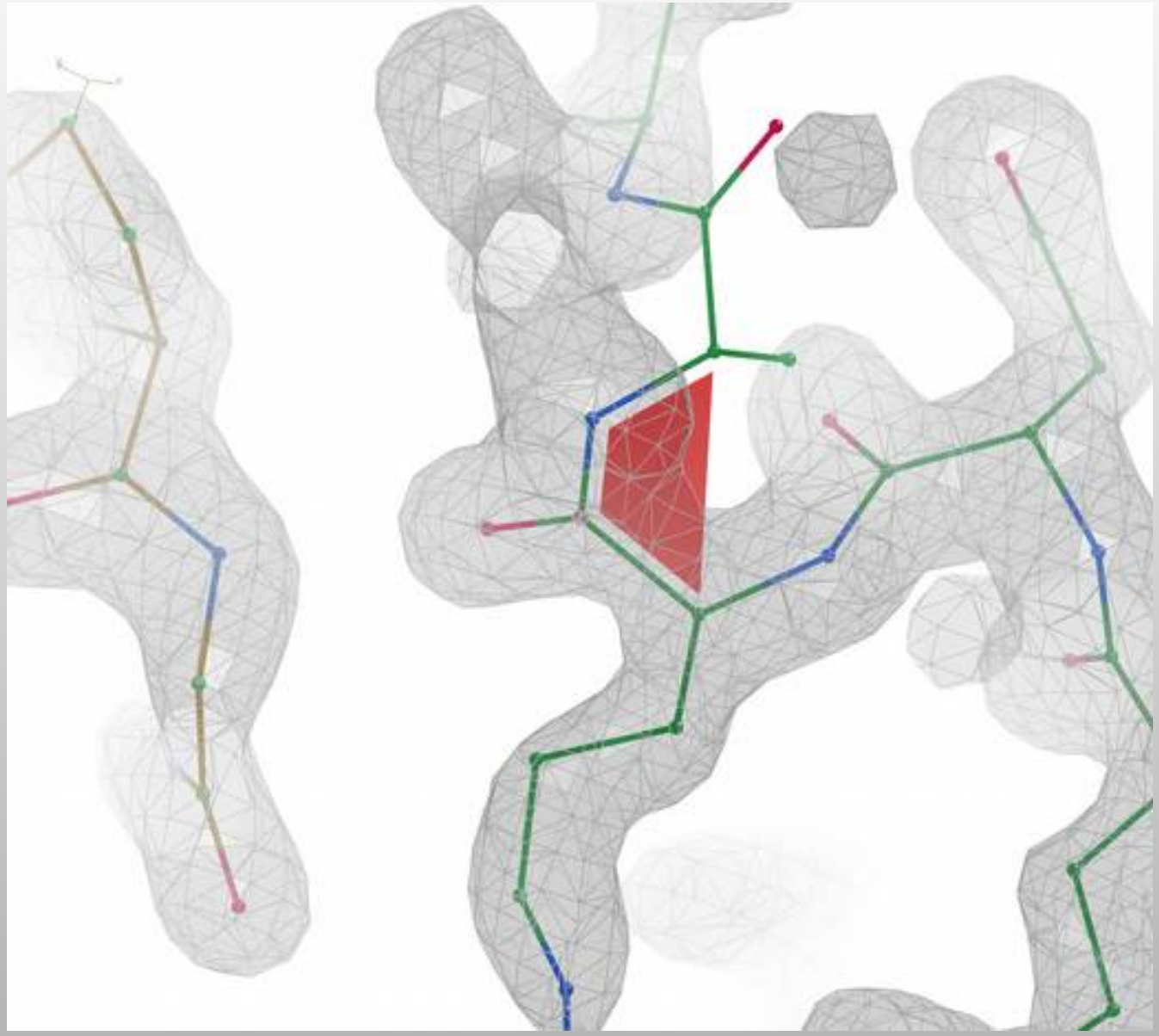


cis-peptide
with plane restraints



trans-peptide
with plane and trans restraints

cis-peptide Representation



Pre-PRO

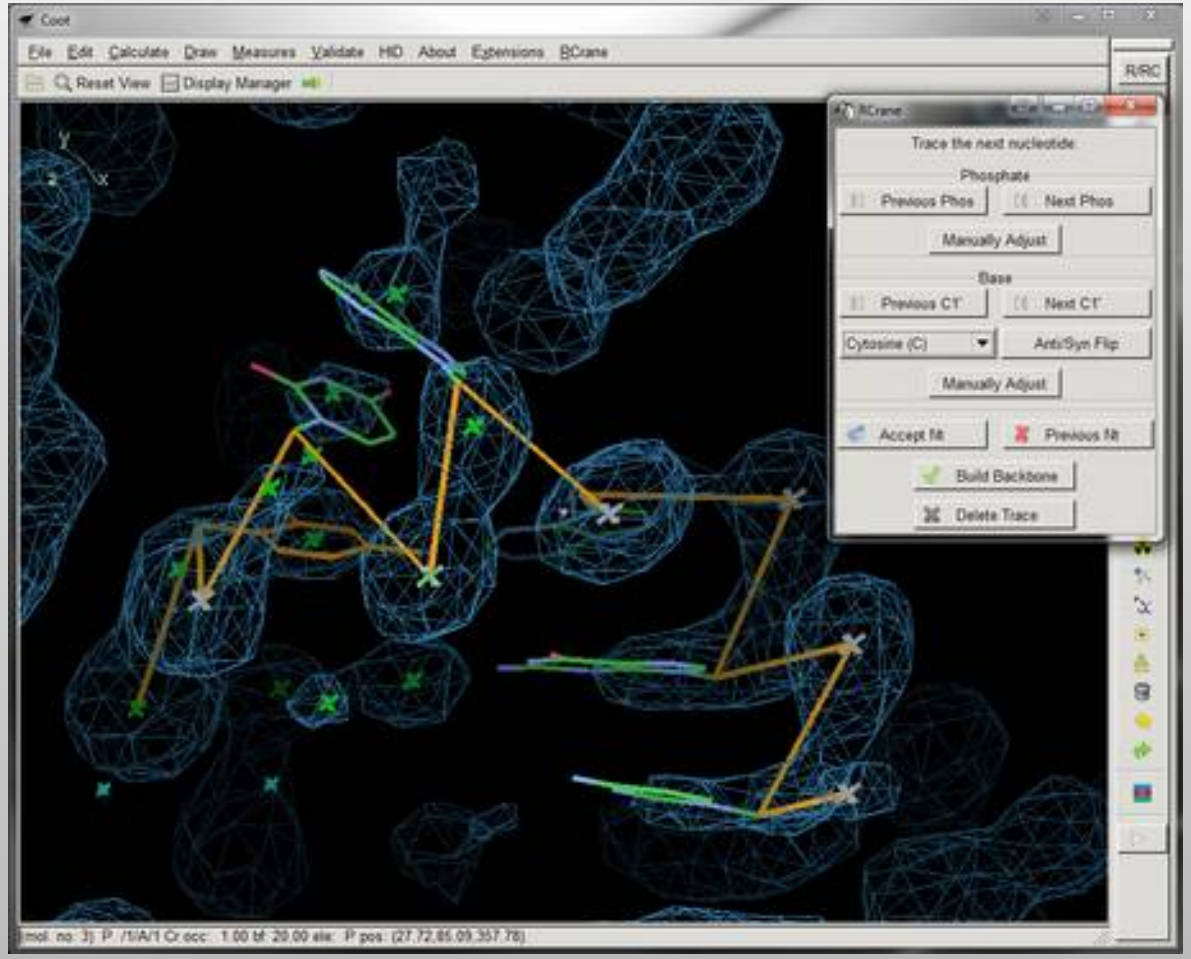
Twisted-trans

Non-pre-PRO

A Sample of Tools

- A few extra tools...

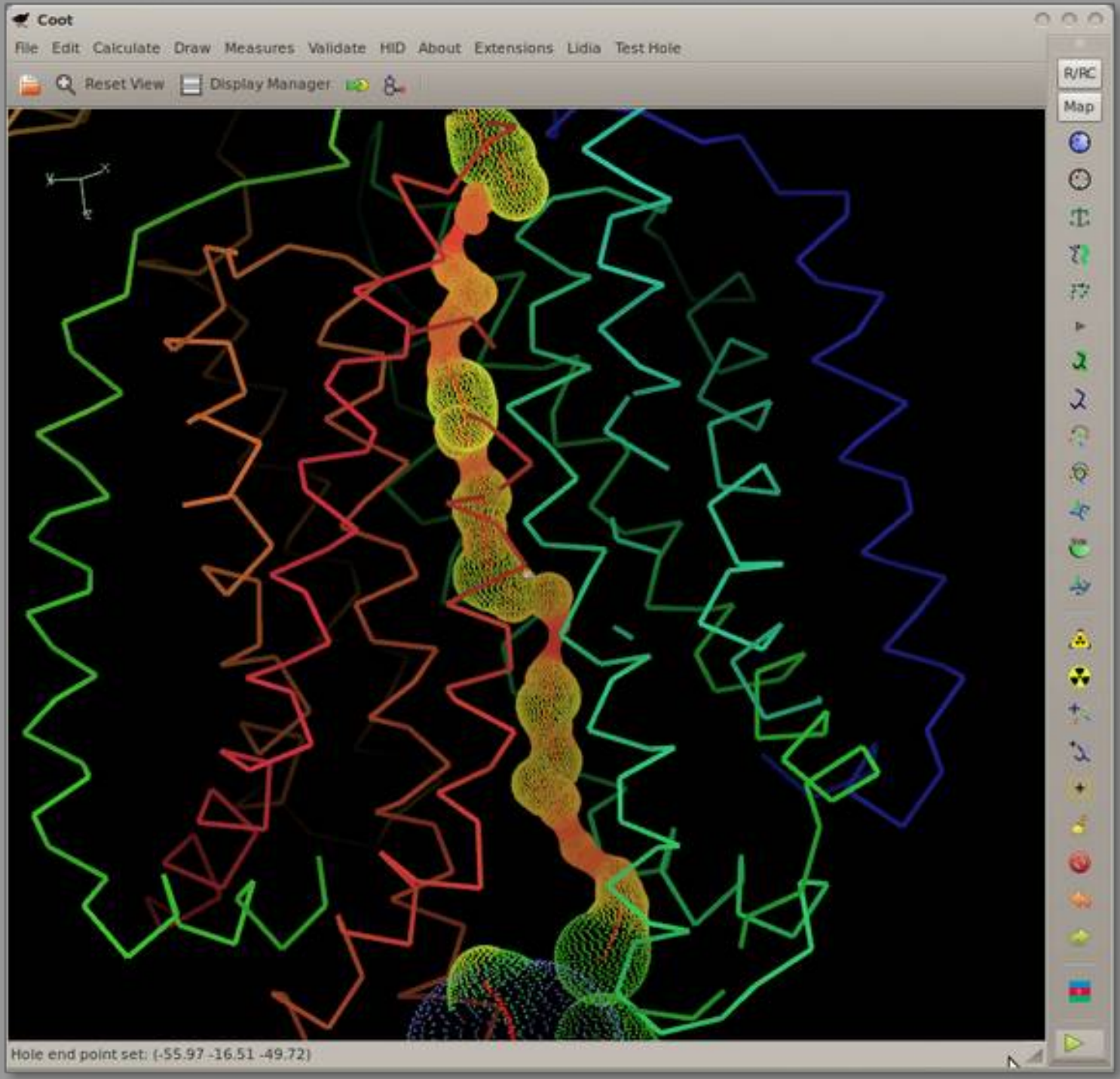
RCrane: Semi-automated RNA building



Kevin Keating

Finding Holes

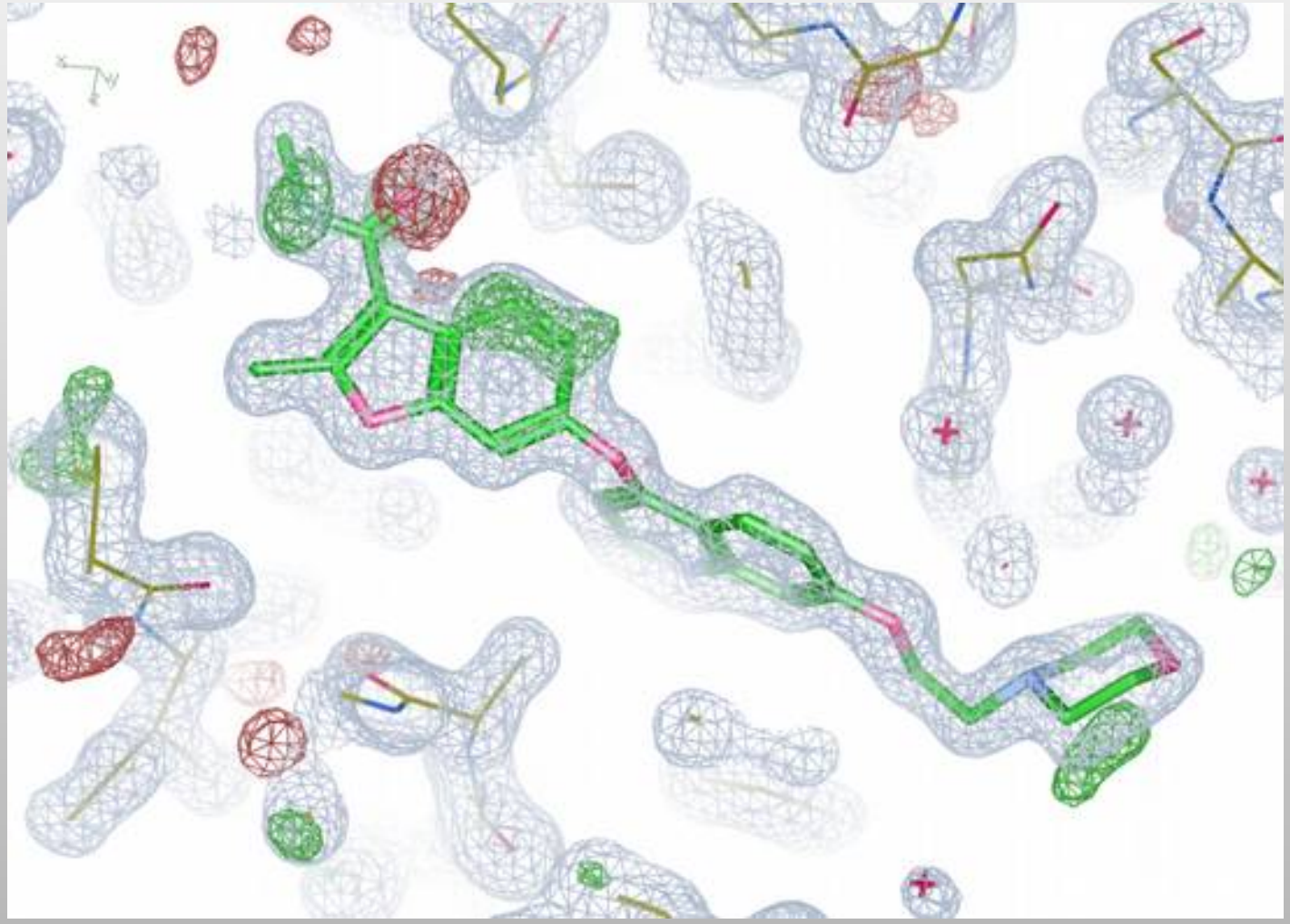
- An implementation of
  - Smart, Goodfellow & Wallace (1993) Biophysics Journal **65**, 2455
  - Atomic radii from AMBER
  - I used
    - radii from CCP4 monomer library
    - sans simulated annealing


Coot
File Edit Calculate Draw Measures Validate HID About Extensions Lidia Test Hole
Reset View
Display Manager
R/RC
Map
Hole end point set: (-55.97 -16.51 -49.72)

Making Density Slides with *Coot*

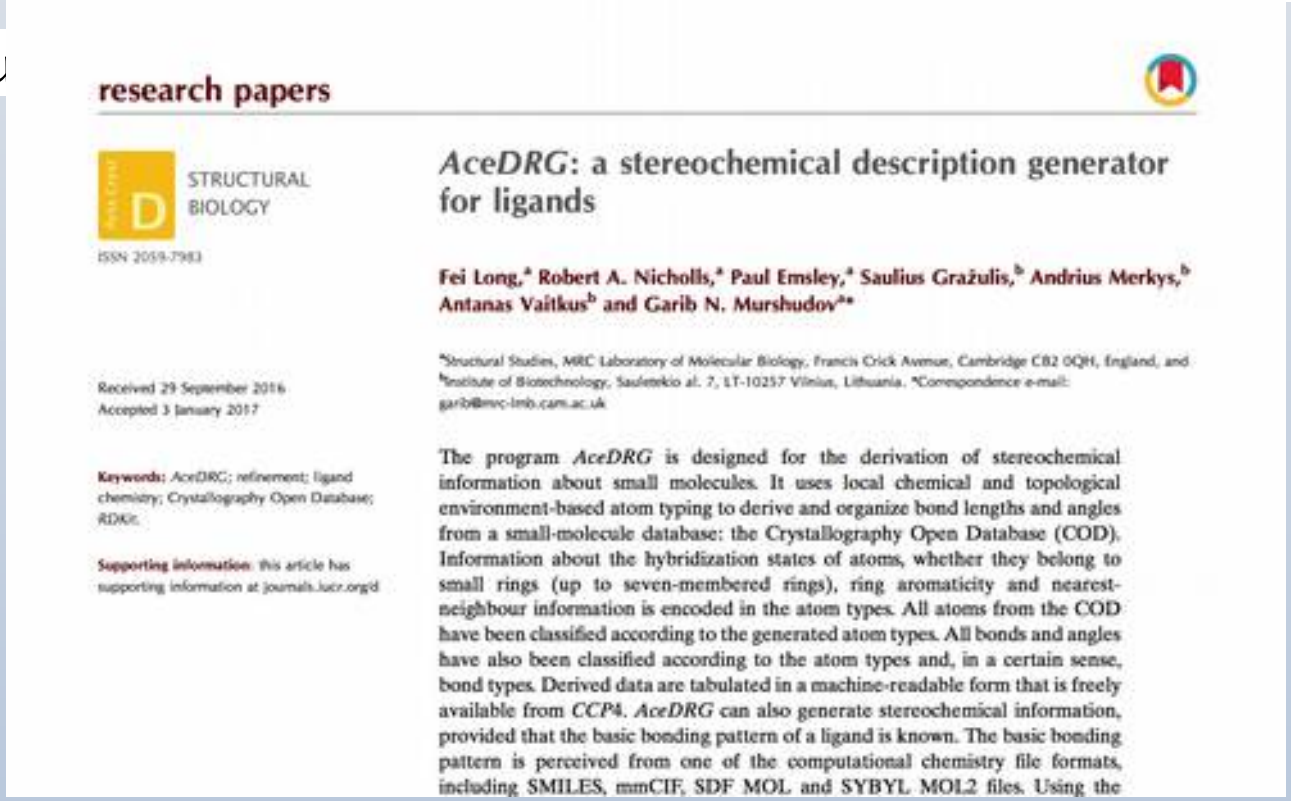
- White background
- “High” Oversampling (2.3x)
- Pale gray (or very pastel) density colour
- Enable Cut-glass mode 5-10%
- Anti-aliased Coot
  - `$ setenv __GL_FSAA_MODE 5`
  - 0.8.3 will do a better job of anti-aliasing out the box
  - (transfer to CCP4-built binaries)

Example Density Slide



New CCP4 Software for Restraints Generation: AceDRG

- A dictionary generator based on geometry derived from structures in COD
- Let's re-write the Refmac/CCP4 Monomer Library
  - canonical sources: wwPBD Chemical Component Dictionary
- Mu



research papers

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Keywords: AceDRG; refinement; ligand chemistry; Crystallography Open Database; RDKit.

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AceDRG: a stereochemical description generator for ligands

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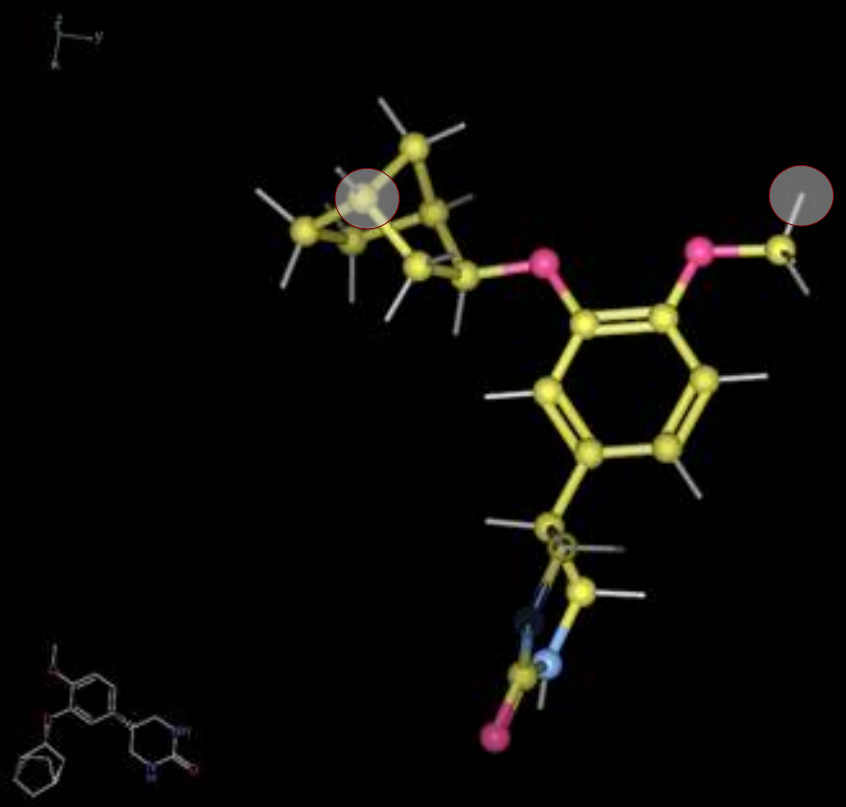
[a]Structural Studies, MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, England, and [b]Institute of Biotechnology, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania. *Correspondence e-mail: garib@mrc-lmb.cam.ac.uk

The program *AceDRG* is designed for the derivation of stereochemical information about small molecules. It uses local chemical and topological environment-based atom typing to derive and organize bond lengths and angles from a small-molecule database: the Crystallography Open Database (COD). Information about the hybridization states of atoms, whether they belong to small rings (up to seven-membered rings), ring aromaticity and nearest-neighbour information is encoded in the atom types. All atoms from the COD have been classified according to the generated atom types. All bonds and angles have also been classified according to the atom types and, in a certain sense, bond types. Derived data are tabulated in a machine-readable form that is freely available from *CCP*4. *AceDRG* can also generate stereochemical information, provided that the basic bonding pattern of a ligand is known. The basic bonding pattern is perceived from one of the computational chemistry file formats, including SMILES, mmCIF, SDF MOL and SYBYL MOL2 files. Using the

Acedrg: COD-Based Atom Types

- COD-based
- 2nd order neighbour-based

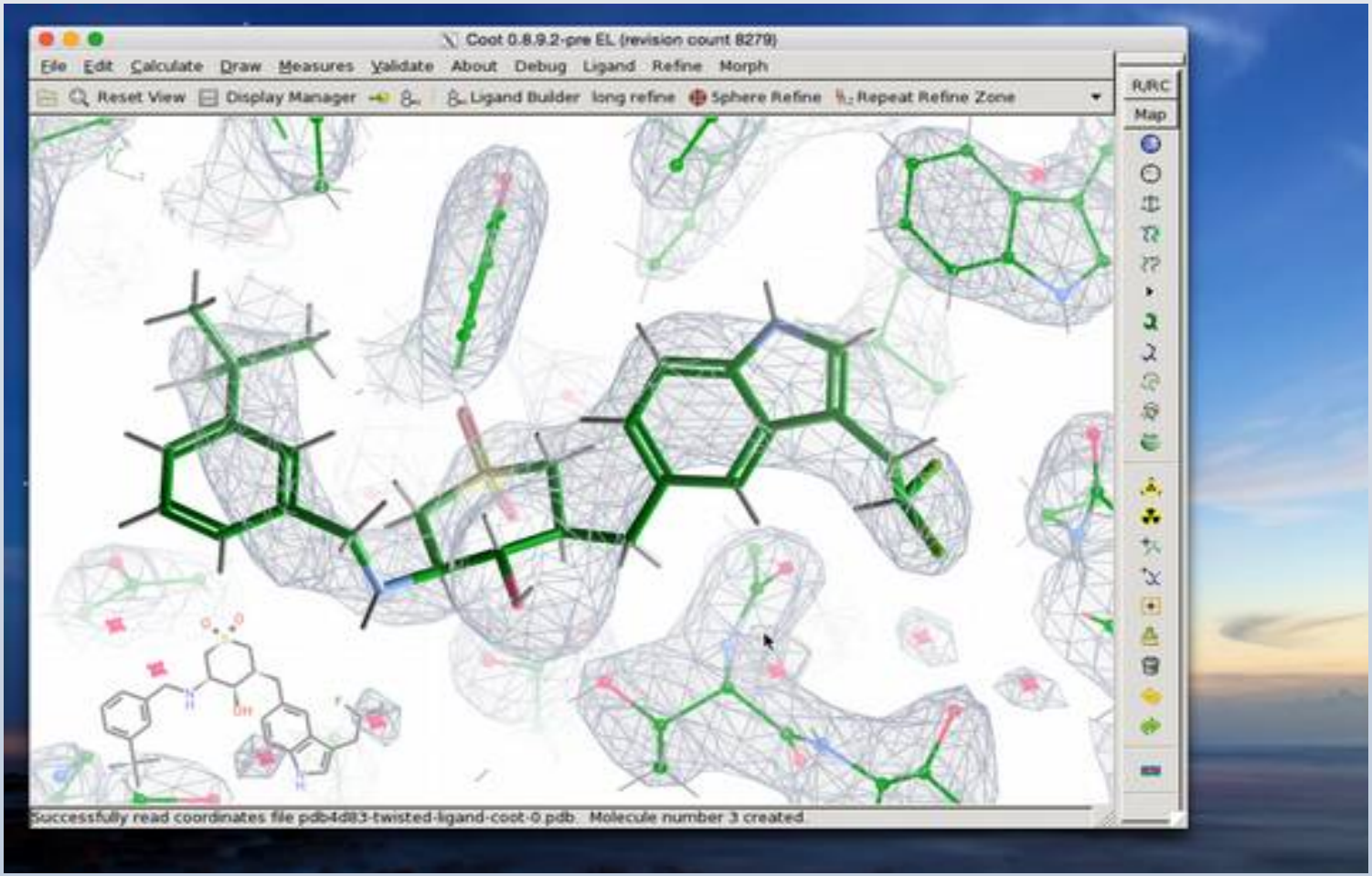


H1B: H(CHHO)
C9: C[5,5,6](C[5,5]CHH)(C[5,6]CHH)(C[5,6]CHO)(H)

Acedrg Link Mode

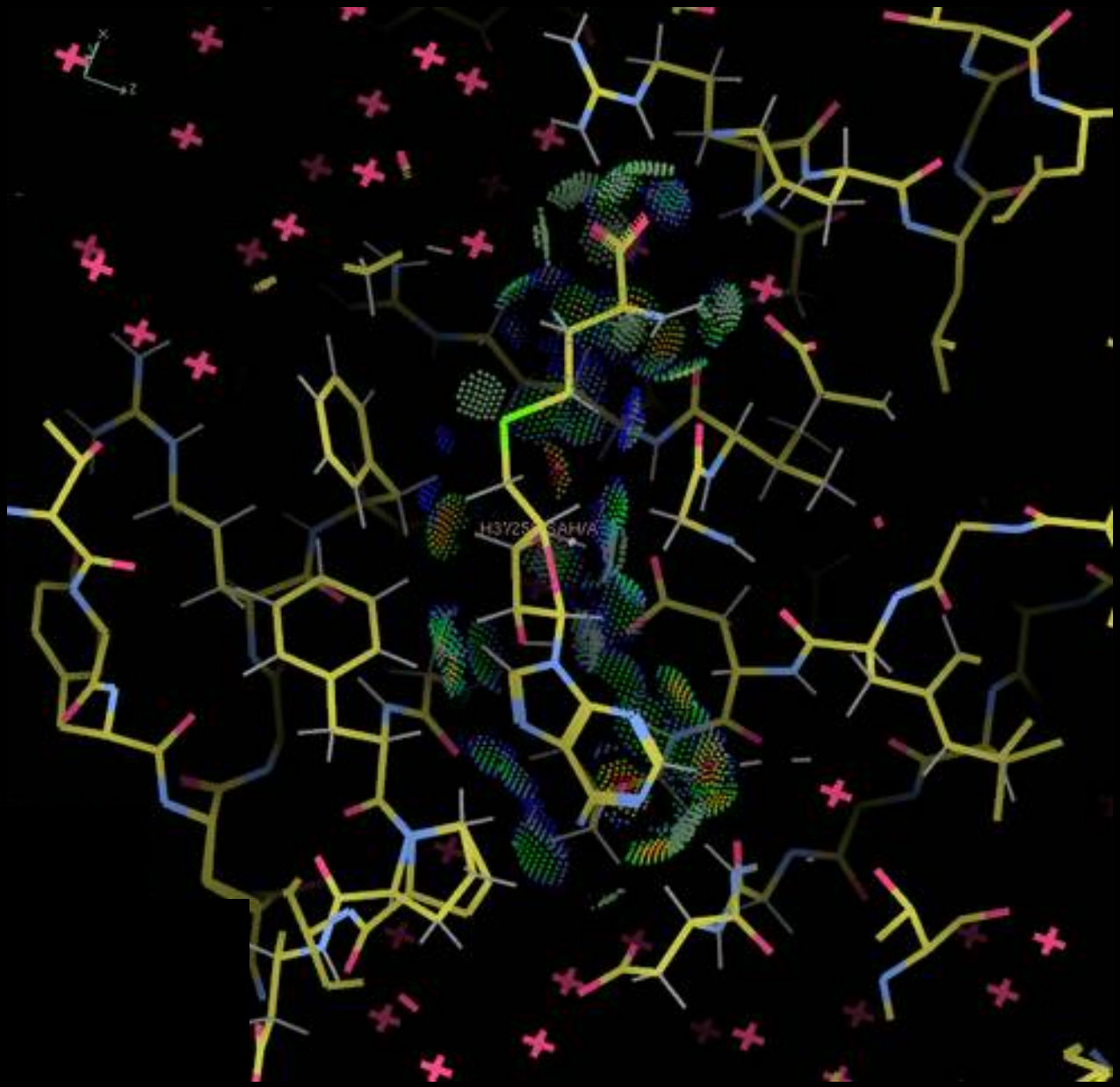
- Links between monomers are made with link dictionary that describes the edits to the chemistry that occurs as a result of the generation of a new covalent bond
- We prefer and recommend the use of Links between (previously know) monomers are preferred to creation of a new chemical entity
- A new interface in Coot to exploit it

Acedrg Link Mode *Coot* Interface

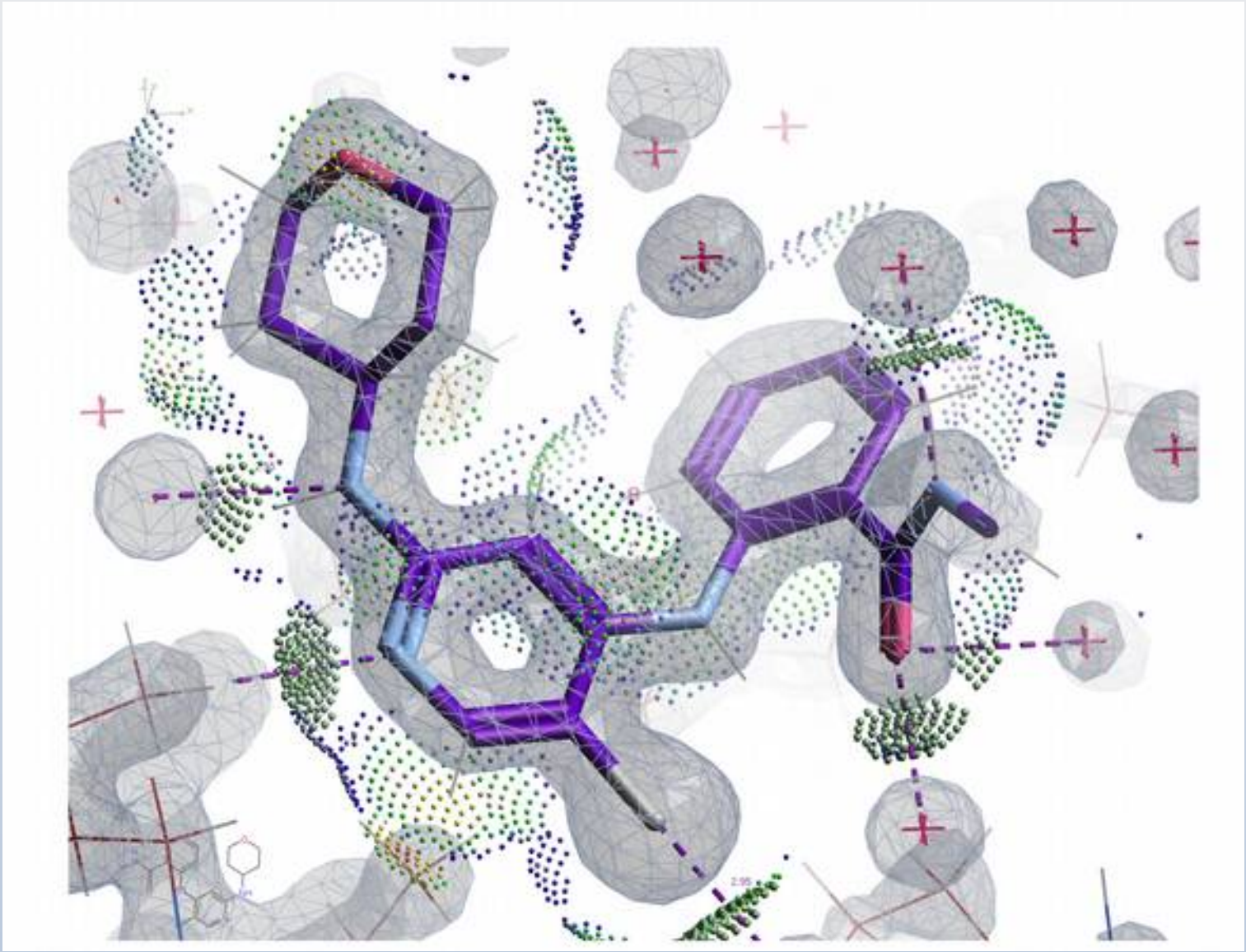


Ligand → Isolated Contact Dots

Probe Contacts



Ligand → Isolated Contact Dots

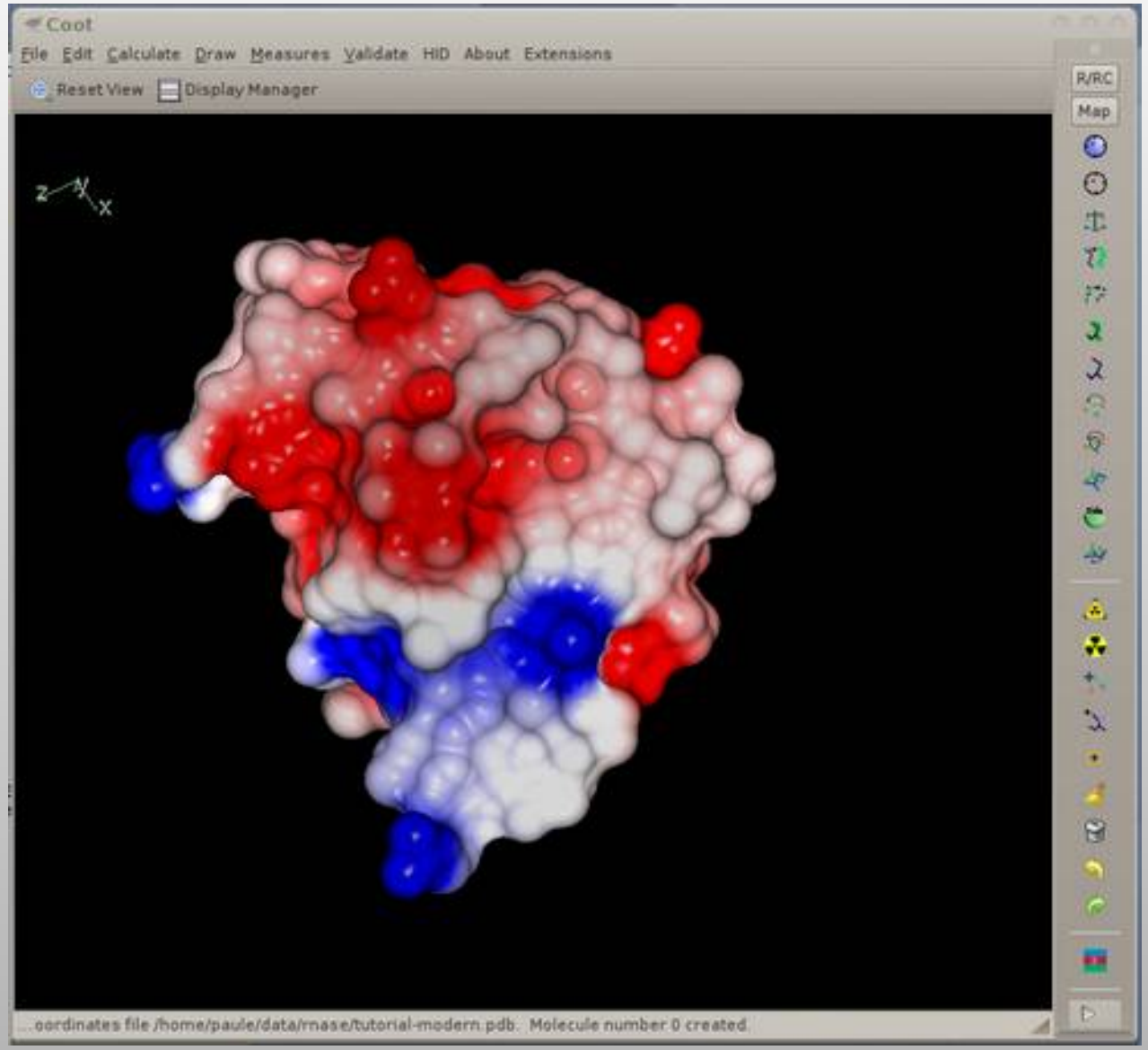


Ligand → Tabulate Ligand Distortions

Example Coot Ligand Distortion Score

```
  Residue Distortion List:
   plane  O3   C19  C20  C18  C16  C15  C17  C13  C14  N2   C4   C5   O1   C3   C6   O2  penalty-score:  36.51
   plane  C2   C7   C8   C9   C10  C11  C12                                              penalty-score:   8.82
   bond  C13 to  C4  target_value:   1.490 d:   1.432 sigma:   0.020 length-devi  -0.058 penalty-score:   8.44
   bond  C4  to  C3  target_value:   1.490 d:   1.436 sigma:   0.020 length-devi  -0.054 penalty-score:   7.21
   bond  O3  to  C19 target_value:   1.362 d:   1.318 sigma:   0.020 length-devi  -0.044 penalty-score:   4.75
   bond  C19 to  C20 target_value:   1.390 d:   1.433 sigma:   0.020 length-devi   0.043 penalty-score:   4.67
   bond  C1  to  C2  target_value:   1.390 d:   1.428 sigma:   0.020 length-devi   0.038 penalty-score:   3.70
   bond  C4  to  C5  target_value:   1.490 d:   1.454 sigma:   0.020 length-devi  -0.036 penalty-score:   3.26
   bond  C13 to  C14 target_value:   1.490 d:   1.456 sigma:   0.020 length-devi  -0.034 penalty-score:   2.91
   bond  C15 to  C13 target_value:   1.490 d:   1.458 sigma:   0.020 length-devi  -0.032 penalty-score:   2.57
   bond  C16 to  C15 target_value:   1.490 d:   1.459 sigma:   0.020 length-devi  -0.031 penalty-score:   2.45
   angle  C13 -  C4  -  C5   target: 108.00 model_angle: 133.80 sigma:  3.00 angle-devi 25.80 penalty-score:  73.93
   angle  O1  -  C5  -  C4   target: 108.00 model_angle: 126.59 sigma:  3.00 angle-devi 18.59 penalty-score:  38.38
   angle  C13 -  C15 -  C16  target: 120.00 model_angle: 102.30 sigma:  3.00 angle-devi 17.70 penalty-score:  34.83
   angle  O2  -  C6  -  N1   target: 108.00 model_angle: 122.80 sigma:  3.00 angle-devi 14.80 penalty-score:  24.34
   angle  O2  -  C6  -  C3   target: 108.00 model_angle: 122.76 sigma:  3.00 angle-devi 14.76 penalty-score:  24.19
   angle  C13 -  C15 -  C17  target: 120.00 model_angle: 133.33 sigma:  3.00 angle-devi 13.33 penalty-score:  19.76
   angle  C4  -  C13 -  C15  target: 120.00 model_angle: 132.99 sigma:  3.00 angle-devi 12.99 penalty-score:  18.76
   angle  N1  -  C5  -  O1   target: 108.00 model_angle: 120.48 sigma:  3.00 angle-devi 12.48 penalty-score:  17.32
   angle  C15 -  C13 -  C14  target: 120.00 model_angle: 110.43 sigma:  3.00 angle-devi -9.57 penalty-score:  10.18
   angle  N1  -  C6  -  C3   target: 108.00 model_angle: 114.28 sigma:  3.00 angle-devi  6.28 penalty-score:   4.38
   angle  C6  -  C3  -  C4   target: 108.00 model_angle: 101.75 sigma:  3.00 angle-devi -6.25 penalty-score:   4.34
Residue Distortion Summary:
   29 bond restraints
   44 angle restraints
   sum of bond  distortions penalties:  59.5697
   sum of angle distortions penalties:  300.405
   average bond  distortion penalty:    2.05413
   average angle distortion penalty:    6.82739
   total distortion penalty:            405.304
   average distortion penalty:          4.93116
```

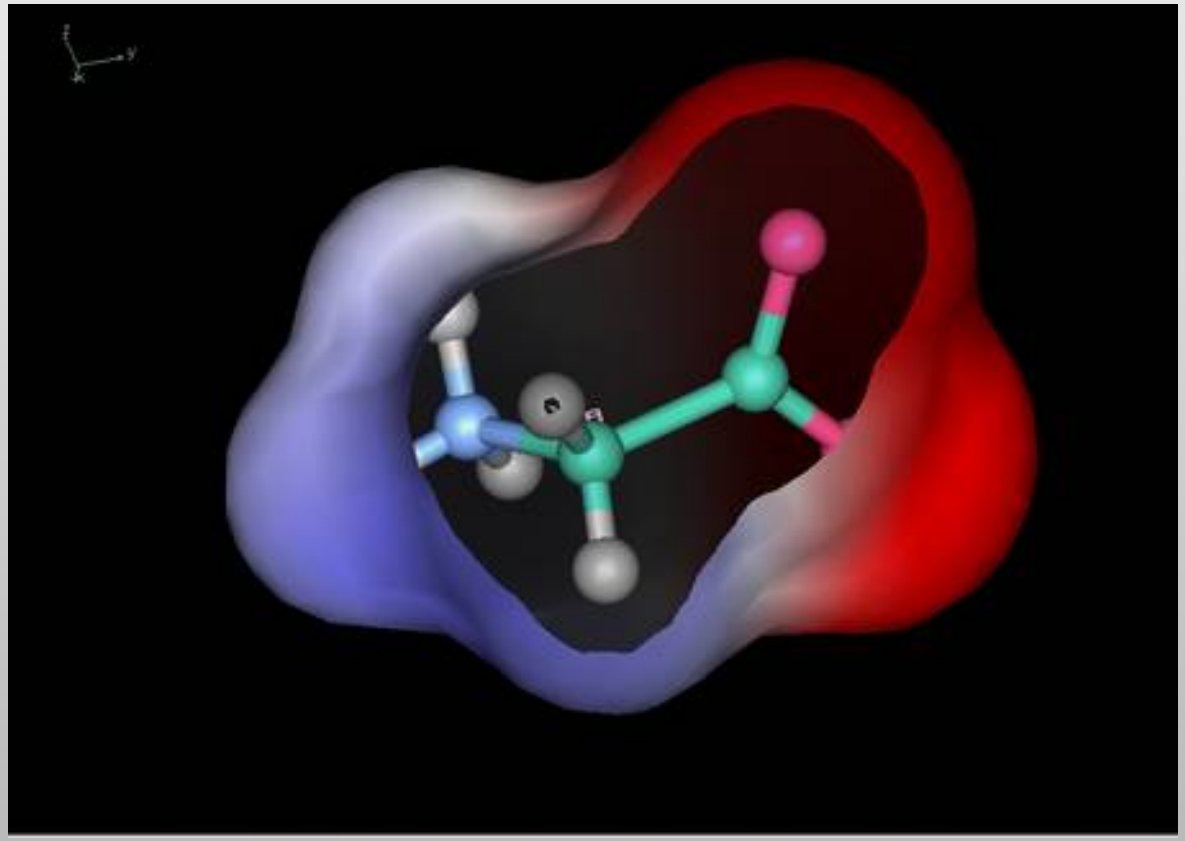
Some Representation Tools



Gruber & Noble (2007)

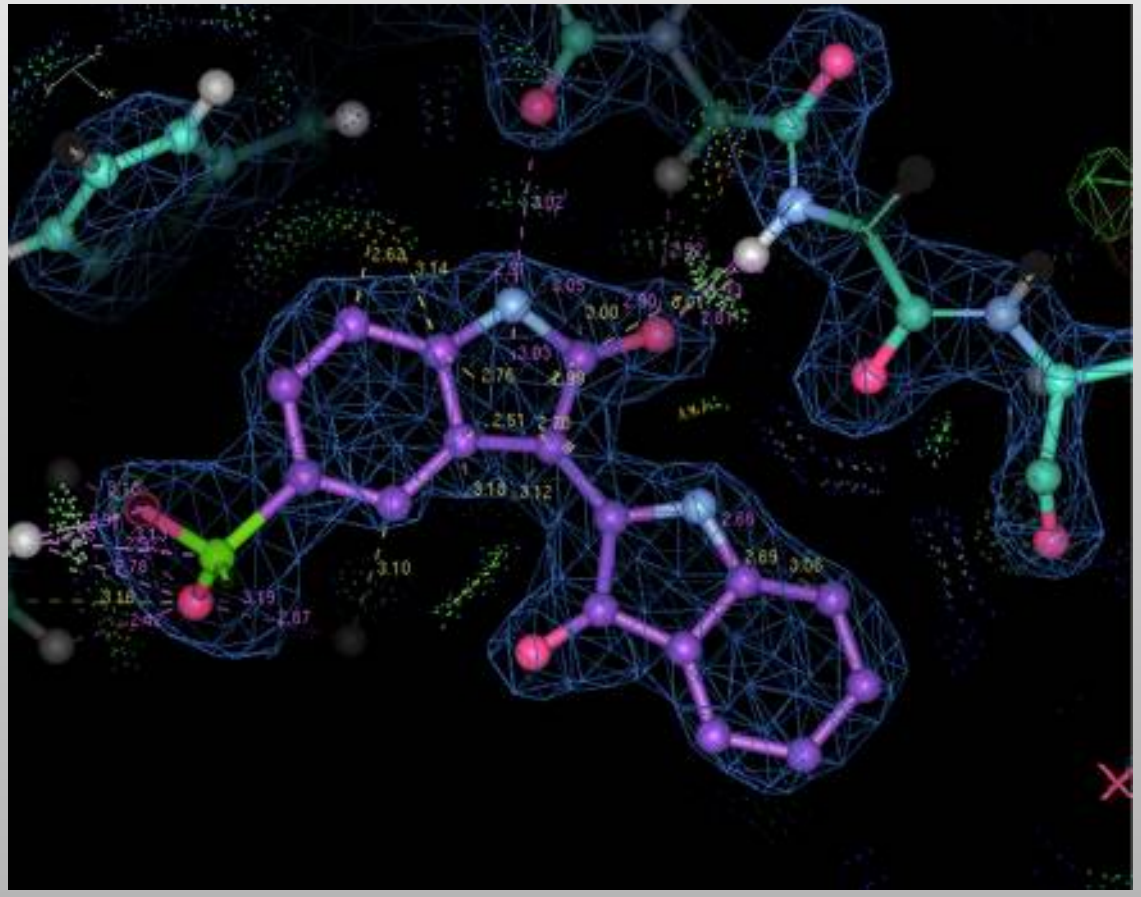
Other Things

- Surfaces that use dictionary partial charges

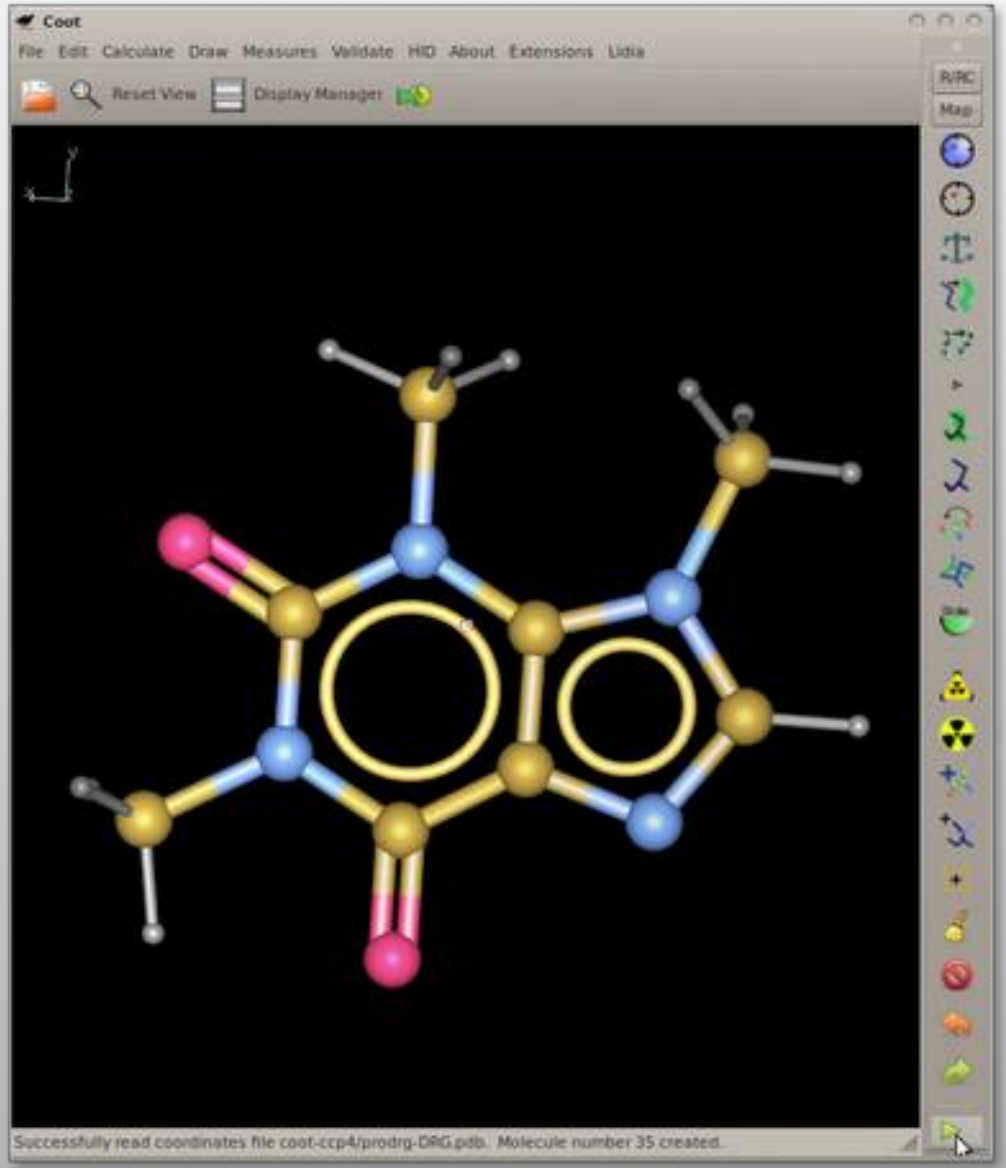


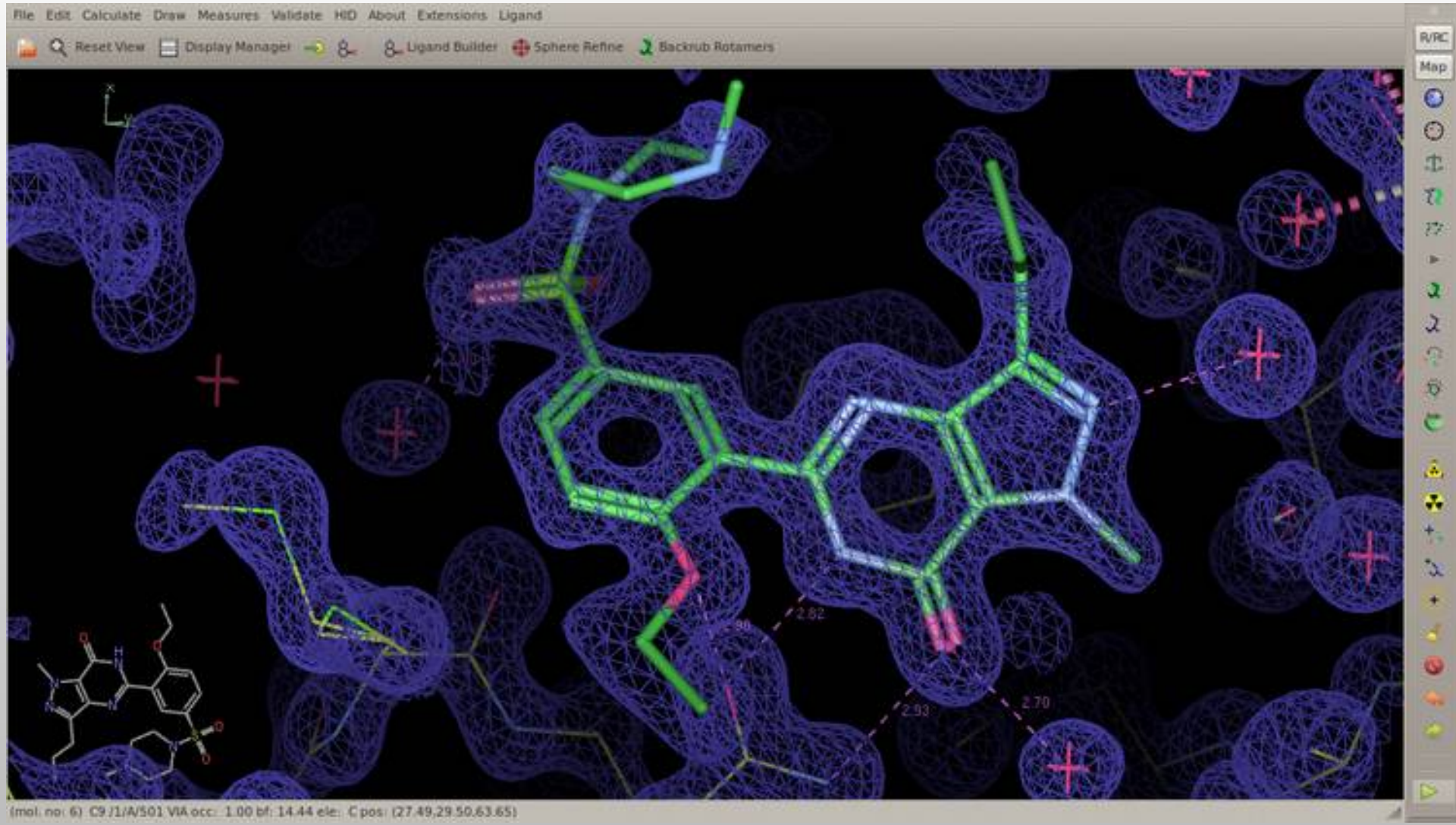
Other Tools

- Molprobity dots for ligands
  - Highlight interesting site



Representing Bond Orders

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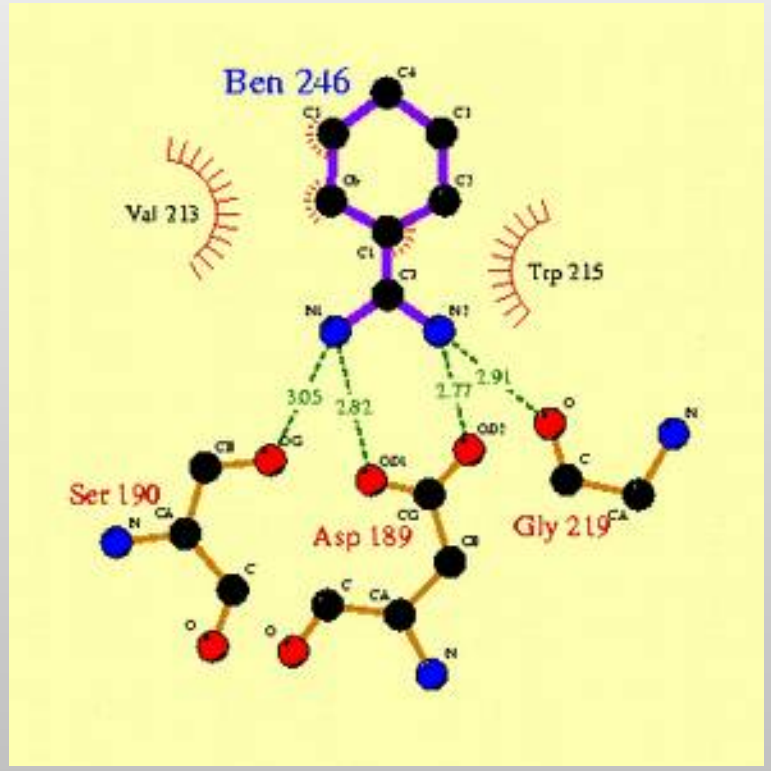
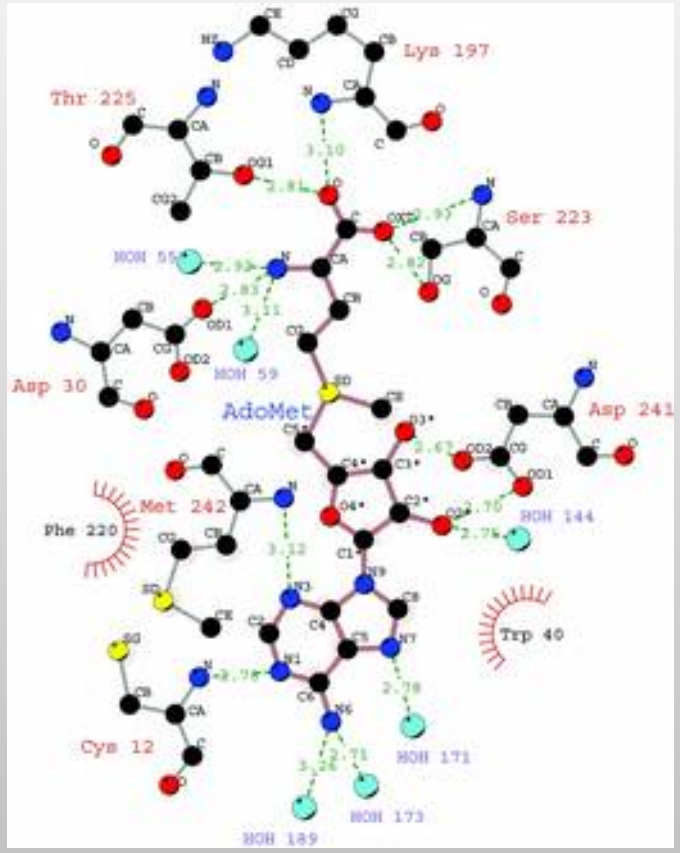
Reset View
Display Manager
Ligand Builder
Sphere Refine
Backrub Rotamers
R/RC
Map
2.82
2.93
2.70
(mol. no: 6) C9 /1/A/501 VIA occ: 1.00 bf: 14.44 ele: C pos: (27.49,29.50,63.65)

Residue Environment Layout

Ligplot





Can we do better?

Residue Environment Layout

- This can't be solved by an “algorithmic/one-pass” procedure
  - Not in the general case
- Introduce “energy penalty terms” for displeasing interactions
- And use 2D energy minimisation to solve

Layout Energy Terms

$$E = \sum\sum w_{ij}(d_{ij}^2 - D_{ij}^2) +$$

Residues match 3D Distances

$$\sum\sum \exp(-\frac{1}{2}d_{ij}^2) +$$

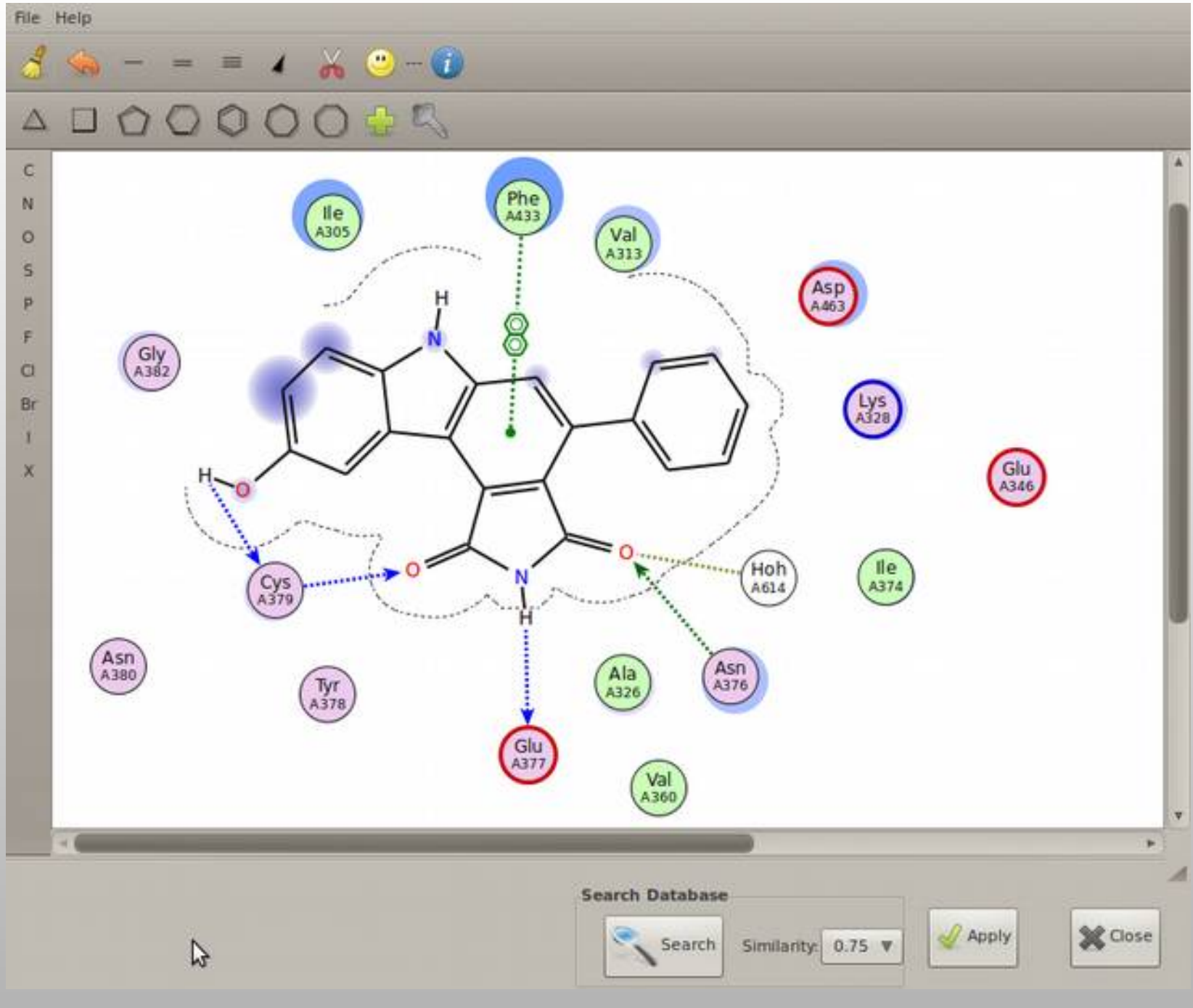
Residues don't overlay each other

$$\sum\sum (d_{ik}^2 - D_{ik}^2) +$$

Residues are close to H-bonding ligand atoms

$$\sum\sum \exp(-\frac{1}{2}d_{ik}^2)$$

Residues don't overlap ligand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Val A313
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Glu A346
Cys A379
Hoh A614
Ile A374
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Search
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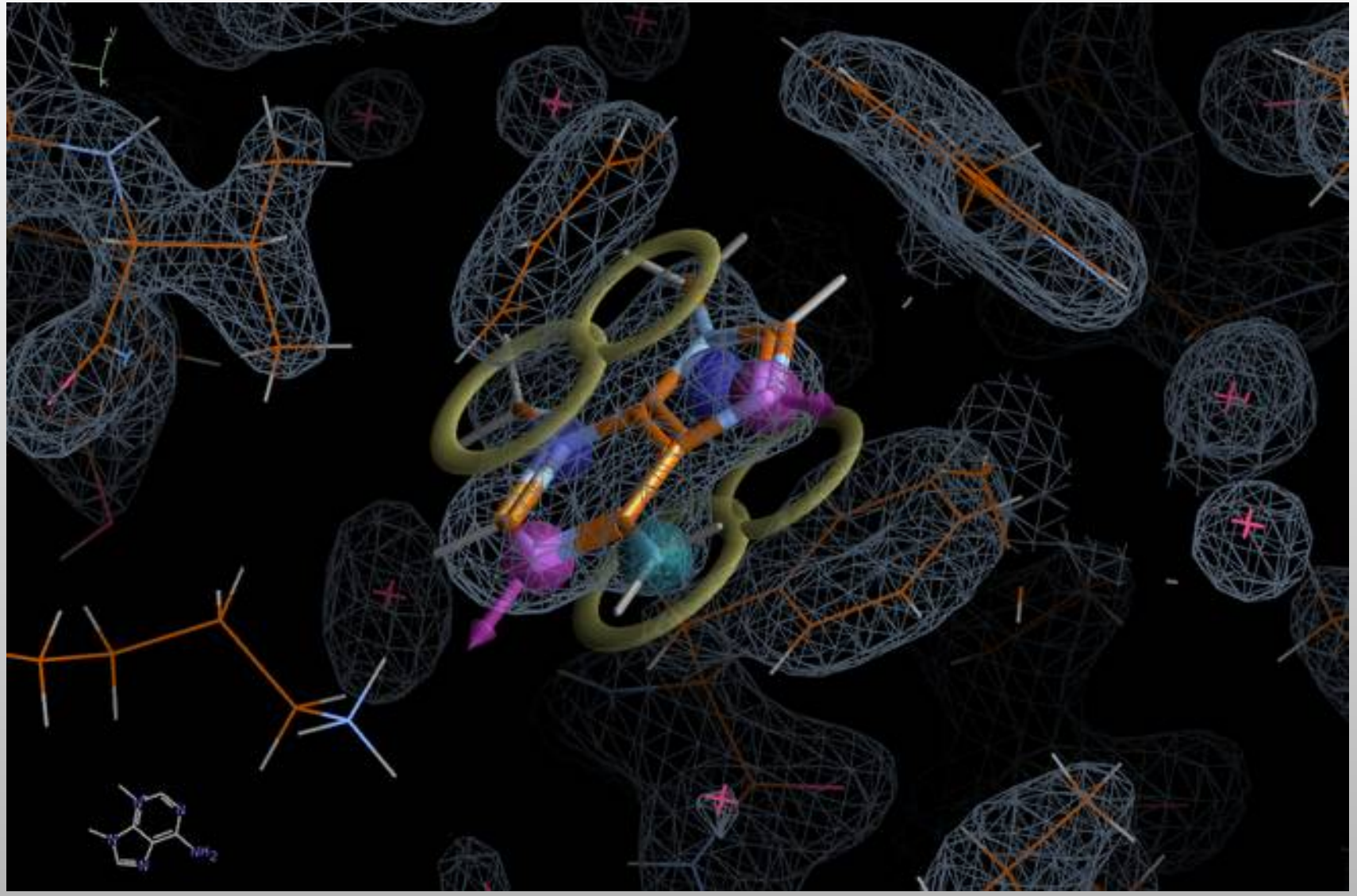
Apply
Close

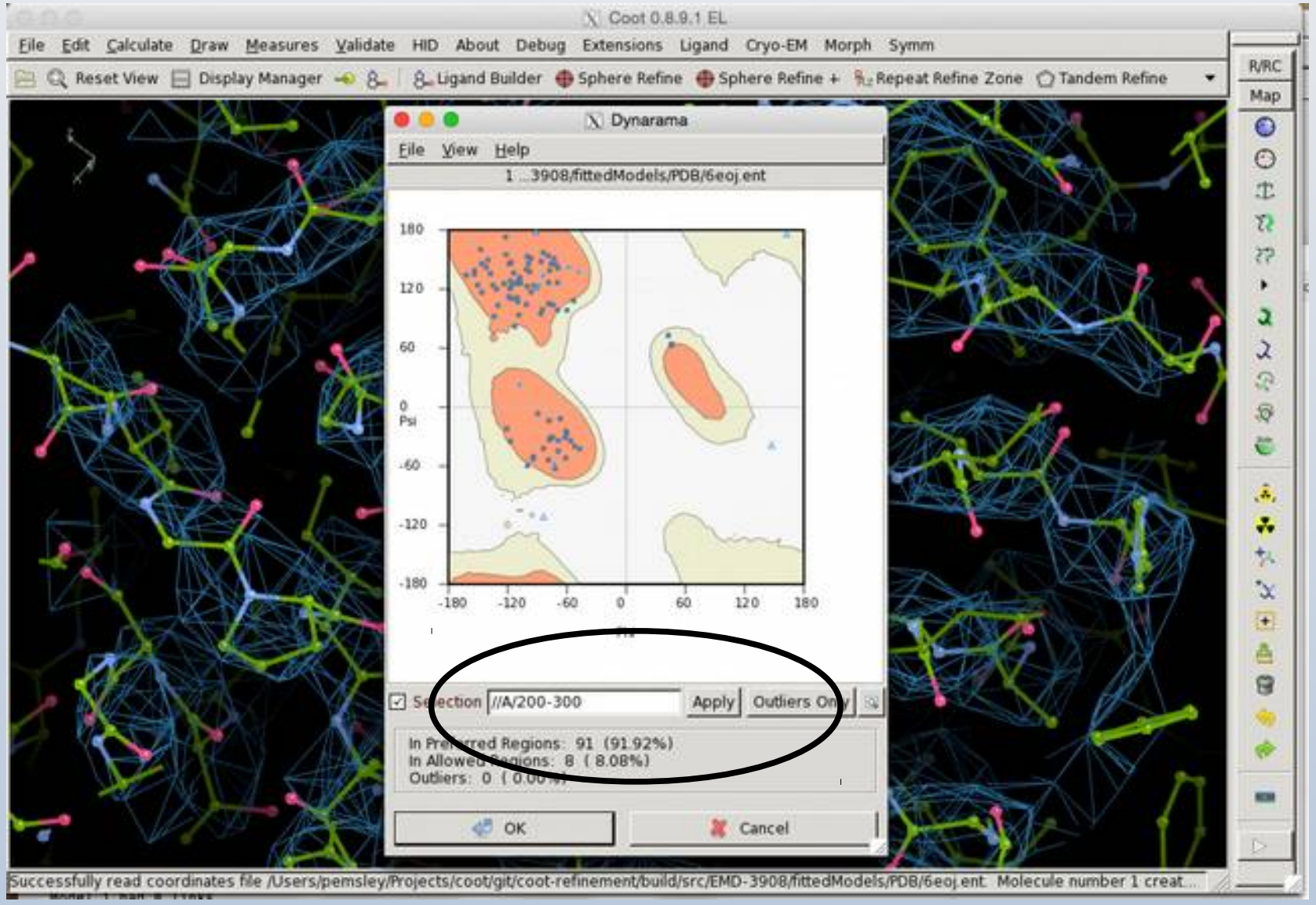
Chemical Features



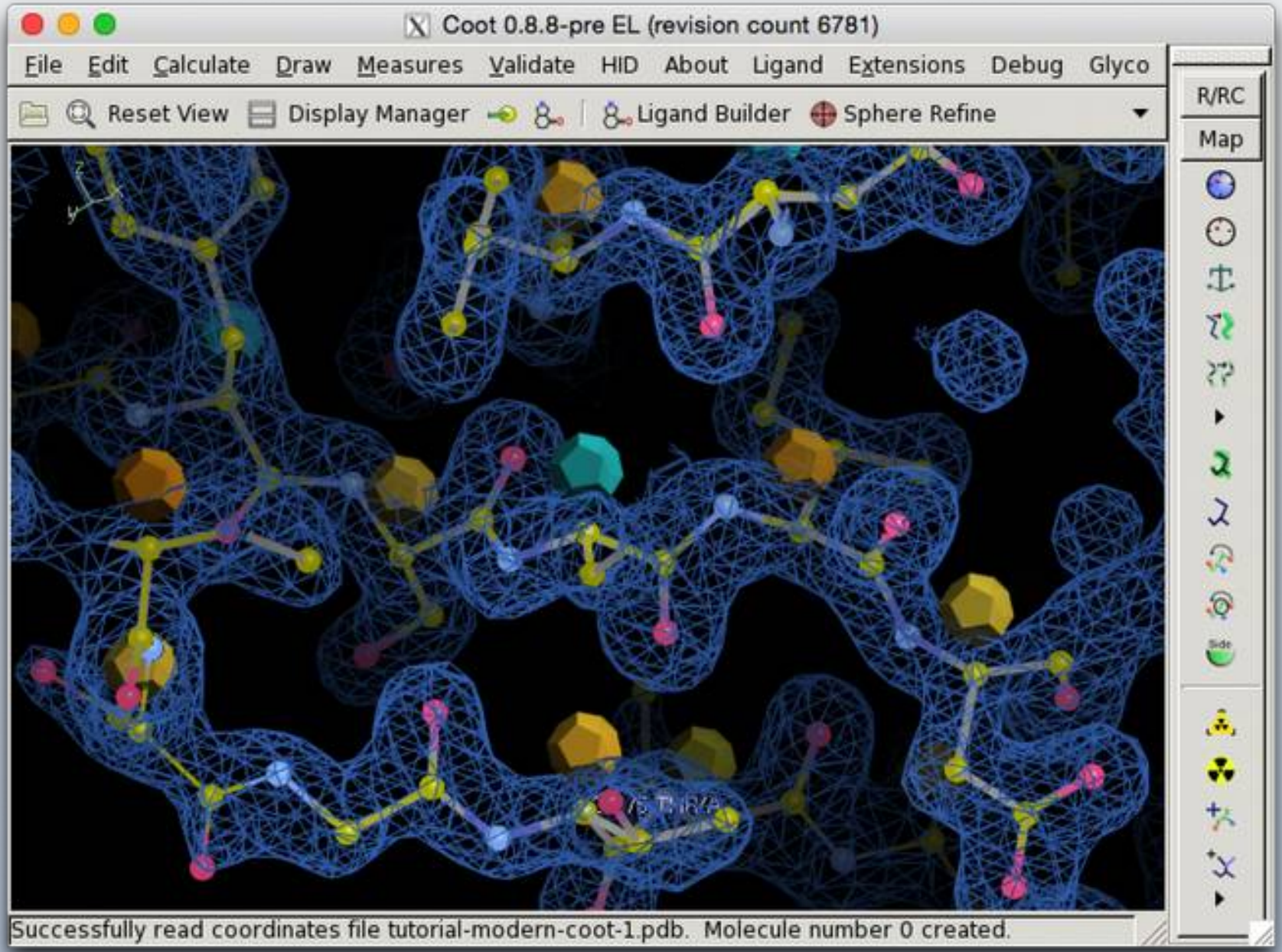
GUI Updates

Map Properties

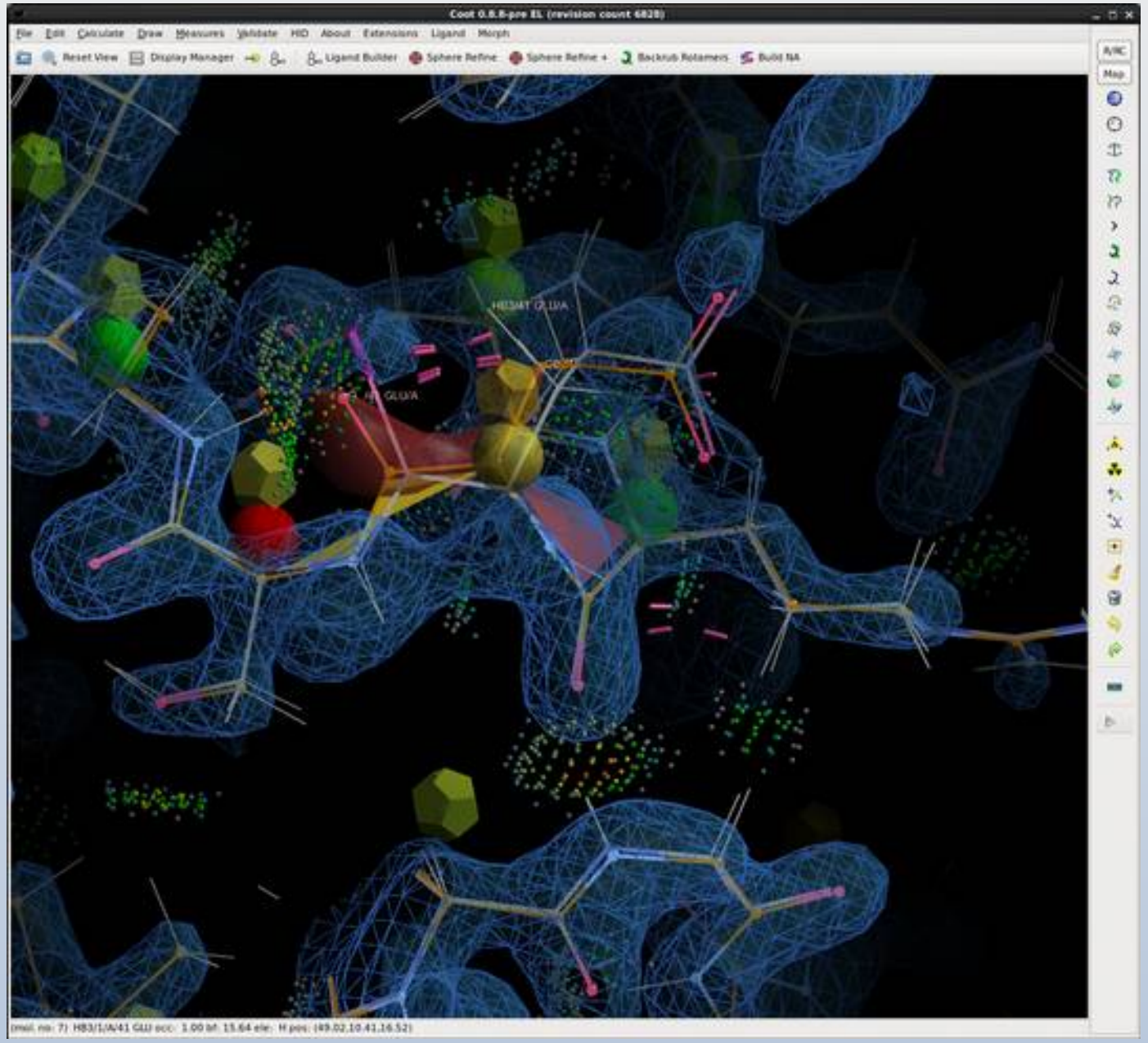
The New Ramachandran Plot



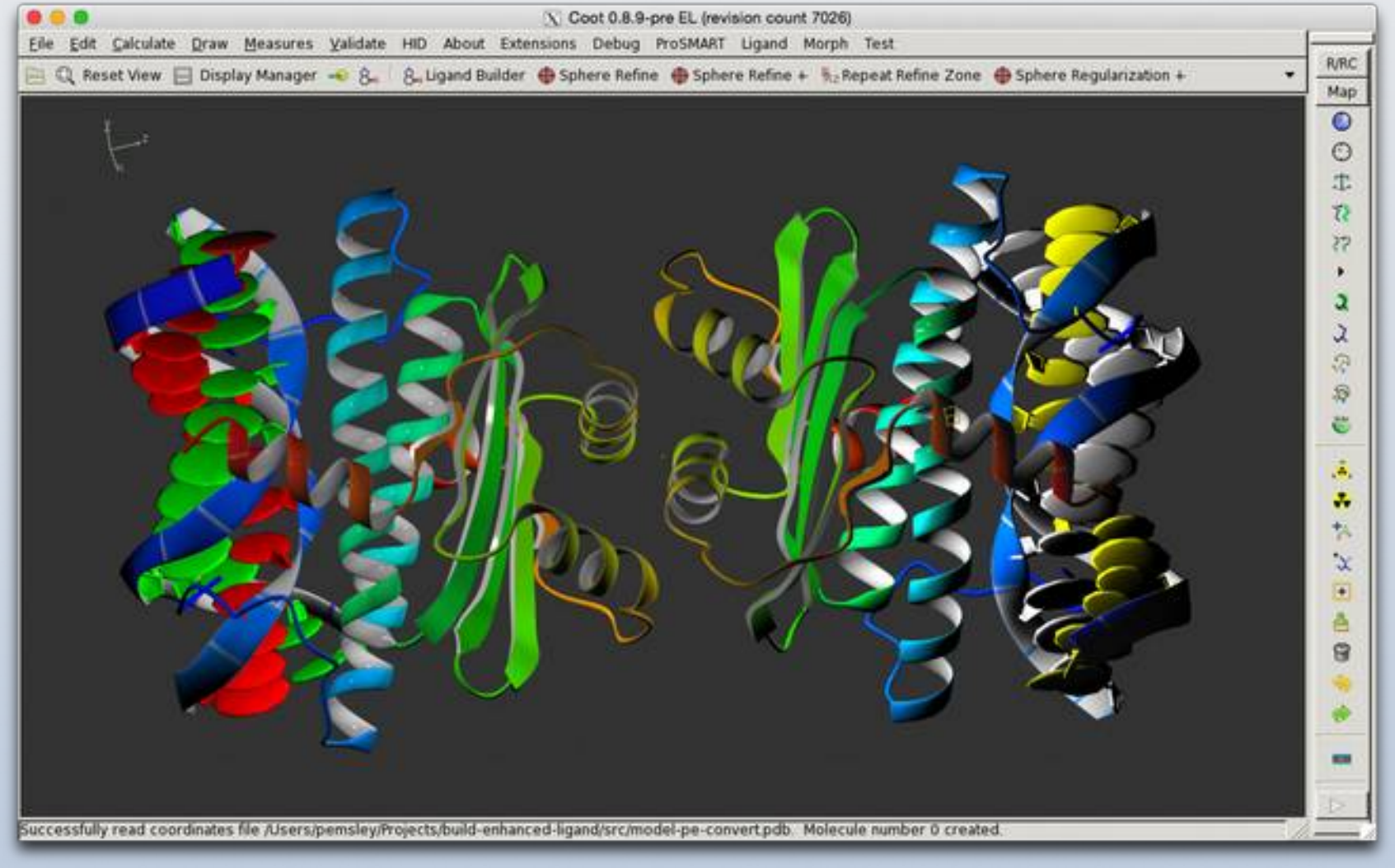
Interactive Rotamer Goodness



Multi-Criteria Markup



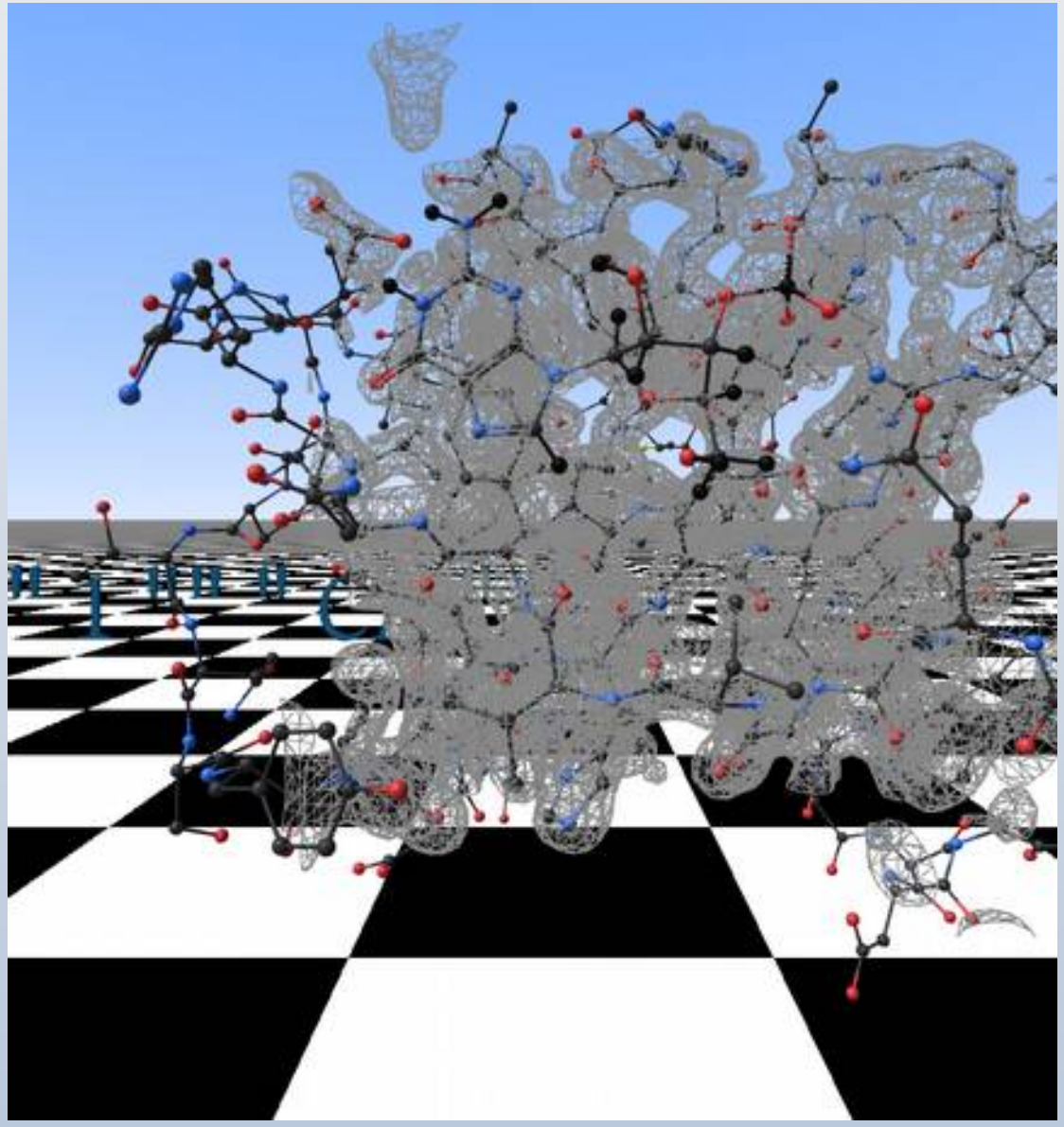
Coot Futures: GPU Ribbons



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

A Few Tools More...

- Fitting Low-Resolution/EM maps
- Ligands:
  - dictionaries
  - ligand-fitting
  - analysis
- Carbohydrate-fitting
  - N-linked glycosylation

Acknowledgements

- Kevin Cowtan
- Bernhard Lohkamp
- Eleanor Dodson
- Keith Wilson

- Libraries, dictionaries
  - Alexei Vagin, Eugene Krissinel
  - Richardsons (Duke)

- Funding
  - BBSRC, CCP4 & MRC

Non-Crystallographic Symmetry

What is 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

Handling NCS

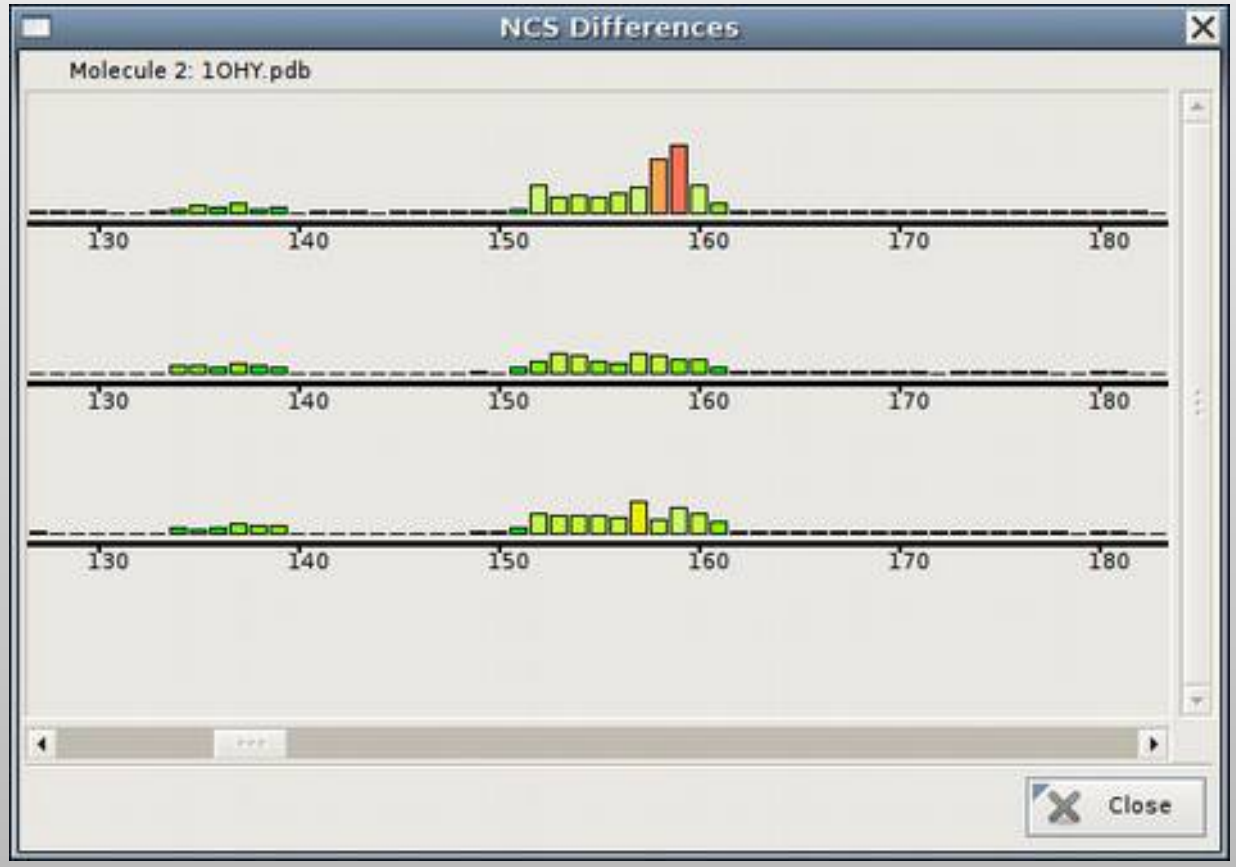
- What are the Problems?
- Strict NCS:
  - NCS should appear like crystallographic symmetry does [exact copies]
- Non-Strict NCS:
  - Molecules are different
  - How to cope with differences, but minimize unnecessary rebuilding?

Handling NCS

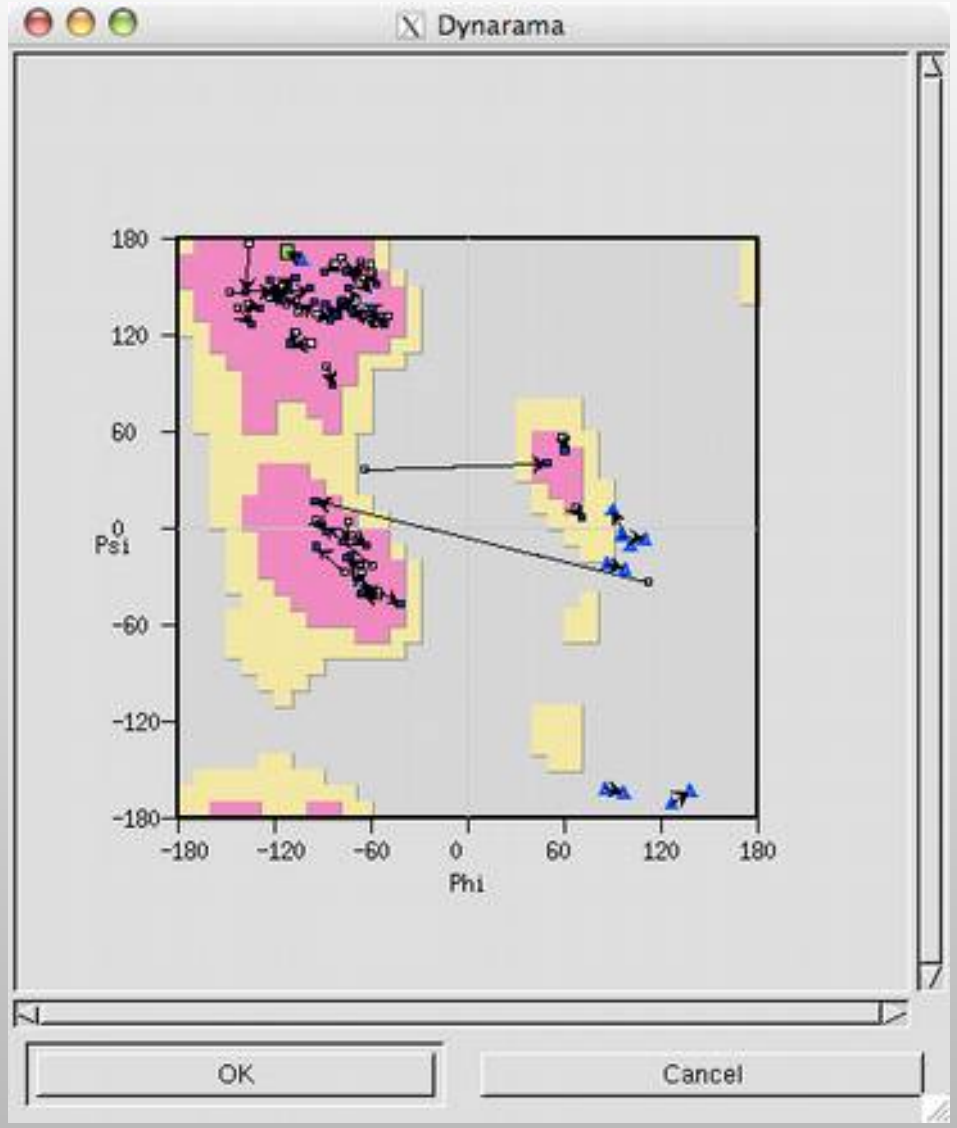
Typical Scenario:

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

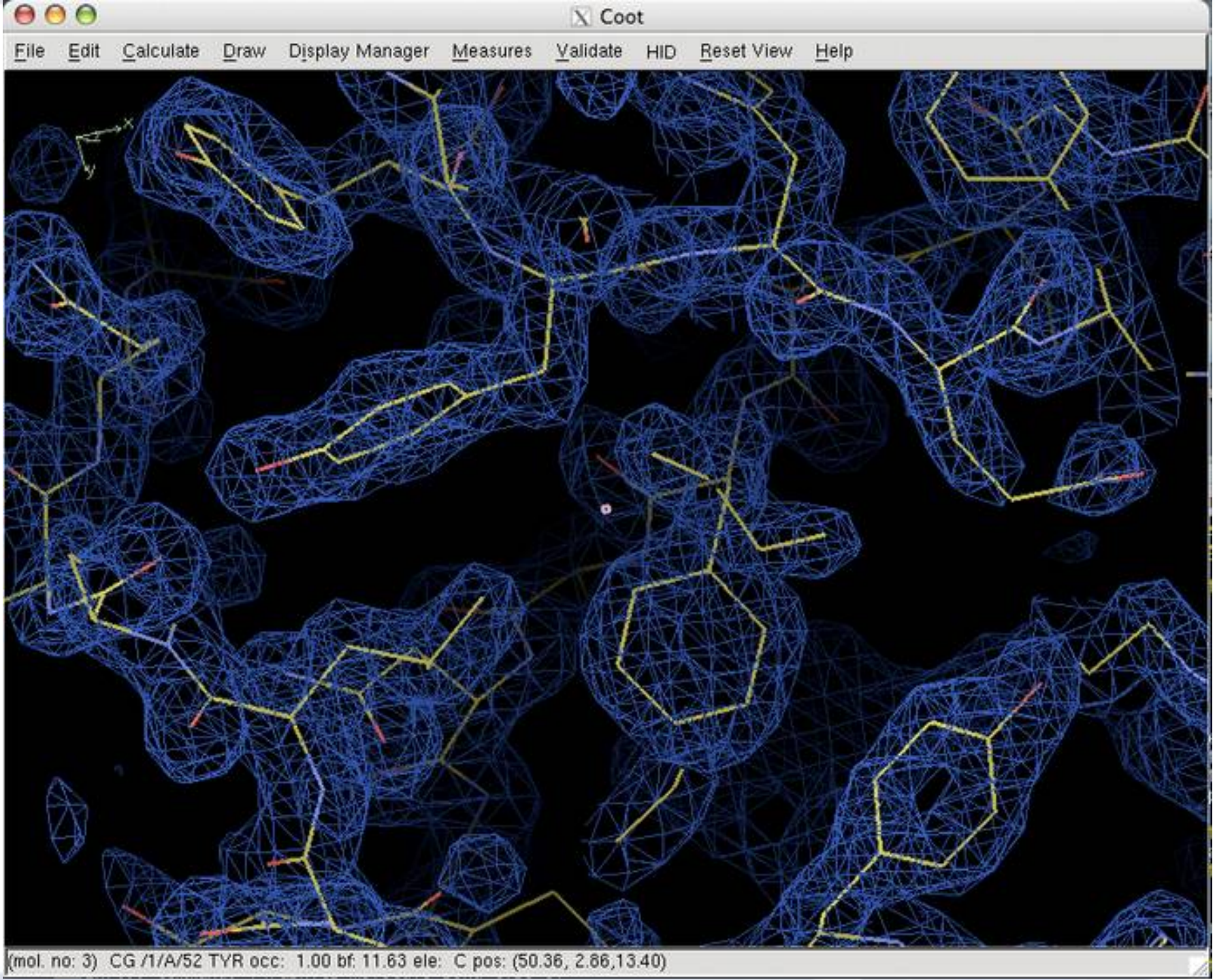
...or new NCS Differences graph



…or Kleywegt Plots[*]



[*] Named by George Sheldrick


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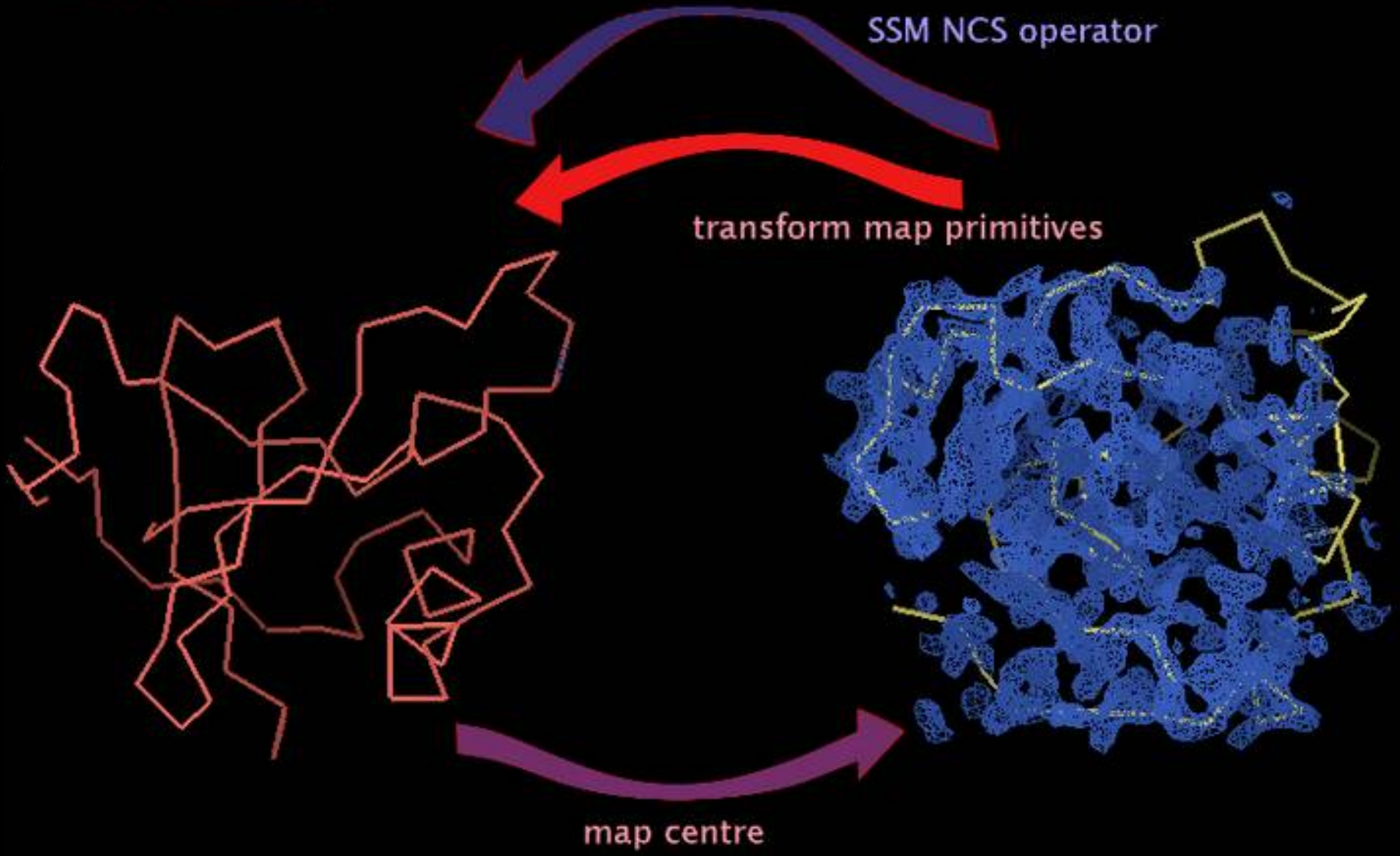
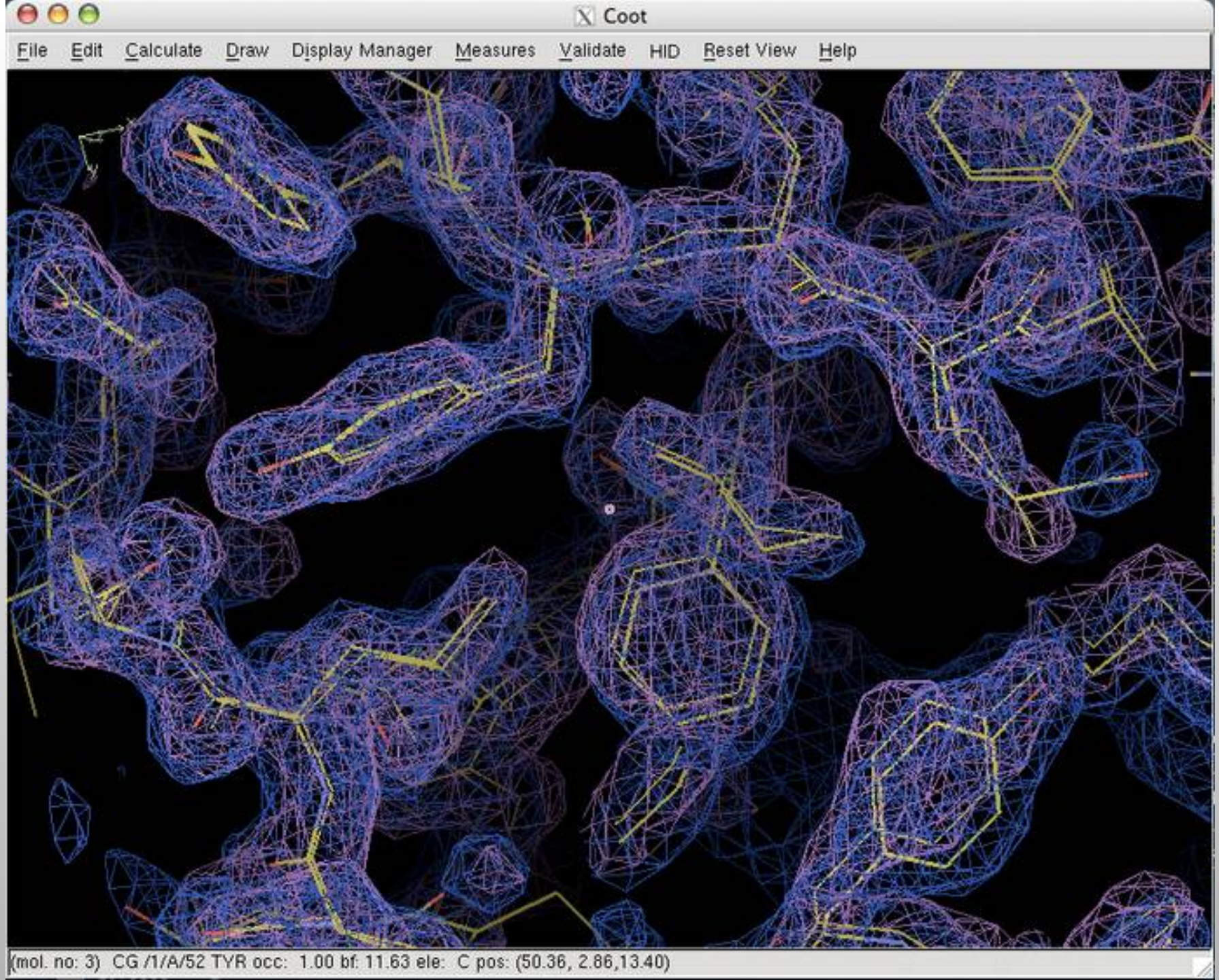
NCS Overlays

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Note to self

- Expand rotamers, (trans/eclipsed/gauche torsions)
- Expand phi, psi
- Discuss Rama restraints