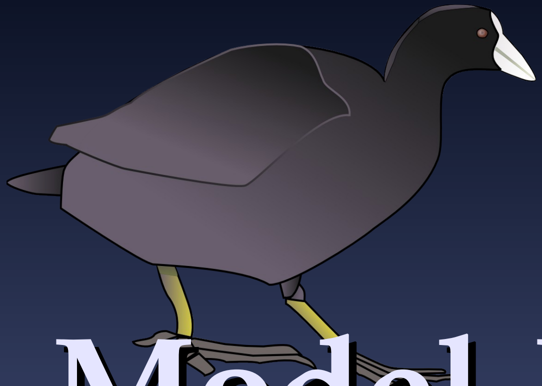
Eurasian Coot
(*Fulica atra*)

September 2009 Třešť



Model-Building with Coot

Low Resolution Tools, Refinement Extensions, Hints & Tips

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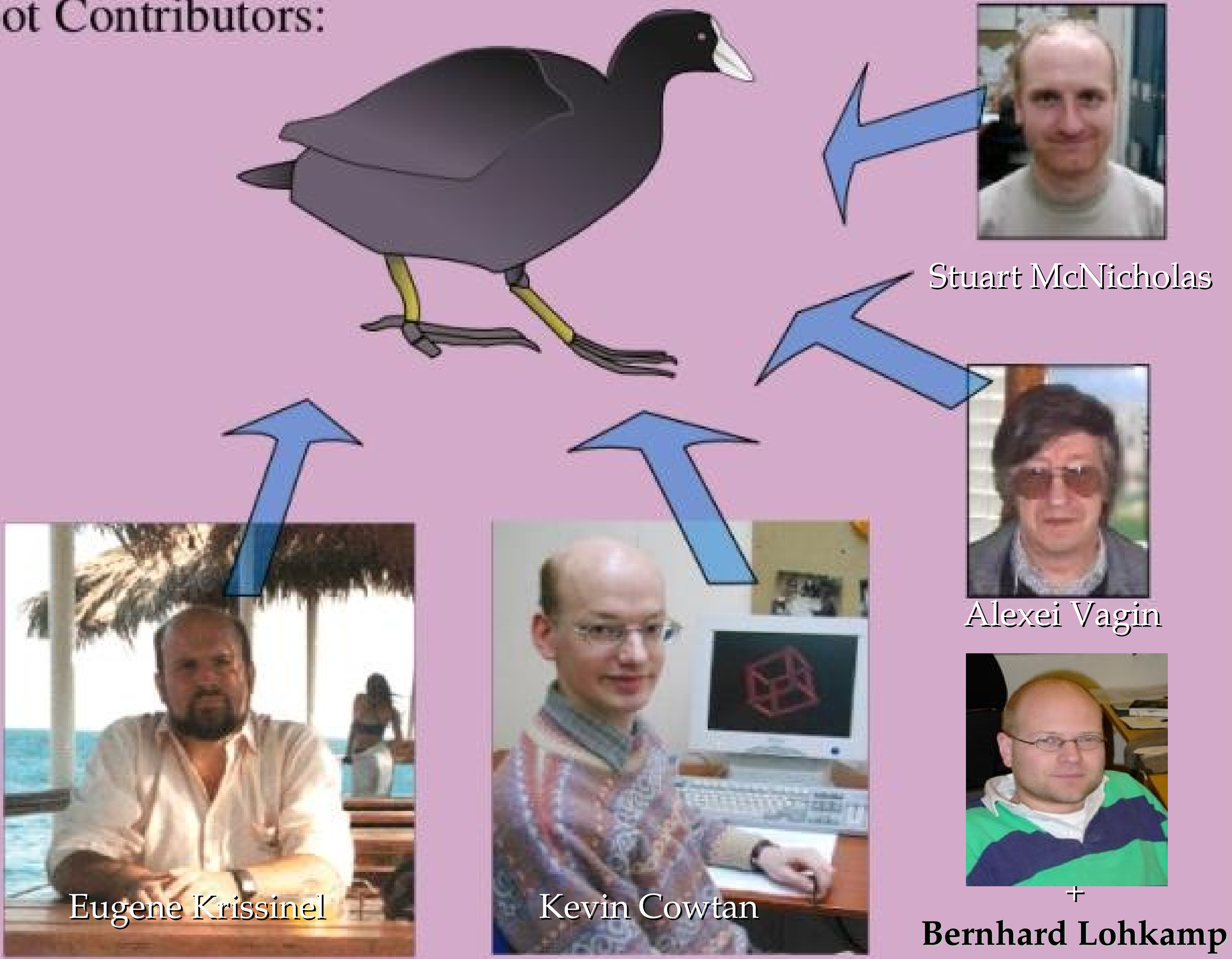
Coot Contributors:



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Kevin Cowtan

\+

Bernhard Lohkamp

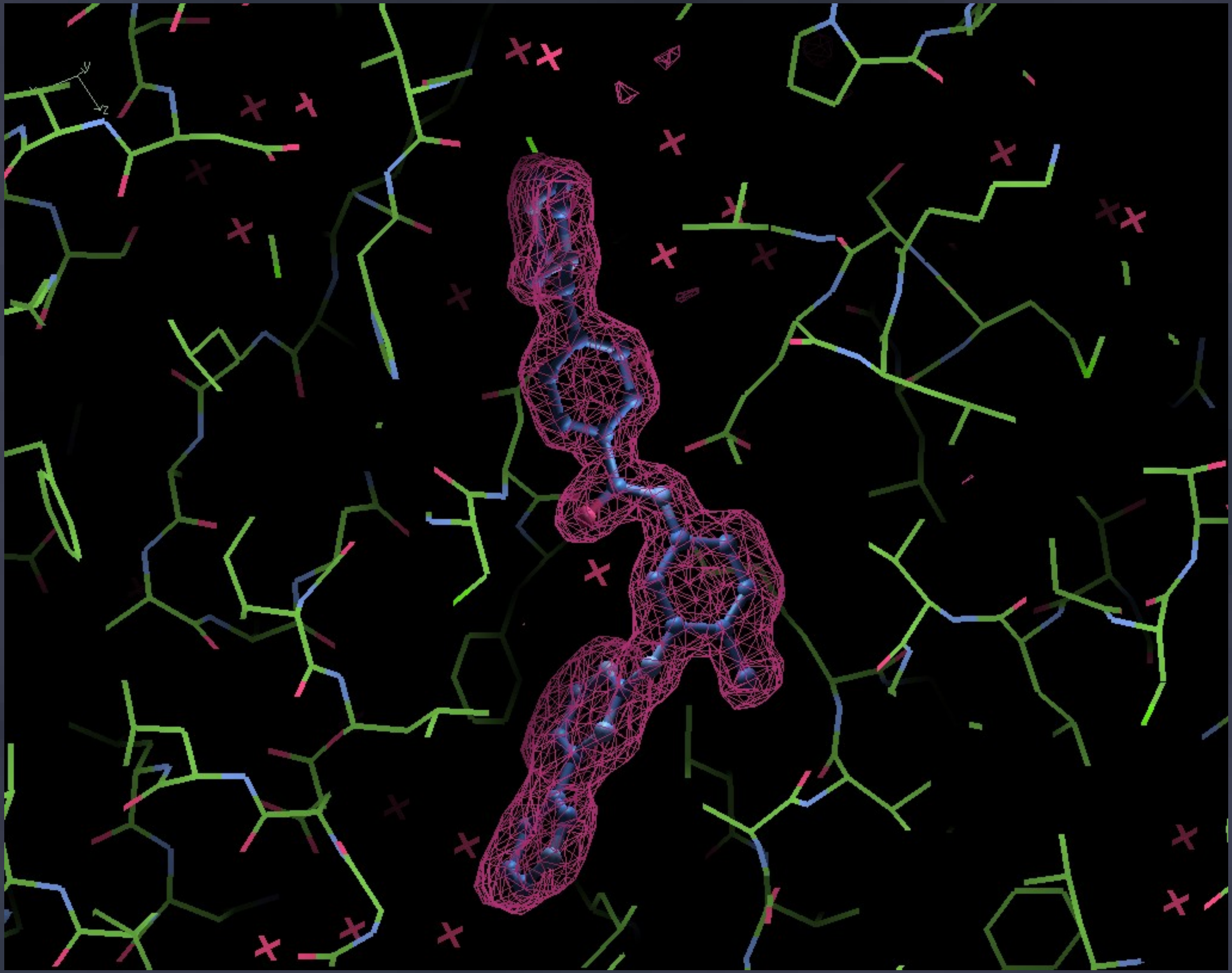
Coot

- Molecular Graphics
    - protein crystallographic model-building
    - Aim at “slick and powerful” interface to CCP4
- Interface to other programs: SHELXL, Refmac, libcheck, Molprobity EBI, EDS, Povray, Raster3d, PHENIX, What_check...

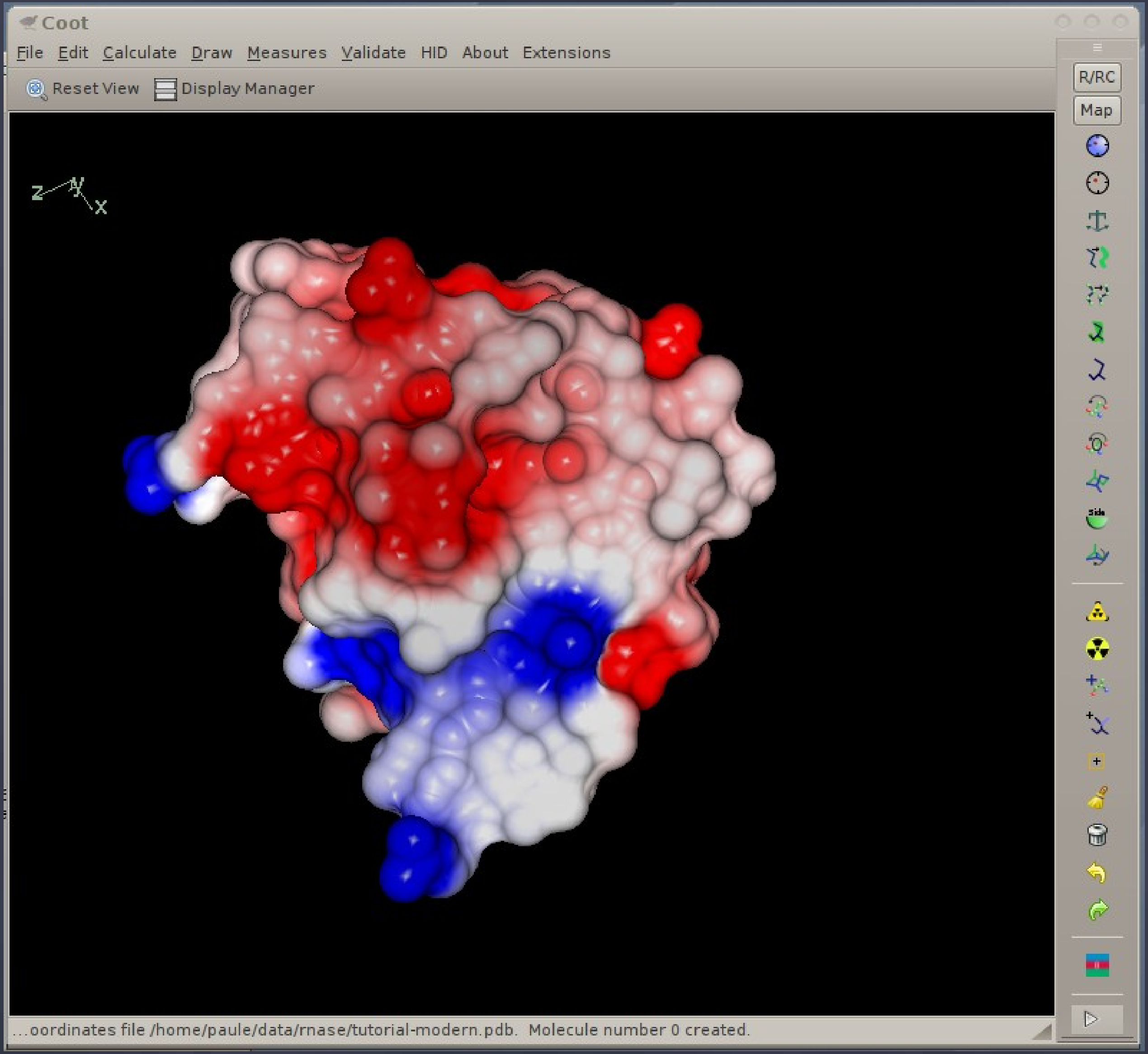
why a graphics program?

- Automated (complete) model-building still impractical
  - Extremely demanding
    - some time before it appears
  - It takes a brain to validate
- Low resolution & RNA structures
  - Still need substantial manual intervention
- Coot is built with Novice users in mind
  - (but not exclusively)

Some Representation Tools

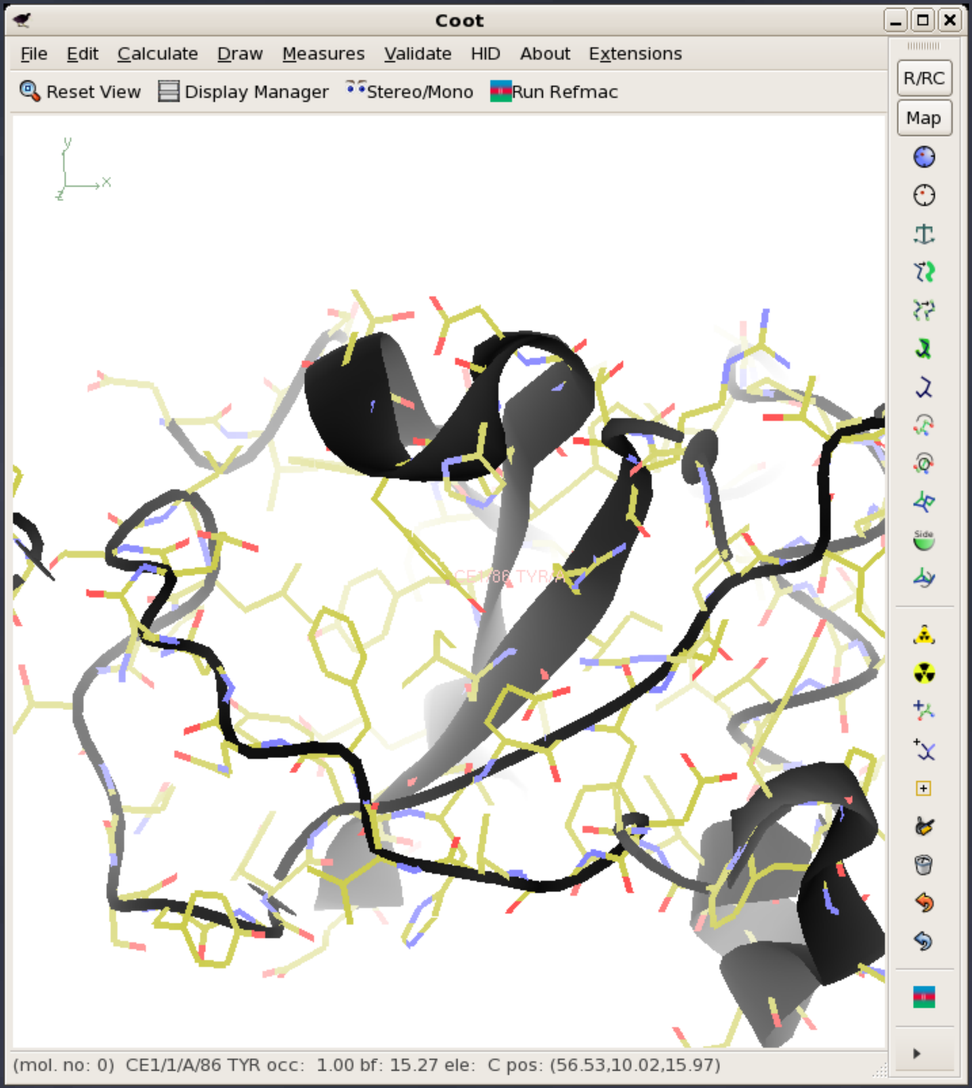


Some Representation Tools



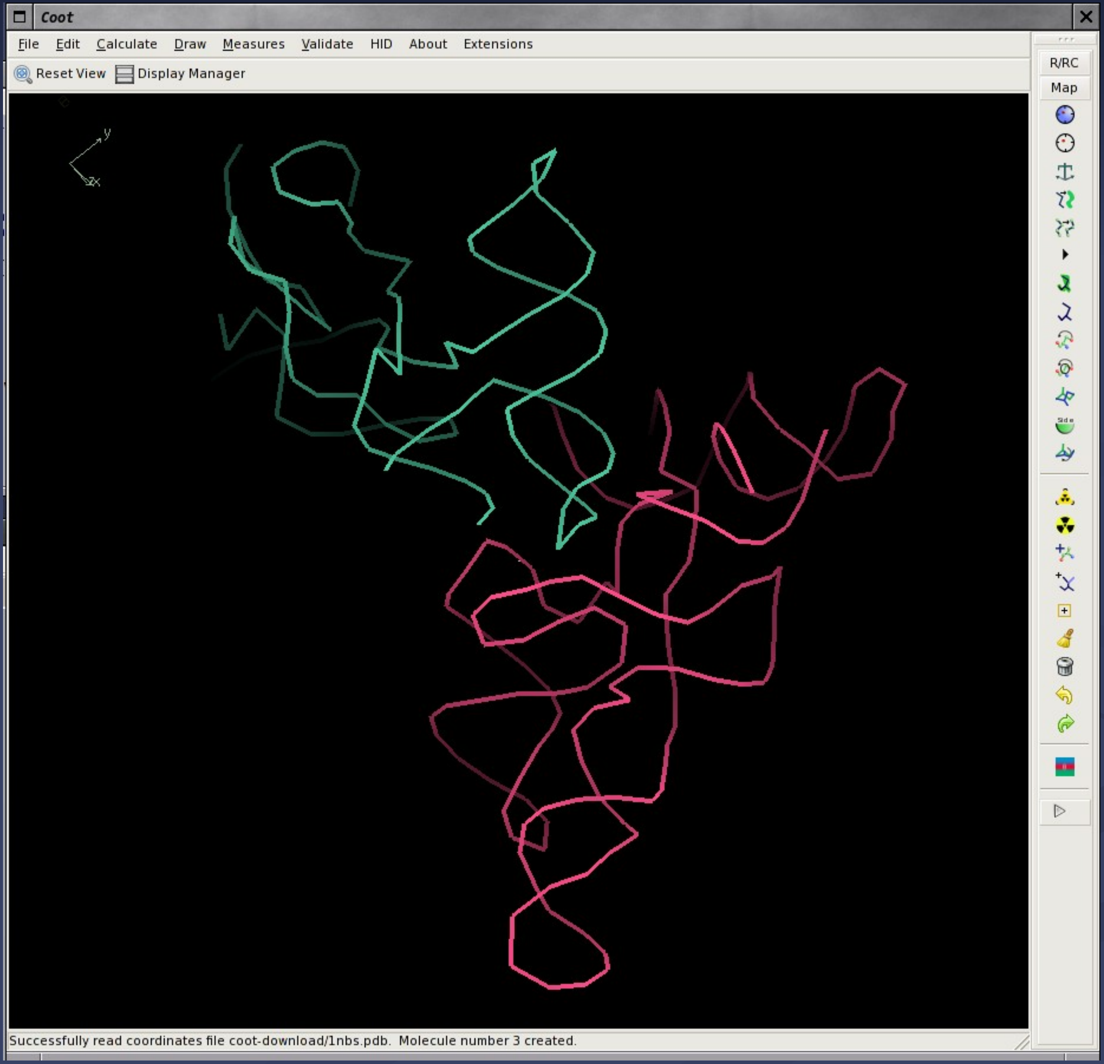
...oordinates file /home/paule/data/rnase/tutorial-modern.pdb. Molecule number 0 created.

Some Representation Tools



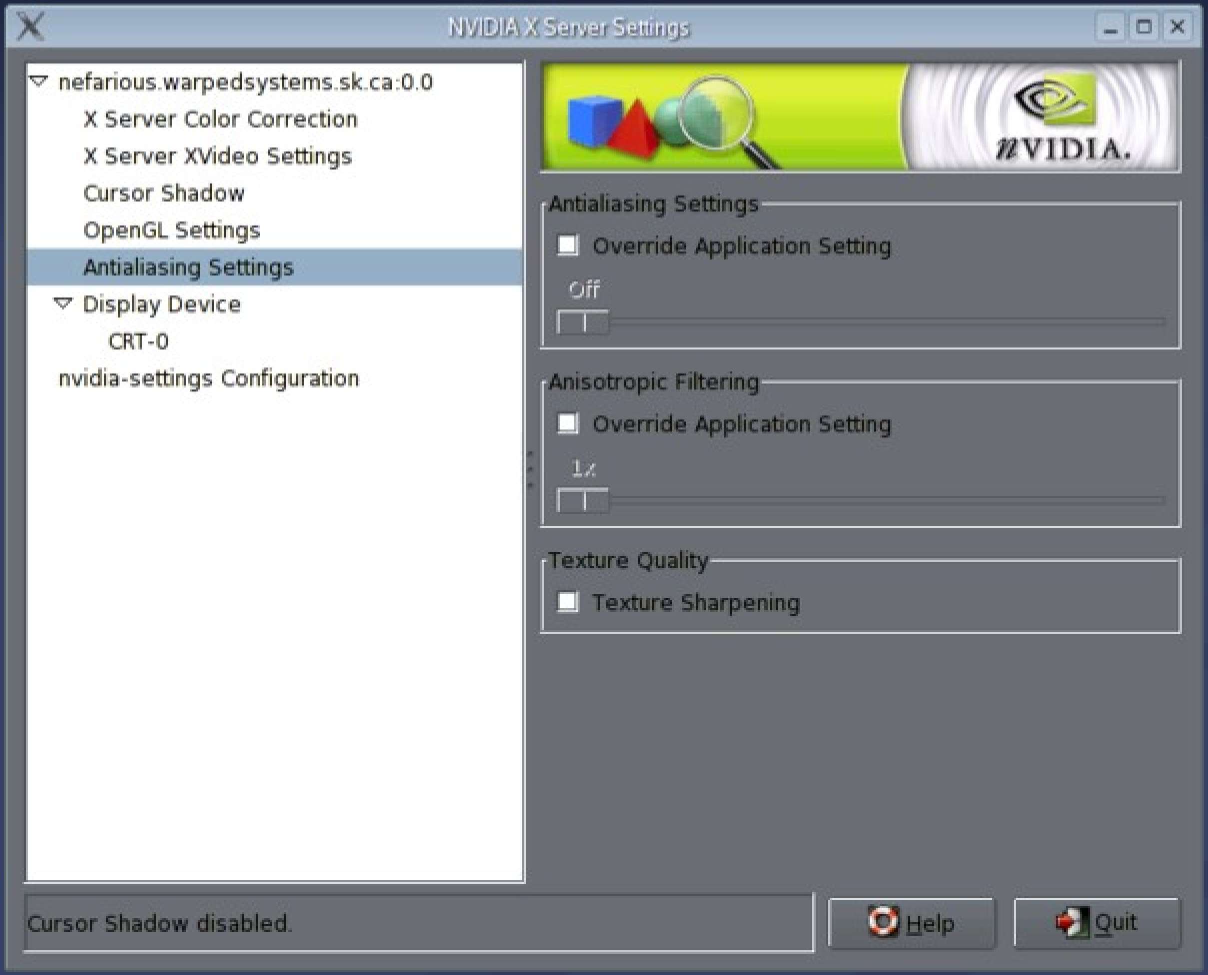
(mol. no: 0) CE1/1/A/86 TYR occ: 1.00 bf: 15.27 ele: C pos: (56.53,10.02,15.97)

Phosphate Backbone mode



Successfully read coordinates file coot-download/1nbs.pdb. Molecule number 3 created.

Antialiasing



Antialiasing Settings

☐ Override Application Setting

Off

Anisotropic Filtering

☐ Override Application Setting

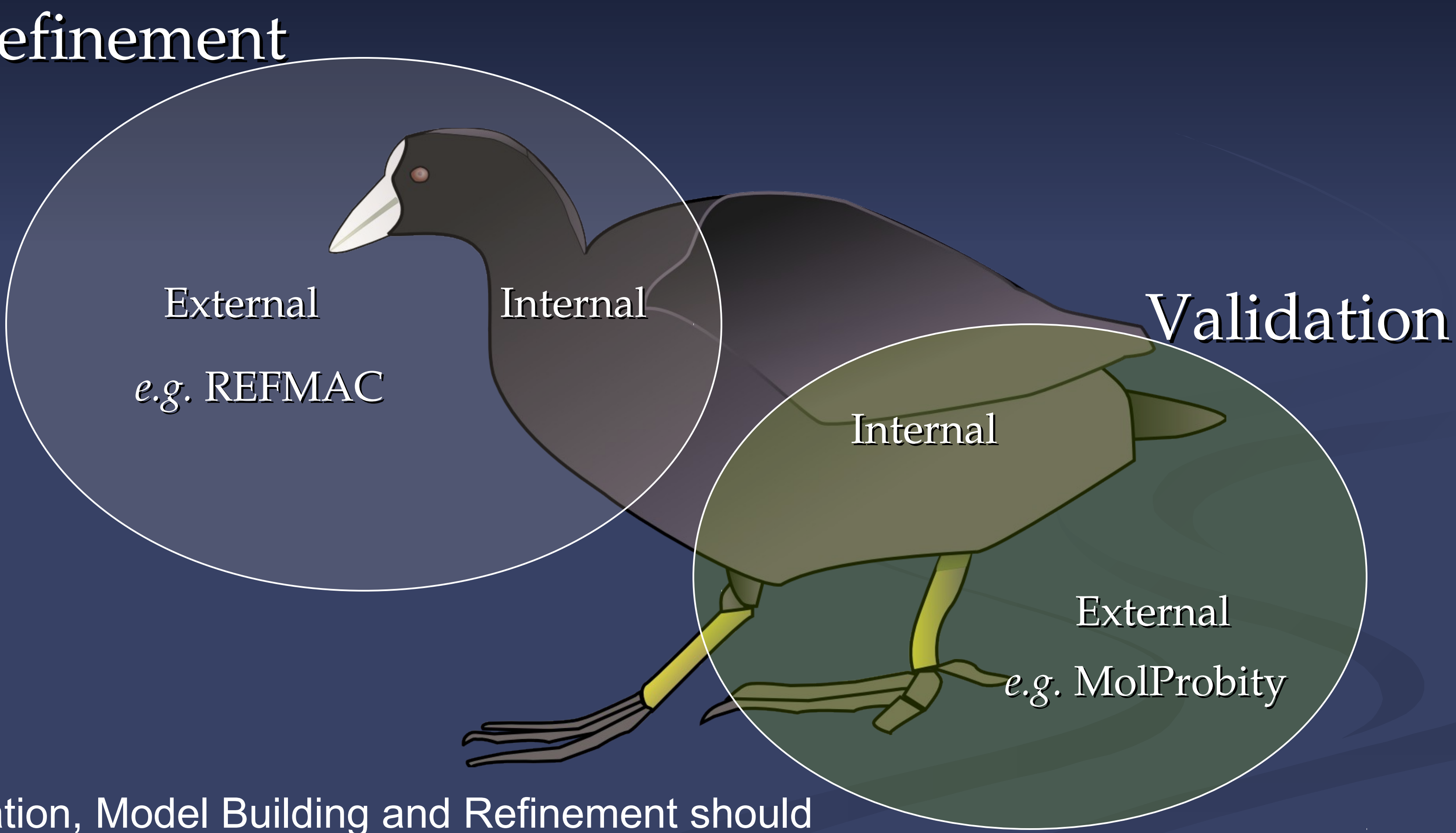
1x

Texture Quality

☐ Texture Sharpening

Cursor Shadow disabled.

Feature Integration



Refinement

External

e.g. REFMAC

Internal

Validation

Internal

External

e.g. MolProbity

Validation, Model Building and Refinement should be used together

Real Space Refinement

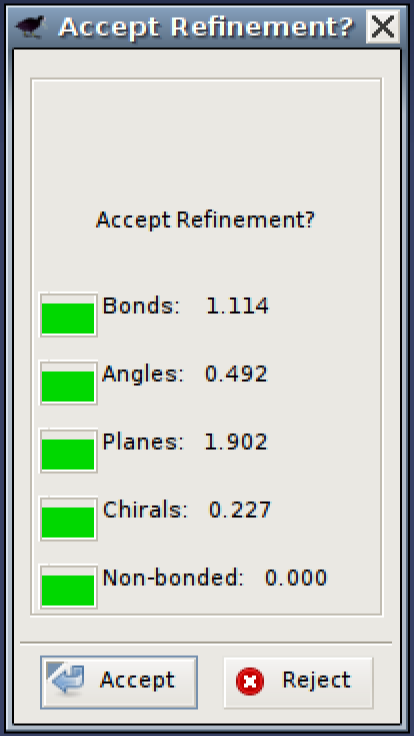
Diamond, R. (1971). *Acta Cryst*. A 27, 436-452.

- Major feature of Coot
  - Gradient minimizer (BFGS derivative)
  - Based on mmCIF standard dictionary
  - Minimizing bonds, angles, planes, non-bonded contacts, torsions, chiral volumes
  - `link_list` and `chem_link` data items
- Provides "interactive refinement"
- Different minimizer to Refmac…
  - …means "nice & tight" geometry
    - Chi squareds

Refinement has been extended in several ways.

Refinement "Traffic Lights"

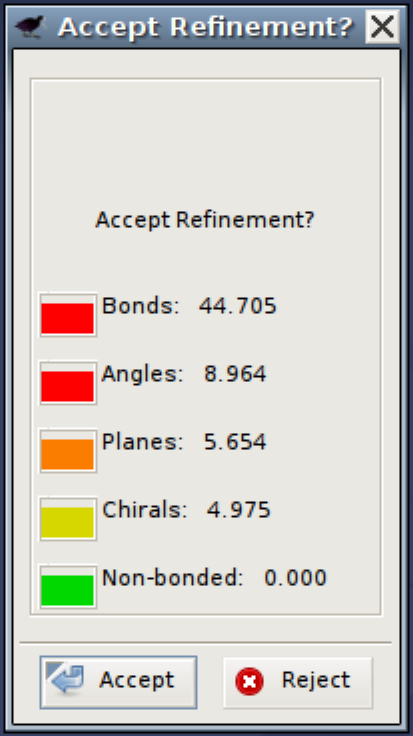
"Traffic Lights" represent the
RMS d values for each of the refined
geometry types



Accept Refinement?

🟩 Bonds: 1.114
🟩 Angles: 0.492
🟩 Planes: 1.902
🟩 Chirals: 0.227
🟩 Non-bonded: 0.000

Good refinement



Accept Refinement?

🟥 Bonds: 44.705
🟥 Angles: 8.964
🟧 Planes: 5.654
🟨 Chirals: 4.975
🟩 Non-bonded: 0.000

Bad refinement

Real Space Refinement (technical note)

- Auto-Zone mechanism
    - The "A" key:
    - Typically have a range of 3 residues to refine, with the middle of the 3 at the centre of the screen
    - Click central atom and "A" key to start the refinement
    - No need to click 2 atoms away from centre

Refinement Techniques

- Auto-zone
- Single-Atom Drag
- Over-dragging
- Sphere Refinement
- Key-bindings:
  - Triple Refine
  - Single Residue Refine with Auto-accept
- Ramachandran Refinement
- Coming Soon..?
  - Wii Refinement

Wii Refinement?

Wii Refinement?

Wii Refinement?

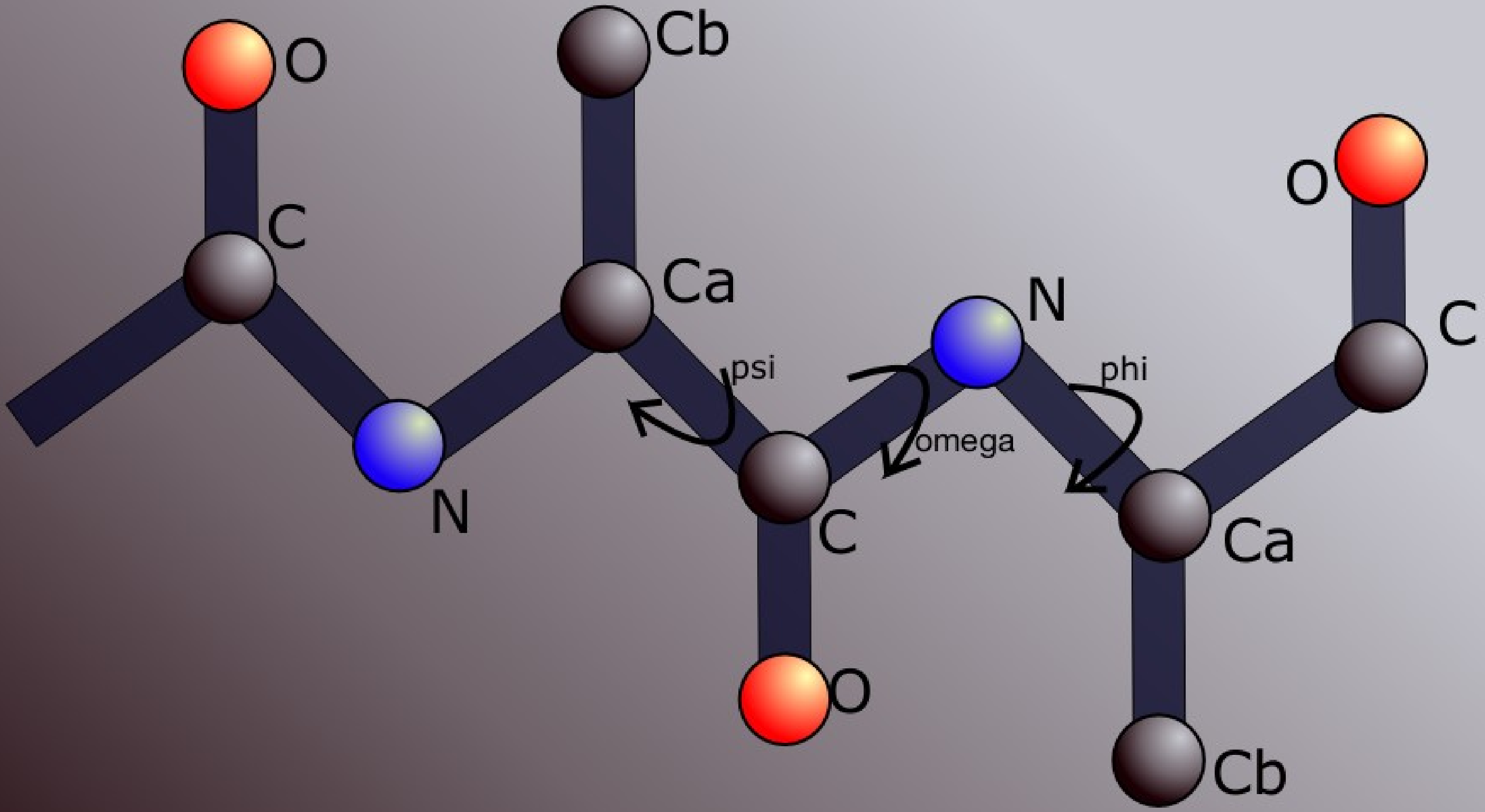
“Sphere” Refinement

- Given an “Active” Residues
  - (The one at the centre of the screen)
  - Fine a sphere of residues around it and use them all for refinement
    - NOT just a linear selection
  - Residues from different chains (or different parts of the same chain) interact
  - Make CYS-CYS or glycosylation links as you find them
    - Use the group and link_list chem_link in the dictionary

Ramachandran Restraints

- Scenario:
  - I have a loop, with poor density, I know the atoms are there somewhere and I want to provide a “reasonable” model
- Controversial Feature?
  - Ramachandran Plots have been used for “validation” - but here we are deliberately optimizing them
- Ramachandran Plots can be added to the geometry target function

Peptide Torsion Angles



Ramachandran Distributions

- Distributions from:
    - Cowtan
    - Otwinowski
    - Richardsons
- Cowtan distribution only included currently
    - Log-likelihood, “negatively enhance disallowed”
    - Secondary-structure-type independent

1 Torsion Angle Derivatives

If we have a torsion angle, θ, say, then the derivates needed are

$$\frac{\partial\theta}{\partial x_1} \tag{1}$$

for the atom positional parameters y_1, z_1, x_2 and so on. These Coot already has in analytical form.

2 Ramachandran Derivatives

Kevin Cowtan provides a "Log Ramachandran" plot, $R(\phi,\psi)$, where the value of the plot should be added to the target function and minimized.

$$R(\phi,\psi), \quad \frac{\partial R(\phi,\psi)}{\partial\phi} \quad \text{and} \quad \frac{\partial R(\phi,\psi)}{\partial\psi} \tag{2}$$

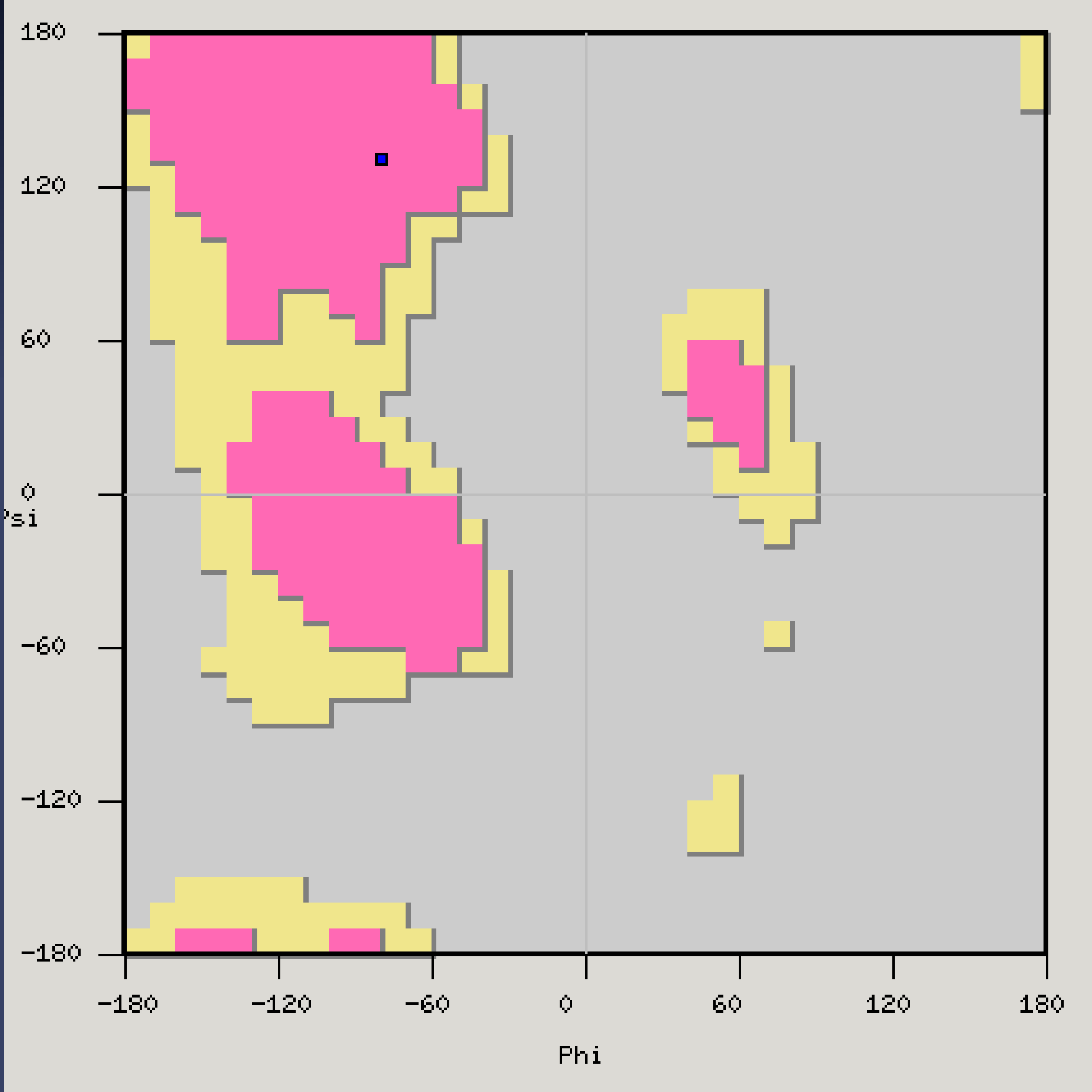
To use this in refinement, I need:

$$\frac{\partial R(\phi,\psi)}{\partial x_1} \quad (\text{and so on}) \tag{3}$$

And this can be calculated from:

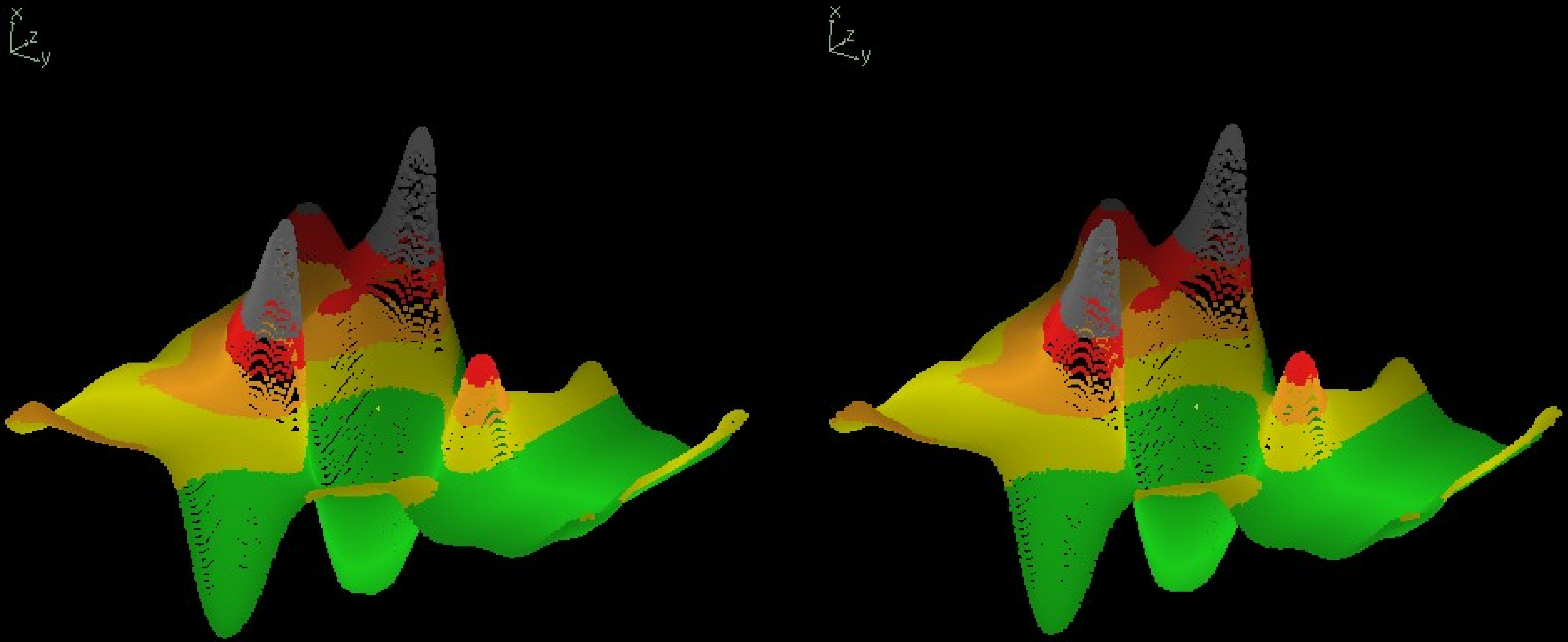
$$\frac{\partial R(\phi,\psi)}{\partial x_1} = \frac{\partial R(\phi,\psi)}{\partial\phi}\frac{\partial\phi}{\partial x_1} + \frac{\partial R(\phi,\psi)}{\partial\psi}\frac{\partial\psi}{\partial x_1} \tag{4}$$

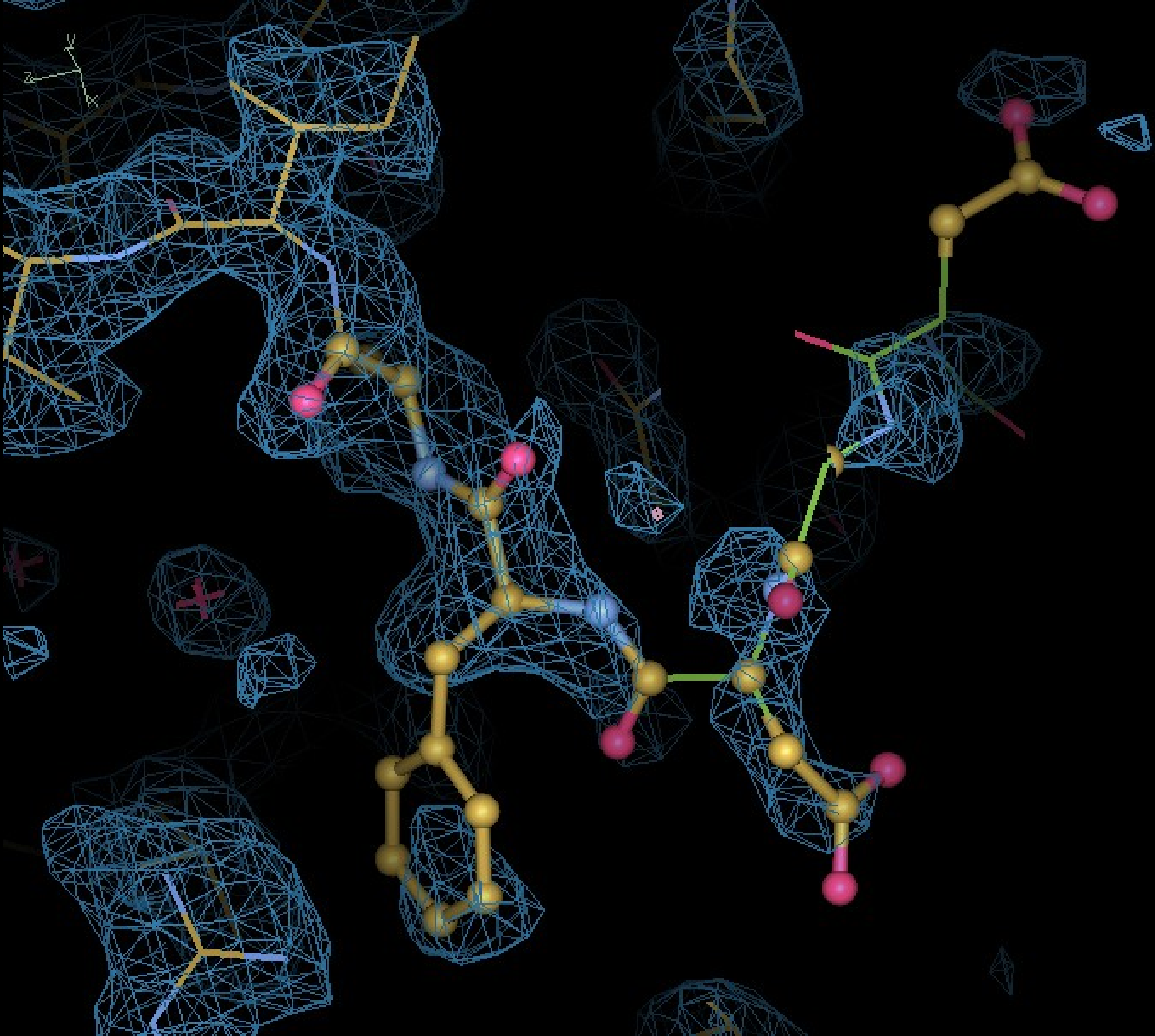
Conventional Ramachandran Plot As Rendered by Coot



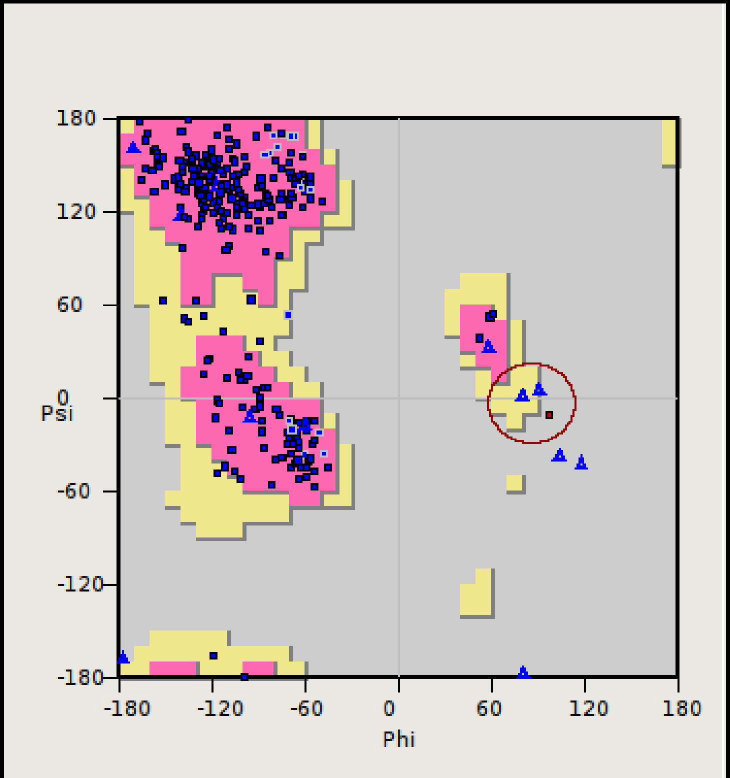
Based on Richardsons “Top500”

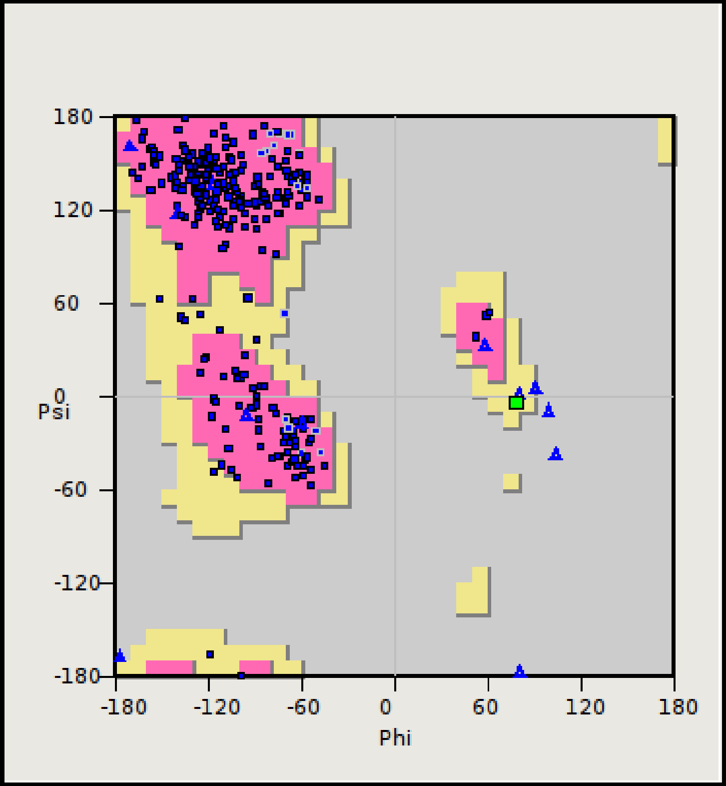
Negatively enhanced disallowed regions



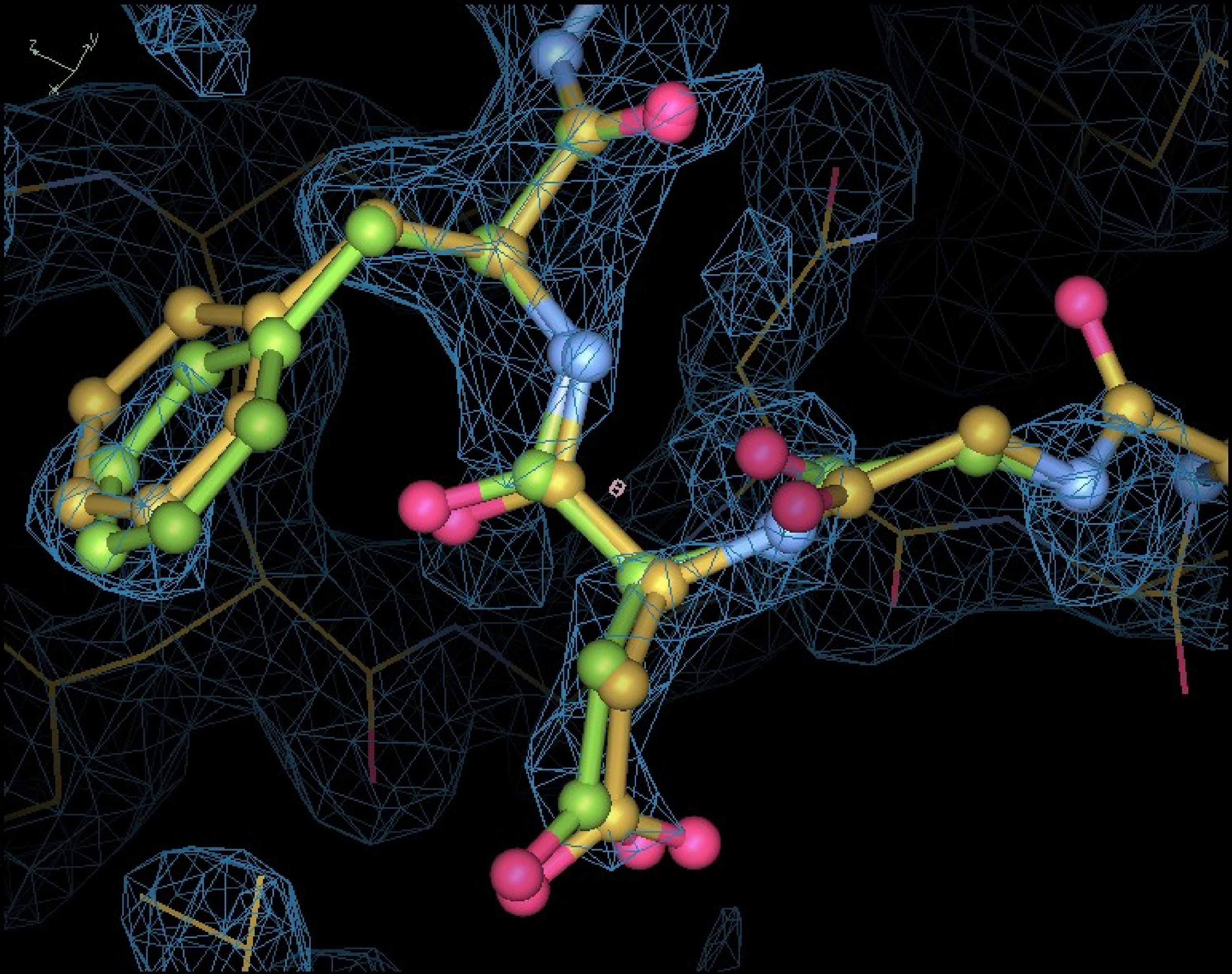

y
z
x

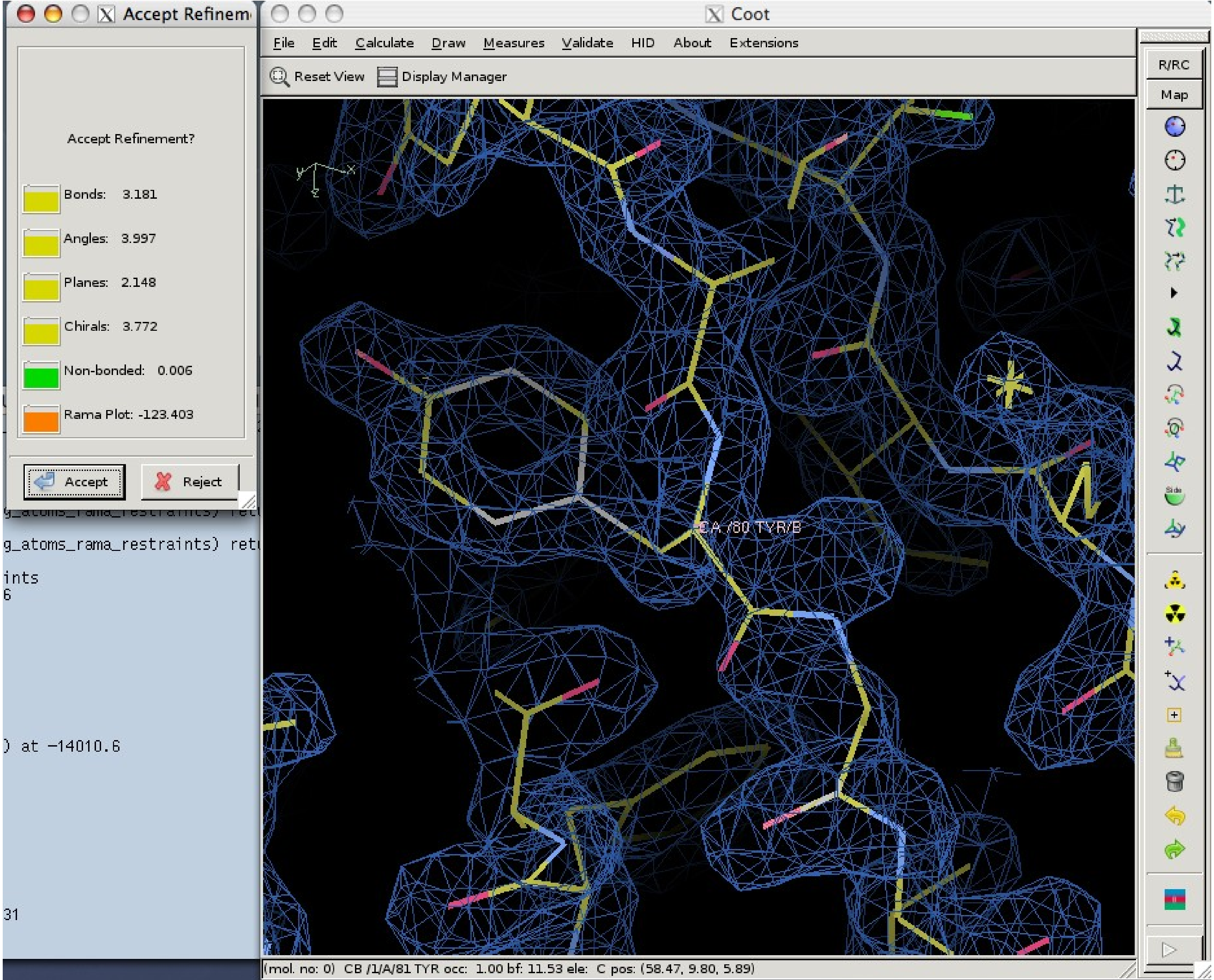
Tweaking a Ramachandran Outlier





Tweaking Phi and Psi





Accept Refinement?

🟨 Bonds: 3.181

🟨 Angles: 3.997

🟨 Planes: 2.148

🟨 Chirals: 3.772

🟩 Non-bonded: 0.006

🟧 Rama Plot: -123.403

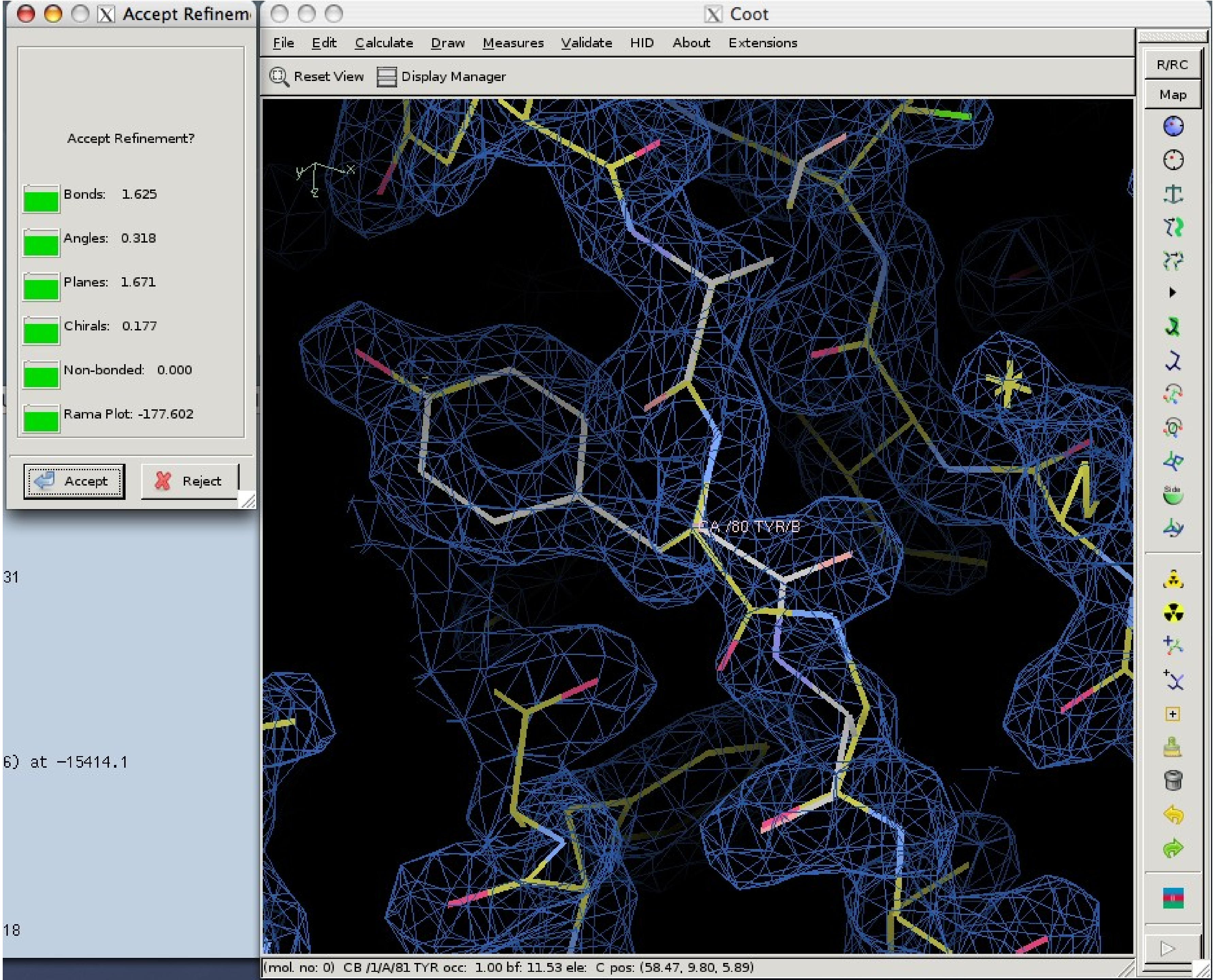
```
g_atoms_rama_restraints) ret

ints
6

) at -14010.6

31
```

```
(mol. no: 0)  CB /1/A/81 TYR occ:  1.00 bf: 11.53 ele:  C pos: (58.47, 9.80, 5.89)
```



Accept Refinement?

🟩 Bonds: 1.625

🟩 Angles: 0.318

🟩 Planes: 1.671

🟩 Chirals: 0.177

🟩 Non-bonded: 0.000

🟩 Rama Plot: -177.602

```
31

6) at -15414.1

18
```

```
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```
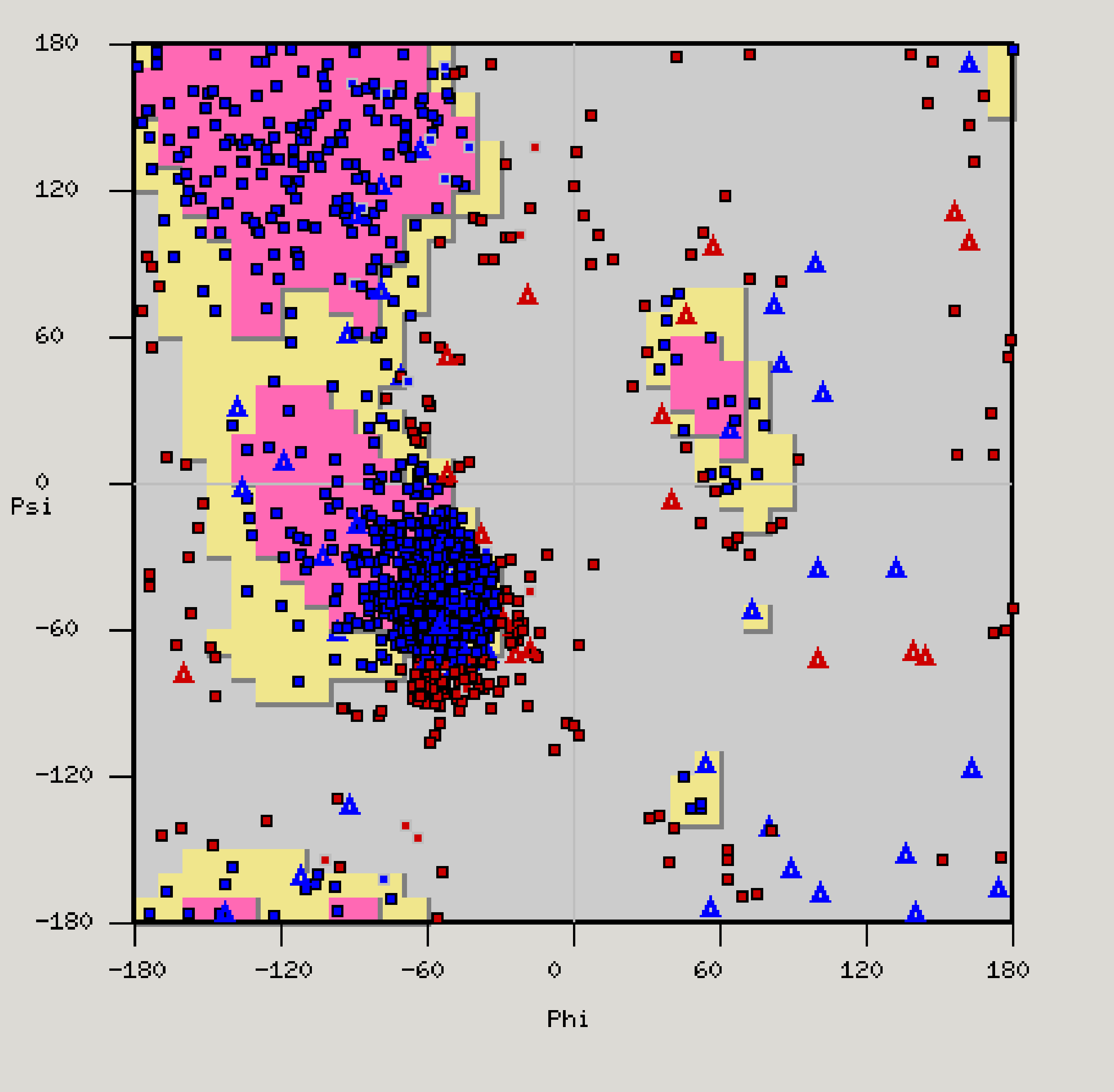
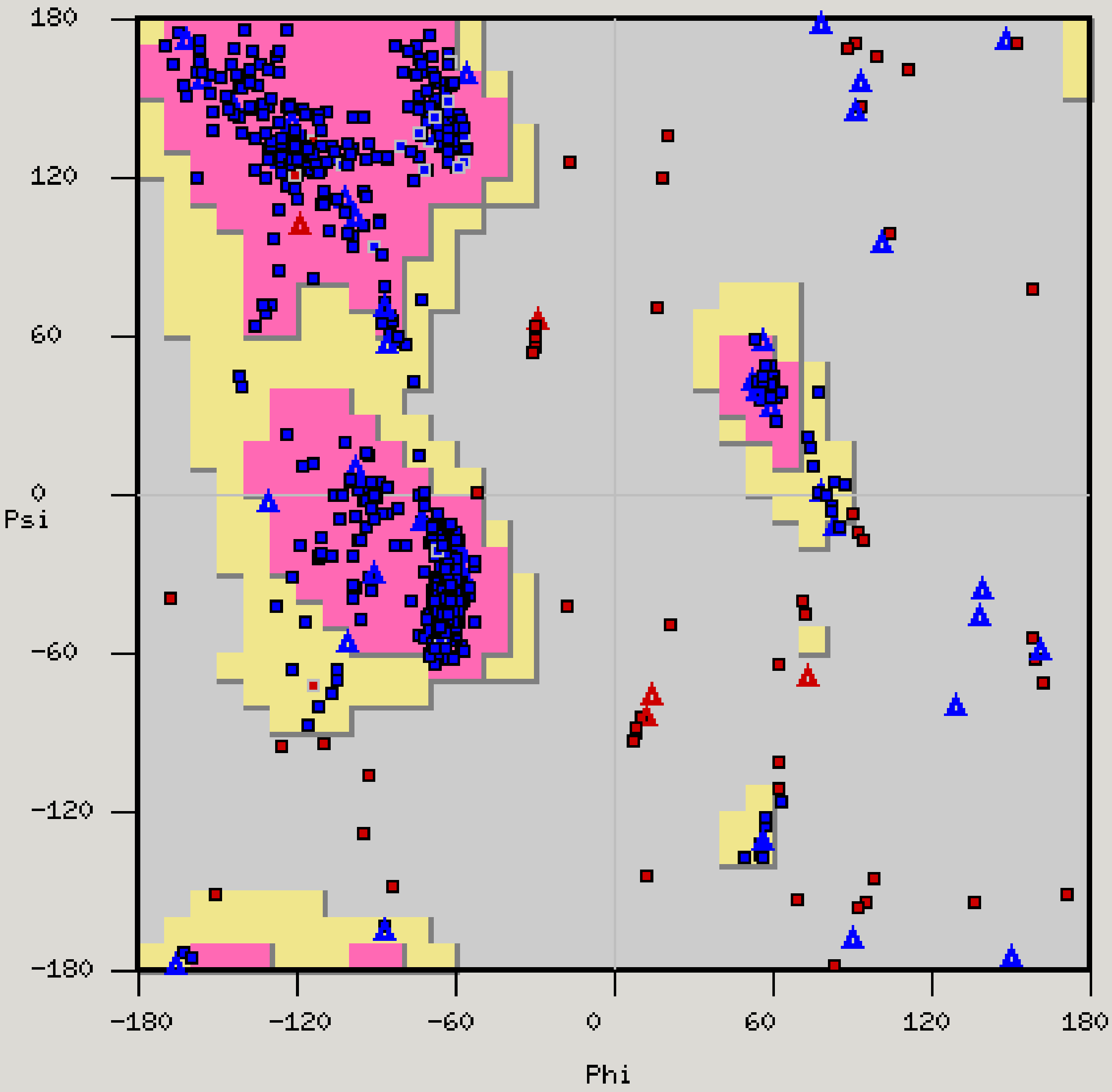
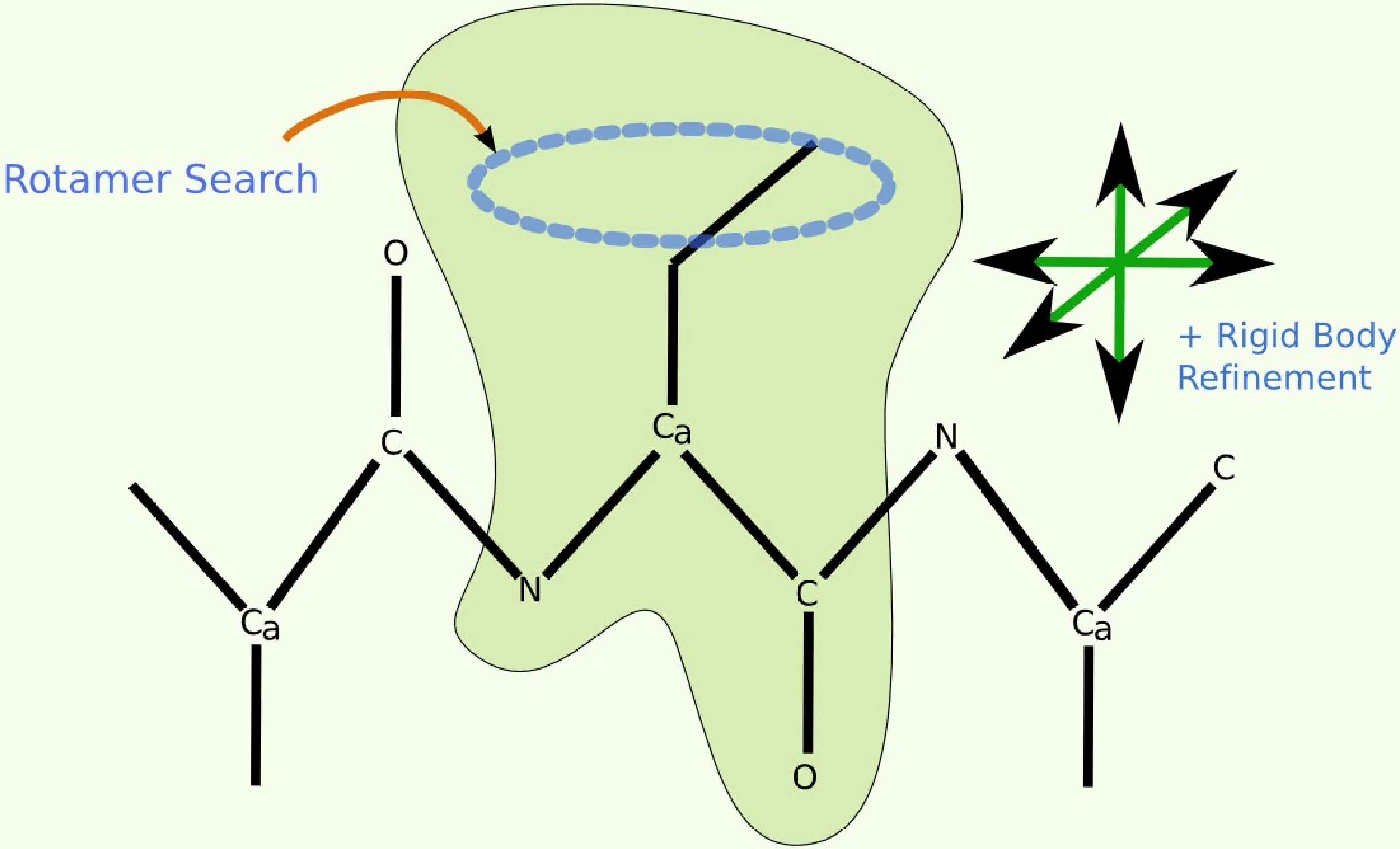


180
120
60
0
Psi
-60
-120
-180
-180
-120
-60
0
60
120
180
Phi


180
120
60
0
Psi
-60
-120
-180
-180
-120
-60
0
60
120
180
Phi

Low Resolution Tools

- Backrub rotamers

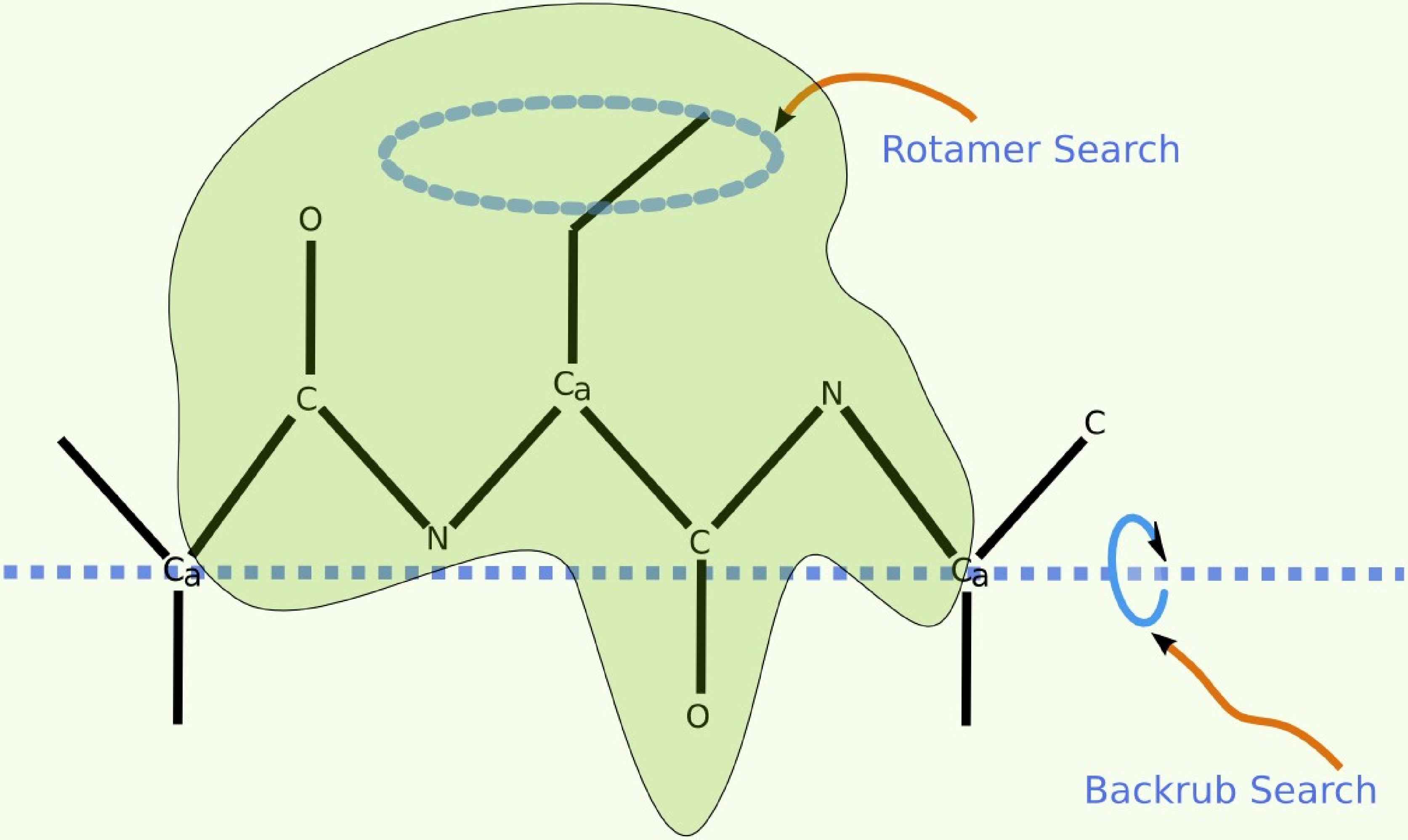
Current Low Resolution Rotamer Search



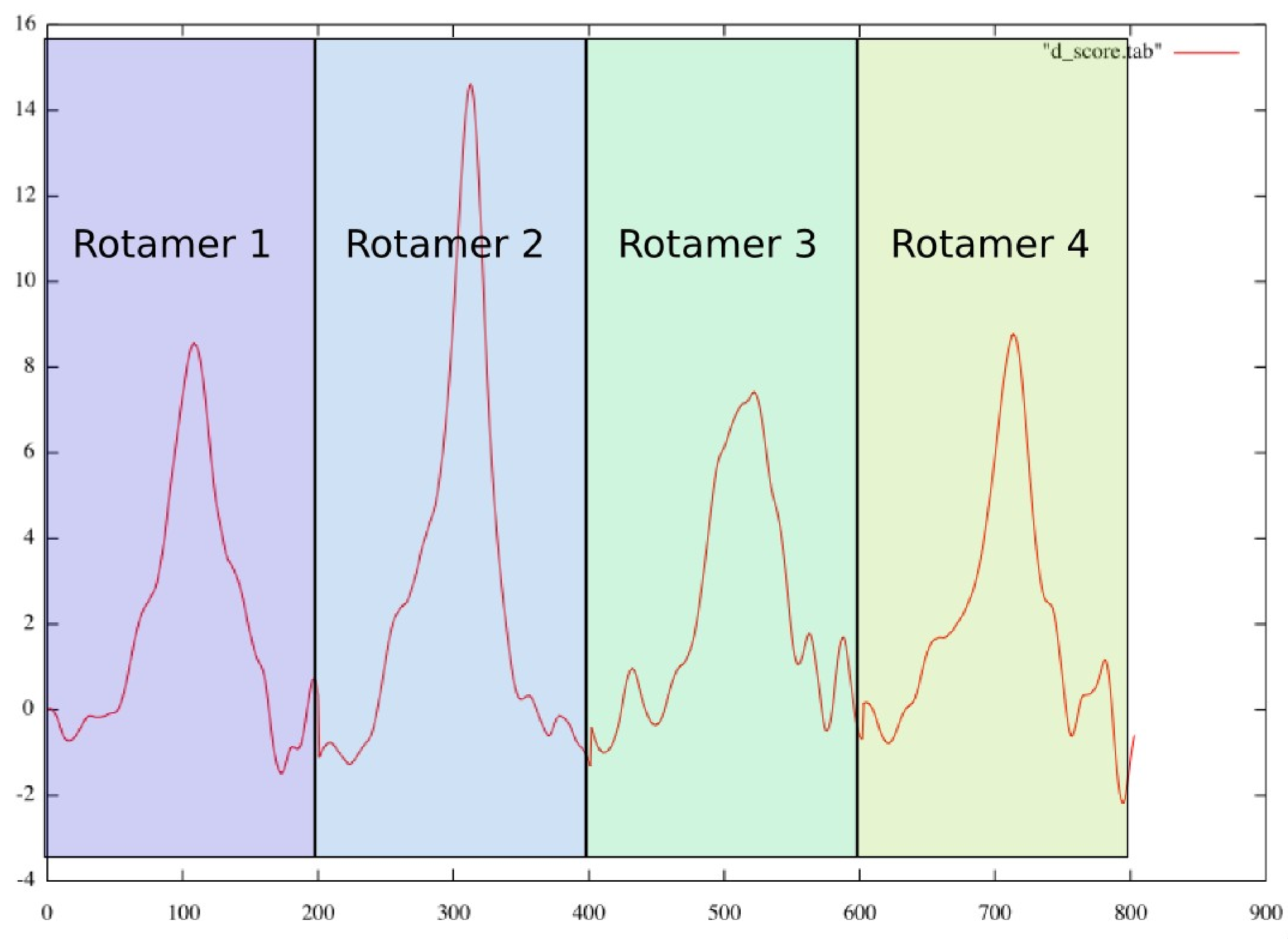
Rotamer Search

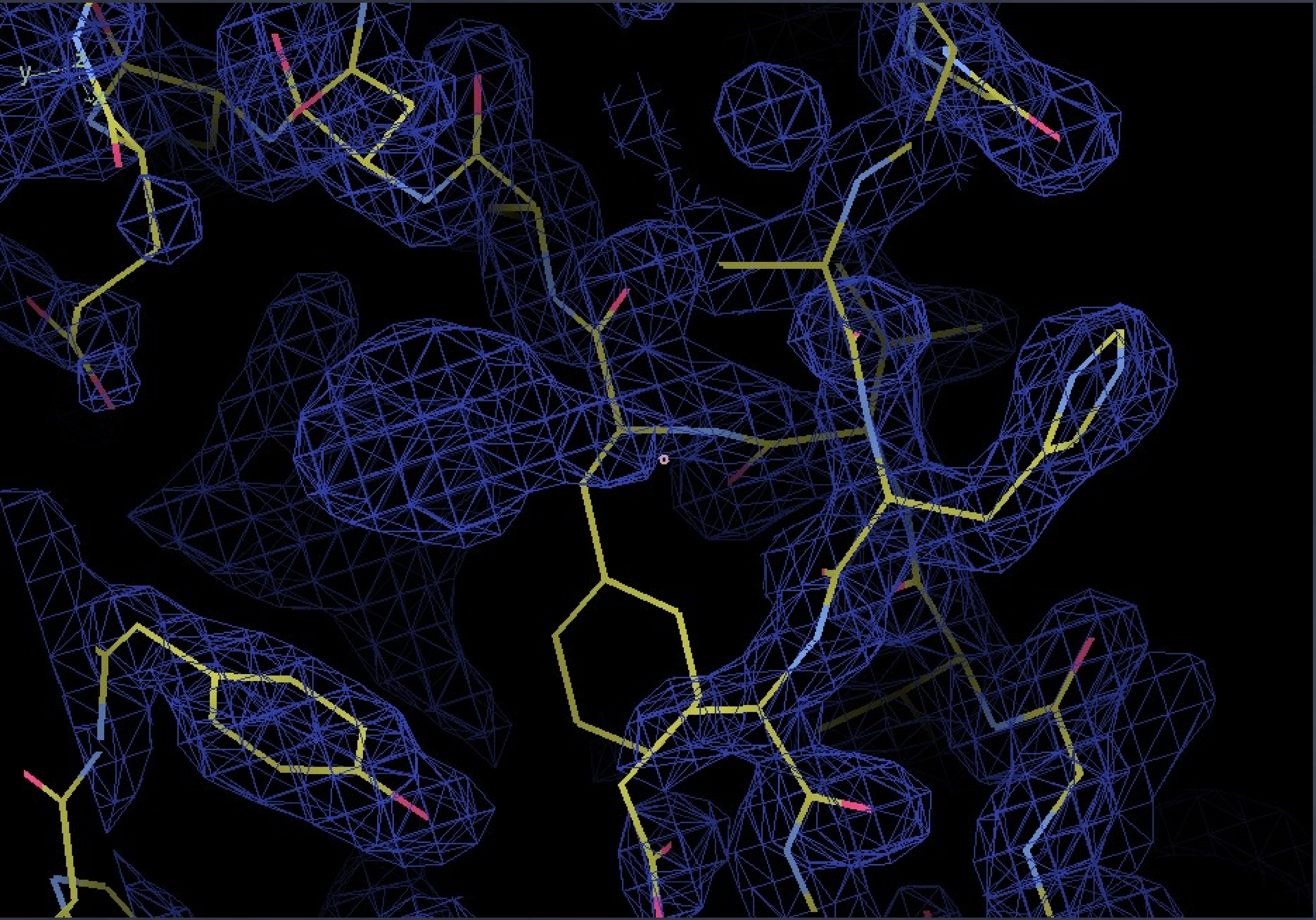
+ Rigid Body Refinement

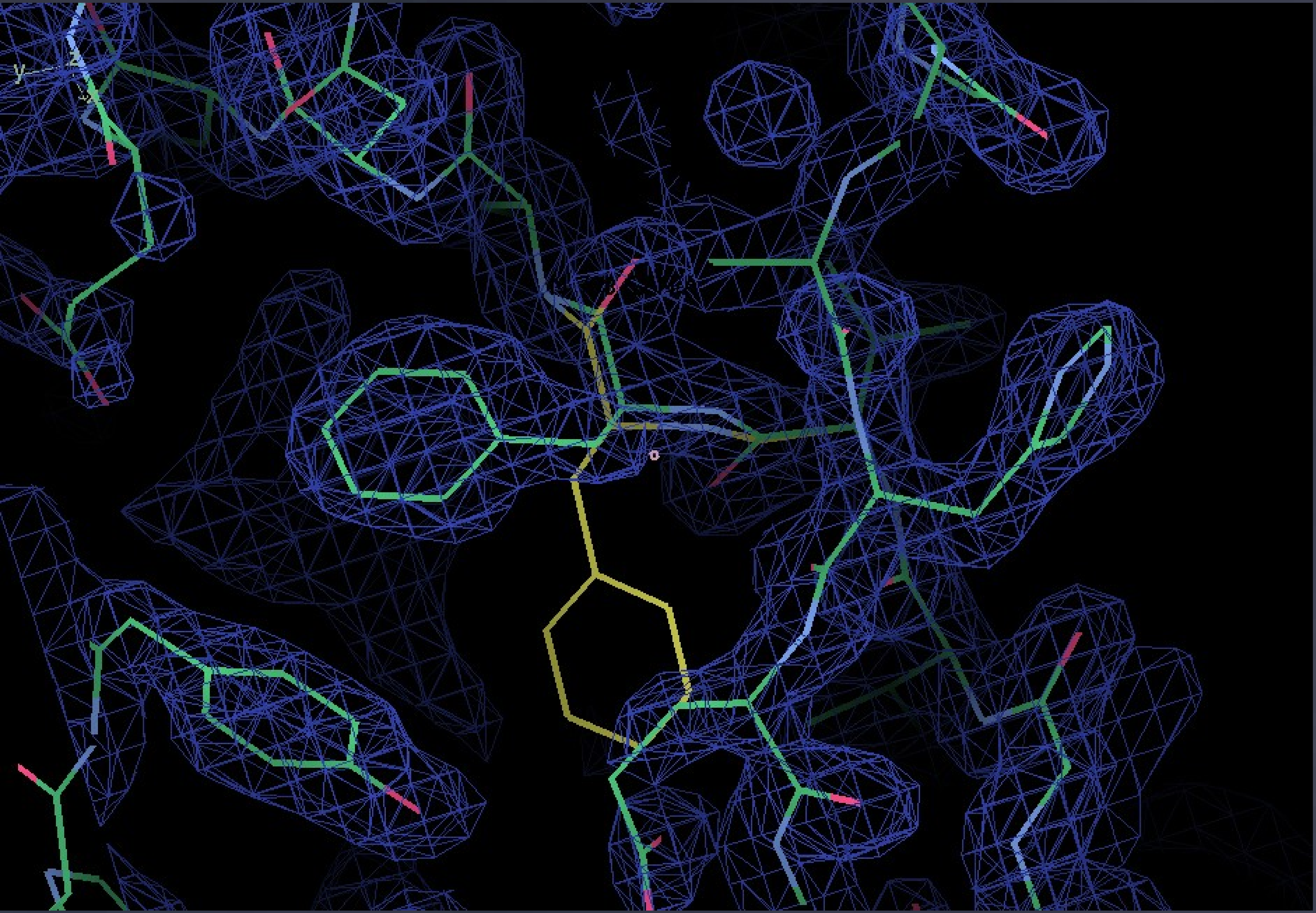
New Low Resolution Rotamer Search

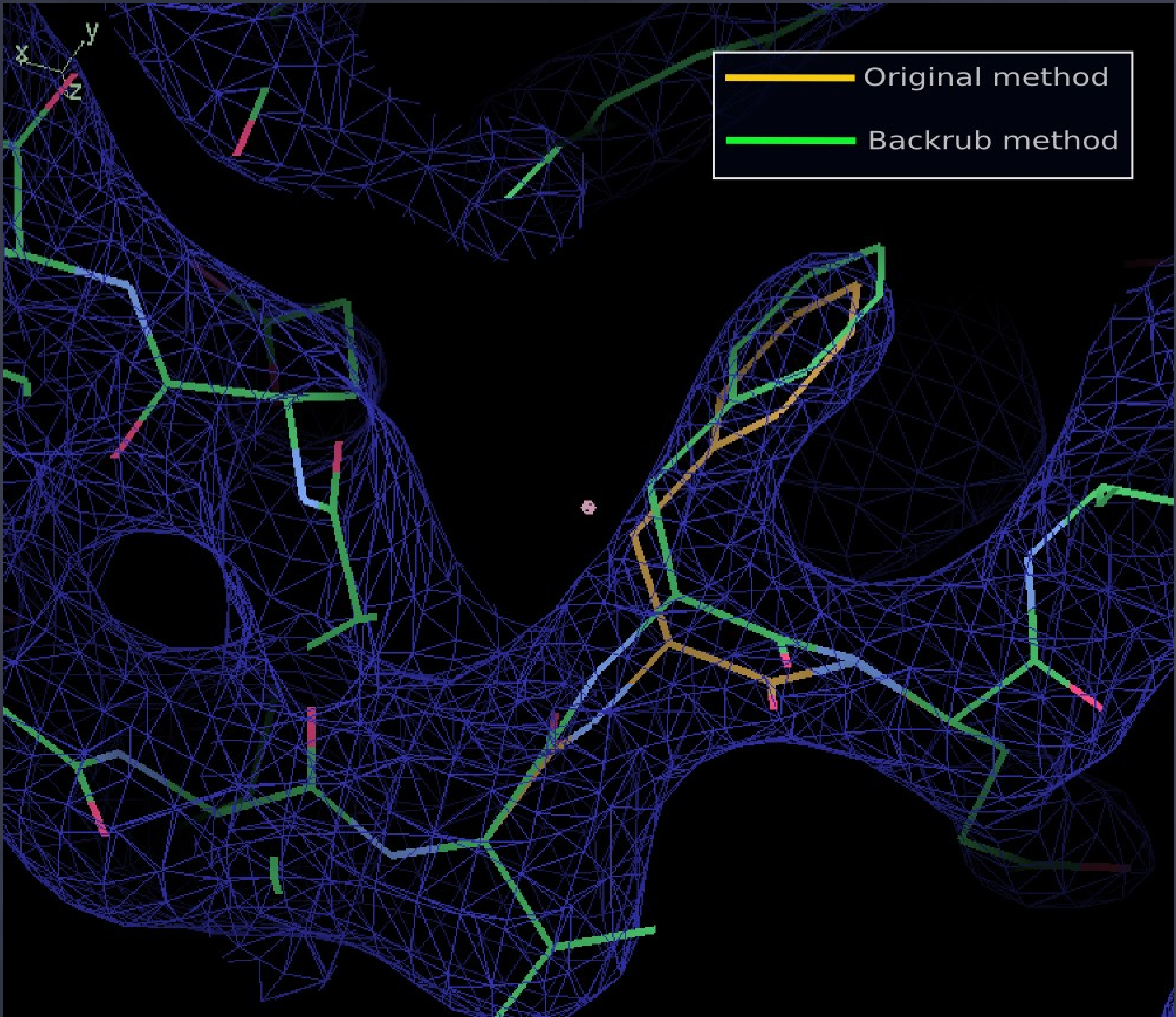


After Fitting Tools in KING/Molprobity


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






x
y
z
Original method
Backrub method

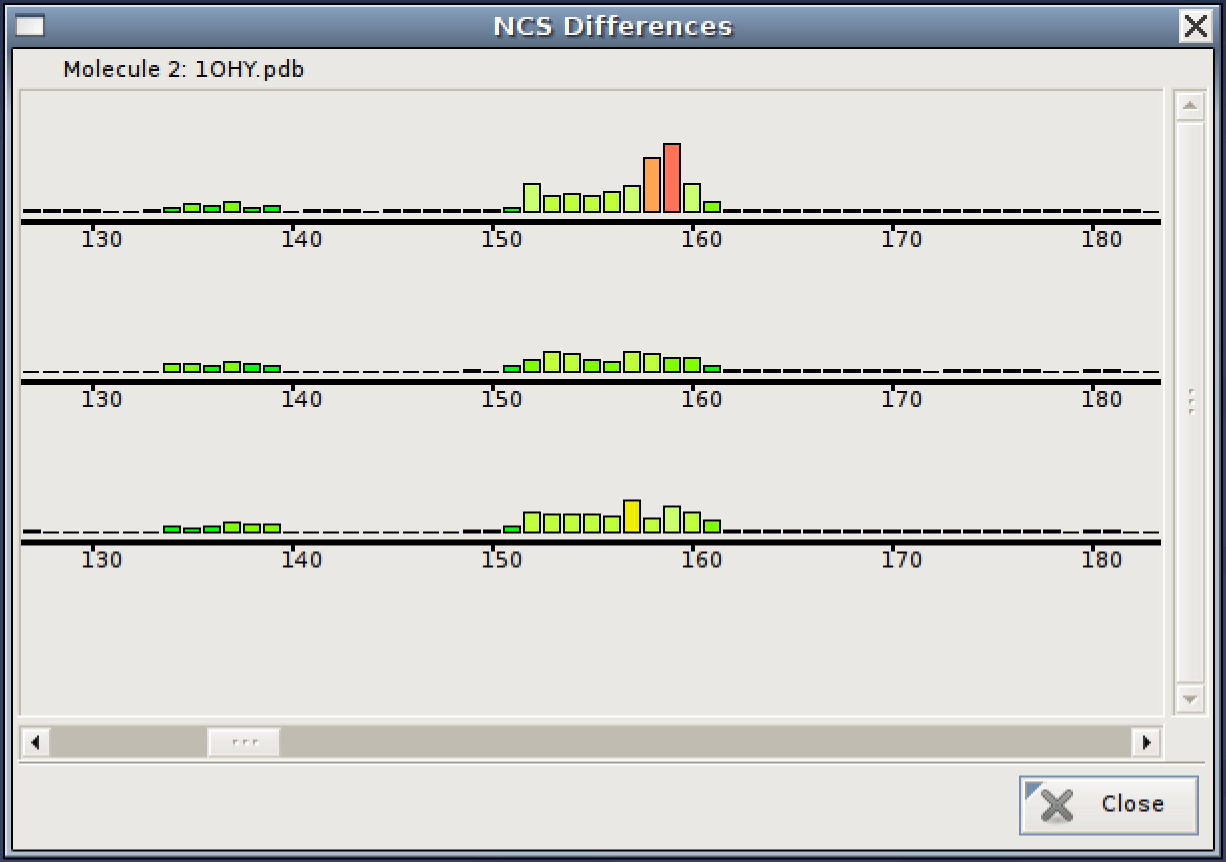
Handling NCS...

Handling NCS

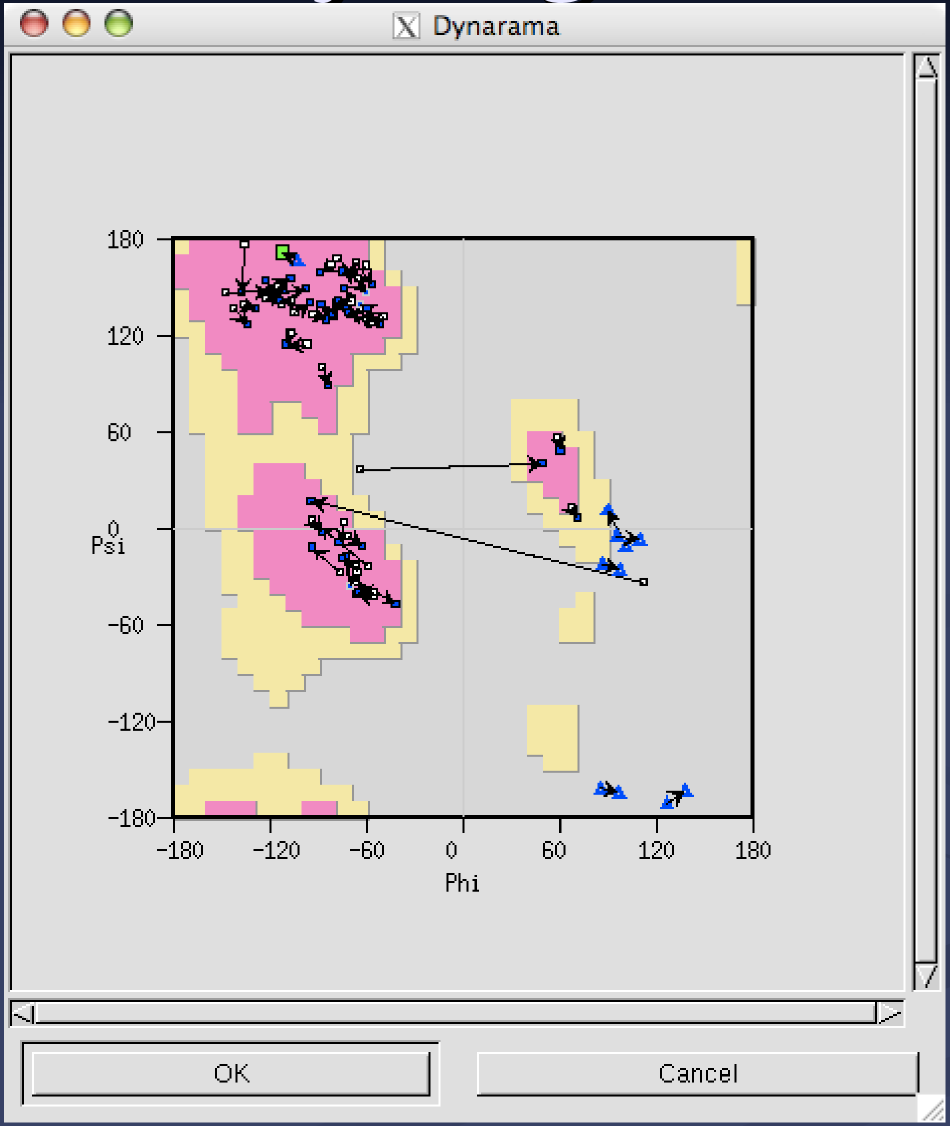
Typical Scenario:

- 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?

… NCS Differences graph

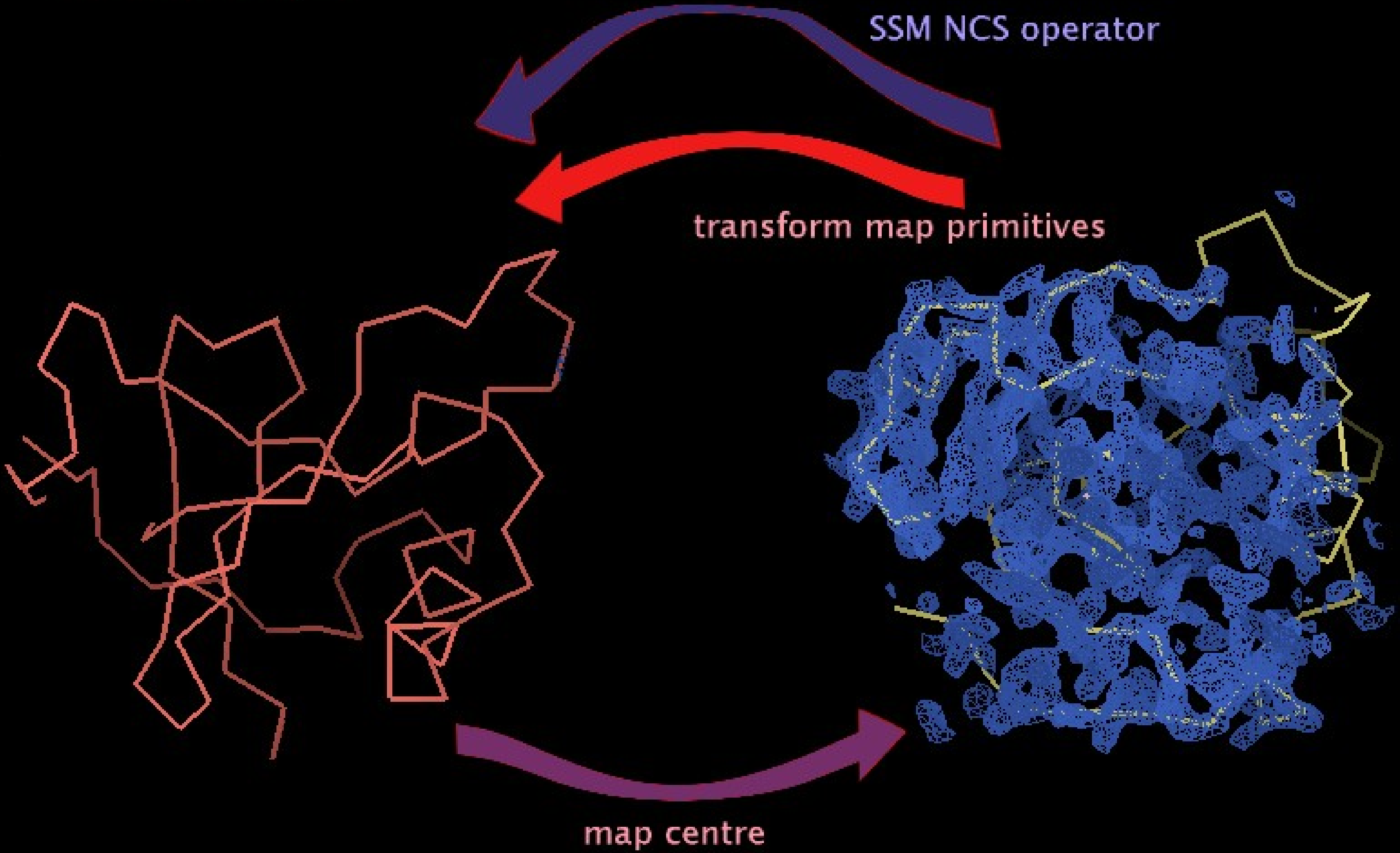


…or Kleywegt Plots[*]



[*] Named by George Sheldrick

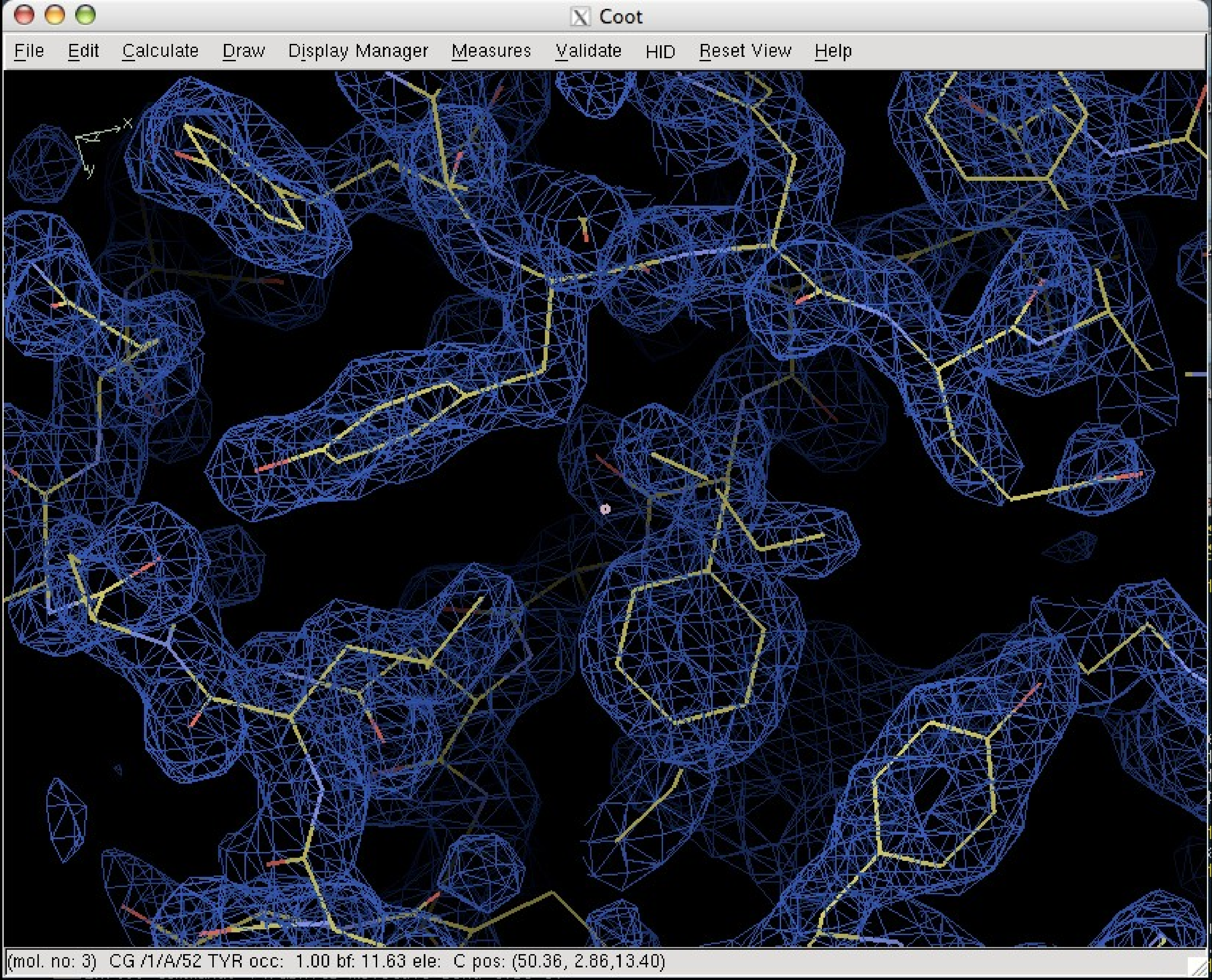
NCS Overlays



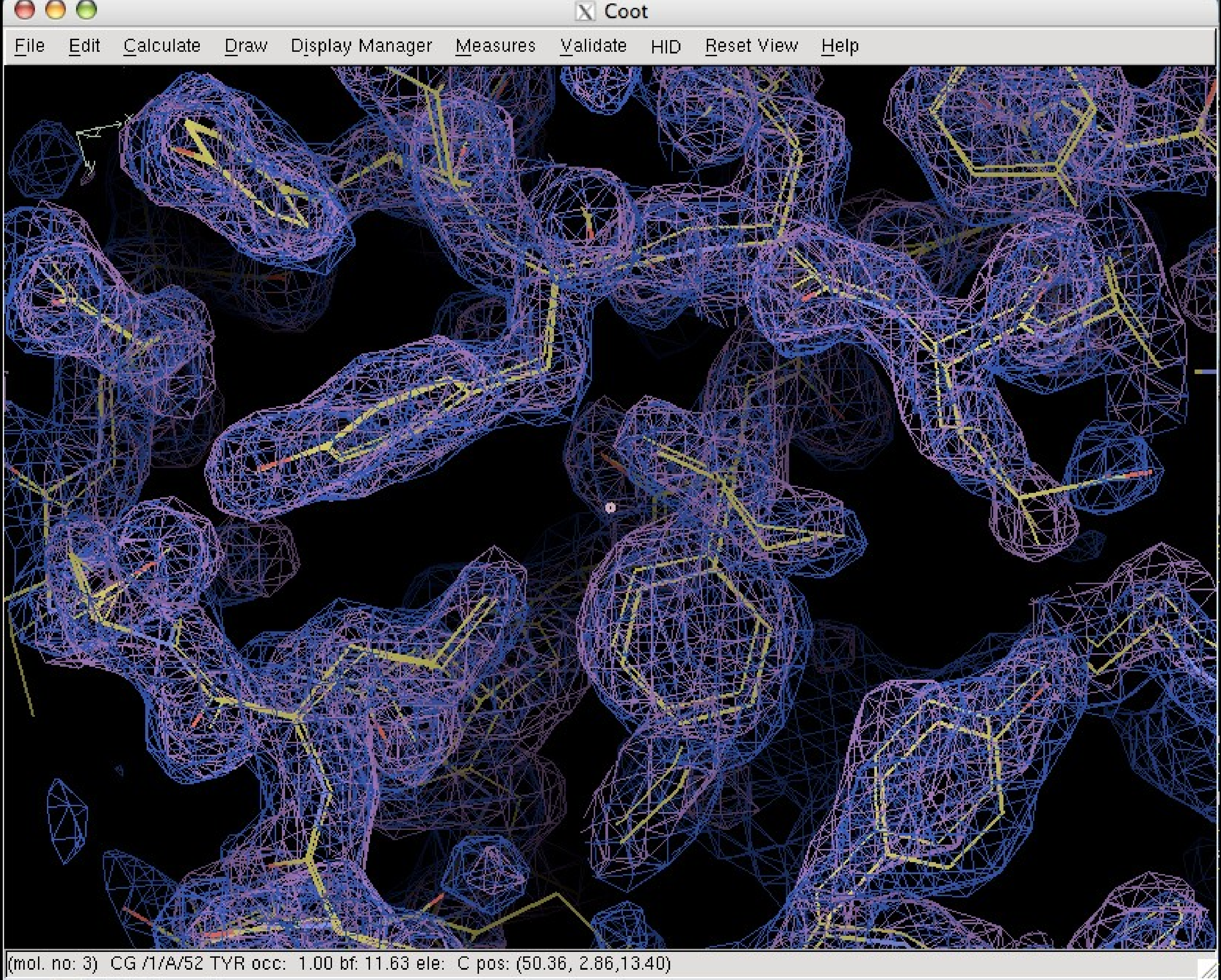
SSM NCS operator

transform map primitives

map centre



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



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

NCS Model-modification Tools

- Automatic detection of NCS
    - And their operators
- Copy Master NCS molecule to others
    - Applies NCS transformation
- Copy NCS Master residue-range
- Change NCS Master chain
- NCS Skipping
- NCS Ligands

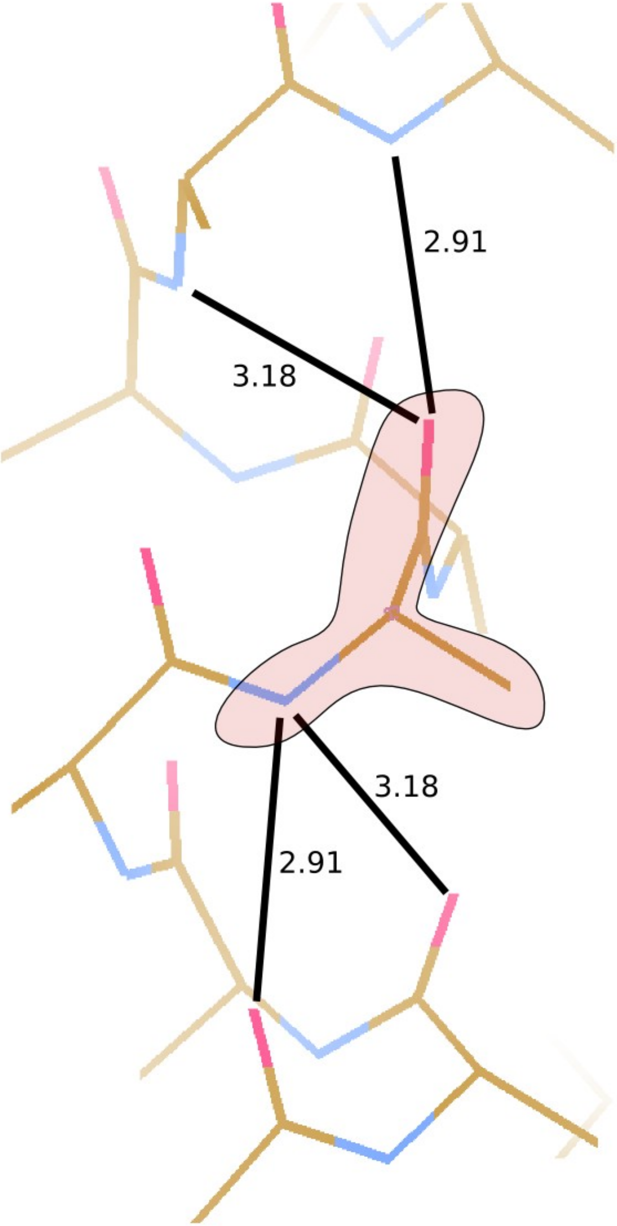
Other Low Resolution Tools

Adding Torsion Angle Restraints

- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”
    - i.e. trapped in Local Minimum

- Add Pseudo-bonds (c.f. Brigcogne & Buster)

Alpha Helix pseudo-bond restraints



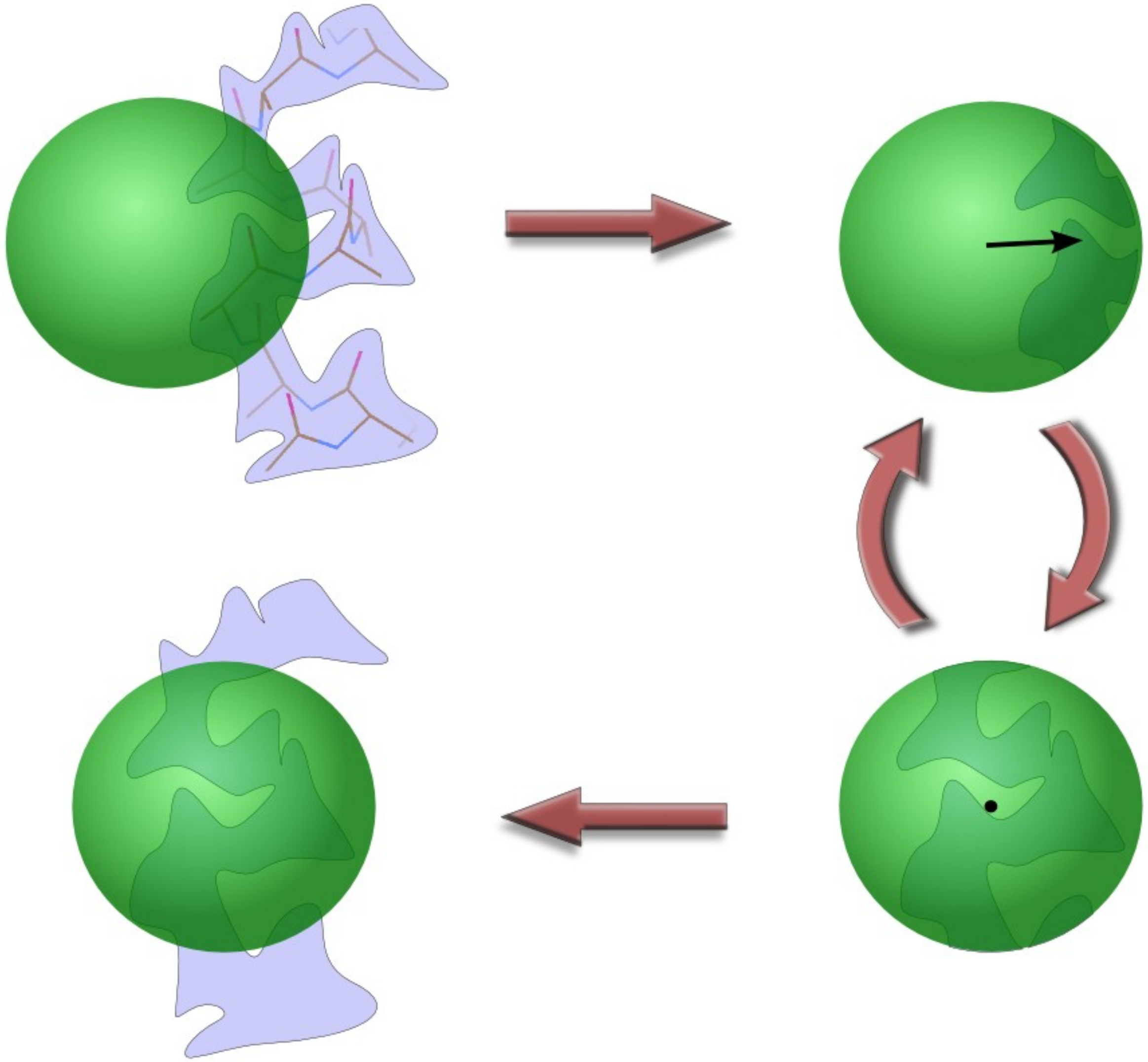
Restrain the Hydrogen-bonding atom distances

Helix-Building

Alpha Helix Placement

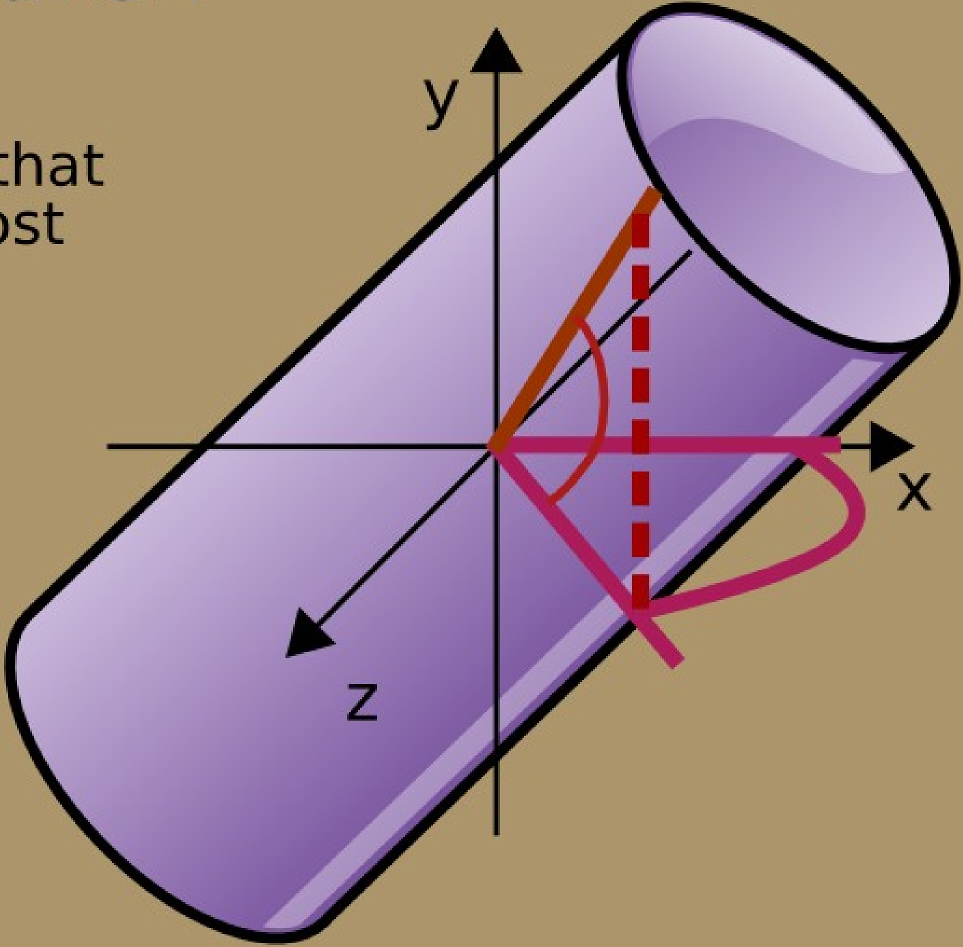
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

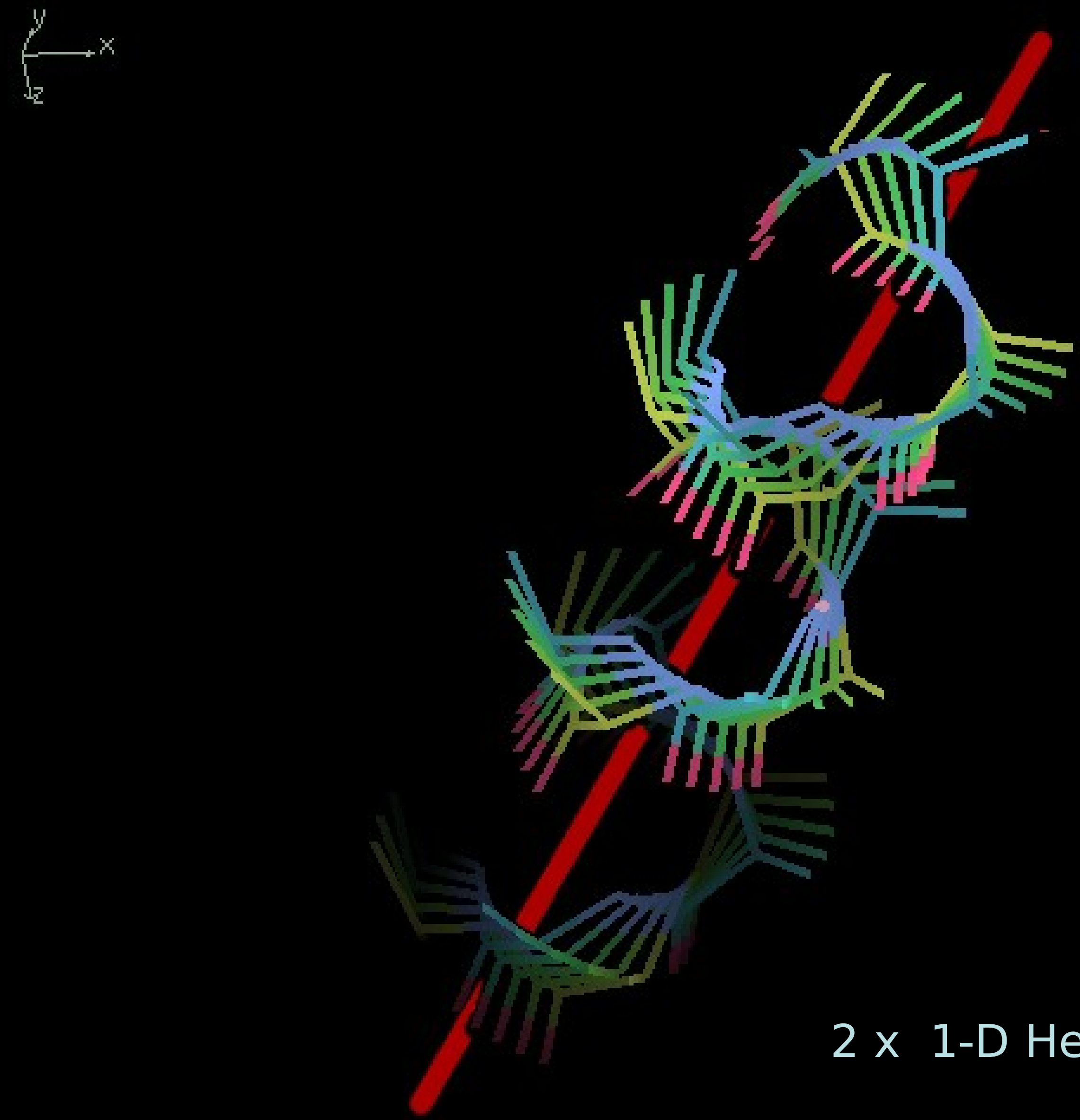


Cylinder Search

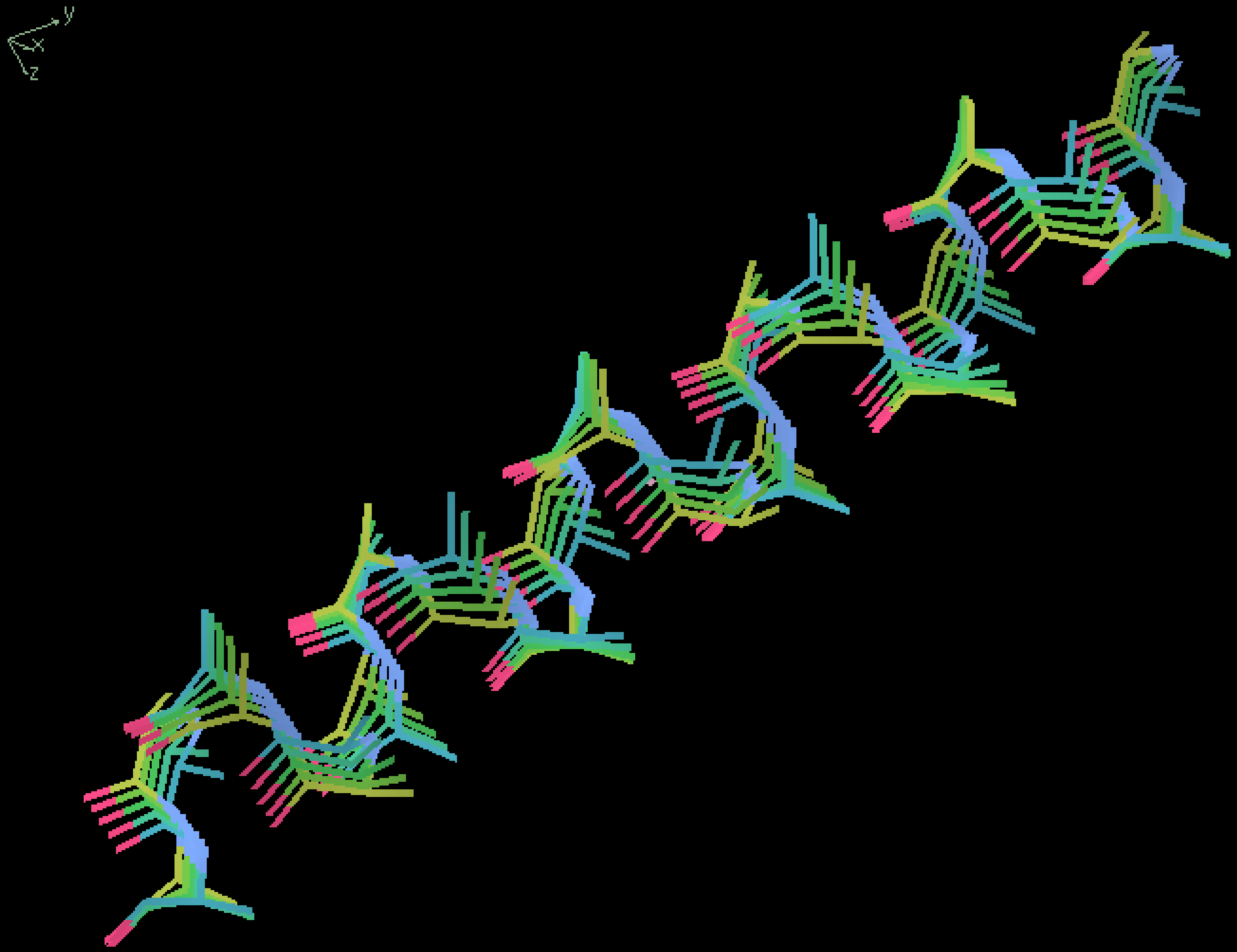
Pick the orientation that encapsulates the most electron density

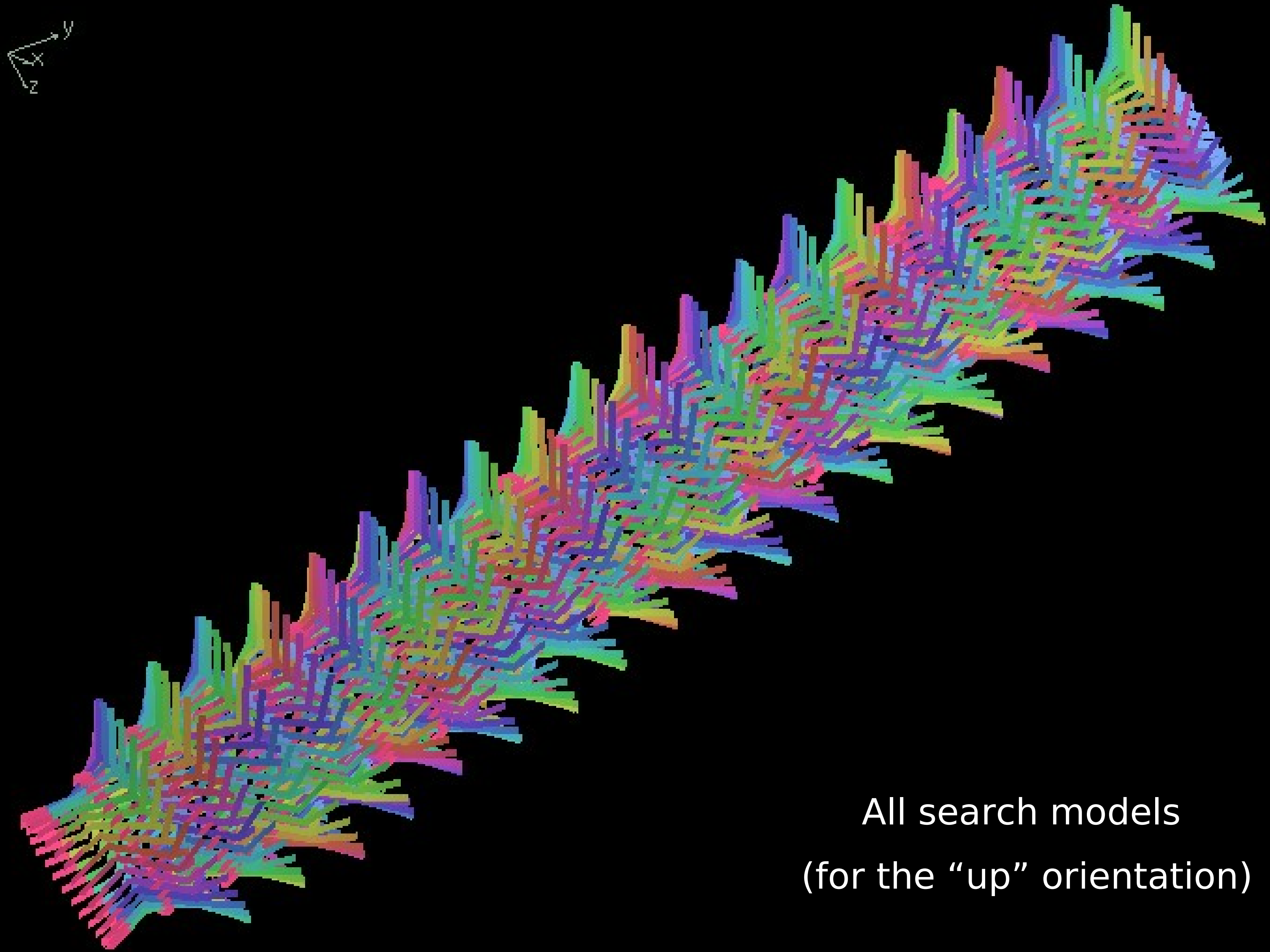


2 orientation axes



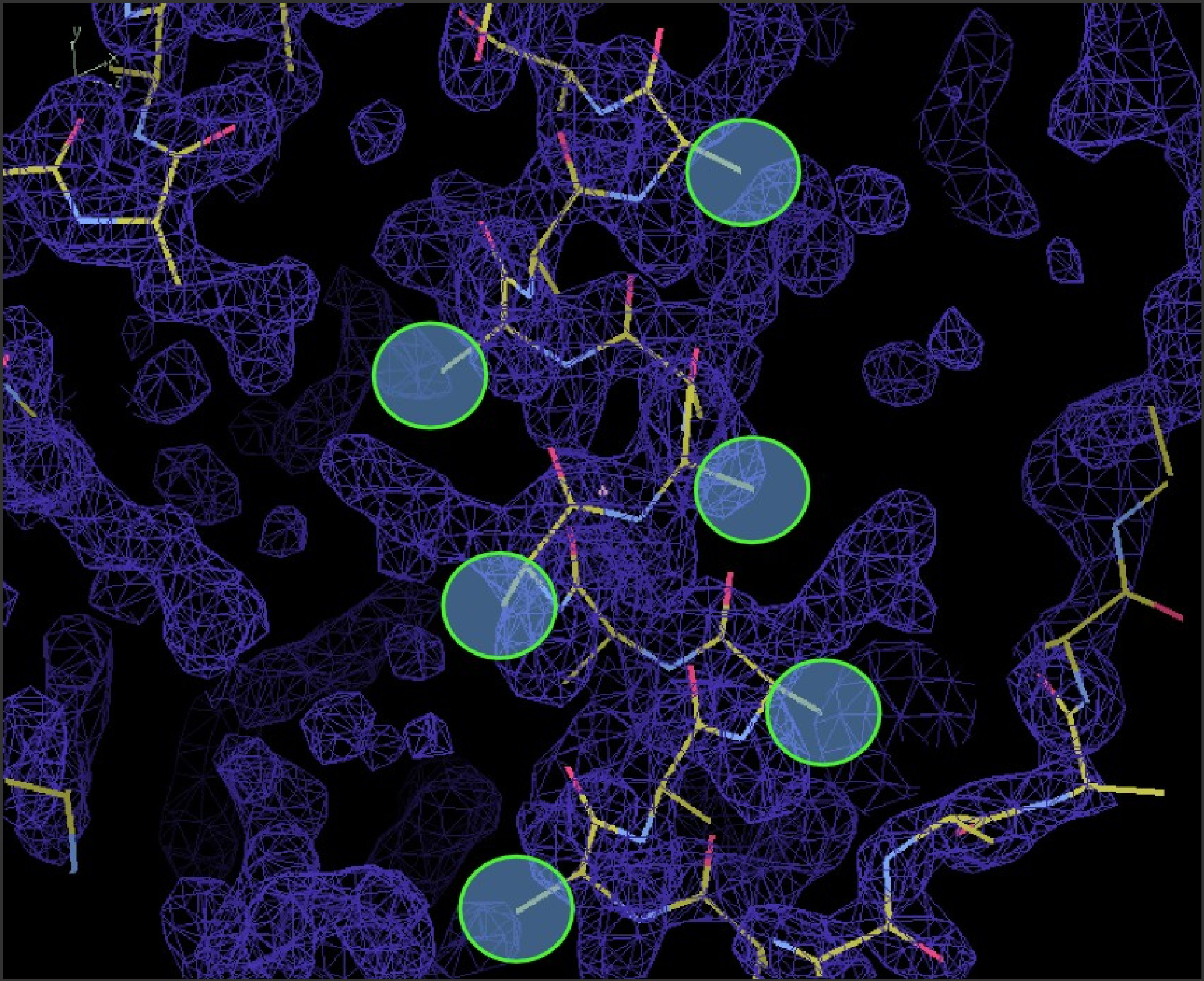
2 x 1-D Helix orientation searches

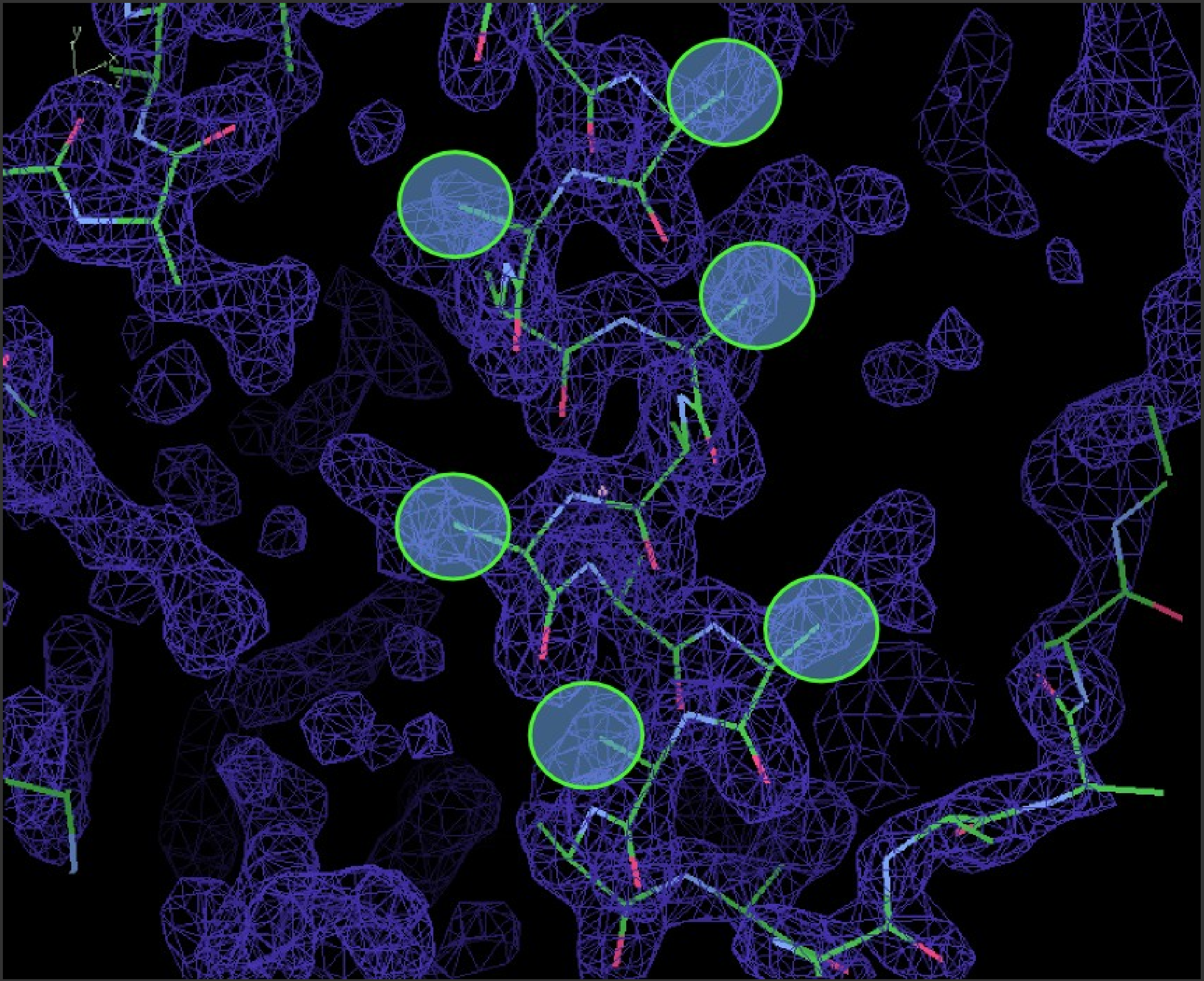

y
x
z



All search models

(for the “up” orientation)


y
x
z


y
x
z

Association of Sequence

- Coot now reads a PIR sequence file
- Use for:
  - Validation
    - Does the model match the required sequence?
  - Loop fitting
  - Terminal residue addition
  - Align&Mutate
    - e.g. Post MR
  - Sequence assignment
    - Likelihood-based “slider”

“Ease of Use” *vs* “Easy to Learn”

“Ease of Use” fights back!

- Any function can be bound to a key
  - Allows for personalization/customization
- Here's how you do it:
- (add-key-binding *name key function*)
- Makes Coot easy to use
  - (but harder to learn)
- See Coot Wiki for more details
- Note: Improved navigation:
  - Ctrl g *num* <Return>

Example Keybindings

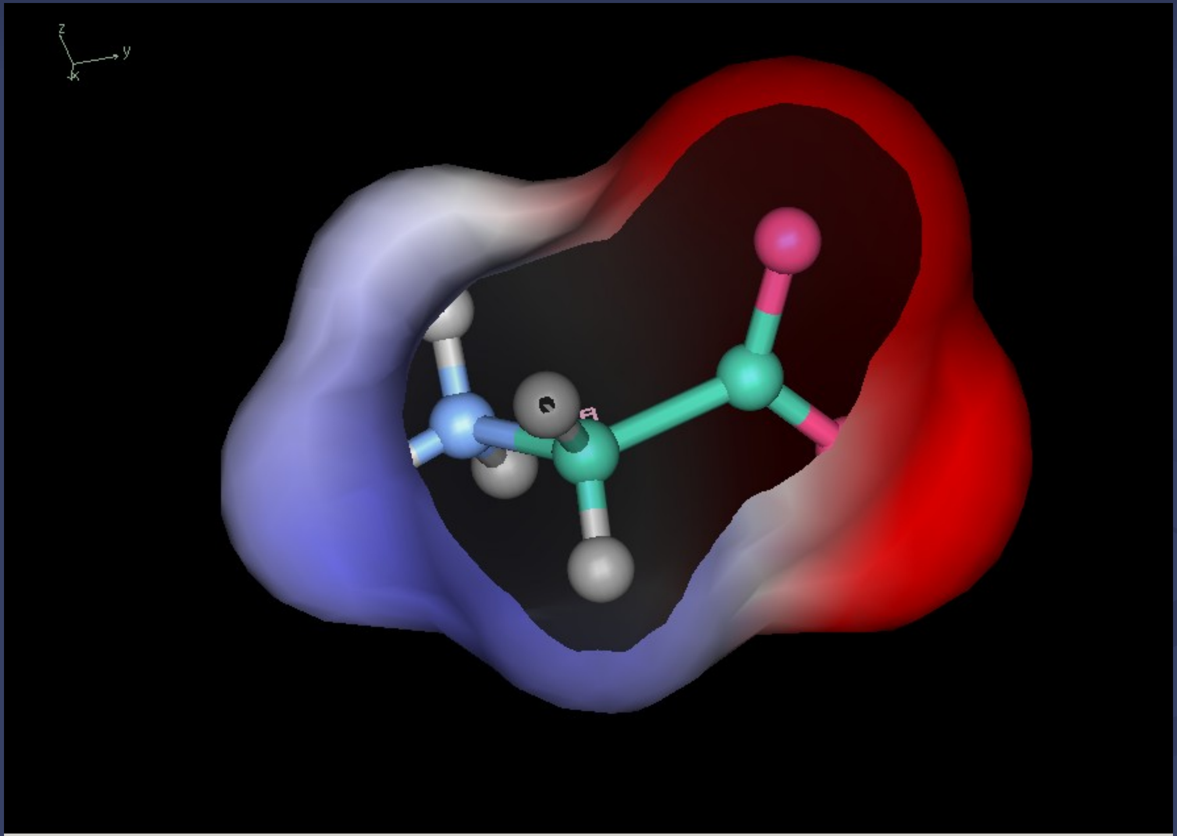
- (add-key-binding "Refine Active Residue AA" "x" refine-active-residue)
- (add-key-binding "Refine Active Residue" "r" (lambda () (manual-refine-residues 0)))
- (add-key-binding "Triple Refine" "t" (lambda () (manual-refine-residues 1)))
- (add-key-binding "Triple Refine AA" "h" (lambda () (refine-active-residue-triple)))

Association of Sequence

- Coot now reads a PIR sequence file
- Use for:
  - Validation
    - Does the model match the required sequence?
  - Loop fitting
  - Terminal residue addition
  - Align&Mutate
    - e.g. Post MR
  - Sequence assignment
    - Likelihood-based “slider”

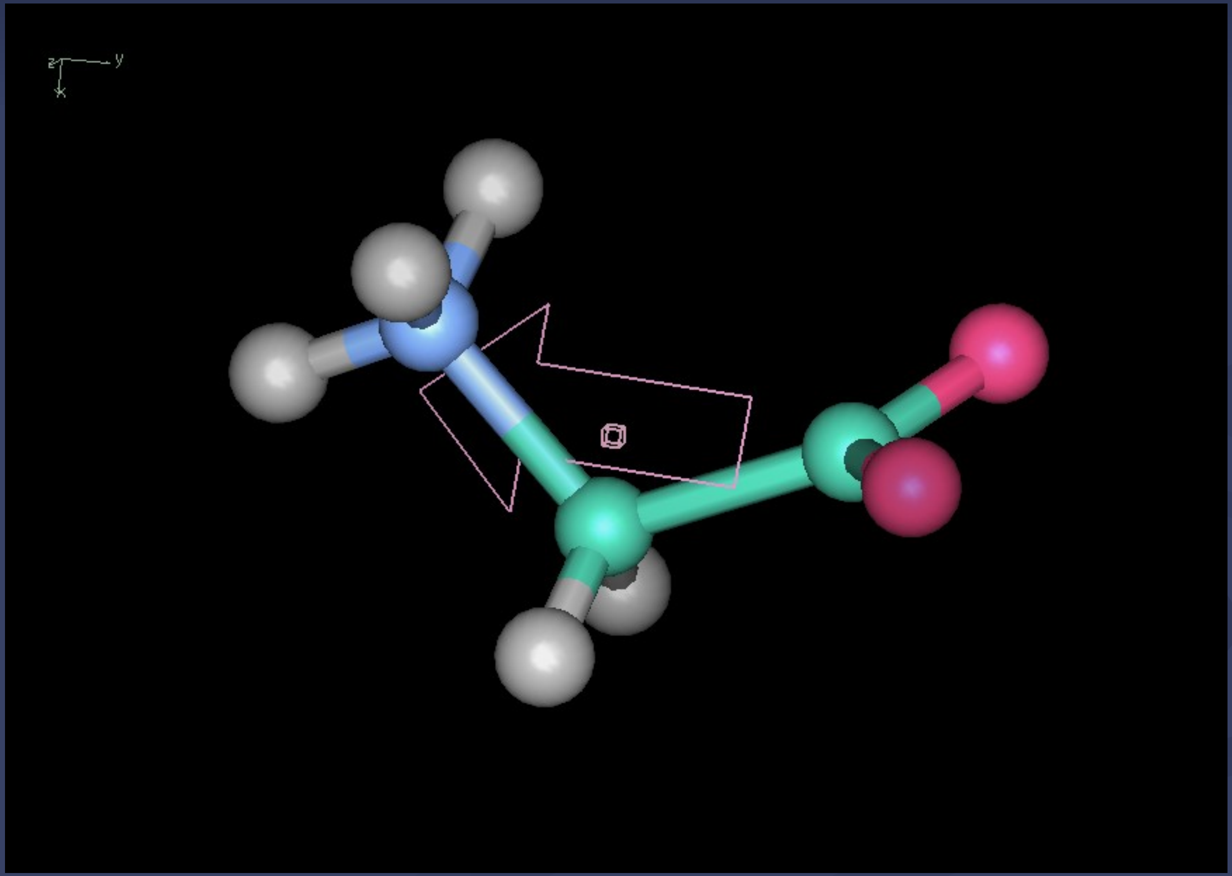
Other Things

- Surfaces that use dictionary partial charges



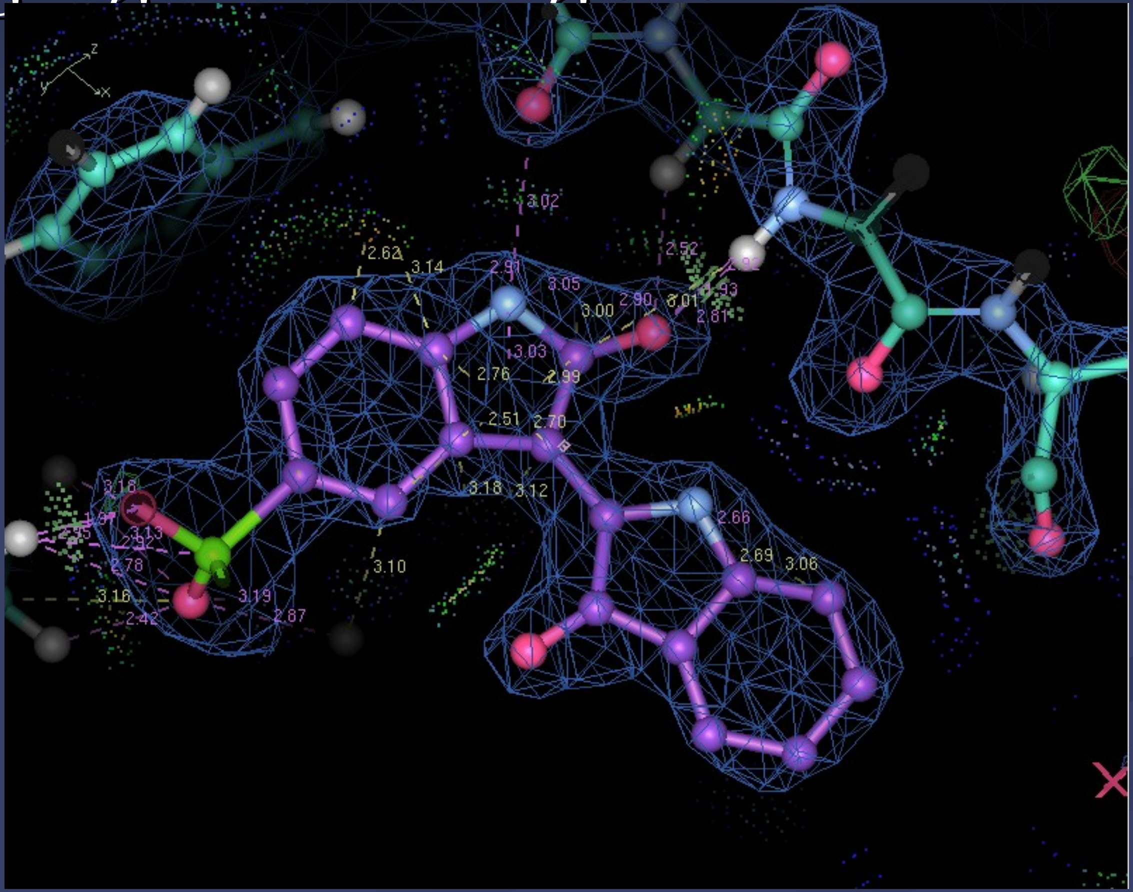
Other Things

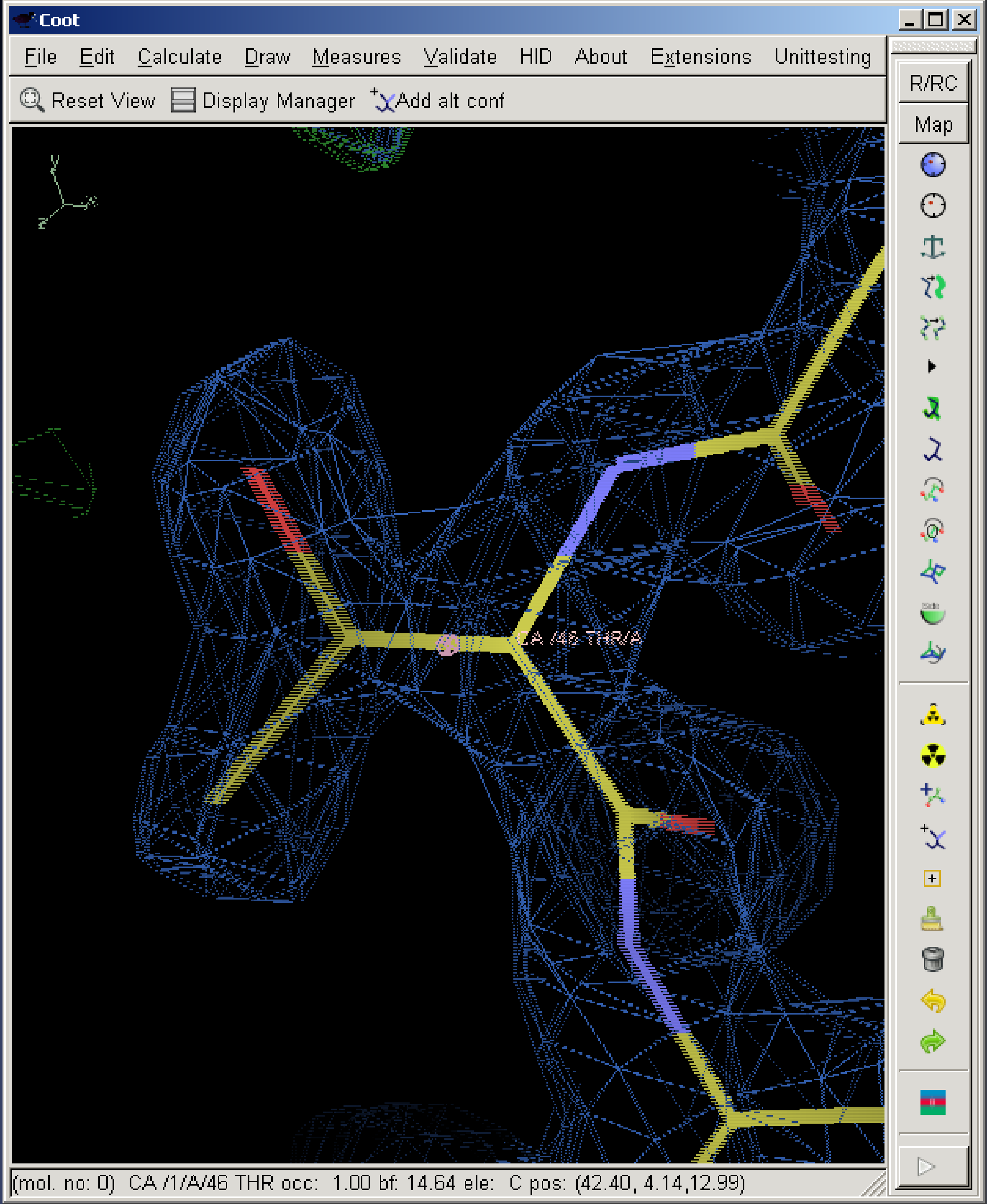
- Partial charges provide dipoles now too



Other Things

- Molprobity dots for ligands
  - Highlight interesting site





(mol. no: 0) CA /1/A/46 THR occ: 1.00 bf: 14.64 ele: C pos: (42.40, 4.14,12.99)

A Sample of Tools

- A few tools and tricks have been described here
  - There are many others...
    - CCP4, Refmac, Libcheck, Molprobity, What_check, Raster3D, SHELXL...

Thanks to...

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