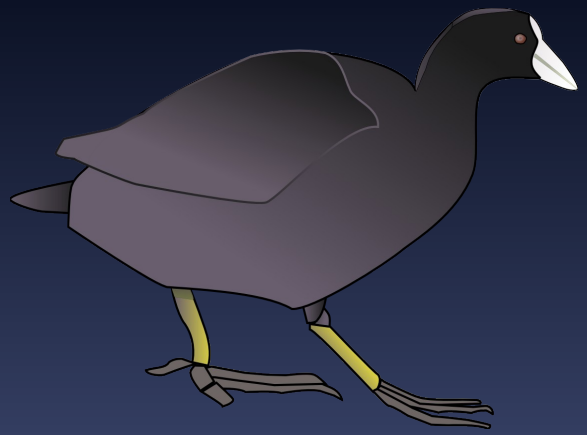
June 2010 Chicago



Model-Building with Coot

An Introduction

(Paul Emsley)

(University of Oxford)

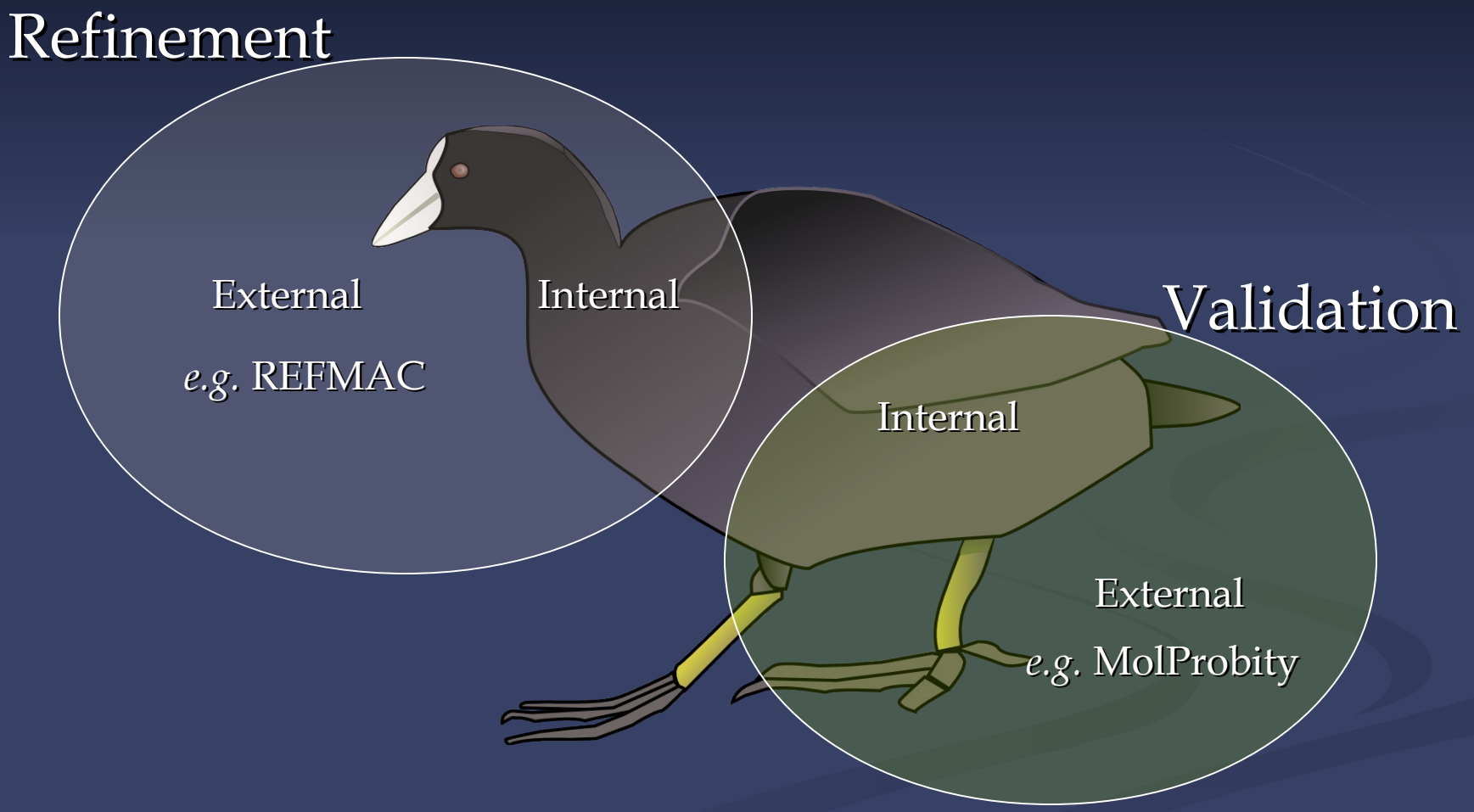
Bernhard Lohkamp

Karolinska Institutet

Coot

- Molecular Graphics application
  - Protein Crystallographic model-building tools (Crystallographic Object-Oriented Toolkit)
  - Aim: “Slick and powerful” interface to CCP4
- Interface to other programs: SHELXL, Refmac, Libcheck, Probe&Reduce (Molprobity), EBI, EDS, Povray, Raster3D, PHENIX
- Several model-building and validation tools

Feature Integration



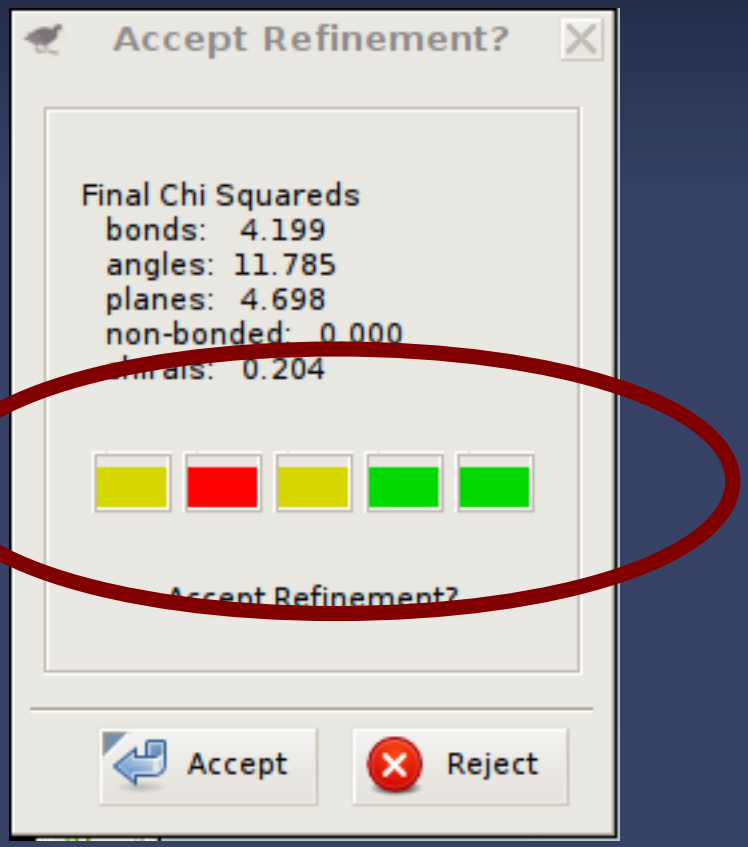
Real Space Refinement

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]
- Provides "interactive refinement"
- Different minimizer to Refmac…
  - …means "nice & tight" geometry
    - Chi squareds

Faster & Animated

Refinement "Traffic Lights"



"Traffic Lights" represent the chi-squared values for each of the refined geometry types

Refinement Techniques

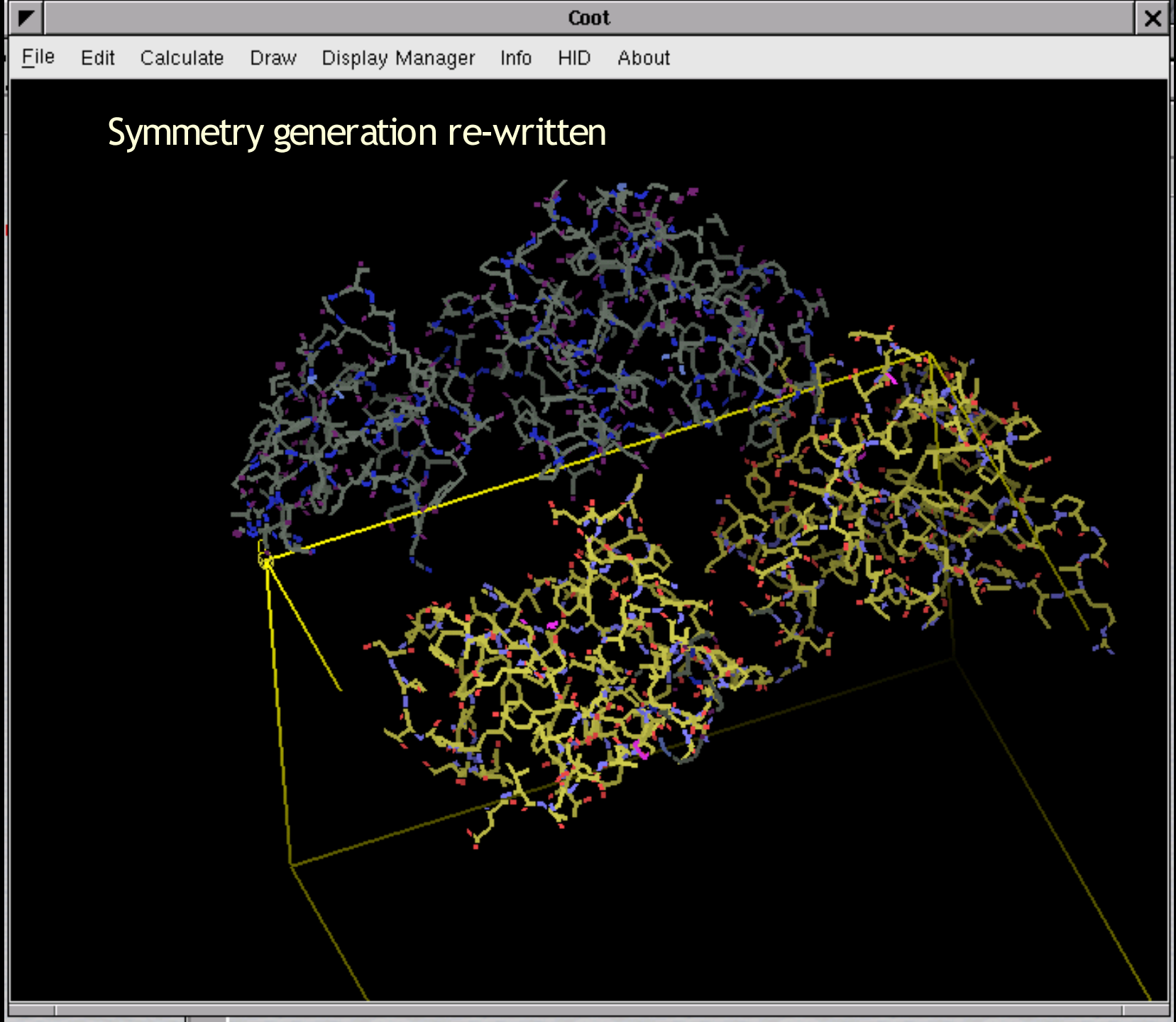
- Auto-zone
- Single-Atom Drag
- Over-dragging
- Ramachandran Refinement
- Sphere refinement
- Coming Soon..?
  - Dials, PowerMate, spaceballs
  - Wii Refinement

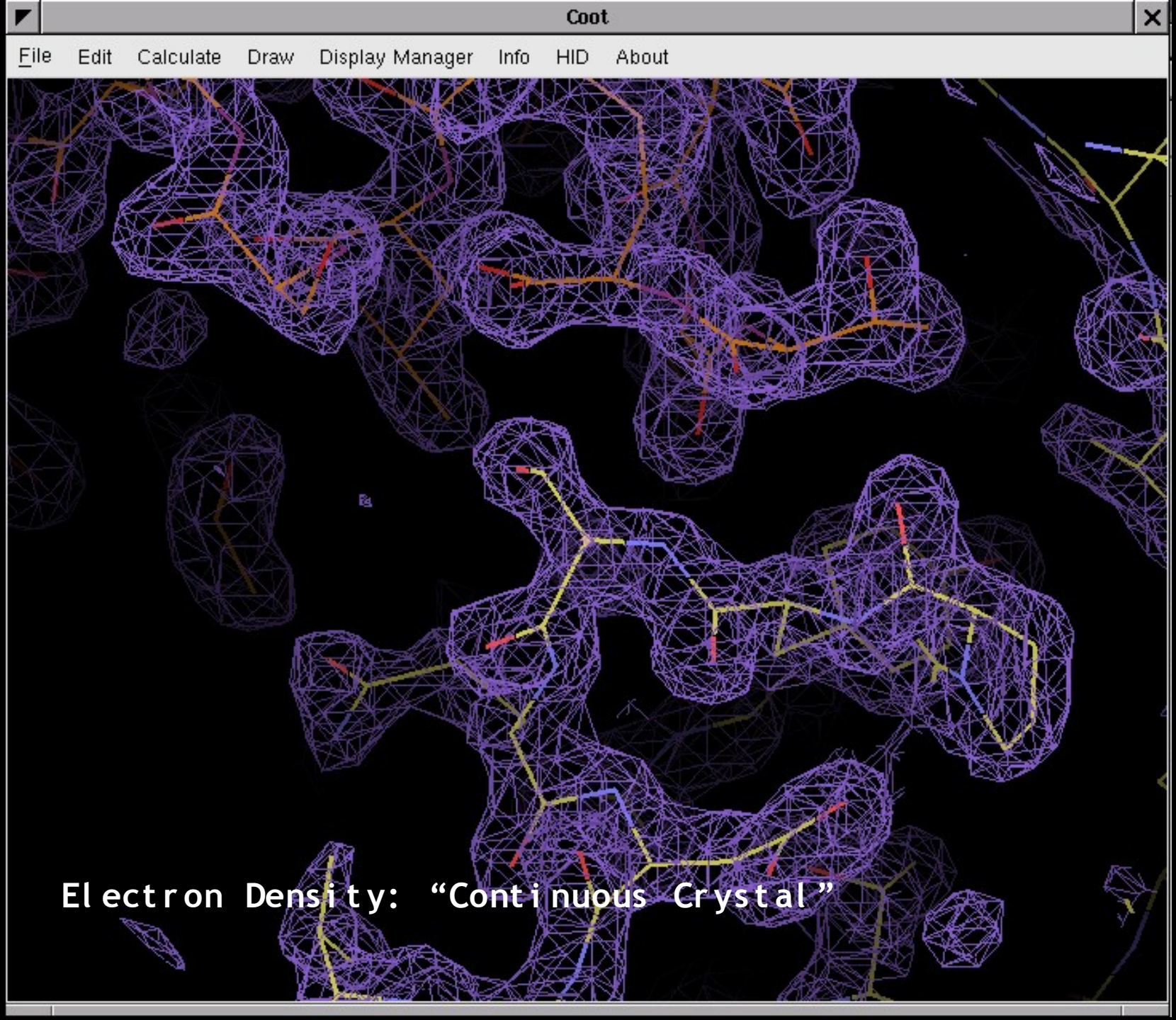
Generic Objects

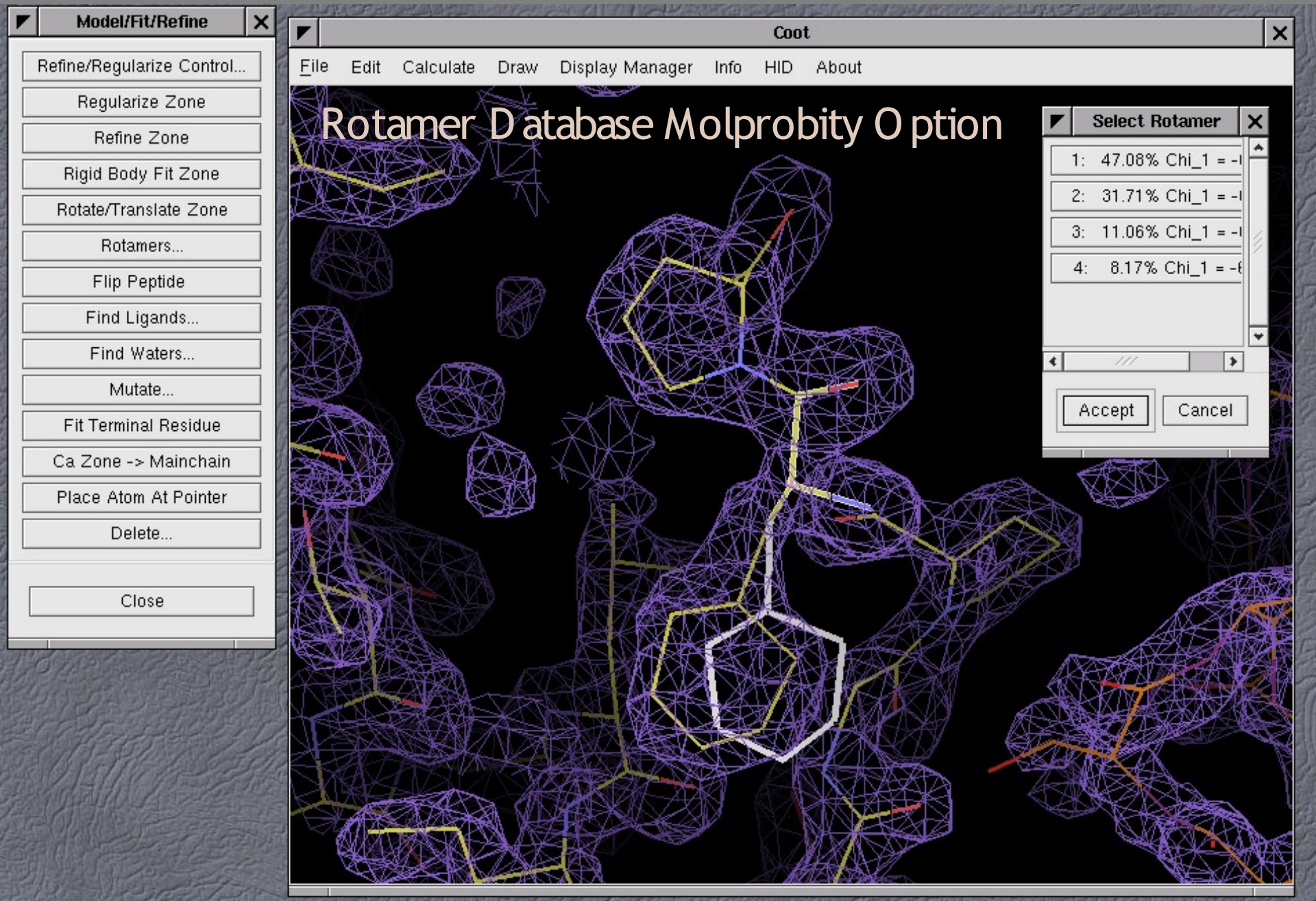
- A generic object can be any set of coloured lines or points
- Mechanism to display and close
  - (not “clickable”)
- Generic Objects from .vu files
- MAPMAN “Bones”
- MolProbity Dots

Some more Coot Tools…

- Alternate Conformations
- Ligand fitting
- Rigid-body Fitting
  - Steepest Descent
  - Simplex (slower but better)
- “Move Molecule Here”
- Water Search


Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About
Symmetry generation re-written


Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About
Electron Density: “Continuous Crystal”

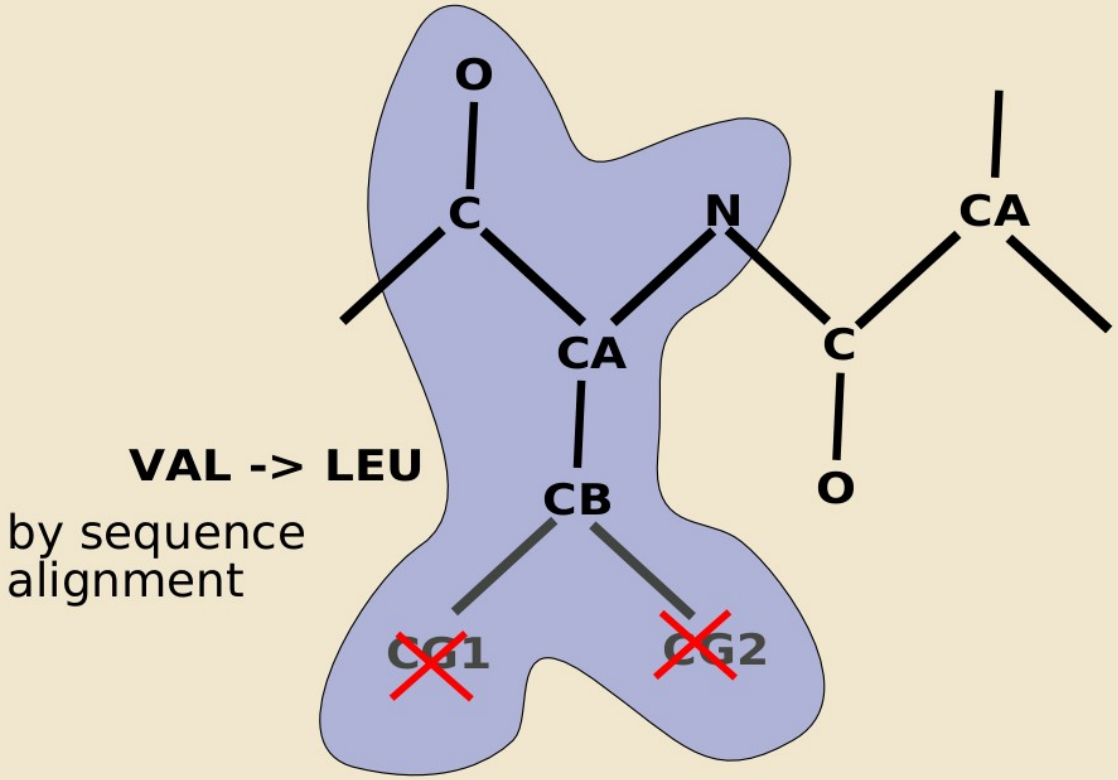

Model/Fit/Refine
Refine/Regularize Control...
Regularize Zone
Refine Zone
Rigid Body Fit Zone
Rotate/Translate Zone
Rotamers...
Flip Peptide
Find Ligands...
Find Waters...
Mutate...
Fit Terminal Residue
Ca Zone -> Mainchain
Place Atom At Pointer
Delete...
Close
Coot
File
Edit
Calculate
Draw
Display Manager
Info
HID
About
Rotamer Database Molprobity Option
Select Rotamer
1: 47.08% Chi_1 = -
2: 31.71% Chi_1 = -
3: 11.06% Chi_1 = -
4: 8.17% Chi_1 = -
Accept
Cancel

Other Tools

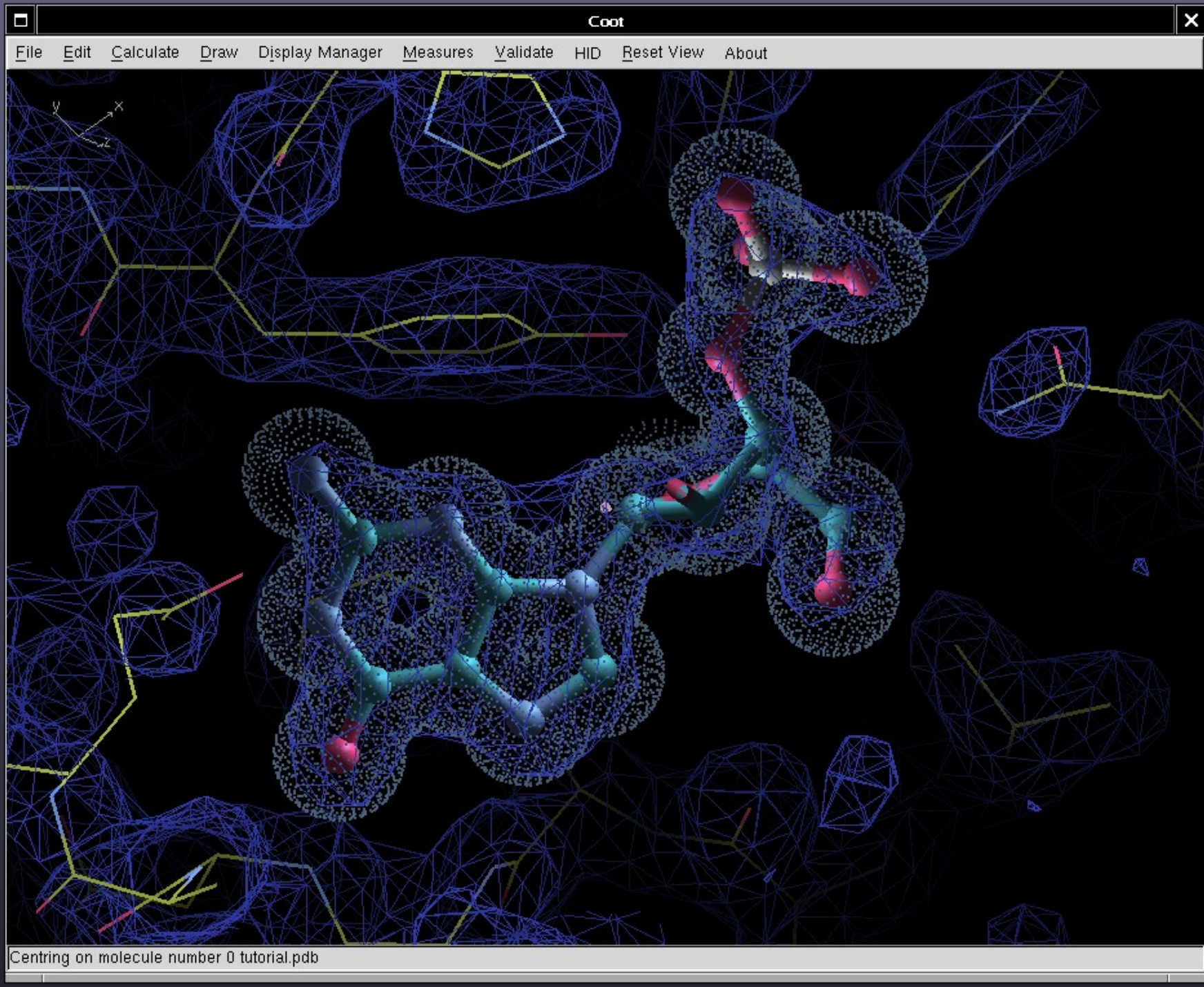
- Reverse chain direction
- 180° side-chain flip
- Planar peptide restraints
- “Chi” angles for ligands
- Dots, ball&stick
- Fill-partial-residues (`de-chainsaw`)

Chainsawing

Model-trimming

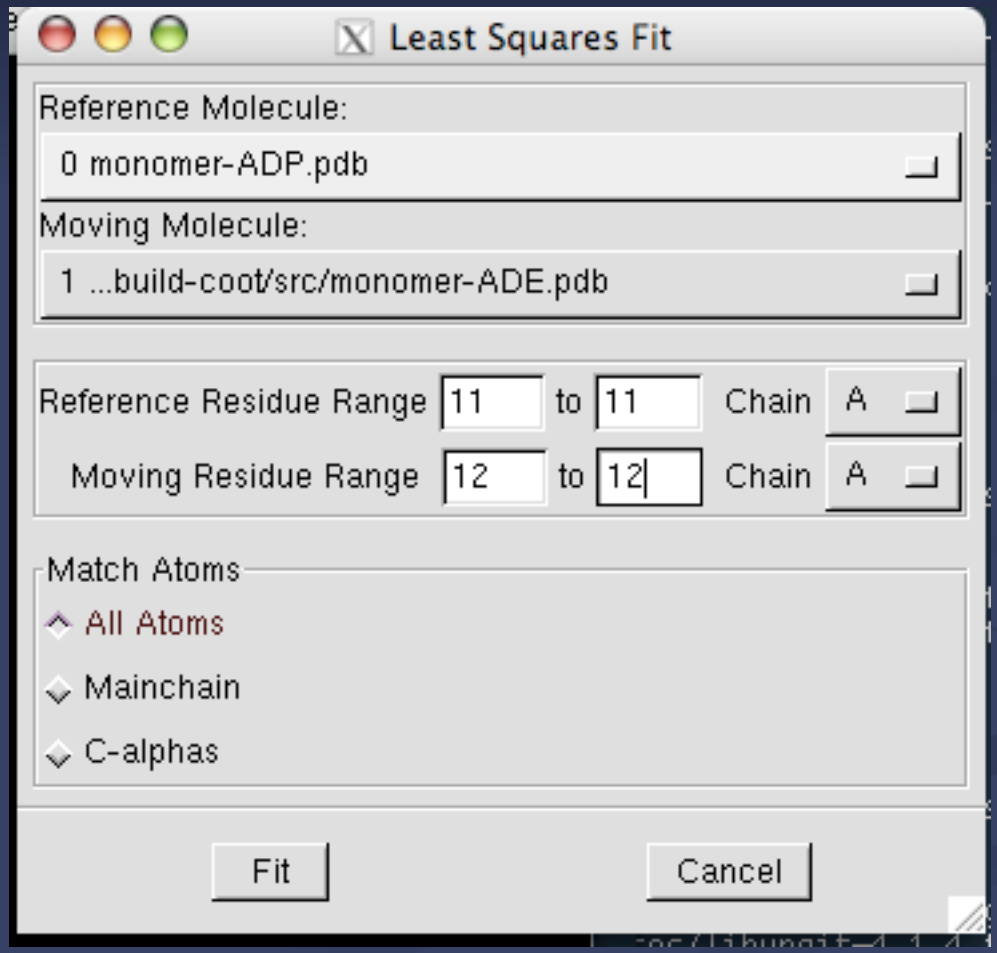


Schwartzenbacker et al. (2004) Acta Cryst D60 1229


Coot
File
Edit
Calculate
Draw
Display Manager
Measures
Validate
HID
Reset View
About
Centring on molecule number 0 tutorial.pdb

Least Squares Fitting

- Least Squares Superposition:

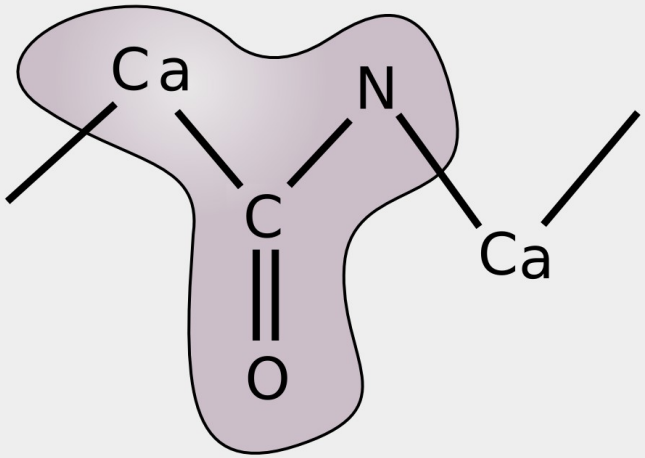


Low Resolution Tools

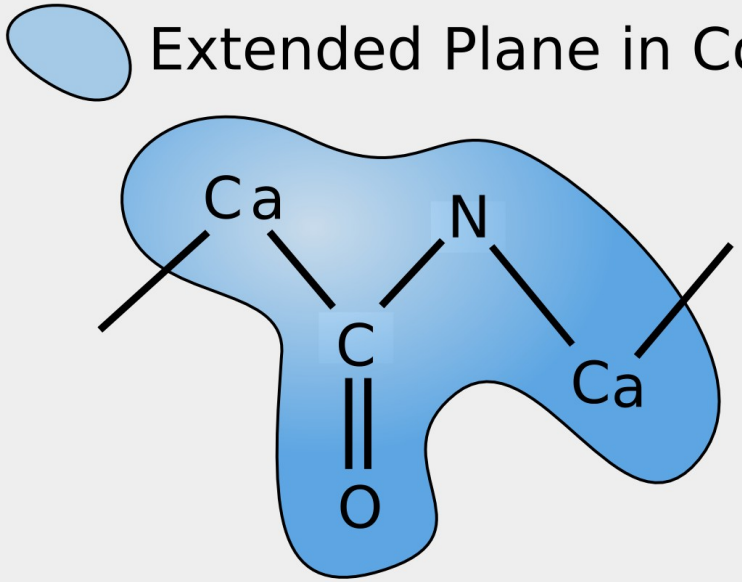
Extra Restraints....

Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane



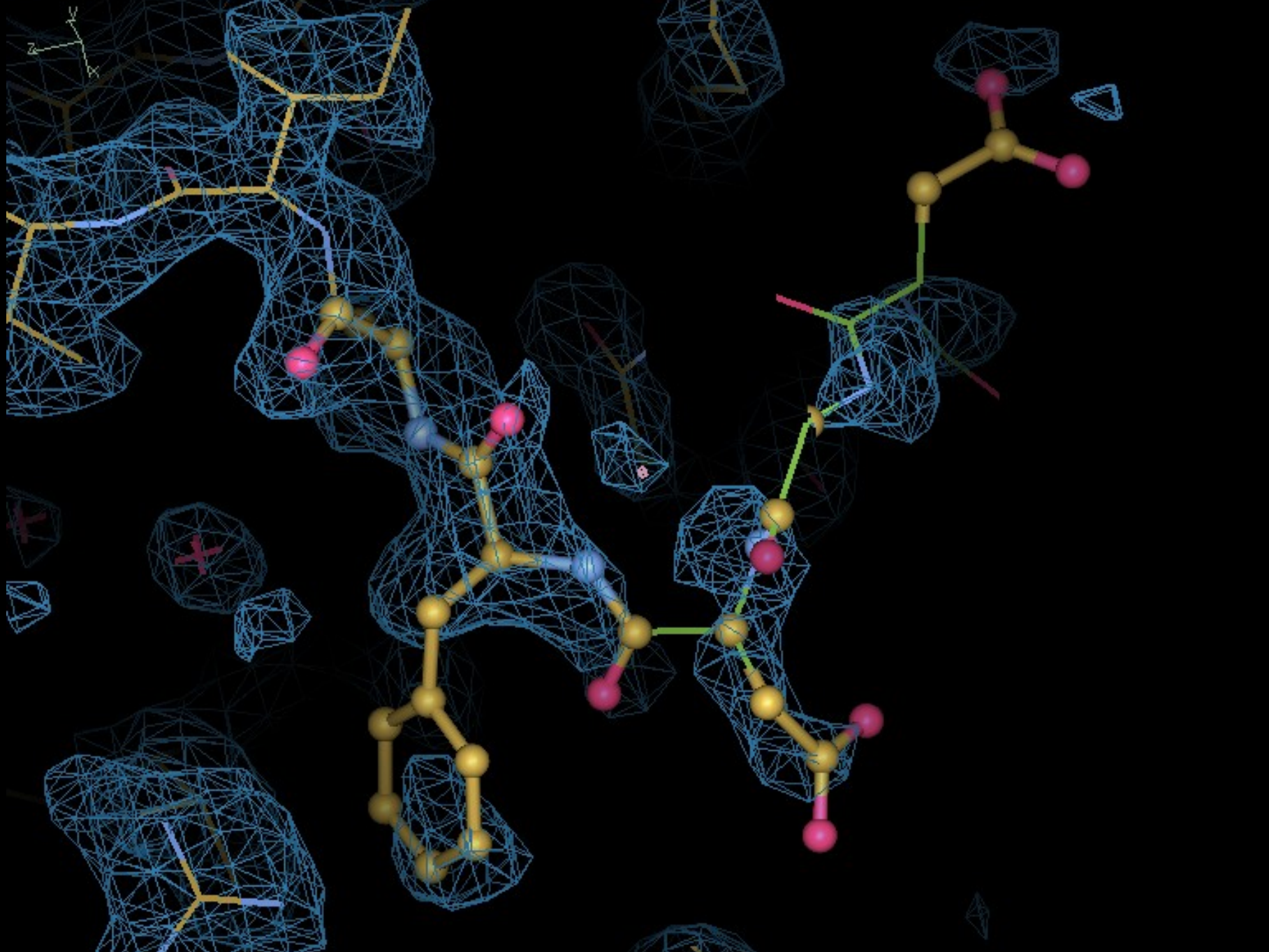
Extended Plane in Coot



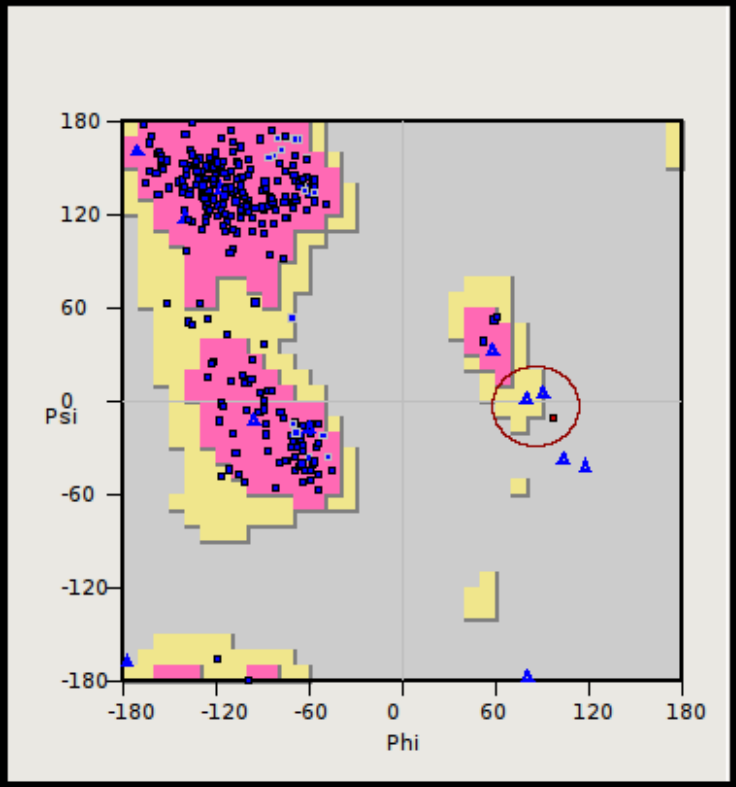
(add-planar-peptide-restraints)

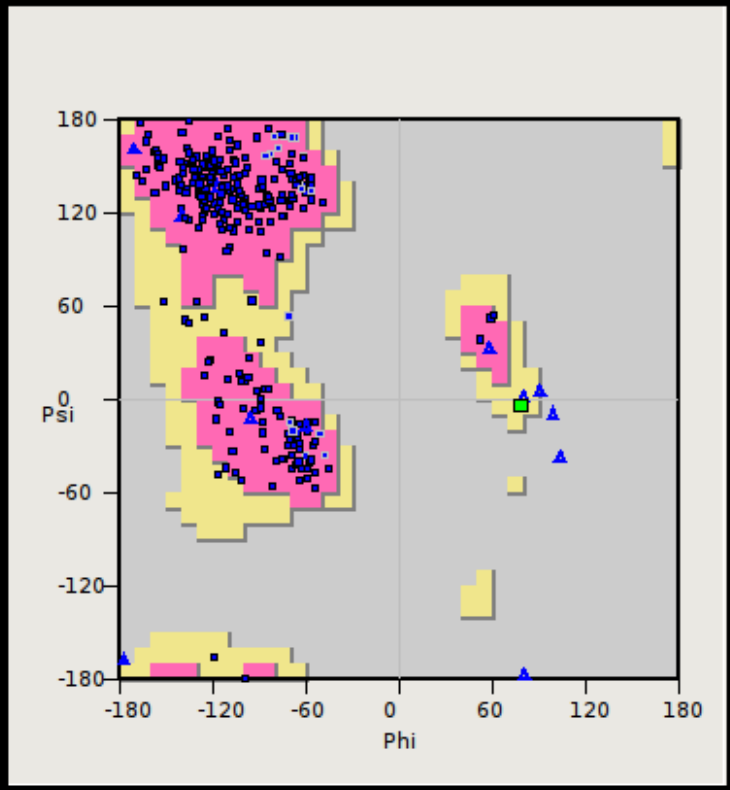
Ramachandran Restraints

- 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

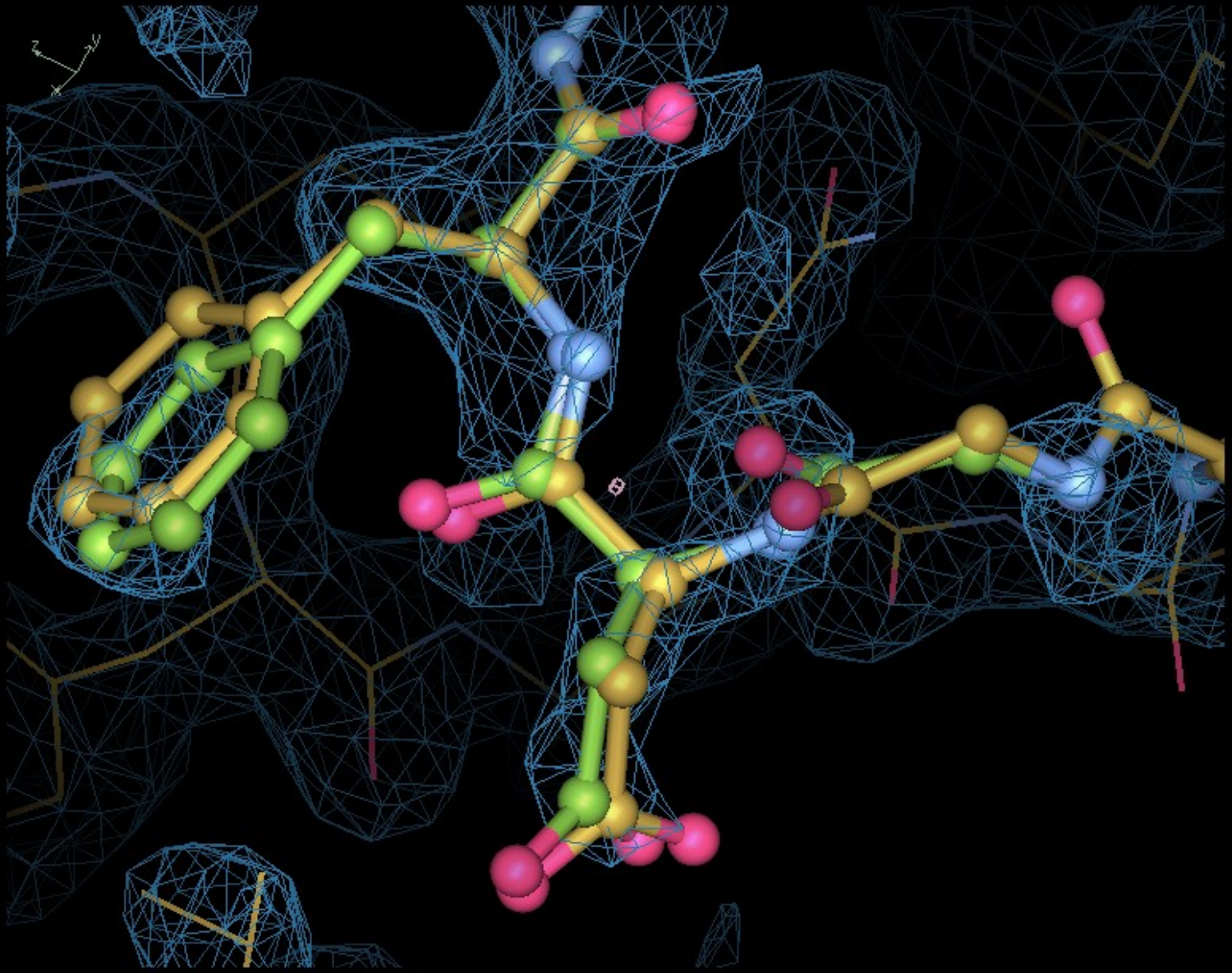

Y
Z
X

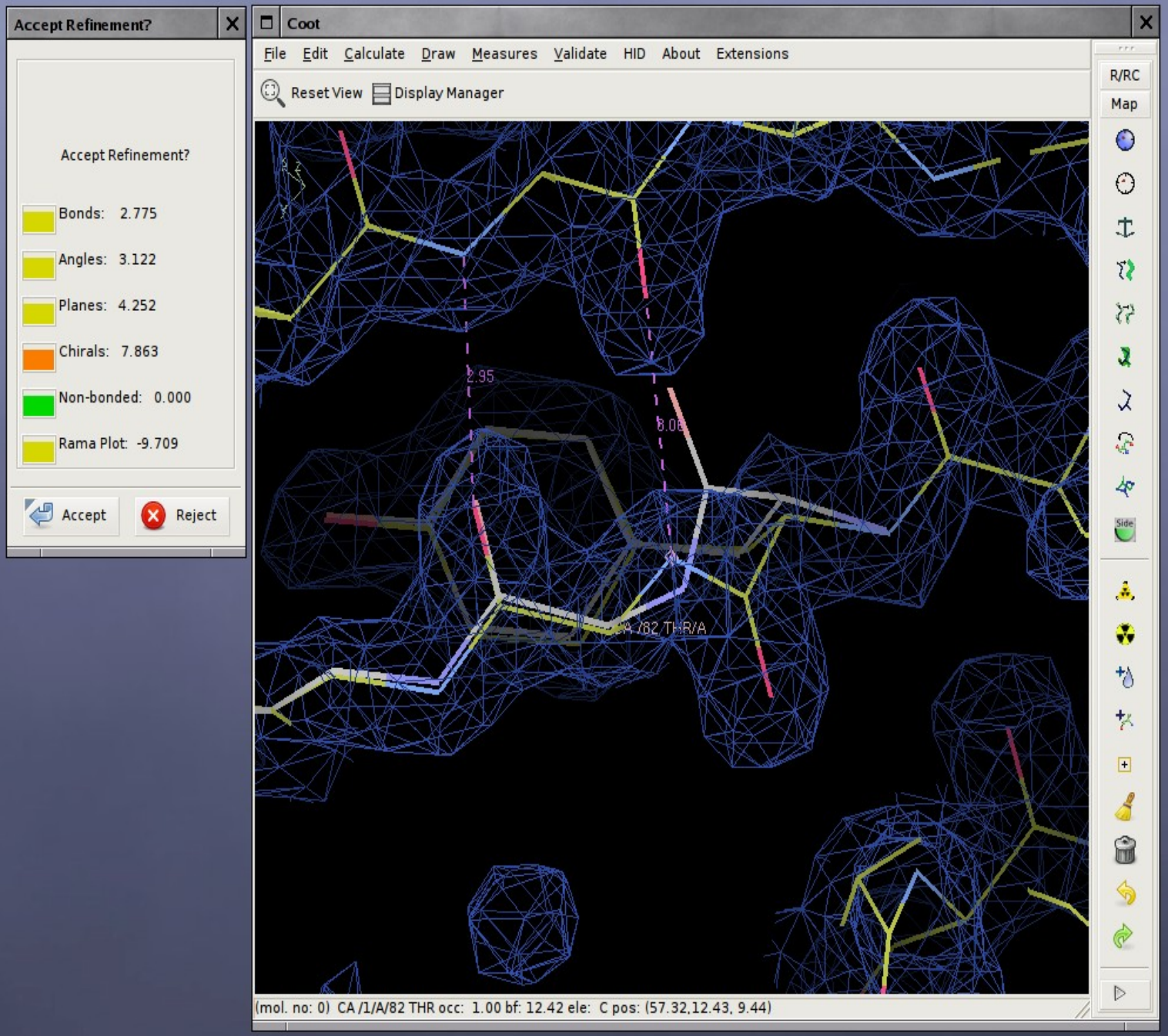
Tweaking a Ramachandran Outlier





Tweaking Phi and Psi




Accept Refinement?
Accept Refinement?
Bonds: 2.775
Angles: 3.122
Planes: 4.252
Chirals: 7.863
Non-bonded: 0.000
Rama Plot: -9.709
Accept
Reject
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View
Display Manager
R/RC
Map
Side
2.95
6.08
A /82 THR/A
(mol. no: 0) CA /1/A/82 THR occ: 1.00 bf: 12.42 ele: C pos: (57.32,12.43, 9.44)

Ramachandran Restraints

- Controversial?
  - "... the Ramachandran Plot is one of the simplest and most sensitive means for assessing the quality of a protein model..."
    - Gerard Kleywegt & Alwyn Jones (1996)
- But to quote Jane Richardson:
  - Do you want a better structure – or a better idea of the quality of your structure?

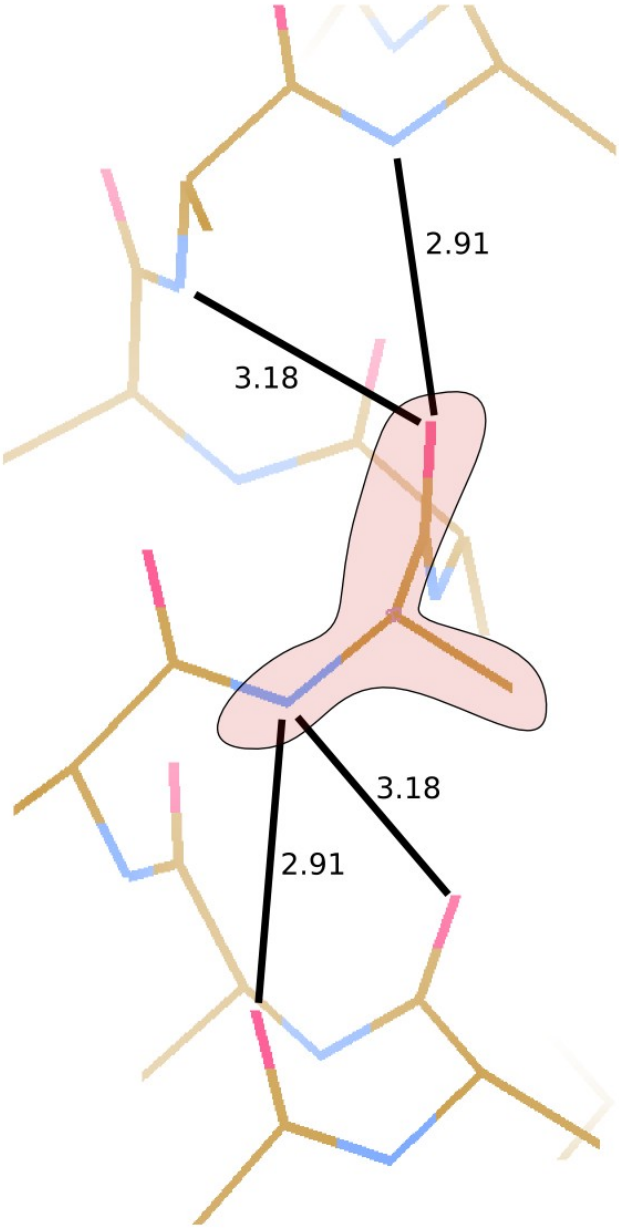
Adding Torsion Angle Restraints

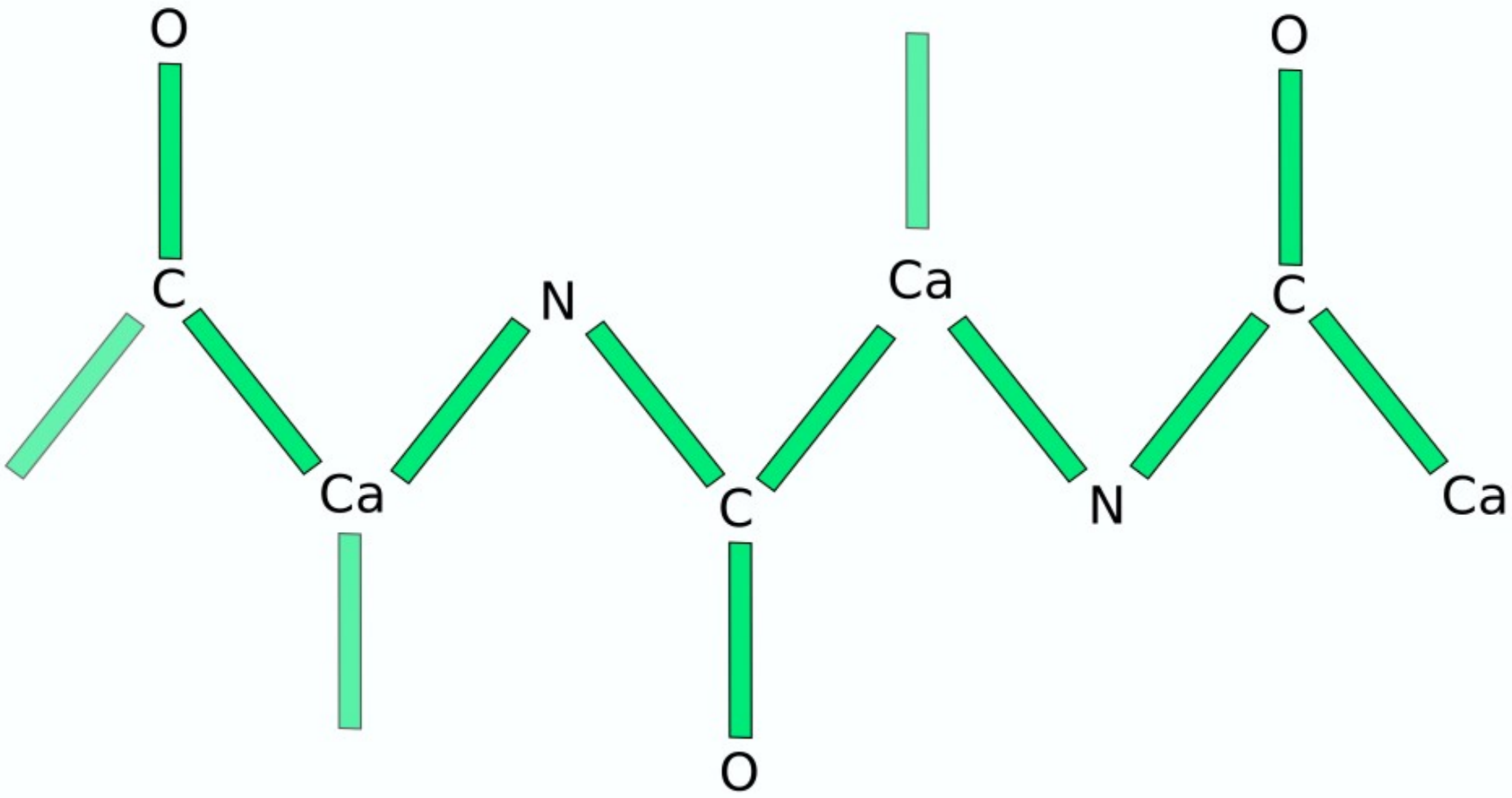
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”

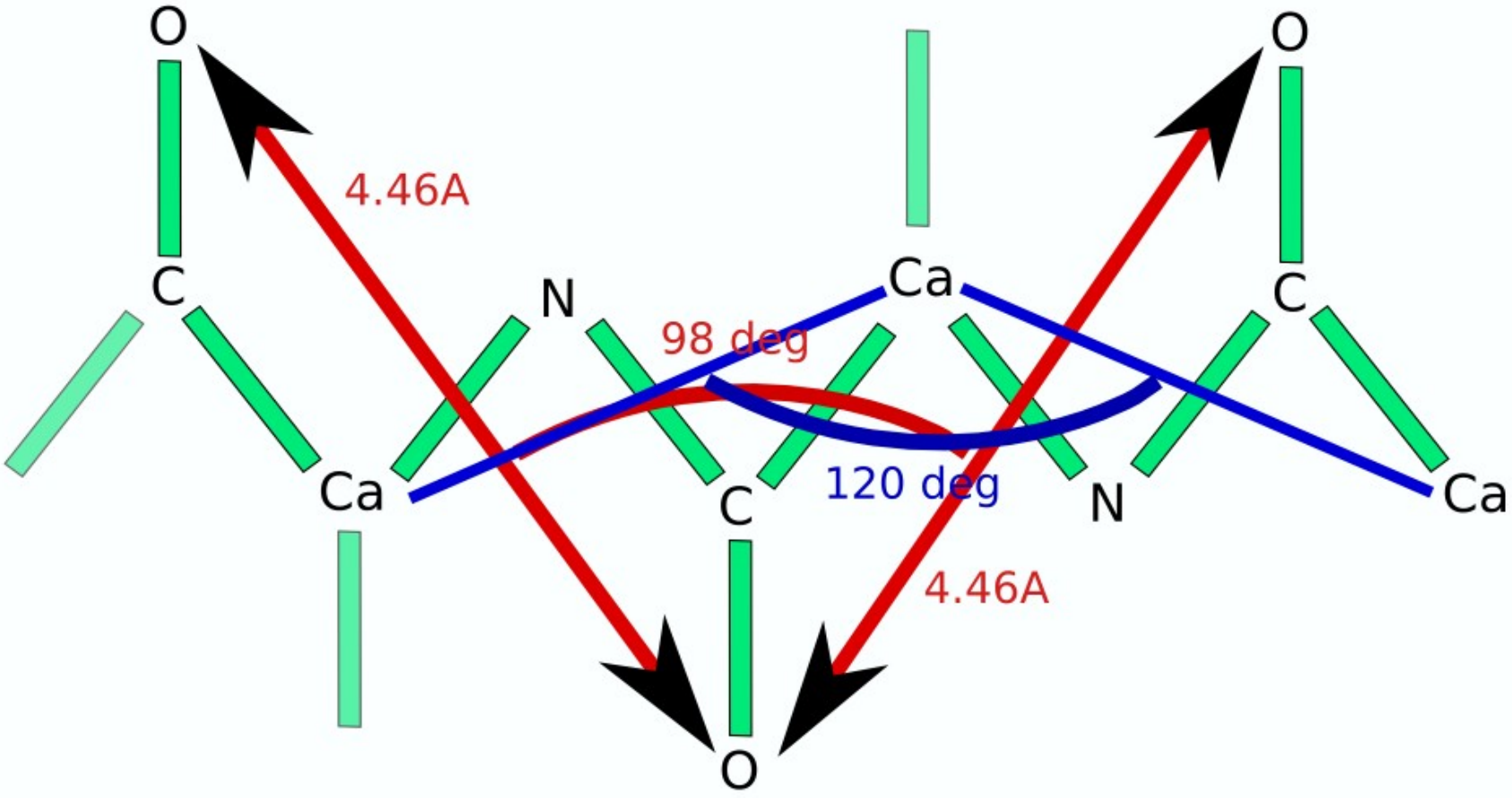
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints

Restrain the Hydrogen-bonding atom distances




O
C
Ca
N
Ca
C
O
O
C
Ca
N
Ca

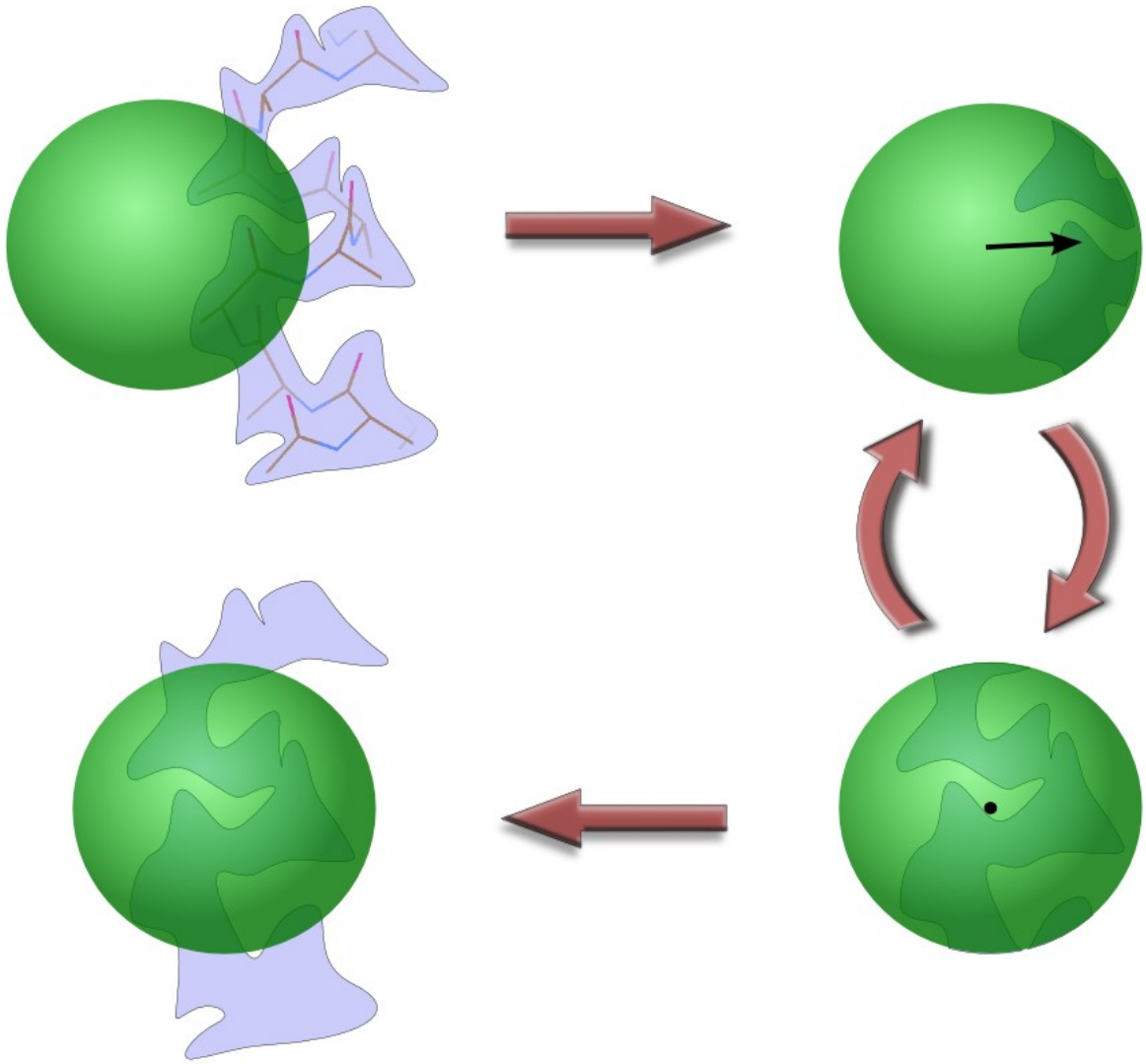

O
4.46A
C
N
Ca
98 deg
O
C
Ca
C
120 deg
N
Ca
4.46A
O

Helix-Building

Alpha Helix Placement

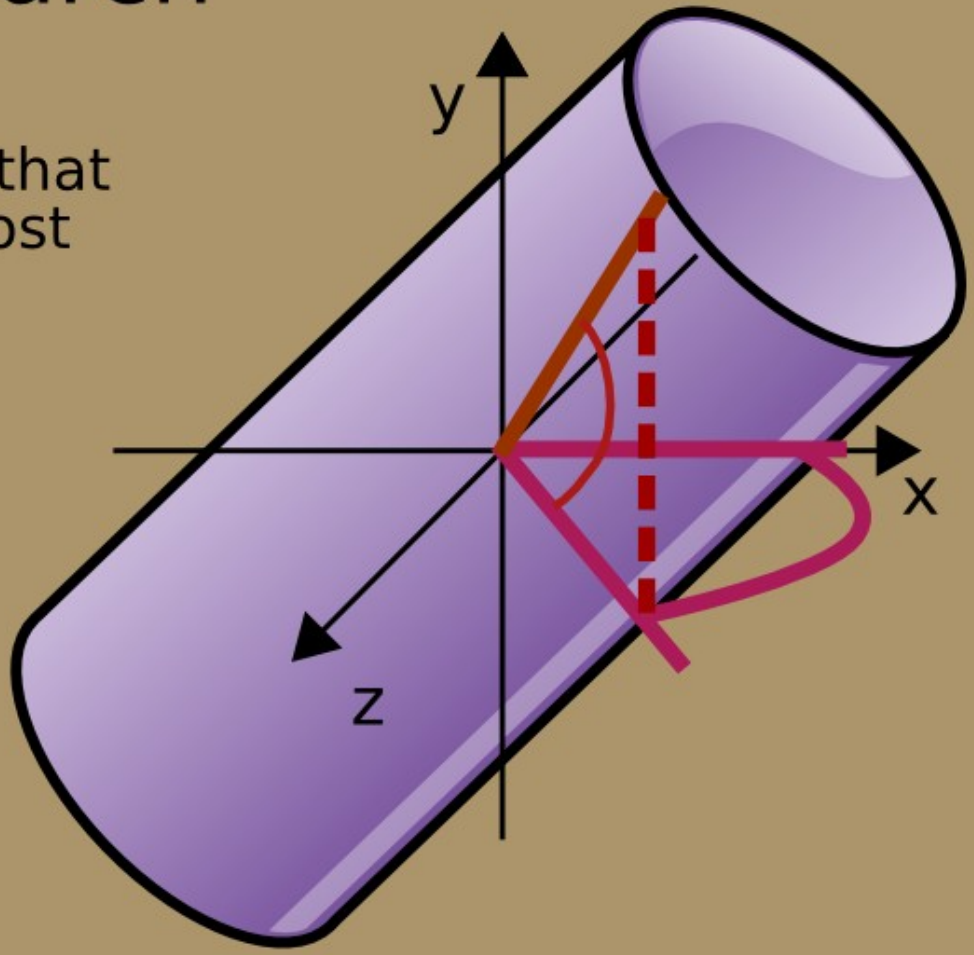
- Scenario: Looking at a new map, not built with automatic tools:
  - “I can see that there’s a helix here - build it for me!”
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point



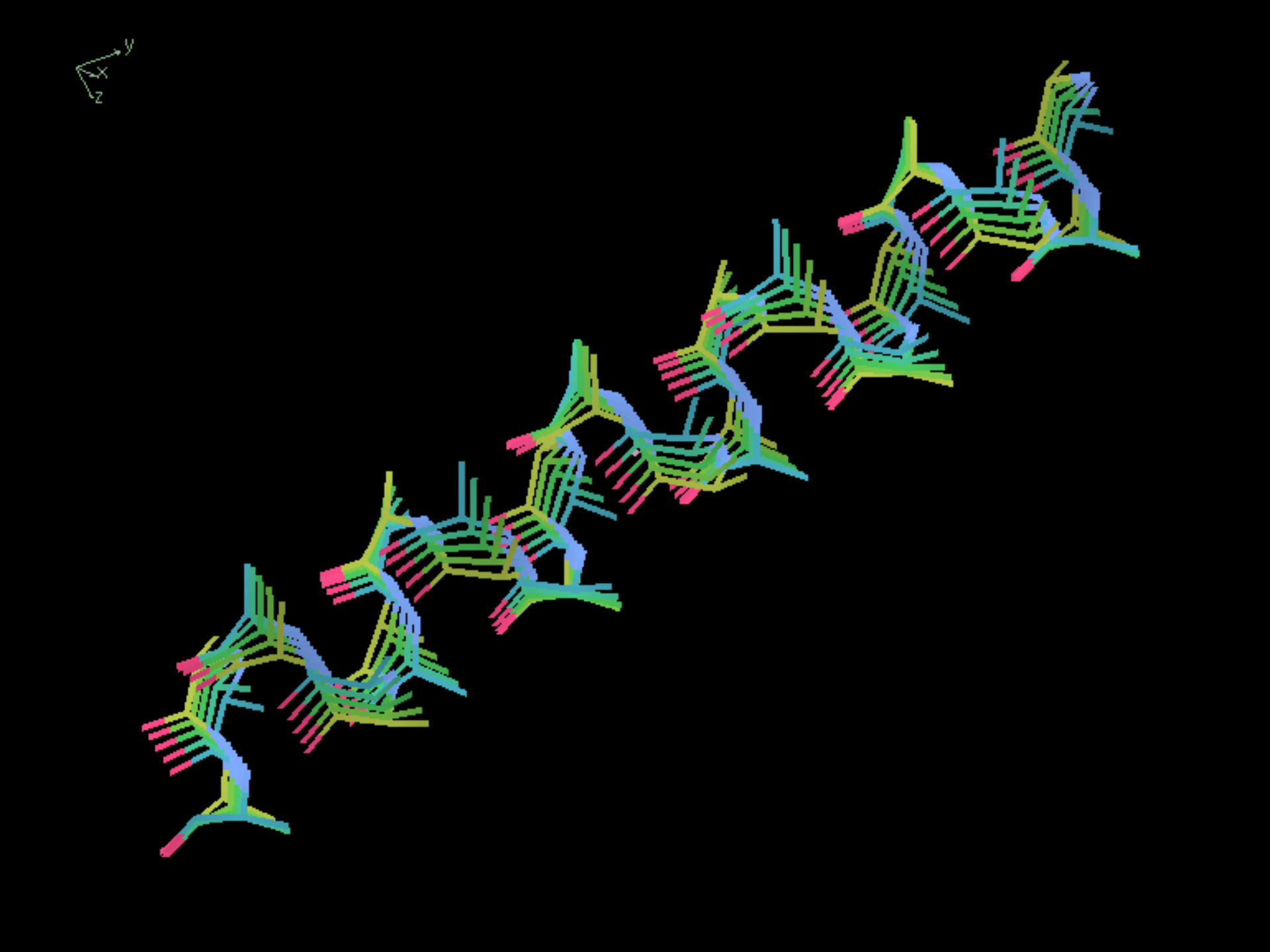
Cylinder Search

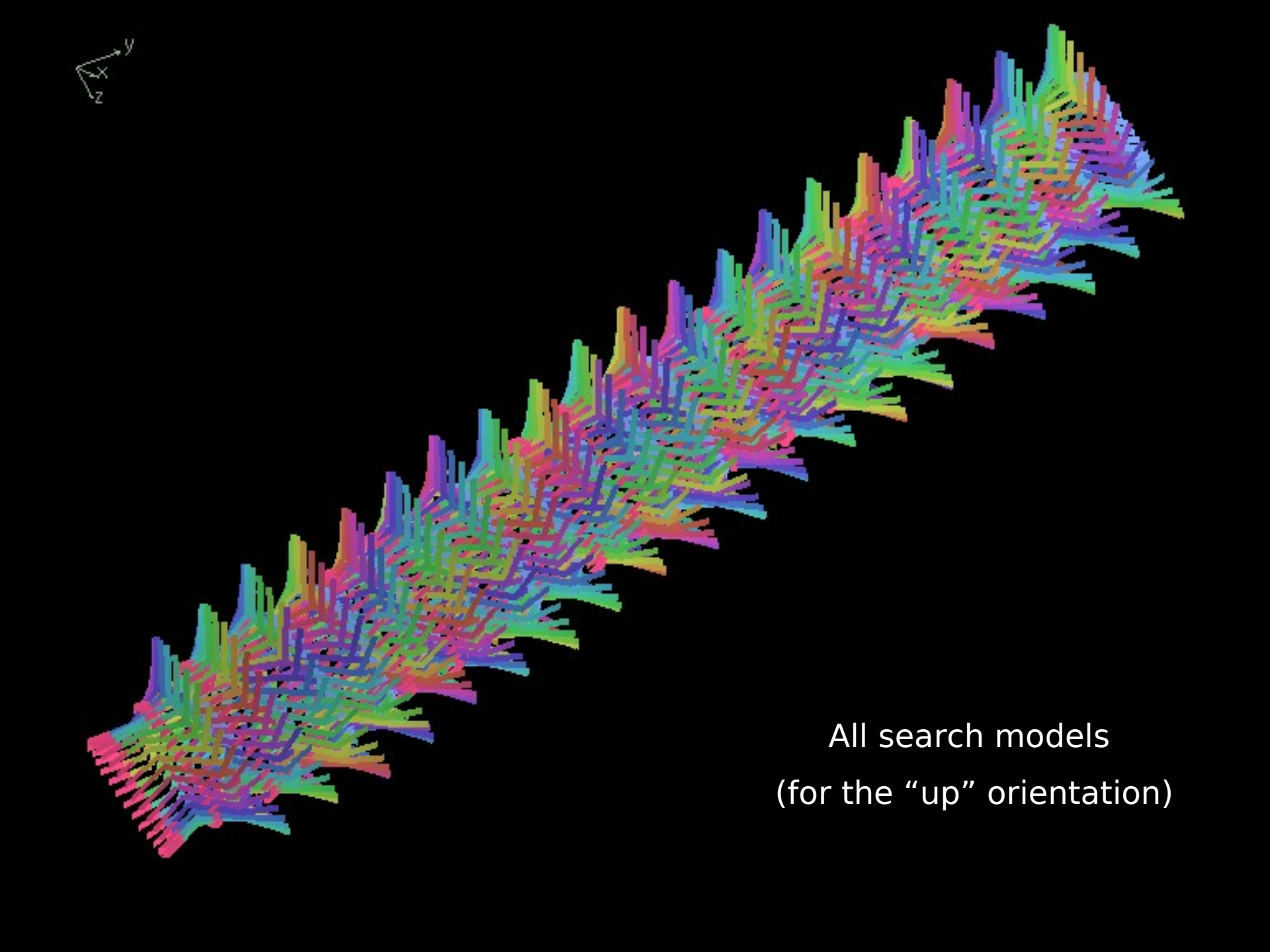
Pick the orientation that encapsulates the most electron density



2 orientation axes

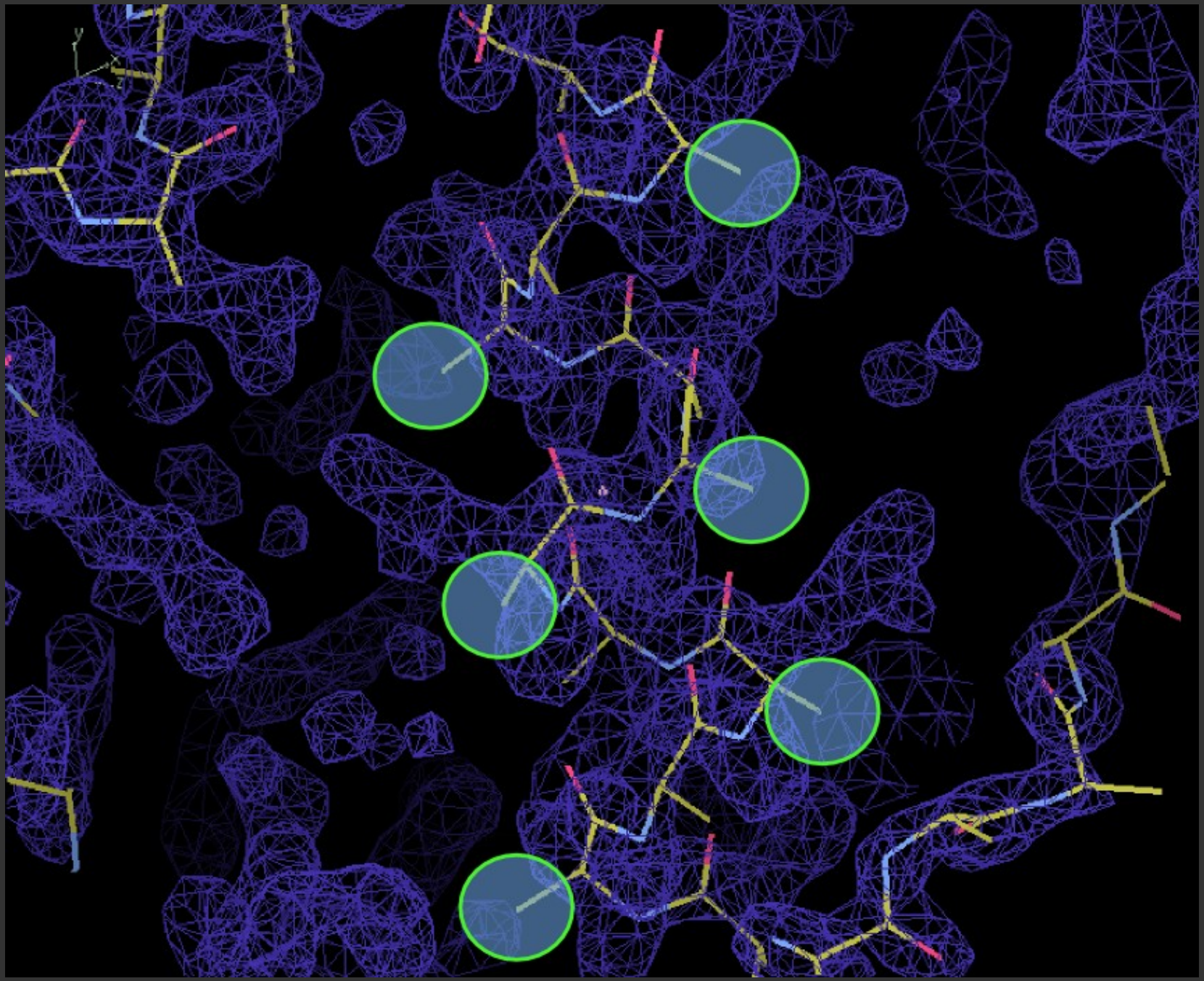
2 x 1-D Helix orientation searches

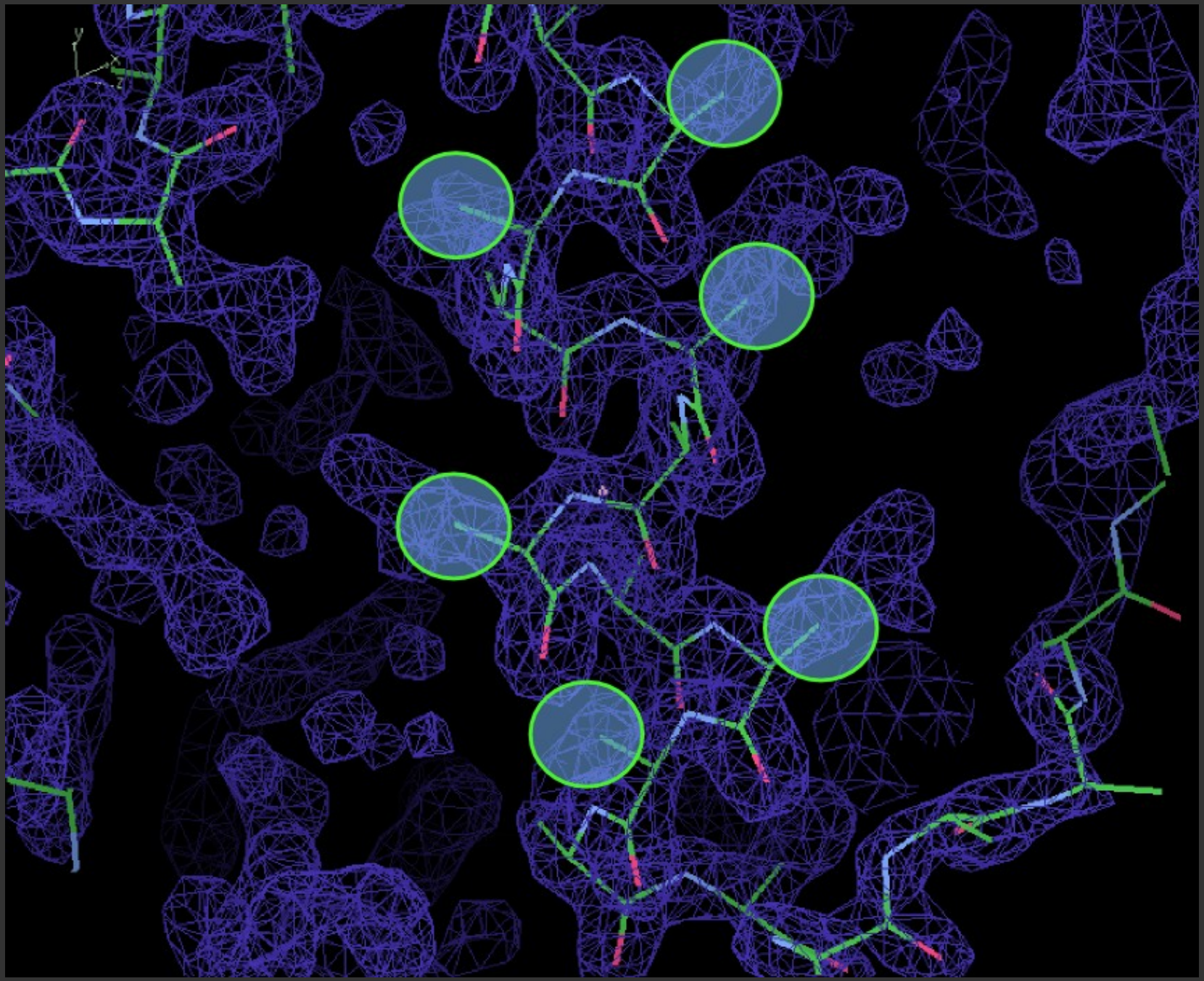

y
x
z



All search models

(for the “up” orientation)





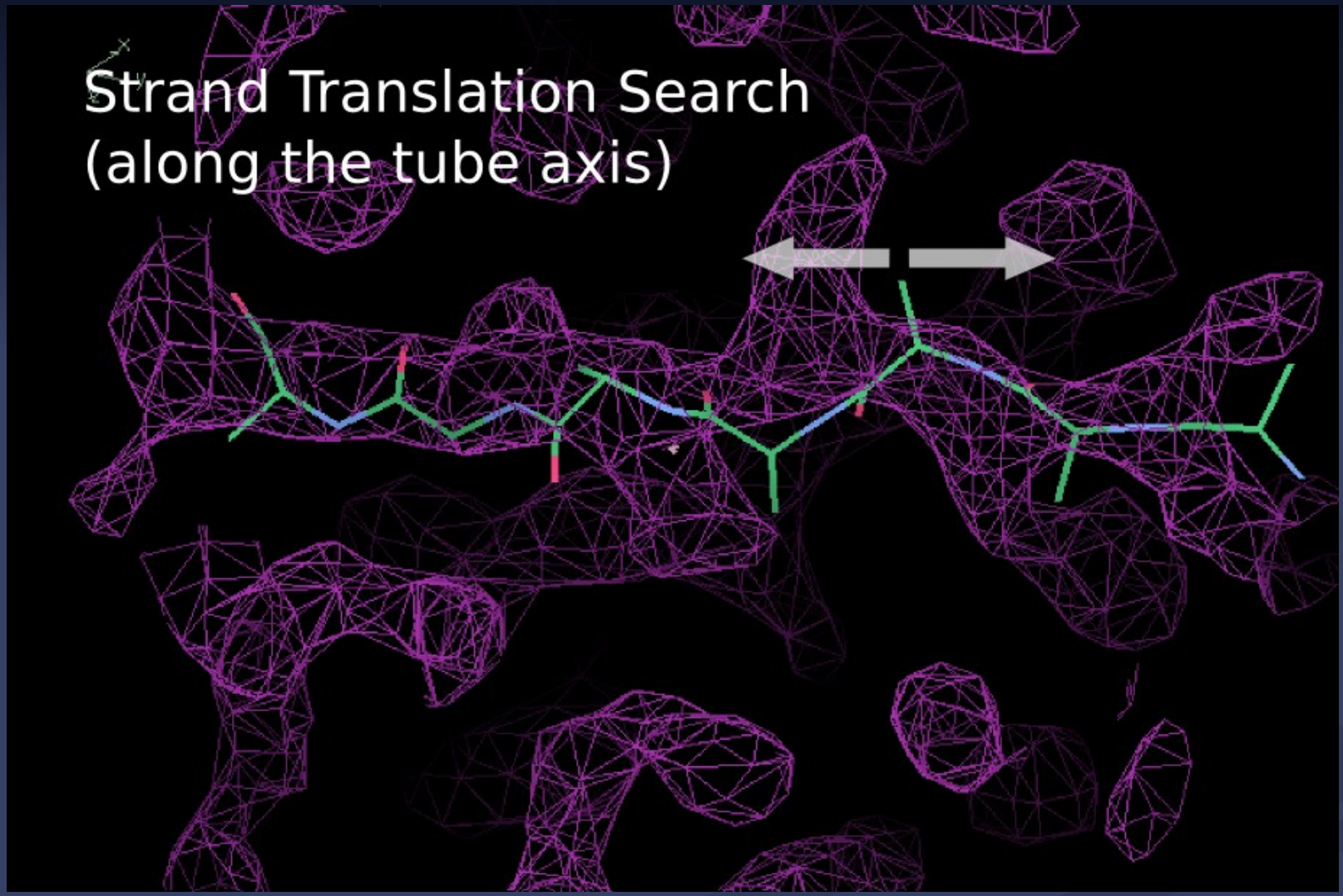
Fitting Strands

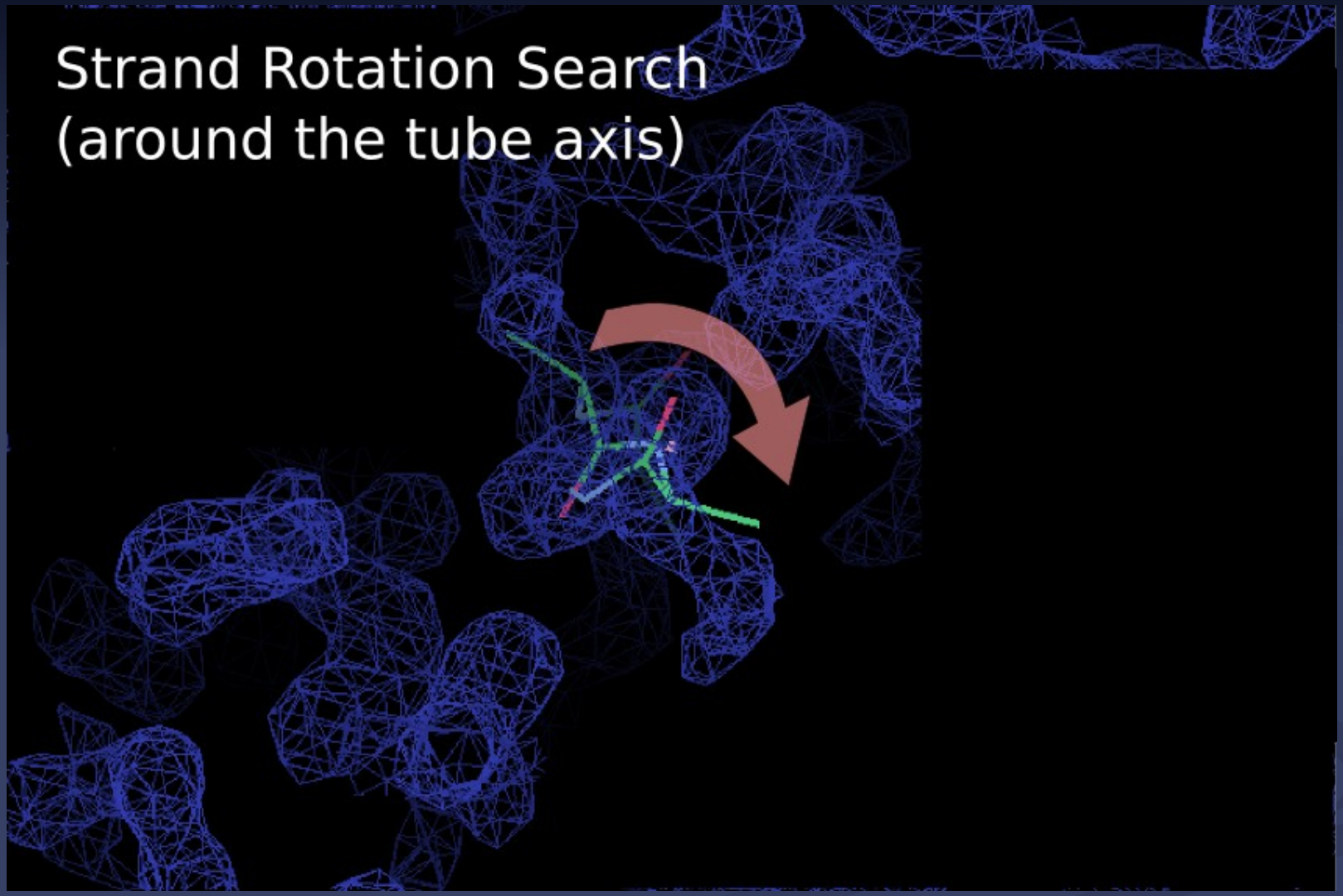
Placing Strands

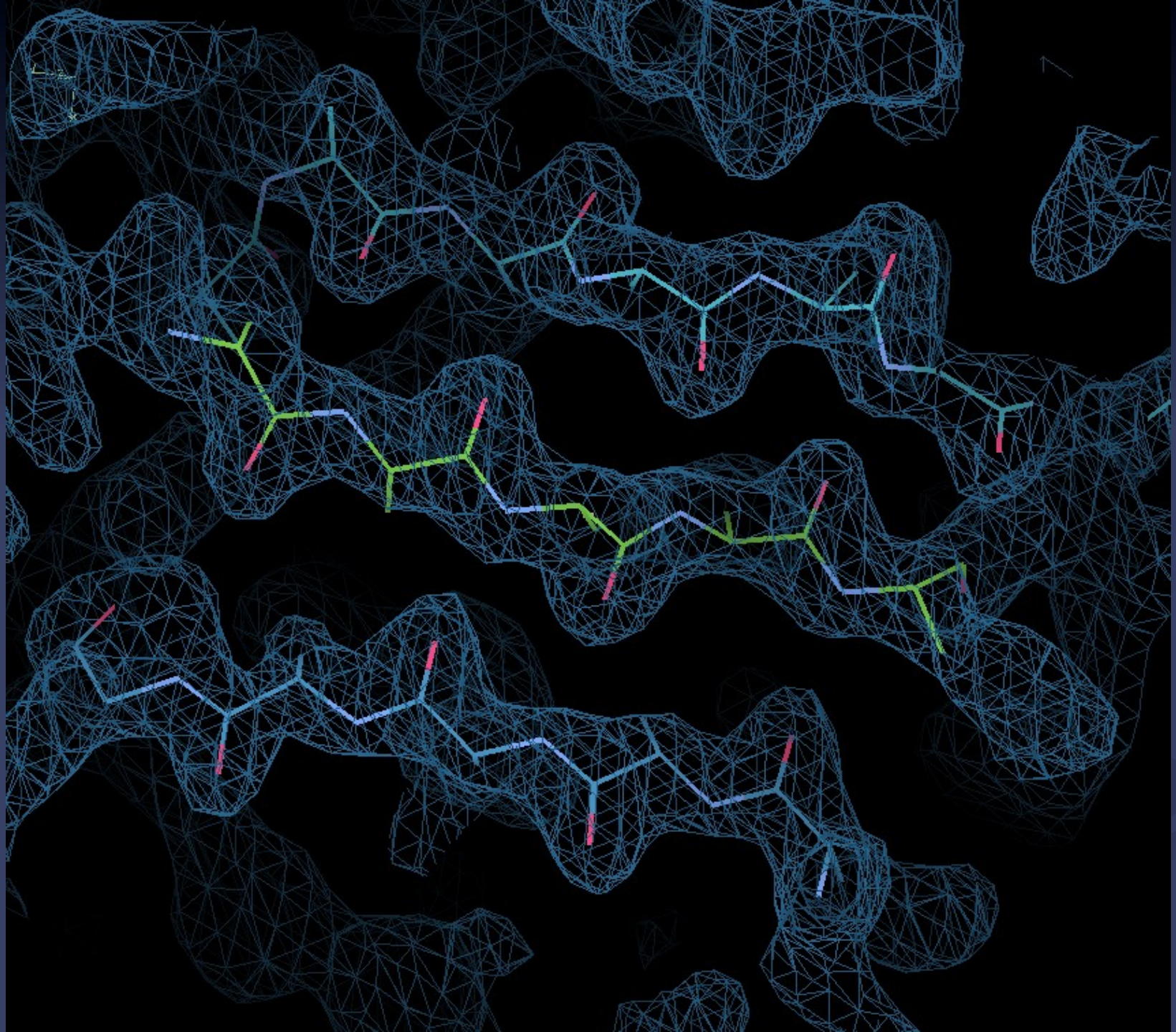
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

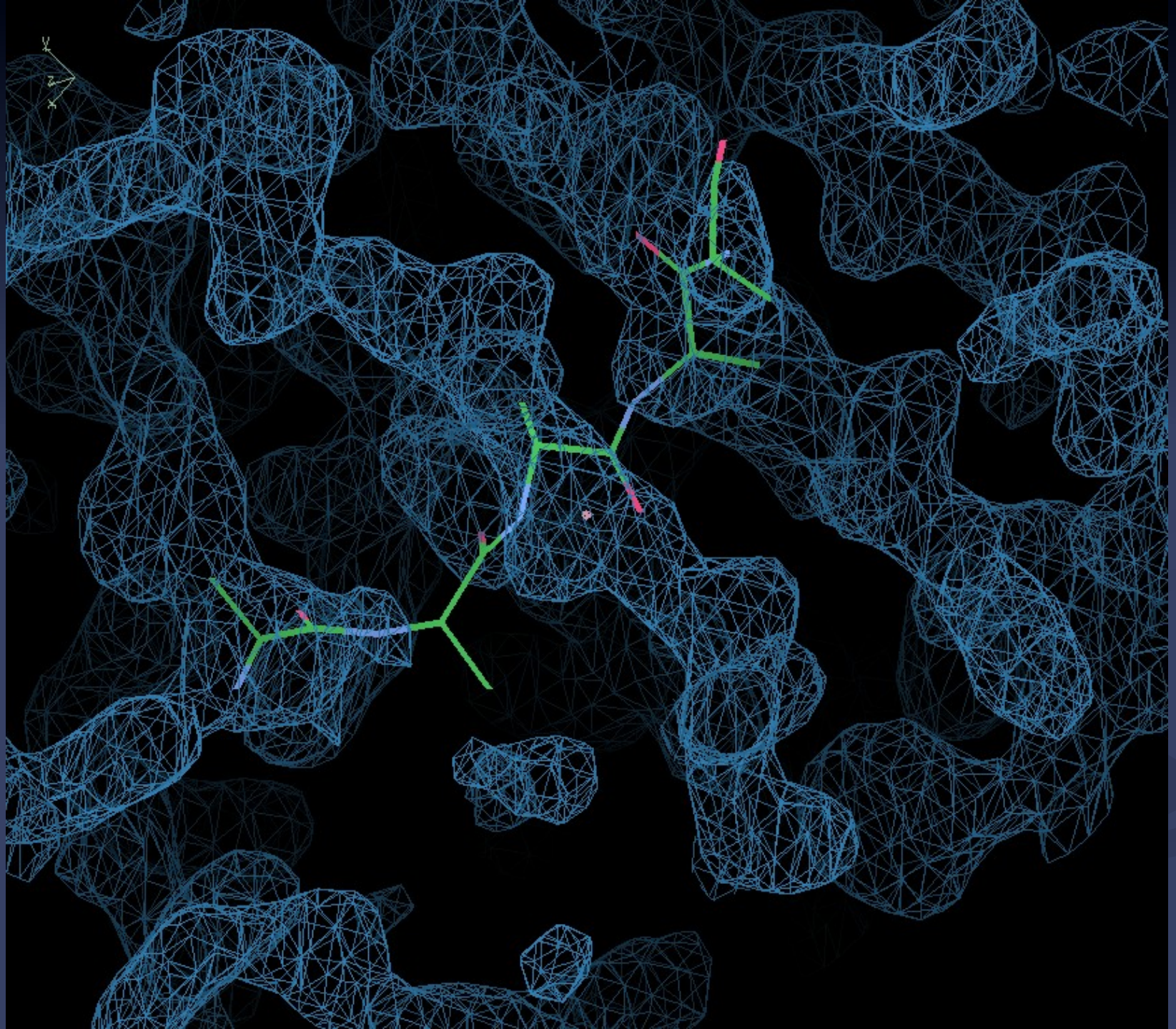
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution


Strand Translation Search
(along the tube axis)


Strand Rotation Search
(around the tube axis)



Not all is rosy...



Fitting Strands caveat

- In the case of strand-fitting, the initial translation search centring the cylinder is not performed (the search cylinder is too thin)
- The user is responsible for centring the search point “in the middle of the tube”
- Not at a C-alpha position

Handling NCS...

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

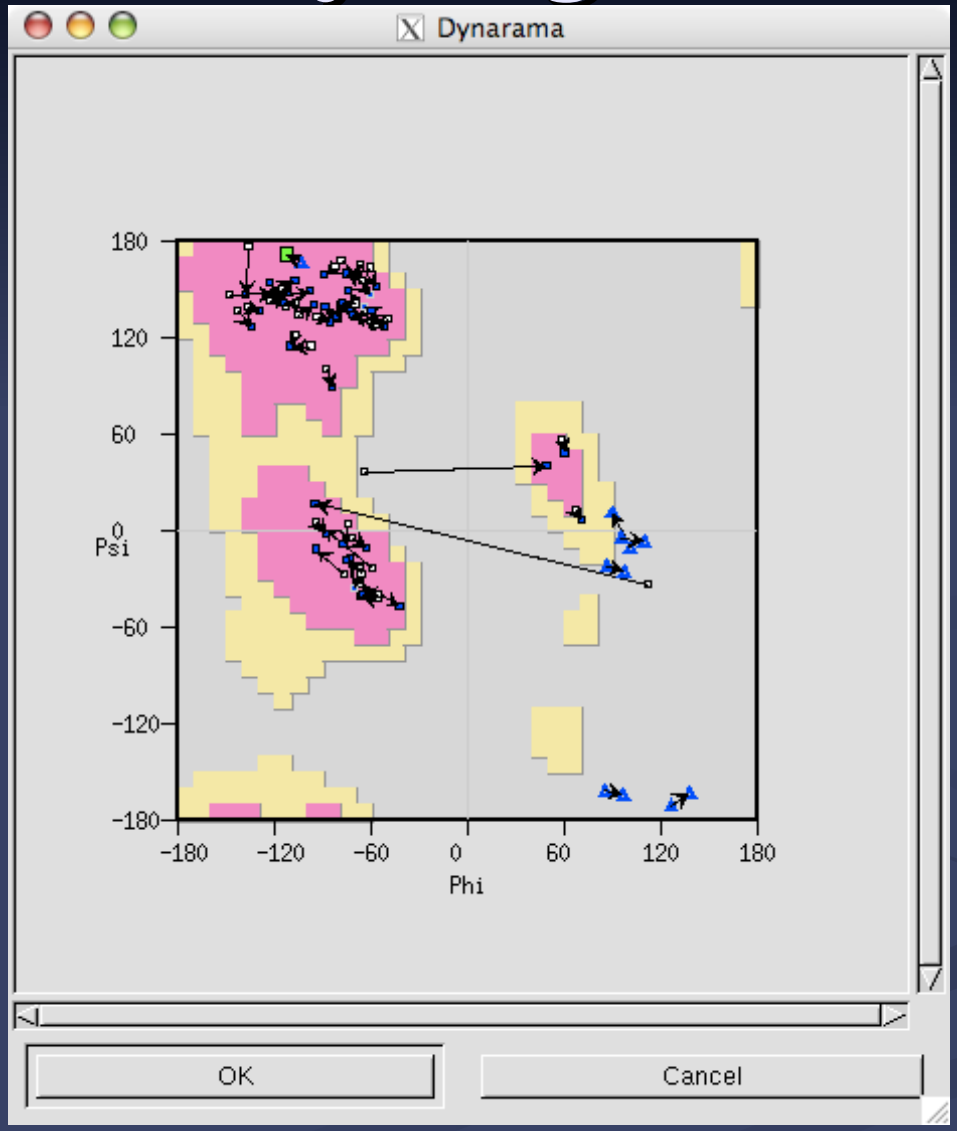
- What are the Problems?
- Strict NCS:
  - NCS should appear like crystallographic symmetry does [exact copies]
- Non-Strict NCS:
  - Molecules are different
  - How to cope with differences, but minimize unnecessary rebuilding?

Handling NCS

Typical Scenario:

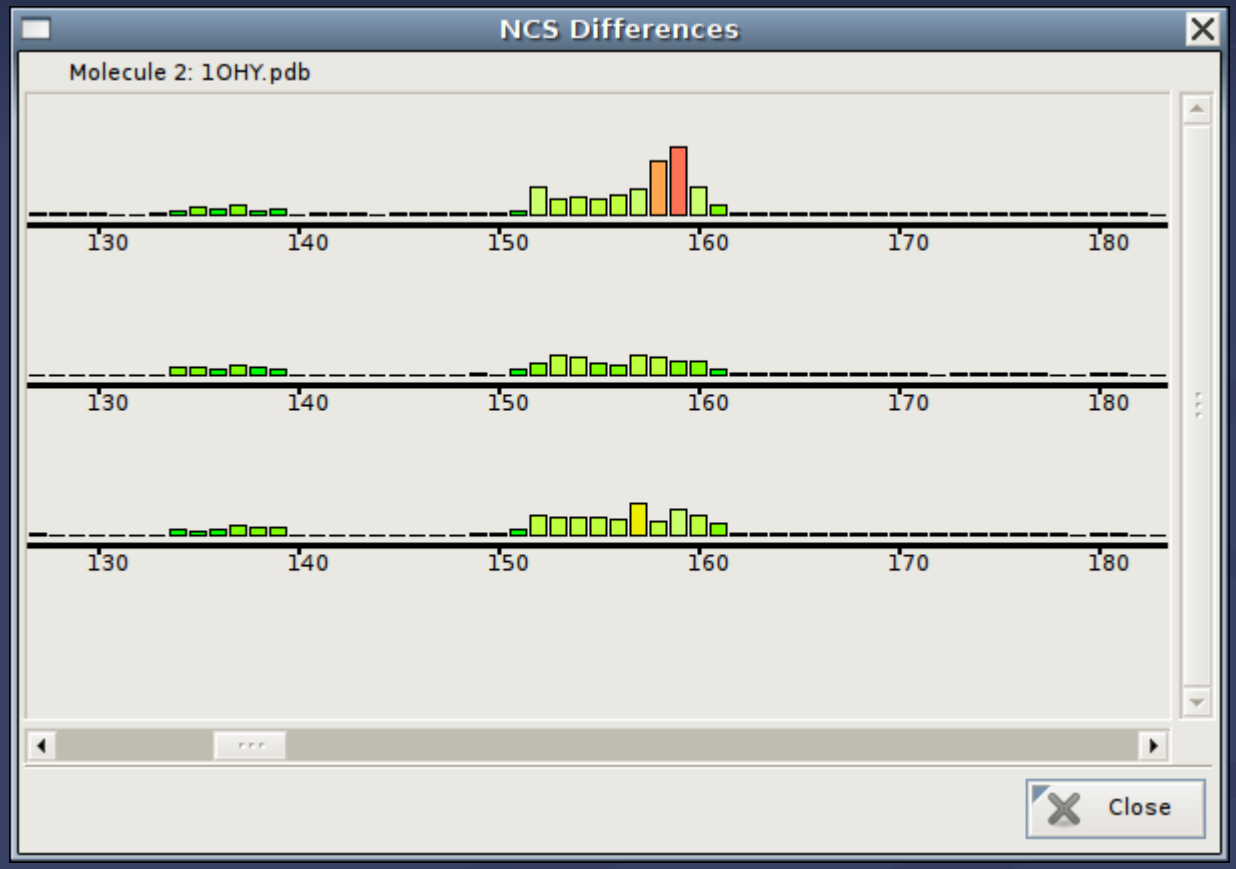
- I have done an LSQ overlap of my NCS-related molecules and from the graph, have seen significant deviations in the positions of some side-chains.
- Why are they different?

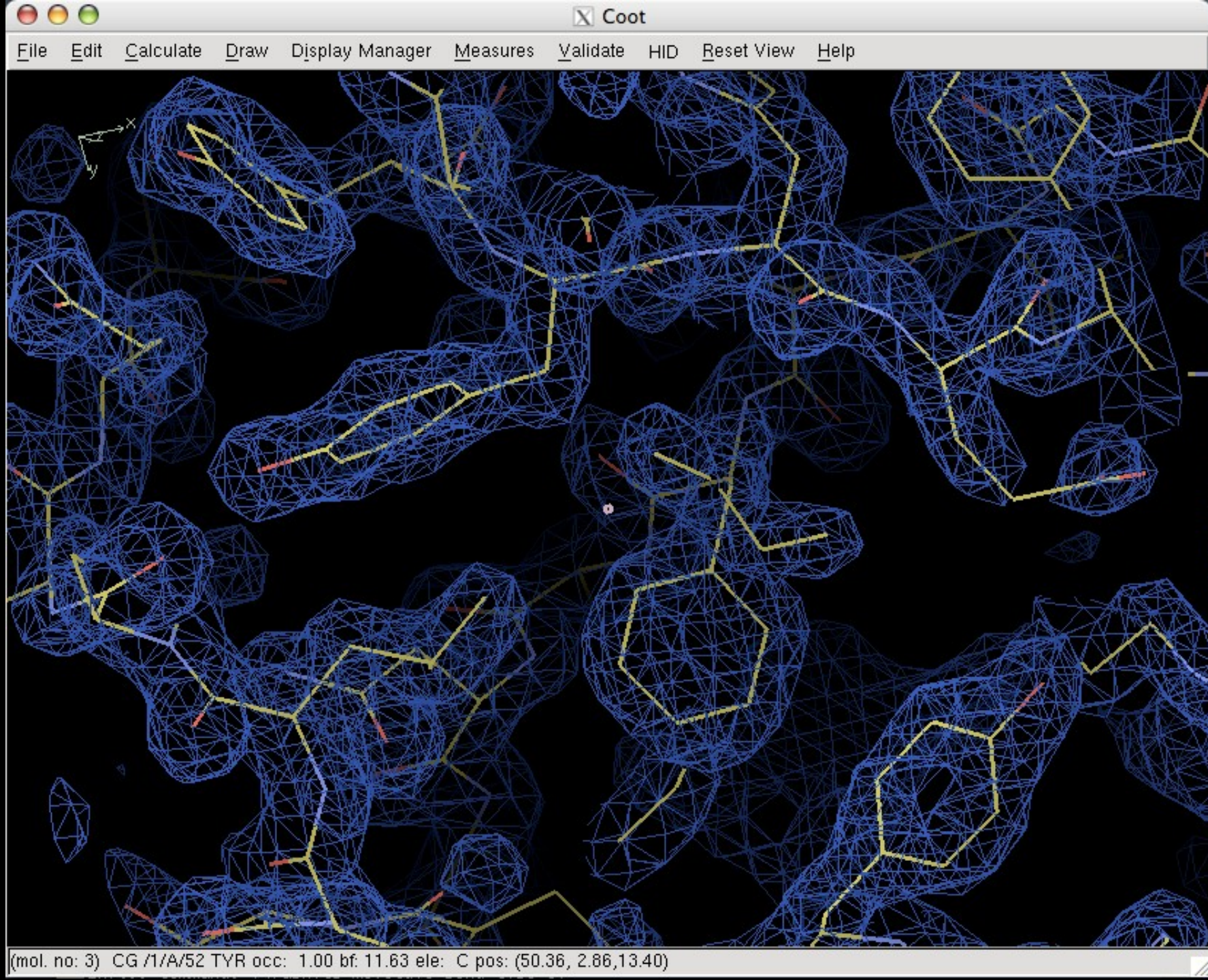
…or Kleywegt Plots[*]



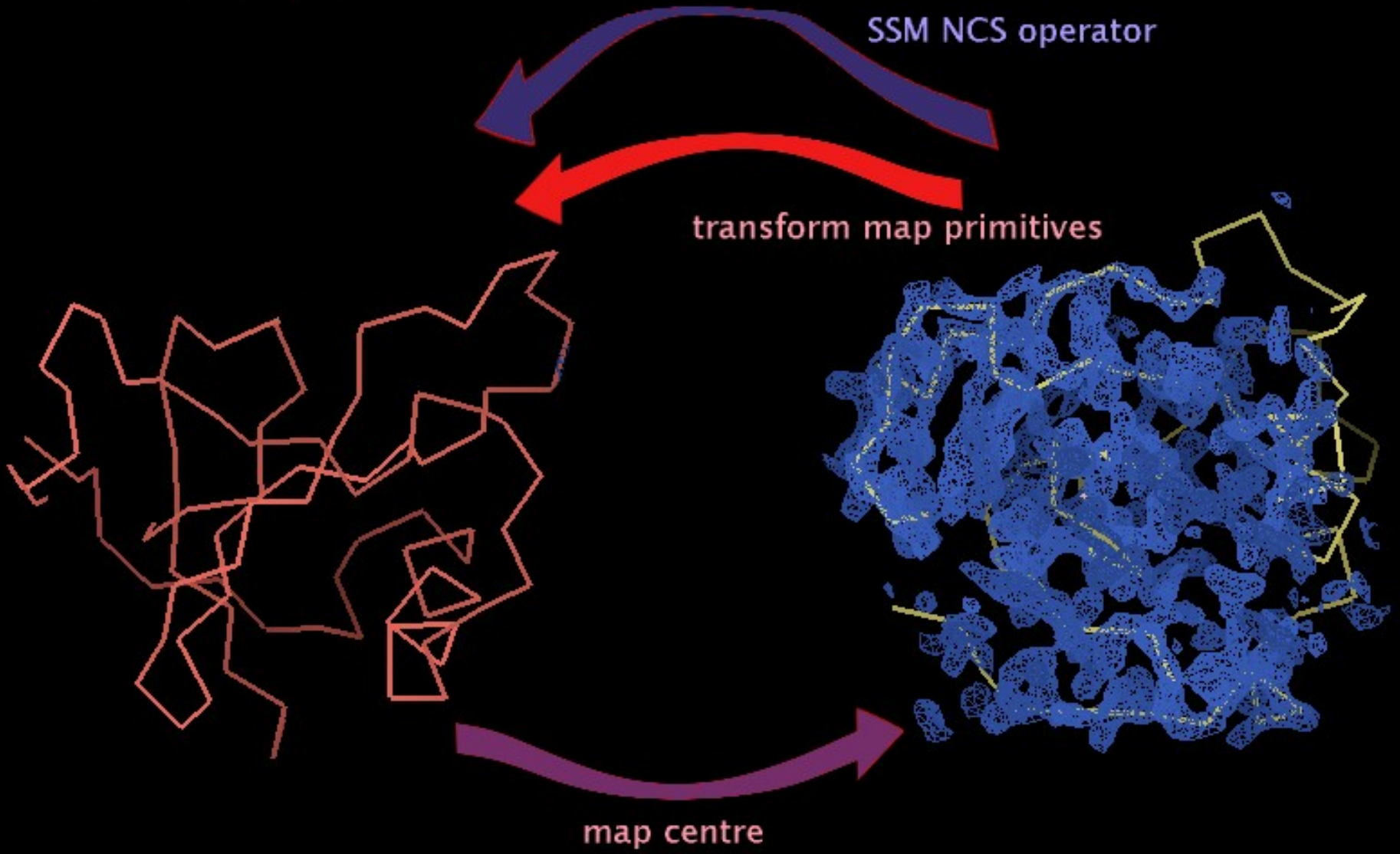
[*] Named by George Sheldrick

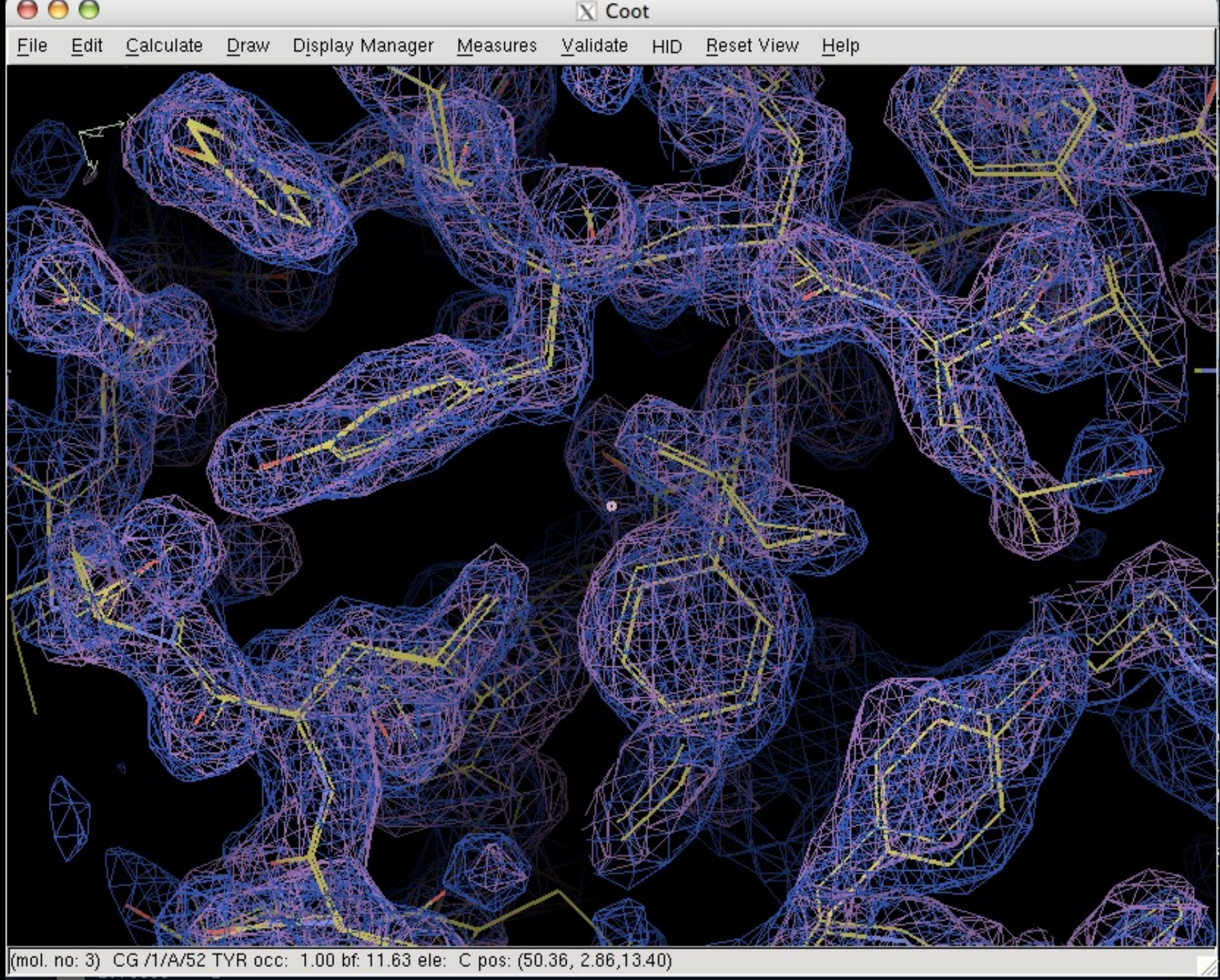
...or new NCS Differences graph




Coot
File
Edit
Calculate
Draw
Display Manager
Measures
Validate
HID
Reset View
Help
(mol. no: 3) CG /1/A/52 TYR occ: 1.00 bf: 11.63 ele: C pos: (50.36, 2.86,13.40)

NCS Overlays




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

Coot Futures…

- Aim:
  - Slick, easy to use
  - Powerful
  - Smooth interface to external applications
- Under Development
  - Interesting things move quickly
  - There may be bugs