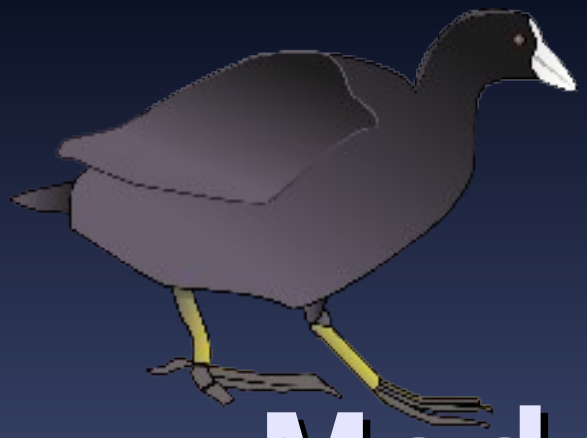

Common
Coot
(*Fulica atra*)

April 2008 Oulu



Model-Building with Coot

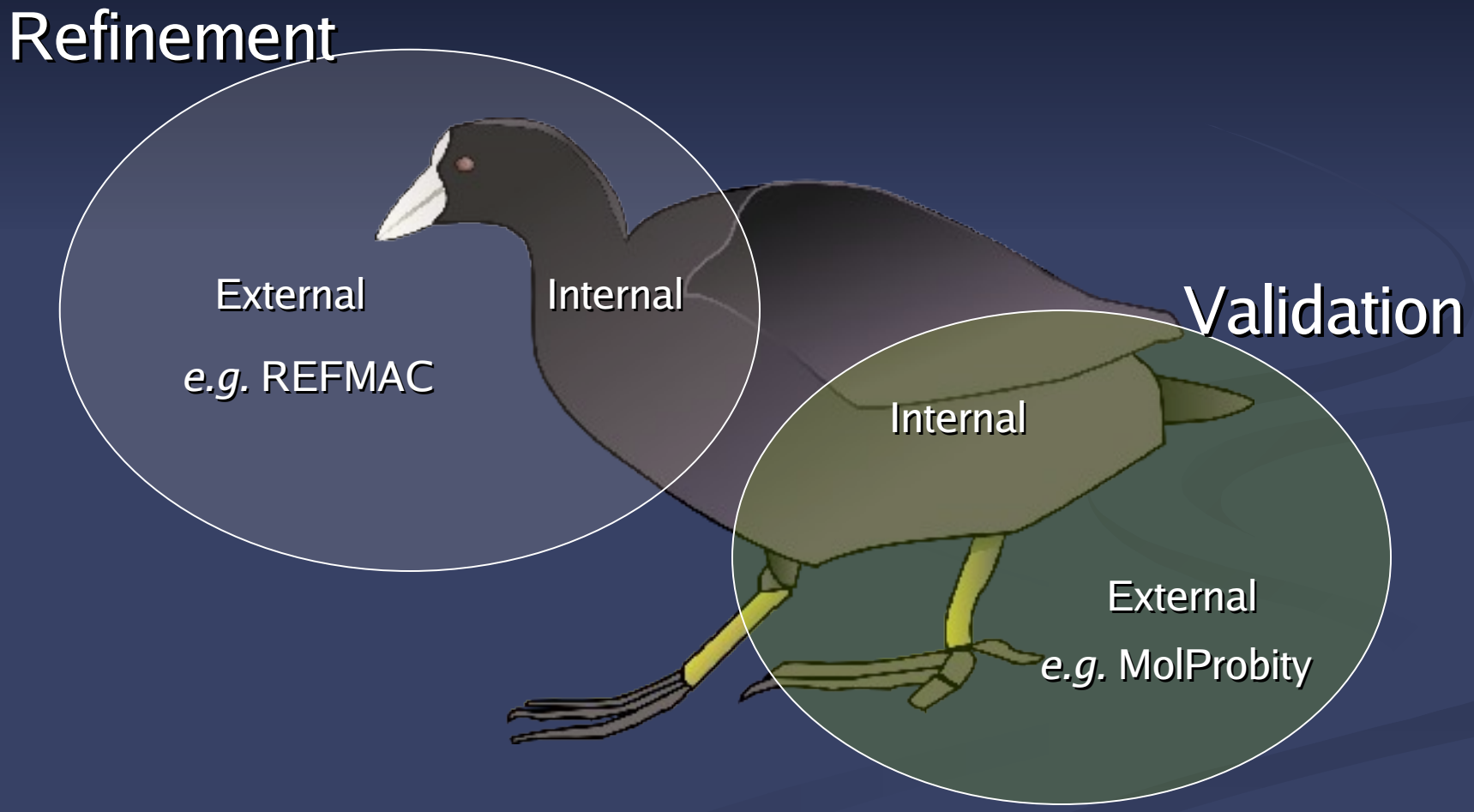
Ligands, NCS, Validation, Low Resolution Tools

Paul Emsley
University of Oxford

Coot

- Molecular Graphics application
  - Protein Crystallographic model-building tools
  - 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

Real Space Refinement (technical note)

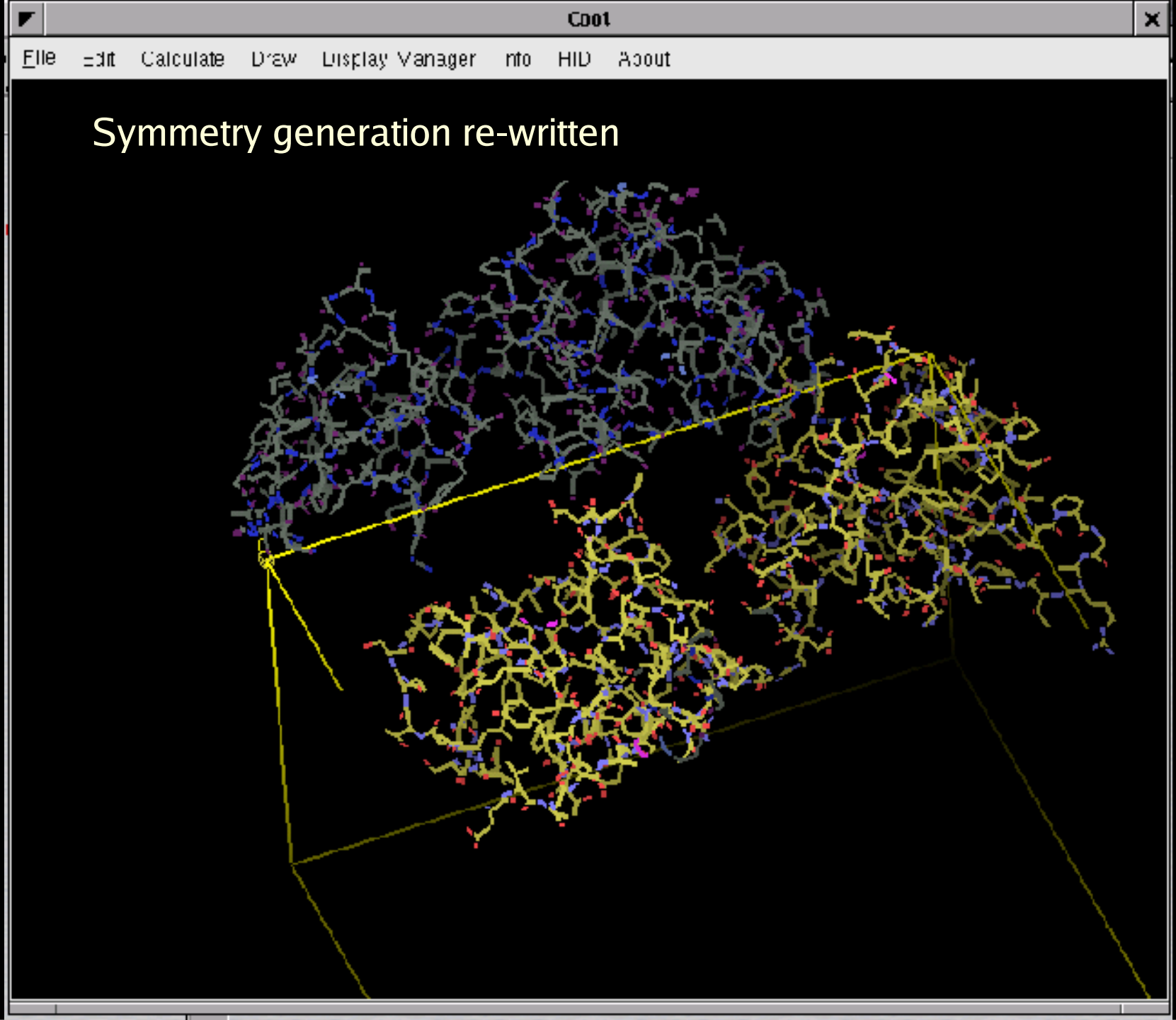
- Auto-Zone mechanism
  - The “A” key:
  - Typically have a range of 3 residues to refine, with the middle of the 3 at the centre of the screen
  - Click central atom and “A” key to start the refinement
  - No need to click 2 atoms away from centre

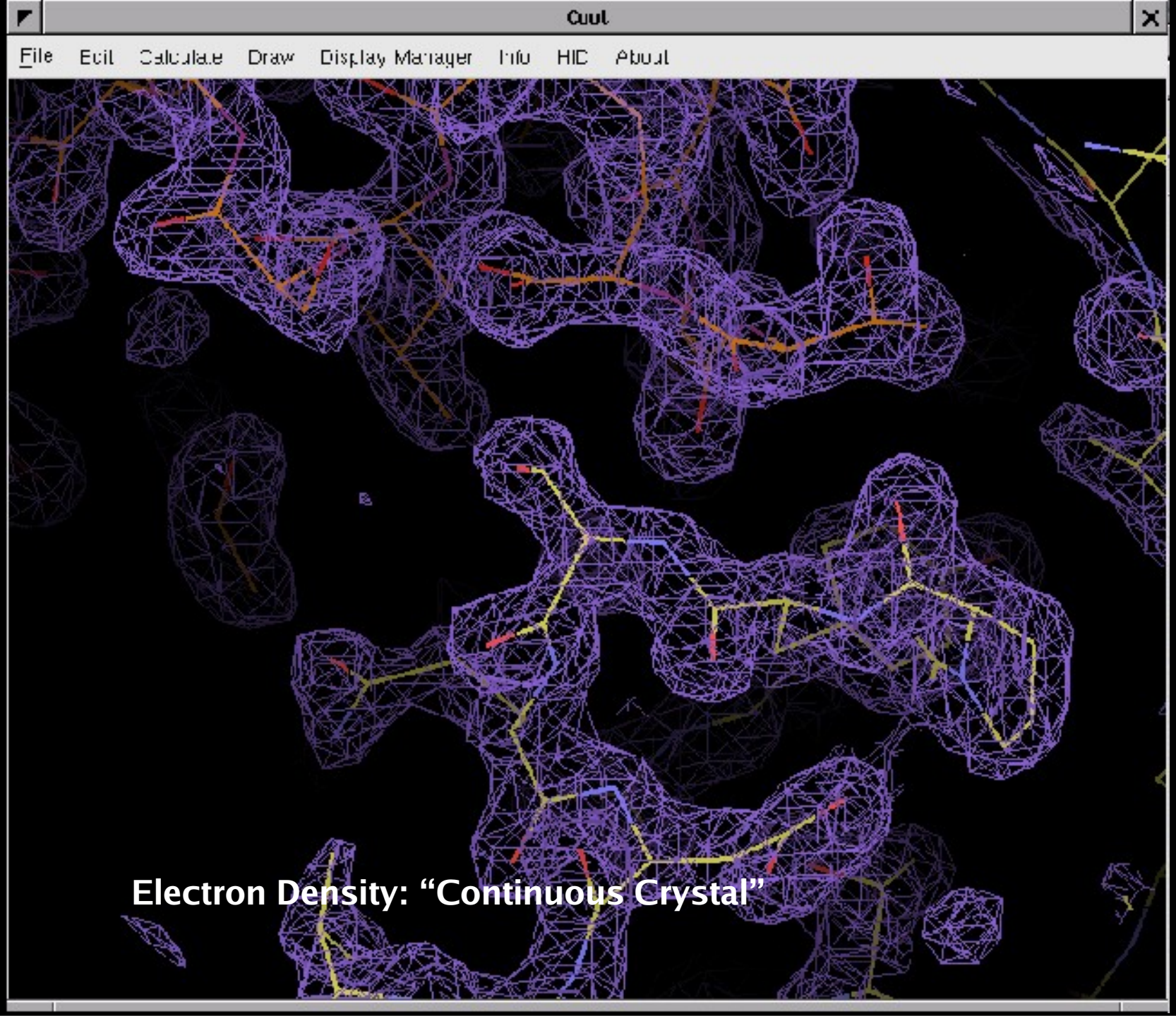
Generic Objects

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

Some more Coot Tools…

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

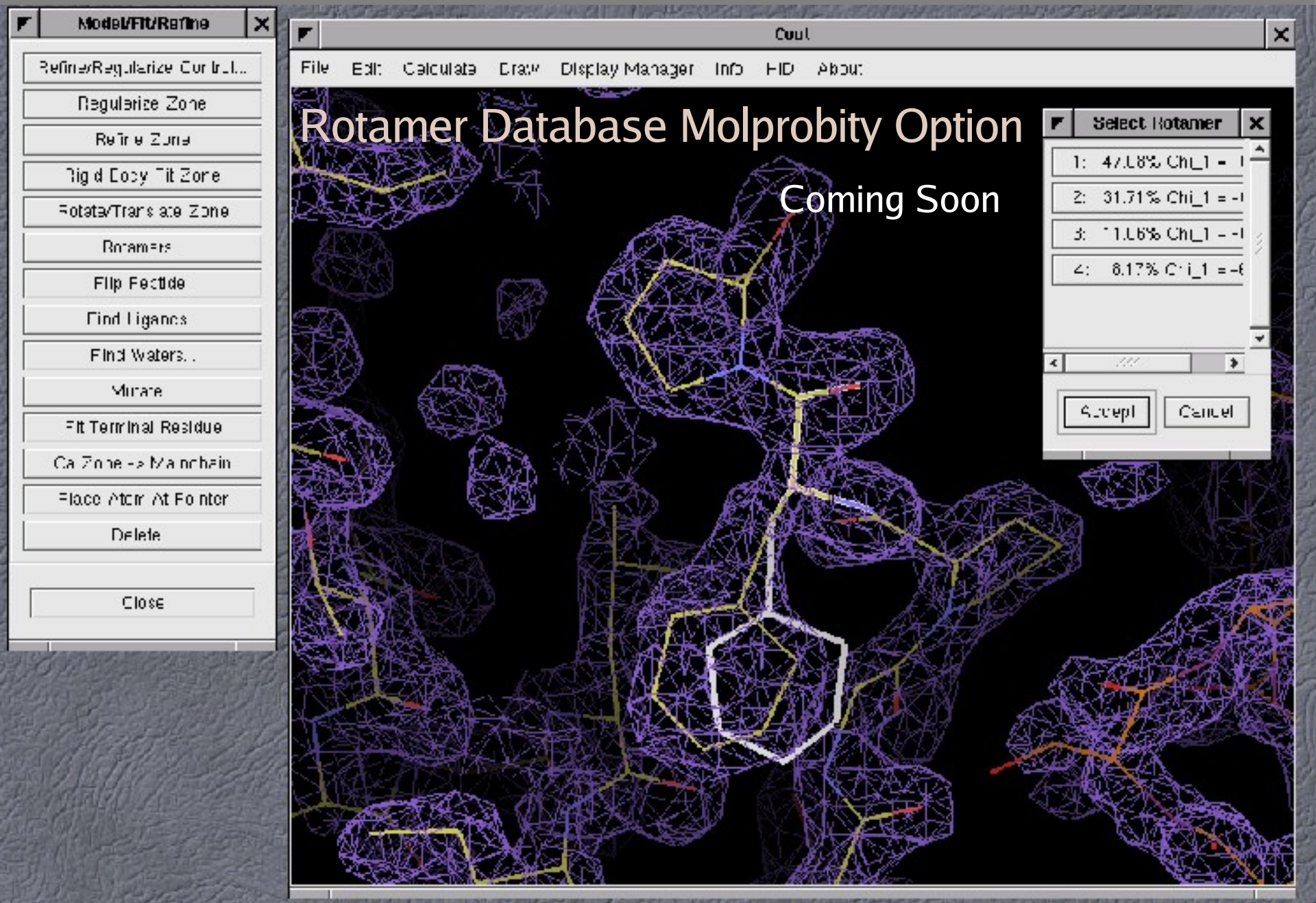

Coot
File Edit Calculate Draw Display Manager Info HID About
Symmetry generation re-written



Electron Density: “Continuous Crystal”

Rotamer Database Molprobity Option

Coming Soon

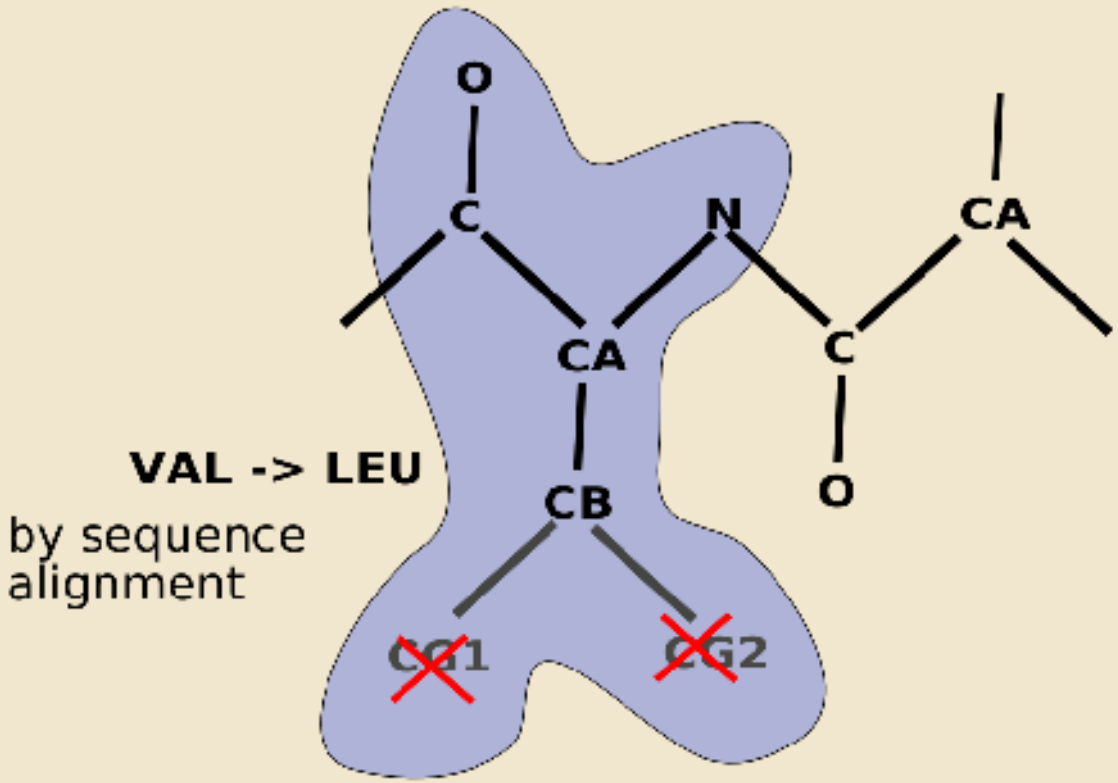


Other Tools

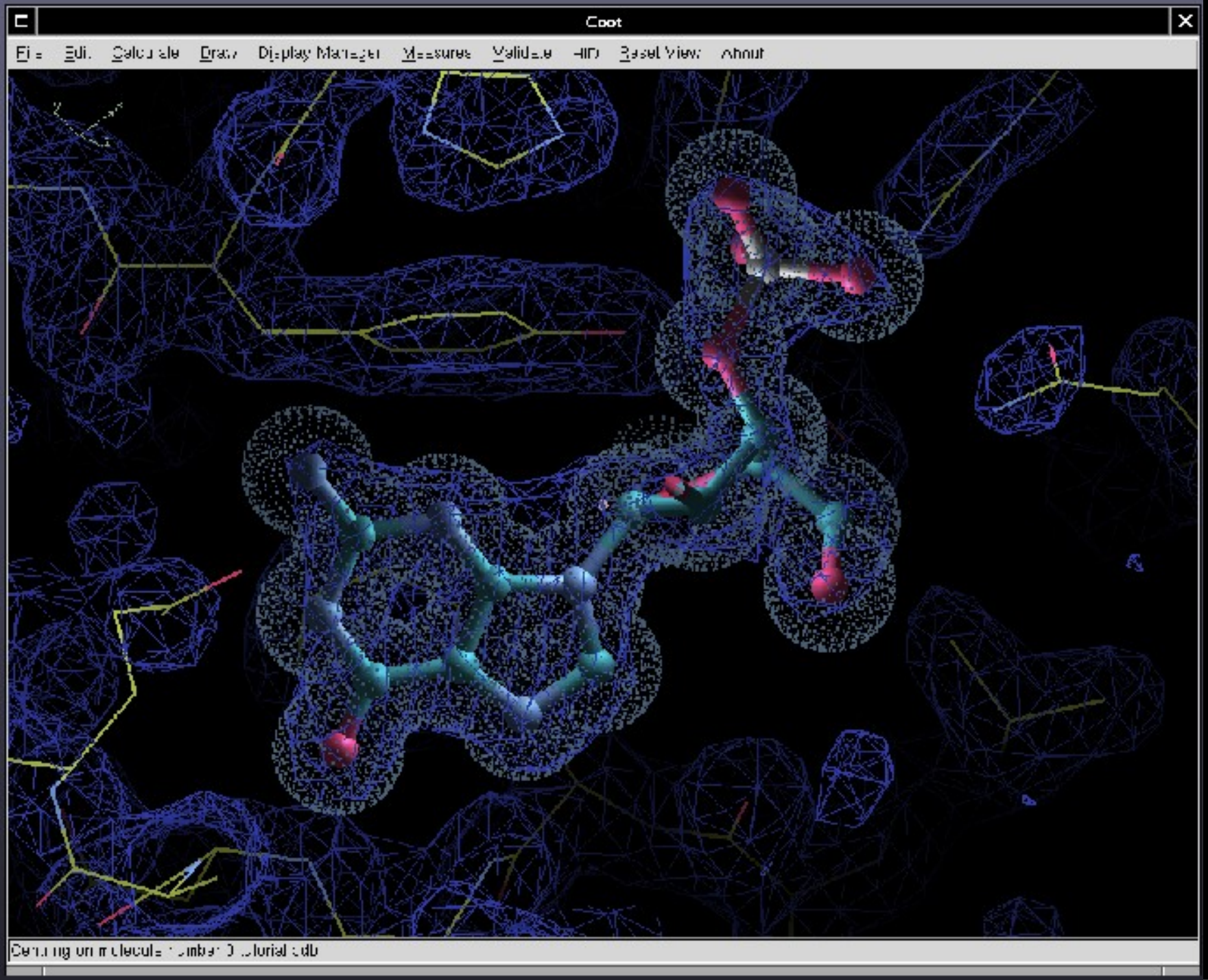
- Reverse chain direction
- 180° side-chain flip
- Planar peptide restraints
- “Chi” angles for ligands
- Dots, ball&stick
- Fill-partial-residues (`de-chainsaw`)

Chainsawing

Model-trimming



Schwartzenbacker et al.
(2004) Acta Cryst D60 1229


Coot
File
Edit
Calculate
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Measures
Validate
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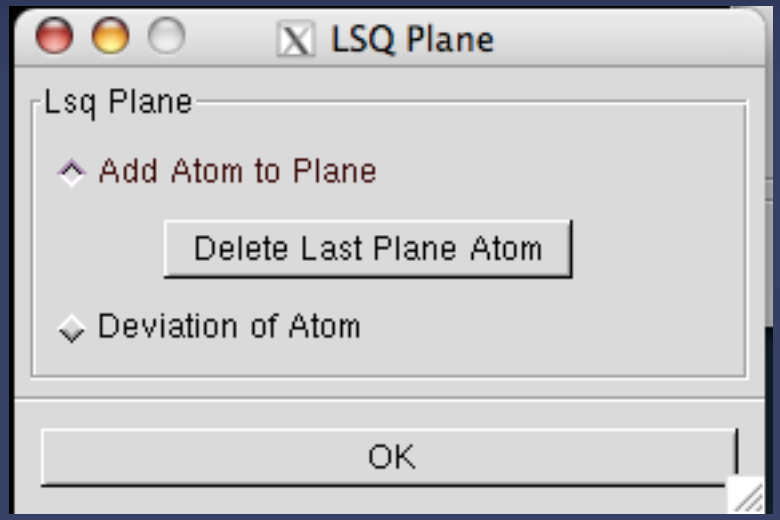
Reset View
About

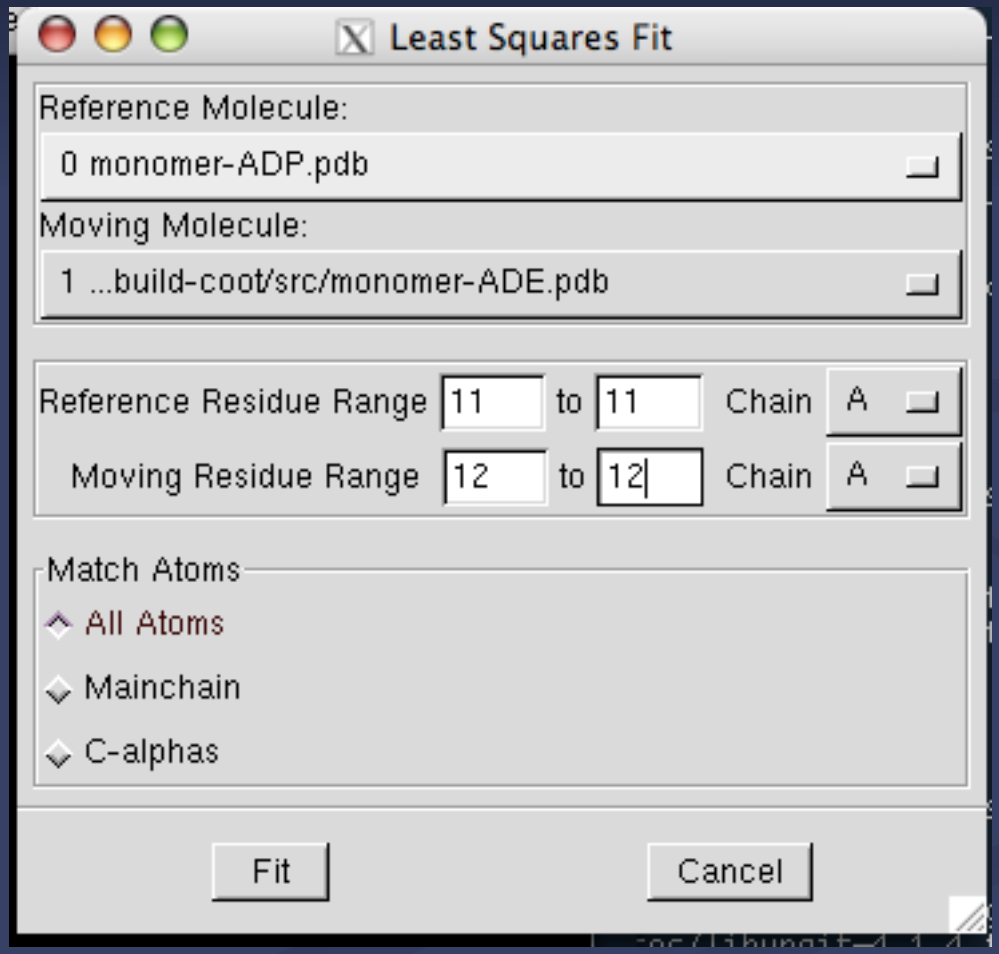
Least Squares Fitting

- Least Squares Plane deviation:



Least Squares Fitting

- Least Squares Superposition:



Scripting

- Python or scheme
- 100s of functions are scriptable
- Accessed via:
  - the command line: **`--script`**
  - the GUI: Calculate -> Run Script...
  - Interactive: Calculate -> Scripting
- Use **`- no-graphics`** for “batch mode”

SSM Overlay by Scripting

- (superpose-with-atom-selection *imol1 mol2 atom-selection-string-1 atom-selection-string-2 move-copy-flag*)
- e.g. `(superpose-with-atom-selection 0 1 "//A/20-120" "//B/30-130" 0)`

Fitting Ligands

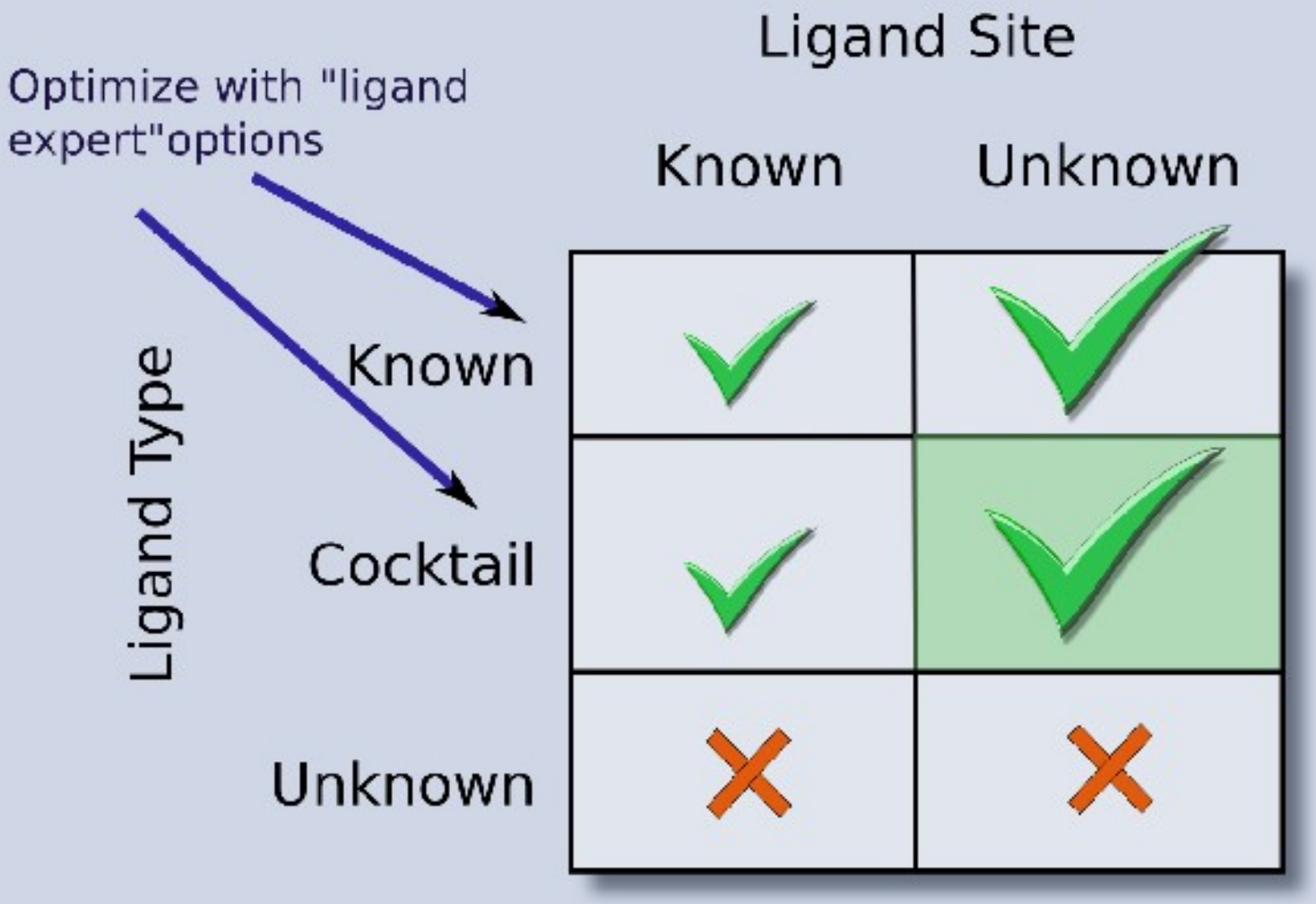
REFMAC Monomer Library chem_comp_bond

```
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      CB   HB1     single     0.960    0.020
 ALA      CB   HB2     single     0.960    0.020
 ALA      CB   HB3     single     0.960    0.020
 ALA      CA   C       single     1.525    0.021
 ALA      C    O       double     1.231    0.020
```

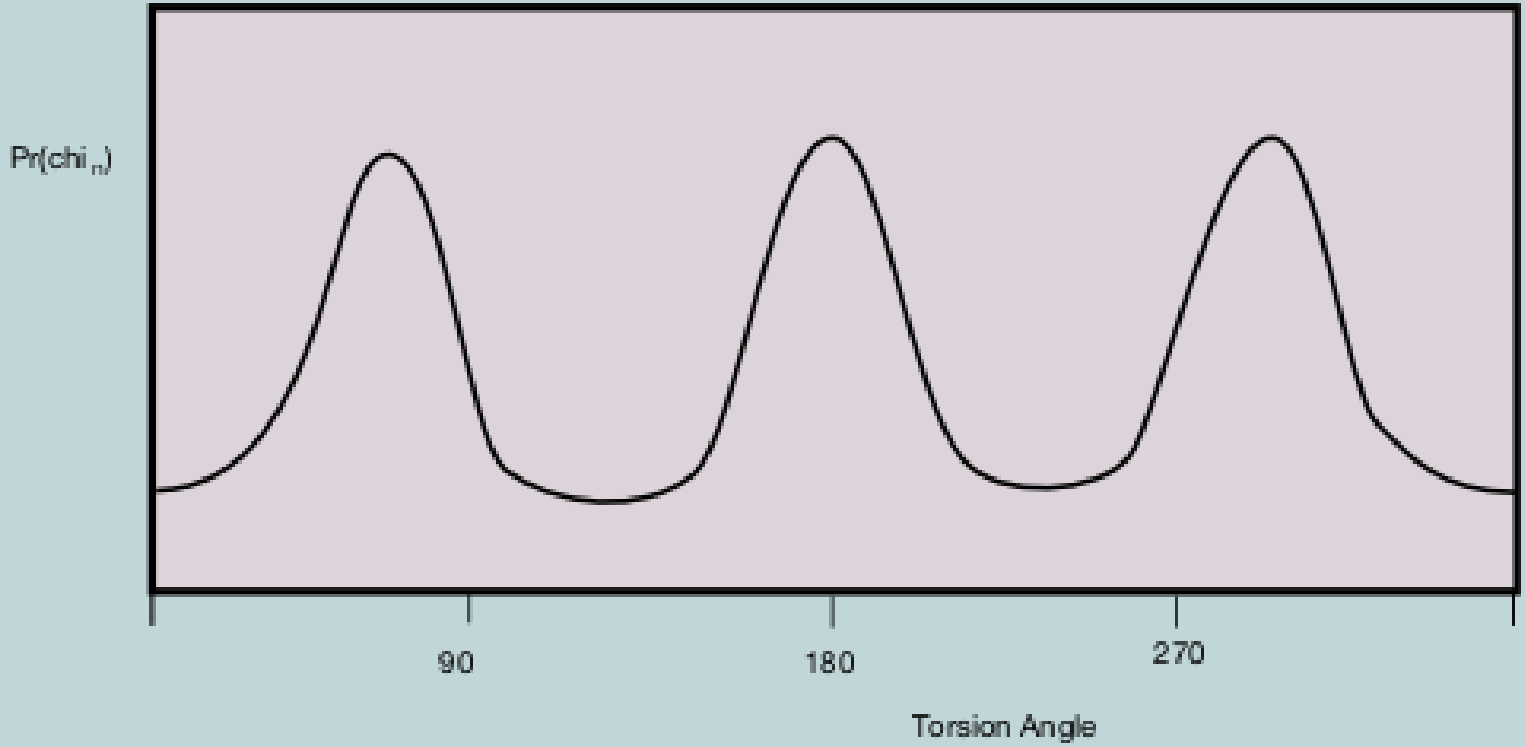
Ligand Fitting

- *c.f.* Oldfield (2001) *Acta Cryst. D* X-LIGAND
- Somewhat different torsion search algorithm
- Build in crystal-space

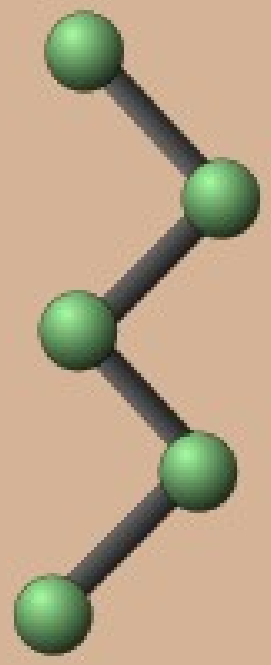
Ligand Type \ Ligand Site	Known	Unknown
Known	✓	✓
Cocktail	✓	✓
Unknown	✗	✗


Ligand Site
Optimize with "ligand expert"options
Known
Unknown
Ligand Type
Known
Cocktail
Unknown

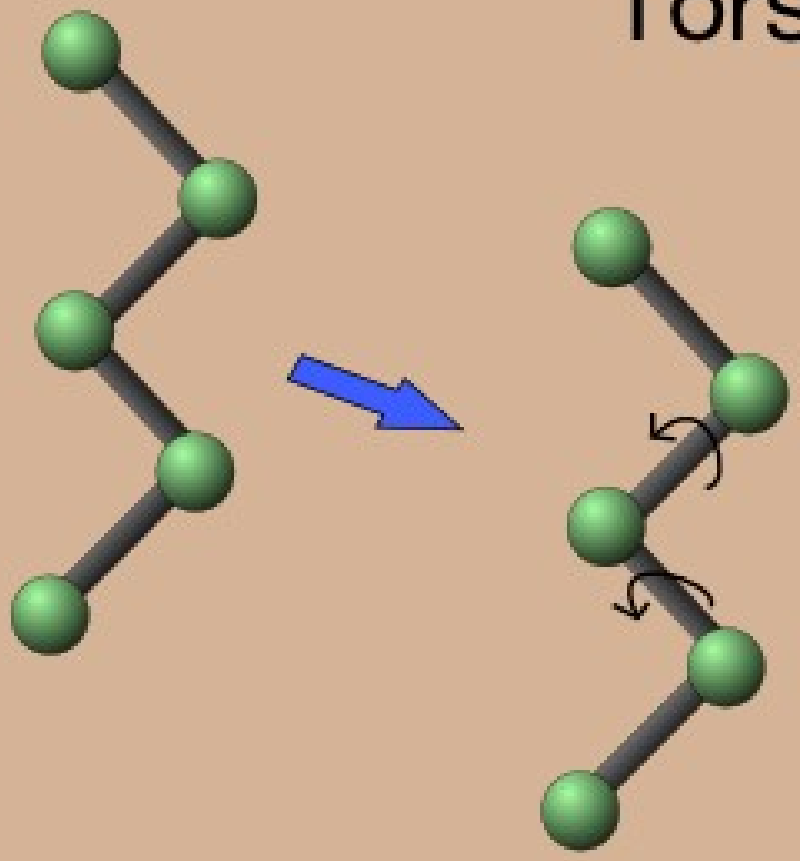
Ligand Torsionable Angle Probability from CIF file



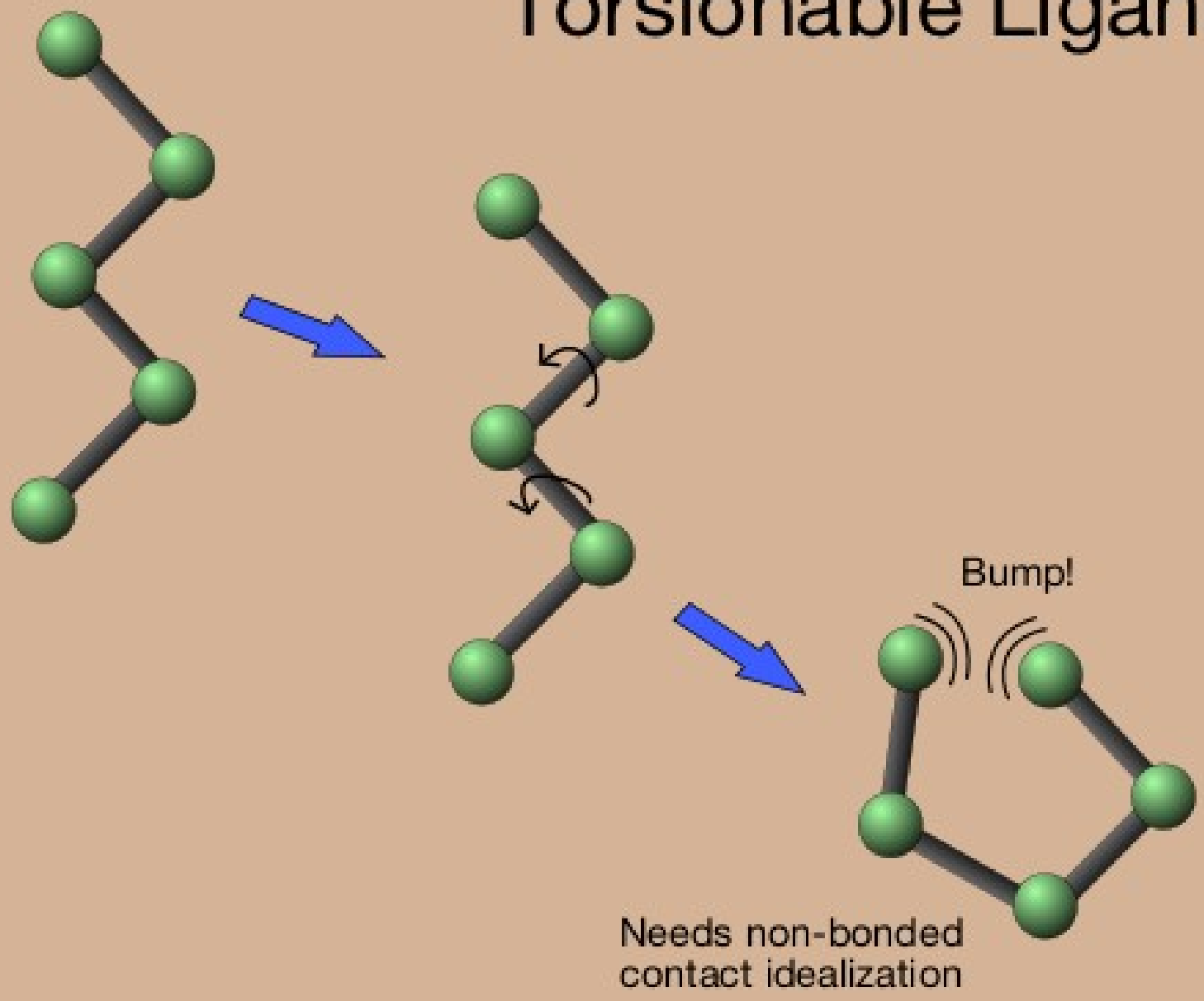
Torsionable Ligands



Torsionable Ligands

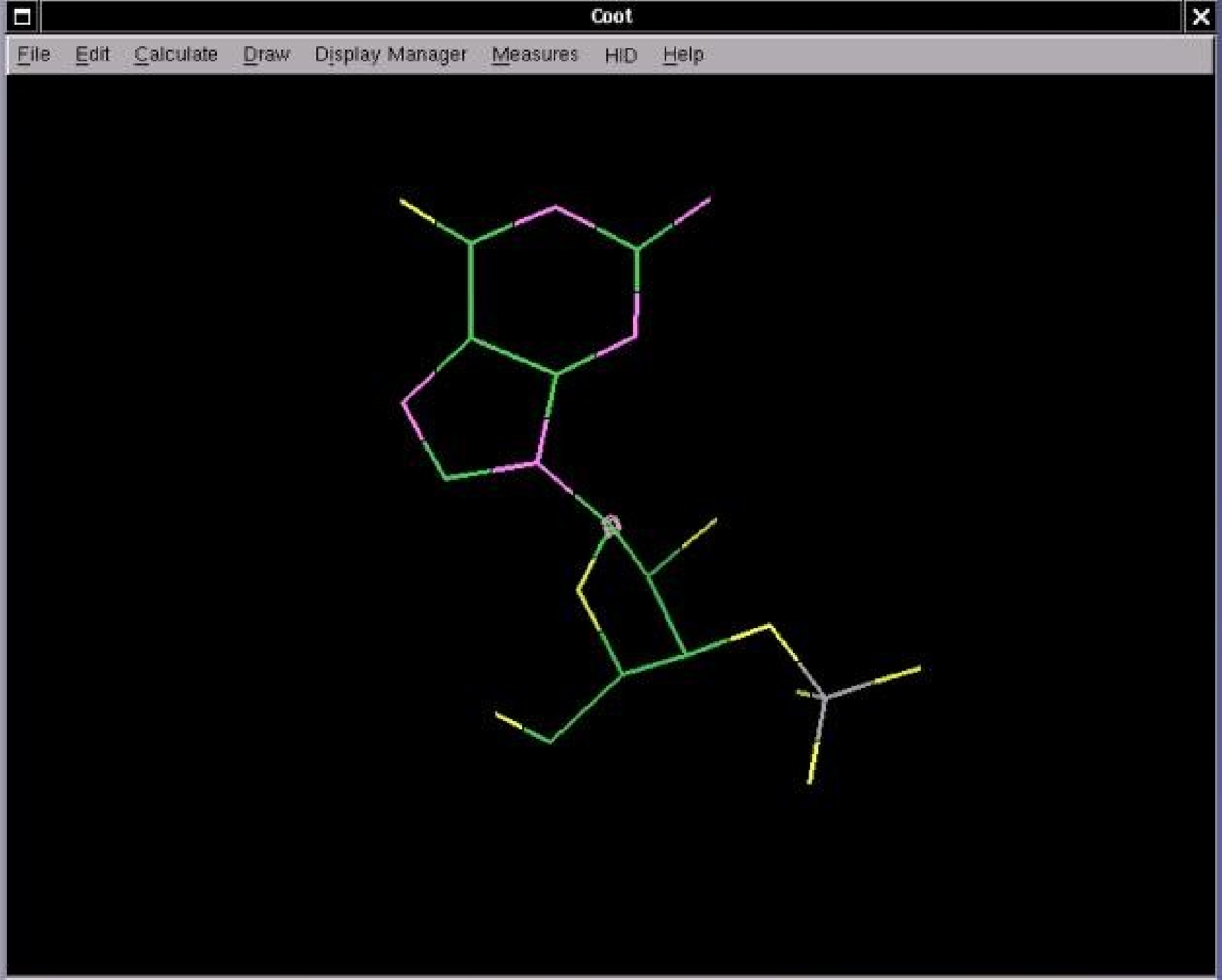


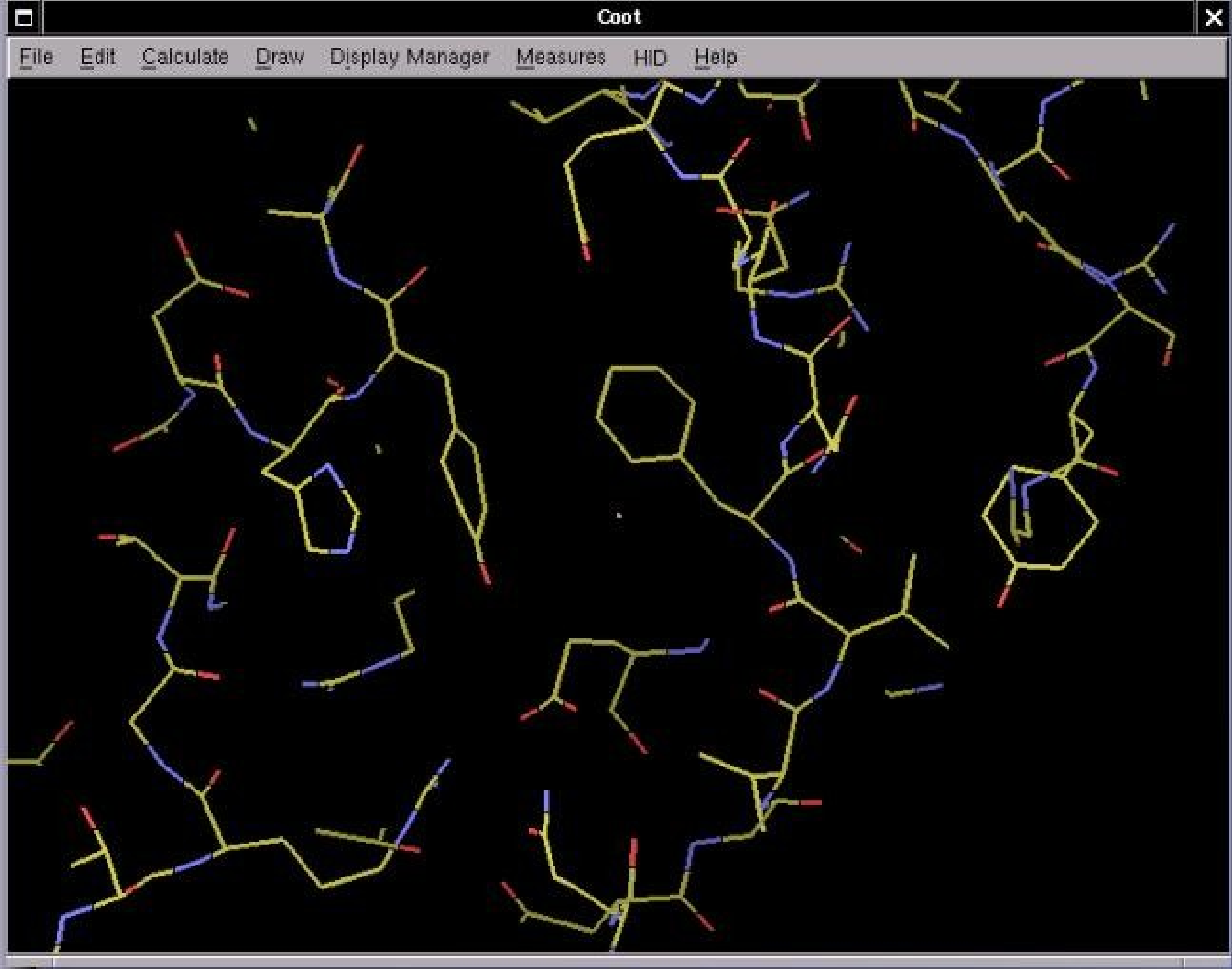
Torsionable Ligands

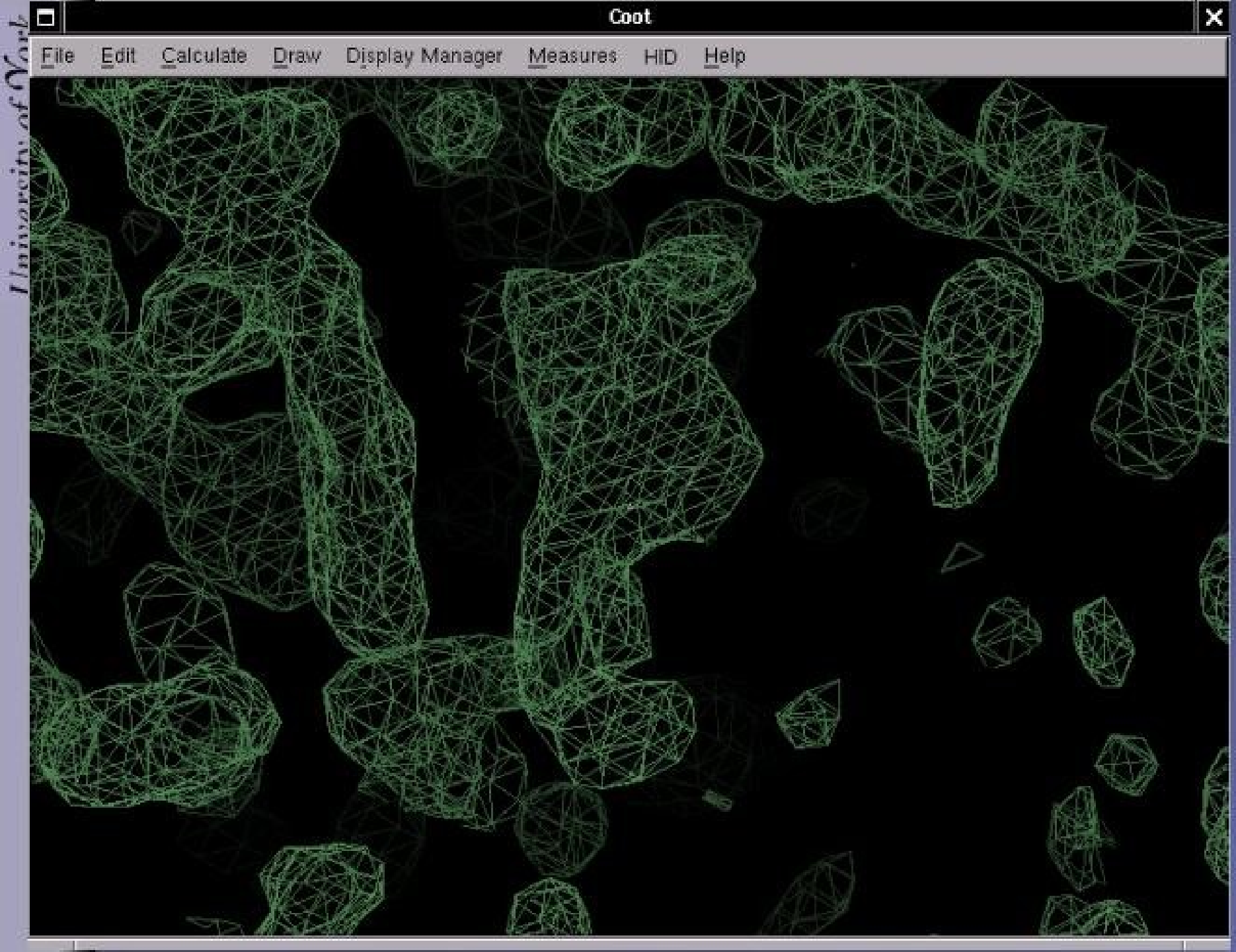


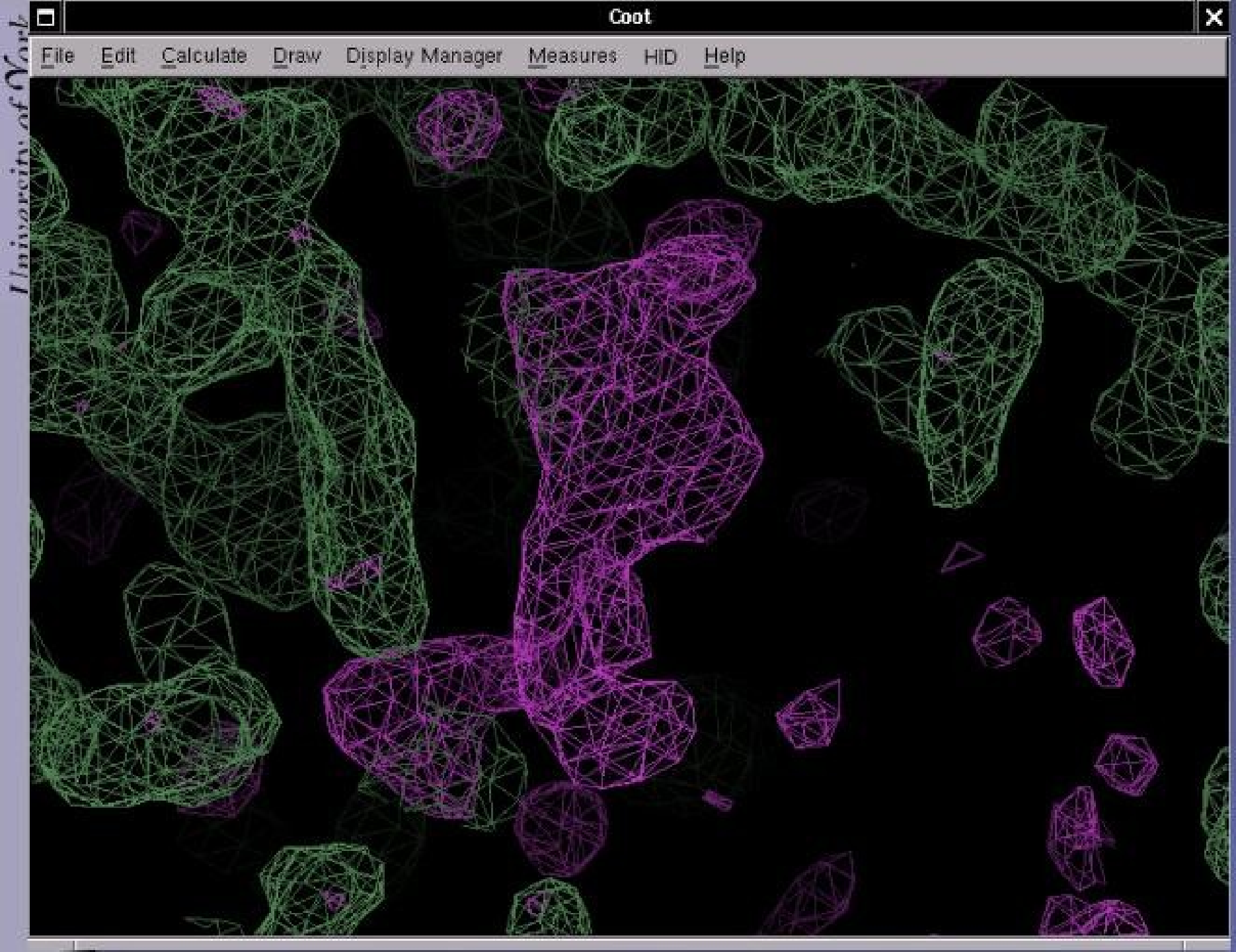
Crystal Space

- Build in “crystal space”
  - Like real-space, but wrapped by crystal symmetry
    - Like “Asteroids”
  - Assures only one real-space representation of map features
    - Build everything only once,
    - No symmetry clashing
  - However, more difficult to calculate real space geometries
    - …such as bonds, torsions



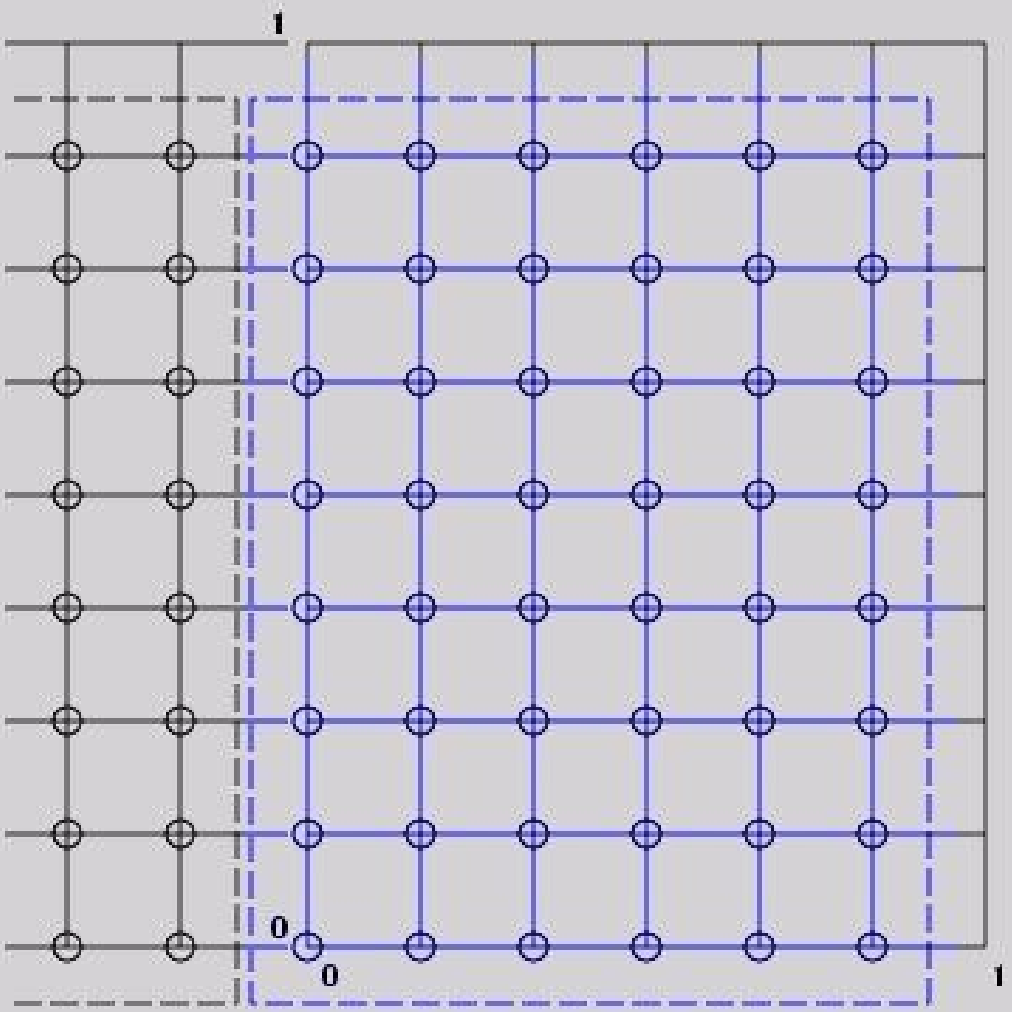


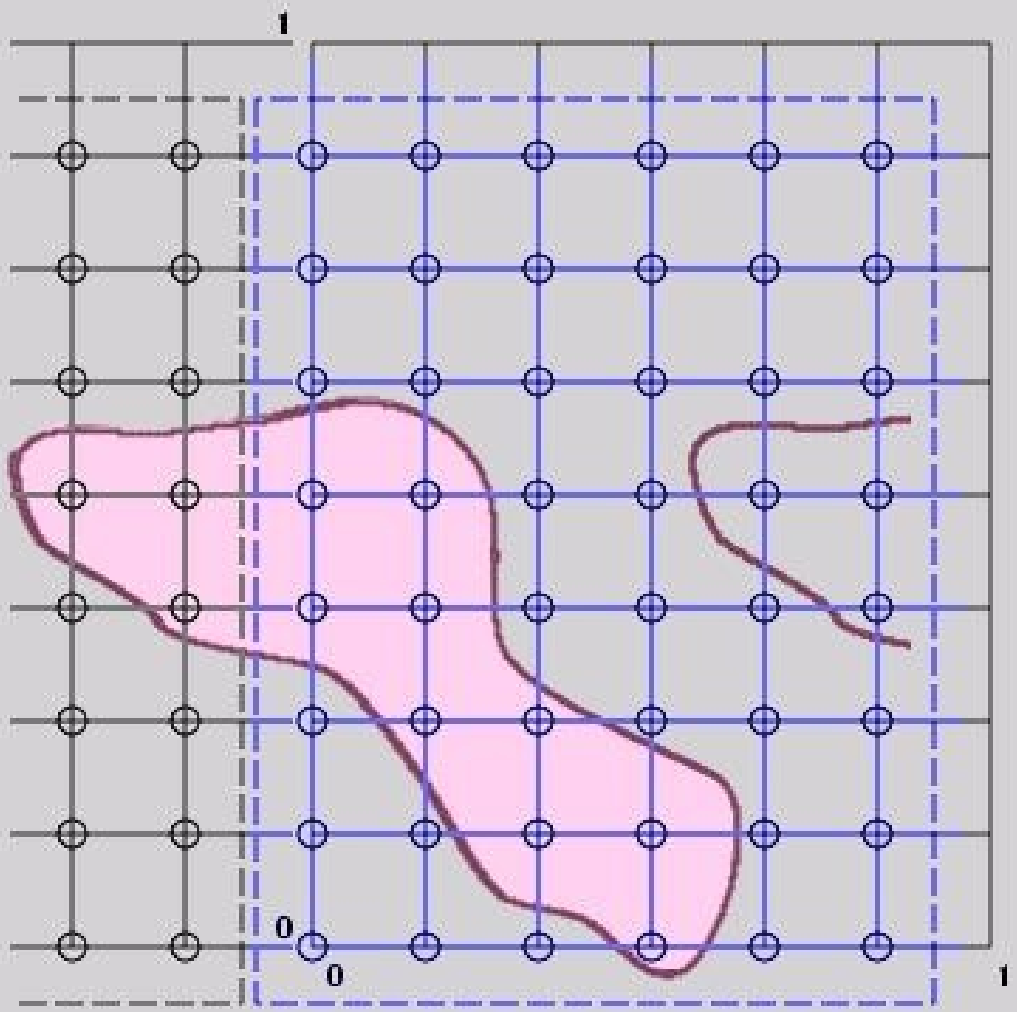


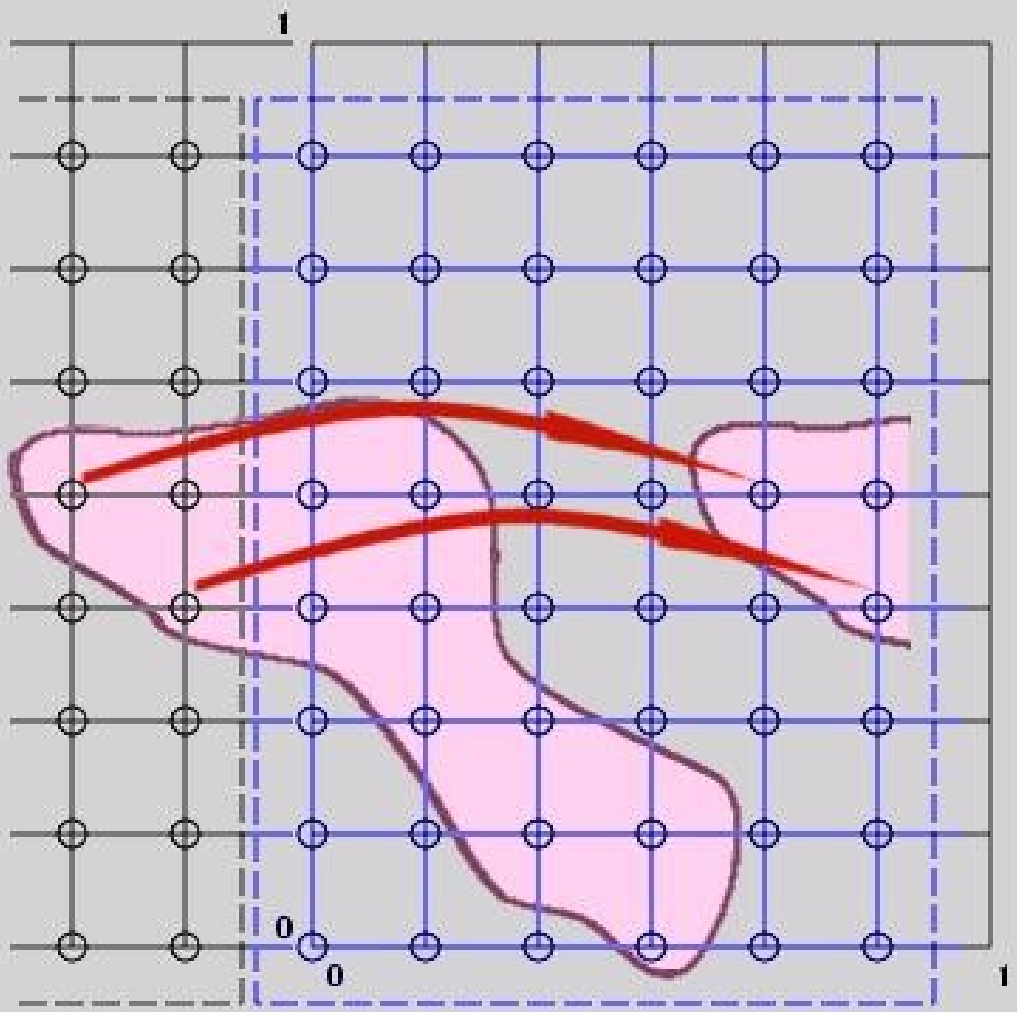


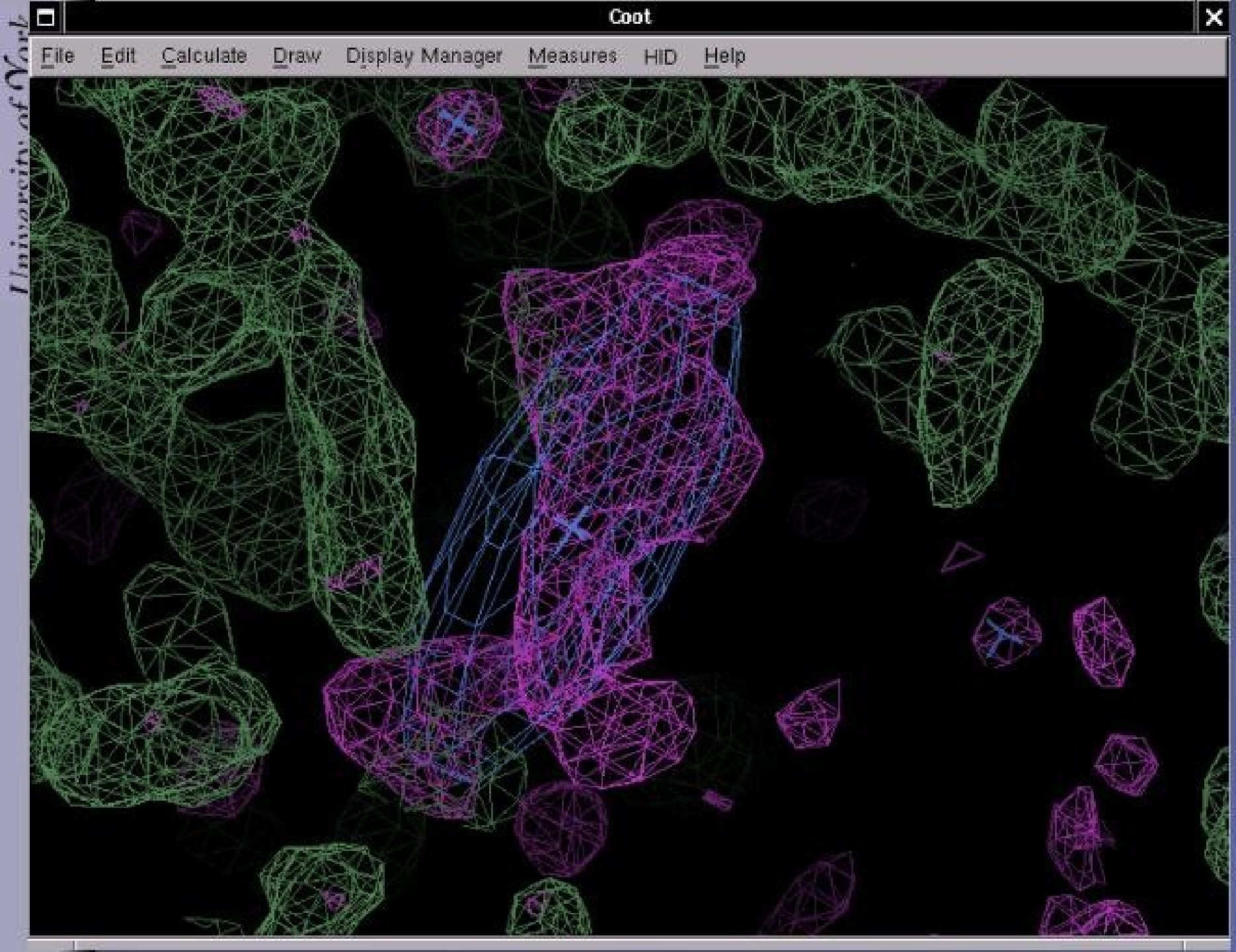
Clipper Map Mapping

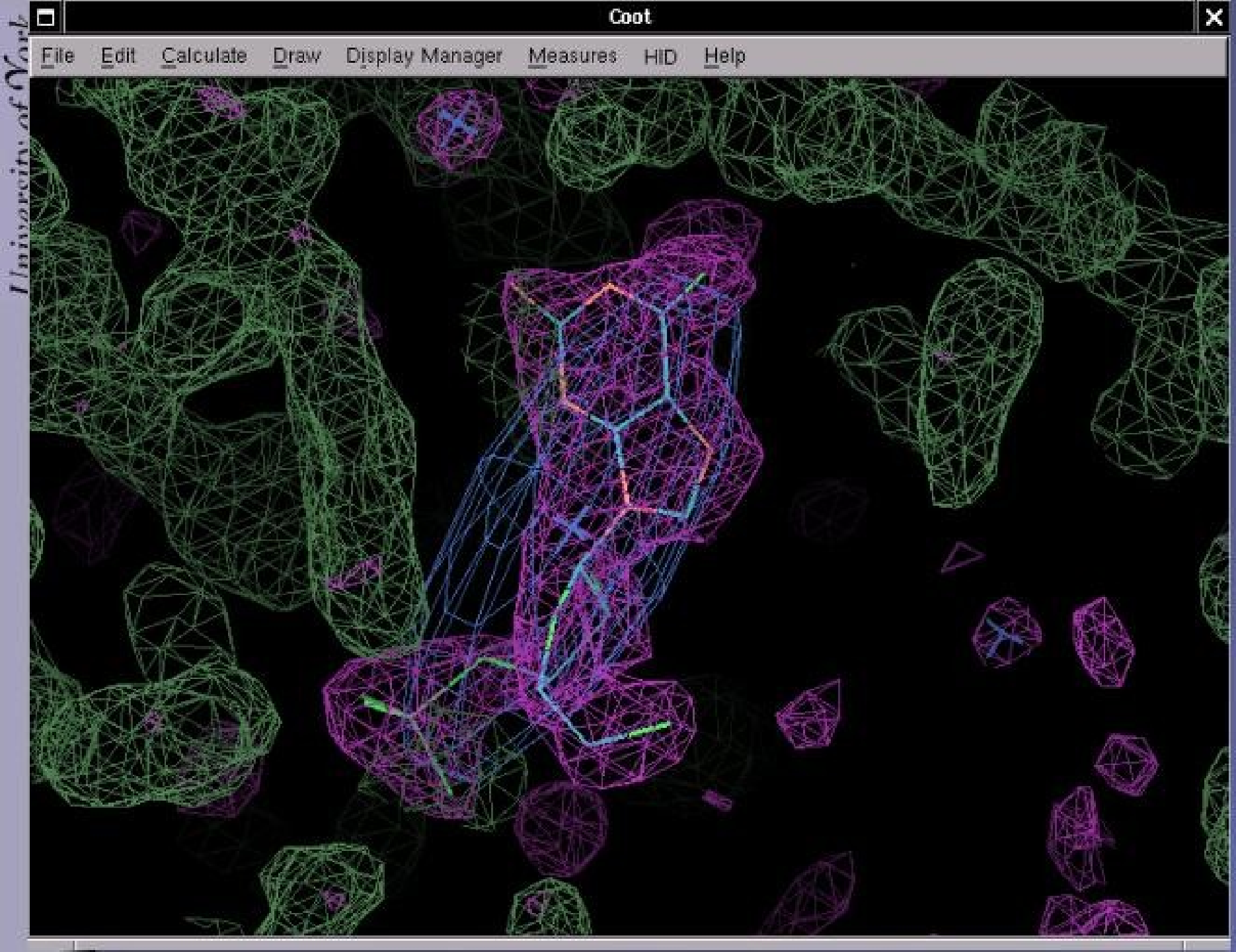
- Clipper maps
  - Appear to be “infinite”
  - Density value can be queried anywhere in space

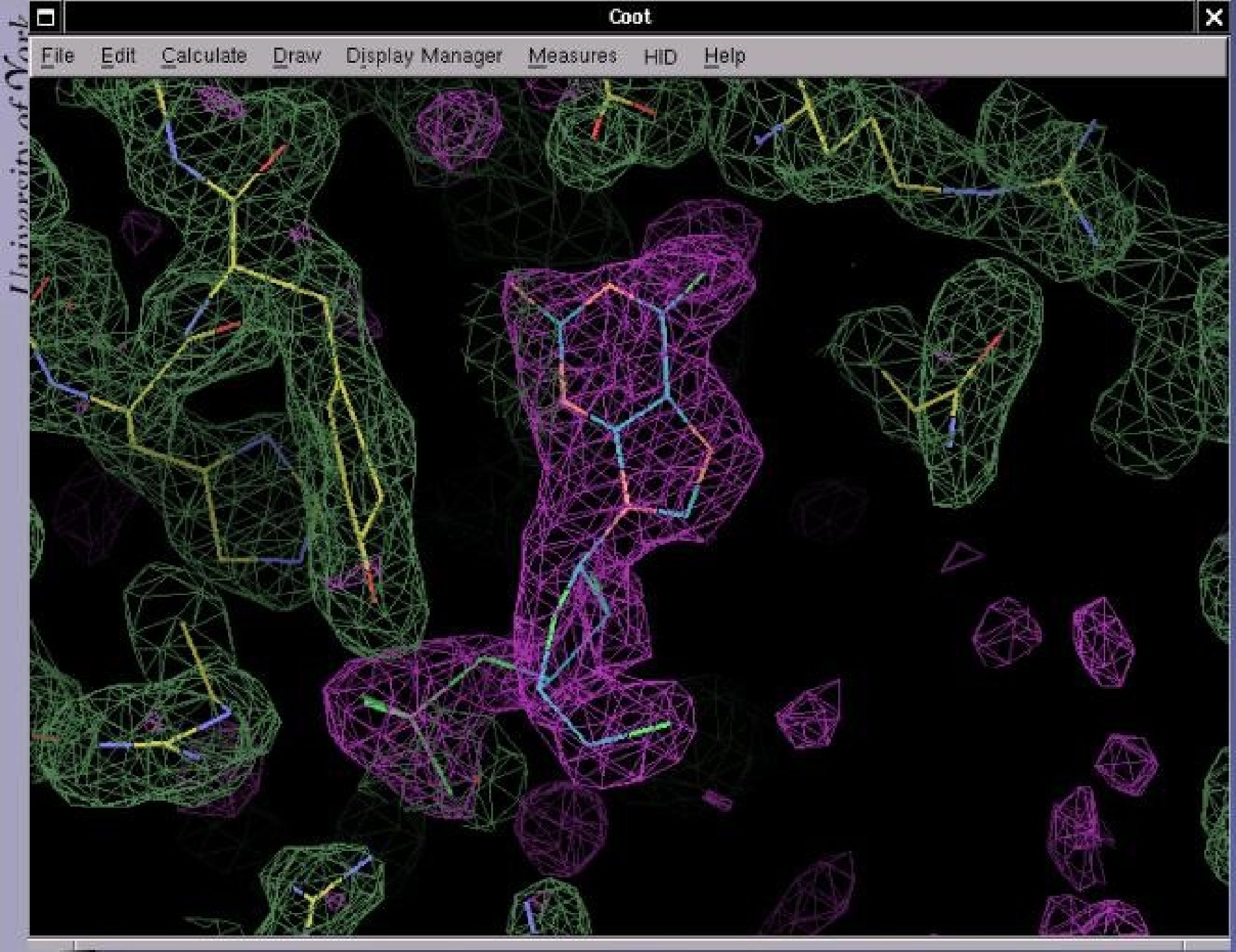












Conformation Idealization

- Each conformer is passed through the “Regularization” function of Coot
  - Non-bonded terms included
- Better to have hydrogen atoms on the model
- Slows slows things down a good deal…
  - May not be the best method to explore conformational variability for many rotatable bonds

Ligand Overlay

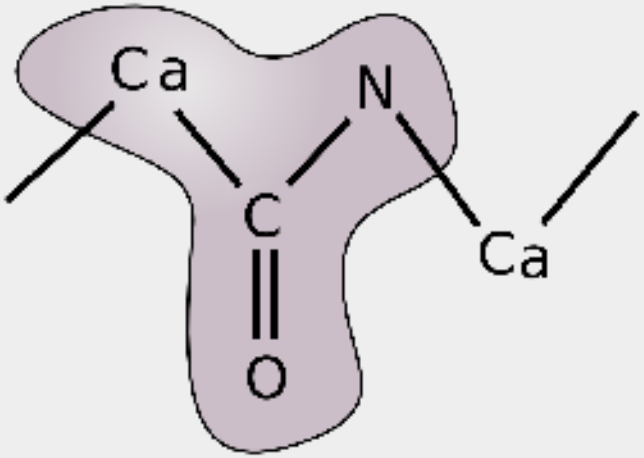
- Algorithm and Code by Eugene Krissinel
- Tries to overlay different ligands/monomers by graph matching
- Useful for “database” ligands where atom names are not selected by hand
- Has been used as the basis of the function which “mutates” residues to alternative monomer types
  - e.g. phosphorylation

Low Resolution Tools

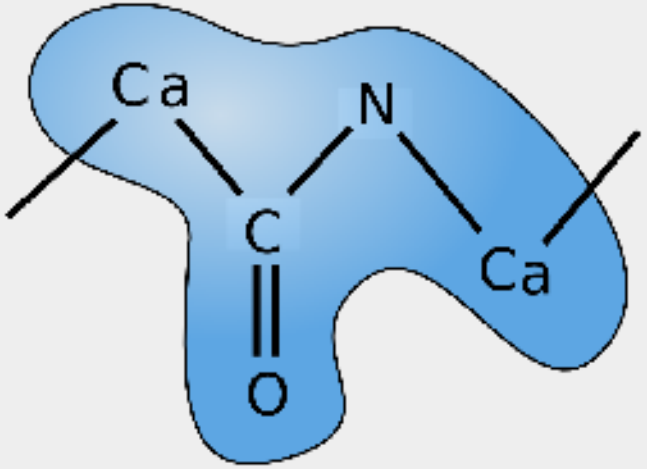
Extra Restraints….

Coot's Extra Peptide Plane Restraint

Default Refmac Peptide Plane



Extended Plane in Coot



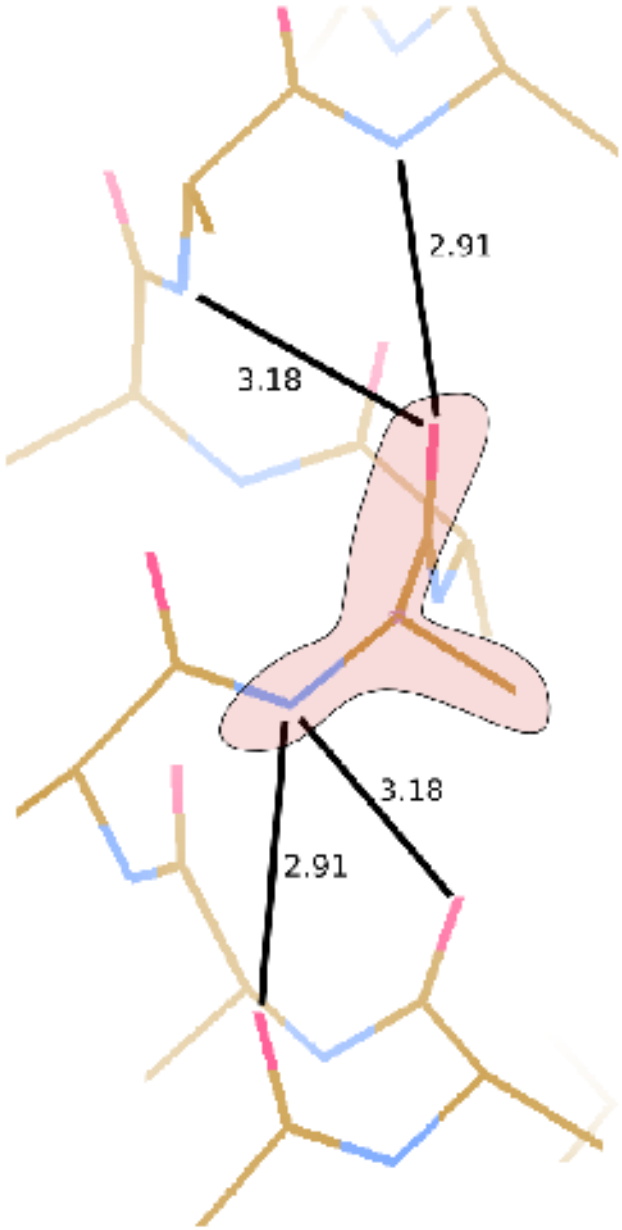
(add-planar-peptide-restraints)

Adding Torsion Angle Restraints

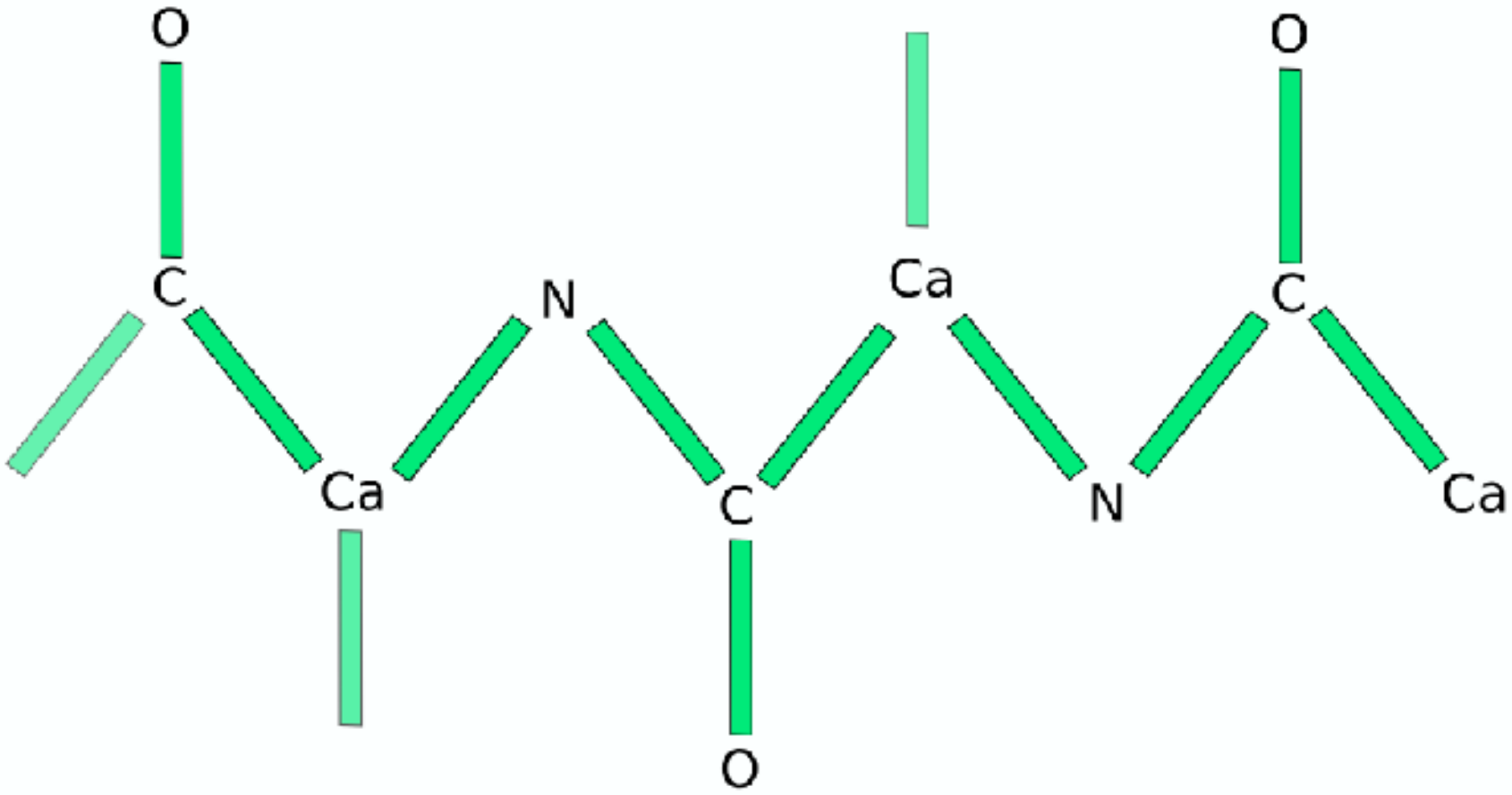
- Torsion angle refinement is slow (relatively)
  - Simple addition of these restraints to the geometry target function
    - often makes the region “stuck and unsatisfied”

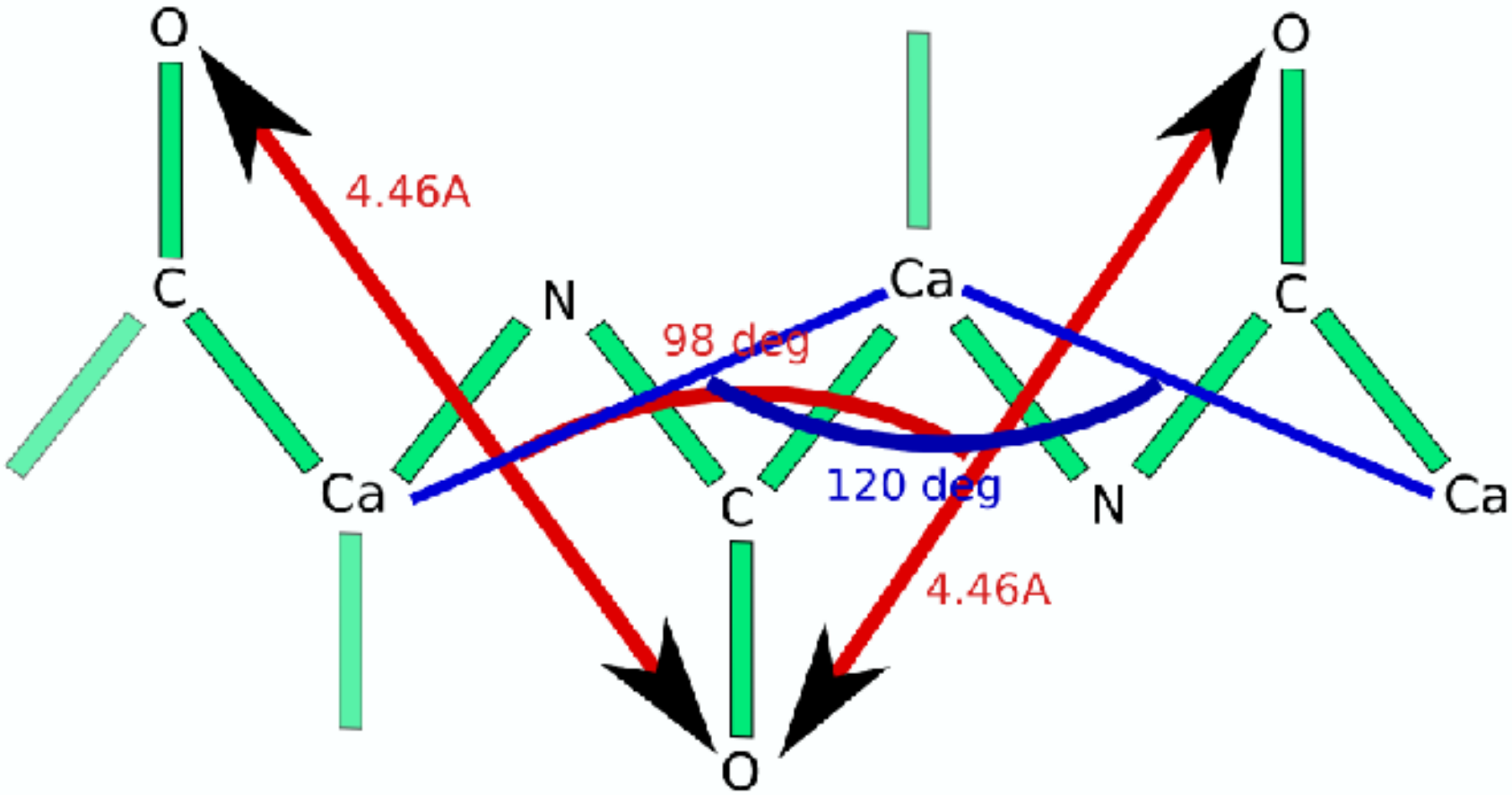
- Add Pseudo-bonds

Alpha Helix pseudo-bond restraints



Restrain the Hydrogen-bonding atom distances


O
C
Ca
N
C
O
Ca
N
O
C
Ca

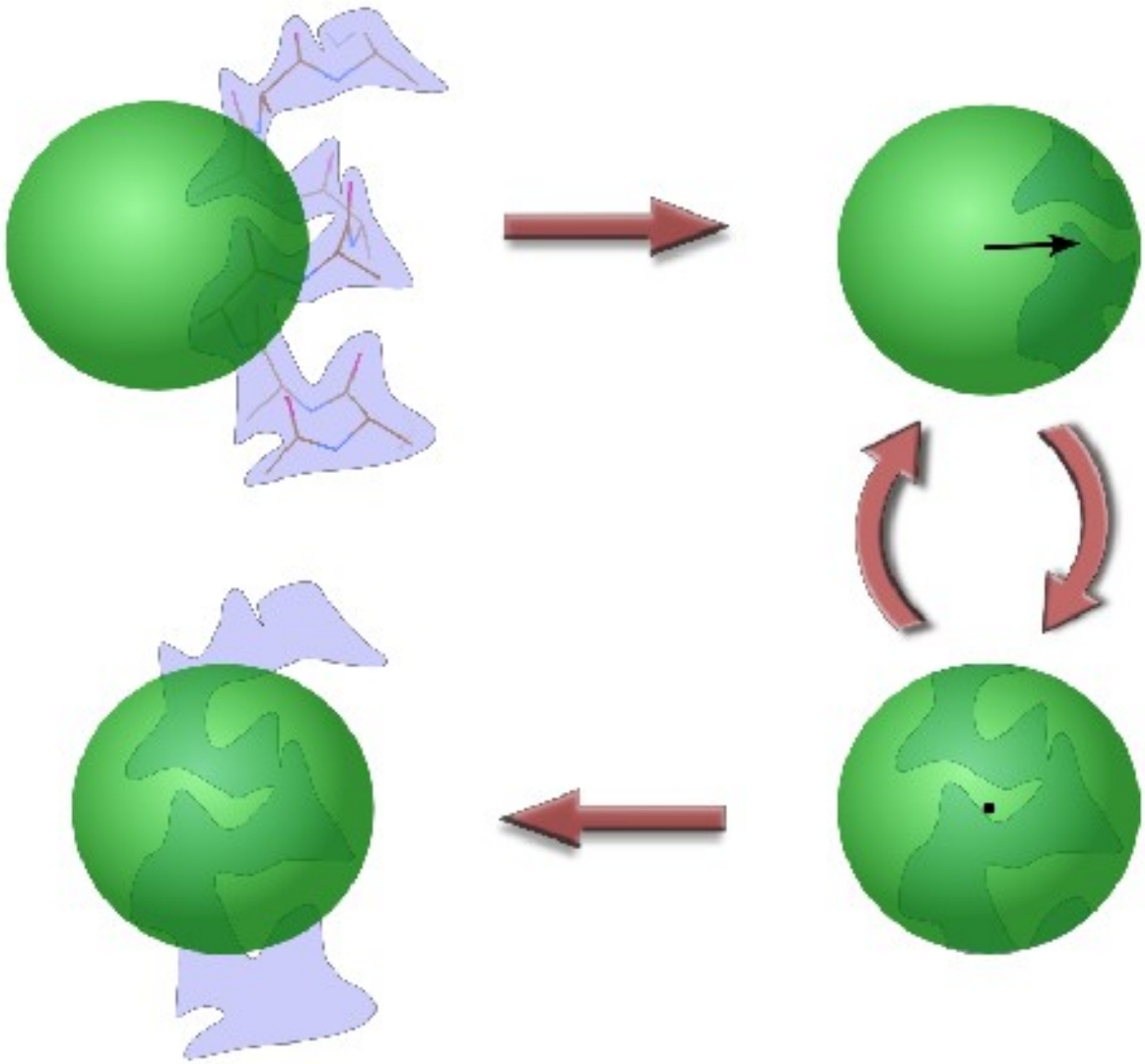

O
C
Ca
N
C
O
4.46A
98 deg
120 deg
Ca
C
N
Ca
O
4.46A

Helix-Building

Alpha Helix Placement

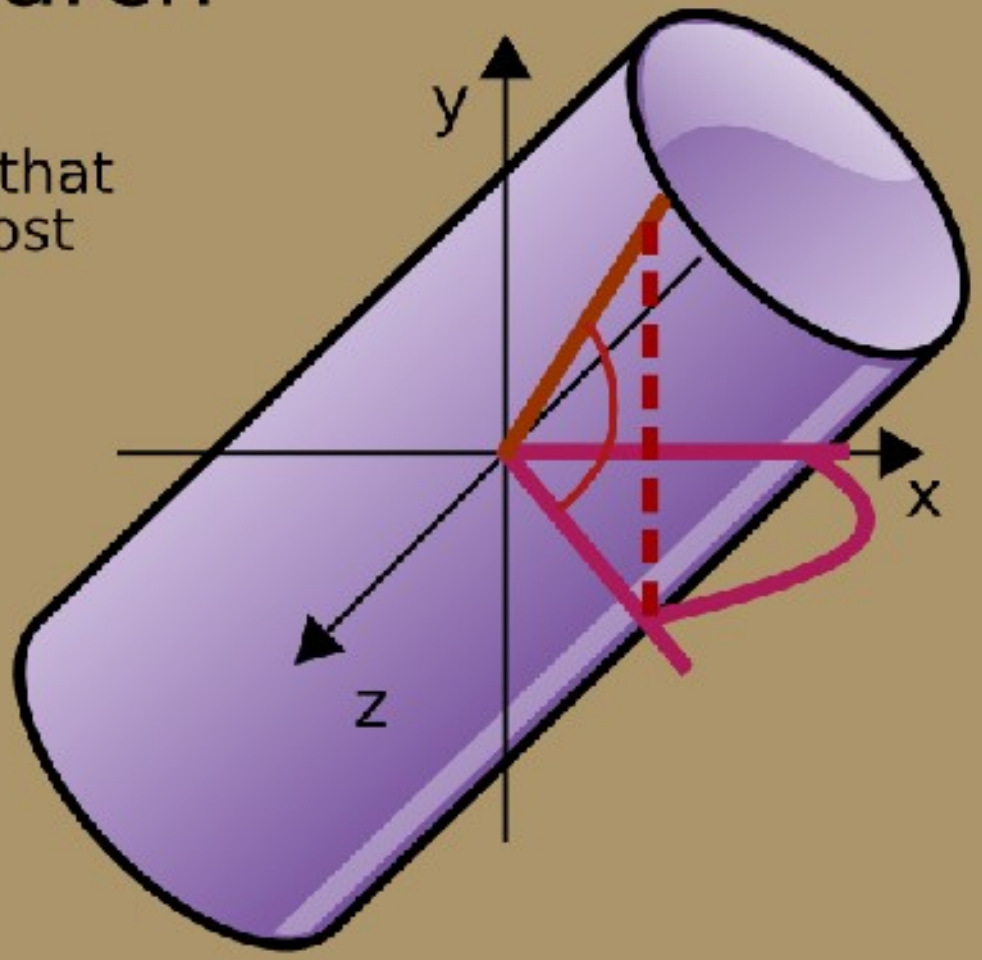
- Scenario: Looking at a new map, not built with automatic tools:
  - "I can see that there's a helix here - build it for me!"
- From a given point:
  - Move to local averaged maximum
  - Do a 2D MR-style orientation search on a cylinder of electron density
  - Build a helix (both directions)
  - 1D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

Centering the Rotation point

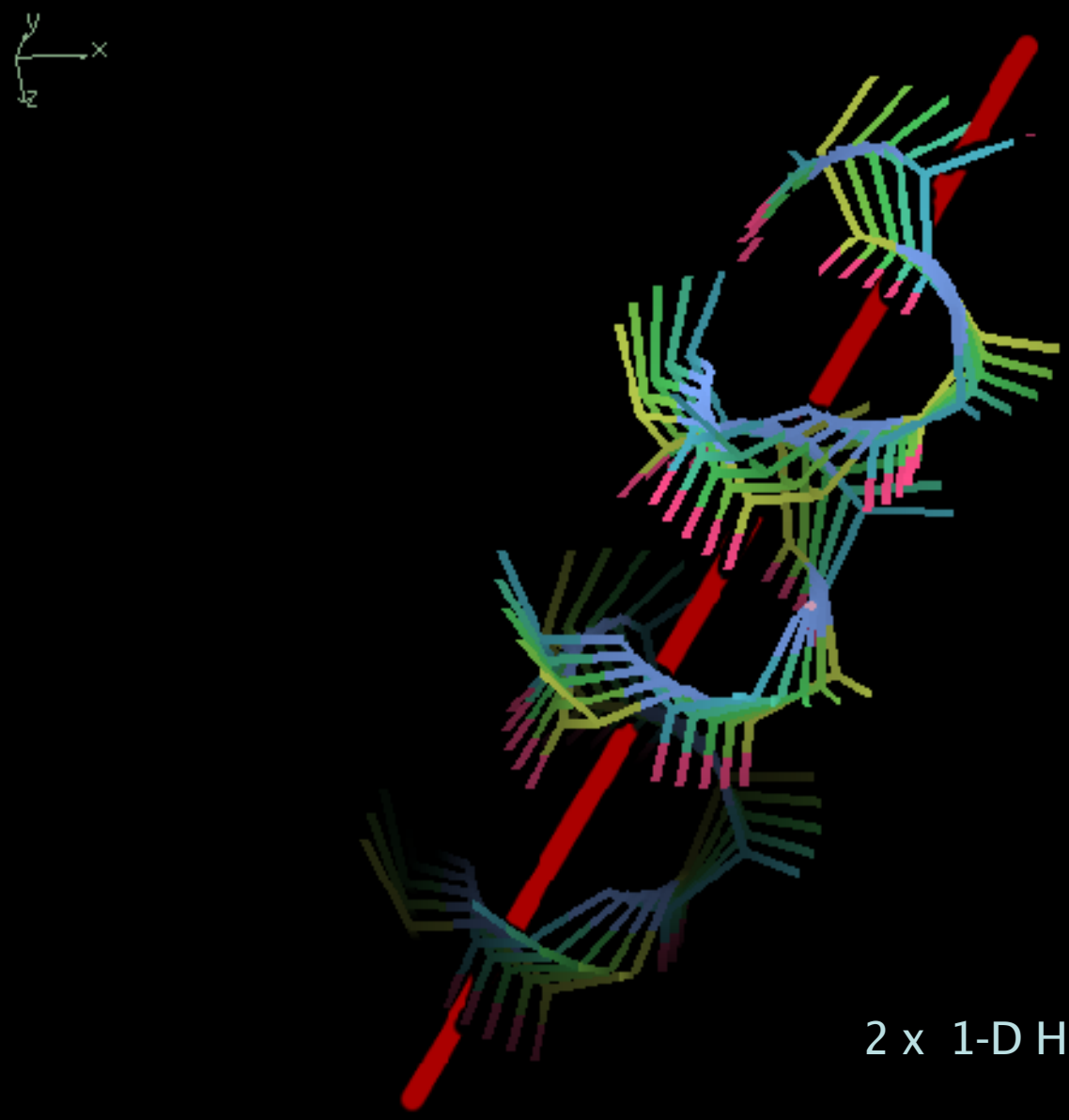


Cylinder Search

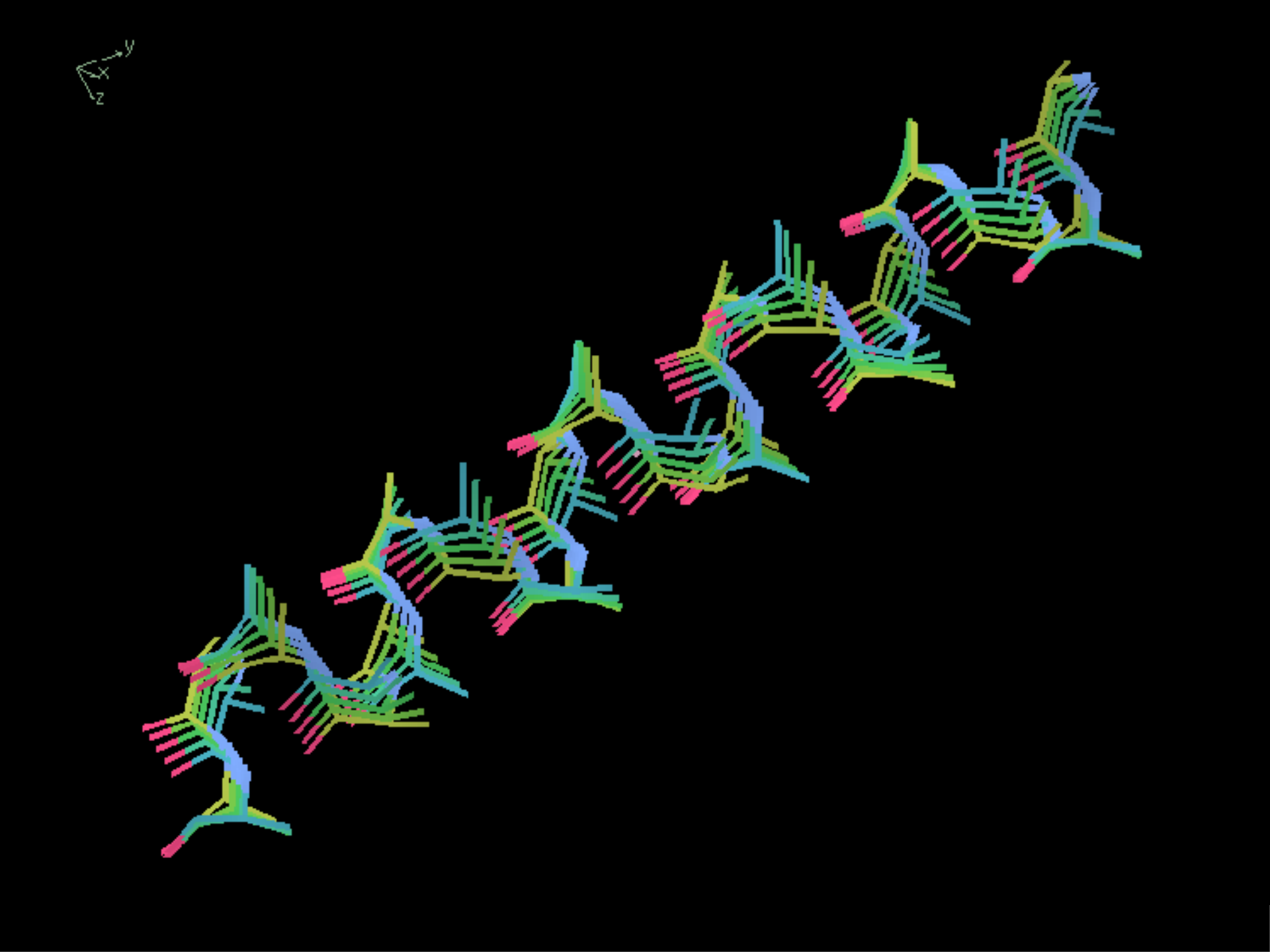
Pick the orientation that encapsulates the most electron density

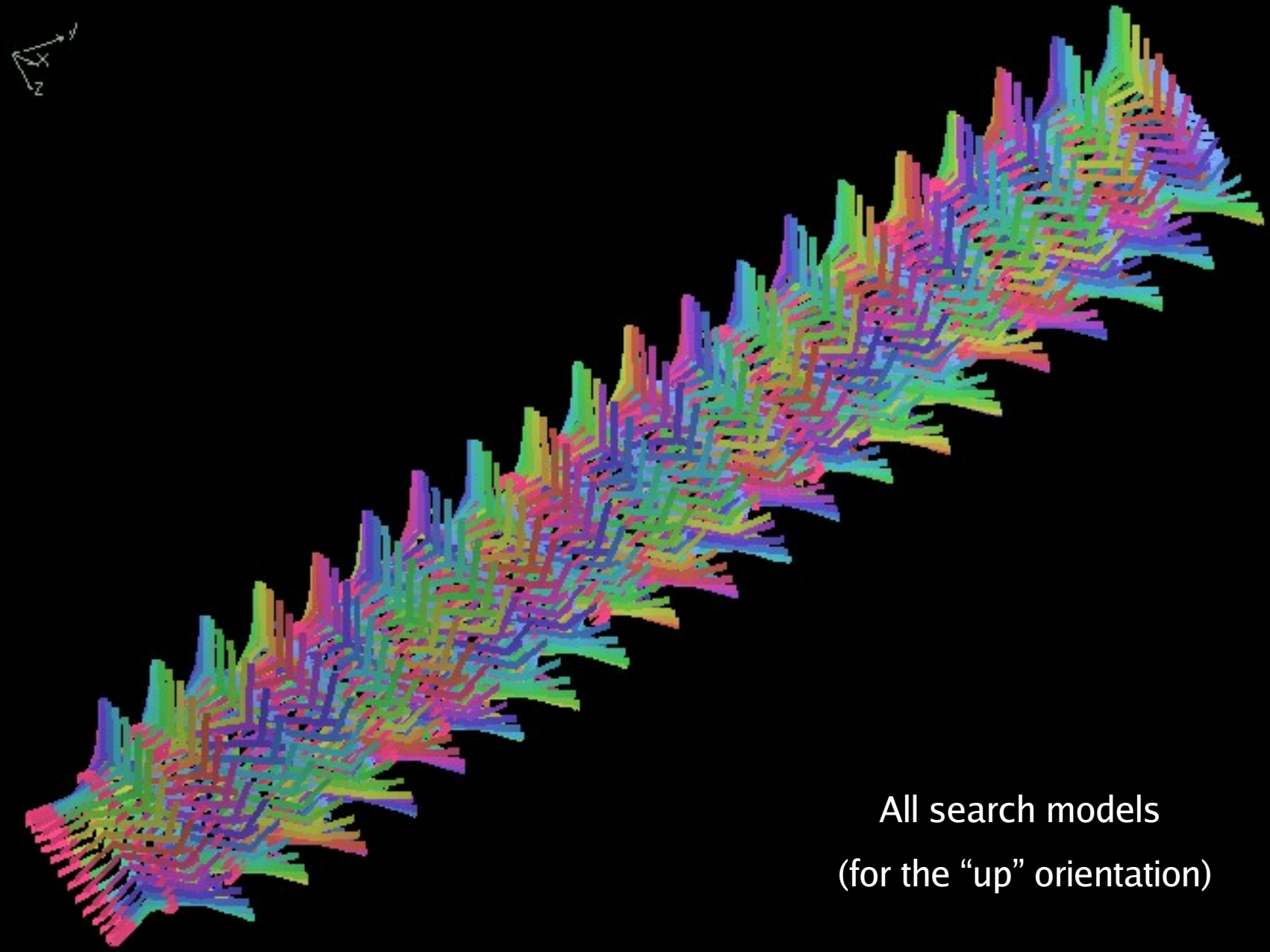


2 orientation axes



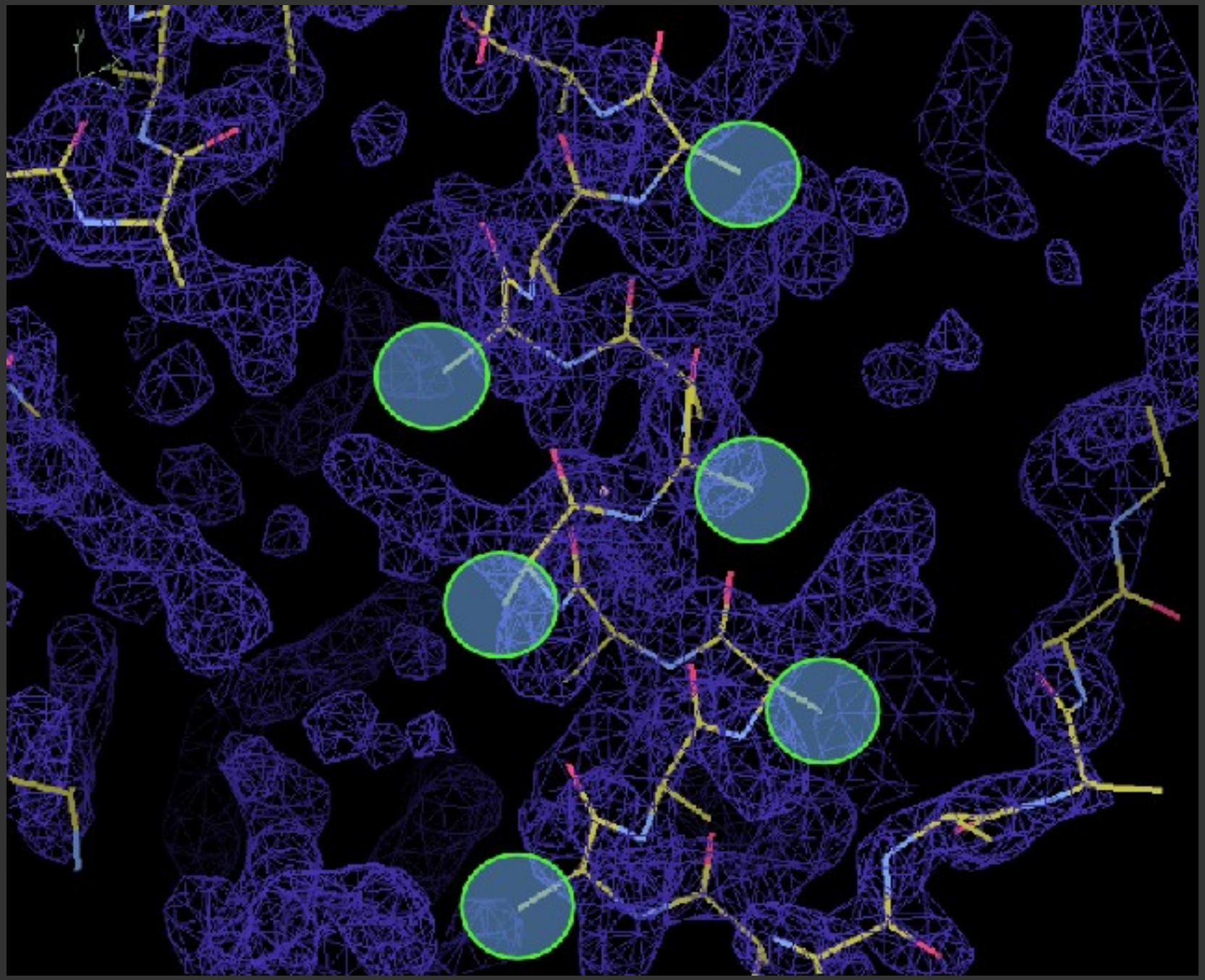
2 x 1-D Helix orientation searches

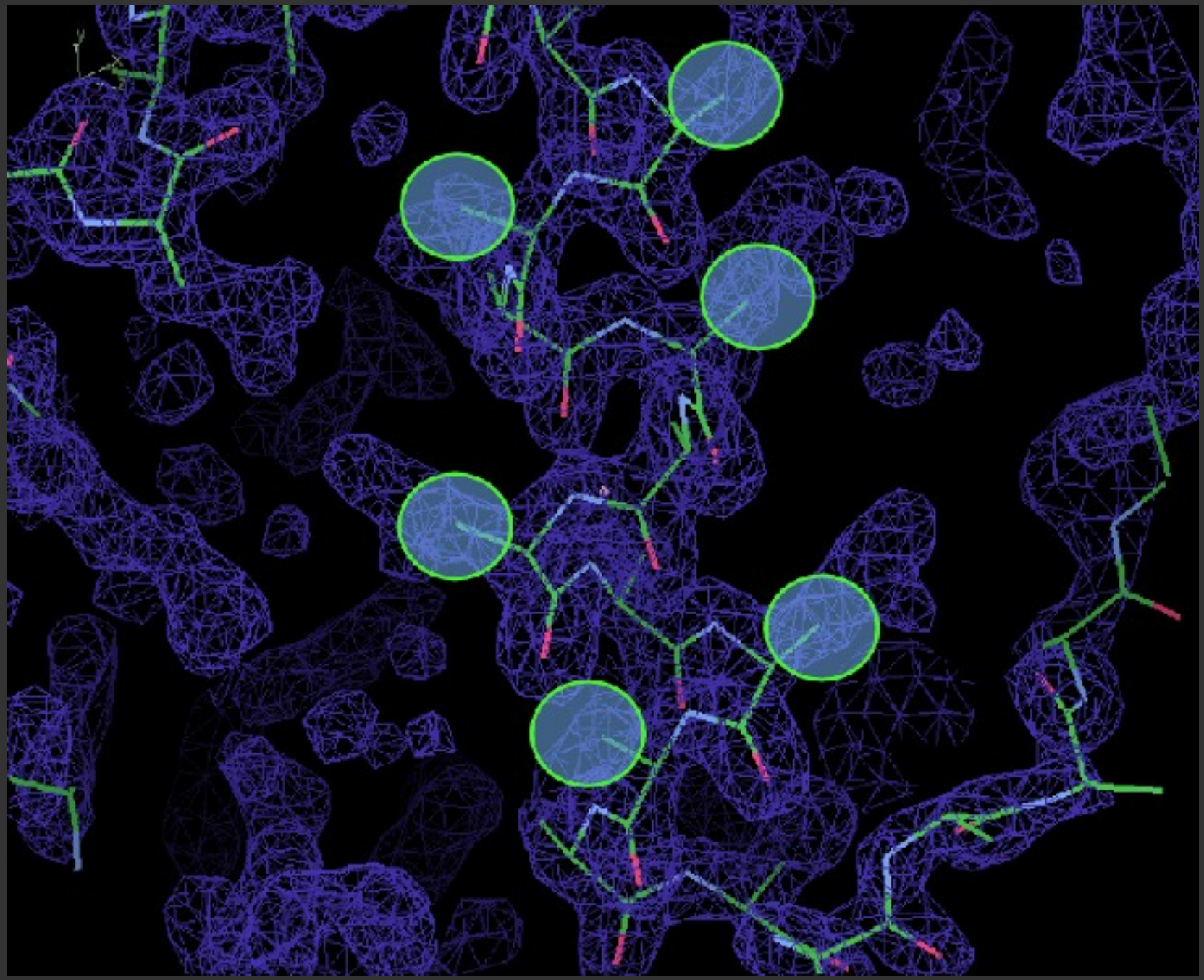

y
x
z



All search models

(for the “up” orientation)





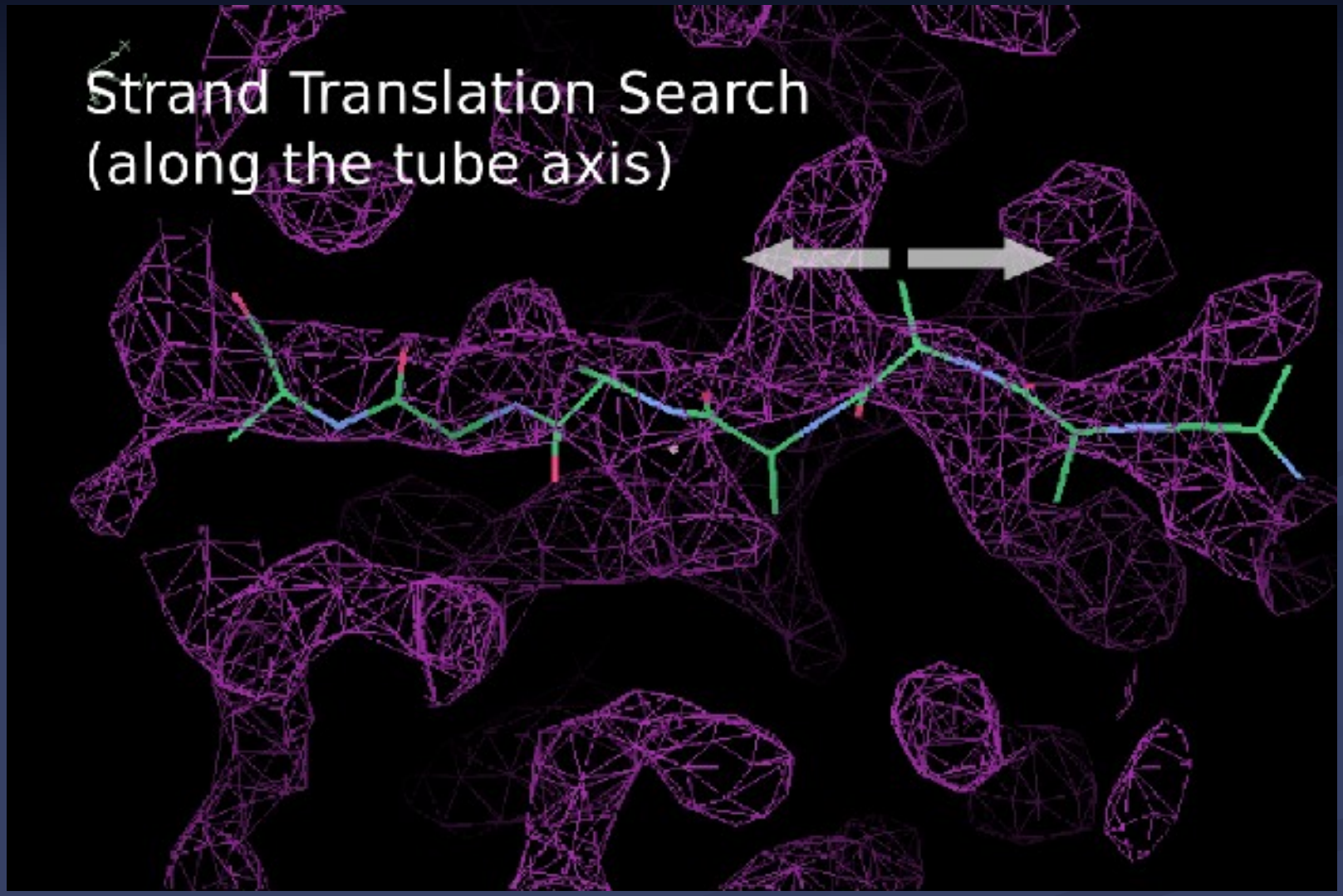
Fitting Strands

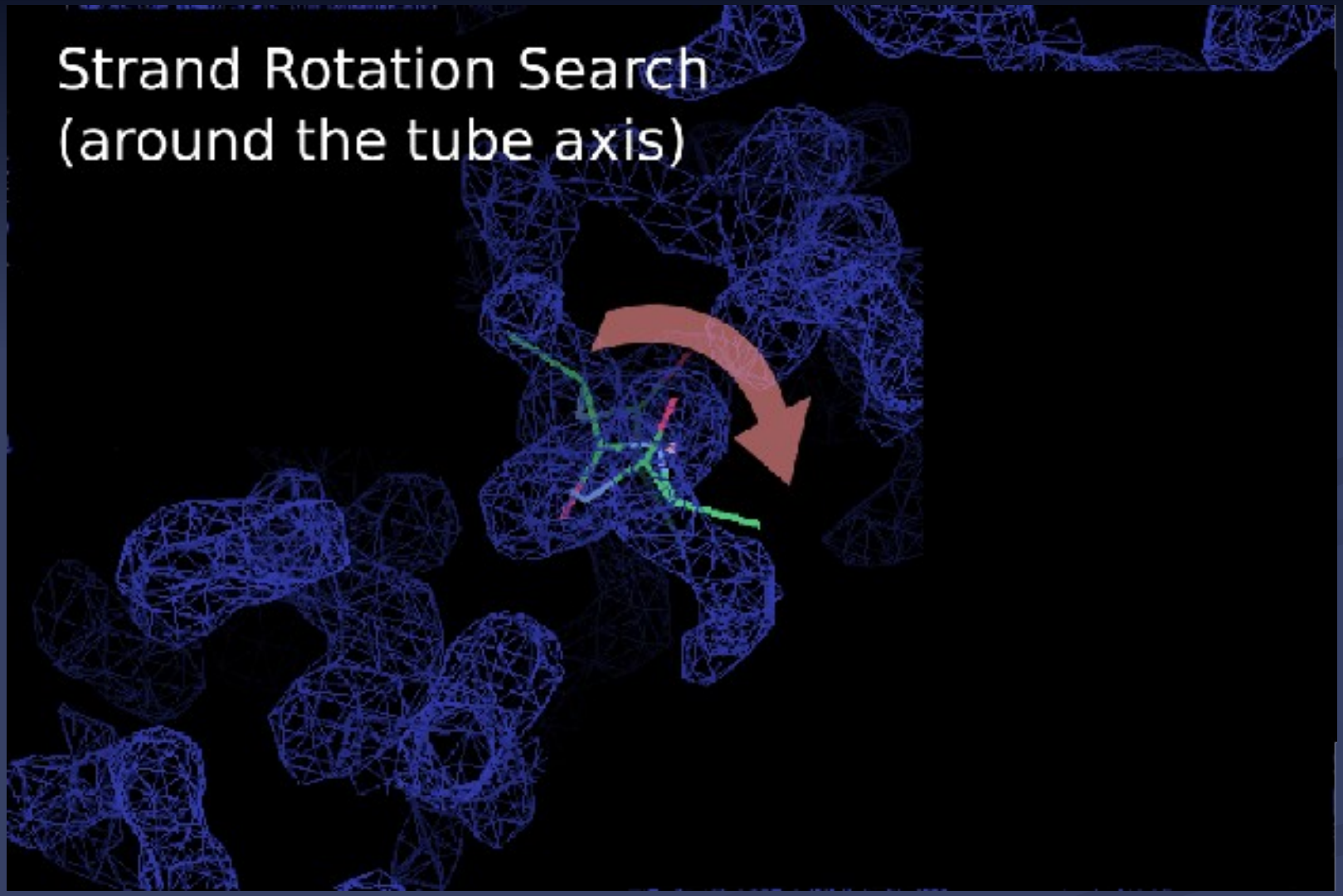
Placing Strands

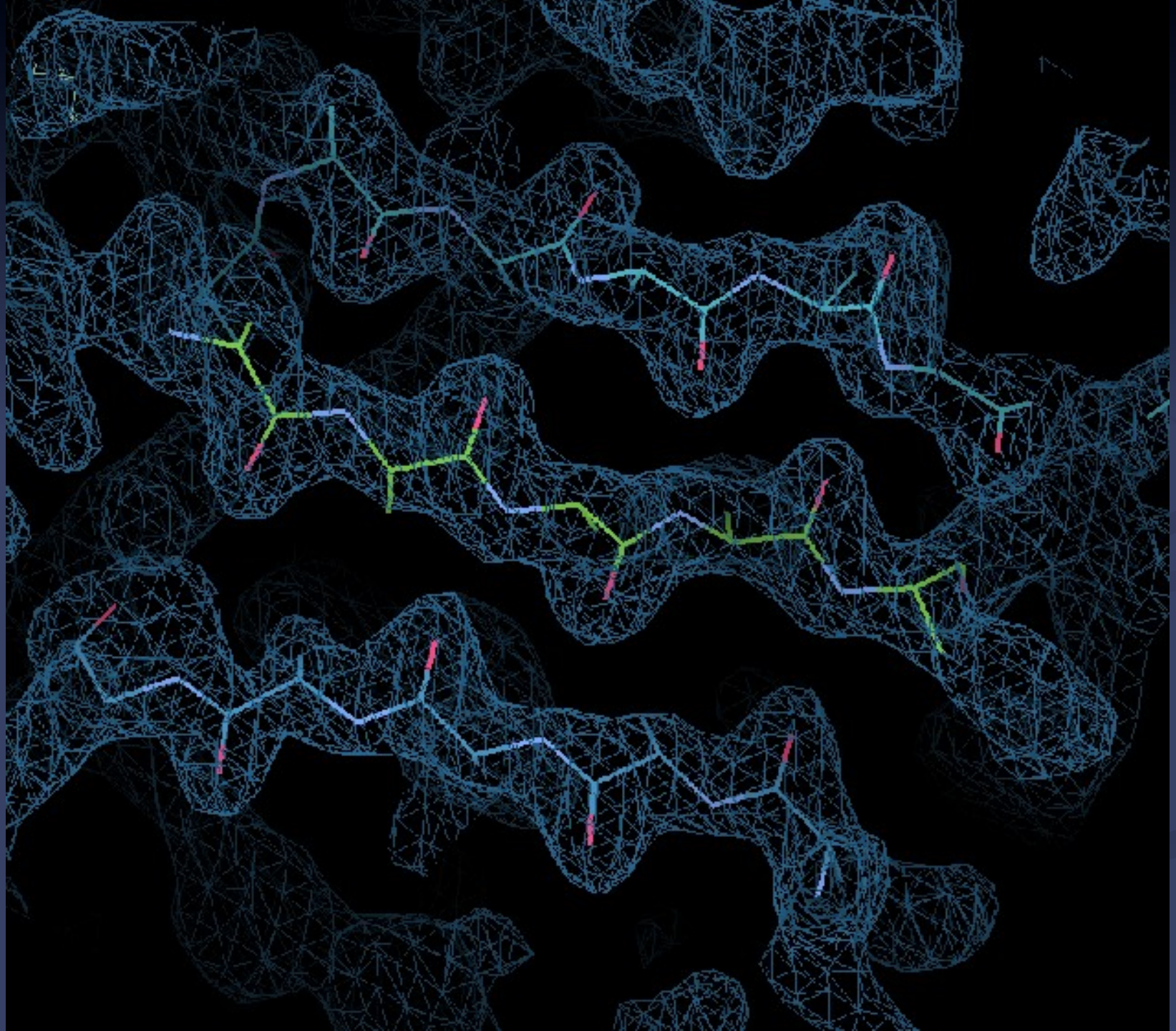
- Unlike Helices, Strands have to be treated as non-idealized
  - Repeating a single phi/psi value doesn't make a structure that fits “real-world” density
- Curvature of strands should be taken into account
  - Use selections from a “database” of good structures

Strand fitting algorithm

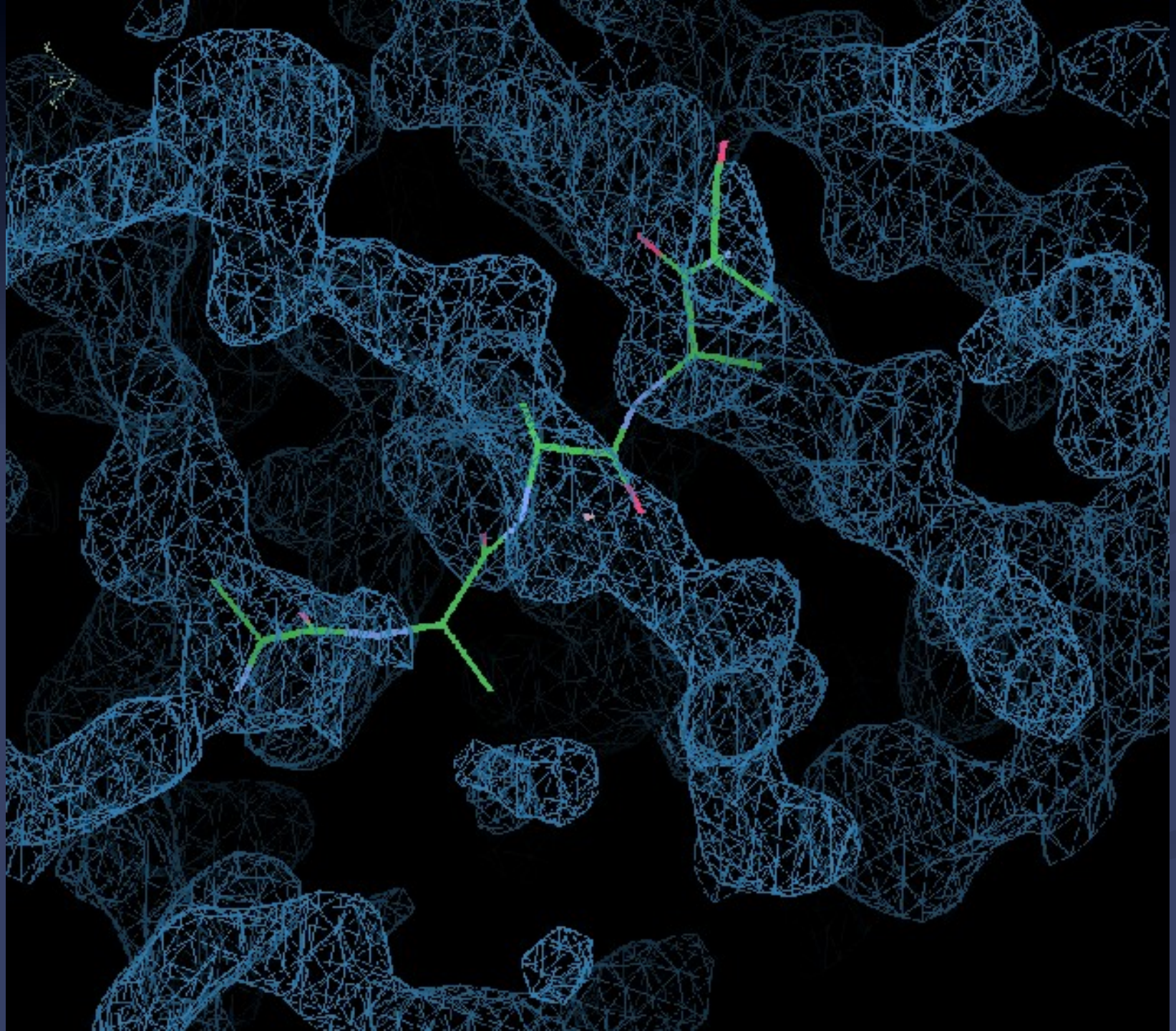
- Cylinder search
- Get N fragments of length l from database
  - 1-D Translation search along the tube
    - 1-D Rotation search around the tube
    - Direction flip search
- Rigid body refine best solutions
- Real-space refine best solution


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

Coot Futures...

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