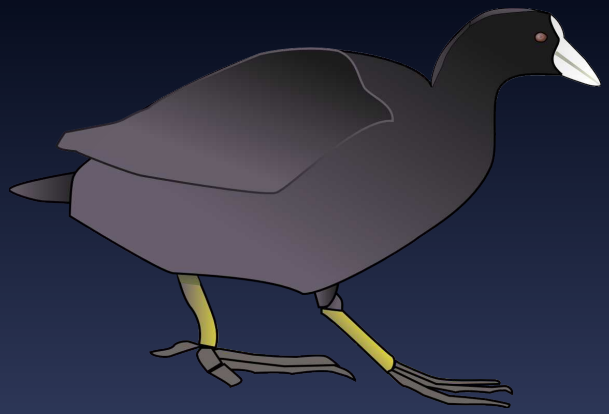


December 2011 OIST

Ligands and validation with Coot

Bernhard Lohkamp

Karolinska Institutet

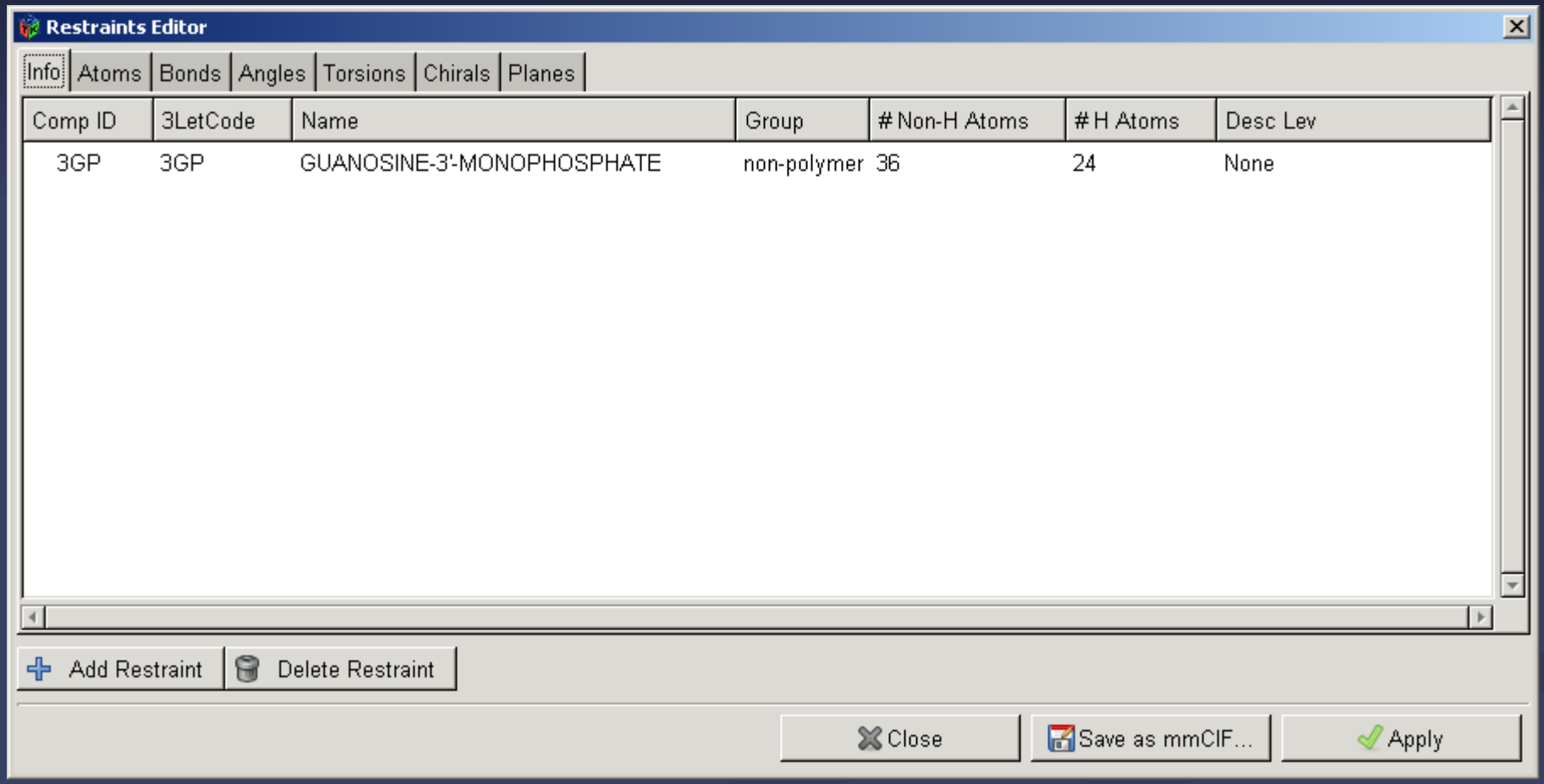
Ligands in Coot

- Fitting ligand
- Ligand validation
- Ligand representation
- Ligand builder

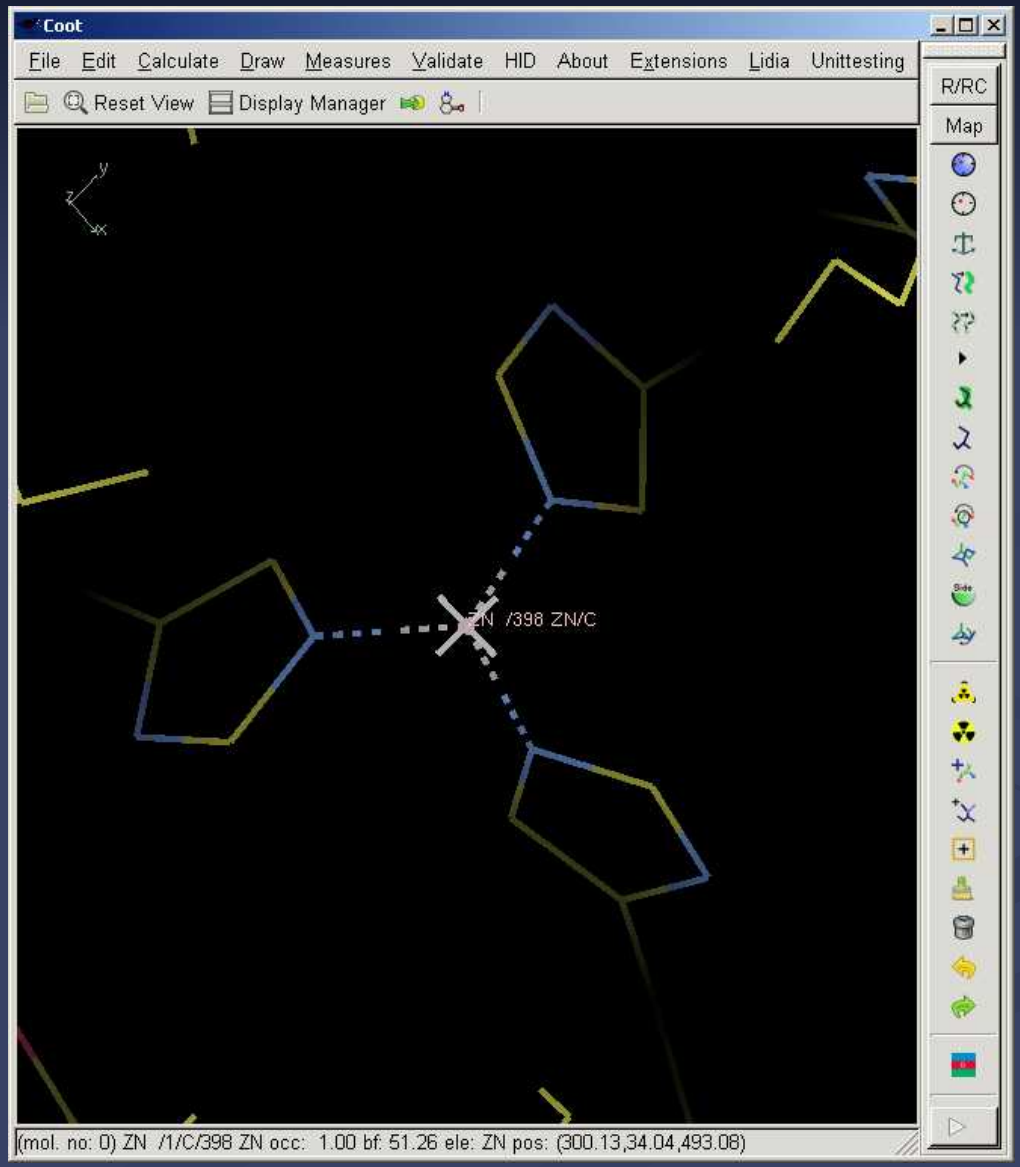
Ligand Overlay

- 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

Restraint Editor



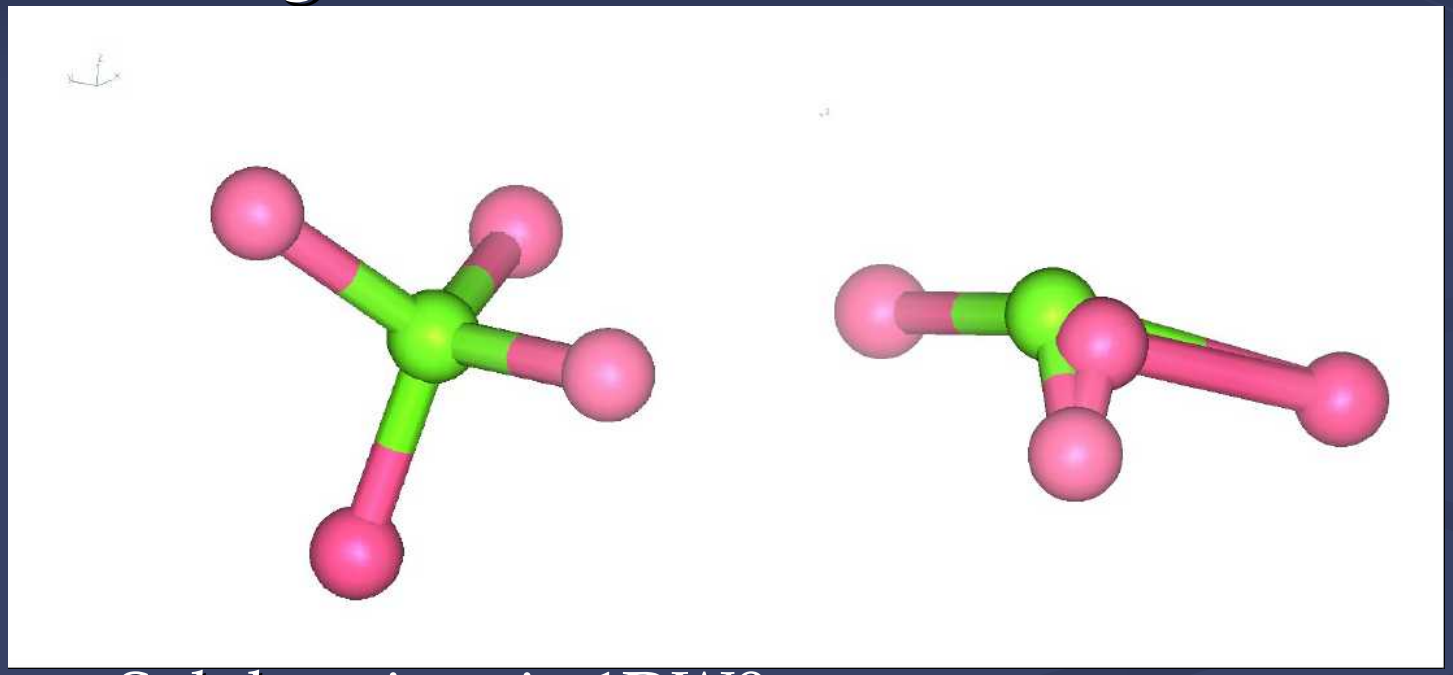
Displaying LINKs



Ligand validation

Ligand validation
Why validate?

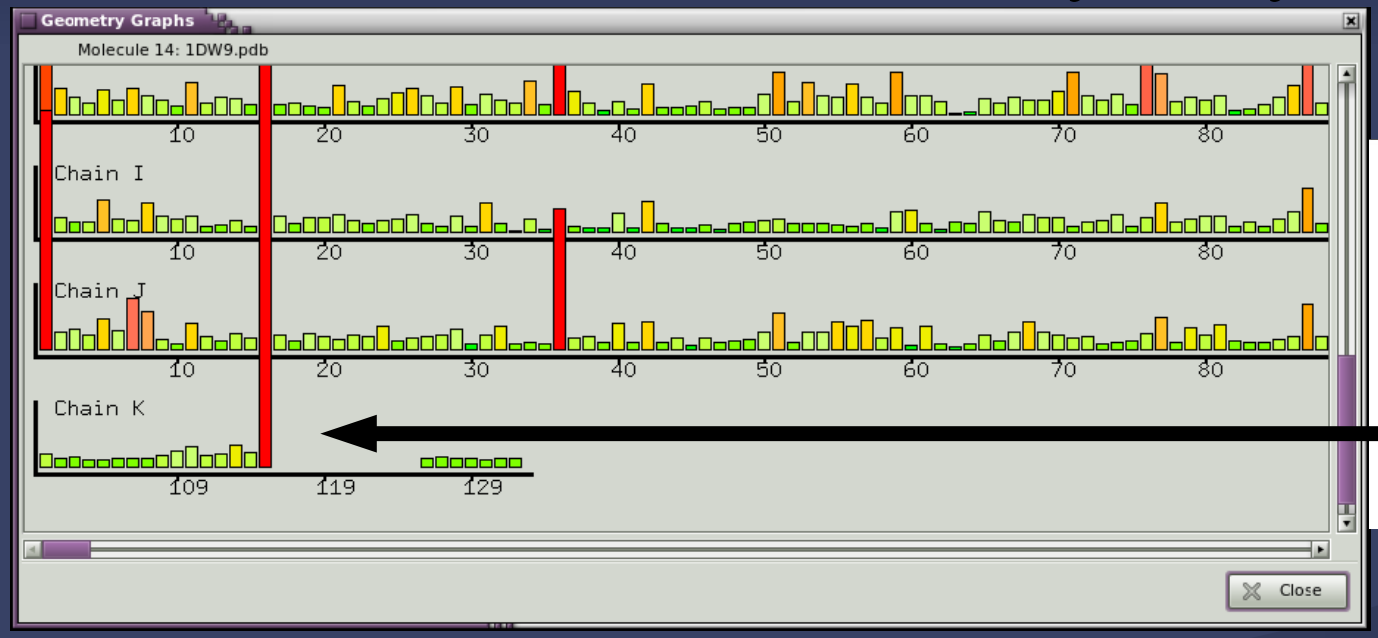
- 70.000 structures in PDB
- 11.000 'ligands' in 50.000 structures

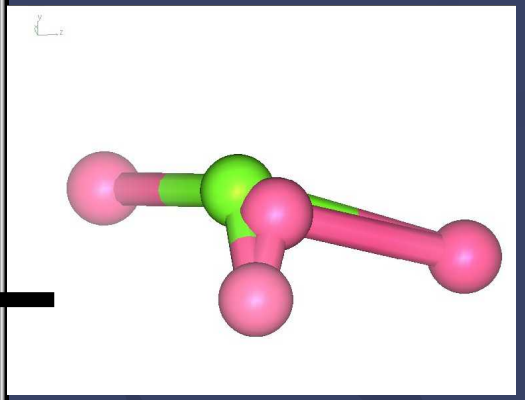


  - Sulphate ions in 1DW9
  - 1.65 A resolution
  - R/Rfree 0.15/0.19

Ligand validation

- How to validate ligand geometry?
  - Compare observed structure to restraints
    Coot: Validate->Geometry analysis





  - But what if parametrization (dictionary) is wrong?

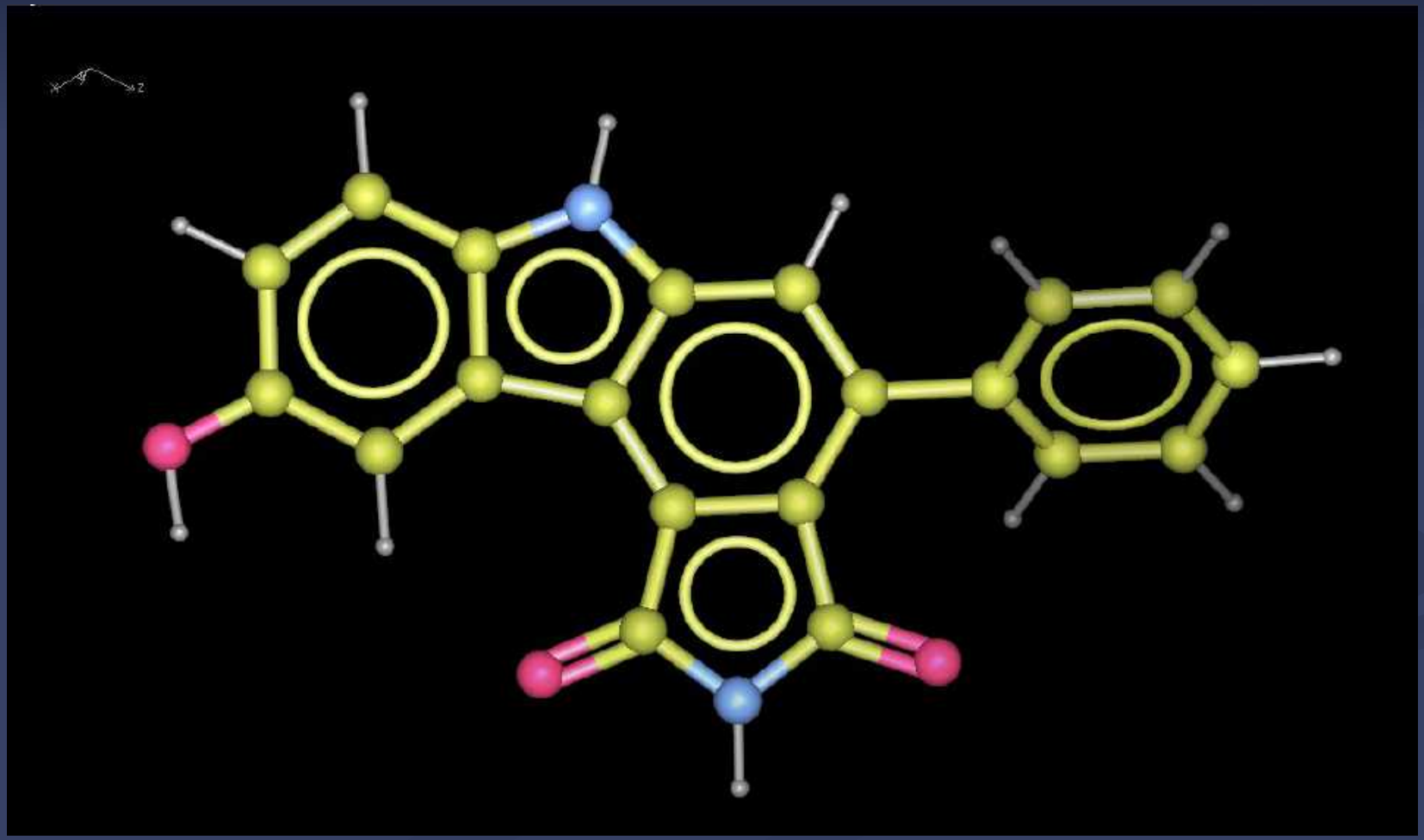
How to validate ligand geometry?

- QM
  - CPU hungry
  - In vaccuo
  - Low energy ≠ (?) bound ligand
- PDB (e.g. ValLigURL)
  - Good cofactor structures
  - Less useful for novel ligands
  - (occ.) questionable quality
- Cambridge Structural Database (CSD)
  - Using small molecule geometries
  - e.g. Mogul

Ligand representation

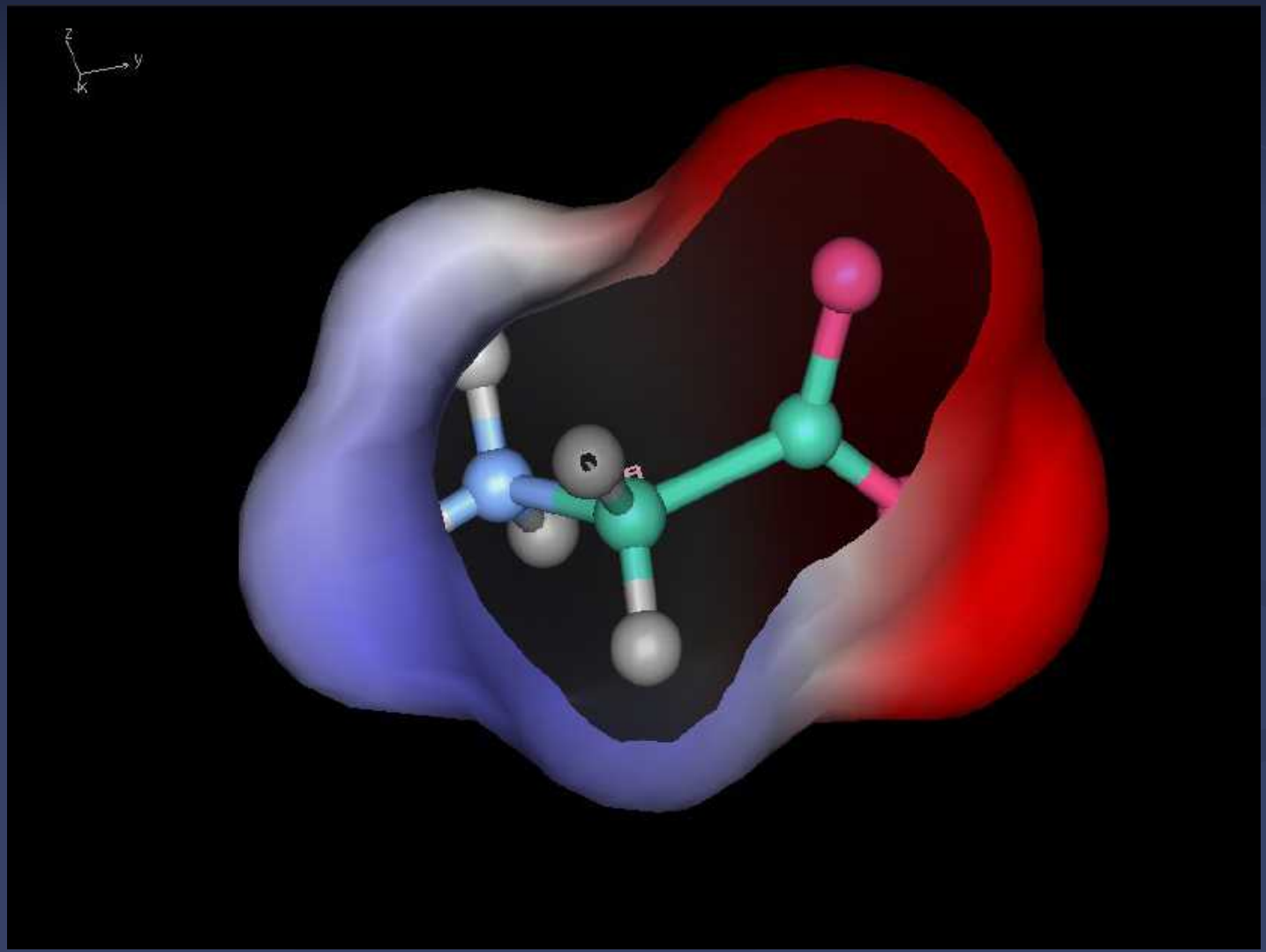
Ligand representation

- Bond order representation from dictionary files



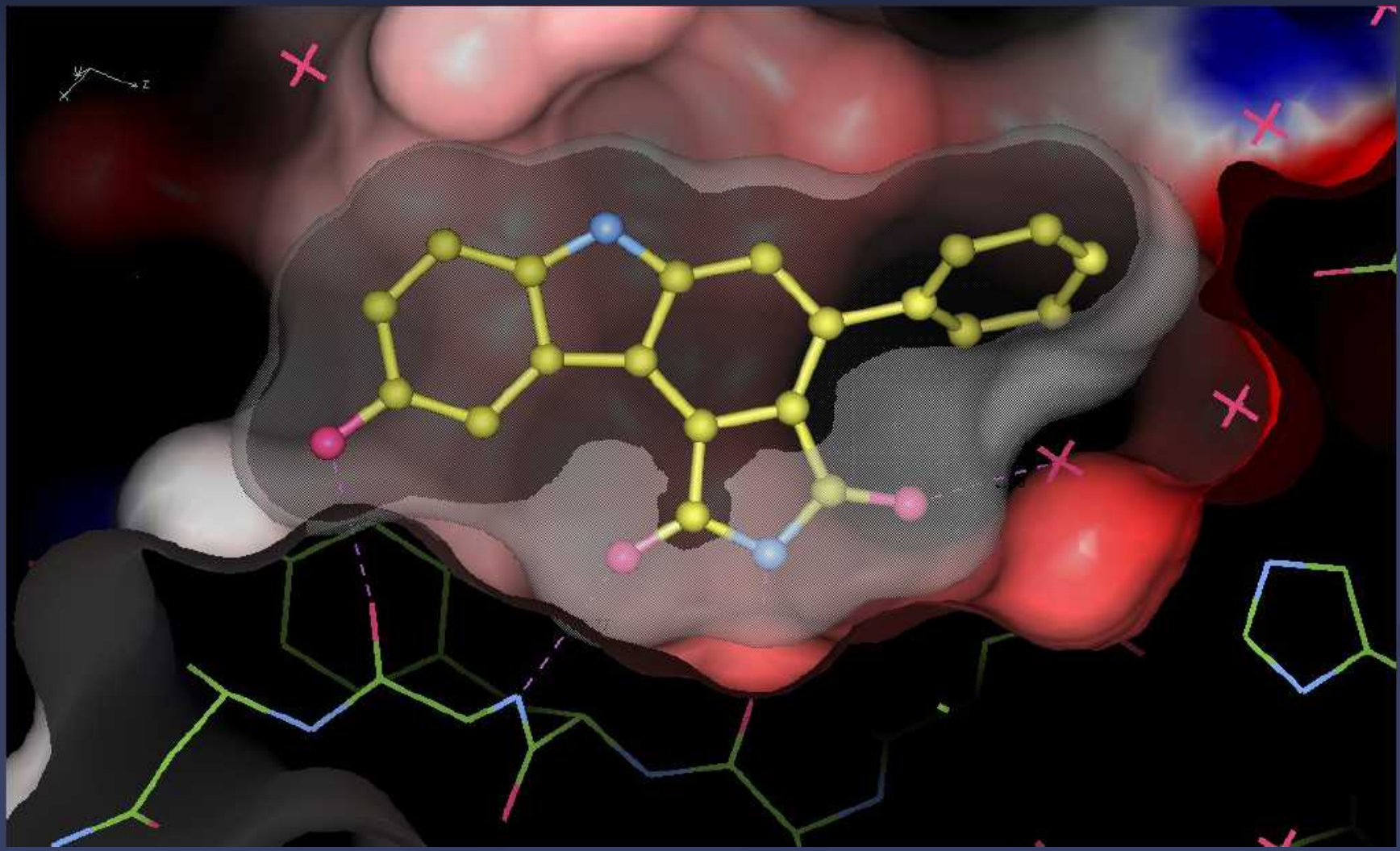
Surfaces

- Partial charges



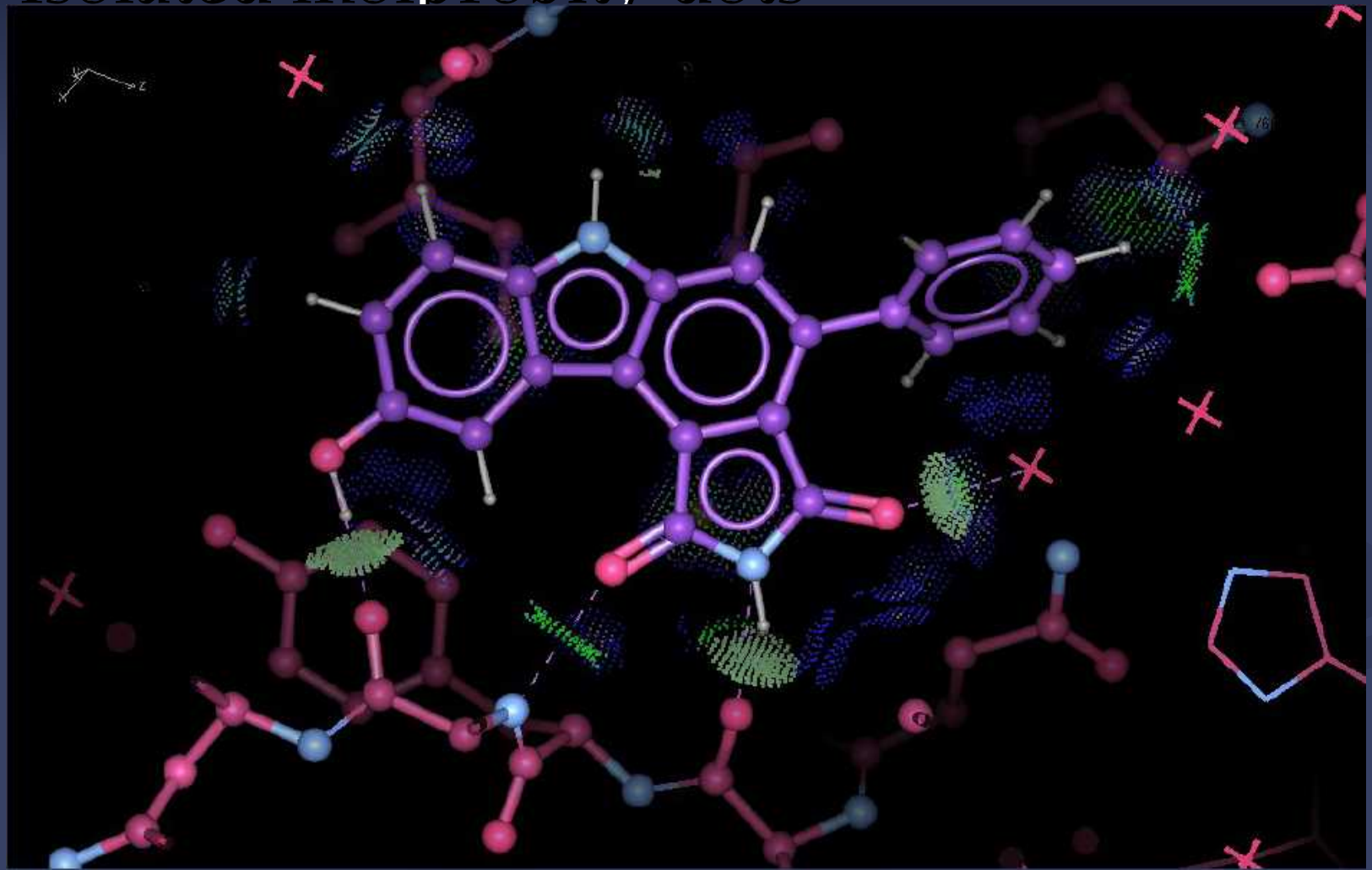
Surfaces

- Transparent surfaces – surface complementarity



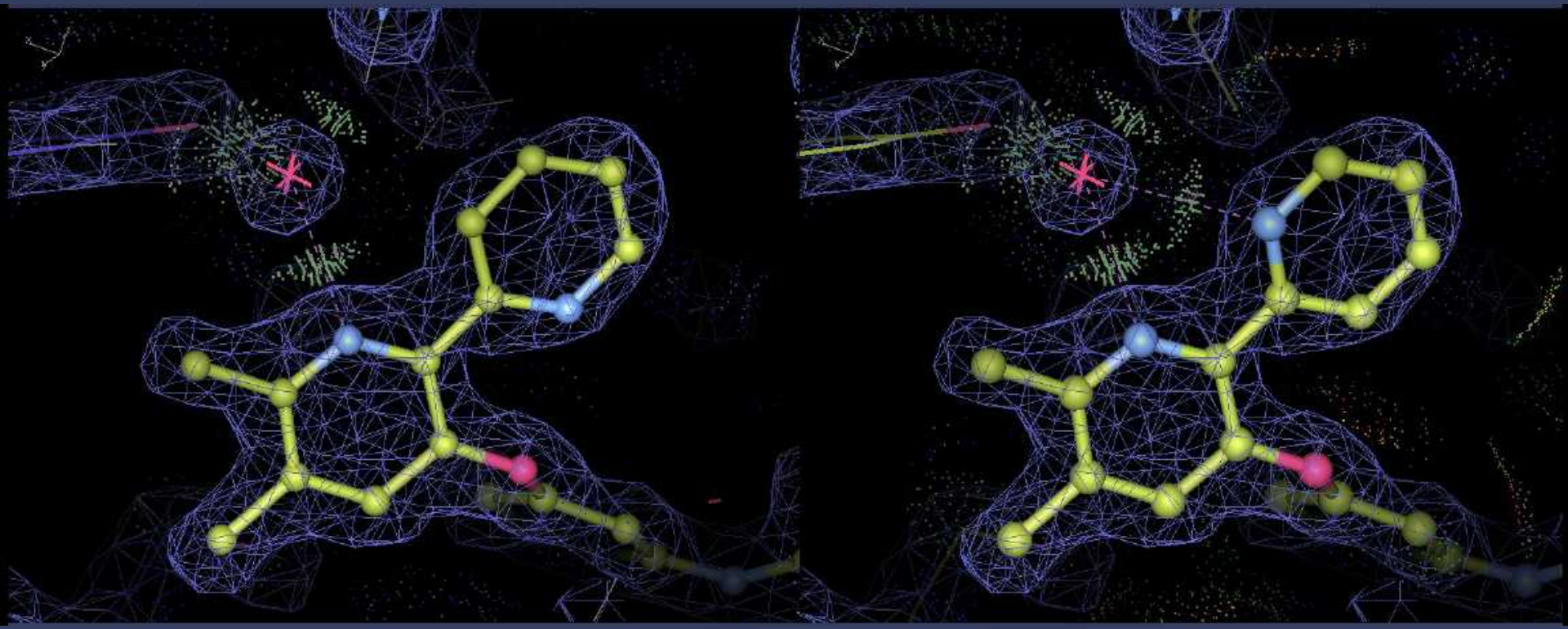
Binding mode analysis

- Binding site highlighting
- Isolated molprobity dots



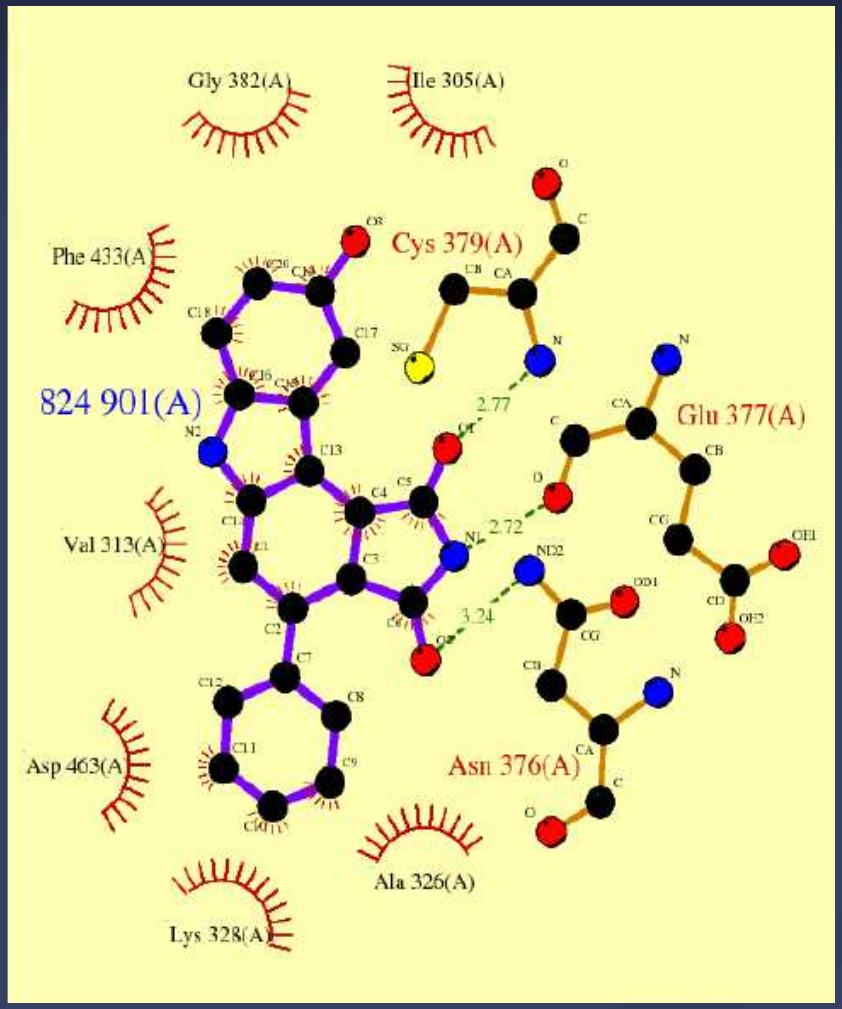
Binding mode analysis

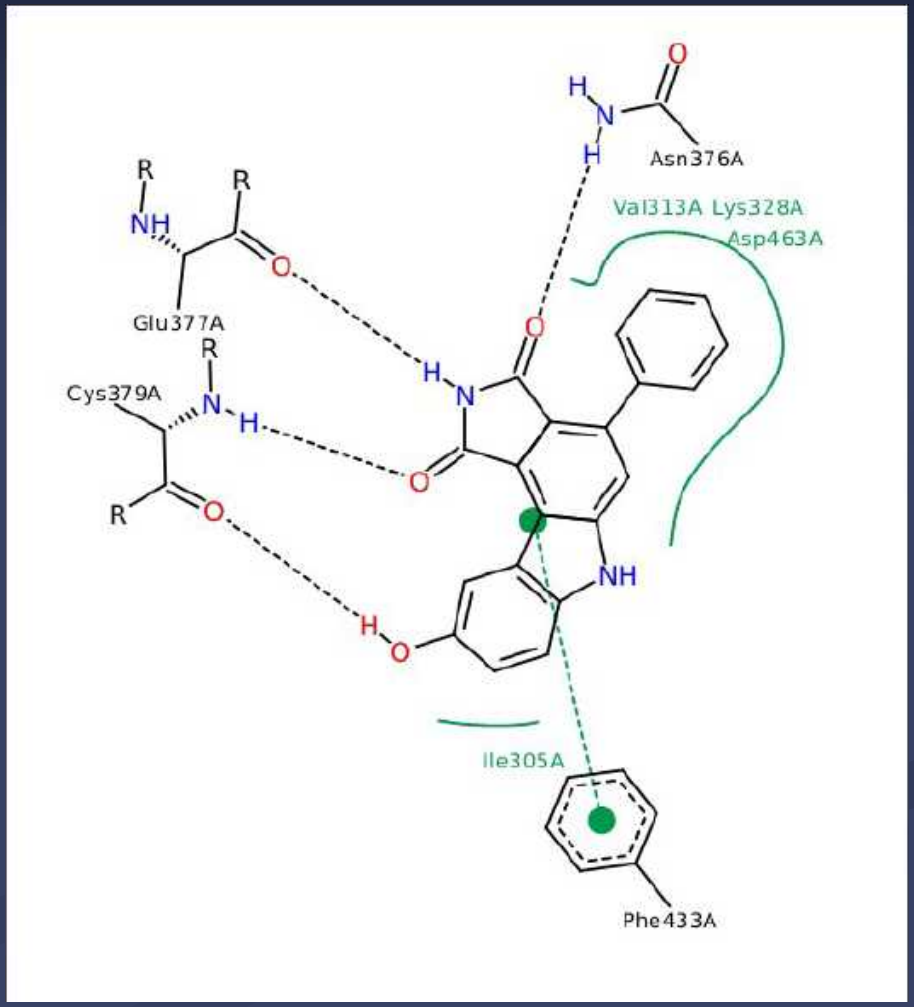
- Binding site highlighting
- Isolated molprobity dots



Ligand environment

- 2D ligand pocket layout (ligplot, poseview)





Can we do better?

Ligand environment layout

- Binding pocket residues
- Interactions
- Substitution contour
- Solvent accessible halos

Work in progress!!!!!

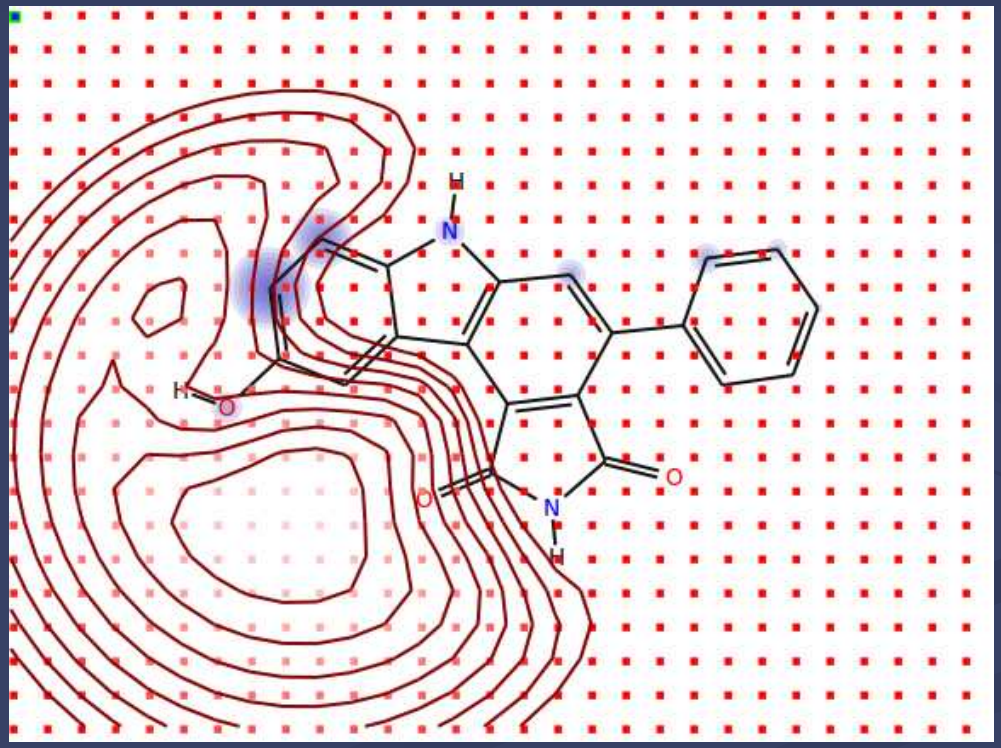
Ligand environment layout

- Considerations
  - 2D placement and distances should reflect 3D metrics
    - As much as possible
  - Residues should not overlap the ligand
  - Residues should not overlap each other
  - H-bonded residues should be close to atoms to which they are bonded
  - Etc.
  - c.f. Clark & Labute (2007); poseview

Work in progress

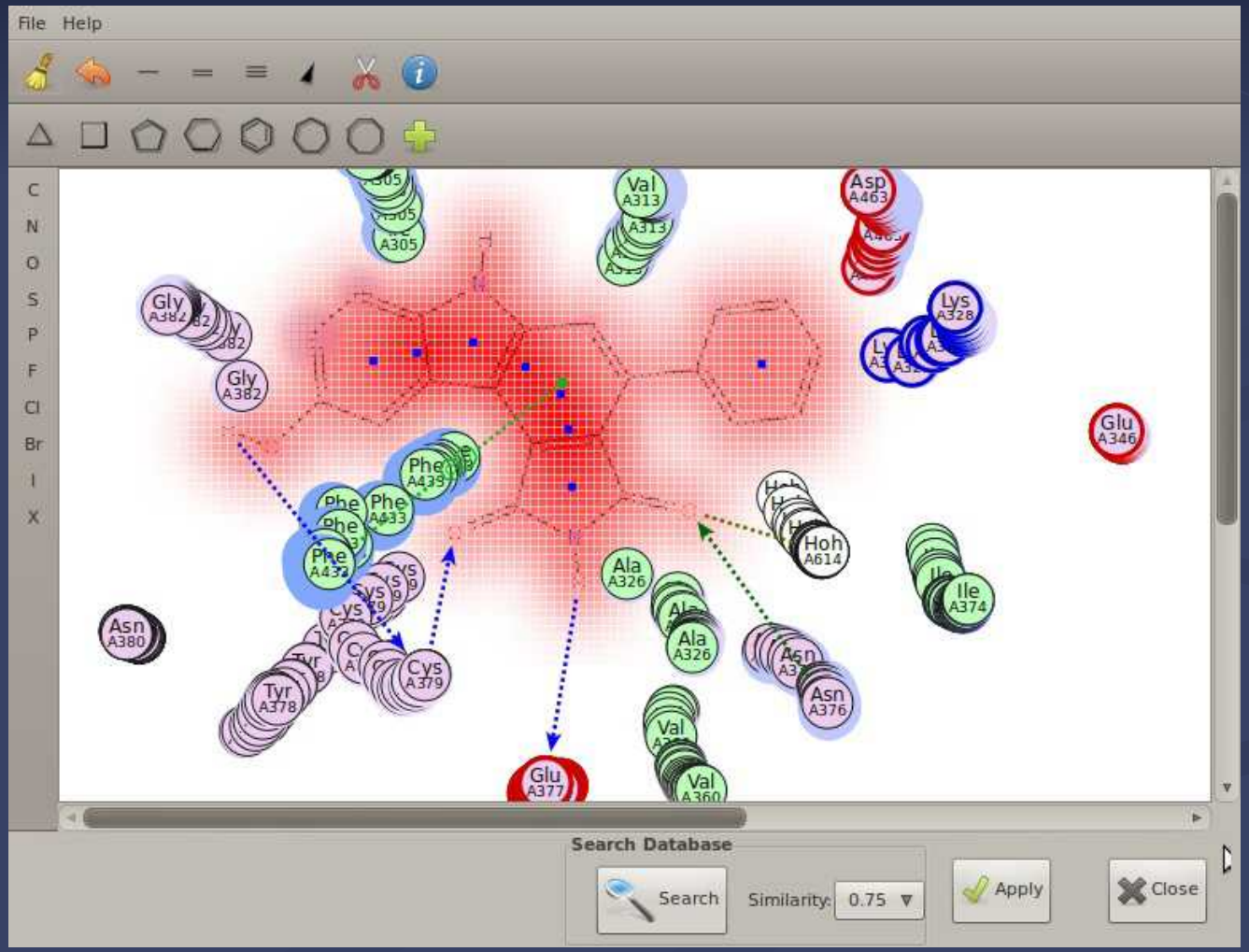
Ligand environment layout

- Initial residue placement



Ligand environment layout

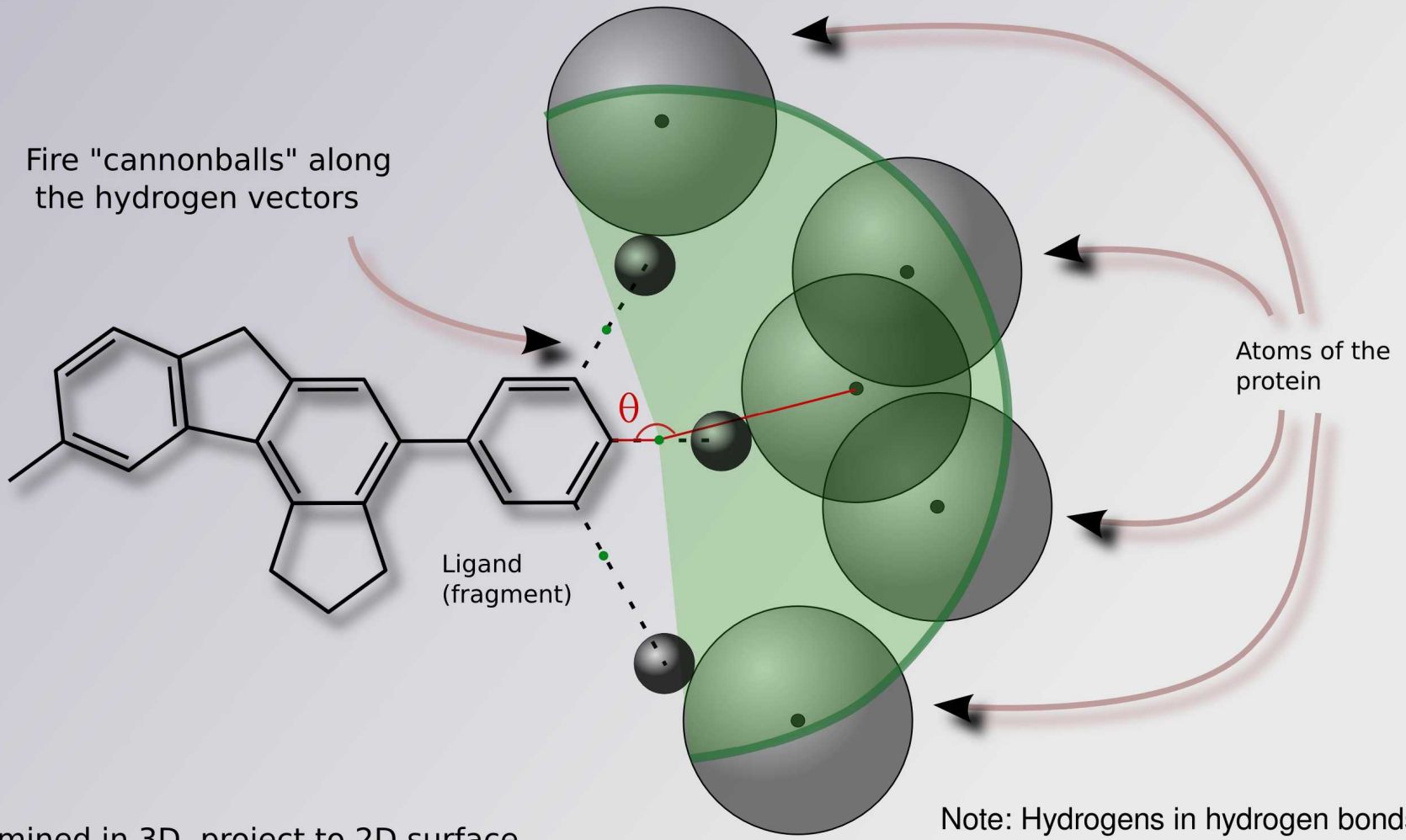
- Residue position minimization



Determination of the Substition Contour

c.f. Clarke & Labute (2007)

How far can we go (in the direction of the hydrogens) before hitting atoms of the protein?

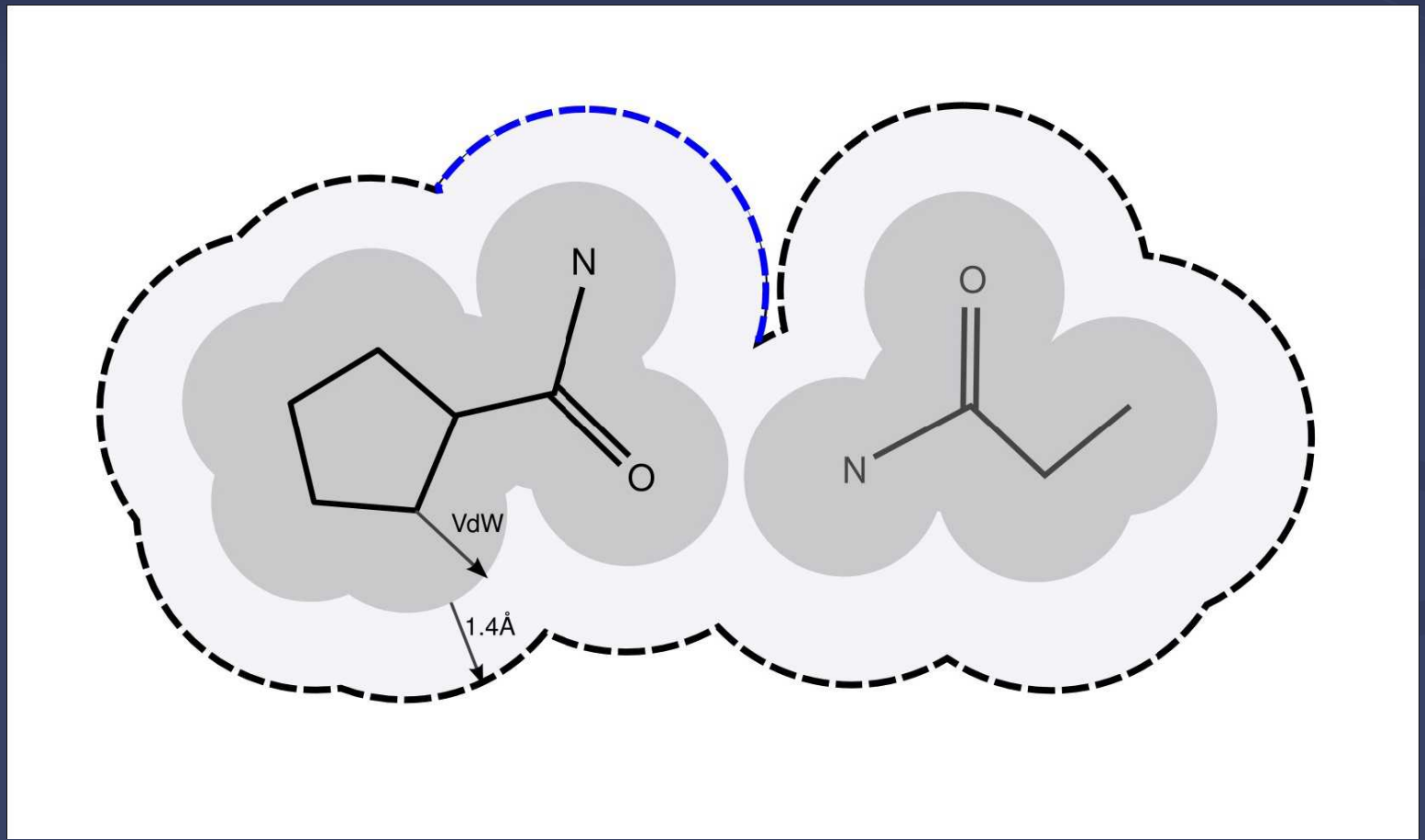


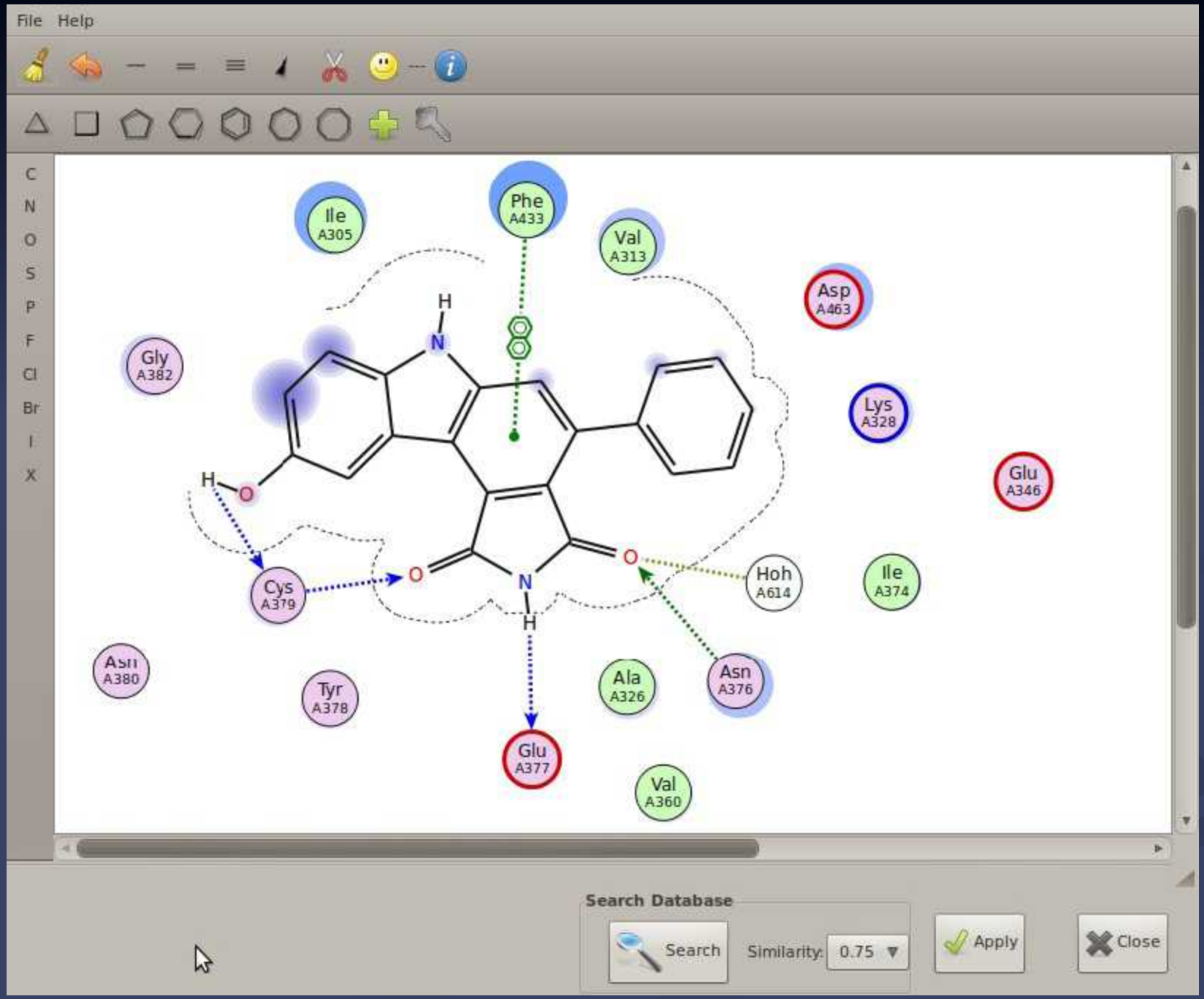
Determined in 3D, project to 2D surface

Note: Hydrogens in hydrogen bonds are a confounding factor

Solvent exposure calculation

- Identification of solvent accessible atoms
- Different from substitution contour




File Help
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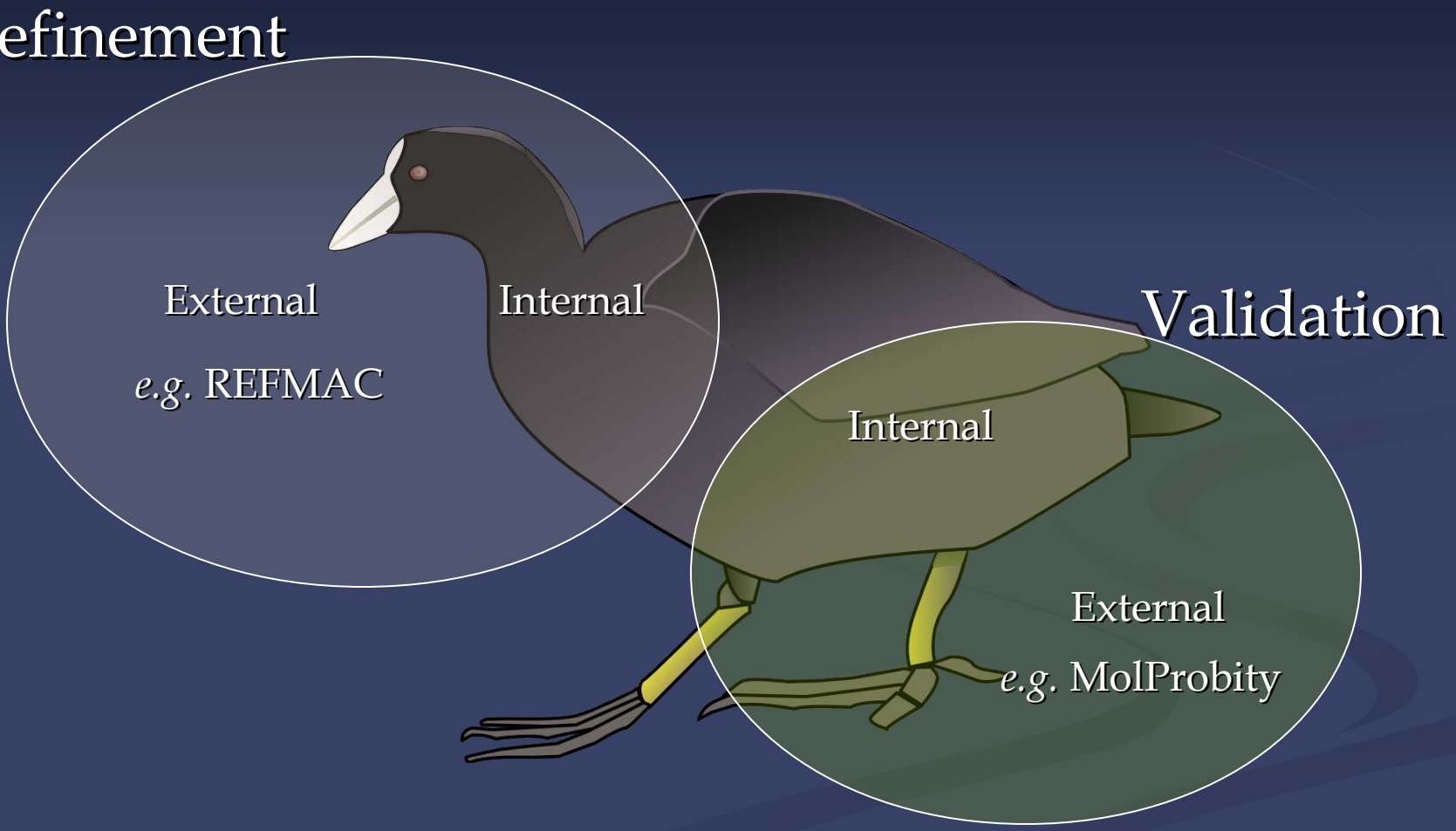
Glu A346
Cys A379
Hoh A614
Ile A374
Asn A380
Tyr A378
Ala A326
Asn A376
Glu A377
Val A360
Search Database
Search
Similarity: 0.75
Apply
Close

Ligand futures

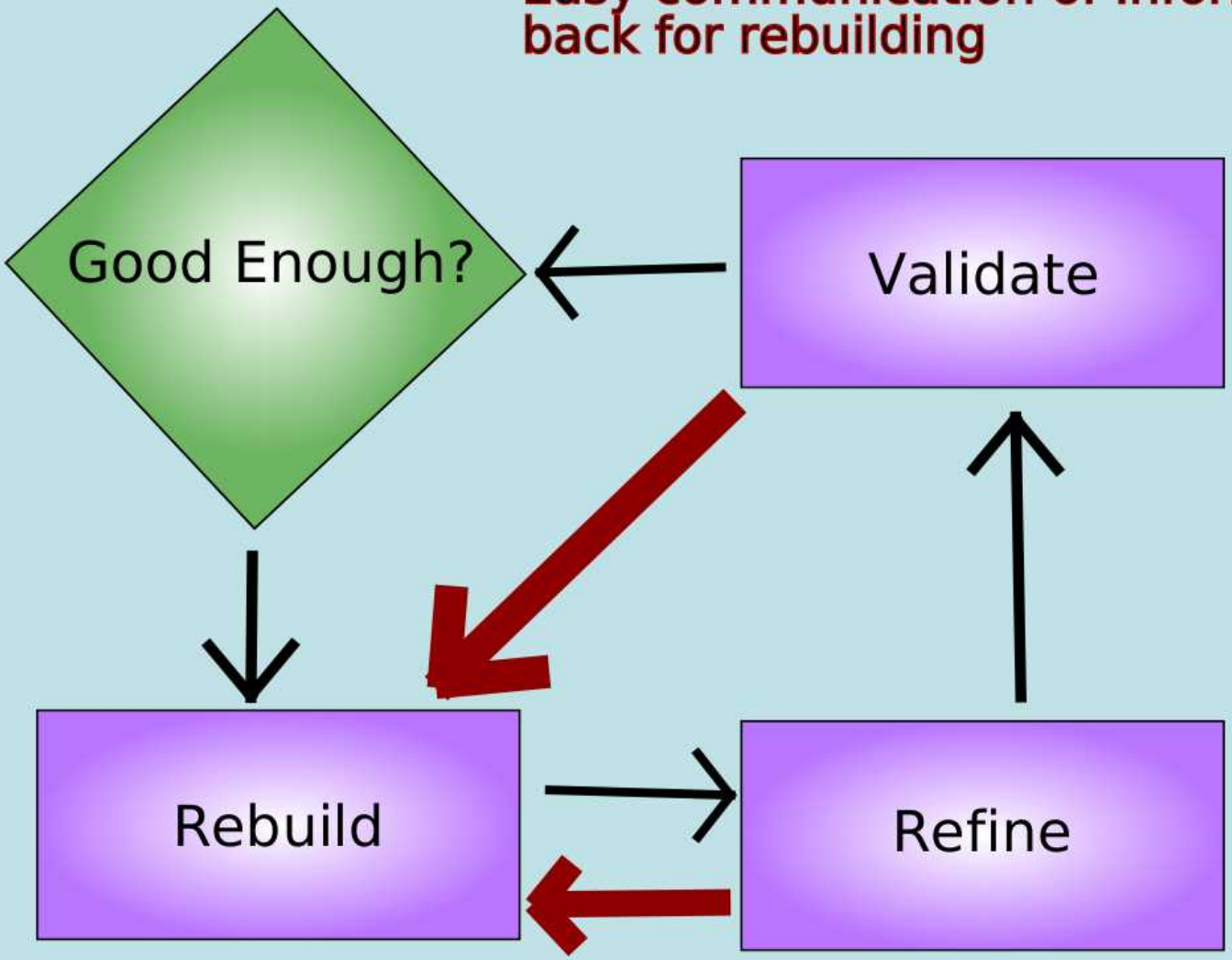
- Make ligand builder (and environment layout) available
- Interface to JLigand
- Ligand validation with CSD (Mogul or similar)

Validation

Feature Integration



Easy communication of Information back for rebuilding



What is Validation?

- Comparison of various aspects of the model with pre-conceived notions of "good quality"
  - Includes **unrestrained** and **restrained** criteria
  - Many aspects of validation overlap with refinement and model-building
  - Global and local indicators

Why Validate?

- Model-building is error-prone
  - (although automated methods seem to do better)
- Map interpretation is subjective
  - Someone else did the model-building
- The model was built several years ago
  - and the notion of "good quality" has changed
- Deposition requires validation

A “good” model

- Makes statistical sense
  - The reciprocal space representation agrees tolerably well with the observations (R-factor)
  - No meaningful difference map peaks (no under- or over-fitting)
- Makes chemical sense
  - Model geometry is consistent with the restraints
  - Ramachandran Plot has less than 1% outliers
  - A good **clashscore**
- Make physical sense
  - Crystal packing and contacts
- Makes biological sense
  - Residues in chemically sensible environment
  - Is consistent (on the whole) with external biochemistry observations (active site residues)

Quick Bayes

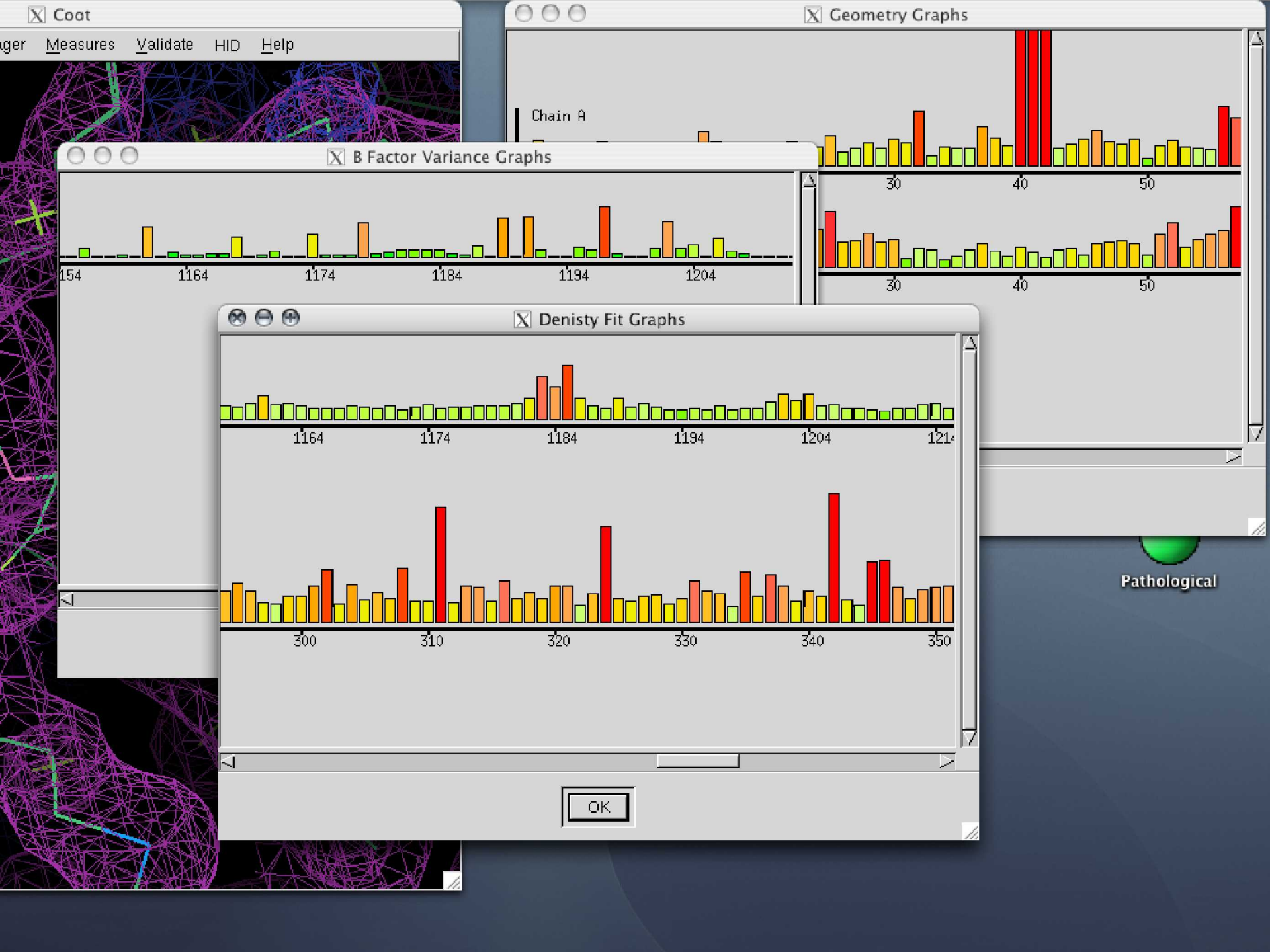
- Bayes Eq:
- $\Pr(\text{model} \mid \text{data}) \propto \Pr(\text{data} \mid \text{model}) * \Pr(\text{model})$
- $\Pr(\text{data} \mid \text{model})$ is also called the model likelihood, $L_M(\text{model} \mid \text{data})$

Validation Tools - Pr(model)

- Ramachandran Plot
  - Kleywegt Plot (NCS differences)
- Geometry Analysis
- Peptide ω Analysis
- Temperature Factor Analysis
- Rotamer Analysis
- Clashes

Validation Tools - Pr(data|model)

- Density Fit Analysis
- Difference Map Peaks
  - Variance analysis at water positions
- Unmodelled blobs


Coot
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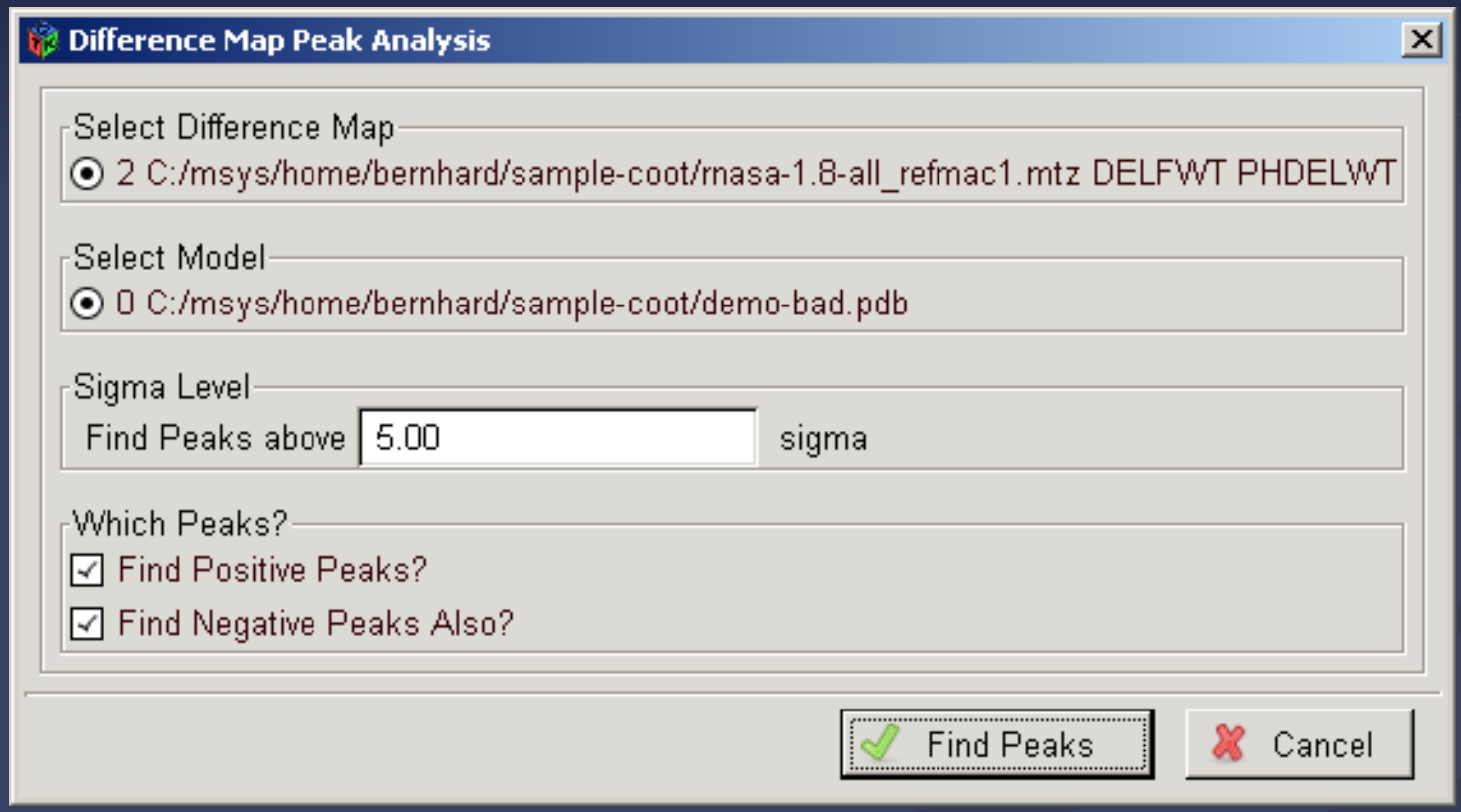
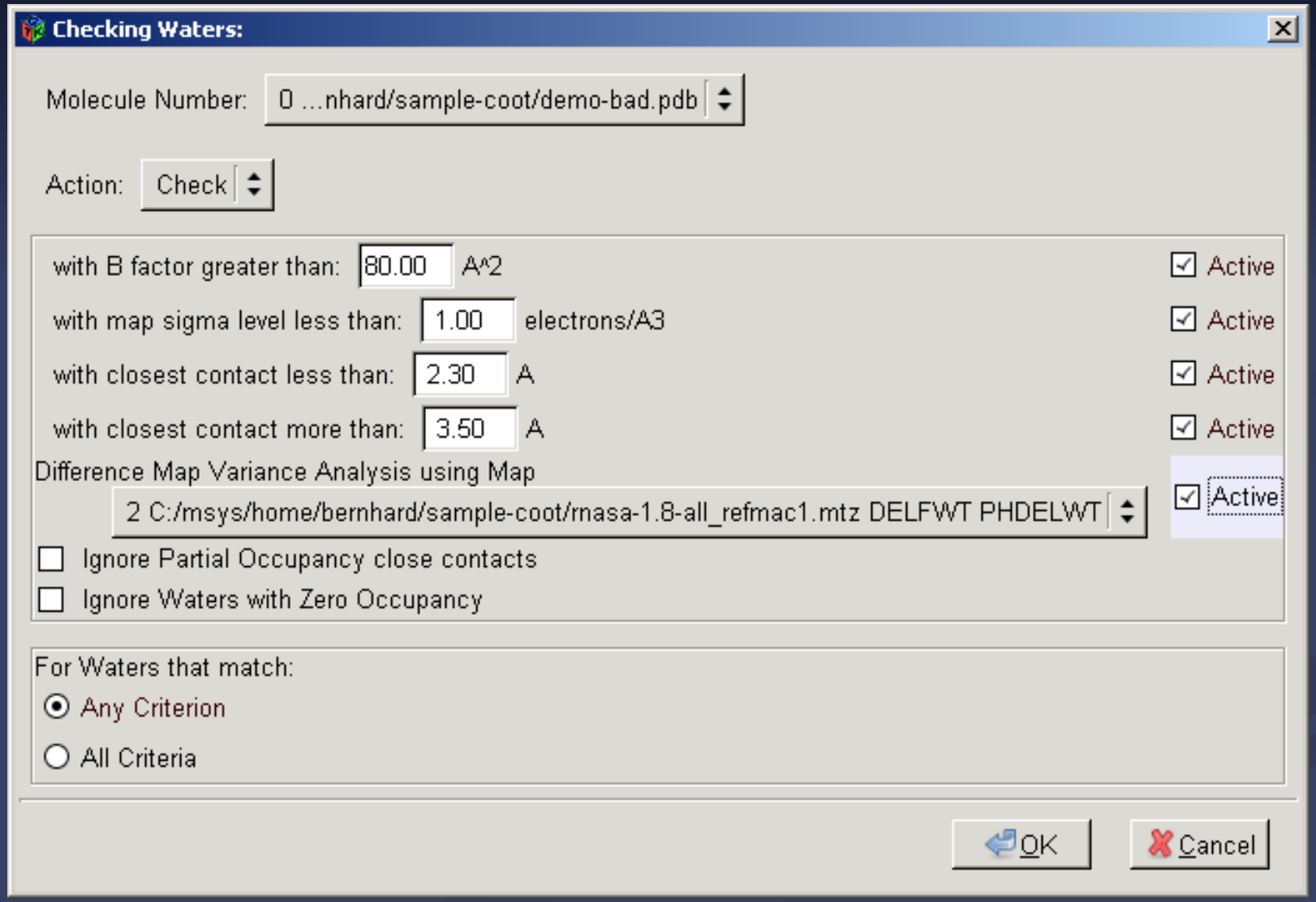
OK
Pathological

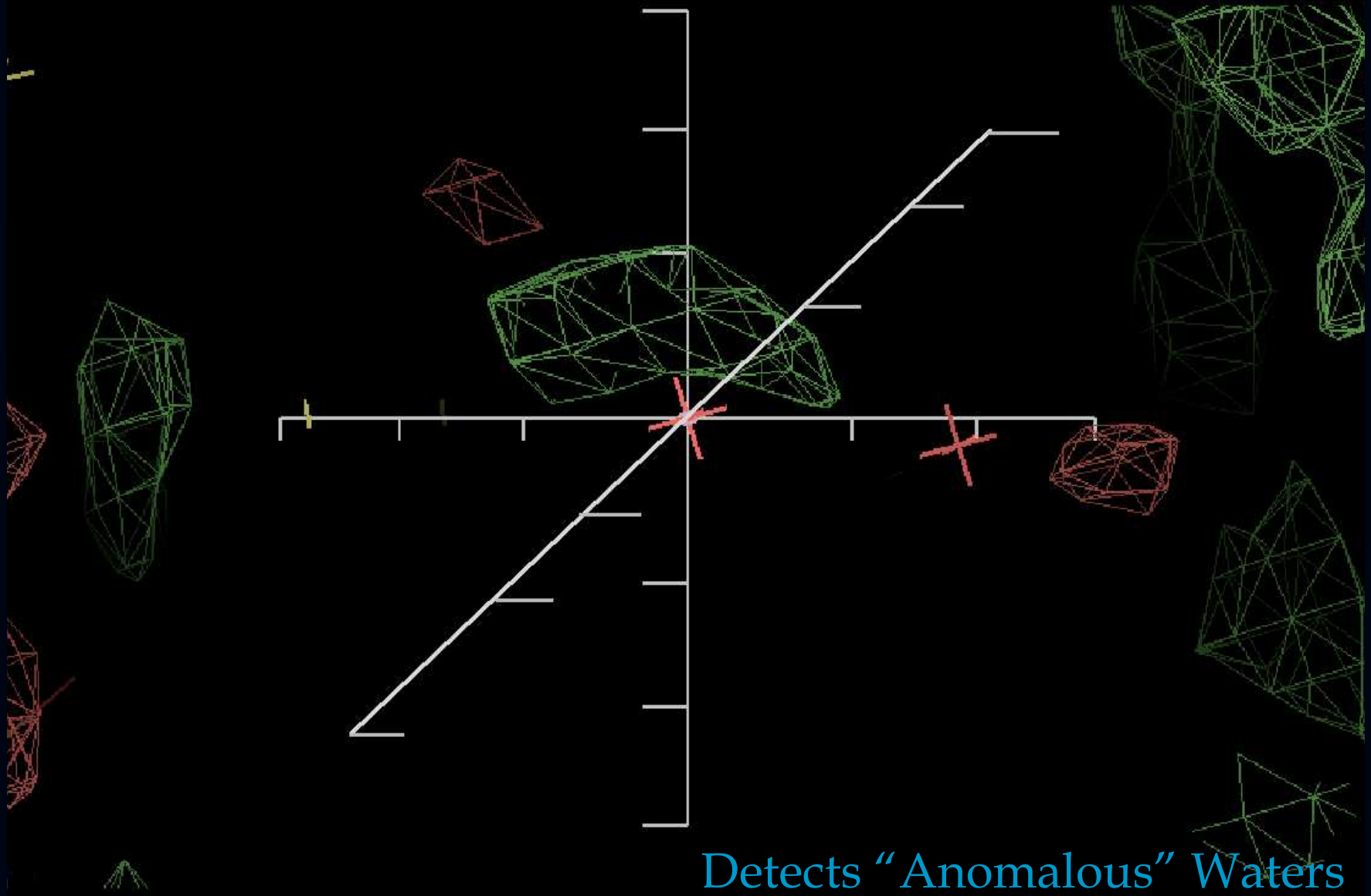
Find difference map peaks



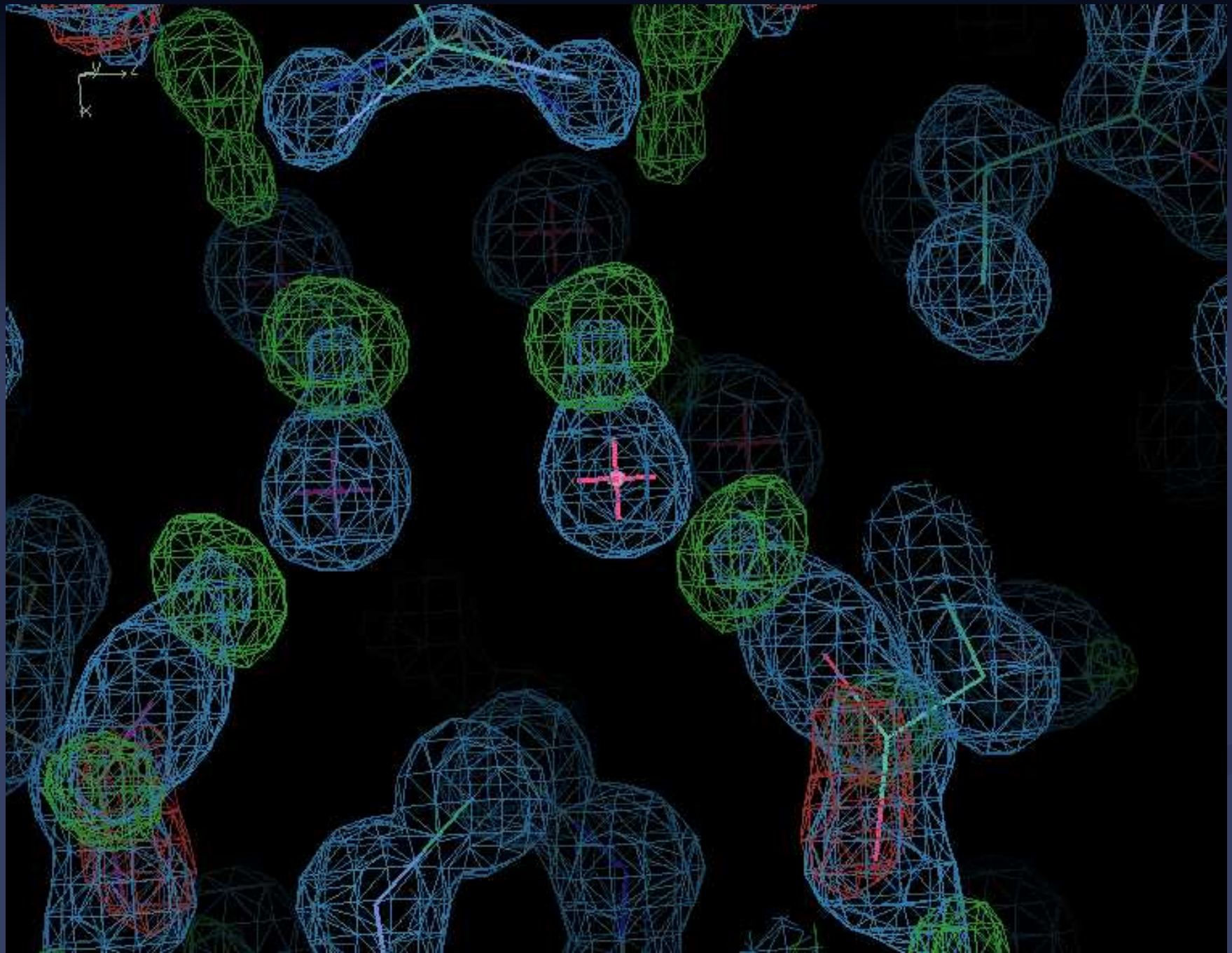
Check/Delete Waters

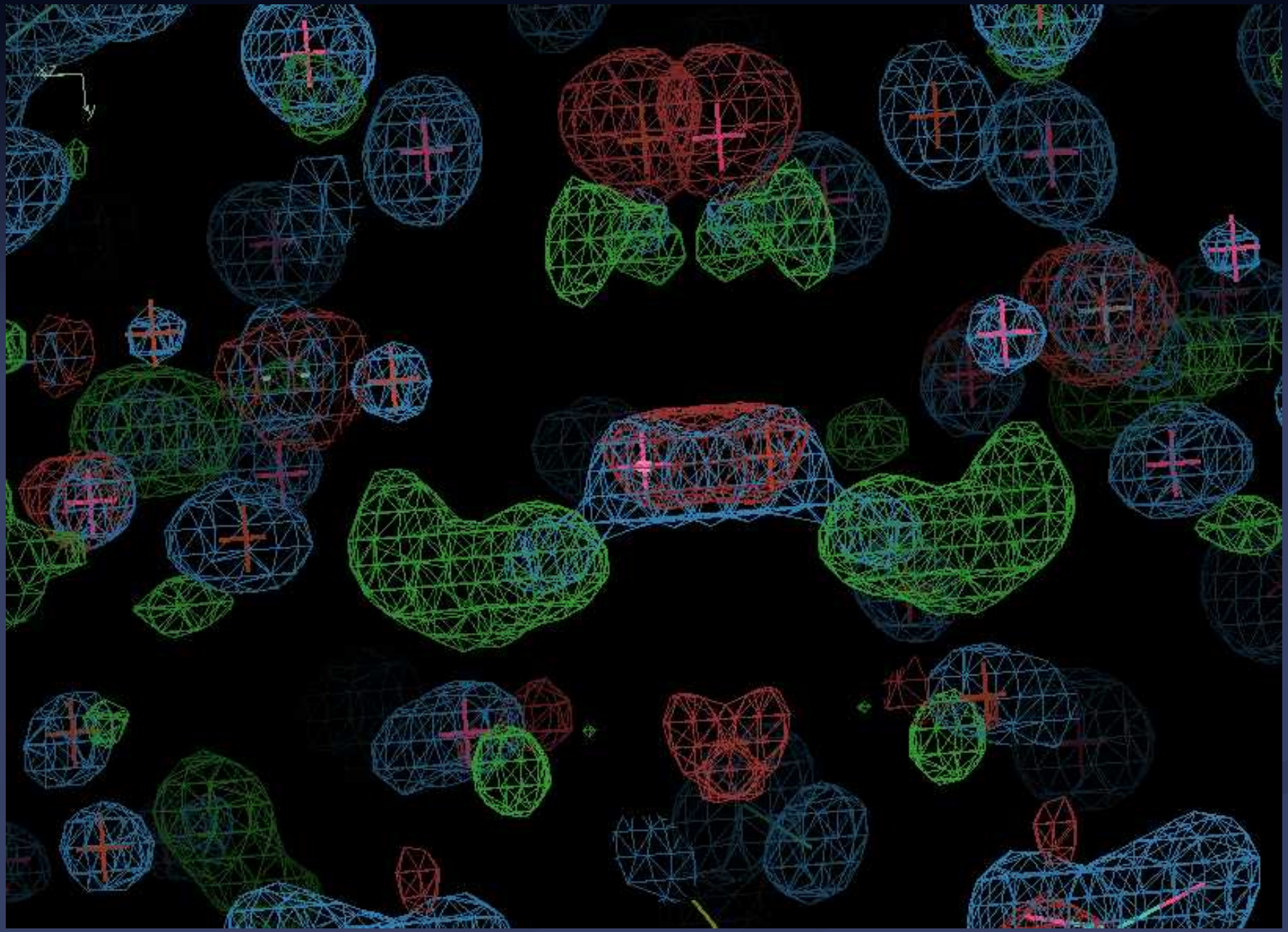


Difference Map Sampling

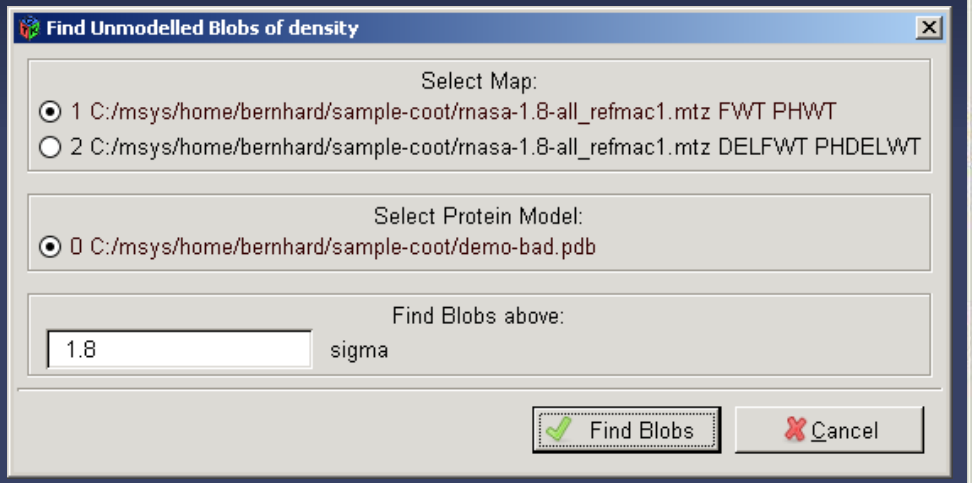


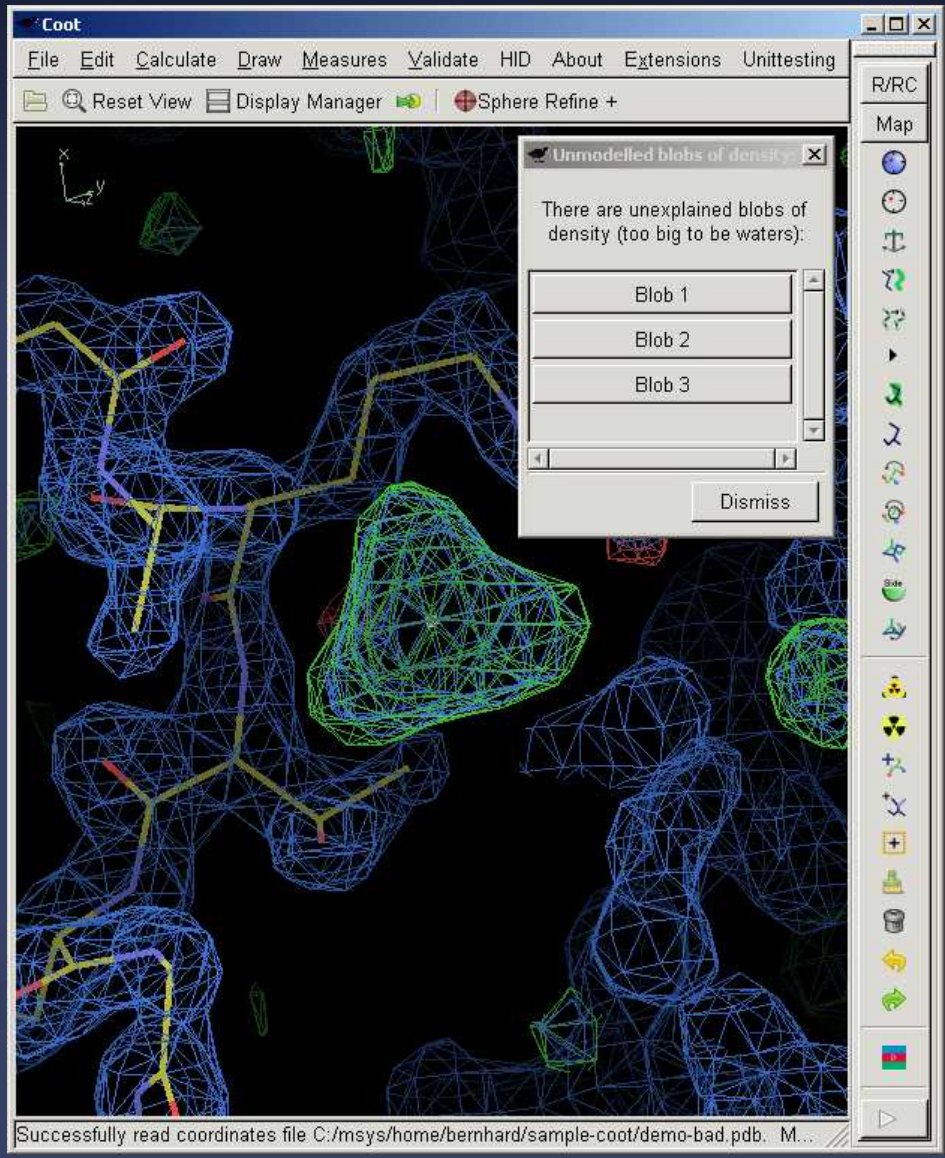
Detects “Anomalous” Waters





Find unmodelled blobs

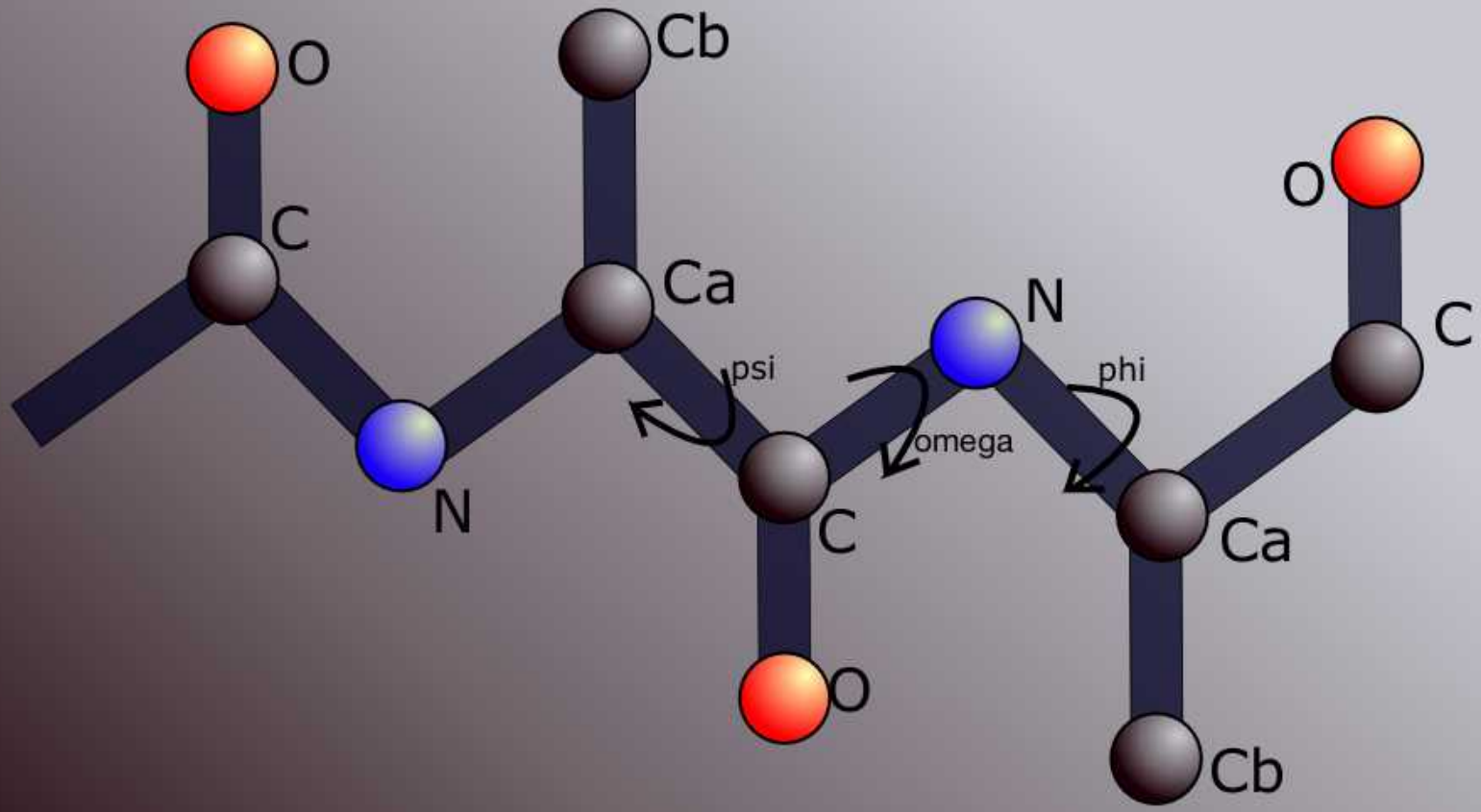




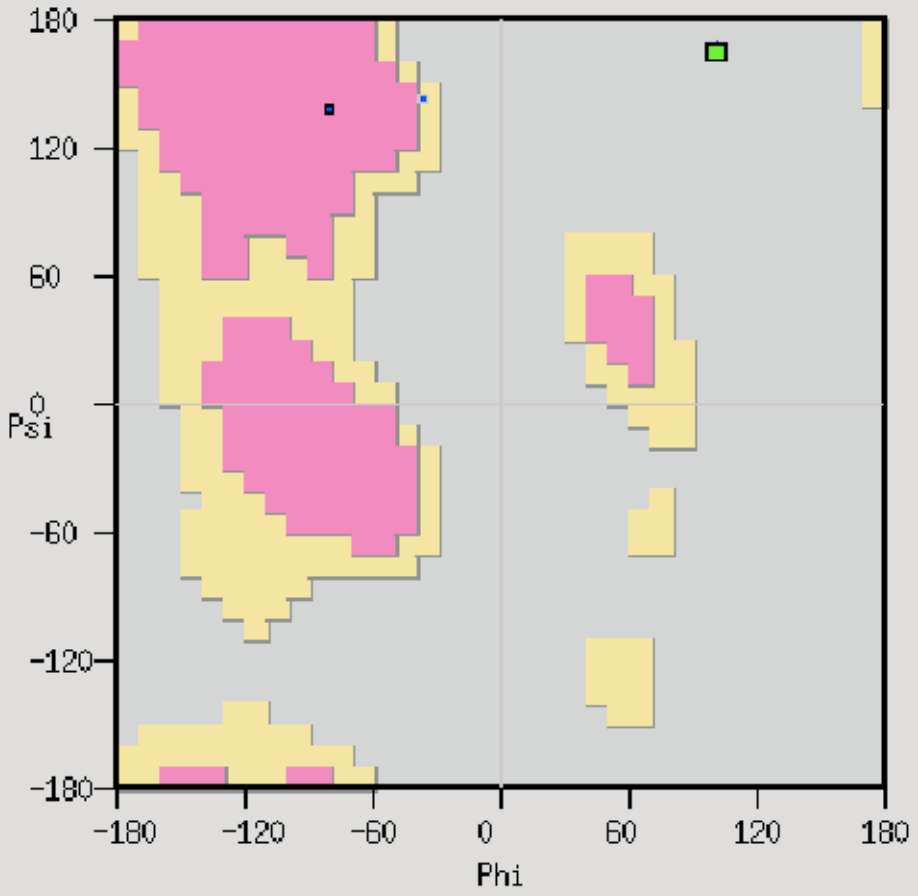
Pr(model)...

- Ramachandran Plot
  - Kleywegt Plot (NCS differences)
- Geometry Analysis
- Peptide ω Analysis
- Temperature Factor Analysis
- Rotamer Analysis
- Sequence analysis
- Clashes

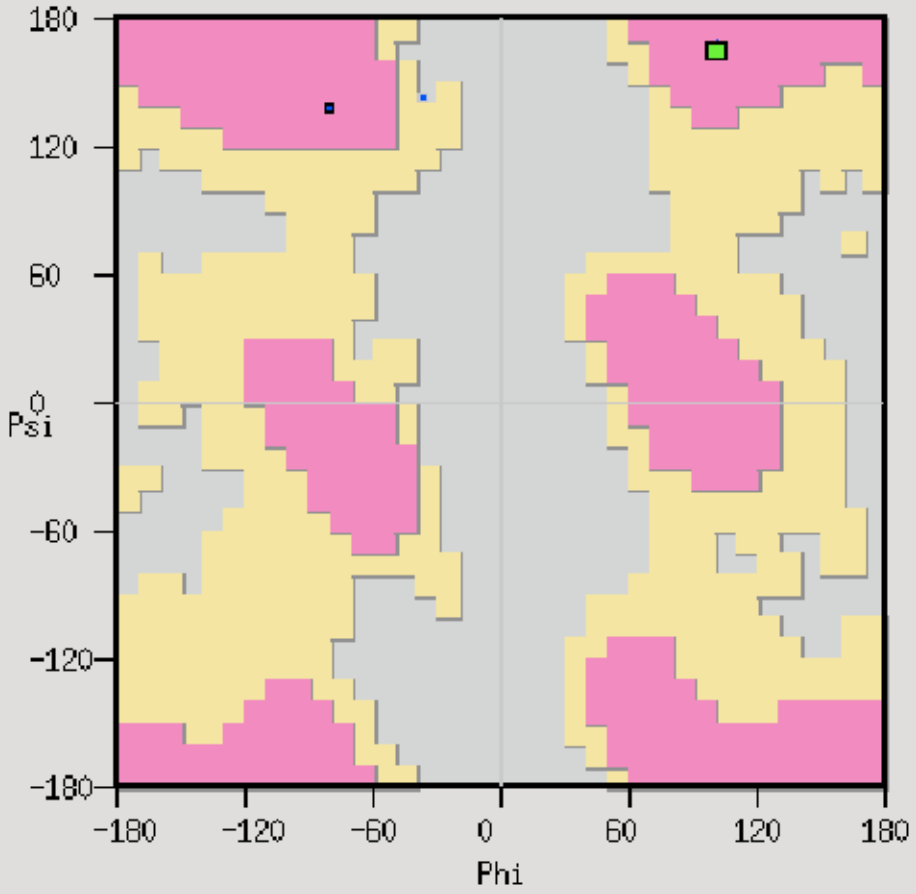
Peptide Torsion Angles



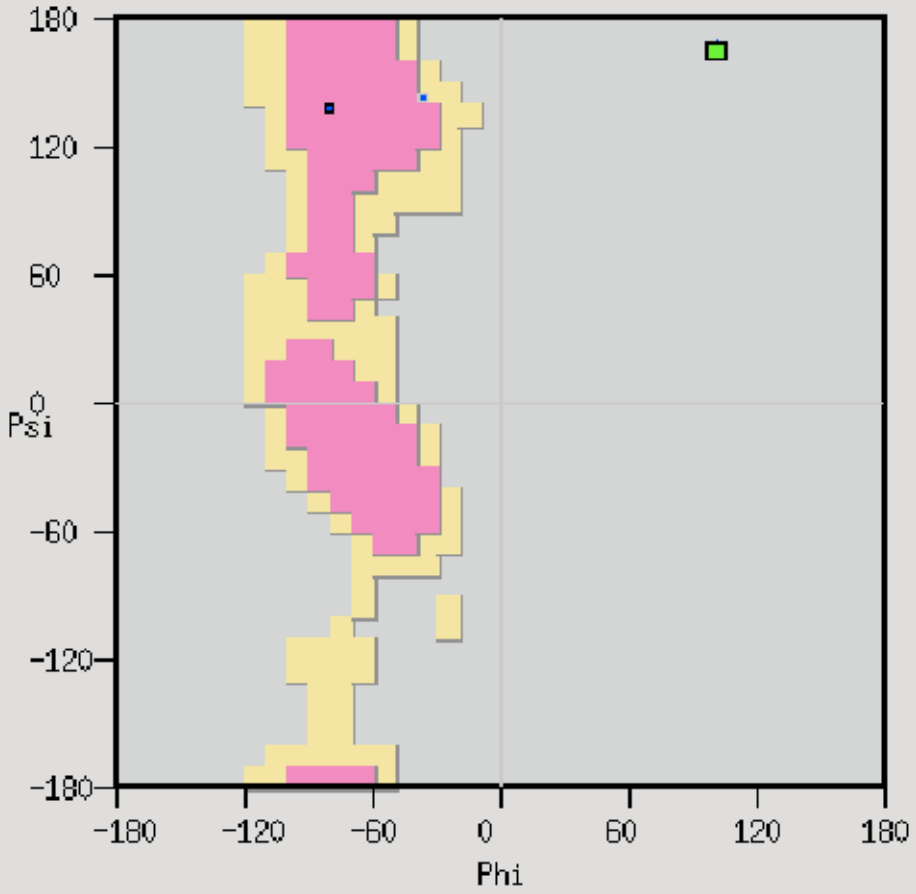
Ramachandran Plot for residues with CB

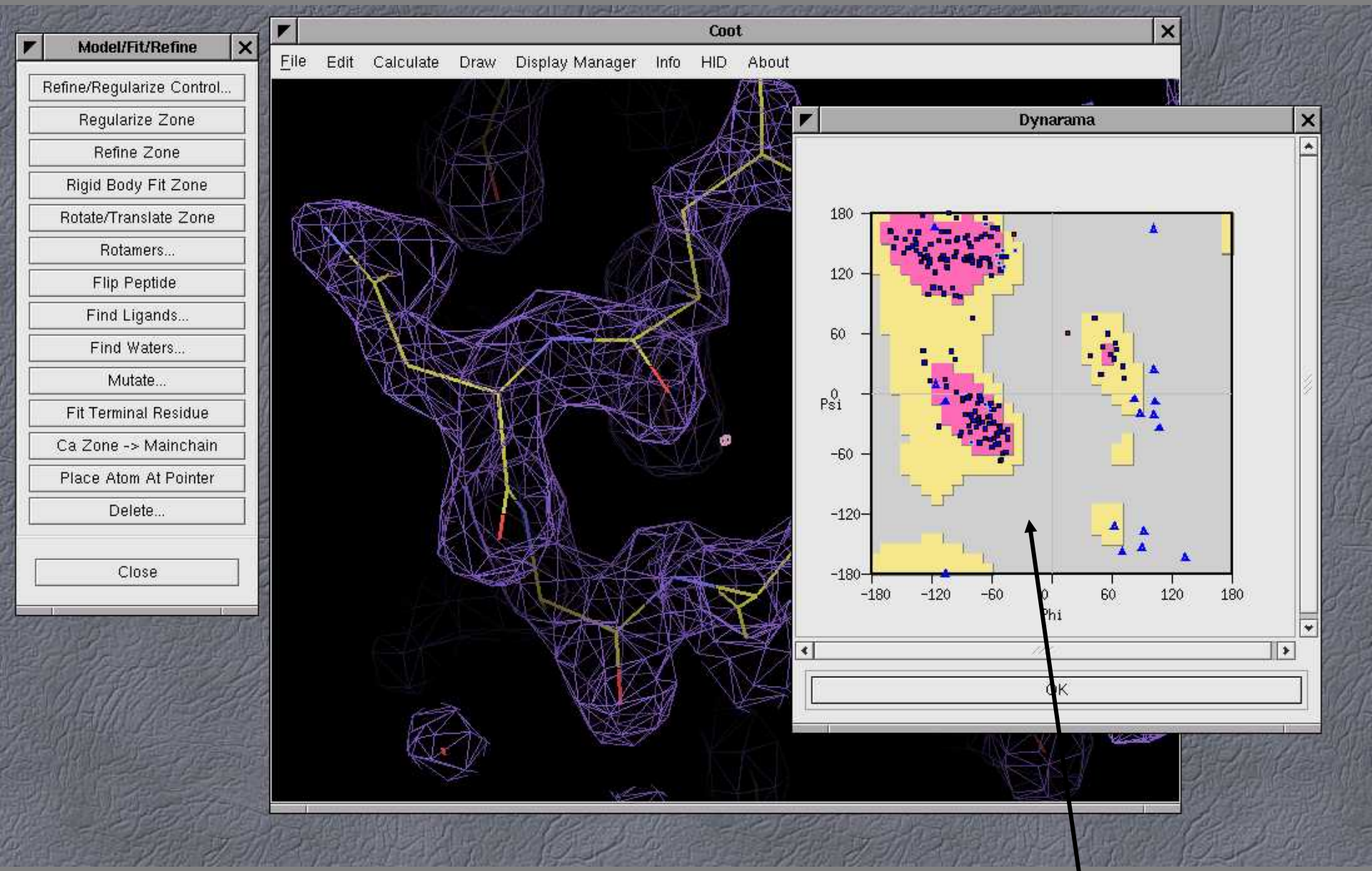


Ramachandran Plot for GLY



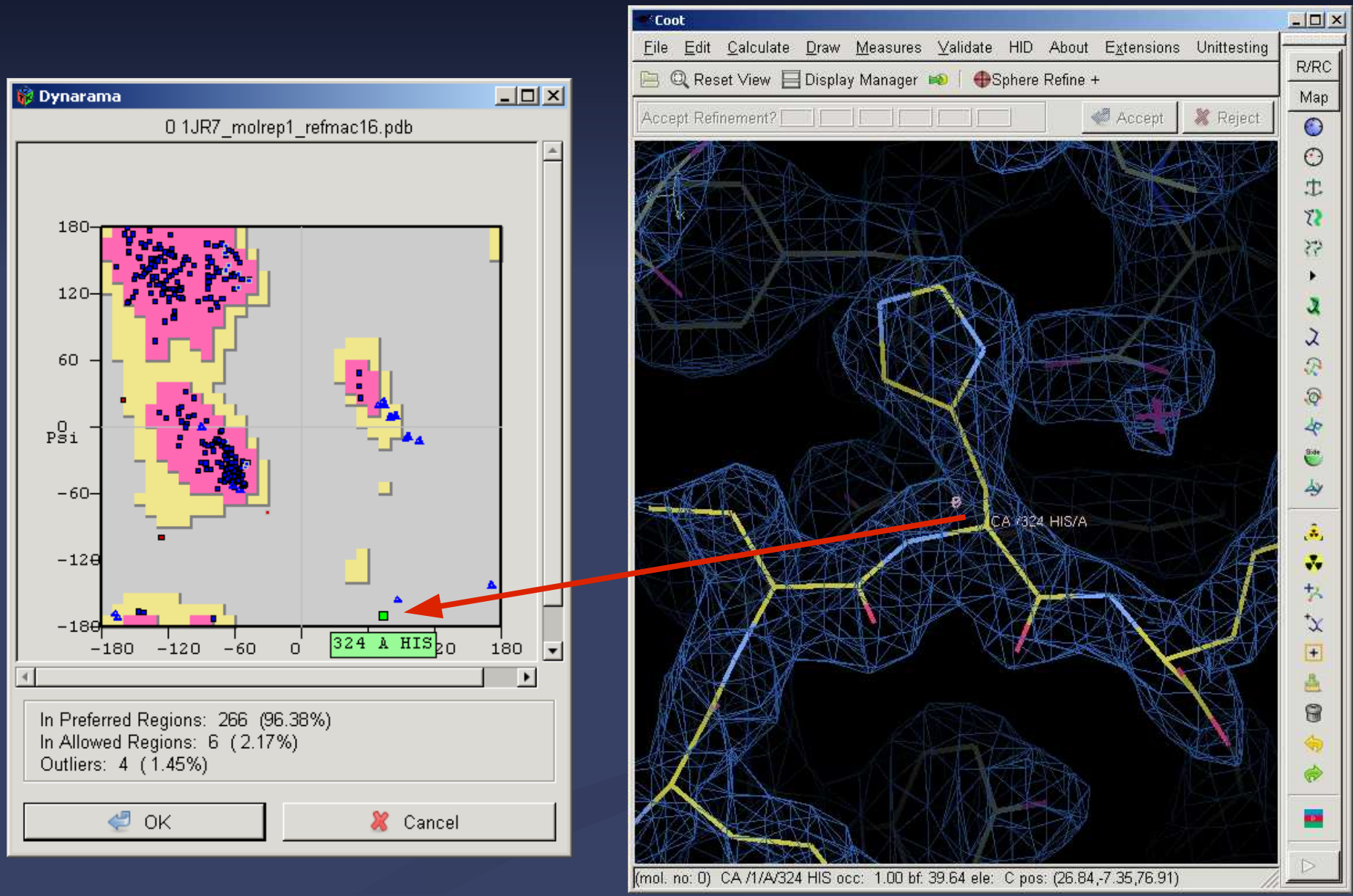
Ramachandran Plot for PRO



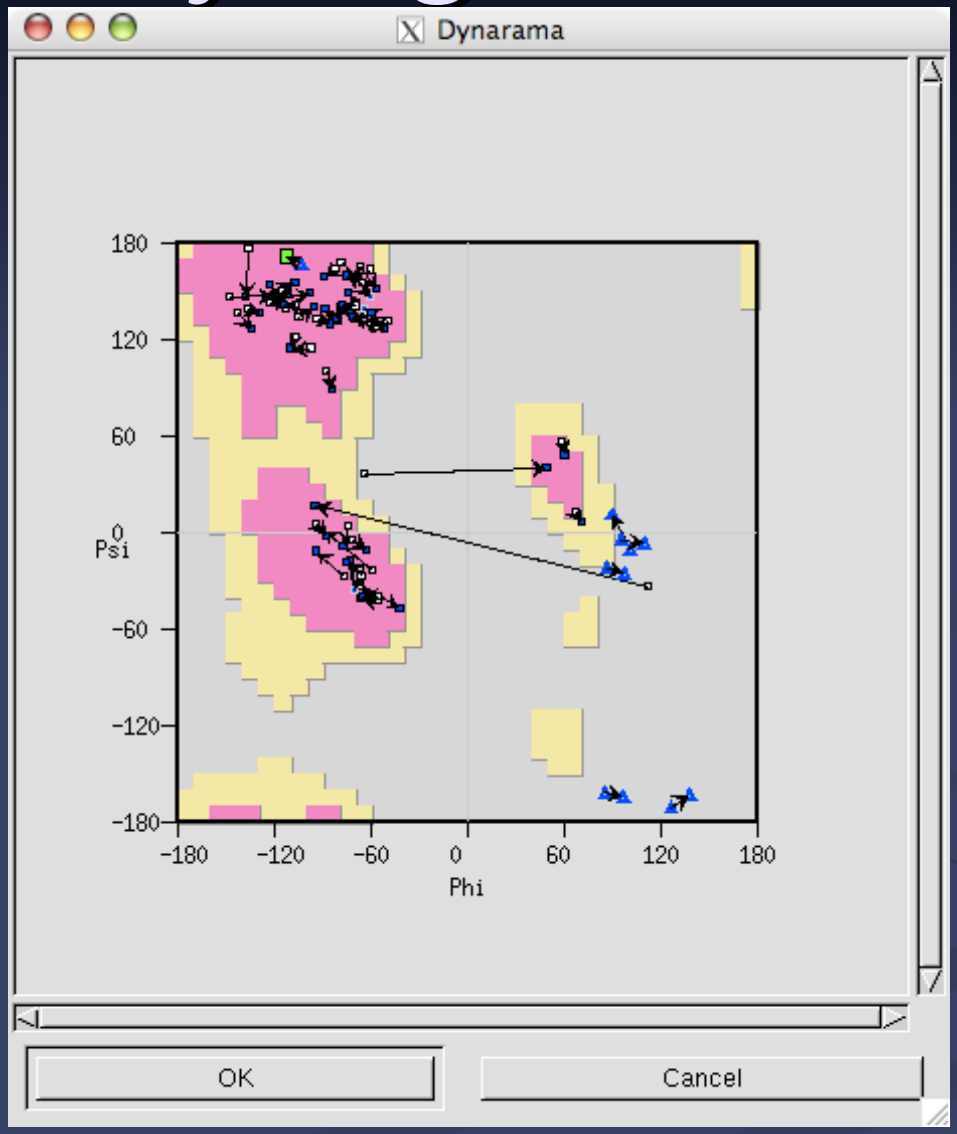


Top500-based distribution

Not all outliers are bad

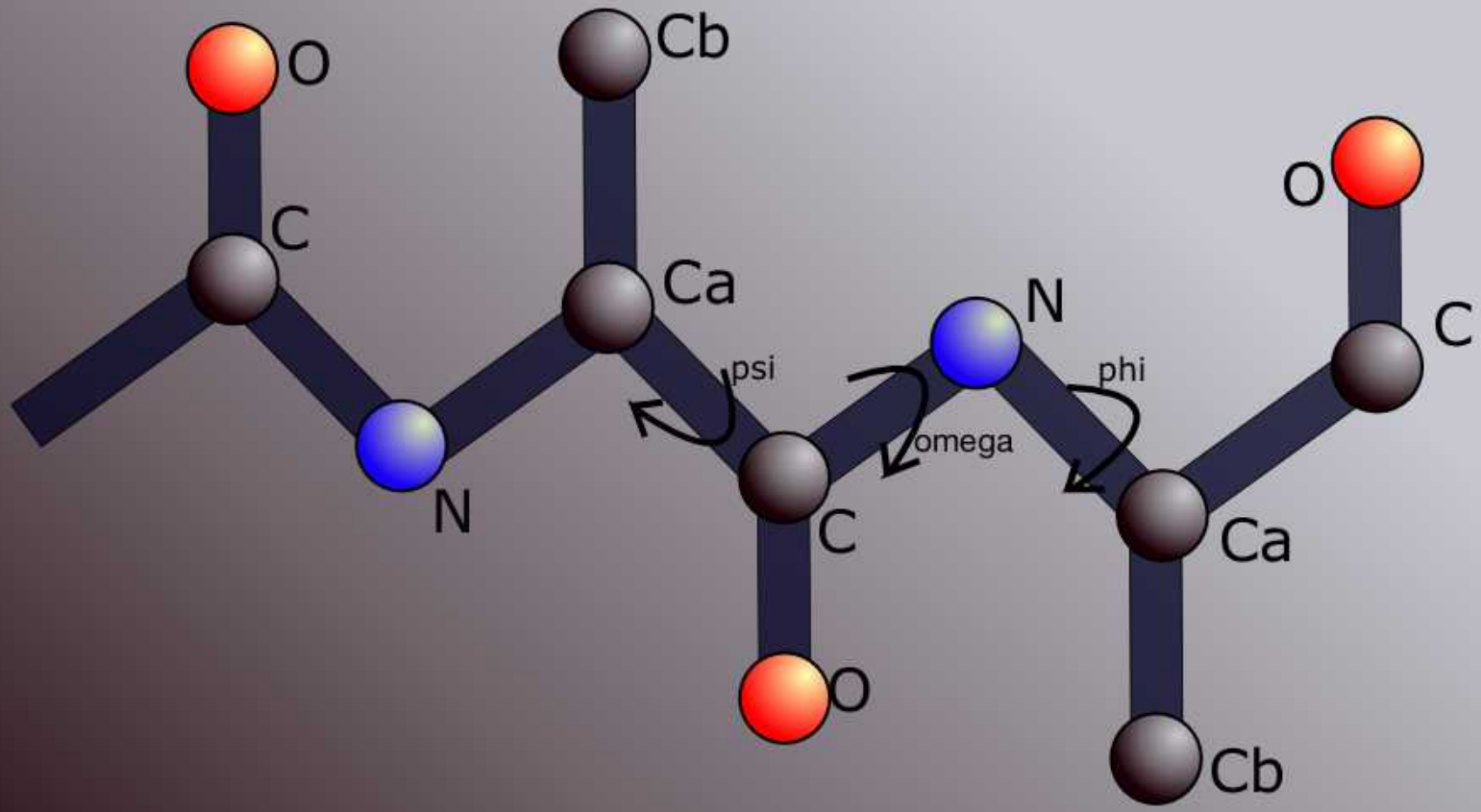


Kleywegt Plots[*]



[*] Named by George Sheldrick

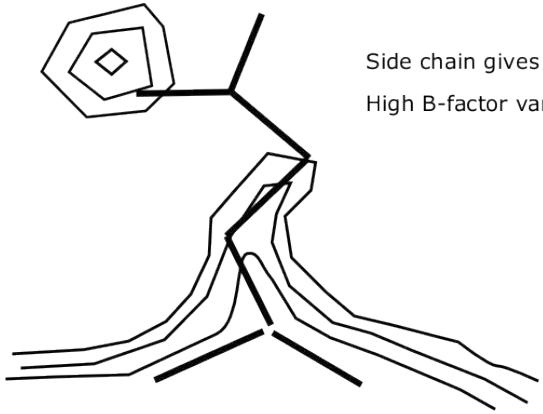
Peptide Torsion Angles



Peptide ω

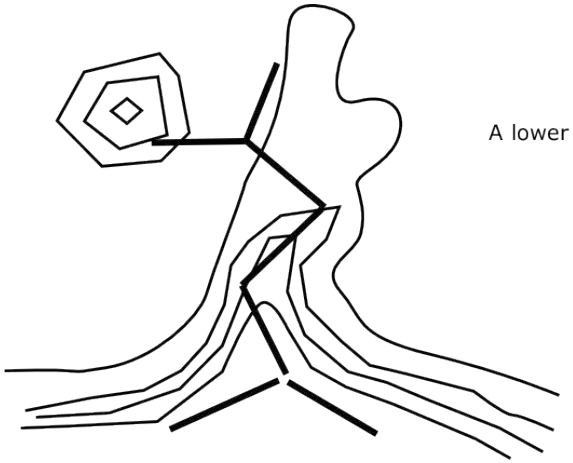
- Needed to check the planarity of the peptide link
  - At low resolutions it is possible to give the protein lots of (too much) freedom to optimize the fit to the density
- Can accidentally create CIS peptides
  - When discovered they are easily reconverted using the CIS<->TRANS peptide tool
- Less accidents happen when peptide plane restraints are applied

B-factor variance

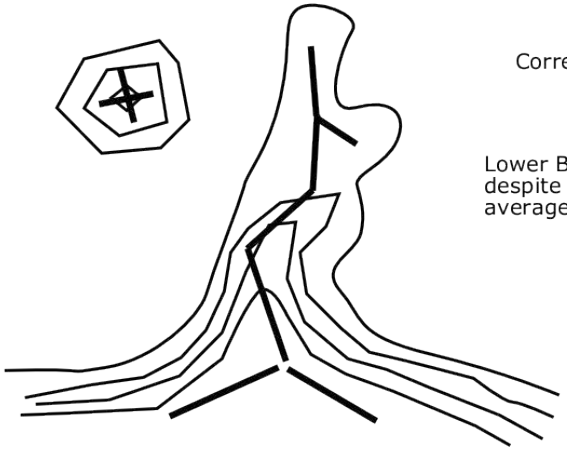


Side chain gives us concern

High B-factor variance



A lower contour level



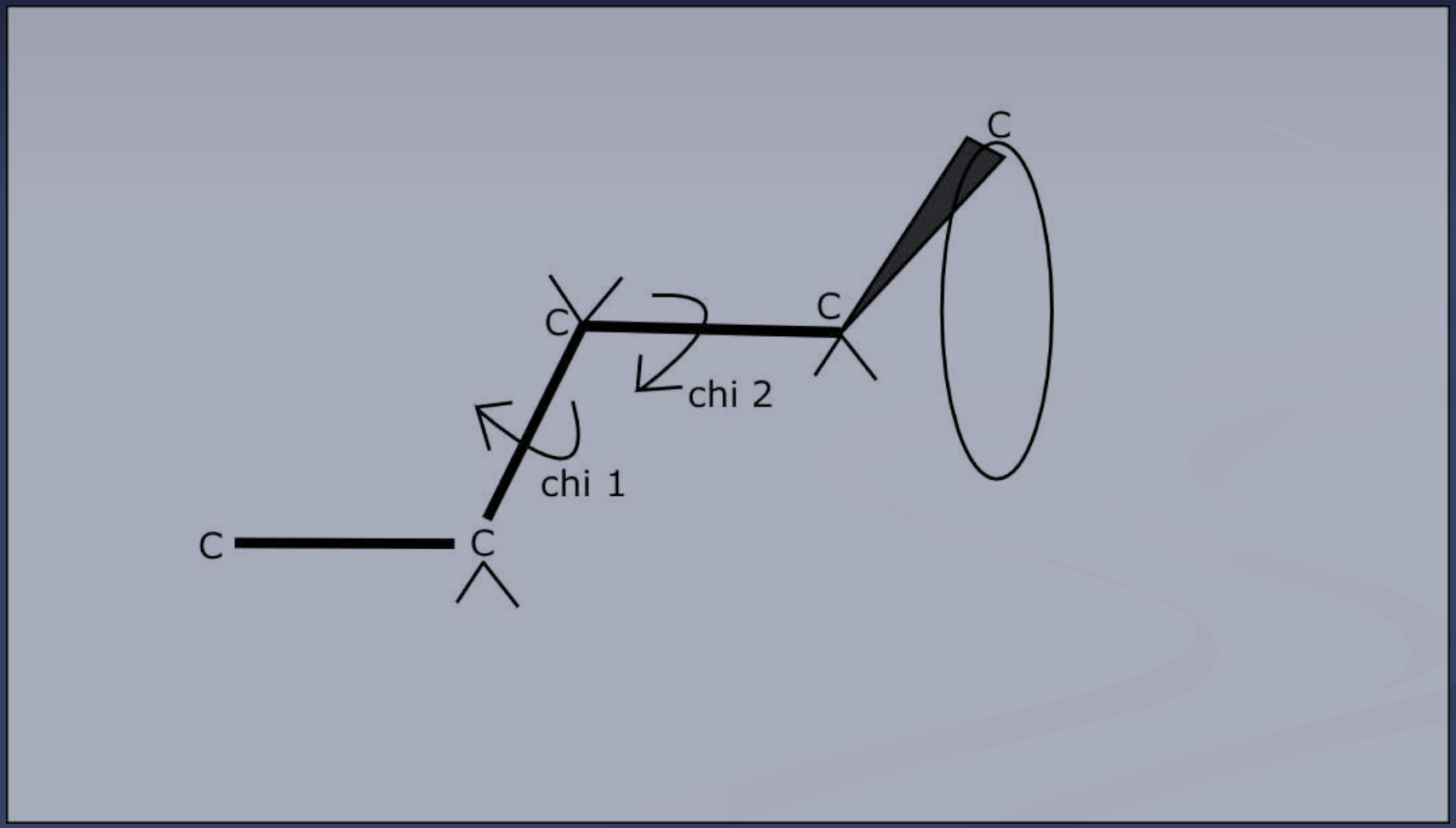
Correctly modelled side-chain

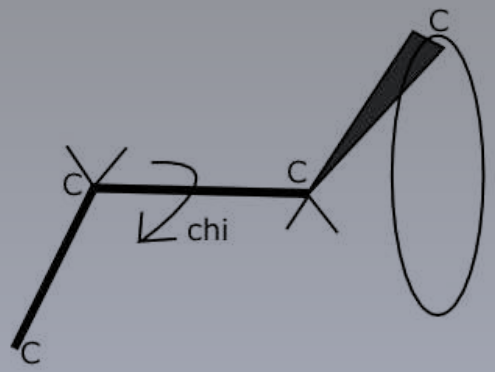
Lower B-factor variance
despite potentially higher
average B-factor

Rotamers

- Side-chains have certain preferred combinations of torsions round their rotatable bonds
- An analysis (batched around the staggered conformations) will give rotamer occurrence

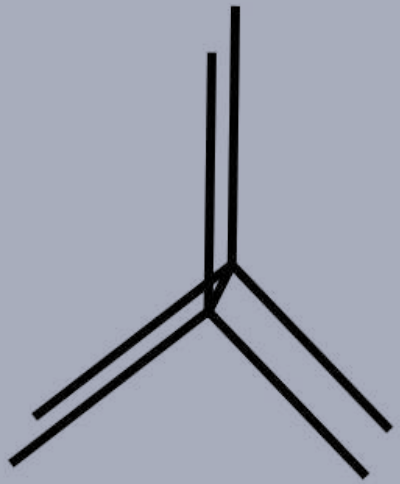
Torsion-based Validation





In principle, there is free rotation

In practice, staggered is energetically more favoured

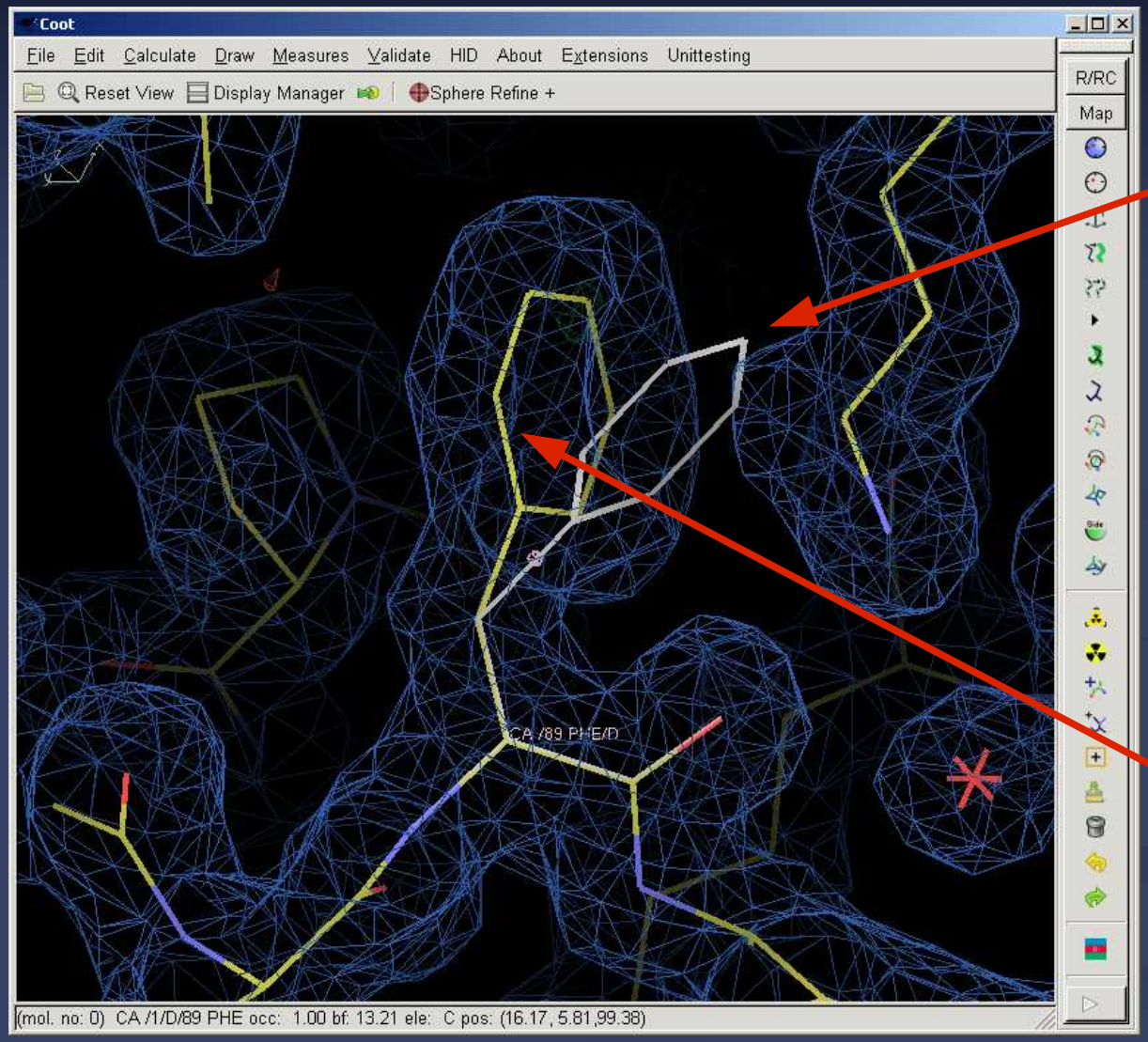


Eclipsed

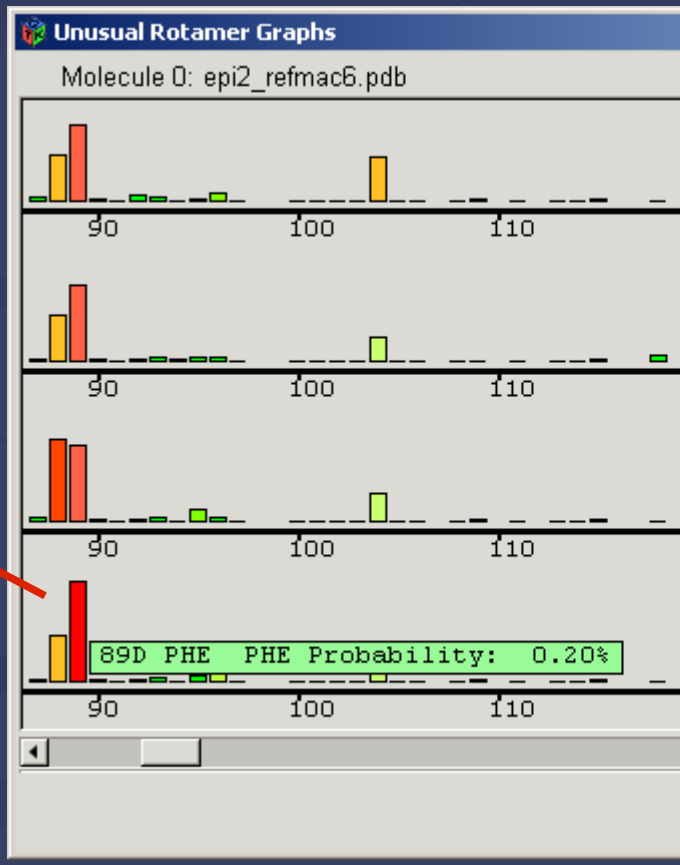
Staggered

Most favoured staggering angles 60, 180, -60 degrees

Not all outliers are bad

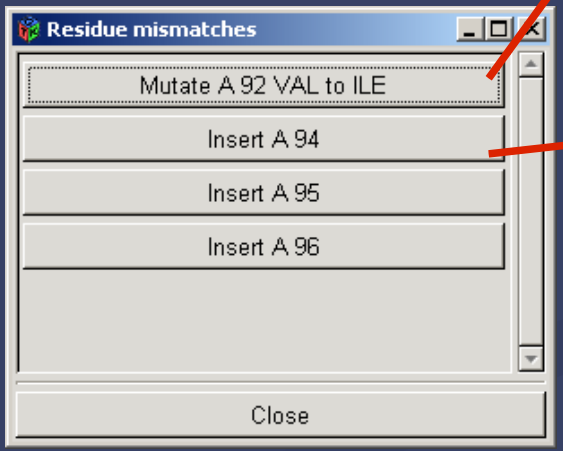


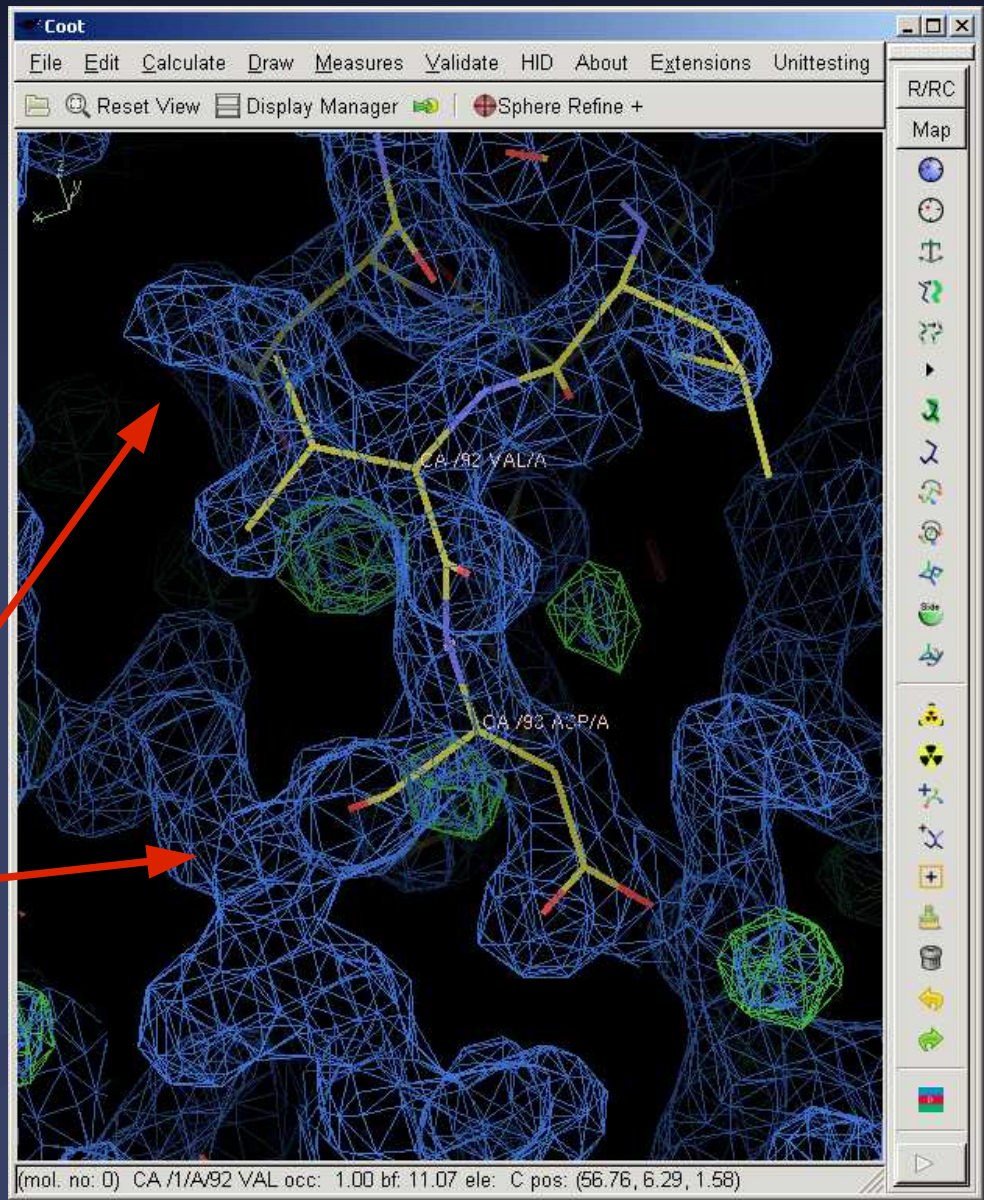
Correct rotamer(?!)



Sequence validation

- Assign sequence (Extensions → Dock sequence → Associate sequence)
- Sequence alignment of model to given sequence (Validate →Alignment vs PIR)





More Validation Pr(model)

- Coot has interface to Molprobity
  - (Molprobity is the widely regarded as the best model validation suite)
- Uses identical Ramachandran plot
- Uses identical Rotamer library
- Coot reads probe dots directly

Analysis output: all-atom contacts and geometry for 1bkrFH.pdb

Summary statistics

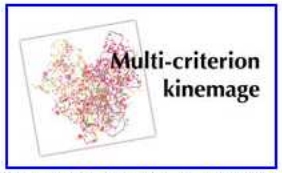
All-Atom Contacts	Clashscore, all atoms:	8.76	38th percentile* (N=222, 1.10Å ± 0.25Å)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	6.19%	Goal: <1%
	Ramachandran outliers	0.00%	Goal: <0.2%
	Ramachandran favored	98.11%	Goal: >98%
	Cβ deviations >0.25Å	2	Goal: 0
	MolProbity score^	2.07	18th percentile* (N=932, 1.10Å ± 0.25Å)
	Residues with bad bonds:	0.00%	Goal: 0%
	Residues with bad angles:	0.00%	Goal: <0.1%

* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst.

^ MolProbity score is defined as the following: 0.42574*log(1+clashscore) + 0.32996*log(1+max(0,pctRotOut-1)) + 0.24979*log(1+max(0,100-pctRamaFavored-2)) + 0.5

By adding H to this model and allowing Asn/Gln/His flips, you have already improved your clashscore by 3.51 points. **Make sure you download the modified PDB to take advantage of these improvements!**

Multi-criterion visualizations



View in KiNG | Download (633 Kb)

View (232 Kb)

Scheme Script Download (8.6 Kb) | Python Script Download (6.2 Kb)

Open this in Coot 0.1.2 or later using Calculate | Run Script...

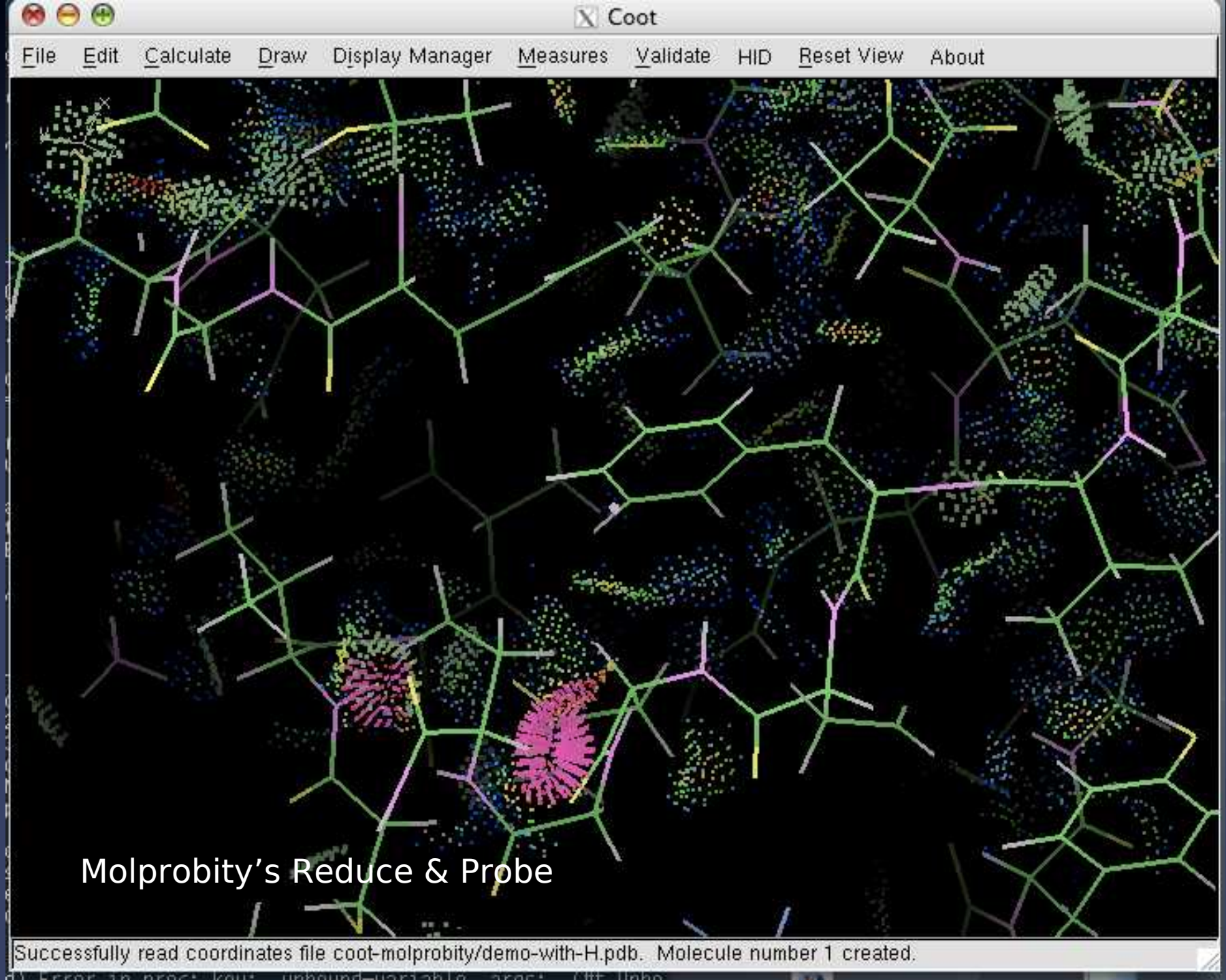
Single-criterion visualizations

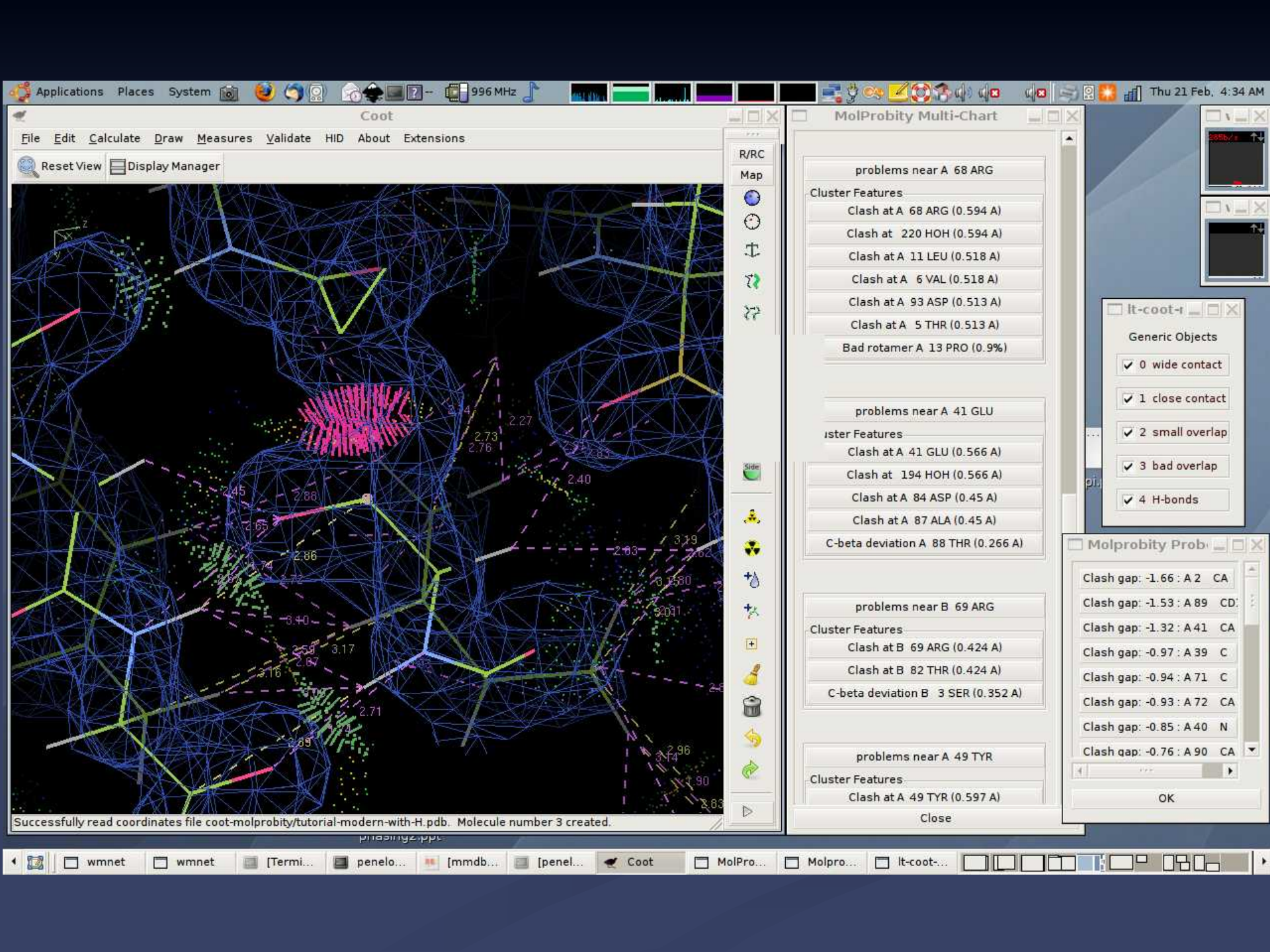
- **Clash list** (1.1 Kb): View
- **Ramachandran plot kinemage** (339 Kb): View in KiNG | Download
- **Ramachandran plot PDF** (187 Kb): View
- **Cβ deviation scatter plot** (16 Kb): View in KiNG | Download

REMARK 40

You can download your PDB file with REMARK 40 inserted, or the same without hydrogens.

```
REMARK  40
REMARK  40 MOLPROBITY STRUCTURE VALIDATION
REMARK  40  PROGRAMS     : MOLPROBITY  (KING, REDUCE, AND PROBE)
REMARK  40  AUTHORS      : I.W.DAVIS,V.B.CHEN,
REMARK  40               : R.M.IMMORMINO,J.J.HEADD,W.B.ARENDALL,J.M.WORD
REMARK  40  URL          : HTTP://KINEMAGE.BIOCHEM.DUKE.EDU/MOLPROBITY/
REMARK  40  AUTHORS      : I.W.DAVIS,A.LEAVER-FAY,V.B.CHEN,J.N.BLOCK,
```


Coot
File Edit Calculate Draw Display Manager Measures Validate HID Reset View About
Molprobity’s Reduce & Probe
Successfully read coordinates file coot-molprobity/demo-with-H.pdb. Molecule number 1 created.


Applications Places System 996 MHz Thu 21 Feb, 4:34 AM
Coot
File Edit Calculate Draw Measures Validate HID About Extensions
Reset View Display Manager
R/RC
Map
Side
Successfully read coordinates file coot-molprobity/tutorial-modern-with-H.pdb. Molecule number 3 created.
MolProbity Multi-Chart
problems near A 68 ARG
Cluster Features
Clash at A 68 ARG (0.594 A)
Clash at 220 HOH (0.594 A)
Clash at A 11 LEU (0.518 A)
Clash at A 6 VAL (0.518 A)
Clash at A 93 ASP (0.513 A)
Clash at A 5 THR (0.513 A)
Bad rotamer A 13 PRO (0.9%)
problems near A 41 GLU
ıster Features
Clash at A 41 GLU (0.566 A)
Clash at 194 HOH (0.566 A)
Clash at A 84 ASP (0.45 A)
Clash at A 87 ALA (0.45 A)
C-beta deviation A 88 THR (0.266 A)
problems near B 69 ARG
Cluster Features
Clash at B 69 ARG (0.424 A)
Clash at B 82 THR (0.424 A)
C-beta deviation B 3 SER (0.352 A)
problems near A 49 TYR
Cluster Features
Clash at A 49 TYR (0.597 A)
Close
lt-coot-r
Generic Objects
0 wide contact
1 close contact
2 small overlap
3 bad overlap
4 H-bonds
Molprobity Prob
Clash gap: -1.66 : A 2 CA
Clash gap: -1.53 : A 89 CD
Clash gap: -1.32 : A 41 CA
Clash gap: -0.97 : A 39 C
Clash gap: -0.94 : A 71 C
Clash gap: -0.93 : A 72 CA
Clash gap: -0.85 : A 40 N
Clash gap: -0.76 : A 90 CA
OK
wmnet wmnet [Termi... penelo... [mmdb... [penel... Coot MolPro... Molpro... lt-coot-...

Other Programs

- Molprobity Suite
  - molprobity.biochem.duke.edu
- WHATCHECK
- VERIFY-3D

Acknowledgements

- Paul Emsley
- Kevin Cowtan
- Eleanor Dodson
- Keith Wilson

http://www.biop.ox.ac.uk/coot/

or

Google: Coot

or for WinCoot

http://www.ysbl.ac.uk/~lohkamp/coot

- Libraries, dictionaries
  - Alexei Vagin, Eugene Krissinel, Stuart McNicholas
  - Dunbrack, Richardsons
- Coot Builders and Testers
  - William Scott, Ezra Peisach
  - York YSBL, Dundee, Glasgow (early adopters)
  - Coot Mailing List subscribers