Model Refinement and Validation

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Goal of model refinement:

To create a set of coordinates that

1) explains the data as best we can, but

2) also conforms with what we know about proteins in general

Model Refinement vs Protein Folding Funnel

$\Delta G_{unfolding}$

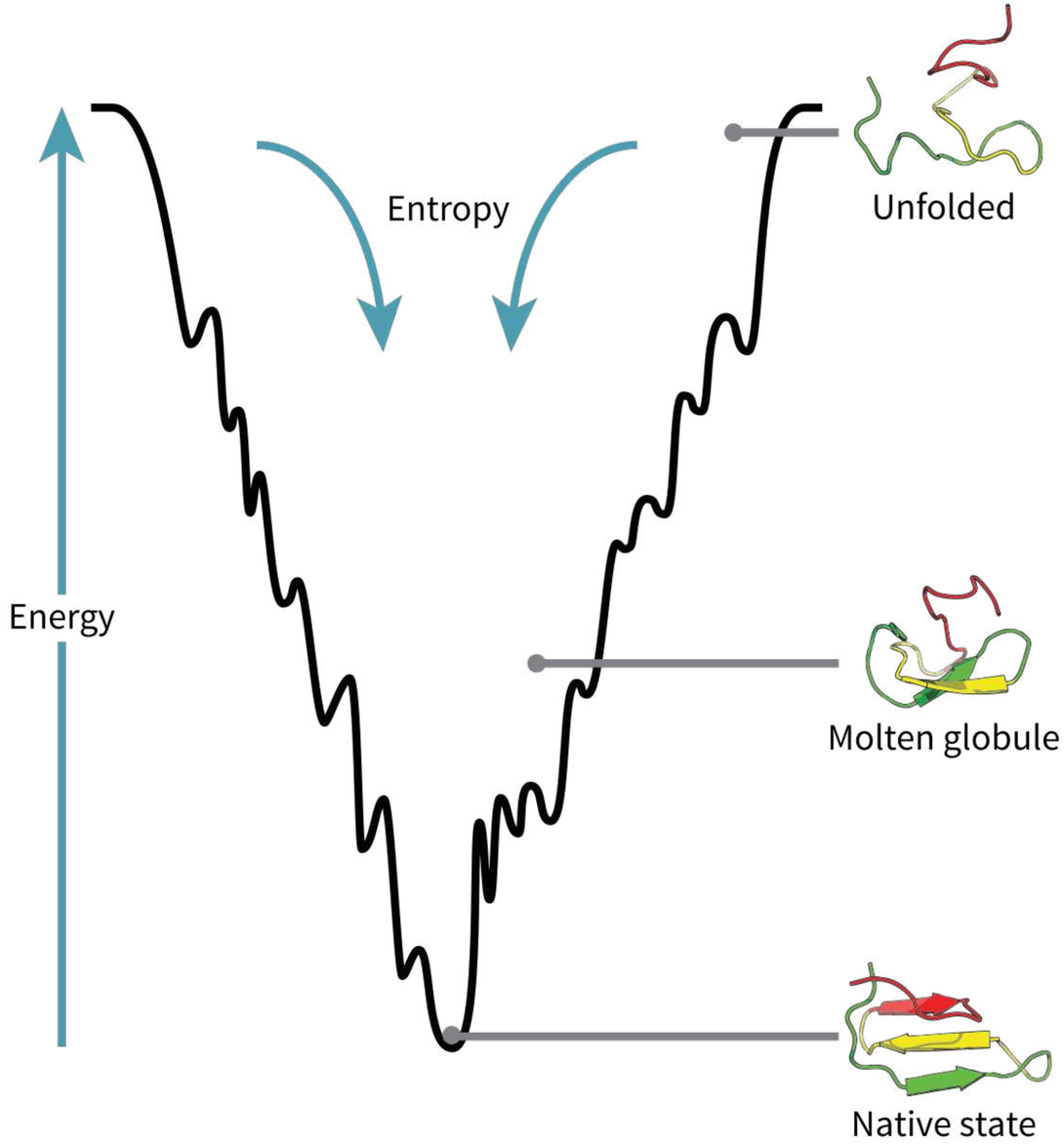


Image: Thomas Splettstoesser

Refinement target describes differences between model and data

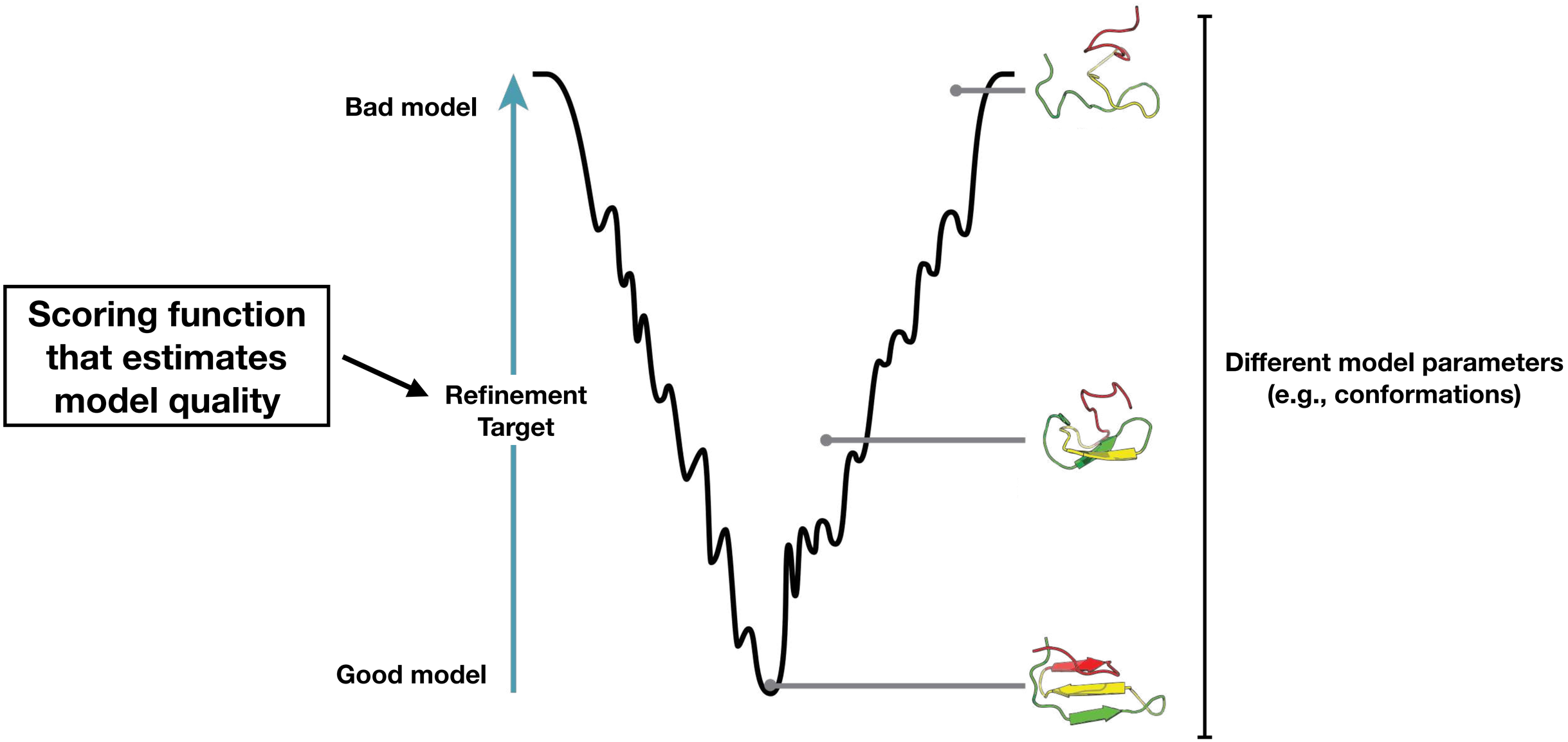
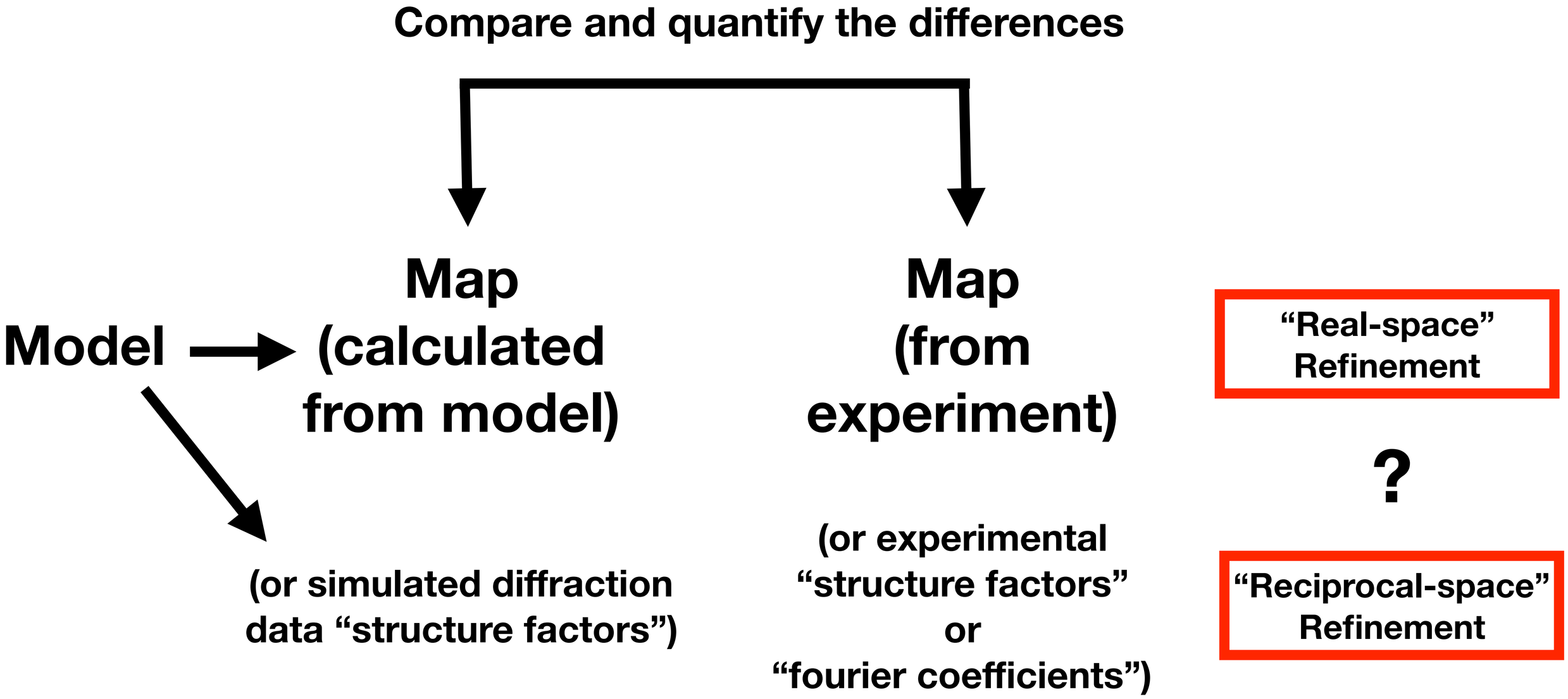
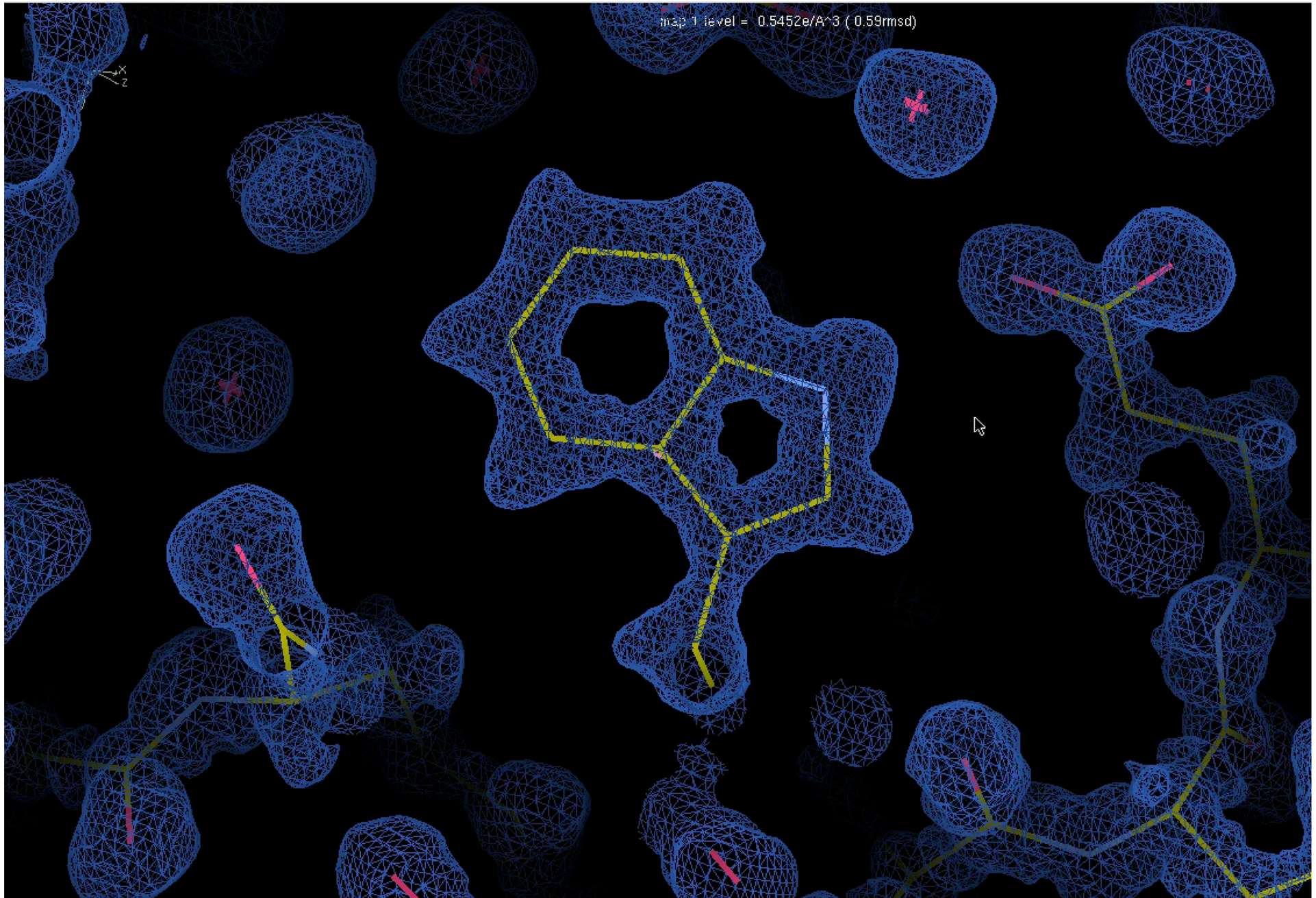


Image (adapted): Thomas Splettstoesser

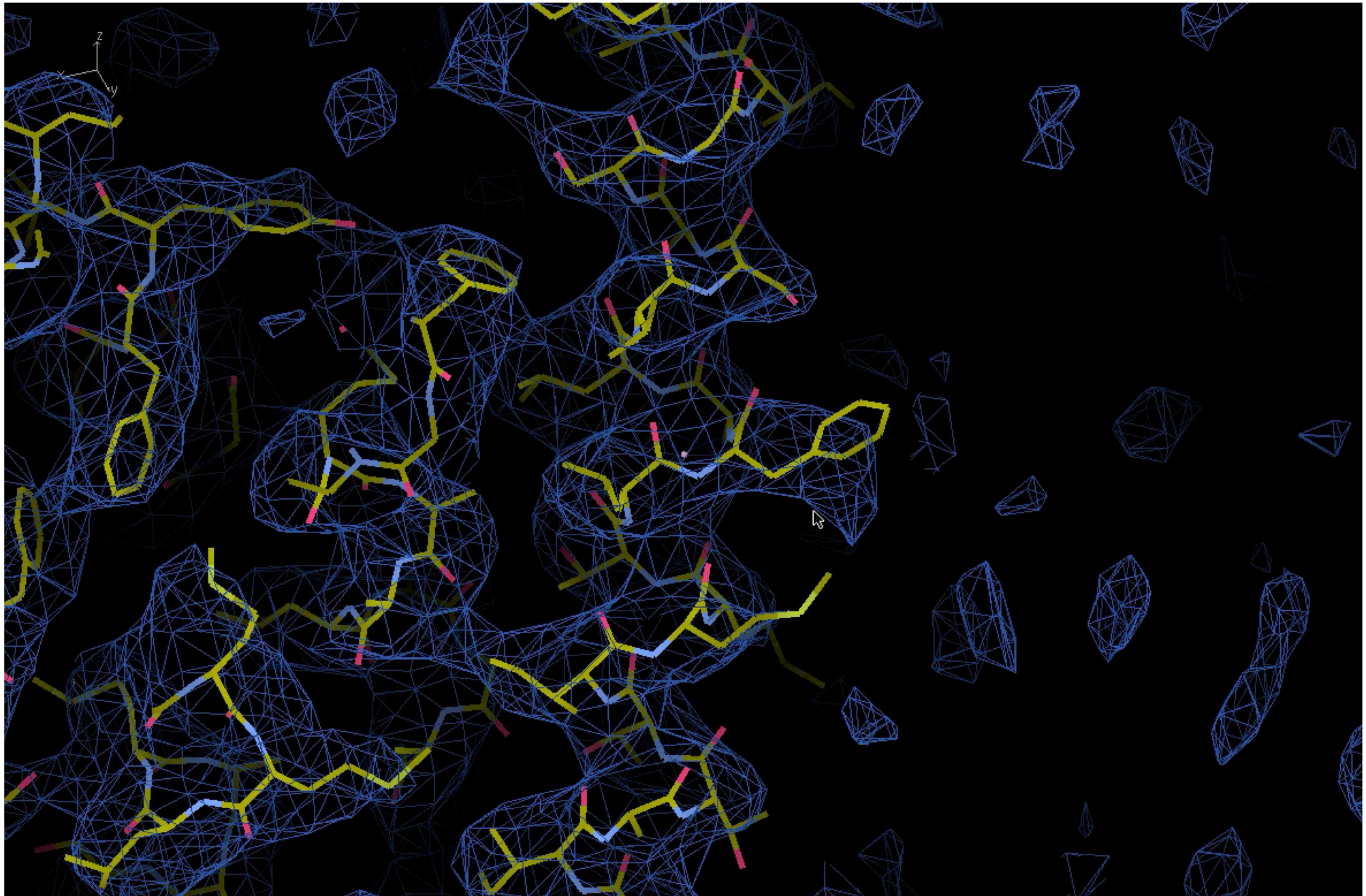
Simplest: Refinement Target = (Model vs Data)



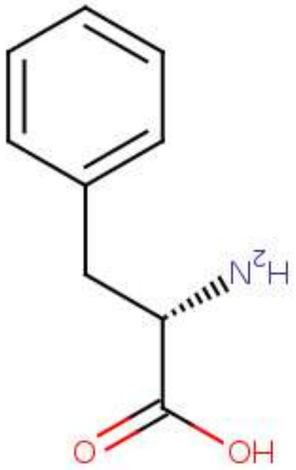
At atomic resolution, position of individual atoms is well-defined

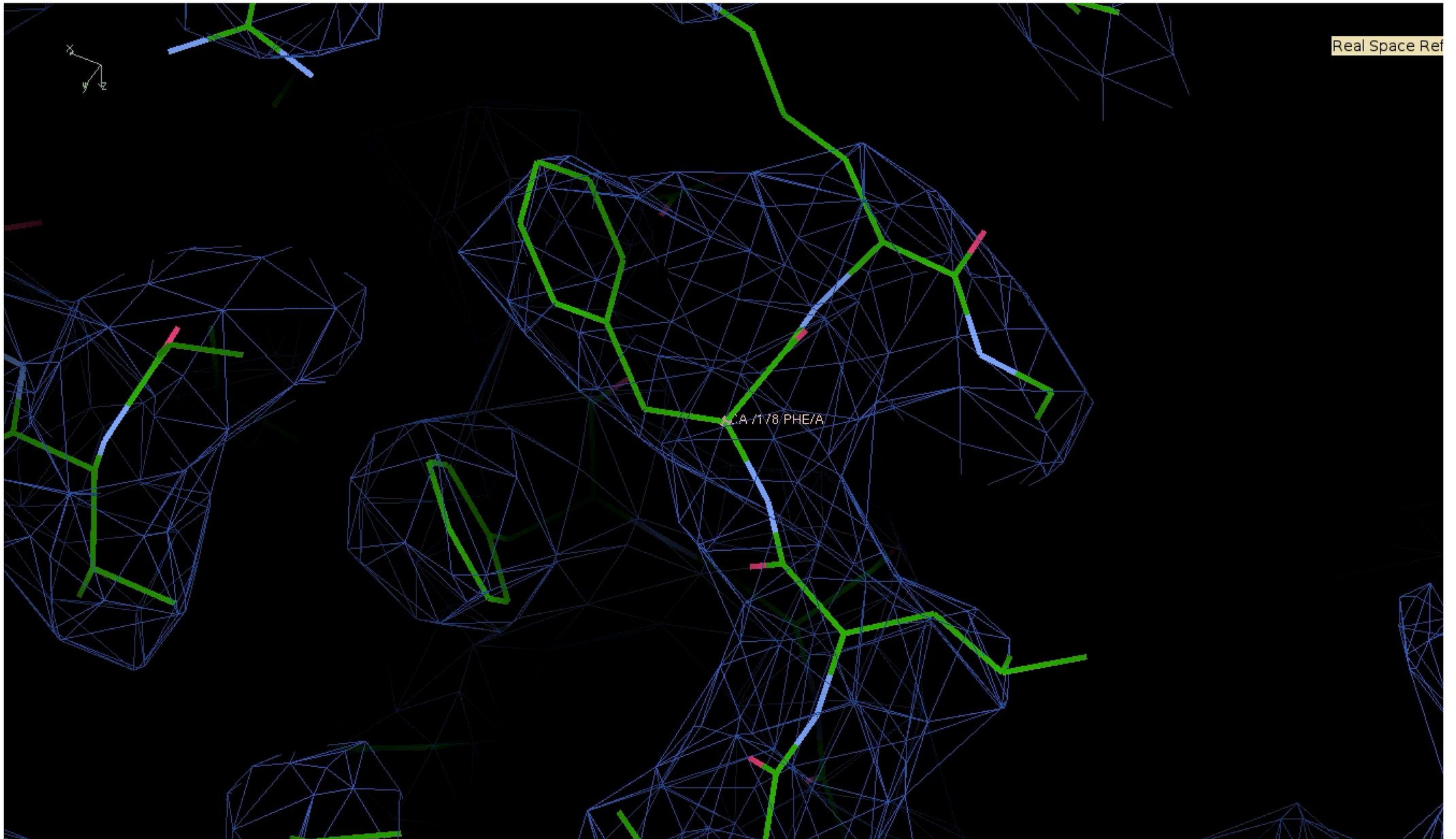


But at “near-atomic” resolution, the position of residues and side chains is not always clear

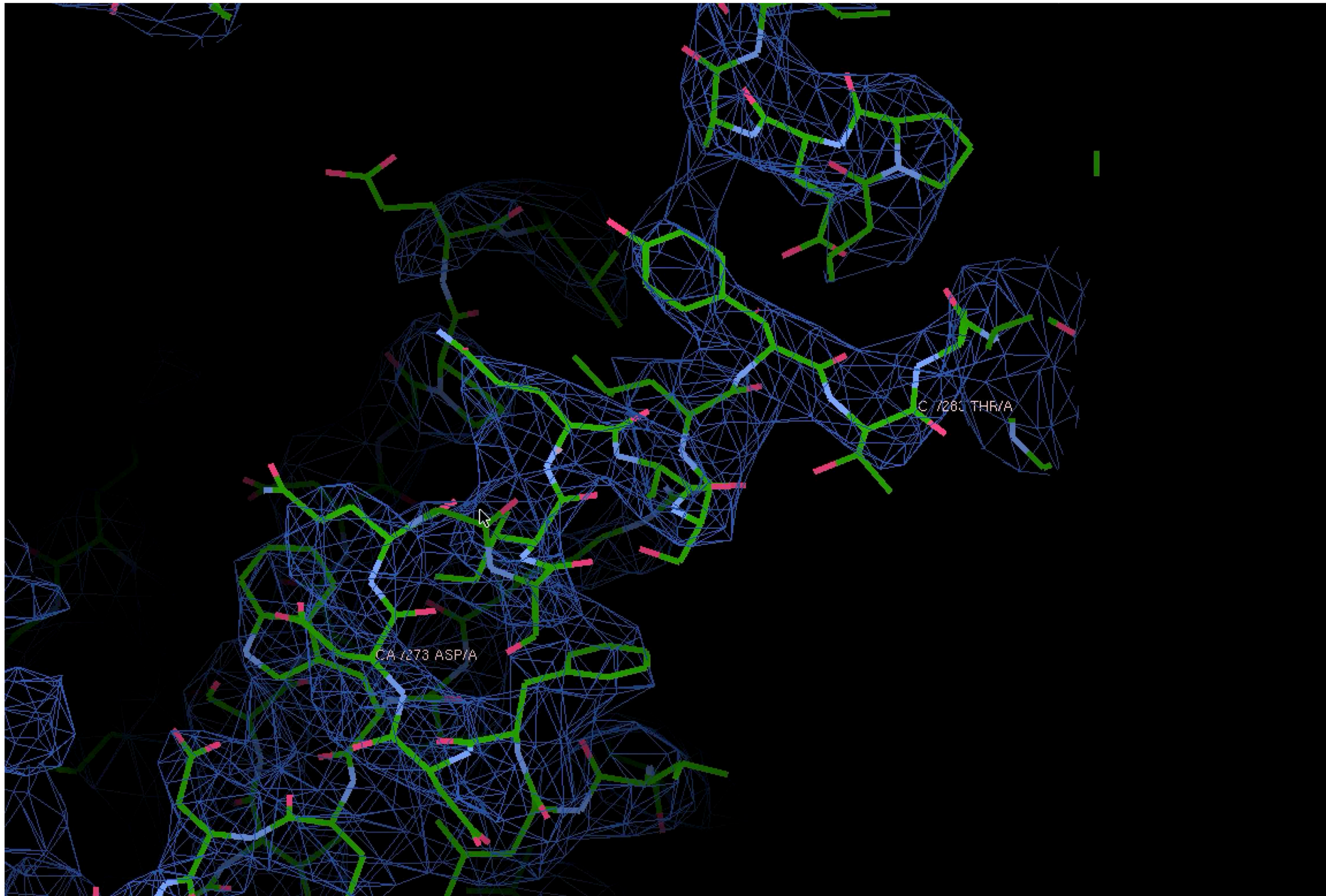


Refinement using <u>only data</u>

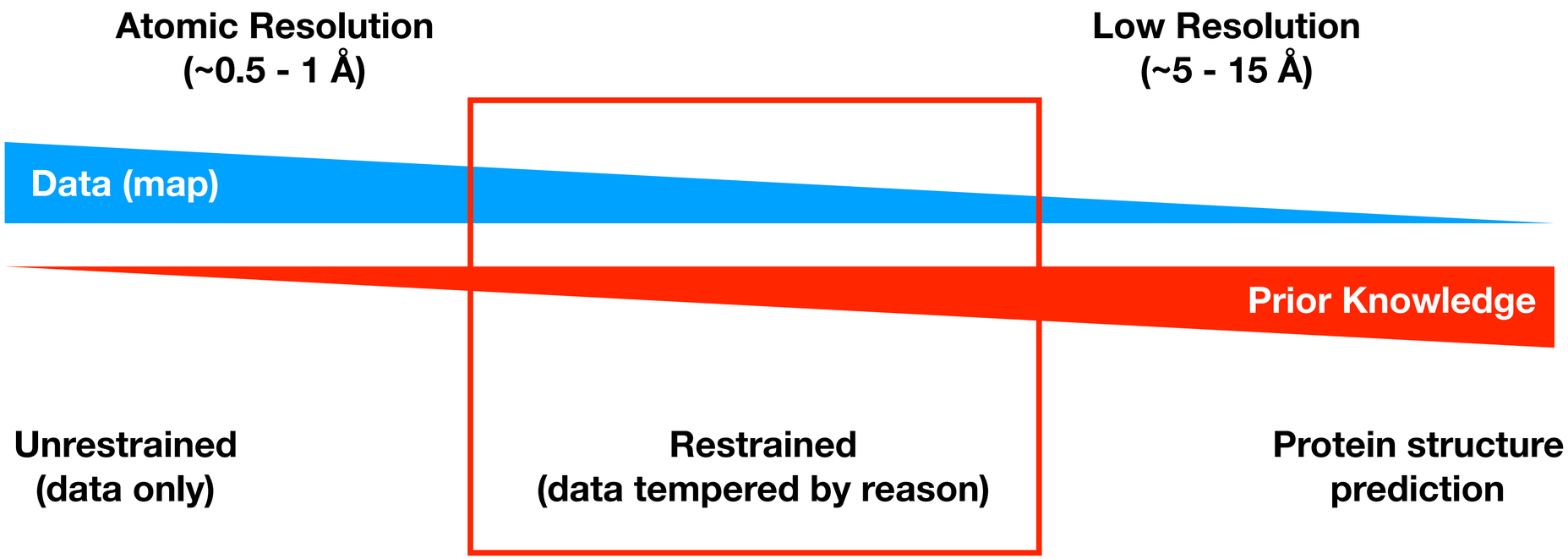




Refinement using only data

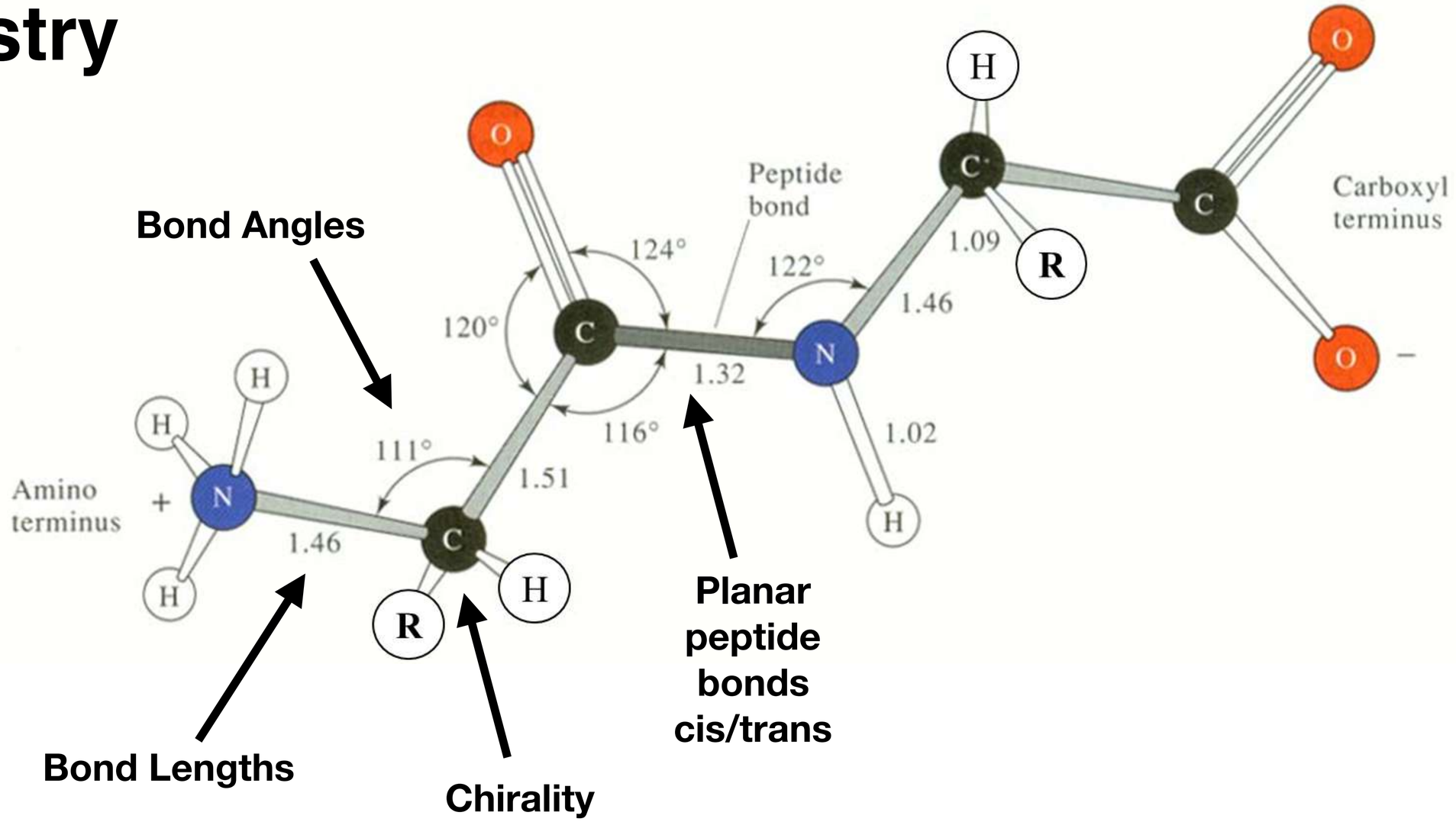


Harnessing prior knowledge of protein structure to bridge the gap

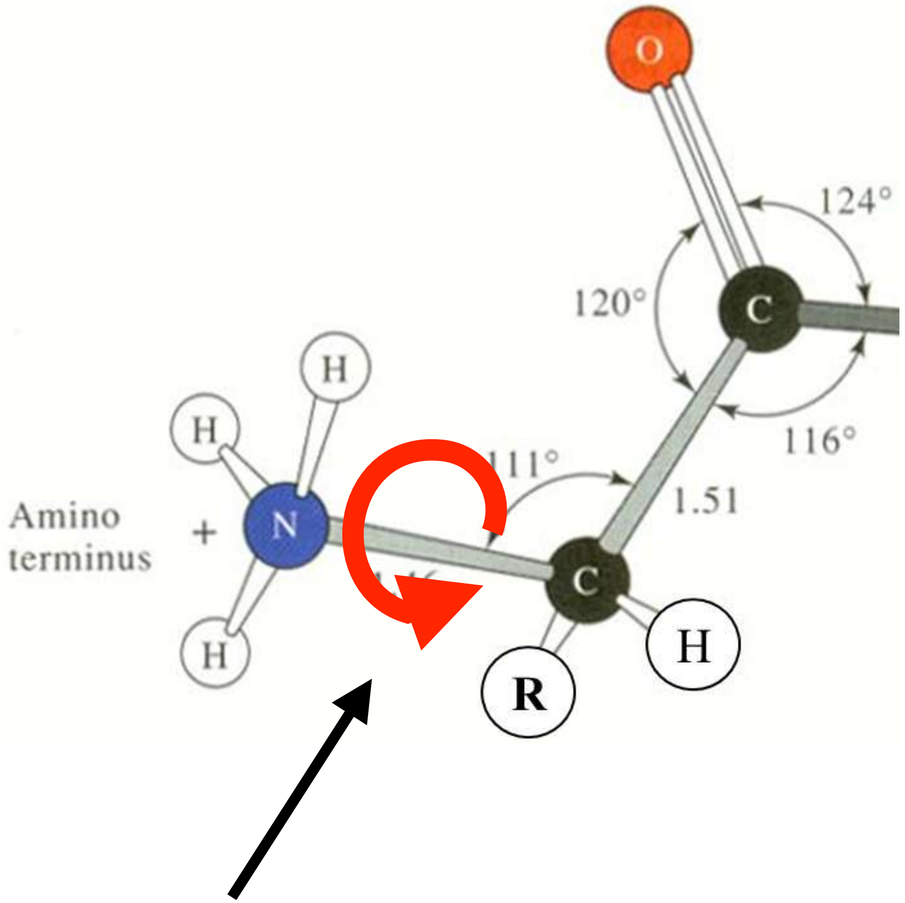


$$\text{Refinement Target} = (\text{Model vs Data}) + w_1(\text{Model vs PriorKnowledge})$$

Stereochemistry

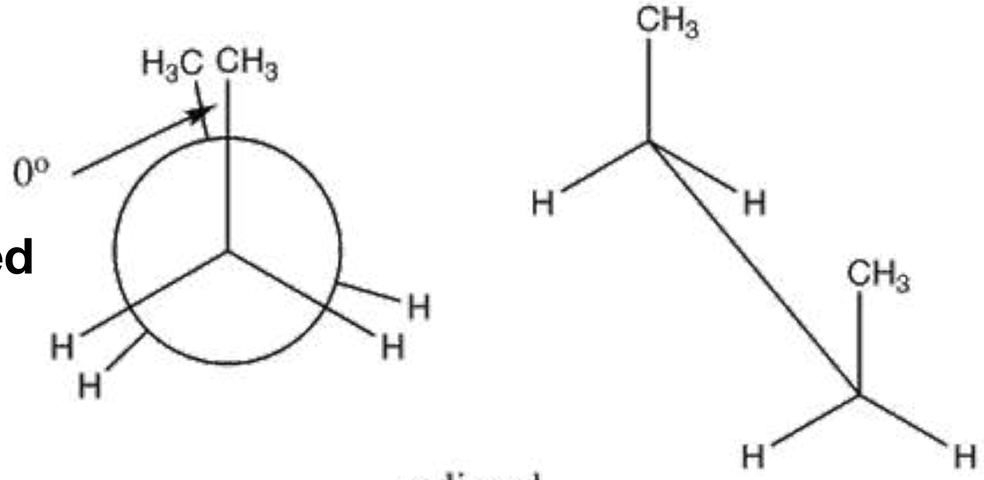


Stereochemistry



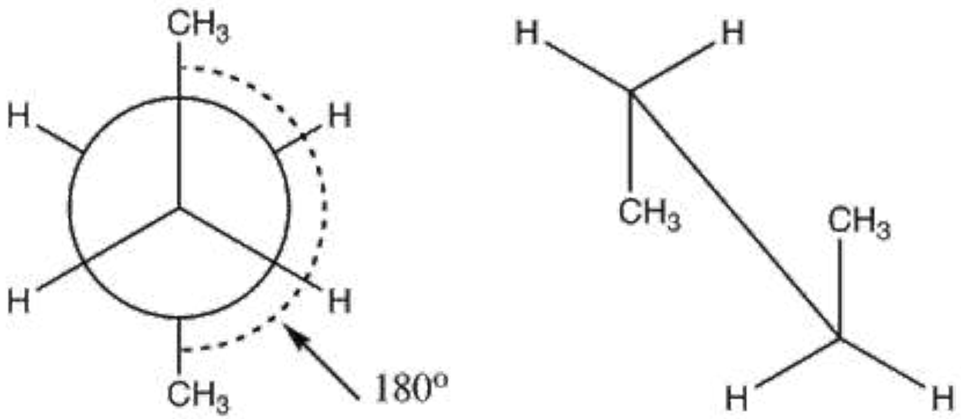
Torsion/dihedral Angles

Disfavored



eclipsed

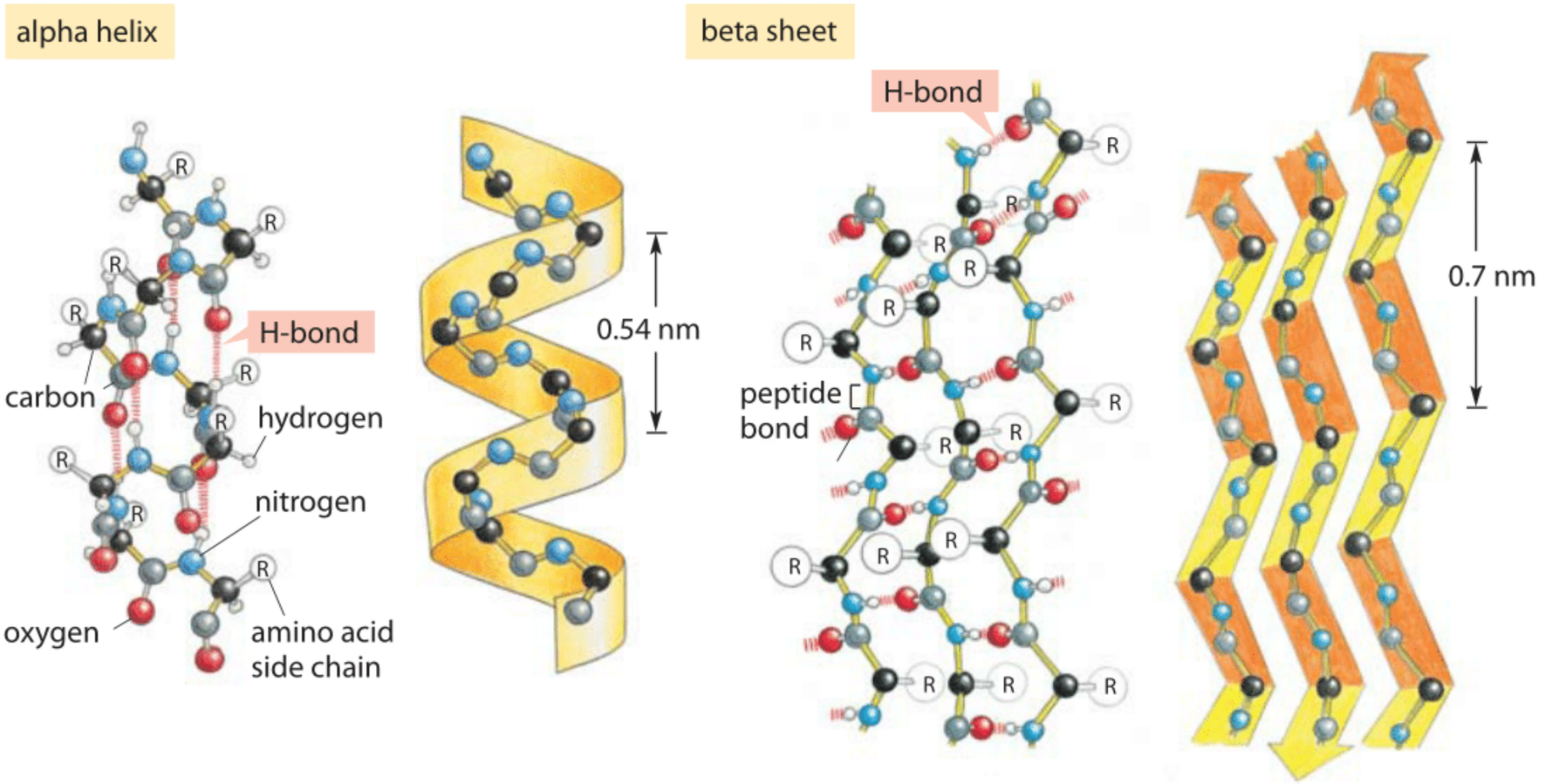
Favored



anti

Constraints backbone conformations as well as side chain rotameric states

Secondary Structure and Hydrogen Bonds

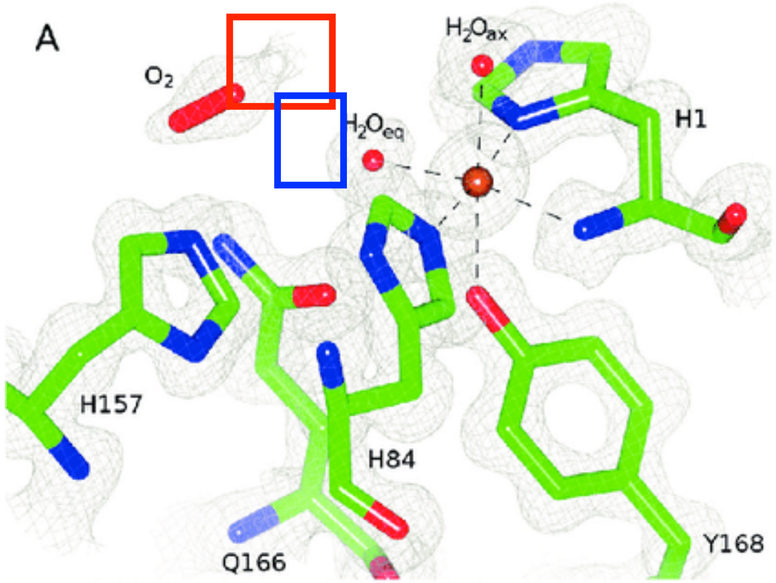


Distance restraints between H-bonding atoms
Torsion angle restraints to maintain appropriate backbone conformation

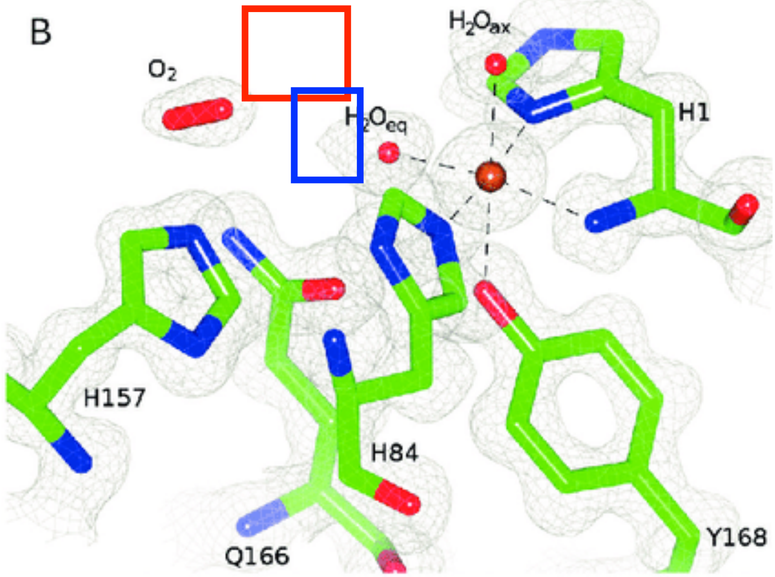
Image: http://book.bionumbers.org/what-is-the-energy-of-a-hydrogen-bond/

“Non-crystallographic symmetry” (NCS), reference model

Active Site #1



Active Site #2



<u>Restrain</u> to be similar
<u>Constrain</u> to be identical

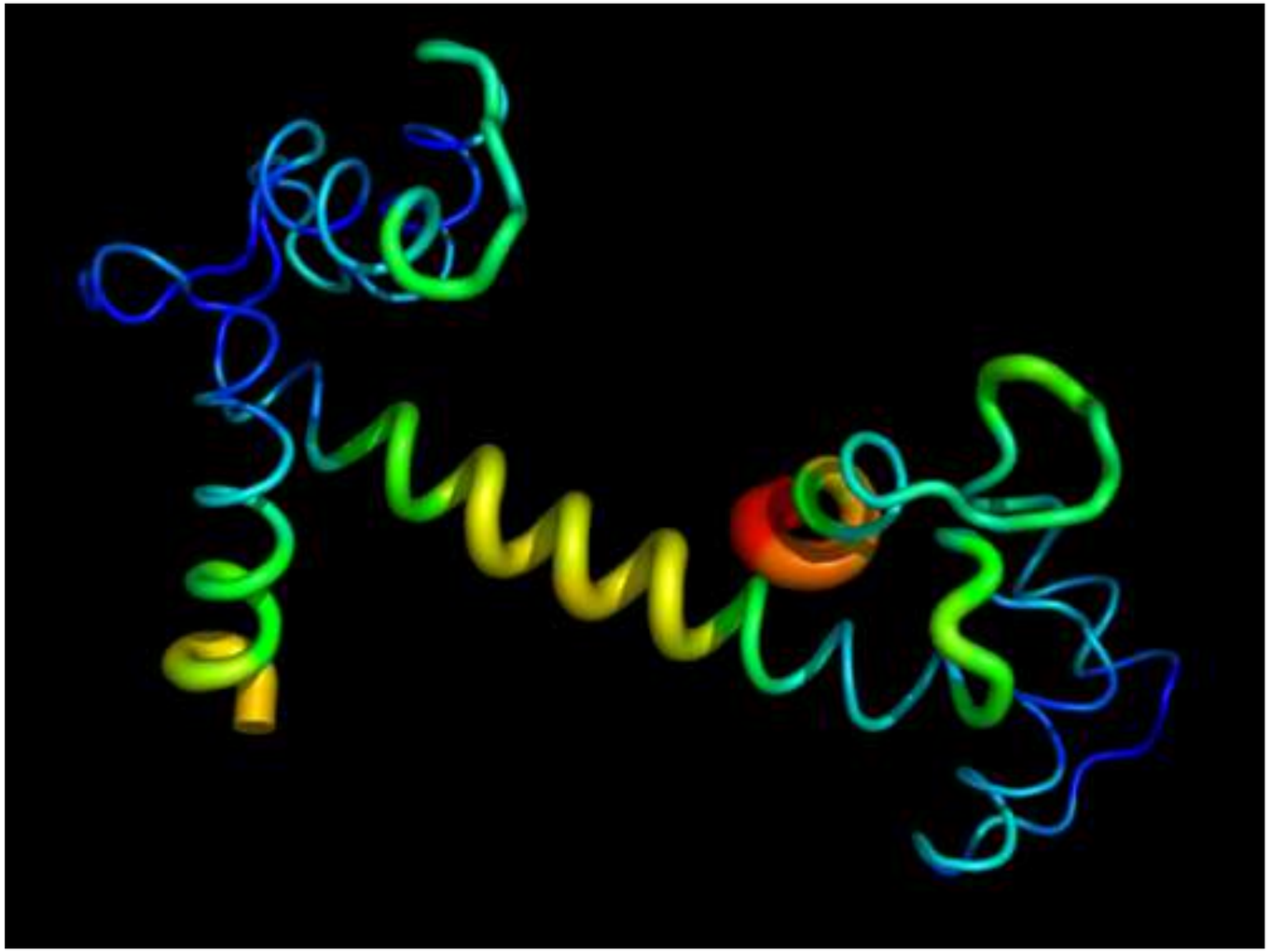
Especially helpful at lower resolution with non-symmetrized maps

Chains can be restrained to be similar to other chains in structure, or a “reference”, higher resolution structure (or the starting model)

Image: O’Dell, et al. Angewandte Chemie (2016).

B factor / ADP restraints

Higher B factor = **fatter ribbon**, warmer color



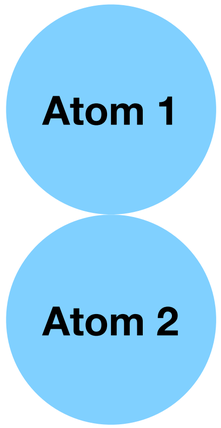
B factors are not randomly distributed

B factor of a particular residue is a good predictor of the residue just before and after

Therefore, we can retrain B factors such that connected atoms/residues must have similar B factors

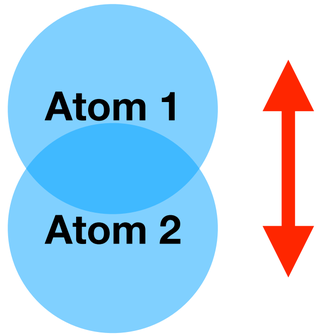
Image: Harry Jubb, https://github.com/arose/ngl/issues/291

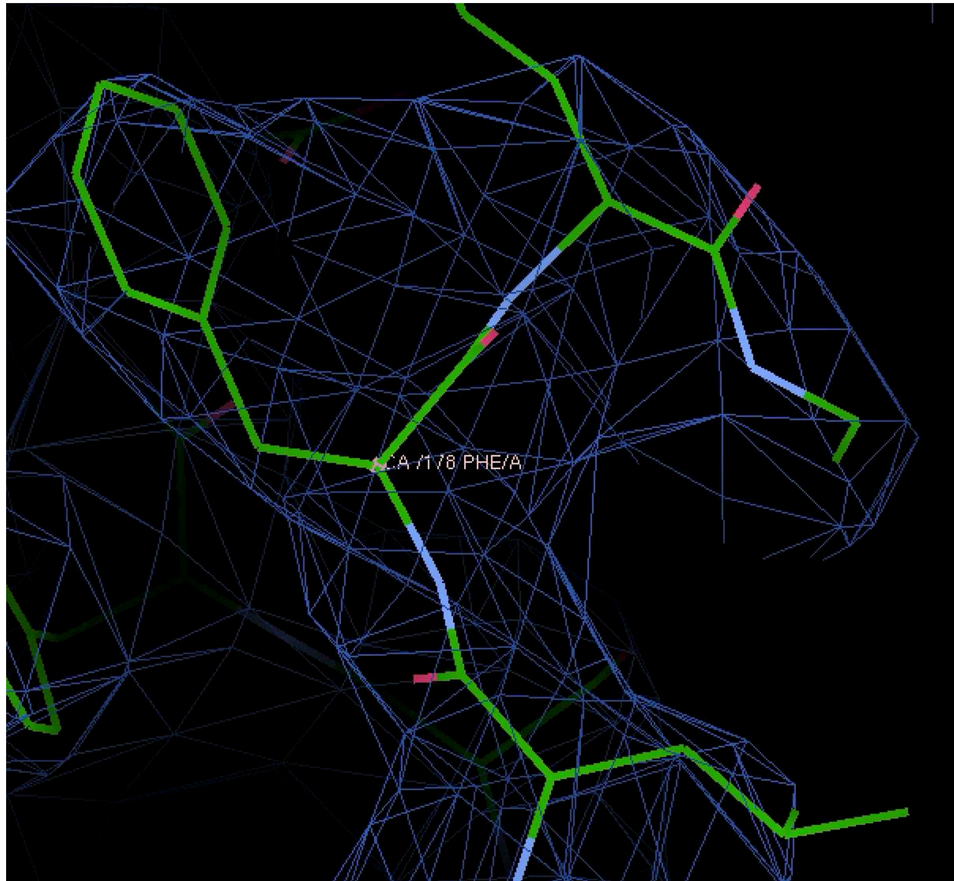
Steric repulsion



Optimal center-to-center distance ~ sum of VDW radii

If atoms get too close together, need a force to push them apart

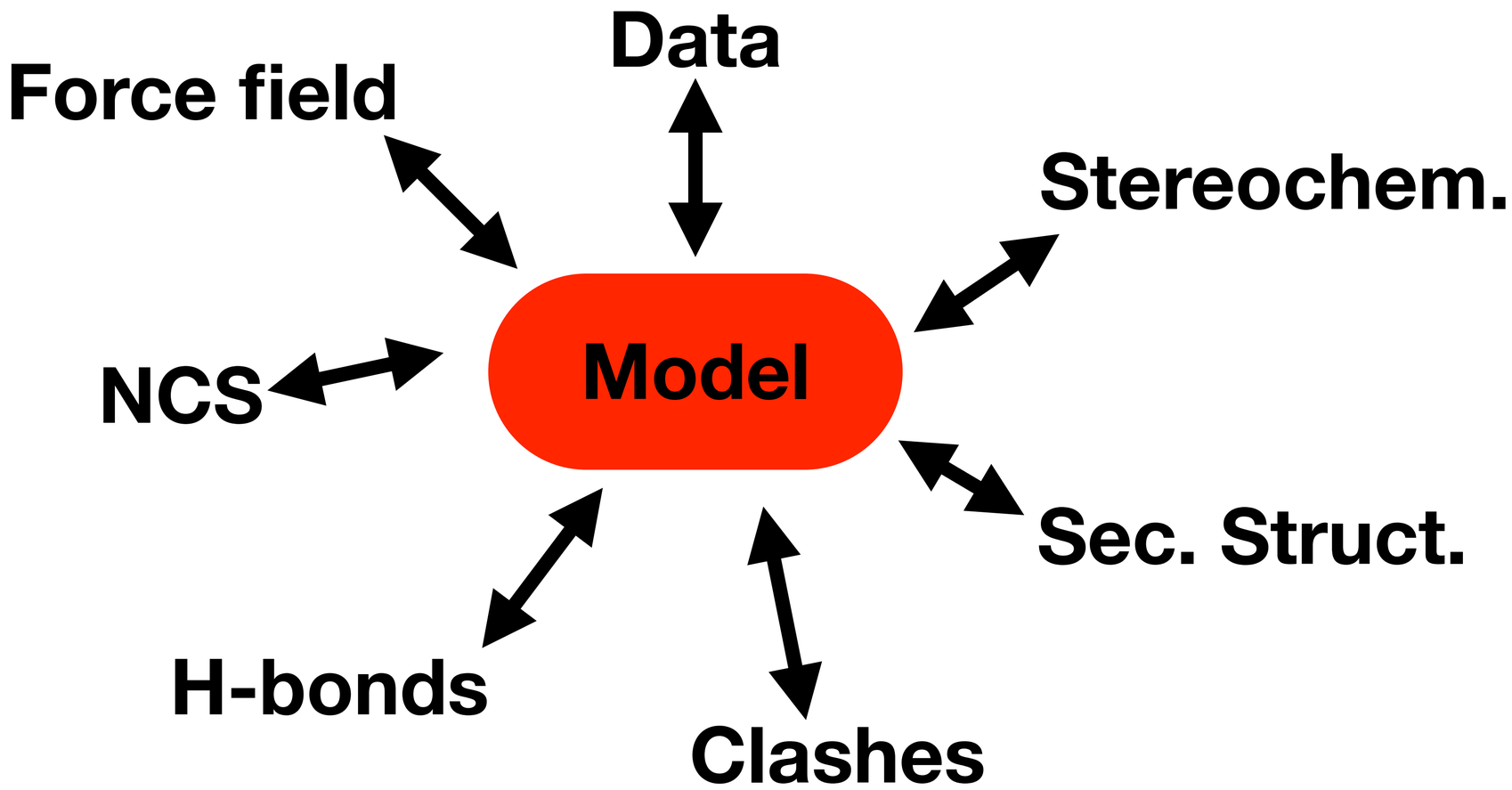




Force fields

- Historically, X-ray refinement has relied “repulsive” forces (steric clashes) to reduce atomic overlap, but also tends to push things apart
- More complex force fields commonly used elsewhere:
    - NMR structure calculations
    - Molecular dynamics
- Growing interest in using more complex force fields, including “attractive” terms that may help stabilize model at lower resolutions
    - Rosetta energy
    - Implementation of Amber force field in latest version of Phenix
    - Especially helpful at lower resolutions!

More complete refinement target includes many terms

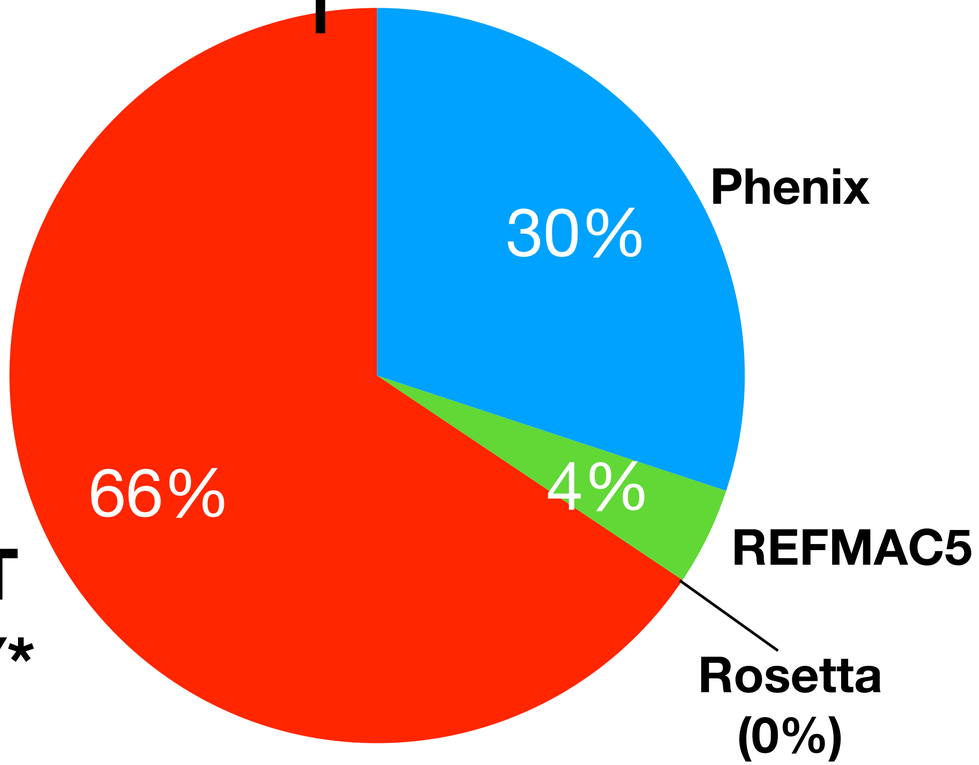


Refinement Target = (Model vs Data) + w_1(Model vs Stereo) + w_2(Model vs ForceField) + w_3(Model vs NCS) + …

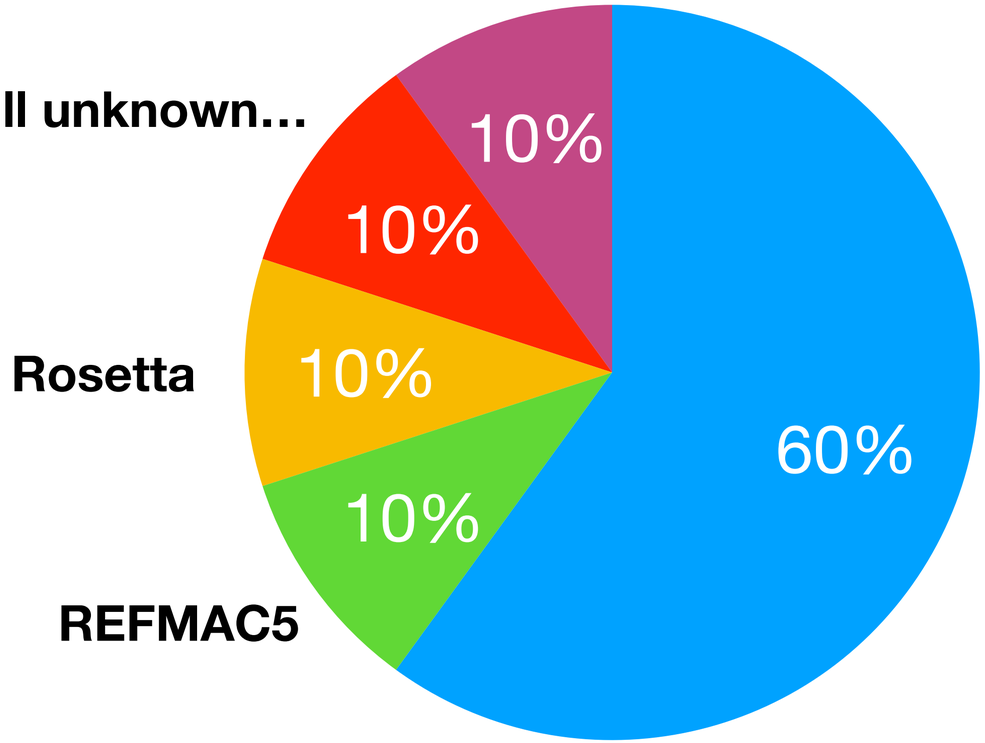
What refinement software should I use?

Field is rapidly changing: Keep trying new things and see what works best for your dataset!

Selected 10 from this group and checked methods section



DID NOT SPECIFY*



*Please don’t be this person
Always give credit to software developers!

Some Highlights

	Refinement Target	GUI	Notes
PHENIX (phenix.real_space_refine)	Real-space	Yes	Optional Amber force field; many restraint options; bundled with many other useful tools
CCP-EM (REFMAC5)	Reciprocal-space	Yes	Many restraint options, including Jelly Body; bundled with many other useful tools; most widely used reciprocal space package for EM?
Rosetta	Real-space	No	Extremely powerful Rosetta force field, might be the best at lower resolution; computationally more demanding

Refinement moves model towards local minimum

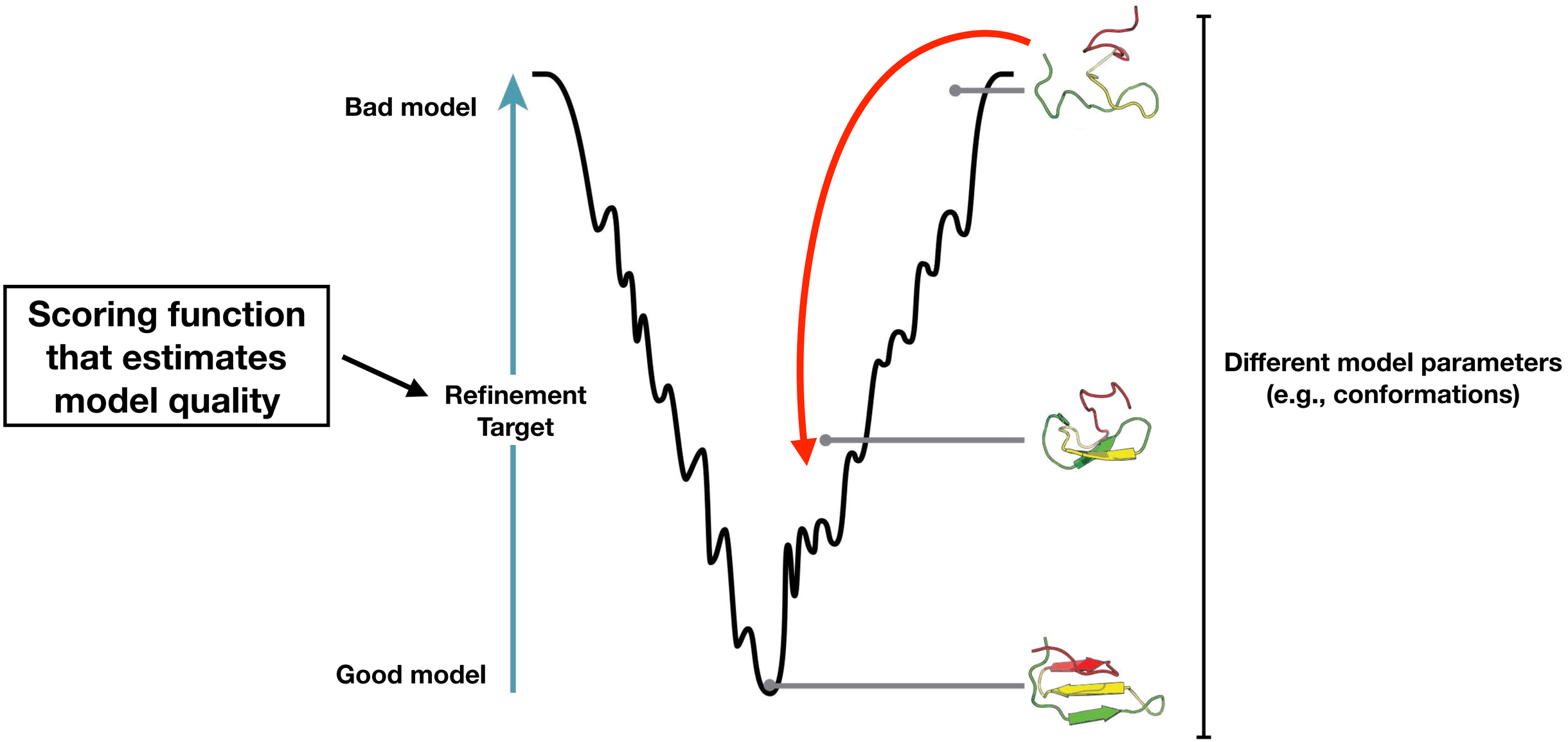


Image (adapted): Thomas Splettstoesser

Optimization protocols: Rigid body refinement

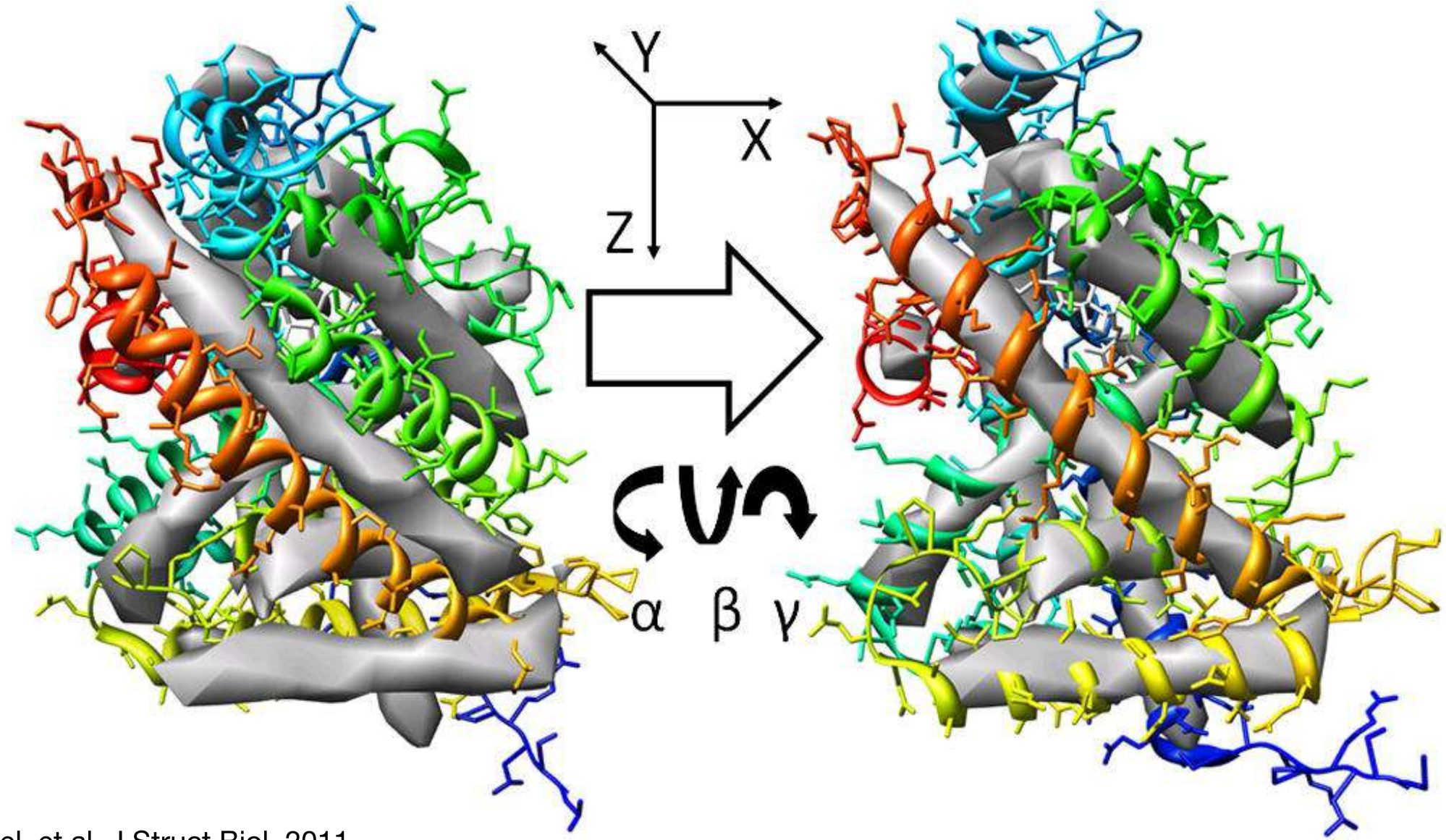
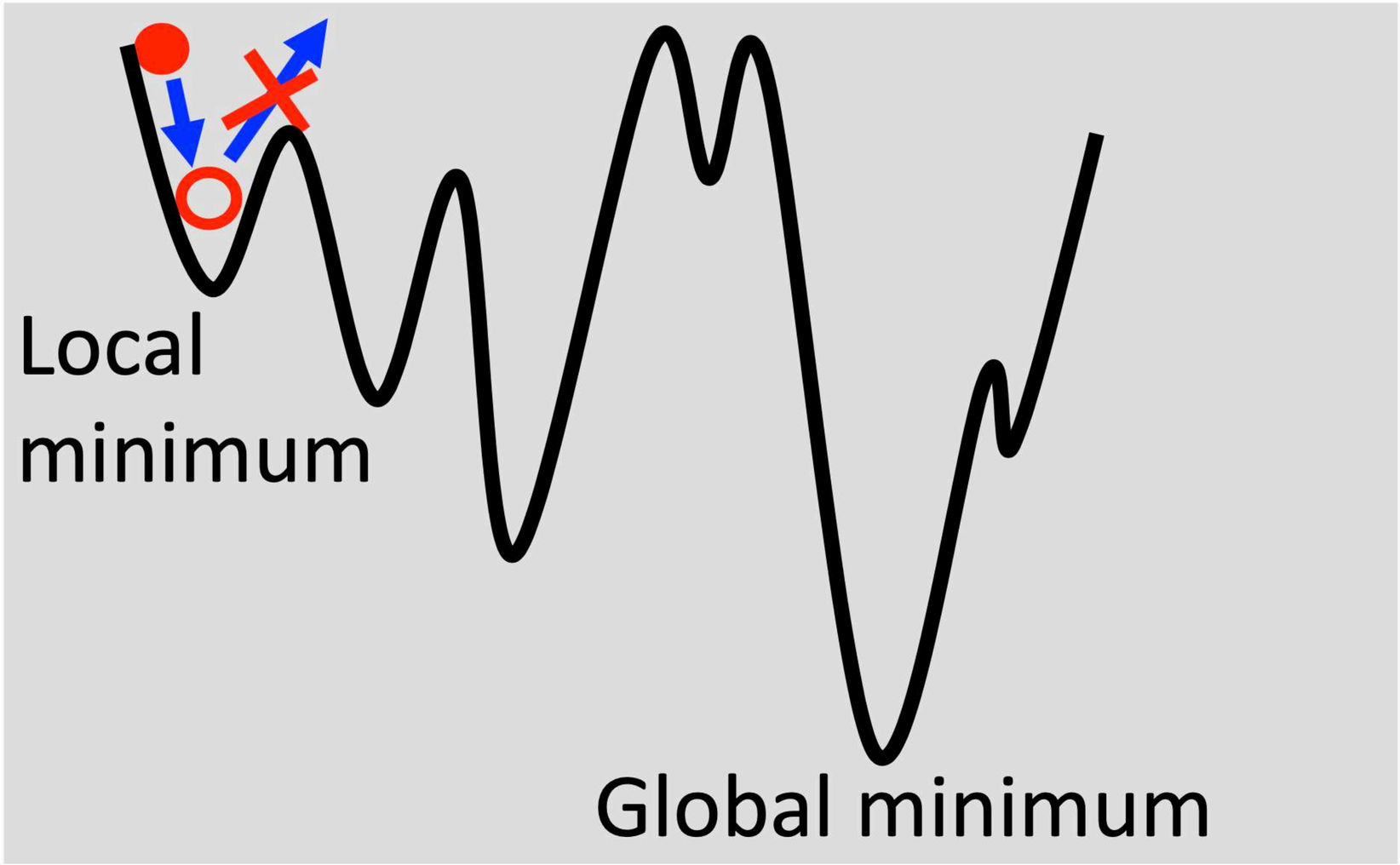


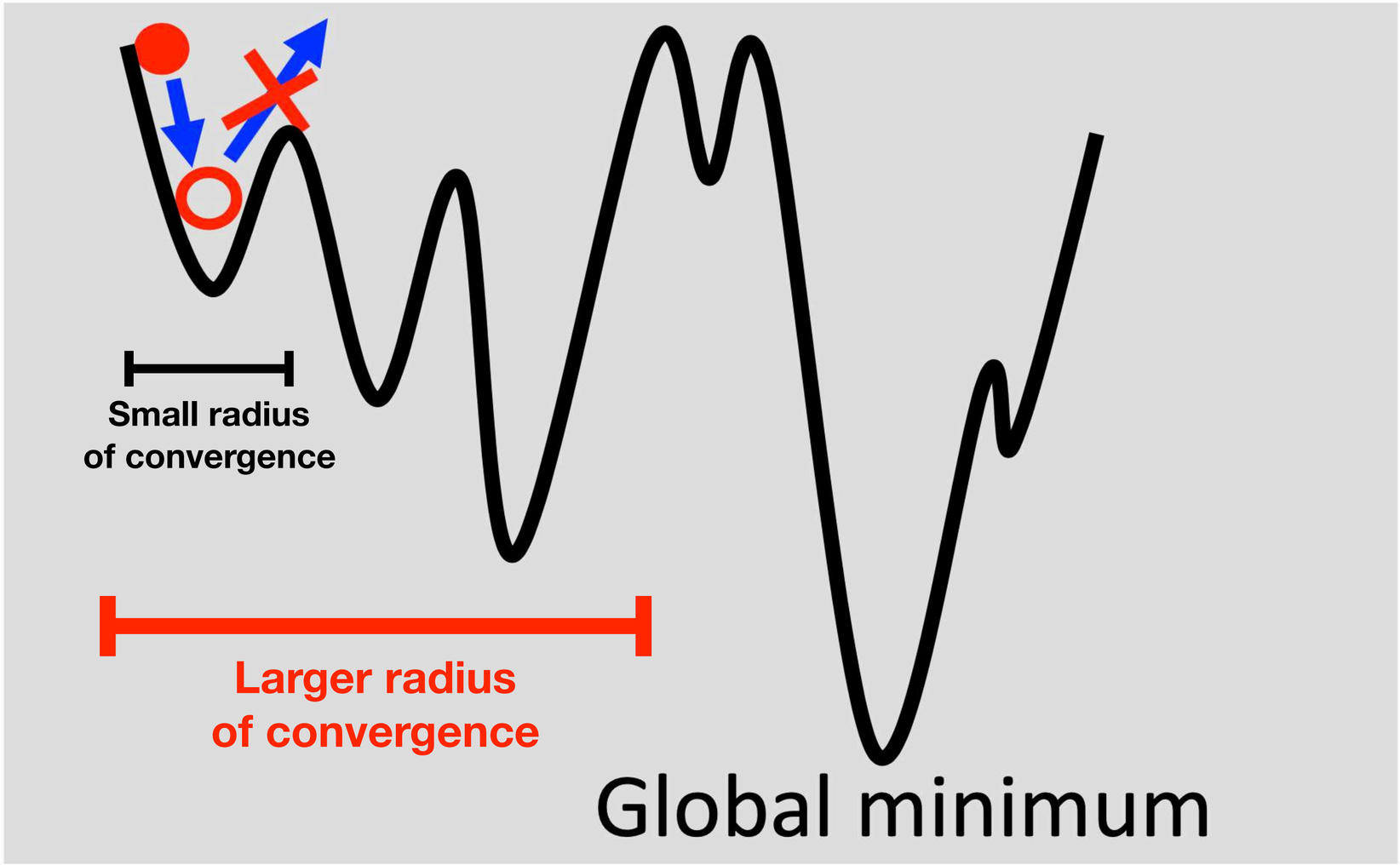
Image: Woetzel, et al. J Struct Biol. 2011.

Optimization protocols: Gradient driven minimization

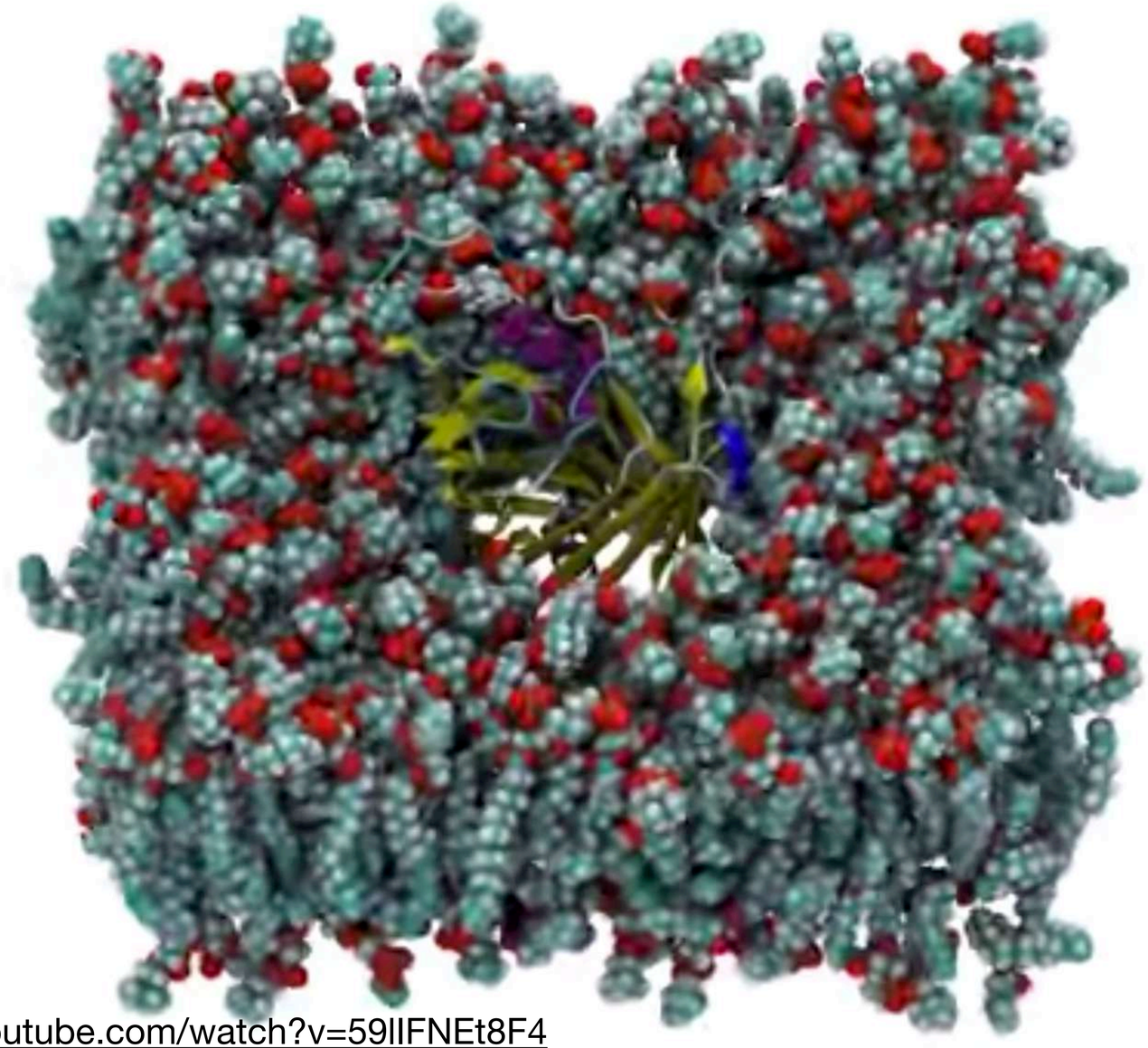


Slide adapted from: Pavel Afonine, LBNL (Phenix)

Refinement “radius of convergence”

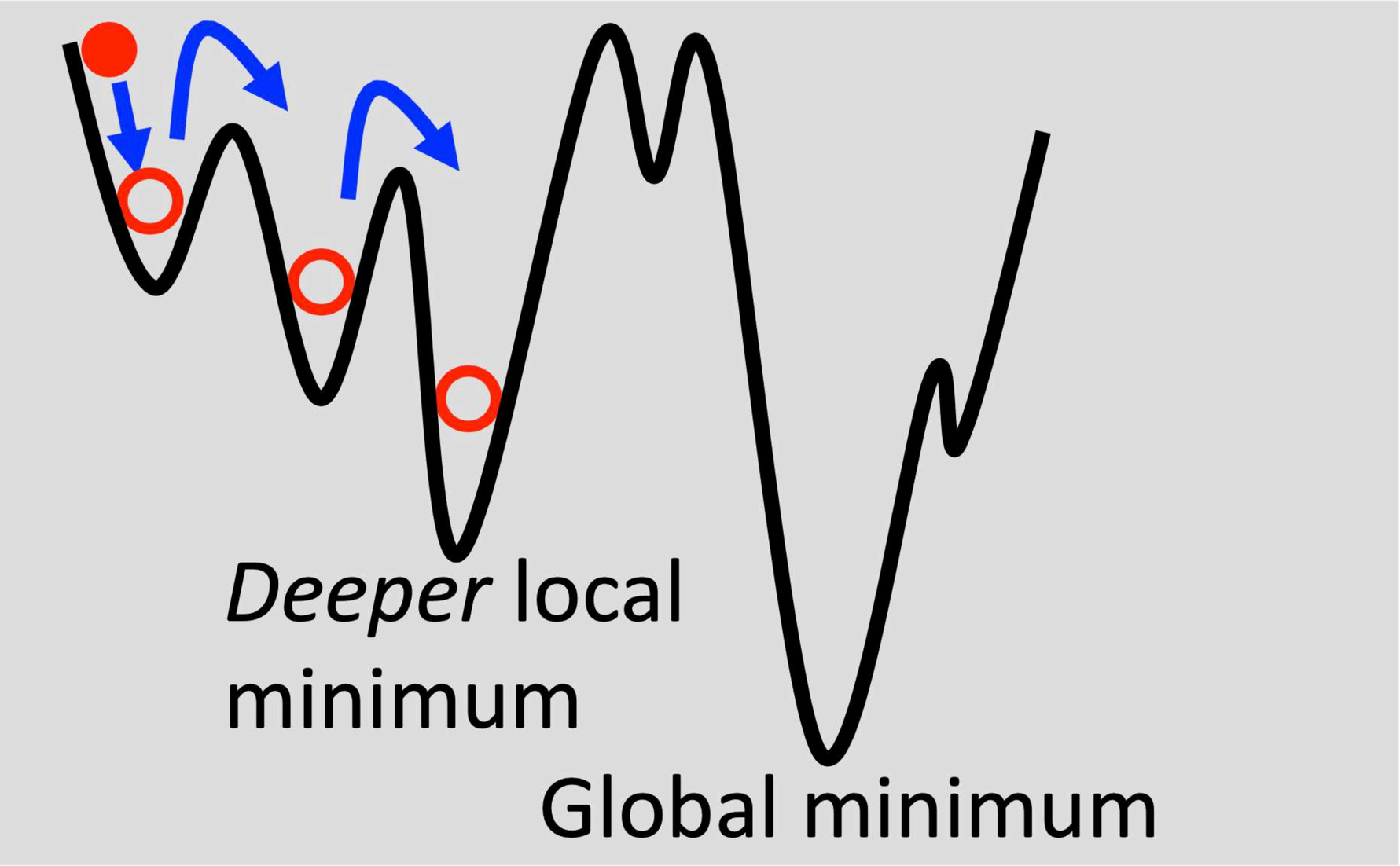


Optimization protocols: Simulated annealing



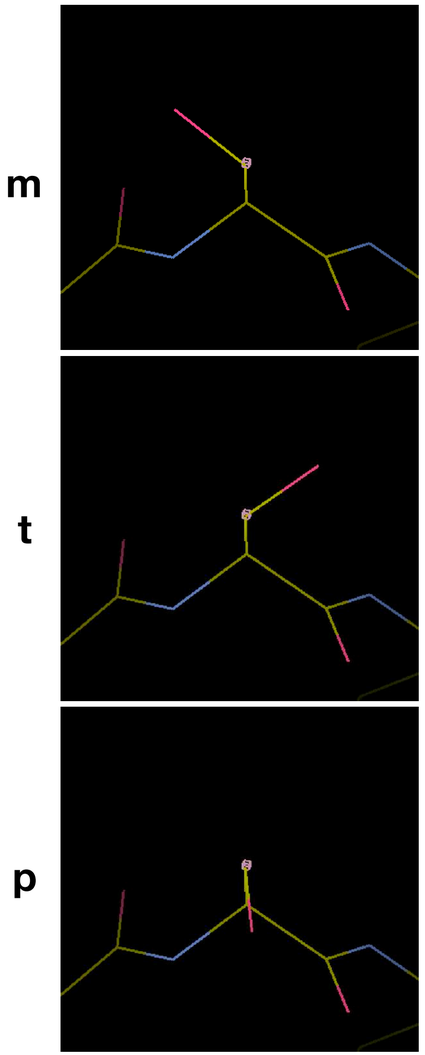
Video: Darrell Hurt, https://www.youtube.com/watch?v=59lIFNEt8F4

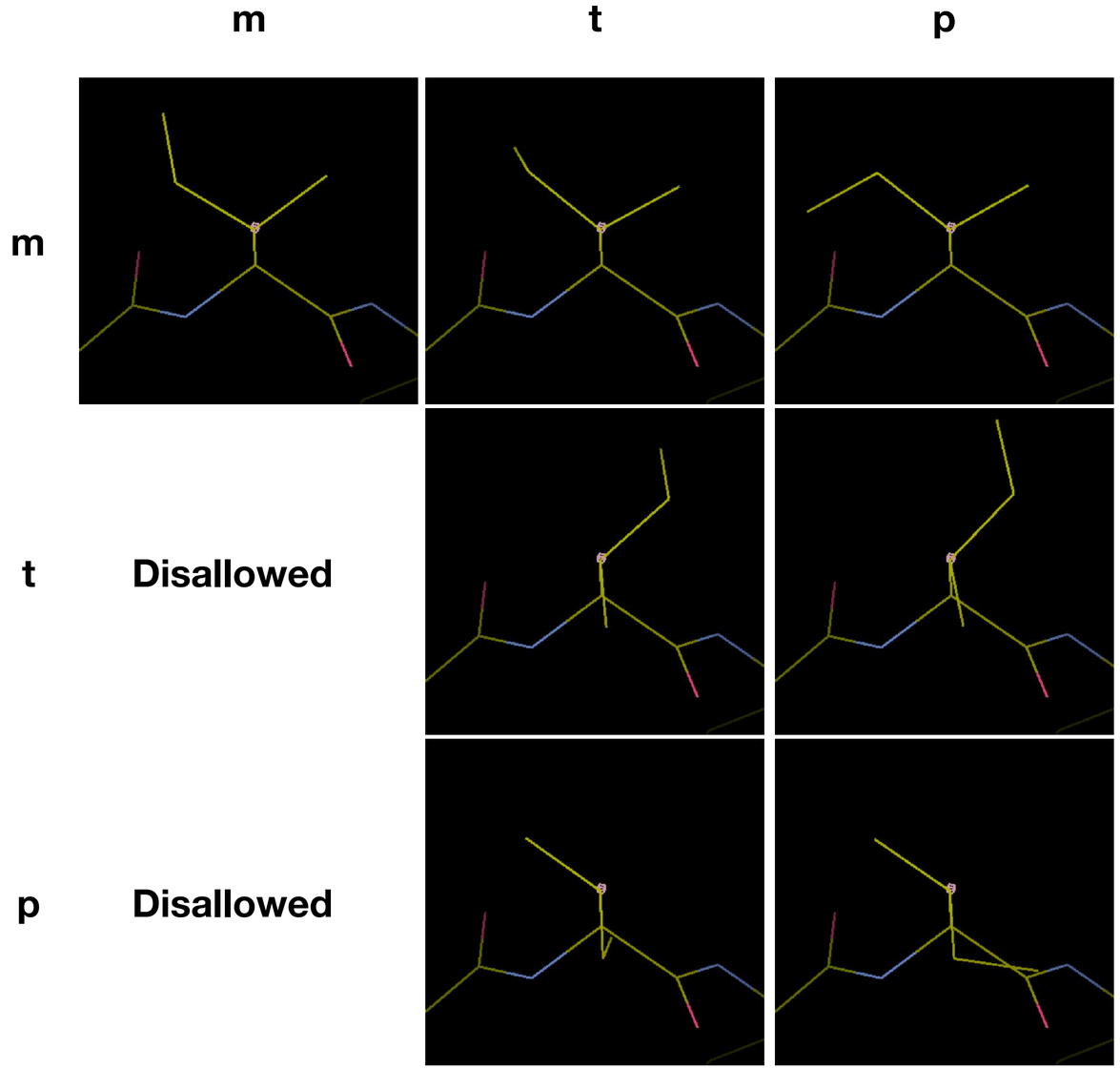
Optimization protocols: Simulated annealing



Slide adapted from: Pavel Afonine, LBNL (Phenix)

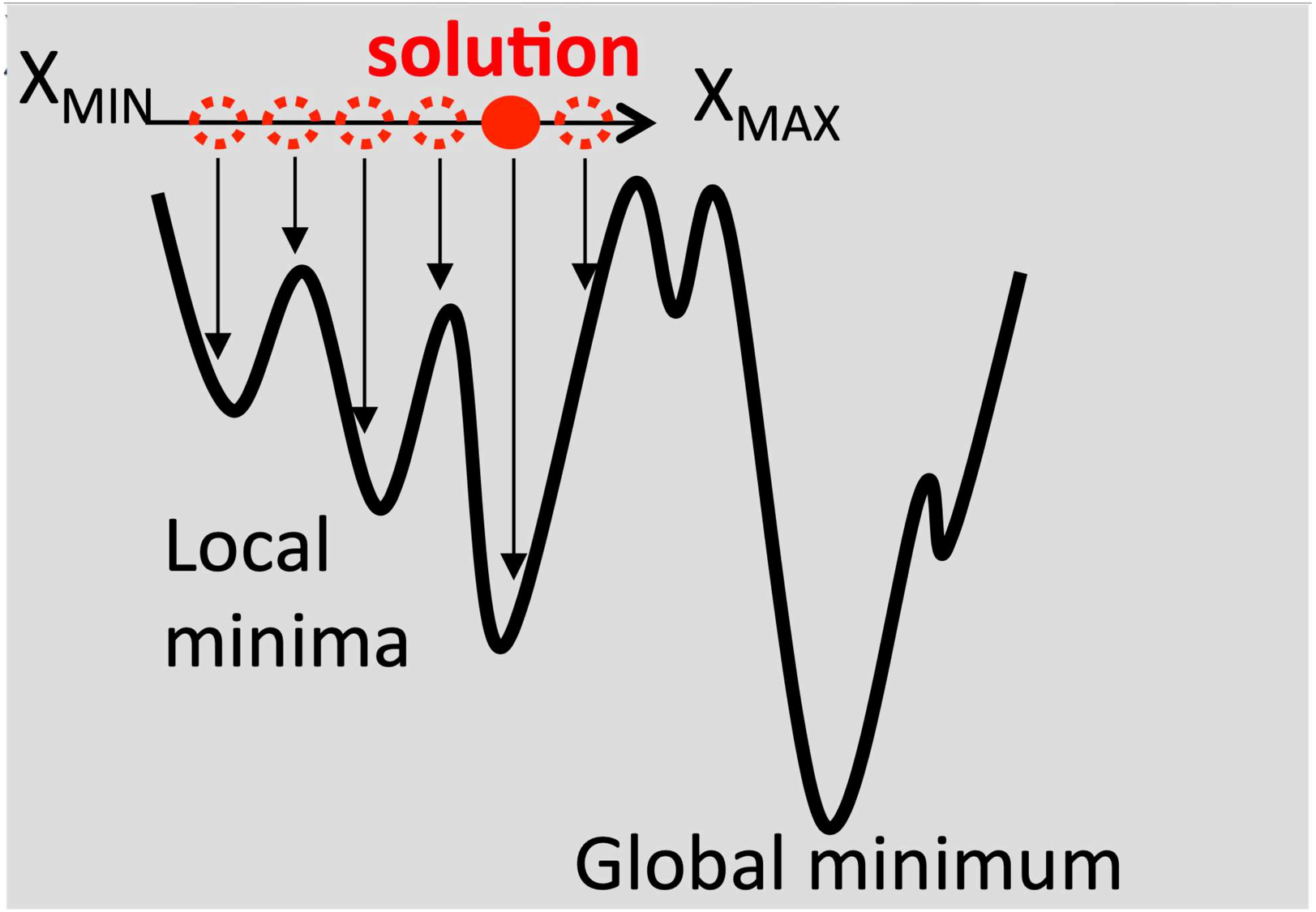
Optimization protocols: Torsion-angle Grid Search





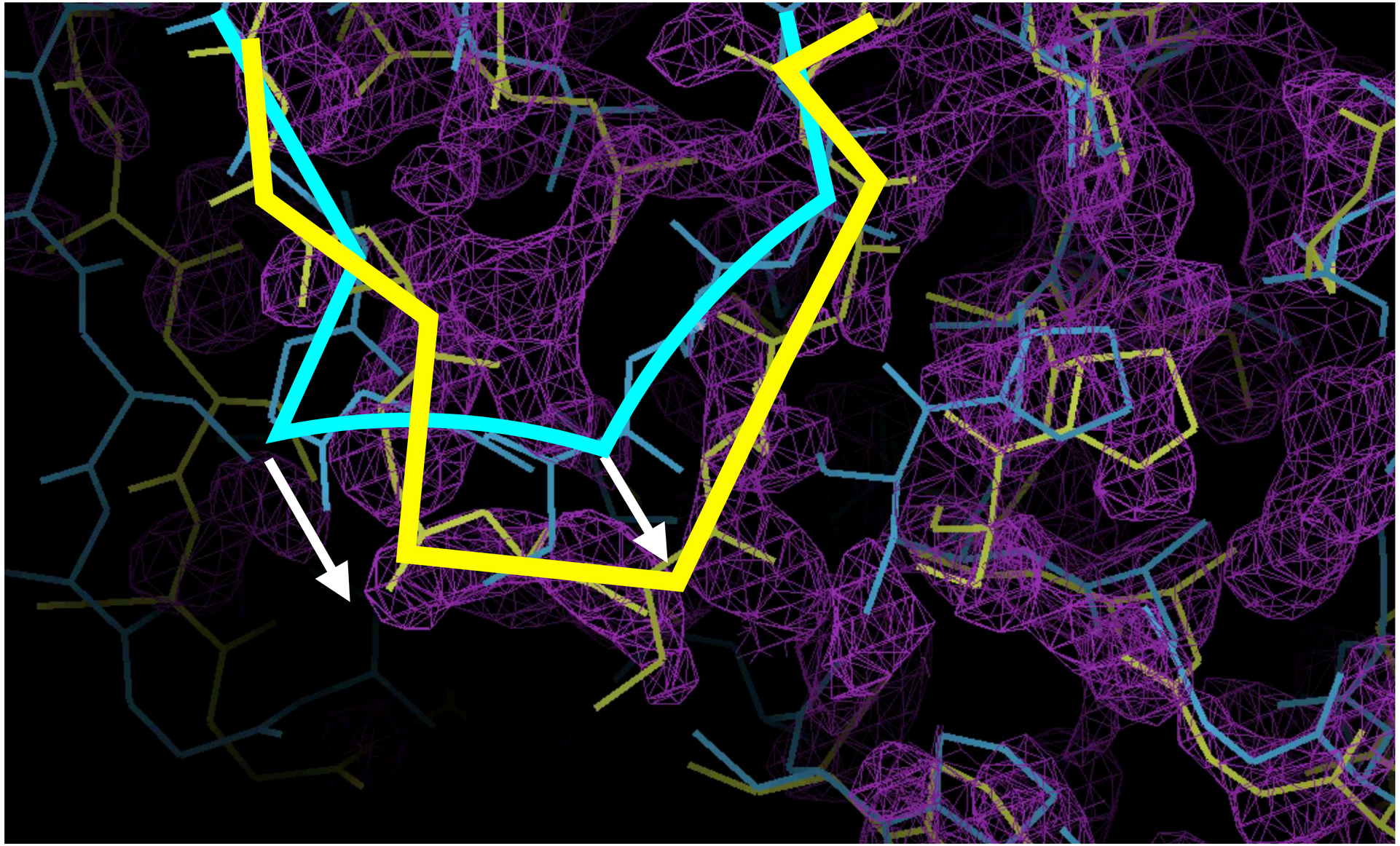
- **Can allow for larger shifts in model than simple minimization**

Optimization protocols: Torsion-angle Grid Search



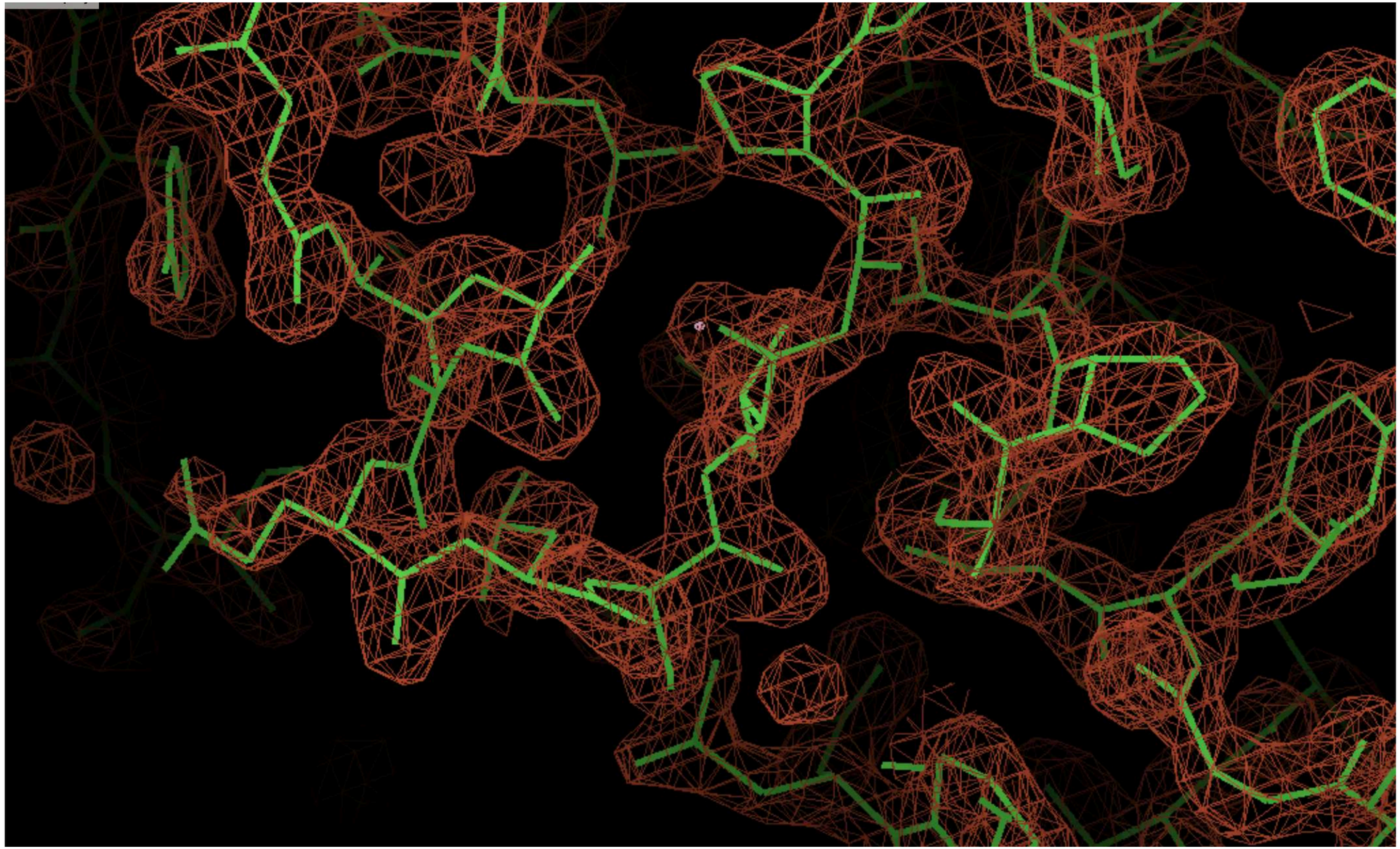
Slide adapted from: Pavel Afonine, LBNL (Phenix)

Optimization protocols: Morphing



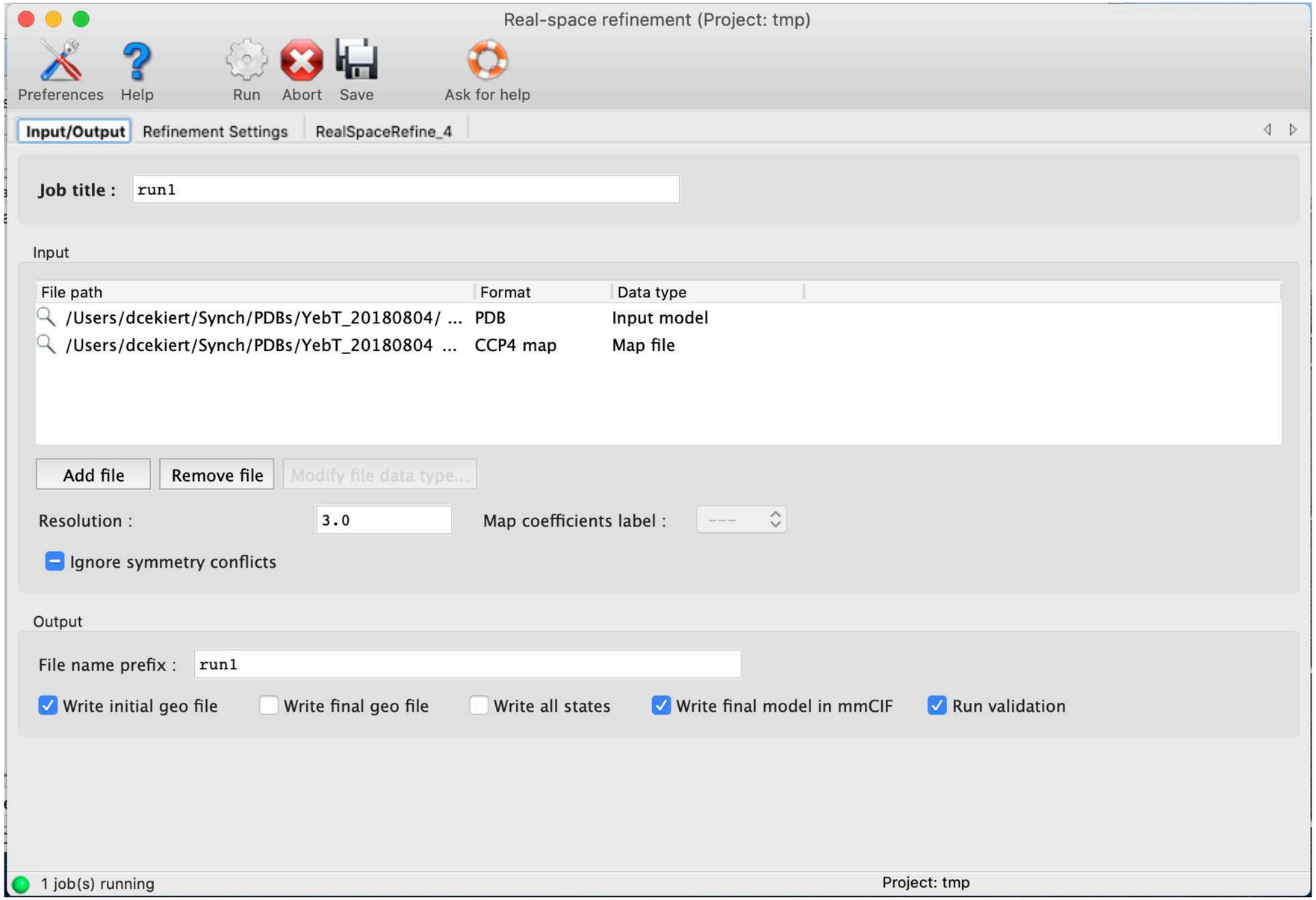
Slide adapted from: Tom Terwilliger, Los Alamos (Phenix)

Optimization protocols: Morphing



Slide adapted from: Tom Terwilliger, Los Alamos (Phenix)

Examples from Phenix GUI



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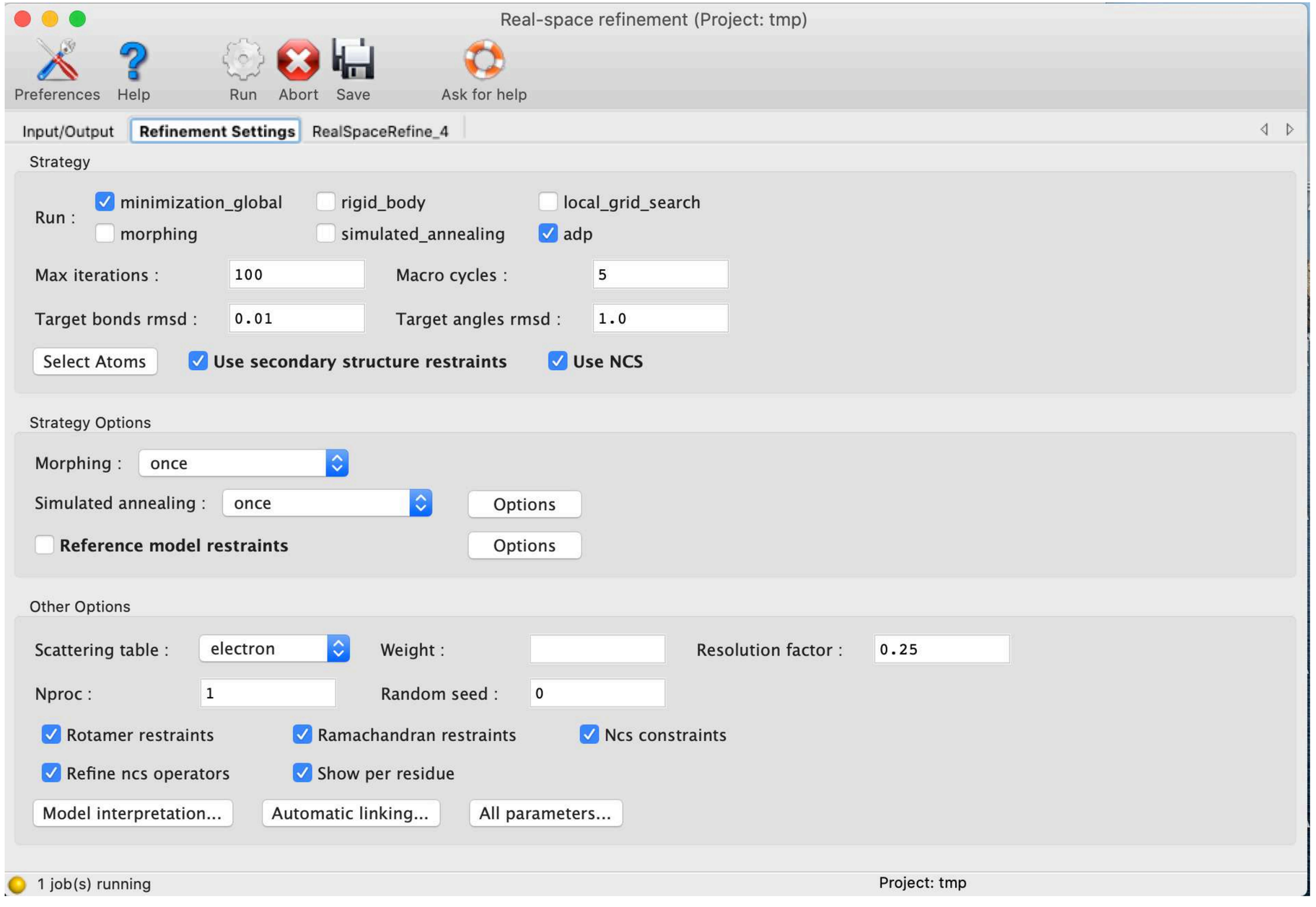

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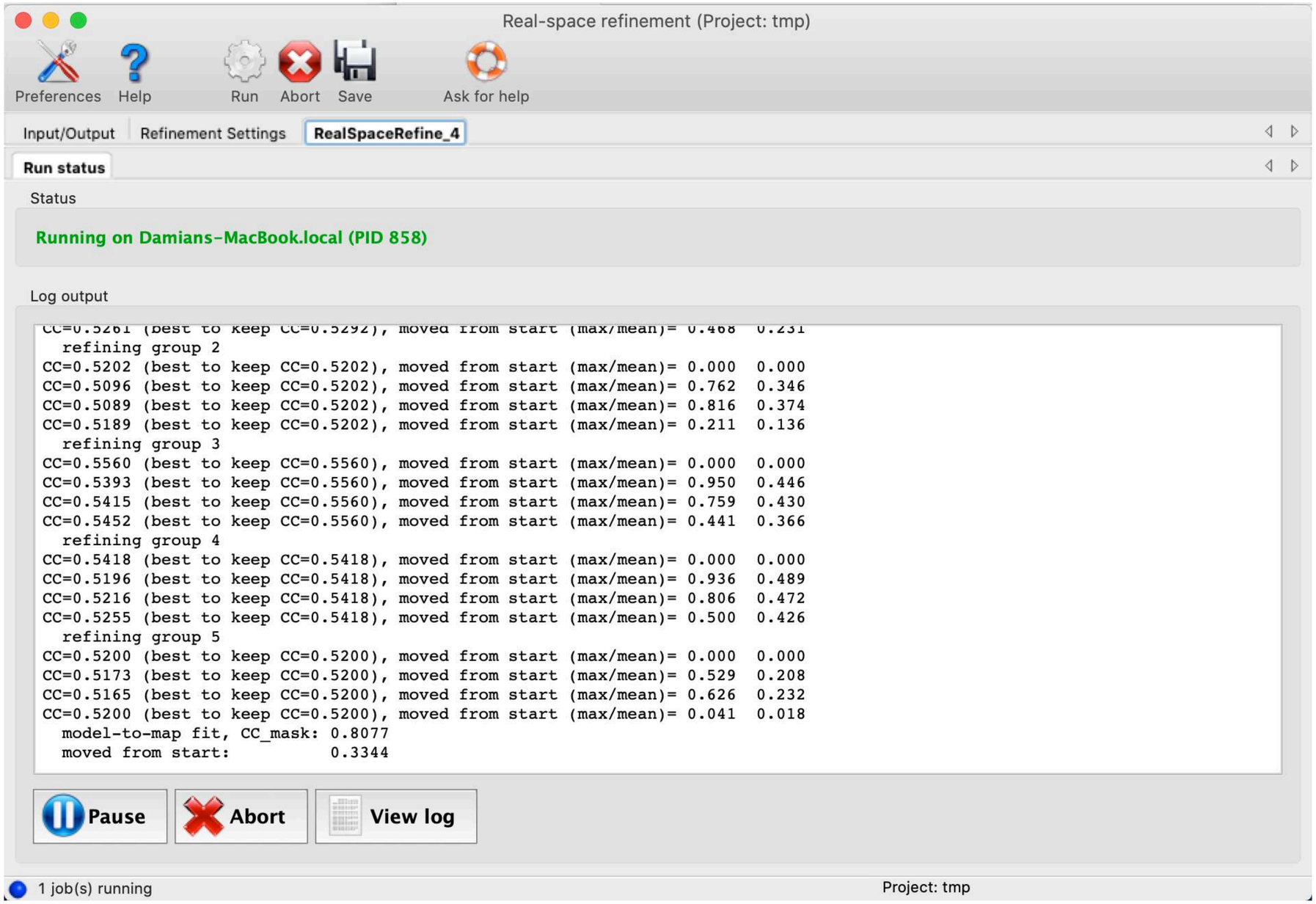

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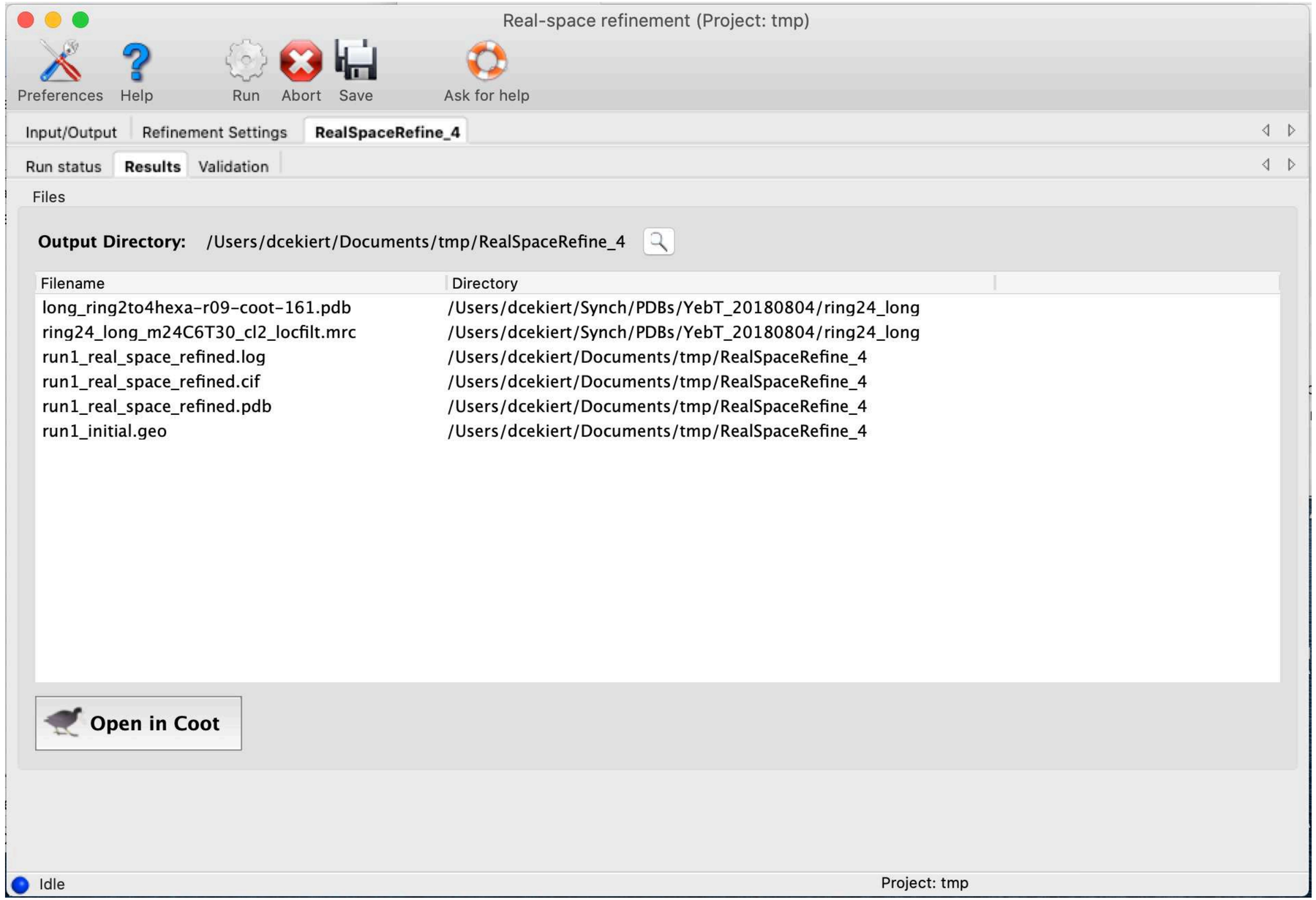
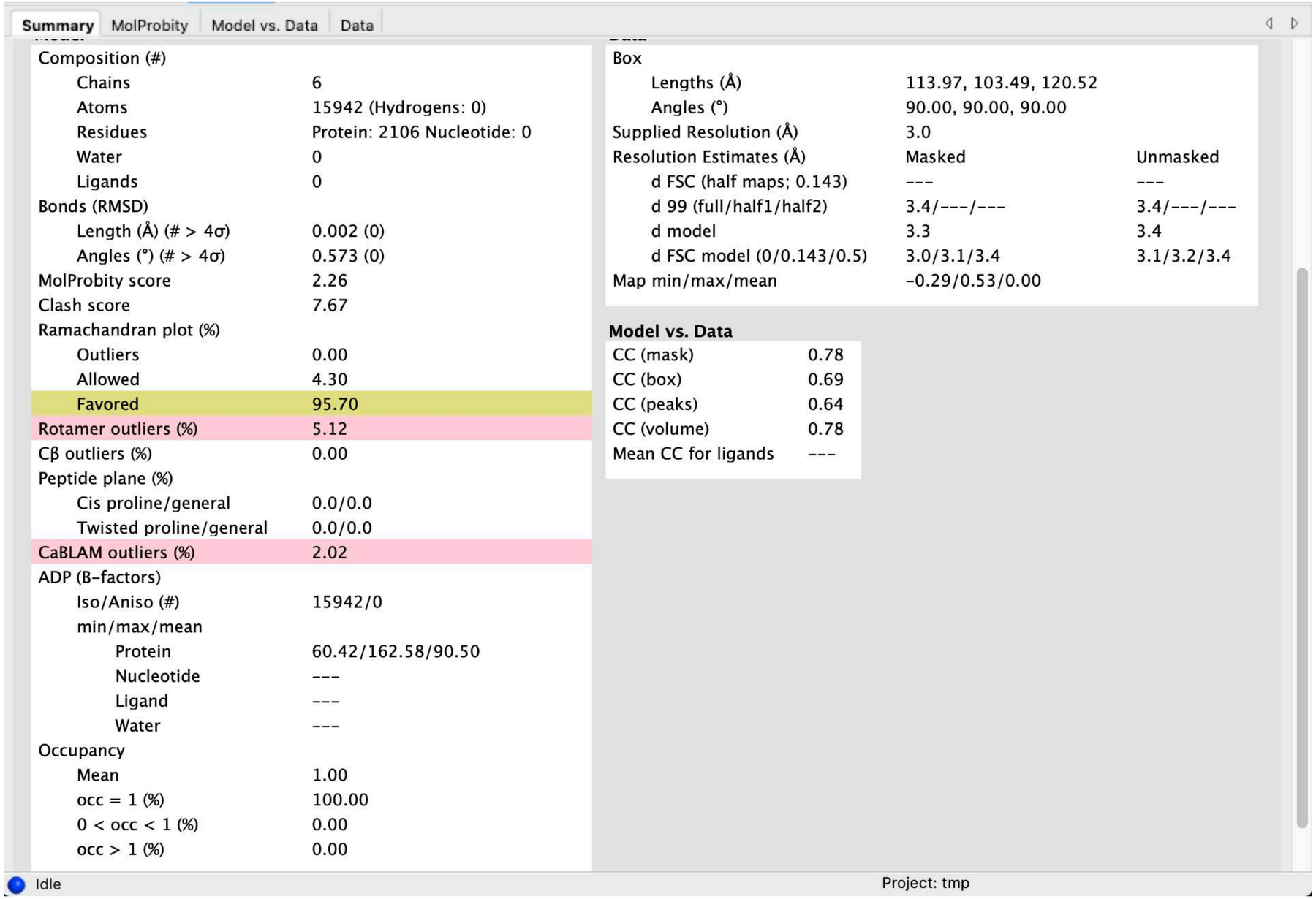


What does the output look like?



Summary | MolProbity | Model vs. Data | Data

Composition (#)	
Chains	6
Atoms	15942 (Hydrogens: 0)
Residues	Protein: 2106 Nucleotide: 0
Water	0
Ligands	0
Bonds (RMSD)	
Length (Å) (# > 4σ)	0.002 (0)
Angles (°) (# > 4σ)	0.573 (0)
MolProbity score	2.26
Clash score	7.67
Ramachandran plot (%)	
Outliers	0.00
Allowed	4.30
Favored	95.70
Rotamer outliers (%)	5.12
Cβ outliers (%)	0.00
Peptide plane (%)	
Cis proline/general	0.0/0.0
Twisted proline/general	0.0/0.0
CaBLAM outliers (%)	2.02
ADP (B-factors)	
Iso/Aniso (#)	15942/0
min/max/mean	
Protein	60.42/162.58/90.50
Nucleotide	---
Ligand	---
Water	---
Occupancy	
Mean	1.00
occ = 1 (%)	100.00
0 < occ < 1 (%)	0.00
occ > 1 (%)	0.00

Box		
Lengths (Å)	113.97, 103.49, 120.52	
Angles (°)	90.00, 90.00, 90.00	
Supplied Resolution (Å)	3.0	
Resolution Estimates (Å)	Masked	Unmasked
d FSC (half maps; 0.143)	---	---
d 99 (full/half1/half2)	3.4/---/---	3.4/---/---
d model	3.3	3.4
d FSC model (0/0.143/0.5)	3.0/3.1/3.4	3.1/3.2/3.4
Map min/max/mean	-0.29/0.53/0.00	

Model vs. Data

CC (mask)	0.78
CC (box)	0.69
CC (peaks)	0.64
CC (volume)	0.78
Mean CC for ligands	---

Idle

Project: tmp

Goal of model validation:

1) To assess refinement strategies and progress

2) To identify problem areas requiring manual intervention

3) To assess overall and local model quality/reliability

“Self-assessment”: We want to create the most accurate and reliable model we can, and validation stats clue us in to regions of the mode that may have issues

Model validation metrics - By Problem type

- Overall Quality Indicators
    - Model/Map CC
    - RMS deviations
    - Unmodeled densities
    - Molprobity score and clash score
- Backbone issues
    - Ramachandran plot
    - Cis peptide bonds
- Side chain issues:
    - Rotamer outliers (EM ringer?)
    - Cbeta deviations
- B factor / ADP outliers

Model validation metrics - By source of problem

- Model building problem
    - Unmodeled densities
    - Cbeta deviations
    - Ramachandran plot
    - Cis peptide bonds
    - Model/Map CC
- Refinement problem
    - RMS deviations
    - B factor / ADP outliers
- Either/both?
    - Molprobity score and clash score
    - Rotamer outliers (EM ringer?)

Model/Map CC

Table 3
Summary of map correlation coefficients used in this work.

Metric	Region of the map used in calculation	Purpose
CC_{box}	Whole map	Similarity of maps
CC_{mask}	Jiang & Brünger (1994) mask with a fixed radius	Fit of the atomic centers
CC_{volume}	Mask of points with the highest values in the model map	Fit of the molecular envelope defined by the model map
CC_{peaks}	Mask of points with the highest values in the model and in the target maps	Fit of the strongest peaks in the model and target maps
CC_{vr_mask}	Same as CC_{mask} but atomic radii are variable and function of resolution, atom type and ADP	Fit of the atomic images in the given map

Afonine, et al. “New tools for the analysis and validation of cryo-EM maps and atomic models” Acta Cryst. 2018

Root mean square (RMS) deviations

Covalent bond lengths and angles exhibit known, narrow distributions

Typical RMS Bonds for protein structure:
0.005 - 0.015 Å

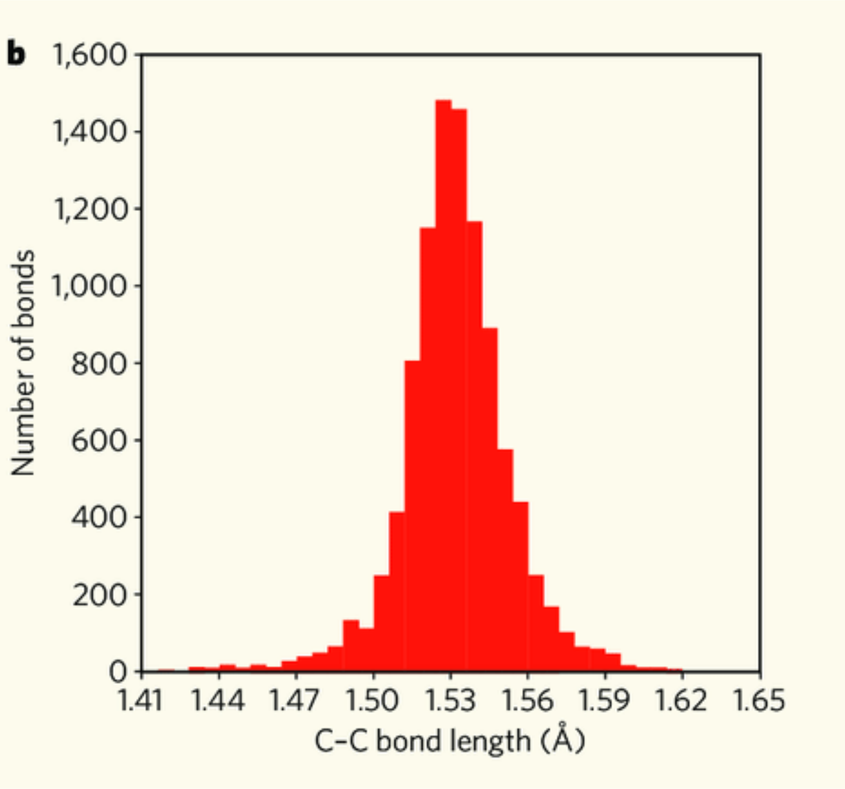
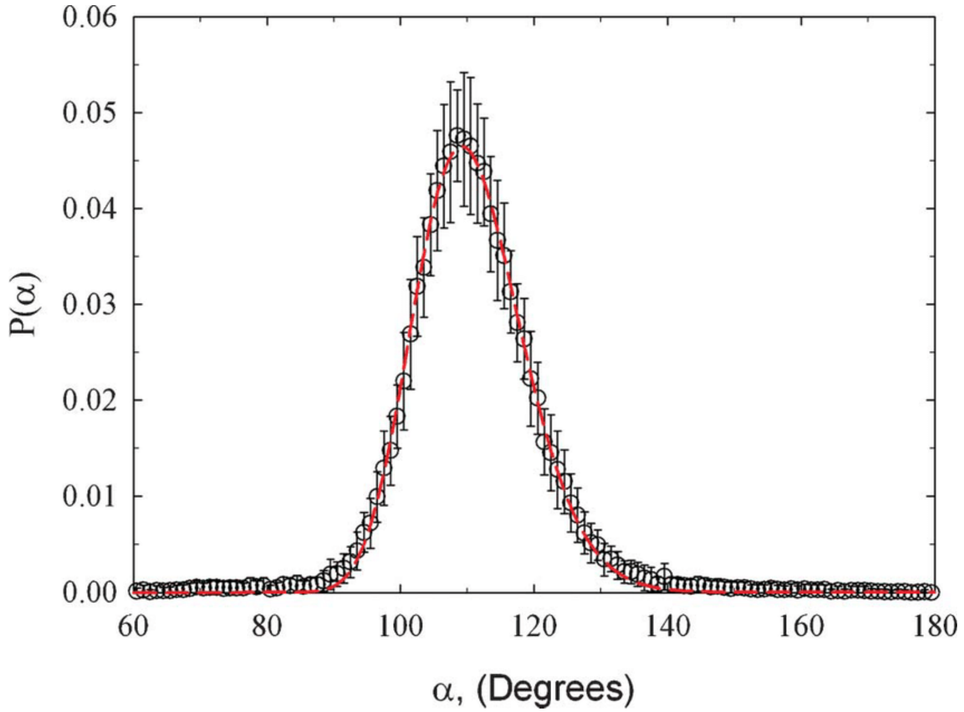


Image: Janez Stepisnik

Typical RMS Angles for protein structure:
0.5 - 1.5 Å



C-C-C angle distribution of sp3 carbon
Kowalczyk, et al. RSC Advances, 2012.

Root mean square (RMS) deviations

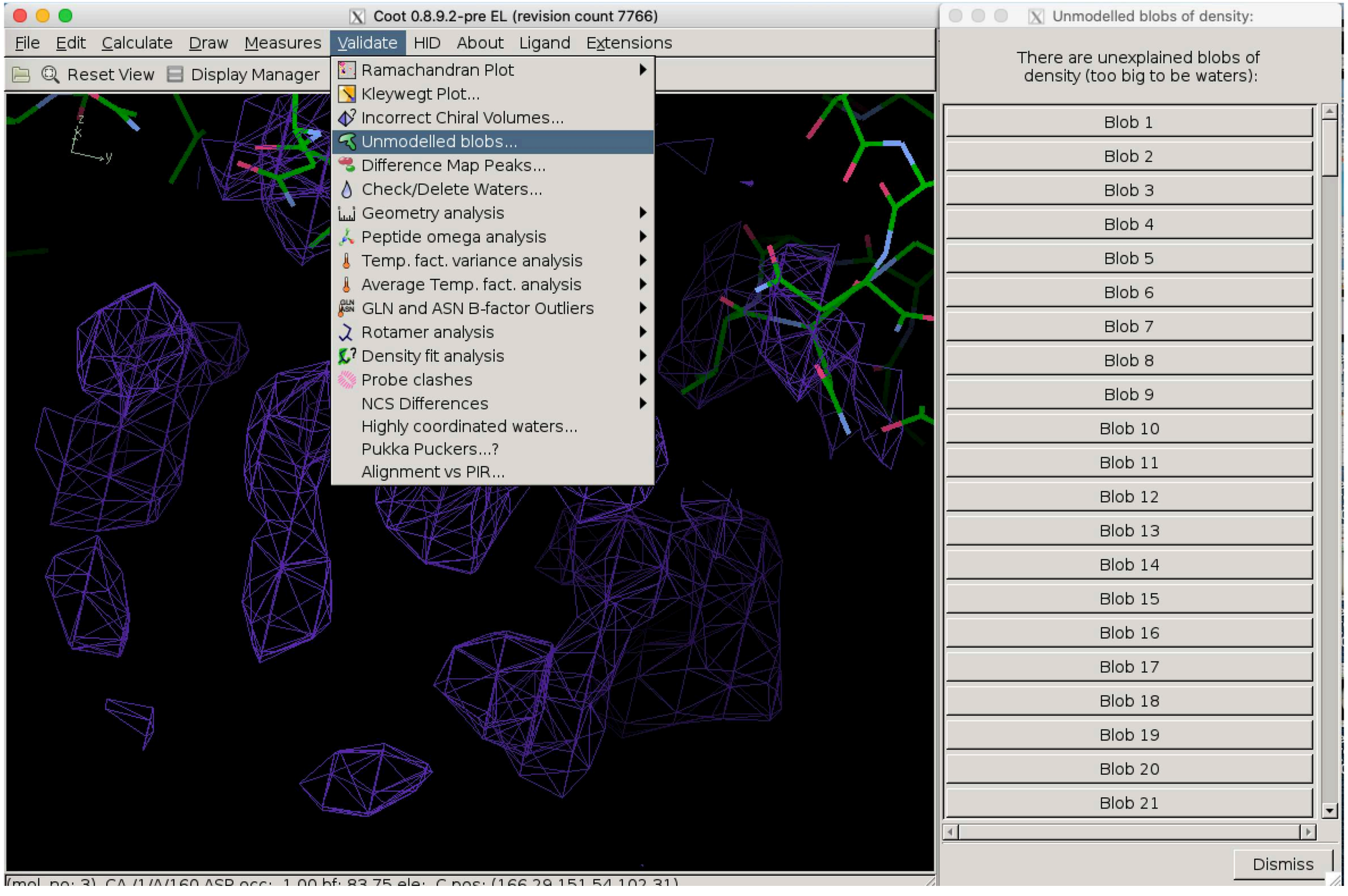
Covalent bond lengths and angles exhibit known, narrow distributions

Typical RMS Bonds for protein structure:
0.005 - 0.015 Å

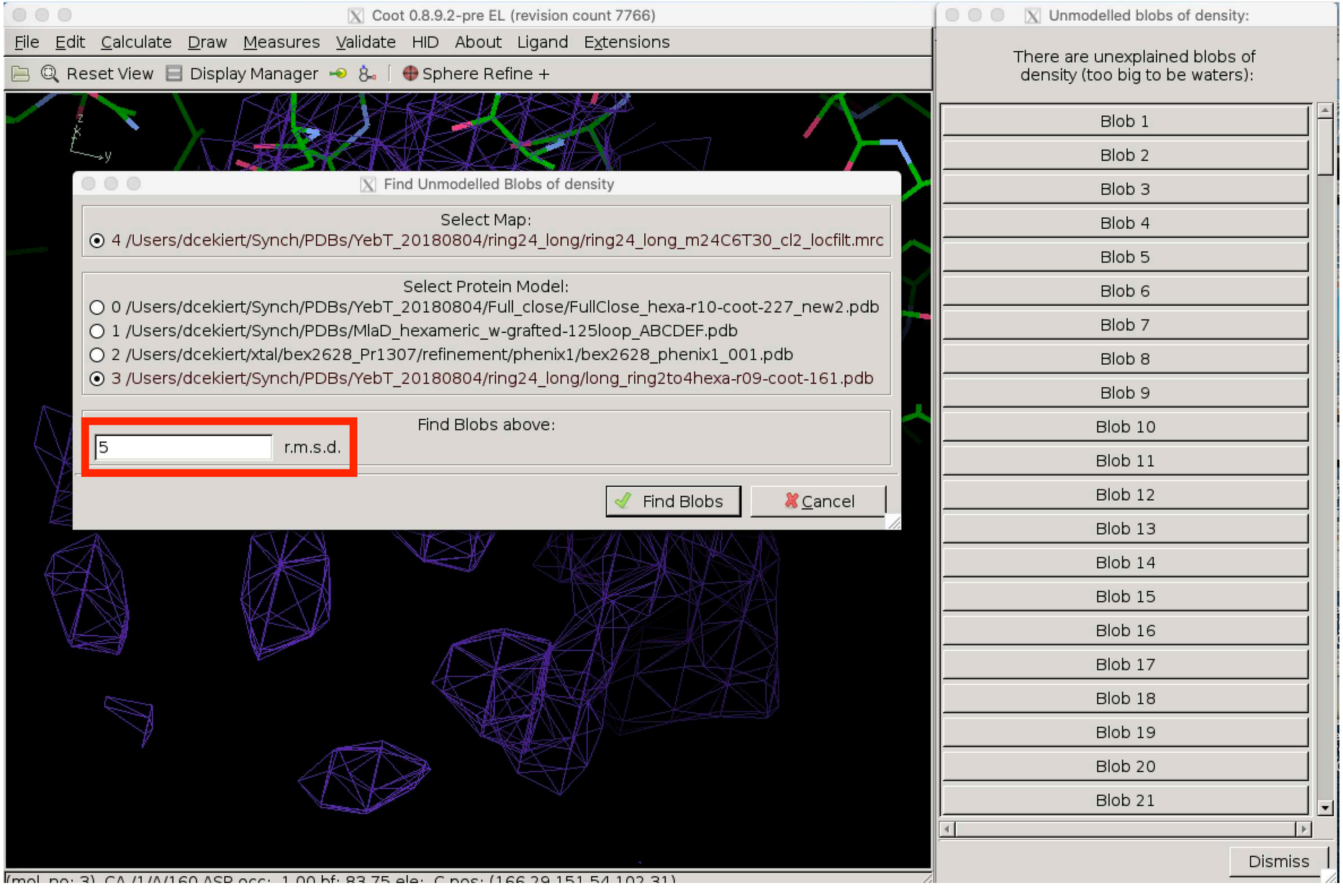
Typical RMS Angles for protein structure:
0.5 - 1.5 Å

```
REMARK   3
REMARK   3  DEVIATIONS FROM IDEAL VALUES.
REMARK   3                    RMSD      MAX   COUNT
REMARK   3   BOND       :    0.010    0.098   16212
REMARK   3   ANGLE      :    1.328   19.912   21996
REMARK   3   CHIRALITY  :    0.074    0.294    2586
REMARK   3   PLANARITY  :    0.009    0.061    2856
REMARK   3   DIHEDRAL   :    9.311  179.980    9852
REMARK   3   MIN NONBONDED DISTANCE : 2.433
REMARK   3
```

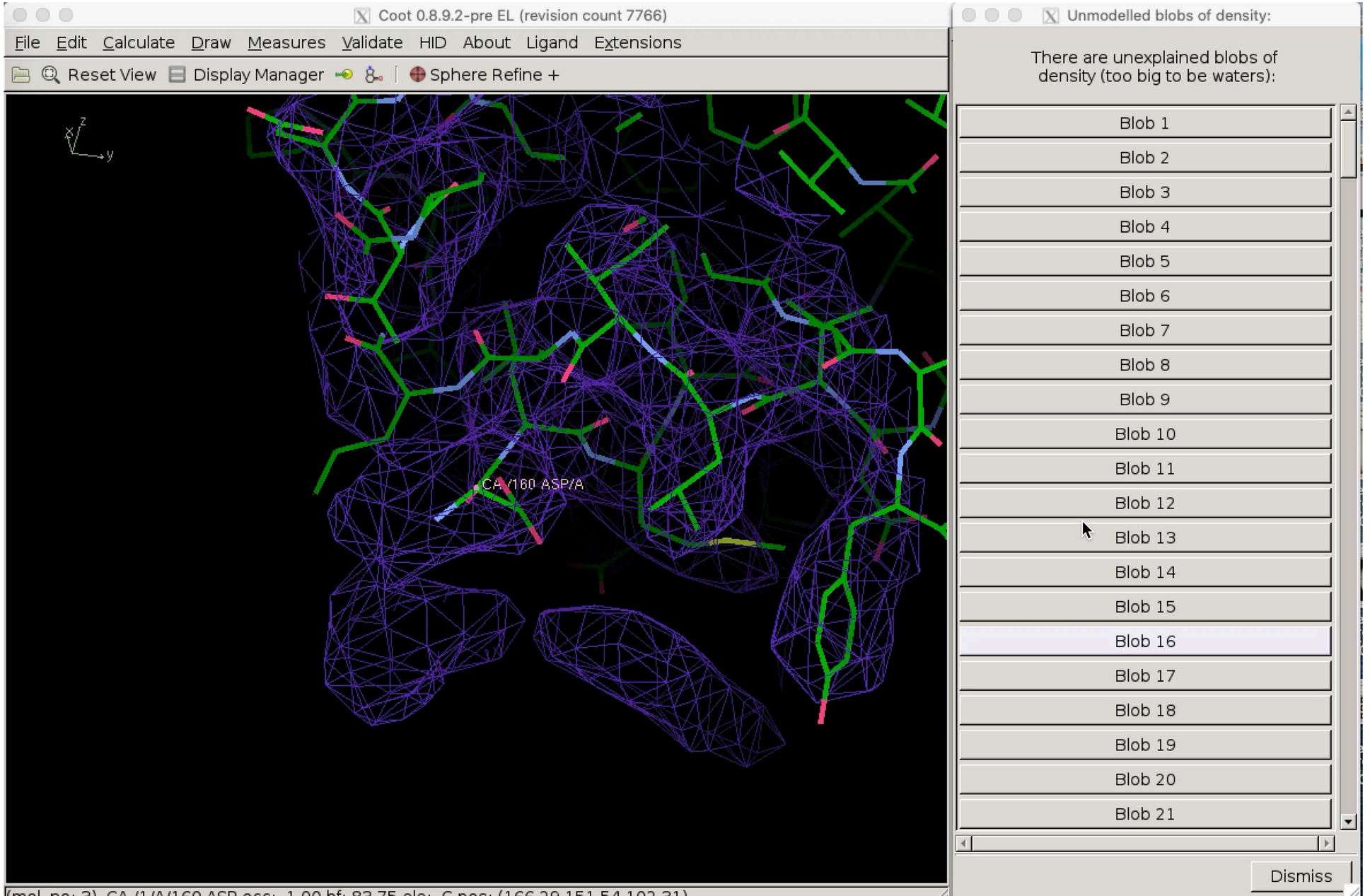
Unmodeled densities



Unmodeled densities



Unmodeled densities



Molprobity score and clash score

Summary statistics

All-Atom Contacts	Clashscore, all atoms:	3.82		96th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	12	0.68%	Goal: <0.3%
	Favored rotamers	1674	95.22%	Goal: >98%
	Ramachandran outliers	0	0.00%	Goal: <0.05%
	Ramachandran favored	2070	98.85%	Goal: >98%
	MolProbity score^	1.17		99th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	6	0.32%	Goal: 0
	Bad bonds:	0 / 16212	0.00%	Goal: 0%
	Bad angles:	24 / 21996	0.11%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 114	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	42	2.0%	Goal: <1.0%
	CA Geometry outliers	24	1.15%	Goal: <0.5%
Additional validations	Pseudochiral naming errors	6		
	Waters with clashes	0/0	0.00%	See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.

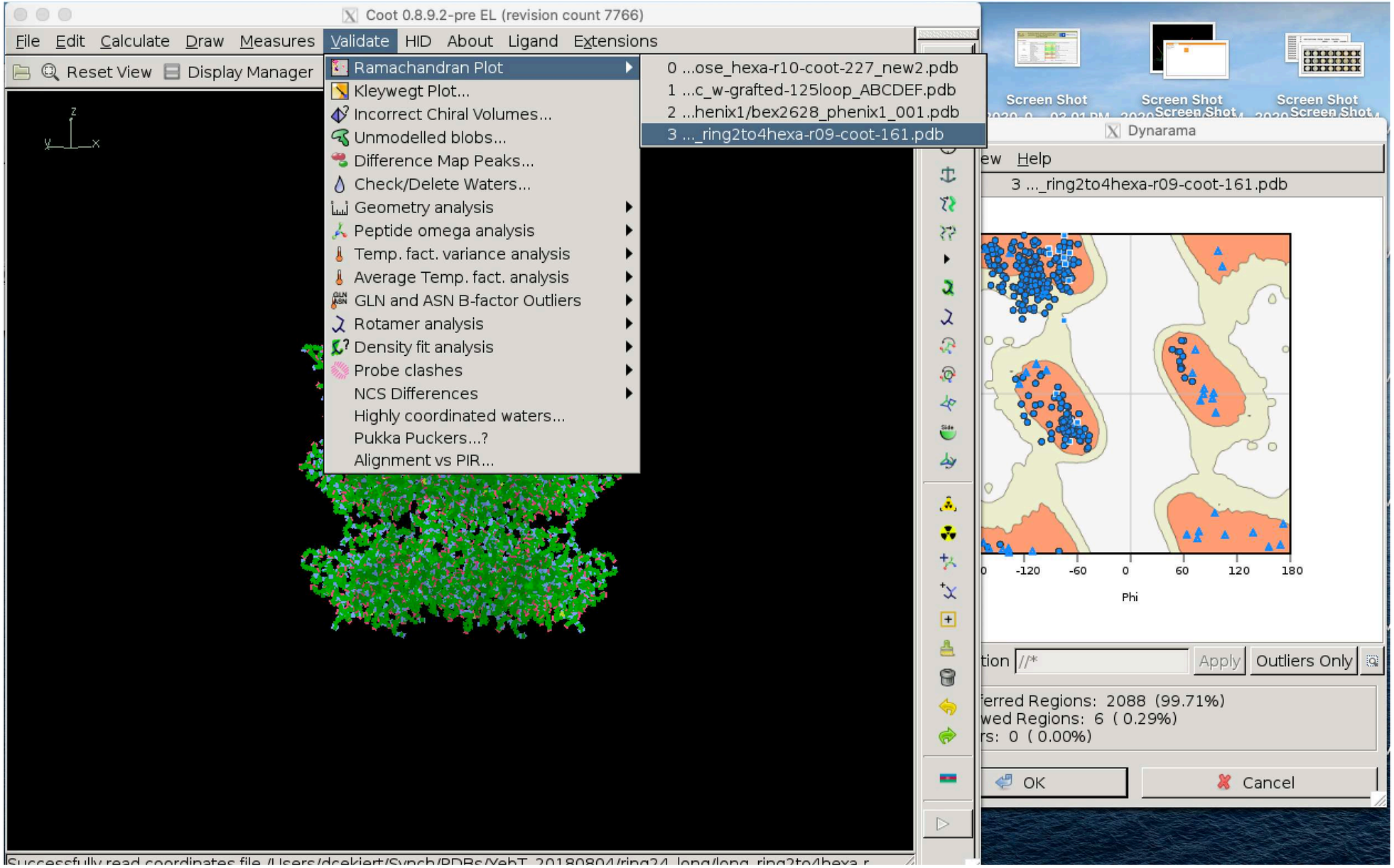
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

^ MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

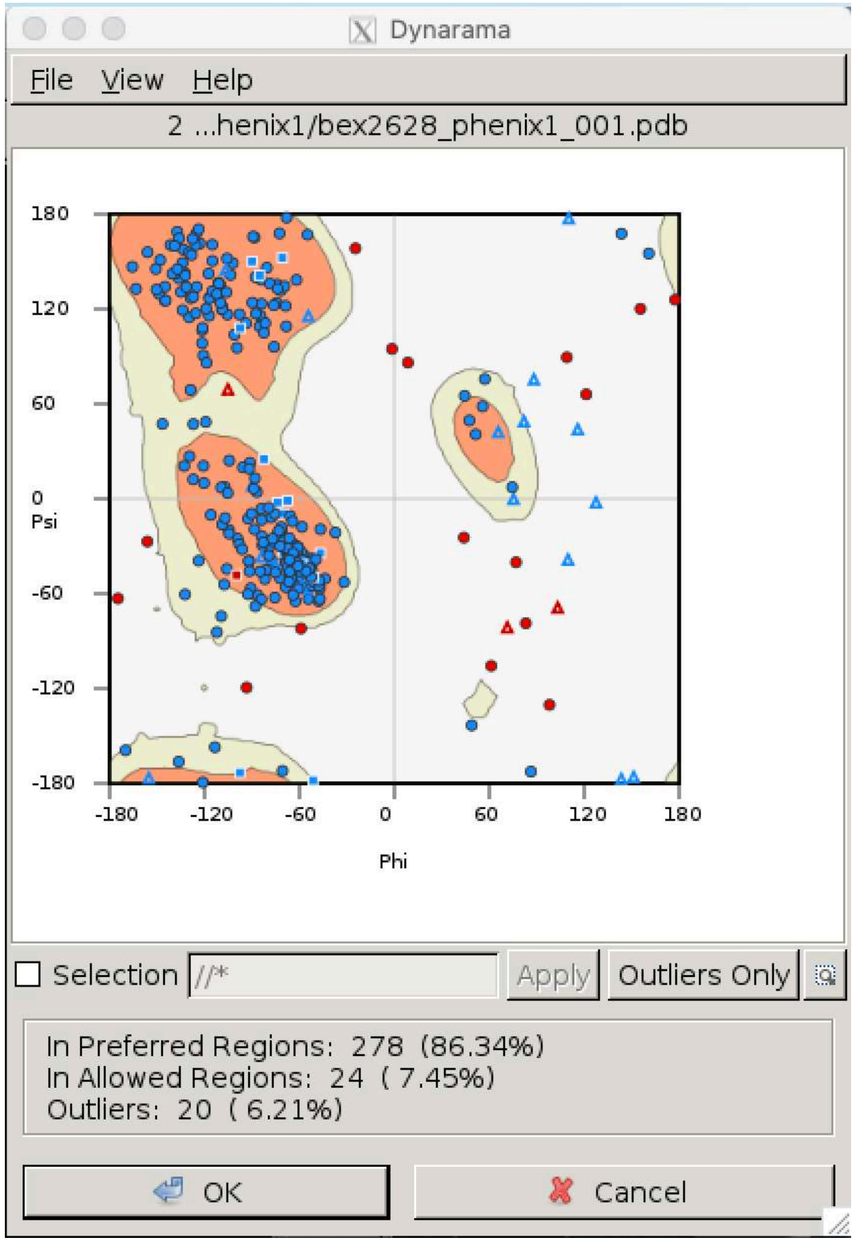
http://molprobity.biochem.duke.edu/

Williams et al. Protein Science (2018).

Ramachandran plot

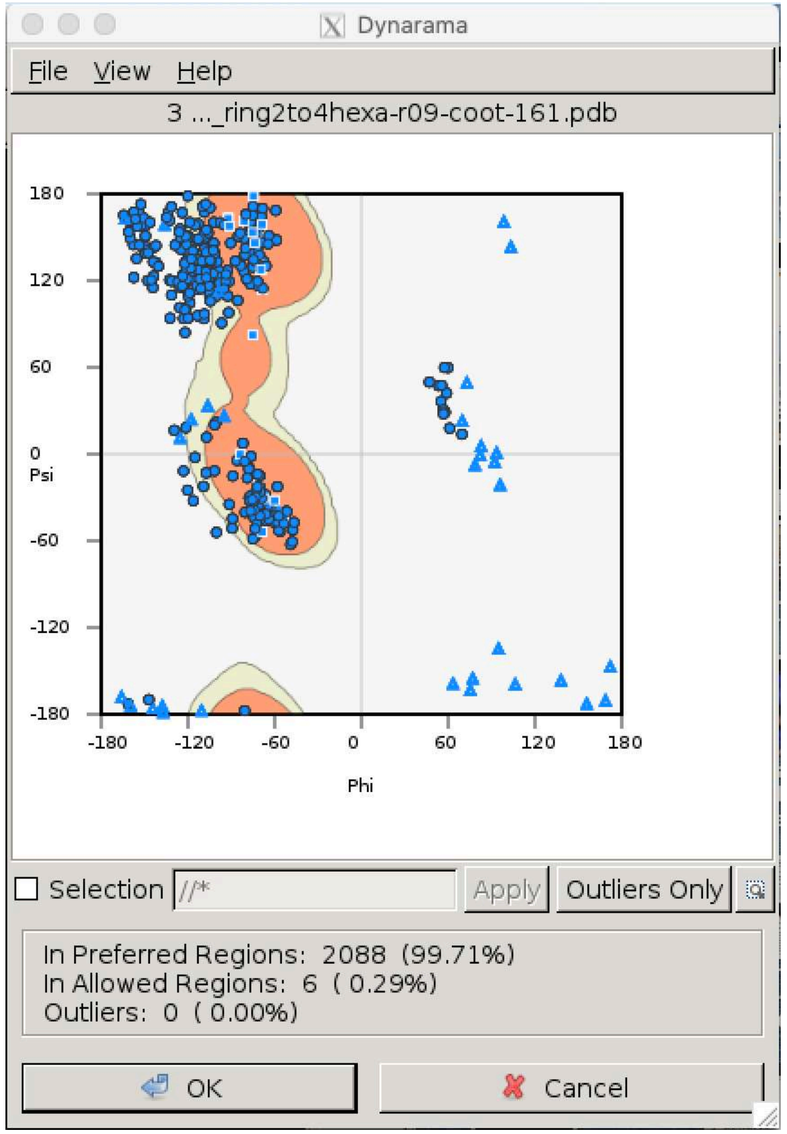


Ramachandran plot

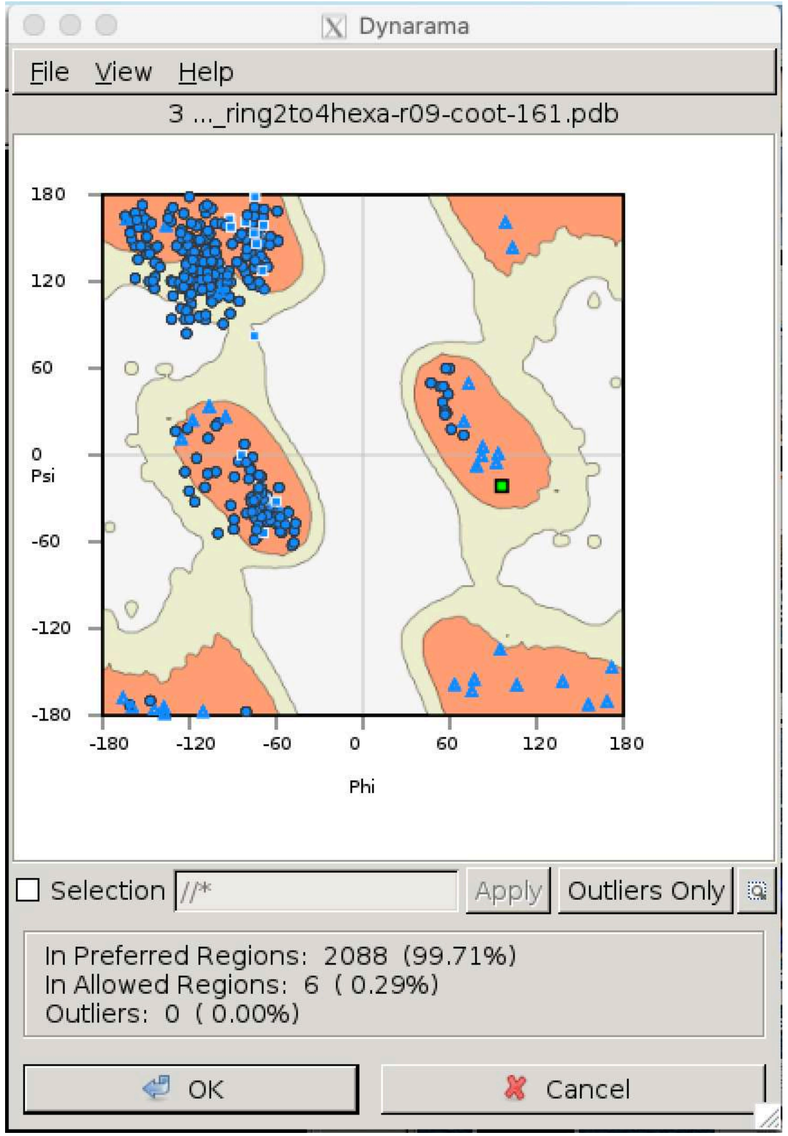


Ramachandran plot

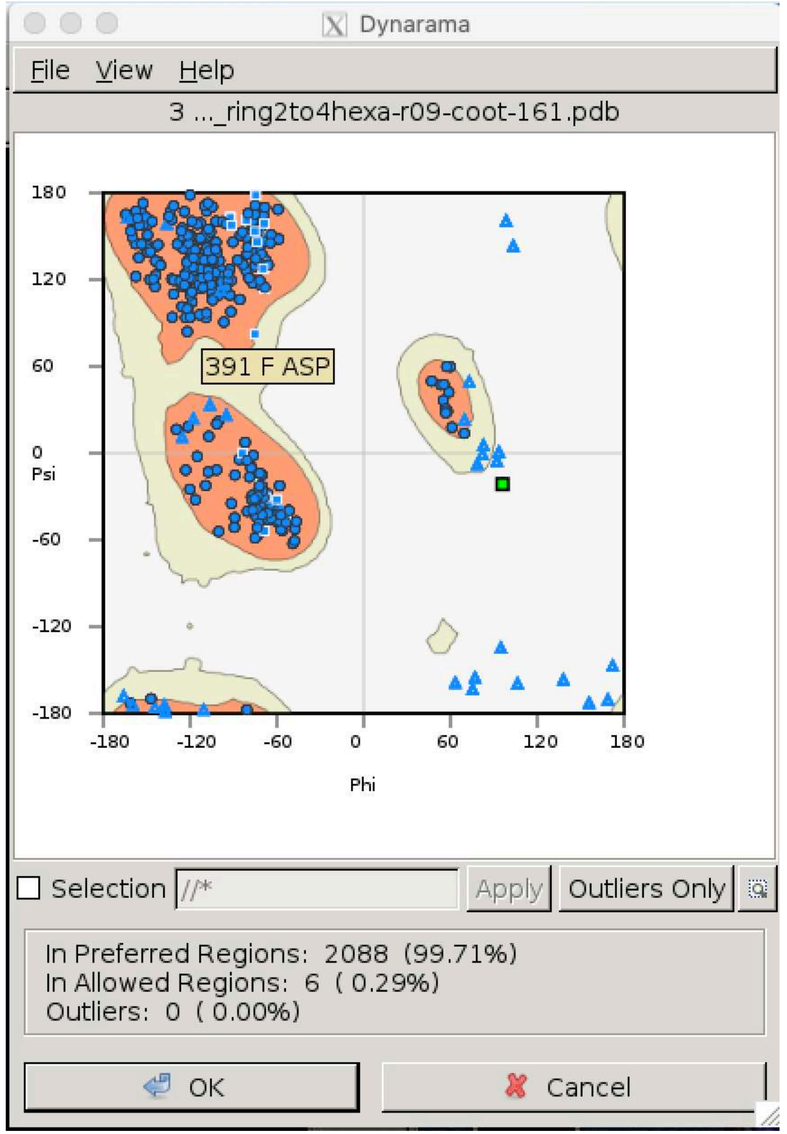
Proline Allowed Regions



Glycine Allowed Regions



Allowed Regions (non-Pro/Gly)



Cis peptide bonds

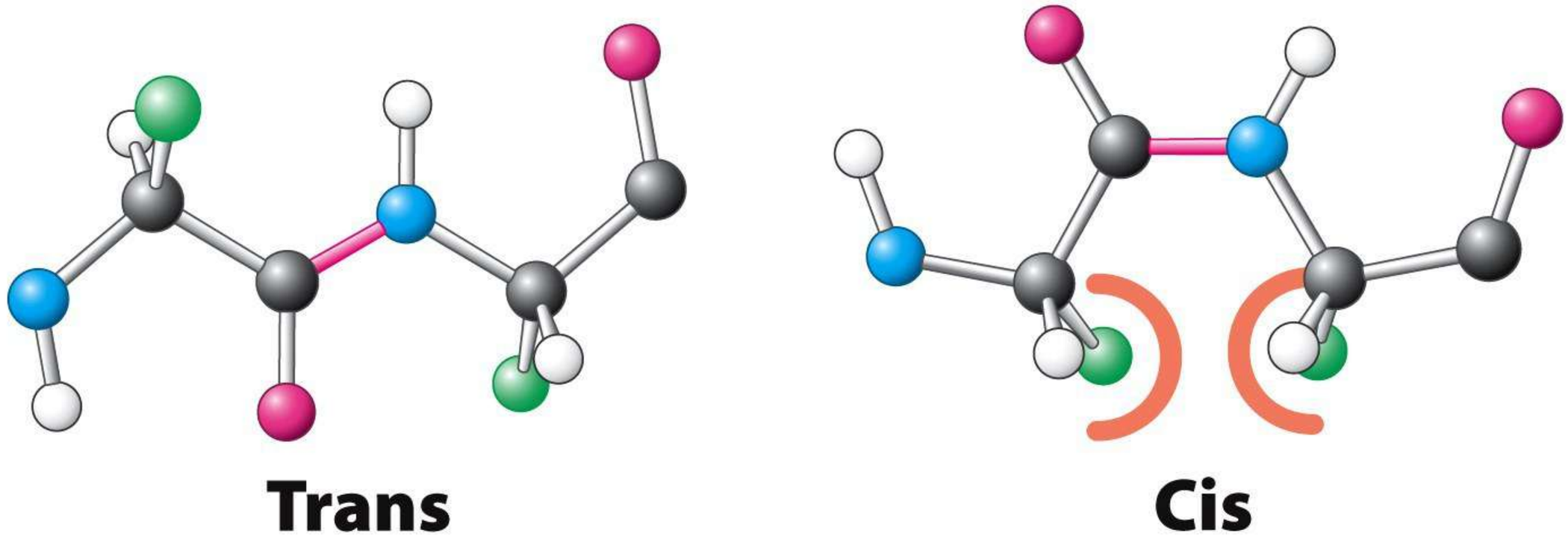


Figure 2.20
***Biochemistry*, Seventh Edition**

Cis peptide bonds

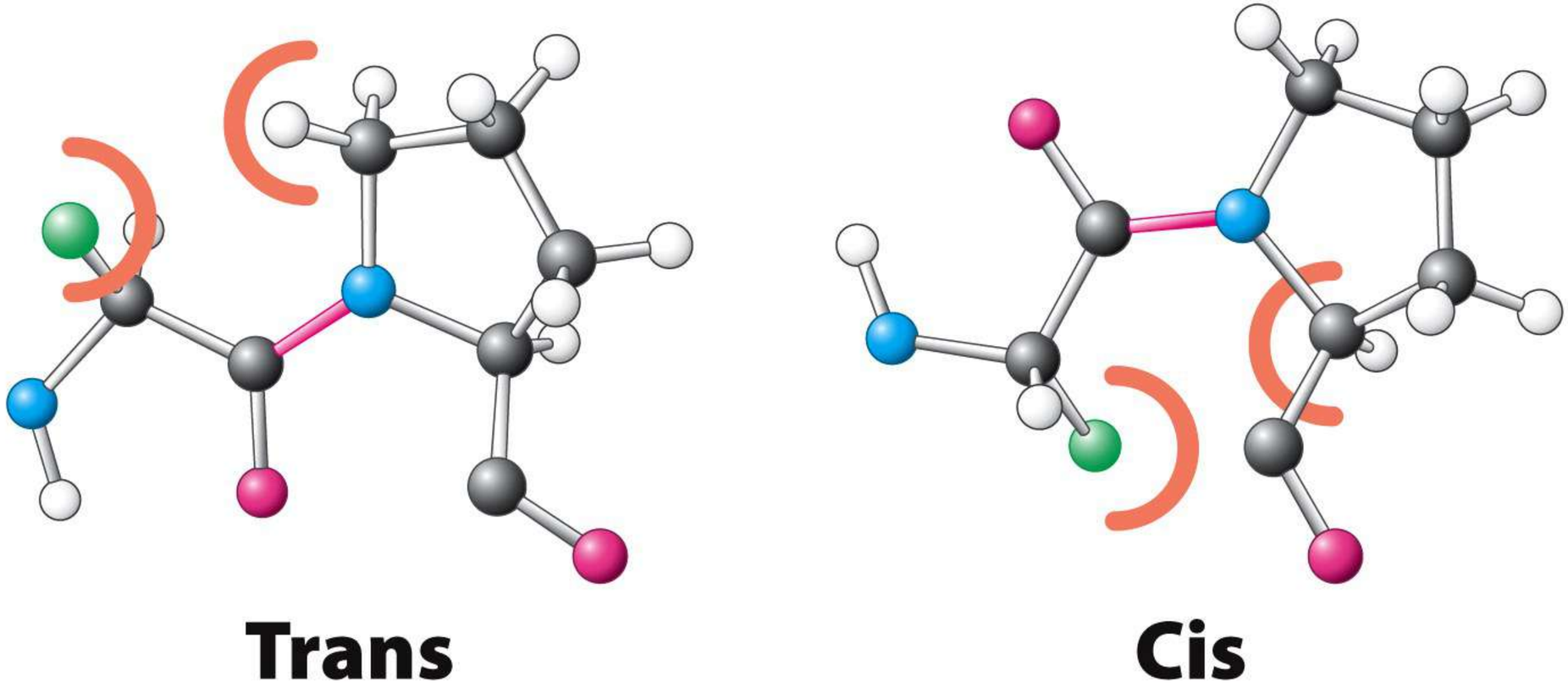
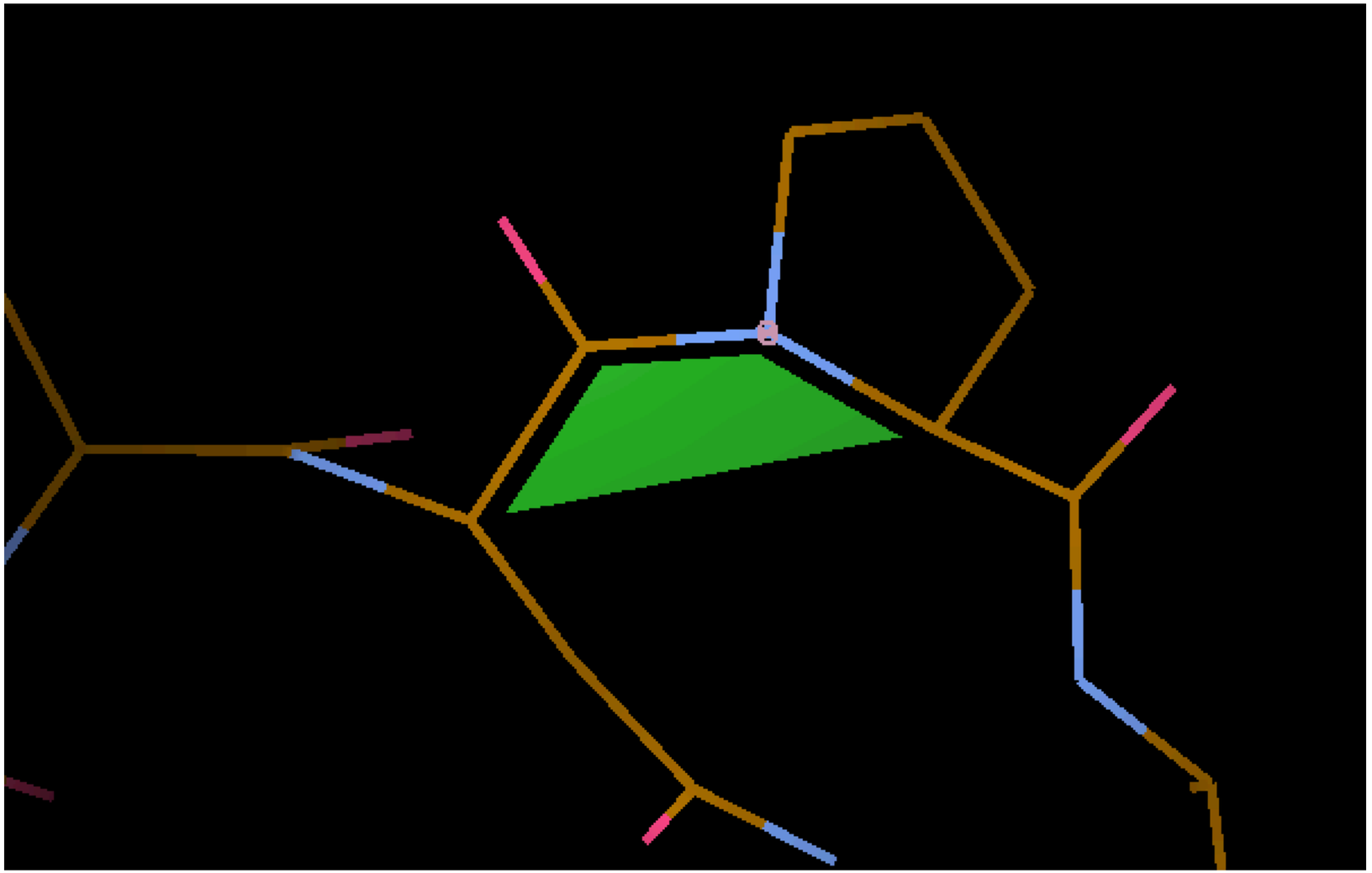


Figure 2.21
***Biochemistry*, Seventh Edition**

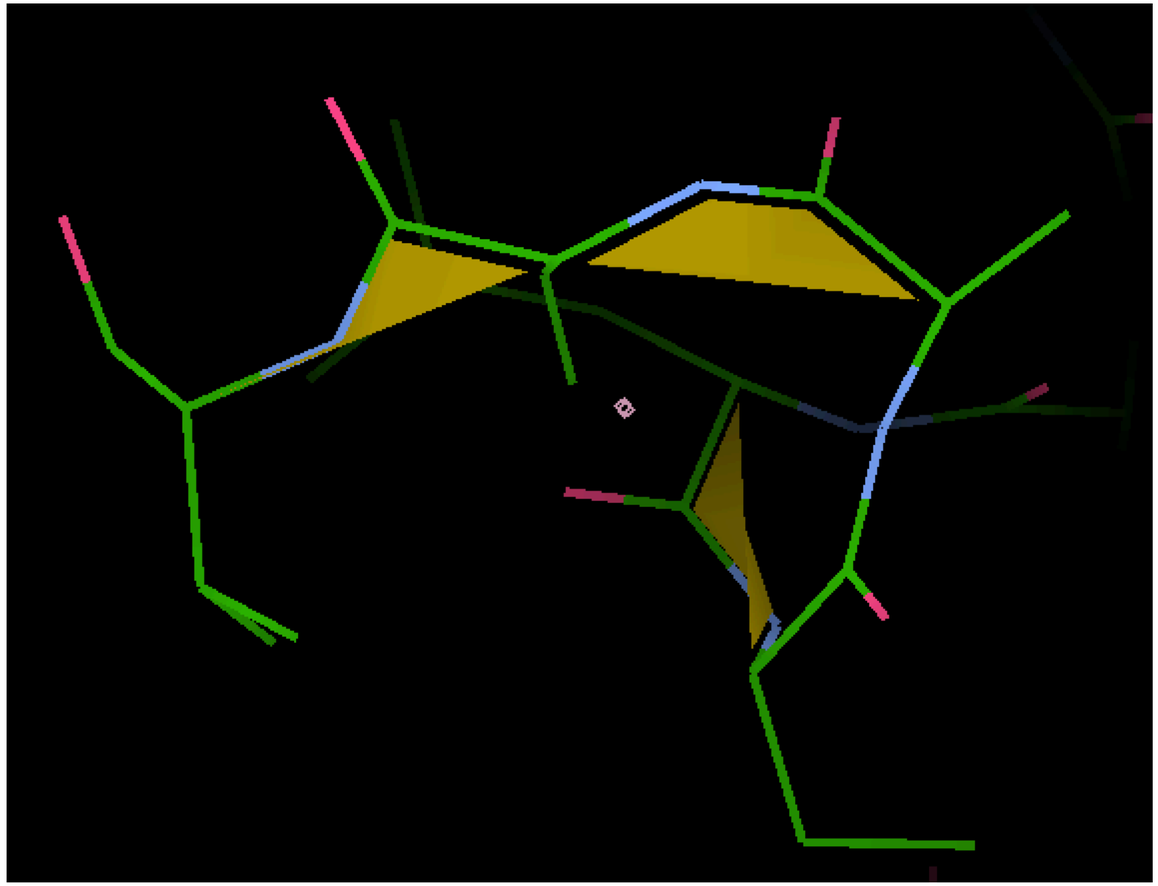
Cis peptide bonds

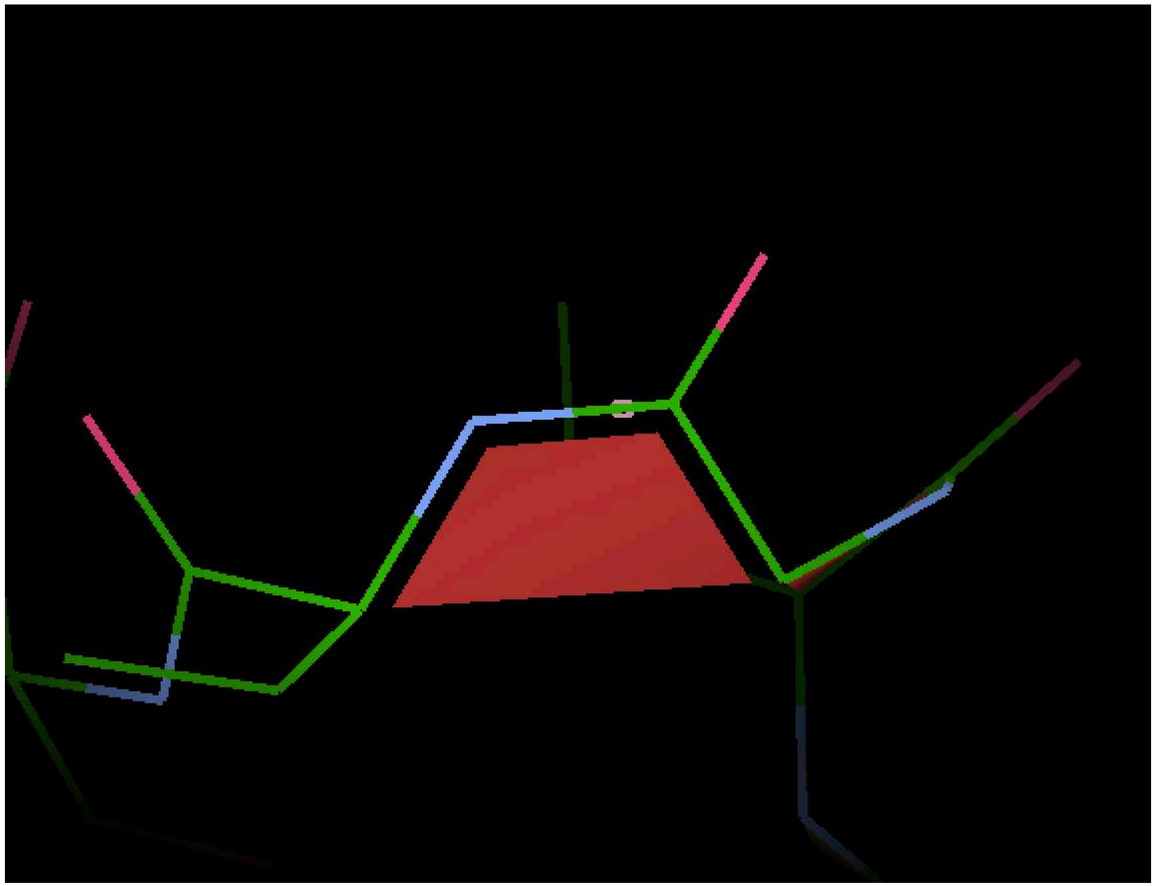


Coot highlights all cis and non-planar peptide bonds, and color codes them to make potential problems easy to ID

Green = cis-Proline (probably OK); Yellow = non-planar peptide bond (check!); Red = non-proline cis peptide bond (check!)

Cis peptide bonds

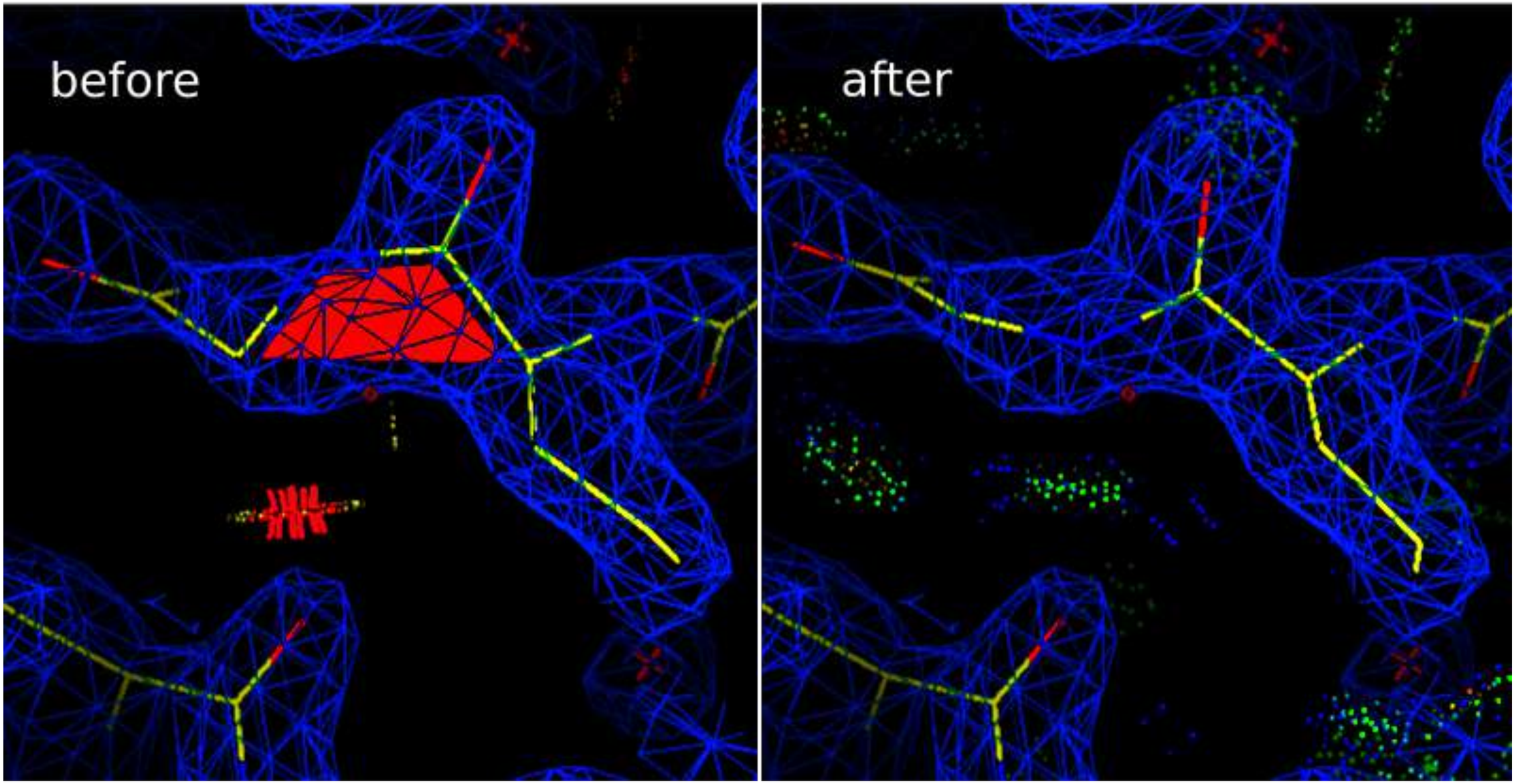




Coot highlights all cis and non-planar peptide bonds, and color codes them to make potential problems easy to ID

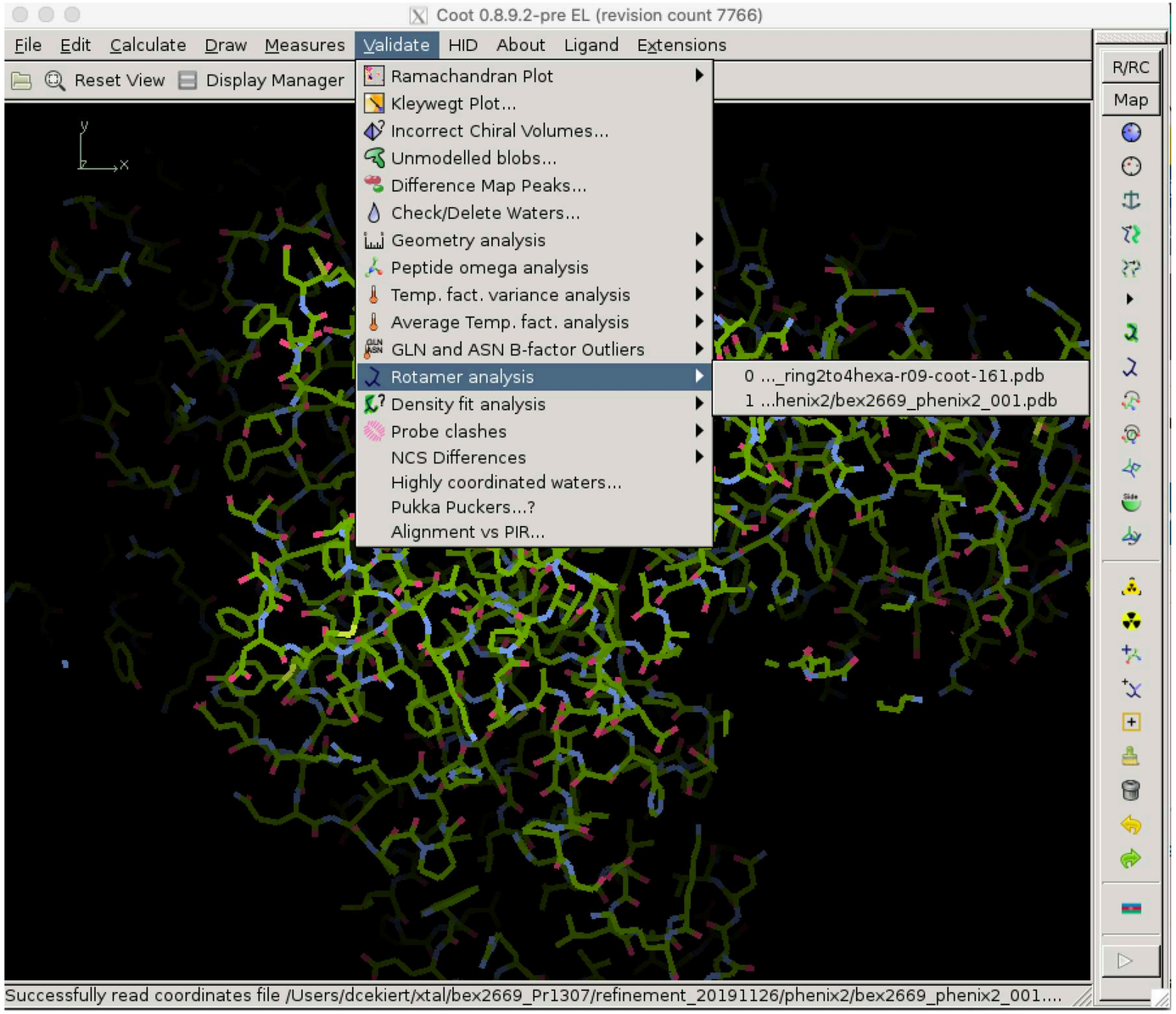
Green = cis-Proline (probably OK); Yellow = non-planar peptide bond (check!); Red = non-proline cis peptide bond (check!)

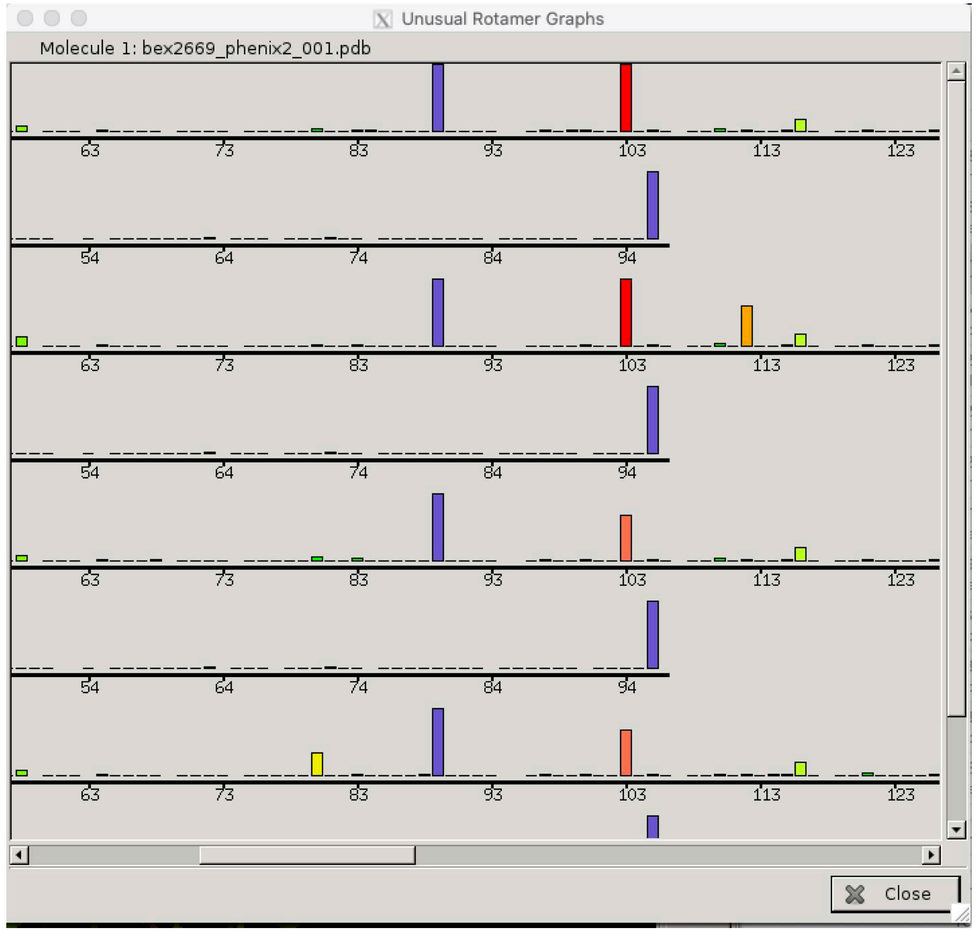
Cis peptide bonds



https://www.phenix-online.org/documentation/tutorials/molprobity.html

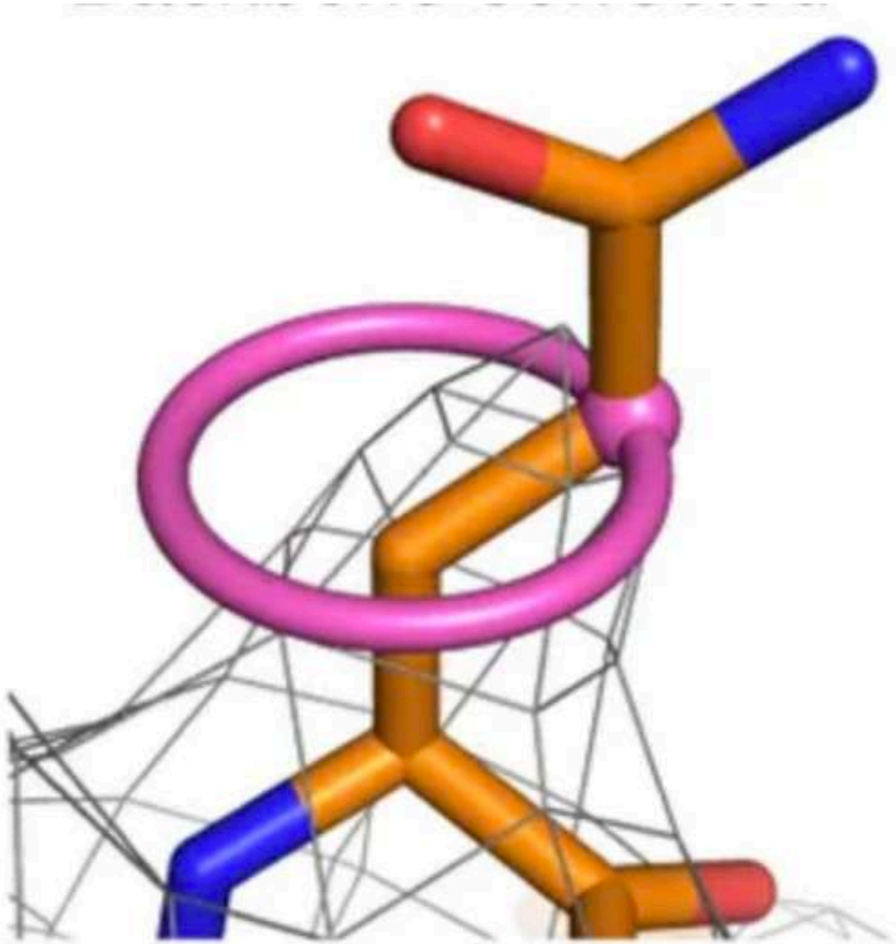
Rotamer outliers: Coot

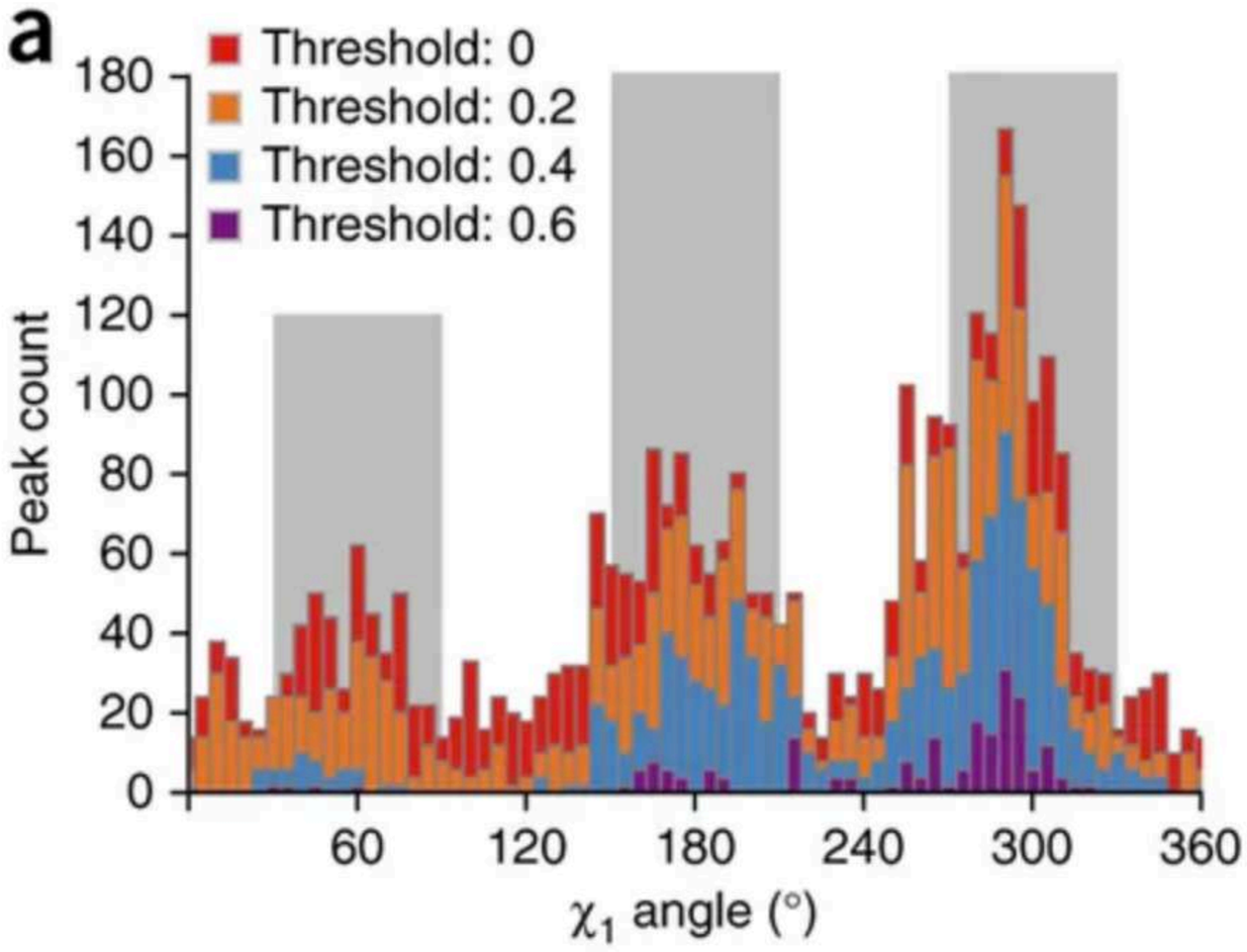




Red/Tall - Rotamer outlier
Green/Short - Happy rotamer
Lilac(?): Missing side chain atoms

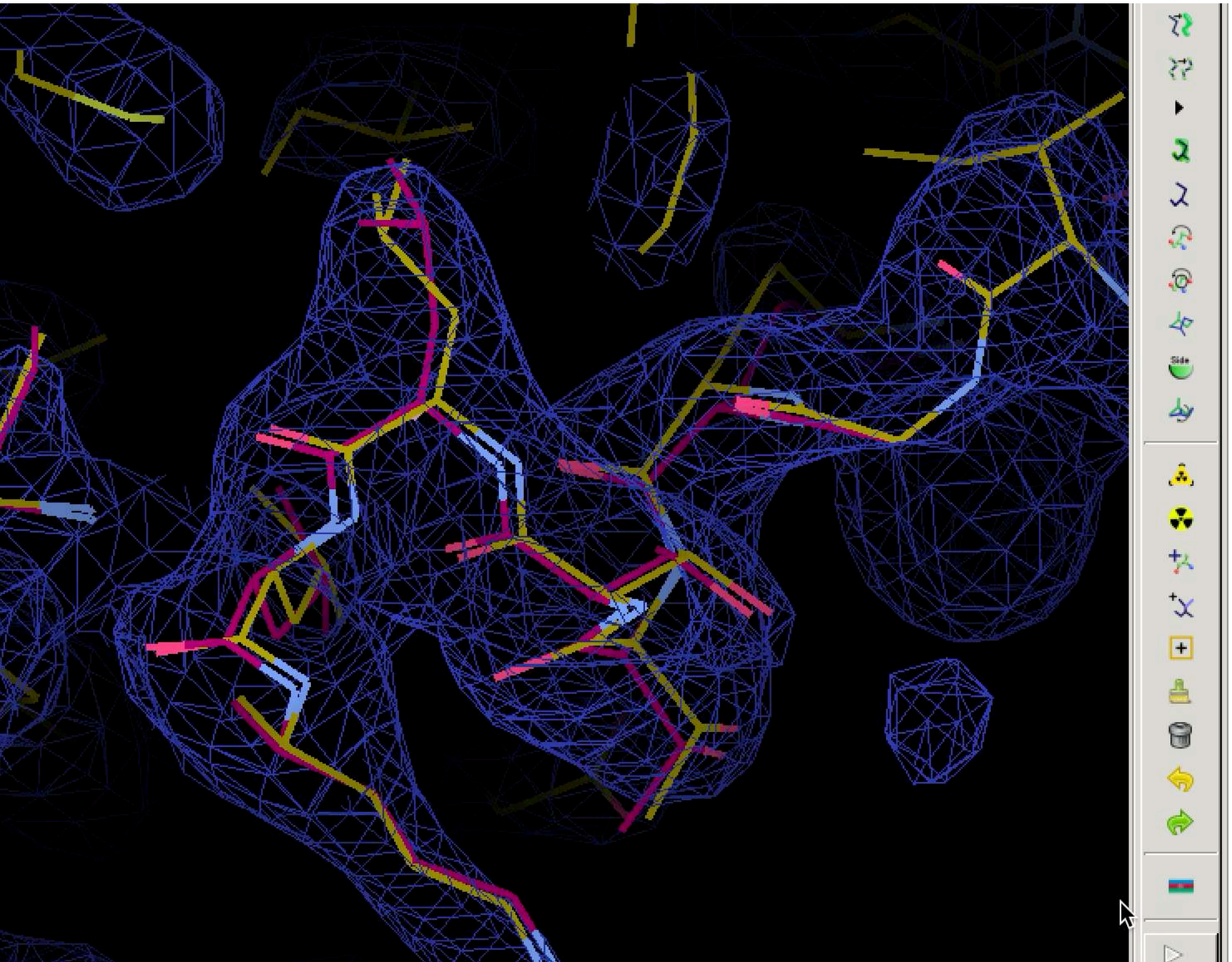
Rotamer outliers: EM ringer





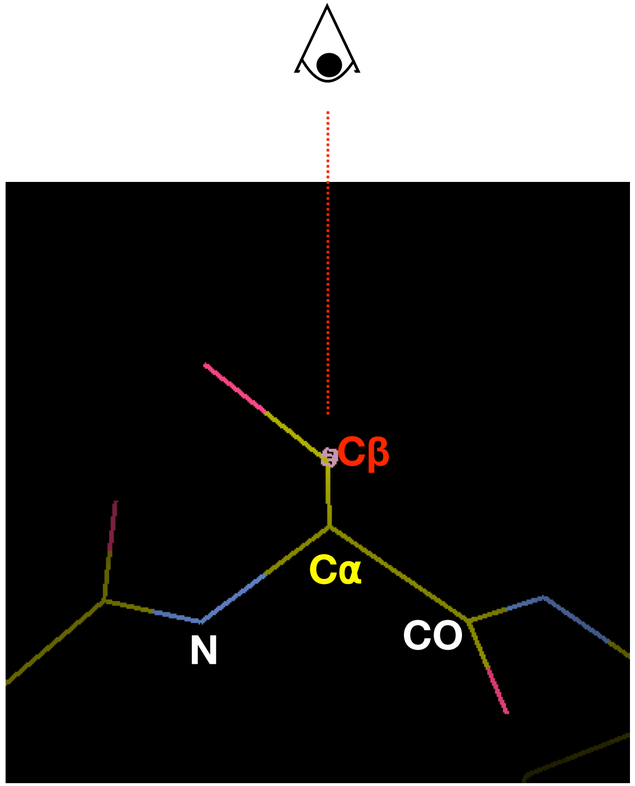
Fraser, et al. Nature 2009.
Barad, et al. Nature Methods 2015.

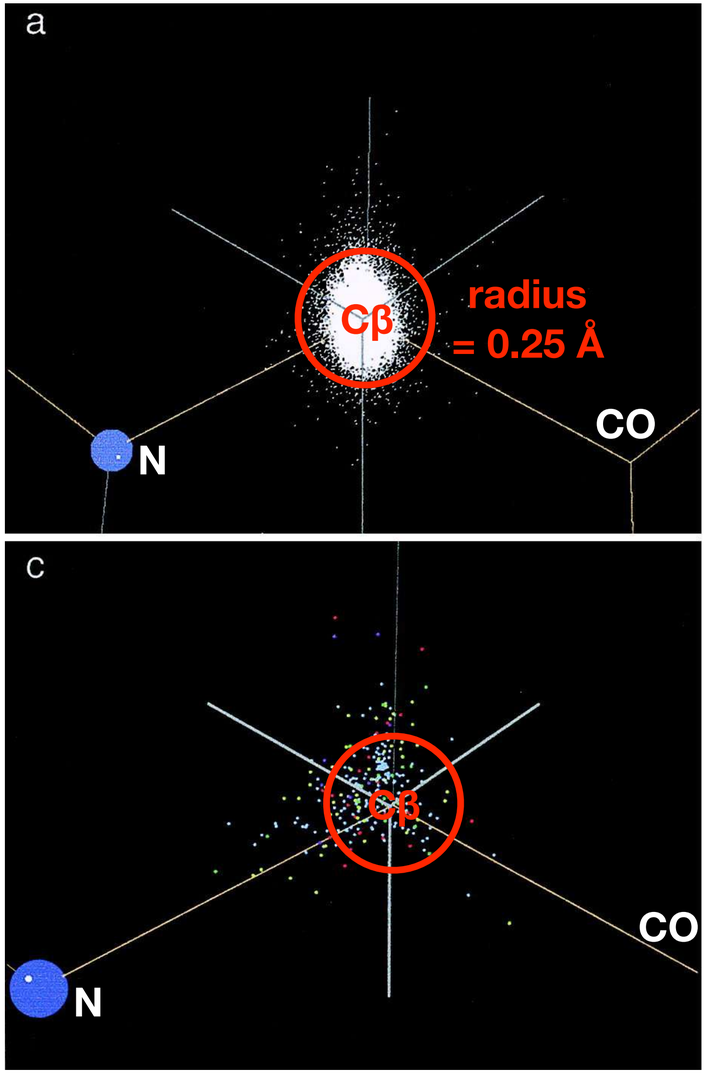
Cbeta deviations



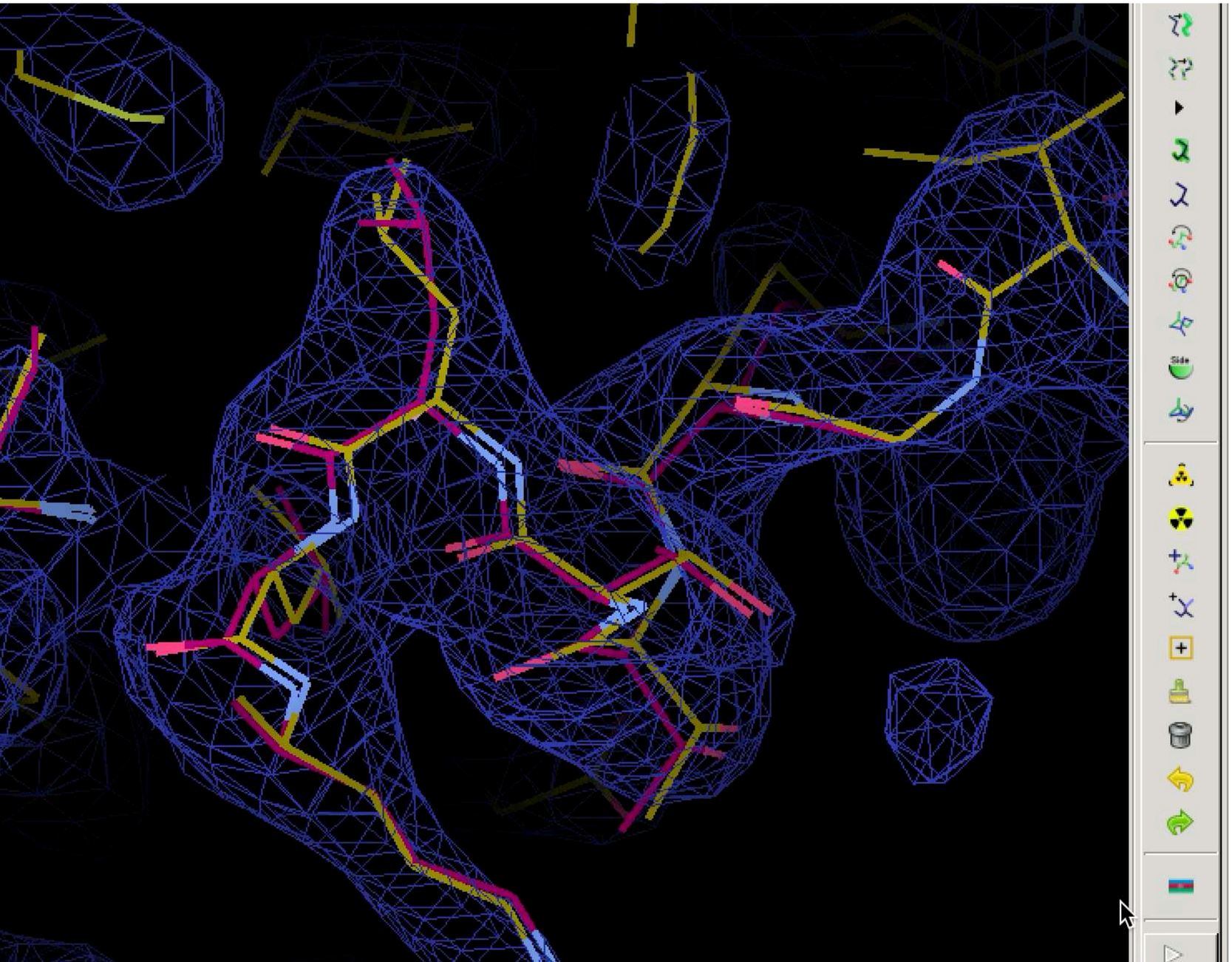
Which Leu rotamer is correct???

Cbeta deviations





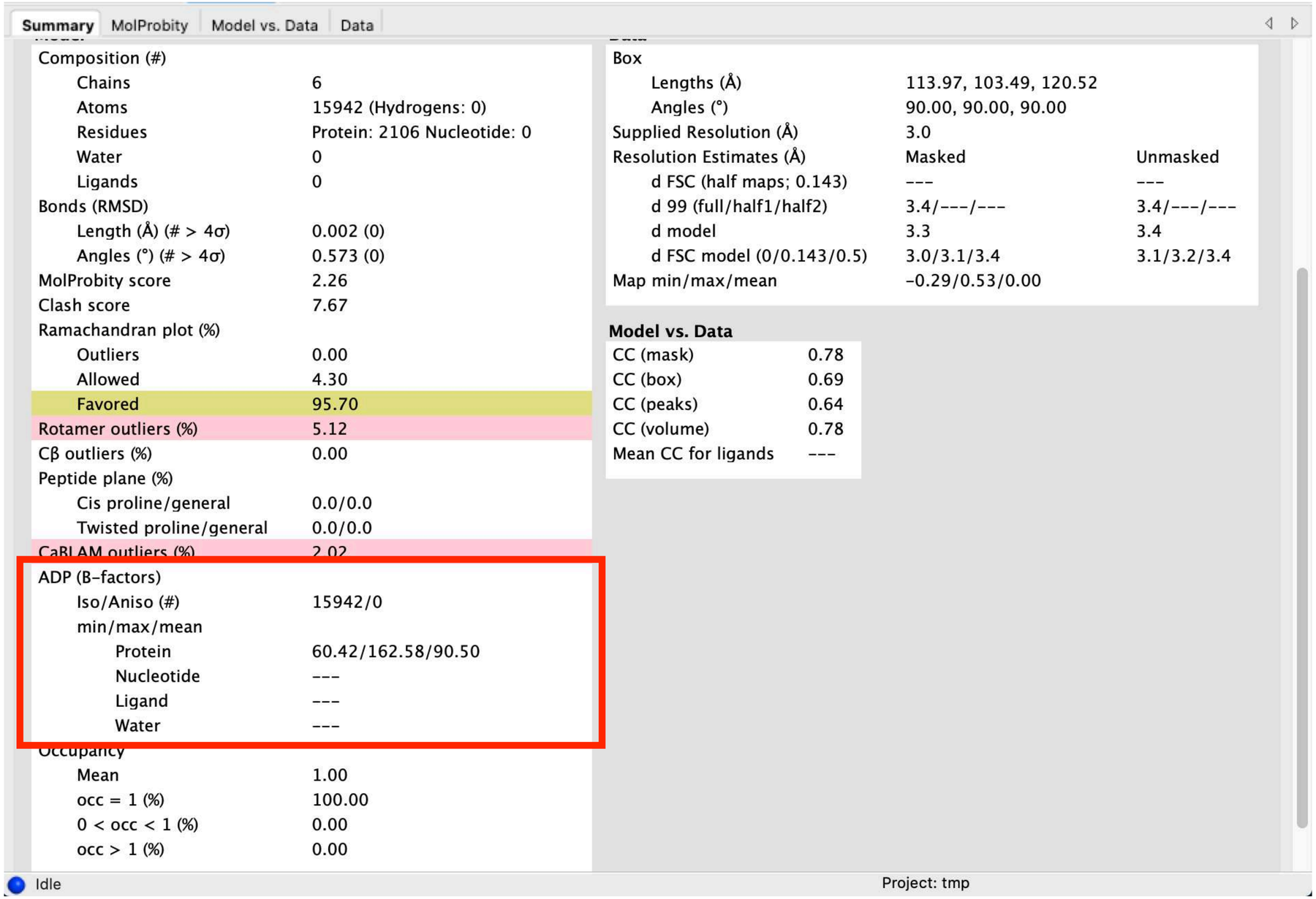
Cbeta deviations



Which Leu rotamer is correct???

Correct answer:
YELLOW

ADP outliers



Full wwPDB/EMDataBank EM Map/Model Validation Report (i)

Dec 2, 2019 – 05:08 PM EST

PDB ID	:	6Q04
EMDB ID:	:	EMD-20542
Title	:	MERS-CoV S structure in complex with 5-N-acetyl neuraminic acid
Authors	:	Park, Y.J.; Walls, A.C.; Wang, Z.; Sauer, M.; Li, W.; Tortorici, M.A.; Bosch, B.J.; DiMaio, F.D.; Veesler, D.; Seattle Structural Genomics Center for Infectious Disease (SSGCID)
Deposited on	:	2019-08-01
Resolution	:	2.50 Å(reported)
Based on PDB ID	:	6BN3

This is a Full wwPDB/EMDataBank EM Map/Model Validation Report for a publicly released PDB/EMDB entry.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/EMValidationReportHelp
with specific help available everywhere you see the (i) symbol.

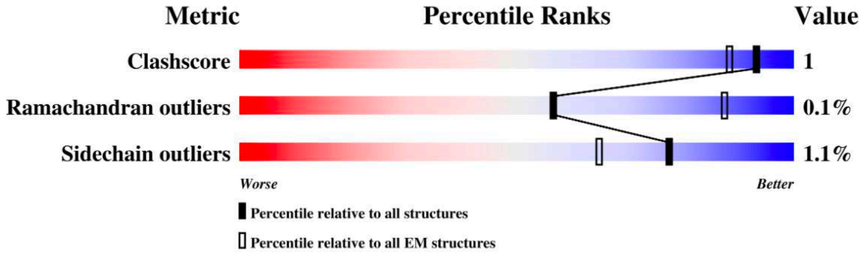
MolProbity	:	4.02b-467
Mogul	:	1.8.0 (224370), CSD as540be (2019)
Percentile statistics	:	20171227.v01 (using entries in the PDB archive December 27th 2017)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et. al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.4

1 Overall quality at a glance

The following experimental techniques were used to determine the structure:
ELECTRON MICROSCOPY

The reported resolution of this entry is 2.50 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	EM structures (#Entries)
Clashscore	136327	1886
Ramachandran outliers	132723	1663
Sidechain outliers	132532	1531

The table below summarises the geometric issues observed across the polymeric chains. The red, orange, yellow and green segments on the bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5%

Mol	Chain	Length	Quality of chain
1	A	1359	78% 6% • 15%
1	B	1359	78% 6% • 15%
1	C	1359	78% 7% • 15%

More Resources

- http://molprobity.biochem.duke.edu/
- https://www.phenix-online.org/documentation/index.html
- https://www.ccpem.ac.uk/
- https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
- Afonine, et al. “New tools for the analysis and validation of cryo-EM maps and atomic models” Acta Cryst. 2018
- Wang, et al. “Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta” eLife 2016
- Nicholls, et al. “Current approaches for the fitting and refinement of atomic models into cryo-EM maps using CCP-EM” Acta Cryst. 2018