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

$\Delta G_{unfolding}$

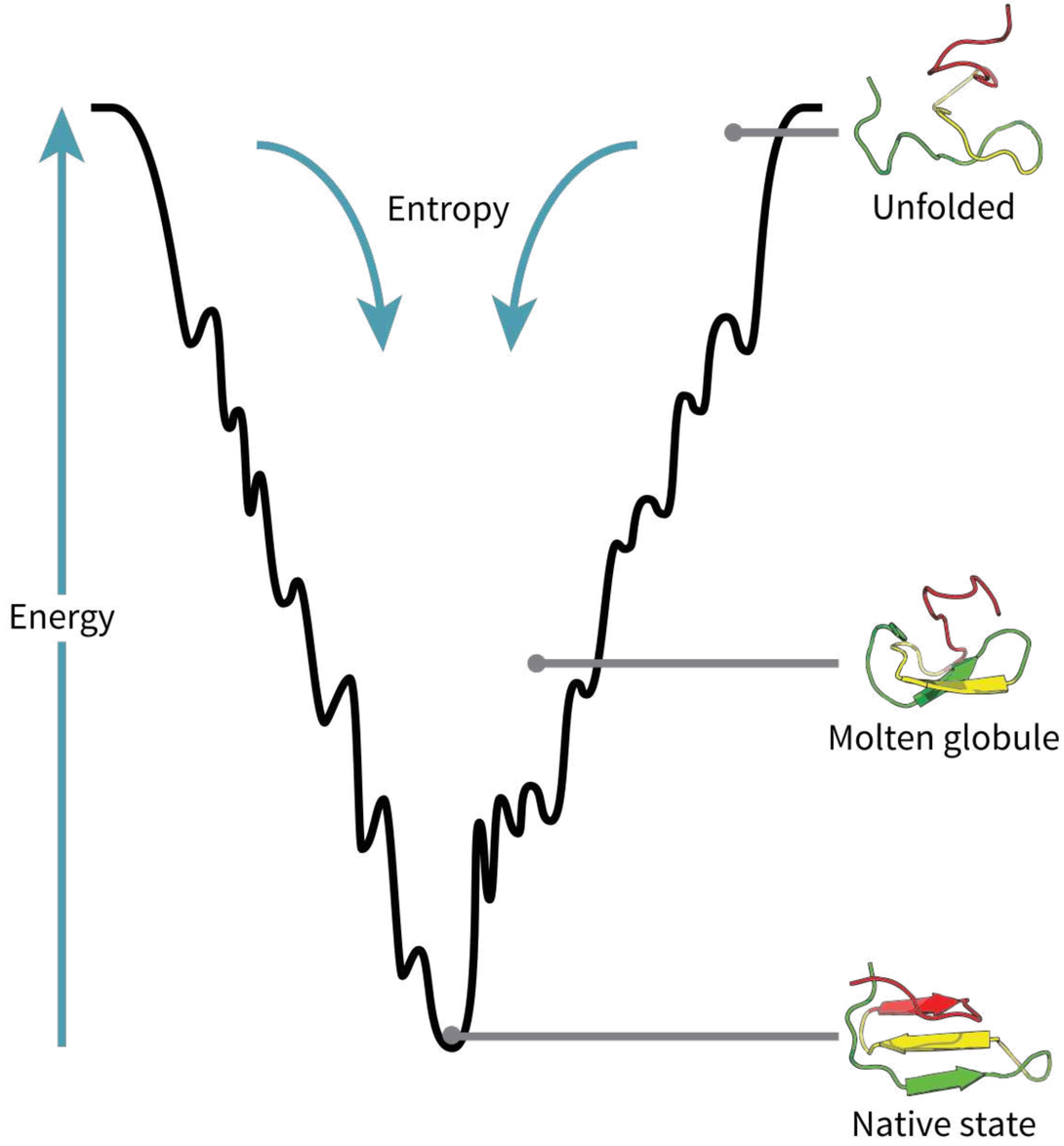


Image: Thomas Splettstoesser

Refinement target describes differences between model and data

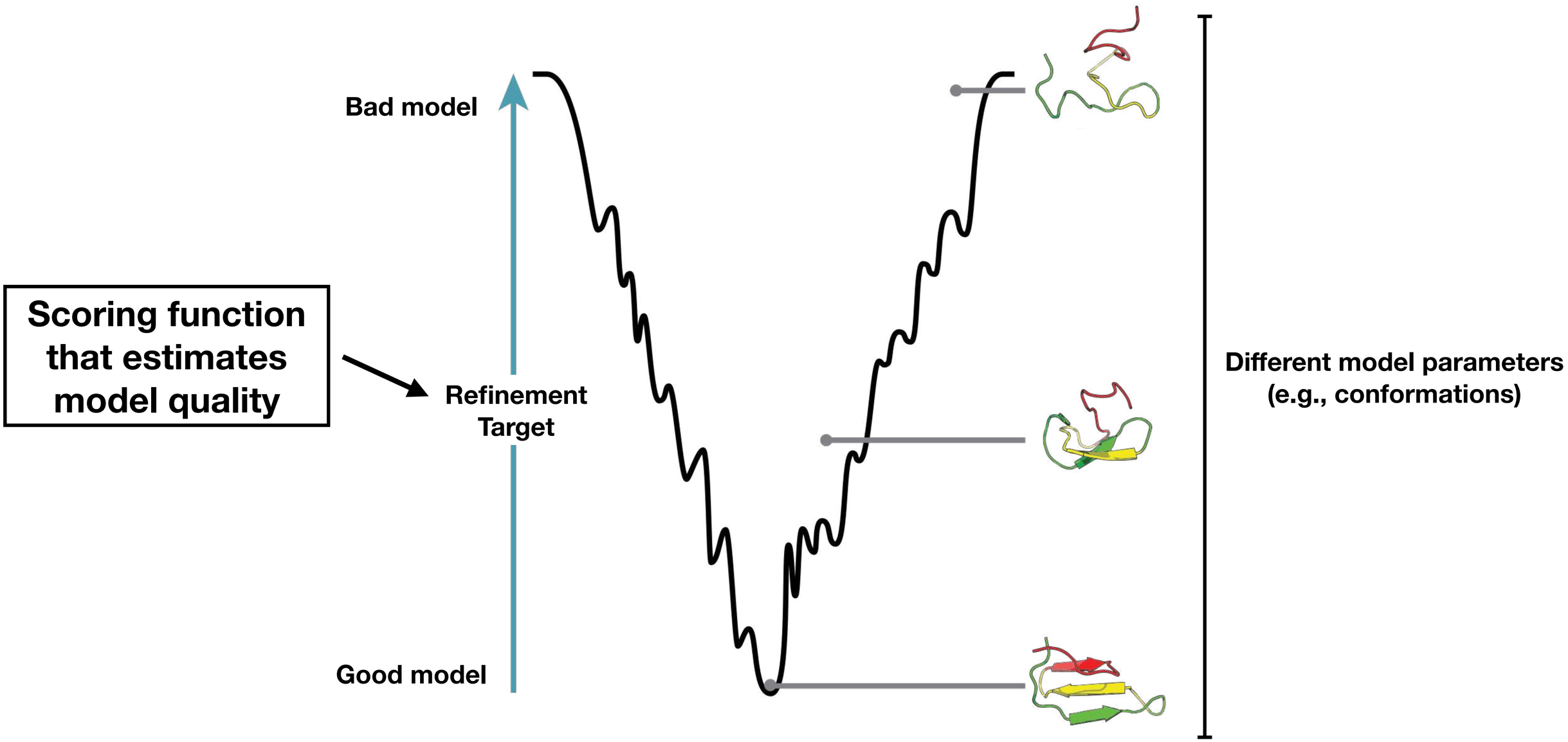
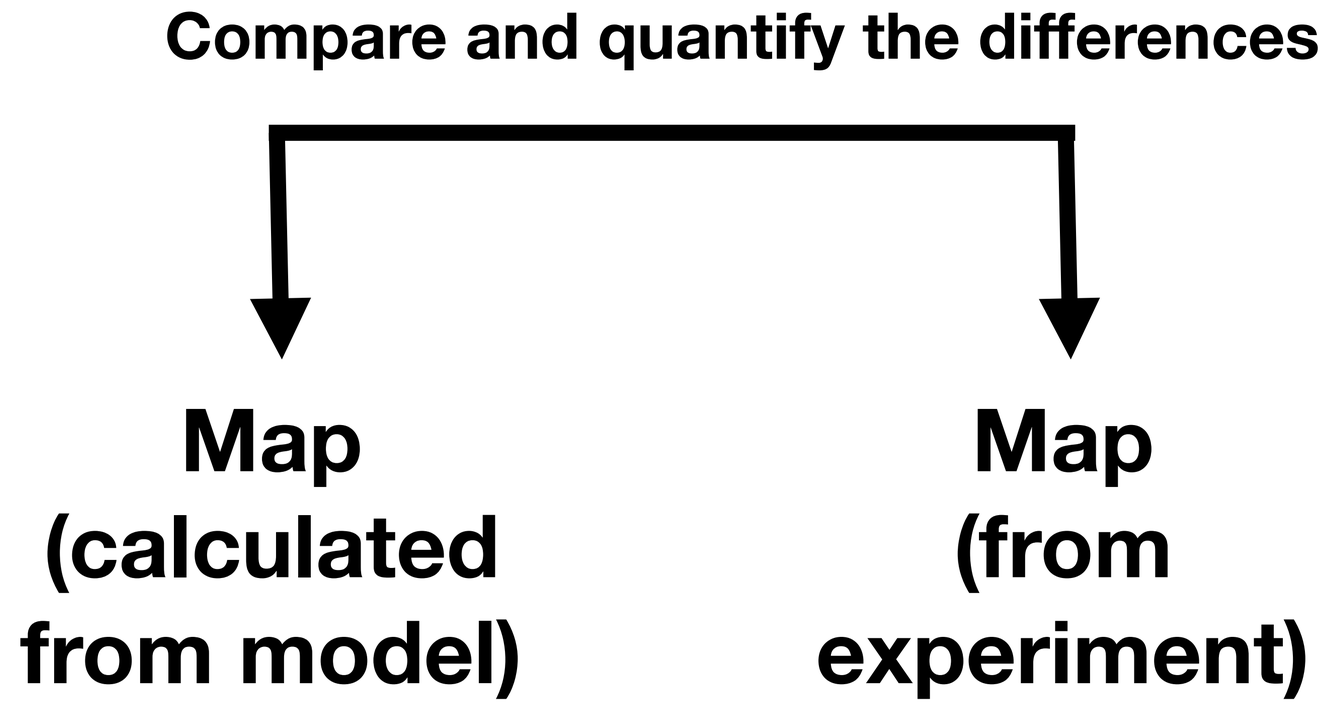
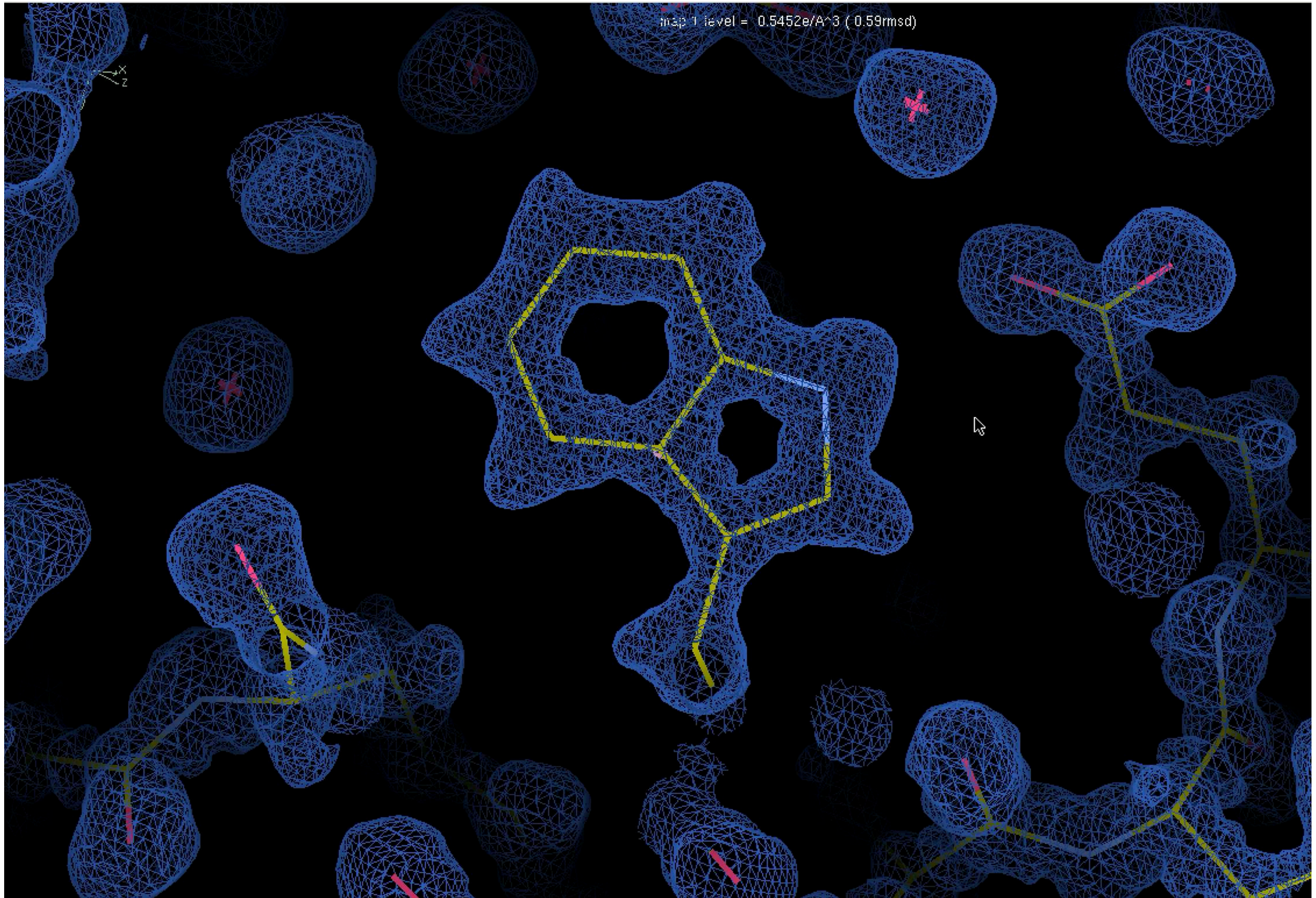


Image (adapted): Thomas Splettstoesser

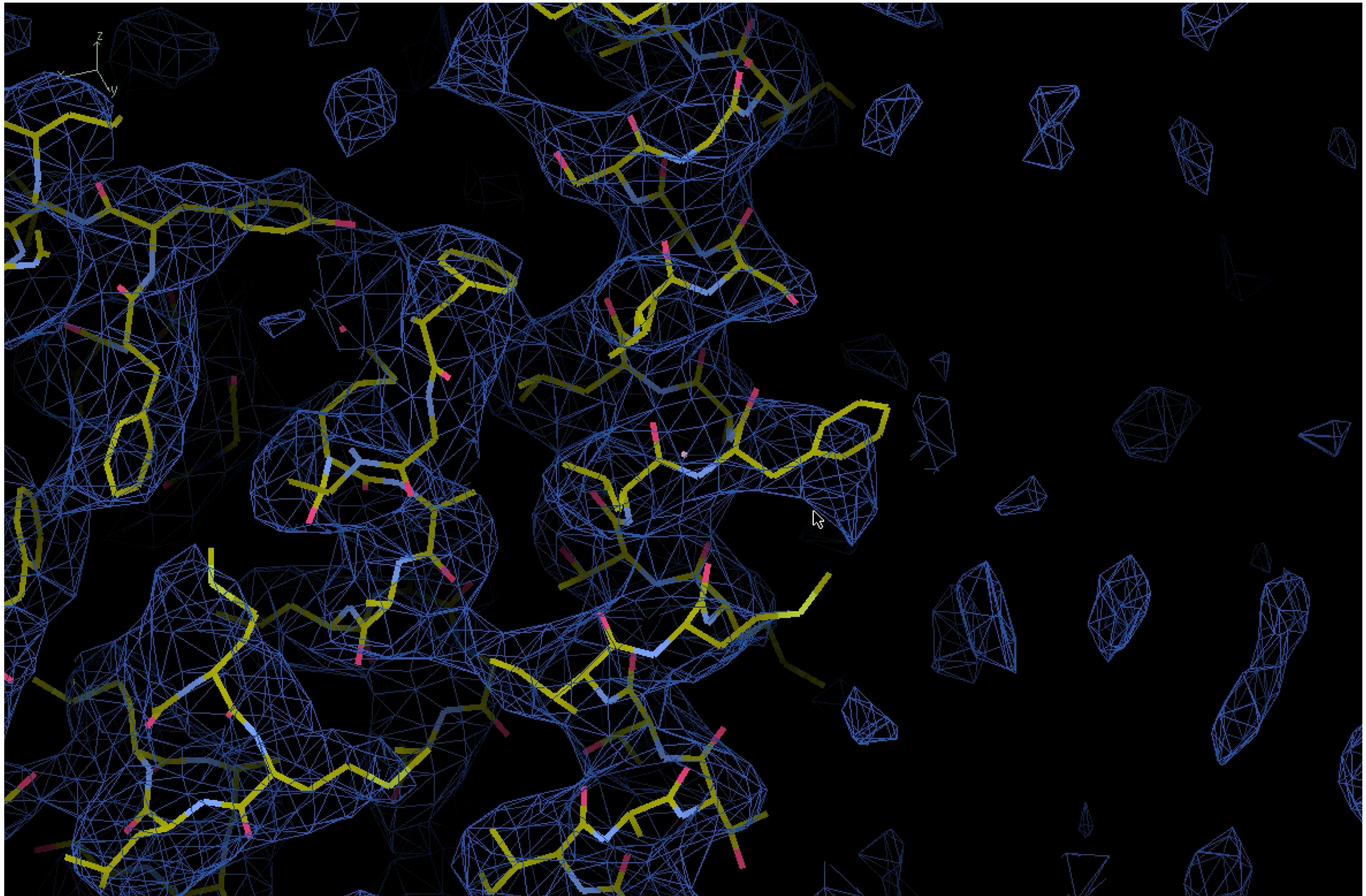
Simplest: Refinement Target = (Model vs Data)



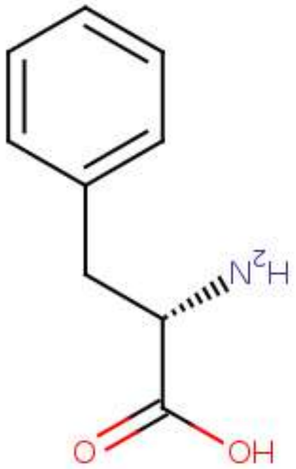
At atomic resolution, position of individual atoms is well-defined

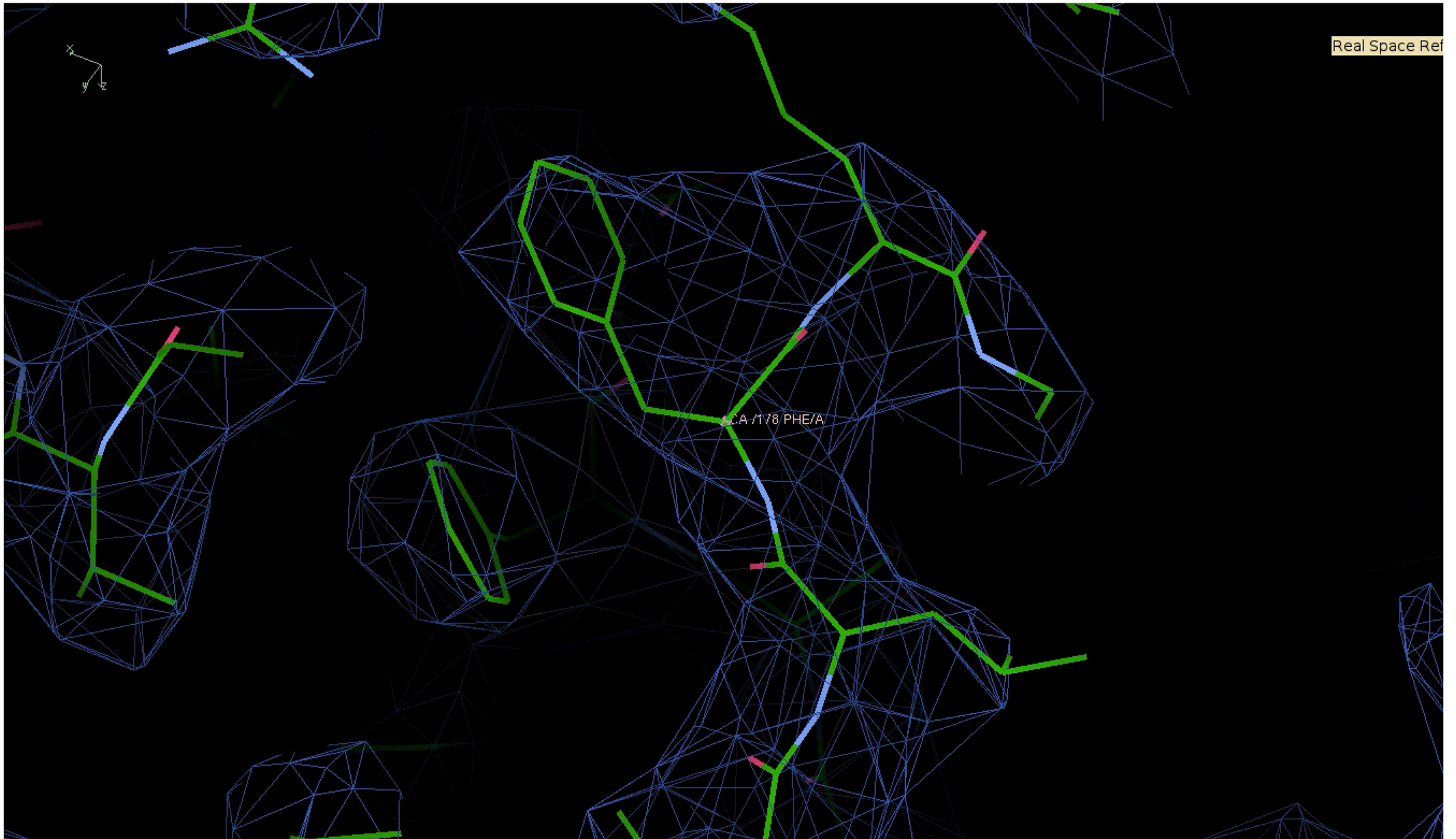


But at “near-atomic” resolution, the position of residues and side chains is not always clear

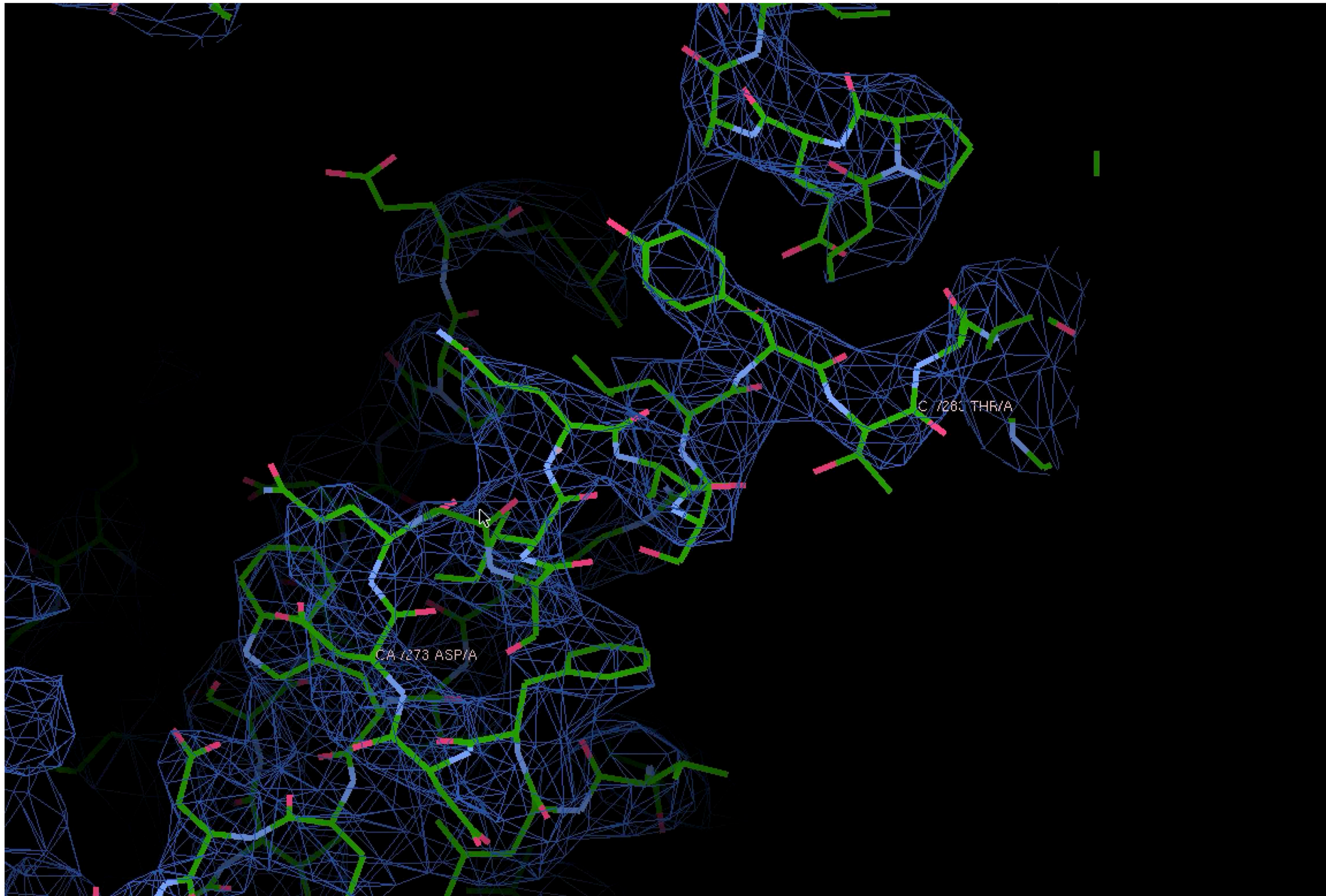


Refinement using only data

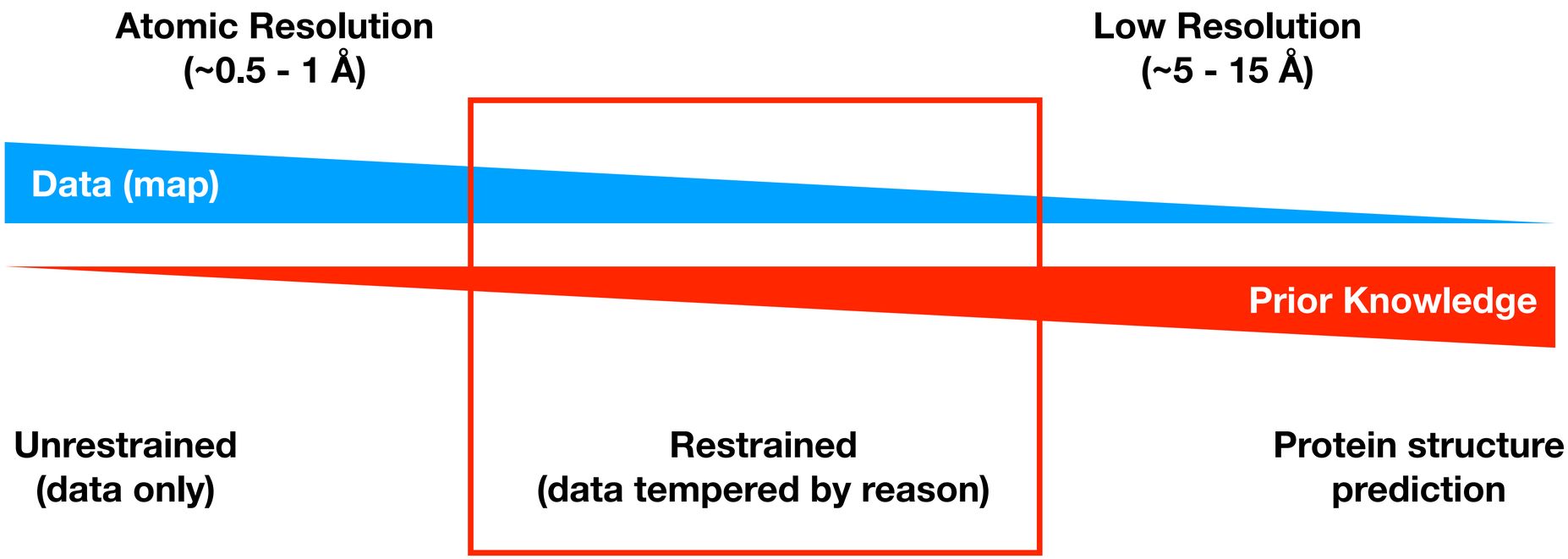




Refinement using only data

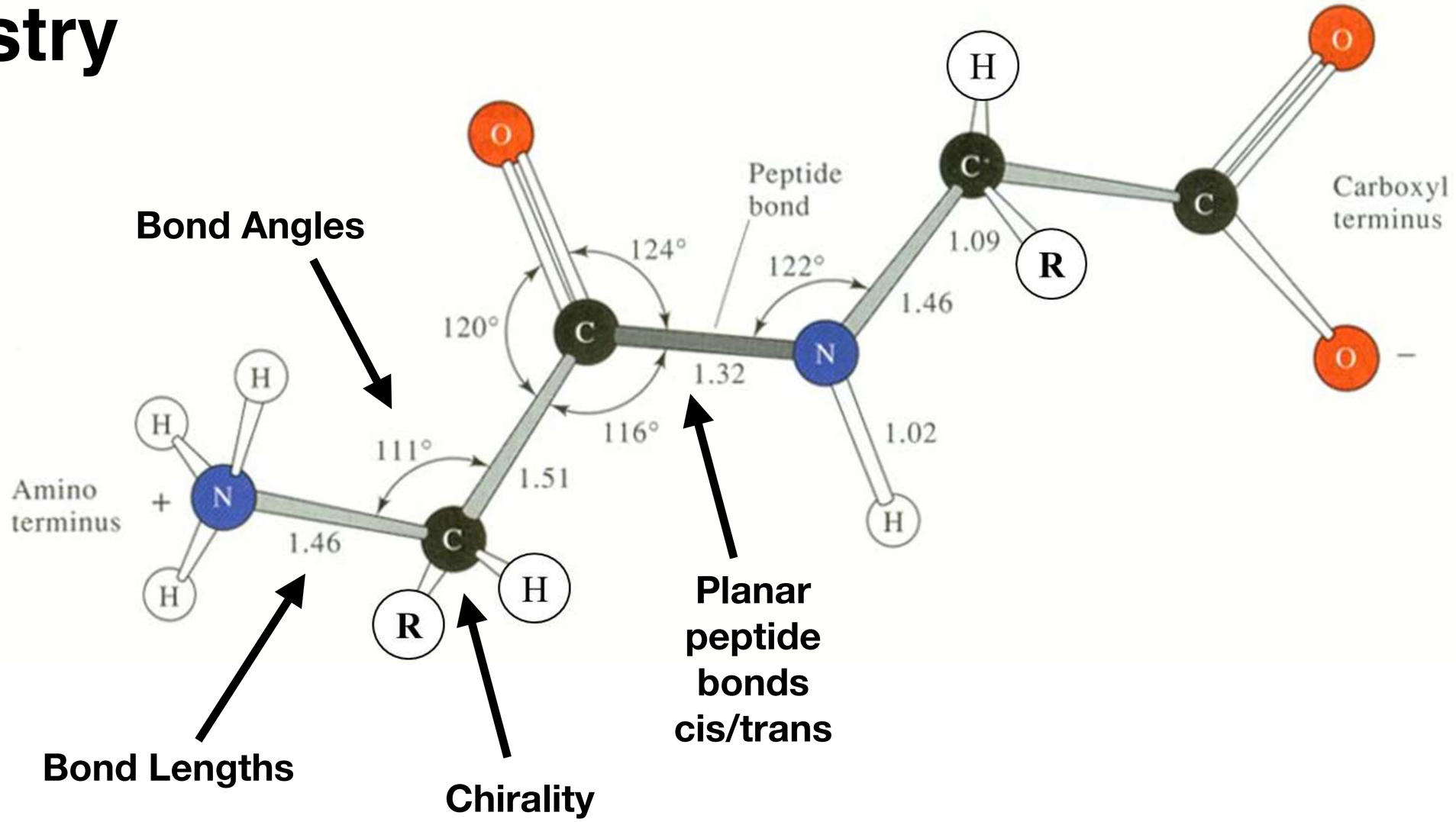


Harnessing prior knowledge of protein structure to bridge the gap

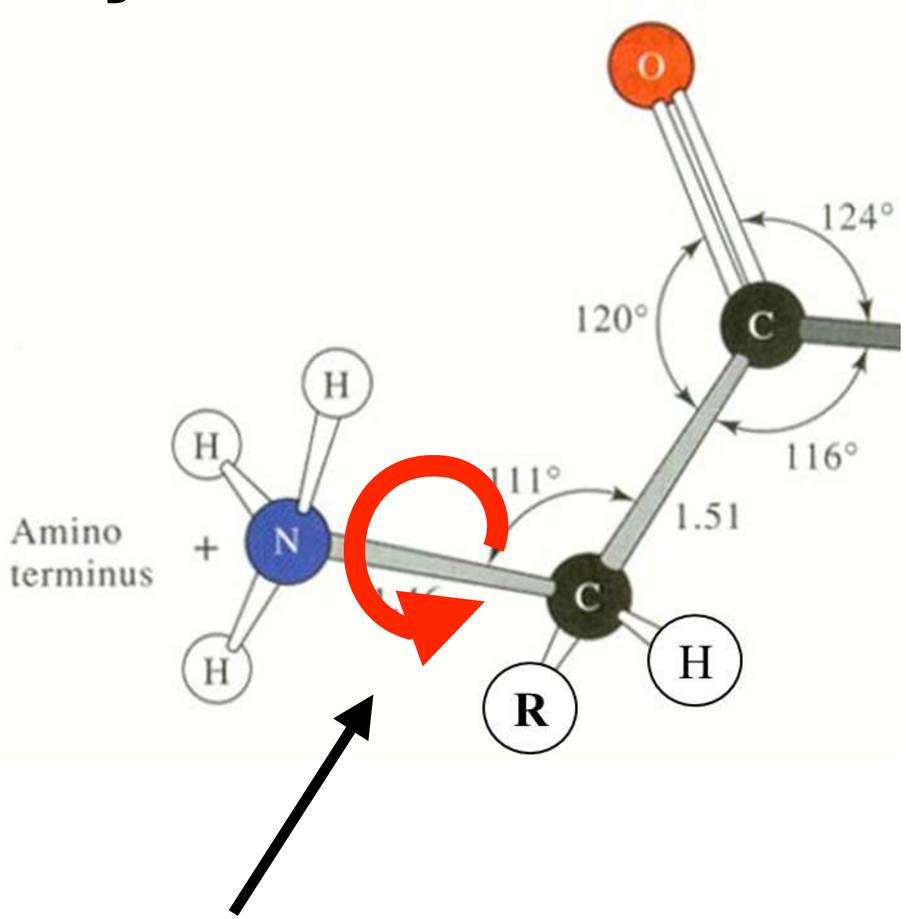


$$\text{Refinement Target} = (\text{Model vs Data}) + w_1(\text{Model vs PriorKnowledge})$$

Stereochemistry



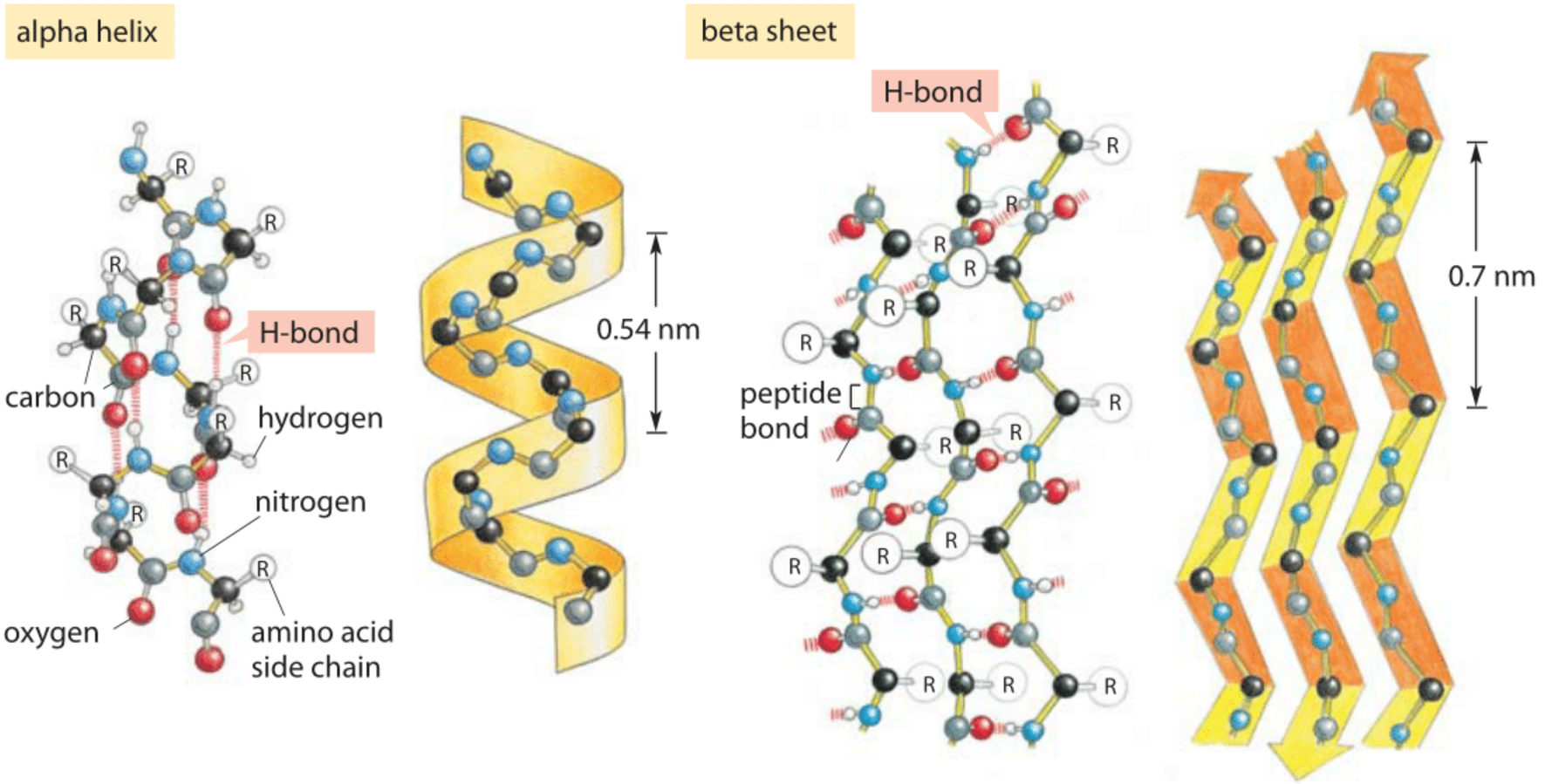
Stereochemistry



Torsion/dihedral Angles

Constraints backbone conformations as well as side chain rotameric states

Secondary Structure and Hydrogen Bonds

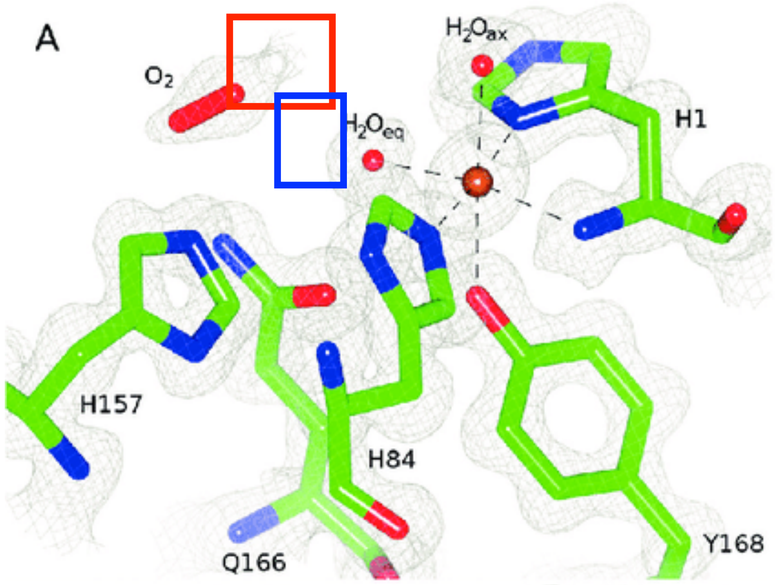


- **Torsion angle restraints to maintain appropriate backbone conformation**
- **Distance restraints between H-bonding atoms**

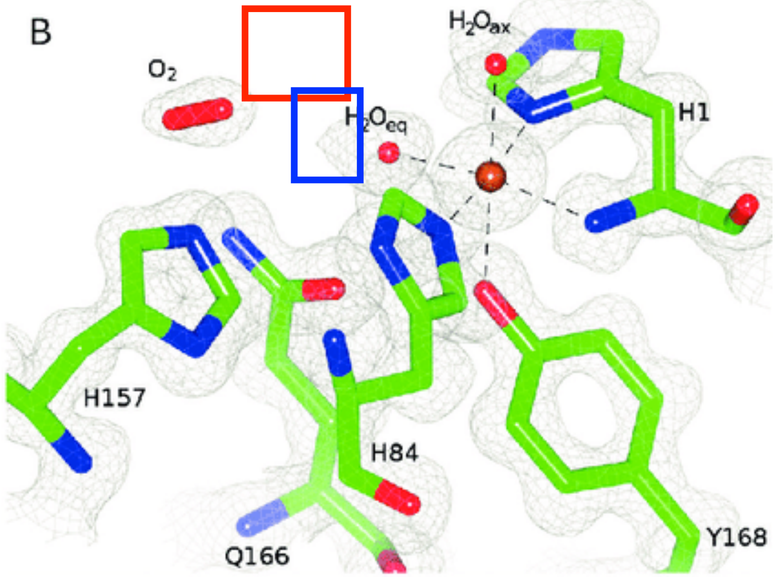
Image: http://book.bionumbers.org/what-is-the-energy-of-a-hydrogen-bond/

“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

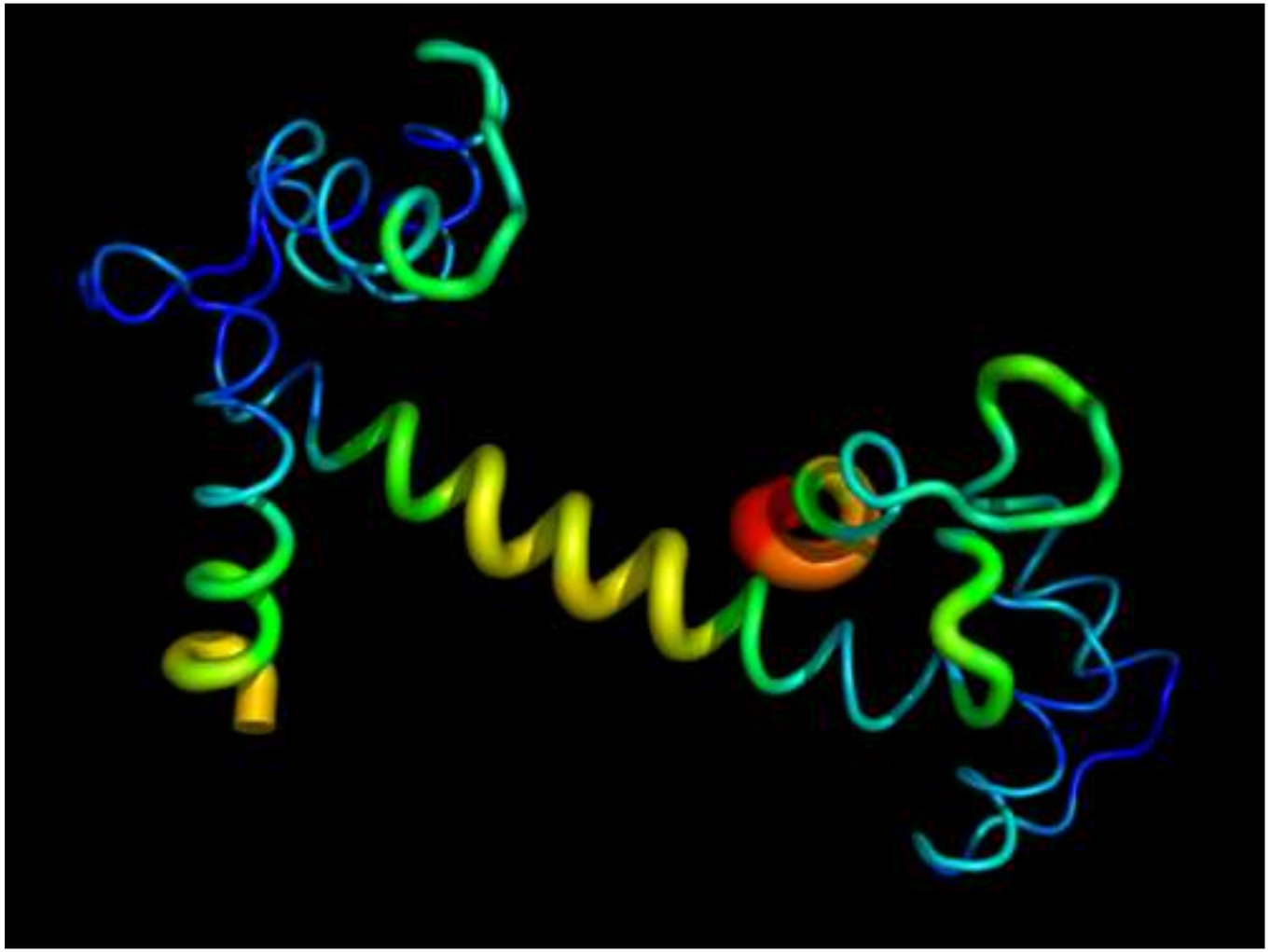


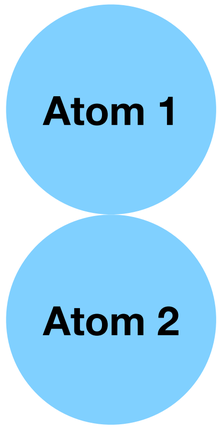
Image: Harry Jubb, https://github.com/arose/ngl/issues/291

B factors are not randomly distributed

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

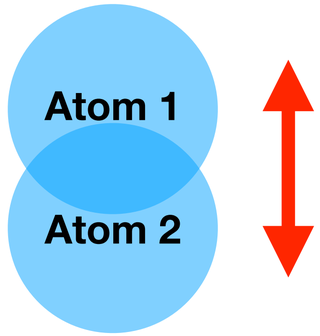
Therefore, we can retrain B factors such that connected atoms/residues must have similar B factors

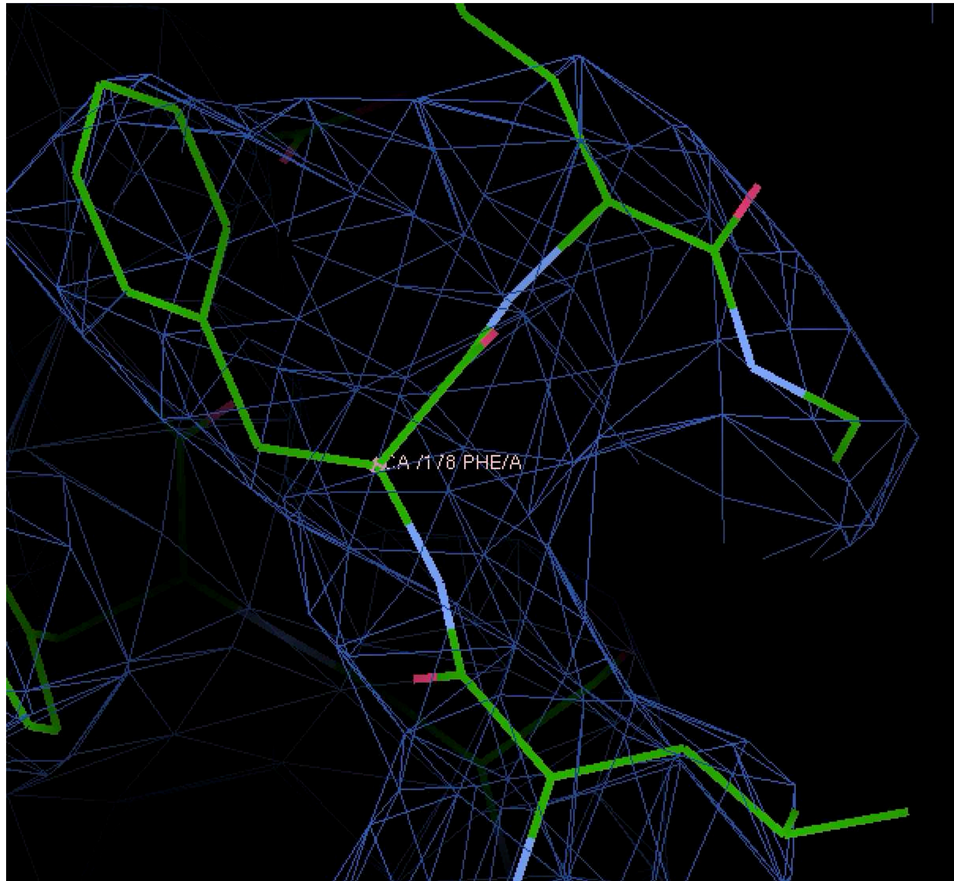
Steric repulsion



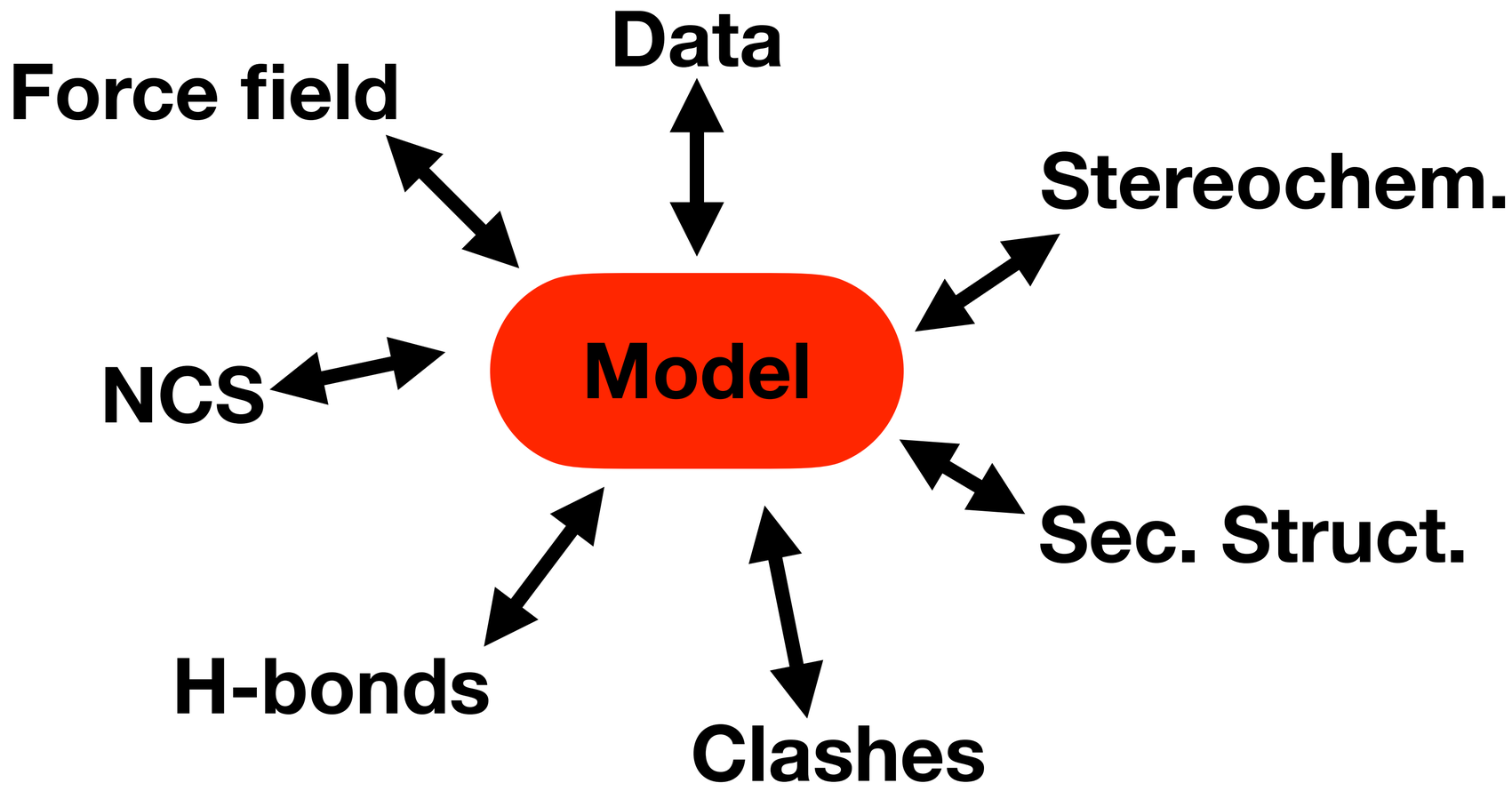
Optimal center-to-center distance ~ sum of VDW radii

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





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) + …

Refinement moves model towards local minimum

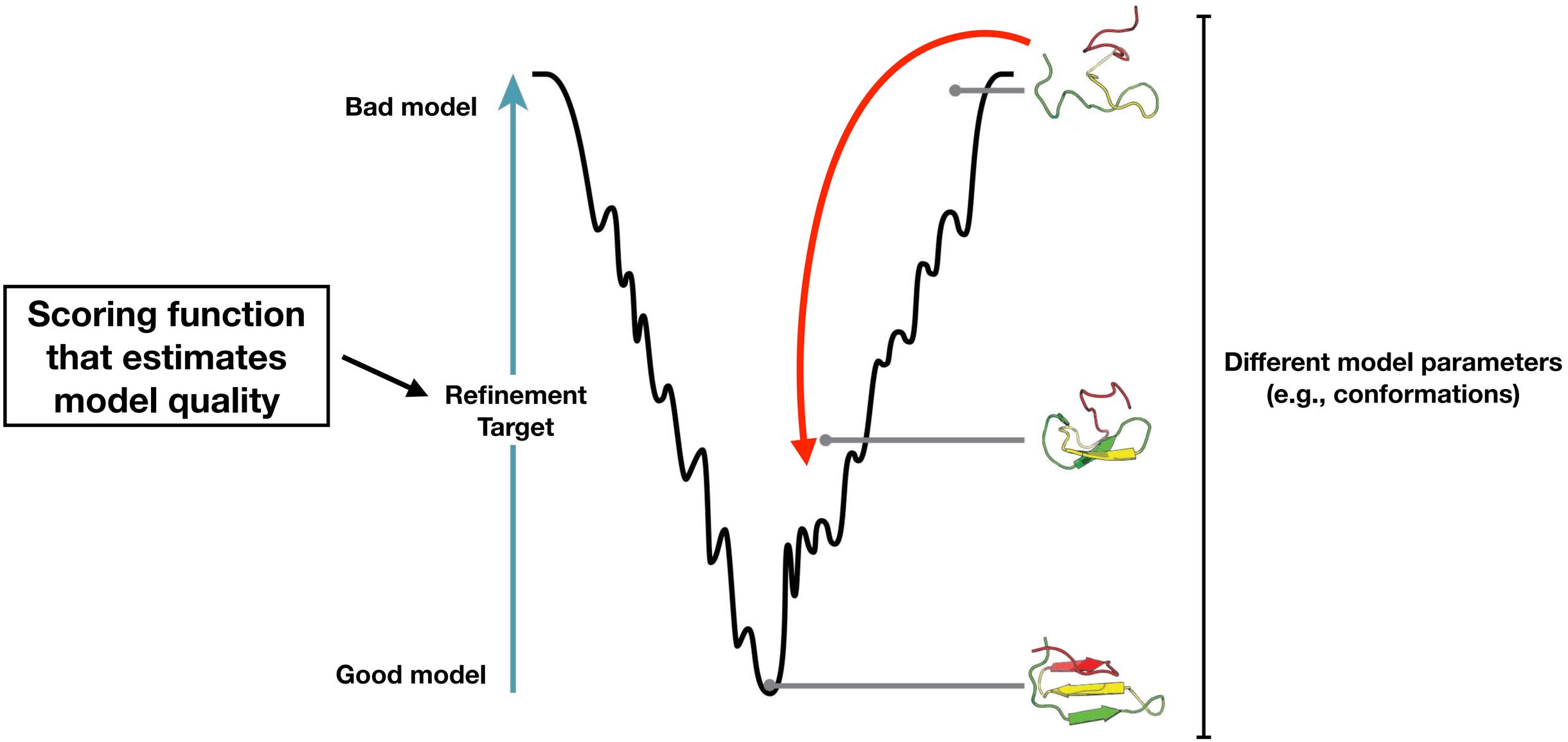
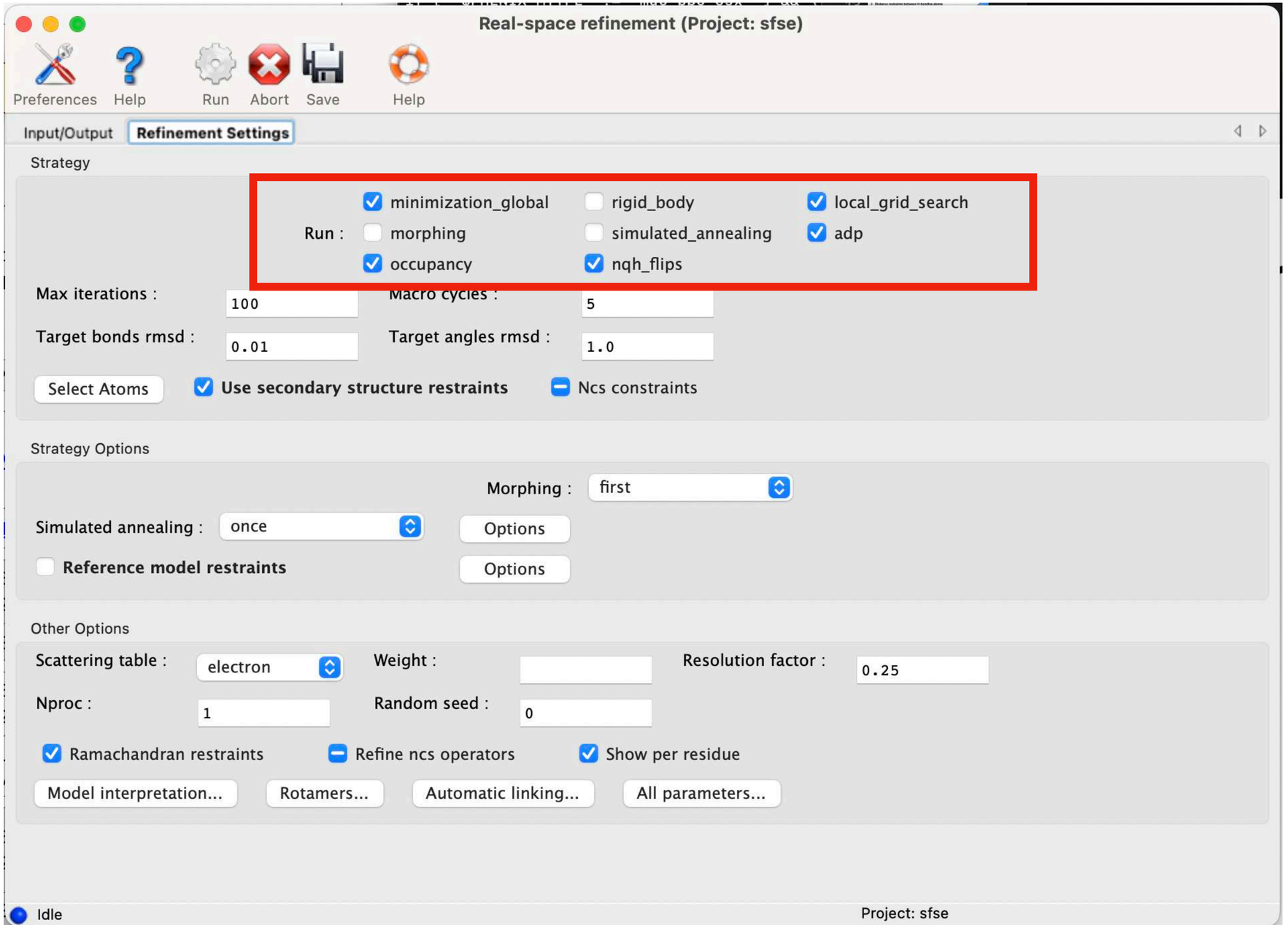


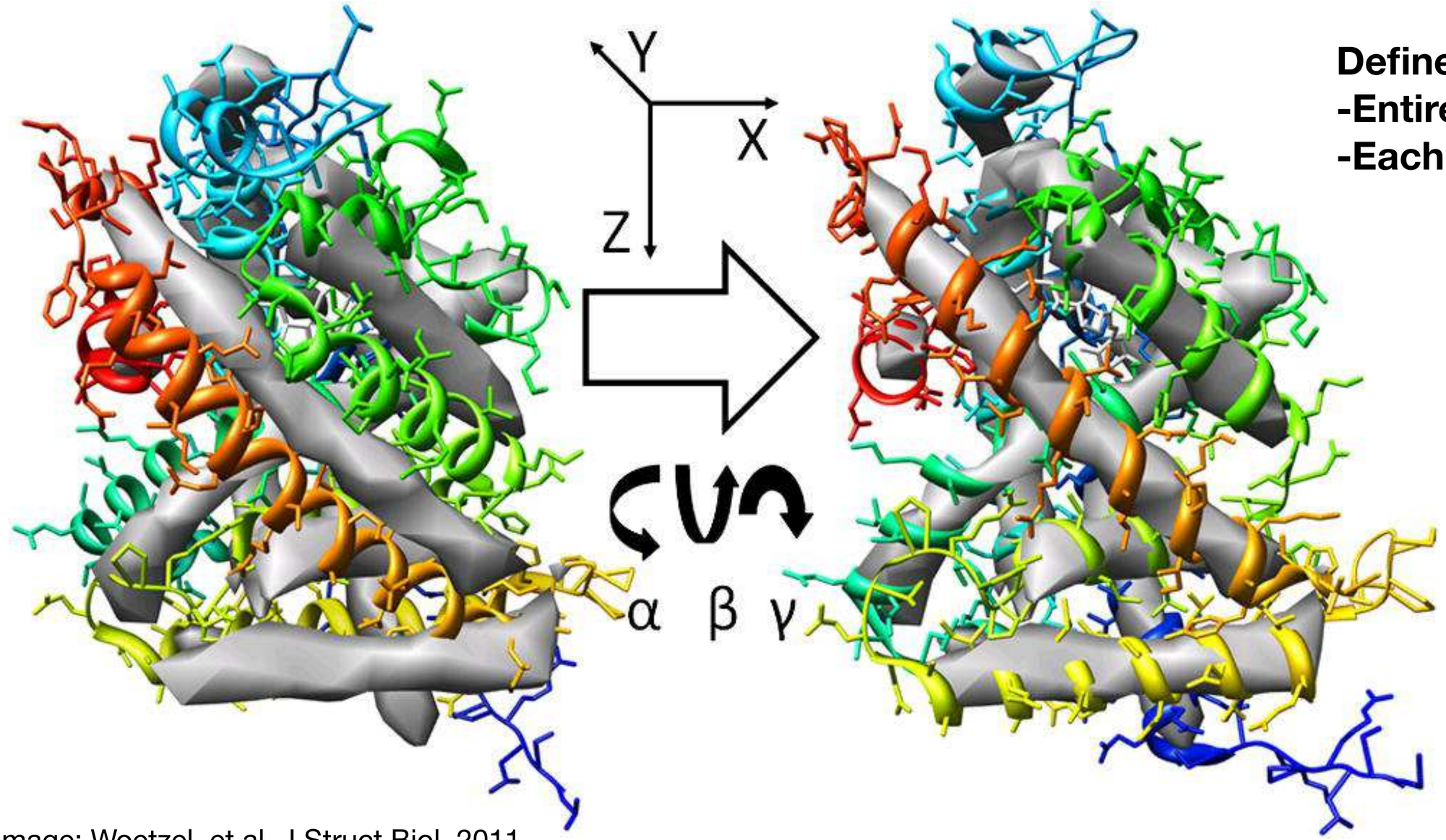
Image (adapted): Thomas Splettstoesser

There are many refinement options to choose from!



phenix.real_space_refine

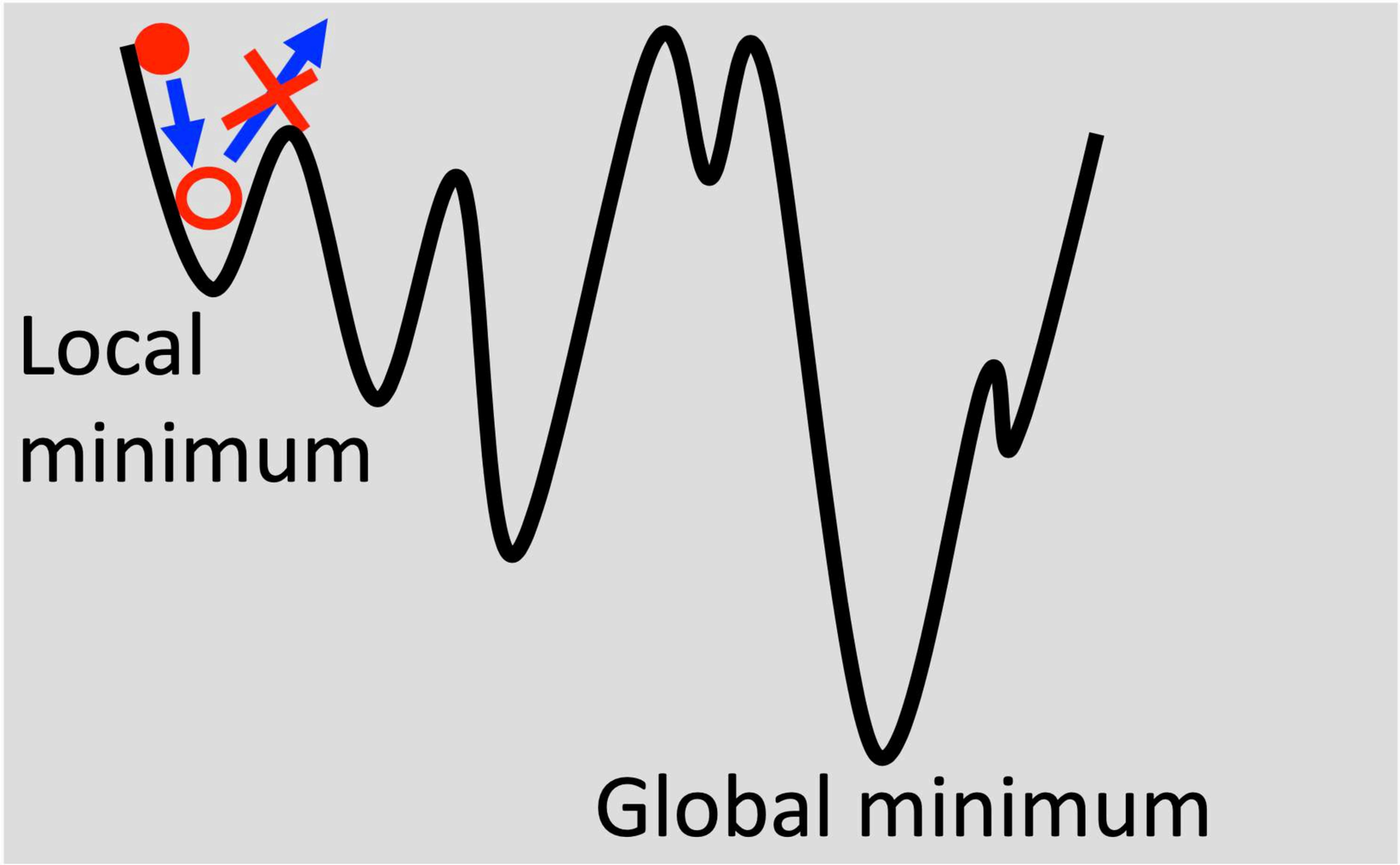
Optimization protocols: Rigid body refinement



Define rigid bodies:
-Entire model?
-Each chain separately

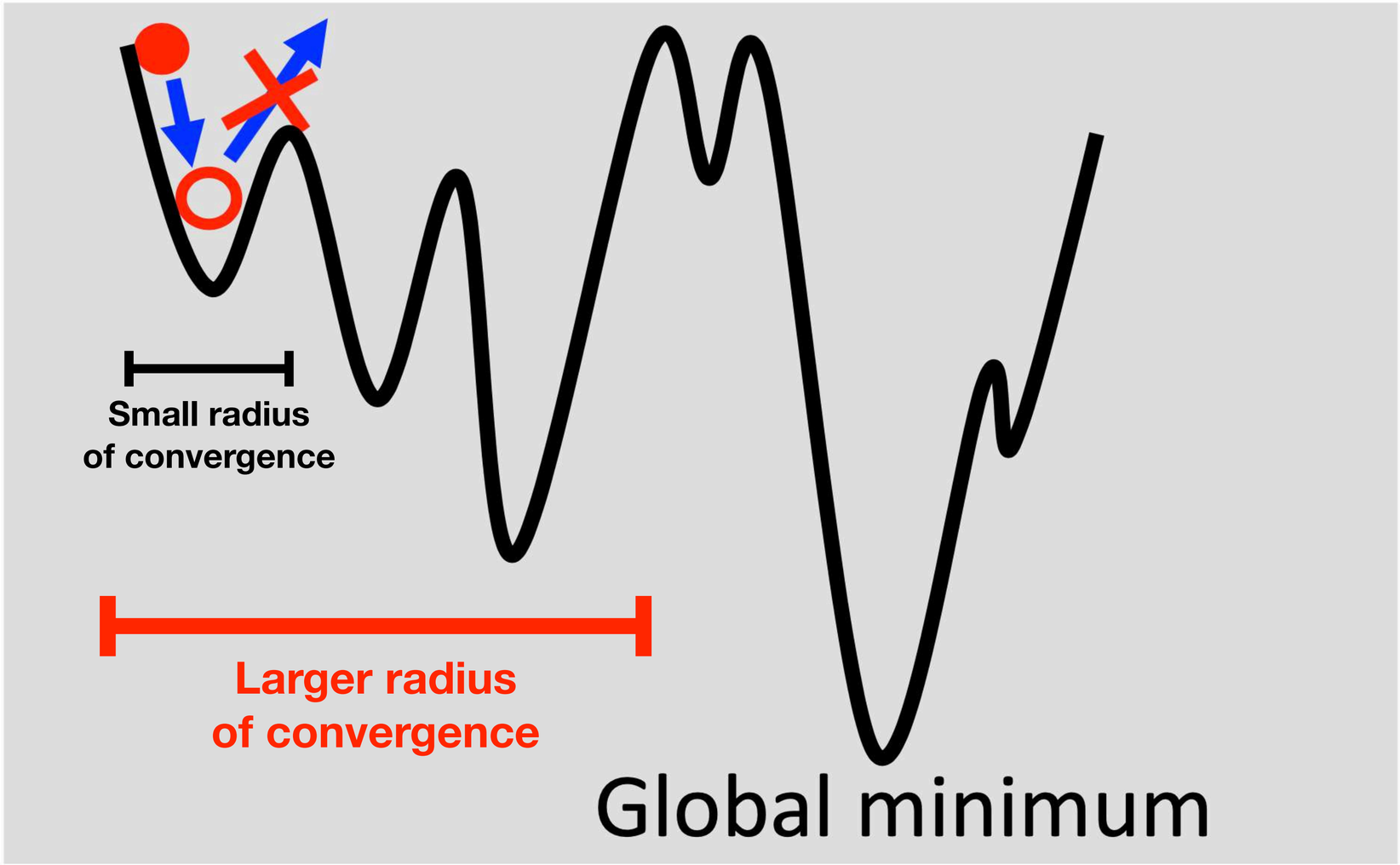
Image: Woetzel, et al. J Struct Biol. 2011.

Optimization protocols: Gradient driven minimization

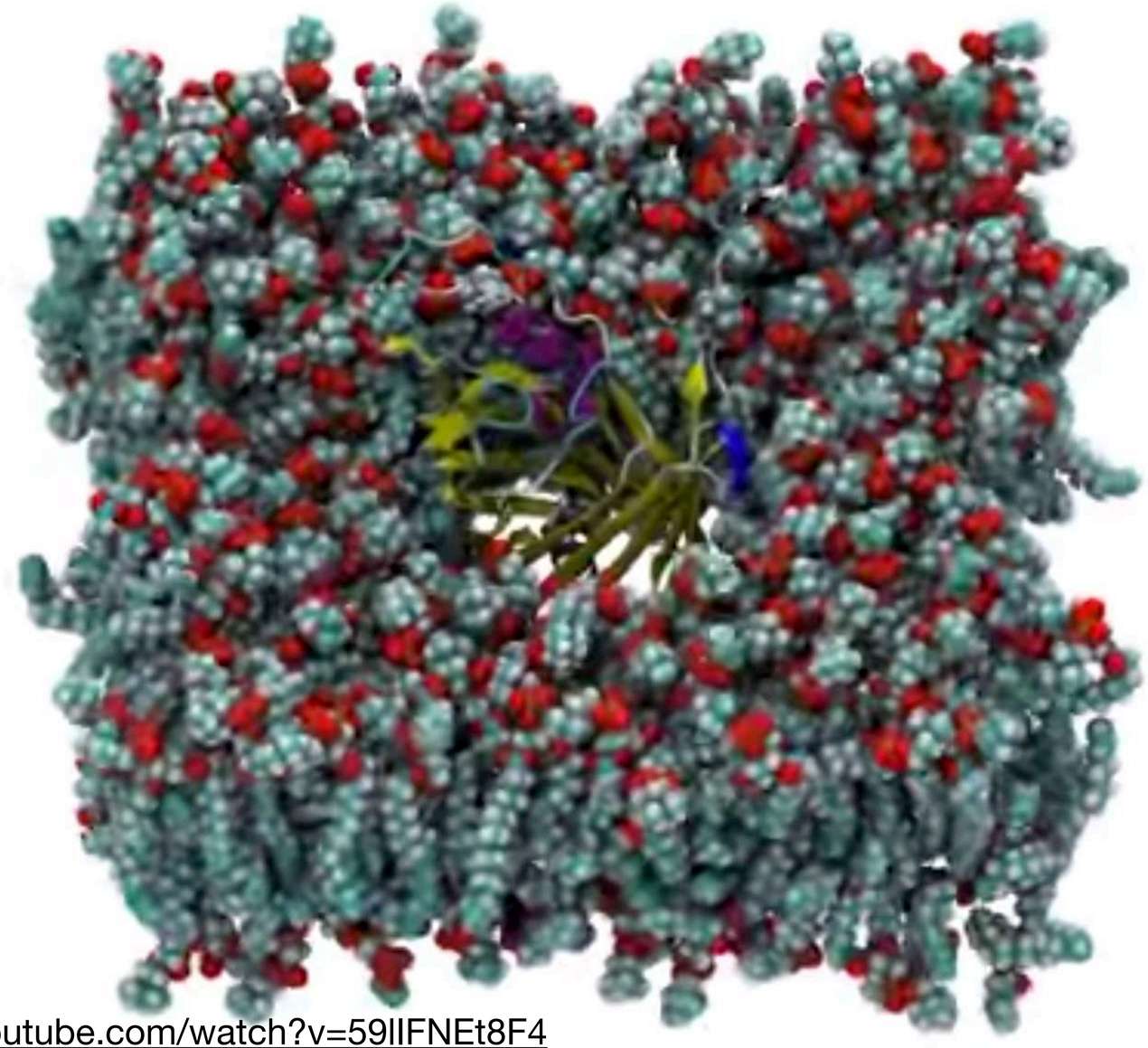


Slide adapted from: Pavel Afonine, LBNL (Phenix)

Refinement “radius of convergence”

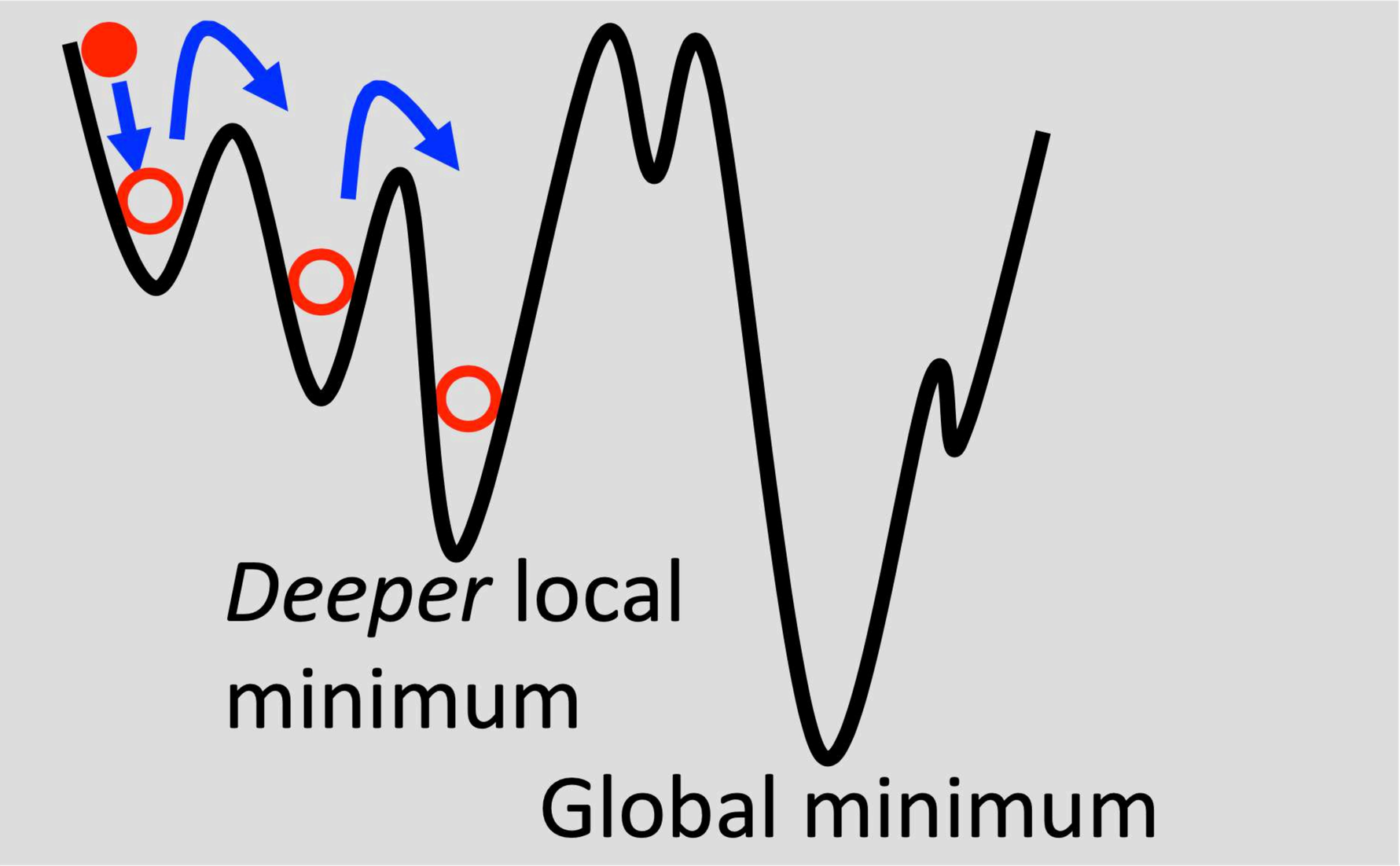


Optimization protocols: Simulated annealing



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

Optimization protocols: Simulated annealing



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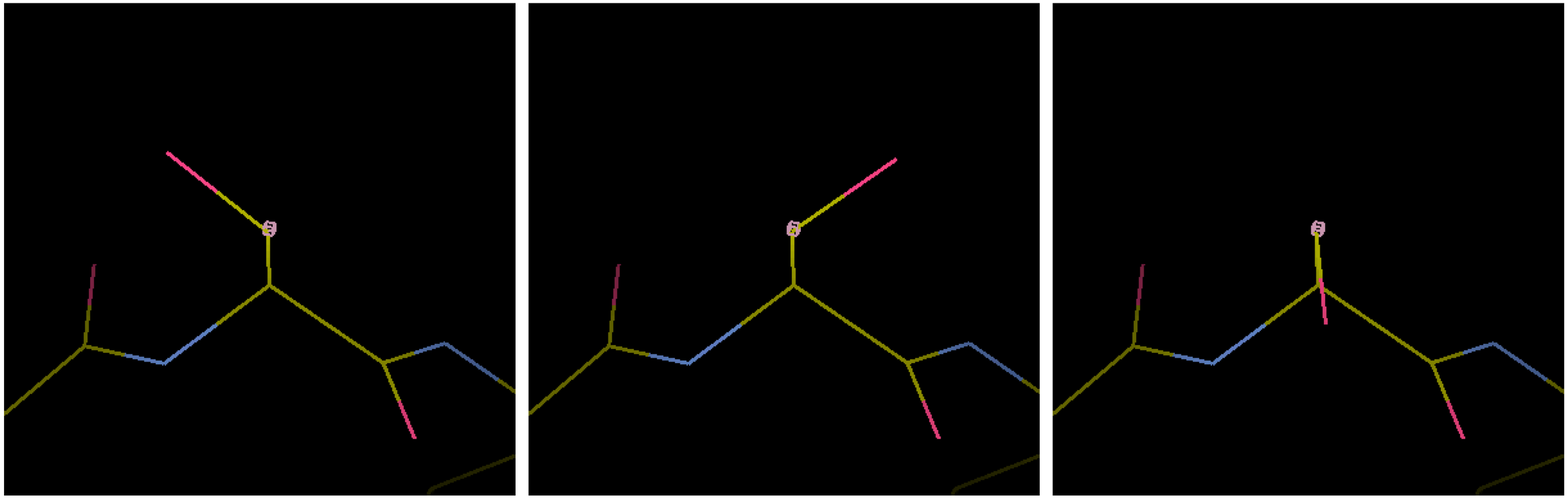
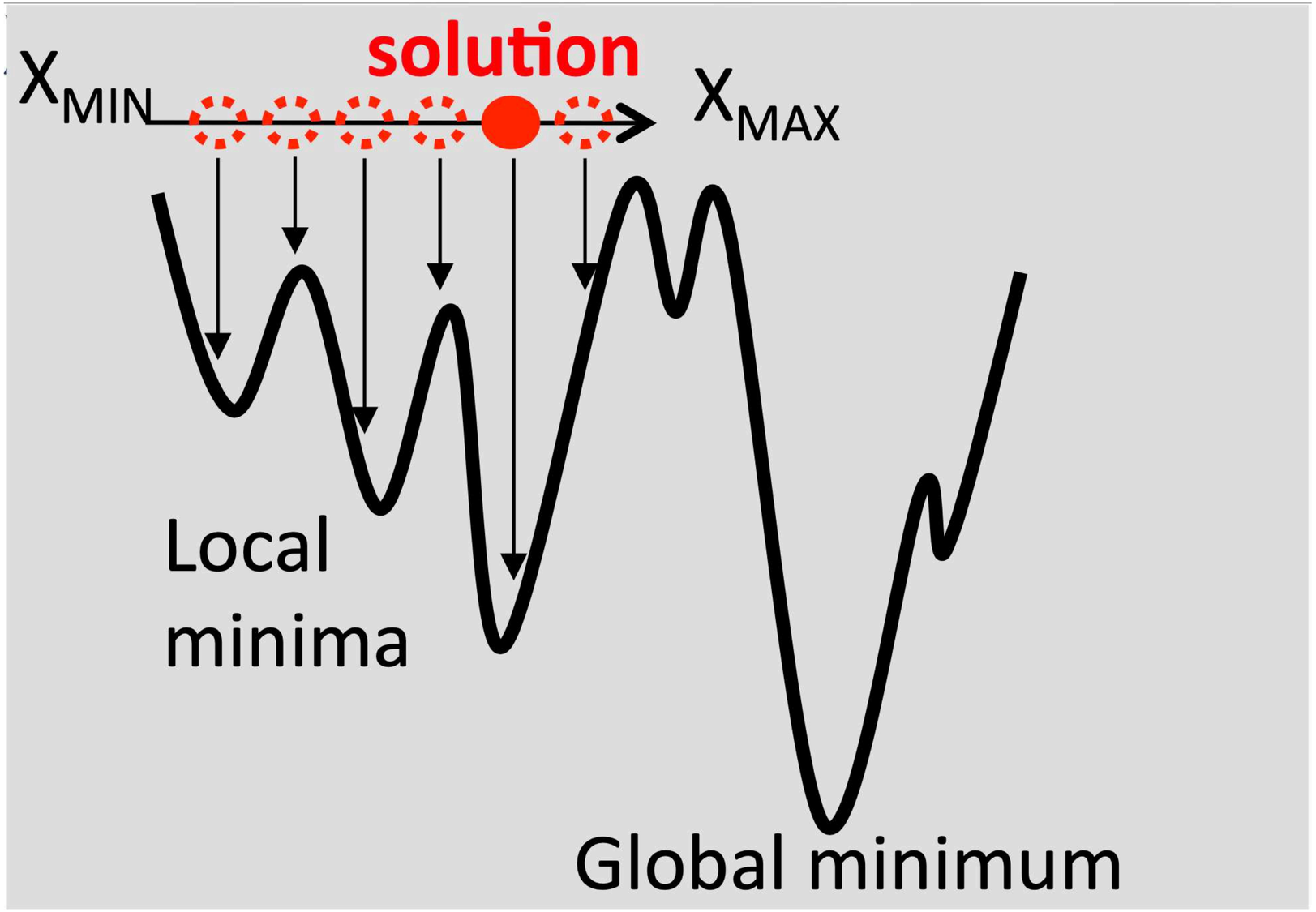
Optimization protocols: Torsion-angle Grid Search



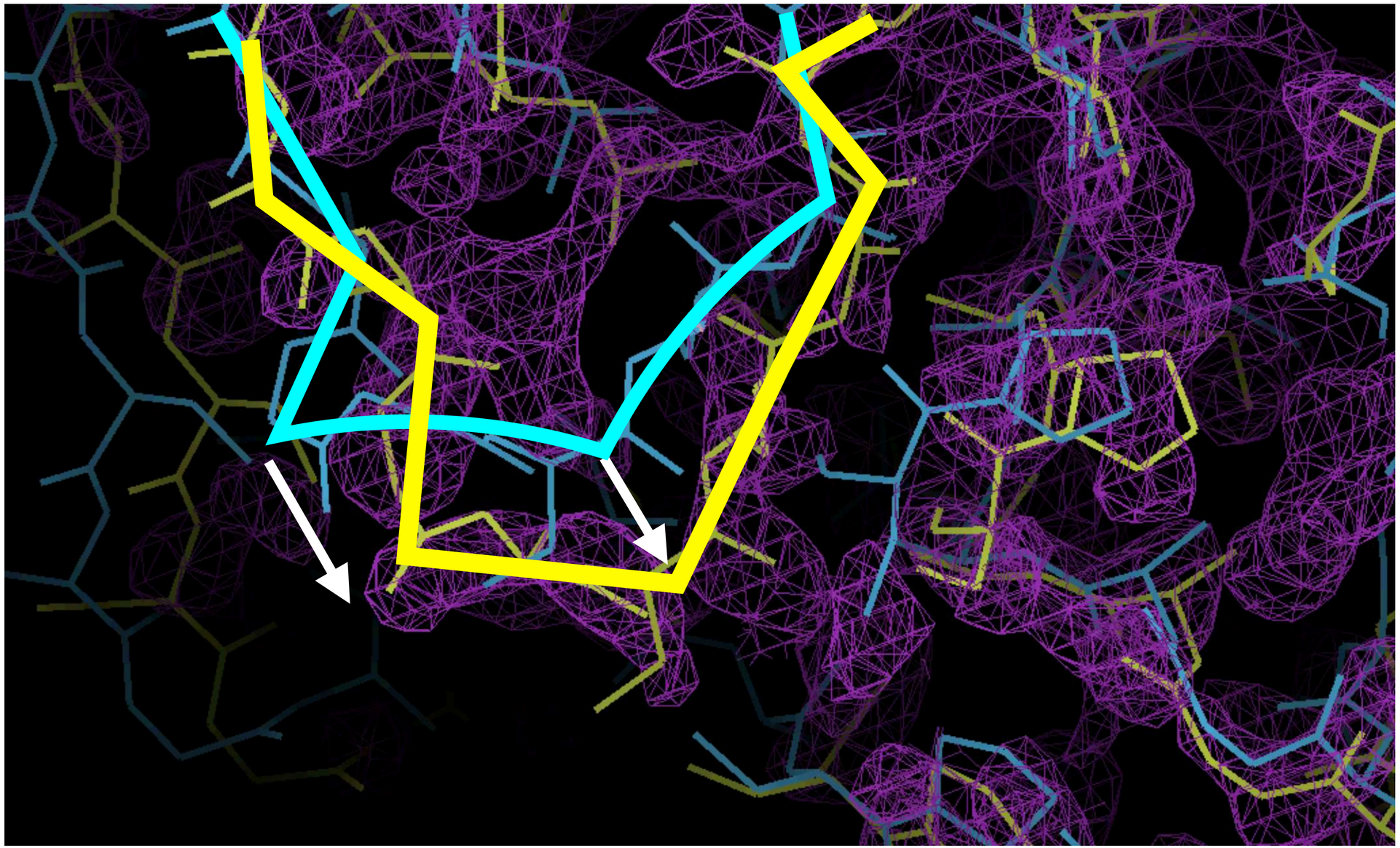
- **Can allow for larger shifts in model than simple minimization**

Optimization protocols: Torsion-angle Grid Search



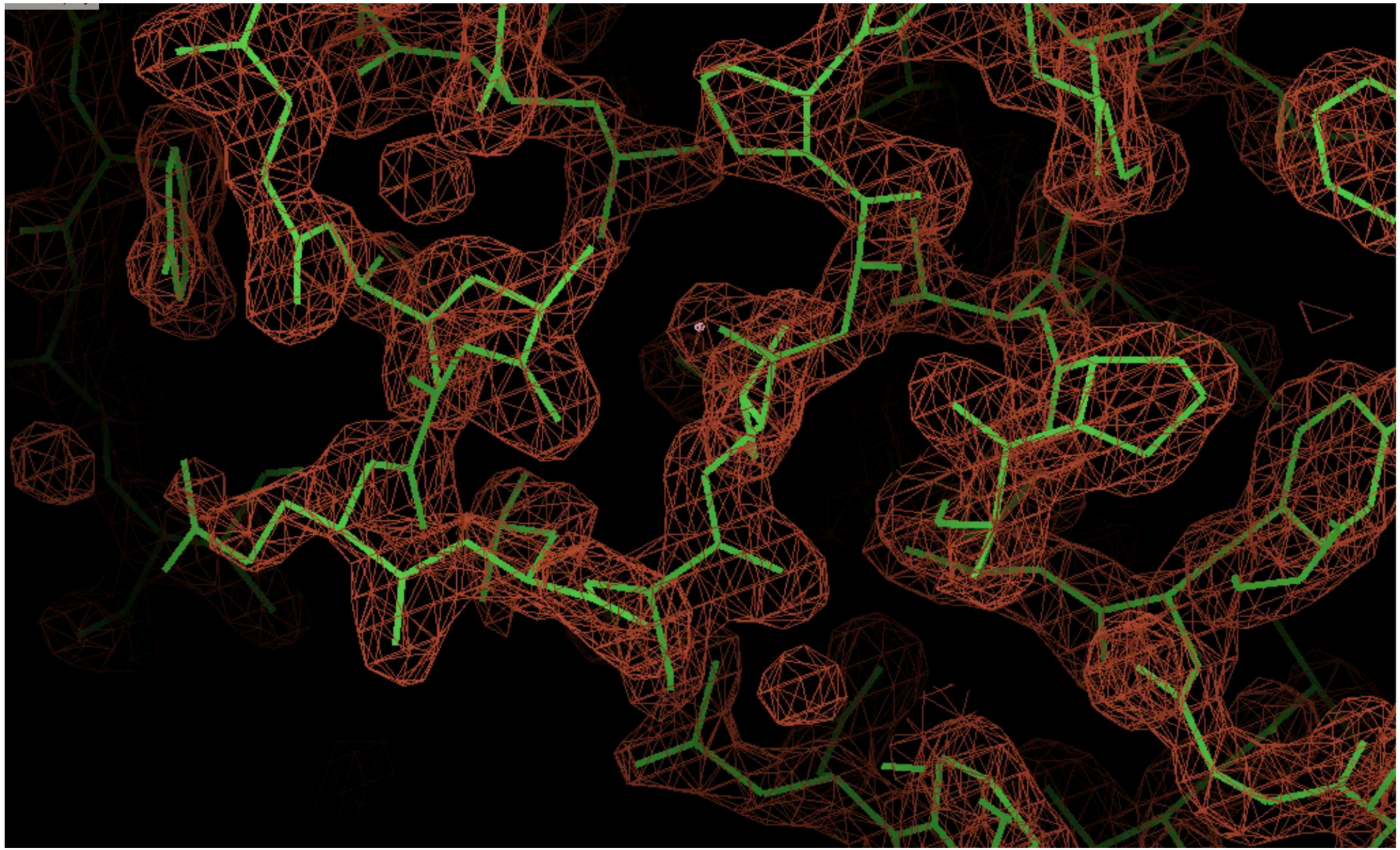
Slide adapted from: Pavel Afonine, LBNL (Phenix)

Optimization protocols: Morphing



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

Optimization protocols: Morphing



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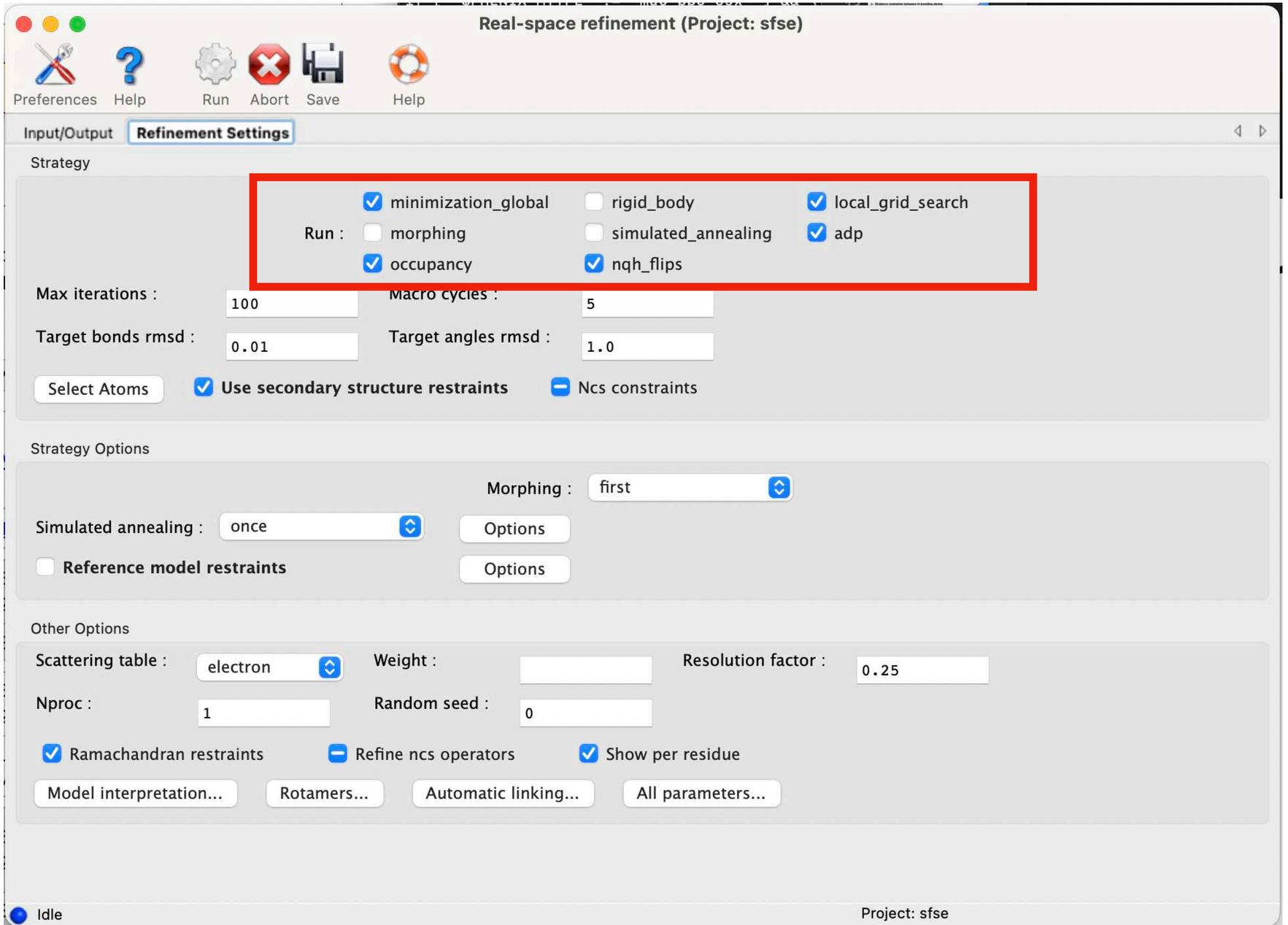
There are many refinement options to choose from!



phenix.real_space_refine

What parameters should I use?

How aggressive do I want to be in refinement?

--How much do I trust my starting model vs my data?

--How different is my starting model from my data?

More aggressive = larger radius of convergence, potentially less manual rebuilding; but changes the model a lot (morphing, grid search, simulated annealing)

Less aggressive = smaller radius of convergence, will change model less (rigid body, minimization_global)

What parameters should I use?

Before doing anything: rigid body refinement (overall, individual domains)

Early stages:

-Target structure very similar to starting model: minimization_global, adp, grid_search(?)

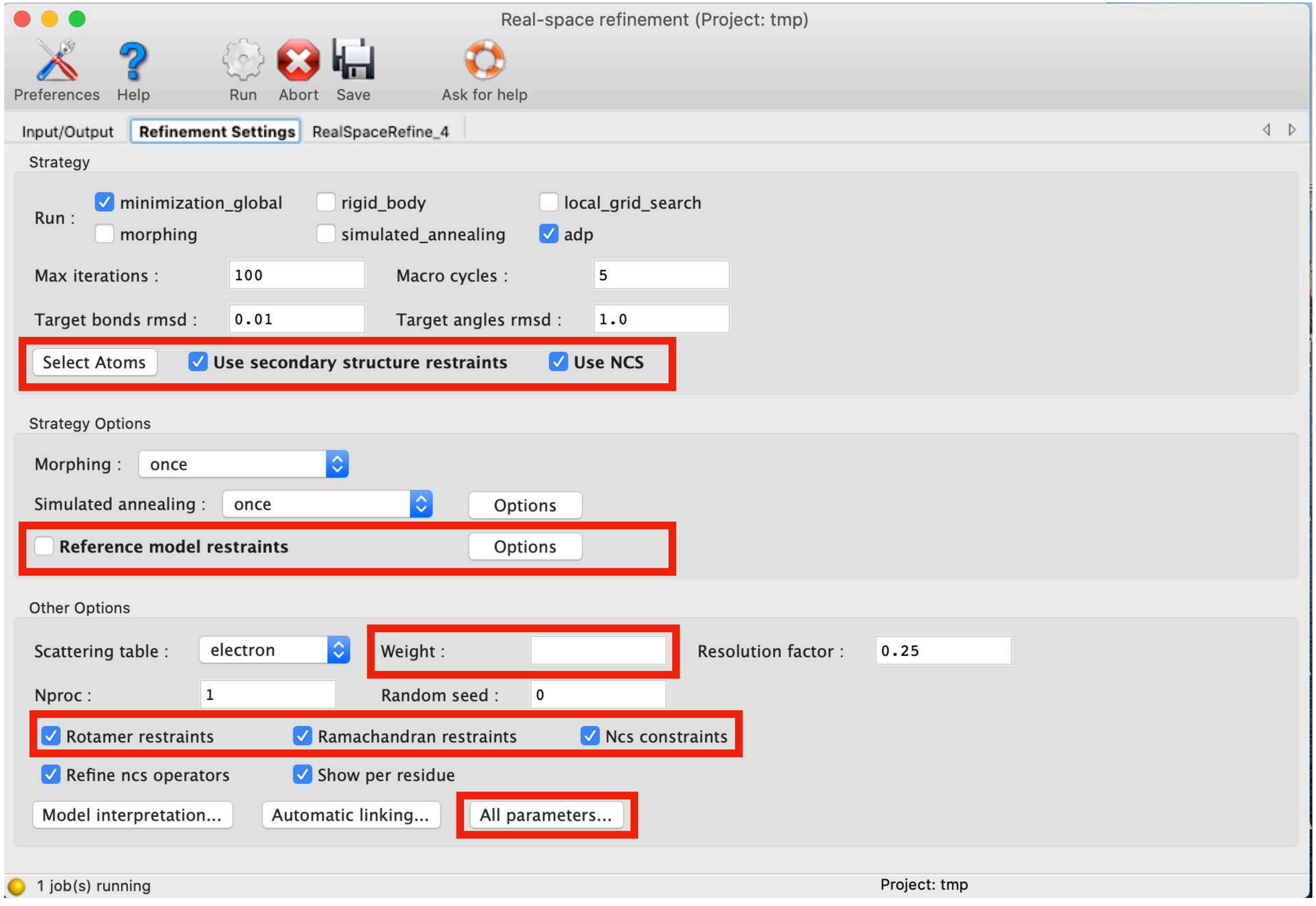
-Target structure very different: minimization_global, adp, grid_search, morph, simulated annealing(?)

Late stages:

-Go easy: minimization_global, adp

Restraints

https://phenix-online.org/documentation/reference/real_space_refine.html



What restraints should I use?

Resolution dependent:

—High res, use less restraints and trust map more

—Low res, use more restraints and trust map less

High res: Often only basic sterochemical restraints are sufficient

Low res: Try different combinations of secondary structure restraints, ncs, reference model, rotamers (ramachandran(?))

—Sometimes using too many restraints can prevent efficient refinement because model can’t move; experiment and see what results in best fit to map while maintaining good geometry

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 model 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
    - 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

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

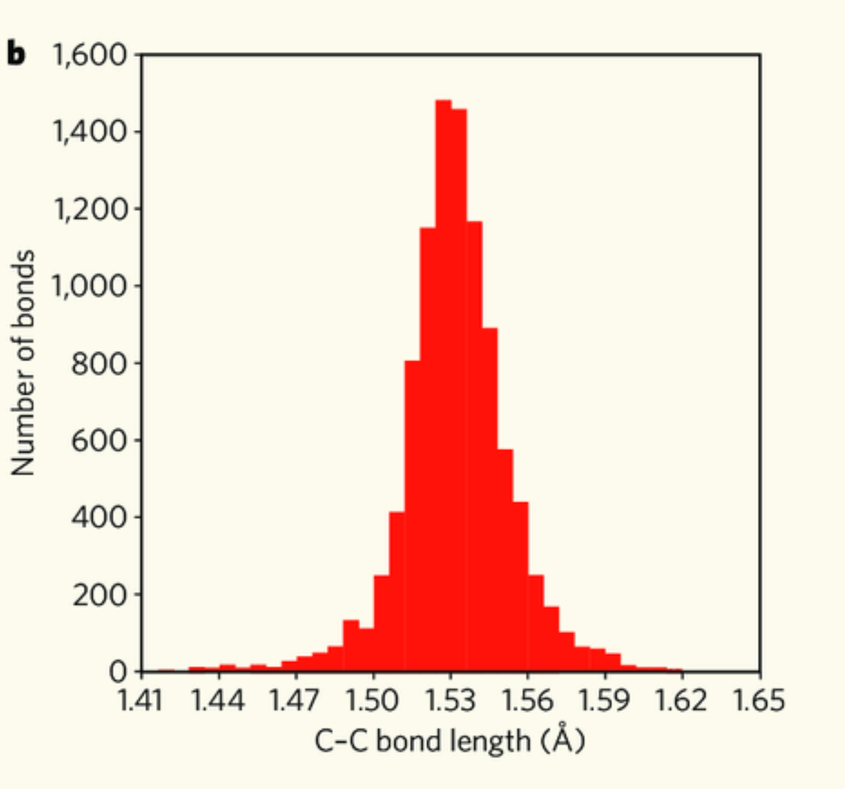
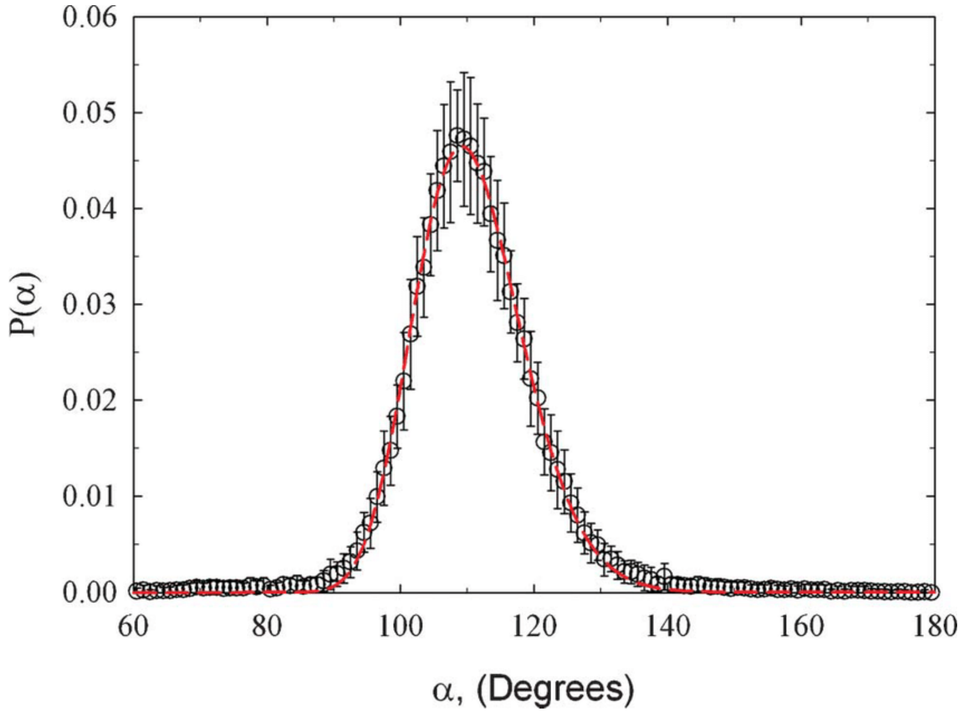


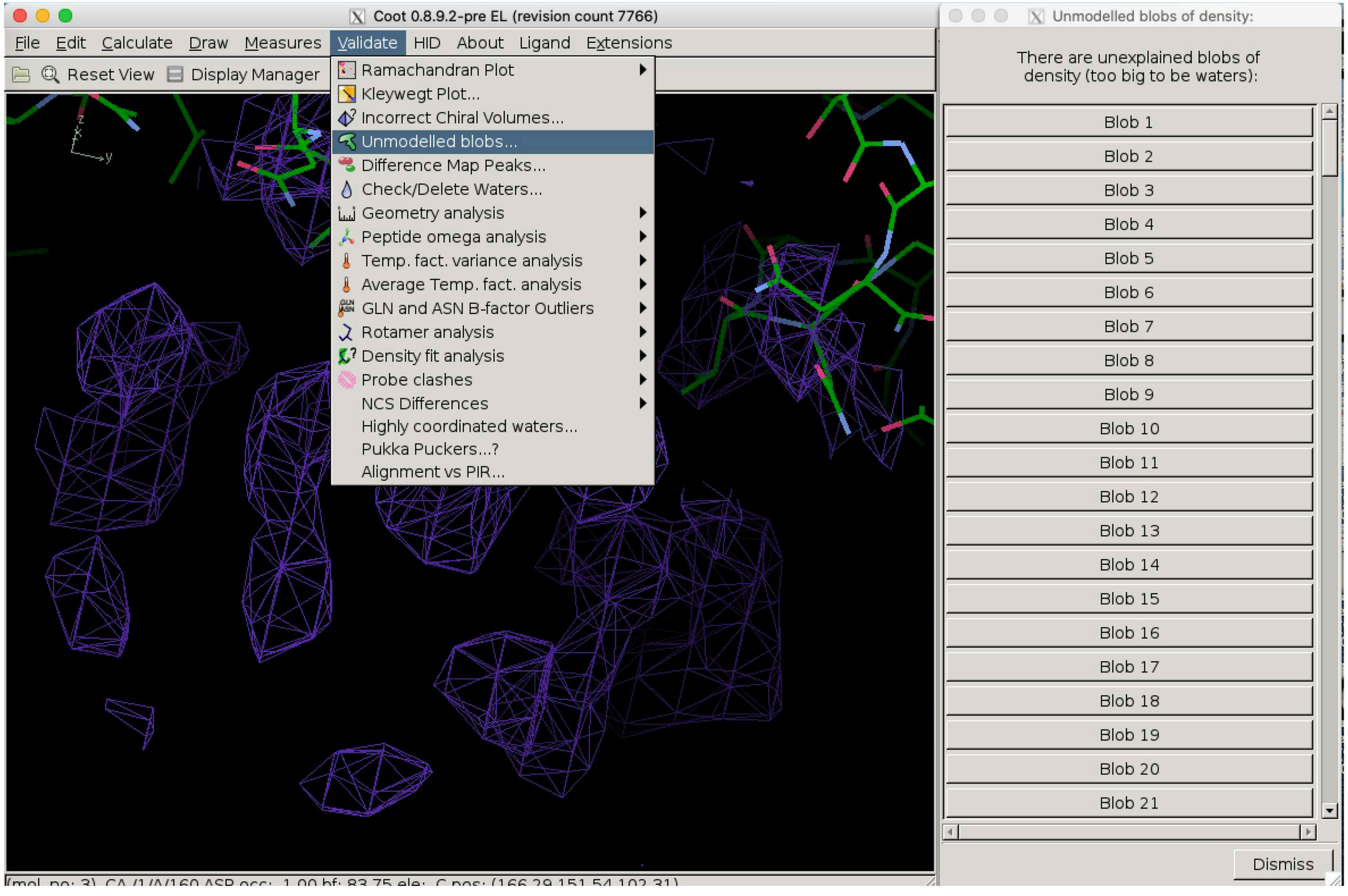
Image: Janez Stepisnik

Typical RMS Angles for protein structure:
0.5 - 1.5 Å

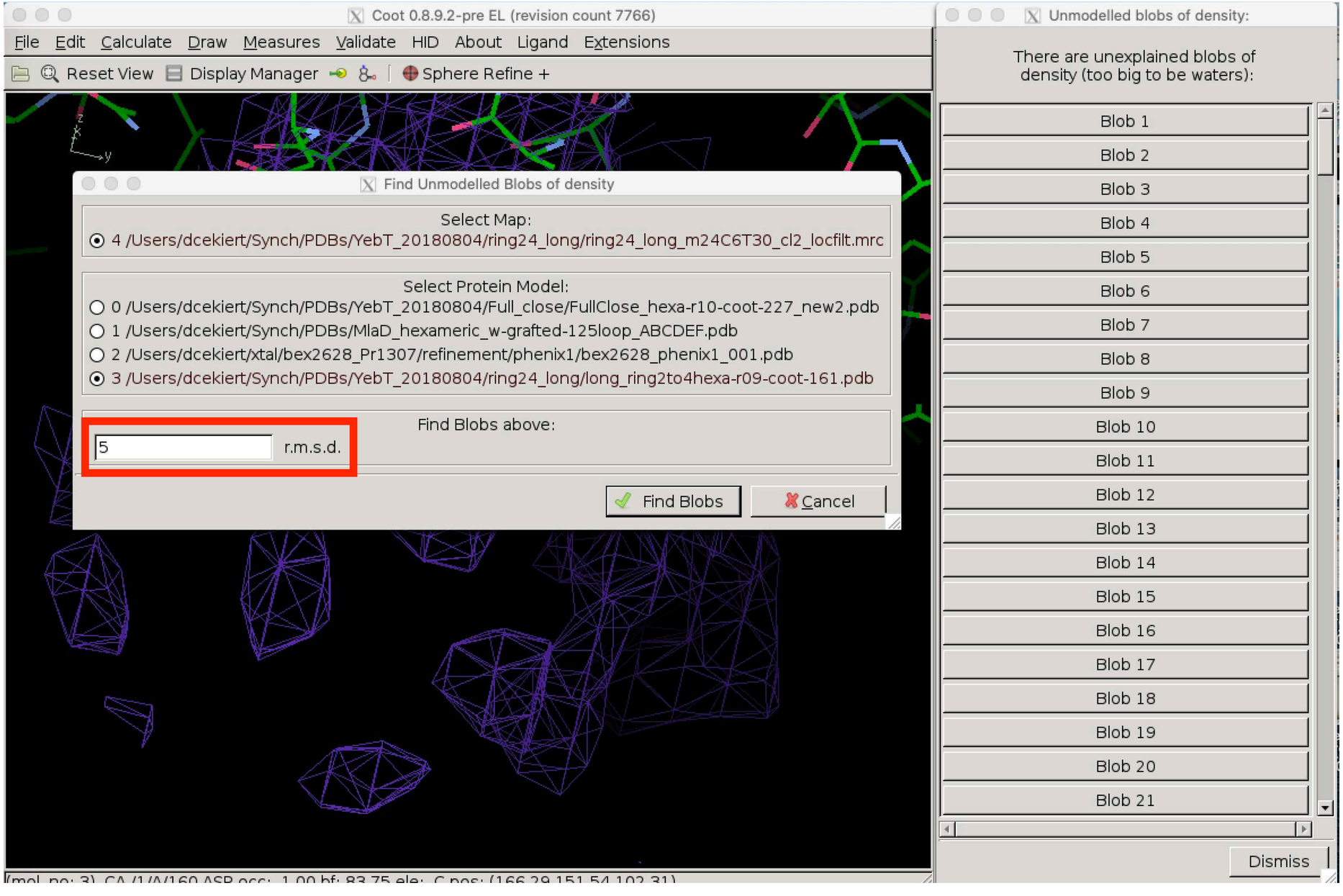


C-C-C angle distribution of sp3 carbon
Kowalczyk, et al. RSC Advances, 2012.

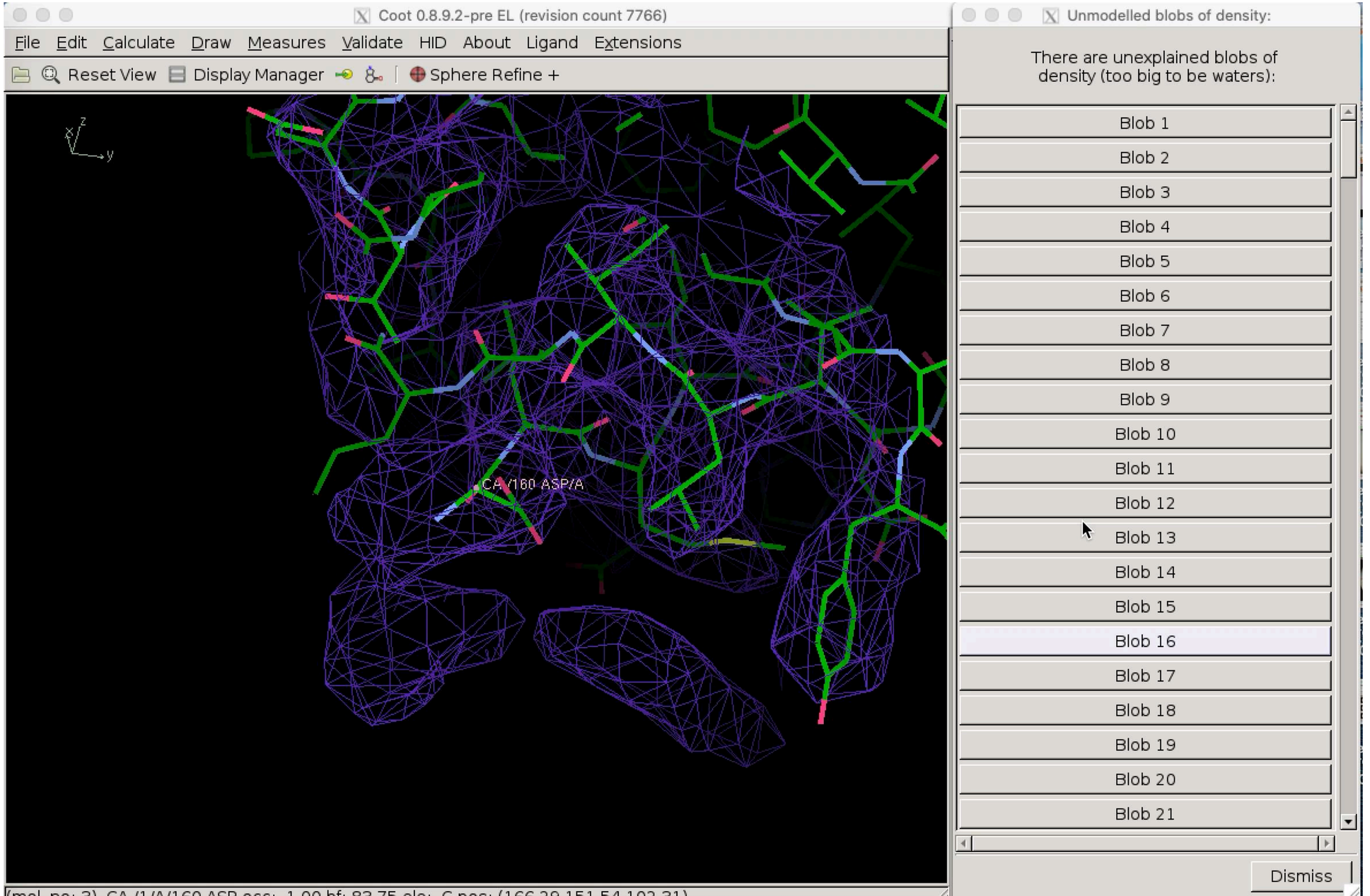
Unmodeled densities



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.

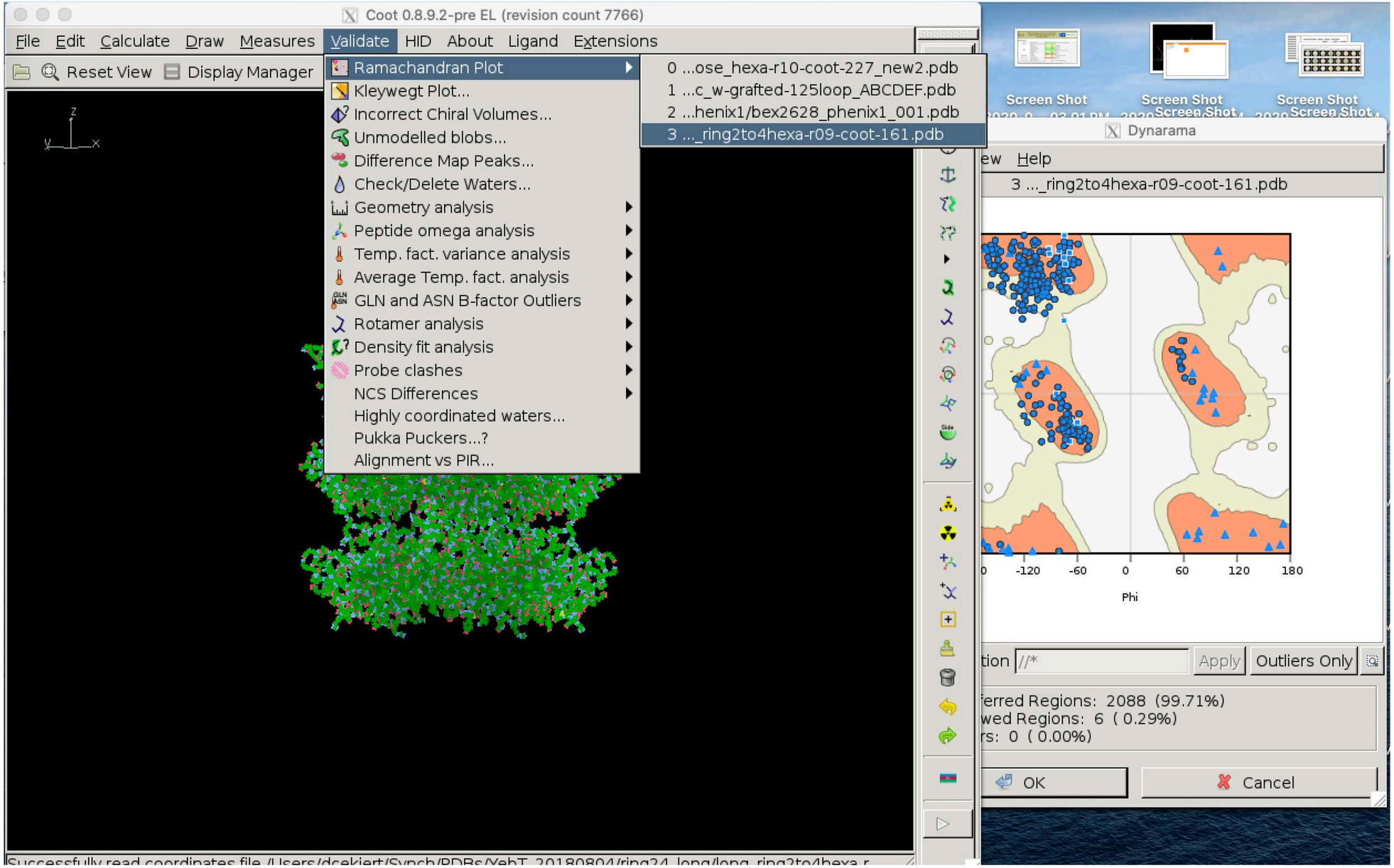
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

^ 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/

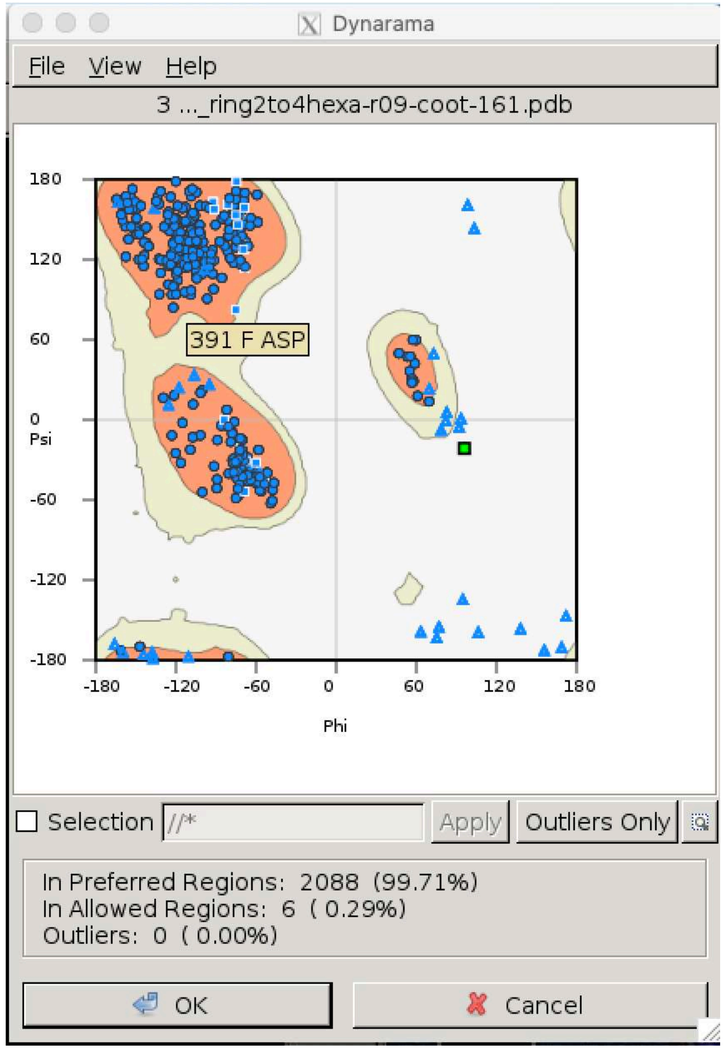
Williams et al. Protein Science (2018).

Ramachandran plot

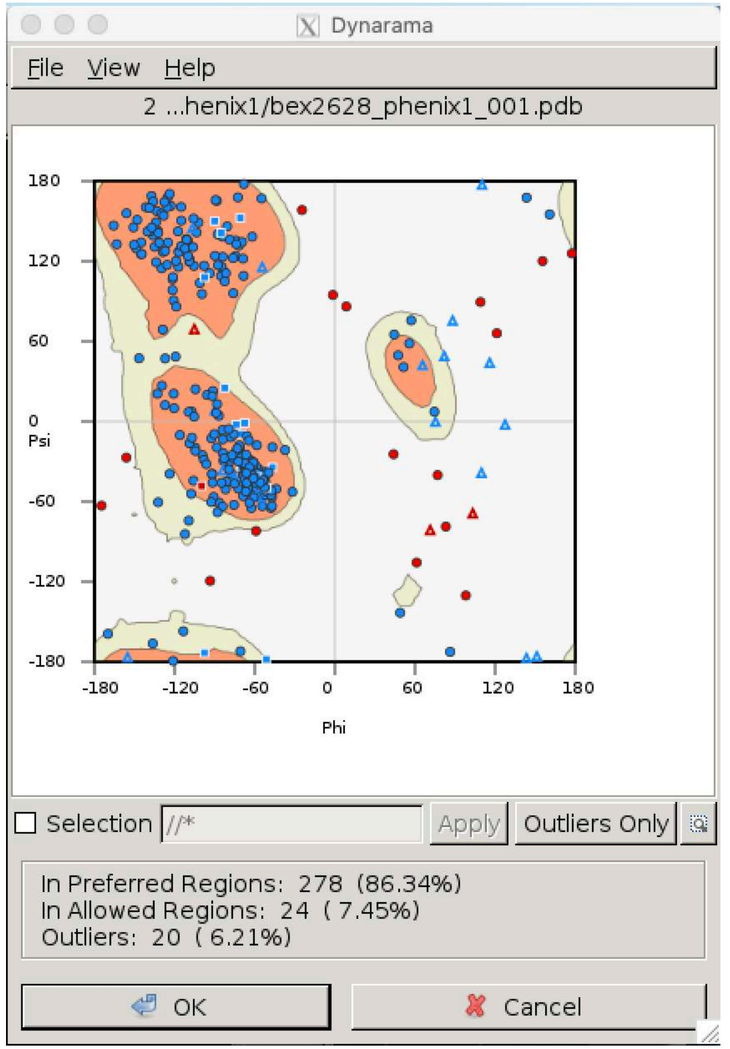


Ramachandran plot

Ideal plot

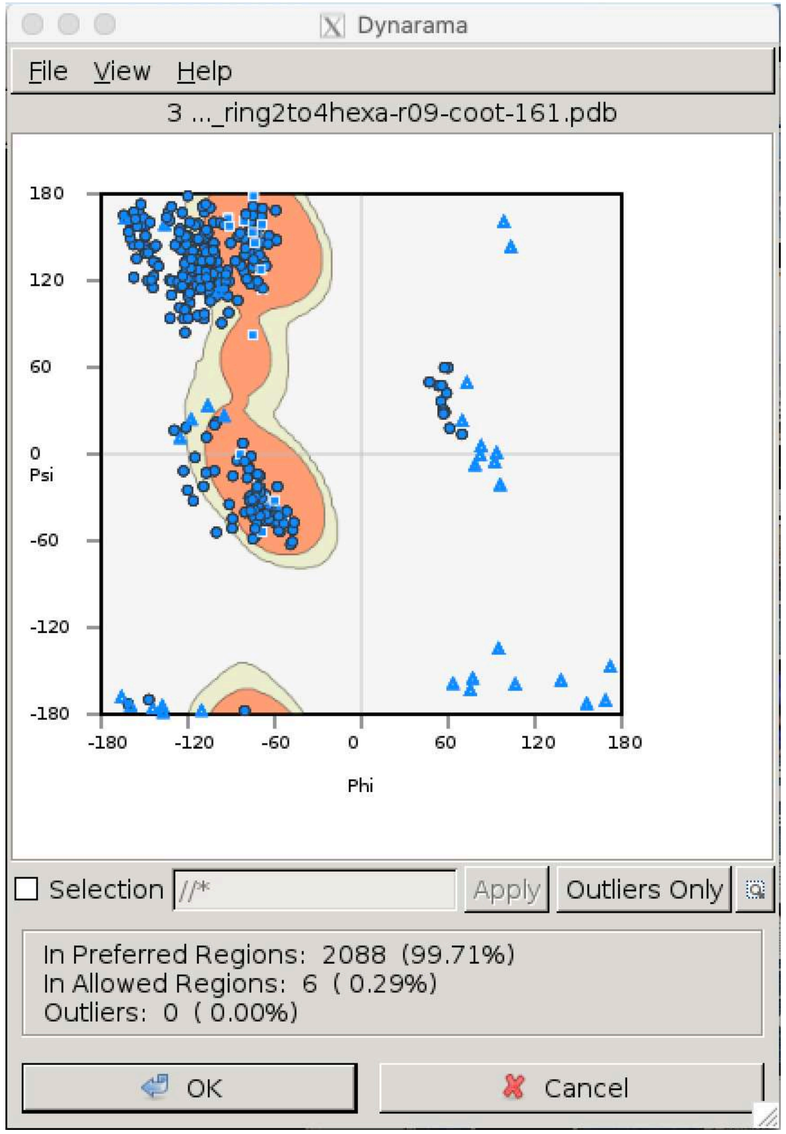


Problematic plot

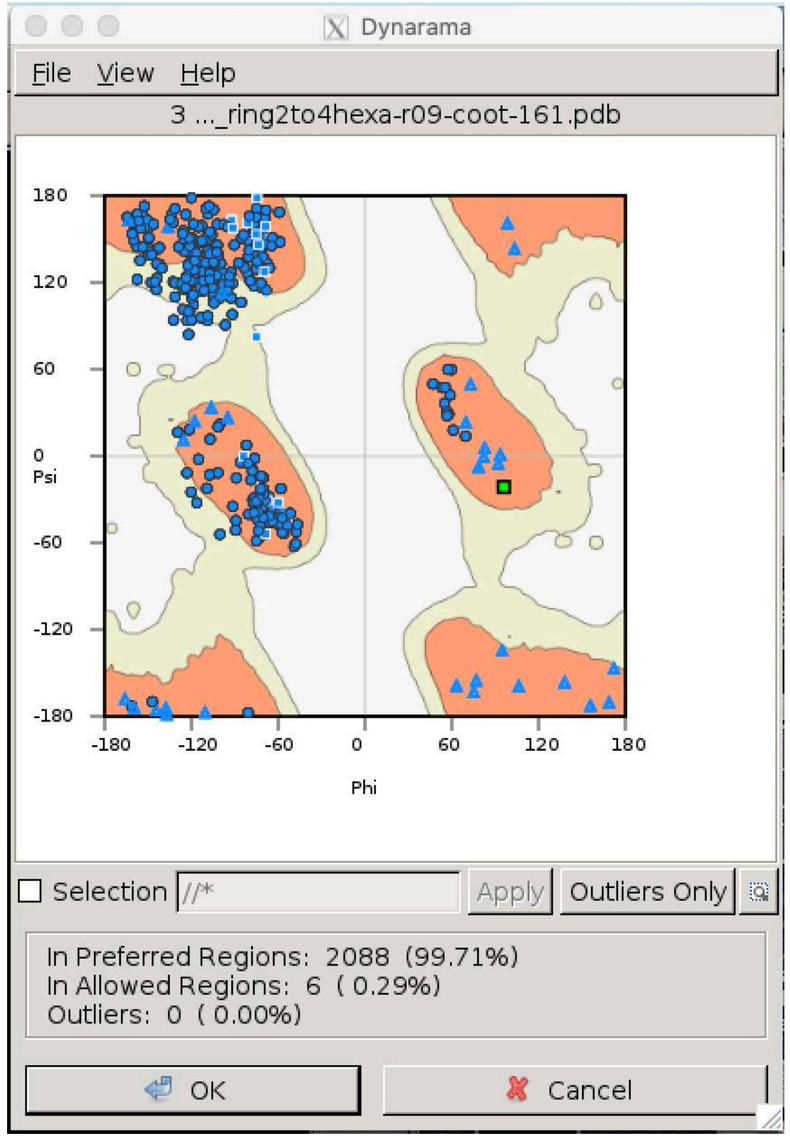


Ramachandran plot

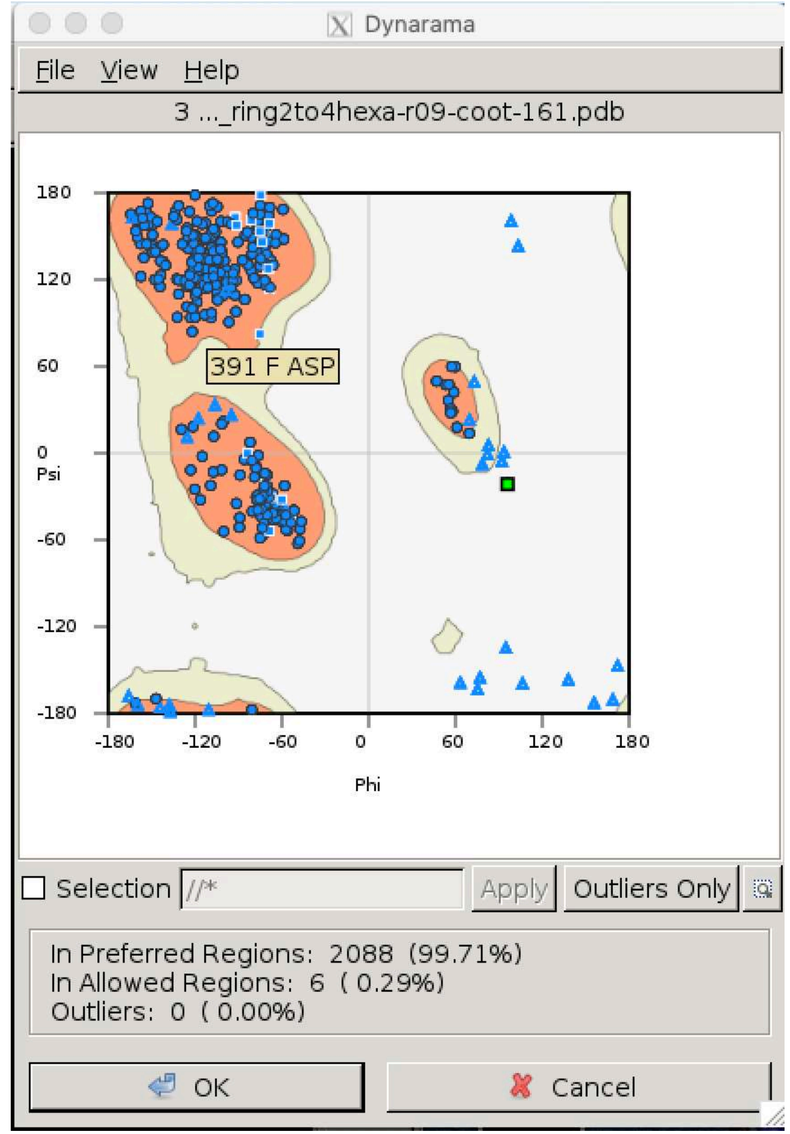
Proline Allowed Regions



Glycine Allowed Regions



Allowed Regions (non-Pro/Gly)



Cis peptide bonds

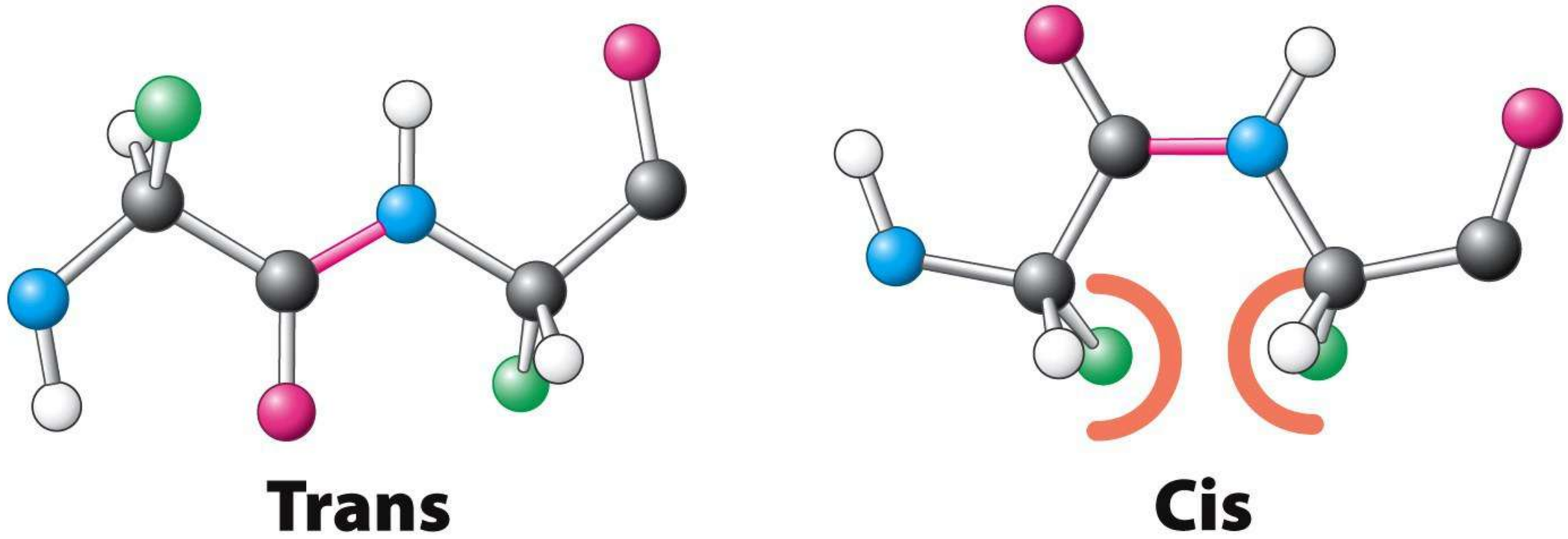


Figure 2.20
***Biochemistry*, Seventh Edition**

Cis peptide bonds

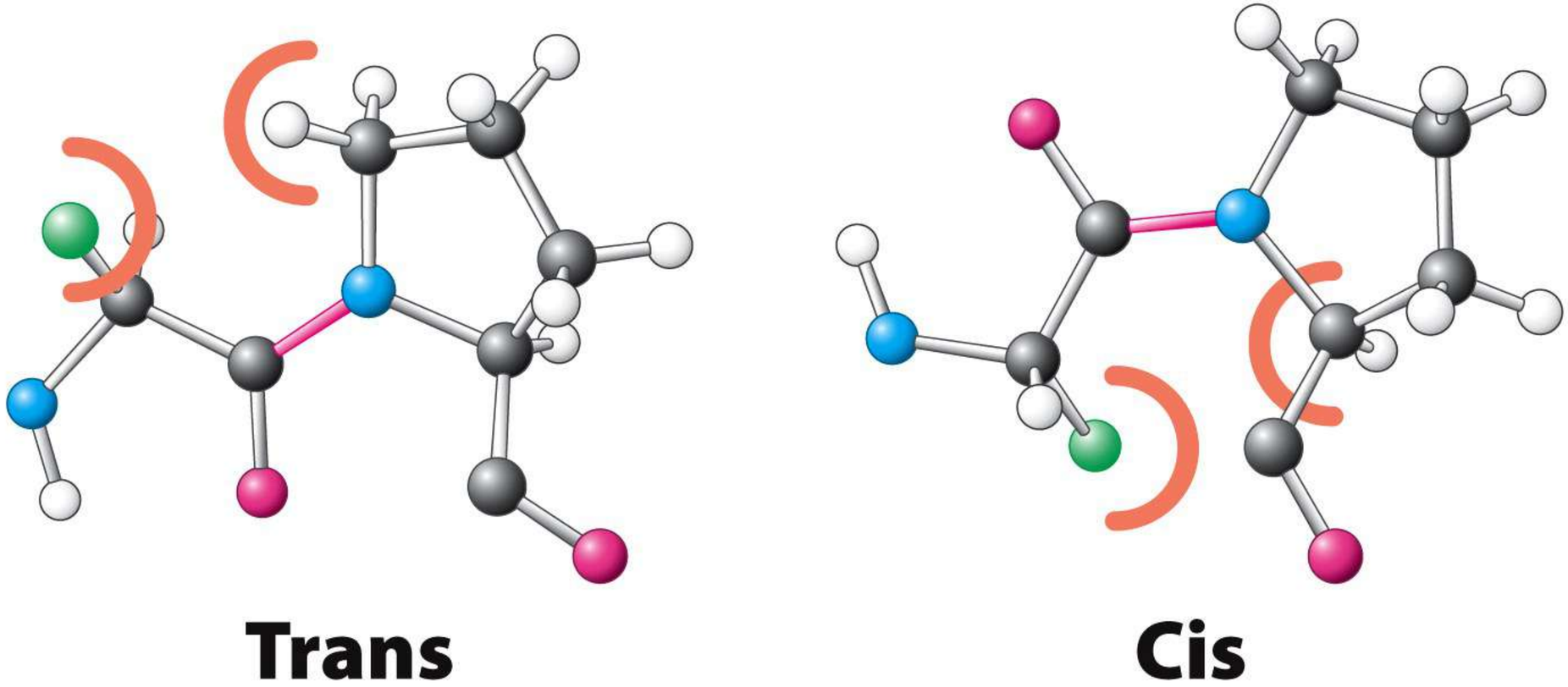
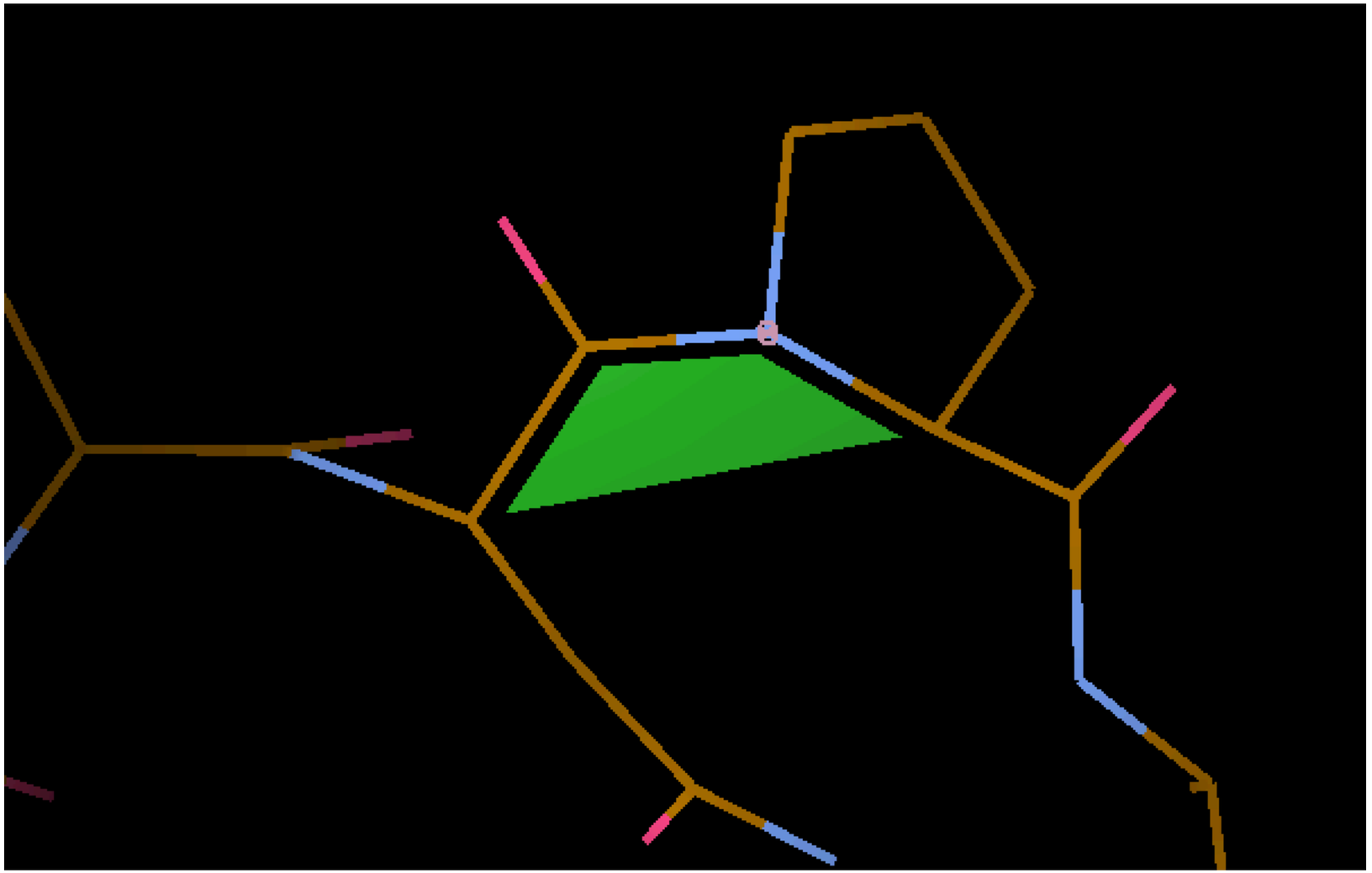


Figure 2.21
***Biochemistry*, Seventh Edition**

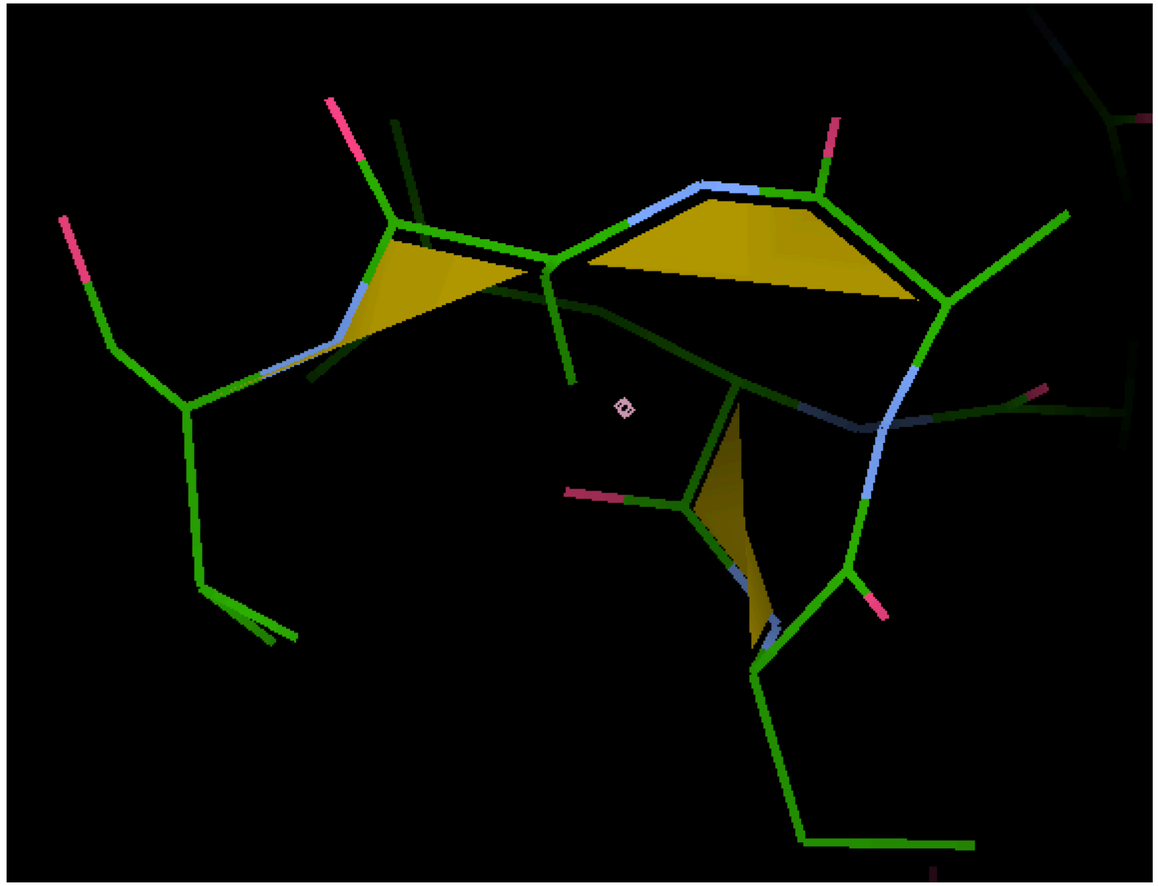
Cis peptide bonds

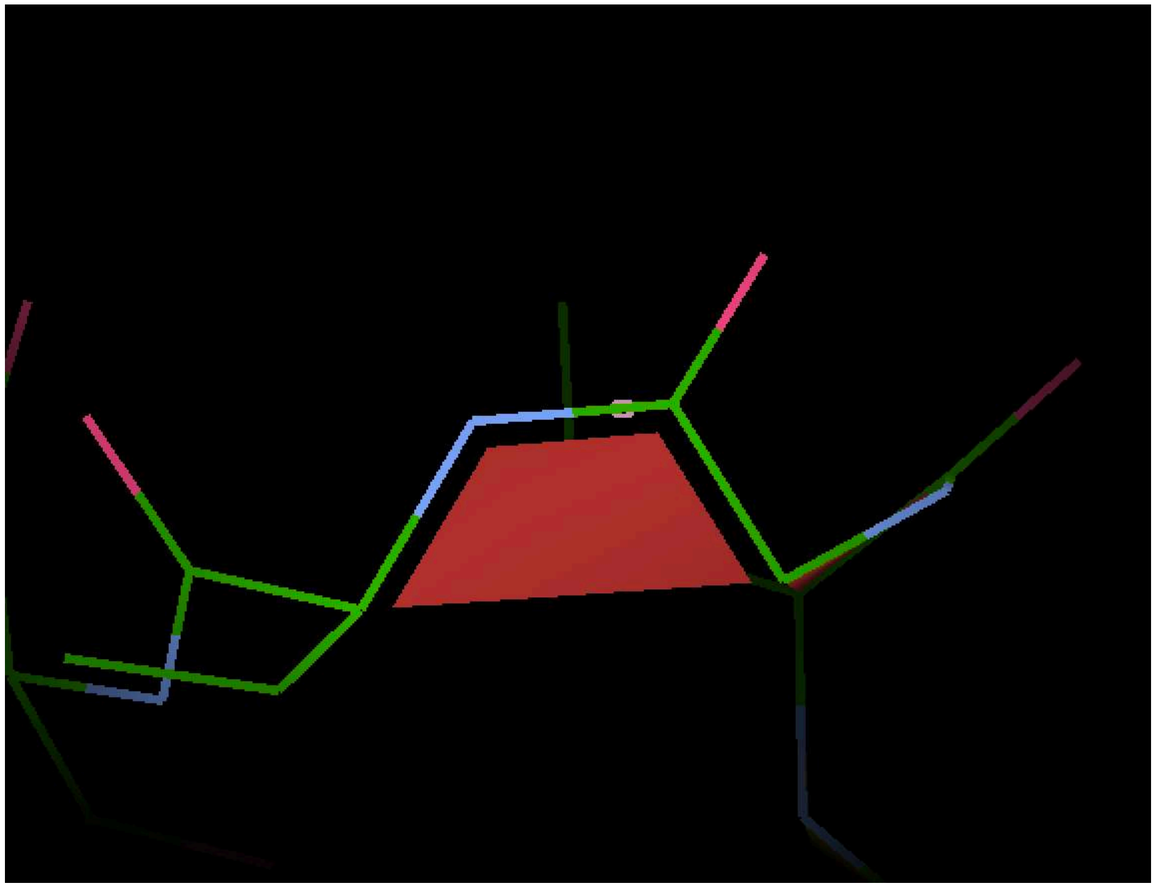


Coot highlights all cis and non-planar peptide bonds, and color codes them to make potential problems easy to ID

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

Cis peptide bonds

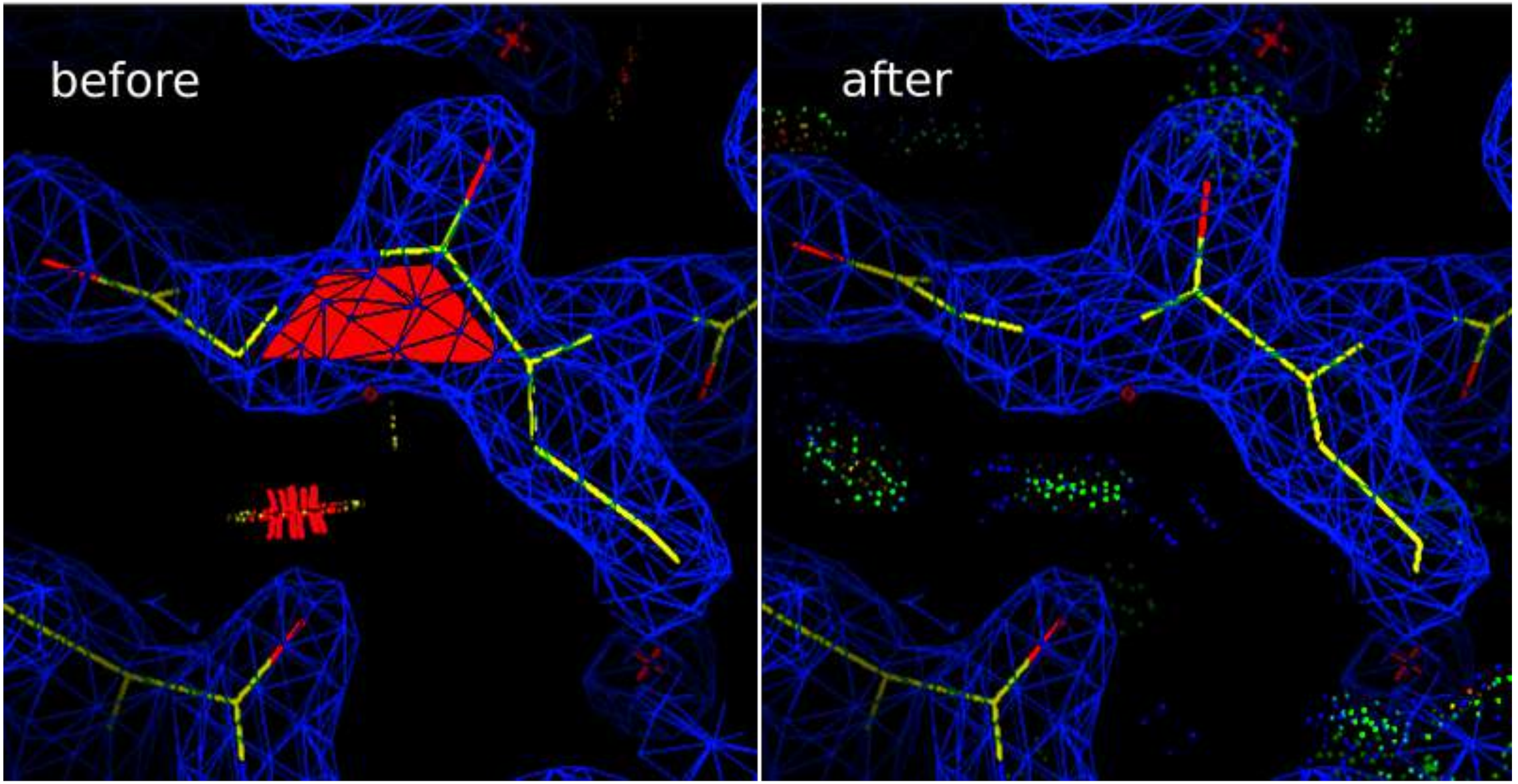




Coot highlights all cis and non-planar peptide bonds, and color codes them to make potential problems easy to ID

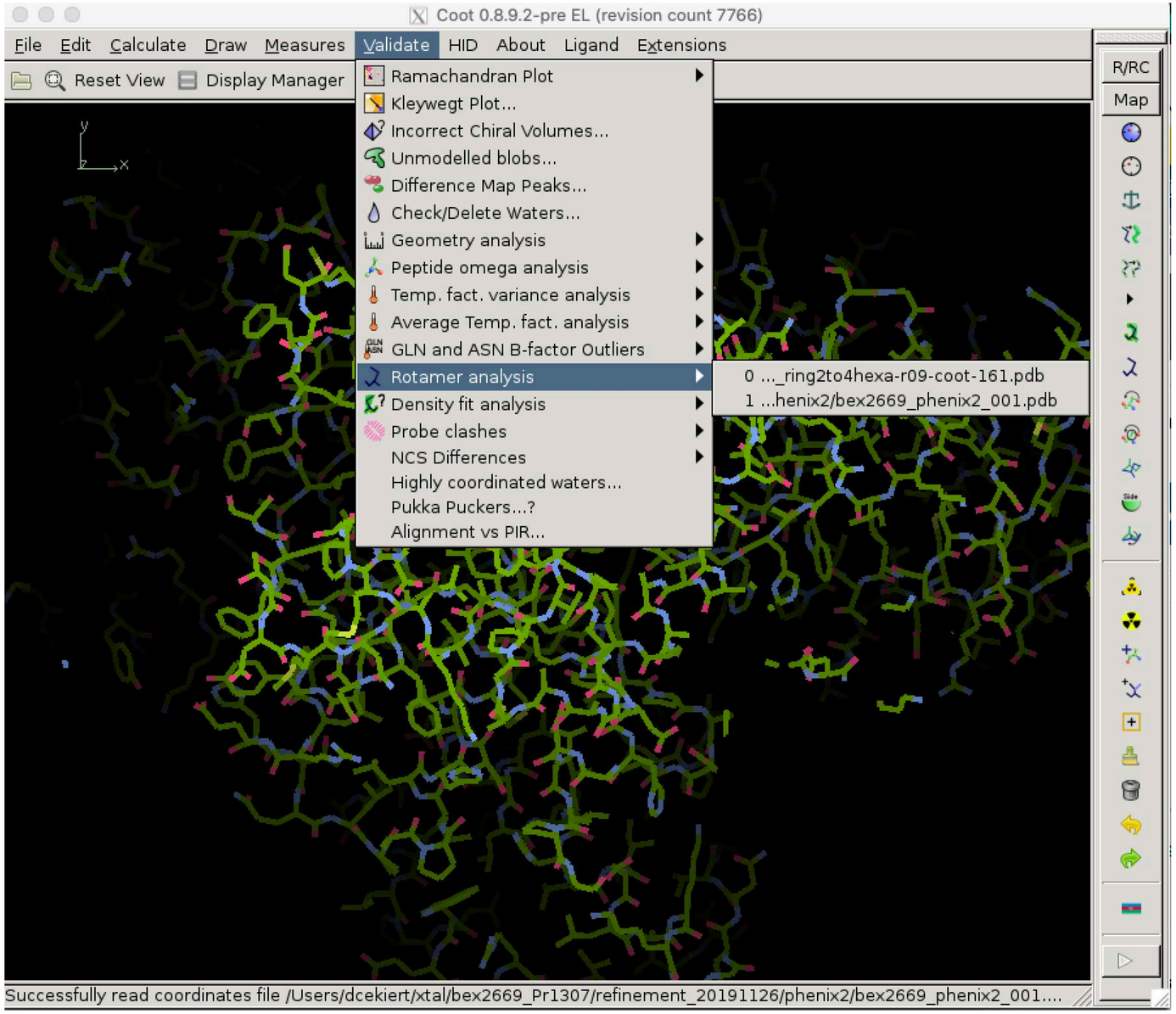
Green = cis-Proline (probably OK); Yellow = non-planar peptide bond (check!); Red = non-proline cis peptide bond (check!)

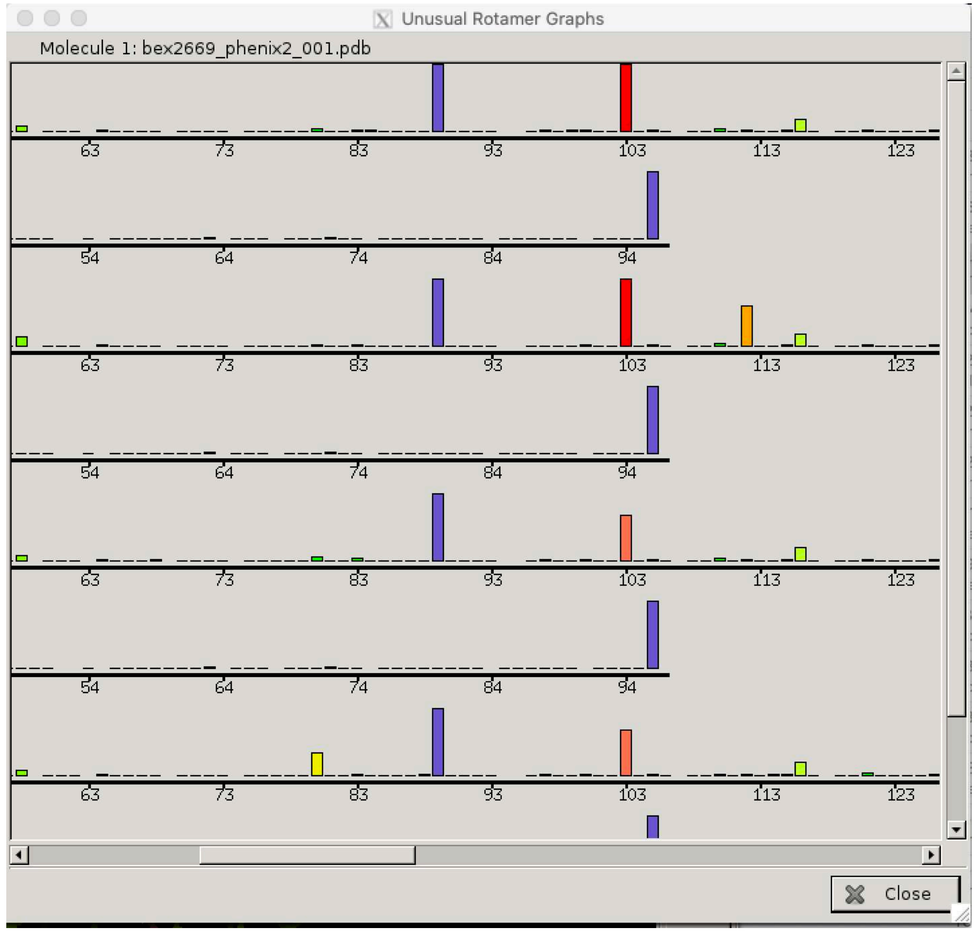
Cis peptide bonds



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

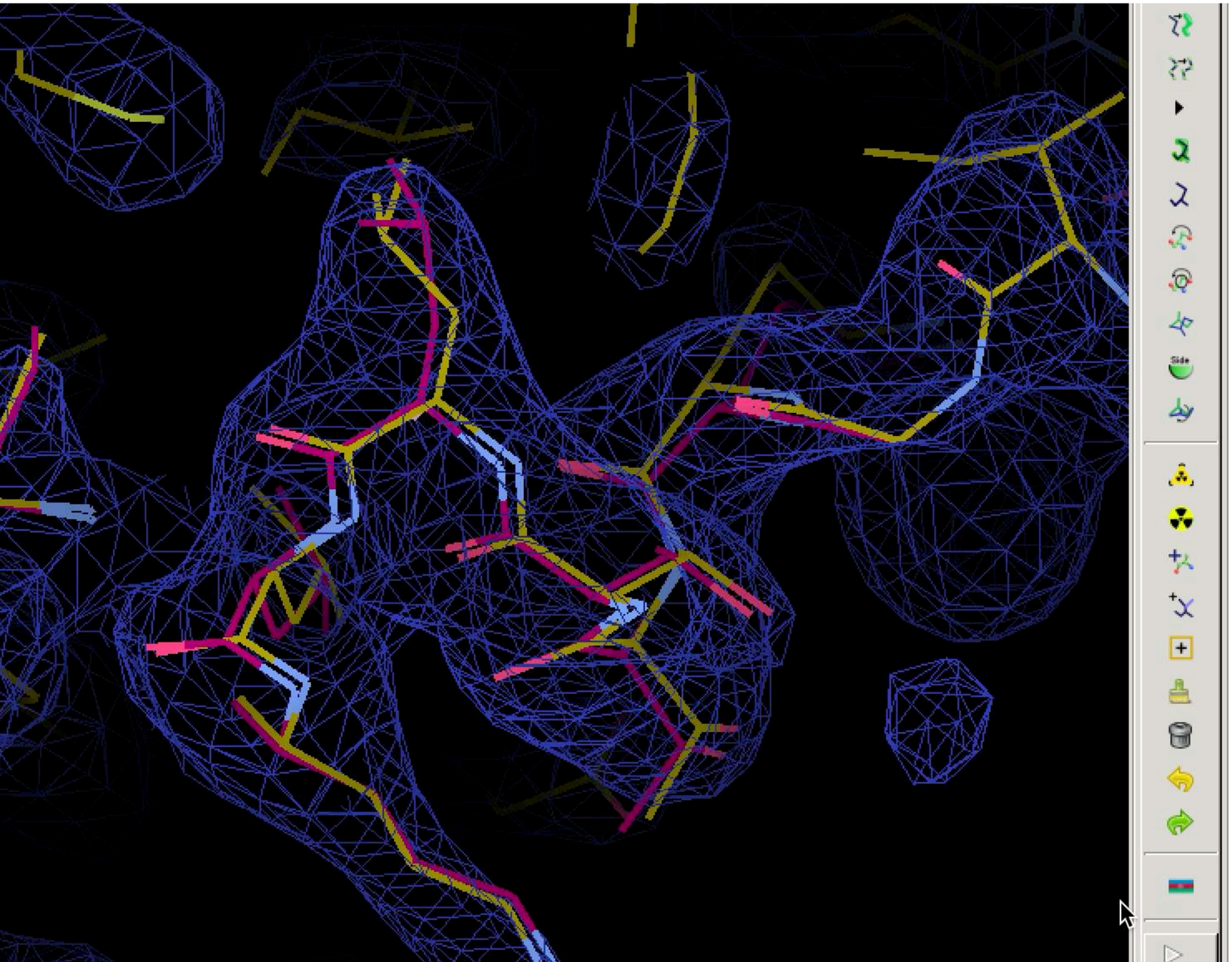
Rotamer outliers: Coot





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

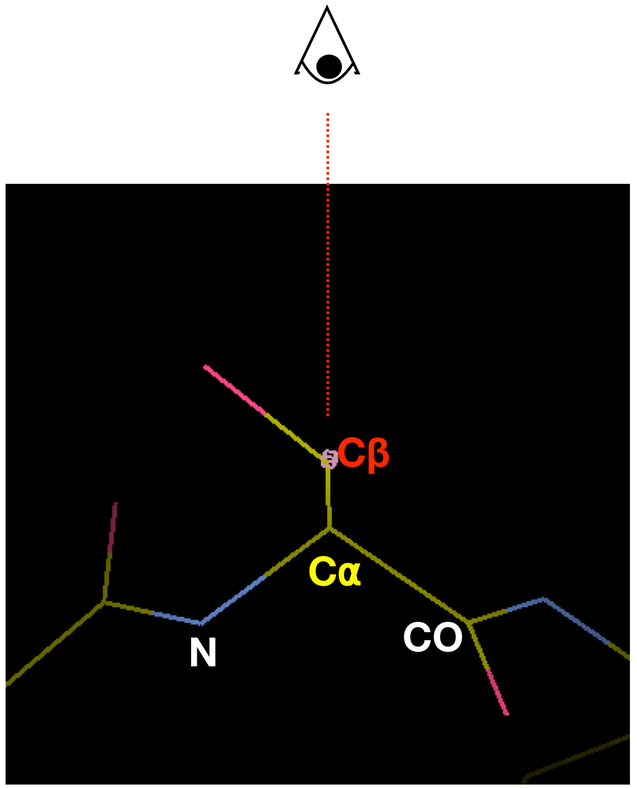
Cbeta deviations

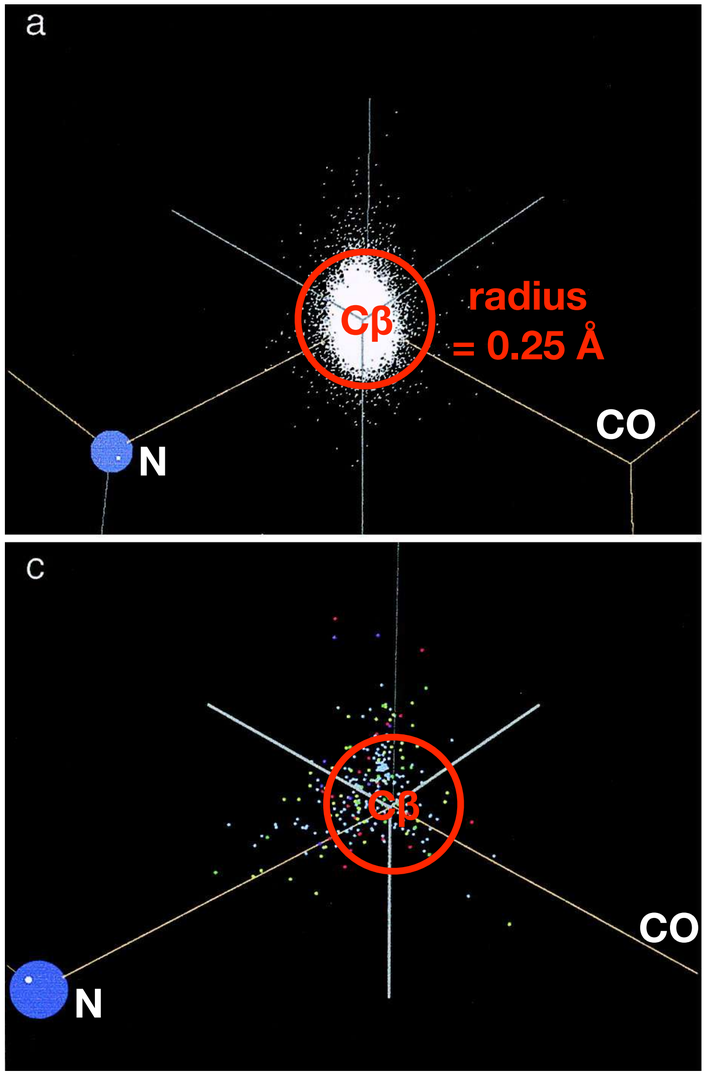


Which Leu rotamer is correct???

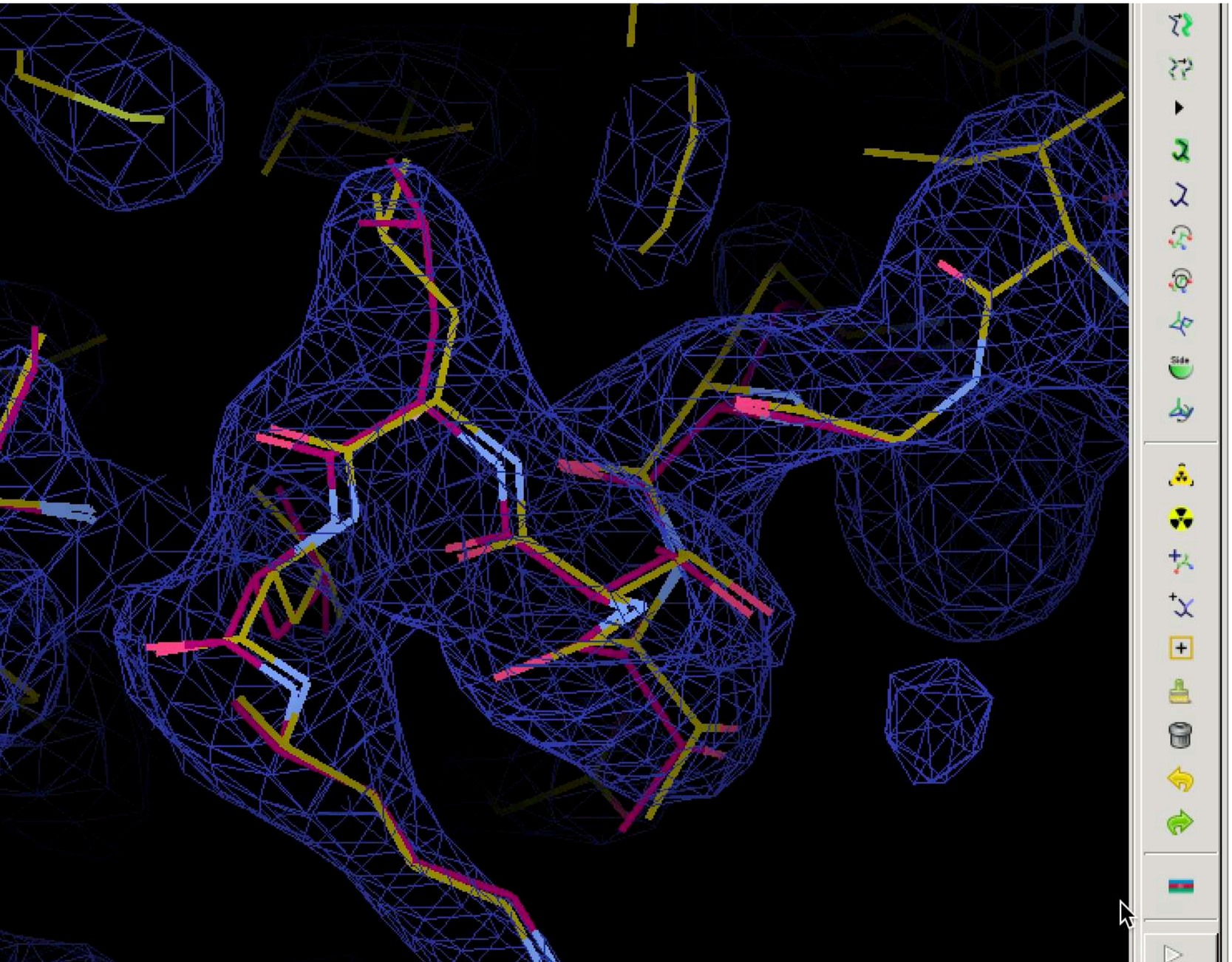
Cbeta deviations

Cbeta deviations report on a combination of backbone and side chain problems, frequently when an incorrect side chain rotamer is leading to a distortion of the backbone conformation as well.





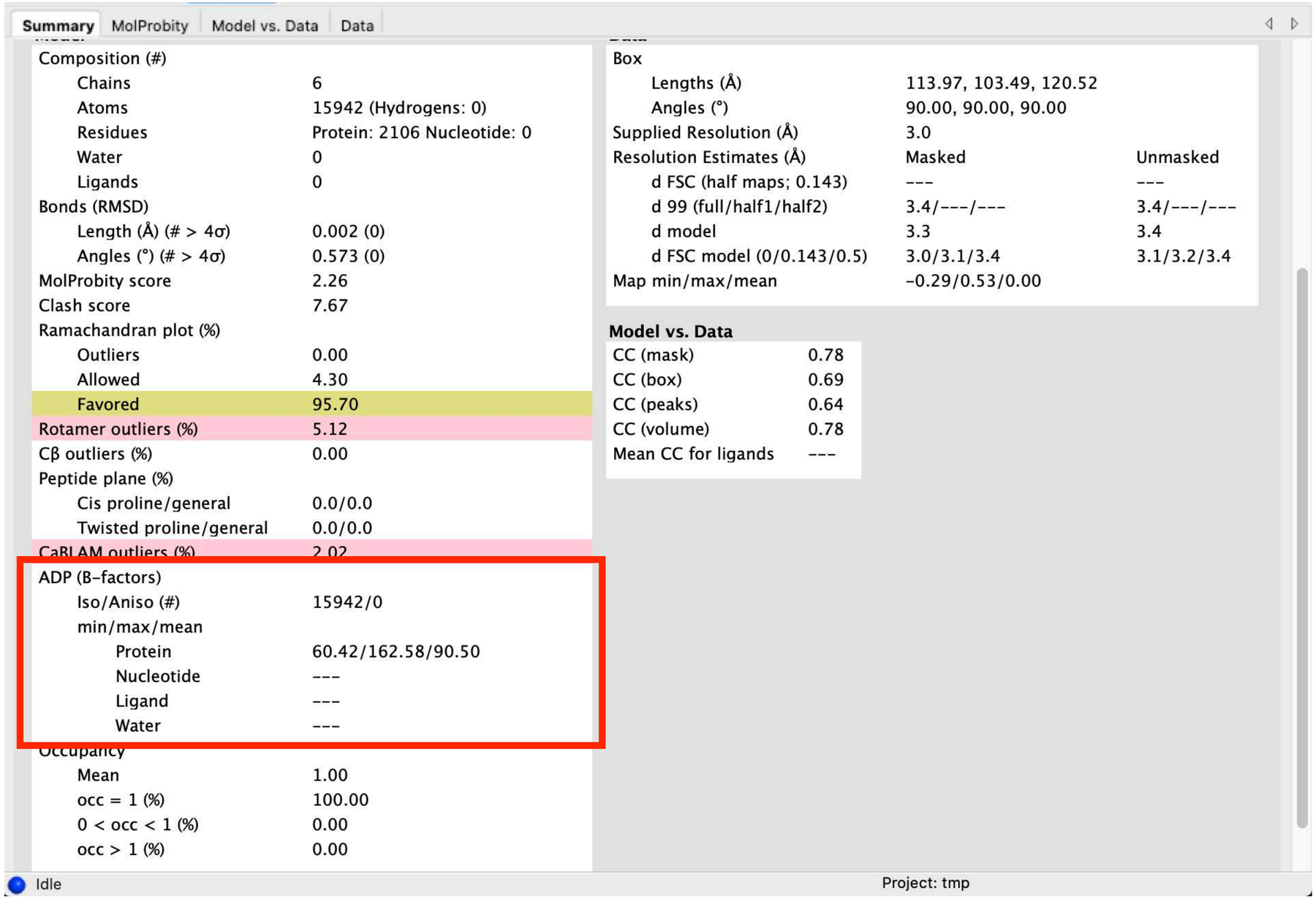
Cbeta deviations



Which Leu rotamer is correct???

Correct answer:
YELLOW

ADP outliers



Expected mean B-factor at 3-4 Å resolution: Roughly 100-200 ?

Currently, ability to adjust B-factor parameterization is limited (e.g. group B was individual atoms; TLS)

If your B factors seem very high or low:

- **Check for regions with very high B's; out of density? Weak density? Delete?**
- **Try reseting all B's to same low value (e.g., 30) and try refining again.**

JCSG QC Server

Date: 03/17/22 16:22:07

The Quality Control Check was developed at the Joint Center for Structural Genomics. It is Maintained by Bridge Structural Biology Center and CARC at USC.
If you use this tool in preparing a structure, please reference this URL: https://qc-check.usc.edu

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Refinement Stats
PDB Check
Nomenclature Check
ADIT Results
Molprobity Results
NCS Check
Sequence Check
Real Space CC

https://qc-check.usc.edu/QC/qc_check.pl

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