# Model Refinement and Validation

Damian Ekiert and Gira Bhabha NYU School of Medicine March 18, 2022

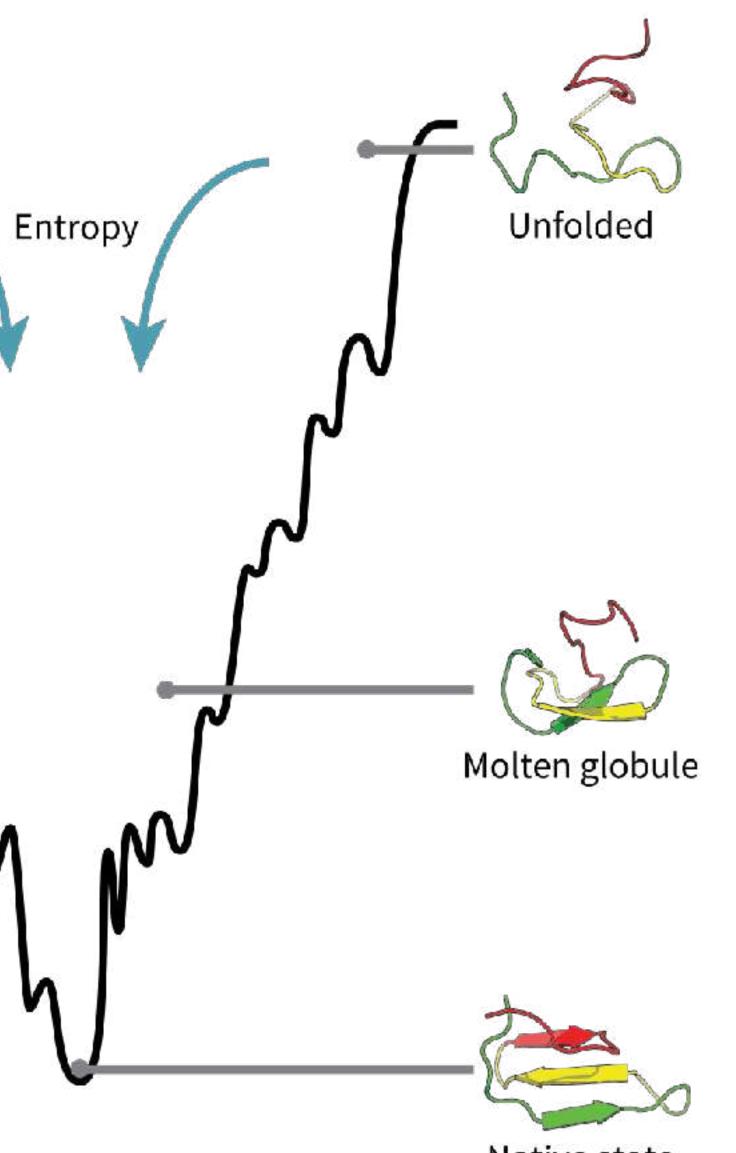
Goal of model refinement: To create a set of coordinates that 1) explains the data as best we can, but 2) also conforms with what we know about proteins in general

# Model Refinement vs Protein Folding Funnel

#### $\Delta G_{unfolding}$

Energy

Image: Thomas Splettstoesser



Native state

#### Refinement target describes differences between model and data

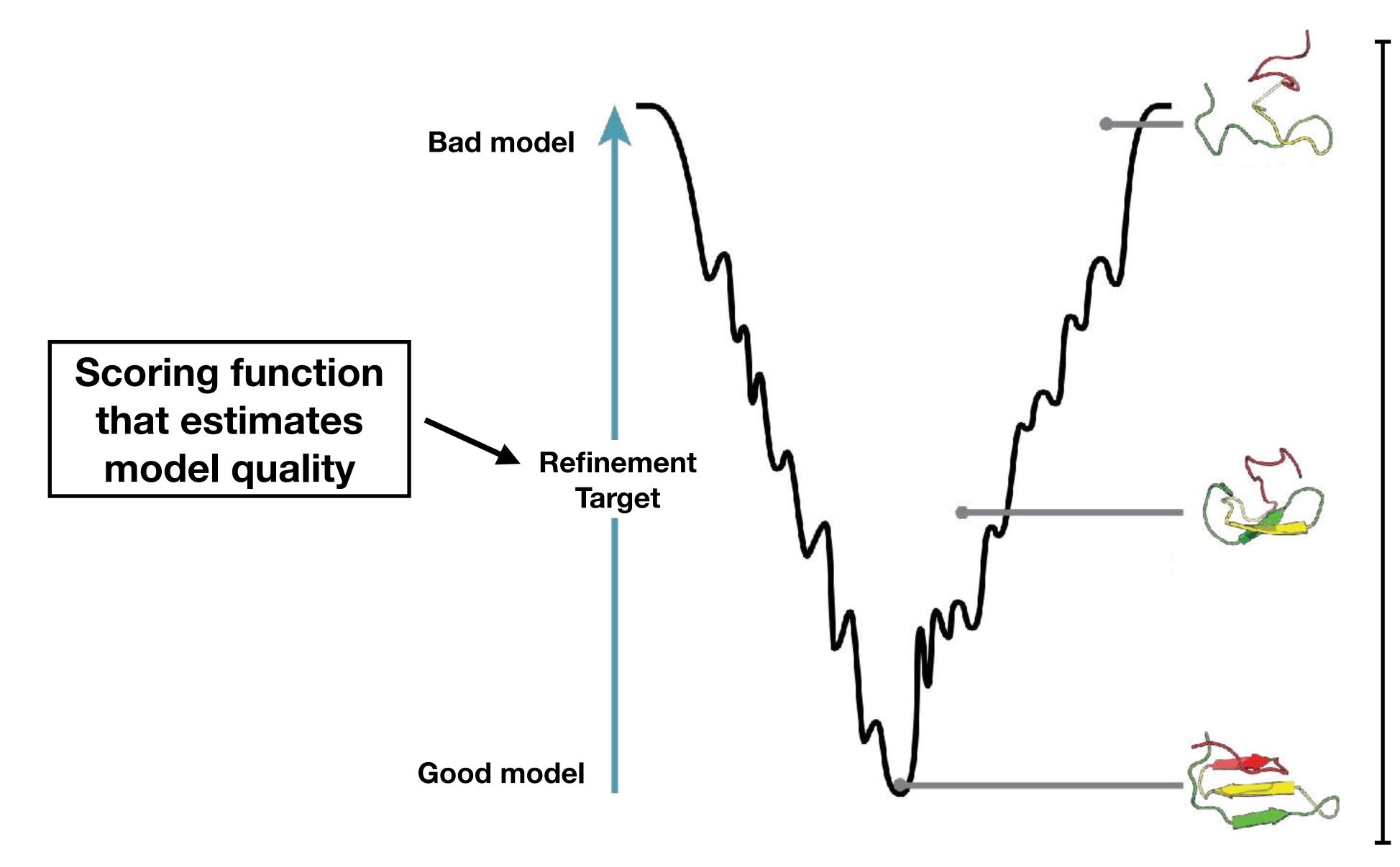


Image (adapted): Thomas Splettstoesser

**Different model parameters** (e.g., conformations)



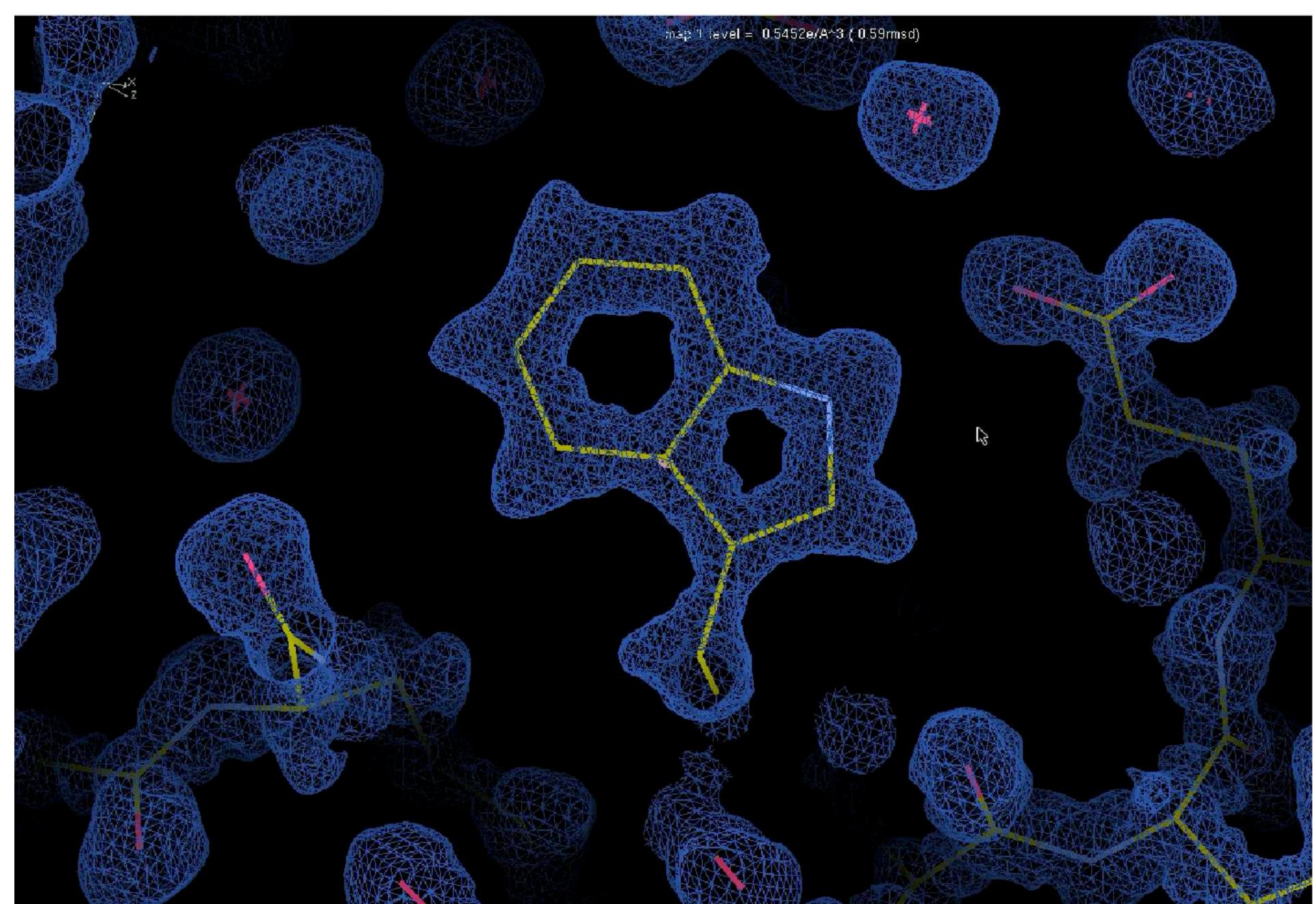
#### Simplest: Refinement Target = (Model vs Data)

#### **Compare and quantify the differences**

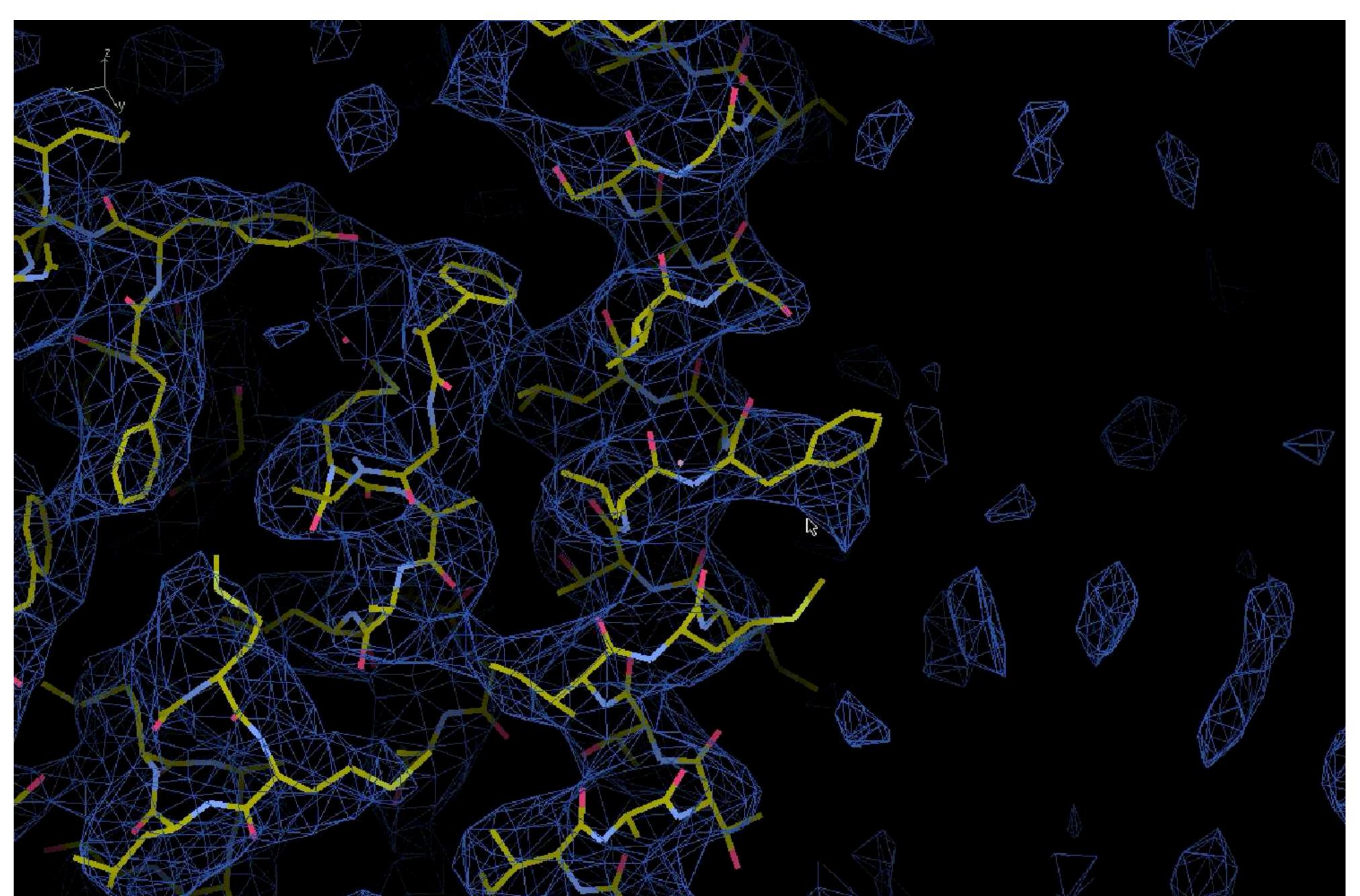
# **N**ap (calculated from model)

# Map (from experiment)

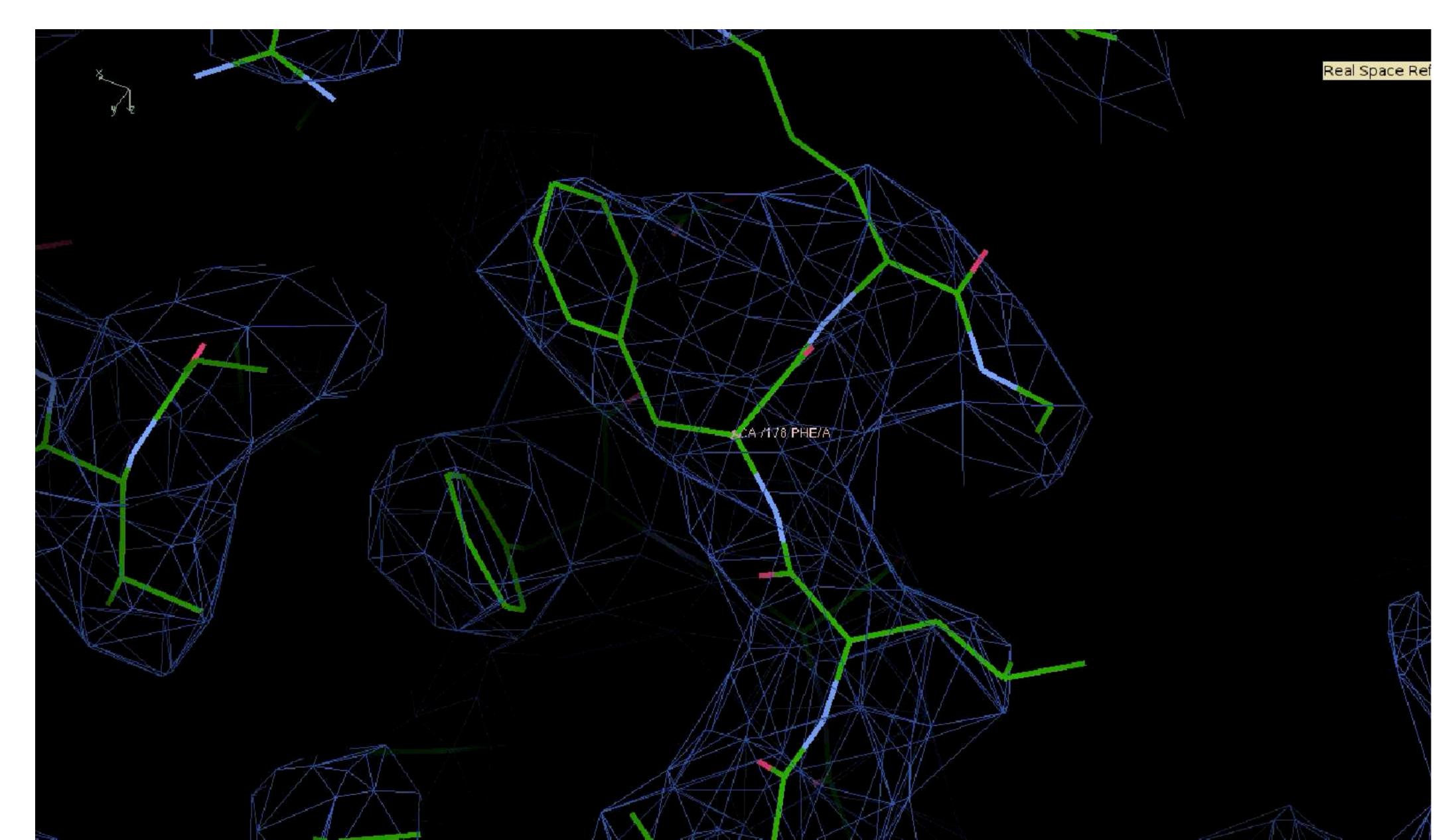
#### At atomic resolution, position of individual atoms is well-defined

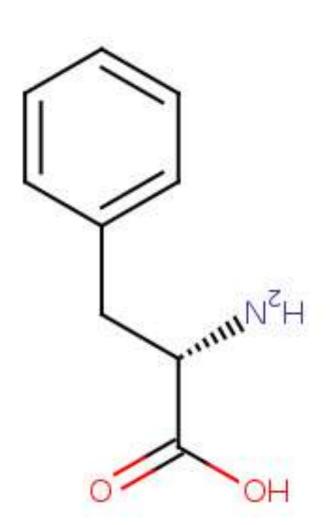


#### But at "near-atomic" resolution, the position of residues and side chains is not always clear

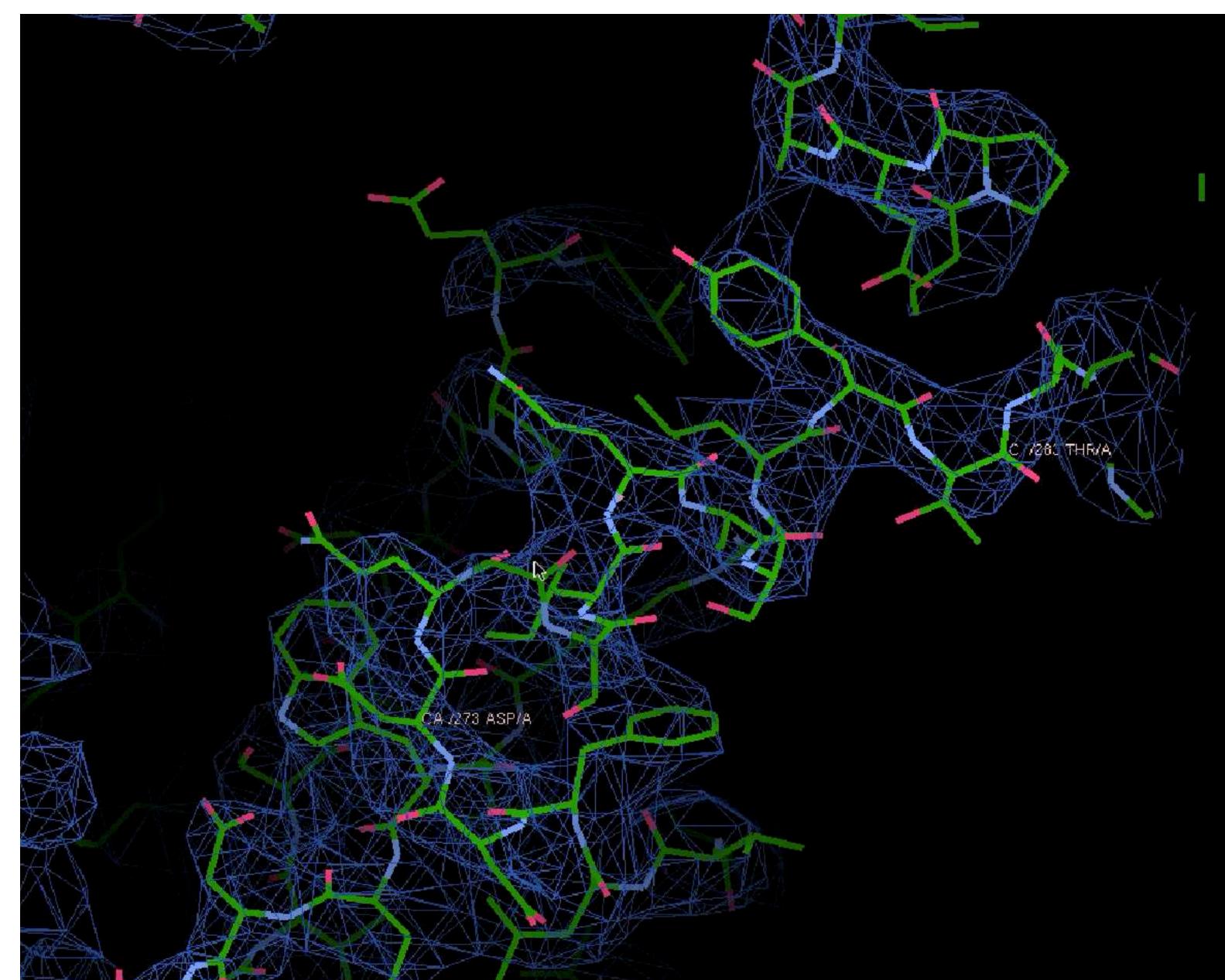






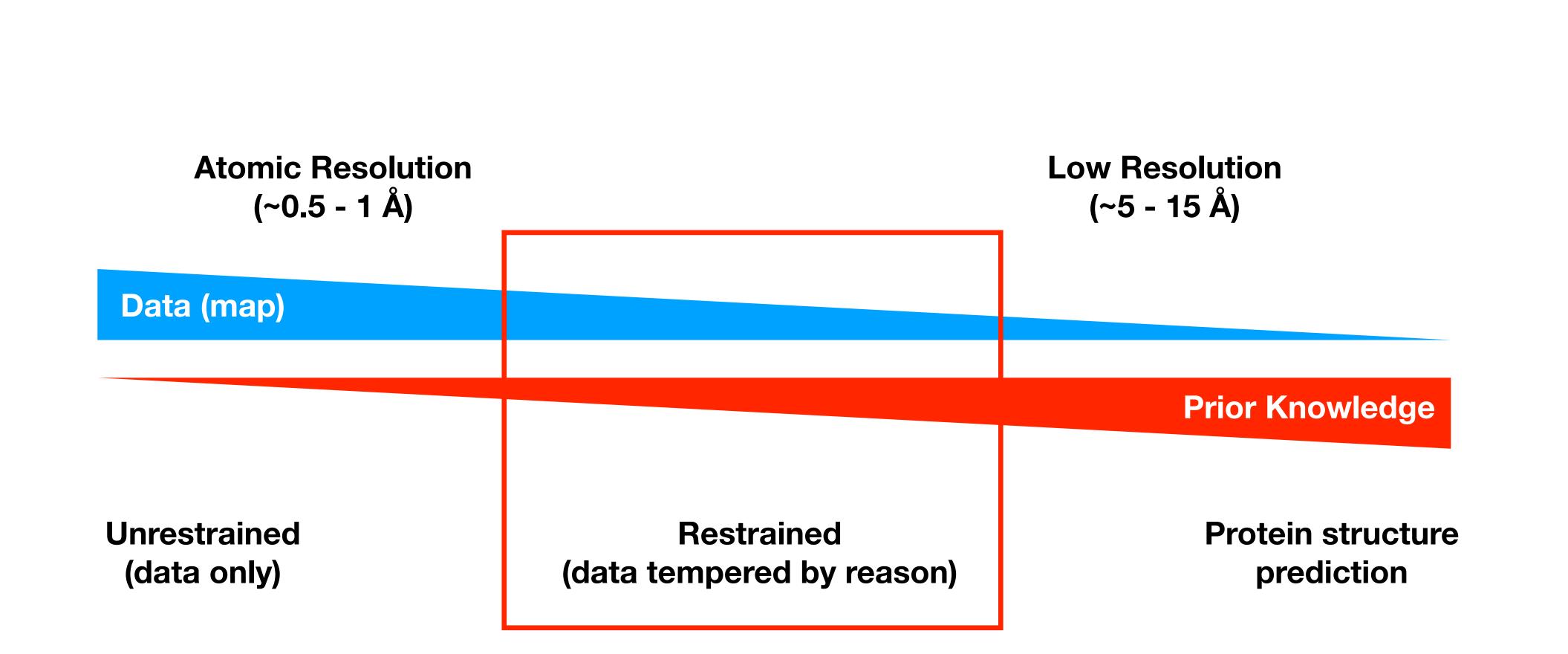


#### Refinement using only data



#### Refinement using <u>only data</u>

#### Harnessing prior knowledge of protein structure to bridge the gap

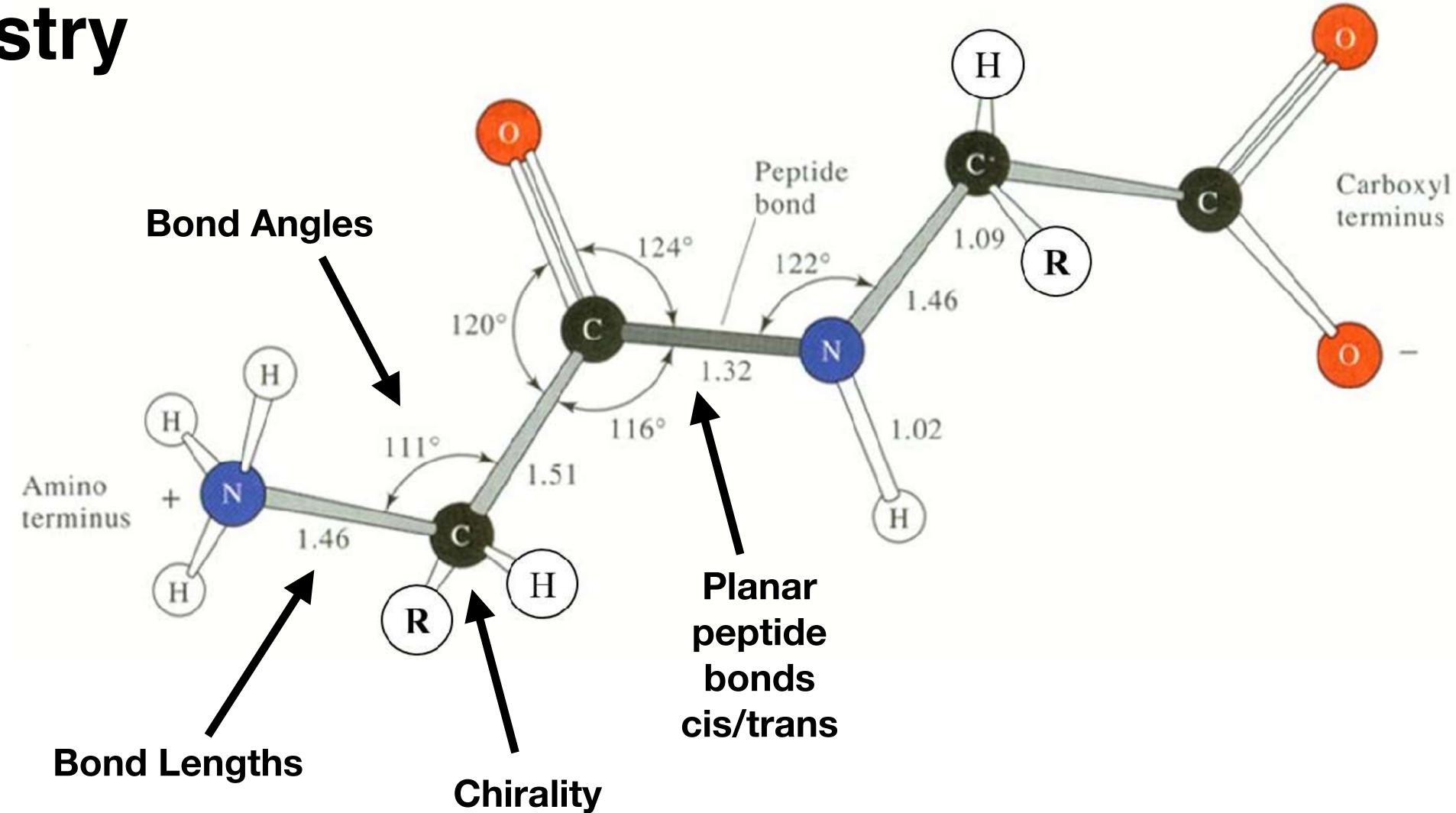


Refinement Target = (Model vs Data) + w<sub>1</sub> (Model vs PriorKnowledge)

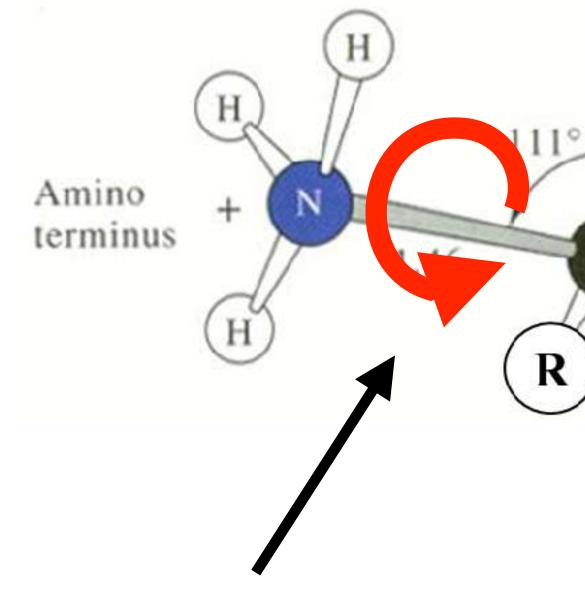




#### Stereochemistry

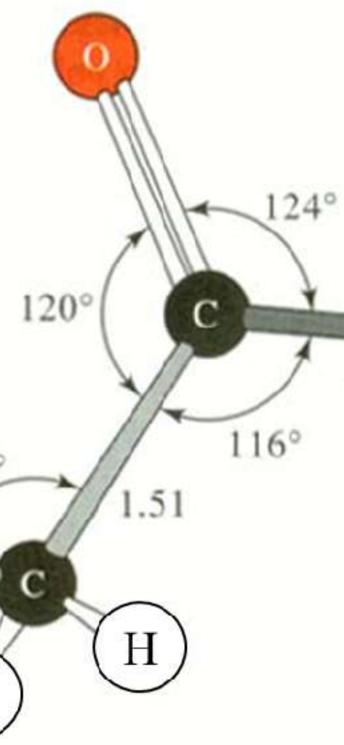


#### Stereochemistry

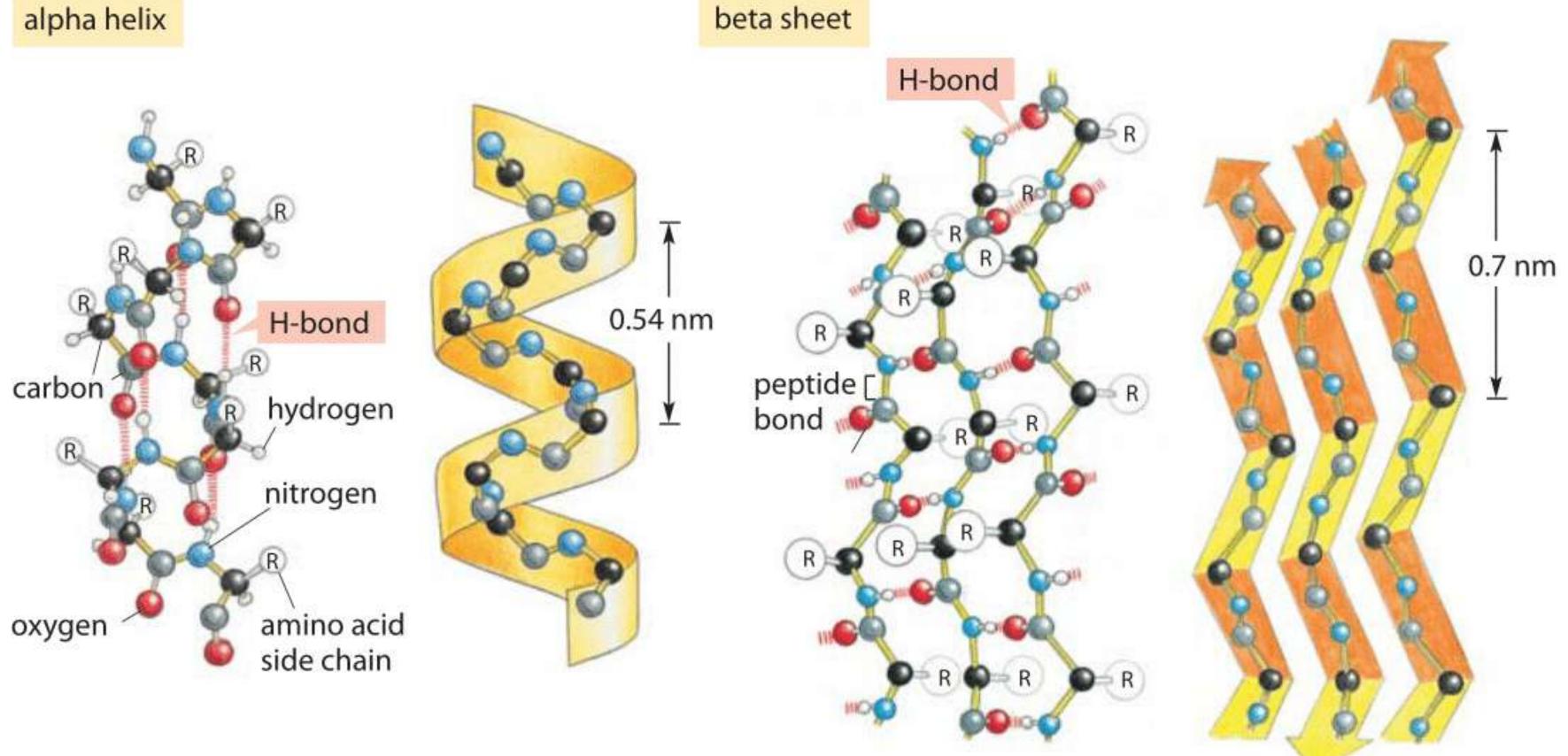


**Torsion/dihedral Angles** 

# Constraints backbone conformations as well as side chain rotameric states



#### **Secondary Structure and Hydrogen Bonds**



#### $\bullet$ **Distance restraints between H-bonding atoms**

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

**Torsion angle restraints to maintain appropriate backbone conformation** 

#### "Non-crystallographic symmetry" (NCS), reference model

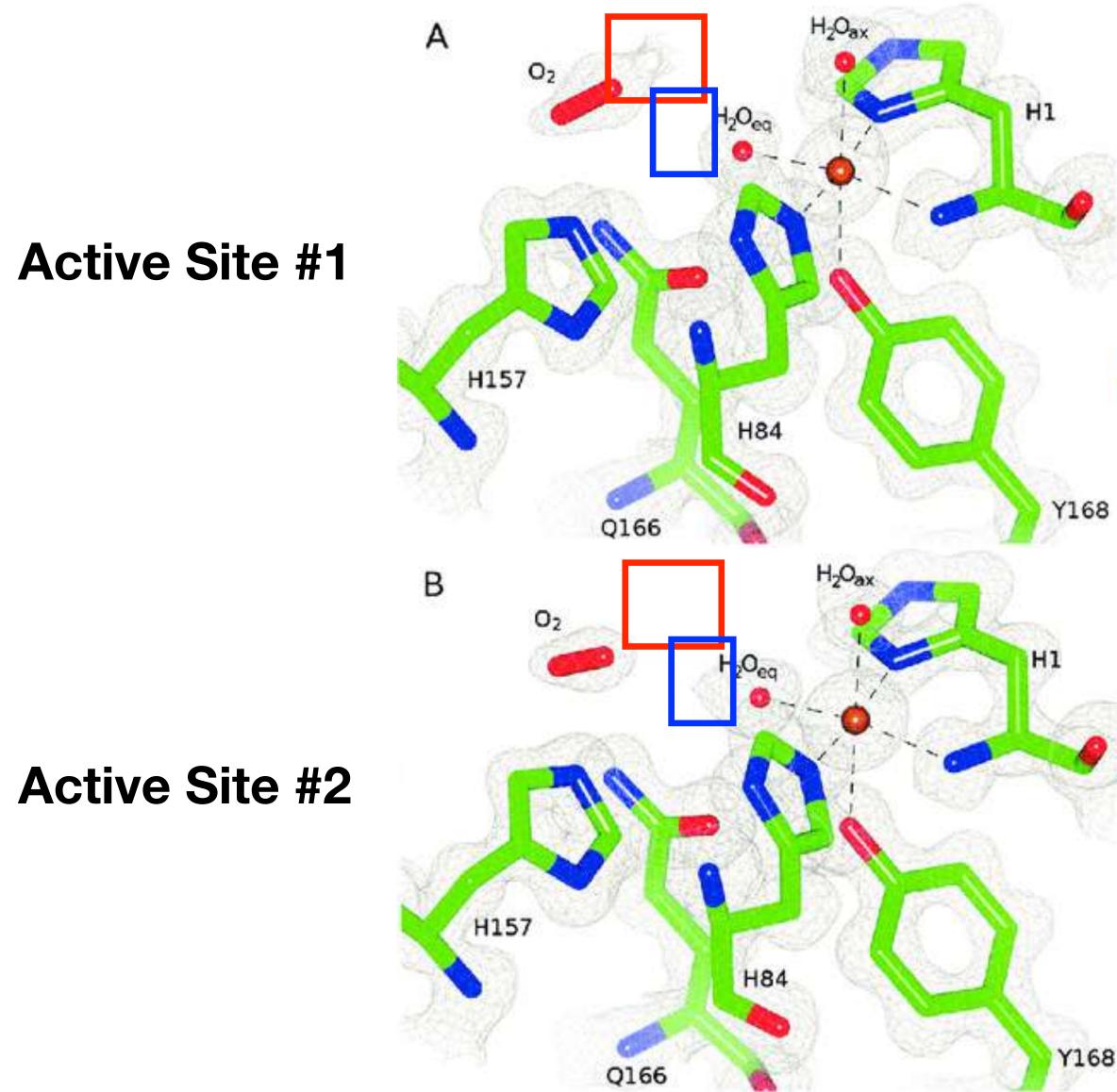


Image: O'Dell, et al. Angewandte Chemie (2016).

#### **Restrain to be similar Constrain to be identical**

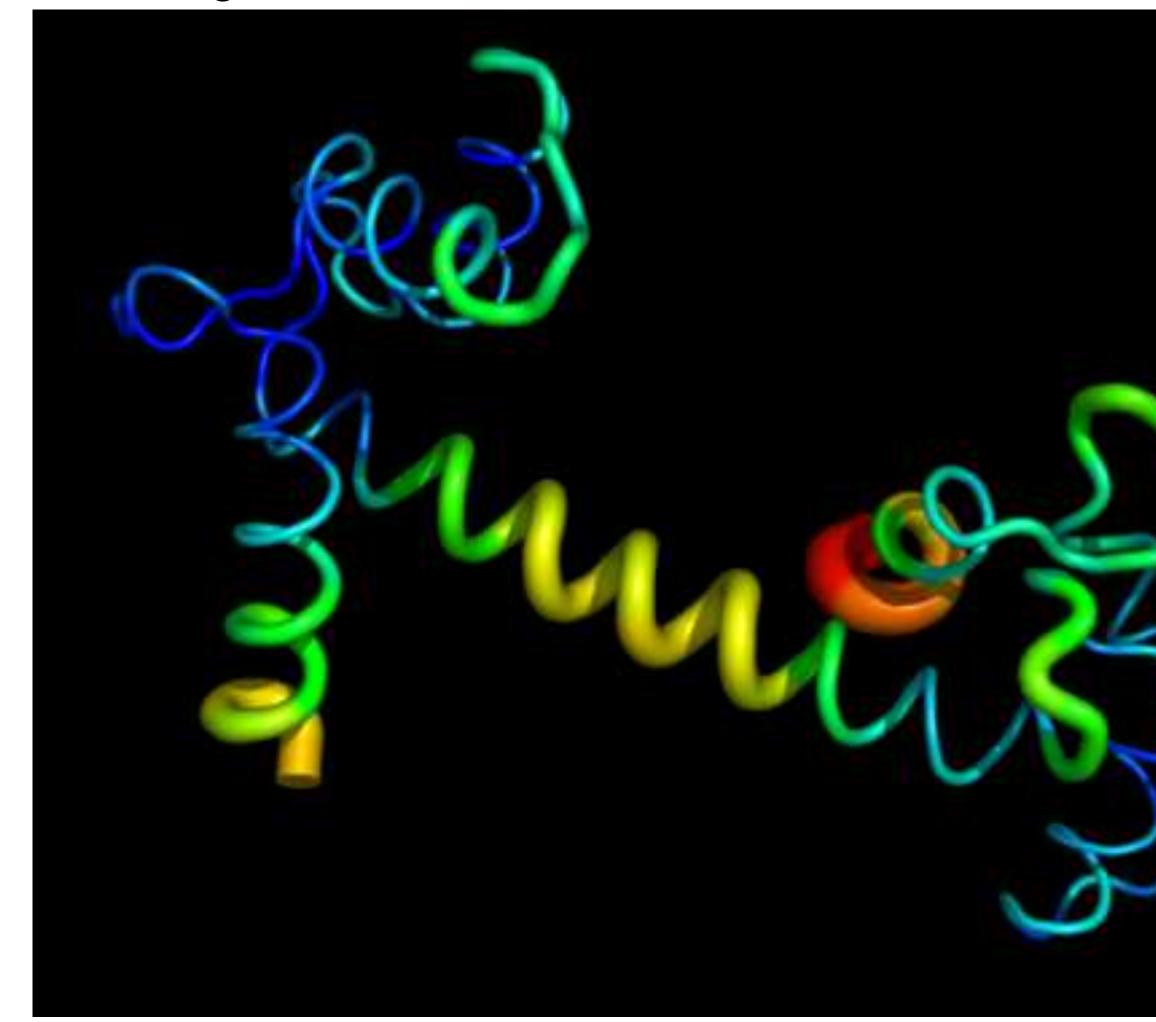
#### **Especially helpful at** lower resolution with non-symmetrized maps

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



#### **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



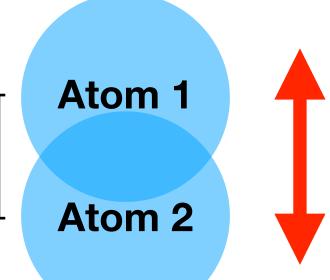
#### Atom 1

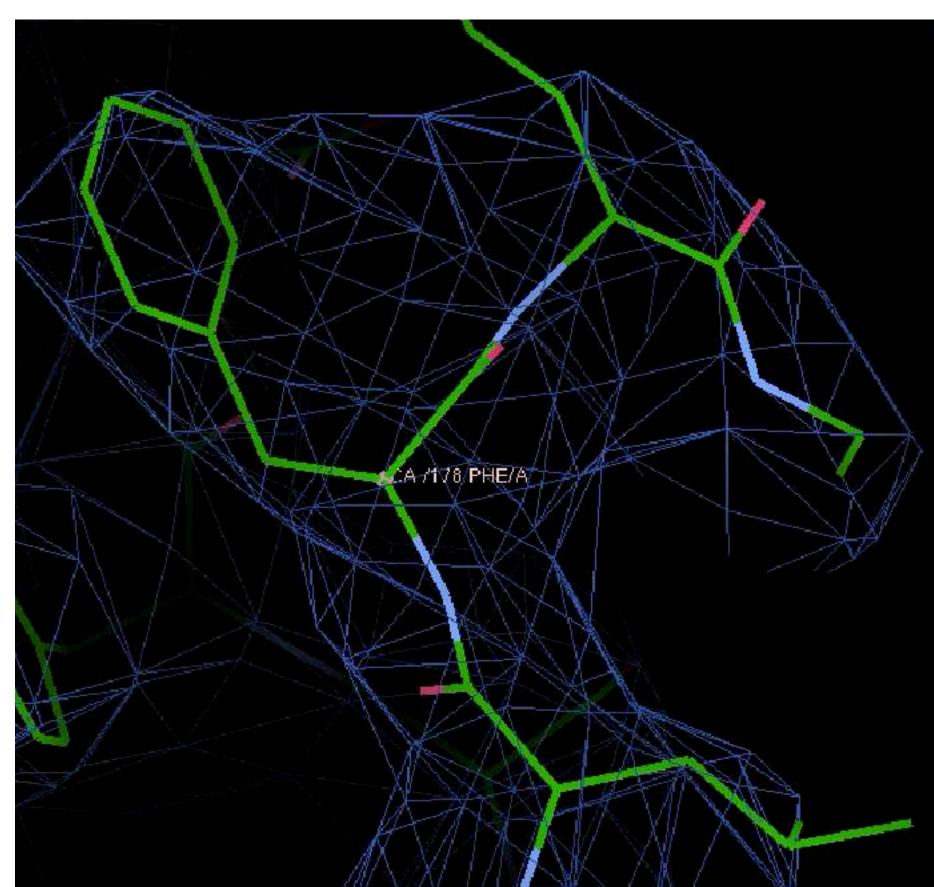
Atom 2

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

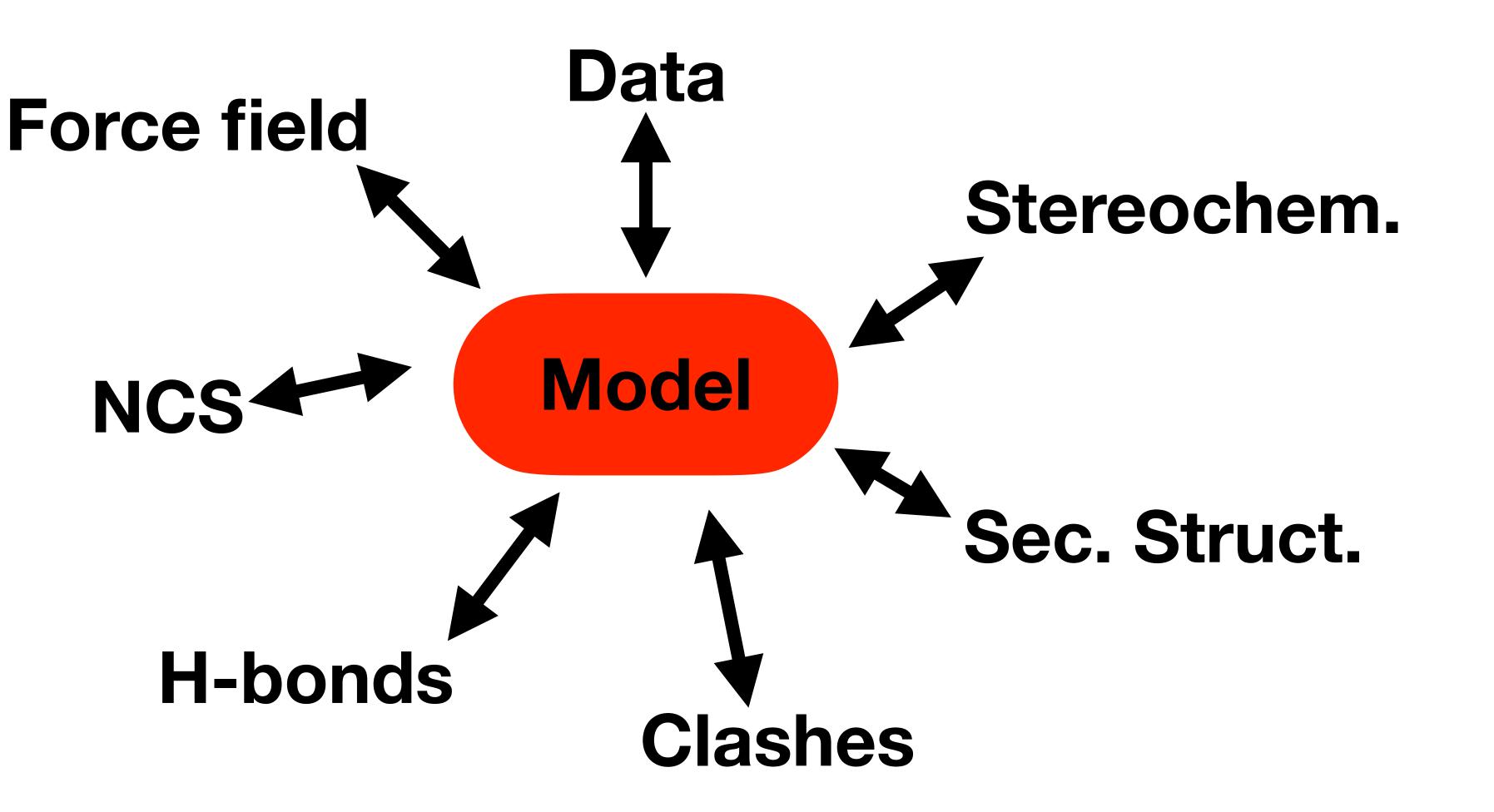
If atoms get too close together, need a force to push them apart

#### Steric repulsion





#### More complete refinement target includes many terms



Refinement Target = (Model vs Data) + w<sub>1</sub>(Model vs Stereo) + w<sub>2</sub>(Model vs ForceField) + w<sub>3</sub>(Model vs NCS) + ...

#### Refinement moves model towards local minimum

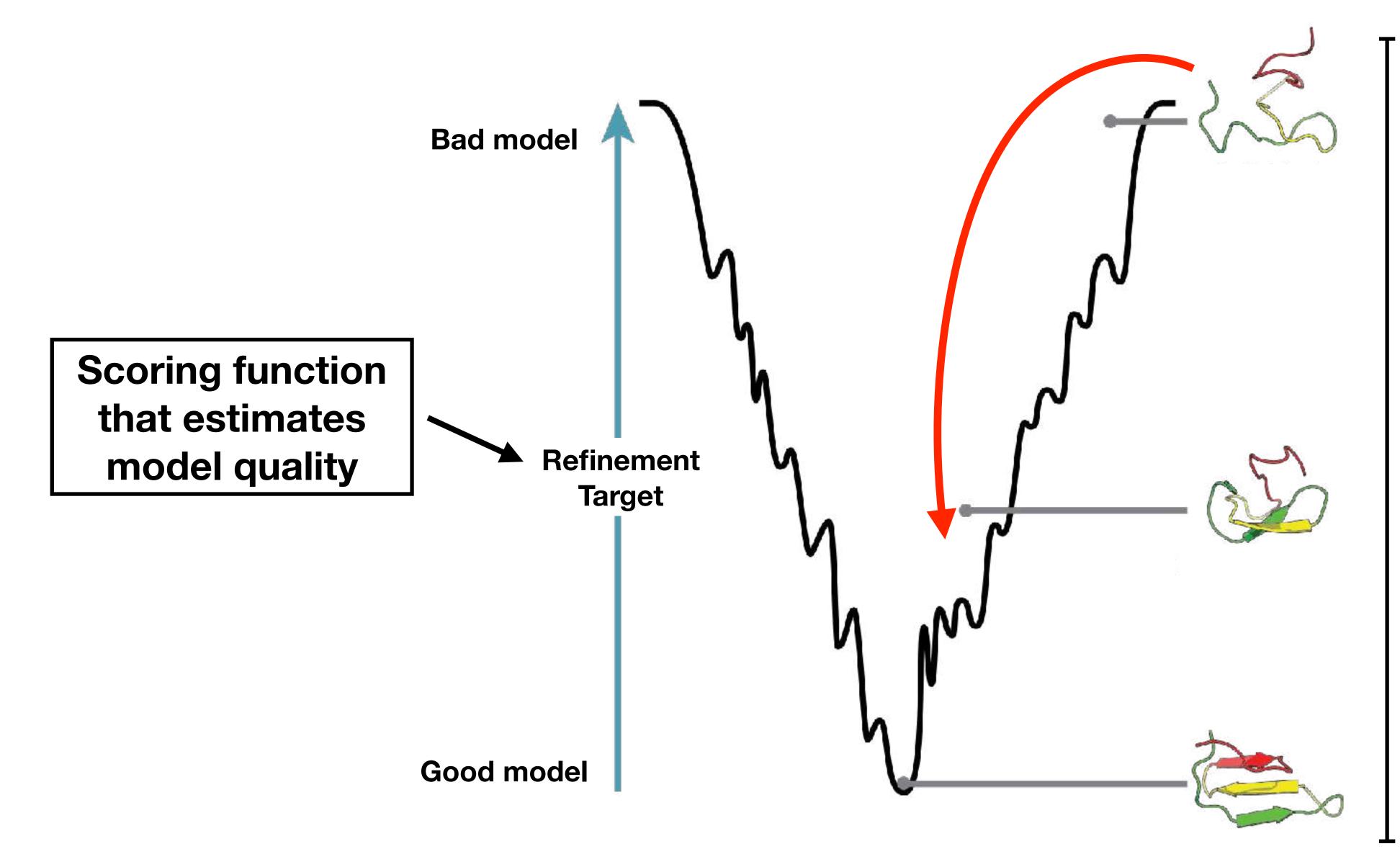


Image (adapted): Thomas Splettstoesser

**Different model parameters** (e.g., conformations)

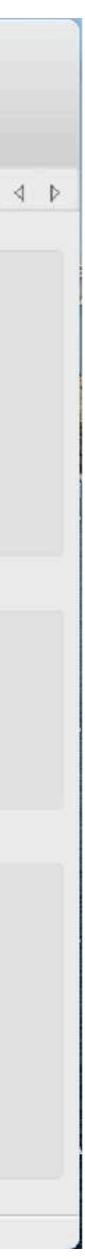


#### There are many refinement options to choose from!

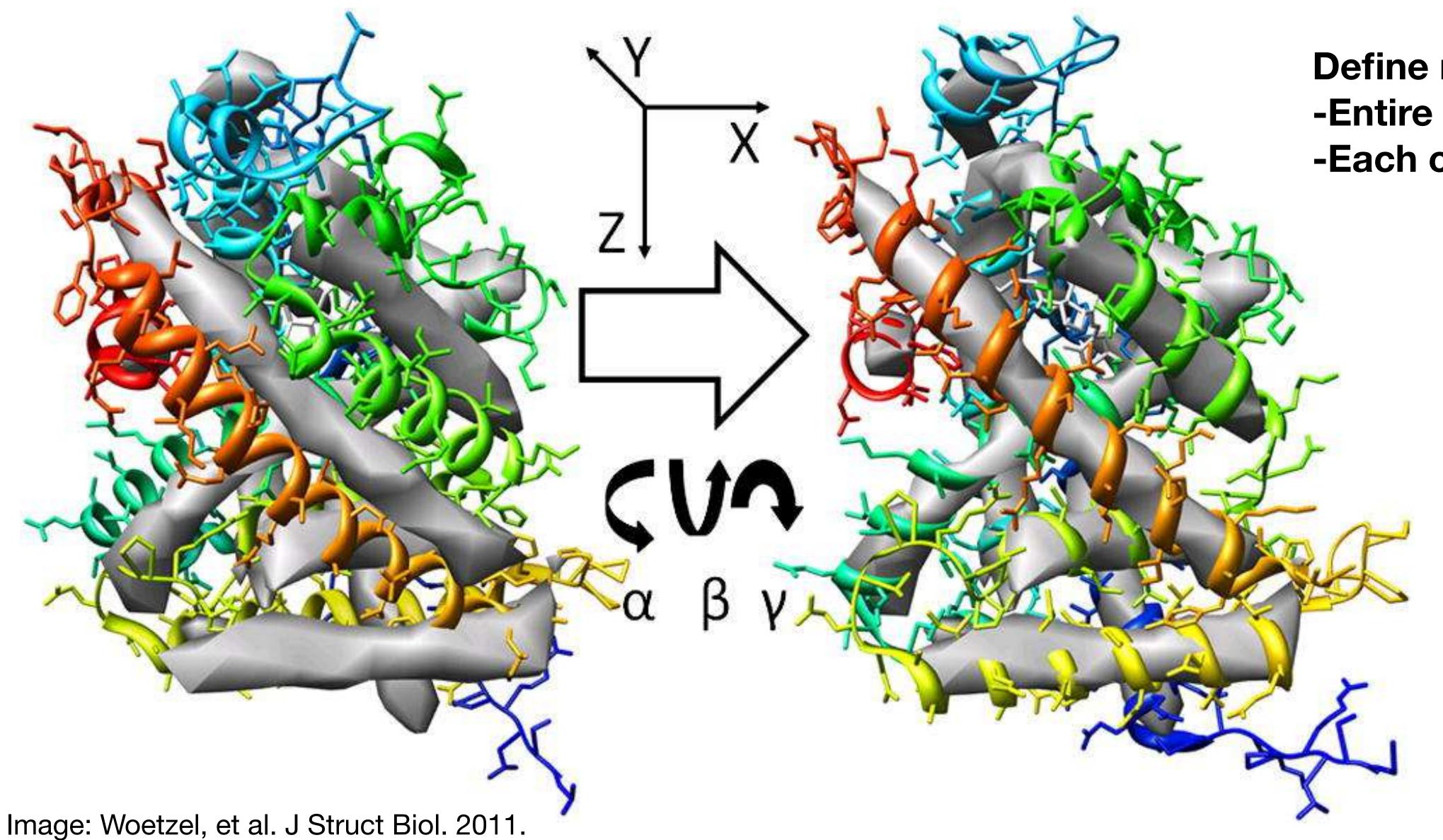
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morphing imulated_annealing 🗹 adp							
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Select Atoms 🛛 🔽 Use secondary structure restraints 🔂 Use NCS							
Strategy Options							
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Simulated annealing : once Options							
Reference model restraints Options							
Other Options							
Scattering table : electron 🔇 Weight : Resolution factor : 0.25							
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Refine ncs operators Show per residue							
Model interpretation Automatic linking All parameters							

#### phenix.real\_space\_refine

😑 1 job(s) running



#### **Optimization protocols: Rigid body refinement**

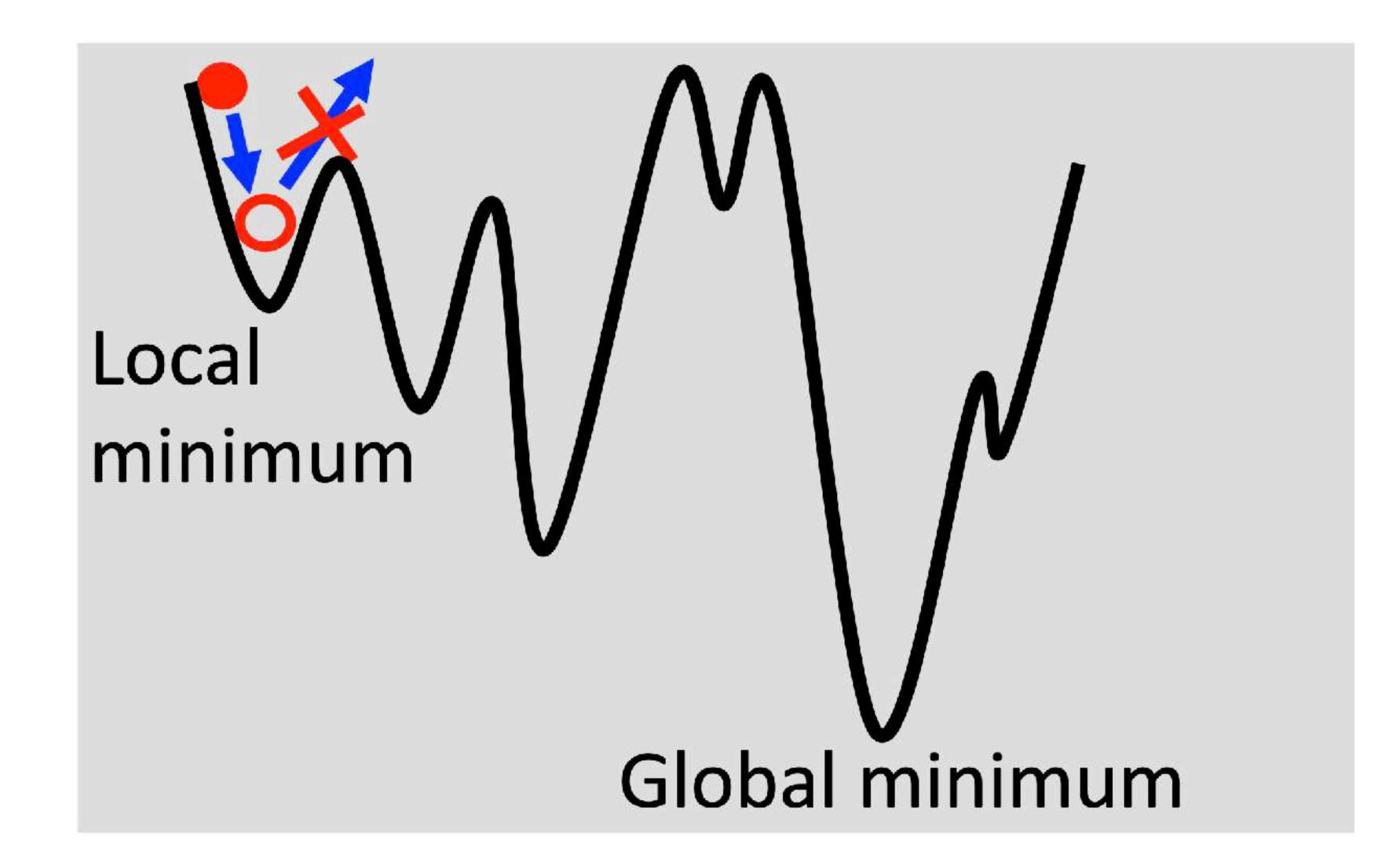


**Define rigid bodies:** -Entire model?

-Each chain separately

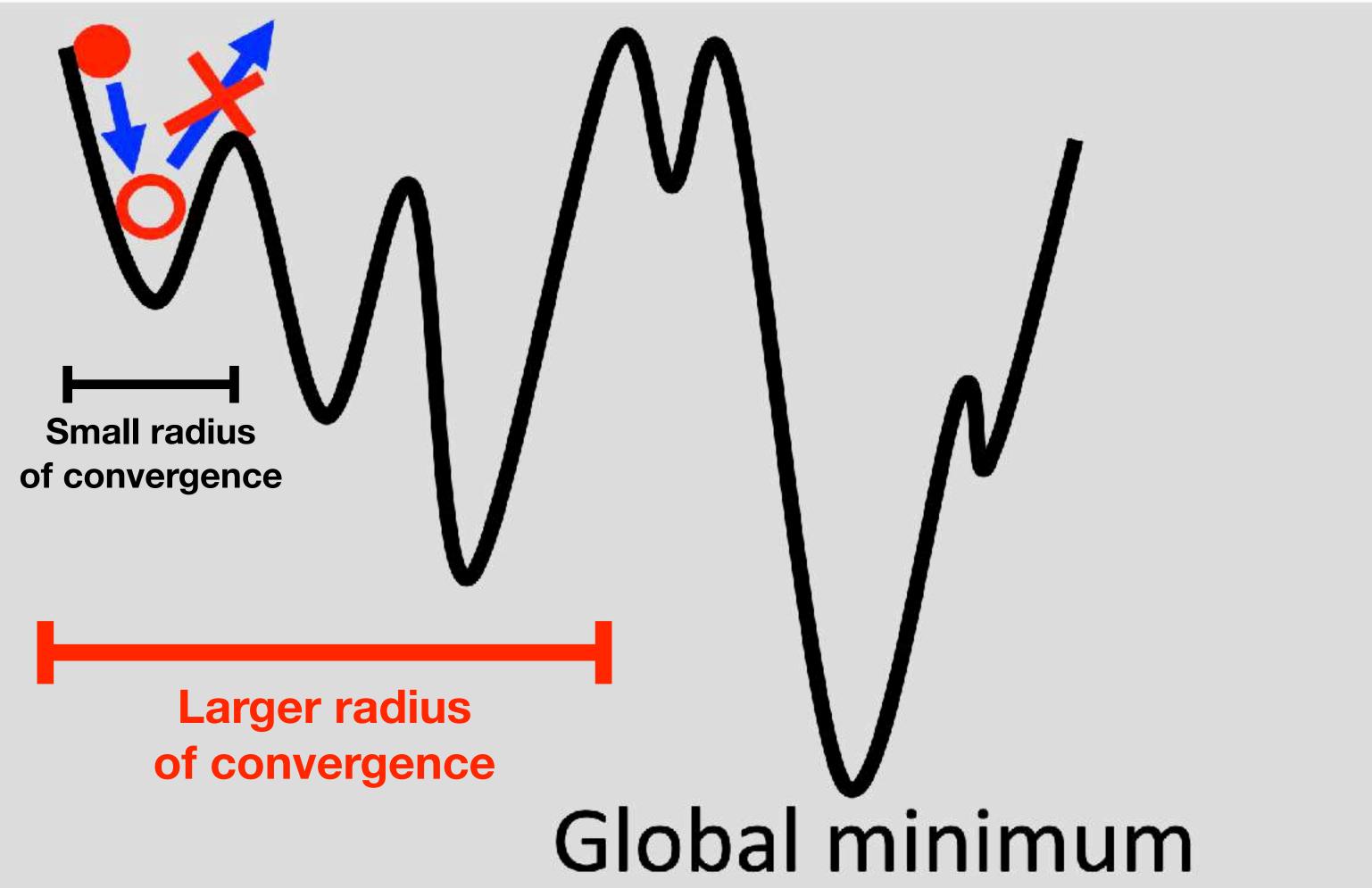


#### **Optimization protocols: Gradient driven minimization**

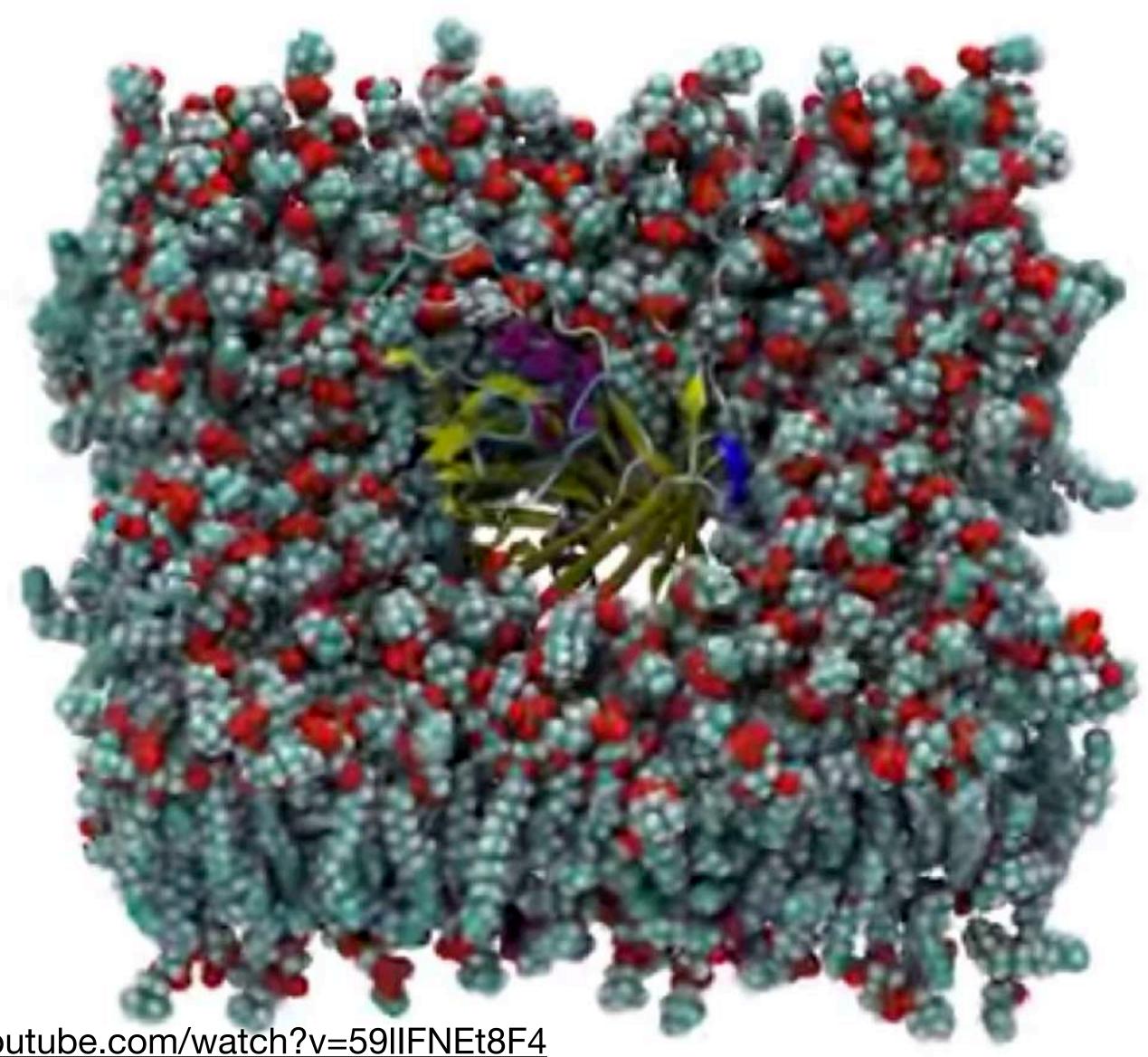


Slide adapted from: Pavel Afonine, LBNL (Phenix)

#### Refinement "radius of convergence"

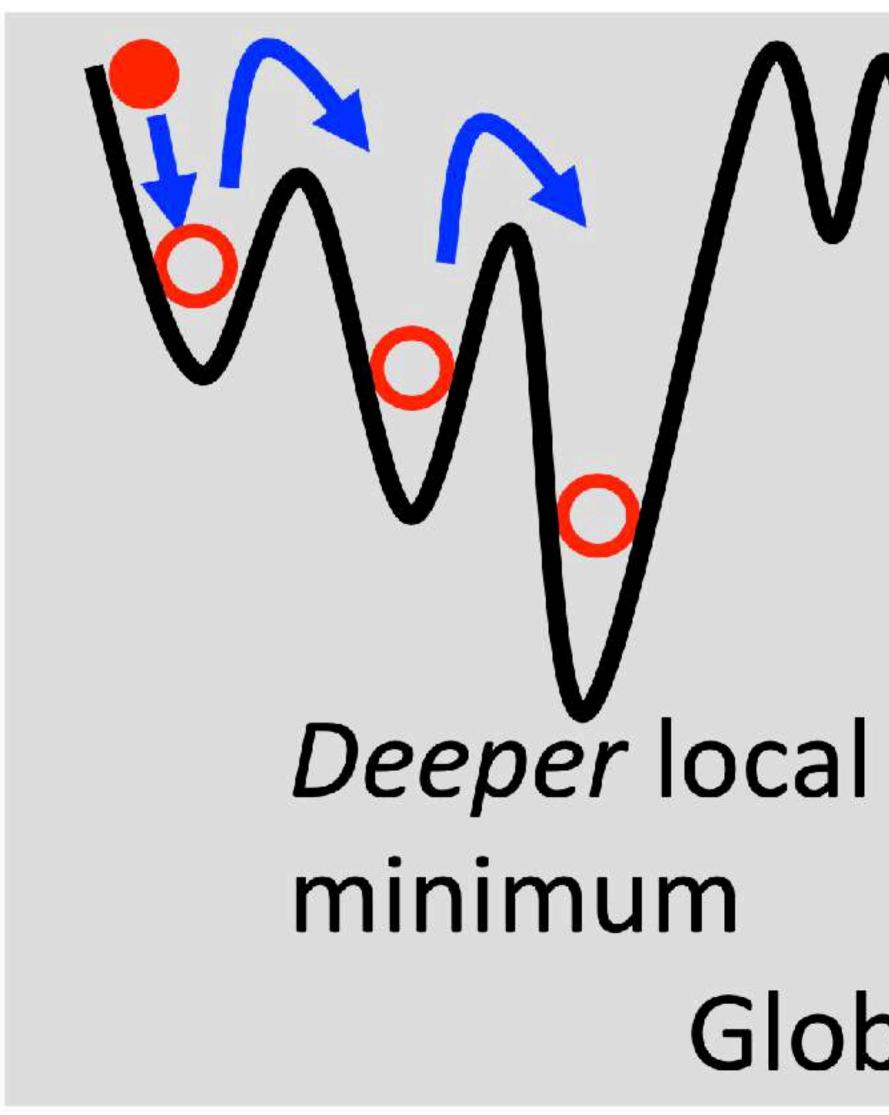


#### **Optimization protocols: Simulated annealing**



Video: Darrell Hurt, <u>https://www.youtube.com/watch?v=59IIFNEt8F4</u>

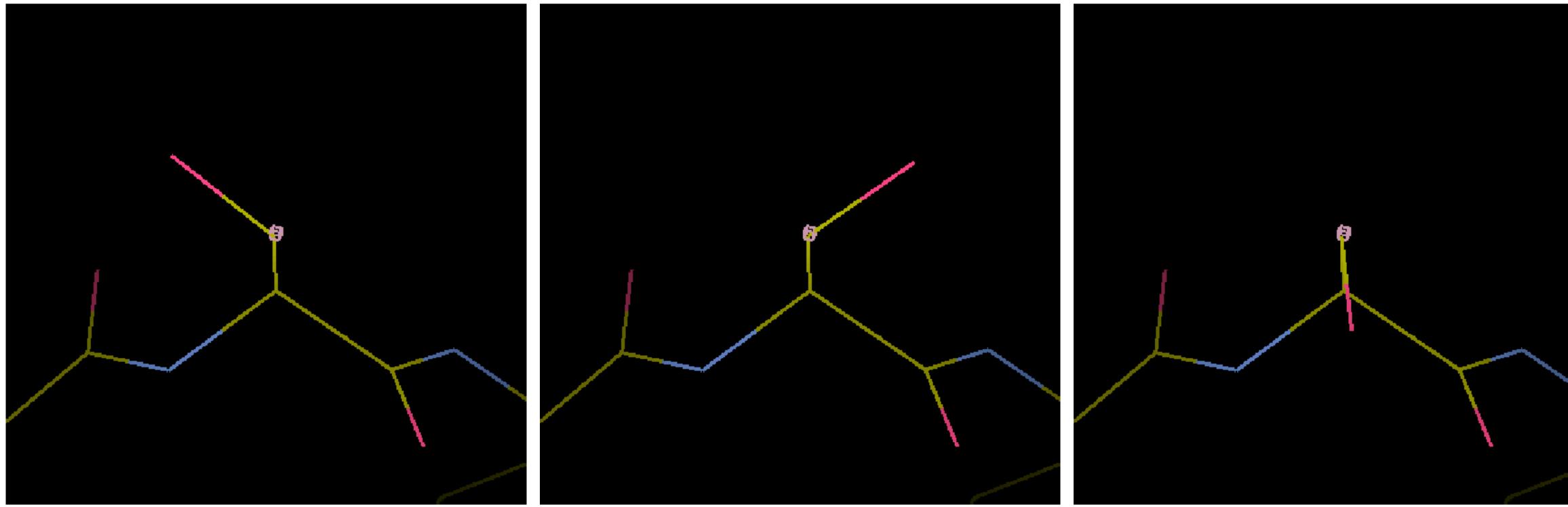
#### **Optimization protocols: Simulated annealing**



Slide adapted from: Pavel Afonine, LBNL (Phenix)

# Global minimum

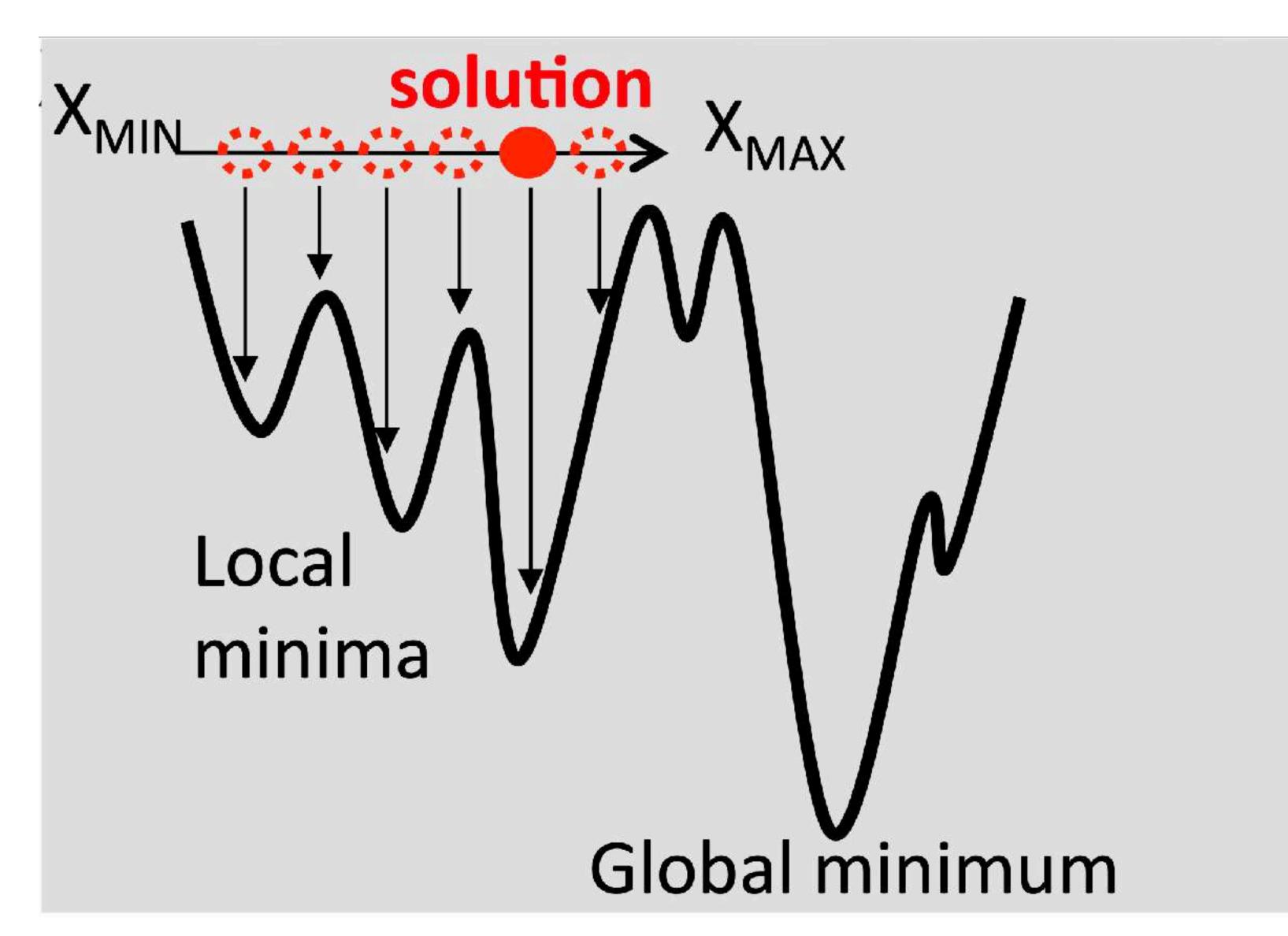
#### **Optimization protocols: Torsion-angle Grid Search**



#### Can allow for larger shifts in model than simple minimization

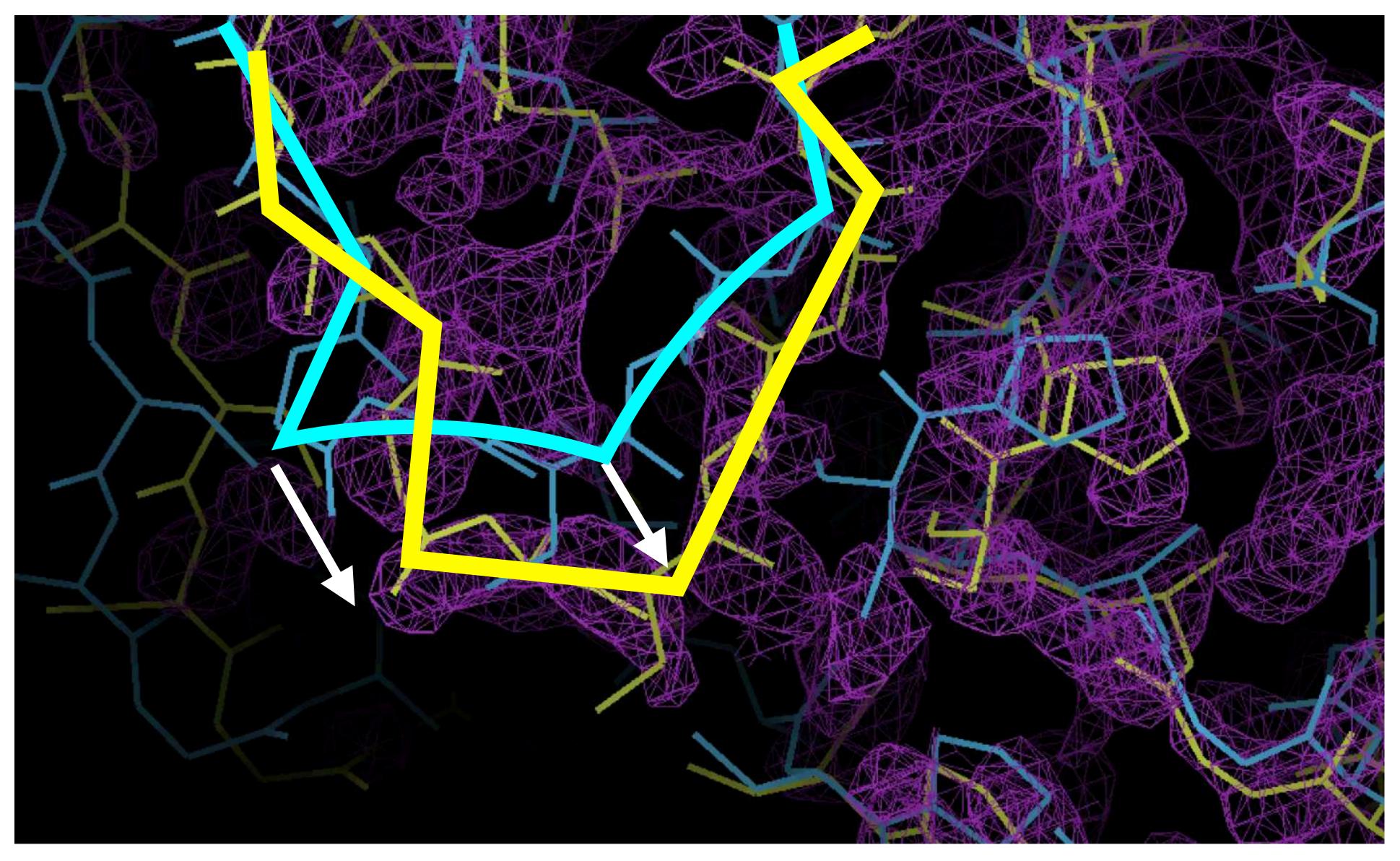


#### **Optimization protocols: Torsion-angle Grid Search**



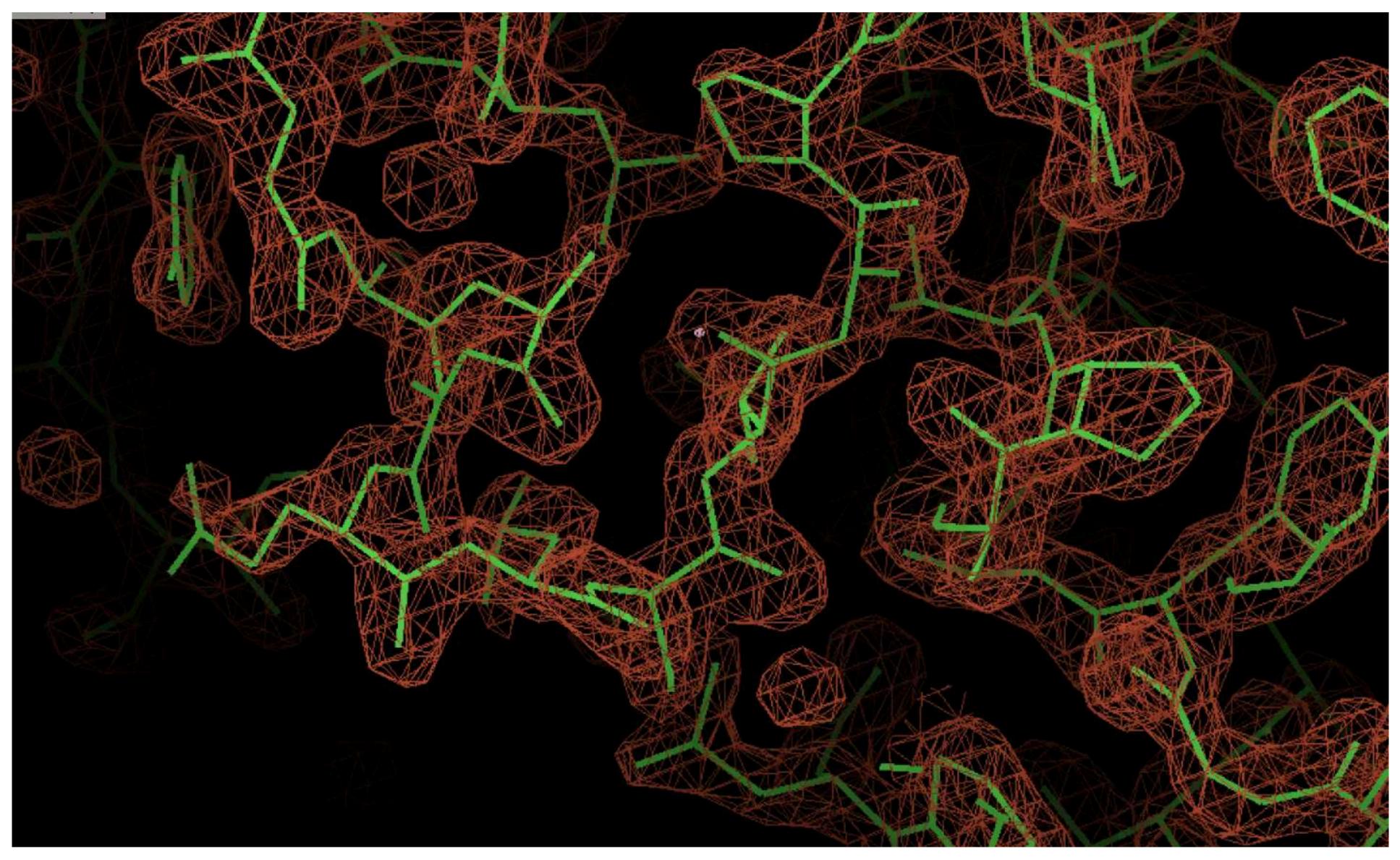
Slide adapted from: Pavel Afonine, LBNL (Phenix)

# **Optimization protocols: Morphing**



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

## **Optimization protocols: Morphing**



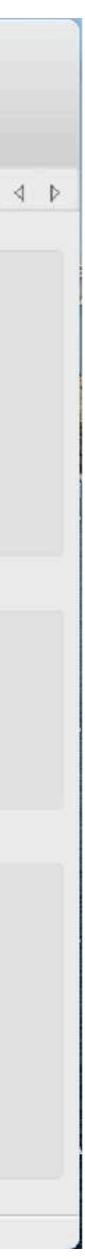
Slide adapted from: Tom Terwilliger, Los Alamos (Phenix)

#### There are many refinement options to choose from!

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Refine ncs operators Show per residue							
Model interpretation Automatic linking All parameters							

#### phenix.real\_space\_refine

😑 1 job(s) running



# <u>What parameters should I use?</u>

- 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?

annealing)

(rigid body, minimization\_global)

- More aggressive = larger radius of convergence, potentially less manual rebuilding; but changes the model a lot (morphing, grid search, simulated
- Less aggressive = smaller radius of convergence, will change model less

#### 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(?)

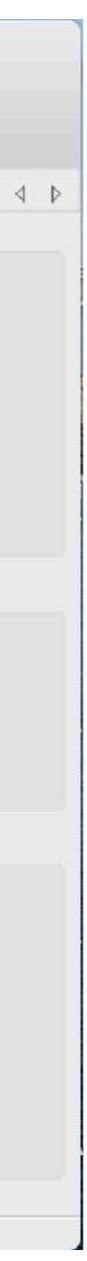
Late stages:

-Go easy: minimization\_global, adp

#### Restraints

#### <u>https://phenix-</u> <u>online.org/</u> documentation/ <u>reference/</u> real space refine.html

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# 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 3) To assess overall and local model quality/reliability

- 2) To identify problem areas requiring manual intervention

"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

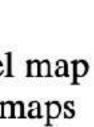
- Model building problem
  - Unmodeled densities
  - Cbeta deviations
  - Ramachandran plot
  - Cis peptide bonds
  - Model/Map CC
- Refinement problem
  - RMS deviations
  - B factor / ADP outliers
- Either/both?
  - Molprobity score and clash score
  - Rotamer outliers

## Model/Map CC

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

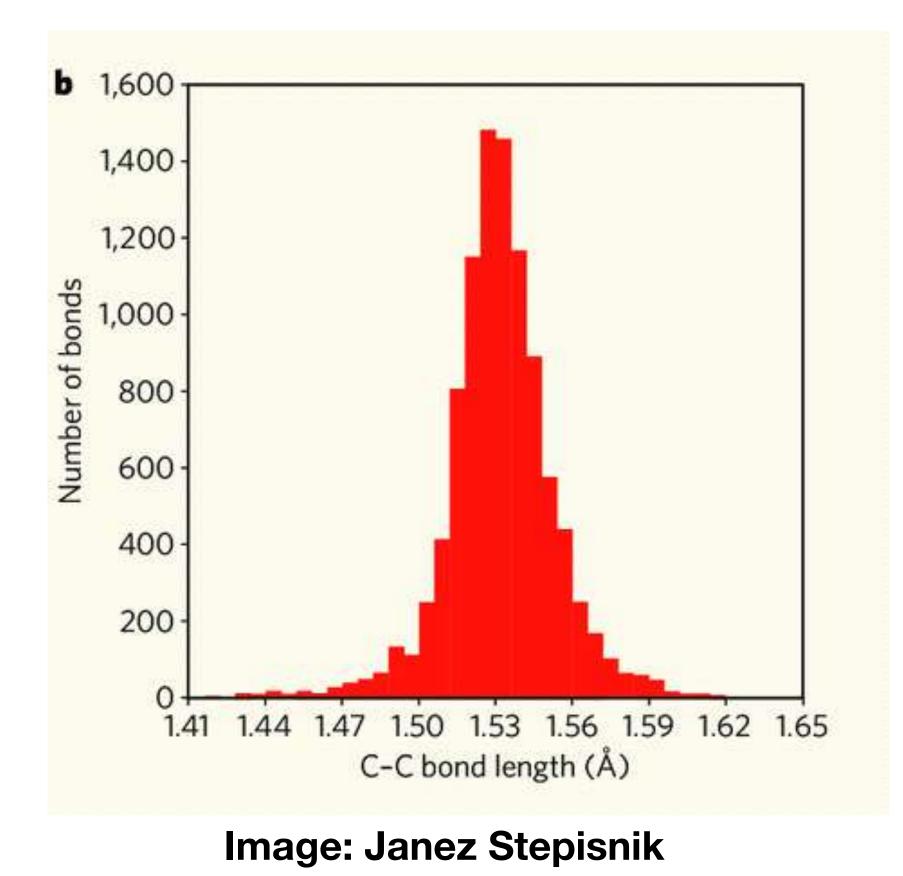
Metric	Region of the map used in calculation		
CC <sub>box</sub>	Whole map		
CC <sub>mask</sub>	Jiang & Brünger (1994) mask with a fixed radius		
CC <sub>volume</sub>	Mask of points with the highest values in the mod		
CCpeaks	Mask of points with the highest values in the model target maps		
CC <sub>vr_mask</sub>	Same as $CC_{mask}$ but atomic radii are variable and resolution, atom type and ADP		

	Purpose		
	Similarity of maps		
	Fit of the atomic centers		
del map	Fit of the molecular envelope defined by the model		
el and in the	Fit of the strongest peaks in the model and target r		
function of	Fit of the atomic images in the given map		



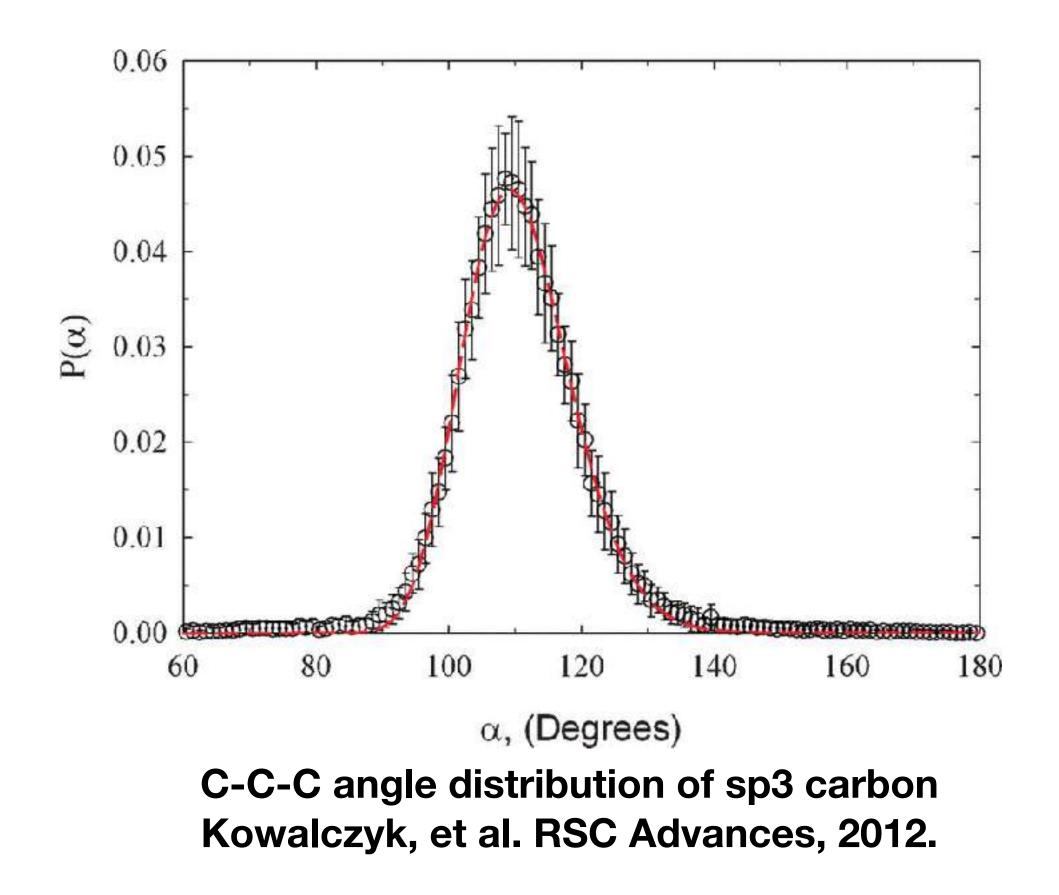
### Root mean square (RMS) deviations

**Typical RMS Bonds for protein structure:** 0.005 - 0.015 Å



#### **Covalent bond lengths and angles exhibit known, narrow distributions**

#### **Typical RMS Angles for protein structure:** 0.5 - 1.5 Å



# **Unmodeled densities**

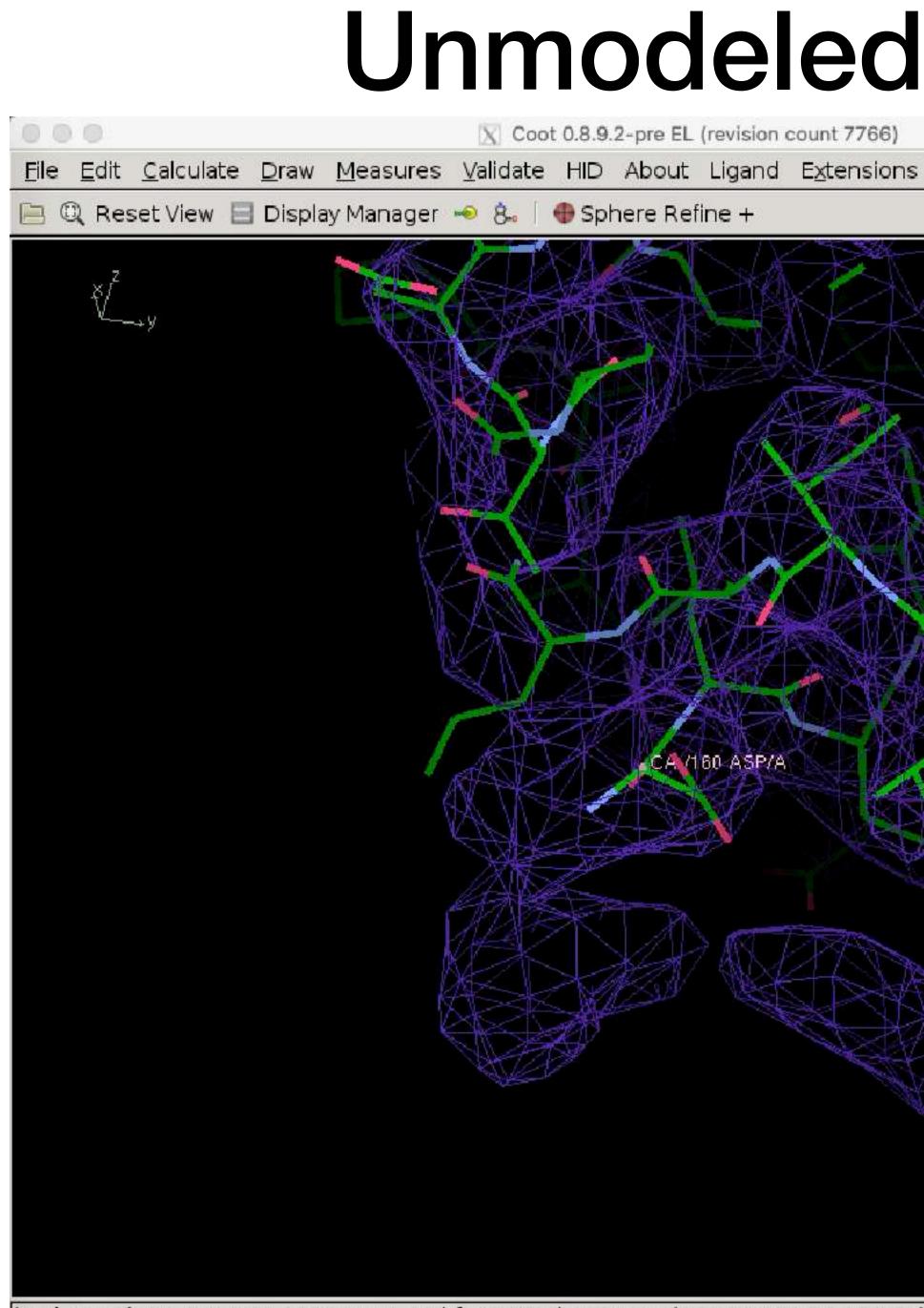
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## **Unmodeled densities**

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#### **Unmodeled densities**

Unmodelled blobs of density: There are unexplained blobs of density (too big to be waters): Blob 1 Blob 2 Blob 3 Blob 4 Blob 5 Blob 6 Blob 7 Blob 8 Blob 9 Blob 10 Blob 11 Blob 12 P. Blob 13 Blob 14 Blob 15 Blob 16 Blob 17 Blob 18 Blob 19 Blob 20 Blob 21

Dismiss

# Molprobity score and clash score

#### **Summary statistics**

All-Atom	Clashscore, all atoms:	3.82		96 <sup>th</sup> percentile <sup>*</sup> (N=1784, all resolutions)		
Contacts	Clashscore is the number of serious st	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.				
	Poor rotamers	12	0.68%	Goal: <0.3%		
	Favored rotamers	1674	95.22%	Goal: >98%		
	Ramachandran outliers	0	0.00%	Goal: <0.05%		
Protein	Ramachandran favored	2070	98.85%	Goal: >98%		
Geometry	MolProbity score^	<b>1</b> .17		99 <sup>th</sup> percentile <sup>*</sup> (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: $\leq 1$ per chain, or $\leq 5\%$		
Low-resolution Criteria	CaBLAM outliers	42	2.0%	Goal: <1.0%		
Low-resolution Criteria	CA Geometry outliers	24	1.15%	Goal: <0.5%		
Additional validations	Pseudochiral naming errors	6				
Additional validations	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.

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

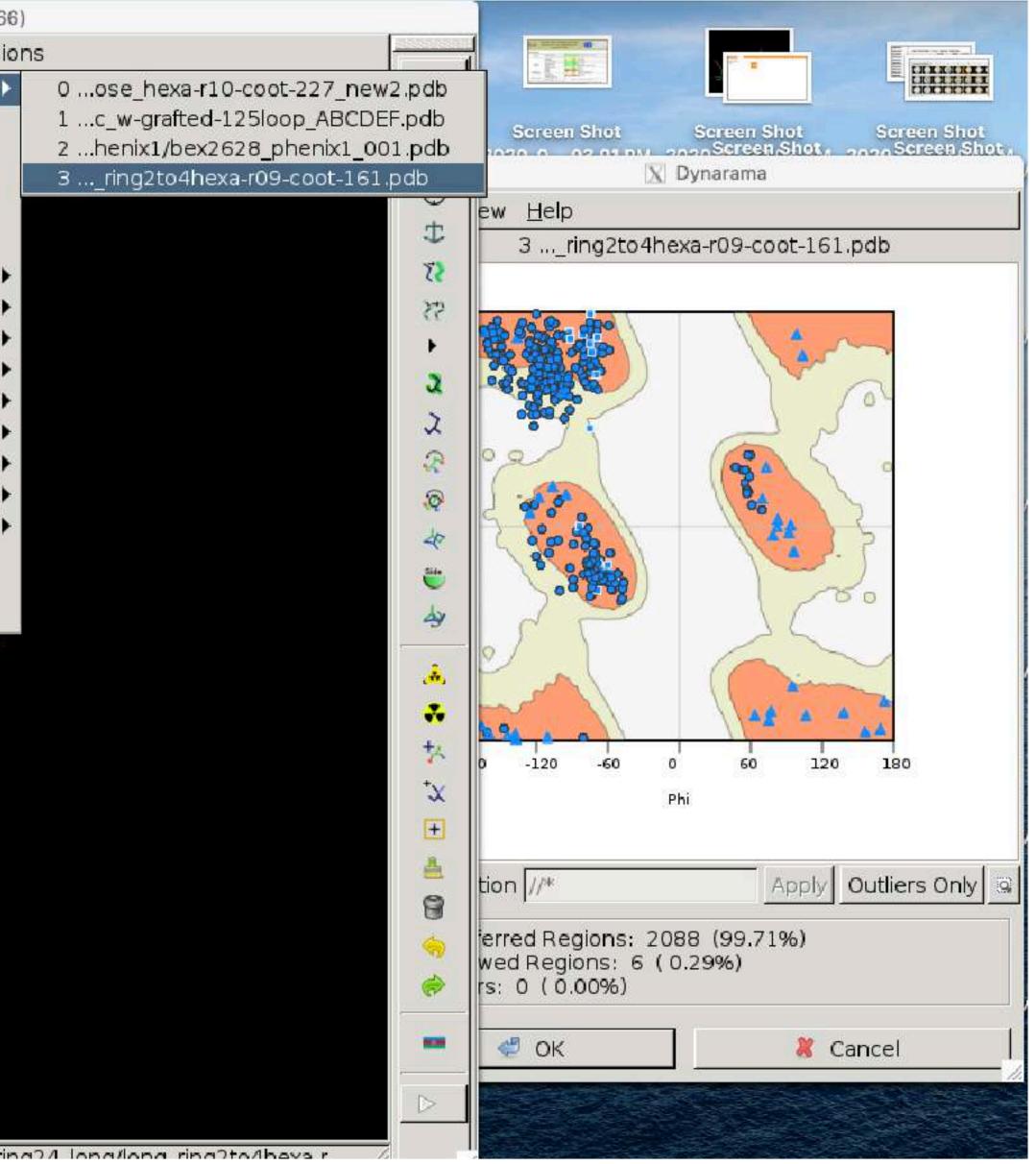
^ MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

#### http://molprobity.biochem.duke.edu/ Williams et al. Protein Science (2018).

## Ramachandran plot

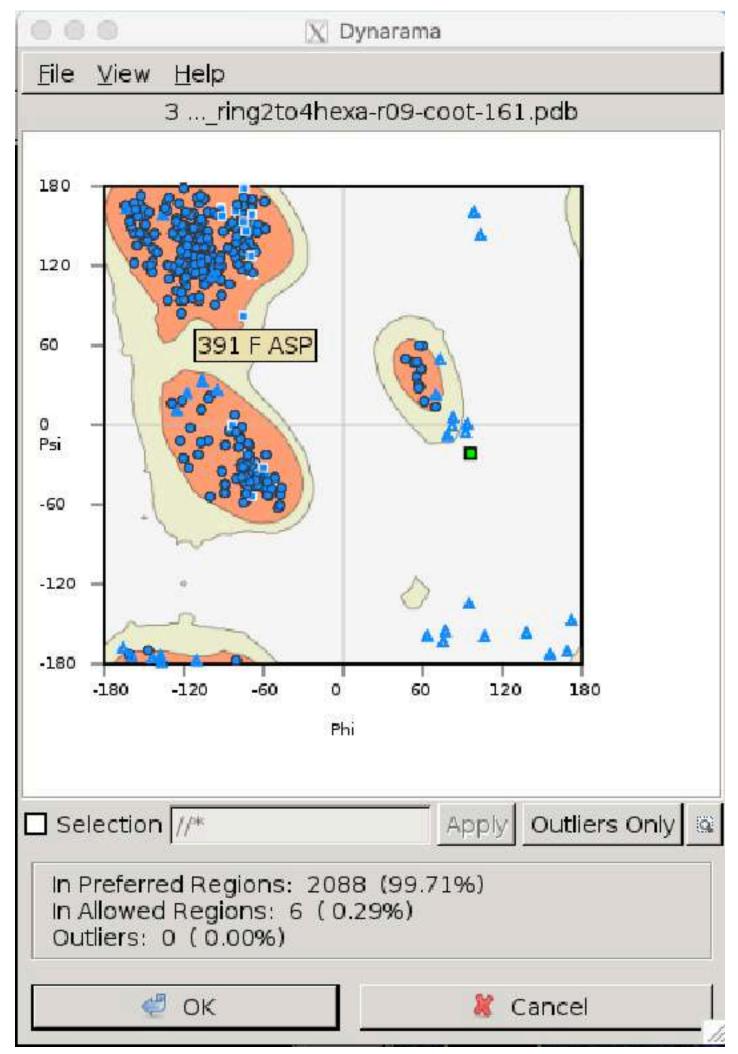
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	<ul> <li>Geometry analysis</li> <li>Peptide omega analysis</li> <li>Temp. fact. variance analysis</li> <li>Average Temp. fact. analysis</li> <li>GLN and ASN B-factor Outliers</li> <li>Rotamer analysis</li> <li>Probe clashes</li> <li>NCS Differences</li> <li>Highly coordinated waters</li> <li>Pukka Puckers?</li> <li>Alignment vs PIR</li> </ul>	* * * * * * * * *

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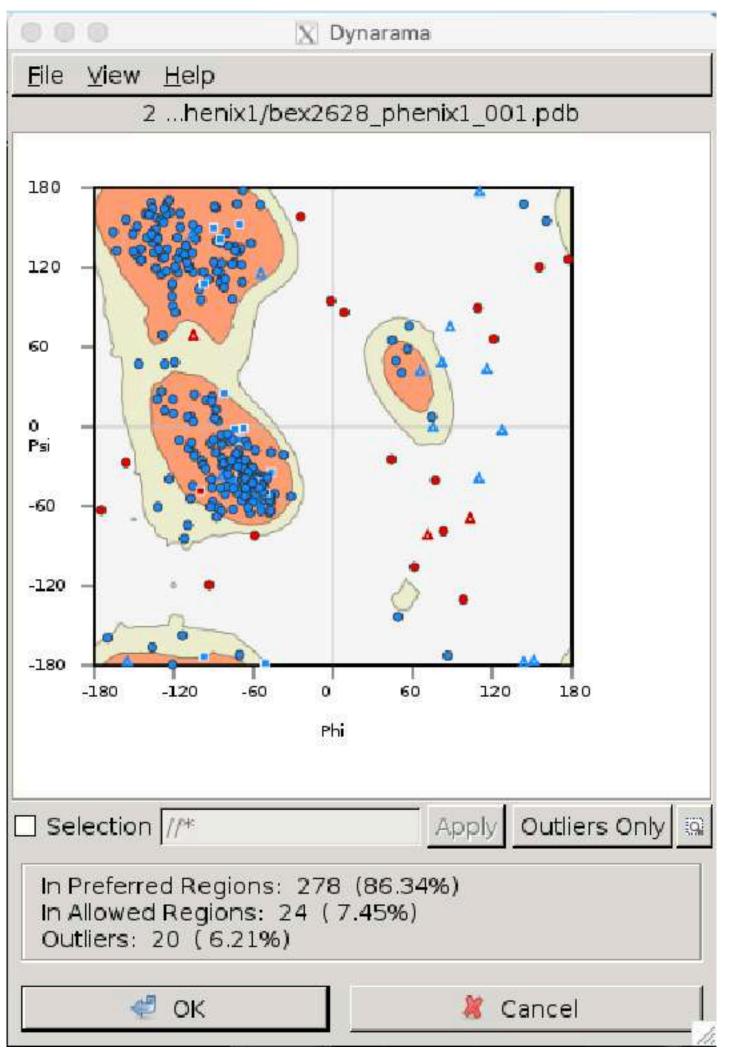


### Ramachandran plot

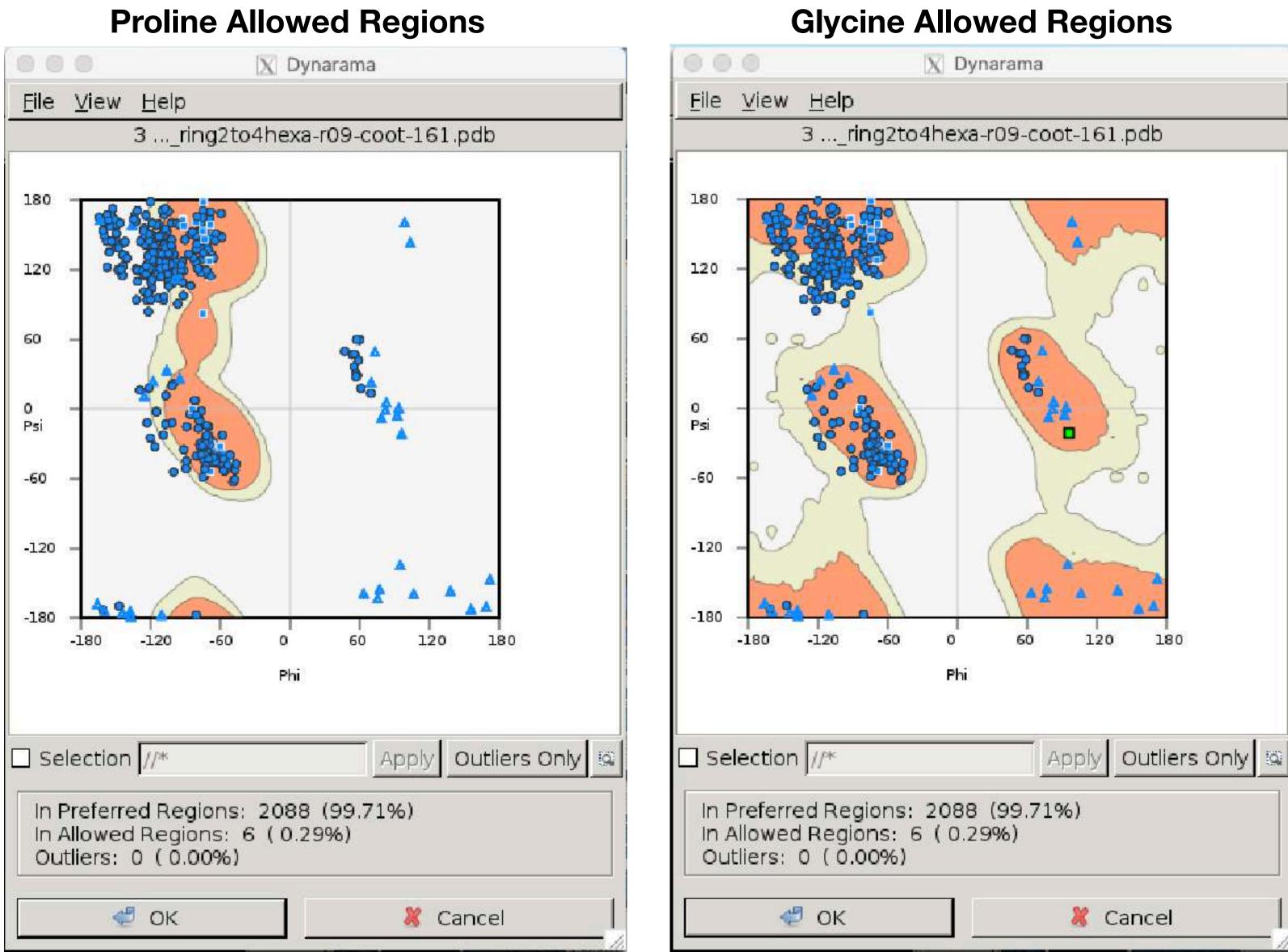
#### Ideal plot



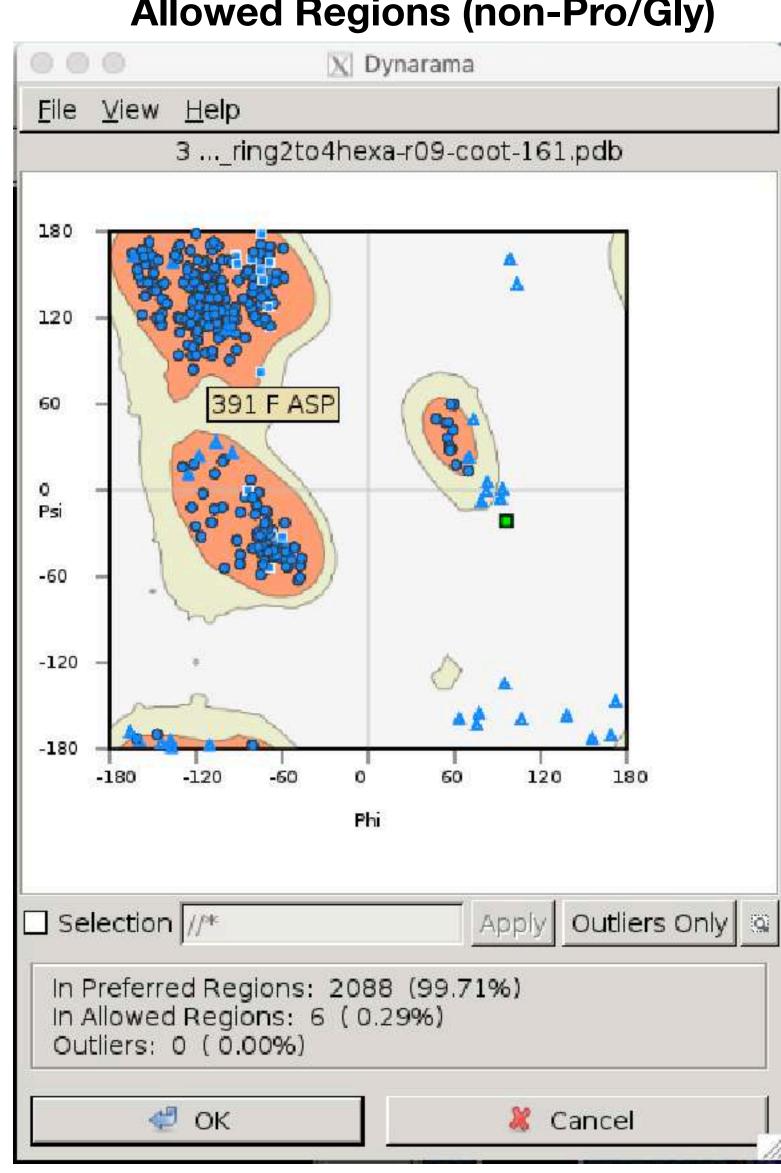
#### **Problematic plot**

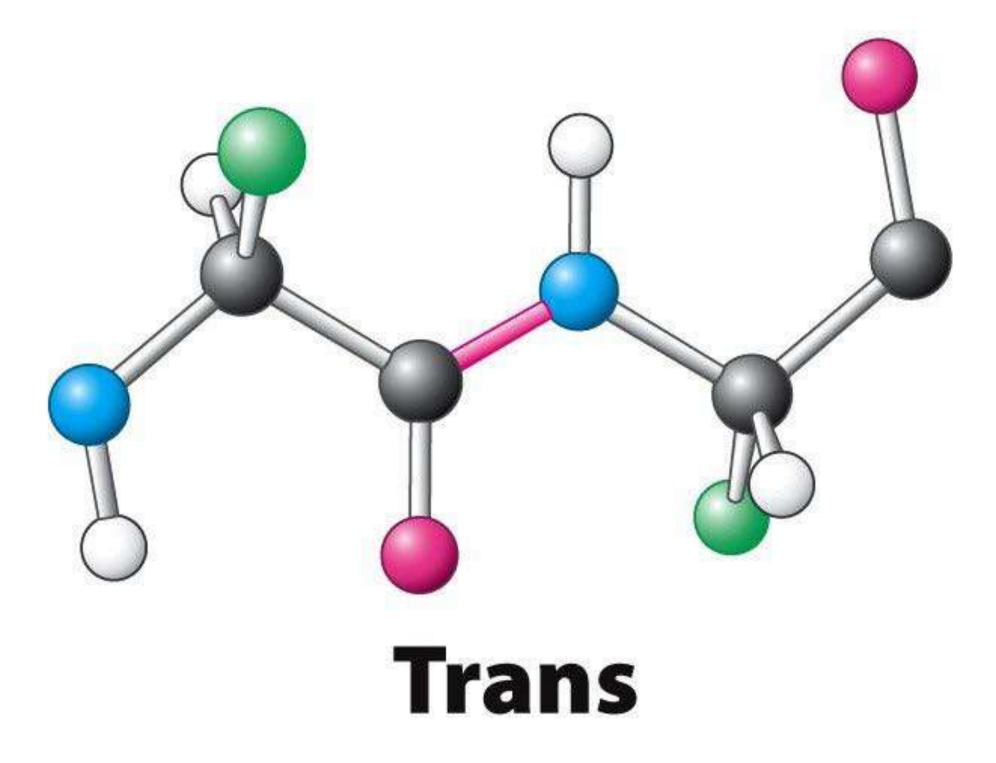


# Ramachandran plot

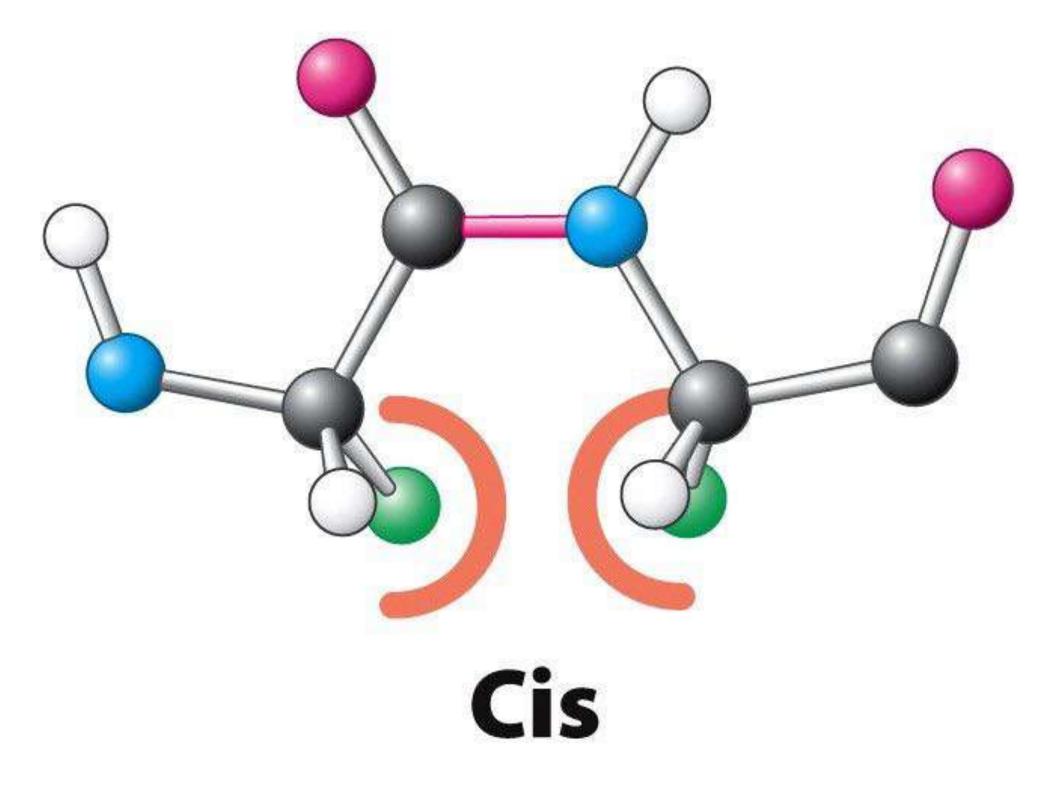


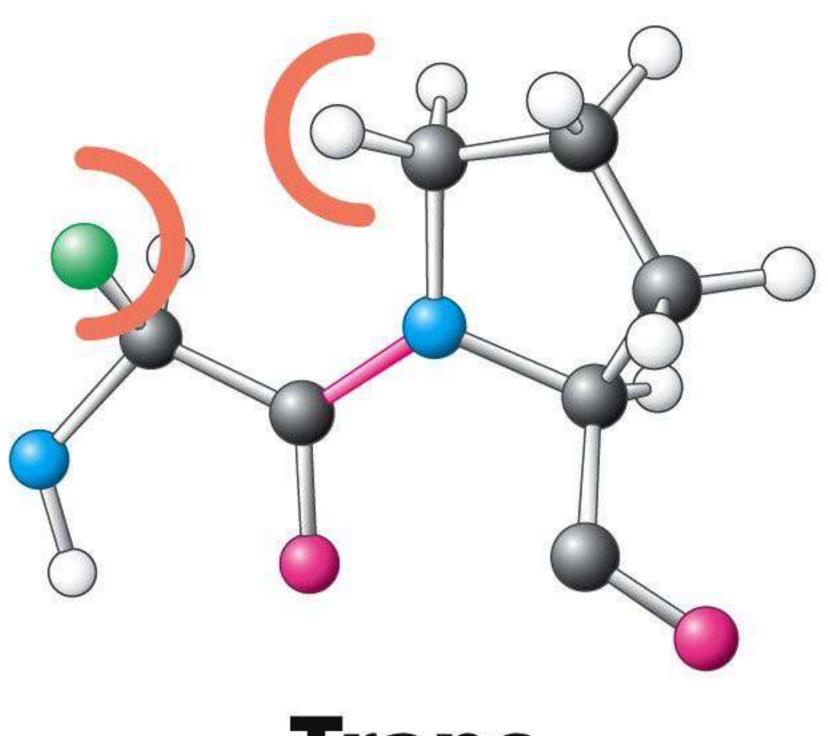
#### Allowed Regions (non-Pro/Gly)





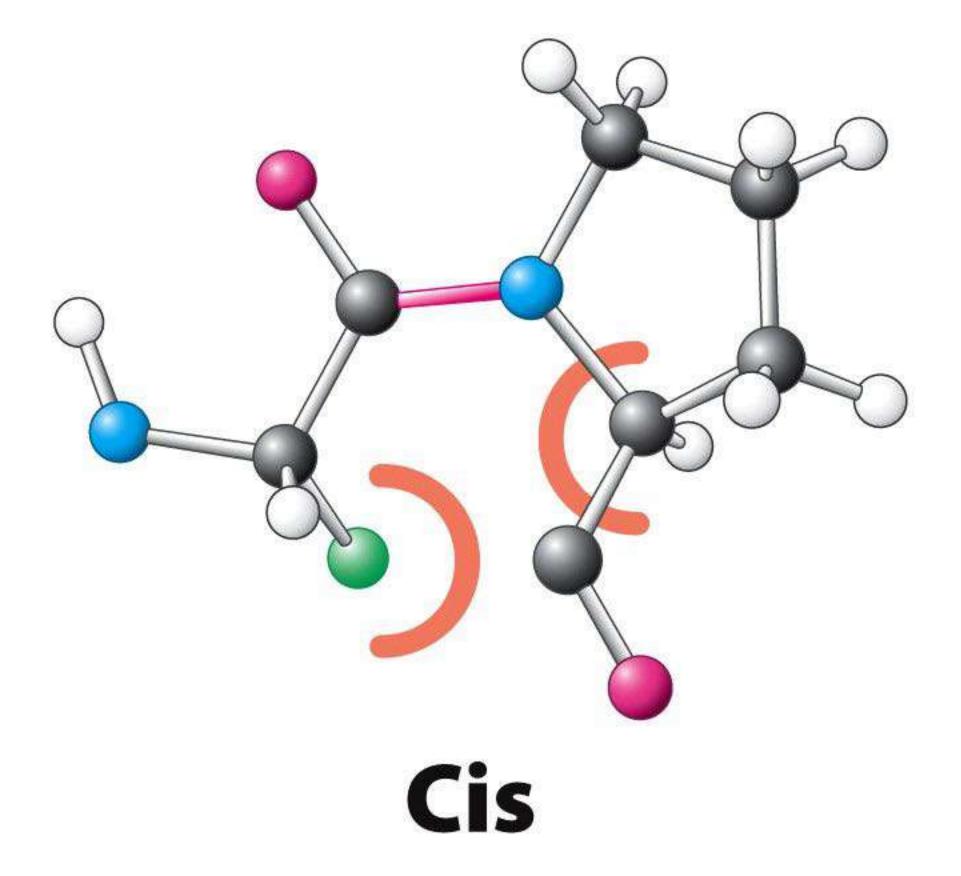
#### Figure 2.20 Biochemistry, Seventh Edition © 2012 W. H. Freeman and Company





#### Trans

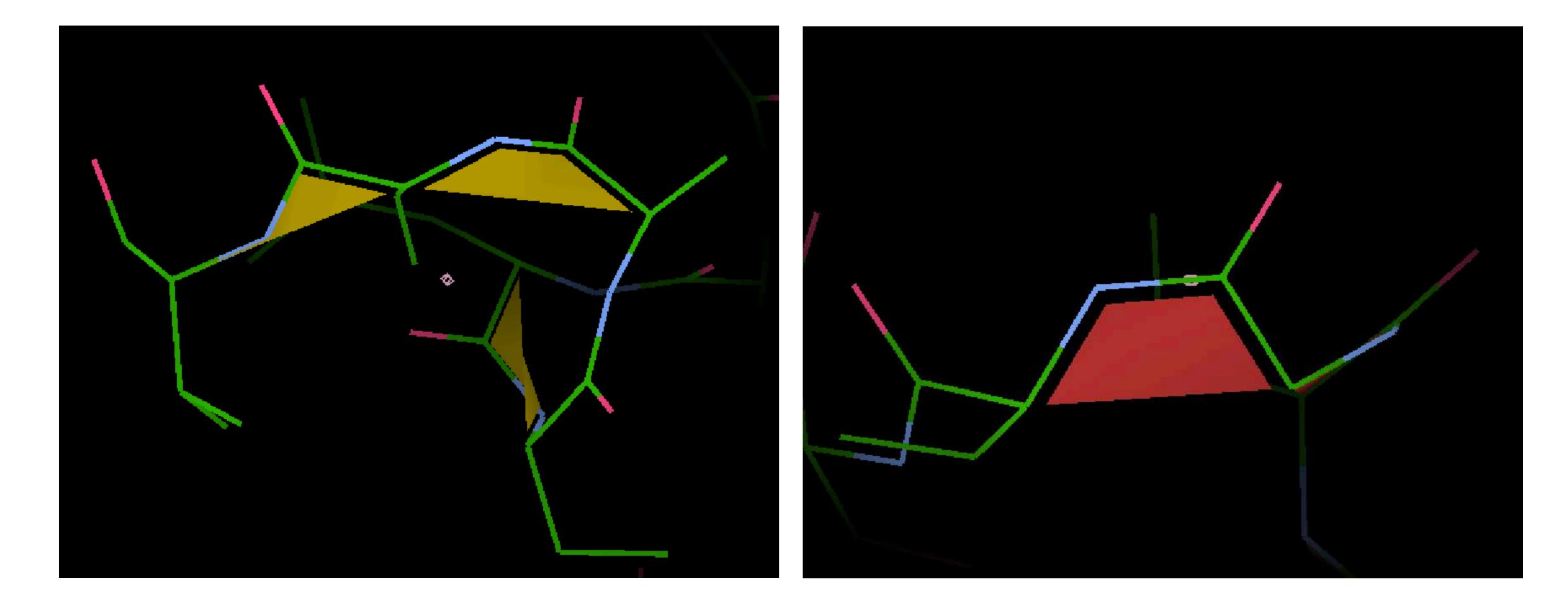
#### Figure 2.21 Biochemistry, Seventh Edition © 2012 W. H. Freeman and Company





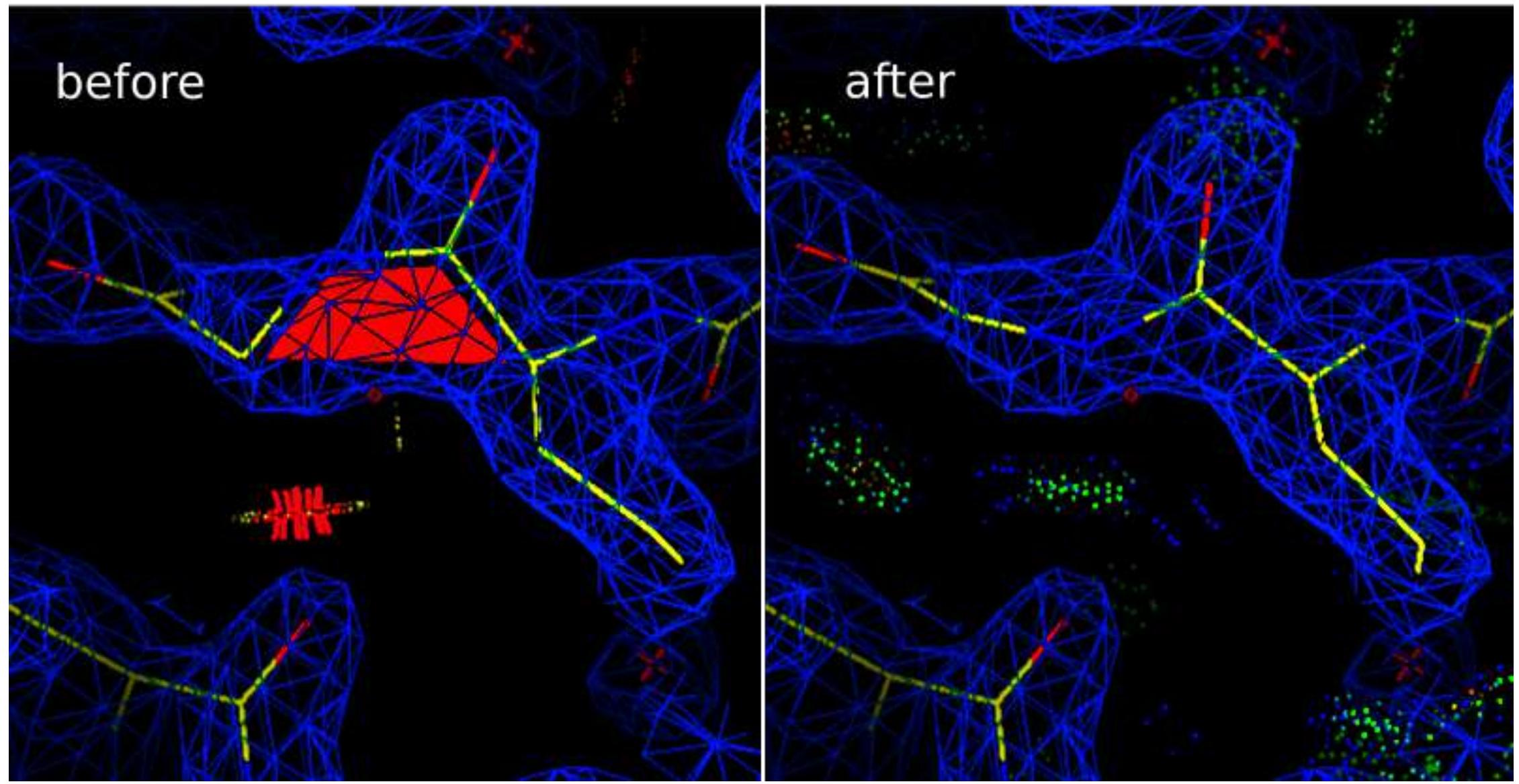
Coot highlights all cis and non-planar peptide bonds, and color codes them to make potential problems easy to ID Green = cis-Proline (probably OK); Yellow = non-planar peptide bond (check!); Red = non-proline cis peptide bond (check!)





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!)



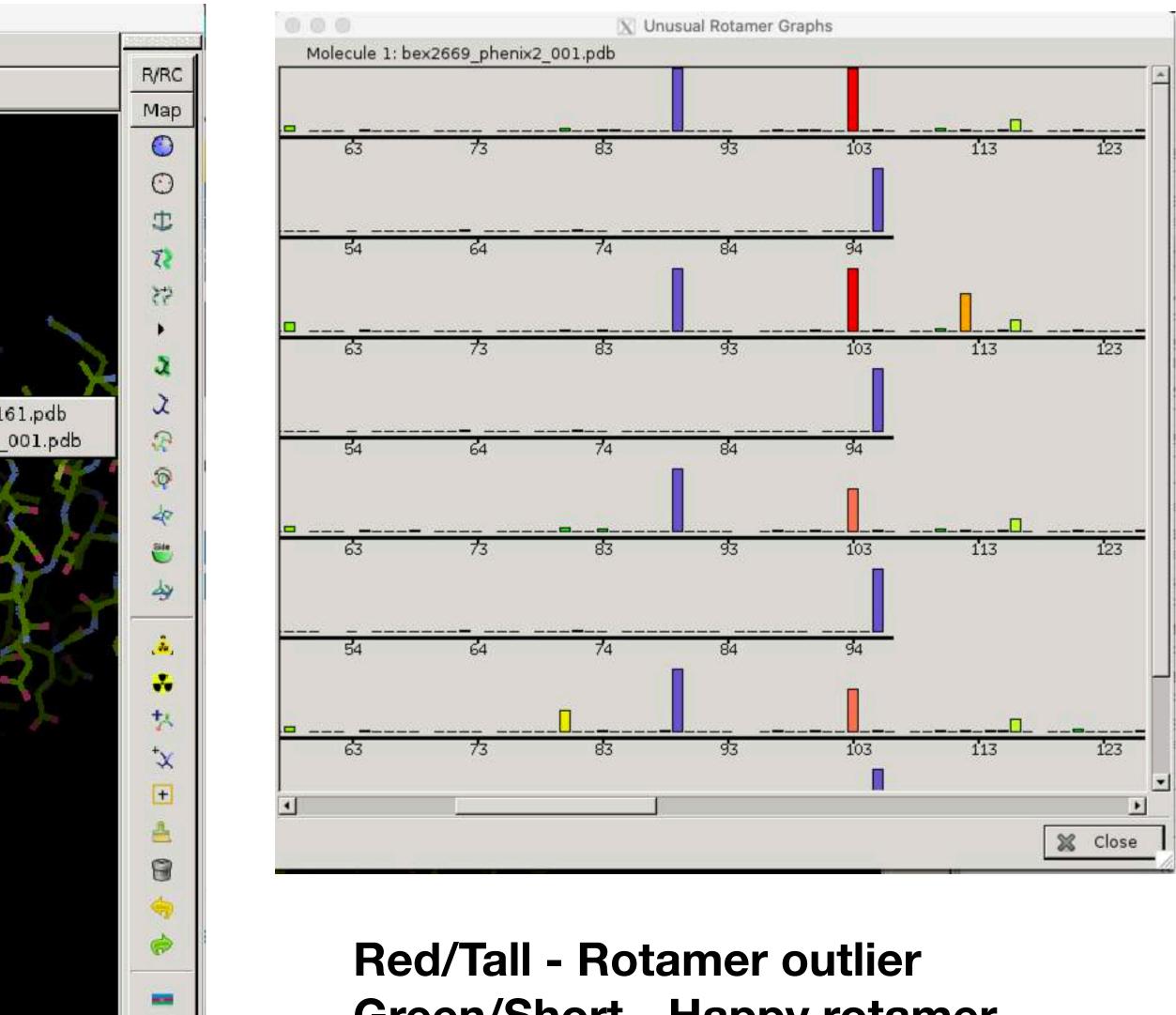


https://www.phenix-online.org/documentation/tutorials/molprobity.html

## Rotamer outliers: Coot

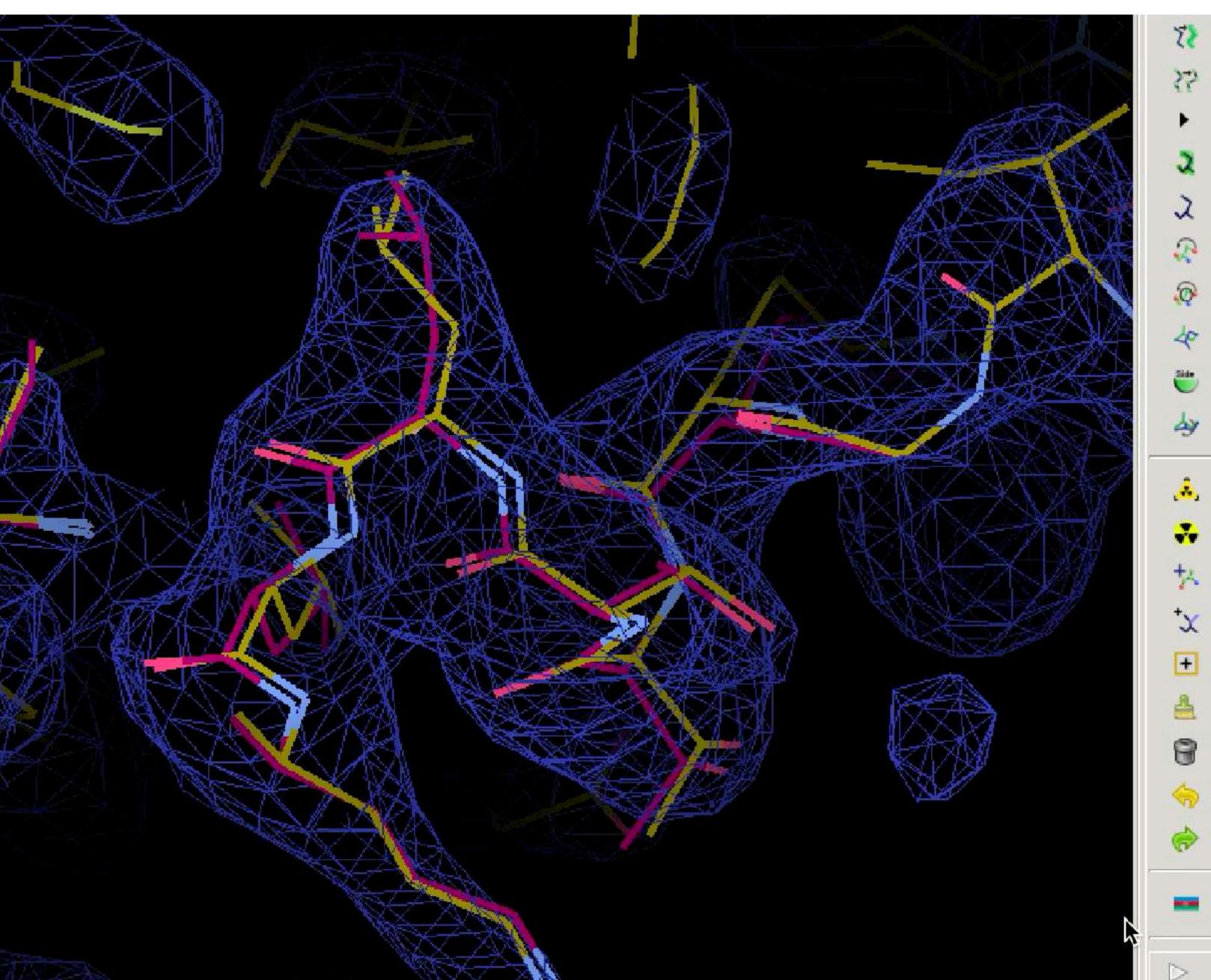
000	X Coot 0.8.9.2-pre EL (revision count 7766)
<u>F</u> ile <u>E</u> dit <u>C</u> alculate <u>D</u> raw <u>M</u> easures	<u>V</u> alidate HID About Ligand E <u>x</u> tensions
Eile Edit Calculate Draw Measures	Validate       HID       About       Ligand       Extensions         Ramachandran Plot <ul> <li>Kleywegt Plot</li> <li>Incorrect Chiral Volumes</li> <li>Unmodelled blobs</li> <li>Difference Map Peaks</li> <li>Check/Delete Waters</li> <li>Geometry analysis</li> <li>Peptide omega analysis</li> <li>Average Temp. fact. variance analysis</li> <li>Average Temp. fact. analysis</li> </ul> <ul> <li>Rotamer analysis</li> </ul> <ul> <li>menix2/bex2669_phenix2_0</li> </ul> Probe clashes             NCS Differences             Highly coordinated waters             Pukka Puckers?             Alignment vs PIR
Current illusion and in the state of the sta	cekiert/xtal/bex2669_Pr1307/refinement_20191126/phenix2/bex2669_phenix2

Successfully read coordinates file /Users/dcekiert/xtal/bex2669\_Pr1307/refinement\_20191126/phenix2/bex2669\_phenix2\_001....



Green/Short - Happy rotamer Lilac(?): Missing side chain atoms

## **Cbeta deviations**

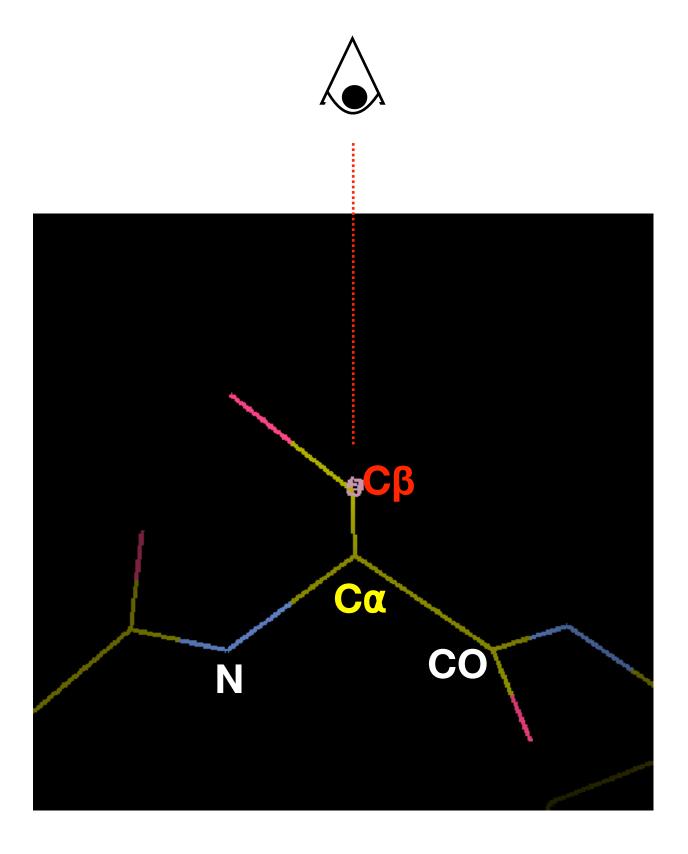


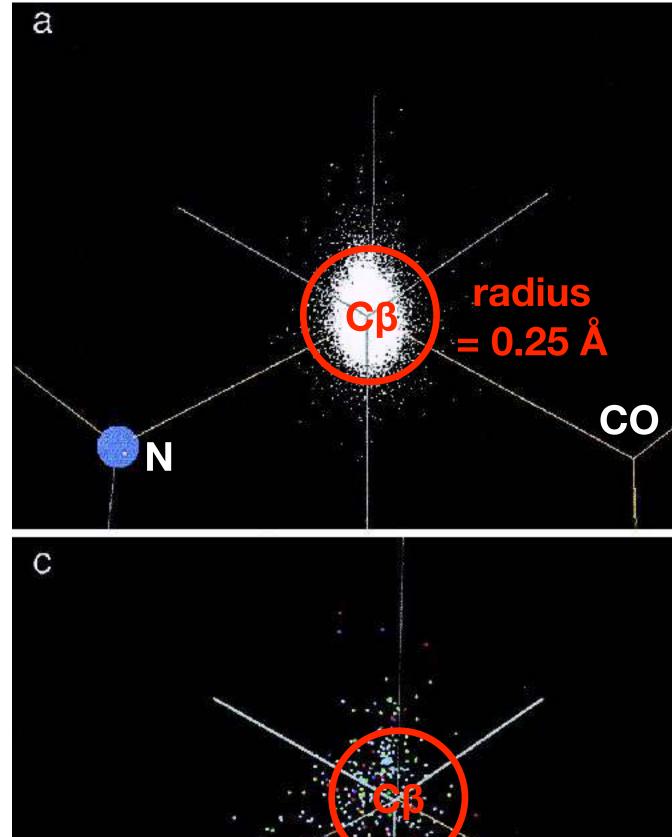
#### Which Leu rotamer is correct???



#### **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.

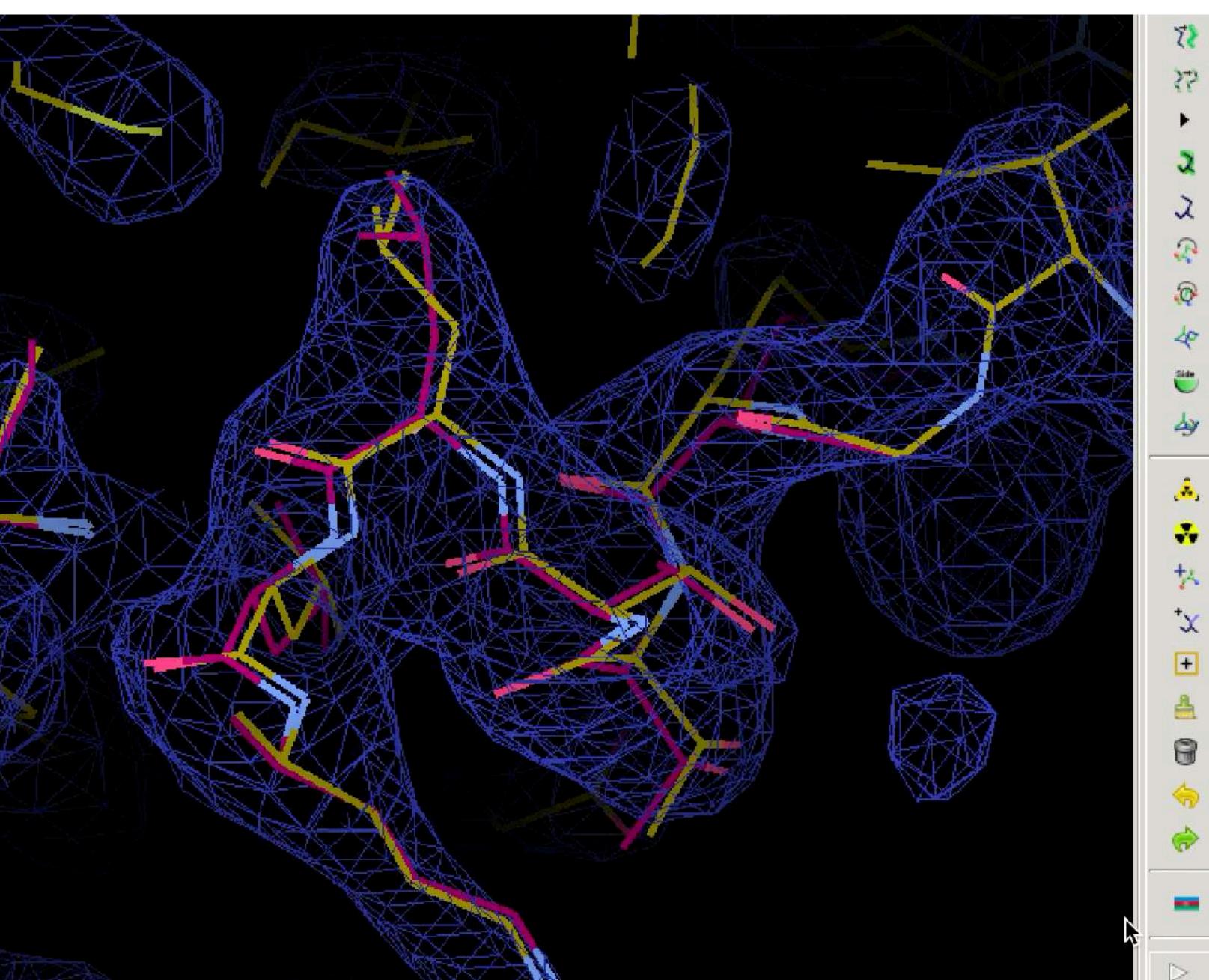




Ν



## **Cbeta deviations**



#### Which Leu rotamer is correct???

#### **Correct answer:** YELLOW



Composition (#)		Box			
Chains	6	Lengths (Å)		113.97, 103.49, 120.52	
Atoms	15942 (Hydrogens: 0)	Angles (°)	Angles (°)		
Residues	Protein: 2106 Nucleotide: 0	Supplied Resolution (A	N)	3.0	
Water	0	Resolution Estimates (	Å)	Masked	Unmasked
Ligands	0	d FSC (half maps;	0.143)		
Bonds (RMSD)		d 99 (full/half1/h	alf2)	3.4//	3.4//
Length (Å) (# > 4σ)	0.002 (0)	d model		3.3	3.4
Angles (°) (# > 4σ)	0.573 (0)	d FSC model (0/0	.143/0.5)	3.0/3.1/3.4	3.1/3.2/3.4
MolProbity score	2.26	Map min/max/mean		-0.29/0.53/0.00	
Clash score	7.67				
Ramachandran plot (%)		Model vs. Data			
Outliers	0.00	CC (mask)	0.78		
Allowed	4.30	CC (box)	0.69		
Favored	95.70	CC (peaks)	0.64		
Rotamer outliers (%)	5.12	CC (volume)	0.78		
Cβ outliers (%)	0.00	Mean CC for ligands			
Peptide plane (%)					
Cis proline/general	0.0/0.0				
Twisted proline/general	0.0/0.0				
CaBLAM outliers (%)	2.02				
ADP (B-factors)					
lso/Aniso (#)	15942/0				
min/max/mean					
Protein	60.42/162.58/90.50				
Nucleotide					
Ligand					
Water	1000000				
Occupancy					
Mean	1.00				
occ = 1 (%)	100.00				
0 < occ < 1 (%)	0.00				
occ > 1 (%)	0.00				

#### **ADP outliers**

Project: tmp

**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)

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If your B factors seem very high or low:

- **Check for regions**  $\bullet$ 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

https://qc-check.usc.edu/QC/qc\_check.pl

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

## 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