Model Refinement

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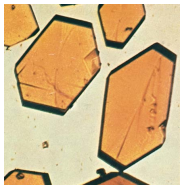
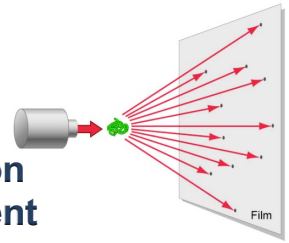
Solving structure by crystallography

Pure sample

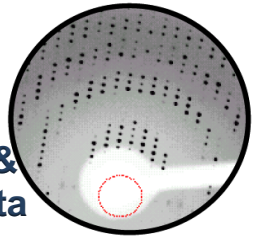
Crystals



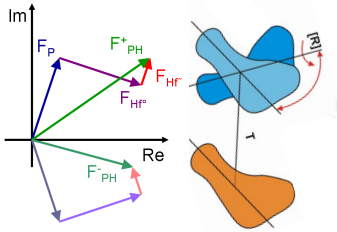
Diffraction experiment



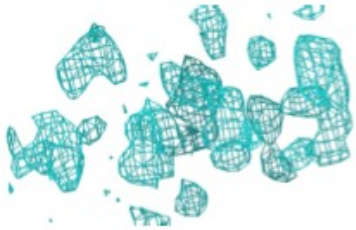
Process & check data



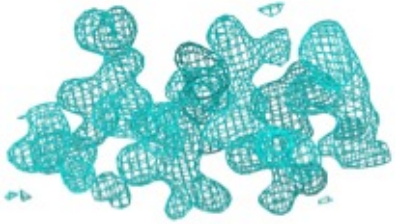
Solve phase problem



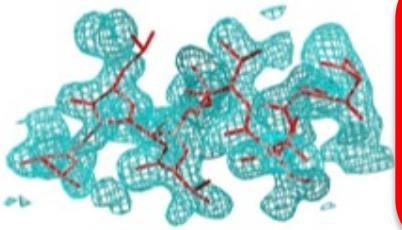
Initial map



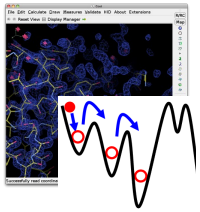
Improved map



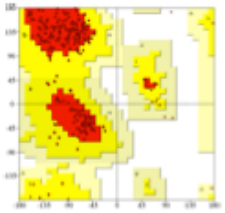
Build model



Refine model



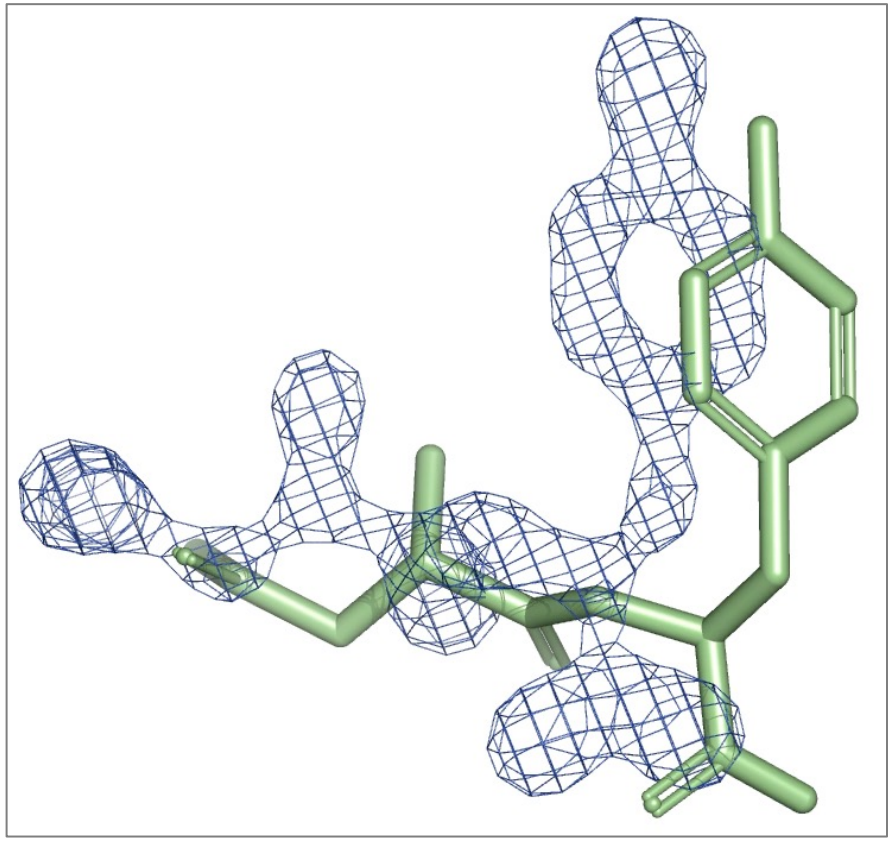
Validate model

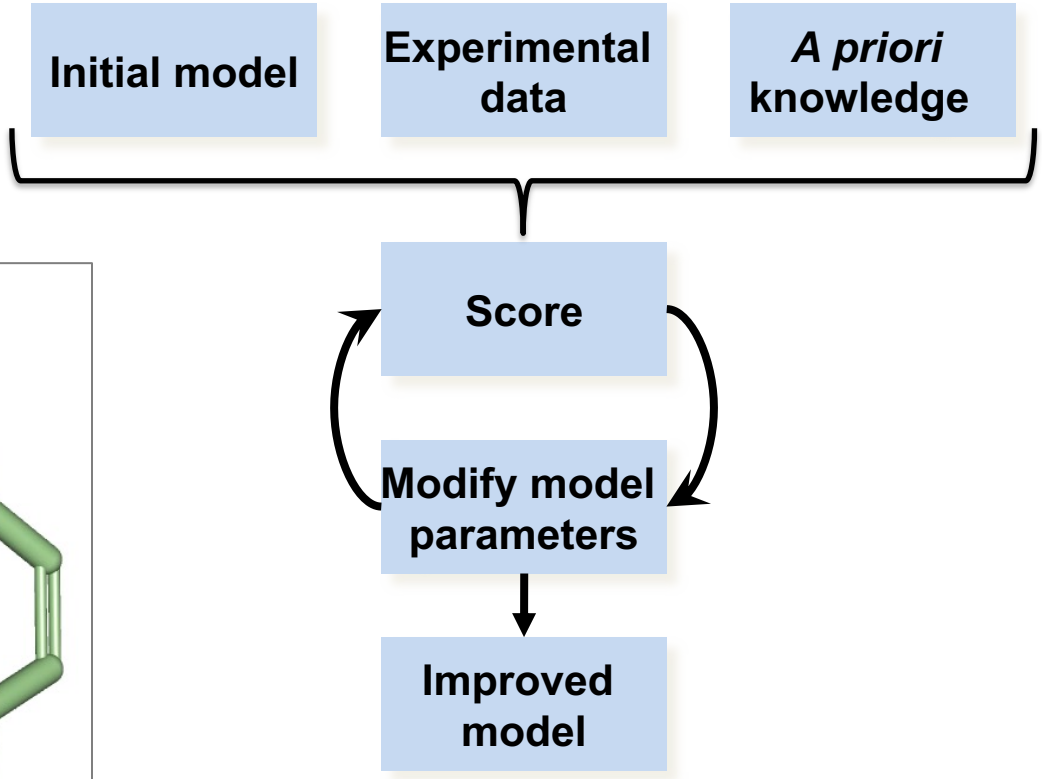


Publish & deposit

- Process is not as ‘linear’ as shown
- Each step has numerous sub-steps
- Crystals may not grow or exhibit pathologies
- Stuck solving phase problem

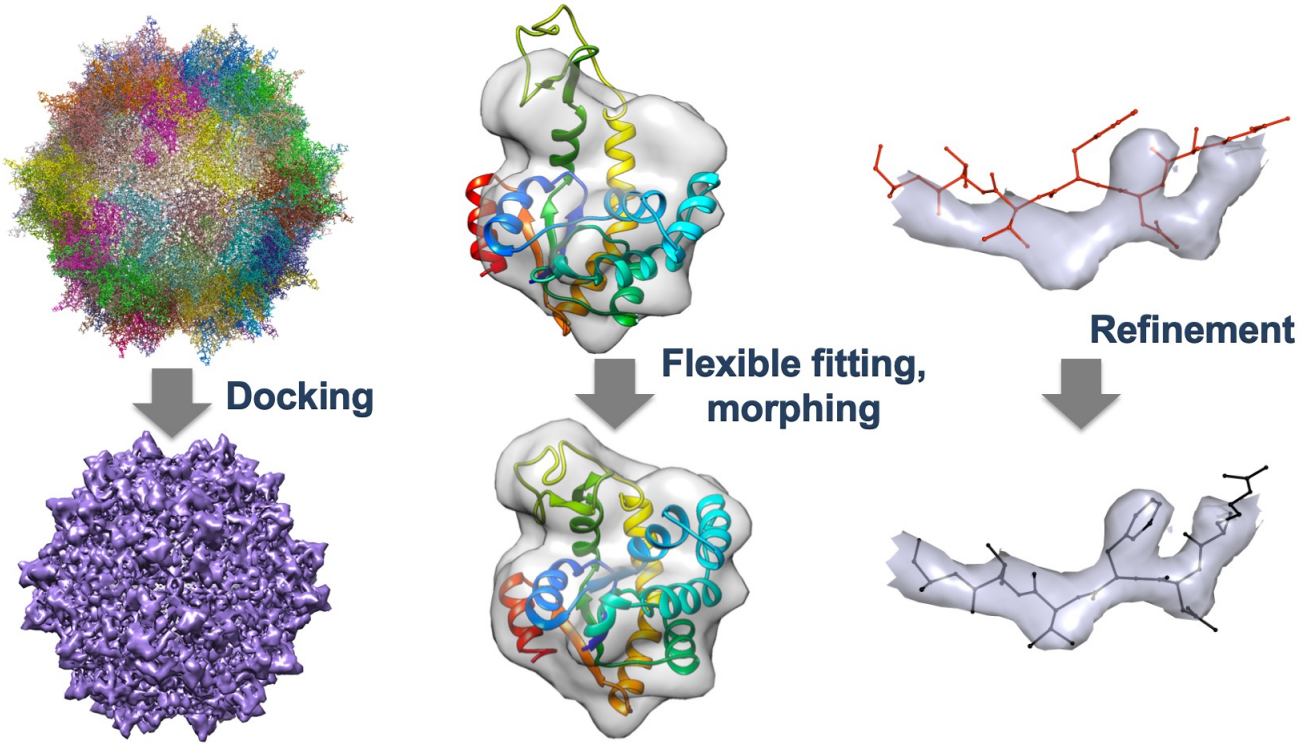
Model refinement





Optimization process of fitting atomic model parameters to experimental data

Not all model-to-data fitting is refinement



- Docking, flexible fitting, morphing are ***not*** refinement
- Refinement is to fine-tune an already fine atomic model
  - Refinement does only small changes to the model (within *convergence radius of refinement*, ~ 1Å)

Solving structure in the past

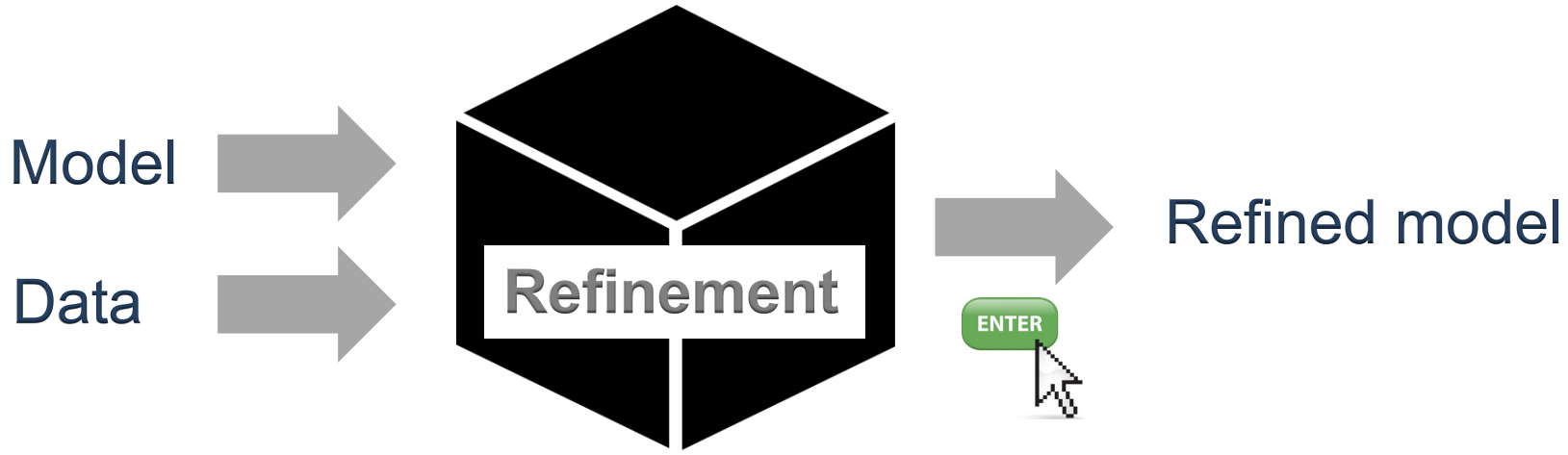
- Familiar with many software packages (often with “orthogonal” philosophies)
- Mutually incompatible file formats for common data exchange
- Coding experience was a must (typically using arcane languages FORTRAN or C)
- No GUIs. Command line expertise (Unix)
- Reading thick books (no Google, YouTube or ChatGPT!)
- Limited online forums
- Don’t expect your questions answered quickly by email
- Slow computers (with sometimes limited access)

Solving structure in the past

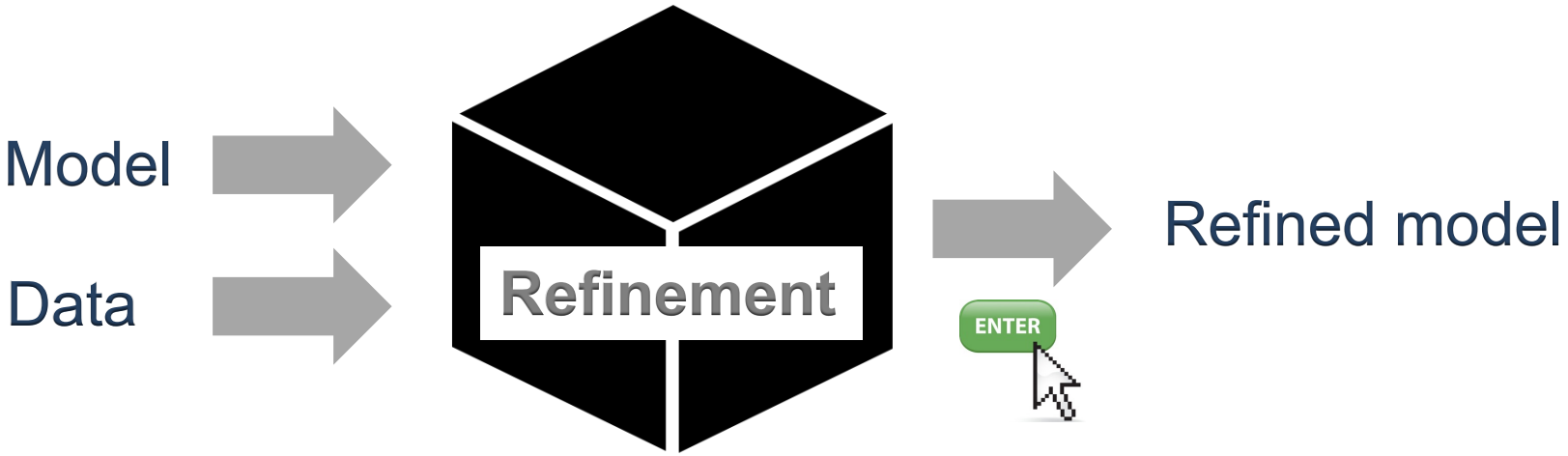
- From many months to years
  - Spend days on graphics (manual atomic model building)
  - Run computations overnight

Solving my first structure back in 1997

Model refinement: black box



Model refinement: black box



- Does it always work?
- Is it always as easy as *poor model in, better model out*?

Model refinement: black box

- No. Because:
  - Refinement parameterization isn't easy (next slide)
  - Default settings suit most common scenario
    - Typical resolution data, model reasonably fits data
  - Less typical situations need customizations
    - Low or high resolution data
    - Incomplete models
    - Final models
    - AlphaFold predicted models
    - Novel ligands

Model refinement: lot of stuff to know…

Reference model?

TLS?

Rotamer fixing?

AltLocs?

Group B vs individual?

Local minima?

ADP?

Clashes?

NCS?

IAS?

tNCS?

Grid search?

Weights?

CDL?

SA?

Minimization?

Rama plot restraints?

f’ & f”?

Hydrogens?

Restraints?

Bulk-Solvent?

Rigid body?

Rama-Z?

Anisotropy?

NQH flips?

SS restraints?

Twinning?

Model refinement: black box

- What to do when the ‘black box’ does not work?
  - **Your decision-making** is needed (and it is not always easy!)

Model refinement: decision-making variables

- **Crystal**
  - Disorder
  - Twining, tNCS
  - Solvent content
  - Symmetry
- **Data**
  - Resolution
  - Errors
  - Completeness
  - Processing
- **Model**
  - Stage
  - Source
  - Parameterization
  - Fit to data

How you know…

- … refinement worked ?
- … you did it correctly ?
- … the model is good enough to publish ?

How you know…

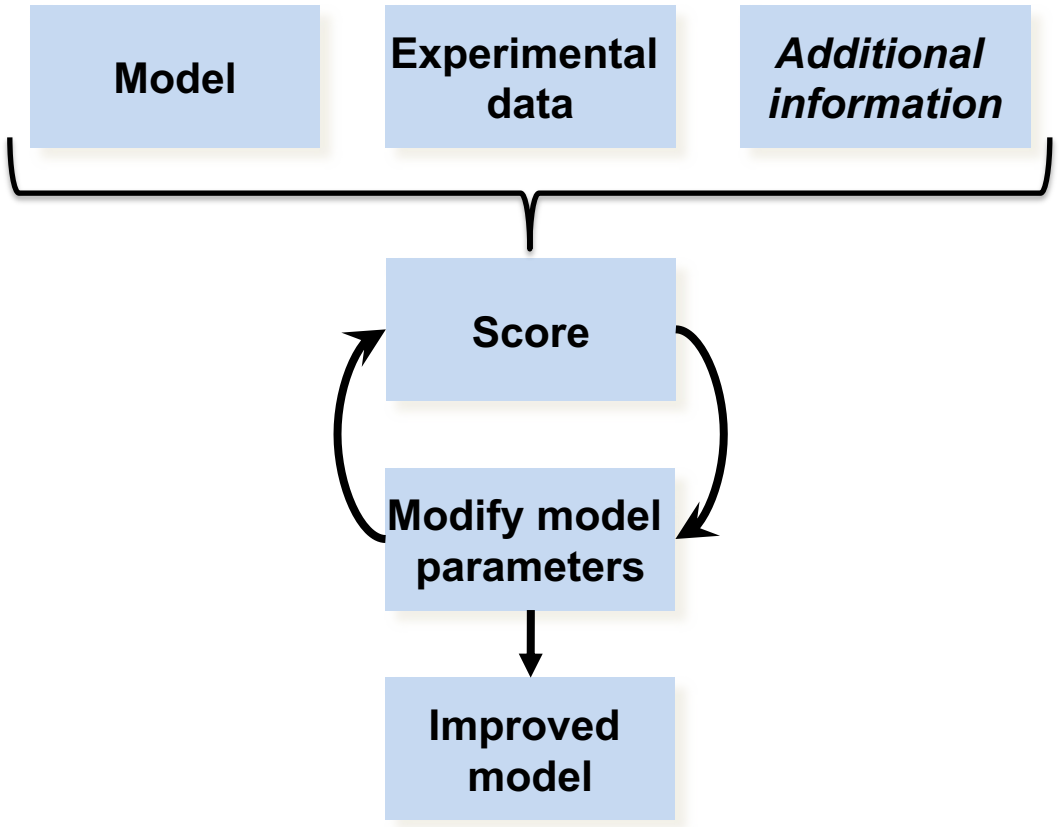
- … refinement worked ?
- … you did it correctly ?
- … the model you got is good enough to publish ?

- Do validation!

Standard validation protocols are designed to answer these questions

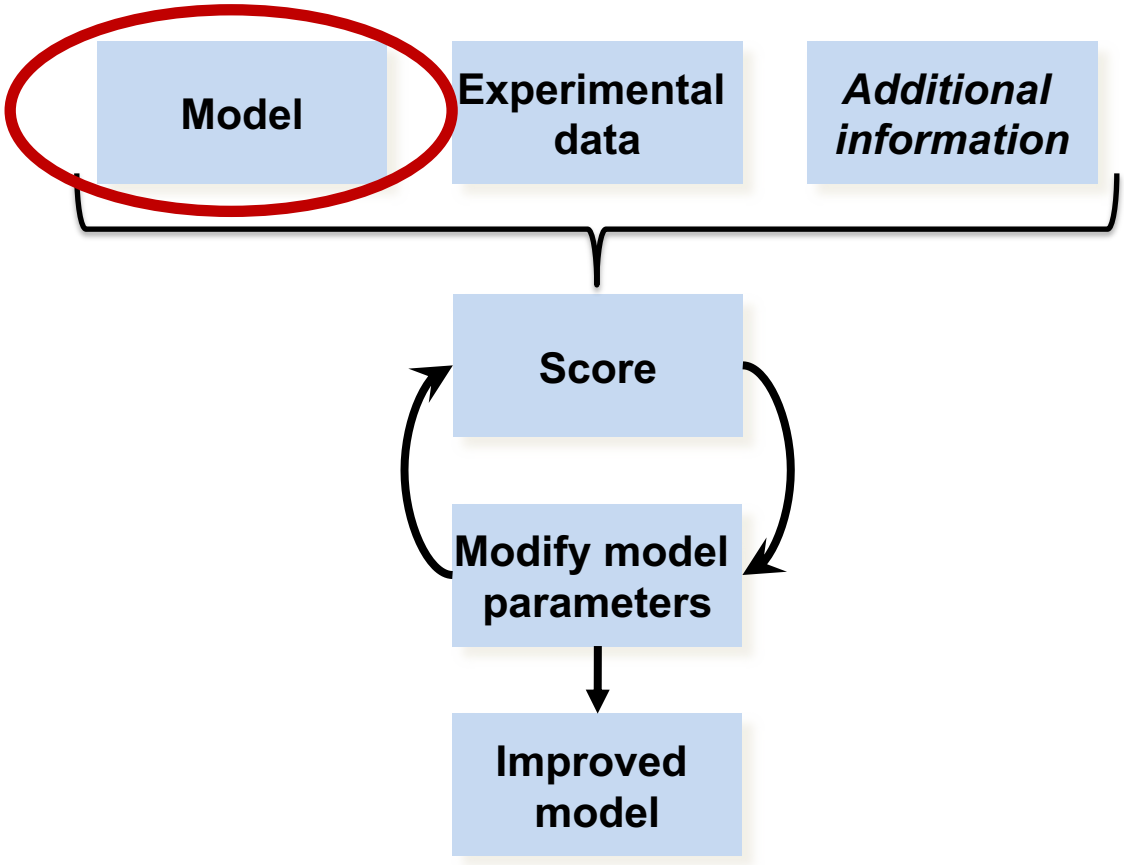
Refinement: a closer look

Model refinement



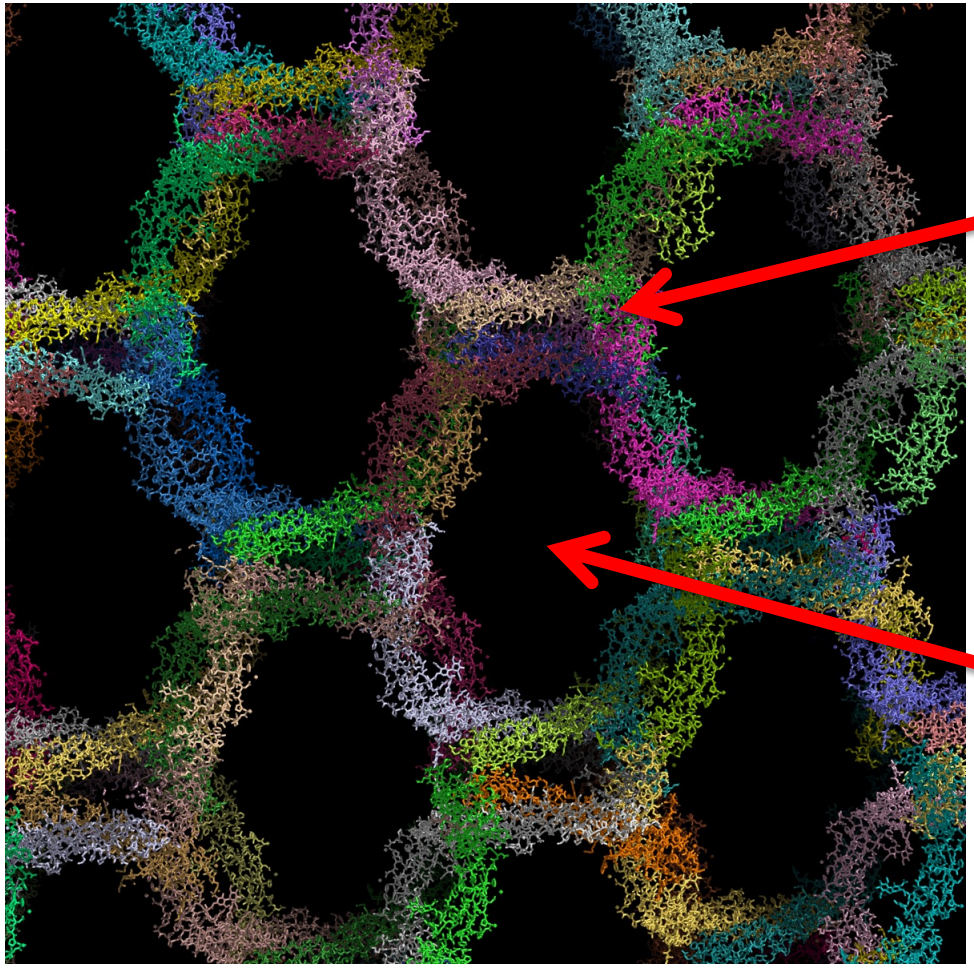
Refinement – optimization process of fitting model parameters to experimental data

Model refinement



Crystal structure model

PDB code: 1QUB



Macro-molecule

Bulk-solvent:
~ 50% of unit cell volume

Crystal model: $\rho_{crystal} = \rho_{atoms} + \rho_{bulk\ solvent}$

Atomic model

Position *Larger-scale disorder*

```
ATOM     25  CA  PRO A   4      31.309  29.489  26.044  1.00 57.79           C
ANISOU   25  CA  PRO A   4    8443   7405   6110   2093    -24    -80       C
```

Local mobility (harmonic vibrations)

$$\mathbf{F}_{\text{MODEL}} = k_{\text{OVERALL}} \left(\mathbf{F}_{\text{CALC (ATOMS)}} + \mathbf{F}_{\text{BULK}} \right)$$

Occupancy `1.00` `57.79` ADP (B-factor)

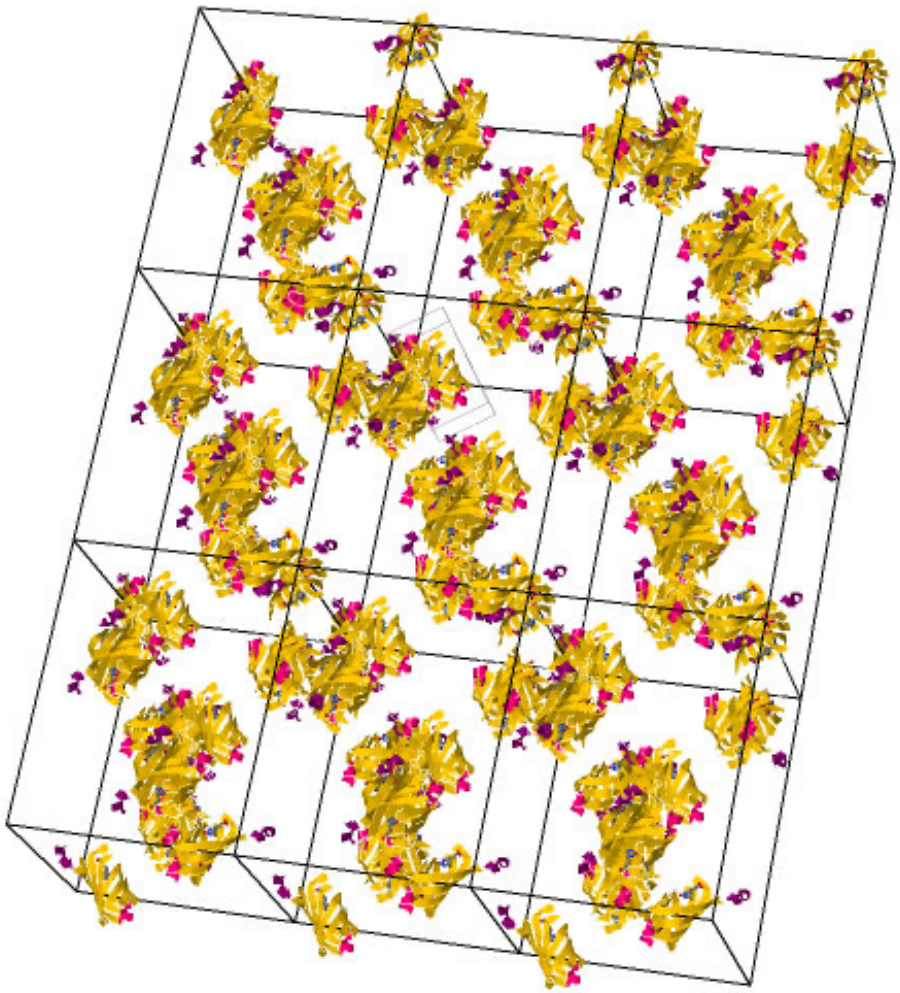
$$\mathbf{F}_{\text{CALC (ATOMS)}}(h,k,l) = \sum_{n=1}^{Natoms} q_n f_n(s) \exp\left(-\frac{B_n s^2}{4}\right) \exp\left(2i\pi \mathbf{r}_n \mathbf{s}\right)$$

Atom type `C`

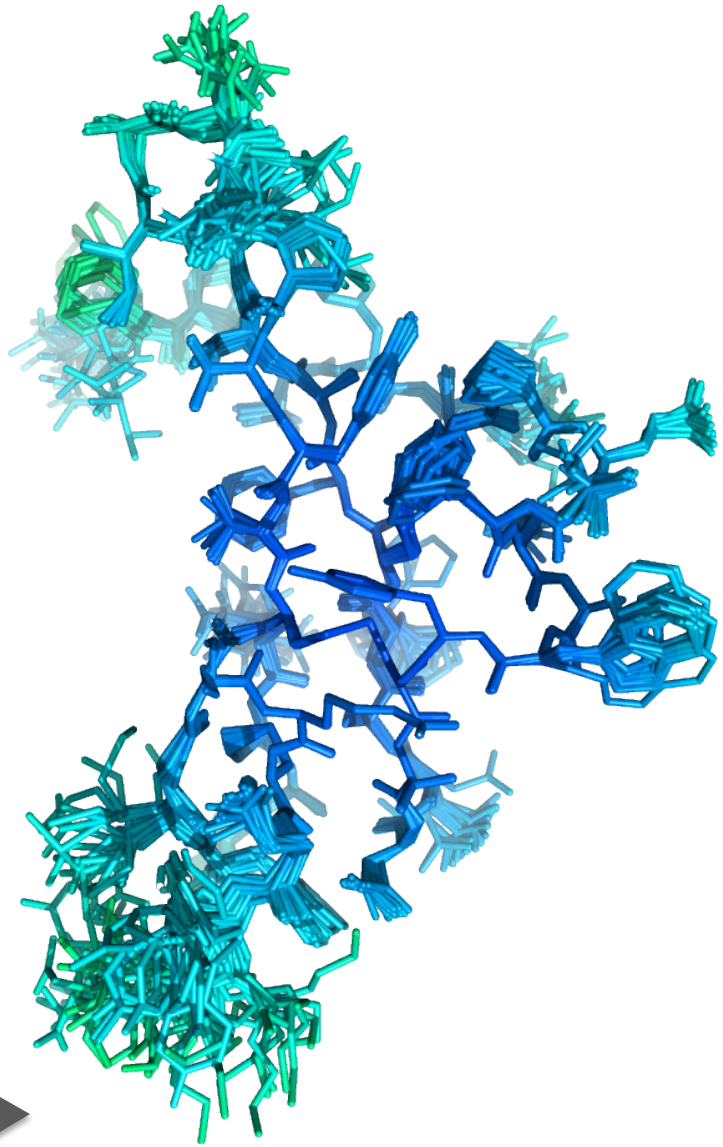
`31.309 29.489 26.044`

Atomic coordinates

Atomic model: disorder

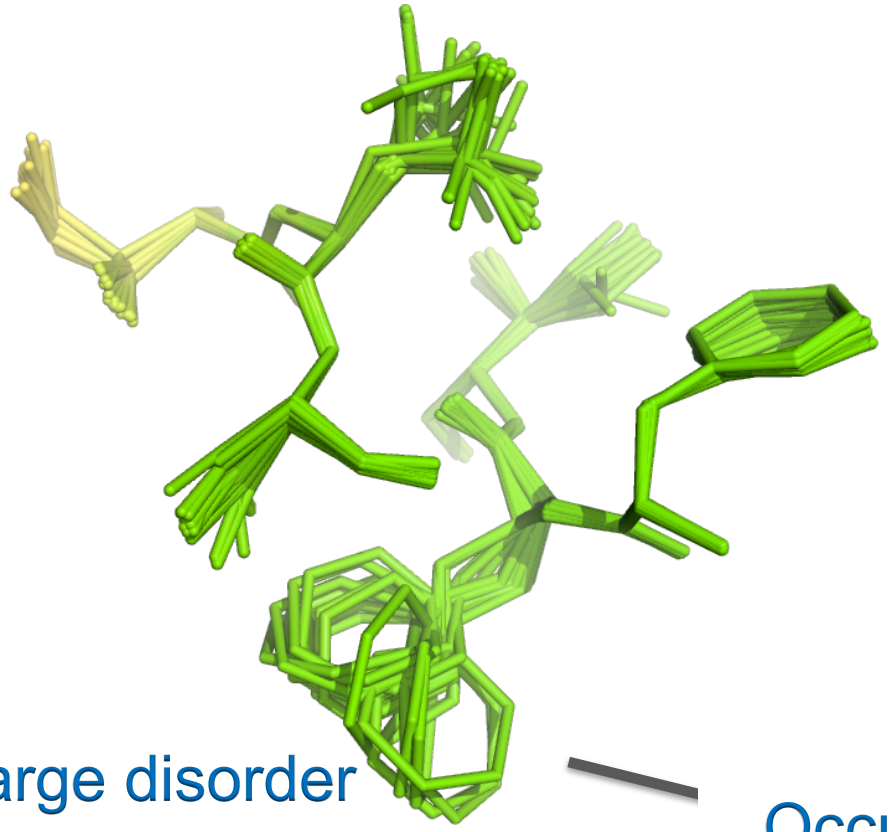


Crystal = many unit cells



Superpose all structures
from each unit cell

Atomic model: disorder



Small disorder

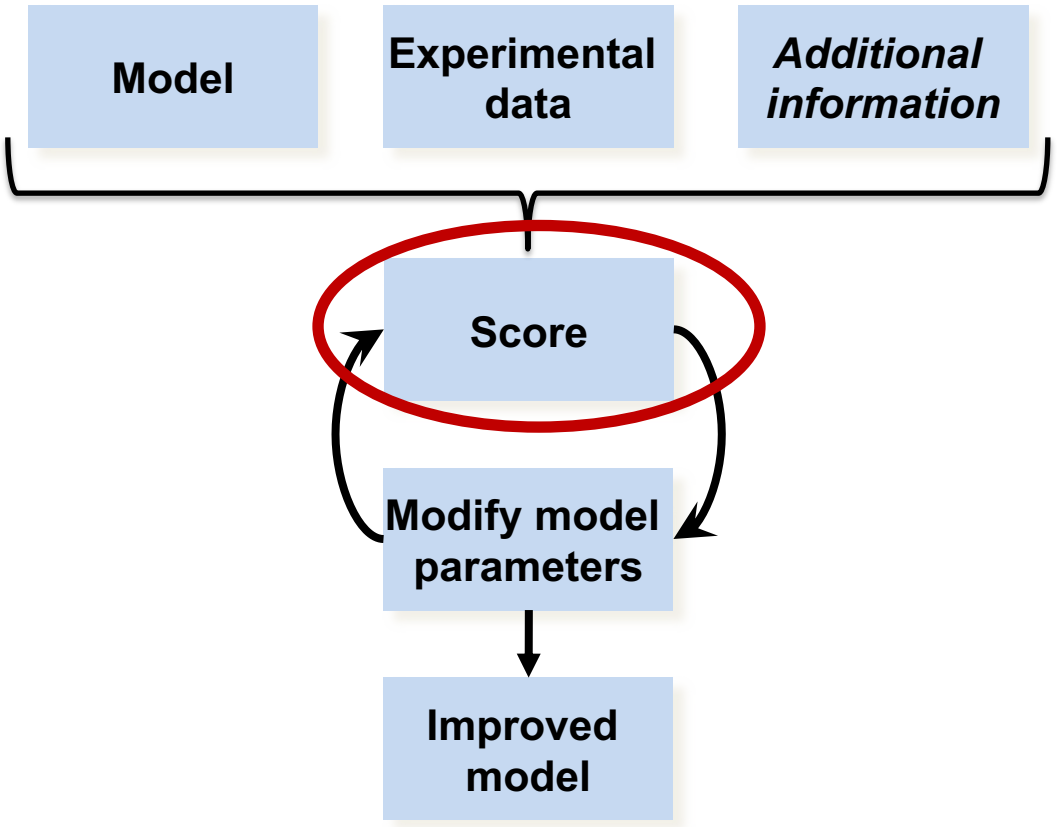
ADP (B-factor)

Large disorder

Occupancy

```
ATOM     25  CA  PRO A   4      31.309  29.489  26.044  1.00 57.79           C
ANISOU   25  CA  PRO A   4    8443   7405   6110   2093    -24    -80           C
```

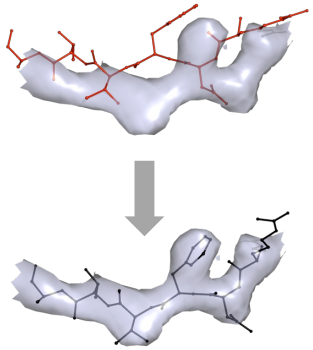
Refinement target function (score)

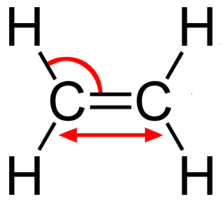


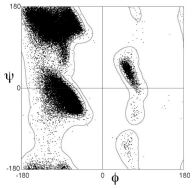
Model refinement target (score)

T = **T_{DATA}** + **w * $T_{RESTRAINTS}$**

Optimize consensus between model-to-data fit and... common sense







Bonds, angles, planes, torsions, chirality, non-bonded repulsion

$$\sum_{hkl} (F_{obs} - F_{model})^2$$

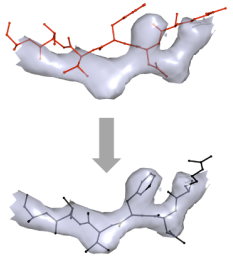
$$\sum_{hkl} \frac{||F_{obs}| - |F_{model}||}{|F_{obs}|}$$

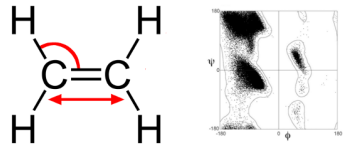
Maximum-Likelihood

Restraints and data resolution

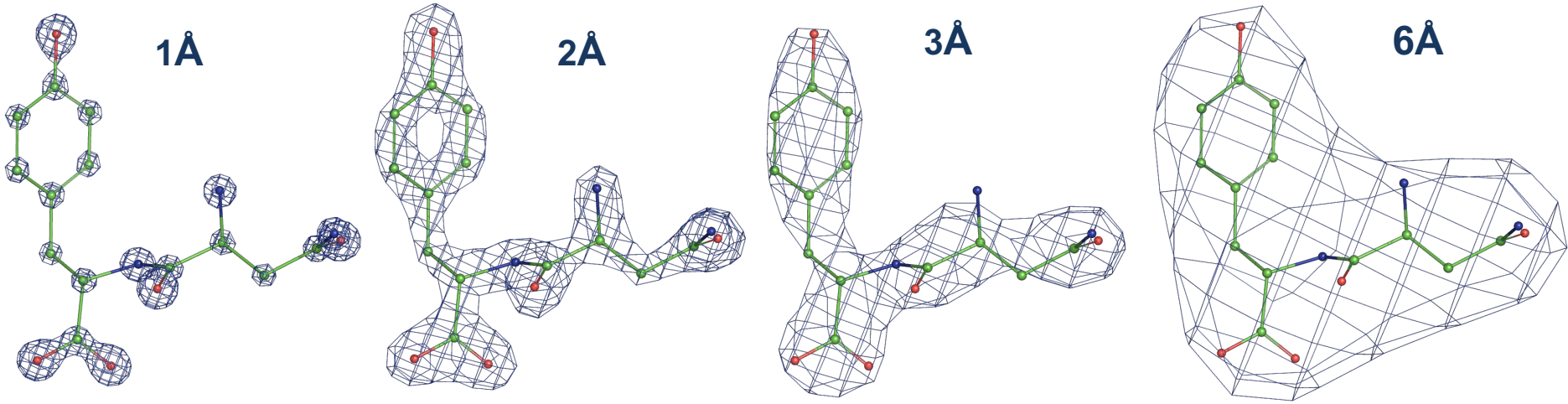
$T = T_{DATA} + w * T_{RESTRAINTS}$

Optimize consensus between model-to-data fit and... common sense



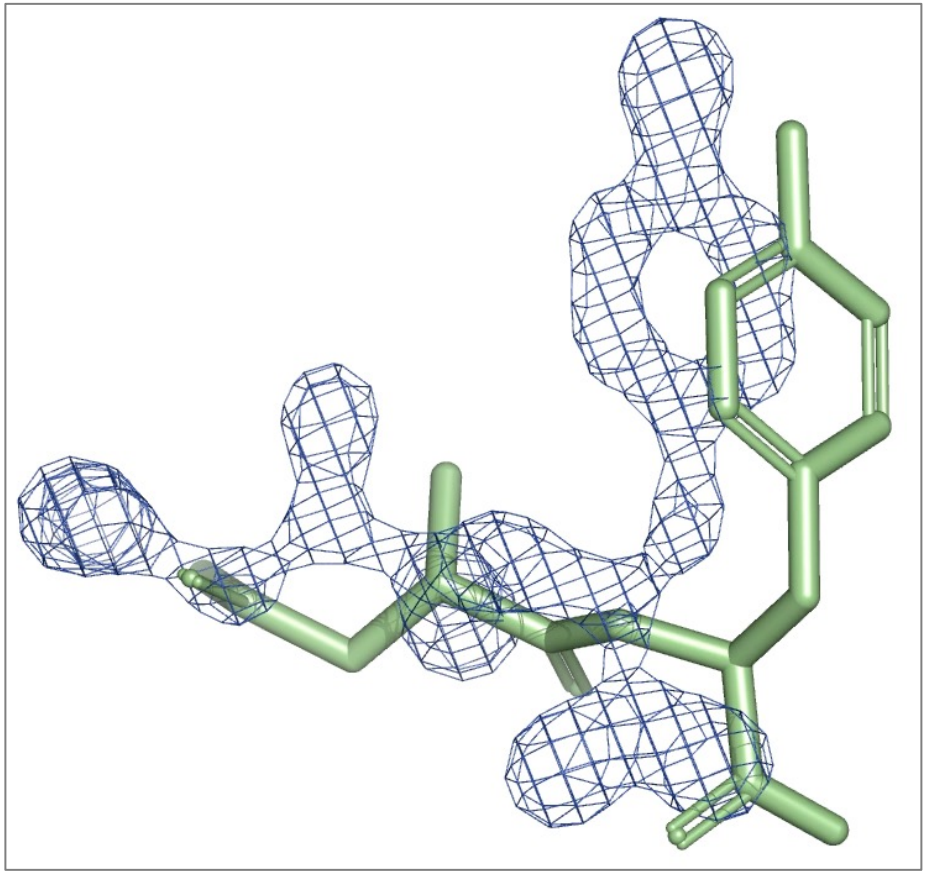


Bonds, angles, planes, torsions, chirality, non-bonded repulsion

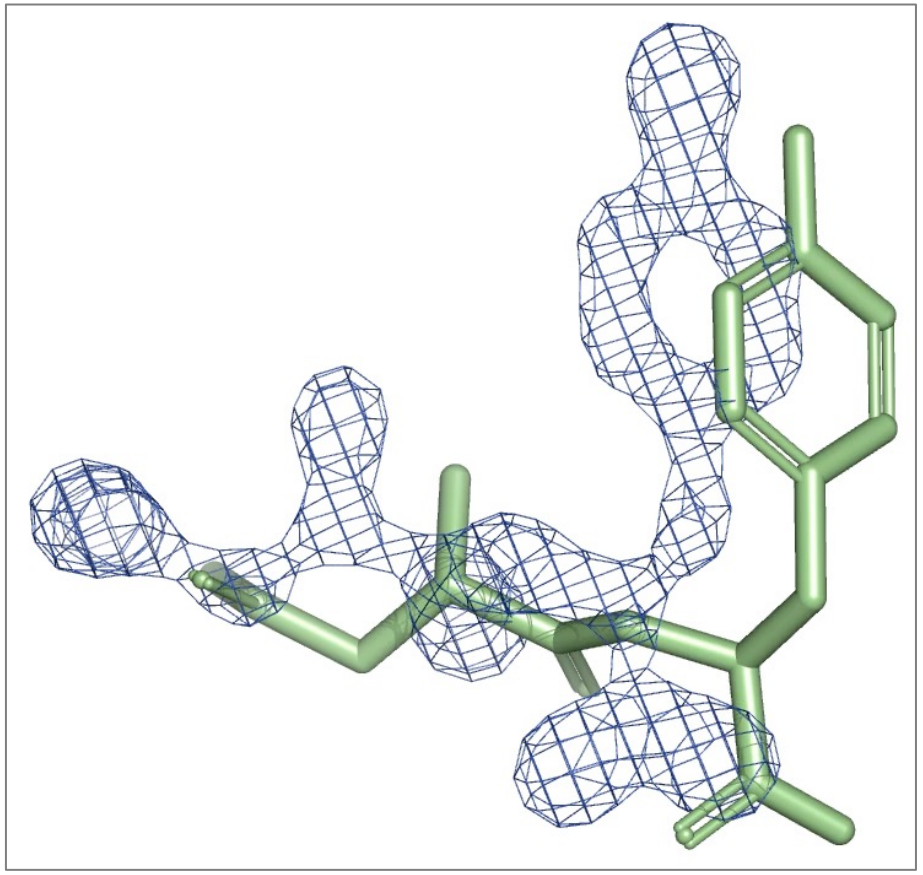


Model refinement with *vs* no restraints: high resolution

$$T = T_{DATA} + w * T_{RESTRAINTS}$$



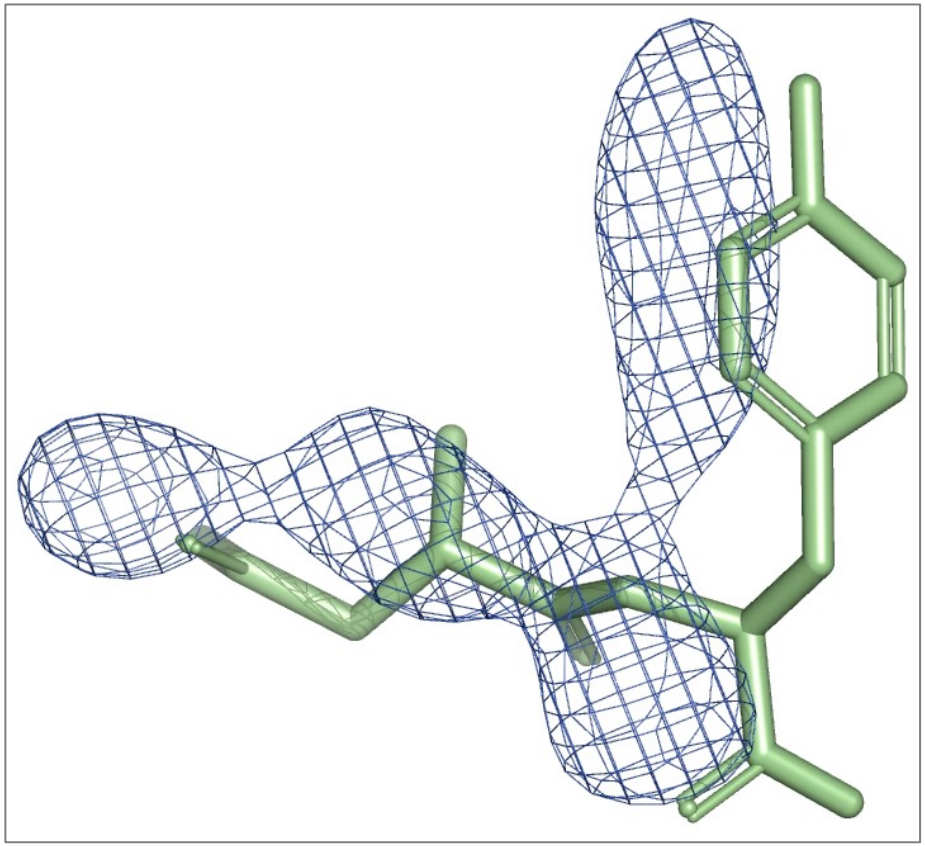
Using restraints



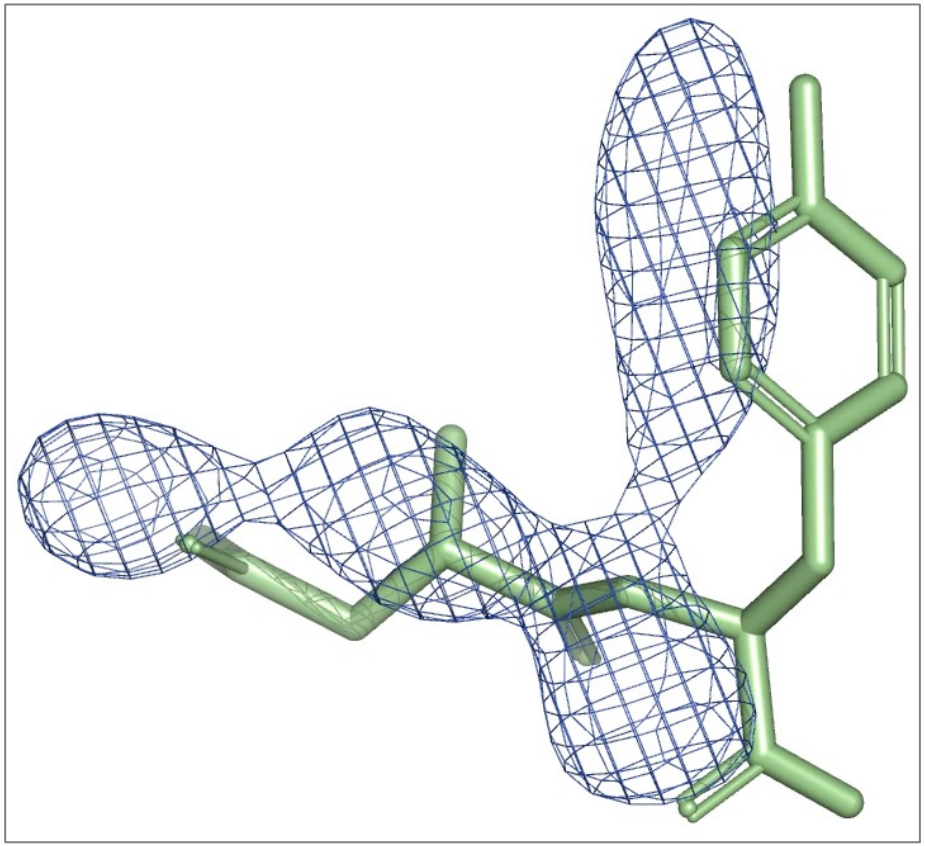
No restraints

Model refinement with *vs* no restraints: low resolution

$$T = T_{DATA} + w * T_{RESTRAINTS}$$



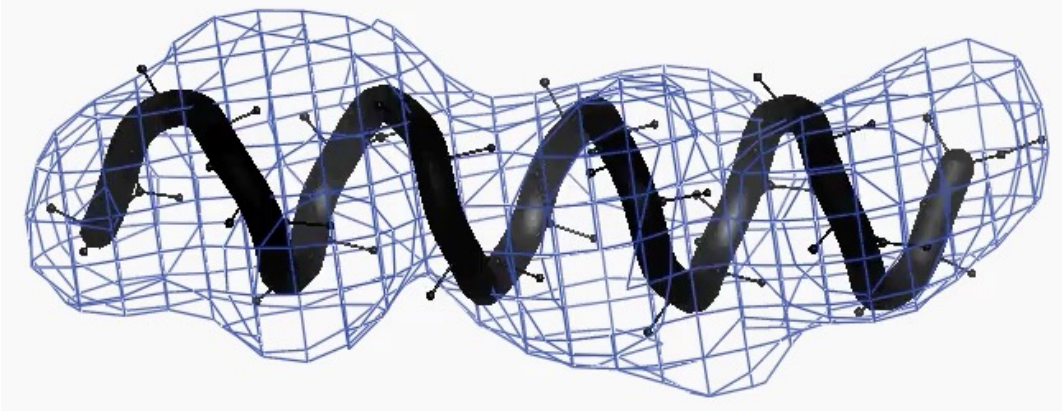
Using restraints



No restraints

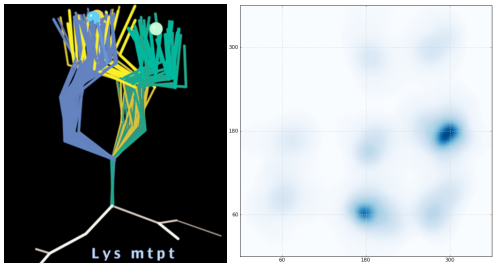
Model refinement with insufficient restraints

- Refinement of a perfect α-helix into low-res map
  - Using simplistic (standard) restraints on covalent geometry
    - Model geometry deteriorates as result of refinement

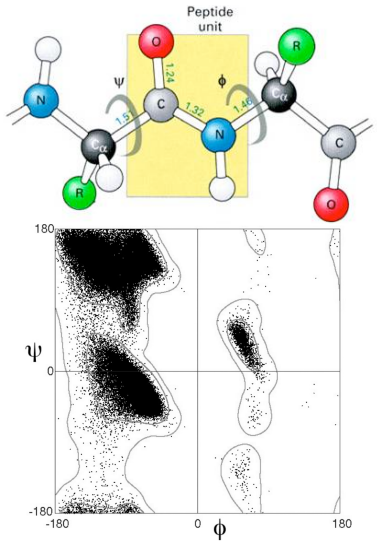


More restraints for low resolution

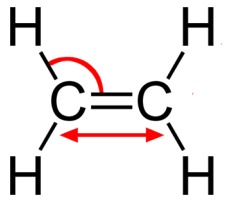
Side chain distributions



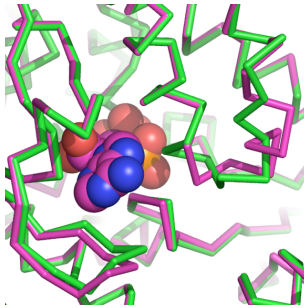
Main chain distributions



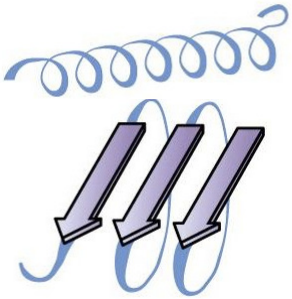
Covalent geometry



Internal symmetry (NCS)

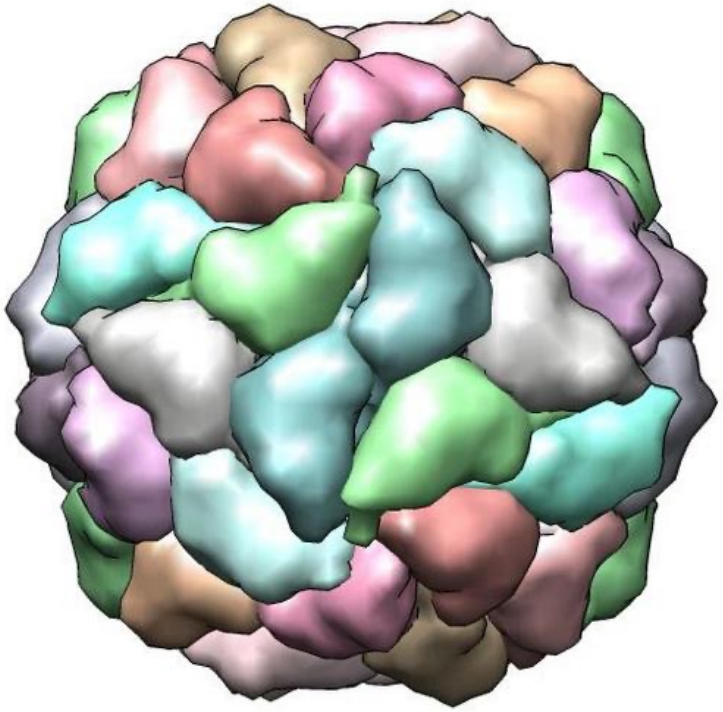


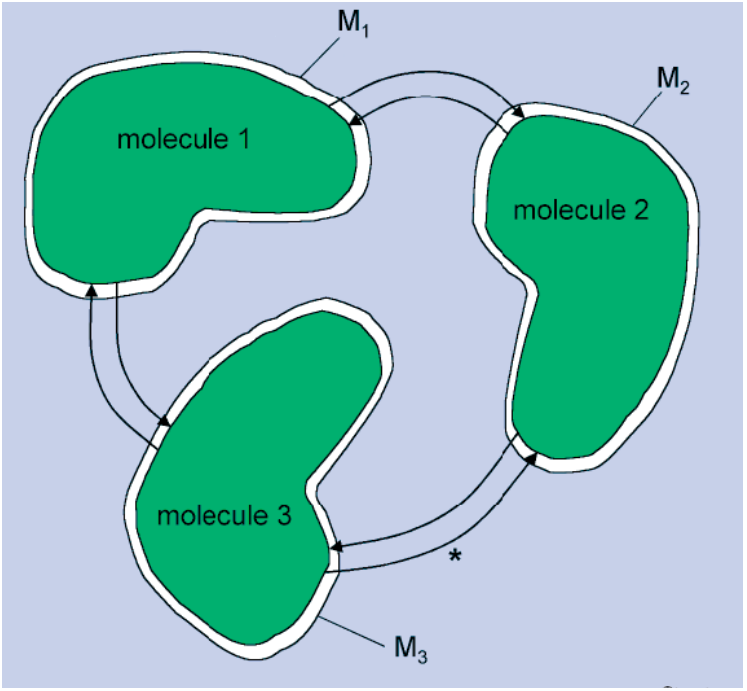
Similar (homologous) structures (reference model restraints)



Secondary structure

NCS (internal symmetry): constraints vs restraints



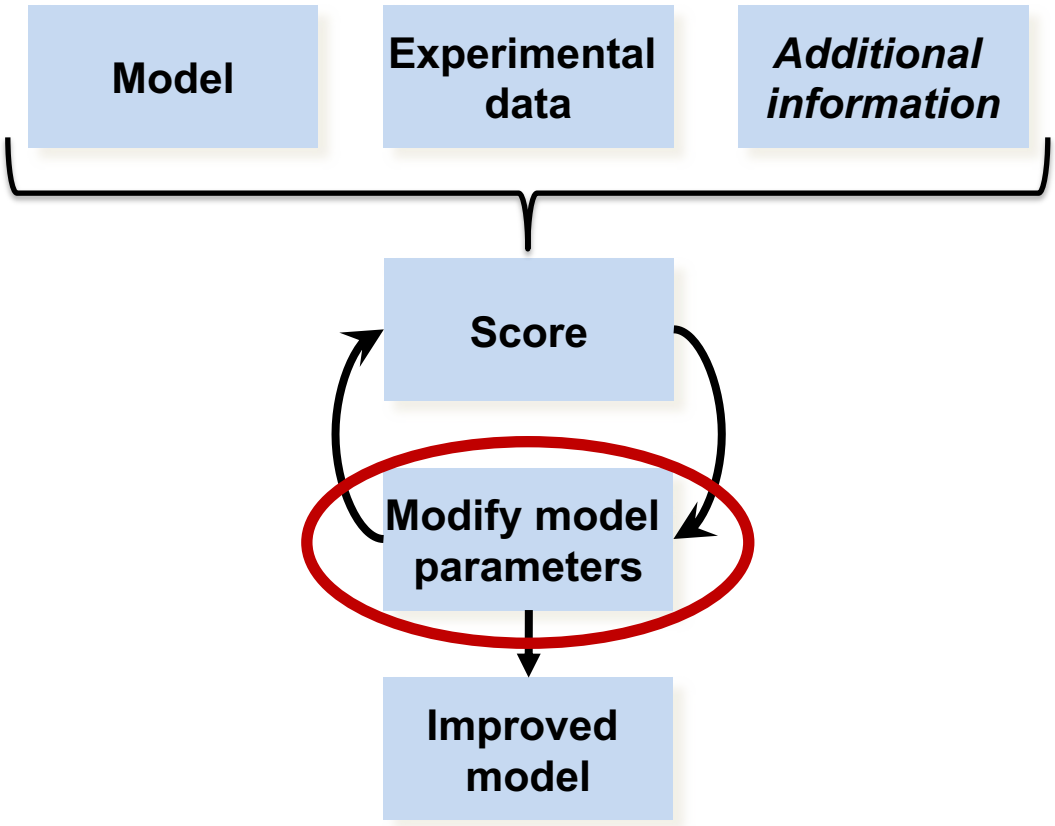


Source: Internet

- **Constraints**: molecules 1, 2 and 3 are required to be identical

- **Restraints**: molecules 1, 2 and 3 are required to be similar but not necessarily identical

Refinement

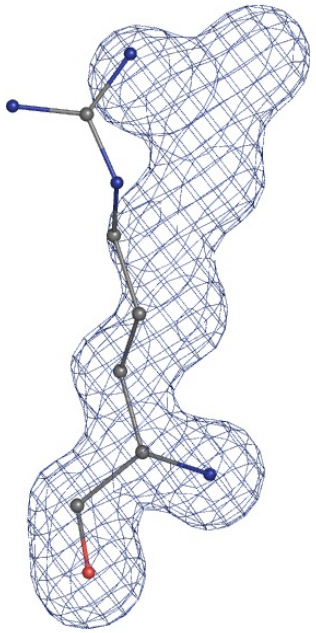

Model
Experimental data
Additional information
Score
Modify model parameters
Improved model

Choices of optimization method

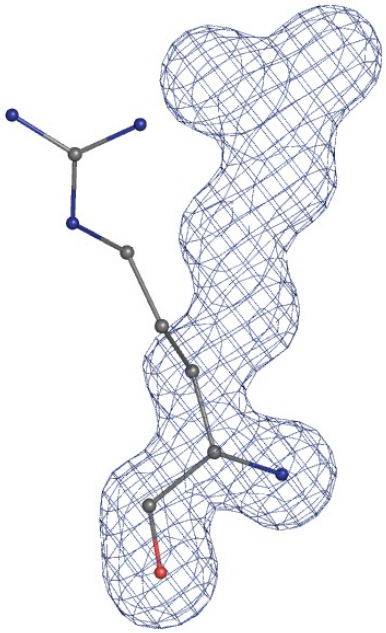
- Gradient-based minimization
- Simulated annealing
- Grid (systematic) searches
- Manual using molecular graphics programs (Coot, Chimera,…

Choice of refinement method and refinement convergence

Minimization

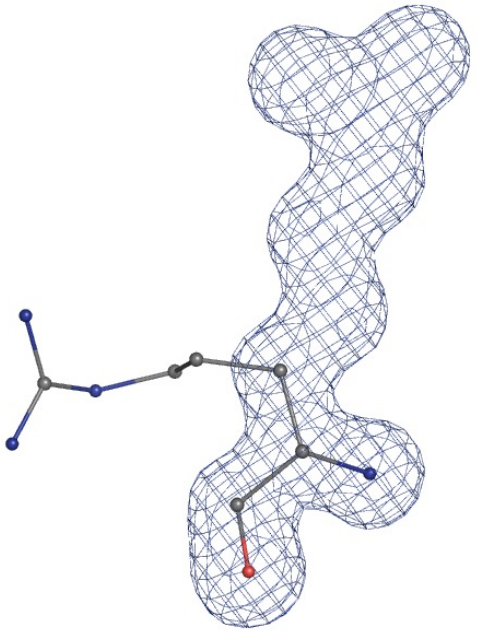


Simulated Annealing



Beyond convergence radius of minimization

Real-space grid search

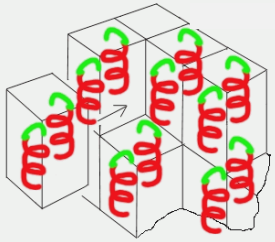


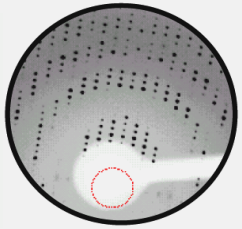
Beyond convergence radius of minimization and SA

Phenix tools for model refinement

Refinement

Crystallography





Initial model

Experimental data

A priori knowledge

Score

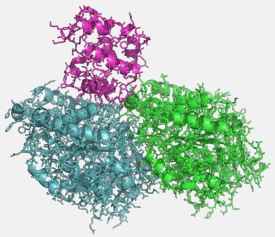
Modify model parameters

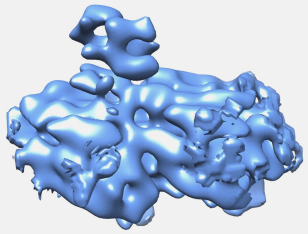
Improved model

phenix.refine

Available since 2005

Cryo-EM





Initial model

Experimental data

A priori knowledge

Score

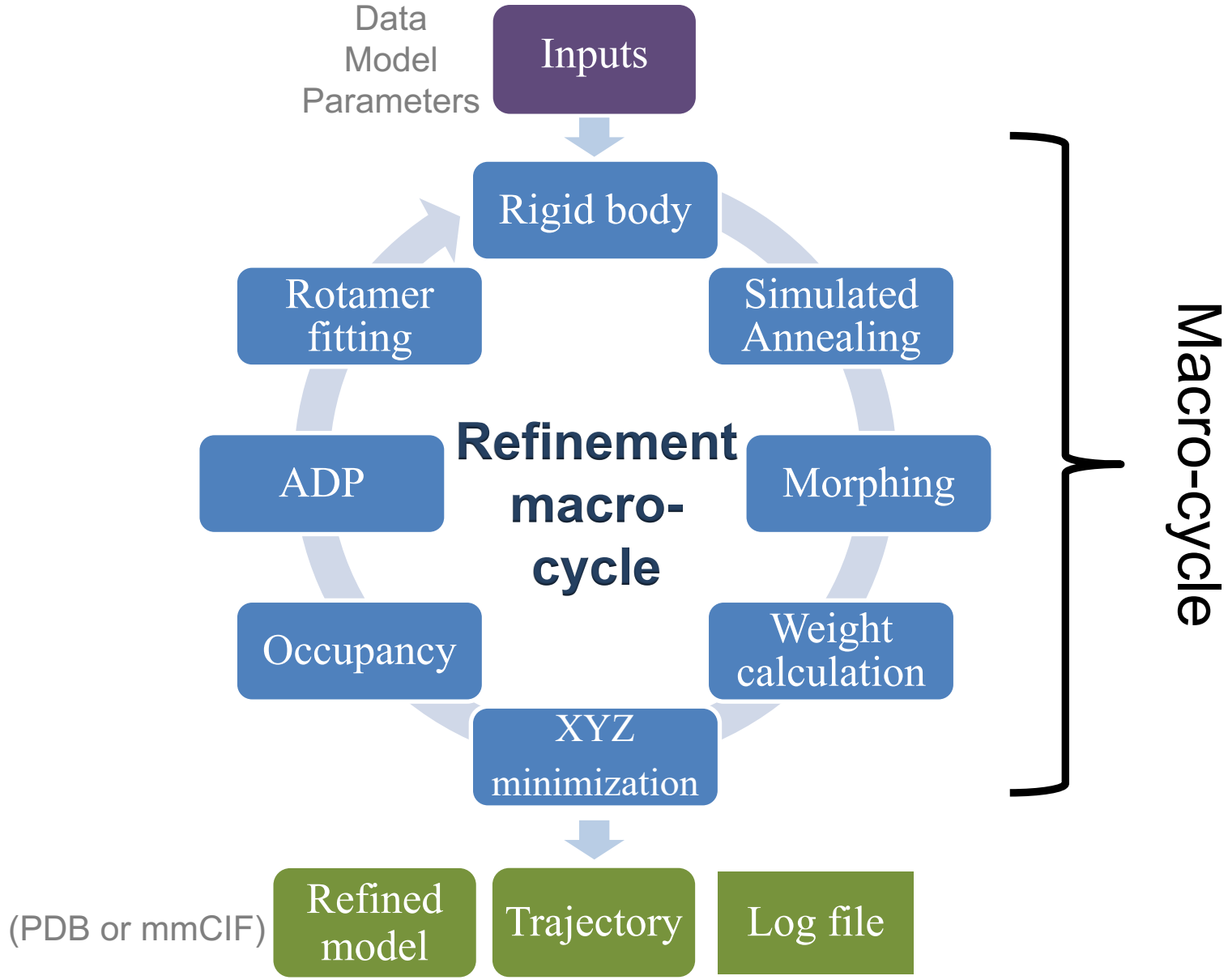
Modify model parameters

Improved model

phenix.real_space_refine

Available since 2013

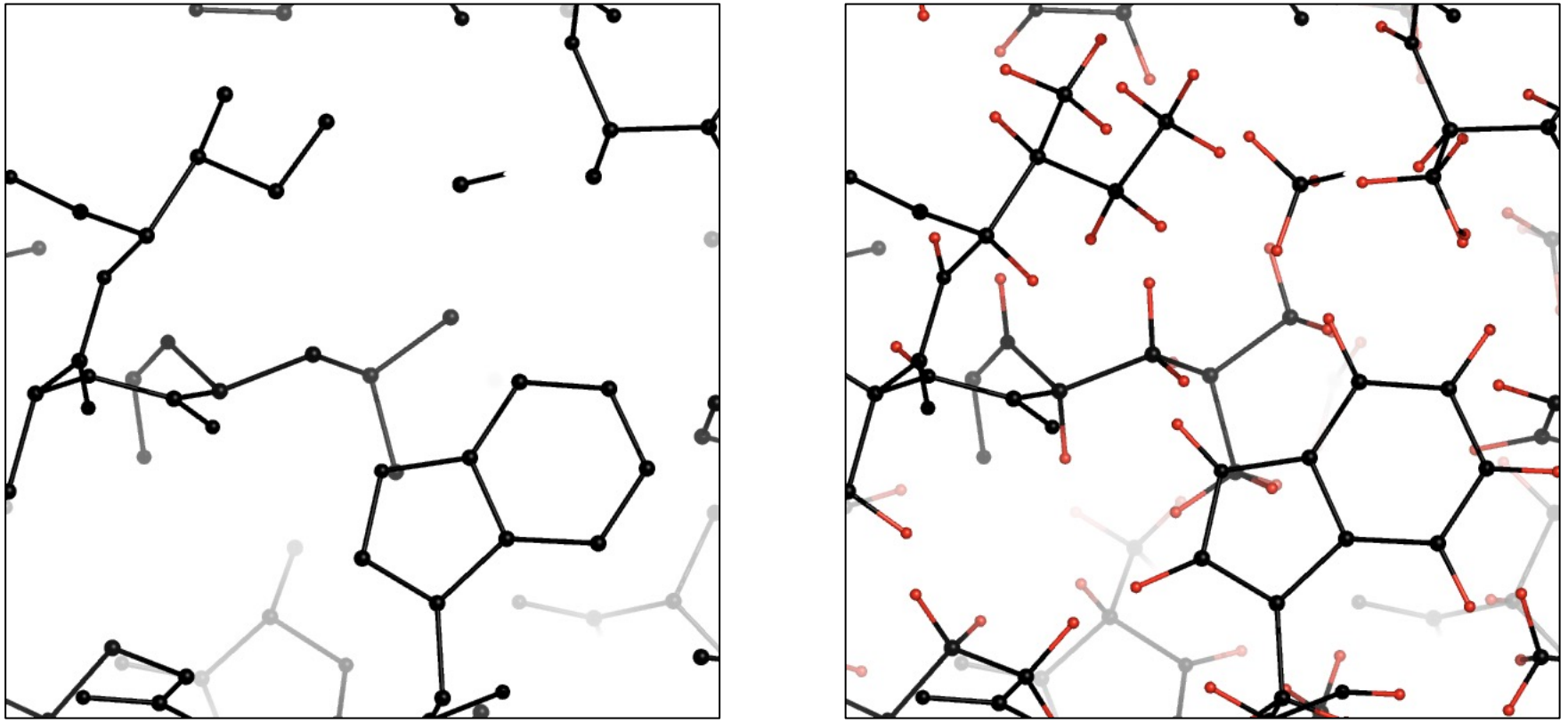
Refinement protocol



Refinement: practical considerations

Use Hydrogen atoms

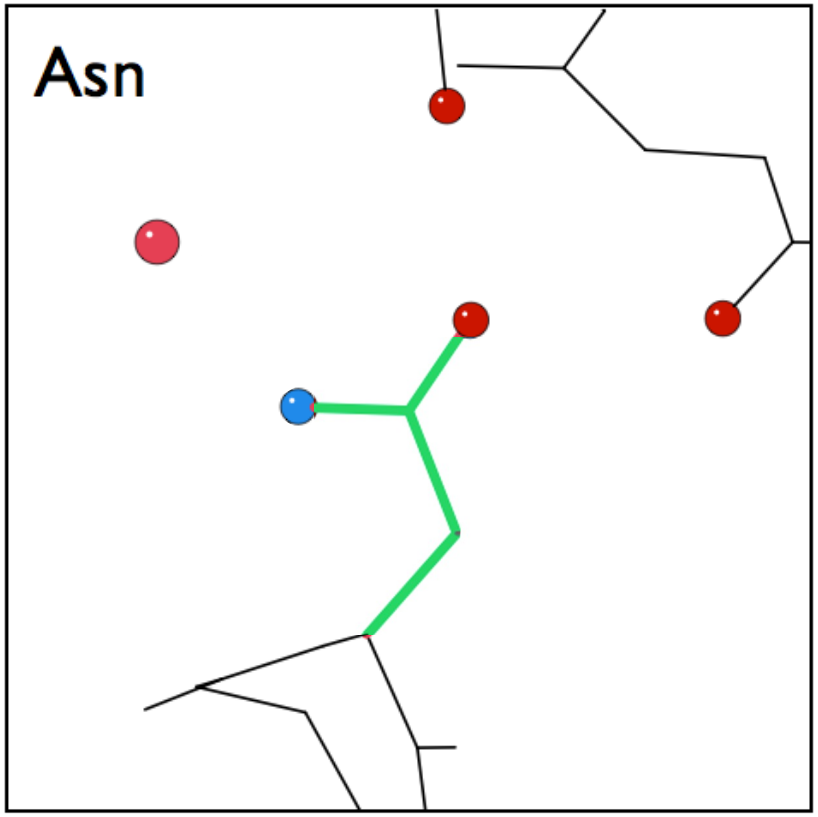
- Half of the atoms in a protein molecule
- Make most interatomic contacts
- Add to model towards the end, data resolution does not matter
- Once added, do not remove before the PDB deposition
- H do contribute to R-factors (expect 0.1-2% drop in R)

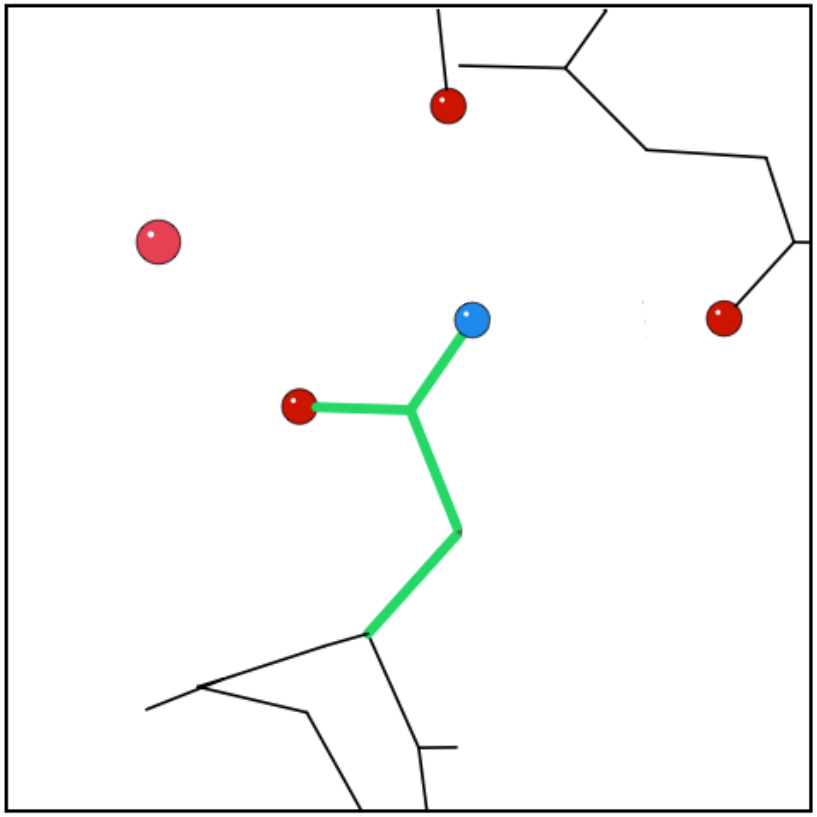


A structure without (left) and with (right) hydrogen atoms

Use Hydrogen atoms

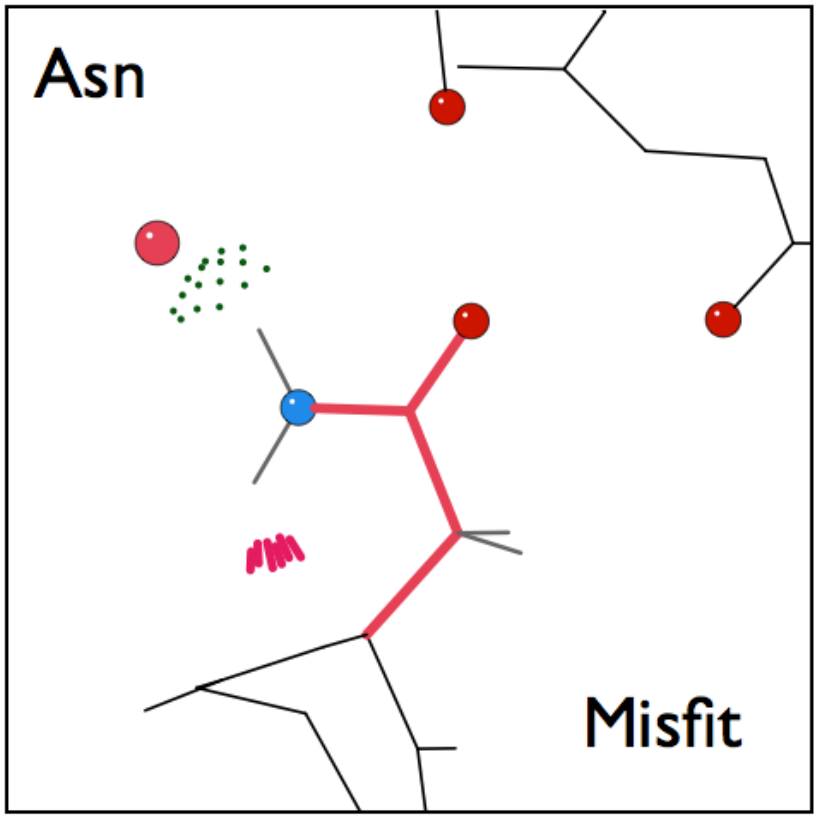
- N/Q/H flips (asparagine/glutamine/histidine)
  - Based on clash analysis
  - Requires H present

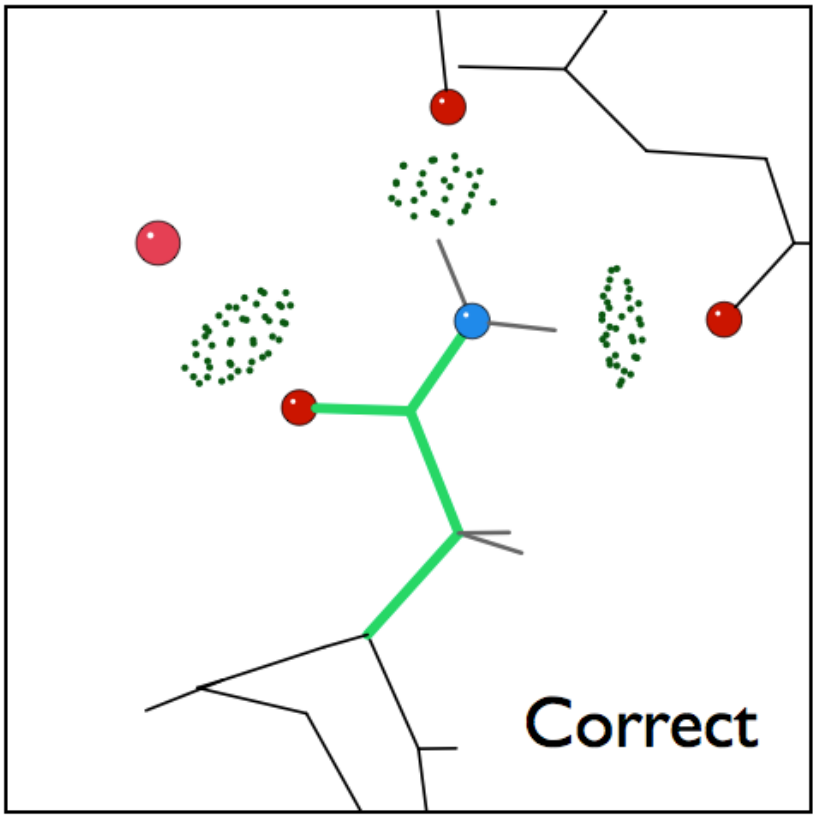




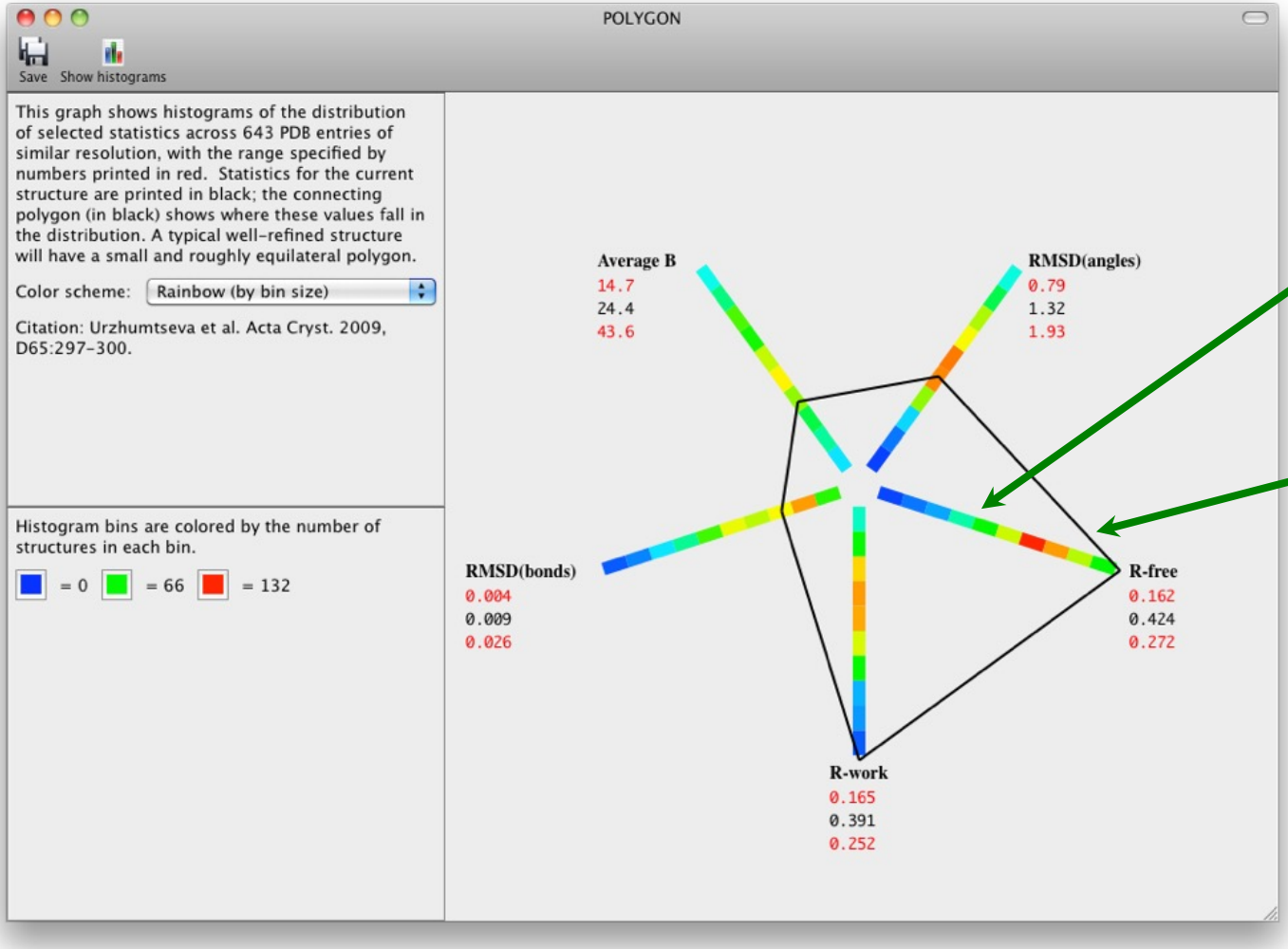
Use Hydrogen atoms

- N/Q/H flips
  - Based on clash analysis
  - Requires H present





Know when to stop



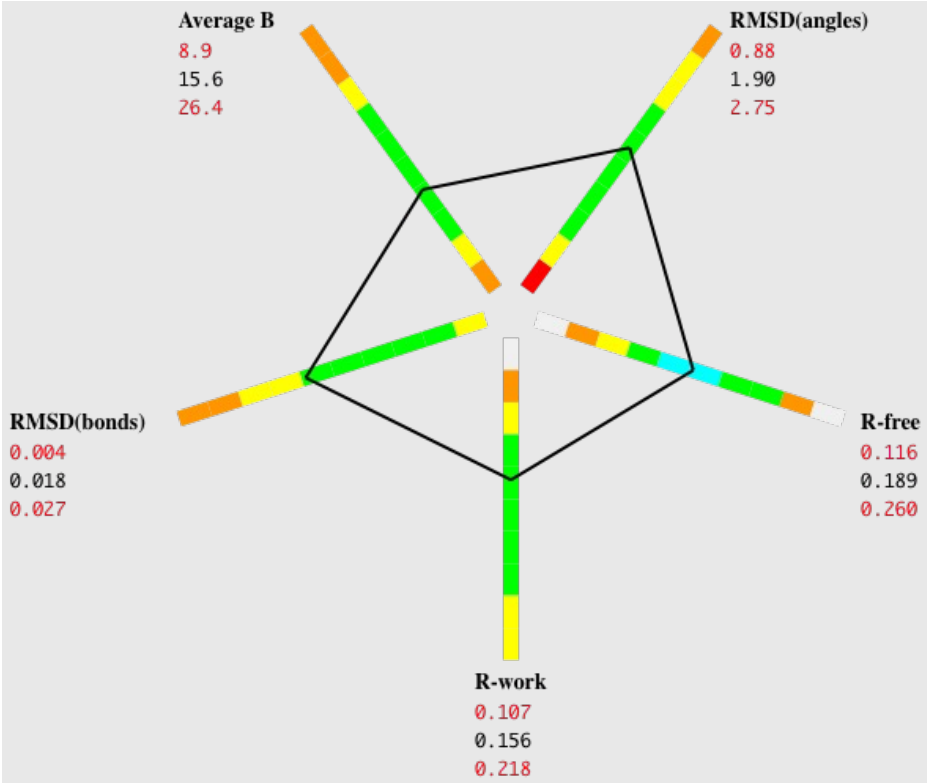
Colored bars are histograms showing distribution of values for structures at similar resolution

The black polygon shows where the statistics for the user's structure fall in each histogram

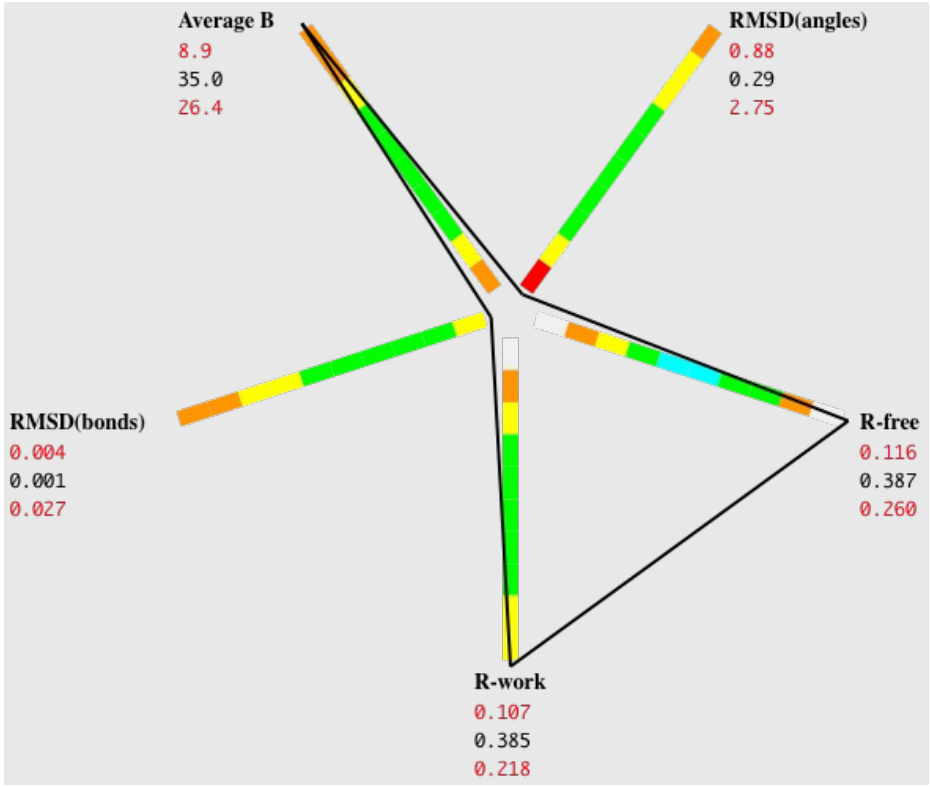
Crystallographic model quality at a glance.
L.Urzhumtseva, P.V.Afonine, P.D.Adams & A.Urzhumtsev. *Acta Cryst*. **D**65, 297-300 (2009)

Know when to stop

Likely overall good model

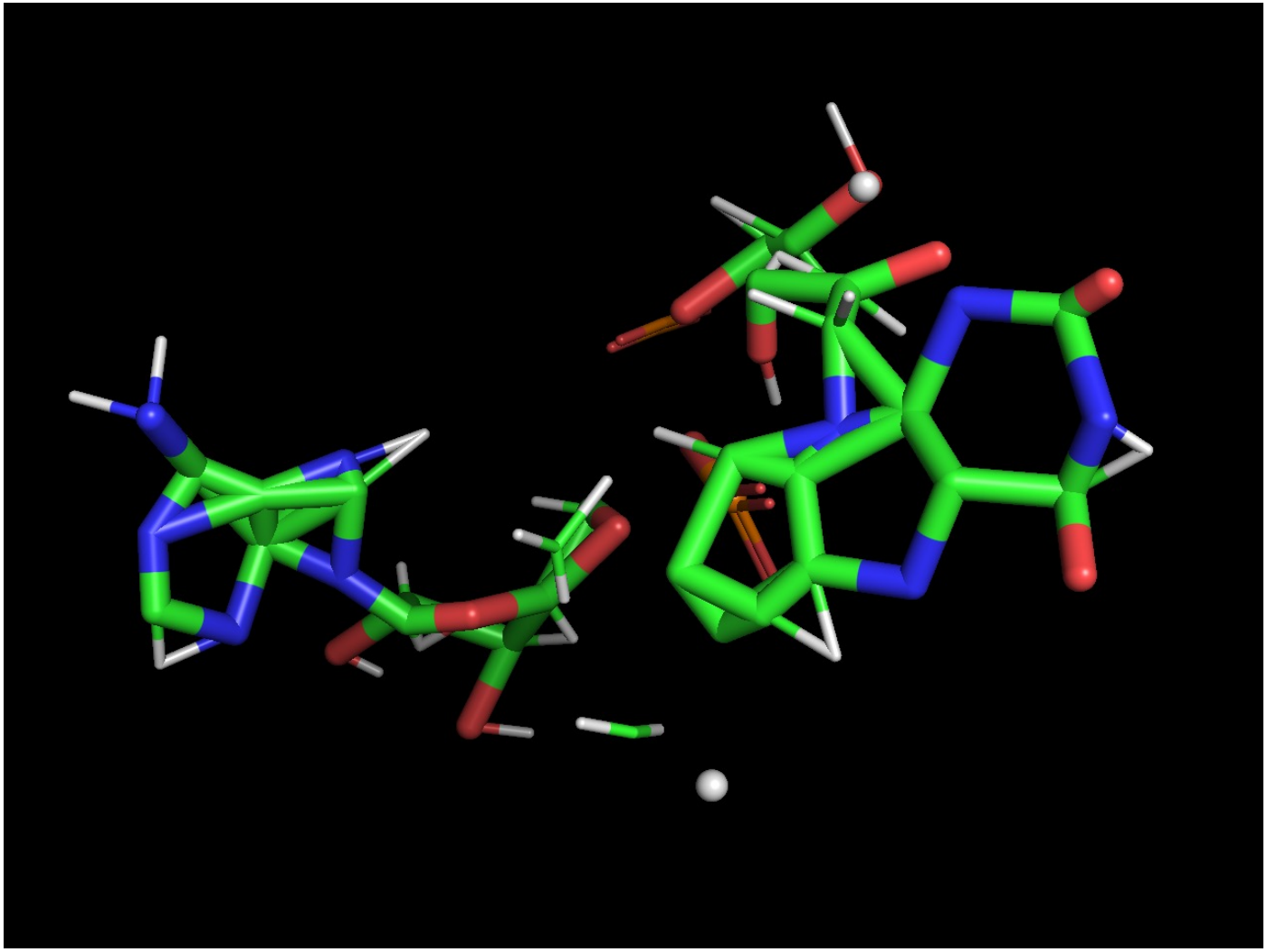


Clearly there are problems

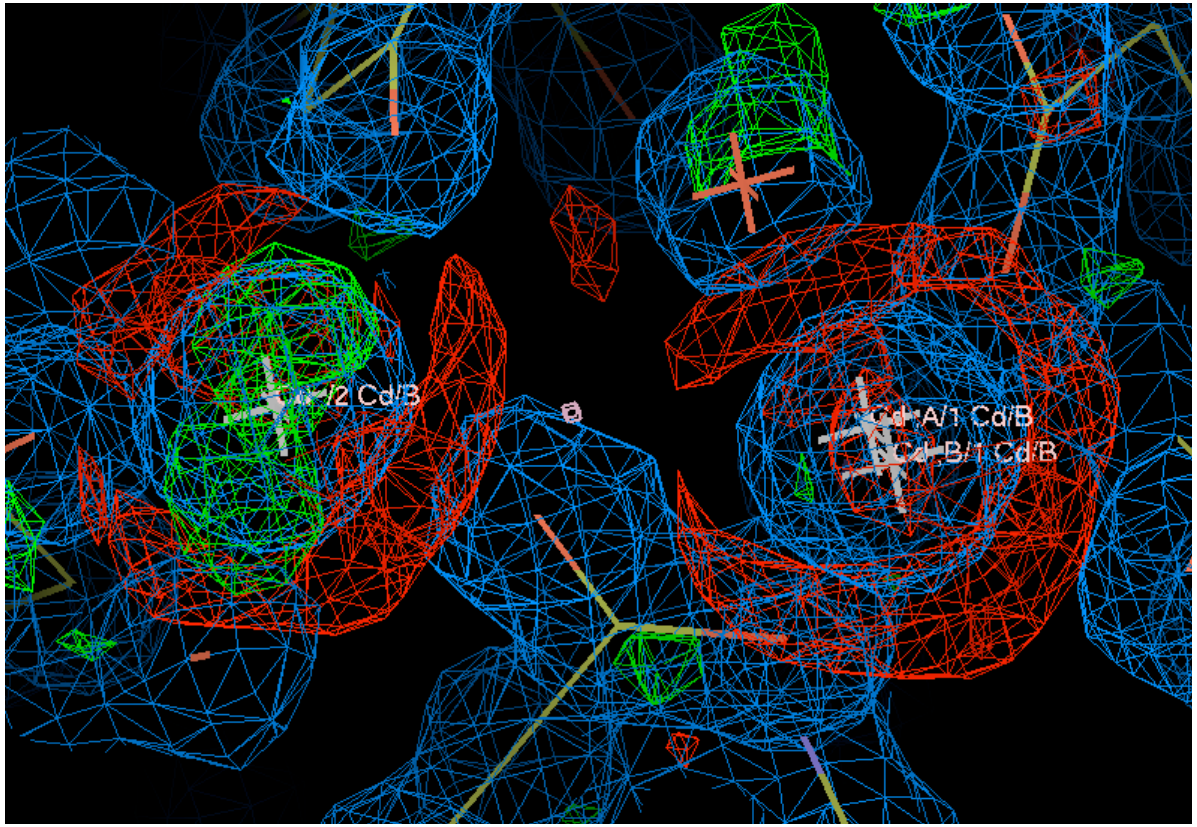


Local *vs* Global

- R_{WORK}/R_{FREE} , bond/angle RMSDs etc do not report on local errors



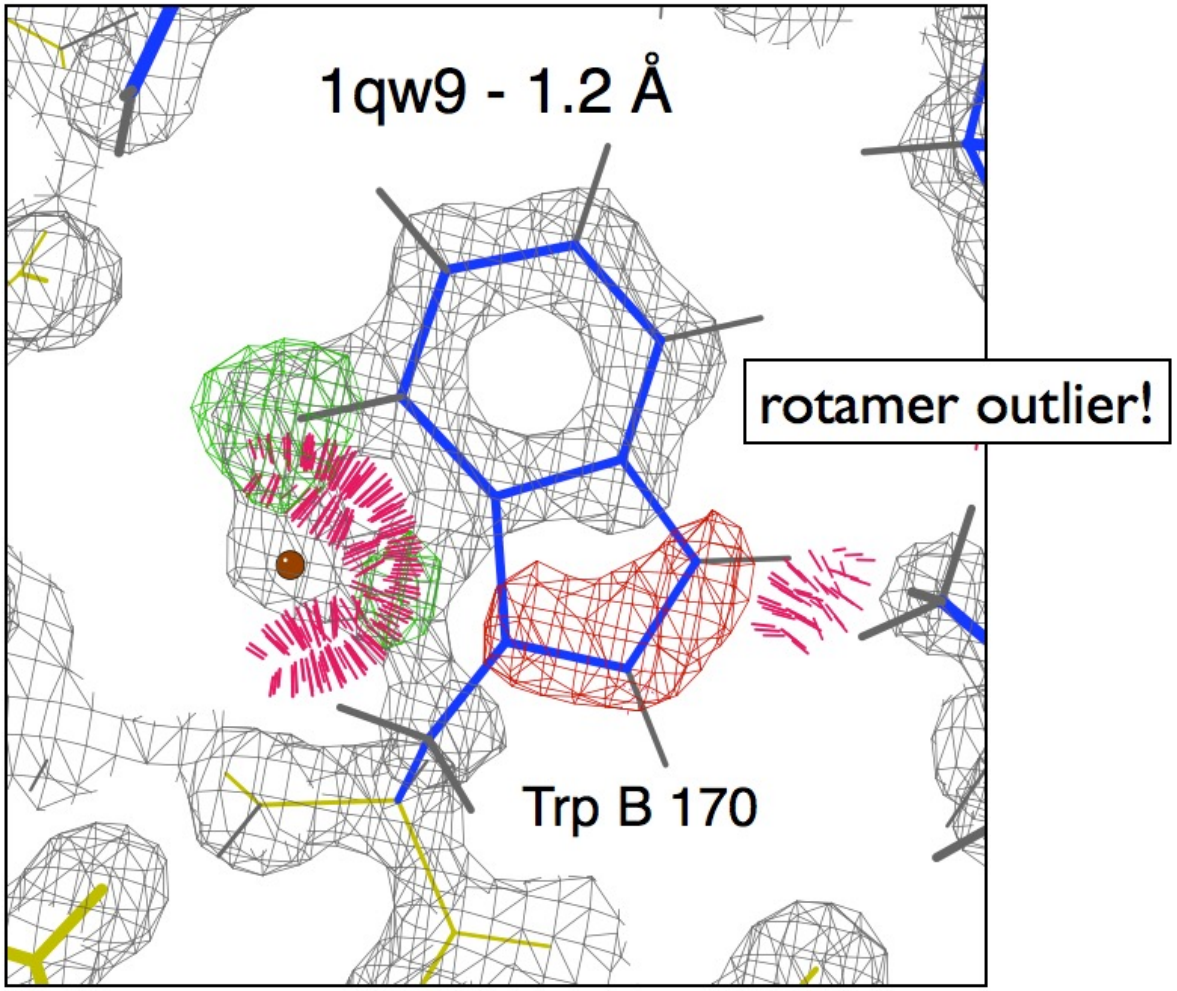
Map and model errors



Reasons for +ve/-ve density:

- Suboptimal xyz, occupancy, ADP, anomalous f' & f'', charge
- Refinement has not reached convergence
- Wrong atom (ion)
- Suboptimal ADP (B-factor) type: isotropic vs anisotropic
- **NEW** `phenix.oat is the new tool to help with this`

Not all modeling errors can be fixed by refinement



Low resolution (3Å or worse)

- Use:
  - Ramachandran plot restraints
  - Secondary structure restraints
  - Reference model restraints (if quality homology model is available)
  - NCS (restraints or constraints)

Aggressive optimization methods

- Simulated annealing (SA)
- Model morphing
  - Only use if model has gross errors (correction requires large movements)
  - Do not use if model is relatively good and only needs small corrections

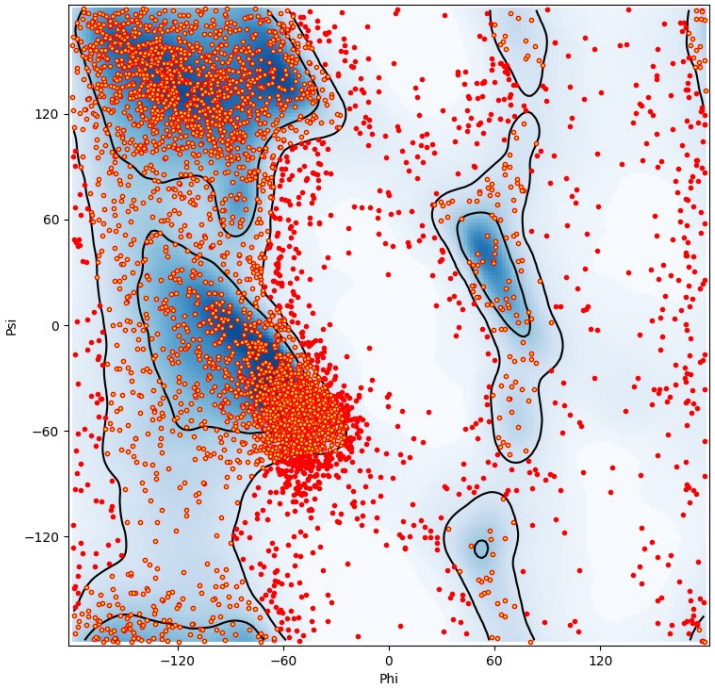
Ramachandran plot restraints

- Likely need at about 3Å and worse
- Better than 3Å: use if needed (preserve good initial model from deterioration)
- Check Ramachandran plot regularly
- Don’t use to fix outliers. Fix outliers first (manually), then use Ramachandran plot restraints to stop re-occurring outliers

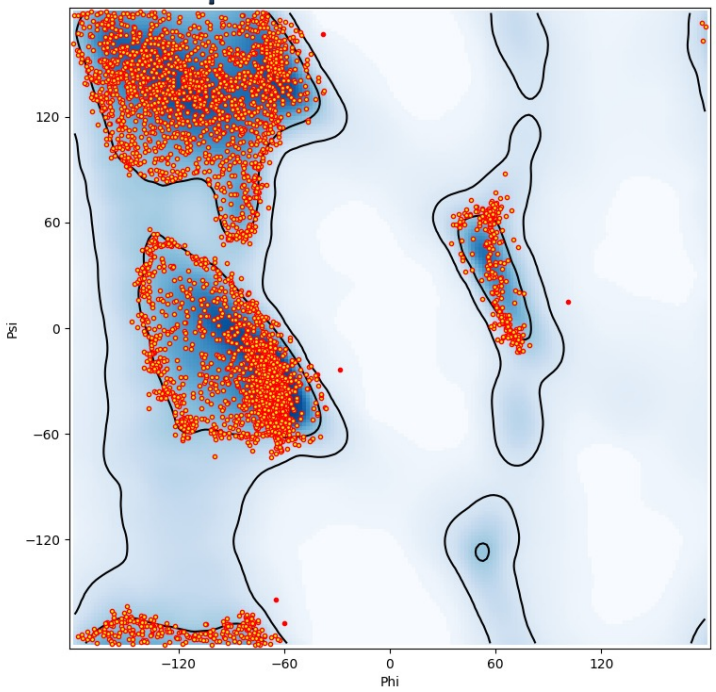
Ramachandran plot restraints

PDB code: 5a9z

Original



Refined with Ramachandran plot restraints

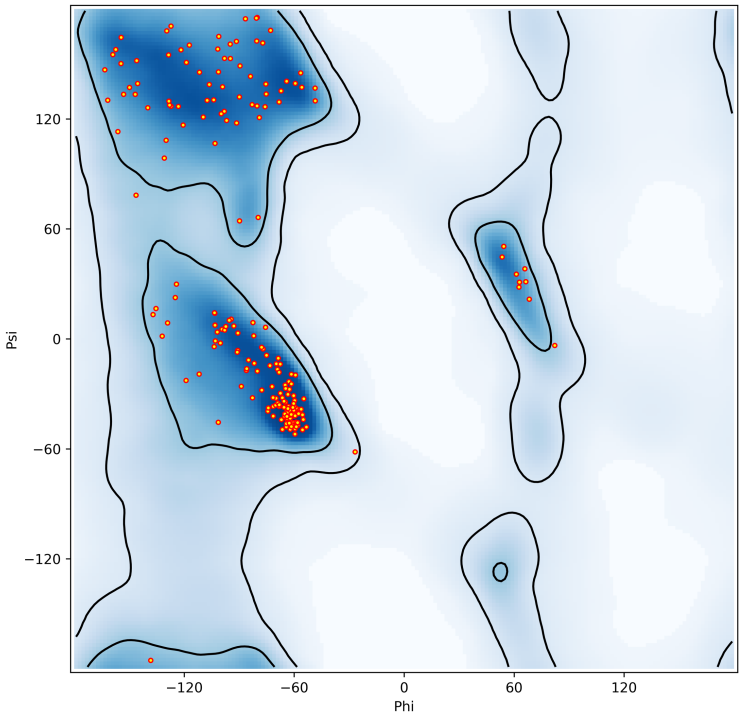


Bad idea to use Ramachandran plot restraints in this case. Fix outliers first!

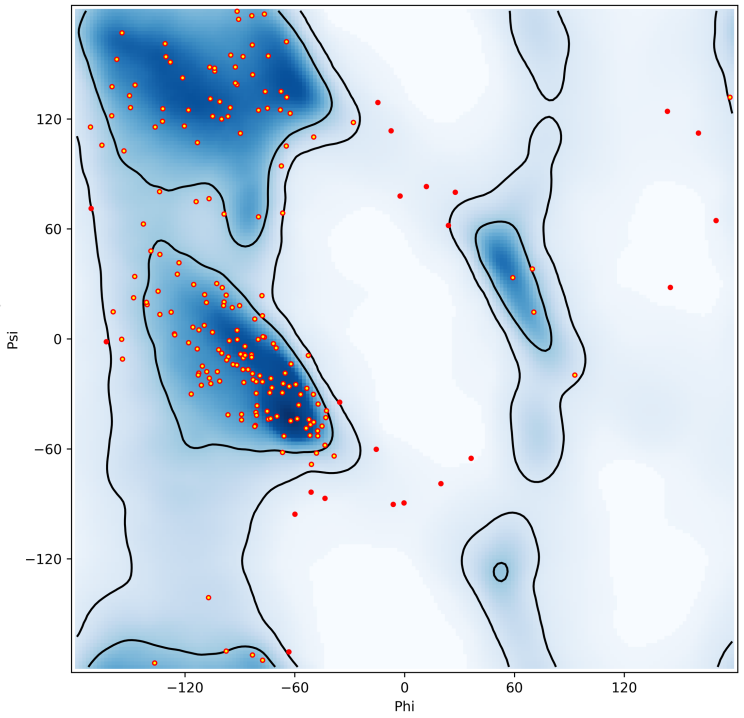
Ramachandran plot restraints

- Ramachandran plot restraints
  - Use to stop outliers from occurring

Before refinement



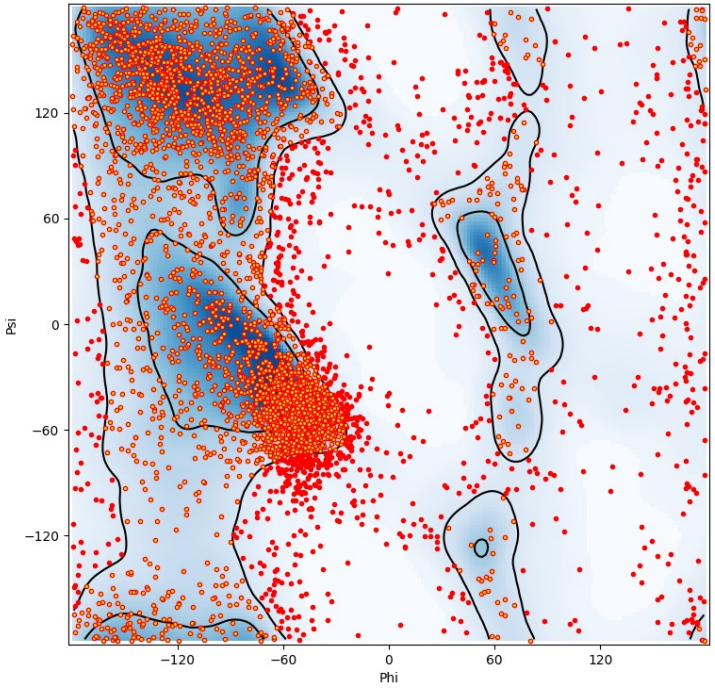
After refinement (No Ramachandran plot restraints)



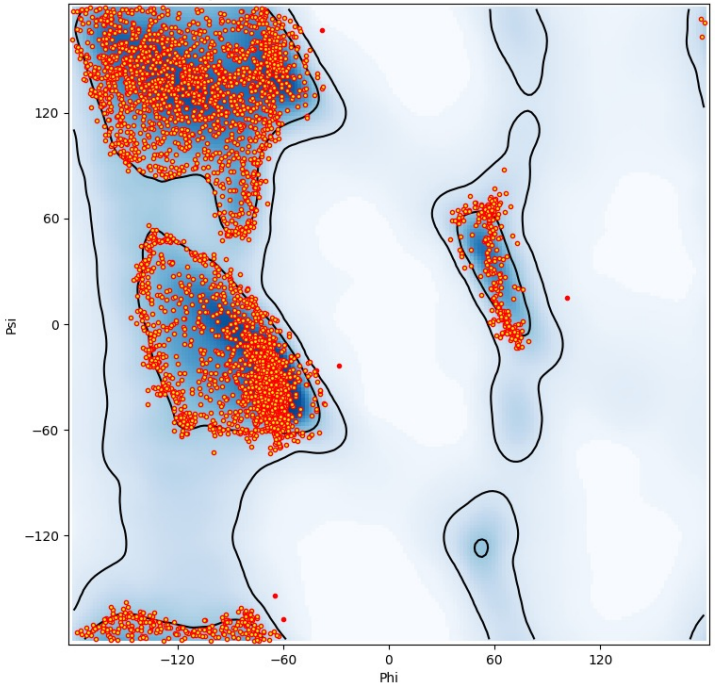
Ramachandran plot restraints

- What is wrong with this plot?

Original

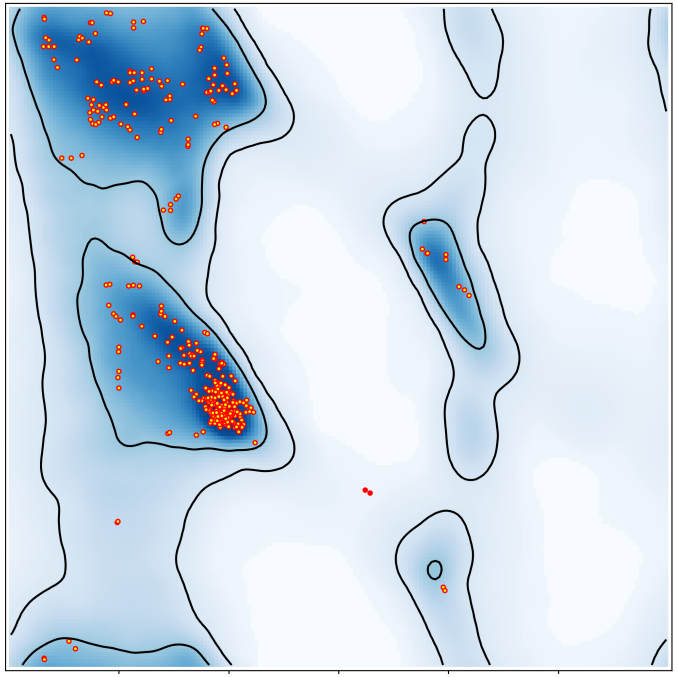


Refined with Ramachandran plot restraints



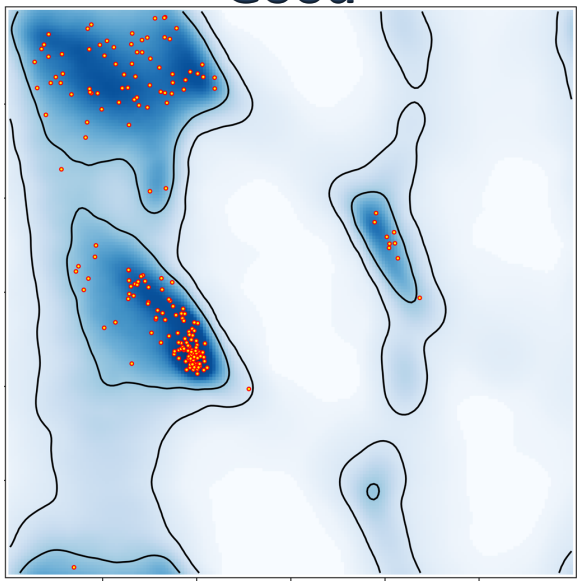
Ramachandran plot restraints

- It is very different from what we expect!

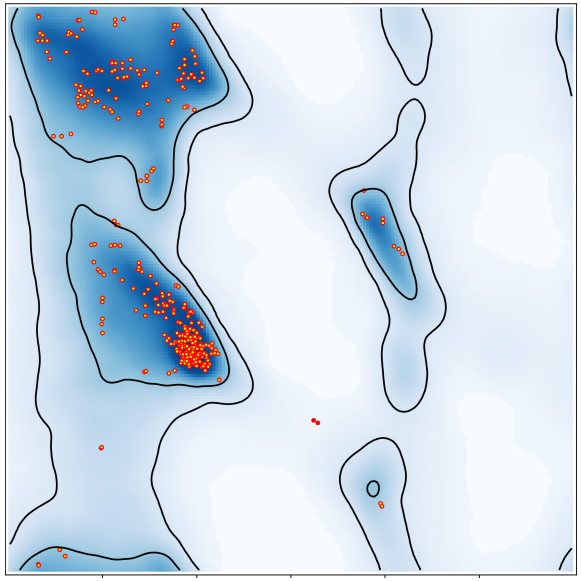


How you can tell good vs bad plot?

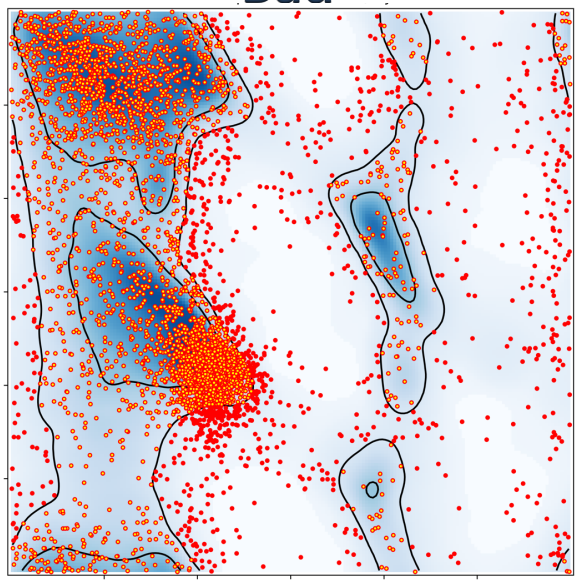
Good



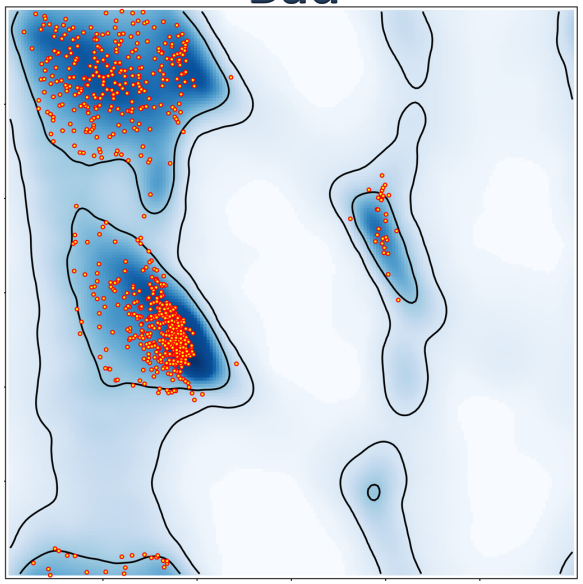
Good



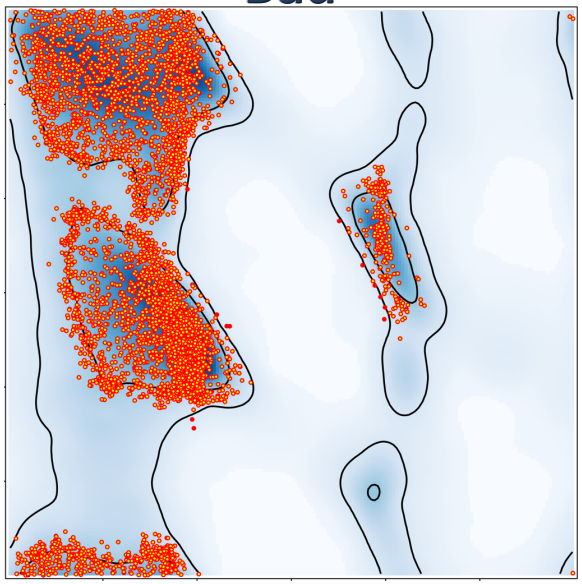
Bad



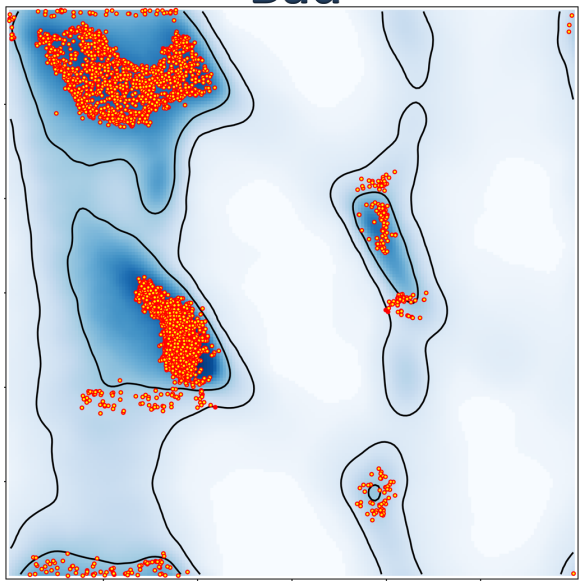
Bad



Bad



Bad



Ramachandran plot Z-score

CABIOS

Vol. 13 no. 4 1997
Pages 425–430

Objectively judging the quality of a protein structure from a Ramachandran plot

Rob W.W.Hooft, Chris Sander and Gerrit Vriend

- Good at spotting odd plots
- One number, simple criteria:
  - Poor: $|Z| > 3$ Suspicious: $2 < |Z| < 3$ Good: $|Z| < 2$

Structure

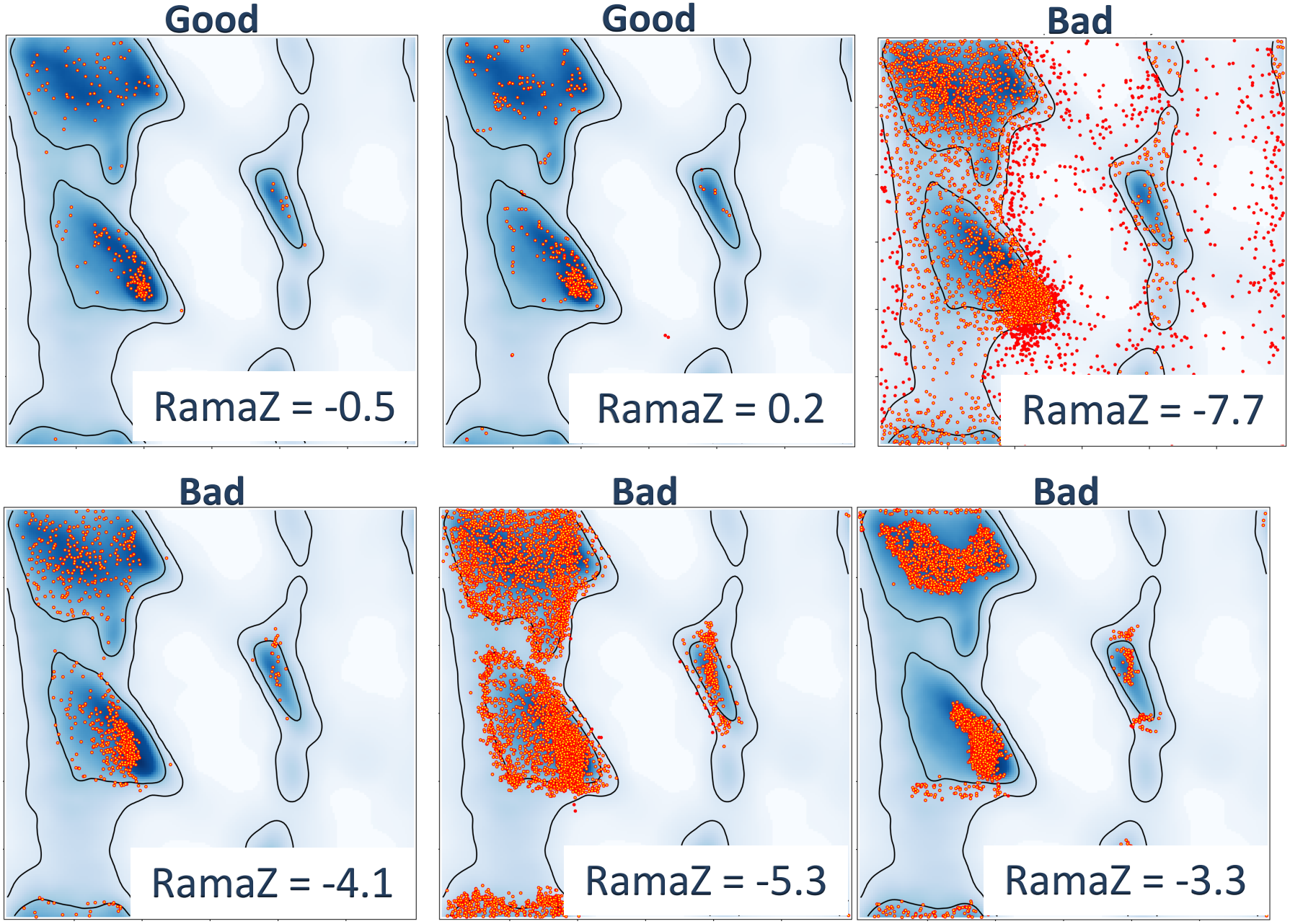
CellPress

Resource

A Global Ramachandran Score Identifies Protein Structures with Unlikely Stereochemistry

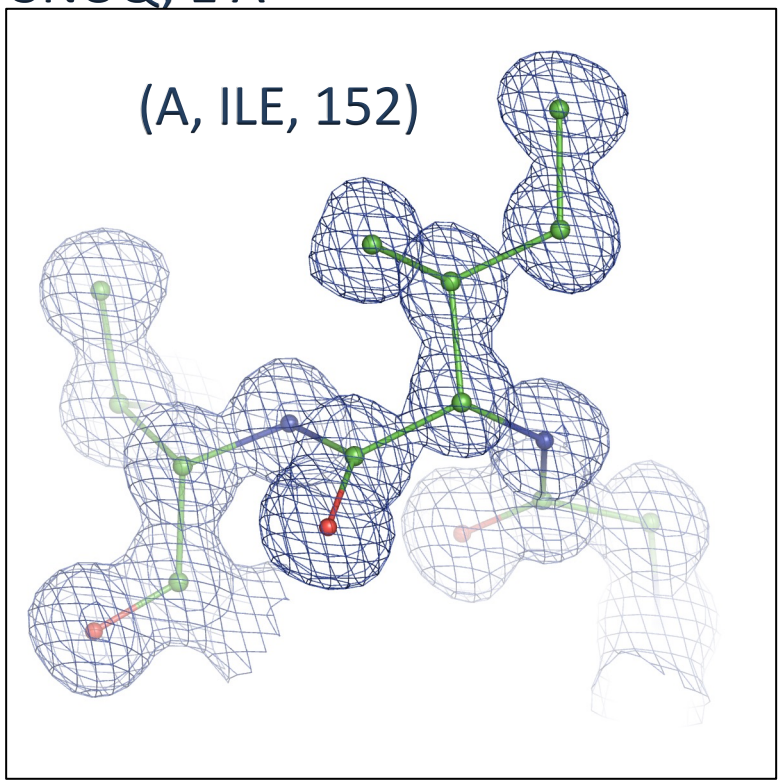
Oleg V. Sobolev,[1,5,*] Pavel V. Afonine,[1] Nigel W. Moriarty,[1] Maarten L. Hekkelman,[2,3] Robbie P. Joosten,[2,3,*] Anastassis Perrakis,[2,3] and Paul D. Adams[1,4]

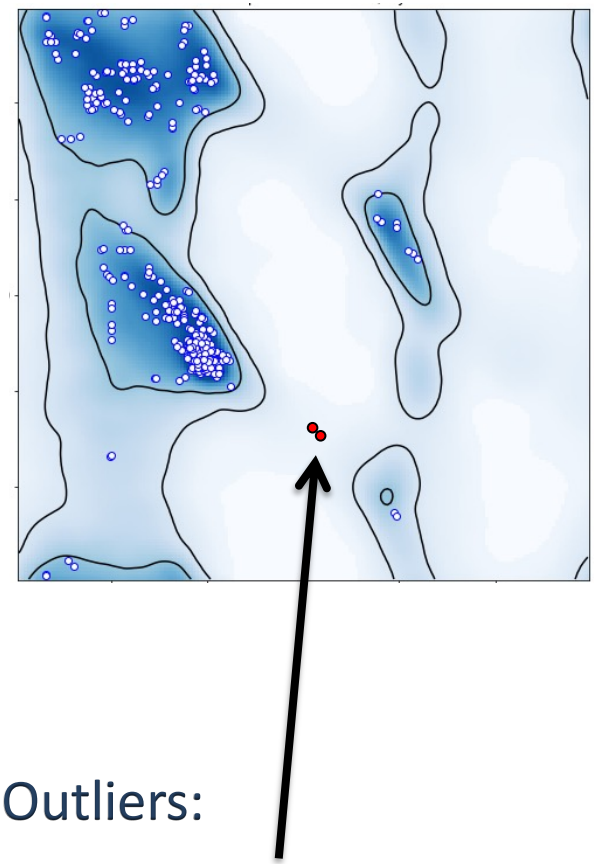
Model validation: *Ramachandran plot Z-score*



An outlier ≠ wrong

3NOQ, 1 Å





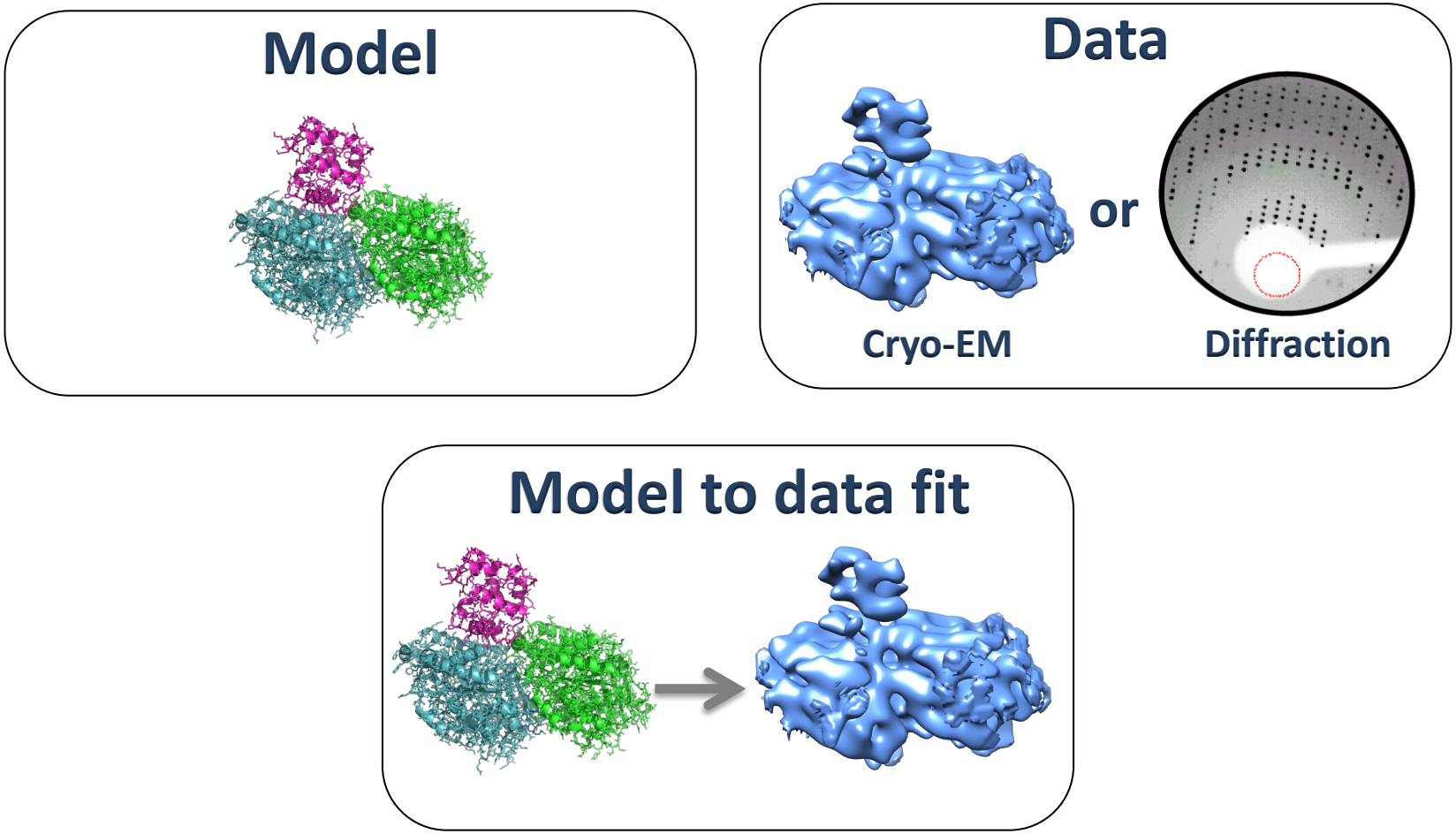
Outliers:

(A, ILE, 152), (B, ILE, 154)

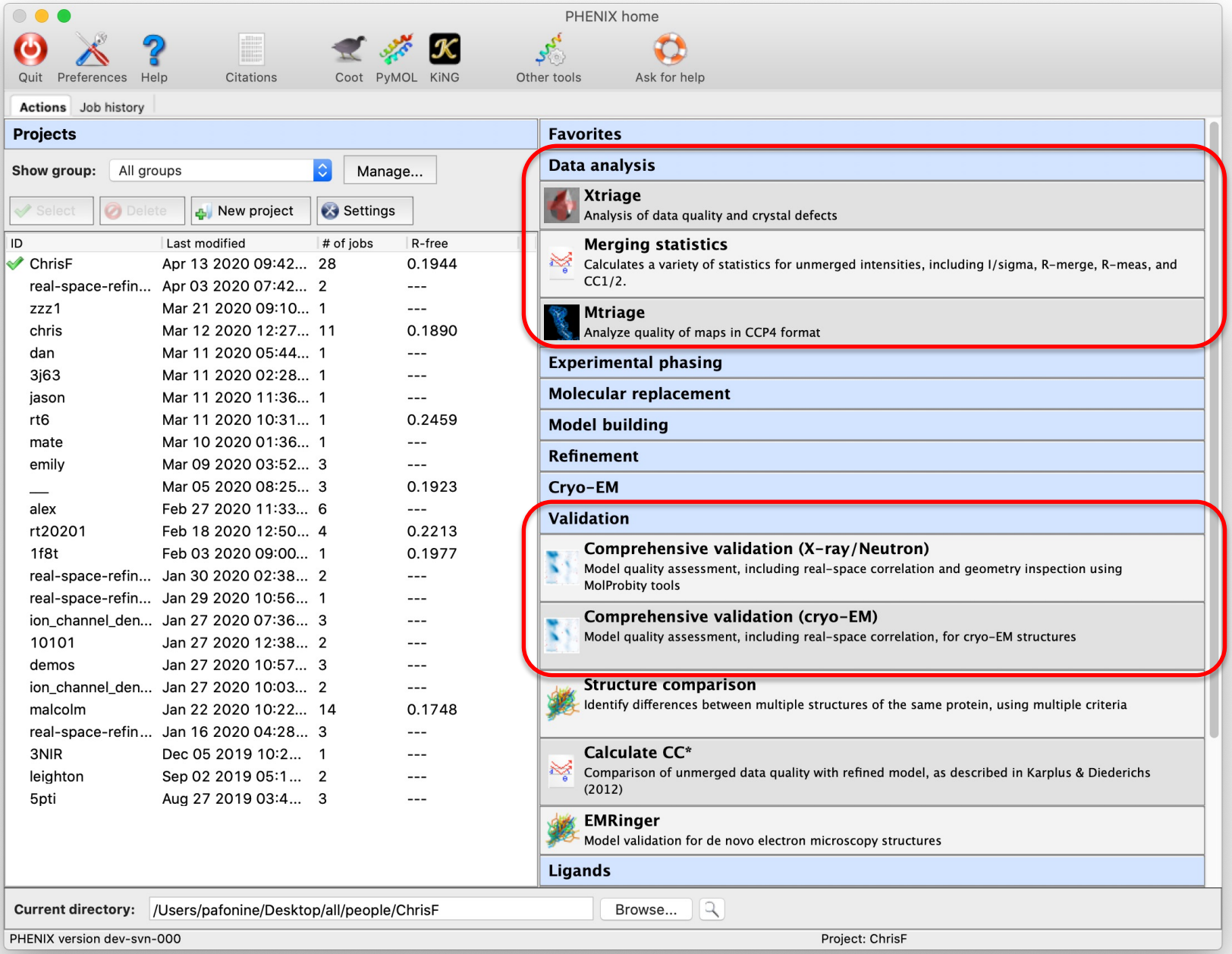
- All outliers need to be explained (supported by the data)

Refinement success is function of data quality

- Do validation



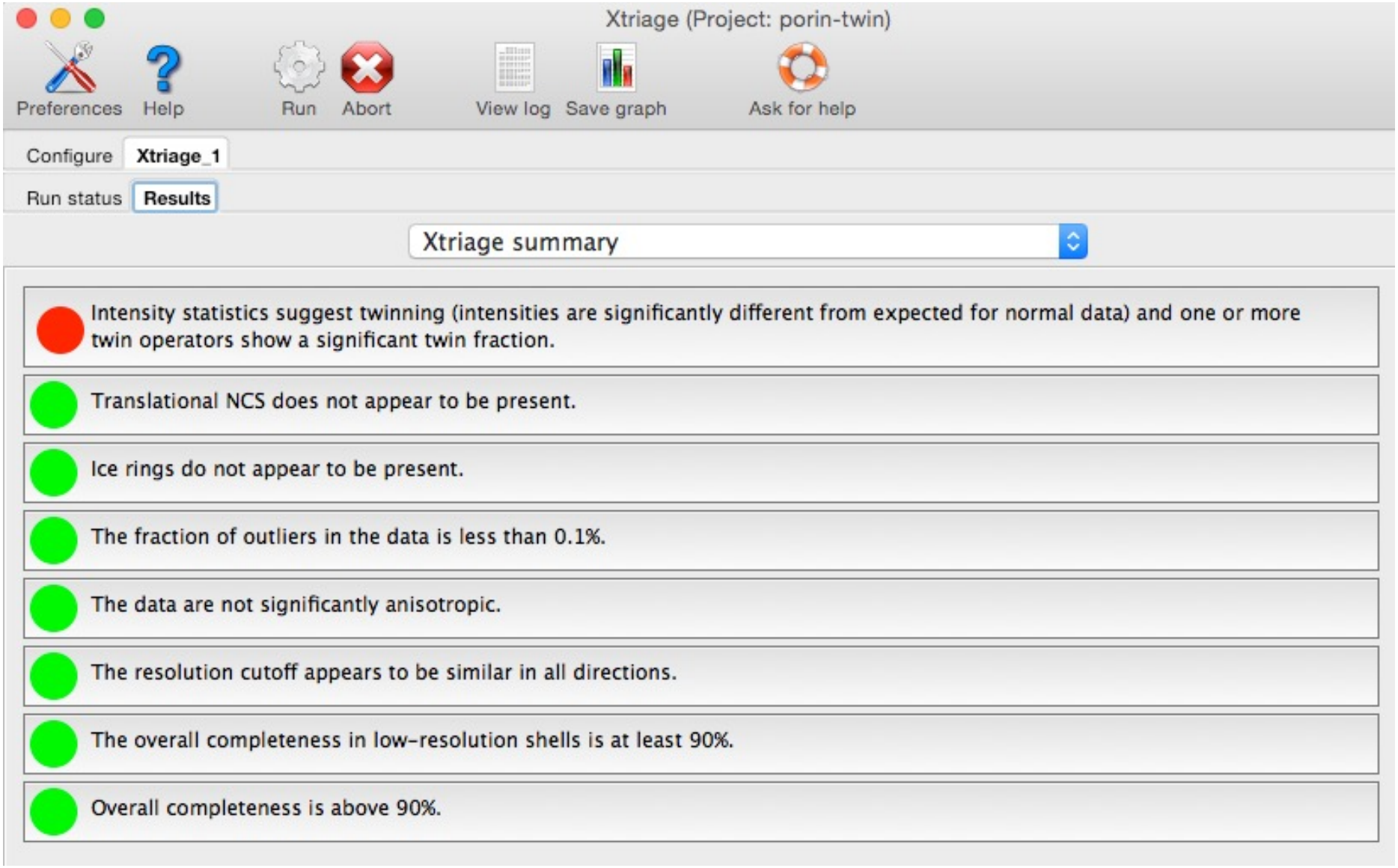
Validation tools in Phenix



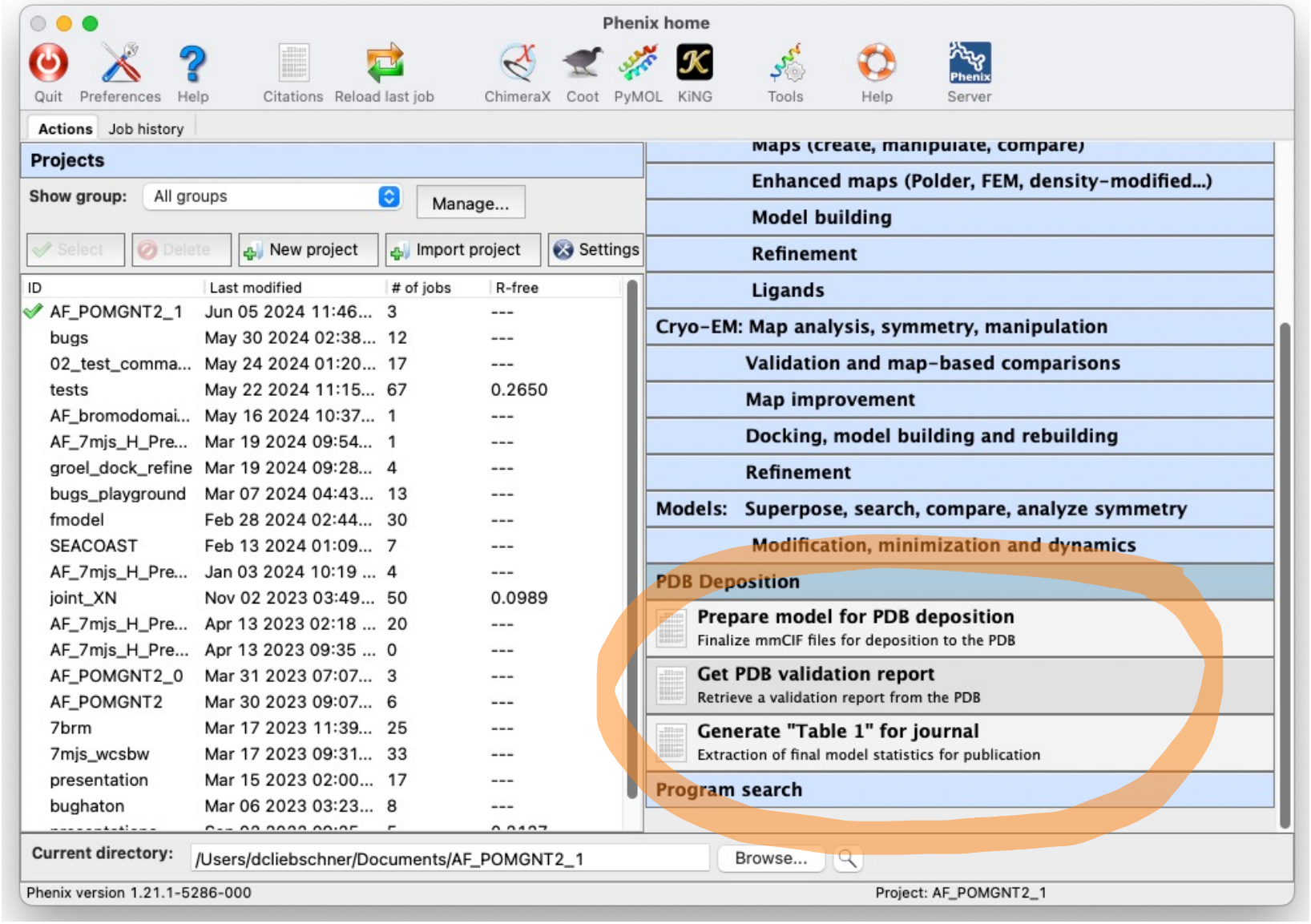
Xtriage: all about your diffraction data

- Matthews coefficient probabilities
- Completeness by resolution
- Wilson plot sanity
- Detection of translational NCS (tNCS)
- Analysis of systematic absences and combination of tNCS with current space group
- Anomalous signal from measurability analysis
- Symmetry and twinning analyses
- Alternative point-group symmetry (can be detected on the basis of an R-value analyses)

Xtriage


Xtriage (Project: porin-twin)
Preferences
Help
Run
Abort
View log
Save graph
Ask for help
Configure
Xtriage_1
Run status
Results
Xtriage summary
Intensity statistics suggest twinning (intensities are significantly different from expected for normal data) and one or more twin operators show a significant twin fraction.
Translational NCS does not appear to be present.
Ice rings do not appear to be present.
The fraction of outliers in the data is less than 0.1%.
The data are not significantly anisotropic.
The resolution cutoff appears to be similar in all directions.
The overall completeness in low-resolution shells is at least 90%.
Overall completeness is above 90%.

PDB deposition



PDB deposition

mmCIF format is mandatory for deposition as of 2019

ISSN 2059-7983

Announcing mandatory submission of PDBx/mmCIF format files for crystallographic depositions to the Protein Data Bank (PDB)

Paul D. Adams,[a,b] Pavel V. Afonine,[a] Kumaran Baskaran,[c] Helen M. Berman,[d] John Berrisford,[e] Gerard Bricogne,[f] David G. Brown,[g] Stephen K. Burley,[d,h,i]* Minyu Chen,[j] Zukang Feng,[d] Claus Flensburg,[f] Aleksandras Gutmanas,[e] Jeffrey C. Hoch,[k]* Yasuyo Ikegawa,[j] Yumiko Kengaku,[j] Eugene Krissinel,[l] Genji Kurisu,[j]* Yuhe Liang,[d] Dorothee Liebschner,[a] Lora Mak,[e] John L. Markley,[c]* Nigel W. Moriarty,[a] Garib N. Murshudov,[m] Martin Noble,[n] Ezra Peisach,[d] Irina Persikova,[d] Billy K. Poon,[a] Oleg V. Sobolev,[a] Eldon L. Ulrich,[c] Sameer Velankar,[e]* Clemens Vonrhein,[f] John Westbrook,[d] Marcin Wojdyr,[f,l] Masashi Yokochi[j] and Jasmine Y. Young[d]

Received 21 February 2019
Accepted 3 April 2019

Edited by R. J. Read, University of Cambridge, England

PDB deposition: mmCIF facts

- Contains a lot more information than PDB
- Not intended to be human editable
  - You can read it but it is (much) harder than PDB
- Phenix tools generally produce output in mmCIF format
- Avoid editing by hand
  - Easy to make hard-to-recover mistakes

PDB deposition: CIF file confusion

- CIF is a file format

- CIF file can contain:
  - Ligand information
  - Atomic model
  - Reflection data
  - Any mixture of three above

PDB deposition: dos and don’ts

- Do not change the content of files from refinement for any reason:
  - Add/remove atoms (hydrogens, water)
  - Edit labels, header information

- Run Comprehensive validation (Phenix GUI) to address all outstanding issues before deposition

- Don’t panic if validation statistics reported by Phenix does not match PDB validation report
  - If that happens and presents a problem – start conversation with PDB stuff and involve Phenix developers

- Once all is deposited and up on the web – check everything: mistakes at PDB end happen

User support

- **Feedback, questions, help**

  Mailing list (anyone signed up): phenixbb@phenix-online.org
  Bug reports (developers only): bugs@phenix-online.org
  Ask for help (developers only): help@phenix-online.org

- **Reporting a bug or asking for help:**
  - We can't help you if you don't help us to understand your problem
  - Make sure the problem still exist using the latest *Phenix* version
  - Send us all inputs (files, non-default parameters) and tell us steps that lead to the problem
  - All data sent to us is kept confidentially

The Phenix Project

Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine, Dorothee Liebschner, Nigel Moriarty, Billy Poon, Christopher Schlicksup, Oleg Sobolev

Los Alamos National Laboratory
New Mexico Consortium

Tom Terwilliger, Li-Wei Hung

UTHealth

Matt Baker, Corey Hyrc

University of Cambridge

Randy Read, Airlie McCoy, Tristan Croll, Claudia Millán Nebot, Rob Oeffner

Duke University

Jane & David Richardson, Christopher Williams, Vincent Chen

An NIH/NIGMS funded Program Project

Liebschner D, *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. Acta Cryst. 2019 **D75**:861–877