



COMPUTATIONAL CRYSTALLOGRAPHY INITIATIVE

Crystallographic Structure Validation and Relevant Phenix Tools

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PHYSICAL BIOSCIENCES DIVISION



Comprehensive validation — why?

- Problems detected early can save a lot of time later
- Subjectivity in data interpretation
 - **Human interpret the maps:** experience, skills, pressure
- Human program the software
 - **Programs may contain bugs**
- Subjectivity in model building and refinement
 - **Model parameterization, target weights, starting points**
 - **Luck of data = multiple possibilities for interpretation**
- Post-refinement pre-deposition manipulations
 - **Hand editing files:** removing waters, hydrogens, ANISOU
- Misusing quality metrics
 - **Choose single water or decide about twinning using R-factor**
- Fraud or honest mistakes



Comprehensive validation — quality filters

- Crystallographer (you)
 - Software you use
 - Your boss
 - Reviewers (of your paper)
 - PDB deposition (software and people)
 - Community (those who eventually may come across of your structure and use it)
- Unnoticed (intentionally or not) problems
 - Likely to be discovered anyway, sooner or later
 - There may be negative consequences

Retraction: Cocrystal structure of synaptobrevin-II bound to botulinum neurotoxin type B at 2.0 Å resolution

Michael A Hanson & Raymond C Stevens

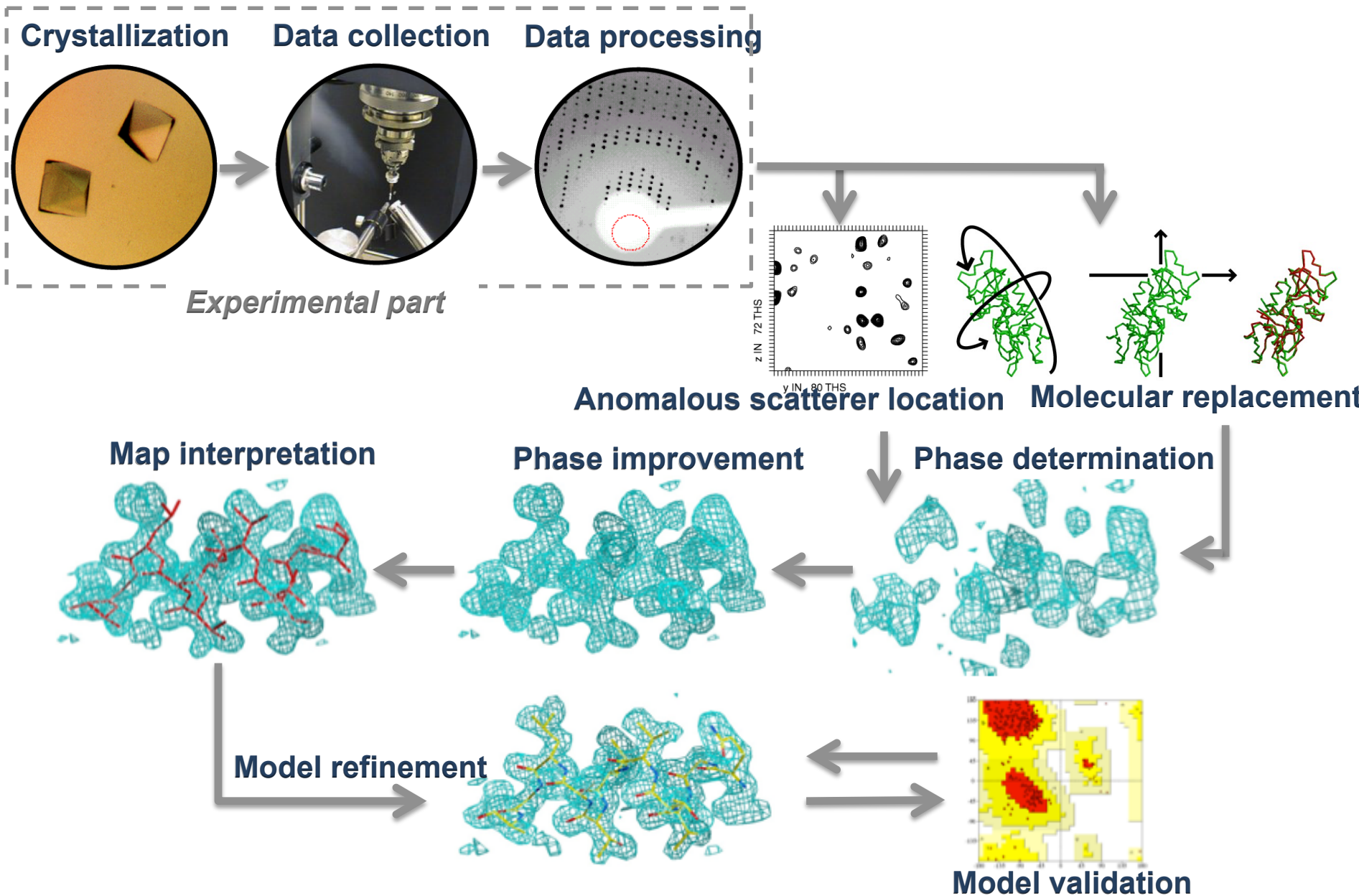
Nat. Struct. Biol. 7, 687–692 (2000); retracted 6 July 2009

In this paper, we described both the three-dimensional crystal structure of a botulinum toxin catalytic domain separated from the holotoxin (BoNT/B-LC, PDB 1F82) and a structure of the toxin catalytic domain in complex with a peptide (Sb2-BoNT/B-LC, PDB 1F83). The complex was later refined and deposited in the Protein Data Bank (PDB 3G94). The apo structure (PDB 1F82) remains valid. However, because of the lack of clear and continuous electron density for the peptide in the complex structure, the paper is being retracted. We apologize for any confusion this may have caused.

- **H.M. Krishna Murthy – Protein Fabrication scandal**

- **12 falsified structures and 10 related papers**
- **1BEF, 1CMW, 1DF9, 2QID, 1G40, 1G44, 1L6L, 2OU1, 1RID, 1Y8E, 2A01, and 2HR0**
- **Murthy's falsified data ended up affecting 449 papers**

Comprehensive validation — what and when?





Comprehensive validation — what and when?

- **What:**
 - **Data**
 - Global: overall R/R_{FREE} , completeness
 - Local: R , completeness, $\langle \text{Fobs} \rangle$ per resolution bin
 - **Model**
 - Global: bond/angle rmsd, rotamer, Ramachandran outliers
 - Local: local (per residue) geometry distortions
 - **Model to Data Fit**
 - Global: overall RSCC
 - Local: RSCC per atom or per residue, map values
- **When:**
 - Throughout the process of structure determination



Comprehensive validation — data (check list)

- **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)**



Comprehensive validation — Xtrriage to check data

Xtrriage (Project: porin-twin)

Preferences Help Run Abort View log Save graph Ask for help

Configure **Xtrriage_1**

Run status **Results**

Xtrriage 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%.

Click on panels to explore data and investigate problems



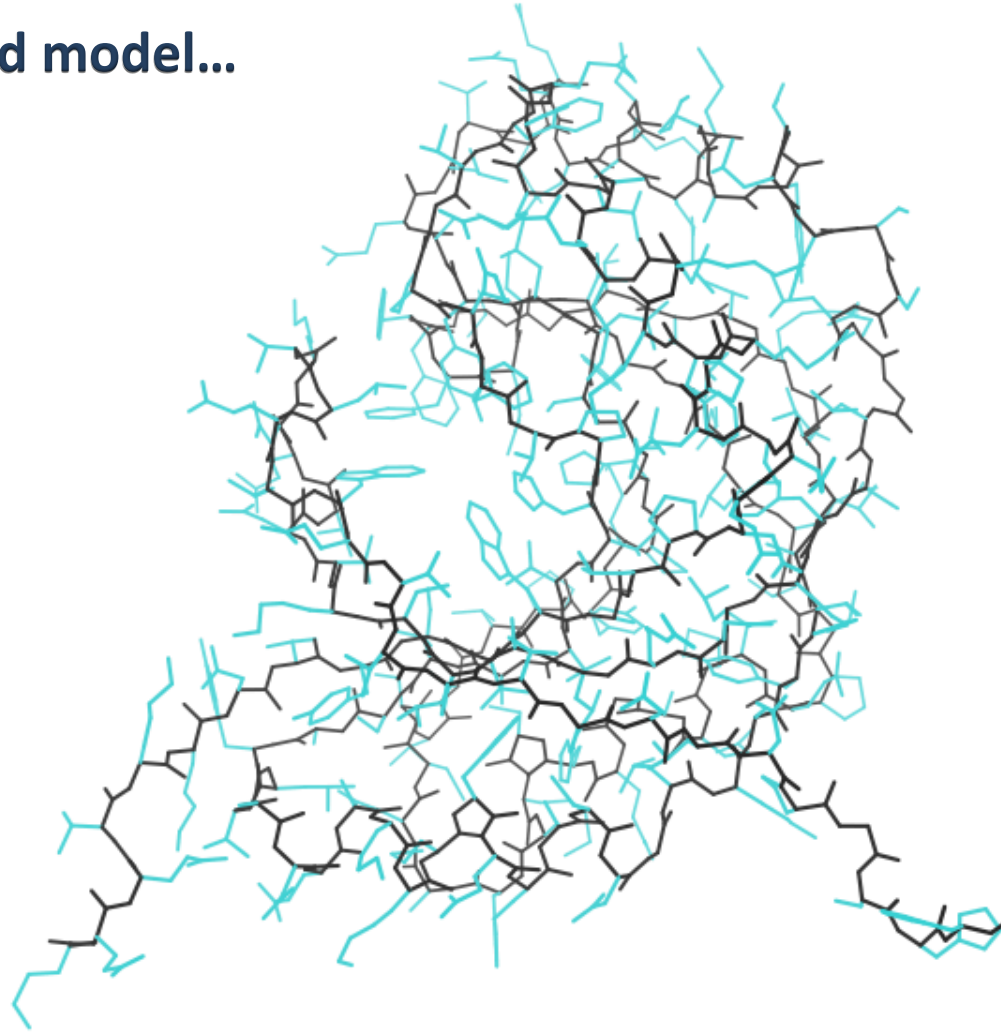
Comprehensive validation — Model

- A good model should be sound in all senses
 - **Physically**
 - packing, contacts, crystal solvent content
 - **Chemically**
 - correct local geometry, bonds, angles, etc
 - **Crystallographically**
 - R-factors, B-factors, map fit, bulk-solvent, symmetry
 - **Statistically**
 - No under-modeling (under-refinement), no over-fitting (over-modeling)
 - Model global quality figures are expected to be in agreement with corresponding values found in similar structures



Comprehensive validation — Model (steric clashes)

Looks like a good model...

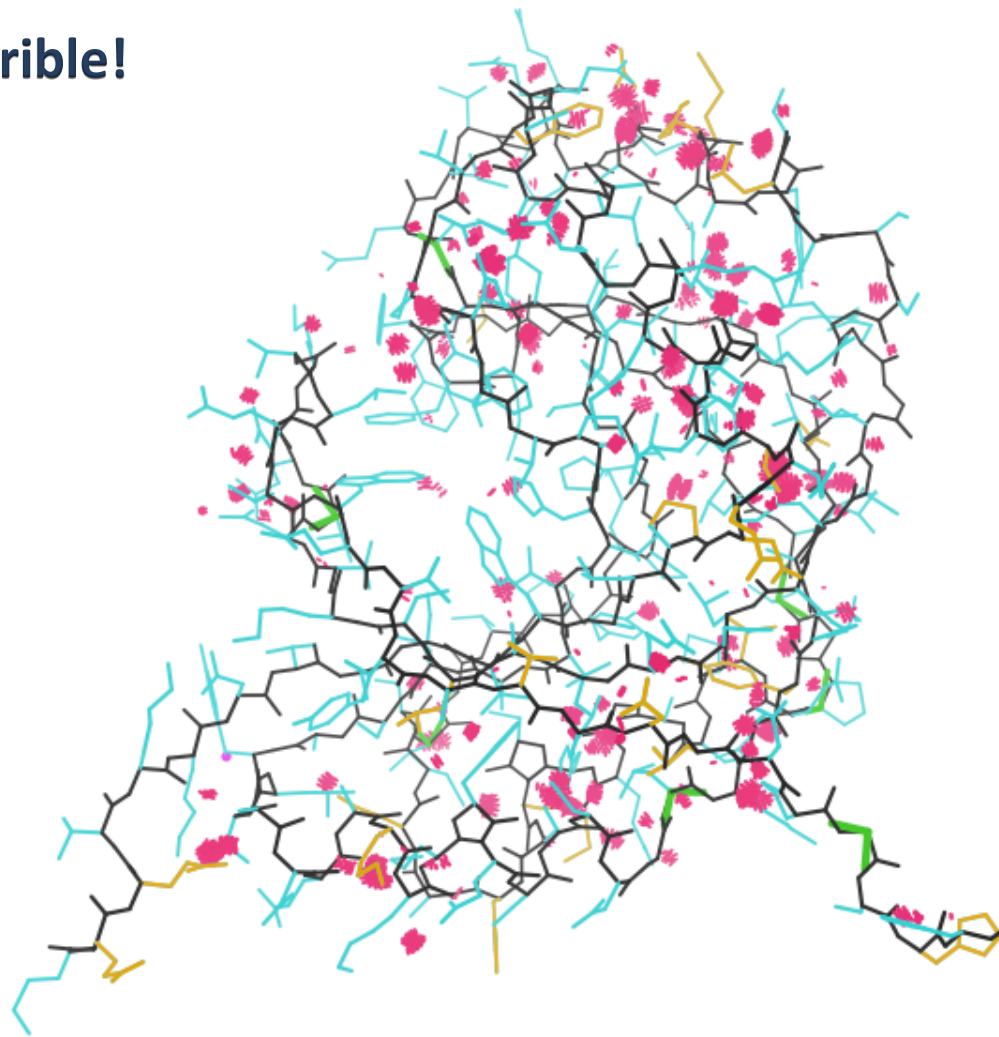


Cyclic Nucleotide Phosphodiesterase (2.4 Å)



Comprehensive validation — Model (steric clashes)

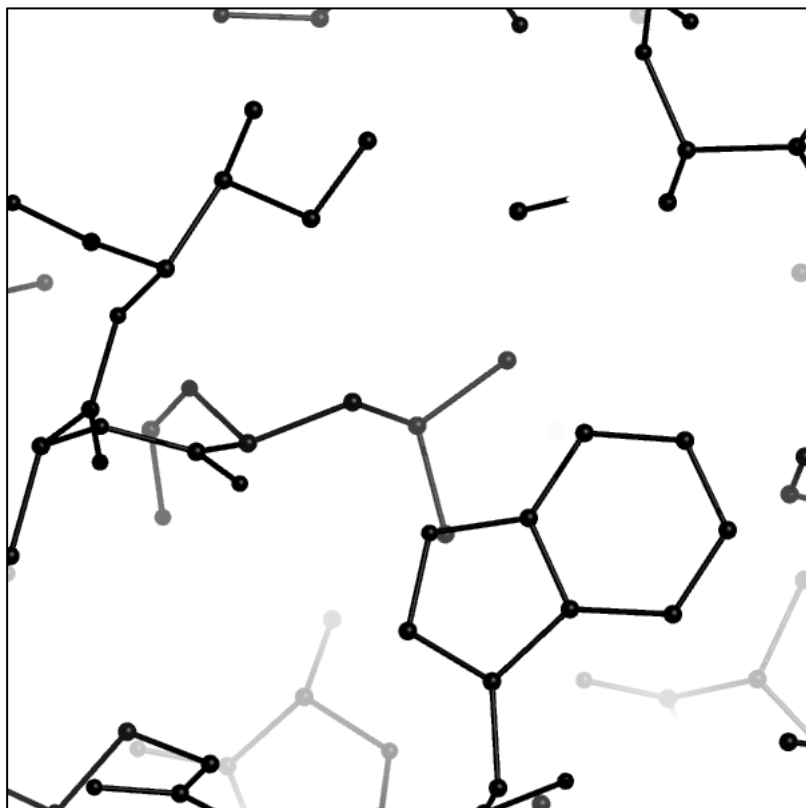
... in fact it is terrible!



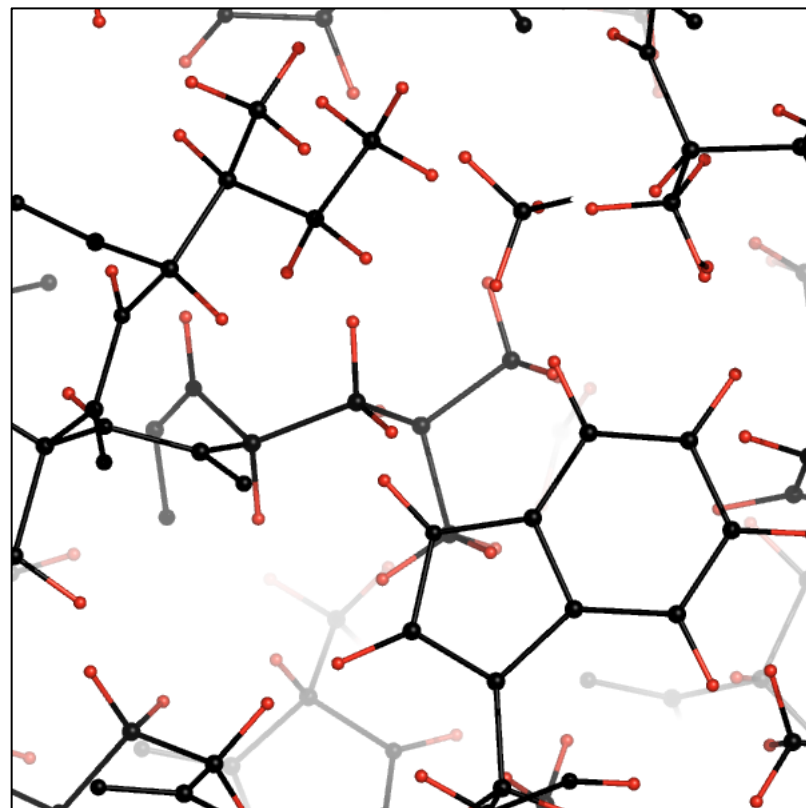
Cyclic Nucleotide Phosphodiesterase (2.4 Å)

Comprehensive validation — Fixing model clashes

- Refinement with H usually helps, because:
 - Half of the atoms in a protein molecule
 - Make most interatomic contacts
- Use weight optimization in refinement
- Resolve severe clashes manually

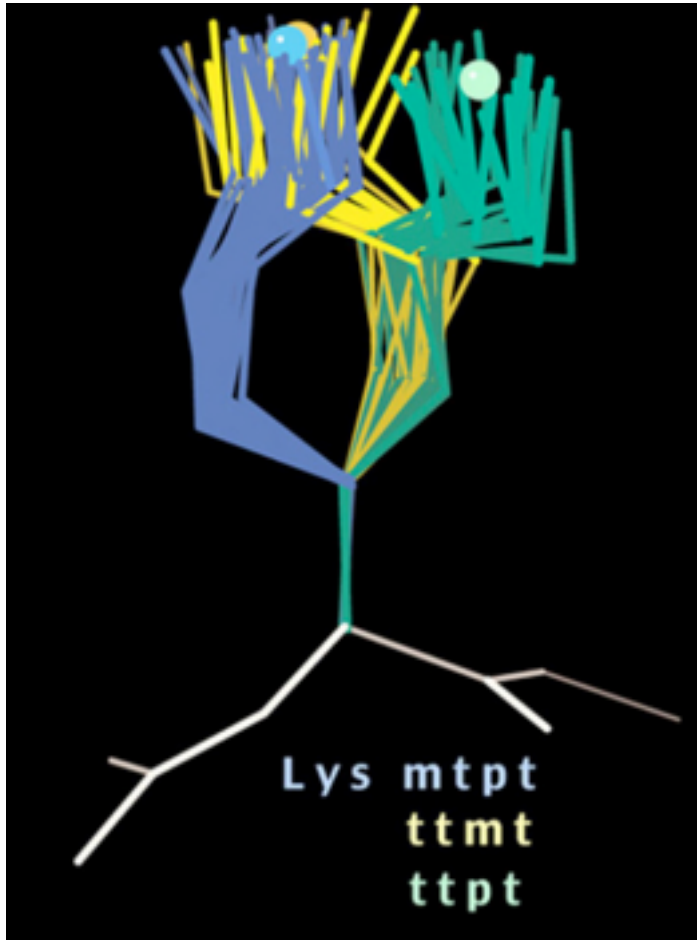


No H atoms



H atoms added

Comprehensive validation — Model (Rotamers)

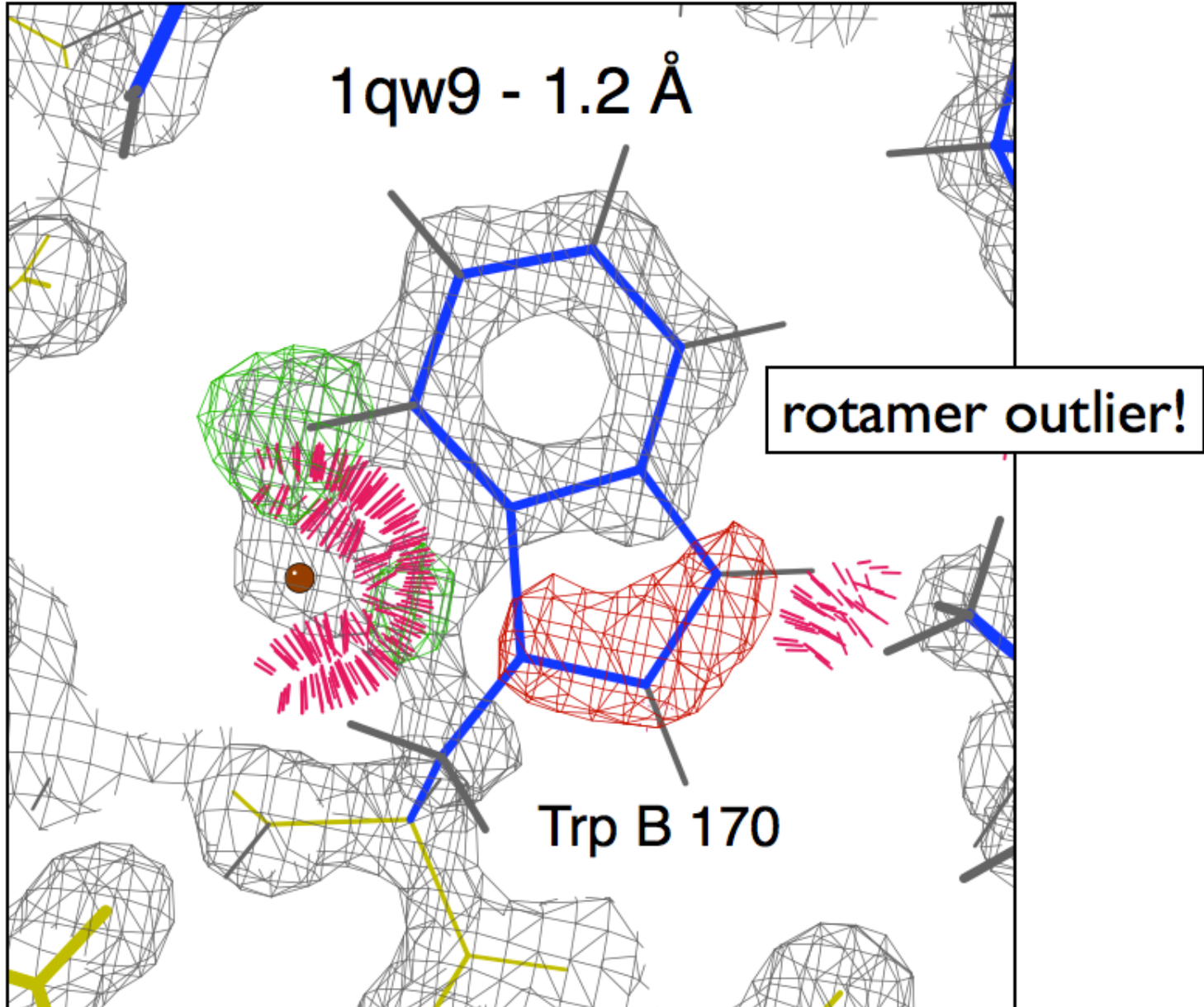


- Steric clashes between atoms within amino acid side chains lead to preferred conformations, called rotamers
- Different rotamers are generated by rotation of side chain torsion angle

Image from Jane and David Richardson, Duke University



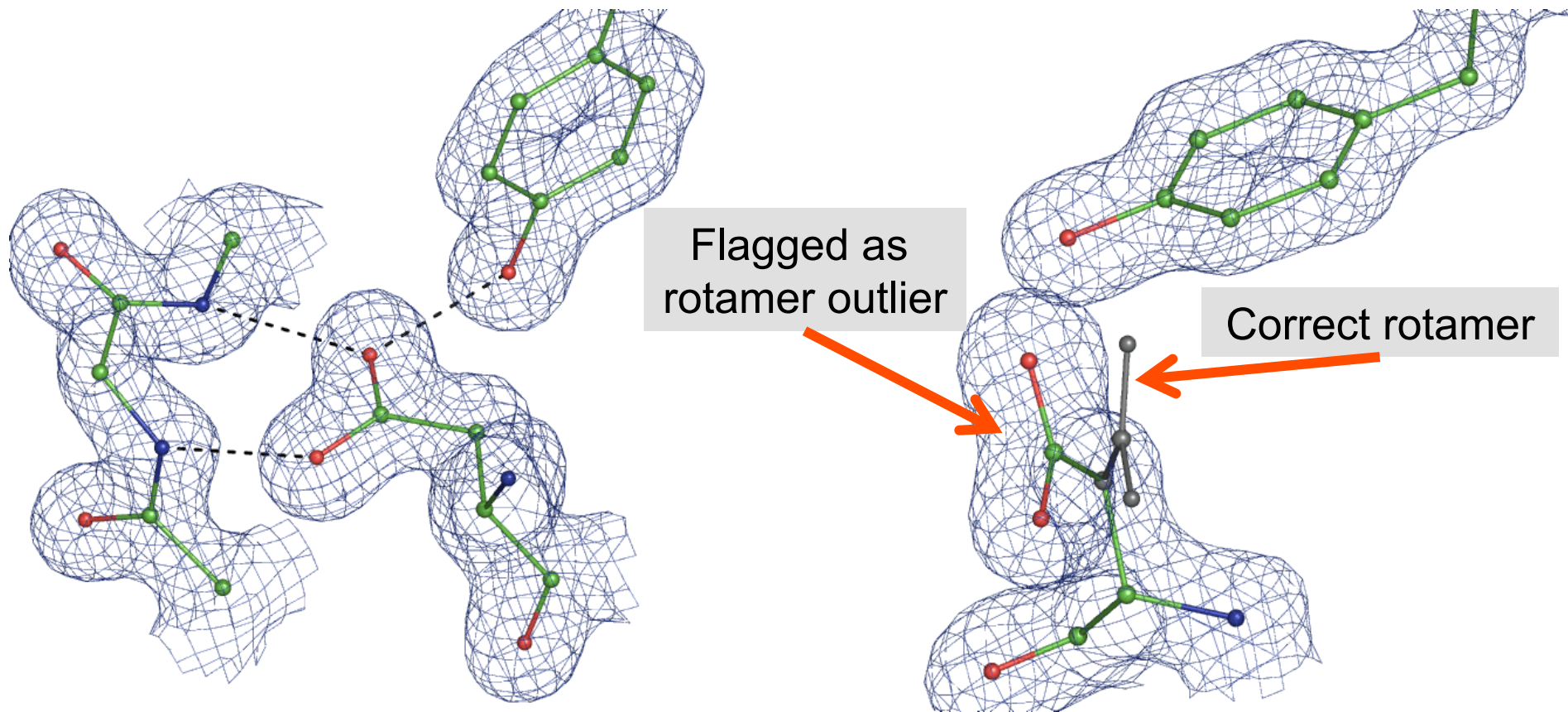
Comprehensive validation — Model (rotamer outliers)



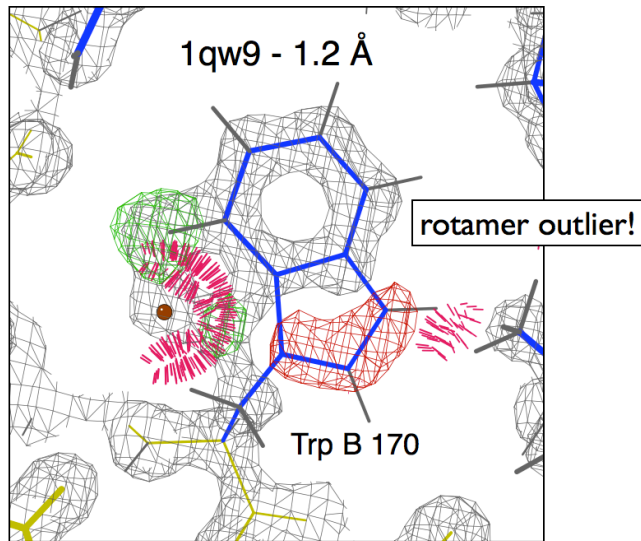


Comprehensive validation — valid rotamer outliers

- Not everything flagged as outlier is actually bad
 - However, each outliers has to be explained



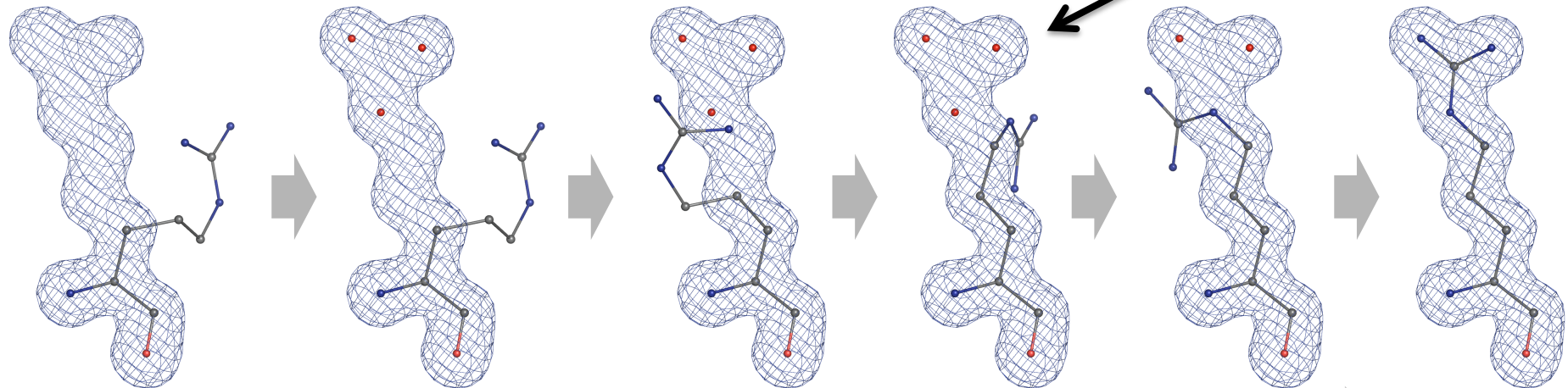
Comprehensive validation — Model (rotamer outliers)



- In proteins 99% of the side chains obey known rotameric conformations
- Often errors are fixed manually but can now be fixed automatically
- A systematic search through rotamer space is combined with a fit-to-density score in Phenix

Find best map fitting rotamer...

... remove water if necessary

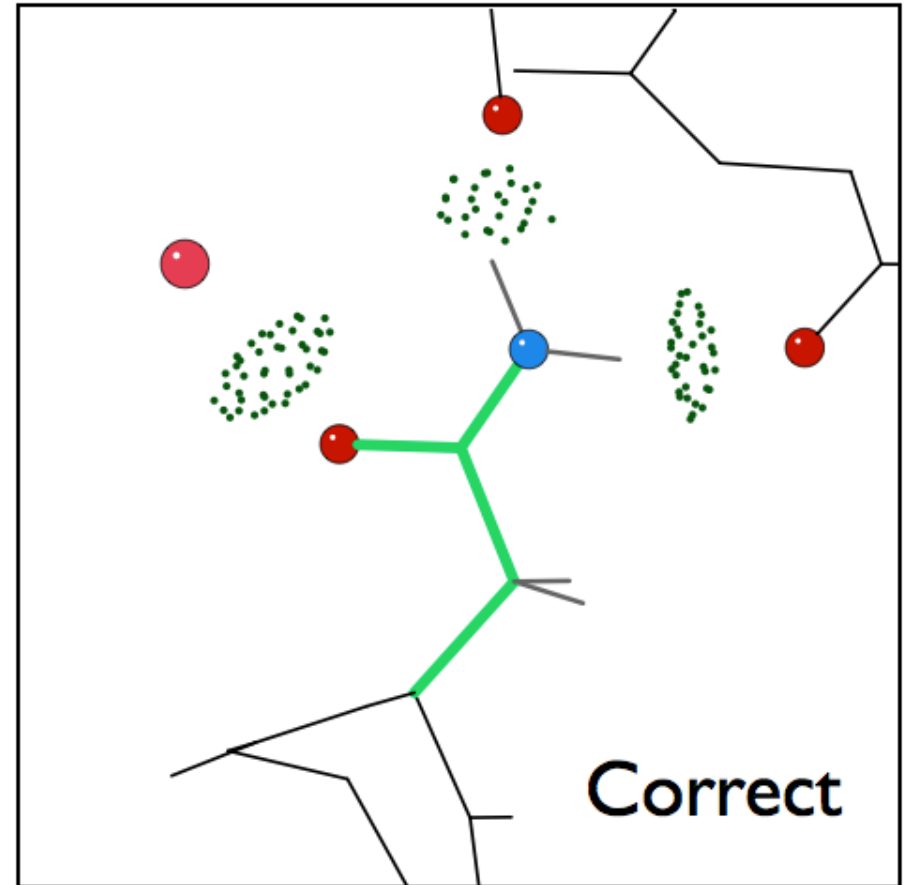
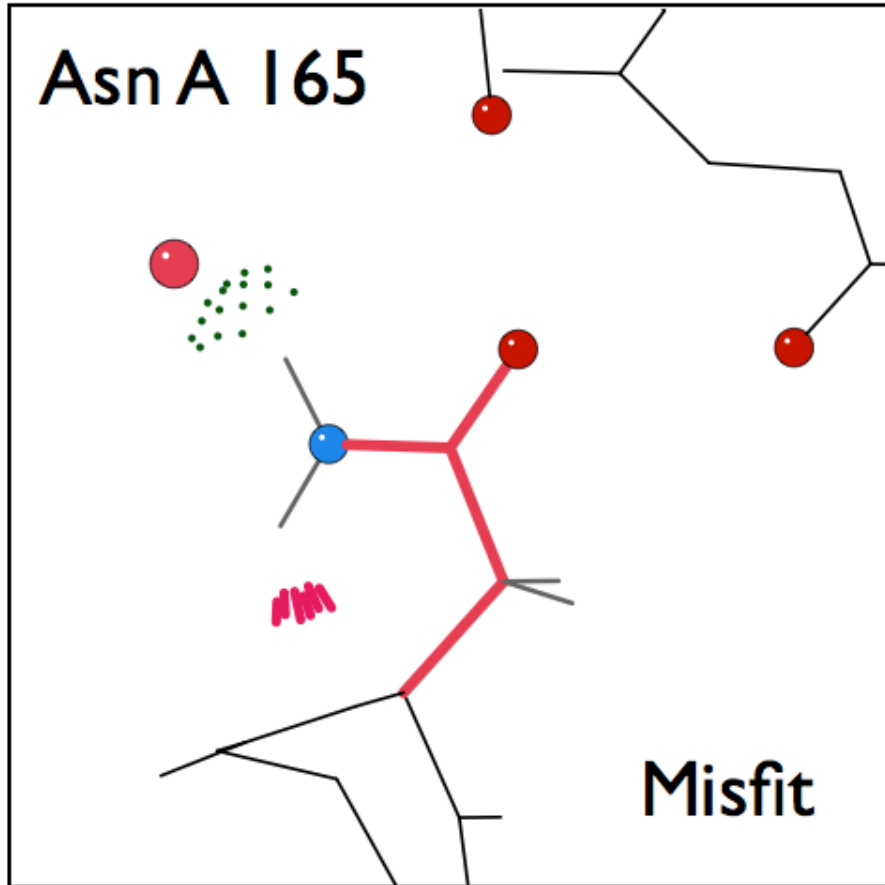


START

~ 1 second for ARG, 1/1000 second for SER

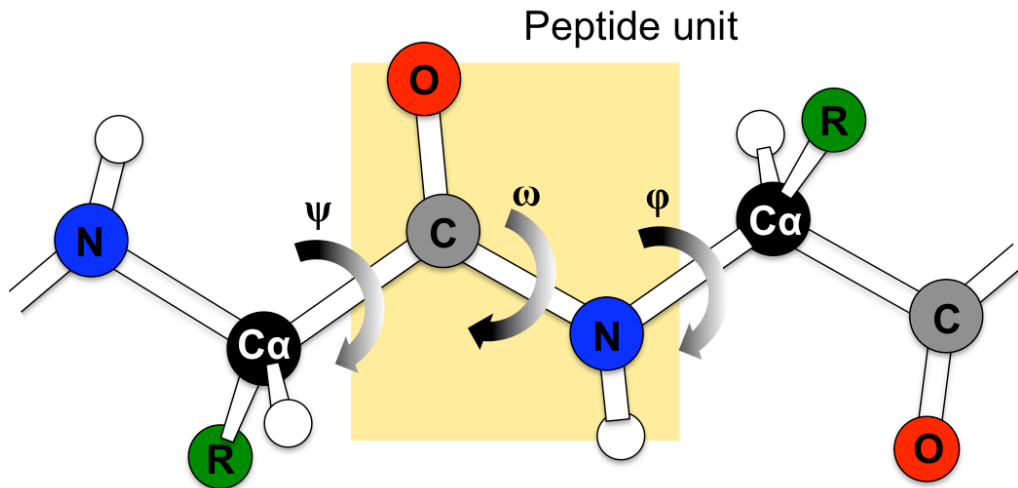
RESULT

N/Q/H flips — H atoms help to resolve ambiguity



- *Phenix.refine* does this correction automatically
- If automation fails, fix manually during validation

Comprehensive validation — Model (Ramachandran plot)

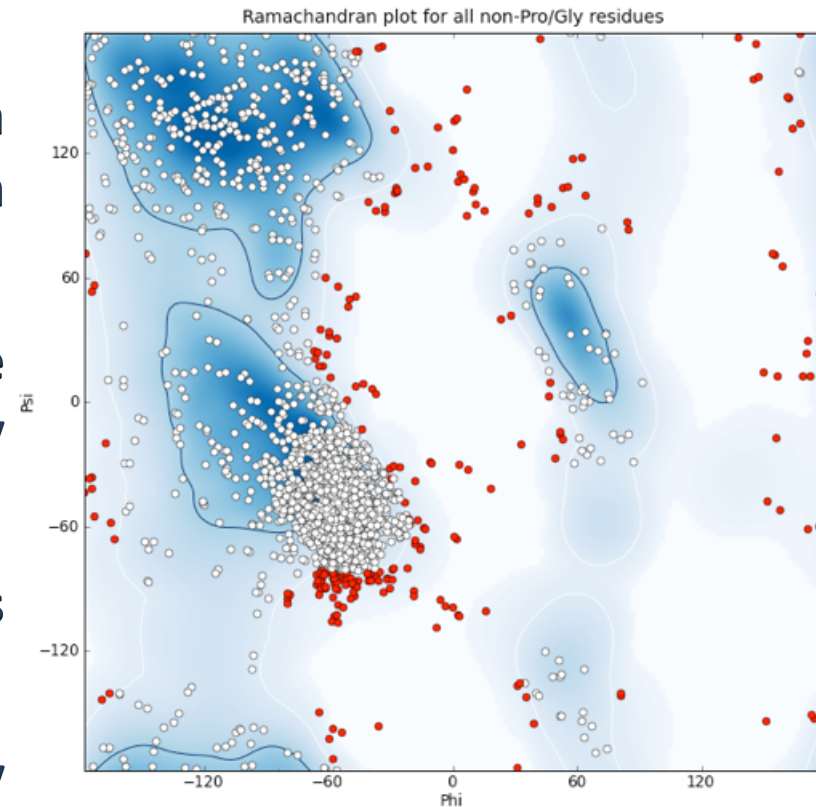


G. N. Ramachandran

The handedness of amino acids, and the steric clashes that occur, given the side chain attachment to the mainchain, results in limits on the distribution of mainchain torsion angles.

Comprehensive validation — Model (Ramachandran plot)

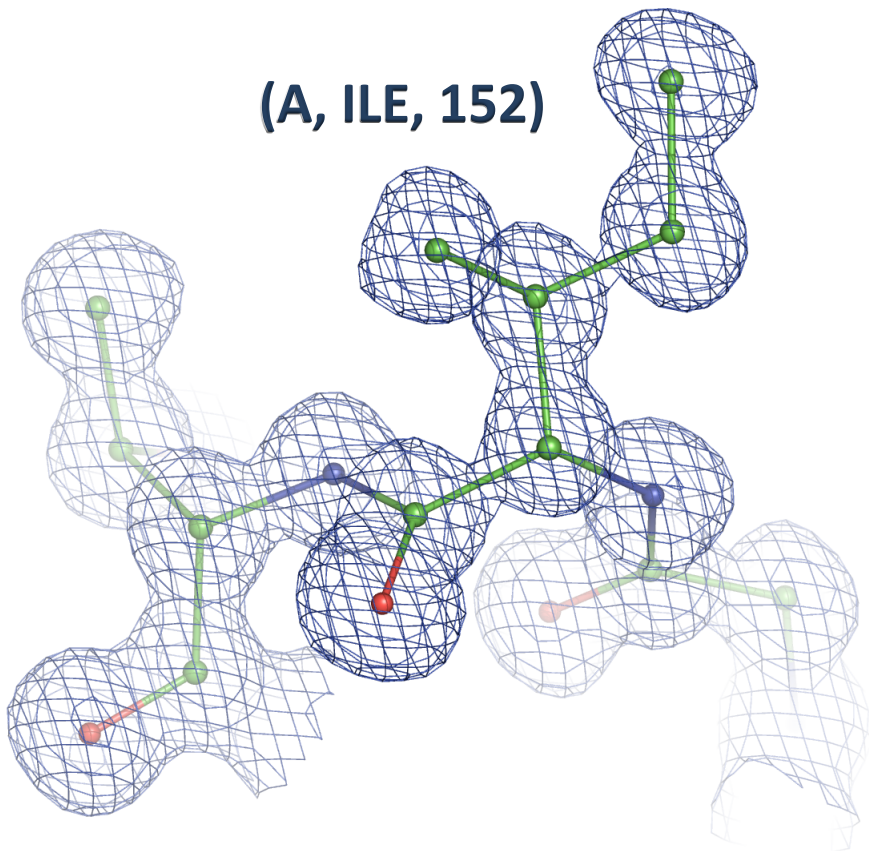
- A protein structure should conform to prior expectations
- Most (98%+) residues should have a mainchain conformation consistent with the Ramachandran distribution
- A small percentage (0.2%) of residue may show Ramachandran outliers (they are not necessarily errors!)
- Outliers can be seen in strained regions of the structure (e.g. in the active site)
- Any outliers need to be confirmed by detailed analysis



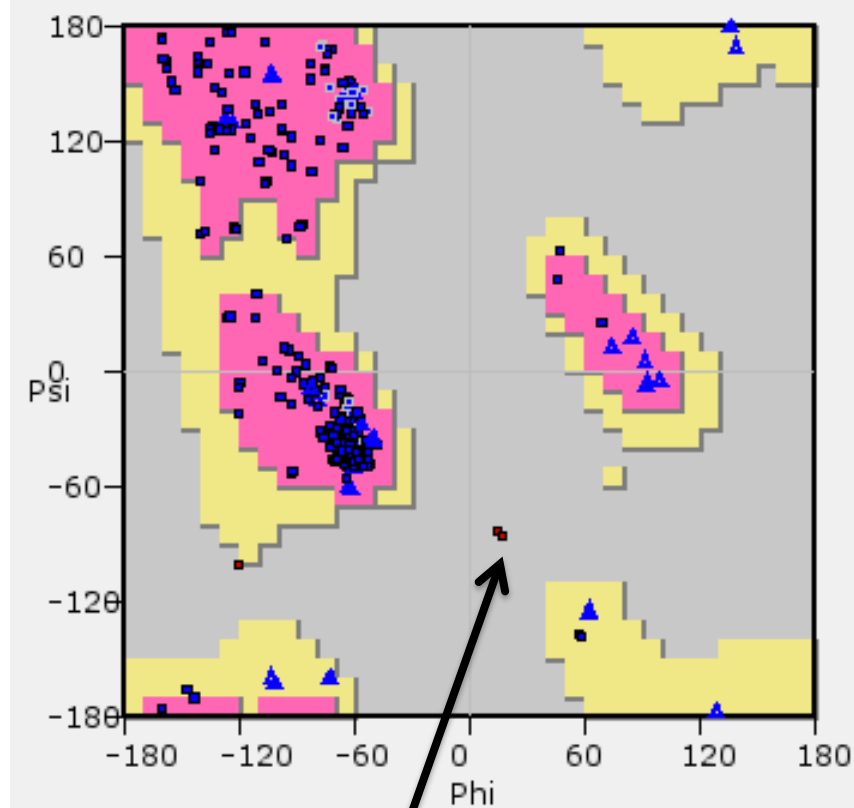
Comprehensive validation — Model (Ramachandran plot)

PDB code 3NOQ, 1 Å

(A, ILE, 152)



Ramachandran plot in Coot

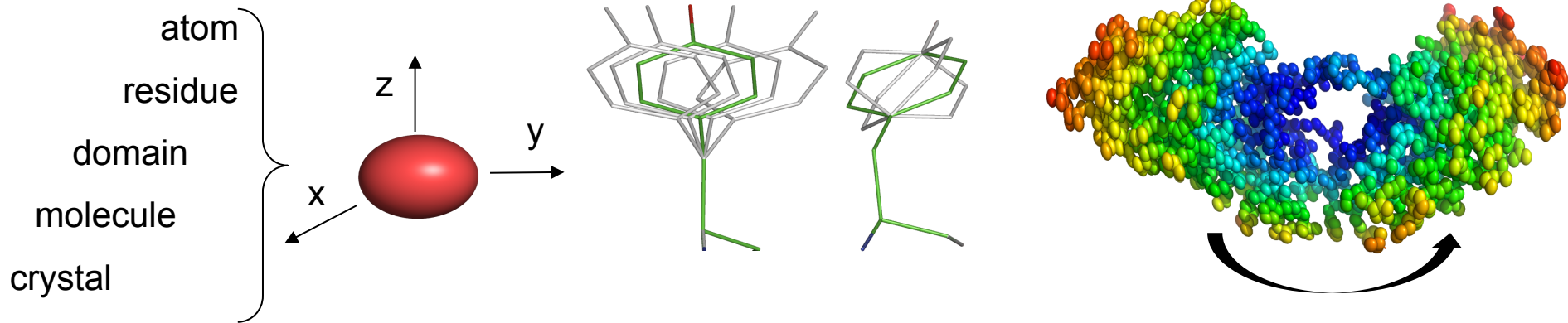


Outliers:

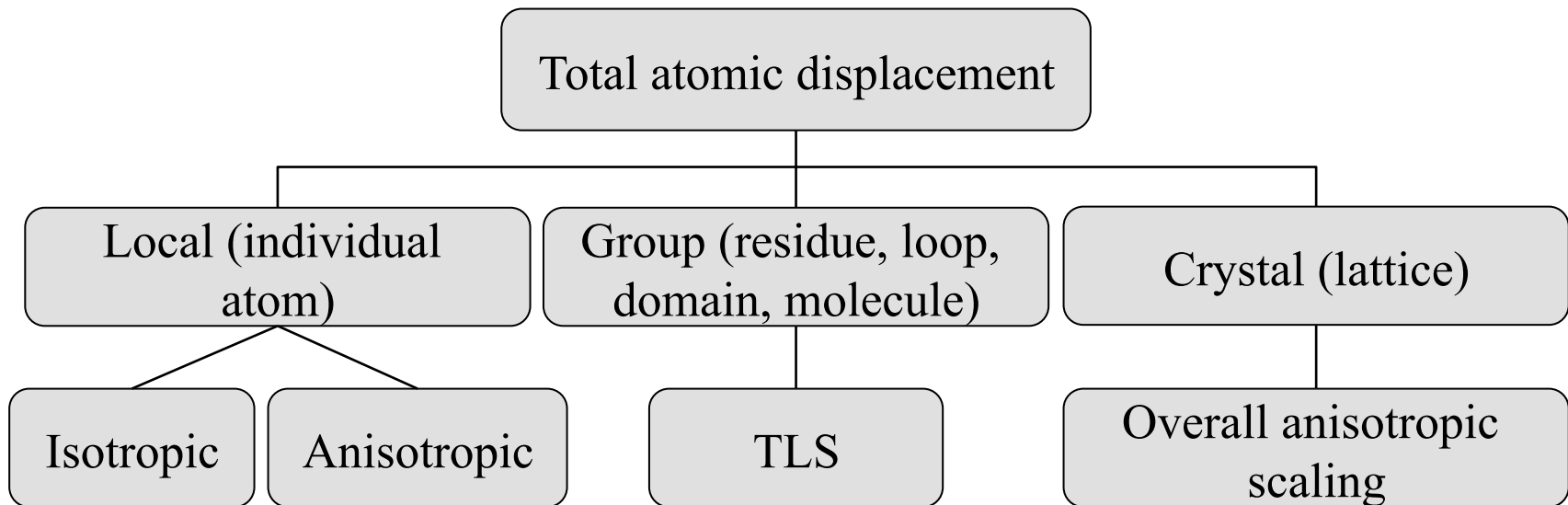
(A, ILE, 152), (B, ILE, 154)

Valid Ramachandran plot outliers: justified by the density map

Atomic Displacement Parameters (ADP, B-factors)



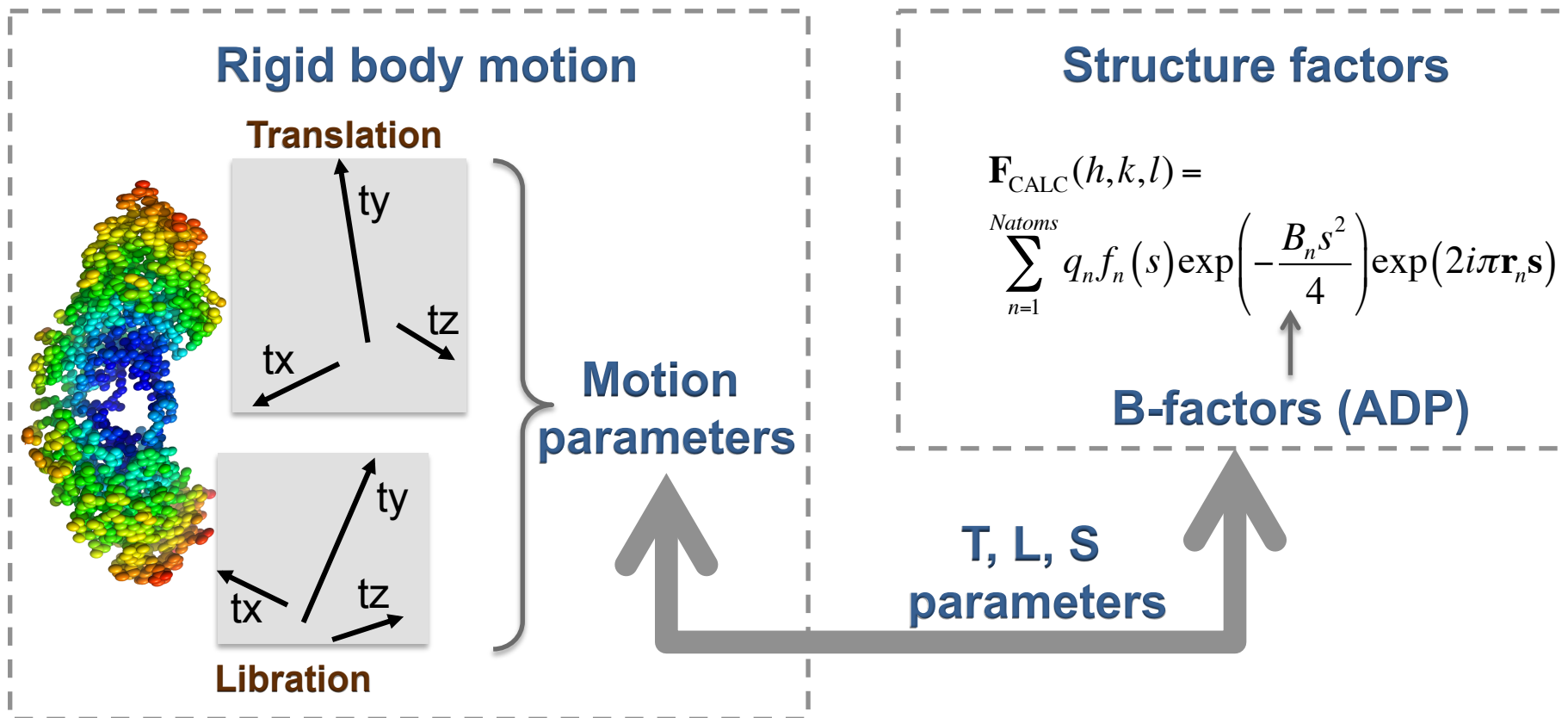
- Parameterization for refinement



$$\mathbf{U}_{\text{TOTAL}} = \mathbf{U}_{\text{CRYST}} + \mathbf{U}_{\text{GROUP}} + \mathbf{U}_{\text{LOCAL}}$$

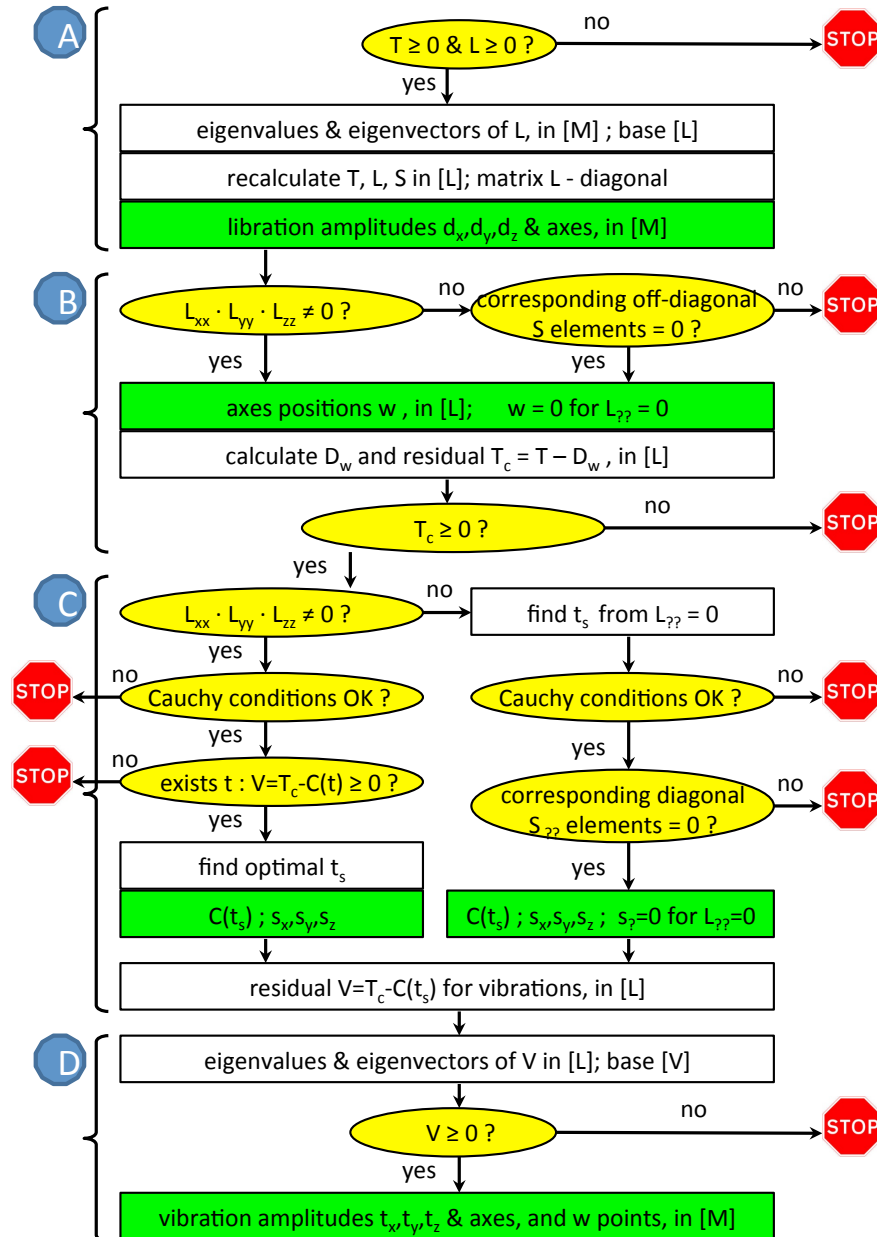
TLS parameters and rigid body motion

- TLS parameters is a way to pack descriptors of rigid-body motion into a form suitable to calculate structure factors

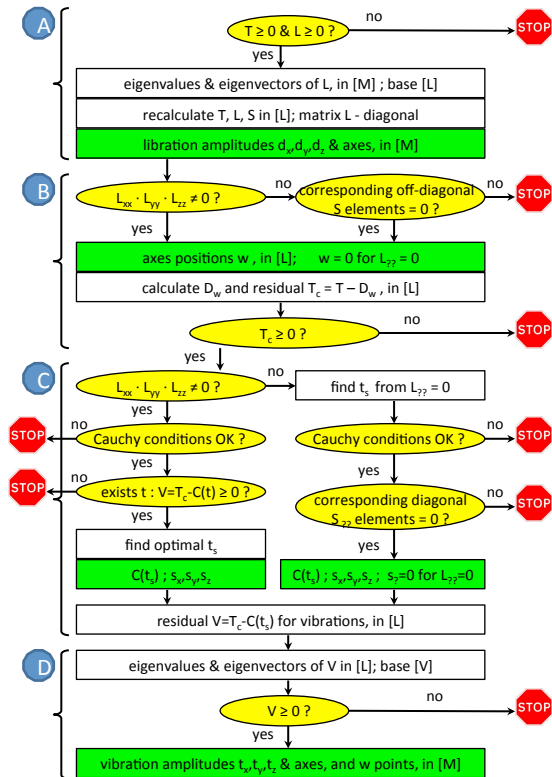


- **TLS do not directly provide parameters of rigid body motion**
 - **Descriptions of motions need to be extracted from TLS matrices**

TLS parameters as descriptors of rigid body motion



TLS parameters as descriptors of rigid body motion



- To describe rigid-body motion TLS matrices must satisfy several conditions
- Broken condition means TLS cannot describe rigid-body motion and therefore do not make physical sense
- PDB survey:
 - Total models: 105,000
 - Contain TLS: 25,284
 - Files with bad TLS: 21,540 (85%)

From deep TLS validation to ensembles of atomic models (...)

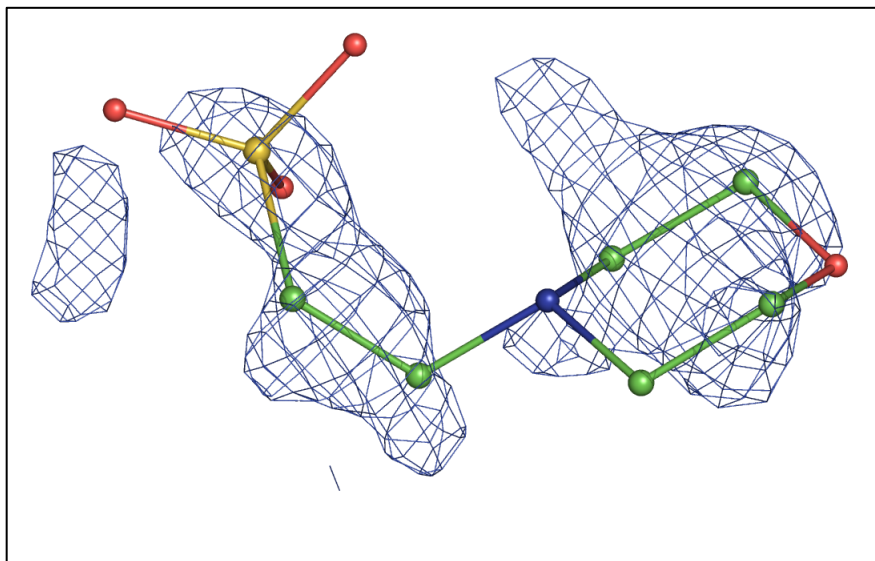
A. Urzhumtsev, P. Afonine, A. Benschoten, J. Fraser & P. Adams
 Acta Cryst. D, 2015 (accepted)

Available in Phenix (work in progress)

Comprehensive validation — Ligands

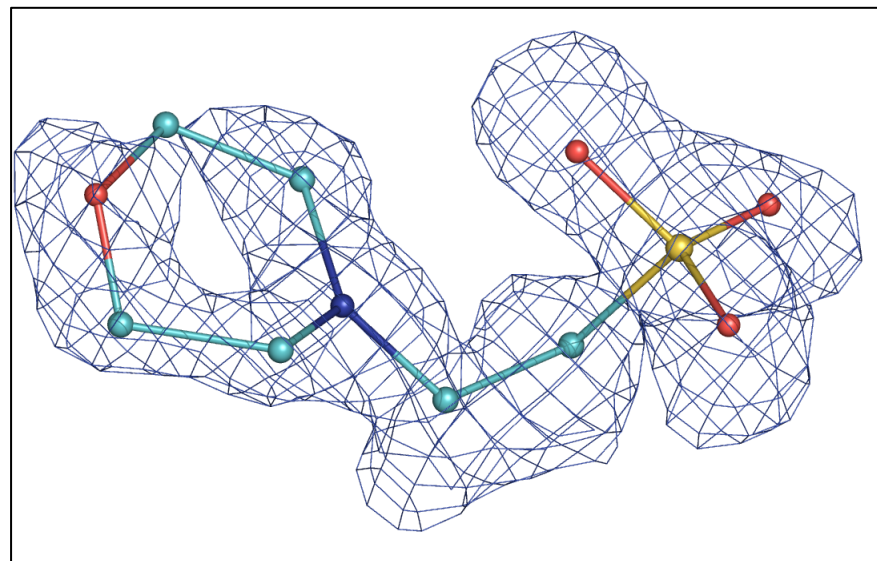
- PDB code: 1SE6, resolution 1.6Å
 - Techniques, tools and best practices for ligand electron-density analysis and results from their application to deposited crystal structures.
Pozharski, Weichenberger and Rupp, Acta Cryst D69, 150-167 (2013)

Wrong



2mFo-DFc

Correct



FEM

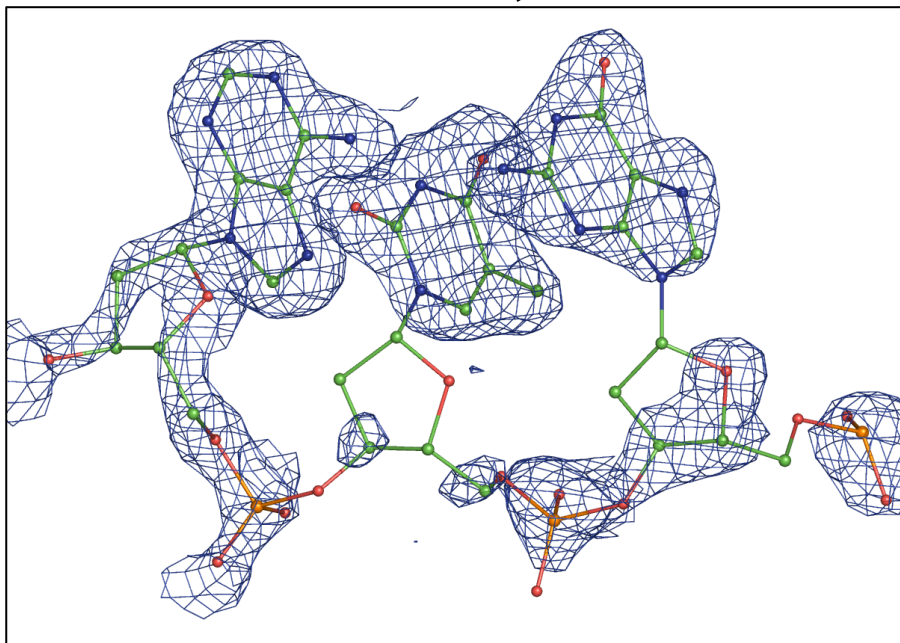
- **phenix.composite_omit_map**
 - **With or without Simulated Annealing refinement**
- **Simple OMIT residual map**
 - **Remove ligand, then compute mFo-DFc map**
 - **There are caveats**
- **Feature Enhanced Map (FEM)**
 - **Compute FEM with and without ligand**



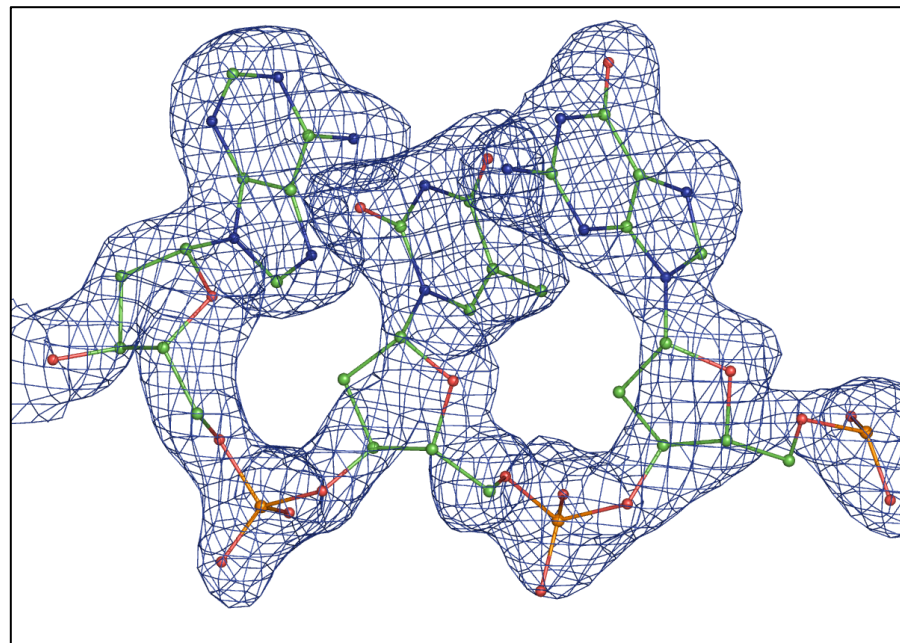
Comprehensive validation — Ligands and maps

- PDB code: 1NH2, resolution 1.9Å, showing E6-E8

2mFo-DFc , 1σ



FEM, equivalent 1σ



FEM





Comprehensive validation — Ligands and maps

Phenix tools to compute maps

PHENIX home

Quit Preferences Help Citations Coot PyMOL KING Other tools Ask for help

Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Settings

ID	Last modified	# of jobs	R-free
✓ porin-twin	Apr 15 2015 06:25 ...	6	---
rnase-s_0	Apr 10 2015 08:21 ...	1	0.2125
iris	Apr 10 2015 07:07 ...	18	---
junk1	Apr 02 2015 08:07 ...	0	---
andre	Mar 31 2015 11:27 ...	1	---
dave	Mar 23 2015 11:32 ...	2	---
p9-sad_0	Mar 21 2015 04:23 ...	0	---
rnase-s	Mar 05 2015 02:32 ...	0	---
ssss	Feb 25 2015 03:12 ...	3	---
zzz	Feb 25 2015 03:10 ...	6	---
fsdghdg	Feb 23 2015 12:15 ...	4	---
teresa	Feb 06 2015 05:55 ...	2	---

Data analysis

Experimental phasing

Molecular replacement

Model building

Refinement

Validation

Ligands

Reflection tools

Maps

Model tools

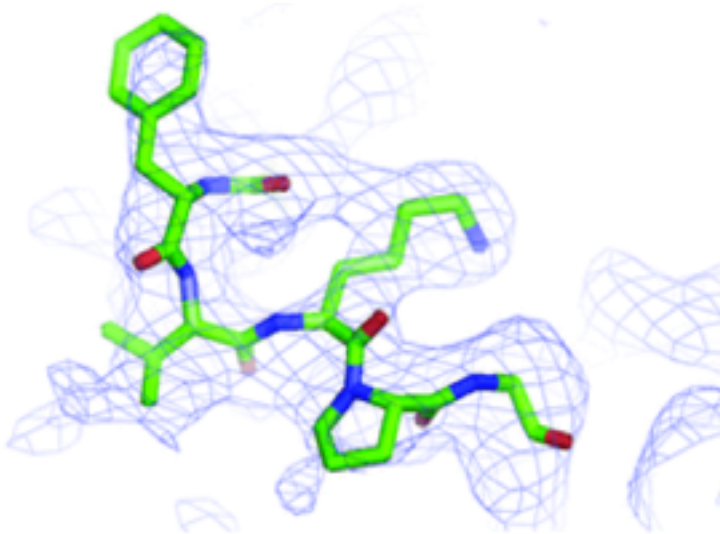
Other utilities

Current directory: /Users/pafonine/Documents/porin-twin Browse...

PHENIX version dev-1988-000 Project: porin-twin

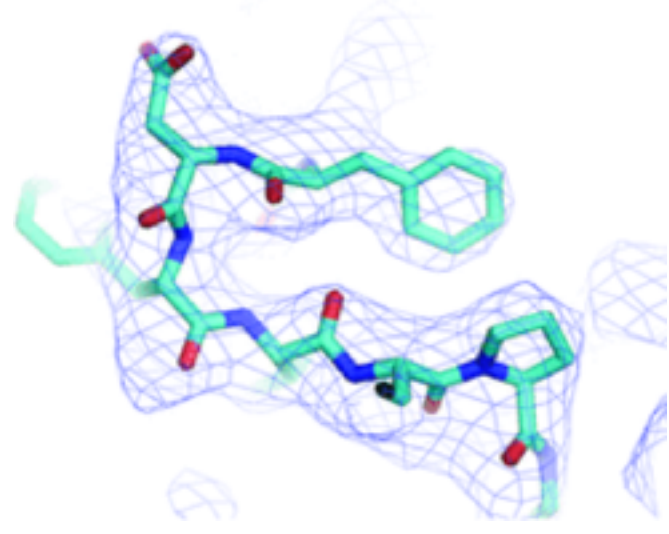


Comprehensive validation — Sequence register errors



**2mFo-DFc model biased map
and incorrect model**

1ZEN



**Corrected model in iterative
build OMIT map**

***Phenix* iterative build OMIT map**

Terwilliger et al., Acta Cryst D64, 515-524, 2008

Comprehensive validation — Sequence register errors

- Check actual sequence against derived from PDB file:

- Extract sequence from PDB file:

```
phenix.print_sequence model.pdb > model.seq
```

- Align actual sequence with model.seq

- Example of a problem:

```
MASTER  GFVDLTLHDQVSMEHPVKLLFGKCVEGMVEIVYTFLSSTLKSLE
Chain A  GFVDLTRHDQVSMEHPGKLLFGK--EGMVEIVYTF-----KSLE
Chain B  GFVDLTRHDQVSMEHPGKLLFGK--EGMVEIVYTFVSSTLKSLE
Chain C  GFVDLTRHDQVSMEHPGKLLFGKKVEGMVEIVYTFVSSTLKSLE
Chain D  GFVDLTRHDQVSMEHPGKLLFGKKVEGMVEIVYTFLSSTLKSLE
*****  *****  *****  *****  *****
```



Local errors obscured by global numbers

- Resolution 2.5Å: $R_{\text{WORK}}/R_{\text{FREE}} = 17.1/21.2\%$ bonds = 0.01Å angles = 1.6°
 - R-factors are great, overall geometry is great, but...

Local errors obscured by global numbers

- Resolution 2.5Å: $R_{\text{WORK}}/R_{\text{FREE}} = 17.1/21.2\%$ bonds = 0.01Å angles = 1.6°
 - R-factors are great, overall geometry is great, but...

Histogram of deviations from ideal values

Bonds				Angles		
0.000 - 0.035:	2645			0.000 - 9.313:	4208	
0.035 - 0.070:	19			9.313 - 18.626:	9	
0.070 - 0.106:	13			18.626 - 27.939:	3	
0.106 - 0.141:	5			27.939 - 37.252:	4	
0.141 - 0.176:	3			37.252 - 46.565:	0	
0.176 - 0.211:	0			46.565 - 55.878:	0	
0.211 - 0.246:	0			55.878 - 65.191:	2	
0.246 - 0.281:	0			65.191 - 74.504:	1	
0.281 - 0.317:	2			74.504 - 83.817:	0	
0.317 - 0.352:	18			83.817 - 93.130:	8	

- Problem with a few atoms, while the rest is ok
 - Poor ligand geometry



Are you done with refinement?

- Say you are refining a structure at 1.0 Å resolution and R-factors are:
 $R_{\text{WORK}} = 18\%$ and R_{FREE} is 23%.

Question: Are these values good? Is refinement completed?

Are you done with refinement?

- Say you are refining a structure at 1.0 Å resolution and R-factors are:
 $R_{\text{WORK}} = 18\%$ and R_{FREE} is 23%.

Question: Are these values good? Is refinement completed?

- **PDB statistics: compare your structure with similar structures in the database**

R_{WORK} at 0.9-1.1Å

0.10 - 0.12:	68
0.12 - 0.14:	94
0.14 - 0.16:	73
0.16 - 0.18:	17 <<<
0.18 - 0.20:	12
0.20 - 0.21:	3
0.21 - 0.23:	5
0.23 - 0.25:	0
0.25 - 0.27:	0
0.27 - 0.29:	2

R_{FREE} at 0.9-1.1Å

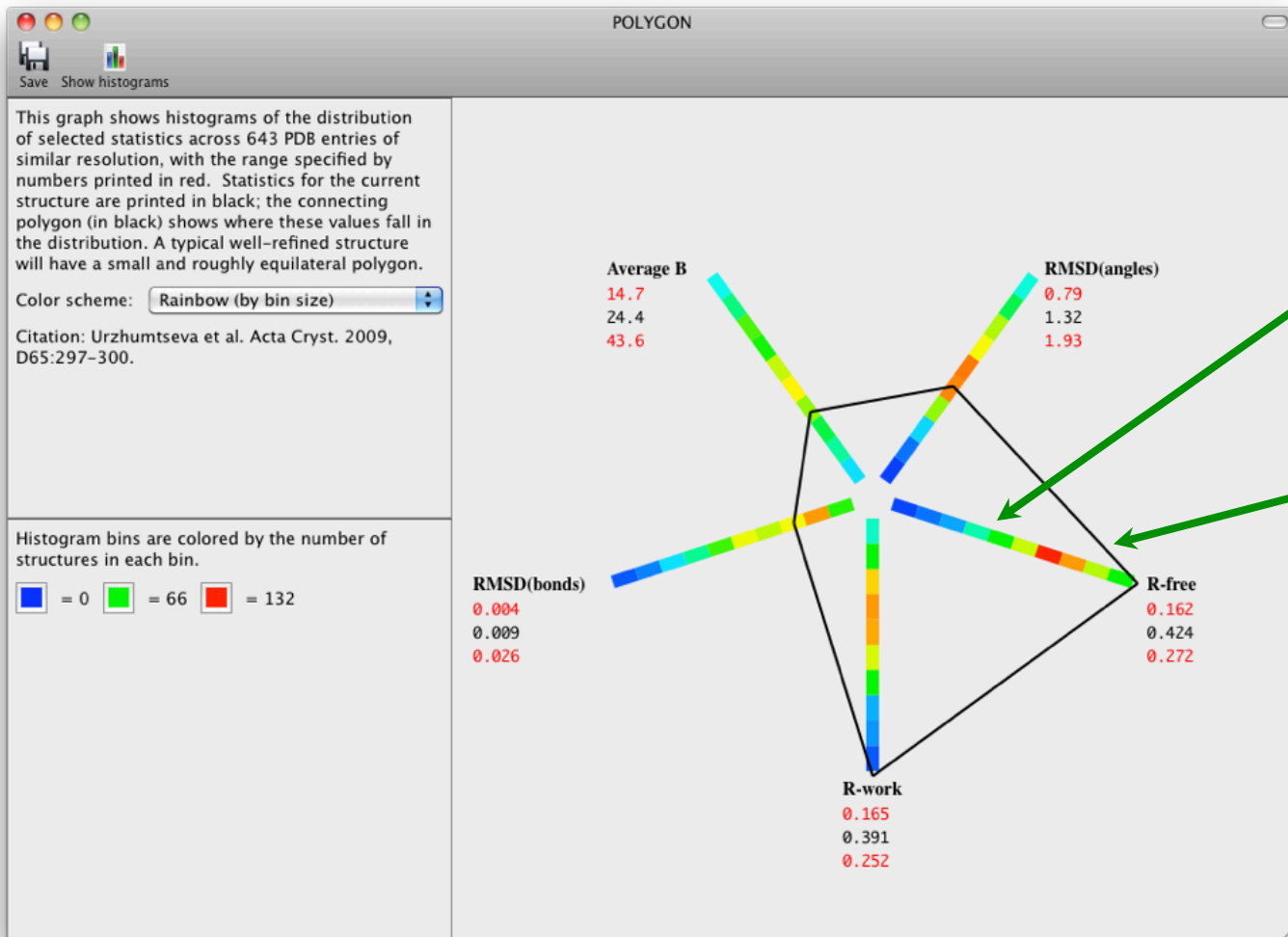
0.11 - 0.13:	16
0.13 - 0.15:	56
0.15 - 0.17:	97
0.17 - 0.18:	69
0.18 - 0.20:	14
0.20 - 0.22:	12
0.22 - 0.24:	3 <<<
0.24 - 0.26:	4
0.26 - 0.28:	1
0.28 - 0.30:	2

$R_{\text{FREE}} - R_{\text{WORK}}$ at 0.9-1.1Å

0.00 - 0.01:	8
0.01 - 0.01:	22
0.01 - 0.02:	56
0.02 - 0.03:	62
0.03 - 0.03:	58
0.03 - 0.04:	29
0.04 - 0.04:	14
0.04 - 0.05:	10 <<<
0.05 - 0.06:	6
0.06 - 0.06:	9

- **Answer: the R-factors are not good, the structure needs some more work**

Are you done with refinement? POLYGON will tell!



Colored bars are one-dimensional 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

The structure used to generate this figure has good geometry relative to the PDB, but very poor R-factors.

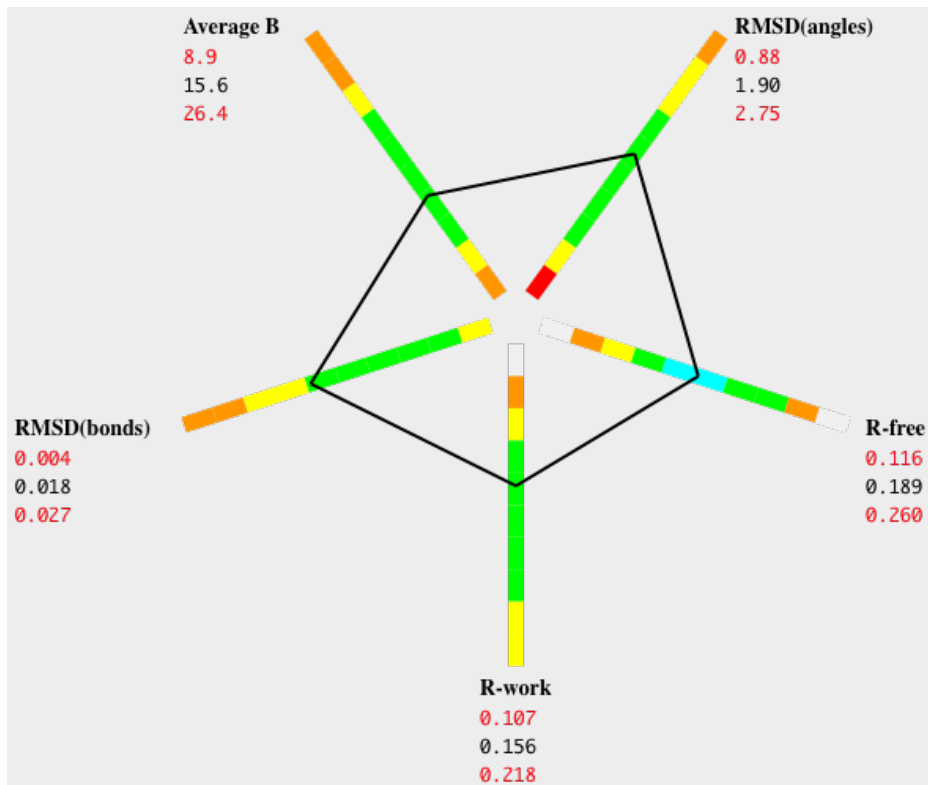
Crystallographic model quality at a glance.

L.Urzhumtseva, P.V.Afonine, P.D.Adams & A.Urzhumtsev. *Acta Cryst.* D65, 297-300 (2009)

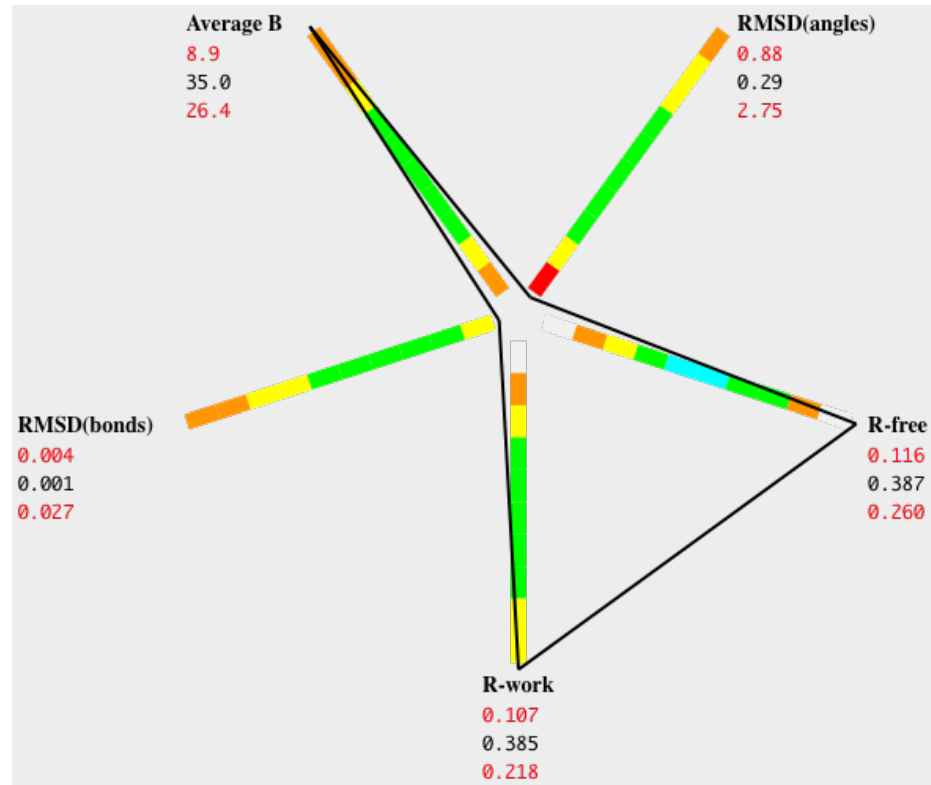


Are you done with refinement? POLYGON examples

Likely overall good model



Clearly there are problems





Are you done with refinement? POLYGON examples

PDB: 1eic

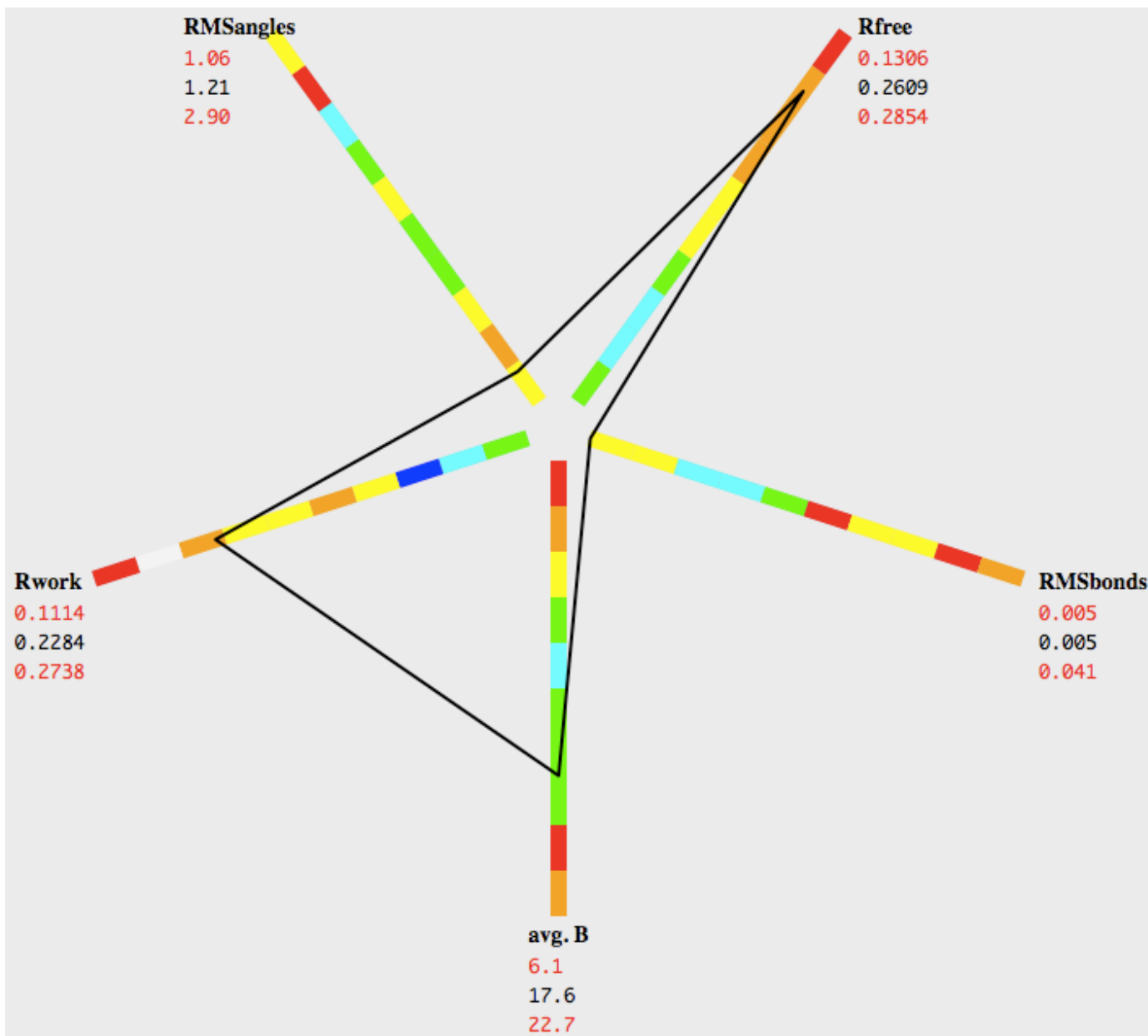
Resolution: 1.4Å

**Deposition year:
2000**

PUBLISHED:

Rwork = 20%

Rfree = 25%



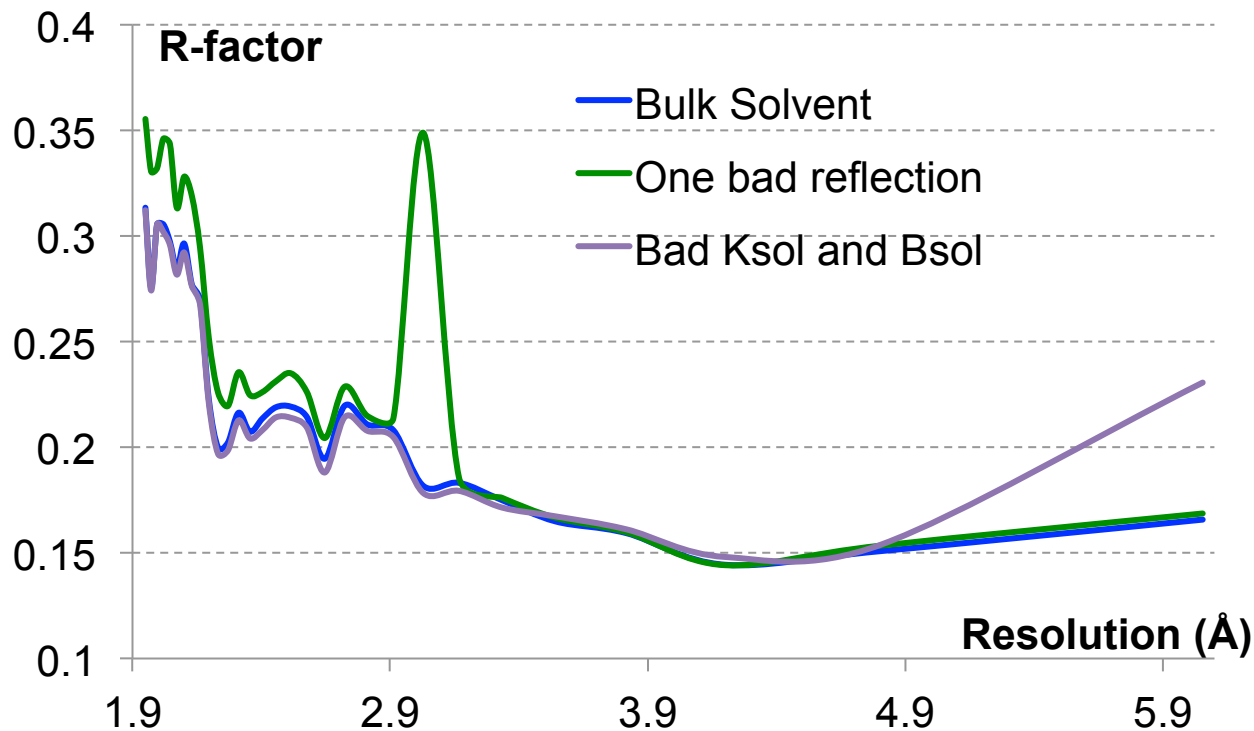


Are you done with refinement? **POLYGON** examples

- Structure from PDB: 1eic (resolution = 1.4Å; deposition year: 2000)
 - **PUBLISHED: Rwork = 20% Rfree = 25%**
- Problems:
 - **No 'riding' H atoms;**
 - **All atoms are isotropic;**
 - **Suboptimal weights, refinement is not converged, incomplete solvent**
- Fixing the model with PHENIX
 - Add and refine H as riding model
 - Update ordered solvent
 - Refine atoms as anisotropic (except H and water)
 - Optimize X-ray/Restraints weights

FINAL MODEL: Rwork = 14% Rfree = 17%

Statistics per resolution

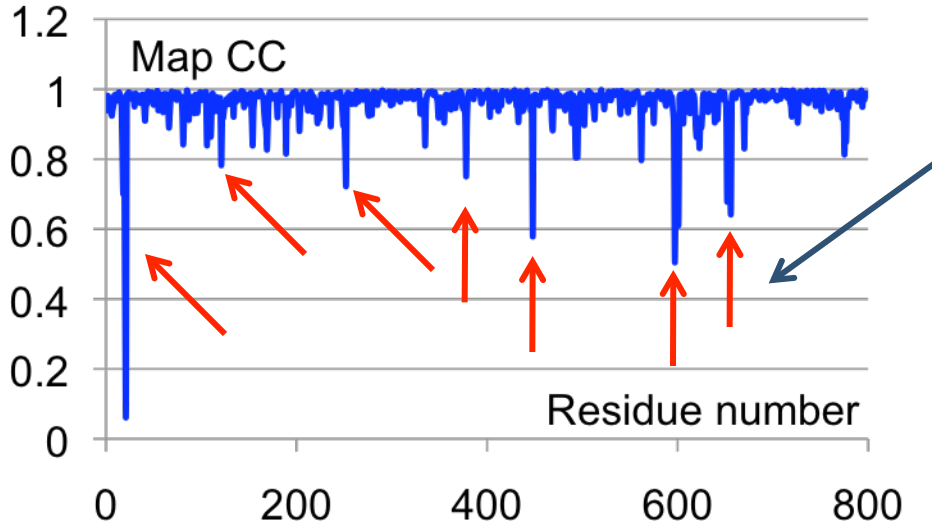
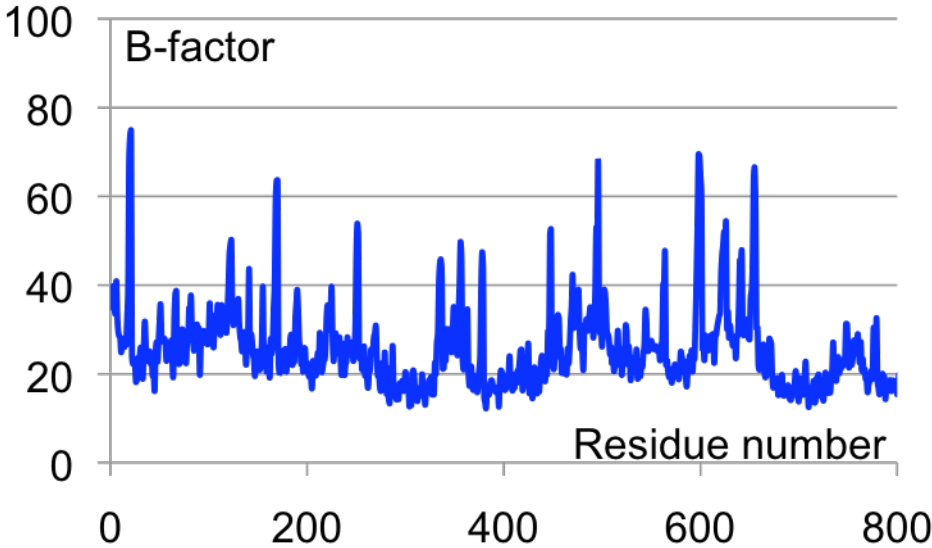


- **R-factor in resolution bins helps to identify:**
 - **Problem with bulk-solvent modeling**
 - **Problems at high resolution**
 - **Artifacts (green line):**

```
INDE      3      5  -42 IOBS= 99999.999 SIGIOBS=      0.000
```



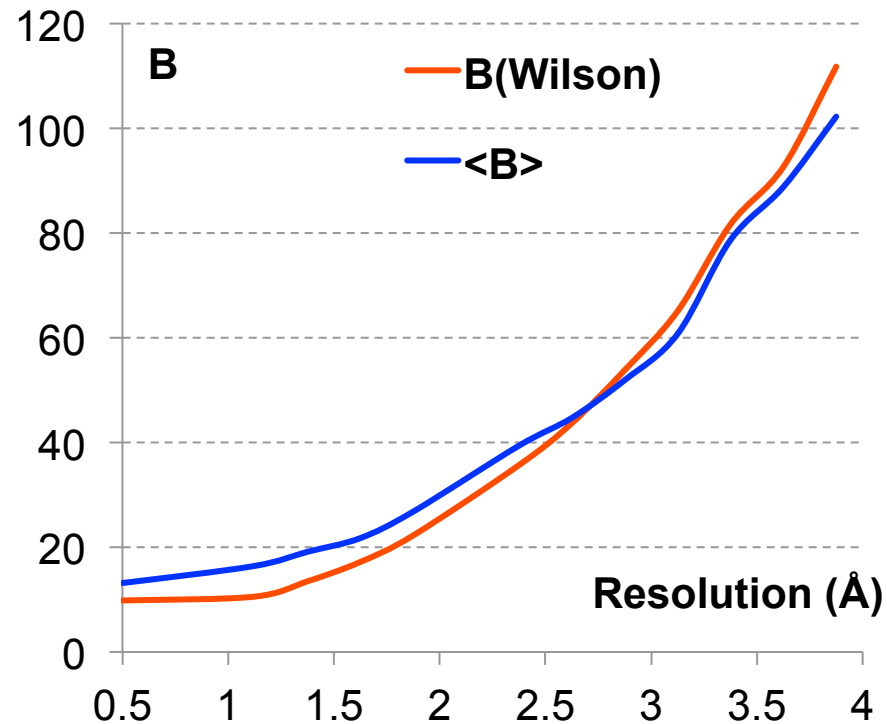
Local model quality — β -factor and map CC per residue



Indicates problem places

Mean and Wilson B-factor

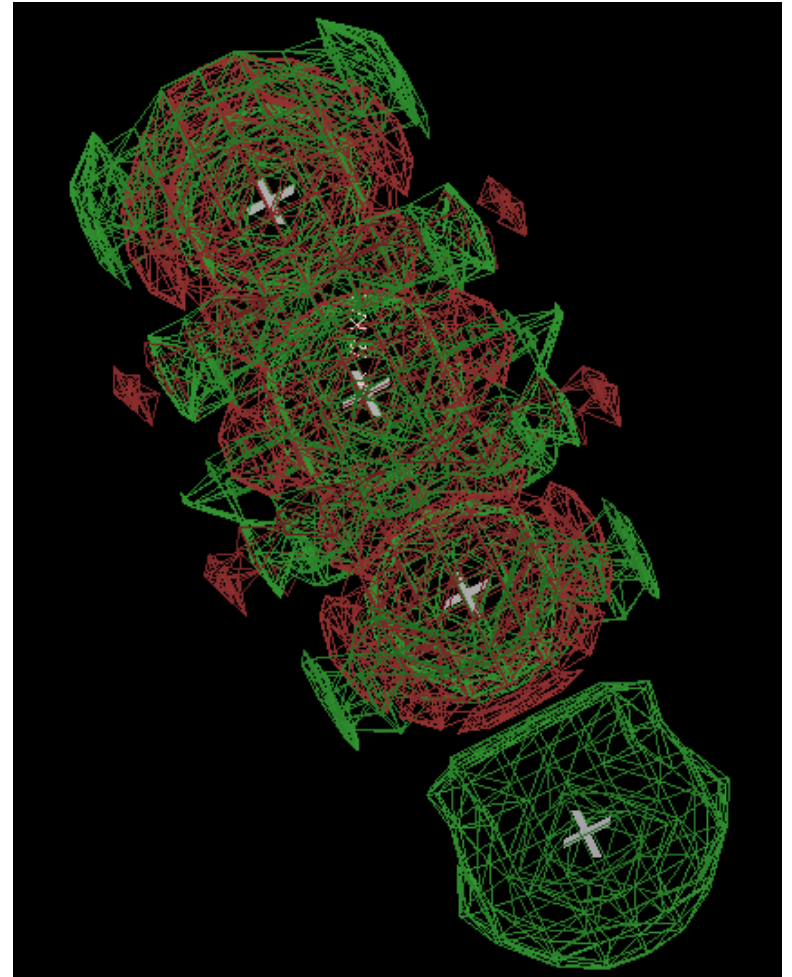
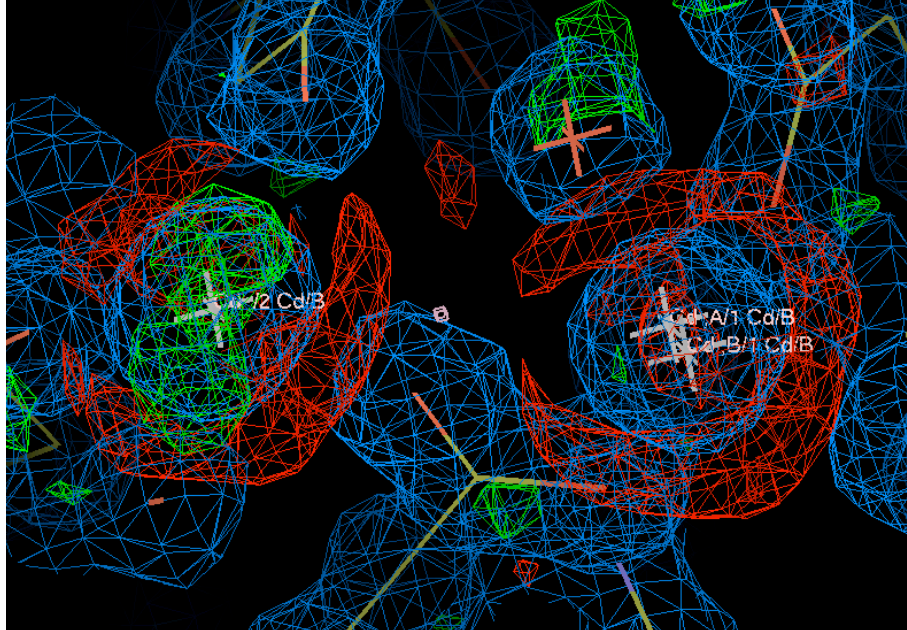
Resolution	B (Wilson)	$\langle B \rangle$	Models
0.00-1.00	9.77	13.11	94
1.00-1.25	10.58	16.44	401
1.25-1.50	13.50	19.14	1050
1.50-1.75	17.20	21.76	3600
1.75-2.00	22.27	26.82	5510
2.25-2.50	35.70	39.42	3385
2.50-2.75	43.71	44.73	2844
2.75-3.00	53.86	51.94	1628
3.00-3.25	65.11	60.76	780
3.25-3.50	81.69	78.70	165
3.50-3.75	92.67	88.84	100
3.75-4.00	111.83	102.29	30



- Mean B and Wilson B are usually similar
 - Wilson B is dominated by strongly diffracting (lower B) atoms that contribute more to high-res reflections
 - Wilson B represents the lower end of the range of B-factors
 - Discrepancy between Wilson B and mean B is not important



Fourier truncation artifacts



- Don't attempt to build water into noise !

Phenix tools for validation

Validation tab

PHENIX home

Click or drag-and-drop files onto a program to launch it. To switch to a project, click the "Choose this project" button.

ID	Last modified	# of jobs	R-free
1rc9	Jul 17 2010 04:34 PM	3	0.2352
✓ lysozime	Jul 17 2010 03:29 PM	2	0.3218
AF	Jul 17 2010 02:48 PM	1	0.4791
rama2	Jul 14 2010 04:10 PM	3	0.1894
rama1	Jul 14 2010 12:31 PM	1	0.1780
industry_MTP	Jul 12 2010 12:17 PM	0	None
rnase	Jul 12 2010 12:14 PM	0	None

Switch project Delete project

Output directory : /Users/afonine/Desktop/AUSTRALIA_SCHOOL_JUL2010/Lys Browse...

PHENIX version 1.6.2-432 Project: lysozime

Reflection tools

Model tools

Experimental phasing

Molecular replacement

Building and refinement

Maps

Ligands

Validation

Comprehensive validation
Model quality assessment, including real-space correlation and geometry inspection using Molprobit tools

POLYGON
Graphical comparison of validation statistics and the PDB

PDB Statistics Overview
Histograms of selected statistics for structures in the PDB (same data as POLYGON, in a different format)

Utilities



Phenix tools for validation

Data analysis - Xtrriage



PHENIX home

Quit Preferences Help Citations Coot PyMOL KiNG Other tools Ask for help

Actions Job history

Projects

Show group: All groups Manage...

Select Delete New project Settings

ID	Last modified	# of jobs	R-free
✓ porin-twin	Apr 15 2015 06:25 ...	6	---
rnase-s_0	Apr 10 2015 08:21 ...	1	0.2125
iris	Apr 10 2015 07:07 ...	18	---
junk1	Apr 02 2015 08:07 ...	0	---
andre	Mar 31 2015 11:27 ...	1	---
dave	Mar 23 2015 11:32 ...	2	---
p9-sad_0	Mar 21 2015 04:23 ...	0	---
rnase-s	Mar 05 2015 02:32 ...	0	---
ssss	Feb 25 2015 03:12 ...	3	---
zzz	Feb 25 2015 03:10 ...	6	---
fsdgsdg	Feb 23 2015 12:15 ...	4	---
teresa	Feb 06 2015 05:55 ...	2	---

Data analysis

- Xtrriage**
Analysis of data quality and crystal defects
- Merging statistics**
Calculates a variety of statistics for unmerged intensities, I/sigma, R-merge, R-meas, and CC1/2.
- Experimental phasing**
- Molecular replacement**
- Model building**
- Refinement**
- Validation**
- Ligands**
- Reflection tools**
- Maps**

Current directory: /Users/pafonine/Documents/porin-twin Browse...

PHENIX version dev-1988-000 Project: porin-twin

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Phenix NEW [Development release of PHENIX version 1.4 now available](#)
Python-based Hierarchical ENvironment for Integrated Xtallography

PHENIX is a new software suite for the automated determination of macromolecular structures using X-ray crystallography and other methods.

Citing PHENIX:
 PHENIX: building new software for automated crystallographic structure determination P.D. Adams, R.W. Grosse-Kunstleve, L.-W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter and T.C. Terwilliger. *Acta Cryst.* D58, 1948-1954 (2002)

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Using PHENIX (release 1.4-3): [Full Documentation](#) [PDF](#)

- Assessing data quality with [phenix.xtriage](#)
- Automated structure solution with [AutoSol](#)
- Automated molecular replacement with [AutoMR](#)
- Automated model building and rebuilding with [AutoBuild](#)
- Automated ligand fitting with [LigandFit](#)
- Structure refinement with [phenix.refine](#)
- Generation of ligand coordinates and restraints with [elbow](#)
- The [PHENIX Graphical User Interface](#)

[Documentation for 1.3-final](#)

The PHENIX system also includes SOLVE/RESOLVE, Phaser, Textal, the CCI Applications (phenix.xtriage, phenix.refine, elbow and many more), components from Molprobit, and the Computational Crystallography Toolbox in a Python framework.




Funding for PHENIX: [Protein Structure Initiative \(NIH General Medical Sciences\)](#)

The PHENIX Industrial Consortium [Information](#)

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