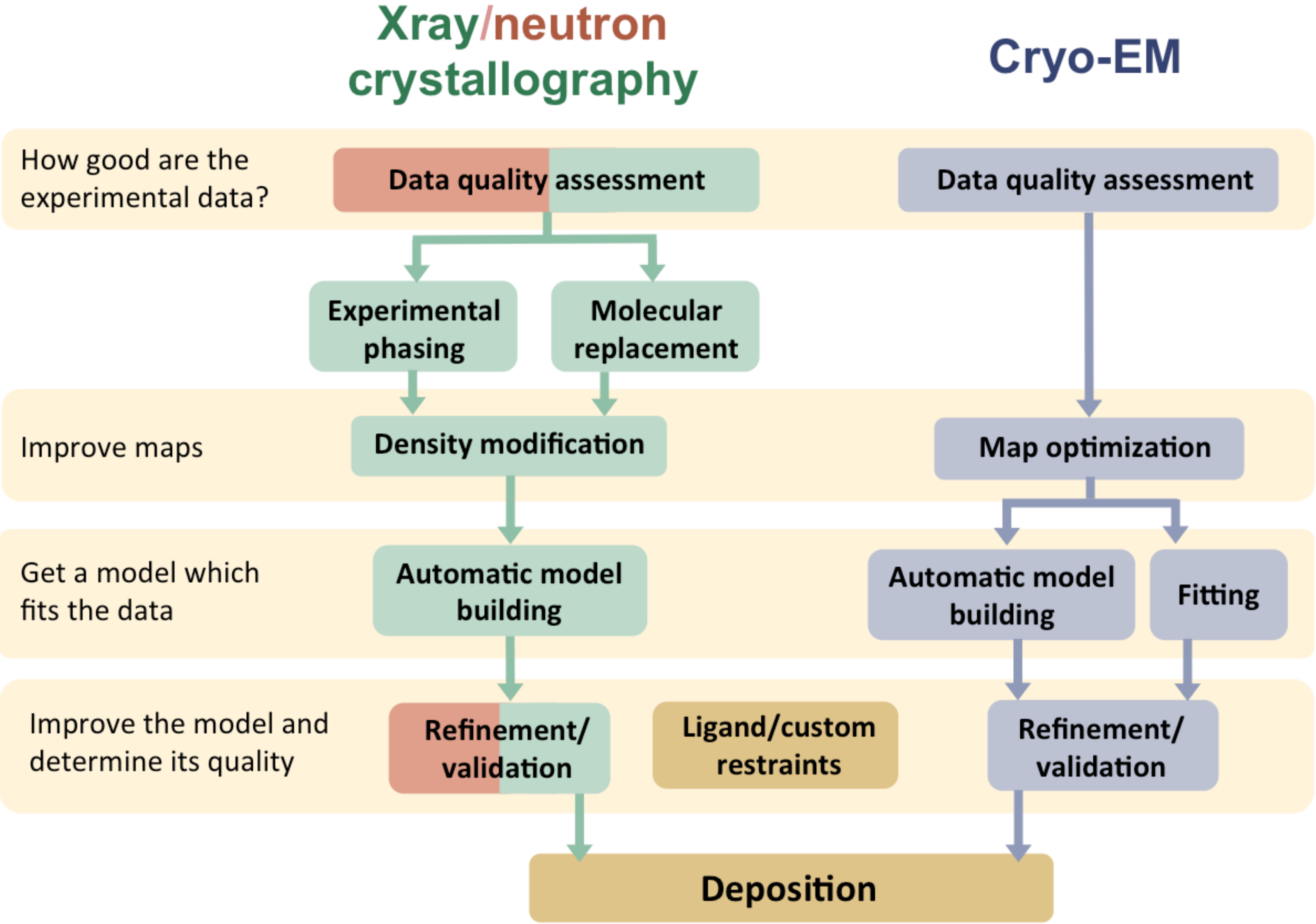
Validation: data analysis

Pavel Afonine

Lawrence Berkeley National Lab, California, USA

KU, November 9th 2023

Phenix: tools for crystallography and cryo-EM



Xray/neutron crystallography

Cryo-EM

How good are the experimental data?

Data quality assessment

Data quality assessment

Experimental phasing

Molecular replacement

Improve maps

Density modification

Map optimization

Get a model which fits the data

Automatic model building

Automatic model building

Fitting

Improve the model and determine its quality

Refinement/ validation

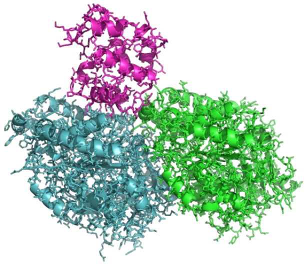
Ligand/custom restraints

Refinement/ validation

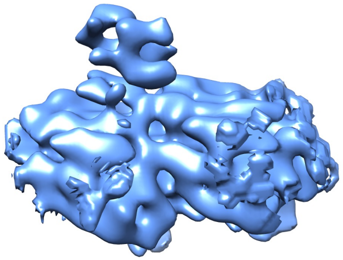
Deposition

Validation

Model

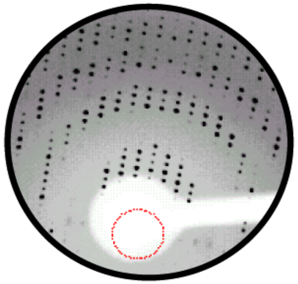


Data



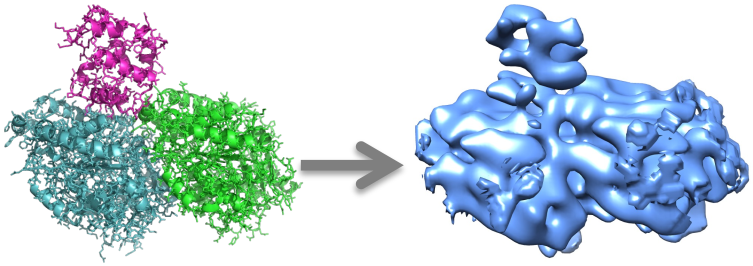
Cryo-EM

or



Diffraction

Model to data fit

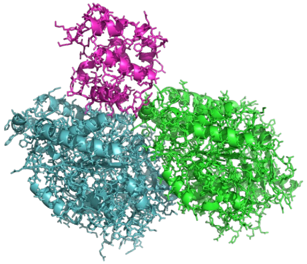


Validation = checking model, data and model-to-data fit are all make sense and obey to prior expectations

Validation tools: *Crystallography vs Cryo-EM*

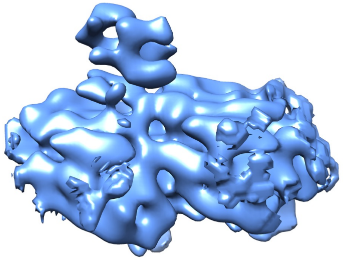
Exact same

Model



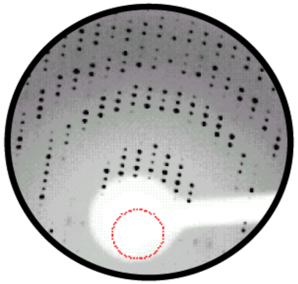
Different

Data



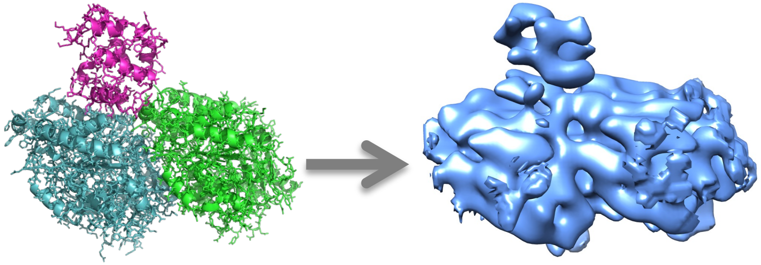
Cryo-EM

or



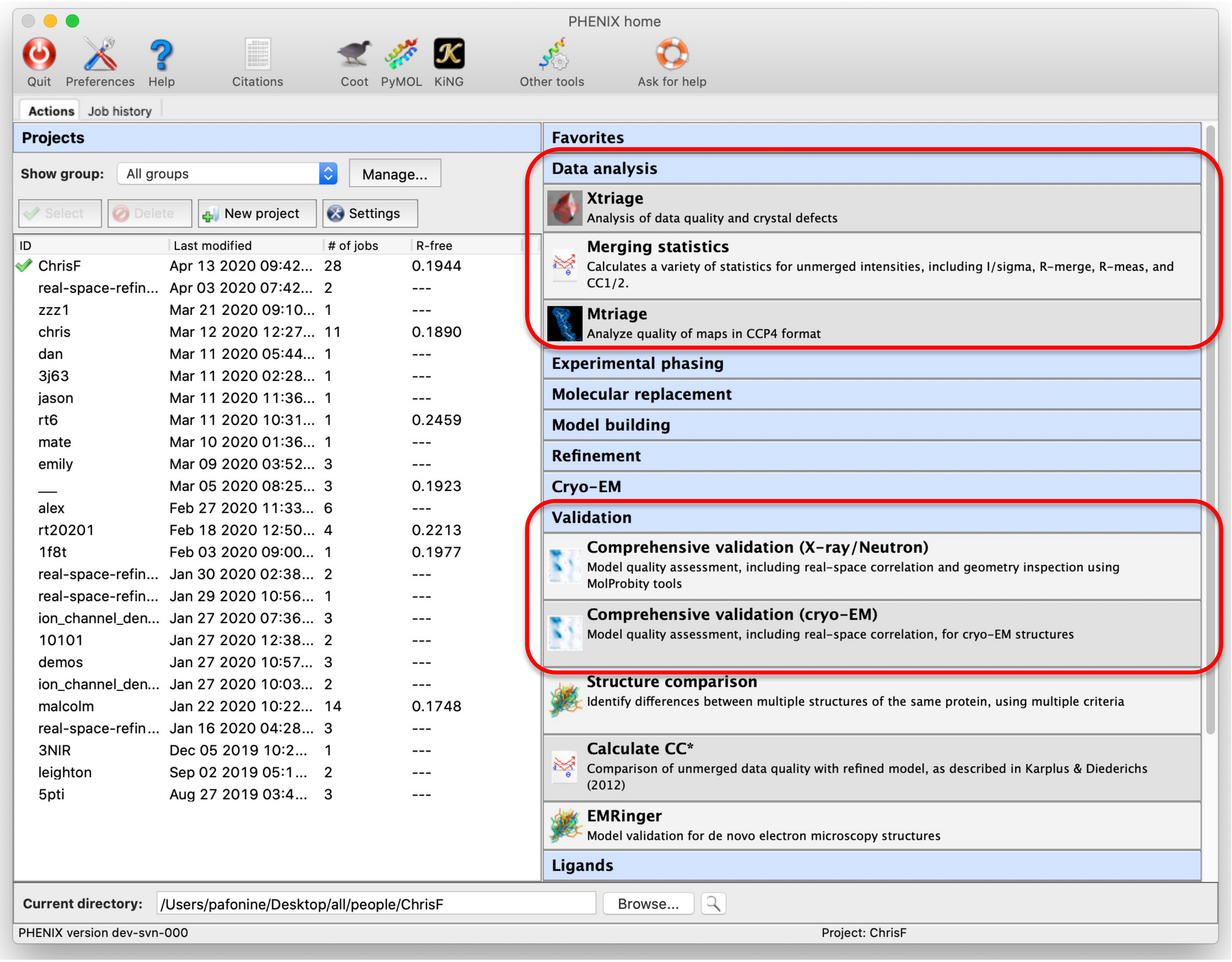
Diffraction

Model to data fit



Similar

Validation tools in Phenix



ID	Last modified	# of jobs	R-free
✓ ChrisF	Apr 13 2020 09:42...	28	0.1944
real-space-refin...	Apr 03 2020 07:42...	2	---
zzz1	Mar 21 2020 09:10...	1	---
chris	Mar 12 2020 12:27...	11	0.1890
dan	Mar 11 2020 05:44...	1	---
3j63	Mar 11 2020 02:28...	1	---
jason	Mar 11 2020 11:36...	1	---
rt6	Mar 11 2020 10:31...	1	0.2459
mate	Mar 10 2020 01:36...	1	---
emily	Mar 09 2020 03:52...	3	---
__	Mar 05 2020 08:25...	3	0.1923
alex	Feb 27 2020 11:33...	6	---
rt20201	Feb 18 2020 12:50...	4	0.2213
1f8t	Feb 03 2020 09:00...	1	0.1977
real-space-refin...	Jan 30 2020 02:38...	2	---
real-space-refin...	Jan 29 2020 10:56...	1	---
ion_channel_den...	Jan 27 2020 07:36...	3	---
10101	Jan 27 2020 12:38...	2	---
demos	Jan 27 2020 10:57...	3	---
ion_channel_den...	Jan 27 2020 10:03...	2	---
malcolm	Jan 22 2020 10:22...	14	0.1748
real-space-refin...	Jan 16 2020 04:28...	3	---
3NIR	Dec 05 2019 10:2...	1	---
leighton	Sep 02 2019 05:1...	2	---
5pti	Aug 27 2019 03:4...	3	---

Data analysis

Xtriage
Analysis of data quality and crystal defects

Merging statistics
Calculates a variety of statistics for unmerged intensities, including I/sigma, R-merge, R-meas, and CC1/2.

Mtriage
Analyze quality of maps in CCP4 format

Experimental phasing

Molecular replacement

Model building

Refinement

Cryo-EM

Validation

Comprehensive validation (X-ray/Neutron)
Model quality assessment, including real-space correlation and geometry inspection using MolProbity tools

Comprehensive validation (cryo-EM)
Model quality assessment, including real-space correlation, for cryo-EM structures

Structure comparison
Identify differences between multiple structures of the same protein, using multiple criteria

Calculate CC*
Comparison of unmerged data quality with refined model, as described in Karplus & Diederichs (2012)

EMRinger
Model validation for de novo electron microscopy structures

Ligands

Current directory: /Users/pafonine/Desktop/all/people/ChrisF

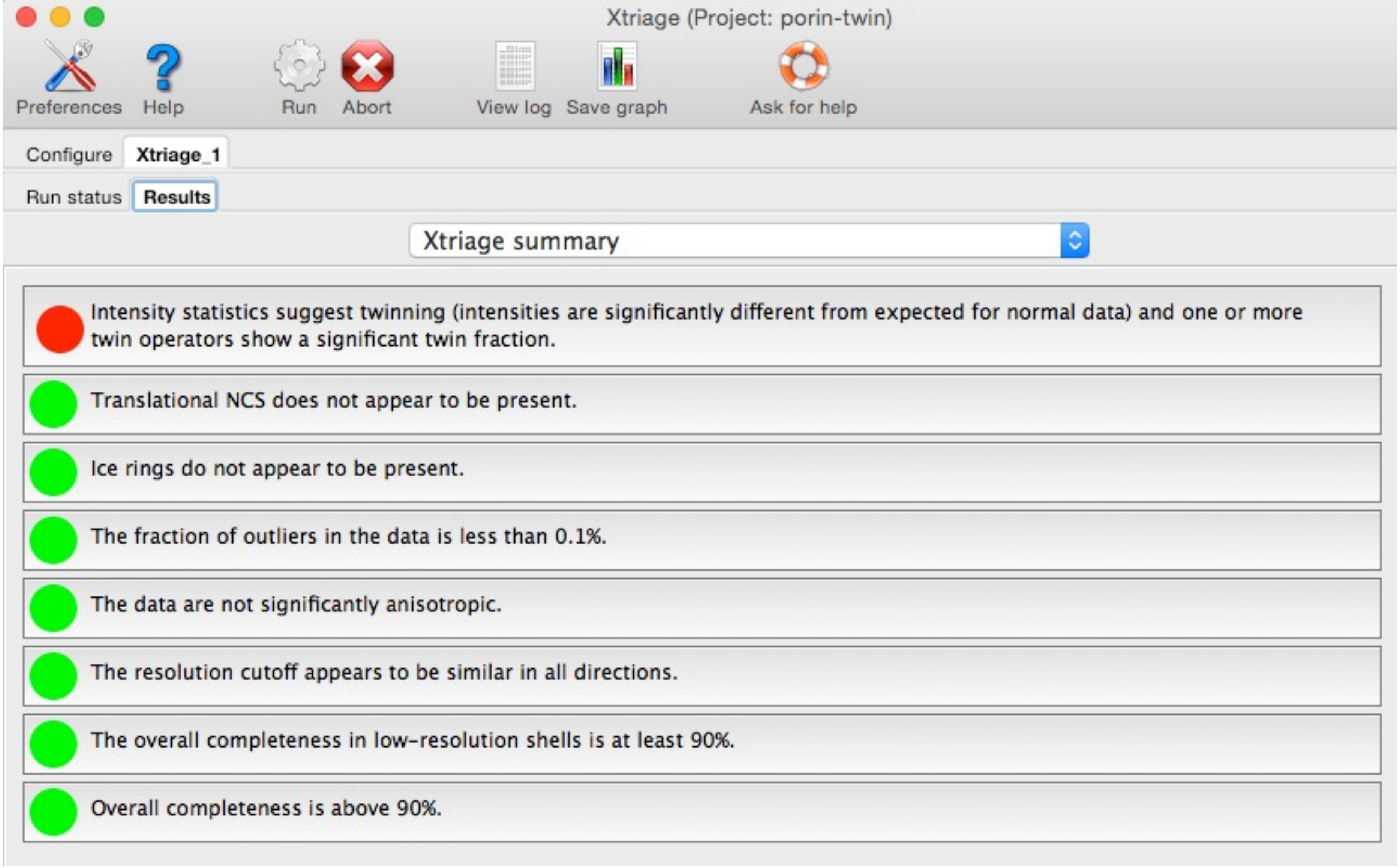
PHENIX version dev-svn-000

Project: ChrisF

Xtriage: all about your Xtal 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



🔴 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%.

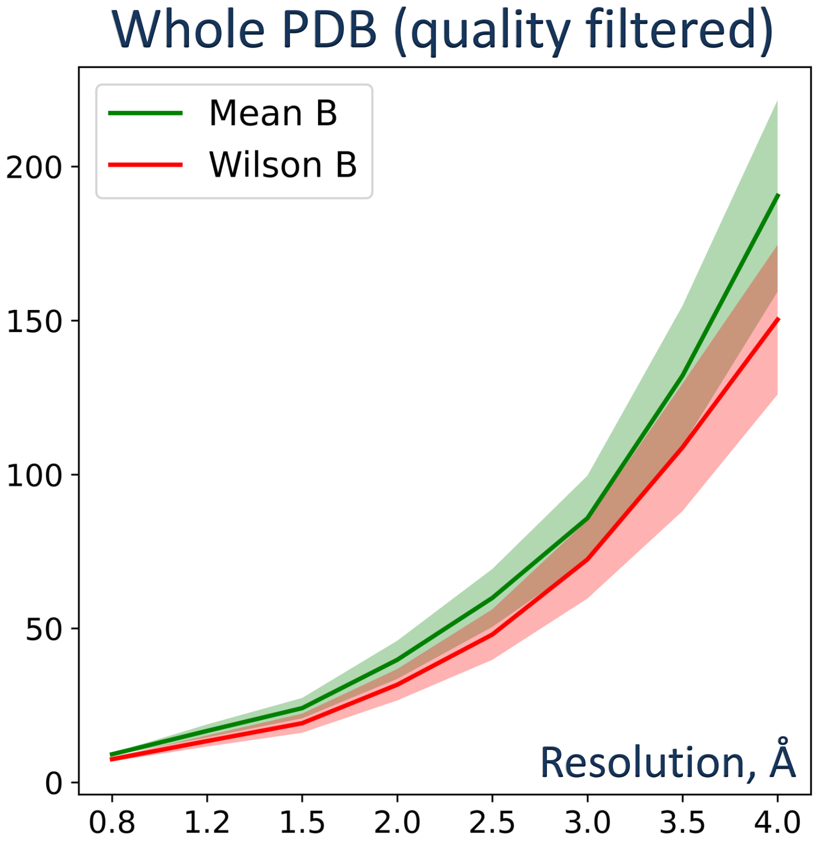
🟢 The data are not significantly anisotropic.

🟢 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%.

Wilson B

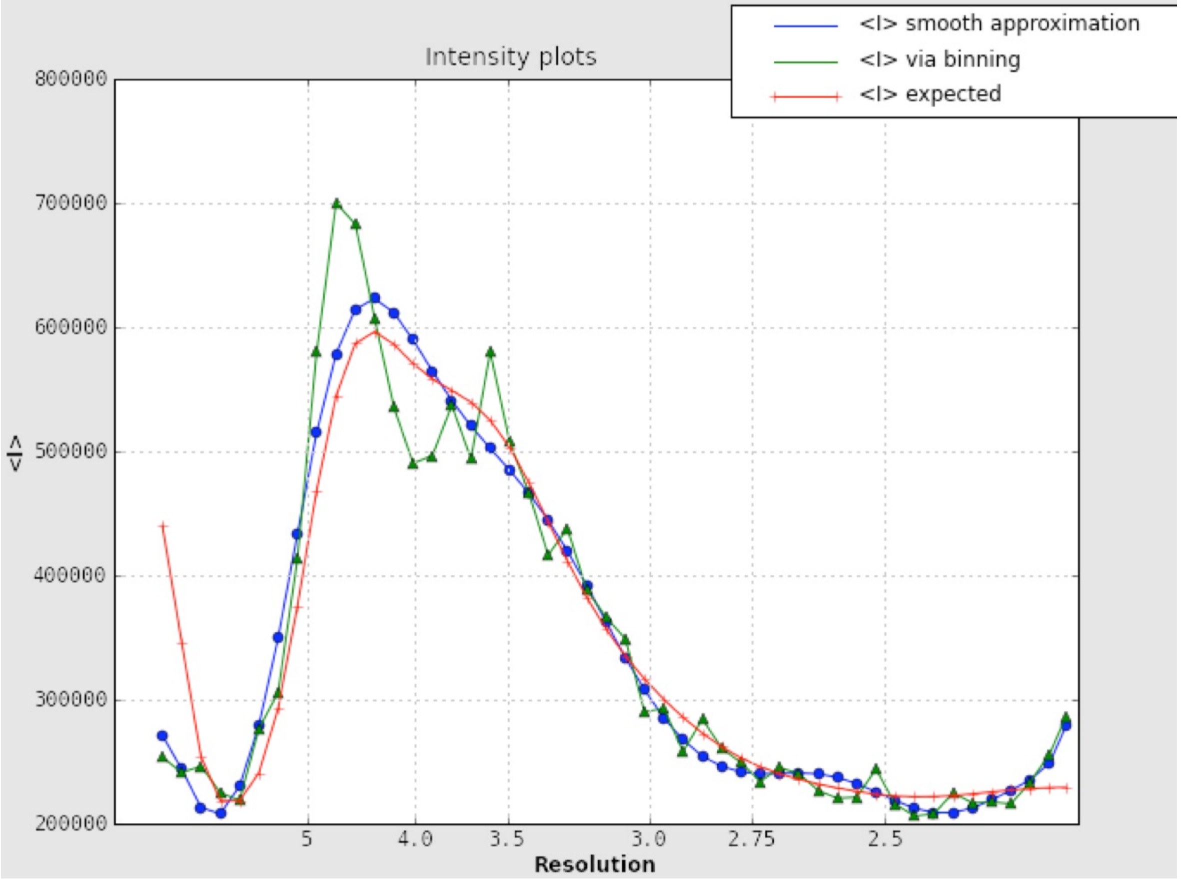


Wilson statistics assumes atoms of the same kind are randomly distributed in the unit cell and have the same isotropic B-factors

- 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

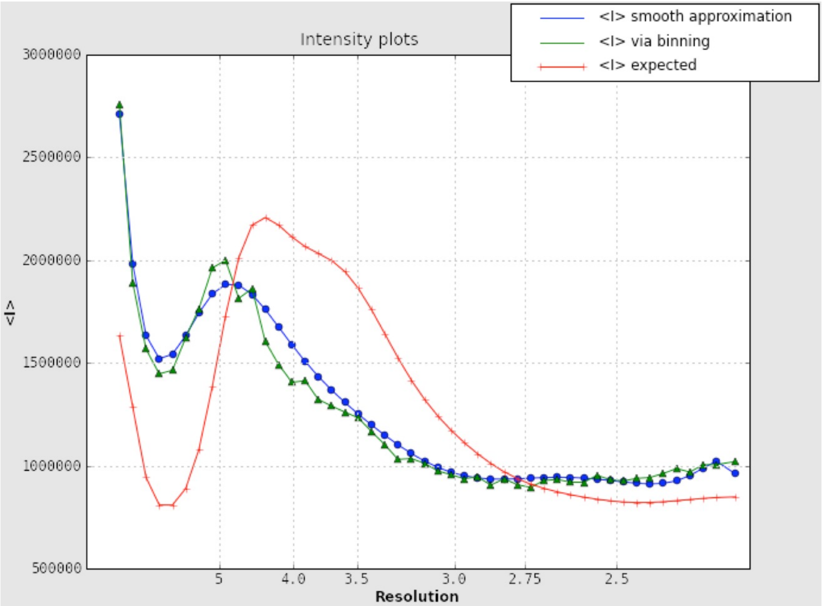
Wilson plot (mean intensity vs resolution)

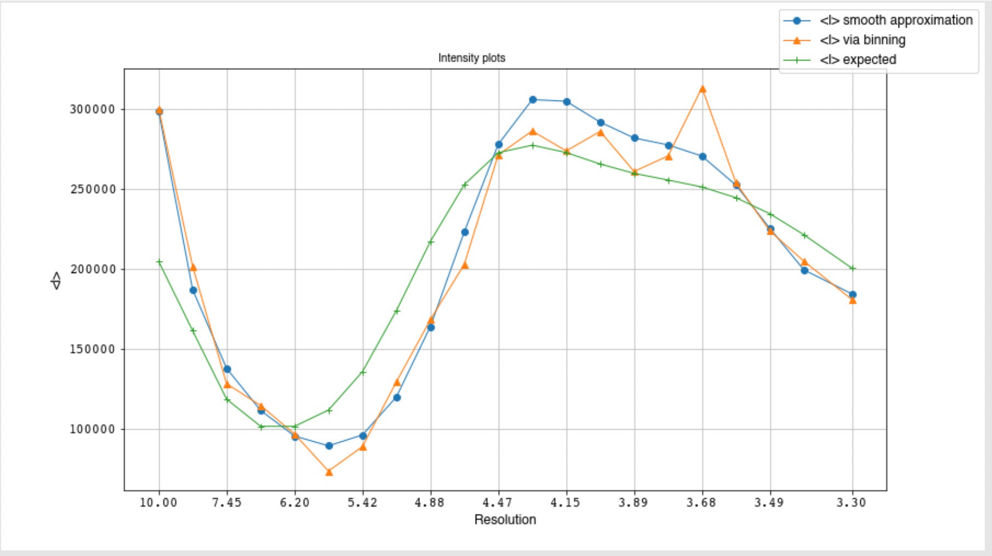
- The Wilson plot looks at mean intensity of diffraction by resolution, a curve which has a predictable shape



Wilson plot (mean intensity vs resolution)

- Main reasons for deviations from expected distribution
  - Bad data (e.g., ice rings or poor data processing
  - Macromolecule that doesn’t look like the average protein
  - Looking at only a part of the plot (e.g., low-resolution data)

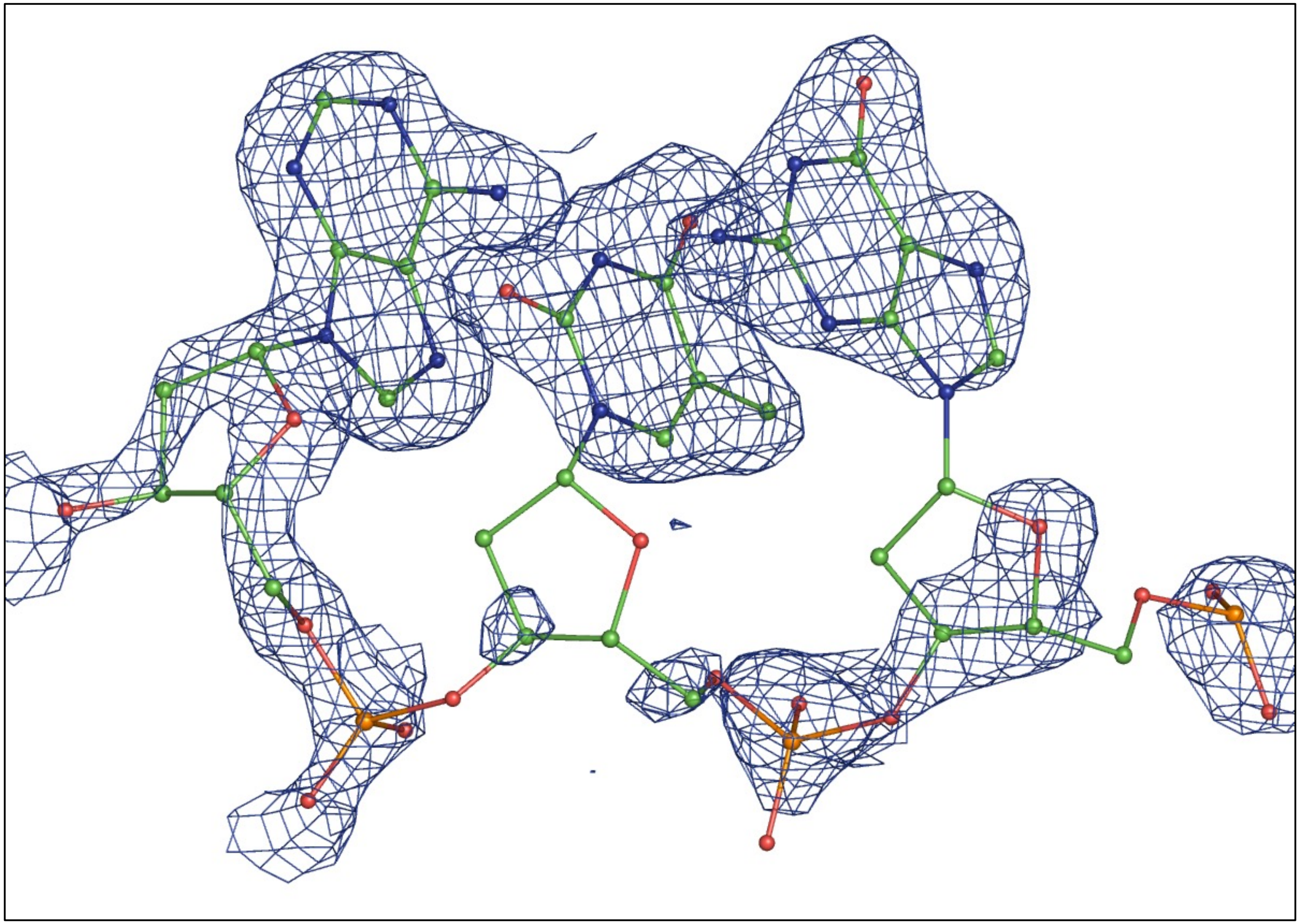




Data completeness

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

2mFo-DFc , 1σ



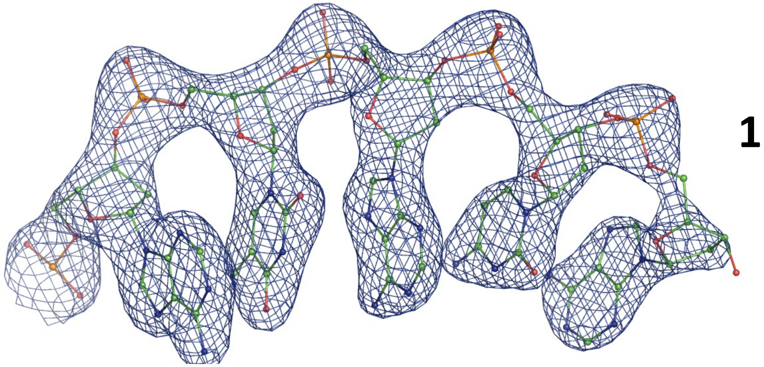
Data completeness

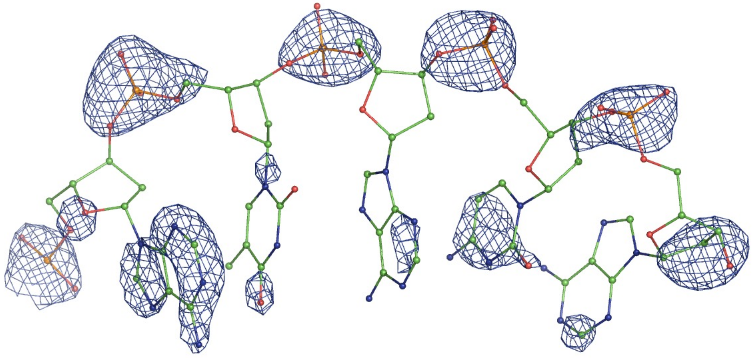
```
Completeness by resolution:
 19.9274 -  3.2441 0.78
  3.2441 -  2.5767 0.99
  2.5767 -  2.2515 1.00
  2.2515 -  2.0459 1.00
  2.0459 -  1.8993 0.99
Overall completeness in dmin-inf: 0.95
```

Fcalc maps, full set d_{min}-inf

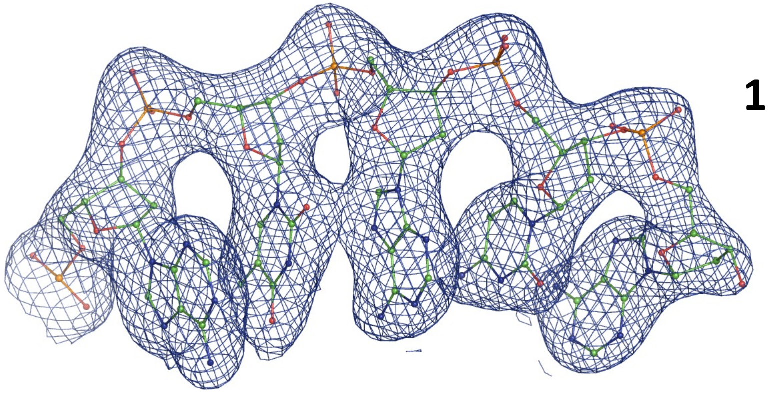
Fcalc maps, incomplete set

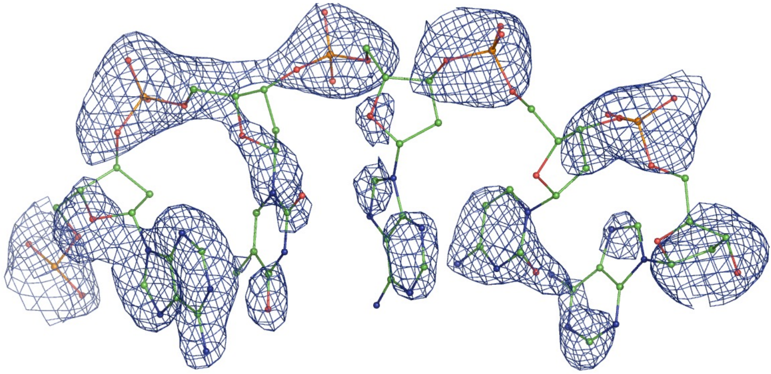
1.5σ map cutoff





1σ map cutoff

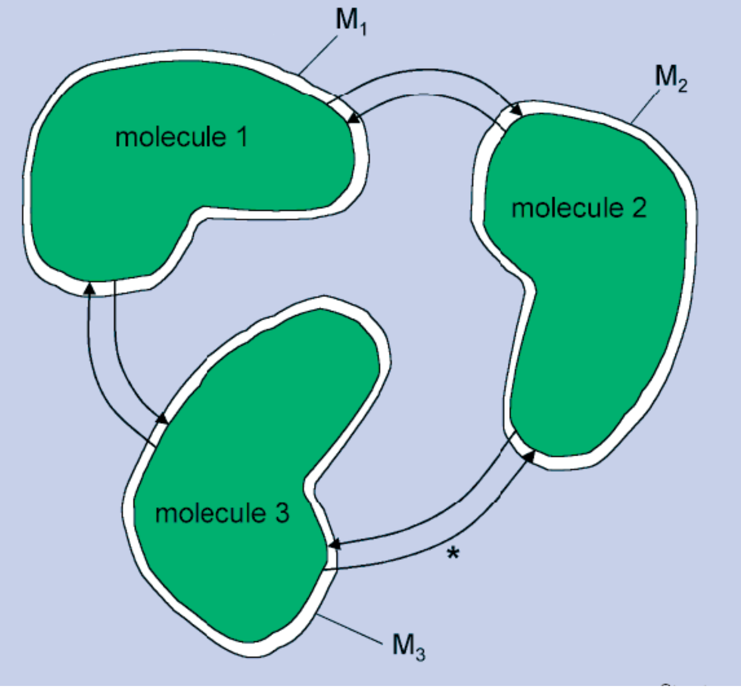




Systematic data incompleteness can distort maps

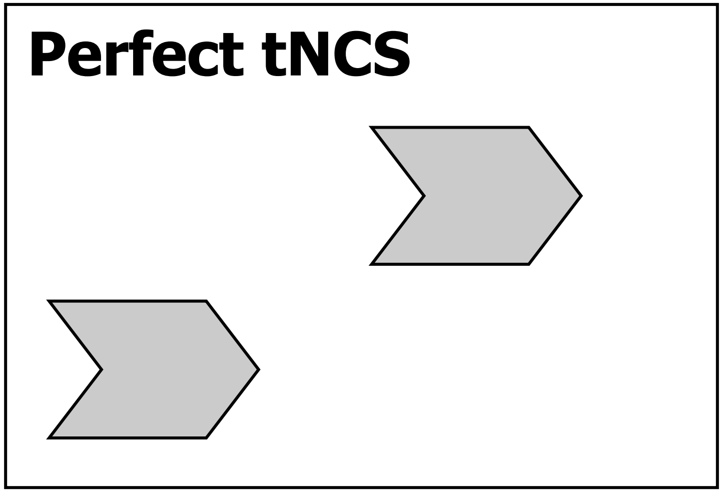
Non-crystallographic symmetry NCS

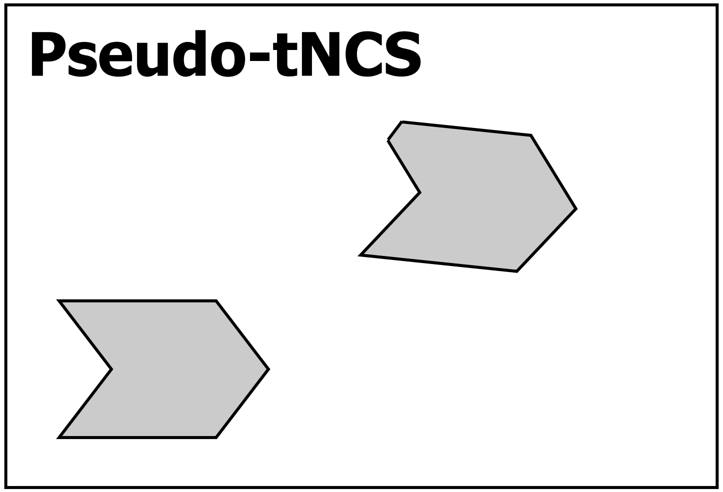
- Two or more molecules in the ASU related by rotation-translation
- NCS is found in about 1/3 to 1/2 of crystal structures
- Usually helps solving/refining models at medium-to-low resolution
- A special case of NCS, translational NCS (tNCS) leads to complications



Translational NCS (tNCS)

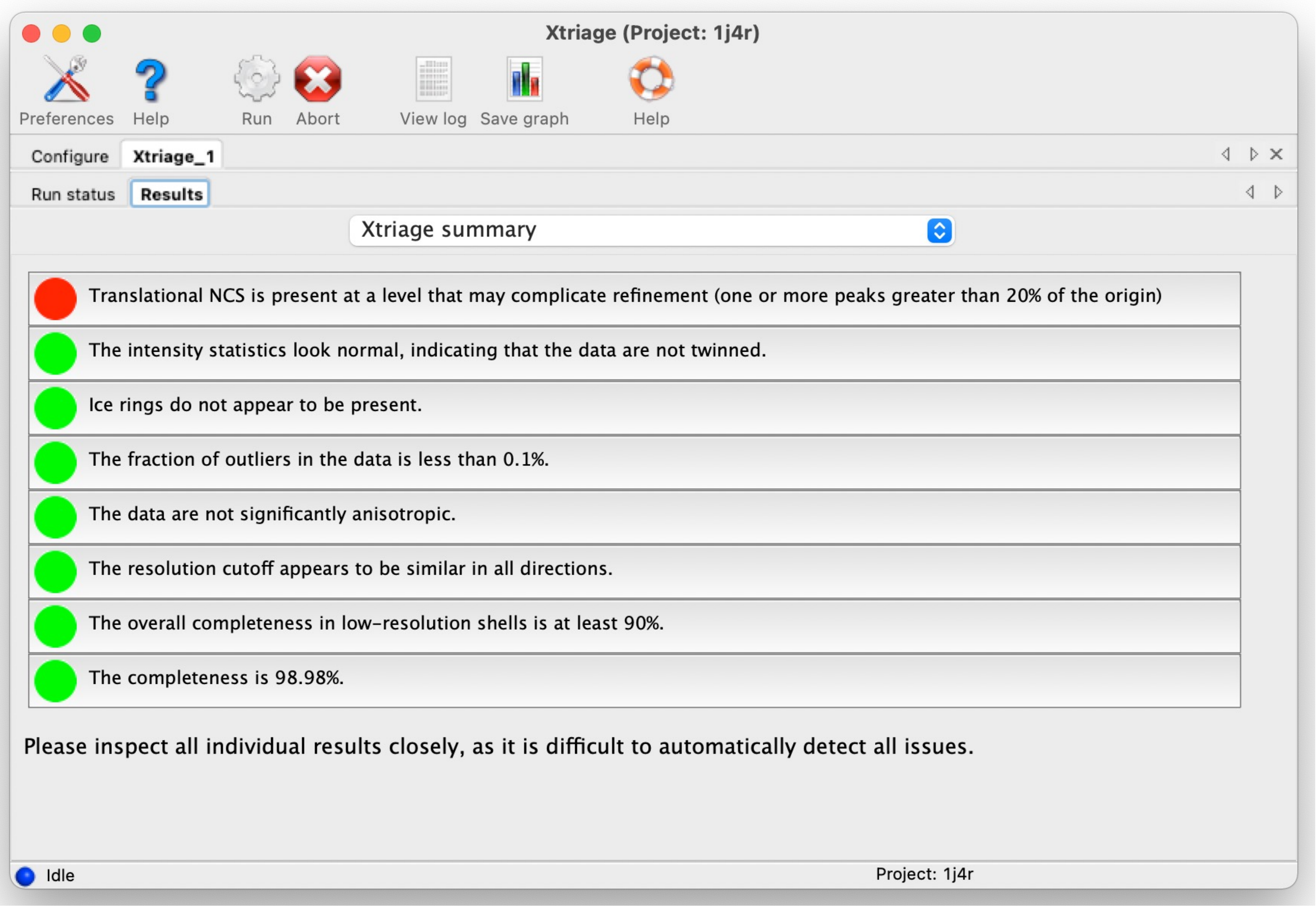
- tNCS arises when the ASU contains components that are oriented in (nearly) the same way and can be superimposed by a translation that does not correspond to any symmetry operation in the space group.





- Used to complicate MR (no it is taken care of)
- Risk to bias OMIT map

Translational NCS (tNCS)



🟥 Translational NCS is present at a level that may complicate refinement (one or more peaks greater than 20% of the origin)

🟩 The intensity statistics look normal, indicating that the data are not twinned.

🟩 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.

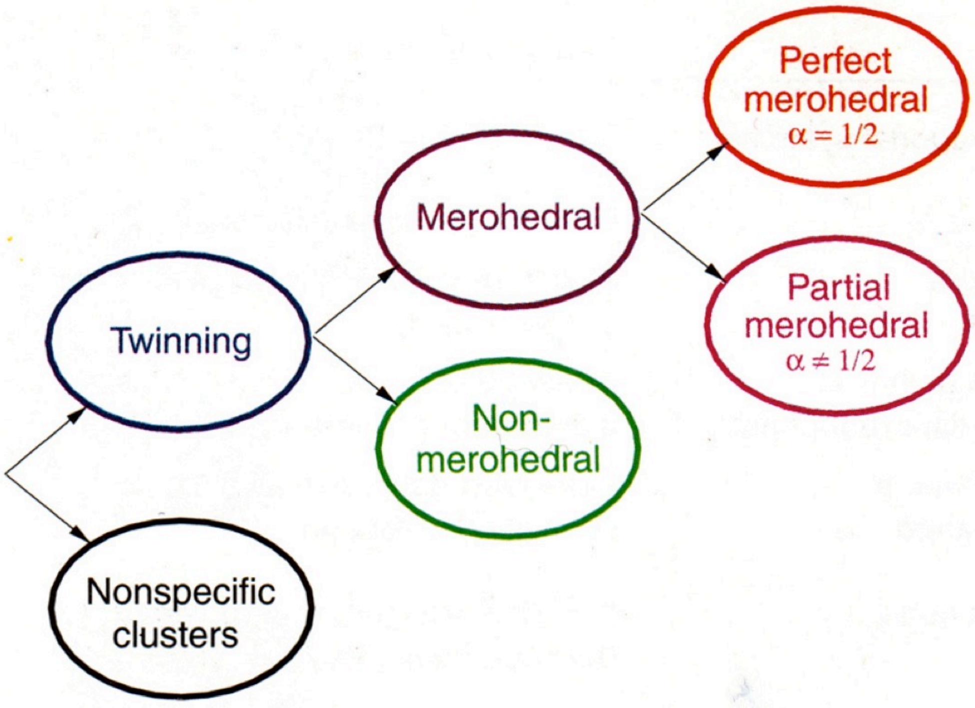
🟩 The overall completeness in low-resolution shells is at least 90%.

🟩 The completeness is 98.98%.

Please inspect all individual results closely, as it is difficult to automatically detect all issues.

Twinning

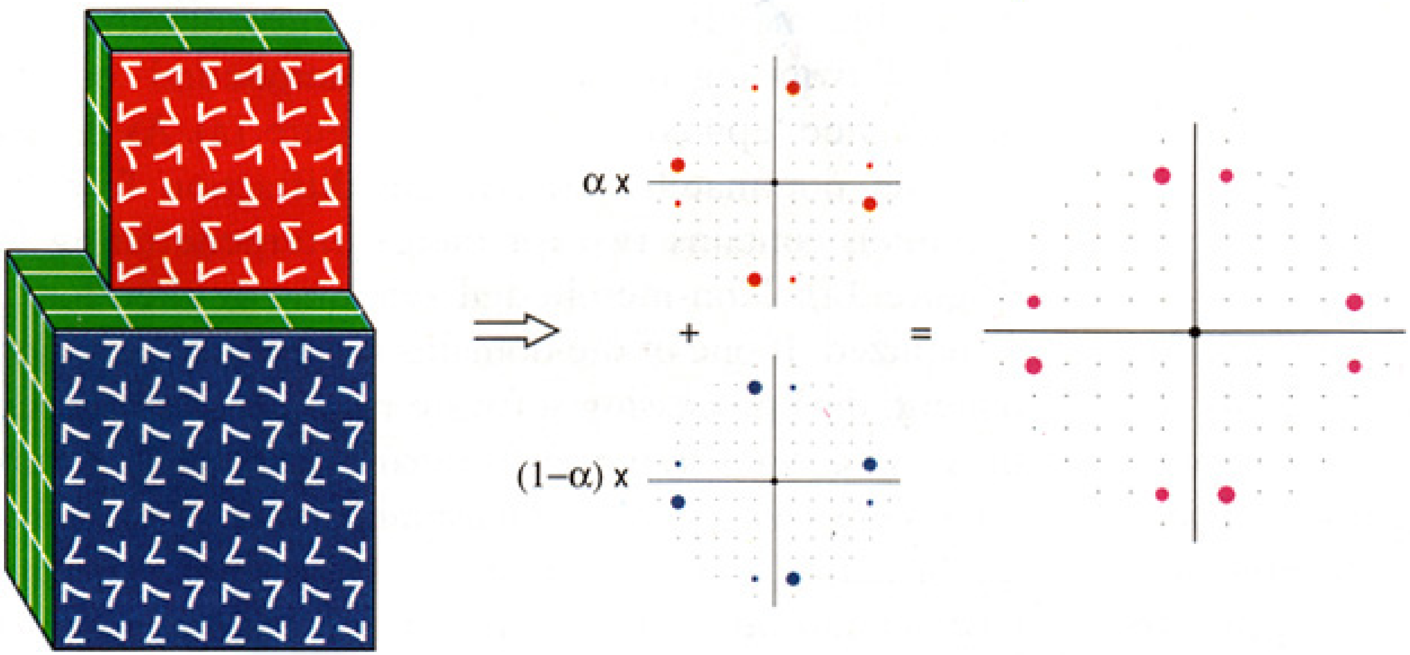
- Twinning is a crystal growth disorder



Typically only merohedral twinning is dealt with in a meaningful way in macromolecules

Twinning

- Merohedral twining occurs when your crystal is composed of identical but rotated crystals combined together such that their lattices matching



- Observed intensity is a weighted sum of individual intensities:

$$I_{\mathrm{OBS}}(\mathbf{h}) = \alpha_1 I(\mathbf{h}) + \ldots + \alpha_N I(\mathbf{T}_N \mathbf{h})$$

$$\alpha_1 + \ldots + \alpha_N = 1$$

Twinning

- Twinning parameterization
  - **Twin law** describes orientation of different species relative to each other (rotation matrix T that transforms hkl indices of one species into the other)
  - **Twin fraction (α):** fractional contribution of each component
    - Estimated by Xtriage
    - Refined by phenix.refine

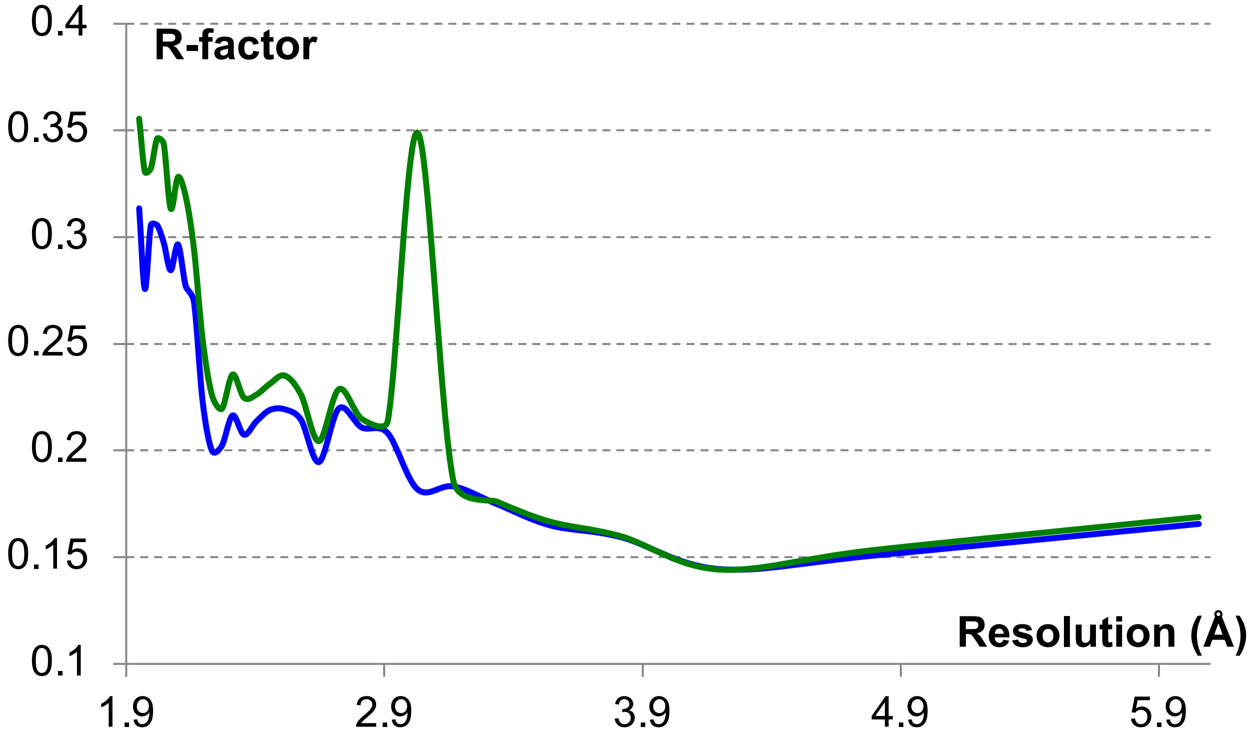
$$I_{\mathrm{OBS}}(\mathbf{h}) = \alpha_1 I(\mathbf{h}) + \ldots + \alpha_N I(\mathbf{T}_N \mathbf{h})$$

$$\alpha_1 + \ldots + \alpha_N = 1$$

Twinning

- tNCS can mask effects of twinning
- If both are present, intensity distributions may look like normal
    - First check for tNCS and use different test for twinning (L-test)
- If crystal is twinned, you have lost information
- Maps going to have model bias that is worse than usual
- Experimental phasing may be difficult
- False symmetry may appear

Watch for outliers



- 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
```