

Phenix user workshop, July 29 2022

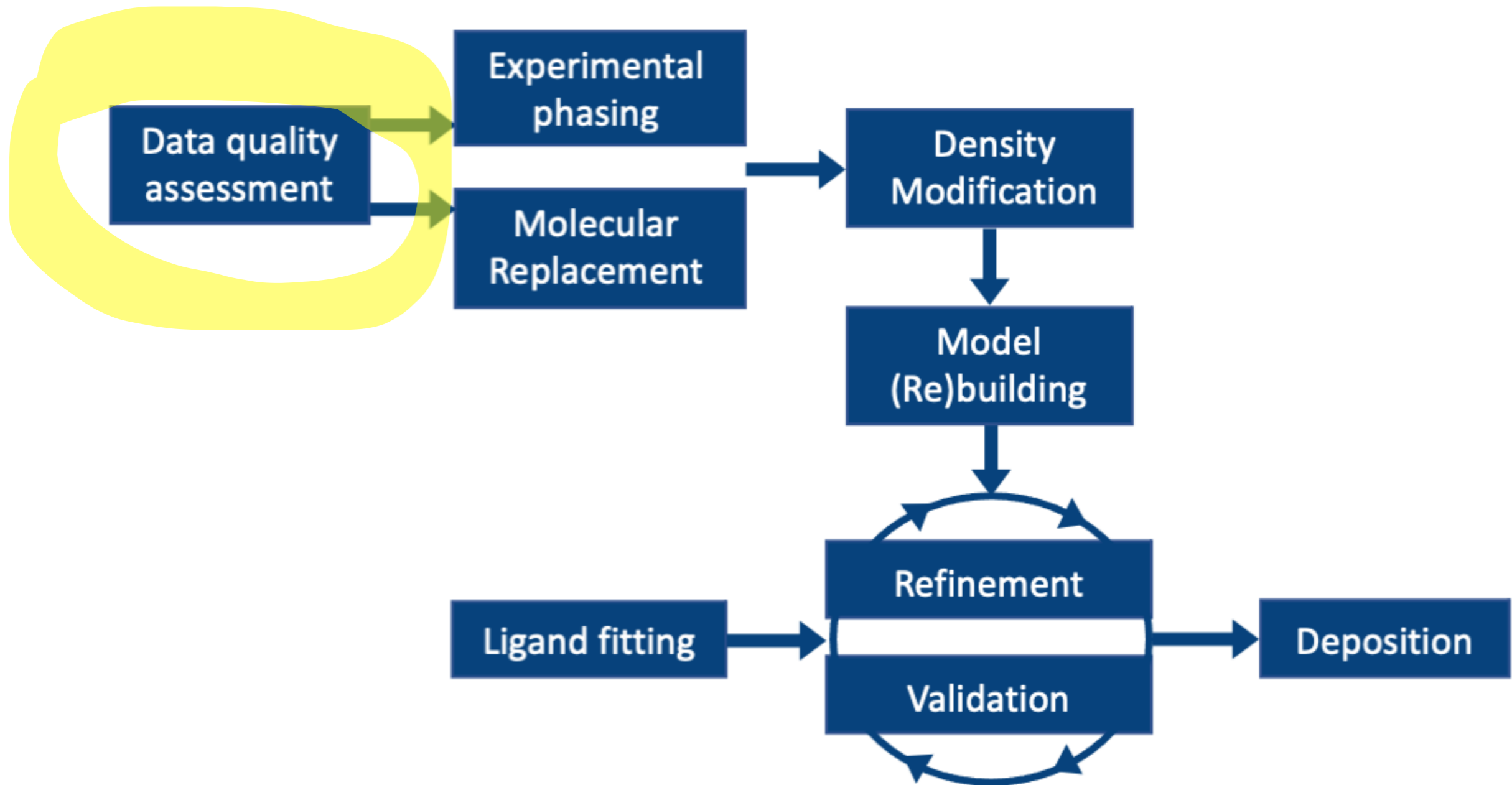


Xtrriage

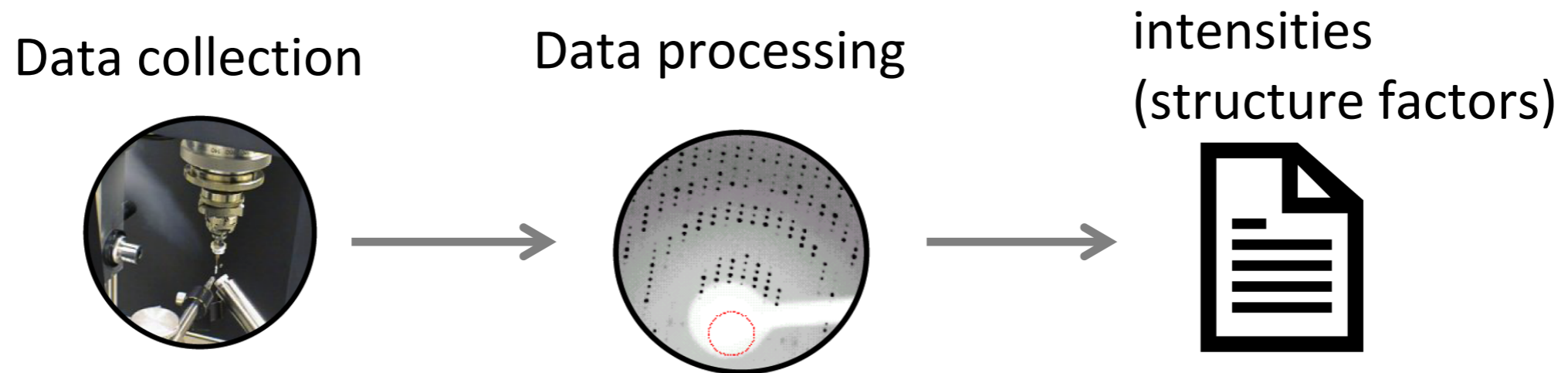
Dorothee Liebschner
Lawrence Berkeley Laboratory

Data validation is the first step!

Before doing anything else, you should validate your data!



Data quality assessment



Macromolecular crystals are prone to pathologies:

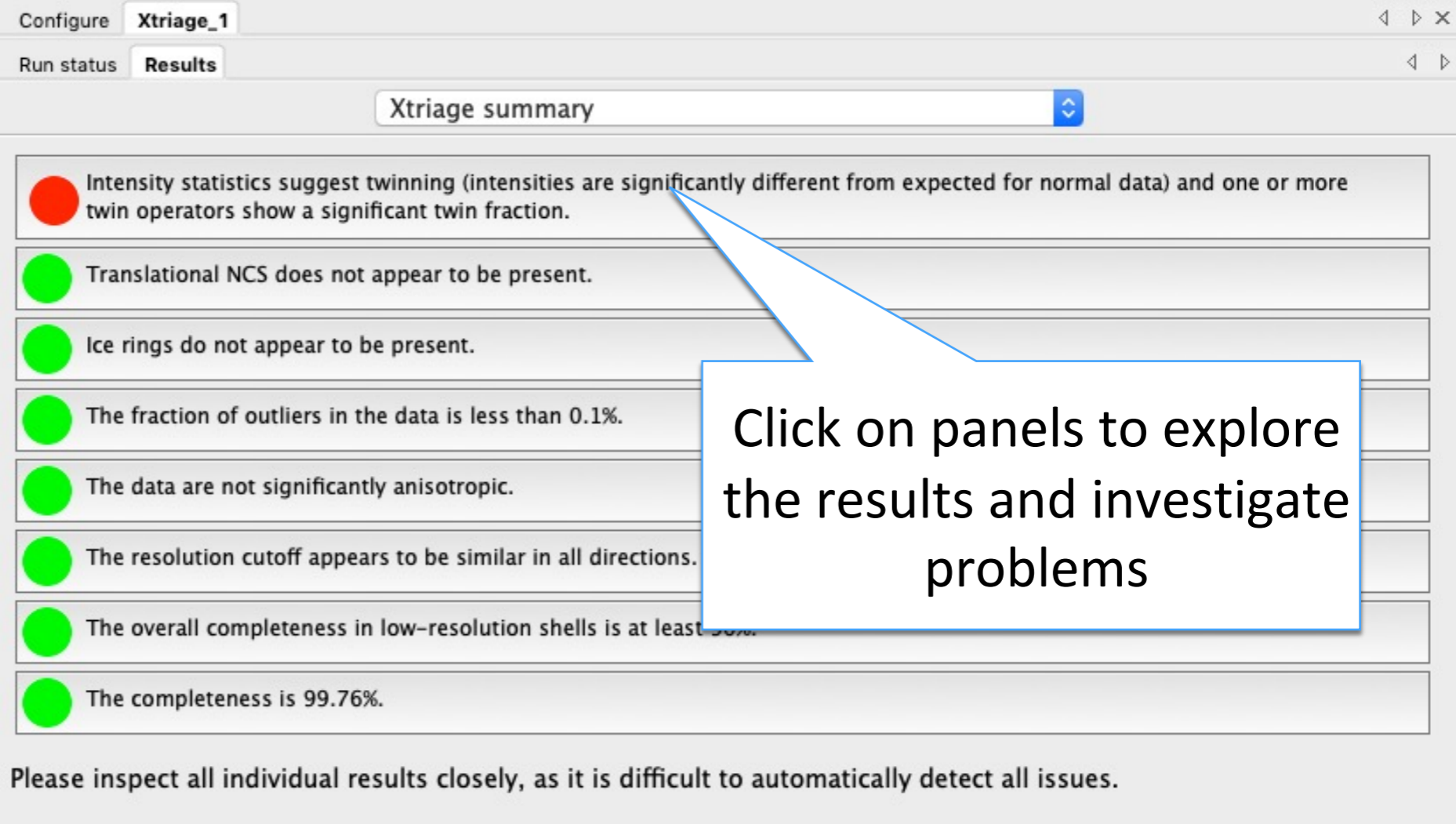
- Twinning: two or more crystals are intergrown (orientations are related by twin operation)
- tNCS: more than one copy of a molecule is in a similar orientation in the asymmetric unit

Data quality assessment

Data anomalies can prevent structure solution!

→ It is important to check your data before phasing, model building and refinement.

Xtrriage does diagnostics for major pathologies and data properties (Wilson plot, completeness, symmetry).



The screenshot shows the Xtrriage software interface. At the top, there are tabs for 'Configure' and 'Xtrriage_1', and 'Run status' and 'Results'. Below this is a search bar containing 'Xtrriage summary'. The main area displays a list of diagnostic results, each with a colored circle icon and a text description:

- Intensity statistics suggest twinning (intensities are significantly different from expected for normal data) and one or more twin operators show a significant twin fraction. (Red circle)
- Translational NCS does not appear to be present. (Green circle)
- Ice rings do not appear to be present. (Green circle)
- The fraction of outliers in the data is less than 0.1%. (Green circle)
- The data are not significantly anisotropic. (Green circle)
- The resolution cutoff appears to be similar in all directions. (Green circle)
- The overall completeness in low-resolution shells is at least 50%. (Green circle)
- The completeness is 99.76%. (Green circle)

A callout box with a blue border and a white background points to the first red circle, containing the text: 'Click on panels to explore the results and investigate problems'. At the bottom of the interface, a note reads: 'Please inspect all individual results closely, as it is difficult to automatically detect all issues.'

Checking data quality with Xtrriage

Are the data going to be useful?

- How accurate are the data?
- Is there an anomalous signal?

Do the data have any unusual characteristics?

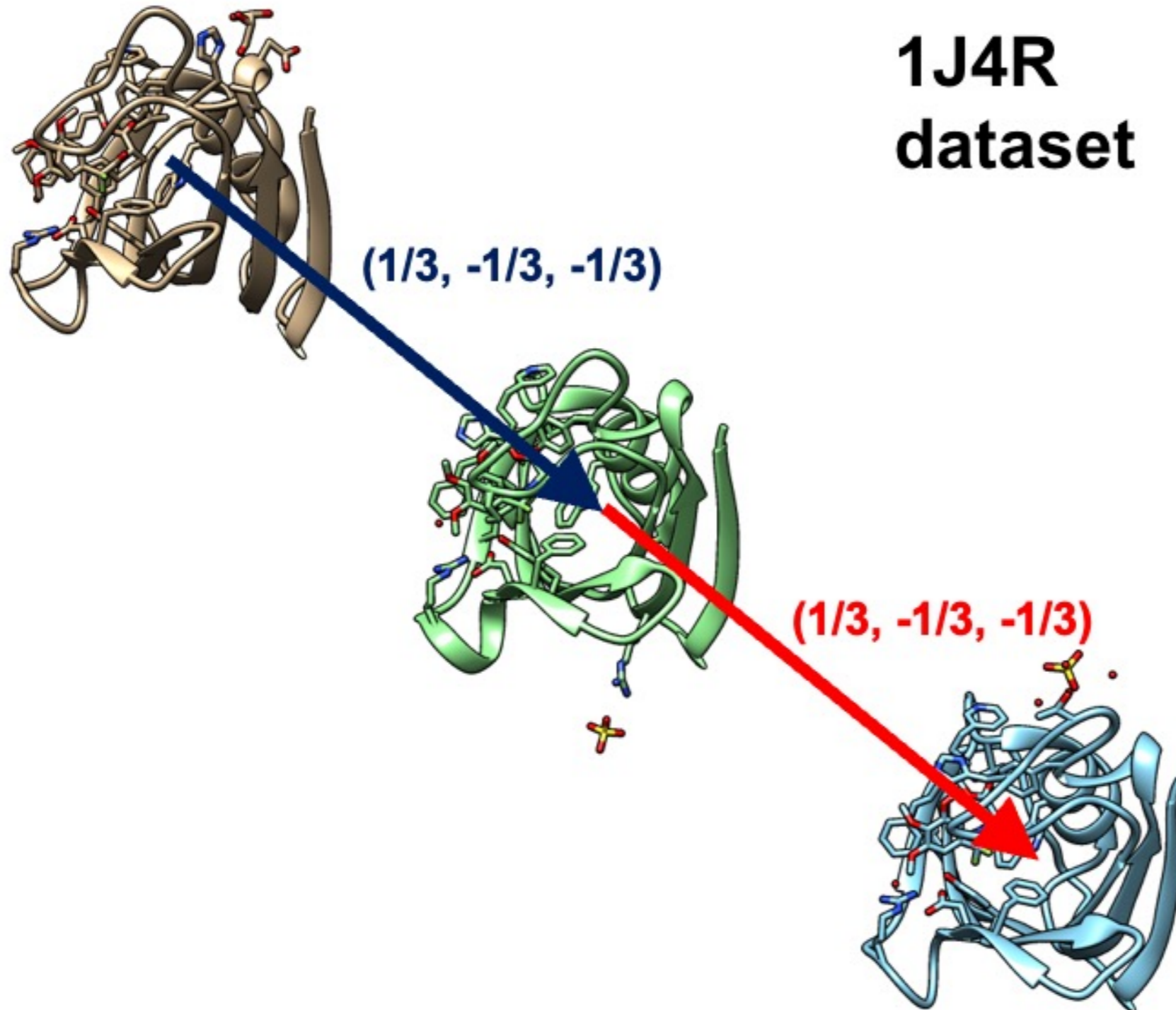
- Are there ice rings?
- Is translational NCS present?
- Is twinning present?

Other things to check with Xtrriage

- Can my data be indexed in a different crystal lattice?
- Do the systematic absences in my data match the space group?

Translational NCS

Two or more copies of the molecule are related by pure translation.



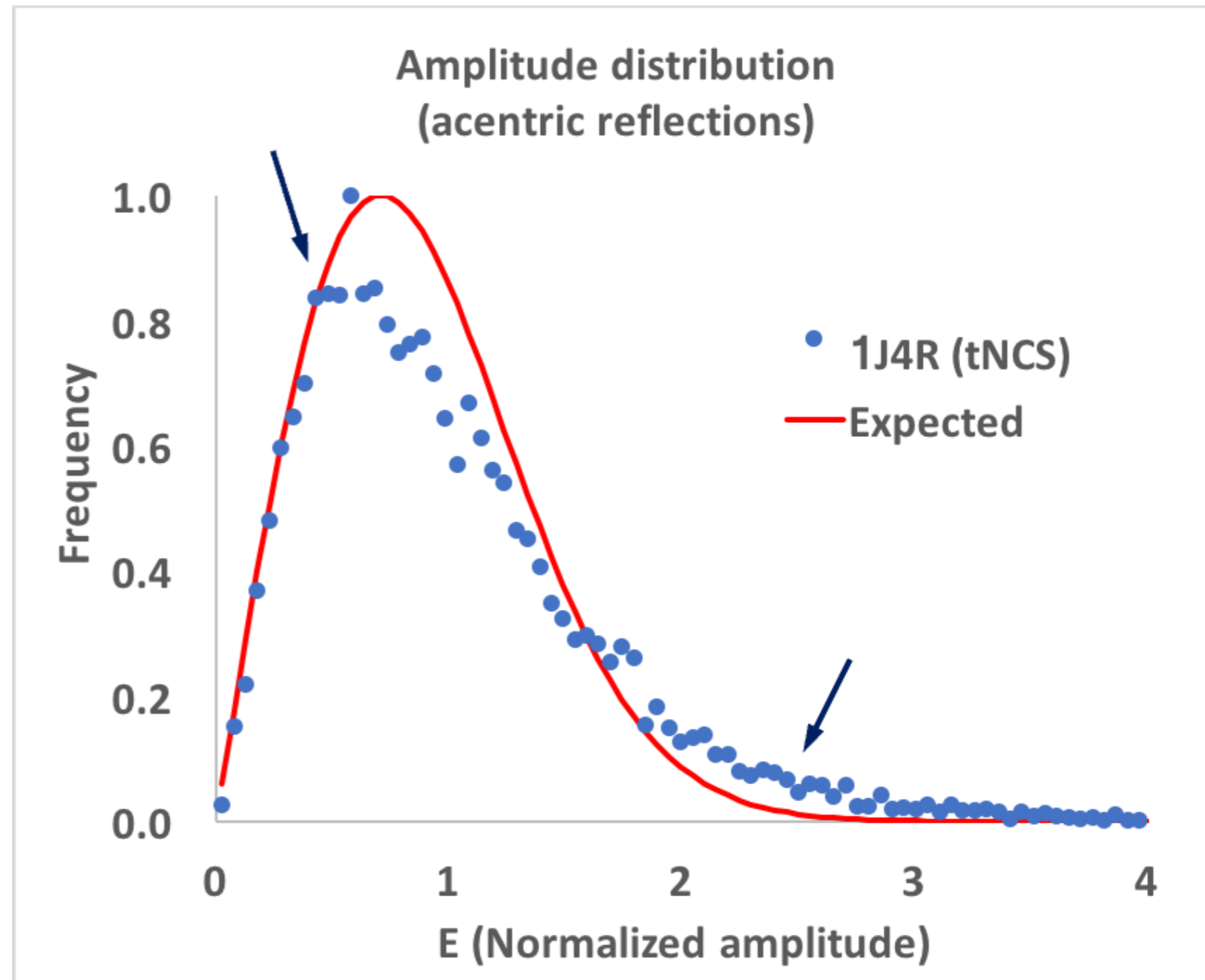
Strong peak in
Patterson function

In this case at
 $(1/3, -1/3, -1/3)$

Translational NCS

Effect of tNCS:

- Pattern of strong/weak reflections
- Broadening of intensity and amplitude distributions

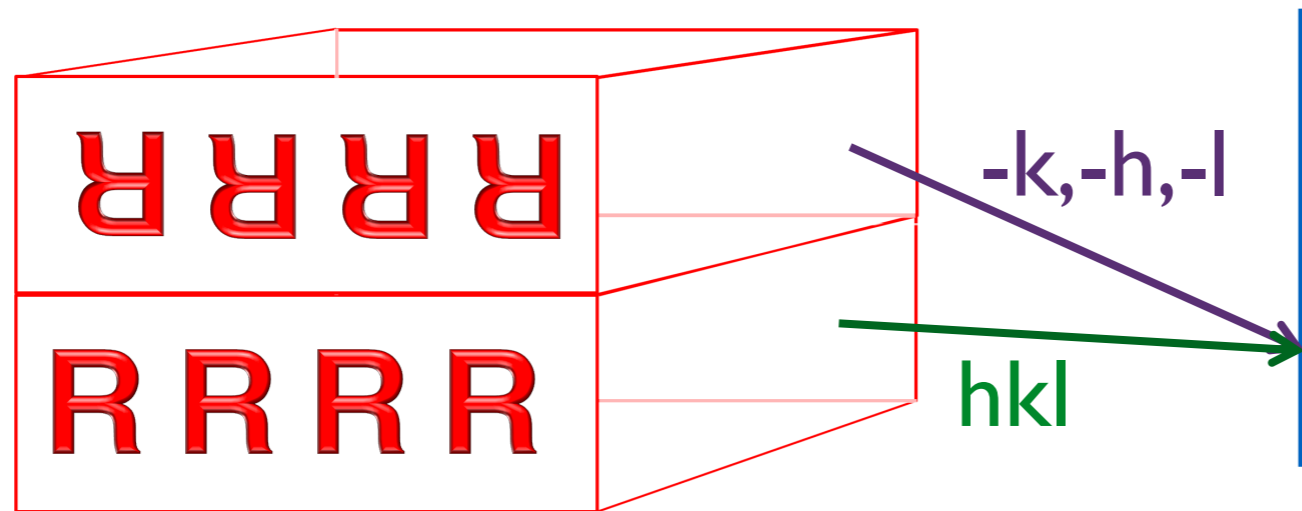


If your crystal has tNCS

- Use L-test for twinning
- Use tNCS-corrected likelihood target in molecular replacement
- Delete corresponding parts of all copies in omit maps

Twinning

Identical but rotated crystals sandwiched together



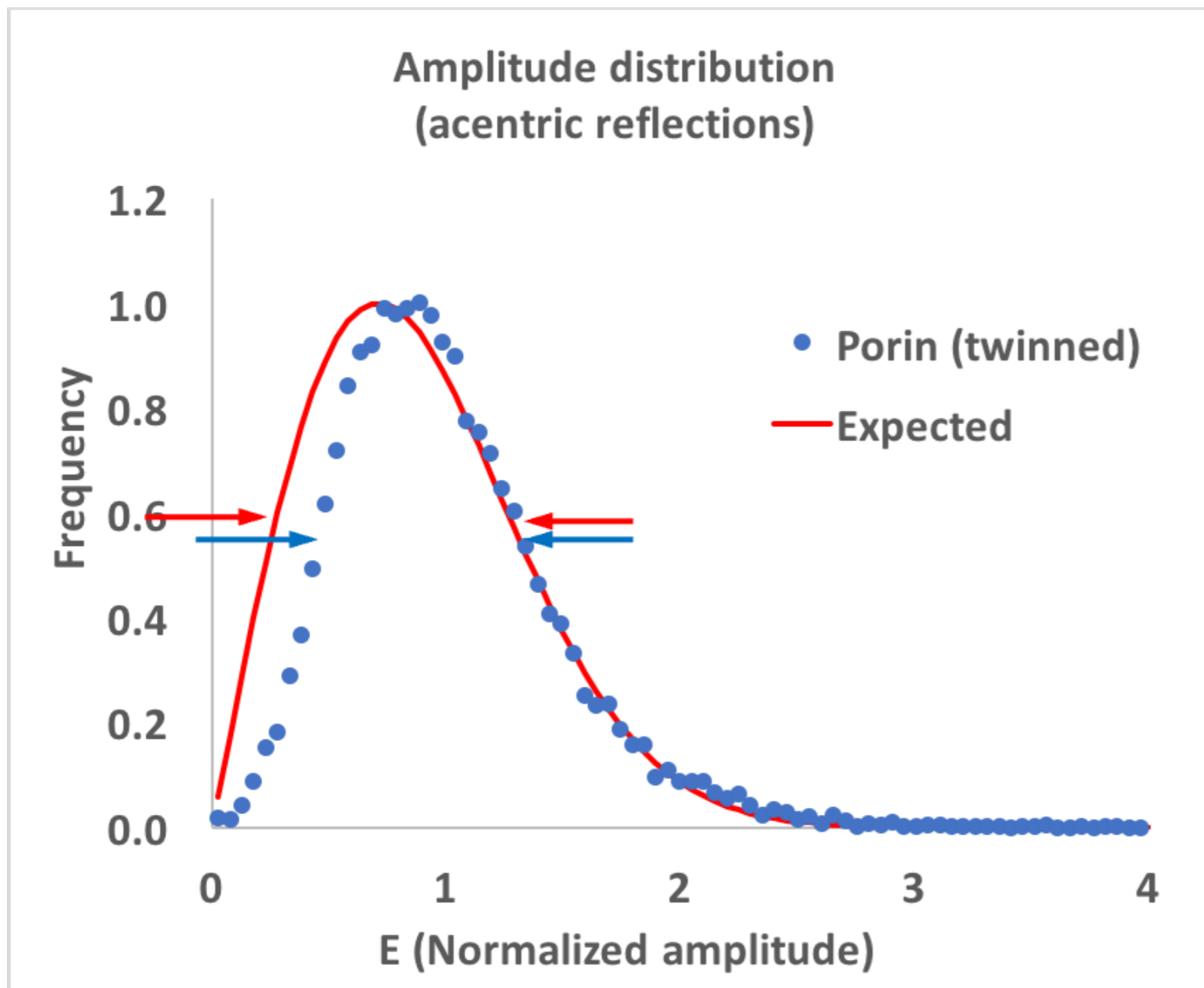
...is mixed with twin-law related reflection (e.g., $-k,-h,-l$)

Diffraction spot for (h,k,l) reflection...

Possible twin laws depend on your crystal symmetry and cell dimensions

Effect of twinning

- Mixing of intensities
- Intensities become more average
- Narrowing of intensity and amplitude distributions



tNCS can mask
twinning effects
on intensities and
amplitudes

Are the data twinned?

Is translational NCS present?

No



Check Wilson ratio: $\langle I^2 \rangle / \langle I \rangle^2$
2 for untwinned
1.5 twinned
(acentric reflections)

Yes



Check L-test*
(corrected for NCS)

*Padilla, J. E. & Yeates, T. O. (2003). *Acta Crystallogr. D Biol. Crystallogr.* **59**, 1124–1130.

The Project



Lawrence Berkeley Laboratory

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



University of Cambridge

Randy Read, Airlie McCoy,
Tristan Croll, Rob Oeffner



Los Alamos National Laboratory New Mexico Consortium

Tom Terwilliger, Li-Wei Hung



UTHealth

Matt Baker, Corey Hyc



Duke University

Jane & David Richardson,
Christopher Williams,
Vincent Chen



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