BERKELEY LAB

U.S. DEPARTMENT OF ENERGY

UNIVERSITY OF CALIFORNIA

AsCA meeting,
6 December 2025

Molecular Replacement

Dorothee Liebschner

Lawrence Berkeley Laboratory

Crystallography: Phase problem

$$\rho(\boldsymbol{r}) = \frac{1}{V_{cell}} \sum_{hkl} |F(\mathrm{hkl})| e^{i\alpha} e^{-2\pi i \boldsymbol{hr}}$$

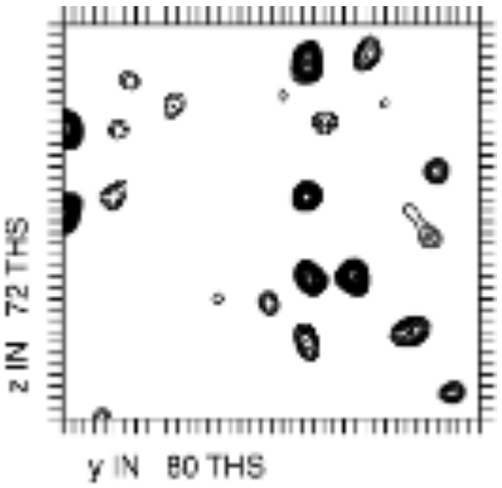
amplitude

phase

Measured in diffraction experiment

Not measured

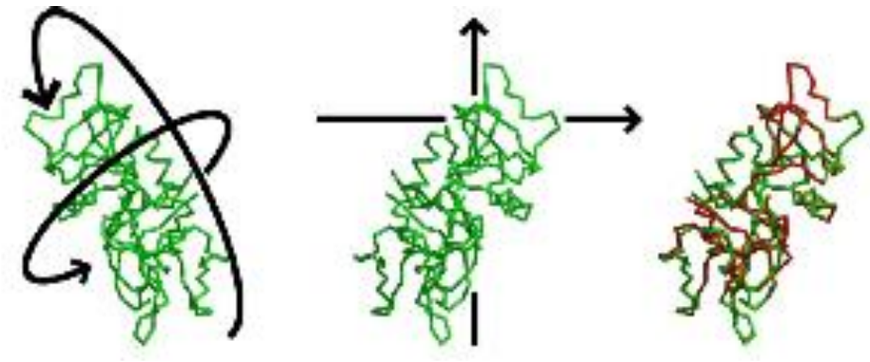
How to recover phases in Crystallography



Experimentally

Exploit the properties of a few special atoms:

- Anomalous scattering
- A large number of electrons



Computationally

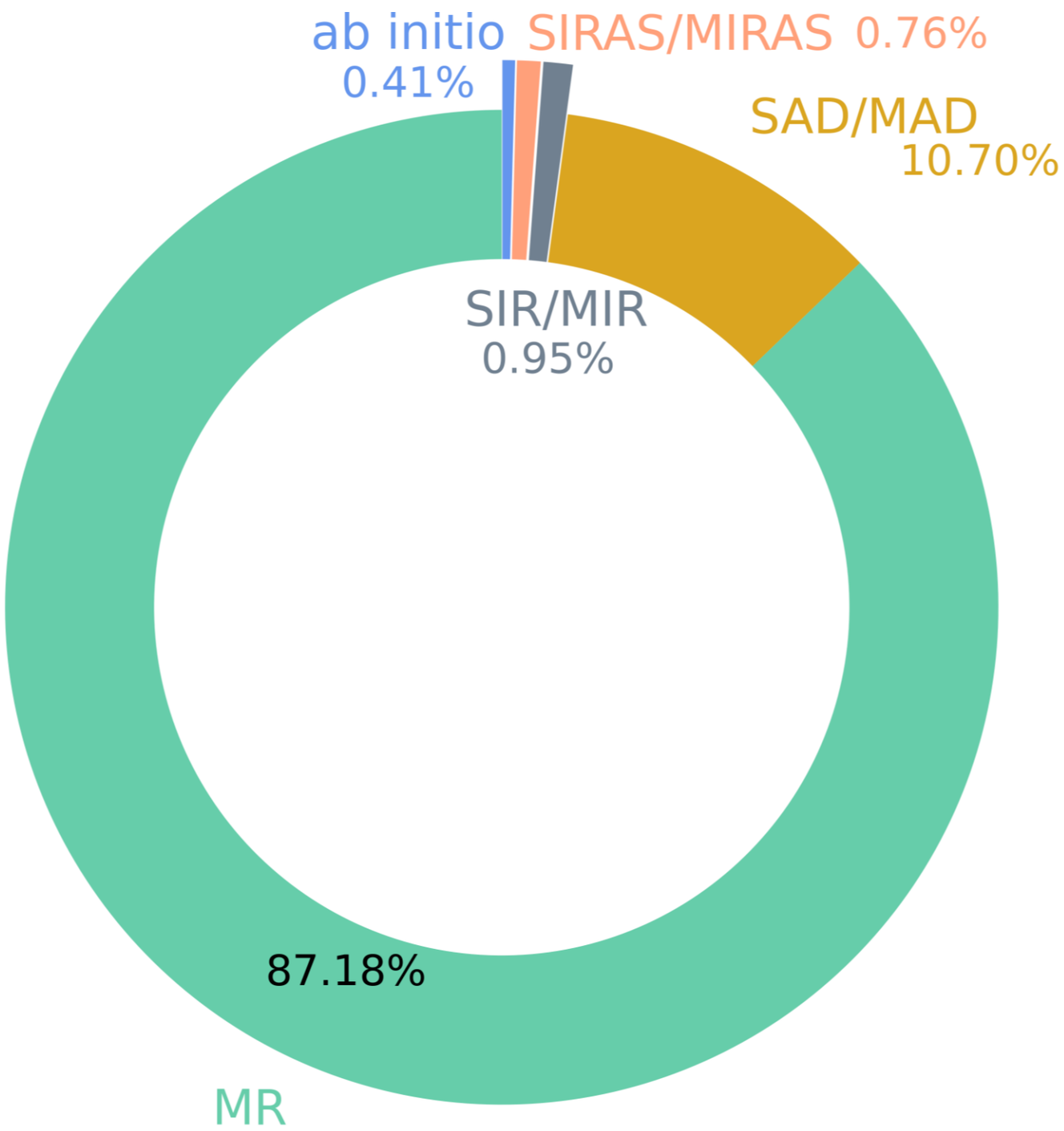
- *Molecular Replacement (MR)*
  A previously known structure provides initial phase estimates for a new structure

- *Direct Methods*
  Phase relationships can be formulated by assuming the positivity and atomicity of the electron density

How to recover phases in Crystallography

Method	Source of phasing information
SIR – single isomorphous replacement	A few heavy atoms (e.g., Hg, Au) in "derivative" contribute to differences from "native"
SAD – single-wavelength anomalous diffraction	A few atoms (e.g., Se, I, Hg atoms) contribute to "anomalous" differences in diffraction between spot *h* and spot *-h*
MAD – multiple-wavelength anomalous diffraction	A few atoms contribute to anomalous and wavelength-dependent "dispersive" differences
SIRAS, MIR	Combinations of SIR and SAD
Molecular replacement	Molecular location and phases are found using a related molecule as a template
Direct methods	Guess where atoms are, good guesses match the measured structure factors

Phasing methods in the PDB



MR method:

- Fast
- Cheap
- Highly automated

Known structure:

- Large number of deposited structures (PDB: 234k)
- Predicted models

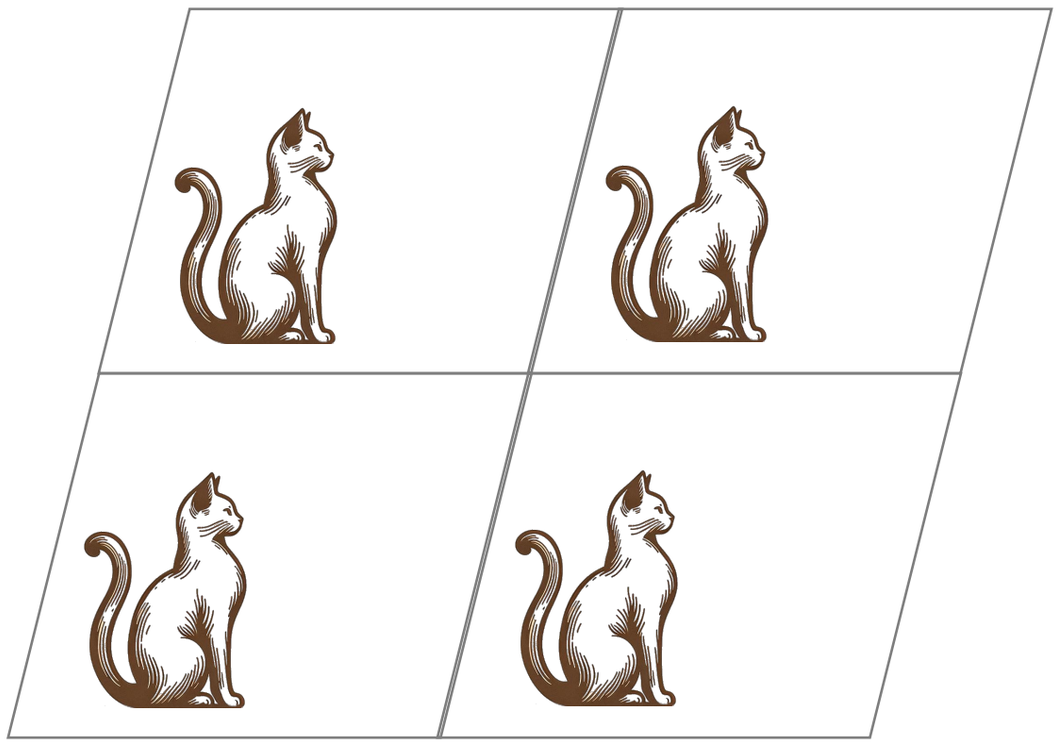
Note: Not all models in the PDB have info, and even if present, it may not represent major phasing method.

Molecular Replacement (MR)

MR = solve the unknown crystal structure of a molecule using a related known molecular model.

Known model

Crystal of unknown structure

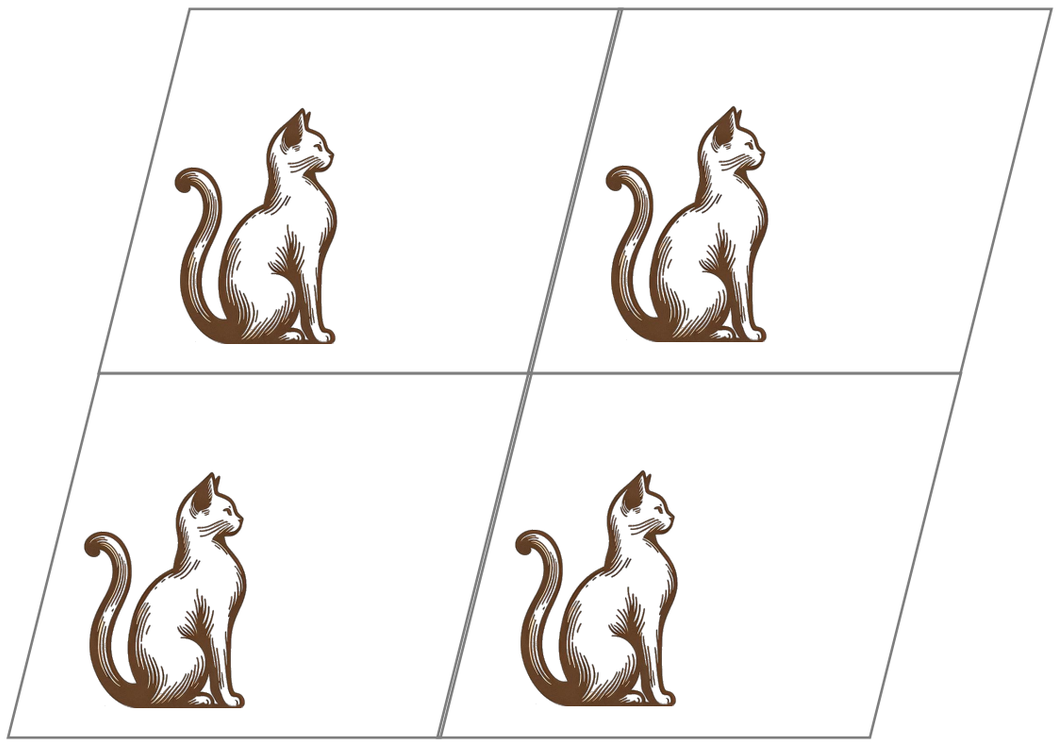


Molecular Replacement (MR)

MR = solve the unknown crystal structure of a molecule using a related known molecular model.

Known model

Crystal of unknown structure

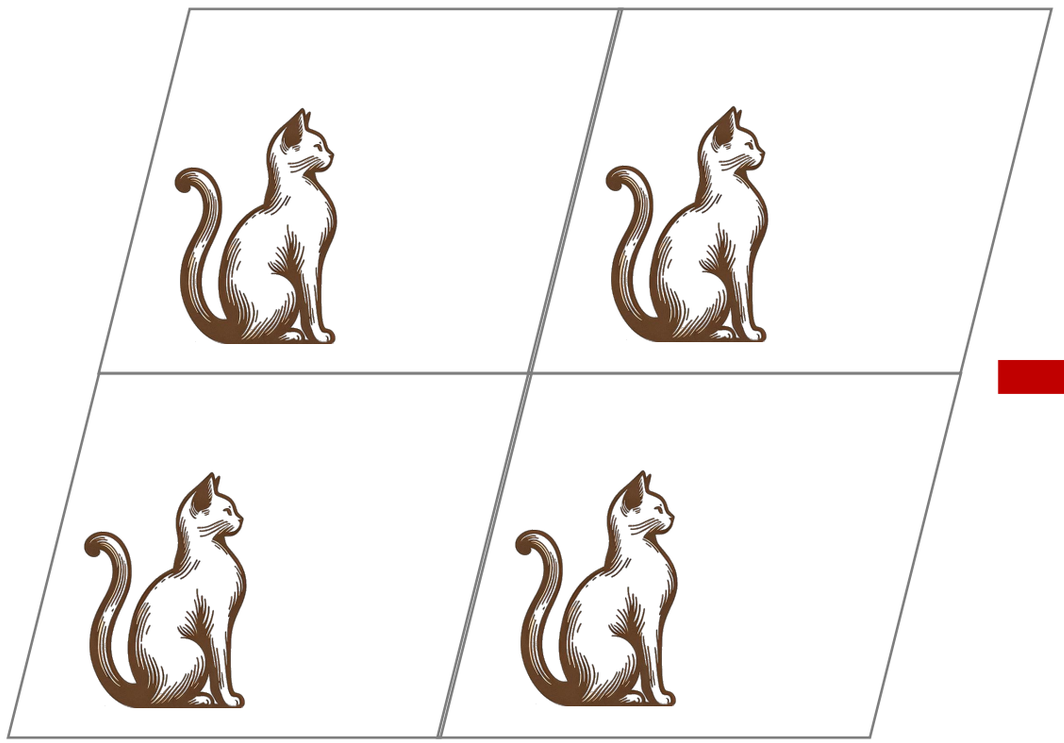


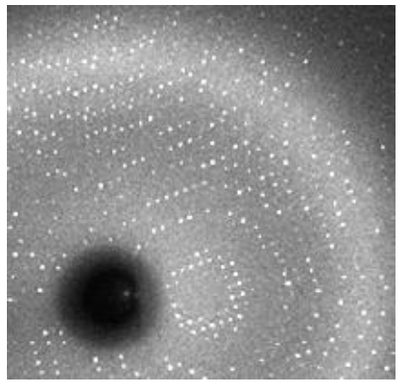
Known model provides initial estimates of the phases of the unknown structure.

Molecular Replacement (MR)

Known model

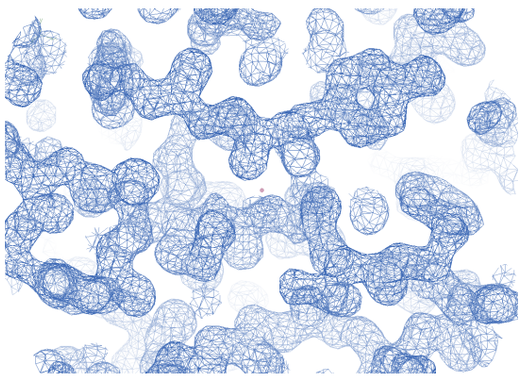
Crystal of unknown structure





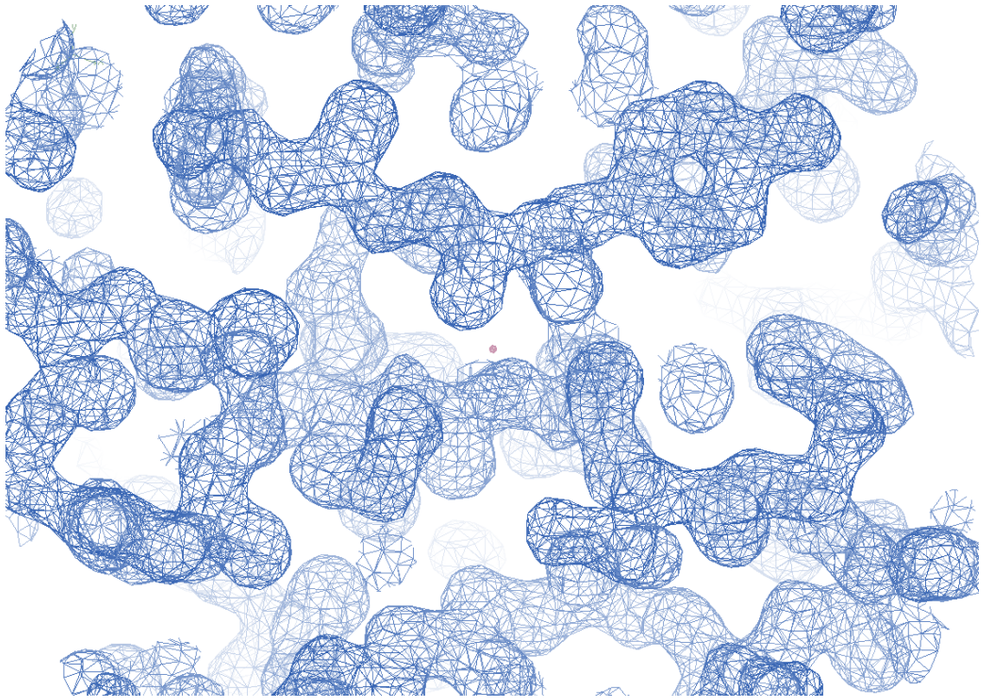
Intensities (hkl)

$|F|\ e^{i\phi}$



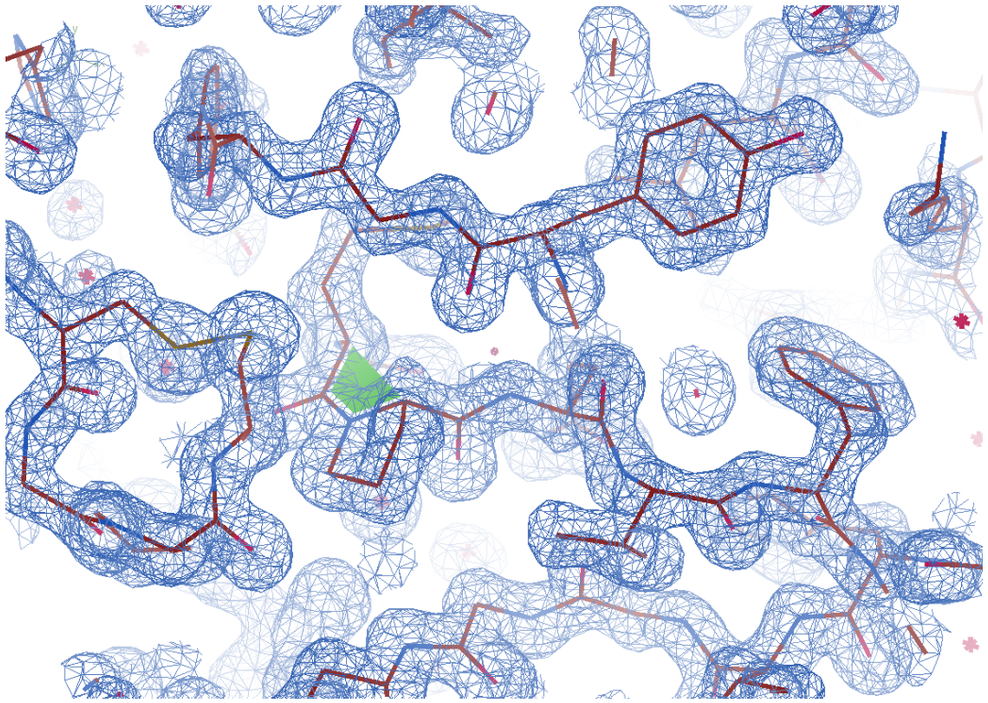
Density map

Molecular Replacement (MR)



If we know the density…

Molecular Replacement (MR)



If we know the density…

… then we can
determine the structure

MR will solve most structures

Try to solve recent SAD structures with MR and AF model.

STRUCTURAL BIOLOGY

ISSN: 2059-7983

Volume 79 | **Part 3** | March 2023 | Pages 234-244
https://doi.org/10.1107/S205979832300102X
OPEN ACCESS
Cited by 50
Part of a special issue

Accelerating crystal structure determination with iterative *AlphaFold* prediction

Thomas C. Terwilliger,[a,b*] Pavel V. Afonine,[c] Dorothee Liebschner,[c] Tristan I. Croll,[d] Airlie J. McCoy,[d] Robert D. Oeffner,[d] Christopher J. Williams,[e] Billy K. Poon,[c] Jane S. Richardson,[e] Randy J. Read[d] and Paul D. Adams[c,f]

[a]New Mexico Consortium, Los Alamos, NM 87544, USA, [b]Los Alamos National Laboratory, Los Alamos, NM 87545, USA, [c]
[d]Depa
United
Univer
*Corre

90% of 215 data sets solved with MR

STRUCTURAL BIOLOGY

ISSN: 2059-7983

Volume 80 | **Part 11** | November 2024 | Pages 766-779
https://doi.org/10.1107/S2059798324009380
OPEN ACCESS
Cited by 2
Part of a special issue

The success rate of processed predicted models in molecular replacement: implications for experimental phasing in the *AlphaFold* era

Ronan M. Keegan,[a,b‡] Adam J. Simpkin[a‡] and Daniel J. Rigden[a*]

[a]Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool L69 7ZB, United Kingdom, and [b]UKRI–STFC, Rutherford Appleton Laboratory, Research Complex at Harwell, Didcot OX11 0FA, United Kingdom
*Correspondence e-mail: drigden@liv.ac.uk

97% of 406 data sets solved with MR

(6% are “hard” cases)

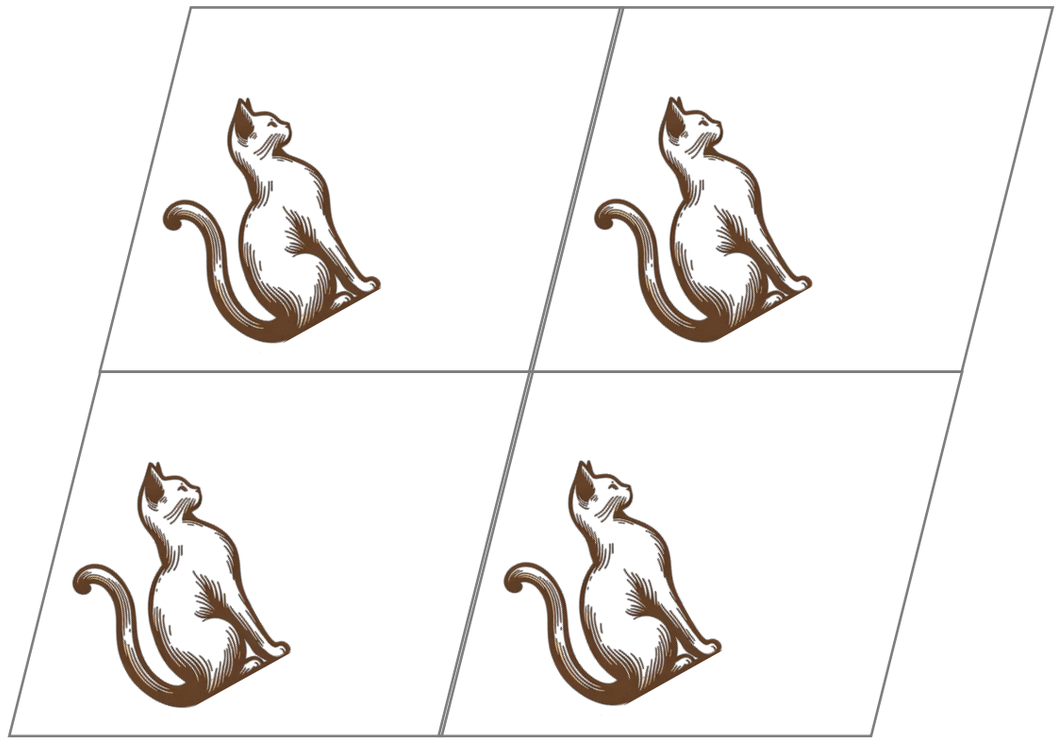
Terwilliger, T. C. *et al*. *Acta Crystallogr. D Struct. Biol.* **79**, 234–244 (2023).
https://doi.org/10.1107/S205979832300102X
Keegan, R. M., Simpkin, A. J. & Rigden, D. J. *Acta Crystallogr. D Struct. Biol.* **80**, 766–779 (2024).
https://doi.org/10.1107/S2059798324009380

Molecular replacement: Approach

Try to match the known model with the unknown structure.

Search model

Crystal of unknown structure



Find the rotation and translation of the search model so that it matches the unknown structure.

The search model

- Finding a suitable search model is critical step in MR.
- Should provide a high proportion of the scattering from the target structure with high accuracy (low r.m.s.d.).

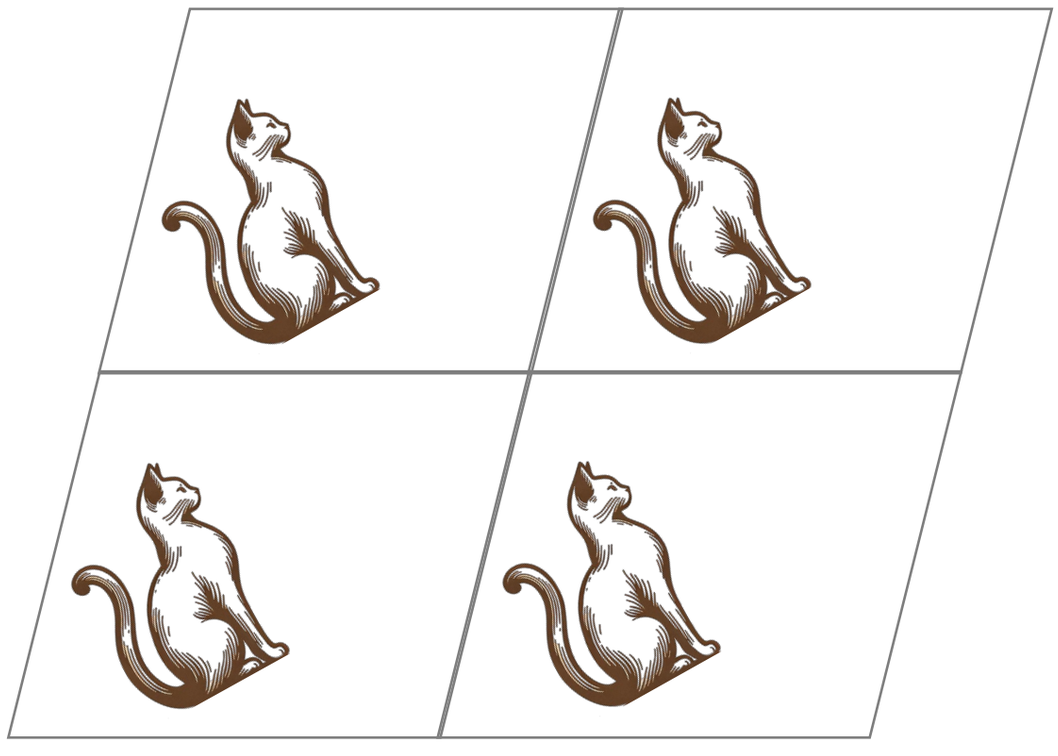
Crystal of unknown structure

The search model

- Finding a suitable search model is critical step in MR.
- Should provide a high proportion of the scattering from the target structure with high accuracy (low r.m.s.d.).

Only small part of actual model

Crystal of unknown structure



The search model

- Finding a suitable search model is critical step in MR.
- Should provide a high proportion of the scattering from the target structure with high accuracy (low r.m.s.d.).

Not similar to the target

Crystal of unknown structure

What can be used as search model?

Search model should be similar to the target structure.

1) **Homologue structure**
   If the sequence is similar → structures may be similar
   - Sequence comparison search
   - Prune regions of large sequence diversity
   - Truncate side-chains

Search model

Unknown structure

What can be used as search model?

Search model should be similar to the target structure.

1) **Homologue structure**
   If the sequence is similar → structures may be similar
   - Sequence comparison search
   - Prune regions of large sequence diversity
   - Truncate side-chains

Search model

Unknown structure

What can be used as search model?

Search model should be similar to the target structure.

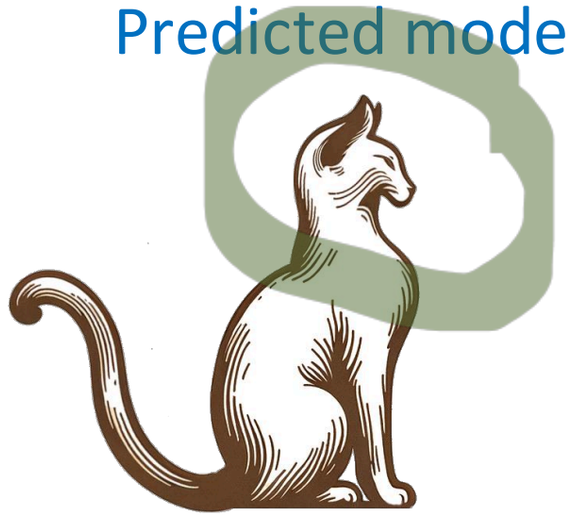
2) Predicted structure (AlphaFold, RosettaFold)

Process the predicted model before MR:

- Remove low pLDDT regions
- Split into domains if necessary
- Convert pLDDt to B-factors

Unknown structure

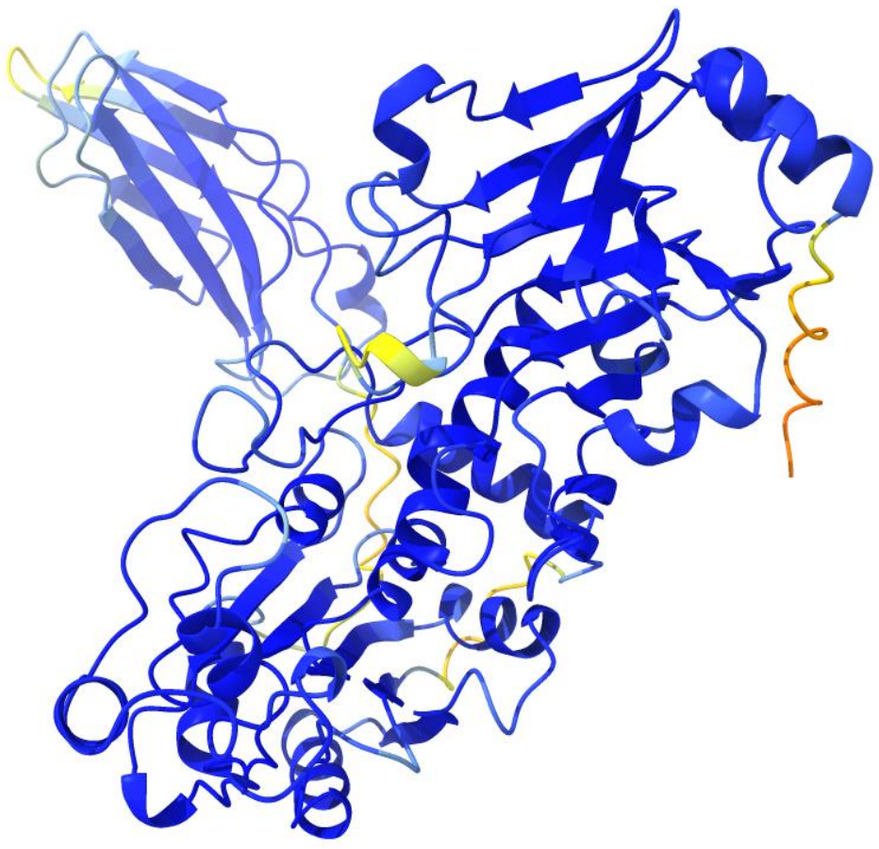
Predicted model



May have
- Distortions
- Incorrect domain relationships

Using predicted models: B-factors

Color by pLDDT



Color by B-factor

high pLDDT (high confidence)

low pLDDT (low confidence, uncertain)

high B-factor (disordered, uncertain)

low B-factor (ordered)

Oeffner RD, Croll TI, Millán C , Poon BK, Schlicksup CJ, Read RJ, Terwilliger TC. Acta Cryst. D, 2022 (78):1303-1314; https://doi.org/10.1107/S2059798322010026

Using predicted models: B-factors

high pLDDT (high confidence)

low pLDDT (low confidence, uncertain)

high B-factor (disordered, uncertain)

low B-factor (ordered)

B-factor may be used in downstream calculations, e.g. to calculate weights for docking. Residues with high B-factors are downweighed.

→ Convert pLDDT to pseudo B-factors.

$$\Delta = 1.5 \exp[4(0.7 - \text{pLDDT})] \qquad B = \frac{8\pi^2\Delta^2}{3}$$

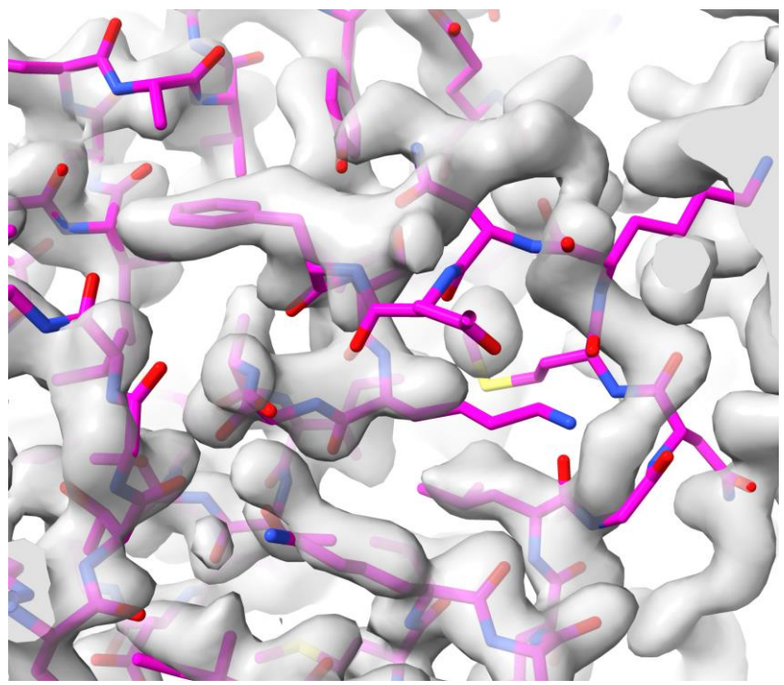
Oeffner RD, Croll TI, Millán C , Poon BK, Schlicksup CJ, Read RJ, Terwilliger TC. Acta Cryst. D, 2022 (78):1303-1314; https://doi.org/10.1107/S2059798322010026

Limitations of predicted models

<u>AI/ML model predictions</u>

- RNA/DNA not as accurate yet.
- High pLDDT regions are not necessarily correct.
- Know almost nothing about physics and chemistry.
- Generate a single structure, but no conformational changes (influenced by pH, temperature, ions, ligands, other proteins).

Wrong prediction



Molecular replacement: Scoring

Compare observed and calculated diffraction.

Poor score

Good score

Different approaches:

- Patterson function
- Maximum-likelihood Methods (Phaser)

MR Scoring: Maximum Likelihood Method

"For any postulated orientation and position of the model, what is the probability of obtaining the structure amplitudes that we observe?"

Explicitly models errors
- Experimental uncertainties
- r.m.s. coordinate error of the search model

→ Likelihood methods are more robust and generally give clearer solutions in difficult cases

Maximum Likelihood Scoring in Phaser

LLG = Log Likelihood gain

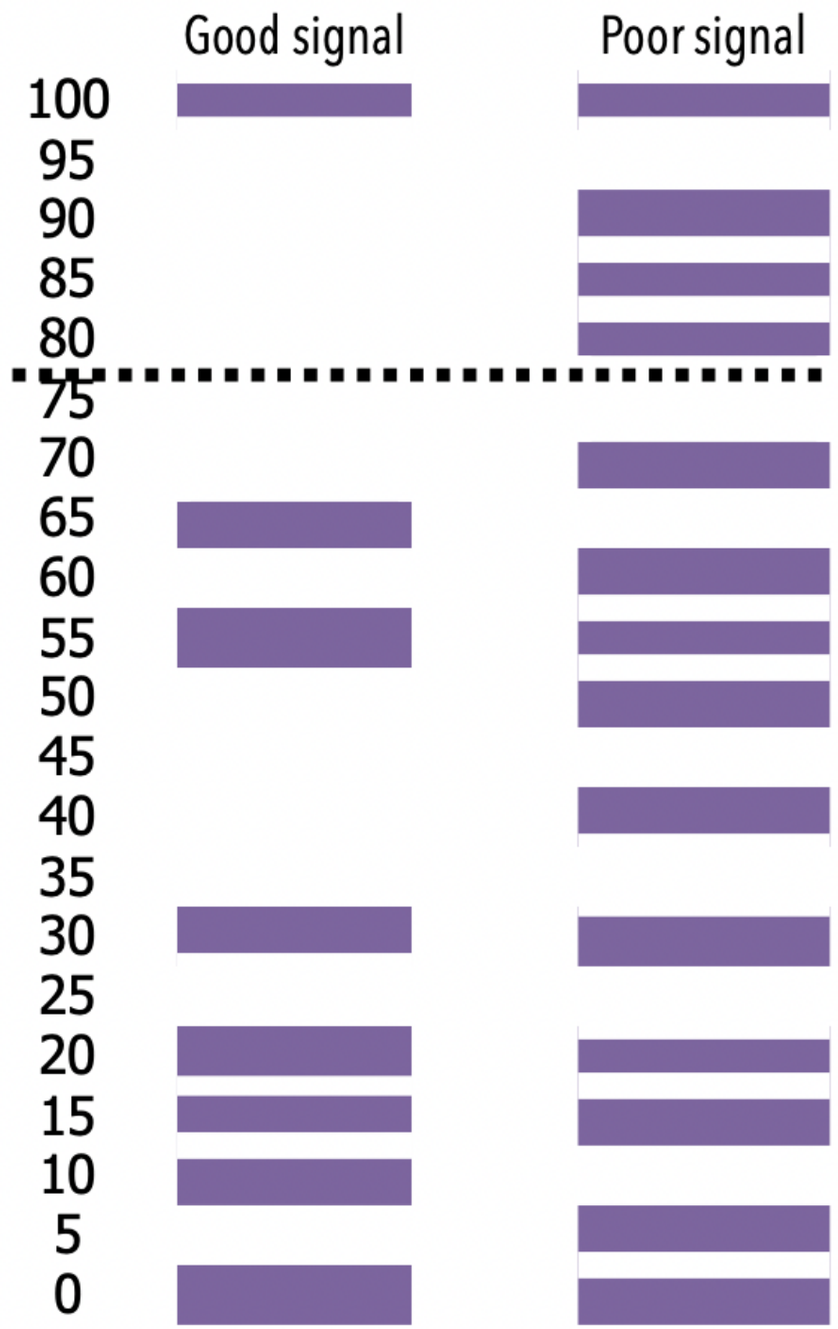
→ It measures how much better the data can be predicted with the search model than with a random distribution of the same atoms.

TF-Z = how many standard deviations your solution is above the mean (the higher the better).

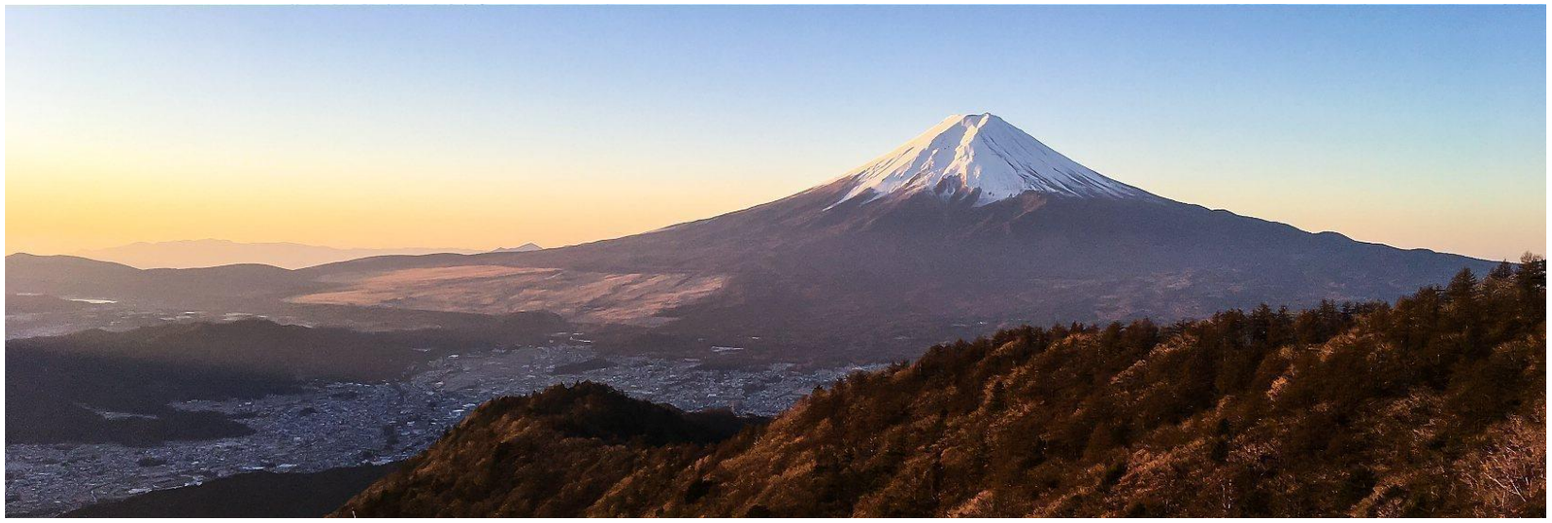
Maximum Likelihood Scoring in Phaser

Select solutions that are over 75% of the difference between the top peak and the mean.

- Good signal, few potential solutions
- Poor signal, many potential solutions

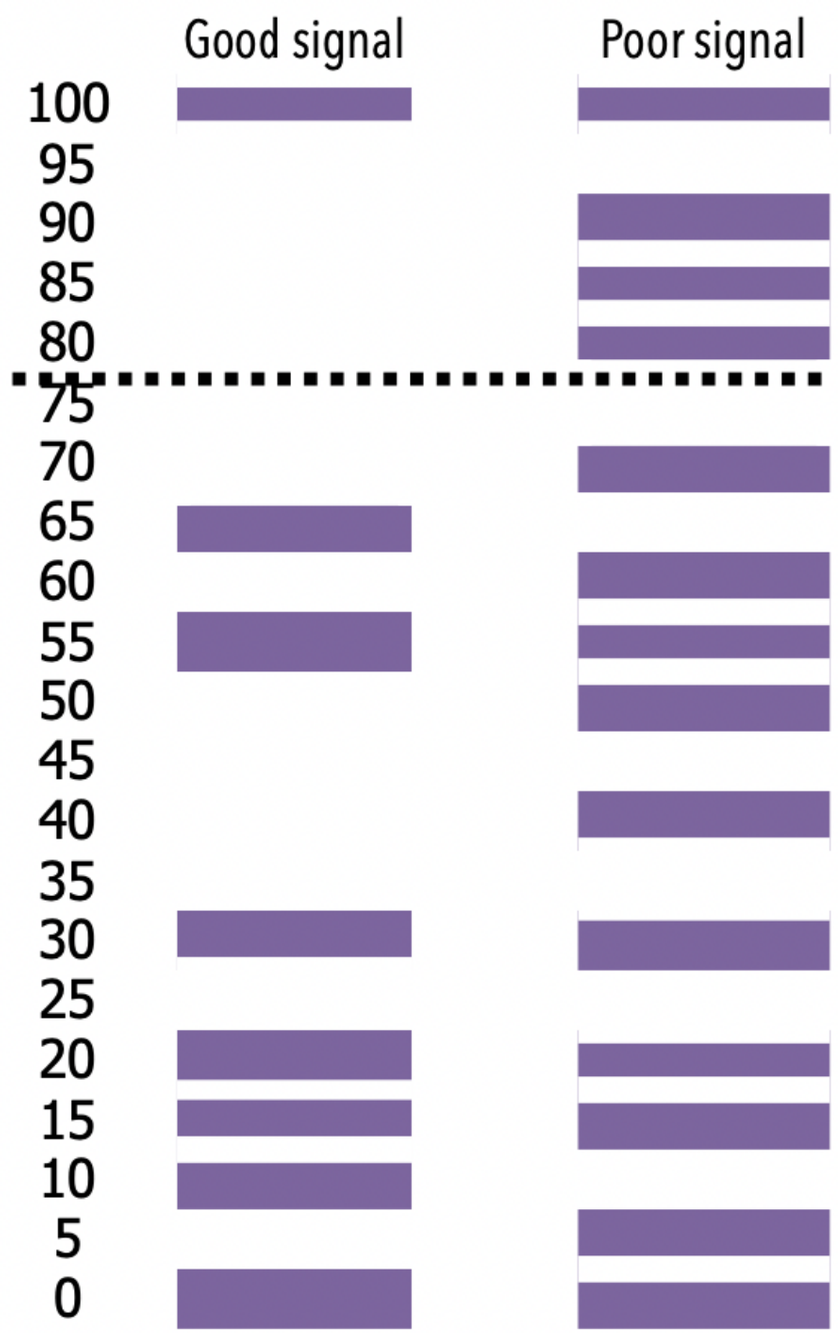


Maximum Likelihood Scoring in Phaser



Good signal

Poor signal



Maximum Likelihood Scoring in Phaser

TF Z-score	LLG score	Solved?
< 5	< 25	no
5 - 6	25 - 36	unlikely
6 - 7		
7 - 8		
> 8		

Summary

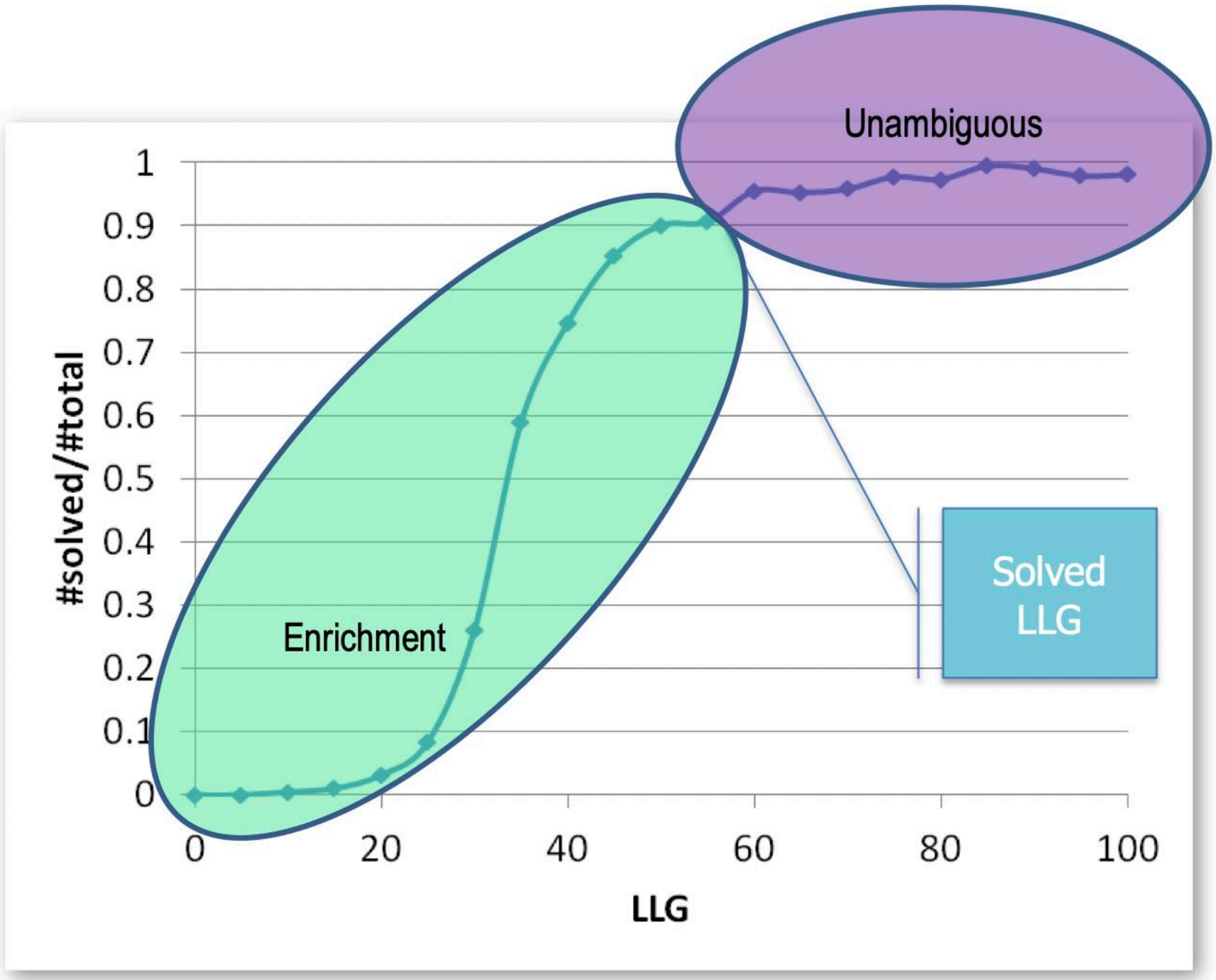
Phaser has found 1 MR solution(s).

Top LLG: 11480.402

Top TFZ: 68.1

Spacegroup: C 1 2 1

Maximum Likelihood Scoring in Phaser



Plot of LLG versus success in structure solution

Database of over 23000 MR problems

R.D. Oeffner

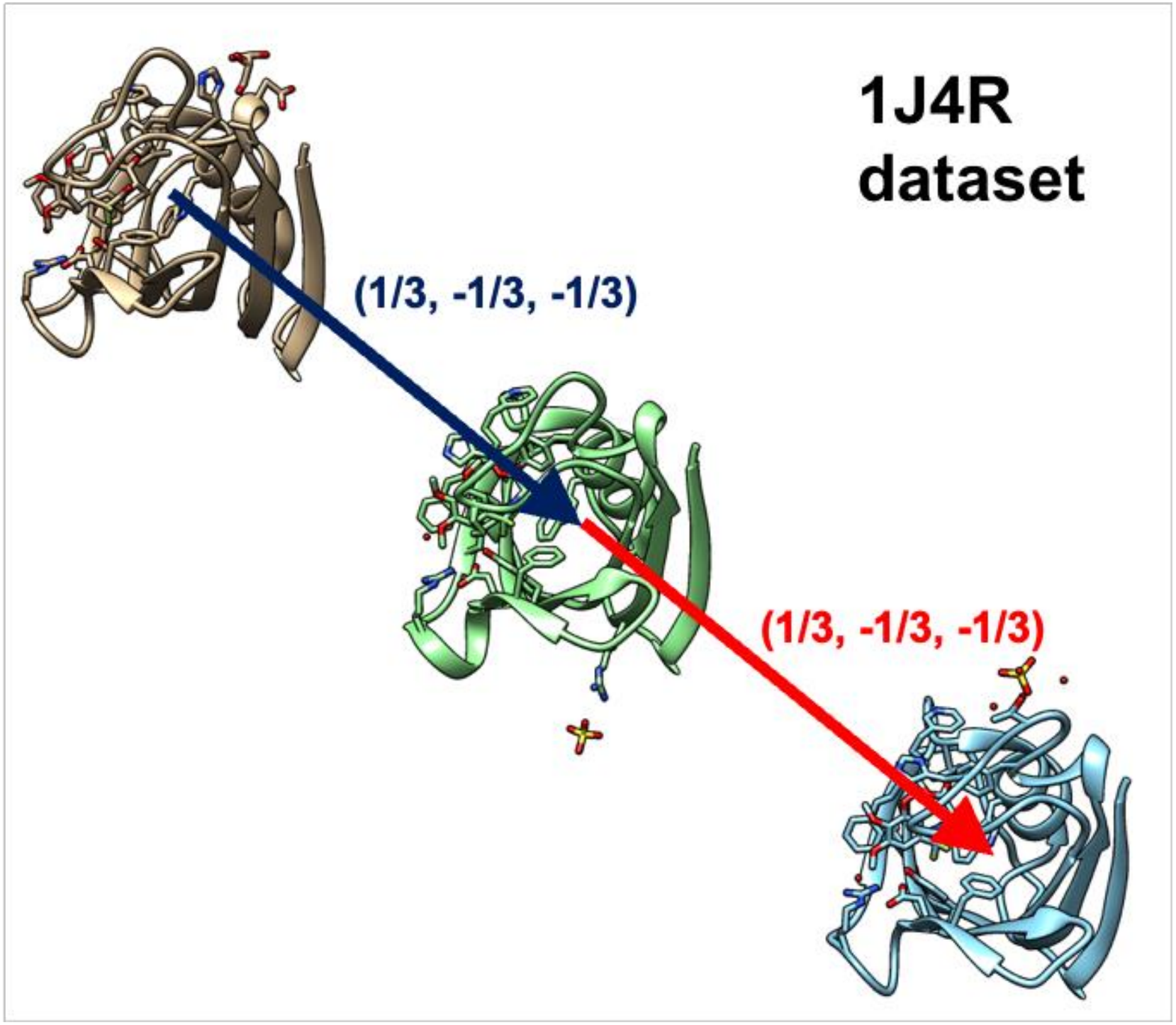
Likelihood is sensitive...

- ...to correct orientation and position of molecular replacement model
  - successful in solving structures with distant relatives, small fragments, or many copies in asymmetric unit
- ...to violations of assumptions
  - data implicitly assumed to be isotropic
    → important to account for anisotropy
  - tNCS: intensity distribution is modulated
  - components may not be equally well-ordered
    →important to correct for differences in overall B-factors

Slide from Randy Read

Translational NCS

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

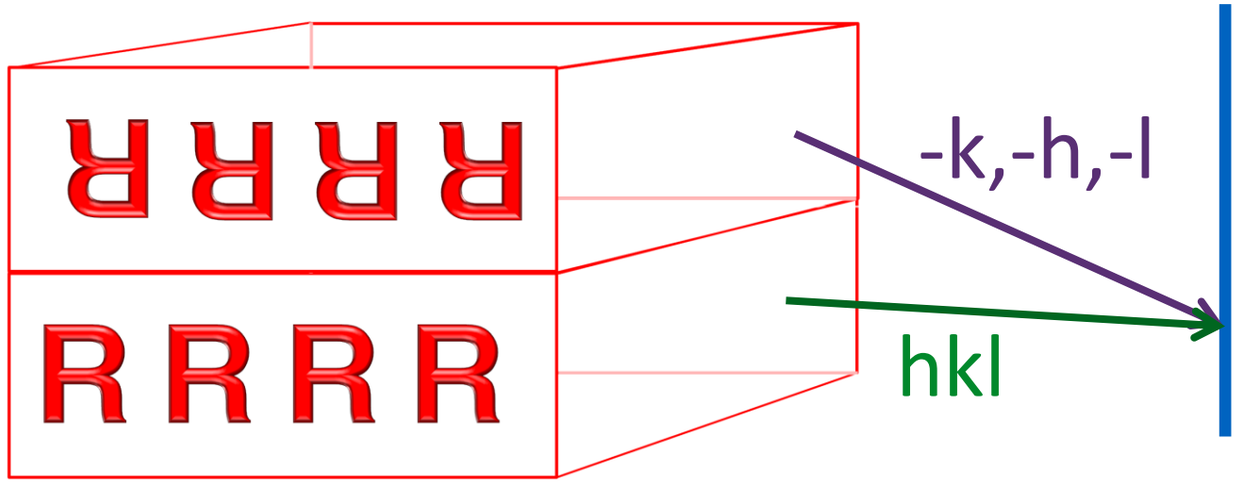


Strong peak in Patterson function

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

Twinning

Identical but rotated crystals sandwiched together



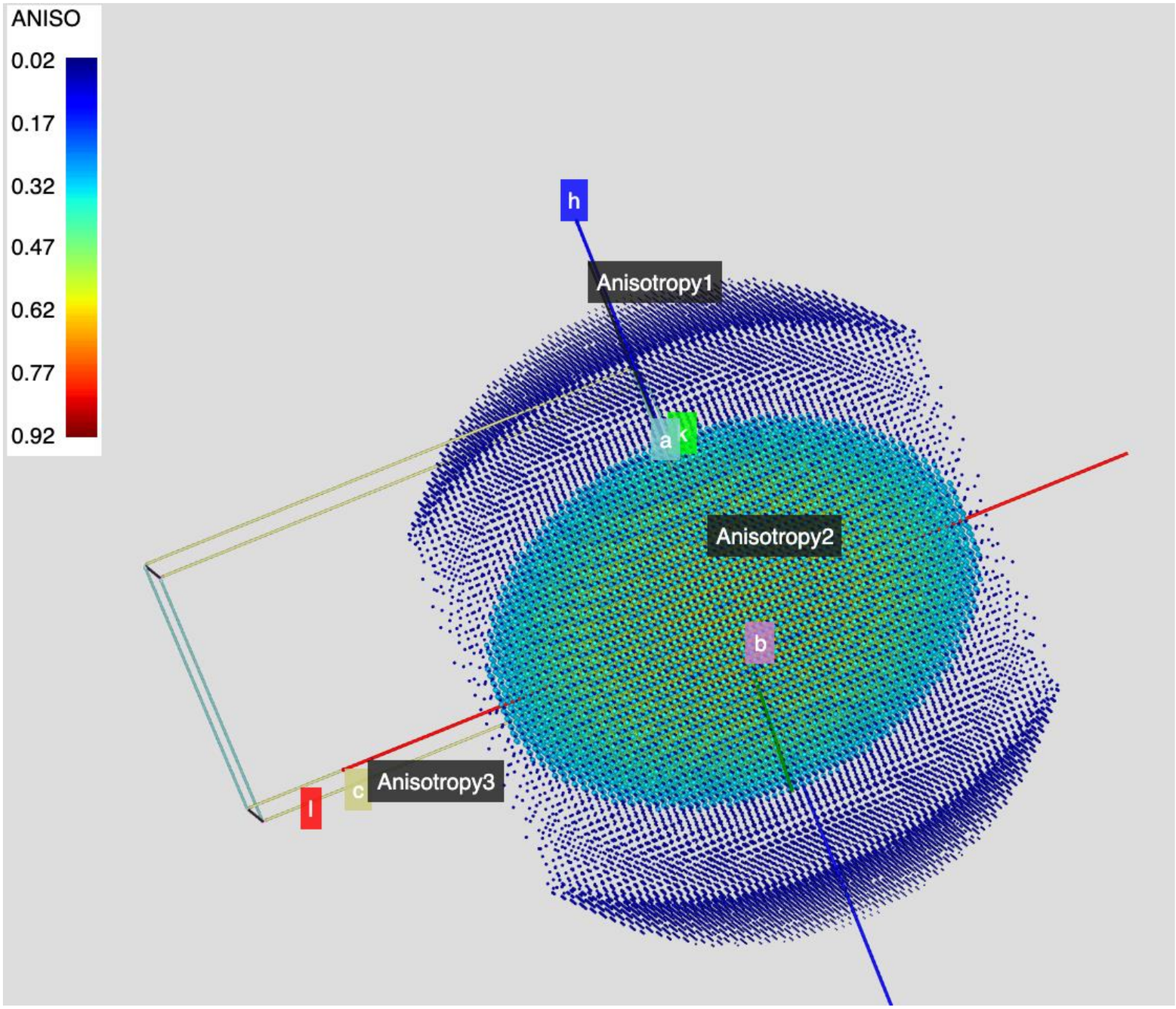
…is mixed with twin-law related reflection (e.g., -k,-h,-l)

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

Possible twin laws depend on your crystal symmetry and cell dimensions

Anisotropic data

Crystal does not diffract equally well in all directions.



Phaser takes care of anisotropy corrections → No need to truncate (Truncating may confuse Phaser's intensity analyses)

What is needed to run Phaser MR

- Reflection data
- Search model
- Error estimation of the search model
  - Homologue: sequence identity
  - Predicted model: r.m.s.d. (1.0 Å)
- ASU content:
  - Sequence of the construct
  - How many copies of the molecule(s)
- Awareness of data pathologies:
  - Twinning
  - tNCS
  - anisotropy

If MR does not work

- Double check space group (twinning).
- Does your data set have uncurable pathologies? (low resolution, high mosaicity)
- Is the content correct (e.g., impurities)?

  Is it the correct sequence file?

- Can the search model be improved?
  - is the search model complete enough (AF multimer)?
  - use other prediction software.
  - use a different splitting method.
- Use secondary structure based software
  - ARCIMBOLDO, AMPLE
- Discuss with your colleagues; ask at bulletin boards (ccp4bb); ask software developers

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

University of Cambridge

Randy Read, Airlie McCoy, Alisia Fadini

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