Phenix User Workshop, Missoula, Montana

Phenix Tools for crystallography

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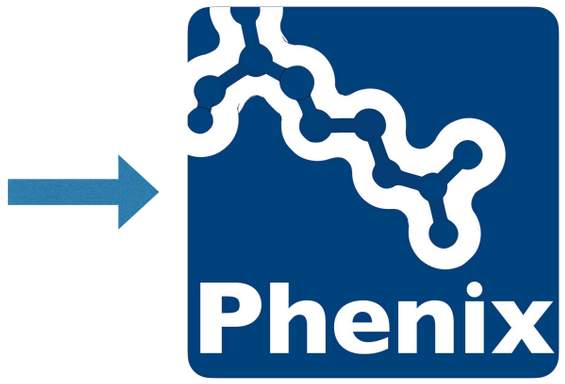
Lawrence Berkeley Laboratory

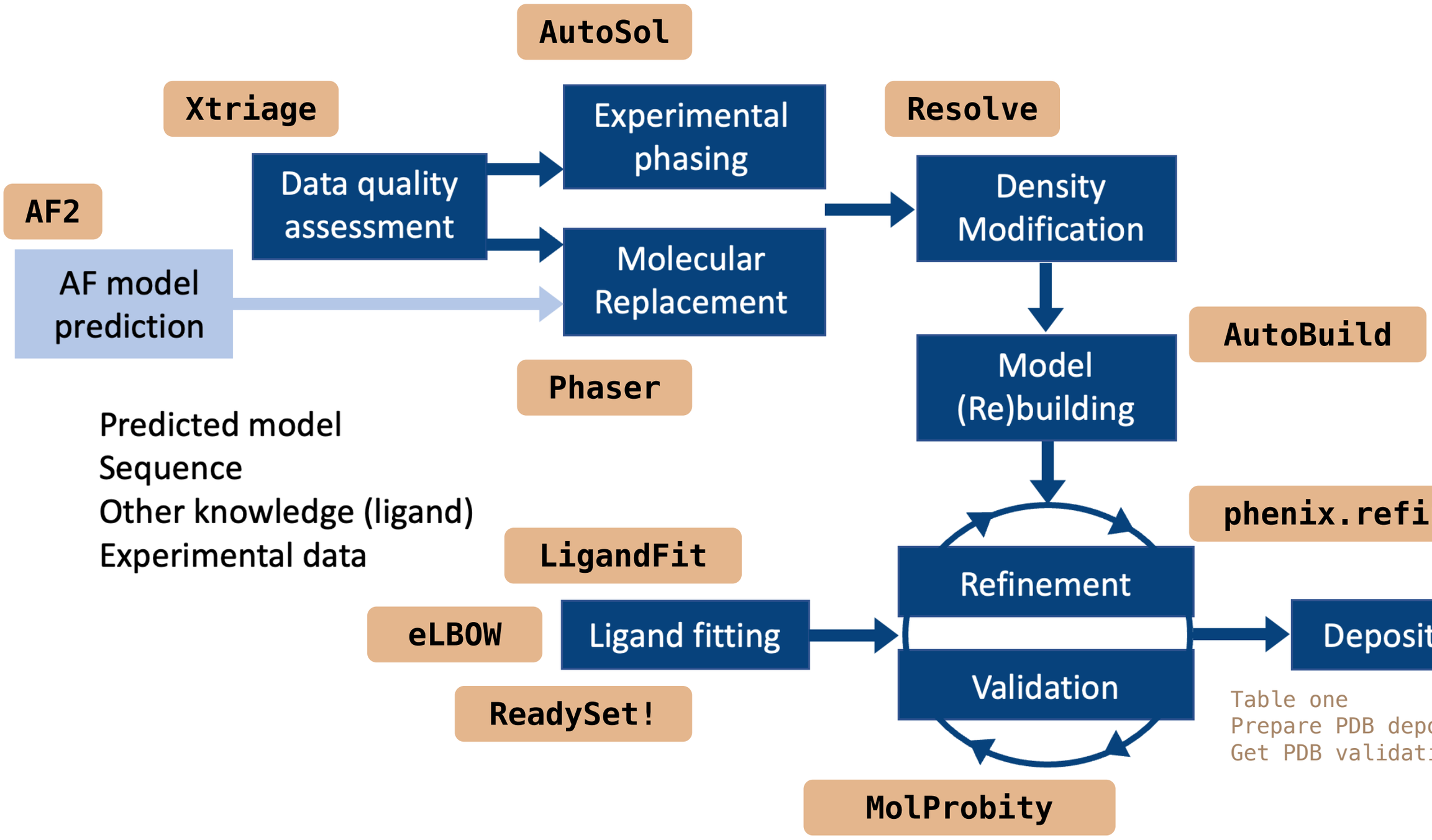
Steps in crystallography

Diffraction experiment

Predicted model
Sequence
Other knowledge (ligand)
Experimental data

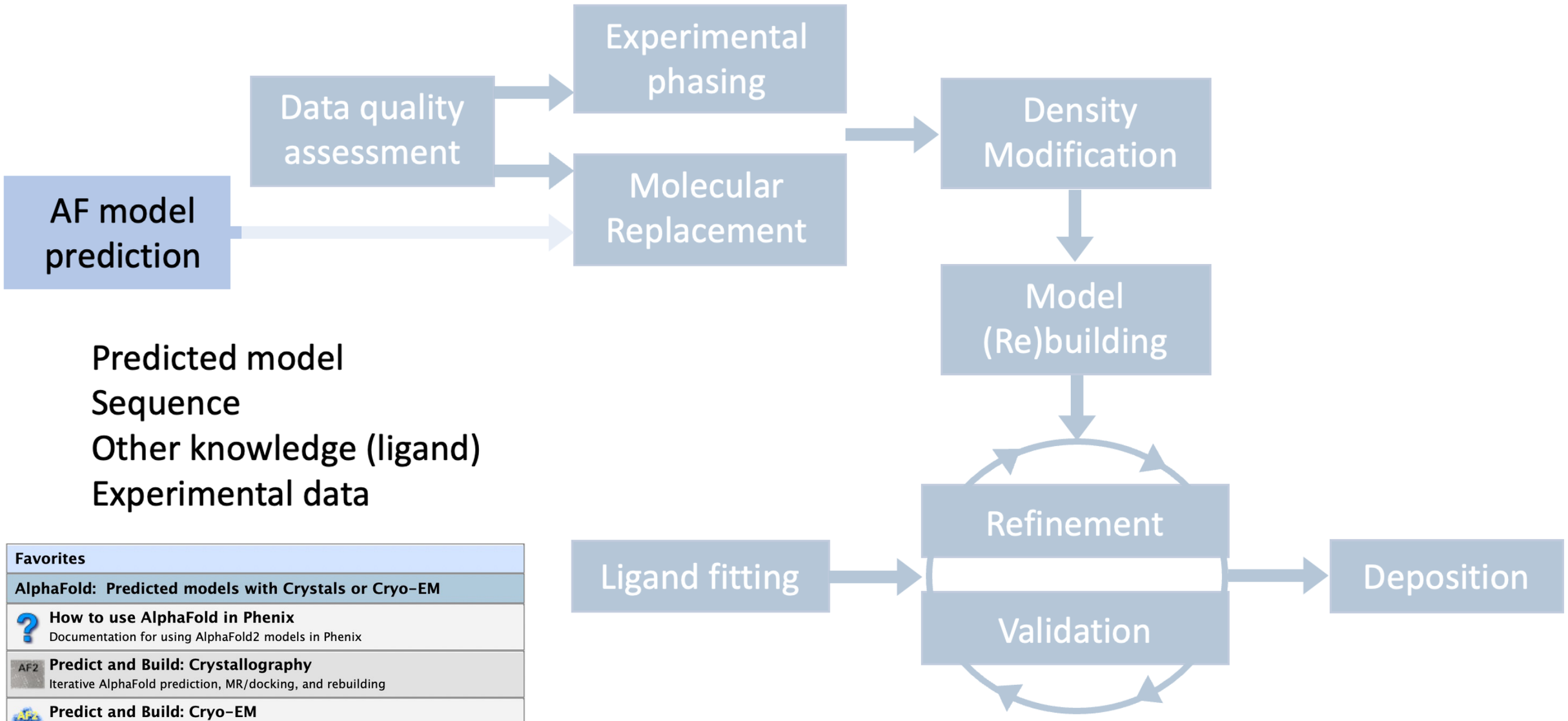


Steps in crystallography



Acta Cryst. 2002, D58:1948-1954 (Phenix)
J. Appl. Cryst. 2002, 35:126-136 (cctbx)
Acta Cryst. 2010, D66: 213-221 (Phenix)
Acta Cryst. 2019 D75:861–877 (Phenix)

Steps in crystallography



Predicted model

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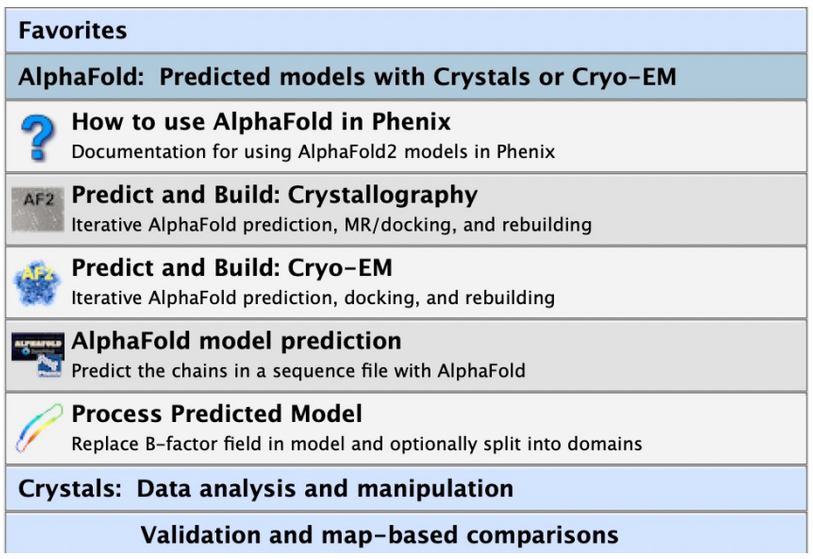
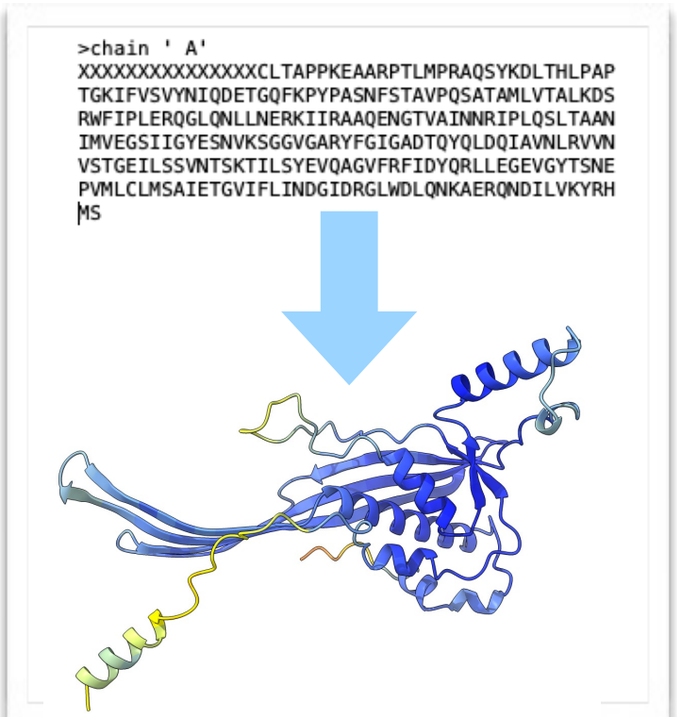
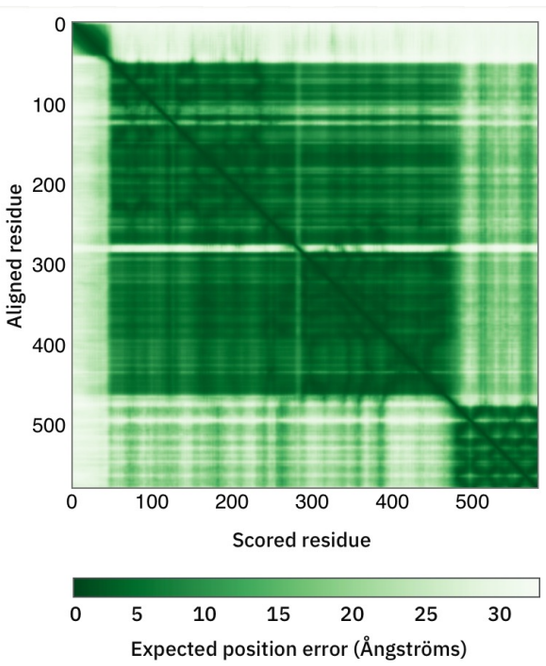


Tools for predicted models in Phenix

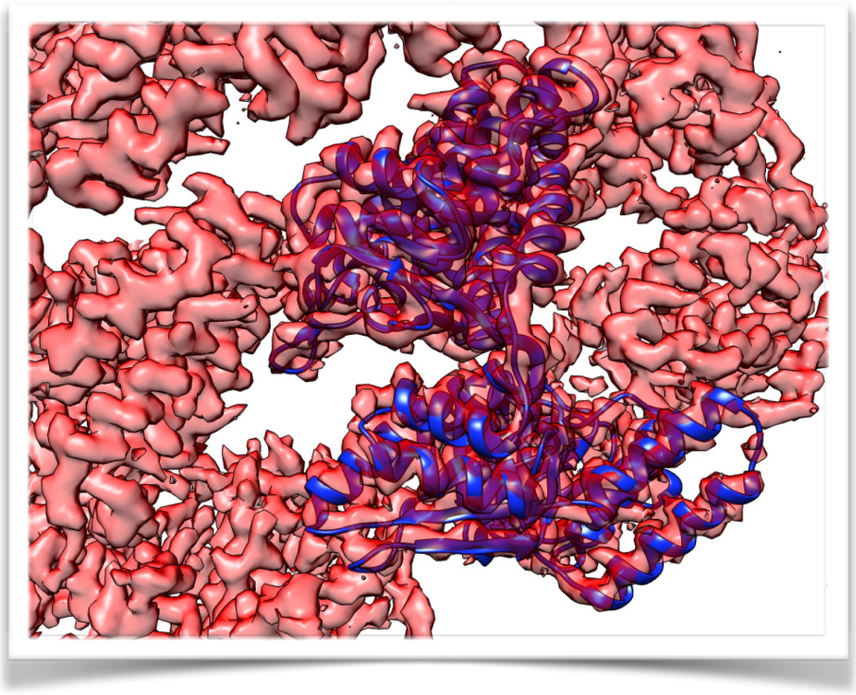


AlphaFold model prediction

Phenix server, no need to have AF2 installed locally



Process predicted model

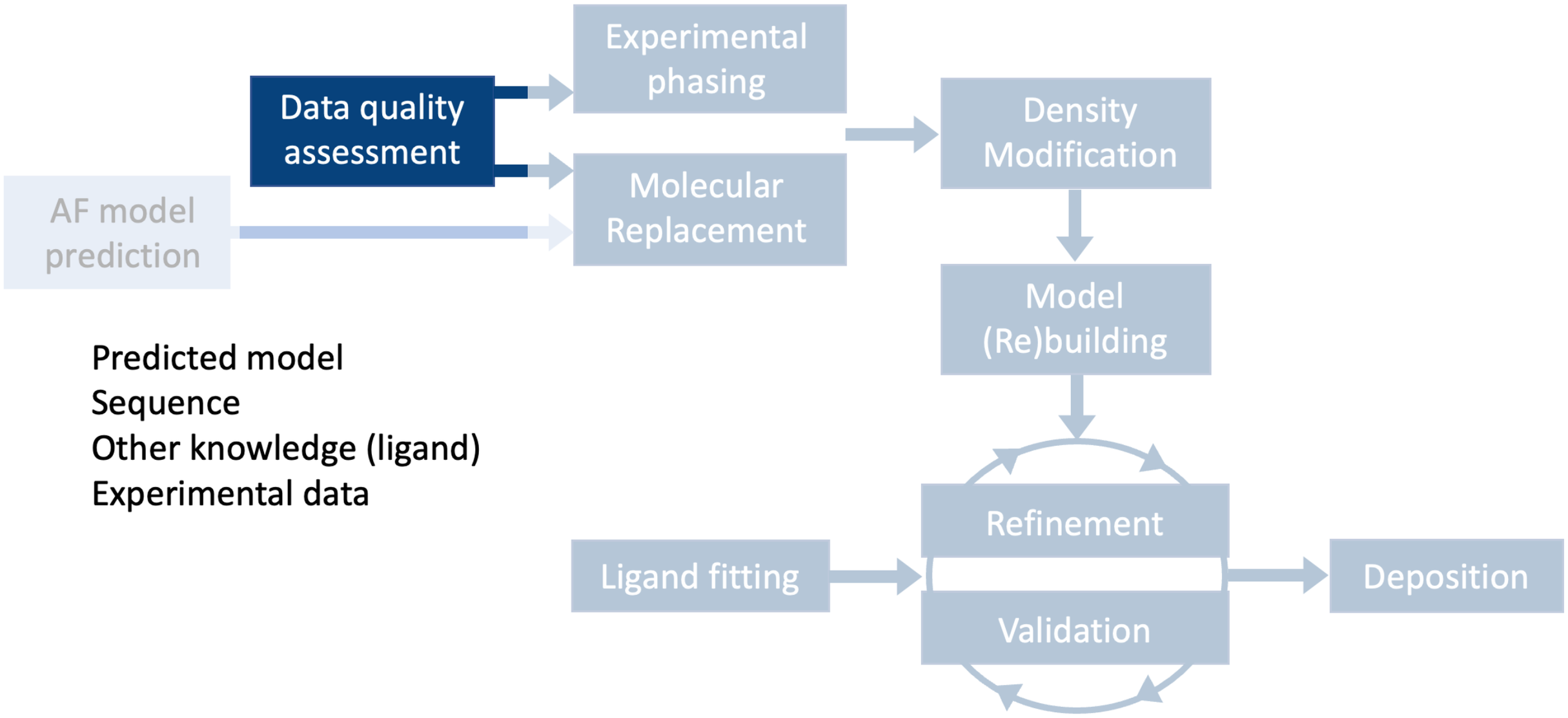


Predict and Build

Iterative AlphaFold prediction, MR/docking, and rebuilding

Fully automatic!

Steps in crystallography



Predicted model
Sequence
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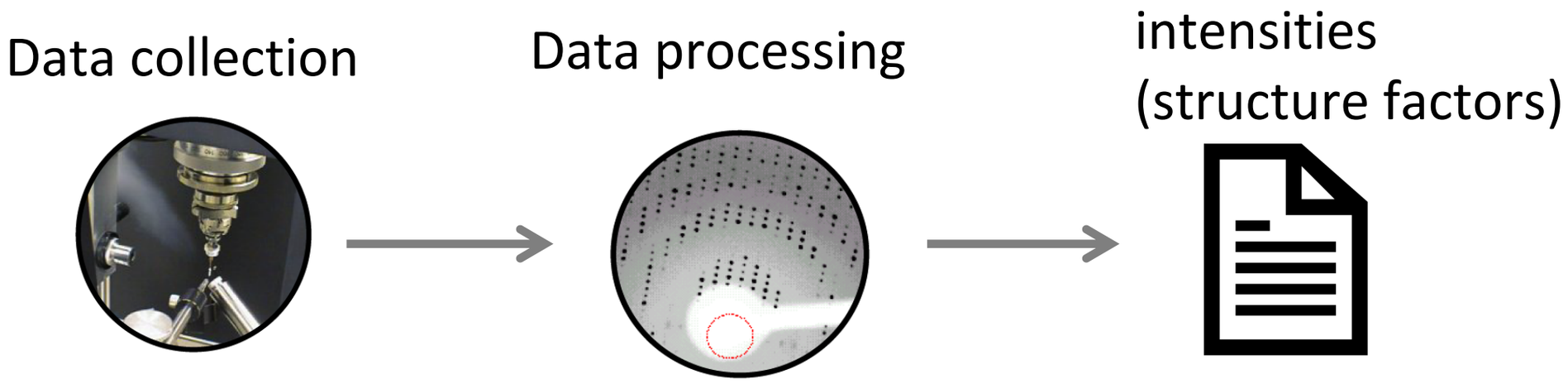
Data Quality Assessment



Macromolecular crystals are prone to pathologies:

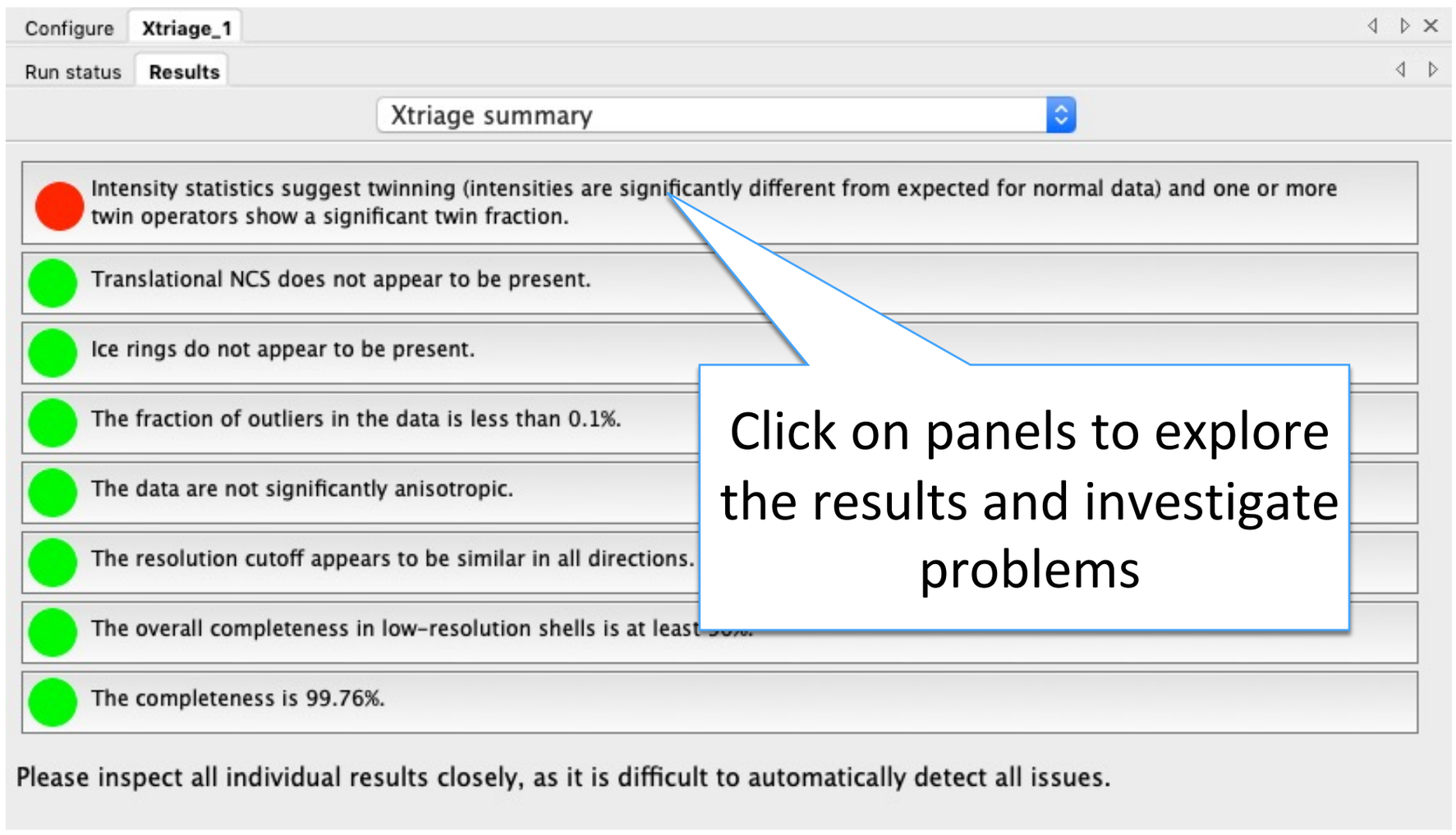
- Twinning: two or more crystals are intergrown (orientations are related by twin operation)
- tNCS: more than one copy of a molecule is in a similar orientation in the asymmetric unit

Data Quality Assessment

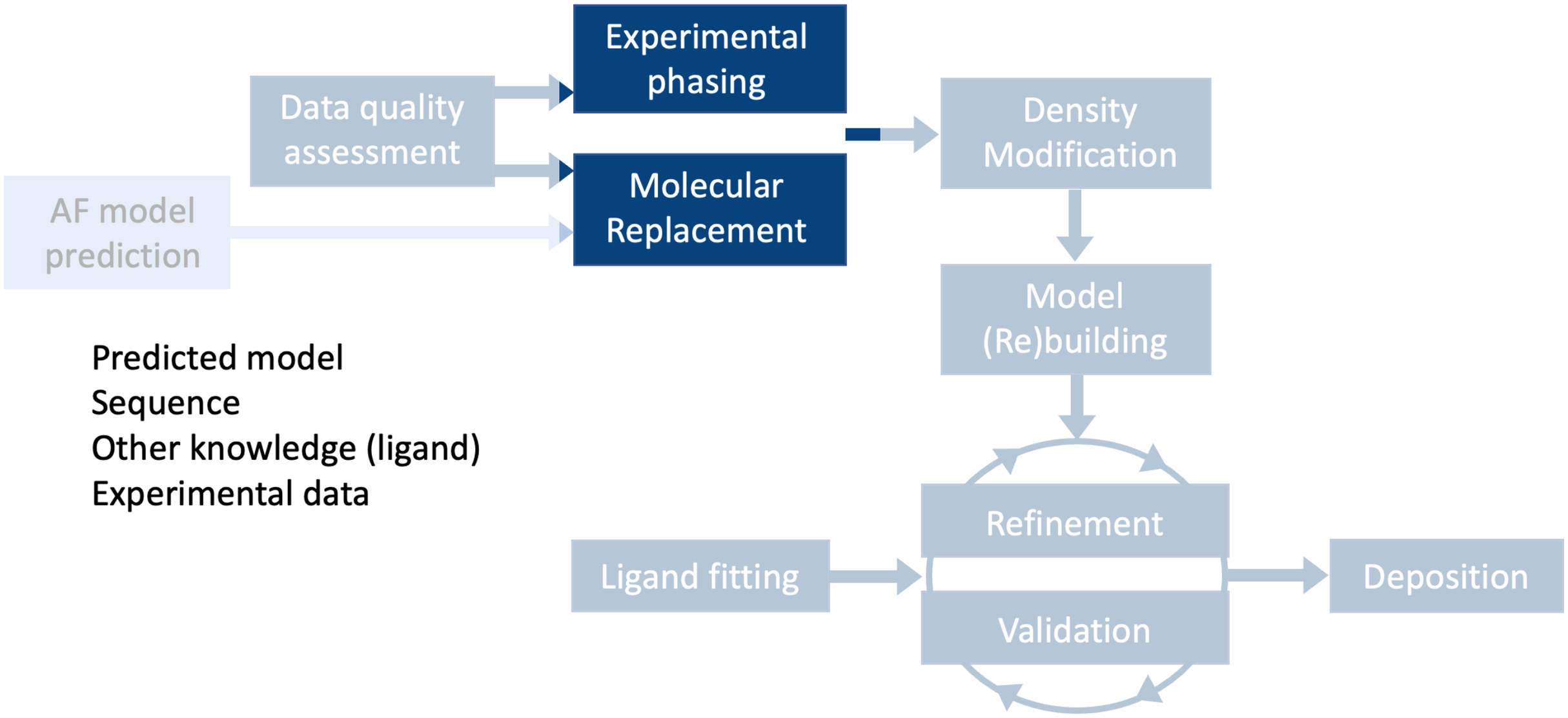
Data anomalies can prevent structure solution!

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

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



Steps in crystallography



Predicted model
Sequence
Other knowledge (ligand)
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Goal of crystallographic experiment

Typically, the goal is to determine the **structure**.
(arrangement of atoms in space)

The electron density in the unit cell is related to the Fourier transform of the **amplitude and phase of the scattered X-rays**.

$$\rho(\vec{r}) = FT\left(\vec{F}(\vec{H})\right) = \frac{1}{V}\int \vec{F} \cdot e^{-2i\pi\vec{H}\cdot\vec{r}}$$

Goal of crystallographic experiment

Typically, the goal is to determine the **structure**.
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The electron density in the unit cell is related to the Fourier transform of the **amplitude and phase of the scattered X-rays**.

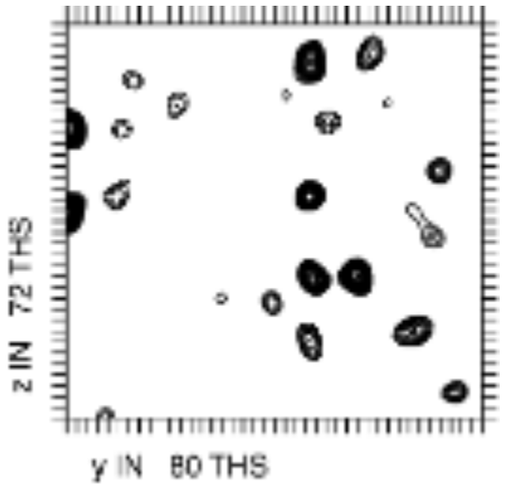
Unfortunately:

ϕ is lost: phase problem

$$\rho(\vec{r}) = FT\left(\vec{F}(\vec{H})\right) = \frac{1}{V}\int |F| e^{i\phi} \cdot e^{-2i\pi\vec{H}\cdot\vec{r}}$$

obtained from the experiment: $I \propto |F|$

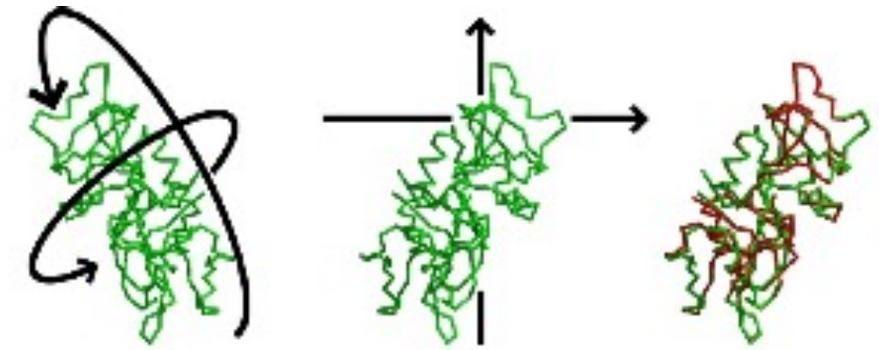
How to recover phases



Experimentally

Exploit the properties of a few special atoms:

- anomalous scattering
- a large number of electrons



Computationally

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

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

Experimental Phasing with AutoSol

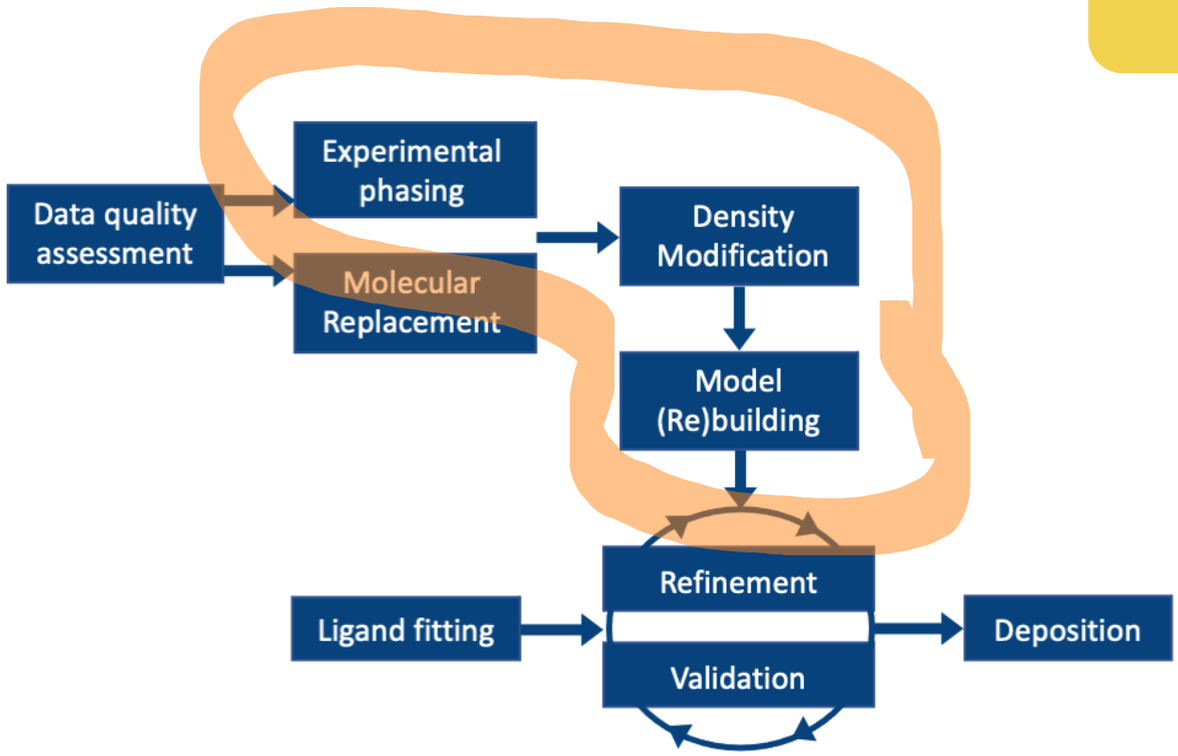
Experimental data
Sequence
Special atom
Wavelength(s)

1. Determine the substructure

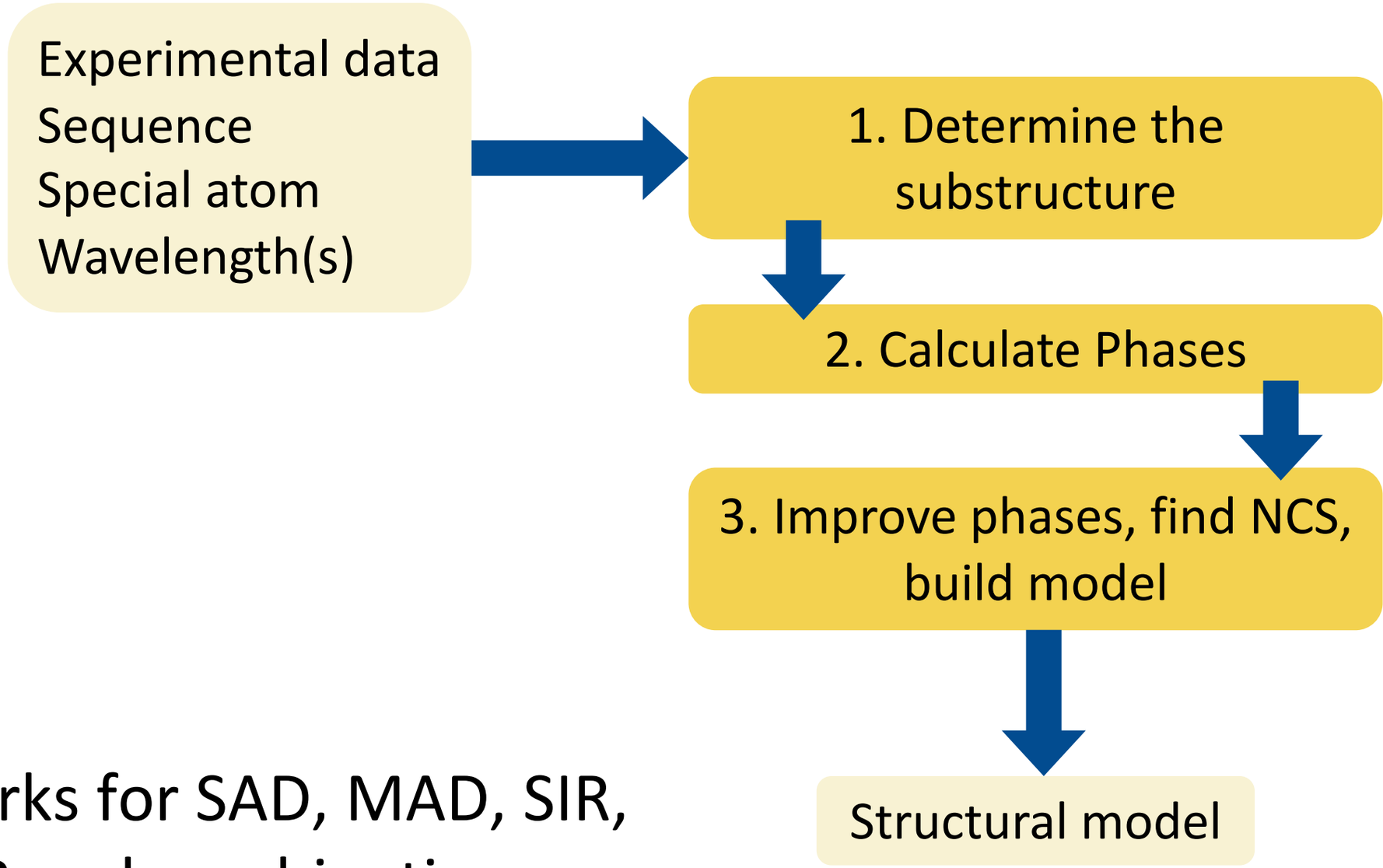
2. Calculate Phases

3. Improve phases, find NCS, build model

Structural model



Experimental Phasing with AutoSol

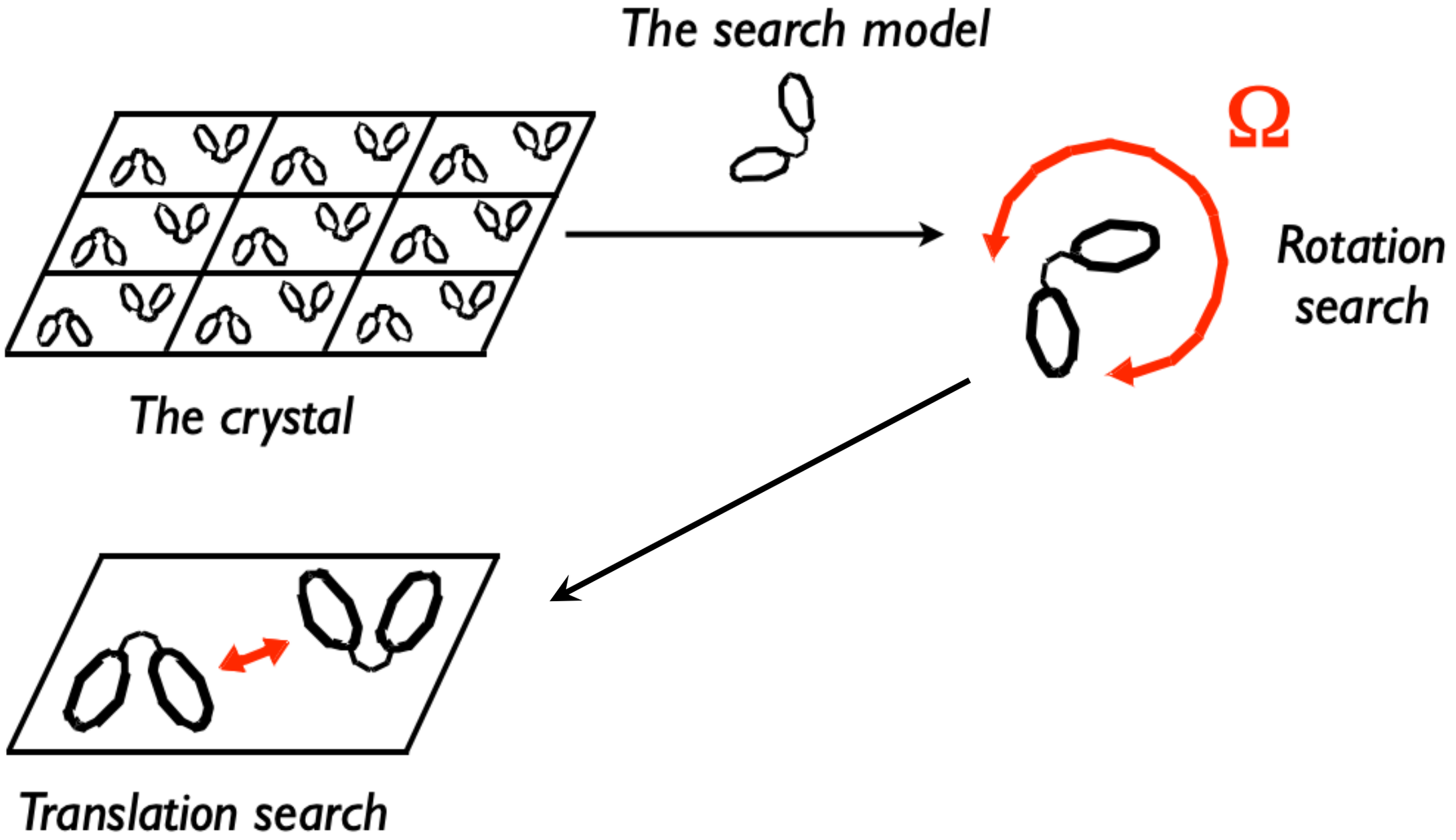


Works for SAD, MAD, SIR, MIR and combinations.

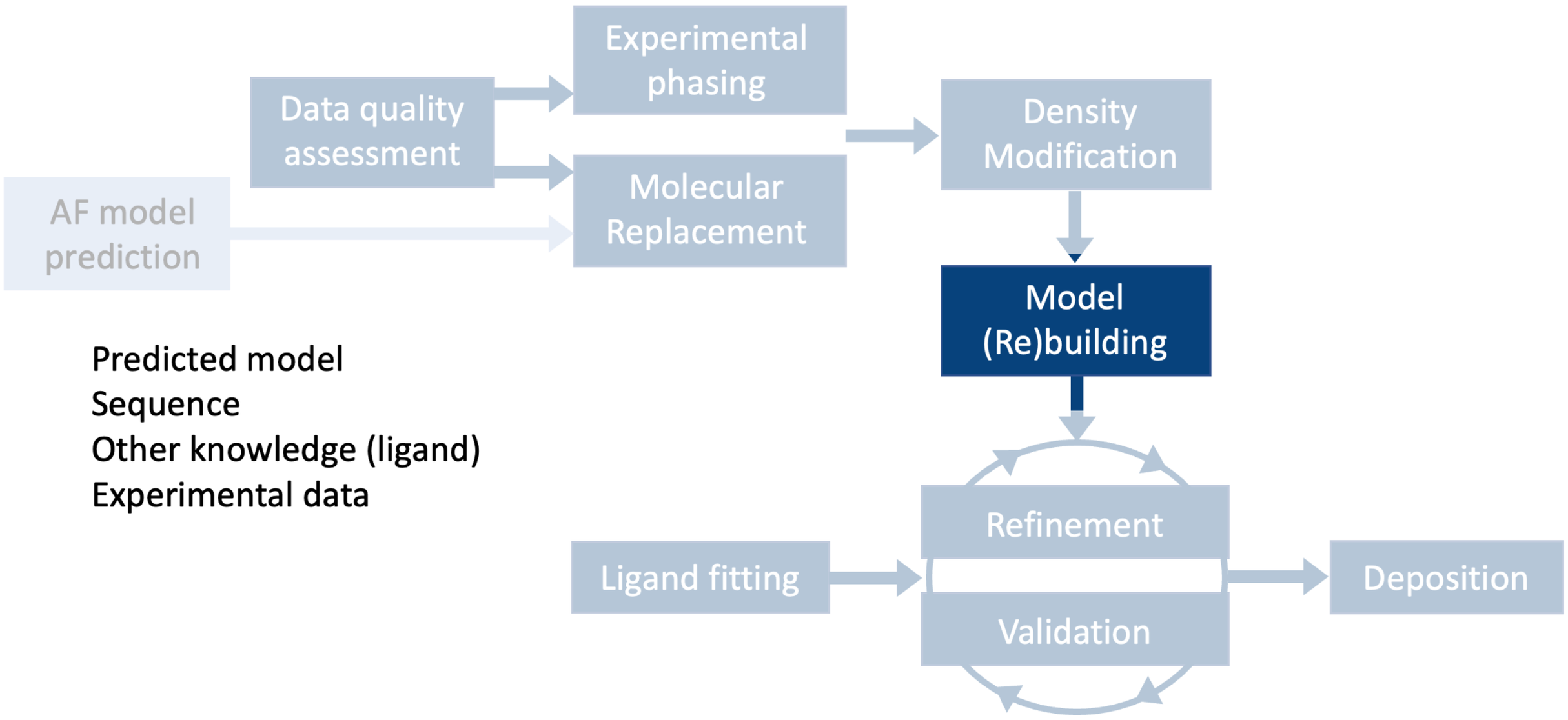
This procedure is fully automatic!

Molecular replacement (MR)

Use a previously known structure to get phase estimates



Steps in crystallography



Predicted model
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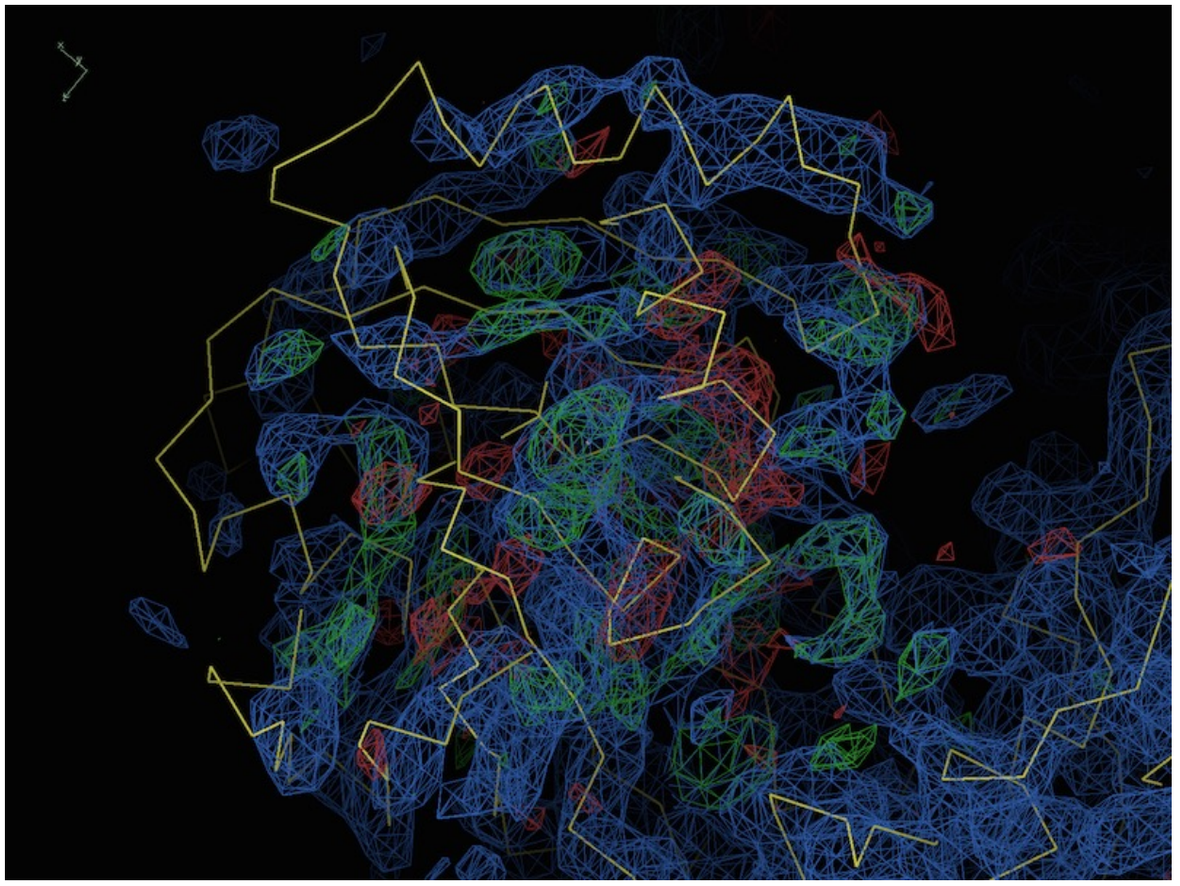
Model building

After phasing, we have an initial model. But sometimes it is not very good yet.

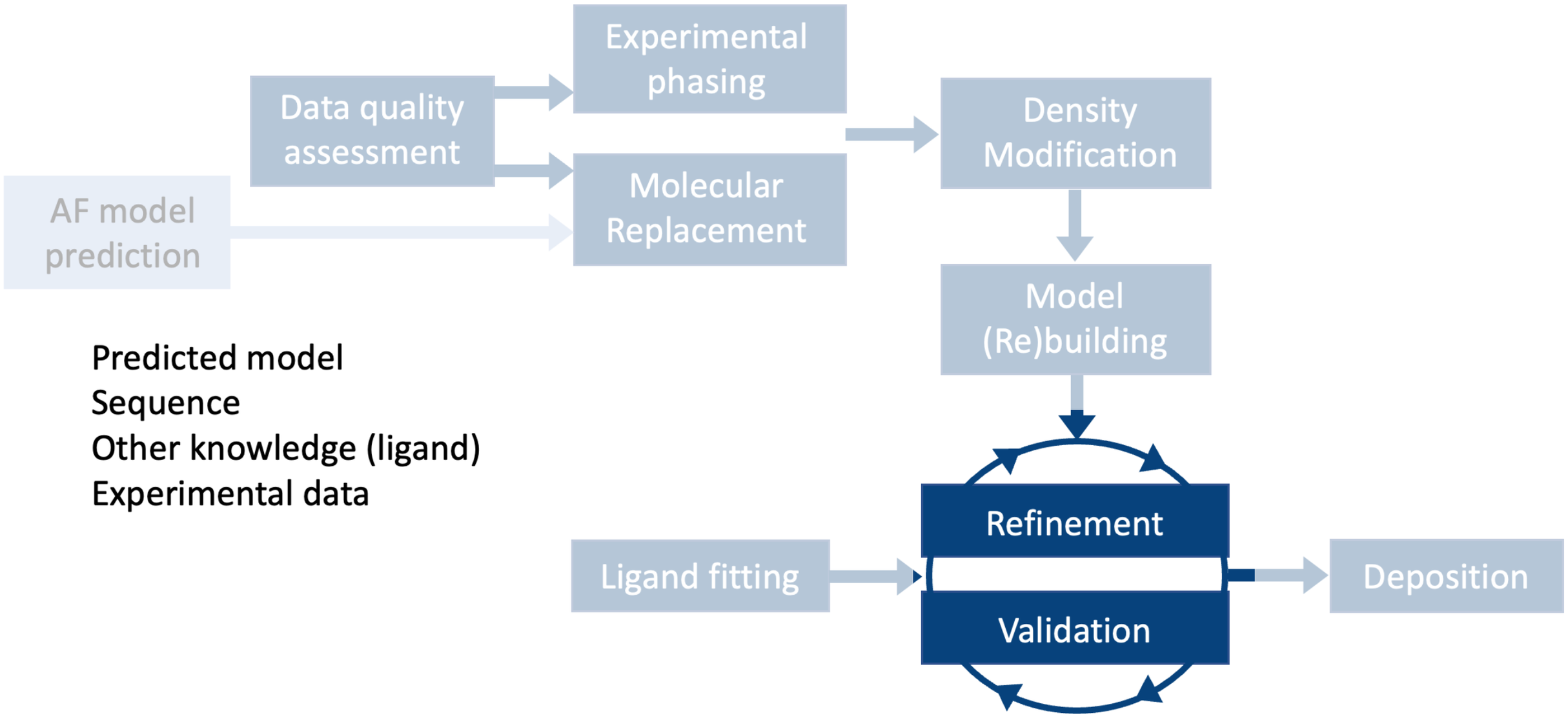
Experimental phasing:
The AutoSol model can be most likely improved by doing a more thorough approach with AutoBuild.

Molecular replacement:
If the search model is of a significantly different protein and/or if there are large conformational changes, run AutoBuild.

→ Run **AutoBuild**



Steps in crystallography



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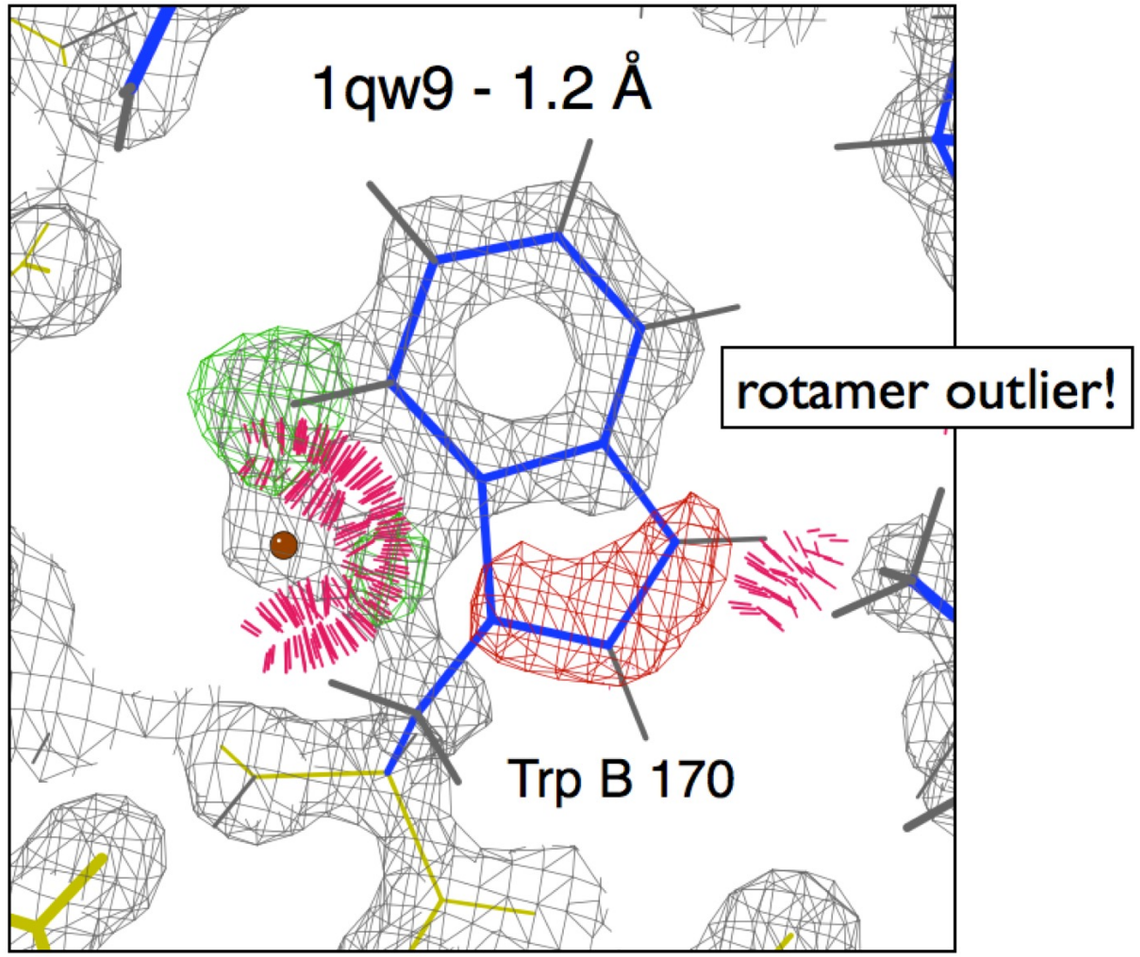
Refinement/Validation

Refinement = Use an *optimization* algorithm to minimize a *target function* by changing the *parameters* of the model

Optimize a model so that it optimally explains the data.

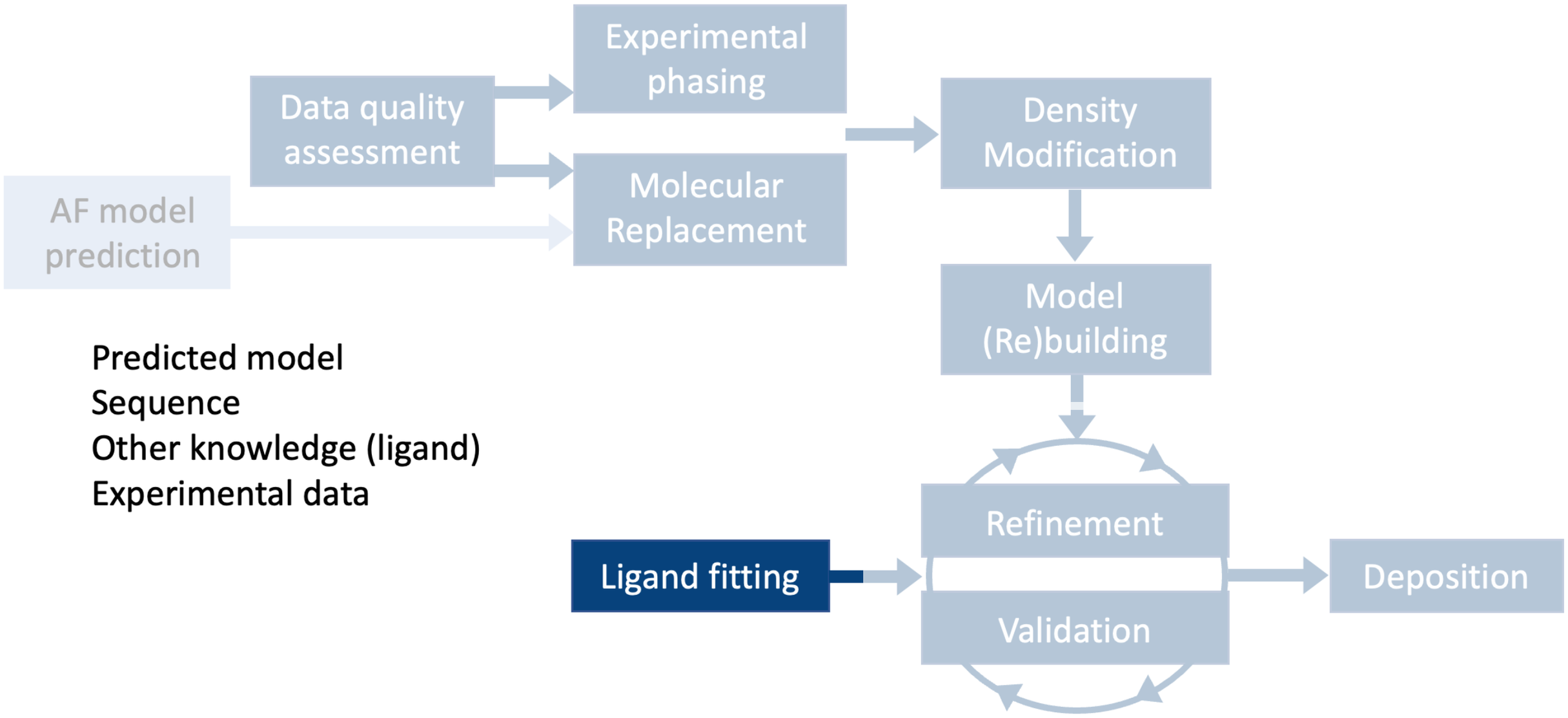
The model should be already quite “good”.

After each refinement run, check the model for outliers/oddities.



*Tronrud, Dale. E. (2004). *Acta Cryst. D*. **60**, 2156–2168.

Steps in crystallography



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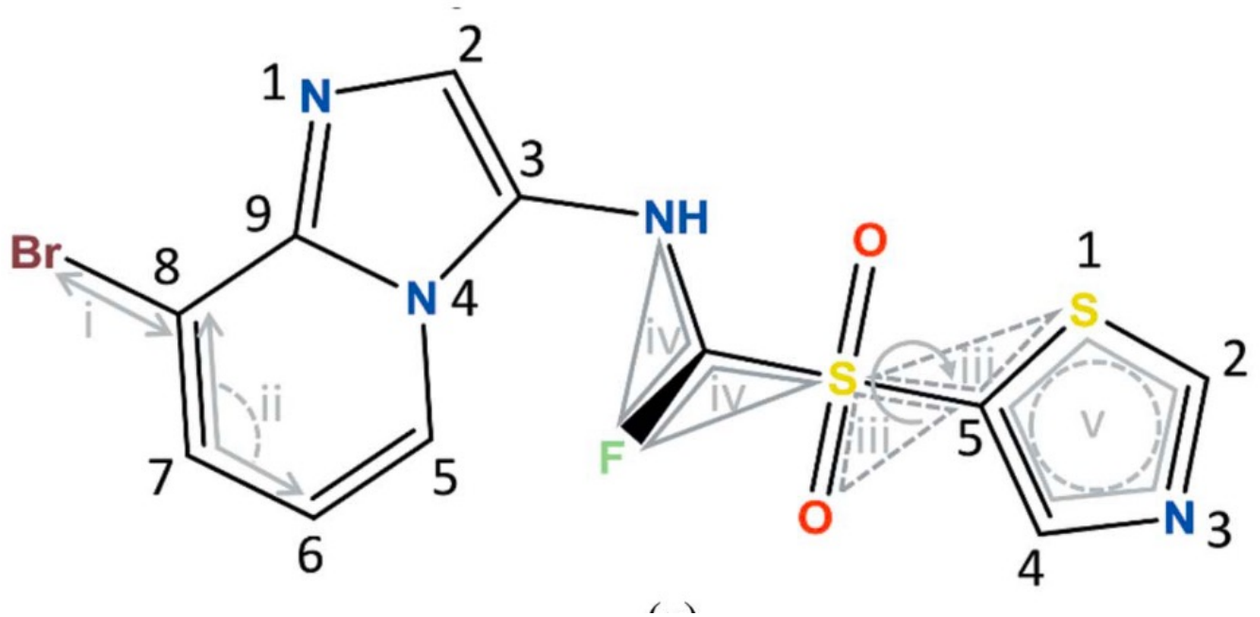
Ligands need restraints

Models (usually) cannot be refined using diffraction data alone: high-resolution information is typically missing.

Use restraints to obtain chemically plausible structures.

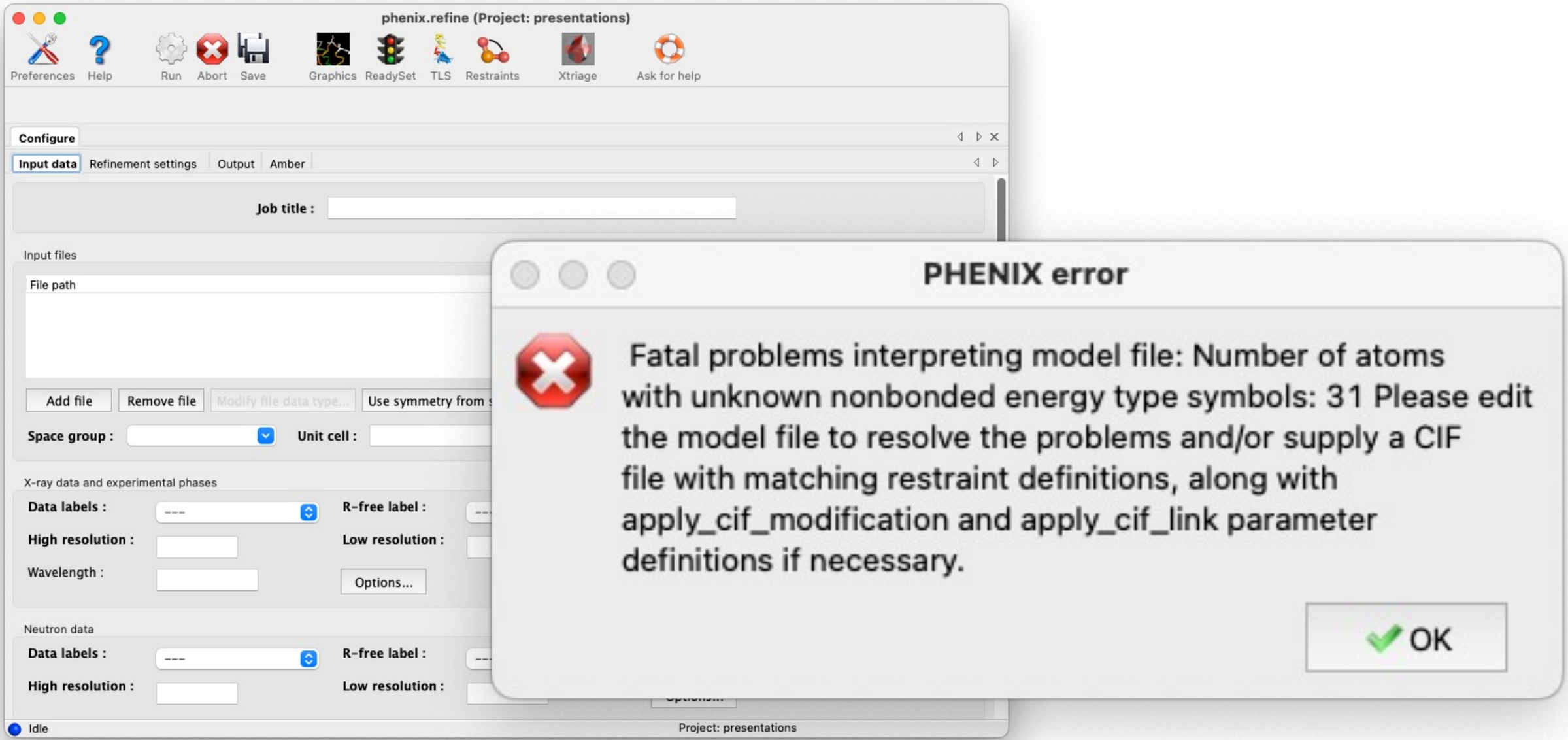
Restraints = prior knowledge

Stereochemical information (e.g. bond distances, angles) about the ligand.



Ligands need restraints

If you try to run phenix.refine and a ligand has no restraints…



(This message also occurs if atom names don’t match!)

Other tools for ligands in Phenix

- **LigandFit**: Flexible ligand fitting into difference maps.

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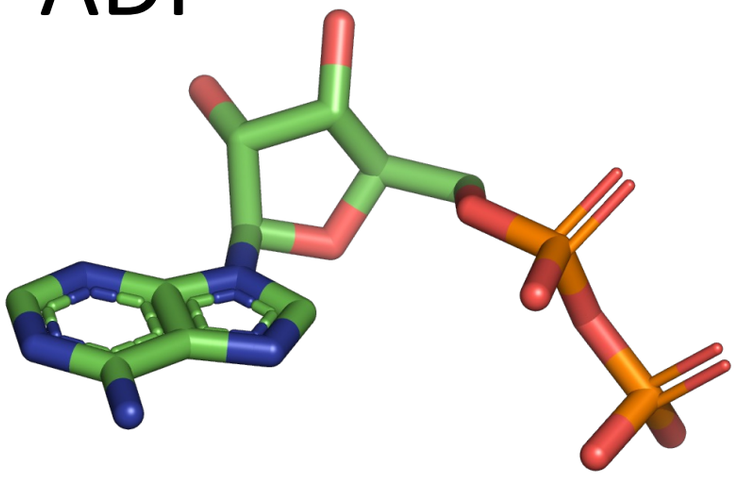
Automated ligand fitting by core-fragment fitting and extension into density

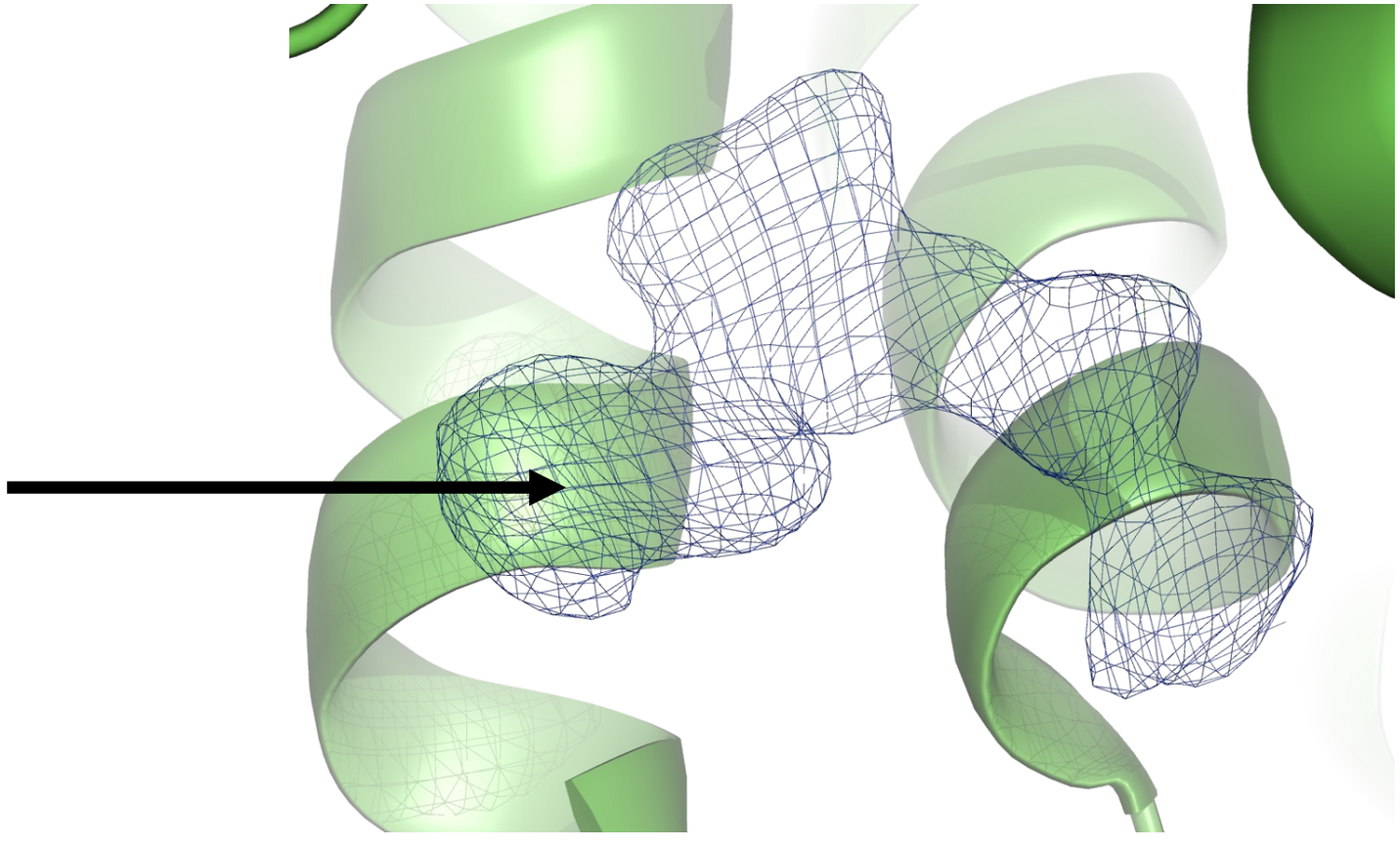
Thomas C. Terwilliger,[a]* Herbert Klei,[b] Paul D. Adams,[c] Nigel W. Moriarty[c] and Judith D. Cohn[a]

A procedure for fitting of ligands to electron-density maps by first fitting a core fragment of the ligand to density and then extending the remainder of the ligand into density is presented. The approach was tested by fitting 9327 ligands

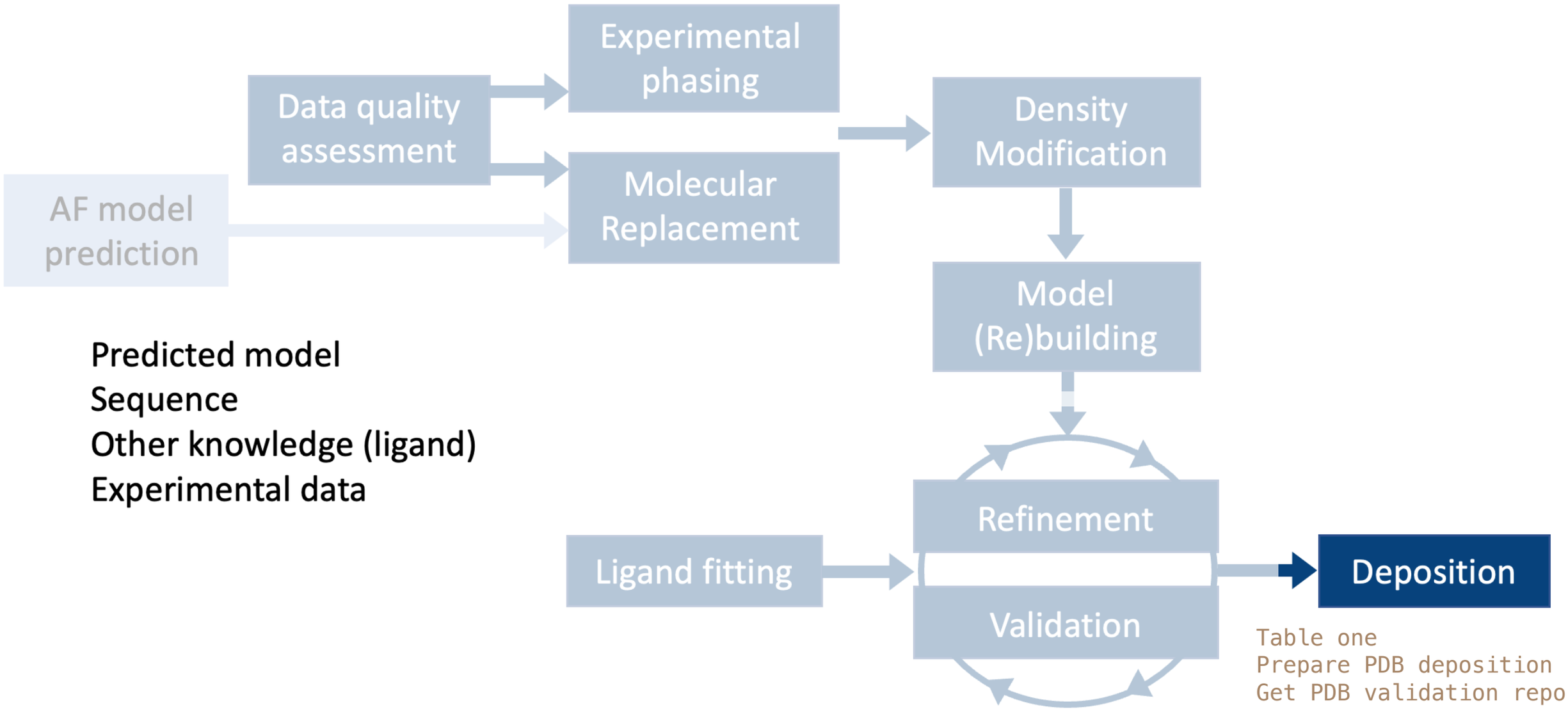
Received 26 January 2006
Accepted 9 May 2006

ADP





Steps in crystallography



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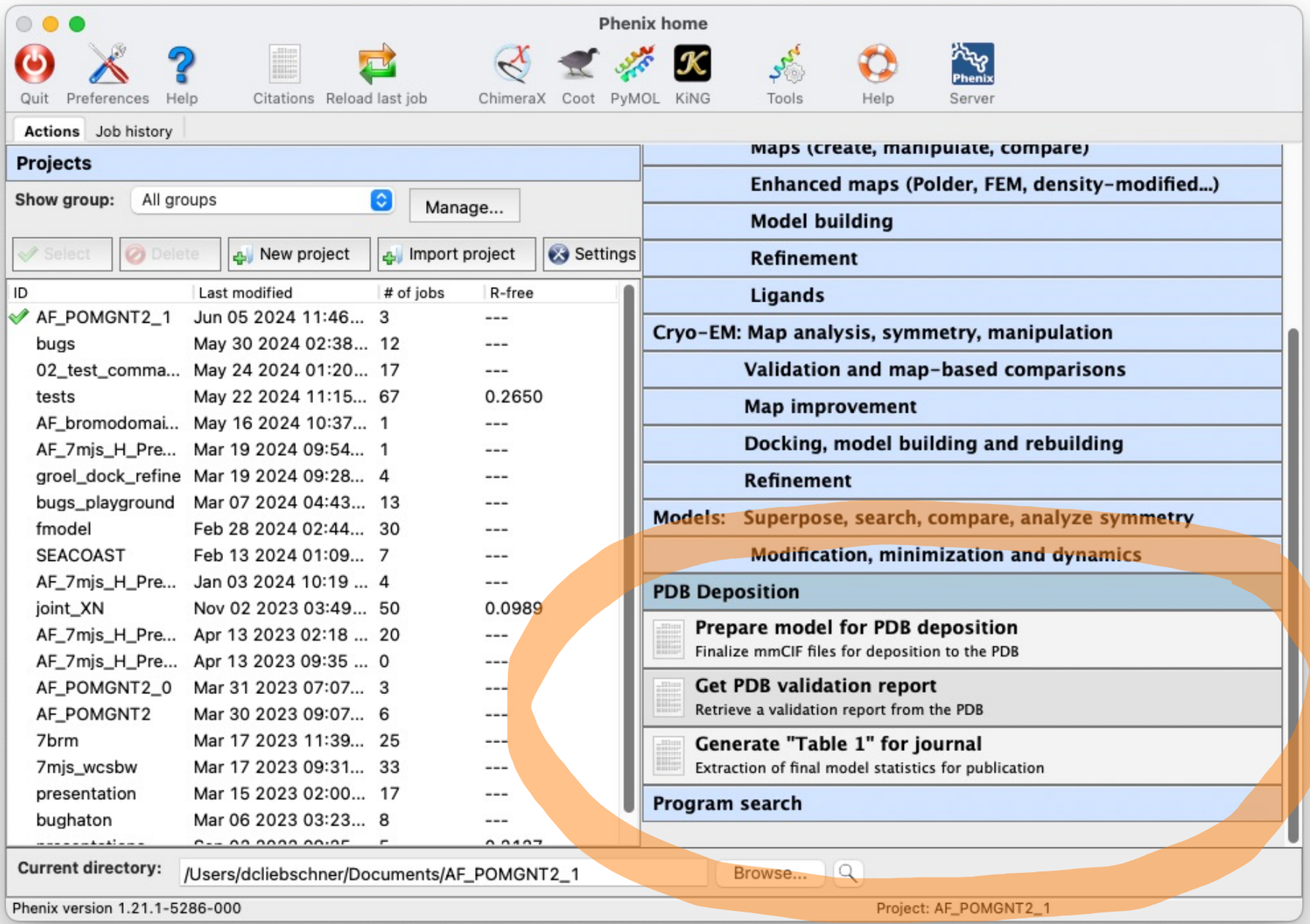
PDB deposition



The Phenix Project

Lawrence Berkeley Laboratory

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New Mexico Consortium

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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