

Phenix User Workshop, Missoula, Montana



Phenix Tools for crystallography

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Lawrence Berkeley Laboratory

Steps in crystallography

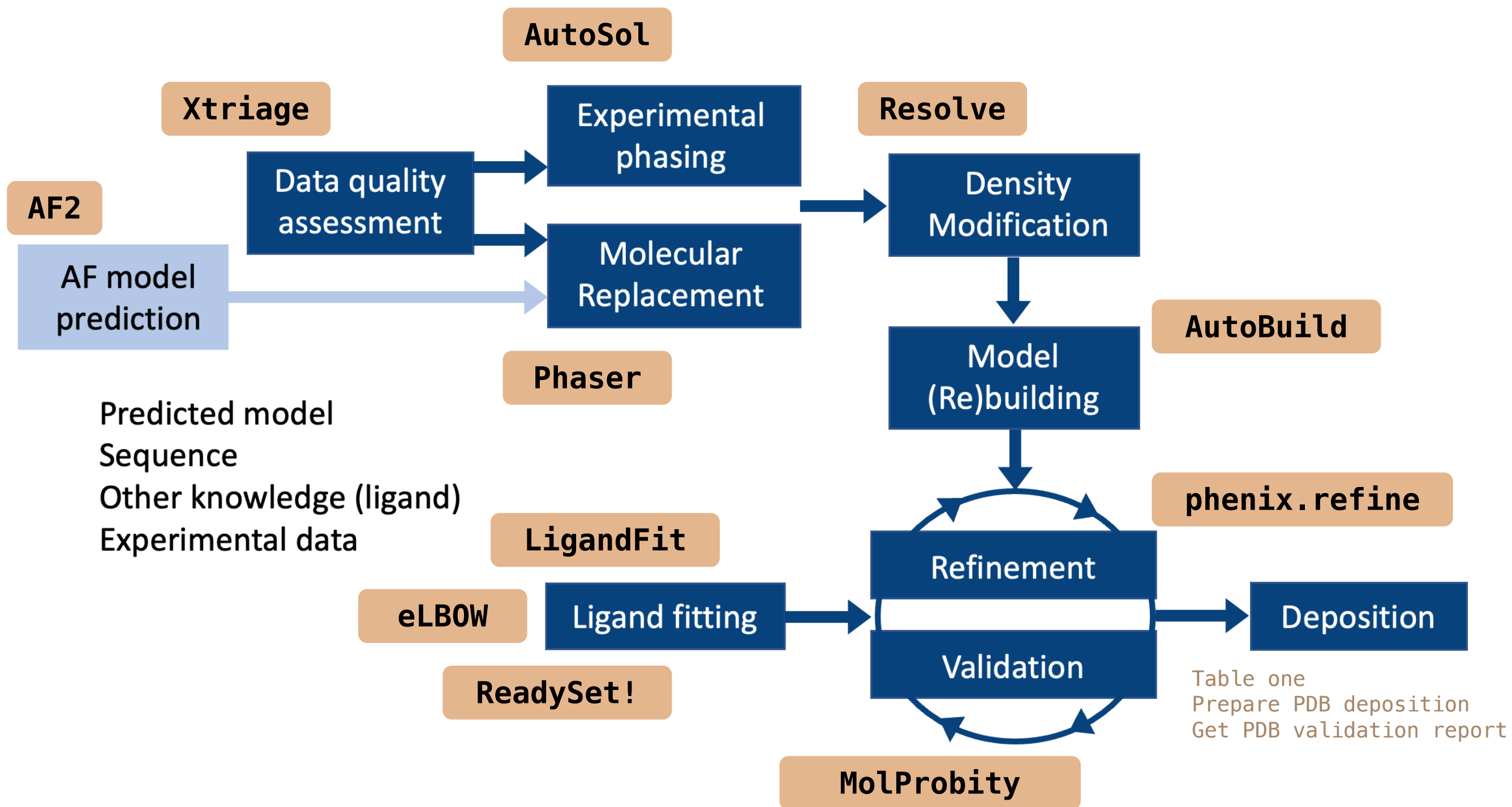
Diffraction
experiment



Predicted model
Sequence
Other knowledge (ligand)
Experimental data



Steps in crystallography

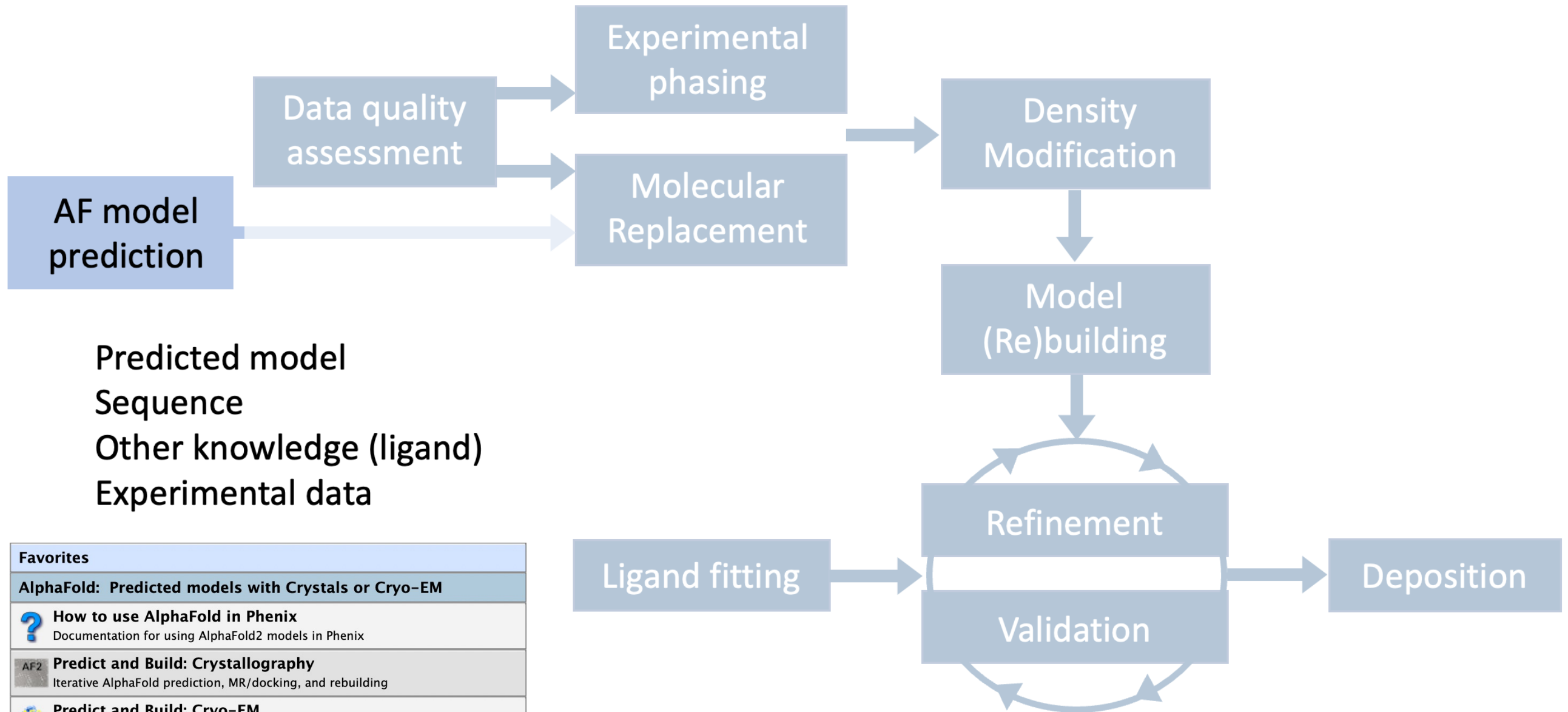


Acta Cryst. 2002, D58:1948-1954
J. Appl. Cryst. 2002, 35:126-136
Acta Cryst. 2010, D66: 213-221
Acta Cryst. 2019 D75:861-877






(Phenix)
(cctbx)
(Phenix)
(Phenix)

Table one
Prepare PDB deposition
Get PDB validation report

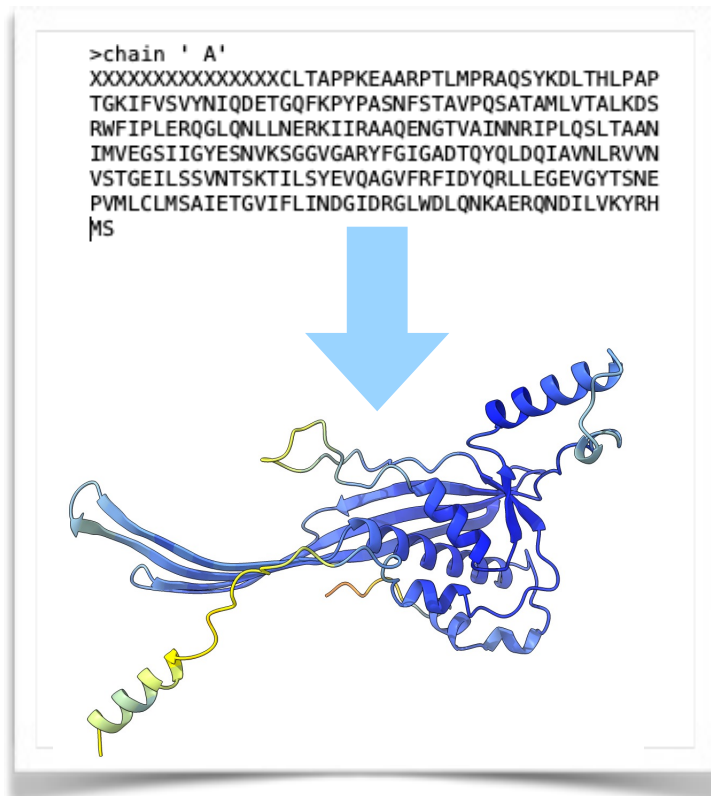
Steps in crystallography



Predicted model
Sequence
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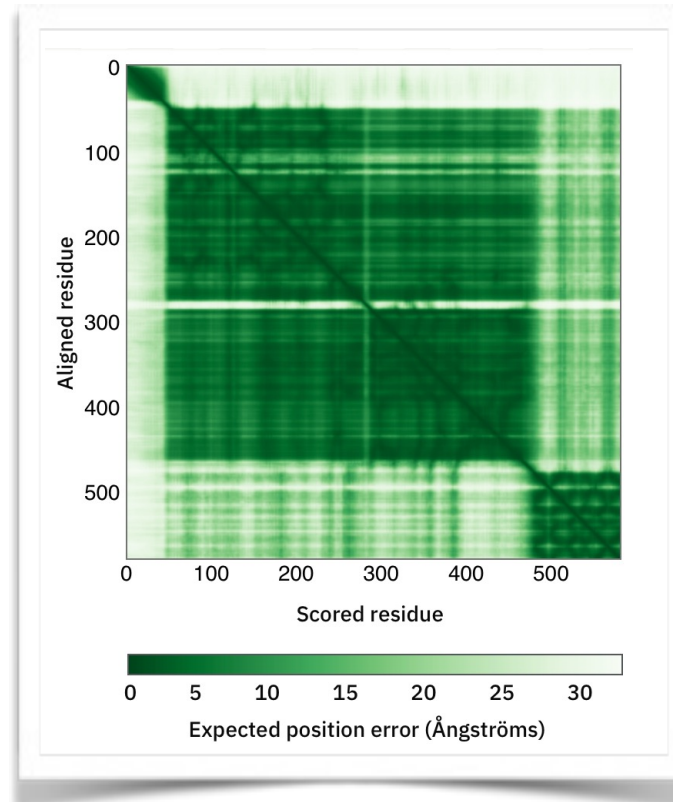
Favorites	
AlphaFold: Predicted models with Crystals or Cryo-EM	
	How to use AlphaFold in Phenix Documentation for using AlphaFold2 models in Phenix
	Predict and Build: Crystallography Iterative AlphaFold prediction, MR/docking, and rebuilding
	Predict and Build: Cryo-EM Iterative AlphaFold prediction, docking, and rebuilding
	AlphaFold model prediction Predict the chains in a sequence file with AlphaFold
	Process Predicted Model Replace B-factor field in model and optionally split into domains
Crystals: Data analysis and manipulation	
Validation and map-based comparisons	

Tools for predicted models in Phenix

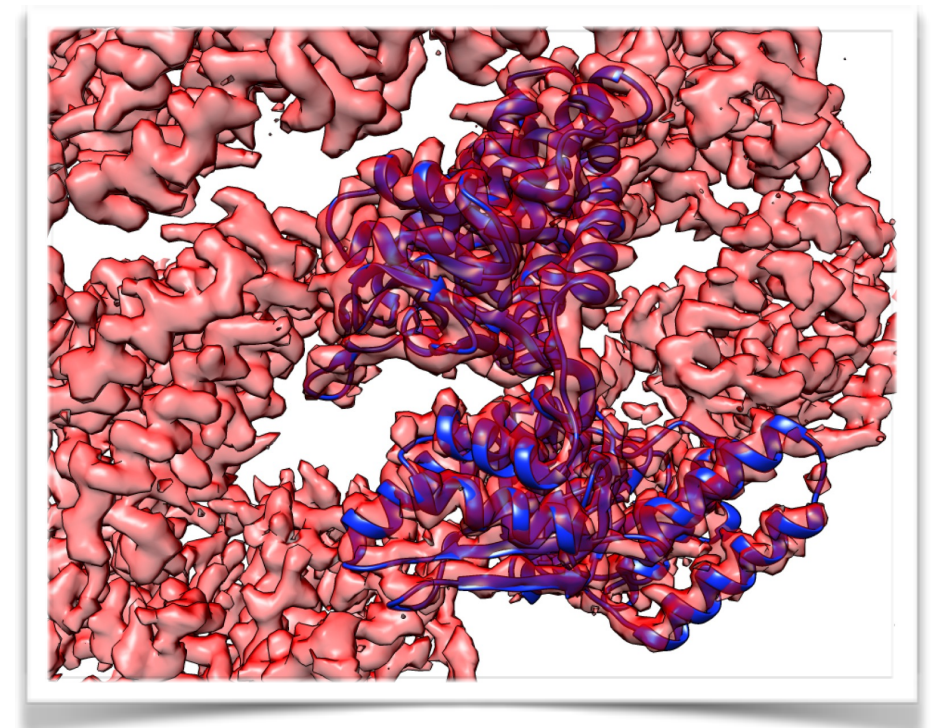


AlphaFold model prediction

Phenix server, no need to have AF2 installed locally



Process predicted model

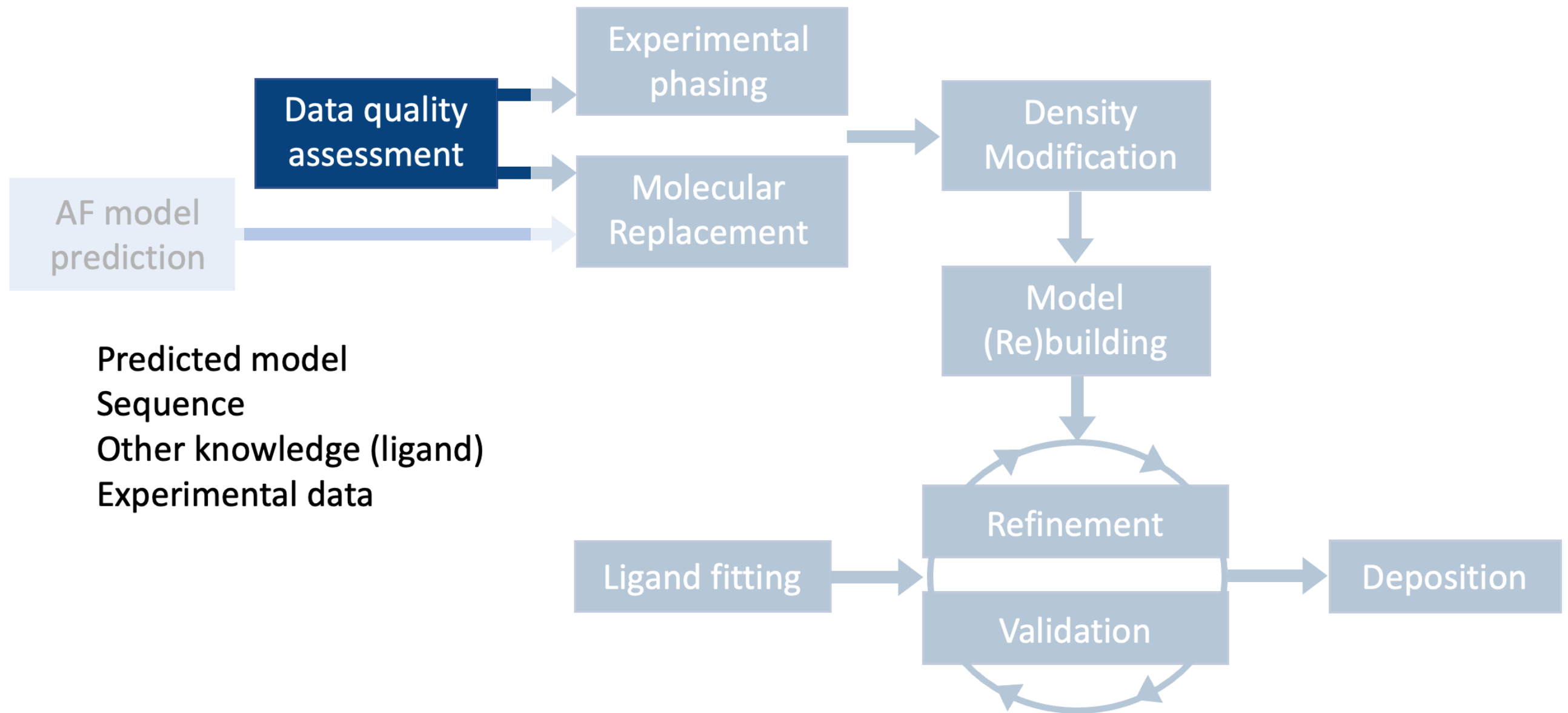


Predict and Build

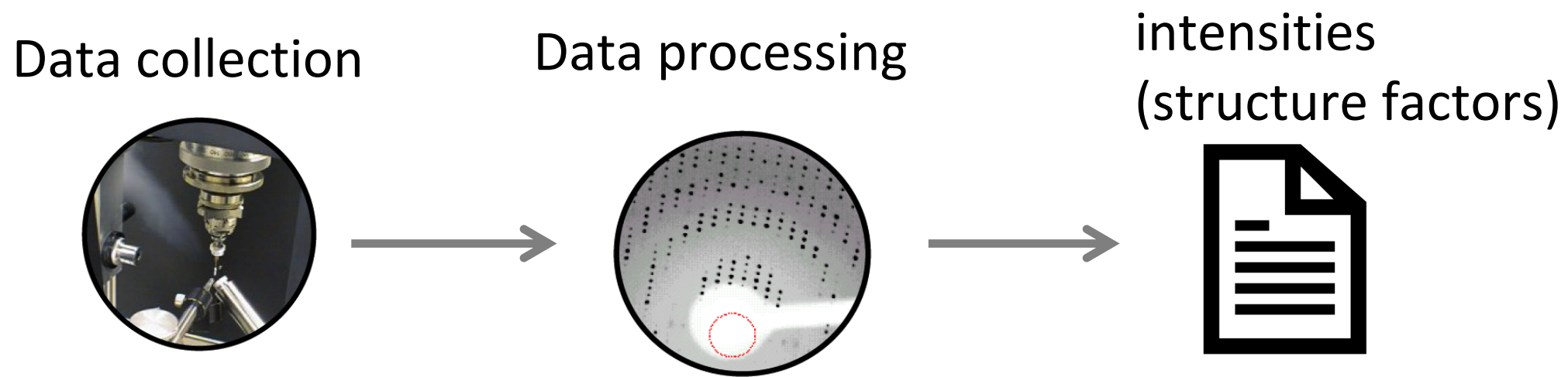
Iterative AlphaFold prediction, MR/docking, and rebuilding

Fully automatic!

Steps in crystallography



Data Quality Assessment



Macromolecular crystals are prone to pathologies:

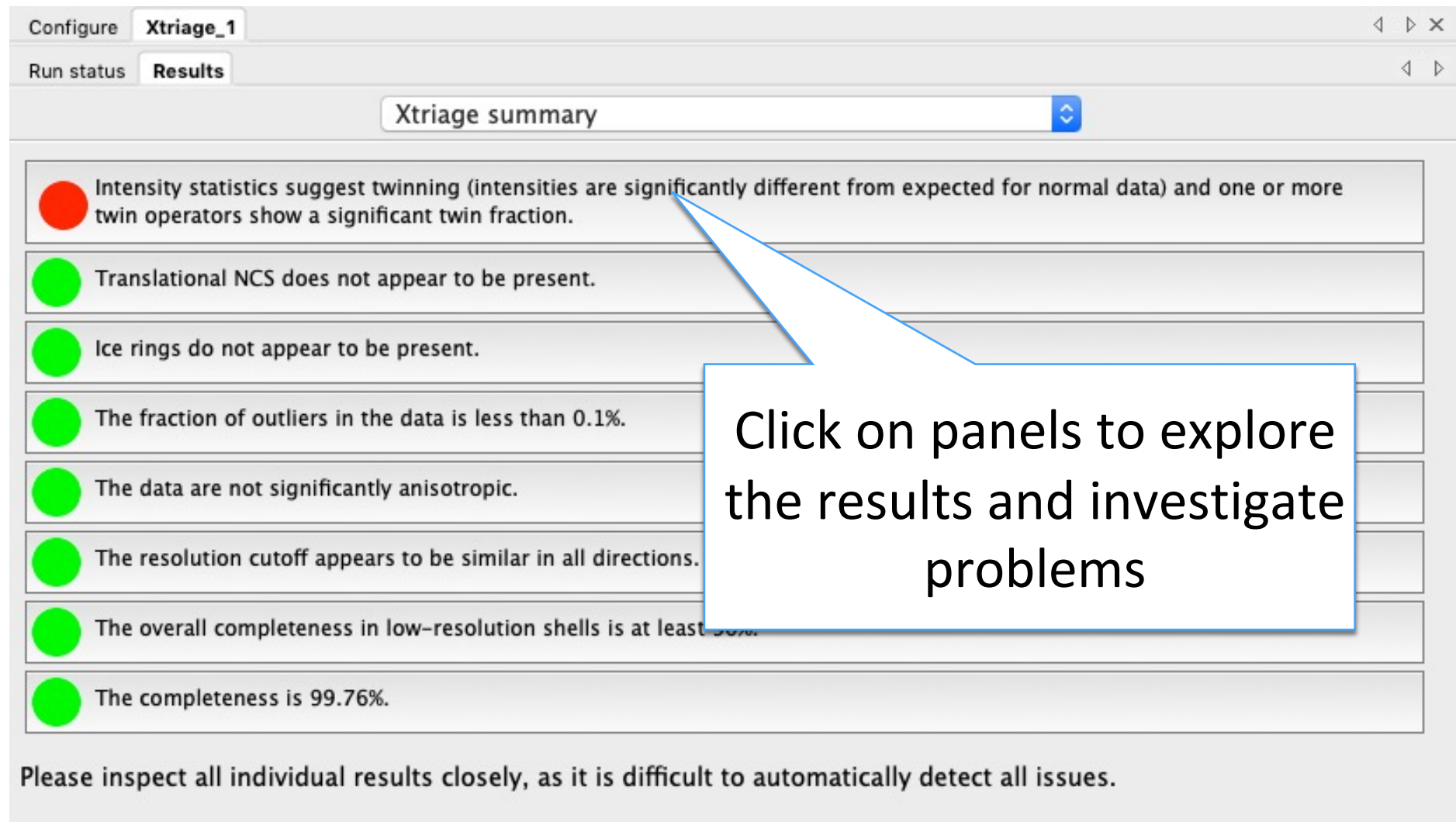
- Twinning: two or more crystals are intergrown (orientations are related by twin operation)
- tNCS: more than one copy of a molecule is in a similar orientation in the asymmetric unit

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).

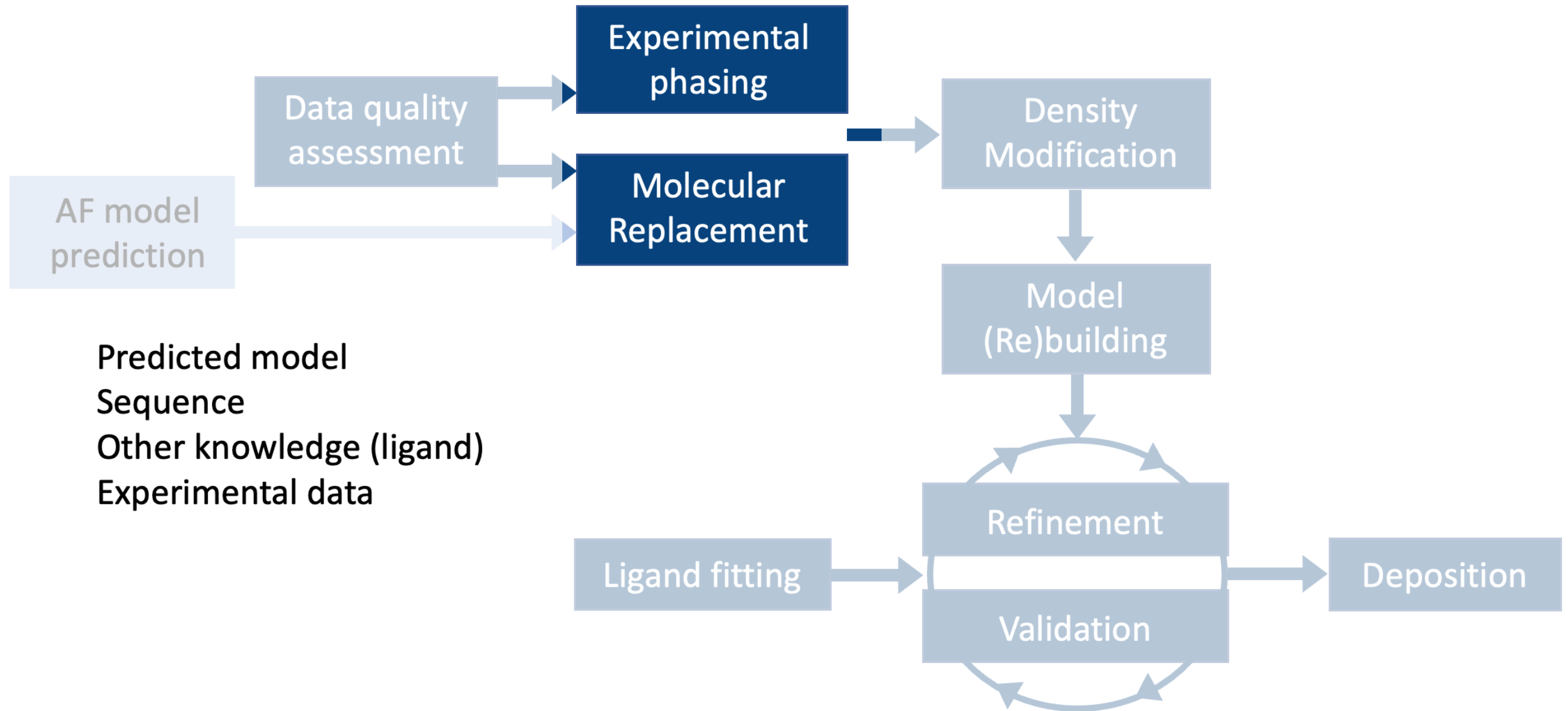


The screenshot shows the Xtrriage software interface. At the top, there are tabs for 'Configure' and 'Xtrriage_1', and 'Run status' and 'Results'. Below this is a search bar containing 'Xtrriage summary'. The main area displays a list of diagnostic results, each with a colored circle icon and a text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

Steps in crystallography



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**.

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

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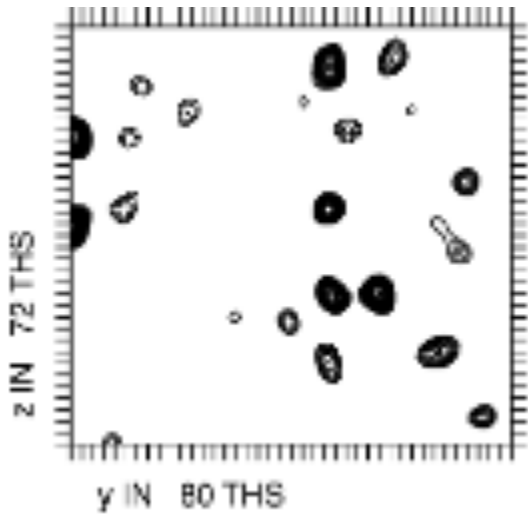
Unfortunately:

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

ϕ is lost: phase problem

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

How to recover phases



Experimentally

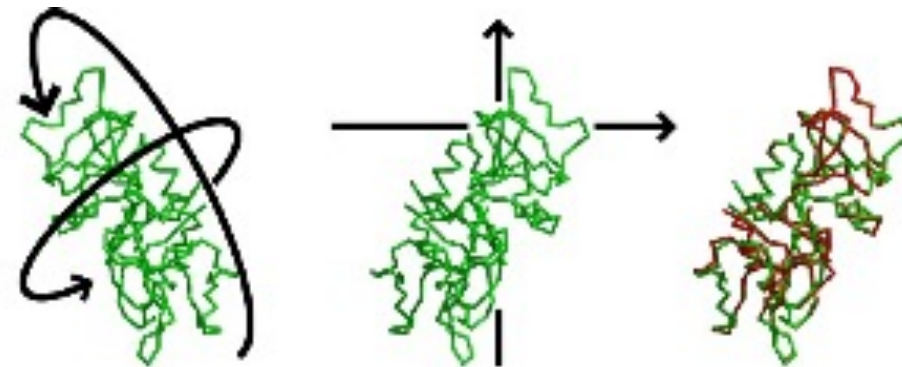
Exploit the properties of a few special atoms:

- 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

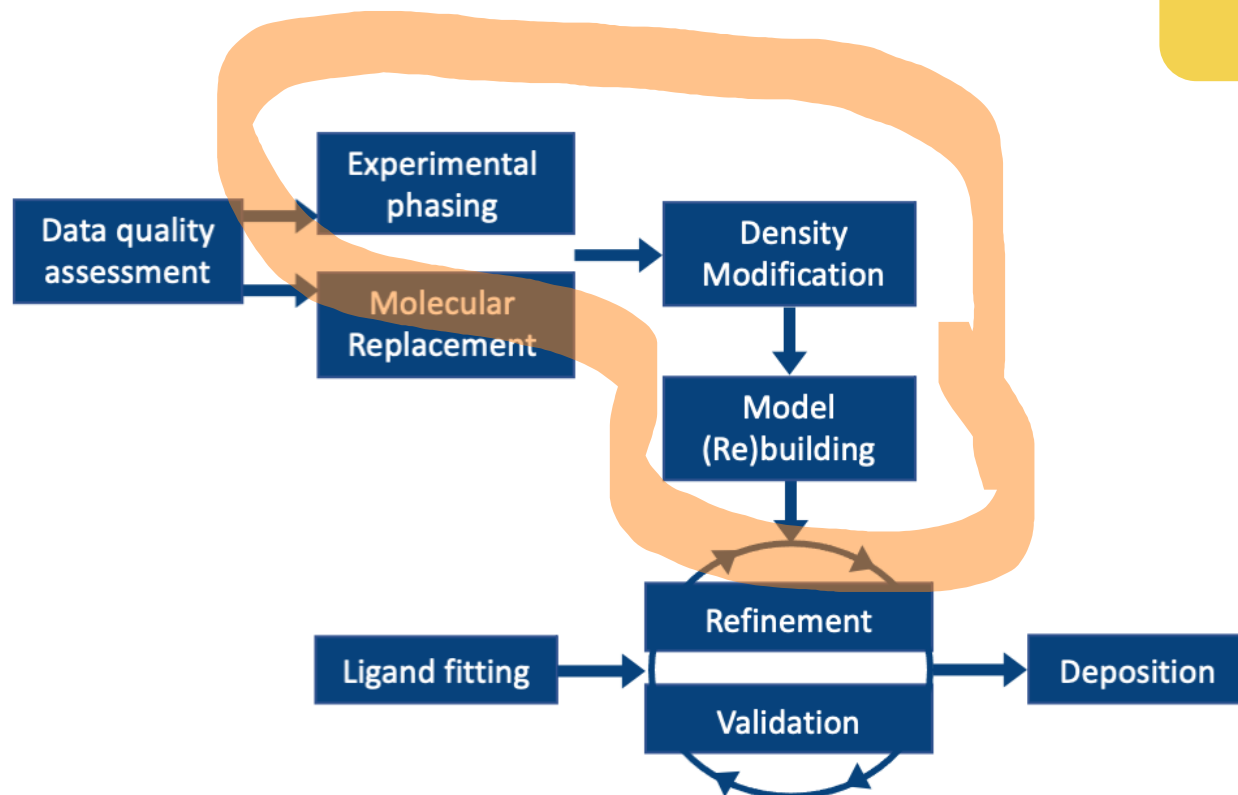
Experimental data
Sequence
Special atom
Wavelength(s)

1. Determine the
substructure

2. Calculate Phases

3. Improve phases, find NCS,
build model

Structural model



Experimental Phasing with AutoSol

Experimental data
Sequence
Special atom
Wavelength(s)

1. Determine the
substructure

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build model

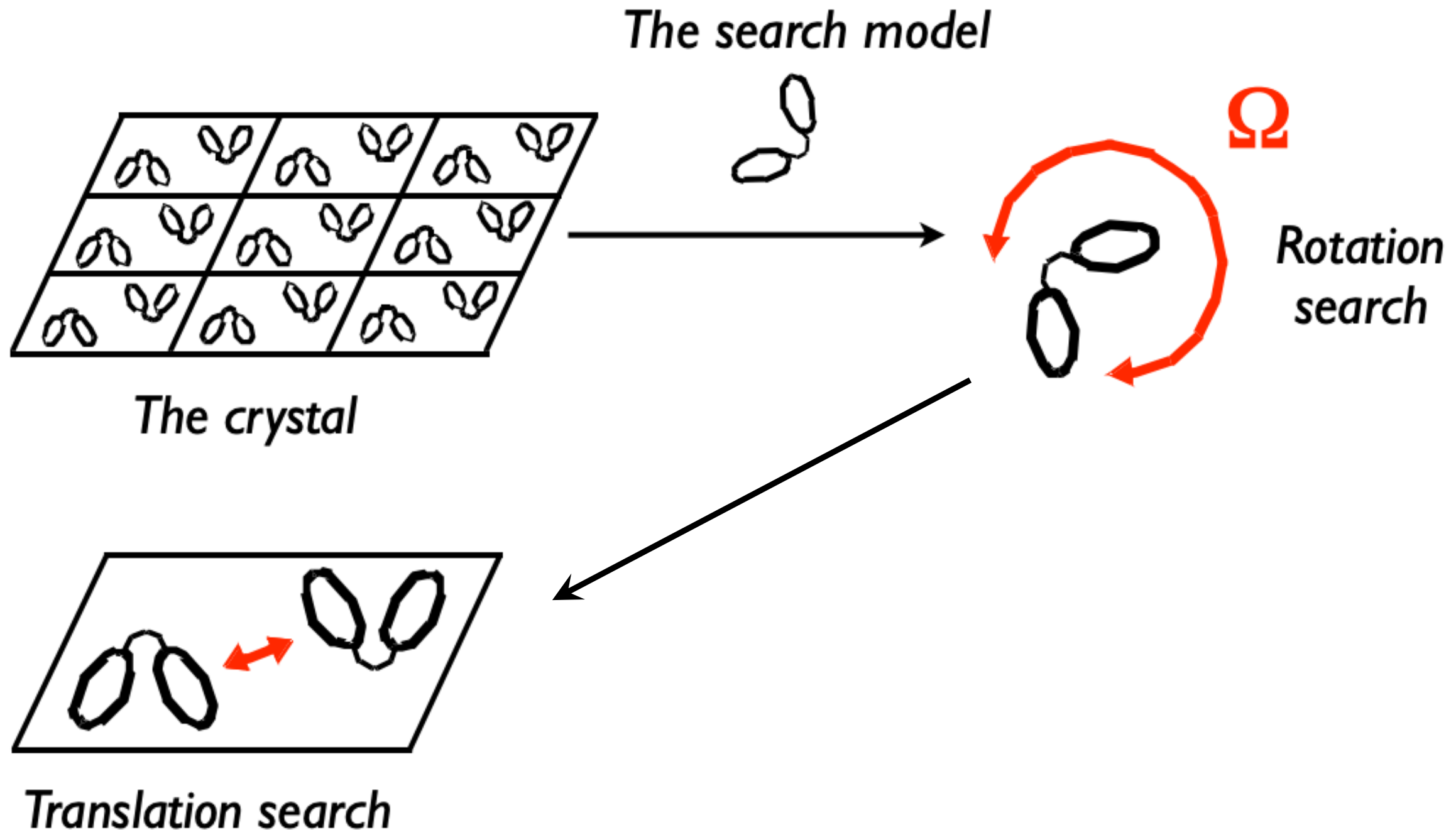
Structural model

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

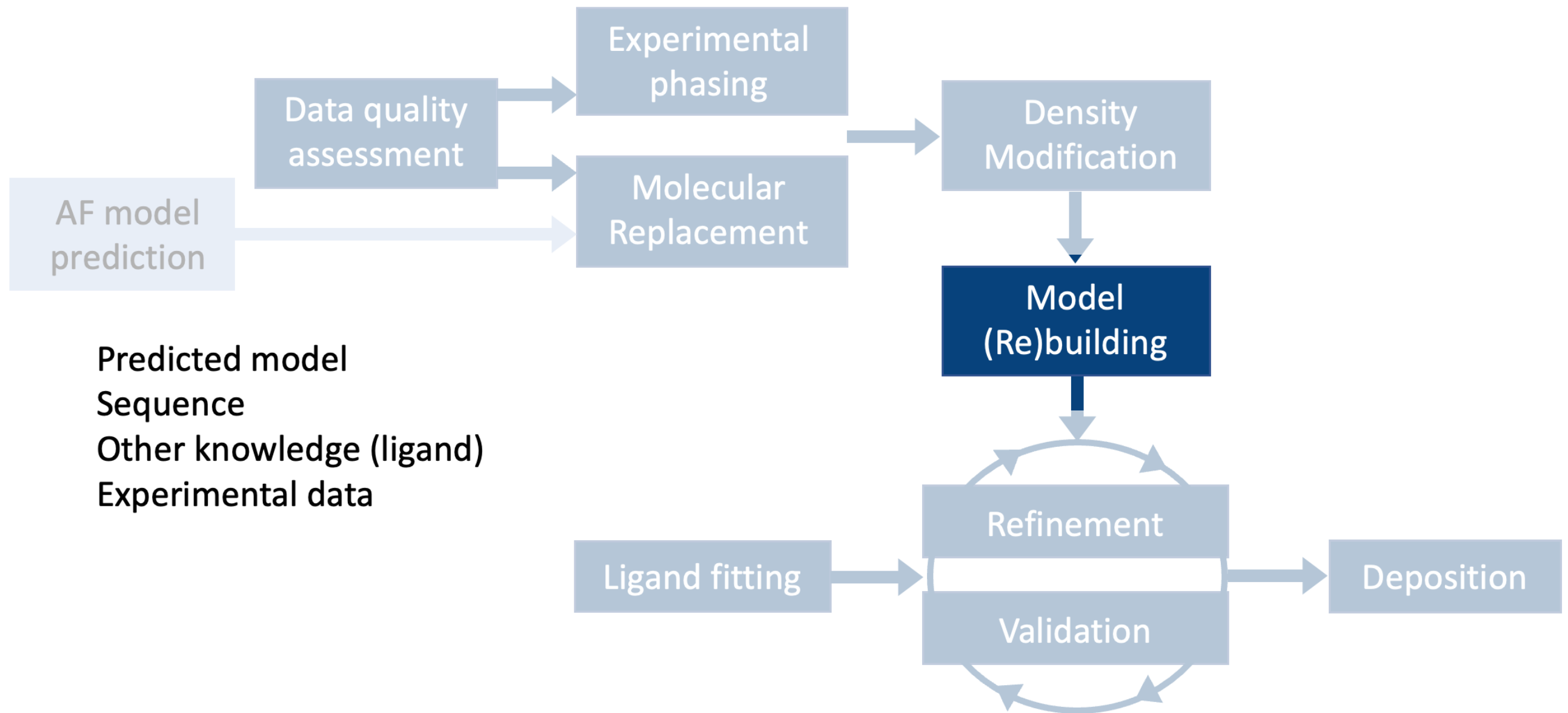
This procedure is fully automatic!

Molecular replacement (MR)

Use a previously known structure to get phase estimates



Steps in crystallography



Model building

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

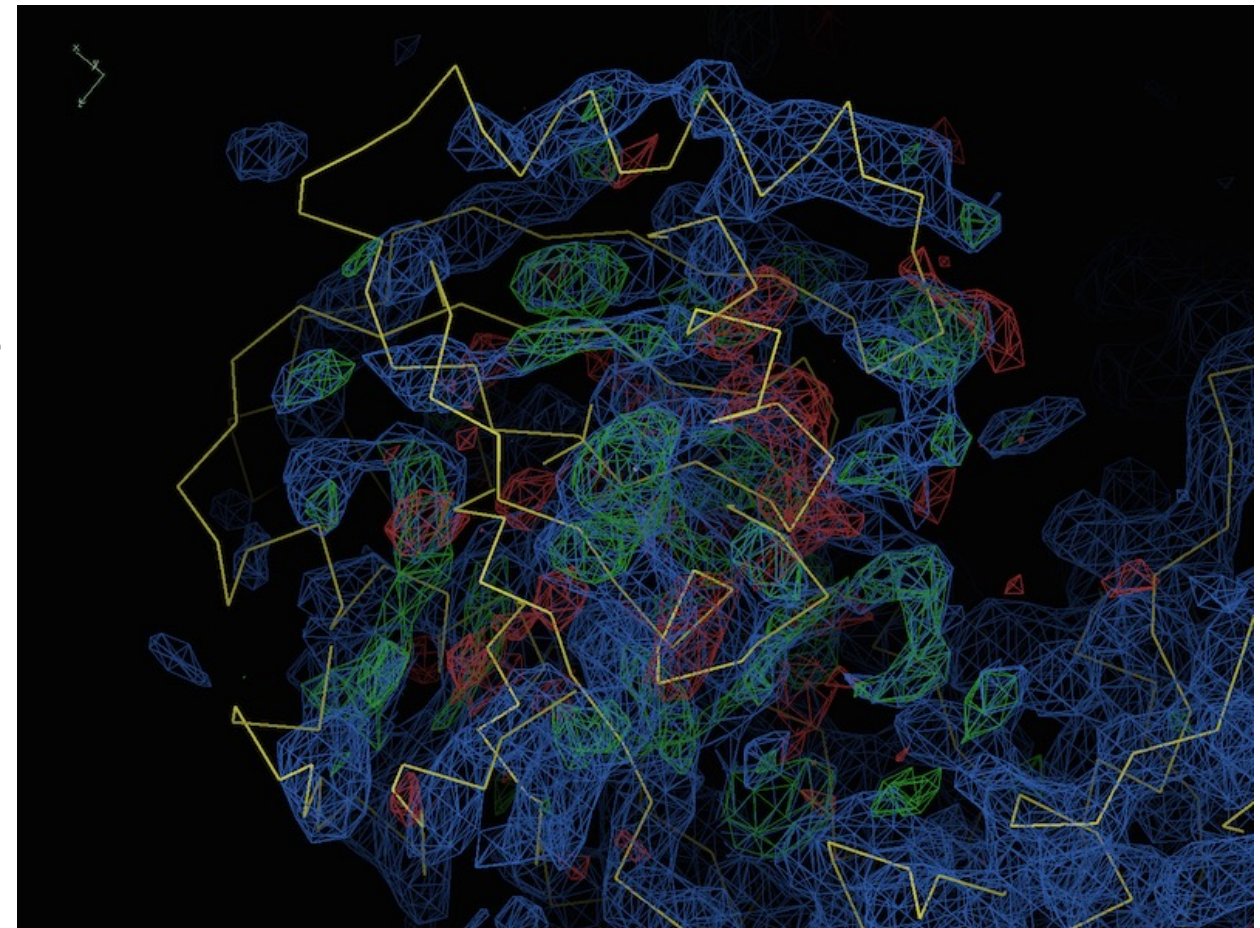
Experimental phasing:

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

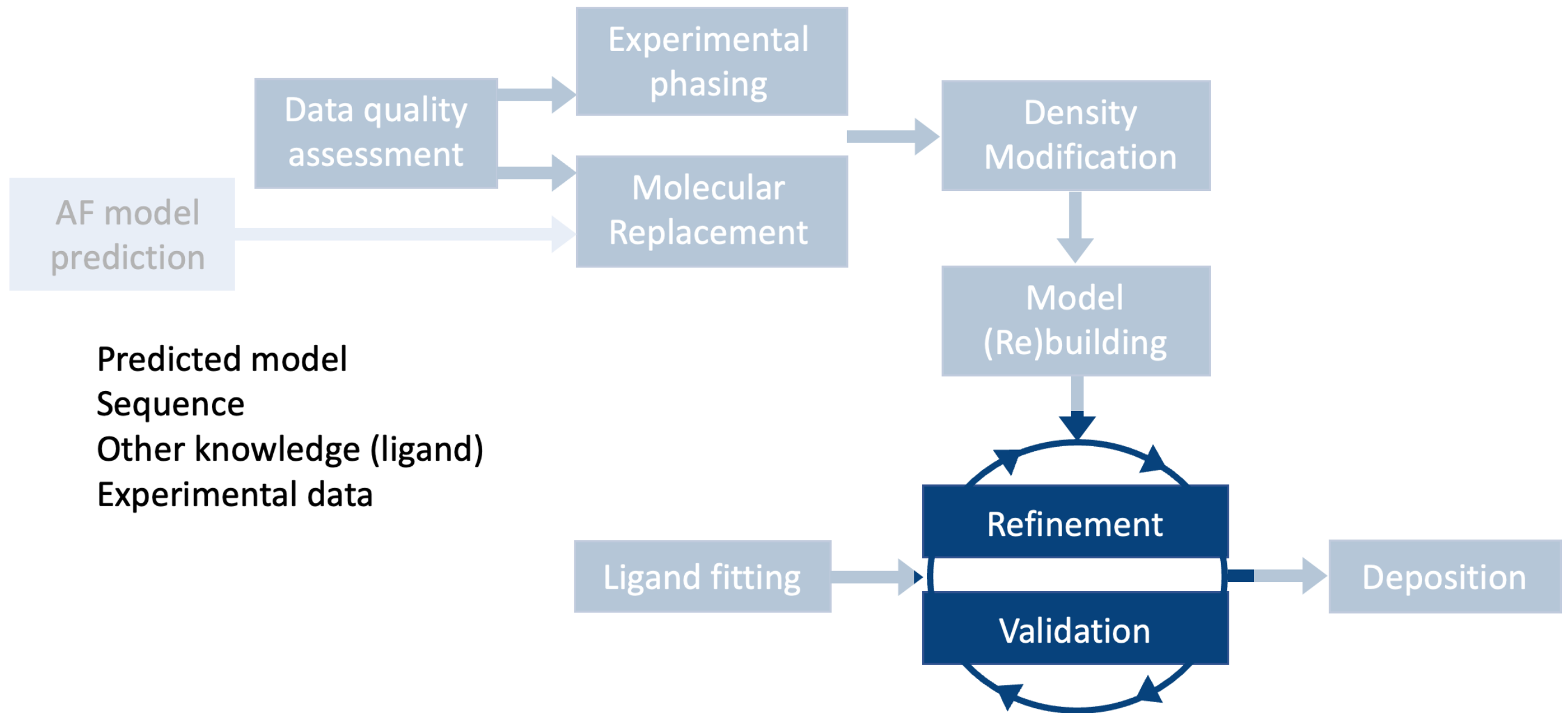
Molecular replacement:

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



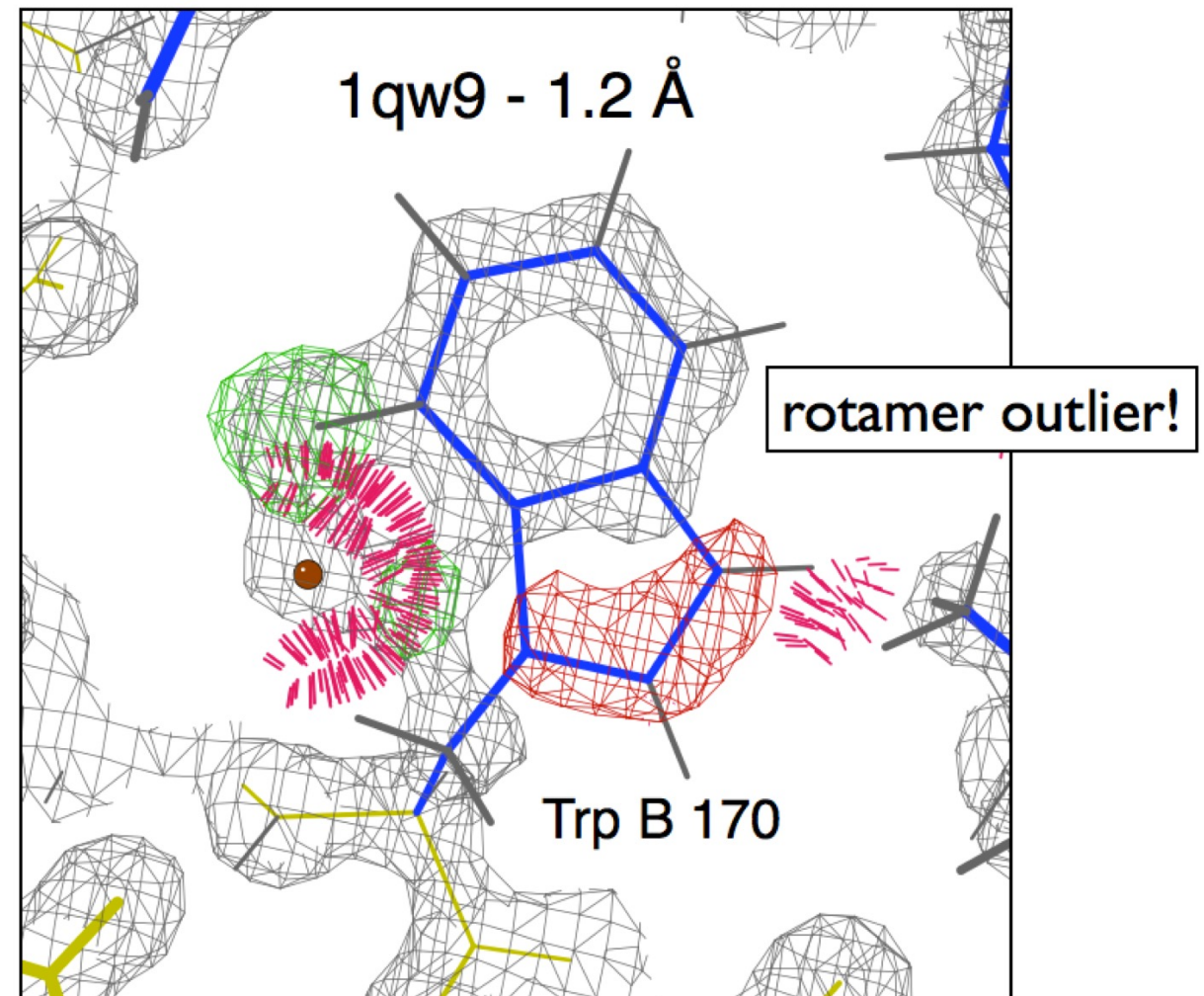
Refinement/Validation

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

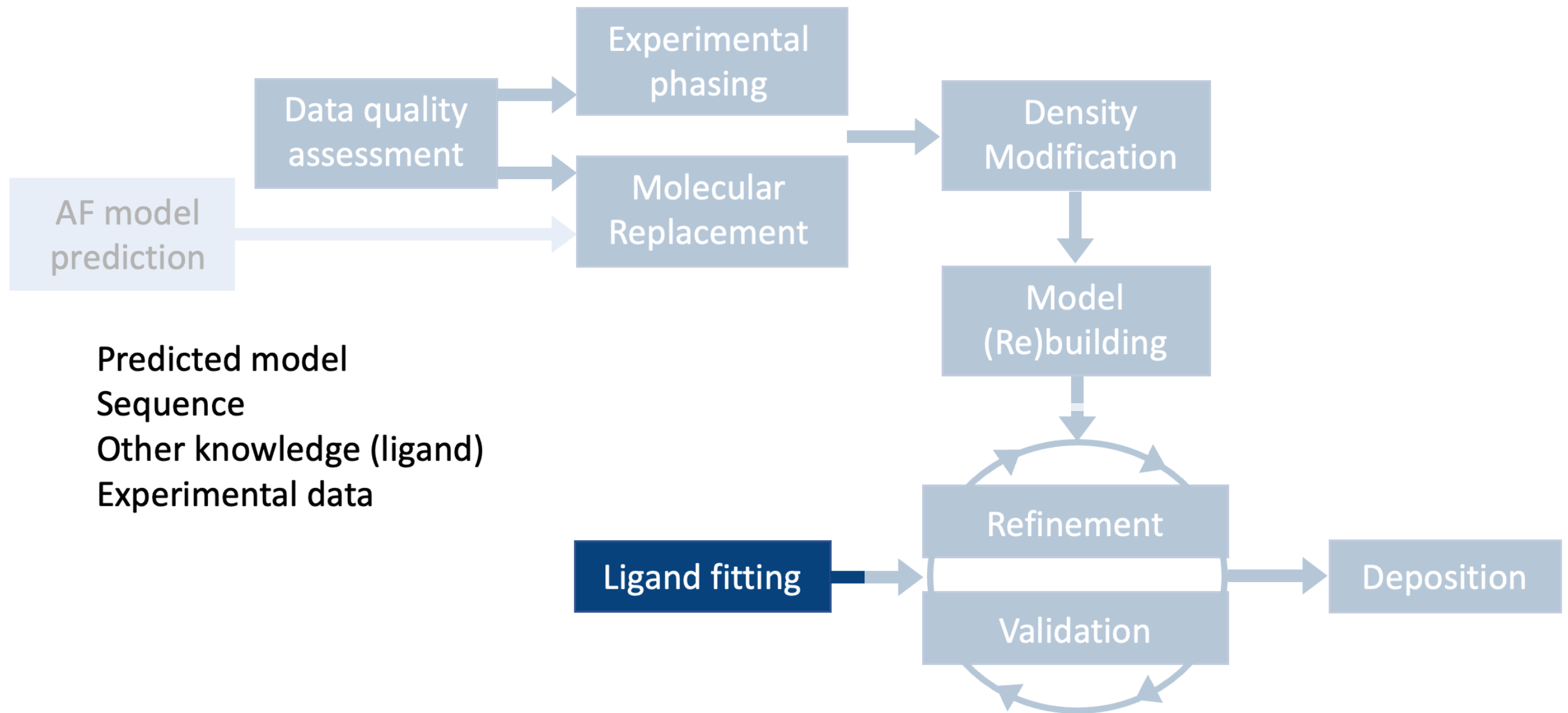
Optimize a model so that it optimally explains the data.

↑
The model should be
already quite “good”.

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



Steps in crystallography



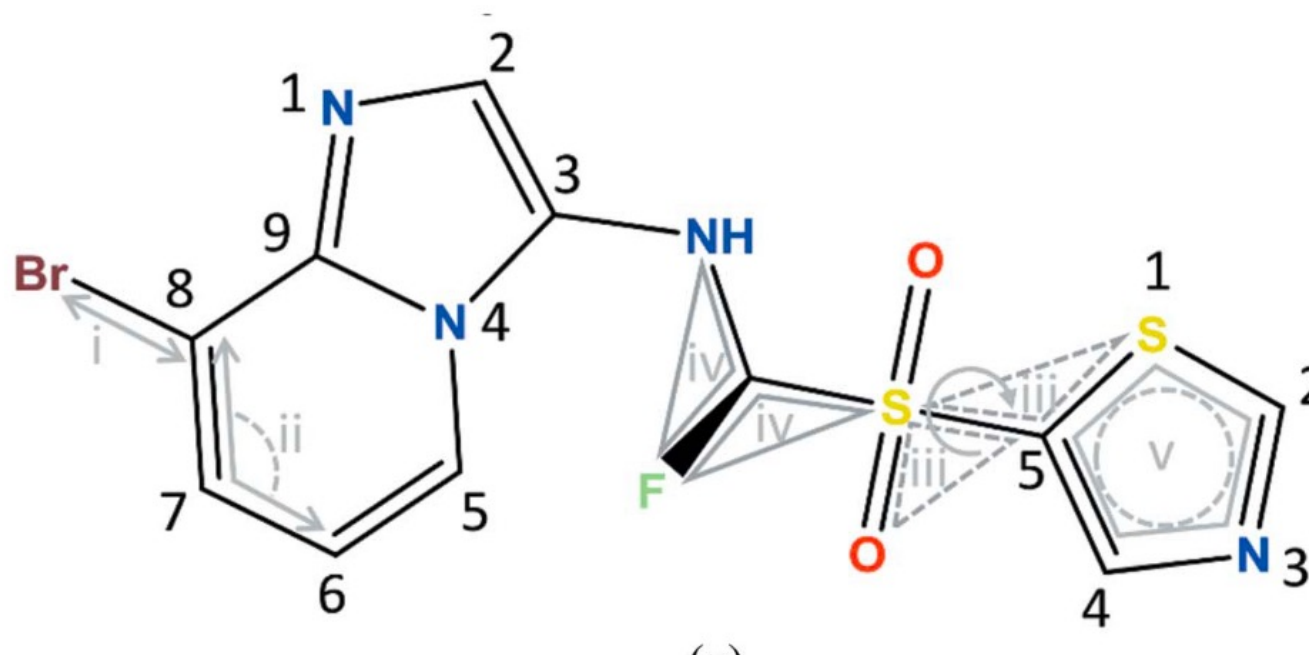
Ligands need restraints

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

Use restraints to obtain chemically plausible structures.

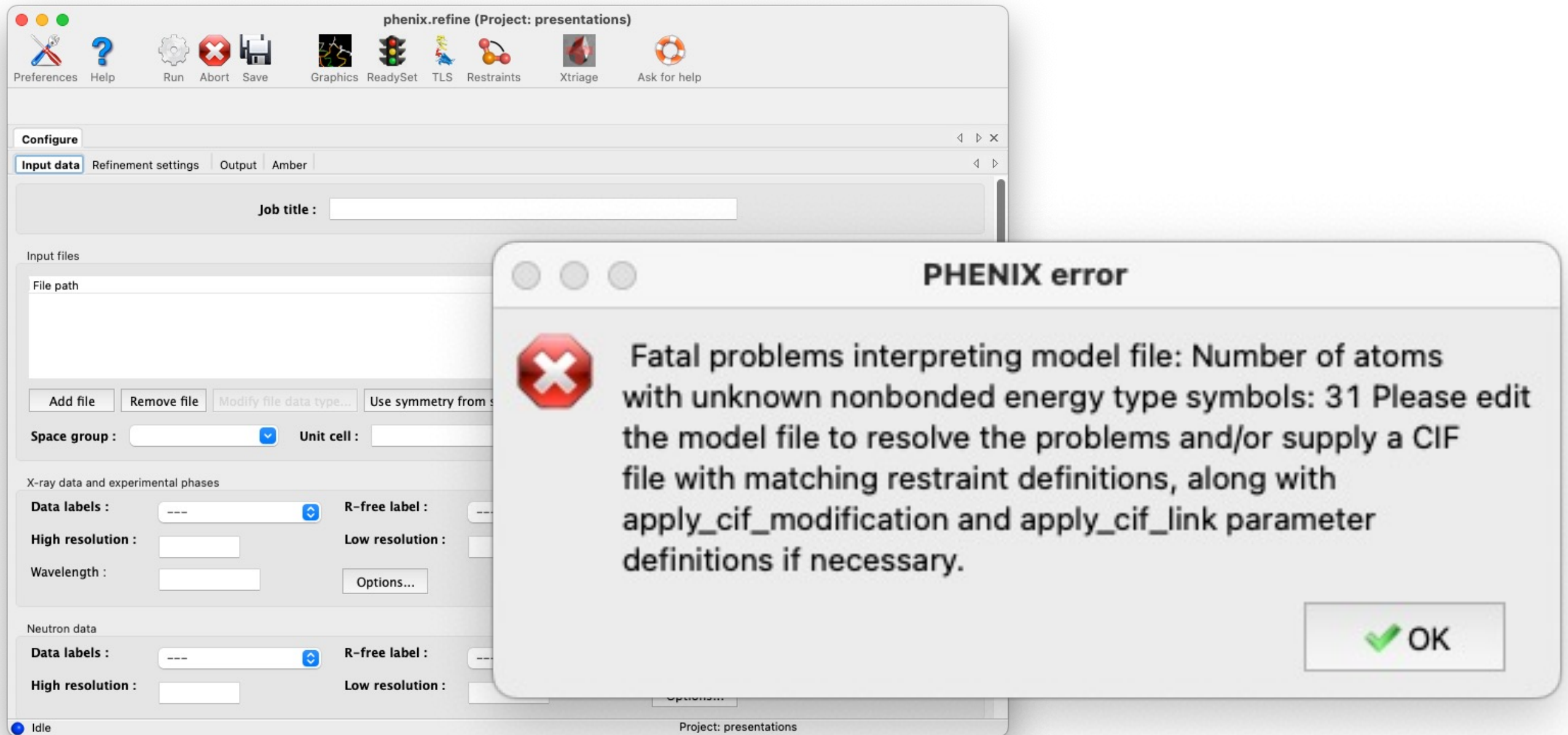
Restraints = prior knowledge

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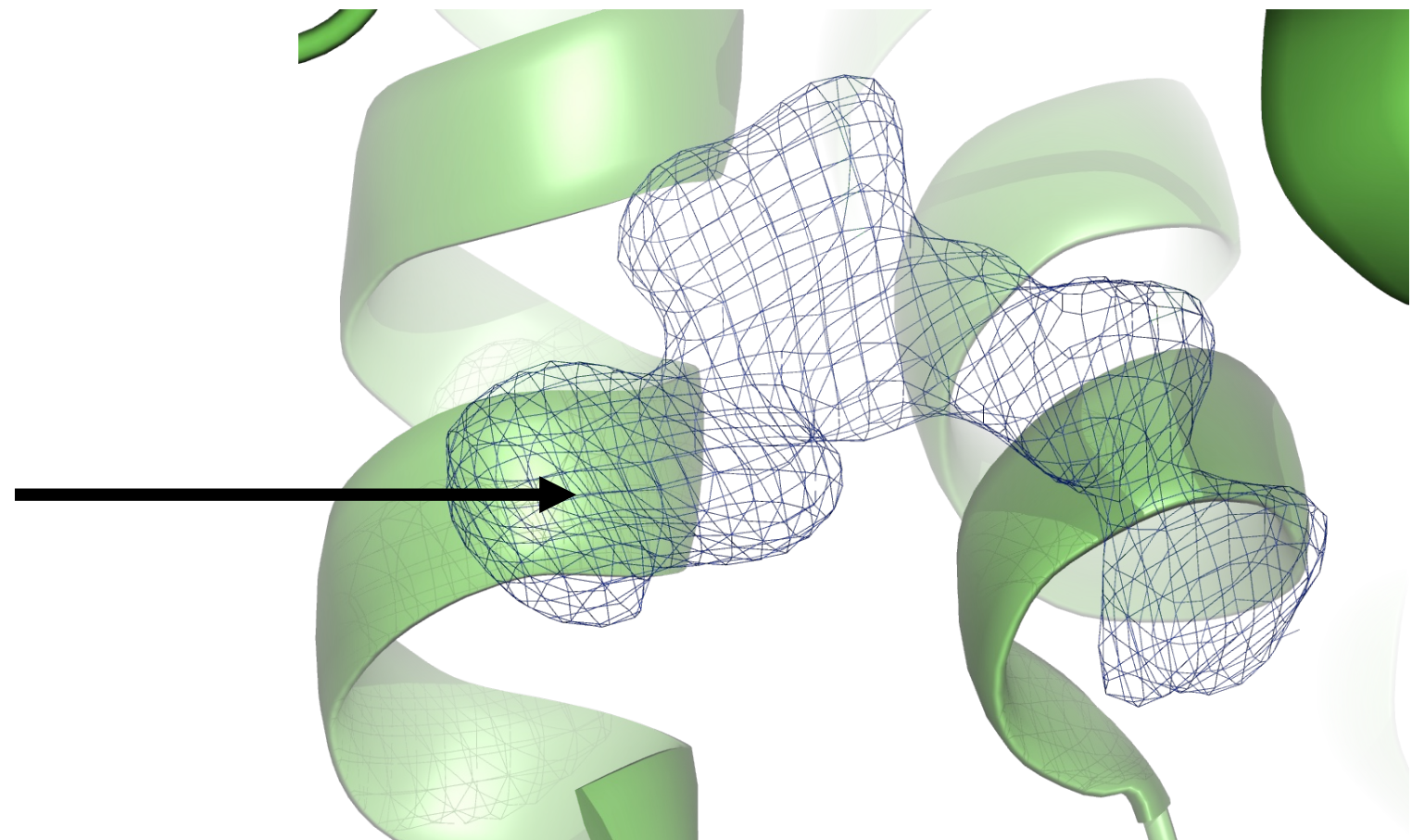
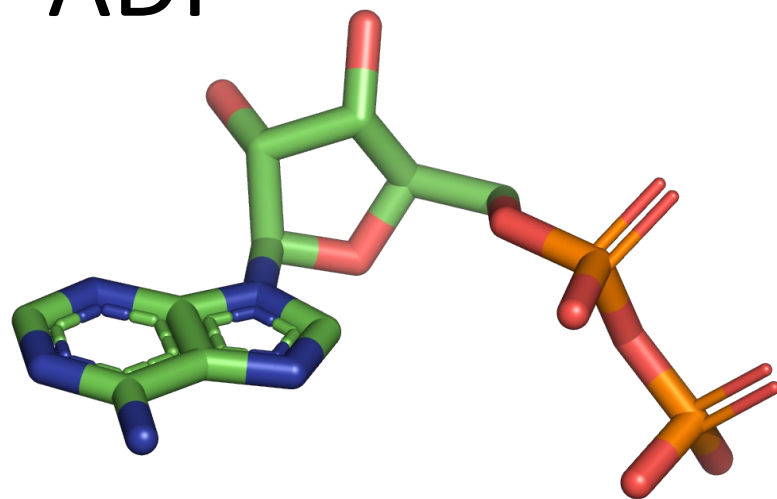
Automated ligand fitting by core-fragment fitting and extension into density

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

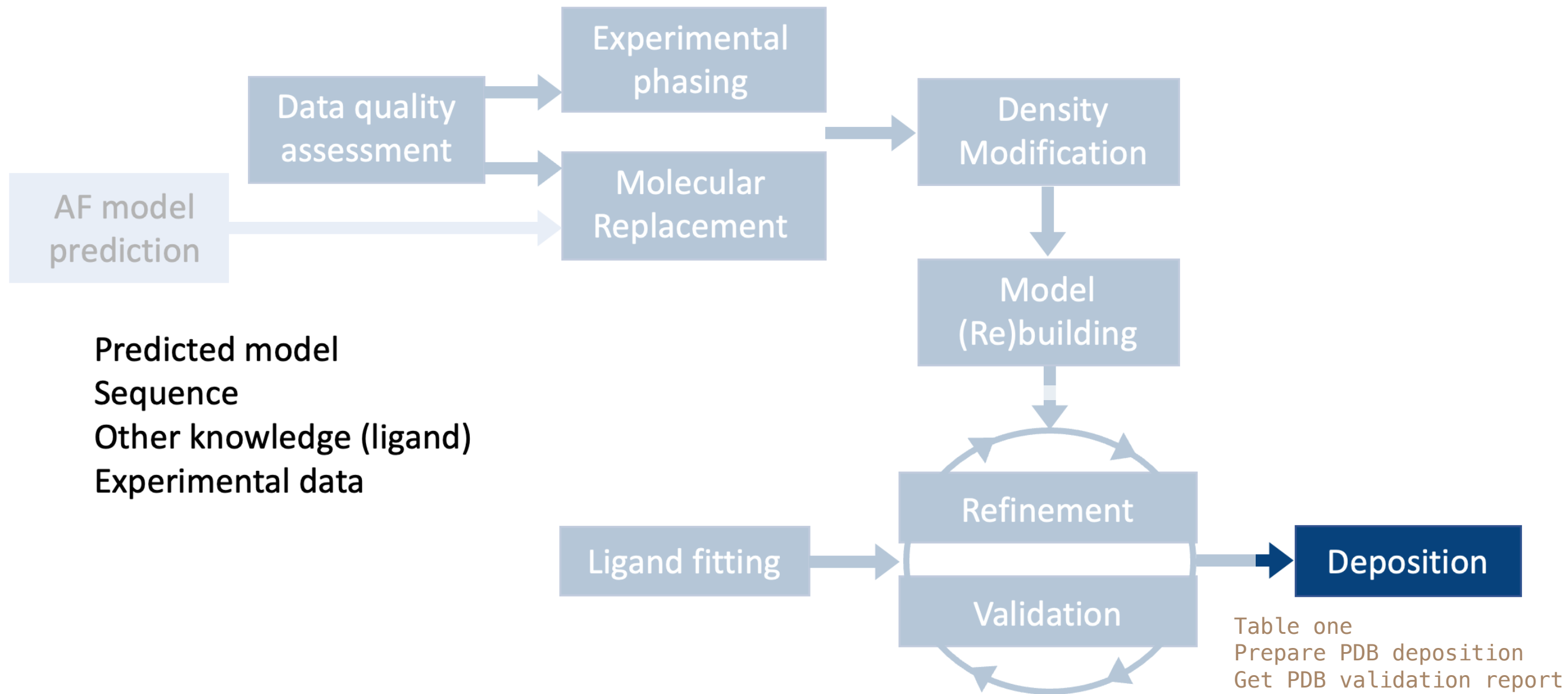
A procedure for fitting of ligands to electron-density maps by first fitting a core fragment of the ligand to density and then extending the remainder of the ligand into density is presented. The approach was tested by fitting 9327 ligands

Received 26 January 2006
Accepted 9 May 2006

ADP



Steps in crystallography



PDB deposition

The screenshot displays the Phenix software interface. At the top, there is a menu bar with icons for Quit, Preferences, Help, Citations, Reload last job, ChimeraX, Coot, PyMOL, KiNG, Tools, Help, and Server. Below the menu bar, there are tabs for 'Actions' and 'Job history'. The main window is divided into two panes. The left pane, titled 'Projects', contains a table with columns for ID, Last modified, # of jobs, and R-free. The right pane shows a list of actions, with 'PDB Deposition' highlighted in orange. Below the 'PDB Deposition' section, there are three sub-items: 'Prepare model for PDB deposition', 'Get PDB validation report', and 'Generate "Table 1" for journal'. The status bar at the bottom shows the current directory and the project name.

ID	Last modified	# of jobs	R-free
✓ AF_POMGNT2_1	Jun 05 2024 11:46...	3	---
bugs	May 30 2024 02:38...	12	---
02_test_comma...	May 24 2024 01:20...	17	---
tests	May 22 2024 11:15...	67	0.2650
AF_bromodomai...	May 16 2024 10:37...	1	---
AF_7mjs_H_Pre...	Mar 19 2024 09:54...	1	---
groel_dock_refine	Mar 19 2024 09:28...	4	---
bugs_playground	Mar 07 2024 04:43...	13	---
fmodel	Feb 28 2024 02:44...	30	---
SEACOAST	Feb 13 2024 01:09...	7	---
AF_7mjs_H_Pre...	Jan 03 2024 10:19 ...	4	---
joint_XN	Nov 02 2023 03:49...	50	0.0989
AF_7mjs_H_Pre...	Apr 13 2023 02:18 ...	20	---
AF_7mjs_H_Pre...	Apr 13 2023 09:35 ...	0	---
AF_POMGNT2_0	Mar 31 2023 07:07...	3	---
AF_POMGNT2	Mar 30 2023 09:07...	6	---
7brm	Mar 17 2023 11:39...	25	---
7mjs_wcsbw	Mar 17 2023 09:31...	33	---
presentation	Mar 15 2023 02:00...	17	---
bughaton	Mar 06 2023 03:23...	8	---
...

Current directory: /Users/dcliebschner/Documents/AF_POMGNT2_1

Phenix version 1.21.1-5286-000

Project: AF_POMGNT2_1

The Project



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Christopher Schlicksup,
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UTHealth

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Duke University

Jane & David Richardson,
Christopher Williams,
Vincent Chen



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Program Project

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