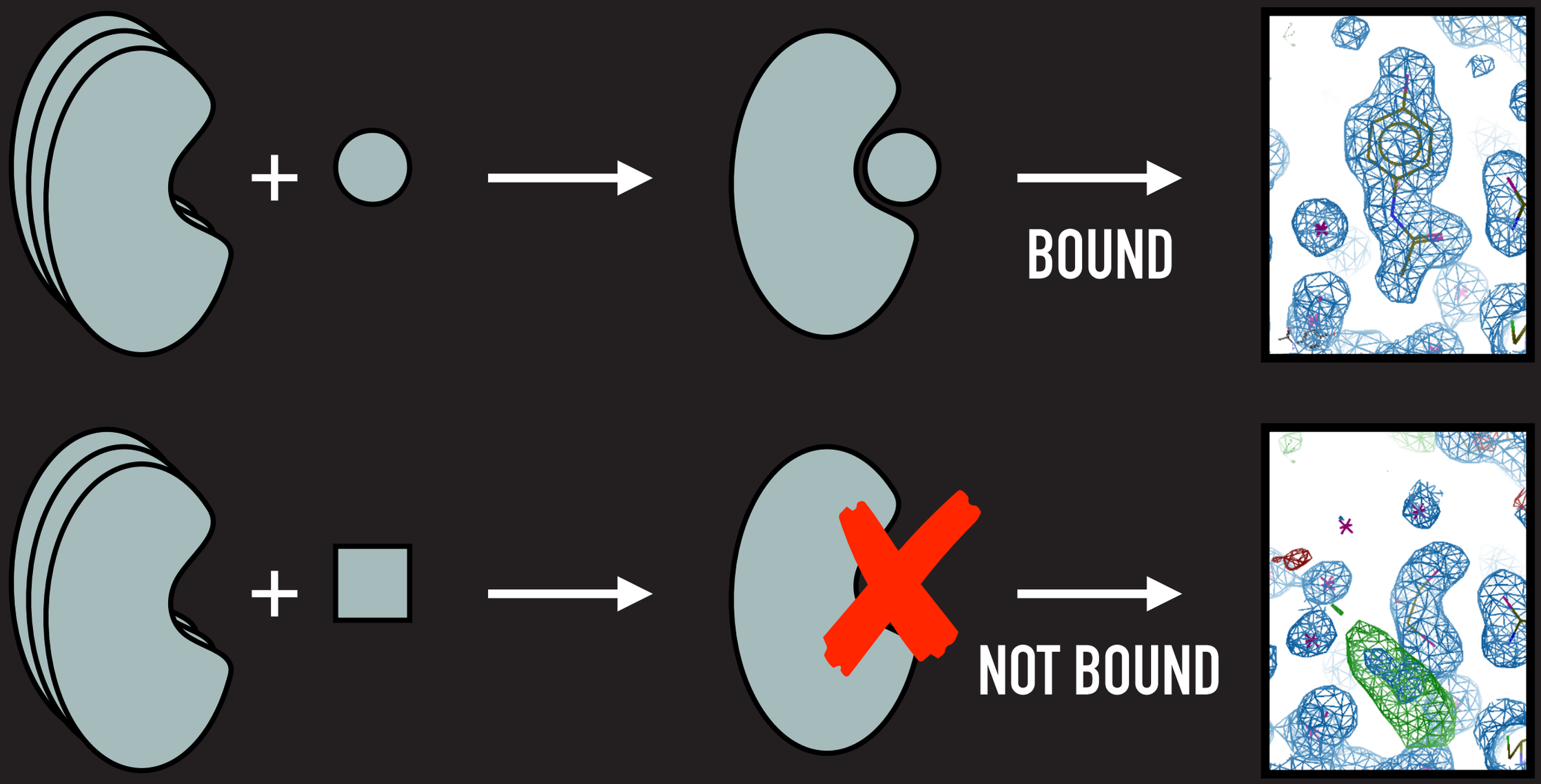
Re-modelling multi-state models

PARTIAL OCCUPANCY FEATURES

LIGAND BINDING IN CRYSTALLOGRAPHY

- Study ligand-binding in protein crystals — does it bind?
- If ligand binds, it appears in the electron density.



PROBLEMS: PARTIAL OCCUPANCY

What if the ligand only binds to a fraction of the crystal?

Crystal:

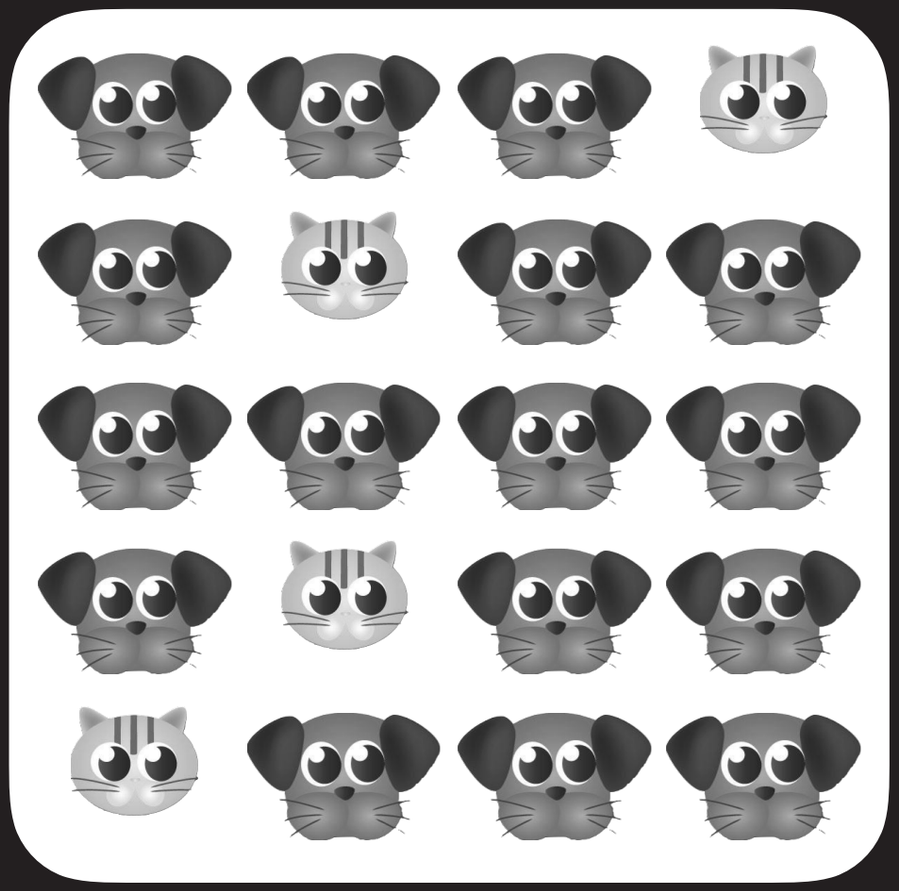
Contains bound (cat) and unbound (dog) states

Diffraction:

Average over the crystal (80% dog + 20% cat)

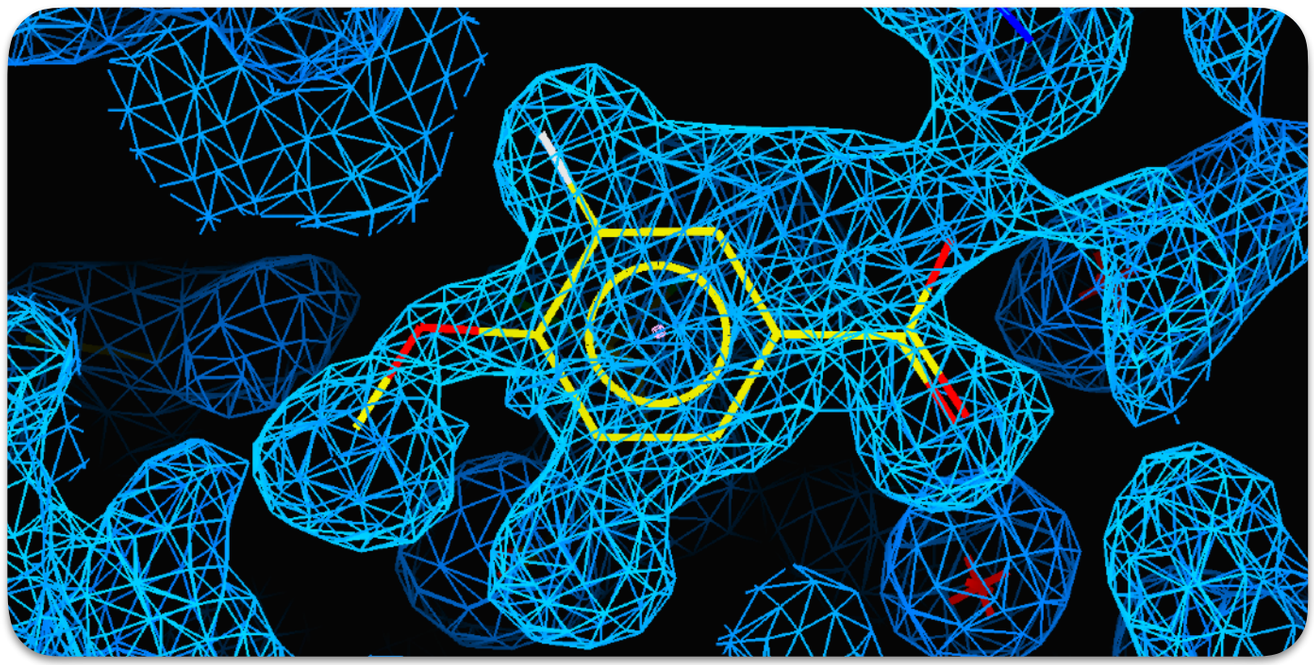
Density:

Looks like the dominant state



EXAMPLE: OBSCURED LIGAND

Standard $2mF_o$-DF_c Map (1.39Å; 0.3rmsd)



Normal density maps show only the superposition of the full crystal.

They are not useful for identifying partial occupancy features.

REVISITING PARTIAL OCCUPANCY

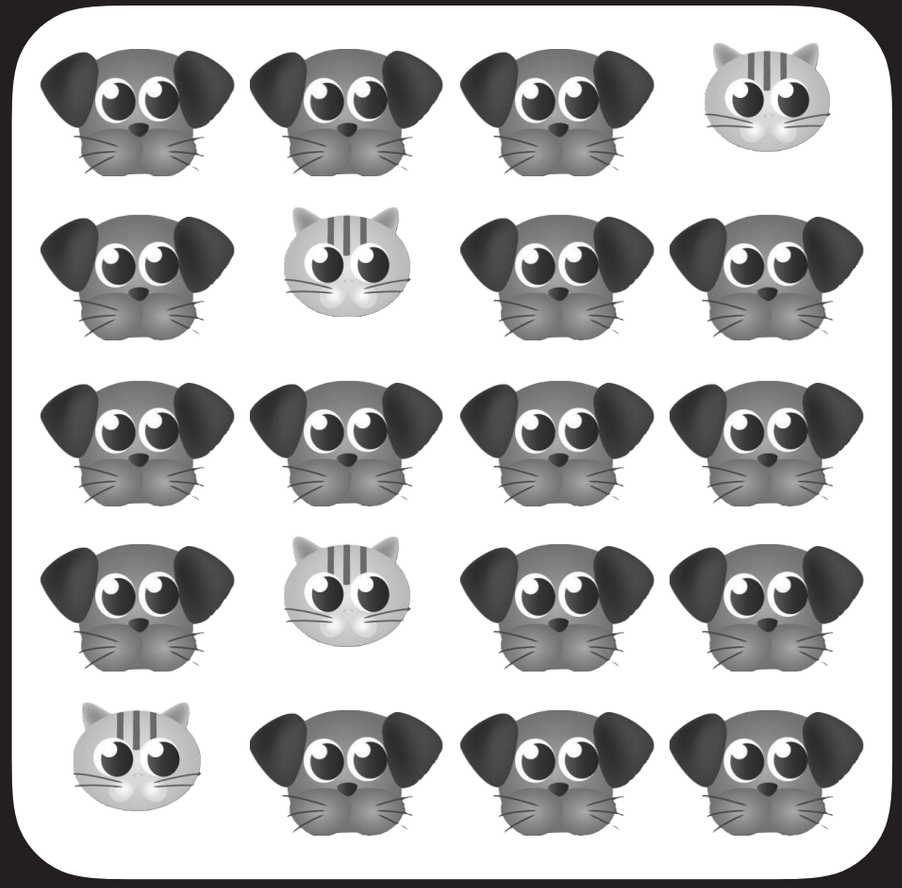
The ligand is bound to a fraction of the crystal:

80% dog + 20% cat

The model should reflect the crystal content:

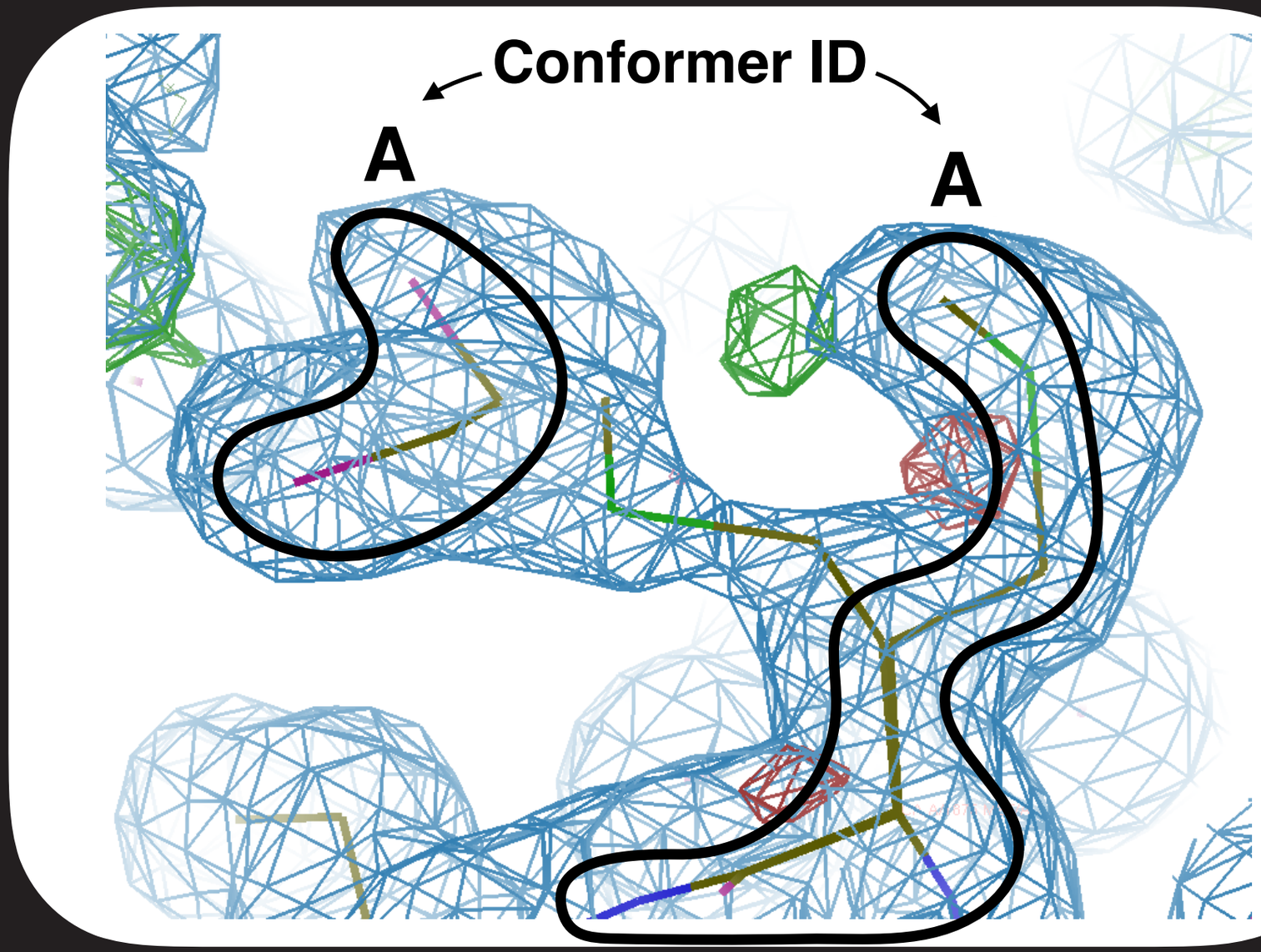
80% dog + 20% cat

or 20% dog + 80% cat !



NOTE ON MULTI-CONFORMER MODELS

- Crystallographic density is an average over many states
- “Alternate conformers” model these different crystal conformations



$2mF_o$-DF_c
blue
@ 1rmsd

mF_o-DF_c
green/red
@ ±3rmsd

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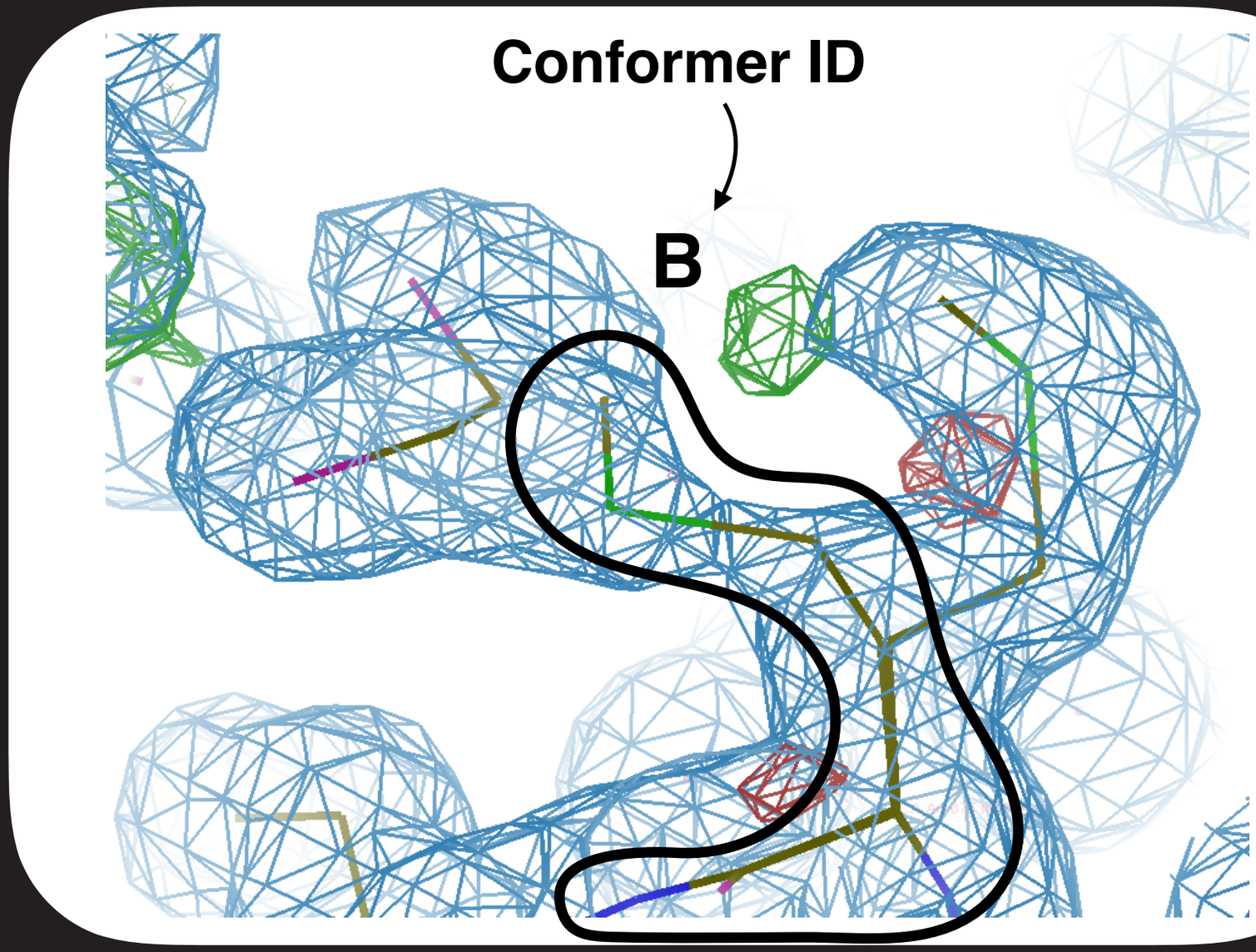


$2mF_o$-DF_c
blue
@ 1rmsd

mF_o-DF_c
green/red
@ ±3rmsd

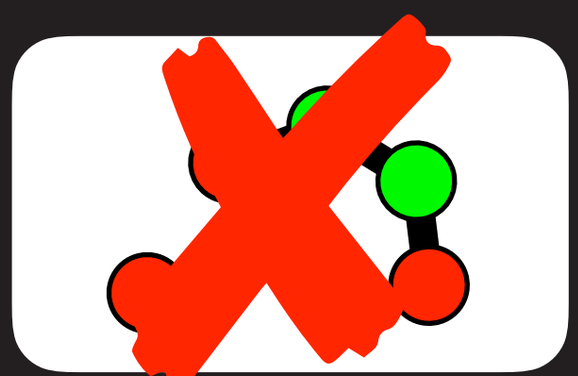
MULTI-STATE REFINEMENT PROTOCOL

From PanDDA ⇝ Multi-state models

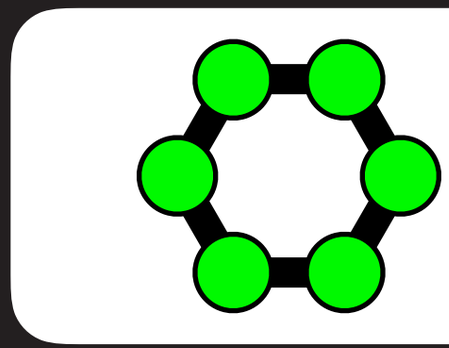
PARTIAL-OCCUPANCY MODELLING & REFINEMENT

NORMAL APPROACH

DELETE INPUT MODEL



MODEL LIGAND



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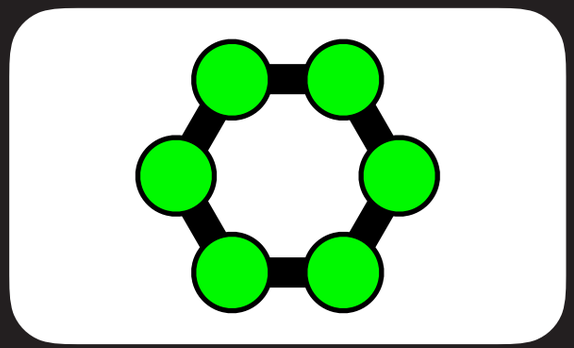
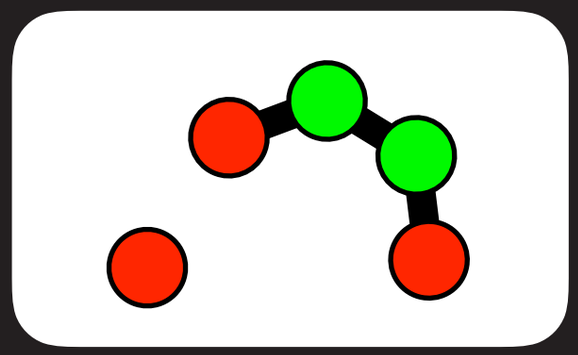
FINAL MODEL



CORRECT APPROACH

COMBINE INPUT MODEL AND LIGAND MODEL



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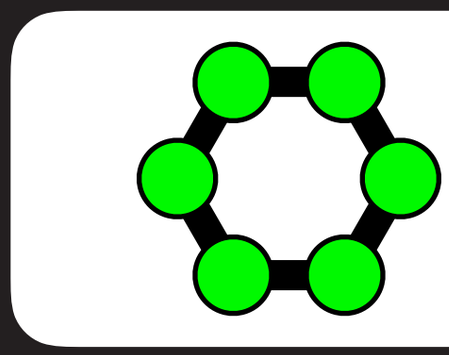

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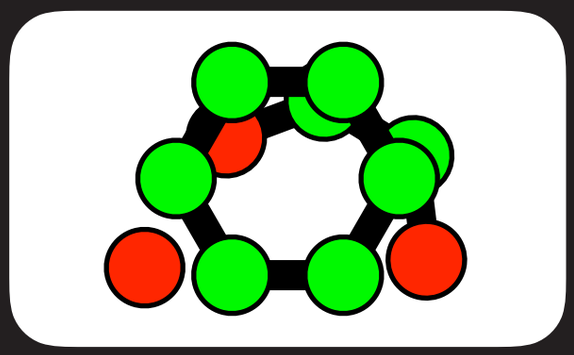
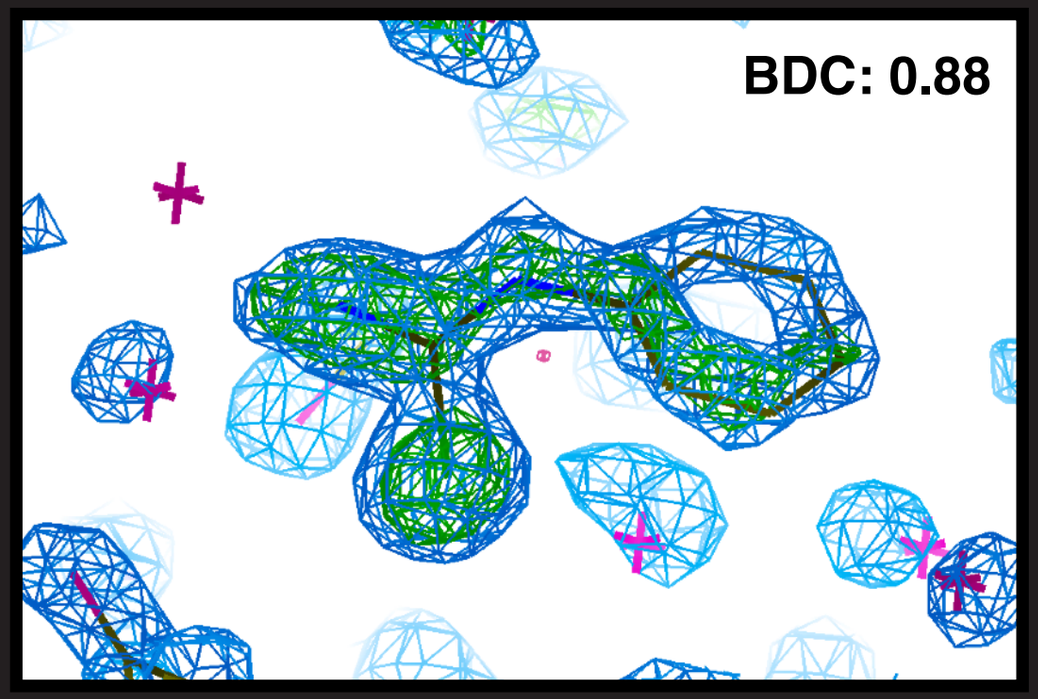
ENSEMBLE MODEL



PARTIAL-OCCUPANCY MODELLING & REFINEMENT

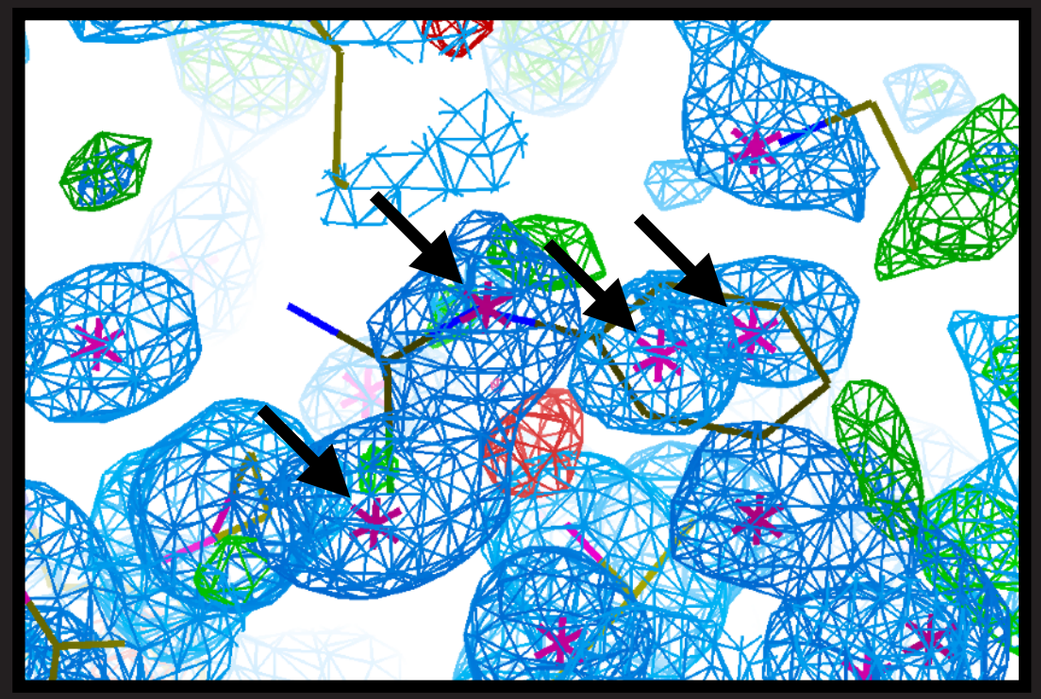
Resolution: 1.39Å; Occupancy: 0.23

PANDDA



EVENT (blue, 2rmsd)
ZMAP (green/red, ±4)

REFINED

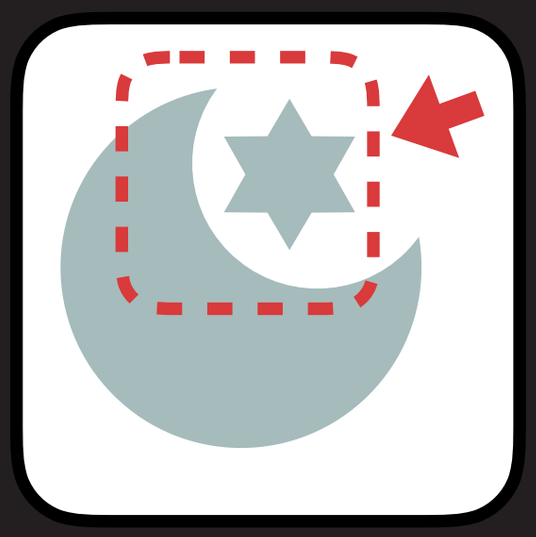


2FOFC (blue, 0.5rmsd)
FOFC (green/red, ±3rmsd)

- Merging the solvent model from another dataset creates a good model
- Ligands do not “appear” in the maps after refinement (nor should they!)

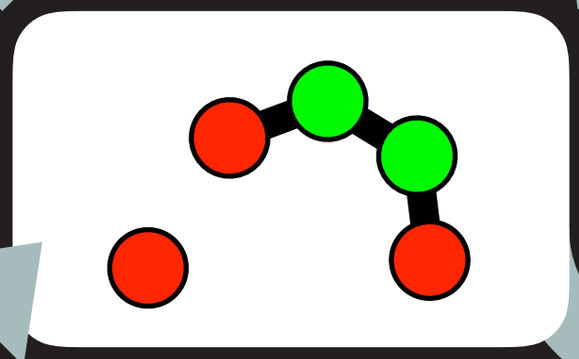
MULTI-STATE REMODELLING

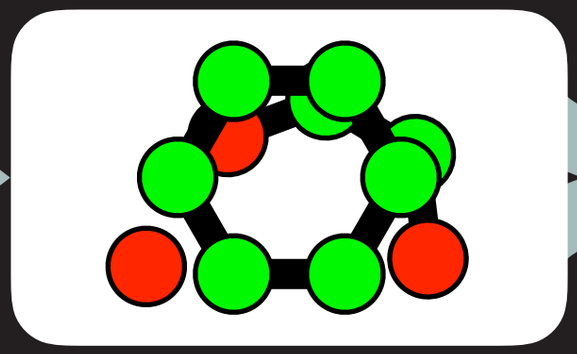
MULTI-STATE MODELLING PROTOCOL



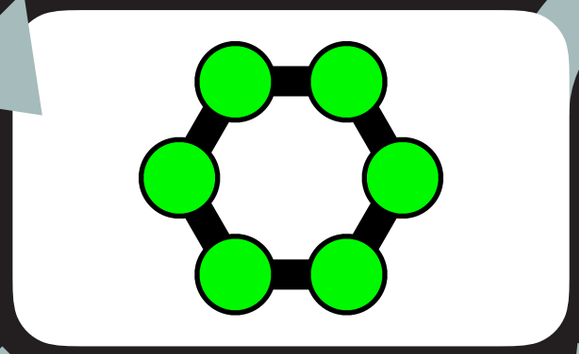
Modelling the ligand-bound region

(re)model “apo” state
— ground-state map
from pandda

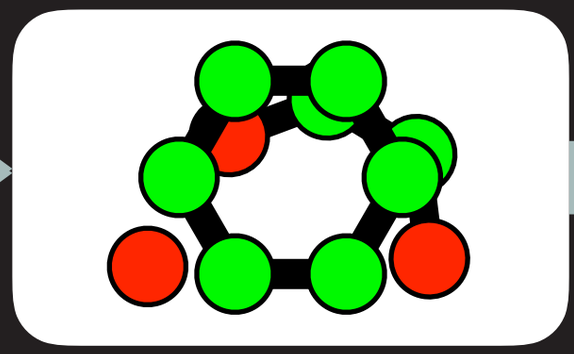




split structure from
pandda.inspect or
refinement (automated)

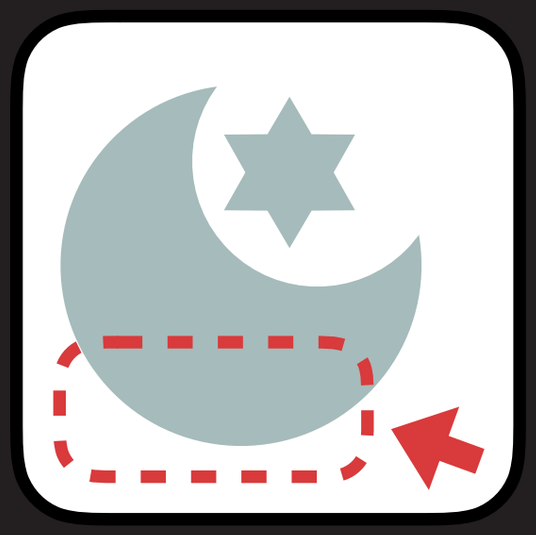


(re)model bound
state — event maps



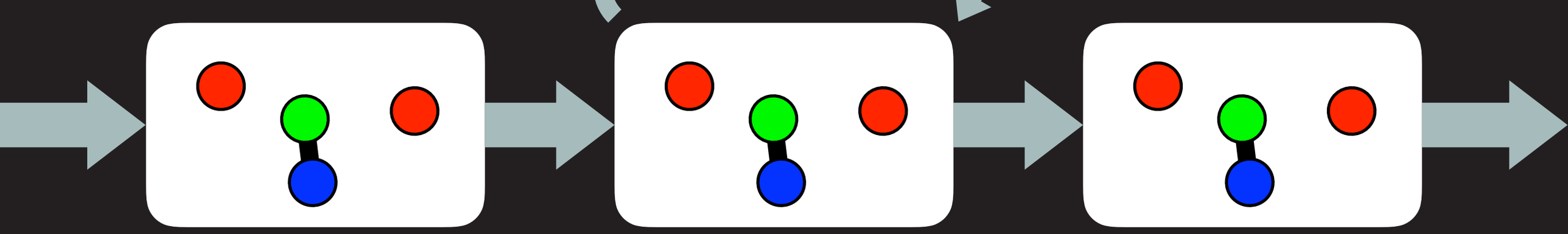
remerge the
two structures
(automated)

MULTI-STATE MODELLING PROTOCOL



Modelling a conserved ground-state region

Normal modelling
(refined maps)



XCHEMEXPLORER OVERVIEW

XChem Explorer

Diffraction Data Review

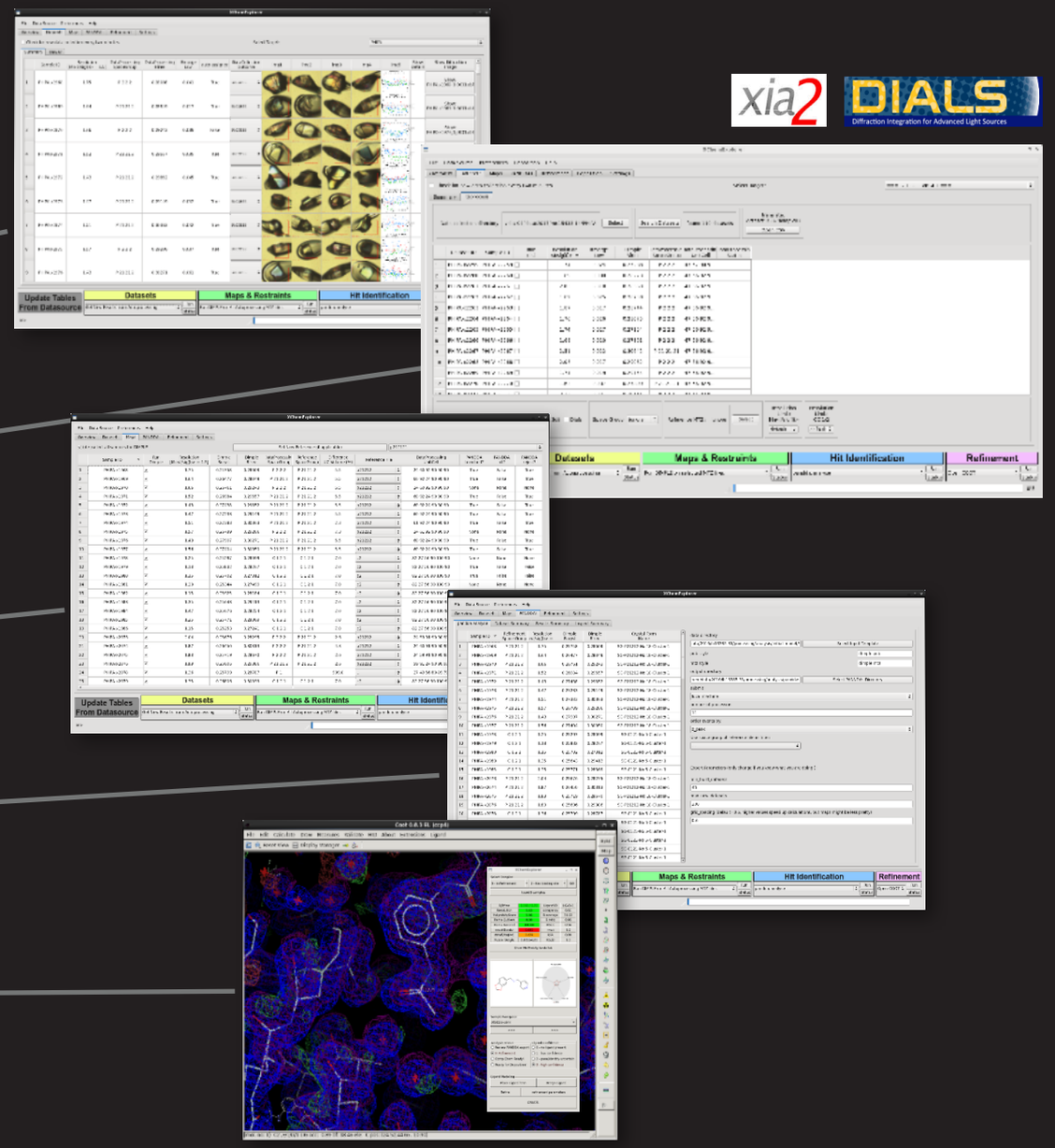
Diffraction Data Processing

Initial Refinement + Restraints

Hit Identification (PanDDA)

Modelling + Refinement

WORLDWIDE PDB PROTEIN DATA BANK + deposition !

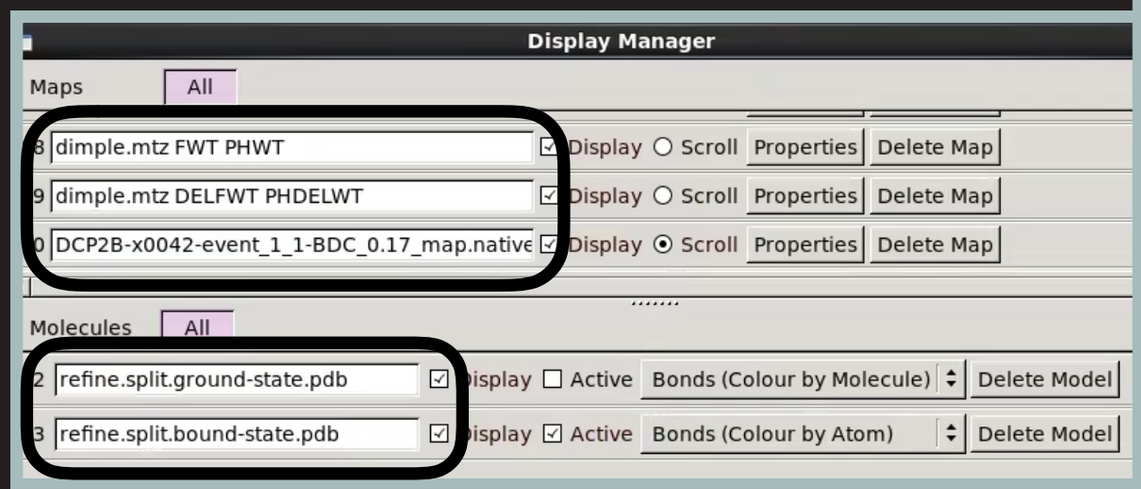


Krojer, T. et al. (2017) 'The XChemExplorer graphical workflow tool for routine or large-scale protein–ligand structure determination', Acta D Cryst.

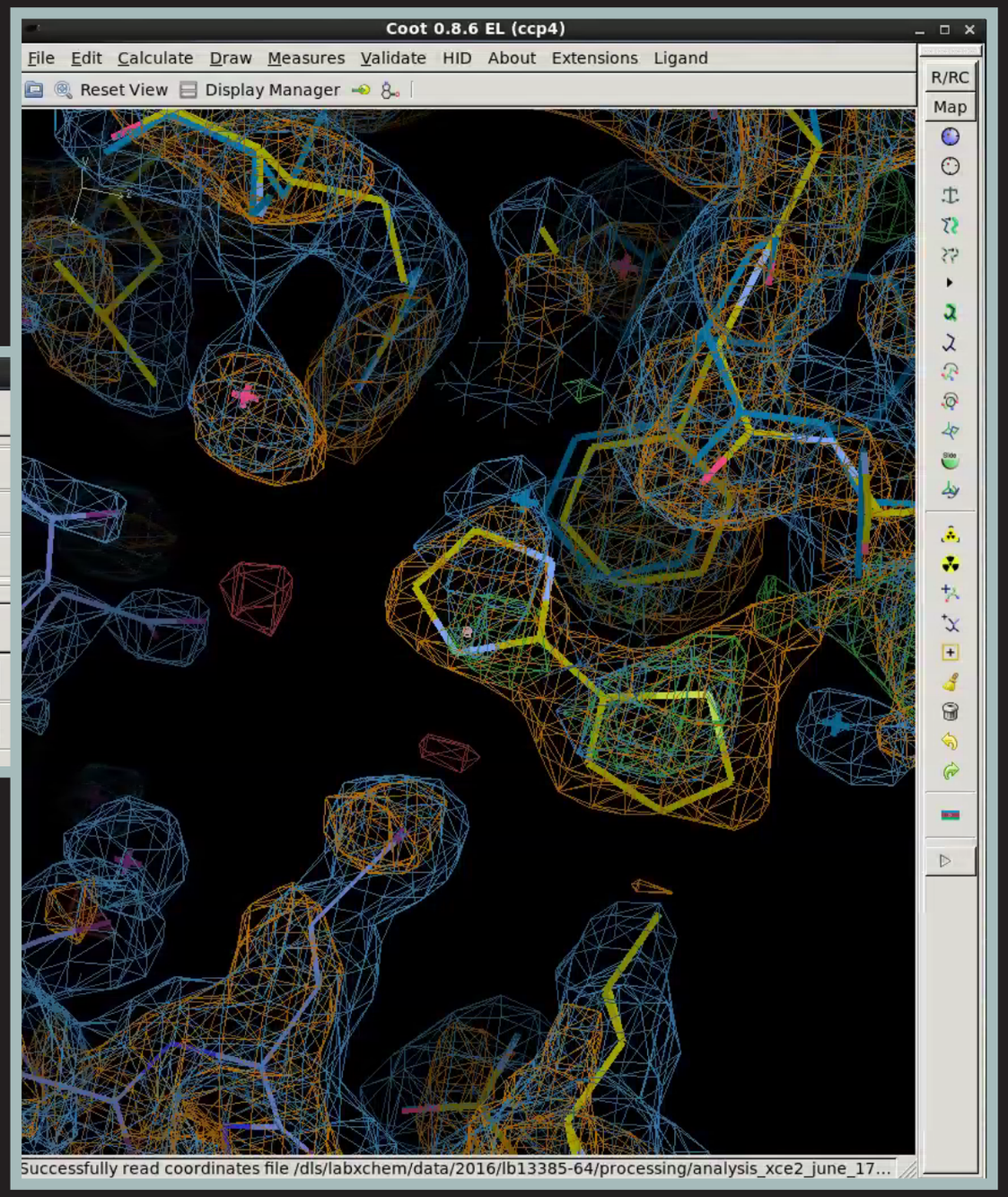
...IN XCHEM EXPLORER

modelling becomes more complex

MULTIPLE MAPS



TWO STRUCTURES



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