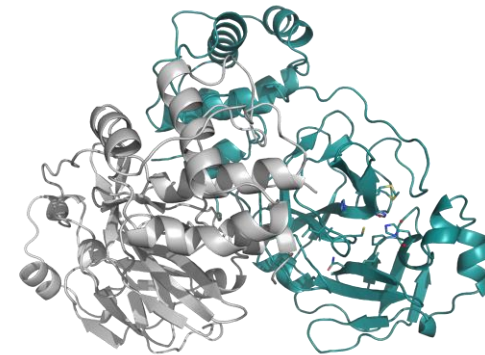




Ligand Fitting with CCP4 Cloud & Coot Tutorial

Tutorial – Introduction



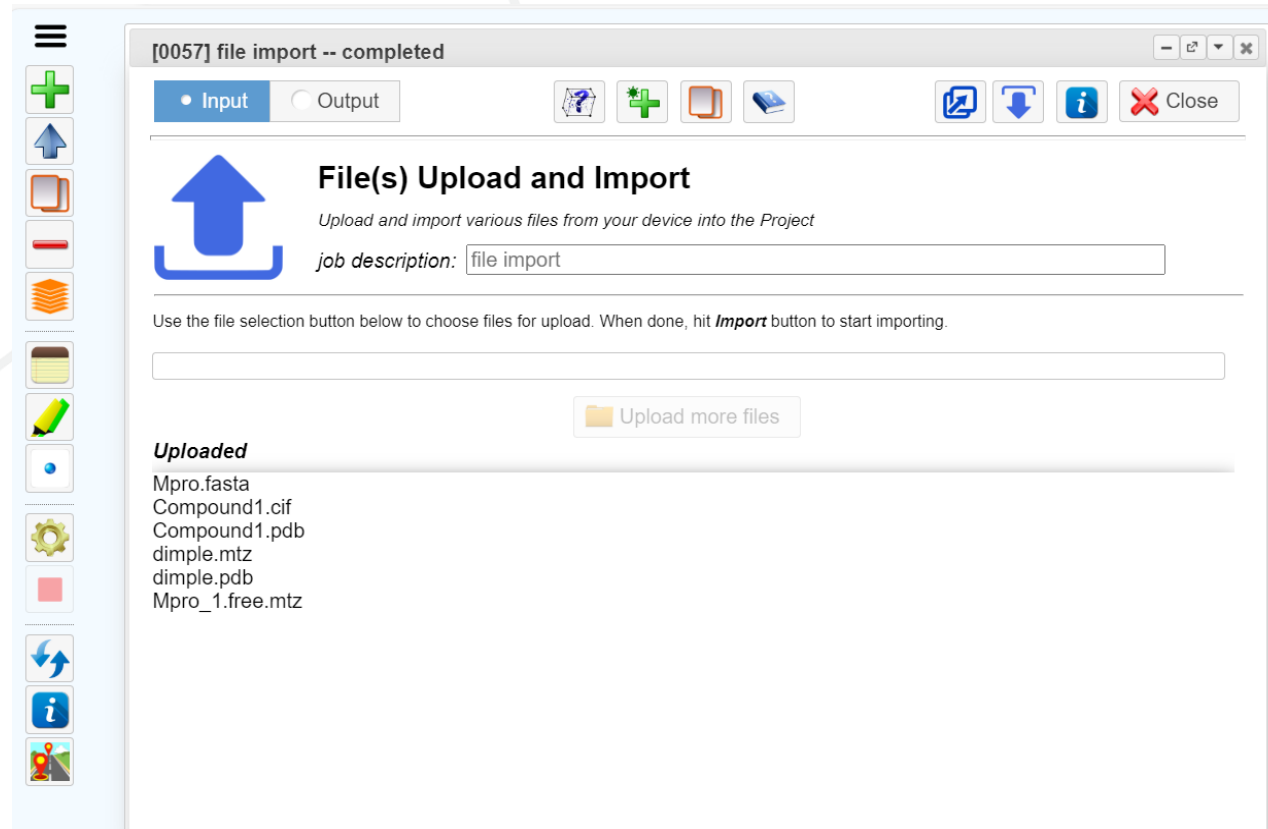
- SARS-CoV-2 Main protease (Mpro) is one of two cysteine proteases that are essential for viral replication, making it an attractive drug discovery target for the treatment of COVID-19.
- We have provided X-ray diffraction data collected from crystals soaked with inhibitors from the COVID Moonshot – a crowdsourced open drug discovery initiative
- In this tutorial we will identify the ligand binding site, generate ligand restraints, model the ligand, refine and validate our model
- Files:
 - DIMPLE output (pdb/mtz)
 - Reflection data (mtz)
 - SMILES string
 - Ligand coordinates (pdb) and restraints (cif)

Tutorial – Part 1: Ligand fitting in CCP4 Cloud

- Copy data: /dls/i04/data/2024/mx39148-1/processing/DLS-CCP4_Covid_Moonshot
- You will see four folders Mpro_1/2/3 (**ignore 4 for now**)
- Login to CCP4 Cloud (cloud.ccp4.ac.uk) and create a new project called:
 - CCP4_Covid_Moonshot (Specify what you want the name and ID to be)

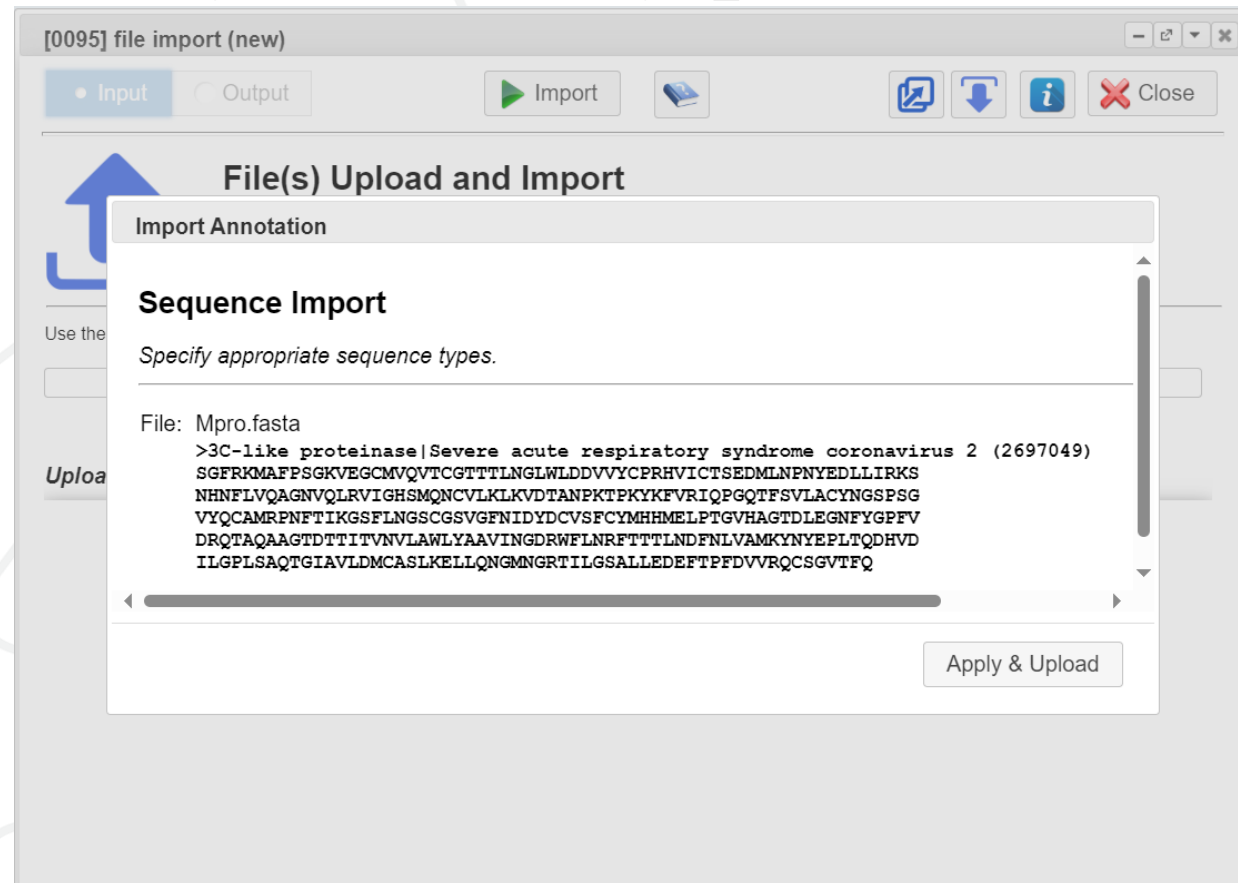
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

1. Import files for respective folder Mpro_1, Mpro_2, Mpro_3
 - You can import all files from Mpro_1



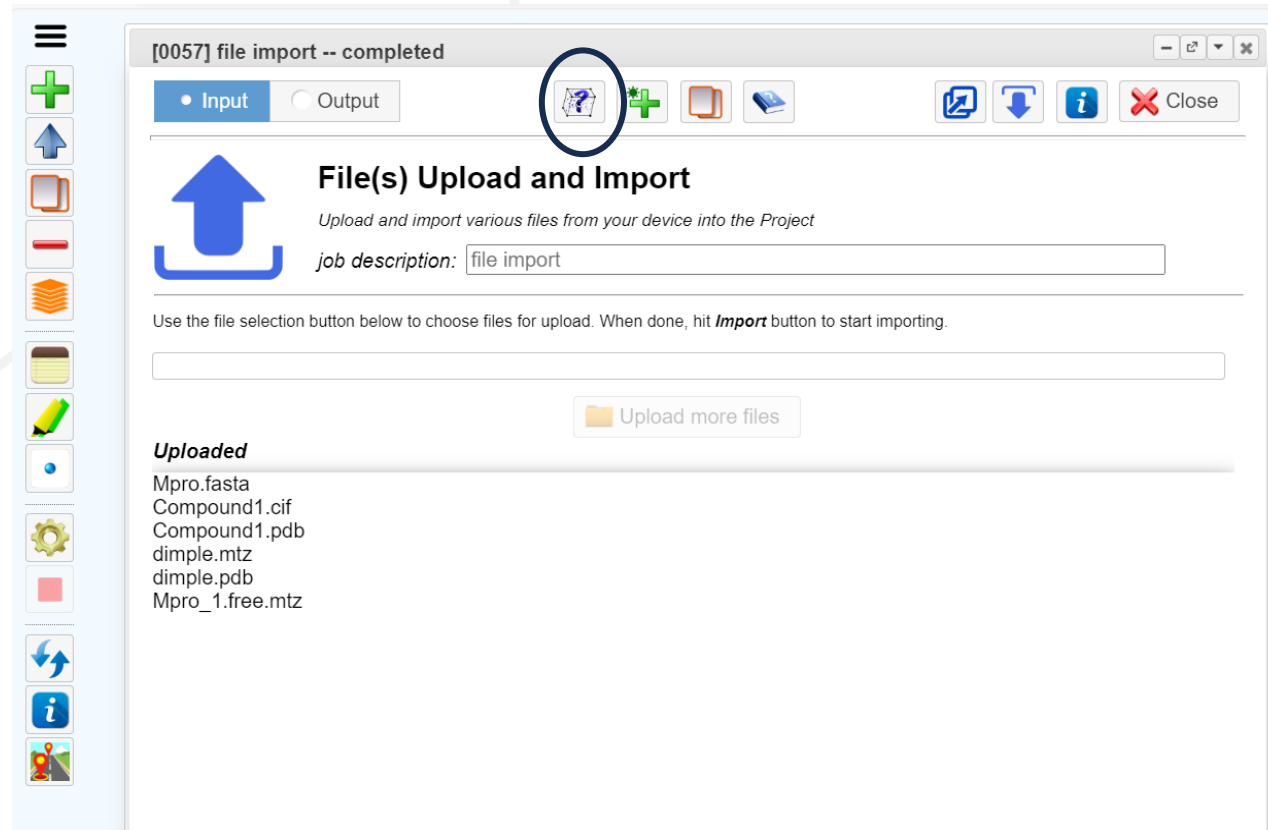
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

1. Import files for respective folder Mpro_1, Mpro_2, Mpro_3
 - You can import all files from Mpro_1



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

2. Define ASU contents



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

3. Run dimple-MR

[0058] define asymmetric unit contents -- completed

Input Output

Report Main Log Service Log Errors

CCP4 v.9.0.004; CCP4 Cloud v.1.8.002
Started: 2024-11-26 11:37:31
Finished: 2024-11-26 11:37:31
CPU: 00.004, Disk: 0.04M

[0058] Asymmetric Unit Contents

User-suggested ASU contents (hypothesis)

| | N_{copies} | Structural unit components | Type | Size | Weight |
|------------------------|---------------------|-----------------------------------|---------|------|---------|
| 1 | 1 | [0057-06] Mpro /sequence/protein/ | PROTEIN | 306 | 33778.8 |
| Total residues/weight: | | | | 306 | 33778.8 |

[0058] Results

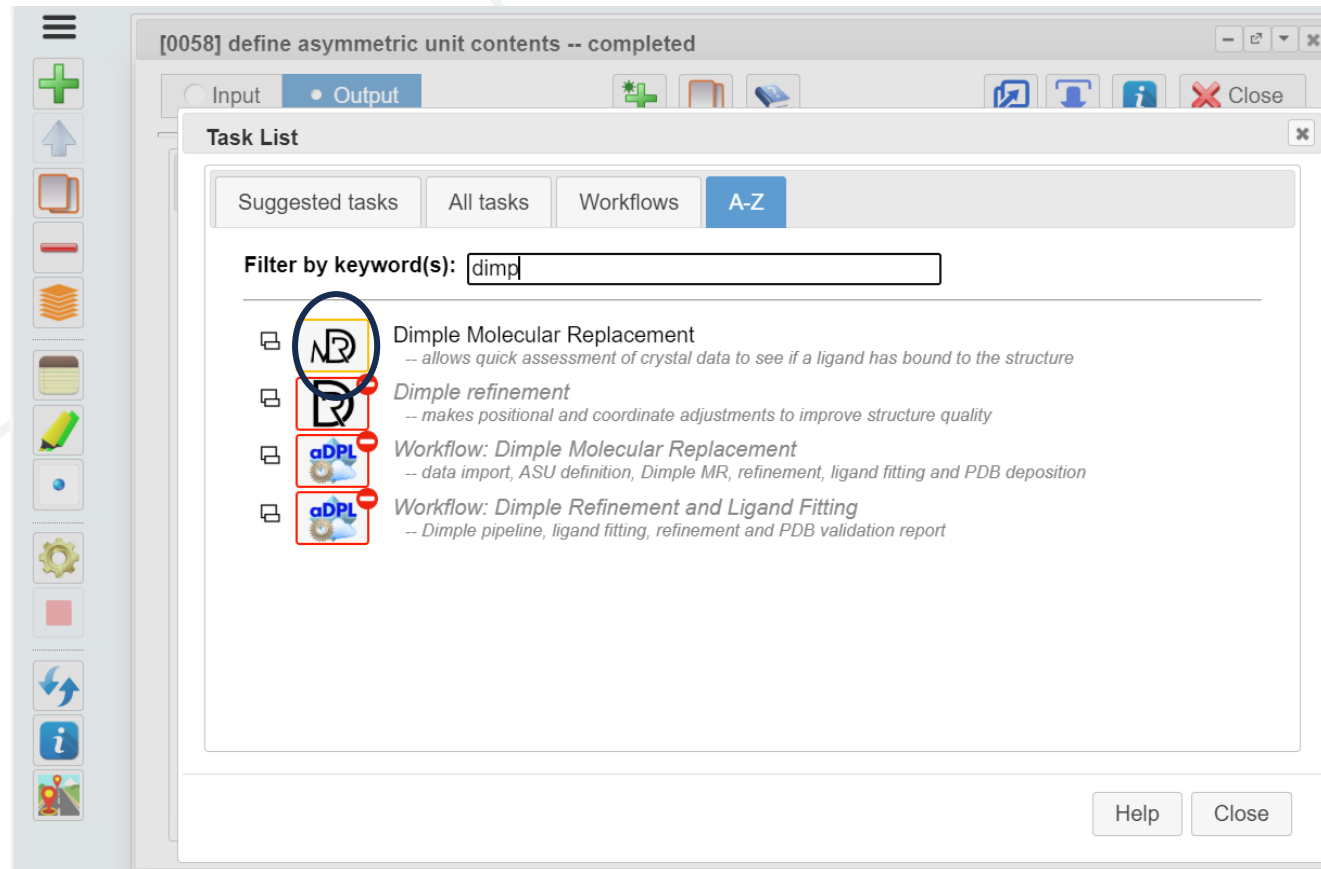
Cell volume: 258878.078 Å³

Molecule fitting statistics

| N_{mult} | Matthews | % solvent | P_{matthews} |
|-------------------|----------|-----------|-----------------------|
| * 1 | 1.92 | 35.84 | 1.000 |

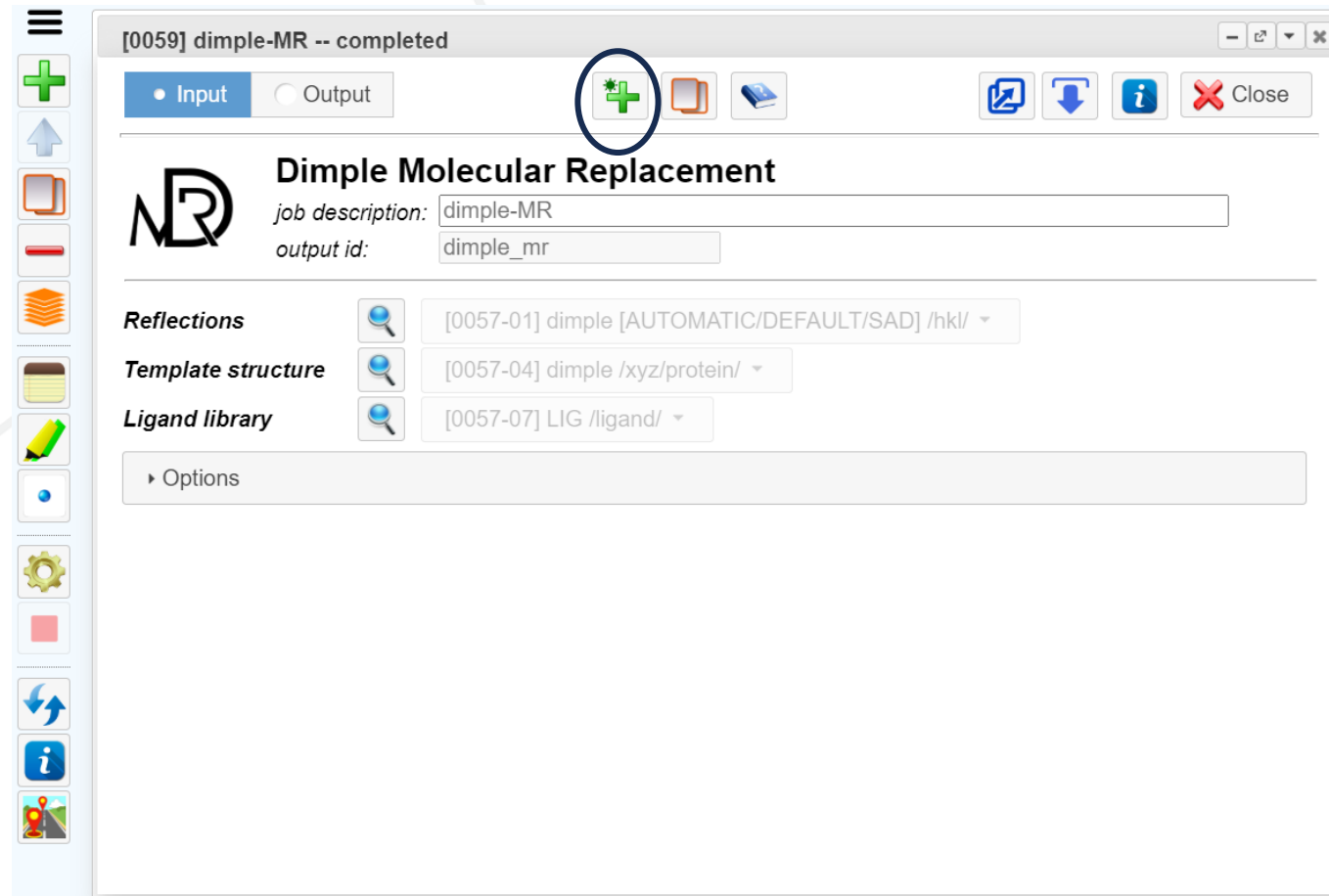
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

3. Run dimple-MR



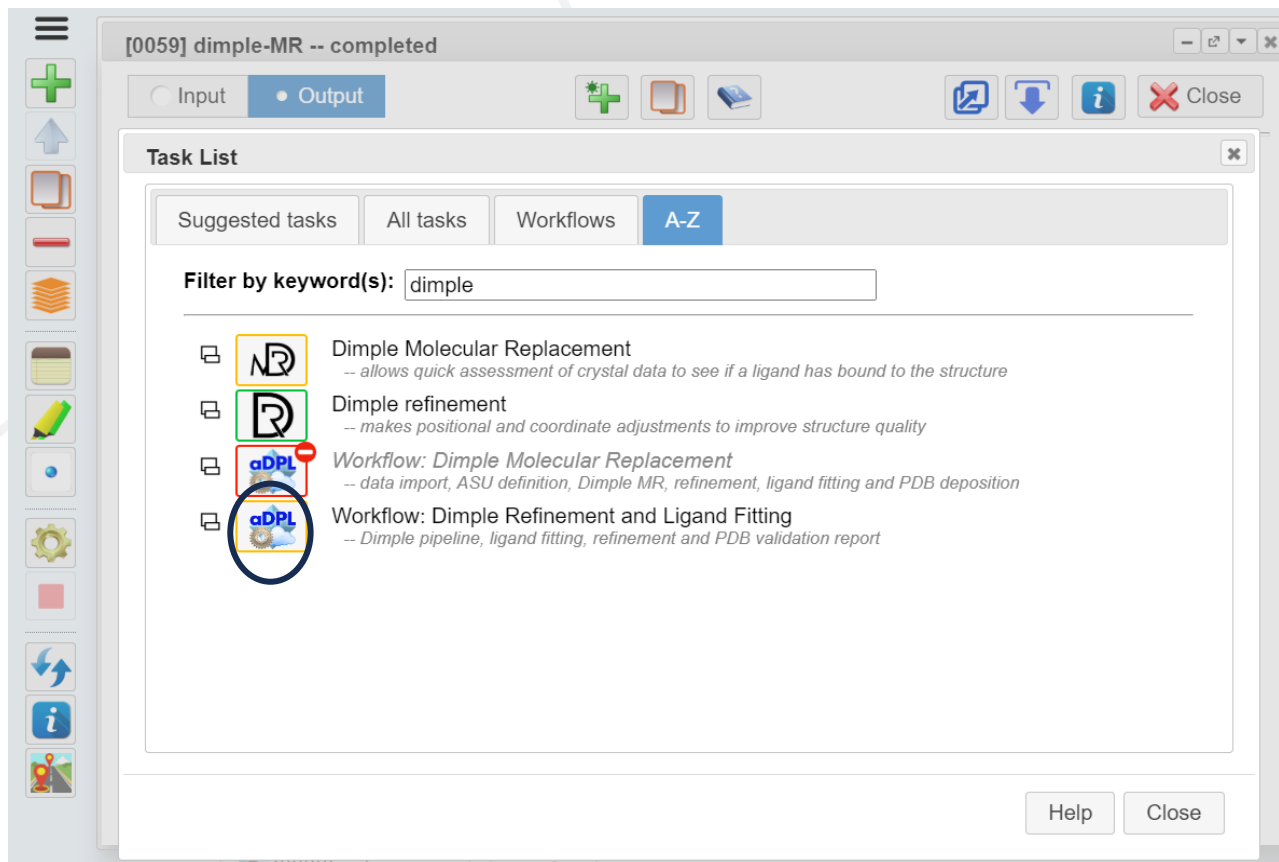
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

4. Run auto-DPL (refinement and ligand fitting)



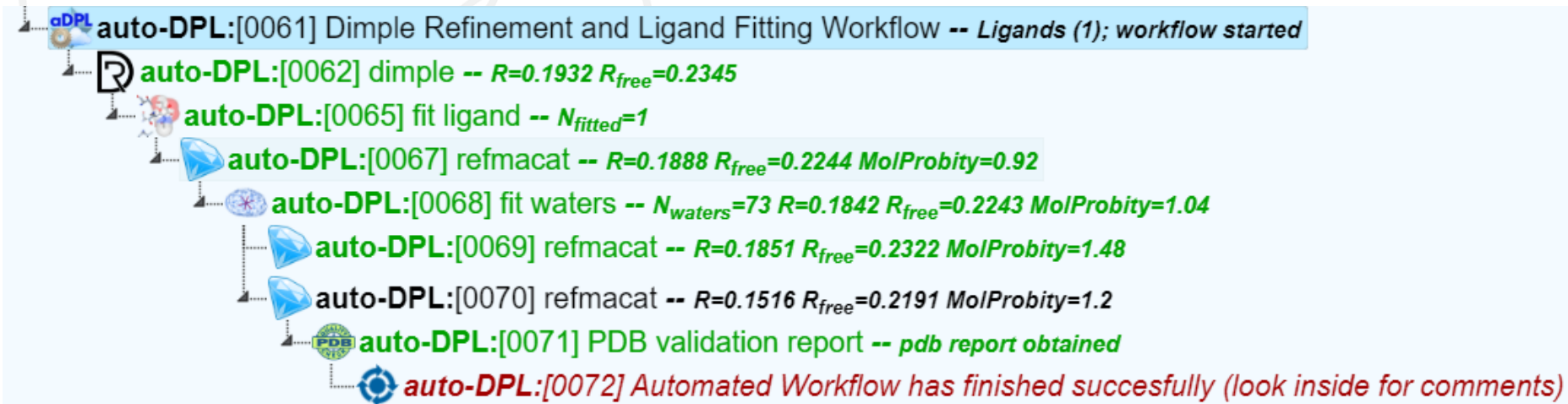
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

4. Run auto-DPL (refinement and ligand fitting) - this requires the ligand and its generated restraints which we already have.



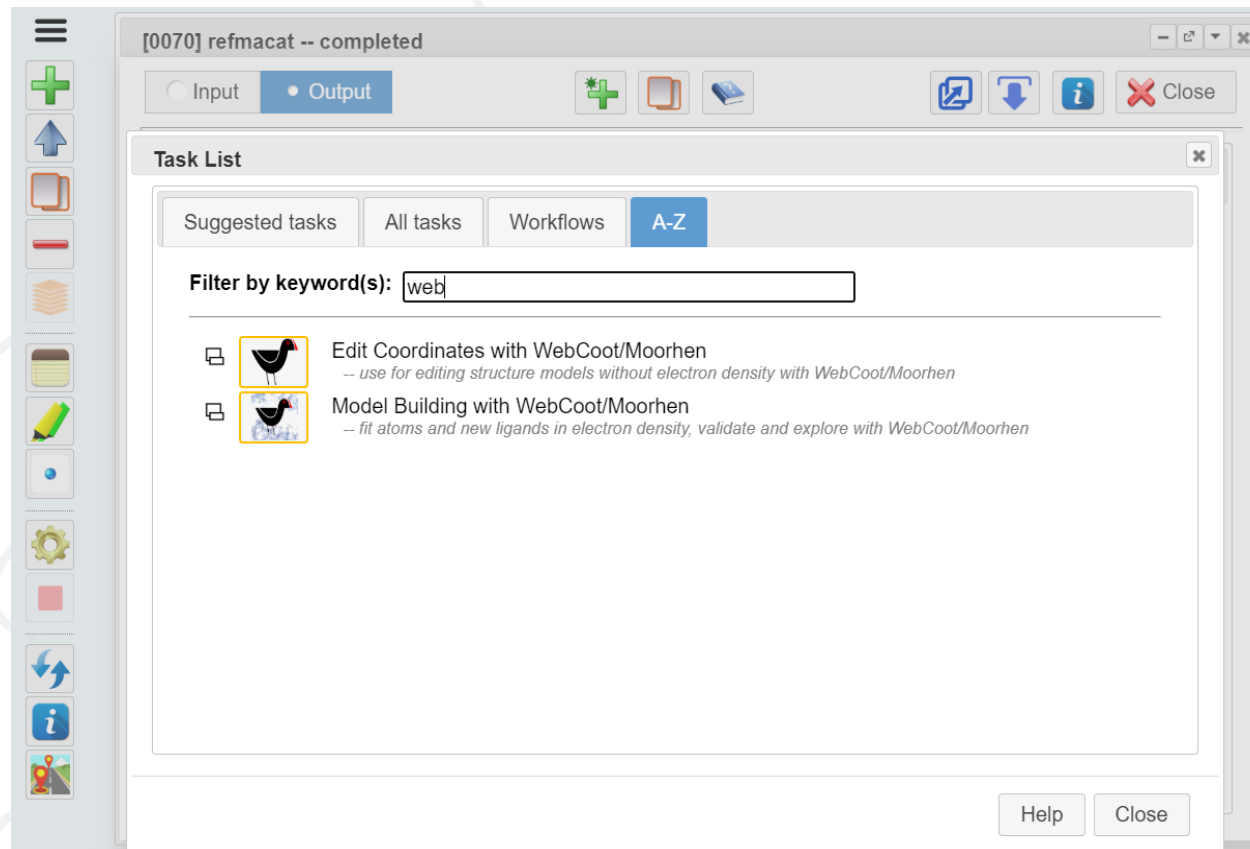
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

4. Wait a few minutes for the workflow to complete (in the meantime start uploading the next Mpro data under a separate job in the project)



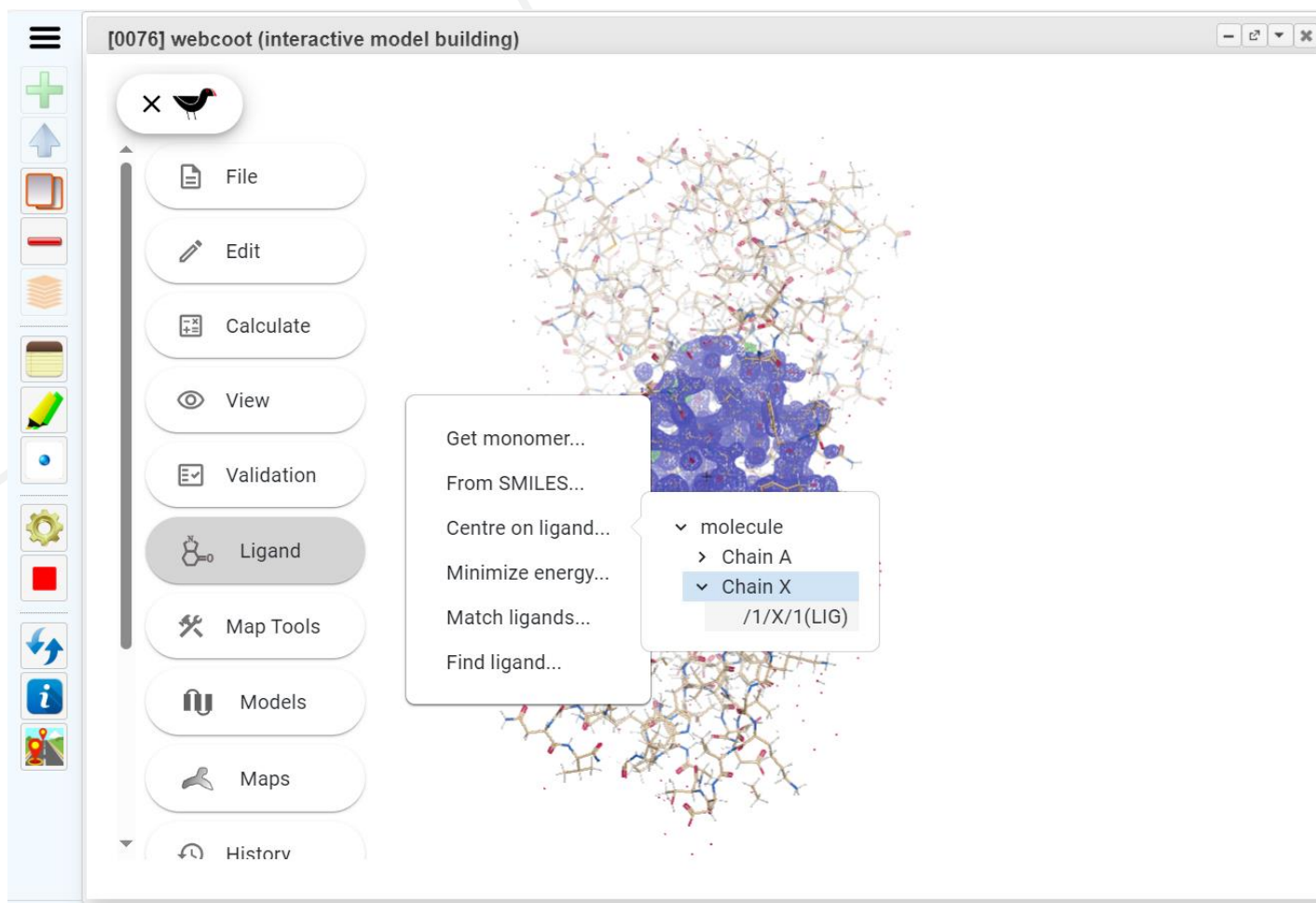
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

5. Once your first job is complete, open the last refmacat and launch *Model build with webcoot*



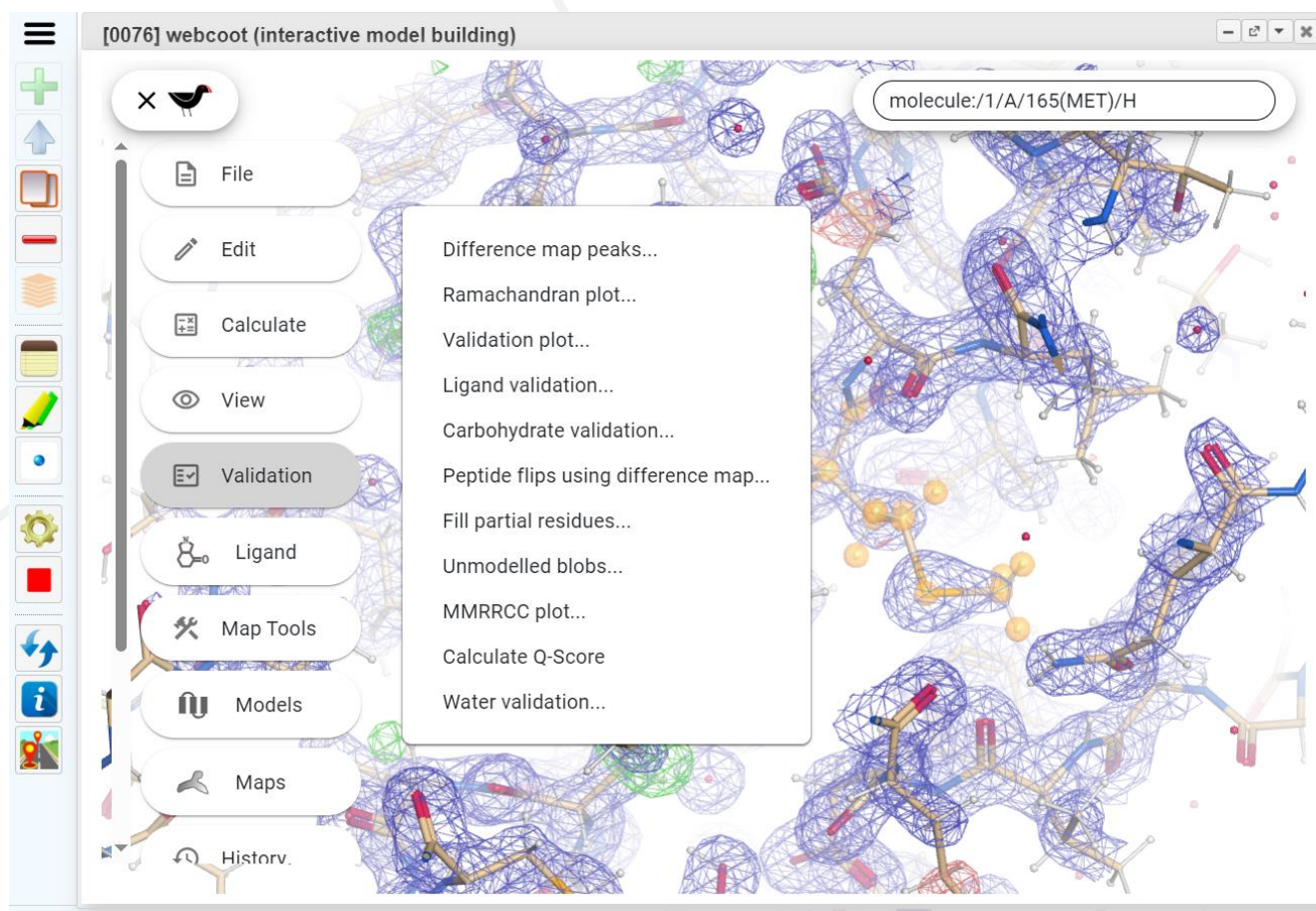
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

5. Centre on ligand and assess how well the ligand was fit (visual inspection)




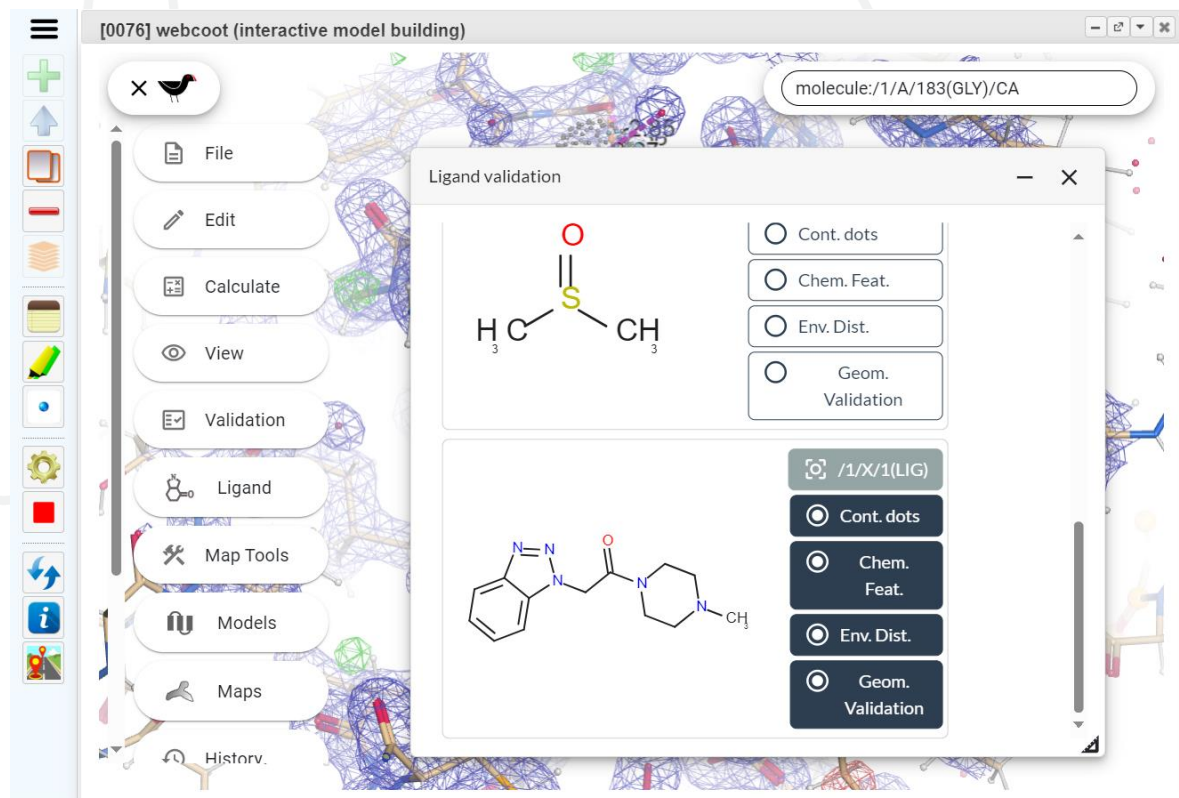
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

5. Assess ligand validation



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

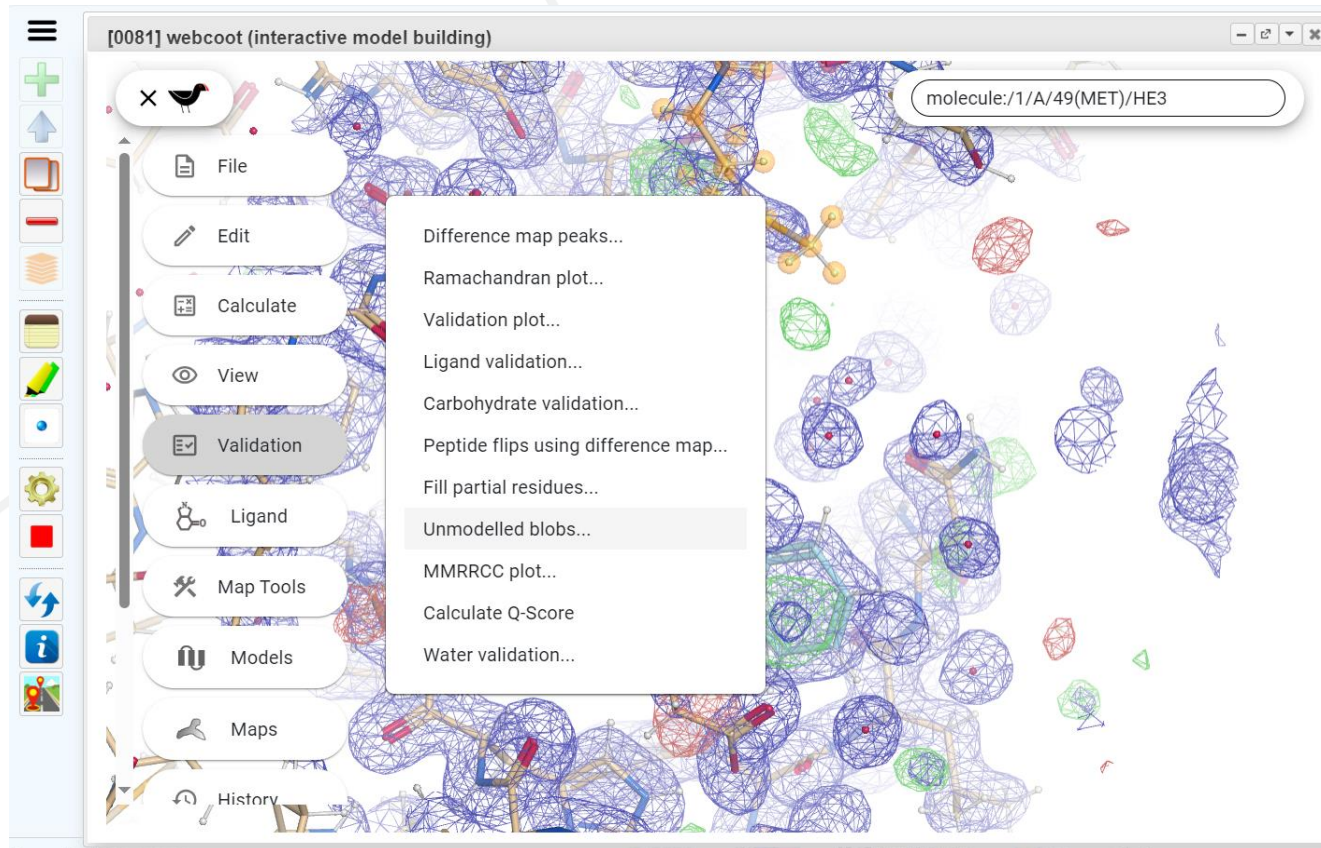
5. Assess ligand validation and check PDB validation report



The screenshot shows the CCP4 PDB validation report for molecule 0071. The report is titled '[0071] PDB Validation Report' and indicates that 80 hydrogens with zero occupancy have been removed from the structure. The report is generated by CCP4 v.9.0.004 and CCP4 Cloud v.1.8.002, with a start time of 2024-11-26 11:48:08 and a finish time of 2024-11-26 11:51:17. The report is displayed in a web browser interface with a 'Report' tab selected. A large 'Review' watermark is visible across the report content.

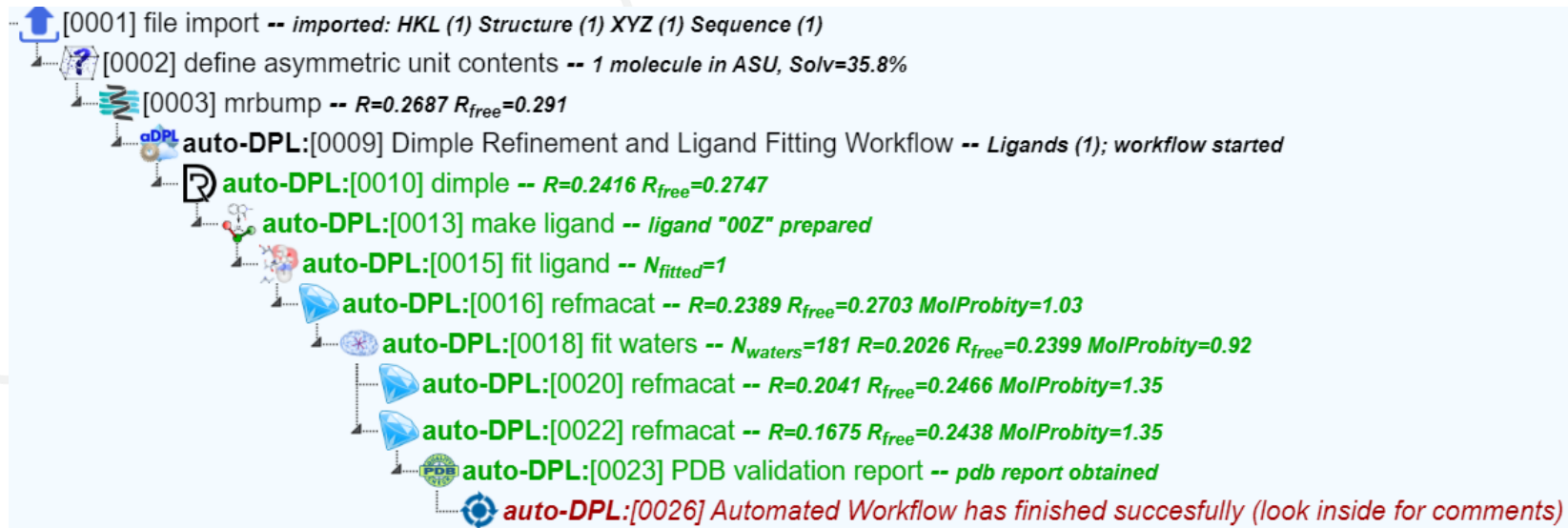
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

6. Inspecting for other potential ligands/unmodelled regions?



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

6. Alternatively, you can upload files then do MR, followed by auto-DPL (refinement and ligand fitting)



Tutorial – Part 1: Identify binding site

- Copy data: /dls/i04-1/data/2023/mx37045-5/processing/DLS-CCP4_Covid_Moonshot
- Select a dataset Mpro_1/2/3 (ignore 4 for now)
- Start Coot and load protein model and reflection data
 - Open Coordinates: dimple.pdb
 - Auto open MTZ: dimple.mtz
- Validate -> Unmodelled blobs...
- Select difference map and protein model
- Click “Find blobs” and search through identified blobs
- Did you find a blob near Cys145?

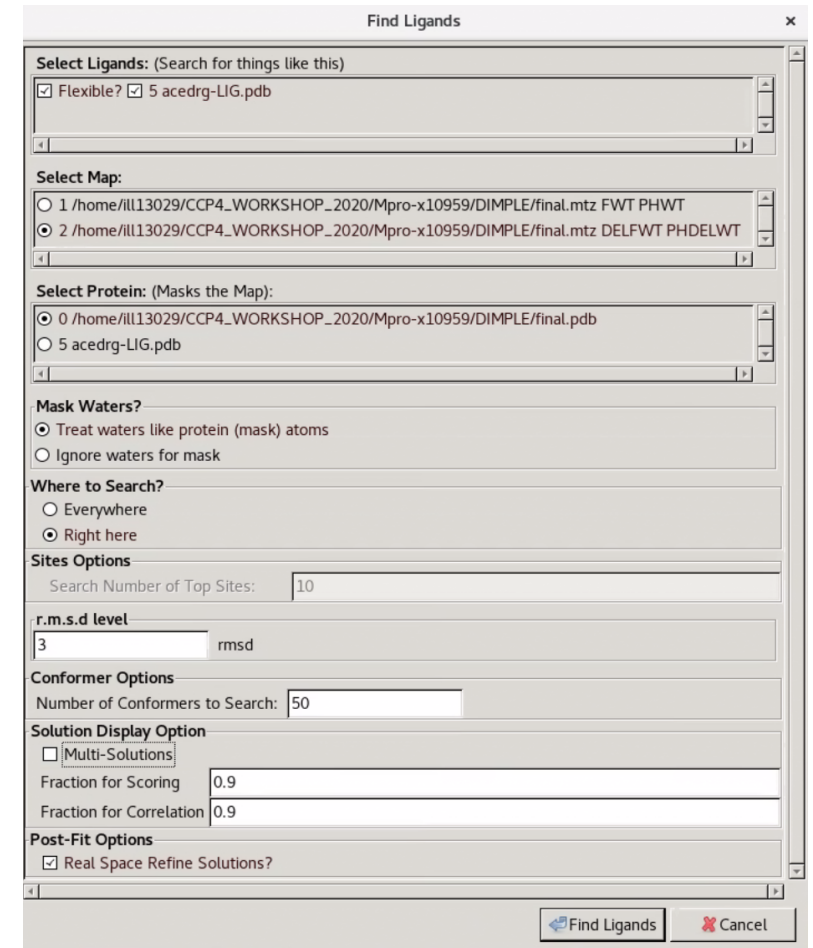
Tutorial – Part 2: Generating restraints

You can either...

- Generate restraints in Coot:
 - Select SMILES -> 2D from Ligand menu
 - Enter provided SMILES string and click “Send to 2D viewer”
 - Check structure in Lidia then click “Apply”
 - This will generate the ligand coordinates and restraints file
- Generate restraints using CCP4 Cloud (“Make Ligand with Acedrg”)
- Use pre-prepared compound pdb and cif files (from Grade webserver)

Tutorial – Part 3: Fitting ligand

- We now know where to place the ligand and have the ligand restraints
- *Run Ligand -> Find Ligands*
 - Select Ligand (Flexible), difference map and protein model
 - Search “Right here” with r.m.s.d. level set to 3
 - Click Find Ligands – do you find a hit?
- Use real-space refinement/Jiggle fit to optimize ligand fit and then **merge molecules**
 - Edit -> merge molecules
- Tidy up any surrounding residues/solvent molecules



The screenshot shows the 'Find Ligands' dialog box with the following settings:

- Select Ligands:** (Search for things like this)
 - ☒ Flexible? ☒ 5 acedrg-LIG.pdb
- Select Map:**
 - ☐ 1 /home/ill13029/CCP4_WORKSHOP_2020/Mpro-x10959/DIMPLE/final.mtz FWT PHWT
 - ☒ 2 /home/ill13029/CCP4_WORKSHOP_2020/Mpro-x10959/DIMPLE/final.mtz DELFWT PHDELWT
- Select Protein:** (Masks the Map):
 - ☒ 0 /home/ill13029/CCP4_WORKSHOP_2020/Mpro-x10959/DIMPLE/final.pdb
 - ☐ 5 acedrg-LIG.pdb
- Mask Waters?**
 - ☒ Treat waters like protein (mask) atoms
 - ☐ Ignore waters for mask
- Where to Search?**
 - ☐ Everywhere
 - ☒ Right here
- Sites Options**
 - Search Number of Top Sites: 10
- r.m.s.d level**
 - 3 rmsd
- Conformer Options**
 - Number of Conformers to Search: 50
- Solution Display Option**
 - ☐ Multi-Solutions
 - Fraction for Scoring: 0.9
 - Fraction for Correlation: 0.9
- Post-Fit Options**
 - ☒ Real Space Refine Solutions?

Buttons at the bottom: Find Ligands, Cancel

Tutorial – Part 4: Validation

- Measures -> Environment Distances -> Show Residue Environment?
 - Are molecular interactions sensible?
- Ligand -> FLEV this residue -> Show Env. Residues
- Ligand -> Isolated Molprobit dots for this ligand
 - Do you see any significant clashes?
- Ligand -> Display Ligand Distortions
 - How does the geometry look?
- Ligand -> Quick Ligand Validate

Tutorial – Part 5

- Dataset Mpro_4 contains DIMPLE files and SMILES strings for 3 ligands
- Using the methods described previously, identify which ligand is bound to the protein

