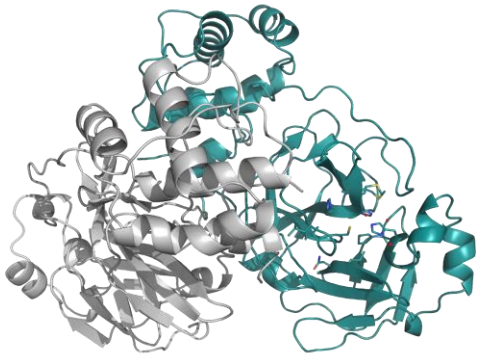
Ligand Fitting with CCP4 Cloud & Coot Tutorial

diamond

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)

https://doi.org/10.1101/2020.10.29.339317
https://doi.org/10.1126/science.abo7201

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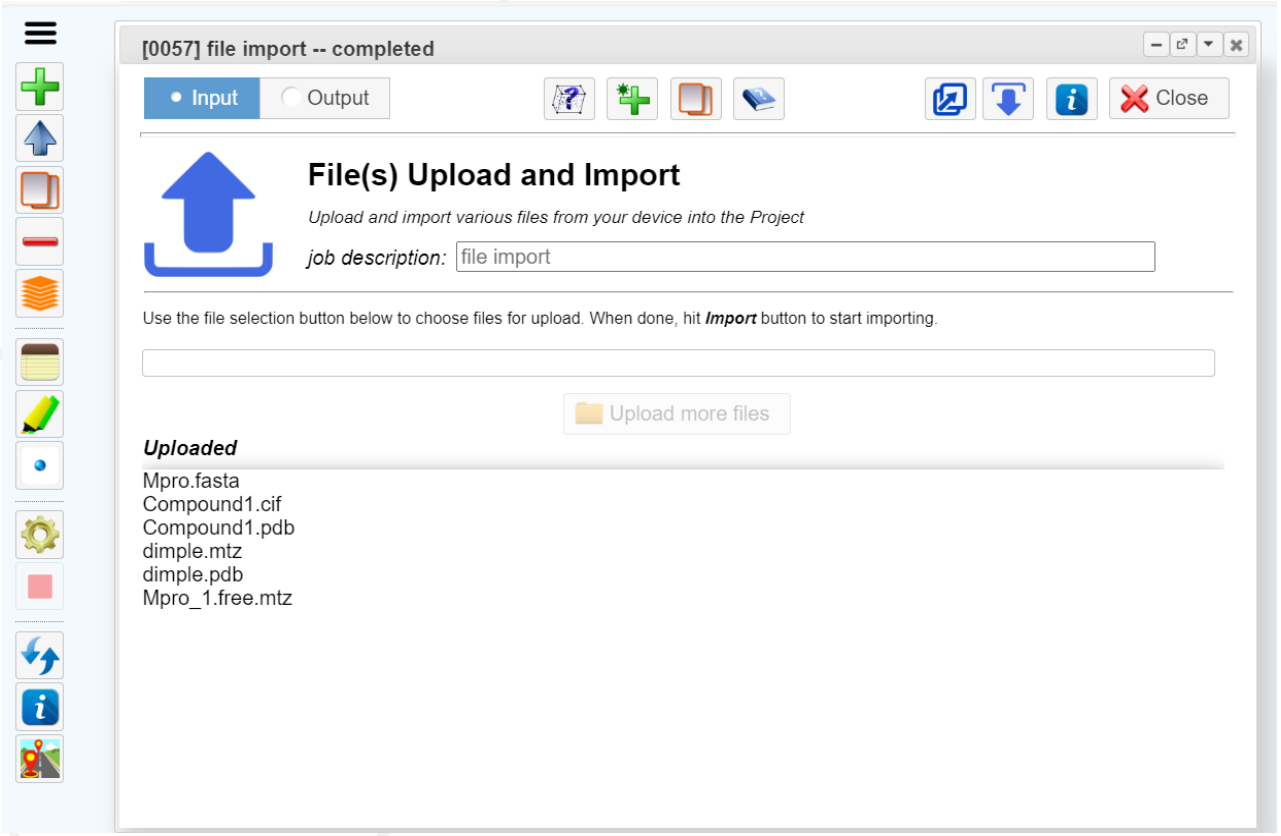
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

- 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



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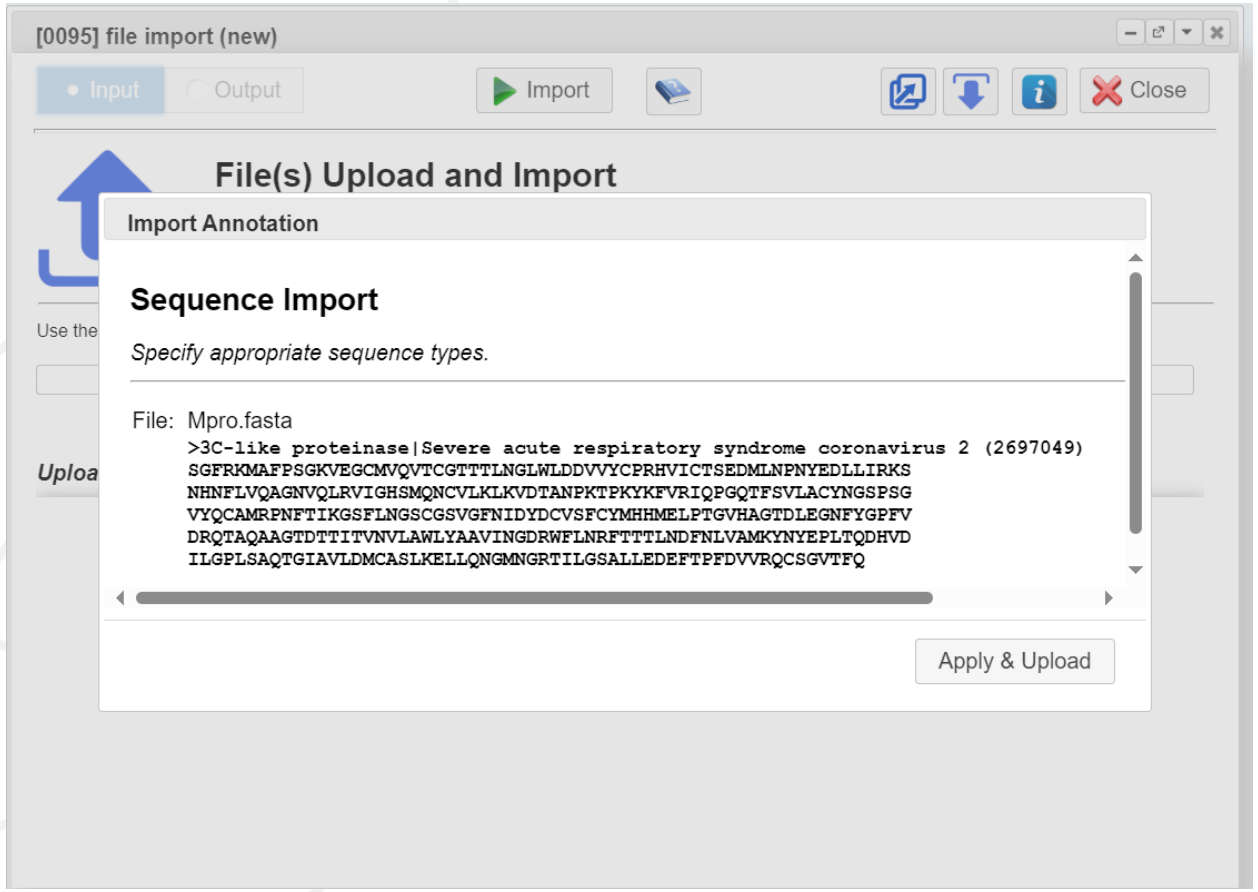
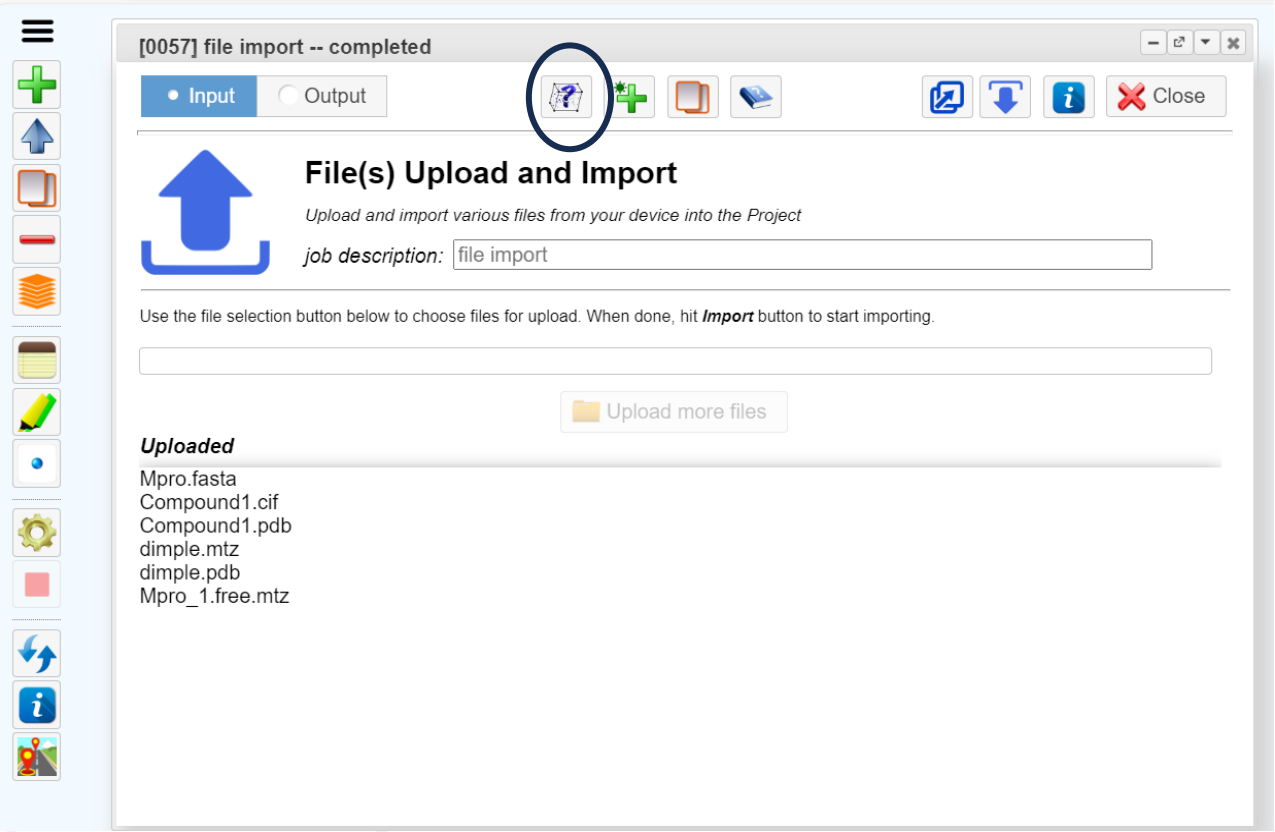


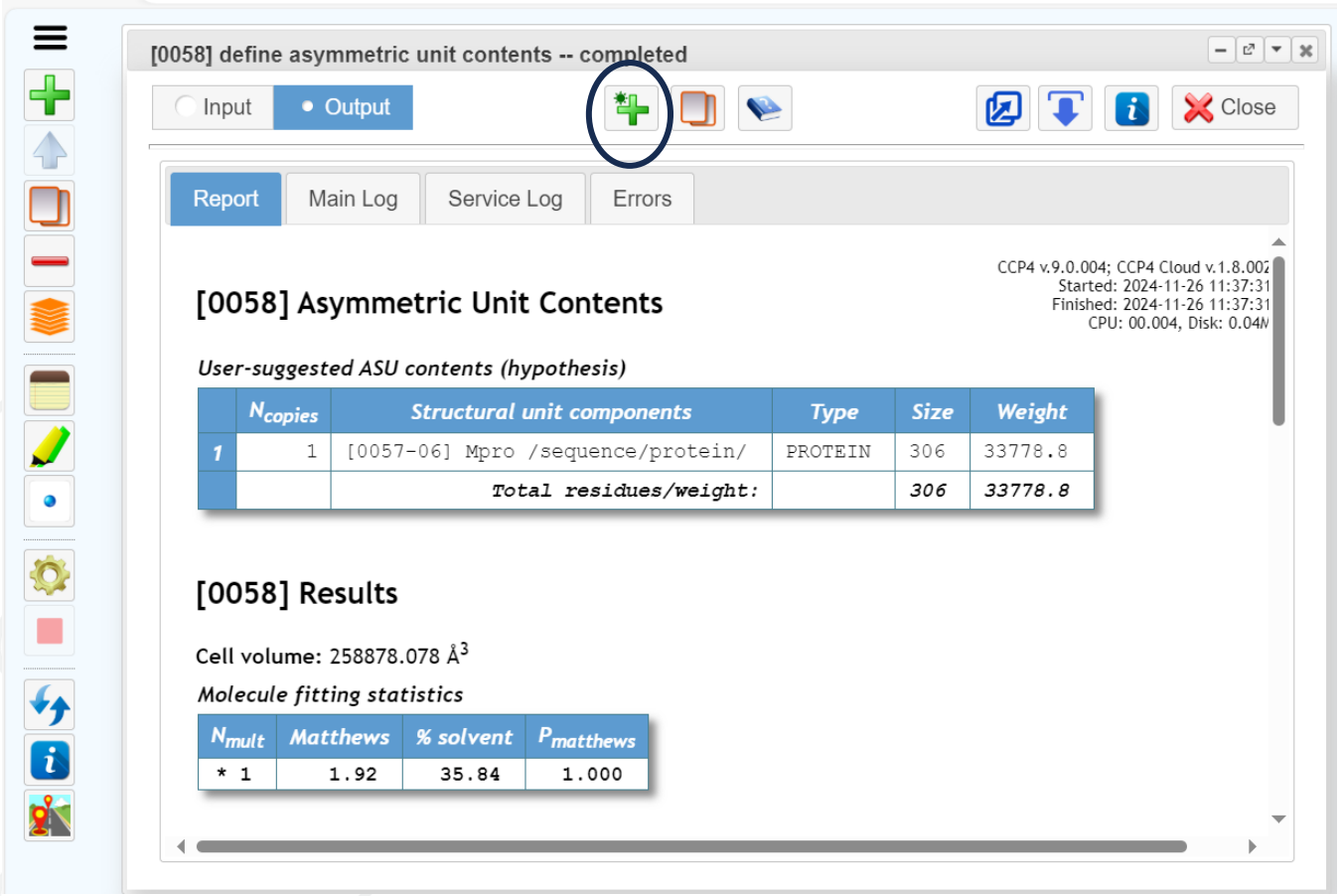
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

2. Define ASU contents



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

3. Run dimple-MR



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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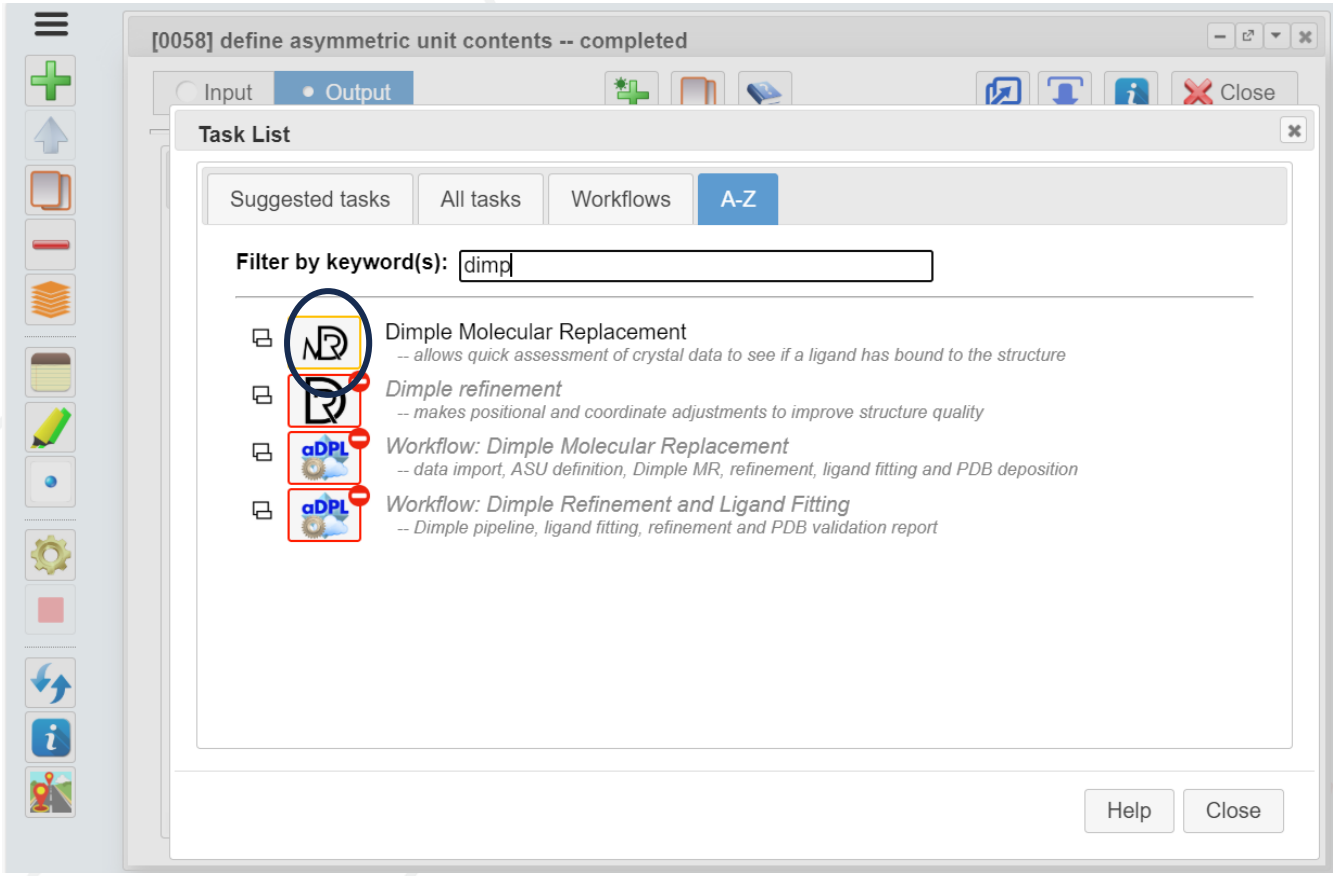
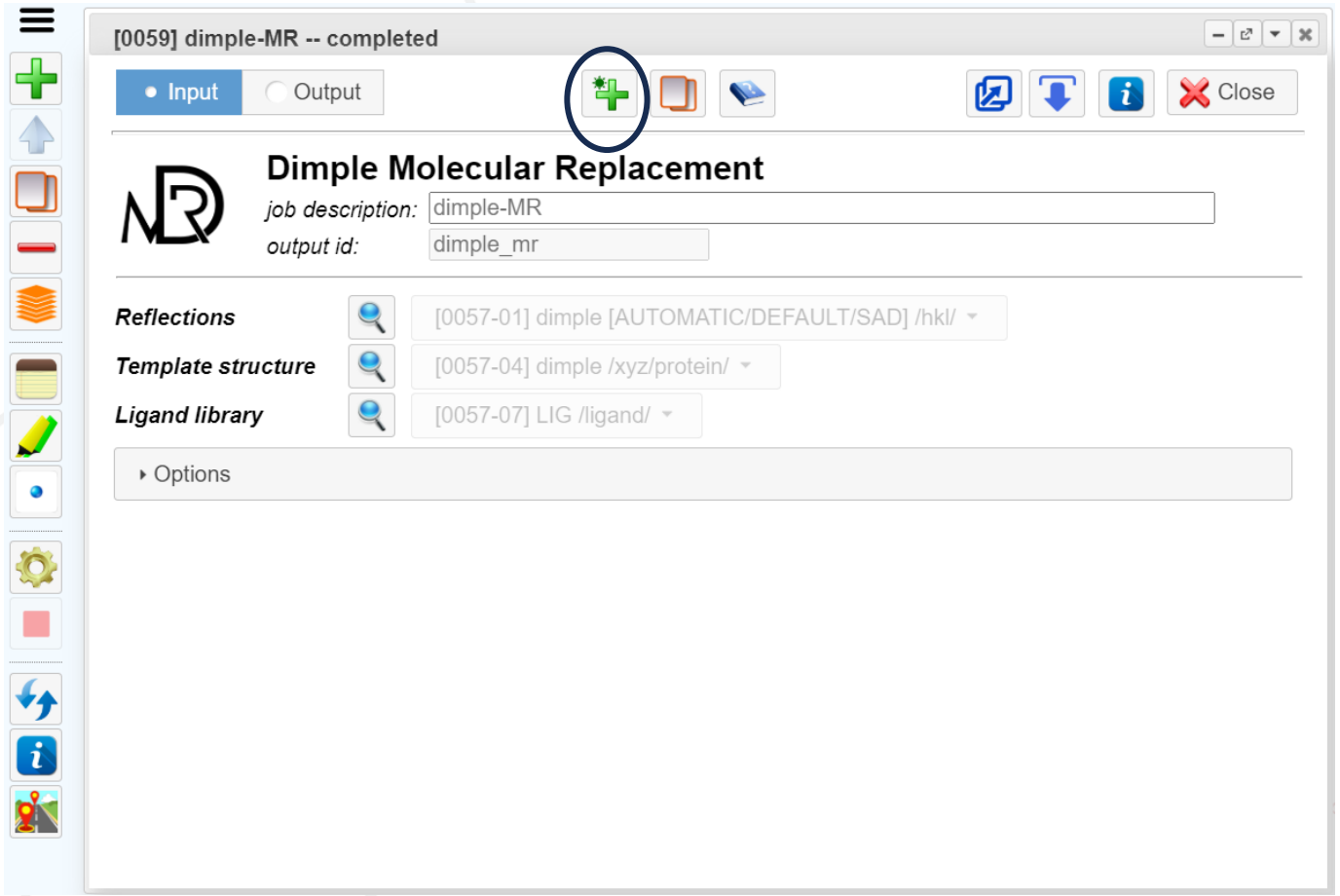


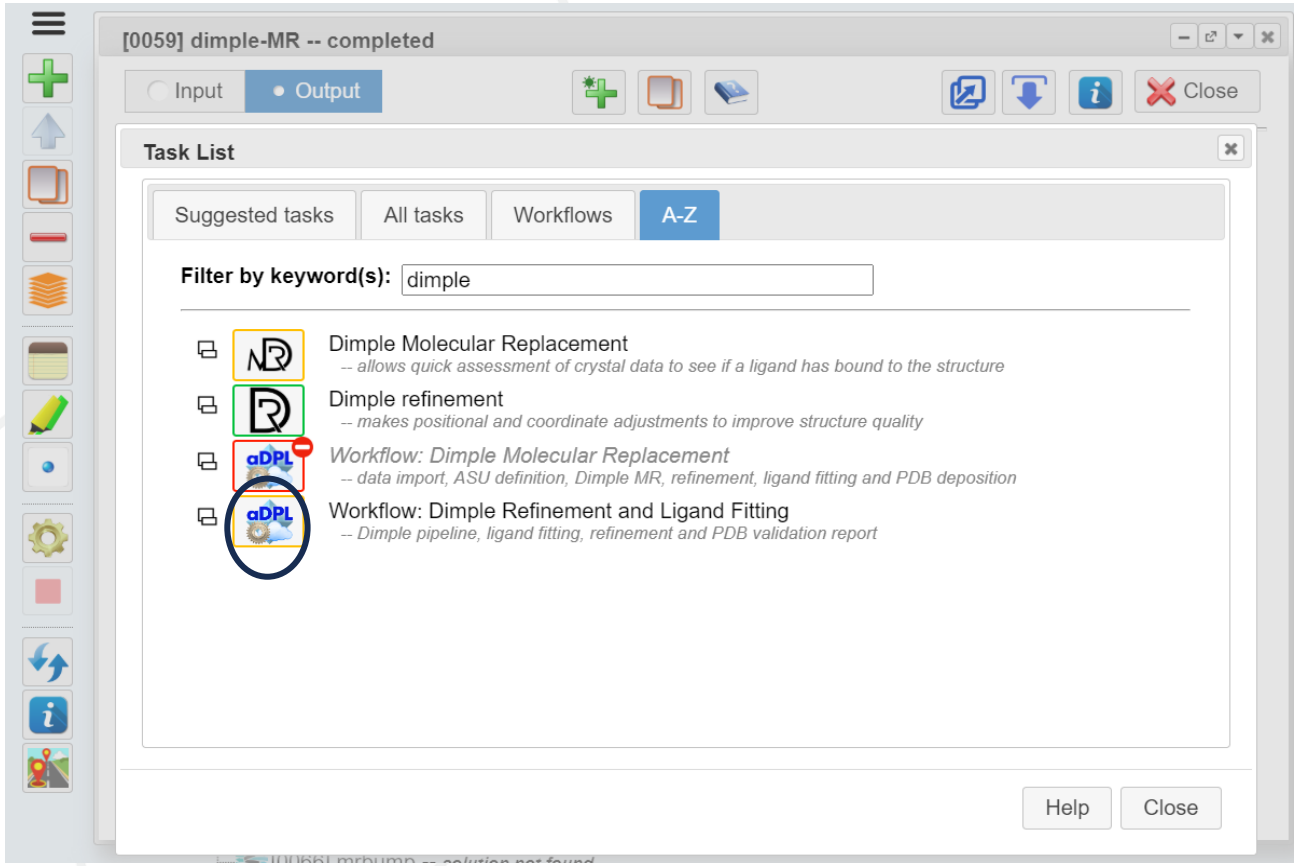
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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



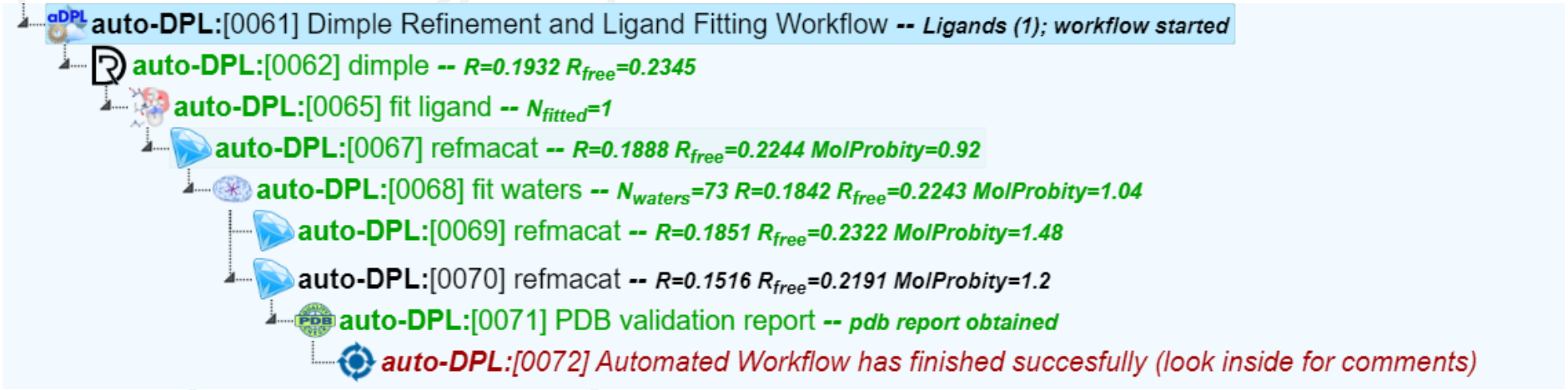
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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



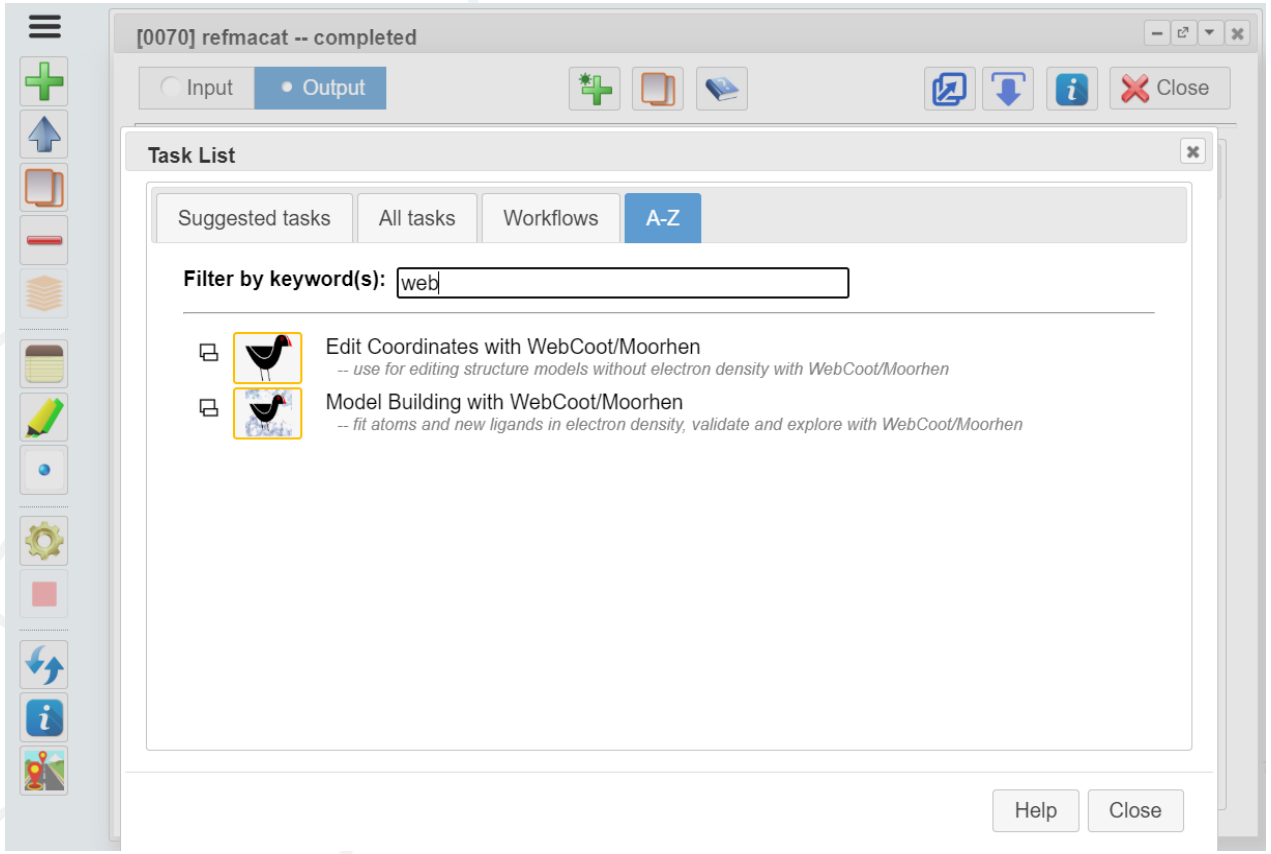
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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)



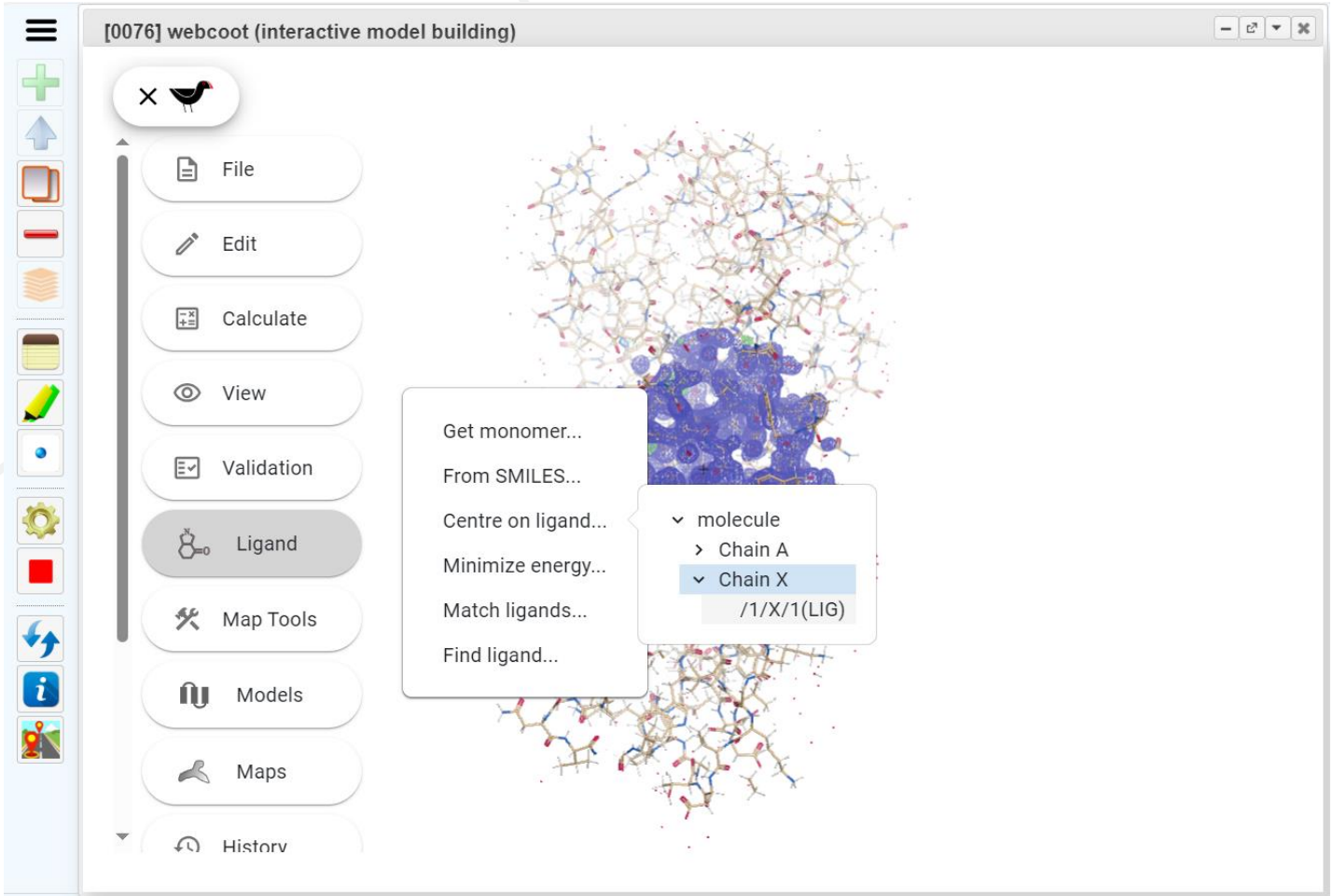
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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



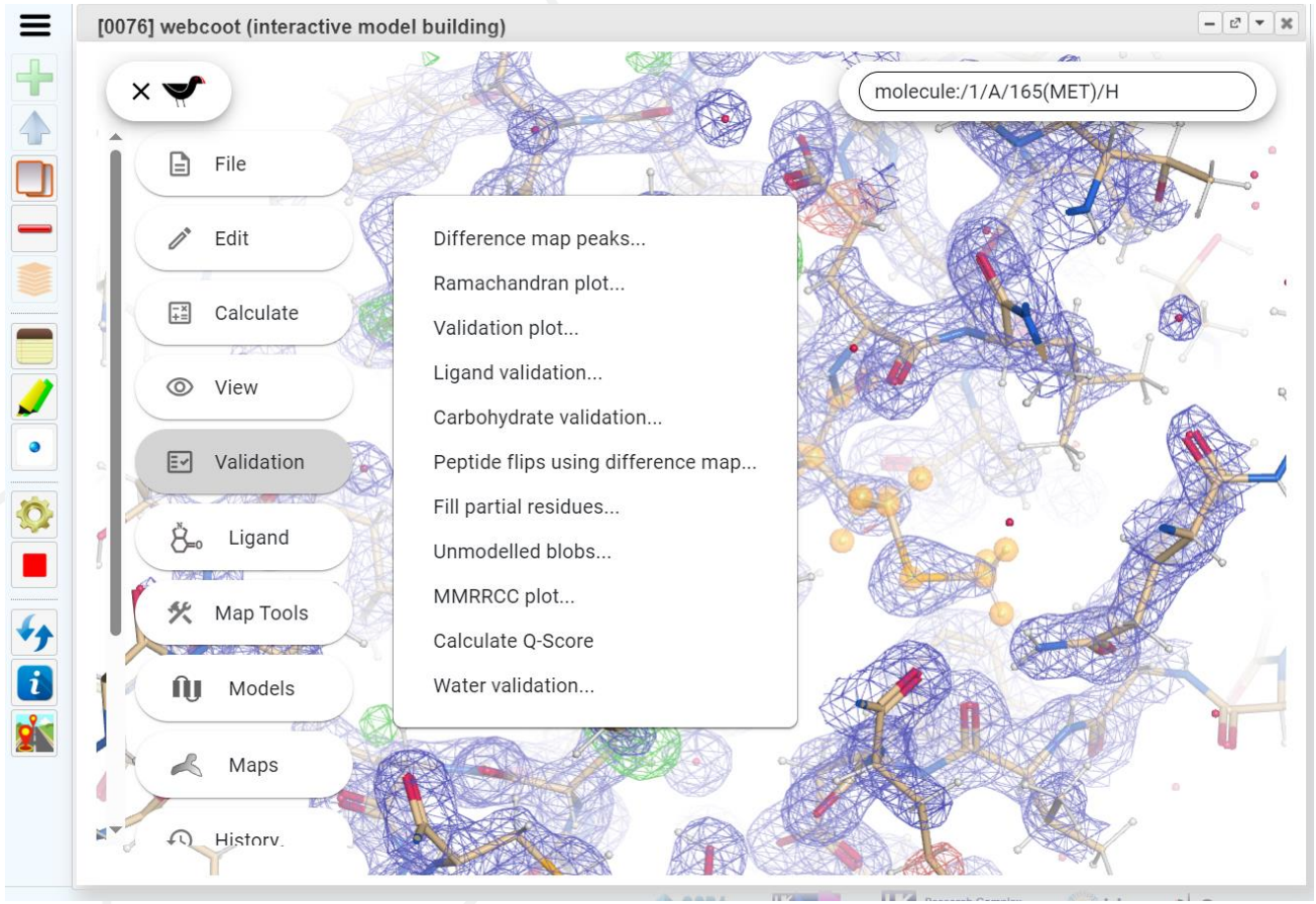
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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



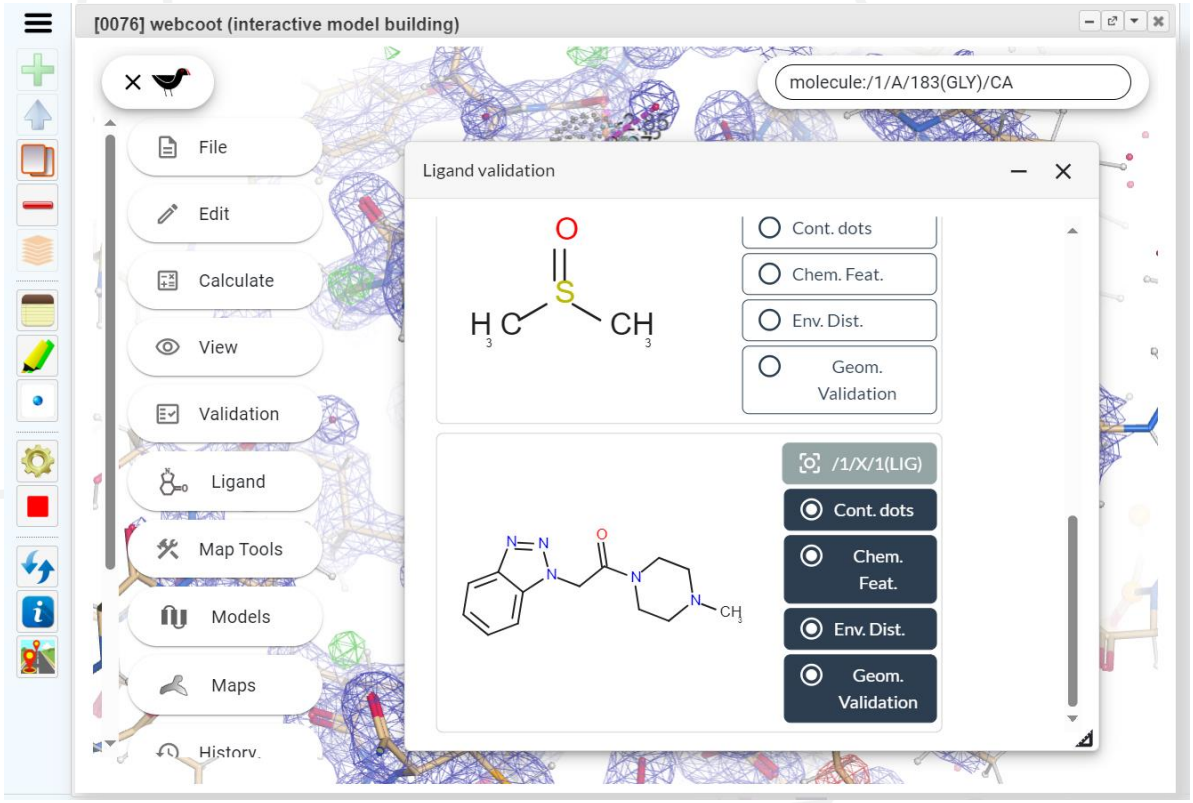
Tutorial – Part 1: Ligand fitting in CCP4 Cloud

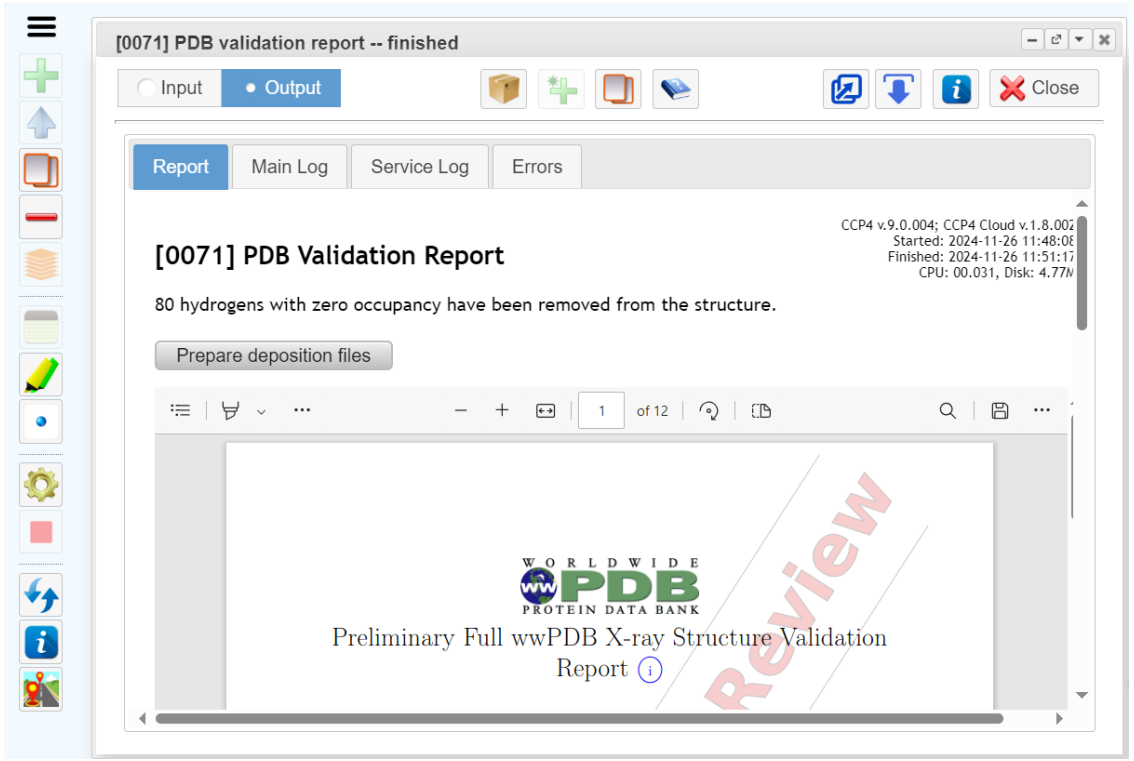
5. Assess ligand validation



Tutorial – Part 1: Ligand fitting in CCP4 Cloud

5. Assess ligand validation and check PDB validation report



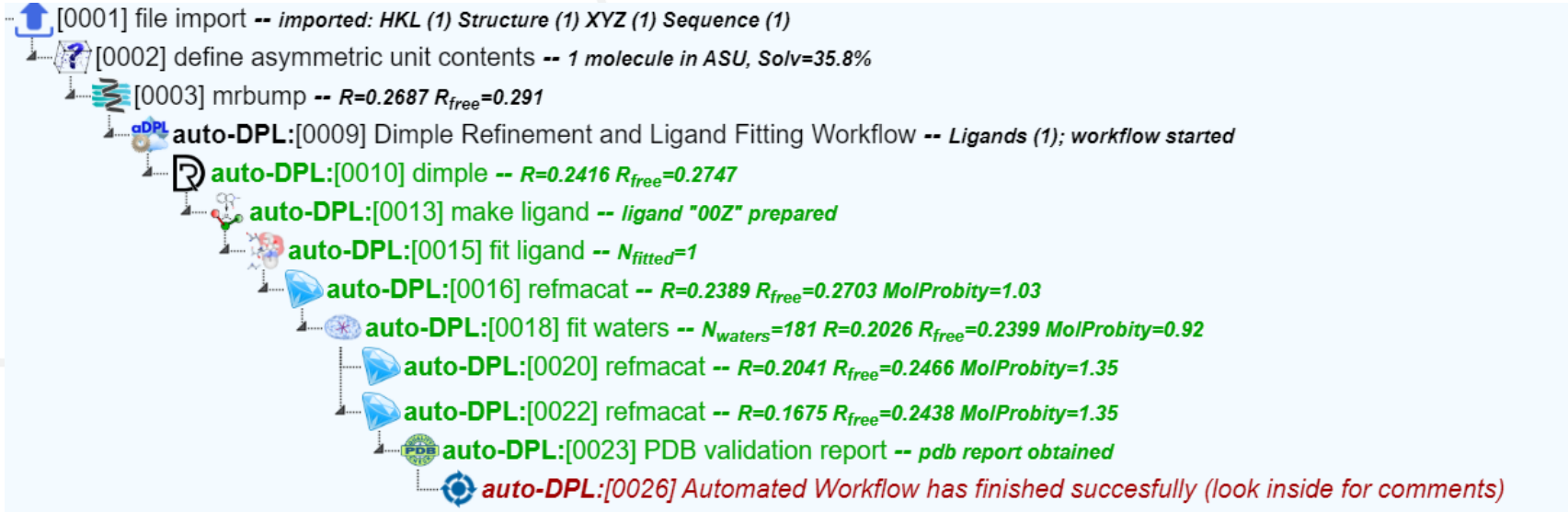


Tutorial – Part 1: Ligand fitting in CCP4 Cloud

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?

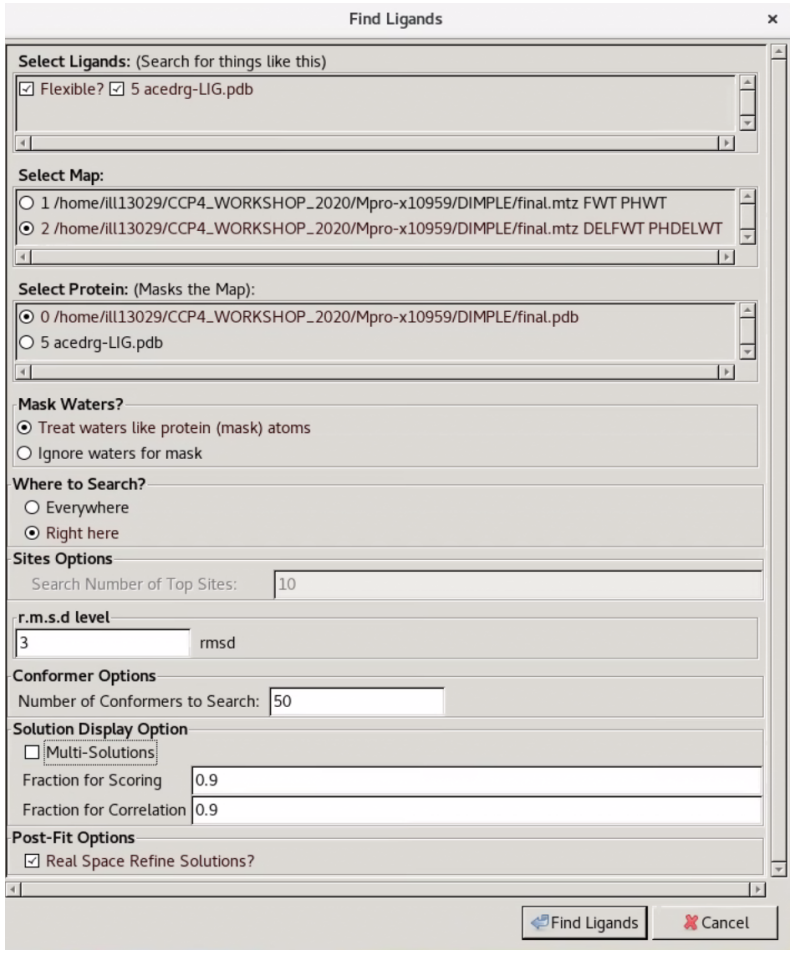
Tutorial – Part 2: Generating restraints

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

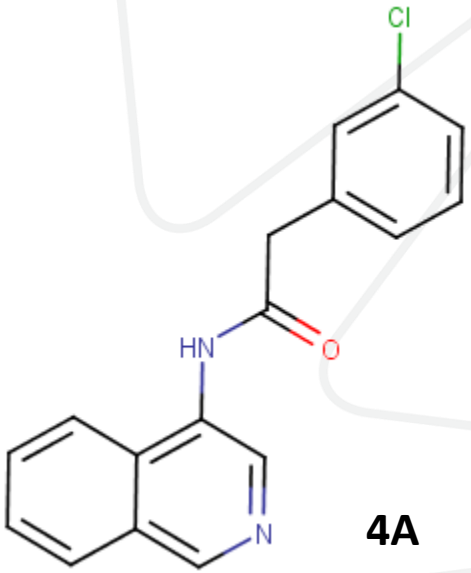


Tutorial – Part 4: Validation

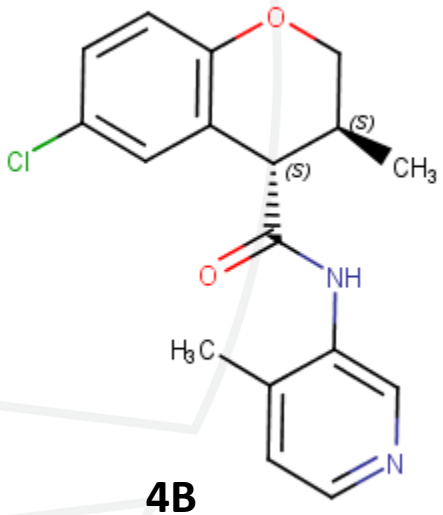
- Measures -> Environment Distances -> Show Residue Environment?
  - Are molecular interactions sensible?
- Ligand -> FLEV this residue -> Show Env. Residues
- Ligand -> Isolated Molprobity dots for this ligand
  - Do you see any significant clashes?
- Ligand -> Display Ligand Distortions
  - How does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

Tutorial – Part 5

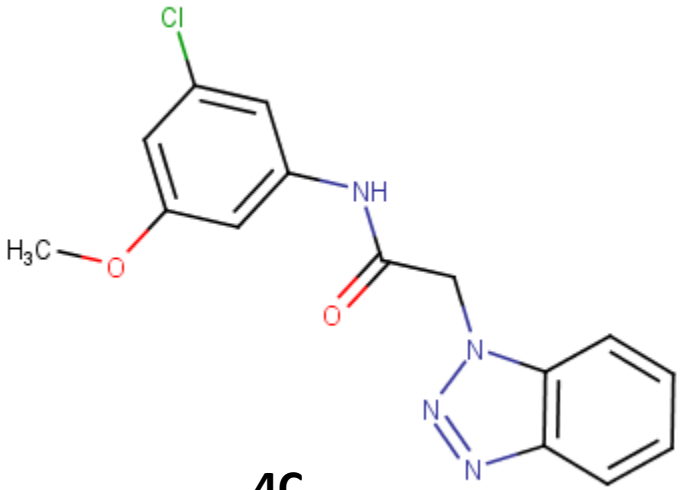
- Dataset Mpro_4 contains DIMPLE files and SMILES strings for 3 ligands
- Using the methods described previously, identify which ligand is bound to the protein



4A



4B



4C

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