CCP4: Electron density map generation

- Download from the PDB database the **coordinates** for the model (*.pdb , *ent or *.cif), the file with the structure factor **amplitudes** (mmCIF, *.cif) and the **validation report**
- Convert the structure factor amplitudes to the .mtz format. Import the same R-free set if included in deposited mmCIF file, otherwise generate a "Free R" subset
    - CCP4i : "Reflection Data Utilities" menu → Convert to/modify/extend MTZ, choose "Import reflection file in" → mmCIF
    - CCP4i2 : "Import merged data, sequences, alignments or coordinates" task →
        - "Import merged reflection data"
        - "Import a coordinate set"
- Generate model-based maximum likelihood maps (corresponding to σ_A-weighted electron density and difference electron density maps):
    - CCP4i : "Refinement" menu → "Run REFMAC5"
        - load the coordinates and the converted *.mtz file
        - in the "Refinement Parameters" tab set the number of cycles to 0
    - CCP4i2 : "Refinement" task → "Refinement – REFMAC5"
        - select the "Import merge reflection data" task as input in "Use data from job"
        - select the imported coordinates as the input in "Atomic model"
        - in "Options" tab change the number of refinement cycle to 0

In Phenix (if you wish to actually REFINE):

- Start the Phenix gui, 0pen a new project, select its folder. Sequence file is optional, necessary only for automated building.
- Select the project and go to 'Refinement' tab, select 'phenix.refine', load the coordinate file and the structure factor file. Phenix does accept the cif file format and if no free R-flags are available they can be generated using an option available from the GUI.
- Some Phenix versions then complain that they do not find a certain restraint file. To make this work, also load (add file) the missing monomer cif file from PDB or the ccp4 monomer library (location: C:\CCP4-7\7.0\lib\data\monomers\y\YXX.cif).
- In refinement settings tab, select parameters as necessary

In Phenix (if you wish to make maps ONLY):

- Start the Phenix gui, 0pen a new project, select its folder. Sequence file is optional, necessary only for automated building.
- Select the project and go to 'Maps' tab, select 'calculate maps', load the coordinate file and the mtz structure factor file.
- When finished, COOT can be opened directly from the results window, proceed as below for **COOT: Electron density map inspection**
- Alternatively view in PyMol, but no validation tools there.

Phenix itself offers a series of stand-alone validation tools, including MolProbity and electron density tools. The polygon tool provides a reasonable visualization of major parameters. Don't forget to load the missing cif files.

COOT: Electron density map inspection

- Open the PDB model and the electron density maps in COOT
  - CCP4i : "View Files from Job" menu → "View result of refinement in COOT"
  - CCP4i2 : in the "Results" tab of the REFMAC5 job select "Manual COOT"

Two validation areas: direct evidence (electron density) **and prior expectations** (stereochemistry)

- Using the PDB Validation Report as a guideline and check the model using COOT's validation tools (accessible through the "Validate" menu):
  - "Difference Map Peaks Analysis": check all the difference density map peaks (recognizable shape? Environment?) higher than 5σ (maybe 4.5 or 4 in later stages of model correction)
  - "Density fit analysis": click in the plot any features of the model which does not properly fit the electron density map
  - "Ramachandran plot": validate (density?) or correct any outliers present in the model
  - "Model geometry": select the model and check high RMSZ (> 4 or 5) outliers
  - (specific for water molecules) "Check/delete waters": compile a list of poorly defined/incorrectly placed water molecules – density shape, environment?
- Save the modified model
  - CCP4i : "File" menu → "Save Coordinates…" : the project directory will show up on the left panel; select it and save the modified coordinates
  - CCP4i2 : "CCP4i2 extensions" menu → "save to CCP4i2"; close COOT
- Run a few minimization cycles
  - CCP4i : "Refinement" menu → "Run REFMAC5", setting the number of refinement cycles to 10-20
  - CCP4i2 :
    - "Refinement" task → "Refinement – REFMAC5"
    - Run the new REFMAC5 job using the latest "Manual model building – COOT" as input, set the number of refinement cycles to 10-20 in the "Options" tab
- Repeat the COOT manual building/REFMAC5 refinement until satisfied

N.B.: For real-space refinement to work in presence of *cis* peptides select the "R/RC" icon on the top right of the COOT window and unselect "Use Trans Peptide Restraints"
N.B.: If unusual compounds are present, then the restraints for the ligand can be obtained in COOT:

- (if part of the REFMAC5 monomer library) "Get Monomer" → enter the ligand's 3-letter code
- "Calculate" menu → "Ligand Builder": Lidia, COOT's ligand build tool, accepts SMILES codes (can be downloaded for example from Wikipedia or other portals) as input. The new ligand information can be saved using the ccp4i2-extension tab.
- generating the coordinates through an external service (e.g. the ProDrg server http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg)
- or add monomer via : Extensions > model building > add other solvent molecule

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