

**1.b What are current best practices for selecting an initial target ligand atomic model(s) for structure refinement from X-ray diffraction data?**

- Visual analysis:
  - Identification of ligand density from appropriately contoured difference density maps (with confirmation in 2Fo-Fc maps)
  - Use of interactive fitting tools that measure fit of ligand to density
- Automated analysis:
  - Use of objective fitting algorithms based on significance of electron density levels and fit of ligand to density
  - Use of methods that screen difference density against libraries of potential ligands
- General:
  - Fitting of ligands should take into account what a priori information such as buffer composition, routes of chemical synthesis and biochemistry of the macromolecule

- Challenges:
  - The actual chemistry of the ligand may not be well determined depending on the physical situation
    - Ligand in isolation may be different from in complex with macromolecule
    - Covalent modifications
    - Radiation damage
    - Ambiguity in chemistry - may be known at the time of synthesis
  - Arriving at the initial conformation for new ligands can be challenging in cases with poor density and conformation variability in ligand
  - A poor starting model will impact the final structure

- Recommendation:
  - Data items are needed to record the ligand(s) added to the crystal (vs what was actually modelled)
  - A record is needed of the method used to introduce the ligand (soak, co-crystal, endogenous)

**1.b What are current best practices for generating restraints for modeling and refinement?**

- Best practice:
  - Currently the use of information obtained from high resolution small molecule structures (e.g. Mogul/CSD, COD)
  - Alternative approaches make use of semi empirical or higher basis set QM calculations
- There are newer refinement programs that can use MD, QM/MM or other force-field methods instead of using researcher specified restraints (examples include DivCon/Phenix, AFITT/Buster/Phenix, AMBER/Phenix)
  - Information about these kinds of refinements needs to be presented to wwPDB end users
  - Validation programs should take account of the source of the restraints/methods used in refinement

- Challenges:
  - Existing small molecule databases can lead to bias in restraint generation
    - Small numbers of observations in some cases
    - Variable redundancy in the CSD
  - Multiple methods should be considered to generate the restraints
  - What to do when user generated restraints differ from the wwPDB internal information?

- Recommendation:
  - The method used to refine the ligand should be itemized in the deposition
  - Validation metrics for non-traditional methods (QM/MM, FF, CDL) need to be investigated
  - Restraints information deposited by the user should be compared to other sources of the same information (e.g. Mogul)
  - Strain energy is not currently a good tool for validation



**2. What are current best practices for validating the ligand(s) coming from such a structure refinement?**

- Best Practice:
  - Current tools look at relatively crude measures such as bond RMSD(-Z) scores, and local fit to density
  - Ligands are also increasingly validated against information derived from small molecule databases (e.g. Mogul/CSD)
- There is a need for a better, validated, metric for ligand/density fit
  - Reciprocal space CC plots (c.f. BusterReports)
  - The shape of the density and the ligand could be other criteria
- Mogul/CSD analysis needs to account for redundancy or small N effects on sigmas

- Challenges:
  - The source of validation information needs to be considered
    - How to have metrics that are universal given different approaches to ligand refinement?
    - There can be limitations to the experimental databases (ligands may have different chemistry when interacting with a macromolecule)
  - Testing and evaluating alternative solutions may be needed to determine the most likely solution, taking into account:
    - What was added to the crystallization vs endogenous
    - Purity of the compound
  - Industrial access to validation tools?

- General recommendation:
  - Software tools for validation should be available for use by the community
- Recommendation:
  - Internal ligand geometry should be validated with standard approaches (bonds, angles, torsion, planarity, chirality etc)
  - Distributions of library values should be shown (visually) when possible

- Recommendation:
  - Enhance existing, and develop new, tools for assessing interaction with the macromolecule, and/or other ligands
    - MolProbity could be adapted to provide more information about ligand geometry and clashes with macromolecule
    - Simple measures such as clashes, acceptor/donor mismatches are a primary target
    - More complex metrics should be implemented later
      - Group/Chemical type interactions (going beyond atom/atom)
      - Charge/charge, VdW, etc
  - Visualization tools should be modified to display this validation information

**3. What new information pertaining to X-ray co-crystal structures should be required for PDB depositions going forward?**

- The origin of the restraints should be provided (i.e. what methods were used to obtain restraints and/or refine the ligand geometry)
- An “Omit” map should be calculated - giving evidence of ligand
  - A best practice should be defined
- A “Best” density can be provided by the author - that shows the ligand density as interpreted
  - A best practice should be defined

- Spectroscopic data (on crystal, on sample, on ligand) could be provided
- Response to validation reports
  - Should users have to explain the outliers?
  - Can responses be formalized to aid understanding by wwPDB users?
  - Where are the boundaries between borderline and significant outliers/deviations?



- Recommendation:
  - Chemical description and restraints (mandatory for new ligands)
    - In CIF format

- Recommendation
  - A user supplied ligand map (optional)
  - An omit map:
    - Remove the ligand (rather than setting occupancy to zero)
    - Need to determine limits for excluding ligands (i.e. all simultaneously or one-by-one, depends on % of structure)
    - Need a systematic test of strategies for making the maps
    - Should be calculated by the wwPDB from deposited information
  - Calculate a measure of fit between model and map:
    - Real space or reciprocal space CC
      - Against user supplied map if available, and against omit map

**4. What information should accompany journal submissions reporting X-ray co-crystal structure determinations? What supplementary materials should accompany publication of X-ray co-crystal structure determinations?**

- Response to validation reports could be provided in reports
  - It would be very helpful if researchers had to justify the outliers in the validation
- In general information should be provided to journal method sections to enable others to reproduce experiments where possible
- A paradigm shift in the review process may be needed:
  - Data (model/maps) provided at review time

- Post deposition:
  - Annotation of changes to ligands (and the deposition in general) should be provided to wwPDB users
  - Deposition authors should be contacted with changes to ligands
    - Let users register to be notified about changes
- The 3-character limit on ligand names is limiting, can this be increased (i.e. by moving to mmCIF)
- Information needs to be provided to give provenance of ligand dictionaries and the specific entries
  - By naming the library/file
  - Recommend use of community tools and clear annotation (c.f. Grade)

- Recommendation
  - The validation report should be more comprehensive
    - Including real space and/or reciprocal space fit of ligand to map
  - Images of the electron density and the model (e.g. Animated GIF or orthogonal views)
    - Calculated for omit and user supplied map
  - Visual display of Mogul analysis of geometry (c.f. BusterReport)
  - Depositor need to define the ligand(s) of interest so they can be highlighted in the validation report

**5. What do you recommend be done to improve descriptions of ligand chemistry in the PDB archive?**

- Ligand restraints (including any links to the macromolecule) need to be provided
  - At the wwPDB these restraints need to be versioned
- Tools/approaches need to be developed to define chemical diversity within a compound
  - A mechanism needs to be available to more completely describe protonation, and tautomeric states.
    - wwPDB has a solution for amino acids, but can this be reasonably extended to ligands?
- Overall we need a better description of ambiguity
  - Guidelines for how to deal with (best practices)
    - Radiation damage - how to best model? (hydrogen vs radical)
      - Use alternate conformations (at the whole ligand level)



- Recommendation
  - mmCIF data items need to be created to identify which atoms are modelled but not by fit to density (data)
  - Visualization tools need to be modified to display this information

**6. What do you recommend be done with existing X-ray co-crystal structures in the PDB archive?**

- All current and future validation tests should be performed where data availability allow
- Much of the nomenclature has been corrected already, with some specific areas such as carbohydrates and metals remaining - these should be pursued
- The different instances of molecules/structures should be versioned
  - Would help with people cleaning up their own structures
  - But what if the sequence/ligands change with new versions
  - Authentication of depositors may be necessary
  - How to deal with highly related structures (e.g. a series of re-refinements of structures that probe different parameterizations)
- One approach could be Jamboree/Hackathons to remediate structures with community buy in

- Recommendation:
  - All current and future validation tests should be performed where data availability allow
  - Much of the nomenclature has been corrected already, with some specific areas such as carbohydrates and metals remaining - these should be pursued
  - In the future it might be useful to have a community coordinated improvement of models (including ligands)
    - E.g. Jamboree/Hackathons to remediate structures with community buy in