Things that are not protein, RNA/DNA and some small molecules in Phenix

Generating & modifying for all scenarios

Nigel W. Moriarty
Ligands? Water?

- Be careful when inheriting a model
- Can contain ligands and water molecules
- Must be able to “see” them in the map
Restraints in Action

• Libraries
  • Monomer Library
  • GeoStd

• Algorithms
  • Polymer
  • Links
GeoStd

- All standard amino acids
- Current list of non-standard amino acids
- All standard RNA/DNA
- Current list of non-standard RNA/DNA
- Others
CIF

- Crystallographic Information File
- mmCIF – macro-molecular CIF
- Used for
  - Model
  - Data
  - Maps
  - Ligands
    - Information
    - Restraints
Confusion

- All depositions of X-ray model use mmCIF from 1 July 2019

- “I need a CIF file.”

  - But what do you really need?
Restraints?

- Provide a reasonable geometry during refinement particularly at low resolution
  - Bonds, angles, dihedrals, chirals, planes, …
- Must be weighted against the experimental information
Overview

- eLBOW - electronic Ligand Builder & Optimisation Workbench
- ReadySet! - One-stop preparation for your refinement needs
- REEL - Restraints Editor Essentially Ligands
Ligands in crystallography

Generate ligand restraints

Fit ligand to density

Refine macromolecule and ligand
**eLBOW goals**

- Fast, simple and flexible procedure to include ligands
- Reduce the tedium of building 3D ligand models
- Automate generation of restraints for ligands
- Comparison of ligand structures

**Chemical input**

- Chemical restraints (CIF)
- Cartesian coordinates (PDB)

**Reflection data**

- Protein information

**refinement**

Topology

- PDB
- xyz
- Auto bond connections
- Auto bond orders
- Generate angles, dihedrals, planes & chirals
- Generate 3D geometry
- Three letter code
- MOL3D
- CIF
- SMILES
- phenix
Optimisation

- Topology information - Atoms, bonds, angles, ...
- Construct Z-Matrix
- Simple force field geometry optimisation
- Add hydrogens
- AM1 geometry optimisation
- Output geometry (PDB) and restraints (CIF)
Getting ready to refine

- Many details needed to prepare for structure refinement

Chemical input

- Chemical restraints (CIF)
- Cartesian coordinates (PDB)

Protein Information

Experimental data

ReadySet!

refinement
ReadySet!

- Add hydrogens
  - Default: adds hydrogens to protein, ligands
    - Protein - Reduce
    - Ligands - eLBOW
  - Add hydrogens to water
  - Add deuteriums instead of hydrogens
    - Add hydrogen & deuteriums appropriately
  - Generate restraints
ReadySet!

Protein → Reduce → D → PDB
Ligand → eLBOW → H → CIF
H₂O → H → edits
Metal →
ReadySet!

- Restraints CIF filename
- Restraints CIF directory
- LINKS to “edits”
- --dry-run to show ligand process pathway
- Metal coordination
Restraints in phenix.real_space_refine

- LINK records have no impact
- Automatically accesses the “standard” residues restraints
- Automatically links the “standard” residues
- Parameter “link_all=True” links
  - Covalent ligands
  - Carbohydrates
  - Metal ions
phenix.real_space_refine (continued)

- RNA/DNA restraints
- Base pair hydrogen bonding
- Base pair planarity
- Base stacking (parallelity)
- Secondary Structure restraints
- NCS restraints
- Custom bonds & angles using edits
- Restraints are written to .geo file including non bonded interactions
Summary

- eLBOW & ReadySet! perform better when provided with better input. (GIGO)
- Need to know something about the ligand
  - Hierarchy of input file value
- Check your .geo file for confirmation of restraints