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PDB2INS – For Setting Up Macromolecular Refinement With SHELXL

PDB2INS – Why use SHELXL for refinement?

SHELXL facilitates the refinement of macromolecules against high resolution data :

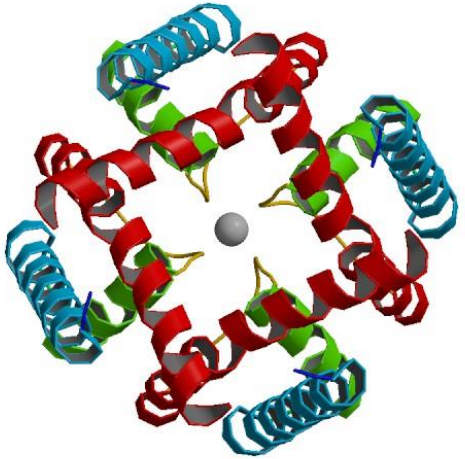
- Enormous flexibility, even refinement of Laue and neutron data.
- Anisotropic refinement (if data:parameter ratio sufficiently high).
- Refinement of (multiple domain) twin data (non – merohedral).
- Refinement against intensities.
- Occupancy refinement.
- Modelling of complicated disorder.
- Calculation of standard uncertainties (esds) for small structures.
- Calculation of $R_{complete}$ in addition to R_{free}.

Sheldrick G.M. Acta Cryst. **2015**, *C71*, 3-8.
Gruene T. Hahn, H.W., Luebben A.V. Meilleur F. and Sheldrick, G.M., J. Appl. Cryst **2014**, *47*, 462-466.
Luebben, J. and Gruene, T., PNAS **2015**, *112 (29)*, 8999-9003.

PDB2INS – Why use SHELXL for refinement?

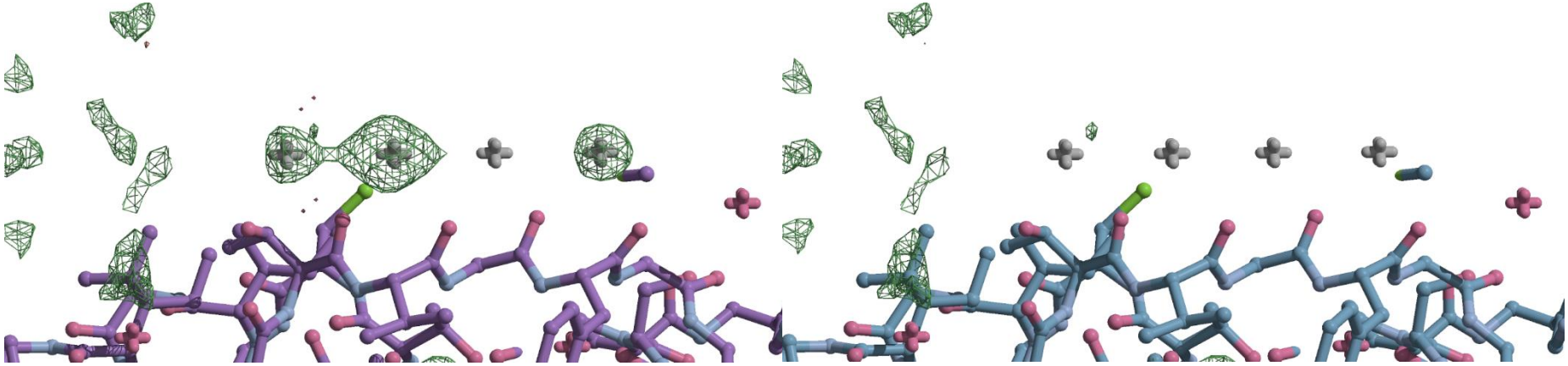
MthK pore, with kind permission from T. Gruene:

- 3LDC: MthK pore with 100mM K+, dmin = 1:45Å.
- SHELXL: calculation of standard uncertainties and correlation coefficients with full matrix least squares.



Fixed occupancies:
50% K^+ and 50% H_2O

Refined occupancies:
(S4)1.00 (S3)1.00 (S2)0.80 (S1)0.92



Köpfer, Song, Gruene, Sheldrick, Zachariae, de Groot, Science **2014**, *346*, 352–355.

PDB2INS - overview

PDB2INS prepares .ins and .hkl files required for SHELXL:

- .ins file contains crystal data, atomic coordinates and instructions.
- .hkl file contains reflection data.

PDB2INS requires files available from the RCSB protein data base:

- .pdb file containing atom coordinates and general information.
- -sf.cif file containing reflection data.

PDB2INS - about

- A command line program written in Python.
- Comes as a packaged version.
- Can be used in **interactive modus** where the user is guided through the creation of the file.
- Extensive **command line parser**.
- Fetches files from the **RCSB PDB** server or from the **PDB_REDO** server.
- **Automatically creates restraints** for natural amino acids, nucleotides and the most common ligands.
- Detection of disulphide bridges, incomplete residues and atoms in alternate locations with appropriate handing by **addition of specific restraints**.
- Making use of the new implementations of **chain IDs in SHELXL** (chainID:Residuenumber):

A:1234

PDB2INS – command line options

Usage: pdb2ins <filename|@pdbcode> [options]

Available options (with - as a prefix):

w	followed by the **wavelength**.
h	followed by 3 or 4 to enter **HKLF** manually.
c	and six coordinates (a,b,c,alpha,beta,gamma) in Angstroms and Degree (no spaces) to enter the **cell**.
a	use **anisotropic** displacement data if available.
o	followed by a filename to specify the **output filename**.
r	if a PDB code was give with the prefix ‘@‘, the **PDB_REDO** file is fetched
i	to skip user input.
b	to **create a .hkl file** (structure factor file).
d	followed by a filename for a structure factor file (cif format).

```
pdb2ins myPDBfile.pdb -w 1.54178 -h 4 -c 100,20.5,30.5,90,90,90 -i -a -o myFile.ins
```

PDB2INS - workflow

PDB file

- **Reads PDB file** and extracts all lines with important information.
- For each atom, residue and chain an object is created to allow individual manipulation afterwards without loosing relationship.

User input

- Asks to **verify data** extracted from PDB file or **add missing information**, such as HKLF, wavelength, cell, Z value or space group.
- Asks **additional setting options** such as reset water occupancy or use anisotropic atom data and output file name.

Interpret

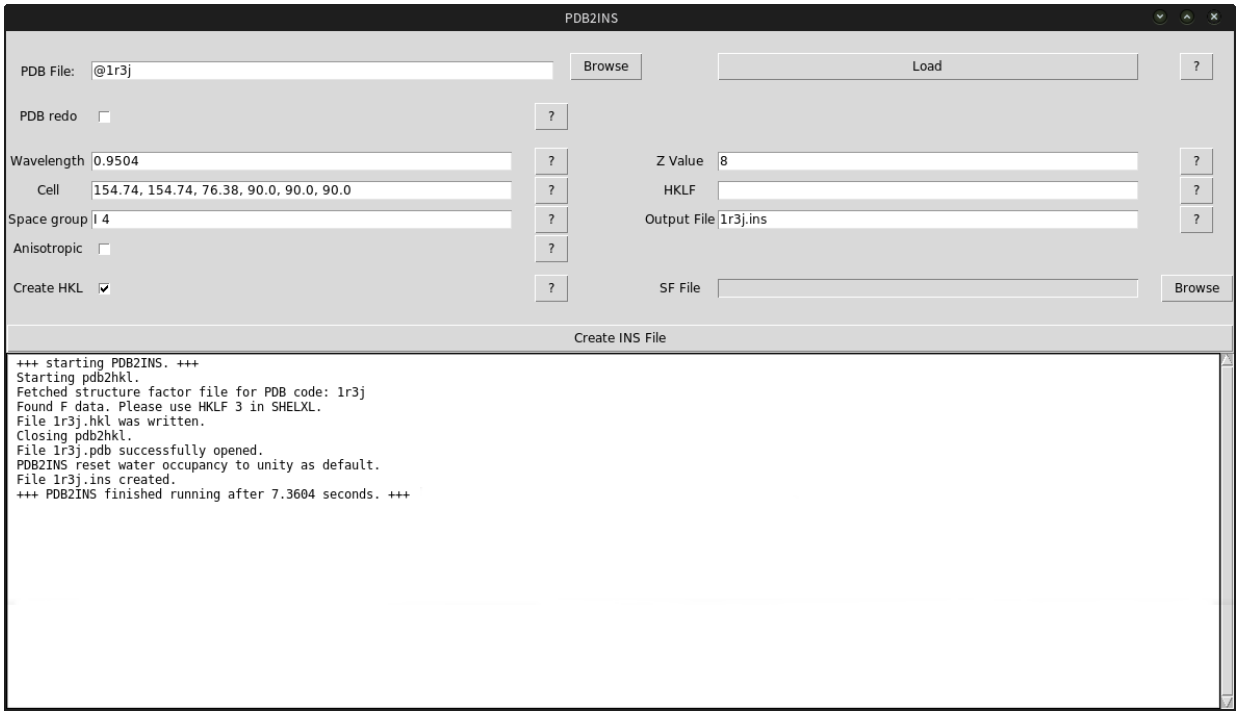
- **Searching** for **disulphide bridges** and **terminal residues** by calculating distances between atoms.
- **Calculating** the **symmetry operations** from the point group and stored symmetry generators.
- **Finding** incomplete residues and the last atom present in them to give appropriate restraints.

INS file

- In different steps the **INS file** is created by first writing a header **with all required crystallographic and residue specific restraints** including a list of residues missing those.
- The main part of the file consists of all **atom data sorted into Residues**. Since SHELXL now recognizes chain IDs all atoms are sorted into residues in the format ‘chainID:Residuenumber’.

PDB2INS - future

- Creating restraints from one PDB file as model for e.g. an wild type homologue or neutron data set.
- PDB2INS GUI is available as test version and will be developed into a stable program.



using interactive mode:

pdb2ins

skipping user input interrupts:

pdb2ins @1r3j –i

pdb2insGUI

Thank you for your kind attention

Myspecial thanks to:

George M. Sheldrick

Jens Lübben, Claudia Wandtke, Massimo Sammito

Tim Gruene

All PDB2INS test users

PDB2INS available at:

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