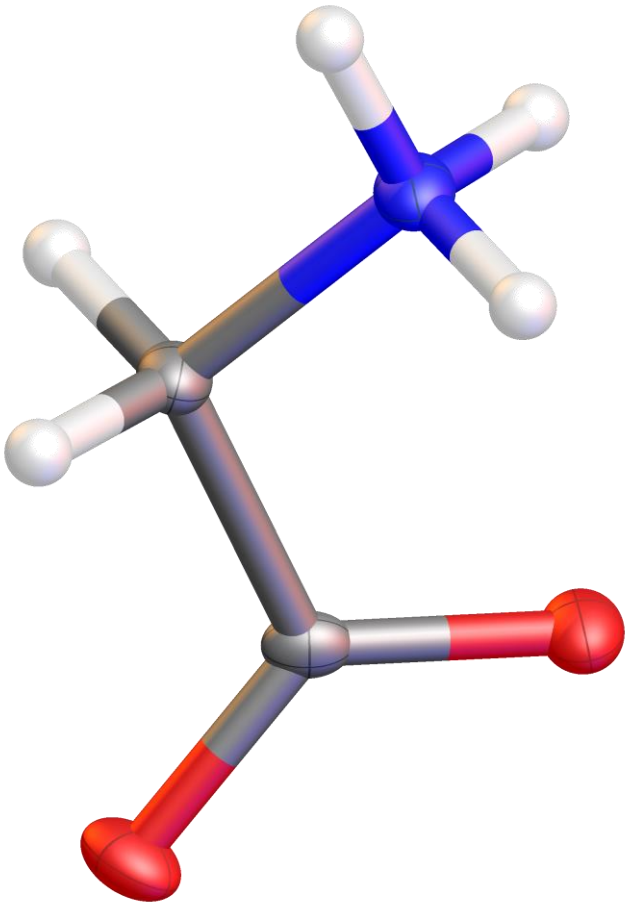
UK National Crystallography Service



Solving and Refining a Crystal Structure using Olex²

Solving and Refining Crystal Structures Using Olex^2

NB. The Olex^2 software can be obtained, free, from http://www.olexsys.org/

Open the Olex^2 software.

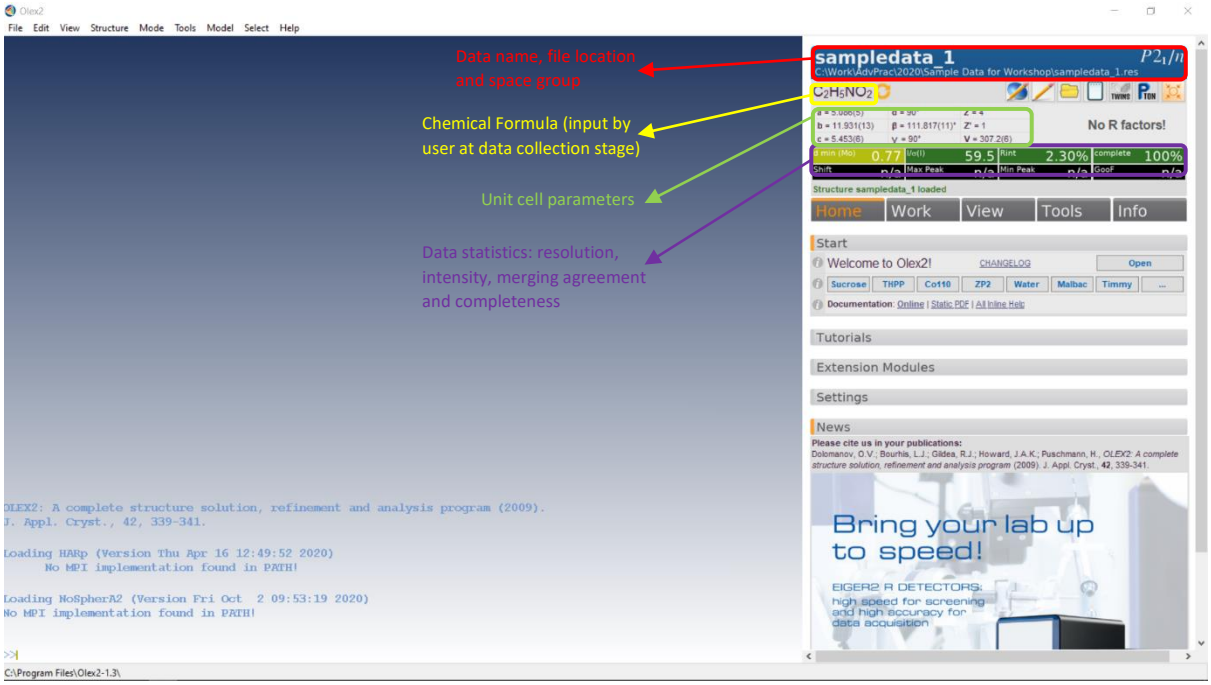
Use either:

File > Open, or

Home tab (on the right) > Open existing structure or data file

and navigate to the folder on your computer containing the sample data (.res file).

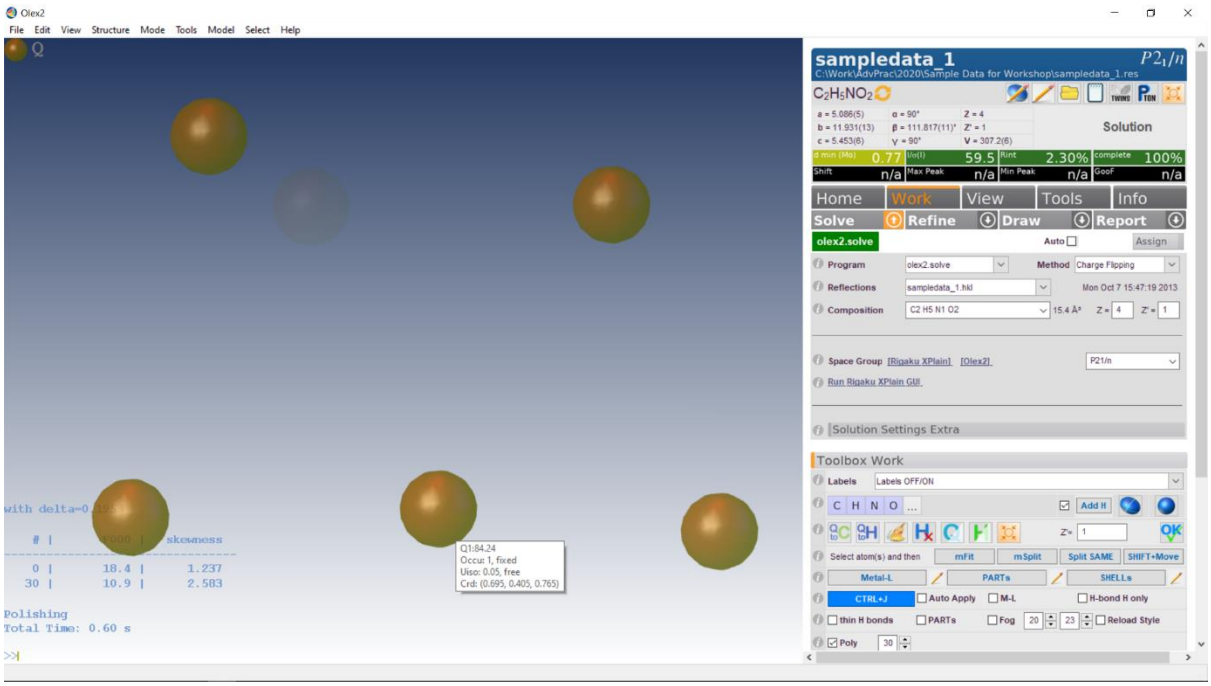
This will open the structure file and provide some initial information about the data (data file name, space group, chemical formula, unit cell parameters).



Structure Solution.

Open the Work tab. Ensure that 'olex2solve' is selected in the solution program drop down menu then click 'Solve' (ensure that 'Auto' is checked off when the dialogue pops up).

This will generate brown spheres (known as Q peaks) on the screen which represent maxima of electron density, ie atomic positions. This is a 3D representation and can be rotated by holding down the left mouse button and moving the mouse.



Hovering the mouse cursor over any Q peak will display information. The Q peaks are numbered sequentially according to amount of electron density (the 'highest peaks' have the most electrons i.e. the biggest will be assigned Q1). This information can also be found in the 'Info' tab under 'Electron Density Peaks'. Use the slider under this menu, or the mouse scroll wheel, to filter which peaks are visible (the scroll wheel will start to remove those with the least density first).

Assign atom types to the Q peaks.

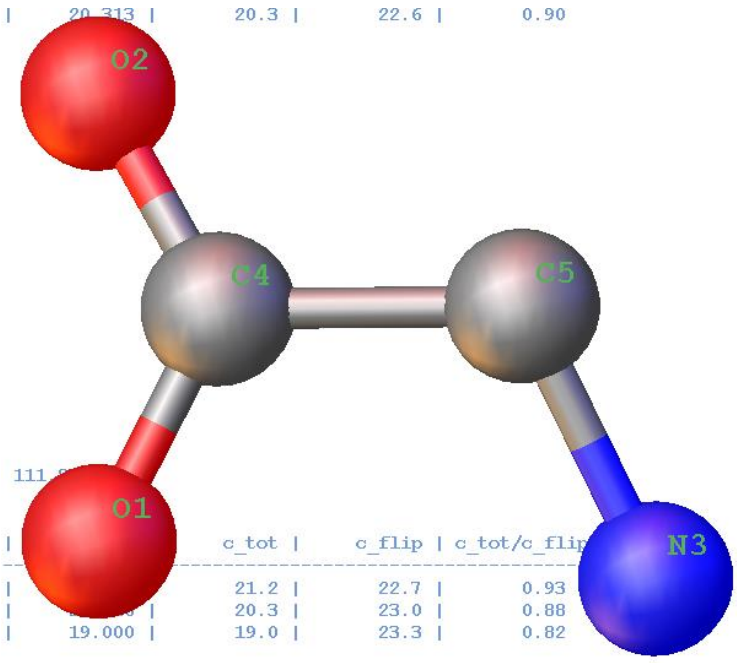
Assigning element types can be done in a number of ways:

1. Select all atoms of the (presumed) same type (click the Q peak and it will turn green), then using the Toolbox Work section of the Work tab, pick the element you wish to assign to these Q peaks.
2. Select the element in Toolbox Work and then pick all atoms you believe to be of that element type
3. Select Q peak (turns green) and type 'name [atom label]', eg name N1

In general the greater electron density (lower Q peak number) indicates a heavier atom type (with more electrons). This Q peak numbering is not definitive and elements very close in the periodic table (similar numbers of electrons, such as nitrogen and oxygen) may appear slightly

out of 'order' in the Q peak list. At this point, hydrogen atoms are not visible as they contain only one electron.
If you cannot make chemical sense of the Q peaks displayed, you can try 'Solve' again, which may give an alternate view of the solution that is easier to interpret. Alternatively, try typing 'compaq –a' or use the  button in the top right corner of the right-hand panel, which can help to complete the structure and connect the Q peaks more sensibly.

You should use chemical knowledge of atoms types and bonding to aid your assignment decisions. The distance between two Q peaks (i.e. a putative bond length) can be displayed by selecting both (click on them and they will turn green) and then hovering the mouse over one of the peaks. Also consider the expected chemical formula and that the bonding makes sense so that, when hydrogen atom placement is considered, all atom types are in accordance with their possible bonding coordination number and geometry.

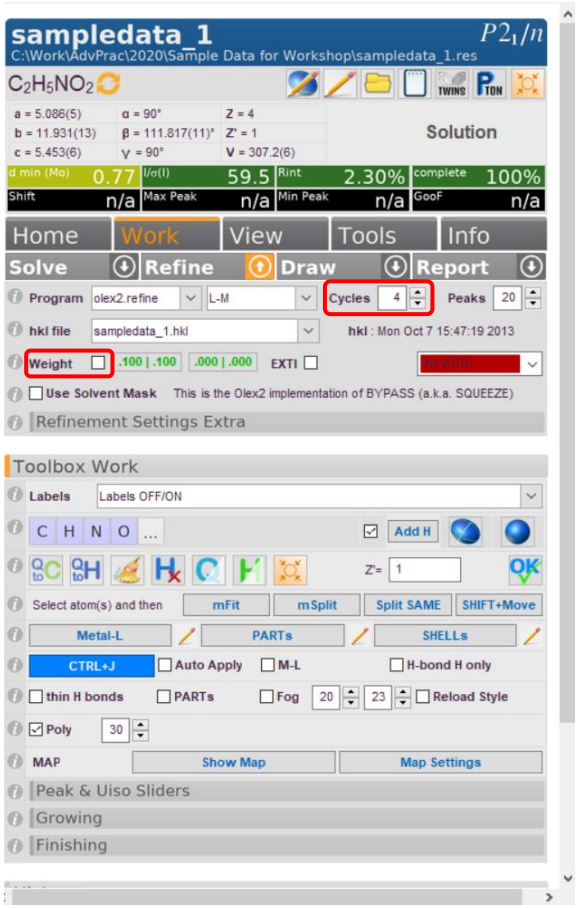


Labelling.
The labels can be changed to specific atom names or turned off using the Labels drop down menu in the Toolbox Work section.

Assign atom types as far as possible (it is not essential at this stage to assign all, or 100% correctly, but try to assign all major Q peaks). Note that some very small, feint peaks may be artefacts of the data rather than true atom positions.

Structure Refinement.

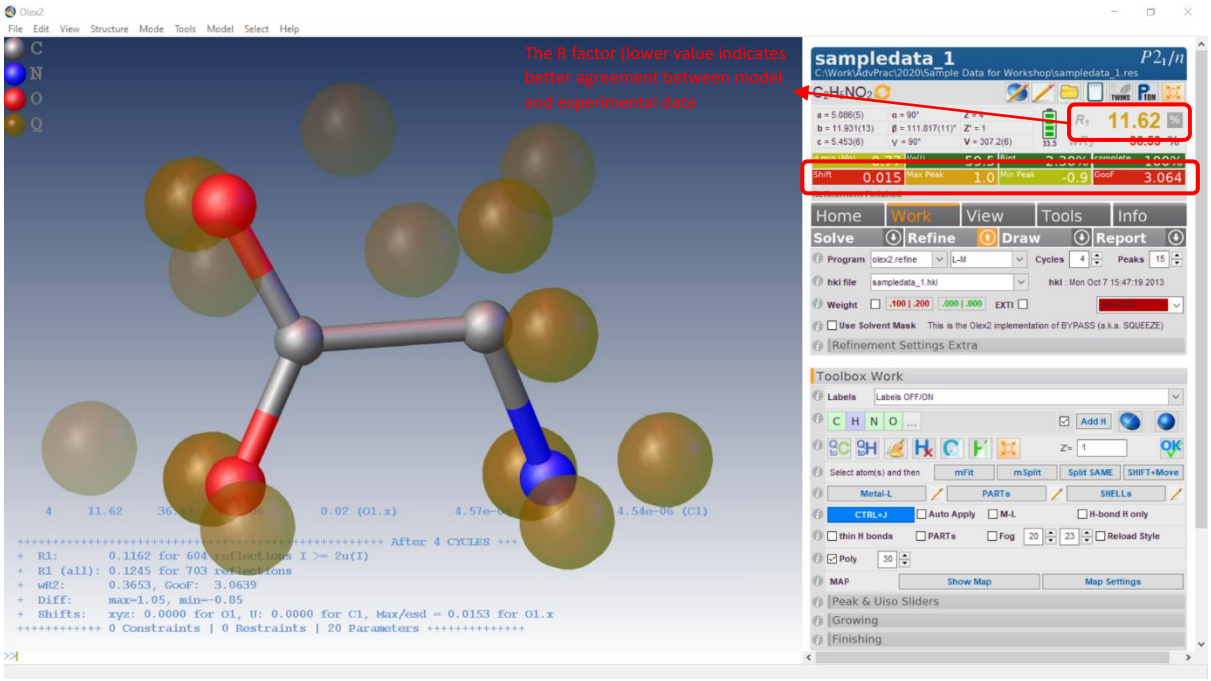
Click the down arrow next to 'Refine' to see the options before running. You can adjust the number of refinement Cycles and should select the box to auto update the weights (as indicated below). Initially 5-10 cycles is sufficient, however when you are close to the final model you may wish to increase this to ensure the model settles with no small shifts occurring. Press the 'Refine' button once you have set the parameters and wait for it to finish.



Refining calculates structure factors based on the current structural model and then compares these to the experimentally measured values. You should refine every time you make a change (or every few changes), as this updates the structure and ensures you are working with the most up to date model (a bit like saving a document).

After the refinement, you will notice the coloured spheres for the atoms reduce in size and further Q peaks appear. In the right hand pane an R1 value will appear along with other statistics about the refinement.

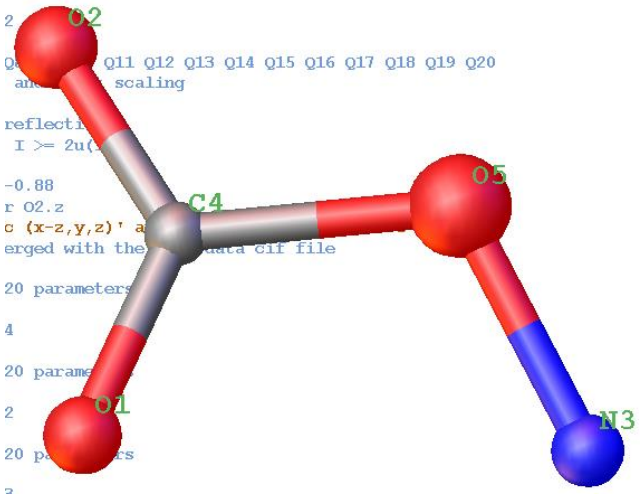
If you wish to remove the Q peaks from the display to get a better view you can use the scroll wheel to reduce the number / remove all, the keyboard shortcut 'Ctrl+Q' which will toggle between showing the Q peaks, showing the peaks with connections and no Q peaks. Alternatively, the Q peak icon  in Toolbox work does the same operation.



The refinement statistics have a traffic light colouring scheme (red = bad, green = good). Dark green is the best and what you should aim for in your final model, but this is unrealistic in some cases. Pale green is equally adequate and generally amber or worse indicates a problem or a situation that would need some justification if you are sure of the model. This is an early-stage model and these statistics and colours will improve as we refine in subsequent stages. 'Shift' represents the greatest change of all refined parameters (between pre and post refinement values) – in a final model these values should be essentially zero and not changing significantly; Max/min peaks are the main residual electron density, Q peaks, yet to be assigned; GOOF is the statistical Goodness of Fit of the model to the data (should converge to 1).

The R factor and statistics are not the only measures of the structure quality. Always ensure that your structure makes chemical sense, i.e. the configuration and connectivity should be chemically correct (element, valence, etc) with reasonable geometry (bond angles and lengths).

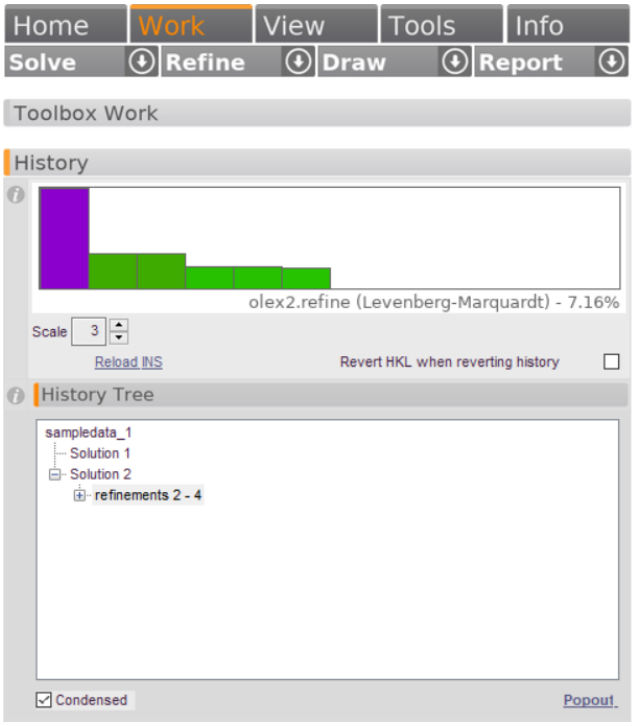
At this stage, you may find that you have unassigned or wrongly assigned atom types. Any Q peaks greater than ~3 units are likely to be unassigned atom – try to assign these. Incorrectly assigned atoms are not always obvious, but can be identified at this stage if the sphere is larger or smaller than the rest, particularly in comparison to any other atoms of the same type. The image below shows a carbon incorrectly assigned as an oxygen atom (O5) and appears significantly larger than the other oxygens (or indeed other spheres in the model).



If a sphere appears too small in comparison to others in the structure it is a heavier atom (more electron density) than that assigned (eg an oxygen assigned as a carbon). Conversely, if a sphere appears too large the atom assigned is too heavy for the atom actually present (as above, carbon assigned as oxygen). Repeat cycles of altering atom types and refining until all spheres are a similar size and you are confident that correct assignment of all appropriate Q peaks is made. Also, look at the electron density peak information (Info tab), if all remaining Q peaks have an intensity below 3 it is likely that all non-hydrogen atoms are assigned.

Undoing Mistakes.

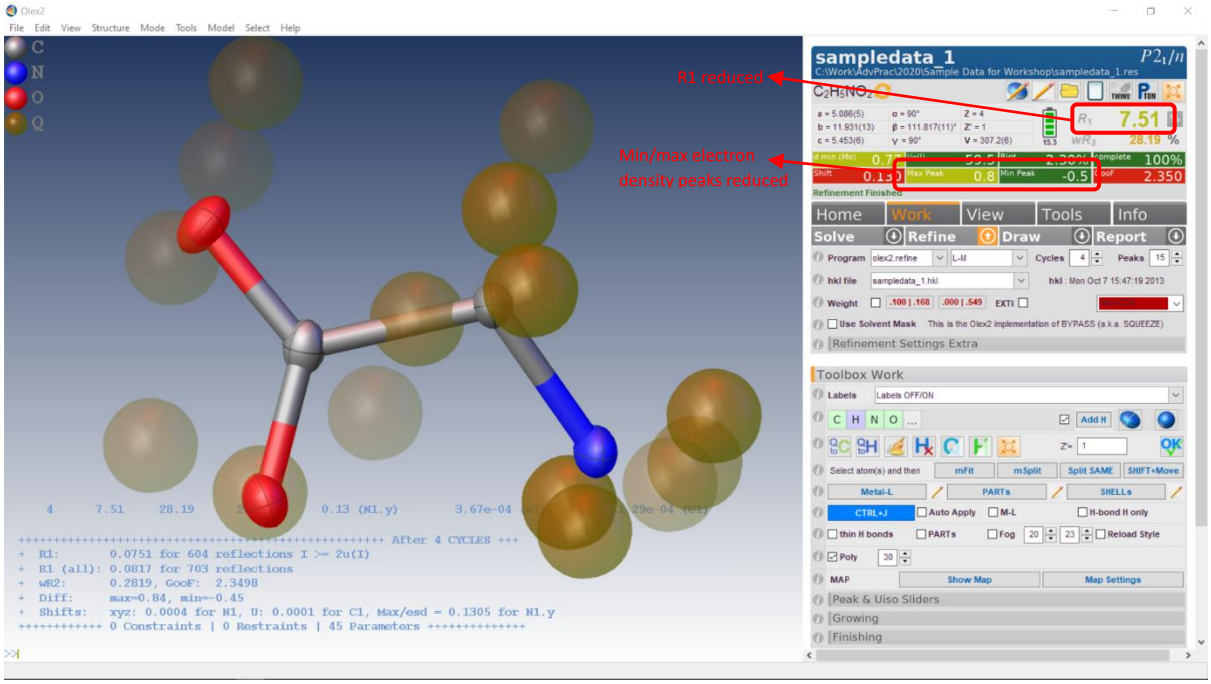
If you make a mistake, or the refinement goes in the wrong direction (making the model worse) and you wish to go back to the previous version, use the histogram displayed in the History section of the Work tab to view the different R1 values for previous models in order. Clicking on a particular bar and doing a Refine job will take you back to that model.



Anisotropic Refinement.

This converts the spheres into ellipsoids (rugby ball shapes) allowing for atomic displacement due to thermal motion and give 6 parameters for an atom, rather than one. Clicking the rugby ball-shaped icon on the right-hand pane converts all atoms to anisotropic and conducts a refinement cycle.

Anisotropic displacement modelling should (significantly) improve the model as electron density around the atoms is incorporated. This will generally result in fewer Q peaks, particularly around the atom centres. The max / min electron density is reduced (here shown within acceptable limits and coloured green) and the R1 has reduced (also green).



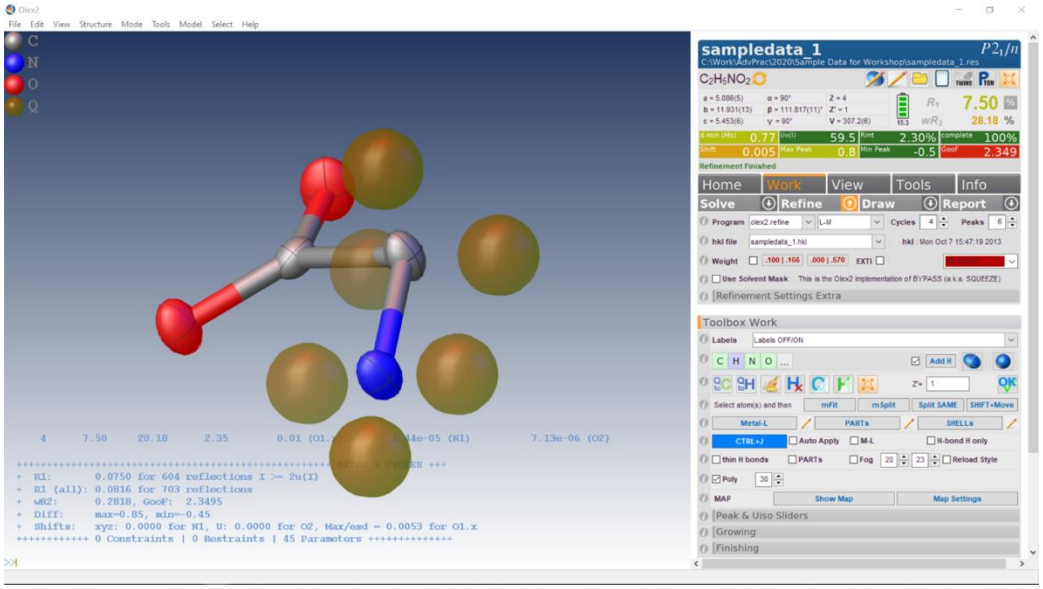
As with the spheres, the rugby balls should be of similar size and shape. If there are any incorrect atom assignments, they will be displayed as smaller ellipsoids or tetrahedral-shaped atoms if the atom should be heavier than the assignment, or they would have larger ellipsoids if the atom should be lighter than the assignment given. If reassignment is required it is best to reset the atoms to spheres (select the sphere next to rugby ball icon) to give isotropic atoms again, make the adjustments and refine before continuing with an anisotropic refinement.

Improving the model.

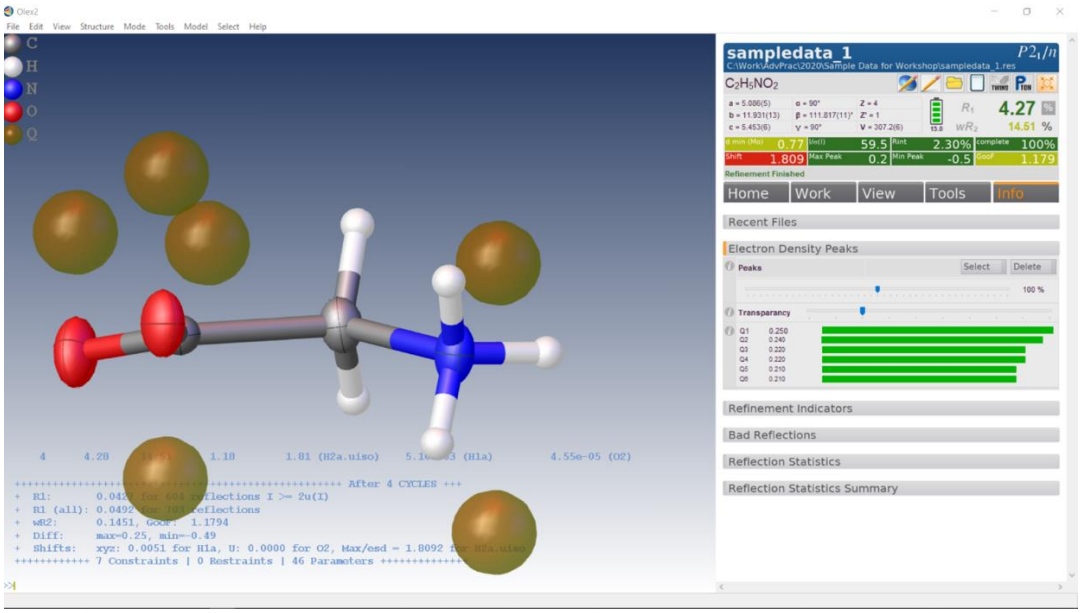
The model shown still displays some areas for improvement, e.g. the shift and weights are red. Improvements can be made by simply doing further refinement cycles as it is clear from the shift value that the model is being modified significantly in the refinement.

Hydrogen Atoms.

As this is a small molecule and the data are very good, we can see all hydrogen atom positions in the electron density map - this is not always the case however! Therefore, all hydrogen atoms are generally added in calculated, fixed positions. This can be done by clicking the box 'Add H' in Toolbox Work. This will add hydrogens to the selected atoms, or if no atoms are selected it will consider all atoms based on atom types and bond distances and add as appropriate. It is also possible to assign hydrogens from the electron density map (select the Q peak and assign as H), but this is only generally done in more complicated cases.



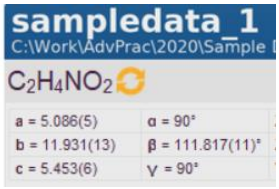
After adding all hydrogen atoms and completing a number of refinement cycles (to reduce the shift towards 0 and allow all indicators to settle), we should see a significant improvement in the model through a drop in R1 and all indicators turning towards green/dark green. The Q peak intensities should be checked to ensure that the remainder are now negligible (small values and all green under the Electron Density Peaks section in the Info tab, see image below). These arise from 'noise' in the data and can be ignored - use 'Ctrl+Q' to change the view so they are no longer visible.



Finalising the Structure.

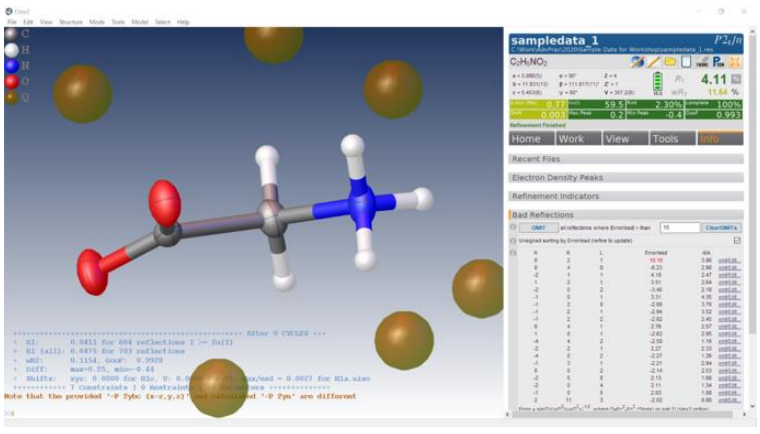
You now have your basic model for the crystal structure that visually looks fine, however it needs tidying up and checking that it is both chemically and crystallographically correct (termed validation). The output files required also need to be created.

The chemical formula will still be based upon the original estimate made back at the point of data collection on the diffractometer. This is sometimes incorrect. If there are circular arrows next to the formula (very top of the right-hand pane, under the data file name) it is wrong and clicking the arrows will update it. Since the update is based on a multiplier of the atoms currently in the structure, it is best to first ensure you are confident that the model and atoms present are correct, and that no hydrogens are missing / extra added.



The formula should update to that expected. However, if you have an incorrect value of Z' in the box next to the OK/tick button in Toolbox Work of the Refine tab, you may have a multiple of the expected formula. Z' is the number of molecules in the asymmetric unit and in the case of this example it is 1. It can be a whole number or fraction depending on space group and symmetry of the molecules present. Once certain that Z' is correct, click the OK/tick button to update the formula. Afterwards run a further refinement cycle.

Your data may include reflections affected by the way they were measured on the instrument. To identify this, use the Info tab > Bad Reflections. Any reflections with large $|F_c^2-F_o^2|$ / esd values, may need to be omitted - they will be obviously different to the rest of the values and often coloured red. Use the 'omit' to remove any as required and refine the structure again. Check again, as there may be more bad reflections arising after the first set have been removed. Note: this should only be carried out once the structure is complete, otherwise you could remove perfectly good reflections. Also, you should never have to remove more than a few reflections (most commonly none at all) from good data.



Quality, 'Correctness' and Validation of the Structure.

To get the best structure and most accurate representation of the compound from the experimental data there are certain values which should be monitored throughout the solving and refining process. These have been briefly mentioned in the process and can be found under the Info tab and are always displayed in the 'refinement indicators' header. The main indicators are: R1, wR2, Max / min electron density map peaks, GooF, Hooft y, Flack x.

R1 and wR2 are an indication of the agreement between the observed and calculated structure factors (F_c and F_o) and will reduce as the refinement progresses and the agreement improves. High values (>0.4 & 0.7, 40 & 70% respectively) indicate that there are problems with the solution and you should look to rectify these.

GooF is the goodness of fit and should converge towards 1 towards the end of the refinement.

The last two, Hooft y and Flack x are applicable only for non-centrosymmetric space group structures (with no inversion centre). These are displayed with an error and should be close to 0 if the solution is correct. A value ~1 indicates that the structure should be inverted and those very different to 0 or 1 show a racemic twin.

Additionally:

- the error on parameters (standard uncertainty – the figure in brackets after a value) should be as low as possible,
- all anisotropic displacement parameters should be meaningful (no negatives),
- the observed reflections : no of refined parameters ratio should be at least 10 : 1
- most importantly, the **model should make chemical sense / be chemically correct**.

Possible areas for errors and a model not being as good as expected can include:

- Wrong space group
- Wrong symmetry
- Wrong atom type assignment
- Insufficient reflections / weak diffraction data
- Extinction correction required
- Twinning
- Disorder present
- Unassigned solvent molecules

An extinction correction may be required and this may be indicated in the lines of text behind the structure which detail information on the refinement cycles. If this is the case (generally for larger crystals) tick the box next to EXTI in the top section of the refine menu (next to Weights) and do a set of refinement cycles. Inspect the value returned (next to the box you ticked) and if the correction is greater than the error, and improves the model, then keep this setting. If there is no model improvement or the error is of similar size to the value, untick the box and refine again to remove any changes made.

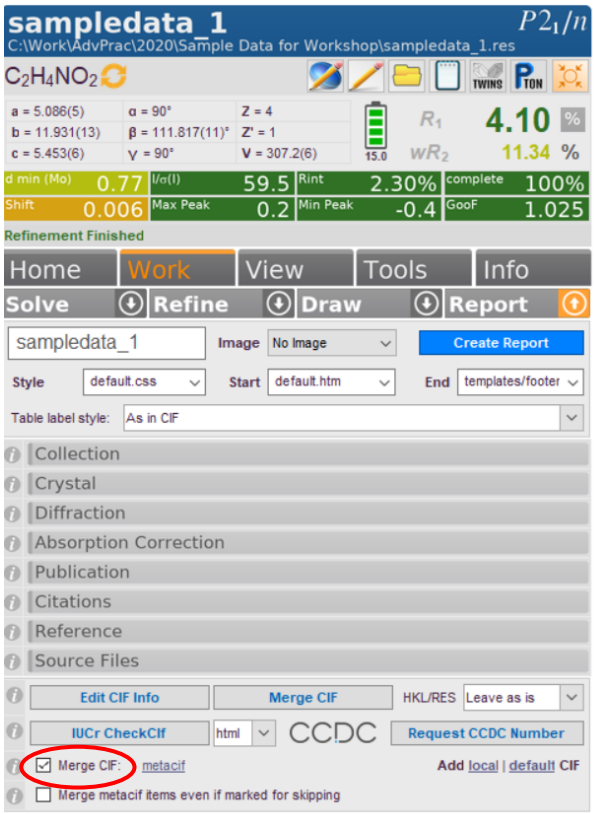
Results generation.

The final output will be a .cif file (this can be used for structure visualisation and checks). To ensure the .cif file is written, in the main Refine tab, the drop down menu below Peaks, needs to be changed from No ACTA to ACTA (note, background colour will change from red to green). After running a further refinement cycle, a .cif file will be generated in the working file directory and will be updated each time further refinement cycles are carried out

Reporting.

You can do a number of checks and add information using the Report tab. Click the arrow next to Report to display the report sections. The main sections of interest are Crystal (add crystal colour, size, description) and Collection (add sample name). You should add a data collection temperature under Diffraction. Ensure the Merge CIF box is ticked and run a refinement by clicking Refine.

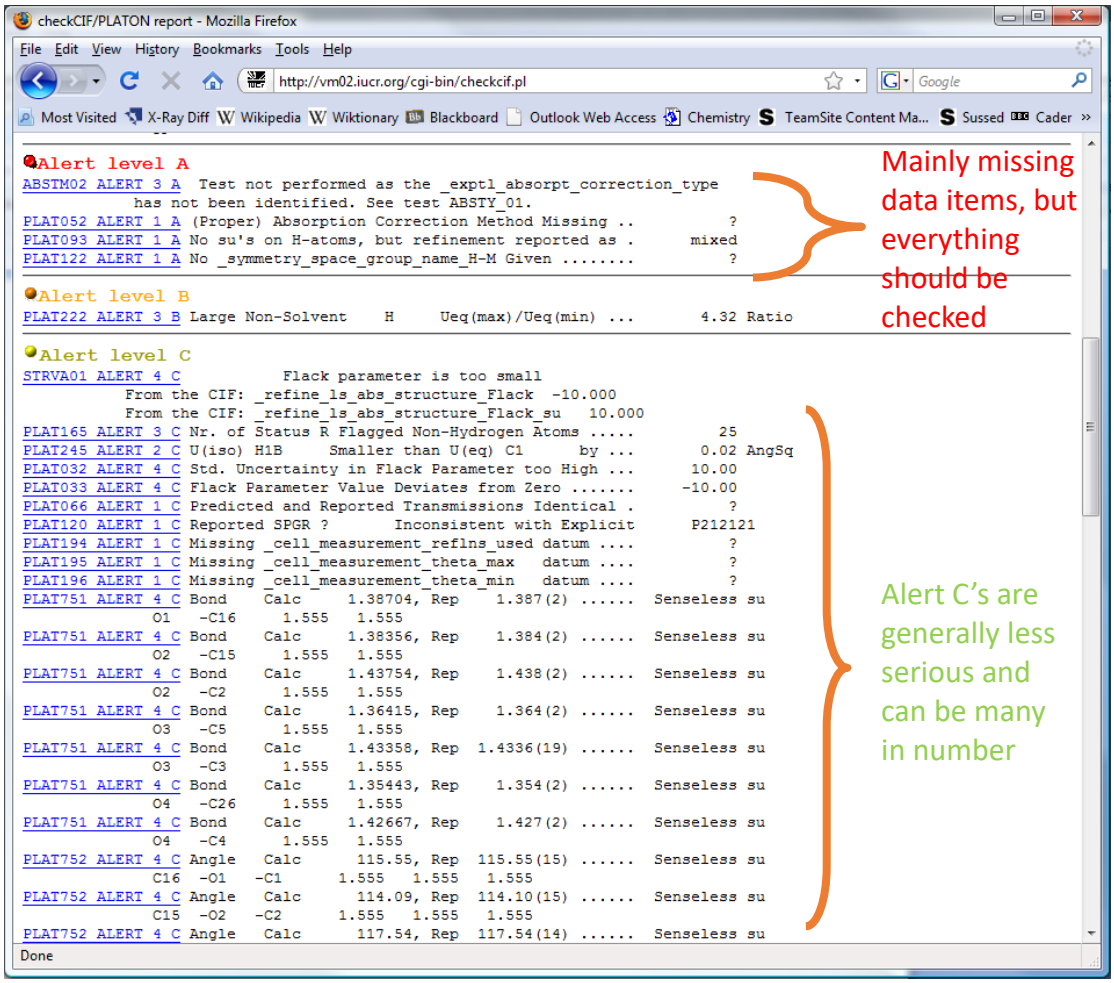
Some of this information should have been automatically inserted, as it will have been read from the original .cif_od file



A check for errors in the model can be made using the IUCr (International Union of Crystallography) CheckCIF service. To run a CheckCIF report, either click IUCr CheckCIF (check html or pdf format pull down) or go to http://checkcif.iucr.org/ and upload your .cif file (see figure below).

This will produce a guidance report with alerts of varying levels indicating areas that may need looking at or reviewing. Alert level A's should always be resolved or have good reason behind their occurrence. B and C levels alerts should also be resolved if possible. It is not always possible to resolve all alerts, in fact sometimes an alert could highlight something interesting about the structure. So this system is merely indication that there is something that needs to be investigated further.

A typical output of some CheckCIF alerts is shown below. There are hundreds of different possible alerts. For more information about each alert/error, in the html report, click on the blue alert itself to bring up an explanatory window. This will provide extra reasoning behind the alert and could allow you to fix the issue.

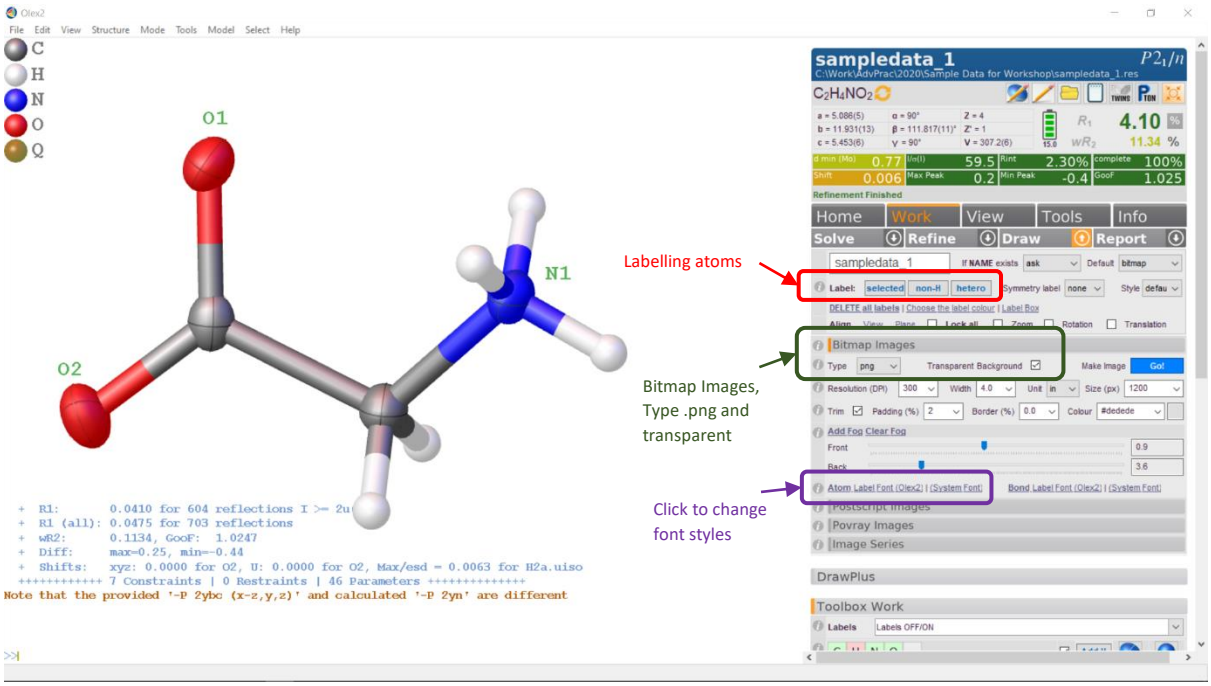

checkCIF/PLATON report - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://vm02.iucr.org/cgi-bin/checkcif.pl
Most Visited X-Ray Diff Wikipedia Wiktionary Blackboard Outlook Web Access Chemistry TeamSite Content Ma... Sussed Cader
Alert level A
ABSTM02 ALERT 3 A Test not performed as the _exptl_absorpt_correction_type has not been identified. See test ABSTY_01.
PLAT052 ALERT 1 A (Proper) Absorption Correction Method Missing .. ?
PLAT093 ALERT 1 A No su's on H-atoms, but refinement reported as . mixed
PLAT122 ALERT 1 A No _symmetry_space_group_name_H-M Given ..... ?
Alert level B
PLAT222 ALERT 3 B Large Non-Solvent H Ueq(max)/Ueq(min) ... 4.32 Ratio
Alert level C
STRVA01 ALERT 4 C Flack parameter is too small
From the CIF: _refine_ls_abs_structure_Flack -10.000
From the CIF: _refine_ls_abs_structure_Flack_su 10.000
PLAT165 ALERT 3 C Nr. of Status R Flagged Non-Hydrogen Atoms 25
PLAT245 ALERT 2 C U(iso) H1B Smaller than U(eq) C1 by ... 0.02 AngSq
PLAT032 ALERT 4 C Std. Uncertainty in Flack Parameter too High ... 10.00
PLAT033 ALERT 4 C Flack Parameter Value Deviates from Zero ..... -10.00
PLAT066 ALERT 1 C Predicted and Reported Transmissions Identical . ?
PLAT120 ALERT 1 C Reported SPGR ? Inconsistent with Explicit P212121
PLAT194 ALERT 1 C Missing _cell_measurement_reflns_used datum .... ?
PLAT195 ALERT 1 C Missing _cell_measurement_theta_max datum .... ?
PLAT196 ALERT 1 C Missing _cell_measurement_theta_min datum .... ?
PLAT751 ALERT 4 C Bond Calc 1.38704, Rep 1.387(2) ..... Senseless su
O1 -C16 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.38356, Rep 1.384(2) ..... Senseless su
O2 -C15 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.43754, Rep 1.438(2) ..... Senseless su
O2 -C2 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.36415, Rep 1.364(2) ..... Senseless su
O3 -C5 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.43358, Rep 1.4336(19) ..... Senseless su
O3 -C3 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.35443, Rep 1.354(2) ..... Senseless su
O4 -C26 1.555 1.555
PLAT751 ALERT 4 C Bond Calc 1.42667, Rep 1.427(2) ..... Senseless su
O4 -C4 1.555 1.555
PLAT752 ALERT 4 C Angle Calc 115.55, Rep 115.55(15) ..... Senseless su
C16 -O1 -C1 1.555 1.555 1.555
PLAT752 ALERT 4 C Angle Calc 114.09, Rep 114.10(15) ..... Senseless su
C15 -O2 -C2 1.555 1.555 1.555
PLAT752 ALERT 4 C Angle Calc 117.54, Rep 117.54(14) ..... Senseless su
Done
Mainly missing data items, but everything should be checked
Alert C's are generally less serious and can be many in number

Generating a Report

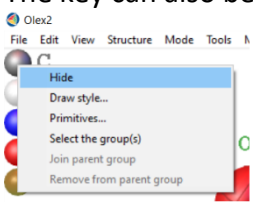
Once satisfied that you have the best structure possible, although all necessary information is in the .cif file, a human readable report should also be generated.
It is essential to have a high quality, labelled thermal ellipsoid diagram in the report to aid correctness and quality assessment. This can be created by using the Draw tab. Click the arrow next to Draw to display the draw sections.

Firstly, rotate and move the structure (click and hold left mouse button) until it is in an appropriate position i.e. with as few atoms obscured as possible while displaying the full geometry of the molecule.

You can decide which atoms you wish to label by selecting one of the Label options (note: if you have any bonds selected when you click selected those bonds will also be labelled!). Generally, hydrogen atoms should not be labelled unless they are vital to explain the structure (such as involved in a vital hydrogen bond). Once labels have appeared, the font, size and colour can be changed. It is possible to move each of the resulting labels around by holding the left mouse button down whilst clicking on the label and holding the mouse button down until you have moved it where you want. If you find a single label you do not want, simply left click on it to highlight it then press the delete key

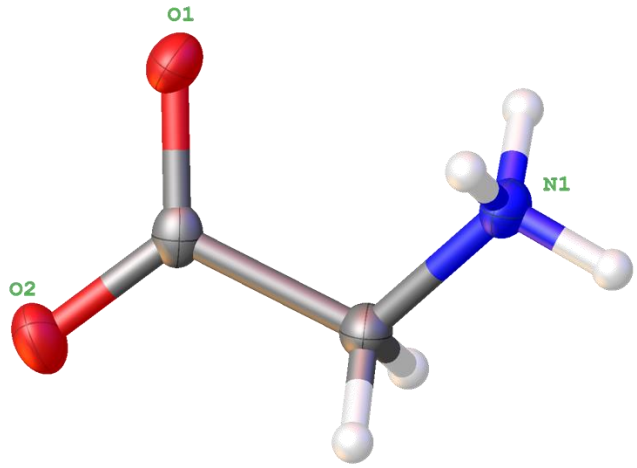


The key can also be removed for a cleaner figure by hiding it (right click on it and select hide).



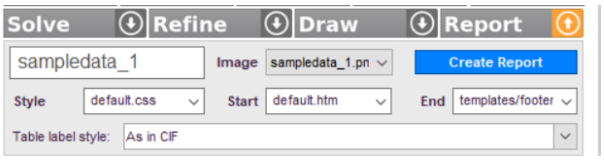
The standard background can be toggled between white, blue and shaded using the function keys F2 and F4. The background writing will not appear on the images generated. For the image a white background is generally considered to be best for viewing features e.g. thermal ellipsoids.

When the image is displayed as you finally want it, in the BITMAP image section set to .png and make sure transparent background is ticked, then click the Go button. This will generate a .png file of the same name as the structure (if you generate further images it will allow you to rename them).



A typical .png output, with just the hetero atoms labelled.

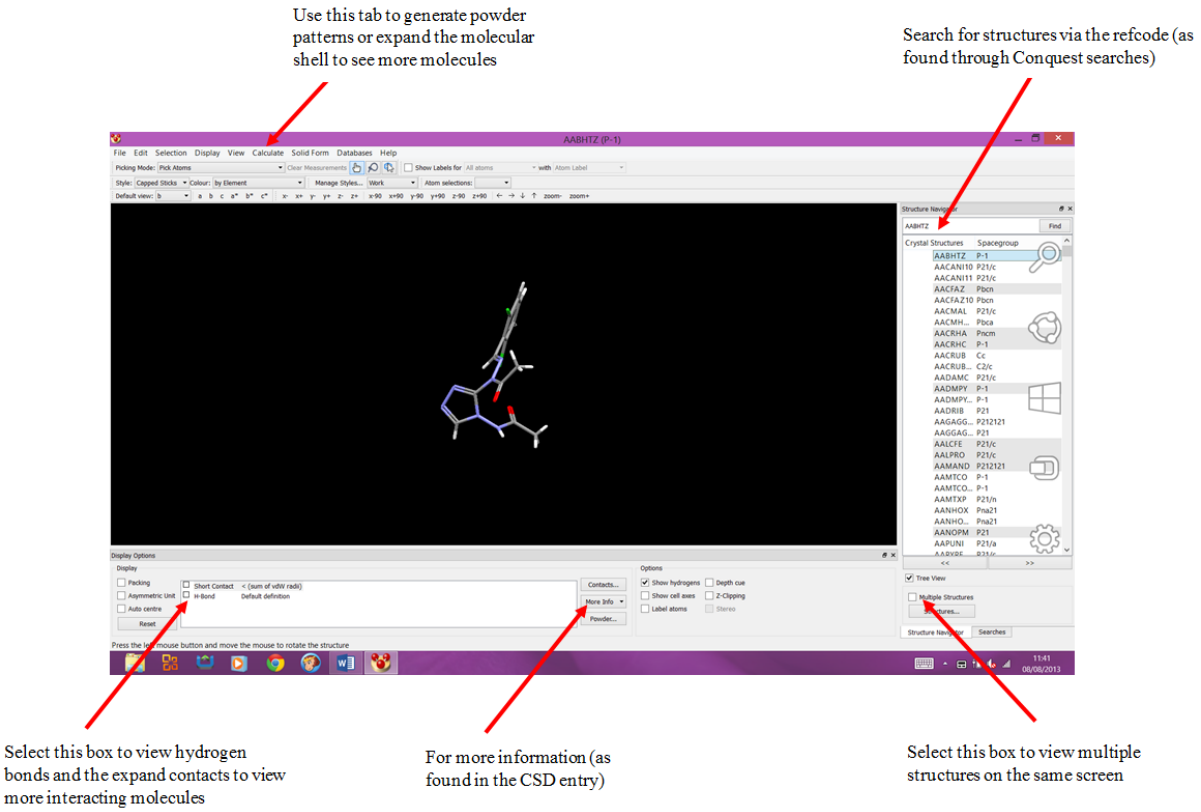
Once the .cif and .png image are complete you are in a position to generate a suitable report. In the Report tab make sure the image you created is selected in the Image drop down menu. Then click Create Report to generate a basic report that can be viewed in a web browser (html format).



Further Structure Visualisation.

Your final model can then be used in analysis to investigate bond distances, angles, interactions and packing and to relate other experimental data and findings to the structure. Structure visualisation and interrogation can be performed by opening the .cif file in the Mercury software. Mercury is part of the CCDC (Cambridge Crystallographic Data Centre) Software suite and is used for visualising crystal structures and investigating some of their properties. Mercury is freely available to all UK academics and can be downloaded from http://www.ccdc.cam.ac.uk/Solutions/CSDSystem/Pages/Mercury.aspx .

Features include measuring bond lengths and angles, atomic distances, inter-and intra-molecular interactions, similarity of molecules and packing in the solid state. Multiple structures can be displayed at once and overlaid to assess similarity/differences.
Some of the more advanced features are only available if you register with CCDC, but that only requires submitting your Uni email address.



To measure bond lengths or angles, change the option in 'Picking mode' (top left drop down menu), where you will be able to measure distances (between any atoms, not just those bonded to one another). There are a variety of other options in this menu which allow further analysis of the crystal structure.

For analysing and comparing structures use the options at the bottom of the display (eg short contacts, packing etc).