

Example: Abiraterone acetate

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Data	Topic	Level
Electron diffraction	Determination of absolute structure with dynamical refinement	Beginner

Introduction

In this example, we start at the stage after structure solution of the pharmaceutical compound abiraterone acetate ($C_{26}H_{33}NO_2$) and we are only interested in the determination of the absolute structure. First, we do not refine any structural parameters (only scale factors and thickness) of the two enantiomorphs. Even with “slow” computers, this step should be only a matter of minutes. We then refine coordinates and isotropic displacement parameters. Data reduction, structure solution and kinematical refinement are not part of this example.

Input data

Data

Electron diffraction data were measured on a transmission electron microscope FEI Tecnai G² 20 (LaB₆) with acceleration voltage of 200 kV ($\lambda = 0.2508 \text{ \AA}$). The sample temperature was 100 K. Diffraction patterns were recorded analogous to the rotation method implemented in most modern X-ray diffractometers in 0.4° steps, i.e. the goniometer performed a rotation of 0.4° while the diffraction patterns were recorded (“continuous rotation”). Several data sets were measured. Data reductions were performed with PETS2 (not part of this tutorial). Here, two data sets were combined for the structure solution and one of them is used for the dynamical refinement.

Input files

abiraterone_acetate_solution.cif: Structure solution generated with SIR 2014
abiraterone_acetate_03_dyn.cif_pets: output file from PETS2
constraints.txt: list of constraints

Additional information

- folder “refinements”: intermediate states of Jana files for comparison and as reference
- used software: Jana2020 (jana.fzu.cz), VESTA (jp-minerals.org/vesta/en/download.html)

References

- L. Palatinus et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: theory and implementation. Acta Cryst. A71: 235–244 (2015).
- L. Palatinus et al. Structure refinement using precession electron diffraction tomography and dynamical diffraction: tests on experimental data. Acta Crystallogr B71: 740–751 (2015)
- terminology of absolute structure: https://dictionary.iucr.org/Absolute_structure

Instructions

1. Create new structure

Start Jana2020

In the Main menu bar, use “Structure → New” and open new structure “abiraterone_acetate”
OPEN

2. Import Wizard

[On the screen: Specify type of the file to be imported]

Select “Structure: from CIF”; NEXT

Specify type of the file to be imported

Single crystal:

- ☐ known diffractometer formats
- ☐ reflection file corrected for LP and absorption
- ☐ start with indexing procedure

Powder data:

- ☐ various CW formats
- ☐ various TOF/ED formats

Structure:

- ☐ from SHELX
- ☒ from CIF

[On the screen: Data reduction file from]

Locate “abiraterone_acetate_solution.cif”; OPEN

CIF file does not contain any reflection block. Do you want to import data from file?

YES

[On the screen: Specify type of the file to be imported]

Specify type of the file to be imported

Single crystal:

- ☒ known diffractometer formats
- ☐ reflection file corrected for LP and absorption

Select “Single crystal: known diffractometer formats”; NEXT

Select “Pets electron diffractometer”

“Browse” for the file “abiraterone_acetate_03_dyn.cif_pets”; OPEN

Data reduction file from:

Input file name: abiraterone_acetate_03_dyn.cif_pets [Browse]

- ☐ Nonius-CCD
- ☐ Bruker-CCD
- ☐ Bruker-CCD (raw)
- ☐ Oxford Diffraction-CCD
- ☐ Rigaku-CCD
- ☐ IPDS Stoe
- ☐ D9-ILL, D23 or Tris-Zebra
- ☐ HeDi
- ☐ ILL-Vivaldi
- ☐ ISIS SXD
- ☐ TOPAZ
- ☐ Koala at ANSTO
- ☐ SCD-LANL
- ☐ HasyLab F1
- ☐ HasyLab HUBER
- ☐ HasyLab XDS
- ☐ 6T2 LBB
- ☒ Pets electron diffractometer
- ☐ SENJU TOF
- ☐ Polarized neutrons
- ☐ SHELX on I - abs.correction needed

☒ Make the reflection file for dynamical refinement

Check “Make the reflection file for dynamical refinement”; NEXT

Note the wavelength of 0.0251 Å.

Set temperature to 100; NEXT

Leave all settings unchanged (no twinning, no modulation); NEXT

The program reads 19797 reflections.

OK

For absorption correction select “None or done before importing”; NEXT

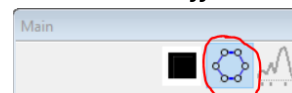
FINISH; OK; NEXT

6960 observed and 19797 reflections in total imported.

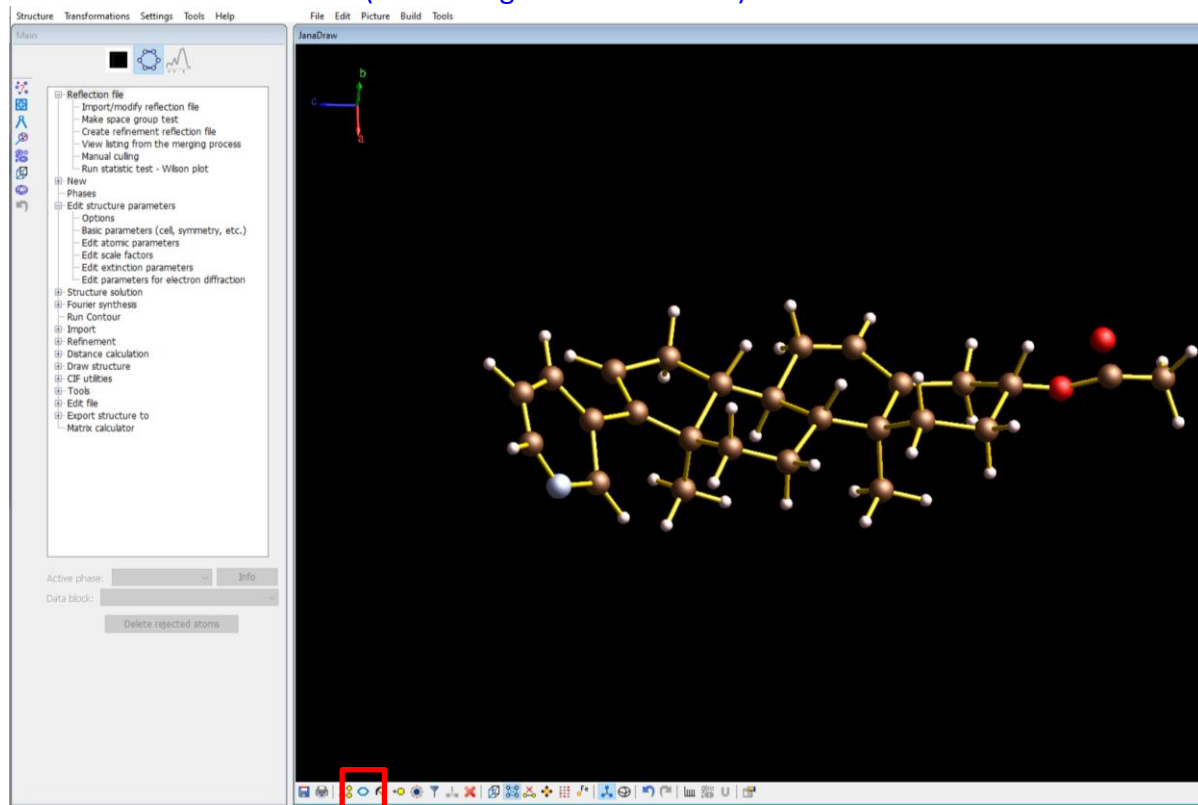
OK; OK; FINISH

We now finished providing the initial structure model, the reflections list and the diffraction geometry. We can have a look at the structure with JanaDraw.

Start JanaDraw by clicking on the six-ring above the command tree



Click “Build molecule” icon (blue hexagon at the bottom)



3. Setup of dynamical refinement

In the command tree (left), expand “Edit structure parameters”
 → “Edit parameters for electron diffraction” (double click)

In the top section on the left:

Set “Maximal diffraction vector $g(\max)$ ” to 1.0

Set “ $RSg(\max)$ ” to 0.6

Set “ $DSg(\min)$ ” to $0.0025 \text{ (\AA}^{-1}\text{)}$

Set “Number of threads” to the number of physical cores of your processor

In the top section on the right:

Set “Number of integration steps” to 96

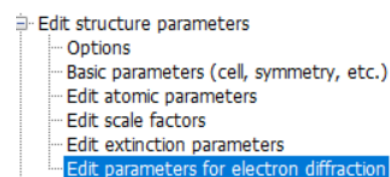
Set “Geometry” to “rotation”

In the section in the middle:

“except of scale, optimize also”: check “Thickness”

Click “Run optimizations”

The dependence of R factors of individual virtual frames on the thickness is determined. This step with an 8-core processor takes ~3 minutes. The initial thickness estimation is necessary to get a stable starting point for the dynamical refinement and to avoid getting stuck in a local minimum.



Orientation matrix: U11	0.0391	U12	0.08833	U13	-0.01231
U21	0.12489	U22	-0.02613	U23	0.00495
U31	0.00477	U32	-0.04121	U33	-0.02957

Maximal diffraction vector g(max):	1.0	Number of integration steps:	96	Geometry:	<input type="radio"/> precession
Maximal excitation error (Matrix):	0.01	Use dynamic approach:	<input checked="" type="checkbox"/>		<input checked="" type="radio"/> rotation
Maximal excitation error (Refine):	0.1	Use twin version:	<input type="checkbox"/>		
RSg(max):	0.6	Apply correction for crystal tilt:	<input checked="" type="checkbox"/>		
DSg(min):	0.0025	For Fourier rescale to Fcalc:	<input type="checkbox"/>		
Number of threads:	8				
Dyngo commands:					

Select zones for refinement

Define zones of equal thicknesses

Run optimizations

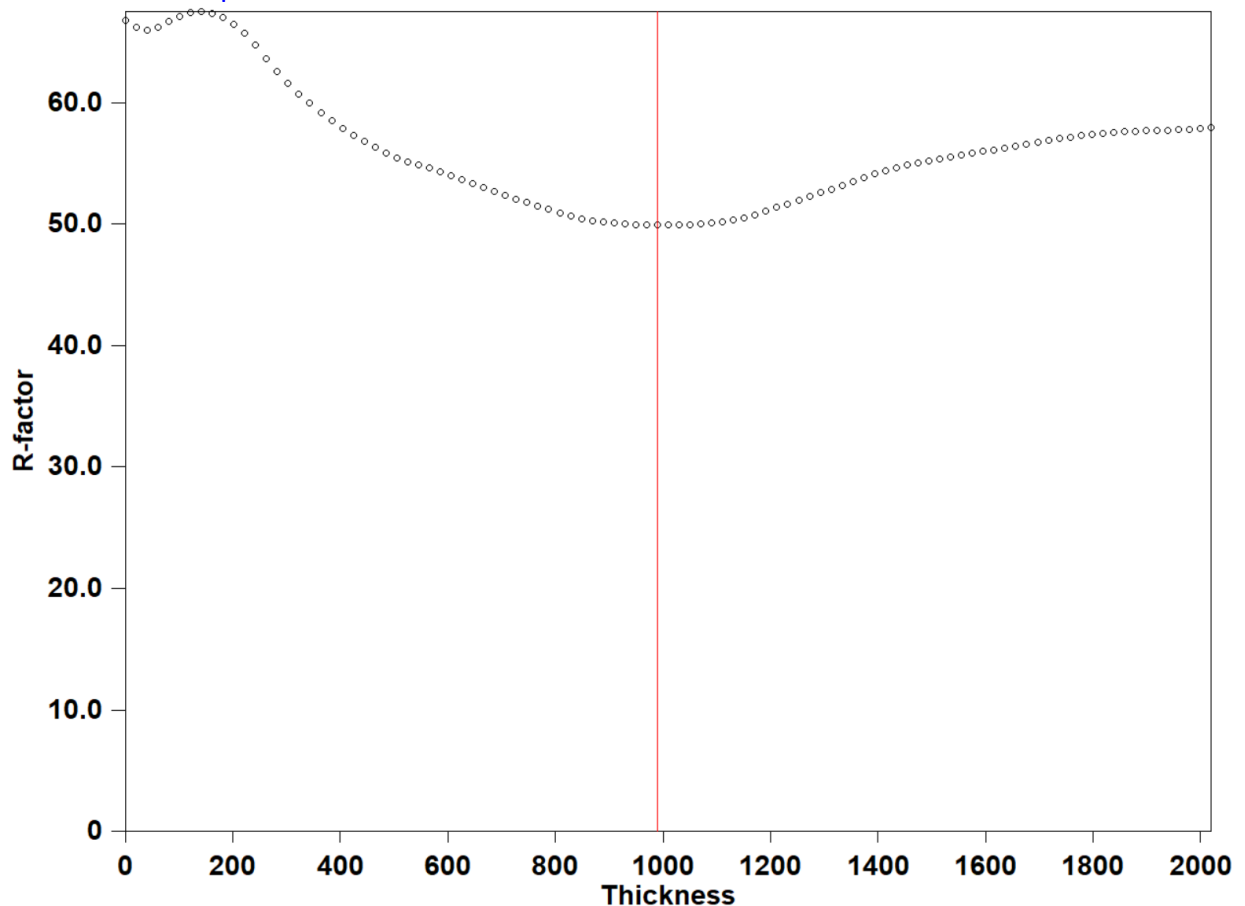
except of scale, optimize also:

☒ Thickness

☐ Orientation

Show thickness plots

“Show thickness plots”



Close the plot (red X).

For frames with a well defined minimum, typical thickness values are between 800 and 1200 Å. We may set the initial thickness to 1000 Å. A larger thickness means that the rocking curve width according to dynamical diffraction theory gets smaller. The numerical integration thus requires a finer sampling. Thin crystals (thickness < 400 Å) are sufficiently sampled with about 30 integration steps per degree (semiangle), but for this case more steps are needed. The dependence of the refinement was tested with 30 to 256 integration steps. Convergence was observed for about 48 steps.

Set "Number of integration steps" to 48.

Click on "Select zones for editing"; "Select all"; OK.

The EDThick box is yellow and locked because the parameter is not the same for all selected zones.

Click EDThick box; click Unlock and change the value to 1000.

This changes EDThick for all zones to 1000 Å.

Click on "Select zones for editing"; "Refresh"; OK

By changing number of zone by "Zone#" textbox, we can see that all zones have EDThick 1000 Å

Uncheck the checkbox "Thickness"

Click on "Run optimizations"

This will optimize the scale factor for each frame based on a thickness of 1000 Å.

OK; YES to save m42 file.

[The current state of the Jana files is refinements/step+3_setup]

4. Initial determination of absolute structure

In the command tree (left), expand "Refinement" → "Refinement commands" (double click)

Set "Number of cycles" to 5

"Check for convergence": set "stop if" to 0.4 in 1 consecutive cycles

Uncheck "Disable atoms having too large ADP parameter"

Go to Tab "Restrains/Constraints"

Click "Fixed commands"

Enter "*" (asterisk) in the field "Atoms/parameters"

Click "Rewrite"; OK

This will fix all structural parameters and thus shorten the refinement cycles significantly.

OK

[On the screen: "Do you want to save new commands?"]

YES+START

The refinement starts. Only the thickness and scales are optimized. With 8-cores, one refinement cycle lasts about 30 seconds.

```
R factors : [4745=1925+2820/80], Damping factor: 1.0000
GOF(obs)= 7.65 GOF(all)= 5.10
R(obs)= 23.93 wR2(obs)= 45.78 R(all)= 34.36 wR2(all)= 47.74
Last wR2(all): 47.79 47.75 47.74 47.74
Maximum change/s.u. : -0.2997 for EDThick[Zone#1]
```

[The current state of the Jana files is refinements/step+4_refine_thickness]

Structure (top main menu bar) → Save as

Save the structure as "inverted_model"; SAVE

[On the screen: "Do you want to continue with the new structure?"]

YES

Transformations (top main menu bar) → Change enantiomorph

Select "rewrite the old structure"

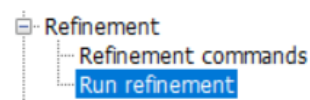
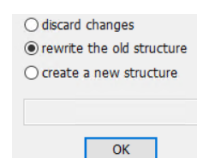
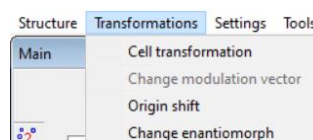
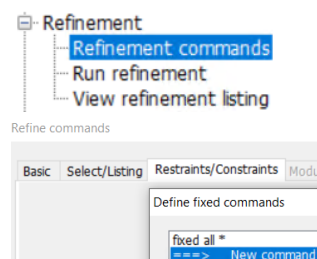
OK

Start JanaDraw (six-ring icon); Build molecule (blue hexagon)

The shown molecule now corresponds to the other enantiomer and the structure model corresponds to the other enantiomorph.

Double-click "Run refinement" in the command tree

A single refinement cycle takes about 30 seconds. The scale parameters and the thickness change significantly and converge after 3 cycles.




RFactors overview

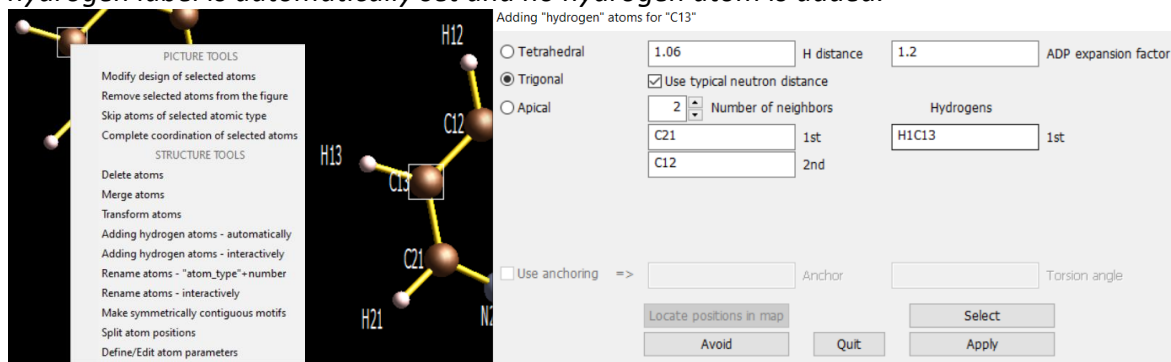
R factors : [4745=1925+2820/80], Damping factor: 1.0000
 GOF(obs)= 6.75 GOF(all)= 4.56
 R(obs)= 20.84 wR2(obs)= 40.39 R(all)= 32.07 wR2(all)= 42.62
 Last wR2(all): 44.15 42.76 42.62
 Maximum change/s.u. : -0.2918 for EDScale[Zone#11]

[The current state of the Jana files is refinements/step+4_refine_thickness_inverted]

In comparison to the *R* factors from before, *R*(obs) reduced by about 3%, *R*all by 2.3% and *wR*2(all) by about 5%. The overall *R* factors are high because of the limited accuracy of the structure solution and thus the change is, on a relative scale, rather small. Nevertheless, the overall refinement time was about 6 minutes and the preliminary result is that the structure solution provided the wrong absolute structure. In the next step, we will implement constraints on the H atoms and refine the coordinates and isotropic displacement parameters of non-hydrogen atoms. Note that refinement cycle times will increase significantly (to about 10 minutes) as we increase the number of refinement parameters.

5. Hydrogen constraints

With JanaDraw you can usually easily setup constraints for H positions automatically by selecting the relevant atoms and choosing “Adding hydrogen atoms – automatically” from the right-click menu. Click the “Add atom labels” icon  to see the site labels. However, the structure solution is rather distorted so that the automatic procedure fails to correctly set the constraints as the distances and bonding angles deviate too much from the ideal geometry on which the automatic procedure is based. You may try the manual approach or a combination of automatic and manual approach with a right click on a C atom. Then choose “Adding hydrogen atoms – interactively”, click OK in the subsequent menu and then check if the environment was correctly detected. In the screenshot below, the geometry and the labels of the carbon atoms was correctly determined, but the already placed H site (H13) was not correctly associated. In that case, Jana would add a new atom H1C13. If the H atom is correctly associated, the hydrogen label is automatically set and no hydrogen atom is added.



Here, we will skip this part and simply take a prepared set of constraints to quickly start the refinement.

Open the tutorial file “constraints.txt” with a text editor of your choice

Select all; Copy (e.g. CTRL + c, or right click and select copy)

40 lines of “keep” commands should now be in the clipboard.

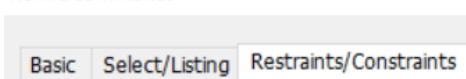
In the command tree of Jana2020: Refinement → Refinement commands

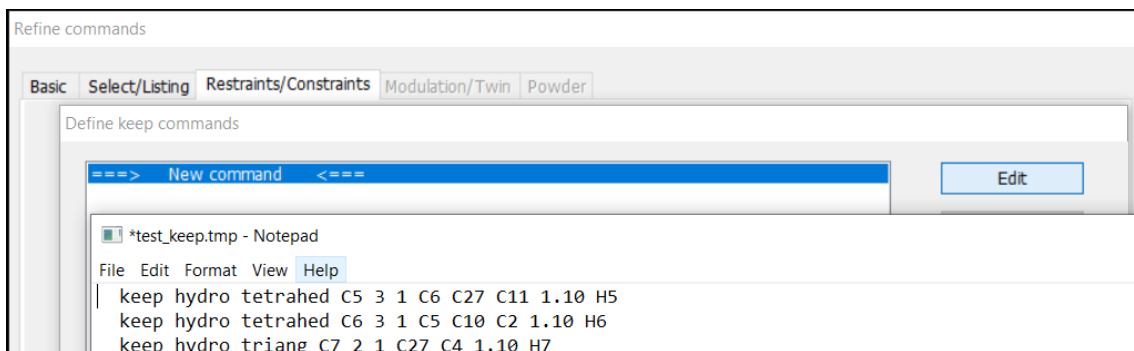
Switch to the tab “Restrains/Constraints”

Click “Keep commands”

Click “Edit

Refine commands





A text editor window opens. It should be empty. If not, remove all lines.

Text editor: Paste the copied lines from constraints.txt

With the default notepad text editor, you can use Edit -> Paste (Shortcut: CTRL+v) to do so.

Text editor: File -> Save

Text editor: File -> Exit

The "Define keep commands" window of Jana2020 now lists the keep commands.

OK

Note that the "Keep commands" button now is now labelled "Keep commands[40+0]"

This indicates that there are 40 active "keep" commands and 0 disables ones.

Click "Fixed commands[1+0!]"

1+0! Indicates that there is one active "fixed" command and 0 disables ones.

Select the command line "fixed all *"

Click "Disable"

A "!" (exclamation mark) is placed before the command indicating that it is disabled.

OK; OK

[On the screen: Do you want to save new commands?]

YES

[The current state of the Jana files is refinements/step+5_constraints]

6. Dynamical refinement of correct enantiomorph

The refinement of the last step only took a few minutes because we limited the number of refinement parameters. Refining the coordinates and isotropic displacement parameters of 29 atoms strongly increases the number of refinement parameters and thus the time for a refinement cycle. Like for the previous steps, the Jana files of each step are provided in the folder refinements if you only want to check the result without having to wait for the end of your own refinement.

If you follow the final steps of this tutorial, you may expect overall refinement times of at least one hour depending on the hardware and on how many refinement cycles you want to see.

Structure (top main menu bar) → Save as

Save the structure as "inverted_model_refine"; SAVE

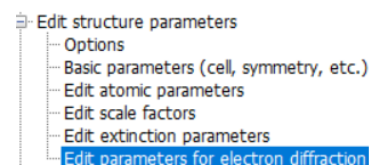
[On the screen: "Do you want to continue with the new structure?"]

YES

Expand "Edit structure parameters" → "Edit parameters for electron diffraction" (double click)

Set "Number of integration steps" to 32

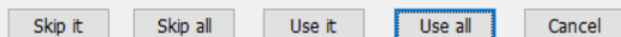
This number is usually too small. However, for this tutorial the convergence behaviour was tested in the range 16 to 128 and 32 is acceptable for demonstration purposes to speed up the refinement.



Make sure that you see the molecule in JanaDraw to follow the structural changes after each refinement cycle. The changes are most pronounced after the very first cycle.

Expand "Refinement" in the command tree → Double-click "Run refinement"

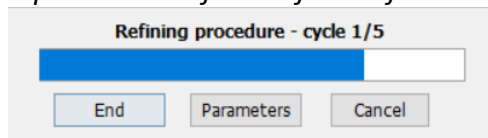
Significant discrepancy 109.47/108.20 between starting and desired value of the angle : H20a-C20-C4



Jana2020 informs us that constraints will change some angles and distances. In all cases the deviation is small. You may look at each message individually by clicking "Use it" until the refinement starts. Do not click "Skip it" or "Skip all" because this will disable the respective "keep" command(s) for the refinement.

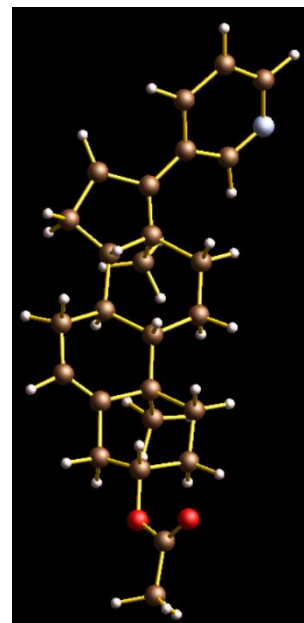
Click "Use all" to start the refinement

The $wR2(all)$ after the first cycles are 42.48, 25.09, 23.02, 22.29, 22.28, 22.23, 22.27, 22.25, 22.22, 22.21 and each cycle lasts about 11 minutes using 8 cores. Obviously, the largest drop is observed after the very first cycle. Though several cycles are needed to achieve a fully converged refinement, the R factors of the fourth cycle is already representative for the final refinement.



You may stop the refinement anytime you want by clicking "End". Jana will not stop immediately! The current cycle will continue and structural parameters refined accordingly. In a (very quick) final run the R factors are calculated based on this model. If you click "Cancel", the refinement stops immediately.

[The current state of the Jana files is
refinements/step+6_refine_correct_enantiomorph]



7. Dynamical refinement of wrong enantiomorph

We now want to determine the R factors of a refined model of the enantiomorph corresponding to the structure solution, which in our initial determination gave a higher R factor. There are three ways to do so:

- Save as new structure. Invert structure. Set number of integration steps: 48. Start refinement.
 - open "abiraterone_acetate", repeat step 5 and 6
 - open "abiraterone_acetate", "copy in" constraints from "inverted_model"
- Here we describe the steps of option c).

Structure (top main menu bar) → Open

Select "abiraterone_acetate"

Structure (top main menu bar) → Save as

Save the structure as "abiraterone_acetate_refine"

[On the screen: "Do you want to continue with the new structure?"]

YES

Structure (top main menu bar) → Copy in

Select "inverted_model"

We can now overtake certain Jana files from another refinement. We are now only interested in the constraints, which are written in the M50 file. Overwriting M90 and M95 has no effect, because the refinements are based on the same data set and data import. M42 contains the frame parameters like scales, thickness, their orientation and other parameters of the dynamical

refinement. M40 contains the atomic parameters and thus the information on the absolute structure. We want to keep the current M40 and M42 file and only overwrite the M50 file. Check "Overwrite it?" in the line "m50". Uncheck all other checkboxes.

File	Source	Target	
m40	exists	exists	<input type="checkbox"/> Overwrite it?
m41	empty	empty	<input type="checkbox"/> Overwrite it?
m42	exists	exists	<input type="checkbox"/> Overwrite it?
m50	exists	exists	<input checked="" type="checkbox"/> Overwrite it?
m70	exists	exists	<input type="checkbox"/> Overwrite it?
m90	exists	exists	<input type="checkbox"/> Overwrite it?
m95	exists	exists	<input type="checkbox"/> Overwrite it?
jdht	exists	exists	<input type="checkbox"/> Overwrite it?

We are now ready to start the refinement. Make sure to plot the model with JanaDraw if you want to follow the evolution of the structural changes during the refinement.

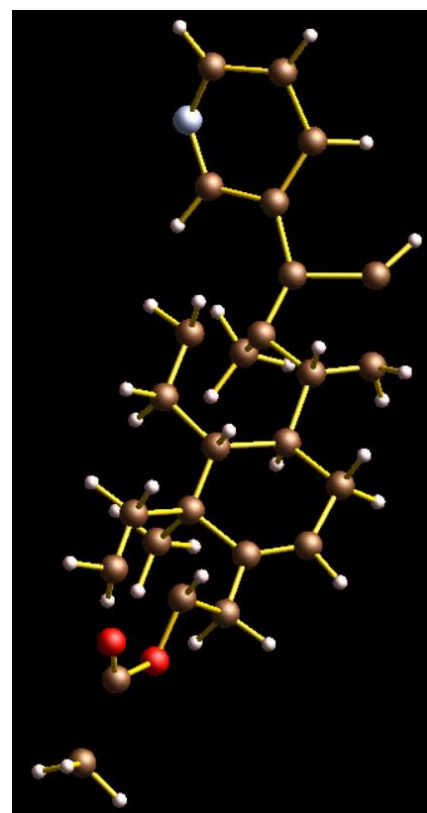
"Run refinement"

Information from refinement:

RFactors overview	
R factors : [4745=1925+2820/196],	Damping factor: 1.0000
GOF(obs)= 5.29	GOF(all)= 3.46
R(obs)= 15.42	wR2(obs)= 30.66
Last wR2(all): 47.69	33.87
Maximum change/s.u. : 3.9365 for EDThick[Zone#1]	

The wR2(all) after the first cycles are 47.69, 33.87, 31.94, 31.44, 31.39, 31.30. With more cycles it drops below 31%. Note that the structural model is visibly distorted in contrast to the model obtained by refining the correct enantiomorph.

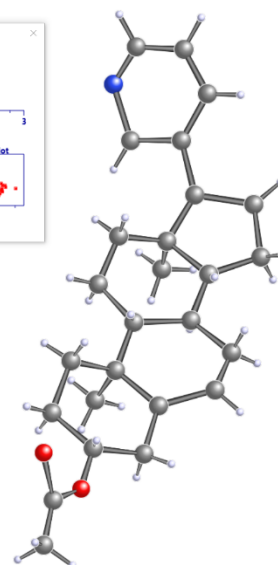
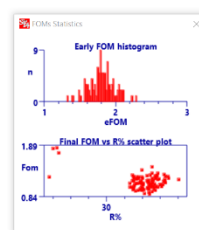
[The current state of the Jana files is refinements/step+7_refine_wrong_enantiomorph]



8. Final comparison of R factors

Model	Enantiomorph	R_{obs}	R_{all}	$wR2_{\text{all}}$
Solution	A	23.93%	34.36%	47.74%
	B	20.84%	32.07%	42.62%
Refined	A	15.91%	25.99%	30.63%
	B	10.65%	20.39%	22.14%

Within the kinematical approximation, the structure was solved by SIR (direct methods, screenshot on the right) with an R factor of 23.5% based on two data sets. A corresponding kinematical refinement (based on the same two data sets) converges with $R(\text{obs}) = 23.74$ and $wR2(\text{all}) = 42.39$. The absolute structure cannot be determined if the kinematical approximation is applied because the R factors do not change if the enantiomorph is changed. The dynamical refinement is intrinsically sensitive to the absolute structure because the dynamical effects, i.e. the interaction of several beams being simultaneously excited, depend on the absolute structure. This effect is completely different to the XRD-based determination of the absolute structure ("anomalous dispersion").



a b c