Apex 3/D8 Venture Quick Guide

Login

Sample → Login

Enter in Username (group name) and Password

Create New Sample

Sample → New

Enter in sample name, be sure to check white board or cards to establish next number

Directory under sample's name will be created in the folder D:\frames*Username**SampleName*

Center Crystal

Note: The instrument doors MUST be closed before moving the goniometer or detector in Mount or Center positions. Door can be left open during Phi rotations.

Use the APEXServer program and camera on the monitor inside the instrument. Ensure the sample is oriented for centering by clicking the Center button.

Using the goniometer tool, first adjust the crystal up-to-down in the middle of the crosshairs before adjusting left-to-right

Use the Phi 90 and Phi 180 buttons to rotate the crystal and continue to adjust the position of the crystal. Ensure that the central point of the crystal remains in the same spot on the screen during rotation.

Determine Unit Cell

I. Collect Data
   A. Select Evaluate Menu in left sidebar, select Determine Unit Cell icon
   B. Select Collect Data in list on the right side of the screen
   C. Select Collect in the lower right corner

Note: Pay attention to the frames as the data is collected. Spots should be well-defined circles and should not overlap. Samples that are twins or multi-crystals may have spots that are smeared and have spots very close to each other and possibly overlapping. If the sample is stable, it is worth trying to find other crystals that may give better diffraction patterns.

II. Harvest Spots
   A. Select Harvest Spots in list on the right side of the screen
   B. Select matrix frames from data collection, change Number of Runs and Images per Run from to reflect the number of matrix files
   C. Adjust the Min I./sigma(I) value to 5
      i. Values can be adjusted by manually entering number in the box or by moving the slider
   D. Click Harvest button at the bottom of the page

Note: After the spots have been harvested, take a look at the Expected Resolution table in the lower right corner. Crystals that give an expected resolution higher than 1 Å for 20 s/° exposure time are generally too weak to give a good enough structure for publication. Other crystals should be tried.

III. Calculating the Unit Cell

The unit cell can be calculated by two different methods. Index is built into the Apex software and works well for well-defined single crystals but will have issues with twins and multi-crystalline samples. Cell Now is an external program that can be accessed using the command prompt and will calculate possible unit cells and help determine the orientation of the additional components for twinned or multi-crystalline samples. It is best to use Index first and resort to Cell Now if Index fails.

A. Index
    i. Use default Methods selected by program (Difference Vectors and Fast Fourier Transform)
    ii. Click Index button at the bottom of the page
    iii. Program will select "best" unit cell based upon calculated score. Click Accept button at the bottom of the page

Note: Pay attention and compare the two calculated unit cells. Did one method fail? Do both methods give the same unit cell? Are the scores comparable? Are the HKL histograms comparable? Are the HKL histograms all around or above 90%? Do the calculated spot circles overlay on a large number of observed spots? If a method failed, two different calculated unit cells were found, there is one very long cell length, and/or there appear to be a large number of overlapping spots, use Cell Now to calculate unit cell (see below)

    iv. Refine the unit cell parameters
        a. Adjust the Tolerance slider all the way to the left (selects all of the reflections for refinement)
        b. Click the Refine button on the lower half of the page
            i. The values and errors for the unit cell parameters will adjust upon refinement
            ii. Pay attention to RMS angle values, values around or below 1 are a good sign
        c. Click the Refine button a couple times until unit cell parameters and RMS angle values remain relatively consistent between refinements

Note: Pay attention to the overlay of calculated spots versus observed spots. Are there a large number of bright, observed spots that are not included in the calculated unit cell?

    v. Determine Lattice type
        a. Select Bravais on right side of the screen
        b. Program will calculate the lattice type. Green text suggests possible lattice, red text suggests discouraged lattice
        c. Select Triclinic P lattice and click Accept button at the bottom of the page

Note: Often the correct lattice type cannot be accurately determined from Bravais especially when the data gives weak reflections, or poorly-defined spots. Even well-diffracting samples may appear to be higher symmetry than they actually are. It is best to collect as Triclinic to ensure plenty of data is collected that a good structure solution can be determined. If the initial

data diffracted strongly and Bravais gives a fairly clear indication as to the lattice, or you are performing a quick screening of the crystal for a preliminary structure solution, higher symmetry than Triclinic can be selected.

B. Cell Now
   i. Export harvested spots into formatted P4P file
      a. **Sample → Export → P4P file**
      b. Automatic file name of *SampleName*.p4p, file name can be edited
      c. Select CELL_NOW for export file format
      d. Click OK button at bottom of window
   ii. Open Command Prompt
      a. **Sample → Run Command**
      b. Type "cell_now" <enter>
      c. Type in file name, default of "*SampleName*.p4p" <enter>
      d. Enter file name of Cell Now file. Typing <enter> accepts default name [*SampleName*._cn]
      e. To calculate unit cell, press <enter> to begin search
      f. Accept default for superlattice threshold, <enter>
      g. Adjust minimum and maximum cell lengths if longer than default of 5-45, otherwise press <enter> to initial unit cell calculation
         i. A table of possible unit cells will be generated and ordered by calculated Figure of Merit (FOM)
         ii. Many unit cells will be mathematically related with very similar cell lengths and cell angles. All that are mathematically related would Bravais to the same cell
      h. The top unit cell calculation is selected by default. Press <enter>.
      i. If a different cell is wanting to be selected, type in the number from the list, otherwise accept the current cell by pressing <enter>
      j. Write file name for P4P: "cell#a.p4p" <enter>
      k. If a large number of observed spots does not fit into the calculated unit cell (second column in table), can search for reorientation of selected unit cell if the sample is a multicrystal or non-merohedral twin. Save resulting orientation as "cell#b.p4p"
   iii. Import resulting P4P file into APEX
      a. **Sample → Import → P4P/SPIN file**
      b. Select appropriate P4P file created from Cell Now. Click Open button
      c. Click OK button

Note: The lattice type from Cell Now (P, C, etc.) is not transferred when importing the P4P file. If Bravais does not recognize the unit cell as having the same lattice type as found in Cell Now (often Monoclinic P vs Monoclinic C), this can be changed by selecting the Edit button next to the Unit Cell Box, and selecting the appropriate Bravais lattice type. You can skip the Bravais step if this is the case.

IV. Refine Unit Cell
   A. Select Refine on the right side of the screen
   B. Adjust the Tolerance slider all the way to the left to select all of the reflections for refinement

C. Refine the unit cell a couple times until the unit cell parameters and RMS angle stay consistent
D. Click Accept button at the bottom of the page

V. Determine Exposure Time
A. At the bottom of the page, the Expected Resolution table lists expected resolution of the data for set exposure times. These values are to be taken into consideration when setting up the data collection in the next step.

Note: The expected resolution values are determined from the Min I./sigma(I) value used when harvesting spots earlier. These suggested exposure times should be used as a guideline when creating your data collection strategy. When in doubt, use a longer exposure time.

VI. Examine your Unit Cell
A. Check your unit cell versus known materials
i. Check versus samples on the X-ray facility website
ii. Check unit cell versus published structures on Cambridge Database
B. Calculate the density and Z value for your sample
i. Use formula $density = \frac{MW}{V} * 1.66 * z$ where MW = molecular weight (g/mol) and V = unit cell volume (Å^3). Reasonable density should be 1.2 – 1.8 g/cm^3.

Note: Use the following list of Z values for common Lattice types:
Triclinic P: Z = 2, 1 (if P1 or molecule on inversion center)
Monoclinic P: Z = 4, 2 (if chiral space group or on special position)
Monoclinic C: Z = 8, 4, 2
Orthorhombic P: Z = 8, 4, 2

<u>Set Up Data Collection</u>

I. Calculate Collection Strategy (Fig. 1)
A. Select Calculate Strategy icon

B. Adjust resolution **(1)**
i. If light atom structure (nothing heavier than S or Si), enter 0.83 Å
ii. If heavy atom structure (includes S, Si, or heavier), enter 0.77 Å
C. Adjust symmetry **(2)**
i. Match symmetry to that of Bravais lattice from determining the unit cell
a. Recommended to use Centrosymmetric (-1)
b. If compound is known to be chiral and is triclinic select Chiral (1)
D. Click Determine Strategy button **(3)**
i. Adjust detector distance
a. Detector distance will automatically adjust for unit cells with long cell axis
b. If possible twin or multi-crystal, increase the detector distance to 50 mm or 60 mm

Note: If frames still show close double spots or overlapping spots during the data collection, stop the data collection and return to Calculate Strategy and increase the detector distance and recalculate the collection strategy.

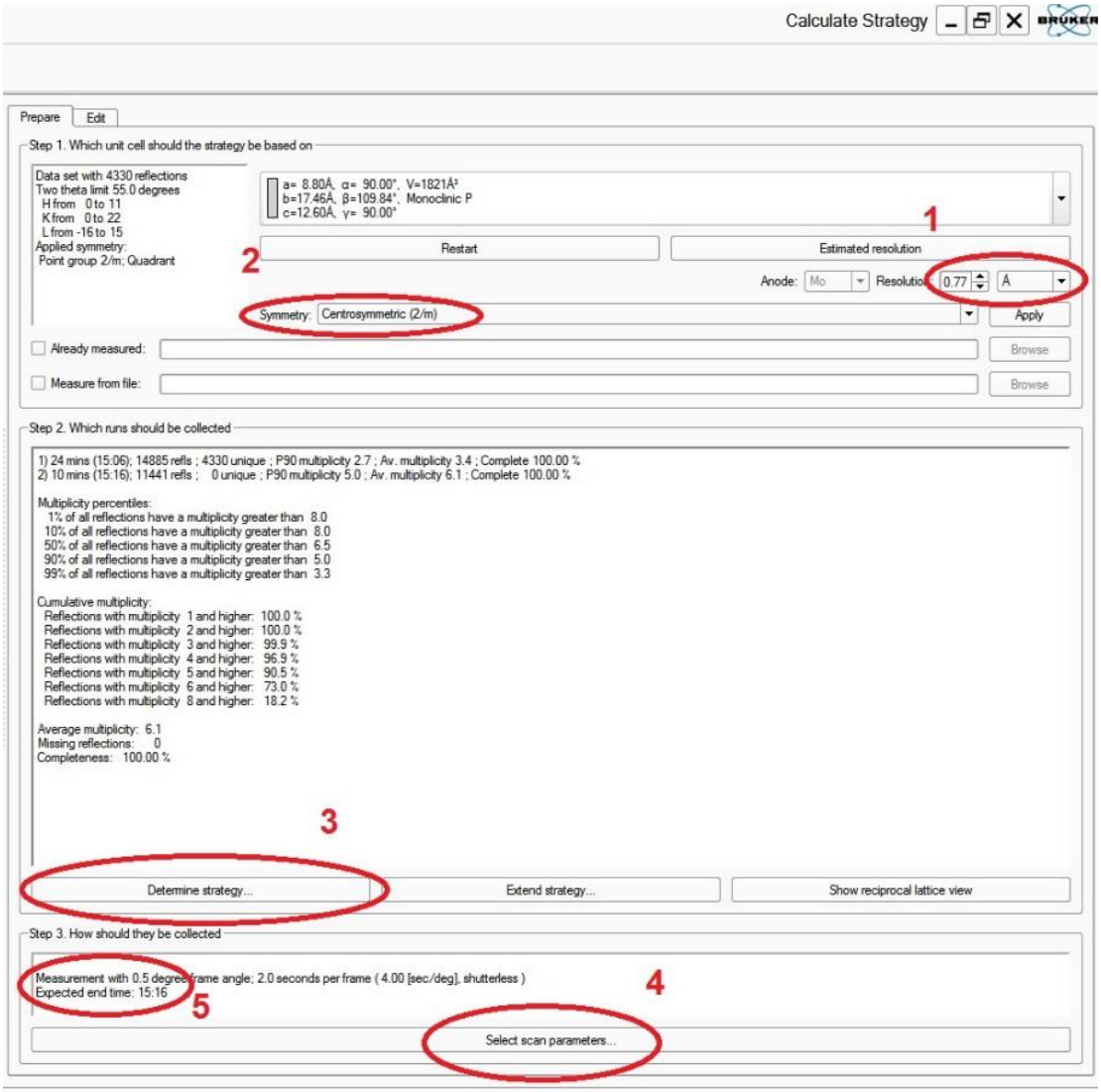
ii. Adjust multiplicity to “1.00”, may need to adjust value later
E. Click Scan Parameter button **(4)**

i. Adjust Frame Angle to 0.50
ii. Adjust Frame Time (exposure time) accordingly. Recommended maximum exposure time of 30 seconds. Collecting higher multiplicity can help with weaker diffractions more than longer exposure times.

F. Adjust Multiplicity (**5**)
   i. In Determine Strategy, increase the multiplicity such that the calculated average multiplicity is between 4-6 for well diffracting crystals
   ii. Weak crystals are recommended to collect with high multiplicity over exposure times greater than 45 seconds

Note: Take note of the expected end time listed at the bottom of the page. The expected end time is listed in 24 hour time. The presence of "+1" indicates data collection will finish the next day. Adjust multiplicity and exposure time for experiment to end at a reasonable hour such that a new sample can be readily collected

Figure 1. Collection Strategy for Apex 3 using Queen



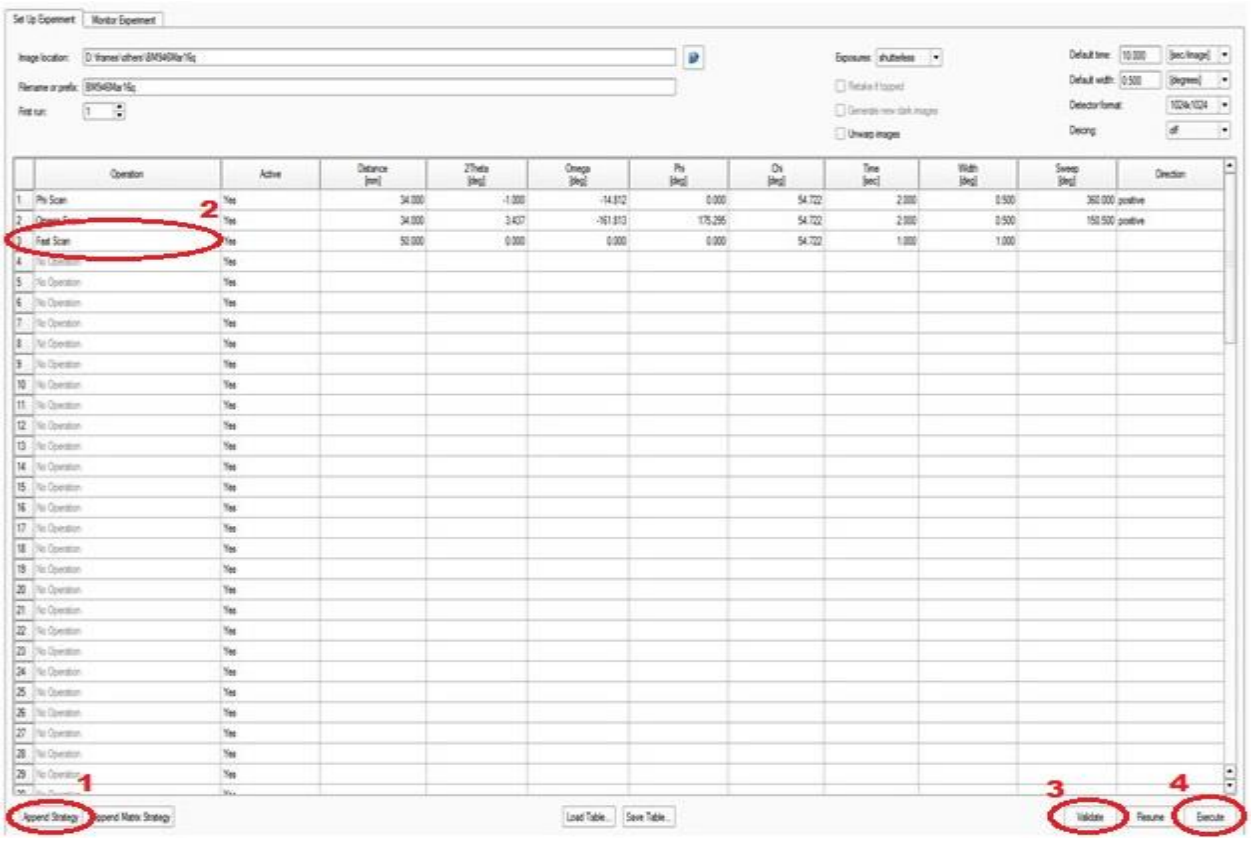
II. Select Run Experiment (Fig. 2)

A. In first row, add Fast Scan **(1)**
   i. Adjust time and width according to the exposure Frame Time of the data collection
      a. If Frame Time is 10 seconds or longer
         i. Fast Scans should have an exposure time of 10%-20% of data collection Frame Time
      b. If Frame Time is less than 10 seconds
         i. Exposure time for Fast Scans should be left at 1 second, but scan width should be increased to 2 or 3 degrees depending on how strong the sample diffracts

Note: When the Fast Scans are collecting, open the BIS window and pay attention to the number of topped reflections that are recorded. If the Fast Scans results in topped reflections, stop the data collection and adjust either the exposure time or frame width for the Fast Scans.

B. Select Append Strategy button in lower left corner **(2)**
C. If data collection will end at night or over the weekend with no new sample to be mounted afterwards, add Thermostat Off at the end
D. Click Validate button in lower right corner **(3)**
   i. If error occurs, resolve error
   ii. If all operations valid, click OK
E. Click Execute button in lower right corner **(4)**

Figure 2. Set Up Experiment Table



Integration of Data

Note: Twinned and Multi-crystalline samples require extra steps when integrating. Consult an expert for integrating non-standard samples.

Select Reduce Data Menu in left column, select “Integrate Images”

I. Set Resolution Limit value located in upper, right corner of the screen
   A. For light atom structures, a value of 0.83 Å should be used
   B. For heavy atom structures, a value of 0.77 Å should be used
II. Integration Options and Refinement Options
   A. If spots are well defined and the sample is not twinned/multi-crystalline
      i. Do Not adjust anything in Integration Options or Refinement Options
   B. If reflections are smeary or the sample is likely twinned/multi-crystalline
      i. Refinement Options
         a. Unselect “Enable Box Size Refinement”
      ii. Integration Options
         a. Select “Blend Profiles from All Detector Regions”
III. Select Runs for Integration
   A. Click Find Runs button in lower right corner of the screen, a new window will appear
   B. If needed, change the file directory to D:\frames*Username**SampleName* to list the images
   C. In the right column of the window, check the boxes for the runs you want to integrate. Place the Fast Scans at the bottom of the run list. This is best done by choosing data collection scans and reopening Find Runs and select just the Fast Scan
   D. The selected image files will be listed in the table
IV. Click the Start Integration button in the lower right corner of the screen to begin integration
   A. Pay Attention to:
      i. Spot Shape Correlation
         a. The average correlation coefficient values should remain high and consistent if a reasonable lattice symmetry has been selected
         b. Random dips that last for a few frames do occur and should not raise any concern
         c. Sudden and sustained drops in the correlation coefficient suggests something is wrong from the collected frames. Possible issues could be a change in the temperature of the cold stream, rings occurring from diffraction from the goniometer head has occurred, or the crystal has moved unexpectedly. Inspect the images where the drop off occurred to determine the possible cause
      ii. Spot Shape Profile
         a. The spot should be well defined and ideally near spherical
         b. If the spot is smeared in shape or has a dumbbell shape, integration without the box size being refined should be considered (see below)
   B. Estimated time for integration can be observed by selecting the Integration Progress box in the column on the right side of the window
   C. Integration process is finished when the Close button in the lower right corner is highlighted and can be clicked on

Absorption Correction

Note: Twinned and Multi-crystalline samples require extra steps for absorption corrections. Consult an expert for non-standard samples.

In Reduce Data Menu in left column, select Scale

I. Set Up
   A. Select appropriate RAW files
      i. Can use individual file or single merged file (*SampleName*_0m.raw)
   B. Select appropriate Laue and Point Group
   C. Click Start button in lower right corner
II. Parameter Refinement
   A. Click Refine button in lower right corner
   B. Examine display on left side of the screen
      i. Mean Weight should plateau by the end of the cycles. Values around and above 0.90 are good
      ii. R(%) values should level out by the end of the cycles. Lower R(%) are better.
      iii. Adjust number of refinement cycles if needed and rerun
         a. Increase value in Number of Refinement Cycles, click Refine button to rerun refinement
   C. Click Next button in lower right corner
III. Error Model
   A. If a Fast Scan was used
      i. Select the Fast Scan box for the appropriate scan
      ii. Click Repeat Parameter Refinement

Note: The program will try to preselect Fast Scans during the refinement, but many not select any scans as Fast Scan or may select the wrong scans as Fast Scan. If this occurs, assign the correct files as Fast Scan and repeat the refinement. The ordering of the scans reflect the order used during integration.

   B. Look at R(int) values for individual batches
      i. Single, high R(int) batches can be unselected. Click Repeat Parameter Refinement button
   C. Look at Number of Reflections bar graph
      i. Large number of rejected reflections suggest wrong Laue group or other underlying problem
   D. Click Finish button
IV. Diagnostics
   A. ABS file will be created
   B. Clicking on tabs at the bottom of the page allows for reviewing of previous steps
   C. Click Exit button

Generate INS File

Select Examine Data Menu on left column. Two different programs can be used to generate the INS file

I. Determine Unit Cell
   A. Setup
      i. HKL and P4P files will be automatically loaded
      ii. Edit name of INS file. Default name automatically loaded
      iii. If formula is not present, insert formula
      iv. Click Next button in lower right corner
   B. Lattice Expectations
      i. Lattice Type in green
      ii. Lattice type can be changed in lower left corner
      iii. Click Next button in lower right corner
   C. Space Group Determination
      i. Bravais Lattice
         a. Calculated lattices will be listed with the “best” option already selected
         b. Pay attention to R(sym) values to help in selecting lattice
      ii. Space Group
         a. Calculated space groups for “best” lattice will already be calculated, with space group with lowest CFOM value already selected
      iii. Choosing different lattice/space group
         a. If the space group is known but not displayed, check the box for “Choose different space group” in lower left corner. Select known space group in drop down menu in lower center of the screen.
         b. If current lattice group is correct but the wrong space group is selected, click new space group
         c. If the lattice needs to be changed, select different lattice and click Repeat button in lower right corner to calculate space groups in the new lattice
      iv. Click Next button in lower right corner to continue
   D. Statistics
      i. Make note of the %Completeness and Redundancy values in the lowest row, completeness will need to be above 95% for publication
      ii. Click Next button in the lower right corner
   E. Unit Cell Contents
      i. Formula, density, and Z values are calculated
      ii. If formula or Z needs to be changed, click Update Formula or Update Z Units button
      iii. Click Next button in lower right corner
   F. Set Up Files
      i. Accept default name for INS file or enter in custom name, corresponding HKL will be generated
      ii. Click Write Instruction File in lower right corner
      iii. INS file will be displayed. File cannot be edited in program
      iv. Click Accept button in lower corner
   G. Click Exit button in lower right corner

II. Analyze Data (XPREP)

A. Select files
   i. Select P4P file
   ii. Select HKL or RAW file
   iii. Click OK button
B. In XPREP window
   i. Select default options
      a. Pay attention to R(sym) values for determining higher symmetry
      b. Pay attention to CFOM values as well as systematic absences values for space group determination
      c. Make note of Completeness, Redundancy, Mean I/s values, and Rmerge/Rsigma values when merging data sets
         i. Completeness should be above 95% for publication
      d. Enter formula as single line of text
         i. Formula is text-sensitive
         ii. Number pad on right side of keyboard cannot be used
         iii. Pay attention to Z value and calculated density
      e. Write output file name
         i. Default name is fine, can write custom name if testing multiple space groups
   ii. Press <enter> until program is quit and window closes

<u>Structure Solution</u>

Two different but related programs can be used to structure solution

I. Autostructure

A. Select appropriate HKL file, will detect correct INS file

Note: Even though an HKL is loaded, open the folder and reselect the latest HKL file. Failure to do so will result in the program using the original unit cell and Bravais type determined from Determine Unit Cell. Reloading the latest HKL file will update the unit cell parameters and lattice type as determined from written INS.

B. If needed, insert correct formula
   i. Program will have difficulty if the wrong atom types are given

Note: Autostructure uses a combination of previous autostructure software and XT so the space group may be different from calculated when writing the INS file.

Symmetry related atoms will have similar naming scheme and a slightly paler color scheme from asymmetric unit.

II. Solve Structure

A. Select Appropriate HKL and INS files
B. Enter formula if not present
C. Select Method for solving the structure

i. Intrinsic Phasing
    a. Select unit cell determined from INS file

Note: Be sure that the unit cell and Bravais type from the INS file match those from Xprep and not the initial unit cell and Lattice group from Determine Unit Cell.

    b. Uses XT, resulting RES file has all atoms isotropic and corresponding HKL file
    c. May generate more than one RES file, each solving the structure in a different, but related space group.
        i. Select the appropriate RES file based on both R values as well as Flack parameters (avoid space groups that give a Flack value of ~0.50). The LXT file will list the preliminary R values, Flack patameters and space groups of each of the generated RES files.
        ii. Be sure to select corresponding HKL and RES files. The output files will have an additional "_a", "_b", and "_c"

ii. Direct Methods
    a. Uses XS, reads original space group determined in XPrep and recorded in INS file
    b. Output RES file will have some heavy atoms labelled and many Q-peaks present

iii. Patterson Methods
    a. Only recommended for difficult structures that didn't work with previous methods
    b. Uses original space group determined in XPrep and recorded in INS file
    c. Output RES file will only have heavy atoms assigned and no Q-peaks present, refinement required for Q-peaks

Resulting RES and HKL files can be copied and transferred for use in crystal solving program of choice