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XChem for dummies

Handbook

Part 1 - From crystal plate imaging to
remote data collection



XChem team

DIAMOND LIGHT SOURCE LIMITED

CONTENTS

XChem is 10 steps (yes 10!) to be completed in total.

- 1 step is done in the Crystallisation lab at the Research Complex (RCAH),
- **8 steps have to be completed in the XChem lab and in the MX data room,**
- 1 step, the last one, is the remote data collection from the i04-1 beamline

Here they are:

1) Imaging plates (RCAH)

2) Pins & pucks recycling (XCHEM LAB)

3) Target xtal drops using TexRank (MX DATA ROOM, XCHEM LAB)

4) Prepare soak experiment (MX DATA ROOM, XCHEM LAB)

5) Dispense using Echo (XCHEM LAB)

6) Prepare crystal harvesting (XCHEM LAB)

7) Crystal harvesting using shifter (XCHEM LAB)

8) Full puck scan (XCHEM LAB)

9) ISpyB upload and communicating puck location (XCHEM LAB)



If you do not complete all the tasks up to that stage, we still don't know where your samples are. Please make sure you do not miss this step 9.

10) X-RAY DATA COLLECTION (I04-1, NX REMOTE)

1) IMAGING PLATES (RCAH)

- a. Open **Rockmaker**, username: XChem_user, no password
- b. Left panel, Project, SwissCi 3drops or 2 drops, copy-paste any existing plate, name it: "lbXXXXX-yourname-targetacronym-plate number", right-click and print barcode
- c. Stick the barcode onto the plate at the opposite side of the row letters. **Remove barcode sticker top strip, make sure the barcode doesn't hang out.**
- d. Open the R11000-0276 20C imager door, put the plate in, barcode facing inward.
- e. On the corresponding computer, open **RockImager**, Load Port tab/Scan load port. Right-click on your plate(s): image plate. Note your plate(s) 4 digit barcode(s). It takes about 6min to image a plate and 20min to get it ranked.

2) PINS & PUCKS RECYCLING (XCHEM LAB)

- a. Scan-in pucks about to be recycled. Click on the **XChem puck scan** shortcut on the corner-window computer, scan the "XChem-IN" QR code directly on the screen and scan-in the pucks you are going to recycle.
- b. Remove puck base (small bit) using the puck holder and the hedgehog.
- c. Grab all the puck pins using the Uniwand.
- d. Switch on the sonicator. Press the On/Off button then the start button just above.
Just plunge the extremity of the pins (the loops) in the bath and swirl the Uniwand, 15-20 seconds per puck.
- e. Put the pins back in one (choose any) tray and sort the pins by size (**blue=35um**, uncoloured=75um, **red=150um**, **green=300um**).
- f. Put the tray to dry on the shelf, on the left-hand side.


3) TARGET XTAL DROPS USING TEXRANK (MX DATA ROOM, XCHEM LAB)

- a. Open **TexRank** on a local Windows computer or through the **tserver**.
- b. Select your plate in the bottom-left table. You might need to auto-hide the Windows taskbar to access the last plate.
- c. Switch to one-subwell mode
- d. **Switch to "Formulatrix" imager**
- e. On the subwell image, right-click away from your crystal, to target the drop
- f. Click on the bottom-left button "Echo 1 target" and save the CSV file in your visit/lab36/crystal-target/ directory.
Do not change the file name. To be read by SoakDB, the file name has to be like barcode_date_imagename-3drop/2drop_targets.csv (i.e. "90yz_2018-09-24_R11000-0082-3drop_targets.csv")

4) PREPARE SOAK EXPERIMENT (MX DATA ROOM, XCHEM LAB)

- a. Open **SoakDB** by clicking on lab36/SoakDB.bat. All the fields in green needs your input. **Keep the habit to copy-paste “as value” in SoakDB.**
 - i. Update the **protein acronym** at the top, beamline visit, expected space group and unit cell parameter in the **Data Collection section**. The beamline visit LBXXXX-XX is the i04-1 data collection visit. It thus differ from the XChem visit you’ve opened SoakDB from.
 1. Or click on “Import data file (first thing to click)”
 - ii. **Crystal section** – Update drop volume > Update plate type > click on “Load Crystal Plate(s)” and import the TexRank CSV file.
 - iii. **Source (Library/Solvent) section**
 1. Solvent test - Manually enter values in the table. E.g. If 3H DMSO soak: Library plate=SOLVENT, Source well=Chosen 384PP well (e.g. H12), Library Name=DMSO_3H. No smiles. Code appears in I SpyB so can be DMSO5_3H (for DMSO 5%)
 2. Fragment soak – Select DSIpoised_001_Set1/2 in the drop-down menu. It will load the entire set(s) automatically.
 3. Own library soak – Copy-paste your library info directly in the corresponding columns.
 - iv. **Batches section**
 1. **SoakDB will export as many CSV files as there are batch names. Batch number + Xtal plate name = batch name. The Echo will need one CSV file per combination of source plate format + transfer mode + destination plate format.** You will need:
 - a. One batch name/CSV per xtal plate to soak multiple xtal plates with the same source plate and transfer mode. Otherwise the Echo won’t request a plate change.
 - b. Two different batch names/CSV files if you soak EG and DMSO from the same source plate to the same xtal plate: one CSV for an AQ-CP transfer protocol, another one for an AG-DMSO transfer protocol (see 4.b)
 - c. Multiple batches names/CSV file for a same xtal plate if you have too many crystals in that plate. You could then soak the first batch and soak the second batch only when you are about to mount the first batch, etc. This will keep the soaking time consistent.
 - d. Use a different batch number per duplicated soak.
 2. If you manually change batch numbers, click on “refresh names” to refresh their names.
 - v. **Soak section**
 1. Solvent test – Manually enter the gradient of % solvent you want directly in the column
 2. Screen – In the top cell, just type the fragment source plate concentration (500mM if you are using DSIpoised) and the amount of solvent your crystal can handle
 3. Click on Recalculate and add to pending.
 4. In SaokDB, as long as something is in “pending” status, it can be exported (by clicking on the button “export”).
 5. Click on export
 6. Do the soak using the Echo – **See 5)**
- b. Prepare some quick seals for your xtal plates, a.k.a destination plates, and some foil seal for the library plate.
- c. Prepare source plate
 - i. Solvent tolerance test
 1. Use 384PP plate, pipette solvent(s) in the plate well(s) chosen in 3.a.iii. 384PP plates takes 15uL to 50uL. 30ul is good.
 2. Spin plate
 3. After the soak is done, mark the well you have used.
 - ii. Fragment soaking,
 1. Take the available DSI Poised plate from the plate container.
 2. Spin it
 - iii. If other soaks, remember the Echo-compatible plate volume ranges:
 1. 1536LDV > 0.5uL to 5uL
 2. 384LDV > 2.5uL to 12uL

5) DISPENSE USING ECHO (XCHEM LAB)

- a. Open **Echo Plate Reformat** and click “New”
- b. Select “XChem_SwissCi-3drop” or “-2drop” as destination plate and select:
 - i. 384PP as source plate with “AQ-DMSO2” transfer mode for DMSO tolerance test
 - ii. 384PP + “AQ-CP” for EG, PEG200, reservoir solution soaking
 - iii. 384PP + “AQ-GP” for 50% stock glycerol soaking.
 - iv. 1536 LDV + “AQ-DMSO2” for fragment library soaking
 - v. 384 LDV + “AQ-DMSO2” for other DMSO compound library soaking
- c. Select “custom mode”
- d. Main window, select source plate and click on File/Import region definition (or on the  button) and load the lab36/echo/.csv file
 - i. Add a destination plate if required. Make sure the right one is selected (✓) before you assign the corresponding destination plate .csv. If the “import region definition” option disappear, just click back once on the source plate.
- e. Click on run (▶) at the top-left corner of the main window, save the protocol, click on “Run” in the pop-up windows end then on “Start” in the bottom-right corner
- f. Depending if your protocol is using 384PP plates or LDV plates, a first pop-up windows may show up asking you to change the INSET (the Echo source plate holder). Remove the current insert and put the one which correspond to your source plate type (PP or LDV). Both inserts are next to the Echo in their respective black case.
 - i. The Echo won't show you which destination plate ID to load. You can always refer to SoakDB but you can also pile up your xtal plates on the bench in the same order you are going to load them
- g. Follow the Echo pop-up windows instructions. Don't panic. A1 plate corner always go on the front-left hand side.
- h. **No need to rush to load the xtal plate when you soak the fragment library. Look at the little blue screen on the Echo instrument and wait for the source plate survey to finish first.**
- i. If library soaking, **turn on the argon gas diffuser and only open the library plate underneath it.** Mark how many times you used/opened the plate.
- j. **SoakDB - Soak section.** Click on “Done” and on “yes” only for the batches you have actually done the soak for. This will also automatically flag as “pending” the harvesting status in the **harvesting section**.

6) PREPARE CRYSTAL HARVESTING (XCHEM LAB)

- a. **SoakDB - Harvesting section.** Export the CSV file for the shifter once the harvesting status=pending by clicking on the “export pending” button.
 - i. Using the Alpha/Old shifter? Tick the “use old shifter” box before exporting.
- b. **Pick pucks and scan them out using the XChem scan-in/out webpage** (shortcut on the corner-window computer desktop). Type your proposal number and/or your name in the first field then click in the puck field and scan the pucks you take. Your pucks will now appear in the separated list.

Purple pucks are for industrial use only.

- c. Go to a Shifter bench and log in on its computer.
- d. Click on the **OLT Crystal Shifter Workflow** icon and go to the “Workflow” tab.
- e. Top-right corner, load your Shifter csv file by clicking on “Open”. A pop-up window will appear telling you how many puck this loaded batch is requiring.
- f. Top-middle, assign it to the left plate using the drop down menu
- g. Click in the puck table and scan your puck in again. Click back on the first puck in the list to select it.
- h. Freeze pucks
- i. Choose pin trays.

Choose the suitable size for your pins! They will be auto-centred so your crystal has to sit in the pins, not float in the middle.

“IND” labelled tray are for industrial use only.

7) CRYSTAL HARVESTING USING SHIFTER AND FULL PUCK SCAN (XCHEM LAB)

- a. In the **OLT Crystal Shifter Workflow** windows, click on “Load to left bay” and slot-in your plate. It has to sit evenly in the rack. Unit20 has a locking spring you need to open when you put in the plate. Well A1 is front-left.
- b. Click on Start,
- c. Mount samples and either click on option1 (compound), option2 (crystal) then “Mount” or “Fail” or just on “Mount” or “Fail”. Always keep mounting samples in the next available puck position: do not skip/jump over a position because you have clicked on “fail”.
- d. Mounting data is automatically saved. Once first batch done, either load the next CSV file in or go to the puck scanning station to scan your first batch of pucks.

8) FULL PUCK SCAN (XCHEM LAB)

- a. Go to the puck scanning bench.
- b. Place your puck in the **XChem puck scanner**. The groove should only fit in a given orientation when you push it in the slot. Make sure it fits properly.
- c. Wait 10sec for the scan to finish. Incomplete pin scan is fine.
- d. Seal the puck(s) and store it(them) in a storage dewar canne.
 - i. **Please make sure the pucks fits perfectly in the cane: they should “click in” and not stick out the cane. Otherwise they could fall into the storage dewar, not being found and thus not being collected.**



AND NO, STORING YOUR PUCKS IS NOT THE LAST STEP. TWO MORE STEPS TO GO!



9) ISPYB UPLOAD AND COMMUNICATING PUCK LOCATION (XCHEM LAB)

- a. **SoakDB – Harvesting section.** Load the mounting data by clicking on “import mounting”. Select the shifter CSV file now updated with the mounting data (in lab36/shifter)
 - i. **Do not import the shifter csv data file before scanning the puck, complete step 8 first.**
 - ii. Using the Alpha/Old shifter? It still needs a quick fix. Once the harvesting is finished:
 1. Close the shifter software,
 2. Open the shifter/harvesting csv file in Excel,
 3. Select the last 10 empty columns which follow the last one filled with harvesting data and delete them (right click on one of the column letter and delete)
 4. Save the fixed file (same format: as CSV comma delimited).
 5. Import the saved csv file in SoakDB
- b. **SoakDB – Data collection section.**
 - i. Once the harvesting data are imported, click on “update ISpyB status” which should change to “pending”
 - ii. Export CSV file for ISpyB. Better is to export all the mounting in one go.
- c. Open **Putty** software and log in ssh.diamond.ac.uk with your FedID and password.
 - i. Now in the Linux world, go to your visit/lab36/ispyb.
 - ii. Run the command: `csv2ispyb yourispybcsvfile.csv` (if you have generated multiple ispyb csv files, run the command for each files).

- d. **SoakDB – Puck tab:** Go back to SoakDB and click on the second tab at the bottom called “Puck”
 - i. At the top of the table, first edit the required field in green with the following info:
 1. Instruction=To collect,
 2. Done=No,
 3. Data collection=Standard (or please specify),
 4. What/Who=Your target acronym/Your name,
 5. Priority=High (solvent tolerance). Medium (pre-screen), Low (screen), or please precise if different.
 - ii. Click on the “Update” button and click on “Yes” on the pop-up windows. This will generate the table.
 - iii. **Update the missing information: puck location (cane numbers) and visit number (LBXXXXXX)**
- e. Open the [XChem Queue Google spreadsheet](#) (desktop shortcut) and copy-paste the content of the SoakDB puck table here. Now, the XChem team knows where your pucks are and what to do with them.
- f. Open an [ISpyB webpage](#) and double-check it is “clean”
 - i. Log in your visit, click on the “assign container” button.
 - ii. Check if your pucks appear in their container there
 - iii. Unassign all the previous pucks from the robot dewar if you see some.

10) X-RAY QUEUE DATA COLLECTION (I04-1, NX REMOTE)

- a. If you are in charge of loading your pucks in the i04-1 robot.
 - i. load the puck in the robot by scanning them (again) using the laser tracking scanner. The laser indicate where to load the pucks.
 - ii. Put the puck “lid” (big bit) in the white tray at its corresponding position
 - iii. Lock the beamline experimental hutch
- b. Log in a computer in the control hutch and check the puck assignment in ISPyB.
- c. Open a **GDA client**
 - i. Select your LBXXXXX-XX visit in the pop-up list.
 - ii. Go to Data Collection Input tab and **check that the data collection path is right:**
 1. Visit folder: /dls/i04-1/data/year/YOURVISIT
 2. Default folder: \${proteinacronym}/\${samplename}
 3. Default prefix: \${samplename}
 - iii. In the data collection table, edit the data collection parameter in the first row.
 - iv. Click on the “Sample ID” cell of this first row.
 - v. Select all of your samples in the opened window and click on “OK”. GDA will freeze while it loads your samples. Don’t panic.
 - vi. Select all sample rows in the Data Collection Table and click on “Queue Selected”
 - vii. Insert a auto-realign command in the queue table every three pucks, especially after beam dump or shutdown. Click on (+) “add jython command to queue” shortcut at the top of the queue table and type: run “realign.py”. Drag the entry up in the queue.
 - viii. At the top of the queue table, click on (▶) to run the queue.

LIST OF XCHEM PROGRAMS, SCRIPTS & WEBPAGES

- RockMaker**[®] Imaging instructions (“project”) and barcode printing for xtal plates. Web version available.
- RockImager**[®] Load, retrieve and image plates in Formulatrix imagers
- Preparevisit** Create the visit directory structure (*home-made*, Patrick Collins @ Diamond i04-1).
- TexRank** Crystal drop image ranker and viewer for Echo targeting (*home-made*. Ng, Jia Tsing *et al.*, 2014). It is dependent on Ranker and on the Luigi pipeline (Rachael Skyner @ Diamond OxXChem)
- SoakDB** Prepare, record and coordinate instruments from xtal targeting to xtal harvesting (*home-made*, Patrick Collins @ Diamond i04-1)
- Echo Plate Reformat**[®] Echo transfer & Echo plate design
- XChem scan-in/out webpage** Scan in or out XChem pucks (*home-made*, Mark Williams @ Diamond i03)
- OLT Crystal Shifter**[®] Control the Shifter for harvesting crystals (*home-made*, Nathan Wright @ SGC Oxford)
- XChem puck scanner** Scan full puck to link puck ID to pin ID (*home-made*, Urszula Neuman @ Diamond SSE)
- Putty**[®] SSH client for Windows.
- Csv2ispyb** Import sample location from the ispyb.csv file to ISpyB (*home-made*, Patrick Collins @ Diamond i04-1).
- XChem Queue Google spreadsheet**[®] Communicate puck locations to XChem team
- GDA** Control experimental hutch and data collection queue
- Queue** Monitor beamline for unattended data collection (*home-made*, Patrick Collins @ Diamond i04-1)
- XChemExplorer (XCE)** X-ray data-management and workflow tool for large-scale simultaneous structure analysis (*home-made*. Krojer, T. *et al.* 2017)
- PanDDa suite** Panda.analyse to find hits, panda.inspect to build hits, panda.export to create and refine ground-state and bound-state model (*home-made*. Pearce N. M. *et al.*, 2017)
- Tserver** Windows remote desktop for Linux system