Symphony X Quick Start Guide

This quick start guide is for reference use only and is an inadequate substitute for formal training. Please contact Zach Nett (zjnett@ucsb.edu) for training and scheduling.

General Information

The Symphony X is a unique automated synthesizer that can independently control 12 reaction vessels (RVs). Up to 24 syntheses can occur simultaneously with the inclusion of the 12 PV positions that operate in tandem with the RVs.

The Symphony X is operable via a touchscreen. A nearby keyboard can be connected through USB to facilitate navigation of the software.

Empty RVs, cleavage vessels (CVs), solvent and amino acid (AA) bottles are installed on the instrument at all times. Select RVs, CVs, and solvent and AA bottles can be replaced by the users for their run.

Only resins of mesh size 200 or lower are allowed. Smaller size resins (larger mesh) will cause clogging and damage the instrument.

Only DMF or NMP can be used within the AA bottles. Use of other solvents can damage the instrument.

The symphony operates using a specific sign-in to design and run experiments. All menu options will be grayed out if the user is not signed-in:

> Username: DESIGNER, Password: (Contact Zach for latest password)

Always use the password WILDCAT for saving files. Failure to use this password can make a file unmodifiable. Be careful to type the password accurately while using the touchscreen.

Associated Hazards

Always wear personal protective equipment (PPE = safety glasses/goggles, flame-retardant lab coat, and nitrile gloves) when working on the Symphony X or preparing solutions.

All solvent and amino acid bottles are operated under pressure. Use with caution. Never remove a bottle without venting. Always use safety-coated glass bottles to prevent bodily harm should a glass bottle break under pressure.

N,N'-Diisopropylcarbodiimide (DIC) is a common reagent and a NFPA category 4 health hazard with acute toxicity that can cause serious eye injury, skin sensitization (future allergic reactions), and can be fatal if excessively inhaled. All DIC handling should be done in a well-ventilated fume hood to avoid breathing fumes.

Various primary amines are used as submonomers in peptoid synthesis and can be toxic, corrosive, or present other health hazards. Always consult the MSDS to learn the specific hazards for your reagent.

All solutions should be prepared in an open fume hood and transported to the Symphony X efficiently.

Be careful while transferring waste solutions from the waste tank to a waste bottle. Make sure the spout is securely connected to the waste tank to prevent leakage of poured waste.

Operation Steps

1) Assess the state of the instrument: open the nitrogen gas cylinders (setting 70-90 PSI), check that there is adequate vacuum (–10 to –26 in Hg), and ensure the waste tanks are not full.

    A. There are two control knobs which adjust the flow of nitrogen through the RVs for mixing: one directly under the RV1 and one on the lower bottom of the instrument for. **Caution:** Adjusting the mix flow controller too high can cause resin to stick to the top of the RV and cause draining issues and incomplete reactions.

    B. There are two gas cylinders connected in a daisy chain, such that one cylinder has a slightly higher output pressure than the other. Open both tanks to ensure an adequate supply of nitrogen throughout the run.

    C. Safety shield doors must always be closed when the Symphony X is running.

2) The **Bottle prep** menu on the main screen sets the current amino acid and solvent file. It also allows you to pressurize, prime, vent, and backflush nitrogen gas and DMF solvent to the solvent and amino acid bottles.

    A. Load the appropriate amino acid (.aa) and solvent files (.slv) – see solvent bottle *system overview.*

    B. If you need to use a new monomer or reagent, you can (1) create a new .aa or .slv file, or (2) simply use the monomer or reagent position in a pre-created file for your own. For monomers and solvents, you will need to remember the symbol/designation in order to properly set-up the sequence and program files.

3) There are three files to create for each run and RV position: **Program** (.prg), **Sequence** (.seq), and **Synthesis** (.syn). The **Setup** menu leads to the **program editor** and **sequence editor**. When saving a file, click the button in the bottom left-hand corner of the save screen to "select/create a new folder" for your collection of files. This will make finding the correct files easier.

    A. **Program** defines each reaction step (*i.e.*, add/mix/drain, etc.).
        a. Name the file with details specific to the synthesis. Examples include:
            i. "Pre_50uM.prg" – indicates pre-synthesis step and reaction scale
            ii. "PEP_1h_50uM.prg" – indicates synthesis protocol for peptoids at 50 µM reaction scale with 1 h amine addition.
            iii. "Post_50uM.prg" – indicates post-synthesis step and reaction scale
        b. Available actions include:
            i. **AA delivery** – delivers the AA to RV in 1000 or 1500 aliquots (or as single-shot)
            ii. **Bottom delivery** – delivers fluid to RV from bottom. Valid for solvents 7 and 8 (2500 uL increments), and CV 1-12 when **Collect Mode** set to **Single Shot AA** on the Settings screen. CVs are delivered as Single-Shots and primary solvent must be in solvent bottle 7.
            iii. **Cleave & Collect** – Delivers solvent 8 to RV from bottom. Only available when **Collect Mode** set to **Cleavage** on the Settings screen. In cleavage mode, DCM must be in bottle 7 and TFA in bottle 8.
            iv. **Cleave Mix** – nitrogen burst for 1 second with 2 minute delay to prevent TFA evaporation over the course of the cleavage step.
            v. **Collect** – empties the RV to the collect vial
            vi. **Drain Dry** – empties the RV to waste, time to dry.
            vii. **Mix** – mixes then empties RV to waste. Nitrogen bursts for 1 second with 10 second delay between bursts.
            viii. **Pause** – programmed pause. Operator must press Resume to continue synthesis.
            ix. **Top delivery** – delivers fluid to the RV from the top. Select from solvents 1-6 or AA 1-28 (1000 or 1500 µL increments) or as single shot for AA 1-28.

B. **Sequence** defines the targeted chemical structure of the peptoid or peptide. Filenames should be descriptive and include the user's initials (e.g., MWB – 6Nme)

C. A **synthesis** defines the pre-synthesis (e.g., swelling/deprotection), synthesis, and post-synthesis (e.g., washing/drying) steps used to build a particular sequence.

a. Pre- and post-synthesis steps cannot be associated with any amino acid additions.

b. Filenames should be descriptive (user initials, notebook ID, and sequence)

4) Load the **synthesis** file in each desired RV position under the menu **RV Automated Operations**. Go to **Tools** menu and select **Calculations**. This tool will calculate the volumes of amino acid and bottle solutions that are required for the runs.

5) Check that there is enough DMF in the stainless steel tank for your synthesis. Only use HPLC grade DMF (>99.5%) on the instrument and for your reagents. ACS grade DMF can be used for cleaning bottles.

6) Prepare, install, pressurize, and prime the AA and bottle solutions on the instrument.

7) Add resins to the RVs and load on the instrument. Click on start in **RV Automated Operations** for each desired RV position to begin the synthesis.

A. Note that RV position 1 is for heated reactions only and must use a glass RV. Do not use a plastic RV. The heating switch (located at the back of the instrument) is turned to off by default.

Cleaning RVs after a run

1) Exchange the RV used in the synthesis for an empty RV.
2) Under **manual operations**, rinse the RV 3x with a **bottom delivery** of 5 mL DCM and drain.
3) Under **manual operations**, **vent wash (**with DMF) 5x.
4) Under **manual operations**, rinse the RV 5x with a **top delivery** of 5 mL DMF and drain.

Cleaning CVs after a run (if applicable – DMF and DCM must be installed in bottles 1 and 7, respectively. Additional DMF or DCM should be installed in bottles 5 or 6, respectively)

1) Carefully exchange the CV used in the synthesis for an empty CV. Use a kimwipe to hold the dangling line so TFA doesn't spray at you.
2) From the main menu, select the **Cleaning** menu button and then select the tab at the top named **Collect Clean**.
3) Select a solvent for cleaning (use DMF installed in bottle 5 or DCM installed in bottle 6 depending on configuration).
4) Click **Start Clean**. An automated process will clean the RV and collect vial lines.
5) Carefully remove the CV and dispose of its contents to waste. Reinstall the CV in its position.

System Cleaning after Synthesis

1) Nitrogen backflush all AA positions (clean up the lines, assuming a different monomer will use the line next time). Remove the AA bottle and discard contents in a waste container. Use a Kimwipe to wipe the line and stop dripping upon removal.
2) Reinstall the AA bottle, and solvent backflush the position three times. Discard the DMF in the AA bottle after each flush. Clean the AA bottle with solvent (e.g., DMF, methanol, and acetone), and leave uncapped to dry in a fume hood. Reinstall a dummy bottle in its place on the instrument.
3) Nitrogen backflush all solvent lines. Pour out the remaining contents as waste for all bottles except DCM and DMF.

4) Remove the pure DCM (bottle 6 or 7) and DMF (bottle 5) and wrap their solvent lines in kimwipe or paper towels. Pure DCM and DMF bottles can be capped and stored for later use.
5) Reinstall the reagent bottles (from step 3) and back flush with solvent. Pour out contents and repeat solvent backflush and disposal two times.
6) Remove the solvent bottles and wrap their solvent lines in kimwipe or paper towels. Pure DCM (bottle 6 or 7) and DMF (bottle 5, if installed) bottles can be capped and stored for later use.
7) Rinse the reagent bottles clean (3x) with DMF (or DCM for bottle 8). To thoroughly clean, rinse bottle with another methanol and acetone (3x) leave the bottles in fume hood to dry.
8) Transfer waste container contents to a labeled waste bottle via the spout attachment.
9) Close nitrogen gas cylinders.

Solvent Bottle System Overview

Bottle positions 1-6 are located on the same valve block, and solvent 1 (DMF) is used to rinse that block after top-down solvent deliveries. A metering loop enables 1000 µL or 1500 µL top deliveries.

Bottle 7 and 8 are located on a separate valve block, and solvent 7 (DCM) is used to rinse that block after deliveries. A metering loop enables 2500 µL bottom deliveries.

A typical configuration for organic solvents bottles in peptoid synthesis (see file PEPTOIDS.slv):

Bottle 1 = DMF

Bottle 2 = 20% (v/v) 4-methylpiperidine in DMF (deprotectant)

Bottle 3 = *N,N'*-Diisopropylcarbodiimide in DMF (typically, 0.8-2 M)

Bottle 4 = Bromoacetic acid in DMF (typically, 0.8-2.5 M)

Bottle 5 = Empty

Bottle 6 = Empty (or DCM – may be used for several syntheses)

Bottle 7 = DCM (may be used for several syntheses)

Bottle 8 = Trifluoroacetic acid (or *empty* if not doing cleavage on the instrument)

A typical configuration for organic solvents bottles in peptide synthesis (see file standard.slv):

Bottle 1 = DMF

Bottle 2 = 20% (v/v) 4-methylpiperidine in DMF (deprotectant)

Bottle 3 = HBTU

Bottle 4 = HCTU

Bottle 5 = 0.4 M NMM in DMF

Bottle 6 = DCM (may be used for several syntheses)

Bottle 7 = DCM (may be used for several syntheses)

Bottle 8 = Trifluoroacetic acid (or *empty* if not doing cleavage on the instrument)