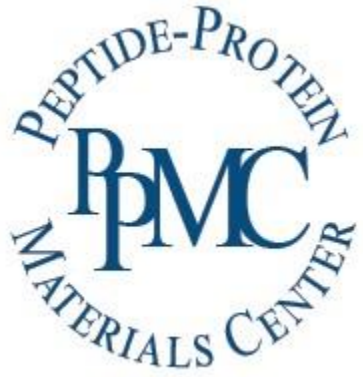
Gyros Protein Technologies PurePep Chorus Peptide Synthesizer Standard Operating Procedures



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About

The Gyros Protein Technologies PurePep Chorus Peptide Synthesizer is an all-in-one scalable peptide synthesizer that utilizes solid-phase peptide synthesis. Induction heating, oscillation mixing, and UV monitoring are available on all four reaction vessels to deliver higher yield, purity, and flexibility. The four reaction vessels can be run in parallel in 10-mL, 25-mL, and 40-mL reaction vessel sizes. Heating conditions in all four reaction vessels can be independently set for customized reactions. Real time UV monitoring ensures complete deprotection of amino acids to improve yields and lower the occurrence of deletion sequences. Eight different solvent/reagent bottles allow for customization of coupling and washing steps. The system includes active solvent level sensing and detection of a full waste container.

Point of Contact

Primary Contact: Nolan Petrich – Peptide Synthesis and Instrumentation Specialist, PPMC

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Instrument Location

Ammon Pinizzotto Biopharmaceutical Innovation Center

590 Avenue 1743, 2nd Floor, Room 255

Newark, DE 19713

Note: Swipe access to the building, floor, and lab are required. Contact Joe Chubbs (jrchubbs@udel.edu) to coordinate swipe access.

Preparation Checklist

Before Your Scheduled Synthesis Time Slot:

1. Set the synthesis method for the peptide(s) you will be synthesizing.
2. Take note of the solvent, resin, coupling reagent, and amino acid solutions required for the synthesis.
3. Prepare amino acid and coupling reagent solutions.
4. Weigh out required resin.

Right Before the Synthesis:

1. Fill out the sign-in sheet.
2. Set the RV Size and Op Times file for the synthesis.
3. Load solvents and coupling reagent solutions on instrument.
4. Prime Solvent/Reagent bottles.
5. Load resin into reaction vessel(s).
6. Load amino acids.
7. Prime amino acid bottles.
8. Begin the synthesis.

When You Are Done:

1. Unload resin from the reaction vessel(s).
2. Backflush the amino acid bottles with solvent.
3. Unload amino acid bottles, dispose of extra reagent, and replace the amino acid bottles with the “Empty” amino acid bottles.
4. Backflush the coupling reagent bottles with solvent.
5. Empty coupling reagent bottles.
6. Logout. If the last user for the day, Shutdown.

A. Preparing Amino Acid and Coupling Reagent Solutions

1. Turn on the tablet and open the PurePep Chorus software to login.
    a. Note: See **Section M** to perform the software calculations remotely.
    b. Username: PPMC User
    c. Password: purepep255
2. If the synthesis and protocols already exist for the desired synthesis, open the synthesis by clicking on the drop-down menu labeled **Synthesis Def** in the upper right-hand corner of the screen. If the desired synthesis does not exist, skip to **Section C** to create a synthesis.
    a. Select **Open** > **SynthesisFiles** and navigate to the folder that contains the desired synthesis.
    b. Select the synthesis and select **Open**.
3. Ensure that the following tabs have the proper inputs: **Grouping**, **Chemistry**, **Sequence**, **Setup**, and **Protocol**.
    a. For more information about each tab, see **Section C Step 2**.
4. Select the **Calculation** tab.
5. Select **Re-Calc** at the bottom of the screen.
6. Write down the required **Solvents**, **Reagents**, **Amino Acids**, and **RV Products** for the synthesis.
    a. **Solvents** – This screen displays the solvents assigned to each bottle position and the respective calculated and suggested volumes.
        i. The calculated volumes depend on the protocol steps.
        ii. The suggested volume of each solvent is the compensation percent above the calculated volume, rounded to the nearest higher 20 mL.
    b. **Reagents** – This screen displays the reagents used in the synthesis and the calculated weight and/or volume of reagent needed, along with the actual total volume of the solution needed to make the calculated concentration value.
        i. The standard protocols were designed for 1000 mM reagent concentrations, unless using a scale greater than 0.25 mmol.
        ii. For solid reagents, the calculated `Weight (g)` is what should be added to the amount of `Act Vol (mL)` of the main solvent, usually DMF, to make the desired concentration.
        iii. For liquid reagents, the `Vol (mL)` is what should be measured out. Then, the main solvent, usually DMF, should be added till the `Act Vol (mL)` is reached to make the desired concentration.
    c. **Amino Acids** – This screen displays the amino acid concentration and the suggested volumes and weight of amino acid that must be weighed out to obtain the desired concentration.
        i. The standard protocols were designed for 200 mM amino acid concentrations, unless using a scale greater than 0.25 mmol.

ii. **Note: It is important to ensure that the `Suggest Vol (mL)` is higher than the `Calc Vol (mL)`. Sometimes the software miscalculates. The `Suggest Vol (mL)` should be 5 mL greater than the `Calc Vol (mL)`.**
    1. `Weight (g)` of amino acid is calculated from the **AA Concentration** and the **Suggested** volume.
    2. If you must adjust the suggested volume, be sure to select **Re-Calc** so that the weights of amino acids are recalculated.

d. **RV Products** – This screen displays the amount of resin that needs to be weighed out and it is calculated using the scale and loading.
    i. The **Yield** column indicates the theoretical yield based on the scale and the peptide's molecular weight.

7. Selecting the "**Done**" button takes the user to the next step in the workflow, which is the **Run** screen.
8. Take the required amino acids out of the freezer to defrost. Wait 15 minutes.
9. Weigh out each amino acid into the specified GPT PurePep Chorus amino acid container. This can often be done days in advance of booking the PurePep Chorus peptide synthesizer. Also weigh out the solid coupling reagents (typically Oxyma).
10. On the day of synthesis, add the required amount of N,N-Dimethylformamide (DMF) to the specific amino acid container to dissolve the amino acids. Additionally, add the correct amount of DMF to the solid coupling reagents.
    a. Solid coupling reagents, such as Oxyma and HCTU, should be weighed out in the hood. DMF can be measured in a graduated cylinder, and the DMF and solid coupling reagent can be combined and mixed in the reagent container attached to the instrument.
11. Using the same graduated cylinder that was used for measuring DMF, measure out the volume of the liquid coupling reagent (usually N,N'-Diisopropylcarbodiimide, DIC or N,N-Diisopropylethylamine, DIPEA). Add DMF to this graduated cylinder to reach the required final solution volume.
12. Weigh out the required amount of resin needed for each reaction vessel using a weighing boat.
    a. Amino acids, coupling reagents, and resin are now ready for synthesis.
13. Proceed to **Section B** to set up and run the synthesis.

B. Setting Up and Running Syntheses

1. After preparing your amino acids, reagents, and resin, as well as logging into the PurePep Chorus software, switch to the **Tools** menu.
    a. This is done by selecting it from the drop-down menu at the top left-hand corner.
2. To select the **RV Size** for the synthesis, navigate to the **Settings** menu and select the **System** tab. Next, check the proper **RV Size**. Select **Save** at the bottom of the screen.
    a. Also, go to the **Op Times** tab, and ensure that the **Op Times** file in the top right-hand corner matches the proper sized RV.
        i. If it doesn't, select the dropdown and select the proper file. Click **Save** at the bottom of the screen.
3. To load solvents and reagents to the instrument, navigate to the **Bottle Prep** screen. Here, solvent/reagent and amino acid bottles can be pressurized and vented.
    a. Ensure that all the bottles under the **Solvents/Reagents** tab are not pressurized. If they were pressurized the Pressurized circle next to the bottle would be orange.
        i. If pressurized, select the box next to the bottle and at the bottom of the screen select **Vent**.
    b. Once all bottles are vented, fill the bottles with the proper solvents and reagents for the synthesis. It is usually only important to fill bottles one through six. To remove the bottle, unscrew the cap while carefully guiding the tubing and filter out of the bottle.
        i. To reinstall the bottles, verify that the O-ring is properly installed on the cap insert and that the insert is in the cap. Insert the line so that it is straight and at the bottom of the bottle. Attach the cap and tighten to a firm hand tight.
    c. The standard (**Std**) solvent/reagent assignments are as follows:
        i. Bottle 1: DMF
        ii. Bottle 2: Dichloromethane (DCM)
        iii. Bottle 3: 20% Piperidine in DMF (v/v) (Displayed as "20% Pip")
        iv. Bottle 4: Methanol (MeOH)
        v. Bottle 5: OxymaPure
            1. Sometimes users may clean this bottle out to use DIPEA instead. **Users are responsible for cleaning these bottles after the synthesis if something other than OxymaPure is used.**
        vi. Bottle 6: DIC
            1. Sometimes users may clean this bottle out to use HCTU instead. **Users are responsible for cleaning these bottles after the synthesis if something other than DIC is used.**
        vii. Bottle 7: DMF
        viii. Bottle 8: TFA (Cleavage Cocktail)

4. Prime the solvent/reagent bottles by selecting all the boxes next to the bottles that will be used. Next, select "**Prime**". This will pressurize and prime the lines for those bottles.
5. To load the resin in the reaction vessel, begin by removing the RV from the instrument.
    a. Cam levers allow the operator to remove and install reaction vessels. Apply slight downward pressure to the RV with one hand while lifting the cam lever with the other hand until the lever locks into the vertical position.
    b. Pull the RV gently up and out of the bottom seat.
        i. A slight twisting motion may help release the RV from the bottom seat.
6. Once the RV has been removed from the instrument, the weighed-out resin can be added to the RV. It helps to use DMF to ensure that all the resin is added to the RV.
    a. Rinsing the sides of the RV helps ensure that all the resin is at the bottom of the RV.
7. Install the RV back on the instrument by first placing the reaction vessel in the bottom RV seat hole. Be sure to line the RV up with the upper seat. Once this is done, hold the reaction vessel with one hand while slowly lowering the upper RV seat with the cam lever to the horizontal position to lock the RV in place.
    a. To ensure that the reaction vessel is properly in place, rotate the reaction vessel to ensure that the black O-ring has been set.
8. Navigate to the **Manual Ops – Individual** tab screen to drain the reaction vessel. This is done by:
    a. Under **Operations**, select the drop-down menu and select `Drain/Dry`.
    b. Next, start the operation by selecting the **Start** button at the bottom of the screen.
9. Once the RV has been drained, it is often helpful to deliver DMF from the top of the reaction vessel to ensure all resin is washed off the sides and located at the bottom of the RV.
    a. Under **Operations**, select the drop-down menu and select `Top Delivery`.
    b. Ensure that the selected **Bottle** is `Solv1`.
    c. Select a **Volume (µL)** of solvent to be added. 5000 µL is usually a good amount for the 10-mL RV, and 10000 µL is usually good for the 25-mL and 40-mL RVs.
    d. Deselect the box labeled **N2** toward the bottom of the screen to prevent Nitrogen bubbling.
    e. Select the box labeled **Drain** in the upper right-hand corner for the RV to automatically be drained after the solvent delivery.
    f. Start the operation by selecting the **Start** button at the bottom of the screen.
10. With the solvents/reagents and resin now loaded, the amino acids are the last thing that must be loaded. Navigate to the **Bottle Prep** screen and to the **Amino Acids** tab.

a. At the top of the screen is a **Select All in Synthesis** button. Clicking on this will select all the amino acids that will be used in the synthesis that is set up in the **Synthesis** tab.
b. Ensure that the amino acid bottles are vented. If not, select the **Vent** button at the bottom of the screen.
    i. It will be grayed out if the bottles have already been vented.
c. To release the bottle, hold the amino acid bottle with one hand while pushing in the metal slide with the other. Carefully slide the bottle off the tubing and filter.
d. To load the amino acid bottle, make sure the metal slide is pushed all the way in. Insert the bottle filter and tube into the bottle and push the amino acid bottle upward. The metal slide is spring-loaded and will pop out when the bottle is properly in place. Make sure to check that the bottle filter is resting against the lower rear of the bottle to ensure that that all the reagent in the bottle will be used.

11. Once all the amino acid bottles have been loaded, click the **Select All in Synthesis** button and select **Prime** at the bottom of the screen to prime all the amino acids.
12. With everything primed, the synthesis can now be started. Select the **Synthesis** tab in the upper left-hand corner of the screen and proceed to the **Run** tab after checking that the proper synthesis is selected from the drop-down menu in the upper right-hand corner of the screen. Select **Start** to start the synthesis.

C. Creating a New Synthesis

1. When creating a new synthesis, users have two options. Start by selecting the **Synthesis Def** drop-down menu in the upper right corner.
    a. To create an entirely new synthesis, select **New**.
        i. If this is selected, a dialog box is shown. Users must input the path, which should be under Data\SynthesisFiles\User's_Name, as well as a synthesis name.
        ii. **Be sure to select your own named folder for the synthesis path.**
    b. To use a previously created synthesis as a template, select **Create from**.
2. Once the new synthesis has a name and a location it will be saved, users can go through the eight submenu buttons on the left side of the screen and input the required information, saving as they go:
    a. **Grouping**: The **Grouping** screen allows reaction vessels to be configured to run simultaneously (same group) or in different groups to run sequentially, as well as apply **Synthesis Settings** for scale, excess, and RV size.
        i. It is only valuable to separate reaction vessels into other groups when using a different resin or when different protocols for coupling/deprotection must be used.
        ii. Users can add and remove RVs from any group using the "**Add RV**" and "**Remove RV**" buttons.
        iii. Groups can be added by clicking on the "**Insert Group**" button at the bottom of the screen. Groups can be removed by selecting the group and then clicking the "**Delete Group**" button.
        iv. No RVs can be duplicates within multiple groups.
        v. **Synthesis Settings** Box: Users specify the:
            1. **Scale**: The synthesis scale in mmol in each reaction vessel.
            2. **Excess**: The amino acid excess that will be used for the synthesis. This is used later in the **Calculations** screen.
            3. **Installed RV Size**: The volume of the installed reaction vessel.
                a. For a 0.10 mmol scale, users should use the 10-mL reaction vessels.
                b. For a 0.25 mmol scale, users should use the 25-mL reaction vessels.
                c. For a 0.26-1.0 mmol scale, users should use the 40-mL reaction vessels.
        vi. Selecting the "**Done**" button takes the user to the next step in the workflow.
    b. **Chemistry**: The **Chemistry** screen allows users to assign the group parameters for ratios of amino acids (AA) to **Activator**, **Base**, as well as for an **Additive**. Users can also identify the name, type, and loading of the resin used in each reaction vessel.

i. **AA/Activator/Base/Additive** numbers are in relation to the previously defined **Excess**.
ii. In most cases the **AA Ratio** will be set to 1.
iii. When using DIC and Oxyma, it is common to use the following conditions:
    1. **AA Ratio**: 1.000
    2. **Activator**: 2.00
    3. **Base**: 1.00
    4. **Additive**: 0.00
iv. **Resin Type**: Allows the choice of resin from a drop-down list defined in the **Resins** screen.
v. A **Preloaded** check box is used to designate that the first amino acid in a sequence is already attached to the resin.
vi. **Resin Substitutions**: Allows the user to input the resin loading in mmol/g.
vii. To add different settings for each RV, scroll using the arrows under **Individual RV Parameters** on the bottom right of the screen.
    1. The "**Apply to All**" button under the resin type, copies the resin types, preloaded option, and resin substitution to other RVs in the group.
    2. The "**Copy to All Groups**" button at the bottom of the screen can be used to apply to all groups.
    3. The user can navigate to different groups by selecting a tab at the top of the screen.
viii. Selecting the "**Done**" button takes the user to the next step in the workflow.

c. **Sequence**: The **Sequence** screen allows users to assign a sequence to each RV, as well as add or remove single shots, no amino acid, and idle cycles.
    i. On the top of the screen the sequence is written, left to right, from N-terminus to C-terminus. The instrument will run the synthesis from right to left (C-terminus to N-terminus). The order in which amino acids are coupled is denoted by the numbers above the amino acid residue.
    ii. When the resin for the synthesis is marked as **Preloaded** on the **Chemistry** screen, the first amino acid in a sequence will be underscored with a red line. The first synthesis cycle will be shifted to the next amino acid in a sequence and the rest of the sequences in the same group will be aligned.
    iii. The bottom part of the screen will show the name and full-length of the currently selected sequence. The numbers presented in this view show the sequence of the amino acids from N-terminus to C-terminus (opposite to the cycle numbers according to which the synthesis will progress).

iv. Users can click on the **Add** tile or "**Add/Edit**" button, depending on whether a sequence is already assigned to an RV, to load or create a sequence for the selected RV.

v. **Sequence Editor**:
    1. The **Sequence Name** drop-down menu allows the user to create a **New** sequence, **Open** and browse an existing one, or load one from the list of recently opened files.
    2. A sequence can be typed in by selecting the amino acid tiles in the N to C terminus format.
    3. Users can click on the "**Library**" button on the bottom to open and allow selection of any member of the library.
    4. The **Sequence Editor** allows the user to create a new sequence from an existing one with the "**Save As**" function.
    5. If the user has a sequence that they wish to load from another device, this can be done with the "**Import**" functionality. This can be done from a CSV or TEXT file.

vi. The user has the option to specify amino acid actions for particular cycles:
    1. *Single Shots* – This delivers all the contents of the collection vial to the reaction vessel at one time.
        a. This would be useful for when you have a small amount of a non-natural amino acid that you don't want to waste.
        b. This is accomplished by selecting the appropriate residue tile within a sequence and clicking the "**Single Shot**" button.
        c. A green border will appear around the selected amino acid when a *Single Shot* has been selected.
        d. To clear the *Single Shot*, the amino acid has to be selected and the "**Clear Single Shot**" button pressed.
    2. *Insert No AA* – Generally used for a special protocol not involving the coupling of an amino acid is to be run at that position of the synthesis.
        a. This is accomplished by selecting the appropriate residue tile within a sequence and clicking the "**Insert No AA**" button.
        b. A "*" will appear on the selected amino acid when an *Insert No AA* has been selected.
        c. To clear the *No AA*, the amino acid has to be selected and the "**Remove No AA**" button pressed.
        d. If a standard protocol is used for this cycle, all protocol steps will be performed except for amino acid addition.

3. *Insert Idle* – This inserts idle cycles, or pauses, that make the RV idle during that cycle.
    a. This is accomplished by selecting the appropriate residue tile within a sequence and clicking the "**Insert Idle**" button.
    b. A "#" will appear on the selected amino acid when an *Insert Idle* has been selected.
    c. To clear the *Idle*, the amino acid has to be selected and the "**Remove Idle**" button pressed.
    d. When special protocols need to be run on particular RVs, pauses can be used to keep others idle.

vii. The **Report** button is used to export all the information about the sequences into a PDF file.

viii. Selecting the "**Done**" button takes the user to the next step in the workflow.

d. **Setup**: The **Setup** screen allows users to assign solvents and reagents to timed or metered delivery bottles, as well as the amino acid bottles and CV/single shots used in the synthesis**.**

i. **Solvents/Reagents** can load saved solvent files by utilizing the top drop-down menu.
    1. Under this menu, there is also a "**New**" option available, along with a "**Search**" function for adding or locating solvent files, respectively.
    2. Std should be the typical solvents and reagents used for standard syntheses.
        a. Users can also use the HCTU_DIPEA solvent file in the case of using HCTU and DIPEA as coupling reagents instead of DIC and Oxyma.
        b. ***Users must get permission to use other solvents.***
    3. Users can use the drop-down menus at each bottle position to set as a specific **solvent**, **reagent**, or **Coupling Combo**. However, these shouldn't be adjusted.

ii. **Amino Acids** allows users to specify which amino acid is at each bottle position.
    1. The natural amino acids should all have assigned locations (1-20) and should not be changed.
    2. AA21 is saved for lysine with an Alloc protecting group (K_{alloc}).
    3. Positions 22-27 are free to be used for different amino acids.
        a. To load an amino acid to a position, press on the desired location to highlight it, then press on the amino acid icon from the bottom box. The amino acid should now be loaded into the correct location.

b. To move any amino acid to a new location, highlight the amino acid and press the "**Clear**" button at the bottom right side. The amino acid should now be available in the bottom box again.
c. Variant and other non-standard amino acids/monomers are marked red.

4. Single shots may also be assigned to any position, indicated by a green border.
    a. Only single shots may be assigned to the six *CV/Single Shot* positions found in the white box in the center of the screen.

iii. Selecting the "**Done**" button takes the user to the next step in the workflow.

e. **Protocol**: The **Protocol** screen allows users to assign pre-defined protocols or create new ones and copy them to cycles in a group, as well as the default temperatures for heated deprotections and coupling reactions.

i. On this screen, sequences are displayed horizontally, and cycles are numbered from the C-terminus (first cycle on the right) to the N-terminus (last cycle on the left).

ii. The bottom left part of the screen displays a list of the protocols assigned to each cycle.
    1. A long press or double click on any cycle tile from the list of protocols or selecting the cycle radio button and pressing the "**Add/Edit**" button, will open the *Protocol Editor* associated with that cycle.
    2. See **Section D** for creating new protocols.
    3. After choosing a protocol for any cycle, the "**Copy**" functionality gives the user the flexibility to copy the protocol to any cycle/cycles of user choice. Selecting the checkbox at the top of the *Copy dialog* will select/unselect all the cycles.

iii. The bottom right part of the screen displays a preview box of the selected protocol.

iv. **Temps Screen** – The *Protocol Temperatures* screen can be accessed by using the "**Protocol/Temps**" toggle on the top left corner of the screen.
    1. The top boxes for coupling (T_c) and deprotection (T_d) are used to set the RV-global temperatures in Celsius to be used in a particular RV during the synthesis. The user can also specify temperature exceptions for T_c and T_d for any AA cycle.

v. **Automatic Cleavage** – Users can have the software perform automated cleavage immediately after synthesis is complete. ***This should not be used unless special permission has been given.***

vi. **Pre and Post Protocols** – Users can assign *Pre and Post synthesis protocols* that are not attached to an amino acid within the sequence.
    1. Use the PRE and POST checkboxes at the top of the screen to turn these protocols ON/OFF accordingly.
    2. This utilizes the same process for creating synthesis protocols.
    3. The PRE protocol will run before the first cycle and is often valuable for swelling the resin.
    4. The POST protocol will run after the last cycle and is often valuable for a final deprotection and washing of the resin.
vii. Selecting the "**Done**" button takes the user to the next step in the workflow.

f. **Calculation**: The **Calculation** screen displays calculations for solvents, reagents, amino acids, and reaction vessel products.
    i. For more information on this tab, see **Section A Step 6**.
    ii. Selecting the "**Done**" button takes the user to the next step in the workflow.

g. **Run**: The **Run** screen displays the horizontal amino acid sequence with the running amino acid highlighted, as well as current running steps, reps, source and destination bottles, and action logs and graphs.
    i. "**Start Cycle**" allows the synthesis to start from any cycle and step. A popup dialog will appear allowing the user to choose which cycle, step, and repetition the synthesis needs to start/continue from.
    ii. By toggling the **Action/Graph** slide on the right, the user can display a preview graph of temperature or UV data.

h. **Run History**: The **Run History** screen displays the temperature and UV individual and summary data from previous jobs for exporting.
    i. Users can load previously recorded data for any completed synthesis by selecting Synthesis name from the drop-down menu at the top right part of the screen.
    ii. At the top left part of the graph a user can select which of the data sets will be loaded: **Temperature**, **UV Individual**, or **UV Summary.**
    iii. Data can be exported to a CSV file by using the "**Export**" function at the bottom of the screen.
        1. UV data can be exported as transmittance or absorbance values, or both.

3. Once a user has gotten to the **Calculation** screen, they have completed the creation of a new synthesis. **Run** and **Run History** are valuable for starting the synthesis and collecting data from previous syntheses.

D. Creating a New Protocol

The **Protocol Editor** allows the user to create a **New** protocol, **Open** and browse an existing one, or load one from the list of recently opened files.

1. Users can create a new protocol from an existing one with the "**Save As**" function. They can adjust the following settings:
    a. **Ops Page** – Users can select the type of operation that will happen at each *Step* within a protocol.
        i. Available operations**:** `Top Delivery`, `Bottom Delivery`, `AA Building Block`, `PV to RV`, `Drain/Dry`, `Collect`, `Mix`, `Cleave Mix`, `Pause`, `E-mail notification`
        ii. Users can choose the bottle position from which the operation takes place (`Source`), followed by the `Volume`.
        iii. Users can assign the `Time` for the operation to take place in hours:minutes:seconds format.
        iv. Users can select if a `Drain` should follow the operation and if the operation will take place at a pre-activation vessel (`PV`) position.
            1. **Note: Users must ensure that there is not too little, or too much, solvent present in the reaction vessels at one time. If the reaction vessel is not drained in a step, then the volumes must be added and must not be greater than the volume range. The following table provides the volume, shaker speed, and temperature ranges for the three reaction vessel sizes:**

	10-mL RV	**25-mL RV**	**40-mL RV**
Volume Range (mL)	3 – 6	6 – 13	13 – 20
Shaker Range (rpm)	300 – 375	300 – 375	350 – 400
Temperature Range (°C)	35 – 90	35 – 90	35 – 90

            2. ***Assume that every 100 mg of resin, when swelled, equates to 1-mL of solvent. Ensure you account for this in your volume additions.***
        v. The UV detection mode (`UVDM`) column specifies the use of the UV for the step.
            1. `None` – Without UV monitoring.

2. `Basic` – Records UV data during the step but does not adjust time or repetitions.
3. `Extend` – Uses real-time UV data to control the time of reaction. The reaction is considered complete when consecutive changes of transmittance readings approach zero.
4. `Extend + Reps` – Uses real-time UV data to control the time and number of repetitions of reaction. The reaction for a particular repetition is considered complete when consecutive changes of transmittance readings approach zero ***and*** the overall transmittance exceeds a set threshold value.

vi. The last column assigns the number of repetitions (`Reps`) of each step.

vii. **Delivery of AA-to-RV from Protocol** – It is possible to deliver a desired volume from an amino acid bottle without assigning the amino acid within a sequence. This is particularly useful for special reagents that are used sparingly throughout the synthesis.

1. To do this type of delivery, select the `Bottom Delivery` operation for the required step, press the `Source` box, select the `Amino Acid` checkbox, and choose the desired AA source.
2. Press the "**Ok**" button to confirm or "**Cancel**" to discard any changes.

b. **Mix Page** – Users can select the type of mixing.

i. Types of mixing:

1. `N2` – Nitrogen bubbling
    a. ***If performing a reaction that generates gas, Nitrogen bubbling must be on so that the RV is properly vented.***
2. `Shake` – Shake with a specific speed (`RPM`).

ii. Users can also select if `Heat` will be used during a particular step, as well as mark a deprotection step by selecting the `Deprotect` checkbox. The software will then apply the temperature that is defined for the deprotections.

c. **Comments Page** – Users can add, delete, or edit relevant comments to each cycle within the protocol.

2. The "**Report**" button will create a PDF file with all the steps and relevant information about the selected protocol.
3. Once the protocol has been saved, users can then open those files when creating a synthesis, as in **Section C Step 2e**.

E. Shutdown

This shutdown routine should be performed after every synthesis.

1. Once the synthesis has been completed, begin by removing the RV from the instrument and removing the resin from the reaction vessel.
    a. To remove resin from the RV, use a DCM squirt bottle to suspend the resin and pour it into another container.
    b. **Important: Users may NOT use any form of spatula to remove the resin. This can damage the reaction vessel.**
2. Once the resin has been removed from the reaction vessel using DCM, rinse the reaction vessel three times with DMF before placing it back on the instrument.
3. Perform a **Solv. Backflush** on all the amino acid bottles used in the synthesis.
    a. In the **Tools** tab under **Bottle Prep – Amino Acids** click the **Select All in Synthesis** button. At the bottom of the screen select **Solv. Backflush**.
4. After the amino acids have been backflushed, unload the amino acid bottles and dispose of extra reagent and DMF that has been added to the bottle.
    a. This waste should be disposed of in a properly labeled waste container.
5. After emptying the amino acid bottles, replace the amino acid bottles with the amino acid bottle that is labeled “Empty”.
6. Perform a **Solv. Backflush** on the two coupling reagent bottles that were used in the synthesis.
    a. In the **Tools** tab under **Bottle Prep – Solvents/Reagents** select the two boxes next to the coupling reagent bottles (5 and 6) and select **Solv. Backflush**.
7. Vent the coupling reagent bottles (5 and 6), and the DMF bottle (1) by selecting the three boxes next to the bottles and pressing the **Vent** button.
8. Dispose of the excess reagent and DMF in the coupling reagent bottles.
9. In the upper left-hand corner of the screen select the drop-down menu and select **Logout**. If you are the last user for the day, select **Exit App**, and shutdown the tablet.

F. Troubleshooting

- If you receive the error “The system has paused due to a shaker motor error.”, this is due to a brief error in the communication between the shaker motor controller card and the backend computer. To fix this, perform a full power cycle of the instrument. This requires you to power off both the Main and Aux power switches on the right side of the instrument, unplug both power supply cables, wait about 30 seconds, and then connect both power cables and turn on the two power switches. This should resolve the issue.
- If you lose resin during a synthesis, this most likely means the Nitrogen bubbling is too strong. Users should not touch the knob to control the Nitrogen (on the right-most side of the instrument) unless they are turning it down (clockwise to tighten).
- To emergency stop the instrument, push in the big red button at the top right-hand corner of the instrument. This should only be done in extreme emergencies. Once stopped, the button must be twisted clockwise to address errors.
- If the tablet disconnects from the instrument, exit the software by swiping from left to right on the screen and exiting the tab (selecting the desktop). Reopening the software will reconnect the tablet. Allow the software to make changes to the tablet.
- If the synthesis needs to be started from a particular point, go to “**Start Cycle**” and select the cycle and step.

G. File Management

The **File Mgr (File Manager)** menu contains four tabs: **Synthesis Manager**, **Libraries**, **Back Up/Restore**, and **Settings**. The **Synthesis Manager** and **Libraries** tabs are useful for managing files used in syntheses.

File Mgr – Synthesis Manager Tab:

- This tab facilitates the editing and import of *Synthesis*, *Protocol*, and *Sequence* definitions/files using the "**Edit**" and "**Import**" buttons, respectively.
- Users can create a new definition/file from an existing one with the "**Save As**" function.
- Using the "**New**" button, the **Protocol** and **Sequence** screens also allow to create new definitions/files via their respective editors.
- The "**Rename**" button allows the user to change the name of a definition/file.
- The "**Move**" button allows the user to rename and select another location, either a new or existing folder, to move and save the file.
- Importing/Exporting Files:
    - CSV Files:
        - The **Synthesis** import/export function will import/export the full synthesis file together with all associated *Protocols*, *Sequence*, and *Solvent* files.
        - To import/export to an external drive, a USB must be plugged into the tablet.
    - TXT Files:
        - The "**Export**" button from the **Synthesis Manager > Sequence** screen allows the user to export a sequence into a text (.txt) file.
        - The "**Import**" button from the same screen can import one or multiple sequences via a single text file.
        - When writing sequences into a text file, a valid sequence adheres to the following rules:

            1. Valid Formats:
                a. `Name:SingleLetters:`
                b. `Name::Abbreviations`
            2. Single-letter format is written as a string without any spaces or dashes in between.
            3. Abbreviations must be at least three letters, and each amino acid is separated by a − (dash).
            4. Defining a sequence using both one-letter and abbreviations is not allowed.
            5. All the amino acids must be part of the amino acid library.
            6. Each sequence must be defined on a separate line.

7. The sequence name is allowed to have dashes (−) and spaces.

- Two Examples:

```
4QC-ACP Sequence: VQAAIDYING:

GLHRH::Phe-Gly-His-Ile-Gln5-Gln6-Ala1-Gly
```

File Mgr – Libraries Tab:

- This tab includes **Solvents/Reagents**, **Amino Acids**, and **Resins**. On this screen, users can add additional solvents, reagent solutions, amino acids (other monomers), and resins to the instrument libraries that are available through the synthesis setup.
- These can be added directly using the "**Add**" button or using "**Import**" to import data from a CSV file.
- Use the "**Export**" button to export a library into a CSV files.

H. Data Extraction

The **Reports** menu contains **Job Reports** and **Audit Reports** screens. The **Job Reports** tab allows for the creation of a *Job Report* that corresponds to all the information from a synthesis run. **Audit Reports** allow the creation of detailed instrument log reports. To save a *Job Report* from a synthesis:

1. Select any job report from the drop-down menu.
2. Press "**Load**" to refresh the report and its contents.
3. Select which details the user would like included in the PDF report. The options include **Summary**, **Details**, **Amino Acid Usage**, **Solvent/Reagent**, **Protocol Summary**, and **Protocol Details**. An option to add an electronic signature is also provided.
4. Once the user selects the details they want in the report, the **Preview/Print** button can be clicked.
5. Exit the PurePep Chorus application and open Windows File Explorer.
6. Open the tablet's C drive and open the folder labeled "PTI" and then the folder labeled "PurePepChorus". Finally open the folder labeled "Reports".
    a. Note: The data path is: This PC\Local Disk (C:)\PTI\PurePepChorus\Reports
7. Locate the particular report and transfer to a USB drive connected to the tablet.
    a. Note: The PDF labeled with "JobReport" at the end is the file that should be transferred.

I. UV-Vis Monitoring

With the real-time UV monitoring system, it is possible to monitor the extent of the Fmoc removal in the deprotection step. There are three UV-Monitoring modes on the PurePep Chorus.

- **Basic** – Records UV data during the step but does not adjust the time or repetitions. The reaction uses the time set by the user.
- **Extend** – Uses real-time UV data to control the time of reaction. The reaction is considered complete when consecutive changes of transmittance readings approach zero. If this criterion is not met, the time of reaction is extended automatically up to a maximum of twice the originally programmed time.
- **Extend + Reps** – Uses real-time UV data to control the time and number of repetitions of reaction. The reaction for a particular repetition is considered complete when consecutive changes of transmittance readings approach zero and the overall transmittance exceeds a set threshold value. If these criteria are not met, the time and/or the number of repetitions extend automatically. Repetition extensions will be carried out up to a maximum of nine repetitions.

The **UV Graphs** screen displays the transmittance graphs for individual deprotection reactions as well as overall syntheses. This can be accessed by selecting the **Synthesis** menu at the top left of the Main Menu, then selecting the **Run History** button at the bottom left. To select a particular synthesis, a dropdown menu is available at the top right of the **Run History** screen. The controls, buttons, and switches on the screen are as follows:

1. **Dataset Type Dropdown** – Loads selected dataset, available types are `Temperature`, `UV Individual`, and `UV Summary`.
2. **RV Colored Circles** – Switches individual RV data ON/OFF.
3. **Top Slider** – Displays numerical UV values/data points for a specific time.
4. **Transmittance/Absorbance** – Alternates between transmittance and absorbance data.
5. **Bottom Scroll Bar** – Scrolls through the graph when the full data set does not fit on the screen.
6. **Synthesis Time** – Displays the date and time of the selected reading/repetition.
7. **RV** – Displays UV monitoring data for the selected RV.
8. **Cycle** – Displays UV monitoring data for the selected cycle.
9. **Step** – Displays UV monitoring data for the selected step.
10. **Rep** – Displays UV monitoring data for the selected repetition.
11. **-/+ Buttons** – Zoom graph in and out.
12. "**Export Options**" Button – Exports the absorbance and/or transmittance data as an external CSV file.

The *UV Summary Graph* displays a summary of the transmittance data for a total synthesis. In a Summary Graph, each peak represents an individual repetition in a UV monitored step. The darker portion represents the minimum transmittance measured during that repetition,

and the lighter portion represents the maximum absorbance measured during that repetition. This summary can be useful to get a rough estimate of troubling deprotections. For a more in-depth analysis of the UV-Vis data, see **Section J**.

J. UV-Vis Data Analysis

For more detailed analysis of UV-Vis data compared to that described in **Section I**, it is valuable to export the *UV Individual* data and process it. This section details this process.

1. Begin by exporting the data from the instrument. This is done in the **Synthesis** tab under the **Run History** screen.
    a. To select a particular synthesis, a dropdown menu is available at the top right of the **Run History** screen. Select the synthesis.
    b. In the upper left-hand corner, select **UV Individual**.
    c. Select **Export** at the bottom of the screen. Export both **Transmittance** and **Absorbance**.
2. The next step is to open the CSV file on your computer. Copy the entire exported .csv sheet by right clicking on the grey triangle in the upper left-hand corner of the Microsoft Excel file. Then, select copy.
    a. When opening the UV Individual data, a pop up may ask if you want to permanently keep conversions of removing leading zeros. You should click "Convert".
3. Download the "Chorus UV-Vis Template" on the PPMC website.
4. Open the "Chorus UV-Vis Template" and paste the data in the green box on the sheet labeled "Raw Data".
5. Next, scroll through the raw data and determine where the first set of data ends.
    a. Each raw data set will have a block of data for Percent Transmittance on top of a block of data for Absorbance on top of a block of data for Raw Transmittance.
6. For each RV that was used, check that the date column goes to the correct row, but does not go past it. Delete any extra rows that reference the next block of data.
7. One last edit that must be made for every RV used is the formulas for column J and K
    a. Going back to the raw data, scroll down to see the beginning of the next data table, which is the absorbance associated with column J in the RV sheet. Click on the highlighted Absorbance cell in the RV sheet and alter the equation to equal 'Raw Data'F and the cell number that data starts on.
        i. Note: For other RVs the letter will automatically be changed and only the number needs to be altered.
    b. Click enter and then hover the mouse over the bottom right-hand corner of the cell until a plus sign appears. Double right click, and this will apply that formula to the rest of the column.

    c. A similar process must be done for the Raw Transmittance column, which is column K. This time, the row that should be looked at is for the next data set after the Absorbance data set.
8. Modifications can then be made to the plots, and they can be analyzed.
    a. When using Extend + Reps, residues that have multiple added deprotection reps may have issues with deprotection (or issues with coupling for the previous residue).
        i. An extended deprotection for this residue, as well as the one before it, may be helpful.
    b. When using Basic monitoring, the percent transmittance may help indicate whether two deprotections is enough per residue.
        i. A downside of this UV system is that the first deprotections often have no useful data because the detector is not adequately sensitive for very high concentrations of the adduct. Therefore, when you look at other deprotection reps, you are seeing that a percentage of transmittance has been achieved, but this does not indicate the extent of deprotection that has been achieved because the initial deprotection data provides no indication of how much Fmoc has initially been removed from the growing peptide.
        ii. Despite this, for peptides that are not properly synthesized (as characterized by mass spectroscopy), looking at this data may help indicate where issues in the synthesis originated.

K. Calibration

The **Calibration** section contains three calibration tabs. Here are instructions for the two most important calibrations.

Solvent Calibration:

1. This screen allows the user to calibrate the volume deliveries of the timed solvent/reagent bottles (1-4, 8). The user should first check that the proper **Op Time** file is selected for the given **RV Size** in the upper right-hand corner.
2. The **Solvent Bottle** section is where users can select a solvent bottle for calibration. To select a bottle, press the radio button of the appropriate level (for top deliveries: Solvent 1 or Solvent 2, for bottom deliveries: Solvent 1 – Solvent 4, or Solvent 8).
    a. Only one solvent bottle may be selected at a time.
    b. When going from Solvent 1 calibration to Solvent 2 Top Delivery calibration, run the calibration three times without measuring the volume delivered to equalize the vapor pressure difference between DMF and DCM. This will save the user time.
3. Set the **Target Delivery Volume (µL)** which will determine the calibration delivery volume.
    a. For best results, this volume should be the same as the volume (in microliters) that will be delivered by the selected bottle during a synthesis.
    b. The **Number of Deliveries** box is where a user can enter the number of times the volume will be delivered to an RV(s) during the calibration. This can usually be kept at 1.
4. Select the RV positions to be tested by pressing the box to the left of the desired RV(s) in the **Test RV** column. Press the box a second time to deselect an RV.
    a. Alternatively, select the **Copy To** feature. This copies the calibration factor for a single RV to all others selected. It is often easiest to calibrate RV1 while copying the calibration to the other three RVs.
5. Click the **Run** button at the bottom of the screen to start running the calibration. Make sure RVs and Collect Vials are in place.
    a. A user may press **Cancel** to cancel the calibration.
6. After the calibration is complete, measure the volumes in the collection vial(s) using a graduated cylinder.
7. Enter the values (in microliters) in the **Actual Volume (µL)** column next to the appropriate RV.
8. Click the **Refactor** button to calculate new calibration factors.
9. Repeat these steps until the **Actual Volume (µL)** and **Expected Collect Volume (µL)** values match. Then, click the **Save Factors** button.
10. After the calibration is complete, the fluid system may be contaminated. Perform a **Wash All Blocks** followed by a **Clear All Blocks** operation in the **Cleaning** tab.

UV Calibration:

1. This screen allows users to calibrate UV modules. In the **Operation** drop down menu, the user can find three calibration operations: **Calibrate UV High Value**, **Calibrate UV Low Value**, and **Wash RV**. The **Calibrate UV High Value** operation is used to calibrate maximum transmittance/minimum absorbance values of the UV module based on the pure deprotection solution. Select **Calibrate UV High Value**.
    a. **Wash RV** operation can be performed to wash the UV module cell.
    b. The **Calibrate UV Low Value** operation is used for references only. ***Users should not perform this calibration.***
2. From the **Bottle** drop down menu, the user should designate the solvent bottle in which the deprotection solution is placed (Bottle 4).
3. Next, users should ensure that the proper **Op Time** file is selected for the **RV Size** that is in place on the instrument.
4. Then, users should select the UV modules they would like calibrated by selecting the box next to UV1, UV2, UV3, and UV4 at the right of the screen.
5. The operation can then be performed by pressing the **Start** button.
6. Once the operation has completed, select **Sav Cal** to save the calibration.

L. Recommended Consumables

The following products are recommended from Fisher scientific. These can be purchased at a discount through UD exchange or through the chemistry store. The Fisher catalog numbers are also provided below.
N,N-Dimethylformamide (DMF): D119-4
Dichloromethane (DCM): D37-4
Methanol: A412-4
Oxyma Pure: 50-187-7780
N,N'-Diisopropylcarbodiimide (DIC): D0254250G
N,N-Diisopropylethylamine (DIPEA): D1599100ML
HCTU: NC0576737

Piperidine must be purchased through Millipore Sigma. The recommended catalog number is 8222990500.

It is recommended that amino acids and resin be purchased through ChemPep Inc. The recommended resins and amino acids, with their associated catalog numbers, are provided below.

Rink Amide Resin: 151801
Fmoc-Ala-OH: 100101
Fmoc-Arg(Pbf)-OH: 100202
Fmoc-Asn(Trt)-OH: 100302
Fmoc-Asp(OtBu)-OH: 100402
Fmoc-Cys(Trt)-OH: 100502
Fmoc-Gln(Trt)-OH: 100602
Fmoc-Glu(OtBu)-OH: 100702
Fmoc-Gly-OH: 100801
Fmoc-His(Trt)-OH: 100902
Fmoc-Ile-OH: 101001
Fmoc-Leu-OH: 101101
Fmoc-Lys(Boc)-OH: 101202
Fmoc-Met-OH: 101301
Fmoc-Phe-OH: 101401
Fmoc-Pro-OH: 101501
Fmoc-Ser(tBu)-OH: 101602
Fmoc-Thr(tBu)-OH: 101702
Fmoc-Trp(Boc)-OH: 101805
Fmoc-Tyr(tBu)-OH: 101902
Fmoc-Val-OH: 102001
Fmoc-Lys(Alloc)-OH: 101244

M. Remote Software Access

The PurePep chorus does NOT have the capability to be run or monitored remotely. However, users can download the same software on their personal computer to design **Syntheses**, **Protocols**, and **Sequences** that can be transferred with a USB drive to the instrument. The following steps should be taken to install the software on a personal computer.

1. Begin by downloading a copy of the "PurePepInstaller.exe" file. This can be requested by contacting the PPMC primary contact for the instrument.
2. Once you have downloaded this file on your personal computer, place the file in a folder on the PC's "C" drive.
3. Double click to run the "PurePepInstaller.exe" file. If prompted with a "User Account Control" dialog, select "Yes".
4. After launching the installer, you will notice a screen to choose the location of the installation. ***The "Editor" option should only be used when installing the editor utility on a PC other than the instrument control tablet.*** After the selection, click "Next".
5. The next screen sets the desired configuration of the instrument. These fields will be blank by default and need to be filled by the user based on the configuration of the instrument. For the PPMC's PurePep Chorus the following fields should be filled and then "Next" should be clicked:
    a. The "IP address" can be left blank.
    b. Using the dropdown, select that there are 4 RVs.
    c. Check the box next to "Heat".
    d. Check the boxes next to "UV1", "UV2", "UV3", and "UV4".
    e. The "Physical Keyboard" option will be checked by default and should be left checked.
6. A **Ready to Install** screen will confirm that the user is ready to set up the installation. Click "Install" to start the installation. The installer will perform various system checks before proceeding with the installation.
7. On the last screen, it is highly recommended to allow the computer to restart after installation. Select "Yes, restart the computer now" and then click "Finish".
8. On restart, navigate to the *C:\PTI\PurePepChorus* folder and find the **PurePepChorus.exe** to launch the software. A shortcut can also now be created.
    a. ***The login credentials for the editor software are "editor" and "editor".***
    b. It's recommended to export both **Solvent/Reagent** and **Amino Acid** libraries from the instrument and import them to the editor to ensure you have the same library items.
        i. To do this, navigate to **Tools** > **File Mgr** > **Libraries** on the instrument and export both **Solvent/Reagent** and **Amino Acid** libraries. From the same screen on the editor, choose to import both libraries.

N. Facilities Use Acknowledgements

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If you use this instrument, then the following acknowledgement statement must be included in related publications:

"The authors acknowledge the use of facilities and instrumentation supported by the National Science Foundation through the University of Delaware Materials Research Science and Engineering Center, DMR-2011824."