PurePep Chorus

User Guide

GYROS PROTEIN
Technologies

Purity
Productivity
Power

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Tucson, AZ 85714 USA

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WARNING ALL REACTION VESSELS MUST BE IN PLACE AT ALL TIMES.

WARNING COLLECTION VIALS MUST BE IN PLACE AT ALL TIMES.

WARNING SAFETY SHIELD DOORS MUST BE CLOSED WHILE A SYNTHESIS OR CLEAVAGE IS RUNNING.

WARNING SYNTHESIS WILL HALT IF WASTE CONTAINER IS FULL.

WARNING DO NOT ATTEMPT TO MOVE THE INSTRUMENT WHILE ANY OF THE SOLVENT OR WASTE CONTAINERS CONTAIN LIQUIDS.

WARNING THIS INSTRUMENT CONTAINS SOLVENTS AND CHEMICALS THAT SHOULD BE HANDLED CAREFULLY. MANY ARE EASILY ABSORBED THROUGH THE SKIN AND CAN CAUSE ADVERSE HEALTH EFFECTS. WEAR SAFETY GLASSES, PROTECTIVE CLOTHING AND RUBBER GLOVES AT ALL TIMES. FOLLOW MSDS HANDLING GUIDELINES PROVIDED WITH THE INDIVIDUAL REAGENTS. RESPIRATORS AND ABSORBENT SHOULD BE AVAILABLE IN THE EVENT OF A SPILL.

WARNING: FLAMMABLE LIQUIDS

INSTRUMENT MAY CONTAIN FLAMMABLE LIQUIDS. SEE SDS BEFORE

WARNING: BURN HAZARD

TOUCHING THIS SURFACE OR SURFACES INSIDE THE RV ENCLOSURE COULD RESULT IN BODILY INJURY.

WARNING: PINCH POINT

KEEP HANDS CLEAR DURING OPERATION.

AVERTISSEMENT : TOUS LES RÉCIPIENTS DE REACTION DOIVENT ÊTRE EN PLACE À TOUT MOMENT.

AVERTISSEMENT: LES FLACONS DE COLLECTION DOIVENT ÊTRE EN PLACE À TOUT MOMENT.

AVERTISSEMENT : LE COUVERCLE DE SÉCURITÉ DOIT ÊTRE TOUJOURS FERMÉ PENDANT LA SYNTHÈSE OU LE CLIVAGE.

AVERTISSEMENT: LA SYNTHÈSE S’ARRETERA SI LE CONTENEUR DE DECHETS EST PLEIN.

AVERTISSEMENT: NE PAS TENTER DE DÉPLACER L’INSTRUMENT PENDANT QU'UN DES SOLVANTS OU DES CONTENEURS DE DÉCHETS CONTIENNENT DES LIQUIDES.

ATTENTION : CET INSTRUMENT CONTIENT DES SOLVANTS ET DES PRODUITS CHIMIQUES QUI DOIVENT ETRE MANIPULÉS ATTENTIVEMENT. NOMBREUX SONT FACILEMENT ABSORBÉS PAR LA PEAU ET PEUVENT PROVOQUER DES EFFETS NÉFASTES SUR LA SANTÉ. PORTER DES LUNETTES DE SÉCURITÉ, VÊTEMENTS DE PROTECTION ET DES GANTS DURANT LEUR UTILISATION.

SUIVRE LES DIRECTIVES DE MANIPULATION FOURNIS AVEC LES REACTIFS INDIVIDUELS. DES RESPIRATEURS ET ABSORBANT DOIVENT ÊTRE DISPONIBLE EN CAS DE FUITE.

AVERTISSEMENT: LIQUIDES INFLAMMABLES

L'INSTRUMENT PEUT CONTENIR DES LIQUIDES INFLAMMABLES. VOIR SDS AVANT CHAQUE UTILISATION.

WARNING PINCH POINT

KEEP HANDS CLEAR DURING OPERATION

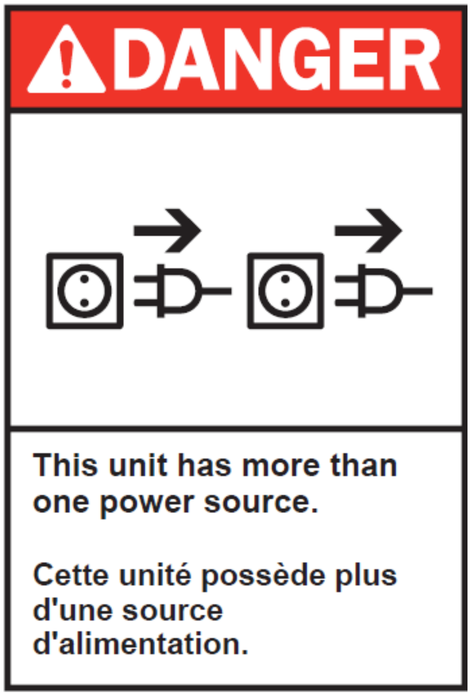
AVERTISSEMENT: RISQUE DE BRÛLURE

TOUCHER CETTE SURFACE OU SURFACES À L'INTÉRIEUR DE LA CLOTÛRE POURRAIENT RESULTANT DE BLESSURES CORPORELLES.

POUR RÉDUIRE LE RISQUE DE BLESSURES,
PERMET DE REFRIGER AVANT DE TOUCHER.

AVERTISSEMENT: POINT DE PINCEMENT

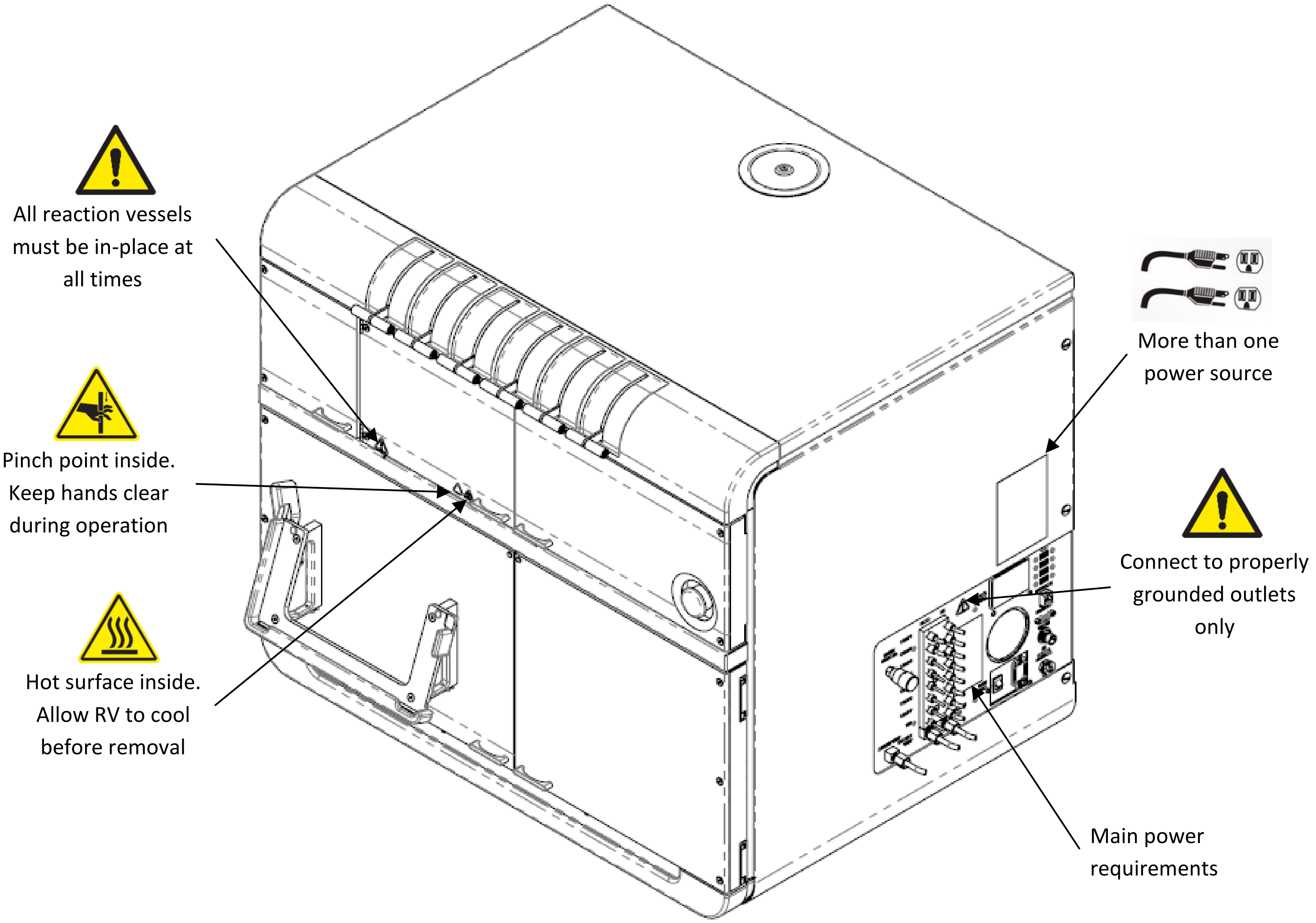
GARDER LES MAINS DURANT LE FONCTIONNEMENT



DANGER

This unit has more than one power source.

Cette unité possède plus d'une source d'alimentation.



All reaction vessels must be in-place at all times

Pinch point inside. Keep hands clear during operation

Hot surface inside. Allow RV to cool before removal

More than one power source

Connect to properly grounded outlets only

Main power requirements

Introduction

Thank you for purchasing your new peptide synthesizer from Gyros Protein Technologies. **PurePep™Chorus** is an all-in-one scalable and field upgradable peptide synthesizer. Induction heating, oscillation mixing, and UV monitoring are available on every reaction vessel to deliver uncompromised purity, yield, and flexibility, creating the most complete peptide synthesis solution available. Heating conditions in up to six reaction vessels can be independently set to enable flexible, customized reactions. Real time UV monitoring with available Single-Shot™ amino acid delivery ensures complete deprotection and the subsequent coupling using a special amino acid won't be wasted. Combined with automatic cleavage and flexible pre-activation options, **PurePep Chorus** enables researchers to synthesize routine and difficult peptides with unparalleled speed and efficiency.

I.2 About the Company

Gyros Protein Technologies (GPT) (formed by the merger of Gyros AB and Protein Technologies Inc. (PTI) in 2016) is built on the belief that our products and services are of the highest possible quality. With over 30 years dedicated to peptide chemistry service and technical support, we have the expertise to develop and manufacture high quality peptide synthesizers that deliver uncompromising purity and yield with the highest quality peptide reagents (FMOC-amino acids – pre-packed or bulk, Resins, Activators and Starter kits). Founded in 1985 by researchers affiliated with the University of Arizona, our first peptide synthesizer was launched in 1990. Since then, we have manufactured and sold the world's finest solid-phase peptide synthesizers. Today, we are growing and innovating to serve the needs of researchers utilizing solid-phase peptide synthesis (SPPS). If you have any questions concerning your GPT synthesizer, please feel free to contact us:

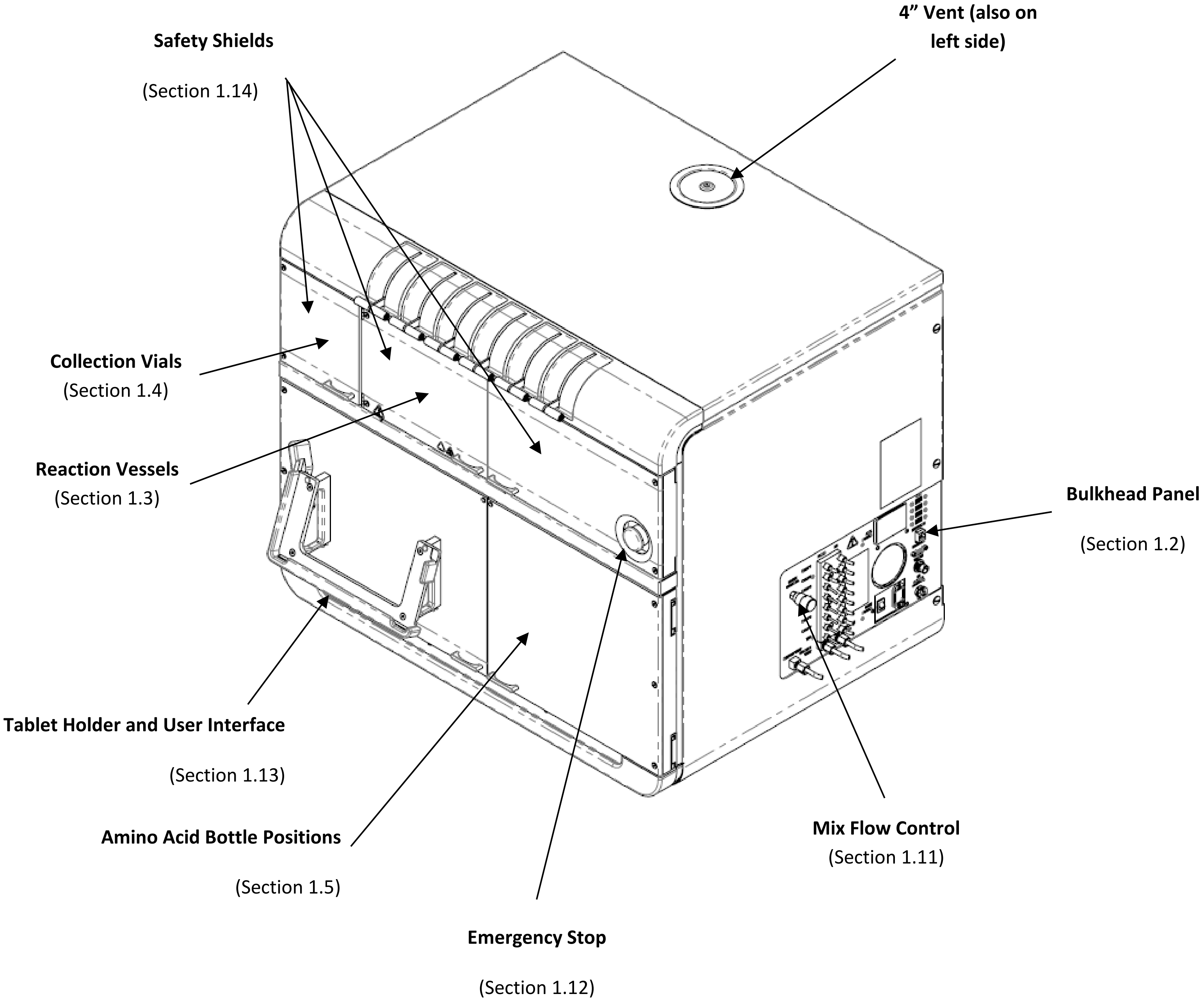
Tel: 520-629-9626 | 800-477-6834 | Email: peptides@gyrosproteintech.com

www.gyrosproteintechnologies.com/protein-technologies

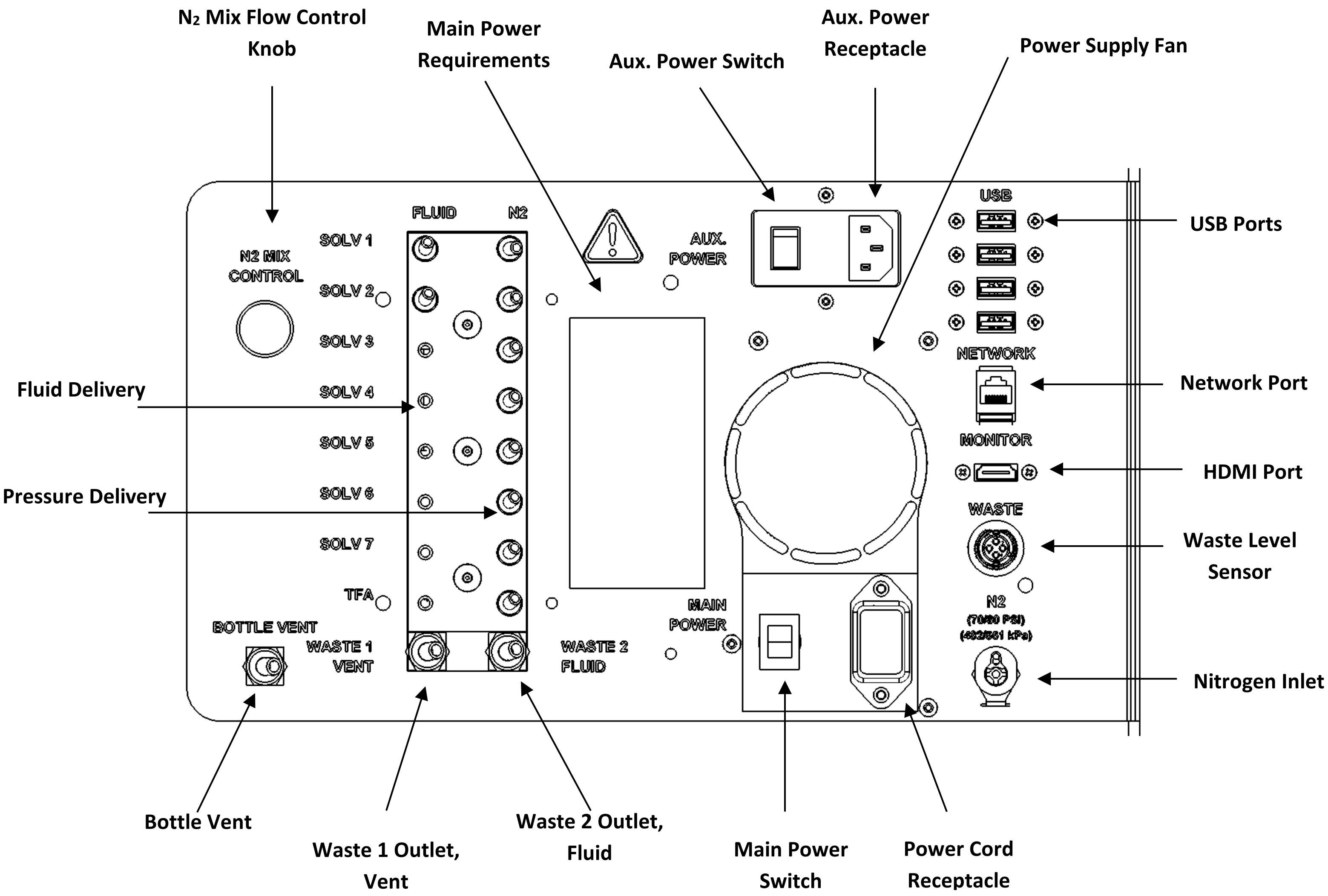
Chapter 1. General System Description

CAUTION Please read and understand all pre-cautions with Symbol 14 ⚠ in the front of this guide.

1.1. PurePep Chorus Front



1.2. Bulkhead Panel



1.3. Reaction Vessel System

PurePep Chorus can be configured with 2, 4 or 6 reaction vessels and can be upgraded at any time (see Section 3.1). The reaction vessel system on **PurePep Chorus** is designed around a simple and reliable quick release mechanism. Cam levers allow the operator to remove and install the reaction vessels (plastic for non-heated protocols or glass to be used with induction heating - see Section 1.3.1) quickly and easily (see section 2.3). An 'In-Place' detection sensor verifies that all RVs are present prior to executing a synthesis.

PurePep Chorus is also available with an induction heating and shaking option. Induction heating makes it possible to assign unique heating temperatures to each reaction vessel position.

CAUTION All RV positions that are being heated must use an induction compatible reaction vessel. Failure to use the correct RV will cause the heater to time out and result in a system error. See Section 1.3.1 for a listing of available reaction vessels and accessories.

NOTE All RV positions (including unused positions) must have an RV present for the instrument to function.

1.4. Cleavage Collection System

The collection system for **PurePep Chorus** accepts 50 mL polypropylene vials. The system is made of materials resistant to the aggressive reagents associated with cleavage solutions. A proper seal between the vial and instrument will ensure cleavage solution vapors are vented (see section 2.4 for vial installation instructions). An 'In-Place' detection sensor verifies that a vial is present at all times. These tubes can also be used as Single-Shot AA. Only VWR tubes should be used, any other type of vessel could be prone to leaking.

NOTE All six collection vials must be in place for the instrument to function.

CAUTION The collection tubing is not rinsed automatically since it exits into the collection vials. Therefore, it is important to perform a cleaning process after each collection operation is completed and the collection vials have been replaced. The cleaning operation is denoted as a **Collect Back Flush** under **Cleaning** in the **Tools** menu. .

1.5. Amino Acid Bottle System

27 amino acid bottle positions are located on the front of **PurePep Chorus**. Amino acid bottles are available in 10, 120 and 400 mL capacities and can be connected or detached quickly and easily (see section 2.5). Pressurizing the bottles with nitrogen accomplishes solution transfer, and this and other operations are controlled using the **Bottle Preparations** screen. Each amino acid bottle has a bottle filter to prevent particulates from entering the fluid system. These filters should be changed on a regular basis depending on the quality and concentration of reagent utilized. See Section 4.2.4, **Bottle Filter Replacement** for instructions.

<u>CAUTION</u> DO NOT install or remove amino acid bottles when they are pressurized.

<u>IMPORTANT</u> All 27 amino acid bottles are vented or pressurized together. For this reason, all amino acid positions must have bottles in place for the pressurization to occur. Empty bottles should be placed in any unused positions.

<u>IMPORTANT</u> The amino acid manifold seals are not affected by DMF or NMP. Contact the Technical Support Department at GPT if alternative solvents are desired. Under no circumstances should TFA be used in the amino acid manifold system—destruction of the seals will occur! See Section 2.5 **Amino Acid Bottle Seal Replacement** for replacement procedures.

1.6. Solvent/Reagent Bottle System

The eight solvent and reagent bottles are located outside the unit and are attached to **PurePep Chorus** via the Bulkhead Panel. These glass bottles are pressurized with nitrogen to accomplish solution transfer. For the safety of the user, safety-coated glass bottles should always be used. The solvent and reagent bottles are controlled using the **Bottle Preparations** screen.

<u>CAUTION</u> Safety-coated glass bottles are supplied by GPT with each instrument and should always be used with this instrument. Using regular glass bottles may result in serious bodily injury.

Bottle positions 1 – 4 are intended for solvents, and volumes are measured out by timed deliveries. These positions are appropriate for the primary and secondary wash solvents, deprotect and capping reagent. Bottle positions 5 – 7 are intended for reagents that have a requirement for a specific stoichiometry when combined, and can precisely measure volumes in 250, 500 and 1000 µL aliquots using a metering loop. These positions are appropriate for coupling solutions. Bottle position 8 is specifically intended for the delivery of cleavage solution. A typical solution composition of each bottle position for Fmoc chemistry would be as follows:

> Btl 1 or DMF: Two daisy-chained 4 L safety-coated glass bottles for the primary wash solvent, typically reagent grade DMF. DMA or NMP may also be used. Because this solvent is quite stable, the 4 L containers may be installed and left in place throughout several sets of syntheses. This solvent is utilized in the automated cleaning operations for the valve fluid system. Therefore, Btl 1 must be in place for normal operation of the instrument. This solvent position is also utilized during the **Bottle Back Flush**, **Wash All Blocks, Wash RVs, and System Clean** cleaning operations.

Btl 2 or DCM: safety-coated glass bottle (1 to 4L) for a secondary wash solvent, such as DCM to wash the peptide-resin in preparation for automated cleavage. This bottle position is utilized during **Collect Block Backflush** cleaning operations. DCM may be installed and left in place for several sets of syntheses.

Btl 3 or Dep: 1L safety-coated glass bottle for deprotectant to remove the N-terminal Fmoc protecting group. The standard composition is 20% (v/v) piperidine in DMF. This solution is also quite stable and may be installed and used for several sets of syntheses. Other reagents may be loaded for alternate chemistries.

Btl 4 or Cap: 1L safety-coated glass bottle for capping solution to permanently block any unreacted amino groups following a coupling reaction or to acetylate the N-terminus of a completed peptide. Typical compositions include 1:1:3 acetic anhydride/pyridine (or DIPEA)/DMF. Other reagents may be loaded for alternate chemistries.

Btl 5 or Base: Safety-coated glass bottle (1 to 4L) for reagent solution. This could be, for example, a base solution if separation of base and coupling reagent is desired. A typical base composition is 0.4 M NMM in DMF. Bottle 6 or 7 should then contain a coupling reagent such as 0.2 M HCTU in DMF. Alternatively, base and coupling reagent may be combined into a single activator solution for short syntheses, or if the solution is to be prepared fresh every day.

Btl 6 or Act1: 1L safety-coated glass bottle for activator solution to form the activated Fmoc amino acid for the coupling reaction. A typical composition is 0.2 M HCTU in DMF. Other reagents may be loaded for alternate chemistries.

NOTE The activator solution should usually be prepared in a concentration such that when adding the desired volumes it is equimolar with the amino acid added. It is recommended that the activator solution be prepared fresh for each synthesis, or according to the stability of the reagent in the solvent used.

Btl 7 or Act2: Safety-coated glass bottle (1 to 4L) for additional reagent.

Btl 8 or CLEAV: Safety-coated glass bottle (1 to 4L) of cleavage reagent for cleavage of the peptide from the resin after synthesis is complete. This position is specifically designed to handle the caustic TFA cleavage solution.

NOTE The cleavage solution should be prepared fresh for each synthesis.

NOTE The **On Demand** feature must be selected for Btl 8 when this bottle is being used for cleavage. However, the **On Demand** feature should be deselected for Btl 8 during **Solvent Calibration**.

Each solvent position has a bottle filter to prevent particulates from entering the fluid system. For replacement procedures, see Section 4.2.4. An encapsulated O-ring in the bottle cap insert establishes the bottle seals and is inert to the reagents. Damage to the insert or O-rings will result in nitrogen leakage and potential loss of reagent (volatiles like TFA, DCM). For bottle seal replacement procedures see Section 4.2.6.

Custom bottle configurations and assemblies can be arranged through Technical Support (peptides@gyrosproteintech.com).

1.7. Waste System

The only exit for the closed fluid flow paths of the instrument is through the waste system. Waste exits **PurePep Chorus** to the waste container through three ports on the Solvent Feedthrough Panel. The waste container is vented through a fourth tube attached to a fitting on the 4" vent duct. The waste container is a 5-gallon carboy fitted with a waste level sensor to prevent overfilling. If the waste container is full, all operations in the instrument will stop automatically and all the bottles will vent. No operations will be allowed until the container is emptied and reconnected.

IMPORTANT The waste container being full is a critical error on **PurePep Chorus**. To prevent overfilling, the instrument will automatically pause all operations and vent all bottles. To resume operations, first empty and reconnect the waste container, then re-pressurize all the bottles using the **Bottle Preparations** screen. Go to the **Run** screen and press the **Resume** button to continue the operations on the paused reaction vessels.

The waste level sensor is wired in a normally closed (NC) configuration so if the switch is disconnected, it is the same as if the container is full. This logic prevents waste from being delivered when the container is not connected. The connectors are resistant to the corrosive waste solutions. Do not attempt to disassemble the switch connector assembly.

CAUTION Be sure to backflush bottles before removing the waste level sensor connector. If the sensor connector is removed from the waste container while any bottles are primed, the bottles will vent, and fluid may remain in the lines.

1.8. Ventilation System

PurePep Chorus has two 4-inch vent holes—one on top and one on the left side panel. It comes equipped with an adjustable angle adaptor for one hole, and a vent cover for the other. The adaptor has a tube fitting for attaching the waste container vent line. **PurePep Chorus** should be connected to lab ventilation with a 4-inch (10 cm) duct supplied by the user. The ducting should be made of a chemically resistant material (PVC or urethane, but no rubber). A minimum flow of 100 cubic feet/min (CFM) must be maintained at the instrument.

1.9. Nitrogen System

The nitrogen inlet is located on the Bulkhead Panel (Section 1.2). A minimum of 80 psi (70-80 psi / 482-551 kPa) must be supplied for the instrument to operate. The lack of nitrogen is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. No operations will be allowed until the supply is restored.

The high-pressure nitrogen is diverted into three regulators:

1. **Valve Pressure** – Used to seal the valve membranes. Should be set to 30-40 psi. User should not adjust.
2. **Nitrogen Pressure** – Used for mixing and delivering fluid. Should be set to 5 psi. User should not adjust.
3. **Bottle Pressure** – Used to pressurize the bottles. Should be set to 9 psi. Contact GPT Technical Support for assistance in adjusting this value.

CAUTION Timed delivery volumes from solvent bottles 1 - 4 and 8 are dependent on the **Bottle Pressure** setting. Any adjustments made to this regulator will require reverification of **Solvent Calibration**.

The intensity of the mixing and fluid deliveries are controlled using the following two flow controls:

1. **Mix Flow Control** – Controls the nitrogen flow during a mix. Located on the Bulkhead Panel (See Section 1.2). Counter clockwise to increase, clockwise to decrease (See Section 1.11).
2. **Nitrogen Push Flow Control** – Controls the nitrogen flow during fluid delivery. Control is only accessible by trained field service personnel.

CAUTION The **Nitrogen Push Flow Control** is factory set. Adjusting the **Nitrogen Push Flow Control** will affect the way the instrument delivers fluid to the reaction vessels. If it is adjusted too low, it may cause the instrument to fail in its delivery and error out. If you feel an adjustment is needed, please contact GPT Technical Support for proper instructions prior to adjusting this control.

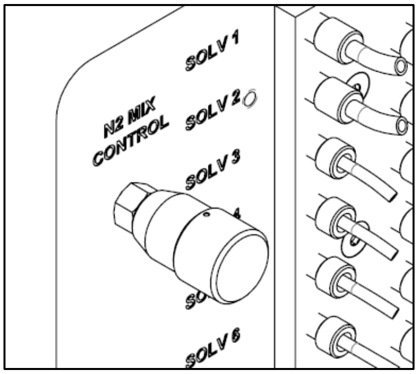
IMPORTANT Adjusting the mixing or delivery flows too high can cause resin to stick to the top of the reaction vessels and possible reagent loss. This can lead to incomplete reactions.

1.10. Vacuum System

Vacuum is supplied by a vacuum pump located inside the instrument. The normal operating range is 10 – 22 in Hg. When the vacuum drops to 10 in Hg, the vacuum pump will turn on. The vacuum is diverted directly to the valve blocks and is used to lift the valve membranes to allow fluid flow from different locations. The lack of vacuum is a critical error, and the instrument will pause all operations, vent all bottles, and display an error message. This occurs when the vacuum pump fails to bring the vacuum >10 in Hg. No operations are allowed until the vacuum is restored.

1.11. Mix Flow Control

The N_2 Mix Flow knob controls the nitrogen flow during a mix. Counter clockwise to increase, clockwise to decrease.



1.12. Emergency Stop Button

Press down to stop **PurePep Chorus** in the event of an emergency. All actions will cease and all bottles will be vented. To release the Emergency Stop Button, open the right-side RV door and twist the button clockwise. This button should only be used in the event of an emergency and is not a viable way to pause an operation during a synthesis. Pressing the Emergency Stop Button can result in a loss of reagents and/or the current synthesis.

1.13. User Interface and Computer System

PurePep Chorus has an internal computer that operates **PurePep Chorus** core instrument software and a touchscreen tablet containing the user interface. Extra USB ports on the Bulkhead Panel (Section 1.2) allow data to be transferred from the computer via a user-supplied memory stick. The network connection allows the instrument to be connected to an available network (optional) and the HDMI port provides a means for service personnel to connect an alternate instrument interface. Contact GPT Technical Support for network or user interface assistance.

1.14. Safety Shields

Safety doors are installed for the protection of the user. The doors in front of the reaction and collection vessels MUST BE CLOSED when **PurePep Chorus** is running. Before opening the doors in front of the reaction vessel, all of the reaction vessels must be in a non-operational state (i.e. paused or completed) and drained of all fluids. Opening the doors while a synthesis is running or before performing the above operations will result in an error and the synthesis will be paused.

IMPORTANT Minimum safety equipment to be used at all times (e.g. lab coat, chemically resistant gloves). Follow your institution’s safety requirements.

Chapter 2. Instrument Setup

2.1. Laboratory Requirements

Ensure a flat sturdy surface capable of safely supporting 136Kg (300 lbs) and to allow for easy access for loading reagents and viewing and operating the user interface. The surface should be near a primary power outlet, a fume hood and a nitrogen source. The power outlet needs to be clear at all times. If main power needs to disconnected, unplug BOTH cords at the power outlet. Only use the power cords supplied with the instrument. Ambient temperature should be 10 – 37.8°C (50 –100ºF) with relative humidity below 90%. Do not place the instrument where it can be exposed to extreme temperatures, e.g. near heating or cooling ducts, near open windows or in direct sunlight. The elevation should be between sea level and 2000 meters.

In order to install and run **PurePep Chorus**, a laboratory must be able to supply the following:

1. **Ventilation System**
   PurePep Chorus has a 4-inch vent equipped with an adjustable angle adaptor. **PurePep Chorus** should be connected to a lab ventilation system with a 4-inch (10 cm) duct supplied by the user. The ducting should be made of a chemically resistant material

(PVC or urethane, but no rubber). A minimum flow of 100 CFM must be maintained at the instrument.

2. **Nitrogen Supply**
   A relatively pure (>99.9%) and dry source of pressurized nitrogen is recommended. The system uses nitrogen for solution transfers and agitation/mixing. Alternative gases can be utilized if desired, e.g., Argon. The user must supply all necessary regulators and nitrogen tanks. One male and one female 1/4" NPT fittings are provided with the unit to connect it to the tank.

IMPORTANT Securely fasten cylinders with safety straps to prevent them from falling, and do not move a cylinder or undo safety straps unless the safety cap is in place.

3. **Power**
   A power source capable of 110-230 VAC 50/60Hz

   - For 110V installations:
     Main Power: 1.0A (Internally Fused), Heater Power:10.0A (Fuse Value = 10.0A Ceramic 110V Slo-Blo)

   - For 230V installations:
     Main Power: 0.5A (Internally Fused), Heater Power: 5.0A (Fuse Value = 5.0A Ceramic 230V Slo-Blo)

IMPORTANT If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired. Only trained Service Personal Only to replace fuses in power entry module. Please call service if instrument fails to power up.

4. **Memory Stick (optional)**
   Files may be transferred from **PurePep Chorus** computer to an external computer using the USB port and a memory stick.

2.2. Instrument Installation Procedure

IMPORTANT Installation of **PurePep Chorus** should be performed by trained personnel only. Improper installation may result in damage to the instrument or operators. Please contact GPT if the instrument needs to be moved after installation.

- Uncrate main unit, safely (bend at the knees) lift off pallet (use caution >200 lbs).

- Remove all materials from crate. Check off list:

(1)	User Manual
(1)	Instrument Guide
(2)	4L safety-coated glass bottles
(7)	1L safety-coated glass bottles
(1)	5 gal. waste container
(12)	10 mL reaction vessel assemblies
(12)	Package of collection tubes
(27)	Package of amino acid bottles
(1)	Waste duct assembly
(1)	Nitrogen tubing assembly
(1)	Flow meter assembly
(1)	Bottle tray
(1)	Bottle cap and tubing assembly
(1)	Waste tubing assembly
(1)	Waste cable assembly
(1)	Power cord (supplied with (2) when optioned w/ heat)
(1)	Tablet (user interface)
(1)	Stylus
(1)	Plastic fitting, 1/4" FPT x 1/4" tube
(1)	Peptide Predictor software
(1)	AA replacement filters (100 pack)
(1)	Solvent bottle filter (100 pack)

- Remove top panel and side panel where vent will be installed. Install adjustable vent adaptor and bend flaps out to fasten. Place vent cap in other side panel vent hole. Once the vent is installed, replace the top panel and side panel.

- Attach waste level sensor cable connector (4 pin) to Bulkhead Panel (Section 1.2).

- Place waste tank in user-provided secondary container and dress cleanly. Attach the other end of the connector to the waste level sensor on the waste container by lining up the red dots, then pushing down.

- Attach the three shorter 1/4" waste lines from waste tank to waste fittings on Bulkhead Panel (Section 1.2).

- Attach the long 1/4" vent line from waste tank to vent duct adaptor fitting.

- Install RV's and collection tubes into positions (Sections 1.3 & 1.4).

- Install Solvent 1 through Solvent 8 bottles (Section 1.6) fittings to solvent feedthrough panel connectors

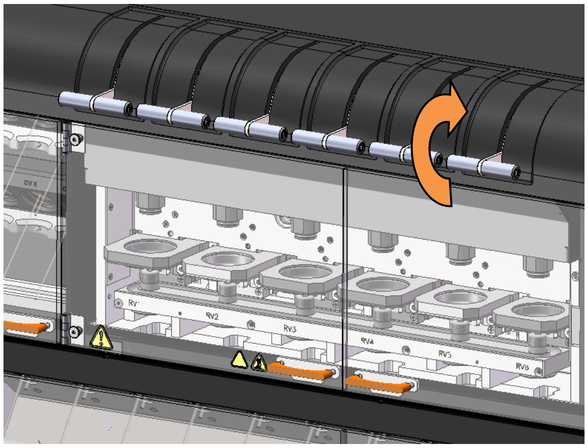
- Install 27 empty amino acid bottles (Section 1.5).

- Attach supplied power cord(s) to power cord receptacle(s) on Bulkhead Panel (Section 1.2) and plug into correct rating wall outlet. Do not plug power cords in a power strip or other extension type outlets. These cords must be accessible by the user in case of emergency.

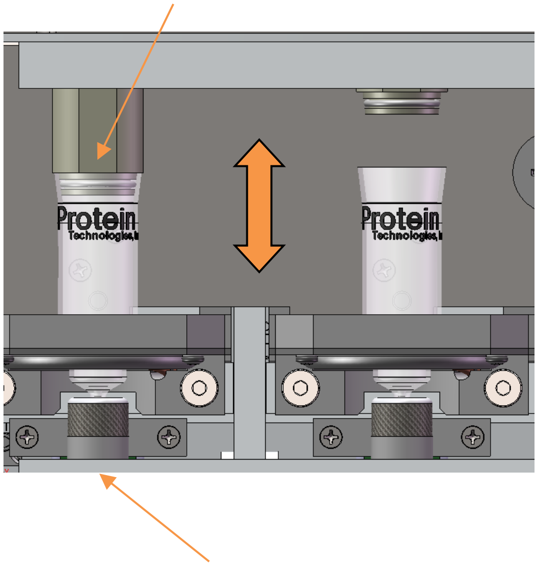
- Attach nitrogen supply lines to Bulkhead Panel (Section 1.2)
- Turn on main power switch and heater power switch.
- Turn on tablet
- Verify all systems alarms are OK. (4 icons on lower right corner of main screen are green)
- Perform **Nitrogen Leak Check** (see Section 4.2.2).
- Back flush Solvent 1 to all 27 amino acid lines and verify liquid delivery to each amino acid bottle using the **Bottle Prep** screen.
- Use the **Manual Operations** screen (See section 2.8) to deliver 1000 µL of Solvent 1 to all RV's. Verify liquid delivery and drain into waste (check for leaks on all waste line fittings).
- Pressurize amino acid bottles using the **Bottle Prep** screen and check for leaks.

2.3. RV & O-Ring Installation

To install or remove reaction vessel:



Upper RV Seat (moves with lever)



Bottom RV Seat

1. To remove an RV, apply slight downward pressure to the RV with one hand while lifting the cam lever with the other until the lever locks into the vertical position. Pull the RV gently up and out of the bottom seat. A slight twisting motion may help release the RV from the bottom seat.

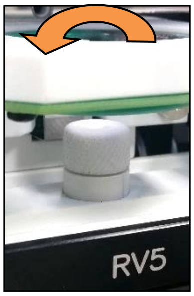
2. When installing an RV, gently insert the RV into the bottom seat. Be sure to line the RV up with the upper seat. Hold the RV with one hand while carefully lowering the cam lever to the horizontal position to lock the RV in place.

3. Gently twist the RV to ensure a proper O-ring seal.

CAUTION Make sure the RV bottom is pressed through the bottom O-ring in the lower RV seat, or the RV may leak. Be cautious when lowering the RV cam lever. It can snap closed and break the RV if it is not guided down.

To install reaction vessel O-rings:

1



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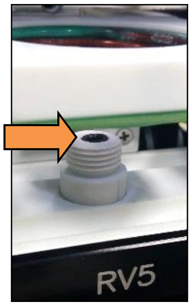

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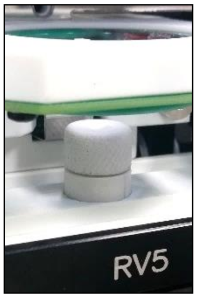

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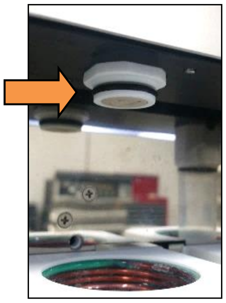
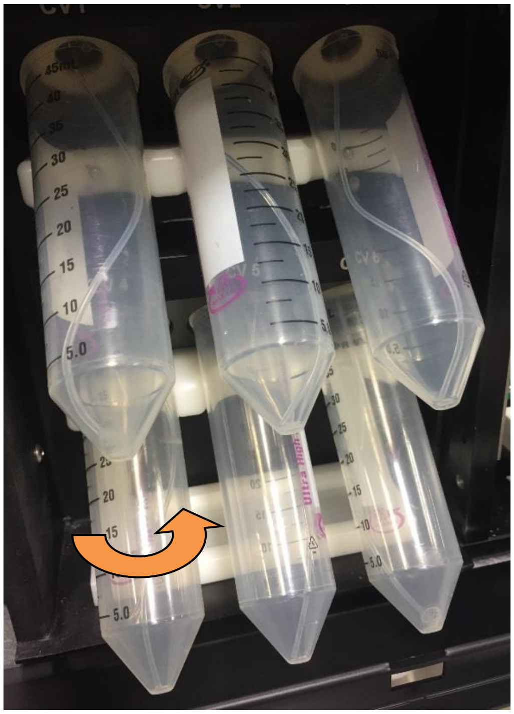


1. Unscrew the cap from the lower RV seat on the instrument.
2. Place a reaction vessel bottom O-ring in the center of the lower RV seat.
3. Screw the cap on over the O-ring until tight.
4. Slide a reaction vessel top O-ring into the groove on the upper RV seat.
5. Test for leaks by installing an empty reaction vessel (below) and performing a **DMF Top Wash** using the **Manual Operations** screen

2.4. Collect Vial Installation



The collection vials install into a threaded port on the machine. Install by turning the vial clockwise, by hand, until the top of the vial is seated firmly against the seal inside the threaded port. To remove, turn the vial counterclockwise. Only VWR tubes (Available from GPT, See Section 3.4) should be used, any other type of vessel could be prone to leaking.

IMPORTANT It is not recommended to have cold ether in the collection vial when the cleavage solution is collected. The vial may overfill during the collection of the product causing both loss of the product and potential damage to the instrument from the TFA solution. Rather, collect cleavage solution, remove collection vial from instrument, then precipitate peptide with cold ether (< 0°C).

2.5. Amino Acid Bottle Installation

To install an amino acid bottle, first make sure the bottle position is vented and if necessary, back flushed with nitrogen (**Bottle Preparations)** then:

1

2

1. 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.

2. The metal slide is spring-loaded and will pop out when the bottle is in place.

NOTE Check that the bottle filter is resting against the lower rear of the bottle. This will ensure that all of the reagent in the bottle will be used.

To release the bottle, make sure the bottle position is vented. 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.

CAUTION Failure to hold the bottle while releasing will cause the bottle to fall and spill, which may result in personal injury, loss of reagent and/or damage to the instrument.

2.6. Solvent/Reagent Bottle Installation

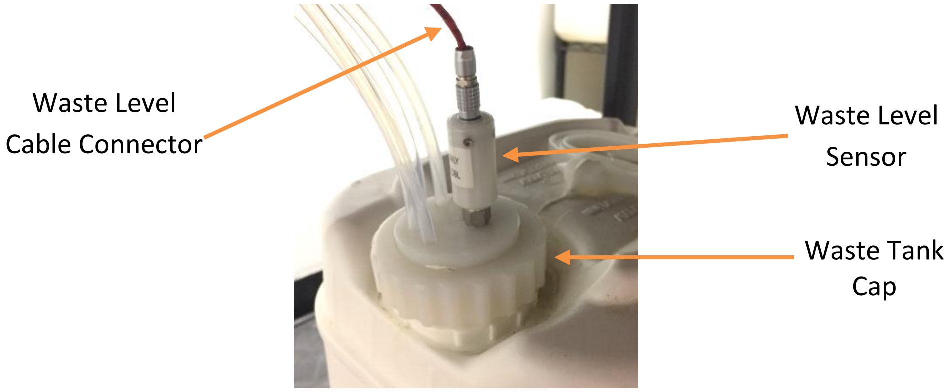
To install a solvent/reagent bottle:

1. Make sure the solvent/reagent bottle position is vented (See **Bottle Preparations** screen).
2. Verify the O-ring is properly installed on the cap insert and that the insert is in the cap. Also, verify that the fluid line has a bottle filter with frit attached.
3. Place the bottle in the bottle container. Insert the line so that it is straight and at the bottom of the bottle (the tubing can be 'molded' by gentle bending—Do not 'kink' or the tubing integrity will be compromised).
4. Attach the cap and tighten to a firm hand tight.

To remove the bottle, make sure the bottle position is vented and unscrew the cap while carefully guiding the tubing and filter out of the bottle.

2.7. Waste Container Installation

To install the waste container:



1. Place the waste container into a secondary containment vessel (if available).
2. Align both red dots to properly insert the waste level cable connector into the waste level sensor located at the top of the waste container. Plug the other end of the waste level cable connector into the waste level sensor port located on the utility panel.
3. Connect the three shorter 1/4" waste lines to the waste fittings on the solvent feedthrough panel and insert them into the waste tank cap.
4. Connect the longer 1/4" vent line to the exhaust vent adaptor fitting and insert the other end into the waste tank cap.
5. Screw the cap onto the waste tank. The waste cap must be properly installed and tightened or waste fumes will be vented into the lab.

To empty a full container:

1. Carefully disconnect the waste level sensor connector by grasping the knurled area of the fitting firmly and pulling directly up. Unscrew the cap.
2. Empty the waste container and place it back into the secondary containment vessel.
3. Screw the cap back on and reconnect the waste level sensor, being careful to line up the red dots before applying pressure. Do not force.

2.8. System Startup

Only after verifying that the system has been setup properly should the instrument be powered on. The Main Power and Heater Power Switches (if the instrument is optioned with induction heat) are located on the Bulkhead Panel (Section 1.2) and should be switched to the on position.

Chapter 3. Upgrades & Accessories

3.1. Instrument Upgrades

IMPORTANT: Any service or upgrades to **PurePep Chorus** should be performed by trained personnel only. Improper installation or servicing may result in damage to the instrument or harm to operators. Only Trained personnel. Please contact GPT.

PurePep Chorus is field upgradable, allowing the instrument to scale and match the evolving needs of each user. Upgrades are available to add RV positions (up to 6), add induction heating to the RVs and add UV monitoring on up to all 6 available RV positions. To discuss these possibilities please contact us today:

Tel: 520-629-9626 | 800-477-6834 | Email: peptides@gyrosproteintech.com
www.gyrosproteintechnologies.com/protein-technologies

3.2. Reaction Vessels & O-Rings

Description	Single	Pkg. of 6	
10 mL Reaction Vessel, Induction Compatible	PPX-FGRV10-1	PPX-FGRV10-6	
25 mL Reaction Vessel, Induction Compatible	PPX-FGRV25-1	PPX-FGRV25-6	
40 mL Reaction Vessel, Induction Compatible	PPX-FGRV40-1	PPX-FGRV40-6	

Description	Single	Pkg. of 10	
10 mL Reaction Vessel, Glass	TPS-GRV10-1	TPS-GRV10-10	
40 mL Reaction Vessel, Glass	TPS-GRV40-1	TPS-GRV40-10	

Description	Pkg. of 30	Pkg. of 90	Pkg. of 180	
10 mL Reaction Vessel, Disposable	PPS-R10-030	PPS-R10-090	PPS-R10-180	
45 mL Reaction Vessel, Disposable	PPS-R45-030	PPS-R45-090	PPS-R45-180	

Reaction Vessel O-Rings:

- Top, Premium:
  Cat#: PPS-ORING-TK-06, Pkg. of 6
- Bottom, Premium:
  Cat#: PPS-ORING-BK-06, Pkg. of 6
- Top, Premium:
  Cat#: PPS-ORING-TK-06, Pkg. of 6

3.3. Amino Acid Bottles

10 mL Single-Shot^{TM}

Cat#: AAR-SSI, 1 ea

120 mL

Cat#: SMP-VX-20, Pkg. of 20

400 mL

Cat#: AAR-400-I, 1 ea.

3.4. Collection Vials

50 mL

Cat#: CLT-050-030, Pkg. of 30
Cat#: CLT-050-090, Pkg. of 90
Cat#: CLT-050-180, Pkg. of 180

3.5. Amino Acids & Reagents for Peptide Synthesis

Gyros Protein Technologies supplies high quality, pre-tested **PurePep** N-Fmoc-protected amino acids preweighed in 5 mmol, 10 mmol and 20 mmol quantities in synthesizer-ready bottles, as well as bulk N-Fmoc-protected amino acids preweighed in 25 g, 100 g and 1kg quantities. We recommend using our **PurePep** amino acids for all your synthesis needs.

Gyros Protein Technologies also supplies coupling reagents, resins and solvents (solvents only shipped in the US) for peptide synthesis on **PurePep Chorus**.

3.6. Replacement Parts & Additional Accessories

Gyros Protein Technologies supplies a full line of replacement parts for **PurePep Chorus**, as well as various accessories, including solvent/reagent bottles and waste containers. For additional part and accessory information, please contact us today:

Tel: 520-629-9626 | 800-477-6834 | Email: peptides@gyrosproteintech.com

www.gyrosproteintechnologies.com/protein-technologies

Chapter 4. Cleaning, Maintenance, Decommissioning

4.1. Cleaning & Maintenance Schedule

Every Synthesis	• **Wash RVs** • **Bottle Position Flush** used bottles • **Collect Back Flush** (Only After Cleave) • **Cleave Bottle Solvent Back Flush** (Only After Cleave)
Every Two Weeks	• **System Clean** • Cleaning the Instrument cabinet (Section 4.2.1)
Quarterly or When switching solvents	• **Solvent Calibration**
Annually	• Amino Acid Bottle Seal Replacement (Section 4.2.4) • Replacement of RV Top Upper valve block membrane (must be performed by GPT Service personnel) • Calibration of UV and IR components
As Needed	• Nitrogen Leak Check (Section 4.2.2) • Bottle Filter Replacement (Section 4.2.3) • Amino Acid Bottle Seal Replacement (Section 4.2.4) • Solvent Bottle Seal Replacement (Section 4.2.5)

4.2. Cleaning Operations

The following cleaning operations are available on **PurePep Chorus**

1. System Clean
2. Bottle Back Flush
3. Collect Block Backflush
4. Wash All Blocks
5. Clear All Blocks
6. Wash RVs

4.2.1. Cleaning the Instrument Surfaces

Accidental spills should be wiped up immediately to have a clean environment to work.

4.2.2. Nitrogen Leak Check

It is recommended to routinely check the sealing of all the reagent supply bottles.

NOTE Do not allow the nitrogen tank gauge to fall below 75 psi during the test, or the bottle positions that are pressurizd for the test will be automatically vented.

Test A: Regulator & QC Test

1. Remove the nitrogen quick connect, i.e., no nitrogen line connected to unit.
2. Turn off the nitrogen tank valve.
3. Watch the nitrogen tank gauge on the tank for a drop in pressure within 15 minutes, then turn on the nitrogen tank valve.
4. If the gauge on the nitrogen tank regulator drops, there is a leak. If this is the case, check the tank regulator and tank fitting for leaks. Also, check the tank regulator outlet fitting and gauges.
5. If the gauge does not drop, there is no leak. If there are no leaks, reconnect the nitrogen quick connect.

Test B: Internal Nitrogen System Test

1. Connect a nitrogen flow meter between the nitrogen tank and the nitrogen inlet to the unit.
2. If the flow is greater than 25 cc/min, there is a leak in the internal nitrogen system. Call GPT Technical Support at 1-800-477-6834.
3. If the flow is less than 25 cc/min, proceed to Test C.

Test C: Solvent System Test

1. Connect the nitrogen flow meter and pressurize all solvent bottles in the Bottle Preparations screen.
2. Allow the system to stabilize for 10-15 minutes.
3. Check the nitrogen flow meter to see if there is any flow.
4. If the flow is greater than 25 cc/min, there is a leak in one of the bottles. If the flow is below 25 cc/min, proceed to Test D.
5. To identify the leaky bottle, vent all bottles and pressurize one at a time checking the nitrogen flow meter.
6. Continue this process until the leaking bottle is identified.
7. Check the bottle cap, insert, and O-ring.
8. Check the supply tubing for cracks or leaks.
9. Pressurize the bottle and re-test. If the flow is still above 25 cc/min, call GPT Technical Support at 1-800-477-6834. If the flow is below 25 cc/min, proceed to Test D.

Test D: Amino Acid System Test

1. Make sure all 27 amino acid bottles are in place.
2. Pressurize the amino acid bottles, and let the system stabilize for 5-10 minutes.
3. Check nitrogen flow meter.
4. If the flow is greater than 25 cc/min, vent the system and examine the amino acid bottle seals for solids, cracking, tears or other damage that would interfere with sealing. Pressurize the amino acid bottles again, let stabilize and check the nitrogen flow meter. If the flow is still greater than 25 cc/min, the amino acid bottles seals might need replacement (see Section 4.2.3).
5. If the flow is still greater than 25 cc/min after replacing the seal, call GPT Technical Support at 1-800-477-6834.

4.2.3. Filter Replacement

The bottle filter should be replaced on a regular basis; the frequency depends upon the quality and concentration of the reagents utilized. Always replace filters for reagents that show any precipitation. If a specific reagent cannot be delivered, replacement of the bottle filter should be the first solution.

The bottle filter consists of a filter housing and a frit. The frit is press fit into the housing. On the other side of the housing is a partially threaded entrance for the tube. To thread the filter housing onto a bottle tube, gently twist the housing clockwise while pushing it onto the tube. Be certain to thread the assembly completely onto the tubing or bubbles may be introduced between the top of the housing and the tubing. The filter assemblies are easily removed by gently twisting counterclockwise while pulling down. To remove the filter frit, either press the frit out with a swab, etc., from the top or lift the frit out with a spatula or dental pick. To replace the frit, put the new frit on a clean, flat surface and press the filter housing firmly over the frit.

CAUTION Always wear protective clothing, safety glasses and gloves when working on the filter assemblies.

Replacement Procedure:

1. From the Bottle Preparations screen, select the positions that need replacement filters.
2. Press the Nitrogen Back Flush button to blow out the reagent from the lines and filter housing.
3. When the operation is complete, remove bottle(s) and wipe exterior reagent off the filter assembly.
4. Unscrew the filter assembly from the tube and remove filter frit from filter housing.
5. Clean and rinse housing with desired solvent and dry.
6. Install new frit by pressing housing over frit.
7. Screw filter assembly back onto tubing.

IMPORTANT When installing the filter assembly onto the tubing, be sure the tube is threaded into the filter housing as far as it will go to prevent nitrogen bubbles from being introduced when the reagent level goes below the top of the filter housing.

NOTE To expedite the replacement procedure, it is best to have extra filter assemblies. The clean filter assembly can be used and the dirty filter can then be cleaned while the instrument is running

4.2.4. Amino Acid Bottle Seal Replacement

1. The amino acid bottle seal should be replaced annually or as needed (see 4.2.3 for nitrogen leak check procedures).
2. To remove, use forceps or tweezers to grab the seal and pull it out of the manifold. Caution should be used to not damage the tubing lines for the backing plate.
3. To replace, remove filter housing, then put new seal over tube and start by feeding one corner into the manifold using a DULL instrument or fingernail to prevent cutting or tearing the surface of the seal. The seal can then be pushed into the manifold in small increments. An AA bottle can be used to push the seal firmly in place.
4. The metal backing disk 'floats' and can be pressed upward to allow entry of the seal.
5. Reinstall filter housing and frit (see 4.2.4)

4.2.5. Solvent Bottle Seal Replacement

The solvent bottle seal consists of an encapsulated O-ring seated in a bottle cap insert. The O-ring can be damaged if not handled properly and should be replaced if a nitrogen leak is noted. Extra caution should be taken not to damage the insert when replacing the O-ring. To remove the O-ring, simply lift the O-ring off the insert with your fingertip. The protective gloves will assist in preventing damage to the inserts by cushioning against fingernail damage.

IMPORTANT Never use sharp or pointed objects to remove the O-rings from the inserts. Even small nicks may cause a nitrogen leak. Never use a razor blade or knife to cut off the O-rings.

CAUTION Always wear protective clothing, safety glasses and gloves when working on bottle seals.

4.3. Decommissioning

4.3.1. Decommissioning the Instrument

The instrument was new when sold unless stated on the original purchase order. When the decision is made to decommission the instrument, Gyros Protein Technologies made the commitment to return the instrument as part of REACH and RoHS guidelines. Please contact Gyros Protein Technologies for removal.

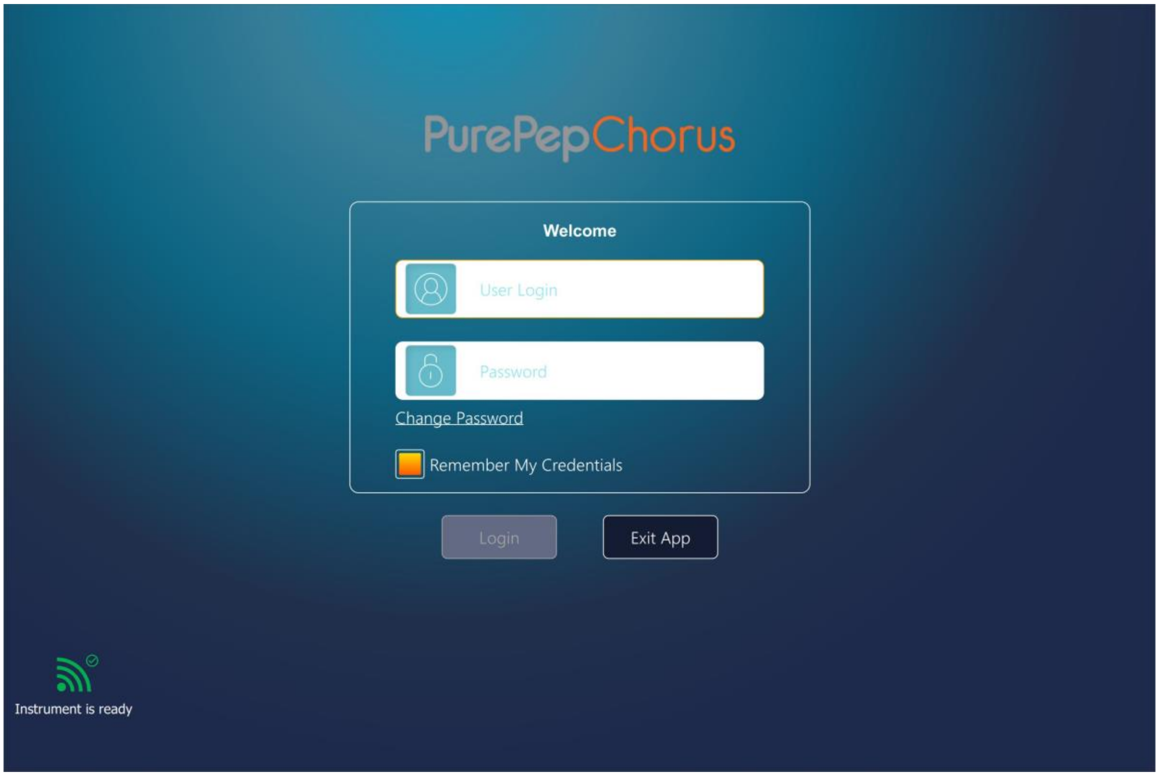
Chapter 5. Software

5.1. Launching the software and logging in

For first-time login, a user can log in using the main login screen.

See section 0 for details on how to set up new and additional users. If the software was installed on a separate PC as the editor utility, type `editor` as the user login and `editor` as the password.

WARNING: do NOT use the editor utility on a tablet that is connected to a synthesizer. To avoid loss of data, the editor utility shall be used on a separate PC only. Use the import/export functionality to move files and libraries from editor utility to instrument tablet or vice versa.

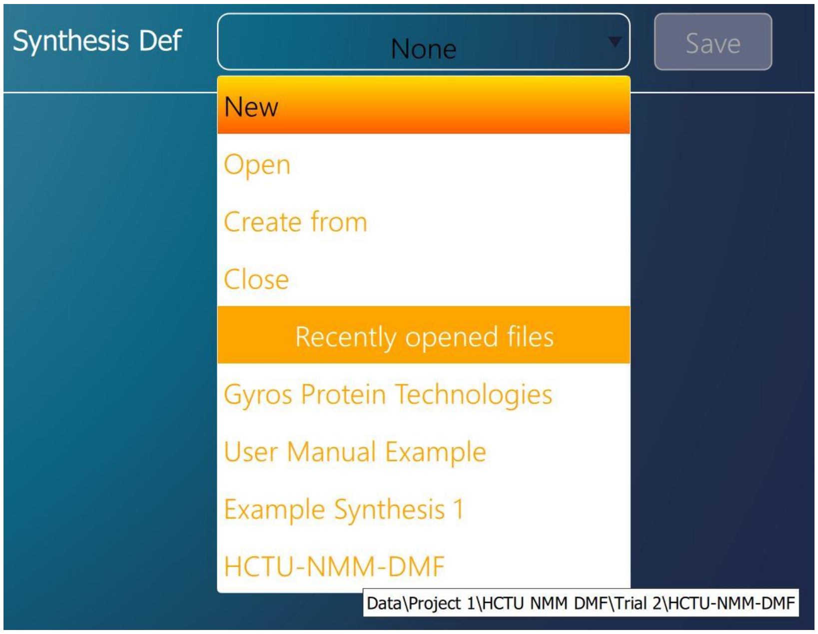


The icon in the lower left corner indicates whether the connection to the instrument is active. When the icon turns green, the software is ready to login.

5.2. Synthesis Menu

After a user log in, the software will open to the empty screen of the **Synthesis** menu. To begin the process of setting up or editing a synthesis, the user should use the **Synthesis Def** drop-down menu in the upper right corner and select:

- **New** – to create an entirely new synthesis,
- **Open** – browse the **Data** folder to select and load a previously created synthesis,
- **Create from –** to use a previously created synthesis as a template,
- **Close** – unload a currently loaded Synthesis, or
- **Recently opened files** – choose a name from the list of the previously created and recently opened syntheses. If using a mouse (or laptop option) and hovering over a name, a tooltip of the location and filename will be displayed.



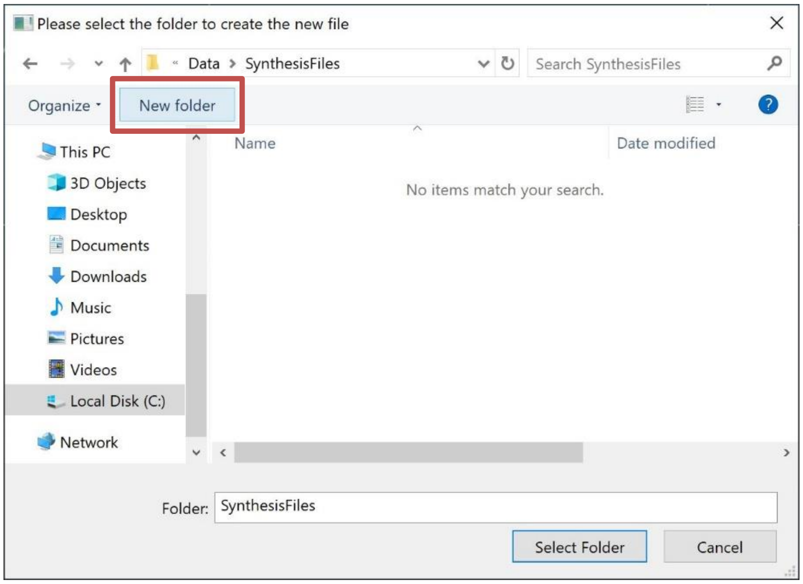
If **New** is selected, the following dialog is shown. A path and a synthesis name are required.

Path: Data\SynthesisFiles

Synthesis Name: Enter the file name

By clicking on the 🔍 icon, users can open the *system folder dialog* to select or create folder structures for organizing Synthesis, Sequence, and Protocol files. A valid path must be inside the **Data** folder and within 10 levels of this (root) folder.

Folder structures can be created by using the **New folder** button.



Please select the folder to create the new file

Data > SynthesisFiles

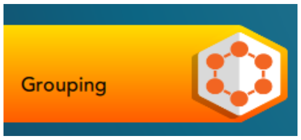
Search SynthesisFiles

No items match your search.

Folder: SynthesisFiles

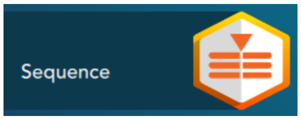
WARNING: do not delete, move, or rename any files using this *system folder dialog* or the Windows File Explorer. Instead use the software **File Manager** (see section 5.3.6 File Mgr) to perform any of these file operations.

Eight submenu buttons are located on the left side of the screen below the main menu. They open the following screens:



- insert or delete groups
- add or remove RVs
- **Synthesis Settings** for all groups: scale, excess, and RV size

- **Group Parameters**: AA, activator, base, and additive ratios
- **Individual RV Parameters**: resin type, loading, and substitution



- sequence assigned to each RV
- add or remove single shots, No AA, and idle cycles

Setup

- solvents/reagents assigned to timed or metered delivery bottles
- amino acids bottles and CV/single shots

Protocol

- protocols assigned to each cycle
- pre, post, and cleave protocols
- Tc and Td temperatures and enable/disable UV per RV

Calculation

- calculations for solvents, reagents, amino acids, and RV products
- calculated/suggested volumes, concentrations, and calculated time
- Molecular weight, density, weight, volume, and percentage calculator

Run

- horizontal sequence with running AA highlighted
- current running step, reps, source/destination bottles
- action log and graphs

Run History

- temperature and UV individual/summary data from previous jobs
- export data to CSV

5.2.1. Grouping

The **Grouping screen** allows reaction vessels to be configured to run simultaneously (same group) or in different groups to run sequentially.

Synthesis Def: Gyros Protein Technologies

Scale: 0.1 mmol

Excess: 5

Installed RV Size: 10 mL

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Users can add and remove RVs from any group using the “**Add RV**” and “**Remove RV**” buttons. Groups can be added by clicking on the “**+**” symbol on a new tab or “**Insert Group**” button at the bottom of the screen. Groups can be removed by selecting them and then clicking the “**Delete Group**” button

Any combination of RVs can be specified by the user with the only limitations being that there can be no duplicates of RVs within a group or groups belonging to the same Synthesis, and a limit of 6 RVs and/or groups overall within a Synthesis. If a user deletes a group, RVs within a deleted group become available to be used within other groups or new groups.

In the **Synthesis Settings** box at the bottom of the window, a user can specify the **Scale** in mmol and the amino acid **Excess** that will be used for the synthesis, this information is later used in the calculations screen. The **Installed RV Size** can be selected as well. The information about the installed RV size is extremely important when using heat during the synthesis. Based on the choice, specific heat profile is applied and choosing the wrong RV size can result with heating errors. These settings will apply to all groups.

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

5.2.2. Chemistry

The top part of the **Chemistry** screen allows the user to assign the **Ratios** of **AA/Activator/Base** and **Additive**. These numbers are in relation to previously defined **Excess**. In most cases **AA Ratio** will be set to 1, with the ratio of **Activator**, **Base**, and **Additive** relative to this number, and entered manually. For example, in typical synthesis with 5 equivalents of amino acids and HCTU/DIPEA used as coupling reagents in ratio 1:2 fields would be filled as follows: **Excess: 5.00, AA: 1.00, Activator: 1.00, Base: 2.00** and **Additive: 0.00**.

AA Ratio: 1.000

Ratio — Activator: 1.00, Base: 2.00, Additive: 0.00

Resin Type: Fmoc-Gly-Wang

Preloaded: ☑

Resin Substitution: 0.31 mmol/g

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The user can also identify the name, type, and loading of the resin used in each RV and copy to other RVs in any group. **Resin type** field allows to choose the resin from a drop-down list defined earlier on the **Resins** screen (see section 5.3.6 File Mgr – Libraries – Resins). The **Preloaded** check box is used to designate that the first amino acid in a sequence is already attached to the resin. To add different settings for each RV, scroll using the arrows under **Individual RV Parameters** on the bottom right of the screen. The "**Apply to All**" button under the resin type, copies the resin type, preloaded option and resin substitution to other RVs in the group. The "**Copy to All Groups**" button at the bottom of the screen can be used to apply to all groups.

The user can navigate to different groups by selecting a tab at the top of the screen. The user can not add or delete a group or an RV from this screen. If the user wants to adjust the Grouping, they simply press on the Grouping tab and go back to make the adjustments.

5.2.3. Sequence

Multiple features are built into the **Sequence** screen, giving the user the flexibility to create, review, and edit each sequence by RV and Group. This is also the screen in which the user can specify a **Single Shot**, insert a **No AA** or **Idle (Pause)** cycles for an RV.

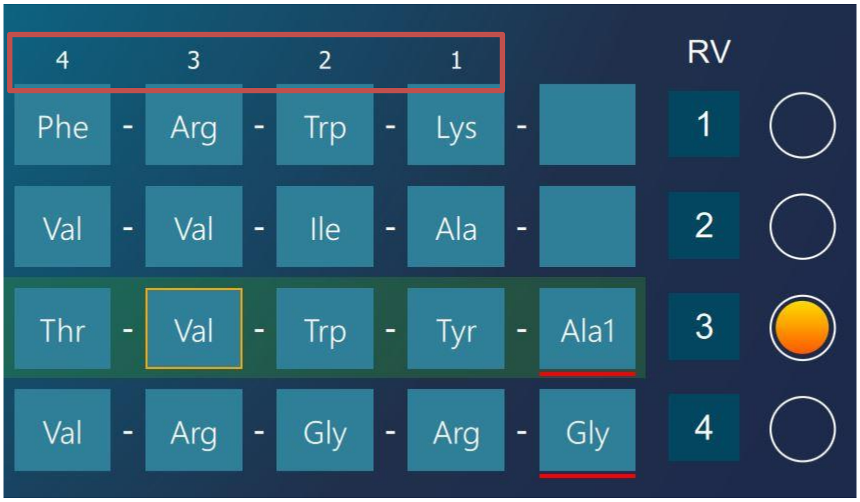
Sequence name: UserManual-01

Ala^{1} - Cys^{2} - Asp^{3} - Glu^{4} - Phe^{5} - Gly^{6} - His^{7} - Gln^{8} - Pro^{9} - Asn^{10} - Met^{11} - Leu^{12} - Lys^{13} - Ile^{14} - Arg^{15} - Ser^{16} - Thr^{17} - Val^{18} - Trp^{19} - Tyr^{20} -
Ala1^{21}

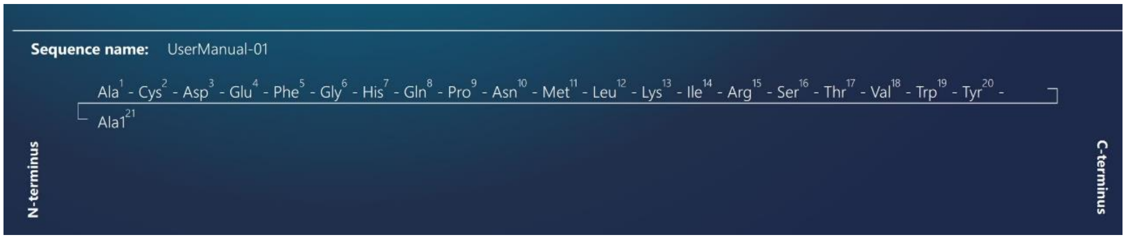
The sequence is represented from the C-terminus at the right part of the screen moving to the N-terminus at the left part of the screen (the way the instrument will run the synthesis – defined by cycles numbers above all sequences).

When the resin used 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.

The bottom part of the screen will show the name and full-length of the currently selected sequence. The numbers presented in this view are showing the sequence of the amino acids from N-terminus to C-terminus (opposite to the cycle numbers according to which the synthesis will progress).



4	3	2	1		RV	
Phe	Arg	Trp	Lys		1	○
Val	Val	Ile	Ala		2	○
Thr	Val	Trp	Tyr	Ala1	3	●
Val	Arg	Gly	Arg	Gly	4	○



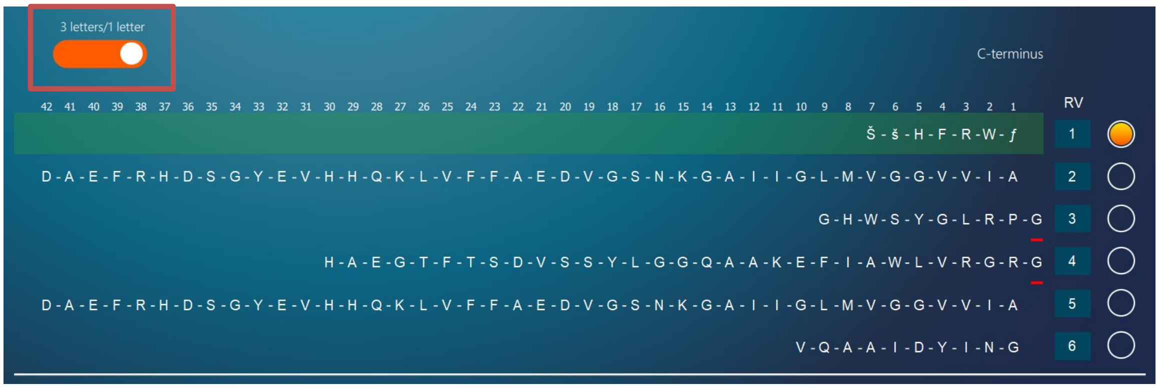
Sequence name: UserManual-01

Ala^{1} - Cys^{2} - Asp^{3} - Glu^{4} - Phe^{5} - Gly^{6} - His^{7} - Gln^{8} - Pro^{9} - Asn^{10} - Met^{11} - Leu^{12} - Lys^{13} - Ile^{14} - Arg^{15} - Ser^{16} - Thr^{17} - Val^{18} - Trp^{19} - Tyr^{20} -
Ala1^{21}

A click on an **Add** tile (when no sequence is assigned) or “**Add/Edit**” button (after selecting an RV with the orange radio button) will allow the user to load or create a sequence for the selected RV.

Once the sequence is assigned to the RV, long pressing or double clicking on any of the amino acid tiles or pressing the “**Add/Edit**” button (after selecting an RV with the orange radio button) at the bottom of the screen will open the Sequence Editor. This editor allows users to edit a sequence or replace it with another one for the selected RV.

The toggle in the upper left corner allows the user to view the AAs within the sequence in its default 3 letter abbreviation or in a single letter representation. The 3-letter code may actually use anywhere from 2 to 5 characters. The 1-letter code view will also allow user to view more cycles on the screen. The sequences can be scrolled horizontally with a touch gesture using the tablet or by holding the left mouse button and moving the mouse horizontally.

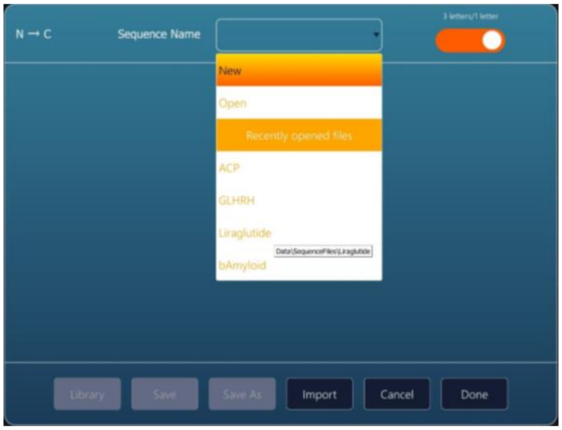


	RV
Š - š - H - F - R - W - ƒ	1
D - A - E - F - R - H - D - S - G - Y - E - V - H - H - Q - K - L - V - F - F - A - E - D - V - G - S - N - K - G - A - I - I - G - L - M - V - G - G - V - V - I - A	2
G - H - W - S - Y - G - L - R - P - G	3
H - A - E - G - T - F - T - S - D - V - S - S - Y - L - G - G - Q - A - A - K - E - F - I - A - W - L - V - R - G - R - G	4
D - A - E - F - R - H - D - S - G - Y - E - V - H - H - Q - K - L - V - F - F - A - E - D - V - G - S - N - K - G - A - I - I - G - L - M - V - G - G - V - V - I - A	5
V - Q - A - A - I - D - Y - I - N - G	6

The groups can be navigated by selecting one of the desired group tabs at the top of the screen.

Sequence Editor

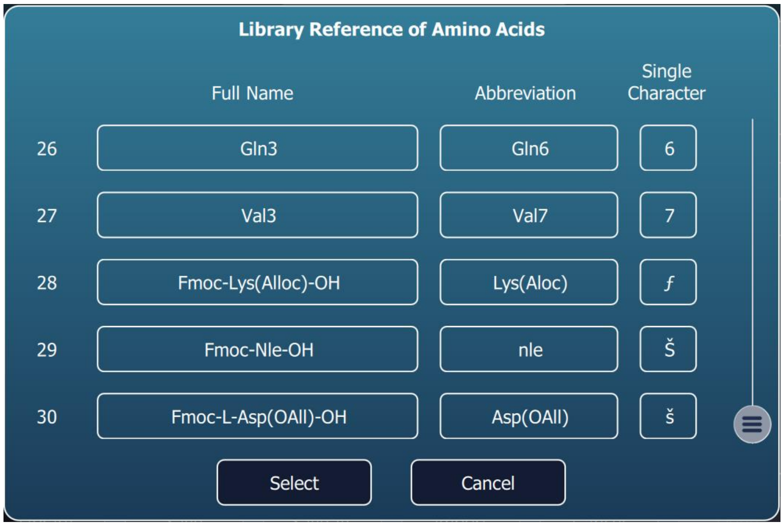
If a sequence is not yet assigned to the selected RV, the user will be prompted with an empty editor window. The *Sequence Name* drop-down menu allows the user to create a **New** sequence, **Open** and browse an existing one, or load one from the list of recently opened files. If the user is editing a sequence, the screen will display in full edit mode with the sequence visible.




Sequence Name bAmyloid

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

A sequence can be typed in by selecting the amino acid tiles in the N -> C format (left to right). The user can scroll down using the scroll bar on the right to see all AAs (or other monomers) available from the library. Alternatively, clicking on the “**Library**” button on the bottom will open and allow selection of any member of the library.



Library Reference of Amino Acids

	Full Name	Abbreviation	Single Character
26	Gln3	Gln6	6
27	Val3	Val7	7
28	Fmoc-Lys(Alloc)-OH	Lys(Aloc)	ƒ
29	Fmoc-Nle-OH	nle	Š
30	Fmoc-L-Asp(OAll)-OH	Asp(OAll)	š

After a variant or other non-standard amino acid/monomer is inserted, it is distinguished by a > symbol below the respective letter on the sequence. An amino acid can be defined as a `Variant` using the *AA Library* located at the **Amino Acids** screen (see section 5.3.6 File Mgr – Libraries – Amino Acids).

Sequence Name: MT-II

```
ƒŠHFRW
>>   >
```

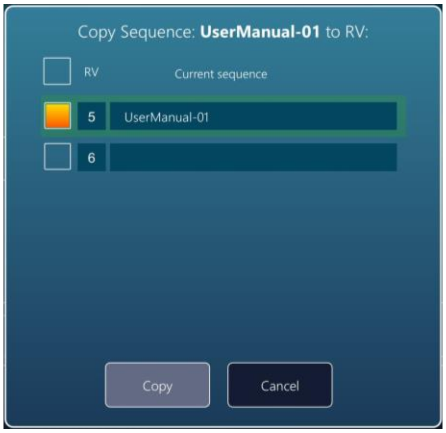
It is also possible to cut, copy, or paste amino acids into the sequence editor. This can be done by opening the *Cut/Copy/Paste* dialog with a long press or right-click (if using a mouse) anywhere within the input field in the sequence editor.

The *Sequence Editor* allows the user to create a new sequence from an existing one with the "**Save As**" function – this will reuse an existing sequence as a starting template and modify without changing the original sequence.

If the user has a sequence that they wish to load from another device (editor utility for example), this can be done with the "**Import**" functionality. Sequences can be imported from

CSV or TEXT files (see section 5.3.6 Import/Export – CSV files and Import/Export – TXT files (Sequences only) for more information).

On the **Sequence** screen, it is possible to copy a sequence to other RVs in the same group using the *Copy* dialog. Once the "**Copy**" button is clicked, a popup dialog will appear that will allow copying sequences to other RVs in the group.



Copy Sequence: **UserManual-01** to RV:

☐	RV	Current sequence
☑	5	UserManual-01
☐	6	

The user has the option to specify amino acids for particular cycles to be added as *Single Shots* – delivering all of the contents of the AA bottle to the RV. This is accomplished by selecting the appropriate residue tile within a sequence and clicking the "**Single Shot**" button. A green border will appear around the selected AA when a *Single Shot* has been selected. To clear the *Single Shot*, the AA has to be selected and the "**Clear Single Shot**" button pressed.

15	14	13	12	11	10	9	8	7	6	5	4	3	2	1		RV
							Ala	Cys	Asp	Glu	Phe	Arg	Trp	Lys		1 ○
Lys	Gly	Ala	Ile	Ile	Gly	Leu	Met	Val	Gly	Gly	Val	Val	Ile	Ala		2 ●
					Gly	His	Trp	Ser	Tyr	Gly	*	Leu	Arg	Pro	Gly	3 ○
His	Gln	Pro	Asn	Met	Leu	Lys	Ile	Arg	#	Ser	Thr	Val	Trp	Tyr	Ala1	4 ○

Sequence name: bAmyloid

Asp^{1} - Ala^{2} - Glu^{3} - Phe^{4} - Arg^{5} - His^{6} - Asp^{7} - Ser^{8} - Gly^{9} - Tyr^{10} - Glu^{11} - Val^{12} - His^{13} - His^{14} - Gln^{15} - Lys^{16} - Leu^{17} - Val^{18} - Phe^{19} - Phe^{20} -
Ala^{21} - Glu^{22} - Asp^{23} - Val^{24} - Gly^{25} - Ser^{26} - Asn^{27} - Lys^{28} - Gly^{29} - Ala^{30} - Ile^{31} - Ile^{32} - Gly^{33} - Leu^{34} - Met^{35} - Val^{36} - Gly^{37} - Gly^{38} - Val^{39} -
Val^{40} - Ile^{41} - Ala^{42}

A user may press the “**Insert No AA**” button to insert *No AA* cycles for any RV within any group, specified by the * symbol. This function is generally used when a special protocol not involving the coupling of an AA is to be run at that position. If a standard protocol is used for this cycle, all protocol steps will be performed except for amino acid addition.

A user may also press the “**Insert Idle**” button to insert idle cycles (*pauses*) – these make the RV idle during that cycle. When special protocols need to be run on particular RVs, pauses can be used to keep others idle. Within the sequence, *pauses* are specified with a # symbol.

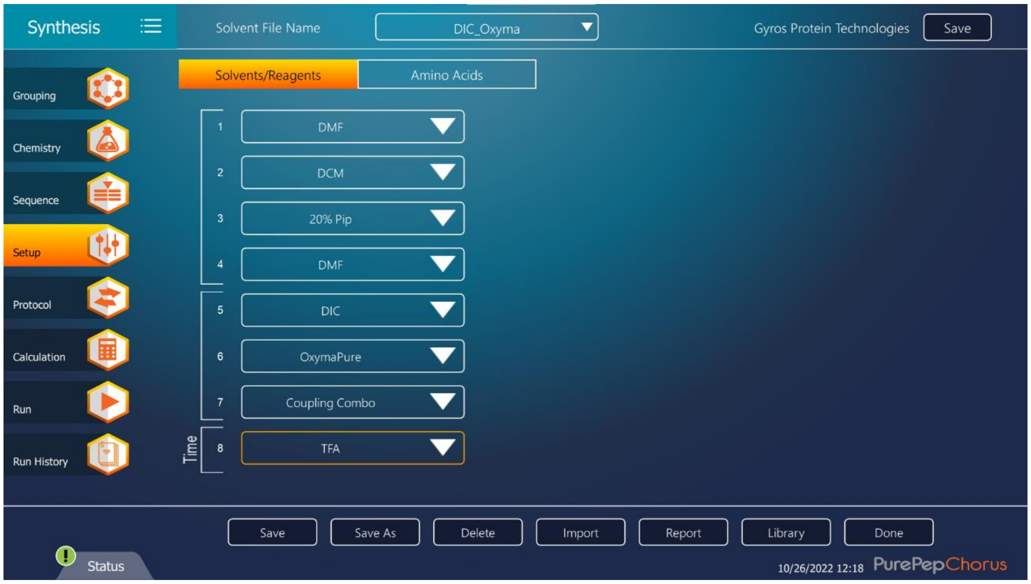
The “**Report**” button is used to export all the information about the sequences into a PDF file. Selecting the “**Done**” button will take a user to the next step in the workflow.

5.2.4. Setup

The **Setup** screen allows the user to specify a solvent/reagent or AA at each bottle position.

Setup – Solvents/Reagents

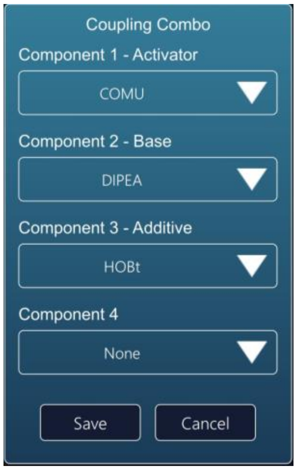
Solvent files can be saved and thus can be accessed at the top drop-down menu, where a “**New**” option will be available. The “**Search**” function is also available.



Solvent File Name: DIC_Oxyma

1 DMF
2 DCM
3 20% Pip
4 DMF
5 DIC
6 OxymaPure
7 Coupling Combo
8 TFA

A drop-down menu at every bottle position allows the user to set a bottle position as a specific **solvent**, **reagent**, or **Coupling Combo**. After selecting a **Coupling Combo** option, a combo selection dialog is displayed, where the user can specify the composition of activators, bases, additives, and others. To edit a previously assigned **Coupling Combo**, long press the white down arrow to open the **Coupling Combo** dialog. This dialog allows to choose 4 mixture components.


Coupling Combo

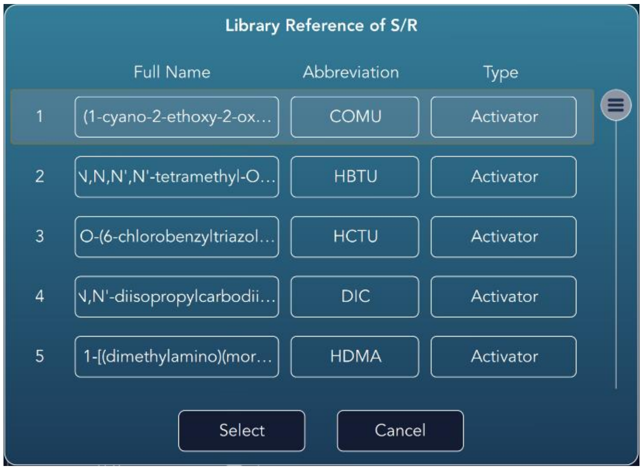
Component 1 - Activator: COMU

Component 2 - Base: DIPEA

Component 3 - Additive: HOBt

Component 4: None

Pressing the “**Library**” button opens the *Library Reference of Solvent/Reagent* dialog. This dialog allows the user to select directly from the SR Library instead of using the drop-down menus. The contents of the drop-down menus are the same as the SR Library.


Library Reference of S/R

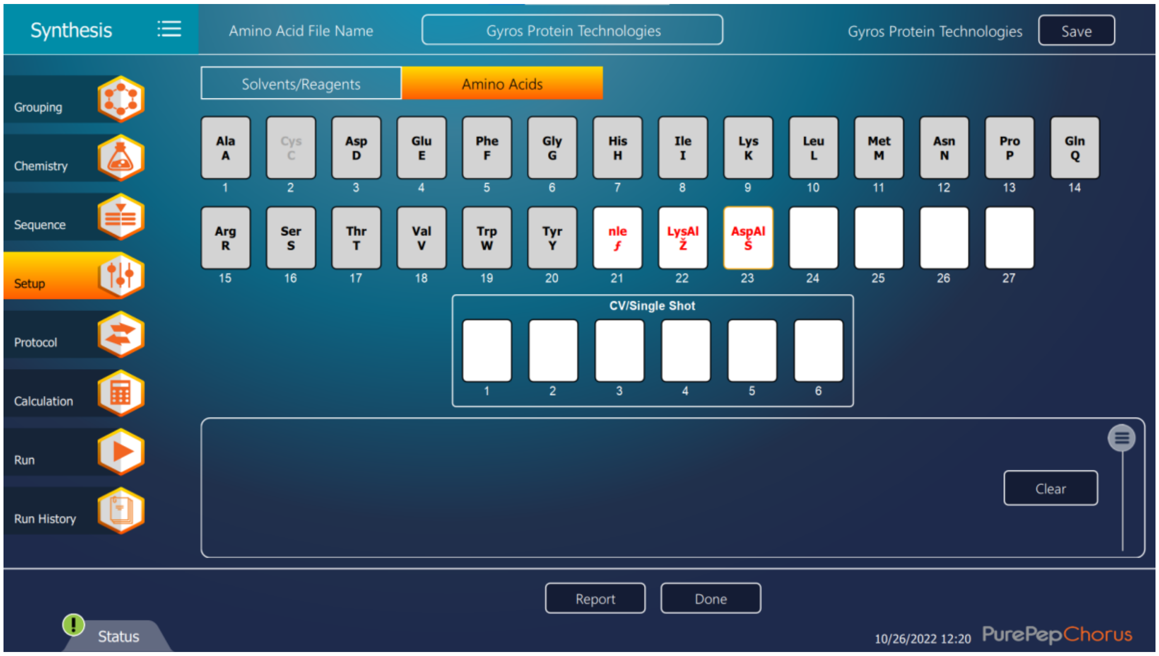
	Full Name	Abbreviation	Type
1	(1-cyano-2-ethoxy-2-ox…	COMU	Activator
2	N,N,N',N'-tetramethyl-O…	HBTU	Activator
3	O-(6-chlorobenzyltriazol…	HCTU	Activator
4	N,N'-diisopropylcarbodii…	DIC	Activator
5	1-[(dimethylamino)(mor…	HDMA	Activator

Setup – Amino Acids

This screen allows the user to specify which amino acid is at each bottle position.

The first 20 positions are pre-filled with the natural 20 AAs in alphabetical order by single letter code, as suggestions, but the user can modify this arrangement. The codes for any amino acids not used in the sequences loaded will remain grayed out. All other AAs/monomers will populate the box at the bottom. To load an AA to a position, press on the desired location to highlight it, then press on the amino acid icon from the bottom box. The AA should now be loaded into the correct location.

To move any AA to a new location, highlight the AA and press the “**Clear**” button at the bottom right side. The AA should now be available in the bottom box again.



Amino Acid File Name: Gyros Protein Technologies

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

Variant and other non-standard amino acids/monomers are marked red.

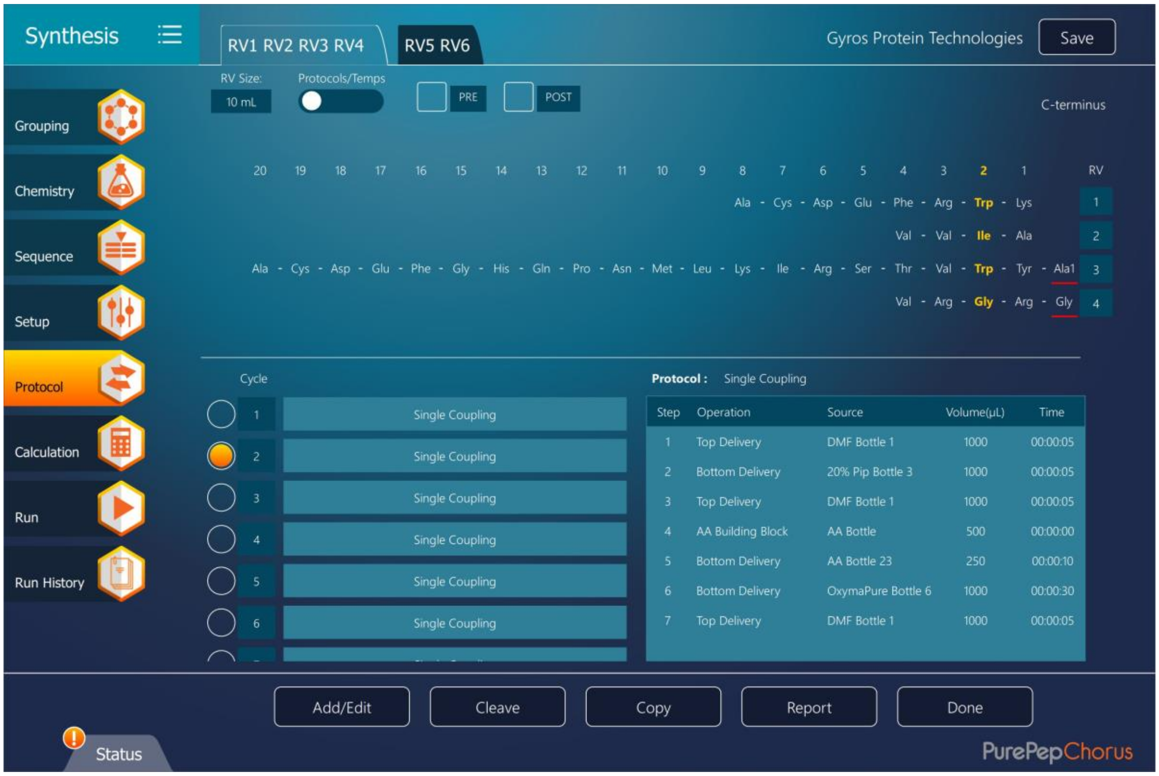
Once complete, the user can move to the next screen in the workflow by pressing “**Done**”.

5.2.5. Protocol

In the **Protocol** screens, the user can assign pre-defined protocols or create new ones and copy them to particular cycles in a group. Also, within the **Protocol** screens, the default temperatures for heated deprotections and coupling reactions can be set, as well as temperature exceptions for any cycle of any RV.

On this screen, sequences are displayed horizontally, and cycles are numbered from the C-terminus (fist cycle on the right) to the N-terminus (last cycle on the left). At the bottom left part of the screen, the user can find a list of the protocols assigned to each cycle. At the bottom right part of the screen, there is a preview box of the selected protocol. When a cycle is selected, amino acids in all sequences corresponding to that cycle will become highlighted.

The sequences can be scrolled horizontally with a touch gesture using the tablet or by holding the left mouse button and moving the mouse horizontally. The list of protocols and preview box can be scrolled vertically with a touch gesture using the tablet or by holding the left mouse button and moving the mouse vertically.



Ala - Cys - Asp - Glu - Phe - Arg - **Trp** - Lys 1
Val - Val - **Ile** - Ala 2
Ala - Cys - Asp - Glu - Phe - Gly - His - Gln - Pro - Asn - Met - Leu - Lys - Ile - Arg - Ser - Thr - Val - **Trp** - Tyr - Ala1 3
Val - Arg - **Gly** - Arg - Gly 4

○ 1 Single Coupling
● 2 Single Coupling
○ 3 Single Coupling
○ 4 Single Coupling
○ 5 Single Coupling
○ 6 Single Coupling

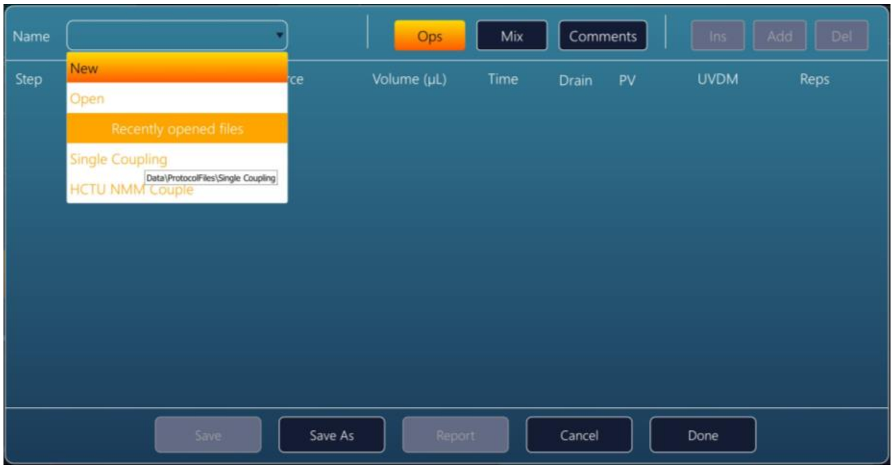
Protocol : Single Coupling

Step	Operation	Source	Volume(µL)	Time
1	Top Delivery	DMF Bottle 1	1000	00:00:05
2	Bottom Delivery	20% Pip Bottle 3	1000	00:00:05
3	Top Delivery	DMF Bottle 1	1000	00:00:05
4	AA Building Block	AA Bottle	500	00:00:00
5	Bottom Delivery	AA Bottle 23	250	00:00:10
6	Bottom Delivery	OxymaPure Bottle 6	1000	00:00:30
7	Top Delivery	DMF Bottle 1	1000	00:00:05

A long press or double click on any cycle tile (where the protocol name is displayed) 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.

Protocol Editor

The top left drop-down menu from this 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. The *Protocol Editor* also allows the user to create a new protocol from an existing one with the "**Save As**" function – this will reuse an existing protocol as a starting template and modify without changing the original protocol. This editor can be used to modify or delete *Steps* (rows) within the protocol.



The *Protocol Editor* has several pages associated with it. These pages are: **Ops** (short for *Operations*), **Mix**, and **Comments**.

Protocol Editor – Ops Page

On this page the user can select the type of operation that will happen at each *Step* within a protocol. The available operations are: `Top Delivery`, `Bottom Delivery`, `AA Building Block`, `PV to RV`, `Drain/Dry`, `Collect`, `Mix`, `Cleave Mix`, `Pause`, and `E-mail notification`. In the following column, the user can choose the bottle position from which the operation will take place (`Source`), followed by the `Volume (µL)`. The next parameter is the `Time` assigned for the operation in the HH:MM:SS (hours:minutes:seconds) format. Select if a `Drain` should follow the operation and if the operation will take place at a pre-activation vessel (`PV`) position. The UV detection mode (`UVDM`) column specifies the use of the UV for the particular step. The number of repetitions (`Reps`) for each step is assigned in the last column. Depending on the operation selected, RVs can be drained in parallel.

Name: Single Coupling

Step	Operation	Source	Volume (µL)	Time	Drain	PV	UVDM	Reps
1	Top Delivery	DMF [Btl1]	1000	00:00:05	■	☐	None	1
2	Bottom Delivery	20% Pip [Btl3]	1000	00:00:05	■	☐	Basic	2
3	Top Delivery	DMF [Btl1]	1000	00:00:05	■	☐	None	3
4	AA Building Block	AA Bottle	500	00:00:00	☐	☐	None	1
5	Bottom Delivery	AA23	250	00:00:10	☐	☐	Extend+Reps	9
6	Bottom Delivery	OxymaPure [Btl6]	1000	00:00:30	■	☐	None	1

Certain protocol operations may be performed using any of the following UV detection modes:

- `None` – Without UV monitoring.
- `Basic` – Records UV data during step but does not adjust 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 (change below a set value). If this criterion is not met, the time of reaction is extended automatically. See Appendix E: Intellisynth UV Monitoring for more information.
- `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 (change below a set value), 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. See Appendix E: Intellisynth UV Monitoring for more information.

In order to add or insert a step, the top right buttons “**Ins**” and “**Add**” can be used. The “**Ins**” button will insert a new step preceding the selected one. The “**Add**” button will add the new step at the end of the list. Steps can also be deleted using the “**Del**” button located at the top right corner.

Protocol Editor – Mix page

On this page the user can select the type of mixing:

- `N2` – nitrogen bubbling, and/or
- `Shake` – shaking with specific speed (`RPM`).

Name: Single Coupling

Step	Operation	Source	Time	N2	Deprotect	Shake	RPM	Heat
1	Top Delivery	DMF [Btl1]	00:00:05	☑	☐	☑	150	☐
2	Bottom Delivery	20% Pip [Btl3]	00:00:05	☑	☑	☑	200	☑
3	Top Delivery	DMF [Btl1]	00:00:05	☑	☐	☐	0	☐
4	AA Building Block	AA Bottle	00:00:00	☐	☑	☐	0	☐
5	Bottom Delivery	AA23	00:00:10	☐	☐	☑	150	☐
6	Bottom Delivery	OxymaPure [Btl6]	00:00:30	☑	☐	☑	250	☑

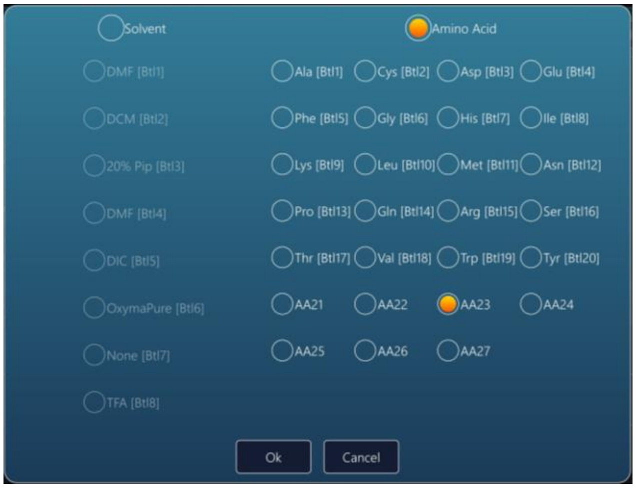
Users can also select if `Heat` will be used during a particular step. Any step using nitrogen bubbling needs a non-zero mix time (**Ops** page). On this page users can also mark a deprotection step by selecting the `Deprotect` checkbox. For the steps where deprotection is selected, the software will apply the temperature that is defined for the deprotections (see **Temps screen** below).

Delivery of AA-to-RV from protocol

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

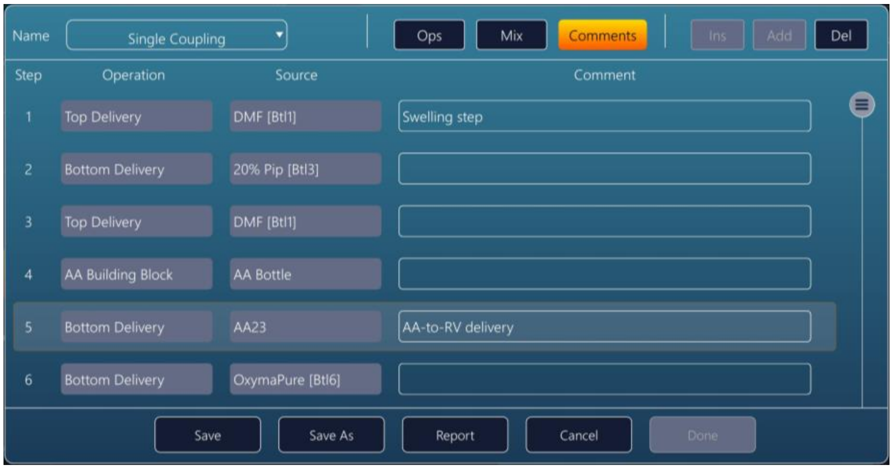
This feature supports the AA-to-RV delivery from any step. To do this type of delivery, select the `Bottom Delivery` operation for the required step, press the `Source` box, select the `Amino Acid` checkbox, and choose the desired AA source.

Press the “**Ok**” button to confirm or “**Cancel**” to discard any changes.



Protocol Editor – Comments page

This page allows the user to add, delete, or edit relevant comments to each cycle within the protocol. The comments can work as a description of the intended action during the protocol.



Name: Single Coupling

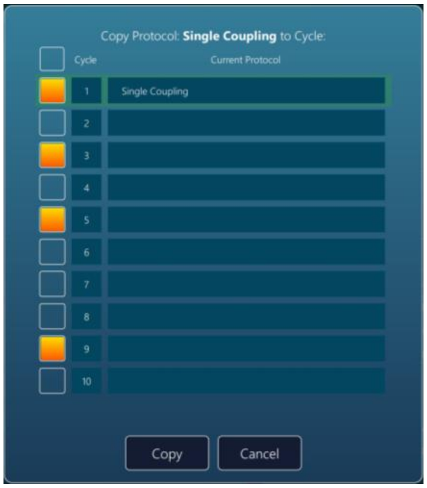
Step	Operation	Source	Comment
1	Top Delivery	DMF [Btl1]	Swelling step
2	Bottom Delivery	20% Pip [Btl3]	
3	Top Delivery	DMF [Btl1]	
4	AA Building Block	AA Bottle	
5	Bottom Delivery	AA23	AA-to-RV delivery
6	Bottom Delivery	OxymaPure [Btl6]	

To save all the protocol changes use the “**Save**” button. After that, pressing “**Done**” will return the software to the main **Protocol** screen. The cycle that was used to open the *Protocol Editor* will get assigned the saved protocol.

The “**Report**” button will create a PDF file with all the steps and relevant information about the selected protocol. Pressing the “**Cancel**” button discards any unsaved changes and takes the user back to the main **Protocol** screen.

After creating or selecting an existing protocol, the empty box (for the selected cycle) will be filled with the assigned protocol’s name.

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. From the *Copy dialog*, use the checkbox next to each cycle to copy the protocol to any other cycle(s). Use the checkbox at the top to select/unselect all the cycles. After the selection of cycle(s) is done, press the “**Copy**” button to perform the action or “**Cancel**” to discard.

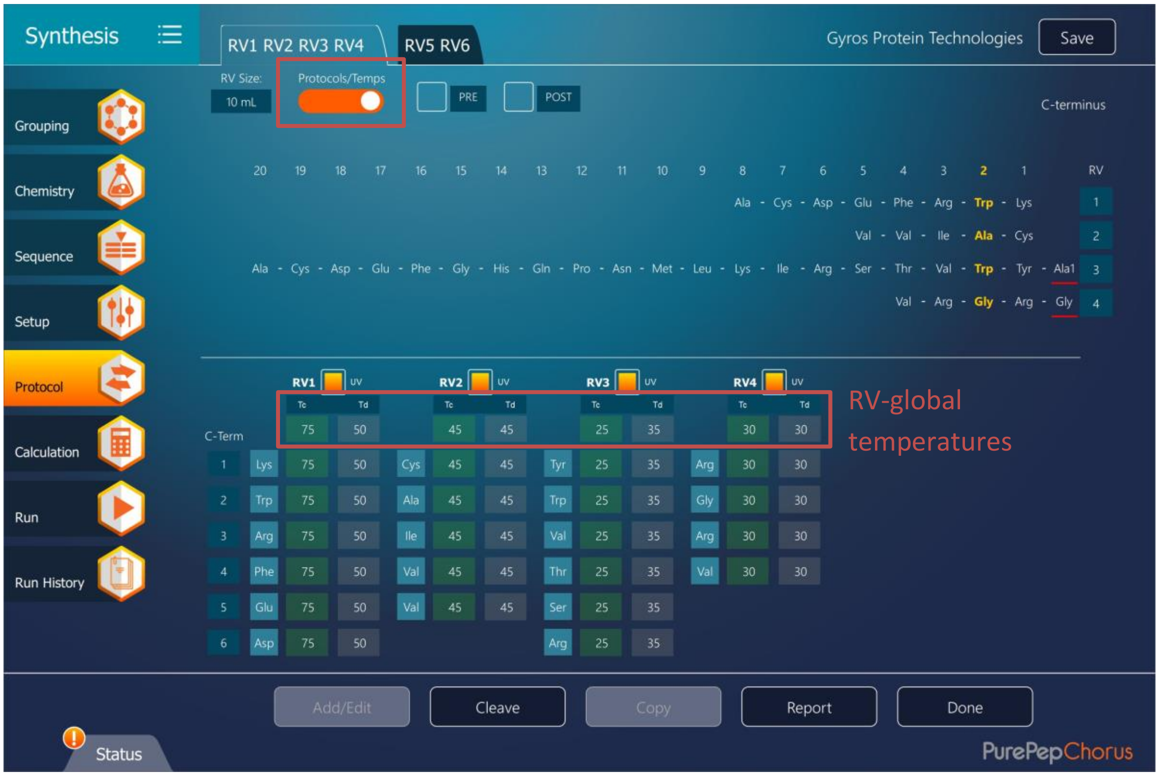


Copy Protocol: **Single Coupling** to Cycle:

☐	Cycle	Current Protocol
☑	1	Single Coupling
☐	2	
☑	3	
☐	4	
☑	5	
☐	6	
☐	7	
☐	8	
☑	9	
☐	10	

Temps screen

The *Protocol Temperatures* screen can be accessed by using the “**Protocol/Temps**” toggle on the top left corner of the screen. This screen allows to input both the RV-global temperature settings and cycle exceptions. The top boxes for coupling (`Tc`) and deprotection (`Td`) are used to set the RV-global temperatures to be used in a particular RV during the synthesis. The user can also specify temperature exceptions for `Tc` and `Td` for any AA cycle (e.g. Cys/His residues regularly use a lower coupling temperature to prevent racemization).



RV Size: 10 mL

Protocols/Temps

☐ PRE ☐ POST

C-terminus

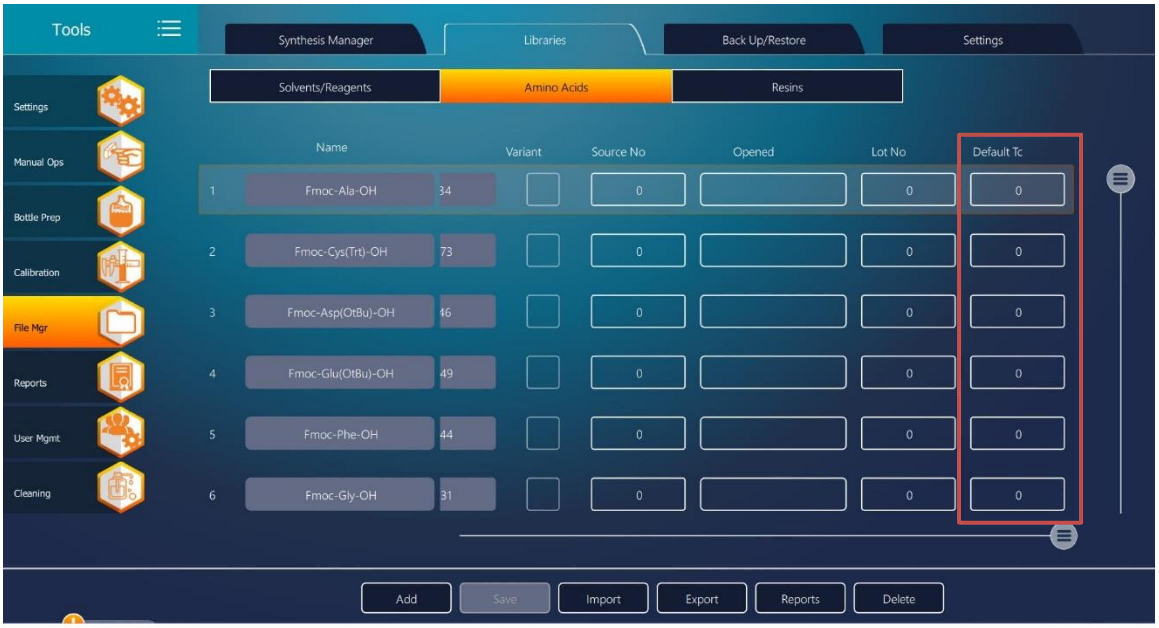
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 **2** 1 RV

Ala - Cys - Asp - Glu - Phe - Arg - **Trp** - Lys 1
Val - Val - Ile - **Ala** - Cys 2
Ala - Cys - Asp - Glu - Phe - Gly - His - Gln - Pro - Asn - Met - Leu - Lys - Ile - Arg - Ser - Thr - Val - **Trp** - Tyr - Ala1 3
Val - Arg - **Gly** - Arg - Gly 4

	RV1	Tc	Td	RV2	Tc	Td	RV3	Tc	Td	RV4	Tc	Td
C-Term		75	50		45	45		25	35		30	30
1	Lys	75	50	Cys	45	45	Tyr	25	35	Arg	30	30
2	Trp	75	50	Ala	45	45	Trp	25	35	Gly	30	30
3	Arg	75	50	Ile	45	45	Val	25	35	Arg	30	30
4	Phe	75	50	Val	45	45	Thr	25	35	Val	30	30
5	Glu	75	50	Val	45	45	Ser	25	35			
6	Asp	75	50				Arg	25	35			

RV-global temperatures

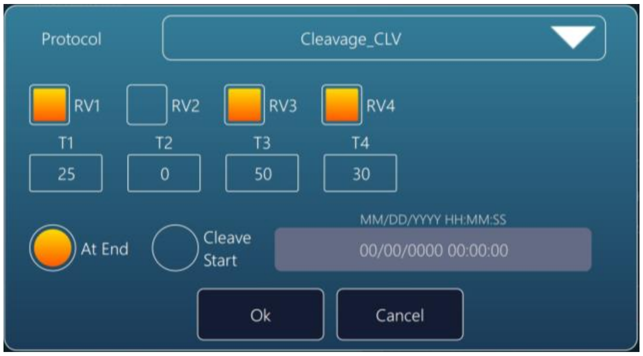
All temperatures are measured in Celsius with a range from room temperature to 90 degrees. A `Default Tc` (coupling) temperature for each amino acid can be defined on the *AA Library* located at the **Amino Acids** screen (see section 5.3.6 File Mgr – Libraries – Amino Acids). When creating new syntheses that use heat, the `Default Tc` values will be automatically populated on the respective AA/cycles. Otherwise, if none of these temperatures are defined, then the reactions are conducted at room temperature.



	Name		Variant	Source No	Opened	Lot No	Default Tc
1	Fmoc-Ala-OH	34	☐	0		0	0
2	Fmoc-Cys(Trt)-OH	73	☐	0		0	0
3	Fmoc-Asp(OtBu)-OH	46	☐	0		0	0
4	Fmoc-Glu(OtBu)-OH	49	☐	0		0	0
5	Fmoc-Phe-OH	44	☐	0		0	0
6	Fmoc-Gly-OH	31	☐	0		0	0

Automatic cleavage

The software can perform automated cleavage immediately after a synthesis is complete or at a time requested by the user. To use this function, click the "**Cleave**" button (from the **Protocol** screen) to open the *Cleave dialog*. At the top, the drop-down menu allows to load or **Open** a previously created cleave protocol, or to create a **New** one. Creating a *Cleave protocol* is similar to the creation of a synthesis protocol. Checkboxes for each RV can be used to indicate in which RVs the cleavage will take place. If heating is to be applied to the cleavage, use the **T#** boxes to specify the temperature to be used in each RV. This temperature will only be used for steps marked as heated in the *Cleave protocol*.



Protocol: Cleavage_CLV

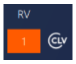
☑ RV1 ☐ RV2 ☑ RV3 ☑ RV4

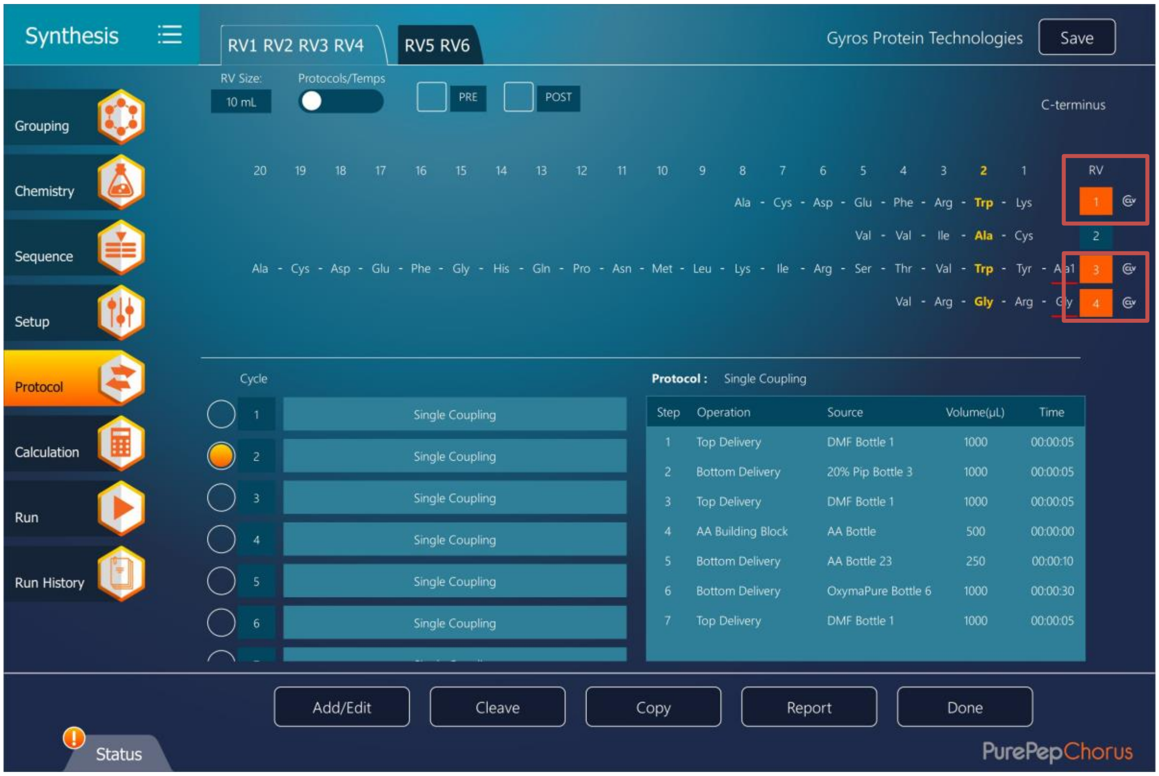
T1	T2	T3	T4
25	0	50	30

● At End ○ Cleave Start

MM/DD/YYYY HH:MM:SS
00/00/0000 00:00:00

At the bottom of the dialog, the user can choose **At End** or **Cleave Start**. If **At End** is selected, the cleave will begin after the last cycle or `POST` protocol (if one is defined). For **Cleave Start**, select the date and time for the cleave to begin. Press “**Ok**” to save or “**Cancel**” to discard the changes.

After a *Cleave protocol* is assigned, the box background of the RV number will change to orange and a symbol  will appear next to the RV number.



Protocol : Single Coupling

Step	Operation	Source	Volume(µL)	Time
1	Top Delivery	DMF Bottle 1	1000	00:00:05
2	Bottom Delivery	20% Pip Bottle 3	1000	00:00:05
3	Top Delivery	DMF Bottle 1	1000	00:00:05
4	AA Building Block	AA Bottle	500	00:00:00
5	Bottom Delivery	AA Bottle 23	250	00:00:10
6	Bottom Delivery	OxymaPure Bottle 6	1000	00:00:30
7	Top Delivery	DMF Bottle 1	1000	00:00:05

This process should be repeated for each group for which automated cleavage is desired.

Pre and Post protocols

Users can assign *Pre* and *Post synthesis protocols* that are not attached to an amino acid within the sequence. Use the `PRE` and `POST` checkboxes at the top of the screen to turn these protocols ON/OFF accordingly.

Creating *Pre* and *Post synthesis protocols* follows the same process as creating a synthesis protocol. Use the `PRE` cycle box to assign a *Pre synthesis protocol*. This protocol will run before the first cycle. Use the `POST` cycle box to assign a *Post synthesis protocol*. This protocol will run after the last cycle of the group and before the cleave protocol.

In the following example, this feature is used to run `Swell` and `Final deprotection` protocols respectively.

RV Size: 10 mL

Protocols/Temps

3	2	1	RV
	Val -	Ile -	Ala 5
Val -	Ile -	Ala	6

Cycle

PRE	Pre-Synthesis: Swell_PRE
1	Single Coupling
2	Single Coupling
3	Single Coupling
POST	Post-Synthesis: Final Deprotection_POST
Cleave	Cleavage_CLV

Protocol : Final Deprotection_POST

Step	Operation	Source	Volume(µL)	Time
1	Bottom Delivery	20% Pip Bottle 3	5000	00:02:00
2	Top Delivery	DMF Bottle 1	5000	00:01:00
3	Top Delivery	DCM Bottle 2	5000	00:01:00
4	Drain/Dry	None	0	00:30:00

The “**Report**” button is used to export all the protocols into a PDF file. Selecting the “**Done**” button takes the user to the next step in the workflow.

5.2.6. Calculations

Calculation – Solvents

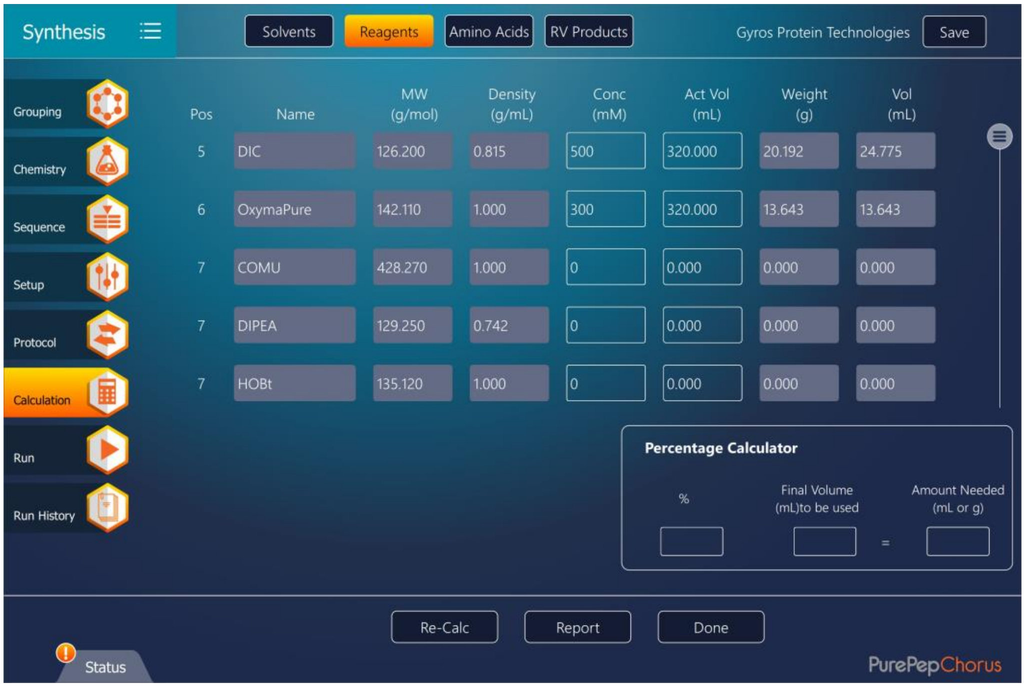
This screen displays the solvents assigned to each bottle position and the respective calculated and suggested volumes (measured in mL).

The calculated volumes depend on all the protocol steps (delivery volumes and repetitions). The suggested volume of each solvent is the compensation percent above the calculated volume, rounded to nearest higher 20mL. The compensation percent volume is used for bottle priming and can be set in the **Tools->Settings->System** screen (see section 5.3.2 Settings – System).

Pos	Name	Calc Vol (mL)	Sugg Vol (mL)
1	DMF	90.000	100.000
2	DCM	0.000	0.000
3	20% Pip	36.000	40.000
4	DMF	0.000	0.000
5	DIC	0.000	0.000
6	OxymaPure	18.000	20.000
7	Coupling Combo	0.000	0.000
8	TFA	0.000	0.000

Calculation – Reagents

The initial reagents concentration value will be calculated automatically based on the scale, excess, and volume information provided in earlier screens. The **Reagents** Calculation screen also allows the user to change the activator or additive concentration (mM) and activator volume (mL). The molecular weight and density are pulled from the Solvents/Reagents Library.



Pos	Name	MW (g/mol)	Density (g/mL)	Conc (mM)	Act Vol (mL)	Weight (g)	Vol (mL)
5	DIC	126.200	0.815	500	320.000	20.192	24.775
6	OxymaPure	142.110	1.000	300	320.000	13.643	13.643
7	COMU	428.270	1.000	0	0.000	0.000	0.000
7	DIPEA	129.250	0.742	0	0.000	0.000	0.000
7	HOBt	135.120	1.000	0	0.000	0.000	0.000

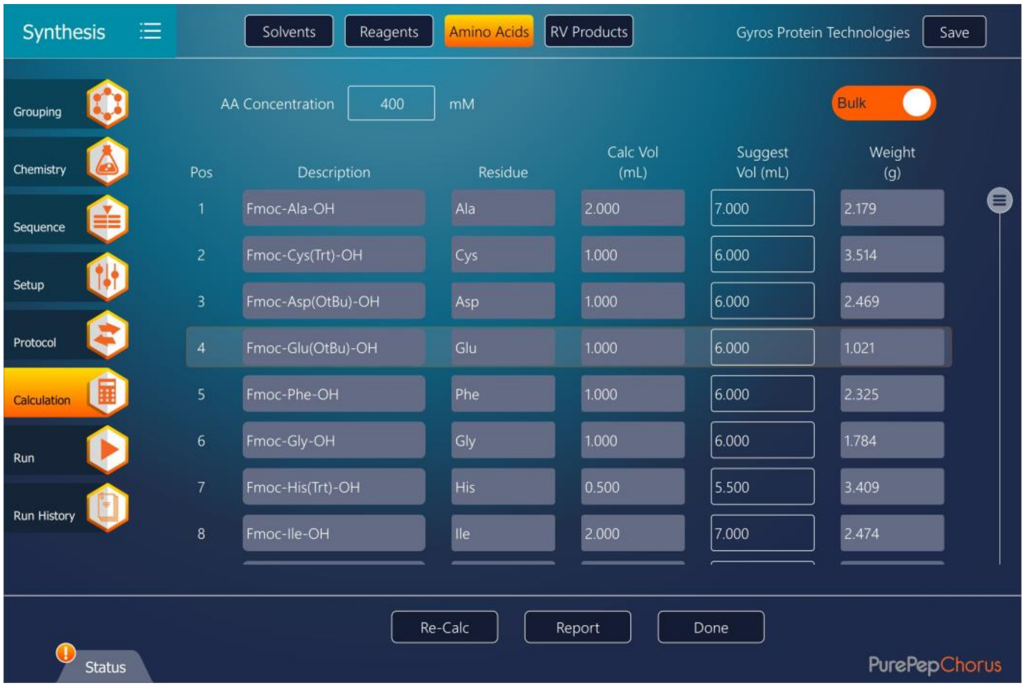
Percentage Calculator

%	Final Volume (mL)to be used		Amount Needed (mL or g)
☐	☐	=	☐

Calculation – Amino Acid

This screen has its fields populated from previously entered information. A user can modify the amino acid concentration and suggested volume of any AA. The **AA Concentration** and **Suggested** volume are used to calculate the amount of AA to be weighed out (**Weight** column). The initial **AA Concentration** value will be calculated automatically based on the scale, excess, and volume information provided in earlier screens. To change this value, enter into the box at the top of the screen and press the "**Re-Calc**" button at the bottom of the screen. All AA weights will be automatically recalculated.

The toggle on the upper right corner is used to change from bulk AA calculations to the SMP bottle calculations for use with pre-packed bottles. This calculation screen will show the appropriate pre-pack size and volume to make the desired concentration.



AA Concentration 400 mM

Pos	Description	Residue	Calc Vol (mL)	Suggest Vol (mL)	Weight (g)
1	Fmoc-Ala-OH	Ala	2.000	7.000	2.179
2	Fmoc-Cys(Trt)-OH	Cys	1.000	6.000	3.514
3	Fmoc-Asp(OtBu)-OH	Asp	1.000	6.000	2.469
4	Fmoc-Glu(OtBu)-OH	Glu	1.000	6.000	1.021
5	Fmoc-Phe-OH	Phe	1.000	6.000	2.325
6	Fmoc-Gly-OH	Gly	1.000	6.000	1.784
7	Fmoc-His(Trt)-OH	His	0.500	5.500	3.409
8	Fmoc-Ile-OH	Ile	2.000	7.000	2.474

Calculation – RV Products

This screen is pre-loaded from previous **Sequence** and **Grouping** screens. The **Resin** column indicates the amount of resin that needs to be weighed out and it is calculated using the scale and loading. The **Yield** column indicates the theoretical yield based on the scale and the peptide's molecular weight, which is calculated using the sequence and the deprotected molecular weights available in the AA library.

	Sequence Name	Preloaded	Sub (mmol/g)	Scale (mmol)	Resin (mg)	Yield (mg)	MolWt (g/mol)	Time
1	MT-II	CONH2	0.31	0.100	322.6	59.1	589.78	2 d 3:03:54
2	bAmyloid	CONH2	0.31	0.100	322.6	451.5	4513.73	2 d 3:03:54
3	GLHRH	COOH	0.31	0.100	322.6	112.9	1129.38	2 d 3:03:54
4	Liraglutide	COOH	0.31	0.100	322.6	331.2	3312.10	2 d 3:03:54
5	bAmyloid	CONH2	0.31	0.100	322.6	451.5	4513.73	2 d 3:03:54
6	ACP	CONH2	0.31	0.100	322.6	106.3	1062.32	2 d 3:03:54

Total Calculated Time 2 d 3:03:54

Selecting “**Done**” allows the user to navigate to the next screen in the workflow.

5.2.7. Run

Run screens allow the user to keep track of the progress of an on-going synthesis and provide a live feed of the RV temperatures and UV monitoring graphs.

This screen summarizes the current status of the instrument and each RV. The current RV cycle, action, and protocol are displayed.

Synthesis Def: Gyros Protein Technologies

15	14	13	12	11	10	9	8	7	6	5	4	3	2	1		RV	Temp
							Ala	Cys	Asp	Glu	Phe	Arg	Trp	Lys		1	73°C
												Val	Ile	Ala		2	72°C
Gly	His	Gln	Pro	Asn	Met	Leu	Lys	Ile	Arg	Ser	Thr	Val	Trp	Tyr	Ala1	3	48°C
											Val	Arg	Gly	Arg	Gly	4	76°C

RV Size: 10 mL Automatic Backflush: ✓

Shaker [RPM]: 150 Actual: 0 Set

Active Cycle 2 of 20

Step : 3 of 7 | Reps : 2 of 3

From : DMF | To : RV4

Remaining Operation Time: 00:00:00

Protocol : Single Coupling

Operation	Source (Bottle Position)	Volume	Reps
Top Delivery	DMF (1)	1000	1
Bottom Delivery	20% Pip (3)	1000	2
Top Delivery	DMF (1)	1000	3
AA Building Block	AA Bottle	500	1
Bottom Delivery	AA (23)	250	9
Bottom Delivery	OxymaPure (6)	1000	1
Top Delivery	DMF (1)	1000	1

Action

Date	Time	Operation
Wed, 05 Jul 2023	17:41:43	Pushing N2
Wed, 05 Jul 2023	17:41:44	Flushing Top Block
Wed, 05 Jul 2023	17:41:44	Venting RV4
Wed, 05 Jul 2023	17:41:46	Filling RV4
Wed, 05 Jul 2023	17:41:47	Pushing N2
Wed, 05 Jul 2023	17:41:48	Mixing
Wed, 05 Jul 2023	17:41:53	Finishing N2 mix
Wed, 05 Jul 2023	17:41:53	Draining RV1 RV2 RV3 RV4

If the system is paused the text on the original “**Start**” button is replaced by “**Resume**”.

The “**Start Cycle**” and “**Manual**” options are available prior to starting a synthesis and become enabled when the system is paused.


Cycle: 6

Step: 2 - Bottom Delivery

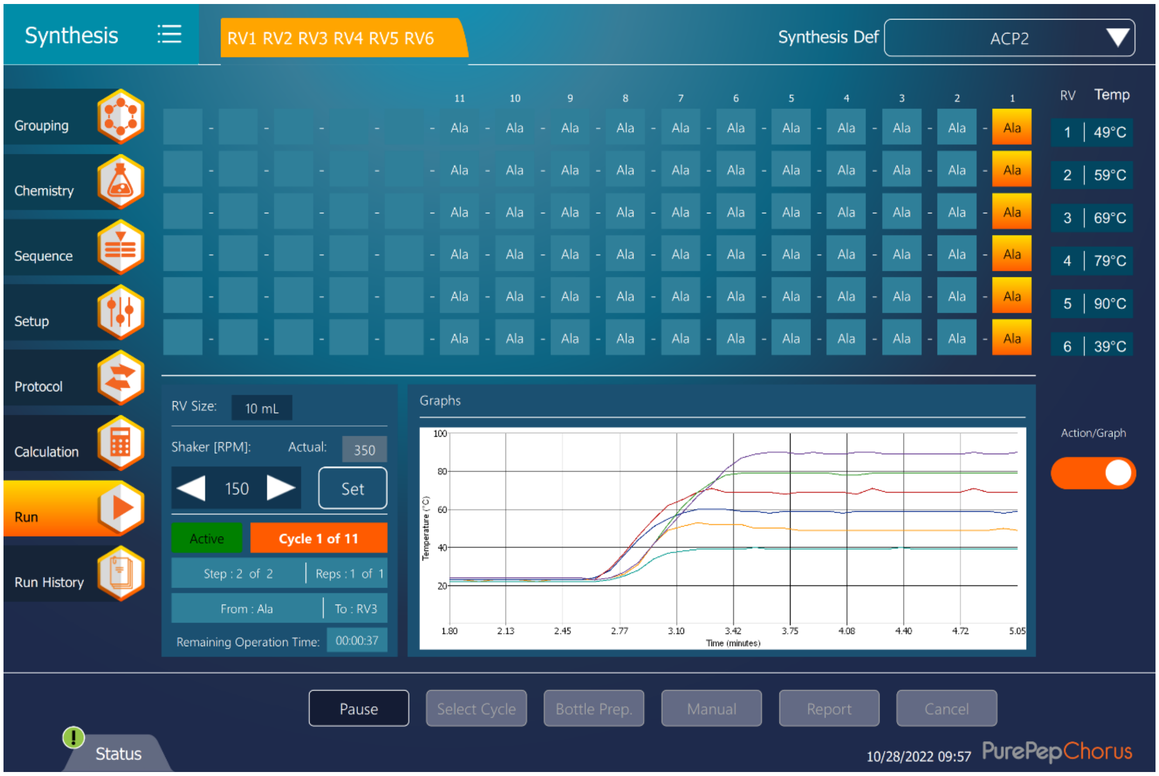
Rep: 1

“**Start Cycle**” allows the synthesis to start from any cycle and step. When the “**Start Cycle**” is chosen a popup dialog will appear allowing the user to choose which cycle, step, and repetition the synthesis needs to start/continue from. After that the “**Select Cycle**” button will reflect the choice. The “**Start**” button needs to be clicked to restart the synthesis.


C:6,S:2,R:1

The action box is displayed all throughout the different run screens as to keep the live feed of the instrument visible on all screens.

By toggling the **Action/Graph** slide on the right, the user can display a preview graph of temperature or UV data.



Synthesis Def: ACP2

11	10	9	8	7	6	5	4	3	2	1
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala

RV	Temp
1	49°C
2	59°C
3	69°C
4	79°C
5	90°C
6	39°C

RV Size: 10 mL

Shaker [RPM]: 150 Actual: 350

Active

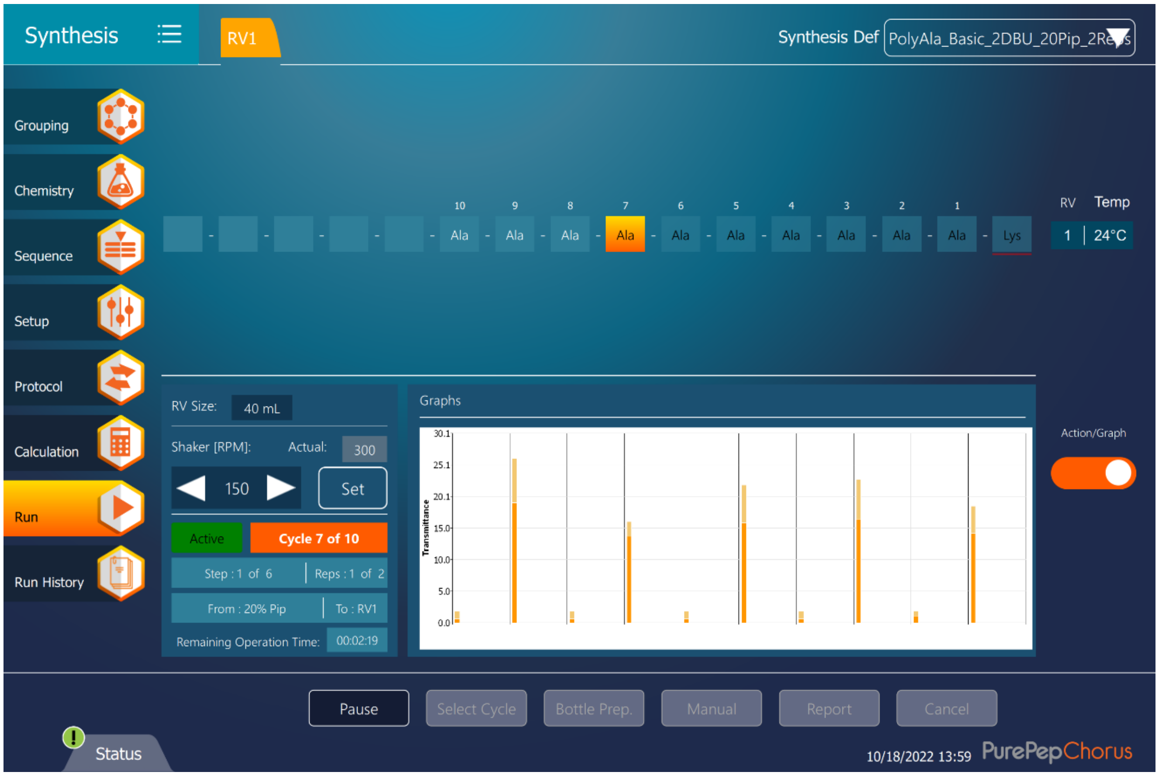
Cycle 1 of 11

Step : 2 of 2 | Reps : 1 of 1

From : Ala | To : RV3

Remaining Operation Time: 00:00:37

10/28/2022 09:57



RV Size: 40 mL

Shaker [RPM]: Actual: 300

150 Set

Active

Cycle 7 of 10

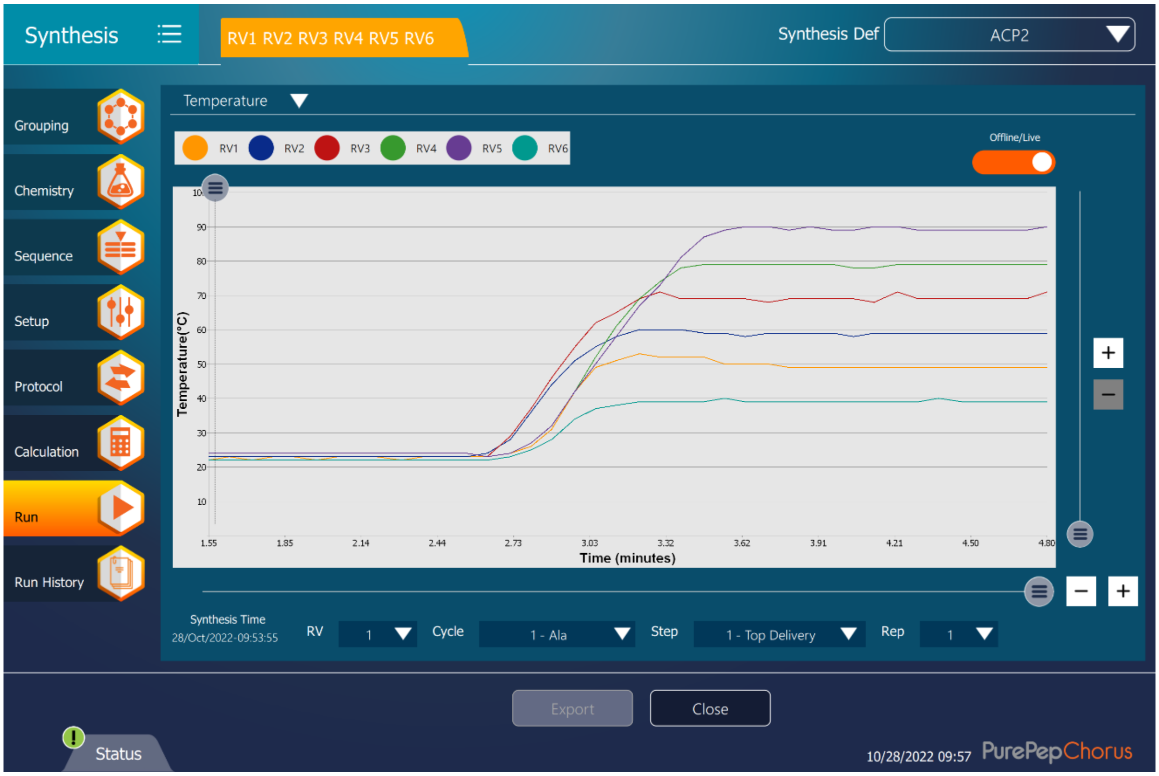
Step : 1 of 6 | Reps : 1 of 2

From : 20% Pip | To : RV1

Remaining Operation Time: 00:02:19

10/18/2022 13:59

Pressing the preview graph will open the graph in a full window mode. After the full window is closed, the preview graph will show the data chosen on the full view.



Synthesis Time 28/Oct/2022-09:53:55

RV 1 | Cycle 1 - Ala | Step 1 - Top Delivery | Rep 1

10/28/2022 09:57

Synthesis Def: PolyAla_Basic_2DBU_20Pip_2Re

Synthesis Time 18/Oct/2022-11:20:50

RV: 1

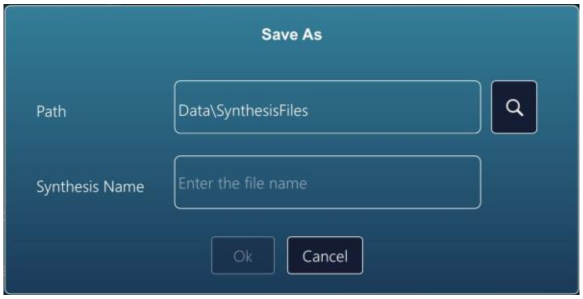
Cycle: 1 - Ala

Step: 2 - Bottom Delivery

Rep: 1

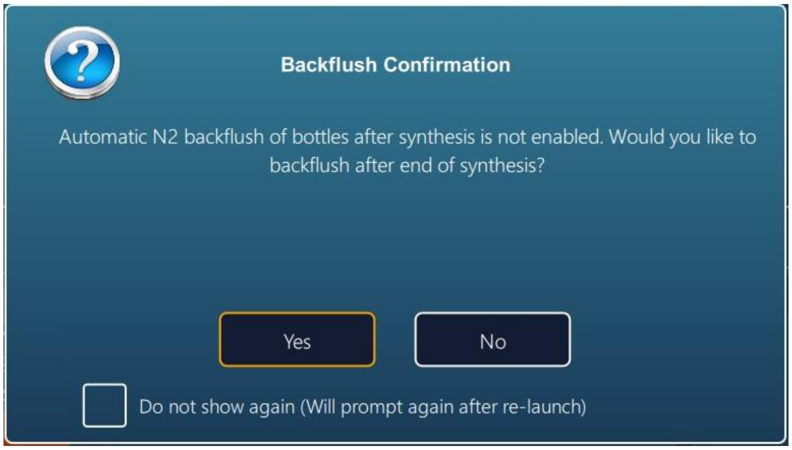
To switch between **Temperature** and **UV data** (**Summary** or **Individual** readings), the user can use the dropdown menu at the top left corner of the graph. Individual RV data can be switched ON/OFF by pressing the colored circle related to the specific RV. The UV data can be exported to the external CSV file by choosing "**Export Options**" at the bottom of the screen. Pressing the "**Close"** button closes the full window and shows the standard run screen. For more information about the graph controls, buttons, and switches see Appendix E: Intellisynth UV Monitoring, **UV Graphs Screen** section.

Once the run is started users have the option to **Save As** and modify the synthesis within the *Synthesis* screens. To accomplish this, go back to the **Grouping** screen, select the **Synthesis Def** drop-down menu, and choose **Save As**.



Path: Data\SynthesisFiles

The software allows for automatic N2 backflush of the bottles after the completion of every synthesis run. To enable this setting see section 5.3.2 Settings – System. If this global setting is not enabled, a popup window will appear before a synthesis run to confirm if bottles need to be N2 backflushed when the synthesis completes.

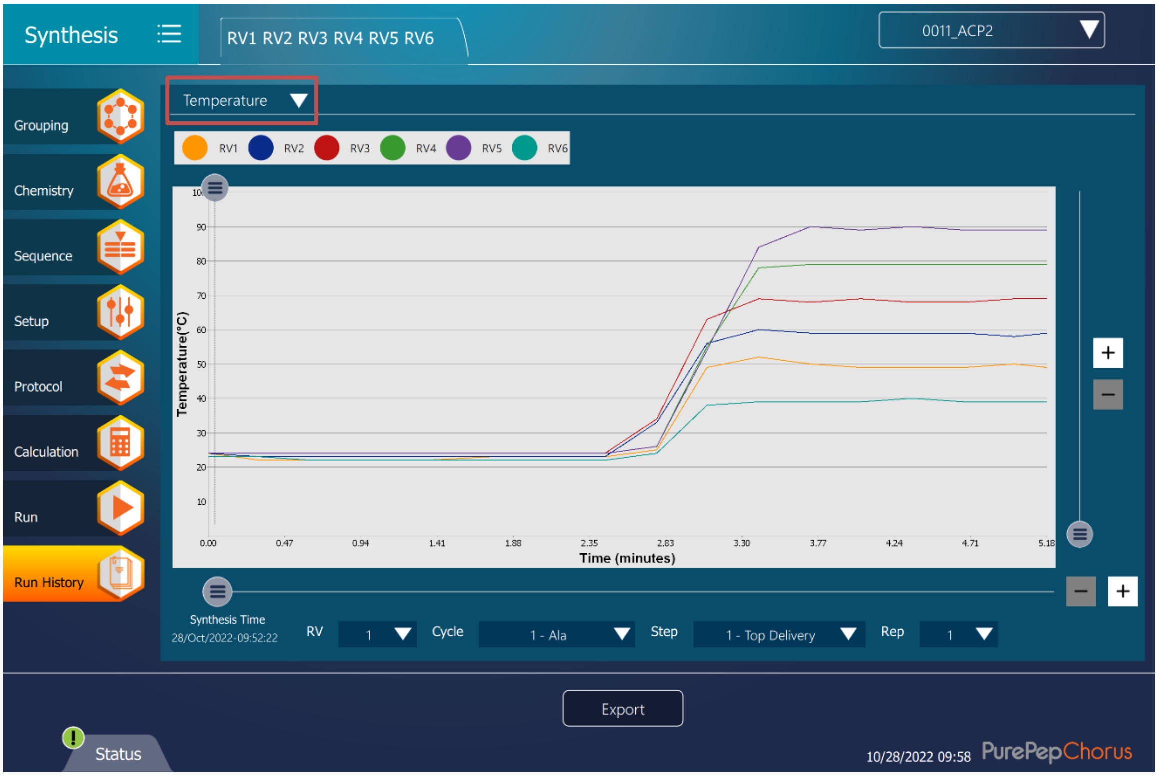


Backflush Confirmation

Automatic N2 backflush of bottles after synthesis is not enabled. Would you like to backflush after end of synthesis?

5.2.8. Run History

In the **Run History** screen, the user can load previously recorded temperature or UV data for any completed synthesis. This can be done by selecting the Synthesis name from the drop-down menu at the top right part of the screen. 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**.



0011_ACP2

Temperature

Synthesis Time 28/Oct/2022-09:52:22

RV 1

Cycle 1 - Ala

Step 1 - Top Delivery

Rep 1

In the **Temperature** screen all or individual RV graphs can be shown by clicking RV related color circles. The range of the data shown on the graph can be change by clicking on +/- buttons on the horizontal/vertical axis. By using the sliders, a user can move through the time of the data acquisition or the temperature range.

At the bottom of the screen a user can also select a specific cycle, step, and repetition for which the data should be displayed. By moving the graph top slider, a numerical temperature value will be shown for a specific time.

The temperature data can be exported to a CSV file by using the "**Export**" function at the bottom of the screen.

The **UV Individual** screen displays individual UV data reads (each bar represents a single read within a given time frame). In the **UV Summary** screen each bar is a rollup of 1 to many reads indicating the low and high reads. The UV data can be displayed as **Transmittance** or **Absorbance** and can be switched using the toggle at the top right part of the graph.

Synthesis Time
12/Apr/2022-18:13:17

RV 1
Cycle 19
Step 9
Rep 1

Synthesis Time
12/Apr/2022-18:15:45
RV 1
Cycle 21
Step 9
Rep 1

Similar to the temperature graph the range of the displayed data can be adjusted by using the +/- buttons, slider, and drop-down menus. The UV data (individual or summary) can also be exported to a CSV file by pressing the “**Export Options**” button at the bottom of the screen. UV data can be exported as transmittance or absorbance values, or both. The exported data also contains the raw data produced by the module. For more information about the graph controls, buttons, and switches see Appendix E: Intellisynth UV Monitoring, **UV Graphs Screen** section.

5.3. Tools

5.3.1. Main Menu

After a successful login the software will open to the **Synthesis** menu. To switch to the **Tools** menu, select it from the drop-down menu at the top left corner.

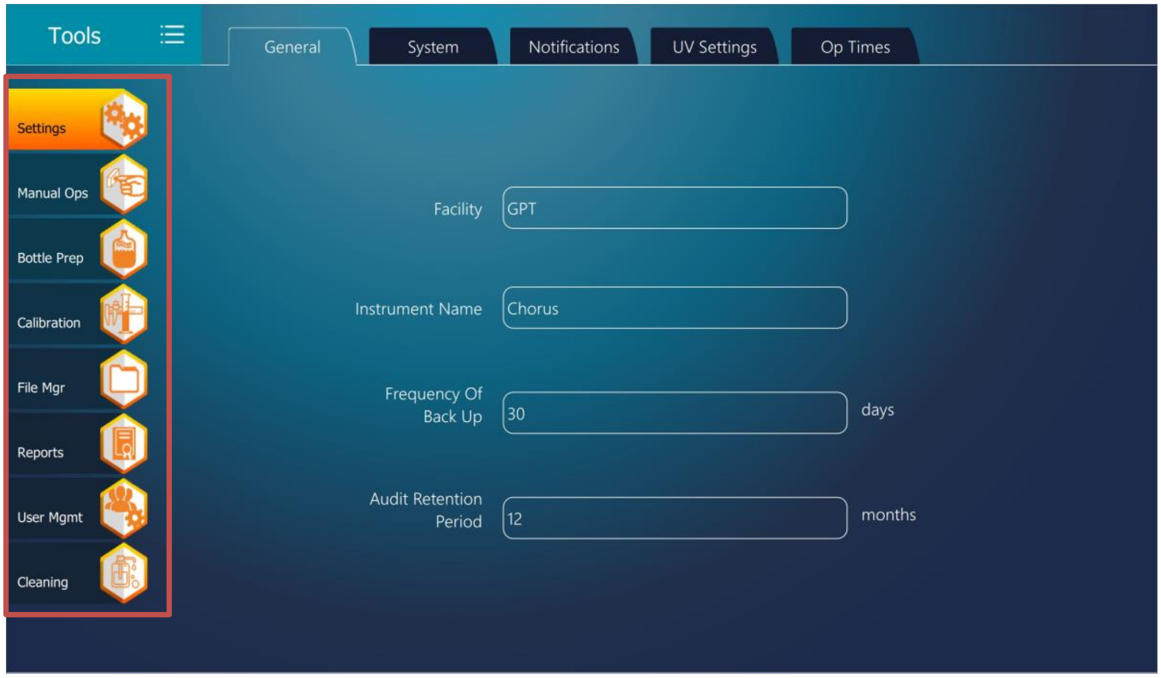
Synthesis Def: Gyros Protein Technologies

Scale: 0.1 mmol

Excess: 5

Installed RV Size: 10 mL

The following screens are available: **Settings**, **Manual Ops**, **Bottle Prep**, **Calibration**, **File Mgr**, **Reports**, **User Mgmt**, and **Cleaning**.



Facility: GPT

Instrument Name: Chorus

Frequency Of Back Up: 30 days

Audit Retention Period: 12 months

5.3.2. Settings

The **Settings** screen contains five tabs: **General**, **System**, **Notifications**, **UV Settings**, and **Op Times.**

Settings – General

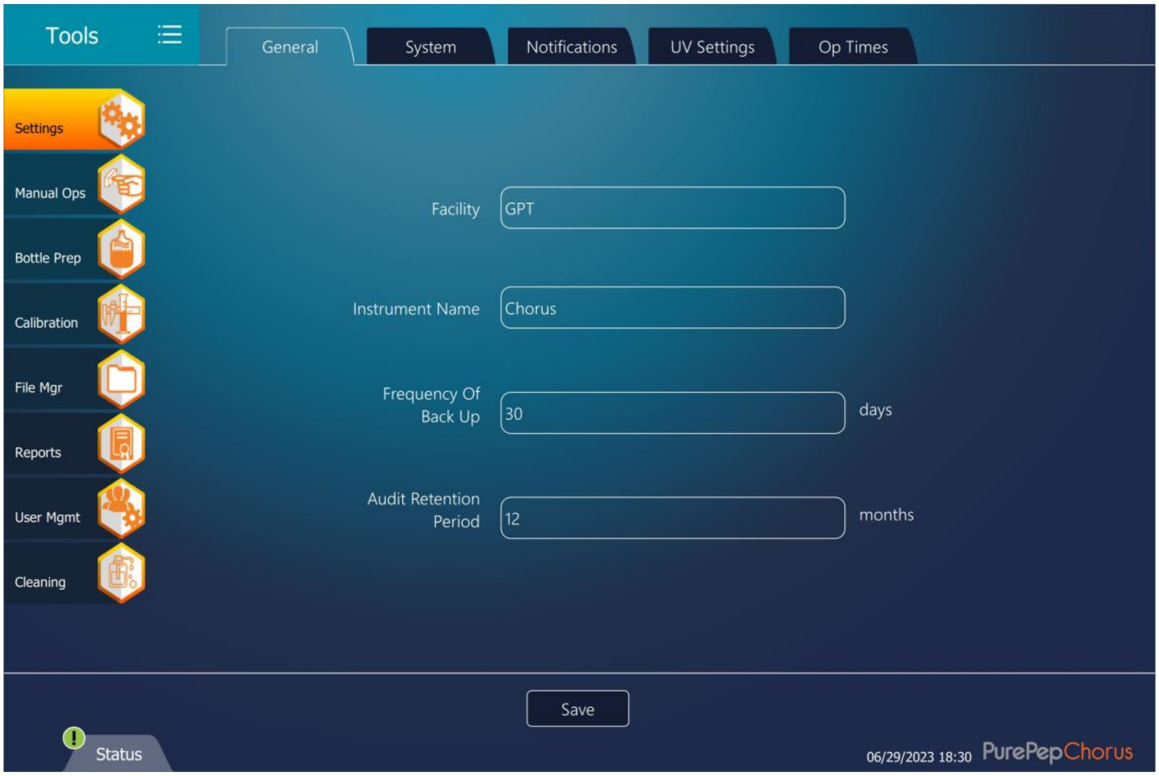
This screen includes the following information:

Facility – Enter name of facility

Instrument Name – Enter name for **PurePep Chorus** computer

Frequency of Back Up – The default setting is 30 days

Audit Retention Period – The default setting is 12 months



Facility: GPT

Instrument Name: Chorus

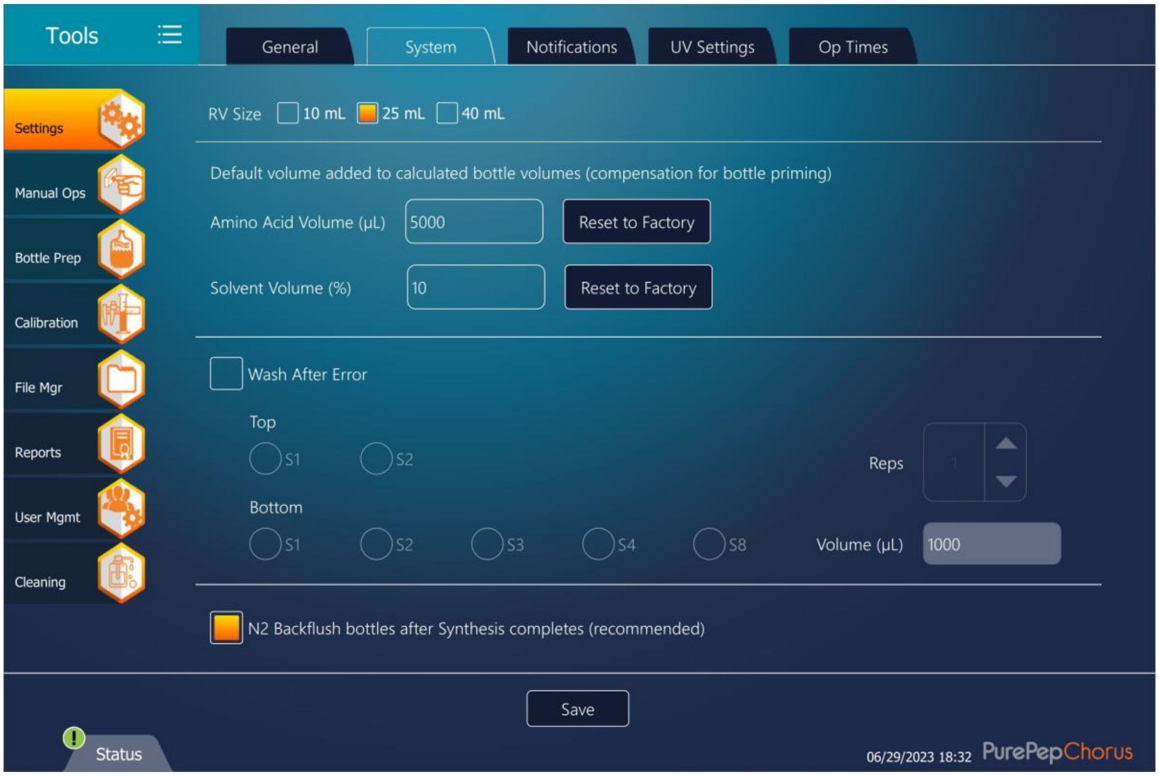
Frequency Of Back Up: 30 days

Audit Retention Period: 12 months

Settings – System

This screen allows the user to **Set RV Size** (**10 mL**, **25 mL**, or **40 mL**). This will adjust the calibration curve, *Wash After Error* wash volume, and maximum delivery volume based on RV size for manual operations. The maximum single delivery volume is 5,000 µL for the 10 mL RV, 13,000 µL for the 25 mL RV, and 20,000 µL for the 40/45 mL RVs. The change goes into effect instantly.

On this screen the user can edit and set excess volumes. These volumes are used to account for priming volumes in calculations.



RV Size ☐ 10 mL ☑ 25 mL ☐ 40 mL

Default volume added to calculated bottle volumes (compensation for bottle priming)

Amino Acid Volume (µL) 5000

Solvent Volume (%) 10

☐ Wash After Error

Top ○ S1 ○ S2

Reps 1

Bottom ○ S1 ○ S2 ○ S3 ○ S4 ○ S8

Volume (µL) 1000

☑ N2 Backflush bottles after Synthesis completes (recommended)

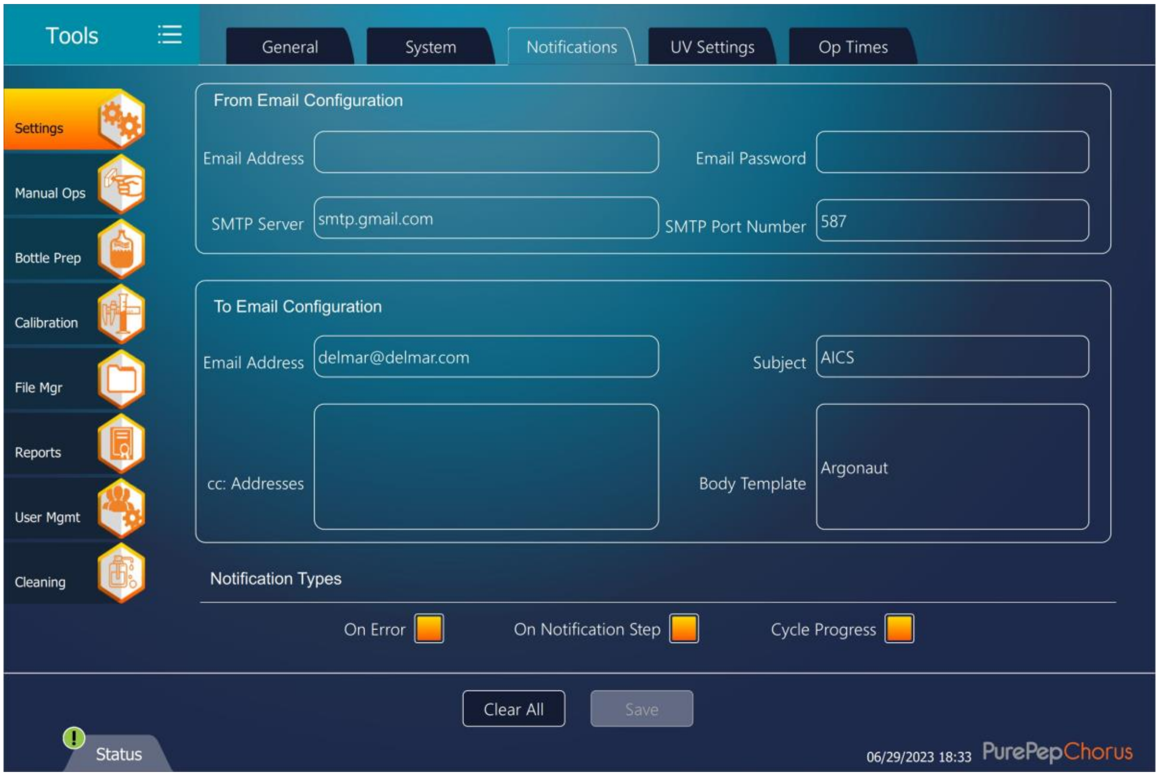
The **System** screen also allows the user to **Wash After Error** – When enabled, the software will wash all RVs with the entered solvent for the entered repetitions if an error occurs. A user would need to select a wash solvent, mode of delivery (top/bottom) if applicable, the number of repetitions in the **Reps** box (1-9), and the volume.

At the bottom of the screen, users can set **Automatic Nitrogen (N2) Backflush** of the bottles after *every* synthesis completes. When this setting is not enabled, every time a synthesis is started, a popup window will appear asking to backflush the bottles after completion of the synthesis. This allows users to select which syntheses require an N2 backflush after completion. The automated backflush is recommended as a preventative measure against unnecessary downtime due to system clogs, amino acid precipitation, etc…

Settings – Notifications

This screen allows the user to add contact information and preferences for email notifications. The **From Email Configuration** at the top requires the sender's email address and password, as well as the SMTP server and port number. All of these fields are mandatory and the software doesn't allow the user to leave them blank. The **To Email Configuration** at the bottom, requires a receiver's email address, email's subject and body template. The cc addresses are optional and can be used to add more receiver email addresses.

Checkboxes at the bottom can be selected to have email notifications **On Error**, **On Notification Step**, and for **Cycle Progress.**



From Email Configuration

Email Address:

Email Password:

SMTP Server: smtp.gmail.com

SMTP Port Number: 587

To Email Configuration

Email Address: delmar@delmar.com

Subject: AICS

cc: Addresses:

Body Template: Argonaut

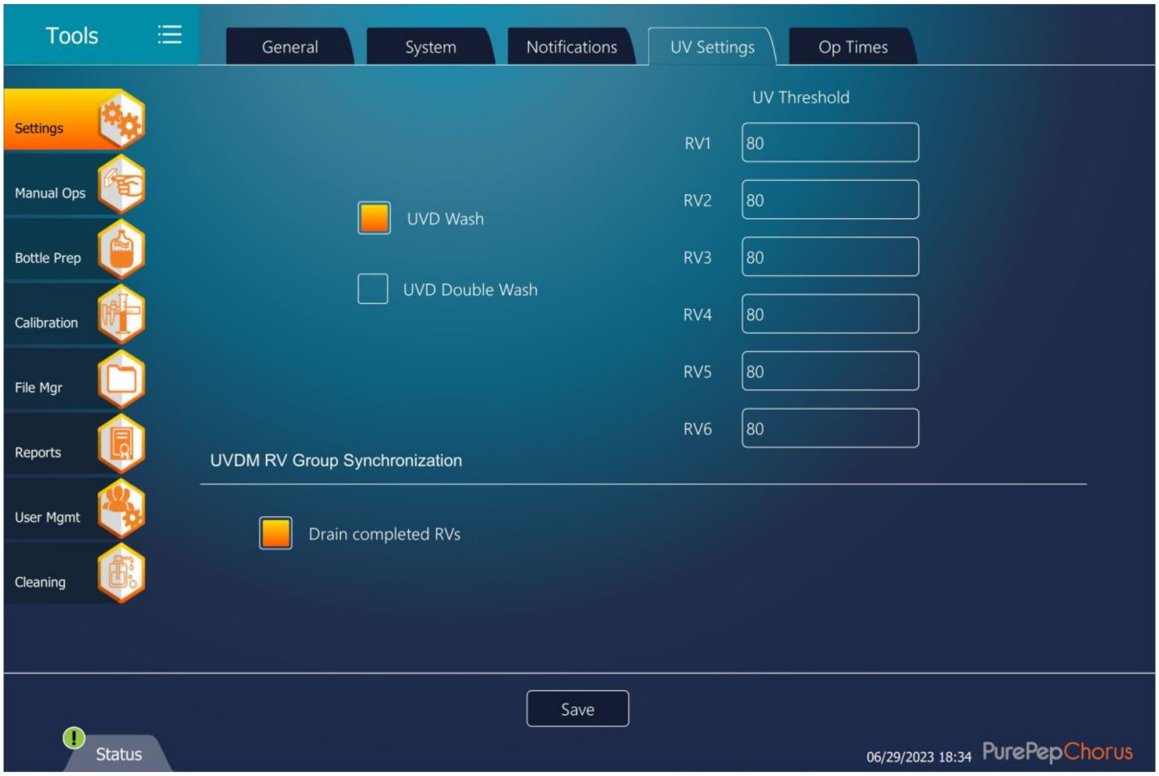
Notification Types

On Error ☑ On Notification Step ☑ Cycle Progress ☑

Settings – UV Settings

This screen allows a user to choose whether one or two washes will be performed between repetitions for protocol steps using UV Extend with Reps. UV threshold values can be changed from this screen. These are the transmittance values, in percentage units, above which UV monitoring data must be recorded multiple times before a **UV Extend with Reps** protocol step is considered complete. The absorbance threshold values will be automatically calculated by the software.

The bottom section of this screen concerns **UVDM RV Group Synchronization**. When this box is selected, each RV within a group running a **UV Extend with Reps** protocol step will drain after a repetition when it is determined to be complete, regardless of the status of other RVs in the group.



UV Threshold

RV1	80
RV2	80
RV3	80
RV4	80
RV5	80
RV6	80

☑ UVD Wash

☐ UVD Double Wash

UVDM RV Group Synchronization

☑ Drain completed RVs

Settings – Op Times

This screen allows *Admin* and *factory* users to adjust instrument operation times. The software allows a 24-hour factory access. Please contact Gyros Protein Technologies to obtain a 24-hour factory password.

These users can save modified operation times in a separate *OpTime* file by using the "**Save As**" button at the bottom of the screen. The software allows to save several *OpTime* files but only one can be used at a time.

Users can store calibration values for different RV sizes using multiple *OpTime* files. The currently loaded *OpTime* file is indicated at the top right part of this screen, as well as at the top right corner of the **Calibration** screens (see section 5.3.5 Calibration).

Op Time File: optimes

	Parameter Name	Value (ms)	Notes
1	Solvent:press_time	5000	Pressurize time for solvents
2	Solvent:venttime	1000	Vent time for solvents
3	Solvent:backflush_n2_time	20000	N2 Backflush time for solvents
4	Solvent:backflush_solv_time	13000	Backflush solvent time
5	Solvent:prime_wait_time_top	80000	Prime wait timeout for top block
6	Solvent:prime_wait_time_solv	20000	Prime wait timeout for solvent block
7	Solvent:prime_clear_time	10000	Prime clear timeout for solvents

06/29/2023 18:34

5.3.3. Manual Ops

The **Manual Ops – Individual** screen allows a user to perform any instrument operations/actions without creating a protocol or synthesis.

Operations	Bottle	Op Time	Volume (µL)	UV Mode	Reps	Drain
Top Delivery	Solv1	00:00:00	0	None	1	

RPM	Heat	T1	T2	T3	T4	T5	T6
150		25	25	25	25	25	25

Idle Thu, 29 Jun 2023 - 18:35:16 - System Idle

Manual Ops – Cleavage

This screen allows users to run *Cleave Protocols* without attaching it to a synthesis. The **Synthesis Manager->Protocol** screen can be used to create a *Cleave Protocol* (see section 5.3.6 File Mgr – Synthesis Manager tab).

Protocol: Cleavage_CLV*

☑ RV1 ☑ RV2 ☐ RV3 ☑ RV4 ☐ RV5 ☐ RV6

Actual/Set

T1	T2	T3	T4	T5	T6
25	25	25	25	25	25

Idle Thu, 29 Jun 2023 - 18:35:16 - System Idle

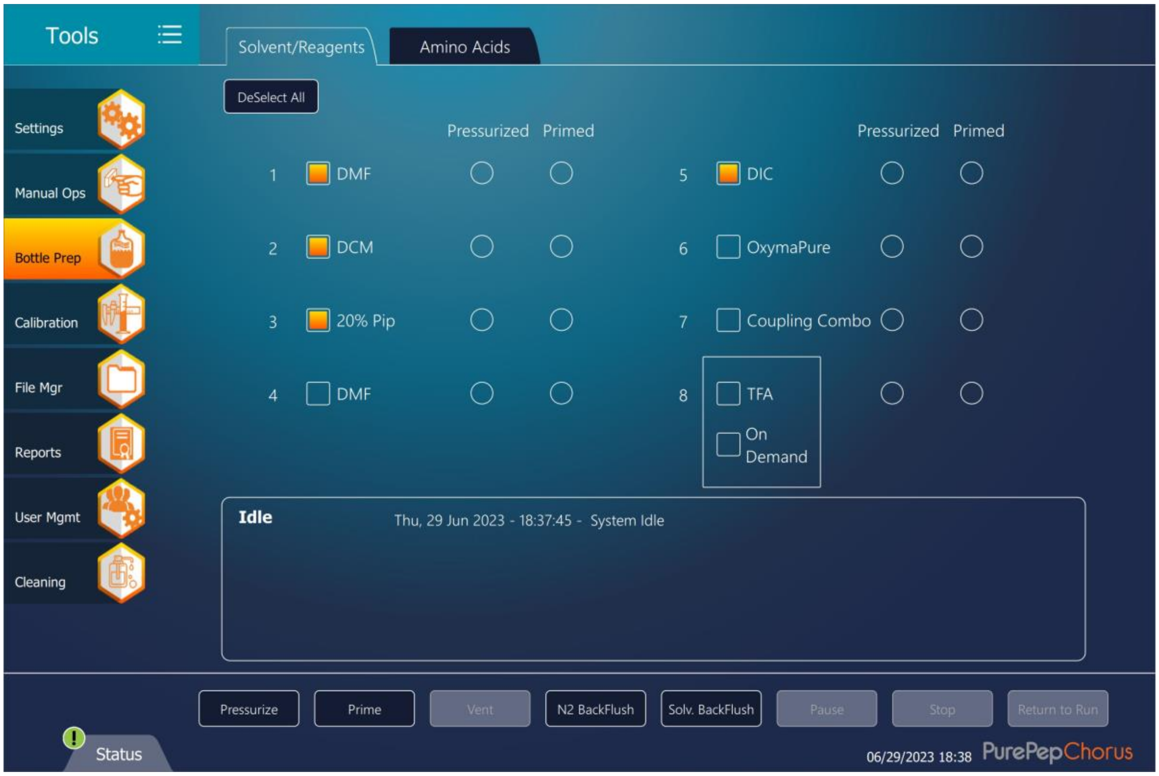
5.3.4. Bottle Prep

In the **Bottle Prep** screens, solvent/reagent and amino acid bottles can be pressurized and primed. Generally, these actions should be performed before starting a synthesis, but amino acids/monomers for **Single Shot additions should not be primed**.

Bottle Prep – Solvent/Reagents tab

On this screen, **Solv 8** has the option to select **On Demand.** This means that this bottle will only be pressurized and primed at the time of use during the course of a synthesis and backflushed and vented after its use. This position is typically used for TFA cleavage solution. The on-demand function is a safety feature to prevent the bottle containing TFA from being unnecessarily pressurized for long periods of time.

If a synthesis is loaded on the **Synthesis** screens, and a *Solvent file* was selected (section 5.2.4 Setup – Solvents/Reagents), then the solvent and reagent bottle names will be displayed on this screen.



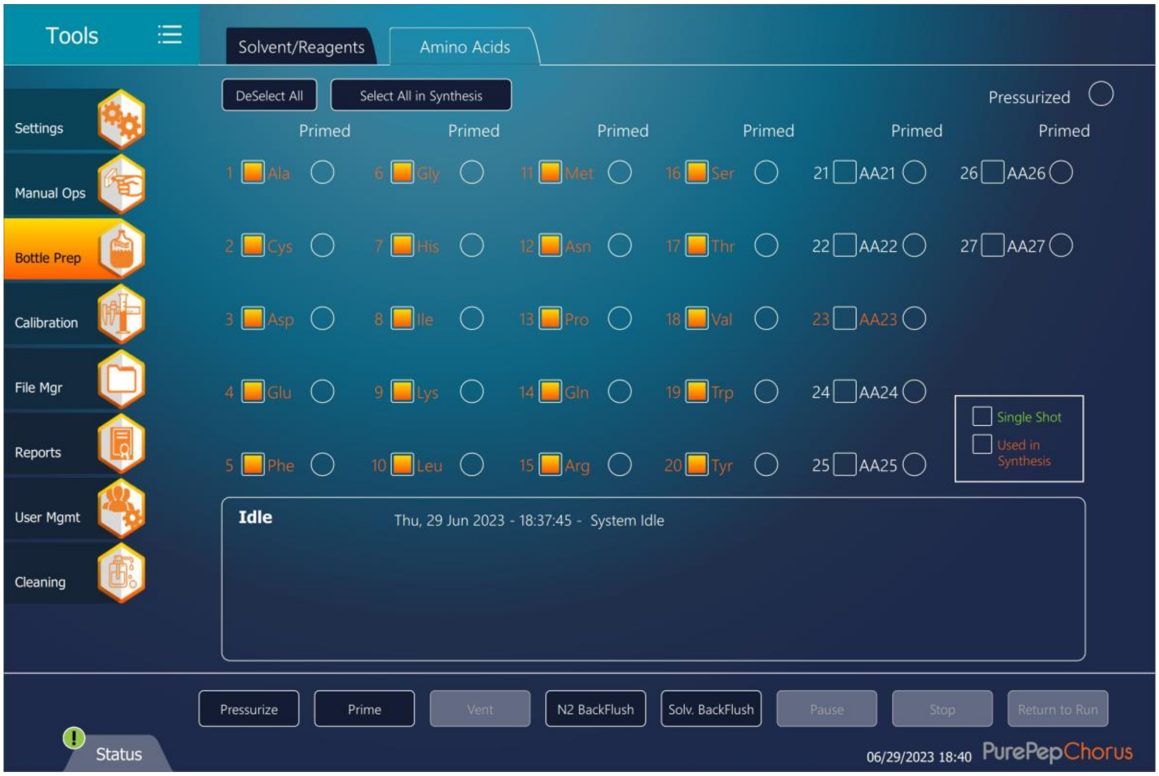
		Pressurized	Primed			Pressurized	Primed
1	☑ DMF	○	○	5	☑ DIC	○	○
2	☑ DCM	○	○	6	☐ OxymaPure	○	○
3	☑ 20% Pip	○	○	7	☐ Coupling Combo	○	○
4	☐ DMF	○	○	8	☐ TFA ☐ On Demand	○	○

Idle Thu, 29 Jun 2023 - 18:37:45 - System Idle

06/29/2023 18:38

Bottle Prep – Amino Acids tab

This screen allows pressurizing and priming of all amino acid bottle positions. Amino acid/monomers to be added as Single Shots should **not** be primed.



Pressurized ○

	Primed		Primed		Primed		Primed		Primed		Primed
1 ☑ Ala	○	6 ☑ Gly	○	11 ☑ Met	○	16 ☑ Ser	○	21 ☐ AA21	○	26 ☐ AA26	○
2 ☑ Cys	○	7 ☑ His	○	12 ☑ Asn	○	17 ☑ Thr	○	22 ☐ AA22	○	27 ☐ AA27	○
3 ☑ Asp	○	8 ☑ Ile	○	13 ☑ Pro	○	18 ☑ Val	○	23 ☐ AA23	○		
4 ☑ Glu	○	9 ☑ Lys	○	14 ☑ Gln	○	19 ☑ Trp	○	24 ☐ AA24	○		
5 ☑ Phe	○	10 ☑ Leu	○	15 ☑ Arg	○	20 ☑ Tyr	○	25 ☐ AA25	○		

☐ Single Shot
☐ Used in Synthesis

Idle Thu, 29 Jun 2023 - 18:37:45 - System Idle

06/29/2023 18:40

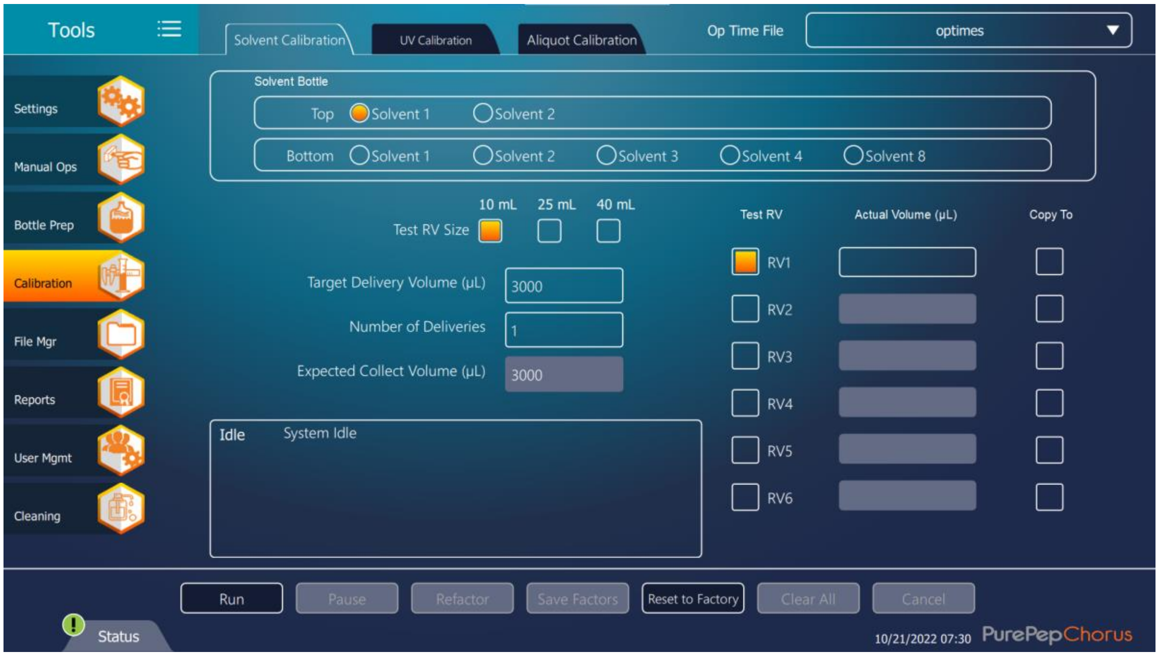
If a synthesis is loaded on the **Synthesis** screens, and the amino acids have been assigned to bottle positions (section 5.2.4 Setup – Amino Acids), then the AA bottle names that are used will be displayed on this screen.

5.3.5. Calibration

The **Calibration** section contains three calibration tabs: **Solvent Calibration**, **UV Calibration**, and **Aliquot Calibration**

Calibration – Solvent Calibration

This screen allows the user to calibrate the volume deliveries of the timed solvent/reagent bottles (1-4, and 8). To save calibration values in a separate file, the user should first create a new *OpTime* file (section 5.3.2 Settings – Op Times), and then choose the desired file at the top right corner of this screen.



Op Time File: optimes

Top: ● Solvent 1

Test RV Size: 10 mL ☑

Target Delivery Volume (µL): 3000

Number of Deliveries: 1

Expected Collect Volume (µL): 3000

Idle System Idle

☑ RV1

10/21/2022 07:30

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 label (for top deliveries: Solvent 1 or Solvent 2, for bottom deliveries: Solvent 1 – Solvent 4, or Solvent 8). Only one solvent bottle may be selected at a time.

The **Target Delivery Volume (µL)** box is where the calibration delivery volume can be entered. 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.

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. The higher this value, the more accurate the calibration.

The software calculates the theoretical volume that should be in the collection vial at the end of the calibration and displays it in the **Expected Collect Volume (µL)** box.

The **Test RV** column allows to select the RV positions to be calibrated. Check the box to the left of the desired RV(s) to select an RV. Press the box a second time will deselect the RV.

The **Copy To** selection copies the calibration factor for a single RV to all others selected. Using the **Copy To** feature significantly shortens the calibration time but may result in a delivery volume variation of up to 10%. The greatest accuracy is obtained when all RVs are selected during a calibration. This is recommended for reagent deliveries that require greater accuracy.

At the bottom of the screen, the following buttons are available:

1. "**Run**" – Starts running a calibration.
2. "**Pause**" – Pauses the actions currently in progress after any deliveries are complete.
3. "**Refactor**" – Calculates new calibration factors based on **Expected Collect Volume (µL)** and **Actual Volume (µL)** values.
4. "**Save Factors**" – Saves new calibration factors. Press the "**Save Factors**" button when the **Actual Volume (µL)** matches the **Expected Collect Volume (µL)**.
5. "**Reset to Factory**" – Restores default calibration factors for the selected solvent.
6. "**Clear all**" – Removes all entered values.
7. "**Cancel**" – Cancels a running calibration.

RVs and collection vials should be installed on the instrument for the selected RV positions before running a calibration.

After running a calibration, the **Actual Volume (µL)** column becomes active. Measure the actual volumes delivered to the collection vial(s) and enter them in the **Actual Volume (µL)** column.

Steps to calibrate a bottle:

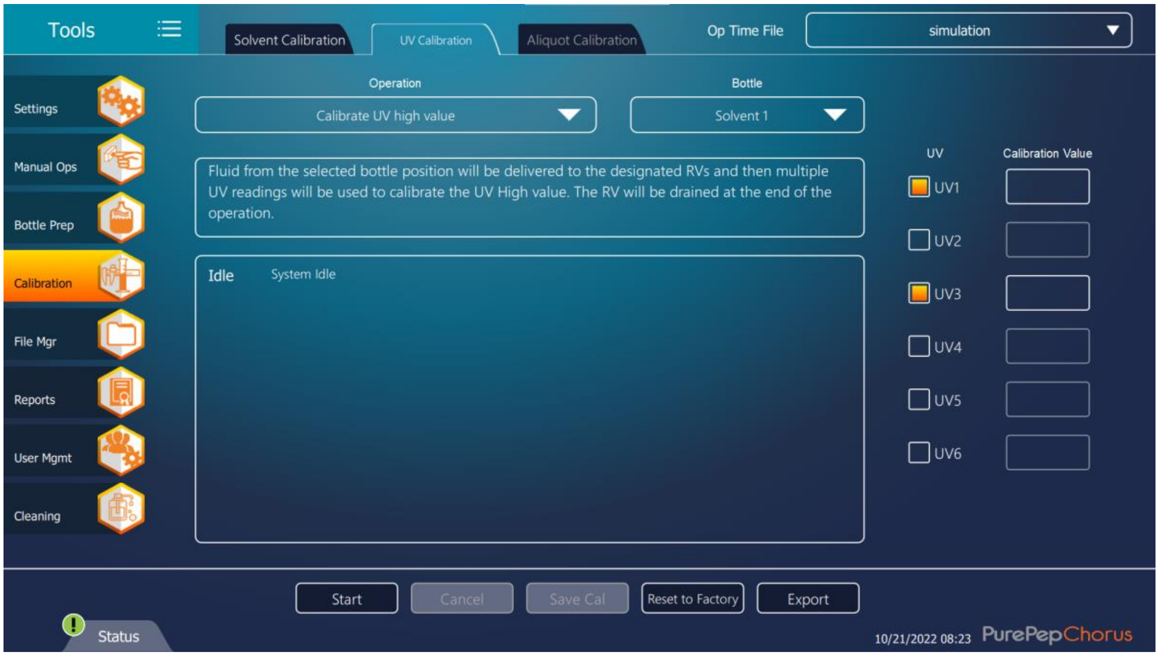
1. Select a solvent bottle in the **Solvent Bottle** section by pressing the desired radio button.
2. In the **Target Delivery Volume (µL)** box enter the volume (in microliters) that will be delivered to an RV from the selected bottle during a synthesis.
3. In the **Number of Deliveries** box enter the number of times the volume will be delivered to the selected RV(s).
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. Alternatively, select the **Copy To** feature.
5. Click the "**Run**" button. Make sure RVs and Collect Vials are in place. A user may press "**Cancel**" to cancel the calibration.
6. After the calibration is complete, measure the volumes in the collection vials(s) using a calibrated collection vial or a graduated cylinder, and enter the values (in microliters) in the **Actual Volume (µL)** column next to the appropriate RV.
7. Click the "**Refactor**" button to calculate new calibration factors.

8. Repeat steps 1 – 7 until the **Actual Volume (µL)** and **Expected Collect Volume (µL)** values match. Then, click the “**Save Factors**” button.

After the calibration is complete, the fluid system may be contaminated. Perform a **Wash All Blocks** followed by a **Clear All Blocks** operation to clean the instrument (section 5.3.9 Cleaning).

Calibration – UV Calibration

This screen allows users to calibrate UV modules.



Op Time File: simulation

Operation: Calibrate UV high value

Bottle: Solvent 1

Fluid from the selected bottle position will be delivered to the designated RVs and then multiple UV readings will be used to calibrate the UV High value. The RV will be drained at the end of the operation.

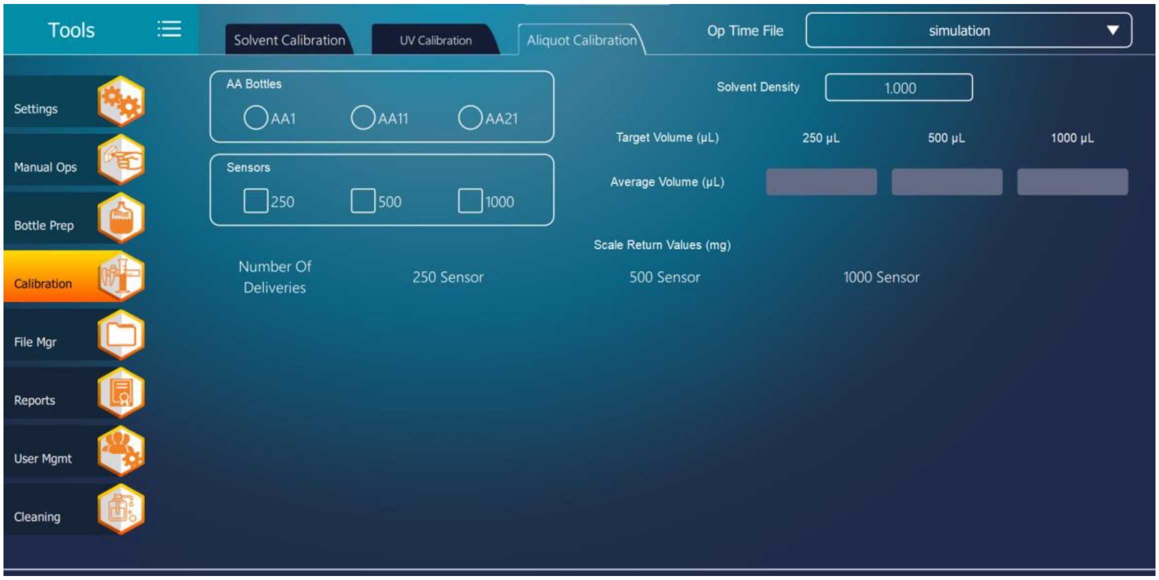
Idle System Idle

☑ UV1
☐ UV2
☑ UV3
☐ UV4
☐ UV5
☐ UV6

In the **Operation** drop down menu the user can find 3 calibration operations: **Calibrate UV High Value**, **Calibrate UV Low Value**, and **Wash RV**. The **Calibrate UV High** operation is used to calibrate maximum transmittance/minimum absorbance value of the UV module based on the pure deprotection solution. From the **Bottle** drop-down menu, the user should designate the solvent bottle in which the deprotection solution will be placed and press “**Start**” to perform the operation. The **Wash RV** operation is used to wash the UV module cell. The **Calibrate UV Low** operation is used for reference only. The values obtained during this operation do not impact any functionality of the instrument. It can be used to test the response for certain solutions. For instance, Fmoc protected amino acid can be placed in one of the amino acid bottles to test the response for certain concentrations. The operation will be performed after pressing the “**Start**” button.

Calibration – Aliquot Calibration

Users with appropriate permissions (24-hour factory access – please contact Gyros Protein Technologies) can also perform aliquot sensor calibrations from the **Aliquot Calibration** screen.



Op Time File: simulation

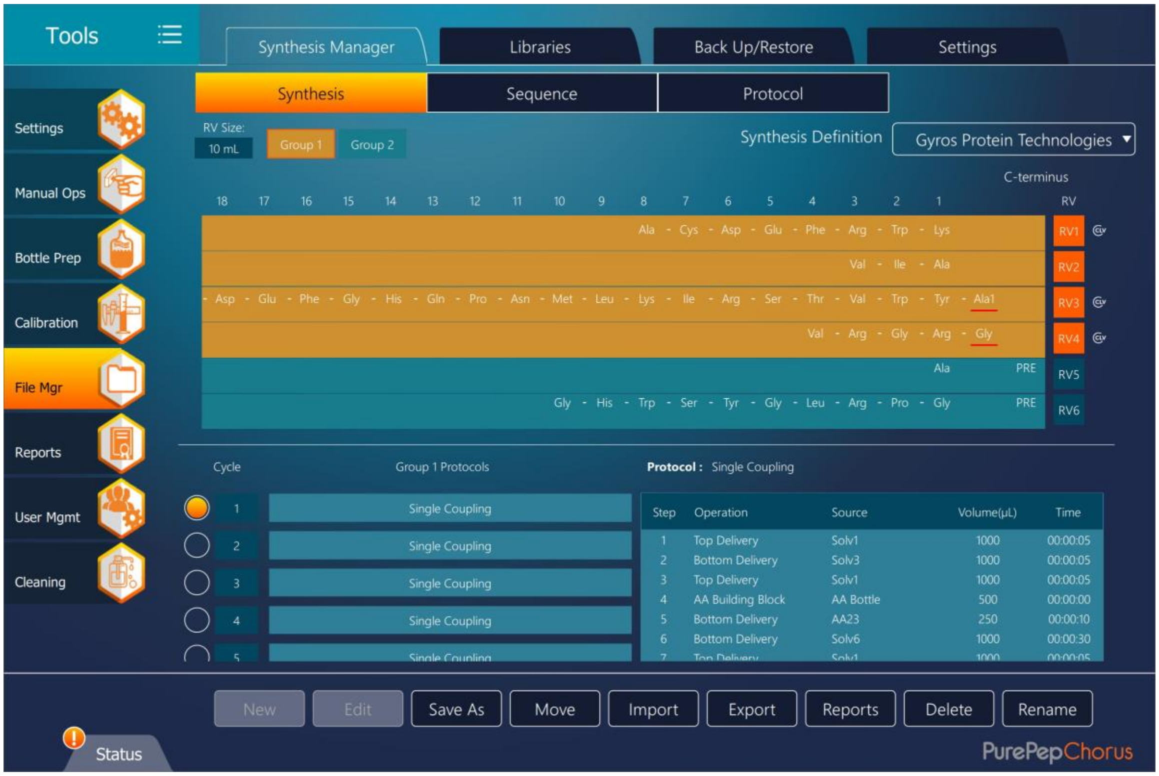
Solvent Density: 1.000

5.3.6. File Mgr

The **File Mgr (File Manager)** menu contains four tabs: **Synthesis Manager**, **Libraries**, **Back Up/Restore**, and **Settings**.

File Mgr – Synthesis Manager tab

The **Synthesis Manager** facilitates the editing and import of *Synthesis*, *Protocol*, and *Sequence* definitions/files using the "**Edit**" and "**Import**" buttons respectively.



Protocol : Single Coupling

Step	Operation	Source	Volume(µL)	Time
1	Top Delivery	Solv1	1000	00:00:05
2	Bottom Delivery	Solv3	1000	00:00:05
3	Top Delivery	Solv1	1000	00:00:05
4	AA Building Block	AA Bottle	500	00:00:00
5	Bottom Delivery	AA23	250	00:00:10
6	Bottom Delivery	Solv6	1000	00:00:30
7	Top Delivery	Solv1	1000	00:00:05

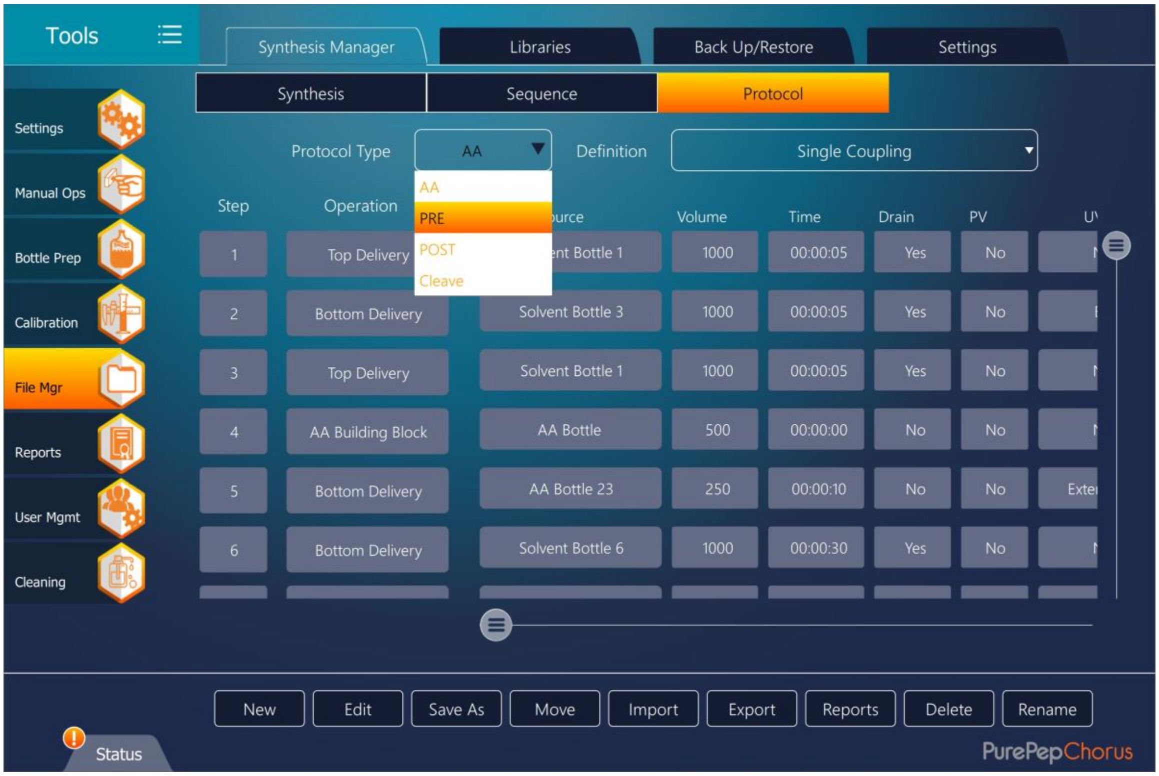
Users are able to create a new definition/file from an existing one with the “**Save As**” function – this will reuse an existing file as a starting template and modify without changing the original one. Using the “**New**” button, the **Protocol** and **Sequence** screens also allow to create new definitions/files via their respective editors. The “**Rename**” button allows to change the name of a definition/file. The “**Move**” button allows to rename and select another location (either a new or existing folder) to move and save the file.

Import/Export – CSV files

The **Synthesis** import/export function will import/export the full synthesis file together will all associated *Protocols*, *Sequences*, and *Solvent* files. There is no need to import/export these files separately. When exporting a *Synthesis* file, the application will request to select a folder and all the files will be exported to the selected folder. When importing a Synthesis file, all the synthesis related CSV files need to be in the same folder.

The software allows to import/export files from and to the local drive, external drive (e.g. USB drive), or network drive. In order for the software to access a network drive, the tablet must have a dynamic IP and an ethernet cable must be plugged in to the right side of the synthesizer near the power switches and solvent lines. For help please contact Gyros Protein Technologies.

The application allows to import multiple CSV files at once, provided they are of the same type. For example, a user can select several protocol CSV files and import them at the same time (e.g. bulk import). This feature allows the user to import large quantities of data (i.e. definitions/files) into the software quickly and efficiently.



Protocol Type: AA

Definition: Single Coupling

Step	Operation	urce	Volume	Time	Drain	PV	U
1	Top Delivery	nt Bottle 1	1000	00:00:05	Yes	No	
2	Bottom Delivery	Solvent Bottle 3	1000	00:00:05	Yes	No	
3	Top Delivery	Solvent Bottle 1	1000	00:00:05	Yes	No	
4	AA Building Block	AA Bottle	500	00:00:00	No	No	
5	Bottom Delivery	AA Bottle 23	250	00:00:10	No	No	Exte
6	Bottom Delivery	Solvent Bottle 6	1000	00:00:30	Yes	No	

If document password protection is enabled (see File Mgr – Settings tab) the software will request a password for each file being imported. Users have the option to define a **Common password for all** imported files or **Unique passwords** for each file.

Would you like to define a common password for all imported files or a unique password for each file?

● Common password for all

○ Unique passwords

NOTE: If password-protected files are created using an editor utility, the passwords will not be included when they are exported. When importing these files into the tablet that is connected to the synthesizer, the software will ask to assign new passwords (assuming document password protection is enabled).

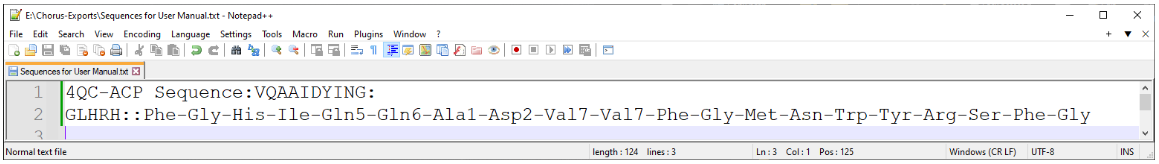
Import/Export – TXT files (Sequences only)

The “**Export**” button from the **Synthesis Manager->Sequence** screen also allows the user to export a sequence into a text (.txt) file. The “**Import**” button from the same screen has the ability to import one or multiple sequences via a single text (.txt) file.

When writing sequences into a text (.txt) file, a valid sequence adheres to the following rules:

1. Valid formats:
   `Name:SingleLetters:`
   `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 AA is separated by a – (dash).
4. Defining a sequence using both one-letter and abbreviation is not allowed.
5. All the amino acids must be part of the AA library.
6. Each sequence must be defined on a separate line.
7. The sequence name is allowed to have dashes (–) and spaces.

The following is an example of two valid sequences saved into a text (.txt) file that can be imported into the software.



```
4QC-ACP Sequence:VQAAIDYING:
GLHRH::Phe-Gly-His-Ile-Gln5-Gln6-Ala1-Asp2-Val7-Val7-Phe-Gly-Met-Asn-Trp-Tyr-Arg-Ser-Phe-Gly
```

Sequence Definition: bAmyloid

3 Letter

Asp	Ala	Glu	Phe	Arg	His	Asp	Ser	Gly	Tyr	Glu	Val
His	His	Gln	Lys	Leu	Val	Phe	Phe	Ala	Glu	Asp	Val
Gly	Ser	Asn	Lys	Gly	Ala	Ile	Ile	Gly	Leu	Met	Val
Gly	Gly	Val	Val	Ile	Ala						

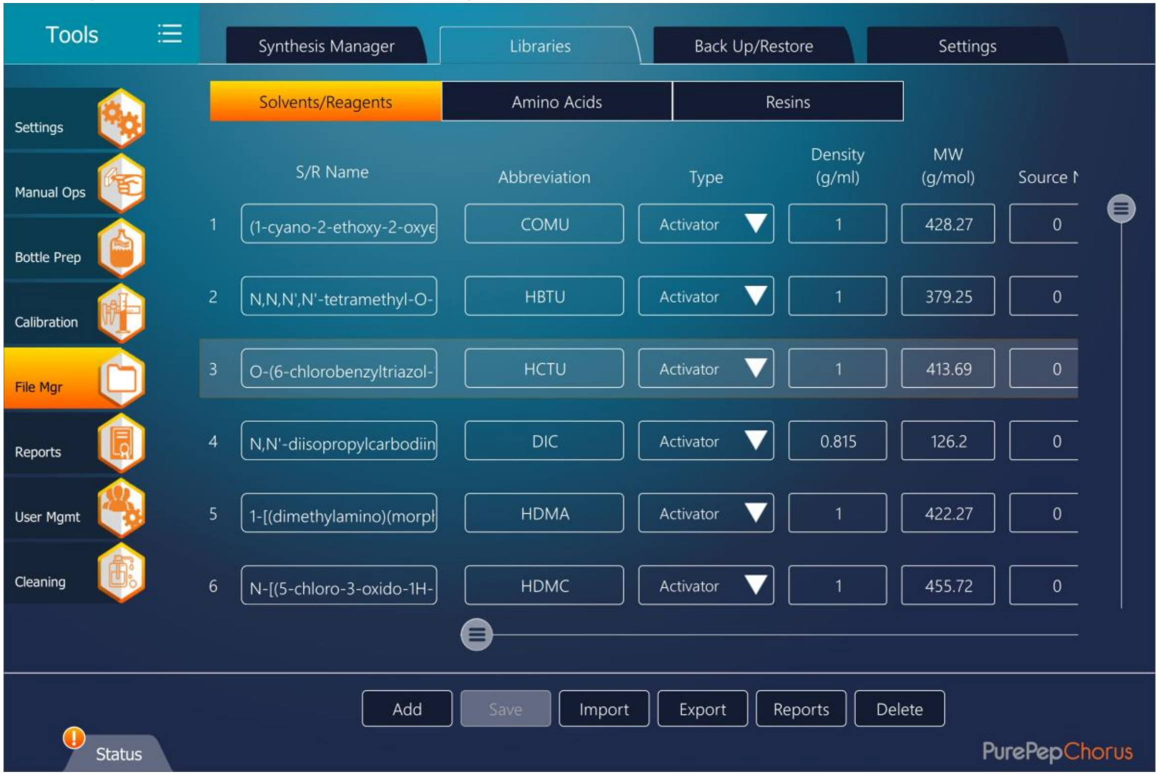
Sequence Length: 42

The **Sequence** screen presents the peptide from N-terminus being at the top left and C-terminus being in the bottom right part of the window.

File Mgr – Libraries tab

This screen 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 throughout 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 file. Information such as density or molecular weight is then used for the calculations.

File Mgr – Libraries – Solvents/Reagents



	S/R Name	Abbreviation	Type	Density (g/ml)	MW (g/mol)	Source N
1	(1-cyano-2-ethoxy-2-oxye	COMU	Activator	1	428.27	0
2	N,N,N',N'-tetramethyl-O-	HBTU	Activator	1	379.25	0
3	O-(6-chlorobenzyltriazol-	HCTU	Activator	1	413.69	0
4	N,N'-diisopropylcarbodiin	DIC	Activator	0.815	126.2	0
5	1-[(dimethylamino)(morpl	HDMA	Activator	1	422.27	0
6	N-[(5-chloro-3-oxido-1H-	HDMC	Activator	1	455.72	0

Solvent/Reagents available fields:

- **S/R Name** – chemical name of the solvent/reagent
- **Abbreviation** – up to 9-letter abbreviation of the solvent/reagent
- **Type** – type of the solvent/reagent chosen from: `Activator`, `Base`, `Additive`, `Cleave`, `Deprotect`, `Capping`, or `NA`
- **Density** – density of the solvent/reagent (if available)
- **MW** – molecular weight of the solvent/reagent
- **Source No** – origin of the solvent/reagent
- **Opened** – date the solvent/reagent was opened
- **Lot No** – lot number of the solvent/reagent

When importing a Solvent/Reagent library (`SRLIB.csv`), the following restrictions are enforced:

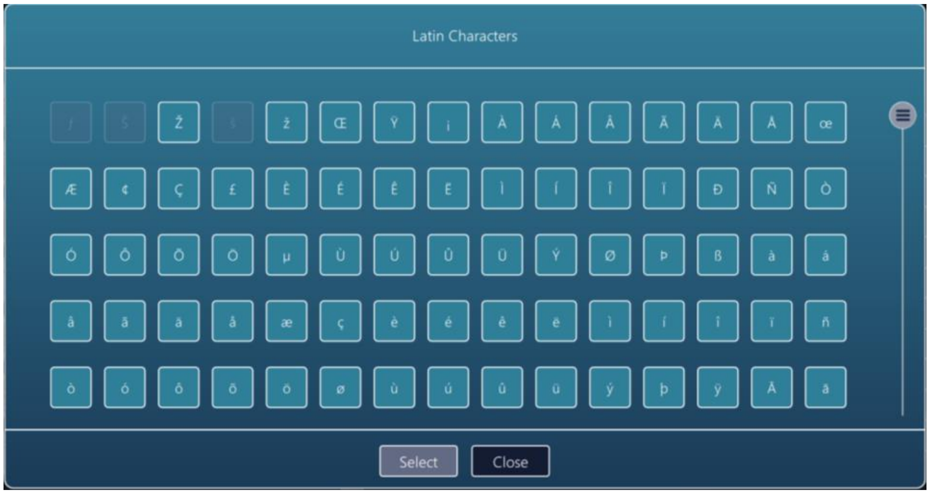
- repeated abbreviations are not allowed,
- maximum number of entries is 200.

File Mgr – Libraries – Amino Acids

	Name	Abbreviation	1 Letter		Dep MW (g/mol)	Prot MW (g/mol)	Var
1	Fmoc-Ala-OH	Ala	A		89.09	311.34	
2	Fmoc-Cys(Trt)-OH	Cys	C		121.16	585.73	
3	Fmoc-Asp(OtBu)-OH	Asp	D		133.1	411.46	
4	Fmoc-Glu(OtBu)-OH	Glu	E		147.13	425.49	
5	Fmoc-Phe-OH	Phe	F		165.19	387.44	
6	Fmoc-Gly-OH	Gly	G		75.07	297.31	

Amino Acids available fields:

- **Name** – name of the amino acid/monomer/building block
- **Abbreviation** – abbreviation of the amino acid/monomer/building block of up to 9 letters. (**CAUTION**: in the current version of the software, 5-letter abbreviation is recommended, 3-letter code cannot start with a capital letter)
- **1 Letter** – 1 letter code of the amino acid/monomer/building block. The first 20 natural amino acids are already assigned. The rest can be chosen from the library of special characters (**Latin Characters**) available after pressing the keyboard icon that is being activated while cursor is on the 1-letter code field. The 1-letter code must be unique

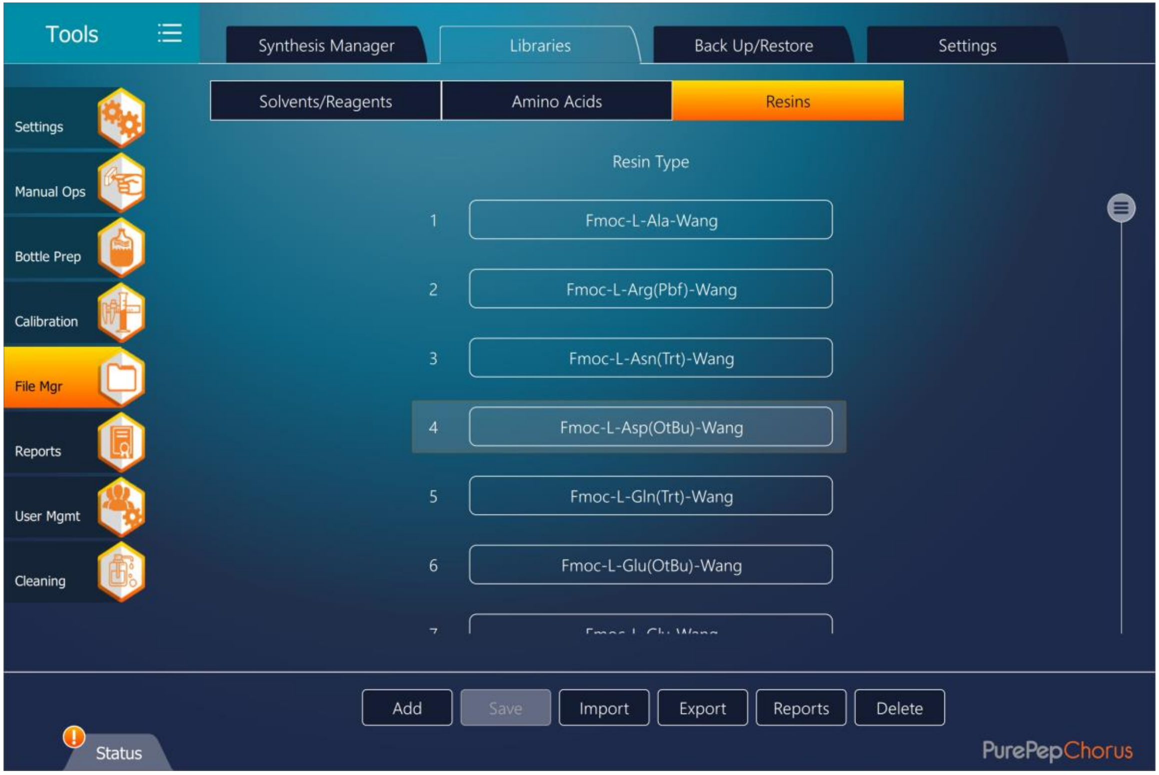


- **Dep MW** – molecular weight of acid/monomer/building block without protecting groups (used in the calculation of the final MW of the product)
- **Prot MW** – molecular weight of acid/monomer/building block with all protecting groups (used in the calculation of the weight needed to prepare the acid/monomer/building block solution)
- **Variant** – set/unset a non-standard amino acid/monomer as a variant
- **Source No** – origin of the amino acid/monomer/building block
- **Opened** – date the amino acid/monomer/building block was opened
- **Lot No** – lot number of the amino acid/monomer/building block
- **Default Tc** – default coupling temperature of the amino acid/monomer/building block (used when creating new syntheses that use heat)

When importing an Amino Acid library (`AALIB.csv`), the following restrictions are enforced:

- repeated abbreviations are not allowed,
- repeated 1-leter codes are not allowed,
- maximum number of entries is 250.

File Mgr – Libraries – Resins



Resin Type

1 Fmoc-L-Ala-Wang
2 Fmoc-L-Arg(Pbf)-Wang
3 Fmoc-L-Asn(Trt)-Wang
4 Fmoc-L-Asp(OtBu)-Wang
5 Fmoc-L-Gln(Trt)-Wang
6 Fmoc-L-Glu(OtBu)-Wang

Resins available fields:

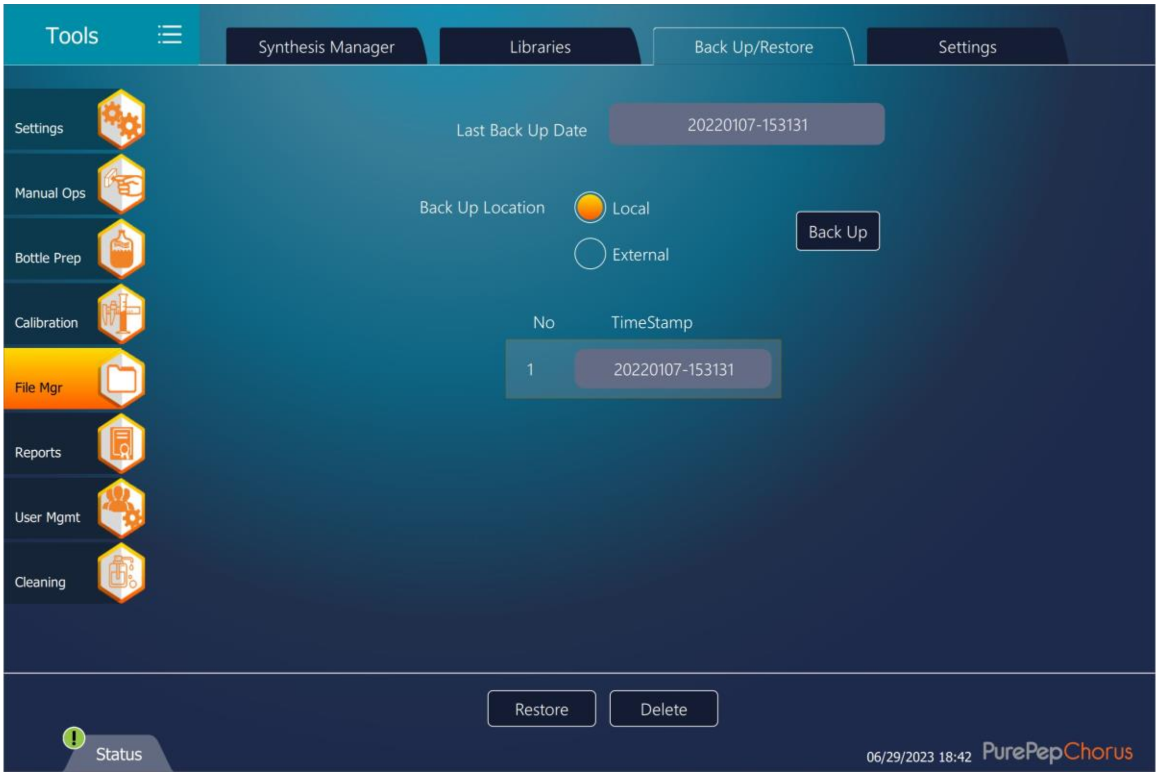
Resin Type – name of the resin

When importing a Resin library (`RESINLIB.csv`), the following restrictions are enforced:

- repeated names are not allowed,
- maximum number of entries is 128.

File Mgr – Back Up/Restore tab

This screen enables a user to create an encrypted system back-up on a local or external drive using the "**Back Up**" button. The "**Restore**" button will allow a user to restore to a previous backup point. **To complete backups to or restores from an external drive, this drive must be connected to the instrument interface rather than the tablet.**



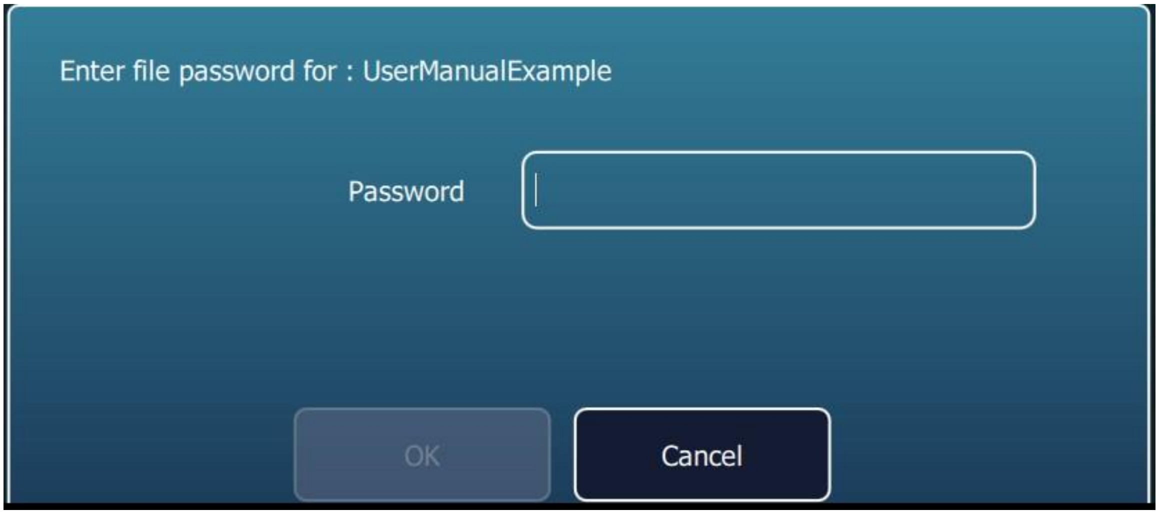
Last Back Up Date: 20220107-153131

Back Up Location: ● Local ○ External

No	TimeStamp
1	20220107-153131

File Mgr – Settings tab

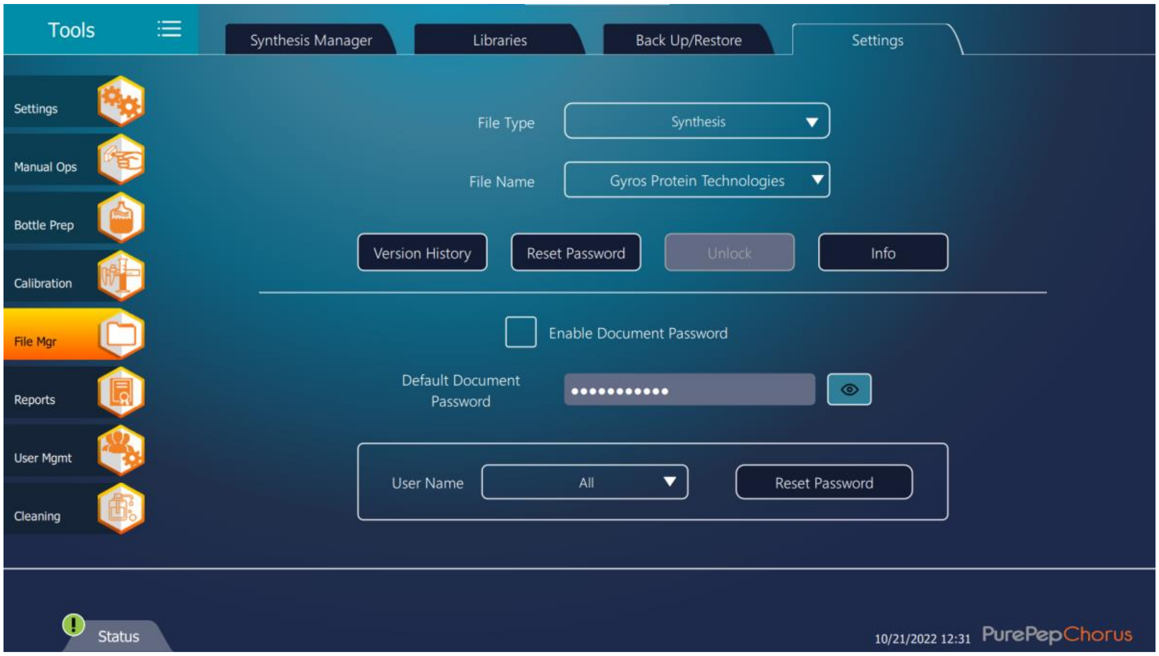
This screen enables a user to modify file settings or view version histories for various file types**.** Document passwords can also be enabled/disabled or reset using this screen. The bottom part of the screen allows to enable/disable document password protection. If enabled, every *Synthesis*, *Protocol*, and *Sequence* file will be password protected. The software will then ask for the password each time a save is requested (after file modification) through a popup which will also show the name of the document.



Enter file password for : UserManualExample

Password

Default Document Password is the default password that will be assigned to a document when its password is reset.



File Type: Synthesis

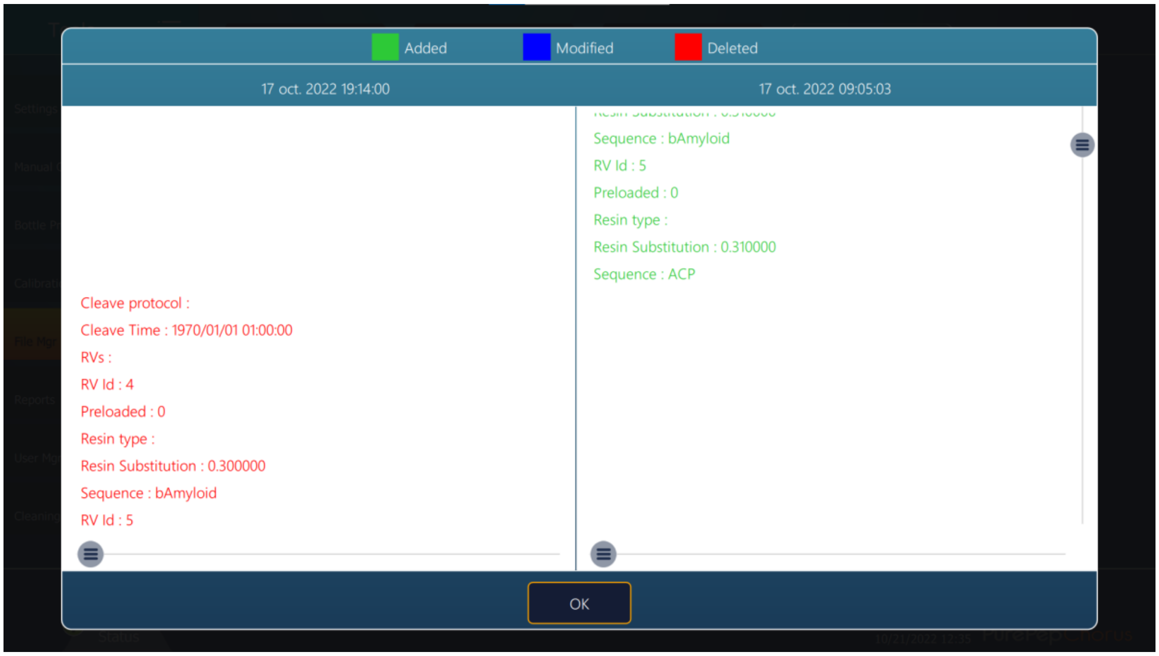
File Name: Gyros Protein Technologies

☐ Enable Document Password

Default Document Password: ••••••••••••

User Name: All

Version History allows the user to compare two different versions of a file and see who, when, and how it was modified. Different type of modifications are displayed with different colors (Additions, Modifications, Deletions).



🟩 Added 🟦 Modified 🟥 Deleted

17 oct. 2022 19:14:00

Cleave protocol :
Cleave Time : 1970/01/01 01:00:00
RVs :
RV Id : 4
Preloaded : 0
Resin type :
Resin Substitution : 0.300000
Sequence : bAmyloid
RV Id : 5

17 oct. 2022 09:05:03

Sequence : bAmyloid
RV Id : 5
Preloaded : 0
Resin type :
Resin Substitution : 0.310000
Sequence : ACP

5.3.7. Reports

The **Reports** menu contains **Job Reports** and **Audit Reports** screens.

Reports – Job Reports tab

A *Job Report* corresponds to all the information from a synthesis run. Users can select any job report from the drop-down menu. The most recently generated reports will be displayed first. Pressing "**Load**" refreshes the report and its contents. It also displays the number of RVs used and the number of protocol steps.

Users can select which details they would like to include in a PDF report, with options for **Summary**, **Details**, **Amino Acid Usage**, **Solvent/Reagent**, **Protocol Summary**, **and Protocol Details.** An option to add an electronic signature is also provided.

Job#	Date	Time	Synthesis Name
11	2022-10-20	19:52:10	Gyros Protein Technologies

☑ Summary
☑ Details
☑ Amino Acid Usage
☑ Solvent/Reagent
☑ Protocol Summary
☑ Protocol Details

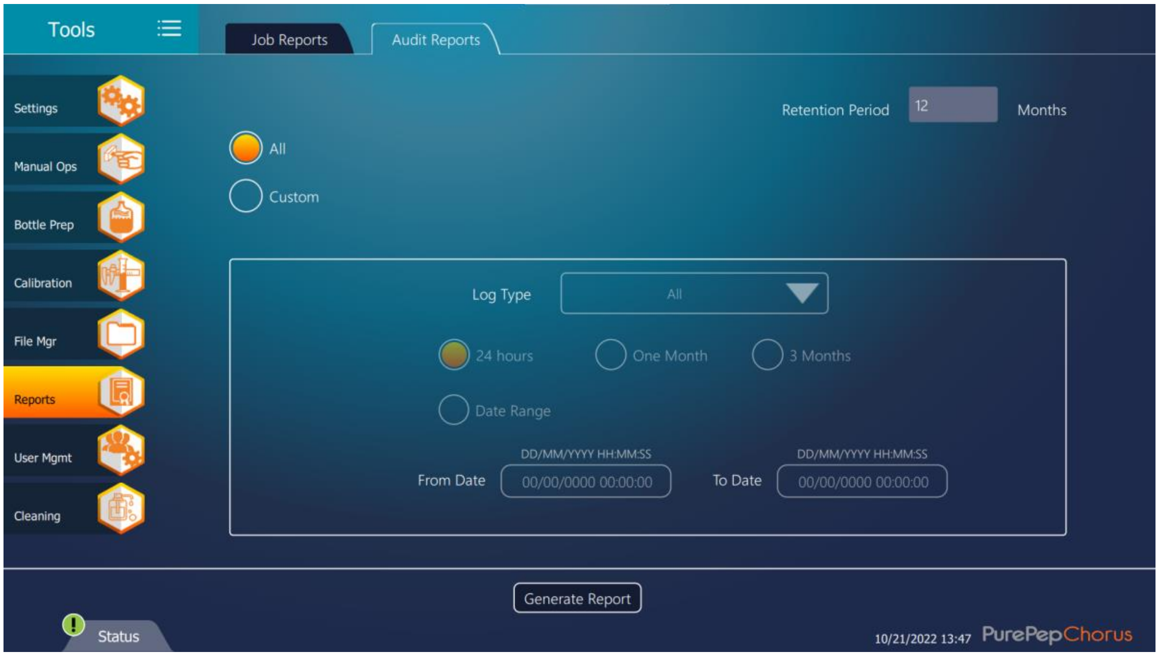
Protocol Steps 11
Of RVs Used 6

☐ Add Electronic Signature

10/21/2022 13:46

Reports – Audit Reports tab

This screen allows the creation of detailed instrument log reports. A user can choose **All** or create a **Custom** report for a specified date and time range.



Retention Period 12 Months

● All
○ Custom

Log Type: All
● 24 hours ○ One Month ○ 3 Months
○ Date Range

From Date (DD/MM/YYYY HH:MM:SS) 00/00/0000 00:00:00
To Date (DD/MM/YYYY HH:MM:SS) 00/00/0000 00:00:00

10/21/2022 13:47

User Mgmt

The **User Mgmt** menu includes **User Management** and **User Settings** screens.

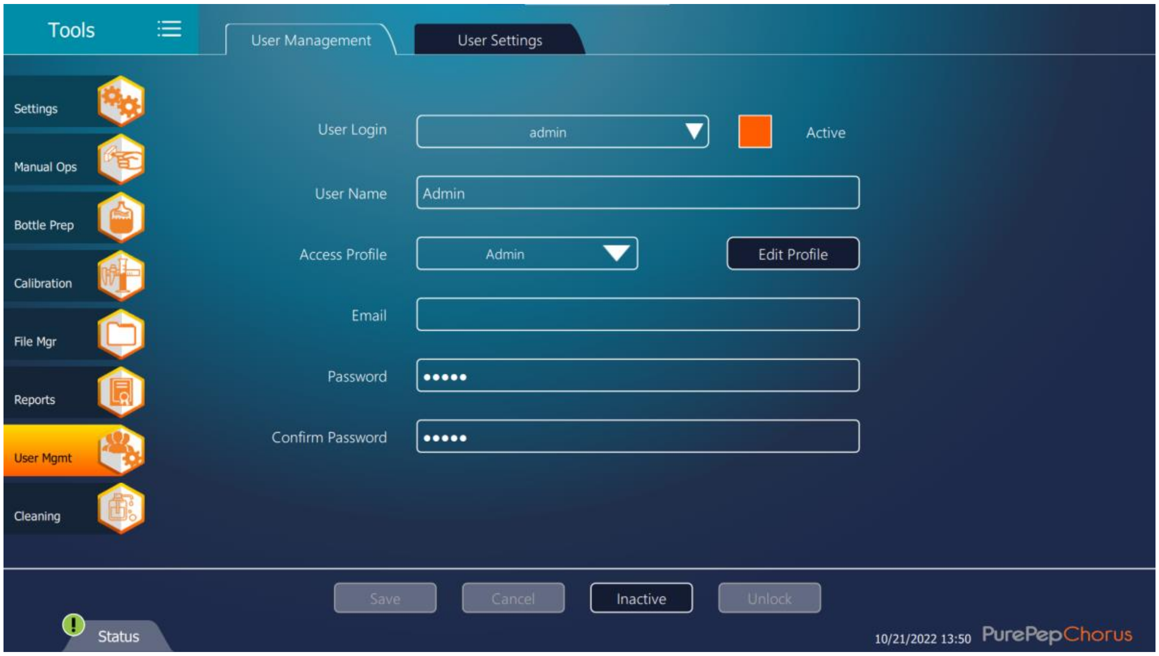
User Mgmt – User Management tab

Using this screen, an administrator can create a new user and select their profile type. The standard user profiles and their default associated privileges are as follows:

Profile	View	Run	Delivery Cal	Op Times	Edit	Diagnostics	Settings	Import	Reports	Exit To OS	Export	User Mgmt	Log Mgmt
Runner	✓	✓	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖	✖
Designer	✓	✓	✓	✖	✓	✖	✖	✓	✓	✖	✓	✖	✖
Admin	✓	✓	✓	✖	✓	✓	✓	✓	✓	✓	✓	✓	✓
Service	✓	✓	✓	✖	✓	✓	✓	✓	✓	✓	✓	✓	✓
Factory	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

The permissions associated with a particular profile may be adjusted by the administrator and need not align with one of the default profiles listed above.

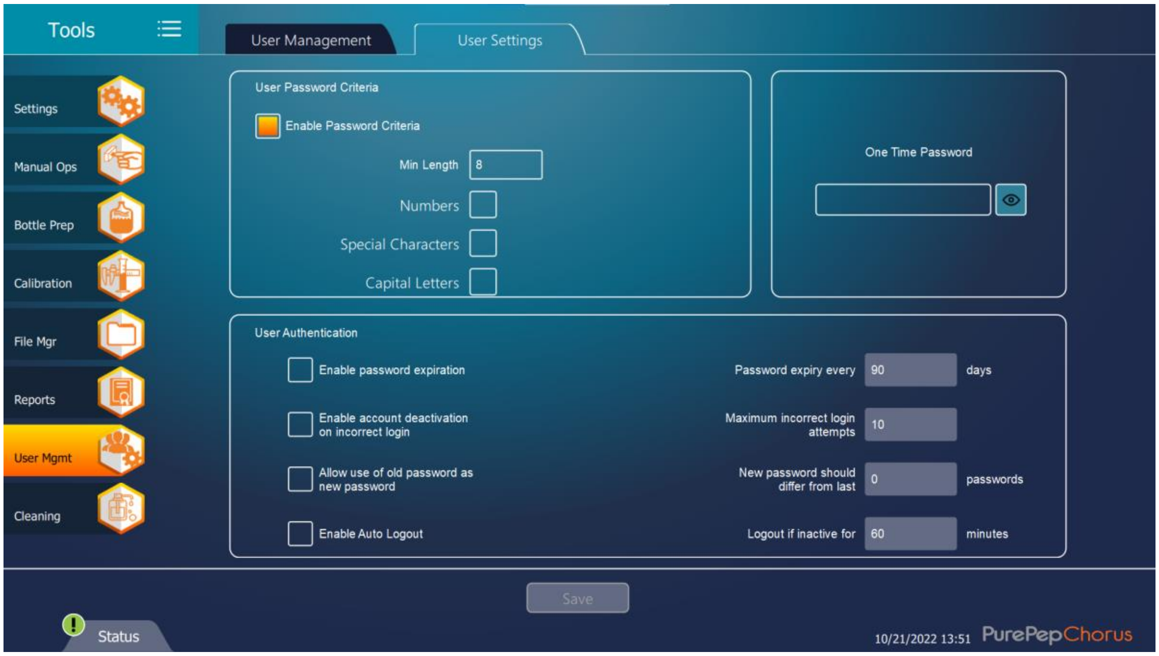
An administrator may inactivate or unlock individual user profiles using the "**Inactive**" and "**Unlock**" buttons.



User Login: admin
Active
User Name: Admin
Access Profile: Admin
Email:
Password: •••••
Confirm Password: •••••
10/21/2022 13:50

User Mgmt – User Settings tab

This screen contains settings pertaining to user passwords and authentication. This includes requirements for password length, special characters, frequency of changes, and maximum login attempts. The **Enable Auto Logout** and **Logout if inactive for** setting determines the number of minutes of inactivity in the software after which a user would be logged out and required to log in with a password again. Any active syntheses running on the instrument will not be affected by this setting.



User Password Criteria

☑ Enable Password Criteria

Min Length 8

Numbers ☐

Special Characters ☐

Capital Letters ☐

One Time Password

User Authentication

☐ Enable password expiration — Password expiry every 90 days

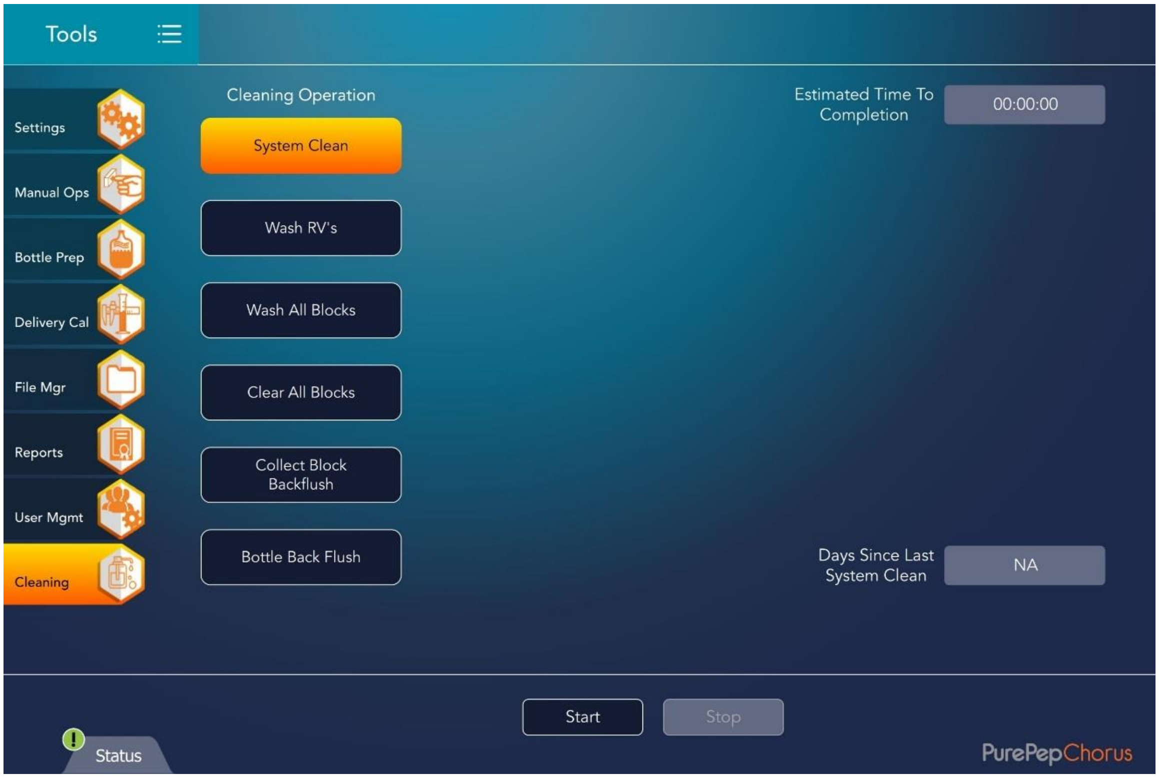
☐ Enable account deactivation on incorrect login — Maximum incorrect login attempts 10

☐ Allow use of old password as new password — New password should differ from last 0 passwords

☐ Enable Auto Logout — Logout if inactive for 60 minutes

5.3.8. Cleaning

The **Cleaning** menu includes all options for cleaning operations on the system. A full **System Clean** includes all of the other individual cleaning operations listed. These are **Wash RVs**, **Wash All Blocks**, **Clear All Blocks**, **Collect Block Backflush**, and **Bottle Backflush**.



Cleaning – System Clean

The **System Clean** flushes the entire fluid system with solvents. A system clean performs Wash RVs, Wash All Blocks, Clear All Blocks, and Bottle Backflush operations using Solvent 1, and Collect Block Backflush using Solvent 2.

1. To perform a **System Clean**, place empty amino acid and solvent/reagent bottles in all positions. Place empty RVs and collection vials in position.
2. Place DMF in the Solvent 1 bottle and DCM or methanol in the Solvent 2 bottle. Alternatively, Premium Wash Solvent may be used for one or both positions. Please contact Gyros Protein Technologies to order Premium Wash Solvent.
3. Select **System Clean** and press "**Start**".

After the cleaning operation is complete, remove the bottles and collection vials, dispose of the rinse solution, and replace the bottles and collection vials with clean ones for the next synthesis.

> If Premium Wash Solvent is used for the System Clean, it is recommended to perform a second System Clean with DMF or NMP in the Solvent 1 bottle and DCM in the Solvent 2 bottle in order to remove the Premium Wash Solvent from the lines. Any remaining Premium Wash Solvent in the system can adversely affect chemistry.

Cleaning – Wash RVs

The **Wash RVs** rinses the reaction vessels and lines by delivering Solvent 1, generally DMF, from the top of reaction vessels through the vessels to waste. After draining the solvent, nitrogen is delivered from the top to dry the RVs.

1. To perform a **Wash RVs**, first pressurize and prime Solvent 1 using the **Bottle Prep** screen.
2. Select **Wash RVs** and press “**Start**”**.**
   Click the “**Pause**” button to pause the operation. Click “**Resume**” to resume a paused operation. Click the “**Stop**” button to cancel the operation.

After the cleaning operation is complete, remove the reaction vessels and replace with clean reaction vessels.

Cleaning – Wash All Blocks

The **Wash All Blocks** rinses each block with Solvent 1, usually DMF, then flushes each block with nitrogen to remove residual fluid. It does so without venting the bottles to prevent contamination of the amino acids, solvents, and reagents. **Wash All Blocks** is performed using as part of a **System Clean**, but it can also be performed alone.

1. To perform a **Wash All Blocks** operation, pressurize and prime Solvent 1 using the **Bottle Prep** screen.
2. Select **Wash All Blocks** and press “**Start**”.
   Click the “**Pause**” button to pause the operation. Click “**Resume**” to resume a paused operation. Click the “**Stop**” button to cancel the operation .

Cleaning – Clear All Blocks

The **Clear All Blocks** function flushes each block with nitrogen gas to remove residual fluid without venting the bottles and contaminating the amino acids, solvents, and reagents. **Clear All Blocks** is performed as part of a **System Clean**, but it can also be performed alone as follows:

1. Select **Clear All Blocks** and press “**Start**”.
   Click the “**Pause**” button to pause the operation. Click “**Resume**” to resume a paused operation. Click the “**Stop**” button to cancel the operation.

Cleaning – Collect Block Back Flush

The **Collect Block Back Flush** cleaning procedure flushes Solvent 2, DCM, through the cleave system and collection lines into the collection vials to remove TFA solution and any residual peptide from the system. **Collect Block Back Flush** should be performed after every collection to prevent contamination of the next synthesis product. **Collect Block Back Flush** is performed as part of a **System Clean**, but it can also be performed alone.

To perform a **Collect Back Flush**:

1. Install empty collection vials to receive the rinse solvent.

2. Pressurize and prime Solvent 2, DCM, using the **Bottle Prep** screen.
3. Select **Collect Back Flush** and press “**Start**”.
   Click the “**Pause**” button to pause the operation. Click “**Resume**” to resume a paused operation. Click the “**Stop**” button to cancel the operation.

After the cleaning operation is complete, remove the collection vials, and discard the rinse solution. Place clean, empty collection vials on the instrument.

Cleaning – Bottle Back Flush

The **Bottle Back Flush** flushes selected amino acid and/or solvent/reagent bottle lines with Solvent 1, DMF, or nitrogen. **Bottle Back Flush** should be performed when changing reagents or to clear a clog caused by amino acid precipitate in the line. **Bottle Back Flush** is performed as part of a **System Clean**, but it can also be performed alone as follows:

1. Replace amino acid and/or solvent/reagent bottles to be flushed with empty bottles.
2. Select **Bottle Back Flush** and press “**Start**”.
3. If a *Solvent Back Flush* will be performed, Solvent 1, DMF, must be pressurized and primed first. A *Nitrogen Back Flush* operation does not require Solvent 1, DMF, to be pressurized and primed.
4. Check the box(es) next to the bottle(s) that will be flushed.
5. To back flush bottle(s) with Solvent 1, DMF, click on the “**Solv. BackFlush**” button. To back flush bottle(s) with nitrogen, click on the “**N2 BackFlush**” button.

After the cleaning operation is complete, empty the flushed bottles of any rinse fluid, and replace with clean bottles.

When changing reagents, it is suggested to perform a *Nitrogen Back Flush* to flush reagent back into the bottle. Replace the bottle with an empty bottle and perform *Solvent Back Flush* to flush residual reagent from the line. Wipe excess fluid off the bottle tubing and load the new reagent bottle. When trying to loosen a clog, remove the bottle filter and use a *Solvent Back Flush*. Different flushing solvents may be used by placing them in the Solvent 1 bottle. However, under no circumstances should TFA be used in the amino acid manifold system—destruction of the bottle seals will occur!

5.4. Services

This screen is accessible by factory, service, and admin users. Here, the diagnostics screen, the complete valve map, and log files can be accessed. This section will be useful for troubleshooting aided by our Service and Technical Support teams. If you have any questions or comments contact us at:

Tel: 520-629-9626 | 800-477-6834 | Email: peptides@gyrosproteintech.com

5.5. About

The product license, software version number, and other relevant copyright information is referenced in the About screen.

5.6. Logout and Instrument Shutdown

Selecting **Logout** will logoff the current user and asks for user login and password to log in again. From the login screen the user can exit the software.

For proper instrument shutdown select **Shutdown** from the main menu. This will logout of the software and puts the instrument in a shutdown ready state when the instrument can be powered off. To restart the instrument, power on the instrument followed by login into the software.

Appendix A: Reagents For Peptide Synthesis

A.1 PurePep Chorus Pre-Packed N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Amino Acid	Quantity
SMP-05-A SMP-10-A SMP-20-A	Fmoc-L-Ala-OH	5 mmol 10 mmol 20 mmol
SMP-05-RBF SMP-10-RBF SMP-20-RBF	Fmoc-L-Arg(Pbf)-OH	5 mmol 10 mmol 20 mmol
SMP-05-NT SMP-10-NT SMP-20-NT	Fmoc-L-Asn(Trt)-OH	5 mmol 10 mmol 20 mmol
SMP-05-DB SMP-10-DB SMP-20-DB	Fmoc-L-Asp(OtBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-CT SMP-10-CT SMP-20-CT	Fmoc-L-Cys(Trt)-OH	5 mmol 10 mmol 20 mmol
SMP-05-EB SMP-10-EB SMP-20-EB	Fmoc-L-Glu(OtBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-QT SMP-10-QT SMP-20-QT	Fmoc-L-Gln(Trt)-OH	5 mmol 10 mmol 20 mmol
SMP-05-G SMP-10-G SMP-20-G	Fmoc-Gly-OH	5 mmol 10 mmol 20 mmol
SMP-05-HT SMP-10-HT	Fmoc-L-His(Trt)-OH	5 mmol 10 mmol

Catalog No.	Amino Acid	Quantity
SMP-20-HT		20 mmol
SMP-05-I SMP-10-I SMP-20-I	Fmoc-L-Ile-OH	5 mmol 10 mmol 20 mmol
SMP-05-L SMP-10-L SMP-20-L	Fmoc-L-Leu-OH	5 mmol 10 mmol 20 mmol
SMP-05-KBC SMP-10-KBC SMP-20-KBC	Fmoc-L-Lys(Boc)-OH	5 mmol 10 mmol 20 mmol
SMP-05-M SMP-10-M SMP-20-M	Fmoc-L-Met-OH	5 mmol 10 mmol 20 mmol

Catalog No.	Amino Acid	Quantity
SMP-05-F SMP-10-F SMP-20-F	Fmoc-L-Phe-OH	5 mmol 10 mmol 20 mmol
SMP-05-P SMP-10-P SMP-20-P	Fmoc-L-Pro-OH	5 mmol 10 mmol 20 mmol
SMP-05-SB SMP-10-SB SMP-20-SB	Fmoc-L-Ser(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-TB SMP-10-TB SMP-20-TB	Fmoc-L-Thr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-WBC SMP-10-WBC SMP-20-WBC	Fmoc-L-Trp(Boc)-OH	5 mmol 10 mmol 20 mmol
SMP-05-YB SMP-10-YB SMP-20-YB	Fmoc-L-Tyr(tBu)-OH	5 mmol 10 mmol 20 mmol
SMP-05-V SMP-10-V SMP-20-V	Fmoc-L-Val-OH	5 mmol 10 mmol 20 mmol

A.2 Bulk N-Fmoc-Protected Amino Acids, Preweighed

Catalog No.	Description	Quantity
FLA-5-A FLA-25-A FLA-100-A FLA-1KG-A	Fmoc-L-Ala-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-RBF FLA-25-RBF FLA-100-RBF FLA-1KG-RBF	Fmoc-L-Arg(Pbf)-OH	5 g 25 g 100 g 1 kg
FLA-5-NT FLA-25-NT FLA-100-NT FLA-1KG-NT	Fmoc-L-Asn(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-DB FLA-25-DB FLA-100-DB FLA-1KG-DB	Fmoc-L-Asp(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-CT FLA-25-CT FLA-100-CT FLA-1KG-CT	Fmoc-L-Cys(Trt)-OH	5 g 25 g 100 g 1 kg
Catalog No.	**Description**	**Quantity**
FLA-5-EB FLA-25-EB FLA-100-EB FLA-1KG-EB	Fmoc-L-Glu(OtBu)-OH	5 g 25 g 100 g 1 kg
FLA-5-QT FLA-25-QT FLA-100-QT FLA-1KG-QT	Fmoc-L-Gln(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-G FLA-25-G FLA-100-G FLA-1KG-G	Fmoc-Gly-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-HT FLA-25-HT FLA-100-HT FLA-1KG-HT	Fmoc-L-His(Trt)-OH	5 g 25 g 100 g 1 kg
FLA-5-I FLA-25-I FLA-100-I FLA-1KG-I	Fmoc-L-Ile-OH	5 g 25 g 100 g 1 kg
FLA-5-L FLA-25-L FLA-100-L FLA-1KG-L	Fmoc-L-Leu-OH	5 g 25 g 100 g 1 kg
FLA-5-KBC FLA-25-KBC FLA-100-KBC FLA-1KG-KBC	Fmoc-L-Lys(Boc)-OH	5 g 25 g 100 g 1 kg
FLA-5-M FLA-25-M FLA-100-M FLA-1KG-M	Fmoc-L-Met-OH	5 g 25 g 100 g 1 kg
FLA-5-F FLA-25-F FLA-100-F FLA-1KG-F	Fmoc-L-Phe-OH	5 g 25 g 100 g 1 kg
FLA-5-P FLA-25-P FLA-100-P FLA-1KG-P	Fmoc-L-Pro-OH	5 g 25 g 100 g 1 kg

Catalog No.	Description	Quantity
FLA-5-SB	Fmoc-L-Ser(tBu)-OH	5 g
FLA-25-SB		25 g
FLA-100-SB		100 g
FLA-1KG-SB		1 kg
FLA-5-TB	Fmoc-L-Thr(tBu)-OH	5 g
FLA-25-TB		25 g
FLA-100-TB		100 g
FLA-1KG-TB		1 kg
FLA-5-WBC	Fmoc-L-Trp(Boc)-OH	5 g
FLA-25-WBC		25 g
FLA-100-WBC		100 g
FLA-1KG-WBC		1 kg

Catalog No.	Description	Quantity
FLA-5-YB	Fmoc-L-Tyr(tBu)-OH	5 g
FLA-25-YB		25 g
FLA-100-YB		100 g
FLA-1KG-YB		1 kg
FLA-5-V	Fmoc-L-Val-OH	5 g
FLA-25-V		25 g
FLA-100-V		100 g
FLA-1KG-V		1 kg

A.3 Reagents & Kits

Gyros Protein Technologies offers a wide selection of high-quality coupling reagents, resins, and solvents, as well as pseudoprolines and other specialty reagents that can be found at www.gyrosproteintechnologies.com.

Catalog No.	Start-Up Kits	Quantity
CHR-STARTKIT	Fmoc Amino Acid Start-up Kit for the PurePep Chorus. Contains: 30 x 10 mL disposable RVs, 6 x 10 mL coated glass RVs, 6 x 25 mL coated glass RVs, 6 x 40 mL coated glass RVs, 0.9 L of 20% Piperidine/DMF, 0.9 L of 0.4M NMM, 0.1 mmol scale Rink amide resin, 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles for running test peptides.	1 ea.
CHR-STARTKIT-I	Fmoc Amino Acid Start-up Kit for the PurePep Chorus. Contains: 30 x 10 mL disposable RVs, 6 x 10 mL coated glass RVs, 6 x 25 mL coated glass RVs, 6 x 40 mL coated glass RVs, 0.1 mmol scale Rink amide resin, 0.1 mmol scale Fmoc-Gly-Wang resin, twenty 5 mmol and twenty 20 mmol prepacked AA bottles (one of each amino acid), 100 g HCTU. Assorted 5 mmol prepacked AA bottles for running test peptides.	1 ea.

Catalog No.	Cleavage Kits	Quantity
CLEAVEKIT-U	**GPT Universal Cleavage Kit**. Suitable for cleaving peptides containing all 20 standard amino acids. Contains 95 mL TFA, 2 mL water, 2 mL anisole, 1 mL EDT. Makes 100 mL	1 ea.
CLEAVEKIT-R	**Reagent K Cleavage Kit.** Suitable for cleaving peptides containing all 20 standard amino acids. Contains 82.5 mL TFA, 5 mL thioanisole, 5 mL water, 5 g phenol, and 2.5 mL EDT. Makes 100 mL	1 ea.

Appendix B: Reagent Shelf Life & Handling

CAUTION This instrument contains solvents and chemicals that should be handled carefully. Many are easily absorbed through the skin and can cause adverse health effects. Wear safety glasses, protective clothing and rubber gloves at all times. Follow MSDS handling guidelines provided with the individual reagents. Respirators and adsorbent should be available in the event of a spill.

A.4 Reagent Shelf Life

Proper handling and storage of peptide synthesis reagents is important for the successful performance of your instrument. Please review the table on the following pages to be certain that your reagents are properly stored. Be sure to rotate your stock of reagents using a “first in, first out” method so that their shelf-life is not exceeded before use.

Reagent	Temperature	Shelf Life
Amino Acids [Solid, except Fmoc-Trp(Boc)-OH]	20-25°C	Stable
Fmoc-Trp(Boc)-OH (Solid)	-20°C	Stable
Amino Acids [in DMF solution, except Fmoc-Cys(Trt)-OH]	20-25°C	7-14 Days
Fmoc-Cys(Trt)-OH (in DMF solution)	20-25°C	1 Day
N,N-Dimethylformamide	20-25°C	Stable
Methylene Chloride	20-25°C	Stable
20% Piperidine/DMF (v/v)	20-25°C	Stable
Acetic Anhydride	20-25°C	Stable
0.2M HCTU in DMF	20-25°C	5-7 Days
0.4M 4-Methylmorpholine in DMF	20-25°C	5-7 Days
0.1M HBTU/0.4M 4-Methylmorpholine in DMF	20-25°C	5-7 Days
Trifluoroacetic Acid	20-25°C	Stable
TFA Cocktail	20-25°C	1 Day

Appendix C: Accessories

Catalog No.	Accessories	Quantity
PPS-R10-030 PPS-R10-090 PPS-R10-180	Reaction Vessel, 10 mL PP	Pkg of 30 Pkg. of 90 Pkf of 180
PPS-R45-030 PPS-R45-090 PPS-R45-180	Reaction Vessel, 45 mL PP	Pkg. of 30 Pkg. of 90 Pkg. of 180
PPX-FGRV10-6	Reaction Vessel, 10 mL Coated Glass (required for heat)	Pkg. of 6
PPX-FGRV25-6	Reaction Vessel, 25 mL Coated Glass (required for heat)	Pkg. of 6
PPX-FGRV40-6	Reaction Vessel, 40 mL Coated Glass (required for heat)	Pkg. of 6
AAR-SSI AAR-SSX	Bottle, 10 mL Single-Shot AA	1 ea. Pkg. of 10
SMP-VX-20 SMP-VX-100	Bottle, 120 mL AA	Pkg. of 20 Pkg. of 100
AAR-400-I AAR-400-X	Bottle, 400 mL AA	1 ea. Pkg. of 10
CLV-050-030 CLV-050-090 CLV-050-180	Vial, 50 mL Collection	Pkg. of 30 Pkg. of 90 Pkg. of 180

Appendix D: Induction Heating System

Heat has been used to aid in the syntheses of difficult peptides for the last 30 years. Believed to be first used in 1984 by Janda and colleagues, heating methods range from a simple oil bath, to specially designed heated reaction blocks, to infrared and induction heating today.

Advantages of the Induction Heating System

The induction heating system from GPT offers extremely fast time to temperature, as well as accurate temperature sensing without overshooting or overcorrecting. Vortex mixing is used to ensure even temperature profiles. With independent control and a compact design, the use of induction heating allows parallel, independent heating of up to six reaction vessels.

Induction Heating Parameters

The recommended parameters to use for induction heating on ***PurePep Chorus***.

Depending on the RV size and volumes used, it is important that the shaking speed is set to an appropriate value within the RPM range described in the table below. The recommended volume range is the apparent volume with the resin.

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

NOTE Make sure that when using induction heating, the RV has a metal band.

Appendix E: Intellisynth UV Monitoring

With the real-time UV monitoring system, it is possible to monitor the extent of the deprotection reaction (Fmoc removal), and use that data to control deprotection times and repetitions.

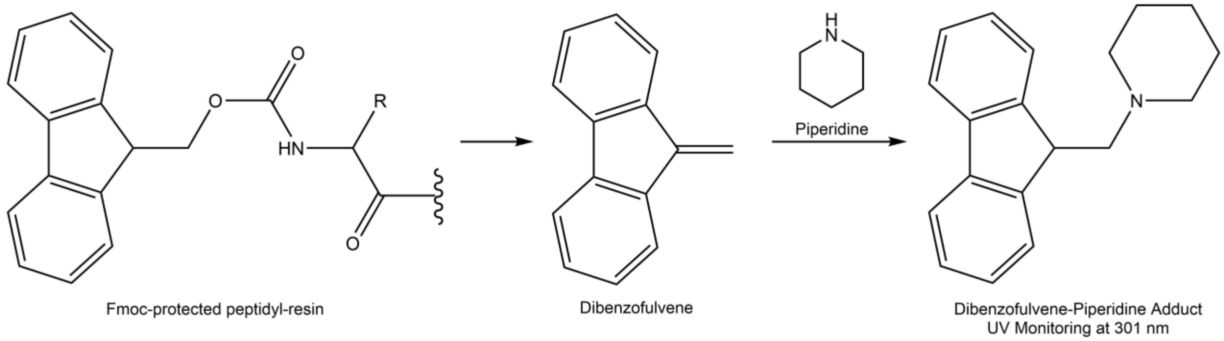
There are three UV-Monitoring modes on the PurePep® Chorus.

1. **Basic** – Records UV data during step but does not adjust time or repetitions. The reaction uses the time set by the user.
2. **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 (change below a set value). If this criterion is not met, the time of reaction is extended automatically.
3. **Extend + Reps** – Uses real-time UV data to control the time and number of repetitions of reaction. The reaction for a particular repetition considered to be complete when consecutive changes of transmittance readings approach zero (change below a set value), and the overall transmittance exceeds a set threshold value. If these criteria are not met, the time and/or the number or repetitions extend automatically.

How UV Monitoring Works

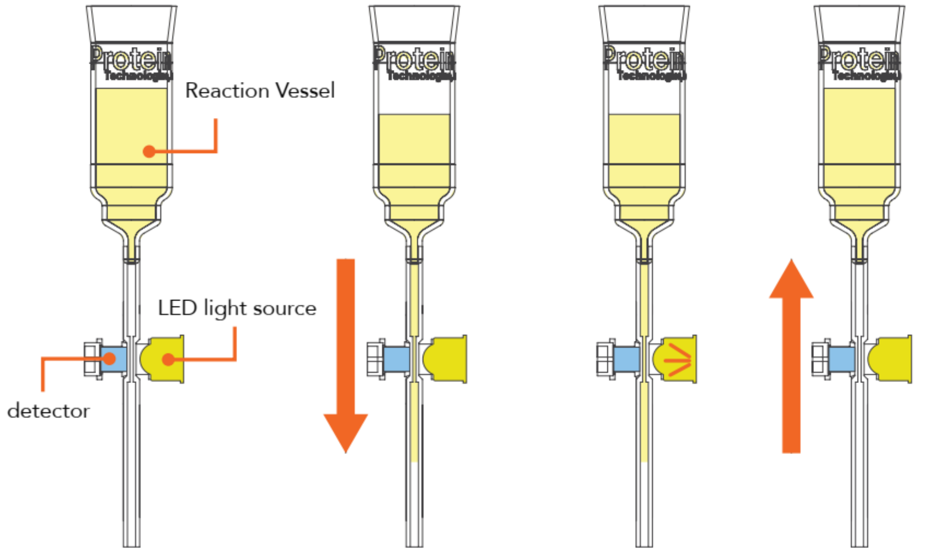
Chemistry

During the deprotection reaction, piperidine removes the Fmoc group and forms a piperidine-dibenzofulvene adduct with the byproduct (See below.



This by-product will affect the transmittance of the deprotecting solution: as the reaction moves forward, the transmittance will decrease. The IntelliSynth UV-Monitoring System monitors the transmittance of the deprotecting solution at 301 nm during the deprotection reaction.

The IntelliSynth UV Monitoring System



1. Starting point

2. Liquid is pushed down to the measuring cell

3. Light source is on and measurement is taken

4. Liquid is pushed back to the reaction vessel

The IntelliSynth UV Monitoring System consists of a light source and detector encased in a 1 3/8” x 1 3/8” x 1 ¾” (3.5 cm x 3.5 cm x 4.5 cm) housing which measures the absorbance of the fluid in the tubing directly below the reaction vessel. During a mix, part of the fluid is pushed down into the section of tubing exposed to the light source and detector and a measurement is taken. The fluid is then pushed back up into the reaction vessel to resume mixing (right diagram). This process occurs once every 10 seconds during a monitored mix.

UV Graphs Screen

The UV Graphs screen displays the transmittance graphs for individual deprotection reactions as well as overall syntheses. The **UV Graphs** screen 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. There are two graph types that can be displayed on the UV Graphs screen: *UV Individual* and *UV Summary*. These can be selected at the top left of the graph area.

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. In this example, RVs 1, 2, 3, and 6 are ON and RVs 4 and 5 are 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 full data set does not fit on screen.
6. **Synthesis time** – Displays the date and time of the selected reading/repetition.
7. **RV** – Displays UV monitoring data for 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 or out.
12. "**Export options**" button – Exports the absorbance and/or transmittance data as an external CSV file.

RV1: 60
RV2: 67
RV3: 12
RV6: 12

UV Thresholds
RV1: 83
RV2: 83
RV3: 83
RV6: 83

Synthesis Time 22/Jun/2023-18:03:18
RV 1
Cycle 1 - Thr
Step 1 - Bottom Delivery
Rep 2

UV Graph data updates after a UV Monitored step is complete, giving access to view the Synthesis Graph and Individual Graph data of that step. These semi-real time graphs provide information about difficult deprotection cycles can be viewed during the course of the synthesis or reviewed after the synthesis.

UV Summary Graph

A 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, where the darker portion represents the minimum transmittance measured during that repetition, and the lighter portion represents the maximum absorbance measured during that repetition.

0011_PolyAla-basic

UV Thresholds
RV1: 80

Synthesis Time 23/Sep/2022-18:15:24

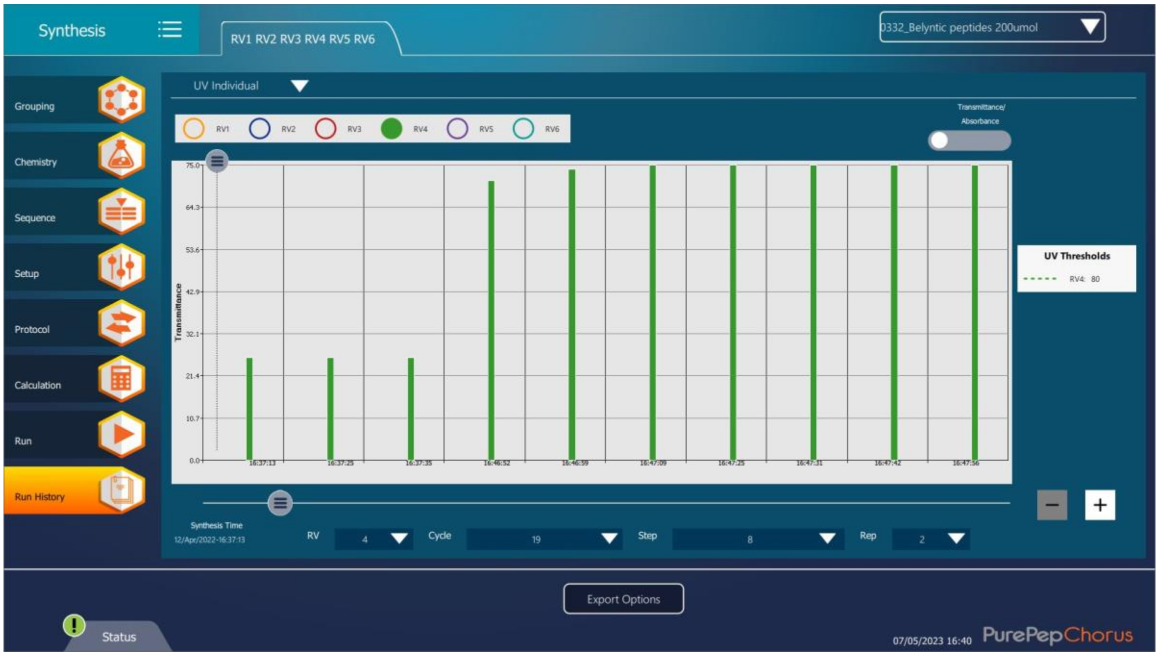
RV 1 Cycle 3 - Ala Step 1 - Bottom Delivery Rep 1

In this experiment, three deprotections (2 min at room temperature) were carried out for each amino acid. It is possible to observe that the transmittance for the first deprotection of each amino acid is low (below the defined threshold), while the transmittance of the second and third repetitions are above the defined threshold. This means that the third deprotection is obsolete, as deprotection is complete after the second repetition.

UV Individual Graph

An Individual Read Graph displays UV data for Individual Repeats of UV Monitored steps. A peak is recorded once every 10 seconds when a UV reading is taken.

In an Individual Read graph, each peak represents an individual transmittance reading. Individual readings are shown for each UV Monitored step.



Synthesis Time 12/Apr/2022-16:37:13

RV 4

Cycle 19

Step 8

Rep 2

Basic Monitoring Mode

Basic monitoring takes absorbance readings every 10 seconds during the UV Monitored step, but it does not interfere with the synthesis. Two types of graphs can be plotted from this data: UV Summary Graph and UV Individual Graph.

Extend Mode

This mode is the same as the Basic Monitoring mode; however, if the readings have not reached a plateau by the end of a deprotection step, the software will extend the deprotection time up to a maximum of twice the originally programmed time. In more detail, if consecutive changes between a number of individual readings are above a set threshold, another individual reading will be added to that repetition. If the transmittance changes less than the threshold for a set number of readings, the repetition will end.

Extend+Reps Mode

This mode uses the Extend function, described above, i.e., will prolong the deprotection step until a plateau is reached. However, in this case, the software also takes into consideration the transmittance value in which the readings plateaued: if this value is below the defined threshold (defined in **Tools->Settings->UV Settings**), another repetition of the same step will be carried out until it reaches the threshold, up to a maximum of 9 repetitions.

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