Supplementary Information for:

Convergence of multiple synthetic paradigms in a universally programmable chemical synthesis machine

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1 General Experimental Remarks

Solvents and reagents were used as received from commercial suppliers unless otherwise stated.

NMR measurements were performed with Bruker Avance III HD 600 spectrometer operating at 600.1 and 150.9 MHz for ^{1}H and ^{13}C, respectively. Spectra were collected at 298 K, chemical shifts are reported in ppm and were calibrated for the (residual) NMR solvent signal (multiplicities are given as s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, with coupling constants reported in Hz). The spectra were processed with MestReNova 14.0.0.

HPLC-UV/Vis (MS) analysis was performed on a Thermo Dionex Ultimate 3000 equipped with a LPG-3400 RS pump, a WPS-3000TRS autosampler, a TCC-3000SD column compartment and a DAD-3000 diode array detector. 10 µL of each sample was injected on to an Agilent Porodhell 120 EC-C18 2.7 µm, 4.6 x 150 mm column, eluting at 1mL/min with mobile phase A being water + 0.1% formic acid and mobile phase B acetonitrile + 0.1% formic acid, detecting using UV (λ = 190, 214, 220 and 254 nm). The total run time was 26 minutes, with the LC method as follows - 0 min- 0% B, 4min – 10% B, 16 min – 70% B, 19 min – 100% B, 23 min – 0% B, 26 min – 0% B. Column compartment was set at 30 °C. The Thermo Dionex Ultimate 3000 HPLC was connected to a Bruker MaXis Impact quadrupole time-of-flight mass spectrometer with an electrospray source, operating exclusively in negative mode. The instrument was regularly calibrated using Agilent ESI-L Low Concentration Tuning Mix. Samples were introduced into the MS at a dry gas temperature of 200 °C. The ion polarity for all MS scans recorded was negative, with the voltage of the capillary tip set at 4500 V, end plate offset at − 500 V, nebuliser at 1.6 bar, dry gas at 8.0 l/min, funnel 1 RF at 400 Vpp, funnel 2 RF at 400 Vpp, isCID energy at 0 eV, hexapole RF at 100 Vpp, ion energy 5.0 eV, low mass at 50 m/z, collision energy at 5 eV, collision cell RF at 200 Vpp, transfer time at 63.5 µs, and the pre-pulse storage time at 1.0 µs. The mass range was set to 50 – 2000 m/z. Data was analysed using the Bruker DataAnalysis v4.1 software suite or MestReNova 14.0.0.

Preparative HPLC was performed on an Agilent Technologies 1260 Infinity system (consisting of two pump modules, an autosampler, a UV flow cell and a fraction collector) equipped with a Reprosil Gold 200 C18 250 x 40 mm column from Dr Maisch GmbH. The injection volume was 5.0 mL, the flowrate was 30.0 mL/min and the UV (at 214 nm) threshold was set to 25 mAU. The gradient started at 20 % A (5 % acetonitrile in 95 % water plus 0.1 % TFA) in B (5 % water in 95 % acetonitrile plus 0.1 % TFA) and ran to 70 % A in B over 25 min.

3D-Printing was performed on a Connex 500 printer from Stratasys using the Fullcure 720 translucent resin for the major body of the printed parts and VeroBlack as the coloured resin in cases where two-coloured prints were required. Once the print was complete, the parts were cleaned first by scrapping away the bulk of the support material manually, followed by thoroughly washing them in a water jet cleaning station (Quill Vogue Polyjet). Finally, the parts were placed in a 0.1 M aq NaOH bath for 30 min and then cleaned again in the water jet cleaning station. Particular attention was paid to small holes for screws and magnets to ensure that no residual support material was present.

Laser Cutting was performed on a Monster1060 CO2 Laser system (ML1060 130 W) from Radecal with the RDWorksV8 software. The applied parameter sets are summarized in the Table 1 below.

Table 1 Settings for the laser cutter.

Material	6 mm acrylic	4 mm acrylic
Laser power	70 % – 85 %	50 % – 65 %
Speed	10 mm / s	17 mm / s
Air	On	On
Laser through mode	Enabled	Enabled
Air pressure	0.3 MPa	0.3 MPa
Flow rate	33 L/min	33 L/min

Soldering was performed with a Tenma Digital Soldering Station (60 W) set to 270 °C. The used solder wire was Loctite (60EN alloy, 0.7 mm diameter, 2C core, X39 flux) with extra Amtech NC-559-TF flux. The electronic components of the Chemputer are under constant development aiming to to avoid most of the soldering. The latest version of design files and schematics for all electronic components is available on request.

Solid Phase Peptide Synthesis was performed on a Biotage Initiator+ Alstra system (part number: 356017) to obtain a benchmark against which the performance of the Chemputer can be measured.

2 Abstraction and Programming Language Ontology of the Chemputer

As already described in a previous publication[1], an arbitrary chemical synthesis can be carried out in steps where each step represents one of the 4 key sub-steps: 1) reaction; 2) workup; 3) isolation; 4) purification. Each of the 4 sub-steps cannot be generalized further except by breaking down into smaller steps. Each chemical synthesis has, indeed, a particular set of basic instructions or unit operations recurring in each of them:

1. move reagents/solvents from a source to a destination
2. stir the mixture
3. heat/cool
4. separate (liquid – liquid extraction)
5. dry-out (desiccate)
6. deoxygenate
7. evaporate
8. filter
9. chromatography

These above listed unit operations can be executed by appropriate hardware abstractions or chemical classes which have a capability to provide that type of action (i.e: a stirring hotplate can stir and heat) (Figure 1).

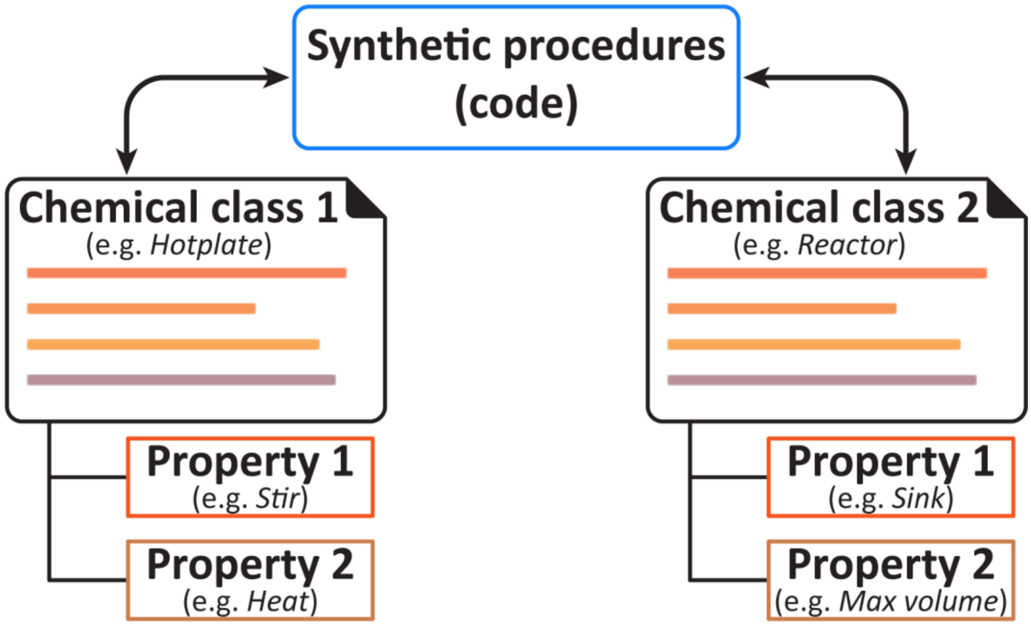


Figure 1. Semantic Ontology representing an example of synergic interactions of connected classes. Each class comprises properties which define the capabilities of the class.

The set of chemical classes needed to perform a specific chemical synthesis may vary substantially because of specific synthesis demands, as well as their arrangement, thus a universal way to represent the map of the hardware setup is necessary as we will see in the next chapter.

2.1 Chemical Ontology

The framework used in the current study is a derivation of the previously developed language (ChASM)[1] directly implemented in Python language. The advantages are all those that a well-established language like Python can offer: a vast selection of built in functions and routines along with extensive standard library to yield concise and readable synthetic procedures (Figure 2).

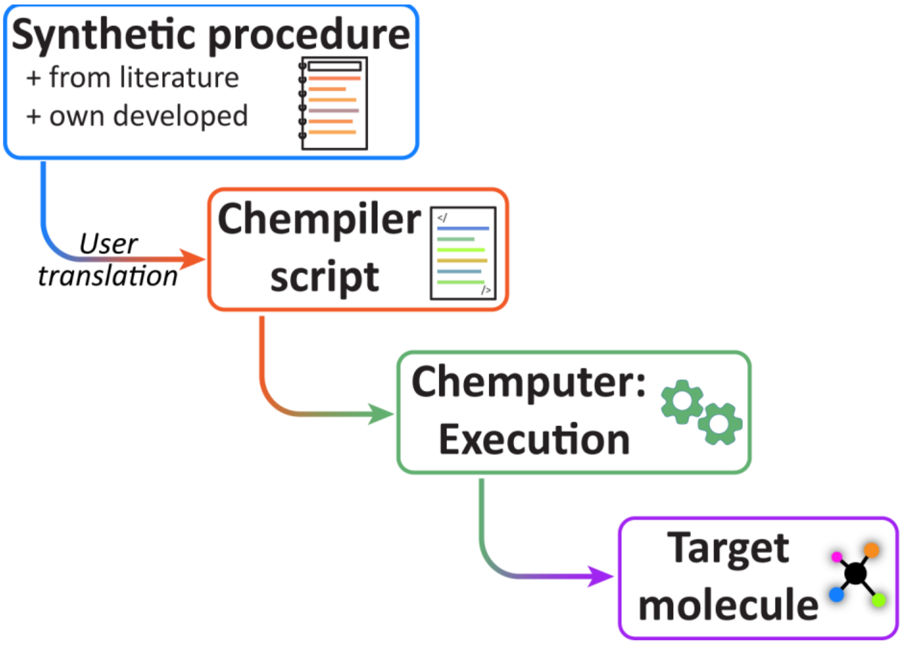


Figure 2. Flow diagram demonstrating the process by which the workflow of carrying out a published synthesis and producing a compound can be automated.

Unlike previous versions of chemical synthesis scripting language (i.e: ChASM) the Chempiler can execute mathematical operations taking into account runtime variables, therefore giving the user the capability to write a more flexible execution of the code.

3 The Chemputer Software Architecture

3.1 Chempiler Software Suite

The Chempiler software suite is consists of three Python 3 libraries which can be installed using PIP. The current version includes the Chempiler core (v2.0.3), the SerialLabware (v1.1) and the ChemputerAPI (v2.0). It is written entirely in Python 3.6. The full source code and documentation is available as supplementary files in Software\Chemputer_Python_Modules. The Chempiler code rely on a file describing the topology of the physical machine. To make the topology creation and description easier, a WebApp called Chemputer Graph has been developed (Figure 3).

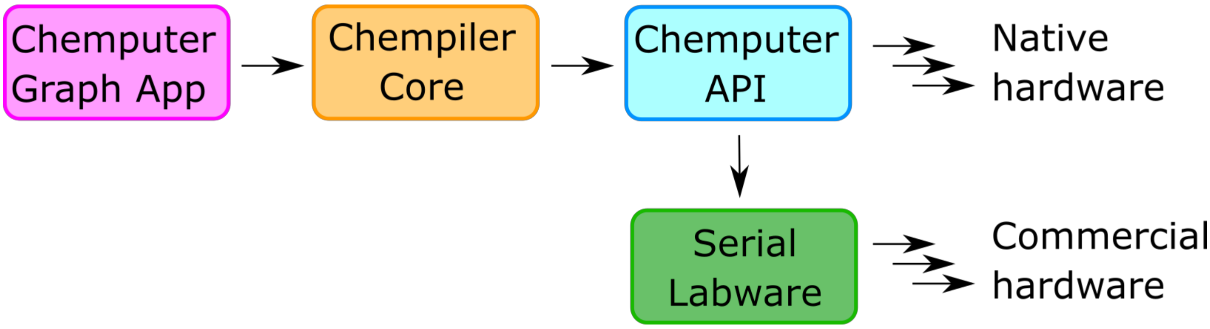


Figure 3. The Chempiler core maps script's commands to the required pieces of hardware using the topology file created with graph editor. Finally, the Chempiler invokes corresponding methods from ChemputerAPI/SerialLabware to execute the synthesis steps in order.

3.2 The Chempiler core code

All the syntheses' codes have been developed and debugged using the functions and tools available in the Chempiler software suite. An excerpt of Chempiler commands used to run the syntheses is presented below.

```
# volume in mL, speed equals transfer flow rate in mL/min,
# src_port and dest_port to be specified if the source or/and destination
node has more than one port.
move(source_node, destination_node, volume, speed, src_port, dest_port)
# stirring_speed value in rpm
stirrer.set_stir_rate(node_name, stirring_speed)
stirrer.stirr(node_name)
stirrer.stop_stir(node_name, stirring_speed)
# temperature value in ºC
stirrer.set_temp(node_name, temperature)
stirrer.heat(node_name)
# temperature value in ºC
chiller.set_temp(node_name, temperature)
chiller.start_chiller(node_name)
chiller.wait_for_temp(node_name)
chiller.stop_chiller(node_name)
vacuum.start_vacuum(node_name)
vacuum.stop_vacuum(node_name)
```

```python
# vacuum value in mbar
vacuum.set_vacuum_set_point(node_name, vacuum)
```

As described above, the Chempiler core needs a knowledge about the concrete platform topology to transform abstract step instructions into hardware-bound actions. A directed multigraph has been chosen as an appropriate ontological abstraction to yield a compromise between complexity and flexibility. A dedicated web-based application with graphical interface (Chemputer Graph) has been created to help easily set up initial graph creation. Each graph node represents a unique physical device with node properties matching device properties and capabilities. Each graph edge represents a physical liquid transfer path between devices which is necessary for the Chempiler core to calculate the optimal delivery paths for every synthetic step performed (automated liquid routing).

3.3 Error handling

While there is still room for improvements several layers of error handling are already in place:

1) A simulation mode catches any syntax or and conceptual errors (e.g. synthetic operations that are incompatible with the hardware topology).
2) Warnings are issued when vessels would be overfilled or emptied.
3) Runtime errors such as a lost connection with a device are handled. The system re-establishes lost connections automatically.
4) All pump and valve movements are tracked. The next operation is only initiated when the previous operation terminated successfully. For example, if a pump stalls due to a clogged tube, the synthesis is stopped.
5) The system considers all error messages issued by peripheral devices (e.g. an error raised due to an open lock on the stirrer) and stops the synthesis as needed.
6) The system makes use of all feedback available from the peripheral devices (e.g. waiting until the chiller reaches the target temperature, start rotation only when the rotavap lift is down).

3.4 Chemputer topology description

The Chemputer Graph application is available at the following website: https://croningroup.gitlab.io/chemputer/graphapp. After creation/editing, the platform topology graph is saved as a JSON file to a user's PC. This file contains the description of each graph node with all parameters as well as the connectivity information. Below is an example of a single graph node description in the JSON format:

```
{
  "id": "valve_column",
  "type": "valve",
  "x": 680,
  "y": 160,
  "internalId": 10,
  "label": "valve_column",
  "class": "ChemputerValve",
  "name": "valve_column",
  "address": "192.168.1.39"
},
```

The key-value pairs describing the node show the graph node's name, position in the graphical representation ("x", "y"), the relevant API class (to bind the node to a proper type of Python object), name of the node (this must be a unique name to distinguish the node from others) and the address used for communicating with the physical device (if the node is a remote controllable device).

The connectivity matrix is defined in separate top-level JSON elements. Below an example of the connectivity specification between the nodes with internal IDs 108 and 110 is given. The direction of the graph edge goes from the source to the target.

```
{
  "id": 121,
  "sourceInternal": 108,
  "targetInternal": 110,
  "source": "valve_carousel_21",
  "target": "purification_cartridge_a",
  "port": "(0, inlet)"
}
```

4 The Chemputer Hardware Modules

Since the original paper where the Chemputer concept was introduced[1] a significant effort was made to improve the hard- and software such that it becomes more robust, reliable and easier to use and build. Here the Chemputer hardware modules used for this publication are described with a special emphasis on new developments and improvements.

4.1 General System Specification and Operational Range

The single components of the platform have different specifications and operational limits. For convenience, the specifications of the main hardware components are provided in Table 2.

Table 2: Specifications and operational range of the system.

HARDWARE COMPONENT	SPECIFICATIONS & OPERATIONAL RANGE	SECTION
Switch	48 ports; 30 W per port	4.3
Boards	Max 57 V, 30 W	4.4
Hall sensor	Sensitivity: 12.5 mV/mT; Range: ±169 mT	4.5
Pump	Available syringe size: 5, 10, 25, 50 mL; Max flowrate: 200 mL/min; Min flowrate: 4 µL/min;	4.6
Valve	6 ports; Max pressure: 60 bar; Valve head materials: PEEK, PTFE	4.7
Programmable Manifold	Operating pressure: 14 mbar to 5 bar; Available gases: Air, Nitrogen, Argon	4.9.2
Reagent module	Volume range: 50 to 2500 mL; Max stirring speed: 800 rpm; Chiller temperature range: -40 ºC to +190 ºC;	4.10
Reactor	Operating stirring speed range: 50 to 1700 rpm; Chiller temperature range: -40 ºC to +190 ºC; Hotplate temperature range: RT to 340 ºC; Volume: 100, 250, 500, 1000 mL; Inline filtration;	4.11.1
SPPS reactor	Volume: 20 mL; Operating temperature range: RT to 75 ºC; Agitation modes: Overhead stirring or gas bubbling;	4.11.2
Jacketed filter	Stirring speed range: 10 to 800 rpm; Chiller temperature range: -40 ºC to +190 ºC; Max volume: 200 mL; Dead volume: ~10 mL	4.12
Separator	Max volume: 300 mL; Min volume: 50 mL; Stirring speed range: 10 to 800 rpm; Conductivity sensor range: ~10 MΩ to ~100 kΩ	4.13
Rotary evaporator	Temperature range: RT to 75 ºC	4.14
Cartridge carousel	6 positions; Cartridge size: 5 g to 10 g;	4.15
Column Chromatography	6 positions; Cartridge size: 5 g to 150 g; Maximum fractions: 5	4.16

The Chemputer system was designed to support a wide range of chemistries. Where possible, highest degrees of chemical compatibility were ensured. In general, limitations of the system result from missing modules (e.g. for photochemistry) or the hardware specifications described above. The current key restrictions of the hardware set-up are:

- Minimal temperature limit is -40 °C (given by the recirculating chiller used)
- Modules are manually charged with solids, solid additions are not automated
- Untested for reactive gases (chemical resistance has yet to be confirmed)

Work addressing these points is in progress.

4.2 Shelving

The shelving unit is a crucial bit of setup as it provides the frame to support not only the pumps/valves forming the liquid-handling backbone, but also other optional components. Initial design suggested the use of existing mounts in the fumehood back panel (provided by fumehood manufacturers to support lattice rack). However, this solution depends heavily on the each fumehood dimensions, which can be quite variable, thus it was not possible to provide a unified set of instructions/parts. Consequently, the new design utilizes a custom-made frame to support the shelves making it possible to unify shelving design to fit almost any fumehood.

The shelving consists of three major components: a frame, an upper shelf for the pumps and a lower shelf for the valves. Dimensions are given to fit the shelving unit in a 1000 x 1700 mm x 600 mm (H x L x D, internal) fume cupboard. For fume cupboards of different size, the frame dimensions might be adjusted accordingly, while the shelves' dimensions can be kept the same.

4.2.1 Frame

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0370	Drop In Tee Nuts M5 Thread size	Ooznest	VSLOT-H-DT-M5	32
CHEMP0371	M5 Low Profile Bolts	Ooznest	VSLOT-S-B-LP-M5-8	32
CHEMP0372	Universal L brackets single clear anodise	Ooznest	VSLOT-B-UL-S-C	12
CHEMP0379	V-Slot Linear Rail – 20x40mm black anodize, 770 mm	Ooznest	VSLOT-2040-O-B-700-799	6
CHEMP0380	V-Slot Linear Rail – 20x40mm black anodize, 540 mm	Ooznest	VSLOT-2040-O-B-500-599	4
CHEMP0381	V-Slot Linear Rail – 20x40mm black anodize, 1640 mm	Ooznest	VSLOT-2040-O-B-1600-1699	5
CHEMP0382	5 Hole T Joining Plate	Ooznest	VSLOT-M-P-T-5-C	2

Table 3 Bill of materials for the Chemputer shelving frame.

The frame was built from OpenBuilds® parts (see Table 3). The individual V-Slot rails were connected with L brackets and drop in tee nuts. To make a connection, the tee nut is simply placed in the V-Slot. When tightening the screw, the tee nut will turn inside the bottom slot and make a no-slip connection. More details on construction with OpenBuilds® parts can be found under https://openbuilds.com/. The fully assembled frame is shown in Figure 4.

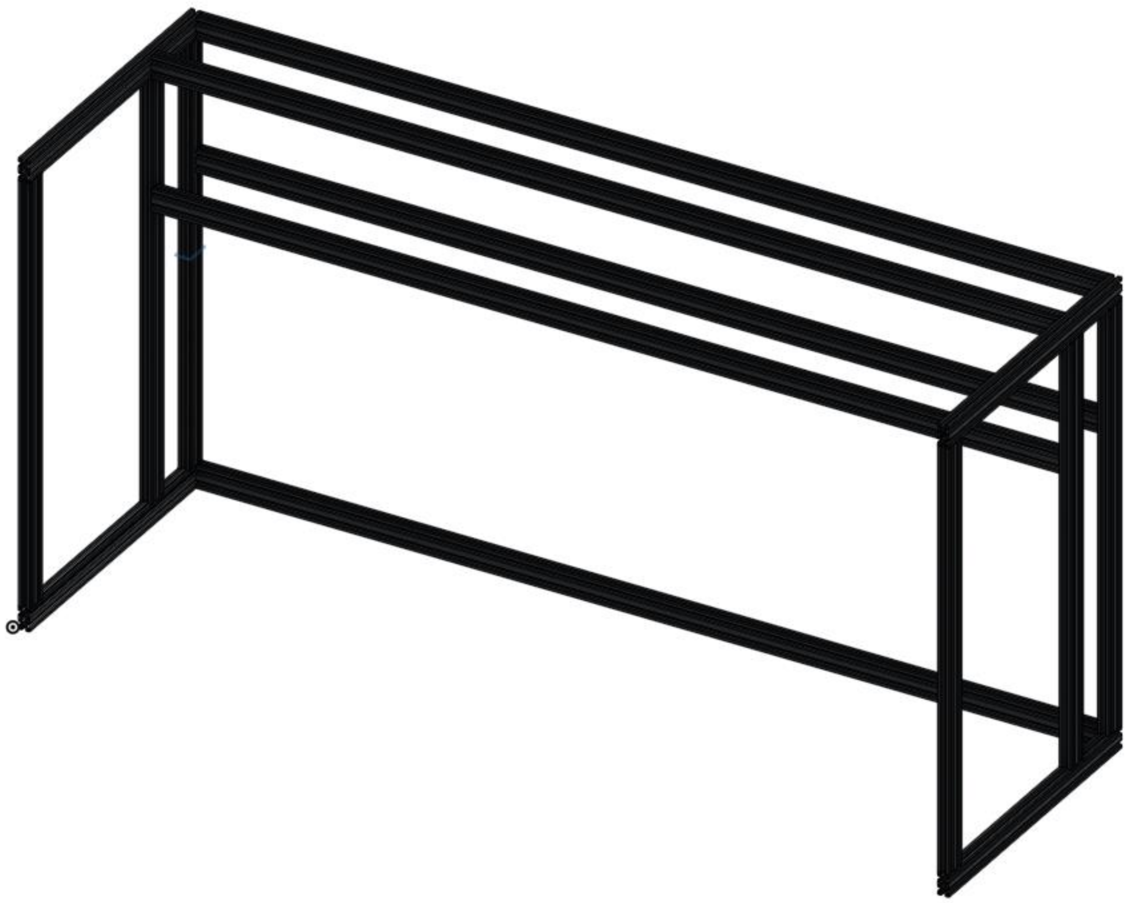


Figure 4 Fully assembled frame for the Chemputer shelving. The upper shelf is placed on the top two horizontal beams, the lower shelf on the lower two horizontal beams. The vertical position of the shelves can be adjusted.

4.2.2 Upper Shelf

Table 4 Bill of materials for the upper shelf assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0118	Pump shelf front panel, 6 mm	Polypropylene custom cut. The	From	1.00

	thickness	drawing is available on Various\Shelving\Pumps.	jetcut.com per quote	
CHEMP0119	Pump shelf top half, 12 mm thickness	Polypropylene custom cut. The drawing is available on Various\Shelving\Pumps.	From jetcut.com per quote	1.00
CHEMP0120	Pump shelf bottom half, 12 mm thickness	Polypropylene custom cut. The drawing is available on Various\Shelving\Pumps.	From jetcut.com per quote	1.00
CHEMP0121	M5 x 30mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M5-30-A4	12.00
CHEMP0122	M5 x 25mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M5-25-A4	4.00
CHEMP0013	M5 Form C Flat Washers (BS4320) - A4 Stainless Steel	AccuGroup	HLDW-M5-A4	16.00
CHEMP0124	M5 Form A Flat Washers (DIN 125) - A4 Stainless Steel	AccuGroup	HPW-M5-A4	16.00
CHEMP0125	M5 Cap Nuts (DIN 917) - A4 Stainless Steel	AccuGroup	HCN-M5-A4	12.00
CHEMP0127	M4 x 10mm Low Head Cap Screws (DIN 7984) - A4 Stainless Steel	AccuGroup	SSCL-M4-10-A4	8.00
CHEMP0128	M4 Form A Flat Washers (DIN 125) - A4 Stainless Steel	AccuGroup	HPW-M4-A4	8.00
CHEMP0129	Cable Clip Black Screw Nylon Cradle Clip	RS Components	424-778	8.00
CHEMP0130	Cable Clip Black PVC Retaining Clip	RS Components	425-989	8.00
CHEMP0126	M5 Hexagon Nuts (DIN 934) - A4 Stainless Steel	AccuGroup	HPN-M5-A4	4.00

1. Using an M5 30 mm cap head screw (CHEMP0121), form A (CHEMP0124) and form C (CHEMP0013) washers and an M5 cap nut (CHEMP0125), join the top and bottom shelving sections together as shown in Figure 5, securing the cap nut with an 8mm spanner and tightening the screw with a 4mm driver.

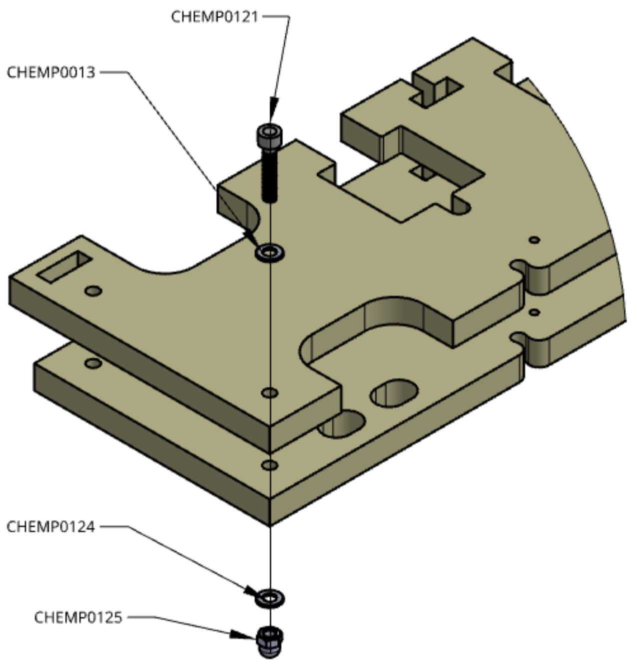


Figure 5: Joining top and bottom shelving sections.

2. Repeat this process until the shelving sections are secured together in all 12 positions (Figure 6).

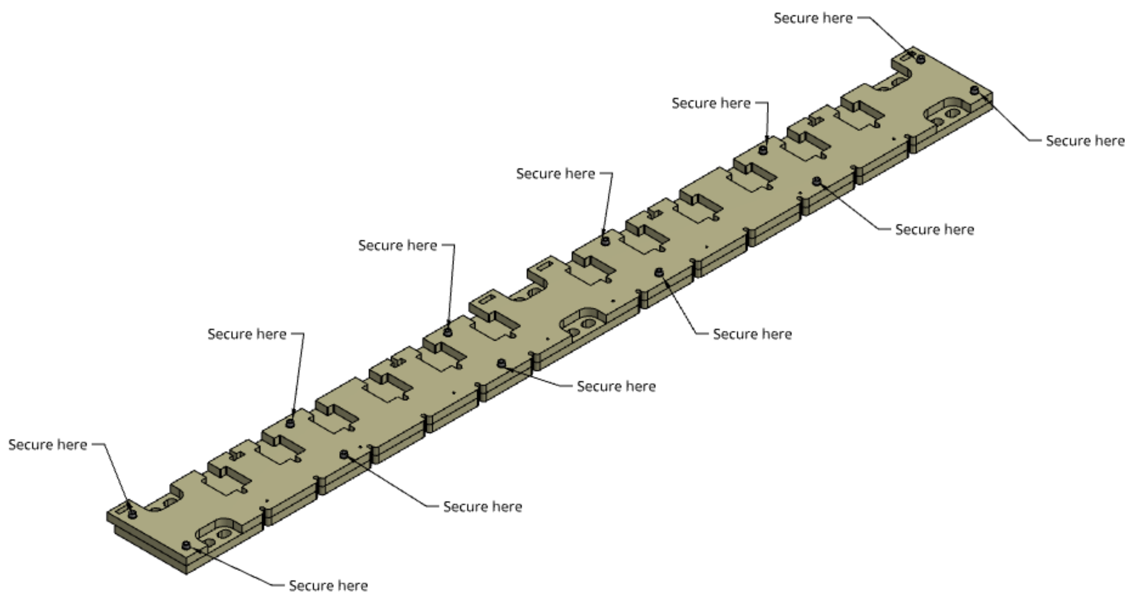


Figure 6: Securing shelving sections.

3. Insert the tabs of the front panel into the slots on the shelf (Figure 7).
4. Using an M4 tap, tap threads into all the screw holes in the bottom shelf plate for attachment of the cable clips. See Figure 9 for assistance in identifying the correct holes.

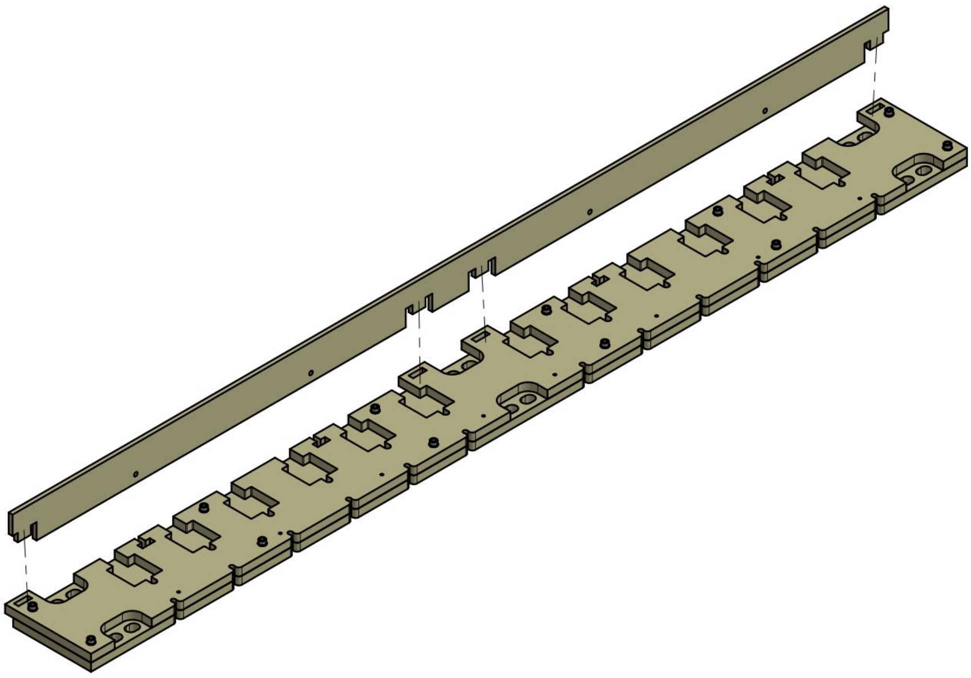


Figure 7: Inserting front panel.

5. Using an M5 25 mm long cap head screw (CHEMP0122), form A (CHEMP0124) and form C (CHEMP0013) washers and an M5 hexagonal nut (CHEMP0126), secure the front panel to the shelf as shown in (Figure 8), securing the nut with an 8mm spanner and tightening the screw with a 4mm driver.

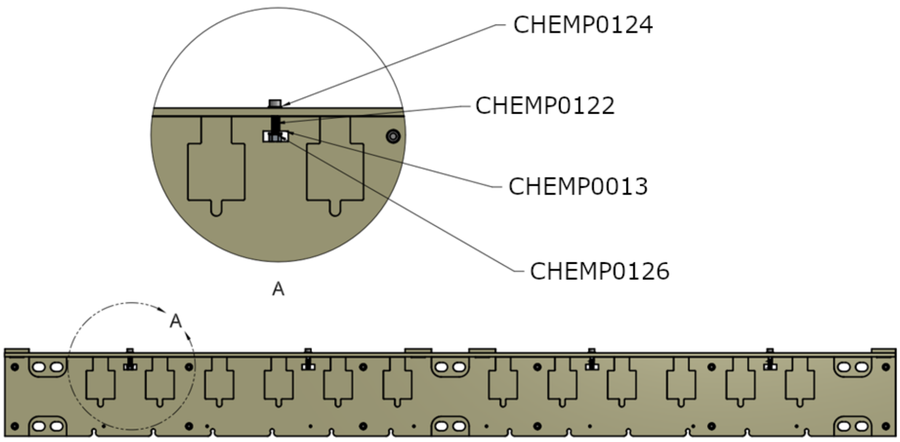


Figure 8: Front panel mount screws. Assembly top view.

6. Attach the cable clip (CHEMP0129) to the underside of the shelf using an M4 x 10mm low head cap screw (CHEMP0127) equipped with M4 Form A flat washer (CHEMP0124) and tighten using a 3.5 mm hex driver (Figure 9).

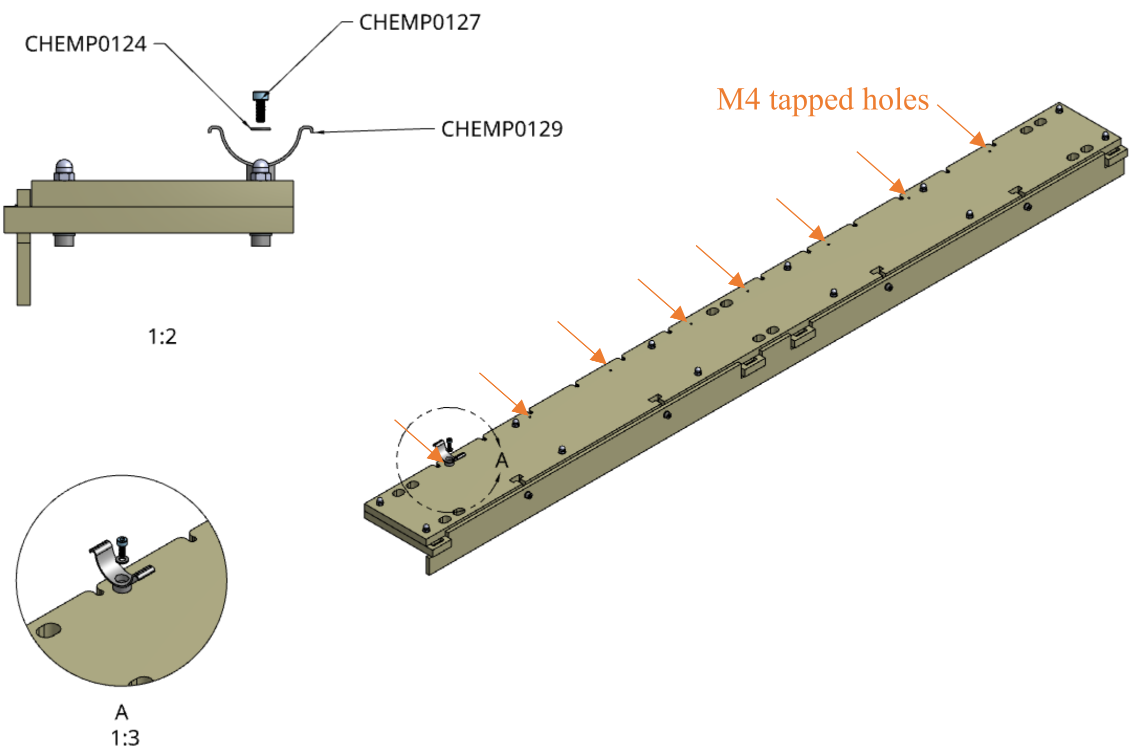


Figure 9: Cable clip attachment. Please note that the shelve is facing upside down in this figure to reflect the appropriate orientation for this construction step.

7. Repeat, attaching the remaining 8 cable clips (Figure 10).

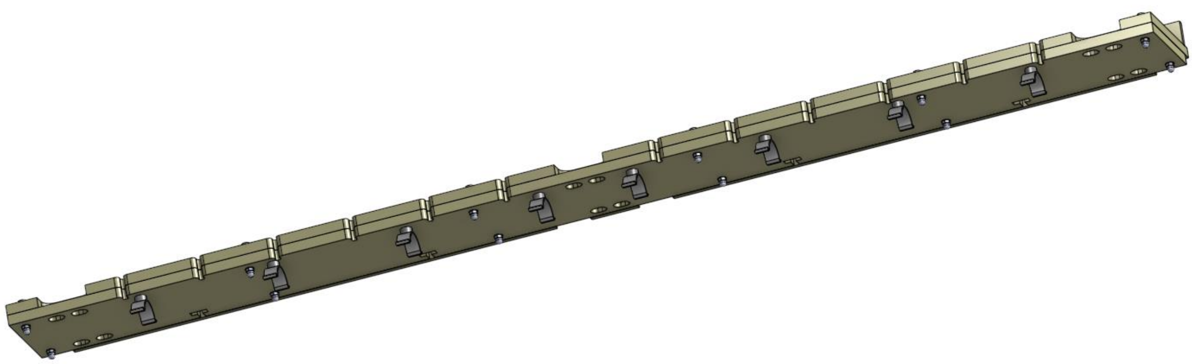


Figure 10: All eight cable clips fitted.

8. Hook the retaining rings (CHEMP0130) around the cable clips (Figure 11).

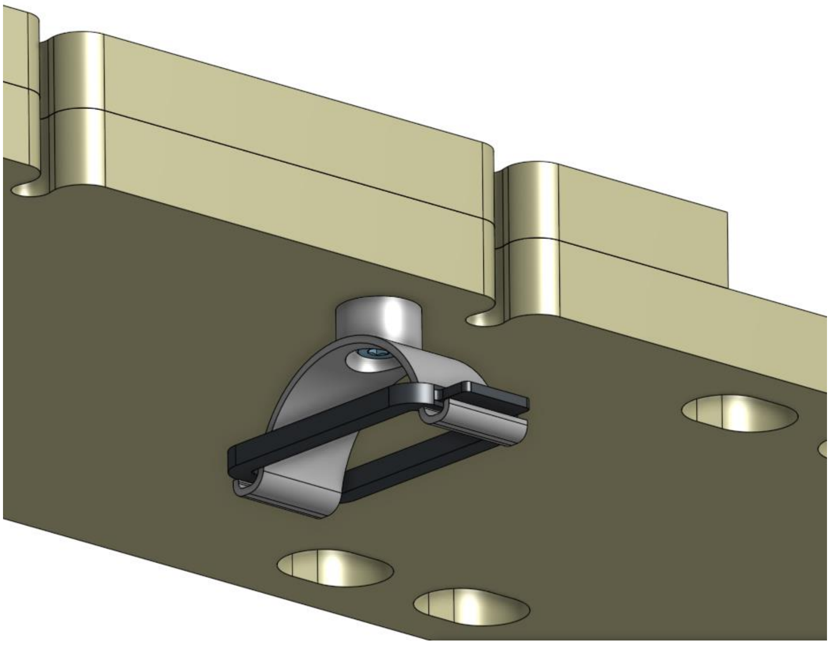


Figure 11: Attachment of retaining rings.

4.2.3 Lower shelf

Table 5 Bill of materials for the lower shelf assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0209	Valve shelf top half, 12 mm thickness	Polypropylene custom cut. The plan is available on Various\Shelving\Valves.	From jetcut.com per quote	1.00
CHEMP0120	Shelf bottom half, 12 mm thickness	Polypropylene custom cut. The plan is available on Various\Shelving\Valves.	From jetcut.com per quote	1.00
CHEMP0121	M5 x 30mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M5-30-A4	12.00
CHEMP0013	M5 Form C Flat Washers (BS4320) - A4 Stainless Steel	AccuGroup	HLDW-M5-A4	12.00
CHEMP0124	M5 Form A Flat Washers (DIN 125) - A4 Stainless Steel	AccuGroup	HPW-M5-A4	12.00
CHEMP0125	M5 Cap Nuts (DIN 917) - A4 Stainless Steel	AccuGroup	HCN-M5-A4	12.00
CHEMP0127	M4 x 10mm Low Head Cap Screws (DIN 7984) - A4 Stainless Steel	AccuGroup	SSCL-M4-10-A4	8.00
CHEMP0128	M4 Form A Flat Washers	AccuGroup	HPW-M4-	8.00

	(DIN 125) - A4 Stainless Steel		A4	
CHEMP0129	Cable Clip Black Screw Nylon Cradle Clip	RS Components	424-778	8.00
CHEMP0130	Cable Clip Black PVC Retaining Clip	RS Components	425-989	8.00

The lower shelf assembly proceeds through very similar steps as the upper shelf assembly. For clarity, instructions will be repeated here:

1. Using an M5 30mm cap head screw (CHEMP0121), form A (CHEMP0124) and form C (CHEMP0013) washers and an M5 cap nut (CHEMP0125), join the top and bottom shelving sections together as shown in Figure 5, securing the cap nut with an 8mm spanner and tightening the screw with the 3.5mm driver.

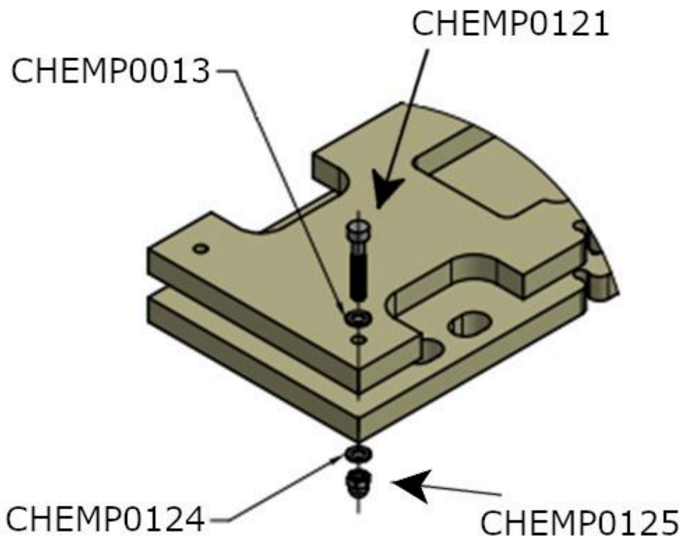


Figure 12: Joining top and bottom shelving sections.

2. Repeat this process until the shelves are secured together in all 12 positions (Figure 13).

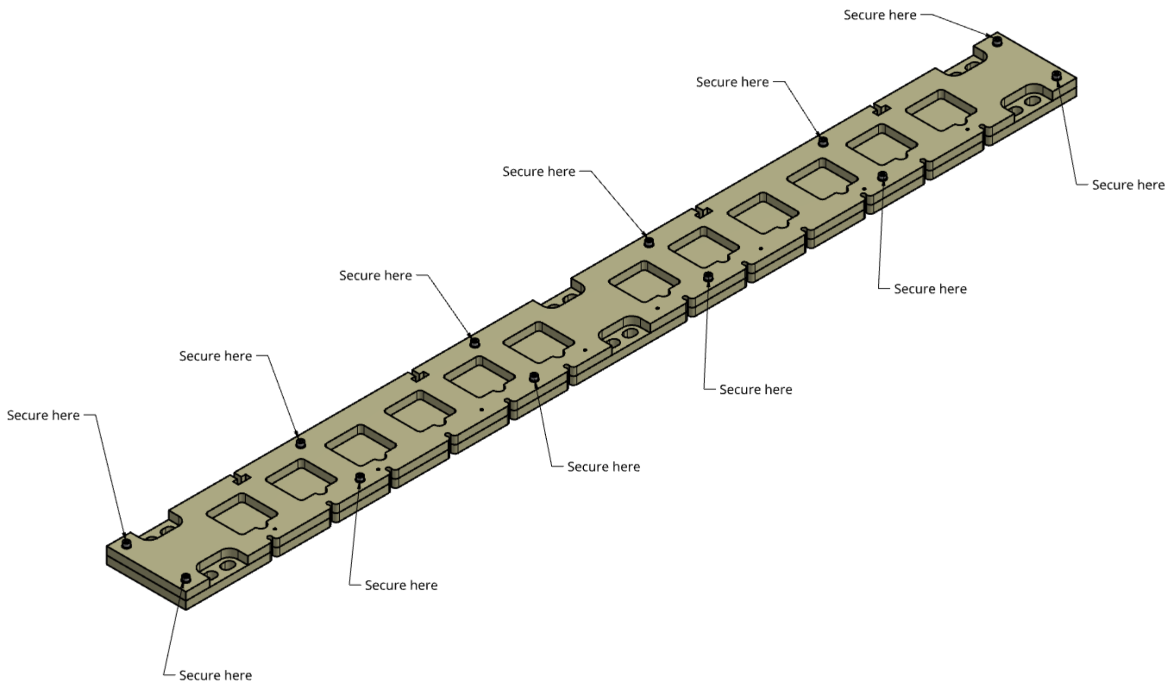


Figure 13: Securing shelving section.

3. Using an M4 tap, tap threads into all the screw holes for attachment of the cable clips. See Figure 10 for assistance in identifying the correct holes.
4. Attach the cable clip (CHEMP0129) to the underside of the shelf using an M4 Form A flat washer (CHEMP0128) and an M4 x 10mm low head cap screws (CHEMP0127) and tighten using a 3.5mm hex driver (Figure 14).

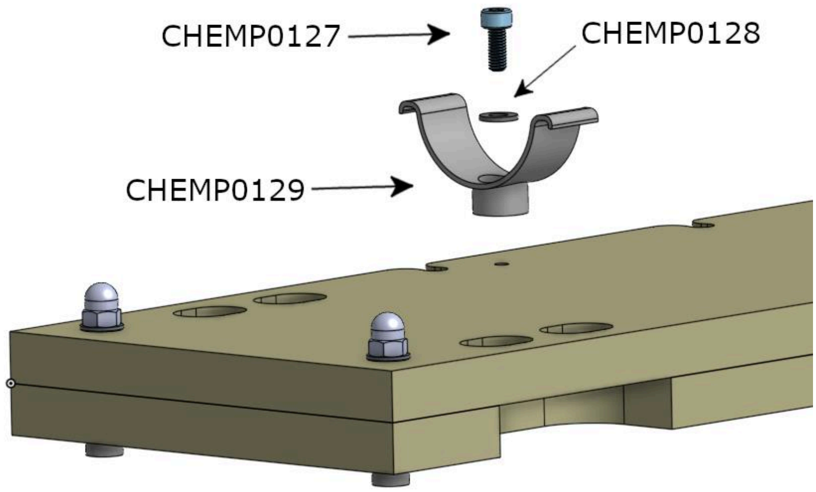


Figure 14: Cable clip attachment

5. Repeat, attaching the remaining 8 cable clips (Figure 15Figure 10).

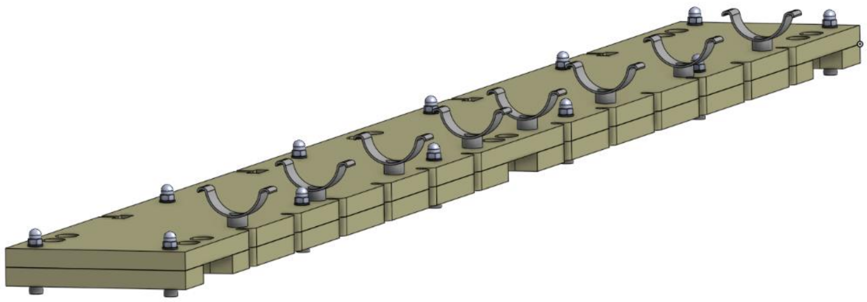


Figure 15: All eight cable clips fitted

6. Hook the retaining rings (CHEMP0130) around the cable clips (Figure 16).

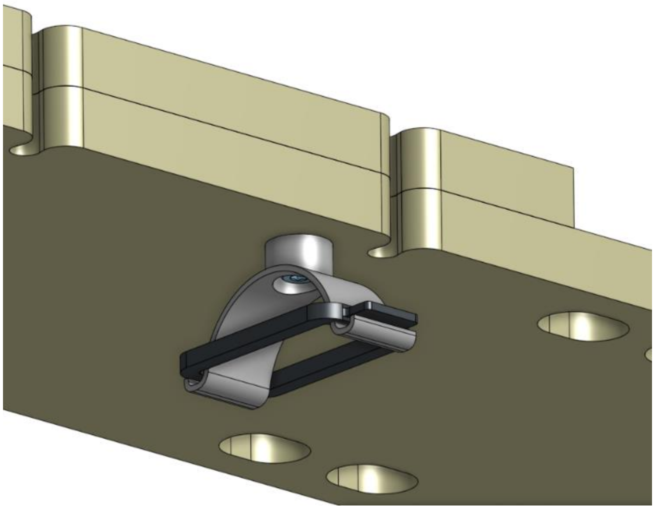


Figure 16: Attachment of retaining rings.

4.3 Switch

The connectivity between the PC running the software and the chemical hardware is achieved by the PoE switch. The switch can provide power and Ethernet connection over the same cable. The PoE switch of preference is the Netgear GS752TPP, providing 48 ports and a maximum of 750 W of power at 48 V. Other types of switches might be viable alternatives; however, their compatibility has not been tested.

4.4 Electronics

Custom-made electronics were used to drive the pumps and valves in the liquid handling back-bone of the Chemputer. These are powered and controlled over Ethernet (PoE). The electronic assembly for a single pump or valve is composed of two boards: the controller and the stepper driver. At the heart of the controller board is a microcontroller (Atmel XMega128A4U) which is programmed with a specific firmware (see Firmware section below). The controller board is powered over PoE with a dedicated DC-DC step-down converter. The Ethernet connectivity is provided by a W5500 embedded Ethernet controller from WizNet. The controller board also carries two rows of standard 100 mil pitch pin headers along edges serving as mezzanine connectors for the stepper driver board. The stepper driver board is plugged on top of the controller board. It gets unconverted 48V PoE voltage to supply the stepper motor as well as 5V from the controller board to supply the stepper driver chip. The chip used is a TMC262 stepper motor controller from Trinamic which is controlled by the main MCU via STEP/DIR interface.

4.4.1 Manufacturing

The manufacturing of all the custom-made electronics can be carried out by any ECM/EMS company which in the current case has been SOUMAC Ltd. The manufacturing company was provided with Gerber files, a bill of materials and pick and place instruction files, which are available on request.

4.4.2 Firmware

The firmware was flashed onto the control boards using an Atmel ICE mkIII (firmware version 1.27) programmer. A simple batch script invoking avrdude (an open source tool for flashing/reading Atmel AVR microcontrollers which can be found here - https://www.nongnu.org/avrdude/) was used for the actual flashing process for convenience. Alternatively, the firmware can be flashed from the Atmel Studio software package freely available from Microchip website. For the Atmel ICE mkIII programmer to work correctly both with avrdude and Atmel studio, libusb filter drivers have to be installed first. All necessary files can be found in the supplementary folder Electronics\Pump_and_Valve_Controller_and_Driver\Firmware\chemputerfirmware-master. For the installation of the Atmel ICE drivers follow the instructions in the README file.

In the following process of flashing the control board, attention must be payed to the order of connecting and disconnecting cables.

1. The connector of the Atmel ICE ribbon cable was plugged into the port labelled "AVR". The controller board was placed on a non-conducting surface and the Atmel ICE ICSP connector was plugged into the six pin header (labelled "ICSP HEADER" in the inset of Figure 17) with the small tab facing the edge of board, after which the board was connected to a PoE switch (see Section 4.3) as shown in Figure 17.

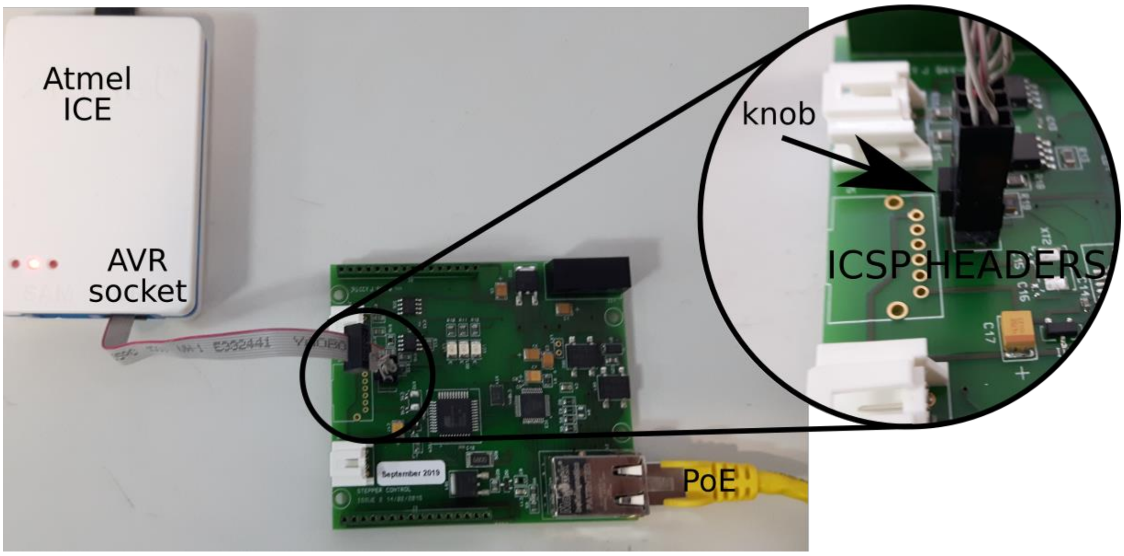


Figure 17 The controller PCB connected and ready for the firmware being flashed.

2. The flash.bat file (available as a supplementary document in Electronics\Pump_and_Valve_Controller_and_Driver\Firmware\chemputer_firmware) was started. The batch file presents a simple question-answer dialogue interface to guide the user through the flashing process. Please note that a different configuration of the firmware would be flashed on the board depending on whether it is to be used in a pump or a valve.
3. Once the firmware was successfully flashed on the control board, first the Ethernet cable was disconnected followed by the removal of the programmer.
4. Finally, the driver board was mounted on the controller board.

4.4.3 Configuration

After fully assembling the pumps and valves (as described in Sections 5.1.4 and 5.1.5) and flashing the firmware, the devices were configured and tested. For this purpose, a custom Python script (available as a supplementary file in Electronics\Pump_and_Valve_Controller_and_Driver\Firmware) was executed and the dialogue was followed. The firmware comes with default settings, which must be adjusted depending on the physical configuration of the device instance. In the case of a pump, for example, the installed syringe size must be specified. For both pumps and valves, the default IP address after flashing the firmware is 192.168.1.99. The user should change this address to a unique IP address inside the Chemputer network. After configuration, the script automatically tests the device by executing a few simple movement commands.

```
config_utility.py - Shortcut
Please enter the current IP address: 192.168.1.99
The current IP address is 192.168.1.99
Do you want to assign a new IP address? y/n y
Please enter the IP address you're going to assign: 192.168.1.121
The new IP address will be 192.168.1.121
Please enter p for pump or v for valve: v
It's a valve.
Starting UDP keepalive broadcast...
2019-11-08 14:34:32,041 ; DEBUG ; Clear errors DONE.
2019-11-08 14:34:32,166 ; DEBUG ; microsteps 0
2019-11-08 14:34:32,166 ; DEBUG ; positive_direction -1
2019-11-08 14:34:32,166 ; DEBUG ; home_magnet_direction -1
```

Figure 18: Screenshot of the dialogue window for the pump and valve configuration and testing.

4.5 Hall Sensor Board

The Hall sensor board is used both in the pump and in the valve to calibrate the home position. It is built by soldering a Hall sensor (Texas Instruments part number: DRV5055A4QDBZR) unit and wires onto a bespoke circuit board (the scheme is available as a supplementary file in Electronics\Pump_and_Valve_Controller_and_Driver\Circuit_Boards). In this section, a general guide on soldering and assembling the boards is given. Alternatively, the fully assembled sensor boards can be obtained from a chosen EMS/ECM provider (necessary design files are available on request). Below, the manual assembly of the Hall sensor is described starting from the bare PCB.

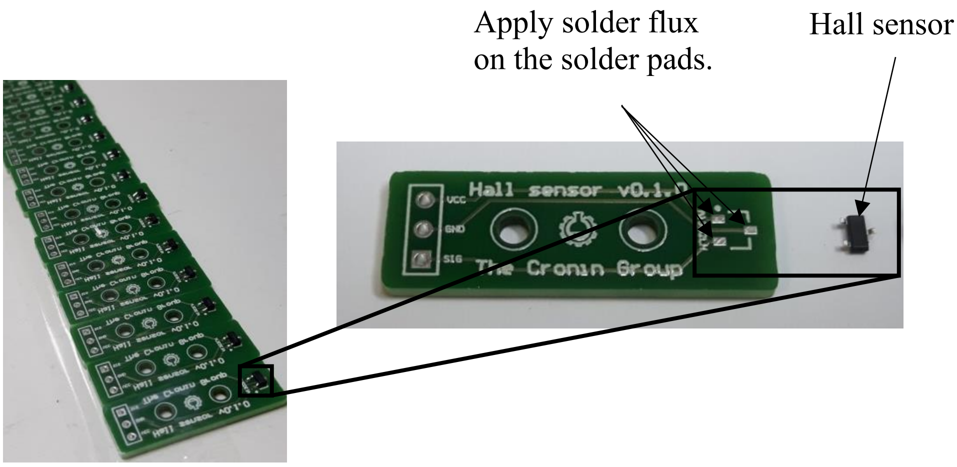


Figure 19: Soldering of the Hall sensors onto the Hall sensor boards.

1. As many sensor boards as required were placed on a desk and fixed with adhesive tape (Figure 19).

2. Solder flux was applied to the solder pads of the sensor followed by a tiny amount of solder to form solder pillows on the pads.
3. The Hall sensor was positioned and held in place with tweezers while the first pin was soldered onto the board (Figure 20).
4. The sensor was slightly pressed onto the board with tweezers and the other two legs were soldered.
5. Finally, while pressing the sensor onto the board, the first leg was resoldered again to relieve any potential strain.

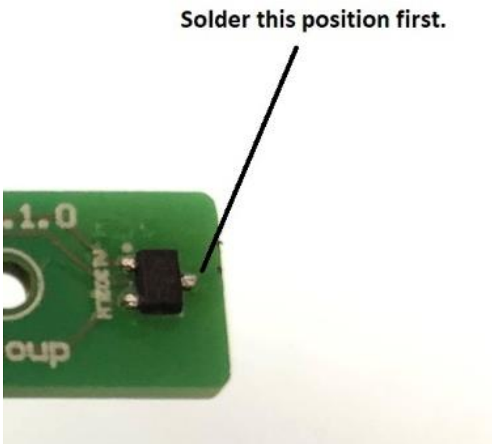


Figure 20. The central pin of the Hall sensor was soldered first.

6. The sensor boards were then removed from the table surface and fixed at the table edge as shown in **Error! Reference source not found.**.

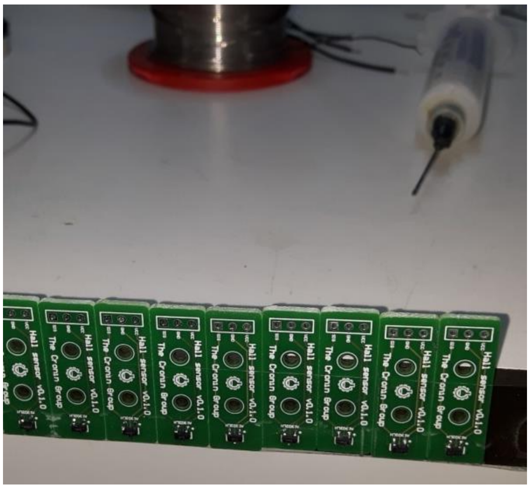


Figure 21: Hall sensor boards attached to the table edge, ready for soldering the connecting wires.

7. The commercially available cable (MicroClasp 4 position receptacle 17.7 ", 450 mm, Farnell part number: 15136-0405) was cut in halves, the individual wires were

removed from the MicroClasp receptacle (Figure 22) and ca 7 mm of the isolation was removed from the cut end.

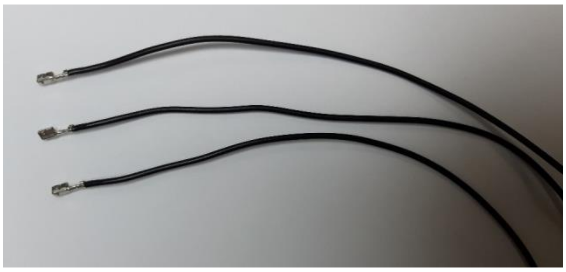


Figure 22 Pre-crimped wires to be soldered on the Hall sensor board.

8. The end with bare wire was inserted into the first hole of the Hall sensor board from the bottom side (the opposite side to where the Hall sensor is mounted). Solder flux was applied, and the wire was moved back and forth to ensure a good distribution of the flux. Then solder was applied and again the wire was moved back and forth while applying heat to ensure even solder distribution. Solder should be visible on both sides of the hole and should be evenly distributed all around the circumference of the wire (Figure 23).

Figure 23: Soldering of the connection wires to the Hall sensor board.

9. After finishing the soldering, the protruding sharp ends were cut off (Figure 24).

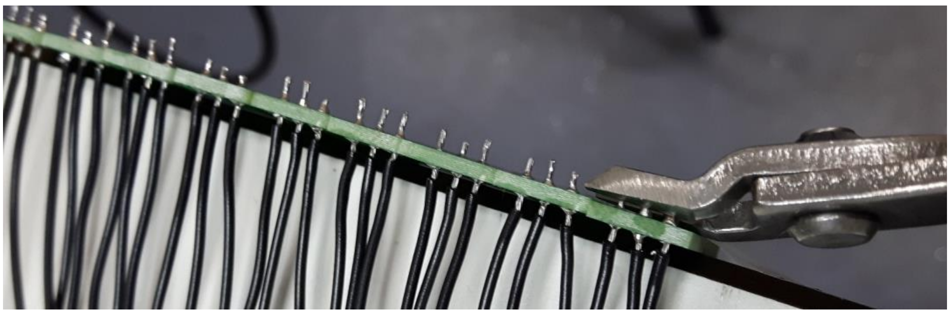


Figure 24 The protruding sharp ends of the soldered wires were cut off even with a nipper.

10. The final boards were wiped clean from soldering flux with isopropanol and the connections between the pins of the Hall sensor and the wire ends were tested with an ohmmeter. The reading should be below 0.2 Ohms for a properly soldered connection.
11. The crimps were then inserted into the MicroClasp receptacle following the schematics in Figure 25. Attention must be payed to ensure correct wiring depending on whether the sensor will be used in a valve or a pump.

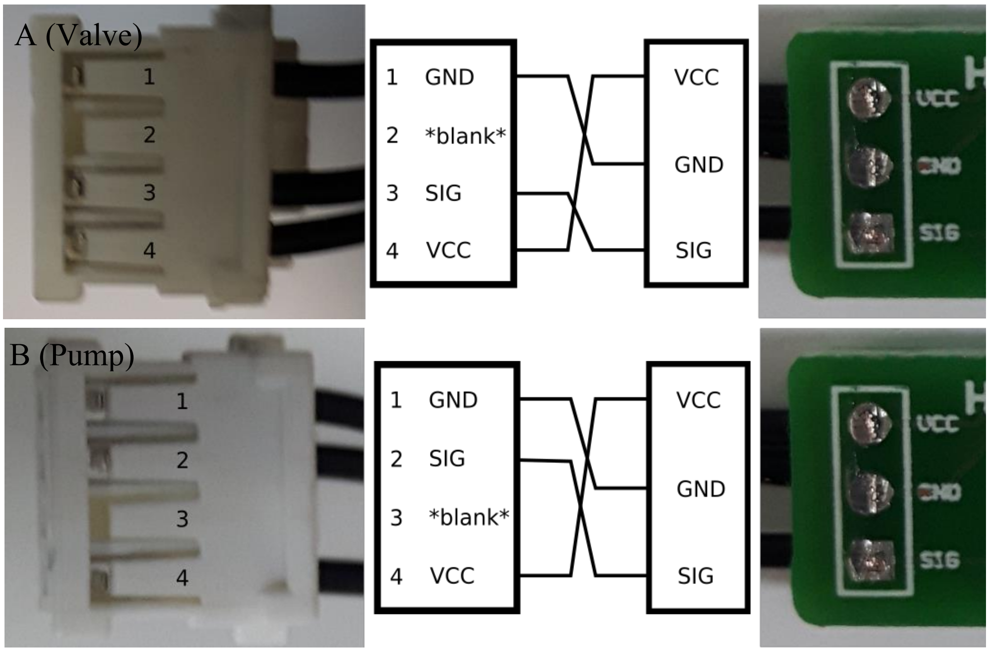


Figure 25: Connection between the Hall sensor board and the MicroClasp receptacle for the valve (A) and for the pump (B).

4.6 Pump

The Chemputer uses custom-made pumps (Figure 26) in the liquid handling backbone. These are controlled and powered over Ethernet. The pumps were equipped with either 10 mL or 25 mL syringes (as specified for each instance of the Chemputer).

Figure 26 Chemputer pump

4.6.1 **3D-Printing**

The standard instructions for 3D printing with the Connex 3D printer were followed (consult the relevant manufacturers manual). The parts were cleaned according to the standard procedure. The STL files specifying the parts are available as supplementary files in 3D_Printed_Parts\Pump.

4.6.2 **Motor Assembly**

Table 6 Bill of materials for the motor assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0195	NEMA23 motor with D-cut shaft	Longs Motors	23HS0420	1
CHEMP0305	MTA-156 Series 3.96mm Pitch Right Angle Cable Mount IDC Connector, Socket, 4 Way, 1 Row, for 24 AWG wire	RS Components	535-5535	1

1. The motors are supplied with bare leads and the connectors need to be attached to a suitable IDC connector.
2. Using the wire cutters remove the exposed ends of the wires (Figure 27).

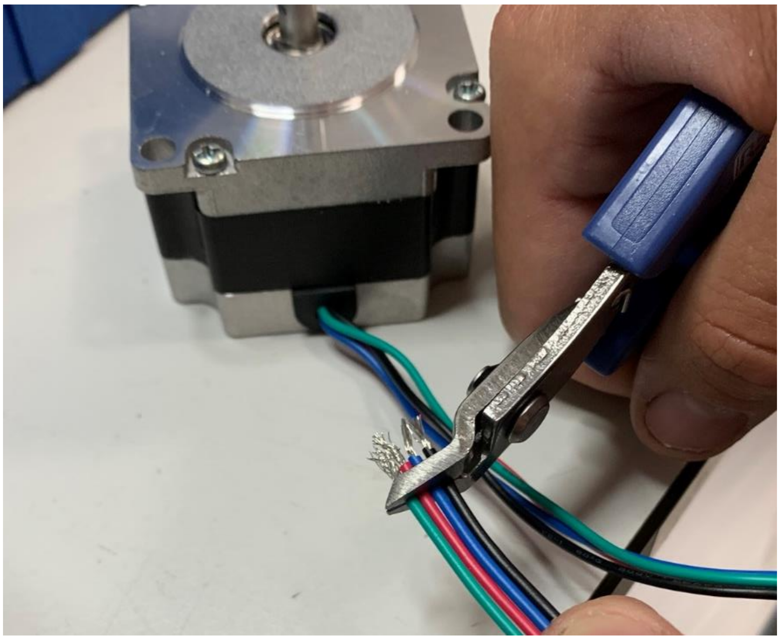


Figure 27: Preparing the motor wires

3. Refer to Figure 28 for guidance on the correct sequence to connect the wires.

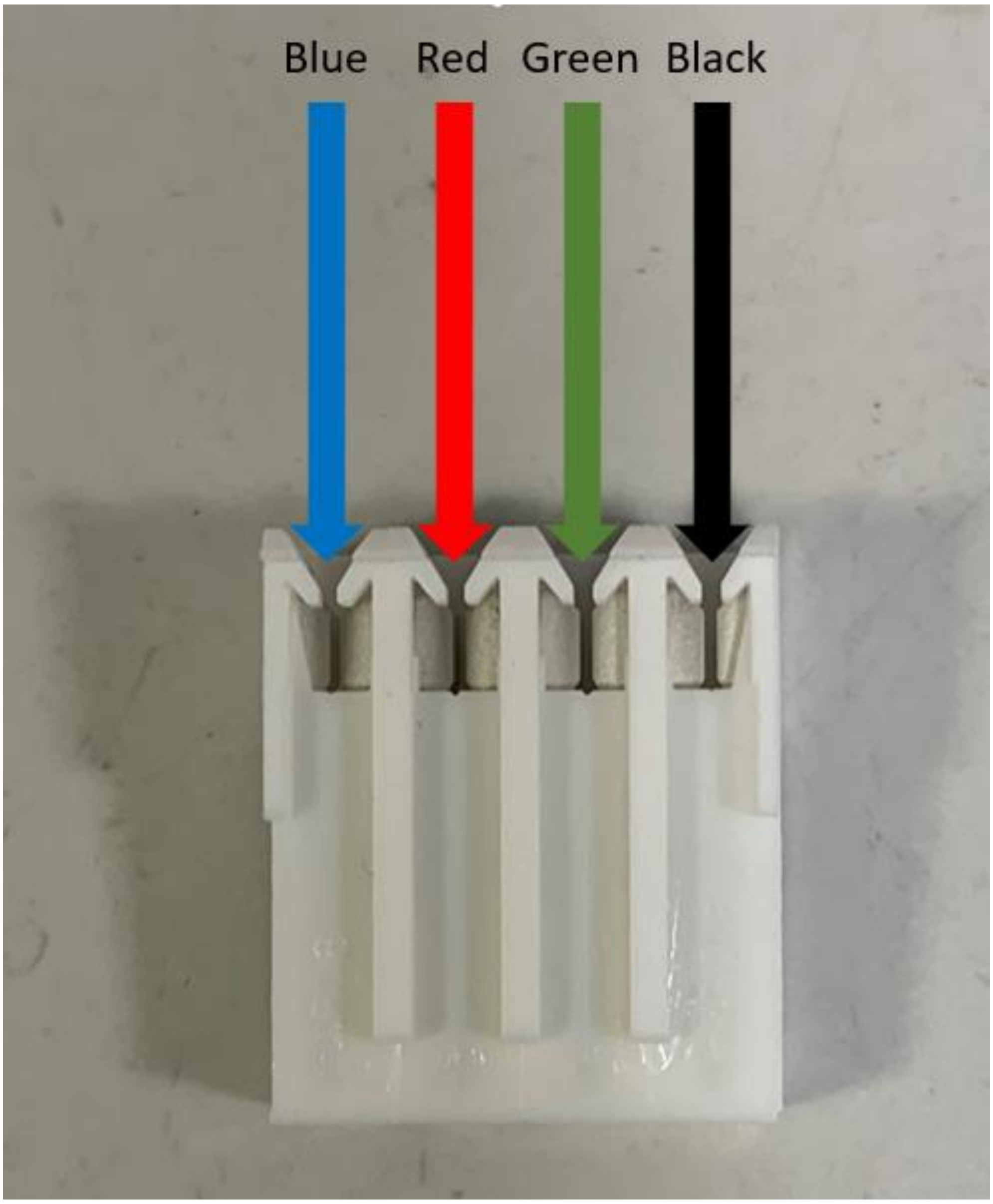


Figure 28: Wire connection

4. Braid the cables by (Figure 29):
   a. Pairing the black and green wires and braiding.
   b. Pairing the red and blue cables.
   c. Braiding the two pairs of braided cables together.

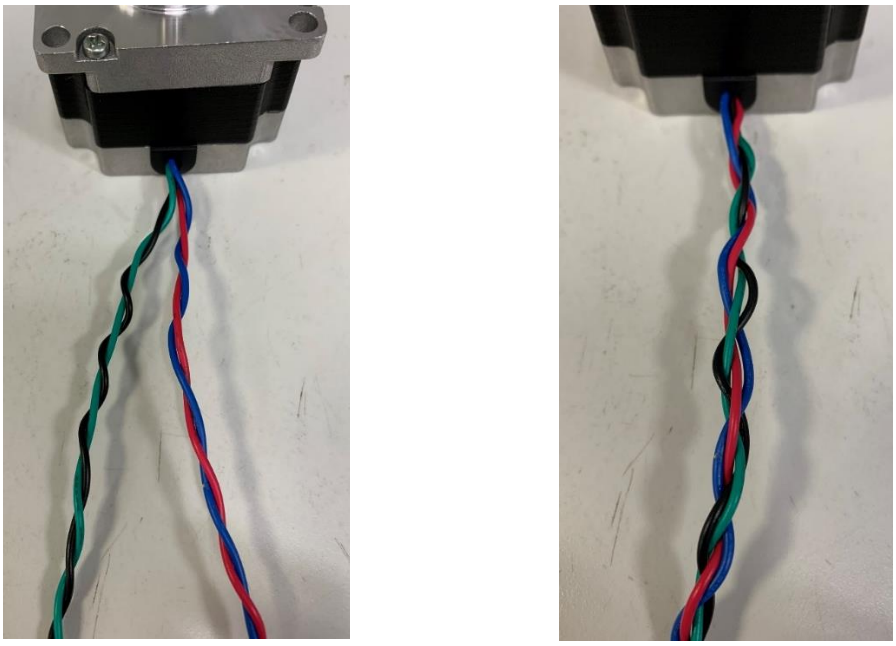


Figure 29: Braided cables

5. Place the connector block on a flat surface or suitable support to prevent possible rocking when inserting the wire.
6. Position wire over the contact in the connector. Make sure the end of the wire does not extend over the shoulder of the connector. Start the wire into contact with your finger (Figure 30).

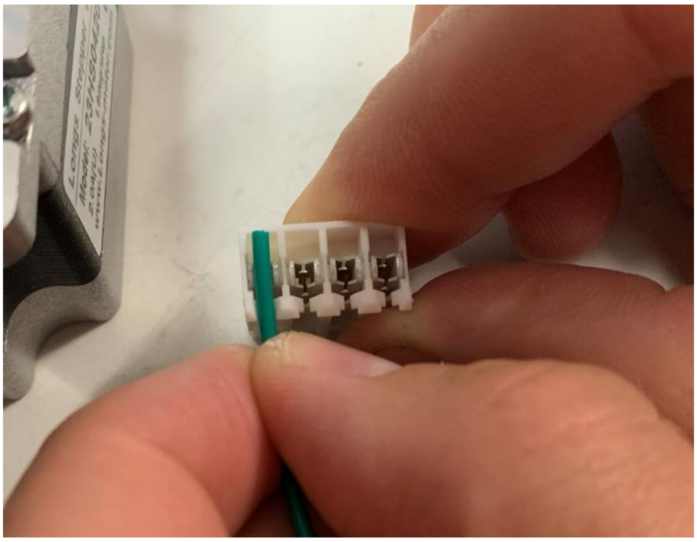


Figure 30: Inserting wire into the connector

7. Place the appropriate tool on the wire over the contact so that the centreline of the tool matches the contact. The tool must be positioned as shown in Figure 31. The tool can only be inserted in one orientation.

Figure 31: Positioning of insertion tool on connector

8. Holding the tool handle perpendicular to the contact, apply a constant, direct pressure until the wire is terminated with the contact.
9. Remove tool and inspect contact for proper wire insertion (Figure 32). If necessary, repeat the operation.

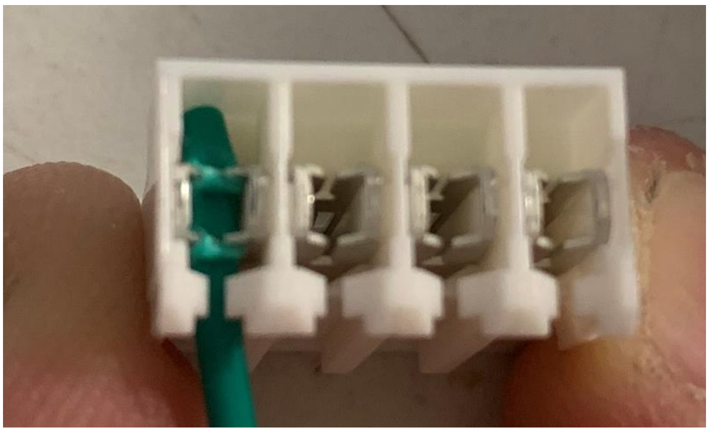


Figure 32: Correct insertion of wire

10. You may find it easier to gently clamp the IDC connector in a vice to provide greater stability.

4.6.3 **Pump Assembly**

Table 7 Bill of materials for the pump assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0092	Chemputer Pump Motor Connector	In-house - 3D printed	N/A	1.00
CHEMP0093	Chemputer Pump Carriage	In-house - 3D printed	N/A	1.00
CHEMP0094	Chemputer pump 10mL plate	In-house - 3D printed	N/A	1.00
CHEMP0095	Chemputer pump 10ml sleeve	In-house - 3D printed	N/A	1.00
CHEMP0236	Syringe, 10ml	ILS	2624076-HT	1.00
CHEMP0097	Carriage	Moore International	MGN12H-ZOHM	1.00
CHEMP0098	Guide rail	Moore International	MGR12R-275mm	1.00
CHEMP0099	Stepper motor Tr8*4(P2)	Longs Motors	17HSL5415-252-1	1.00
CHEMP0100	Lead screw nut	Longs Motors	17HSL5415-252-2	1.00
CHEMP0102	Magnet	Farnell	1800035	2.00
CHEMP0106	M3 x 10mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M3-10-A4	7.00
CHEMP0107	M3 x 12mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M3-12-A4	4.00
CHEMP0108	M3 x 20mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M3-20-A4	4.00
CHEMP0109	M3 x 30mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M3-30-A4	4.00
CHEMP0110	M4 x 30mm Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSC-M4-30-A4	4.00
CHEMP0111	M3 Hexagon Nuts (DIN 934) - A4 Stainless Steel	AccuGroup	HPN-M3-A4	5.00
CHEMP0112	M3 External Fine Tooth Locking	AccuGroup	HLFW-M3-	15.00

	Washers (DIN 6798A) - A4 Stainless Steel		A4	
CHEMP0113	M4 Hexagon Insert Press Nuts - A2 Stainless Steel	AccuGroup	HPNI-M4-A2	4.00
CHEMP0114	6-32 x 3/4 inch UNC Cap Head Screws (ANSI B18.3) - A4 Stainless Steel	AccuGroup	SSC-6-32-3/4-A4	1.00
CHEMP0116	Female Connector Housing - SL-156, 3.962mm Pitch, 4 Way, 1 Row	RS Components	756-7027	1.00
CHEMP0104	M2 x 10mm Socket Button Screws (ISO 7380) - A4 Stainless Steel	AccuGroup	SSB-M2-10-A4	2.00
CHEMP0178	M3 x 8mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M3-8-A4	2.00
CHEMP0233	Molex Female Connector Housing - MICROCLASP, 2mm Pitch, 7 Way, 1 Row	RS Components	188-181	1.00
CHEMP0234	Molex Female Connector Housing - SL, 2.54mm Pitch, 3 Way, 1 Row	RS Components	670-3890	1.00
CHEMP0078	M20-1180046 HARWIN M20 Female Crimp Terminal Contact 22AWG	RS Components	681-2887	7.00
CHEMP0235	Cable Assembly, MicroClasp 4 Position Receptacle, 17.7 ", 450 mm	Farnell	15136-0405	1.00
CHEMP0207	SL-156 Female Crimp Terminal Contact 18AWG	RS Components	660-6039	4.00

1. Locate the pump carriage (CHEMP0093) and orientate it as shown in Figure 33. Insert 4 x M3 external fine-tooth locking washers (CHEMP0112) as shown.

Figure 33: Serrated washers in pump carriage

2. Insert 4 x M3 x 10mm full thread cap head screws (CHEMP0106) (Figure 34).

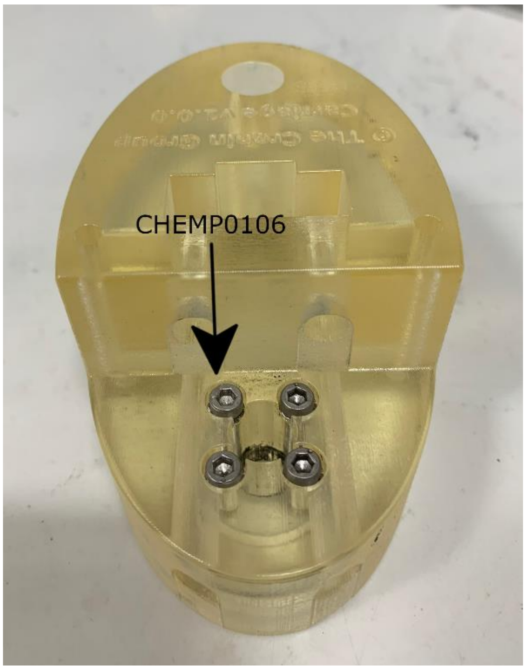


Figure 34: Screws in pump carriage

3. Carefully lay the pump carriage on its side and using the screws previously inserted, secure the lead screw nut (CHEMP0100) into the recess provided using the M3 driver to tighten the bolts (Figure 35).

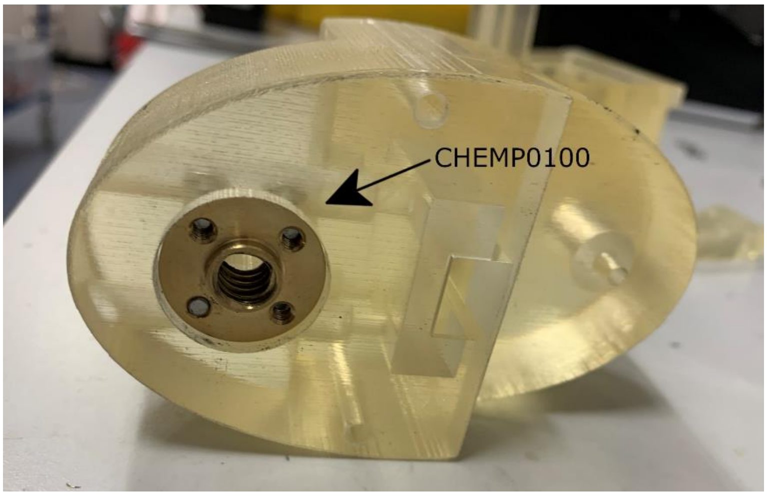


Figure 35: Lead screw nut insertion

4. Insert the magnets (CHEMP0102) as shown in Figure 36, taking care to orient them to the correct polarity using the magnet pole identifier.

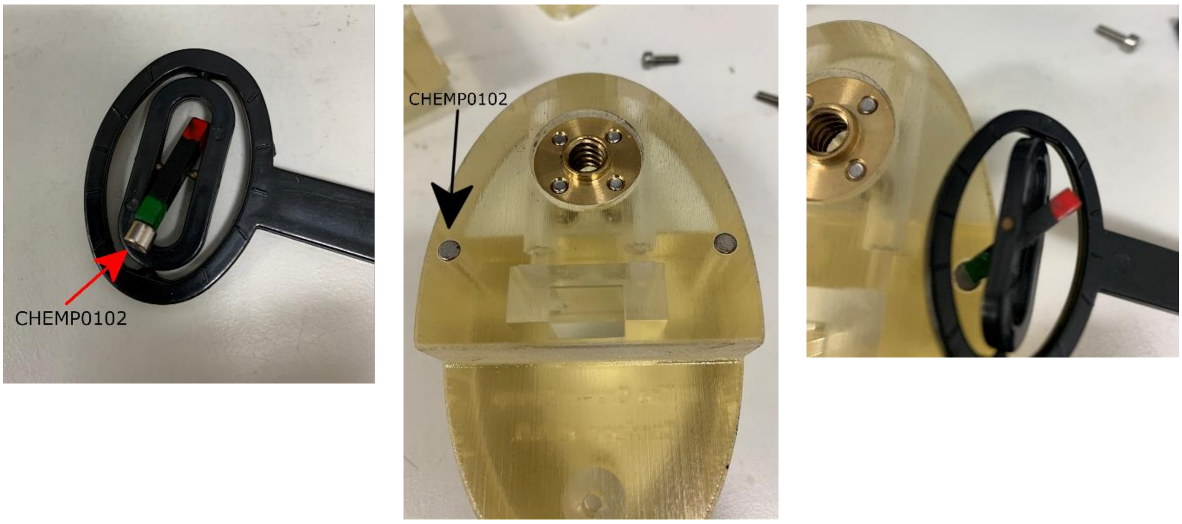


Figure 36: Magnet insertion

5. Carefully insert the carriage (CHEMP0097) into the socket (Figure 37). Taking care that none of the balls from linear bearings fall out. If any are lost the carriage will not work and will need to be replaced.

Figure 37: Carriage insertion

6. Gently place the pump carriage assembly on its side as shown in Figure 38 and apply a piece of adhesive tape across the base. This will prevent the plastic blank that secures the ball-bearings from falling out during assembly.

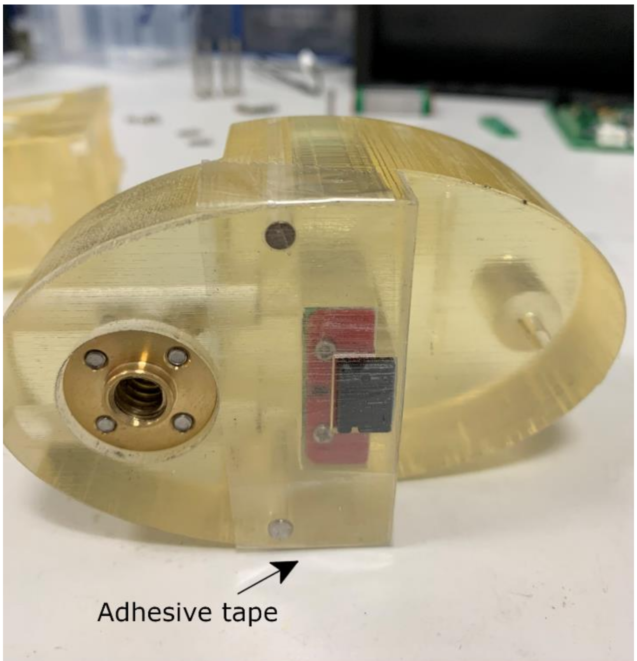


Figure 38: Carriage secured with tape

7. Insert 4 x M3 external fine-tooth locking washers (CHEMP0112) as shown in Figure 39.

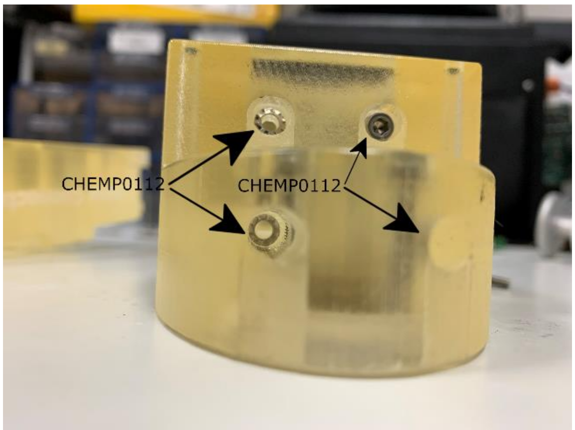


Figure 39: Washers for securing carriage

8. Insert 4 x M3 x 10mm full thread cap head screws (CHEMP0106) to secure the carriage to the pump carriage assembly (Figure 40). Tighten using a 3mm hex screwdriver.

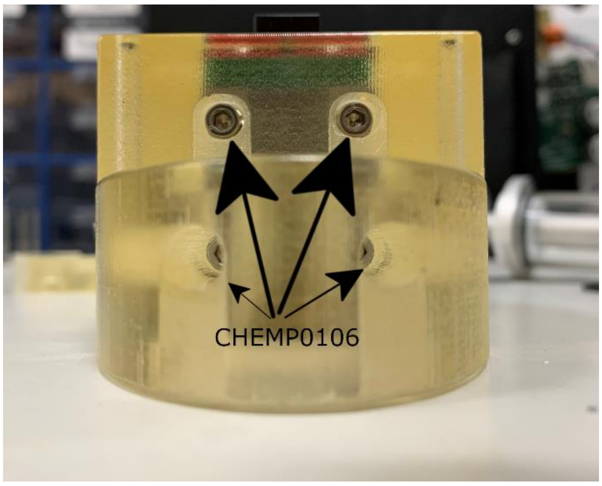


Figure 40: Screws fixing the carriage

9. Put the pump carriage aside.
10. Secure the stepper motor (CHEMP0099) to the pump motor connector (CHEMP0092) using 4 x M3 x 30mm Cap Head Screws (CHEMP0109) and 4 x M3 External Fine-Tooth Locking Washers (CHEMP0112) (Figure 41), using the M3 driver to tighten the bolts (Figure 42).

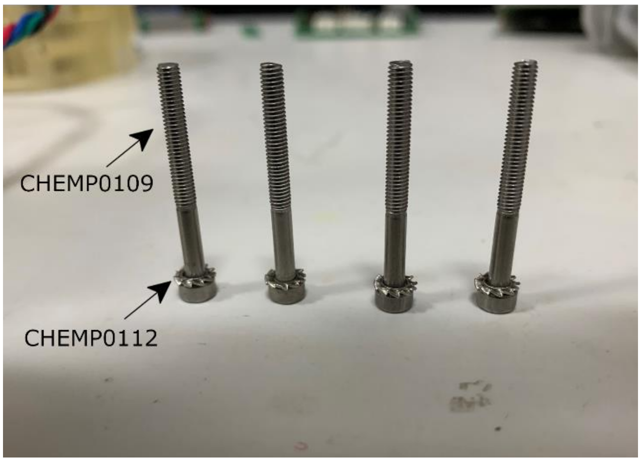


Figure 41: Bolts and washers for securing motor

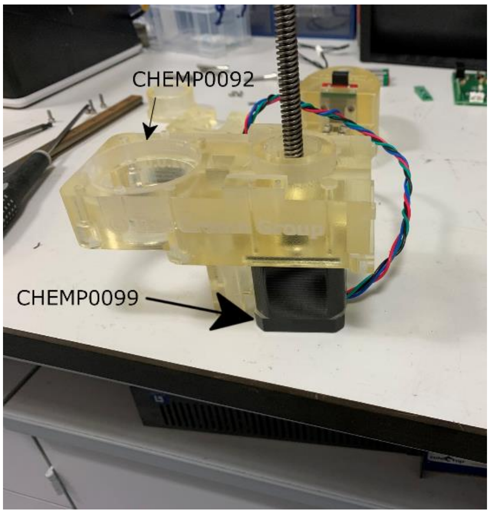
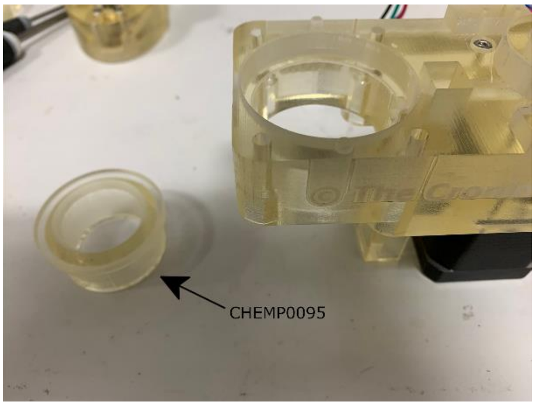


Figure 42: Motor positioning and assembly

11. Insert the sleeve (CHEMP0095) into the pump motor connector, ensuring that it is flush with the motor connector (Figure 43).



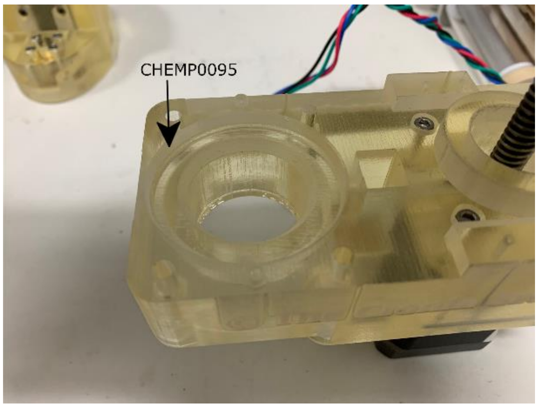


Figure 43: Sleeve insertion

12. Secure the guide rail (CHEMP0098) to the pump motor connector using 2 x M3 x 10mm Full Thread Cap Head Screws (CHEMP0106), 2 x M3 External Fine Tooth Locking Washers (CHEMP0112) and 2 x M3 Hexagon Nuts (CHEMP0111) as shown in Figure 44, using the M3 driver to tighten the bolts (Figure 45).

Figure 44: Fixing the guide rail

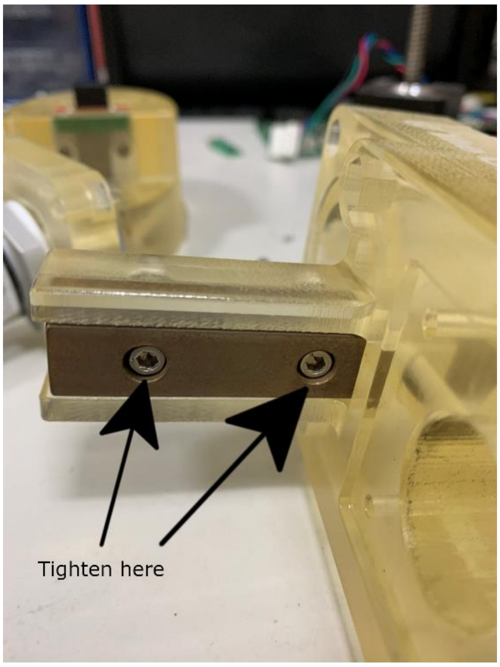


Figure 45: Fixing the rail

13. Insert the syringe (CHEMP0236) and secure in place using the pump plate (CHEMP0094) (Figure 46).

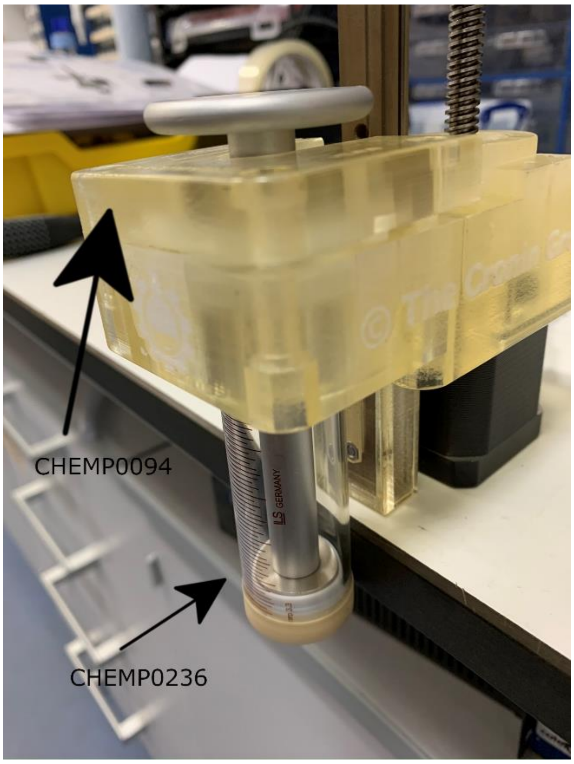


Figure 46: Syringe insertion

14. Secure the pump plate to the motor assembly using 4 x M3 x 30mm Cap Head Screws (CHEMP0109), 4 x M3 External Fine-Tooth Locking Washers (CHEMP0112) and 4 x M3 Hexagon Nuts (CHEMP0111) as shown in Figure 47, using the M3 driver to tighten the bolts.

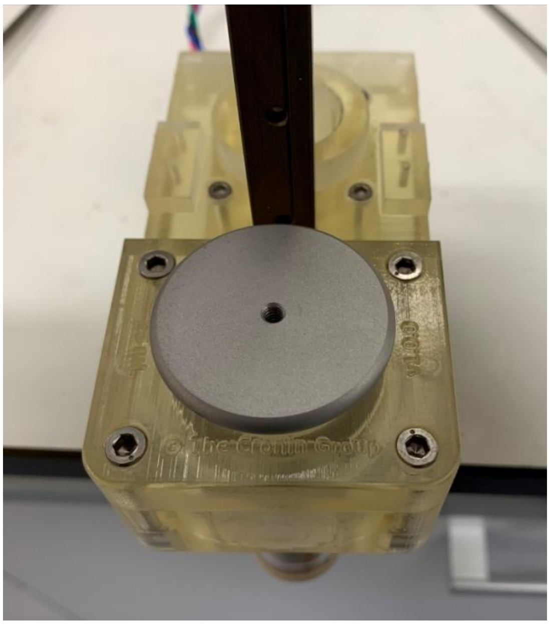
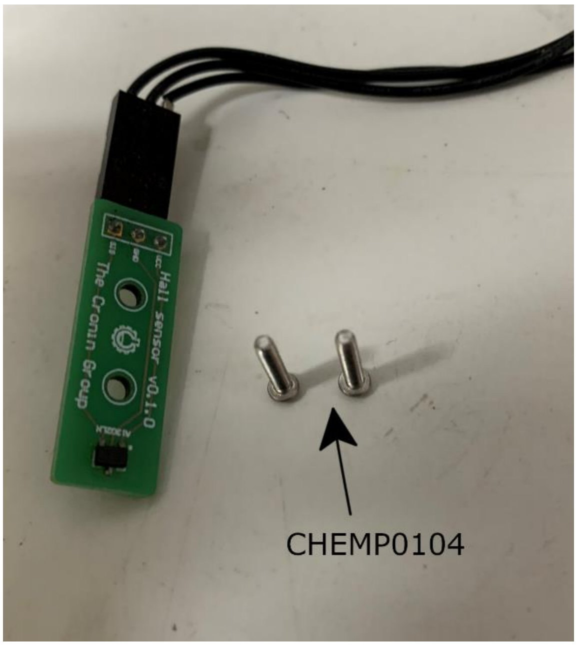


Figure 47: Fixing the syringe in place

15. Attach the Hall sensor assembly to the top of the pump motor assembly using 2 x M2 x 10mm Socket Button Screws (CHEMP0104) (Figure 48). The Hall sensor assembly must be securely attached. If the screw holes were insufficiently cleaned after printing, there may support material trapped in the holes which will prevent the screws being fully inserted. If this is the case, then excess support material can be removed using a syringe needle.



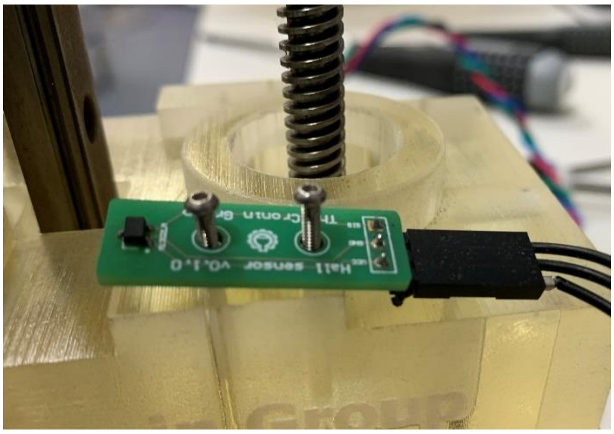


Figure 48: Attaching the Hall sensor.

16. Remove the tape from the base of the pump motor assembly.
17. Insert the pump carriage onto the top of the lead screw and begin to turn the lead screw by hand. This will begin to pull the pump carriage down the shaft of the lead screw (Figure 49). As it does so, the blank in the carriage will be pushed out by the guide rail. Once it has been completely pushed from the carriage, it can be discarded.

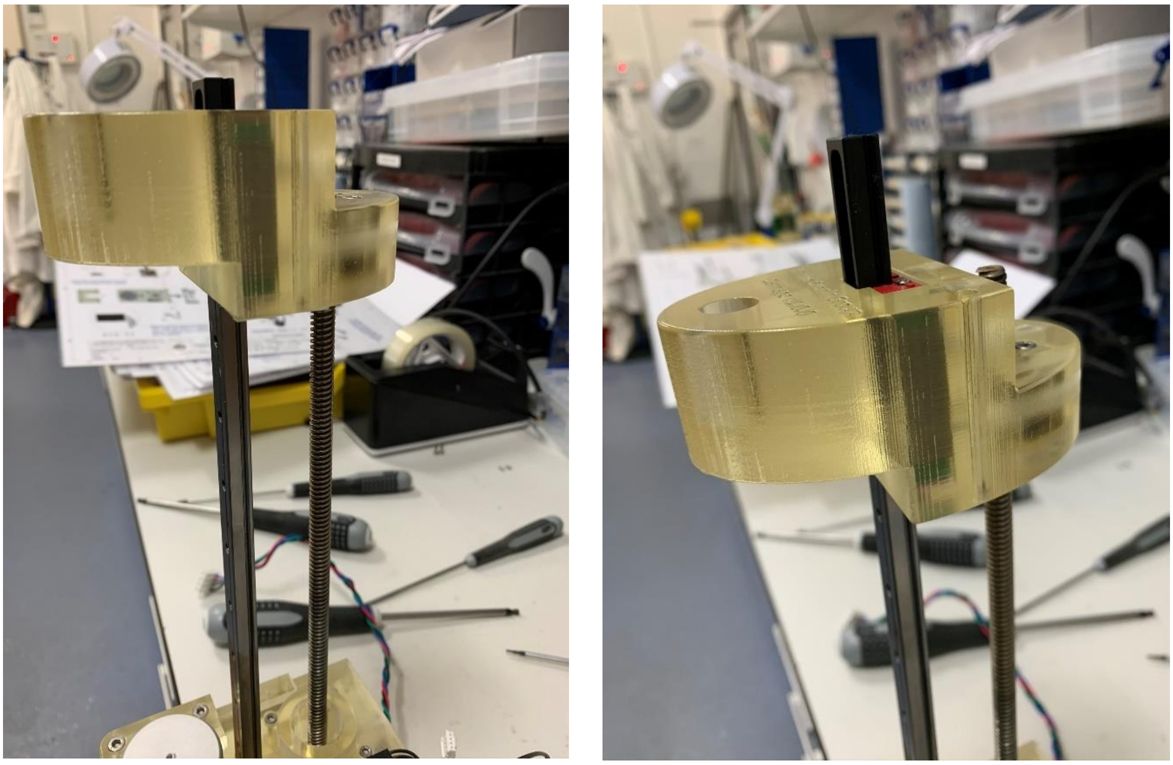


Figure 49: Attaching the pump carriage to the lead screw

18. Continue to screw the pump carriage down until it sits flush with the top of the syringe.
19. Fix the syringe to the pump carriage using a 6-32 x 3/4-inch UNC cap head screw (CHEMP0114), securing with a 6-32 hex head driver (Figure 50).

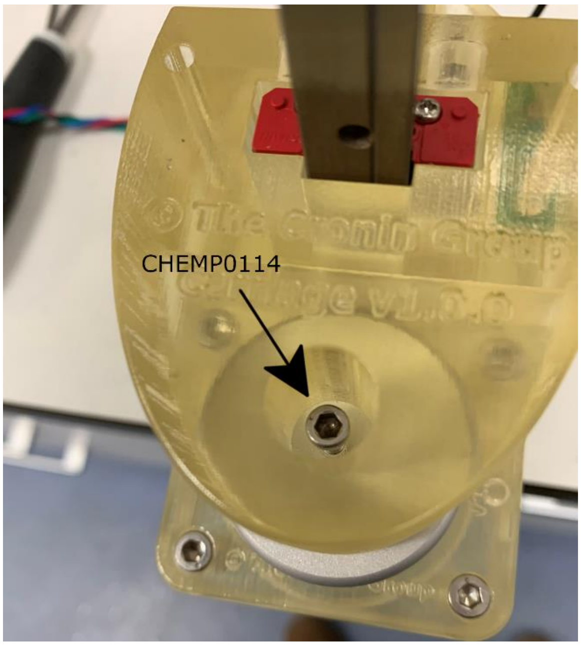


Figure 50: Screwing the syringe into place

20. Insert 2 x M3 Hexagon Nuts (CHEMP0111) into the sides of the pump motor connector (Figure 51).

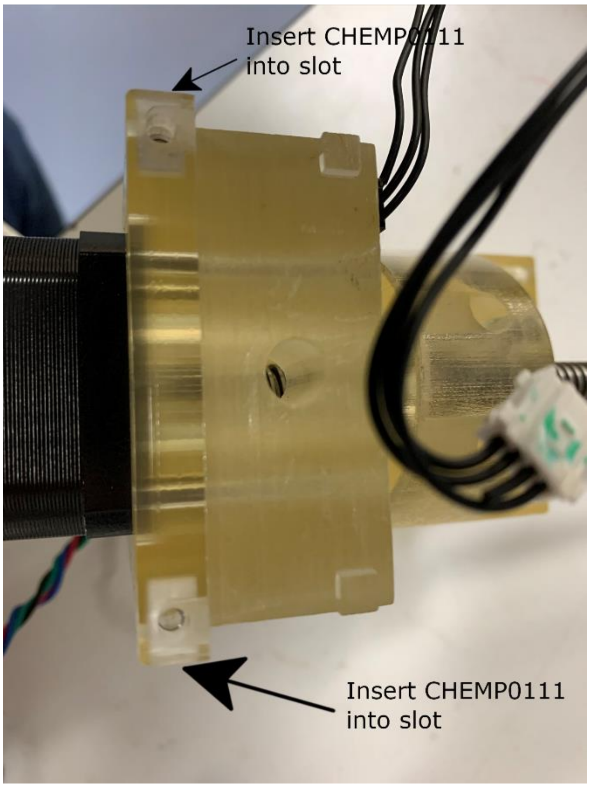


Figure 51: Insertion of nuts into pump motor connector

21. Using the M3 screwdriver, secure the control PCB (CHEMP0240) to the valve housing using the final 2 M3 screws (CHEMP0178) (Figure 52).

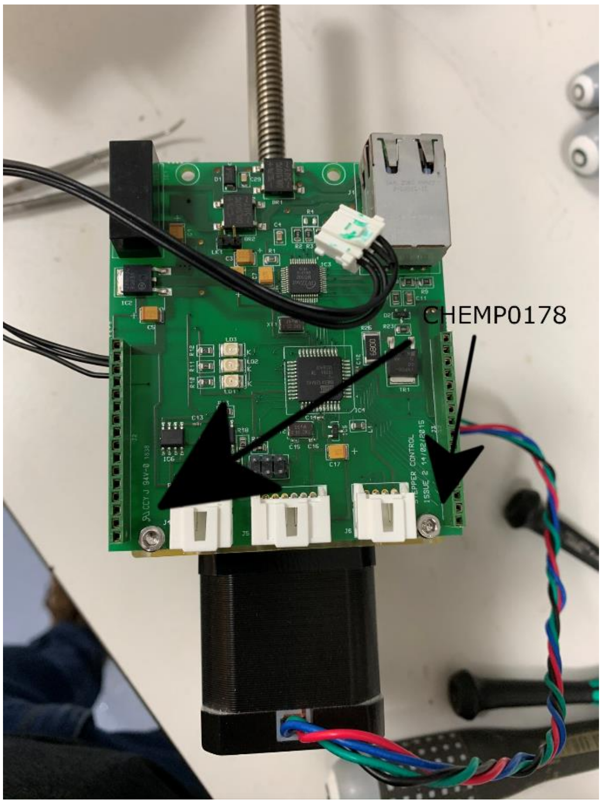


Figure 52: Attaching the control PCB

22. Gently mate the driver PCB (CHEMP0198) with the controller PCB, taking care to ensure that the pins are correctly aligned and that the wires are not sandwiched between the two boards and insert the Hall sensor and motor connectors (Figure 53).

Figure 53: Driver PCB and final connections

4.6.4 **Optional Re-Gluing of Syringes**

The commercially available syringes (ILS part number: 2624093 {for 10 mL syringes}, 2624076-HT {for 25 mL syringes}) may fail after long-term use in the Chemputer (though durability strongly depends on what chemicals are handled). Instead of replacing broken syringes, it was found that they can be repaired rather easily, and good results were obtained (i.e. low failure rate in long-term use). For critical applications, it may even be considered to perform the following re-gluing process as a preventive maintenance.

The syringe cap was disconnected from the glass cylinder and re-glued as follows.

1. The end of the syringe (Figure 54 A) was immersed in toluene for 24 h to soften the factory adhesive. Then the cap was removed, the parts were dried, and all residual glue was removed mechanically (Figure 54 B).
2. The surface on the inner side of the syringe cap was roughened with sandpaper.

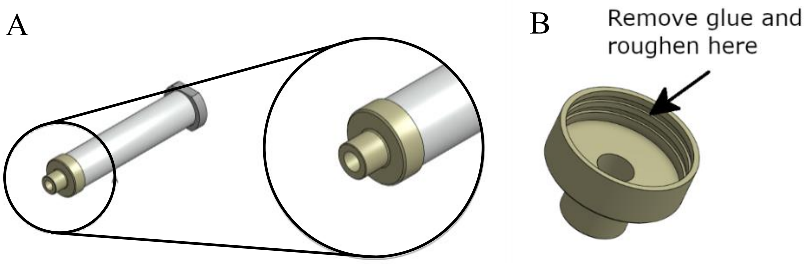


Figure 54 The cap of the syringe was disconnected from the glass cylinder.

1. Two-component slow curing epoxy adhesive (UHU Plus Endfest 300 glue) was applied to flat surface and interior edges of the syringe cap.
2. The syringe was reassembled. Care was taken to ensure that the syringe hole was not blocked with adhesive.
3. The syringe was fixed inside a custom made clamping block (Figure 55) for 24 h (for a drawing of the clamping block see Figure 56 and Figure 57).

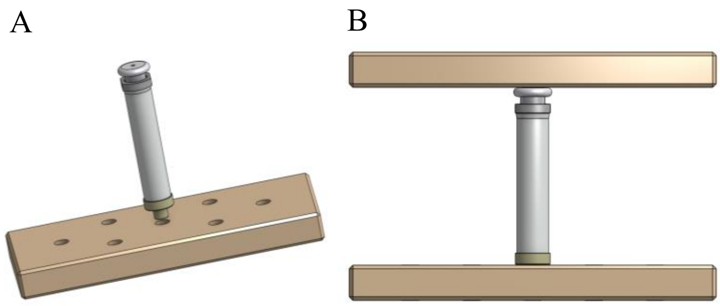


Figure 55 (A) The re-glued syringe was inserted into a hole on the bottom place of a custom-made clamping block. (B) The assembly was then fixated by the top plate of the clamping block.

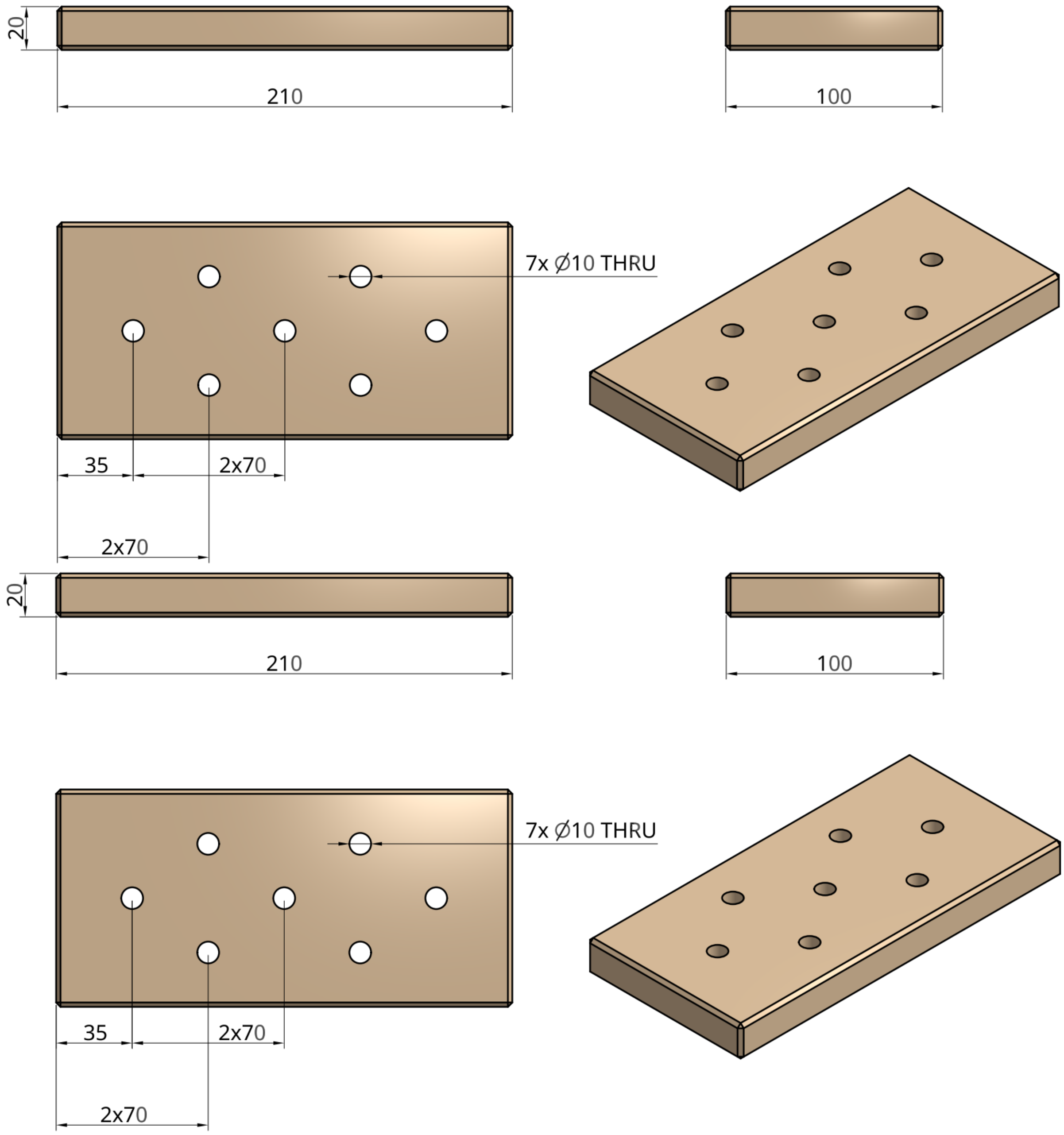


Figure 56 Bottom plate of the clamping block used to re-glue the syringes.

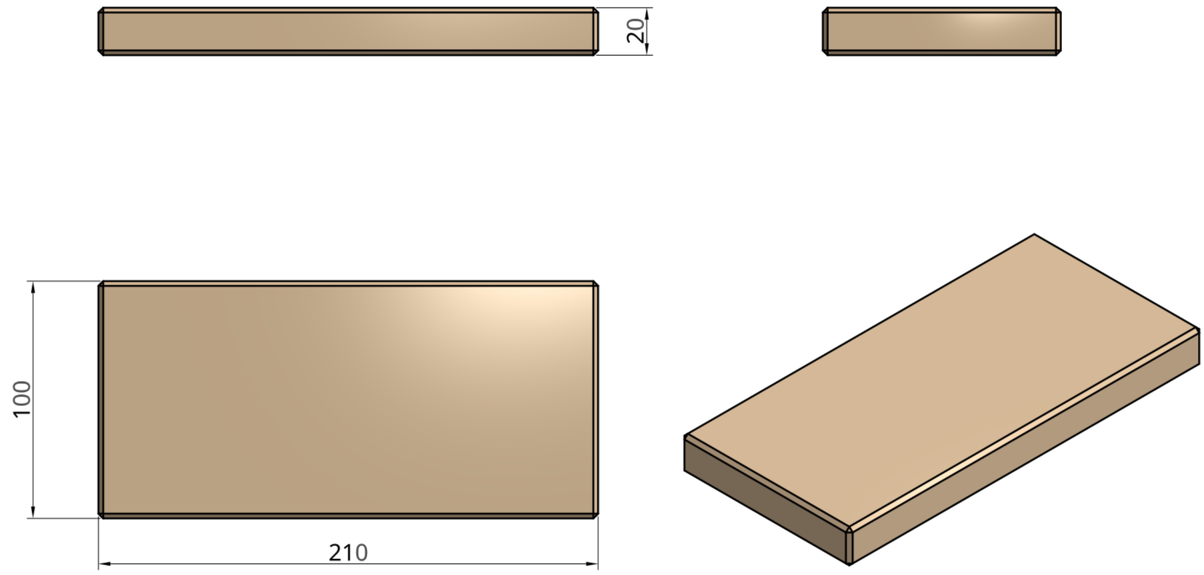


Figure 57 Top plate of the clamping block used to re-glue the syringes.

4.6.5 Pump Calibration Results

In the solid-phase peptide synthesis (SPPS) it became necessary to prepare the cleavage mix containing small amounts (< 1.0 mL) of triisopropylsilane (TIPS) and water (< 1.0 mL). Since the Chemputer pumps have been designed to operate with volumes larger than 1.0 mL, it became necessary to test their accuracy when smaller volumes are handled. In order to do so, a short test script was written that dispensed a small amount of water into a tared vial. From the measured mass of dispensed water, the volume was calculated based on the density of 0.99823 g/mL at 20 °C. The measurement was repeated 10 times with a target volume of 0.35 mL and 10 times with a target volume of 0.45 mL. The results are shown in Table 8 and Table 9. In both series repeatability was high. However, the dispensed volume was systematically too small by 0.0807 mL and 0.0856 mL when dispensing 0.35 mL and 0.45 mL, respectively. Hence, if small volumes (<1.0 mL) are dispensed an appropriate correction should be made to account for this systematic error.

Table 8 Dispensing a target volume of 0.35 mL of water.

Entry	Mass (mg)	Calc. Volume (mL)
1	270.0	0.2705
2	272.1	0.2726
3	270.1	0.2706
4	272.3	0.2728
5	266.1	0.2666
6	257.1	0.2576

7	276.7	0.2772
8	265.6	0.2661
9	269.7	0.2702
10	273.5	0.2740
Mean	269.3	0.2693
STD	5.131	0.005140
% error	1.9	
Deviation from target value		-0.0807

Table 9 Dispensing a target volume of 0.45 mL of water.

Entry	**Mass (mg)**	**Calc. Volume (mL)**
1	352.4	0.3530
2	360.3	0.3609
3	385.8	0.3865
4	366.3	0.3669
5	360.3	0.3609
6	354.0	0.3546
7	364.1	0.3647
8	356.2	0.3568
9	374.8	0.3755
10	363.5	0.3641
Mean	363.8	0.3644
STD	9.590	0.009607
% error	2.6	
Deviation from target value		-0.0856

4.7 Valve

The Chemputer uses custom-made valves (Figure 58) in the liquid handling backbone. These are controlled and powered over Ethernet.

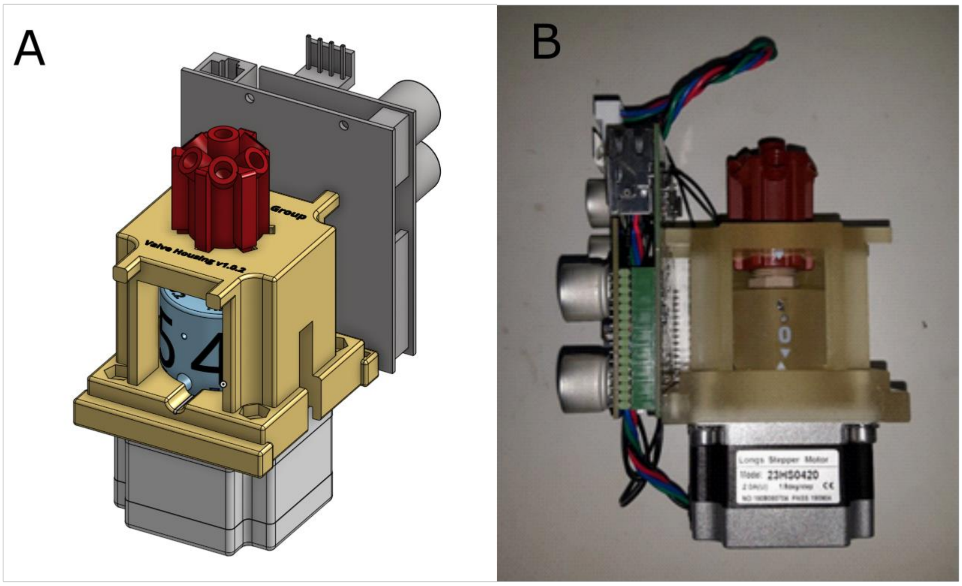


Figure 58: Chemputer valve. CAD render (A) & as built (B)

4.7.1 3D-Printing

The standard instructions for 3D printing with the Connex 3D printer were followed (consult the relevant manufacturers manual). The parts were cleaned according to the standard procedure. The STL files specifying the parts are available as supplementary files in 3D_Printed_Parts\Valve.

4.7.2 Motor Assembly

The motor is assembled as detailed in section 4.6.2

4.7.3 Valve Assembly

Table 10 Bill of materials for the valve assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0192	Valve Housing	In-house - 3D printed	N/A	1.00
CHEMP0193	Valve Motor Connector	In-house - 3D printed	N/A	1.00
CHEMP0194	6-port selection valve 0.063 red	Thames Restek	UPV-340	1.00
CHEMP0195	NEMA23 motor with D-cut shaft	Longs Motors	23HS0420	1.00
CHEMP0197	2mm dia x 4mm thick N35 Neodymium Magnet - 0.13kg Pull	First4Magnets	F412-N35	6.00
CHEMP0240	Controller PCB	SOUMAC	N/A	1.00
CHEMP0178	M3 x 8mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M3-8-A4	2.00
CHEMP0107	M3 x 12mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M3-12-A4	3.00

CHEMP0201	M4 x 8mm Cone Point Set / Grub Screws (DIN 914) - A4 Stainless Steel	AccuGroup	SSO-M4-8-A4	1.00
CHEMP0202	M5 x 16mm Full Thread Cap Head Screws (DIN 912) - A4 Stainless Steel	AccuGroup	SSCF-M5-16-A4	4.00
CHEMP0111	M3 Hexagon Nuts (DIN 934) - A4 Stainless Steel	AccuGroup	HPN-M3-A4	5.00
CHEMP0204	M4 Flat Square Nuts (DIN 562) - A4 Stainless Steel	AccuGroup	HFSN-M4-A4	1.00
CHEMP0126	M5 Hexagon Nuts (DIN 934) - A4 Stainless Steel	AccuGroup	HPN-M5-A4	4.00
CHEMP0206	M5 External Fine Tooth Locking Washers (DIN 6798A) - A4 Stainless Steel	AccuGroup	HLFW-M5-A4	4.00
CHEMP0207	SL-156 Female Crimp Terminal Contact 18AWG	RS Components	660-6039	4.00
CHEMP0116	Female Connector Housing - SL-156, 3.962mm Pitch, 4 Way, 1 Row	RS Components	756-7027	1.00
CHEMP0234	Molex Female Connector Housing - SL, 2.54mm Pitch, 3 Way, 1 Row	RS Components	670-3890	1.00
CHEMP0235	Cable Assembly, MicroClasp 4 Position Receptacle, 17.7 ", 450 mm	Farnell	15136-0405	1.00
CHEMP0078	M20-1180046 HARWIN M20 Female Crimp Terminal Contact 22AWG	RS Components	681-2887	7.00
CHEMP0198	Driver PCB	SOUMAC	N/A	1.00

The shaft coupling (a 3D-printed part) makes the physical connection between the motor shaft and the valve head. It contains six 2 mm magnets embedded above each position sign. These are used to track and re-calibrate the position of the valve during operation.

1. Six magnets (CHEMP0197) were lined up as shown in Figure 59.

Figure 59 The magnets were lined up to help tracking their orientation.

2. A pole identifier was used to identify the north pole of the magnets (Figure 60).

Figure 60 The absolute orientation of the magnets was determined with a pole identifier.

3. The north pole of the magnet was dipped into adhesive (UHU Plus Endfest 300) and inserted into the hole at positions 0, 1, 2, 4 and 5 of the shaft coupling such that the north pole faced inwards. No magnet is put into the hole at position 3 at this stage.
4. Position 3 is used as the homing position during operation of the valve. To distinguish it from the other positions, the orientation of the magnet was inverted. The last magnet was dipped in adhesive on the south pole and was inserted into the hole at position 3 such that the south pole faced inwards.
5. The orientation of the magnets was checked with the pole identifier.
6. Three hexagon nuts (CHEMP0111) were inserted into the bottom rectangular slots of the shaft coupling (Figure 61).

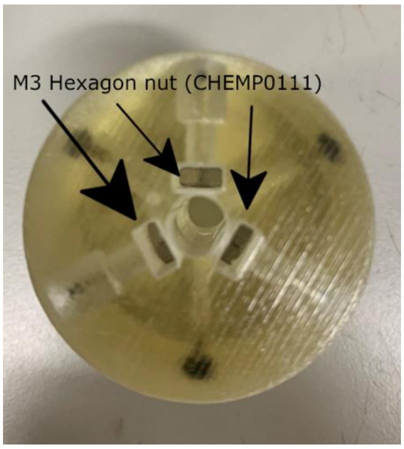


Figure 61 M3 Hexagon nuts were inserted into the bottom rectangular slots of the shaft coupling.

7. Three screws (CHEMP0107) were inserted from the side into the hexagon nuts. It is important that the screw ends do not protrude into the central channel at this stage.
8. The shaft coupling was mounted onto the shaft of the stepper motor (CHEMP0195). A gap of ca 1 mm was left between the shaft coupling and the motor housing.
9. The M3 screws were tightened.
10. A square nut (CHEMP0204) was inserted into the top rectangular slot of the shaft coupling (Figure 62).

Figure 62 A square nut was inserted into the top rectangular slot of the shaft coupling.

11. The Hall sensor board was inserted into the valve housing (3D-printed part) as shown in Figure 63.

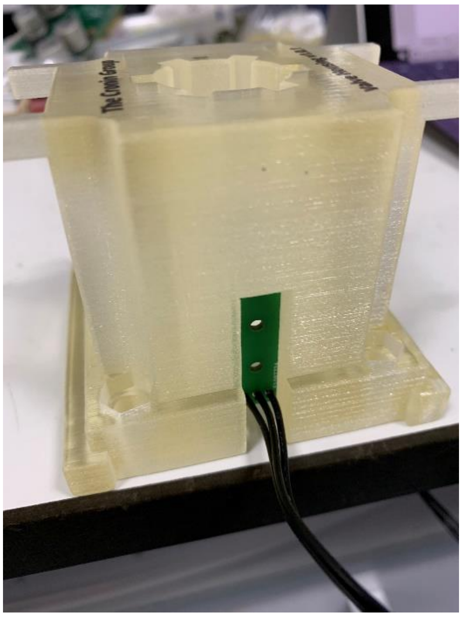


Figure 63 Insertion of the Hall sensor board into the valve housing.

12. The valve housing was mounted onto the motor with shaft coupling. The housing was oriented such that when the motor wires face away from the viewer, the Hall sensor is located on the right.
13. Hexagonal nuts (CHEMP0126) were inserted into the valve housing (Figure 64).

Figure 64 Hexagonal nuts were placed in the dedicated slot of the valve housing.

14. Socket cap head screws (CHEMP0202) with locking washers (CHEMP206) were inserted through the motor mount holes into the hexagonal nuts sitting in the valve housing (Figure 65) and tightened.

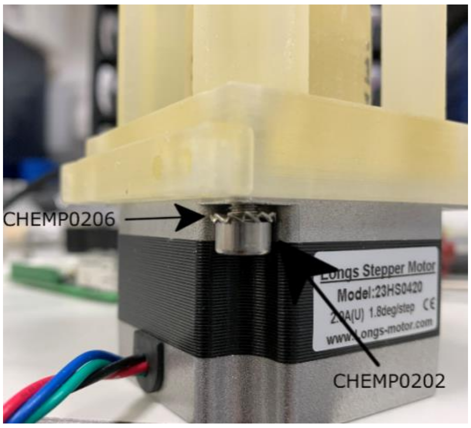
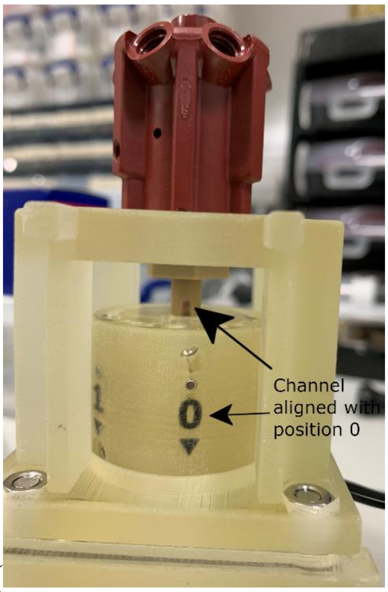


Figure 65 Cap head screws and locking washers were used to connect the valve housing and the motor housing.

15. The shaft coupling was rotated until position 0 (‘zero’) faced the opposite way from the cables of the motor. Then the valve head (CHEMP0194) was inserted. The groove

in the shaft of the valve head was aligned with the position 0 as shown



(

16. Figure 66).

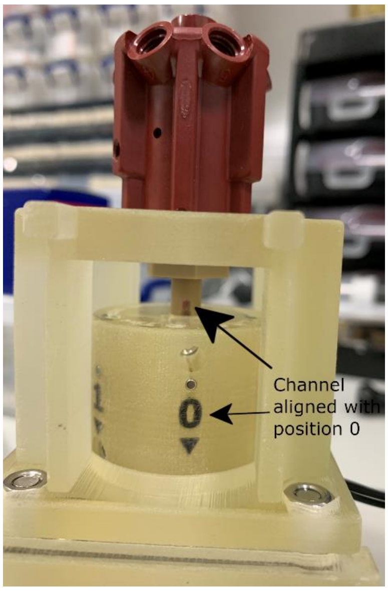


Figure 66 The shaft coupling was rotated such that it faced the opposite way than the cables connecting the motor. The groove in the shaft of the valve head was aligned with position 0.

17. The shaft coupling was secured onto the shaft of the valve head by inserting a grub screw (CHEMP0201) into the dedicated hole above position 0.
18. M3 Nuts (CHEMP0111) were inserted into the slots in the base of the valve housing (Figure 67).

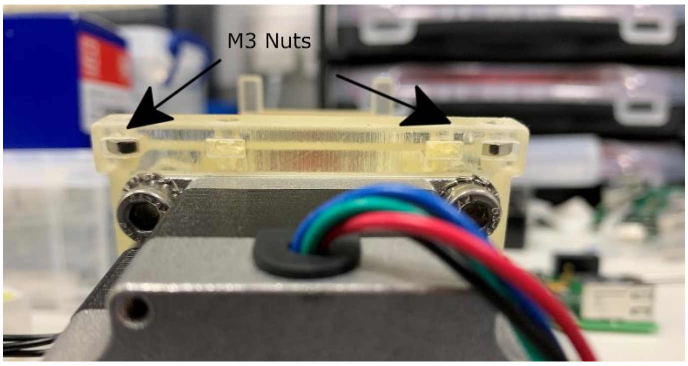


Figure 67 M3 Nuts were inserted in the dedicated slots on the bottom of the valve housing.

19. The control board was secured to the valve housing with M3 screws (CHEMP0178) as shown in Figure 68.

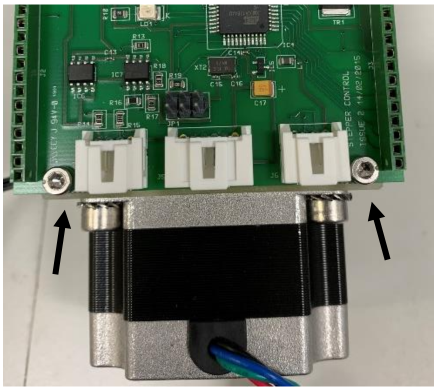


Figure 68 The control PCB was fixed to the valve housing with two M3 screws.

20. The driver board was inserted, and the shaft coupling, and Hall sensor connector were plugged in (Figure 69).

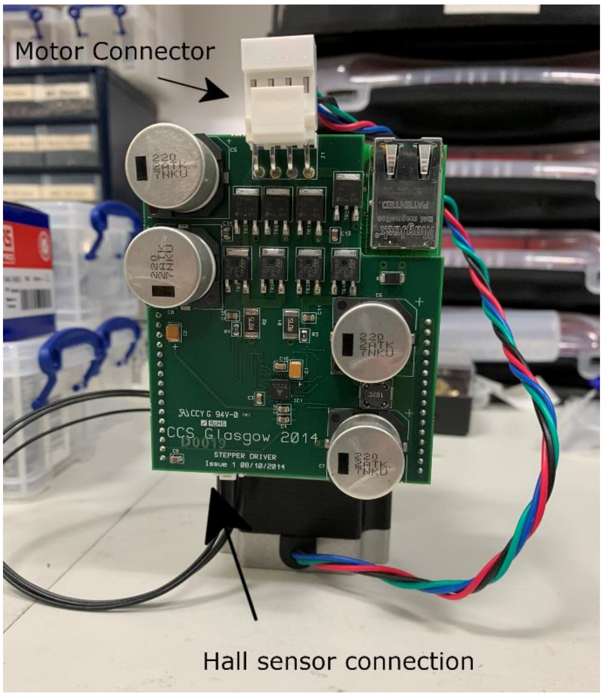


Figure 69 The complete valve with the driver board connected to the control board.

4.8 Liquid Handling Backbone

The pump and valves were connected to form the liquid handling backbone – the core of the physical instance of the Chemputer. The Chemputer architecture allows for much flexibility in terms of how the liquid handling backbone is designed. First the liquid routing is planned and visualized as a graph using a suitable graph editor (an in-house developed web app was used, as described in the Software section). This helps to highlight any possible problems with liquid routing and provides a reference schematic for the following assembly. During assembly, all tubing must be cut with an appropriate tube cutter. Do not use scissors as these squeeze the end of the tubing, which may result in leakages.

A bill of materials for the construction of the liquid handling backbone is provided, however the quantities of the parts have been omitted as the required number depends on the size and complexity of the backbone.

Table 11 Bill of materials for the liquid handling backbone assembly.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	N/A
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for	Thames Restek	UPXP-320	N/A

	1/8" OD			
CHEMP0333	Snap On Cable Marker, Pre-printed 0 9 assorted colours 2.8 3.8mm Dia.	RS Components	408-4670	N/A

1. As many pumps and valves as required (typically seven pieces each) were placed on the shelving and connected to the switch via one of the Ethernet cables (see Section 4.1).
2. A 125 mm piece of tubing (CHEMP0149) was cut and fittings and ferrules (CHEMP0220) were mounted on both ends. One such piece of tubing was prepared per neighbouring valves (typically six pieces are required). It was found very helpful to add cable markers (CHEMP0333) on each end of the tubing to indicate the number of the valve port (Figure 70).

Figure 70 Short tubing to connect valves in the liquid handling backbone.

3. The valves were connected as shown in Figure 71 with the tubes from the previous step connecting ports 4 and 5 on adjacent pumps. The port numbering convention of the valves is shown in Figure 72.

Figure 71 Connecting up the valves in the liquid handling backbone.

B

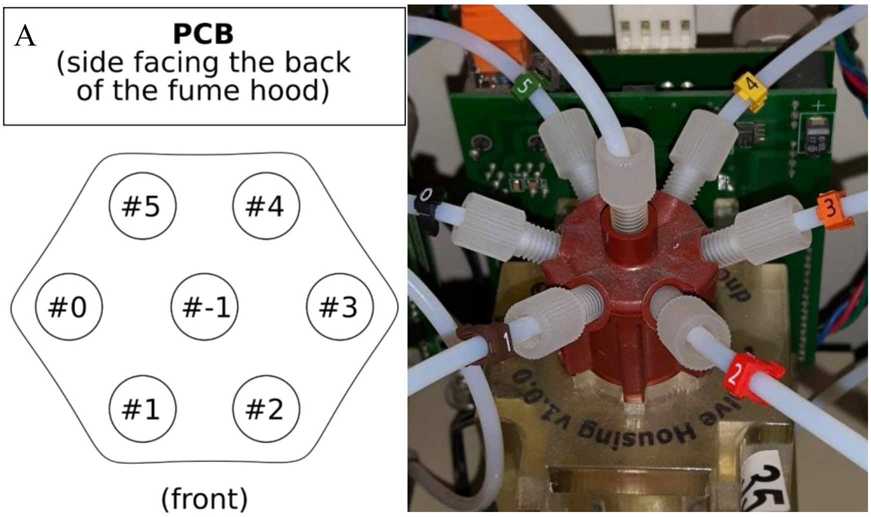


Figure 72 Port numbering convention of the Chemputer valve (A) as a scheme and (B) physically implemented. Port # -1 is the central port and typically connected to a pump.

4. A second piece of tubing (CHEMP0149) ~240 mm long was cut and fittings and ferrules (CHEMP0220) were mounted on both ends. One such piece was required per pump (typically seven).
5. The pumps were connected to port -1 of the valves as shown in Figure 73 using the tubes from the previous step.

Figure 73 Connecting up the pumps with the valves in the liquid handling backbone.

6. Tubing (CHEMP0149) for waste is cut to appropriate lengths (typically between 300 mm and 600 mm) was prepared and a fitting and ferrule (CHEMP0220) was mounted on one end. One piece of tubing was required per valve (Figure 74). Next, the tubing for the waste port was attached to the port 'zero' on each valve in the backbone (see Figure 72 for the port numbering convention). This is the only current design restriction to the configuration of the Chemputer backbone – each valve that is attached to a pump is required to have its position 'zero' connected to the waste as during the initialization of each valve and pump they switch to the home position which might cause contamination unless a waste port is used for that purpose.
7. The waste tubing is bundled and inserted into a suitable waste container (Figure 74).

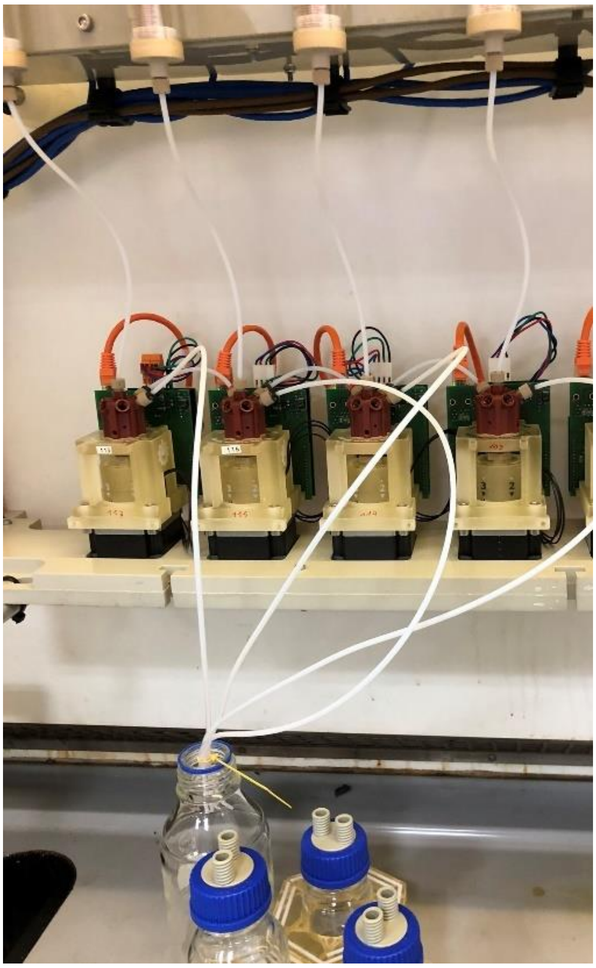


Figure 74 The port 'zero' of each valve in the backbone was connected to the waste bottle.

4.9 Inert Gas Supply

Establishing an inert atmosphere is a key step in many synthetic transformations. Hence, inert gas supply is a critical feature of any automated synthesis platform. The Chemputer architecture provides two solutions for inert gas supply. In the first approach a constant positive pressure of a protective gas is provided, while the second approach consists of a programmable pneumatic manifold (4.8.2) that functions analogous to a Schlenk line.

4.9.1 Positive Pressure Inert Gas Supply

Table 12 Bill of materials for the positive pressure inert gas supply.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0087	Speed Controller, R 3/8 Male Inlet Port x 8mm Tube Outlet Port	RS Components	815-5313	1
CHEMP0090	Air Hose Blue Nylon 8mm x 30m	RS Components	483-5030	1.2 m
CHEMP0082	10 Outlet Ports PBT Pneumatic Manifold Tube-to-Tube Fitting, Push In 4mm Outlet	RS Components	245-3052	1
CHEMP0334	Pneumatic Tubing, General Air Pressure, PUR (Polyurethane), Black, 4 mm, 2.5 mm, 0.8 MPa	Farnell	TU0425B-20	Varies
CHEMP0083	Non Return Valve, 4mm Tube 4mm Tube, -100 kPa → 1 MPa	RS Components	367-0618	Varies
CHEMP0085	Pneumatic Straight Tube-to-Tube Adapter, Plug In 4 mm	RS Components	227-5650	Varies
CHEMP0335	Tygon® ND-100-65, Non-DEHP, 1/8" ID x 3/16" OD	Cole-Parmer	WZ-95666-05	Varies
CHEMP0148	Idex CV-3324 Nonmetallic Inline Check Valve, Inlet, 1 psi, 0.06" ID, 1/4-28 (F) to 1/4-28 (M) Flat Bottom	VWR	CV-3324	Varies
CHEMP0336	Viton Tubing, 1/8"ID x 3/16"OD	Cole-Parmer	WZ-06434-02	Varies
CHEMP088	Polymer Blanking Plug	RS Components	722-047	Varies
CHEMP0084	Non Return Valve, 8mm Tube 8mm Tube, -100 kPa → 1 MPa	RS Components	367-0630	1
CHEMP0086	Pneumatic Straight Tube-to-Tube Adapter, Plug In 8 mm	RS Components	227-5688	1
CHEMP0337	PVC Flexible Tubing, Transparent, 12mm External Diameter, Reinforced, 40mm Bend Radius	RS Components	368-0182	0.6

1. The standard nozzle of the inert gas supply in the fume hood was replaced with a flow controller (CHEMP0087) and attached to *ca.* 600 mm blue nylon tubing (CHEMP0090) as shown in Figure 75.

Figure 75 Reduction valve in the fume hood to control the gas supply.

2. The inert gas was distributed using a 10-port distribution manifold (CHEMP0082) (see Figure 76). The 8mm tubing was connected to the input port of the manifold. In cases where more than one manifold was required, multiple manifolds were daisy-chained with ca 200 mm appropriate 8 mm tubing (CHEMP0090).
3. As shown in Figure 76 *ca.* 200 mm polyurethane tubing (CHEMP0334) was inserted in a free port on the distribution manifold. A high-pressure check-valve (CHEMP0083) was attached to the end of this tubing (this type of check valve has a relatively limited chemical resistance). At the other side of the check-valve a barb hose adapter (CHEMP0085) was inserted. This adapter was connected to Tygon tubing (CHEMP0335) of appropriate length (depending on the arrangement of the Chemputer in the fume hood *ca.* 300 to 600 mm). The Tygon tubing was mounted on a second low-pressure check-valve with good chemical resistance (CHEMP0148) but lower backpressure resistance. The outlet of this check-valve was attached to ca 300 mm of Viton tubing (CHEMP033). Two check-valves were used in series to achieve both good chemical resistance and high minimal tolerated backpressure. Care must be taken when mounting the check valves such that the direction of flow is away from the manifold.
4. Unused ports on the distribution manifold were blocked with a blanking plug (CHEMP088).
5. The required parts are shown in Figure 76.

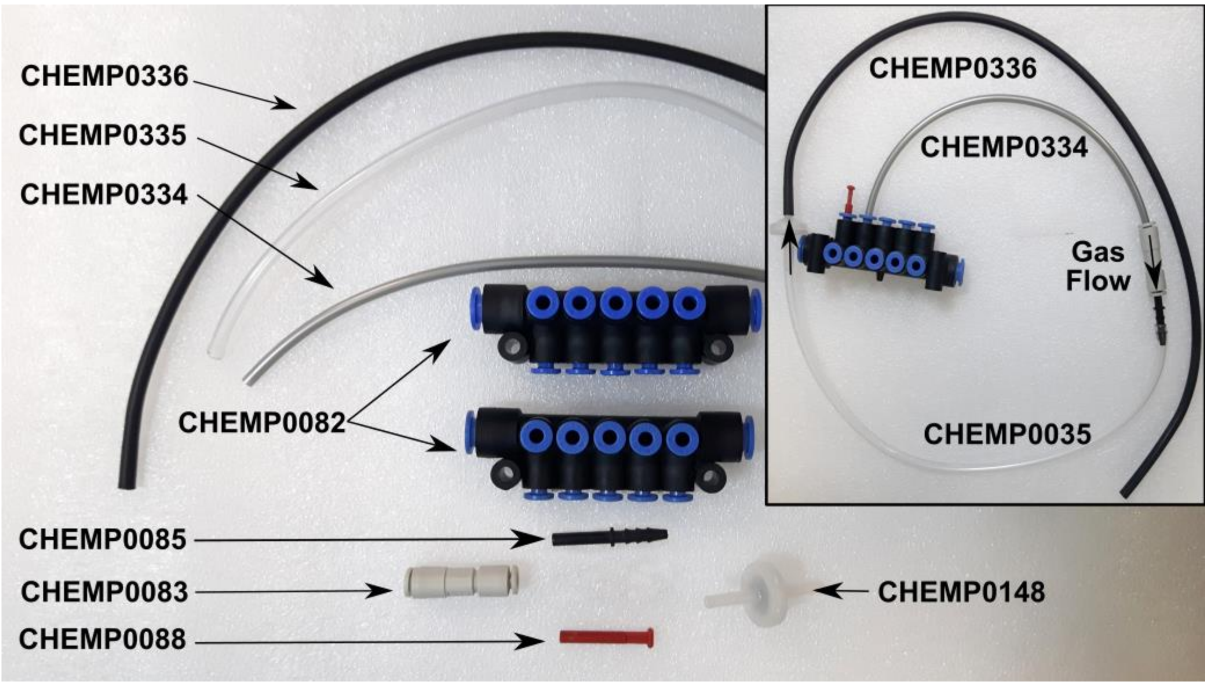


Figure 76 Assembly of the tube connections between the distribution manifold and the target vessel.

6. The Viton tubing was attached to a reagent flask cap (or analogously to a glass joint of a reactor, see Section 4.11 below) as shown in Figure 77. For the assembly of the cap see Section 4.10.

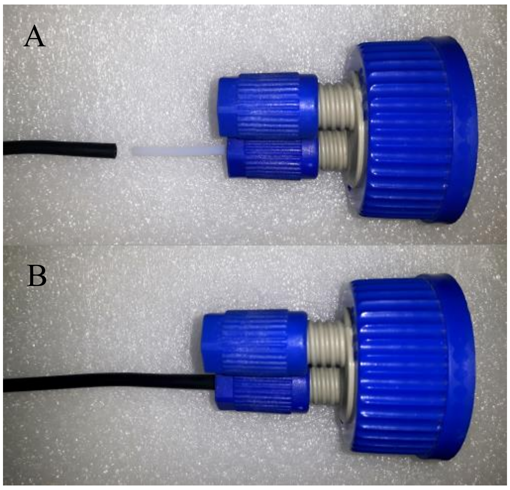


Figure 77 Connection of the inert gas tubing to a reagent bottle cap. A) The Viton tubing is slid over the protruding PTFE tubing B) to give the finished assembly.

7. The outlet of the distribution manifold was connected to a standard oil bubbler as follows (Figure 78). Approximately 600 mm nylon tubing (CHEMP0090) was attached to the outlet of the distribution manifold. A check valve (CHEMP0084) was attached to the end of the tubing. At the other side of the check valve a barb hose

adapter (CHEMP0086) was inserted. Finally, *ca.* 600 mm of PVC tubing (CHEMP0337) was used to connect the barb hose adapter to the standard bubbler.

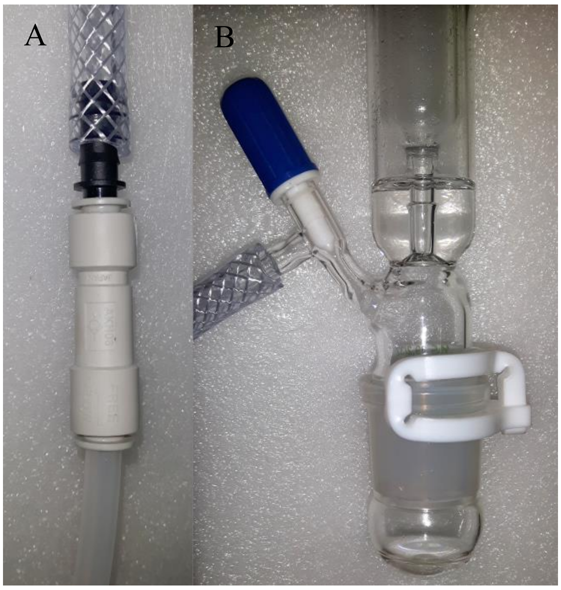


Figure 78 A) Connection of the distribution manifold to B) a standard bubbler.

4.9.2 **Programmable Manifold**

Table 13 Bill of Materials for the programmable manifold

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0280	Series V100, S41 Type 6-position Manifold	SMC	VV100-S41-06-M5	1
CHEMP0281	3 Port Direct Operated Solenoid Valve - Large flow	SMC	V114A-6LOU	6
CHEMP0282	One-touch Fitting Nickel Plated - 4 mm Hexagon socket head male connector	SMC	KQ2S04-M5N	12
CHEMP0284	One-touch Fitting White Color - 6 mm Male Elbow	SMC	KQ2L06-M5N	3
CHEMP0283	One-touch Fitting White Color - 4 mm Male Elbow	SMC	KQ2L04-M5N	7
CHEMP0285	3 Port Direct Operated Solenoid Valve - Large flow - with backplate	SMC	V114A-6LOU-M5	6
CHEMP0286	One-touch Fitting White Color - 6mm -6mm- M5 Male Branch Tee	SMC	KQ2T06-M5A	4
CHEMP0287	One-touch Fitting White Color - 6-6-8 Different Diameter Tee	SMC	KQ2T06-08A	1
CHEMP0338	FEP Fluoropolymer tubing 6mm	RS Components	2550255657	Varies

CHEMP0291	One-touch Fittings Manifold Series - Port A One-touch Fitting, Port B One-touch Fitting	SMC	KM11-04-08-10	1
CHEMP0339	Pneumatic Straight Tube-to-Tube Adapter, Plug In 6 mm	RS Components	771-5614	2
CHEMP0331	M2.5 x 12mm Hex Socket Cap Screw Black, Self-Colour Steel	RS Components	281-653	12
CHEMP0110	M4 x 30mm Cap Head Screws (DIN 912) - A4 Stainless Steel	Accu	SSC-M4-30-A4	4
CHEMP0128	M4 x 10mm Low Head Cap Screws (DIN 7984) - A4 Stainless Steel	RS Components	186-636	
CHEMP0289	Finger Valve 6-6	SMC	VHK3-06F-06F	1
CHEMP0293	In-line Air Filter with One-touch Fitting	SMC	ZFC54-B	1
CHEMP0127	M4 x 10mm Low Head Cap Screws (DIN 7984) - A4 Stainless Steel	Accu	SSCL-M4-10-A4	4
CHEMP0174	D-Link DPE-301GS PoE Splitter	Insight	6338009	1
CHEMP0303	PCB hex spacer 20 mm	RS Components	125-6018	4
CHEMP0309	Rotameter, 0.04-0.5 LPM air, with valve	Omega	FL-2011	1
CHEMP0310	Rotameter, 0.4-5 LPM air, without valve	Omega	FL-2013-NV	1
CHEMP0341	Threaded-to-Tube Elbow Connector Uni 1/8 to Push In 6 mm	RS Components	771-5386	4
CHEMP0311	Rotameter spacer for pneumatic panel	In-house - laser cut	N/A	10
CHEMP0295	Hex socket head cap screw M5x0.80 x 45	Accu	SSCF-M5-45-A4	4
CHEMP0249	M4 x 20mm Penny Washers - A2 Stainless Steel	Accu	HYW-M4-20-A2	4
CHEMP0290	Regulator Single Unit Type	SMC	ARM5SA-08-A	2
CHEMP0294	One-touch Fitting White Color - Different diameter union "Y" 8-6-6	SMC	KQ2U06-08A	1
CHEMP0302	Regulator spacer for pneumatic panel	In-house - laser cut	N/A	6
CHEMP0297	Hex socket head cap screw M3x0.50 x 50 x 18	Accu	SSC-M3-50-A2	2
CHEMP0340	M3 x 16mm Full Thread Cap Head Screws (DIN 912) - A2 Stainless Steel	Accu	SSCF-M3-16-A2	2
CHEMP0292	Speed Controller, One-touch Fitting, In-line, Compact	SMC	AS2002F-06	1

CHEMP0288	Shuttle Valve with One-touch Fitting Series	SMC	VR1210F-06	1
CHEMP0342	Non Return Valve, 6mm Tube 6mm Tube, -100 kPa → 1 MPa	RS Components	367-0624	1
CHEMP0088	Polymer Blanking Plug	RS Components	722-047	Varies
CHEMP0308	SMC Cable, Plug, 600mm	RS Components	701-3019	12
CHEMP0418	Arduino Mega Rev3	RS Components	715-4084	1
CHEMP0343	M4 x 8mm Socket Button Screws (ISO 7380) - Black A2 Stainless Steel	Accu	SSB-M4-8-A2-BL	4
CHEMP0299	Hex spacer 60 mm	RS Components	664-3303	4
CHEMP0344	M4 x 8mm Vented Socket Cup Point Set / Grub Screws (DIN 916) - Black A2 Stainless Steel	Accu	SSUV-M4-8-A2-BL	4
CHEMP0300	Thumb screw	Accu	SKT-M4-12-A1	4

The programmable manifold was designed with the typical synthetic chemists' needs in mind:

- Providing positive inert gas overpressure
- Providing an option to evacuate the vessel for filtration / inert gas re-fill
- Providing pressure differential between arbitrary outlets to perform cannula transfer-type manipulations

In order to provide all the above, the system was designed as a stack of two rows of six 2/3 electromagnetic valves. The frontend row (referred to as frontend valve manifold hereafter) of valves switches between vacuum (normally closed) and the output of the backend row of valves (referred to as backend valve manifold hereafter). The backend valve manifold switches between low-pressure (normally open) and the high-pressure inert gas supply. Additionally, the high-pressure inert gas supply can be manually switched between the high-pressure gas and ambient air for vacuum relief/drying purposes. When all valves are de-energized, the system provides low pressure inert gas on all 6 outputs. Excessive pressure is vented to the atmosphere through the outlet flow meter and the check valve. The valves are controlled by an Arduino board with custom-made shield hosting 12 MOSFETs and an Ethernet-to-serial converter. The programmable manifold can be supplied with up to 8 bar of inert gas.

1. The back panel was laser cut from 6mm acrylic. The front panel was cut from the 4mm acrylic to save the weight. To align the knobs of the regulators flush with the front panel, stacks of small rectangular shims were put between the regulators and the

back panel during assembly. The shims were cut from the same 6mm thick acrylic sheet. All DXF files can be found as supplementary files in Laser_Cut_Parts\Programmable_Manifold.

2. The holes in the back panel were tapped with M2.5, M3 and M4 threads according to the scheme shown in Figure 79. The four unmarked larger holes are through-holes for M5 screws and were not tapped.

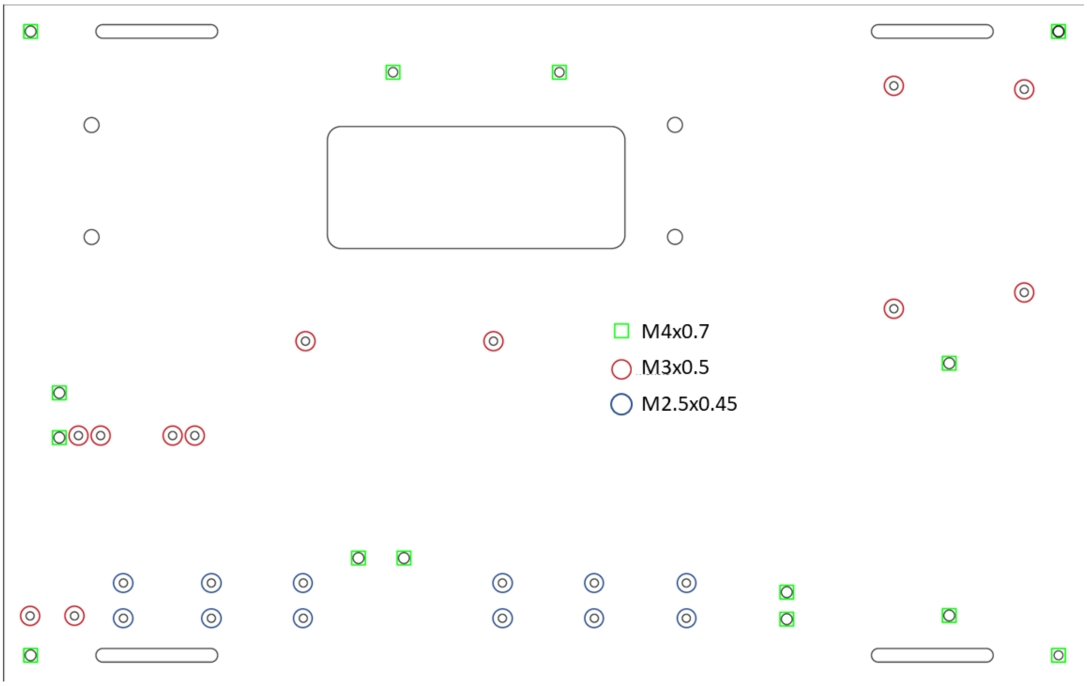


Figure 79 Tapping scheme for the back panel of the programmable manifold.

3. For the backend valve manifold, six valves without backplates (CHEMP0281) were mounted on a manifold base (CHEMP0280) as shown in Figure 80.

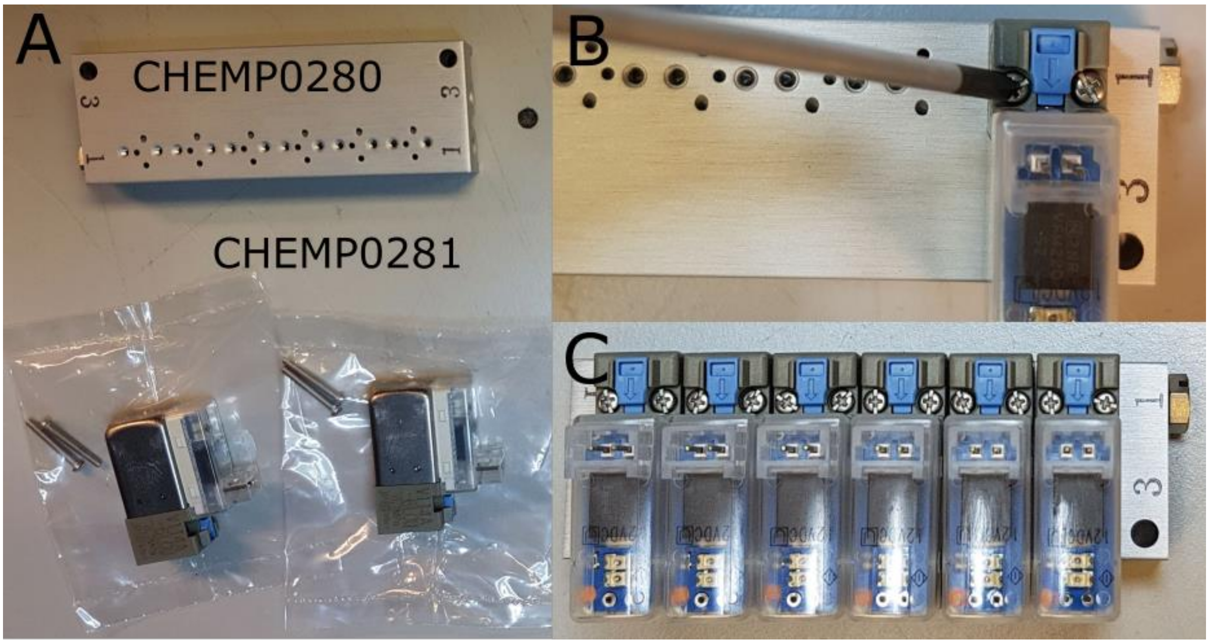


Figure 80 Assembly of the backend valve manifold: A) the source parts, B) mounting of the first valve on the manifold base and C) the fully assembled valve manifold.

4. Next, six straight fittings (CHEMP0282) were mounted to the manifold base "port 2" outlets as shown in Figure 81.

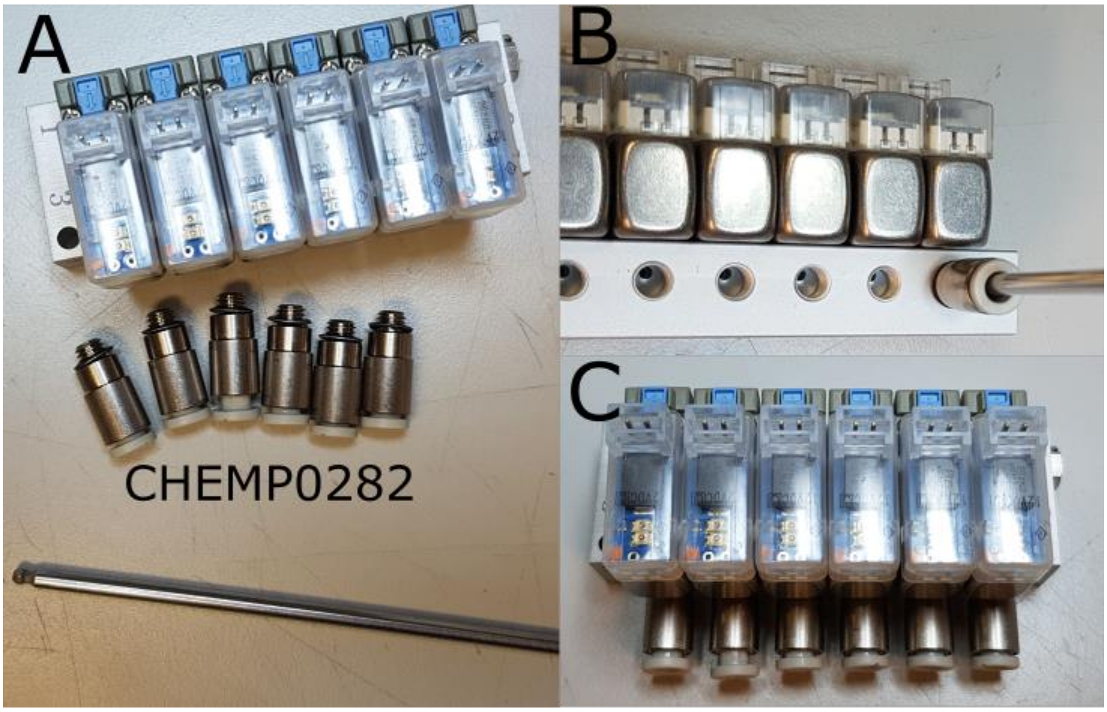


Figure 81 Installation of straight fittings to the backend valve manifold: A) the source parts, B) mounting of the first fitting on the manifold base and C) the finished assembly with all fittings.

5. Then the manifold inlets were installed as shown in Figure 82. On the left-hand side an angled 6 mm M5 fitting (CHEMP0284) was attached to "port 1" and on the right-

hand side an angled 4 mm M5 fitting (CHEMP0283) was attached to “port 3”. The unused ports were sealed with blanking plugs, which can be made from short M5 screws and O-rings (Figure 82 C and D).

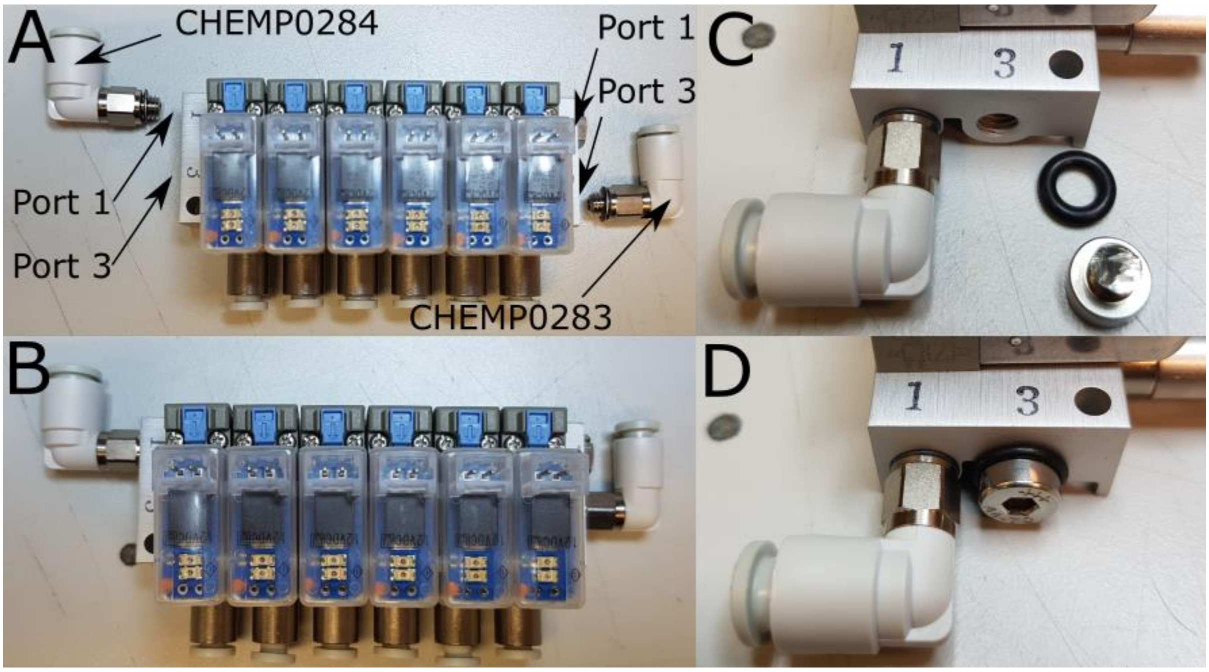


Figure 82 Assembly of the connections to the backend manifold (A and B) and sealing unused ports with a blanking plug (C and D).

6. The frontend valve manifold was assembled as shown in Figure 83. The four central valves with backplate (CHEMP0285) were fitted with T-pieces (CHEMP0286) on “port 1”, straight fittings (CHEMP0282) on “port 2”, and angled fittings (CHEMP0283) on “port 3”, as shown in Figure 83 A and B. The two side valves were assembled in the same way as the four central valves but with angled fittings (CHEMP0284) instead of the T-pieces (Figure 83 C) at “port 1”. The assembled valves were connected with six ca 15 mm bits of polyurethane tubing (CHEMP0338) to a central T-piece (CHEMP0287) as shown in Figure 83 D.

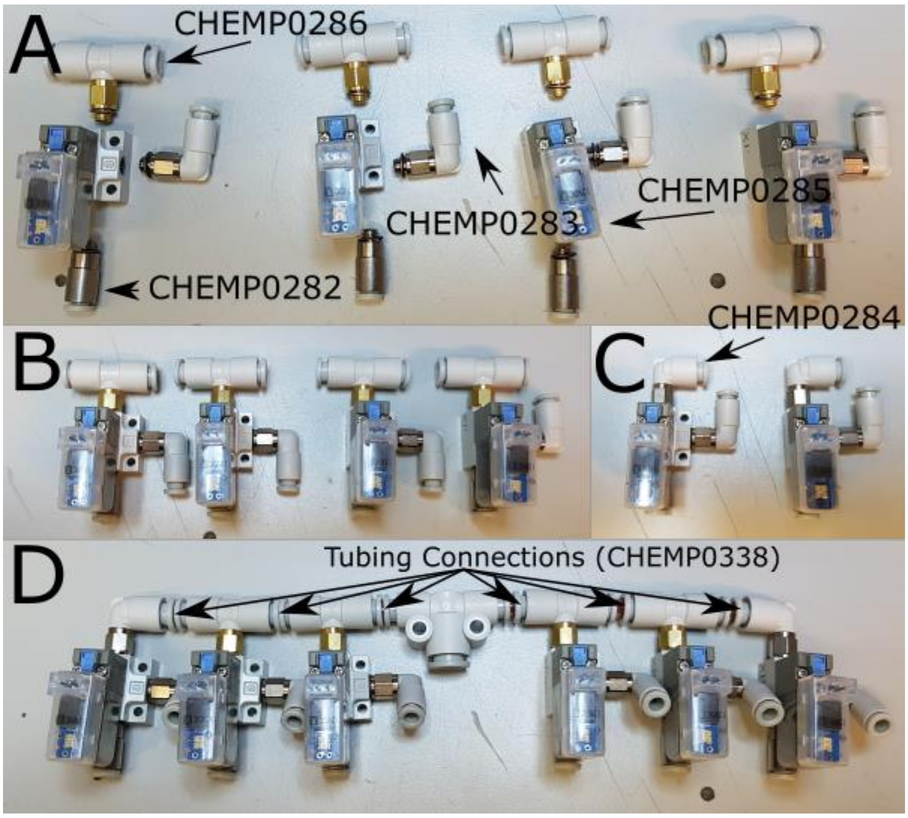


Figure 83 Assembly of the frontend valve manifold: A) source parts for the four central valves, B) the four central valves assembled, C) all six fully assembled valves and D) the finished fronted valve manifold.

7. The inlet ports of the low-pressure inert gas distribution manifold (CHEMP0291) were fitted with straight tube-to-tube 8-to-6 mm reducer adaptors (CHEMP0339) as shown in Figure 84.

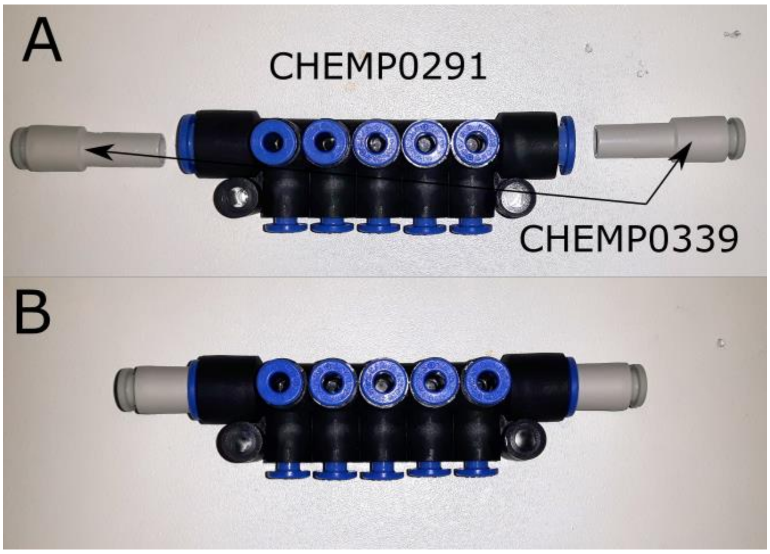


Figure 84 Assembly of distribution manifold: A) the source parts and B) the finished assembly.

8. The backend and frontend valve manifold were mounted on the back panel using M2.5 (CHEMP0331) and M3 (CHEMP0340) screws, respectively, as shown in

Figure 85. Next, the distribution manifold was mounted using M4 x 30 mm screws (CHEMP0110) with M4 washers (CHEMP0128), a high-pressure gas shut-off valve (CHEMP0289) was mounted using M4 x 30 mm screws (CHEMP0110) with M4 washers (CHEMP0128) and a holder for the air filter (CHEMP0293) was mounted using M4 x 10 mm screws (CHEMP0127). At this stage also a PoE splitter (CHEMP0174) was attached to the back panel using M4 x 10 mm screws (CHEMP00127) and M4 washers (CHEMP0128). Last, four PCB spacers (CHEMP0303) were screwed into the holes above the splitter.

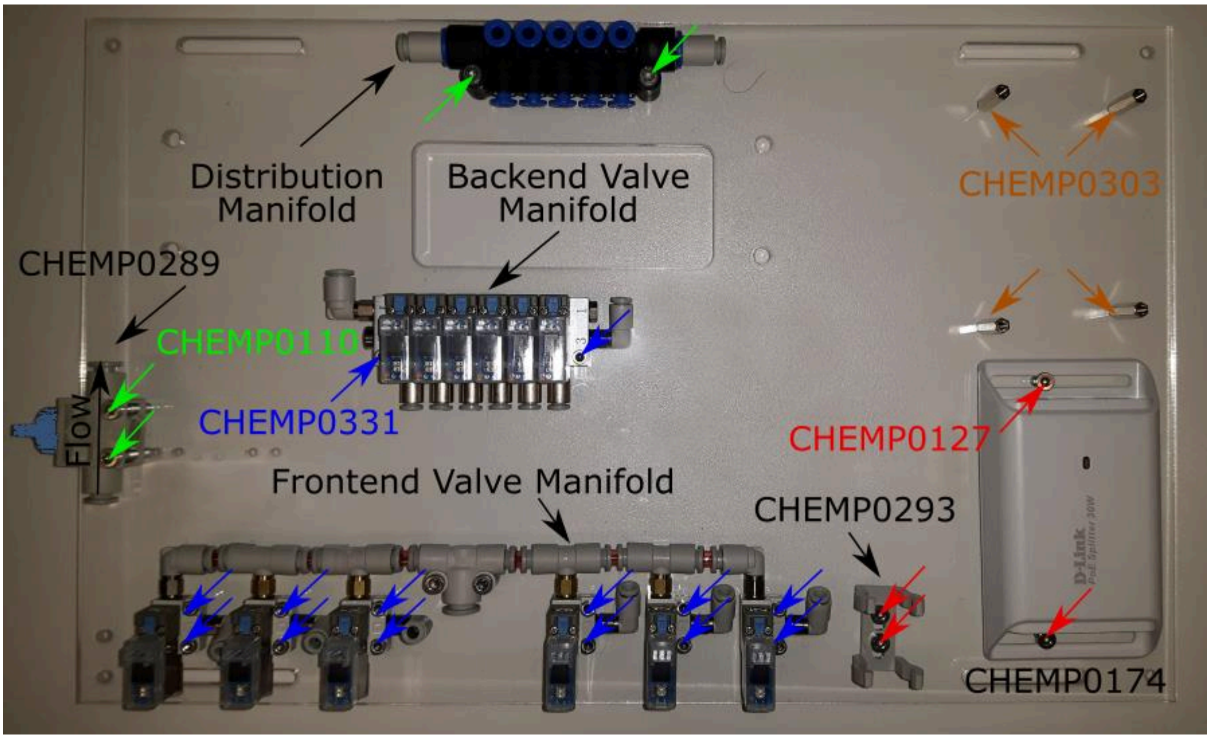


Figure 85 The previously assembled backend and frontend valve manifolds were mounted on the back panel along with other parts as shown.

9. One acrylic flow meter with valve (CHEMP0309) - which serves to adjust the low-pressure inert gas inlet flow - and one without valve (CHEMP0310) – which serves to monitor the outlet gas flow – were equipped with angled fittings (CHEMP0341) as shown in Figure 86.

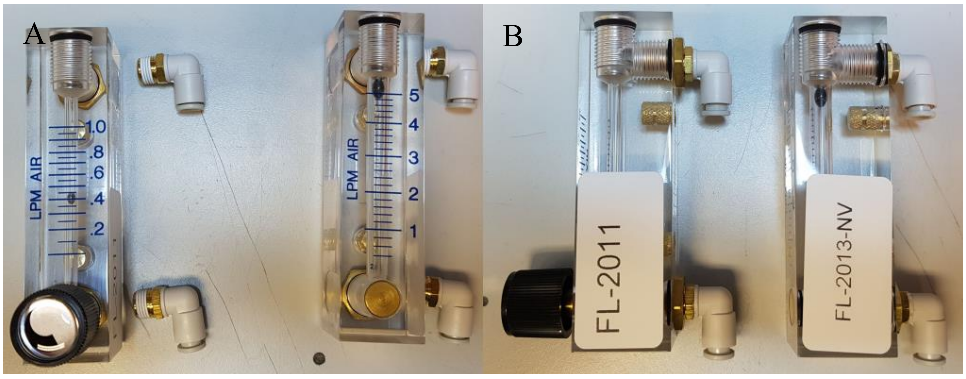


Figure 86 Assembly of the two acrylic flow meters: A) the source parts and B) the finished assemblies.

10. The flow meters were mounted on the back panel (Figure 87). These were fixed using 5 x 6 mm acrylic spacers (CHEMP0311, the DXF file is available as supplementary material in Laser_Cut_Parts\Programmable_Manifold) and M5 x 45 mm screws (CHEMP0295) with washers (CHEMP0249) as shown in inset A and B of Figure 87.

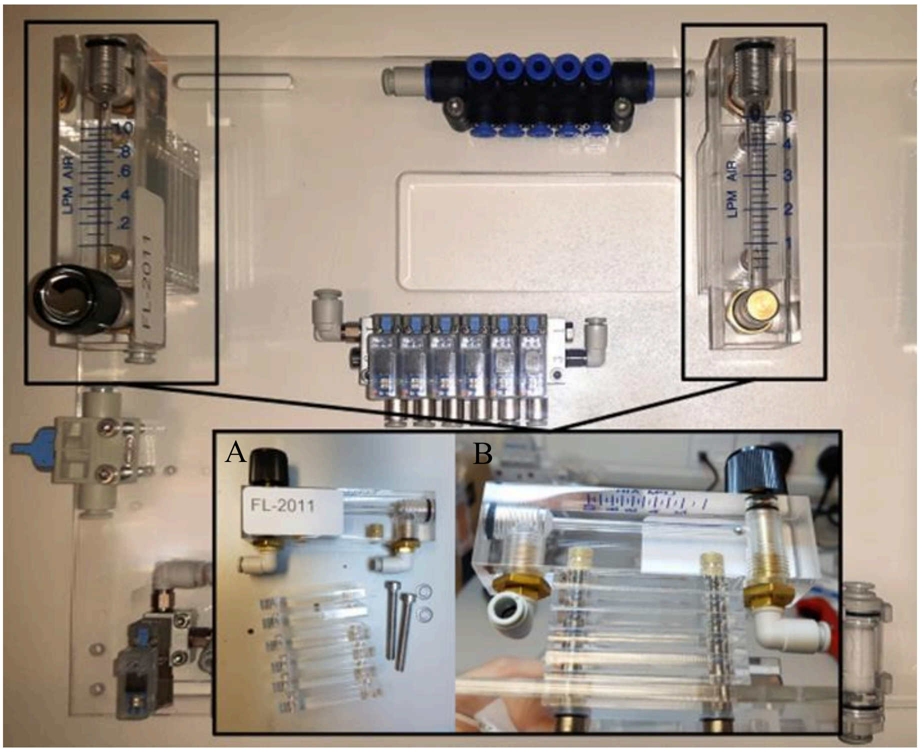


Figure 87 Mounting the two flow meters onto the back panel.

11. Next, two pressure regulators (CHEMP0290) and one Y-piece (CHEMP0294) were assembled with tubing (CHEMP0338) as shown in Figure 88 A including the length of individual pieces. Three 6mm thick acrylic spacers (CHEMP0302, the DXF file is available as supplementary material in Laser_Cut_Parts\Programmable_Manifold) were used to position the pressure regulators flush with the front panel. 2 x M3 x 50 mm screws (CHEMP0297) were used to secure the regulators through the spacers.

The tubing from the low-pressure line regulator was connected to the left-hand side flow meter and the regulator was screwed to the back panel (as shown in Figure 88 B and inset). Then the long tubing from the inlet of the high-pressure line regulator was connected to the outlet of the high-pressure gas shut-off valve and the regulator was screwed to the back panel (Figure 88 C). Finally, the short tubing from the Y-piece was connected to the inlet of the high-pressure gas shut-off valve, the long tubing was connected to the inlet of the low-pressure line regulator and the Y-piece was attached to the back panel with M3 x 16 mm screws (CHEMP0340).

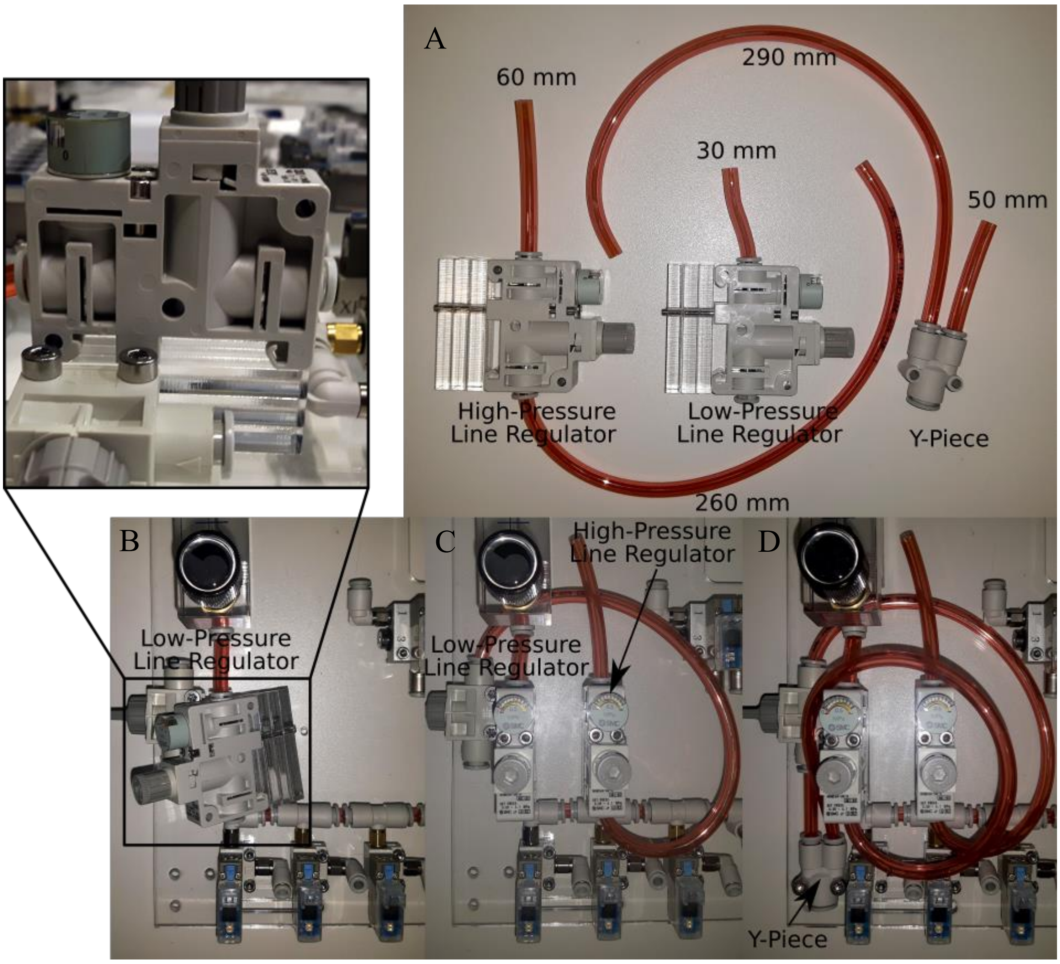


Figure 88 Assembly of the pressure regulators for the high- and low-pressure inert gas lines. A) Assembly of the two pressure regulators and the Y-piece with tubing of appropriate length. B) to C) shows mounting and connectivity between the two pressure regulators and the Y-piece.

12. The backend and frontend valve manifolds were connected with polyurethane tubing (CHEMP0338) of appropriate length as shown in Figure 89. After that, the "port 3" of the backend valve manifold was connected to the black distribution manifold with 120 mm of the same type of tubing. The outlet of the left-hand side flow meter was connected to the inlet of the distribution manifold with 90 mm of 6 mm polyurethane

tubing (CHEMP0338). Then the air filter (CHEMP0293) was mounted in the dedicated holder.

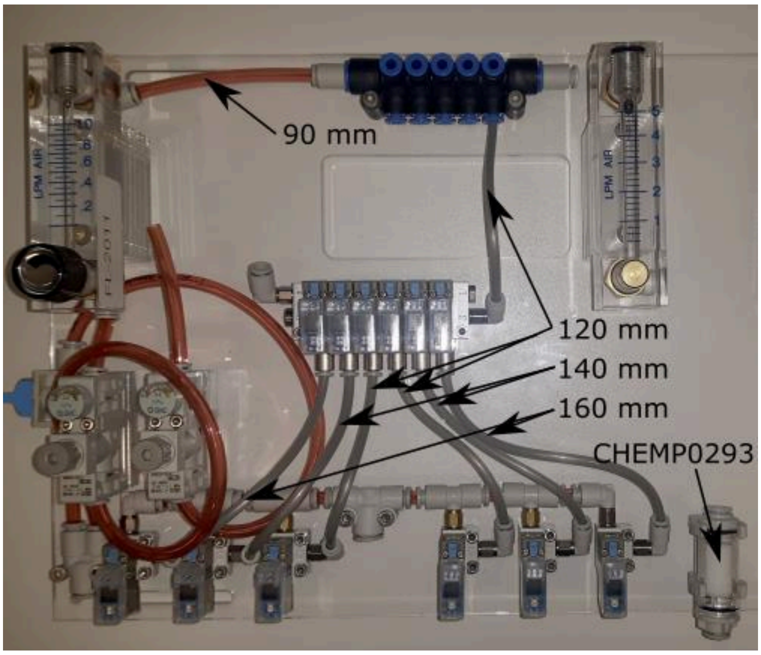


Figure 89 Connections between the backend and frontend valve manifold.

13. The outlet of the high-pressure flow regulator (CHEMP0292) was connected to the bottom inlet of the shuttle valve (CHEMP0288) with 6 mm polyurethane tubing of appropriate length as indicated in Figure 90A. The inlet of the flow regulator was connected to the outlet of the high-pressure line regulator, the outlet of the shuttle valve was connected to the "port 1" of the backend valve manifold and the second inlet of the shuttle valve was connected to the air filter (Figure 90 B). The outlet of the distribution manifold was connected to the inlet of the exhaust flow meter with 6 mm polyurethane tubing. A check-valve (CHEMP0342) was attached to the outlet of this flow meter via a short piece of tubing. Unused ports in the distribution manifold were plugged using a blanking plug (CHEMP0088).

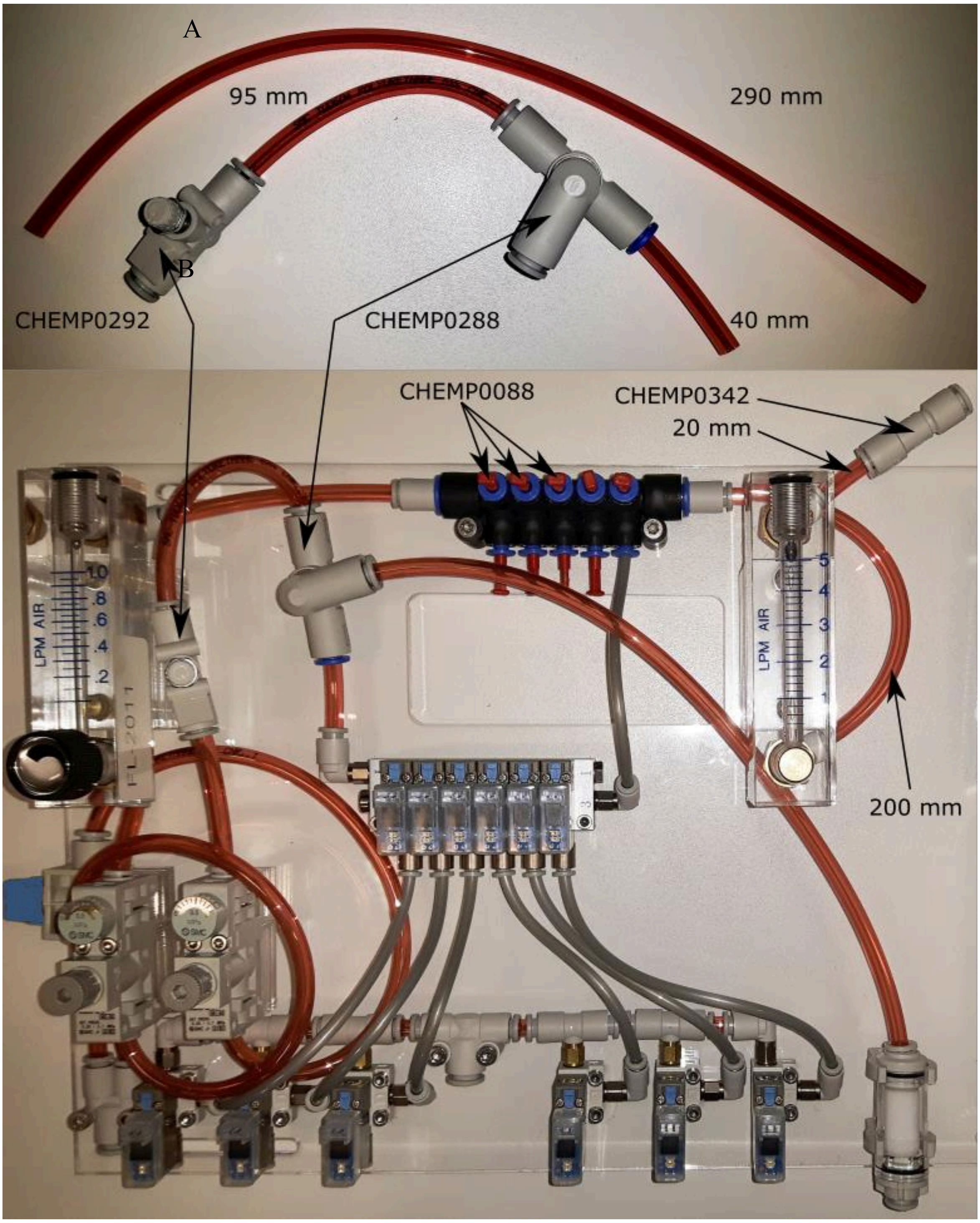


Figure 90 Assembly of the high-pressure line flow regulator and shuttle valve. If the high-pressure gas shut-off valve is closed, the shuttle valve connects to the air filter. If the high-pressure gas shut-off valve is open, the shuttle valve connects to the high-pressure inert gas supply.

14. In order to control the valve switching, an Arduino (CHEMP0035) fitted with a custom-designed MOSFET shield was used. The Arduino was secured using 4 x 8mm screws (CHEMP0343). The shield was carrying 12 MOSFETS along with an Ethernet-to-serial converter (full details on the Arduino firmware, the design of the

MOSFET shield and detailed assembly instructions are available on request). As long as the designed used was an early prototype with some flaws discovered during testing, exact build & assembly instructions are not presented here, however they are available from authors upon request. As a fully functional replacement, 12 Sparkfun MOSFET modules (https://www.sparkfun.com/products/12959) along with a standard Arduino Ethernet shield (https://www.sparkfun.com/products/11166) can be used.

15. Each valve was equipped with the connection cable (CHEMP0308). The cables were routed along the panel to the location of the MOSFET connectors, cut to length if required and connected.
16. In each corner, a 60 mm M4 hexagonal spacer (CHEMP0299) was attached using socket set screws (CHEMP0344) as shown in Figure 91 (highlighted with blue rectangles). Further, two Openbuilds screws with nuts (Ooznest) were inserted into the dedicated slots at the top of the back panel (Figure 91 – highlighted with blue rectangles). The Openbuilds screws would allow the manifold to be attached to the shelving frame of the Chemputer (see Section 4.1).

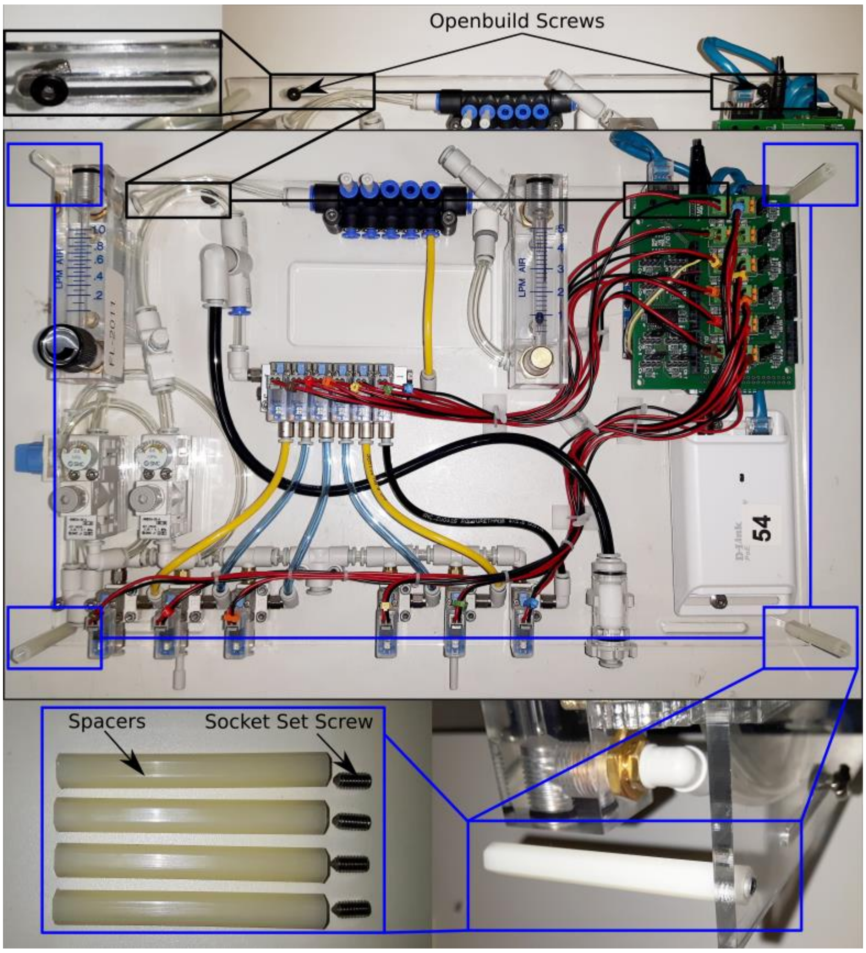


Figure 91 Mounting of hexagonal spacers in each corner and Openbuilds screws with nuts in the dedicated slots.

17. Finally, the manifold was mounted in the fume hood (Figure 92). The back panel was attached to the shelving frame at two points as shown. The inert gas supply was attached to the Y-piece at the bottom left corner of the manifold and the vacuum line was attached to the central T-piece in the frontend valve manifold. The output lines from the frontend valve manifold (bottom) were connected to the target vessel according to the corresponding graph file. Unused valve outlets were blocked with blanking plug (CHEMP088). The inert gas distribution manifold was connected to the reagent vessels in the same way and again unused ports were blocked. Finally, the front panel was attached to the four hexagonal spacers with four thumb screws (CHEMP0300).

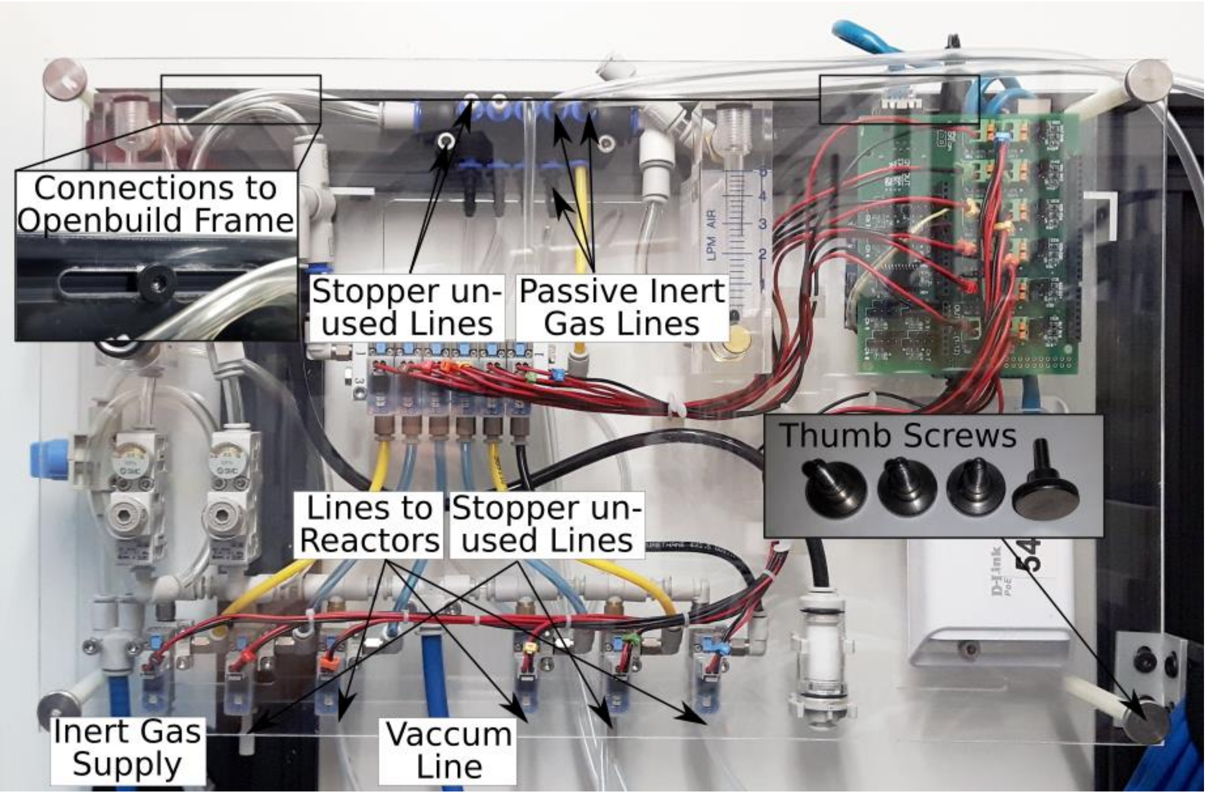


Figure 92 The programmable manifold was attached to the shelving frame. The inert gas supply and the vacuum line were connected as shown. All other connections were made as specified in the corresponding graph file. Unused ports were blocked with stoppers.

4.10 Reagent Bottles

Two types of reagent bottle setups were used. The standard setup allows for keeping the reagent under a positive pressure of argon if needed. In the advanced setup, the flask can additionally be chilled and stirred (that is used, for example, when a solution needs to be freshly prepared from a solid reagent just before use).

4.10.1 Standard Reagent Bottle Setup

Table 14 Bill of Materials for the standard reagent bottle

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0229	Screw cap, GL 45, PP, with two ports GL 14	VWR	554-3000	1 per bottle
CHEMP0256	Laboratory bottles, round, with screw cap, DURAN, 100ml	VWR	215-1514	Varies
CHEMP0257	Laboratory bottles, round, with screw cap, DURAN, 250ml	VWR	215-1515	Varies
CHEMP0258	Laboratory bottles, round, with screw cap, DURAN, 500ml	VWR	215-1516	Varies
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID	Kinesis	008T32-150-100	Varies
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	1 per bottle
CHEMP0156	Insert for screw cap GL14, 3,2 mm inner diameter	VWR	554-3007	2 per bottle
CHEMP0052	GL14 screw cap for tube connection, blue	VWR	554-3004	2 per bottle

1. One reagent bottle cap port (CHEMP0229) was connected to a free valve port in the liquid handling backbone according to the definition in the graph file. PTFE Tubing (CHEM0149) of appropriate length (ca 600 mm) was prepared and a fitting and ferrule (CHEMP0220) was mounted on one end. The tubing was attached to the valve port as specified in the graph file of the Chemputer instance that was to be built.

2. The tubing coming from the valve from the previous step was routed to the reagent flask. Then it was fed through a GL 14 screw cap (CHEMP0052) and insert (CHEMP0156) as shown in Figure 93. The GL 14 screw cap was mounted onto the GL 45 screw cap (CHEMP0229), which was screwed on a bottle of appropriate size. The tubing must reach the bottom of the flask.

Figure 93 Tubing connection to a standard reagent bottle.

3. A second assembly of tubing (ca 50 mm), GL 14 screw cap and insert, was attached to the remaining GL14 port on the GL 45 screw cap. The tubing must only reach into the headspace of the flask and ca 30 mm should protrude from the screw cap assembly. This outlet was attached to a passive low-pressure argon line as specified in Section 5 if the contents of the reagent bottle were air sensitive.

4.10.2 Advanced Reagent Bottle Setup

Table 15 Bill of Materials for the advanced reagent bottle

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0229	Screw cap, GL 45, PP, with two ports GL 14	VWR	554-3000	1 per bottle
CHEMP0256	Laboratory bottles, round, with screw cap, DURAN, 100ml	VWR	215-1514	Varies
CHEMP0257	Laboratory bottles, round, with screw cap, DURAN, 250ml	VWR	215-1515	Varies
CHEMP0258	Laboratory bottles, round, with screw cap, DURAN, 500ml	VWR	215-1516	Varies
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID	Kinesis	008T32-150-100	Varies
CHEMP0220	Flangeless Fitting Natural,	Thames Restek	UPXP-320	1 per bottle

	Polypropylene,1/4-28 Flat-Bottom for 1/8" OD			
CHEMP0156	Insert for screw cap GL14, 3,2 mm inner diameter	VWR	554-3007	2 per bottle
CHEMP0052	GL14 screw cap for tube connection, blue	VWR	554-3004	2 per bottle
CHEMP0347	Cooling jacket for advanced reagent bottle	In-house glassware	N/A	1
CHEMP0348	Fisherbrand microstirrer magnetic stirrer	Fisher Scientific	11765694	1

The advanced reagent bottle setup builds on the standard setup. It provides additional stirring with a magnetic stirring plate, and low/high temperature storage capability.

1. A standard reagent bottle was assembled as detailed above.
2. The bottle was placed inside a cooling jacket (CHEMP0347; drawings can be found in the supplementary folder Various\).
3. The cooling jacket with bottle was placed on a magnetic stirrer (CHEMP0348) as shown in Figure 94. The stirrer cannot be controlled remotely and was therefore switched on before the automated run was started and left on for the rest of the synthesis.

Figure 94 Advanced reagent bottle setup.

4. The cooling jacket was connected to a recirculating chiller (see Section 4.12 for details).

4.11 Chemputer Reactor

The Chemputer used the three-neck round bottom flask as the standard reactor based on the Chemputer paradigm to mimic the manual organic chemistry workflow. In addition to the usual round bottom flask as the reaction vessel, a reactor more suitable for solid-phase peptide synthesis (SPPS) was developed.

4.11.1 Standard Reactor

Table 16 Bill of Materials for the standard reactor

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0055	IKA RET control-visc, hotplate stirrer	IKA	0005020002	1.00
CHEMP0045	Bosshead, 8x8mm	VWR	241-0128	1.00
CHEMP0058	H 16 V Support rod	IKA	0001545100	1.00
CHEMP0060	Flask clamp, zinc die-cast, powder coated 0 - 80 mm	VWR	241-7549	1.00
CHEMP0137	Reflux Condenser	Asynt	ASYNGB-C-350-B24	1.00
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	120.00
CHEMP0350	GL18 screw cap for tube connection, blue	VWR	215-2082	2.00
CHEMP0053	Insert for screw cap GL 18, 3,2 mm inner diameter	VWR	215-2077	2.00
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	1.00 (optional)
CHEMP0135	250 ml 3-neck flask - 24/29 (Centre), 14/23 (side)	Fisher Scientific	N/A	1.00
CHEMP0061	GL18 to B14 connector	VWR	201-1642	2.00
CHEMP0226	PTFE Sleeve	VWR	201-0027	2.00
CHEMP0134	DrySyn Scholar, kit with 250 ml base and insert for 100 ml flasks, with 2 heat resistant handles	VWR	442-1238	1.00
CHEMP0136	Magnetic stirring bar 25x8mm	VWR	COWI001.525.RE	1.00
CHEMP0223	9 way D male to female RS232 serial	Farnell	MXT10050	1.00

	cable, 0.5 m		CMBK	
CHEMP0260	Serial to ethernet converter assembly	In-house - Assembly	N/A	1.00
CHEMP0349	Flasks, round bottom, with three necks and standard ground joints, 100ml, 24/29, 14/23	VWR	201-1177	
CHEMP0351	Filter Assembly, UHMWPE/ETFE, 10 µm, 1/8" OD Tubing	Cole-Parmer	WZ-42711-47	1 (optional)

The reactor of choice in the Chemputer is the three-neck round-bottom flask as in the manual organic chemistry workflow. The flask size was chosen according to the needs of the synthesis at hand. The size was either 250 mL or 100 mL. For simplicity the instruction below relate to a 250 mL flask.

1. The hotplate stirrer (CHEMP0055) was connected to the the power supply and to the serial-to-Ethernet converter (CHEMP0260) using a suitable RS232 serial cable (CHEMP0223), which in turn was connected to the switch (Section 4.3) via an Ethernet cable.
2. A 250 mL heating mantle (CHEMP0134) was placed on the hotplate stirrer.
3. A 250 mL three-neck round-bottom flask (CHEMP0135) with a 25 mm stir bar (CHEMP0136) was placed inside the heating mantle and secured with a clamp (CHEMP0060) to a suitable support rod (CHEMP0058) via a boss head (CHEMP0045).
4. Two GL18 adapters were assembled as shown in Figure 95. A PTFE sleeve (CHEMP0226) was mounted on a GL18 adapter (CHEMP0061) joint cone. A screw cap (CHEMP0350) with insert (CHEMP0053) was attached to the thread. The screw cap was only mounted loosely (this eased the insertion of the PTFE tubing through the cap later).

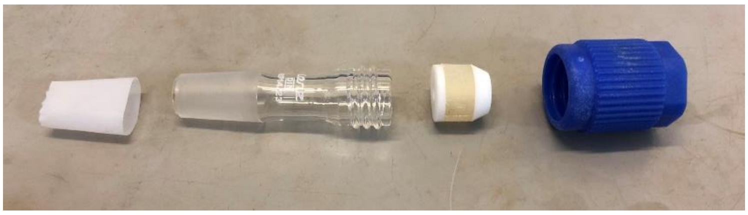


Figure 95 Assembly of the GL18 adapter.

5. PTFE tubing (CHEMP0149) was fed through the GL18 assembly.
6. Optionally, an in-line filter (CHEMP0351) was attached to the end of the tubing as shown in Figure 96 using one ferrule and fitting (CHEMP0220).

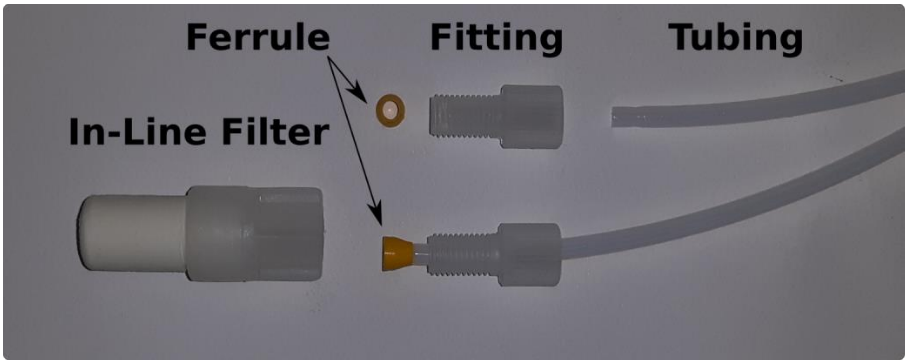


Figure 96 In-line filter assembly.

7. The GL18 adapter assembly from step 4 was inserted into a side neck or the central neck of the reactor flask as suitable and secured with a Keck clip of appropriate size. Attention was payed that the end of the tube (or the optional in-line filter) reached the bottom of the reactor flask.
8. The adapter assembly with the tubing already inserted was connected to a valve port in the Chemputer backbone (as described in Section 4.8). The other adaptor assembly was connected to the inert gas supply (as described in Section 4.9).
9. For reactions requiring reflux conditions, an air-cooled reflux condenser (CHEMP0137) was inserted in the central neck of the reactor flask. If no reflux was required, the remaining neck of the reactor flask was simply plugged with a glass stopper, PTFE sleeve and a clip. The finished assembly is shown in Figure 97.

Figure 97 Completed assembly of the standard reactor flask.

4.11.2 SPPS Reactor

Table 17 Bill of Materials for the SPPS reactor

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0352	SPPS filter frit reactor	In-house glassware	n/a	1
CHEMP0354	60 W heating mat (2 x 6 in, 60 W, 12 V)	RS Components	181-2061	1
CHEMP0355	digital temperature sensors (DS18B20+, TO-92)	RS Components	189-7723	2
CHEMP0033	STP36NF06L TO-220 MOSFET	RS Components	486-5671	1
CHEMP0356	Resistor R1 (2.4 kΩ)	RS Components	739-8786	1
CHEMP0357	Resistors R2 and R3 (4.7 kΩ)	RS Components	707-7726	2
CHEMP0358	LED (VD1, yellow)	RS Components	LTL-307Y	1
CHEMP0359	LED (VD2, red)	RS Components	LTL-307P	1
CHEMP0360	LED (VD3, green)	RS Components	L-1503GC	1
CHEMP0079	Molex MicroClasp connectors, single row female connector, 3-way	RS Components	679-5909	2
CHEMP0361	Pin headers, one-row, through-hole, straight	RS Components	251-8632	6
CHEMP0362	Ultra-Fit right-angle header, single row, 2-way	RS Components	895-1880	1
CHEMP0363	Ultra-Fit tangles receptacle housing, single row, 2-way	RS Components	187-3673	1
CHEMP0080	Pin headers, one-row, through-hole, right angle	RS Components	251-8654	2
CHEMP0078	Female crimp terminals	RS Components	681-2887	8
CHEMP0353	IKA MICROSTAR 15 control stirrer	IKA	25001986	1
CHEMP0066	stirrer shaft (PTFE, 300 mm x 8 mm)	VWR	441-0253	1
CHEMP0067	Stir blade	VWR	441-9561	1
CHEMP0364	NS29->NS24 expansion adapter	Fisher Scientific	12156220	1
CHEMP0035	Arduino Duo with headers A000062	RS Components	769-7412	1
CHEMP0365	Male USB A to male micro USB B cable	RS Components	901-5064	1
CHEMP0224	Heidolph STIRRER GUIDE NS 29/32 PTFE	Heidolph	509-09000-00	1
CHEMP0226	PTFE Sleeve	VWR	201-0027	2
CHEMP0061	GL18 to B14 connector	VWR	201-1642	2
CHEMP0350	Screw cap for tube connection, blue, GL18	VWR	215-2082	3
CHEMP0053	Insert for screw cap GL 18, 3,2 mm inner diameter	VWR	215-2077	3
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	Varies
CHEMP0227	Chemputer Valve	In-house -	N/A	1

		Assembly		
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	3
CHEMP0273	Screw cap GL45, 2 port GL14	VWR	554-3000	Varies
CHEMP0261	Screw cap, GL 45, PP, with 3 ports GL 14	VWR	554-3001	Varies
CHEMP0366	Tubing connectors, complete with screw cap, straight	VWR	SCOT28623 0005	1
CHEMP0151	250ml Reusable Glass Media Bottles with Cap GL45	Fisher Scientific	15456113	1

The solid-phase peptide synthesis (SPPS) reaction is most conveniently performed on a filter frit reactor in order to retain the resin during the solvent exchange steps. Several reactor models and stirring options were investigated[2]. It was found that the dead volume under the filter frit is a critical parameter and must be minimized. Further, overhead stirring was found to be adequate in these studies. Knowing this, a bespoken glass frit reactor was commissioned with a dead volume of 0.9 mL in contrast to 18.5 mL as in the first-generation filter frit reactor (Figure 98)[2].

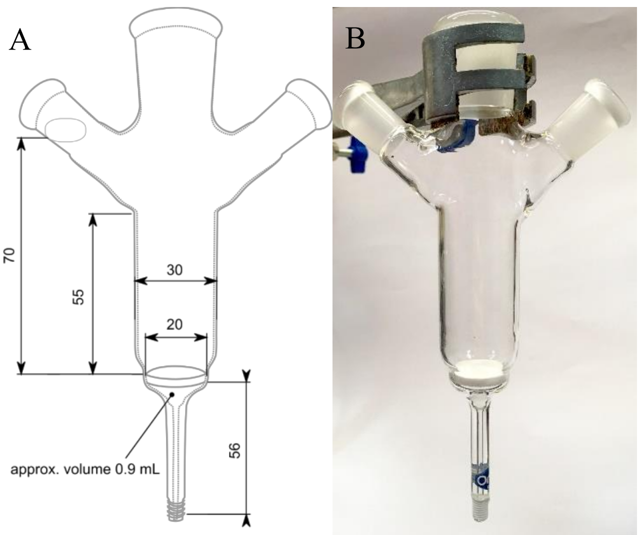


Figure 98 SPPS filter frit reactor (A) schematics and (B) the actual built.

Assembly of the Heating Element

Resistive heating was chosen for the SPPS reactor as it allows for quicker response times than the recirculating heating and chilling system used for the jacketed filter (see Section 4.12). A 60 W heating mat was used as a heating element. The heating output was controlled via an Arduino Duo board. The board was connected to two digital temperature sensors (DS18B20). The sensor S1 was placed in intimate contact with the heating mat and its readings were fed to a PID loop running on the Arduino. The output signal of the PID regulator was the PWM level, which was used to switch the MOSFET. Thus, the heating power output was directly proportional to the PWM level. The red LED was switched on when the PID regulation was active. The intensity of the yellow LED was directly proportional to the actual heating power output. The green LED was switched on when the measured temperature of the heating mat reached the target temperature +/- 0.5 °C.

1. A shield was assembled on a prototype board according to the circuit scheme shown in Figure 99. The connections to the sensors S1 and S2 were made with a Molex MicroClasp connectors (CHEMP0079). The connection to the heating mat (CHEMP0354) was made with a single row 2-way 2.54 mm Molex connector (CHEMP0362).

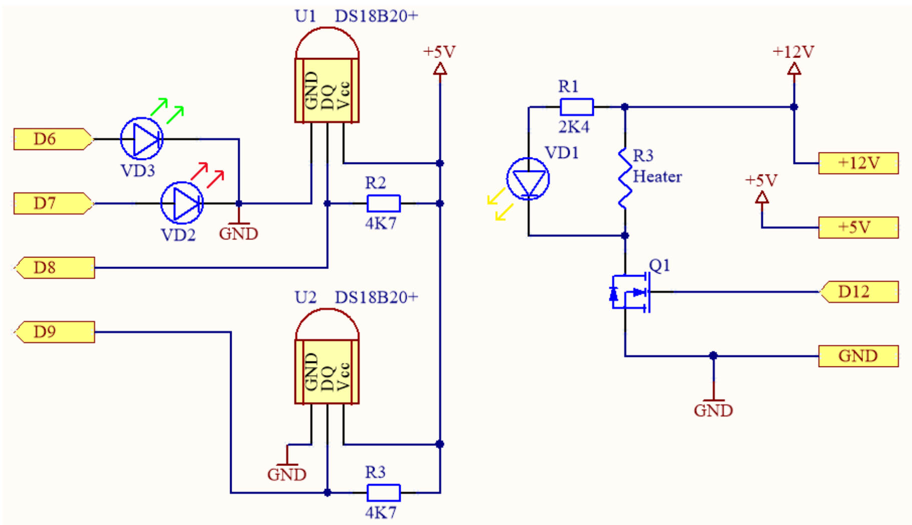


Figure 99 Circuit scheme for the heating element.

2. The shield was mounted on an Arduino Duo board running the appropriate sketch (the sketch is available as a supplementary file in Electronics\SPPS_Reactor_Heating_Element\Heating_Element_Sketch).

Assembly of the Reactor Module

1. A standard laboratory stand was placed in the fume hood serving both as a stirrer stand and as a hold for the SPPS filter frit reactor.
2. The stirrer (CHEMP0353) was attached to the stand approximately 650 mm from the base of the fume cupboard.

3. The USB cable (CHEMP0365) was attached to the rear of the stirrer and connected directly to the computer.
4. A stirrer shaft (CHEMP0066) with blade (CHEMP0067) was inserted into a PTFE stirrer guide (CHEMP0224).
5. The stirrer guide with stirrer shaft was inserted in an NS29->NS24 expansion adapter (CHEMP0364).
6. The assembly consisting of the reduction adapter with stirrer shaft with blade and stirrer guide was mounted on the SPPS filter frit reactor.
7. Then the stirrer shaft was inserted in the overhead stirrer and the reactor was centered directly below the stirrer using a clamp mounted on the same stand as the stirrer.
8. The position of the stirrer shaft was adjusted such that the stirrer blade is positioned *ca.* 5 mm above the sintered glass frit (Figure 100).

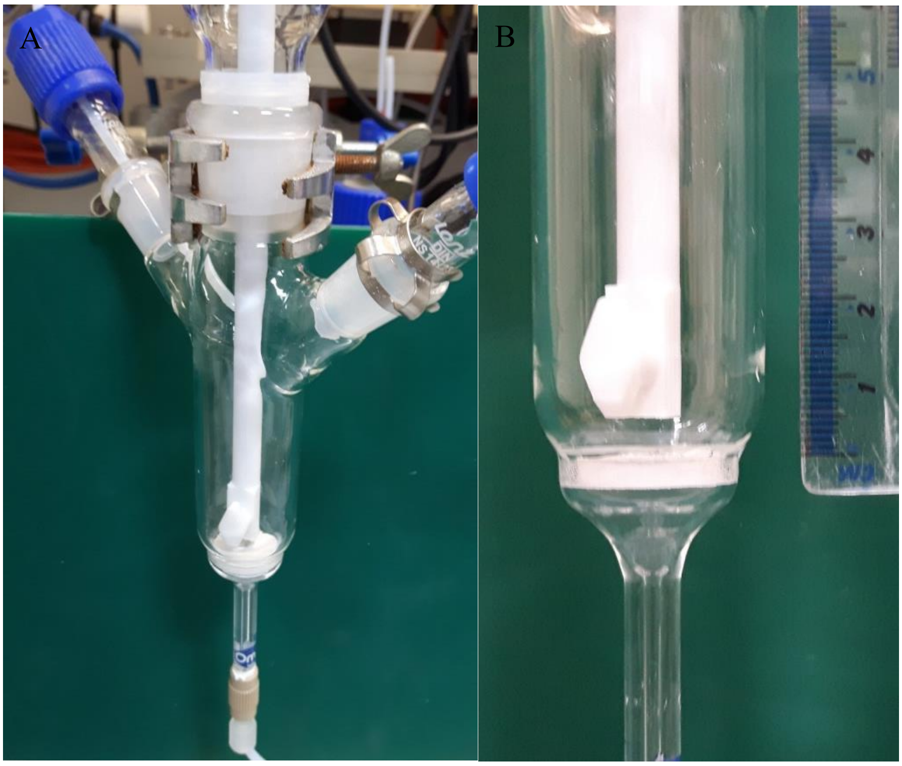


Figure 100 SPPS filter frit reactor with adjusted stir blade (A) from an angle and (B) from the side with a ruler (mm).

9. A mark was taken for the adjusted height of the stir blade. Then the reactor was removed from the clamp again and wrapped with the heating mat (CHEMP0354) as shown in Figure 101. The temperature sensor S1 was placed between the outer surface of the reactor vessel and the heating mat. The heating mat was fixed with four cable ties.

10. The reactor was insulated by a layer of cotton wool wrapped in aluminium foil, which was fixed with adhesive tape (Figure 102).

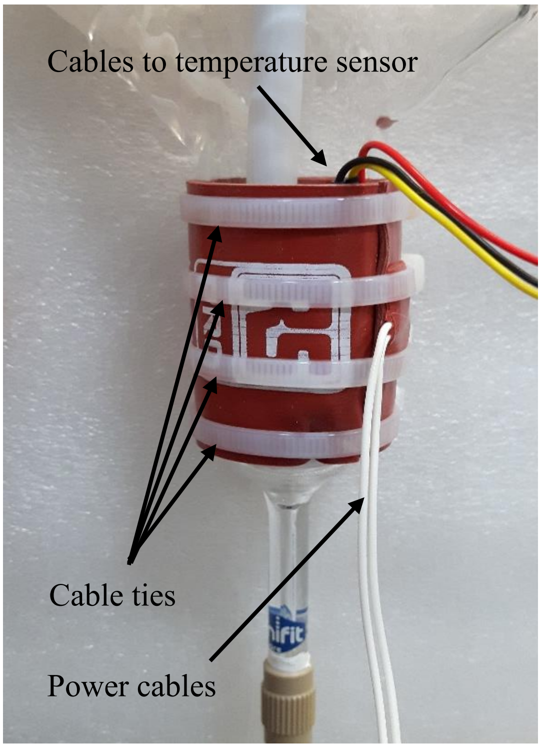


Figure 101 The SPPS reactor was wrapped with a heating mat.

Figure 102 The reactor was isolated with cotton wool and aluminium foil.

11. The reactor with the fully assembled heating mat and insulation was mounted back on the laboratory stand and the stir shaft was inserted according to the previously taken mark to ensure proper alignment of the stir blade.
12. Two GL18 adapters were assembled as described above (Figure 95).
13. The adapters assemblies from step 9 were inserted into the NS14 side-necks of the SPPS filter frit reactor.
14. One adapter assembly was connected to a dedicated valve port in the Chemputer backbone (as described in Section 4.8).
15. The outlet of the reactor was attached to a dedicated Chemputer valve (CHEMP0227), which was used to switch between vacuum, positive gas supply (used for stirring the reaction mixture, as required) and the Chemputer backbone (as described in Section 4.8) liquid handling backbone with PTFE tubing (CHEMP0149) and a flangeless tube fitting (CHEMP0220) as shown in Figure 103. It is important to wrap PTFE tape around the glass threading of the SPPS filter frit reactor outlet before the flangeless tube fitting is mounted.

Figure 103 Connection of the SPPS filter frit outlet to PTFE tubing.

4.11.3 Connection to Vacuum and Liquid Handling Backbone

During the synthesis it was necessary to be able to apply vacuum to the filter frit as well as removing liquids via the liquid handling backbone. In order to be able to switch between vacuum and liquid handling backbone a dedicated valve (see Section 4.7) and a Woulff-type bottle were placed between the reactor outlet and the liquid handling backbone.

1. PTFE tubing (CHEMP0149) was inserted into a screw cap (CHEMP0350) with insert (CHEMP0053) as shown in Figure 104. One or two of these assemblies, as required, were mounted on a larger screw cap (3 ports - CHEMP0261 or 2 ports - CHEMP0273 as required) together with a screw cap with PTFE barbed connector (CHEMP0366).

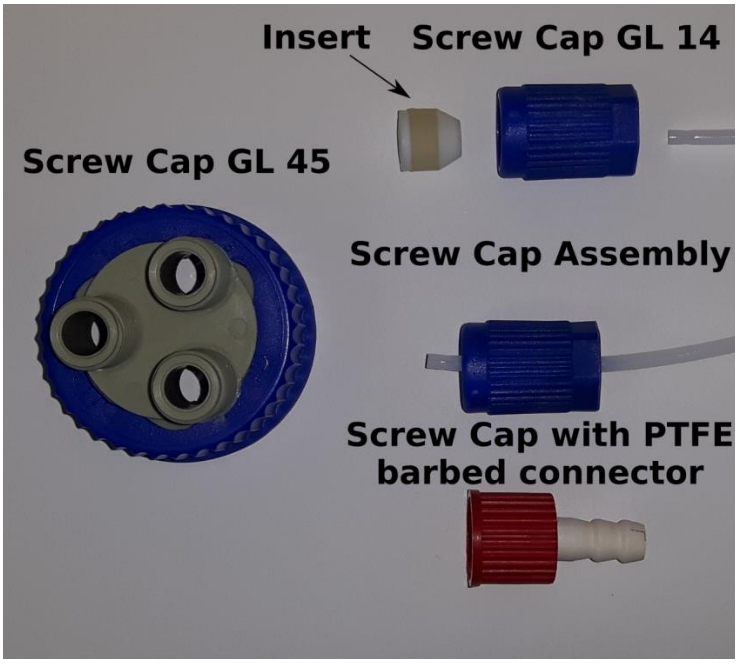


Figure 104 Assembly of the cap for the vacuum bottle (solvent trap).

2. The assembly of the cap for the vacuum bottle was attached to a 250 mL Duran glass bottle (CHEMP0151).
3. The end of the PTFE tubing was inserted into the side-port of the dedicated valve (see Section 4.7) using ferrule and fitting (CHEMP0220) as shown in Figure 105.

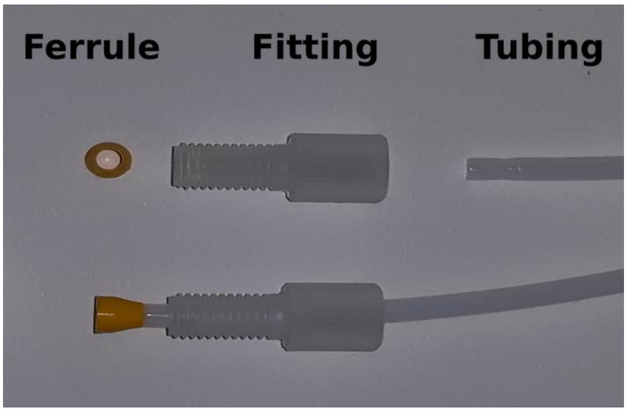


Figure 105 Assembly of the ferrule and fitting to the end of the PTFE tubing.

4. The screw cap with PTFE tubing was connected to the house vacuum supply using appropriate vacuum tubing.
5. The outlet tubing of the SPPS filter frit reactor (or any other module that needs a connection to the house vacuum) was connected to the top port of the dedicated valve using ferrule and fitting (CHEMP0105) as shown in Figure 105. The final assembly

for two devices (requires the 3-port screw cap) connected to the vacuum bottle (serving as solvent trap) is shown in Figure 106.

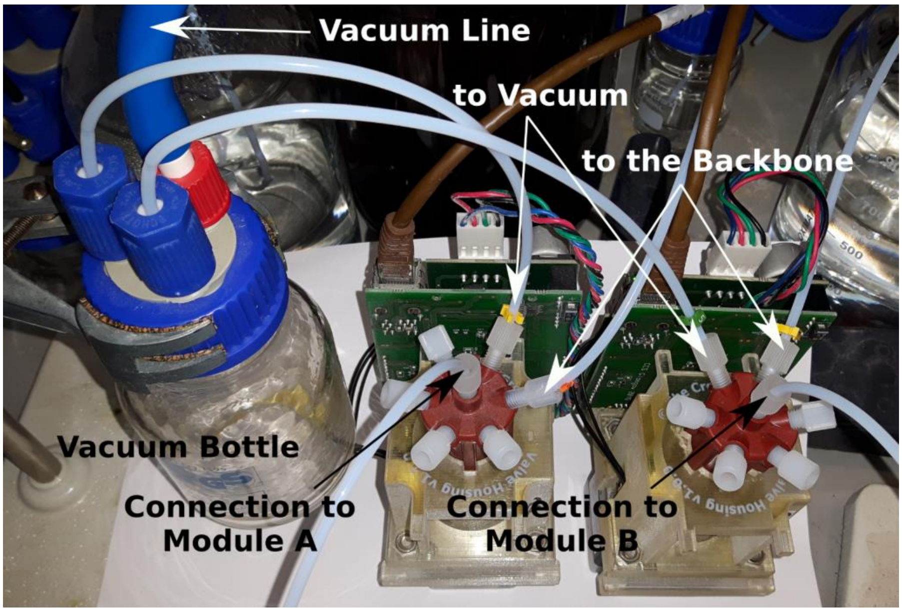


Figure 106 Connection of two modules to the vacuum bottle (solvent trap).

4.11.4 Configuration

When connecting the IKA Microstar 15 stirrer to the USB port of the PC, it is recognized in the system as a virtual serial port COM*n*. The port number has to be included in the graph file describing the Chemputer topology in the format 'COMn'. The number was easily retrieved as follows.

1. The "Device Manager" was opened.
2. The menu "Ports (COM & LPT)" was expanded.
3. The COM port associated with the IKA Microstar 15 stirrer was identified.
4. This number (e.g. COM4) was added to the graph element symbolising the IKA Microstar 15 stirrer in the "port" field.

Note that the COM port number is re-assigned upon device connection/disconnection and thus may change after PC reboot.

4.12 Jacketed Filter

Table 18 Bill of Materials for the Jacketed Filter

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY

CHEMP0056	Chemputer jacketed filter (custom glassware)	In-house - glassware	N/A	1.00
CHEMP0044	Heidolph™ Stand S2 XXL	Heidolph	570-12200-00	1.00
CHEMP0059	Double bossheads with nickel plated steel screws Zinc die-cast, powder coated	VWR	241-7223	1.00
CHEMP0060	Flask clamp, zinc die-cast, powder coated 0 - 80 mm	VWR	241-7549	1.00
CHEMP0061	GL18 to B14 connector	VWR	201-1642	2.00
CHEMP0052	GL14 screw cap for tube connection, blue	VWR	554-3004	6.00
CHEMP0053	Insert for screw cap GL 18, 3,2 mm inner diameter	VWR	215-2077	6.00
CHEMP0065	Heidolph Hei-TORQUE 100 Precision Base overhead stirrer	Heidolph	501-61020-00	1.00
CHEMP0066	Stirring shaft, 300 x 8 mm	VWR	441-0253	1.00
CHEMP0067	Stirrer blade, square ends 52(w) x 3(d) x 14(h) mm	VWR	441-9561	1.00
CHEMP0073	Clamp for ground joints 14/23	VWR	201-1079	1.00
CHEMP0043	Union, Threaded, 1/4"-28 (Flat Bottom), 1.5mm bore, PEEK™	Kinesis	002307	1.00
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	10.00
CHEMP0165	Glass stoppers, standard taper, hexagonal, hollow, 14/23, with drip tip	VWR	217-9105	1.00
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	100.00
CHEMP0226	PTFE Sleeve	VWR	201-0027	2.00
CHEMP0227	Chemputer Valve	In-house - Assembly	N/A	2.00
CHEMP0242	5m ethernet cable, blue	Insight	83165	2.00
CHEMP0243	5m, ethernet cable, orange	Insight	83607	1.00
CHEMP0228	Chemputer Pump 10ml	In-house - Assembly	N/A	1.00
CHEMP0261	Screw cap, GL 45, PP, with 3 ports GL 14	VWR	554-3001	1.00
CHEMP0151	250ml Reusable Glass Media Bottles with Cap GL45	Fisher Scientific	15456113	1.00
CHEMP0054	Smiths Medical™ Portex™ Coloured PVC Tubing	Fisher Scientific	13180863	1.00
CHEMP0245	5m, ethernet cable, brown	Insight	83677	1.00

CHEMP0262	Hose connectors, with nut, GL14 thread, 8mm tube diameter	VWR	BOHLD581-02	5.00
CHEMP0263	Support rod, polished, M10 thread, 12mm diameter, 500mm length	VWR	241-0236	2.00
CHEMP0264	Retort base, threaded M10 × 1,5 mm, pressed steel finished in matt black textured acrylic paint	VWR	NICK6891	3.00
CHEMP0022	Julabo CF41 chiller	Cole-Parmer	WZ-12150-72	1.00
CHEMP0265	Plug Tefzel - 1/4-28	Kinesis	P-311	4.00
CHEMP0266	1m Viton tubing (-35'C to 200'C) 8930108, accessory suitable for selected Julabo units	Fisher Scientific	10097961	3.00
CHEMP0260	Serial to ethernet converter assembly	In-house - Assembly	N/A	1.00
CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050CMBK	1.00
CHEMP0246	5m ethernet cable, yellow	Insight	83245	2.00
CHEMP0267	Insulation for CR® and Viton® tubing (I.Ø 12 mm)	VWR	JULA8930412	3.00
CHEMP0224	Heidolph STIRRER GUIDE NS 29/32 PTFE	Heidolph	509-09000-00	1.00
CHEMP0270	D-sub Adapter Null Modem Adapter , For use with 9 Way D-Sub Connector	RS Components	243-0374	1.00
CHEMP0366	Tubing connectors, complete with screw cap, straight	VWR	SCOT286230005	2
CHEMP0271	Buerkle™ Worm-Threaded Hose Clips	Fisher Scientific	10595624	4.00

The jacketed filter module (Figure 107) is used in steps where the reaction product has to be separated as a solid such as in a re-crystallisation or a precipitation step. It can even be used for simple reactions where the product is formed as a solid. The reactor part of the module is a custom glass-blown device.

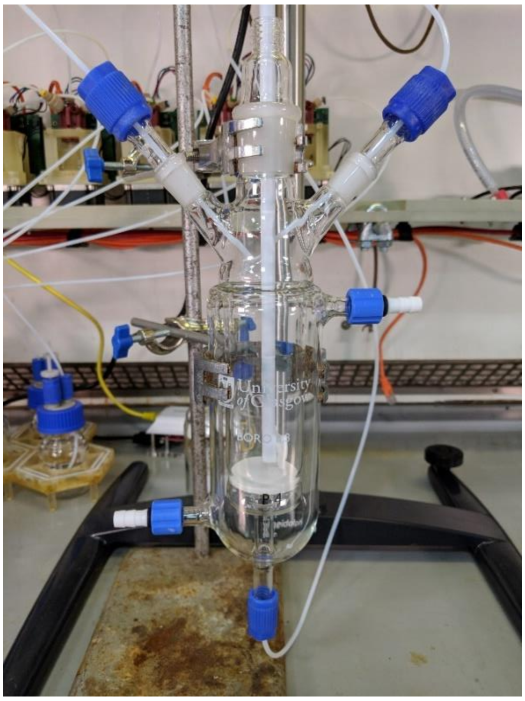


Figure 107 Complete jacketed filter module.

4.12.1 Assembly

1. An overhead stirrer stand was placed in the fume cupboard (CHEMP0444).
2. The stirrer (CHEMP0065) was attached to the stand approximately 650 mm from the base of the fume cupboard to the bottom of the stirrer.
3. The serial cable (see Table 27 for specifications) (CHEMP0223) was attached to the RS-232 connector at the back of the stirrer. The other side of the cable was connected to the serial-to-Ethernet converter (see Section 4.15) (CHEMP0260), which in turn was connected to the switch via an Ethernet cable.
4. A stirrer shaft (CHEMP0066) was inserted in a PTFE stirrer guide (CHEMP0224).
5. The stirrer blade (CHEMP0067) was attached to the stirrer shaft.
6. The assembly consisting of the stirrer shaft, stirrer guide and stirrer blade was mounted on the jacketed filter (CHEMP0056, bespoken glassware, see Figure 108 for plans).

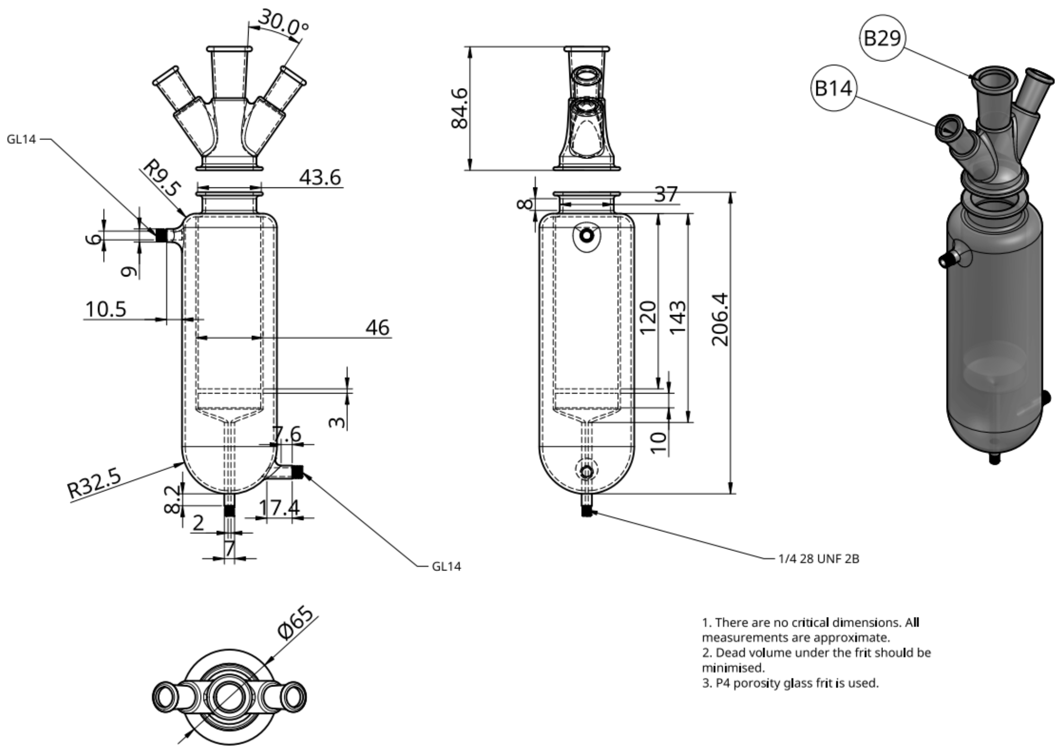


Figure 108 Design of the jacketed filter.

7. Then the stirrer shaft was inserted in the overhead stirrer and the jacketed filter was centred directly below the stirrer with a standard laboratory stand.
8. The position of the stirrer shaft was adjusted such that the stirrer blade is ca 1 mm above the filter frit (see Figure 109).

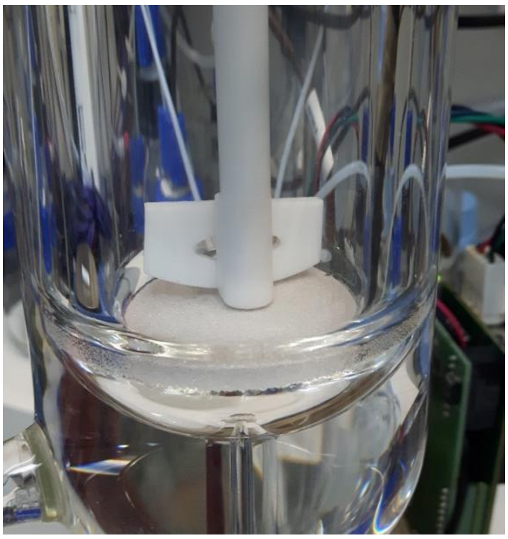


Figure 109 The stirrer blade was positioned as close as possible to the sintered filter frit without actually touching it (ca 1 mm gap).

9. Two GL18 adapters were assembled as described above (Figure 95).

10. The adapter assemblies from step 9 were inserted into the NS14 side-necks of the jacketed filter.
11. One adapter assembly was connected to a free valve port in the Chemputer backbone (as described in Section 4.8) the other adaptor assembly was connected to the inert gas supply (as described Section 4.9).
12. The outlet of the jacketed filter was attached to a dedicated valve (which was used to switch between vacuum and the liquid handling back-bone, see the corresponding sub-section in Section 4.11) with PTFE tubing (CHEMP0149) and a flangeless tube fitting (CHEMP0220) as shown in Figure 110. It is important to wrap PTFE tape around the glass threading of the jacketed filter outlet before the flangeless tube fitting is mounted.

Figure 110 Connection of the jacketed filter outlet to PTFE tubing

4.12.2 Installation and Connection of the Recirculating Chiller

1. A Julabo recirculating chiller (CHEMP0022) was set-up according to the manufacturer's operating manual.
2. A null modem adapter (CHEMP0270) was attached to the RS232 port at the rear of the chiller.
3. The serial cable (CHEMP0223) (see Table 27) was attached to the null modem adapter (see Figure 111).

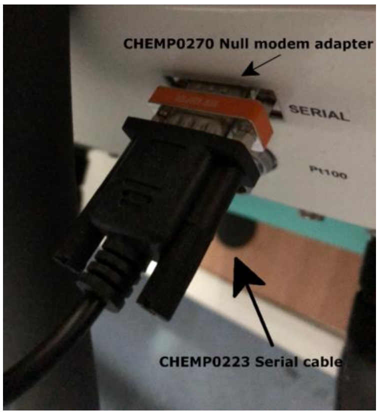


Figure 111 Connection of the null modem adapter and serial cable to the Julabo CF41 chiller.

4. The serial cable was connected to a serial-to-Ethernet converter (CHEMP0260) (see Section 4.15), which in turn was connected to the switch (see Section 4.1) via an Ethernet cable (CHEMP0246).
5. Viton® tubing (CHEMP0266) was wrapped with the insulation (CHEMP0267).
6. The tubing with insulation was attached to the in- and outlet of the chiller and secured with hose clips (CHEMP0271) as shown in Figure 112.

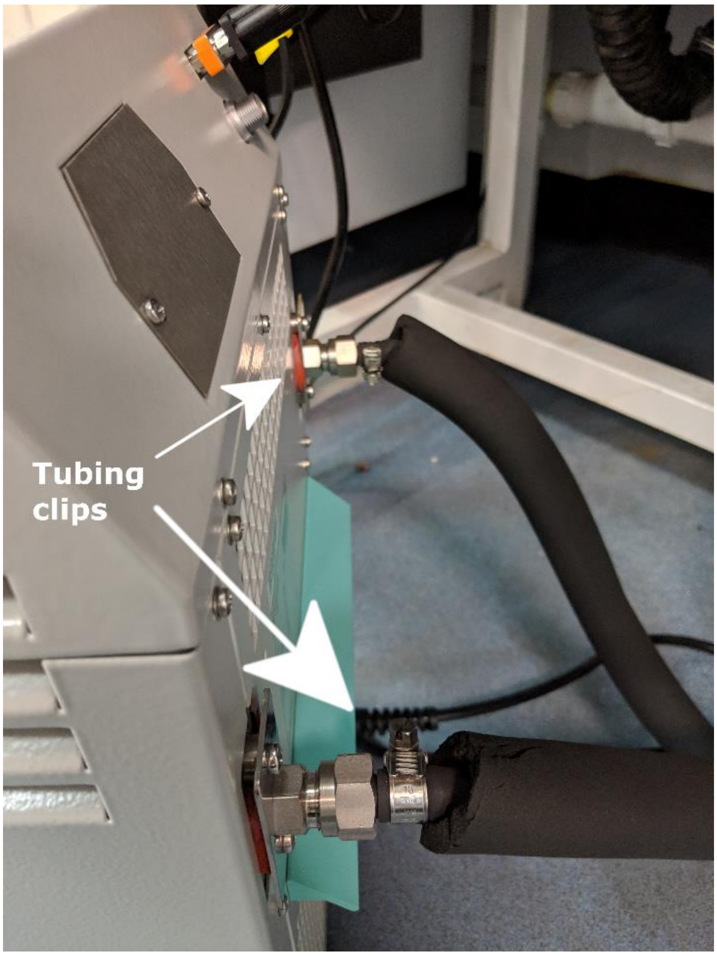


Figure 112 Tubing attachment to the recirculating chiller.

7. A hose connector (CHEMP0366) was attached to the ends of both tubing strands and secured with tubing clips (CHEMP0271).
8. The hose connectors were attached to the jacketed filter as shown in Figure 113.

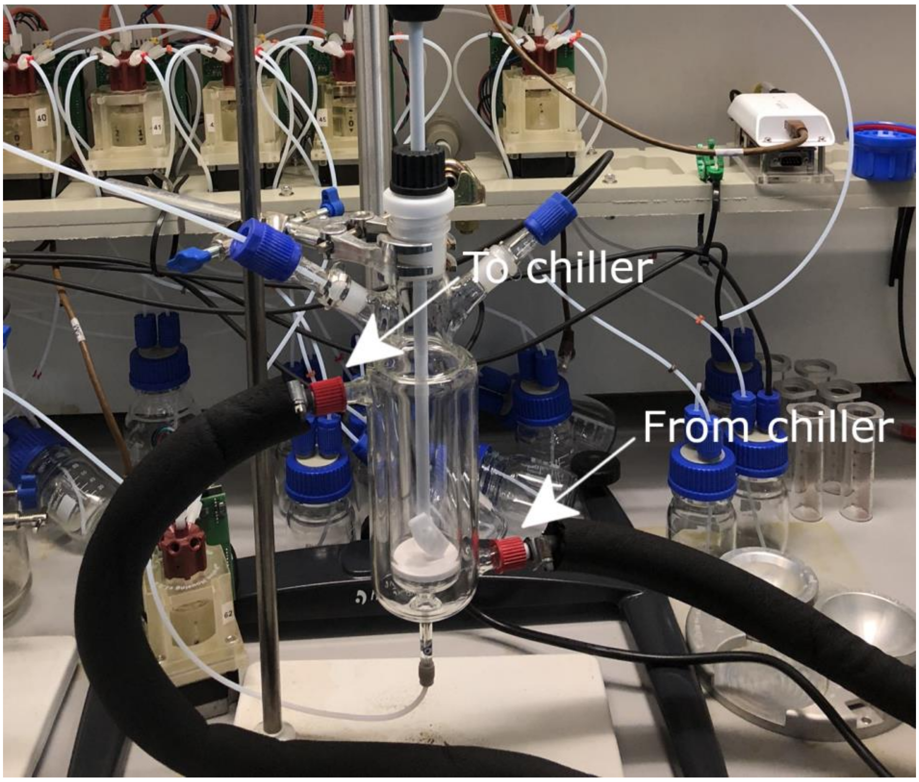


Figure 113 Attachment of the tubing from the recirculating chiller to the jacketed filter.

4.13 Separator

Table 19 Bill of Materials for the Separator

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0027	Custom Arduino shield.	In-house	N/A	1.00
CHEMP0032	Proto shield extension Arduino MEGA Rev3	RS Components	769-7399	1.00
CHEMP0033	N-Channel MOSFET, 30 A, 60 V STripFET, 3-Pin TO-220	RS Components	486-5671	1.00
CHEMP0034	Axial Thin Film Fixed Resistor 10MΩ ±1% 0.6W ±50ppm/°C	RS Components	683-2945	1.00
CHEMP0035	Arduino Duo with headers A000062	RS Components	769-7412	1.00
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	4.00
CHEMP0221	Red Equipment Wire BS4808, 0.52 mm^2 CSA , 1 kV 20 AWG	RS Components	748-2178	1.00
CHEMP0222	Insulated Crimp Ring Terminal, M4 Stud Size, $0.26mm^2$ to $1.65mm^2$ Wire	RS Components	457-5104	2.00

	Size, Red			
CHEMP0065	Heidolph Hei-TORQUE 100 Precision Base overhead stirrer	Heidolph	501-61020-00	1.00
CHEMP0066	Stirring shaft, 300 x 8 mm	VWR	441-0253	1.00
CHEMP0067	Stirrer blade, square ends 52(w) x 3(d) x 14(h) mm	VWR	441-9561	1.00
CHEMP0061	GL18 to B14 connector	VWR	201-1642	2.00
CHEMP0052	GL14 screw cap for tube connection, blue	VWR	554-3004	2.00
CHEMP0053	Insert for screw cap GL 18, 3,2 mm inner diameter	VWR	215-2077	2.00
CHEMP0044	Heidolph™ Stand S2 XXL	Heidolph	570-12200-00	1.00
CHEMP0059	Double bossheads with nickel plated steel screws Zinc die-cast, powder coated	VWR	241-7223	1.00
CHEMP0060	Flask clamp, zinc die-cast, powder coated 0 - 80 mm	VWR	241-7549	1.00
CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050 CMBK	1.00
CHEMP0224	Heidolph STIRRER GUIDE NS 29/32 PTFE	Heidolph	509-09000-00	1.00
CHEMP0225	B45->B29 reduction adapter	Fisher Scientific	12478876	1.00
CHEMP0226	PTFE Sleeve	VWR	201-0027	2.00
CHEMP0227	Chemputer Valve	In-house - Assembly	N/A	1.00
CHEMP0228	Chemputer Pump 10ml	In-house - Assembly	N/A	1.00
CHEMP0155	Screw cap with bore, for tube connection, GL 14, blue	VWR	554-3004	1.00
CHEMP0156	Insert for screw cap GL14, 3,2 mm inner diameter	VWR	554-3007	1.00
CHEMP0229	Screw cap, GL 45, PP, with two ports GL 14	VWR	554-3000	1.00
CHEMP0230	Laboratory bottles, round, with screw cap, DURAN, 500 ml	VWR	215-1516	1.00
CHEMP0161	Chemputer separator	In-house - glassware	N/A	1.00
CHEMP0260	Serial to ethernet converter assembly	In-house - Assembly	N/A	1.00
CHEMP0246	5m ethernet cable, yellow	Insight	83245	2.00

The separator module was used to perform liquid-liquid extractions (Figure 114). It consists of an overhead stirrer, a separating funnel and a conductivity sensor, which detects the phase-boundary.

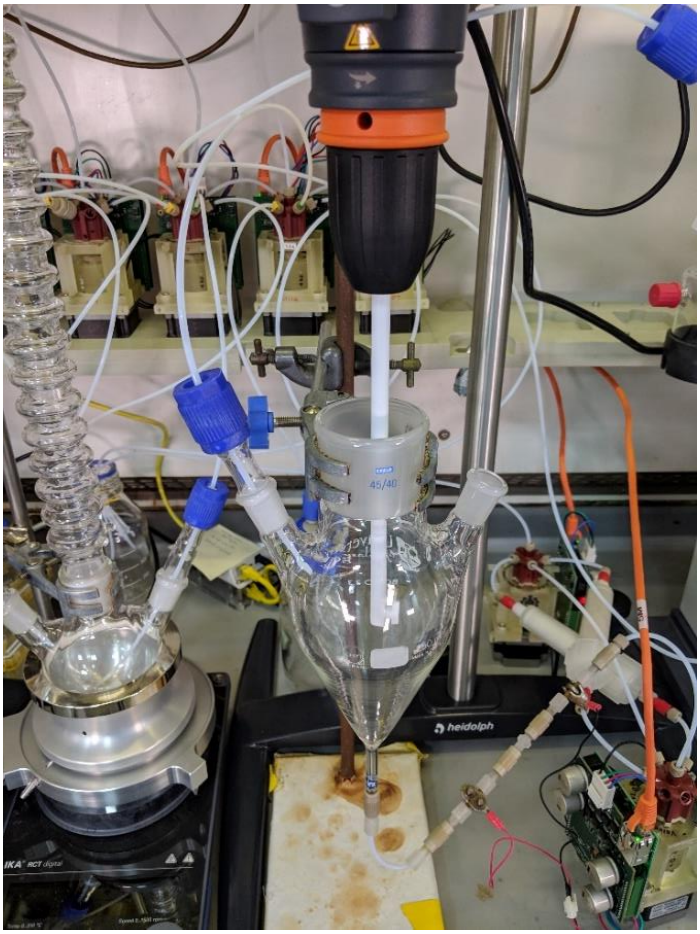


Figure 114 Separator Module.

1. An overhead stirrer stand (CHEMP0044) was placed in the fume cupboard.
2. The stirrer (CHEMP0065) was attached to the stand approximately 650 mm from the base of the fume cupboard.
3. A serial cable (CHEMP0223) (see Table 27 for specifications) was attached to the RS-232 connector at the back of the stirrer and connect to a serial-to-Ethernet convertor (CHEMP0260, see Section 4.15), which in turn was connected to the switch via an Ethernet cable (CHEMP0246).
4. A stirrer shaft (CHEMP0066) was inserted in a PTFE stirrer guide (CHEMP0224) and then into a reduction adaptor (CHEMP0225).
5. The stirrer blade (CHEMP0067) was attached to the stirrer shaft.

6. The assembly consisting of the reduction adapter with stirrer shaft, stirrer guide and stirrer blade was mounted on the Chemputer separator funnel (CHEMP0161, see Figure 115).

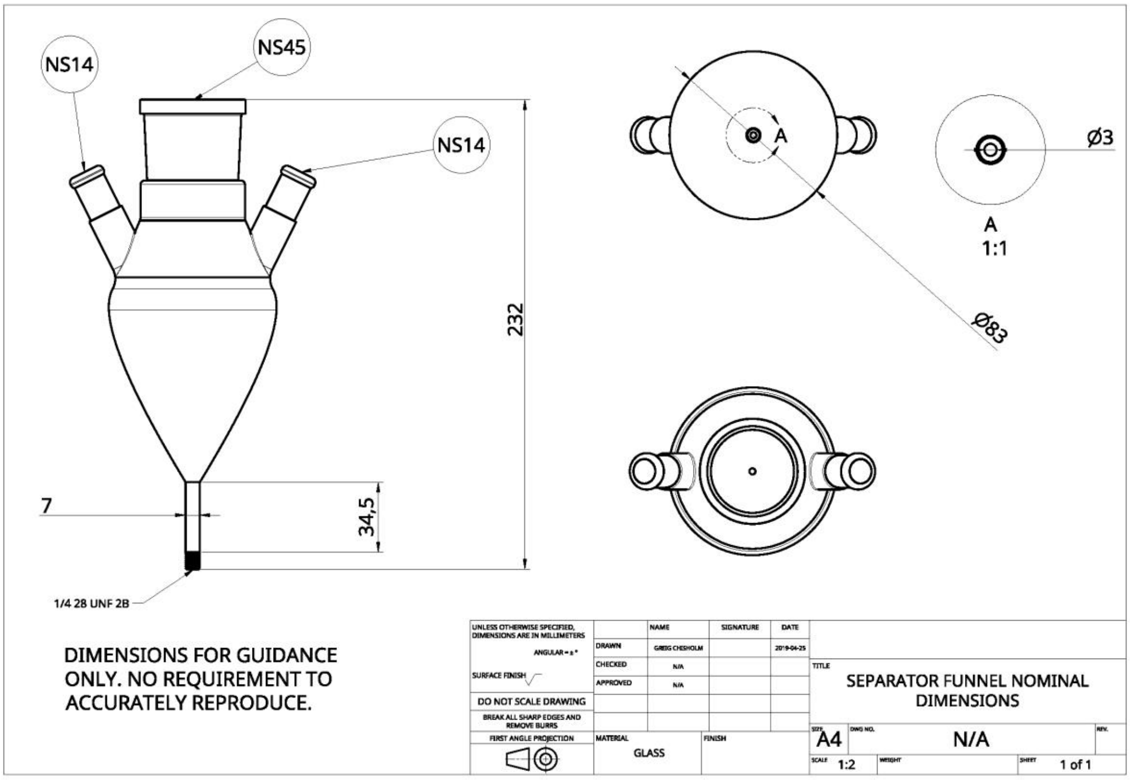


Figure 115 Schematics of the Chemputer separating funnel. The only key difference to a standard separating funnel is the ¼ 28 UNF glass thread at the outlet instead of a stopcock.

7. The stirrer shaft was inserted in the overhead stirrer and the separating funnel was centred directly below the stirrer with a standard laboratory stand.
8. The position of the stirrer shaft was adjusted such that the stirrer blade was in the bottom third of the separating funnel (see Figure 116).

Figure 116 The stirrer blade positioned in the bottom third of the separating funnel.

9. A GL18 adapter was assembled as shown in Figure 117. A PTFE sleeve (CHEMP0226) was mounted on a GL18 adapter (CHEMP0061) joint cone. A screw cap (CHEMP0052) with insert (CHEMP0053) was attached to the thread. The screw cap was only mounted loosely yet (this eased the insertion of the PTFE tubing through the cap later).

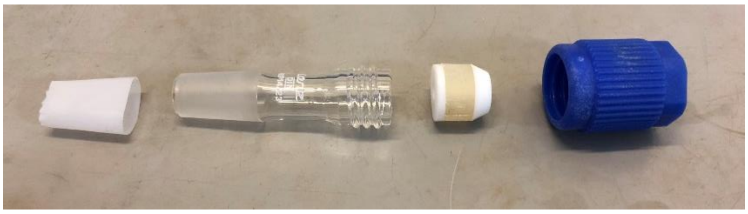
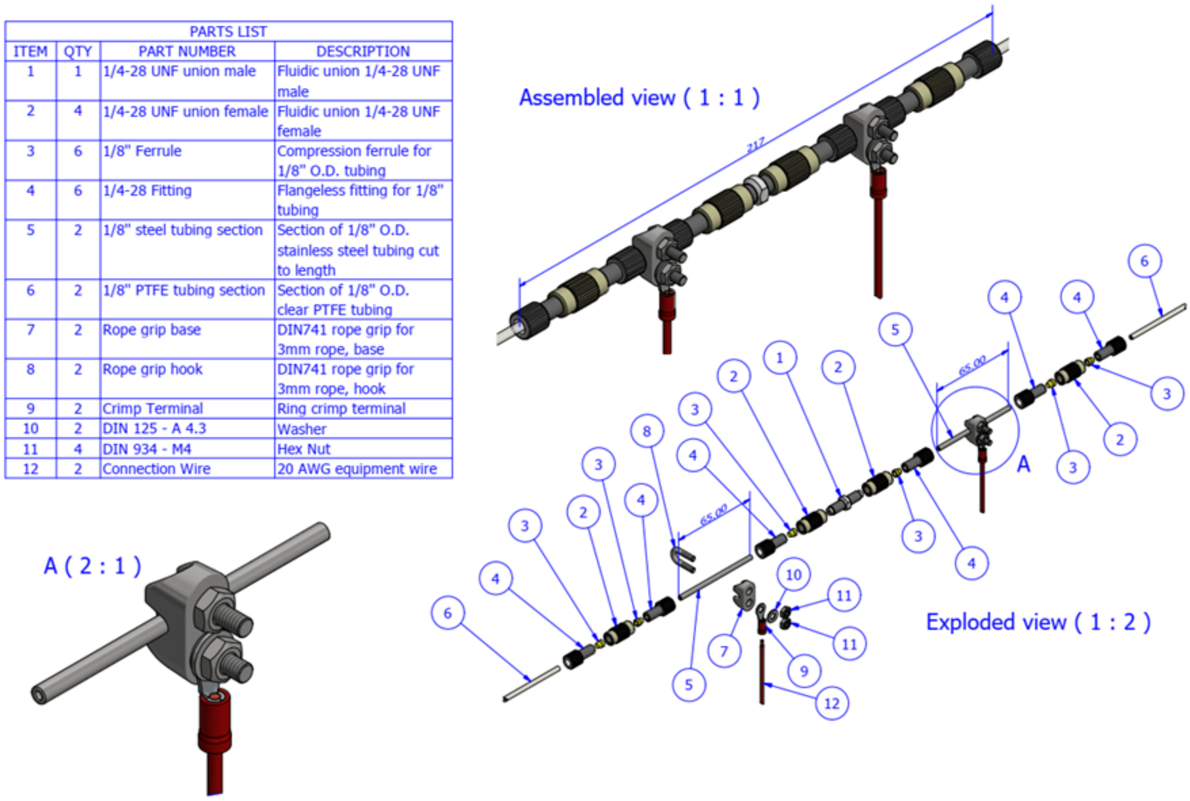


Figure 117 Assembly of the GL18 adapter.

10. The adapters assemblies from step 10 were inserted into the NS14 side-necks of the separator funnel.
11. One adapter assembly was connected to the dedicated valve port in the Chemputer backbone (as described in Section 4.8).
12. For the conductivity sensor assembly, two AWG20 wires (CHEMP0221) were crimped with ring terminals (CHEMP0222).
13. These wires were used as attachments to the conductivity sensor flow tube as shown in Figure 118. A detailed bill of materials for the flow tube itself can be found in Table 20.



PARTS LIST			
ITEM	QTY	PART NUMBER	DESCRIPTION
1	1	1/4-28 UNF union male	Fluidic union 1/4-28 UNF male
2	4	1/4-28 UNF union female	Fluidic union 1/4-28 UNF female
3	6	1/8" Ferrule	Compression ferrule for 1/8" O.D. tubing
4	6	1/4-28 Fitting	Flangeless fitting for 1/8" tubing
5	2	1/8" steel tubing section	Section of 1/8" O.D. stainless steel tubing cut to length
6	2	1/8" PTFE tubing section	Section of 1/8" O.D. clear PTFE tubing
7	2	Rope grip base	DIN741 rope grip for 3mm rope, base
8	2	Rope grip hook	DIN741 rope grip for 3mm rope, hook
9	2	Crimp Terminal	Ring crimp terminal
10	2	DIN 125 - A 4.3	Washer
11	4	DIN 934 - M4	Hex Nut
12	2	Connection Wire	20 AWG equipment wire

Figure 118 Assembly of the conductivity sensor.

Table 20 Bill of materials for the assembly of the conductivity sensor flow tube.

PART NO.	ITEM NUMBER IN SCHEMATIC	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0028	1	Adapters & Connectors: Union, Threaded, 1/4"-28 (Male), PCTFE	Kinesis	P-645	1.00
CHEMP0029	2	Dibafit™ Threaded Coupling, 1/4"-28 UNF(F) flat bottom, PEEK	Cole-Parmer	WZ-21939-99	4.00
CHEMP0220	3 & 4	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	6.00
CHEMP0030	5	Premium Grade 304 Stainless Steel Tubing	Sigma	20526-U	Cut to length
CHEMP0149	6	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	Cut to length
CHEMP0031	7, 8 & 11	Stainless Steel Wire Rope Clamp, DIN 741	RS Components	183-5891	2.00
CHEMP0222	9	Insulated Crimp Ring Terminal, M4 Stud Size, 0.26mm² to 1.65mm² Wire Size, Red	RS Components	457-5104	2.00
CHEMP0179	10	M3 Form A Flat Washers (DIN	AccuGroup	HPW-	2

		125) - A4 Stainless Steel		M3-A4	
CHEMP0221	12	Red Equipment Wire BS4808, 0.52 mm² CSA , 1 kV 20 AWG	RS Components	748-2178	Cut to length

14. The conductivity sensor assembly was attached to the outlet of the Chemputer separating funnel with PTFE tubing (CHEMP0149) and a flangeless tube fitting (CHEMP0220) as shown in Figure 119. It is important to wrap PTFE tape around the glass threading of the Chemputer separating funnel outlet before the flangeless tube fitting is mounted.

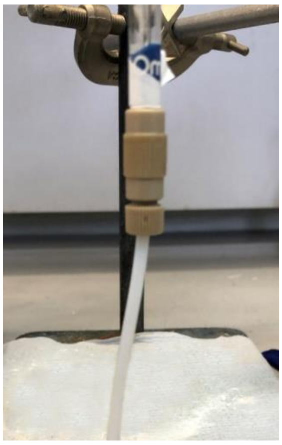


Figure 119 Connection of the Chemputer separating funnel outlet to PTFE tubing.

15. The connection wires of the conductivity sensor were attached to a custom-made Arduino shield (the circuit schematics is shown in Figure 120).

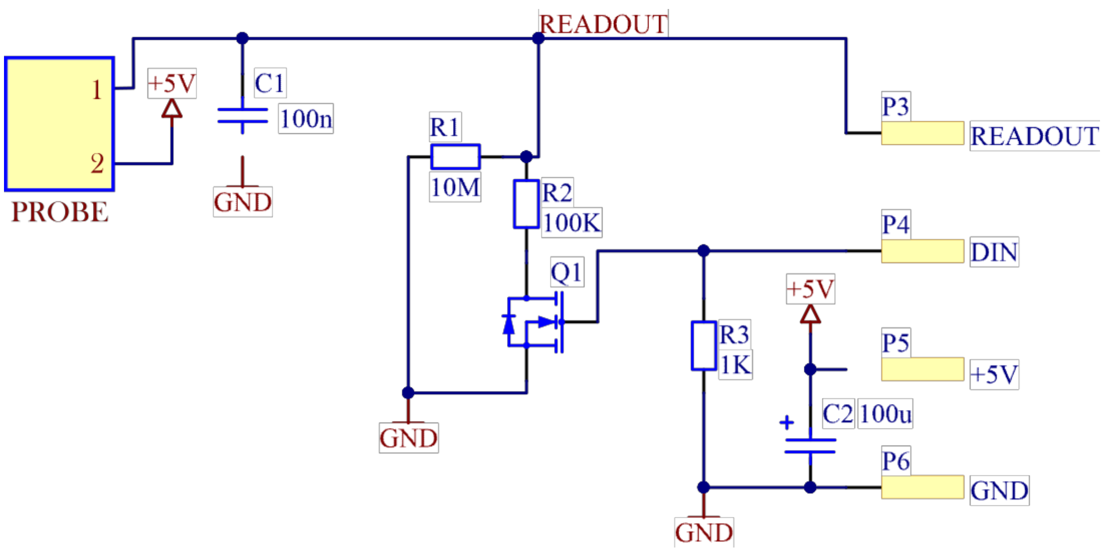


Figure 120 Circuit schematics of the Arduino shield. V_{READ}: read-out voltage level, +5V: supply voltage, PWM: input to switch the MOSFET, GND: signal ground, Probe: conductivity sensor.

16. The shield from step 28 was connected to an Arduino Mega, which was connected to the computer via USB. The sketch to program the board can be found as a supplementary file in Electronics\Conductivity_Sensor\Arduino_Sketch. The BoM is given in Table 21.

Table 21 Bill of materials for the assembly of the Arduino shield.

PART NO.	SYMBOL	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0032		Proto shield extension Arduino MEGA Rev3	RS Components	769-7399	1.00
CHEMP0033	Q1	N-Channel MOSFET, 30 A, 60 V STripFET, 3-Pin TO-220	RS Components	486-5671	1.00
CHEMP0374	R1	10MΩ 0.25W Metal Glaze Resistor ±5%	RS Components	484-4595	1.00
CHEMP0375	R2	100kΩ 0.125W Metal Film Resistor ±1%	RS Components	830-7668	1.00
CHEMP0361		Pin headers, one-row, through-hole, straight	RS Components	251-8632	1.00
CHEMP0376	R3	1kΩ 0.25W Carbon Film Resistor ±5%	RS Components	707-7666	1.00
CHEMP0377	C1	100µF Polymer Capacitor 20V dc, Through Hole	RS Components	795-5531	1.00
CHEMP0378	C2	100nF Multilayer Ceramic Capacitor MLCC 50V dc ±10% Through Hole	RS Components	538-1310	1.00

4.13.1 Testing of the Conductivity Sensor

The circuit scheme shown in Figure 120 emerged from the search for a versatile and robust conductivity sensor. In an initial built of the Chemputer separator module, the phase boundary was detected with a simple voltage splitter[1]. In the course of this work, however, it was found that this set-up is not able to reliably detect the phase boundary for very conductive solutions (e.g. when high concentrations of organic salts are formed or when iodine/iodide mixtures are present, as encountered during the NHS-diazirine synthesis). This was temporarily mitigated by the use of the switchable voltage divider with two resistance values; however, the search was continued to find a potentially more robust solution. Three test mixtures were selected to assess the performance of alternative conductivity sensor designs:

Case A (low conductivity extreme):

- aquatic phase: 50 mL of ultrapure water.
- organic phase: 50 mL of petroleum ether.

Case B (standard situation):

- aquatic phase: 50 mL of tap water.
- organic phase: 50 mL of a 0.5 M solution of paracetamol (3.78 g, 25.0 mmol) in ethyl acetate (50 mL).

Case C (high conductivity extreme, akin to the biphasic mixture formed during one of the workups in the NHS-diazirine synthesis):

- aquatic phase: 50 mL of a solution of potassium hydroxide (5.85 g, 104 mmol) in 70 mL of tap water.
- organic phase: 50 mL of a solution of iodine (12.9 g, 50.7 mmol), valeric acid (10.9 mL, 100 mmol) and triethylamine (2.79 mL, 20.0 mmol) in ethyl acetate (132 mL).

The solutions as described above were loaded in a separating funnel and stirred. Once the phases were separated, the separating funnel was drained through the conductivity sensor probe driven by gravity. The conductivity was measured in short intervals (as specified below for each individual set-up) by an Arduino Mega board connected to the appropriate conductivity sensor circuitry. The Arduino read-out was monitored in real time and recorded with the SerialPlot software or a Python script (available as a supplementary file in Electronics\Conductivity_Sensor) and visualized with Origin. In general, triplicates were run for each test case.

Multi-Reference Circuit Scheme

The aim of this design was to try to accommodate to the situations of biphasic mixtures with very different conductivities by changing the reference voltage while using a single resistance reference. The Arduino AVR-based boards allows use of internal 1.1 V, 2.56 V and Vcc as ADC reference voltage levels, thus allowing “zooming” into the bottom region of the whole ADC range when operating from the +5V Vcc. A reading was taken every 0.1 s. During preliminary testing a composite 11.2 MΩ resistor was found to give the best discrimination while resistors with higher or lower resistances performing worse either in situations with low conductivity mixtures or high conductivity mixtures, respectively. A bypass capacitor was added close to the measurement point to reduce the error from noise. It was found to give adequate filtering while maintaining satisfactorily short response times. The circuit scheme is shown below (Figure 121).

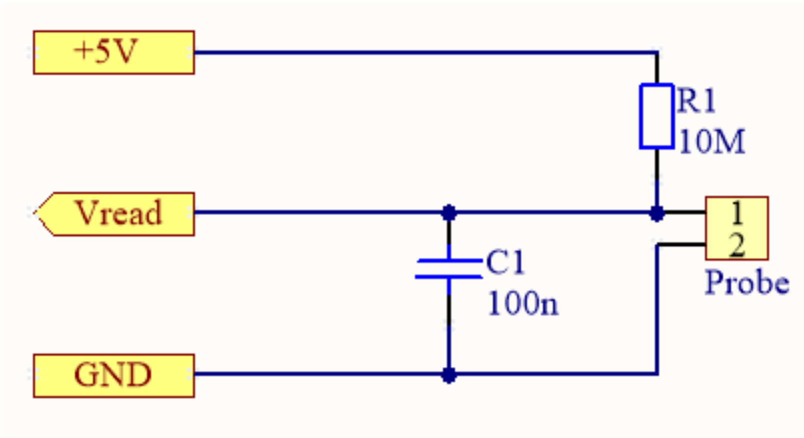


Figure 121. Multi-reference circuit scheme. VREAD: read-out voltage level, VCC: reference voltage level, Probe: conductivity sensor.

The results obtained with the multi-reference circuit scheme are shown in Figure 122. In case A the ADC Vref was set to 1.1 V for the detection of the phase boundary. To illustrate the "zoom" effect of the ADC Vref on the read-out, the Vref value was temporarily changed to 2.56 V and 5.0 V while the conductivity sensor was filled with the organic phase (as indicated on the green curve). One can see that at 1.1 V Vref the largest possible ADC reading drop can be expected (in ADC units).

A lower Vref (1.1V instead of 5V) increases a drop amplitude in the test Case B (Figure 122). However, in Case A the phase boundary produced only a small change in the read-out, which would be difficult to capture algorithmically. In case C it was impossible to perform a run at Vref set to 1.1V as the high conductivity of the solution yielded ADC saturation even after the phase change. In principle, allowing for a broader range of the Vref settings in combination with different resistor values may give satisfactory results over the range of test cases. This avenue was not further explored.

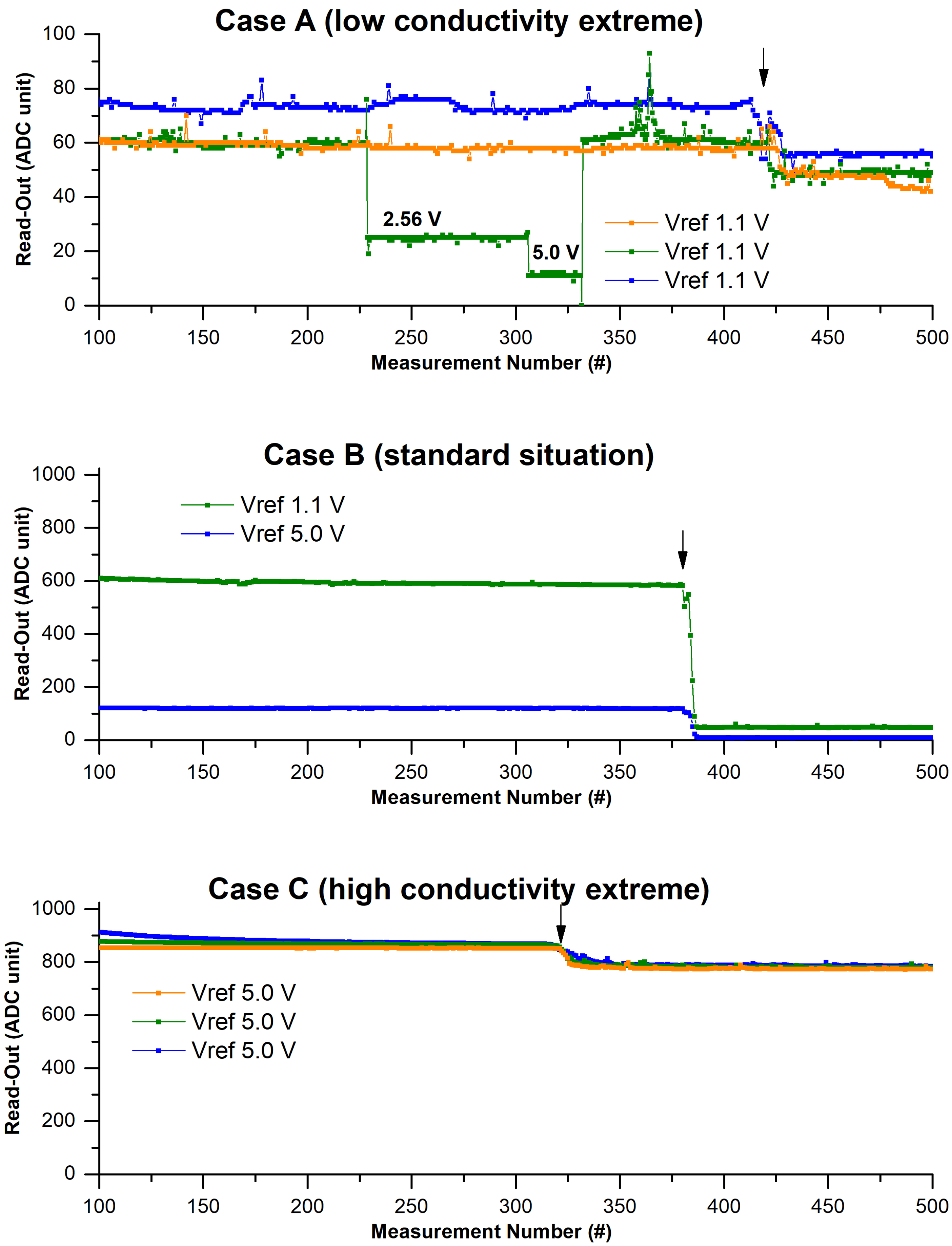


Figure 122. The black arrow indicates the region where the phase boundary passed through the conductivity sensor.

Multi-Resistor Circuit Scheme

Since it was not possible to achieve reliable phase discrimination in all three test cases with the multi-reference circuit above (given the available selection of internal reference voltage levels) we turned back to the previously used approach. Instead of varying the ADC reference voltage, two resistors with different values were included in the divider design. The high-resistance leg can be shunted with a lower resistance by switching on the MOSFET, thereby the total resistance drops from 10 MΩ to about 100 KΩ. This approach was augmented with a bypass capacitor from the experiments above, as it proved to give more stable readings. The values of the resistors R1 and R2 were chosen based on preliminary experiments with similar multi-resistor set-ups and experience gained with the multi-reference set-up discussed above. An extra filtering capacitor C1 was added to compensate for the effect of long wires between the sensor and the Arduino board. The ADC reference voltage was fixed to 2.56 V (generated internally on Arduino). One reading was taken with the MOSFET switched off, then the MOSFET was switched on and another reading was taken. These two readings were summed up and sent back to the PC. The whole cycle was repeated every 200-1000 ms yielding one data point per cycle. The circuit schematic used is shown in Figure 123.

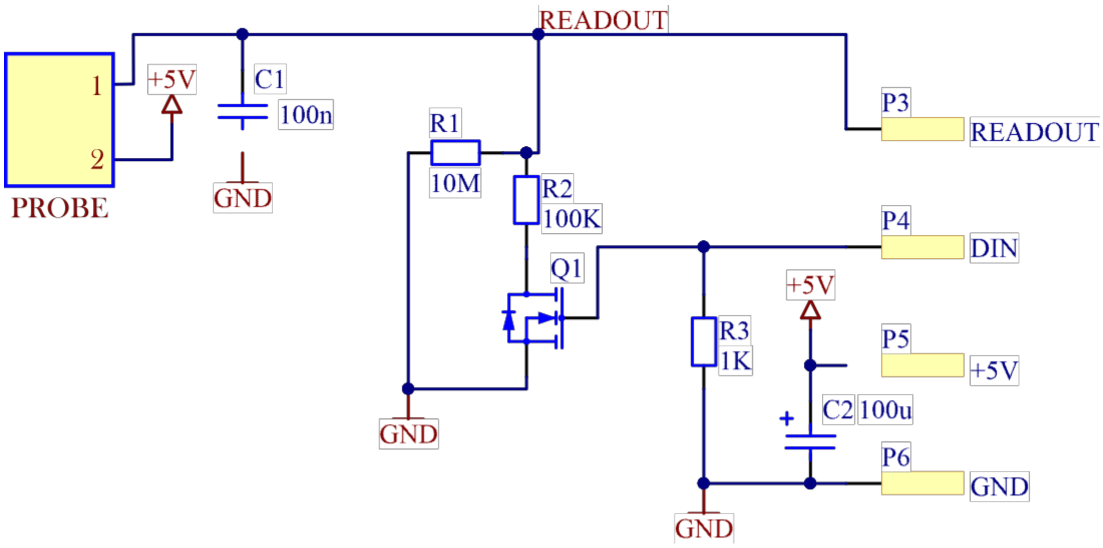


Figure 123 Multi-resistor circuit scheme. V_{READ}: read-out voltage level, +5V: supply voltage, PWM: input to switch the MOSFET, GND: signal ground, Probe: conductivity sensor. (Figure 120 reprinted for convenience).

The results obtained with the multi-resistor circuit scheme are shown below in Figure 124. In all three cases a distinct change of the read-out was observed when the phase boundary passed through the conductivity sensor. An interesting case was observed for the first test mixture (Figure 124, A) - while changing the sampling rate, we observed a clear dependence of the former on the absolute signal value. This might have arised due to parasitic inductance from the connection wires between the conductivity sensor and the PCB forming a tank circuit. The fast-enough signal change rate under free-flow draining was obtained with a sampling time of 0.5 s, so this was chosen as a final value.

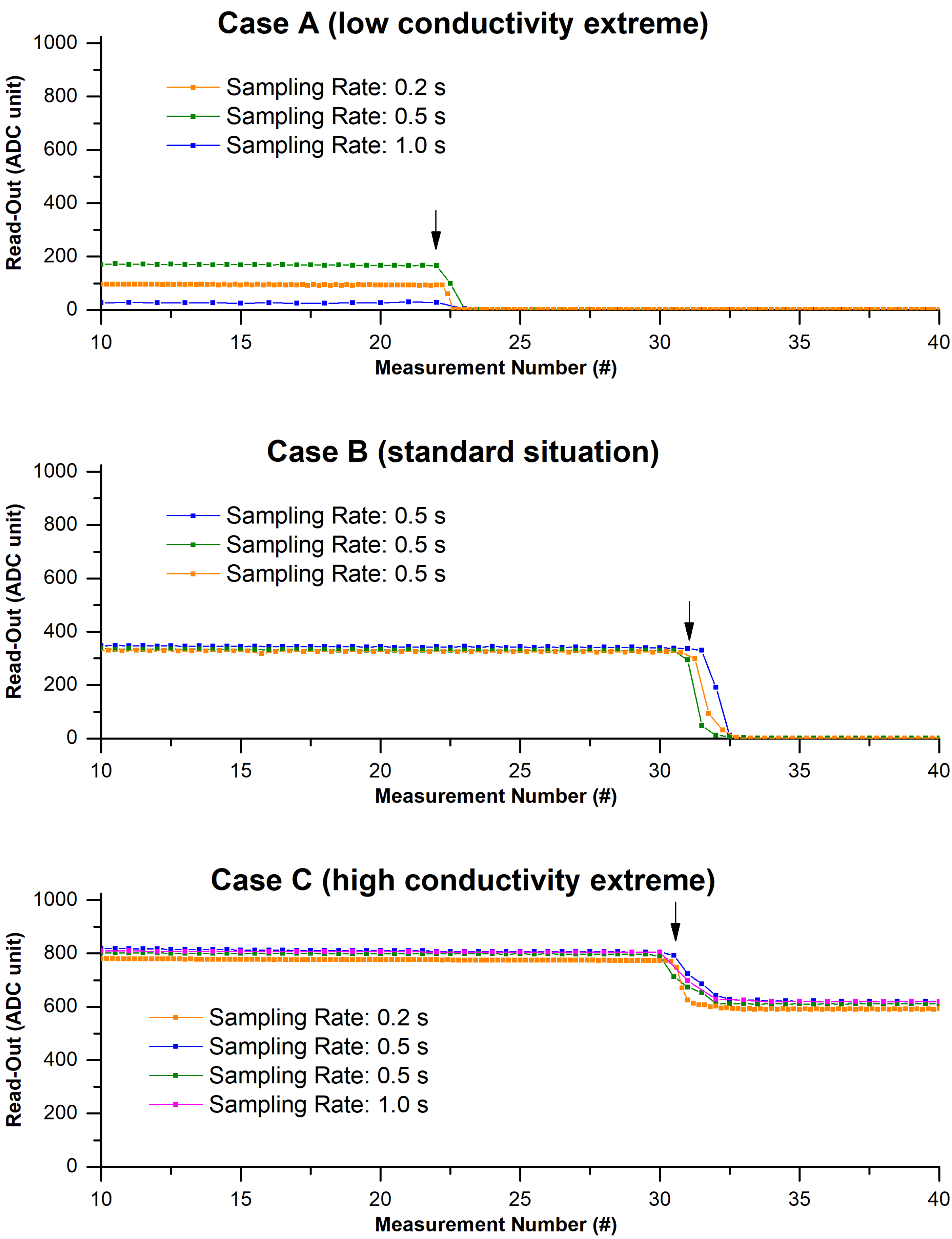


Figure 124. The black arrow indicates the region where the phase boundary passed through the conductivity sensor.

The difference in amplitude drops (ADC units) for the multi-reference circuit and the multi-resistor circuit is presented in the Figure 125. It can be clearly seen, that the multi-resistor setup (A) gave satisfactory results in all three cases with either a high-value or the low-value resistor giving the drop amplitude high enough to be reliably detected.

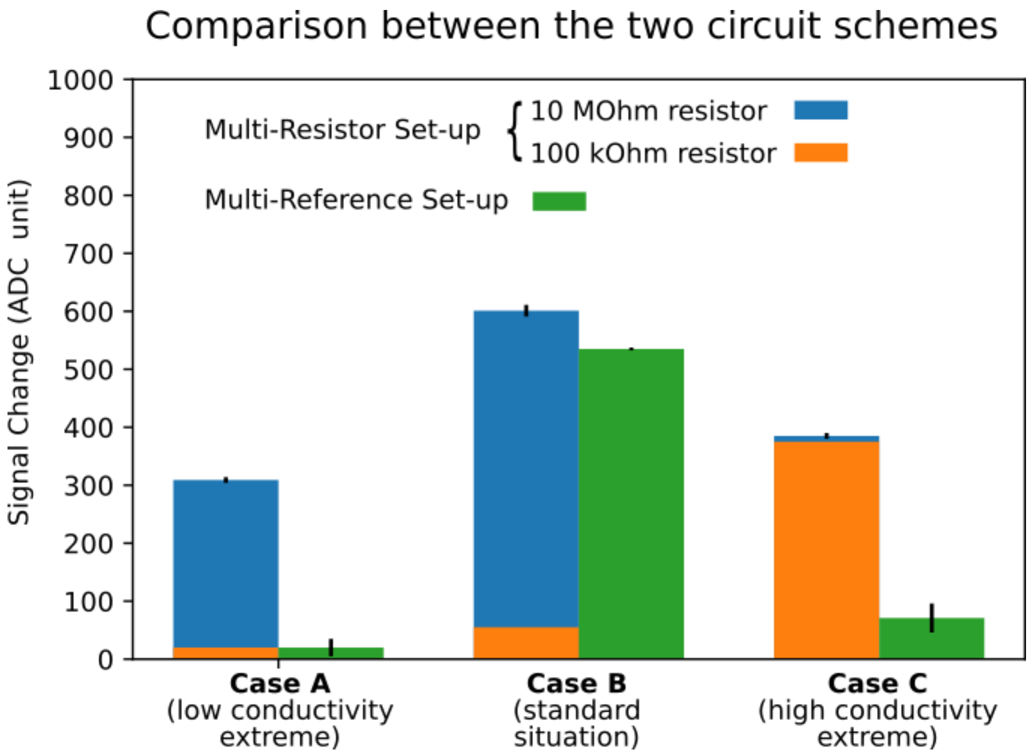


Figure 125 Comparison of the absolute signal obtained with the multi-resistor scheme (A) and the multi-reference scheme (B). For the mutli-resistor scheme the measurement with each resistor is shown. The error bars indicate the standard deviation of the last ten data points before the phase change.

4.14 Rotary Evaporator

The rotary evaporator module (Figure 126) consists of the rotary evaporator with heating bath, jack, and rotator. Additionally, it includes a separate vacuum pump. Two types of rotary evaporators have been integrated in the Chemputer module library so far: an IKA RV10 digital rotary evaporator combined with a Vacuubrand Vario Plus vacuum pump and the modern Büchi R-300 rotary evaporator with Büchi V-300 vacuum pump and F-308 chiller.

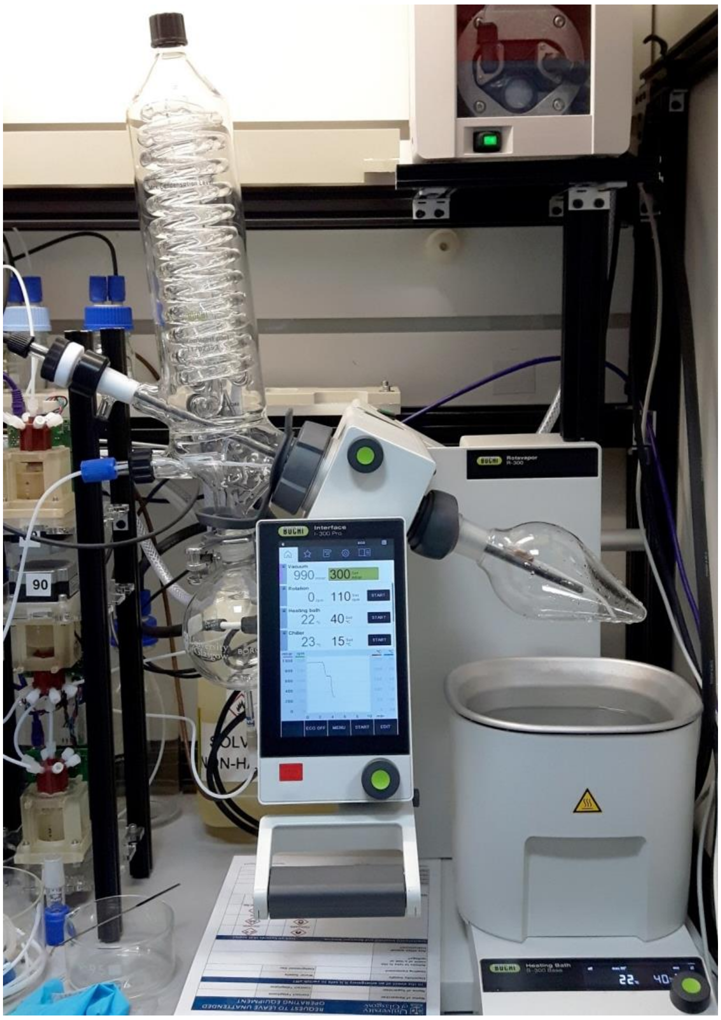


Figure 126 Rotary evaporator module.

Table 22 Bill of materials for the assembly of the rotary evaporator module.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0036	IKA RV10 digital, rotary evaporator	IKA	0010004800	1.00
CHEMP0037	Vacuubrand MD1C vario PLUS	Asynt	ASY-696662PLUS	1.00
CHEMP0023	Serial Cable Assembly 1.8m Male to Female, DB9 to DB9	RS Components	777-621	1.00
CHEMP0041	Receiver flask with connector	In-house - glassware	N/A	1.00
CHEMP0042	Distillation flask	VWR	201-1045	1.00
CHEMP0043	Union, Threaded, 1/4"-28 (Flat Bottom), 1.5mm bore, PEEK™	Kinesis	002307	1.00
CHEMP0045	Bosshead, 8x8mm	VWR	241-0128	2.00
CHEMP0048	30 x 30 x 15mm thick x 6.5mm c/s	First4Magnets	F303015NS	1.00

	N42 Neodymium Magnet - 30kg Pull		-1	
CHEMP0050	Clear No 3.2:1, Heat Shrink Tubing 9.5mm Sleeve Dia. x 1.2m Length	RS Components	170-6305	1.00
CHEMP0052	GL14 screw cap for tube connection, blue	VWR	554-3004	1.00
CHEMP0053	Insert for screw cap GL 18, 3,2 mm inner diameter	VWR	215-2077	1.00
CHEMP0054	Smiths Medical™ Portex™ Coloured PVC Tubing	Fisher Scientific	13180863	2.00
CHEMP0260	Serial to ethernet converter assembly	In-house - Assembly	N/A	2.00
CHEMP0227	Chemputer Valve	In-house - Assembly	N/A	1.00
CHEMP0228	Chemputer Pump 10ml	In-house - Assembly	N/A	1.00
CHEMP0242	5m ethernet cable, blue	Insight	83165	1.00
CHEMP0243	5m, ethernet cable, orange	Insight	83607	1.00
CHEMP0246	5m ethernet cable, yellow	Insight	83245	2.00
CHEMP0268	Zinc Plated Mild Steel Threaded Rods & Studs, M4, 20mm	RS Components	175-6970	1.00
CHEMP0132	Black Nylon Cable Tie, 200mm x 4.6 mm	RS Components	170-3902	1.00
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Kinesis	008T32-150-100	150.00
CHEMP0269	1m 9 Way D RS232 Serial Null Modem Cable - Female to Female	Farnell	SCNM9FF1MBK	1.00
CHEMP0263	Support rod, polished, M10 thread, 12mm diameter, 500mm length	VWR	241-0236	1.00
CHEMP0264	Retort base, threaded M10 × 1,5 mm, pressed steel finished in matt black textured acrylic paint	VWR	NICK6891	1.00
CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050CMBK	1.00
CHEMP0197	2mm dia x 4mm thick N35 Neodymium Magnet - 0.16kg Pull	First4Magnets	F214-500	3.00
CHEMP0155	Screw cap with bore, for tube connection, GL 14, blue	VWR	554-3004	1.00
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Thames Restek	UPXP-320	3.00
CHEMP0265	Plug Tefzel - 1/4-28	Kinesis	P-311	1.00
CHEMP0350	Screw cap for tube connection, blue, GL18	VWR	215-2082	1.00
CHEMP0029	Dibafit™ Threaded Coupling, 1/4"-28	Cole-Parmer	WZ-21939-	1.00

	UNF(F) flat bottom, PEEK		99	

4.14.1 **Vacuum Pump (for the IKA Rotary Evaporator Only)**

1. A serial-to-Ethernet converter (CHEMP0260, Section 4.15) was connected to the pump with a serial cable (CHEMP0023, see Table 27 for specifications of the cable).
2. The pump (CHEMP0037) was mounted on the shelving (Section 4.1).
3. The serial-to-Ethernet converter was attached to the switch (Section 4.3) with an Ethernet cable (CHEMP0246).

4.14.2 **Rotary Evaporator Assembly**

Most steps in the assembly of the rotary evaporator were independent of the actual brand of the rotary evaporator. In the following description, specific instructions are given for each particular brand where required. If the brand is not specified this means that the instructions are general.

1. The rotary evaporator (CHEMP0036) or a Büchi R-300 rotary evaporator with an I-300 Pro interface with VacuBox, a V-300 vacuum pump and a F-308 recirculating chiller) was assembled according to the manufacturer's manual. The rotary evaporator and the pump were placed in the fume hood while the recirculating chiller was placed below the fume hood.
2. For the IKA rotary evaporator a serial-to-Ethernet converter (CHEMP0260, Section 4.15) was connected to the Rotavap RS-232 port with a serial cable (CHEMP0223, see Table 27 for specifications of the cable). The converter was connected to the switch (Section 4.3) with an Ethernet cable (CHEMP0246). The Büchi rotary evaporator was directly connected to the switch with an Ethernet cable taking advantage of the inbuilt Ethernet interface of the I-300 controller.

To remove the concentrated material from the rotary evaporator flask it was necessary to place for the PTFE tubing to reach the very bottom of the evaporator flask. In order to avoid uncontrolled transfer of material through that tubing when the rotary evaporator is vented, a simple system was devised that would raise the tubing slightly when the evaporator flask is lifted:

1. A standard laboratory stand with boss head (CHEMP0045, 0263 & 0264) and a steel rod (CHEMP0268) was positioned close to the neck of the evaporator flask (Figure 127).

2. A magnet (CHEMP0248) was attached to the steel rod with a cable tie (CHEMP0132, Figure 127).

Figure 127 Neodymium magnet placed above the evaporator flask.

3. Tubing that was sufficiently long to reach from the Chemputer backbone (Section 4.8) to the base of the evaporation flask (approximately 1.5 m of tubing, CHEMP0149) was prepared.
4. Three neodymium magnets (CHEMP0197) were positioned approximately 12 cm from the end of the tubing and fixed with ca 5 cm of heat shrink (CHEMP0050) as shown in Figure 128.

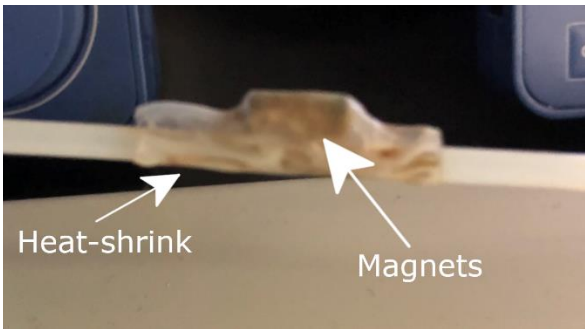


Figure 128 Fixing three neodymium magnets to the PTFE tubing using heat shrink.

5. To be able to fully drain the collection flask, it was necessary to bend the end of the tube after the magnets to an angle of approximately 30° (see Figure 129). Some experimentation was required from case to case to ensure the best angle and length of tubing. The tubing was bent while being gently heated with a heat gun.

   Important: With the Büchi rotary evaporator it is important to place the tubing below the foam detector (if installed) to avoid false readings from the detector. Büchi provided the rotary evaporator with a custom-made additional port and connector on the glassware assembly to accommodate this tube.

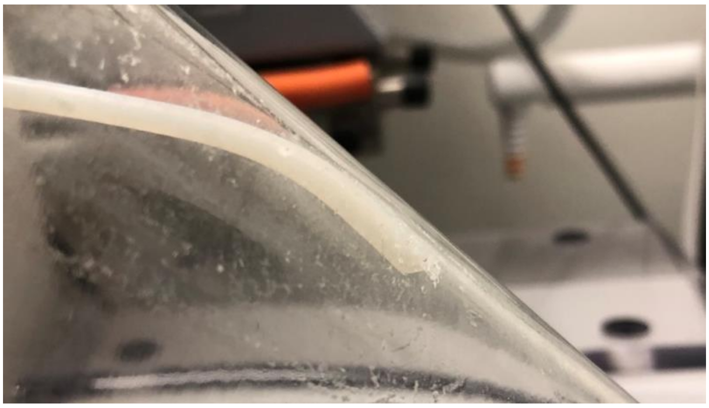


Figure 129 The tubing reaching into the evaporator flask was bent to an angle of approximately 30°.

6. It was ensured that when the evaporator flask was lifted out of the heating bath, the tubing would be attracted to the external magnet (see Figure 130) and away from the bottom of the evaporator flask.

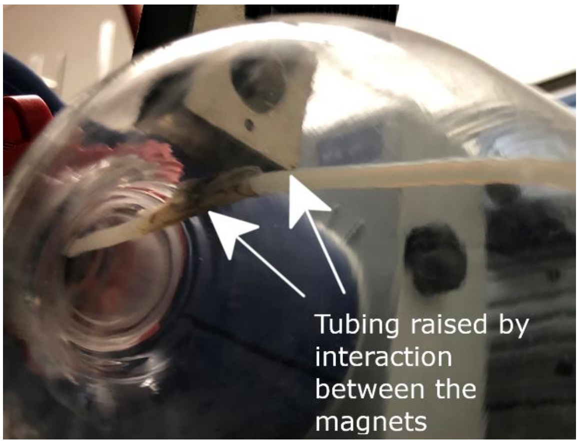


Figure 130 When the evaporator flask was lifted out of the heating bath, the tubing was pulled towards the external magnet.

7. For the IKA rotary evaporator the other end of the tubing from above steps was fed through a NS19 insert (CHEMP0053) with GL14 adaptor (CHEMP0350) as shown in Figure 131 A (disassembled) and B (assembled). For the Büchi rotary evaporator the tubing was fed through the custom-made connector port (which was provided by Büchi on request), which consisted of a bespoken glass tube with GL14 thread and GL14 cap with inset (see Figure 131 C).

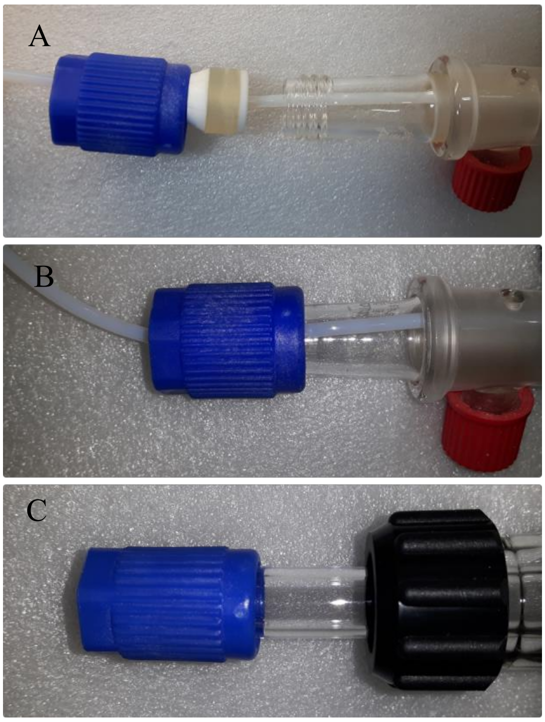


Figure 131 Assembly of the tubing adaptor for the IKA rotary evaporator (A - disassembled and B - assembled) and for the Büchi rotary evaporator (C).

8. The free end of the tubing coming from the evaporator flask was attached to the liquid handling backbone with fitting and ferrule (CHEMP0220).
9. A custom-made solvent receiving flask with a threaded glass tube extension was connected to the condenser in lieu of the standard receiving flask to enable the automated draining of the distilled solvent (see Figure 132 for details).

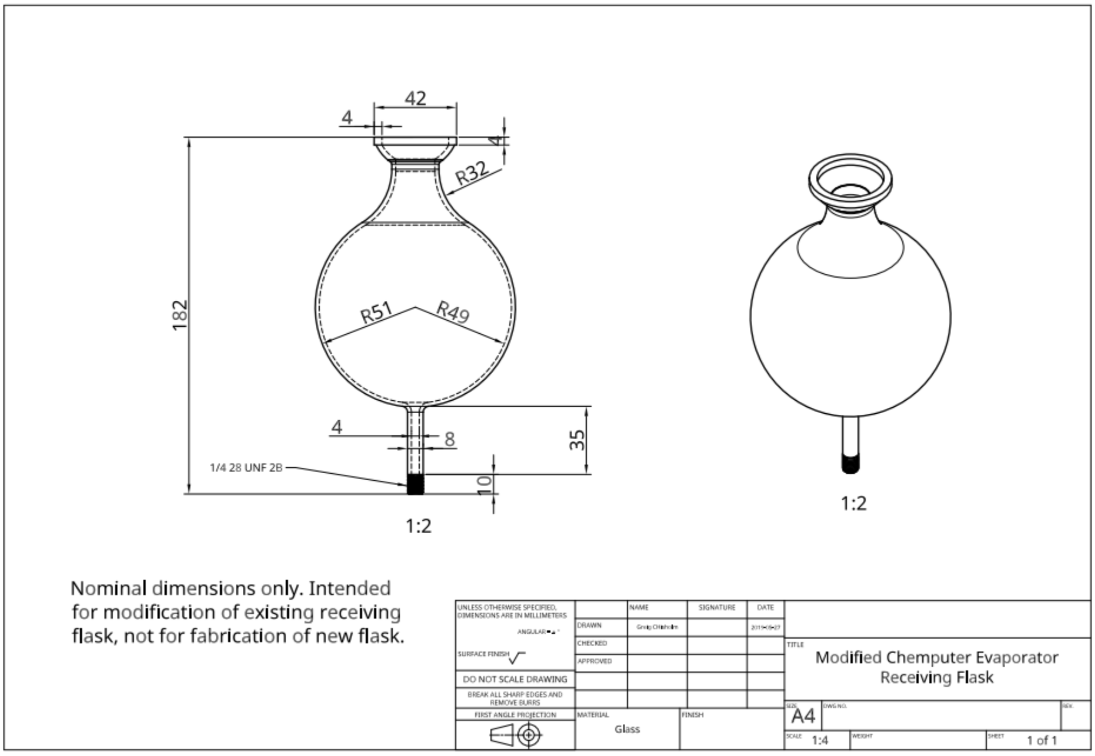


Figure 132 Modified solvent receiving flask with threaded glass tube extension.

10. PTFE tape was wrapped around the glass thread of the glass tube outlet and a threaded union (CHEMP0029) was attached.
11. The outlet of the solvent receiving flask was connected to the liquid handling backbone using approximately 1 m of PTFE tubing (CHEMP0149) with ferrule and fitting (CHEMP0220) on both ends (Figure 133).

Figure 133 Connection of the solvent receiving flask to the liquid handling backbone.

4.15 Cartridge Carousel

A simple module was developed that allows for switching between up to six different cartridges filled with solid reagents, which, depending on the content, can be used for filtering or drying operations. The module consists of a standard valve that is attached to 6-way manifold. The assembly is made of a custom laser-cut cartridge holder (the DXF file is available as a supplementary document in Laser_Cut_Parts\Cartridge_Carousel) and Openbuilds V-slot rails acting as a frame.

The bill of materials is shown in Table 23.

Table 23 Bill of materials for the assembly of the cartridge carousel.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0227	Chemputer Valve	In-house - Assembly	N/A	1.00
CHEMP0367	Cartridge holder	In-house - laser cut	SCOT286230006	1.00
CHEMP0148	Idex CV-3324 Nonmetallic Inline Check Valve, Inlet, 1 psi, 0.06" ID, 1/4-28 (F) to 1/4-28 (M) Flat Bottom	Thames Restek	CV-3324	6.00
CHEMP0368	Low-Pressure Manifold Body, Natural PEEK, 6 Port, 0.062" Bore, 1/8" OD Tubing, 1/4-28 Flat Bottom	Cole-Parmer	WZ-02023-09	1.00
CHEMP0369	Port Plug, Standard Knurl, Natural PCTFE, 1/4-28 Coned For 1/8"OD Tubing	Cole-Parmer	WZ-02018-32	6.00
CHEMP0370	Drop In Tee Nuts M5 Thread size	Oozenest	VSLOT-H-DT-M5	8.00
CHEMP0371	M5 Low Profile Bolts	Oozenest	VSLOT-S-B-LP-M5-8	8.00
CHEMP0372	Universal L brackets single clear anodise	Oozenest	VSLOT-B-UL-S-C	4.00
CHEMP0373	V-Slot Linear Rail – 20x20mm black anodise	Oozenest	VSLOT-2020-O-B-2900-3000	Cut to size
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Kinesis	008T32-150-100	Varies
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Thames Restek	UPXP-320	Varies

1. The custom laser-cut cartridge holder (CHEMP0367) shown in Figure 134 was attached to the V-Slot rail (CHEMP0373) via the L brackets (CHEMP0272), drop in tee nuts (CHEMP0370) and M5 bolts (CHEMP0371) as shown in Figure 135.

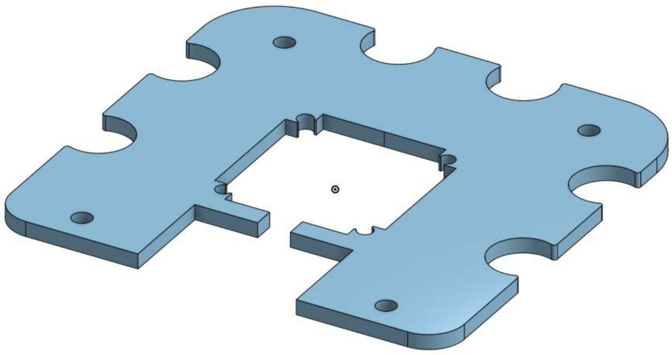


Figure 134 CAD model of the custom laser-cut cartridge holder.

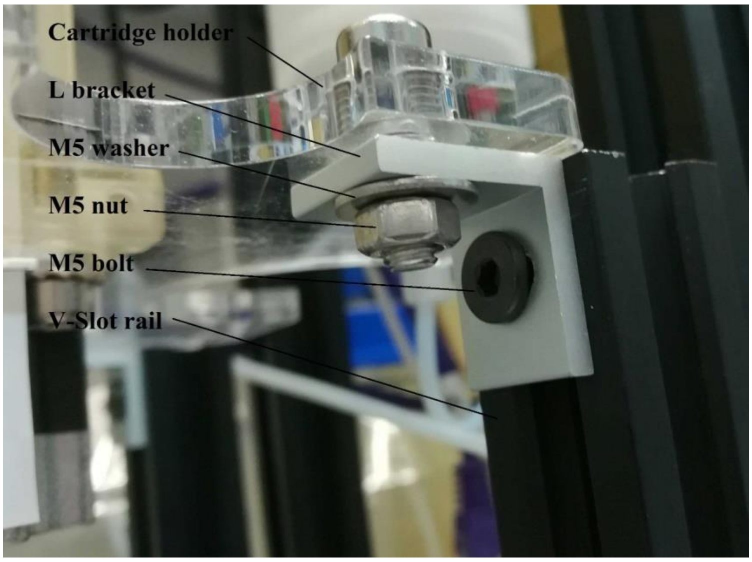


Figure 135 Assembly of the cartridge carousel stand.

2. The valve was inserted in the custom laser-cut cartridge holder (CHEMP0367). Optionally, the valve can be attached to the cartridge holder through the motor mount holes with 30 mm long M5 screws (not shown).
3. The PEEK manifold (CHEMP0368) was assembled with the appropiate number of check valves (CHEMP0148) and PCTFE plugs (CHEMP0369) as shown in Figure 136.

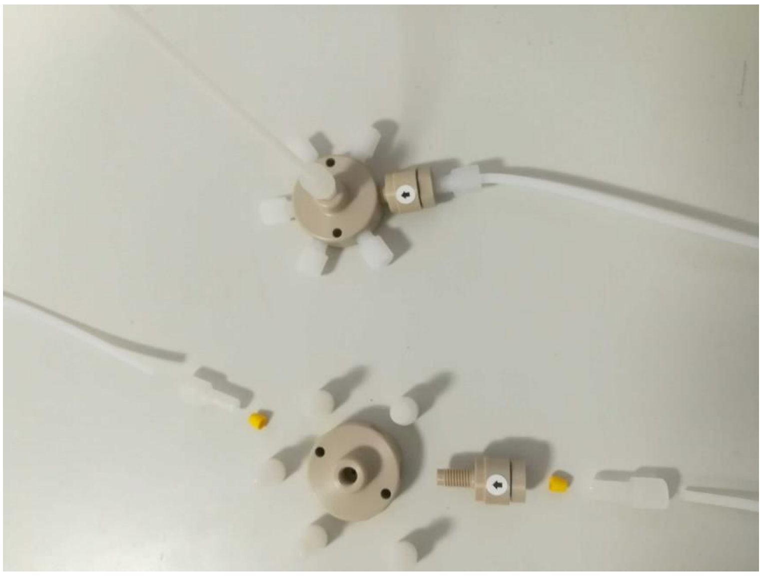


Figure 136 Assembly of the manifold for the cartridge carousel module.

4. Cartridges were mounted and connected via the standard PTFE tubing (CHEMP0220), flangeless fittings and connectors (CHEMP0149) (Figure 137).

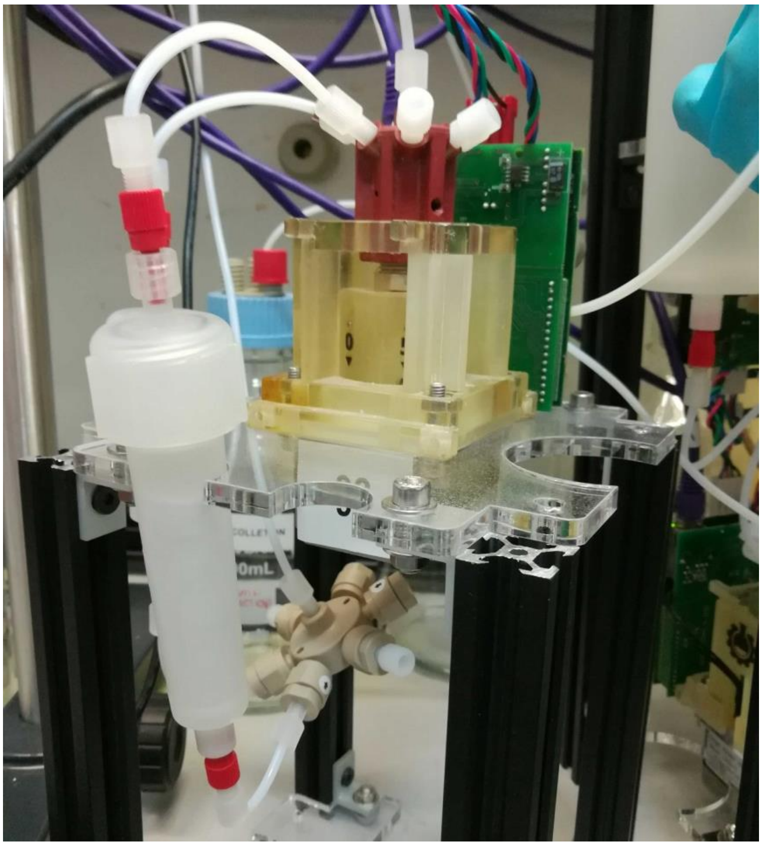


Figure 137 Example of a fully assembled cartridge carousel module.

4.16 Column Chromatography Module

The column chromatography module is an extension of the cartridge carousel (the modified DXF file is available as a supplementary file in Laser_Cut_Parts\Chromatography_Module). However, the manifold was replaced with additional valves that allow for switching between different cartridges as well as different collection flasks and vacuum for dry-loading purposes. In this development the module doesn't have any spectroscopy feedback to detect the desired product for collection, however it relies on the sole elution volume as a parameter for determining the collection of the desired fraction and which proved to be a very reliable method. The bill of materials for the assembly of the column chromatography module is shown in Table 24.

Table 24 Bill of materials for the column chromatography module.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0227	Chemputer Valve	In-house - Assembly	N/A	3.00
CHEMP0367	Cartridge holder	In-house - laser cut	SCOT28623 0006	3.00
CHEMP0370	Drop In Tee Nuts M5 Thread size	Oozenest	VSLOT-H-DT-M5	32.00
CHEMP0371	M5 Low Profile Bolts	Oozenest	VSLOT-S-B-LP-M5-8	12.00
CHEMP0372	Universal L brackets single clear anodise	Oozenest	VSLOT-B-UL-S-C	4.00
CHEMP0373	V-Slot Linear Rail – 20x20mm black anodise	Oozenest	VSLOT-2020-O-B-2900-3000	Cut to size
CHEMP0220	Flangeless Fitting Natural, Polypropylene,1/4-28 Flat-Bottom for 1/8" OD	Kinesis	008T32-150-100	Varies
CHEMP0149	Tubing, PTFE, 1/8" (3.2mm) OD x 1.5mm ID, 100M	Thames Restek	UPXP-320	Varies

1. Valve, cartridge holder and stand were assembled as described for the cartridge carousel.
2. The manifold was replaced with two valves (CHEMP0227) facing each other, connected via the central port, each attached to the stand via a separate custom laser-cut cartridge holder (CHEMP0367) as shown in Figure 138.

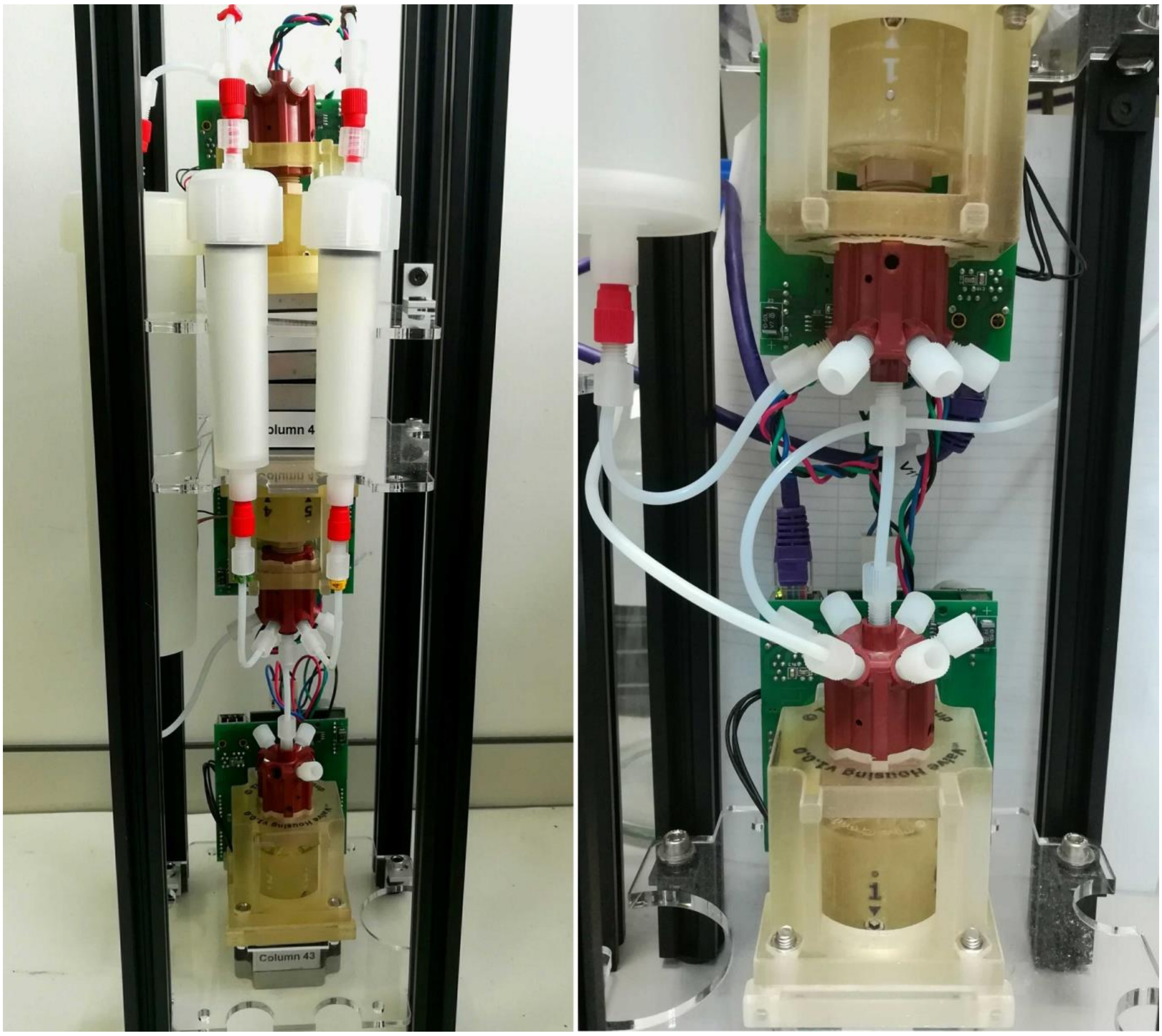


Figure 138 Example of a fully assembled column chromatography.

4.17 **Serial-to-Ethernet Converter**

One of the key incentives during the further development of the Chemputer was to make the system more uniform by reducing the number of unique hardware components. One step towards this end was to include as many third-party devices as possible available over the Ethernet network, thus removing the need to have different custom interface modules. As many of the used devices had a serial port as the standard mean of communication, a serial-to-Ethernet converter (Figure 139) was needed to make the transition from serial to Ethernet.

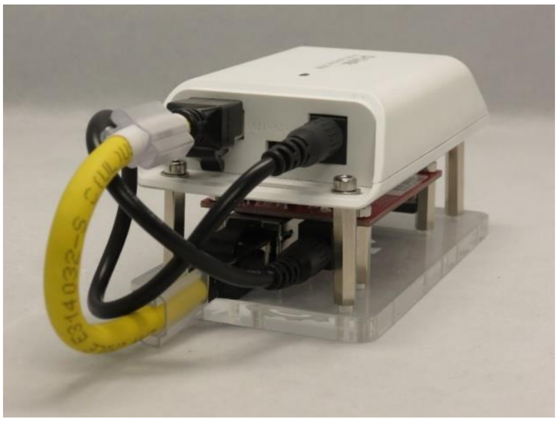


Figure 139 The serial-to-Ethernet converter fitted with PoE splitter used to connect third-party devices with serial connection to the Chemputer Ethernet network.

Table 25 Bill of materials for the serial to ethernet converter.

PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE	QUANTITY
CHEMP0172	Mounting panel for serial to Ethernet convertor	In-house - laser cut	N/A	1.00
CHEMP0173	D-sub, 9-Pin (Serial) to RJ45 Network Adapter	RS Components	419-968	1.00
CHEMP0174	D-Link DPE-301GS PoE Splitter	Insight	6338009	1.00
CHEMP0175	STP Cat6a Cable 200mm, Yellow, RJ45	RS Components	123-5549	1.00
CHEMP0176	Male/Female PCB spacer, M3, 15mm length	RS Components	161-3631	4.00
CHEMP0177	BRACE F/M 25MM	RS Components	125-6024	4.00
CHEMP0178	M3 x 8mm Full Thread Cap Head Screws (DIN 912) - A2 Stainless Steel	AccuGroup	SSCF-M3-8-A2	8.00
CHEMP0179	M3 Form A Flat Washers (DIN 125) - A4 Stainless Steel	AccuGroup	HPW-M3-A4	4.00

4.17.1 Assembly

1. The mounting panel (CHEMP0172) was cut from acrylic (transparent, colourless, 6 mm) with a laser cutter. The DXF file can be found in Laser_Cut_Parts\PoE_Splitter. Note that the Chemputer logo was engraved and not cut.
2. After cutting any material that was still sticking inside the holes was removed by pressing the mounting panel on a flat surface or alternatively with the pointed end of a pair of scissors.
3. PCB spacers (CHEMP0176) were screwed in the four central holes (Figure 140 A).

4. The serial-to-Ethernet board (CHEMP0173) was mounted on the four PCB spacers and secured with four screws (CHEMP0179) as shown in Figure 140 B. Note that the sockets on the board should point towards the acrylic mounting panel.

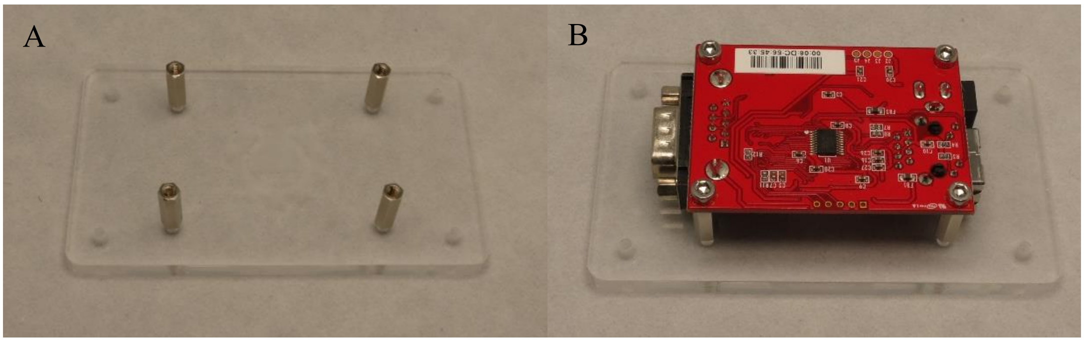


Figure 140 (A) 15 mm PCB spacers were screwed in the mounting panel and (B) the Ethernet-to-serial board was mounted.

5. PCB spacers (CHEMP0177) were screwed in the four remaining holes (Figure 142 A).
6. The used PoE splitter (CHEMP0174) can output three different voltages: 5 V, 9 V and 12 V. It is important to set the splitter to the 5 V output (Figure 141). Otherwise the serial-to-Ethernet board will be destroyed when powered up.

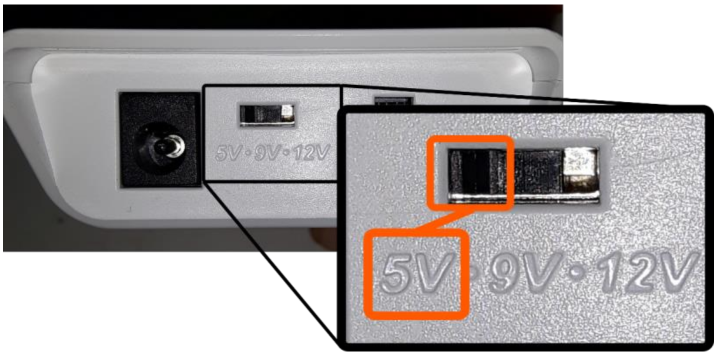


Figure 141 Set the PoE splitter to the 5 V output before use.

7. The PoE splitter was mounted on outer four PCB spacers and secured with four screws (CHEMP0178) and washers (CHEMP0179) as shown in Figure 142 B. The washers were placed between the PoE splitter and the screw heads.

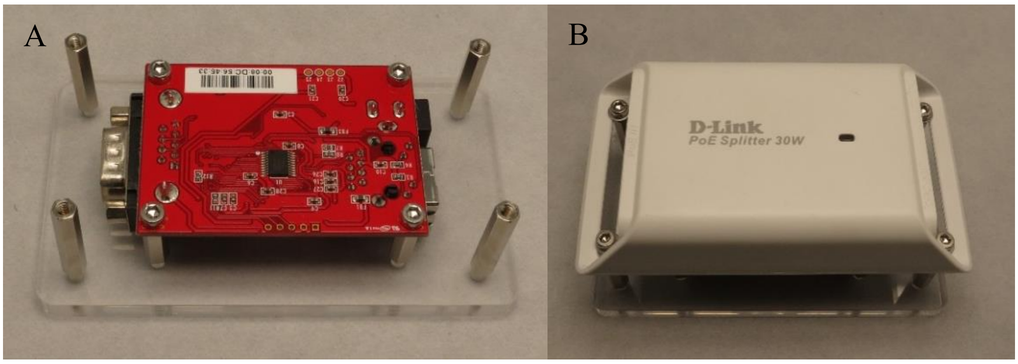


Figure 142 (A) 25 mm PCB spacers were screwed in the remaining four holes and (B) the PoE splitter was mounted.

8. The Ethernet cable (yellow) and the power cable (black, included with the PoE splitter) were attached as shown in Figure 143. Attention must be payed to bend the Ethernet cable as gently and as evenly as possible.

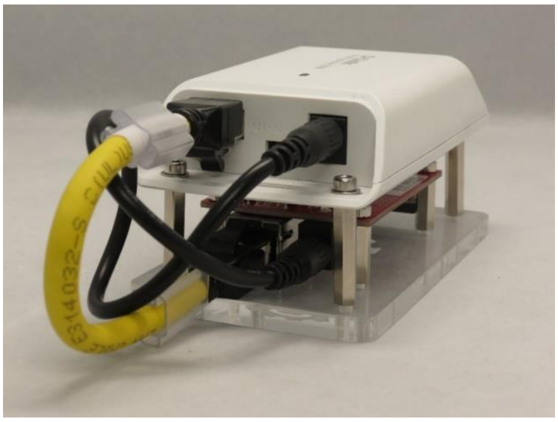


Figure 143 Cable connections between the PoE splitter and the serial to Ethernet board.

4.17.2 Settings

1. The WIZnet configuration tool was installed (open https://www.wiznet.io/product-item/wiz110sr/ and scroll down to Config-Tool) and opened.
2. The serial-to-Ethernet converter was attached to a free port on the switch (see Section 4.1). Alternatively, a direct temporary connection between the PC and the converter can be established.
3. The "Direct IP Search" box was ticked, the factory IP address was inserted (192.168.11.1) and "Search" was clicked (Figure 144). The search returned the MAC address of the corresponding board in the "Board list". The board was selected, and the "Network" parameters were set as follows:
    a. "IP Configuration Method" was set to "Static".
    b. The "Local IP" was set to 192.168.1.50 in the example shown. It is advised to increment the IP addresses of all serial-to-Ethernet converters starting from

192.168.1.50 upwards. The “Port” was set to 5000. It is advisable to make a well-visible note of the IP address on the casing of the device.

c. The “Subnet” mask was set to 255.255.0.0.

d. The “Gateway” was set to 192.168.1.1.

e. The “Server IP” was the same as the local IP (i.e. in the example shown it was 192.168.1.50). The “Port” was set to 5000.

f. The “Operation Mode” was set to “Server”.

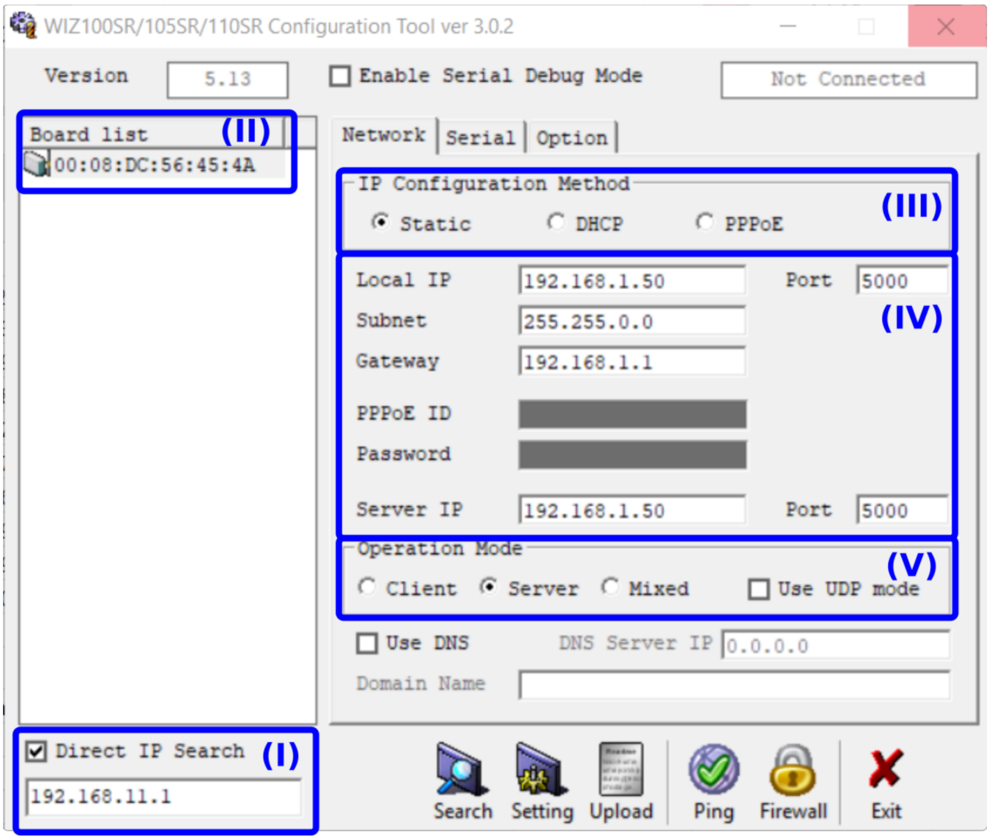


Figure 144 Connecting to the serial-to-Ethernet board and configuring the network settings.

4. The “Serial” tab was selected next (Figure 145) and the serial settings were configured according to the manufacturer’s specifications of the device the serial-to-Ethernet converter will be connected to. Full details are given in Table 26.

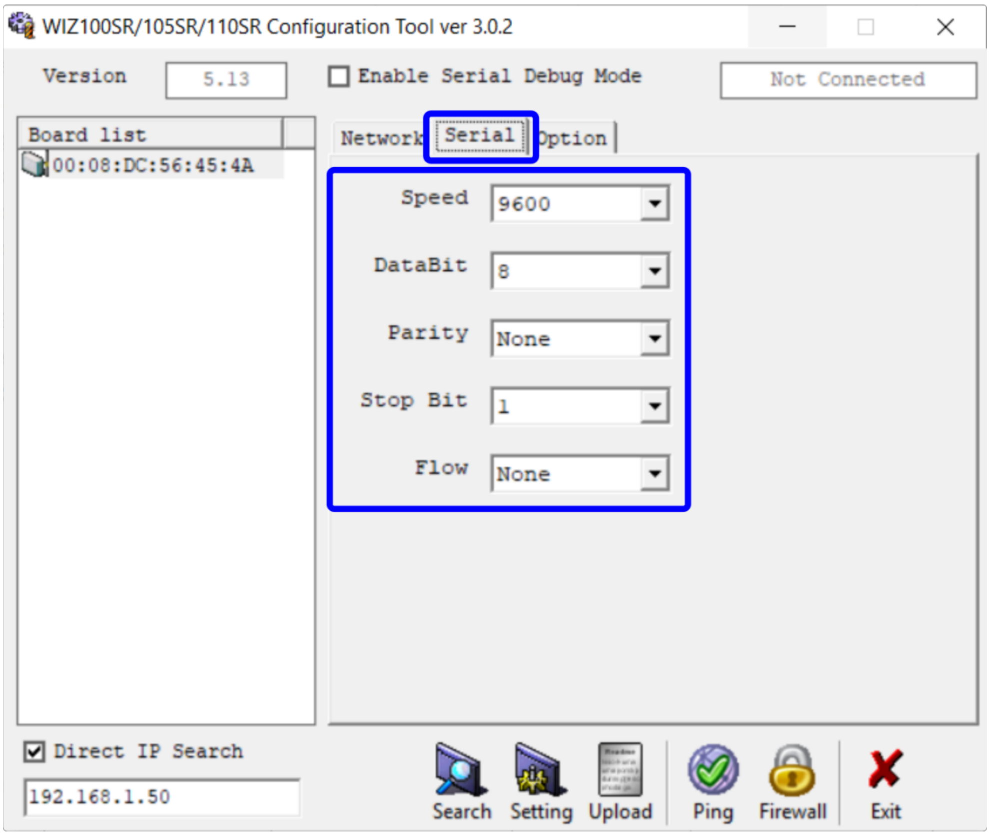


Figure 145 The serial settings must be made based on the requirements of the device the serial-to-Ethernet connector will be attached to.

Table 26 Serial configuration for the used third-party devices.

Device	Speed	DataBit	Parity	Stop Bit	Flow
IKA RET Control-Visc Hot Plate	9600	7	Even	1	None
IKA RCT Digital Hot Plate	9600	7	Even	1	None
CF41 Chiller	9600	7	Even	1	RTS/CTS
CVC 3000 Vacuum Pump	19200	8	None	1	RTS/CTS
RV 10 Digital Rotavap	9600	7	Even	1	None
RZR 2052 Control Stirrer	9600	8	None	1	None
Heidolph Torque 100 Stirrer	9600	8	None	1	None

5. Under the “Option” tap the “Time” for the “Data Packing Condition” was set to 500 ms (Figure 146). All other parameters were left at their default values.
6. The settings were saved to the PoE splitter board by clicking “Setting”.

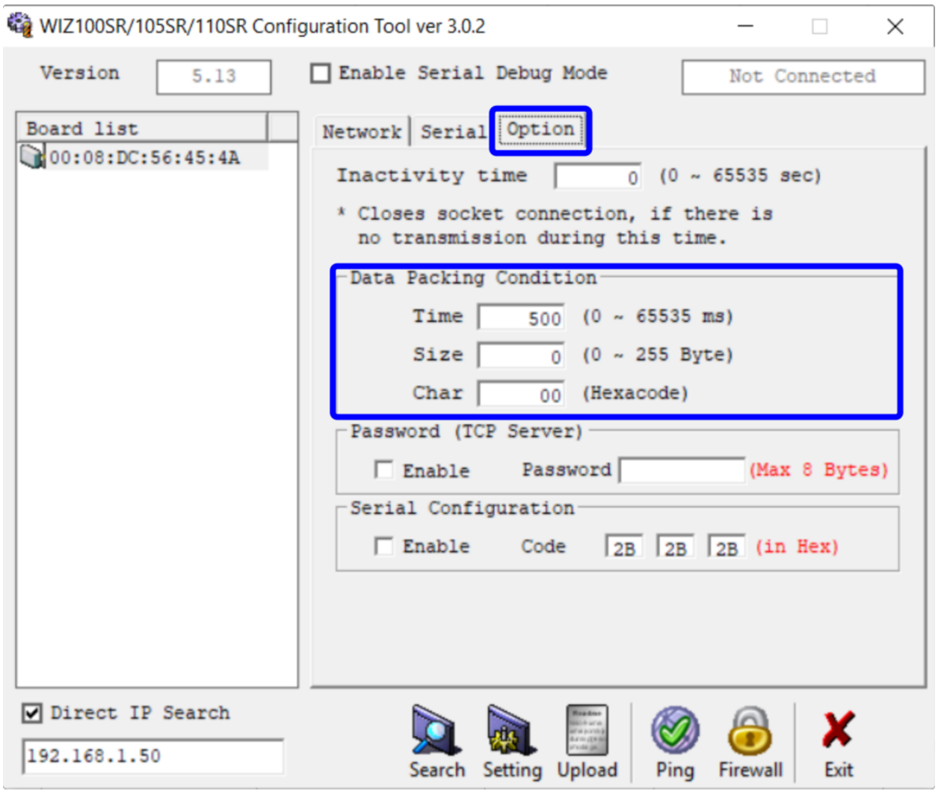


Figure 146 Under “Option” the “Time” in “Data Packing Condition” was set to 500 ms.

7. If not already done so, the serial-to-Ethernet converter was connected to the switch (see Section 4.1).
8. The serial-to-Ethernet converter was connected to its dedicated device via a serial cable. In the case of the CF41 Chiller a null modem adapter (D-sub 9 way null modem adapter, RS Components part number: 243-0374) was required for the connection. A list of the serial cables used can be found in Table 27. Consider varying the length of the serial cable to minimise unnecessary loose cabling cluttering the fume cupboard.

Table 27 Serial cables used to connect the third-party devices.

Device	PART NO.	PART DESCRIPTION	SUPPLIER	ORDER CODE
IKA RET Control-Visc Hot Plate	CHEMP0223	9 way D male to female RS232 serial cable, 1 m	Farnell	MXT10050CMBK
IKA RCT Digital Hot Plate	CHEMP0223	9 way D male to female RS232 serial cable, 1 m	Farnell	MXT10050CMBK
CF41 Chiller	CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050CMBK
CVC 3000 Vacuum Pump	CHEMP0269	9 way D female to female RS232 serial null modem cable, 1 m	Farnell	SCNM9FF1MBK
RV 10 Digital	CHEMP0223	9 way D male to female RS232 serial cable,	Farnell	MXT10050CMBK

Rotavap		0.5 m		
RZR 2052 Control Stirrer	CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050CMBK
Heidolph Torque 100 Stirrer	CHEMP0223	9 way D male to female RS232 serial cable, 0.5 m	Farnell	MXT10050CMBK

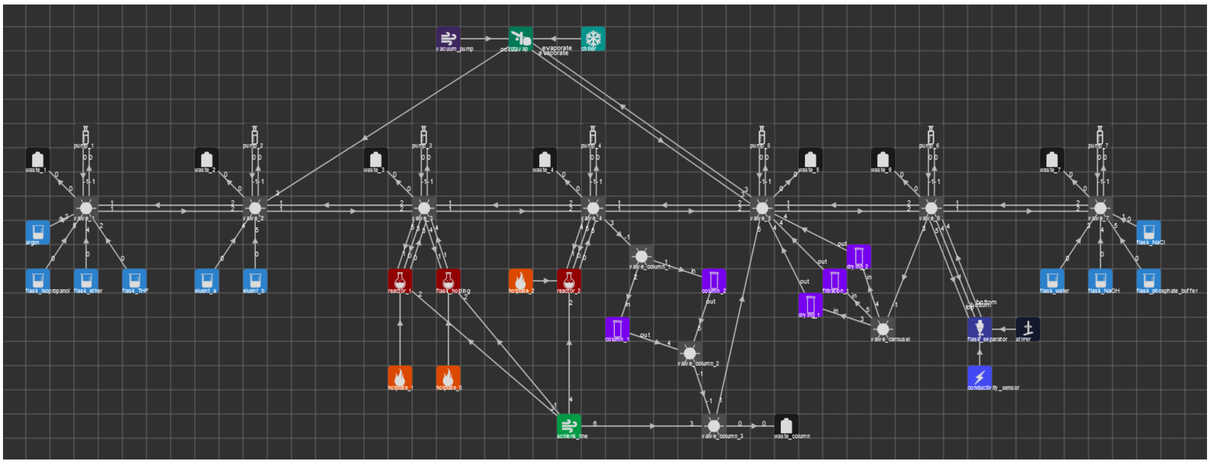
5 Hardware Instances

5.1 Chemputer Hardware Instance for the Iterative Cross-Coupling Sequence of MIDA-Boronates

The Chemputer hardware instance for the iterative cross-coupling (ICC) sequence of MIDA-boronates (Figure 147) consisted of two reactor modules equipped with in-line filters, one rotary evaporator, one separator, one holding flask, one cartridge carousel and one column chromatography module. All reagent flasks were connected to the passive inert gas supply of the programmable manifold and were kept under a constant positive pressure of argon. The reactor and holding flasks and the rotary evaporator were connected to the valve outlets. The cartridge carousel and the column chromatography module were equipped with EasyVarioFlash® cartridge 10 g Luer with seals, frits, filters and 17 mm internal diameter. The cartridge carousel was equipped with two drying cartridges containing a homogenous mixture of magnesium sulfate and sand (3:1 w/w) and one cartridge filled with Celite. The column chromatography module was equipped with two cartridges filled with silica gel (40 µm – 63 µm, 60 Å mean pore size, 500 m^2/g surface area) for the catch-and-release purification. The two reactors were connected to the backbone through a cartridge containing 4 Å molecular sieves. The modules were connected via the liquid handling backbone, which was composed of 7 pumps with 25 mL syringes and 7 valves. A graph representation is shown in Figure 148. The Python script detailing the synthesis and the graph file are available as supplementary files in Software\Syntheses_Scripts_and_Graph_Files\MIDA.

Figure 147 Chemputer hardware instance for the MIDA boronate ICC.

Figure 148 The graph representation of the MIDA Boronate rig.



5.2 Chemputer Hardware Instance for the Synthesis of NHS-Diazirine

The Chemputer hardware instance (Figure 149) used for the synthesis of NHS-diazirine consisted of one reactor, one jacketed filter, one phase separation module, one chromatography module loaded with a 150 g Easy Vario Cartridge filled with silica gel (40 µm – 63 µm, 60 Å mean pore size, 500 m^2/g surface area), a cartridge carousel and one evaporation module. All flasks, but those for aqueous solutions, and all the modules were maintained under constant, positive pressure of argon. The solvents (methanol, dichloromethane and diethyl ether) were stored over anhydrous magnesium sulphate (which was wrapped in filter paper to avoid clogging of the tubes). The hydroxylamine sulfonic acid and EDCI were stored as solids in 'advanced reagent flasks' and were dissolved at runtime. The modules were connected via the liquid handling backbone, which was composed of 8 pumps with 10 mL syringes and 8 valves. A graph representation is shown in Figure 150. The Python script detailing the synthesis and the graph file are available as supplementary files in Software\Syntheses_Scripts_and_Graph_Files\NHS-Diazirine.

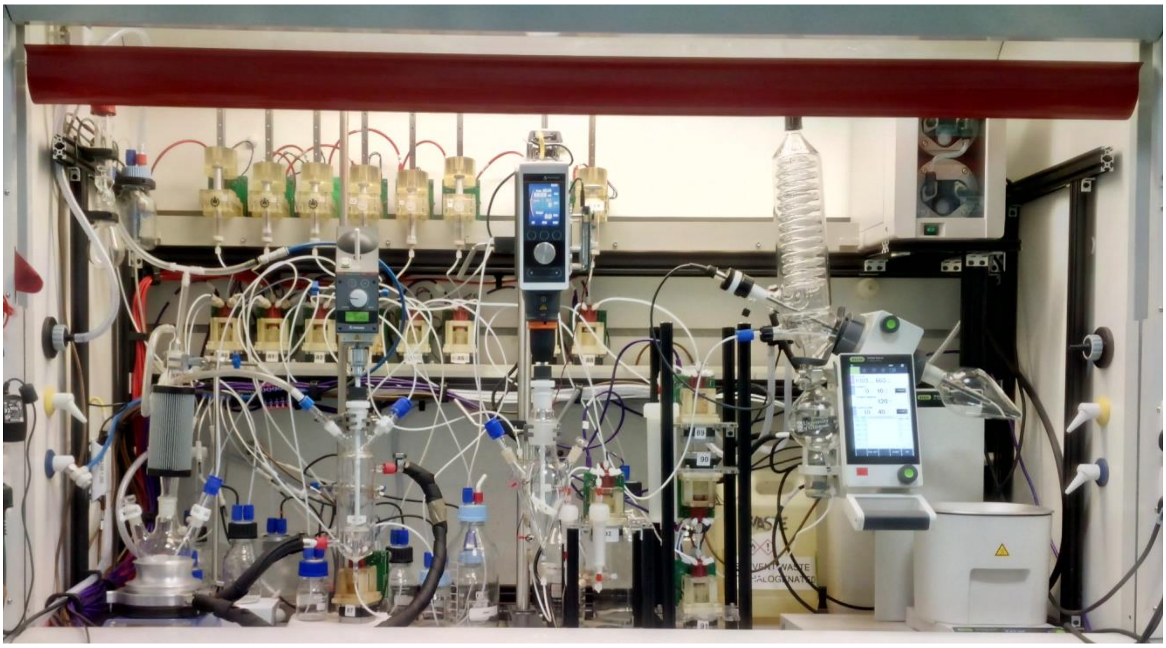


Figure 149 Chemputer hardware instance for the NHS-Diazirine synthesis.

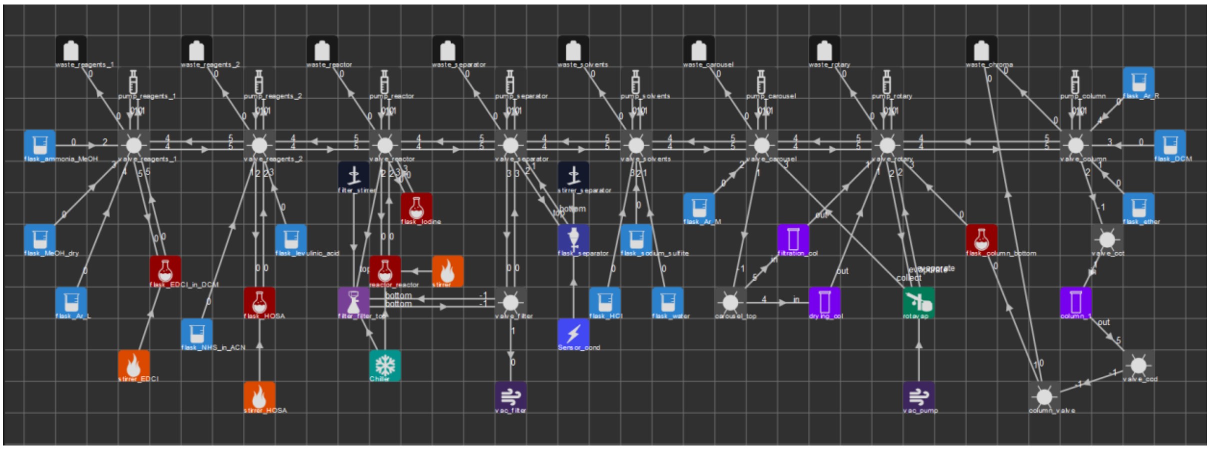


Figure 150 The graph representation of the NHS Diazirine rig.

5.3 Chemputer Hardware Instance for the Solid-Phase Peptide Synthesis

The Chemputer hardware instance (Figure 151) used for the solid-phase peptide synthesis (SPPS) consisted of the SPPS reactor module and one jacketed filter. The filter cartridge and the jacketed filter were both shielded from light with aluminum foil and kept under a positive pressure of argon. Also, the following reagents were kept under a positive pressure of argon: all amino acid solutions, NHS-diazirine, HBTU solution in DMF, DIPEA solution in DMF, piperidine solution in N-methyl-2-pyrrolidone, trifluoracetic acid, triisopropylsilane, DMF, diethyl ether, and dichloromethane. The NHS-diazirine stock flask was shielded from light. The modules were connected via the liquid handling backbone, which was composed of 8 pumps, 7 of which were fitted with 10 mL syringes and one fitted with a 25 mL syringe; and 8 valves. A graph representation is shown in Figure 152. The Python script detailing the synthesis and the graph file are available as supplementary files in Software\Syntheses_Scripts_and_Graph_Files\SPPS.

Figure 151 Chemputer hardware instance for the SPPS, cleavage, deprotection and purification by precipitation of the peptide.

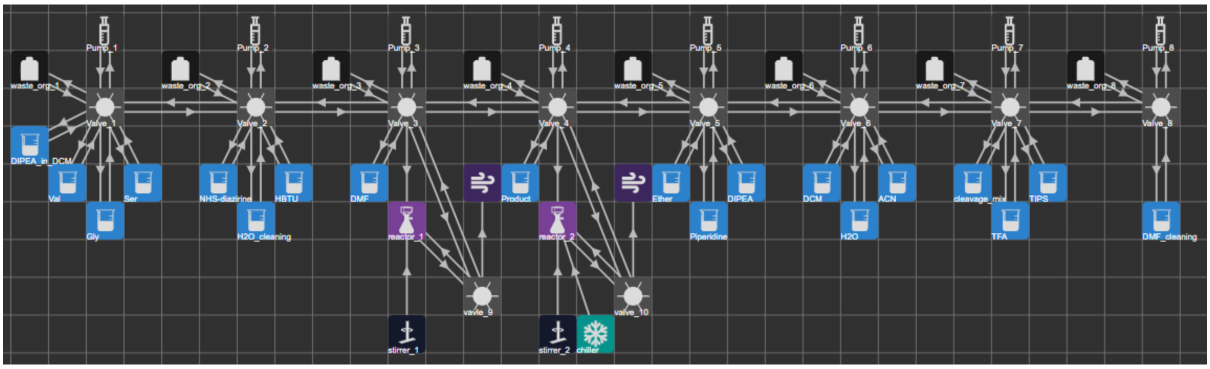


Figure 152 The graph representation of the physical set-up shown in Figure 151.

6 Manual and Automated Syntheses

6.1 Iterative Suzuki–Miyaura Cross-Coupling Sequence

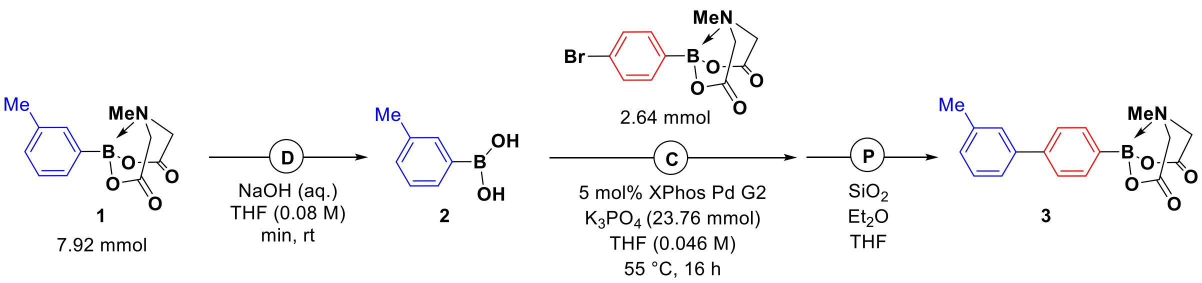
6.1.1 Key Optimisation Runs

The development of the synthesis script for the Iterative Suzuki–Miyaura Cross-Coupling sequence required 40 test runs out of which 10 runs successfully executed the full sequence. The Table 28 below summarizes the key experiments. Due to safety concerns test runs were typically performed using stabilized solvent resulting in low purity product since the stabilizer (BHT) accumulated during the process. For the final runs, inhibitor-free THF was used and safely disposed after execution of the automated procedure.

Table 28 Suzuki–Miyaura Cross-Coupling key optimisation runs

Run	**Yield**	**Purity**	**Comment**
1	58 %	< 80%	Minor issues with automatic evaporation. Manual concentration.
2	55 %	> 80 %	Resolved issues with automatic evaporation. BHT/THF impurities
3	50 %	~ 76 %	In-line filtration implemented to replace liquid-liquid separation. BHT/THF impurities
4	54 %	> 90 %	Fully automated run with inhibitor-free THF
5	52 %	> 95 %	Repeat of fully automated run with inhibitor-free THF and updated hard- and software

6.1.2 6-Methyl-2-(3'-methyl-[1,1'-biphenyl]-4-yl)-1,3,6,2-dioxazaborocane-4,8-dione 3



The separator module was manually charged with 6-methyl-2-(*m*-tolyl)-1,3,6,2-dioxazaborocane-4,8-dione **1** (1.96 g, 7.92 mmol, 3.0 eq.) and the reactor module was charged with 4-bromophenylboronic acid MIDA ester (823 mg, 2.64 mmol, 1.0 eq.), 2nd generation XPhos Buchwald precatalyst (104 mg, 0.132 mmol, 0.05 eq.) and K_3PO_4 (5.04 g, 23.8 mmol, 9.0 eq.).

The automatic procedure started with the dissolution of the MIDA building block, *m*-tolylboronic acid MIDA ester **1**, in THF (100 mL) and aqueous NaOH (24 mL, 1 M added at 2 mL/min). The mixture was stirred for 40 min. After stirring, potassium phosphate buffer (0.5 M, pH = 6.0, 24 mL) was added, followed by diethyl ether (30 mL). The phases were separated, and the organic layer was washed with brine (24 mL). The organic layer was passed through a drying cartridge and concentrated in the rotary evaporator to give the free boronic acid **2**. The rotary evaporator, the backbone and the holding flask were then deoxygenated. The free boronic acid was dissolved in 20 mL THF, transferred to the holding flask and deoxygenated. Under stirring, the reactor was deoxygenated, THF (28 mL) was added and the resulting suspension was deoxygenated (3x switching between vacuum and argon for 60 seconds each) and heated to 55 °C. The solution of the free boronic acid **2** was slowly added over 4 hours. The holding flask was rinsed with THF (10 mL), which was subsequently added to the reactor. The reaction mixture was stirred for additional 8 hours. The reaction mixture was pre-filtered using the in-line filter, passed through the filtration cartridge filled with Celite and concentrated in the rotary evaporator. The crude product was dissolved in THF (26 mL). The resulting solution was loaded onto a silica column in nine portions of ca 3 mL, each. After each portion was loaded, the column was dried under vacuum for 30 min. The column was then washed with diethyl ether (150 mL). Then the product was eluted with THF (150 mL). The THF solution was transferred to the rotary evaporator and concentrated to dryness to give the pure product **3** as an off-white solid (445 mg, 52%). The NMR spectra were in agreement with those reported in the literature[3].

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 – 7.62 (m, 2H), 7.54 – 7.50 (m, 2H), 7.50 – 7.44 (m, 2H), 7.35 (dd, J=7.6, 1H), 7.20 – 7.16 (m, 1H), 4.35 (d, J=17.1, 2H), 4.14 (d, J=17.1, 2H), 2.55 (s, 3H), 2.38 (s, 3H).

^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6) δ 169.4, 140.6, 140.1, 138.0, 133.0, 128.8, 128.1, 127.3, 125.9, 123.7, 61.8, 47.6, 21.1.

6.2 Succinimidyl 4,4′-azipentanoate (NHS-Diazirine) 7

6.2.1 Key Optimisation Runs

The development of the NHS-diazirine **7** synthesis was constituted of a total of 32 test runs with approximately 10 of them executing the script without mechanical/electronic failures. A total of 5 runs, resumed in Table 29, gave the final product with reasonable purity.

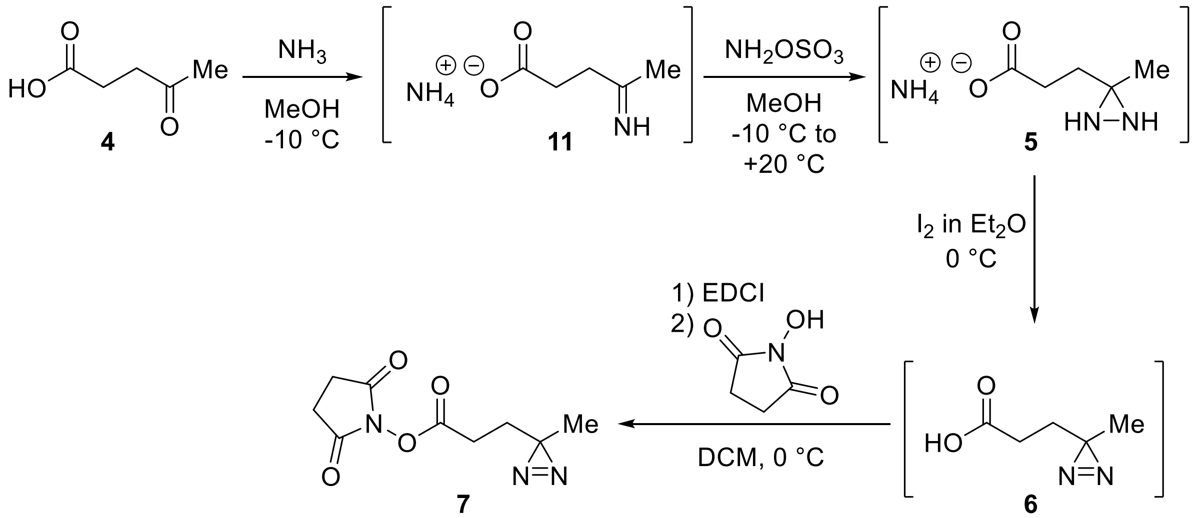
Table 29 NHS-Diazirine key optimization runs

Run	**Yield***	**Purity* via ^{1}H-NMR (‡)**	**Comment**
1	23 %	90 %	Adjust time to dissolve the crude before column needed
2	21 %	95 %	Fully automated with minor interventions if needed
3	21 %	95 %	Fully automated
4	17% (8 %)	85 % (95 %)	Human error: Levulinic acid stock not refilled
5	(5.4 %)	(95 %)	Increased methanol volume used in step 2

* In parenthesis the values after recrystallization in diethyl ether at -20 ºC
‡ Occasional purity essay performed via HPLC UV 220 nm was performed providing better purity compared to ^{1}H-NMR essay.

6.2.2 **Automated Synthesis**

The whole four-step synthesis of succinimidyl-4,4'-azipentanoate was run without human intervention. For documentary purposes, the synthesis was repeated to obtain a sample of all intermediates for characterisation.



Setup of the automated run

The jacketed filter of the platform was charged manually with 2 g of anhydrous $MgSO_4$. One liter of anhydrous methanol, 2.5. L of dichloromethane and 1 L of diethylether were kept in Chemputer reagent bottles under argon and dried over anhydrous $MgSO_4$. Hydroxylamine-*O*-sulfonic aicd (HOSA) (11.5 g, 102 mmol), methanolic ammonia solution (80 mL, 7 M, 560 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (11.5 g, 74.1 mmol) were charged in Advanced Reagent Bottles and kept under argon at 5 °C. A solution of *N*-hydroxysuccinimide (NHS) (5.66 g, 49.2 mmol) in acetonitrile (15 mL) and a solution of iodine (13.9 g, 54.8 mmol) in diethyl ether (100 mL) were prepared. All the other reagents were used in large excess as they coould be stored for future synthesis without risk of decomposition.

Step 1 - Ammonium 3-(3-methyldiaziridin-3-yl)propanoate ***5***

The automated sequence started with a general procedure for cleaning the liquid handling backbone with water, dry methanol and finally dry diethyl ether. Then the dead volume under the filter frit of the jacketed filter was filled with methanol and the jacketed filter was cooled to -10 °C. The ammonia solution (80 mL, 7 M, 560 mmol, 6.8 eq.) was transferred into the jacketed filter followed by a solution of levulinic acid **4** in methanol (9.3 mL, 8.83 M, 82 mmol, 1.0 eq.). The reaction was stirred for 3 h at -10 °C. Then HOSA (11.5 g, 102 mmol, 1.2 eq.) was dissolved in methanol (60 mL) and then slowly added to the reaction mixture in the jacketed filter over 30 min. After the addition was complete the temperature of the filter was raised to 20 °C and and the reaction was stirred for 15 h. Then the reaction mixture was filtered and concentration in vacuo (at a temperature below 27 °C) to give crude ammonium 3-(3-methyldiaziridin-3-yl)propanoate **5** as white crystals in quantitative yield. In a repetition of the synthesis, the process was stopped at this stage and an aliquot of **5** was taken for anaylsis.

^{1}H NMR (600 MHz, Methanol-*d*$_4$) δ 2.28 (t, *J*=7.7, 2H), 1.82 (t, *J*=7.8, 2H), 1.33 (s, 3H).

^{13}C{^{1}H} NMR (151 MHz, Methanol-*d*$_4$) δ = 180.9, 56.5, 49.8, 35.6, 33.9, 22.2.

*Step 2 - 3-(3-Methyl-3H-diazirin-3-yl)propanoic acid **6***

The crude ammonium 3-(3-methyldiaziridin-3-yl)propanoate **5** obtained in the previous step was dissolved in aqueous KOH (45 mL, 0.1 M) and moved into the reaction flask (reactor), which was previously charged with an aqueous KOH solution (15 mL, 9 M KOH) and cooled to 0 °C. A solution of I_2 in diethyl ether (13.9 g of I_2 in 80 mL of diethyl ether) was added dropwise over 30 min and the resulting mixture was stirred for additiona 1.5 h at 0 °C. The excess of iodine was quenched by the addition of aqueous sodium sulfite (15 mL of 15 % w/w) and the phases were separated. The organic layer was passed through a drying cartridge (filled with anhydrous $MgSO_4$ and sand, 3:1 w/w) and transferred to the rotary evaporator. The aqueous layer was moved into the reaction flask where it was acidified with aqueous HCl (32 mL, 3 M). The mixture was transferred back into the separator and extracted with diethyl ether (4 x 40 mL). The organic phases were passed through the drying cartridge to the rotary evaporator. Concentration in vacuo gave 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid **6** as a pale yellow oil (6.5 g, 62 %). In a repetition of the synthesis, the process was stopped at this stage and an aliquot was taken for analysis. The NMR spectra were in agreement with those reported in the literature[4, 5].

^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.22 (t, *J*=7.7, 1H), 1.71 (t, *J*=7.7, 1H), 1.03 (s, 1H).

^{13}C{^{1}H} NMR (151 MHz, Chloroform-*d*) δ 178.7, 29.4, 28.6, 25.2, 19.8.

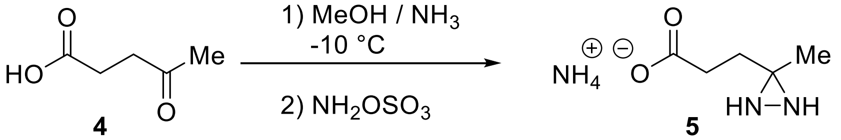
*Step 3 - Succinimidyl 4,4'-azipentanoate **7***

The crude product **6** obtained in the previous automated was dissolved in dichloromethane (20 mL) and transferred to the reactor. The the solution was cooled to 0 °C. Then EDCI (11.5 g, 60 mmol) was dissolved in dichloromethane (120 mL) and was added to the reaction mixture over 10 min. Next, a solution of *N*-hydroxysuccinimide in acetonitrile (15 mL, 30.5 M) was added to the reaction mixture. After the addition was complete, the reaction was stirred for 15 h at 20 °C. The reaction mixture was then concentrated in vacuo. A flash chromatography column (150 g, 40 μm - 63 μm, irregular silica) was equilibrated with dichloromethane (550 mL). The crude product was re-dissolved in dichloromethane (60 mL) and loaded on the flash chromatography column. The crude material was re-dissolved in dichloromethane (60 mL) and loaded onto the column and eluted with dichloromethane. The fraction from 150 mL to 330 mL was collected, concentrated and dried in vacuo to give the pure product succinimidyl 4,4'-azipentanoate **7** (3.9 g, 21 % over three steps) as a white solid. The spectra were in agreement with those reported in the literature[6].

^{1}H NMR (600 MHz, Chloroform-*d*) δ 2.83 (s, 4H), 2.52 (app. dd, *J*=8.4, 7.3, 2H), 1.80 (app. dd, *J*=8.4, 7.2, 2H), 1.07 (s, 3H).

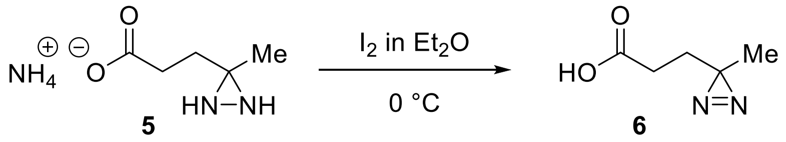
^{13}C{^{1}H} NMR (151 MHz, Chloroform-*d*) δ = 169.0, 167.7, 29.6, 25.9, 25.7, 24.9, 19.6.

6.2.3 **Manual Synthesis[6]**



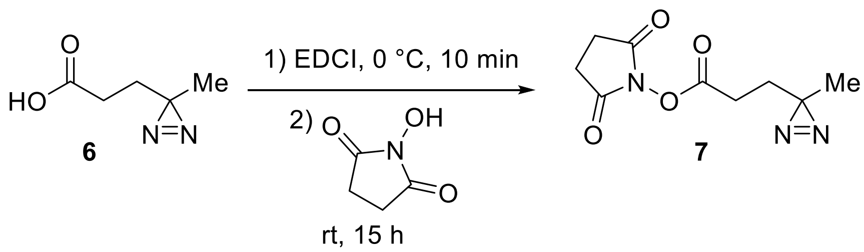
*Ammonium 4,4’-diaziridinepentanoate **5***

In a 250 mL round bottom flask charged with molecular sieves (3 Å), levulinic acid (9.5 g, 81.8 mmol, 1 eq.) was dissolved in ammonia in methanol (75 mL, 525.0 mmol, 6.41 eq., 7 M). The reaction mixture was stirred in a brine/ice bath at ca -6 °C for 3 hours. Next, a solution of hydroxylamine-*O*-sulphonic acid (60 mL, 101.6 mmol, 1.24 eq., 1.7 M in dry methanol) was added dropwise to the mixture over 20 min. During the addition of hydroxylamine-*O*-sulphonic acid a white precipitate formed. The reaction was allowed to warm up to room temperature and was vigorously stirred for 15 h. The obtained mixture was decanted and concentrated in vacuo to give the pure ammonium 4,4’-diaziridinepentanoate **5** as a white solid (11.6 g). This material is unstable and was used in the next step without further purification. The NMR spectra of the crude product were in agreement with the data obtained from the automated run.



3-(3-Methyl-3H-diazirin-3-yl)propanoic acid ***6***

A 250 mL round bottomed flask was charged with ammonium 4,4’-diaziridinepentanoate **5** (11.6 g, 78.8 mmol, 1 eq.) and aqueous KOH (45 mL, 3 M, 135.0 mmol, 1.71 eq.) and the mixture was cooled in an ice bath. Then a solution of iodine (13.9 g, mmol, eq.) in diethyl ether (80 mL) was added dropwise over 30 min and stirring was continued for another 1.5 h at ice bath temperature. The excess of iodine was quenched by the addition of solid sodium sulfite (2.5 g, 18 mmol, 23 eq) in portions until the mixture in the round bottom flask turned from dark brown to colourless. The aqueous layer in the reaction flask was acidified with aqueous HCl to ca pH 2 and extracted with diethyl ether (4 x 40 mL). The combined organic layers were dried over magnesium sulfate and concentrated in vacuo to give pure 3-(3-methyl-3*H*-diazirin-3-yl)propanoic acid **6** as a pale-yellow oil (5.05 g, 39.4 mmol, 50 %). The NMR spectra were in agreement with those reported in the literature[4, 5].



Succinimidyl 4,4'-azipentanoate ***7***

A 250 mL round bottomed flask was charged with 4,4’-azipentanoic acid (5.05 g, 39.4 mmol, 1 eq.) and cooled in an ice bath. Then a solution of *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI) in dichloromethane (120.0 mL, 0.5 M, 60.0 mmol, 1.52 eq.) was added dropwise over 10 min, followed by the addition of *N*-hydroxosuccinimide (5.1 g, 46 mmol, 1.16 eq.). The reaction was allowed to warm up to room temperature and was stirred for 15 h. The reaction mixture was concentrated in vacuo and the crude product was purified by chromatography (isocratic elution with DCM) to give pure succinimidyl 4,4'-azipentanoateas **7** as a white solid (5.16 g, 22.9 mmol, 58 %). The overall yield for the three steps was 28 %. The NMR spectra were in agreement with those reported in the literature[6].

6.3 **SPPS**

6.3.1 **Key Optimisation Runs**

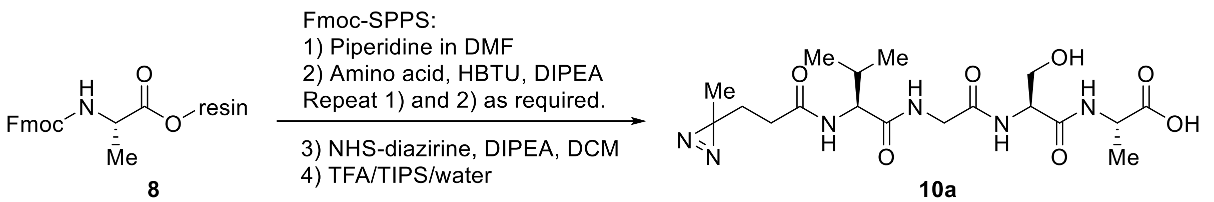
In total the peptide synthesis has been executed 15 times for the optimisation of the synthesis script. Table 30 below summarises the key experiments. The first major challenge was to perform the precipitation and washing of the crude peptide automatically (Entry 2). Further, the cleaning routine of the liquid handling backbone was critical in order to achieve good purity of the peptide product (Entries 3 and 4). Finally, it was observed that the diazirine building block decomposes to a significant extent to the ketone derivative. Thus, preparative HPLC was required to obtain the pure product (Entry 5). (Importantly, the decay of the diazirine building block also occurred during the manual synthesis, hence, this was not a *Chemputer*-specific feature).

Table 30 SPPS key optimisation runs.

Run	Yield	Purity	Comment
1	53 %	75 % (by ^{1}H-NMR)	Precipitation step performed manually.
2	45 %	74 % (by ^{1}H-NMR)	Implemented automatic precipitation.
3	33 %	44 % (HPLC UV 220 nm)	Changed backbone cleaning routine.
4	33 %	76 % (HPLC UV 220 nm)	Changed backbone cleaning routine.
5	10 %	95 % (HPLC UV 214 nm)	Purified product by preparative HPLC.

6.3.2 **(3-(3-Methyl-3H-diazirin-3-yl)propanoyl)-NH-Val-Gly-Ser-Ala-OH 10a**

Automated



The following stock solutions were manually charged into reagent flasks before the automated synthesis was started (excess volumes were used so that several runs could be performed without the need to remake the solutions):

- 0.500 M solutions of amino acids in ultrapure DMF:
    - Fmoc-*O-tert*-butyl-L-serine (19.2 g, 50.0 mmol) in ultrapure DMF (100 mL).

    - Fmoc-L-glycine (14.9 g, 50.0 mmol) in ultrapure DMF (100 mL).
    - Fmoc-L-valine (17.0 g, 50.0 mmol) in ultrapure DMF (100 mL).
- 0.457 M solution of *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) (9.01 g, 23.8 mmol) in ultrapure DMF (50.0 mL).
- Piperidine in DMF (250 mL, 20 % v/v, commercially available as a solution).
- 2.00 M solution of *N,N*-Diisopropylethylamine (DIPEA) (174 mL, 1.00 mol) in *N*-methyl-2-pyrrolidone (total volume 500 mL).

The following reagents and solvents were provided manually before the automated run (excess amounts were used so that several runs could be performed without the need to recharge the reagent flasks):

- Succinimidyl 4,4′-azipentanoate **7** (NHS-diazirine, 338 mg, 1.50 mmol) as a solid. This reagent was shielded from light.
- Trifluoroacetic acid.
- Triisopropylsilane (TIPS).
- Ultrapure *N,N*-dimethylformamide (DMF), which was dried over 3 Å molecular sieves for at least 24 h before the synthesis.
- Reagent grade *N,N*-dimethylformamide (DMF).
- Reagent grade dichloromethane (DCM), which was dried over magnesium sulphate for at least 24 h before the synthesis.
- Reagent grade diethyl ether.
- Reagent grade acetonitrile (ACN).
- De-ionised water.

The reactor flask was manually charged with Fmoc-Ala-Wang resin **8** (0.75 g, 0.50 mmol, 1.0 equiv., 0.67 mmol/g). All subsequent steps were performed automatically (the Python script controlling the synthesis is available as a supplementary file in Software\Syntheses_Scripts_and_Graph_Files\SPPS\Diazirine-NH-VGSA-OH):

1. Ultrapure DMF (9 mL) was added to the reactor and stirred for 1 h at room temperature to swell the Fmoc-Ala-Wang resin.
2. Then a two stage deprotection was performed. The solution of piperidine (9 mL, 20 % v/v in DMF) was added and the reaction was stirred at room temperature for 3 min. Then the solution was drained and fresh piperidine (9 mL, 20 % v/v in DMF) was added. The reaction was stirred at room temperature for 12 min and then the solution was drained.

3. Ultrapure DMF (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times.
4. Then the appropriate amino acid solution (4 mL) was added, followed by the solution of HBTU (4 mL), followed by the solution of DIPEA (2 mL). The reaction was stirred at room temperature for 1 h. Then the reagents were drained, and the resin was washed as described in step 3. Then the coupling of the amino acid followed by washing of the resin was repeated one more time.
5. The deprotection was performed as detailed in step 2.
6. Steps 4 and 5 were repeated for each amino acid.
7. Then the resin was washed as detailed in step 3.
8. Next, DCM (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times.
9. The solid NHS-diazirine **7** was dissolved in DCM (8 mL). Then the solution of DIPEA (2 mL, 0.5 M, 1.0 mmol, 2.0 eq.) in DCM was added to the reactor, followed by the solution of NHS-diazirine **7** (4 mL, 0.19 M, 0.75 mmol, 1.5 eq.) in DCM. The reaction was stirred at room temperature for 1 h. Then the reagents were drained.
10. The resin was washed as detailed in step 8 and dried under a flow of argon for 15 min.
11. The cleavage mix was prepared by adding TFA (19 mL) to a mixing flask followed by the addition of TIPS (0.6 mL, see Section 4.6) and water (0.6 mL, see Section 4.6). 10 mL of the cleavage mix were transferred to the reactor and the reaction was stirred at room temperature for 3 h.
12. Diethyl ether (150 mL) was added to the jacketed filter and the temperature was adjusted to -25 °C. The cleaved peptide product was then transferred from the main reactor to the jacketed filter and precipitated. The main reactor was washed three times with 0.5 mL of the cleavage mix and the liquid of these washings was added to the jacketed filter. The product was precipitated for 1 h at -25 °C. Then the jacketed filter was drained. The crude material was re-suspended in diethyl ether (30 mL) and filtered four times.
13. The material was dissolved in ACN (1.5 mL) and water (6.0 mL) and transferred to a receiver vial. The jacketed filter was washed once with ACN (1.5 mL) and water (6.0 mL) and this liquid was added to the receiver vial.

The crude product was obtained in 44 % purity (measured by HPLC-UV/vis at 214 nm). Further manual purification by preparative HPLC (three injections, the product peak eluted at 12.2 min) gave (3-(3-methyl-3H-diazirin-3-yl)propanoyl)-NH-Val-Gly-Ser-Ala-OH **10a** (21.4 mg, 48.4 µmol, 10 % yield, 95 % purity {measured by LC-MS at 214 nm}) as a white solid.

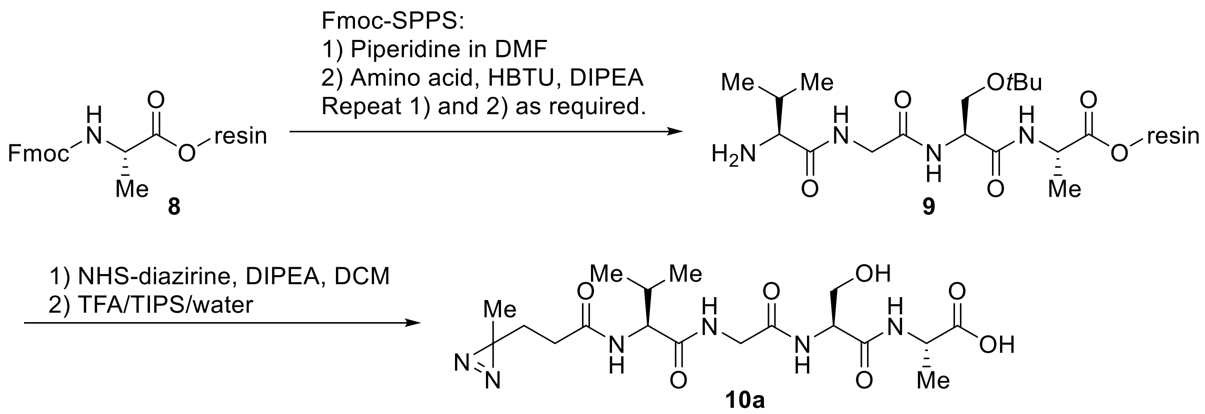
^{1}H NMR (600 MHz, DMSO-*d$_6$*) δ 8.18 (t, *J*=5.8, 1H), 8.10 (d, *J*=7.3, 1H), 7.96 (d, *J*=8.4, 1H), 7.81 (d, *J*=8.1, 1H), 4.32 (ddd, *J*=8.1, 6.4, 4.9, 1H), 4.24 – 4.17 (m, 1H), 4.11 (dd, *J*=8.4, 6.7, 1H), 3.74 (d, *J*=6.0, 2H), 3.57 (dd, *J*=10.9, 4.9, 1H), 3.54 (dd, *J*=10.9, 6.3, 1H), 2.15 – 2.02 (m, 2H), 2.00 – 1.93 (m, 1H), 1.55 (t, *J*=7.8, 2H), 1.27 (d, *J*=7.3, 3H), 0.98 (s, 3H), 0.88 – 0.84 (m, 6H).

^{13}C{^{1}H} NMR (151 MHz, DMSO-*d$_6$*) δ 173.9, 171.5, 171.3, 169.6, 168.7, 61.8, 58.1, 54.9, 47.5, 42.0, 30.1, 29.9, 29.5, 25.8, 19.3, 19.2, 18.2, 17.2.

HPLC-UV/Vis retention time: 10.172 min, purity: 95 % (at 214 nm).

ESI-HRMS $(M+H)^+$ expected: 443.2249 Da, observed: 443.2230 Da.

Manual



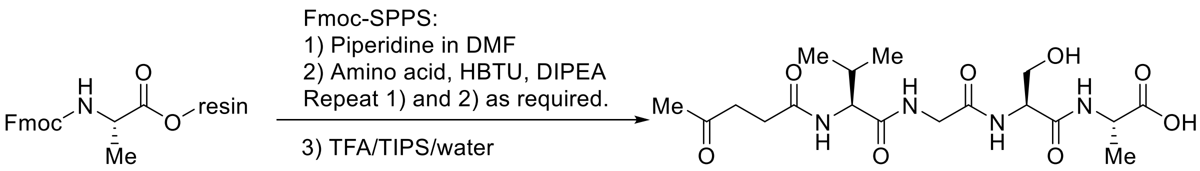
The synthesis of the NH_2-Val-Gly-Ser-Ala-OH peptide core was performed with a Biotage Initiator peptide synthesizer as detailed in Section 6.3.1 – Manual Synthesis. The reactor was manually charged with Fmoc-Ala-Wang resin **8** (0.75 g, 0.50 mmol, 1.0 equiv., 0.67 mmol/g). All subsequent steps were then performed automatically (the Biotage report and the method-file controlling the synthesis is available as a supplementary file in Software\Syntheses_Scripts_and_Graph_Files\SPPS\Diazirine-NH-VGSA-OH\Biotage).

Then the reactor filter frit was removed from the Biotage Initiator and all subsequent operations were performed manually. A solution of NHS-diazirine **7** (169 mg, 0.750 mmol, 1.50 equiv.) in DCM (6.0 mL) was added followed by DIPEA (174 µL, 1.00 mmol, 2.00 equiv.). The reaction was stirred by oscillation at room temperature while being shielded from light for 1 h. Then the reaction mixture was drained from the reactor filter frit. The solid phase resin was washed with DCM (5 x 9 mL) and dried under suction of air for 20 min. Then the cleavage mix {TFA (14.3 mL), TIPS (0.38 mL), and water (0.38 mL) TFA/TIPS/water (95/2.5/2.5 v/v/v)} was added and the reaction was stirred by oscillation at room temperature and shielded from light for 3 h. After that time the cleavage solution was drained from the reactor filter frit and collected. The filter frit was washed with the cleavage mix (3 x 0.5 mL). The combined cleavage mix containing the peptide product was diluted with diethyl ether (150 mL). The product was precipitated at -20 °C for 1 h and pelleted by centrifugation (10 min at 4’400 rpm). The pellet was re-suspended in diethyl ether (30 mL), cooled to -20 °C for 5 min and pelleted by centrifugation again (5 min at 4’400 rpm). This washing process was repeated three more times. The crude product was obtained in 64 % purity (measured by LC-MS at 214 nm). The material was re-dissolved in ACN/water (14 mL, 1/4 v/v) for further manual purification by preparative HPLC (three injections, the product peak eluted at 12.2 min), which gave (3-(3-methyl-3H-diazirin-3-yl)propanoyl)-NH-Val-Gly-Ser-Ala-OH **10a** (39.1 mg, 88.4 µmol, 18 % yield, 93 % purity {measured by LC-MS at 214 nm}) as a white solid. The NMR spectra were consistent with the data recorded for the product from the automated synthesis.

HPLC-UV/Vis retention time: 10.167 min, purity: 93 % (at 214 nm).

6.3.3 (4-Oxopentanoyl)-NH-Val-Gly-Ser-Ala-OH 10b

Automated



8 **10b**

The same stock solutions were used as in the synthesis of **10a**. The following reagents and solvents were provided manually before the automated run (excess amounts were used so that several runs could be performed without the need to recharge the reagent flasks):

- Levulinic acid (5.14 mL, 50.0 mmol) as a solution in 100 mL ultrapure DMF.
- Trifluoroacetic acid.
- Triisopropylsilane (TIPS).
- Ultrapure *N,N*-dimethylformamide (DMF), which was dried over 3 Å molecular sieves for at least 24 h before the synthesis.
- Reagent grade *N,N*-dimethylformamide (DMF).

- Reagent grade dichloromethane (DCM), which was dried over magnesium sulphate for at least 24 h before the synthesis.
- Reagent grade diethyl ether.
- Reagent grade acetonitrile (ACN).
- De-ionised water.

The reactor flask was manually charged with Fmoc-Ala-Wang resin **8** (0.75 g, 0.50 mmol, 1.0 equiv., 0.67 mmol/g). All subsequent steps were performed automatically (the Python script controlling the synthesis is available as a supplementary file in Software\Syntheses_Scripts_and_Graph_Files\SPPS\Levulinic_acid-NH-VGSA-OH):

14. Ultrapure DMF (9 mL) was added to the reactor and stirred for 1 h at room temperature to swell the Fmoc-Ala-Wang resin.
15. Then a two stage deprotection was performed. The solution of piperidine (9 mL, 20 % v/v in DMF) was added and the reaction was stirred at room temperature for 3 min. Then the solution was drained and fresh piperidine (9 mL, 20 % v/v in DMF) was added. The reaction was stirred at room temperature for 12 min and then the solution was drained.
16. Ultrapure DMF (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times.
17. Then the appropriate amino acid solution (4 mL) was added, followed by the solution of HBTU (4 mL), followed by the solution of DIPEA (2 mL). The reaction was stirred at 70 °C for 30 min. Then the reagents were drained, and the resin was washed as described in step 3.
18. The deprotection was performed as detailed in step 2.
19. Steps 4 and 5 were repeated for each amino acid.
20. Then the resin was washed as detailed in step 3.
21. Next, DCM (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times.
22. The solid NHS-diazirine was dissolved in DCM (8 mL). Then the solution of DIPEA (2 mL, 0.5 M, 1.0 mmol, 2.0 eq.) in DCM was added to the reactor, followed by the solution of NHS-diazirine (4 mL, 0.19 M, 0.75 mmol, 1.5 eq.) in DCM. The reaction was stirred at room temperature for 1 h. Then the reagents were drained.

23. The resin was washed as detailed in step 8 and dried under a flow of argon for 15 min.
24. The cleavage mix was prepared by adding TFA (19 mL) to a mixing flask followed by the addition of TIPS (0.6 mL, see Section 4.6) and water (0.6 mL, see Section 4.6). 10 mL of the cleavage mix were transferred to the reactor and the reaction was stirred at 50 °C for 1 h.
25. Diethyl ether (150 mL) was added to the jacketed filter and the temperature was adjusted -25 °C. The cleaved peptide product was then transferred from the main reactor to the jacketed filter and precipitated. The main reactor was washed three times with 0.5 mL of the cleavage mix and the liquid of these washings was added to the jacketed filter. The product was precipitated for 1 h at -25 °C. Then the jacketed filter was drained. The crude material was re-suspended in diethyl ether (30 mL) and filtered four times.
26. The material was dissolved in ACN (1.5 mL) and water (6.0 mL) and transferred to a receiver vial. The jacketed filter was washed once with ACN (1.5 mL) and water (6.0 mL) and this liquid was added to the receiver vial.

The solution of product was manually lyophilised for 48 h to give the product (4-oxopentanoyl)-NH-Val-Gly-Ser-Ala-OH **10c** (102 mg, 0.238 mmol, 48 % yield, 90 % purity {measured by LC-MS at 214 nm}) as a white solid.

^{1}H NMR (600 MHz, DMSO-d_6 δ = 8.15 (t, J=5.8, 1H), 8.09 (d, J=7.3, 1H), 7.91 (d, J=8.4, 1H), 7.81 (d, J=8.1, 1H), 4.35 – 4.30 (m, 1H), 4.23 – 4.18 (m, 1H), 4.09 (t, J=7.6, 1H), 3.74 (d, J=5.7, 2H), 3.61 – 3.52 (m, 2H), 2.63 (qt, J=17.9, 6.9, 2H), 2.46 – 2.31 (m, 2H), 2.00 – 1.93 (m, 1H), 1.27 (d, J=7.3, 3H), 0.86 (dd, J=7.0, 3.6, 7H).

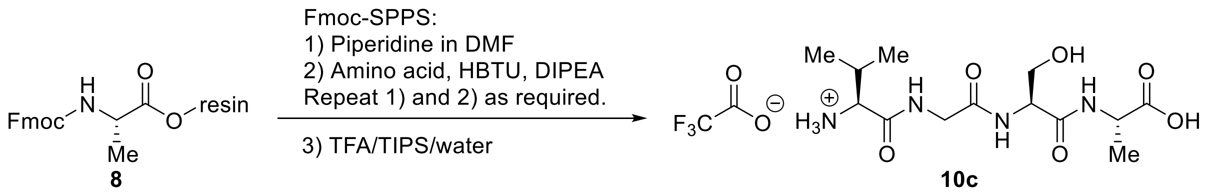
^{13}C{^{1}H} NMR (151 MHz, DMSO-d_6) δ = 207.4, 173.9, 171.7, 171.5, 169.6, 168.7, 61.7, 58.1, 54.8, 47.5, 42.0, 30.1, 29.6, 29.0, 19.2, 18.2, 17.2.

HPLC-UV/Vis retention time: 11.494 min, purity: 90 % (at 214 nm).

ESI-HRMS $(M+H)^+$ expected: 431.2136 Da, observed: 431.2093 Da.

6.3.4 H_3N-Val-Gly-Ser-Ala-OH Trifluoroacetate 10c

Automated



The same stock solutions were used as in the synthesis of **10a**. The following reagents and solvents were manually charged into reagent flask before the automated synthesis was started (excess amounts were used so that several runs could be performed without the need to recharge the reagent flasks):

- Trifluoroacetic acid (250 mL).
- Triisopropylsilane (TIPS, 10 mL).
- Ultrapure *N,N*-dimethylformamide (DMF, 2.5 L), which was dried over 3 Å molecular sieves for at least 24 h before the synthesis.
- Reagent grade *N,N*-dimethylformamide (DMF, 1.0 L). This solvent is used solely for washing the Chemputer backbone.
- Reagent grade dichloromethane (DCM, 250 mL).
- Reagent grade diethyl ether (1.0 L).
- Reagent grade acetonitrile (ACN, 100 mL).
- De-ionised water (1.0 L for washing and 100 mL for re-dissolving the final peptide product).

The reactor flask was manually charged with Fmoc-Ala-Wang resin **8** (0.75 g, 0.50 mmol, 1.0 equiv., 0.67 mmol/g). All subsequent steps were performed automatically (the Python script controlling the synthesis is available as a supplementary file in Software\Syntheses_Scripts_and_Graph_Files\SPPS\TFA-NH2_VGSA-OH):

1. Ultrapure DMF (9 mL) was added to the reactor and stirred for 1 h at room temperature to swell the Fmoc-Ala-Wang resin.
2. Then a two stage deprotection was performed. The solution of piperidine (9 mL, 20 % v/v in DMF) was added and the reaction was stirred at room temperature for 3 min. Then the solution was drained and fresh piperidine (9 mL, 20 % v/v in DMF) was added. The reaction was stirred at room temperature for 12 min and then the solution was drained.
3. Ultrapure DMF (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times.
4. Then the appropriate amino acid solution (4.0 mL, 0.5 M, 2.0 mmol, 4.0 eq.) was added, followed by the solution of HBTU (4.0 mL, 0.46 M, 1.8 mmol, 3.7 eq.), followed by the solution of DIPEA (2.0 mL, 2.0 M, 4.0 mmol, 8.0 eq.). The reaction was stirred at room temperature for 1 h. Then the reagents were drained,

and the resin was washed as described in step 3. Then the coupling of the amino acid followed by washing of the resin was repeated one more time.

5. The deprotection was performed as detailed in step 2.
6. Steps 4 and 5 were repeated for each amino acid.
7. Then the resin was washed as detailed in step 3.
8. Next, DCM (9 mL) was added and the reaction was stirred for 45 s before the solvent was drained. This washing cycle was repeated five times. Then the resin was dried under a flow of argon for 15 min.
9. The cleavage mix was prepared by adding TFA (19 mL) to a mixing flask followed by the addition of TIPS (0.6 mL, see Section 4.6 for the required correction to dispense small volumes accurately) and water (0.6 mL). 10 mL of this cleavage mix were then transferred to the reactor and the reaction was stirred at room temperature for 3 h.
10. Diethyl ether (150 mL) was added into the jacketed filter followed by cooling it down to -25 °C. The cleaved peptide product was then transferred from the main reactor to the jacketed filter and precipitated. The main reactor was washed three times with 0.5 mL of the cleavage mix and the liquid of these washings was subsequently added to the jacketed filter. The product was precipitated for 1 h at -25 °C. Then the jacketed filter was drained. The crude material was re-suspended in diethyl ether (30 mL) and filtered four times.
11. The material was dissolved in ACN (2 mL) and water (8.0 mL) and transferred to a receiver vial. The jacketed filter was washed twice with ACN (2 mL) and water (8.0 mL) and this liquid was added to the receiver vial.

The solution was manually freeze-dried for 48 h to give the final product H_3N-Val-Gly-Ser-Ala-OH trifluoroacetate **10c** (112 mg, 0.251 mmol, 50 %) as a white solid.

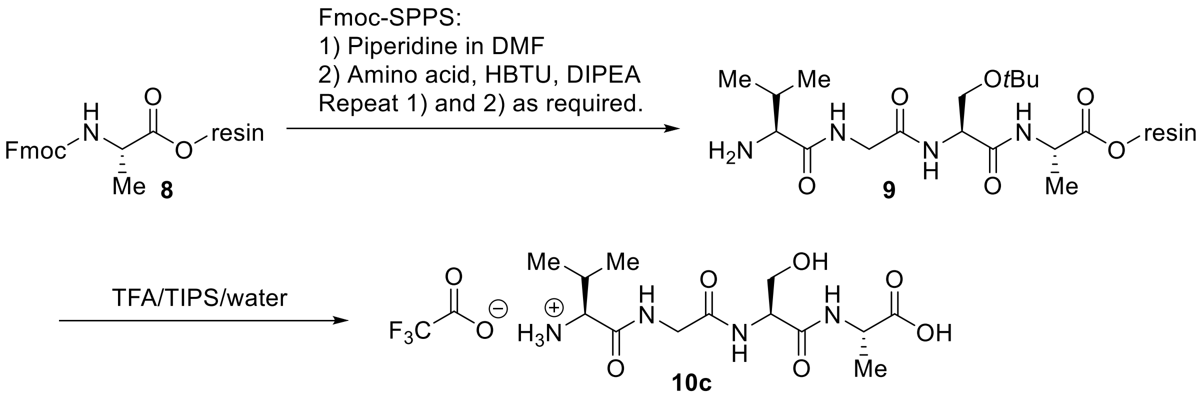
^{1}H NMR (600 MHz, DMSO-*d$_6$*) δ 8.62 (t, *J*=5.7, 1H), 8.19 (d, *J*=7.3, 1H), 8.12 (s, 3H), 8.08 (d, *J*=8.1, 1H), 4.36 (ddd, *J*=8.1, 6.7, 4.8, 1H), 4.25 – 4.17 (m, 1H), 3.96 (dd, *J*=16.7, 6.0, 1H), 3.82 (dd, *J*=16.7, 5.4, 1H), 3.64 (d, *J*=6.0, 1H), 3.59 (dd, *J*=10.9, 4.9, 1H), 3.54 (dd, *J*=10.9, 6.6, 1H), 2.11 – 2.02 (m, 1H), 1.27 (d, *J*=7.3, 3H), 0.95 (dd, *J*=6.9, 3.8, 6H).

^{13}C{^{1}H} NMR (151 MHz, DMSO-*d$_6$*) δ 173.9, 169.6, 168.1, 168.1, 61.8, 57.4, 55.0, 47.6, 41.8, 29.8, 18.3, 17.7, 17.2.

HPLC-UV/Vis retention time: 4.319 min, purity: 96 % (at 214 nm).

ESI-HRMS $(M+H)^+$ expected: 333.1769 Da, observed: 333.1753 Da.

Manual



The reference synthesis was performed with a Biotage Initiator system followed by manual cleavage and deprotection of the peptide and manual precipitation with ether. The same stock solutions were used as for the automated synthesis on the Chemputer. The following reagents and solvents were manually charged into reagent flask before the automated synthesis was started:

- Ultrapure *N,N*-dimethylformamide (DMF, 10 L).
- Reagent grade dichloromethane (DCM, 250 mL).

The reactor filter frit was manually charged with Fmoc-Ala-Wang resin **8** (0.75 g, 0.50 mmol, 1.0 equiv., 0.67 mmol/g). All subsequent steps were performed automatically on a Biotage Initiator peptide synthesizer (the Biotage report and the method-file controlling the synthesis is available as a supplementary file in Software\Syntheses_Scripts_and_Graph_Files\SPPS\TFA-NH2_VGSA-OH\Biotage):

1. Ultrapure DMF (9 mL) was added to the reactor and was stirred through oscillation for 1 h at room temperature to swell the Fmoc-Ala-Wang resin.
2. Then a two stage deprotection was performed. The solution of piperidine (9 mL, 20 % v/v in DMF) was added and the reaction was stirred through oscillation at room temperature for 3 min. Then the solution was drained and fresh piperidine (9 mL, 20 % v/v in DMF) was added. The reaction was stirred through oscillation at room temperature for 12 min and then the solution was drained.
3. Ultrapure DMF (9 mL) was added and the reaction was stirred through oscillation for 45 s before the solvent was drained. This washing cycle was repeated five times.
4. Then the appropriate amino acid solution (4.0 mL, 0.5 M, 2.0 mmol, 4.0 eq.) was added, followed by the solution of HBTU (4.0 mL, 0.46 mmol, 3.7 eq.),

followed by the solution of DIPEA (2.0 mL, 2 M, 4.0 mmol, 8.0 eq.). The reaction was stirred through oscillation at room temperature for 1 h. Then the reagents were drained, and the resin was washed as described in step 3. Then the coupling of the amino acid followed by washing of the resin was repeated one more time.

5. The deprotection was performed as detailed in step 2.
6. Steps 4 and 5 were repeated for each amino acid.
7. Then the resin was washed as detailed in step 3.
8. Next, DCM (9 mL) was added and the reaction was stirred through oscillation for 45 s before the solvent was drained. This washing cycle was repeated five times. Then the resin was dried under a flow of air for 15 min.

The reactor filter frit was removed from the Biotage Initiator. All subsequent operations were performed manually. The cleavage mix {TFA (14.3 mL), TIPS (0.38 mL), and water (0.38 mL) (95/2.5/2.5 v/v/v)} was added and the reaction was stirred at room temperature in a shaker for 3 h. After that time the cleavage solution was drained from the reactor filter frit and collected. The filter frit was washed with the cleavage mix (3 x 0.5 mL). The combined cleavage mix containing the peptide product was diluted with diethyl ether (150 mL). The product was precipitated at -20 °C for 1 h and pelleted by centrifugation (10 min at 4’400 rpm). The pellet was re-suspended in diethyl ether (30 mL), cooled to -20 °C for 5 min and pelleted by centrifugation again (5 min at 4’400 rpm). This washing process was repeated three more times. The final product was freeze-dried for 48 h to give the final product H_3N-Val-Gly-Ser-Ala-OH trifluoroacetate **10c** (155 mg, 0.351 mmol, 70 %) as a white solid. The NMR spectra were consistent the with the data recorded for the product from the automated synthesis.

HPLC-UV/Vis retention time: 4.271 min, purity: 97 % (at 214 nm).

6.4 Training

The required “training” for automating each new synthesis may vary based on three levels:

1) New hardware operations required substantial changes and further development of the existing hardware. Often entirely new modules were created to provide the required functionality. For example, for the coupling step in the MIDA synthesis the programmable manifold had to be developed to establish inert atmosphere. In the case of the peptide synthesis, preliminary experiments were needed to optimize the dimensions of the SPPS

module. For the diazirine synthesis, preliminary experiments identified the need for improvements in reagent storage and phase separation. This led to the extension of the reagent module and to the improved conductivity sensor design, respectively. All these improvements can now be reused with relative ease in all other syntheses as well.

2) Training specific to synthesis conditions is required for the chromatography. The chromatographic separation requires prior examination of the chromatographic behaviour of the specific synthesised compound (retention factor, peak width). This step has to be repeated for every new compound that is purified by chromatography on the Chemputer, unless the molecules have very similar behaviour. Chromatography is the only module that requires compound-specific training.

3) In most cases only minor adjustments of a literature protocol are required to execute it on a Chemputer if the fundamental hardware functionality is available. For example, in the MIDA synthesis the deoxygenation step had to be optimised (i.e. how many cycles of evacuation/refill are required to establish sufficiently low oxygen levels in the reaction flask). In the SPPS synthesis, the liquid handling protocol had to be tuned to ensure accurate transfer of microliter volumes. The knowledge gained from these optimisation studies can be reused in related syntheses (e.g. the established deoxygenation protocol will be appropriate for most palladium catalysed cross-coupling reactions). The evaporator and separator module have been optimised to the point that they require no prior experimental training. The drying cartridges content have been optimized for the right ratio of desiccant/inert filling mixtures to maximise the drying potential whilst preventing clogging of the cartridge upon the passage of solutions.

It is our expectation that the training required for each new reaction will further decrease as new hardware modules are developed and existing modules further refined. A key outcome of this work is to show, once a new module has been developed, that it is relatively easy to run the module and transfer the bench chemistry into that module with minimal training.

7 NMR Spectra

The NMR spectra are available as processed spectra in separate PDF document and as the unprocessed files in the folder Analysis\.

8 References

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