

Solid phase peptide synthesis

Purpose:

To prepare peptide sequences for incorporating in the tissue specific hydrogels.

Training and reservation:

Queries regarding equipment or training: Li-Wei Chang (mask2ferrero@gmail.com), Dr. Sarah Perry (perrys@engin.umass.edu). Reserve the peptide synthesizer in Google Calendar through the 'Perry's Group Peptide Synthesizer' calendar. Fill in the Excel log sheet after the run is complete stating your name, lab name, date, peptide(s) synthesized, reagents used and refilled etc.

Overview:

The recommended maximum length of peptide is ~20 amino acids and the recommended maximum number of peptides run is ~4 (Check with Dr. Sarah Perry before proceeding). Running one peptide of 10 amino acid length would take approximately 12-15 hours.

Special notes:

1. Ventilate the chemical hood during the process of introducing all the reagents into the system.
2. Make sure the nitrogen tank is available before setting experiment.
3. It is advised to make the piperidine solution last (Prepare 5-10% extra).
4. Always keep the reagent place protected by an empty clean bottle when the reagent is not in place.
5. In case of cleaning glass bottles with residues of chemical make sure to rinse with acetone to remove any traces of odor.
6. Use appropriate PPE and gloves (<https://www.fishersci.com/shop/products/fisherbrand-powder-free-nitrile-exam-gloves-medium/191301597c>). Change the gloves if some chemical spills on the gloves to reduce odor.
7. It is advised to flush the system before and after the synthesis is performed. Finish with an extended rotary wash to remove traces from the reactor vessel.

Materials:

1. Amino acids (Farkas 4°C refrigerator)
2. Resin (Farkas 4°C refrigerator)
3. Piperidine (Peyton/Arcaro Flammable cabinet)
4. Oxya base
5. Diisopropylcarbodiimide (DIC) (Perry Flammable cabinet)
6. Dimethyl Formamide (DMF) (Peyton/Arcaro Flammable cabinet)
7. Dichloromethane (DCM) (Peyton/Arcaro Corrosive cabinet)
8. 50 mL centrifuge tubes specifically used for peptide synthesis (http://www.pipettipsandtubes.com/mm5/merchant.mvc?Screen=PROD&Product_Code=3181-345-008-9)

Safety and quality control:

Handling Dichloromethane:

1. Use in a well-ventilated chemical hood.
2. In case of skin contact, immediately wash with water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Get medical attention.
3. In case of inhalation, get access to fresh air. If not breathing, give artificial respiration. Get medical attention.
4. If ingested, do not induce vomiting unless directed to do so by medical personnel. If large quantities are swallowed, call a physician immediately.

5. In the event of spill or leak, remove personnel from area. Secure and control entrance to the area. If safe, stop or reduce the spill/leak. Absorb liquids in a material such as dry sand or similar material, and place into sealed containers. Ventilate area of spill or leak. Do not wash into sink or sewer.

Handling Dimethylformamide:

1. In case of eye contact, rinse immediately with water, including under the eyelids, for at least 15 minutes.
2. In case of skin contact, immediately wash with water for at least 15 minutes. Immediate medical attention is required.
3. In case of inhalation, get access to fresh air. If not breathing, give artificial respiration. Get medical attention.
4. Ensure adequate ventilation. Use personal protective equipment. Keep people away from and upwind of spill/leak. Evacuate personnel to safe areas. Remove all sources of ignition. Take precautionary measures against static discharges.
5. This chemical should not be released into the environment.
6. Methods for containment and cleanup is to soak up with inert absorbent material. Keep in suitable and closed containers for disposal. Remove all sources of ignition. Use spark-proof tools and explosion proof equipment.

Handling Piperidine:

1. This chemical is extremely hazardous in case of skin contact, eye contact, ingestion or inhalation. Liquid or spray mist may produce tissue damage particularly on mucus membranes of eyes, mouth and respiratory tract. Skin contact may produce burns. Inhalation of the spray mist may produce severe irritation of respiratory tract, characterized by coughing, choking, or shortness of breath. Severe over-exposure can result in death.
2. In case of eye contact, remove any contact lense. Immediately wash eyes with water for at least 15 minutes, including under the eyelids. Seek medical attention.
3. In case of skin contact, gently and thoroughly wash the contaminated skin with water and non-abrasive soap. Remove contaminated clothing and shoes as quickly as possible, protecting your own hands and body. Place the victim under a deluge shower.
4. If ingested, do not induce vomiting. Examine the lips and mouth to ascertain whether the tissues are damaged, which is a possible indication of ingestion the toxic material.

Procedure:

Note: Use gloves to touch the equipment, and the computer.

Method creation:

1. Open the Liberty Blue Software.
2. Select the 'Method Editor'.
3. Open the folder (Peyton Lab) or subfolder in the 'Methods' box where the new method is to be created.
4. Write the amino acid sequence that will be synthesized in the 'Sequence' box.
5. Select the 'Bottle Setup' (Standard).
6. Select the 'Synthesis Scale' (0.10 M).
7. Select the 'C-Terminus' (Amide or Acid).
8. Select the 'Resin Type' (Not preloaded). Using a preloaded resin will result in addition of the resin-attached amino acid to the resultant peptide.
9. Select the 'Resin Swelling' (Standard).
10. Write the 'Resin Name' (Rink Amide). This step is optional.
11. Select the 'Resin Cycle' (0.10-Resin Swelling).

12. Select the 'Final Deprotection Cycle' (0.10-Final Deprotection).
13. In the 'Cycles' section, select '0.10-Single Coupling' for half of the amino acids attached to the resin and select '0.10-Double Coupling' for the other amino acids that are distant from the resin. For arginine (R), select '0.10-Double Arg 75°C Coupling' and for cysteine (C), select '0.10-Single 50°C Coupling'.
14. When the method is complete, click 'Save' to save the method.

Reagent calculation:

1. Select the 'Usage Calculator' from the 'Calculators' menu.
2. From the 'Methods' box at the left of the 'Usage Calculator', right-click the methods to be included in the calculation and select 'Add to Calculation'. The method will then appear in the table at the top of the 'Usage Calculator'.
3. The calculator will calculate the total mass of each amino acid required, volume of each reagent and estimate the total waste produced.

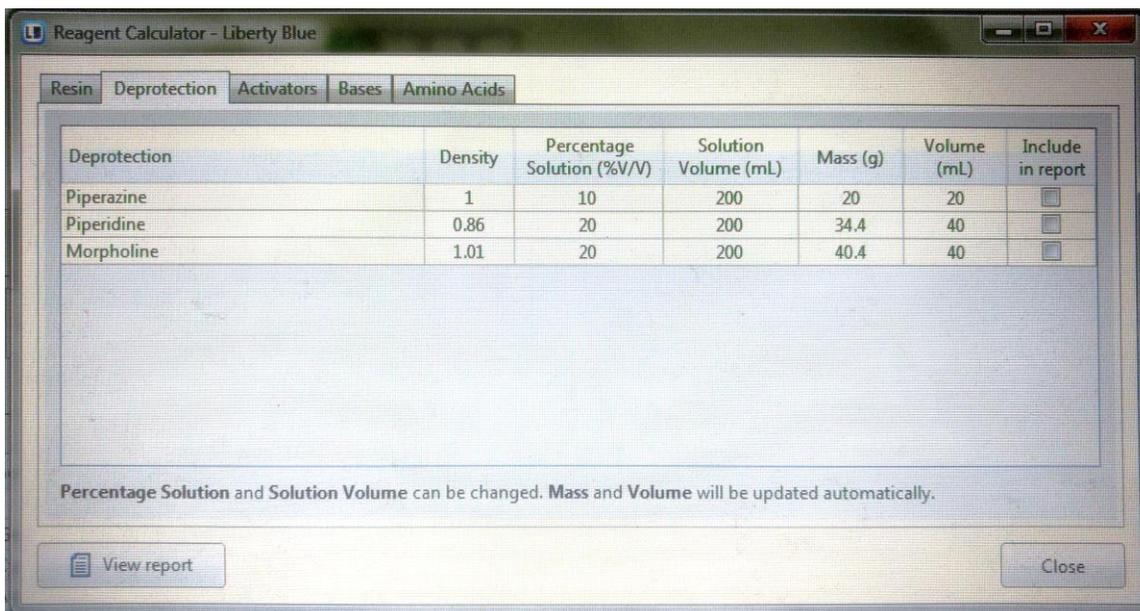
The screenshot shows the 'Usage Calculator - Liberty Blue' window. On the left, under 'Methods', 'Collagen IV' is selected. Below it, a tree view shows a folder 'Aritra Nath Kundu' containing 'Collagen IV', 'Elastin 1', 'Elastin 2', 'Fibrinogen gamma 1', 'MMP 1', 'MMP 11', 'MMP 2', and 'MMP 3'. On the right, the 'Required Reagent Amounts' section shows an 'Amino acid concentration' of 0.2 M. A table lists reagents with their 'Volume (mL)' and 'Mass (g)'. The 'Total Waste Volume' is 388 mL and 'Resin mass required' is N/A. Buttons for 'View report' and 'Close' are at the bottom.

Reagent	Volume (mL)	Mass (g)
Lysine	0	0
Methionine	0	0
Phenylalanine	27	2.1
Proline	0	0
Serine	0	0
Threonine	0	0
Tryptophan	0	0
Tyrosine	14	1.29
Valine	0	0
External-1	0	
External-2	0	
External-3	0	
External-4	0	
External-5	0	
External-6	0	
External-7	0	
Main Wash (DMF)	188	176.73
Deprotection (Piperazine)	35	
Activator (DIC)	40	
Activator Base (Oxyma)	20	
Carminic Acid Anhydride	0	0

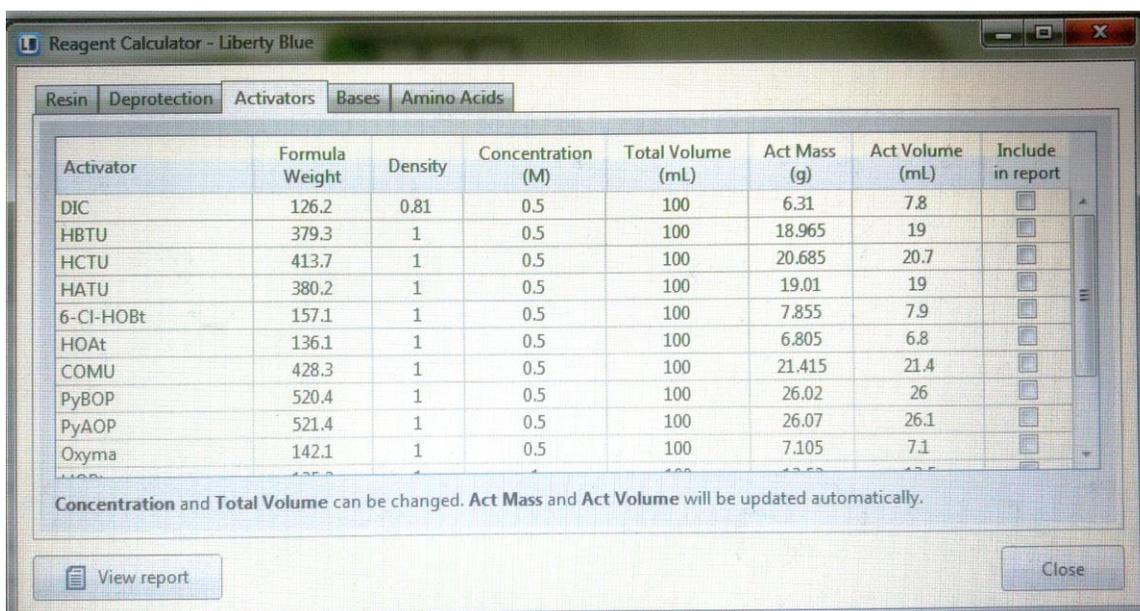
Total Waste Volume: 388 mL
Resin mass required: N/A

4. Select the 'Reagent Calculator' from the 'Calculators' menu to determine the preparation of the appropriate concentrations of the reagent. There are five tabs: Resin, Deprotection, Activators, Bases and Amino Acids.
5. Resin Calculator:
 - a. Enter the scale for the synthesis (0.10 mM).

- b. Enter the resin substitution value in the 'Resin Substitution' box, which can be found on the label of the resin bottle.
 - c. The mass of the resin required is calculated automatically (~0.313 g).
6. Deprotection Calculator:
- a. Select the desired deprotection reagent (Piperidine) from the list.
 - b. Enter the desired concentration (20% v/v).
 - c. Enter the total volume of the deprotection solution required.
 - d. The volume of the deprotection required is calculated automatically.



7. Activator Calculator:
- a. Select the desired activator (DIC) from the list.
 - b. Enter the desired concentration (0.5 M).
 - c. Enter the total volume of the activator solution required.
 - d. The volume of the activator required is calculated automatically.



8. Base Calculator:

- Select the desired activator base (Oxyma) from the list.
- Enter the desired concentration (1.0 M).
- Enter the total volume of the activator base solution required.
- The mass of the activator base required is calculated automatically.

Base	Formula Weight	Density	Concentration (M)	Total Volume (mL)	Base Mass (g)	Base Volume (mL)	Include in report
Oxyma	142.1	1	1	100	14.21	14.2	<input type="checkbox"/>
HOBt	135.2	1	1	100	13.52	13.5	<input type="checkbox"/>
HOBt monohydrate	153.1	1	1	100	15.31	15.3	<input type="checkbox"/>
DIEA	129.25	0.74	2	100	25.85	34.9	<input type="checkbox"/>
NMM	101.15	0.92	2	100	20.23	22	<input type="checkbox"/>
DIC	126.2	0.81	0.5	100	6.31	7.8	<input type="checkbox"/>
6-Cl-HOBt	157.1	1	0	100	0	0	<input type="checkbox"/>
HOAt	136.1	1	0	100	0	0	<input type="checkbox"/>

Concentration and Total Volume can be changed. Base Mass and Base Volume will be updated automatically.

- Approximately 5 mL of DCM: DMF (50:50) solution is added to the resin. Leave the resin for at least 5 minutes in the centrifuge tubes to allow swelling of the resin.
- DMF is used as solvent for all the amino acids and all the reagents except the resin. Prepare the solutions by adding the appropriate volumes of DMF as calculated before. Prepare piperidine last.

Reagent loading:

- Select 'Maintenance' from the 'Options' menu. Select the 'Extended Rotary Wash' from 'Extended Wash' folder to perform final rinse and click 'Run Operation'.
- Select 'Maintenance' from the 'Options' menu. Select the 'Wash' folder and choose the amino acid positions of interest and activator and activator base positions and click 'Run Operation' to clean the positions in the synthesizer.
- Connect amino acid centrifuge tubes to the appropriate positions on the synthesizer. Connect empty centrifuge tubes to all the unused positions. Screw the
- Connect activator and activator base bottles to the appropriate positions on the synthesizer.
- Check the level of solvent remaining in the solvent bottle. Use the following steps to replace the solvent bottle.
 - Select 'Maintenance' from the 'Options' menu.
 - Select the 'Change Bottle Main' operation from the 'Change Bottle' folder and click 'Run Operation'.
 - Follow the onscreen instructions to depressurize and remove the existing bottle from the 'Change Bottle' window.
 - Add the required volume of the solvent to the bottle. Ensure that the dip tube filter is in place, the connect the cap to the bottle.
 - Click 'Next' to continue with the 'Change Bottle' procedure. The synthesizer will automatically pressurize the bottle and prime the lines.
- Check the level of deprotection solution remaining in the deprotection bottle. Use the following steps to replace the deprotection bottle.

- a. Select 'Maintenance' from the 'Options' menu.
 - b. Select the 'Change Bottle Deprotection' operation from the 'Change Bottle' folder and click 'Run Operation'.
 - c. Follow the onscreen instructions to depressurize and remove the existing bottle from the 'Change Bottle' window.
 - d. Add the required volume of the deprotection solution to the bottle or replace the empty bottle with a filled bottle. Ensure that the dip tube filter is in place, then connect the cap to the bottle.
 - e. Click 'Next' to continue with the 'Change Bottle' procedure. The synthesizer will automatically pressurize the bottle and prime the lines.
7. Before loading the resin, make sure the HT loader where the product is received is clean.
- a. Select 'Maintenance' from the 'Options' menu.
 - b. For all the positions of interest in the HT loader, select 'Transfer Solvent to HT Position' operation from 'HT Loader' folder and click 'Run Operation'.
8. Add resin to the resin holder (HT loader).
9. Add DCM: DMF (50:50) solution to the bottle kept on top of the HT loader.
10. Check the level of waste in the waste container to see if enough space is left for the estimated waste volume. If it is filled, replace with a new waste container.

Method loading and running:

1. Open appropriate folder (Peyton Lab) and subfolder in the 'Methods' tab.
2. Right click on the desired method and select 'Queue for Run'. The method name will appear in the 'Loaded Method' indicator. The indicator will turn blue, which indicates that the method is loaded and ready to run.
3. Click the Start button at the top of the Software main screen. The 'Loaded Method' indicator will turn yellow, which indicates that the method is running. The lights in all the amino acid positions will turn on.



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on.

4. After the completion of the run, the 'Loaded Method' indicator will turn green.
5. Remove the peptide products and replace with empty conical tubes.
6. Select 'Maintenance' from the 'Options' menu. Select the 'Wash' folder and choose the amino acid positions of interest and activator and activator base positions and click 'Run Operation' to clean the positions in the synthesizer.
7. Select 'Maintenance' from the 'Options' menu. Select the 'Extended Rotary Wash' from 'Extended Wash' folder to perform final rinse and click 'Run Operation'.