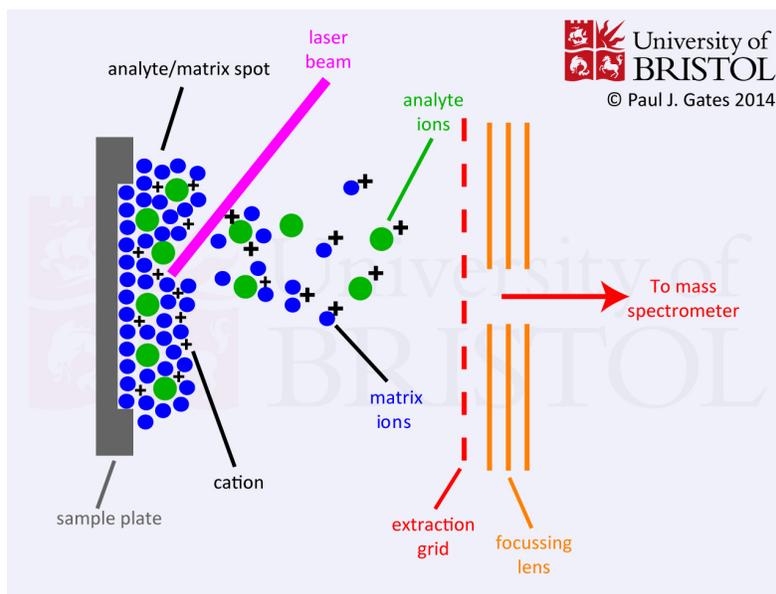


## Matrix Assisted Laser Desorption/Ionization (MALDI) Time of Flight (TOF) Mass Spectrometry



MALDI is an ionization technique that uses a laser directed into the matrix/sample to create ions. It can be applied to DNA, proteins, **peptides**, sugars, and polymers.

### For MicroFlex MALDI-TOF training:

- Sign up for an account on the FOM system (fom.umass.edu) and request training through there.
- Any questions regarding equipment or training: Stephen J. Eyles (eyles@biochem.umass.edu)
- For information about the Mass Spectrometry center click this link:  
<https://www.umass.edu/masspec/>

### Matrix to use:

- For peptides:  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA, MW189.04 Da), a good matrix choice for samples with MW <10,000Da, preferably 2000-3000 MW
- For large peptides and proteins: Sinapinic Acid (MW 224.07Da), a good choice for samples with MW >10,000Da.
- For polymers: Gentisic acid

### Notes:

- Matrix are available to use for the users
- Make fresh matrix everyday (since they are light sensitive)
- Two targets or sample plate: Ground (aqueous, for peptides) and polish (organic solvents, for polymers)

### Matrix and sample preparation:

Note: This protocol is for low molecular weight peptides

1. Make matrix solution 50:50 water/acetonitrile with 0.1% TFA (trifluoroacetic acid) final concentration (e.g.: 250 ml ionized water + 250 ml acetonitrile + 0.5 ml TFA). Note: You can storage this solution at room temperature and reuse this solution.
2. Weigh out 10 to 20 mg of CHCA, then add 1.0 ml of matrix solution (or 2 to 4 mg of CHCA in 200  $\mu$ l of matrix solution).
3. After mixing CHCA with the matrix solution, vortex 1 minute to dissolve (this is a saturated solution, hence some undissolved matrix will remain).
4. Next, centrifuge for 20 second, then transfer the clear solution part to another tube
5. Dilute your sample (**peptide**) with matrix solution at about 10 ~ 100 pmol/ $\mu$ l, then mix with matrix at sample to matrix ratios of 1:10, 1:20 or 1:30  
E.g.: Note: You just need a little bit of your sample (a few milligrams is okay)  
-Weigh 0.0035 g of peptide HAIYPRH and add 1 ml of matrix solution to have a concentration of 4000 pmol/ $\mu$ l.  
-Make a 20x dilution to have a final concentration of 100 pmol/ $\mu$ l  
Calculations:  
 $0.0035 \text{ g} / (867.97 \text{ g/mol}) = 0.000004 \text{ mol} = 4000000 \text{ pmoles}$   
 $4000000 \text{ pmoles} / 1000 \mu\text{l} = 4000 \text{ pmol}/\mu\text{l}$   
20x Dilution:  
Take 5  $\mu$ l of 4000 pmol/ $\mu$ l and add 195  $\mu$ l of matrix solution for a final concentration of 100 pmol/ $\mu$ l  
-Take 1  $\mu$ l of 100 pmol/ $\mu$ l and add 10  $\mu$ l of matrix (Ratio 1:10)
6. Then transfer 1  $\mu$ l to the target. Let the sample dry

#### Equipment:

Note: Use gloves to touch the equipment (The IN/OUT can also be controlled with the software)

1. Press IN/OUT. It will go to 'progress' (orange color). This indicates that the equipment is under vacuum.
2. Once the equipment indicates 'access' (green color) we can open the compartment.
3. Put the target and close it gently (barcode in the left corner). Close the lide of the compartment
4. Press IN/OUT again. It will go to progress (orange color). This indicates that the equipment is under vacuum.
5. Once indicate access (green color) we can start running the software

#### Flex analysis software:

Note: Log in to be able to use the equipment (They start charging at this time)

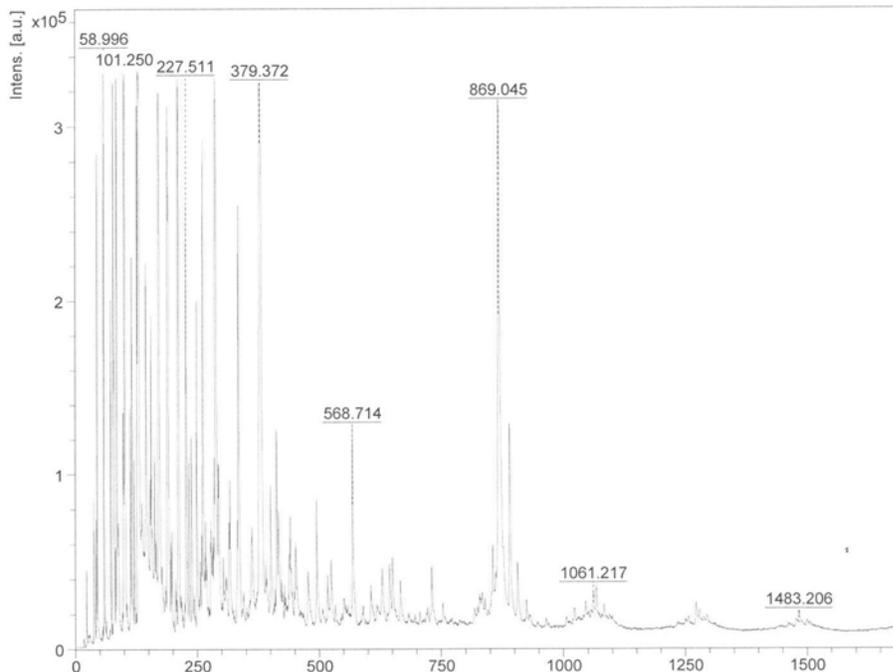
1. Select method: LP 66, LP 35000 (polymers and peptides), LP 18000, and RP
2. Laser stand by (Sample is preparing and is in yellow), wait for green, which means ready
3. In the method: Check spectrometer (check range of MW)
4. For peptides start laser small, around 10%, and then increase to 20%. Note: Do not go to high, you do not want fragmentation.
5. Once you start and finish with one sample, 'save as' in the proper directory (Bring your usb, so you can get your spectra. We have the software in computer 3, so you can play later with the spectra) (Note: Log off the software)

E.g: Spectra sample from previous peptide

D:\Data\Specs\Service\_YMMDD\THOMPSON\Bugielski\6-17-2011\Maria\_2nd peptide\_MW867.97\_0617201'

Comment 1

Comment 2



### Equipment shut down:

Note: Use gloves to touch the equipment

1. Press IN/OUT. It will go to progress (orange color). This indicate that the equipment is under vacuum.
2. Once indicate access (green color) we can open the compartment.
3. Remove the target and close it gently
4. Press IN/OUT again. It will go to progress (orange color). This indicate that the equipment is under vacuum.

Notes:

- Clean the target with an organic solvent/followed by water.
- Make sure the equipment is under vacuum before you leave