MALDI Sample Preparation

Peptides:

Peptides in water based solvent

Matrix solution:

saturated α-cyano-4-hydroxycinnamic acid (CHCA) in 70:30 acetonitrile: water or 50:50 acetontrile:water or 50:50 acetonitrile:TFA(0.1%)/water or 50:50 acetonitrile:NH_4OAc(10 mM)/water

Mix equal volumes of peptide solution and matrix solution.

Comment: One drop of an HPLC solution and one drop of matrix solution usually produces nice spots.

Proteins:

Protein in water based solvent (20 μ M in 0.1% TFA or 10 mM ammonium acetate)

Matrix solution:

saturated sinapinic acid (SA) in 1:2 acetonitrile:water (v/v) [water or 0.1% TFA or 5 mM NH_4OAc] [30% acetonitrile: 70% water]

Mix equal volumes of protein solution and matrix solution. If that doesn't work, mix 1 μL of protein solution with 9 μL of matrix solution, then spot 1 μL of mixture.

Comments: SA is more organic soluble than water soluble. Proteins crash out of solution in organic solvents. To make sure the protein is surrounded by matrix, one has to keep it in solution long enough for the matrix to find it.

Solvent-free sample preparation:

Grind together sample and anthracene until mixture is like talcum powder. The idea is to surround each sample molecule with anthracene and avoid clumps of just sample and just anthracene. A convenient way to grind small amounts of material is to put the compounds in a 2-mL glass vial along with a clean glass ball (boiling point balls) and vortex the mixture until it is very fine. This usually takes several minutes. Press the fine powder onto a piece of double-sided tape. Invert target to lose loose material.

Calibration:

For MALDI, two calibrants are needed. One should be larger than the sample, and one should be smaller. The mass range between the two calibrants can then be calibrated. In general, peptides should be used to calibrate peptides, proteins to calibrate proteins, lipids to calibrate lipids, and so forth. This generates peaks with similar shapes making it easier for the eye and the computer to assign masses.

PEG or PPG calibration spot preparation:

Place a μL of matrix solution on the target. After it dries, place 1 μL of a methanol polymer solution on the dried spot. Not recommended for peptides and proteins.

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Everyday Matrix Mixing:

Because of the 337 nm component in laboratory light, it is a good idea to make up fresh matrix solutions every day. Several procedures are described below.

Method A.

In the bottom of a 1.5-mL Eppendorf tube place a small amount of α-cyano-4-hydroxycinnamic acid. Add 30 µL of water and 70 µL of acetonitrile or methanol. Vortex for 90 sec. Centrifuge. Use the solution above the solid.

Method B.

Weigh out 1 mg of matrix and dissolve in 100 µL of solvent.

Method C.

Weigh out several mg of matrix and dissolve in a solvent. Aliquot the solution into 1.5-mL Eppendorf tubes. Lyophilize or Speed Vac™ the tubes to dryness. Store in the dark until needed. This method produces "pre-weighed" small samples of matrix.

Solvent Suggestions:

30% water, 70% acetonitrile

30% water, 70% methanol

50% water, 50% ethanol

45% water, 45% isopropanol, 10% formic acid

30% 0.1% TFA (trifluoroacetic acid in water), 70% acetonitrile

40% water, 40% acetonitrile, 20% acetone

30% 20mM diammonium citrate, 70% acetonitrile

- Ideally the analyte and the matrix should be soluble in the same solvent so that they dry well-mixed together.
- Solvents with some water content stay as droplets on stainless steel targets (i.e. water containing solvents do not sheet all over the target.)

Matrix Suggestions (337 nm laser):

α-cyano-4-hydroxycinnamic acid

2,5-dihydroxybenzoic acid

sinapinic acid

HABA

6-aza-2-thiothymine

2',4',6'-trihydroxyacetophenone

dithranol

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Handling Calibration Standards for MALDI-TOF-MS

Peptides and proteins make good calibration standards for MALDI-TOF-MS for several reasons:

- They give both positive ion and negative ion spectra.
- They are available in a wide range of molecular weights.
- Samples free of detergents and buffers are commercially available.
- One milligram of sample can be used hundreds of times.

Here is a suggested protocol for handling calibrants - for getting your money's worth out of one milligram. Bradykinin will be the example. The following procedure makes bradykinin solutions quickly and conveniently available just before any MALDI run.

- Add 1.000 mL of water (18 MΩ) to 1 mg of bradykinin [Bradykinin can be purchased from Sigma Chemical Co. in 1 mg vials.] Vortex carefully trying to keep water off the gray plug stopper. This plug contains zinc ions among other things (*).
  (*) "Rubber stoppers as sources of contaminants in electrospray analysis of peptides and proteins," X. K. Zhang, R. C. Dutky, H. M. Fales, *Anal. Chem*. **68**: 3288-3289 (1996).
- Aliquot 50 µL into 1.5-mL polypropylene Eppendorf tubes. The number of tubes prepared seems to depend on patience.
- Speedvac™ or lyophilize the aliquots to dryness. [Note: if a nearby -80° C freezer has space, the peptide aliquots can be stored safely as solutions. Peptide solutions stored at -20°C do not do well.]
- Store the aliquots in a plastic bag in a -20°C freezer. Mark on the bag how much water should be added to each tube to make a specific concentration of bradykinin.

<u>Sample Arithmetic</u>:

1 mg of bradykinin is 943 nanomoles of bradykinin (or 943,000 picomoles of bradykinin)

Goal: 1-20 picomoles of peptide on a target for MALDI-TOF-MS.

(1.0 mg/1060 g/mole) x 1/1 mL H_2O = 943 µM	Prepare 50 µL aliquots. Dry.
(50 µL x 943 µM)/100 µM = 472 µL	Reconstitute with 472 µL water. Now have 100 µM solution. Aliquot 25 µL quantities, dry, store.
(25 µL x 100 µM)/2 µM = 1,250 µL	Reconstitute with 1,250 µL water. Now have 2 µM solution.

(0.5 µL x 2 µM) = 1 pmol

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Calibrants

name	formula, MH^+	monoisotopic MH^+	average MH^+
leu⁵-enkephalin	$C_{28}H_{38}N_5O_7$	556.28	556.64
angiotensin II	$C_{50}H_{72}N_{13}O_{12}$	1,046.5	1,047.2
bradykinin	$C_{50}H_{74}N_{15}O_{11}$	1,060.6	1,061.2
angiotensin I	$C_{62}H_{90}N_{17}O_{14}$	1,296.7	1,297.5
substance P	$C_{63}H_{99}N_{18}O_{13}S$	1,347.7	1,348.7
bombesin	$C_{71}H_{111}N_{24}O_{18}S$	1,619.8	1,620.9
neurotensin	$C_{78}H_{122}N_{21}O_{20}$	1,6729	1,674.0
ACTH [18-39]	$C_{112}H_{166}N_{27}O_{36}$	2,465.2	2,466.7
glucagon	$C_{153}H_{226}N_{43}O_{49}S$	3,481.6	3,483.8
insulin, bovine	$C_{254}H_{378}N_{65}O_{75}S_6$	5,730.6	5,734.6
ubiquitin	$C_{378}H_{630}N_{105}O_{118}S$	8,560.6	8,565.9
cytochrome c, equine	$C_{560}H_{874}N_{148}O_{156}S_4Fe$		12,360
apomyoglobin, equine	$C_{769}H_{1213}N_{210}O_{218}S_2$		16,952.5
soybean trypsin inhibitor	$C_{892}H_{1393}N_{238}O_{278}S_6$		20,092
trypsinogen, bovine	$C_{1039}H_{1627}N_{286}O_{338}S_{14}$		23,982
glyceraldehyde-3-phosphate dehydrogenase, porcine	$C_{1590}H_{2513}N_{436}O_{472}S_{13}$		35,706
enolase, yeast	$C_{2079}H_{3307}N_{570}O_{637}S_6$		46,672
BSA (bovine serum albumin)	$C_{2935}H_{4583}N_{780}O_{899}S_{39}$		66,431
phosphorylase b rabbit muscle	$C_{4367}H_{6814}N_{1211}O_{1249}S_{30}$		97,228
BSA-dimer	$C_{5870}H_{9163}N_{1560}O_{1798}S_{78}$		132,859

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Getting Started with **mMass**: An Open Source Mass Spectrometry Tool

Go to www.mmass.org and download the appropriate version of **mMass** for your computer.
Versions are available for Linux, Windows, and Mac OSX.

File → Open
mMass will open its own files (.msd) and ASCII files (.txt). When you open an ASCII (.txt) file, save it as (save as) a .msd file.

Control-click (right-click) the cursor over an area of the spectrum to enlarge that area. Double-click on the spectrum to return to the full spectrum.

Tools → Label Peak : Cursor is now labeled with a small green +. Pull the cursor across the top of a peak, and a label appears on the spectrum and in a list to the right of the spectrum.
To delete a label, highlight it in the peak list, and hit delete.

View → Canvas Properties
m/z precision, intensity precision, bars height, gel height, canvas font size, label font size, notation length

Tools → Mass Calculator → Isotope Pattern window

File → Export → Export Spectrum Image
Choose size, resolution, font scale, line scale, format (PNG, JPEG, or TIFF).

To change color of spectrum: control-click (right-click) on the colored dot for that spectrum → colour

Other:

View → Peak Labels → vertical/horizontal

View → Peak List → lots of choices

Sequence → New → Sequence Editor (This is all about peptides and proteins.)

Processing → Peak Picking → wrench button Check and see if your data fits any of the types listed.
Processing means treating a batch of spectra all the same way.

If you use images generated with mMass in publications, reports or posters, be sure to say you used mMass and cite it.

mMass 3: A Cross-Platform Software Environment for Precise Analysis of Mass Spectrometric Data Strohalm M, Kavan D, Novák P, Volný M, Havlíček V *Anal Chem* 82 (11), 4648-4651 (2010).

mMass Data Miner: an Open Source Alternative for Mass Spectrometric Data Analysis Strohalm M, Hassman M, Košata B, Kodíček M *Rapid Commun Mass Spec* 22 (6), 905-908 (2008).

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