MALDI-TOF Sample Preparation

Matrix Assisted Laser Desorption Ionization (MALDI) requires that a relatively small amount of **sample** (1-100 pmol) be mixed with a relatively large amount of **matrix** compound (mole ratio of $1:10^3 - 10^4$ of sample : matrix) and deposited onto a **target plate**.

Choosing a matrix solution.

1. Ideal solvents are volatile and dissolve both the matrix and analyte. This will allow for an even co-crystallization.
2. Select an **appropriate matrix** for your sample. The suggestions below are starting points only. There are numerous matrixes available and the correct choice for your sample can depend greatly on the MW and functionality of your analyte.
    a. Small molecules
        - "**2,5-DHB**" (2,5-dihydroxybenzoic acid) – works well for polar analytes.
        - "**DTH**" (dithranol) – works well for non-polar analytes, organometallic and metallic samples.
        - Typical solvents are methanol, isopropanol, acetonitrile, acetone, chloroform, dichloromethane, and tetrahydrofuran.
        - **DBH** and **DTH** solutions are usually prepared at ~10mg/mL.
    b. Peptides and proteins
        - "**Alpha-cyano," "HCCA," or "CHCA"** (α-cyano-4-hydroxycinnamic acid) – works well for MW < 5 kDa, can be used for some analytes up to 20 kDa.
        - "**Sin**" or "**SA**" (Trans-3,5-dimethoxy-4-hydroxy cinnamic acid) – works well for MW > 5 kDa.
        - Typical solvent is TA; 30-50% acetonitrile in water with 0.1% TFA (trifluoroacetic acid). Higher acetonitrile percentages can be used to assist solubility.
        - **CHCA** and **SA** are typically prepared fresh daily as saturated solutions. Mix well and spin down excess for ~30 seconds using the benchtop centrifuge.
    c. Polymers
        - Choice of matrix depends greatly on the MW, solubility, hydrophilicity, and end-group chemistry of the specific polymer. Some suggestions are below.

Matrix	**Polymer**	
DHB	Polyethylene glycol	**Hydrophilic**
HCCA	Polydimethylsiloxane	↑
Ferulic Acid	Polytetramethylene glycol	|
IAA	Polymethylmethacrylat	|
Dithranol	Polystyrene	↓
DCTB	Polyisoprene	**Hydrophobic**

        - Choose an appropriate ionization agent (100 mM in water).
            - Use an alkali salt (NaI, NaTFA) for polar polymers containing heteroatoms (Polyether, Polyester, Polyacrylates, Polyamides).
            - Use an Ag or Cu salt for nonpolar polymers with no heteroatoms (Polystyrene, Polybutadiene, Polyisoprene).
        - Typical solvents are water and tetrahydrofuran.
        - Typical mix ratios for sample : matrix : salt are 5 : 25 : 1 or 5 : 50 : 1.
        - Additional recipes for polymer analysis by MALDI can be found at

http://maldi.nist.gov/

B. Sample Preparation

1. If your sample contains **salts, detergents, or certain buffers** they likely need to be removed.
2. Ideally, use the same solvent for both matrix and analyte.
3. Sample preparation can be highly dependent on the analyte and choice of matrix. Following are several recipes that can be used as starting points, but they are not guaranteed to give the best results.
    a. **Small Molecules with DTH/2,5-DHB Matrix**
        i. Dissolve a small amount (the very tip of a glass pasteur pipette full ~1mm) of your analyte in 100 µL of an appropriate solvent such as chloroform.
        ii. Dilute 1 µL of this analyte solution in 20 µL of a 10 mg/mL matrix solution.
        iii. Dilute 1 µL of this analyte/matrix mixture in 20 µL of the same matrix solution.
        iv. Spot both analyte/matrix mixtures and use the one that gives preferred signal/resolution.
    b. **Small Peptides/Proteins (<10 kDA) with CHCA Matrix**
        i. Prepare an approximately 1 µM solution of your analyte in water or TA solvent.
        ii. Prepare a saturated CHCA matrix solution in TA33.
        iii. Mix 1 part analyte solution with 1 part matrix solution.
    c. **Larger Proteins (>10 kDA) with SA Matrix**
        i. Prepare a 1-50 µM analyte solution in water of TA solvent.
        ii. Prepare a saturated SA matrix solution with TA33.
        iii. Spot 1 µL of the matrix solution on the target plate and allow to dry.
        iv. Spot 1 µL of the analyte solution on top of the dried matrix and allow to dry.
        v. Spot a second layer of the matrix solution on top of the dried analyte (creating a sandwich) and allow to dry.

Notes

- Solvents have different cohesive properties. 1 µL of water or TA can neatly fill a spot but 1 µL of chloroform will easily overflow into other spots. Reduce spotting volume as necessary (0.6 µL works fairly well for chloroform).
- Higher analyte concentration will not always improve your spectrum. Optimizing matrix:analyte ratio sometimes means lowering analyte concentration.
- Matrix:analyte concentration/ratio is not the only parameter that can be tuned to improve signal. In the MALDI instrument software, increasing laser power can increase ionization to give better signal, while conversely lowering laser power can reduce noise. Alternatively, weak signals can be amplified by increasing the total number of laser shots (either directly or by adding several spectra together).