Sample preparation guidelines for MALDI ToF mass spectrometry

Short description of the technique

MALDI (Matrix Assisted Laser Desorption/Ionisation) is a soft ionisation technique in biological mass spectrometry. It is generally combined with Tof-MS (Time-of-flight mass spectrometry) technology to determine the molecular mass or better mass-to-charge ratio (m/z) of analytes. For a very simple introduction see http://en.wikipedia.org/wiki/MALDI. In general, singly charged molecular ions are produced without fragmentation. The sensitivity and mass accuracy of MALDI are high, analysis is rapid and spectral interpretation is relatively uncomplicated. It is suitable for the analysis of peptides, proteins, oligoribonucleotides, oligonucleotides, carbohydrates, metabolites and other small molecules. (If requested, the BioCentre can perform fragmentation analysis by MALDI to generate peptide sequence data. This requires longer acquisition and analysis time and is charged accordingly. If sequence data is required, users should discuss different MS sequencing options with the BioCentre staff.) Successful MALDI analysis depends very largely upon good sample preparation. In the MALDI preparation process, a microlitre volume of analyte solution is mixed with a matrix solution and both are allowed to co-crystallise. The following seven factors should be considered:

1. **Compound class and mass range**
2. **Salt and small molecule contamination**
3. **Purity**
4. **Choice of matrix**
5. **Sample charge state**
6. **Sample concentration**
7. **Storage**

1. **Compound class and mass range**

The likelihood of successful MALDI analysis, and the utility of the data obtained is heavily influenced by the class of compound being analysed. Experimenters should bear this in mind when requesting service. **MALDI is well-suited for the analysis of basic peptides in the mass range 600 Da -3500 Da.** In this way it is most frequently used to achieve identification of a protein previously digested with trypsin. **See BioCentre recommended in-gel digestion protocol.** Analysis of larger proteins is perfectly feasible, but requires more material of a higher purity. High mass spectra are low in resolution such that they reveal less useful information. Sequence information is not obtained from intact protein analysis.

2. **Salt and small molecule contamination**

Although MALDI is more tolerant to contamination than other MS methods, the salts and detergents commonly used in biomolecular science can still interfere with either crystallisation or ionisation or both. **Particular attention should therefore be paid to using sample purification and storage solvents which do not utilise buffers, salts or detergents such as SDS.** A final purification step which includes de-salting, such as reversed phase separation or which utilises a volatile salt buffer, such as ammonium acetate, usually gives good results. If desalting is required, this can be performed by the BioCentre at additional cost, or we may recommend an alternative MS analysis method. Samples which are rich in lipids, or which contain glycerol may also fail to crystallise.

3. **Purity**

In general, the higher the purity of your sample the better the MALDI spectrum which will be obtained. Mixtures are commonly analysed, but since the ionisation process is competitive, different components in the mixture are ionised to a greater or lesser degree dependent upon their size and chemistry. This has two important implications: (1) The intensity (peak height on the y-axis) of the resulting MALDI spectrum is not quantitative, only the mass is obtained. (2) If the mixture is very complex, only a few of the most abundant, easy to ionise species will be observed. For this reason, **complex biological samples are normally**
fractionated by chromatography or electrophoresis prior to MALDI analysis. Experimenters should bear in mind that MALDI may be considerably more sensitive and precise than other techniques available to them. Impurities, chemical heterogeneity and degradation are frequently found in spectra obtained from “pure” samples.

4. **Choice of matrix**

A range of excellent matrices have been empirically determined for each compound class commonly analysed by MALDI and for particular mass ranges. Please advise the BioCentre staff of the expected compound class and mass range of interest, so that they may determine a suitable matrix. If a compound not commonly analysed by MALDI is supplied, please make available any information you have regarding successful MALDI analysis elsewhere. If the BioCentre is required to determine the best matrix experimentally, this will add considerably to the time and cost of analysis.

5. **Sample charge state**

As already indicated, ease of MALDI ionisation is dependent upon sample chemistry and hence charge state. In acidic matrix solution most proteins and peptides (especially tryptic peptides) are basic and are best observed in positive ion mode. Other compounds such as phosphopeptides and oligonucleotides, however, are acidic, and may be best observed in the less sensitive negative ion mode. Unless specifically requested otherwise, routine analysis will only be conducted in positive ion mode.

6. **Sample concentration**

The amount of material required varies. In general, a few picomoles (often only femtomoles) of sample lyophilised or dissolved in 5-10μL is sufficient for most analyses. In the case of proteins and nucleic acids, this should be considered a minimum. For proteins isolated by electrophoresis, a band which is visible by Coomassie staining is usually sufficient when subjected to in-gel tryptic digestion.

7. **Storage**

Samples for MALDI analysis may be supplied either in solution or lyophilised. Ideally samples should be resuspended in an aqueous solution of 50% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid using ultra high purity water. If any other solution is used, BioCentre staff must be supplied with comprehensive details. Oxidation or methylation of amino acid side chains can occur if peptides or proteins are stored in an alcohol. Please do not resuspend your protein in PBS (see salt and small molecule contamination). Lyophilisation is not recommended when resuspension may prove difficult, in the case of large proteins for example, or when very small molar quantities are supplied.