Sample Submission Guidelines

1. The Mass Spectrometry Facility is dedicated to highly accurate molecular weight measurement (chemical compounds, peptides and proteins), protein identification and sequence analysis using oMALDI/ESI QqTOF mass spectrometer.

In protein identification using oMALDI QqTOF, proteins are initially digested with trypsin (cleaves at R-X and K-X except when X is Pro). The digest is analyzed in "MS only mode" and the masses of all the peptides recorded. From this mass list, a number of peptides are selected for MS/MS analysis. The mass spec is then switched to "MS/MS mode" and a single peptide is fragmented in the collision cell by undergoing collisions with nitrogen or argon. This process produces a number of fragment ions that are used to search a protein or a translated DNA sequence database, which has been theoretically processed to generate mass numbers of peptides and peptide fragment ions. The experimentally generated mass list is compared to theoretically calculated database to find a match. Obviously, this does not apply to the protein that is not in the sequence database.

On ESI QqTOF, the peptide digest is analysed as above on oMALDI QqTOF. In addition, the fragment ions of each peptide from ESI QqTOF can be used to call the sequence of the fragmented peptide (*de novo* sequencing). The average peptide sequence generated using this method is 6 to 14 amino acids. We can usually sequence 2 to 5 peptides per protein.

2. To submit a protein sample, a request form has to be filled out **in full** by the sender and signed by the PI. Radioactive samples are **NOT** accepted.

3. Samples can be submitted as liquid, solid or gel pieces. The sample should be placed in an Eppendorf tube. Screw cap type tubes are not recommended since consistent polymer contamination has been found from this type of tube.

4. Keratin contamination is a significant problem when analyzing samples by MS. All care should be taken to ensure keratin is not introduced (wear gloves and long-sleeved lab coats, frequently wash your gloves, use clean tubes, tips etc.).

5. For samples in a liquid form, the guidelines below should be followed :

i. Detergent must be avoided where possible, or removed completely after use.
ii. The buffer concentration should be kept low (< 5mM) and a desalting procedure using ZipTip™ might be required by the analysis.
iii. A small volume (up to 20 µl) is sufficient for the analysis. If the sample is too dilute, we will need to concentrate it using ZipTip™.

6. PAGE gel samples should be excised and placed in Eppendorf tubes. Cut the gel as close to the band/spot as possible to reduce the volume of gel to be processed. The gel should be stained with a proper staining protocol. The recommended staining methods are Coomassie Blue, colloidal Coomassie Blue, negative staining (Zn^{2+} or Cu^{2+}) and SYPRO Ruby. Silver stain often gives poor results of mass spectrometry. If you have to use silver stain, we recommend using one of the following techniques :

i. Shevchenko A, Wilm M, Vorm O, Mann M: Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. *Analytical Chemistry* 1996; 68(5) : 850-858.
ii. Blum H, Beier H, Gross HJ: Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 1987; 8 : 93-99.

If you intend to use other staining methods, please discuss the staining protocol with the Facility operators.

For protein identification/sequencing, with **a clear Coomassie Blue stained gel band/spot** there is a good chance of identifying the protein. Please handle gels with gloves and destain gels extensively with clean

buffers. It would be very helpful if a control gel band/spot of same size is excised from a blank region of the gel (or from an equivalent size region of a 'control' lane) and submitted in a separate tube with the sample.

7. Membrane blotted samples are not recommended although PVDF can be used for the analysis. However, the peptide recovery is much lower than that from PAGE gels.

8. The service is on a first-come-first-serve policy. The sample turnaround time is less than a week for molecular weight determination and 1 to 2 weeks for protein identification/sequencing. These times are dependent on the number of samples received and our workload at the time.

9. Please feel free to contact the Facility for any inquiry on sample preparation, analysis and result interpretation.

10. Please note that it is kindly requested to acknowledge the Mass Spectrometry Facility, HKUST in your publications.