Guidelines of Sample Submission for MS Analysis

Mass spectrometry is a highly sensitive analytical method for many different biological and chemical compounds. Therefore, a sample should always be as pure or clean as possible and a proper sample preparation is critical for a successful MS analysis. In the following, a few guidelines are described, how to prepare samples for MS measurements. These guidelines should be strictly followed. If there are any open questions regarding your samples, please get in contact with the facility staff before you submit them.

SYNTHETIC & SMALL ORGANIC COMPOUNDS

If possible, submit samples as solids. Usually, microgram amounts (100-200µg) are sufficient. An appropriate solubility information should be noted in “comments” in the sample submission form. If samples are provided in solution, please note the following instructions.

Solvents

- In general, aqueous solvents are preferred.
- Viscous solvents should be avoided, including **DMSO, DMF and THF**.
- **Hydrocarbon solvents, such as hexane, are not practicable for ESI**, but can be used for MALDI analysis.
- Please specify if particular solvents should **not** be used.
- Two MALDI matrices (HCCA and DHB) are available in the facility. If your compound needs a different matrix, you have to provide it together with your sample.
- Please indicate on the sample submission form, which molecular weight you expect.

Concentration & Volume

- Good quality spectra can typically be obtained from samples at 20-50 micromolar concentrations (e.g. 20-50 micrograms/mL for a compound of MW 1000). The volume should be 20µl for MALDI-MS or 100µl for ESI-MS.

Reactivity

- Please indicate if the compound is sensitive to acidic or basic conditions, as small amounts of acid (formic, acetic) or base (ammonium hydroxide, triethylamine) are normally added in order to enhance sample ionization.

PEPTIDES & PROTEINS

Concentration & Volume

- For routine intact mass analysis, the minimum amount of sample depends on the MW of the peptide or protein. Good results have been obtained with 25 pmol at 5 kD, 100 pmol at 20 kD, 200 pmol at 40 kD, and 500 pmol at 60 kD.

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- Sample concentration should be such that the appropriate amount of protein is contained in 20-25 ul.

Salts & Buffers

- Concentrations of non-volatile components (e.g. NaCl, Na_2HPO_4 etc.) should be kept below 10mM, if not eliminated completely.
- Concentrations of Glycerol, DMSO or DMF should be kept under 1%.
- Detergents like **Tween, Triton etc. should be avoided**, since they give strong signals in MS.
- Volatile buffers such as ammonium acetate and formate are tolerable below 20 mM.
- In case of ESI measurements, samples should not contain TFA (e.g. from a reverse-phase HPLC run); therefore, **TFA has to be removed by lyophilization** (don´t use Speedvac).
- The salt and buffer limits become increasingly stringent as the MW of the protein increases. To maximize the likelihood of a successful analysis, **proteins greater than 20 kD should ideally be submitted in deionized water only**.

PROTEOMICS

Proteins in polyacrylamide gel

- Samples for protein identification by proteolytic digestion and LC-MS/MS are commonly submitted as Coomassie stained or silver stained bands or spots in polyacrylamide gels. Optimized staining protocols for samples for MS analysis can be found on the facility homepage (http://www.proteomics-facility.uni-konstanz.de/protocols/). **Unstained gels will not be analyzed**!
- The gels should be handled as little as possible, to minimize contamination by dust and keratin.
- The spot or band of interest should be excised precisely, excluding all of the surrounding blank gel.
- Cut the excised gel into small pieces (1-2 mm square) and place it in a clean eppendorf vial without addition of buffers/solvents/liquids.
- If a specific protein should be analyzed in detail (e.g. identification of posttranslational modifications), the protein **should be isolated** (e.g. by affinity purification) before MS analysis.

Proteins in solution

- If protein samples are submitted for in-solution digest, the concentration should be at least 20pmol in 1µl (e.g. 0.2µg/µl for a protein with a MW of 10kD). The volume should be 25µl.

GENERAL REMARKS

Samples have to be submitted together with the **sample submission form (**http://www.proteomics-facility.uni-konstanz.de/sevices/sample-submission-form/**).** Each sample has to be labeled as follows:

☑ Sample name

☑ Name of the applicant

☑ Date