Sample preparation for mass spectrometry

In order to interpret the results one has to be familiar with the "history" of the sample. One has to know where it was isolated from, what other proteins it was in contact with, what kind of chemicals were used, what did the control show, etc. For example, the antibody may be contaminated with other proteins; bovine serum albumin from the medium may find its way into the protein mixture isolated; the affinity column prepared for biotinylated substrates may leak avidin. Under oxidative conditions (maybe just air is present) the methionines will yield sulfoxides, cysteines may yield cysteic acids, even the triptophanes may pick up an oxygen or two. Long storage or heating in urea-solution may lead to the carbamoylation of the N-termini as well as the side-chains of Lys-residues. Isolation performed in the absence of protease inhibitors may yield cleaved proteins, while leaving out the phosphatase inhibitors may lead to complete phosphate losses.

Let's get together and make a detailed plan before starting the project!

General recommendations

Do not store low level peptide or protein samples in glass vials!
For low level sample storage, digestion, and/or derivatization use a conical plastic vial of the lowest necessary volume, such as 0.6 mL Eppendorf vials.
We found the Costar microcentrifuge vials of better quality than some others, they contained less plastic softener soluble by acetonitrile.
Not properly maintained lyophilizers or vaquum centrifuges may be a source of polymer-contamination.
One has to use chemicals of the highest purity, HPLC-quality water, TFA in ampules etc.

Everything that gets in contact with your sample has to be considered as a potential source of contamination or side-reactions.

We are able to identify proteins only if they are listed in the database (usually the NCBI nonredundant protein database), and if they are not contaminated with a series of other proteins.

In order to characterize a post-translational or other covalent modification we have to know the amino acid sequence of the modified protein. Such analysis requires much larger sample quantities than a protein identification experiment, and the presence of any other protein seriously hinders the analysis.

1) Peptides, proteins, carbohydrates in solution

The solutions submitted for analysis should contain a few picomoles/µl of peptides; 10-20 picomoles/µl of proteins; and a few 100 picomoles/µl of sugars.
The samples must not contain any detergents, salts are not welcome either. From our point of view the best solvent is water containing the minimally necessary amount of organic solvent (acetonitrile, MeOH etc.). Acid in the solution usually helps. 0.1-1% formic acid is the best.
The most realiable method for protein quantitation is amino acid analysis. Colorimetric reactions usually lead to overestimation of the real quantity by a factor of 10.

2) Proteins in-gel

In 2D separations we recommend blocking the sulfhydrils with iodoacetamide after isoelectric focusing. This will prevent acrylamide addition onto the Cys-residues as well as aid in peptide recovery by preventing the random reoxidation of the -SH groups.

Use MS compatible staining procedure (e.g.: CBB, Colloidal CBB, modified silver staining)
Modified Coomassie-staining: J. Rosenfeld et al. Anal. Biochem. 203, 173-179 (1992)
Modified silver staining: Shevchenko et al. Anal. Chem. 68, 850-858 (1996).

Use powder free latex gloves! It contains ~8x less water-soluble protein than the powdered ones. Good quality latex gloves contain less than 50 µg of water-extractable protein. Nitrile gloves are even better!

Human keratins are the most frequent contaminants of gel-preparations. Working in a cleanroom is the best resolution. Otherwise prepare the solutions wearing gloves, plastic headcover, labcoat or at least plastic sleeves! Everything that can be done (staining, destaining, spot selection, cutting the gels etc.) under a laminal flow hood should be done there after proper cleaning.

The gel-pieces may be stored covered with water in Eppedorf vials in a refrigerator.