Sample preparation for mass spectrometry

In order to interpret the results reliably we have to know how the sample was prepared. We have to know from what species/cell type/organelle/tissue were the proteins isolated, what 'foreign proteins', chemicals might have been encountered during the process, and we also need appropriate controls. For example, the antibody used for an IP might be contaminated by other proteins; bovine serum proteins of the media are very hard to get rid of; the column used to catch biotinylated substrates may leak avidin. Methionine will be transformed into sulfoxide in the presence of oxidizing agents, cysteine could be turned into cysteic acid, and tryptophane also could pick up an oxygen or two. If we heat or store proteins in urea solution we may find carbamylated N-termini and lysine side-chains. Isolation performed without protease inhibitors may lead to extensive non-specific protein cleavages; the lack of phosphatase inhibitors may lead to significant phosphopeptide losses.

General recommendations

- Do not store low level peptide or protein samples in glass vials!
- For sample storage/submission always use conical plastic vials for example, 0.5 ml eppendorf vials! Based on our experience, Eppendorf Protein LoBind and Safe-Lock vials are of good quality.
- Not properly maintained/contaminated lyophilizers and vacuum concentrators may introduce volatile polymers into the samples.
- Use chemicals of the highest purity during your sample preparation.
- Please, pay attention to everything that may come in contact or surrounds your samples, including the lab, the workbench as well as the glassware/or plastic vials used to store your solutions.
- We can identify proteins only if they are included in our database (we use UniProt most frequently). Whenever we have to work with proteins from some rarely studied species, providing us with sequences from specific, perhaps even proprietary databases is extremely helpful.
- In order to characterize post-translational or other covalent modifications we have to know the accurate amino acid sequence of the protein studied. In addition, we need significantly more material for such analyses than for routine protein identification, and having minimal contamination from other proteins enables us much more likely to succeed.

Peptides, proteins in solution - Recommended concentrations:
Peptides: a few picomole/µl; proteins 10-20 picomole/µl are 'ideal'. Detergents, salts and other additives, such as glycerol, in the sample solutions make our task harder, sometimes they will prevent successful analysis. Remember, we do not care about the preservation of the biological activity, we will 'destroy' your protein anyway. Dissolve your peptides/proteins in the appropriate volume of water, add a bit of acetonitrile if necessary. Adding 0.1-1% formic acid is also a good idea. (Water, ACN, HCOOH should be of HPLC-quality).

Amino acid analysis is the most reliable method for protein quantification. Unfortunately, colorimetric reactions may yield highly erroneous numbers.

Proteins in-gel

- In 2D-gel fractionation, after isoelectric focusing the blocking of free sulfhydryls is highly recommended, for example, with iodoacetamide. Otherwise acrylamide might modify some cysteine side chains, or disulfide bridges may form randomly.
- Use MS-compatible staining – for example, Pierce™ Silver Stain for Mass Spectrometry, EZBlue™ Gel Staining Reagent (SigmaAldrich).
- Never use powdered latex gloves! They release ~8x as much protein than the unpowdered ones. Good gloves do not contain more than 50 µg water extractable protein/g. Or even better, switch to nitrile gloves!
- Human keratin is the most frequent impurity in gels that may interfere with the analysis of low level samples. We have a few ideas how to minimize it:
  * prepare your solutions when you are alone in the lab!
  * from the beginning wear gloves, surgent hat, and rinsed plastic sleeve or lab coat!
  * similarly perform the staining and cutting the gel when there is not much traffic in the lab, or even better, under a carefully cleaned (with (m)ethanol) laminal flow hood.
  * gel slices should be stored in eppendorf vials, under a minimal amount of HPLC-quality water, at 4 °C.
  * Do not freeze the gels!

Please discuss your project with us prior to sample preparation!