FROM THE DIRECTOR’S DESK

Happy New Year! At IMSU, we are committed to helping you with experimental design, providing a first consultation free of charge, working on data interpretation, and establishing strategies for result validation. A well-designed experiment is crucial in the process of generating new knowledge and everything relies on the proficiency and efficiency of sample preparation. Therefore, we have developed two training programs at IMSU directed to increase competence and understanding of the strengths and limitations of mass spectrometry-based proteomics. The training programs are tailored for graduate students, postdoctoral fellows, and faculty. You could also consider these training programs as part of the curriculum or career development activities in training grants or fellowships applications. We will be happy to work with you on developing that component of your application or finding ways to insert the training program in the graduate program curriculum. As an example of the basis for these training programs, please read these book chapters that I wrote several years ago, but that is still relevant as introduction to mass spectrometry-based proteomics. We wish you a productive 2019!!

TECHNICAL DISCUSSION – PROTEIN PREPARATION

Bottom-up proteomic sample preparation begins by removing proteins from lipids, polynucleotides, and cellular debris. Extraction methods vary depending on the sample type (cell culture, fluids, tissues, etc.), but most employ a combination of shearing force, heat, and chemical lysis that works to break apart cell membranes and release proteins into solution.

After the cells are lysed, proteins are purified from other biomolecules by precipitation with cold acetone, methanol and chloroform, or TCA. For targeted proteomic studies, immunoprecipitation, 1D and 2D gels, or multiple stages of preparatory separations help can help isolate the proteins of interest from biomolecules and other background proteins.

Intact proteins can be injected directly into the mass spectrometer, but usually better sequence coverage and more confident identifications are obtained by first cleaving the proteins into peptide
fragments. In order to facilitate protein cleavage, the secondary and tertiary protein structures are denatured with heat and chemical stabilizers, usually urea. Urea non-covalently binds the protein backbones, forcing them into a roughly linear geometry. Cysteine bonds are then cleaved through a reversible reaction with dithiothreitol. Iodoacetamide is added to alkylate the exposed cysteines which prevents them from reforming bonds. Now that the proteins are exposed, they can be cleaved into peptides using digestive enzymes. The serine protease Trypsin is used for 95% of protein digestion experiments, but other enzymes may be appropriate if you plan to target proteins rich in lysine and arginine.

These general digestion procedures can be modified through the addition of buffers, organic solvents, changes in pH, time, heat, etc. The finer points of protein digestion and how to obtain the “perfect” amount of digestion is still hotly debated.

**IMSU SUGGESTED ARTICLES**

A new publication demonstrating many of the sample preparation techniques described above.


If you feel like jumping into the debate of exactly how you should optimize your tryptic digestions, here is a good place to start.