MASS SPECTROMETRY IN PROTEOMICS AND METABOLOMICS

Advances in mass spectrometry and data analysis have provided new opportunities to comprehensively investigate protein expression in complex biological systems. CYTOMICS has been providing mass spectrometry services for the analysis of proteins and proteomes since 2015 and today we make use of extremely sensitive and accurate quantitative MS instrumentation and platforms to comprehensive survey even the most complex of biological systems. We can assess alterations to key biological pathways which might be brought about by changes in disease state, condition, treatment, or a stimulus. We currently use a range of state-of-the-art mass spectrometry platforms including Q-tof, Orbitrap and Q-trap technologies coupled to nano-LC and UHPLC systems to provide extremely sensitive detection and quantitation capabilities. We provide access to this high-end LC-MS instrumentation and work closely with researchers in industry, academia, and government organisations to support multidisciplinary projects which span the chemical and biological sciences, biomedicine and health, food and agriculture, and microbiology. Our core MS-expertise is in quantitative proteomics and protein identification and characterisation. We also specialise in small molecule characterisation and quantitation.

Relative Quantitation: SWATH

SWATH is a relative quantitation approach which is well suited to large scale proteomic projects (tens to hundreds of samples). CYTOMICS offers SWATH services using nanoflow LC which provides superior sensitivity and concentration-response outcomes for your project. SWATH is well suited to most protein sample types including cells, tissue, biofluids, bacteria, fungi, and plants. Our service provider has recently developed bioinformatics tools to enhance SWATH analysis using our own SWATH Extend approach.

Chemical Labelling and Sample Multiplexing: Tandem Mass tags (TMT)

The multiplexed nature of Tandem Mass Tags (TMT) provides an efficient and cost-effective approach for quantitative proteomic projects. When combined with sample fractionation using high pH offline reverse-phase HPLC, TMT quantitation provides in depth sample analysis and differential expression profiling. TMT can be used for larger sample numbers, and is well suited to cell culture studies, microbiological applications, and plant-based studies. Cytomics has recently established 10plex-TMT protocols for organelle profiling, including exosomes and microparticles, phosphorylation studies, and for depleted and undepleted plasma proteomic studies.

Targeted Quantitation: SRM, PRM and MRM-HR

Targeted mass spectrometry approaches such as Selected Reaction Monitoring (SRM), Parallel reaction Monitoring (PRM) and Multiple Reaction Monitoring – High Resolution (MRM-HR) are used for the specific quantitation of a smaller numbers of target proteins in complex samples. Targeted approaches yield improved sensitivity, specificity, and reproducibility, and are commonly used in conjunction with stable isotope standards for the absolute quantitation of target proteins. CYTOMICS uses Q-tof, orbitrap, and Q-trap technology for targeted quantitative studies using SRM, PRM, or MRM-HR.

Application:

- Quantitative and Comparative Proteomics
- Protein Identification and Profiling

Platform:

- Thermo Scientific LTQ Orbitrap™ XL Mass Spectrometer
- Thermo Scientific Q-Exactive™ Hybrid Quadrupole Orbitrap™ Mass Spectrometer
- Thermo Scientific Q-Exactive™ Plus Hybrid Quadrupole Orbitrap™ Mass Spectrometer

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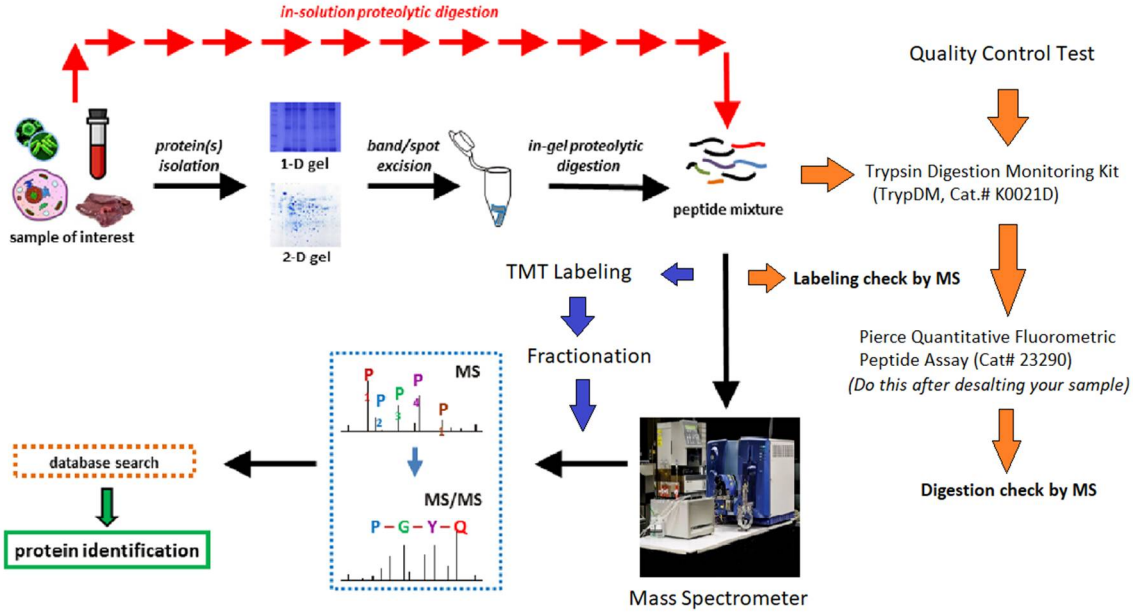
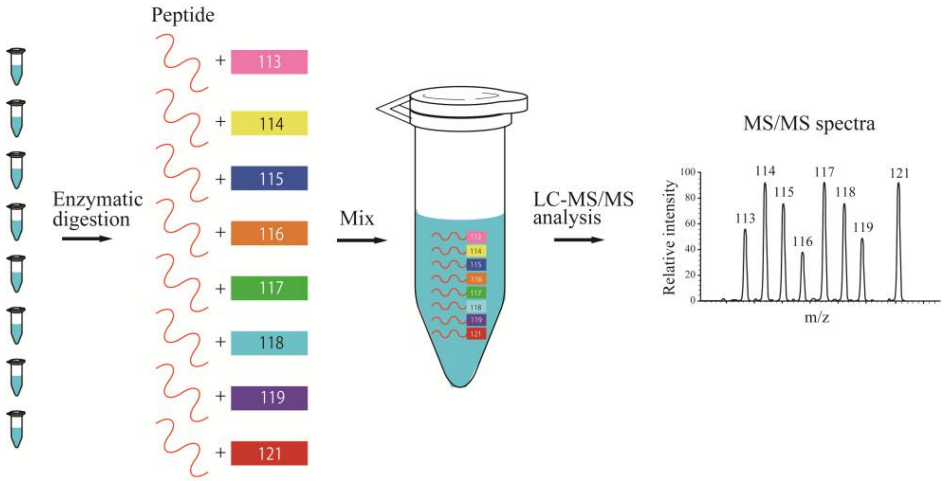
Overview

I) Sample Preparation



II) TMT-Labelled Protein for Comparison and Quantitation



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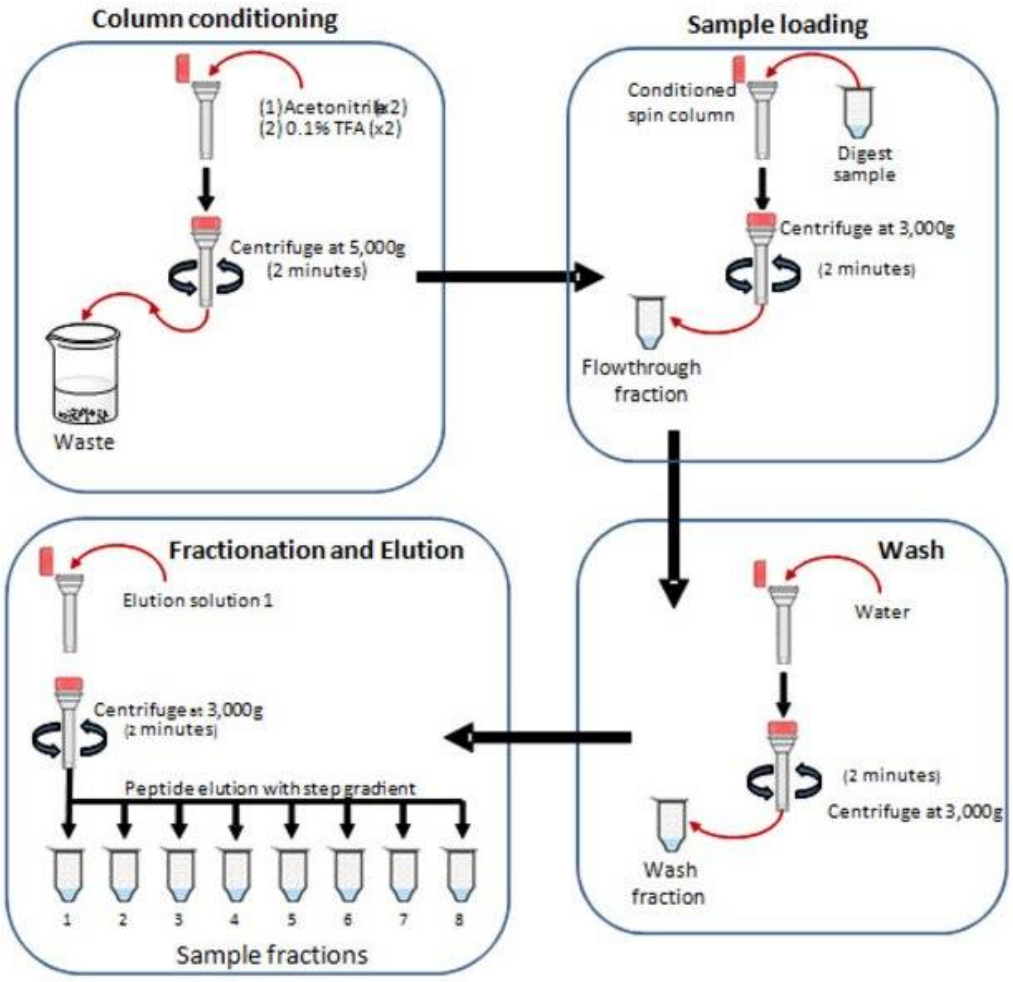
III) High pH Reversed-Phase Peptide Fractionation



Procedure Summary

Peptides are bound to the hydrophobic resin under aqueous conditions and desalted by washing the column with water by low-speed centrifugation. A step gradient of increasing acetonitrile concentrations in a volatile high pH elution buffer is then applied to the columns to elute bound peptides into eight different fractions collected by centrifugation. Each fraction is then dried in a vacuum centrifuge and stored until analysis by mass spectrometry. During LC-MS analysis, peptides in each high pH fraction are further separated using a low pH gradient, thus reducing the overall sample complexity and improving the ability to identify low-abundant peptides.

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