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Summary

Mass spectrometry services are critical to the characterization of protein therapeutics in the development state. Expanded applications of mass spectrometry offerings include molecule weight determination, peptide mapping, impurity characterization, and identification of modifications.

BIOLOGICS TESTING SOLUTIONS

Large Molecule Mass Spectrometry Services

Our staff, many with over 15 years of experience, is proficient in the management of diverse and complex protein characterization projects including large molecule comparability studies. Our state-of-the-art mass spectrometry laboratory consistently delivers excellent structural analysis services to our clients.

Molecular Weight Determination

Various complementary approaches are available to determine protein molecular weights, ranging from the analysis of intact proteins under native or denaturing conditions, to the use of enzymes and/or reducing conditions to provide subunit-level mass analysis using electrospray ionization mass spectrometry (ESI-MS) or MALDI-TOF MS.

Glycosylation Analysis and Glycosylation Site Mapping/Occupancy Determination

A glycoprotein is often initially analyzed in intact form using LC-MS with high-resolution mass spectrometry (HRMS). Based on the masses resolved, several attributes may be confirmed, including the amino acid sequence, N- and C-termini, the glycosylation status, and certain other post-translational modifications (PTMs) such as phosphorylation and oxidation. For highly heterogeneous glycoproteins, however, MALDI-TOF MS may be required to assign the overall protein average molecular weight; protein subunits might also be generated and several glycosidase (glycan) trimming steps added. LC separation is carried out by size-exclusion chromatography (SEC) or reversed phase high performance liquid chromatography (RP-HPLC) with HRMS.

After cleaving proteins into peptides and glycopeptides for analysis by HRMS, a more detailed understanding of glycosylation may be undertaken at the protein level as a component of peptide mapping; in the case of O-linked glycan site determination, this may involve the use of special proteases. Protein level glycosylation analysis may involve hydrolysis into monosaccharides, or mild hydrolysis of labile sialic acids for compositional profiling. N-glycans can be enzymatically released in an intact form from the protein; whereas, O-glycans are chemically released for LC-MS profiling.

Detailed structural analysis of N- and O-linked glycans may require further analysis involving additional exoglycosidase enzyme treatments, or mass spectrometry based fragmentation analysis. An overview of the analytical strategies employed for N- and O-linked glycoproteins is provided in the table below.

Peptide Mapping

Peptide mapping is initiated by determining optimal protein digestion conditions including choosing between enzymatic or chemical digestions and the best chromatography conditions. This analysis is first performed by *in silico* digestion, with conditions aligned to the protein of interest and the overall project goals. After performing the protein digest, peptides and glycopeptides are separated by HPLC with detection by MS or MS/MS, obtaining both accurate mass analysis and MS/MS, as necessary for sequence confirmation and identification of any modification sites. Detection may be conducted concurrently using UV absorbance and/or fluorescence. Through peptide mapping, various post translational modifications can be identified and, if appropriate, quantified. These include disulfide linkages, glycosylation, oxidation, deamidation, N-terminal cyclization or acylation, integrity of N- and C-termini, phorphorylation and covalent addition of lipids.

De Novo Sequencing

De novo sequencing is required when the protein sequence is either unavailable or can only be inferred from the nucleotide sequence. For *de novo* sequencing, the protein is digested using multiple individual protease treatments, and the resolved peptides are all analyzed by MS/MS. When there is ambiguity within the obtained amino acid sequence, the peptides are collected and analyzed by Edman degradation using automated N-terminal sequencers.

Antibody Drug Conjugate (ADC) Analysis

Several ADC mass spectrometry services are offered including determination of drug antibody ratio (DAR), mapping of the drug linkage site(s) and, if appropriate, percent occupancy at each linked site(s). Analysis and quantitation of any free antibody, drug, linker, or degradants may be provided depending on the scope of work.

Analysis of PEGylated Proteins

Pegylation is a type of modification introduced into proteins to extend their circulatory half-life. Several mass spectrometry approaches have been developed for these unique proteins, including intact mass determination, mapping of pegylation sites within the protein primary structure, and the quantification of occupancy at pegylation sites.

Other Services

Proteomic analyses can be performed using two different approaches: shotgun and targeted. For either strategy, a protein mixture such as human serum or a monoclonal antibody in serum is proteolytically digested and separated by liquid chromatography, followed by MS/MS analysis. In the shotgun approach, database searches are used to identify proteins in the sample. Identifications, once made, may be further refined into targeted analyses (e.g., quantitation) of the specific peptides and proteins of interest.

Glycoprotein Analytical Strategy				
Glycan Composition	**Sugar Composition:** 1. Monosaccharide analysis 2. Sialic acid analysis			
N-linked Glycans	**Profiling/Characterization:** 1. Release *N*-glycans with PNGase-F 2. Derivatize with 2AB, 2AA, or Rapi-Fluor 3. Analyze by HILIC or HIAX UPLC 4. Detect by fluorescence, MS, and MS/MS	**Detailed Structure Analysis:** 1. Exoglycosidase sequencing 2. Sequential mass spectrometry (MS^n)	**Glycan Heterogeneity:** Protein & subunit intact LC/MS	**Glycan Site-Occupancy Mapping:** 1. Protease cleavage 2. LC-MS/MS (glyco) peptide mapping
O-linked Glycans	**Profiling/Characterization:** 1. Release by reductive beta elimination 2. Native or permethylated *O*-glycans 3. Analyze by RP-LC and detect by CAD, MS, and MS/MS			

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