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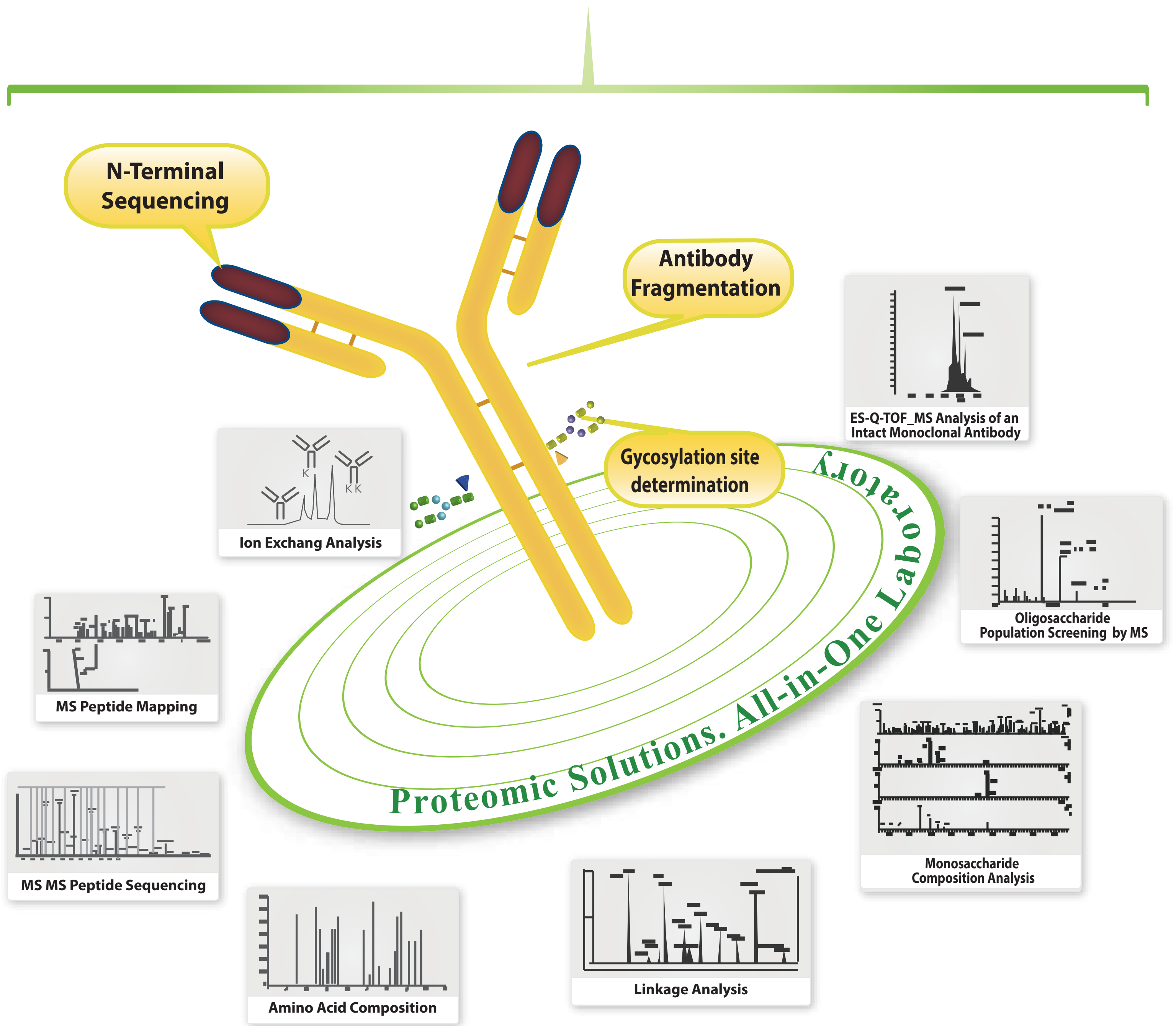
Antibody Characterization



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MOLECULAR MASS ANALYSIS OF ANTIBODIES

Mass analysis of recombinant proteins is a key characterization method used to evaluate the entire amino acid sequence of the molecule. It also allows determination of the presence of post-translational modifications. Intact mass analysis supports the characterization package for regulatory filings as part of the protein drug development process and may be used to evaluate lot-to-lot consistency on the whole molecule level.

Mass analysis of intact or reduced antibodies has been used to evaluate the degree of processing of C-terminal lysine on the heavy chain subunit (1), evaluate N-terminal heterogeneity, such as pyroglutamic acid formation (2, 3), profile N-linked carbohydrate heterogeneity (3, 4), detect instabilities in the molecule such as oxidation (5), succinimide formation from aspartic acid (6), glycation (7), internal cleavage (8), as well as thioether formation (9).

Brady et al. 2008 (10) report the use of a commercially available polyhydroxyethyl aspartamide column to operate in SEC mode (pHEA-SEC) for the rapid on-line analysis of antibodies. This chromatographic material has been originally developed for the separation of polar compounds by hydrophilic interaction chromatography (11). The column is operated in 0.1% formic acid under isocratic conditions and organic solvents are introduced by post-column addition. A robust and fast separation method that is highly flexible and tolerates numerous sample buffer components was developed. Additionally, this new approach differs from other reported SEC-MS methods, achieving a rapid separation in the absence of salts or organic modifiers. They were able to interface this method with an ESI-TOF mass spectrometer and obtained very high quality mass data with experimental to theoretical mass agreements below 25 ppm for several intact antibodies. Furthermore, since this method is highly tolerant of different sample buffer components, they were able to use the same method, without modification, for the analysis of reduced antibody samples. Chakraborty et al., 2007 described a on-line LC-UV/ESI-TOF-MS method for the analysis of reduced monoclonal antibodies. A Waters ACQUITY UPLC system with a LCT Premier XE mass spectrometer was used. The use of a BioSuite pPhenyl-RPC column (2.0 x 75 mm, 10 m, 1000 Angstroem) a gradient buffer system based on 0.1% formic acid in water (Buffer A) and in acetonitrile (Buffer B) at a flow rate of 0.2 ml/ml allowed the separation of light and heavy chain and the determination of the whole mass for both proteins. Adamczyk et al., 1999 reported the use of an API 100 single quadrupole mass spectrometer operated on-line with a microbore HPLC for the analysis of lot-to-lot heterogeneities of a monoclonal antibody. A PLRP-S and a C18 column were used to analyze the whole antibody as well as the reduced heavy and light chain. Da Reyna et al, 2008, reported the use of a diphenyl column to separate post-translationally modified intact monoclonal antibodies from the unmodified antibodies.

Bio–Synthesis Offers comprehensive antibody/protein analyses for biopharmaceutical analytical technologies. The following individual analyses, or a full antibody characterization package, tailored to the FDA and ICH guidelines and used under GLP/GMP guidelines, may include:

- **Amino acid sequence analysis**
- **Amino acid composition analysis**
- **N– and C– terminal amino acid sequencing**
- **Peptide MS Mapping analysis**
- **Disulfide bridge analysis**
- **Carbohydrate structure analysis**
- **Determination of intact molecular weight**
- **Electrophoretic and isoform pattern analysis**
- **Liquid chromatographic patterns**
- **Identification of post–translational modifications**
- **Spectroscopic profiling**
- **Identification of product related impurities**

Reference

1. D.Bondarenko et al., J. Chromatogr. A 2004, 1053, 299-305, 2004.
2. D. Chelius et al., Anal. Chem., 2006, 78 (7), pp 2370-2376.
3. B. Yan et al., Journal of Chromatography A Volume 1164, Issues 1-2, 14 September 2007, Pages 153-161.
4. D. Thomas et al., Journal of Chromatography Volume 1120, Issues 1-2, 7 July 2006, Pages 112-120
5. J. Yanga et al., Journal of Chromatography A Volume 1156, Issues 1-2, 13 July 2007, Pages 174-182. Abstract
6. G.Chu et al., Pharmaceutical Research Volume 24, Number 6 / June, 2007, 1145-1156.
7. L. Brady et al., Anal. Chem., 2007, 79 (24), pp 9403-9413.
8. A. Cordobaa et al., Journal of Chromatography B Volume 818, Issue 2, 25 April 2005, Pages 115-121.
11. A.J. Alpert; J. Chromatogr. A 4999 (1990), pp. 177–186.