

Leveraging Mass Spectrometry Capabilities to Study Protein Degradation

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Outline

1. Mass Spectrometry
 - Instrumentation
 - Capabilities
 - Analytical use for biopharmaceutical drugs
2. Protein Degradation
3. Mass Spectrometry Applied to Protein Degradation
 - Protein sequence analysis
 - Amino acid modification analysis
 - Glycosylation analysis
 - Aggregation

Mass Spectrometry

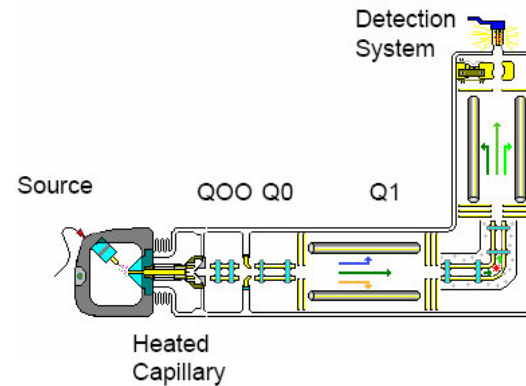
A mass spectrometer can be defined as “*A machine used to weigh molecules*” from “What is Mass Spectrometry?”, on-line vulgarization poster at www.asms.org

“Mass spectrometry is the art of measuring atoms and molecules to determine their molecular weight. Such mass or weight information is sometimes sufficient, frequently necessary, and always useful in determining the identity of a species. To practice this art one puts charge on the molecules of interest, i.e., the analyte, then measures how the trajectories of the resulting ions respond in vacuum to various combinations of electric and magnetic fields.”

John Fenn, 2002 Nobel laureate

Mass Spectrometry

A large range of instruments from multiple vendors
Increased performance, sensitivity, automation
From floor print systems to benchtops units



Thermo TSQ Quantum

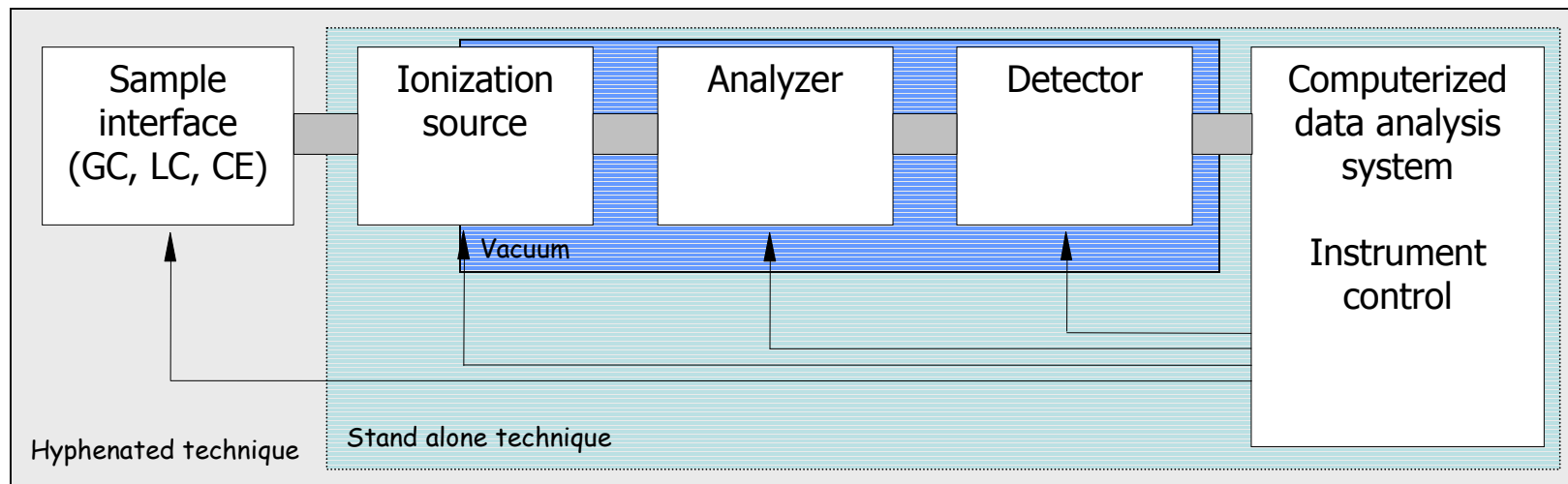
c/o Thermo Finnigan Corp.



Mass Spectrometry

A limited number of techniques

A common concept for mass spectrometers



Ionization sources

Electron Impact – 1918 – GC/MS and GC/MS/MS

Dempster A.J., Phys. Rev. 11, 316 (1918)

Chemical Ionization – 1966 – GC/MS and GC/MS/MS

Munson M.S.B., Field F.H. J. Am. Chem. Soc., 1966, 88(12), 2621

Fast Atom Bombardment – 1982

M. Barber, R. S. Bordoli, G. J. Elliot, R. D. Sedgwick, and A. N. Tyler, Anal. Chem., 54, 645A (1982)

Atmospheric Pressure Chemical Ionization - 1975

Carroll D.I., Dzidic I., Stillwell R.N., Haegele K.D., Horning E.C. Analytical Chemistry, 1975, 47(14), 2369

Matrix-Assisted Laser Desorption/Ionization – 1988

Karas M., Hillenkamp F. Analytical Chemistry, 1988, 60, 2299; Tanaka K., Waki H., Ido Y., Akita S., Yoshida Y., Yoshida T. Rapid Commun. Mass Spectrom., 1988, 2(8), 151

ElectroSpray Ionization applied to biomolecules – 1989

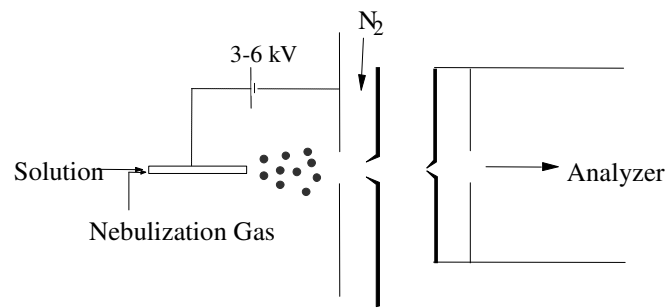
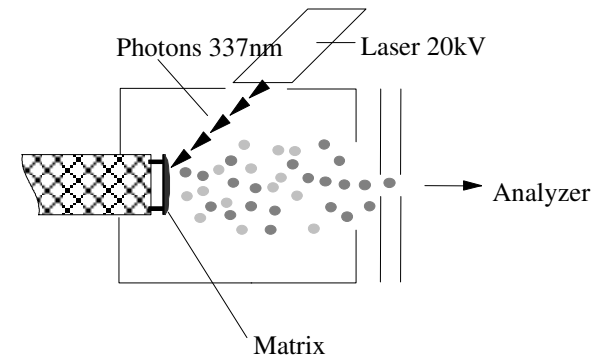
Fenn J.B., Mann M., Meng C.K., Wong S.F., Whitehouse C.M. Science, 1989, 246(4926), 64

Desorption ElectroSpray Ionization – 2004

Takats Z., Wiseman J.M., Gologan B., Cooks R.G. *Science*, **2004**, 306(5695), 471

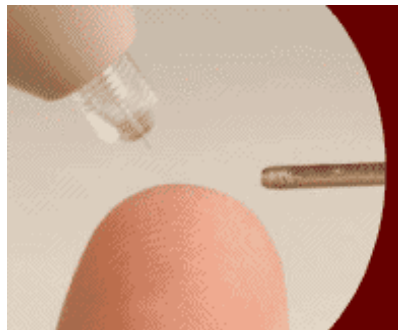
Ionization sources

Matrix-Assisted Laser Desorption/Ionization



ElectroSpray Ionization

Desorption ElectroSpray Ionization



Analyzers

Analysis based on space

Magnetic sector

Quadrupole (Q)

ESI, APCI

Time-of-Flight (TOF)

MALDI, ESI

Analysis based on time

Ion Traps

ESI, APCI, MALDI

3D or quadrupole – Paul traps (QIT)

ICR – Penning traps

FTMS

2D – Linear ion traps (LIT)

Orbitrap

Combination of Analyzers

Triple quadrupoles (QQQ)

Quantitation

Quadrupole Time of Flight (QTOF)

Identification

TOF/TOF

Magnetic sectors,
Proteomics

IT/TOF

LIT/TOF

LIT/FTICRMS








High resolution,
Identification,
Structural analysis

LIT/Orbitrap

<http://masspec.scripps.edu/MSHistory/mshisto.php>

Analyzer Comparison

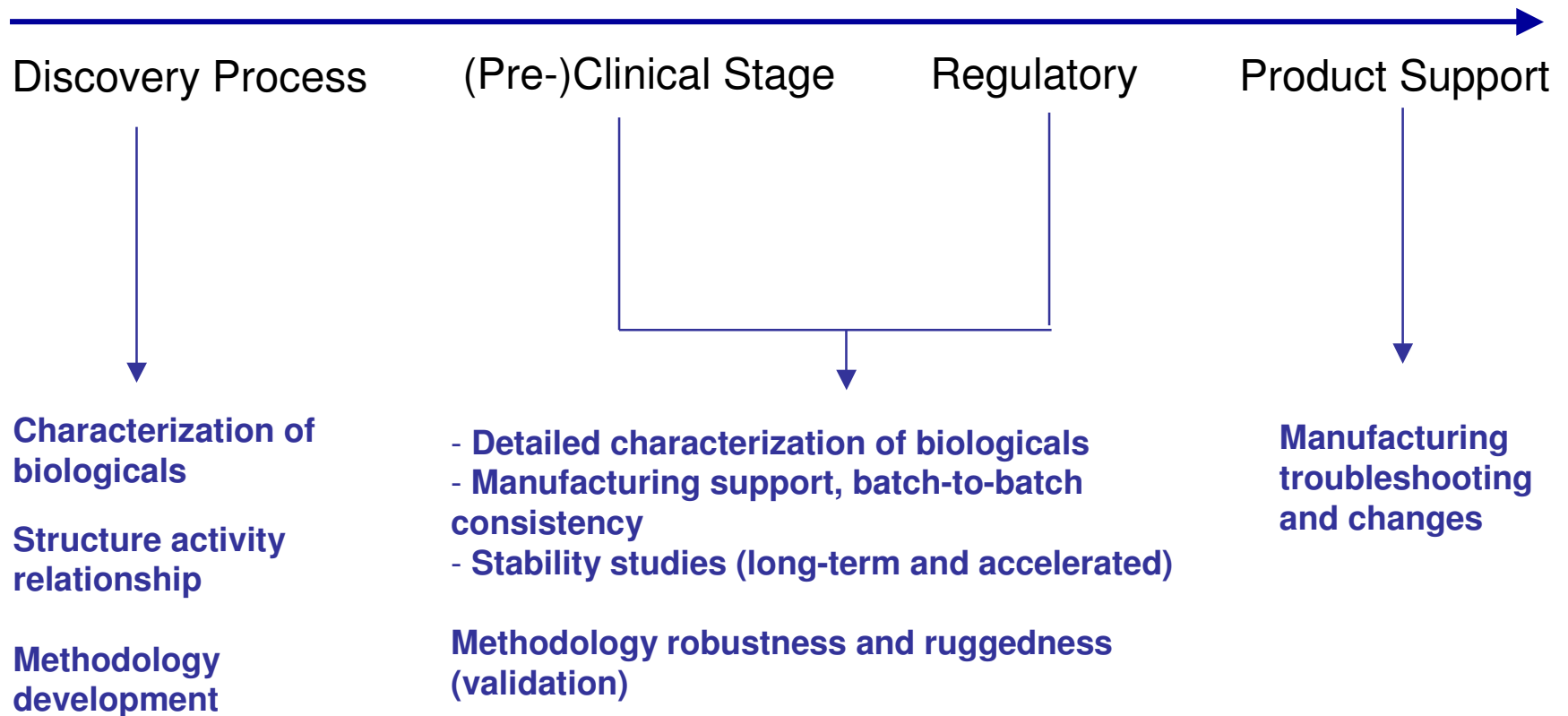
Parameters to consider:

- Resolution  FTMS > TOF, QTOF, LIT/Orbitrap
- Accuracy  FTMS > QTOF, LIT/Orbitrap
- m/z range  FTMS, TOF, QTOF
- Structural analysis (MSⁿ)  Ion traps (FT/MS)
- Price  Quadrupole and triple quadrupole, QIT, TOF
- Installation, maintenance  FTMS
- Throughput  FTMS

The choice of an instrument (and its price) is based on your needs. *The most expensive system may not be the best choice for you!*

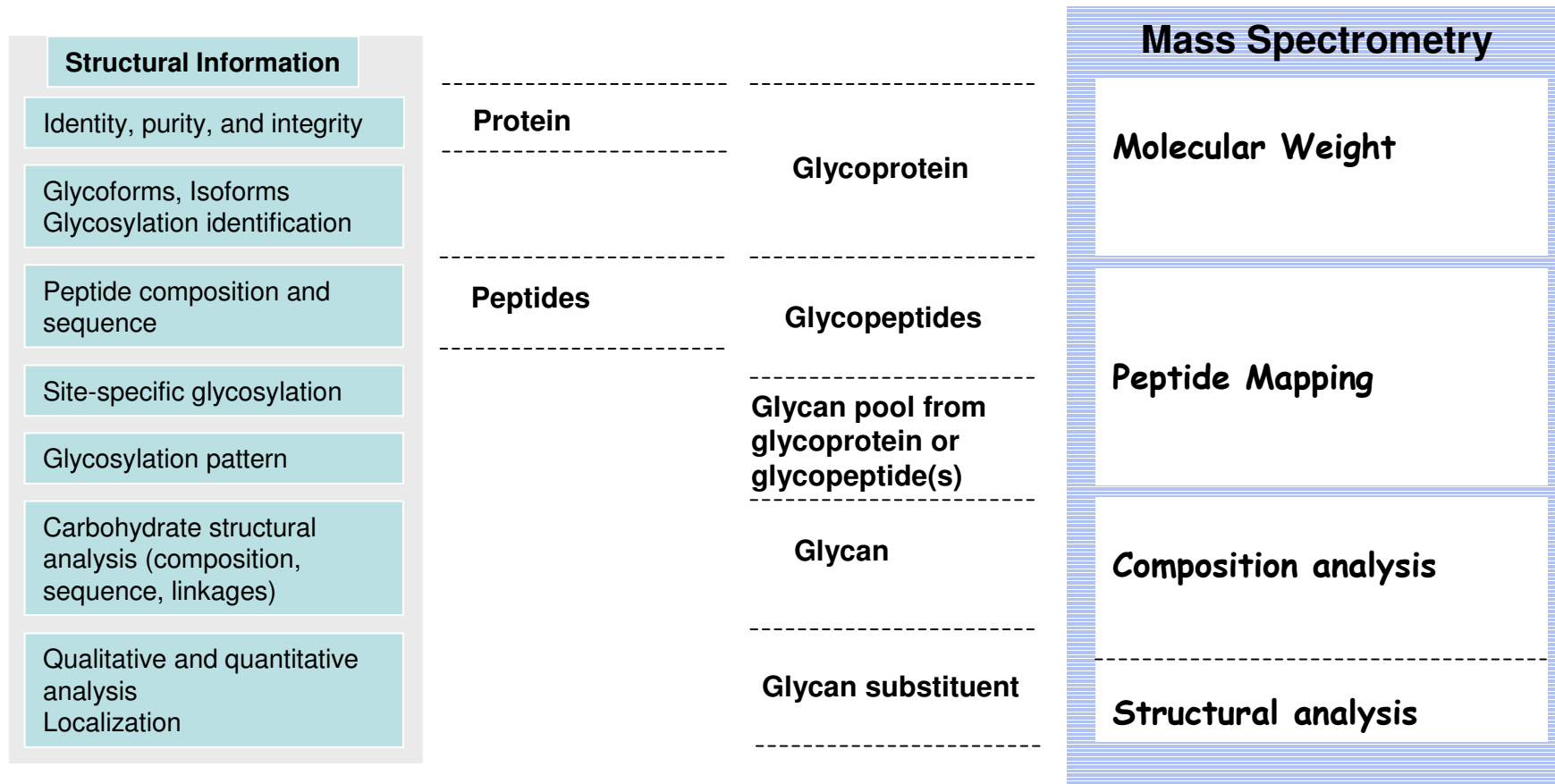
Analytical Support

Development of a biopharmaceutical drug



Biomolecule Structural Analysis

The depth of characterization sought defines the type of analysis



Protein Degradation

Protein backbone degradation, amino acid modifications

- deamidation
- oxidation
- fragmentation (clips)
- exchange of disulfide bonds

Protein substitutions

- glycosylation

Aggregation

Protein Primary Structure Analysis

From Edman degradation to LC/UV, LC/MS and LC/MS/MS

Peptide sequencing and protein mass fingerprinting

Biemann K., Cone C., Webster B.R., Arsenault G.P. J. Am. Chem. Soc., 1966, 88(23), 5598

Henzel W.J., Billeci T.M., Stults J.T., Wong S.C., Grimley C., Watanabe C. PNAS, 1993, 90(11), 5011

Henzel W.J., Watanabe C., Stults J.T.

Journal of the American Society for Mass Spectrometry, 2003, 14, 931

Glycopeptide mapping

Conboy J.J., Henion J. Biol. Mass Spectrom. (1992), 21, 397-407

Huddleston M.J. et al. Anal. Chem. (1993) 65, 877-884

Peptide Mapping

Pivotal method for protein identification by confirmation of the primary (amino acid sequence) and secondary structures (disulfide bridges)

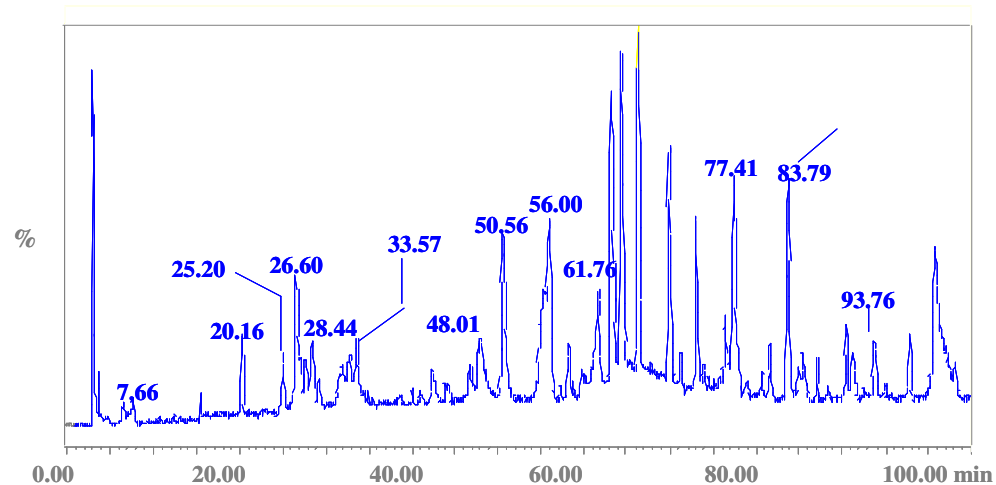
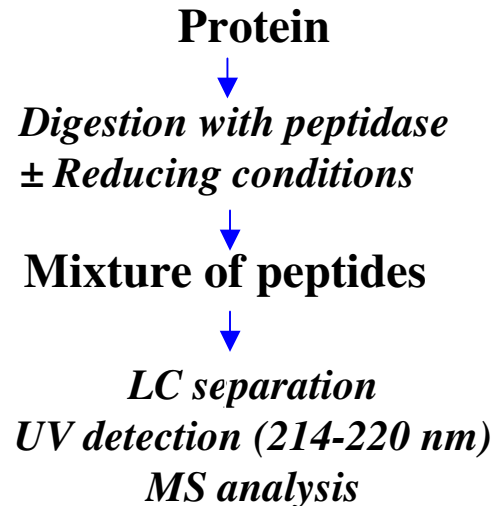
- bottom-up or shotgun proteomics
- identification and lot release method for recombinant proteins

Complex method - sample preparation, LC and MS set up, references

Informative, robust, precise, aiming at detecting single amino acid substitutions and modifications, benefits from improved instrumentation and data processing systems

Stability-indicating assay?

Peptide Mapping as Stability-Indicating Assay



Choice of enzymatic conditions → ability to cleave and obtain the most sequence coverage (complexity of the protein and its substitutions, incomplete or non specific cleavages)

Choice of LC conditions → ability to separate the peptides potentially object of changes

Peptide Mapping for Monoclonal Antibodies

(18)O Labeling Method for Identification and Quantification of Succinimide in Proteins. Xiao G, Bondarenko PV, Jacob J, Chu GC, Chelius D. *Anal Chem.* 2007 Feb 22 (electronic publishing)

Formation of Pyroglutamic Acid from N-Terminal Glutamic Acid in Immunoglobulin Gamma Antibodies

Dirk Chelius, Kay Jing, Alexis Lueras, Douglas S. Rehder, Thomas M. Dillon, Alona Vizel, Rahul S. Rajan, Tiansheng Li, Michael J. Treuheit, and Pavel V. Bondarenko. *Anal Chem.* 2006 Apr 1;78(7):2370-6.

Peptide Mapping as Stability-Indicating Assay

Validation

Most methods with UV detection, MS used as support for identification of changes

MS analysis

Mire-Sluis AR (ed): State of the Art Analytical Methods for the Characterization of Biological Products and Assessment of Comparability. Dev Biol (Basel). Basel, Karger, 2005, vol 122, pp 29-47.

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Validation of Peptide Mapping with Electrospray Mass Spectrometry for Recombinant Proteins of Biopharmaceutical Interest and its Applications as an Identity Test and a Characterization Tool

Z. Wei, G. Tous, A. Yim, J.N. Hope, J.R. Casas-Finet, G. Folena-Wasserman, M.A. Schenerman

MedImmune, Inc., Gaithersburg, MD, U.S.A.

Peptide Mapping and Quantification

Mass Spectrometry is based on the ability of producing ions

Ionization efficiency resulting from the source type and conditions is directly influenced by the composition of the molecule to ionize

A peptide

- native
- modified

presents differences in ionization yield

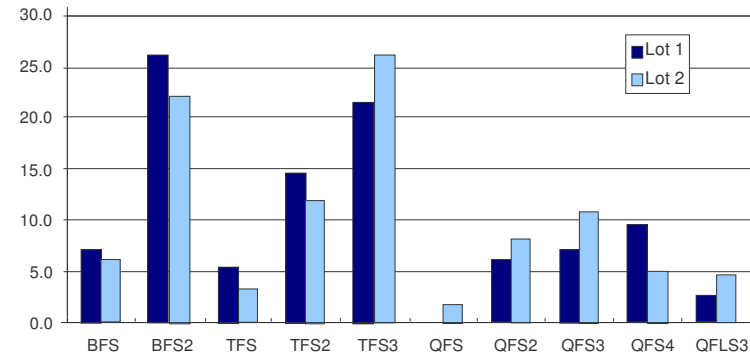
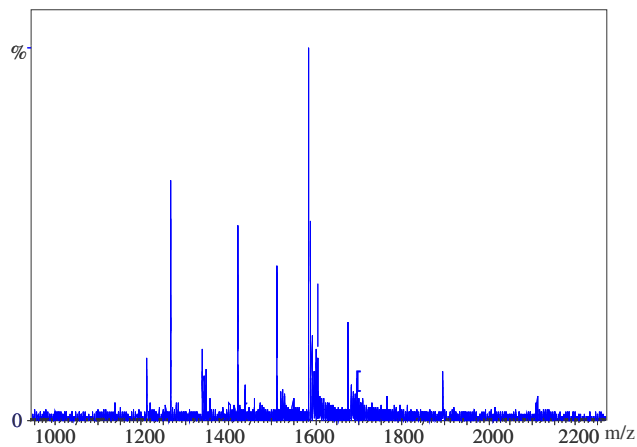
→ Absolute quantitation of peptide or particular modification can be very tricky

→ Relative abundance is usually reported

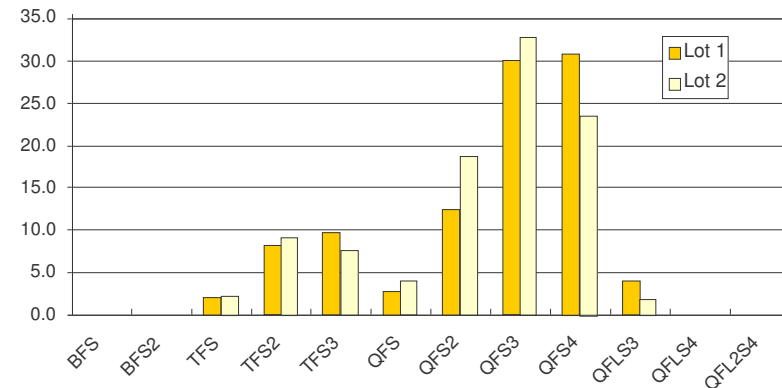
Peptide Mapping and Glycosylation Analysis

Glycosylation site 1

Mass spectra of glycopeptide



Glycosylation site 2



Semi-quantitative glycosylation profiles can be retrieved from peptide maps

Comparison can be done between batches or stability samples

Glycoprotein Sialylation



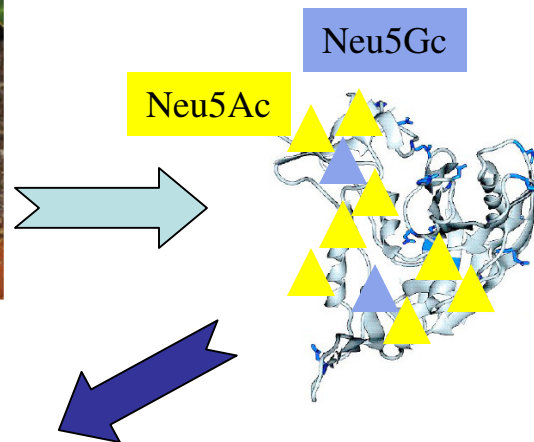
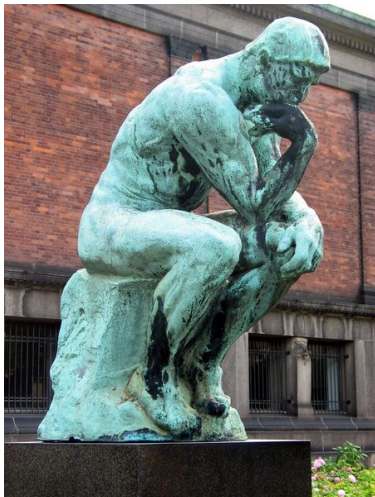
Sialic acids: monosaccharides decorating the outside of the glycans

Pivotal role in biological activity through interactions

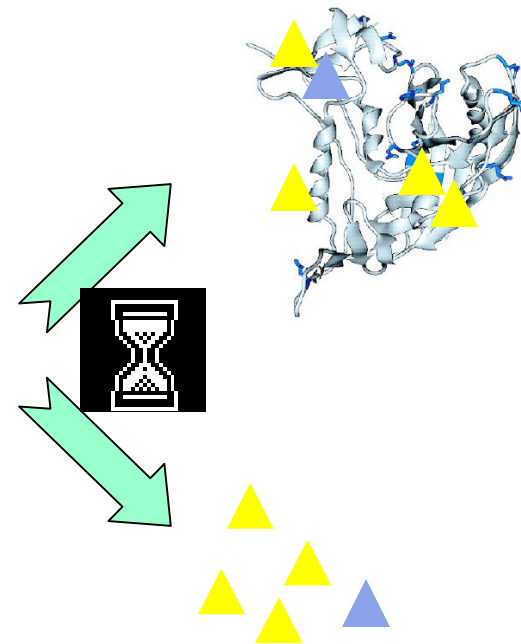
The loss of sialic acids is a stability-indicating parameter of glycoproteins

Sialic Acid Analysis

Goal: Absolute quantitation of sialic acids
⇒ potential immunogenicity

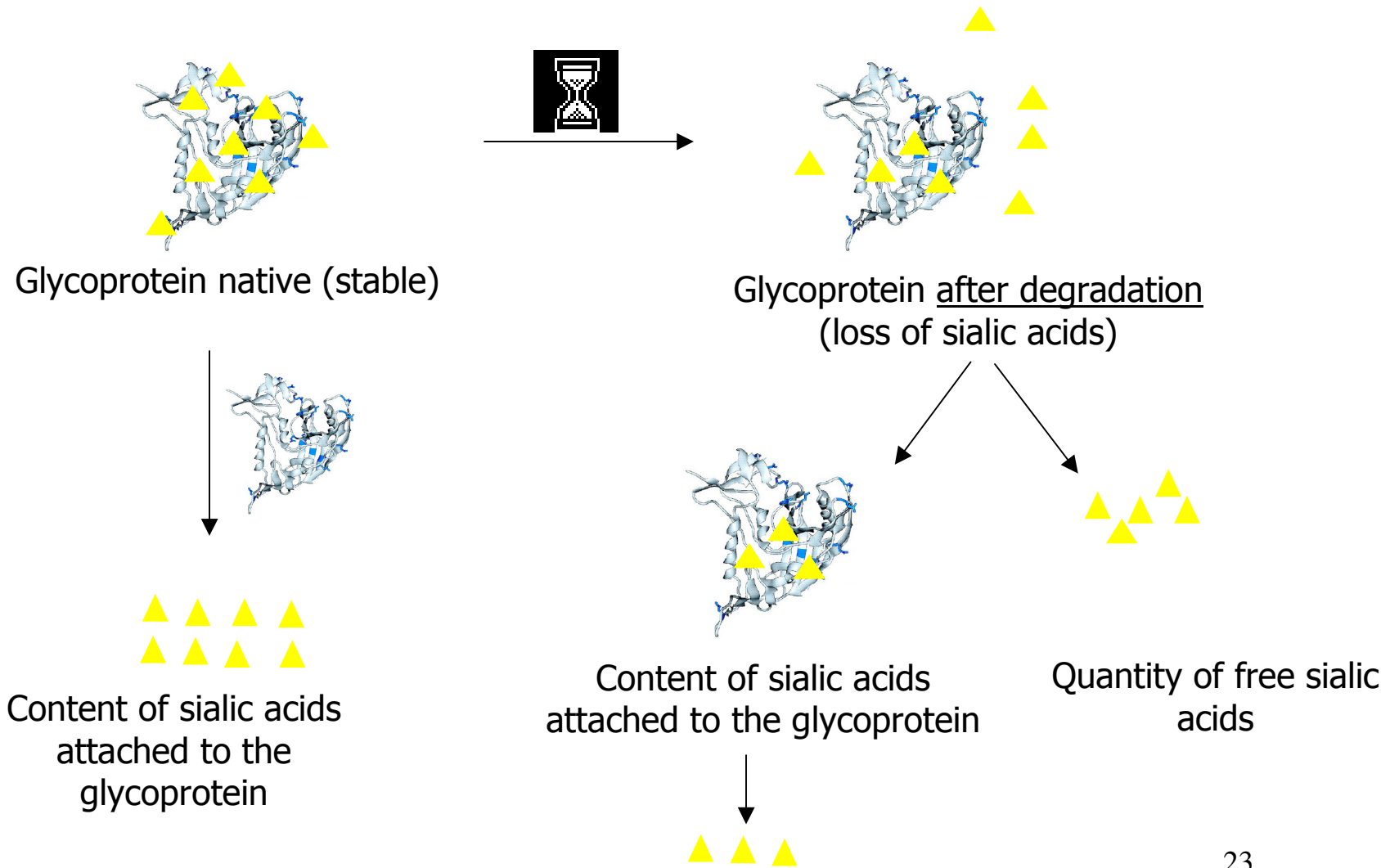


⇒ protein stability



Chromatographic methods:
HPAEC-PAD or LC with FLD after DMB derivatization

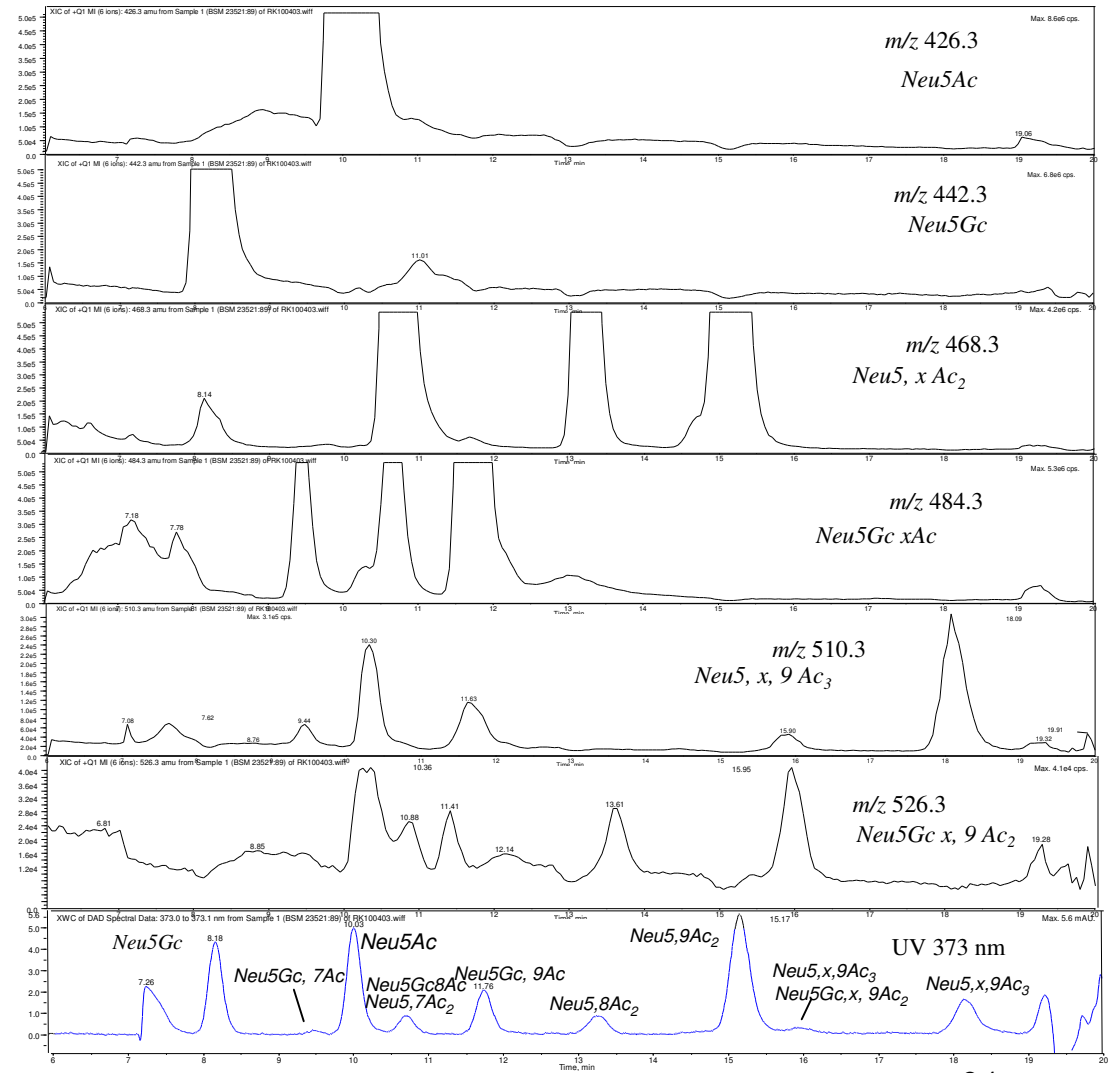
Stability-Indicating Analysis of Sialylation



LC/MS Analysis of Sialylation

LC/FLD method

- Quantitative
- Validated
- Sensitivity
- Differentiation of sialic acids
- Interface with MS



Bovine Submaxillary Mucin

Protein Aggregation

Classical Approaches

- Chromatography: SEC, RP with UV, RI or MALLS detection
- Analytical ultracentrifugation (sedimentation velocity)

Mass Spectrometry

- Hyphenation requirements
- Analysis of molecular complexes such as non covalent complexes by ESIMS

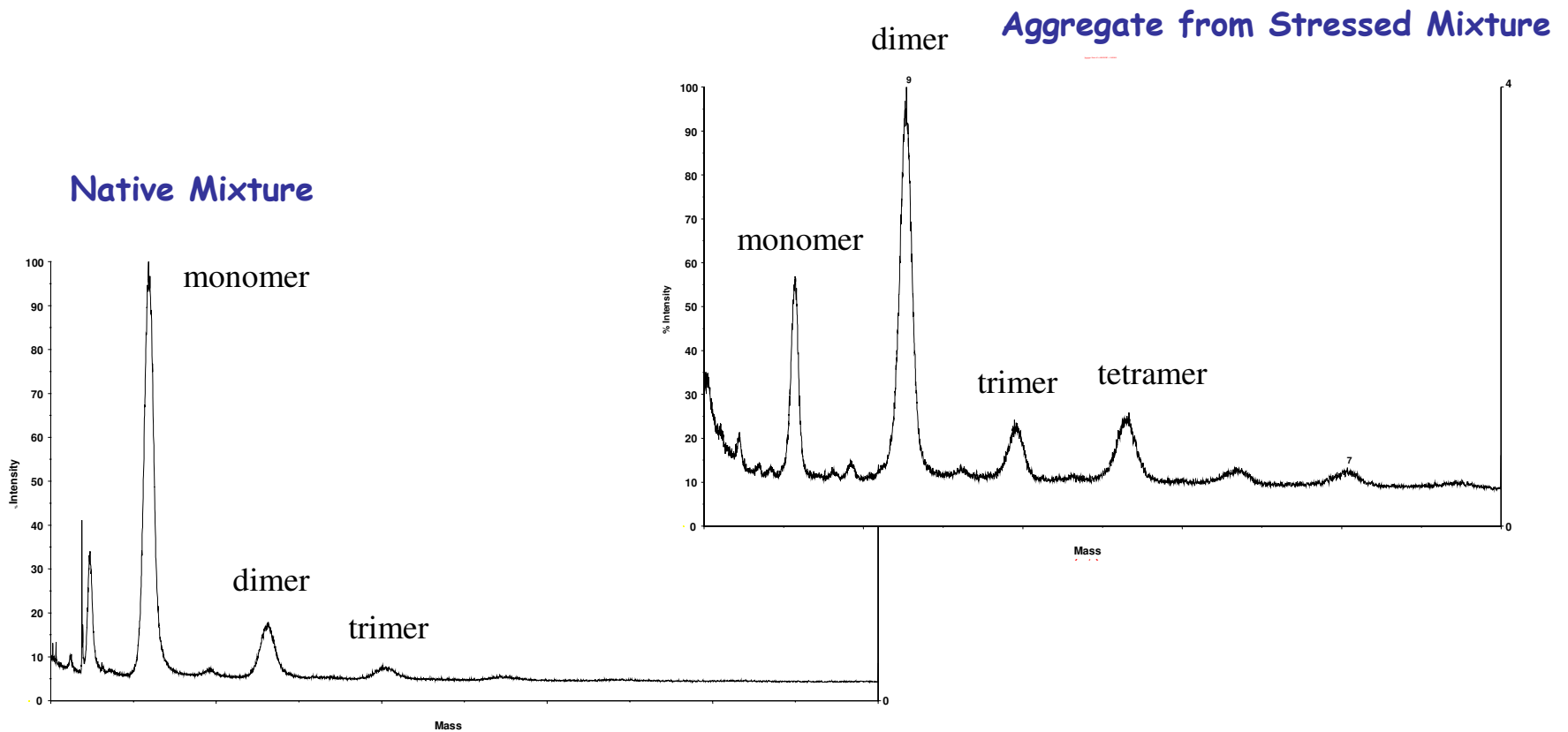
Ganem B., Li Y.T., Henion J.D. Journal of the American Chemical Society, 1991, 113(16), 6294

Katta V., Chait B.T. Rapid Communications In Mass Spectrometry, 1991, 5(4), 214;
Journal Of The American Chemical Society, 1991, 113(22), 8534

- MALDI MS

MALDIMS Analysis of Aggregates

Mixture of 2 glycoproteins (A, B) that produced aggregates under stressed conditions (AA, AB, BB)



Conclusion

Mass Spectrometry

- Pivotal analytical technique
- Used as stability analysis tool either as a primary or secondary technique

Peptide mapping

Glycosylation and sialylation analysis

Aggregation

- Formulation impact
- One more approach in a stability study plan

Acknowledgments

Mass spectrometry inventors and pioneers

Mass spectrometer vendors

Article authors

Baxter Healthcare

Min Xie (Biomolecule Structural Analysis)

Duane Reiber (Physical and Chemical Sciences)