

Mass Spectrometry a Multi-purpose Tool in Cancer Research

Stephen Barnes, PhD

**Director, Center for Nutrient-Gene
Interaction in Cancer Prevention**

**Director, Mass Spectrometry Shared
Facility**

Cancer Research and Mass Spectrometry

**Protein target
identification and
modifications**

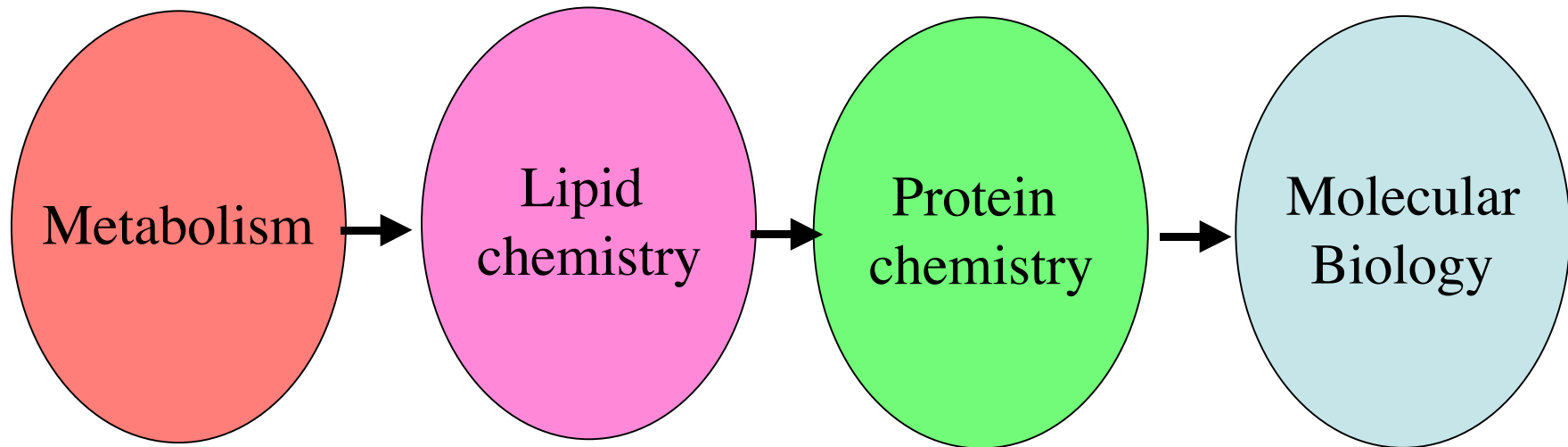
**Proteome imaging
of tissues**
Dr. Pierre Chaurand

**SELDI analysis of
body fluid proteomes**
Dr. David Chhieng

Synopsis of this talk

- **How did mass spec emerge?**
- **What can mass spec (and proteomics) bring to your research?**
- **Time to change the research paradigm?**

Direction of NIH research for the past 50 years



Where did the interest in mass spectrometry come from?

Fundamental experiments on the nature of the matter



Lord Rutherford

In the early 20th century, discovery of isotopes of the elements led to atomic weapons (U^{235}/U^{238})

Ernst Chain
Niels Bohr
Ben Gurion
Linus Pauling

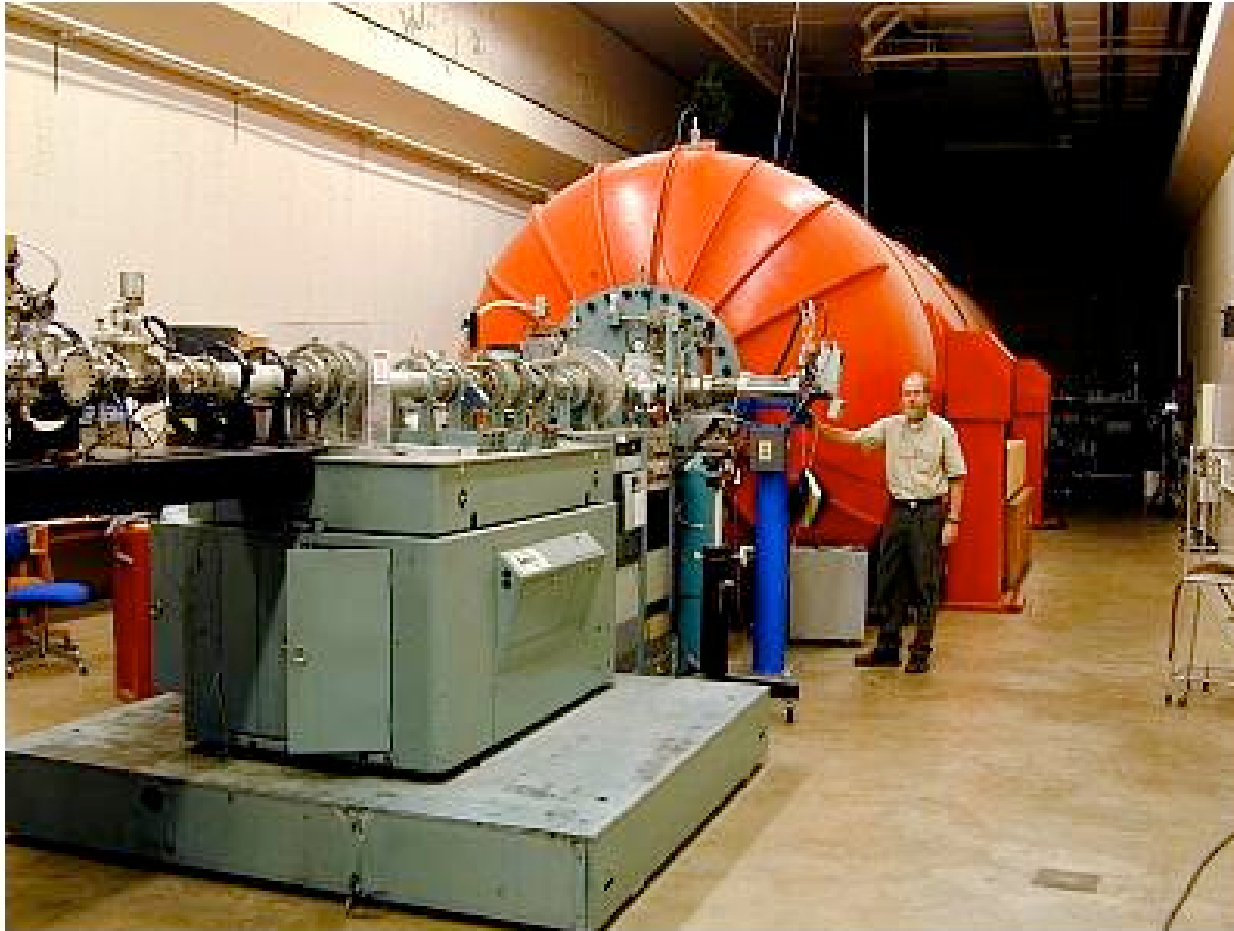


Post-World War II, generation of isotopes of hydrogen, carbon, nitrogen, phosphorus and sulfur created biomedical research as we know it today



David Rittenberg

Accelerator MS in PRIME Lab



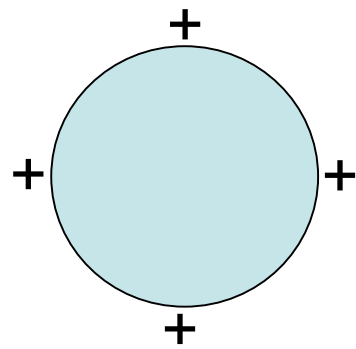
**Dr. David
Elmore next to
10 MV
accelerator**

What is mass spectrometry?

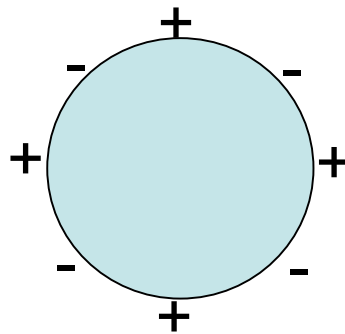
Compounds have to be converted to ions



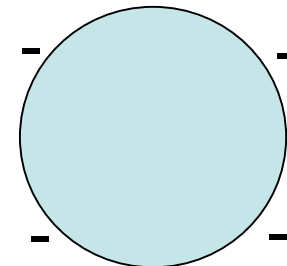
and then analyzed in the gas phase



pH 1



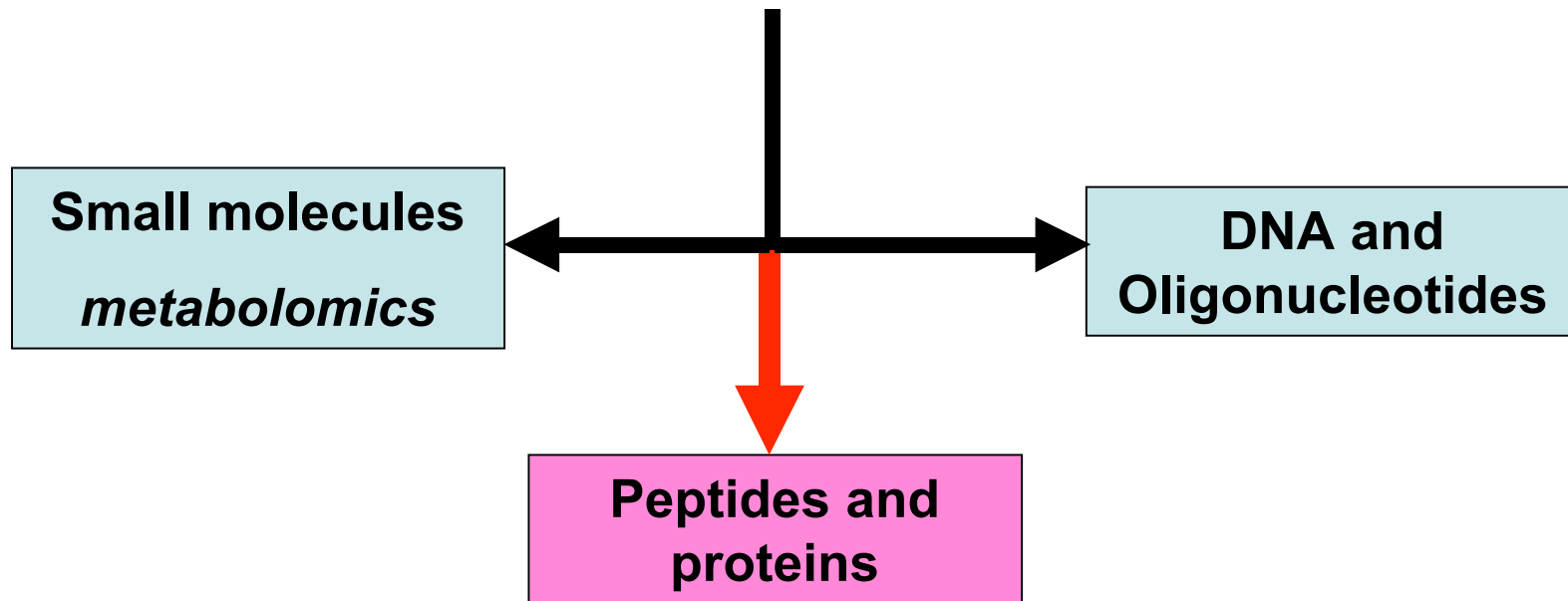
pH 6



pH 10

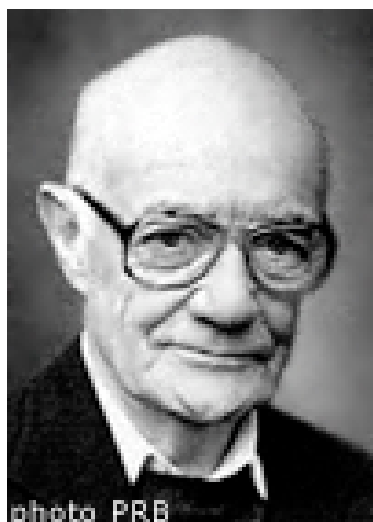
Charge on a protein at different pHs

What can mass spectrometry measure in 2003?



How can we evaporate peptides and proteins?

Nobel Laureates in Chemistry - 2002



John Fenn



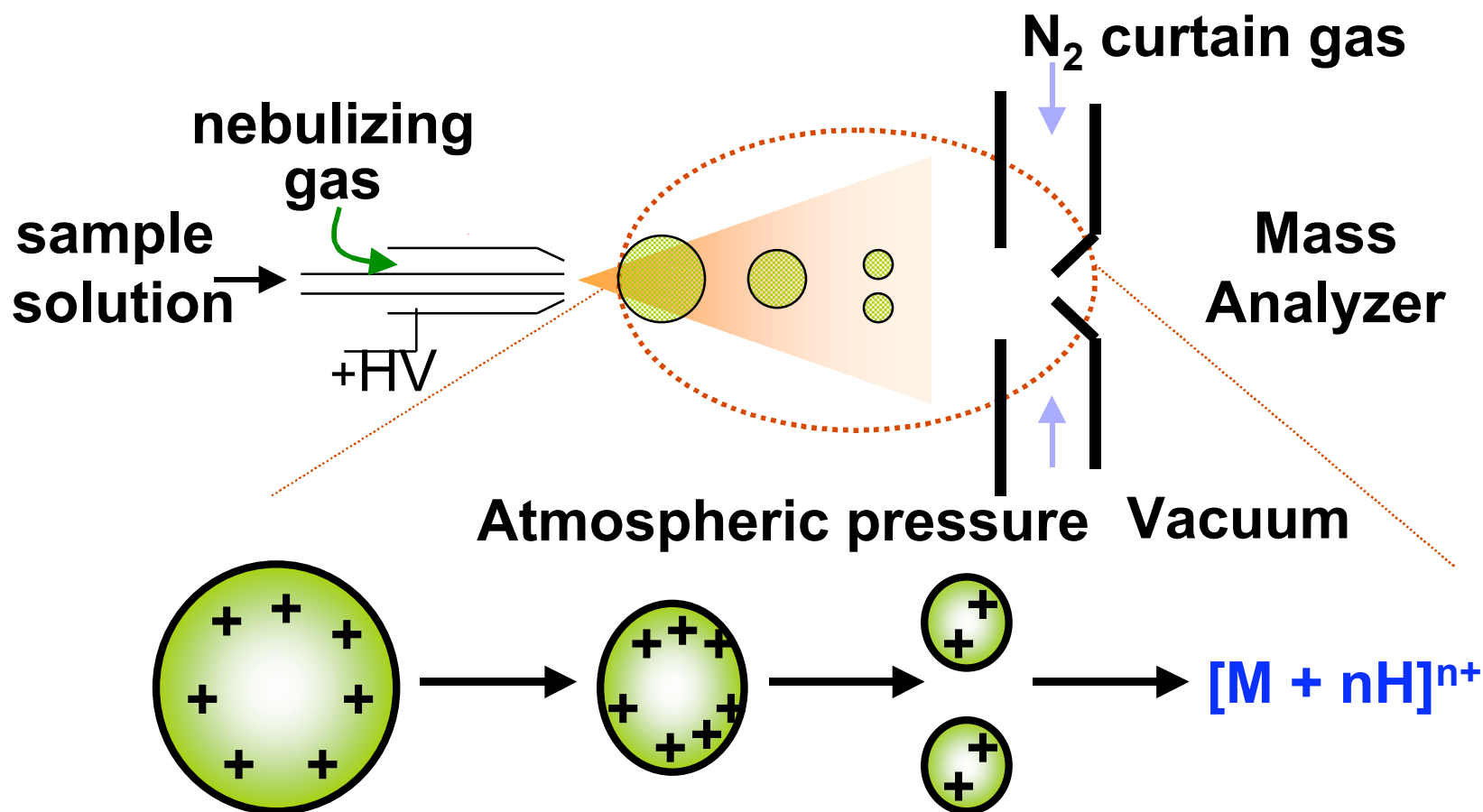
Koichi Tanaka

"for the development of methods for identification and structure analyses of biological macromolecules"

and

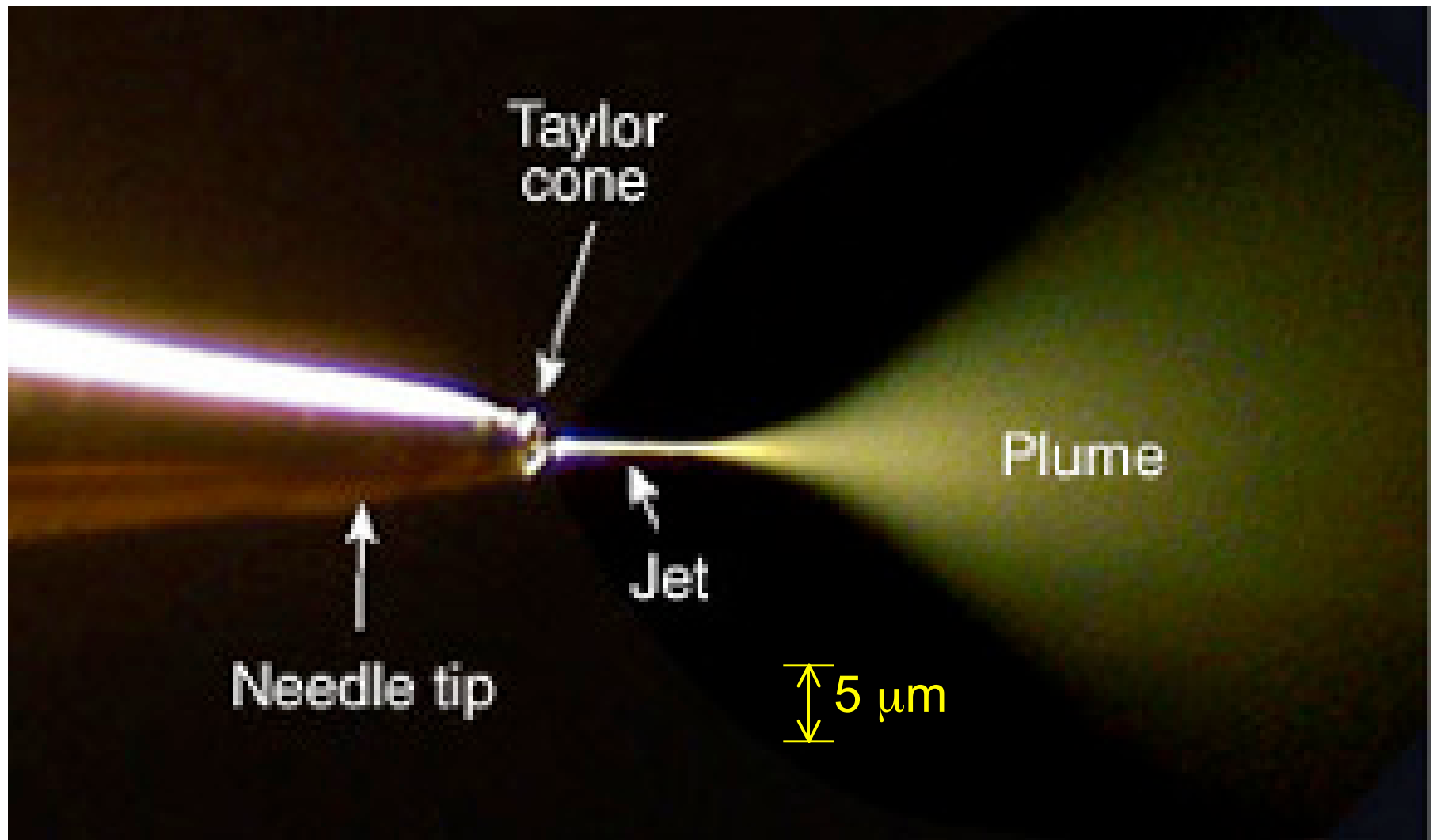
"for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules"

Electrospray Ionization (ESI)

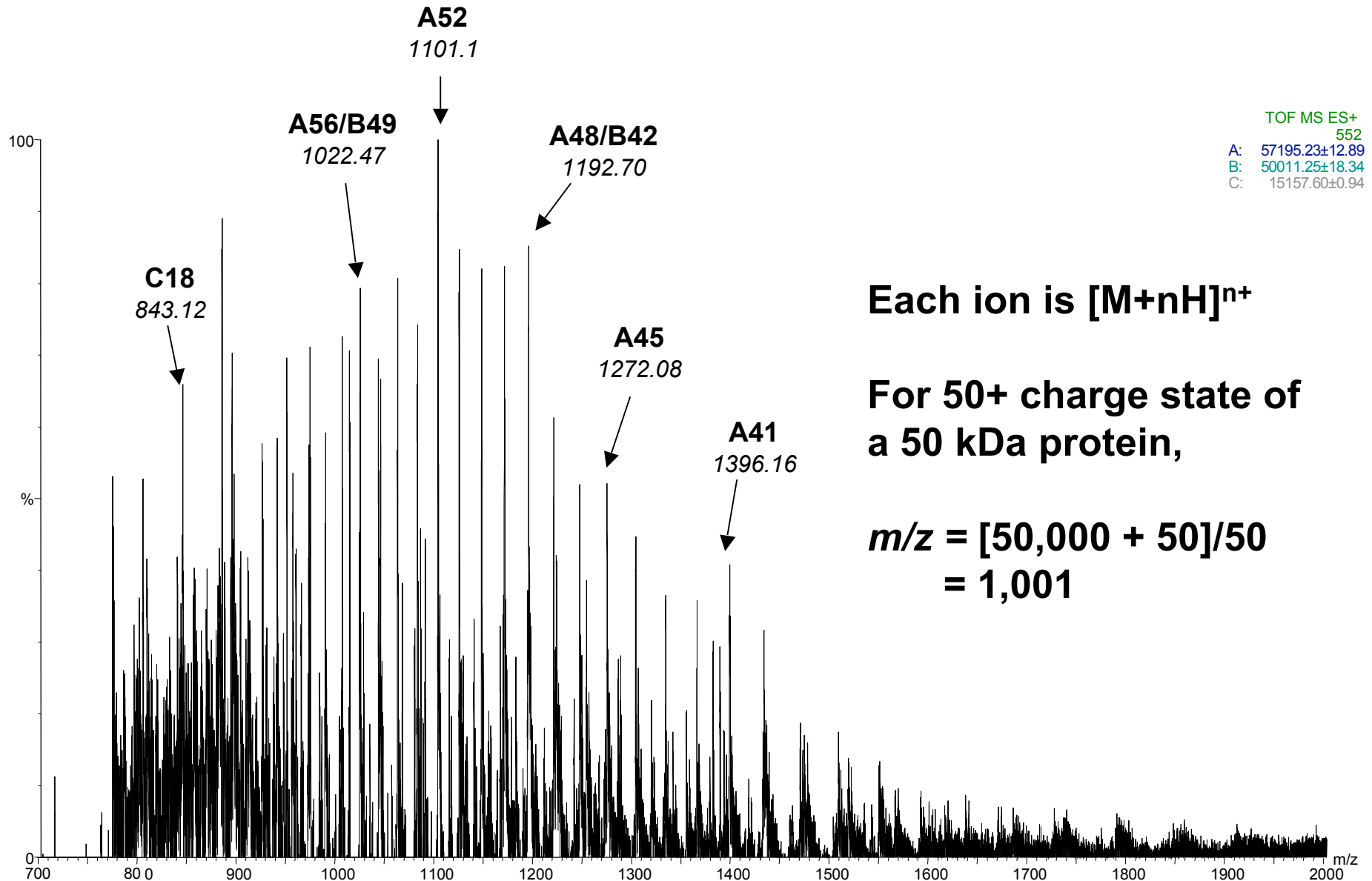


1. Solvent evaporation
2. Coulombic repulsion

NanoElectrospray

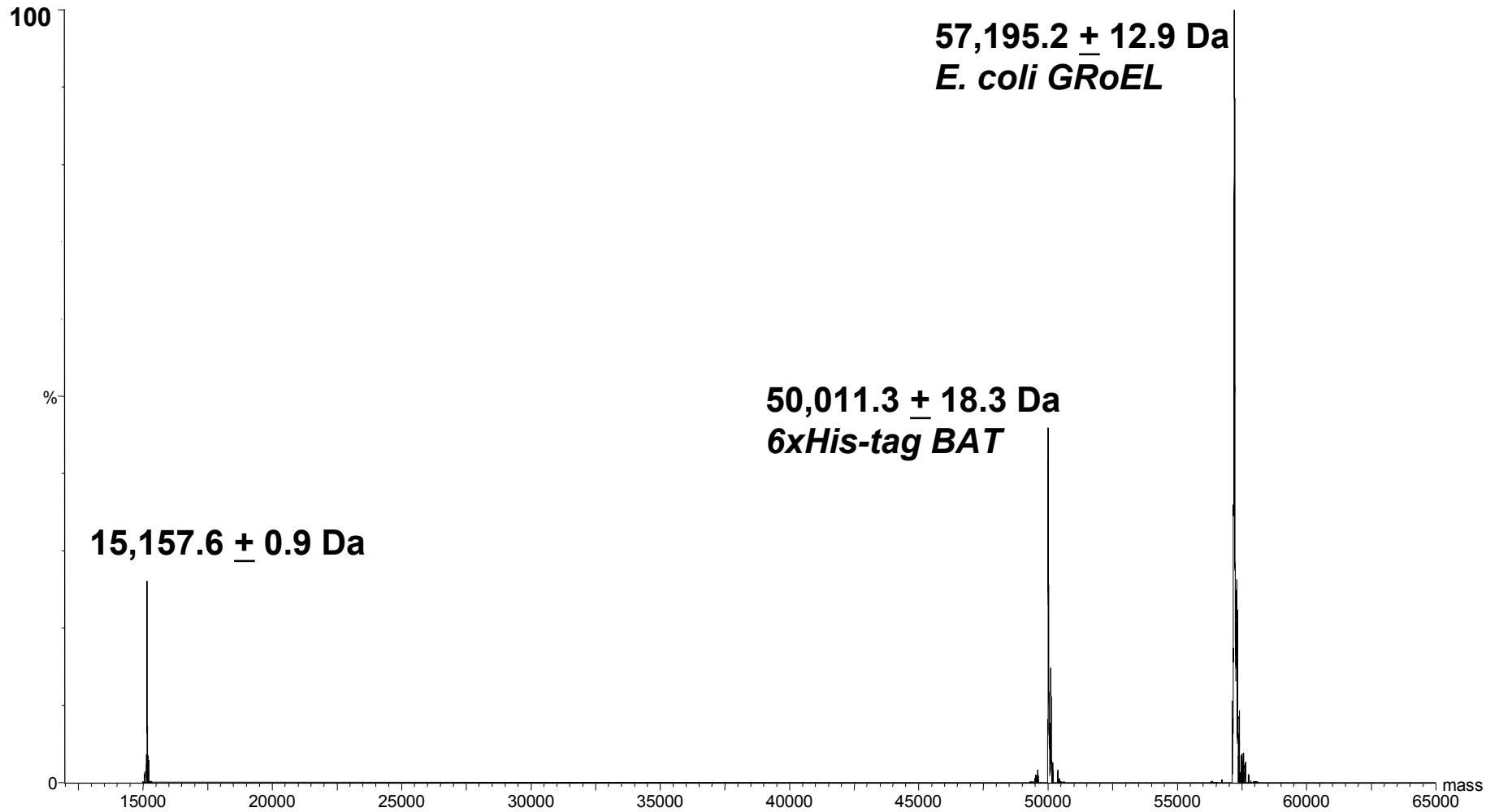


ESI spectrum of bacterially expressed protein



Courtesy of Mindan Sfakianos

MaxEnt deconvolution of ESI-MS data

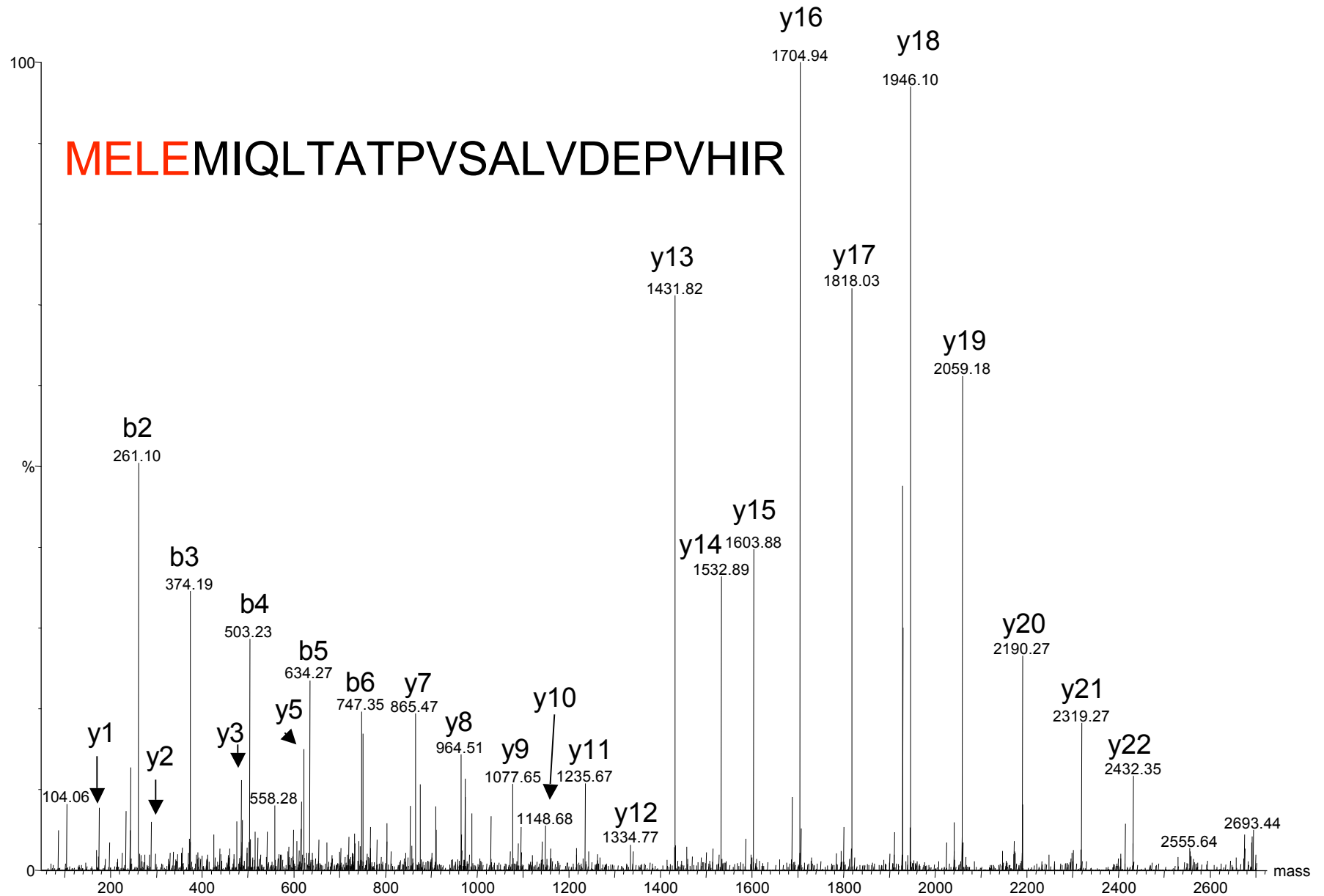


Courtesy of Mindan Sfakianos

How can ESI-MS of proteins help you?

- **Establish that an expressed or isolated protein is authentic**
 - *C. elegans* proteins expressed in bacteria
- **Measure the post-translational changes to protein caused by biochemical or chemical modifications**
 - e.g., changes in the mol wt of antibodies (Ahmad Safavy - attachment of radiometal complexing agents)
 - Glycation of antibodies in diabetes

Unexpected peptide from a bacterially expressed protein

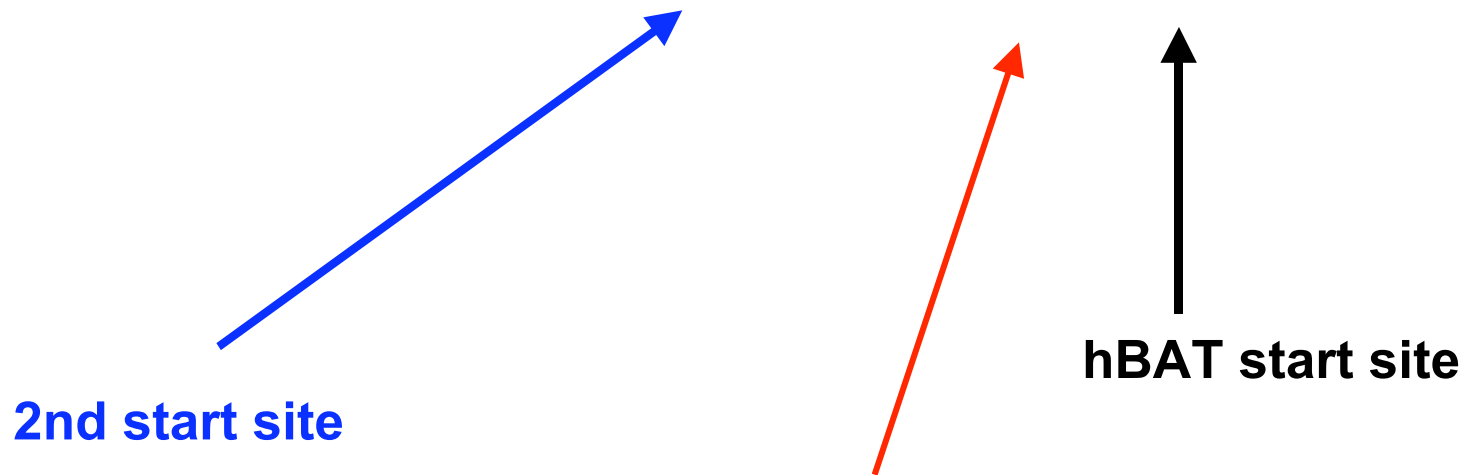


N-terminal extension revealed

Antisense 3' -CATCTCGAGCTCCATGGT-5'

Reversed 5' -TGGTACCTCGAGCTCTAC-3' -hBAT

Sense 5' -ACCATGGAGCTTCGAGATG-3' -hBAT

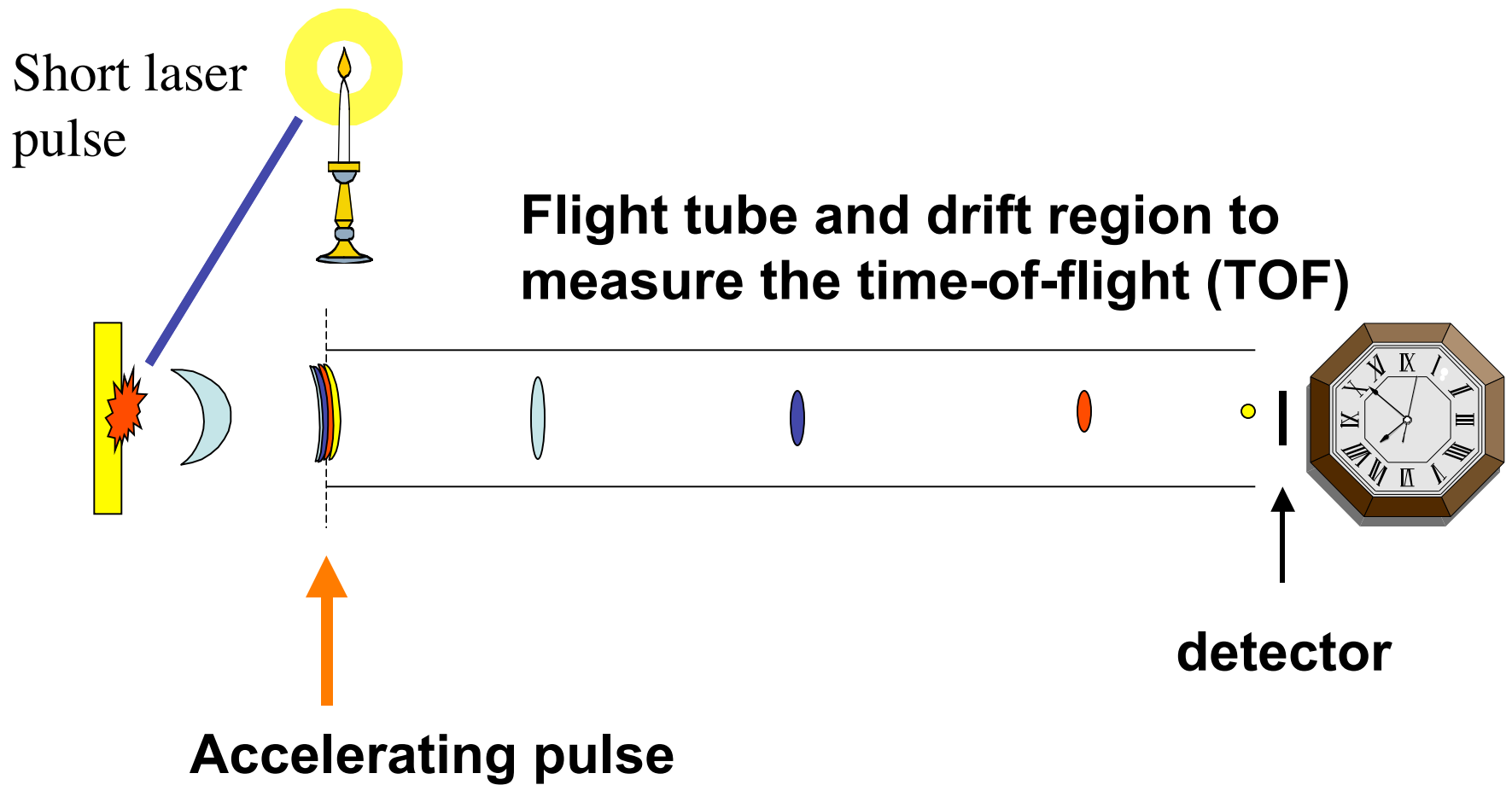


Xho1 site where hBAT was inserted

MALDI - shining the light on your research

- A focused laser beam, either in the UV or infra-red ranges, can “evaporate” compounds from the solid phase
- The resulting ions are injected into a tube (1 - 2 m in length), accelerated and allowed to drift towards a detector. Their *time-of-flight* is proportional to their $(MW)^{1/2}$

Matrix-Assisted Laser Desorption Ionization (MALDI)



Protein analysis 2003

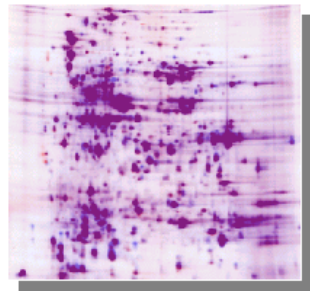
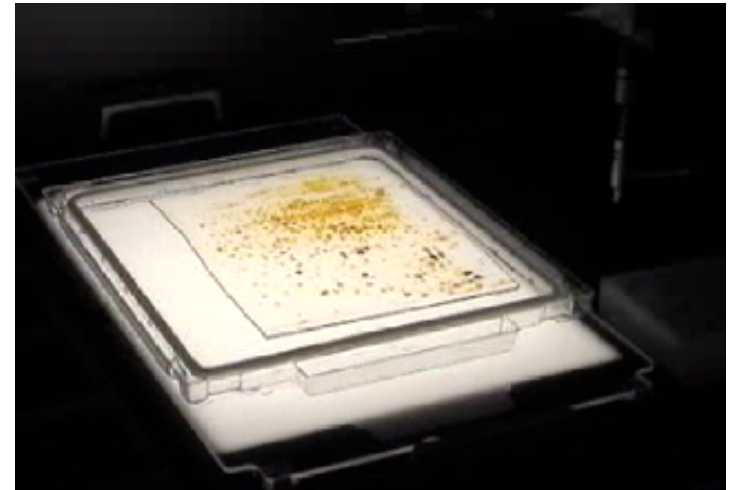
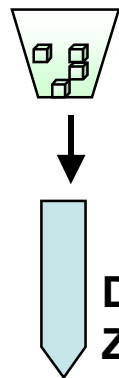


Image analysis

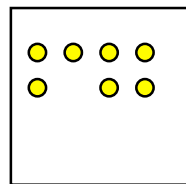
Robotic spot picking



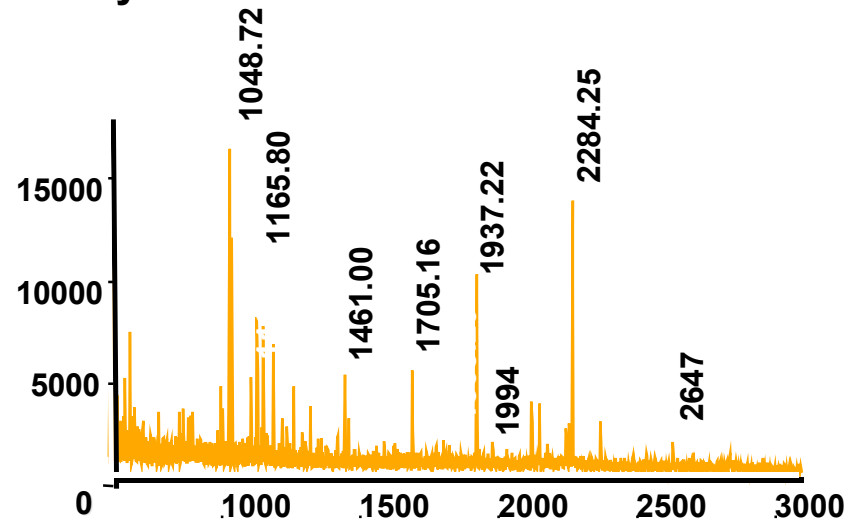
Destaining, drying and trypsinolysis



MALDI plate

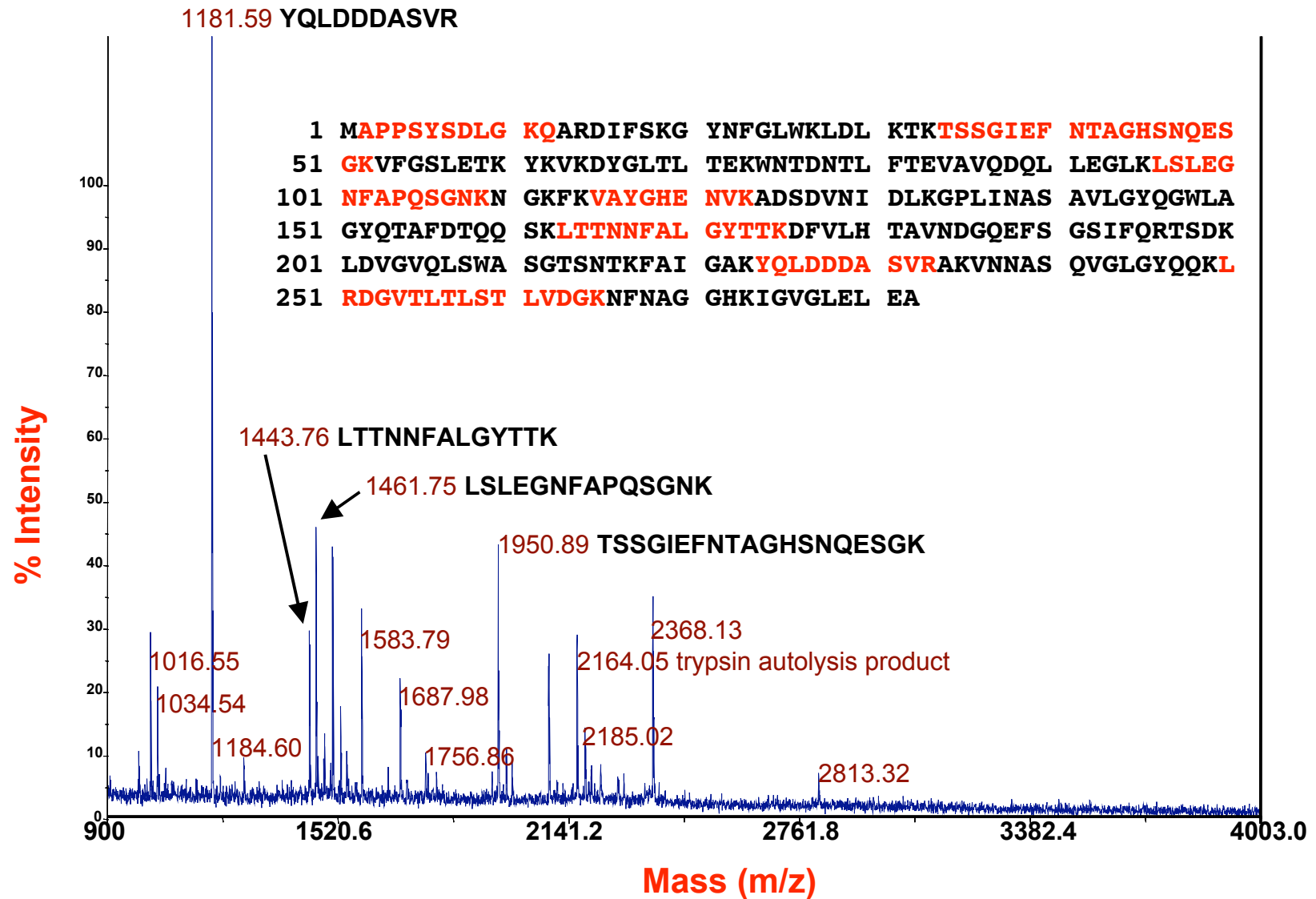


Desalting Ziptip



Mass (m/z)

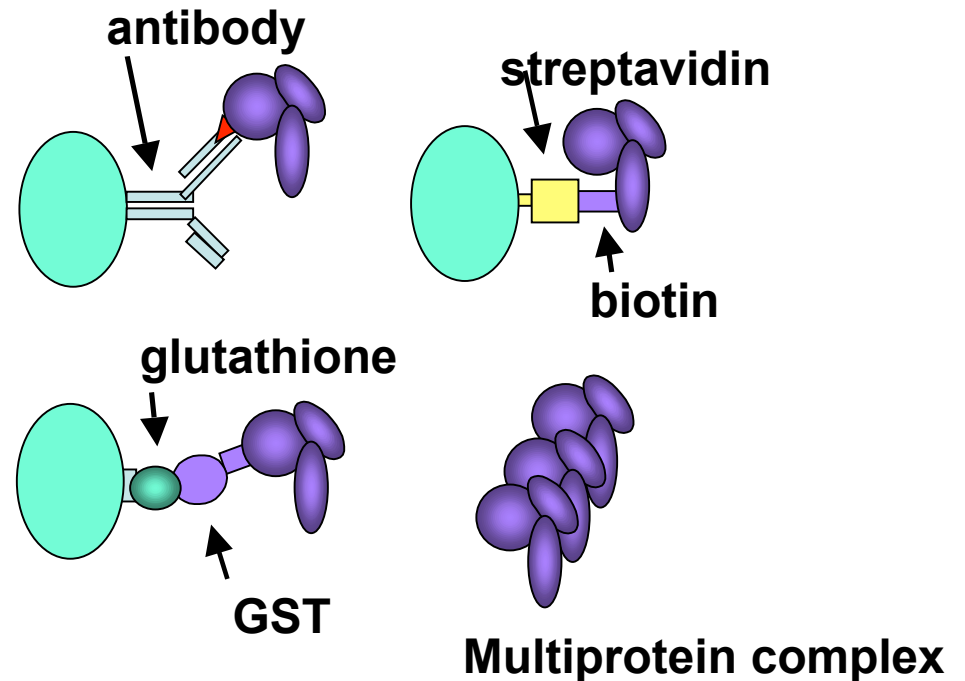
Tryptic mass fingerprint of a porin from *Drosophila*



Other Applications of Mass Spec in Cancer Research

Mapping the protein:protein
network by antibody or
affinity isolation

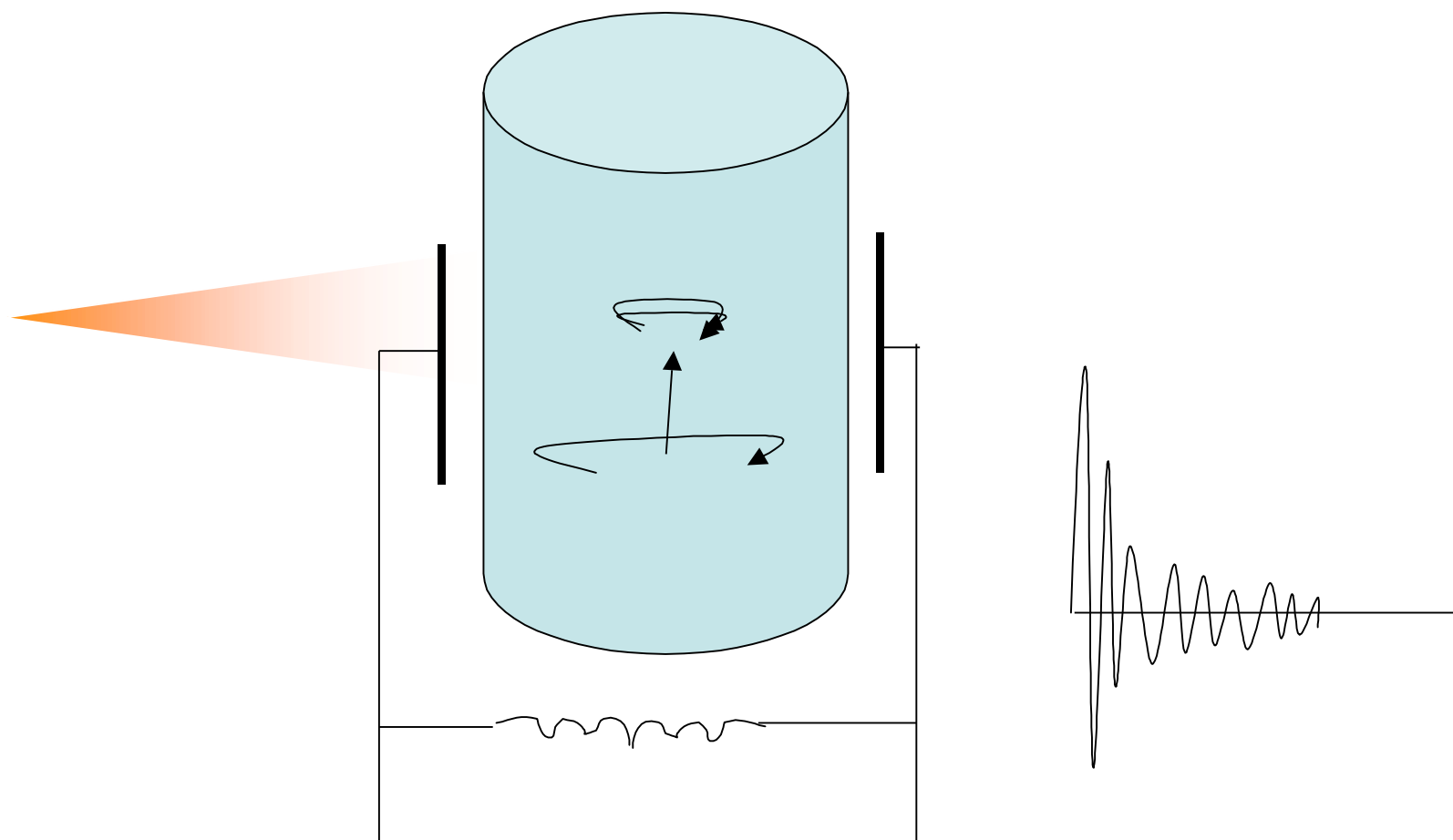
- Reproducibly sub-dividing the proteome in meaningful ways - cell-to-cell (see [Pierre Chaurand's talk this afternoon](#))
- or within cells (see [Huang-Ge Zhang's work on the composition of the exosome](#))



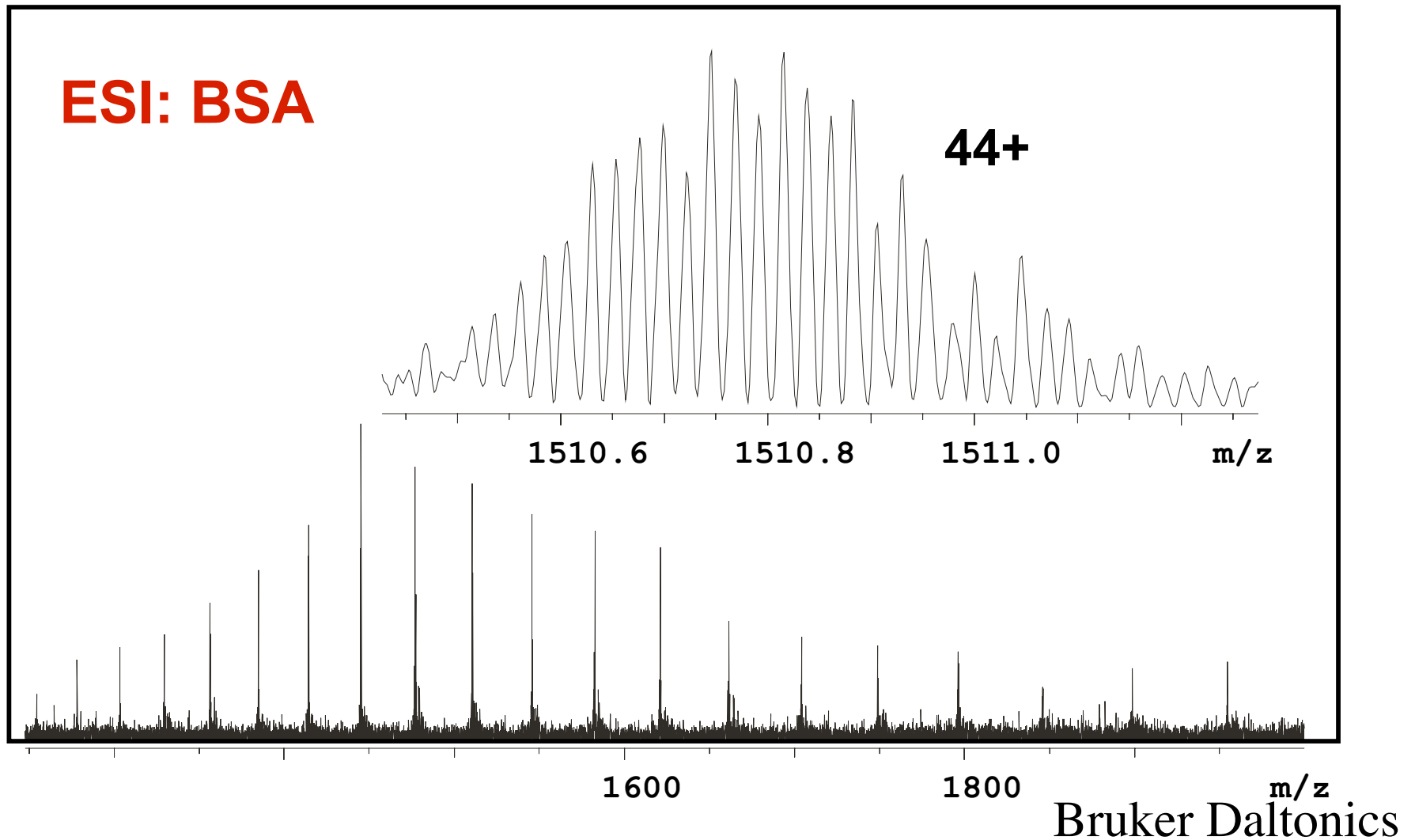
Emerging or emerged mass spec methods in proteomics

TOF-TOF	High speed peptide sequencing - some limitations because of light sensitivity
MUDPIT	Automated proteomics based on 2D-LC tandem mass spectrometry
H/D exchange	An alternative to NMR for the study of protein structure in solution
LC-MRM-MS	An old method revamped for quantitation in proteomics
FT-ICR-MS	An exotic method whose time has come

Detection in the FT-ICR cell

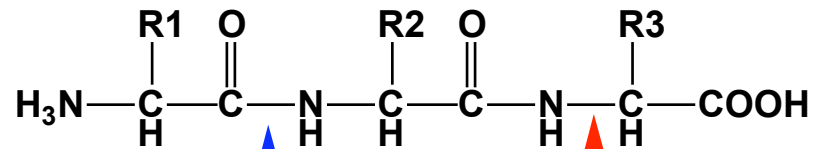
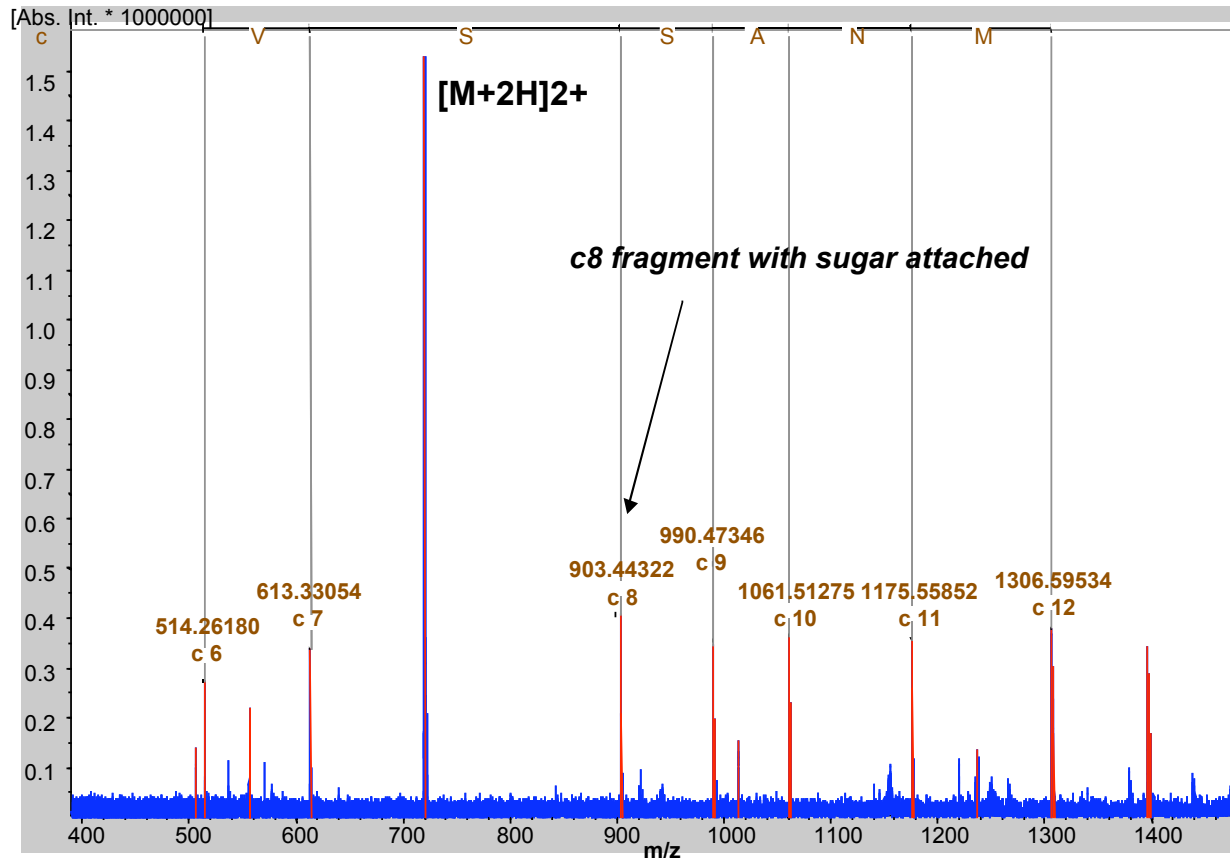


Bovine Serum Albumin (66 kDa) 4.7 T Act. Shielded Magnet



Sequencing O-GlcNAc peptides by ECD FT-ICR-MS

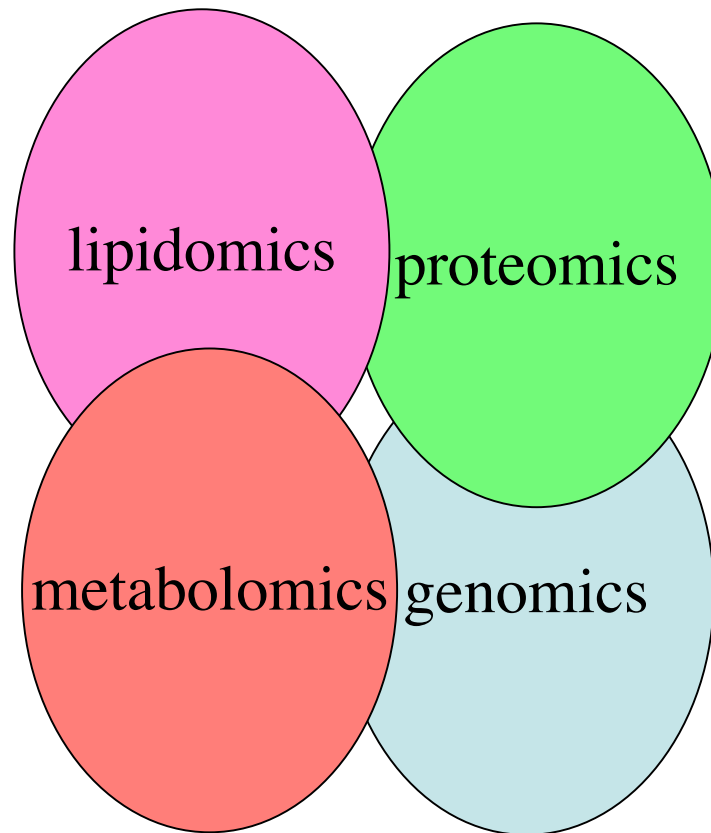
Casein kinase II - AGGSTPVSSANMSG



b ion cleavage

c ion cleavage

The future of NIH research

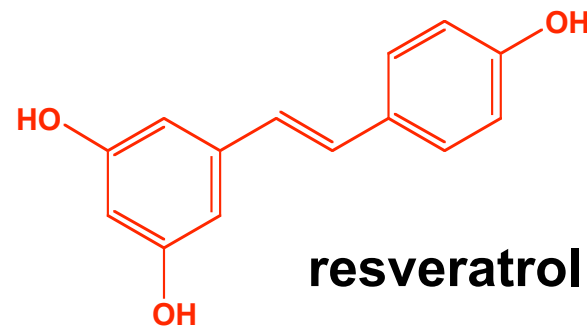
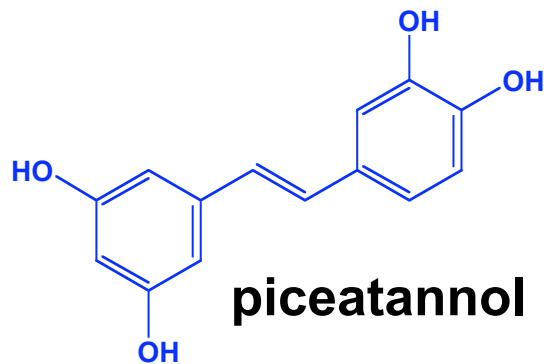


Integration for life extension in yeasts

Caloric restriction increases yeast life span - dependent on the SIR-2 gene (a sirtuin) - a NAD⁺-dependent deacetylase - calorie restriction increases NAD⁺

Human equivalent SIRT1 - deacetylates p53 (used K382)

Sinclair et al. screened a library of compounds - quercetin and piceatannol emerged as activators of deacetylase



Resveratrol increased life span of the yeast by 70% in the absence of caloric restriction, decreased p53 K382 acetylation, and reduced the frequency of repetitive DNA recombination

Conclusions

- **Mass spectrometry and proteomics are good partners (aided by the genome effort and computers)**
- **MALDI identification of proteins from 1D- and 2D-gels is routine (and available in the Cancer Center - see <http://www.uab.edu/proteomics>)**
- **Mass spectrometry can probe protein:protein interactions and protein structure**
- **Mass spectrometry is poised for the question of quantitation in proteomics, the prelude to integration of data from several dimensions (and consistent with the roadmap espoused by NIH Director, Dr. Zerhouni)**

Acknowledgments

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David
Rittenberg
1906-1970