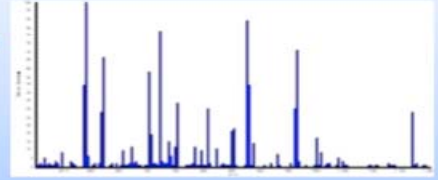




Mass spectrometry Core Lab KUMed Scientific report 2010



Mass spectrometry (MS) is a well-established technique that historically has been important for characterizing small molecules; however, rapid advances since the late 1980's have made MS applicable to large biomolecules, such as proteins, nucleic acids, and their complexes. Today, MS plays an important role in the health sciences and is an integral part of the proteomics and drug discovery processes. In addition, MS can provide relevant information about the structure and dynamics of proteins and their complexes.

The key to forming gas-phase ions from large molecules, a prerequisite for MS analysis, lies in the development of electrospray ionization (ESI) (Fenn, 1989) and matrix-assisted laser desorption/ionization (MALDI) (Hillenkamp, 1991; Nakanishi, 1994). With these ionization techniques, MS has been increasingly applied to the analysis of complex biological systems, providing biological information in the form of molecular mass. The success of MS-based proteomics is the result of advances in other areas, such as the design of improved mass spectrometers, the development of alternative fragmentation methods, the availability of protein and genomic databases, the development of new computer algorithms that can use MS information to interrogate these databases for protein and peptide identification, and the application of new chemistries to obtain quantitative information of a protein present in very complex mixtures

The interdisciplinary nature of MS and the cost of acquisition and operation of modern mass spectrometers require specialized laboratories, and today MS laboratories specialized in proteomics analysis are common in all major scientific research institutions. The MS/Proteomics Core Laboratory (MSPCL) of KUMC was established in 2004 as a research unit to provide technical support and expertise for protein research. Since then, members of this facility, some times in collaboration with other labs, have established a diverse array of methods for the analysis of proteins, protein complexes and protein structure and dynamics.

Equipment: The MSPCL Laboratory operates high end mass spectrometers: a hybrid electrospray Ion Trap/IonCyclotron Resonance Fourier Transform (LTQ FT), a triple quadrupole (Quantum AM), both from ThermoFinnigan, and a MALDI TOF TOF (4700 proteomics analyzer, Applied Biosystems). In addition, the MSPCL is equipped with automatic equipment for sample analysis and processing: a Typhoon scanner, a gel cutter, in gel digester and MALDI spotter. A variety of software tools and computer equipment is available for analyses of the large and complex data sets collected on the diverse instrument operated at the facility. This includes a dedicated computer cluster to perform protein identification using Sequest, Mascot, Peaks, X! Tandem and OMSSA algorithms are also available, as well as the PPT from the Systems Biology Institute.



The LTQ Rigged for HD MS

Methodologies. The facility has established standard methodologies for protein identification from SDS-PAGE, 2D PAGE or from simple to very complex protein mixtures in solution using a variety of chromatographic methods. Information on the applications of the facility can be found on the lab web pages (www.kumc.edu/mspc). In addition to protein identification, other applications include analysis of protein posttranslational modifications, protein-protein interactions and protein dynamics by a combination of deuterium/hydrogen exchange and mass spectrometry (DH MS).



The Nobel Prize in Chemistry 2002 John B. Fenn, Koichi Tanaka, Kurt Wüthrich



John B. Fenn



Koichi Tanaka



Kurt Wüthrich

The Nobel Prize in Chemistry 2002 was awarded "for the development of methods for identification and structure analyses of biological macromolecules" with one half jointly to John B. Fenn and Koichi Tanaka "for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules" and the other half to Kurt Wüthrich "for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution".

"The Nobel Prize in Chemistry 2002". Nobelprize.org. 21 Mar 2011
[Http://nobelprize.org/nobel_prizes/chemistry/laureates/2002](http://nobelprize.org/nobel_prizes/chemistry/laureates/2002)

Method development. Some specialized applications require the development of instruments and computer software. This includes an innovative pressure-loading device with a transparent chamber for sample loading. For the analysis of protein dynamics we have designed an efficient cooling box to reduce the back exchange of deuterium during analysis, and we are in the process of implementing a set of web-based computer applications to assist in the analysis of the highly complex set of data generated by DH MS.

Functions of the Mass Spectrometry Core Lab	
Maintain and operate instrumentation	<ul style="list-style-type: none"> • Identification of gel-separated proteins by MS • Identification of proteins in complex mixtures • Mass measurements of peptides and protein
Research projects	<ul style="list-style-type: none"> • Discuss the project and • Find application to specific biological problems, or • Develop a mass spectrometry procedure
Educational	<ul style="list-style-type: none"> • Workshops, teaching • Techniques, possibilities & limitations of proteomics approaches

Projects/Service. Since its establishment, the MSPCL has contributed to the success of many research projects. Among others, we have applied proteomic methodologies to: identifying proteins in the arteriosclerotic plaque (H.H. Hsu, Pathology), characterizing protein dynamics and allosteric regulation of pyruvate kinase (A. Fenton, Biochemistry), differential protein expression during fetal organ damage by hypoxia, (Y. Dong, Gynecology), differential protein expression in small and large islets of Langerhans (I. Smirnova, Physical Therapy), structural proteomics of phosphorylase kinase (G. Carlson and O. Nadeau, Biochemistry), protein folding of aspartate aminotransferase isoenzymes (A. Artigues, Biochemistry), mitochondrial protein modification by xenobiotic agents in liver toxicity (A. Cooper, Biochemistry, NY Medical College and H. Jaeschke, Pharmacology), and analysis of protein phosphorylation of Gads (T. Yankee, Microbiology). We are currently developing methodologies for protein quantification utilizing a variety of labeling agents.

Training. Since 2008 a series of workshops has been presented to introduce the diverse applications of this methodology and to discuss specific applications/needs of the researchers primarily at KUMC. The next workshop to take place this year will be announced on the lab web pages (www.kumc.edu/mspc/seminars). Stay tuned! If you are interested in specific MS-based proteomics methodologies, please contact us for their inclusion in our workshop series.

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Recent Publications:

- Jeyasingham, M.D., Artigues, A., Nadeau, O.W. and Carlson, G.M. (2008). Structural evidence for co-evolution of the regulation of contraction and energy production in skeletal muscle. *J. Mol. Biol.* 377, 623-629. PMID: PMC2293304. [Link](#)
- Hsu, H.H., Artigues, A. and Villar, M.T. (2008). Induction of calcification by serum depletion in cell culture: a model for focal calcification in aortas related to atherosclerosis. *Lipids Health Dis.* 7, 2. PMID: PMC2248577. [Link](#)
- Nadeau, O.W., Wyckoff, G.J., Paschall, J.E., Artigues, A., Sage, J., Villar, M.T., and Carlson, G.M. (2008). CrossSearch, a user-friendly search engine for detecting chemically cross-linked peptides in conjugated proteins. *Mol. Cell. Proteomics* 7, 739-749. PMID: PMC2401330. [Link](#)
- Boulatnikov, I.G., Nadeau, O.W., Daniels, P.J., Sage, J.M., Jeyasingham, M.D., Villar, M.T., Artigues, A. and Carlson, G.M. (2008). The regulatory beta subunit of phosphorylase kinase interacts with glyceraldehyde-3-phosphate dehydrogenase. *Biochemistry* 47, 7228-7236. PMID 18549242 [Link](#)
- C. Saito, H-M Yan, A. Artigues, M.T. Villar, A. Farhood and H. Jaeschke(2010): Mechanism of protection by metallothionein against acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol.* 242, 182-190. PMID: PMC2789886. [Link](#)
- Cooper AJ, Krasnikov BF, Niatsetskaya ZV, Pinto JT, Callery PS, Villar MT, Artigues A, Bruschi SA. (2010): Cysteine S-conjugate beta-lyases: important roles in the metabolism of naturally occurring sulfur and selenium-containing compounds, xenobiotics and anticancer agents. *Amino Acids.* PMID: PMC2898922. [Link](#)

References:

- Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989). Electrospray ionization for mass spectrometry of large biomolecules. *Science.* 246(4926), 64-71
- Hillenkamp F, Karas M, Beavis RC, Chait BT. (1991) Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers. *Anal Chem.* 63(24), 1193A-1203A.
- Nakanishi T, Okamoto N, Tanaka K, Shimizu A. (1994) Laser desorption time-of-flight mass spectrometric analysis of transferrin precipitated with antiserum: a unique simple method to identify molecular weight variants. *Biol. Mass. Spectrom.* 4, 230-233.

√ John Fenn, who received the Nobel Prize in Chemistry in 2002 for his innovations in Electrospray Ionization, passed away in December last year at the age of 93. According to one* who knew him well, "John was a visionary person - intelligent, driven, and a very warm human being. After 2002, John made himself very available to the scientific community even though he was 85-years old when he received the Nobel Prize. He traveled around the world at nearly every invitation that he received to speak. He was a gentleman, a scholar, a role-model, a leader and always had the time to talk with anyone and everyone about his love for science and life. With his strong sense of humility, John inspired those around him as a mentor and friend. He will be deeply missed."

*From remarks by Scott A. McLuckey (Wetherill Laboratory)