



Albert Einstein College of Medicine  
OF YESHIVA UNIVERSITY

# PROTEOMICS SHARED FACILITY NEWSLETTER

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**L M A P**  
LABORATORY FOR  
MACROMOLECULAR  
ANALYSIS &  
PROTEOMICS



Ruth Hogue Angeletti, Ph.D.  
Scientific Director

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## MESSAGE FROM THE DIRECTOR: RUTH HOGUE ANGELETTI, PH.D.

The quality of results from proteomics experiments is of utmost importance to the LMAP and this is why the LMAP encourages discussions in the early stages of experimental design to ensure the best

data acquisition and analysis for your project. We hope that these newsletters will assist the Einstein community to keep abreast of the types of proteomic experiments their colleagues are per-

forming within the LMAP and help increase understanding of proteomics, leading to mass spectrometric results of exceptional quality.

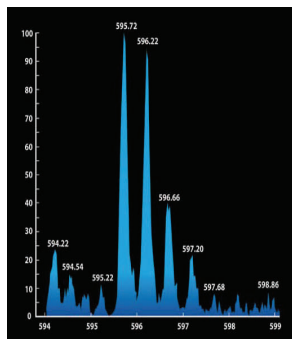
## LMAP UPDATE PROTEIN ID: IMPROVED TURNOVER - REDUCED COSTS!

The LMAP is an Einstein resource center providing mass spectrometric, chromatographic & gel services together with training, consulting, and collaborations. Our goal is to use our resources to assist the Einstein community with their proteomic research. After consulting with Kathy Stone, Co-Director of Mass Spectrometry Services at Yale's Biotechnology Resource Laboratory, the LMAP has incorporated improvements for protein identification, specifically the use of robotics for automation. This will allow us to decrease the turn-

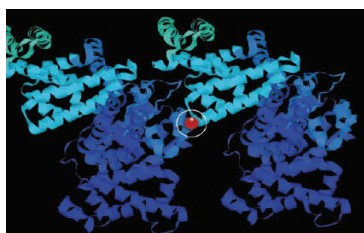
over-time for protein identification (one week or less) and substantially lower the cost (\$50 total per gel band/spot). This automation will allow rapid identification of the most abundant proteins identified in a gel band/spot. In most cases this will be sufficient. If further data analysis is required an extra charge will be included. Controls to check for contamination during sample handling on our end and for sensitivity will be included. If no protein is identified from the gel spot/band there will be no charge. However, if client-introduced contami-

nating proteins are detected, the \$50 charge per sample/spot will still apply. An additional cost saving feature is available for those performing their own digestion. In these cases the LMAP can provide a MALDI target for the samples to be spotted. Data acquisition and protein identification will be usually performed within 48-72 hours. These improvements are to be incorporated starting April 2009.

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ESI-MS was utilized to identify crosslink peptides in a protein complex (above). In order to find which proteins these linked peptides belong to, researchers map the peptides against a library consisting of all possible protein combinations. Once the interacting proteins are identified, a three-dimensional model of the protein complex can be created (below). This model shows two homodimeric proteins crosslinked at a contact point (below, red circle).



## SERVICES AND CONTACT INFORMATION

The LMAP offers access to analytical technologies on a fee-for-service basis. If protein ID, or any other type of proteomic, mass spectrometric or analytical experiment is required by your laboratory, please contact us to discuss your

project, so that we can work together as a team to achieve results. Contact the LMAP Administrator at X 2218, for answers to questions and/or to schedule a meeting to discuss your project.

Contact us via Email at [imap@aecom.yu.edu](mailto:imap@aecom.yu.edu).

Visit our web page, [www.aecom.yu.edu/imap](http://www.aecom.yu.edu/imap) for further information & costs.

## PROTEOMICS AT EINSTEIN— PROTEIN ID AND POST-TRANSLATIONAL MODIFICATION

Mass spectrometry's most important contribution to the field of proteomics is its ability to detect low quantities of peptides (down to attomoles or  $10^{-18}$  moles) with high specificity. The mass spectrometer not only detects peptides but also other components that ionize easily and may be present in the protein or peptide sample. These components include reagents that are normally used during protein/peptide purification and which can partially or completely mask the desired signal from the protein or peptide samples (such as salts, detergents or chaotropic agents). This is one reason why we request detailed information in our Sample Information Form. The information provided will assist us to obtain an accurate molecular mass measurement by using the appropriate sample clean-up method for your particular sample. In some cases we can suggest the use of "compatible" solvents, buffers, salts or deter-

gents that can be used with mass spectrometry. A recent LMAP publication describes the removal of detergents from protein digests prior to MS analysis (Anal. Biochem.; 2008 Nov 15; 382(2), 135-7). So please provide us with as much information on sample preparation as you can.

Post-translational modifications (PTMs) of proteins can also be identified using mass spectrometry based proteomic methods. One PTM of special interest is phosphorylation. Identification of PTMs of proteins using mass spectrometry is not trivial. One review highlights some of the current strategies used for PTM identification by mass spectrometry (Witz, et.al., Mapping Protein Post-translational Modifications with Mass Spectrometry; Nature Methods; Vol.4 No.10; October 2007 pp.798-806). This review also contains a flow chart describing the mass shifts observed for phosphorylation, acetylation, cysteine oxidation,

cysteine nitrosylation and ubiquitination along with recommended enrichment strategies. Many of the strategies discussed in this review can be performed with the mass spectrometers available within the LMAP.

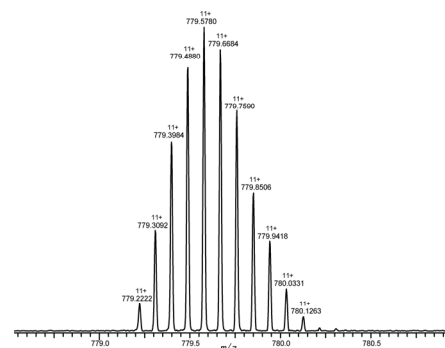
At Einstein, the identification of protein phosphorylation sites with mass spectrometry has been carried out in several laboratories. One example is in the laboratory of Dr. A. Wolkoff, where the sites of phosphorylation of the organic anion transporting protein were determined with tandem mass spectrometry. Another example is found in the laboratory of Dr. E.R. Stanley, where some phosphorylation and ubiquitination sites of the CSF-1 receptor have been confirmed or determined.

Some aspects of proteomics are routine while others, such as PTMs, are more challenging. Our goal is to overcome these challenges and we can do so by working with you to render your analysis practical.

## L MAP INSTRUMENTS

- Typhoon 9400 - a highly sensitive gel imager
- Ettan Spot Picker - a robotic gel spot picker
- Ettan Digester - a robotic instrument for performing in-gel digestion
- 4800 MALDI-tof/tof – replaces the 4700 MALDI-tof/tof providing better sensitivity and long-term stability (a problem with the 4700 when operating continuously for over 2 days)\*
- An FT-ICR mass spectrometer
- A DE-STR MALDI-tof mass spectrometer
- For ESI (electrospray ionization) the LCQ, LTQ and QSTAR
- Other available instruments are a variety of HPLC for analytical, cap, micro and nano flow rates

\* We are grateful to the Einstein Administration for funding this replacement



ESI Ultra High Resolution Measurement

## RECENT PUBLICATIONS

Basu J, Romney SL, Angeletti RH, Vermund SH, Nieves E, Kadish AS, Mikhail MS, Orr GA. Human Immunodeficiency Virus (HIV) Antigens and RNA in HIV-Seronegative Women with Cervical Intraepithelial Neoplasia. [AIDS Res Hum Retroviruses. 2009 Mar 17.](#)

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Hugonnet JE, Tremblay LW, Boshoff HI, Barry CE 3rd, Blanchard JS. Meropenem-clavulanate is effective against extensively drug-resistant Mycobacterium tuberculosis. [Science. 2009 Feb 27;323\(5918\):1215-8.](#)

Verdier-Pinard P, Pasquier E, Xiao H, Burd B, Villard C, Lafitte D, Miller LM, Angeletti RH, Horwitz SB, Braguer D. Tubulin proteomics: towards breaking the code. [Anal Biochem. 2009 Jan 15;384\(2\):197-206.](#)

Madrid-Aliste CJ, Dybas JM, Hogue Angeletti R, Weiss LM, Kim K, Simon I, Fiser A. EPIC-DB: a proteomics database for studying Apicomplexan organisms. [BMC Genomics. 2009 Jan 21;10\(1\):38.](#)

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Du P, Stolovitzky G, Horvatovich P, Bischoff R, Lim J, Suits F. A noise model for mass spectrometry based proteomics. [Bioinformatics. 2008 Apr 15;24\(8\):1070-7.](#)

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Cao E, Zang X, Ramagopal UA, Mukhopadhyaya A, Fedorov A, Fedorov E, Zencheck WD, Lary JW, Cole JL, Deng H, Xiao H, DiIorenzo TP, Allison JP, Nathenson SG, Almo SC. T cell immunoglobulin mucin-3 crystal structure reveals a galectin-9-independent ligand-binding surface. [Immunity. 2007 Mar;26\(3\):311-21.](#)

Hugonnet JE, Blanchard JS. Irreversible inhibition of the Mycobacterium tuberculosis beta-lactamase by clavulanate. [Biochemistry. 2007 Oct 30;46\(43\):11998-2004.](#)



4800 MALDI tof/tof mass spectrometer

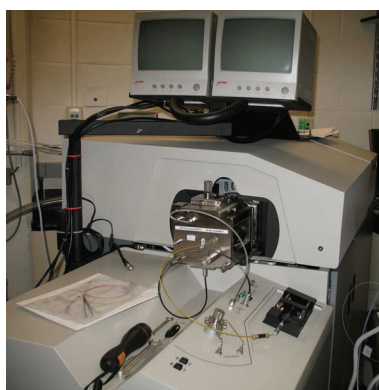
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submitting samples & cost.](#)



LMAP QSTAR Pulsar i ESI mass spectrometer

## RECENT PUBLICATIONS- CONTINUED

Nacharaju P, Friedman JM, Prabhakaran M, Acharya SA, Manjula BN. Combining the influence of two low O(2) affinity-inducing chemical modifications of the central cavity of hemoglobin. [Biochemistry. 2007 Apr 17;46\(15\):4554-64.](#)

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Che FY, Zhang X, Berezniuk I, Callaway M, Lim J, Fricker LD. Optimization of neuropeptide extraction from the mouse hypothalamus. [J Proteome Res. 2007 Dec;6\(12\):4667-76.](#)

Fricker LD. Neuropeptidomics to study peptide processing in animal models of obesity. [Endocrinology. 2007 Sep;148\(9\):4185-90](#)

Luo Q, Siconolfi-Baez L, Annamaneni P, Bielawski MT, Novikoff PM, Angeletti RH. Altered protein expression at early-stage rat hepatic neoplasia. [Am J Physiol Gastrointest Liver Physiol. 2007 May;292\(5\):G1272-82](#)

Sudha R, Kawachi N, Du P, Nieves E, Belbin TJ, Negassa A, Angeletti RH, Prystowsky MB. Global proteomic analysis distinguishes biologic differences in head and neck squamous carcinoma. [Lab Invest. 2007 Aug;87\(8\):755-66](#)

McFadden DC, Fries BC, Wang F, Casadevall A. Capsule structural heterogeneity and antigenic variation in *Cryptococcus neoformans*. [Eukaryot Cell. 2007 Aug;6\(8\):1464-73](#)

Sturm MB, Roday S, Schramm VL. Circular DNA and DNA/RNA hybrid molecules as scaffolds for ricin inhibitor design. [J Am Chem Soc. 2007 May 2;129\(17\):5544-50](#)

Du P, Sudha R, Prystowsky MB, Angeletti RH. Data reduction of isotope-resolved LC-MS spectra. [Bioinformatics. 2007 Jun 1;23\(11\):1394-400.](#)

Puan KJ, Jin C, Wang H, Sarikonda G, Raker AM, Lee HK, Samuelson MI, Märker-Hermann E, Pasa-Tolic L, Nieves E, Giner JL, Kuzuyama T, Morita CT. Preferential recognition of a microbial metabolite by human  $\gamma$ 2Vdelta2 T cells. [Int Immunol. 2007 May;19\(5\):657-73.](#)

Proteomics: Power To The Proteins, by Larry Katzenstein: [The Winter 2008 edition of the Einstein Edge](#) describing the Albert Einstein Biodefense Proteomics Research Center. This article discusses how scientists with different scientific backgrounds overcame the challenges of using proteomics to identify and characterize proteins of *T. gondii* and *C. parvum*.

## NEWSLETTER TUTORIAL: MASS SPECTROMETRY'S ROLE IN PROTEOMICS

In our last tutorial ([September 2007 Newsletter](#)) we discussed resolving power and charge state determination. In this tutorial we will briefly discuss the time-of-flight analyzer, one of the most common and simplest analyzer used in proteomics.

A mass spectrometer's main component is the analyzer, where the actual separation of gaseous ions, mainly positive ions, occurs according to their mass/charge ( $m/z$ ) ratio. There are many different types of analyzers used in mass spectrometry. In proteomics however, the analyzers utilized are mainly time-of-flight (tof), ion-trap, a quadrupole and a quadrupole ion-trap combination, ion cyclotron resonance (ICR) and a new analyzer, the OrbiTrap. Each type of analyzer has its unique advantages and disadvantages. It is important to match the analyzer with the experimental goals of the project, thus enabling one to take full advantage of the analyzer's best properties. Let us first look at one type of MS analyzer used by the LMAP and its role in mass accuracy.

The MALDI mass spectrometers, DE-STR & 4800, utilize the time-of-flight (tof) analyzer. Briefly, all ions are accelerated into the tof analyzer having the same kinetic energy (which is equal to the applied accelerating voltage, typically 20,000 volts) and allowed to separate (drift) according to their  $m/z$  ratio within the flight tube/path. Ions with lighter masses will reach the detector before the heavier ions according to this formula:

$$t^2 = \frac{d^2}{2U} * \frac{m}{z}$$

$t$  – ion's drift time in seconds (s)  
 $d$  – drift/flight path length in meters (m)  
 $U$  – accelerating voltage applied to the ion (volts)  
 $m$  – ion's mass in kilograms (kg)  
 $z$  – ion's charge or  $1.602 \times 10^{-19}$  coulomb (C; note that this ion/particle will be accelerated and thus have units of  $\text{kg m}^2/\text{volts s}^2$  and have a +1 charge that is typical for MALDI-tof)

Therefore, the flight/drift time for an ion is proportional to the square root of its mass. For a positive ion with the amino acid sequence DRVYIHPFHL (angiotensin I), the predicted monoisotopic protonated mass  $[M+H]^+$  is 1296.6848 Da (or  $1296.6848 \text{ Da} * 1.672621 \times 10^{-27} \text{ kg/Da} = 2.169 \times 10^{-24} \text{ kg}$ ). If one were to measure this peptide using the 4800 MALDI-tof mass spectrometer the predicted time of flight/drift would be about 50 microseconds (the 4800 consists of a 2.7 m flight path and this ion would experience a 20,000 acceleration voltage potential).

$$t^2 = \frac{(2.7m)^2}{2(20,000 \text{ volts})} * \frac{(1296.6848 \text{ Da} * 1.672621 \times 10^{-27} \text{ kg/Da})}{1 * 1.602 \times 10^{-19} \text{ C}}$$

$$t^2 = 2.467 \times 10^{-9} \text{ s}^2$$

$$t = 49.67 \mu\text{s}$$

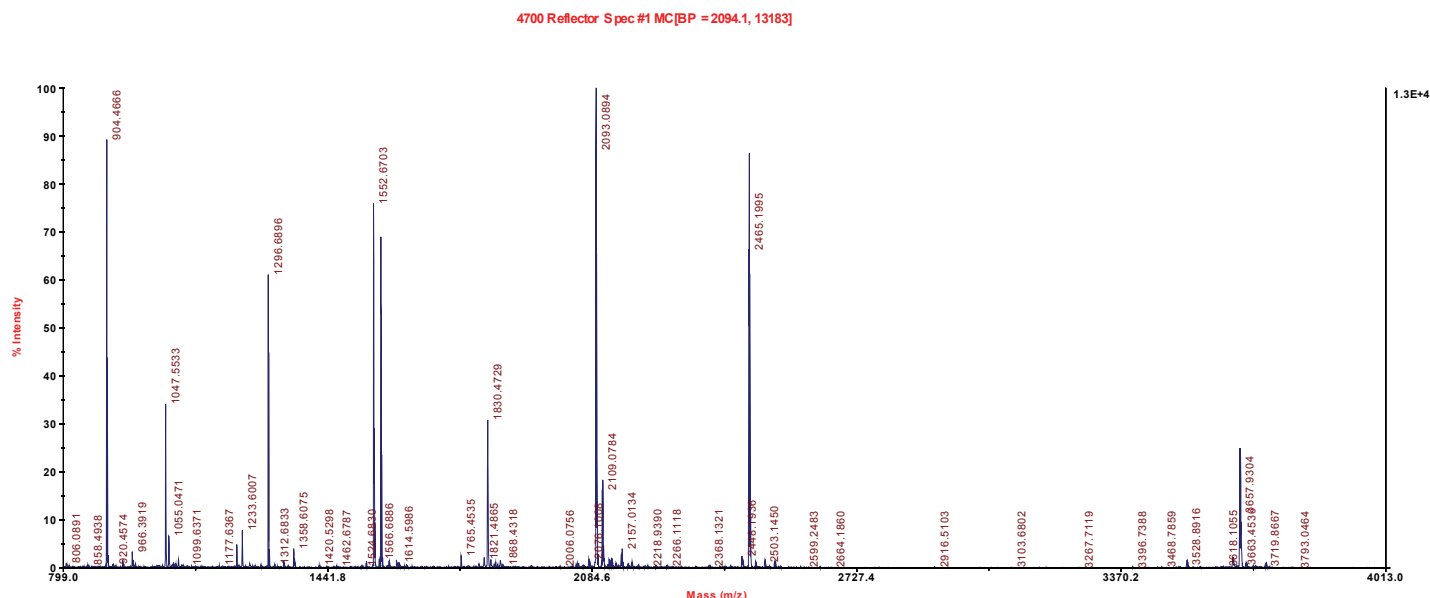


Figure 1: MALDI-tof mass spectrum of a known calibration mixture including angiotensin I, 1296.6896 m/z predicted to have a flight time of 49.67 μs. The low intensity ion at m/z 1312.6833 predicted flight time is 50.00 μs or a difference of 0.33 μs.

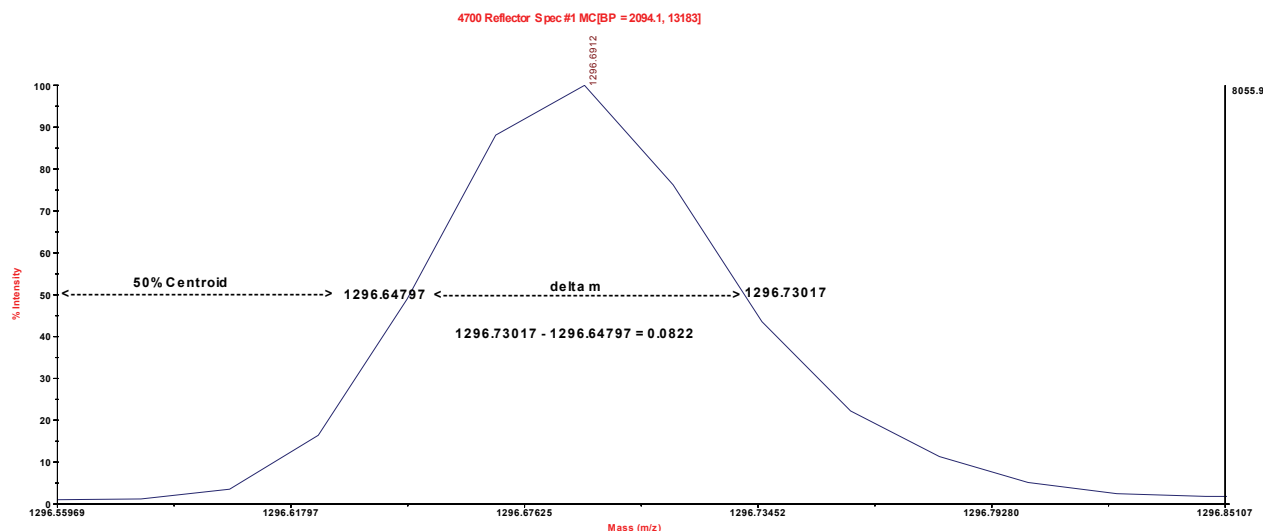


Figure 2: MALDI-tof mass spectrum from Figure 1 displaying the  $m/z$  range only from 1296.55972 to 1296.85111  $m/z$ .

Resolving power is defined as the ability to distinguish between ions differing in the quotient mass/charge by a small increment and can be calculated as  $m/\Delta m$ . Typically for MALDI-tof, the resolution is calculated using  $\Delta m$  at 50% centroid or FWHM (full width at half maximum). The calculated FWHM resolution for angiotensin I is  $1296.6896 / (\Delta m = 1296.73017 - 1296.64797 \text{ or } 0.0822) = 15,775$  (Figure 2). The peak shape for angiotensin I is not symmetrical and if the ion was labeled at its apex rather than its centroid it would be 1296.6912  $m/z$ , a difference of 0.0016  $m/z$  or 1.2 ppm (parts per million). A hundred fold  $m/z$  difference, 0.16  $m/z$  or 120 ppm, would be too high an error in mass accuracy. This example highlights the importance of mass accuracy and resolution in mass spectrometry. Our next tutorial will discuss mass accuracy and resolution in regards to proteomics.

In closing I want to quote from a section of the Journal of The American Society Mass Spectrometry's guidelines for authors:

“The Use of High Resolution Mass Spectral Data for Formula Confirmation

For publication of exact-mass data that are used to confirm identities of synthetic and natural products, report the uncertainty in the accurate-mass measurement used for formula verification along with the result. The acceptable uncertainty in a measurement by any analytical method must be adequate for the intended use of the data.

Evaluate the uncertainty of accurate mass measurement by any statistically valid method. Determine, for example, the precision and accuracy of replicate measurements or evaluate the performance characteristics of the mass spectrometer (see, for example, Sack, T. M., Lapp, R. L., Gross, M. L., and Kimble, B. J. *Int. J. Mass Spectrom. Ion Proc.* 1984, 61, 191-213). Consider all candidates fitting the experimentally determined value and its reported uncertainty when the result of accurate mass measurement is used for formula confirmation. Do not set fixed acceptable error limits for exact mass measurement. Consider the following when using accurate mass data.

When valence rules and candidate compositions encompassing  $C_{0-100}$ ,  $H_{3-74}$ ,  $O_{0-4}$ , and  $N_{0-4}$  are considered at nominal parent  $m/z$  of 118, there are no candidate formulae within 34 ppm of each other. When the ion is of  $m/z$  750.4 and the formulae are in the range  $C_{0-100}$ ,  $H_{25-110}$ ,  $O_{0-15}$ , and  $N_{0-15}$ , there are 626 candidate formulae that are possible within 5 ppm. Thus, for a measurement at  $m/z$  118, an error of only 34 ppm uniquely defines a particular formula, whereas at  $m/z$  750, an error (and precision) of 0.018 ppm would be required to eliminate all extraneous possibilities.”

Eddie Nieves