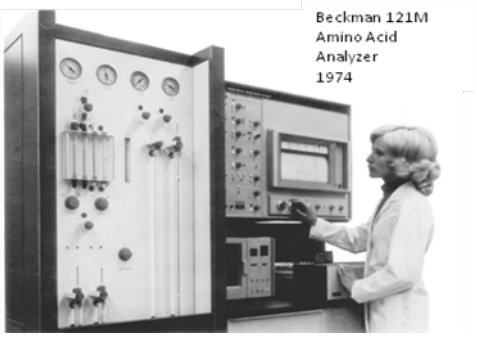
# History of the Keck MS & Proteomics Resource

The current W.M. Keck Foundation Biotechnology MS & Proteomics Resource Laboratory originated from the expertise and long history of both the Yale Protein Chemistry Facility and the Yale Mass Spectrometry Resource. The Yale Protein Chemistry Facility was established by Dr. Kenneth R. Williams (currently a Professor (Adjunct) of Research in Molecular Biophysics and Biochemistry and Director of the W.M. Keck Biotechnology Resource Laboratory) in 1980. His vision was to bring cutting edge biotechnology to all investigators, both at Yale and outside the University. (1) The facility was based on a single instrument, the Beckman 121M Amino Acid analyzer (Picture on right), which at the time, was considered cutting edge.




The history of mass spectrometry in the Yale Medical School has its origin in 1965 in the laboratory of Dr. Sandy Lipsky in the Department of Medicine. Professor Lipsky was an expert in gas chromatography which he used to separate and analyze complex mixtures of lipids in the gas phase. To facilitate analysis and identification of the lipids, he coupled a mass spectrometer detector to a gas chromatograph – becoming one of the early laboratories to utilize the new technique of gas chromatography-mass spectrometry (GC-MS). At that time this was not an easy technique to implement since it required interfacing a high pressure separation technique (GC) to a high vacuum and high voltage instrument (MS). NASA provided funding to purchase an AEI MS-9 mass spectrometer and Drs. Sandy Lipsky and Walter McMurray (retired Co-Director of the Keck MS Resource) used this instrumentation to analyze Apollo 11 lunar samples. As part of this research, they developed the technique to electrically record rapidly scanned mass spectra. Prior to this advance, spectra were recorded on photographic paper or photographic plates and the masses were determined by manually counting the peaks in the spectrum. Simultaneously with the development of the GC-MS technique, Drs. Lipsky and Csaba Horvath in the Department of Chemical Engineering, Yale University, were developing the technique of High Pressure Liquid Chromatography (HPLC) which is now one of the most widely used techniques for separating and analyzing complex mixtures of proteins, peptides, amino acids, and other biomolecules. Figure 1 shows Walt during his post doctoral years in Dr. Klaus Beimann's lab, along with a state of the art CEC 21-110 high resolution mass spectrometer.

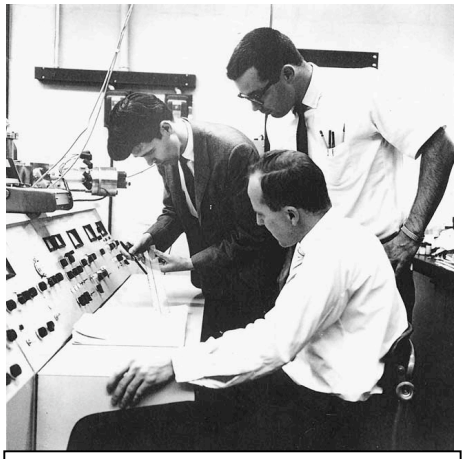


**Figure 1**. Klaus Beimann (standing left) discussing a photographicplate with Walter McMurray and Peter Bommer (seated) in front of the operator panel of the CEC 21-110 high-resolution mass. spectrometer (photo ca. 1982/1983). J Am Soc Mass Spectrom 2002, 13, 1254-1272

The Yale Comprehensive Cancer Center (YCC) Mass Spectrometry Resource was founded in January, 1984 with Dr. Walter McMurray as the Resource Director. At this time the YCC Mass Spectrometry Resource had only a VG 16F single focusing mass spectrometer equipped with a Varian gas chromatograph. Utilizing funding provided by a NIH Shared Instrumentation Grant (SIG) and by the YCC, the MS Resource purchased a VG ZAB-SE. This double focusing mass spectrometer was equipped with gas and liquid chromatographic interfaces. In the early 90's, the YCC Resource was awarded a second SIG which funded the purchase of a VG Quattro triple quadrupole mass spectrometer equipped with an electrospray ion source. Electrospray, developed by Dr. John Fenn and graduate student Craig Whitehouse, Department of Chemical Engineering, Yale University, revolutionized (concurrently with the development elsewhere of

Matrix Assisted Laser Desorption Ionization (MALDI)) the mass spectral analysis of biological molecules - particularly proteins.

In 1993 the Keck Laboratory Mass Spectrometry Resource was founded by Kathryn Stone and was equipped initially with a loaner TofSpec MALDI-MS from Micromass, Inc. which was replaced in 1995 by a research-grade TofSpec SE instrument funded with a joint NIH/NSF Shared Instrumentation Grant (RR09167/BIR9319358). From the late 1980's there had always been close interactions between the YCC Mass Spectrometry Resource and several of the Resources that make up the Keck Laboratory. One nice example of this collaborative effort were the studies these two laboratories carried out to solve the structure of the "unknown" Standard Test Peptide 3 (STP-3) that was specifically designed (*e.g.*, it included a branched structure in which the two branches were of unequal lengths, an acylated epsilon lysine amino group, a disulfide linkage, two methionine residues susceptible to air oxidation, and whose sequence was chosen to be refractory to enzymatic cleavage) to challenge state-of-the-art capabilities of protein chemistry and mass spectrometry laboratories around the world. This sample was distributed to 180 registrants who requested it in conjunction with the 1988 Protein Society Meeting. Together, the YCC Mass Spectrometry and Keck Laboratories produced one of only three correct answers for the structure of this challenging peptide - with these studies being described in a publication by Elliott *et al* (Elliott et al, 1989).

The YCC and Keck Laboratory Mass Spectrometry Resources formally merged in July, 1998 - with the Directors of each Resource (Walt McMurray, and Kathy Stone respectively) becoming Co-Directors of the joint Resource. The first instrumentation grant application submitted by the joint Resource resulted in an award (NSF987111) that was made jointly by the National Science Foundation and the Howard Hughes Medical Institute (HHMI). This award funded the purchase of one of the first quadrupole/time-of-flight mass spectrometers (a Micromass Q-Tof) to be placed in academia. The NIH Shared Instrumentation Grant application (RR015837) submitted in 2001 by the joint Resource resulted in funding that was used towards the purchase of two mass spectrometers. The first system purchased was a Micromass M@LDI-R mass spectrometer that was the initial platform used for high throughput peptide mass-based protein identification. This system included a Micromass MassPrep (Packard Multiprobe II) robot which is highly programmable and is currently still being used to automatically ZipTip serum/plasma and other biological fluids. The M@LDI-R was subsequently upgraded to the M@LDI-L/R configuration (linear/reflectron modes) with funding from HHMI. The second system that was partially funded by this NIH Shared Instrumentation Grant was a very high throughput Sequenom SNP genotyping system that utilized a single base extension/MALDI-MS detection methodology and was capable of analyzing >20,000 genotypes/day.

The Yale/NHLBI Proteomics Center was created within the MS & Proteomics Resource on October 1, 2002 by the awarding of one of 10 NHLBI contracts to Yale University (N01-HV-28186, principal investigators Drs. Kenneth Williams and William Sessa). This contract brought together 21 Yale faculty in 12 departments with highly regarded research programs in vascular biology, hematopoiesis, and hypertension with faculty who are leaders in designing the cell permeable synthetic biomolecule delivery systems that hold enormous promise for developing entirely new strategies for disease treatment. The MS & Proteomics Resource provided state-of-the-art mass spectrometry, protein profiling, and peptide synthesis biotechnology expertise as well as instrumentation in the Keck Laboratory. In addition, the Center has been further supported by Biostatistical and Bioinformatic faculty who are developing new approaches to the study of proteomics and who are experts in building the databases needed to effectively analyze, archive, and interpret the enormous amounts of protein expression data that has been produced by this research. The contract enabled an expansion of the MS & Proteomics Resource, with the addition of an Applied Biosystems QSTAR-XL mass spectrometer equipped with a LC Packings Ultimate HPLC system, and additional staff (Dr. Christopher Colangelo). This mass spectrometry system was set up initially for isotope coded affinity chromatography (ICAT) but has been more heavily utilized for isobaric tagging reagents for absolute and relative quantiation (iTRAQ). Dr. Colangelo obtained his doctorate at the University of Georgia where he helped develop a novel technique for whole cell MALDI analysis which enables the monitoring of protein expression in whole cell bacteria (Easterling, et. al., Anal Chem. 1998, 70, 2704-09). In 2000, Dr. Colangelo was hired by CuraGen Corporation to work in the Advanced Engineering group where he developed

new technologies for both genomic and proteomic analysis, work for which he obtained patent applications in both areas.

In 2002, a Waters Q-Tof Ultima was purchased with funds from Yale University and the Keck MS resource. This instrument at the time was one of the most advanced protein identification instruments available. A Waters Q-Tof Micro was leased in 2003 for lipid analysis and currently is heavily used for analysis of small molecules, intact proteins, oligos and lipids.

Differential gel (fluorescence ) gel electrophoresis or DIGE was established in 2003 with the lease of the GE Healthcare (Amersham) Typhoon 9410 imager, the Ettan Spot Picker, TA Digested, DATtwelve system and the IPGphor. This protein expression technique has been heavily utilized with ~ 220 gels analyzed per year. A Shared Instrumentation Grant (2005) was used to purchase this system. (grant # PAR-05-028)

In the fall of 2003, grant # U54 AI057158 I (Ian Lipkin, Columbia Univ., PI) established this Regional Center of Excellence (RCE) for Biodefense And Emerging Infectious Disease Research, with the Proteomics Core of the Northeast Biodefense Center (NBC) located within the Keck Proteomics Resource. This Center provides a broad range of mass spectrometry, protein chemistry analyses, MS and Edman sequencing, protein profiling, peptide synthesis expertise and analytical capabilities supporting basic science, preclinical and clinical research programs of the 200 faculty in this multi-institutional Northeast Biodefense Center (NBC). Dr. Erol Gulcicek is the PI of the Proteomics Core.

In August, 2004, the Yale/NIDA Neuroproteomics Center was established (grant # 1 P30 DA018343-01) with a theme "Proteomics of Altered Signaling in Addiction" under principal investigators Drs. Kenneth Williams and Angus Nairn. This Center was again located within the Keck MS & Proteomics Resource, and provided for further expansion of the MS staff with the addition of Dr. Erol Gulcicek(currently, Deputy Director of the W.M. Keck Biotechnology Resource and the Director of the Northeast Biodefense Proteomics Core). Erol has been in both mass spectrometry product development and proteomics applications for over 16 years, with 11 years spent at Analytica of Branford, Inc. (founded by Nobel Laureate John B. Fenn and Craig M. Whitehouse) developing ion sources and mass spectrometers. These efforts led to several peer reviewed publications and to the assignment of 6 key US Patents (including one for linear traps) that provide the key technological components of most present day, commercialized mass spectrometers. Before leaving Analytica, (moving to Cellular Genomics, Inc. (CGI)) he was a project manager for Analytica's ESI TOF MS product line. At Cellular Genomics, Inc., Erol lead the biological mass spectrometry applications, with an emphasis on the phosphoproteome.

Drs. Ken Williams and Angus Nairn discuss NIDA Neuroproteomics projects

As an example of the Yale School of Medicine's strong support of the MS & Proteomics Resource, in 2004 they provided an additional 5,470 $ft^2$ of custom-designed laboratory space to house this Resource. This laboratory is located within 1 block of the main medical school at 300 George Street.


In the fall of 2004, con-current with our move into this new lab space, we installed a Bruker 9.4T Apex-Qe Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) . The instrument was purchased with a $1.4 million dollar award from the NIH High End Instrumentation Program (RR17266-01). This ultra-high resolution and high mass accuracy mass spectrometer is equipped with both Electrospray and MALDI ion sources, with IRMPD (InfraRed MultiPhoton Dissociation), CID (Collisional Induced Dissociation), and ECD (Electron Capture Dissociation) MS/MS fragmentation capabilities. This instrument is being used, under Dr. TuKiet Lam's supervision, to carry out several types of analyses including determining exact/accurate masses on a wide range of peptides/proteins and other biomolecules, and for analyzing extremely complex digests of whole cell protein extracts for the purpose of identifying protein and protein post-translation

al modifications. Dr. TuKiet Lam received his doctorate in Dr. Alan Marshall's (Co-Inventor of FT-ICR MS) laboratory at the National High Magnetic Field Laboratory / Florida State University and has researched various aspects of biological applications of FT-ICR instrument for 6 years. The 9.4T FT-ICR MS instrument compliments well with other MS instruments currently in use at the Keck Laboratory and provides an added service to research oriented projects which require multiple experimental protocols to comprehend a biological issue (*i.e.* determining the level of phosphorylation in a lysate mixture, and locating site(s) of phosphorylation of an identified protein).

A 2006 commitment by the Yale School of Medicine provided funds for the leasing of an Applied Biosystems 4700 MALDI Tof/Tof Mass Spectrometer (now upgraded through a private gift to a model 4800 MALDI-Tof/Tof system) for identification of proteins found to be differentially regulated using differential (fluorescence) gel electrophoresis (DIGE). This instrument has made a huge improvement with regards to speed and sensitivity of analysis (versus an LC-MS/MS approach), and has enabled protein identifications at sub-attomol levels. The School made another commitment in 2007 to purchase an Applied Biosystems QSTAR Elite, equipped with a Waters nanoAcquity, to advance and improve the very popular iTRAQ protein profiling analyses.

In July 2007, the Yale Center for Clinical Investigation (YCCI) purchased an Applied Biosystems 4000 QTRAP Mass Spectrometer and a Waters nanoACQUITY UPLC system (located in the Keck Laboratory) through its CTSA funding (CTSA Grant Number UL1 RR024139) for the purpose of developing a new proteomic technology to maximally support NIH's "Bench-to-Bedside" Research. Since acquisition of the instrument we have developed, and implemented "Targeted Proteomics" technology, which utilizes a triple quadrupole mass mass spectrometer to perform targeted protein quantification by efficiently obtaining absolute concentrations of pre-selected protein biomarkers on a larger number of samples. Additionally, in early 2008, the 4000 QTRAP Mass Spectrometer was setup to perform Small Molecule Quantitation service and currently offers this service alongside Targeted Proteomics. YCCI also purchased a second Waters nanoACQUITY system for the front end of a Thermo Scientific LTQ-Orbitrap XL mass spectrometer (N CRR, SIG, RR024617-01, PI: E. Gulcicek) installed in Oct 2007. This latter instrument focuses on phosphopeptide analysis, SILAC, Label Free Quantitation and protein identification from complex mixtures.

MS & Proteomics Staff in 2006 in front of the Bruker 9.4T APEX Qe FT-ICR

The MS and Proteomic resource is working to expand its Targeted Proteomic services and also establish a Mass Spectrometric Tissue Imaging Center to further the Keck MS & Proteomic analyses, making these services available to as many investigators as possible.


1. Stone, K., Bjornson, R.D., Blasko, G., Cofrancesco, R., Carriero, N.J., Colangelo, C., Crawford, J., Crawford, M., DaSilva, N., Deluca, J., Elliott, J., Elliott, M., Flory, P., Folta-Stogniew, E., Gulcicek, E., Kong, Y., Lam, T., Lee, J., Lin, A., LoPresti, M., Mane, S., McMurray, W., Tikhonova, I., Westman, S., Williams, N., Wu, T., Zhao, H. and Williams, K. (2007a) Invited Review. Keck Foundation Biotechnology Resource Laboratory, Yale University, Yale Journal Biology & Medicine 80, 195-211