# Facility for Elemental Microchemical Analysis (FEMCA)

**Annual Report 2007-2008**

**Table of Contents**

- Director’s Report ................................................................. 1
- CSUERPB Core Facility Reporting Table .................................. 7
- FEMCA Overview ................................................................. 10
- FEMCA Laboratory relocation ................................................ 12
- FEMCA Technical Staff Hiring ............................................... 15
- FEMCA Workshops ............................................................... 17
- FEMCA Cal PRISSM Initiative ................................................. 21
- FEMCA PPMS Instrumentation Addition .................................. 23
- FEMCA Publication ............................................................... 24
- FEMCA Grants ................................................................... 25
- FEMCA Student Research Projects ....................................... 27
- FEMCA New Research Projects ........................................... 32
Director’s Report
Overview of FEMCA Activities

Facility for Elemental Micro-Chemical Analysis (FEMCA)
Annual Report 2007-2008
Year established 2005

Director’s Report

As the founder Director of the core Facility for Elemental Micro Chemical Analysis (FEMCA), it gives me great pleasure to submit this annual report on the activities of the facility to the Strategic Planning Council (SPC) of the California State University Program for Education in Biotechnology (CSUPERB).

FEMCA was inaugurated in the late summer of 2005 upon the initial approval of a 2-year $106,000 seed grant by CSUPERB. A third and terminal year of funding was provided by CSUPERB for the 2007-2008 academic year. This report summarizes and highlights some of the accomplishments of FEMCA over the past 12 months.

FEMCA is housed within the Institute for Integrated Research in Materials, Environments and Society (IIRMES) in College of Natural Sciences and Mathematics (CNSM) at California State University Long Beach (CSULB). As part of the agreement and memorandum of understanding outlined in the core facility proposal, IIRMES, through the funding provided by CSUPERB, established FEMCA to provide 25% instrument access time (1.5 days per week) to CSU customers. The funded core facility proposal established: (i) the suitability of our instrumentation; (ii) the system wide need and demand for the facility; (iii) the carrying capacity of our facility; (iv) our quality assurance protocols; and (v) our technical expertise. The proposal also included 32 letters of support from faculty and students with affiliations from CSU Los Angeles, Northridge, San Francisco and Fullerton, as well as researchers from the UC system. Letters of support were also included from California biotechnology industries in recognition of the importance of the CSU system in providing an educated, technology-trained workforce.

As outlined in the original proposal, the recognition of FEMCA as a CSUPERB core facility was, and remains, important for two reasons: first, it has increased access and use of our analytical instrumentation and resources for teaching and research to a wider CSU community; second, it has promoted awareness, and consequently, utilization of the facility by fee paying customers outside the CSU community. The generation of this fee-paying customer base is an important priority in ensuring the ultimate fiscal independence of FEMCA.

Mission Statement of IIRMES and FEMCA
Within the general context of the mission of IIRMES, some of the specific goals which identify the purpose and existence of FEMCA, and to which we aspire are listed below:

Promotion of Scholarly and Creative Activity for Faculty
- Promote the cross-application of analytical techniques and foster intellectual exchange between scientists in a broad range of scientific fields
- Facilitate the access to state-of-the-art instrumentation for researchers in all fields of academic inquiry,
- Sponsor colloquia, lecture series and conferences, and promote interdisciplinary workshops
- Collaborate in obtaining funding for the purchase of instruments, supporting their upkeep, and in facilitating access to the instruments by the broader CSU community and collaborating researchers;
- Promote collaborations with other universities including UC campuses, creating research possibilities for our faculty and students in some of the leading Ph.D. programs in the country; and
- Help in the recruitment and retention of new faculty members in the sciences.

Development of Instructional Programs that provide for Student Training and Research
- Allow exposure to an innovative and highly interdisciplinary environment that promotes cross-training in various disciplines;
- Provide access and hands-on training on state of the art instrumentation to enable students to combine theory and the use of analytical techniques to solve problems central to the understanding of the physical, life and
social sciences;
- Provide internships and graduate and undergraduate assistantships;
- Improve connections with regional industry and the private sector thereby creating local career opportunities for our students in the California and other regions of the country;
- Generate funds specifically designated for both graduate and undergraduate student research projects;
- Generate funds to support visiting scholars who will work with students and conduct workshops.

Contribution to Community Service
- Develop outreach programs, involving remote access of instrumentation and video conferencing to promote science education in local schools. IIRMES places special emphasis on targeting inner urban schools that have historically served underprivileged children with limited access to technological resources;
- Provide analytical services for scientists in industry and consulting firms to help support undergraduate and graduate programs and defray the institutional overheads necessary to run the facility.

FEMCA Relocation
The last year can be best described as a year of change and flux, both in terms of personnel and venue. As anticipated, perhaps the biggest event of the year has been the completion of the new IIRMES facility in the Microbiology Building. Over the past year, both IIRMES and FEMCA have been relocated from their original location in Peterson Hall 3 to a renovated 4000 sq ft location on the 2nd floor of the Microbiology Building, which will be its final location for the foreseeable future. The new facility was dedicated by the President of CSULB, King Alexander in May of this year. This relocation was necessary due to demolition and replacement of the old science complex with a new building which, when it opens in 2010, will provide assigned space for 22 research laboratories, 31 teaching laboratories, two 180-seat lecture halls, and two 80-seat lecture halls.

Most of the major reconstruction in the laboratories was completed during April-May of 2007 but confounding issues, related to the air balance in the facility prevented the completion of the transfer of the capital instruments until November. All of the instruments are operational, calibrated and are meeting the guaranteed specifications from the manufacturers. I would like to thank the many people who assisted in relocating these instruments including our IIRMES and FEMCA staff - Chris Mull, John Dudgeon, Nolen Lambert and Ashraf Elamin, amongst others. Special thanks also go to Jim McKibben and Kris Finkel from the CNSM shop who not only helped move some of the larger units, but also provided fabrication support and expertise to modify the facilities to enable us to plumb, wire and install the instruments.

Technical Support and Staff Hiring.
Over the past 12 months there have been some changes in the technical staff. Funding from the CSUPERB grant allowed FEMCA to initially appoint Mr. Chris Mull as an Instructional Support Assistant III. Since the last report, Chris has been promoted to the Microbeam Technician for IIRMES following a national search to fill this vacant position. Chris started at FEMCA in June 2006 and has worked under the general supervision of the FEMCA Director to provide technical support on CSUPERB related samples. Chris has proved himself a capable mass spectroscopist and has provided extensive customer support for CSUPERB clients on the PE6100-DRC and the GBC 8000 Optimass inductively coupled plasma mass spectrometers (ICP-MS’s), providing data for a number of exacting projects that have resulted in both publications and grant proposals. He is also proficient in the operation of the FEI environmental scanning electron microscope (ESEM) and the Oxford Instruments’ energy dispersive X-ray microanalyzer (EDX). Although no longer officially the FEMCA technician, in his new position as the IIRMES Microbeam Technician, Chris is responsible for the direct supervision of his replacement, Mary Blasius, who was hired in June of this year after a regional search. We welcome Mary as the new FEMCA technician. Mary completed her M.S. at CSULB in 2007 on pesticide residue analyses in three local species of pinnipeds- the California Sea Lion, the Harbor Seal and the Elephant Seal and has had extensive experience with GC-MS analyses. Following her M.S. she was retained as a Foundation employee to assist in a number of IIRMES projects funded by the U.S. Navy and the States of Alaska and California. This period of employment provided her with analytical expertise and an understanding of the compliance and quality control measures necessary for the service roles that both FEMC and IIRMES provide as a core facilities for the CSU and the greater scientific community. She is currently receiving training from Chris on ICP-MS and SEM-EDX so that she can assist FEMCA clients on these instruments.
FEMCA Usage and Instrument Access

Table 1 itemizes the usage of FEMCA by CSU faculty and students for research and teaching over the past 12 months. Although the facility has been used by a number of different CSU campuses, for logistical reasons, most of the users who have attended the facility have been from the southern California campuses, although requests for analyses and logistical support have come from as far away as CSU Humboldt. Equal and equivalent access has been provided to all requests for analysis and the total instrument usage has exceeded the 1.5 days originally designated for FEMCA customers.

As in the previous report, although the degree of usage on the different instruments available in FEMCA has varied considerably, the majority of the requests for analyses remain for quantitative elemental or isotopic analyses by ICP-MS. These analyses can be divided into four types: i) simple liquid aspiration and analysis by conventional quantitative ICP-MS; ii) isotopic dilution and isotopic ratio analysis; iii) directly coupled 1- and 2-dimensional HPLC-ICPMS for metalloprotein separation, identification and quantification; and iv) laser ablation ICPMS analysis for the quantitative spatial distribution of elements/isotopes in solid samples/gels.

FEMCA has provided custom sample preparation and, whenever requested, one-on-one user training for each of these different types of analyses. Conventional preparative procedures that have been conducted for clients have varied from total sample digestion using mixtures of concentrated nitric and hydrofluoric acid for liquid sample aspiration analyses to native gel electrophoresis for laser ablation applications. In addition, FEMCA has accommodated, whenever feasible, user-defined requests for specific method and application development. This has included stable isotopic procedures for volatile Hg species determination to the preparation of metal-free and isotopically doped proteins.

Other instruments that have been used to a lesser extent by FEMCA clients have been the environmental scanning electron microscope (ESEM) and the energy and wavelength dispersive X-ray systems. Despite the considerable applications of analytical ESEM in biotechnology, there has been limited interest or recognition of the capabilities of this instrumentation for biotechnological investigation. It is our opinion that there needs to be better dissemination of the virtues of this instrument for high spatial resolution and elemental mapping of hydrated samples. This capability to undertake microscopy of materials in a fully hydrated atmosphere without the need to coat with an electron permissive material is a relatively new concept and needs to be better communicated to users in the CSU system. We seek assistance from CSU-PERB to help us accomplish this in accordance with their policy mandate.

Finally, there has been considerable interest and usage of our new Agilent GC-MS for volatile organic compound identification and quantification. The sheer volume of samples and the extended run-times that are typically used for environmental samples has meant that we now have a significant backlog of samples waiting to be analyzed on the instrument. Other than stoppages for cleaning, calibration and routine maintenance, the instrument has been operating essentially around the clock on a variety of research projects. The instrument was installed approximately 3 years ago and, during its brief period of operation, analyses performed on this instru-

"Having access to the FEMCA at CSULB has helped our research group publish at least 4-5 papers and make 5-6 presentations over the past few years. About seven students and postdoctoral associates from my group have used the various instruments in the facility. They benefited tremendously from the experience. Four of these students are currently in top-notch Ph. D. programs (Cornell, University of Illinois-Urbana Champaign, UC-Irvine, and University of Washington). The impact on the training of many talented undergraduate students, many of whom are from minority groups, is immeasurable.

Dr Feimeng Zhou, CSULA
ment have provided sufficient data for the acquisition of a number of grants and contracts, the successful defense of a number of Masters Thesis.

Instrument Acquisition by FEMCA.

One of the mandates of FEMCA is to provide cutting edge technologies to CSU faculty and students. Over the past 12 months two NSF MRI’s were submitted for instrument acquisition. Unfortunately neither proposal was funded although the reviews for both were generally positive.

The FEMCA facility at Long Beach has been a very great advantage to me and my research, since I work on trace elements in mammalian blood and tissues, and by nature, these are impossible to quantify without the kind of mass spectrometric capabilities present there. We have two manuscripts submitted that contain critical work obtained with the collaboration of FEMCA. Several more are anticipated, and I very much look forward to working with FEMCA on a new project to characterize at the molecular level what may be a component of the cell that shuttles iron from one molecular to another. This kind of core facility is essential if we are to compete with researchers at other institutions”.

Maria Linder CSUF

“...the kind of core facility is essential if we are to compete with researchers at other institutions.
ment. The reviewers acknowledged the need for this instrument for high throughput C,H,N isotopic studies in biological samples for trophic standing analyses and dietary source determinations.

Curriculum Development at FEMCA
FEMCA also continues to be involved in undergraduate education and teaching as well as student research. As outlined in this annual report, IIRMES and FEMCA are involved, to varying degrees, in a number of classes. Over 700 students from CSULB, our sister campuses and other academic institutions have visited the facility for training educational and research purposes. Perhaps, at least in part, in recognition of our achievements in this area we are very excited to be invited as a founder team member of the Cal-PRISSM e-consortium that, through the use of cyber-infrastructure, will provide a network of remotely controlled instruments available for teaching, research and outreach. The capabilities and goals of this new initiative are described in this annual report in an article authored by our colleague, Dr. Katherine Kantardjieff from CSU Fullerton who is the Director of CMolS, a sister CSUPERB core facility. The virtues of the cyber-structure were tested fully in Fall 2007 when the CSU Oceans Studies Institute (OSI) comprising the 7 campuses in the Southern California region remotely accessed both the Perkin Elmer 6100 and the Agilent GC-MS in FEMCA from the Wrigley Marine Science Center at Two Harbors on Catalina Island for some of their scheduled classes (OSI 345, Environmental Physiology & Toxicology of Marine Organisms). Data were obtained from both conventional ICP-MS and directly coupled HPLC-ICPMS as well as GC-MS for a variety of laboratory exercises and research projects being conducted by the students on the Island. To the best of our knowledge, this is the first time this type of remote acquisition has been conducted as part of a scheduled undergraduate class. [http://www.screencast.com/t/zi4UPpktl]

Workshop Development in Biotechnology at FEMCA
FEMCA offered a highly successful CSUPERB sponsored workshop via the 2007 Programmatic Workshop Program to fund an introductory workshop for faculty and student training in proteomic analysis. In total 12 faculty and students from around the CSU attended the 1 week workshop, which provided basic skills in 2 dimensional gel electrophoresis, MALDI-MS and MS/MS. Attendees conducted MS analyses on their own samples and most, if not all, managed to successfully annotate proteins from both trypsin digestates and from peptide fragments sequenced de novo in the MS/MS mode. Although somewhat modest in scope, we anticipate the training provided by this workshop will lead to increased awareness of the capabilities of MALDI-MS-MS for protein identification and discovery. Given the success of the workshop, together with the increasing importance of proteomics analysis in biotechnology, we anticipate continuing interest and growth in usage of the facility by our CSU colleagues as the applications of this technology become more well known. A proposal has been submitted to CSUPERB requesting that the proteomics facility become a part of FEMCA and obtain core status. It is anticipated that the development of the laboratory as a CSU core offering subsidized analytical services will provide opportunities for the development of novel research possibilities for both faculty and students. Proteomics can be used to answer fundamentally important questions regarding gene expression and is one of the most rapidly emerging technologies in the life and biotechnologically-related sciences with growing applications in the medical, pharmaceutical as well as in the basic sciences.

Student Research and Training
Without doubt, our most significant accomplishment over the last year has been our involvement with students. This engagement has been in the classroom and in the laboratory. As can be seen for Table 1 a large number of both undergraduate and graduate students have been trained on the instrumentation at FEMCA. Many, if not most, have been given personalized training and the majority have past tests of competence that allow for unattended operation of the instrument. These students have also been given supervision in sample preparation and data interpretation. Their research findings have formed the
basis of presentations, manuscripts and fellowships.

**Student and Faculty Safety**

Safety in the workplace is of the highest priority to FEMCA. This represents a challenge for a multi-user facility involving numerous student and faculty visitors. With the help of CSULB Environmental Health & Safety, and in particular Kristen Hunter and Jeff Mellon from the CNSM safety office, we have developed safety training procedures to comply with the law (Cal/OSHA and Cal/EPA regulations) and University policy to ensure that all activities conducted in and by FEMCA within IIRMES adhere to established health, safety and environmental policies.

Details of the implemented safety policy and procedures can be obtained from the IIRMES website at [http://www.csulb.edu/programs/iirmes/index_files/Page601.htm](http://www.csulb.edu/programs/iirmes/index_files/Page601.htm).

**Perspectives**

Undoubtedly one of the biggest issues for FEMCA in the months ahead will be implementing the migration of FEMCA from being a CSUPERB sponsored and subsidized laboratory into a self-supporting entity. Over the past 3 years, the support provided by CSUPERB has substantially increased our client base both inside and outside of the CSU. Our major effort in the years to come will be to continue to increase the usage of the facility to maximize efficiency and productivity without compromising the levels of service offered to our CSU clients. While the loss of our CSUPERB subsidy will necessitate that we pass on some of the operational costs of the center to our customers, we will try, to the best of our ability to honor our agreement with CSUPERB to maintain our prices at a minimum.

*Respectfully submitted,*

A. Z. Mason, Ph.D.
Director, FEMCA, IIRMES
&
Professor, Department of Biological Sciences &
Professor, Environmental Science and Policy
<table>
<thead>
<tr>
<th>Faculty Name</th>
<th>Institution</th>
<th>Project Description</th>
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<tr>
<td>Dr. Steven Manley</td>
<td>CSULB</td>
<td>Nitric Acid Digestion and Perkin Elmer Quadrapole ICP-MS analysis of Kelp Sieve Tube Sap to Assess the Effectiveness of Kelp as a Bioindicator</td>
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<tr>
<td>Dr. Simon Malcomber</td>
<td>CSULB</td>
<td>Sputter Coating and SEM imaging of grass flowers inferences to examine genic regulation of floral development</td>
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<td>Dr. Kasha Slowinska</td>
<td>CSULB</td>
<td>SEM imaging of Collagen infused with Au-nanoclusters to examine changes in porosity of the collagen matrix: implications for drug delivery</td>
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<td>Dr. Roger Acey</td>
<td>CSULB</td>
<td>Metal Binding Determination of Metallothionein and Al+3 eluted in PBS buffer using Perkin-Elmer Quadrapole ICP-MS</td>
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<td>Dr. Gwen Goodmanlowe</td>
<td>CSULB</td>
<td>Nitric Acid Digestion and Perkin Elmer Quadrapole ICP-MS Analysis of Skim Milk Powders to Identify Characteristic Elements for Location of Origin Validation</td>
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<tr>
<td>Dr. Chad Imoos</td>
<td>CSU San Luis Obispo</td>
<td>Department of Chemistry and Biochemistry Monies Perkin Elmer Quadrapole ICP-MS analysis of Heavy Metals in the Purple Sea Urchin</td>
<td>30</td>
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<tr>
<td>Dr. Corinne Lehr</td>
<td>CSU San Luis Obispo</td>
<td>Department of Chemistry and Biochemistry Monies Nitric Acid Digestion and Perkin Elmer Quadrapole ICP-MS Analysis of Sediments from Los Cerritos Wetlands and Hellman Properties for Heavy Metals</td>
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<td>Dr. Pat Baird</td>
<td>CSULB/Kahiltna Research Group</td>
<td>Nitric Acid Digestion and Perkin Elmer Quadrapole ICP-MS analysis of Panamanian Sand Piper Blood and Feathers</td>
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<td>Dr. Tulin Mangir</td>
<td>CSULB</td>
<td>SEM imaging for the observation of bacteria (Pseudomonas reginosa) in relation to carbon nanotubules</td>
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<td>Dr. Sean Liu</td>
<td>Cal Poly Pomona</td>
<td>Nitric Acid Digestion and Perkin Elmer ICP-MS Analysis of Organic Polymers for Boron Content</td>
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<td>Dr. Vilupanur A. Ravi</td>
<td>Cal Poly Pomona</td>
<td>SEM Imaging and EDX analysis of sputter coated Alloys</td>
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<td>Dr. Vilupanur A. Ravi</td>
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<td>SEM imaging of Corrosion Dynamics of Titanium Alloys</td>
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<td>Dr. Jiyeong Gu</td>
<td>CSULB</td>
<td>SEM Imaging and EDX analysis of MgB2 sputter coating of ceramic conductors</td>
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<td>Dr. Richard Behl</td>
<td>CSULB</td>
<td>SEM imaging, GC/MS and MALDI TOF/TOF analysis of black mat sediment from Arizona for characterization of a previously undescribed organic layer</td>
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<td>Faculty Name</td>
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<td>Dr. Kevin Kelley</td>
<td>CSULB</td>
<td>GC/MS and MALDI TOF/TOF analysis of sediment, invertebrate, and fish tissue from waste water treatment outfall sites for evidence of bioperturbation</td>
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<tr>
<td>Dr. Andrew Mason</td>
<td>CSULB</td>
<td>Analysis of blood from Green Sea Turtles in Mission Bay, San Diego for heavy metal and persistent organic pollutant load</td>
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<td>Dr. Andrew Mason</td>
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<td>HPLC ICP-MS analysis of MT I &amp; II to determine the kinetics of metal binding and transport between proteins</td>
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<td>Dr. Andrew Mason</td>
<td>CSULB/Wrigley Marine Science Center</td>
<td>Nitric Acid Digestion and Perkin Elmer Quadrupole ICP-MS analysis of kelp sieve tube sap to assess heavy metal concentration and fractionation with depth in a southern California Marine Reserve</td>
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<td>Dr. Srinivasan</td>
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<td>Nitric Acid Digestion and FIA Perkin Elmer Quadrupole ICP-MS analysis of benthic worms for lead toxicity</td>
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<td>Dr. Maureen Fitzpatrick</td>
<td>CSUSM</td>
<td>Tooth Fairy: Laser Ablation GBC TOF ICP-MS analysis of metals in pre vs. post natal enamel of teeth from children with behavioral disorders and autism</td>
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<td>Dr. Rebecca Lewison</td>
<td>SDSU</td>
<td>GC-MS analyses of green turtle trophic web</td>
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<td>Dr. Maria Linder</td>
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<td>Cu and Zn analyses of rat milk and HPLC of serum proteins</td>
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<td>Dr. Katherine Kantardjieff</td>
<td>CSUF</td>
<td>Workshop Demonstrations of remote access to Instrumentation</td>
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Table 1. Analysis of Users and Products

(Under “Citation of Publications” designate as: thesis (T), peer-reviewed paper (P), reviewed abstract presented at national meeting (A), technical report (R), intellectual property report (IP) or Submitted Grant (G))
### CEPA CLIENTS SPONSORED THROUGH FEMCA

<table>
<thead>
<tr>
<th>Faculty Name</th>
<th>Institution</th>
<th>Type of Analysis</th>
<th>Instrumentation Technique Used</th>
<th>Title of Research Investigation</th>
<th>Number of products produced (analyses, etc.)</th>
<th>Citation of Publications</th>
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<td>Douglas McAbee</td>
<td>CSULB</td>
<td>2-D gels, protein identification</td>
<td>2-D gels and MALDI</td>
<td>Identification of unknown proteins from rat</td>
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<td>Protein mass determination</td>
<td>MALDI</td>
<td>Studies on lipoproteins</td>
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<td>Young Shon</td>
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<td>Small organic molecule mass determination</td>
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<td>Kevin Kelly</td>
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<td>2-D and protein identification</td>
<td>2-D, MALDI and de novo sequencing</td>
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<td>Kay Lee-Fruman</td>
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<td>Studies mass determination of synthetic organic compounds</td>
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<td>Marquez-Magana</td>
<td>CSUSF</td>
<td>Protein identification</td>
<td>MALDI</td>
<td>Mass determination of a purified and modified proteins</td>
<td></td>
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</tbody>
</table>
FEMCA Core Facility Overview

Instrumentation and Applications.

The facility for elemental micro-chemical analysis (FEMCA) was inaugurated in the spring of 2005 as the newest core facility of CSUPERB. FEMCA is housed within the Institute for Integrated Research in Materials, Environments and Society (IIRMES) at CSULB and marshals a sophisticated array of NSF funded equipment for molecular and elemental analysis; scanning, transmission and atomic force microscopy; as well as purpose-built clean-room facilities for organic and inorganic extractions and sample preparation.

As a CSUPERB core facility, FEMCA offers subsidized analyses at reduced rates for students and faculty researchers associated with CSUPERB. Specific capital equipment currently available for CSUPERB sample analyses includes: (i) a Perkin Elmer™ 6100 DRC, dual quadrupole ICP-MS with hydride generation facility and a flow injection analysis system; (ii) a GBC TOF ICP-MS with an attached NewWave 213LUV laser Ablation system for the analysis of solid materials; (iii) a analytical FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM) with integrated Oxford Energy Dispersive X-ray Spectroscopy (EDS), Wavelength Dispersive X-ray Spectroscopic analyzer (WDS) and cathode-luminescence for element mapping equipped with remote access capabilities through PCI Quartz networking; (iv) a Finnegan MAT Delta-XP Stable Isotope Gas-Ratio Mass Spectrometer for carbon, nitrogen, oxygen, sulfur, and hydrogen isotope ratio determination and (v) a Nanoscope III Multimode Atomic Force and Scanning Probe Microscope (AFM/SPM) capable of contact, and tapping-mode AFM and magnetic and lateral force and scanning tunneling microscopy. Since its inception 3 years ago, FEMCA has sought external funding to update and upgrade instrumentation to keep up with technological advances in the field as mandated in the CSUPERB policy statement for core facilities. Approximately $1.2 million dollars have been acquired from grants from the Keck Foundation, NSF and other funding agencies during this period for the purchase of a MALDI-TOF-TOF, a liquid handling station, a 2D gel system with a robotic imager and spot cutter, a Physical Property Management System (PPMS) and an Agilent 5973 GC-MS with ESI and CI capabilities.

Facilities and Instrumentation

FEMCA is located in IIRMES within the College of Natural Sciences and Mathematics at CSULB. The facility currently comprises five contiguous laboratories covering some 4,000 sq. ft. of space housed in the Microbiology building that has been specifically designed to enhance the capabilities and services offered by FEMCA. The centralization of the instrumentation to this suite will not only promote their effective utilization but will also enhance cross-disciplinary collaboration.

Details of some of the current capital equipment and their applications that are available through FEMCA for CSUPERB subsidized analyses are as follows:

**Perkin Elmer™ 6100 Dynamic Reaction Cell (DRC) ICP-MS**

The Perkin Elmer is primarily used for...
quantitative elemental analysis of solutions. The instrument has a hydride generation capability, robotic autosampler, a flow injection analysis system and an interfaced two-dimensional HPLC for metalloprotein separation and analysis. The double quadrupole is equipped with a dynamic reaction chamber (DRC) in the first quadrupole that removes many of the polyatomic interferences caused by complex matrices that can confound accurate quantification of particular elements by conventional ICP-MS. This NSF funded instrument was installed in 1999, can operate round the clock and has continuously performed at, or better than, the manufacturer’s analytical specifications.

**GBC Optimass orthogonal TOF ICP-MS**

This NSF-funded instrument, installed in 2004, is only the second of its type to be installed in the USA, the other being at the Oak Ridge National Laboratories. The major advantage of the TOF mass discriminator over a quadrupole system is the speed of data acquisition, which provides for essentially simultaneous analysis across the mass spectrum. This form of analysis is advantageous where the element signal is highly transient yet needs to be resolved temporally. The instrument has an attached NewWave 213LUV Laser Ablation system that permits solid sample, in-situ analysis of a wide variety of biological, chemical, or petrographic samples.

**Analytical FEI Quanta 200 Environmental Scanning Electron Microscope (ESEM)**

Installed in 2003 using NSF funds, the FEI ESEM features integrated Oxford Energy Dispersive X-ray (EDS) and Wavelength Dispersive X-ray (WDS) Spectrophotometers and cathodoluminescence for elemental mapping. Operating in environmental mode, the ESEM is capable of imaging and major/minor elemental characterization of large samples at low vacuum without the need for sample over coating eliminating spectral artifacts traditionally associated with sputter-coated materials.

**Veeco Nanoscope III Multimode Scanning Probe Microscope**

This instrument is ideal for nano-scale metrology. It features multimode SPM including contact and tapping-mode atomic force microscope, magnetic force microscope, lateral force microscope, and scanning tunneling microscope. SPM can measure atoms, molecules, and other nanoscale topographic, magnetic, and electric features with accuracy and precision in the nano-spatial scale. The instrument has a sample heater with a fluid cell that provides in-situ temperature control up to 50°C for samples in air and fluids.

**Finnegan MAT Delta-XP Stable Isotope Gas-Ratio Mass Spectrometer**

The NSF-funded Delta-XP, installed in 2004, has dual inlet and continuous flow capabilities. The dual inlet provides economical and reliable determination of carbon, nitrogen, oxygen, sulfur, and hydrogen isotope ratios of gas species containing these elements. Sample preparation and gas extraction is performed off-line in a newly refurbished stable isotope laboratory. Three extraction lines are available: (i) a vacuum line that uses fluorine gas to extract oxygen from silicate materials for oxygen isotope analysis; (ii) a vacuum line that provides for the extraction of carbon dioxide from carbonate materials for carbon and oxygen isotope analysis; and (iii) an oxygen isotope extraction line for the analysis of the oxygen isotopic composition of water. The continuous flow inlet provides for the introduction of H₂ and CO gas produced by reacting liquid or solid sample materials in an elemental analyzer that utilizes carbon reduction to produce these gases, allowing for the measurement of hydrogen and oxygen isotope ratios.
IIRMES, FEMCA and CEPA were relocated from their prior location in Petersen Halls of Science 2 & 3, into new, freshly renovated and purpose-built, contiguous laboratory space on the second floor of the Microbiology building on the CSULB campus. The footprint of the new facility in Microbiology consists of about 4,050 square feet. This relocation was necessitated by the scheduled demolition of Peterson Hall 3, which is currently underway. The move, which was finished in the Fall of 2007, marked the culmination of a two-year, $850,000 initiative to provide IIRMES and the two centers with dedicated permanent space for their operations. The new facility was dedicated in May 2008 by the President of CSULB, King Alexander.

Funds for the renovation were provided by the Division of Academic Affairs and the CNSM at CSULB from Minor Capital Funds. IIRMES and FEMCA staff have worked extensively with the University Architect, Michael Gardner, the College Facilities Coordinator, Dr. Robert Loeschen, and others to design a modern functional analytical laboratory suite capable of undertaking ultra-trace analyses of materials and biological samples. The physical renovation of the facility was conducted by PS-2 Engineering and provides a floorplan that offers efficient utilization of the space with the result that the facility is now able to expand upon the microchemical and analytical services offered to the CSU and the larger academic community. In addition, the space has been designed specifically to enable the facility to be utilized flexibly for diverse applications. In particular, the internal flow of traffic within the facility has been designed to allow for the processing and sequential analysis of specimens totally within the research space, avoiding possible sample contamination issues, as well as mitigating safety concerns. Moreover, the entire facility is isolated from the outside by a series of vestibules that are maintained under positive pressure to act as filtration barriers to keep the facility clean of external contamination that could otherwise compromise the analytical performance of the instruments. The layout of the new facility is shown to scale in
In essence the laboratories have been divided into five functional areas for spatial microchemical analyses, thermo-luminescence analysis, stable isotopic analysis, functional genomic analysis and organics analysis. Each area has been renovated in such a manner as to enhance the functionality and analytical specifications of the facility for quantitative, ultra-trace analytical research. The new facility boasts 5 state-of-the-art Teflon lined, metal-free hoods capable of safely handling the most corrosive vapors. The laboratories also have dedicated instrument exhaust ducts with individual variable induction systems capable of precise control of the exhaust extraction rates to provide optimal performance from the instruments. The entire facility is designed to meet class 10,000 or better standards via the central air HEPA filtration system. For more exacting work, class 100 clean hoods are available. The design specifications also include a gas distribution system that automatically monitor the delivery and usage of cryogenic liquids and compressed gases to the various instruments from the outside gas storage units.
Figure 4.

Top panel: Renovation of thermoluminescence laboratory (behind wall) and completed facility showing dividing wall with CHN analyzer.

Center panel: Proteomics Laboratory with ABI 4800 MALDI-TOF-TOF, and ancillary equipment (left) and Finnegan MAT Delta-XP Stable Isotope Gas-ratio Mass Spectrometer (right).

Bottom panel: FEI ESEM with WDS and EDX analysis systems after installation in new facility being operated by a graduate student (left). The new conference and lecture room (right).
NEW FACES: Mary Blasius, New FEMCA Technician

Mary Blasius was hired in July of this year as the new FEMCA technician. She replaces Chris Mull, who was recently promoted to IIRMES Microbeam technician. Mary is an expert in GC-MS analyses and will be trained over the coming months in many of the other instruments used by our CSUPERB clients.

Mary completed her B.S. from UCI in Biological Sciences in 2002 and received her Master’s from CSULB in 2007. Her M.S. thesis research was conducted in IIRMES on organochlorine (OC) compounds in Californian pinnipeds.

Highly industrialized areas such as the Southern California Bight (SCB) have repositories of OCs, such as polychlorinated biphenyls (PCBs) and DDT, which through seepage, runoff, and dumping can enter freshwater waterways to eventually be discharged into the marine environment.

One of the concerns associated with OCs is that they are lipophilic and are resistant to metabolic processes, and therefore, are biomagnified up the food chain. One way to predict the potential effects of these chemicals on complex food webs is to determine the contaminant burdens of apex predators, such as pinnipeds. Pinnipeds can be useful sentinel species for monitoring lipid-soluble contaminants because many species have a high body fat content, are in a high trophic level, and have long life-spans. These biological traits make pinnipeds vulnerable to the toxic effects of these chemicals.

Although Southern California is well known as a highly industrialized area and for its highly publicized contaminant dumping (i.e.-dumping of DDT from 1940-1970s), there are surprisingly few studies on DDT and PCB levels in upper trophic organisms such as pinnipeds from the SCB compared to those from the northern and central coast of California. Therefore, the present goals of this study were to: (a) evaluate current levels of PCBs and chlorinated pesticides of harbor seals (Phoca vitulina), CA sea lions (Zalophus californianus), and northern elephant seals (Mirounga angustirostris) to ascertain if they exhibited different levels of contaminants in their blubber due to differences in diet and habit use (b) determine if differences exist in contaminant concentrations among the age classes, and between gender due to contaminant build up with age and offloading of contaminants by females to their pups, and (c) examine temporal trends in PCB and DDT concentrations from 1994 to 2006 to evaluate if current contaminant concentrations are decreasing over time after the cessation of production and dumping of DDT and PCBs in the 1970s.

To address these aims, the blubber of 92 CA sea lions, 11 harbor seals, and 43 n. elephant seals obtained from the marine mammal centers located in San Pedro, CA, and Laguna Beach, CA. Contaminants were extracted at high temperatures from homogenized blubber using dichloromethane and were subsequently cleaned by eluting through an Alumina-B/Silica Gel column. For identification and quantification of contaminants, each sample was injected onto an Agilent gas chromatograph (6890N series) equipped with a mass selective detector (GC/MS; Agilent 5973 inert series). The GC column employed was a ZB-5 (Phenomenex; Torrance, CA) fused silica capillary (0.25 mm ID x 60m) column. The analytes were resolved using a temperature profile of the 45°C to 125°C at 20°C/min, then to 295°C at 2.5°C/min and held for 10 min. The mass detector was used in the Electron Ionization (EI) mode and scanned from 45-500 amu at a rate of 1.66 scans/sec.

Mean concentrations of both tDDTs and tPCBs in blubber of the three species were significantly different from each other ($F = 11.61; df = 2, 100; p < 0.001$ for DDTs; $F = 9.31; df = 2, 100; p < 0.001$ for PCBs; Figure 2). CA sea lion and harbor seal blubber samples did significantly higher concentrations of both tDDTs and tPCBs than n. elephant seals. Harbor seal blubber samples did not differ significantly in their concentrations of either tDDTs or tPCBs from CA sea lions (Tukey’s comparisons; $p = 0.99$ for tDDTs; $p = 0.99$ for tPCBs).

Mary Blasius presented the research in this report in partial fulfillment of a M.S degree in Biological Sciences at CSULB. Funding for this project was provided by a CSULB graduate Fellowship from Academic Affairs. Funds for the purchase of the GC-MS was provided by internal contracts through IIRMES (AZM) and matching funds from the

Figure 1: The author with a California sea lion.

Figure 2. Mean and standard deviation for tPCB and tDDT concentrations ($\mu$g/g, lipid weight) in harbor seal, California sea lion, and northern elephant seal blubber (all age classes and sexes combined). No significant differences were found among species that have the same letter above their bars; species that have different letters above their bars are significantly different from each other.
exposure to these contaminants for each likely related to the different sources of concentrations of tDDTs and tPCBs are most concentrations of tPCBs in their blubber than the northern elephant seals from the adult female had significantly lower concentrations of tDDTs and tPCBs, whereas for northern elephant seals there was no significant decline (Figure 4). The decrease in tDDT and tPCB concentrations is particularly clear in highly contaminated areas such as the SCB and has been documented in other highly contaminated regions. This decreasing trend of contaminants in the regions located far from these point sources as a consequence of atmospheric transport and redistribution. The long-term transfer of airborne contaminants from warmer to colder regions can act as sinks for contaminants and allowing for a steady state of contaminants in these regions. Thus, in the SCB, concentrations of tDDT and tPCB are found to be decreasing significantly over time in the more resident CA sea lion versus the transient northern elephant seal where concentrations of tDDT and tPCB are not found to be decreasing significantly over time.

The highly elevated levels of tDDT and tPCB warrant further examination into the relationship of OC exposure to fitness of individual pinnipeds. Because humans often feed on similar fish and cephalopods caught in these regions, studies on pinnipeds could also be relevant to human health and risk assessment as well.

categories and gender were not significantly different from each other. There was a significant interaction between the age and gender categories for tDDT concentration (Figure 3). The blubber samples from the adult female had significantly lower concentrations of tDDTs than the pup females, yearling females, and adult males (Figure 3). There was a significant interaction between the age and gender categories for tPCB concentrations (Figure 3). The adult female class had significantly lower concentrations of tPCBs in their blubber than the yearling female, pup male, yearling male, and adult male classes (Figure 3).

Controlling for the effects of sex, age, and lipid concentration, tDDT and tPCB concentrations were significantly decreasing over time (1994-2006) for the CA sea lion blubber samples (Figure 4). Controlling for the effects of sex, age, and lipid concentration, tDDT and tPCB concentrations were not significantly different across years (1997-2005) for the northern elephant seal blubber samples (Figure 4).

California sea lions and harbor seals exhibited significantly higher levels of tDDTs and tPCBs than northern elephant seals (Figure 2). Differences in concentrations of tDDTs and tPCBs are most likely related to the different sources of exposure to these contaminants for each species. For example, differences in concentration of tDDTs and tPCBs between harbor and elephant seals can be explained by the harbor seal’s permanent residency in the Bight compared to the elephant seal’s dual habitats (Alaska and SCB), as well as the deeper diving habits of elephant seals during foraging. Concentrations of tDDTs and tPCBs in CA sea lions and harbor seals were not significantly different, which is likely linked to the fact that they occupy a similar trophic position in the SCB. Both CA sea lions and harbor seals are opportunistic feeders; hence, contaminants in local prey species will affect the degree of bioaccumulation of OCs in these pinnipeds.

The typical pattern of contaminant concentrations in marine mammals is an increase in males with increasing age throughout their lifetime, while in females, contaminant concentrations increase with increasing age only until reproductive maturity is reached. In other reports of CA sea lions so far studied, the pattern of adult males being significantly more contaminated than adult females and juveniles had yet to be confirmed as has been done in other studies, including other otariids. It has been suggested this may be due the CA sea lion’s habits. Males migrate north of the SCB (as far north as B.C., Canada) for half the year to feed while lactating females remain in the SCB all year round in close proximity to the tDDT hotspot. In this study, it was confirmed for the first time that adult males have significantly higher levels of tDDT and tPCB concentrations than adult females in the CA sea lion. However, adult males did not have higher levels of tDDT and tPCB concentration than any of the juvenile age classes. Because males feed in a relatively more pristine environment by moving north along the coast they may not be exposed to the greater levels of contaminants compared to juveniles and adult females that remain in the SCB. Despite the fact that adult females do remain in the SCB year round, transferred loads appear to be greater than intake loads incorporated via the diet, which is supported by adult females having significantly lower concentrations of tDDT and tPCB than the younger age classes.

In assessing the decline of tPCBs and tDDT from 1994 to 2006 CA sea lions were found to have a significant decline for both tDDTs and tPCBs, whereas for northern elephant seals there was no significant decline (Figure 4). The decrease in tDDT and tPCB concentrations is particularly clear in highly contaminated areas such as the SCB and has been documented in other highly contaminated regions. This decreasing trend of contaminants in the regions where pollution was initially so high could be attributable to the cessation of dumping of contaminants at point sources, and an increasing trend of

Figure 3. Mean and standard deviation for tPCB (a) and tDDT (b) concentrations (mg/kg lipid weight) in California sea lion blubber by age and sex class. No significant differences were found among age and sex classes that have the same letter above their bars; age and sex classes that have different letters above their bars are significantly different from each other.

Figure 4. Mean tPCB and tDDT concentrations in all age classes of a) northern elephant seal and b) California sea lion blubber pooled together by year.
As part of its continuing mission to bring training in high technologies to CSU faculty and students, FEMCA, in coordination with the Center for Education in Proteomics (CEPA) offered a one week, hands-on, workshop in proteomics in June of this year. The CSUPERB sponsored workshop was organized and taught by Dr. Ashraf Elamin, the manager of CEPA and was made available to both students and faculty. Enrollment was limited to 12 registered participants, who came from a variety of campuses including CSDH, CSUF, FSU, CSSB, and CSUEB. Attendees from CSULB were excluded.

Proteomics constitutes the large-scale identification, characterization, and structural analysis of proteins and is performed in basic and applied molecular life science laboratories worldwide. It is a fundamental discipline for understanding the expression, function, and regulation of the entire set of proteins encoded by an organism. Recent developments in mass spectrometry (MS) have revolutionized proteomics. In particular, non-destructive “soft ionization” techniques using MALDI or electrospray ionization (ESI) allow MS analysis of large biomolecules, such as proteins and peptides. MALDI- and ESI-MS can identify peptides or proteins in complex mixtures by comparing masses of fractioned peptides with those from a sequenced genome or proteome library. Moreover, the amino acid sequence of peptides can be derived de novo from fractioned peptides subjected to tandem MS analysis. Due to their mass resolving power, MALDI- and ESI-MS can also identify post-translational modifications (e.g., glycosylation, phosphorylation) and changes in amino acid composition in mutant or engineered proteins.

The CSUPERB workshop was offered June 16th-20th and aimed to introduce attendees to the applications, virtues and limitations of MALDI-TOF-TOF for phenotype profiling. The inherent attributes of a TOF-MS as a procedure for mass discrimination are its excellent and comprehensive sensitivity, an extended mass range and speed of analysis. Since the discovery of matrix assisted laser desorption in 1988, products with molecular weights exceeding hundreds of thousands of daltons, such as polymers, proteins, glycans and nucleotides, have been analyzed by TOF-MS detectors. For analysis, samples are spotted together with a UV absorptive matrix onto a target surface that allows the sample to “softly-ionized” via a pulsed laser beam with minimal molecular fragmentation. The irradiated sample forms ions of the type [M+X]+ (where X= H, Li, Na, K, etc.). In addition to causing ionization, the light energy absorbed by the sample results in rapid heating and expansion that causes sublimation of the matrix and supersonic expansion of the analyte away from the target surface at velocities that varies between ~200 to 1000 meters per second (m/s). These ions are then extracted and accelerated from the laser...
amino acid sequencing will also be used as a strategy for developing degenerate nucleotide primers for functional gene expression through cDNA and genomic DNA library analysis.

In addition to introducing the theory and basic concepts behind conventional 2D-gel electrophoresis, trypsin digestion, MALDI-TOF sample preparation and MS interpretation for protein and metabolite discovery, the proposed workshop also introduced participants to the theory of more refined techniques to quantify protein content and expression and to study post-translational modifications such as glycosylation and phosphorylation and quantitative protein expression using isobaric ITRAQ™ reagents.

One of the primary aims of the workshop was to increase general awareness of the capabilities of CEPA for CSU research and teaching. Educating faculty and students on the virtues and limitations of this emergent technology in proteomics, metabolomics and clinical biomarker studies will increase both its usage for research and its incorporation within the curriculum. It is anticipated that this workshop will promote curriculum development and the systemic adoption and incorporation of this technology into research projects and will introduce new possibilities for faculty, making them more competitive in obtaining external funding and allowing them to diversify into new areas of research within their discipline.

Table 1: Scheduled and Completed Activities for the MALDI-TOF Workshop

<table>
<thead>
<tr>
<th>Day</th>
<th>Morning Activities</th>
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<tbody>
<tr>
<td>Monday</td>
<td>Welcome, Dean College of Natural Sciences and Mathematics and Associate V.P. for Research, CSULB. Tour of the IIRMES facility.</td>
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<td></td>
<td>Theory of MALDI-TOF; Fundamentals and Overview of hardware; Software Overview of Instrument Control, Tuning, Calibration and Basic Maintenance</td>
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<tr>
<td>Tuesday</td>
<td>MALDI-TOF Method development, MALDI, Pro Pic II, GPS Explorer and nonlinear software.</td>
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<td>Sample preparation for a 2-D experiment. Sample cleanup, and running the 1st dimension (isoelectric focusing).</td>
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<tr>
<td>Wednesday</td>
<td>Equilibration of isoelectric focusing strips, running 2nd dimension (SDS-PAGE) and staining.</td>
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<td>Scanning gels, cutting gels plugs, and overnight trypsin digestion.</td>
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<td>Thursday</td>
<td>Extraction of the tryptic peptides from gel plugs, and evaporation.</td>
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<td></td>
<td>Spotting the samples on MALDI plate, running MALDI for analysis, GPS Explorer and de novo sequencing</td>
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<tr>
<td>Friday</td>
<td>Guest lectures: Dr. Tieli Wang (CSUDH), and Dr. Bryan Rourke (CSULB). Concluding statements; Workshop assessment</td>
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<td></td>
<td>Instrument time: Analysis of specimens brought and prepared by attendees.</td>
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</table>

Figure 3. Dr Ashraf Elamin, the workshop organizer, explaining how to interpret MS MALDI spectra to Drs. Calderon-Urrea (CSU Fresno) and Kidan (CSU Dominguez Hills).
Dr. Tilly Wang (CSUDH):

MS/MS Spectrum

Protein identified: -Phosphopyruvate hydratase from human.

Dr. Joy Goto (CSU Fresno).

MS Spectrum

MS/MS Spectrum

Protein identified: -Fructose-bisphosphate aldolase from the fruit fly

**Figure 4.** MS and MS Spectra together with protein confirmation obtained from 2D gels prepared (not shown) by Drs. Wang and Goto at the CSUPERB workshop.
Funding for this research was provided by the W.M. Keck Foundation and CSUPERB.

Dr. Getachew Kidane (CSUDH).

Protein identified: -Tubulin from the mouse

Dr. Alejandro Calderon-Urra (CSU Fresno).

Protein identified: -Ribulose-bisphosphate carboxylase activase from the tobacco plant

**Figure 5.** MS and MS Spectra together with protein confirmation obtained from 2D gels prepared (not shown) by Drs. Kidane and Calderon-Urra at the CSUPERB workshop
FEMCA became a partner in an e-consortium that allows scientists, educators, and other users to remotely access and run its instruments. The California Partnership for Remote Instruments to Study the Structure of Matter (CAL-PRISSM) is a new California e-consortium whose members include several predominantly undergraduate institutions, community colleges and high schools. The effort is led by Katherine Kantardjieff, Director of the W.M. Keck Foundation Center for Molecular Structure (CMoS), a CSUPERB core facility for X-ray diffraction at Cal State Fullerton. CMoS pioneered the use of remote instrumentation access for education and training at predominantly undergraduate institutions, placing its instruments online to the CSU since 1997. In addition to CMoS' X-ray diffractometers and computers, CAL-PRISSM's remotely enabled instruments include remote access to diffractometers at the Stanford Synchrotron radiation Laboratory (SSRL); nuclear magnetic resonance and electron paramagnetic resonance spectrometers at Cal State Fullerton, under the direction of Hal Rogers; atomic force and environmental scanning electron microscopes, as well as the inductively coupled plasma mass spectrometers at IIRMES/FEMCA, under the direction of Zed Mason; a visible/near infrared spectrometer, circular dichroism spectropolarimeter and computer cluster at Cal Poly Pomona’s Center for Macromolecular Modeling and Materials Design (CM3D), under the direction of Phyllis Nelson. A cohort of CAL-PRISSM high school classroom teachers is led by Julie Karjala, a chemistry instructor at Newport Harbor High School and director of LearningBeaker.com, a web resource for high school teachers.

Harnessing the power of end-to-end cyberinfrastructure and building on existing programs and expertise, CAL-PRISSM provides students, college/university faculty and secondary classrooms with real-time remote access and control of specialized scientific instruments from remote locations, together with real-time discourse. CAL-PRISSM aims to not only promote cross-disciplinary collaboration between researchers using these technologies, but also to improve educational quality and student opportunities at the undergraduate and secondary levels in learning about and researching the structure of matter”.....
In July, 2007, the National Science Foundation hosted a two-day workshop in Washington D.C. concerning its Cyberinfrastructure Vision for 21st Century Discovery. This recently released document (March 2007, http://www.nsf.gov), developed in consultation with the wider science, engineering, and education communities, describes an evolving vision to guide future investments by the NSF in cyberinfrastructure (CI) over the next five years. Cyberinfrastructure is defined as the coordinated aggregate of software, hardware and other technologies, as well as human expertise, required to support current and future discoveries (NSF Office of Cyberinfrastructure, OCI). This includes data analysis and management; computationally intensive tasks and process management; data mining and integration; automation (sample and data management); visualization and modeling; and remote collaboration tools (interactive conferencing; remote sensing; remote access/control of instrumentation).

The CI vision is a bold one that encourages the use of cyberinfrastructure-mediated tools to collaborate and communicate in ubiquitous learning environments and virtual organizations. It urges the development of new kinds of learning and research cultures that support peer-to-peer modes of education and enable distributed knowledge communities transcending traditional disciplinary, institutional, geopolitical and cultural boundaries. CI efforts have led to the establishment of Virtual Organizations (VOs), also defined as collaboratories, grid-communities/networks, virtual communities and e-communities. VOs are groups of individuals whose members and resources are dispersed geographically and/or temporally, yet who function as a coherent unit through the use of end-to-end cyberinfrastructure. Virtual organizations, such as CAL-PRISSM, are revolutionizing the conduct of research and education.

CAL-PRISSM was officially launched at the American Chemical Society Western Regional Meeting in San Diego, October 9-13, 2007, where a poster was presented by Katherine Kantardjieff (Cal State Fullerton/CMoIS) and others. The power of the remote accessing capabilities was also demonstrated at the last CSUPERB Symposium held in January 11-13, 2008, at the Oakland Marriott City Center.

Professor Kantardjieff is Director of the W.M. Keck Foundation Center for Molecular Structure, a biophysical chemist and crystallographer. She is also a Participating Guest in the macromolecular x-ray facility of the Biology and Biotechnology Program (BBRP) at Lawrence Livermore National Laboratory (LLNL) and a member of the TB Structural Genomics Consortium. Professor Kantardjieff completed B.S. degrees in Chemistry and Biology at USC (1979), a M.S. in Chemistry (1984), a Ph.D. in Chemistry (1988) at UCLA, where she was a Gold Shield Distinguished Scholar, and conducted postdoctoral studies in structural biology at UCLA.
We are pleased to announce the installation of a Quantum Design Physical Property Measurement System (PPMS), funded through a $248,939 NSF Major Research Instrumentation (MRI) award granted to Dr. Gu, and Co-PI’s, Drs. Kwon and Barbic from the Department of Physics and Astronomy and Co-PI’s Drs. Li and Bu from the Department of Chemistry and Biochemistry.

Promoted as part of the instrumentation offered to other CSU campuses through the CSUPERB funded initiative supporting FEMCA in IIRMES, the instrument was ordered in August, 2006 and delivered/installed in April, 2007 in PH1-205. Since installation, PPMS has been used by a number of CSU faculty to measure various physical properties of materials including thin films, bulk, and powder samples in a wide range of temperature (1.9 – 400 K) and magnetic fields (up to 9 T) which have culminated in the publications of 3 manuscripts. This versatile instrument is the first of its kind in the CSU system and is used to perform a variety of measurements of physical properties that are strongly temperature and/or magnetic-field dependent. The PPMS includes the PPMS Base System with a 9 Tesla Longitudinal Magnet & Power Supply (PPMS-9), Vibrating Sample Magnetometer (VSM) System, AC Susceptibility/DC Magnetization Measurement Option (ACMS), Horizontal Sample Rotator, AC Transport Property Measurement System (ACT), and Multi-Functional Probe. This capability enables researchers to study magnetization (DC and AC), electrical resistance (DC and AC), Hall Effect, current-voltage characteristic, and superconducting critical currents and other physical properties of materials that are strongly temperature and/or magnetic-field dependent.

To date, the PPMS has been used primarily in three research projects. First it has enabled Dr. Xianhui Bu’s group to study the magnetic properties and magnetic susceptibility of a porous metal-organic framework material (MnSO₃)₂en (en = ethylenediamine). The results indicate the presence of a dominant antiferromagnetic interaction in these materials (Figure 2). The results have been published in the Journal of Inorganic Chemistry (C. Austria, J. Zhang, H. Valle, Q. Zhang, E. Chew, D.-T. Nguyen, J. Y. Gu, P. Feng, X. Bu, “Amine-Controlled Assembly of Metal Sulfite Architecture from 1-D Chain to 3-D Framework”, Inorg. Chem., 2007, 46, 6283-6290).

The PPMS has also allowed Dr. Jiyeong Gu’s group to measure superconducting properties of magnesium diboride (MgB₂) thin film samples (Figure 3). AC susceptibility is the standard tool for determining the physics of superconductors, in particular for measuring critical temperature. In normal state (above the critical temperature), superconductors typically have a small susceptibility. In the fully superconducting state, the sample is a perfect diamagnetic and so $\chi' = -1$. Typically, the onset of a significant nonzero $\chi'$ is taken as the superconducting transition temperature.

Finally, Dr. Chuhee Kwon’s research group has used the PPMS to study the magnetic and transport properties of colossal magnetoresistance (CMR) (La,Pr)₀.₆₇Ca₀.₃₃MnO₃ thin films. Figure 4 shows the magnetic field dependent resistance and magnetization measurements of a doped manganite thin film sample.

Funding for this project was provided by an NSF Major Research Instrumentation Award NSF DMR-0619909.
Copper binding components of blood plasma and organs, and their responses to influx of large doses of $^{65}$Cu, in the mouse

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Copper is an obligatory trace element in all living organisms and has been identified as important for the functions of a discrete number of redox enzymes involved in varied aspects of mammalian metabolism. These include mitochondrial cytochrome c oxidase - the terminal step of respiration; dopamine-beta-monooxygenase and alpha-amidating enzyme - required for the formation of specific neurotransmitters; lysyl oxidase - needed for the cross-linking maturation of collagen and elastin in connective tissue; tyrosinase – which helps to form the pigment, melanin; and Cu/Zn superoxide dismutase and ceruloplasmin – which protect against reactive oxygen species.

Most of the information about copper in the various proteins described and the kinetics of its transport and excretion has been obtained from trace analysis of intracellular copper distributions and from radioactive isotopic dosage studies in rats. In humans, there have also been some studies using the stable isotope, $^{65}$Cu. Today, because of its size and ease of genetic manipulation, the mouse is the most widely used organism to model the transport and metabolism of specific nutrients or metabolites in humans; yet Cu metabolism in the mouse is not well studied, particularly with regard to copper transport and blood plasma binding components.

Indeed, earlier experimental work from our laboratory have indicated that there are significant differences in the number of mammalian copper proteins among the human and rat but also differs significantly in some aspects. The most striking difference is that mice have less than half as much copper in the circulating blood plasma as humans and rats. This is consistent with our earlier findings and is also in accord with the observation that mice also have less copper in their livers and kidneys. Thus, others have reported liver concentrations of $2.6 \pm 0.2$ug/g for 10-12 week old mice of the same strain used here, which is significantly lower than the values of $4.6 \pm 1.1$ and $6.2 \pm 0.8$ reported for rats and humans, respectively (means + SD, N=9-23). Similarly, mouse kidney values of $3.8 \pm 0.1$ are significantly less than the values of 7.9 and 12 for rats and humans, respectively. Heart and brain did not show marked interspecies differences.

This technique was also used to monitor the biochemical responses and long term fluxes of copper, into and out of organs of adult female C57CL6 mice before and after i.p. injection of large doses of $^{65}$Cu. Plasma from untreated mice had different proportions of Cu associated with transcuprein/macroglobulin, ceruloplasmin and albumin than in humans and rats, and two previously undetected copper peaks (Mr 700k and 15kDa) were observed. Cytosols had Cu peaks seen previously in rat liver (Mr $>100k$, 28k and 11kDa) plus one of 28kDa. $^{65}$Cu (141mg) administered over 14h, initially loaded plasma albumin and mainly entered liver and kidney (especially 28k and 11kDa components). Components of other organs were less, but still significantly, enriched. $^{63}$Cu/$^{65}$Cu ratios returned almost to normal by 14d, indicating a robust system for excreting excess copper. We conclude that there are significant differences but also strong similarities in Cu metabolism between mice, rats and humans; the liver is able to compensate for imposed changes in copper status and that a large number of mammalian copper proteins remain to be identified.

The results published in Biometals are the first showing that although the major copper cytoplasmic copper binding proteins responsible for coping with large doses of extraneous copper are similar to those previously reported in the rat that the mouse differs considerably from the rat and human with regard to the proportions of copper associated with various plasma proteins. The study also shows that it is possible to obtain significant, if not substantial, changes in the isotopic ratios of copper in proteins in organs peripheral to the liver—establishing the utility of stable isotopic Cu to study the long term metabolism of the metal over physiologically relevant time scales beyond those feasible using radioisotopic forms of the element. Relatively small doses of the stable copper isotope, $^{63}$Cu, have been used successfully in humans to study whole body intestinal absorption, transport and excretion kinetics, with the finding that long term retention and excretion of copper are inversely related to copper status and intake, those with low intakes retaining more and excreting less and vice versa. Studies in humans are obviously limiting, and do not allow the study of copper turnover in specific organs and their proteins, which is the advantage of using experimental animals. Our feasibility studies have shown that stable isotopic copper $^{65}$Cu administered i.p. to mice results in the detectable enrichment of specific copper proteins in all organs but the brain and particularly so in the liver and kidney. Although large doses of $^{65}$Cu were administered, the data indicate that physiologically relevant doses of stable isotopic copper can be used in conjunction with HPLC-quadrupole ICP-MS or, more preferably, multi-collector magnetic sector instruments to successfully model the long-term homeostatic capabilities and turnover of copper in the individual binding components of the tissues more actively involved in the metabolism and processing of this essential, but potentially toxic, trace element.
Proteomics to Develop Biomarkers of Environmental Impacts in Wild Marine Fish of Southern California’s Urban Ocean

Kevin M. Kelley§,¶, Andrew Z. Mason¶, Ashraf Elamin¶,€, and Jeffrey L. Armstrong*. Department of Biological Sciences, §Environmental Endocrinology Laboratory, ¶IIRMES, €CEPA, California State University, Long Beach, *Environmental Assessment Division, Orange County Sanitation District, Fountain Valley, CA.

Collaborative studies between our research groups have uncovered several striking instances of endocrine-disrupted states in marine fish species exposed to impacted environments of the Southern California Bight (SCB), such as near wastewater treatment plant (WWTP) outfalls or urban river outlets. The findings have made it clear that endocrine and physiological disruption is evident in a variety of different fish species, and that different endocrine systems can be significantly and simultaneously impacted dependent upon the study location and types of contaminants present. There is strong concern that disruption of endocrine systems, whose role is to maintain normal physiological functions, may have serious negative consequences to the well being of wild fish populations.

Most studies, including those conducted in our laboratories, have developed and used single or a limited set of biomarkers (e.g., measurement of hormone level, expression of a steroidogenic enzyme gene, etc.) to identify an impact in fish. However, the choice of which biomarker to use can be difficult at the outset of a study or environmental assessment effort. In addition, single endpoint bioassay approaches typically provide little or no insight on the larger physiological status of an impacted animal or on underlying mechanisms of the effects seen. It is the integrative perspective of an animal’s overall biological response to a given environment that is most directly relevant to estimating potential adverse effects.

Consequently, there is a significant need to develop more powerful diagnostic methodologies that will allow an integrated, relevant assessment of environmental effects in wildlife. “...Consequently, there is a significant need to develop more powerful diagnostic methodologies that will allow an integrated, relevant assessment of environmental effects in wildlife.”

Recent technological advances in the areas of genomics and proteomics have provided researchers with new tools for developing biomarkers. Most impressively, these technologies allow simultaneous measurement of multiple biomarkers that, collectively, can reflect the types of chemical (and/or other factors) exposure and the systemic (integrated) biological effects in animals. While genomics is proving useful in developing single and multiple biomarkers, it is important to recognize changes in gene transcription do not necessarily correlate with protein expression or protein activity, and that proteins are the primarily effectors for adaptive changes and cellular and physiological functions in response to environmental stimuli.

Thus, proteomics provides for an assessment of relevant phenotypic alterations, in which changes in the expression of a multitude of proteins can be measured simultaneously. While the individual proteins of interest can be developed as specific biomarkers themselves, the larger protein expression profile (PEP), or “fingerprint”, may also be used as an integrative biomarker of the phenotypic condition. Thus, a comparison of a control vs. altered condition in an animal reveals a set of biomarkers specifically indicative of the altered state and environmental fac-

Figure 1: Results of 2-D gel electrophoretic analysis of hepatic tissues from English sole residing at the EPA reference site (Top Panel) and the OCSD T-I outfall site (Bottom panel). Five proteins (a-e) exhibiting >2-fold changes between fish were excised and analyzed by MALDI TOF/TOF mass spectrometry (see figure 2).

Figure 2: MS spectrum of tryptic peptides from protein “c” (calmodulin), as identified by 2D GE (shown in Fig. 3). Individual peptide mass/charge (m/z) values shown at the top of each peptide peak.
tors at play. Therefore, with the advent of this emergent technology, we are now presented with the exciting opportunity to develop PEPs and new biomarkers as powerful diagnostic tools for classifying chemical exposures, predicting mode(s) of action, and for assessment of environmental biological impacts.

In prior collaborative studies between CSULB and Orange County Sanitation District (OCSD), English sole (Pleuronectes vetulus) collected at the OCSD outfall (off shore of Newport Beach) were found to consistently exhibit significant endocrine and physiologic disruption, as compared with fish from an EPA reference site near Dana Point, CA. Therefore, these two well-documented populations – impacted versus reference – are being compared to identify biomarkers and PEPs reflecting the impacted condition.

Two-dimensional gel electrophoretic (2-D GE) analyses of liver proteins of English sole from the OCSD outfall site and the Dana Point EPA reference site reveals substantial differences in their PEP, pointing to a strong potential for developing biomarker suites reflective of environmental effects (figure 1). Five of the proteins exhibiting >2-fold increases in intensity in the outfall group were initially chosen for study, excised from the gels, and processed for MALDI TOF/TOF mass spectrometry analysis. MS spectra (example shown in Figure 2) of each of the proteins were analyzed using the Mascot search engine and NCBI databases that include Actinopterygian fish protein databases. Four of the five proteins were positively identified using these MS spectra data, with each protein exhibiting very high confidence indices (>99.9%) of identification and reflecting important toxicological responses and physiological alterations in the English sole at the OCSD outfall location.

One of the identified proteins was glutathione-S-transferase (GST), a well known detoxification enzyme involved in catalyzing the conjugation of a wide variety of electrophilic substrates to reduced glutathione, thus protecting tissues from chemically induced damage. Expression of GST is known to be increased by exposure to environmental contaminants such as alachlor, dimethoate, and various others as well as by contaminated environments. Of note, a recent report indicates that the industrial contaminant, PCB 126, induces GST. This PCB congener also disrupts the stress response endocrine system in fish, a type of disruption consistently observed in our studies of English sole from the OCSD outfall location (see articles in this annual reports by Hamilton et al. and Kalman et al.).

A second protein identified was Cu/Zn Superoxide Dismutase (Cu/Zn-SOD), a metalloenzyme that catalyzes the dismutation of superoxide radicals ($O_2^-$) into $O_2$ and $H_2O_2$ (which is typically later reduced to $H_2O$ by glutathione peroxidase or catalase). Cu/Zn-SOD is an indicator of oxidative stress. Environmental exposures to copper in fish increase Cu/Zn-SOD, and also have disruptive effects on the stress response and thyroid endocrine systems in fish.

The third and fourth proteins identified were calmodulin and adalose B. Calmodulin is a ubiquitous, intracellular calcium-binding protein that regulates a variety of protein kinases and phosphodiesterases, serving as a key mediator of calcium-regulated biochemical and physiological events in cells. Its down-regulation in English sole points to a catabolic physiological status in the animals, in which hepatic glucose output (gluconeogenesis, glycolysis) has been activated.

The fifth protein chosen for analysis was not readily identified using the MS spectrum data (protein “e” in Fig. 1), and therefore a de novo sequencing approach was pursued. Using an MS peak from the MS spectrum from sample “e”, representing a peptide mass of 1,314.6 daltons, de novo analysis generated a set of peptide sequences as shown in Figure 3. A BLAST-P search against the NCBI database indicated that all the peptides strongly identified with liver-basic fatty acid binding protein. This signaling protein is involved in communicating the state of fatty acid metabolism from the cytosol of cells to the nucleus, and its increase may reflect additional changes in metabolic physiology of fish from the outfall location.

Thus, from an initial proteomic approach in defining the effects of a WWTP outfall site on English sole, five potential biomarkers of impact, and a simple PEP fingerprint (4 specific proteins up-regulated, 1 protein down-regulated), were identified. These exciting results point to the power of the modern proteomic methodology, and its strong potential in providing an integrative (“systems”) understanding of environmental impact.

In September of 2007, NOAA/Southern California Sea Grant funded a two-year project to continue these studies. As part of the effort, biomarkers and PEPs will be correlated with environmental and bioaccumulated tissue contaminant levels, as well as with physiological and endocrine measures of impact, allowing a highly integrative “systems” analysis. The proposed work takes as a first step toward developing specific phenotypic biomarkers of environmental effects in the SCB, and is at the forefront of environmental physiology and assessment research. This Sea Grant is also funding a CSULB graduate student fellowship, for Ms. Claire Wingard whose thesis research is centered on this work.
Laurel Fink, a new MS. candidate conducting research under the supervision of Dr. Steven Manley from the Department of Biological Sciences has been using the PE ICP-MS in FEMCA to test the hypothesis that the sieve tube sap (STS) of the macro-algae Macrocystis can be used as a bio-sentinel to identify sites of metal pollution and exposure.

Kelp have a primitive translocation system that transports a sugar-based fluid called sieve tube sap (STS) throughout the frond which has been shown by Dr. Manley in his previous research to bioconcentrate metals at levels 1-3 orders of magnitude higher than their total concentration in the ambient surrounding seawater. STS is primarily composed of mannitol (60% dry weight) and amino acids (15%), which are the immediate products of kelp photosynthesis, and inorganic ions (20%), but also contains trace metals such as As, Mn, Cd, Pb, Cr, Cu, and Zn. Many of these trace metals are released in the Southern California Bight (SCB) through runoff as common pollutants off our coast line. It is hypothesized the trace metal concentrations within the STS will reflect the bioavailability of the various metals in the neighboring marine environment. Providing that this relationship is directly robust, then this information can be used to extrapolate the biologically available concentrations of metals and identify potential pollutant outfalls in the coastal waters.

In this study, Laurel aims to use this approach using STS to identify plumes of polluted surface waters here in Southern California to be able to quantify which metals are present, and to determine the extent in which these waters travel along the coast. This information is crucial in risk assessment for different biological populations. ..

Laurel has spent this last summer extracting sap from selected fronds inside kelp populations down the length of the breakwater of the Los Angeles Port and Long Beach Harbor, along the Palos Verdes Peninsula, offshore of Escondido Beach in Malibu, and on the seaward side of Catalina Island (figure 2). The novelty of this research required a unique design of frond selection and extraction of the sap while in the field to ensure natural conditions were present (figure 3). Since the sap extraction procedures are performed offshore on a boat, all samples undergo an automated titration analysis for chloride content to ensure that no seawater contamination occurred during collection. Each month, Laurel collects 50 samples (250uL each) of this substance from her 10 field sites to analyze in the IIRMES lab for trace metal content.

Using the Perkin Elmer 6100 ICP-MS in IIRMES, she has quantified the level of contaminants in the STS. Laurel Fink is a M.S. student in Biological Sciences at CSULB. Laurel has received three scholarships so far to support her research; the SCTC Marine Biology Educational Scholarship, the Dr. Reish Marine Biology Award, and a grant from the PADI Foundation. Funding for this research was provided by NSF grants # OCE-9977564 and funds for the Facility for Micro–Chemical Analysis provided by CSUPERB.
a toxicity level.

For sample preparation prior to metal analysis on the ICP-MS, the sap is reflux digested on a hot plate with 2% HNO₃, then diluted down 20-30x with water containing 2% HNO₃ and 10mL⁻¹ of Gallium, Yttrium, and Thallium (used as internal standards). No less than 5 reagent blanks, and a blank spike are prepared similarly for comparison in each experiment. A calibration curve created with a standard containing a suite of trace metals provides a comparison to find concentrations of relevant metals within the sap.

Results so far have indicated the presence of at least 8 new elements that haven’t formerly been identified within Macrocystis STS including: Rb, Pd, Ag, Sn, and Cs. (figure 4). Table 1 shows values found for several of Laurel’s metals of interest in 5 of her field sites. While further statistical analyses have yet to be conducted to ascertain differences amongst the various collection sites, a cursory examination of the data collected so far shows some very exciting differences among sites, even in close proximity, that demonstrate the potential of STS in spatially identifying sites containing different concentrations of metals.

![Figure 3](image3.png)

Figure 3. Samples are carefully processed on board ship to extract the STS without contaminating the sample with seawater.

<table>
<thead>
<tr>
<th>Metals</th>
<th>LB Harbor: Inside Breakwall</th>
<th>LB Harbor: Outside Breakwall</th>
<th>Bunker Point, PV</th>
<th>Escondido Beach, Malibu</th>
<th>Kelp Point, Catalina Island</th>
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<tbody>
<tr>
<td>V</td>
<td>153.20</td>
<td>217.94</td>
<td>169.64</td>
<td>120.42</td>
<td>226.93</td>
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<tr>
<td>Mn</td>
<td>112.20</td>
<td>89.45</td>
<td>78.80</td>
<td>72.30</td>
<td>76.85</td>
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<tr>
<td>Ni (58)</td>
<td>142.60</td>
<td>187.22</td>
<td>100.02</td>
<td>88.67</td>
<td>97.74</td>
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<tr>
<td>Co</td>
<td>91.14</td>
<td>50.30</td>
<td>47.19</td>
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<td>Cu</td>
<td>392.74</td>
<td>501.81</td>
<td>403.76</td>
<td>325.76</td>
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<td>Fe</td>
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<td>2077.12</td>
<td>1370.10</td>
<td>1107.34</td>
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<td>Zn (64)</td>
<td>1664.99</td>
<td>1643.51</td>
<td>724.52</td>
<td>835.18</td>
<td>609.58</td>
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<tr>
<td>As</td>
<td>33577.08</td>
<td>67824.54</td>
<td>56037.7</td>
<td>71670.50</td>
<td>72916.4</td>
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<td>Se</td>
<td>167.71</td>
<td>299.17</td>
<td>130.26</td>
<td>146.86</td>
<td>105.54</td>
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<td>Rb</td>
<td>1885.79</td>
<td>3373.77</td>
<td>2918.95</td>
<td>3227.88</td>
<td>2038.43</td>
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<tr>
<td>Sr</td>
<td>879.75</td>
<td>2315.21</td>
<td>1239.86</td>
<td>979.33</td>
<td>1269.88</td>
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<tr>
<td>Ag</td>
<td>134.26</td>
<td>97.11</td>
<td>96.99</td>
<td>107.40</td>
<td>94.74</td>
</tr>
<tr>
<td>Cd</td>
<td>510.04</td>
<td>870.97</td>
<td>973.79</td>
<td>1186.76</td>
<td>777.11</td>
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<tr>
<td>Cs</td>
<td>54.63</td>
<td>9.74</td>
<td>8.99</td>
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<td>8.84</td>
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<tr>
<td>Ba</td>
<td>173.45</td>
<td>151.50</td>
<td>126.33</td>
<td>162.41</td>
<td>139.23</td>
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<tr>
<td>Pb</td>
<td>272.33</td>
<td>254.59</td>
<td>259.55</td>
<td>437.63</td>
<td>206.14</td>
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</tbody>
</table>

Table 1: Macrocystis STS collected in August 2007 analyzed by ICP-MS; all values are in ppb, mg/L⁻¹ and represent an average of 5 samples taken.

![Figure 4](image4.png)

Figure 4: An STS sample from the LB Harbor analyzed by ICP-MS in the scanning mode through the periodic table to define the elements present in the sample. Expanded part of the mass spectrum from 100-140 showing elevated Cu, Ag, I, Sn, Cs, and Ba in the sample. The In is from the internal standard while Xe is an impurity in the argon used for the plasma.
Monitoring metals in Soils and Edible Produce in the LA basin

Peter Mack and Dr. Oliver Seely, Department of Chemistry, California State University Dominguez Hills, CA.

Prior to the arrival of European settlers, the population of native Americans in southern California was estimated to have been a few thousand individuals. The arid climate was not conducive to ambitious farming and a large population. That farming which was practiced depended either on the periodic trickle of the half-dozen rivers in the region or on those few places where percolating water from the Coast Mountain Range broke through the surface of the alluvial fan to irrigate small plots of fruits and vegetables of what is today the Los Angeles Basin.

The dream of developers to sell real estate to satisfy the needs of a burgeoning population by transporting water to the LA area, a region which can be properly classified as desert, became a reality with the growth of the California Water Project in the 20th century, first with the Owens Valley Aqueduct, bringing gravity feed water from the eastern watershed of the Sierra Nevada range into the San Fernando Valley and dedicated in 1913. This was followed by the Colorado River Aqueduct, built between 1933 and 1941 and finally the California Aqueduct, on the western watershed of the Sierra Nevada Mountains, dedicated in 1971. With the water came increased agriculture, business, industry, population and pollution including nitrogen oxide, lead and hydrocarbon vapor pollution from automobiles, dispersal of chromium, cadmium, copper and arsenic from the plating and paint industries and chemical waste deposited in landfills with subsequent seepage into the soil and ground water.

The problems associated with the lack of natural dispersal mechanisms for these accumulated pollutants in the off-shore corridor between the breakwater and the L.B./L.A. harbor areas was of concern to Peter Mack, a retired real estate broker, lode and placer miner and returning student at CSU Dominguez Hills (CSUDH). Under the mentorship of Dr. Oliver Seely, of the Department of Chemistry at CSUDH, an expert in urban dust fall, NO2 and soil contamination by chlorinated hydrocarbon solvents, Peter Mack has been using the PE ICP-MS at CSULB to study the concentration of fourteen heavy metals: Chromium, copper, arsenic, selenium, strontium, molybdenum, silver, cadmium, tin, antimony, mercury, lead, bismuth and uranium at 60 sites that were considered to be likely points of contamination because of their proximity to industry and other activities during the last century. The sites included playgrounds, public parks, flood control channels, culverts, oil well footprints, campgrounds and selected locations in the San Gabriel and Los Angeles River channels. Cadmium, chromium and lead, elements that are known to have associated risks to human health, were found at concentrations ranging up to 3000 parts per million in some sites. Uranium was found at two unlikely spots (but near enough to each other to offer some mutual corroboration) at a concentration of 150 parts per million.

Although the data are still preliminary, thirteen sites showing significantly elevated levels of two or more of the elements lead, chromium, arsenic and cadmium or significant trace amounts of uranium have been tentatively targeted for future surveys that will involve the use of undergraduates to study the absorption and bioaccumulation of these metals in plants grown to produce edible fruits and vegetables. This information will be used to evaluate potential human risk factors for residents who grow produce for consumption in these areas.

Peter Mack undertaking soil sample collection for strong acid digestion and metal analysis by ICP-MS

Sample preparation and archiving back in the laboratory

Peter Mack is a student in Chemistry at CSUDH. Funding for this research was provided by NSF grants # OCE-9977564 and funds for the Facility for Microchemical Analysis provided by CSUPERB.
Earth metals served as internal standards to normalize the signals between runs. A preliminary experiment used the results for these 22 samples to calibrate the Oxford Instruments Twin-X Benchtop XRF Spectrometer (Fig. 1). This unit contains two separate detectors: the Focus 5 and the PIN diode. The Focus 5 is more sensitive, but optimized for lower molecular weight elements (S16 to U92). The PIN diode is optimized for higher molecular weight elements (S16 to U92).

Initial XRF runs were performed using 10 whole milk powder (WMP) samples from California Dairy Research Foundation and the CSUPERB, Dairy Management Inc., the California Dairy Research Foundation and the CSU-Agricultural Research Initiative.

Table 1. Measurement conditions for detectable elements of preliminary experiment. Please note that, while detected, no ICP-MS data for Br, P, and Sb were available.

<table>
<thead>
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<tr>
<td>Voltage (kV)</td>
<td>39</td>
<td>19</td>
<td>4</td>
<td>3</td>
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<td>Tube Current (µA)</td>
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<tr>
<td>Live Time (s)</td>
<td>118</td>
<td>180</td>
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<td>180</td>
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<tr>
<td>Analytes</td>
<td>Cl, K, Ca, Zn, Br</td>
<td>P, Sb, Fe</td>
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<td>Mg</td>
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Figure 1. Oxford Instruments Twin-X Benchtop XRF Spectrometer

Non-destructive Energy Dispersive X-ray fluorescence (ED-XRF) is a technique that can be used to determine the mineral profile of samples in their solid state, thus, avoiding sample digestion via toxic and corrosive acids. Such techniques require very little sample preparation and results are obtained in a matter of minutes. ED-XRF generates X-rays using a palladium source, firing them at the sample. The X-ray removes an electron from the innermost shell of the sample, causing an electron from the next closest shell to take its place. The energy emission from this electron jump, in the form of X-rays is characteristic of the emitting element. The spectrophotometer can detect these X-rays and the energy and intensity used for identification and quantification.

Widely used in the soil and cement industries, XRF techniques are logical choices for the food quality assurance - specifically, for flours, spices, dried mixes, and dry milk. The dry milk industry

Verification of ED-XRF as a reliable method for determining the mineral composition of skim milk powders

Salvador Uson III 1, Chad Immoos§, & Rafael Jiménez-Flores†, †Department of Dairy Science, Dairy Products Technology Center, California Polytechnic State University San Luis Obispo, and , §Department of Chemistry, California Polytechnic State University San Luis Obispo

“...while XRF is convenient, it cannot accurately detect trace elements in samples. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), however, is a well-established method for trace elements (Na11 to Fe26). The PIN diode is optimized for higher molecular weight elements (S16 to U92).”

Nonfat dry milk, or skim milk powder (SMP), is made by removing water from pasteurized skim (nonfat or fat free) milk. It contains no more than 5% by weight moisture, and no more than 1.5% by weight milkfat unless otherwise indicated. It contains no more than 5% by weight phosphorus and no more than 1.5% by weight potassium. Calcium is readily available in the food supply. In addition, milk and other dairy products, the major sources of calcium in the US diet, provide more than 70% of the calcium available in the food supply. In addition, milk is also high in phosphorous, magnesium, and potassium. Calcium is readily absorbed by the body, while phosphorus plays a role in the calcium absorption and utilization. Phosphorous is needed in the proper ratio to calcium for bone formation. A calcium deficiency can result in the bone deterioration known as osteoporosis. In the US, those primarily not meeting the daily recommendations for calcium are young and adolescent girls and older adults.

Nonfat dry milk, or skim milk powder (SMP), is made by removing water from pasteurized skim (nonfat or fat free) milk. It contains no more than 5% by weight moisture, and no more than 1.5% by weight milkfat unless otherwise indicated. It has become an important commodity of the dairy industry, also serving as a common ingredient for bakers and confectioners. The US, one of the leading exporters of nonfat dry milk, exports 258 thousand tons of powder.

Salvador Uson III, a masters student from California State University San Luis Obispo, recently visited IIRMES to undertake ICP-MS analyses of trace elements in milk samples. The nutritional benefits of milk are well-known and have been recognized throughout human history. Milk and other dairy products, the major sources of calcium in the US diet, provide more than 70% of the calcium available in the food supply. In addition, milk is also high in phosphorous, magnesium, and potassium. Calcium is readily absorbed by the body, while phosphorus plays a role in the calcium absorption and utilization. Phosphorous is needed in the proper ratio to calcium for bone formation. A calcium deficiency can result in the bone deterioration known as osteoporosis. In the US, those primarily not meeting the daily recommendations for calcium are young and adolescent girls and older adults.

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Figure 1. Oxford Instruments Twin-X Benchtop XRF Spectrometer

Verification of ED-XRF as a reliable method for determining the mineral composition of skim milk powders

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ples as controls for a 74 skim milk powder sample set. Samples for XRF were formed into pellets using 4 g of powder under 15-20 tons of pressure in triplicate. In order to calibrate the spectrometer, initial spectrum scans were performed on one of the samples to determine which elements are detectable by means of XRF. The detected elements are represented as peaks in the spectra, which are quantified with ICP-MS standard values. Measurement conditions for this initial experiment are shown in Table 1. Preliminary data has shown that the best correlation between both XRF and ICP-MS techniques is for calcium.

Statistical analysis was carried out on the XRF results of this initial experiment. Using principle component analysis, the 74 SMP samples were compared against the 10 WMP controls. The score plot (Fig. 4) shows the mineral variability of the SMPs as compared to the clear grouping of the WMPs. The loading plot (Fig. 5) shows where these variations occur. The WMPs appear to have higher Al, Zn, Fe, Ca, K, and Cl contents than the SMPs.

ICP-MS was once again used to derive a more comprehensive elemental profile for a total of 95 powder samples, including trace elements. A CEM Mars 5 microwave digester allowed for a more efficient nitric acid digest of the samples. Samples were once again diluted and spiked with In, Y, Tl, and Ga. The samples were analyzed for the following: Be, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Pb, Na, Mg, K, Ca. Future research will use these values as standards to calibrate the XRF for the determined mineral concentrations. Additionally, the XRF results will be compared to those of ICP-MS to verify capabilities. For example, the nature of milk samples (having extremely high Ca and K signals), make detection of several trace elements impossible. This makes more complete testing methods, such as ICP-MS, necessary to validate and complement our current XRF procedures.

References


The Application of Proteomics for Studying the Differential Expression of Muscle Proteins under Different Physiological States

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Over the last year the Rourke laboratory at CSULB has initiated a proteomics project to study the differential phenotypic expression of proteins and, in particular, the myosin heavy-chain contractile protein that determines the physiology activities and contractile tensile properties of vertebrate skeletal and cardiac muscle. Myosin is the major force producing component of muscle, and has many functionally different isoforms; each protein variant is a distinct gene product and is differentially expressed in individual muscles. The control of myosin protein expression is complex, and very sensitive to activity, temperature, and hormone influences. Skeletal muscle and the heart are both affected by exercise, resistance training, and other factors, which may dramatically change the muscle size and myosin heavy chain composition. Current research in the laboratory aims to elucidate the shifts or transitions in myosin protein expression with increased activity, or conversely, with disuse.

As a proponent of the comparative approach, the Rourke laboratory investigates a wide variety of vertebrates having distinct and sometimes uniquely remarkable aspects of muscle physiology. The principle of comparative physiology employs a methodological approach in which the similarities and differences in the natural adaptations and species-specific physiology of animals are evaluated to elucidate mechanisms and limits of organ systems. In the case of the Rourke laboratory, the specific adaptations that non model animals, have with respect to skeletal muscle often are far more dramatic and therefore elucidating than those of more commonly studied mammals such as humans or rats, and provide greatly expanded understanding of muscle biology.

The techniques involved are elementary to muscle physiology and molecular biology, but are somewhat novel in their application to these diverse organisms. Protein gel electrophoresis is useful for measuring the expression of myosin isoforms in individual muscles, or even individual muscle fibers. Reverse-transcriptase polymerase chain reaction (RT-PCR) allows estimation of the mRNA expression of each myosin gene, and in general, myosin genes from new species are sequenced where possible. The addition of proteomics analyses to the procedural repertoire adds an additional dimension of inquiry and discovery that will enable the broad changes in muscle state that occur between two different temporal conditions to be observed in a way that was previously impossible.

Dr Rourke aims to use 2-D gel electrophoresis and MALDI-TOF-TOF to study the muscle physiology of hibernating. Hibernating mammals present many interesting opportunities to study muscle biology. Many mammals, from orders as diverse as rodents, bats, bears, and even primates, spend months in a state of lowered metabolism and reduced physical activity. In the few instances where it has been investigated, the muscles of these hibernators demonstrate the remarkable ability to withstand disuse atrophy and a loss of oxidative metabolism. This has now been studied in golden-mantled ground squirrels (figure 1), prairie dogs and black bears using SDS-PAGE, and after cloning the myosin genes, RT-PCR. Myosin expression is altered following up to 6 months of inactivity, but in the direction of more oxidative, fatigue-resistant isoforms, uncharacteristic of disuse in non-hibernators. The Comparative Muscle Physiology lab continues to work on the possible transcriptional control and environmental factors that influence hibernating muscle phenotypes. Proteomics data is shortly forthcoming on the changes in protein expression, beyond myosin, that occur with hibernation. It is anticipated that the proposed research will uncover important regulatory proteins and their roles in protecting muscle function throughout hibernation.

An increase in postprandial metabolism has been well documented in carnivorous lizards and snakes. Pythons, for instance, have been reported to undergo a rapid upregulation of many tissues, including an enlargement of the heart, to support the increased metabolic demands after eating. With collaborators James Hicks, Albert Bennett and Johnnie Andersen at UC Irvine, Dr Rourke has demonstrated a remarkable 40% hypertrophy of the python ventricle within two days of consuming a large meal (25% of body mass). His laboratory is now examining the possible hormonal signals that trigger this event, and they are continuing to characterize the cardiovascular contribution to the metabolic response. To date, they have sequenced the myosin genes from the heart of Python molurus (figure 2) and are using molecular techniques to examine the response of other tissues. Proteomics analyses will be suitably employed in an extension of these studies to evaluate the diversity, magnitude and the temporal characteristics in the expression of proteins in the heart muscle of pythons as they transition between the fed to the fasted states.
Grasses are a diverse family of plants comprising approximately 10,000 species, including the cereal crops barley, corn, oats, rice, rye, sorghum, teff and wheat. With the exception of the earliest diverging lineage (subfamily Anomochlooideae), all grasses have a unique floral structure and arrangement that distinguishes them from all other flowering plants.

Grass flower clusters (inflorescences) are comprised of spikelets that, depending on the species, contain from one to forty florets. Each floret typically consists of a pistil, three stamens, two modified petals called lodicules, and two novel outer whorl organs called a palea and lemma (Figure 1). The morphological origins of the palea and lemma have been disputed for centuries and remain controversial to this day. In a research project now supported by the National Science Foundation, our research group is using the FEMCA FEI ESEM to investigate the morphological development of flowers in diverse grasses and immediate grass relatives. These structural studies complement our developmental genetic and molecular evolutionary analyses which, collectively, aim to unravel the complex evolutionary origins of the palea and lemma.

The earliest diverging lineage of grass comprises 4 tropical species and is characterized by floral structures (“spikelet equivalents”) intermediate between the floral structures of all other grasses and the typical monocot flowers of the closest relatives (Joinvillea [Joinvilleaceae] and Ecdeiocolea [Ecdeiocoleaceae]). By using phylogenetic methods and dense sampling within subfamily Anomochlooideae, typical grasses and the closest relatives we

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can reconstruct the pattern of change that resulted in the evolution of the palea and lemma. By incorporating developmental genetic data into the analysis we will test hypotheses for the genes responsible for the evolution of these novel floral structures.

This project is conducive for both undergraduate and graduate student involvement and offers participating students opportunities to be trained in a number of different state-of-the-art techniques ranging from molecular biology to SEM to study the linkages between genotype and phenotype that dictate form and function. Undergraduate researcher Daniel Woods and graduate student Ashley Christensen (Figure 2) have helped capture images on the IIRMES ESEM of early inflorescence development in *Streptochaeta angustifolia* (subfamily Anomocholoideae) and *Joinvillea ascendens* (Figure 3). The *Streptochaeta* images illustrate early inflorescence development and acropetal initiation of six spikelet equivalents (Colors, Figure 2b) ranging from initiation (lilac, Figure 2b) through the formation of bracts I-V (green, Figure 2b). These images indicate that bracts I-V and the abscission zone beneath the spikelet equivalent can be discerned before the other organs. Additional stages will complete the developmental series of this critical early diverging grass. The spiral initiation of *Streptochaeta* is in direct contrast to the distichous branching arrangement in other grasses. Images of *Joinvillea* reveal an unexpectedly complex pattern of inflorescence branching, indicating that the complex branching characteristic of the species is determined at a very early morphological stage.

Graduate and undergraduate researchers in the laboratory will continue to use the IIRMES ESEM to investigate inflorescence and floral development in diverse grasses and immediate grass relatives during the course of the 3-year award from the National Science Foundation.

In addition to being used in research, undergraduate students in the upper level plant morphology class (BIOL 439) will also use the FEMCA ESEM to collect images of developing grass inflorescences as part of semester long group projects investigating flower development in cereals. Using the developmental data collected on the ESEM the students will then use bioinformatics and published mutant genetic analyses to hypothesize which genes are regulating the various developmental stages and how crop yield might be improved by regulating the activity of the different genes. The SEM images collected by the Plant Morphology students will also be posted in Botanical Society of America Online Image database (http://www.botany.org/plantimages/) and used in a collaborative project with the Botanical Society of America and BioQUEST to develop learning modules investigating the developmental and genetic basis of grass floral diversity. Undergraduate students across the nation will use these data collected on the IIRMES ESEM in their inquiry-based learning of the plant morphological development.