

Biotechnology Center Director of Proteomics

Dr. Peter Yau began as Director of Proteomics of the Biotechnology Center at the University of Illinois in February 2003. Dr. Yau received his undergraduate degrees in both Biology and Chemistry at the University of Oregon. His graduate training includes both Washington University (St. Louis) and Liverpool (UK). Dr. Yau worked at the University of California, Davis from 1977–2003 where he was a researcher and equipment manager.

My research has been on the structural and functional relationship of post-translational modifications (PTM) of histones and chromosomal proteins. Histones are basic proteins that form the building blocks on nucleosomes, accounting for the “beads-on-the-string” on chromosomes. In addition to the charge neutralization on DNA, the dynamic change of histones and their modifications also serve as “signal” and “code” to many of the DNA processes such as transcription, DNA replication, damage, repair, spermeogenesis and development etc. Most of the signals in the “histone code” are PTMs with the major ones being acetylation, phosphorylation, methylation, glycosylation and ubiquitination.

As director, my goal is to organize and constitute a proteomics facility at the UIUC over the next year, offering campus researchers improved ability to characterize proteins. This new facility involves both reorganizing several of our facilities as well as expanding our capabilities for protein characterization. The proteomics facility consists of the PSF (Protein Sciences Facility), IRC (Immunological Resource Center) and FCF (Flow Cytometry Facility). The IRC and FCF will remain unchanged in producing antibodies and supporting the UIUC campus need for flow cytometry, both essential tools for proteomic studies. The PSF will continue its service for peptide synthesis, and Edman sequencing. Our goal is to add the ability to handle complex samples, separate them using a variety of state-of-the-art separation methods into relatively pure fractions and

continued on page 2

David Clayton Awarded NIH Songbird Grant

Professor David Clayton of the Department of Cell and Structural Biology was awarded a NIH grant for the songbird neurogenomics initiative, which merges cutting-edge genomic technology with a powerful and unique neurobiological model system, the songbird. With the sequencing of the human genome and the rapid evolution of cost-effective genomic technologies it has now become practical to tackle the ambitious goal of producing a comprehensive description of gene expression in the songbird brain. Songbirds have become an important and productive model for neurobiology, for a number of reasons. In the wild, songbirds are highly visible and available for observation in a natural context. Because they are small, have short generation time relative to other complex vertebrates, and can be bred in captivity, laboratory research on songbirds have surged in recent years. By far the most studied single species is the zebra finch which is native to dry areas of Australia, and is the easiest songbird to breed. Zebra finch males sing a song as part of the courtship ritual and copy their songs from their fathers during an earlier critical period of development. Study of neurons in the neural circuit therefore offers tremendous advantages for understanding the cellular events that open critical periods of development and that underlie synaptic plasticity and learning. This system has become one of a very small group of model systems for the study of the cellular and molecular basis of vertebrate learning. Molecular

continued on page 3



Summer 2003

IN THIS ISSUE

3

Microarrayer for
Functional Genomics

4

Toll-like Receptors:
Essential Mediators of
Innate Immune
Recognition

5

High Throughput
Computation Framework
for Large-scale
Comparative Genomics

6

New Associate Director of
Biotechnology Center

Three UIUC Students are
Sponsored Delegates

7

Cytometric Bead Array
for Cytokine Assay

Dr. Mark Band Wins
CAPE Award

8

Student Science Poster
Competition—Spring 2003

Biotechnology Center Director of Proteomics, continued from page 1



Dr. Peter Yau

then to analyze them using enzymatic and mass spectrometric approaches. We want to automate these procedures as much as possible to allow higher quality data faster.

How do we perform a proteomics measurement? Mass spectrometry has been the tool of choice for characterizing the identity and for studying the modifications of proteins. It has been estimated that there are about 200 possible modifications of a protein with the major ones being phosphorylation, methylation and glycosylation. The use of mass spectrometry for analyzing biological molecules has increased exponentially in the past decade. MALDI and electrospray are the most common ionization methods for protein samples. In MALDI (Figure 1A), samples are crystallized with matrix and subjected to ionization. In electrospray, the samples can be directly injected or continuously fed in an ion-trap quadrupole mass spectrometer from a HPLC. Currently we are using the mass spectrometers in MSF (Mass Spectrometry Facility) under the School of Chemical Sciences.

While most researchers have complex mixtures of proteins, the samples must be purified before analysis to yield

high quality results. The typical purification methods are by fractionation using salt and solvent, gel electrophoresis, column chromatography (ion-exchange, affinity, HILIC and RP), immunological methods etc. Two-dimensional (2-D) electrophoresis is by far the preferred method of separation for biological samples. The first dimension gel is based on isoelectrofocusing and the second dimension gel is SDS polyacrylamide gel electrophoresis. We already have the ability to purify samples using such technology and hope to auto-

mate the separation, identification and processing in the coming months.

As an alternative to the gel-based 2-D system, it is also possible to separate proteins according to their pIs by chromatofocusing. Chromatofocusing uses an ion-exchange column and ampholyte to generate a pH gradient using a HPLC/FPLC system. Proteins are eluted from the column according to their pIs and processed for further analysis. Perhaps the most common alternative method of protein fractionation and analysis is LC-MS (Figure 1B). The protein sample is first digested with trypsin and loaded on a reverse-phase column. The digested peptides are eluted from the column and fed into a mass spectrometer. The peptides are ionized and the mass of the samples are detected and recorded and subjected to a thorough search of the protein database. A new method of analysis called MudPIT (multi-dimensional Protein Identification Technology) was developed by Yates group in UCSD. MudPIT (Figure 1C) allows analysis of complex mixtures of proteins because of the resolving power of the multiple columns. It has been shown to be an excellent complement to samples missed by the 2-D electrophoresis method. We hope to be able to offer this service in the coming year.

The field of proteomics has evolved from measuring protein masses to identification, *de novo* sequencing, structural studies, dynamic changes of post-translational modifications, functional studies and association with other components and factors. These studies in conjunction with the advances in genetic discoveries are becoming the new frontiers of science. I am excited about the opportunities at UIUC for proteomics. One key factor of success for the proteomics efforts at UIUC depends on the support and needs of the users. Please stop my office to discuss your proteomics needs with myself or with one of our staff. My goal is to make the new facility responsive to everyone's measurement challenges.

—Peter Yau

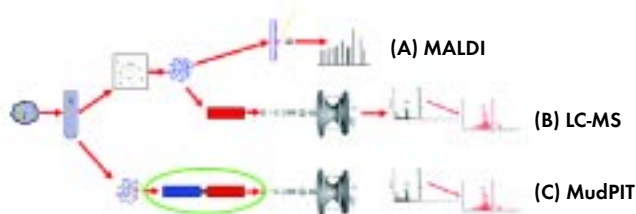


Figure 1. Protein Analysis by Mass Spectrometry (Courtesy of Thermo-Finnigan)

Late Breaking News!

The BTC just received a \$500,000 Roy J. Carver Charitable Trust Scientific Instrumentation Grant to upgrade our proteomics measurement capabilities and throughput! This equipment will automate isolating and preparing protein samples for mass spectrometry, as well as performing analysis

on smaller samples. This is welcome news and puts Peter Yau, our Director of Proteomics, off to a fast start (Dr. Yau is featured in this newsletter). We wish to thank the Roy J. Carver Charitable Trust for this award!. Make sure to stop by the Protein Science Facility this Fall to see the new equipment.

—J. Sweedler

David Clayton Awarded NIH Songbird Grant, continued from page 1

studies of song learning have contributed to an understanding of the functional biology of molecules such as synuclein, which was discovered independently in a screen for genes regulated during song learning and subsequently found to be centrally involved in Parkinson's and other neurodegenerative diseases.

In addition it was found that the adult songbird brain makes new neurons, overturning the universally held belief that neurogenesis does not occur in adults. This discovery of adult neurogenesis led to studies proving that significant neurogenesis occurs daily in the adult rodent and primate brain. It is fair to say that research in songbirds led to a large shift in the field of neurology, so that a major modern goal is to control the development of stem cells so that they can be introduced into the brain to replace or repair damaged neural circuits. The adult songbird remains one of the best models to study the functional significance and control of neurogenesis.

In order to copy his father's song, the young zebra finch receives sensory input and uses that neural activity to sculpt motor output via auditory feedback. This process of auditory processing and sensory-motor integration has explicit parallels to the process of speech learning in humans. The circuits that mediate this have been increasingly appreciated as homologs of mammalian cortical-striatal-cortical pathways. The songbird, which has long been recognized as a principal model of vocal learning, is now emerging as a new model for study of basal ganglia design and function. Although motor learning of song in zebra finches is limited to juvenile males, both sexes use the sound of song throughout life to discriminate among individuals and support various social interactions. A breakthrough came with the discovery that perception

of novel song causes up regulation of gene expression in specific brain regions in adult birds. The study of gene regulation in adult nervous system function in songbirds has led to the realization that gene regulation plays an active and ongoing role in the operation of the nervous system, integrating information on a timescale of minutes-to-hours – a process that has been dubbed the “genomic action potential.”

Songbirds are also a major model for studying the influences of steroid hormones on neural networks. Both brain circuit development and singing behavior have been shown to depend on various gonadal steroids in songbirds. Even more surprising, the songbird brain itself synthesizes estrogen, a steroid normally thought to derive from the gonads and which has a number of beneficial effects on brain function and repair.

All of these studies on the functional biology of songbirds has had dramatic impact on concepts of brain development and function in humans because the concepts developed first from discoveries on songbirds have been subsequently found to apply to mammals. Now, Dr. David Clayton, working with the W.M. Keck Center, will study gene expression in the songbird zebra finch to gain further insight into the molecular and cellular processes that underlie normal brain development and function. Up to 50,000 expressed sequence tags (ESTs) will be sequenced from normalized and subtracted zebra finch brain cDNA libraries derived from combination of adult and juvenile brains of both sexes. The goal will be to create a “unigene” set of up to 20,000 genes, which would represent a majority of the genes being expressed in the songbird brain. Gene expression studies will then be conducted using cDNA microarrays from this unigene library.

—D. Clayton



Microarrayer for Functional Genomics

The Functional Genomics Lab of the W.M. Keck Center has purchased a new OmniGrid Arrayer from GeneMachines for making cDNA and Oligonucleotide microarrays. The arrayer was purchased by joint funding of the BTC, the Provost, and eight departments and colleges. For further information contact Dr. Mark Band, Director of Functional Genomics, at markband@uiuc.edu.

—M. Mikel

Toll-like Receptors: Essential Mediators of Innate Immune Recognition



Dr. Richard
Tapping

Dr. Richard Tapping joined the Department of Microbiology in August of 2002. Dr. Tapping completed a postdoctoral fellowship at The Scripps Research Institute in La Jolla, California where he studied innate immune responses to Gram-negative bacterial endotoxin.

All higher eukaryotes possess an immune system whose ultimate function is to eliminate non-self while retaining self. How the immune system accomplishes this essential task has been a long standing question in biology. It has been appreciated for some time that specialized immune cells produce proteins, such as antibodies, that recognize and remove specific foreign antigens. This adaptive immune response is robust but takes several days or weeks to develop. What protects the host from invading pathogens during the initial stages of infection? Moreover, what triggers and directs

the actions of the adaptive immune response? The answer to both questions is the innate immune system.

Research in my laboratory is focused on a family of cell surface molecules, called Toll-like receptors (TLRs), which have been recently identified as essential components of the innate immune system. Humans possess ten TLR family members

which recognize structural features unique to viral, bacterial and fungal organisms (see figure). In response to these non-self structures, TLRs activate intracellular signaling cascades that result in the cellular production of molecules designed to destroy the invading pathogen, as well as various immune modulators and co-stimulatory molecules that alert and guide the actions of the acquired immune response. Thus, TLRs provide a direct link between pathogens and the cellular immune system of the host. The importance of TLRs in immune defense is underscored by the fact that

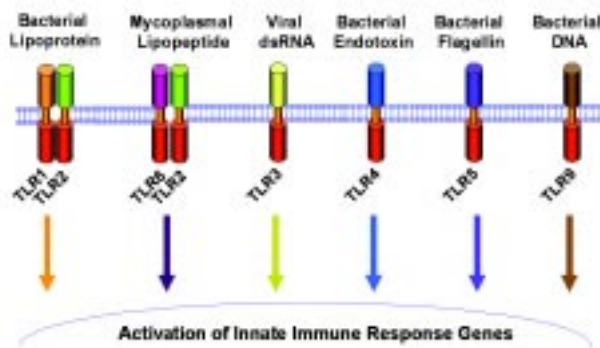
patients with mutations in these receptors, or their downstream signaling components, suffer from recurrent infections.

Despite the rapid advances in this field, the mechanism by which TLRs enable cells to recognize and mediate appropriate responses to a given pathogen is largely unknown. Recent studies indicate that some pathogen-derived products initiate cell activation through the cooperative actions of different TLR family members (see figure). The functional and physical basis for this cooperative signaling is currently being explored in our lab using reconstitution and knock-down assays as well as a variety of molecular, biochemical and fluorescence imaging approaches.

Although all TLRs share the ability to activate a common group of inflammatory genes, there are additional signaling pathways and genes that are uniquely associated with certain TLRs. For example, only stimulation of TLR3 by viral double stranded RNA leads to the potent activation of genes encoding type one interferons, molecules specifically required for antiviral immunity. Thus, each TLR-mediated response appears to be tailored toward eliminating a specific class of pathogen. Identifying TLR specific responses and delineating their intracellular signaling pathways is an important and integrated area of research in our laboratory.

As TLRs are central to initiating cellular innate immune responses that direct the subsequent actions of the acquired immune system, their study has many potential clinical applications. The most obvious is the control of septic shock, an often fatal consequence of infection brought about by uncontrolled stimulation of the innate immune system. Conversely, enhancing TLR-mediated cell activation is being explored as an approach to rationally design more effective vaccine adjuvants, not only to protect the host from future infections, but also to boost immunity to other invasive disease states such as cancer. The study of TLRs attempts to address the core question as to how the innate immune system ultimately discriminates between self and non-self. This question is central to understanding the pathogenesis of a wide variety of deleterious immune conditions.

—R. Tapping



A variety of microbial and viral products directly activate immune response genes through specific Toll-like receptor family members. In some instances certain combinations of TLRs have been shown to cooperate in cell activation. This would presumably enable cellular responses to a much wider variety of pathogen-associated molecules. The microbial agonists for TLRs 7, 8 and 10 are currently unknown.

New Associate Director of Biotechnology Center



Dr. Mark Mikel

We welcome Dr. Mark Mikel the new Associate Director of the Biotechnology Center, Mark started work at the University of Illinois in January and has remained very busy ever since. Mark came from Illinois Foundation Seeds, Inc. (IFSI) where he had worked for twenty years and was Director of Biotechnology. Mark earned his B.S. from Purdue University in 1978, M.S. and Ph.D. from the University of Illinois in 1980 and 1983, respectively.

The work of the BTC is exciting because our mission spans genomics and proteomics, and due to the large number of investigators on campus who use our services. I am enjoying working both with the BTC staff and campus faculty. In all of the BTC facilities it is an ongoing process to modernize our equipment with new technology platforms to increase throughput, quality of data, and reduce cost. In our Functional Genomics Unit, both cDNA and oligonucleotide microarrays have proven a valuable tool in monitoring gene expression. As a result, we have added an OmniGrid microarrayer (featured in this newsletter), which will dramatically increase microarray slide throughput. To sustain this high throughput a robot and another PCR tetrad will be added. Our DNA Sequencing Unit works on numerous sequencing projects for over 200 faculty on campus, plus additional projects for researchers across the country. To continue to support the torrid growth of our Core and High Throughput DNA sequencing a pending NIH equipment grant, if awarded, will enable us to

modernize our DNA sequencers by adding two ABI 3730 sequencers. These will generate longer read lengths, with less chemical input costs and do all of this in 50% shorter run times resulting in better quality data, faster at less cost to the user. We will continue our focus in our oligonucleotide synthesis service on custom oligonucleotides, which several campus researchers depend on for their research efforts.

The Roy J. Carver Charitable Trust recently awarded the BTC an equipment grant dedicated to instrumentation for our Proteomic Facility. As a result, we will be able to modernize our Protein sequencing by increasing 2-D gel electrophoresis throughput to characterize and identify proteins present in complex biological samples. This new equipment coupled with the arrival of Dr. Peter Yau as Director of Proteomics and our continued access to the excellent equipment at the Mass Spectrometry Laboratory on campus will enable us rapidly expand and improve our proteomics facility.

The BTC will continue adjust its services to meet the needs of campus researchers by picking up new techniques and instrumentation platforms. I hope we can continue our close relationship with campus investigators to keep us focused on emerging technology needs. Please contact me at any time with whatever suggestions and concerns you may have.

Three UIUC Students are Sponsored Delegates To Forum

Three students from the University of Illinois, Urbana-Champaign were selected as BioVision.Nxt Fellows to attend the "Tomorrow's Bioleaders" meeting at the BioVision World Life Sciences Forum in Lyon, France. The UIUC attendees were Anne Carpenter Nye, Davyd Chung, and Norman Atkins. Eight delegates represented the United States out of the 100 worldwide BioVision.Nxt Fellows selected to participate.

Attendees participated in workshops focusing on bioethics in the Life Sciences and enjoyed networking opportunities. The meetings ran from April 7th through the 11th with world leaders such as Tony Blair, Kofi Annan, President Mubarek of Egypt and Nelson Mandela attending. In addition, 13 Nobel Prize winners gave presentations. Congratulations to the BioVision Fellows!

—K. Brinkmann

Biotechnology Center at the University of Illinois at Urbana-Champaign

Jonathan Sweedler, PhD • Director

Mark Mikel, PhD • Associate Director and Editor

Administrative Office • (217) 333-1695 phone; (217) 244-0466 (fax)

To add a subscription, change an existing subscription, or be dropped from our mailing list, please contact Mark Mikel at mmikel@uiuc.edu, or the Administrative Office. The Biotech Center News is free of charge.

Cytometric Bead Array for Cytokine Assay

Eric Deszo is a recent graduate of the Department of Animal Sciences having completed his Ph.D. work under the guidance of Dr. Gregory G. Freund. His research focused on the maturation and differentiation of the promyelocyte and the implications of this in the immune dysfunction of Type II diabetes.

Since the discovery of interferon in 1957 by Alick Isaacs and Jean Lindenmann, a burgeoning number of cytokines have been characterized. Indeed, cytokines are important to a wide variety of cellular processes such as proliferation, differentiation, and gene expression. Consequently the ability to accurately quantify cytokines is of great importance. A well-established method used to measure cytokine levels is that of enzyme-linked immunosorbent assay (ELISA). However this process requires the generation of antibody-coated wells, and the need for triplicate interrogation to produce meaningful results. In addition the entire protocol requires several days to complete. The commercial availability of antibody-coated well plates and pre-made reagents has greatly reduced the technical difficulty in ELISA. However the investment of time and increased cost of pre-manufactured reagents still makes this an expensive assay.

The Cytometric Bead Array (CBA) from Becton, Dickinson, and Company (BD) employs a collection of small spheres of discrete fluorescent intensities to detect multiple soluble proteins (See

Figure). This discrete difference in fluorescent intensity allows the detection of several solutes from a single sample through the mixing of different spheres. Each bead in the array provides a capture surface for a specific protein, which is akin to the coated surface of an ELISA well. The combined advantage of flow cytometric detection and the efficient capturing of analytes in suspension provide a significant time saving and increased dynamic range of detection when compared to traditional methods (such as ELISA). Currently, BD produces several CBA kits for both murine and human cytokine detection. Each kit is able to detect 5 to 6 different cytokines from a single, relatively small sample. In addition the sample may be serum, plasma or culture media. The protocol is simple, requiring few steps, and efficient, needing only 3 to 4 hours to generate the samples. Data is collected by flow cytometry and can be analyzed with a simple spreadsheet program such as Excel.

Flow cytometric detection of cytokines is a straightforward technique that has many advantages over that of traditional ELISA. The most advantageous aspect of the CBA assay is the ability to quantify several different cytokines from a single sample. This would require several separate ELISA plates and a significant increase in both assay time and cost. Consequently the CBA assay represents an attractive alternative to ELISA that is both cost effective and simple to perform.

—E. Deszo



Schematic of the Cytometric Bead Array analysis kit from Becton, Dickinson, and Company.

Dr. Mark Band Wins CAPE Award

Dr. Mark Band, Director of the Functional Genomics Unit of the Keck Center was recently awarded the Chancellor's Academic Professional Excellence (CAPE) award. The CAPE award is designed to honor the special accomplishments of academic professionals who perform a wide range of functions on our campus. Annually up to six awards are given. Nominees are judged on the following criteria: work, personal and professional contributions.

Dr. Band is the founding director of the Functional Genomics Unit and his leadership and skills have been instrumental in the success of many

current and future research projects by investigators on campus. Mark's laboratory excels in designing and carrying out projects in custom cDNA microarrays that enable researchers to quantify gene expression between samples for thousands of genes simultaneously. In addition, Mark has developed the popular short course "Microarray Technology" to give investigators on campus hands on training in the production and use of microarrays.

Congratulations Mark, on winning the CAPE award and thanks for your hard work and dedication.

—M. Mikel



Dr. Mark Band, Director of Functional Genomics, W.M. Keck Center.

Student Science Poster Competition—Spring 2003

To reach the editor . . .

Please send comments on the newsletter, news, or questions to the attention of the Editor, Biotech Center News.

You can reach our office by e-mail at mmikel@uiuc.edu and by fax at 217-244-0466. We enjoy hearing from you.

The 2003 Student Science Poster Competition, sponsored by Pierce Biotechnology, Inc., Kimberly-Clark Company and the Biotechnology Center Placement Office, was held in conjunction with the Biotechnology Research-Oriented Job and Information Fair on January 30th. Students from a broad range of scientific disciplines entered the Science Poster Competition in hopes of winning a cash prize of \$200. Five finalists were each selected from the undergraduate and graduate category. Posters were evaluated for visual and verbal presentation, experimental design, relevance, and the student's ability to answer questions and clearly explain their work. The 10 finalists displayed their posters at the Biotechnology Job Fair luncheon attended by company recruiters. The two winners were each awarded a check for \$200, and the finalists were each awarded \$20 gift certificates.

The winner of the undergraduate student science poster contest was Mariya Lazebnik whose poster was entitled "Functional Optical Coherence Tomography for Neural Imaging." The finalists were Freddy E. Escorcía "Investigation of an Iron-Regulated Non-Coding Small RNA in *Escherichia coli*"; Kristoff Homan "Fluorescence Lifetime Imaging: A Real-Time Diagnostic Tool";

Catherine Panozzo "Monitoring the Transmission of *Borrelia burgdorferi* in the Illinois River Valley"; and Pleasant A. Radford, Jr. "How Tropospheric Changes Alter Insect Herbivory & Leaf Chemistry in Soybean."

The winner of the graduate student science poster contest was Dawn Schmidt whose poster was entitled "*In Vitro* Evolution in the Enlase Superfamily: Changing Reactions via Single Amino-Acid Substitutions." The finalists were Sean William Deacon "Dynactin Serves as a Receptor for Kinesin II on *Xenopus* Melanosomes; Robert Kazmierczak "Regulation of Site-Specific Recombination by the Carboxyl Terminus of I Integrase"; Christopher Pierce "What Role does the Phenological Asynchrony of Corn and Soybean Development Play in the Egg-Laying Behavior of the Western Corn Rootworm Variant in East Central Illinois"; and Lili Xie "Post Translational Modifications Involved in Lantibiotic Biosynthesis."

We would like to thank Jeff King of Kimberly-Clark and Joseph LaPointe of Pierce Biotechnology, Inc., for their generous support and Kathleen Brinkmann, for organizing this exciting event.

—K. Brinkmann, M. Mikel



The Biotechnology Center
103 Observatory
901 S. Mathews Avenue
Urbana, IL 61801
PHONE: (217) 333-1695
FAX: (217) 244-0466
WEB: www.life.uiuc.edu/biotech/

Non-Profit Org.
U.S. Postage
PAID
Permit No. 75
Champaign, IL