



Third Programmatic Meeting of the NIAID Biodefense Proteomics Research Centers



Dr. Seong K. Mun, Georgetown University

The Administrative Resource Center (AC) for Biodefense Proteomics Research Centers (PRCs) hosted the third programmatic meeting of the PRC project on May 16 and 17, 2006, at Georgetown University in Washington, DC. This project is funded by the National Institute of Allergy and Infectious Diseases (NIAID).

Dr. Seong K. Mun, a professor of radiology, and director and founder of the Imaging Science and Information Systems Center, welcomed everyone to Georgetown University. Dr. Mun is an active proteomics researcher and Georgetown's Associate Vice President for Special Programs.

Investigators from each of the seven PRCs, along with staff from NIAID and the AC, attended the meeting.

The meeting featured:

- Scientific presentations by each of the PRCs
- A Scientific Working Group session (page 2)
- Progress and accomplishments of the Administrative Resource Center (page 3)
- Information about the NIAID Genomics/Bioinformatics/Proteomics Program (page 4)

For the complete meeting summary, go to <http://www.proteomicsresource.org/Meeting/May2006/default.aspx>.

This newsletter is produced by the NIAID-funded Resource Center for Biodefense Proteomics Research, whose mission is to design, develop, and maintain a publicly accessible Web site containing data and technology protocols generated by each PRC, as well as a catalog that lists reagents and resources developed by the sites and available for public distribution to the scientific community.

Proteomics Research Centers Presentations

Dr. Bruno Sobral (AC) introduced each of the seven PRCs as they presented their scientific and technological advancements of the past year.

Albert Einstein College of Medicine (AECOM)

Dr. Louis Weiss presented an overview of AECOM's study of the proteomics of waterborne parasites. Its goal is to develop an integrated approach for the identification and validation of new therapeutic drug targets for *Toxoplasma gondii* and *Cryptosporidium parvum*, two CDC category B pathogens.

Results are as follows:

- Identified several modifications to tubulin in *T. gondii*.
- Uploaded these data to its private, internal Proteomics Research Database (PORE DB), along with all mass spectrometry and antibody data, allowing researchers to

First Face-to-Face Meeting of the Scientific Working Group (SWG)



Left to right: Dr. Jonas Almeida, Dr. Abdu Azad, Dr. David Roos, and Dr. Reid Townsend

The SWG, introduced by Dr. Cathy Wu (AC), includes eight leading scientists with research expertise in the areas of proteomics, bioinformatics, and biodefense. Dr. Jonas Almeida (University of Texas M.D. Anderson Cancer Center), Dr. Abdu Azad (University of Maryland School of Medicine), Dr. David Roos (University of Pennsylvania), and Dr. Reid Townsend (Washington University School of Medicine), held their first face-to-face meeting. Dr. Roos was elected chair of the SWG for the upcoming year.

The SWG provides advice to the PRCs and the AC on current operations and future directions of the NIAID Biodefense Proteomics Research project, as well as on the needs of the community, technical advancement trends, and relevant scientific issues and efforts. The SWG assists NIAID in evaluating the progress made by the PRCs and the AC in meeting the goals of the project.

integrate predicted and experimental data, facilitate identification and validation of proteins and gene predictions, and provide a feedback mechanism for designing new experiments.

- Produced a set of 50 antibody sera specific to single identified peptides, which are made available to the research community through the Biodefense and Emerging Infectious Disease Research Resources Repository (BEI Resources). This centralized repository acquires, authenticates, stores, and distributes category A, B, and C agents to the scientific community for use in research and product development. It also produces and dispenses reagents, such as DNA clones, body fluids and cells, synthetic peptides, and monoclonal and polyclonal antibodies.

Caprion Pharmaceuticals, Inc.

Dr. Eustache Paramithiotis presented an update on Caprion's study of *Brucella abortus*. In the process of investigating what changes occur if infection were induced by an attenuated bacteria versus a virulent form, Caprion has achieved the following:

- Identified group 3 outer member proteins, which are highly abundant on the outer membrane and functionally related.
- Discovered that if a single protein of this group is deleted, the remaining proteins compensate and return full virulence to the bacteria. In nonvirulent strains, most to all of this family are absent.
- Postulated that virulence in *B. abortus* is a physiological process rather than a consequence of one protein or another.
- Compared expression patterns of differentially expressed proteins.
- Found 42 proteins, 29 of which have not been previously associated with virulence.

Harvard Institute of Proteomics (HIP)

Dr. Joshua LaBaer presented HIP's progress toward its key objectives. The objectives are to build complete clone sets for *Vibrio cholerae* and *Bacillus anthracis*, to build protein microarrays to study serum responses, and to characterize targets for vaccine and drug development. Current progress is as follows:

- HIP has produced a complete clone set for *V. cholerae*; clones are ready and available for distribution either through HIP or the Pathogen Functional Genomics Resource Center (PFGRC) at The Institute for Genomic Research (TIGR). (See page 4 for more on PFGRC.)
- Similar efforts are under way for *B. anthracis*, with approximately 100 clones produced so far.

Myriad Genetics, Inc.

Dr. Jerry Lanchbury presented the overview and update on Myriad's project, which has three parts:

- Directed yeast two-hybrid approach, in which a known organism and its proteins are fragmented into known pieces and run against human expression libraries to find protein-protein interactions that are specific for the known inputs.
- Random yeast two-hybrid approach, in which the digestion is random and generates a wide variety of pieces and sizes. These are inserted into expression vectors that support open reading frames, then run against the entire human proteome.
- Validation: The center has pathogen-specific experts for this phase.

Dr. Grant McFadden is the *vaccinia/variola* collaborator/expert, and he addressed immune system interactions with pox viruses. Findings and progress are as follows:

- Knocking out certain genes results in a virus with zero virulence. Targeting the gene products identified by the knockout study may result in a good therapy.
- The validation phase of this project is under way.

Pacific Northwest National Laboratory (PNNL)/Oregon Health Sciences University

Dr. Josh Adkins presented PNNL's work with *Salmonella* (bacterial model) and the *Orthopox* viruses (viral model). He also spoke about the accurate mass and time tag approach and ongoing work on advanced technologies.

Initial results:

- Identified 315 *Salmonella* and 371 mouse proteins. STM3117 is a newly identified protein that appears to be involved in pathogenesis. Tubulin appears to have a role in later phases of infection.
- Continued work on characterization of the *Orthopox* proteome.
- Focused on estimating false peptide identifications, identifying highly abundant proteins and whether they are unique or shared between the virulent and nonvirulent forms of the virus.
- Continued efforts to advance proteomics capabilities in order to increase data quality, proteome coverage, and proteomics throughput, as well as maximize the use of informatics.

Scripps Research Institute—Functional and Structural Proteomics of SARS-CoV (FSPS) and Related Proteins

Dr. Peter Kuhn presented for the center. The goal of FSPS is to provide a comprehensive molecular characterization and catalog of all SARS-CoV proteins, as well as associated functions at the viral and host levels in order to enable the development of therapeutic interventions for the virus. Scripps endeavors to understand the biology of SARS-CoV and its host by using a combination of cryo-electron microscopy, x-ray crystallography, and NMR spectroscopy, as well as functional proteomics.

This multidisciplinary approach to the project has led to the following advances and milestones:

- Designed and built a compact light source to aid in its structural studies.
- Produced nine publications specific for this project that are either published, in press, or in preparation for submission since 2005.
- Identified or collaborated in identifying 15 structures of the virus.

University of Michigan

Dr. Phil Hanna presented his center's research on the proteomics of anthrax infection and its mission to find new targets for intervention. The center uses microarrays, multidimensional protein identification technology (MudPIT), bioinformatics, genetic (knockout/mutant) confirmations, other *in vitro* analyses, and animal models as tools in its work.

Progress is as follows:

- Study of the early intracellular events of the establishment stage of infection is almost complete.
- Work is ongoing to complete sample collection for all stages and to analyze all samples, arrays, and MudPIT data as well as fill in gaps in the data.
- Efforts are planned to perform data mining, develop a priority target list, and perform detailed molecular validation of selected targets.

Genomic approaches, as presented by Dr. Nick Bergman, have resulted in the following:

- The center made significant findings regarding *Bacillus anthracis* gene expression and regulation of virulence factors inside the host cell.
- The center identified 3,911 macrophage and 990 *B. anthracis* proteins and associated expression levels. This work is ongoing and will include efforts to prioritize potential targets in order to reduce the list to a manageable size.

Progress and Accomplishments of the Administrative Resource Center

Kimberly ("JoJo") Stemple (DMID, NIAID) provided insight on the AC achievements since its inception. The AC is composed of three separate entities (Social & Scientific Systems, Inc.; Georgetown University; and the Virginia Bioinformatics Institute) working together to build a seamless, integrated Web site that contains data and related information generated by the PRCs. The goal of this work is to facilitate effective communication and collaboration among the PRCs, NIAID, and the research community.

Margaret Moore and Dr. Raja Mazumder presented an overview and a case study of how the Web site may be used as a research tool. The Proteomics Project Catalog consists of two directories that provide searchable access to data and reagents from this project. These directories contain a listing of output as well as information regarding where each item resides. The data directory can be searched for summaries of submitted data, the experimental data itself, specific proteins identified, and the associated metadata. The reagent directory lists how and where to obtain a reagent used to generate that (or other) data. For this project, a reagent is any substance used in the production of the data and may be an input or a result of the proteomic investigation. Therefore, solutions, buffers, and enzymes, as well as c-DNA clones, all qualify as reagents in this project. However, only unique reagents will be stored at BEI Resources because most common reagents can be acquired "off the shelf" or modified as detailed in data-associated publications.

Drs. Peter McGarvey (AC) and Stephen Cammer (AC) demonstrated the various tools available on the integrated Web site, including visualization tools, and showed how the scientific community can use them in their own research. Examples of specific data types and some of the ways that the catalog and visualization tools may be used by the PRCs and the user community were displayed.

The PRCs' Progress and Data Dissemination

Dr. Joseph Breen (DMID, NIAID) offered an overview of the seven PRCs' accomplishments and goals. Over the past year, they have transferred data, lists of reagents, and experimental details to the AC for posting on the project Web site as a resource for the scientific community. Dr. Malu Polanski (DMID, NIAID) presented the Data Release Worksheet that NIAID uses for this program and emphasized that the success of this project depends on the expediency of making data, protocols, reagents, and information available to the scientific community via the AC Web site or the linkable repositories.

Immune Epitope Database and Analysis Resource (IEDB)

Dr. Tim Gondré-Lewis, project officer for the IEDB, gave an overview of this NIAID resource, pointing out that the primary objectives of this program are to design, develop, populate, and maintain a comprehensive immune epitope database containing antibody and T-cell epitopes, with an emphasis on epitopes associated with category A, B, and C priority pathogens and their toxins; and to develop and maintain an analysis resource to complement and enhance the IEDB. Dr. Gondré-Lewis believes that this potentially could be a very useful resource for the Proteomics project.

Additional NIAID Genomics/Bioinformatics/Proteomics Programs

Dr. Maria Giovanni (DMID, NIAID) presented a review of the NIAID's Genomics Program. The mission of the program is to support basic and applied research to prevent, diagnose, and treat infectious and immune-mediated illness, including HIV/AIDS and other sexually transmitted diseases, illness from potential agents of bioterrorism, tuberculosis, malaria, autoimmune disorders, asthma, and allergies. NIAID's infectious disease research has a dual mandate to maintain and grow a robust basic and applied research portfolio in microbiology, immunology, and clinical research, and to respond rapidly to new infectious disease threats.

NIAID's program includes:

Pathogen Functional Genomics Resource Center (PFGR) The PFGR is based at The Institute for Genomic Research (TIGR) and offers the following resources: organism-specific microarrays and protocols, protein expression clones, genotyping/genome analysis, development of computational tools for array and comparative genomic data analysis, and technology development. The center's goal is to develop and distribute genomic reagents, resources, and technologies at no cost to the public.

<http://www.niaid.nih.gov/dmid/genomes/pfgrc/default.htm>

Microbial Genome Sequencing Centers There are two genome sequencing centers that have the capacity to sequence genomes for other Government agencies and the scientific community, and to respond to national emergencies. The Microbial Genome Sequencing Centers' goal is to produce high-quality genome sequences of human pathogens and invertebrate vectors of disease rapidly and cost-efficiently.

<http://www.niaid.nih.gov/dmid/genomes/mscs/default.htm>

Bioinformatics Resource Centers (BRCs) The centers provide a robust point of entry for access of genomic and related data to the scientific community, including a database of genomic and related data and an analysis center that develops and provides software tools. The centers have also built a multidisciplinary team of bioinformatics and database experts and biologists and domain experts.

NIAID/DMID site: <http://www.niaid.nih.gov/dmid/genomes/brc/default.htm>

BRCs central site: http://www.brc-central.org/cgi-bin/brc-central/brc_central.cgi

Proteomics Research Centers (PRCs) As highlighted in this newsletter, the project goals are to characterize the pathogen and/or host cell proteome, identify proteins associated with the biology of microbes, and identify the mechanisms of microbial pathogenesis and host response to infection. The PRCs are also involved in developing proteomic technology and discovering potential targets for the next generation of vaccines, therapeutics, and diagnostics. Data, catalogs of reagents, and methods developed by the PRCs are accessible to the public on the project Web site.

Administrative Resource Center site: <http://www.proteomicsresource.org/default.aspx>

Information about each PRC: <http://www.proteomicsresource.org/PRC/About.aspx>