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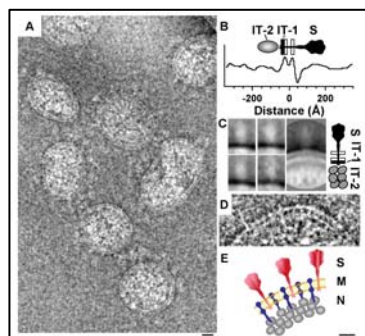
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*"Nothing tends so much to the advancement of knowledge as the application of a new instrument."*

- Sir Humphrey Davy, chemist (1778-1829)

**Proteomics Resource News** is a quarterly newsletter that features the work of the NIAID-funded Biodefense Proteomics Research Centers (PRCs). The PRCs conduct research to characterize proteomes of pathogen and host cells. This includes identifying proteins associated with microbial biology, elucidating mechanisms of microbial pathogenesis, and understanding host responses to infection. The ultimate goal of the PRCs is to discover targets for potential candidates for the next generation of vaccines, therapeutics, and diagnostics. This newsletter is produced by the Biodefense Proteomics Administrative Resource Center whose mission is to design, develop, and maintain a [publicly accessible Website](#) 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 distribution to the scientific community. In this edition, we feature the work of [The Scripps Research Institute](#), one of the Proteomic Research Centers participating in this project.

## Functional and Structural Proteomics analysis of SARS-CoV related proteins (FSPS)



Supramolecular design of SARS-CoV. Cryo-EM of frozen-hydrated pleomorphic SARS-CoV particles

In spring 2003, the world learned of an outbreak of a newly recognized pneumonia that was named Severe Acute Respiratory Syndrome (SARS). The outbreak is thought to have first emerged in southeastern China's Guangdong province in November 2002, with subsequent spread to Hong Kong SAR (February 2003) and other countries including Vietnam, Taiwan, Singapore, Canada and the United States. Epidemiologic investigation showed that the disease disproportionately affected healthcare workers and other close SARS patient contacts, such as family members. Higher mortality has been observed in older patients, and in patients with comorbid conditions such as diabetes, lymphopenia, and liver dysfunction. A novel coronavirus was identified as the cause of SARS (SARS coronavirus or SARS-CoV). In light of the potential re-emergence of the SARS coronavirus (SARS-CoV), a comprehensive functional and structural characterization of the virus is essential.

The FSPS team's goal, funded through NIH-NIAID, is "to provide a comprehensive molecular characterization and cataloging of all SARS-CoV related proteins and associated functions at the viral and host levels." The following approaches are used to attain this goal:

- Determination of 3D structure of the proteins and their complexes using high-throughput X-ray crystallographic techniques. These studies are funded by the NIAID Proteomics Research Centers contract and carried out in the laboratories of Professor Peter Kuhn, Professor Ray Stevens, and Professor Ian Wilson. All three were involved in the initial creation and operation of the Joint Center for Structural Genomics (JCSG), an NIH Protein Structure Initiative sponsored project. FSPS builds upon and extends the automated structural proteomics pipeline pioneered by these investigators. In addition, FSPS benefits from the results of ongoing R&D projects in the Kuhn-Stevens laboratory including the Accelerated Technologies Center for Gene to 3D Structure (ATCG3D) and the Joint Center for Innovative Membrane Protein Technologies (JCIPT).
- Determination of 3D structure of the proteins and their complexes using high-throughput NMR techniques. These studies are carried out in the laboratory of the Nobel Laureate Kurt Wüthrich.
- Determination of the function and biological role of SARS-CoV and related proteins. This includes the characterization of the life cycle and host response, the identification and characterization of protein-protein interactions, and the identification and characterization of binding ligands. These studies are carried out in the laboratory of Professor Michael Buchmeier. In addition, functional studies are using proteomics tools, particularly the nano-calorimeter, developed by the [Scripps-PARC Institute for Advanced Biomedical Sciences](#).

More information about FSPS, including mission, background and how to get involved is available at <http://sars.scripps.edu/index.htm> ◇

**Favorite Site****beiresources**[www.beiresources.org](http://www.beiresources.org)

The National Institute of Allergy and Infectious Diseases (NIAID) conducts and supports research aimed at developing new and improved tools against potential [agents of bio-terrorism](#) or organisms that cause [emerging diseases](#) such as [SARS](#), [West Nile virus](#), and [Lyme disease](#).

BEI Resources was established by NIAID to acquire, authenticate, and produce reagents, provided for the cost of shipping and handling to eligible investigators, that scientists need to carry out basic research and develop improved diagnostic tests, vaccines, and therapies.



## Biomedical structural proteomics at FSPS

### Sample Production

An expression-ready clone library has been created and maintained containing all known and predicted coding regions for most available strains. The library has been constructed using experimentally validated clones for *E. coli*, baculovirus and mammalian expression systems and is updated with publicly released information. Sequencing of clone products ensures quality assurance. Clones from the FSPS library are used for protein production through primary expression strategies and predefined alternate pathways. Sufficient amounts of soluble folded proteins, domains, and other constructs are expressed and purified for functional and structural analysis. Analytical and biophysical characterization approaches are used to ensure high quality products.

### Structural Proteomics

The structures and substructures of SARS-CoV proteins and complexes are being determined by using either NMR or X-ray crystallography. The method used is based on size and protein behavior. Rapid optimization strategies through variation in clones, expression systems, and crystallization conditions enhance the likelihood of successful structure determination. In addition, a set of pathways outside the traditional automated structure determination pipelines have been developed for more difficult structures, such as glycosylated and membrane-bound proteins. All structures are fully validated, annotated, and released according to established standards of the [NIH Protein Structure Initiative](#). To date FSPS has solved structures for six SARS-CoV protein constructs and has assigned fold and/or function to the six Open Reading Frames.

### Functional Proteomics

Several approaches are used to probe the function of each protein and its interactions, such as the characterization of the life cycle and host response; identification and characterization of protein-protein interactions, and identification and characterization of ligands. Cryo-electron microscopy and iterative single-particle image reconstruction is used to produce 2-D and 3-D maps of viral coat protein complexes from formalin-inactivated native and fusion-activated virus preparations. The effects on the viral lifecycle and intracellular host response for each SARS-CoV protein will be defined using a combination of viral cDNA cloning, site-directed mutagenesis, antisense functional mapping and microarray-based functional mapping using live virus produced from cDNA knockouts. In addition, SARS-CoV cellular receptors and the entry process are being identified and characterized using ligand "fishing" techniques (e.g. TAP-TAG approaches).

### Computational Biology

FSPS continues to collect and integrate existing data to derive hypotheses that are being experimentally tested utilizing the methods in sample production, functional proteomics, and structural proteomics. These bioinformatics services support the pipeline of activities from design of primers to structural analysis and are done through sequence analysis that facilitates the *in silico* discovery phase of functional proteins, protein domains and substrates/cofactors/inhibitors, and large scale virtual docking to identify possible inhibitors of solved structures. Bioinformatics will additionally be used to reconstruct essential pathways and important networks involving SARS-CoV proteins, to produce a prioritized list of possible drug/vaccine targets, and to interact with the public data resources to optimize dissemination of data to the public. ♦



Structure of the ADRP domain of SARS-CoV nsp3. FSPS studies confirmed its phosphatase activity



### What's Happening ...

**First installment of data from NIAID Proteomics Research Centers now available.** See our [Special Edition](#) for details or go to the [Project Catalog](#).



Cathy Wu, Co-PI of the Administrative Center and HUPO council member, to speak at HUPO in July. She will report recent bioinformatics database/catalog developments in the NIAID Administrative Resource Center and Proteomics Research Centers programs.

### Upcoming Meetings ...

**17th International Mass Spectrometry Conferences**

Prague, Czech Republic  
August 27-September 1, 2006

**HUPO 5th Annual World Congress: Translating Proteomics from Bench to Bedside**

Long Beach, California  
October 28-November 1, 2006

### PRC Publications ...

**The *ditABCD* operon of *Bacillus anthracis* *sterne* is required for virulence and resistance to peptide, enzymatic, and cellular mediators of innate immunity** - *J Bacteriol.* 2006 Feb; 188(4):1301-4 [PMID: 16452412] University of Michigan

**Characterization of *Bacillus anthracis* germinant receptors *in vitro*** - *J Bacteriol.* 2005 Dec; 187(23):8055-62 [PMID: 16291679] University of Michigan

**In Situ data collection and structure refinement from microcapillary protein crystallization** - *J. Appl Cryst.* 2005 Dec; 38:900-905. The Scripps Research Institute

**Structural basis of severe acute respiratory syndrome coronavirus ADP-ribose-1"-phosphate dephosphorylation by a conserved domain of nsP3** - *Structure.* 2005 Nov; 13(11):1665-75 [PMID: 16271890] The Scripps Research Institute