BIOTECHNOLOGY
CAPABILITIES

Sandia National Laboratories

PROTEOMICS

Membrane Protein System and Structural Proteomics

In the area of Mass Spectrometry for Protein Structure Function Analysis, Sandia has established protein expression, protein chemistry, and separations and mass spectrometry methods for studying membrane protein structure/function relationships and protein/protein interactions. Membrane systems are of particular importance in cell signaling and pathogenesis, but they present severe challenges to existing structure/function approaches. We are able to express proteins, reconstitute them in proteoliposomes, expose them to a variety of commercial and proprietary cross linkers and reagents and a range of proteolysis protocols, and analyze resulting peptide signatures with LC/MS methods. The resulting data allow three-dimensional distances between residues to be derived in different functional states of the protein. These methods are used to study the mechanism of actions of toxins and key signaling proteins, with applications in the areas of bioterrorism countermeasures, pharmaceutical development, biotechnology, and biosensors.

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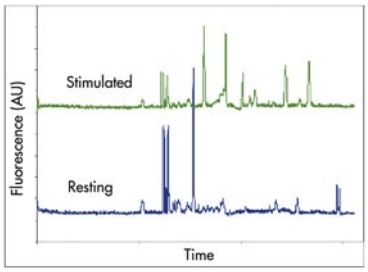
Cellular Signaling

We are building on Sandia's strengths in micro-scale separation and detection techniques (μChem Lab™ Program) and applying these capabilities to the study of the molecular events associated with cell signaling. Such molecular events include changes in the phosphorylation state of key proteins. We are employing a proteomics approach in which we selectively collect the phosphorylated proteins from cultured cells by affinity chromatographic techniques and subject them to a variety of sensitive and micro-scale separation techniques. We seek to use the separation patterns or "fingerprints" of phosphorylated proteins as a diagnostic tool for the state of the cells. A specific application for this work is the development of a rapid diagnostic tool for pathogen exposure, although the fingerprinting of phosphorylated protein levels has broader implications in the cell-signaling and biomedical communities.



Fingerprinting of Signaling Proteins: Capillary gel electrophoresis displays differences in the phosphoprotein populations from resting and stimulated immune cells.

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Microfabricated Integrated Protein Analysis Systems

Microfabricated systems are attracting significant attention in the areas of proteomics, national security, and health care because of their portability, speed of analysis, potential for multiplexing and high throughput, and ability to analyze

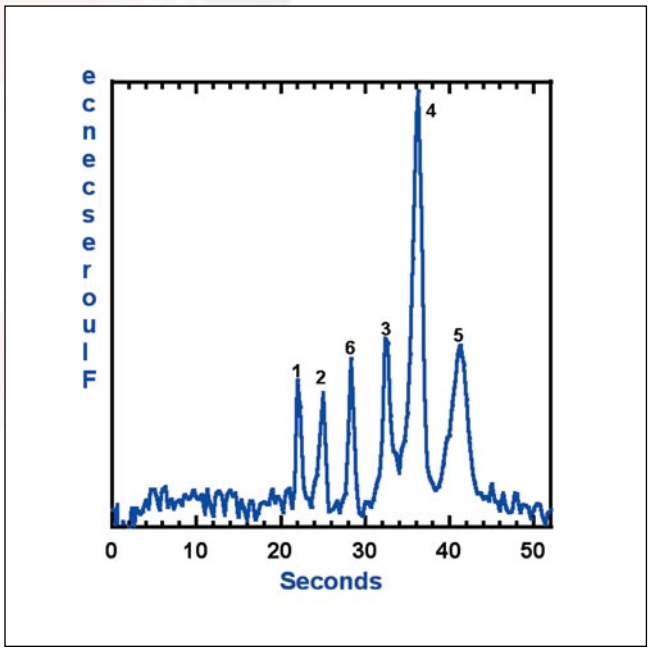
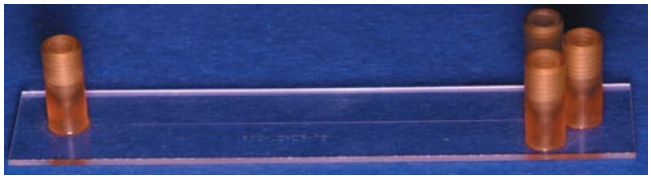


Fig 1: Separation of 6 bioactive peptides in a microchip in less than 45 seconds. (1) papain inhibitor, (2) proctolin, (3) Casein fragment 90-95, (4) Ile-angiotensin III, (5) angiotensin III, (6) Gly-Gly-Gly.

minute sample volumes. Sandia is developing integrated microchips for rapid separation and detection of minute levels of proteins and peptides with the goal of application in biotoxin detection and proteome analysis.

The integrated module will consist of three elements 1) a preconcentrator to concentrate proteins by 2–3 orders of magnitude, 2) a separation channel for fast and efficient separation; and 3) a microfluidic valve to selectively isolate proteins of interest for further analysis by another module. Modules can be combined to achieve multidimensional



A glass microchip for chromatography.

analysis. A number of on-chip approaches have been developed for separation of proteins and peptides including zone electrophoresis, gel electrophoresis, isoelectric focusing, and chromatography.

We have recently demonstrated rapid (6 peptides in less than 45 seconds) and high-efficiency (up to 600,000 plates/m) chromatographic separations in microchips using photopatterned porous polymer monoliths (Fig 1). The chips being used are fabricated in glass and fused silica and UV light-iniated polymerization is used for patterning of polymer in channels for optimal design of multi-functional

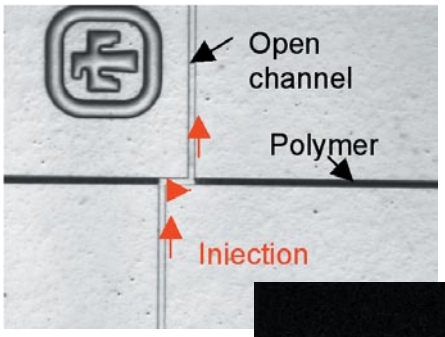


Figure 2

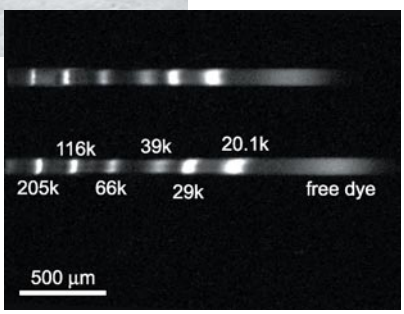


Fig 3: SDS-PAGE separation of six proteins molecular weight markers (20.1-205 kD) in a microchip in 25 seconds using photopolymerized polyacrylamide.

and multi-dimensional analysis systems (Fig 2). Efforts are also under way to miniaturize 2-D gel electrophoresis, the most common method of analyzing complex protein mixtures such as cellular protein content to a microchip format where proteins are first separated by isoelectric focusing (IEF) and then by capillary gel electrophoresis (CGE) in orthogonal parallel channels. We have developed chip-based IEF and CGE (Fig 3) using very short channels (<5 mm) that require less than 30 seconds to perform separation of proteins.

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LOCKHEED MARTIN

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Modeling of 3D Structures of Membrane-Associated Complexes

Sandia/CA has an active research program for modeling the 3D structures of membrane-associated complexes by adapting a technique called MS3D to specifically address integral membrane proteins. MS3D uses distance constraint information derived from intramolecular crosslinking, proteolysis, and mass spectrometry experiments to construct a 3D model of a protein structure. We plan to use MS3D to derive distance constraints within and between proteins in membrane-associated complexes. These distances are integrated with theoretical information, such as helical packing preferences and genomic information, to produce model structures that are consistent with experimental data. The IBIG Grand Challenge and other related LDRD programs at Sandia are currently validating MS3D on a well-characterized integral membrane protein of known structure, bacteriorhodopsin, and are mapping protein-protein interactions in the light-activated rhodopsin-transducin complex.

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Prion Detection and Discrimination by Multi-Spectral Ultraviolet Fluorescence

Sandia has been evaluating the use of multi-spectral UV fluorescence as a means of detecting and distinguishing between different forms of PrPSc, the protein associated with spongiform encephalopathies such as scrapie and BSE (Mad Cow disease). Spectroscopic measurements of fluorescence from PrPSc purified from 263K scrapie strain infected hamsters and ME7 scrapie strain infected mice were made. The spectral signatures from the protein and calculations of the fluorescence cross section indicate that detection and discrimination are feasible by this method. This technique has the potential for rapid, specific, and direct detection and discrimination of the protein at extremely low concentrations.

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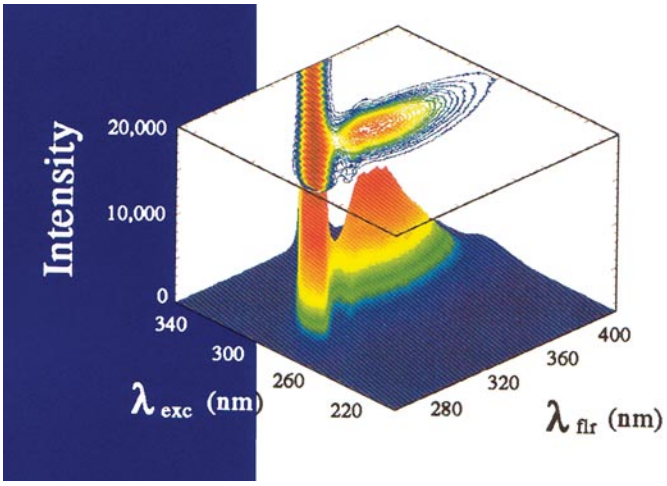
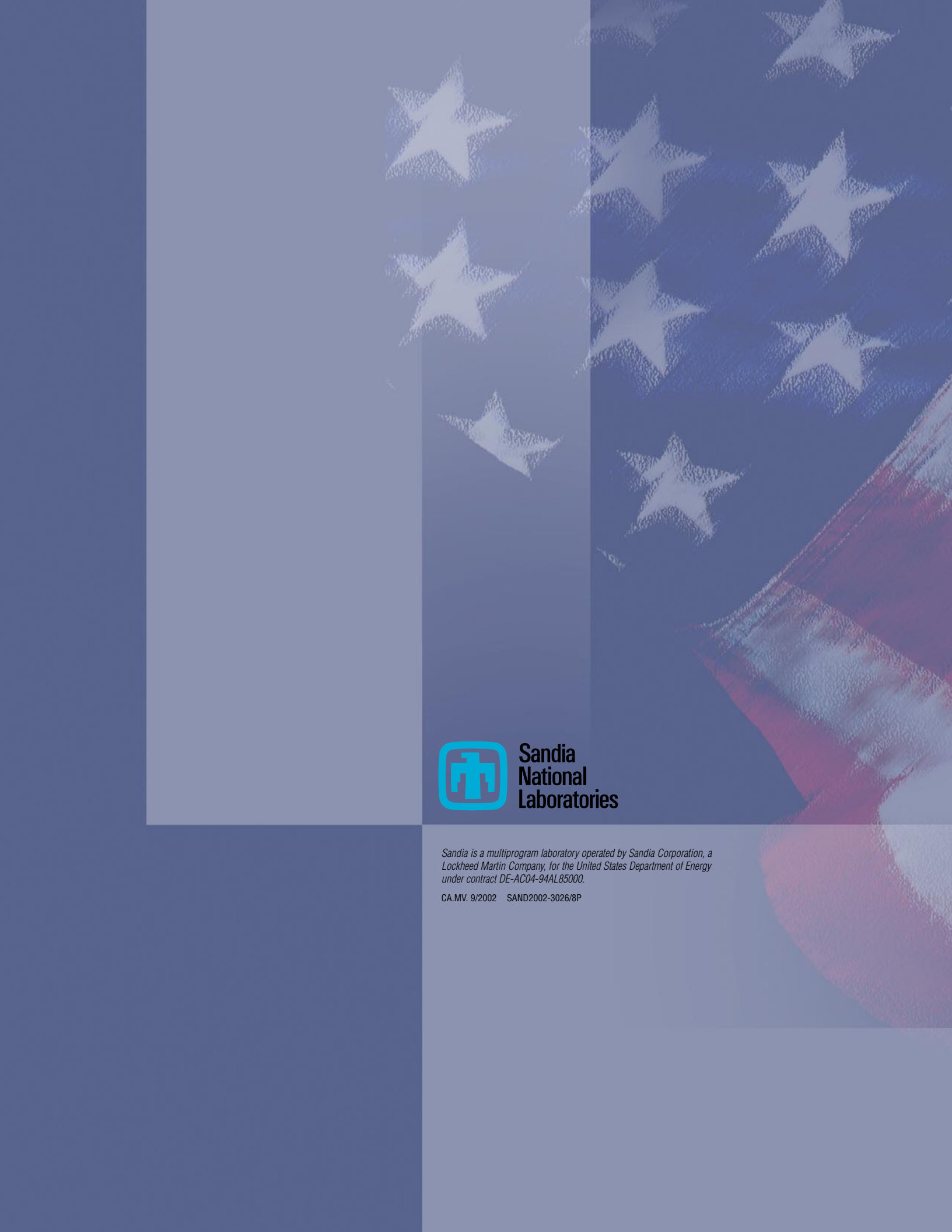


Multispectral ultraviolet fluorescence signature of E. coli suspended in phosphate buffered saline solution.



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